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# Pasteurellosis in farm animals

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**Abstract**. The purpose of this study was to investigate the biological properties of *Pasteurella multocida* strains circulating among farm and wild animals in Kazakhstan, particularly saigas, to optimise the diagnostics and prevention of pasteurellosis. The study employed bacteriological, microscopic, and biochemical methods to analyse 20 samples of biomaterial collected from saigas and farm animals: 10 samples from specimens from Torgai village (Dzangeldy district, Kostanay region, Kazakhstan) and 10 samples from specimens from Kaztalov district (West Kazakhstan region, Kazakhstan). The study found that all isolates (100%, n = 60) belonged to *Pasteurella multocida* biovar *ovis*: greyish-white colonies 1-2 mm in diameter were formed on meat-peptone agar

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without haemolysis, gram-negative bipolar rods were detected in smears, and fermentation of glucose, sucrose, mannose, and trehalose was accompanied by the formation of acid without gas (in 100% of cases). Bioassays on 10 white mice (weight 16-20 g) showed 100% mortality within 48 hours after injection of 0.2 ml of culture, with haemorrhages in the heart, liver necrosis, and exudate in the abdominal cavity. Statistical analysis (Fisher's test) revealed no significant differences between the 2015 and 2023 isolates ($p > 0.05$), confirming the circulation of a stable clone. The findings reflect the key role of saigas as a reservoir of infection and the need for integrated measures: aerosol vaccination during migrations, introduction of polymerase chain reaction diagnostics, and antibiotic resistance monitoring. The data obtained are the basis for the development of regional antiepizootic strategies aimed at reducing risks to agriculture and conservation of wildlife populations in Kazakhstan

**Keywords:** saigas; epizootics; isolates; meat-peptone agar; bioassays

## INTRODUCTION

Pasteurellosis continues to be one of the most dangerous infectious diseases of farm and wild animals, causing extensive economic damages due to massive livestock losses and treatment costs. This problem is particularly acute in regions with intensive livestock production, where outbreaks of the disease can lead to food security issues. In Kazakhstan, where the agricultural sector plays a key role in the economy, pasteurellosis poses an extra threat due to the migration of saigas, a species that is a natural reservoir of *Pasteurella multocida*. The increase in cases of the disease among wild populations, combined with the lack of comprehensive data on the biological properties of circulating strains, limits the development of effective anti-epizootic strategies.

Research in 2020-2025 highlighted the genetic diversity of *Pasteurella* and their ability to adapt to different ecological niches. Z.K. Buienbayeva *et al.* (2023) found that strains isolated from saigas have unique biochemical profiles compared to samples from domestic animals. This fact suggests intraspecific microevolution of the pathogen, which increases its adaptive potential. Such differences, according to S.J. Hasani *et al.* (2024), complicate the creation of universal vaccines. This is of key significance in regions with biodiversity of livestock and wildlife, where the pathogen circulates between different hosts. In Saudi Arabia, F.A. Alarawi and E.M. Saeed (2021) showed that even within the same animal species (e.g., sheep), pasteurellosis can cause distinct clinical forms, which is associated with the specificity of local strains. These findings reflect the need for a territorial approach to the analysis of the pathogen, which accounts for both genetic and epidemiological factors. In Central Asia, particularly in Uzbekistan, I.D. Sheraliyeva (2022) found a link between epizootics among sheep and changes in climatic conditions that intensify the transmission of the pathogen. This highlights another risk factor – global warming, which changes the seasonal dynamics of the spread of infections.

However, existing studies focus mainly on certain aspects of pasteurellosis, from molecular biology to clinical treatment. M. Umitzhanov *et al.* (2024) analysed the biochemical characteristics of *Pasteurella* in birds, while F. D'Amico *et al.* (2024) detailed the pathogenicity of strains in rabbits. However, there is a lack of studies that integrate epizootic, microbiological, and environmental data for concrete regions. For example, in Kazakhstan, where saigas are a key component of the ecosystem, the lack of a comprehensive analysis of their role in the spread of pasteurellosis limits the understanding of the mechanisms of infection transmission. It is particularly significant to investigate the virulence of strains. Experiments on laboratory animals conducted by T.D. Costa *et al.* (2023) revealed that even strains with low pathogenicity can cause systemic complications under certain conditions, especially in the presence of immunosuppression or stress factors. This indicates the potential of *Pasteurella* for latent persistence with subsequent reactivation. At the same time, M. Boulianne *et al.* (2020) noted that the effectiveness of existing diagnostic methods depends on the geographical origin of the pathogen, which confirms the need for localised studies. In Kazakhstan, according to Z.K. Buienbayeva (2024), even standard protocols for bacteriological analysis must be adapted due to the characteristics of local strains. This requires a review of existing methods, particularly in terms of the composition of culture media and test interpretation.

The current epidemiological situation in Central Asia demonstrates that the circulation of the same strain between domestic and wild animals is a real threat of interspecies transmission (Tursumbetov *et al.*, 2024). Particular attention should be paid to the areas of seasonal migration of saigas, which may be a mechanism for the transfer of the pathogen between natural and farmed ecosystems. The absence of systematic monitoring of this process limits the ability to identify epizootic nodes promptly and prevent their consequences. Thus, despite a considerable amount of accumulated data, the issues of interaction of *Pasteurella multocida* with different animal species in the specific environmental conditions of Kazakhstan are still unresolved. Comparison of the obtained findings with the data of reference strains allows determining the biovar affiliation of isolates and assess the level of virulence, which is key to the development of a national strategy for the specific prevention of pasteurellosis in Kazakhstan. The purpose of the present study was to comprehensively investigate the properties of *Pasteurella multocida* isolated from farm animals in Kazakhstan to optimise diagnostics and prevention.

## MATERIALS AND METHODS

The study was conducted in the bacteriology laboratory of the LLP "Kazakh Scientific-Research Veterinary Institute" (KazSRVI LLP, Kazakhstan) from 22 February to 2 March 2023. The object of the study included 20 samples of biomaterial collected from saigas and farm animals: 10 samples from specimens from the village of Torgai (Dzangeldy district, Kostanay region, Kazakhstan) and 10 samples from specimens from Kaztal district (West Kazakhstan region, Kazakhstan). The sample included animals with clinical signs of pasteurellosis (fever, haemorrhagic lesions, necrosis) and excluded samples from healthy individuals or animals with symptoms of other infections. Meat-peptone agar and meat-peptone broth manufactured by Sigma-Aldrich (USA), as well as Hottinger's agar and broth with 1% glucose (Merck, Germany) and 10% bovine serum (Thermo Fisher Scientific, USA) were used. For serological analysis, the study used an erythrocyte pasteurellosis antigenic diagnostic kit (series 010422 B/K No. 210, expiration date: 20.04.2024, manufactured by KazVetPharm, Kazakhstan). Biochemical studies were performed using Hiss kits for carbohydrate fermentation (HiMedia, India). Microscopic analysis was performed using an Olympus CX23 light microscope (Olympus Corporation, Japan) with an immersion system; Gram stain (Coico, 2006) (Bio-Rad, USA), and Romanowsky-Giemsa stain (Kuhlmann, 2020) (Sigma-Aldrich, USA) were used to stain the smears.

The pathological material was inoculated on meat-peptone agar, meat-peptone broth, and Hottinger's medium, and then incubated at 37°C for 24-48 hours. The cultural and morphological properties of Pasteurella (colony shape, presence of capsules) were examined after obtaining isolated cultures. After incubation, Gram-stained smears (Coico, 2006) (to detect Gram-negative bacilli) and Romanowsky-Giemsa microscopy (to visualise bipolar staining) were performed (Kuhlmann, 2020). Biochemical activity was studied by inoculating cultures on Hiss medium with glucose, sucrose, and other substrates; carbohydrate oxidation was assessed by changing the colour of the indicator (acid formation without gas). Catalase activity was determined by applying a 3% hydrogen peroxide solution to the surface of the cultures: a positive reaction was accompanied by the release of oxygen bubbles. To detect hydrogen sulphide, filter paper moistened with lead acetate (Sigma-Aldrich, USA) was used, and to detect indole – paper moistened with a saturated oxalic acid solution (Merck, Germany). Additionally, bacteriological analysis of pathological material from saigas that died during the 2015 pasteurellosis epizootic was conducted. Organ samples (heart, liver, spleen) were taken from each of the 20 animals for the study, a total of 20 samples.

The virulence of the isolated strains of *Pasteurella multocida* was studied in vivo in BALB/c white mice (Charles River Laboratories, USA), which were kept under standard laboratory conditions. Ten outbred mice aged 6-8 weeks and weighing 16-20 g were used. The animals were divided by randomisation into three experimental groups: two experimental and one control. In groups 1 and 2, animals were injected subcutaneously into the back area with 0.2 $cm^3$ of daily broth culture of *Pasteurella multocida* isolates 1 (from a farm animal) and 2 (from a saiga), respectively. The third group was left intact (without inoculation) and served as a negative control. The observation lasted for 10 days with daily clinical monitoring of general condition, activity, appetite, coat condition, mucous membranes, and registration of deaths. Statistical data processing was performed using Fisher's test (to compare the frequency of positive results between groups). Animal experiments were performed following the requirements of the Veterinary Legislation of the Republic of Kazakhstan (Law of the Republic of Kazakhstan No. 339-II, 2002) and the World Veterinary Association Position Statement on the Humane Care of Animals in Biomedical Research (2023). Animal experiments were conducted according to the Directive of the European Parliament and of the Council No. 2010/63/EU (2010).

The study also included archival samples (n = 20) collected during the pasteurellosis epizootic among the saiga population in May 2015 in the Turgai Basin (Kostanay region, Kazakhstan). Biomaterials (liver, heart, spleen) were collected by specialists of KazSRVI LLP. The samples were delivered to the laboratory of KazSRVI LLP (Kazakhstan) and, after initial identification, preserved in liquid nitrogen cryostorage at -196°C. In 2023, these samples were revitalised by inoculation on Sigma-Aldrich meat-peptone agar (USA) and Hottinger agar (Merck, Germany) with bovine serum (Thermo Fisher Scientific, USA), followed by incubation at 37°C for 24-48 hours. The isolated cultures were re-checked for sterility, homogeneity of morphology, and absence of extraneous microflora. Further procedures for morphological analysis, biochemical testing, and bioassays on mice were performed using identical protocols as for the 2023 samples. This ensured comparability of results between the two-time samples, enabling a retrospective analysis of the stability of the biological properties of the isolates in time and geography.

## RESULTS

The first stage of the study was to investigate the culture properties of *Pasteurella multocida* isolates obtained from saiga and domestic animals in 2015 and 2023. After 24-48 hours of incubation, a uniform turbidity of the medium without the formation of sediment or film was observed in meat-peptone broth, which is typical for this type of bacteria and indicates the high adaptability of the microorganism to classical nutrient media (Fig. 1). Round, convex, translucent colonies of greyish-white colour with a shiny surface were formed on meat-peptone agar.

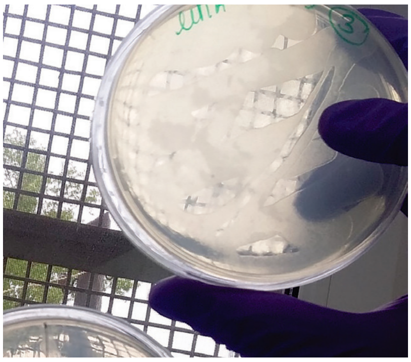

***Figure 1**. Growth of Pasteurella multocida on meat-peptone agar*
***Source:** created by the authors*

Microscopic examination of smears made from daily cultures and stained with Gram stain revealed small Gram-negative rods or coccobacilli with a clear bipolar tinctoriality. The bacteria were mostly isolated or formed small clusters in the form of chains or aggregates, which was also typical for this species. The presence of morphological homogeneity of cells in the field of view indicated the purity of the culture and a high degree of identification reliability. Figure 2 presents a typical microscopic image illustrating these diagnostic features. This tinctoriality, combined with morphological homogeneity, served as a key diagnostic feature for the initial recognition of the culture. Additionally, some Romanowsky-Giemsa-stained smears showed pronounced bipolar staining patterns, which further enhanced the differential diagnostic value of morphological analysis.

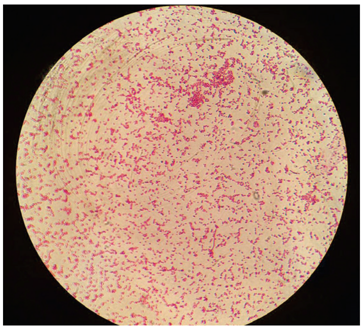

***Figure 2**. Gram-stained daily culture*
***Source:** created by the authors*

A particularly significant diagnostic feature was the absence of zones of haemolysis when the isolates were cultured on blood agar. In contrast to the haemolytic active species *Pasteurella haemolytica*, which forms pronounced zones of β-haemolysis, the isolates recovered from farm animals did not cause any visible changes in the erythrocyte layer. This allowed them to be reliably identified as *Pasteurella multocida* even at the initial stage, before more in-depth biochemical or molecular verification. Cultures isolated from these organs demonstrated stability of culture and morphological properties. Regardless of the type of biological material, all isolates showed a characteristic growth pattern, indicating the absence of microbial contamination by foreign cultures, as well as high conservation of the morphological and physiological properties of the pathogen. The isolation of pure cultures from different sources and animal species showed a wide range of *Pasteurella multocida* carriage, which is a serious threat to both the agricultural industry and biodiversity conservation. Thus, the first stage of the study – isolation and primary characterisation of *Pasteurella multocida* isolates – confirmed the presence of an epizootic strain circulating among animals of different taxonomic groups and demonstrating stable cultural and morphological characteristics inherent in the species.

The study found that all isolated Pasteurella isolates (n = 60) actively fermented a number of carbohydrates: glucose, galactose, sucrose, mannose, fructose, and trehalose. The results of the reaction were accompanied by a change in the colour of the indicator in the test tubes, indicating the formation of organic acids as fermentation products. All isolates demonstrated the same reaction, which was accompanied by a change in the colour of the indicator in the test tubes, reflecting the formation of organic acids as the primary metabolic product. Therewith, no gas was produced as a secondary metabolite, which is a typical property of *Pasteurella multocida* and allows differentiating it from some other enterobacteria capable of gas production. On the contrary, substrates such as lactose, rhamnose, raffinose, maltose, salicin, dulcit, and glycerol in the studied isolates were not fermented. This was confirmed by the preservation of the initial colour of the medium, suggesting the absence of acid production. Such a pattern of biochemical activity helped to clearly define the boundaries of the isolates belonging to the genus Pasteurella and to distinguish them from related species (*Pasteurella haemolytica*, *Pasteurella pneumotropica*), as well as from other Gram-negative facultative anaerobic bacteria that may be present in pathogenic material as concomitant or opportunistic pathogens. The high conservation of the biochemical properties of the isolates was confirmed by their comparative identification with the reference strain *Pasteurella multocida* B-0229, which is stored in microbiological collections. For comparison, two isolates were selected – No. 1 (from a farm animal) and No. 2 (from a saiga), which showed full compliance with the biochemical profile of the reference strain

(Fig. 3). This allowed identifying them as representatives of the species *Pasteurella multocida*, biovar *ovis*. This result is of major epizootological significance, as it confirms the circulation of a single stable strain with identical properties in animal populations in fundamentally different ecological niches.

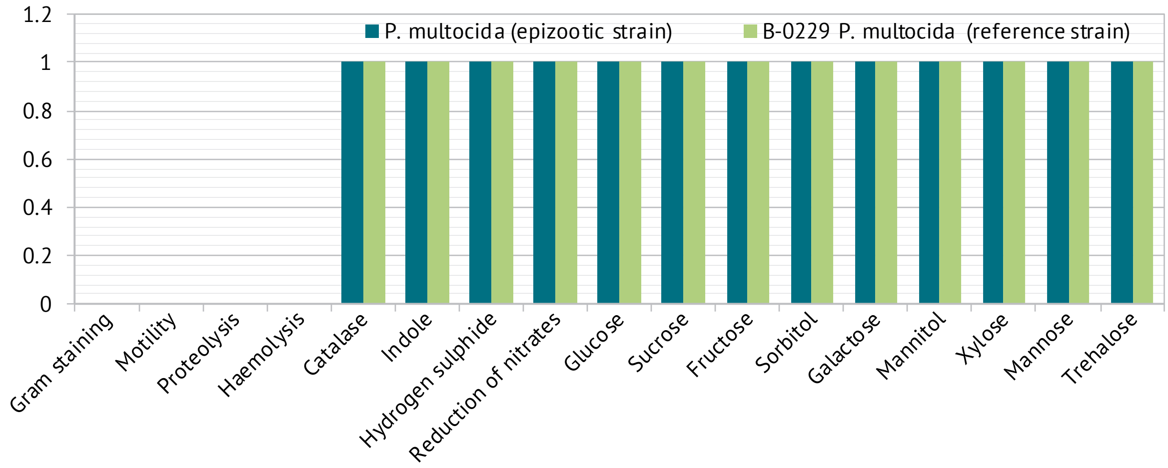


***Figure 3***. *Comparative biochemical characterisation of epizootic and reference strain isolates*
***Source:*** *created by the authors*

The analysis of the biochemical characteristics of *Pasteurella multocida* biovar *ovis* isolates presented in Figure 3 indicates that their metabolic profile closely resembles the reference strain B-0229 stored in microbiological collections. The epizootic isolates were Gram-negative, non-motile, did not show proteolytic activity (did not liquefy gelatine) and did not form zones of haemolysis on blood agar, which enabled a clear differentiation from *Pasteurella haemolytica*, which exhibits β-haemolytic activity. Catalase activity was positive, indole was produced, and hydrogen sulphide was formed. The results of the Voges-Proskauer reaction were negative, which excluded the presence of acetone as a side metabolite. The isolates reduced nitrates to nitrites, which confirmed their belonging to the typical representatives of the genus Pasteurella. As for carbohydrate metabolism, the epizootic isolates fermented glucose, galactose, mannose, fructose, sucrose, xylose, trehalose, sorbitol, and mannitol to produce organic acids without gas, which was confirmed by the change in the colour of the indicator in the medium. This type of fermentation – acid production without gas formation – is a characteristic species feature of Pasteurella. Therewith, the isolates did not ferment lactose, dulcitol, raffinose, rhamnose, glycerol, salicin, maltose, and arabinose, which allowed clearly differentiating them as representatives of *Pasteurella multocida* biovar *ovis*. Specifically, the absence of lactose fermentation is considered a marker diagnostic feature for this biovar. Thus, the biochemical properties of the epizootic strains were typical for the genus *Pasteurella*, particularly *Pasteurella multocida*, and fully corresponded to the characteristics of the reference strain B-0229.

Already on the second day after inoculation of experimental mice with a daily broth culture of isolated Pasteurella, the death of most experimental animals was recorded. Out of ten mice, five died in the first 24 hours, and three died on the second day (Fig. 4). In two cases, the mice stayed alive on the first day, but showed signs of depression: apathy, decreased appetite, and limited motor activity. These data indicate the high virulence of *Pasteurella multocida ovis* isolates both for laboratory models and, probably, for natural hosts.

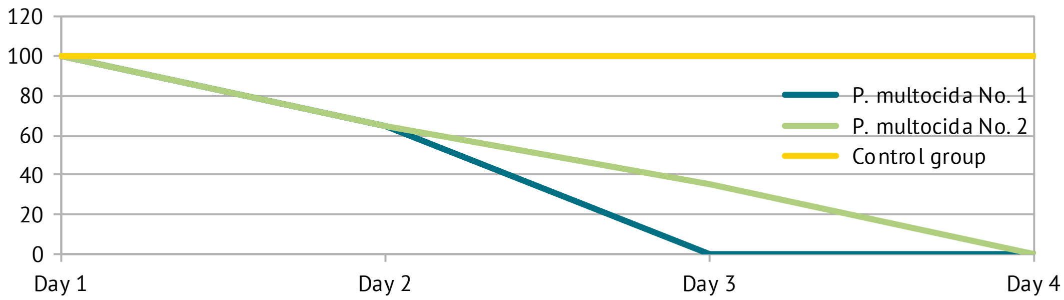


***Figure 4***. *Assessment of pathogenicity (survival)*
***Source:*** *created by the authors*

Pathological examination revealed numerous punctate and striated haemorrhages on the pericardium, serous-exudative peritonitis with clear or turbid exudate in the abdominal cavity, and foci of yellow-grey necrosis in the liver. Cultures of pathological material on meat-peptone agar and broth gave rise to a characteristic culture of *Pasteurella multocida ovis*. Small, transparent, delicate S-type colonies were formed on meat-peptone agar, without contamination with foreign microflora. In the liquid medium, uniform turbidity without ring or sediment formation was observed, a typical sign of *Pasteurella multocida* growth. Repeated microscopy of smears from such cultures confirmed the presence of Gram-negative bipolar rods morphologically identical to the original pathogen.

Thus, the results of bioassays in laboratory mice provided convincing evidence of the high pathogenicity of the isolated strains. They simultaneously served as a diagnostic tool (pathogenicity bioassay) and experimental verification of the species by comparing clinical and anatomical changes and bacteriological results. As a result, the study of the biochemical properties and pathogenicity of *Pasteurella multocida* isolates helped to reliably determine their species characteristics, virulence, and potential for epizootic spread. The identity of biochemical profiles in samples from domestic animals and saigas indicates the circulation of a single clone or stable biovar of the pathogen, which is significant for the development of specific prevention strategies and epidemiological monitoring in mixed livestock and wildlife environments.

As a result of laboratory cultivation, isolates of *Pasteurella multocida* were obtained from these samples, which demonstrated a stable phenotype characteristic of this species. Microscopic examination of smears made from the culture material revealed Gram-negative rods with typical bipolar tinctoriality, which is one of the key differentiating features in the identification of *Pasteurella multocida*. The culture properties were also typical: the isolates did not cause haemolysis when grown on blood agar, which distinguishes them from some other respiratory pathogens such as *Mannheimia haemolytica*. Furthermore, they were characterised by stable growth in meat-peptone broth with the formation of turbidity without the formation of sediment or film, which confirms good adaptation to the culture medium (Fig. 5). Despite considerable environmental and geographical differences in the sampling sites – specifically, the steppe zones of the West Kazakhstan and Kostanay regions – the properties of the isolates stayed almost identical, indicating high stability and adaptability of the strain.

The biochemical properties of the isolates from saigas also confirmed their belonging to *Pasteurella multocida* biovar *ovis*. All strains were catalase-positive, produced indole, formed hydrogen sulphide, and reduced nitrate to nitrite. This set of traits, combined with negative reactions to lactose, rhamnose, and raffinose, allowed distinguishing the isolates not only from related species of the genus Pasteurella, but also from potentially comparable members of the family *Pasteurellaceae*, which may act as companions or contaminants in natural biocenoses. The circulation of the same epizootic strain among geographically distant animal populations, including saigas, cattle, and small ruminants, indicates an extremely high contagiousness and stability of the pathogen. This stability may be related to the genetic conservation of the strain, its ability to persist in the host for a prolonged period without clinical manifestations, and the possibility of transmission through contact and aerogenic routes.

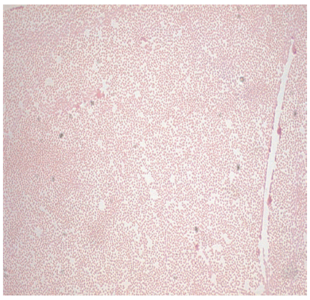

***Figure 5***. *Microscopy of the Pasteurella multocida isolate isolated in 2015 (Gram stain)*
***Source:*** *created by the authors*

A separate threat is posed by the translocation of the pathogen through ecological corridors, particularly the migration routes of saigas that intersect with areas of seasonal grazing of unvaccinated livestock. It was found that during periods of intense migration load, animals can come into contact with pathogen sources at watering holes, pastures, and through mechanical vectors. Such situations contribute to the formation of a natural and anthropogenic epizootic chain, within which the pathogen spreads between wild and domestic populations (Davydovych *et al.*, 2025). Considering the cyclical nature of pasteurellosis outbreaks (with a frequency of 3-5 years) and the presence of numerous latent bacterial carriers, the development of a unified national approach to the prevention and control of the disease is crucial. Particular attention should be paid to the development of vaccine prophylaxis programmes for wild animals, which constitute a natural reservoir of infection (Abutalip *et al.*, 2017). It is advisable to introduce aerosol vaccination of saigas during calving, when the number of animals is the largest and contacts with other populations are minimal. This approach has already demonstrated effectiveness in the fight against other translocated zoonoses in Africa and Central Asia.

Studies confirm that the combination of culture, morphological, tinctorial, and biochemical methods with a bioassay on laboratory animals (white mice) enables a comprehensive assessment of the pathogenicity and taxonomic affiliation of the pathogen. The bioassay showed high sensitivity: after inoculation, mice died within 24-48 hours, which corresponds to a prominent level of virulence of the isolates. This gave the study a high degree of reliability and opens up the prospect for further strain typing using molecular markers of virulence. Statistical analysis showed that the differences between the groups were not statistically significant ($p > 0.05$), suggesting that the biological properties of the isolates were stable regardless of the year of the epizootic and geographical origin. This confirms the circulation of a single conserved epizootic strain of *Pasteurella multocida* biovar *ovis*. Summarising the data obtained, it can be stated that regardless of the type of animal, region of distribution or conditions of keeping, the same stable epizootic strain of *Pasteurella multocida* biovar *ovis* circulates in the objects under study. It is characterised by a stable morphology, typical biochemical characteristics, and high virulence. This indicates the dominance of a pathogen clone that has adapted to many biological niches, and at the same time, a unique opportunity to create universal approaches to specific prevention, diagnostics, and control of this infectious agent. The data obtained are of both practical (for veterinary medicine and wildlife conservation) and theoretical (for evolutionary microbiology and epizootology) significance.

## DISCUSSION

The results of the conducted study reflect that isolates of *Pasteurella multocida* biovar *ovis* isolated from farm animals and saigas in Kazakhstan have a prominent degree of stability of morphological, cultural, and biochemical characteristics. Specifically, all strains demonstrated typical growth on meat-peptone broth and agar, formed transparent S-colonies without haemolysis on blood agar, had Gram-negative bipolar tinctoria and a positive reaction to the fermentation of a series of carbohydrates (glucose, mannose, galactose, sucrose, etc.) with the formation of acid without gas. Such conservatism of the traits is consistent with the data of U. Taubaev *et al*. (2024) who described analogous properties of *Pasteurella multocida* strains isolated in the Western Kazakhstan region, particularly during saiga migrations. Analogous findings were reported by S. Boianovskiy *et al*. (2023), noting that the stability of the biochemical profile of *Pasteurella multocida ovis* is a diagnostically significant feature in outbreaks in mixed-type farms.

The identified virulence of the isolates, confirmed by bioassays in mice (70% mortality in the first 48 h), indicates a high pathogenic potential of these strains. The pathological changes, such as haemorrhages, serous-exudative peritonitis, and liver necrosis, correspond to the typical manifestations of septic pasteurellosis described by Y.A. Mirtneh *et al*. (2022) during experiments with inoculation of *Pasteurella multocida* in laboratory animals. Y.M. Hashem *et al*. (2022) observed an analogous pattern in cases of infection among wild ungulates in the Mediterranean region, which emphasised the universal pathogenesis of the infection regardless of the area. The similarities in pathogenesis found, regardless of host species or geographic location, emphasise the universal nature of infection mechanisms, suggesting the evolutionary stability of the virulence of the pathogen. These data are crucial not only for diagnosing and predicting the course of the disease, but also for developing effective measures to control and prevent pasteurellosis in livestock.

The conservative biochemical profile of the identified strains (negative reaction to lactose, salicin, dulcite fermentation, etc.) helped to reliably differentiate them from other species of the genus Pasteurella, particularly *Pasteurella haemolytica* and *Pasteurella pneumotropica*. This approach is consistent with the recommendations of Y.A. Mirtneh *et al*. (2022), who emphasised the value of classical biochemical tests in the absence of molecular genetic capabilities. The results of the present study also confirmed the findings of D. Abera and T. Mossie (2023), who successfully identified *Pasteurella multocida* serotypes based on carbohydrate metabolism profiles. At the same time, the difference from the results of Z. Peng *et al*. (2022) should be noted, who found significant genetic heterogeneity of *Pasteurella multocida* isolates, even within the same host. In this case, the dominance of a single biochemical and morphological type suggests the presence of a circulating clone adapted to the conditions of the steppe ecosystem of Kazakhstan. A. Pascual-Garrigos *et al*. (2021) recorded an analogous trend in their study of clinical isolates in Europe, and H. Wang *et al*. (2023) – in their study of Chinese farms.

A significant finding was the detection of the same strain among isolates from saigas and domestic animals in different regions of Kazakhstan. Such geographical stability of the strain, combined with high virulence, indicates a strong epizootic potential and confirms the hypothesis of S. Boianovskiy *et al*. (2023) regarding the universality of pathogenic mechanisms of *Pasteurella multocida* biovar *ovis*. A vital role in the transmission of the pathogen is likely to be played by natural ecological corridors, primarily the migration routes of saigas that intersect with areas of seasonal cattle grazing. A. Abbas *et al*. (2023) presented analogous conclusions, studying the impact of joint grazing of wild and domestic animals on the risks of pasteurellosis transmission in Pakistan. S.A. Alemu *et al*. (2023) also demonstrated comparable findings in Ethiopia, where the key factors of epizootic spread included watering holes, mobile contacts between populations, and insufficient vaccination rates. In this case, these factors also play a critical role, which requires a review of anti-epizootic strategies at the state level.

One of the most reliable morphological markers for the primary identification of *Pasteurella multocida* continues to be bipolar tinctoria in Gram or Romanowsky-Giemsa staining, as well as the absence of haemolysis on blood agar (Ospanov *et al.*, 2024). These features are the key differentiating criteria for distinguishing *Pasteurella multocida* from *Mannheimia haemolytica* and other Gram-negative facultative anaerobes, as noted by S. Sahay *et al.* (2020) and J. Xiao *et al.* (2021), which was also confirmed in the present study. In the current study, the absence of β-haemolysis zones was crucial for confirming the species status of the isolates before the use of biochemical analysis. However, the introduction of molecular methods continues to be relevant. Specifically, H. Wang *et al.* (2023) showed that multiplex PCR enables the simultaneous identification of several pathogens in mixed infections, which increases the sensitivity and specificity of laboratory diagnostics. This may be particularly relevant in cases of co-infections or in the early stages of an outbreak.

The obtained results confirm that *Pasteurella multocida* biovar *ovis* isolated from farm animals and saigas in Kazakhstan retains high conservation of cultural, morphological, and biochemical characteristics, which is consistent with the data of I. Cuevas *et al.* (2020) and F. Ahmad *et al.* (2024) on the stability of this pathogen in different biocenoses. The stability of the biochemical profile of the isolates, particularly the ability to ferment glucose, sucrose, and other carbohydrates to produce acid without gas, corresponds to the typical characteristics of the *Pasteurella multocida* biovar *ovis* species. Z. Peng *et al.* (2021) reported analogous results, noting that the biochemical stability of the strains ensures the reliability of classical diagnostic tests. However, the genotyping results obtained by these researchers indicate the presence of high genetic variability between strains isolated from different hosts. The spread of an identical strain among geographically dispersed groups of animals confirms its ability to adapt and transmit widely (Irgashev *et al.*, 2020). This is consistent with the studies of S. Ali *et al.* (2025), who found the circulation of unified strains of *Pasteurella multocida* among cattle and small cattle in Pakistan. A. Akane *et al.* (2022) made analogous conclusions, emphasising the role of mixed animal housing in the transmission of infection. Considering this, the need to implement integrated control measures was confirmed, especially in transboundary regions with intensive grazing.

Clinical and diagnostic features, such as the absence of haemolysis zones on blood agar and bipolar coloured bacilli, continue to be crucial markers for differentiating *Pasteurella multocida* from other pathogens such as *Mannheimia haemolytica* (Verzhykhovsky & Nedosekov, 2024). This is confirmed by the results reported by S. Sahay *et al.* (2020) and I. Cuevas *et al.* (2020), where the diagnostic significance of morphological and culture tests is noted. The researchers also emphasised that the combination of classical methods with modern approaches to identification can improve the accuracy of diagnostics and help prescribe the corresponding treatment promptly. The study found that the use of traditional microbiological methods in combination with bioassays on white mice provides high diagnostic accuracy. However, considering the requirements for pathogen detection, it is advisable to introduce genotyping and whole-genome sequencing to identify virulence genes and resistance markers. Thus, the data obtained indicate the existence of a stable epizootic strain of *Pasteurella multocida* biovar *ovis* circulating in both wild and domestic animal populations. This necessitates an interdisciplinary approach to prevention, which would include elements of environmental monitoring, vaccination and rapid laboratory diagnostics.

## CONCLUSIONS

As a result of the studies, it was found that mass animal deaths in Kazakhstan in 2015 and 2023 were caused by the circulation of a stable strain of *Pasteurella multocida* biovar *ovis*. Cultural and morphological analysis revealed that all isolates had typical characteristics: uniform turbidity was observed on meat-peptone broth within 24-48 hours without precipitation and rounded greyish-white colonies 1-2 mm in diameter were formed on meat-peptone agar. Microscopic examination revealed typical Gram-negative rods with a pronounced bipolar tinctoriality. Biochemical analysis of the isolates showed a high degree of homogeneity. All 20 isolates (100%) actively fermented glucose, galactose, sucrose, mannose, fructose, and trehalose to produce acid, but without gas. Therewith, all of them gave negative results for the fermentation of lactose, rhamnose, raffinose, maltose, salicin, dulcit, and glycerol. Additional tests confirmed the presence of catalase activity, indole production, hydrogen sulphide production, and the ability to reduce nitrate to nitrite in all samples tested. Evaluation of the pathogenic properties of the strain in BALB/c laboratory mice showed high virulence. After infection, 50% of the animals (5 out of 10) died within the first 24 hours and 80% (8 out of 10) – within 48 hours. Pathological examination revealed haemorrhages in 100% of cases and necrotic changes in the liver in 75% of cases, which correlated with the results of repeated isolation of the pathogen from pathological material.

Notably, the complete convergence of all the studied characteristics of the isolates obtained in 2015 and 2023, which indicates the extraordinary stability of the circulating strain. The findings substantiate the need to develop a comprehensive prevention programme, which should include regular vaccination of farm animals covering at least 80% of the population and aerosol immunisation of saigas during the period of their encirclement. Particular attention should be paid to monitoring of common watering places, which are recommended to be inspected 3-4 times a year. Such measures will effectively control the spread of this highly pathogenic strain among both wild and domestic animals. A limitation of this study was the restrictive genetic analysis of isolates, which did not allow definitively determining the intraspecific structure of circulating strains. Furthermore, the study was focused mainly on phenotypic and biochemical characterisation, without broad coverage of other possible carriers in the wild fauna. Prospects for further research include the use of whole-genome sequencing to determine the phylogenetic relationship of strains, immunogenicity assessment for the development of specific vaccines, and the study of antibiotic resistance, which is crucial considering the growing threat of antibiotic resistance in wild and agricultural fauna.

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## CONFLICT OF INTEREST

None.

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## Пастерельоз у сільськогосподарських тварин

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**Анотація**. Метою даного дослідження було вивчення біологічних властивостей штамів Pasteurella multocida, що циркулюють серед сільськогосподарських та диких тварин в Казахстані, зокрема сайгаків, з метою оптимізації діагностики та профілактики пастерельозу. У дослідженні використовувалися бактеріологічні, мікроскопічні та біохімічні методи для аналізу 20 зразків біоматеріалу, зібраного від сайгаків і сільськогосподарських тварин: 10 зразків від особин із села Торгай (Джангельдинський район, Костанайська область, Казахстан) і 10 зразків від особин із Казталовського району (Західно-Казахстанська область, Казахстан). Дослідження показало, що всі ізоляти (100 %, n = 60) належали до Pasteurella multocida biovar ovis: на м'ясо-пептоновому агарі утворилися сірувато-білі колонії діаметром 1-2 мм без гемолізу, у мазках виявлено грамнегативні біполярні палички, а ферментація глюкози, сахарози, манози та трегалози супроводжувалася утворенням кислоти без газу (у 100 % випадків). Біоаналізи на 10 білих мишах (вага 16-20 г) показали 100 % смертність протягом 48 годин після ін'єкції 0,2 мл культури, з крововиливами в серці, некрозом печінки та ексудатом в черевній порожнині. Статистичний аналіз (тест Фішера) не виявив істотних відмінностей між ізолятами 2015 і 2023 років ($p > 0,05$), що підтверджує циркуляцію стабільного клону. Отримані результати свідчать про ключову роль сайгаків як резервуару інфекції та необхідність комплексних заходів: аерозольної вакцинації під час міграцій, впровадження діагностики методом полімеразної ланцюгової реакції та моніторингу антибіотикорезистентності. Отримані дані є основою для розробки регіональних антиепізоотичних стратегій, спрямованих на зменшення ризиків для сільського господарства та збереження популяцій диких тварин у Казахстані

**Ключові слова:** сайгаки; епізоотії; ізоляти; м'ясо-пептоновий агар; біоаналізи

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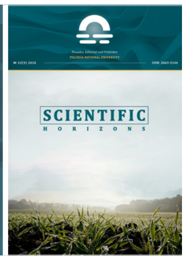


# Influence of corn seed treaters on field germination at low temperatures

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**Abstract**. The relevance of the study was to establish a link between low temperatures, seed treaters and plants, and to develop technological measures to increase plant resistance to possible spring frosts. In accordance with this, the purpose of the study was to determine the effect of fungicidal seed treaters on the ability of corn seedlings to counteract low-temperature stress. Both laboratory and field methods were used to conduct studies to determine the reaction of corn plants to fungicidal seed treaters (Vencedor, Avicenna, Maxim XL, Bastion, and Fever) at low temperatures. Under optimal laboratory temperatures, treatment with all preparations did not negatively affect the germination energy and laboratory germination of seeds of the Onio corn hybrid and line B 831. It was found that despite the same high results of laboratory germination of corn seeds, field germination on the provocative low-temperature background of early sowing dates as a result of the use of different seed treaters was very different. In years with varying moisture levels and low temperatures, high germination rates were observed when using the fungicide seed dressings Vencedor,

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Avicenna, and Maxim XL (at a level of 70-90%). Significantly lower results of field germination were obtained when using bastion and fever preparations – 45-60%. Thus, in the conditions of early sowing, and considering the risk of returning spring cold weather, it is more expedient to use Vencedor, Avicenna, and Maxim XL seed treaters. The results of this study indicate the need for pesticide originators and research institutions to conduct a more detailed study of the reaction of plants to drugs under various weather conditions and are of practical importance in production for more effective use of drugs

**Keywords:** seed treatment; fungicides; early crops; low temperatures; seed germination

## INTRODUCTION

Seed treatment is a mandatory technological element of growing agricultural crops. Fungicidal preparations used as seed treaters negatively affect various biochemical processes in the body of fungi: they suppress the intensity of respiration, disrupt the synthesis of amino acids and other vital reactions. The aggressiveness of fungicide chemicals towards fungi cannot fail to affect the plants themselves. The activity of drugs significantly depends on external conditions, and the reaction of the plants themselves. Testing of new fungicides takes place under close to optimal hydrothermal conditions. But the conditions for growing agricultural crops are often extreme, so it is important to determine the reaction of plants to certain drugs in atypical conditions, in particular at low temperatures.

In a steppe zone with little moisture supply to plants, early sowing guarantees plants the opportunity to obtain more of this important resource. This is especially important for late spring crops. However, there is a negative downside to early sowing dates. In spring, the weather is quite changeable and it is possible to return low temperatures, a short-term cold snap. This can lead to damage to plant seedlings. The return of cold weather is difficult to predict. In order to guarantee to avoid losses from a possible decrease in temperature, farmers are forced to postpone sowing to a later date. But in the steppe, in the conditions of a short spring, when the temperature increases intensively and the top layer of soil quickly loses moisture, a delay in sowing leads to a decrease in seed germination and causes variegation of crops, which negatively affects yields. Due to the warming observed in recent years (the average annual air temperature according to weather forecasters in Ukraine increased by 1.7°C), there is an earlier onset of a stable transition of the average daily temperature through + 5°C (Meteopost, n.d.). This, in turn, forces farmers to start field work earlier. However, the risks of returning cold weather, especially in steppe conditions, increase significantly.

In the scientific literature, much attention was paid to the effect of seed treaters on the field germination of seeds. Recent studies have found that not only the seed treaters themselves can affect seed germination, but also their various compositions (Capo *et al.,* 2020). The effectiveness of seed protectants is not static, it is unstable and largely depends on weather conditions (Semenov, 2024). That is, different drugs manifest themselves differently under different conditions. Accordingly, it is likely to expect that under extreme conditions (such as low temperatures), some drugs will be more effective than others. The study by A. Meng *et al.* (2022) contains information about the negative effect of low temperatures on seed germination, but there is no information about the effect of low temperatures on the plant – seed treaters interaction. Industrial production of crops is difficult to imagine without the use of pesticides (Krachan & Nedilska, 2022). Pesticides that enter production, including seed treaters, usually pass comprehensive tests for the ability to effectively suppress pathogenic microorganisms, harmful insects, not negatively affect the beneficial flora and fauna of the soil, be as safe as possible for humans, do not pollute the environment, etc.

Seed treaters are widely used and it is possible to find many sources about their positive effect on field seed germination and yield (Yatsukh *et al.,* 2023). However, there is also information about the negative impact of seed treaters on germination energy and germination in the laboratory (Medeiros *et al.,* 2023). However, there is no alternative to seed treatment to provide effective protection against pathogens. The etching process is not a technological formality, but as indicated in the scientific and methodological recommendations of V.M. Stefkivskyi *et al.* (2024), is a measure that should be approached based on the phytosanitary condition of seeds and the spectrum of action of the drug. To prevent a decrease in seed quality, inhibit the activity of physiological and biochemical processes during germination, increase the energy of germination and, as a result, increase yield, as some researchers point out, plant growth regulators should be added to seed treaters (Alekseeva, 2019). It is also recommended to use microfertilisers together with seed treaters (Siroshtan *et al.,* 2021) or to use two-component seed treaters (Pospelova *et al.,* 2021). There is also a proposal to treat soybean seeds with Standak Top and Fever seed treaters in conjunction with pesticide-resistant nodule bacteria B. japonicum PC09 (Kukol *et al.,* 2022). According to the researchers, this will increase the activity of legume-rhizobial symbiotic systems. Attention is drawn to the need to preserve biological diversity. According to A. Dubey *et al.* (2020), treatment affects the abundance of both soil and leaf taxa, including predators and parasitoids.

It is known that various chemical preparations used in crop production can affect not only directly for their main purpose, but also indirectly through changes in the metabolic processes of the plant itself. And such changes can be quite significant. According to O. Dermenko (2024), seed treaters can, in addition to their direct function of suppressing microorganisms, affect the physiological state of plants. So the drug Avicenna Plus can significantly stimulate natural resistance to biotic and abiotic factors. However, drug developers are not always able to comprehensively assess the indirect effect of the drug on all economically valuable features.

Seed treaters vary greatly in their intensity of action. They should not only suppress unwanted microflora, but also approach a particular type of cultivated plant as much as possible. As already noted, some of them can reduce the sowing quality of seeds. Thus, the choice of seed treaters should be based on the aggregate characteristic of the drug, which requires obtaining a wide range of information. This is what prompted the study of the indirect effect of seed treaters on the resistance of corn seedlings to a possible decrease in temperature after sowing, which is quite common in the Steppe zone. The purpose of this study was to develop an effective technological measure that contributes to the maximum field germination of corn seeds in conditions of a likely decrease in temperature.

## MATERIALS AND METHODS

To solve this problem, studies of field germination of corn seeds were conducted in the conditions of early sowing dates. Such a provocative background of low temperatures could simulate the return of cold weather. Field experiments with corn were conducted in 2023 and 2024 in the educational farm of the Dnipro State Agrarian and Economic University. Its predecessor was sunflower. The Onio hybrid and the self-pollinating line B 831 were used for sowing. According to the originator, HIKS Ukraine LLC, the Onio hybrid is quite resistant to cold weather and has high initial growth rates, while line B 831, used as the parent form of this hybrid, has, on the contrary, weak growth rates at low temperatures. The line was chosen to compare plants $F_1$, which have heterosis, and corn lines that do not have one. Corn lines are generally less resistant to adverse conditions and are sown at a later date, but it has been included as a sensitive marker. Own laboratory studies of seed germination were determined by germination in a wet chamber in petri dishes on filter paper in accordance with DSTU 4138-2002 (2004); field – directly in the ground when manually sowing 10 treated seeds in four repetitions.

The following fungicidal preparations were used both for the hybrid and for the line: Vencedor, Bastion, Fever, Maxim XL, and Avicenna (the rate of consumption of the drug in accordance with the manufacturer's recommendations). The drugs have the following active ingredients: Vencedor – tebuconazole 25 g/l + Tiram 400 g/l; Bastion – diphenoconazole 30 g/l + ciproconazole 6.25 g/l; Fever – protioconazole, 300 g/l; Maxim XL – fludioxonyl, 25 g/l + mefenoxam, 10 g/l; Avicenna – tebuconazole, 50 g/l + prochlorase, 250 g/l + cresoxime-methyl, 50 g/l. In the field, the number of sprouted seeds was carried out on the 25$^{th}$ day after sowing. At this time, the temperature rose to a favourable temperature for germination. Corn of the line B 831 and the Onio hybrid was sown in two periods. In 2023, they were sown on April 15 and 19, and in 2024 – on April 12 and 19. Such sowing dates can be considered the earliest in comparison with long-term ones in this area (the Northern Steppe zone of Ukraine).

The weather conditions of spring 2023 were special and were characterised by both elevated temperatures and extremely high soil humidity. At the beginning of sowing, the soil moisture content was 139% of HB (or 72% of the total moisture capacity, or 37% of the weight of absolutely dry soil). The soil was not physically mature, so it was impossible to carry out pre-sowing cultivation. Sowing was carried out manually in waterlogged soil. Suitable temperatures for sowing (10-12$^{o}$C) came very early, about a month before the usual dates for late spring crops, but later came to a multi-year norm. At the time of sowing, the air temperature fluctuated during the day from 3 to 12$^{o}$C. It turned out in such a way that the second period of sowing had lower temperatures than the first. As will be shown later, this significantly affected the seed's ability to germinate. The conditions of 2024 differed significantly in their aridity, both for the entire growing season, and in particular for the sowing period. When sowing at an early stage, the soil moisture content did not exceed 20% of the total moisture capacity (38.5% of HB). At the time of sowing, the soil temperature fluctuated between 7-11$^{o}$C. Lack of moisture and low temperatures significantly reduced the field germination of corn seeds. But the decrease in germination largely depended on the seed treater that was used (Meteopost, n.d.).

To objectively assess the effect of various seed treaters on the field germination of corn seeds at low temperatures, their effect on laboratory germination indicators under optimal seed germination conditions was first determined. In accordance with this, the germination energy and laboratory germination of seeds were determined. The determination was carried out as follows: twenty seeds were laid out on wet filter paper in petri dishes. Kept at a temperature of 23-25$^{o}$C and determined the germination energy on the third day, and laboratory germination on the seventh day after the start of germination. The repetition was fourfold. It was also necessary to first establish the sowing qualities of the Onio hybrid and the line B 831. The determination was carried out in accordance with DSTU 2240-93 (1994). All seeds that were used in the experiment met the technical conditions and had a laboratory germination rate of 99% of the hybrid, and 97% of the line. In their research, the researchers adhered to the standards of the Convention for the Protection of Biological Diversity (1992).

## RESULTS AND DISCUSSION

Analysis by seed treater exposure was performed in accordance with DSTU 4138-2002 (2004) and as the results showed (Table 1), there was a slight decrease in the indicators of both germination energy and laboratory germination of corn seeds. Despite a slight decrease in these indicators, the sowing qualities of seeds met the requirements of DSTU 2240-93 (1994). There were no significant differences between seed treaters on the effect of these indicators. The maximum deviation in laboratory germination rates under the conditions of using mordants for the hybrid was 5.0% (Fever), and for the line – 4.0% (Maxim XL).

***Table 1**. Germination energy and laboratory germination of treated seeds, %*

| Seed treater | Onio hybrid | | Line B 831 | |
|---|---|---|---|---|
| | germination energy | laboratory similarity | germination energy | laboratory similarity |
| Vencedor | 89 ± 3 | 96 ± 3 | 84 ± 5 | 94 ± 5 |
| Bastion | 88 ± 3 | 95 ± 4 | 83 ± 3 | 94 ± 5 |
| Fever | 88 ± 7 | 94 ± 5 | 83 ± 5 | 94 ± 5 |
| Maxim XL | 88 ± 3 | 95 ± 4 | 84 ± 5 | 93 ± 3 |
| Avicenna | 90 ± 0 | 96 ± 3 | 86 ± 3 | 94 ± 3 |

***Source:** compiled by the authors*

In contrast to favourable laboratory conditions, seed germination rates in extreme conditions of early sowing periods at low temperatures significantly decreased when using all seed treaters without exception. In addition, in 2023, at low temperatures of early sowing periods, differences in field germination of seeds were found depending on the seed treaters used (Table 2). The discrepancy between the values between the variants of this indicator under the conditions of sowing on 15.04 was 15% for the hybrid, and 52.5% for the line. Sowing on 19.04 was carried out at lower temperatures than the previous one, so the indicators were obtained lower: for the hybrid – 42.5%, and for the line – 25%.

***Table 2**. Field germination of hybrid seeds and corn lines at the earliest sowing dates depending on the seed treaters in 2023, %*

| Drug (B) | Sowing period 15.04.23 (counting 09.05.23) | | Sowing period 19.04.23 (counting 13.05.23) | |
|---|---|---|---|---|
| | hybrid Onio (a) | line B 831 (a) | hybrid Onio (a) | line B 831 (a) |
| Vencedor | 90.0 ± 11.5 | 77.5 ± 12.6 | 77.5 ± 5.0 | 65.0 ± 10.0 |
| Bastion | 75.0 ± 12.9 | 32.5 ± 12.6 | 45.0 ± 12.9 | 40.0 ± 8.2 |
| Fever | 85.0 ± 10.0 | 55.0± 12.9 | 50.0 ± 8.2 | 50.0 ± 11.5 |
| Maxim XL | 90.0 ± 8.2 | 85.0 ± 5.8 | 87.5 ± 12.6 | 47.5 ± 12.6 |
| Avicenna | 85.0 ± 5.8 | 75.0 ± 5.8 | 85.0 ± 10.0 | 62.5 ± 9.6 |
| $LSD_{05}$ | A – 7.0; B – 11.1; AB – 15.7 | | A – 6.6; B – 10.4; AB – 14.7 | |

***Source:** compiled by the authors*

It can also be noted that corn lines are more sensitive to germination conditions at low temperatures than hybrids. Among seed treaters, the best indicators of field seed germination, at conditions of lower temperatures than optimal, were found in the preparations Vencedor and Maxim XL. Thus, when using the drug Vencedor for sowing 15.04, the field germination rate of the Onio hybrid was at the level of 90.0%, and in the line B 831 – 77.5%; in the seed treater Maxim XL – 90.0% and 85.0%, respectively. Similar results were also obtained during sowing on 19.04. In the conditions of sowing on 19.04, Avicenna seed treater also performed well. The greatest decrease in field germination of seeds of both the corn line and hybrid at low temperatures was observed when using Bastion and Fever seed treaters. This decrease in relation to the indicators of Vencedor and Maxim XL seed treaters averaged 15.04 for the hybrid by 10%, and for the line by 37.5%. Under the conditions of sowing on 19.04, these indicators were 35.0% and 11.3%, respectively.

The experiment was continued in the following year 2024 (Table 3). Despite the fact that the conditions during sowing differed significantly in soil moisture, seed treaters at low temperatures showed the same effect on field germinated seeds as in the wet year 2023. At low temperatures, the seed germination rate of both the hybrid and the line decreased with the use of seed treaters such as Bastion and Fever.

When sowing on 12.04.2024, this decrease in comparison with Vencedor and Avicenna preparations on average occurred in the hybrid by 25.3%, and in the line – by 29.8%. The middle position in this gradation is occupied by Maxim XL. Similar results were obtained during sowing on 19.04.23.

***Table 3**. Field germination of hybrid seeds and corn lines at the earliest sowing dates depending on the seed treater in 2024, %*

| Drug (B) | Sowing period 12.04.2024 (counting 07.05.24) | | Sowing period 19.04.2024 (counting 14.05.24) | |
|---|---|---|---|---|
| | hybrid Onio (a) | line B 831 (a) | hybrid Onio (a) | line B 831 (a) |
| Vencedor | 70.5 ± 8.2 | 55.0 ±5.8 | 82.5 ± 9.6 | 50.0 ± 8.2 |
| Bastion | 55.0 ± 10.0 | 45.0 ± 5.8 | 47.5 ± 12.6 | 40.0 ± 8.2 |
| Fever | 50.0 ± 8.2 | 37.5 ± 9.6 | 60.0 ± 11.5 | 37.5 ± 5.0 |
| Maxim XL | 65.0 ± 5.8 | 55.0 ± 5.8 | 72.5 ± 9.6 | 60.0 ± 8.2 |
| Avicenna | 70.0 ± 8.2 | 62.5 ± 9.6 | 77.5 ± 9.6 | 62.5 ± 9.6 |
| $LSD_{05}$ | A – 5.1; B – 8.1; AB – 11.4 | | A – 6.3; B – 10.0; AB – 14.1 | |

***Source:** compiled by the authors*

Seed germination is an extremely important stage in plant life, which largely affects their growth and development throughout the growing season. For seed germination, first of all, the necessary amount of moisture and the appropriate temperature are required. Research by V.A. Doronin *et al.* (2019) found that both insufficient and excessive moisture negatively affects seed germination. The required temperature for seed germination also has certain limitations. According to M.I. Dudka *et al.* (2024) optimal conditions for corn sowing are formed in the early stages at a temperature of 8-10°C. According to the researchers, this allows using productive moisture reserves more efficiently and reducing the impact of a possible drought in the future. In addition to the main factors necessary for seed germination, such technological measures as fertilisers (Voloshchuk *et al.,* 2021) and physiologically active drugs (Kuchmenko & Kurylenko, 2024), which under certain conditions can increase seed germination. Also in the scientific literature, much attention is paid to highlighting the varietal features of corn seed germination (Dziubetsky *et al.,* 2019; Mashchenko & Butenko, 2024).

Obtaining friendly shoots is the primary task of corn cultivation technology. But despite all the efforts of high-quality and timely implementation of all technological measures, it is not always possible to get the desired result. The return of cold weather can be an obstacle, which will lead to inhibition of seed germination and even seed failure. The shoots become liquefied, unproductive. In addition, plants develop unevenly, are more exposed to stress, and after a significant period of being in cold soil, seeds are more susceptible to disease. Since the drugs act indirectly on plants, all drugs, including fungicides, should be examined under cold stress conditions to identify the most effective conditions for these conditions. During the period of seed germination, even a slight decrease in the average daily temperature leads to negative consequences. The minimum temperature during the day is usually observed in the early morning. In addition, the resistance of plants to low temperatures during the day is not the same, the minimum value of this indicator is noted in the morning. It is the coincidence of these two factors in such a period of the day: – a decrease in air and soil temperature and minimal daily resistance of plants to low temperatures lead to a significant loss of field germination of corn seeds.

It is generally accepted that normal corn shoots can be obtained at soil temperature at a seed depth of 10-12°C. However, there are recommendations for possible seeding at a lower temperature – +7°C, but with a subsequent increase in temperature (Nesmachna, 2025). In the event of the return of cold weather, on the contrary, the temperature will decrease and the grain that has already begun to germinate will be subjected to cold stress. One of the ways to prevent the suppression and death of plants in these conditions is to use in advance exactly those chemicals that would contribute to the maximum preservation of the ability of plants to resist negative factors in low temperatures. In the experiments presented in this paper, an attempt was made to find out whether the field germination rate of corn seeds will change under various seed treatment in the event of the return of cold weather immediately after sowing. Under extreme temperature conditions artificially created due to early sowing, it was found that seed treaters significantly and differently affected the ability of plant seedlings to tolerate low temperatures. The results obtained in 2023 and 2024 were averaged and presented as graphs for better analysis. Figure 1 shows the field germination rates of the Onio hybrid for 2023 and 2024. Data for 2023 represent the average of the two early sowing dates – April 15 and 19. In addition, data for 2024 are also average indicators for April 12 and 19. This helps to more objectively assess the effect of seed treaters on the field germination of corn seeds at low temperatures. For comparison, the graph also shows data on laboratory seed germination.

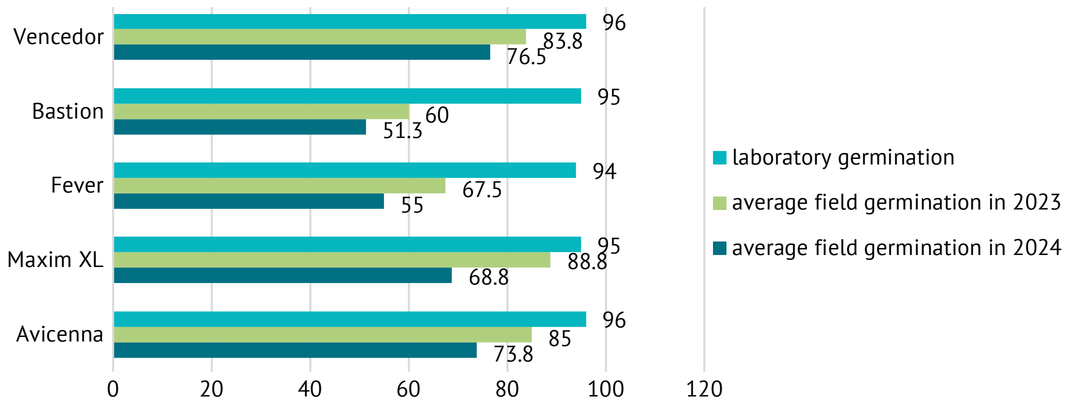


***Figure 1**. Germination of seeds of the Onio corn hybrid on average over two early sowing periods*
***Source:** compiled by the authors*

Under conditions of low temperatures, on average, for two sowing periods, the maximum seed germination rate was obtained in 2023 using the Maxim XL fungicide – 88.8%. In 2024, it was only slightly inferior to the drug Vencedor (by 2.7%). Vencedor seed treater in 2023 had only 1.2% lower indicators than Maxim XL. The same high rates were obtained when using the Avicenna fungicide: in 2023 – 85.0%, in 2024 – 73.8%. Thus, these three drugs: Maxim XL, Vencedor, and Avicenna were most effective at low temperatures. In contrast to these drugs, the use of fungicides Bastion and Fever led to a significant decrease in field germination of seeds of the Onio hybrid. Thus, the germination rate when using bastion fell in 2023 to 60.0%, and in 2024 – to 51.3%. Almost the same results were obtained with Fever – 67.5% and 55.0%, respectively. These drugs negatively affected the germination of corn at low temperatures.

The wet conditions of the study years (2023 with a wet spring, and 2024 with an extremely dry spring) did not significantly affect the identified patterns of dependence of the drugs used on the field germination of corn seeds at low temperatures. A similar graph (Fig. 2) was also represented for line B 831. The results obtained indicate that the line was significantly more vulnerable to low temperatures than the hybrid. These data indicate that early sowing of corn lines, without conditions for normal seed germination, is impractical. The maximum difference between field and laboratory seed germination of line B 831 was 57.7% (when using Bastion in 2023), and the minimum difference was 22.7% (when using Vencedor in 2023). Regarding drugs, there is the same dependence as in the hybrid. The greatest decrease in field germination over the years of research was observed with the use of Bastion and Fever seed treaters. Just as in the case of the hybrid, the slightest decrease in field germination at low temperatures was observed with the use of such fungicides as Vencedor, Avicenna, and Maxim XL.

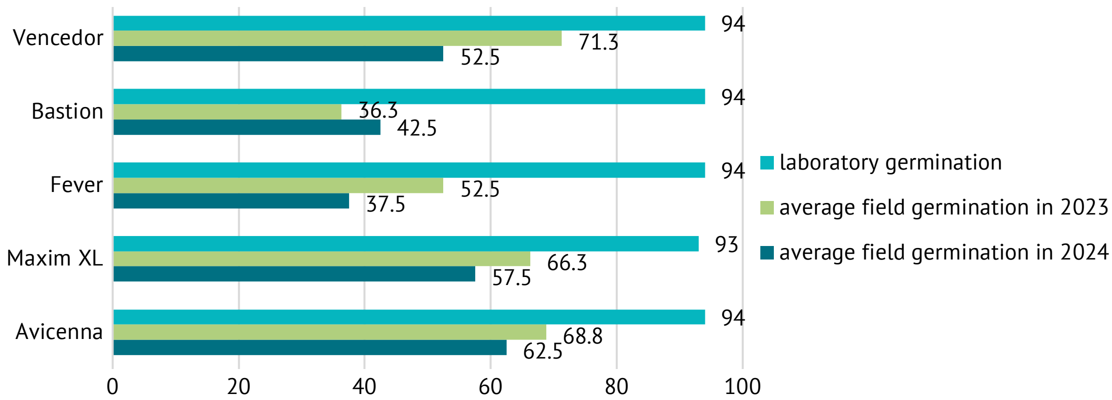


***Figure 2**. Seed germination of the corn line B 831 on average over two early sowing periods*
***Source:** compiled by the authors*

The use of Bastion and Fever preparations caused a decrease in resistance to low temperatures. But this does not mean that Bastion and Fever are unsuitable for treating corn seeds. This only indicates that it is better not to use these drugs when sowing at an early stage. Laboratory tests (Table 1) showed that the seeds treated with them have the same germination energy and laboratory germination as other preparations. It can be assumed that Bastion and Fever will contribute to better germination compared to other drugs at higher temperatures. This assumption can be made based on the fact that the orientation of physiological and biochemical processes that increase the cold resistance of plants is reversed by the processes of intensive growth. If the preparations that are received for use in crop production are accompanied by more detailed information about the effect on the plant under various conditions, this will significantly increase the effectiveness of their use.

The use of fungicides becomes particularly important in conditions of the return of low temperatures, when seedlings are under stress, weakened, and delayed in growth. According to Yu. Kolupaev *et al.* (2023), changes in temperature lead to stress responses of the plant body: damage to proteins, lipids, and nucleic acids. It is likely that low temperatures affect not only the activity of the preparation, but also the reaction of the plant to this substance. Fungicidal seed treaters have a detrimental effect on the pathogen and have a different mechanism of action: blocking the activity of enzymes, violation of respiratory phosphorylation, destruction of cellular structures, etc. Such an active effect of the drug cannot but affect the plant itself.

Chemical preparations, getting into the plant's body, can change the course of biochemical processes, and thereby lead to a change in a certain desired trait. However, this correction of metabolism can automatically lead to more or less significant changes in other signs. Thus, preparations for seed treatment can affect some important signs, which happened in this experiment. Other researchers also observe the indirect effect of chemical preparations on economically important characteristics of cultivated plants (Sobchenko, 2019; Bilousova *et al.,* 2020). The active ingredients of the preparations Vencedor and Avicenna helped to increase the resistance of plants to low temperatures, and the metabolic processes of resistance and active growth are multidirectional. Based on this, bastion and fever preparations probably assume that they will have the advantage at optimal temperatures – to promote more active initial plant development. The decrease in field germination of corn seeds when using bastion and fever seed treaters can be caused both by the loss of activity of the drug due to low temperatures, and by a possible indirect effect. This issue requires further research.

Thus, research has shown that drugs, in addition to their direct action, can affect the course of other important physiological processes in plants. O. Dermenko (2024) held a similar conclusion. The researcher noted that the fungicidal preparation Avicenna Plus can improve the general condition of winter wheat plants, increase winter hardiness, and stimulate the natural resistance of plants. The researcher did not specify how winter hardiness increases, whether due to direct or indirect action. G. Peng *et al.* (2020) conducted research on a similar topic related to seed treaters. When treating rapeseed with Fluopyram, they observed not only the restriction of plant infection, but also the activation of PR proteins. They are specific plant proteins that are synthesised in response to stressful conditions, in particular, low temperatures. This allowed researchers to assume that this drug will be useful in changing weather conditions that delay seed germination. Current research also concerns the effect of seed treaters on the state of plants when weather conditions change. But in this case, the studies were conducted purposefully at low temperatures, by provocative sowing at the earliest possible time, and with various fungicidal preparations. This helped to identify the most effective fungicides. Among them were: Vencedor, Avicenna, and Maxim XL.

The relationship between weather, diseases, and seed treaters was considered by R. Semaškienė *et al.* (2025). The researchers emphasise that unstable weather conditions contribute to the spread of diseases. In addition, they emphasise the need to be extremely careful when choosing fungicides so as not to cause resistance of pathogens to them. Special attention was paid to the need to develop appropriate technological measures to the conditions of environmental change. It was this approach that guided the authors in conducting their research. According to the authors, this will raise the technology of growing agricultural crops to a new level. Unlike the study by R. Semaškienė *et al.* (2025), current experiments without assumptions have established a close relationship between seed treater activity, plant response, and weather conditions. The example of a hybrid and a corn line shows the different reaction of plants to seed treaters at low temperatures. Such knowledge is of great practical importance due to the fact that it allows using the line of mordants most effectively to ensure maximum economic effect.

## CONCLUSIONS

Fungicides, depending on weather conditions, can affect seed germination in different ways. In the conditions of early spring, when there is a high probability of the return of cold weather, preparations should be used for pickling corn seeds, which in these conditions would help to increase field germination. Among the studied drugs, these are Vencedor, Avicenna, and Maxim XL. At low temperatures of early sowing periods, the field germination rate of seeds for the Onio corn hybrid varied depending on the year for the preparation: Vencedor 76.5-83.8%; Avicenna 73.8-85.0%; Maxim XL 68.8-85.0%. Under the same conditions, a significant decrease in germination was observed when using Bastion and Fever seed treaters: 51.3-60.0% and 55.0-67.5%, respectively. This indicates that in the conditions of waiting for a decrease in temperature, it is necessary to refrain from using Bastion and Fever preparations, but this does not mean that they should not be used in other weather conditions.

The value of the chosen method of conducting research in the field using a provocative background is emphasised, which helped to obtain results as close as possible to real ones. Similar studies have the prospect of conducting with all preparations that are used in crop production, and not only for the reaction to low temperatures, but also for other negative factors for plants. This will allow using the potential of both drugs and plants more effectively. The results obtained in the course of research have shown that detailed information on the features of the action of pesticides under various weather conditions is of great importance for the transition to a higher technological level of crop cultivation. Further research should focus on studying the mechanism of action of the preparations and the biochemical processes that occur in the plant under the influence of the preparations under various hydrothermal conditions.

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None.

## CONFLICT OF INTEREST

The authors of this study declare no conflict of interest.

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# Вплив протруйників насіння кукурудзи на польову схожість за низьких температур

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**Анотація**. Актуальність роботи полягає у встановленні зв'язку між низькими температурами, протруйниками і рослинами, а також в розробці технологічних заходів підвищення резистентності рослин до можливих весняних приморозків. Відповідно до цього, метою роботи було визначення впливу фунгіцидних протруйників на здатність проростків кукурудзи протидіяти низькотемпературному стресу. Для проведення досліджень з встановлення реакції рослин кукурудзи на протруйники фунгіцидної дії (Венцедор, Авіценна, Максим XL, Бастіон і Февер) за умов низьких температур використовували як лабораторний, так і польовий метод. В умовах оптимальних температур лабораторних досліджень обробка всіма препаратами не впливала негативно на енергію проростання і лабораторну схожість насіння гібриду кукурудзи Оніо і лінії В 831. Встановлено, що незважаючи на однакові високі результати лабораторної схожості насіння кукурудзи, польова схожість на провокаційному низькотемпературному фоні надранніх строків сівби в результаті використання різних протруйників сильно різнилась. У різні за зволоженням роки за умов низьких температур для проростання високі показники схожості відмічені при використанні фунгіцидних протруювачів Венцедор, Авіценна і Максим XL (на рівні 70-90 %). Значно нижчі результати польової схожості отримані при використання препаратів Бастіон і Февер – 45-60 %. Таким чином, за умов ранньої сівби, а також враховуючи ризик повернення весняних холодів доцільніше використовувати протруювачі Венцедор, Авіценна і Максим XL. Результати даної роботи свідчать про необхідність проведення оригінаторами пестицидів і науково-дослідними установами більш детального вивчення реакції рослин на препарати за різних погодних умов і мають практичне значення у виробництві для більш ефективного використання препаратів

**Ключові слова:** обробка насіння; фунгіциди; надранні посіви; низькі температури; проростання насіння

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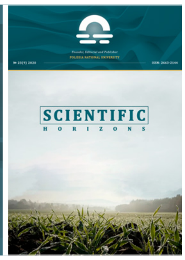


# Causal relationships between wheat productivity and biological activity and soil nitrogen content

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**Abstract**. Coverage of the components of causal dependencies of crop productivity mediated by N-, C-chemical, biological indicators of soil (sizes of pools of relevant available forms, enzymatic activities) in the context of theoretical aspects of resource turnover in it is potentially significant for the design of systems of evaluation criteria for soil health using machine learning (ML) models. The purpose of this study was to find statistically valid and meaningful coordination and causal relationships of grain weight per sown area with available forms of N, C (inorganic and easily hydrolysed N – NIN, NEH; C of labile humus – CLH), in situ proteolytic PA, in vitro urease UA activity in association with the concepts of the turnover of these organogens in the soil under winter wheat under prototypical environmentally friendly fertiliser systems. The

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Stasiv, O., Dubytska, A., Kachmar, O., Dubytskyi, O., & Vavrynovych, O. (2025). Causal relationships between wheat productivity and biological activity and soil nitrogen content. *Scientific Horizons*, 28(8), 30-50. doi: 10.48077/scihor8.2025.30.



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following methods were employed: field, in situ, laboratory-analytical chemical and biochemical, morphometric, statistical, ML-modelling. With a high probability, the increase in GM was caused by an additive increase in NIN, NEH, and a simultaneous decrease in in situ PA, in a way that mediated the effect of in situ PA on GM by the listed forms of N. Statistically less valid was the mediation of the effect of the ratio of NEH to NIN on GM through in situ PA with possibly mutually complementary predispositions of N forms, which, however, provided the basis for substantiating the simultaneous mutually opposite determination of GM by these N forms and in situ PA. It is plausible that CLH and in vitro UA were not involved in the development of GM under these experimental conditions, but it is possible that the latter of these variables may have a more significant effect on the variation of the former than vice versa. Studies in this vein will develop a discourse of theoretical predictions regarding the improvement of the structure of soil quality indices towards revealing aspects of the laws of promoting the balance between the characteristics of agro-ecosystem services, considering the chemical and biochemical properties of N and C turnover in the soil, and crop bioproductivity necessary for the development of sustainable agriculture

**Keywords:** available forms of nitrogen in soil; labile humus carbon; proteolytic and urease activity of soil; winter wheat grain weight; exploratory factor analysis; systems of simultaneous equations; causal determinants

## INTRODUCTION

Nitrogen (N) is a key element for primary production (PP) and soil organic matter (SOM) stabilisation in agroecosystems. Microbial activity (MA) of the soil, especially the enzymatic activity (EA) of N-mineralising enzymes (NMEs), is essential for the release of bioavailable N (BAN) from soil organic matter (OM) (Daunoras *et al.*, 2024; Wang *et al.*, 2024). According to N. Fierer *et al.* (2021), EA is a significant indicator of soil quality and health, characterising its ecological functions and ecosystem services to support bioproductivity. As noted by J. Lehmann *et al.* (2020), the analysis of NME variations is significant for assessing soil health and understanding crop production. However, a series of obstacles, such as interactions between soil components, asynchrony of N (Zhu *et al.*, 2025), etc., complicate the interpretation of changes in EA, N-forms, which also complicates the identification of the relative significance and causality of EA(NME) – BAN – bioproductivity.

Understanding the complex causal relationships between agro-technological interventions, soil characteristics, and crop productivity is key to the development of sustainable agriculture. The literature has identified two principal ways in which such links can be established. The first (*i*) involves the direct or indirect impact of agricultural technologies on soil EA, which leads to changes in SOM and nutrient release, which ultimately affect yields (Hassan *et al.*, 2022). The second (*ii*) focuses on the modification of substrates (S)/intermediates/products by agricultural technologies, which causes changes in EA, which subsequently affect nutrient accumulation, with a possible positive feedback effect and impact on yield (Tian *et al.*, 2020). The following literature review details studies that confirmed and extended these general models by considering the impact of individual factors and processes on EA, N-forms, and the interdependencies between them.

In this context, it is essential to note that S availability and nutrient limitation (LIM) are crucial factors in the regulation of NME and C- and P-metabolism enzymes. The principles of soil ecology and biochemistry expand the understanding of the interdependencies between EA and S, nutrients-LIM. Specifically, X. Tan *et al.* (2021) argued that eco-EA (EEA) of C, N, P cycles, and EE-stoichiometry (EES) depend on the state, nutrient-stoichiometry, relative LIM/availability of microbial resources, soil microbial properties, and environmental conditions. According to the theory of resource allocation, microorganisms can release exoenzymes (increase in EEA) in response to C-, N-, P-LIM to produce LIM nutrients (negative nutrient-efficiency relationships – EEA). However, there may also be an increase in EEA with the addition of S or no clear relationship. The significance of regulating NME and other enzymes is also conditioned by the fact that the uptake of bioavailable N (BAN) by crops is mainly from the soil, particularly from the turnover of SOM and soil organic nitrogen (SON) (Yan *et al.*, 2020). Therefore, the values of the balance sizes of the $N_{IN}$, $N_{EH}$, $C_{LH}$ pools, potentially affecting GM, can be predetermined (regulated) by EA (NME). At the same time, these pools may well be involved in the regulation of isPA, ivUA (feedback loop).

Admittedly, earlier studies by Q. Ma *et al.* (2020), B. Adamczyk (2021), revealed that soil PA is a crucial LIM factor in mineralisation and N availability, although L. Greenfield *et al.* (2020) suggested that the rate of protein supply may have a greater influence on differences in N turnover. PA regulation is a complex process that is influenced by a variety of abiotic and biotic factors, such as NPP, $NO_3^-$ concentration, C:N, and soil height and depth. T. Jesmin *et al.* (2021) noted a $NH_4^+/NO_3^-$-repressive effect on PA, but suggested a stimulating effect of organic C-, N-compounds of plant residues on this NME. Since the SON dynamics, which is determined by SOM and MBC via MA (EA), is vital for the accumulation of mineralised S, it is natural that MBC and initial SOM directly and indirectly through PA and glutaminase affect the SON dynamics.

The mutual regulation between nutrient pools and UA is less understood. Overall, nutrient availability ($NH_4^+$, SOM, SON), C:N are crucial regulators of UA, where $NH_4^+$-sensitivity may vary between distinct groups of bacteria (Chettri *et al*., 2021). K. Ampong *et al*. (2022) also showed that the effect of humic acids on soil UA can be different and depends on their type, origin, and can be modulated by MB, C:N, nitrification/denitrification. UA can be induced by the presence of N and OM but inhibited by high concentrations of $NH_4^+$ as the only N source. Further mineralisation processes are also subject to complex regulation involving S/intermediates/reaction products. The key factors controlling the mineralisation rate are the quantity and quality of detritus and natural fertilisers. Furthermore, C:N in SOM plays a significant role in determining the mineralisation/immobilisation balance (Dalias & Christou, 2022). However, Y. Cao *et al*. (2021) argued that the key role in regulating the response of microbial N immobilisation to OC addition belongs to the characteristics of its chemical quality, which is determined by the ratios of labile, intermediate, and recalcitrant pools.

Nitrification, as one of the final stages of complete N-mineralisation, is also characterised by complex regulation. $NH_4^+$ is S for nitrifying bacteria and therefore accelerates nitrification. On the other hand, high C:N can lead to competition for N among microorganisms, decelerating nitrification. The $NH_4^+$ requirement of plants can also affect the rate of nitrification, as plants are the primary consumers of mineral N. $O_2$ is essential for aerobic nitrifying bacteria and therefore stimulates nitrification while inhibiting anaerobic denitrification. Analysing the relationships between denitrification and readily available SOC (energy source), $O_2$ and $NO_3^-$/mineral N in soil, B. Pan *et al*. (2022) found that the latter contributed to increased $N_2$ emissions. The ratio of $N_2O/(N_2O + N_2)$ increased with increasing soil $O_2$ but decreased with SOC, C:N, and soil pH. Therewith, G.P. Robertson and P.M. Groffman (2024) argued that with sufficient SOC and $O_2$ deficiency, $NO_3^-$ concentration can become a LIM factor in denitrification. Global studies (structure equation modelling, SEM) conducted by Z. Li *et al*. (2020) showed that total nitrogen (TN) and microbial nitrogen (MBN) are positively correlated with nitrification, which is also related to SOM content. An increase in C-LIM (especially microbial) and a decrease in N-LIM (leading to an increase in SON) can stimulate both N-mineralisation and nitrification.

Thus, there is a complex network of interactions/interregulations and causality between biological and chemical factors that control N cycling in soil, including nutrient availability, EA, OM. Mineralisation, nitrification/denitrification are regulated by various biotic and abiotic factors, including C:N, $O_2$, MB. Understanding these relationships is key to assessing the influence of agroecosystems on soil productivity and stability. The purpose of this study was to determine the stochastic (inter)coordination and causal relationships between the soil traits $N_{IN}$, $N_{EH}$, $C_{LH}$, isPA, ivUA and GM of winter wheat under ESFS conditions.

## MATERIALS AND METHODS

The study described in this publication was conducted in 2021-2022 on grey forest surface-gleyed sandy-loam soil under winter wheat (*Triticum aestivum* L.) of the Benefis variety (stationary experiment to investigate the scientific basis of productivity management of short-term crop rotations in the Carpathian region; Institute of Agriculture of the Carpathian Region of the National Academy of Agrarian Sciences of Ukraine). The experimental microplots (area = 1 $m^2$; replication = 3) were arranged systematically; the distance between them was 0.5 m. Before setting up the experiment, the key physicochemical and agrochemical parameters of the soil were determined (depth 0 – 30 cm): $pH_{KCl}$ = 4.78-4.92, hydrolytic acidity (according to Kappen) 23.83-24.61 mg-eq $kg^{-1}$ of the soil, the content of the easily hydrolysed nitrogen ($N_{EH}$; according to Kornfield) 86.22-91.32 mg $kg^{-1}$ of the soil, available phosphorus, available potassium (according to Kirsanov; extraction with 0.2 M HCl) – 105.11-113.04 mg $kg^{-1}$ of the soil and 84.03-90.17 mg $kg^{-1}$ of the soil, respectively, the carbon (C) content of total humus (according to Tyurin) – 1.91-1.96%. All experimental variants (ecologically safe technologies) were divided into 2 groups (each with its own control), depending on the applied precursor: *i*) variants of group A (A-1 – A-7); the precursor was pea (*Pisum sativum* L.); *ii*) variants of group B (B-1 – B-7); the precursor was fodder bean (*Vicia faba* L. var. major Harz). The layout of the typical agrotechnical and ecologically safe factors in the listed variants 1-7 of groups A, B was the same, and was as follows: A-1) control (no mineral or organic fertilisers or ecologically safe factors were used; the precursor was pea); A-2) FPS (field pea straw); A-3) FPS + RMF (recommended rate of mineral fertilisers – $N_{90}P_{60}K_{60}$; A-4) FPS + RMF + BS (biostimulant "Millerplex"); A-5) FPS + RMF + BS + HF (humic fertiliser "BlackJak"); A-6) FPS + RMF + CF (chelated fertiliser of the "Rozalik" range); A-7) FPS + HMF (high rate of mineral fertilisers – $N_{150}P_{120}K_{120}$) + HF; B-1) control (mineral or organic fertilisers or the ecologically safe factors were absent; precursor – fodder beans); B-2) FBS (fodder beans straw); B-3) FBS + RMF (recommended rate of mineral fertilisers – $N_{90}P_{60}K_{60)}$ ; B-4) FBS + RMF + BS (biostimulant "Millerplex"); B-5) FBS + RMF + BS + HF (humic fertiliser "BlackJak"); B-6) FBS + RMF + CF (chelated fertiliser of the "Rozalik" range); B-7) FBS + HMF (high rate of mineral fertilisers – $N_{150}P_{120}K_{120}$) + HF. A brief description, methods, and time of application of the commercial preparations used in the study – components of the ecologically safe factors (bioeffectors), are presented below.

The biostimulant (BS) "Millerplex". Treatment of plants twice during the spring-summer vegetation in the phase of spring tillering, tubing at 0.5 L $ha^{-1}$. Contains natural cytokinins from 3 plant sources, which have enhancing properties for the hormonal regulation of growth of winter cereals at the cellular level, particularly under stressful conditions. Composition of the preparation: N amide – 3.0%, $P_2O_5$ – 3%, $K_2O$ – 30%, algae extract *(Ascophyllum nodosum*); pH – 6.0-6.5; additionally, amino acids, specific carbohydrates (improve the immune system of plants), trace elements (Cu, Fe, Mn, Mg, Zn, B, Co) in chelated form. Built-in adjuvant system for maximum penetration and translocation of biological components into plants. The humic fertiliser (HF) "BlackJak". Spraying on the surface of the root zone twice during the spring-summer growing season in the phases of spring tillering and tubing at 1.0 L $ha^{-1}$. Has a prominent biostimulant effectiveness; apart from humic and fulvic acids, it contains ulmic acids and humin (the most active in plants). Composition: humic acids – 19-21%, fulvic acids – 3-5%, total organic matter – 27-30%; pH – 3.5-5.0. The product is effective both for plants and is most useful in the soil. The chelated fertiliser (CF) of the "Rozalik" range (Zn, P, N, S). Treatment of plants twice during the spring-summer growing season in the phases of spring tillering, tubing at 2.0 L $ha^{-1}$. Composition: N amide – 3%, $P_2O_5$ – 19%, $SO_3$ – 5.3%, Zn in the Zn-EDTA form – 5.9%.

The dynamics of synoptic conditions during the autumn of 2020 and the growing seasons of 2021-2022 was characterised by a combination of the following properties: *i*) the winter and spring-summer intervals of 2020-2021 and 2021-2022 differed in terms of precipitation (PI); in the winter-summer period of 2021, PI was 473.6 mm, while in 2022 it was 318.3 mm (average long-term PI values were 458.0 mm); more than half of these precipitations occurred in the winter-spring interval; *ii*) in 2022 there was a slight lack of moisture during March, May-June, which, however, did not affect biological processes in the soil, plant development, and their final productivity; *iii*) during the entire time interval of the study, the temperature regime of the soil and atmospheric air was predominantly favourable for biological processes in the substrate, for the growth and development of winter wheat; overall, the average monthly temperatures were comparable during the spring and summer periods of 2021 and 2022; the exception was July, when the air temperature was 4.7°C greater than the long-term average. Thus, meteorological conditions during the autumn-winter and spring-summer growing seasons of the above years were generally satisfactory for biological processes in the soil and the development of winter wheat bioproductivity.

After the onset of the ontogenetic stages of spring tillering and wax ripeness (~75% of plants in one of these stages), soil samples were collected (depth 0-30 cm; row spacing – bulk soil) from experimental variants A-1–A-7, B-1–B-7, and prepared for *in vitro* testing of the soil traits described in this study (DSTU ISO 11464-2001, 2003). The content of N-$NO_3^-$ was determined potentiometrically according to DSTU 4725:2007 (2008), N-$NH_4^+$ – using Nessler's reagent (DSTU 4729:2007, 2008); the total content of the 2 listed forms of nitrogen in the soil was denoted as inorganic nitrogen $N_{IN}$ = N-$NO_3^-$ + *N-$NH_4^+$*. The content of easily hydrolysed nitrogen ($N_{EH}$, according to Kornfield) was measured according to DSTU 7863:2015 (2016), the content of carbon of labile humus ($C_{LH}$, soluble in 0.2 M NaOH) was measured according to the Tyurin method – DSTU 4732:2007 (2008). $N_{IN}$, $N_E$H, $C_{LH}$ were calculated at mg $kg^{-1}$ of air-dried soil. To prepare the Nessler's reagent, the study used the corresponding procedure presented in S. Abdelwahed *et al.* (2021), which the authors of the present study modified as follows: 45.50 g of $HgJ_2$ "CHDA" (Chemlaborreactor LLC) was mixed with 35.00 g of KJ "CHDA" (Sphere Seven LLC), 50.00 ml of double-distilled water was added, and gradually stirred in 112.00 g of cold KOH "CHDA" (Sphere Seven LLC) + double-distilled water until the components were completely dissolved; stirring and cooling, the volume was gradually brought to 800.00 ml, and then to 1.00 L; left in the dark for ~72 h. The resulting reagent was filtered (ashless filter "Blue Ribbon"); therewith, the neck of the funnel contained glass wool, previously washed with dilute solutions of HCl, distilled water, and double-distilled water.

To determine the *in vitro* urease activity (urease, urea aminohydrolase, EC 3.5.1.5) in soil (ivUA) (0-30 cm; row spacing – bulk soil), the study employed typical approaches for testing this activity in soils, plant seeds, microorganisms, specifically, soil treatment with toluene (Cheng *et al.*, 2020; Tahir *et al.*, 2023), a reaction mixture based on 0.2-0.5 M phosphate-buffered saline (PBS) with pH ≈ 7.0, incubation temperature 30-38°C, incubation time 24 h (Zusfahair *et al.*, 2021; Magomya *et al.*, 2022), photometric measurement of $NH_4^+$ concentration with Nessler's reagent at $\lambda \approx 400$ nm (Nathan *et al.*, 2020). Briefly, ~5.00 g of air-dry soil, 10.00 ml of PBS (pH 6.7), 0.50 ml of toluene, 10.00 ml of 10.00% urea solution were added to an Erlenmeyer flask, sealed with cork stoppers, incubated at 30°C for 24 h; after incubation, 15.00 ml of 1 M KCl solution were added (reaction stop + $NH_4^+$ displacement); the contents of the tube were centrifuged at $3 \times 10^3$ rpm; an aliquot of the supernatant was significantly diluted with double-distilled water in analytical tubes, and a solution of K, Na-tartrate was added (the last 2 steps were performed in such a way as to leave room only for the Nessler reagent); 5–10 min after the addition of Nessler's reagent, the extinction of the solution was measured at $\lambda = 400$ nm using an electrophotocolourimeter CFC-2. The value of ivUA was calculated in mg $NH_4^+$ $kg^{-1}$ of air-dried soil $hour^{-1}$. The toluene, urea, K, Na-tartrate used in this method were of AR grade (Sphere Seven LLC), the phosphate components of PBS, KCl were of chemically pure grade (Sphere Seven LLC); all solutions were prepared with double-distilled water.

To determine the *in situ* proteolytic activity (isPA) in the field using the method of Mishustin, Nikitin, and Vostrov (described by Khaziev) (Dubytska *et al.*, 2022), a gelatine-containing coating of medical X-ray film was used as a substrate. The film was placed vertically in the soil (up to 30 cm deep from the bottom edge; row spacing) and left for 4–8 days, pressed down from the sides (so that the middle of this time interval was approximately at the beginning of the onset of the ontogenesis phases of spring tillering and wax ripeness, i.e., ~75% of plants in one of these phases). After the film was excavated, it was placed in chilled plastic containers and transported to the laboratory. Before and after excavation, the film was weighed on a Radwag AS 220/R2 analytical balance (Poland) (±0.0001 g); before weighing the film with the already fermented gelatine-containing coating, the substrate carrier was lightly washed from the soil with a moderate stream of tap water and then air-dried for several hours. The isPA was calculated as the ratio of the film weight after and before application, expressed as %%. All the above measurements were performed in 3 analytical (or "field" for isPA) parallels, and in 3 replicates ($n = 6$). The obtained individual data for each indicator in the ontogenetic phases of spring tillering and wax ripeness were averaged among themselves for the spring and summer vegetation in 2021 or 2022. Thus, sets of averaged individual data ($n = 6$) were obtained for the corresponding periods of plant development for years 1 and 2.

In grassroots approaches, such as genetic studies, at least 8–10 random or representative plants per plot or replication are sufficient to record ear productivity of agricultural cereal plants, specifically, grain weight per ear, *etc*. (Zhai *et al.,* 2020). In the present study, after the full grain ripeness stage (~75% of plants), 6 ears of grain were selected from productive shoots of winter wheat plants per replication, and in 3 replications ($n = 18$) to estimate the grain weight per plant. Grain weight was measured on an analytical balance (±0.0001 g) (Radwag AS 220/R2, Poland). Grain mass per sown area (GM, kq $m^{-2}$ of the sowing area) was calculated as the product of grain mass per plant and the number of productive shoots per $m^2$. The final values of each soil and plant trait for the 2 years of research were obtained by averaging the corresponding values (for $n$ = 6 or $n$ = 18; year 1, year 2) for the spring-summer growing season in 2021 and 2022.

The assessment of statistical validity $p$ of pairwise differences between the numerical sizes of the analysed indicators in experimental variants A-1–A-7, B-1–B-7 was assessed using the Student's t-test calculated as described by S. Brown *et al.* (2020); the appraisal of statistical soundness $p$ of differences between the data in the groups of experimental variants A-1–A-7, B-1–B-7, and in the combined category A-1–B-7 were performed using ANOVA; the grades listed were executed in Microsoft Office Excel 14.0.7128.5000. The indicated differences were considered significant if $p < 0.05$. The construction and analysis of the full form of the general linear model (GLM) and its not full form (without factor(s)) for the dependence of GM on the continuous predictors (covariates – random effects of variation $N_{IN}$, $N_{EH}$, $C_{LH}$, isPA, ivUA), categorical variable (factor – fixed effects of variation caused by experimental variants A-1–A-7, B-1–B-7) were performed on non-standardised individual (the continuous predictors) and neighbourhood averages (the categorical variable) in the experimental variation series of each experimental variant using Statistica Version 10 (StatSoft Inc). The corresponding data were presented only for the not full form of GLM. The Pearson product-moment correlation coefficient (PMC; based on the arithmetic means of each experimental variant), the corresponding $p$ values, exploratory factor analysis (EFA) with dependent variable (GM) for 6 and 2 factors (based on the standardised means for each experimental variant; extraction – principal component analysis (PCA); rotation – varimax normalised) were calculated in the same software package. For the above methods of factor extraction and rotation, the study employed the options built into Statistica Version 10 (StatSoft Inc.) (specifically, Statistics → Multivariate exploratory techniques → Factor analysis → Variable selection → … → Advanced → Extracting method: Principal component analysis → … → Factor rotation: Varimax normalised). To obtain a variation series of 6 individual GM data per experimental variant, in the original variation series of 18 individual GM data per variant, each 3 neighbouring individual data were averaged.

The results of the EFA (2 factors) served as the basis for a preliminary prediction of potential causal relationships between the variables under study. For a more detailed assessment of the hypothesised causal relationships between predictors and response functions, simultaneous equations models (Eqs.), designated as SIEMs (from non-standardised averages for each trial) in the GNU Regression, Econometrics and Time-series Library package (GRETL 2021b; a free Unix-like operating system developed by the GNU Project); only recursive SIEMs among them were selected for the categories of resulting SIEMs. Since the 3-stage least squares (3SLS) approach to SIEMs was found to be heteroscedastic in some cases (the Breusch-Pagan test), the authors of the present study employed the weighted least squares (WLS) method (i.e., essentially, the weighted two-stage least squares W2SLS method) with iterative estimation. Additionally, in the case of 3SLS models, significant over-identification (the Hansen-Sargan test) was demonstrated several times. For the final SIEMs, the 2-stage least squares (2SLS) method provided mostly almost the same estimates as WLS, but the latter approach was still more reliable. Only the estimates built into GRETL 2021b were used as typical estimates of the statistical effectiveness of SIEMs. The values of Fisher's Criterion (FC) and their statistical validity for each Eq. in SIEMs were estimated in Microsoft

Office Excel 14.0.7128.5000 according to D.M. Levine *et al.* (2021). During the theoretical analysis, it became necessary to combine 2 Eqs. with semantic and mathematical mutual inverse causality within one system (e.g., $C_{LH}$ → ivUA, ivUA → $C_{LH}$). The analysis of such SIEM using seemingly unrelated regressions (SUR), 2SLS, 3SLS, WLS approaches did not reveal any successful variance models. In this regard, two types of independent Eqs. were found ($C_{LH}$ → ivUA; ivUA → $C_{LH}$) using the Ordinary Least Squares (OLS) method (OLM) in GRETL 2021b. In addition, combining the last of the listed types of Eqs. into a single model within the assumed "subordination" using the 4 methods listed above did not lead to the formation of valid SIEMs. Therefore, the independent Eqs. derived by OLM were fully analysed (including FC) in GRETL 2021b and left for further interpretation. The study followed the standards of the Convention on Biological Diversity (1992) and the Convention on Trade in Endangered Species of Wild Fauna and Flora (1979).

## RESULTS

The study found that under the conditions of the environmentally safe technologies in the soil of variants of groups A (A-2–A-7) and B (B-2–B-7), there was a significant ($p$ < 0.001-0.05) increase in the content of $N_{IN}$ by 8.93-64.36%, 5.37-86.19%, relative to the control variants A-1, B-1, respectively (Fig. 1, a). Analogously, statistically significant ($p$ < 0.001) increases in $N_{EH}$ content in the variants of groups A-2–A-7 and B-3–B-7, compared to the indicated controls, were 5.86-34.59%, 21.26-29.50%, respectively. In the case of B-2, the last of the listed indicators did not undergo statistically significant changes ($p$ > 0.01), compared to B-1. The values of both of the listed agrochemical features of soil N-state were statistically significantly ($p$ < 0.001) greater by 23.68-76.69% (for $N_{IN}$), 21.06-29.49% (for $N_{EH}$) under the conditions of the ecologically safe technologies A-3–A-7, B-3–B-7 *vs.* A-2, B-2, respectively (Fig. 1, a). Furthermore, in the variants A-2–A-7, B-2–B-7, there was an increase in the level of $C_{LH}$ by 5.73–39.17%, 7.93-41.38% ($p$ < 0.001–0.01), relative to A-1, B-1, respectively (Fig. 1, b); in addition, the ecologically safe technologies A-3–A-7, B-3–B-7 caused an increase in the size of this indicator by 3.92-31.63%, 4.15-30.99% ($p$ < 0.001-0.05) compared to A-2, B-2, respectively.

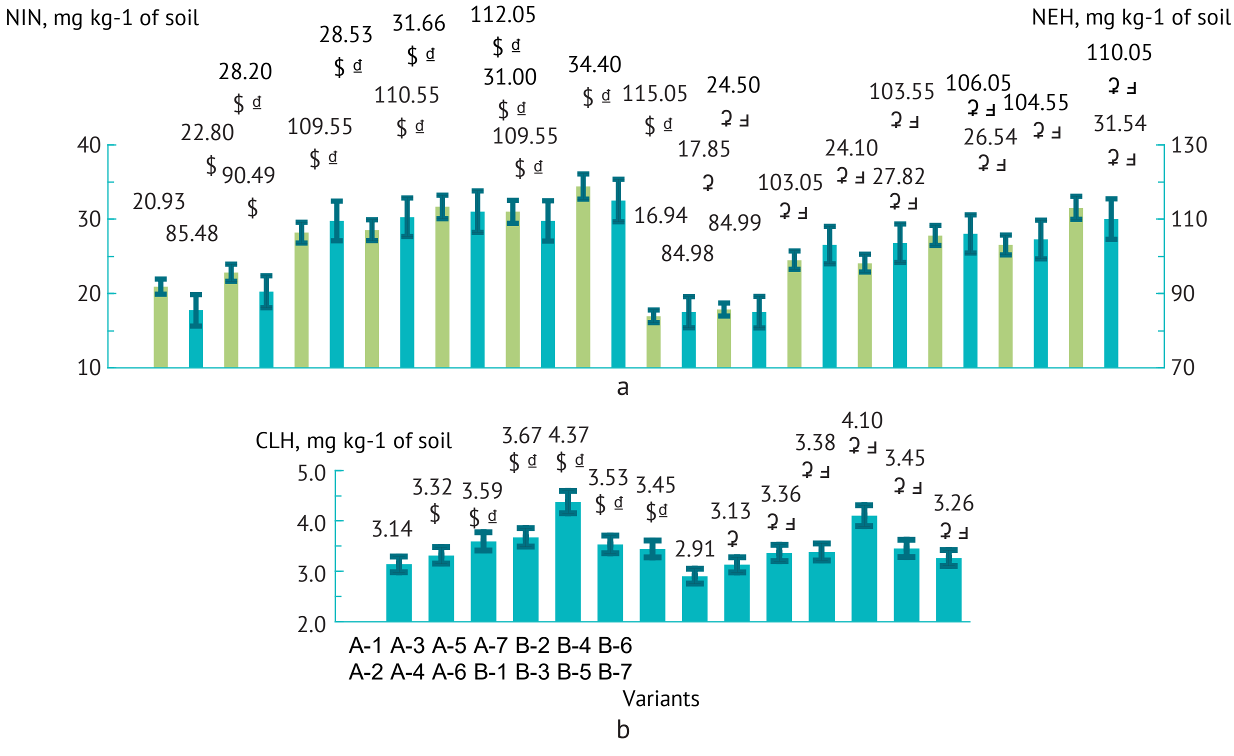


***Figure 1**. Variability of inorganic nitrogen ($N_{IN}$), easily hydrolysable nitrogen ($N_{EH}$) (a) and C of labile humus ($C_{LH}$) (b) in the soil under winter wheat under the research variants*

***Note:** A-1, B-1 – the controls of variant groups A, B with pea and fodder beans as precursors, respectively; A-2–A-7 or B-2–B-7 – the ecologically safe technologies of variant groups A or B, respectively; column height in the diagram = arithmetic mean (mean, M; n = 6; data averaged by ontogenetic stages spring tillering, wax ripeness, and for 2021–2022), error bars = ± standard error (SE, m); numbers next to the levels of M ± m – numerical values of the corresponding means; $ or ʔ – the statistical validity of differences between the study variants A-2–A-7 and A-1 or B-2–B-7 and B-1 – p < 0.001–0.05; đ, ɟ – the statistical reliability of differences between the variants A-3–A-7 and A-2 or B-3–B-7 and B-2 – p < 0.001–0.05; the statistical soundness of differences between the data in ANOVA for A-1–A-7, B-1–B-7 – p < 0.001–0.003, A-1–B-7 – p < 0.001*

***Source:** developed by the authors of this study*

All the ecologically safe technologies used in variants A-2–A-7, B-2–B-7 caused a statistically significant ($p < 0.001$) increase in isPA by 1.50-9.35%, 2.05–9.40% *vs.* A-1, B-1 controls, respectively (Fig. 2, a); at the same time, the increase of the indicated soil EA under conditions A-3–A-7, B-3–B-7 by 3.40-7.85%, 2.91-7.35% (*p* 0.001) was found compared to A-2, B-2, respectively. Additionally, the ecologically safe factors in variants A-2–A-7, B-2–B-7 caused a statistically significant ($p < 0.001$) increase in ivUA by 93.69-404.50%, 120.19-522.12%, relative to controls A-1, B-1, respectively (Fig. 2, b); ivUA increases in A-3–A-7, B-3–B-7 when compared with A-2, B-2, were 74.81-160.47%, 78.78-182.53% ($p < 0.001$), respectively.

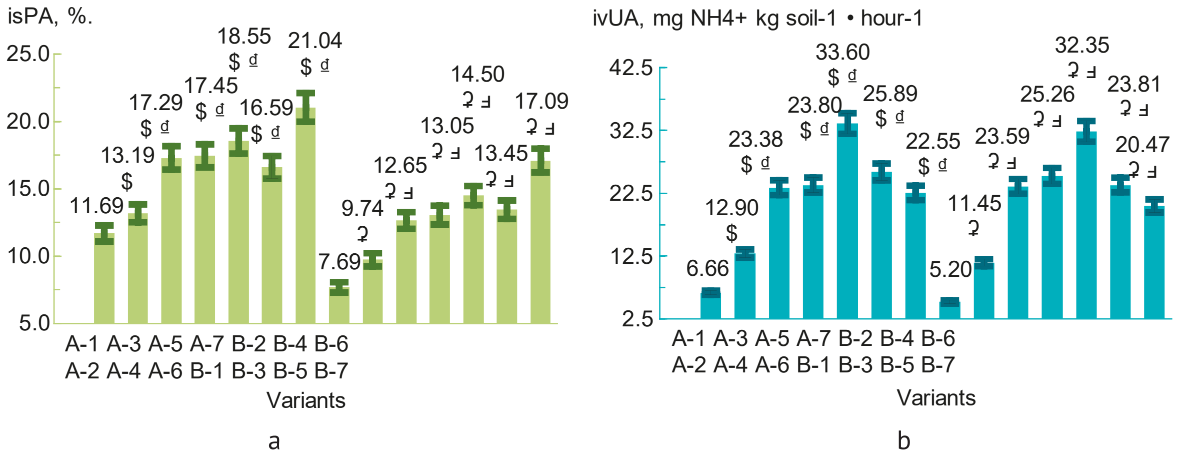


***Figure 2**. Changes in the arithmetic averages of in situ proteolytic activity (isPA) (a) and in vitro urease activity (ivUA) (b) in the soil under winter wheat depending on the research variant*
***Note:** A-1, B-1 – the controls of variant groups A, B with pea and fodder beans as precursors, respectively; A-2–A-7 or B-2–B-7 – the ecologically safe technologies of variant groups A or B, respectively; column height in the diagram = arithmetic mean (mean, M; n = 6; data averaged by ontogenetic stages of spring tillering, wax ripeness, and for 2021–2022), error bars = ± standard error (SE, m); numbers next to the levels of M ± m – numerical values of the corresponding means; $ or ʔ – the statistical reliability of differences between the variants A-2–A-7 and A-1 or B-2–B-7 and B-1 – p < 0.001; đ, ɟ – the statistical validity of differences between the variants A-3–A-7 and A-2 or B-3–B-7 and B-2 – p < 0.001; the statistical soundness of differences between the data in ANOVA for A-1–A-7, B-1–B-7, A-1–B-7 – p < 0.001*
***Source:** developed by the authors of this study*

Each of the ecologically safe technologies used in variants A-2–A-7, B-2–B-7 provided a statistically significant ($p < 0.001$) increase in GM within 14.29-159.11%, 17.55-163.30%, compared to controls A-1, B-1, respectively (Fig. 3); the ecologically safe factors in variants A-3–A-7, B-3–B-7 caused the levels of increment of this indicator by 49.57-126.72%, 50.23-123.98% ($p < 0.001$), relative to A-2, B-2, respectively.

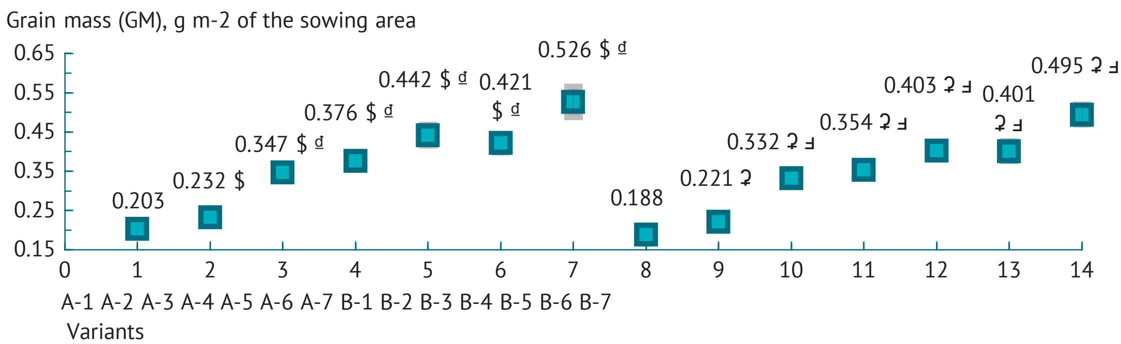


***Figure 3**. Grain mass (GM) per sowing area of winter wheat depending on the research variant*
***Note:** A-1, B-1 – the controls of variant groups A, B with pea and fodder beans as precursors, respectively; A-2–A-7 or B-2–B-7 – the environmentally safe technologies of variant groups A or B, respectively; "shaded square" symbols – the arithmetic mean (mean, M; n = 18; ontogenetic phase of wax ripeness; averaged data for 2021-2022), error bars = ± standard error (SE, m); numbers next to the "shaded square" symbols – numerical values of the corresponding means; $ or ʔ – the statistical validity of differences between the research options A-2–A-7 and A-1 or B-2–B-7 and B-1 – p < 0.001; đ, ɟ – the statistical reliability of differences between the variants A-3–A-7 and A-2 or B-3–B-7 and B-2 – p < 0.001; the statistical soundness of differences between the data in ANOVA for A-1–A-7, B-1–B-7, A-1–B-7 – p < 0.001*
***Source:** developed by the authors of this study*

The one-factor (paired) linear correlation analysis showed the presence of statistically valid ($p \leqslant 0.001$-$0.05$) medium and close interdependencies between GM and $N_{IN}$, $N_{EH}$, $C_{LH}$, isPA, ivUA with PMC values of 0.5500-0.9473 (Table 1). Therewith, the PMC values for the mutual subordinations between the last 5 variables listed were also significant and statistically reliable (0.6055-0.9441; $p \leqslant 0.001$-$0.05$). Such results most likely reflect multicollinearity within the correlation matrix under study. This substantially hinders the development of even the conceptual configuration of the causal relationships between GM and the N- and C-soil indicators presented here.

***Table 1****. Estimates of effect sizes, parameters, power, and efficiency for the not full form of GLM dependence of GM on the studied soil indices and the Pearson product-moment correlation coefficient (PMC) between these traits*

| Index | GLM (n = 6 × 14) | | | | Product-moment correlation coefficient (PMC) | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Index | | | | | | | | |
| | Partial $\eta^2$ | Parameter | $\beta$ | OP ($\alpha$ =0.05) | GM | $N_{IN}$ | $N_{EH}$ | $C_{LH}$ | isPA |
| Intercept | 0.5498æ | 582.202æ | – | 1.0000æ | – | – | – | – | – |
| $N_{IN}$ | 0.1607æ | 24.273æ | 1.217æ | 0.9684æ | 0.9473æ | - | – | – | – |
| $N_{EH}$ | 0.1285æ | -4.871æ | -0.638æ | 0.9176æ | 0.9347æ | 0.9441æ | – | – | – |
| $C_{LH}$ | 0.3232æ | -155.755æ | -0.711æ | 1.0000æ | 0.5500č | 0.6224č | 0.6500č | – | – |
| isPA | 0.0015 | -2.358 | -0.078 | 0.0630 | 0.8607æ | 0.9608æ | 0.8965æ | 0.6055č | – |
| ivUA | 0.4019æ | 10.027æ | 0.743æ | 1.0000æ | 0.7733æ | 0.7553æ+ | 0.8567æ | 0.8669æ | 0.6732ƀ |

| | Separate performance indicators for the not full form of GLM |
|---|---|
| $R^2$ | 0.7564 |
| $R^2$(Adj) | 0.7408 |
| FC | 48.4435 |
| p (GLM) | < 0.01 |

***Note:*** *GLM – General Linear Model (6 data per variant; 14 variants); β – standardised regression coefficient; OP – observed power; $R^2$, $R^2$ (Adj), FC, p (GLM) – the multiple coefficient of determination, adjusted multiple coefficient of determination, the Fisher coefficient, and the statistical reliability of the GLM; GM, NIN, NEH, CLH, isPA, ivUA – the grain mass, the inorganic N, the easily hydrolysed N, the carbon of labile humus, in situ proteolytic activity, the in vitro urease activity; æ, æ +, ƀ, č – the statistical validity of p ⩽ 0.001, p = 0.002, p ⩽ 0.01, p ⩽ 0.05, respectively*
***Source:*** *developed by the authors of this study*

The authors of this study suggested that for a better understanding of the systemic principles of the probable way GM is subordinated to $N_{IN}$, $N_{EH}$, $C_{LH}$, isPA, ivUA, it may be potentially expedient to generate the full form of the GLM (data not presented; the reasons for this are explained below) and/or the not full form of the GLM (without categorical variable); the results of the latter analysis are presented in Table 1. On the other hand, the relevance of this approach is confirmed by the properties of the structure of the used experimental data, namely: *i)* the above-mentioned multicollinearity within the correlation matrix of the data; *ii)* the deviation from the normal distribution of the $N_{EH}$ parameter ($p$ for $H_1$ "there is a deviation from the normal distribution (ND)" in the case of the Doornik-Hansen, Lilliefors, and Shapiro-Wilk W-statistic tests was 0.0130-0.0222; in this situation, only the Jarque-Bera test revealed $p = 0.4017$). Notably, logarithmisation of the data exacerbated the situation with the ND for $\ln(N_{EH})$ and caused deviations from the ND for ln(ivUA) (approximately the same as in the case of the aforementioned mean variable).

In the resulting full form of the GLM ($R^2 = 0.8351$, $R^2_{(Adj)} = 0.7894$, $p < 0.01$; data not presented), only the values of the partial $\eta^2$ for the intercept (= constant = $b_0$) and the categorical variable (effects of variation due to the experimental variants) were statistically valid ($p < 0.001$-$0.05$); the rest of the values of the partial $\eta^2$ for the independent variables were statistically insignificant ($p = 0.7162$-$0.9795$). In this form of GLM, the values of the parameters (regression coefficients $b_0$, $b_i$) for the intercept = $b_0$ and the categorical variable for options A-1, A-2, A-7, B-1, B-2 were statistically valid ($p < 0.001$-$0.05$). Therewith, the statistical significance of the variability of all the variables under study by variants was proved earlier using ANOVA (outside the full form of the GLM) and, on the other hand, the full form of the GLM was unable to characterise the subordination of GM to the predictors presented herein. The analysis of the not full form GLM showed significantly higher values of the partial $\eta^2$, parameter = $b_0$ for the intercept, compared to the partial $\eta^2$, $b_i$ for the other regressors, and at the same time, statistical unreliability of the partial $\eta^2$, $b_4$ for isPA (Table 1). Clearly, this means that GM is more dependent on GM properties than on the regressors, and that GM is not caused by the variation of isPA. The synergistic effect of $N_{IN}$, ivUA, and the antagonistic effect of $N_{EH}$, $C_{LH}$ on GM development (by the signs of the parameter) are also evident

(Table 1). Therewith, certain inconsistencies between the not full form GLM and the correlation matrix of the data, as well as the lack of opportunities to predict the mediating effects of predictors, limit the potential of using this model to establish causality within the analysed system of variables.

The next step towards predicting causality was to apply EFA (with GM as the dependent variable) to find indicators that had the following set of properties: *i)* they shared variance with each other and simultaneously with GM; *ii)* their variance was separated from the unique variance and *iii)* from the error variance (Schreiber, 2021; Watkins, 2021). Furthermore, M.S. Sulaiman *et al.* (2021) demonstrated that regression of variables on the principal components (PCs) or the rotated PCs (essentially, on the factors in the FA) is characterised by a lower level of muticollinearity compared to regression of the original data; moreover, $R^2$ is greater in the second case. Thus, EFA can reduce multicollinearity between variables. For factor extraction in this study, PCA was employed to reduce the number of items to a smaller number of representative uncorrelated components and to account for a larger proportion of the variance. Earlier, N. Shrestha (2021) confirmed that PCA solutions can lead to the emergence of items with cross-loadings; the variance of such an item can be explained by several factors. However, the authors of the current study considered it possible to ignore this drawback to achieve the "essentially" desired purpose of EFA here. Based on the theoretical hypothesis that a simple latent structure should be at least partially based on uncorrelated measurements, the factor loadings matrix was orthogonally rotated using the varimax normalised method. An item was considered a statistically relevant factor identifier if it had a loading of ⩾0.7 (Watkins, 2021).

The EFA results for the 6 factors (= the number of all variables) are presented in Table 2. The eigenvalues (EV) for the factors FA-1–FA-6 were 4.9895, 0.6947, 0.2206, 0.0673, 0.0148, 0.0130, and the corresponding values of the total variance (%) for the eigenvalues were 83.16, 11.58, 3.68, 1.12, 0.25, 0.22. For almost all variables within FA-1–FA-4, cross-loadings were found in the range of debatable values ~0.3-~0.6 (Watkins, 2021), and no such loadings were found with values closer to significant (e.g., 0.60-0.69). As mentioned above, this may be conditioned by the PCA extraction of the factors, but it is also possible that it may be caused by the properties of the original data, specifically, multicollinearity between the analysed features (Kyriazos & Poga, 2023). However, statistically valid loadings occur only in the case of FA-1 and FA-2. Additionally, the EVs and total variance values for FA-1–FA-6 indicated the presence of a "breakpoint" (Schreiber, 2021) immediately after FA-1 or, at least, after FA-2, while the software used suggested leaving only FA-1 and FA-2.

***Table 2**. Loadings of the first six factors (the standardised variables; extraction: PCA; rotation: varimax normalised)*

| Variable | Factor loadings | | | | | |
|---|---|---|---|---|---|---|
| | FA-1 | FA-2 | FA-3 | FA-4 | FA-5 | FA-6 |
| GM | 0.8062* | 0.2590 | 0.4330 | 0.3088 | 0.0018 | 0.0106 |
| $N_{IN}$ | 0.8959* | 0.3398 | 0.2526 | 0.0861 | 0.0152 | 0.1026 |
| $N_{EH}$ | 0.7749* | 0.3739 | 0.4967 | -0.0054 | -0.1140 | -0.0043 |
| $C_{LH}$ | 0.2970 | 0.9453* | 0.1288 | 0.0326 | -0.0229 | 0.0012 |
| isPA | 0.9301* | 0.3371 | 0.1034 | -0.0664 | 0.0204 | -0.0756 |
| ivUA | 0.4059 | 0.7116* | 0.5688 | 0.0494 | 0.0536 | 0.0114 |
| Expl.Var. | 3.1710 | 1.8359 | 0.8489 | 0.1107 | 0.0170 | 0.0165 |
| Prp.Totl. | 0.5285 | 0.3060 | 0.1415 | 0.0184 | 0.0028 | 0.0027 |

***Note:** GM – grain mass (dependent variable); $N_{IN}$, $N_{EH}$, $C_{LH}$, isPA, ivUA – inorganic N, easily hydrolysed N, labile humus carbon, in situ proteolytic activity, in vitro urease activity (independent variables); FA-1–FA-6 – extracted factors; * – loadings > 0.700000*

***Source:** developed by the authors of this study*

Elimination of FA-3 to FA-6 resulted in an increase in the amount of explained variance and the share of total variation for FA-1 and FA-2 (Table 3). The insignificant cross-loadings for FA-1 for the variables $C_{LH}$, ivUA and for FA-2 for the variables GM and $N_{IN}$, $N_{EH}$, isPA were within 0.2833–0.5148. The authors of this study considered it expedient not to involve the last of these elements in the interpretation to preserve the possibility of forming and explaining simple, meaningful and mathematical constructs for the desired causal relationships between the variables. Even though the ratio between the EVs for FA-1 and FA-2 (~7.182 > 3) (Schreiber, 2021) was found to be low (potentially, it may represent a relatively small part of the total variance, which, while not directly attributable to the model, can affect the observed estimates of such a construct in the experimental conditions and in reality). It is likely that in this case, the subordinations between the statistically reliable FA-2 items ($C_{LH}$, ivUA) are at least partially independent of the key causal relationships between FA-1 items (GM, $N_{IN}$, $N_{EH}$, isPA).

***Table 3**. Loadings of the first two factors (extraction: PCA; rotation: varimax normalised), obtained based on the standardised variables*

| **Variable** | **Factor loadings** | |
|---|---|---|
| | **FA-1** | **FA-2** |
| GM | 0.9065* | 0.3370 |
| $N_{IN}$ | 0.9205* | 0.3666 |
| $N_{EH}$ | 0.8604* | 0.4661 |
| $C_{LH}$ | 0.2833 | 0.9364* |
| isPA | 0.8975* | 0.3243 |
| ivUA | 0.5148 | 0.8228* |
| Expl.Var. | 3.5600 | 2.1243 |
| Prp.Totl. | 0.5933 | 0.3540 |

***Note:** GM (dependent variable) – grain mass; $N_{IN}$, $N_{EH}$, $C_{LH}$, isPA, ivUA (independent variables) – inorganic N, easily hydrolysed N, labile humus carbon, in situ proteolytic activity, in vitro urease activity; FA-1, FA-2 – extracted factors; marked (*) loadings > 0.700000*

***Source:** developed by the authors of this study*

Considering that the general organisation of the causal relationships between the variations of N-, C-soil indicators and GM studied herein under conditions of "central" mediation involving $N_{IN}$ (and/or $N_{EH}$) or isPA (and/or ivUA), proposed in the INTRODUCTION section, is partly based on experimental data, partly on theoretical findings, and itself constitutes a certain theoretical generalisation, the following ways to elucidate the detailed structure of such subordination could be possible, namely: *(i)* exploratory structural equation modelling (ESEM) and its variants; *(ii)* confirmatory factor analysis structural equation modelling (CFA/SEM) and its variants; and *(iii)* path analysis models. However, the inclusion of latent constructs, complex types of variances, and combinations of mediator, moderator conditions between them, etc., in ESEM, CFA/SEM is more suitable for creating models on sufficiently large samples with a prominent level of generalisation of the final mathematical construction. Therewith, a series of simplifications in path analysis approaches, again, contribute too much to the "generalisation" of the resulting model. In the case of the more "local" patterns studied herein and the use of rarely used integrated environmentally safe technologies, it would be advisable to first characterise the causal relationships between variables with a somewhat greater level of specificity (as it were, narrowing down generalisations).

In this regard, the resulting factor structure (Table 3) was interpreted in the following 2 stages. Firstly, it is reasonable to assume that between the variables that significantly load (> 0.7) the factor 1, there may, overall, be causal subordination, where (*i*) $N_{IN}$ (or $N_{EH}$) are predetermined and mediate the effects of each other and/or isPA on GM under the conditions of simultaneous action of $N_{IN}$, $N_{EH}$, isPA on GM, or (*ii*) not only $N_{IN}$ (or $N_{EH}$) (mediating each other's effects), but also isPA (mediating the effects of both $N_{IN}$ and/or $N_{EH}$) are predetermined, provided that $N_{IN}$, $N_{EH}$, isPA act simultaneously on GM. Secondly, variables that reliably load (> 0.7) the factor 2 can be involved in semantic and mathematical reverse causality: variation in $C_{LH}$ causes the development of ivUA, and vice versa, variability in ivUA leads to the formation of $C_{LH}$. Clearly, the above concept of causality between the above N- and C-soil condition traits and the trait of final biological productivity of winter wheat differs in a series of general points and nuances from the one presented in the INTRODUCTION section. A brief presentation of the finally proposed systems of causal subordination is as follows. Firstly: (*i*) $N_{IN}$, $N_{EH}$, isPA → *GM*; isPA, $N_{EH}$ → $N_{IN}$ (or isPA, $N_{IN}$ → $N_{EH}$); isPA → $N_{EH}$ (or isPA → $N_{IN}$), respectively; (*ii*) $N_{IN}$, $N_{EH}$, isPA → GM; $N_{IN}$, $N_{EH}$ → isPA; $N_{EH}$ → $N_{IN}$ (or $N_{IN}$ → $N_{EH}$), respectively. Secondly: $C_{LH}$ → ivUA (and/or ivUA → $C_{LH}$).

All the above groups of causality were estimated by 3SLS, 2SLS, WLS, and, if necessary, SUR, OLS (the latter for the group of independent Eqs. (IEs) for the $2^{nd}$ stage) using non-standardised data (specifically, to approximate concrete experimental conditions) to generate mathematically relevant, interpretable SIEMs or IEs. All SIEMs and IEs that characterise the causal and only mathematical relationships, respectively, between the studied soil and winter wheat traits listed in the previous paragraph are presented below. The SIEM-1 contains Eqs. (1), (2), (3):

$$\ln((0.5260 \times 50)/GM) = 15.0370^{a} - 1.4308^{c} \times \ln N_{IN} - 1.6750^{c} \times \ln N_{EH} + 0.6445^{d} \times \ln isPA, \quad (1)$$

$$\ln((34.4050/N_{IN}) \times 100) = 9.9197^{a} - 0.4669^{a} \times \ln isPA - 0.8193^{b} \times \ln N_{EH}, \quad (2)$$

$$\ln((115.0500/N_{EH}) \times 100) = 5.7005^{a} - 0.3667^{a} \times \ln isPA, \quad (3)$$

where $^{a}$, $^{b}$, $^{c}$, $^{d}$ – $p \leqslant 0.001$, 0.01, 0.05, 0.1.

Here and in the following Eqs., the symbol "ln" means natural logarithm.

The SIEM-2 contains Eqs. (1) (see above), (4), (5) as follows:

$$\ln((115.0500/N_{EH}) \times 100) = 6.4714^{a} + 0.1204 \times \times \ln isPA - 0.6349^{b} \times \ln N_{IN}, \quad (4)$$

$$\ln((34.4050/N_{IN})) = 6.9292^{a} - 0.7673^{a} \times \ln isPA, \quad (5)$$

where $^{a}$, $^{b}$ – $p \leqslant 0.001, 0.01$.

The SIEM-3 contains Eqs. (1) (see above), (6), (7) as follows:

$$\ln((21.045/isPA) \times 100) = 7.4790^{a} - 1.4494^{a} \times \ln N_{IN} + 0.4825 \times \ln N_{EH}, \quad (6)$$

$$\ln((34.4050/N_{IN}) \times 100) = 13.3878^{a} - 1.8373^{a} \times \ln N_{EH}, \quad (7)$$

where $^{a}$ is $p \leqslant 0.001$.

The SIEM-4 contains Eqs. (1) and (6) (see above), (8) as follows:

$$\ln((115.0500/N_{EH}) \times 100) = 6.3158^{a} - 0.4887^{a} \times \ln N_{IN}, \quad (8)$$

where $^{a}$ is $p \leqslant 0.001$.

The group of independent Eqs. (IEs) (9), (10) is as follows:

$$\ln((33.5950/ivUA) \times 100) = 8.0052^{a} - 1.6055^{a} \times \ln(\ln^2(\ln^2(\ln^2 C^2_{LH}))), \quad (9)$$

$$\ln((4.3750/C_{LH}) \times 100) = 8.2472^{a} - 0.6816^{c} \times \ln ivUA + 0.0418^{b} \times \ln^4 ivUA - 0.0099^{b} \times \ln^5 ivUA, \quad (10)$$

where $^{a}$, $^{b}$, $^{c}$, $^{d}$ – $p \leqslant 0.001, 0.01, 0.05, 0.1$.

The symbols GM, $N_{IN}$, $N_{EH}$, isPA, ivUA, $C_{LH}$ denote the grain mass per sowing area, the content of inorganic N, easily hydrolysed N, the *in situ* proteolytic and *in vitro* urease activities, the labile humus C content, respectively. Eqs. (1)-(10) presented herein are variants of the linearised bounded exponential function denoted as log-ratio-$Y_{max}$; non-linearised versions of this function, on the one hand, asymptotically approach 0, and on the other hand, are limited to the maximum value of the dependent variable $Y_{max}$ (the value in the numerator of the left-hand side of each Eq.). Depending on the grade $b_i$, this function can describe *i*) an exponential decline to 0; *ii*) an exponential increase, bounded from above by $Y_{max}$ (in the case of an inverse relationship or a certain transformation of the variables). The bounded exponential function can be found, specifically, in the publications of T.C. Han *et al.* (2021), T. Kolokolnikov and D. Iron (2021), M.M.T. Lakshani *et al.* (2023); a non-linearised form of the same Eq. was presented by M. Portnoi *et al.* (2021).

SIEM-1, SIEM-2 characterise the mediation of isPA effect on GM by $N_{IN}$, $N_{EH}$ traits in case of simultaneous action of these indicators of soil N status on the final biological productivity of winter wheat. SIEM-3, SIEM-4 reflect the mediator role of isPA in the additive effect of $N_{IN}$, $N_{EH}$, isPA on GM, provided that the effects of $N_{IN}$ and $N_{EH}$ on the specified trait of the plant production process are mediated by each other, and all soil N-indicators considered here simultaneously affect GM. IEs (9), (10) characterise the independent mathematical determinations of log-ratio-$Y_{max}$ (ivUA) or log-ratio-$Y_{max}$ ($C_{LH}$) by modified variables based on $C_{LH}$ or ivUA, respectively.

Estimates of the statistical efficiency of SIEM-1-SIEM-4, IEs are presented in Table 4. For all Eqs. within SIEMs and IE (10), relatively low values of the regression standard error (RSE) were obtained (0.0332-0.0723), while for IE (9), the value of this characteristic was slightly greater (0.2745). The sizes of the coefficient of determination ($R^2$, the adjusted coefficient of determination ($R^2_{(Adj)}$) for all Eqs. in SIEMs and IEs were significant and amounted to 0.7838-0.9670, 0.7658-0.9610, respectively, while the statistical validity of the Fisher coefficient (FC = 40.2072-162.5170) was $p < 0.001$. The indicated ranges of the SIEMs and IEs estimates were accompanied by rather small values of the MAPE: 0.5612-2.8312 (Table 4). For all Eqs. within the SIEMs and IEs, the statistical reliabilities of the hypotheses $H_1$ "heteroscedasticity occurs" and $H_1$ "there is a deviation of the residuals from normality" were $p > 0.005$, reflecting the absence of heteroscedasticity and violations in the ND residuals. The determinants of the covariance matrices of the residuals for SIEM-1-SIEM-4 were quite close to 0; the corresponding ln-values were within −17.8911-−16.1084, providing compelling evidence of linear dependencies between the individual Eqs. in these SIEMs. For the IE (10), the ln-determinant of the covariance matrix of the regression coefficients was −43.7045, while for the IE (9) it was −8.0508. This may suggest that IE (10) is much more consistent with a linear relationship between the predictors and the response variable than IE (9).

***Table 4**. Statistical performance estimates of the SIEMs and IEs*

| No. of of the SIEMs, IEs and Eqs. | RSE | $R^2$ | $R^2_{(Adj)}$ | FC | MAPE | Heteroscedasticity test (HST) | Residue normality test (RNT) | Ln(det) of the cov-matrix[e] |
|---|---|---|---|---|---|---|---|---|
| SIEM-1 | | | | | | | | |
| 1 | 0.0723 | 0.9499 | 0.9348 | 63.1363[æ] | 1.4285 | 1.0000[ʒ] | 0.2591[ʒ] | -17.8911[ʤ] |
| 2 | 0.0377 | 0.9670 | 0.9610 | 161.1814[æ] | 0.6430 | | | |
| 3 | 0.0479 | 0.7997 | 0.7830 | 47.9096[æ] | 0.7758 | | | |
| SIEM-2 | | | | | | | | |

*Table 4. Continued*

| ***No. of of the SIEMs, IEs and Eqs.*** | ***RSE*** | ***$R^2$*** | ***$R^2_{(Adj)}$*** | ***FC*** | ***MAPE*** | ***Heteroscedasticity test (HST)*** | ***Residue normality test (RNT)*** | ***Ln(det) of the cov-matrix***ɘ |
|---|---|---|---|---|---|---|---|---|
| 1 | -ϕ | -ϕ | -ϕ | -ϕ | 1.4285 | | | |
| 4 | 0.0332 | 0.9039 | 0.8864 | 51.7170æ | 0.5612 | 1.0000ɠ | 0.2888ʒ | -17.8911ʣ |
| 5 | 0.0544 | 0.9312 | 0.9255 | 162.5170æ | 0.98755 | | | |
| SIEM-3 | | | | | | | | |
| 1 | -ϕ | -ϕ | -ϕ | -ϕ | 1.4285 | | | |
| 6 | 0.0664 | 0.9352 | 0.9235 | 79.4192æ | 1.1983 | 1.0000ɠ | 0.5302ʒ | -16.1084ʣ |
| 7 | 0.0662 | 0.8980 | 0.8894 | 105.5880æ | 1.1869 | | | |
| SIEM-4 | | | | | | | | |
| 1 | -ϕ | -ϕ | -ϕ | -ϕ | 1.4285 | | | |
| 6 | -ϕϕ | -ϕϕ | -ϕϕ | -ϕϕ | 1.1983 | 1.0000ɠ | 0.5081ʒ | -17.4327ʣ |
| 8 | 0.03416 | 0.8980 | 0.8894 | 105.5631æ | 0.5742 | | | |
| IE | | | | | | | | |
| 9 | 0.2745 | 0.7838 | 0.7658 | 43.5126æ | 2.8312 | 0.4407–0.7132ɟ | 0.7478ʤ | -8.0508ʤ |
| 10 | 0.0335 | 0.9234 | 0.9005 | 40.2072æ | 0.32962 | 0.5068–0.8590ɟ | 0.7504ʤ | -43.7045ʤ |

***Note:*** *RSE, $R^2$, $R^2_{(Adj)}$, MAPE, FC – the regression standard error, the coefficient of determination, the adjusted coefficient of determination, the mean absolute percentage error, the Fisher coefficient, respectively; ɘ – the natural logarithm of the determinant of the covariance matrix; æ – the statistical reliability of the FC – $p < 0.001$; ϕ or ϕϕ – the same values (sizes) of efficiency estimates, as in the case of the previous Eq. (1), SIEM-1 or Eq. (6), SIEM 3; ɠ, ʒ – the sizes (quantities) of the statistical validity p of the hypothesis $H_1$ (HST (Breusch-Pagan)): "heteroscedasticity occurs", and $H_1$ (RNT (Doornik-Hansen)): "there is a deviation from normality"; ɟ, ʒ – the intervals of sizes (quantities) of the statistical validity p of the hypothesis $H_1$ (HST(White-only squares and the usual full versions, Breusch-Pagan regular and robust versions)): "heteroscedasticity occurs", and $H_1$ (RNT): "there is a deviation from normality", respectively; ɐ – the natural logarithm of the determinant of the covariance matrix of residuals of the SIEM or of the covariance matrix of the regression coefficients of each independent OLM-Eq; ʣ, ʤ – obtained from the covariance matrix of the residuals of different Eqs. of each SIEM or from the covariance matrix of the regression coefficients of each independent OLM-Eq, respectively*

***Source:*** *developed by the authors of this study*

Considering that for Eqs. (2), (3), (4), (5) within SIEM-1, SIEM-2, the RSE values (0.0332-0.0544), the MAPE range (0.5612-0.98755), and the values of the ln-determinant of the covariance matrix of residuals (−17.8911) are smaller compared to the corresponding estimates for Eqs. (6), (7), (8) in SIEM-3, SIEM-4 (0.03416-0.0664; 0.5742-1.1983; −17.4327-−16.1084) (Table 4), it is reasonable to assume that SIEM-1, SIEM-2 may have a certain priority over SIEM-3, SIEM-4. However, the comparison of these Eqs. in the above groups of models by $R^2$, $R^2_{(Adj)}$ (0.7997-0.9670, 0.7830-0.9610 vs. 0.8980–0.9352, 0.8894-0.9235, respectively) (Table 4) rather testifies in favour of the advantages of SIEM-2 over the rest of the analysed SIEMs. Still, it is possible that SIEM-1 will become relevant if and when Eqs. (2), (3) still dominate Eqs. (4), (5) under experimental and/or real field conditions. Nevertheless, SIEM-3, SIEM-4 can make a lot of sense in situations where there are various factors, stochastic disturbances that will cause deviations from the ND residuals of the considered SIEMs (greater *p* values (lower $\chi^2$) for the $H_1$ (residual normality test): "there is a deviation from normality" for SIEM-3, SIEM-4 are more difficult to reject than the corresponding smaller *p* values for SIEM-1, SIEM-2).

Significantly smaller (~ by an order of magnitude) RSE and MAPE, greater $R^2$, $R^2_{(Adj)}$, and substantially smaller values of the natural logarithm of the covariance matrix of the regression coefficients for IE (10), compared to IE (9) (Table 4) argue for the probable advantages of the log-ratio-$Y_{max}$ ($C_{LH}$) dependence on ivUA over the log-ratio-$Y_{max}$ (ivUA) dependence on $C_{LH}$, however, without specifying causality. This assumption is also supported by a comparison of the values of the log likelihood, Akaike, Schwartz, and Hennane-Queen criteria (30.0500, −52.3366 to −49.5438 for IE (10) versus −0.686762, 5.2552 to 6.6516 for IE (9), respectively). Therewith, for each of the regressors in IE (10), structural shifts were found by the Chow test (centre of the sample); the latter may reflect a partially imperfect model specification and, possibly, the effects of multicollinearity on the properties of this model.

Notably, the 3SLS method is typically more consistent and provides more asymptotic estimates for SIEM and SEM compared to 2SLS, OLS. This is not least conditioned by the fact that 3SLS is a combination of 2SLS and SUR (Tetteh, 2024). In this regard, 3SLS jointly estimates the entire model in a structural form and factors in the simultaneous cross-correlations between errors

(Butkus *et al*., 2024). Such modelling properties allow adjusting the weighting matrix for potential heteroscedasticity problems in 3SLS regression equations using the covariance matrix of the residuals from 2SLS; the generalised least squares method is usually used for parameter estimation (Huda & Istiana, 2024). However, 2SLS is effective when there is a secondary predictor that is correlated with the problem predictor (even hypothetically) but not with the error term. In the case when stochastic disturbances in different equations are uncorrelated or there is no heteroscedasticity, the results of 2SLS are typically equivalent to 3SLS estimates (Tetteh, 2024). Notably, the advantages of 2SLS over 3SLS may substantially depend on the level of multicollinearity between the variables (Oduntan, 2023). It would not be unreasonable to assume that the priority of 2SLS, or even OLS *vs*. 3SLS, at least in part, may also be conditioned by contamination of variables by errors, specifically, with small samples, depending on the accuracy of identification of Eqs., the type of distribution, and sample size. Overall, it is possible that the combination of similar multicollinearity and identification problems, in addition to small sample sizes, were significant reasons for the preference of 2SLS (SIEM-1–SIEM-4) and OLS (IEs (9), (10)) over 3SLS.

Therewith, the approach to formulating and evaluating SEM, SIEM (even if it is 2SLS) can be considered as a task for an artificial neural network (ANN), and therefore it makes sense to consider SEM, SIEM as special cases of ANN (Pérez-Sánchez *et al*., 2024). The latter clearly suggests the relevance of using SEM, SIEM to solve various problems, at least not of a large size, based on mathematical and statistical regularities and assumptions about causality between variables. It is natural to assume that the mentioned significance of SEM, SIEM is inherent in biological and agricultural scientific problems.

## DISCUSSION

The authors of the present study assumed that changes in $N_{IN}$, $N_{EH}$, $C_{LH}$ in the soil of variants A-1–B-7 are the outcome of a complex interaction of physicochemical and biological processes initiated by plant residues (PR–FPS/FBS), mineral fertilisers (RMF/HMF) and/or bioeffectors (BS/HF/CF). Considering the following arguments, it is worth noting that the C:N ratio in biomass or straw (STR) of legumes is several times lower than in non-legumes (Watthier *et al*., 2020; Grimm *et al*., 2024) and may approach the threshold of 25:1 for this criterion in the substrate (S) (Cao *et al*., 2021). Thus, it is likely that the addition of legume STRs alone (variants A-2, B-2) stimulated N-releasing microbial activity (MA), N-cycling, and gross mineralisation (Robertson & Groffman, 2024), providing energy for heterotrophic PR-degrading microflora (analogous to priming – PRM (Jesmin *et al*, 2021)), although efficiency may be reduced under N deficiency due to N sequestration in the microbial biomass (MB) (Henneron *et al*., 2020).

Reducing C:N in OM or adding N (also possibly $N_{IN}$) can enhance net N mineralisation and weaken N immobilisation (Sawicka *et al*., 2020; Cao *et al*., 2021; Robertson & Groffman, 2024), which is known as PRM (Meng *et al*., 2020) and was observed in A-3, B-3 (FPS/FBS + RMF). Such intensive treatments can increase PR and $CO_2$-production, but do not promote C-sequestration (Jesmin *et al*., 2021). Mineralisation of OC (especially under N-stimulation) releases C,N-S to MB, enhancing MA, which decomposes PR, increasing N-$NH_4^+$ and N-$NO_3^-$ (observed for $N_{IN}$ in A-3, B-3). The formation of $N_{IN}$ is preceded by the formation of available SON from its turnover in soil and PR (Yan *et al*., 2020). The evident partial overlap of the fraction of dissolved organic nitrogen (DON) with $N_{EH}$ explains the unidirectional changes in $N_{IN}$ and $N_{EH}$ in the soil of the studied variants (Farzadfar *et al*., 2021). Comparable changes in $C_{LH}$ in A-3, B-3 are probably conditioned by the decomposition of SOM and SON, especially with PR.

The increase in the *in situ* PA (isPA) in A-2 and B-2, compared to A-1, B-1, was consistent with stimulated mineralisation due to a decrease in C:N in S and accumulation of $N_{IN}$, $N_{EH}$, $C_{LH}$ (Jesmin *et al*., 2021). Removal of N-limiting (N-LIM) can increase microbial attack on fresh OM (Henneron *et al*., 2020). The levelling of C-LIM by available N (Luo *et al*., 2021) explains the analogous changes in isPA, $N_{IN}$, $N_{EH}$, $C_{LH}$ in A-3, B-3, relative to A-1, B-1, which is also consistent with the dependence of protein mineralisation on S and proteases (Greenfield *et al*., 2020) and the correlation of PA with DON (Farzadfar *et al*., 2021), and thus probably with $N_{EH}$. Unidirectional changes of the *in vitro* UA (ivUA) and isPA (at least in A-1–B-3) are predicted, specifically, due to the participation of UA in N cycling and based on the findings of J. Yang *et al*. (2020), M.I. Khan *et al*. Experiments by P. Tian *et al*. (2020) revealed the effect of STR ploughing depth on ivUA. The application of N fertiliser + rice STR increased ivUA (Sharma *et al*., 2021), while commercial mulch decreased ivUA in long-term experiments (Sun *et al*., 2021).

Changes in soil physicochemical properties in A-4–B-7 are potentially caused by a combination of PRM (N-stimulated mineralisation of crop residues, as in A-3, B-3) and rhizosphere PRM (rPRM), considering the significance of the relationship between the rhizosphere and bulk soil (Sun *et al*., 2021). In A-4–A-6/B-4–B-6, rPRM could be initiated with BS, BS+HF or CF. BSs contain phytohormones and C,N-auxiliary compounds that stimulate metabolism, growth, and nutrient uptake (Franzoni *et al*., 2022), and CFs may act similarly to BSs and HFs (Musych *et al*., 2024). It was assumed that BS, BS+HF, CF stimulated N uptake, photosynthesis, wheat growth, and C-rhizodeposits release, which could enhance N cycling through rPRM degradation of SOM (Henneron *et al*., 2020). This could have been combined with bulk soil N-PRM (A-3, B-3), causing an increase in $N_{IN}$, $N_{EH}$, $C_{LH}$, NME. However, the absence of substantial differences in $N_{IN}$, $N_{EH}$, $C_{LH}$, isPA, ivUA in A-4, A-6, B-4, B-6 *vs*. A-3, B-3 reflects insignificant synergistic effects between the 2 types of PRM and, probably, the predominance of the influence of N-stimulated PR mineralisation. The power and duration of these technologies are clearly insufficient for C and N sequestration.

Conversely, A-5, B-5 had maximal $C_{LH}$, insignificant changes in $N_{IN}$, $N_{EH}$, isPA, and a significant increase in ivUA (the latter complicates the assessment of N sequestration) compared to A-3, B-3. It was assumed that the synergistic effect of BS+HF in A-5, B-5 stimulated the rhizosphere turnover of SOM, probably activating C-cycles throughout the soil. Q. Ma *et al.* (2020) argued that N mineralisation and nitrification increase with decreasing N- and increasing C-LIMs Therefore, the decrease in C-LIMs due to increased SOM turnover led to the accumulation of $C_{LH}$ in A-5, B-5, while the intensity of N-turnover and $N_{IN}$, $N_{EH}$, isPA were comparable to A-3, A-4, A-6 and B-3, B-4, B-6. Based on the hypothetical pattern of C sequestration under chronic N deposition (Lu *et al.*, 2021) and the findings of Q. Ma *et al.* (2020), partial enhancement of C- and weakening of N-LIMs in A-7, B-7 (HMF-apl.) was assumed. The rPRM from HF may not generally compensate for C-LIM in these variants, which was accompanied by a moderate decrease in $C_{LH}$ turnover, but also a moderate increase in N mineralisation ($N_{IN}$, $N_{EH}$, isPA); ivUA co-changed with $C_{LH}$.

Changes in N, C, and NME forms reflect the functioning and interaction of microbes and plants at the soil level, where intermediates (IM) and final products (FP) of these cycles are a source of plastic material and energy. MA, particularly NME, can influence crop productivity, which is consistent with the observed correlation (PMC) ($N_{IN}$, $N_{EH}$, $C_{LH}$) – GM, as well as (isPA, ivUA) – GM. The significance of soil NME for plant productivity was confirmed by studies (Zhou *et al.*, 2020; Yang *et al.*, 2022; Garnaik *et al.*, 2022), which showed $r > 0$ for (ivPA/UA) – (wheat/rice yield). The absence of GM dependence on isPA within the GLM used may be partially consistent with the findings of other studies (Nath *et al.*, 2021), where no significant single-factor dependence (rice yield) – ivPA was found. However, the authors of the present study believe that the results of the incompletely parameterised GLM provide insufficient information to understand the causal relationships between the considered regressors and GM.

The EFA results reflect that GM is dependent on $N_{IN}$, $N_{EH}$, and isPA (factor 1, FA-1), while $C_{LH}$ and ivUA form a separate FA-2, potentially affecting each other but not directly GM, which may reflect the partial decoupling of the N and C cycles and the asynchrony of N cycle elements (Ma *et al.*, 2020). The N-LIM of microorganisms may undergo a deepening under excessive available C (PR), which will stimulate compensatory N-mineralisation (Tan *et al.*, 2021; Qiu *et al.*, 2021; Cui *et al.*, 2022), explaining the spatial and temporal divergence of the N and C cycles. Admittedly, with the depletion of labile fractions of plant SOM/SON, microbes attack N-enriched SOM, activating N-mineralisation, which increases N availability. Conversely, the presence of C in low-energy compounds favours C-LIM. Thus, changes in the energy supply of microorganisms, affecting the C/N balance, limit N mineralisation and break the causality between microbial C and N availability/demand (Zhang *et al.*, 2021).

The link between $C_{LH}$ and ivUA may be mediated by intensification of ureolysis, which increases N availability, promoting the development of microbial communities less capable of degrading recalcitrant C, leading to C sequestration and reduced NME activity. Indirect C-input from plant residues can stimulate EA, particularly UA (Tan *et al.*, 2021). Other studies have not found a direct correlation between rice/maize yields and ivUA (Nath *et al.*, 2021; Kalala *et al.*, 2022). The distribution of variables by FA-1, FA-2 partially revises the hypotheses about the causal relationships between soil characteristics and wheat GM. In method (*i*) (abbreviated: NME → agrochemical/physical-chemical soil characteristics → GM), the simultaneous additive effect of $N_{IN}$, $N_{EH}$, isPA on GM is assumed (Eq. (1)), where each of the pools of $N_{IN}$, $N_{EH}$ depends on isPA and another pool (Eqs. (2), (3) in (SIEM-1, Eqs. (4), (5) in SIEM-2). Thus, the effect of isPA on GM can be mediated through $N_{IN}$ (depending on the ratio of isPA and $N_{EH}$) or through $N_{EH}$ (depending on the ratio of isPA and $N_{IN}$).

To argue for a positive feedback loop (PFL) (method (*ii*); abbreviated: agrochemical/physical-chemical soil characteristics → NME activity → GM), the resource allocation theory is applied, according to which nutrients-LIM for microorganisms stimulates the production of eco-EA (EEA) for their assimilation (Luo *et al,* 2021; Mori *et al.*, 2021), i.e., EEA ∝ 1/(nutrient availability) (negative feedback loop (NFL), possible under C-LIM; L. Luo *et al.* (2021)). C-enrichment can limit microorganisms, causing an increase in EA for other nutrients, particularly N (Tan *et al.*, 2021), which could account for the PFL between EA and N and C forms found by P. Tian *et al.* (2020). Enhanced N mineralisation can stimulate NME more strongly than EA to produce C (Qiu *et al.*, 2021). The size of the hydrolase pool ($V_{max}$) ∝ of the S concentration (Tan *et al.*, 2021). Causality method (*ii*) assumes a combination of simultaneous determination of GM by $N_{IN}$, $N_{EH}$, isPA with the predetermination of isPA by the ratio $N_{EH}/N_{IN}$ and mutual determination of $N_{IN}$ and $N_{EH}$. Hence, Eq. (1) is combined with the mediation of the effect of the $N_{IN}/N_{EH}$ balance through isPA on GM and the mediation of the effect of $N_{EH}$ on GM through $N_{IN}$ (or vice versa) (SIEM-3/SIEM-4). In causality mode (*ii*), GM simultaneously depends on $N_{IN}$, $N_{EH}$, and isPA (Eq. (1)), while isPA is mediated by $N_{EH}/N_{IN}$ (Eq. (6)), while $N_{IN}$ and $N_{EH}$ are mutually determined (Eqs. (7), or (8); SIEM-3/SIEM-4).

Eq. (1) showed the direct effect of $N_{IN}$ and $N_{EH}$ on GM growth and the inverse dependence of GM on isPA.

In SIEM-1, an increase in isPA and $N_{EH}$ mediated an increase in $N_{IN}$, while an increase in isPA alone mediated an increase in $N_{EH}$. In SIEM-2, a decrease in isPA and an increase in $N_{IN}$ could mediate the effect of $N_{EH}$, while an increase in isPA alone could cause an increase in $N_{IN}$. In SIEM-3/SIEM-4, an increase in $N_{IN}$ and a decrease in $N_{EH}$ were influenced by an increase in isPA, while an increase in $N_{EH}$ caused an increase in $N_{IN}$ (and vice versa). All these dependencies are statistically significant. The statistical significance of the relationship between $C_{LH}$ and ivUA (IE 10) reflects correlation rather than causation, and $C_{LH}$ may be influenced by other factors. The expected positive effect of $N_{IN}$ and $N_{EH}$ on GM (Eq. (1)) contrasts with the unexplained inverse relationship between isPA and GM. This contradiction is partially explained by the dependence of $N_{IN}$ growth on isPA and $N_{EH}$, and $N_{EH}$ growth on isPA (Eqs. (2), (3)), as well as the dependence of $N_{EH}$ growth on isPA decline and $N_{IN}$ growth, and $N_{IN}$ growth on isPA increase (Eqs. (4), (5)).

The positive effect of $N_{EH}$ and isPA on $N_{IN}$ (Eq. (2)) suggests that protease-mediated mineralisation of S from $N_{EH}$ (isPA) increases the available forms of $N_{IN}$, which is consistent with the concept of N-mineralisation (Robertson & Groffman, 2024) and the significance of SON/DON (Ma *et al*., 2020; Yan *et al*., 2020). This is also consistent with the role of soil TN (and likely SON) and MBN in controlling nitrification (Li *et al*., 2020). The ability of isPA to stimulate $N_{IN}$ accumulation (Eq. (2)) supports the concept that protein mineralisation rates depend on available S and protease production (Greenfield *et al*., 2020), with the amount of S ($N_{EH}$) being more significant. Interpreting $N_{EH}$ as proteolysis products, its level increases with increasing isPA (Eq. (3)), which is also consistent with N-mineralisation (depolymerisation of SON by isPA leads to the accumulation of $N_{EH}$/DON; X. Sun *et al*., 2021) and the effect of ivPA and other factors on SON (Yang *et al*., 2020). The triggering effect of isPA on $N_{EH}$ (Eq. (3)) and the consolidated effect of isPA and $N_{EH}$ on $N_{IN}$ (Eq. (2)) are manifested in Eq. (1) as additive stimulating effects of $N_{IN}$ and $N_{EH}$ on wheat GM with simultaneous suppressive effect of isPA on GM. It is unclear whether this is a direct effect of isPA on GM or a latent NFL between this EA and $N_{IN}$/$N_{EH}$, which is consistent with the theory of resource allocation in the soil.

In SIEM-2, the effect of isPA on $N_{IN}$ and $N_{EH}$ with the stimulating effect of the latter on GM and the NFL from isPA on GM are complex. The key is the activating effect of isPA on $N_{IN}$ (Eq. (5)), simultaneously with the suppressive effect of isPA and the stimulating effect of $N_{IN}$ on $N_{EH}$ (Eq. (4)). The ability of isPA to increase $N_{IN}$ (Eq. (5)) is consistent with the characteristics of N-mineralisation and proteolysis. The stimulatory effect of $N_{IN}$ on $N_{EH}$ (Eq. (4)) may be conditioned by PFL, which is consistent with the significance of SON turnover (Yan *et al*., 2020) and soil N priority (Anas *et al*., 2020), and is also consistent with FL control by $N_{IN}$ of the BAN pool (Daly *et al*., 2021). Enhanced microbial C supply and activation of N-mineralisation could contribute to the development of PFL control of NME/isPA by $N_{IN}$. The stimulatory effect of $N_{IN}$ on $N_{EH}$ (as a product of isPA) may reflect the stimulation of isPA levels by $N_{IN}$, which is partially consistent with the findings of L. Greenfield *et al*. (2020). The inverse effect of isPA on $N_{EH}$ may reflect a decrease in S with an increase in EA. Therefore, in SIEM-2, isPA stimulates $N_{IN}$ (Eq. (5)), which increases $N_{EH}$ (Eq. (4)), and the growth of both forms of N positively influences GM (Eq. (1)). The simultaneous increase of isPA balances the activating effect of $N_{IN}$ on $N_{EH}$ (Eq. (4)) and counteracts the favourable effects of these forms of N on GM (Eq. (1)).

For SIEM-3 and SIEM-4, the feedback between isPA and its products is significant. The stimulatory effect of $N_{IN}$ on $N_{EH}$ (Eq. (8), SIEM-4, as in Eq. (4), SIEM-2) may reflect the enhancement of isPA by FP proteolysis and N-mineralisation, which is consistent with the activating effect of $N_{IN}$ on isPA (Eq. (6), SIEM-3/4). The direct relationship between $N_{IN}$ and $N_{EH}$ (Eq. (7), SIEM-3) reflects that $N_{IN}$ increases with increasing $N_{EH}$. The inhibitory effect of $N_{EH}$ (as products of isPA) on isPA (Eq. (6), SIEM-3/4) indicates FL. Therefore, in SIEM-3/4, an increase in $N_{EH}$ (the result of isPA) causes an increase in $N_{IN}$ (Eq. (7)), which can enhance isPA (Eq. (6)) and expand $N_{EH}$ (Eq. (8)), while an increase in $N_{EH}$ suppresses isPA. The indirect ratio of the effects of $N_{IN}$ and $N_{EH}$ on isPA increase can cause GM falloff, while the consolidated gain of these N forms is GM gain (Eq. (1)). The stimulatory effect of $N_{IN}$ on $N_{EH}$ can lead to an indirect decrease in GM gain by isPA; on the other hand, the expansion of $N_{IN}$ can cause an increase in isPA, which will lead to GM falloff.

This feedback is different from the one proposed by X. Tan *et al*. (2021). However, the stimulatory effect of $N_{IN}$ on $N_{EH}$ and isPA (Eqs. (4), (8), (6)) is consistent with the ability of $N_{IN}$ to increase ivPA, which is consistent with r > 0 for $N\text{-}NH_4^+$ – ivPA (Sawicka *et al*., 2020), but partially contradicts the r < 0 found by them for $N\text{-}NO_3^-$ – ivPA. These interpretations may be opposed by the effects of ivPA inhibition by high concentrations of $NH_4^+$ or $NO_3^-$ (Jesmin *et al*, 2021) and a moderate r < 0 between the rate of slow phase protein mineralisation and $NO_3^-$ (Greenfield *et al*., 2020), although a possible r > 0 between N-cycle enzyme (NAG) activity and $NO_3^-$ (Błońska *et al*., 2021). The attenuation of isPA by $N_{EH}$ is partially consistent with the resource allocation theory and the concept of decreasing NME activity with increasing available N forms (He *et al*., 2021), where the size of the $N_{EH}$ pool may reflect the availability of N. Additionally, E. Błońska *et al*. (2021) argued that mineralisation dominates immobilisation in the decomposition of N-rich detritus, which may lead to a decrease in NME production and r < 0 between this EA and SON/DON/$N_{EH}$ components, but r > 0 between NAG and N light fractions is possible (Błońska *et al*., 2020).

The statistical efficiency of IE (10) versus IE (9) and the greater nonlinearity of the latter reflect a probable dependence of $C_{LH}$ on ivUA, rather than vice versa.

However, the activating effect of humic acids (HA) and vermicompost on ivUA is known to be accompanied by an increase in microbial population and C:N, while HA from weathered coal/leonardite can inhibit ivUA (Ampong *et al*., 2022). IvUA is nonlinearly dependent on the mass fraction of soil macroaggregates (Zhao *et al*., 2020), but the effect of initial OM and MBC on ivUA, as well as the dependence of SON on the latter, was not confirmed (Yang *et al*., 2020). At the same time, an inverse relationship between ivUA and MBC was found (Sun et al., 2021). There is evidence of the effect of ivUA on SOC components, including a significant effect on fine POC (Liu *et al*., 2021) and participation in the pre-mortemisation of other SOC fractions. IvUA can mediate the effect of organic fertiliser on MBC and its effect on LOC, as well as the effect of organic fertiliser on LOC/UOC and their effect on SOC (Zhang *et al*., 2022).

The obtained results of EFA, SIEMs, IEs do not show a clear direction of changes in ivUA and N conversion/mineralisation, unlike the study of organic tree mulching (Sun *et al*., 2021). However, the PMC between ivUA – $N_{IN}$ is partially consistent with the directly proportional ivUA – $NH_4^+$ relationships (Sun *et al*., 2021; Sawicka *et al*., 2020), the high probability of ivUA – $N_{IN}/NH_4^+/NO_3^-$ relationships (Sharma *et al*., 2021), and the positive effect of $NO_3^-$ on ivUA (Tian *et al*., 2020). Inconsistencies between correlation and multivariate analyses, as well as ambiguities in their agreement with the literature, may be in favour of the expected spatial and temporal divergence of N and C cycle trajectories in all tested variants, which may be conditioned by *i*) the specific features of N- and C-mineralisation, PRM and the role of plants in each variant, *ii*) insufficient number of explanatory variables to reflect the relationships between N- and C-cycles in soil in relation to plant productivity. The application of PR with/without fertilisers and bioeffectors determined the direction of N- and C-mineralisation, where variations in $N_{IN}$, $N_{EH}$, isPA were the probable causes of GM changes. All the analysed causal relationships were characterised by a positive effect of $N_{IN}$ and $N_{EH}$ on GM and a possible suppressive effect of isPA on GM. The increase in GM due to an increase in $N_{IN}/N_{EH}$ can potentially be accompanied by a decrease in isPA (a possible latent NFL), which is partially consistent with the theory of resource allocation and the stoichiometry of N-mineralisation.

Considering the statistical efficiency of SIEMs, mediation of isPA effect on GM via $N_{IN}/N_{EH}$ (SIEM-1/2) is more probable than mediation of N-forms effect on GM via isPA (SIEM-3/4). The most plausible is the stimulating effect of $N_{IN}$ on isPA and the dependence of $N_{EH}$ on the balance between isPA and $N_{IN}$ (SIEM-2). Less relevant are the dependence of $N_{EH}$ on isPA and the conditionality of $N_{IN}$ on the consolidated effect of isPA, $N_{EH}$ (SIEM-1). Statistically, the least reliable isPA control of the $N_{EH}/N_{IN}$ ratio (SIEM-3/4) may be consistent with latent NFL and may be updated under certain conditions.

Simultaneously with the influence of N-characteristics on GM, there may be a separate dependence of $C_{LH}$ on ivUA and other unidentified factors (IE 10), while the dependence of ivUA on $C_{LH}$ (IE 9) is less likely. The reasons for the separation of $C_{LH}$ and ivUA from the N-characteristics continue to be unknown but can hypothetically be related to the specific features of microbial and plant economics in various experimental variants.

## CONCLUSIONS

The expediency of argumentation of changes in N-, C-chemical, N-biological soil indicators ($N_{IN}$, $N_{EH}$, $C_{LH}$, *in situ* PA – isPA, *in vitro* UA – ivUA) in the context of the following hypothetical and theoretically predictable processes in soil is illustrated: (*i*) N-, C-mineralisation/immobilisation, (*ii)* sequestration, (*iii*) priming, (*vi*) aspects of resource allocation. The most effective were the rationales built in the areas (*i*), (*iii*), (*vi*). The statistical validity of the predetermination of grain weight per sown area of GM winter wheat by the interdependencies $N_{IN}$, $N_{EH}$, isPA was proved. The simultaneous significant probability of the interrelationships between $C_{LH}$ and ivUA revealed the disconnect between the availability and demand of microorganisms for C and N under these experimental conditions. Among the crucial properties of the causal relationships between these variables were the stimulating effect of $N_{IN}$, $N_{EH,}$ and the reverse effect of isPA on GM, as well as a more significant mediation of isPA action through $N_{IN}$, $N_{EH}$ (especially when $N_{IN}$ is predetermined by isPA level, and at the same time, $N_{EH}$ size is predetermined by the ratio of isPA and $N_{IN}$), compared to the mediation of the proportion of $N_{IN}$, $N_{EH}$ effects through isPA (considering the predetermination of $N_{IN}$ by $N_{EH}$ size or *vice versa*). In contrast to the first of the above variants of variable premixing, the second helped to partially isolate the elements of the basis for the opposite influence of $N_{IN}$, $N_{EH}$, on the one hand, and isPA, on the other hand, on GM formation. The non-causal conditioning of $C_{LH}$ by ivUA did not take part in the development of GM variation but was probably associated with the effect of unidentified factor variables. The complex structure of the data (deviations from normal distribution, multicollinearity, etc.) and the presence of unidentified predictors herein, hinder the search for reliable simple, yet extensive mathematical formalisations for causality or directionality of relationships between variables. Prioritising combinations of N-, C-chemical, and biological soil indicators, identifying and analysing potential and/or actual causal relationships between them and crop productivity traits is vital for constructing minimum sets of parameters and indices of soil health and quality relevant for predicting potential and actual soil fertility. In the future, it is significant to search for and analyse, using statistical/machine learning methods, supplementary features to those presented herein that may contribute to the formation of the C/N balance in soil and crop bioproductivity, particularly under sustainable farming technologies.


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## CONFLICT OF INTEREST

The authors of this study declare no conflict of interest.


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# Каузальні залежності між продуктивністю пшениці й біологічною активністю, вмістом у ґрунті азоту

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**Анотація**. Висвітлення компонентів каузальних залежностей продуктивності сільськогосподарських культур, опосередкованих N-, C-хімічними, біологічними індикаторами ґрунту (розміри пулів відповідних доступних форм, ферментативних активностей) у контексті теоретичних аспектів обігу ресурсів у ньому, є потенційно важливим для конструювання систем оціночних критеріїв здоров'я ґрунту з використавнням моделей машинного навчання (ML). Мета роботи полягала у відшуковуванні статистично валідних і змістовних координацій і каузальних зумовленостей маси зерна на площу посіву доступними формами N, C (неорганічний і легкогідролізований N – N_IN, N_EH; C лабільного гумусу – C_LH), *in situ* протеолітичною PA, *in vitro* уреазною UA активністю у асоціації з концепціями обігу зазначених органогенів у ґрунті під пшеницею озимою за прототипних екологічно безпечних систем добрив. Використано наступні методи: польові, аплікаційний (*in situ*), лабораторно-аналітичні хімічні й біохімічні, морфометричний, статистичні, ML-моделювання. З високою ймовірністю приріст GM був спричинений адитивним зростанням N_IN, N_EH, одночасним зменшенням *in situ* PA у спосіб, за якого вплив *in situ* PA на GM опосередковувався переліченими формами N. Статистично менш валідним було опосередкування дії співвідношення N_EH до N_IN на GM через in situ PA з, можливо, взаємно доповнюючими предетермінуваннями форм N, що втім, створювало основу для обґрунтування одночасних взаємно протилежних зумовленостей GM цими формами N й in situ PA. Правдоподібно, що C_LH й *in vitro* UA не брали участі у розвитку GM за даних експериментальних умов, проте не виключено, що друга серед цих змінних може більш значуще впливати на варіювання першої, ніж навпаки. Дослідження у такому руслі дозволять сформувати дискурс теоретичних передбачень стосовно удосконалення структури індексів якості ґрунту у напрямку розкриття аспектів закономірностей сприяння необхідному для розвитку стійкого сільського господарства балансу між характеристиками агроекосистемних послуг, з урахуванням хімічних і біохімічних властивостей обігу N і C у ґрунті, й біопродуктивності сільськогосподарських культур

**Ключові слова:** доступні форми азоту у ґрунті; вуглець лабільного гумусу; протеолітична й уреазна активність ґрунту; маса зерна пшениці озимої; експлораторний факторний аналіз; системи одночасних рівнянь; каузальні зумовленості

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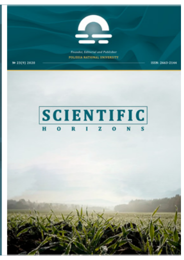


# Efficiency of adjuvants use in the application of pre-emergence herbicides on annual sunflower crops

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**Abstract**. The use of adjuvants in chemical crop protection systems against harmful objects, including weeds, is one of the most substantial innovations in agricultural production. The study aimed to investigate the effectiveness of different rates of Peritera adjuvant in the system of pre-emergence herbicide protection of annual sunflower (*Helianthus annuus* L.) in the western forest-steppe. Research methods: field, laboratory, quantitative, mathematical and statistical to assess the reliability of the data obtained. Many years of research have shown that uneven distribution of precipitation (drought or excessive moisture) during sowing and in the early stages of

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crop development creates obstacles to the effective action of soil (pre-emergence) herbicides, and the addition of adjuvants to them has a positive effect on the growth, development and formation of sunflower seed productivity. In the conditions of the Western Forest-Steppe (Ternopil region), the best results when using pre-emergence herbicide protection of sunflower were recorded in the variant where Soranet, CE (propizochlor, 720 g/l) – 2.0 l/ha + Idaho, KS (terbutylazine, 500 g/l) 1.5 l/ha was applied, and Peritera adjuvant 0.2 l/ha was added to the specified tank mixture. The use of this soil protection scheme for the crop under study ensured the lowest number of segetal vegetation in the agrocenoses of annual sunflower. In addition, the highest chlorophyll content and low phytotoxicity of the used preparations in relation to cultivated plants were noted. The use of surfactants, which, on the one hand, prevent the movement of active substances to the lower horizons and, on the other hand, ensure better penetration into the soil during moisture shortages, can help to mitigate adverse factors. The results obtained are the basis for improving the elements of sunflower cultivation technology and will ensure a seed yield of 3.58 t/ha and can be used in agricultural formations in Ukraine

**Keywords:** *Helianthus annuus* L; segetal vegetation; plant protection products; surfactants; phytotoxicity; productivity; oil content

## INTRODUCTION

Among the limiting factors that affect the yield of sunflowers, as one of the key export-oriented crops, the determining role belongs to the segetal vegetation, which requires mandatory control. The need for herbicide protection as a central agrotechnological measure for obtaining high yields of the crop under study is due to the fact that, as noted by S. Masliiov *et al.* (2021), weeds can significantly reduce its seed productivity, which will negatively affect foreign exchange earnings in the budget. According to A. Shuvar *et al.* (2021), Yu. Tarasovskyi (2025), sunflower oil is central in the structure of agricultural products in Ukraine, 5.1 billion USD or almost 21%, which exceeds the volumes of corn (5.0 billion USD) and wheat (3.7 billion USD).

According to M. Özkil *et al.* (2022), weed infestation of sunflower agrocenoses is especially harmful during the herbicidal period, which is long and falls on the phases V2-V3 to R3-R5. Weeds compete with cultivated plants for light, moisture and nutrients. According to V. Zuza *et al.* (2022), I. Mostoviak *et al.* (2024), control of segetal vegetation in sunflower crops is conducted by applying soil (pre-emergence) and insurance (post-emergence) herbicides. In Ukraine, the most common active substances used to protect agrocenoses of the crop under study from weeds in the pre-germination period are propizochlor, acetochlor, s-metolachlor, dimethanamide-P, terbutylazine, flufenacet, aclonifen and their mixtures (State Register..., n.d.). At the same time, only aclonifen, terbutylazine and flufenacet are used in the European Union (Pesticide Properties Data Base, n.d.).

The effectiveness of pre-emergence herbicides depends on soil, weather and climatic conditions at the initial stages of sunflower growth and development. On light-texture soils with low cation exchange capacity, active substances can move into the lower soil layers and cause phytotoxicity to crops. Thus, according to Z. Pacanoski and A. Mehmeti (2021), oxyfluorfen, aclonifen, acetochlor, dimethanamid-p, and propizochlor do not significantly reduce their effectiveness at low soil moisture, while the effectiveness of linuron, prosulfocarb and petoxamide depends more on soil moisture. One of the ways to increase the effectiveness of soil herbicides under adverse weather conditions and reduce their phytotoxicity is to use adjuvants. Adjuvants are substances that are added to the solution of plant protection products to increase the effectiveness of pesticide active ingredients (Legleiter *et al.*, 2024).

According to H. Gonçalves *et al.* (2024), adjuvants significantly increase the effectiveness of soil herbicides, and this effect can, in some cases, be twice as high as the control without their use. The addition of adjuvants to soil herbicides improves the adsorption of active substances by the soil, which in turn prevents their movement to the lower horizons, keeping them in the 0-5.0 cm layer and protecting them from photolysis under the influence of sunlight. However, according to W. Grichar and J. McGinty (2022), adjuvants do not always increase the effectiveness of soil herbicides, which probably depends on the agroclimatic conditions of the region and the properties of the surfactants themselves. In addition, the study determined that adjuvants do not reduce the evaporation of applied plant protection products but rather increase it in some cases.

Addressing the ambiguity of research results on the use of surfactants in herbicide crop protection technologies, the study aimed to analyse the effectiveness of soil herbicide formulations with Peritera adhesive pre-emergence on annual sunflower crops. The study aimed to study the peculiarities of growth, development and formation of seed productivity of annual sunflower depending on the options for pre-emergence herbicide protection of crops.

## MATERIALS AND METHODS

The field research was conducted in the Western Forest-Steppe of Ukraine at Agrarian Marka Limited Liability Company in Ternopil district, Ternopil region, during 2022-2024. The soil of the experimental field is dark grey podzolised medium loamy with an organic matter content of 3.1%. The scheme of the experiment included the study of six variants of herbicide protection of sunflower hybrid LG 5478 (Table 1).

***Table 1**. Experiment design*

| No. | Experiment variants |
|---|---|
| 1 | Control without herbicides |
| 2 | Tank mix of herbicides SoraNet, CE (propizochlor, 720 g/l) 2.0 l/ha + Idaho, KS (terbutylazine, 500 g/l) 1.5 l/ha |
| 3 | Tank mix of herbicides Soranet, CE (propizochlor, 720 g/l) 2.0 l/ha + Idaho, KS (terbutylazine, 500 g/l) 1.5 l/ha + Peritera adjuvant 0.15 l/ha |
| 4 | Tank mix of herbicides Soranet, CE (propizochlor, 720 g/l) 2.0 l/ha + Idaho, KS (terbutylazine, 500 g/l) 1.5 l/ha + Peritera adjuvant 0.2 l/ha |
| 5 | Tank mixture of herbicides SoraNet, KE (propyzochlor, 720 g/l) 2.0 l/ha + Idaho, KS (terbuthylazine, 500 g/l) 1.5 l/ha + adjuvant Peritera 0.25 l/ha |
| 6 | Tank mix of herbicides Soranet, CE (propizochlor, 720 g/l) 2.0 l/ha + Idaho, KS (terbutylazine, 500 g/l) 1.5 l/ha + Peritera adjuvant 0.30 l/ha |

***Source:** compiled by the authors*

The area of the registered plots is 100 m$^2$. The repetition is four times. The studies were conducted following the generally accepted methods of V. Polozhenets *et al.* (2024). A characteristic feature of the weather conditions in the Ternopil region in 2001-2024 is that they reflect these indicators on a global scale and are manifested in the increase in the aridity of the growing season in 2022-2024 (Table 2).

***Table 2**. Sum of precipitation by months of vegetation of crops in the Ternopil region, mm*

| Years | Months | | | | | | |
|---|---|---|---|---|---|---|---|
| | April | May | June | July | August | September | For the period April-September |
| 2001 | 53 | 45 | 154 | 135 | 51 | 156 | 594 |
| 2002 | 46 | 52 | 82 | 54 | 55 | 70 | 359 |
| 2003 | 22 | 49 | 33 | 137 | 32 | 42 | 315 |
| 2004 | 21 | 63 | 45 | 94 | 87 | 41 | 351 |
| 2005 | 55 | 56 | 67 | 47 | 113 | 11 | 349 |
| 2006 | 40 | 79 | 81 | 61 | 184 | 18 | 463 |
| 2007 | 18 | 59 | 55 | 114 | 80 | 90 | 416 |
| 2008 | 125 | 65 | 39 | 169 | 50 | 138 | 586 |
| 2009 | 33 | 59 | 116 | 43 | 28 | 18 | 297 |
| 2010 | 48 | 130 | 129 | 107 | 63 | 80 | 557 |
| * | **46.1** | **65.7** | **80.1** | **96.1** | **74.3** | **66.4** | **428.7** |
| V, %* | 66.9 | 37.3 | 51.1 | 45.5 | 62.3 | 76.0 | 26.6 |
| 2011 | 32 | 37 | 82 | 93 | 39 | 14 | 297 |
| 2012 | 62 | 64 | 116 | 78 | 84 | 54 | 458 |
| 2013 | 39 | 96 | 137 | 91 | 35 | 78 | 476 |
| 2014 | 35 | 124 | 38 | 70 | 87 | 27 | 381 |
| 2015 | 30 | 65 | 49 | 41 | 17 | 54 | 256 |
| 2016 | 84 | 42 | 151 | 38 | 23 | 73 | 411 |
| 2017 | 52 | 72 | 68 | 53 | 37 | 124 | 406 |
| 2018 | 15 | 49 | 84 | 87 | 35 | 25 | 295 |
| 2019 | 25 | 121 | 54 | 30 | 22 | 10 | 262 |
| 2020 | 16 | 60 | 123 | 94 | 81 | 82 | 456 |
| $\bar{X}_*$ | **39.0** | **73.0** | **90.2** | **67.5** | **46.0** | **54.1** | **369.8** |
| V, %* | 55.2 | 42.3 | 43.7 | 36.9 | 59.2 | 66.7 | 48.7 |
| 2021 | 10 | 53 | 41 | 101 | 48 | 58 | 311 |
| 2022 | 28 | 17 | 13 | 82 | 78 | 107 | 325 |
| 2023 | 73 | 23 | 86 | 84 | 47 | 4 | 317 |
| 2024 | 41 | 23 | 45 | 61 | 35 | 82 | 287 |

*Table 2. Continued*

| Years | Months | | | | | | |
|---|---|---|---|---|---|---|---|
| | **April** | **May** | **June** | **July** | **August** | **September** | **For the period April-September** |
| $\bar{X}_*$ | 38.0 | 29.0 | 46.3 | 82.0 | 52.0 | 62.8 | **310.0** |
| V, %* | 69.9 | 56.0 | 65.0 | 20.0 | 35.2 | 70.1% | 48.9 |
| Long-term averages | 43 | 66 | 80 | 87 | 67 | 58 | 401 |

***Note:*** *$\bar{X}$ – Average value of the indicator for the period; V – coefficient of variation of the indicator for the period*
***Source:*** *compiled by the authors based on meteorological observations*

Thus, in the period 2001-2010, the average amount of precipitation for April-October was 428.7 mm, in 2011-2020, 369.8 mm, and for the same period in 2021-2024, only 310.0 mm, while the long-term average for the study area is 401 mm. In addition to the deterioration of the atmospheric humidification regime, there is an uneven distribution of precipitation over the years of observation, as the coefficient of variation is more than 25%, which indicates a significant variability of this indicator. The most common indicator that characterises the variability of weather elements is the coefficient of deviation, which reflects the value of the current value from the long-term average and is determined by the formula.

$$\mathrm{Kc} = \frac{X_i - \bar{X}}{\sigma}, \tag{1}$$

where Kc – deviation significance coefficient; $X_i$ – current weather elements; $\bar{X}$ – average long-term value indicator; σ – standard deviation.

If the coefficient of significance of deviations is at the level of 0-1, the weather conditions are close to normal; at the value of Kc 1-2, the weather conditions are very different from the long-term average; at Kc > 2, the conditions are close to rare M. Kulbida *et al.* (2009) calculated the coefficients of significance of deviations in precipitation and found that there is a tendency to increase the number of atypical months in terms of atmospheric moisture regime (Table 3).

***Table 3****. Significance coefficients of deviations of precipitation from long-term averages in the Ternopil region*

| Years | Months | | | | | | Number of atypical months, pcs. |
|---|---|---|---|---|---|---|---|
| | **April** | **May** | **June** | **July** | **August** | **September** | |
| 2001 | 0.434 | -0.771 | 2.103 | 1.265 | -0.525 | 2.766 | 23 |
| 2002 | 0.126 | -0.511 | 0.060 | -0.887 | -0.394 | 0.340 | |
| 2003 | -0.932 | -0.622 | -1.331 | 1.318 | -1.147 | -0.450 | |
| 2004 | -0.976 | -0.102 | -0.990 | 0.175 | 0.654 | -0.478 | |
| 2005 | 0.522 | -0.362 | -0.366 | -1.073 | 1.506 | -1.324 | |
| 2006 | -0.139 | 0.493 | 0.031 | -0.701 | 3.831 | -1.127 | |
| 2007 | -1.109 | -0.251 | -0.706 | 0.707 | 0.425 | 0.904 | |
| 2008 | 3.608 | -0.027 | -1.161 | 2.168 | -0.558 | 2.258 | |
| 2009 | -0.447 | -0.251 | 1.025 | -1.180 | -1.278 | -1.127 | |
| 2010 | 0.214 | 2.390 | 1.394 | 0.521 | -0.132 | 0.622 | |
| 2011 | -0.491 | -1.069 | 0.060 | 0.149 | -0.918 | -1.240 | 23 |
| 2012 | 0.831 | -0.065 | 1.025 | -0.250 | 0.556 | -0.111 | |
| 2013 | -0.183 | 1.125 | 1.621 | 0.096 | -1.049 | 0.566 | |
| 2014 | -0.359 | 2.167 | -1.189 | -0.462 | 0.654 | -0.873 | |
| 2015 | -0.580 | -0.027 | -0.877 | -1.233 | -1.638 | -0.111 | |
| 2016 | 1.801 | -0.883 | 2.018 | -1.312 | -1.442 | 0.425 | |
| 2017 | 0.390 | 0.233 | -0.338 | -0.914 | -0.983 | 1.863 | |
| 2018 | -1.241 | -0.622 | 0.117 | -0.011 | -1.049 | -0.929 | |
| 2019 | -0.800 | 2.055 | -0.735 | -1.525 | -1.474 | -1.353 | |
| 2020 | -1.197 | -0.213 | 1.223 | 0.175 | 0.458 | 0.679 | |
| 2021 | -1.461 | -0.474 | -1.104 | 0.361 | -0.623 | 0.002 | 10 |
| 2022 | -0.668 | -1.812 | -1.898 | -0.143 | 0.359 | 1.384 | |
| 2023 | 1.316 | -1.589 | 0.173 | -0.090 | -0.656 | -1.522 | |
| 2024 | -0.095 | -1.589 | -0.990 | -0.701 | -1.049 | 0.679 | |

***Source:*** *compiled by the authors*

In 2001-2010, the number of months with precipitation amounts significantly different from long-term averages was 23, of which 7 were abnormal. For the period 2011-2020, 23 atypical cases were also recorded, of which only 1 was rare. In 2021-2024, there were already 10 months with precipitation amounts that significantly differed from the long-term average. This indicates a tendency to increase the uneven distribution of precipitation during the growing season and necessitates the use of adjuvants in soil (pre-emergence) herbicide technologies to improve their effectiveness. Directly during the years of research (2022-2024), the weather conditions during sowing and application of soil herbicides in the pre-emergence period differed from the average long-term data, which was used to objectively assess the studied factor under different meteorological indicators (Table 4).

***Table 4**. Agrometeorological conditions at the time of sowing and the beginning of the sunflower growing season, mm*

| Year | Months | |
|---|---|---|
| | **April** | **May** |
| | Sum of precipitation, mm | |
| 2022 | 38 | 16 |
| 2023 | 75 | 12 |
| 2024 | 45 | 17 |
| Long-term averages | 43 | 66 |
| | Air temperature, °C | |
| 2022 | 7.1 | 14.9 |
| 2023 | 8.2 | 14.8 |
| 2024 | 12.3 | 16.1 |
| Long-term averages | 8.7 | 14.1 |

***Source:** compiled by the authors based on meteorological observations*

Thus, in 2022, the amount of precipitation in April was 38 mm, and the average daily air temperature was +7.1°C; in 2023 and 2024, respectively, 75 mm and +8.2°C and 45 mm and 12.3°C. The long-term meteorological indicators were 43 mm and +8.7°C, respectively. May 2022-2024 was dry, with 16, 12 and 17 mm of precipitation, respectively, compared to the average long-term norm of 66 mm. The air temperature was +14.9°C in 2022, +14.8°C in 2023 and 16.1°C in 2024. The long-term average was 14.1°C. Thus, April 2022 was cool with a slight deficit of precipitation, 2023 was excessively wet and cold, and May was dry with air temperatures close to the climatic norm. In 2024, April was characterised by sufficient precipitation and above-normal temperatures, while May was dry and excessively warm.

Soil herbicides were applied immediately after sowing the test crop. The working fluid consumption was 200 litres per 1 ha. Weed counts were conducted on permanently fixed plots of 1 $m^2$. The leaf surface area of the crop under study was determined by the calculation method, the essence of which is that this indicator was calculated by multiplying the length of the leaf by its width and by a conversion factor of 0.74. The sample size of the plants was 20 pieces. The chlorophyll content was determined using the N-tester (Germany), analysing the 30 youngest fully developed leaves. The higher the index, the higher the chlorophyll content and, accordingly, the plants are less affected by stress factors. Harvesting was conducted by threshing the entire experimental plot. The oil content was determined using a FOSS Infratec Nova device (Denmark). For the research, average samples were taken from each replication, weighing 2 kg, which were placed in the device and the oil content was displayed on the screen. The use of this device for research is not subject to copyright infringement restrictions. The authors adhered to the standards of the Convention on Biological Diversity (1992). The cost-effectiveness of the research was conducted based on the developed technological maps, addressing current prices for work performed and material resources as of 2025.

## RESULTS AND DISCUSSION

Practical experience in the use of soil (pre-emergence) herbicides shows that one of the main prerequisites for their effective operation and safety for crops is optimal soil moisture. Insufficient moisture partially or completely reduces the effectiveness of soil herbicides, while excessive rainfall can cause the active ingredients to be washed into the lower soil layers, which causes phytotoxicity to crops. However, in actual production conditions, there are rarely favourable conditions for the maximum effectiveness of soil herbicides, as technological operations related to agricultural production are conducted in the open field, where the amount of precipitation is determined by weather conditions.

According to the results of field studies conducted in 2022-2024, a positive effect of the soil adhesive Peritera on the processes of growth, development and formation of seed productivity of annual sunflower was revealed. It was found that the addition of Peritera adjuvant to the tank mixture of herbicides reduced the number of segetal vegetation in the agrocenosis of the studied crop (Table 5). The main representatives of weeds in the experiments were white quinoa (*Chenopodium album* L.) and chicken millet (*Echinochloa crus-galli* L.). The highest weed infestation of sunflower crops was observed in the control without herbicide protection – 35.6 plants per $m^2$. Pre-emergence application of a tank mixture of soil herbicides with the active ingredients propizochlor, 720 g/l at a rate of 2.0 l/ha and terbutylazine (500 g/l), 1.5 l/ha, helped to reduce the number of segetal vegetation to 5.9 pcs/$m^2$. The addition of Peritera adjuvant enhanced the effectiveness of the applied products, as the number of weeds was 1.3-2.6 pcs/$m^2$, which is statistically significant compared to the variant without its use.

A comparative assessment of the application rates of Peritera soil adhesive indicates that it is advisable to use 0.2 l/ha, since further increases do not provide a significant reduction in the number of weeds. Reducing the rate to 0.15 l/ha provides less control of segetal vegetation. It should be noted that the use of adjuvants with soil herbicides is intended not only to enhance the effect of the latter, but also to reduce their possible phytotoxic effects on cultivated plants. One of the indicators that characterise the state of a plant organism is the leaf surface area and the intensity of green colour of the leaves, which in turn is determined by the chlorophyll content. Studies have shown that herbicide protection schemes affected the photosynthetic activity of sunflower plants (Table 6).

***Table 5**. Weed infestation of annual sunflower agrocenoses, depending on the experiment variant, pcs/$m^2$ (average for 2022-2024)*

| Experiment variants | Number of weeds in the 8-leaf stage |
|---|---|
| Control without herbicides | 35.6 |
| Tank mix of herbicides SoraNet, CE (propizochlor, 720 g/l) 2.0 l/ha + Idaho, KS (terbutylazine, 500 g/l) 1.5 l/ha | 5.9 |
| Tank mix of herbicides Soranet, CE (propizochlor, 720 g/l) 2.0 l/ha + Idaho, KS (terbutylazine, 500 g/l) 1.5 l/ha + Peritera adjuvant 0.15 l/ha | 2.6 |
| Tank mix of herbicides Soranet, CE (propizochlor, 720 g/l) 2.0 l/ha + Idaho, KS (terbutylazine, 500 g/l) 1.5 l/ha + Peritera adjuvant 0.20 l/ha | 1.9 |
| Tank mixture of herbicides SoraNet, KE (propyzochlor, 720 g/l) 2.0 l/ha + Idaho, KS (terbuthylazine, 500 g/l) 1.5 l/ha + adjuvant Peritera 0.25 l/ha | 1.5 |
| Tank mix of herbicides Soranet, CE (propizochlor, 720 g/l) 2.0 l/ha + Idaho, KS (terbutylazine, 500 g/l) 1.5 l/ha + Peritera adjuvant 0.30 l/ha | 1.3 |
| $LSD_{05}$, pcs/$m^{2*}$ | 0.6 |

***Note:** Least significant difference (LSD) indicates a significant reduction in weed infestation from herbicide application and its absence between adjuvant application rates*
***Source:** compiled by the authors*

***Table 6**. Main indicators of photosynthetic activity of sunflower crops in the 6-leaf stage, depending on herbicide protection schemes (average for 2022-2024)*

| Experiment variants | Area of sunflower crop leaf surface, thousand $m^2$/ha | Chlorophyll content in leaves, N-tester units |
|---|---|---|
| Control without herbicides | 2.95 | 489 |
| Tank mix of herbicides SoraNet, CE (propizochlor, 720 g/l) 2.0 l/ha + Idaho, KS (terbutylazine, 500 g/l) 1.5 l/ha | 3.25 | 496 |
| Tank mix of herbicides Soranet, CE (propizochlor, 720 g/l) 2.0 l/ha + Idaho, KS (terbutylazine, 500 g/l) 1.5 l/ha + Peritera adjuvant 0.15 l/ha | 3.62 | 511 |
| Tank mix of herbicides Soranet, CE (propizochlor, 720 g/l) 2.0 l/ha + Idaho, KS (terbutylazine, 500 g/l) 1.5 l/ha + Peritera adjuvant 0.20 l/ha | 4.65 | 551 |
| Tank mixture of herbicides SoraNet, KE (propyzochlor, 720 g/l) 2.0 l/ha + Idaho, KS (terbuthylazine, 500 g/l) 1.5 l/ha + adjuvant Peritera 0.25 l/ha | 4.71 | 564 |
| Tank mix of herbicides Soranet, CE (propizochlor, 720 g/l) 2.0 l/ha + Idaho, KS (terbutylazine, 500 g/l) 1.5 l/ha + Peritera adjuvant 0.30 l/ha | 4.75 | 570 |
| $LSD_{05}{}^*$ | 0.63 | 32 |

***Note:** Least significant difference (LSD) indicates a significant reduction in weed infestation from herbicide application and its absence between adjuvant application rates*
***Source:** compiled by the authors*

LSD (least significant difference) indicates a reliable increase in the leaf surface area of plants and chlorophyll content in leaves from the application of herbicides and their absence between the application rates of adjuvants. The use of soil herbicides that control weeds in the study crop contributed to the growth of the leaf area of the sunflower. Thus, in the variant without the use of adjuvants, this indicator was at the level of 3.25 thousand $m^2$/ha, when adding Peritera adhesive to the tank mixture at a rate of 0.15 l/ha, it was 3.62 thousand $m^2$/ha, and when adding 0.2, 0.25 and 0.3 l/ha, it was 4.65, 4.71 and 4.75 thousand $m^2$/ha, respectively. Statistical processing of the research results indicates that there is no significant difference between the application of 0.20, 0.25 and 0.3 l/ha of Peritera adjuvant.

The chlorophyll content in sunflower leaves depended on the herbicide protection scheme. Thus, in the control without the use of plant protection products, it was the lowest and was at the level of 489 units. The use of a tank mix of herbicides with the active ingredients propizochlor, 720 g/l and terbutylazine, 500 g/l, contributed to an increase in the chlorophyll content in the leaves to 496 units due to the reduction of the negative impact of segetal vegetation on cultivated plants. The addition of Peritera adhesive to the tank mixture of herbicide active ingredients led to an increase in the intensity of green colour of sunflower leaves, which was reflected in the increase in the accumulation of green pigments in photosynthetically active organs. Thus, in the variant with the application of 0.15 l/ha, the N-tester readings were 511 units. Further increase in the rate of soil adhesive application contributed to the accumulation of chlorophyll content in the leaves, but its maximum statistically confirmed content was noted in the variant with the use of 0.2 l/ha 551 units. Studies have shown that variants of herbicide protection affected the seed productivity of sunflower (Table 7).

***Table 7**. Key indicators of seed productivity of sunflower crops depending on herbicide protection schemes (average for 2022-2024)*

| Experiment variants | Seed yield, t/ha | Oil content in seeds, % |
|---|---|---|
| Control without herbicides | 1.62 | 42.2 |
| Tank mix of herbicides SoraNet, CE (propizochlor, 720 g/l) 2.0 l/ha + Idaho, KS (terbutylazine, 500 g/l) 1.5 l/ha | 3.31 | 48.9 |
| Tank mix of herbicides Soranet, CE (propizochlor, 720 g/l) 2.0 l/ha + Idaho, KS (terbutylazine, 500 g/l) 1.5 l/ha + Peritera adjuvant 0.15 l/ha | 3.45 | 49.0 |
| Tank mix of herbicides Soranet, CE (propizochlor, 720 g/l) 2.0 l/ha + Idaho, KS (terbutylazine, 500 g/l) 1.5 l/ha + Peritera adjuvant 0.20 l/ha | 3.58 | 49.3 |
| Tank mixture of herbicides SoraNet, KE (propyzochlor, 720 g/l) 2.0 l/ha + Idaho, KS (terbuthylazine, 500 g/l) 1.5 l/ha + adjuvant Peritera 0.25 l/ha | 3.61 | 49.5 |
| Tank mix of herbicides Soranet, CE (propizochlor, 720 g/l) 2.0 l/ha + Idaho, KS (terbutylazine, 500 g/l) 1.5 l/ha + Peritera adjuvant 0.30 l/ha | 3.65 | 49.5 |
| $LSD_{05}$ | 0.11 | 0.51 |

***Note:** LSD (least significant difference) indicates a reliable increase in seed yield and oil content in seeds from the application of herbicides and their absence between the application rates of adjuvants*
***Source:** compiled by the authors*

Among the options studied, the lowest seed yield was observed in the control without herbicides, where, due to the intensive development of segetal vegetation, the seed yield per unit area was 1.62 t/ha. The oil content in the seeds was also the lowest and amounted to 42.2%. The application of a tank mixture of soil herbicides with the active ingredients propyzochlor, 720 g/l, and terbuthylazine, 500 g/l, followed by the application of insurance preparations, contributed to an increase in seed productivity to 3.31 t/ha and oil content to 48.9%. Depending on the application rate of the soil adjuvant, the sunflower seed yield was 3.453.65 t/ha. The minimum application rate of the Peritera adjuvant (0.15 l/ha) ensured seed productivity of the tested crop at 3.45 t/ha with an oil content of 49.0%. This is 0.14 t higher than the production control. In the experiment with the application of 0.2 l/ha of Peritera, an increase in sunflower yield to 3.58 t/ha was noted, which is 0.27 t/ha more than the production control. The oil content was 49.3% (Table 7, option 3).

Further increase in the rate of soil adjuvant application, although it provides an increase in the seed productivity of the crops under study, this increase is within the statistical error. Studies have shown that herbicide protection options provided different economic efficiency (Table 8). As of 1.02.2025, the average selling price of sunflower seeds in Ukraine was 25000 UAH/t, and the cost of Peritera soil adhesive was 780 UAH/l. Depending on the variant of the experiment, the cost of increasing the yield of sunflower seeds relative to the absolute control was 1,692.06 t/ha, and relative to the production control, 0,140.37 t/ha. In monetary terms, these figures amounted to 4225051500 and 35009250 UAH/ha, respectively. The highest yield increases in physical and monetary terms were observed in the variant with 0.3 l/ha of Peritera soil adjuvant, 2.06 t/ha and 51500 UAH/ha relative to the absolute control and 0.37 t/ha and 9250 UAH/ha compared to the production control.

***Table 8**. Main indicators of the efficiency of soil herbicide protection of sunflower (average for 2022-2024)*

| Experiment variants | Seed yield, t/ha | Yield increases to absolute control, t/ha | Yield increases to production control, t/ha | Cost of yield increase to absolute control, UAH/ha | Cost of crop growth to production control, UAH/ha | Cost of adhesive application, GBP/ha | Contingent net profit from the use of adhesive, UAH/ha | Level of profitability of soil adhesive application, % | Sunflower equivalent, application of soil adhesive, kg/ha |
|---|---|---|---|---|---|---|---|---|---|
| Control without herbicides | 1.62 | - | - | - | - | - | - | - | - |
| Tank mix of herbicides SoraNet, CE (propizochlor, 720 g/l) 2.0 l/ha + Idaho, KS (terbutylazine, 500 g/l) 1.5 l/ha | 3.31 | 1.69 | - | 42,250 | - | - | - | - | - |
| Tank mix of herbicides Soranet, CE (propizochlor, 720 g/l) 2.0 l/ha + Idaho, KS (terbutylazine, 500 g/l) 1.5 l/ha + Peritera adjuvant 0.15 l/ha | 3.45 | 1.83 | 0.14 | 45,750 | 3,500 | 117 | 3,383 | 2,891.5 | 4.68 |
| Tank mix of herbicides Soranet, CE (propizochlor, 720 g/l) 2.0 l/ha + Idaho, KS (terbutylazine, 500 g/l) 1.5 l/ha + Peritera adjuvant 0.20 l/ha | 3.58 | 1.96 | 0.27 | 49,000 | 6,750 | 156 | 6,594 | 4,226.9 | 6.24 |
| Tank mixture of herbicides SoraNet, KE (propyzochlor, 720 g/l) 2.0 l/ha + Idaho, KS (terbuthylazine, 500 g/l) 1.5 l/ha + adjuvant Peritera 0.25 l/ha | 3.61 | 1.99 | 0.30 | 49,750 | 7,500 | 195 | 7,305 | 3,746.2 | 7.80 |
| Tank mix of herbicides Soranet, CE (propizochlor, 720 g/l) 2.0 l/ha + Idaho, KS (terbutylazine, 500 g/l) 1.5 l/ha + Peritera adjuvant 0.30 l/ha | 3.68 | 2.03 | 0.34 | 50,750 | 8,500 | 234 | 8,266 | 3,532.5 | 9.36 |

***Source:** compiled by the authors*

Considering the price of 1 litre of Peritera soil adhesive 780 UAH/l and the absence of additional costs for its application, as it is added to the tank mixture of soil herbicides, the cost of treating 1 ha with it is 117234 UAH. In the variant where the application rate was 0.15 l/ha, this figure was the lowest and was at 117 UAH/ha, and when using 0.3 l/ha of the product, it was the highest at 234 UAH/ha. In Ukraine, several studies have been conducted to investigate the impact of soil (pre-emergence) herbicides on the seed productivity of annual sunflower. For example, according to O. Rudik *et al.* (2020), the active ingredients oxyfluorfen, terbuthylazine, acetochlor, promethrin, pendimethalin, S-metholachlor, and propizochlor are used to control segetal vegetation in the agrocenoses of the crop. In addition, according to M. Radchenko *et al.* (2022), a tank mix of herbicides with the active ingredients flumioxazin and fluorochloridone is quite effective.

V. Gurtovenko and O. Tsyuk (2024) established that, for conditions in the Cherkasy region, the best option for weed control in sunflower agrocenoses is the application of 5 l/ha of the herbicide Challenge. At the same time, the highest seed yield of the studied crop was 3.8 t/ha. The high efficiency of the tank mixture of soil herbicides Challenge (2.5 l/ha) + Harness (1.5 l/ha) was proven by V. Hryhoriev and A. Fedchuk (2021) in the conditions of the Khmelnytskyi region, which ensured the highest seed yield of the studied hybrids, 3.03.47 t/ha. When studying the effect of soil herbicides on biometric indicators and sunflower yield, authors S. Mazur and H. Matusevych (2023) found that the application of the preparation Gezagard proved to be the most effective and ensured a seed yield of 2.64 t/ha, which was 23.9% higher than the control.

The use of weed control products during the period of VVSN 00-09 is advisable even in crops of hybrids grown using ExpressSun technology, as proven in the experiments of G. Delchev *et al.* (2020). Notably, according to Z. Pacanoski and A. Mehmeti (2021), in dry conditions, the effect of pre-emergence herbicides is reduced, and in conditions of sufficient moisture, their high efficiency is ensured. In this context, it is necessary to create conditions to ensure the maximum effectiveness of soil herbicides, as F. Önemli and Ü. Tetik (2023) found that post-emergence herbicides used to control segetal vegetation harm the growth, development and formation of sunflower seed productivity.

However, according to the literature, despite a significant number of experiments on the effectiveness of pre-emergence control of segetal vegetation of sunflower agrocenoses, the issue of using adjuvants in herbicide protection technologies has not been studied in Ukraine. At the same time, the State Register of Pesticides and Agrochemicals Approved for Use in Ukraine (State Register... (n.d.) includes a significant number of such products, and they are widely used in production conditions of agricultural enterprises in different regions of the country. However, abroad, in the European Union and other countries, adjuvants are widely used in crop cultivation technologies, in terms of their chemical protection against harmful objects, including weeds. They help to increase the effectiveness of crop protection products and reduce the application rates and the number of chemical treatments.

According to research conducted by scientists around the world, the use of adhesives for soil (pre-emergence) herbicides is an ambiguous issue and therefore requires more detailed study. According to N. Sharma *et al.* (2020), the addition of adjuvants to soil herbicides results in better soil absorption of the active components of herbicides, which improves the environmental component of their use. However, production experience and research results, as noted by W. Grichar and J. McGinty (2022), on the use of these substances in pre-emergence herbicide protection technologies for crops, including sunflower, indicate that the obtained indicators are controversial. This is manifested in the insufficient effectiveness of the applied preparations, the lack of evaporation reduction, or even its increase, as reported by S. Das and K. Hageman (2020). Thus, modern crop cultivation technologies should be based on adaptation to specific soil and climatic conditions to ensure the highest efficiency of agrotechnical measures and create preconditions for obtaining high yields with appropriate quality and economic efficiency indicators.

## CONCLUSIONS

Long-term studies have shown that the addition of adjuvants to soil herbicides has a positive effect on the growth, development and formation of sunflower seed productivity. On average, over the three years of research, the best statistically proven herbicide protection option was the application of a tank mix of Soranet, CE (propizochlor, 720 g/l) 2.0 l/ha + Idaho, KS (terbutylazine, 500 g/l) 1.5 l/ha + Peritera adjuvant 0.2 l/ha. This variant of the experiment showed the lowest weediness of crops and the lowest manifestation of phytoxicity, since the N-tester indicators and, consequently, the chlorophyll content were the highest. The seed yield of the crop under study was 3.58 t/ha, and the oil content was 49.3%. The use of Peritera soil adhesive was profitable in all variants of the experiment, as the low cost of its application was paid off by significant yield increases. The highest profitability was observed in the variant with a rate of 0.2 l/ha of the adjuvant – 42-26.9%.

The use of Peritera soil adhesive was profitable in all variants of the experiment, as the low cost of its application was paid off by significant yield increases. The highest profitability was recorded in the variant with a rate of 0.2 l/ha of adjuvant, which was 42-26.9%. Another indicator of the effectiveness and feasibility of Peritera is the equivalent of the grown product (sunflower seeds) required for its application. Thus, considering the cost of 1 tonne of sunflower seeds at 25,000 UAH/t, the cost of using the adhesive is 4,68-9.36 kg/ha of sunflower seeds, while the yield increase from its application is 140-340 kg/ha. Given that the use of adjuvants in pre-emergence herbicide crop protection technologies in Ukraine is completely unexplored, this issue is highly relevant in the context of climate change and in the context of reducing the pesticide burden on the environment.

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## CONFLICT OF INTEREST

None.

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# Ефективність використання ад'ювантів при застосуванні досходових гербіцидів на посівах соняшнику однорічного

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**Анотація**. Використання ад'ювантів у системах хімічного захисту посівів від шкідливих об'єктів, зокрема і бур'янів є однією із важливих інновацій у сільськогосподарському виробництві. Метою досліджень було вивчити ефективність застосування різних норм ад'юванта Peritera в системі досходового гербіцидного захисту соняшнику однорічного (*Helianthus annuus* L.) в умовах західного Лісостепу. Методи досліджень: польовий, лабораторний, кількісний, математично-статистичний для оцінювання достовірності отриманих даних. Багаторічними дослідженнями було встановлено, на фоні нерівномірного розподілу опадів (посуха або надмірне зволоження) під час сівби та на початкових етапах розвитку сільськогосподарських культур, створюються перешкоди для ефективної дії ґрунтових (досходових) гербіцидів, а додавання ад'ювантів до них позитивно впливає на процеси росту, розвитку та формування насіннєвої продуктивності соняшнику. В умовах Західного Лісостепу (Тернопільська область) найкращі результати при використанні досходового гербіцидного захисту соняшнику зафіксовано на варіанті, де вносилися препарати СораНет, КЕ (пропізохлор, 720 г/л) – 2,0 л/га + Айдахо, КС (тербутилазин, 500 г/л) – 1,5 л/га і в зазначену бакову суміш було додано ад'ювант Peritera 0,2 л/га. Застосування цієї ґрунтової схеми захисту для досліджуваної культури забезпечило найменшу чисельність сегетальної рослинності в агроценозах соняшнику однорічного. Крім цього відмічено найвищий вміст хлорофілу та низьку фітотоксичність використовуваних препаратів у відношенні до культурних рослин. Через застосування поверхнево-активних речовин, які з одного боку запобігають переміщенню діючих речовин в нижні горизонти, а з іншого забезпечують краще їх проникнення в ґрунт під час нестачі вологи можливе нівелювання несприятливих факторів. Отримані результати є основою для вдосконалення елементів технології вирощування соняшнику та забезпечать урожайність насіння на рівні 3,58 т/га і можуть застосовуватися в агроформуваннях України

**Ключові слова:** *Helianthus annuus* L; сегетальна рослинність; засоби захисту рослин; поверхнево-активні речовини; фітотоксичність; продуктивність; олійність

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# Efficiency of leaf fertilisers in complex fertilisation of cereals on southern chornozems of Northern Kazakhstan

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**Abstract**. The study aimed to evaluate the effect of foliar feeding on the physiology, yield and economic efficiency of spring wheat in Northern Kazakhstan. The field trials were conducted during the growing season of 2024 (May-August) at the experimental plot of Kokshetau University named after Sh. Ualikhanov and were aimed to assess the agricultural, ecological and economic efficiency of different schemes of foliar feeding of spring wheat. The study included five variants of the experiment, which differed in the composition and frequency of fertiliser application, including urea, microelements and biostimulants. The variants with foliar treatments showed a stable advantage in agrophysiological parameters, especially in hot and dry weather conditions, at a temperature of about +33°C and air humidity of 30-35%. The highest results were obtained with two-time foliar feeding (variant B5), where the yield reached 43.2 c/ha,

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which exceeded the control by 5.6 c/ha (14.9%). The weight of 1,000 grains increased to 45.6 g, the productive density to 417 stems/m2, and the number of ears per ear to 16.5. The duration of the flag leaf functioning in this variant reached 14 days, and the chlorophyll level remained high during the filling phase. There was a significant reduction in leaf rust (down to 14.5%) and a 15% increase in the share of milled grain. The economic analysis demonstrated that option B5 provided the highest income (129,600 tg/ha) with a payback ratio of 2.1 and an economic effect of 18,000 tg/ha. The other options provided a yield increase of 3.3 to 4.7 cwt/ha with payback periods ranging from 1.6 to 1.9. The data confirm the expediency of integrating phase-selected foliar fertilisation into wheat cultivation technologies in the risky farming zone. Comparing experiments in different years in terms of moisture levels, it can be concluded that, provided there is sufficient moisture, foliar feeding is more effective when combined with the main application of macronutrients (nitrogen and phosphorus)

**Keywords:** physiological activity; productive stems; chlorophyll content; vitality index; microelements; spring wheat; yield

## INTRODUCTION

The research relevance is determined by the need to improve agronomic approaches to soft spring wheat cultivation in Northern Kazakhstan. The agroclimatic features of the region, including frequent periods of drought, sharp fluctuations in vegetation period temperatures and unstable moisture supply, made it difficult to realise the biological potential of varieties. In the context of declining efficiency of traditional cultivation methods, the development of alternative strategies for managing the physiological state of plants became particularly relevant. Foliar feeding was seen as one of the most promising ways to quickly deliver nutrients to critical phases of organogenesis, helping to maintain the photosynthetic apparatus and prolong the functioning of the flag leaf. The problem of the study was the limited adaptability of standard foliar nutrition schemes to the soil and climatic conditions of the North Kazakhstan zone.

According to T.Z. Aidarbekova *et al.* (2024) differentiated mineral nutrition on the chornozems of Northern Kazakhstan, which contributed to the growth of flag leaf photosynthetic activity, which was reflected in an increase in the chlorophyll index and, as a result, in an increase in yield. The greatest effect was observed with a balanced nitrogen supply in the phase of tube emergence. The publication by S. Turebayeva *et al.* (2022) noted that when direct sowing in the arid conditions of Southern Kazakhstan, phased fertilisation provided an economically justified increase in winter wheat yield. Late treatments combined with minimal inputs helped to improve the spike structure and reduce the risk of hollowing. A study by N.O. Turganbayev *et al.* (2023) showed that under no-till conditions, the highest productivity indicators were associated with early micronutrient supply. This reduced the yield variability between replications and increased the protein content in the grain. R. Drăghici *et al.* (2022) described how micronutrient sheet fertilisers on sandy soils improved rye development, especially in conditions of moisture deficit. The benefits were expressed in sustainable biomass growth, slower leaf senescence, and uniform formation of productive shoots.

The analysis of L. Crista *et al.* (2024) revealed that when applying complex foliar fertilisers to maize, the main performance indicators were the Soil Plant Analysis Development (SPAD) index and the weight of the aerial part. The direct relationship between the dosage of trace elements and the uniformity of generative organs indicated the stability of the physiological status of plants. S. Šeremešić *et al.* (2022) emphasised that crop rotation with the use of leaf fertilisers on chornozems led to an improvement in the phytosanitary condition of soybeans. The impact of weather stress was significantly reduced, and productivity remained high even in years with limited rainfall. The review by L. Marcinska-Mazur and M. Mierzwa-Hersztek (2023) emphasised the high efficiency of integrated nutrition schemes for wheat and rapeseed, combining root and foliar feeding. This strategy significantly increased not only the yield, but also the technological parameters of grain, including crude protein content and vitreous content. According to R.A. Paunescu *et al.* (2023), intensive crop rotations with a well-thought-out nutrition system provided an increase in wheat yields by increasing productive bushiness and ear length. In addition, at optimal fertiliser doses, there was an improvement in the morphological characteristics of the stem, which increased lodging resistance.

Materials by G. Macra and F. Sala (2022) recorded a significant variation in nitrogen use efficiency when combining mineral fertilisers with foliar treatments. The maximum return per unit of nitrogen applied was achieved in schemes that included stimulation of the leaf apparatus in the phase of intensive growth. According to the results of S. Dhaliwal *et al.* (2022), bioenrichment through micronutrient foliar treatments contributed to an increase in the content of iron, zinc and selenium in field crops. It is noted that the use of such agricultural technologies helps to increase yields and improve the chemical composition of grain, including the content of protein and trace elements. The study by R. Gaj *et al.* (2023) demonstrated that proper management of nitrogen nutrition in triticale ensured high efficiency of its assimilation, which was reflected in a decrease in the residual nitrate content in the soil and a stable level of yield. Schemes with controlled fractional fertilisation combined with foliar nutrition correction proved to be effective. A synthesis review by I. Varga *et al.* (2022) addressed nitrogen management strategies in sugar beet but notes the universality of approaches applicable to cereals. Among the most effective methods were foliar corrections in the context of a balanced basic nutrition, which help to maximise the nitrogen use factor by plants.

However, the effectiveness parameters of different types of foliar feeding, which differ in composition, time and frequency of application, were not studied in detail. The question of the impact of such treatments on plant resistance to pathogens, photosynthetic activity under stress and the duration of flag leaf functioning remained open. In addition, there was no comprehensive assessment of the agronomic and economic effectiveness of these practices, which prevented the development of sound recommendations for adaptive wheat cultivation in an unstable climate. The study aimed to determine the optimal schemes of foliar feeding of spring wheat to increase its productivity and sustainability in Northern Kazakhstan.

## MATERIALS AND METHODS

The field study was conducted during the growing season of 2024 (May-August) based on the educational and experimental plot of Kokshetau University named after Sh. Ualikhanov in Kazakhstan. The climate is characterised as temperate continental, with a high temperature range and insufficient precipitation during the growing season. The average monthly air temperature ranged from +15.8°C in May to +28.3°C in July, with temperatures rising to +33°C on some days, which, combined with a total precipitation of 138 mm per season, met the criteria for atmospheric drought. Soil conditions of the site are represented by southern chornozems with humus content in the range of 3.6-3.8%, slightly acidic reaction of the environment (pH 6.8), nitrogen content of 14.3 mg/kg, phosphorus of 17.8 mg/kg, and potassium of 38.6 mg/kg. The particle size distribution is light loamy. These characteristics provide a favourable agricultural background subject to additional nutrition.

The study was conducted on soft spring wheat of the Stepnaya 62 variety, zoned in Northern Kazakhstan. The variety Stepnaya 62 was chosen for its high adaptation to the agro-climatic conditions of Northern Kazakhstan and stable productivity. The trial included two factors: main fertilisation and foliar application. The first factor was the application of a complex mineral fertiliser at a dose of $N_{60}P_{40}K_{20}$ for pre-sowing cultivation. The second factor included five variants of foliar treatments: in variant B1, no foliar feeding was carried out, and it was used as a control. In variant B2, a 10% urea solution was applied during the tillering phase. Variant B3 involved the application of a complex microfertiliser containing boron (150 g/l), zinc (75 g/l) and manganese (100 g/l) at the tube stage. Variant B4 included treatment with a biostimulant based on amino acids and humates during the earing phase. Variant B5 combined urea treatment in the tillering stage and a biostimulant in the earing stage. The experiment was designed using the randomised block design in triplicate. The registered area of each plot was 10 m$^2$. All agrotechnical practices were conducted in the same way in all variants: the crop predecessor was pea, the main treatment included ploughing by 25-27 cm followed by cultivation, sowing was conducted at the end of May with a seeding rate of 550 germinating seeds/m$^2$. Herbicidal protection in all variants was provided by a 2.4-D-based preparation at the beginning of tillering.

Productive elements were recorded at full maturity manually at three plots within each plot. The productive density (pcs/m$^2$) and the number of spikelets per plant were determined manually at three sites. The average number of spikelets per plant (pcs.), weight of 1,000 grains (g) and yield (c/ha) were determined. The weight of 1,000 grains were measured on a laboratory balance AXIS ADG-200 (Poland), in triplicate, and the yield was calculated using the formula (1):

$$Y = \frac{M \cdot 10^4}{S} \cdot \frac{1}{100}, \tag{1}$$

where Y – yield; hwt/ha; M – grain weight per plot; kg; S – plot area; m$^2$.

The chlorophyll content in the flag leaf was assessed during the grain filling phase using a portable chlorophyllometer SPAD-502Plus (Konica Minolta, Japan). The readings were taken in the morning (8:00-10:00) from ten plants per plot in the middle of the flag leaf length. The obtained values were averaged and used to analyse the physiological activity of the leaf. Phytosanitary resistance of plants was assessed at the stage of milk ripeness by visual diagnostics. The manifestations of leaf diseases (brown and yellow rust, septoria) were noted, using a 5-point scale, where: 0 – no lesions, 1 – up to 5% of the leaf surface, 2 – up to 15%, 3 – up to 35%, 4 – up to 55%, 5 – more than 75%. The assessment was conducted by a committee of three experts from the Department of Agriculture and Biological Resources, each of whom had experience in phytosanitary diagnostics. The data were averaged over all observations within the same variant. The lesion index was calculated as a weighted average score based on visual assessments using formula (2):

$$I = \frac{\sum(a \cdot b)}{n}, \tag{2}$$

where a – lesion score; b – number of plants with the score; n – number of examined plants.

The damage to the ears (%) was recorded separately by visual inspection during the wax ripeness phase. The delay in the onset of disease symptoms was estimated as the difference in days between the first manifestation and the control variant. The duration of the flag leaf functioning under stressful weather conditions was determined visually, by the time more than 50% of its area remained green. The countdown started from the beginning of milk ripeness to the moment of visual yellowing. The counting was conducted at intervals of 2-3 days in the morning. Variants with longer leaf retention were considered to be physiologically more resistant to weather stress (overheating, drought). The agronomic and economic efficiency was assessed by gross income (tg/ha), incremental costs (tg/ha), net income and return on investment (ROI). The latter was calculated using formula (3):

$$K_r = \frac{D_{var} - D_{contr}}{Z_{var} - Z_{contr}}, \tag{3}$$

where $K_r$ – investment return ratio; $D_{var}$, $D_{contr}$ – gross income in variant and control; $Z_{var}$, $Z_{contr}$ – costs in variant and control. The yield increase (tonnes per hectare) was calculated based on the average market price for wheat, with costs including the cost of inputs, labour and depreciation of equipment. All calculations were made per 1 ha.

The results of the experiments were statistically processed by one- and two-factor analysis of variance using Statistica 12.0 software (USA). The reliability of the differences between the variants was assessed by the significance criterion ($p < 0.05$), and coefficients of variation and standard errors were calculated. The authors adhered to the standards of the Convention on Biological Diversity (1992).

## RESULTS

**Effect of foliar feeding on productivity elements of durum wheat.** Yields varied widely, from 37.6 c/ha in the control (B1) to 43.2 c/ha in the variant with two foliar applications (B5), which corresponded to a 15% increase compared to the background level. A single application of urea in the tillering phase (B2) provided a moderate increase in yield to 40.9 c/ha, or 8.8% higher than the control. The use of a micronutrient complex (boron, zinc, manganese) in the tube stage (B3) resulted in a yield of 41.6 c/ha, which was 10.6% higher than the control. The use of a growth biostimulant, including humates and amino acids (B4), had a pronounced positive effect, with a yield of 42.3 c/ha, or 12.5% higher than B1. The most significant results were obtained with double foliar treatment (B5), which combined early application of urea and subsequent application of a biostimulant, with a maximum yield of 43.2 c/ha.

Leaf fertilisation had a complex effect on the structure of the crop. The number of productive stems per square metre in the treated variants was 18-22 units higher than in the control. Thus, in B1, an average of 383 productive stems/m$^2$ was recorded, while in variants B3, B4 and B5 this value reached 401, 405 and 417, respectively. Thus, the increase in stem density was accompanied by an improvement in the spatial structure of the crops, which had a favourable effect on the formation of the ear. The number of spikelets per ear also varied depending on the nutrition scheme. In the control, it averaged 15.4 spikelets, and in the variants with microelements and biostimulants (B3, B4), 16.1 and 16.3 spikelets, respectively. The maximum value of 16.5 pcs/ear was recorded in variant B5. These data indicate that follicle nutrition stimulated the development of generative organs, especially in the phase of tube formation.

The weight of 1,000 grains, one of the most sensitive indicators of plant nutrition, also changed significantly under the influence of fertilisation. If in the variant without additional treatment this indicator was 43.4 g, then in B3 and B4 it reached 44.8 g and 45.1 g, respectively. The highest weight was recorded in the B5 variant (45.6 g), which indicated a more intensive grain filling. Thus, biostimulants and combined nutrition schemes contributed to the formation of high-grade grains with a high specific weight. Additional confirmation of the positive impact of leaf fertilisers was provided by observations of the uniformity of plant development. In variants B1-B2, significant fluctuations in stem height and heterogeneity in developmental stages were observed, especially under stressful conditions of late tillering and heat. Whereas in variants B3-B5, plants were distinguished by a levelled stem, synchronous development and more uniform grain filling. This reduced intra-field variability and ensured a more uniform maturation of the crop (Table 1).

***Table 1**. Changes in productive traits of soft wheat under the influence of different foliar nutrition schemes*

| Variant | Solution description | Yield, hwt/ha | Productive stems, pcs/m2 | Spikelets per ear, pcs | Weight of 1,000 grains, g |
|---|---|---|---|---|---|
| B1 | Without foliar feeding (control) | 37.6 | 383 | 15.4 | 43.4 |
| B2 | Urea 10% (tillering phase) | 40.9 | 394 | 15.9 | 44.2 |
| B3 | Trace elements (B+Zn+Mn, tube output) | 41.6 | 401 | 16.1 | 44.8 |
| B4 | Biostimulant (amino acids + humates) | 42.3 | 405 | 16.3 | 45.1 |
| B5 | Double application (tillering + mowing) | 43.2 | 417 | 16.5 | 45.6 |

***Source:** compiled by the authors*

Thus, the study confirmed that foliar fertilisation, especially in the form of biostimulants and micronutrient complexes, has a positive effect on yield and its structural parameters. The increase in productive bushiness, ear development and grain filling were significantly improved in the variants with combined or repeated application. Particularly high efficiency was demonstrated by variant B5, which implements the principle of layered plant nutrition in key phases of ontogeny.

**Chlorophyll a content (SPAD) in different foliar application patterns**. One of the key indicators used in assessing the physiological state of crops was the chlorophyll content in the leaves, measured in SPAD units. This parameter is closely related to the functional state of the photosynthetic apparatus and reflects the reaction of plants to the supply of nutrients. In the control variant (B1), where no foliar feeding was applied, the chlorophyll content was 38.7 SPAD, indicating moderate chloroplast activity and possible restriction of access to nitrogen during critical phases of the growing season. The variant with urea application in the tillering phase (B2) provided an increase in chlorophyll content to 42.3 SPAD, indicating partial compensation for nitrogen deficiency and improvement of photosynthetic activity. The use of a microelement mixture in the phase of entering the tube (B3) contributed to an increase in this indicator to 45.2 SPAD, which can be explained by the role of zinc, boron and manganese in the regulation of photosynthetic processes, amino acid synthesis and the functioning of enzymatic systems. In the variant with the use of a biostimulant based on humates and amino acids (B4), the chlorophyll content reached 47.1 SPAD, indicating an increase in metabolism, improved membrane permeability and increased physiological activity of plants. The highest values were recorded at the two-time treatment (B5) – 49.5 SPAD, which is 27.9% higher than the control level. Thus, foliar feeding, especially in combination, provided a significant increase in photosynthetic potential, which is key in the formation of the crop.

The content of nutrients in the aboveground mass of plants also varied depending on the fertilisation scheme. In the control (B1), the content of total nitrogen was 2.65%, phosphorus – 0.32%, and potassium – 1.41%. In the variants with foliar fertilisers, these indicators increased: in B2 to 2.77%, 0.34% and 1.46%, in B3 to 2.89%, 0.35% and 1.49%; in B4 to 2.95%, 0.36% and 1.51%; and in B5 to 3.02%, 0.37% and 1.54%, respectively. This confirms the effectiveness of foliar nutrition in conditions of limited availability of elements in the soil solution, especially in the case of drought and soil compaction in the upper horizons. In addition, an increase in the concentration of elements in tissues indicates a more active transport and assimilation of nutrients in the phases of intensive growth. A complex indicator of the general physiological state of plants was the viability index, which considers a set of parameters of turgor, colour, development, and the presence of stress symptoms. In the control, this index was 0.74. In variant B2 (urea), the index value reached 0.79, with the use of trace elements (B3), it increased to 0.83, with the use of biostimulants (B4) to 0.87, and in variant B5, it reached 0.89. Such values correspond to a high physiological tone of plants and indicate resistance to external influences. The treated variants demonstrated not only better leaf colour but also longer preservation of green mass. In variants B4 and B5, under the conditions of July heat (daytime temperatures up to +33°C), the leaves remained active longer, without showing signs of anthocyanin colouration and premature ageing, which is relevant for full grain filling.

A separate observation concerned the duration of the active growing season. In the variant without foliar treatment, earlier yellowing and death of the leaves of the upper tier were observed, which reduced the life of the photosynthetic apparatus by 4-5 days. In the variants with foliar treatment, especially B5, the period of green activity lasted until the middle of grain filling, ensuring full photosynthesis in the crucial time for yield. In addition, these variants showed a more uniform colour of plants, stem density and the absence of depressed areas, which are typical for the control (Fig. 1).

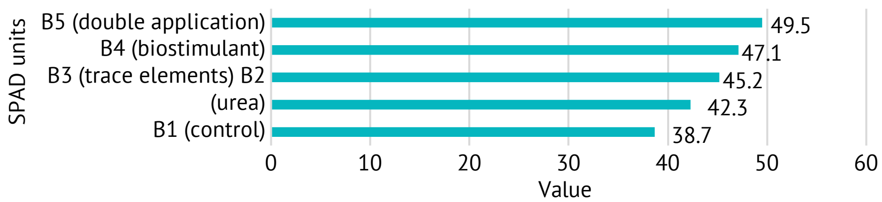


***Figure 1**. Intensity of chlorophyll accumulation depending on the methods of foliar feeding*
***Source:** compiled based on Formula (1)*

Thus, foliar feeding has proven to be efficient for increasing the physiological activity of durum wheat. Biochemical shifts towards an increase in chlorophyll, nitrogen, phosphorus and potassium in plant tissues, as well as visually observed stress resistance, indicate the effective impact of the used products. A particularly pronounced effect was observed when the combined strategy was applied in layers, which provided the highest level of all the physiological and biochemical parameters studied. This makes it possible to consider variant B5 as the most effective in temperate continental climate and southern chornozems, especially in years with limited water supply. The data obtained confirm the need to include folate nutrition in the overall fertilisation system of cereals under intensive cultivation technology.

**The effect of foliar feeding on disease resistance and physiological parameters of leaves**. In the control variant (B1), where leaf nutrition was not carried out, the level of leaf damage by rust and septoria was the highest, with an average of 26.4% of the leaf area showing visually pronounced symptoms. The intensity of the damage was also maximum: the average depth of necrosis on the affected leaf reached 3.2 points on a 5-point scale, and the flag leaf preservation index did not exceed 0.62. The presence of a significant proportion of damaged leaves in the phase of active photosynthesis negatively affected grain formation, reducing the efficiency of the filling phase, as evidenced by a decrease in the weight of 1,000 grains and the number of productive ears in this variant. With a single application of urea (B2), the degree of damage decreased to 21.3%, and the intensity of symptoms decreased to 2.7 points. The flag leaf preservation index increased to 0.7, which indicates a partial increase in physiological resistance due to improved nitrogen nutrition, although the effect cannot be considered stable and complete. The delayed onset of pathogenesis symptoms compared to the control was about 2 days.

A more pronounced reduction in disease incidence was observed with the use of a microelement complex (B3): the proportion of the affected leaf surface was 16.1%, and the visual lesion index decreased to 2.2 points. The flag leaf safety index reached 0.76. This result is due to the fact that zinc and manganese are actively involved in lignin synthesis and strengthening of cell walls, limiting the penetration of fungi, while boron regulates the formation of the epidermis and reduces the number of stomata of the main gates of pathogen penetration. The delay in symptom onset was 4-5 days compared to B1. In the variant with the use of a biostimulant (B4) containing humates and amino acids, the degree of damage was 17.4%, and the intensity of symptoms was 2 points. There was a clear effect of preserving the flag list (index 0.78) and slowing down the development of the lesion: the delay factor was 5 days on average compared to the control. This effect is associated with the activation of the synthesis of phytoalexins and other secondary metabolic compounds with fungistatic effects and regulating the antioxidant activity of tissues.

However, the best results were recorded in the B5 variant with a double application of only 14.5% of the affected surface, with a visual lesion index of 1.7 points and a flagging index of 0.81. This is 11.9 percentage points and 1.5 points below the benchmark, respectively. This decrease indicates a synergy between the early stimulating effect of nitrogen and the subsequent activation of immune and anti-stress mechanisms under the influence of biostimulants. The delay in the onset of primary symptoms was 7 days on average, which completes critical phases of plant ontogeny without significant limitation of photosynthetic activity. In addition to assessing the area of damage, there was a difference in the speed of symptom development. In the control, the first signs of rust were recorded at the end of the tube, and intensive spreading began from the earing phase. In the treatment variants, especially B4 and B5, the onset of symptoms was shifted to later dates (5-7 days). This meant not only a reduction in infection intensity, but also a shift in pathogenesis to later stages, when the impact on yield becomes less critical. Visual assessments also confirmed better preservation of the upper tier of leaves, especially the flag leaf, which remained green in the B5 variant until full maturity in 72% of plants, compared to 49% in the control.

In addition, cases of ear infections (mainly Fusarium) were recorded, especially in conditions of high humidity in July. Although the total number of infected ears did not exceed 4.8% in the control variants, in the plots with foliar nutrition, this indicator remained at the level of 2.4-3.2%. At the same time, the severity of symptoms (discolouration, underdevelopment of grain, hollow spikelet) in variants B3-B5 was significantly lower, which further confirms the strengthening of generative protection. Visually, such ears retained their full shape, and the damage more often affected no more than 1-2 grains, as opposed to 4-5 in the control. The analysis of the inter-variant difference showed that under the same agricultural technology, the main factor limiting the development of pathogens was not the direct fungicidal effect of substances, but an increase in the nonspecific resistance of plants. It was the improvement of nutrition, photosynthesis, and the synthesis of structural and protective metabolites under the influence of microfertilisers and biostimulants that created the conditions for preventing the mass spread of infections. Variant B5, which combined nitrogen and humic stimulation, provided the most complete implementation of this protective mechanism (Table 2).

***Table 2**. Indicators of resistance and leaf viability under different foliar nutrition schemes*

| Variant | Leaf damage, % | Defeat index (points) | Saving the flag list | Delayed onset of symptoms, days | Spikelet damage, % |
|---|---|---|---|---|---|
| B1 (control) | 26.4 | 3.2 | 0.62 | 0 | 4.8 |
| B2 (urea) | 21.3 | 2.7 | 0.7 | 2 | 4.1 |
| B3 (trace elements) | 16.1 | 2.2 | 0.76 | 4 | 2.9 |
| B4 (biostimulant) | 17.4 | 2 | 0.78 | 5 | 3.2 |
| B5 (double application) | 14.5 | 1.7 | 0.81 | 7 | 2.4 |

***Source:** compiled based on Formula (2)*

Thus, phytosanitary monitoring data showed that foliar feeding, especially in a combined scheme (double application), not only increases plant productivity but also contributes to the formation of a sustainable agrophytocenosis. This is especially relevant in conditions where chemical protection may be limited for economic or environmental reasons. The use of physiologically active substances reduces the pesticide load and, at the same time, increases the biological resistance of crops, which makes this technique a promising element of an integrated crop protection system.

**Duration of flag list operation in stressful weather conditions**. In the variants without foliar feeding (B1), plants showed low agroecological stability. Under moisture deficit and high air temperature in the tillering and tube growth phase, yellowing of the lower tier of leaves, reduced turgor and signs of photosynthetic activity inhibition were observed already in the first decade of development. These plants were distinguished by uneven developmental stages, lower height, and pronounced stem spotting. The duration of flag leaf functioning in the earing phase did not exceed 9-10 days, which limited grain filling and reduced the weight of 1,000 grains. In the same weather conditions, the variants with leaf dressing demonstrated significantly higher resistance. This was especially evident in variants B3 (microelements) and B5 (double application), where plants retained turgor even at air temperatures above +32°C, maintained a bright colour, did not show an anthocyanin reaction and less often showed leaf curl. In these variants, the flag leaf remained functional for up to 13-14 days, ensuring active photosynthesis throughout the entire grain filling period.

According to meteorological data, the key climatic stress factors occurred during the earing and earing phase: average daily air temperatures exceeded +29°C, and relative humidity dropped to 30-35%. Under these conditions, the control variant showed a rapid decrease in the SPAD level of chlorophyll and visual signs of leaf apparatus degradation. While in variants B4 and B5, the chlorophyll content remained at a high level, indicating stabilisation of metabolism and maintenance of metabolic activity. The analysis of morphological and physiological reactions showed that the effectiveness of foliar feeding was most pronounced under extreme weather conditions. Under hot and dry weather conditions, the difference in yield between the control variant and B5 was 5-6 c/ha, while under milder temperature conditions, the effect was weaker. This indicates that the effectiveness of foliar nutrition is particularly high in stressful years, when the crop experiences a sharp limitation of water or heat balance.

With a single application of fertiliser (e.g. B2 or B3), a temporary positive effect was observed due to increased nutrition and photosynthesis, but it was less stable compared to variant B5. The latter ensured the maintenance of physiological balance and sustainable realisation of productive potential throughout the growing season. This variant also showed synchronisation of earing and filling, uniformity of plant height and ear maturity, which had a positive effect on grain uniformity and quality during harvesting. An additional factor of agro-ecological sustainability was the ability of plants to compensate for short-term moisture deficits due to increased osmotic activity. In variants B4 and B5, plants retained turgor longer at identical soil moisture, which indicates the influence of biostimulant compounds on the physiological water retention capacity of tissues. It was also noted that such plants moved more slowly into the phase of physiological senescence in the context of of a simultaneous increase in the length of the milk ripeness phase and slower leaf death. Significantly, resistance to weather stress was accompanied not only by an increase in yield, but also by an improvement in its structure: a greater number of productive stems, a higher weight of 1,000 grains, and improved spikelet formation. All this reflects the complex effect of foliar feeding not only on nutrition, but also on the regulation of growth and development under stress. Notably, these effects were achieved without changing the main agronomic practices, which indicates the influence of foliar treatments on physiological processes in critical phases of plant development (Fig. 2).

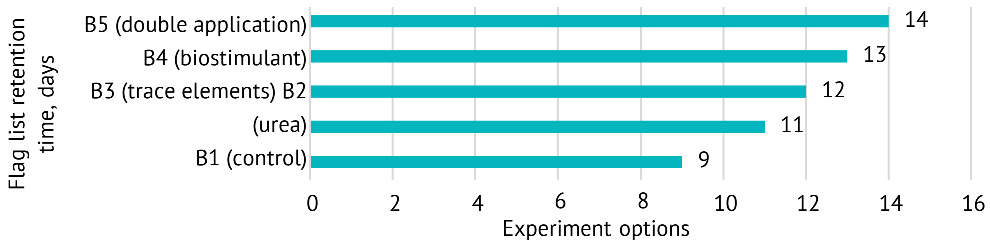


***Figure 2****. Dynamics of flag leaf green mass preservation under temperature and water stress*
***Source:*** *compiled by the authors*

Thus, foliar nutrition in the weather conditions of Northern Kazakhstan proved to be an effective mechanism for increasing the resistance of wheat to weather fluctuations. Maintaining photosynthetic activity, prolonging the functioning of the leaf apparatus, reducing the rate of ageing and maintaining physiological tone ensured the use of limited resources more efficiently and fulfilled their productive potential. Variant B5, which included early and late fertilisation, ensured the most balanced and sustainable development of the crop, compensating for the impact of short-term climatic stress. This approach can be considered as an adaptive element of intensive wheat cultivation technology in the risky farming zone.

**Agronomic and economic efficiency of leaf dressings**. In the control variant (B1), where no foliar feeding was applied, the yield was 37.6 c/ha. At the same time, the structure of the crop was characterised by minimal values: the productive density was 383 stems/m$^2$, the average number of spikelets per ear was 15.4, and the weight of 1,000 grains was 43.4 g. The grain was characterised by a high proportion of small and underdeveloped grains, which reduced its marketability. Gross income was 112,800 t/ha, costs were minimal, but the return on investment remained at zero due to the lack of additional agricultural practices. In the second variant (B2), which included a single foliar treatment with 10% urea solution during the tillering phase, the yield increased to 40.9 c/ha, which corresponded to an increase of 3.3 c/ha or 8.8% compared to the control. The productive density was 394 stems/m$^2$, the number of spikelets per ear was 15.9, and the weight of 1,000 grains was 44.2 g. The gross income increased to 122,700 tg/ha, the additional costs were 5,000 tg/ha, and the economic effect per hectare was 8,900 tg. The payback ratio was 1.6. Visually, the crops were more evenly developed but reacted sensitively to temperature stress during the tube phase.

The third variant (V3), which included the introduction of a microelement complex in the tube phase, provided a yield of 41.6 c/ha. The increase over the control was 4 c/ha (10.6%), the productive density reached 401 stems/m$^2$, the number of ears was 16.1, and the weight of 1,000 grains was 44.8 g. The gross income was 124,800 tg/ha, with costs of 6,500 tg/ha; the economic effect reached 7,500 tg/ha. The return on investment was 1.9. Alongside the yield increase, there was better resistance to leaf infections, longer green mass retention and a 13% increase in the proportion of milled grain. The fourth variant (B4) included foliar treatment with a biostimulant based on amino acids and humates during the earing phase. The yield was 42.3 c/ha, which was 4.7 c/ha (12.5%) higher than the control level. The productive density was 405 stems/m$^2$, the number of ears was 16.3, and the weight of 1,000 grains was 45.1 g. Gross income was 126,900 tg/ha, additional costs were 7,000 tg/ha, and the economic effect was 7,400 tg/ha. The payback ratio reached 1.8. The plants were distinguished by high levels, flag leaf preservation, resistance to overheating and high grain quality at harvest. The share of marketable grain increased by 15%.

The fifth variant (B5), which included two treatments with urea in the tillering phase and a biostimulant in the earing phase, yielded the highest yield of 43.2 c/ha. The increase was 5.6 c/ha or 14.9% compared to the control. The productive density reached 417 stems/m$^2$, the number of ears was 16.5, and the weight of 1,000 grains was 45.6 g. Gross income increased to 129,600 tg/ha, additional costs amounted to 8,500 tg/ha, and the economic effect reached 18,000 tg/ha. The return on investment was 2.1. There was a 4-5-day extension of the flag leaf viability, minimal disease damage and the lowest proportion of substandard grain (Table 3).

***Table 3**. Productivity and profitability indicators of different foliar feeding schemes*

| Variant | Yield, hwt/ha | Productive stems, pcs/ᵐ2 | Spikelets per ear, pcs | Weight of 1,000 grains, g | Crop growth, c/ha | Revenue, tonnes per hectare | Additional costs, tonnes/ha | Economic effect, tg/ha | Payback ratio |
|---|---|---|---|---|---|---|---|---|---|
| B1 (control) | 37.6 | 383 | 15.4 | 43.4 | 0 | 112,800 | 0 | 0 | 0 |
| B2 (urea) | 40.9 | 394 | 15.9 | 44.2 | 3.3 | 122,700 | 5.000 | 8.900 | 1.6 |
| B3 (trace elements) | 41.6 | 401 | 161 | 44.8 | 4 | 124,800 | 6.500 | 7.500 | 1.9 |
| B4 (biostimulant) | 42.3 | 405 | 16.3 | 45.1 | 4.7 | 126,900 | 7.000 | 7.400 | 1.8 |
| B5 (double application) | 43.2 | 417 | 16.5 | 45.6 | 5.6 | 129,600 | 8.500 | 18.000 | 2.1 |

***Source:** compiled based on Formula (3)*

In 2023, production experience in areas with low availability of mobile nitrogen and phosphorus was implemented, sowing spring wheat of the Omskaya 36-3 reproduction variety with pre-sowing seed treatment with Scarlet at a rate of 0.4 litres/tonne of seeds. The sown area was 50 hectares. Different doses of phosphate fertiliser in the form of ammophos NP 10-46 were applied at doses of 125 and 95 kg/ha of physical weight according to the approved experimental scheme. Sowing was conducted on 2 June 2024, with a seeding rate of 3 million units/ha or 140 kg/ha. Phenological observations of grain crops were carried out, field germination and weediness of crops were determined, and herbicide treatment with the following preparations was carried out: Phenomenon 0.5 l/ha + Baron 0.015 g/ha + Shansyugen 0.9 l/ha + Title duo 0.3 l/ha + Punto extra 0.025 g/ha. Leaf diagnostics was conducted on wheat leaves, and a deficiency of macro and microelements was identified in the critical phase. For the deficit, leaf treatments were conducted in the phase of entering the tube and the beginning of mowing with nitrogen-phosphorus liquid fertiliser ZhKU 11-37 in different doses on different variants, both with and without pre-sowing application of basic fertilisers. Doses ranging from N1P4 to N13P60 were applied (Table 4).

***Table 4**. Spring wheat yield formation in the conditions of southern chornozems of Northern Kazakhstan, depending on different fertilisation systems*

| No. | Option name | Green mass, g/m$^2$ (tube phase) | Ear length, mm | Average number of grains per ear, pcs | Yield, t\ha |
|---|---|---|---|---|---|
| 2 | $N_{13}P_{58}$ (NP calc) | 148.5 | 7.2 | 29.3 | 1.16 |
| 3 | Management | 121.5 | 8.3 | 32.3 | 0.75 |
| 5 | Foliar application of $N_2P_7$ | 102.75 | 7.8 | 31 | 0.87 |
| 6 | Foliar application $N_3P_{10}$ | 109 | 7.5 | 31.4 | 0.96 |
| 7 | Foliar application $N_4P_{14}$ | 131 | 7.7 | 30.6 | 0.97 |
| 8 | ($N_{10}P_{41}$) soil application + ($N_2P_7$) foliar fertilisation | 145 | 8.4 | 32.9 | 0.96 |
| 9 | Soil application $N_{10}P_{41}$ | 102 | 8.8 | 33.78 | 0.82 |

***Source:** compiled by the authors*

Given the high humidity of 2023, in Lobanovo LLP (Imantau village, Northern Kazakhstan region), foliar feeding without the main fertiliser application did not show its effectiveness compared to the variant with the full calculated dose of N13P58. However, the yield in the control was significantly lower than in the foliar fertilisation variant, at 0.75 t/ha compared to 0.82-1.05 t/ha. Comparing the experiments in different years of moisture, it is possible to conclude that with sufficient moisture, foliar feeding is more effective when applied in combination with the main application of macronutrients (nitrogen and phosphorus).

Thus, in all variants that included foliar feeding, a stable advantage was recorded in terms of yield, the main elements of the productivity structure and economic parameters of crop cultivation. The yield increase ranged from 3.3 to 5.6 c/ha, with the highest increase achieved with two treatments (B5), indicating the effectiveness of the combination of various physiologically active substances and precise phase positioning of fertilisers. The increase in productive density, the number of spikelets per ear and the weight of 1,000 grains in most variants was accompanied by an improvement in the morphophysiological state of plants, a decrease in the phytosanitary load and the prolongation of the functioning of the assimilation apparatus in the critical phases of crop formation. Summarising the data obtained, it can be concluded that the integration of foliar feeding into cereal crop cultivation technology, especially in the form of phased and combined application, is a highly efficient and economically viable element of the adaptive farming system. This approach simultaneously increased the biological realisation of crop potential, reduced dependence on weather stress and improved the economic return on each hectare of sown area.

## DISCUSSION

The obtained results demonstrated a significant influence of foliar nutrition schemes on the productivity and physiological state of spring wheat under the weather conditions of Northern Kazakhstan. In the context of the same main dose of fertiliser ($N_{60}P_{40}$), the highest yield was recorded in the variant with double foliar application (B5), which indicates the synergistic effect of the sequential application of urea and biostimulant. Significant changes were also observed in the structure of the crop: an increase in the number of productive stems, weight of 1,000 grains and number of spikelets per ear confirmed the improvement of generative development and efficiency of the photosynthetic apparatus. The increase in the content of chlorophyll and nutrients in the tissues, as well as the growth of the viability index, indicates the activation of metabolic processes and the strengthening of the metabolic stability of plants (Pakhomeev & Ibragimova, 2025). The contribution of foliar nutrition to increasing agro-ecological resistance to abiotic and biotic stresses was particularly significant. Delaying the onset of disease symptoms and preserving the flag leaf during the grain filling phase ensured prolonged photosynthesis, which contributes to the formation of a full-fledged harvest (Drobitko *et al.*, 2024).

In addition, publications by R. Bojović *et al.* (2022) and P. Govindasamy *et al.* (2023) considered aspects of agricultural technology and fertilisers in the context of economic efficiency and climate change. R. Bojović *et al.* studied sugar beet productivity management in Serbia and Montenegro, focusing on the systematic use of mineral fertilisers. However, the study did not cover the issues of plant physiological resistance or the impact of nutrition on the response of crops under stressful conditions. P. Govindasamy *et al.* emphasised the importance of increasing nitrogen use efficiency for sustainable agriculture but limited themselves to conceptual approaches without detailed agronomic adaptation to specific climatic regions. In contrast to these studies, the present study was based on a comprehensive empirical framework with an assessment of physiological, biochemical and economic indicators: a yield increase of up to 15%, a decrease in disease incidence by 11.9 percentage points and an extension of the flag leaf viability were recorded, making it more applicable to the conditions of Northern Kazakhstan.

On the other hand, M. Palka *et al.* (2021) and A. Fast *et al.* (2023) analysed the diagnosis and management of nitrogen nutrition. M. Palka *et al.* confirmed the effectiveness of the CCCI-CNI index for assessing the nitrogen status of wheat but did not consider real agronomic actions to correct nutrition. A. Fast *et al.* conducted field studies on Canadian wheat, establishing productive nitrogen doses with the addition of inhibitors, but did not include foliar treatments and assessment of the physiological response of plants. Compared to these studies, the present study was characterised by a holistic approach: it included field SPAD measurements, visual scoring, disease resistance assessment and comprehensive economic diagnostics. This multi-level methodology ensured not only an increase in yields but also an increase in agro-ecological sustainability without changing the basic agricultural technique (Trembitska & Bohdan, 2023; Kalenska *et al.*, 2025).

Publications by C. Mihuț *et al.* (2022) and F. Mocanu *et al.* (2023) addressed the influence of fertilisers and weather conditions on crop productivity. C. Mihuț *et al.* evaluated different nutrition schemes, but without physiological and phytosanitary indicators, which limited the applied value of the conclusions. F. Mocanu *et al.* determined that drought significantly reduced the productivity of sorghum hybrids but did not propose specific ways to compensate for this effect. This study managed to provide proven solutions, confirming the effectiveness of phase foliar nutrition with biostimulants and microelements. By means of an integrated assessment of physiology, chlorophyll and water retention, pathogen resistance and economics, the adaptive value of the developed schemes in the arid conditions of the region was proved.

F. Chețan *et al.* (2023) and K. Pokovai *et al.* (2025) raised the issues of adaptation of agricultural technology to stressful conditions and rational land use. F. Chețan *et al.* addressed no-till tillage to preserve the structure of chornozems under maize cultivation but did not include agrophysiological parameters of plants. K. Pokovai *et al.* found a decrease in yields under warming conditions but did not evaluate the effectiveness of compensating practices. In contrast to these studies, the present research proved that the use of biostimulants and urea in key phases of wheat organogenesis contributed to the prolongation of the flag leaf function and stabilisation of the photosynthetic apparatus. In addition, under drought conditions, an increase in the proportion of productive stems and an increase in the weight of 1,000 grains were achieved, which directly reflected the crops' resistance to weather stress (Boiko *et al.*, 2024).

The publications by H. Domnariu *et al.* (2025) and K. Akchaya *et al.* (2025) analysed sustainable farming systems, including soil microbiota and the role of legumes in agroecosystems. H. Domnariu *et al.* recorded changes in microbial communities under the influence of different tillage practices, without extending the analysis to plants. K. Akchaya *et al.* summarised the benefits of mixed crops but did not provide quantitative data on the effectiveness of foliar treatments in cereals. In contrast to these approaches, this study was based on field data on foliar nutrition in spring wheat, including trace elements and amino acids. Due to the comprehensive evaluation (SPAD, productivity, disease resistance), the results were highly applicable, which is especially relevant for adaptive agriculture in Northern Kazakhstan.

R. Láposi *et al.* (2024) and M. Mirosavljević *et al.* (2024) studied the physiological responses of maize and the effect of sowing dates on the efficiency of wheat varieties, respectively. R. Láposi *et al.* demonstrated that the use of phytoconditioners increased chlorophyll content in maize during drought, but there was no relationship with yield and pathogen resistance. M. Mirosavljević *et al.* determined differences in nitrogen use among modern varieties but did not consider the effect of foliar treatments. In contrast to these publications, this study combined physiological, agronomic and economic parameters: the optimal fertilisation scheme was determined, which prolonged the functioning of the flag leaf, increasing grain weight and reducing disease incidence.

A. Rahman *et al.* (2023) and J. Behr *et al.* (2024) considered various agrophysiological approaches to improving plant health. J. Behr *et al.* described the effect of long-term use of minimal tillage in combination with moderate doses of nitrogen, which enhanced microbial interactions in the rhizosphere and improved the health of winter wheat. However, productivity and economic efficiency were not analysed. At the same time, A. Rahman *et al.* recorded an increase in flax yield and oil content when phosphorus and zinc were applied in foliar form, but the study did not involve cereals and did not include leaf physiological parameters. In contrast to these limitations, the present study includes not only a detailed analysis of leaf activity by SPAD and flag leaf, but also a comprehensive assessment of yield, disease resistance and profitability, making it applicable to North Kazakhstan conditions.

I. Racz *et al*. (2021) and H. Raniro and J. Santner (2024) conducted a variety of studies on phosphorus uptake by crops in contrasting soil systems. H. Raniro and J. Santner described the differences in phosphorus uptake strategies of cover crops in calcium and decalcified chornozem, but the topic of cereals remained outside the scope of the study. At the same time, I. Racz *et al*. conducted a field assessment of the agronomic properties of spring wheat but did not investigate the effectiveness of foliar nutrition under stressful conditions. This study not only determined the agronomic value of foliar treatments but also recorded their impact on the viability of the leaf apparatus, which is critical in conditions of drought and temperature spikes.

The review by S. Ouda and A. Zohry (2024) and E. Daly *et al*. (2025) addressed climate adaptation and environmental risks. E. Daly *et al*. presented modelling of greenhouse gas emissions under different nitrogen fertilisation regimes, emphasising reducing environmental impact, but did not address plant growth or photosynthesis parameters. S. Ouda and A. Zohry emphasised the agro-ecological benefits of rotating legumes and cereals but omitted the topic of foliar feeding and the physiological response of plants. In contrast, this study demonstrated a clear link between foliar nutrition, disease resistance and photosynthetic activity duration, which has direct agrotechnological applicability. In the context of the presented publications, this study is prominent for its applied orientation and breadth of coverage: it includes agrophysiological, phytopathological and economic aspects. The use of foliar feeding not only increased the yield of spring wheat, but also prolonged the functioning of the flag leaf, improved chlorophyll content, disease resistance and ensured the profitability of the technology in the weather conditions of Northern Kazakhstan.

## CONCLUSIONS

During the study, a comprehensive assessment of the effectiveness of different schemes of foliar feeding of durum wheat under the weather conditions of Northern Kazakhstan was conducted. The study determined that foliar nutrition has a complex effect on productivity, physiological condition, disease resistance and adaptive potential of plants under weather stress. Yields increased significantly in all variants compared to the control (37.6 c/ha), reaching 40.9 c/ha with urea (B2), 41.6 c/ha with microelements (B3), 42.3 c/ha with a biostimulant (B4) and a maximum of 43.2 c/ha with two treatments (B5). The increase ranged from 8.8% to 14.9%, depending on the variant. The elements of the yield structure were significantly improved: the number of productive stems increased from 383 pcs/m$^2$ in the control to 417 pcs/m$^2$ in the B5 variant, the number of spikelets per ear from 15.4 to 16.5, and the weight of 1,000 grains from 43.4 g to 45.6 g. These changes indicate stimulation of both vegetative and generative development of the crop.

Indicators of physiological condition also showed a significant increase: chlorophyll content in leaves increased from 38.7 to 49.5 SPAD, the content of total nitrogen, phosphorus and potassium in the aboveground mass increased to 3.02%, 0.37% and 1.54%, respectively. The plant viability index increased from 0.74 in the control to 0.89 in the B5 variant, reflecting an improvement in metabolic activity and stress resistance. The fertilisers contributed to a reduction in disease incidence: the area of affected leaves decreased from 26.4% to 14.5%, and the disease index from 3.2 to 1.7 points. There was also a lower incidence of Fusarium infection of ears, at 2.4% against 4.8% in the control. In conditions of heat and moisture deficit (up to +33°C, humidity up to 30-35%), leaf dressing, especially in variants B4 and B5, ensured the preservation of turgor, bright colour and uniform grain filling. The limitation of the study is the conduct of experiments in one agro-ecological region and during one growing season. It is advisable to expand further research to different soil and climatic zones and a multi-year period to assess the stability of the effect of foliar feeding.

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## CONFLICT OF INTEREST

The authors of this study declare no conflict of interest.

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# Ефективність листових підживлень при комплексному удобренні зернових культур на південних чорноземах Північного Казахстану

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**Анотація**. Мета дослідження полягала в оцінці впливу листових підживлень на фізіологію, врожайність та економічну ефективність ярої пшениці в Північному Казахстані. Польові випробування були проведені у вегетаційний період 2024 року (травень-серпень) на базі навчально-дослідної ділянки Кокшетауського університету ім. Шокана Уаліханова і були спрямовані на оцінку агроекологічної та економічної ефективності різних схем листових підживлень ярої пшениці. Дослідження включало п'ять варіантів досвіду, що відрізняються складом і кратністю внесення добрив, у тому числі карбаміду, мікроелементів і біостимуляторів. Варіанти з фоліарними обробками демонстрували стійку перевагу за агрофізіологічними показниками, особливо в умовах спекотної та сухої погоди, при температурі близько +33 °C і вологості повітря 30-35 %. Найвищі результати отримано при дворазовому листковому підживленні (варіант В5), де врожайність досягла 43,2 ц/га, що перевищувало контроль на 5,6 ц/га (14,9 %). Маса 1 000 зерен збільшилася до 45,6 г, продуктивна густота – до 417 стебел/м2, а кількість колосків у колосі – до 16,5. Тривалість функціонування прапорцевого листка в цьому варіанті досягала 14 діб, а рівень вмісту хлорофілу залишався високим протягом фази наливу. Відзначалося значне зниження ураження листя іржею (до 14,5 %) і підвищення частки кондиційного зерна на 15 %. Економічний аналіз показав, що варіант В5 забезпечив максимальний дохід (129 600 тг/га) при коефіцієнті окупності 2,1 і економічному ефекті 18 000 тг/га. Решта варіантів забезпечили приріст врожайності від 3,3 до 4,7 ц/га при коефіцієнтах окупності від 1,6 до 1,9. Дані підтверджують доцільність інтеграції фазово підібраних листових підживлень у технології вирощування пшениці в зоні ризикованого землеробства. Порівнюючи досліди в різні роки зволоження, можна зробити висновок, що при достатньому зволоженні, листові підживлення більш ефективні при комплексному внесенні з основним внесенням макроелементів (азоту і фосфору)

**Ключові слова:** фізіологічна активність; продуктивні стебла; вміст хлорофілу; індекс життєздатності; мікроелементи; яра пшениця; врожайність

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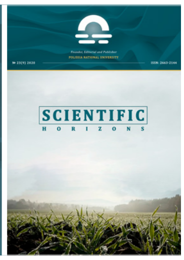


# Biotechnological aspects of the molecular diagnosis of cereal crop viruses

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**Abstract**. The annual decline in cereal crop yields across many regions of the world due to pathogens such as Barley Yellow Dwarf Virus (BYDV), Wheat Streak Mosaic Virus (WSMV), and Soil-borne Cereal Mosaic Virus (SBCMV) underscores the importance of studying their spread and control. The aim of this study was to identify patterns in the distribution of viral infections affecting cereal crops in Ukraine and Azerbaijan. The research utilised reverse transcription followed by polymerase chain reaction (RT-PCR) and loop-mediated isothermal amplification (LAMP) to detect viruses in cereal crop samples. Results revealed a high infection rate in both countries (51.1% in Ukraine and approximately 51% in Azerbaijan), with BYDV being the dominant virus. Regional differences were identified: in Ukraine's forest-steppe zone, BYDV was most prevalent (41.5%), while in the steppe zone, WSMV was more frequently detected (25.9%). In Azerbaijan, BYDV prevalence was lower (≤ 18.7%), but the frequency of

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SBCMV was higher (up to 20.4%). Co-infections were found in 26.1% of infected samples in Ukraine and 18.9% in Azerbaijan, with 23.7% of asymptomatic plants in Ukraine and 18.5% in Azerbaijan testing positive for viral material. The LAMP method proved effective for rapid field diagnostics, demonstrating 100% specificity and over 82% sensitivity. The findings highlighted the need for regionally adapted strategies to control viral diseases in cereal crops. The practical value of this work lies in the development of recommendations for improving plant monitoring and protection systems, taking local conditions into account, thereby contributing to increased yields and food security in the region

**Keywords:** polymerase chain reaction; reverse transcription; loop-mediated isothermal amplification; soil-borne cereal mosaic virus; barley yellow dwarf virus; wheat streak mosaic virus; arable land; agriculture

## INTRODUCTION

Agriculture faces numerous challenges related to the spread of viral infections in cereal crops, leading to economic losses and posing risks to food security. Viral pathogens such as Barley Yellow Dwarf Virus (BYDV-PAV), Wheat Streak Mosaic Virus (WSMV), and Soil-borne Cereal Mosaic Virus (SBCMV) present a serious threat due to their high contagiousness, ability to quickly adapt to diverse agroecological conditions, and potential to develop new, aggressive strains. Therefore, developing effective methods for the diagnosis, monitoring, and control of viral infections is crucial for preventing epidemics and ensuring stable crop yields. Importantly, cereal production occupies a significant share of global arable land, making viral epidemics a serious concern not only for local but also for international agriculture (Andreychenko *et al.*, 2024).

Recent research highlights the importance of molecular diagnostic techniques such as reverse transcription followed by polymerase chain reaction (RT-PCR) and loop-mediated isothermal amplification (LAMP) for the accurate detection of viral pathogens in plants at early stages of infection. For instance, the work by V.C. Chalam *et al.* (2020) demonstrates the advantages of modern biotechnological approaches in diagnostics, including high sensitivity, specificity, and the capacity to detect latent infections. Simultaneously, the study by O.A. Kolade *et al.* (2022) emphasises the need to integrate molecular methods into virus-free plant material exchange systems and phytosanitary control schemes. However, despite progress in this field, key issues remain unresolved concerning regional patterns of virus distribution, interactions with local agroecosystems, and the variable efficacy of diagnostic methods under different climatic conditions.

In the context of Ukraine and Azerbaijan, the problem is particularly relevant, as both countries are major cereal producers in their respective regions, though they differ in agroclimatic conditions, crop rotation structures, and farming systems. The research by H. Snihur *et al.* (2025) points to the role of seed transmission in the spread of cereal crop diseases in Ukraine, where up to 30% of seed material may be infected. Meanwhile, N. Sultanova *et al.* (2024) describe the characteristics of viral infections in Azerbaijan's agricultural crops, which are influenced by the arid climate and specific local agrotechnologies. This highlights the need for similarly comprehensive studies on cereal crops. This highlights the need for similarly comprehensive studies on cereal crops, especially considering that both countries dedicate vast areas of arable land to cereal production, making the sustainability of agriculture directly dependent on effective viral disease management.

Biotechnological tools such as RNA interference and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-based genome editing are being explored as promising strategies to enhance cereal crop resistance to viruses (Khassanova *et al.*, 2024). However, their practical application in Ukraine and Azerbaijan requires further investigation, considering regional virus circulation patterns, the presence of local strains, and agroecosystem specifics. Another notable issue is co-infection, where plants are simultaneously affected by multiple viruses, potentially resulting in synergistic effects and increased pathogenicity (Solomiichuk & Pikovskyi, 2025). Existing studies, such as that by M. Bhanjadeo *et al.* (2023), suggest innovative nanotechnology-based approaches to virus diagnostics, including biosensors and quantum dots. Nonetheless, their practical implementation in Ukraine and Azerbaijan remains under-researched, especially regarding economic feasibility and adaptability to local laboratory conditions. Similarly, the research by L. Aguilar-Marcelino *et al.* (2020) highlights the potential of beneficial microorganisms in viral infection biocontrol, though their effectiveness in local agroecosystems requires further study, particularly under climate change conditions.

A vital aspect is the comparative evaluation of diagnostic method effectiveness in real field conditions. As K. Dhiman *et al.* (2024) note, traditional serological methods often lag behind molecular techniques in sensitivity, especially for detecting new virus strains. At the same time, high-tech methods such as metagenomic sequencing remain inaccessible for routine use in most agricultural laboratories in Ukraine and Azerbaijan. As shown by J. Aghayev and E. Samadova (2022), the high infection pressure within agrocenoses necessitates systematic monitoring, timely diagnosis, and comprehensive phytosanitary protection that accounts for pathogen specificity and climatic factors. Current diagnostic approaches, according to M. Raza *et al.* (2024), involve integrating molecular methods with digital monitoring platforms, enhancing pathogen detection accuracy and enabling rapid responses. Additionally, as noted by O.V. Dubrovna *et al.* (2023), RNA interference technologies offer promising strategies for developing virus-resistant crop varieties, opening new directions for plant breeding.

The identified knowledge gaps stem from the fact that current research lacks comparative analysis of agroclimatic determinants of virus spread in the contrasting regions of Ukraine and Azerbaijan, insufficiently addresses the dynamics of co-infections, and provides limited data on the efficacy of available molecular diagnostic tools under local conditions. The aim of this study was to identify regional characteristics of the spread of viral infections in cereal crops within the contrasting agroecosystems of Ukraine and Azerbaijan. The objectives included: comparing the structure of viral infections in cereal crops across different climatic zones in both countries; assessing the effectiveness of reverse transcription followed by PCR and LAMP methods for diagnostics; analysing the impact of agroclimatic factors on infection dynamics; investigating the frequency of co-infections and asymptomatic pathogen carriage.

## MATERIALS AND METHODS

Sample collection and material preparation. The study was conducted in 2024 at the laboratory of the Institute of Plant Protection of the National Academy of Agrarian Sciences of Ukraine, Kyiv. In Azerbaijan, the research was carried out at the Research Institute of Plant Protection and Technical Crops under the Ministry of Agriculture of the Republic of Azerbaijan. Plant samples (leaves and basal stems) were collected during the vegetation period (May-June 2024) from fields of winter wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), and rye (*Secale cereale* L.) across six agricultural enterprises located in two main agro-climatic zones. In Ukraine, samples were collected from three enterprises in the forest-steppe zone: Vinnytsia region (Agricultural Enterprise "Agro-Vinnytsia"), Cherkasy region (Scientific and Production Centre "Reproductive Grain"), and Sumy region (Farm "SULA-AGRO"); and from three enterprises in the steppe zone: Dnipropetrovsk region (Dnipro Agro Group), Kherson region (LLC "Tavria-Agro"), and Zaporizhzhia region (LLC "Druzhba 5"). In Azerbaijan, sampling was also carried out in two agro-climatic zones: Steppe zone: Beylagan district (LLC "Garabag Tahil"), Ganja district (LLC "Akrolink-Ganja"), and Shirvan district (several grain-focused enterprises, though specific legal names were not confirmed in public sources); Forest-steppe zone: Jalilabad district (LLC "Gyunashli Agro"), Lankaran district (LLC "Chinar"), and Astara district (LLC "Astarachay"). Inclusion criteria: affiliation with target crops (wheat, barley, rye); presence of typical viral symptoms (yellowing, mosaic patterns, dwarfing, necrotic spots) or complete absence of visible symptoms (asymptomatic plants); sampling from various field areas to avoid bias from localised infection hotspots. Exclusion criteria: samples with mechanical damage, fungal or bacterial infection; technical artefacts during analysis (e.g. missing internal amplification control, protocol errors); samples with conflicting detection results between RT-PCR and LAMP, without possibility of verification.

From each field in Ukraine, 15 samples were collected (5 symptomatic, 5 asymptomatic, and 5 random samples selected blindly, without visual pre-assessment), totalling 270 samples (6 enterprises × 3 crops × 15 samples). In Azerbaijan, the same sampling structure was applied, with 135 samples collected in each zone, also totalling 270. Thus, the overall sample size across both countries was 540. Samples were collected by walking diagonally across fields at random. Collected material was immediately frozen in liquid nitrogen and transported to the laboratory on dry ice for analysis. Samples were stored in a -80°C freezer (Sanyo, Japan). Virus-free wheat plants grown in vitro at the institute's cell collection served as negative controls. All procedures for sampling, transportation, and storage were conducted in accordance with the Convention on Biological Diversity (1992).

Total RNA extraction. RNA was extracted from approximately 100 mg of homogenised frozen leaf/stem tissue. The commercial kit "RNeasy Plant Mini Kit" (Qiagen, Germany) was used according to the manufacturer's protocol, which includes lysis with RLT buffer containing β-mercaptoethanol, column precipitation, washing, and RNA elution. Concentration and purity of the isolated RNA were assessed with a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA). Acceptable RNA quality was defined as A260/A280 ratio of 1.8-2.1 and A260/A230 > 2. Samples showing signs of degradation (as evaluated by 1% agarose gel electrophoresis) were excluded from further analysis. Molecular detection of viruses. Reverse Transcription (RT) and Polymerase Chain Reaction (RT-PCR). Reverse transcription was performed on 500 ng of total RNA using the RevertAid Premium Reverse Transcriptase kit (Thermo Fisher Scientific, USA) with random hexamer primers following the manufacturer's protocol (25°C – 5 min, 42°C – 60 min, 70°C – 10 min). The resulting cDNA was used as the template for PCR. PCR was performed using specific primers for the target viruses:

1. BYDV-PAV: Forward: 5′-ACCTAGACGCGCAAATCAAA-3′; expected product: 590 bp (Malmstrom and Shu, 2004).
2. WSMV: Forward: 5′-GATCAAATACCAACCGCGTG-3′; expected product: 493 bp (Jones *et al.*, 2005).
3. SBCMV: F: 5′-GGTAGTCAGCTGTTAGCGTGT-3′; expected product: 773 bp (Marra *et al.*, 2022).

Each reaction mix (25 μL) included: 2.5 μL of 10 × Taq polymerase buffer, 1.5 μL of 25 mM $MgCl_2$, 0.5 μL of 10 mM dNTPs, 0.5 μL of each primer (10 μM), 0.25 μL

(1.25 U) of Taq DNA polymerase (Thermo Fisher Scientific), 2 μL of cDNA template, and nuclease-free water. Amplification conditions were: initial denaturation at 94°C – 3 min; 35 cycles of denaturation at 94°C – 30 sec, annealing at 58°C (BYDV, SBCMV) / 60°C (WSMV) – 30 sec, extension at 72°C – 45 sec; final extension at 72°C – 5 min. Amplification was performed using a T100 Thermal Cycler (Bio-Rad, USA). PCR products were analysed by 1.5% agarose gel electrophoresis (Agarose I, Amresco, USA) with ethidium bromide (0.5 μg/mL) in TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8) at 100 V for 45 min. Visualisation was carried out using a Gel Doc XR + system (Bio-Rad, USA) under UV light. A sample was considered positive if a DNA fragment of the expected size was present. Each run included a negative control (water instead of template) and a positive control (RNA from previously confirmed infected plants).

Coinfections were identified based on RT-PCR data: a sample was considered coinfected if two or all three target viruses were detected simultaneously. The coinfection rate was calculated as the percentage of infected samples carrying multiple viruses. Additionally, the distribution of infections between symptomatic and asymptomatic plants was assessed. Samples were grouped by symptom presence, and infection rates were compared using the $\chi^2$ test. LAMP. Virus detection was also performed using the LAMP-based "Plant Virus Detection Kit" (OptiGene, UK), with primers specific to the same target viruses (BYDV-PAV, WSMV, SBCMV), according to the manufacturer's instructions. The specific LAMP primer sequences are proprietary and not disclosed. The reaction mixture (25 μL) contained: 12.5 μL of ready-to-use lyophilised reaction mix (including Bst DNA polymerase, dNTPs, buffer, and fluorescent intercalating dye), 1 μL each of outer primers (F3, B3; 10 μM), 1 μL each of inner primers (FIP, BIP; 40 μM), 2 μL of extracted RNA, and nuclease-free water. Amplification was conducted on a Genie III device (OptiGene, UK) at 65°C for 30 minutes (amplification), followed by 98°C for 5 minutes (enzyme inactivation). Results were detected in real time via fluorescence and analysed using the amplification curves generated by the instrument. The time to positivity (Tp) was recorded. A sample was considered positive if a characteristic sigmoidal amplification curve appeared with Tp < 30 minutes. Negative and positive controls were included in each run. Data were processed using IBM SPSS Statistics v.29. The virus prevalence (P) in each zone and overall was calculated using the following formula (1):

$$P = \left(\frac{\text{Npositive}}{\text{Ntotal}}\right) \times 100\%, \qquad (1)$$

where Npositive – number of positive samples; Ntotal – total number of tested samples.

To compare the frequency of virus detection between agroclimatic zones, between symptomatic and asymptomatic groups, as well as to assess the effectiveness of RT-PCR and LAMP methods, the $\chi^2$ (Chi-squared) test for contingency tables was employed. The level of statistical significance was set at $p < 0.05$. To evaluate the agreement between RT-PCR and LAMP results, Cohen's kappa coefficient (κ) was used. The Cohen's kappa coefficient (κ) was interpreted according to the Landis & Koch scale: $\kappa < 0$ – poor agreement; 0-0.2 – slight; 0.21-0.4 – fair; 0.41-0.6 – moderate; 0.61-0.8 – substantial; 0.81-1 – almost perfect agreement. The percentage of agreement was calculated using formula (2):

$$\% \text{ Agreement} = [(A + D)/N] \times 100\% \qquad ,(2)$$

where A – number of samples with a positive result in both tests (RT-PCR$^+$ and LAMP$^+$); D – number of samples with a negative result in both tests (RT-PCR$^-$ and LAMP$^-$); N – total number of samples examined.

The data is presented in tables and diagrams created in Microsoft Excel.

## RESULTS

Overall virus detection frequency. The analysis results revealed a high overall infection rate, calculated using formula 1 – more than half of the samples examined, specifically 51.1% (138 out of 270) in Ukraine, contained at least one of the target viruses. Among these, the dominant pathogen was found to be BYDV-PAV, RNA of which was detected in 34.8% of the samples (94/270), indicating its widespread distribution and potentially key role in the viral pathology of cereals in the studied agroecosystems. Significantly lower, but still notable, frequencies were recorded for WSMV, detected in 19.3% of the samples (52/270), and SBCMV, whose RNA was detected in 12.6% of cases (34/270). The results showed that the trends in virus prevalence across Azerbaijan were similar: the overall infection rate ranged between 50-52%, with BYDV-PAV as the dominant pathogen. WSMV and SBCMV were also consistently detected in Azerbaijan samples, indicating their persistence in the pathogen complex of cereals in the region. Thus, the total infection rate among all examined samples from both countries, according to calculations (formula 1), was approximately 51% (276 out of 540), confirming the stable circulation of the studied viruses under different agroecological conditions.

It is important to note that, in addition to individual infections, several cases of mixed infections were recorded: almost a third of the infected samples (27%, or 37 out of 138 in the Ukrainian sample) contained genetic material from two or three viruses simultaneously. Similar mixed infection rates were observed in samples from Azerbaijan (18.9%, 26 out of 138 infected samples), indicating favourable conditions for polyetiological damage to crops. Such a situation can complicate clinical diagnosis, intensify the harmful effects of viral damage, and also hinder the selection of an effective plant protection strategy. The co-infection level increases the risk of synergy between viruses and requires further research on its impact on the physiological-biochemical parameters of plants and crop yields. Regional distribution of viruses. BYDV-PAV demonstrated the highest spatial variability, being more frequently detected in samples from the forest-steppe zone (41.5%, 56/135) than from the steppe zone (28.1%, 38/135). The difference was statistically significant ($\chi^2$, $p = 0.021$), indicating real differences in the ecological or agronomic factors affecting the spread of this virus across regions. This difference is partly explained by the milder climatic conditions in the forest-steppe, which support the survival of insect vectors, particularly aphids, which are the primary vectors of BYDV.

In contrast, WSMV showed the reverse trend: its detection rate in the steppe zone was 25.9% (35/135), almost twice as high as in the forest-steppe zone (12.6%, 17/135). This difference was statistically significant ($p = 0.005$). Given that the primary vector of WSMV is the mite *Aceria tosichella*, known for its high adaptability to dry conditions, this territorial difference aligns with the ecological characteristics of the steppe zone, where higher temperatures and lower rainfall create favourable conditions for the spread of this pest. For SBCMV, the prevalence was more uniform across both zones: 14.1% in the forest-steppe (19/135) versus 11.1% in the steppe (15/135). This difference was not statistically significant ($p = 0.452$), suggesting a relatively stable, non-region-specific spread of this pathogen, likely driven more by soil conditions than climatic factors. The overall detection rate for any of the three viruses was similar between agroclimatic zones: 53.3% in the forest-steppe and 48.9% in the steppe zone, with the difference not being statistically significant ($p = 0.458$). Thus, while overall infection rates with crop viruses are comparable, the distribution of individual viral pathogens clearly reflects regional specificity, which should be considered when planning monitoring systems and virus control measures for each zone. Summary data is presented in Table 1.

***Table 1**. Virus detection frequency in cereal samples by agroclimatic zones in Ukraine (RT-PCR)*

| Virus | Forest-steppe zone (n = 135) | Steppe zone (n = 135) | Total (n = 270) | p-value ($\chi^2$) |
|---|---|---|---|---|
| BYDV-PAV | 56 (41.5%) | 38 (28.1%) | 94 (34.8%) | 0.021 |
| WSMV | 17 (12.6%) | 35 (25.9%) | 52 (19.3%) | 0.005 |
| SBCMV | 19 (14.1%) | 15 (11.1%) | 34 (12.6%) | 0.452 |
| Overall infection | 72 (53.3%) | 66 (48.9%) | 138 (51.1%) | 0.458 |

***Source:** compiled by the authors*

Comparing the virus detection frequency between agroclimatic zones indicates statistically significant spatial variability in the spread of individual pathogens. The most noticeable differences were in the detection of BYDV-PAV, which was more frequently detected in samples from the forest-steppe zone (41.5%) compared to the steppe (28.1%), confirmed by a significant p-value of 0.021. Conversely, for WSMV, the frequency was higher in the steppe (25.9% vs 12.6%; $p = 0.005$), suggesting potential climatic influences on the dominance of certain viruses. While the spread of SBCMV and the overall infection rate did not differ significantly between zones ($p > 0.45$), the differences in BYDV-PAV and WSMV require further investigation into their ecological and agronomic determinants. BYDV-PAV demonstrated a lower prevalence in both Azerbaijani zones (18.7% in the forest-steppe, 15.8% in the steppe) compared to Ukraine, with a significant difference ($p < 0.001$). This is due to several factors: lower humidity, which negatively impacts the survival of aphids – the main vectors of the virus; differences in agronomic practices, such as less frequent early sowing or other practices influencing vector development; and region-specific crop varieties that may be more tolerant to infection.

For WSMV, a similar distribution was found: it predominated in both countries' steppe zones, but with a specific difference – its prevalence in Azerbaijan's forest-steppe zone was lower (4.3%) compared to Ukraine (12.6%). This points to the limited spread of the wheat mite (*Aceria tosichella*) in Azerbaijan's relevant regions or differences in climatic conditions, such as a shorter period of favourable temperatures. Meanwhile, SBCMV, although not demonstrating a statistically significant inter-country difference overall ($p = 0.083$), had the highest prevalence in Azerbaijan's forest-steppe region (20.4%) (Table 2). This correlates with the fact that soil conditions, soil structure, moisture, and vector activity (*Polymyxa graminis*) play a more significant role in the spread of this virus than climatic factors alone.

***Table 2**. Comparison of virus detection frequency in cereal crops in Ukraine and Azerbaijan by agroclimatic zones (RT-PCR)*

| Country | Zone | BYDV-PAV (%) | WSMV (%) | SBCMV (%) | Overall infection (%) | n |
|---|---|---|---|---|---|---|
| Ukraine | Forest-steppe | 41.5 a | 12.6 b | 14.1 | 53.3 a | 135 |
| | Steppe | 28.1 b | 25.9 a | 11.1 | 48.9 a | 135 |

*Table 2. Continued*

| Country | Zone | BYDV-PAV (%) | WSMV (%) | SBCMV (%) | Overall infection (%) | n |
|---|---|---|---|---|---|---|
| Azerbaijan | Forest-steppe (Jalilabad) | 18.7 c | 4.3 c | 20.4 a | 32.2 b | 135 |
| | Steppe (Beylagan/ Ganja) | 15.8 c | 19.6 a | 13.7 | 36.3 b | 135 |
| p-value ($\chi^2$) | | <0.001 | <0.001 | 0.083 | <0.001 | |

***Note:*** *values within a column marked with different letters (a, b, c) differ significantly by the $\chi^2$ test ($p < 0.05$). For example, for BYDV-PAV: Ukraine Forest-steppe (a) > Ukraine Steppe (b) > Azerbaijan. Overall infection = Percentage of samples infected with at least one of the three target viruses. p-value (Country x Virus) reflects the significance of the interaction between "Country" and "Virus" factors in influencing detection frequency*
***Source:*** *compiled by the authors*

The overall infection rate with any of the three target viruses was lower in Azerbaijan (≈32-36%) than in Ukraine (≈49-53%), suggesting lower epidemic pressure in the studied regions of Azerbaijan or more effective local plant protection systems, including soil treatment, crop rotation, variety composition, and potentially lower crop load. Comparative effectiveness of RT-PCR and LAMP. The comparative evaluation of molecular detection methods – RT-PCR and LAMP – showed a noticeable difference in sensitivity between these two approaches. RT-PCR, used as the reference method due to its high analytical sensitivity and specificity, demonstrated superiority over LAMP in detecting all three target viruses: BYDV-PAV, WSMV, and SBCMV. However, the LAMP method showed a high level of agreement with RT-PCR, confirming its potential as a rapid and effective tool for field or routine diagnostics. For BYDV-PAV, RT-PCR detected 94 positive samples, while LAMP detected 82 of them. Thus, LAMP's sensitivity in this case was 87.2%. The overall agreement between the two methods was 91.1%, indicating a high concordance between the results. Cohen's kappa coefficient (κ), which accounts for random agreement, was 0.82, corresponding to "almost perfect agreement" on the Landis & Koch scale, indicating a high degree of correspondence between the methods for this virus.

A similar trend was observed for WSMV. RT-PCR detected 52 positive samples, while LAMP detected 43. The calculated sensitivity of LAMP was 82.7%, meaning approximately 5 out of 6 samples detected by the reference method were confirmed by isothermal amplification. Again, the overall agreement was high – 93.7%, indicating stable reproducibility between the two approaches. The κ value of 0.85 indicates very high agreement, further emphasising the practical value of the LAMP method. For SBCMV, which had a lower prevalence in the sample, a similar trend was observed. RT-PCR detected 34 positive samples, LAMP detected 28. This corresponds to a sensitivity of 82.4%, comparable to the other two viruses. The overall agreement between LAMP and RT-PCR was 96.3%, the highest among the three viruses, possibly due to fewer false-negative results or better primer specificity in the corresponding LAMP kit. The κ value of 0.86 confirms high reliability of the results and almost perfect agreement between the methods. For all three viruses, no cases were found where LAMP produced a positive result while RT-PCR was negative (i.e., no RT-PCR$^-$/LAMP$^+$ cases). This indicates very high specificity of the LAMP method, virtually eliminating false-positive results. Therefore, while LAMP is less sensitive than RT-PCR, it provides reliable results, especially considering the speed of the assay, lower laboratory equipment requirements, and suitability for field conditions. Summary data comparing the two methods are presented in Table 3, providing a quick overview of the effectiveness of both methods in field virus diagnostics in cereals.

***Table 3****. Comparison of virus detection results by RT-PCR and LAMP methods (Ukraine samples)*

| Virus | RT-PCR$^+$/ LAMP$^+$ | RT-PCR$^+$/ LAMP$^-$ | RT-PCR$^-$/ LAMP$^+$ | RT-PCR$^-$/ LAMP$^-$ | LAMP Sensitivity (%) | % Agreement | Kappa (κ) |
|---|---|---|---|---|---|---|---|
| BYDV-PAV | 82 | 12 | 0 | 176 | 87.2 | 91.1 | 0.82 |
| WSMV | 43 | 9 | 0 | 218 | 82.7 | 93.7 | 0.85 |
| SBCMV | 28 | 6 | 0 | 236 | 82.4 | 96.3 | 0.86 |

***Note:*** *no RT-PCR$^-$/LAMP$^+$ cases were observed for any virus*
***Source:*** *compiled by the authors*

The results show very high concordance between RT-PCR and LAMP methods in detecting the three viruses: BYDV-PAV, WSMV, and SBCMV. In all cases, the agreement level exceeded 91%, with Cohen's kappa ranging from 0.82 to 0.86, corresponding to "high" to "almost perfect" agreement on the Landis & Koch scale. The absence of false-positive LAMP results (RT-PCR⁻/LAMP⁺ = 0) for all viruses highlights the specificity of the method, with sensitivity exceeding 82%, demonstrating its effectiveness in field or resource-limited conditions. Comparative evaluation of the effectiveness of molecular virus detection methods in Ukrainian and Azerbaijani samples revealed both similar trends and some interesting differences. For Ukrainian samples, the LAMP method demonstrated a sensitivity of 82.4-87.2% compared to RT-PCR, while for Azerbaijani samples, this figure was slightly lower – 77.8-83.3%. The largest sensitivity difference was observed for WSMV: 82.7% in Ukraine versus 77.8% in Azerbaijan (Table 4).

***Table 4**. Comparative effectiveness of RT-PCR and LAMP for virus detection in Azerbaijani samples*

| Virus | RT-PCR$^+$/ LAMP$^+$ | RT-PCR$^+$/ LAMP$^-$ | RT-PCR$^-$/ LAMP$^+$ | RT-PCR$^-$/ LAMP$^-$ | LAMP Sensitivity (%) | % Agreement | Kappa (κ) |
|---|---|---|---|---|---|---|---|
| BYDV-PAV | 25 | 5 | 0 | 240 | 83.3 | 94.1 | 0.84 |
| WSMV | 21 | 6 | 0 | 243 | 77.8 | 95.6 | 0.82 |
| SBCMV | 34 | 8 | 0 | 228 | 81 | 93.3 | 0.83 |

***Source:** compiled by the authors*

It is worth noting that in all cases, LAMP showed high specificity – no false-positive results were recorded (RT-PCR$^-$/LAMP$^+$). This confirms the reliability of the method regardless of the geographic origin of the samples. The agreement level between the methods remained high in both countries: 93.3-96.3% for Ukraine and 93.3-95.6% for Azerbaijan. Cohen's kappa, which measures the degree of agreement between methods, ranged from 0.82 to 0.86, corresponding to the "almost perfect agreement" category on the Landis & Koch scale. Analysing the results for individual viruses, it is notable that the highest sensitivity of LAMP in both countries was observed for BYDV-PAV. For SBCMV, the highest percentage of agreement was recorded – 96.3% in Ukraine and 93.3% in Azerbaijan. This suggests particularly successful primer selection for this virus or more stable RNA expression in plant tissues. The slightly lower LAMP performance in Azerbaijani samples could be due to several factors: differences in the quality and quantity of viral material in the samples, the impact of climatic conditions on the stability of viral particles during transportation, or sample preparation specifics. However, this difference is not critical and does not significantly affect the practical value of the method.

The results confirm the potential use of standardised LAMP protocols for cross-country monitoring of cereal crop viruses. Despite a slight difference in sensitivity, the LAMP method remains a reliable, rapid, and cost-effective tool for field diagnostics, especially under resource-limited conditions. The high specificity of the method minimises the risk of false-positive results, which is critical for making timely phytosanitary decisions. Coinfections. Molecular diagnostic results revealed differences in the nature of coinfections between the two countries. In Ukraine, among the 138 infected samples, coinfections were detected in 26.1% of cases (36/138), while in Azerbaijan, this figure was slightly lower at 18.9% (26/138). In both countries, mono-infections predominated (73.9% in Ukraine and 81.1% in Azerbaijan), indicating the dominance of individual viral pathogens in the agroecosystems.

The most common combination in Ukraine was BYDV-PAV and WSMV (18 cases), while in Azerbaijan, the most frequent combination was BYDV-PAV and SBCMV (9 cases). This difference could be related to the varying distribution of vectors between the two countries. Triple-virus coinfections were rare in both regions: 2.2% in Ukraine (3/138) and 1.4% in Azerbaijan (2/138), yet their detection is important from an epidemiological and epidemiological perspective, as such combinations can lead to enhanced pathogenicity, complicated symptomatology, and decreased effectiveness of phytosanitary measures. The detection of such coinfections also highlights the complexity of the viral spectrum in agrocenoses and the need for expanded monitoring using multiplex or metagenomic approaches. Virus detection in asymptomatic samples. The ability of diagnostic methods to detect viral infection at early, asymptomatic stages is important for ensuring timely responses and preventing the spread of pathogens in agricultural ecosystems (Havryliuk *et al*., 2024; Serikbaeva *et al*., 2021). The study confirmed that both RT-PCR and LAMP methods can identify viral infection in plant samples that show no external symptoms of damage. This indicates the high analytical sensitivity of both methods and underscores their suitability for early diagnosis in field conditions, which is particularly important for monitoring latent infections.

According to the data obtained, viral pathogens were detected in 23.7% of cases by RT-PCR (32 positive samples out of 135 asymptomatic samples). This number also includes samples taken through "blind" sampling, which, as later revealed, showed no visual signs of infection at the time of collection. Thus, almost a quarter of all asymptomatic plants actually had latent viral infections, which could have gone unnoticed without molecular diagnostics. The most frequently detected pathogen in asymptomatic plants was the BYDV-PAV, present in 15.6% of the samples (21 out of 135). This result indicates the widespread distribution of BYDV-PAV in the population, even in the absence of phenotypic manifestations, which is typical for many flaviviruses that can have a latent course of infection at early stages or under low inoculum pressure. By comparison, the frequency of viral pathogens in samples with clearly expressed disease symptoms was 61.5% (83 positive samples out of 135), which is statistically significantly higher ($\chi^2$, $p < 0.001$) (Table 5). The difference observed between symptomatic and asymptomatic samples indicates a close relationship between viral replication, the accumulation of viral load, and the manifestation of symptoms, although it does not exclude the possibility of effective detection of infection even during its clinically silent course.

The study revealed significant differences in the detection of asymptomatic infections between the countries. In Ukraine, RT-PCR detected viruses in 23.7% of asymptomatic samples (32/135), while in Azerbaijan this figure was 18.5% (25/135). The most common asymptomatic pathogen in both countries was BYDV-PAV: 15.6% of detections in Ukraine (21/135) versus 12.6% in Azerbaijan (17/135). Interestingly, the proportion of virus detection in symptomatic samples was significantly higher in Ukraine (61.5%) compared to Azerbaijan (53.3%). This difference was statistically significant ($p < 0.05$) and may indicate a more aggressive nature of viral infections in Ukrainian agroecosystems or better adaptation of local varieties to viral pressure in Azerbaijan.

***Table 5**. Comparative data on virus detection in symptomatic and asymptomatic samples (RT-PCR)*

| Parameter | Ukraine | Azerbaijan | p-value ($\chi^2$) |
|---|---|---|---|
| **Symptomatic samples (n = 135)** | | | |
| BYDV-PAV (+) | 58 (43%) | 48 (35.6%) | 0.042 |
| WSMV (+) | 41 (30.4%) | 32 (23.7%) | 0.038 |
| SBCMV (+) | 28 (20.7%) | 34 (25.2%) | 0.152 |
| Overall infected | 83 (61.5%) | 72 (53.3%) | 0.028 |
| **Asymptomatic samples (n = 135)** | | | |
| BYDV-PAV (+) | 21 (15.6%) | 17 (12.6%) | 0.251 |
| WSMV (+) | 7 (5.2%) | 5 (3.7%) | 0.382 |
| SBCMV (+) | 4 (3%) | 3 (2.2%) | 0.502 |
| Overall infected | 32 (23.7%) | 25 (18.5%) | 0.041 |

***Source:** compiled by the authors*

Comparative analysis of virus detection in symptomatic and asymptomatic samples indicates higher infection rates in symptomatic plants. All differences were statistically significant ($p < 0.001$), pointing to a close relationship between the presence of symptoms and the likelihood of viral infection. The study revealed a high level of infection in cereal crops with target viruses, with BYDV-PAV dominating among the main pathogens. Regional analysis revealed statistically significant spatial differences in virus prevalence: BYDV-PAV was significantly more prevalent in the forest-steppe zone, while WSMV was more widespread in the steppe. Comparative evaluation of methods confirmed the higher analytical sensitivity of RT-PCR, while the LAMP method demonstrated high specificity and substantial agreement with the reference method, justifying its use for rapid diagnostics. A significant aspect was the detection of a high frequency of coinfections (especially combinations of BYDV-PAV and WSMV) and the presence of latent infections – viruses were detected in a notable proportion of asymptomatic plants, underscoring the capability of molecular methods for early detection. All key patterns were confirmed by statistical significance.

## DISCUSSION

The results provide valuable insights into the epidemiology of key cereal crop viruses in Ukraine and the effectiveness of molecular diagnostic methods. The high overall infection rate (51.1%) with BYDV-PAV (34.8%) confirms its status as one of the most economically significant pathogens of cereals in the region. This finding aligns with global data pointing to BYDV as a major cause of cereal disease, resulting in substantial yield losses due to impaired photosynthesis, dwarfing, and infertility of ears, as studied by L.T. Mishchenko *et al.* (2022). They studied wheat dwarf virus in Ukraine, confirming that viral infections are a serious threat to the country's grain industry. The authors also recorded the widespread occurrence of wheat dwarf virus and its impact on yield, highlighting the complex nature of viral threats, where multiple pathogens may simultaneously circulate in agrocenoses.

The regional specificity of pathogen spread is a key result. The predominance of BYDV-PAV in the forest-steppe zone (41.5% vs 28.1% in the steppe) is a direct consequence of more favourable conditions for its main vectors – aphids (e.g., Rhopalosiphum padi, Sitobion avenae), whose populations thrive in moderate temperatures and higher humidity typical of the forest-steppe, as also highlighted in the study by W.A. Miller and Z. Lozier (2022). Conversely, the significantly higher prevalence of WSMV in the steppe zone (25.9% vs 12.6%) strongly correlates with the range and ecology of its vector – the wheat mite (*Aceria* *tosichella*), which is better adapted to the dry and warm conditions of the steppe, consistent with the results of S. Singhal *et al.* (2021). These findings underscore the critical role of climatic factors and vector dynamics in shaping regional viral landscapes. They align with the data of M. Sõmera *et al.* (2021), who used high-throughput sequencing to show that the diversity and distribution of yellow dwarf viruses (including BYDV) in Europe is often underestimated, with a clear geographical component. Similar regional specificity in cereal virus spread influenced by climate has been described in Kazakhstan by K. Zhambakin and K. Zhapar (2020).

The comparative effectiveness of RT-PCR and LAMP confirmed the expected advantages and limitations of each approach. The higher sensitivity of RT-PCR (detecting an additional 12, 9, and 6 positive samples for BYDV-PAV, WSMV, and SBCMV, respectively) aligns with numerous studies that recognise it as the "gold standard" for plant virus molecular diagnostics due to its high specificity and sensitivity, particularly with the data from J.D. Ibaba and A. Gubba (2020) and J. Sharma *et al.* (2024). However, the results also clearly demonstrate the significant potential of LAMP as an express method for field conditions. The high specificity of LAMP (no false-positive results) and good agreement with RT-PCR ($\kappa = 0.82$-$0.86$, "almost perfect agreement") make it a valuable tool for rapid screening. This is important for early detection of infection hotspots and timely phytosanitary decisions without the need to transport samples to equipped laboratories, which is also discussed in the work of R. Bhat *et al.* (2022). The sensitivity of LAMP (82.4-87.2%) is within the range reported by other researchers for various plant viruses. However, some LAMP modifications, integrated with CRISPR systems (e.g., Cas-PfLAMP), demonstrate potentially higher sensitivity, approaching that of RT-PCR, as noted in the study by Z. Zhu *et al.* (2022), suggesting further development potential.

The significant frequency of coinfections (27% of infected samples), particularly the combination of BYDV-PAV + WSMV, is a serious concern. Polyetiological infections can lead to synergistic effects, worsening symptom severity, accelerating plant death, and increasing yield losses compared to mono-infections (Movsumov *et al.*, 2018; Lyubchyk *et al.*, 2019), which aligns with the findings of U.C. Jha *et al.* (2023). The presence of multiple viruses in a plant complicates diagnosis as symptoms may overlap or mask each other, and also presents challenges for resistance breeding, as the plant must simultaneously cope with several pathogens. Detection of viruses in asymptomatic samples (23.7% by RT-PCR) is a critically important practical result. It confirms the ability of molecular methods, especially highly sensitive ones like RT-PCR, to detect latent or early-stage infections before visible symptoms appear, as confirmed by S. Zhang and A. Vrient (2020). These plants can be an invisible source of inoculum, contributing to local spread through vectors or contaminated seeds, as well as inter-regional/international spread through trade in planting material.

The results regarding the high prevalence of cereal viruses are supported by the work of many researchers, but with significant differences in focus. The study by G. Zahmanova *et al.* (2023) focuses on the potential usefulness of plant viruses for molecular farming, whereas the current study examines them as pathogens. However, both studies agree on the need for a deep understanding of viruses for effective management. In comparison to the work of P. Abrahamian *et al.* (2020) on viral vectors in biotechnology, the results obtained in this study confirm the importance of accurate virus detection methods, especially given their potential as vectors. However, this study focuses on the pathogenic properties, while P. Abrahamian *et al.* emphasise their biotechnological applications. Regarding diagnostic methods, the current conclusions on the effectiveness of RT-PCR and LAMP fully align with the data of S.K. Sharma *et al.* (2021) on the CRISPR-Cas revolution in diagnostics. However, unlike their emphasis on the latest CRISPR technologies, this work demonstrates the practical effectiveness of more traditional but accessible methods.

The work of K. Kalimuthu *et al.* (2022) on point-of-care diagnostics confirms the findings regarding the promise of LAMP for field use. Particularly important is the conclusion by the authors about the need for simple methods in resource-limited regions, which fully supports stance. In the context of early diagnostics, the current results on virus detection in asymptomatic samples are supported by the work of N. Deepa *et al.* (2021), who emphasise the importance of molecular methods for detecting pathogens before symptoms appear. However, their focus on fungal pathogens differs from the current focus on viruses. Innovative detection approaches, such as RT-RPA-CRISPR/Cas12a, described by R. Aman *et al.* (2020), demonstrate potentially higher sensitivity compared to the methods studied. This points to potential areas for further improvement of the diagnostic platform in future research. Reviews of modern detection methods by A. Cassedy *et al.* (2021) confirm RT-PCR as the gold standard but also point to the prospects of new approaches, which could be a subject for future studies. G. Xing *et al.* (2022) in their work on microfluidic biosensors propose promising alternatives to the studied methods, especially for field use. However, at present, these technologies remain less accessible for widespread use in Ukraine. The established patterns and methodological approaches serve as the scientific foundation for developing regionally adapted monitoring strategies based on molecular diagnostics, viral disease management, and the selection of resistant varieties.

## CONCLUSIONS

The study revealed a significant prevalence of viral infections among cereal crops in both Ukraine and

Azerbaijan, with a similar overall infection rate (around 51%). However, some differences were observed in the viral infection structure between the countries. In Ukraine, BYDV-PAV was the dominant pathogen (34.8%), whereas in Azerbaijan, its prevalence was lower (≈17%), which may be related to less favourable conditions for aphid populations and possible differences in agricultural practices. At the same time, Azerbaijan showed a higher infection level of SBCMV (up to 20.4% in the forest-steppe zone) compared to Ukraine (12.6%), which may indicate a more active spread of the soil vector *Polymyxa graminis* under local soil conditions.

Regional analysis in both countries confirmed the impact of climatic conditions on virus spread. In Ukraine, BYDV-PAV predominated in the forest-steppe, and WSMV in the steppe, while in Azerbaijan, a similar but less pronounced trend was observed. This is explained by the more arid conditions in Azerbaijan's climatic zones (especially the steppe areas of Beylagan and Ganja), which affect vector activity. The LAMP method showed slightly lower sensitivity in Azerbaijan samples (77.8-83.3%) compared to Ukraine (82.4-87.2%), which may be related to differences in sample quality or the stability of viral RNA during transportation. However, the high specificity of the method (100%) confirms its suitability for diagnostics in both countries.

An important practical outcome is the detection of a high frequency of coinfections (up to 26.1% in Ukraine and 18.9% in Azerbaijan among infected samples) and significant asymptomatic viral carriage (23.7% and 18.5% of asymptomatic plants, respectively), indicating the need for enhanced monitoring of polyetiological damage and early diagnostics. In this context, the LAMP method confirmed its effectiveness for detecting latent infections and coinfections in field conditions: high specificity (100%), sensitivity >82%, and rapid analysis make it a key tool for implementation in phytosanitary control systems, especially in regions with limited laboratory equipment.

Furthermore, potential genetic variations in the viruses, which may influence detection efficacy, were not considered. Future research perspectives include: expanded analysis of agronomic factors affecting virus spread in different climatic zones; studying the genetic diversity of viruses to identify potential new strains; developing optimised LAMP protocols to improve sensitivity under different regional conditions; and studying the impact of climate change on vector dynamics and virus spread in the long term. Thus, the results highlight the need for regionally adapted approaches to monitoring and controlling viral infections in cereal crops, taking into account the climatic, soil, and agronomic characteristics of each country.

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## CONFLICT OF INTEREST

None.

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## Біотехнологічні аспекти молекулярної діагностики вірусів зернових культур

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**Анотація**. Щорічне зниження врожайності зернових культур у багатьох регіонах світу через такі патогени, як вірус жовтої карликовості ячменю (BYDV), вірус смугастої мозаїки пшениці (WSMV) та вірус мозаїки зернових культур, що передається через ґрунт (SBCMV), підкреслює важливість вивчення їх поширення та контролю. Метою цього дослідження було виявлення закономірностей поширення вірусних інфекцій, що вражають зернові культури в Україні та Азербайджані. У дослідженні використовували зворотну транскрипцію з подальшою полімеразною ланцюговою реакцією (RT-PCR) та ізотермічну ампліфікацію за допомогою петлі (LAMP) для виявлення вірусів у зразках зернових культур. Результати показали високий рівень інфікування в обох країнах (51,1 % в Україні та приблизно 51 % в Азербайджані), причому домінуючим вірусом був BYDV. Були виявлені регіональні відмінності: в лісостеповій зоні України найпоширенішим був BYDV (41,5 %), тоді як у степовій зоні частіше виявлявся WSMV (25,9 %). В Азербайджані поширеність BYDV була нижчою (≤18,7 %), але частота SBCMV була вищою (до 20,4 %). Коінфекції були виявлені в 26,1 % інфікованих зразків в Україні та 18,9 % в Азербайджані, при цьому 23,7 % безсимптомних рослин в Україні та 18,5 % в Азербайджані дали позитивний результат на вірусний матеріал. Метод LAMP виявився ефективним для швидкої діагностики в польових умовах, продемонструвавши 100 % специфічність і понад 82 % чутливість. Отримані результати підкреслили необхідність розробки регіонально адаптованих стратегій боротьби з вірусними захворюваннями зернових культур. Практична цінність цієї роботи полягає в розробці рекомендацій щодо вдосконалення систем моніторингу та захисту рослин з урахуванням місцевих умов, що сприятиме підвищенню врожайності та продовольчої безпеки в регіоні

**Ключові слова:** полімеразна ланцюгова реакція; зворотна транскрипція; петльова ізотермічна ампліфікація; вірус мозаїки зернових культур, що передається через ґрунт; вірус жовтої карликовості ячменю; вірус смугастої мозаїки пшениці; орні землі; сільське господарство

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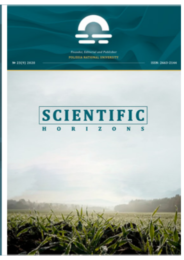


# Hazardous environmental factors and their impact on plant vital activity and biosecurity

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**Abstract**. The aim of this study was to analyse the impact of hazardous environmental factors on plant viability and biosecurity, as well as to develop strategies for mitigating their toxic effects. Experiments were conducted using agricultural crops, including rapeseed (*Brassica napus*), rice (*Oryza sativa*), common wheat (*Triticum aestivum*), potato (*Solanum tuberosum*), and tomato (*Solanum lycopersicum*). The effects of heavy metals (cadmium, lead, mercury), ionising radiation, climatic extremes (high temperature, drought), and biotic pathogens (*Phytophthora infestans*, tobacco mosaic virus) were investigated under laboratory conditions. Quantification of metal concentrations, photosynthetic activity, stress gene expression, and pathogen prediction were performed using spectrometric, fluorometric, molecular-genetic, and machine-learning

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approaches. The results demonstrated that cadmium accumulated in rice grains at concentrations of up to 2.8 mg/kg, exceeding the maximum permissible levels established by the Food and Agriculture Organization of the United Nations and the World Health Organization by 2.3-fold ($p < 0.001$). The combined exposure to cadmium and elevated temperature (+35°C) resulted in a synergistic reduction in plant biomass by 75% compared to individual stress factors ($p < 0.01$). Under radiation exposure, the activity of antioxidant enzymes, such as catalase and superoxide dismutase, increased by 150-220%; however, prolonged stress led to a 275% accumulation of malondialdehyde, a marker of oxidative damage. A machine-learning predictive model achieved 89% accuracy in identifying risk zones for the fungal pathogen *Phytophthora infestans*, with an area under the curve of 0.93. The practical significance of these findings lies in their applicability for environmental monitoring, the development of adaptive agro-technologies, the breeding of stress-resistant cultivars, and the implementation of biosecurity measures in agriculture

**Keywords:** cadmium; oxidative stress; phytotoxicity; heavy metal bioaccumulation; environment; ecosystem

## INTRODUCTION

Under contemporary anthropogenic environmental pressures, the issue of plant biosecurity has gained particular relevance. Heavy metals, ionising radiation, climatic extremes, and biotic pathogens have emerged as key risk factors for agricultural crops, reducing their productivity and facilitating the accumulation of toxic substances in the food chain. Elevated contamination of soils with cadmium, lead, and mercury disrupts plant physiological processes, inducing oxidative stress and genetic mutations, which in turn affect agroecosystems and food safety. Concurrently, global climate change exacerbates the adverse effects of these factors, creating additional risks for crop production and the sustainable development of the agricultural sector. Modern research in biosecurity underscores the necessity of an integrated approach to monitoring and mitigating the impact of hazardous environmental factors on plant organisms. The combination of molecular biology, machine learning, and biotechnological methods enables the development of effective risk-reduction strategies, particularly through the use of genetically modified indicator plants and pathogen spread prediction (Zavertaliuk & Naumovska, 2024). At the same time, the legal and ethical regulation of biosecurity measures remains critical, necessitating in-depth analysis of existing scientific approaches and strategies.

K.M. Kyshko and M.M. Vakerych (2023) explored the interrelation between bioethics, biosafety, and biosecurity, emphasising the need for normative frameworks to protect plants from ecological threats. Their work highlighted the importance of integrating ecological, medical, and agricultural aspects into bioprotection systems to ensure comprehensive, interdisciplinary solutions to biosecurity challenges. The study by M. Kravchuk and I. Metelskyi (2022) focused on the identification and mitigation of biological threats, particularly the concept of biorisks. It stressed the imperative of implementing preventive measures and monitoring potential hazards that could affect the biological security of plant ecosystems. O. Shamsutdinov (2023) analysed socio-legal aspects of biological security, examining conditions conducive to criminal offences in this domain. His work underscored the necessity of strengthening legislative control mechanisms to restrict the spread of harmful agents in agriculture, which is crucial for preventing ecological threats. The study by M. Wondafrash *et al.* (2021) evaluated the role of botanical gardens as both resources and potential biosecurity hazards. The authors noted the risk of uncontrolled dissemination of invasive species and pathogens via botanical gardens, calling for stricter regulatory measures.

The study by L. Symochko *et al.* (2021) focused on the ecological aspects of biosecurity in agroecosystems. It examined the relationship between anthropogenic influence and the level of biological threats to plant life, emphasising the need for an integrated approach to biosecurity monitoring. E. Butucel *et al.* (2022) analysed bioprotective measures on farms, which contributed to reducing the risk of bacterial biofilm dissemination. C. Kantor *et al.* (2024) focused on biological risks associated with nematodes, which may negatively impact food security. An innovative approach to enhancing plant resilience to stress factors was presented in the work of O.R. Devi *et al.* (2023). They explored the potential of using nanofertilisers to improve plant biosecurity and enhance their adaptive capacity. It was established that nanomaterials facilitate better nutrient absorption, which may mitigate the adverse effects of unfavourable environmental conditions. A distinct research direction, concerning the impact of genetically modified (GM) plants on biosecurity, was examined in the study by B.K. Ghimire *et al.* (2023). The authors analysed the risks and benefits of GM crops in the context of their influence on ecosystems and food security. Notably, such plants exhibit increased resistance to abiotic and biotic stress factors – particularly toxic metals and pathogens, which were investigated in this study. This underscores the potential of GM crops as an element of strategies to reduce biological risks in agriculture, while simultaneously highlighting the necessity of ecological monitoring of their impact.

Thus, prior research has confirmed the importance of an integrated approach to ensuring biosecurity, incorporating molecular monitoring methods, biotechnological applications, and the development of adaptive strategies for plants. However, the impact of combined stress factors on the viability of cultivated plants remains insufficiently studied, complicating the development of effective protection methods. The aim of the study was to assess the influence of chemical, physical, climatic, and biotic factors on plant biosecurity and to develop effective approaches to mitigating their adverse effects.

## MATERIALS AND METHODS

The study was conducted at the laboratories of the Institute of Plant Biology, National Academy of Sciences of Ukraine (Kyiv), between March 2024 and February 2025. The research subjects included agricultural crops: *Brassica napus* (rapeseed), *Vicia faba* (broad bean), *Triticum aestivum* (common wheat), *Oryza sativa* (rice), *Solanum tuberosum* (potato), *Solanum lycopersicum* (tomato), and a transgenic *Arabidopsis thaliana* line with the GFP gene. The selection of crops was based on their agricultural significance in Ukraine and globally, differences in physio-biochemical responses to stress factors, and the availability of established protocols for resilience studies. *Arabidopsis thaliana* was chosen as a model system for molecular genetic tests and fluorescence-based toxicity monitoring. To simulate stress conditions, the following chemicals were used: cadmium nitrate ($Cd(NO_3)_2$, Sigma-Aldrich, USA), lead acetate ($Pb(CH_3COO)_2$, Merck, Germany) and mercury chloride ($HgCl_2$, Fluka, Switzerland). The pathogens *Phytophthora infestans* and tobacco mosaic virus (TMV) were obtained from the microorganism collection of the Institute of Phytopathology.

Plants were cultivated under controlled conditions in climate chambers (Binder KBW 240, Germany) at a temperature of +22 ± 2°C, a photoperiod of 16/8 h, and 60-70% humidity. To study the effects of heavy metals, Cd (50 mg/kg), Pb (20 mg/kg), and Hg (0.5 mg/kg) were introduced into the soil. Metal concentrations in tissues were determined via ICP-MS (Agilent 7900, USA) after sample mineralisation in an $HNO_3/H_2O_2$ mixture (3:1). The obtained values were compared with the maximum permissible levels established by the World Health Organization (2011) and Codex Alimentarius (n.d.). Photosynthetic activity (Fv/Fm) was assessed using a portable fluorimeter (OS-30p, Opti-Sciences, USA). To investigate radiation stress, *Vicia faba* seeds were irradiated with gamma rays (doses of 10-20 Gy) using a $^{60}Co$ source ("Gammatron" apparatus, Ukraine). Chromosomal aberrations were analysed in root meristem cells after aceto-carmine staining (Leica DM2500 microscope, Germany). The activity of antioxidant enzymes (CAT, SOD) was measured spectrophotometrically (Shimadzu UV-1800, Japan) using BioVision kits (USA).

Thermal stress was modelled by exposing *Triticum aestivum* plants to a temperature of +40°C for 72 hours. To study drought stress, irrigation was reduced to 30% of the standard rate, and leaf turgor was assessed using a pressure chamber (PMS Instrument, USA). The expression of *HSP70*, *DREB2A*, and *NCED3* genes was analysed via quantitative real-time polymerase chain reaction (qRT-PCR) (Qiagen Rotor-Gene Q, Netherlands) with primers designed in Primer-BLAST. Additionally, the expression of *PIN1, PAO, HSF,* and *PIP2;*1 genes – associated with hormonal balance regulation, stress response, and water transport – was investigated. A minimum of three biological replicates were performed for each gene. For pathogen studies, potato leaves were inoculated with *Phytophthora infestans* spores ($10^5$ spores/ml), while tomatoes were infected with TMV (ELISA test, Agdia, USA). The efficacy of a *Bacillus subtilis*-based biofungicide (strain BS-12, $10^8$ CFU/ml) was evaluated by the reduction in leaf damage index (%) compared to untreated control plants, accounting for standard deviation and significance ($p < 0.05$). To analyse synergistic stress, *Oryza sativa* was cultivated at +35°C with Cd supplementation (5 mg/kg). Grain Zn and Cd content was determined via atomic absorption spectroscopy (PerkinElmer PinAAcle 900T, USA).

For real-time heavy metal detection, GFP-transgenic *Arabidopsis thaliana* plants were used, with fluorescence levels correlating to $Cd^{2+}$ concentration in the root zone ($R^2 > 0.9$). A predictive model for *Phytophthora infestans* spread was developed using the Random Forest algorithm (Python, scikit-learn library), trained on 15,000 data samples (soil, climate, historical outbreaks). Model efficacy was assessed via ROC analysis, calculating area under the curve (AUC), accuracy, recall, and specificity. The final AUC value was 0.93. Plant samples were selected based on uniformity: 21-day-old plants at the 3-4 true-leaf stage. Each experimental variant included 20 plants to ensure sufficient statistical power ($n = 20$). Sampling was randomised from developmentally synchronised plants grown in climate chambers. For metal content analysis, aerial and subterranean organs (root depth ⩽10 cm) were collected, thoroughly rinsed, blotted dry, and weighed. All samples were taken in the morning (9:00-11:00) to minimise diurnal metabolic fluctuations. Plants showing disease or mechanical damage were excluded. Data were processed in GraphPad Prism 9.0. Group comparisons used Student's t-test (normal distribution) or ANOVA with Tukey's correction (multiple comparisons). Pearson's coefficient ($R^2$) was calculated for correlation analysis. Significance threshold: $p < 0.05$. Errors are reported as ±SD ($n \geq 5$). Analytical equipment: ICP-MS (Agilent 7900), AAS (PerkinElmer PinAAcle 900T). Microscopy: Leica DM2500, Leica TCS SP8. Climate chambers: Binder KBW 240. The study complied with DSTU ISO 14001:2015 (2016). All experiments were performed in triplicate biological replicates.

## RESULTS

**The impact of chemical contaminants on plant physiology.** Cadmium (Cd) exhibits high mobility in soil and is actively absorbed by the root system, disrupting the homeostasis of essential ions ($Zn^{2+}$, $Fe^{2+}$) through competition with transporter proteins. This adversely affects the functioning of metal-dependent enzymes responsible for respiration and photosynthesis. The accumulation of Cd in *Brassica napus* roots (Table 1) is attributed to ion sequestration mechanisms, particularly binding with thiol groups of phytochelatins and deposition in vacuoles. Such compartmentalisation limits metal translocation to aerial organs but poses a risk of secondary contamination through rhizodeposition fluxes. Despite reduced Cd transport to leaves and fruits, its concentration in the latter exceeds international safety standards, which is associated with the low selectivity of ABC transporters responsible for xenobiotic homeostasis.

***Table 1**. Heavy metal concentrations in Brassica napus organs under soil Cd contamination (50 mg/kg)*

| Organ | Cd (mg/kg) | Pb (mg/kg) | Hg (mg/kg) |
|---|---|---|---|
| Roots | 38.7 ± 2.1 | 4.2 ± 0.3 | 0.3 ± 0.02 |
| Leaves | 12.4 ± 1.3 | 2.1 ± 0.2 | 0.1 ± 0.01 |
| Fruits | 4.8 ± 0.6 | 0.9 ± 0.1 | ND |

***Source:** compiled by the authors*

Cd accumulation in roots is driven by compartmentalisation mechanisms that restrict its translocation to aerial organs, while excess levels in fruits surpass safety thresholds – particularly critical for food crops such as rice, where cadmium may accumulate in grains. Root growth inhibition (Fig. 1) correlates with the suppression of auxin-dependent processes, particularly the expression of PIN (PINOID) genes regulating polar hormone transport. Cadmium disrupts the $Ca^{2+}$-dependent signalling cascade required for proton pump activation, leading to reduced apoplast acidification and impaired cell elongation.

Correlation analysis revealed a direct relationship between soil Cd concentration and root growth suppression. At 50 mg/kg Cd, root length decreased by 32% ($p < 0.001$), indicating a critical impact of the metal on early morphogenesis stages. Photosynthetic activity serves as an indicator of Cd toxicity, particularly under low soil pH conditions. $Cd^{2+}$ ions compete with $Mn^{2+}$ for binding sites in the oxygen-evolving complex (OEC) of photosystem II, disrupting water photolysis. This is accompanied by reduced electron transport rate (ETR) and accumulation of singlet oxygen ($^1O_2$), inducing photooxidative stress (Table 2).

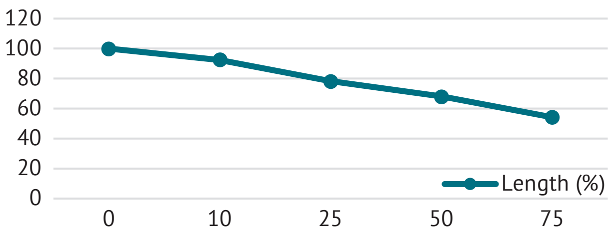


***Figure 1**. Correlation between soil Cd content and root growth inhibition in Brassica napus*

***Note:** control group - plants grown in Cd-free soil; p-values calculated using Student's t-test compared to control*

***Source:** compiled by the authors*

***Table 2**. Effect of soil pH on photosystem II activity (Fv/Fm)*

| Soil pH | Fv/Fм (control) | Fv/Fм (+50 mg/kg Cd) | Activity reduction (%) |
|---|---|---|---|
| 4.5 | 0.82 ± 0.02 | 0.49 ± 0.03 | 40.2 ± 1.5 |
| 5.5 | 0.81 ± 0.01 | 0.65 ± 0.02 | 19.8 ± 0.9 |

***Source:** compiled by the authors*

Chlorophyll degradation (Fig. 2) results from the inhibition of magnesium chelatase, which catalyses $Mg^{2+}$ incorporation into protoporphyrin IX. By displacing $Mg^{2+}$ in the enzyme's active site, Cd disrupts chlorophyll biosynthesis, leading to leaf chlorosis. Concurrently, Cd induces the expression of pheophorbide an oxygenase (PAO) gene, triggering chlorophyll catabolism and reducing the plant's photosynthetic potential.

Cadmium-induced oxidative stress activates plant antioxidant defence systems, including superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD). However, prolonged Cd exposure leads to the depletion of antioxidant reserves, resulting in lipid peroxidation and membrane structure degradation. Protective mechanisms in plants involve enhanced production of glutathione (GSH) and phytochelatins, which form stable complexes with Cd, thereby reducing its toxic effects (Shuvar *et al.*, 2022). Additionally, cadmium disrupts redox homeostasis by inhibiting glutathione reductase (GR) activity and increasing malondialdehyde (MDA) levels, indicating intensified lipid peroxidation. This is accompanied by the degradation of membrane proteins and DNA damage, impairing normal cell division and regeneration. Thus, the impact of cadmium on plants is multifaceted: it disrupts essential ion balance, inhibits key enzymatic processes, and induces oxidative stress. This justifies the need for further research into developing effective phytoremediation methods and breeding resilient cultivars for contaminated areas.

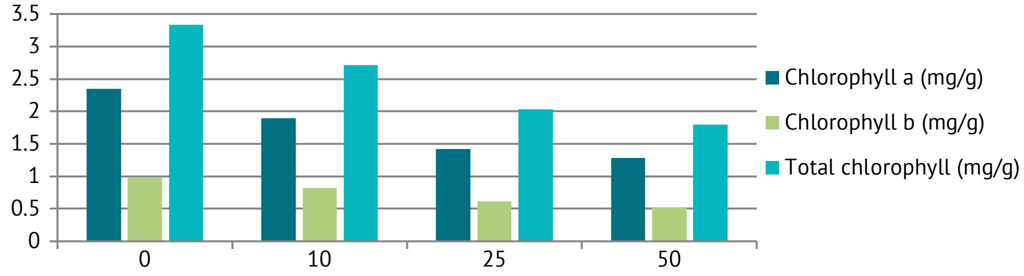


***Figure 2***. *Changes in chlorophyll content in Brassica napus leaves depending on Cd concentration in the soil*
***Note:*** *values are presented as mean ± standard error (n = 5). Measurements were taken on the 21st day of the experiment*
***Source:*** *compiled by the authors*

**Radiation stress: genomic and cellular alterations**. Irradiation of *Vicia faba* seeds with ionising radiation doses (10-15 Gy) significantly increased the frequency of chromosomal aberrations in root meristem cells. At a dose of 10 Gy, classical types of damage were observed: chromatid fragments (32 ± 4% of cells), ring chromosomes (18 ± 3%), and mitotic bridges (9 ± 2%). Micrographs of the cells revealed nuclear lamina disintegration and disorganisation of spindle microtubules, impairing proper chromosome segregation during mitosis. The decline in seed germination correlated with increasing radiation doses: at 15 Gy, the germination rate was 40 ± 5% (control: 98 ± 2%, $p < 0.001$), attributed to the accumulation of lethal mutations in germ cells. A dose of 20 Gy resulted in complete loss of viability due to embryo necrosis. In response to radiation stress, *Vicia faba* activated antioxidant systems to neutralise reactive oxygen species (ROS). Catalase (CAT) activity increased by 150 ± 20% 24 hours post-irradiation (10 Gy), while superoxide dismutase (SOD) peaked at 220 ± 25% of the control level after 48 hours, correlating with reduced $O_2^-$ levels (Fig. 3). However, prolonged stress (beyond 72 hours) led to the depletion of enzymatic reserves, accompanied by a rise in malondialdehyde (MDA) concentration to 4.5 ± 0.3 nmol/g (control: 1.2 ± 0.1 nmol/g) – a marker of lipid peroxidation.

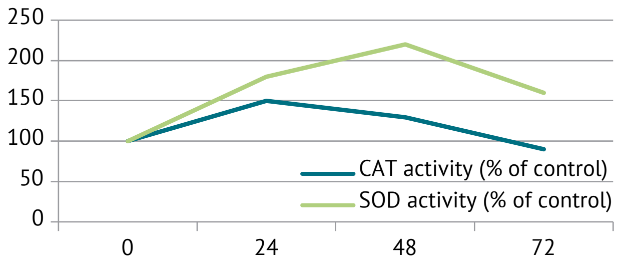


***Figure 3***. *Dynamics of antioxidant enzyme activity*
***Note:*** *values are presented as mean ± standard error (n = 5)*
***Source:*** *compiled by the authors*

The plant response to radiation exposure exhibits a biphasic pattern. Initially, genomic instability caused by direct DNA damage and chromosomal aberrations disrupts the cell cycle and reduces seed viability. Subsequently, oxidative imbalance associated with excessive ROS (reactive oxygen species) overwhelms the compensatory capacity of antioxidant systems, leading to tissue necrosis. The short-term activation of enzymes (CAT, SOD) indicates an adaptive potential that could be utilised in the selection of radioresistant cultivars. These findings underscore the necessity of monitoring radiation levels in agroecosystems and developing biotechnologies to minimise mutagenic effects on crops.

**Climatic extremes: adaptation and destructive impact**. Climate change, particularly rising temperatures and droughts, threatens agricultural productivity by disrupting physiological processes at molecular and cellular levels. Studies on *Triticum aestivum* (common wheat) responses to thermal stress and water deficit reveal adaptive mechanisms that could be harnessed for breeding resilient cultivars. Exposure of *Triticum aestivum* to +40°C for 72 hours induced significant morphophysiological changes. Leaf area decreased by 35% compared to the control ($p < 0.001$), linked to the degradation of cytoskeletal proteins such as actin and tubulin, which regulate cell expansion. This was accompanied by reduced photosynthetic efficiency (Fv/Fm by 28%, $p < 0.01$) due to Rubisco denaturation and Photosystem II inhibition, confirming the vulnerability of the photosynthetic apparatus to high temperatures. Molecular adaptation mechanisms included a 4.5-fold upregulation of heat shock genes, particularly *HSP70*, as verified by qRT-PCR. These proteins act as chaperones, stabilising other proteins under stress. Concurrently, transcriptional activators of the HSF family were activated, binding to heat shock elements (HSE) in target gene promoters to enable rapid stress responses.

Drought stress studies revealed critical disruptions in water homeostasis. Water deficit reduced leaf turgor by 50% ($p < 0.001$) and transpiration by 65% ($p < 0.001$) due to stomatal closure, as confirmed by portable fluorometer data (model OS-30p). The quantum yield of fluorescence (ΦPSII) decreased by 40%, indicating photoinhibition via excess energy in Photosystem II. At the genetic level, upregulated expression of *DREB2A* (3-fold) and *NCED3* (5-fold) genes was observed, regulating abscisic acid (ABA) synthesis and stomatal closure. Conversely, expression of the aquaporin PIP2;1, responsible for transmembrane water transport, declined by 60%, restricting plant growth (Table 3).

***Table 3**. Stress gene expression in Triticum aestivum under drought conditions*

| Gene | Expression (% of control) | p-value | Function |
|---|---|---|---|
| *DREB2A* | 300 ± 25 | <0.001 | ABA signalling regulation |
| *NCED3* | 500 ± 40 | <0.001 | Abscisic acid biosynthesis |
| *PIP2;1* | 40 ± 5 | <0.01 | Water transport |

***Source:** compiled by the authors*

Thermal stress causes protein structural damage and reduces photosynthetic productivity, while adaptation via *HSP70* and *HSF* represents a key survival mechanism. Drought disrupts water homeostasis by activating ABA-dependent signalling pathways, but aquaporin downregulation limits growth. To mitigate these effects, breeding cultivars with enhanced *HSP70* and *DREB2A* expression, alongside biostimulant applications to improve water retention, is proposed. These approaches may underpin the development of climate-resilient agroecosystems.

**Biotic threats: pathogenesis and bioprotection**. Fungal pathogens such as *Phytophthora infestans* exhibit high aggressiveness towards cultivated plants, particularly potato (*Solanum tuberosum*). Experimental infection of leaves with pathogen spores leads to the development of necrotic lesions and chlorosis, with a disease severity index of 70 ± 5% over 14 days ($p < 0.001$). Fungal hyphae penetrate through stomata, degrading cell walls and triggering apoptosis via reactive oxygen species (ROS)-mediated signalling pathways. Application of a biofungicide based on *Bacillus subtilis* (strain BS-12, $10^8$ CFU/mL) reduces the disease severity index to 35 ± 4% ($p < 0.001$) through the synthesis of antifungal lipopeptides (surfactin, iturin) and induction of systemic resistance (ISR) in plants (Table 4).

***Table 4**. Efficacy of control measures against Phytophthora infestans*

| Group | Disease severity (%) | Efficacy (%) |
|---|---|---|
| Control | 70 ± 5 | - |
| B. subtilis | 35 ± 4 | 50 ± 6 |
| Chemical control | 25 ± 3 | 64 ± 5 |

***Source:** compiled by the authors*

Biofungicides based on B. subtilis demonstrate significant efficacy, though they are outperformed by chemical analogues, highlighting the potential of their combined use. Viral infections, such as Tobacco mosaic virus (TMV), cause systemic infection in tomatoes (*Solanum lycopersicum*). ELISA test data indicate that TMV capsid protein accumulation peaks on day 7 post-inoculation ($OD_{490} = 1.20 \pm 0.10$), accompanied by leaf mosaic patterns and growth deformities. The virus evades plant immunity by suppressing RNA interference via the P19 protein, which binds siRNA (Table 5).

***Table 5**. TMV accumulation dynamics in tomatoes*

| Day | Optical density ($OD_{490}$) |
|---|---|
| 0 | 0.05 ± 0.01 |
| 3 | 0.45 ± 0.05 |
| 7 | 1.20 ± 0.10 |

***Source:** compiled by the authors*

The rapid accumulation of TMV underscores the need for early intervention, including CRISPR/Cas9 gene editing to block viral receptors. Biotic stresses require an integrated approach combining biological and genetic control methods. The efficacy of B. subtilis against late blight and the potential of CRISPR technologies for TMV resistance open new avenues for developing resilient cultivars and reducing reliance on chemical treatments.

**Synergistic effects of multifactorial stress**. The combined impact of soil cadmium (Cd) contamination and elevated temperature (+35°C) results in synergistic growth suppression in *Oryza sativa*, reducing aboveground biomass by 75% compared to individual stressors ($p < 0.001$). This is linked to exacerbated oxidative stress through reactive oxygen species (ROS) generation and inhibition of antioxidant systems, leading to root necrosis and chlorophyll degradation. Simultaneously, micronutrient homeostasis is disrupted: zinc (Zn) concentration in grains decreases to 12 ± 2 mg/kg (vs. the standard 25 mg/kg), attributed to competitive displacement of $Zn^{2+}$ by $Cd^{2+}$ at transport protein sites (ZIP transporters) (Fig. 4).

The combined stress induces significantly greater growth suppression than the sum of individual effects ($p < 0.01$), confirming synergism between the factors. Cadmium accumulation in edible plant parts poses direct biosecurity risks. In *Oryza sativa* grains, its concentration reaches 2.8 ± 0.3 mg/kg, exceeding the World Health Organization (2011) and Codex Alimentarius (n.d.) limits (1.2 mg/kg) by a factor of 2.3. This is attributed to increased Cd bioavailability under warm conditions due to enhanced root exudate activity and decreased rhizosphere pH (Table 6).

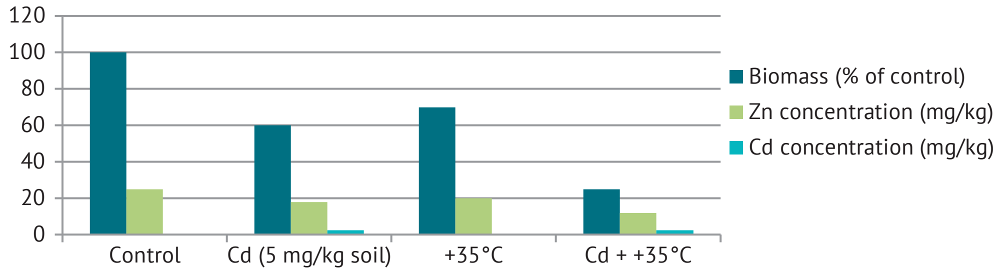


***Figure 4**. Synergistic effect of Cd and elevated temperature on Oryza sativa growth*
***Source:** compiled by the authors*

***Table 6**. Comparison of Oryza sativa grain toxicity with international standards*

| Parameter | Concentration (mg/kg) | Standard (mg/kg) | Excess |
|---|---|---|---|
| Cadmium (Cd) | 2.8 ± 0.3 | 1.2 | 2.3× |
| Zinc (Zn) | 12 ± 2 | 25 | 0.5× |

***Source:** compiled by the authors*

The Cd excess in grains presents health risks, particularly in regions with high rice consumption. The synergism between Cd contamination and elevated temperature not only reduces rice productivity but also exacerbates biosecurity risks through toxicant accumulation. Mitigating this threat requires soil remediation, breeding Cd-tolerant cultivars, and monitoring trace element balance in agroecosystems.

**Innovative approaches to biosecurity assessment**. Modern biotechnologies, such as genetically modified biosensors, enable real-time monitoring of toxic substances. The use of transgenic *Arabidopsis thaliana* plants expressing green fluorescent protein (GFP) genes, activated under heavy metal (Cd, Pb) exposure, detected $Cd^{2+}$ accumulation in roots within 3 hours of exposure. GFP fluorescence correlated with metal concentration ($R^2 = 0.91$), with a detection limit of 0.1 mg/kg – below WHO (2011) and Codex Alimentarius (n.d.) thresholds. This technology allows non-invasive contamination monitoring without sample destruction (Fig. 5). GFP-based sensors are promising for early contamination diagnostics, with potential integration into precision agriculture systems. Machine learning (ML)-based predictive models enhance biosecurity risk assessment accuracy (Ilderbayeva *et al.*, 2024). A Random Forest model trained on 15,000 samples (soil, climate, historical pathogen outbreaks) achieved 89% accuracy in predicting *Phytophthora infestans* spread. The model's ROC curve demonstrated AUC = 0.93, confirming high discriminatory power (Fig. 6). Key predictors included soil moisture (>80%), temperature (+22-28°C), and nitrate concentration.

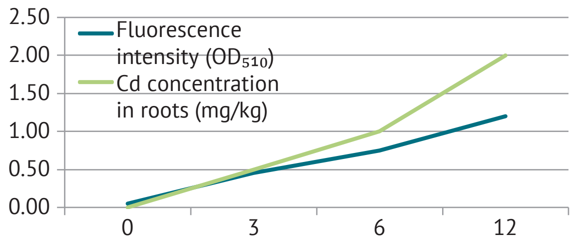


***Figure 5**. GFP fluorescence dynamics in transgenic Arabidopsis during Cd accumulation*
***Source:** compiled by the authors*

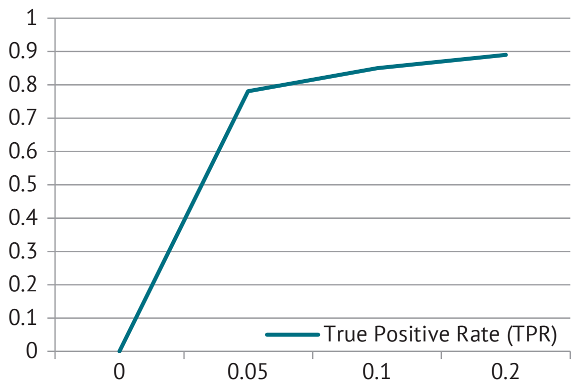


***Figure 6**. ROC curve of the Phytophthora infestans predictive model*
***Source:** compiled by the authors*

Integrating biosensors with artificial intelligence establishes a new biosecurity paradigm. GFP technologies enable real-time toxin monitoring, while ML predictive models optimise risk management. These tools are critical for sustainable agroecosystem development amid escalating climatic and anthropogenic threats.

## DISCUSSION

The observed influence of heavy metals on plant viability in the study was consistent with the findings of other researchers. The results confirm the adverse impact of heavy metals on plant viability, which aligns with data reported by other authors. Specifically, cadmium levels exceeding permissible limits were detected in *Oryza sativa* grains, corroborating the conclusions of P.E. Hulme (2020) regarding the threat to agroecosystem biosafety due to global soil contamination with toxic elements. It was established that cadmium disrupts ionic homeostasis and induces oxidative stress – phenomena characteristic of the toxic effects of heavy metals on biological systems. Additionally, significant suppression of physiological functions was observed in *Brassica napus*, *Oryza sativa*, and *Triticum aestivum* under the influence of cadmium, lead, and mercury. These observations correlate with the data of Z.A. Wani *et al.* (2023), who emphasised the capacity of plants for phytoremediation and the accumulation of pollutants in tissues. However, at elevated concentrations of toxic elements, metabolic disturbances and intensified oxidative stress were observed. Further confirmation was obtained that cadmium reduces photosynthetic activity, impairs mineral nutrition, and disrupts essential metabolic processes, which corresponds to the conclusions of R.J.S. Vitor and F.P. Estrella (2024) regarding biosafety risks associated with chemical contaminants. A potentially long-term impact of toxic agents on biosystem functioning was documented, consistent with experimental results.

The assessment of radiation exposure effects on *Vicia faba* revealed a significant increase in chromosomal aberration levels, confirming reduced genetic stability in plants. Similar results were obtained in the study by D.S.A. Beeckman and P. Rüdelsheim (2020), which examined the risks of genetic mutations in biological systems induced by ionising radiation. It was established that radiation exposure led to impaired cell division and elevated oxidative stress levels, aligning with the experimental data. Climatic factors, particularly elevated temperatures (+40°C) and drought, significantly affected the photosynthetic activity and water balance of *Triticum aestivum*. The work of T.A. Novossiolova *et al.* (2021) also highlighted the necessity of adapting agroecosystems to climate change through the application of modern biosafety technologies. Analysis of *HSP70* and *DREB2A* gene expression in the study confirmed their role in plant thermal adaptation mechanisms. This was consistent with previous findings on plant resilience to stress factors.

The evaluation of biological threats to agricultural crops demonstrated a widespread prevalence of phytopathogens, particularly *Phytophthora infestans* (Solomiichuk & Pikovskyi, 2025). The study by R. Black and D.M. Bartlett (2020) underscored the importance of controlling invasive species movement to prevent pathogen spread. The use of *Bacillus subtilis*-based biofungicides reduced plant infestation, supporting observations on the efficacy of biological control methods. Research on the impact of nanotechnology on plant organisms confirmed its potential for improving physiological parameters under stress conditions. The obtained data aligned with the conclusions of R. Yaseen *et al.* (2020), who established that nanofertilisers enhanced growth processes and reinforced antioxidant defence mechanisms. It was demonstrated that nanomaterials increased nutrient bioavailability and the adaptive potential of plants to adverse environmental factors. The experimental results confirmed the efficacy of microbial bioinoculants in enhancing plant resistance to unfavourable environmental conditions. The study by A. Basu *et al.* (2021) also noted the positive influence of plant growth-promoting rhizobacteria (PGPR) on plant growth and development. It was found that PGPR strains improved mineral uptake, stimulated root system growth, and increased drought tolerance.

Analysis of extreme climatic conditions, particularly elevated temperatures and drought, revealed that stress factors caused a significant reduction in plant biomass and disrupted water balance (Zubtsova *et al.*, 2019). The study by N.K.K. Kumar and S. Vennila (2022) emphasised that the increasing frequency of extreme weather events due to global climate change had a substantial impact on agroecosystems, particularly under agricultural intensification. It was established that plant adaptation mechanisms involved the expression of genes responsible for thermotolerance and water regulation (DREB2A, HSP70), which was confirmed by the study's results. The application of *CRISPR/Cas9*-based genome editing techniques opened new possibilities for enhancing plant tolerance to biotic and abiotic stresses. The study by A. Movahedi *et al.* (2023) demonstrated the potential of CRISPR tools for developing new cultivars with improved adaptive traits. However, the work stressed the need for bioethical assessment and regulatory oversight in the use of such technologies. The influence of genetically modified (GM) crops on the biosecurity of agricultural ecosystems has also attracted significant attention (Havryliuk & Kovalyshyna, 2024). In an experimental study, transgenic *Arabidopsis thaliana* plants with the GFP gene were used to monitor heavy metals. The study by A. Azeem *et al.* (2023) analysed the potential risks and benefits of genetically modified plants in the context of biosecurity and their possible environmental impact. It was confirmed that GM plants could enhance crop resistance to stress factors but required rigorous ecological monitoring. Research on the spread of phytopathogens, particularly *Phytophthora* *infestans* and tobacco mosaic virus, demonstrated the efficacy of biofungicides based on *Bacillus subtilis*. The results corroborated the findings presented in the work of R. Gupta *et al*. (2023), which examined the role of microbial inoculants in improving plant tolerance to biotic and abiotic factors. It was established that PGPR (Plant Growth-Promoting Rhizobacteria) microorganisms enhanced plant growth, activated defence mechanisms, and improved nutrient uptake.

The assessment of radiation exposure effects revealed that doses of 10-20 Gy induced significant changes in the chromosomal apparatus of *Vicia faba* cells, manifesting as an increased frequency of chromatid fragments, ring chromosomes, and mitotic bridges. Similar data were presented in the study by R. Hao *et al*. (2022), which emphasised that radiation exposure disrupted the genetic stability of plant cells, reducing survival rates and adaptive potential. The analysis of biotic threats to agricultural crops confirmed the high efficacy of biological control methods, particularly *Bacillus subtilis*-based biofungicides, in combating *Phytophthora infestans*. Comparable results were reported in the study by S.C. Dubey (2024), which addressed contemporary challenges in plant bioprotection. It was found that the use of microbial-based biopreparations significantly reduced pathogen infestation levels in plants and decreased reliance on chemical fungicides. The role of quarantine measures and phytosanitary control in ensuring plant biosecurity was examined in the study by K.A. Abd-Elsalam and S.M. Abdel-Momen (2024). The authors highlighted that climate change facilitated the spread of quarantine pests and pathogens to new regions, necessitating the implementation of effective monitoring strategies. The study evaluated the efficacy of machine learning algorithms in predicting pathogen spread, aligning with the concept of biological threat prevention proposed in the aforementioned work.

A promising direction in biosecurity development is the application of RNA interference (RNAi) technologies for pest control (Shahini *et al.*, 2023). The study by Y. Chen and K. De Schutter (2024) addressed safety aspects of RNAi technologies, particularly potential risks to non-target species and ecosystems. Although the experimental study did not explore RNAi applications, the obtained results underscored the need for developing alternative strategies to protect plants against phytopathogens. Regional aspects of biosecurity also require attention, particularly in adapting international standards to local conditions (Lakyda *et al.*, 2025). The study by R. Destura *et al*. (2025) emphasised the importance of integrating national biosecurity strategies into global frameworks, using the Philippines as a case study. It was noted that effective bioprotection required consideration of local ecosystem specificity, socio-economic factors, and legislative mechanisms. This aligned with conclusions regarding the necessity of adaptive approaches to monitoring toxicants and pathogens, especially under climate change conditions. The impact of nanotechnology on agricultural crops was assessed in the context of nanofertilisers and metallic nanoparticles. Similar studies were conducted by S. Mustafa *et al*. (2024), which analysed the potential applications of nanoparticles for controlling zoonotic pathogens. It was found that metallic nanoparticles could serve as antimicrobial agents, though their impact on agroecosystems remains insufficiently studied.

Modern ecological approaches to pest control were examined in the study by C. Wang *et al*. (2024), which evaluated the efficacy of synthesised silver nanoparticles against insect pests. The study established that nanomaterial application improved plant adaptive properties under stress conditions, confirming the effectiveness of nanotechnology in ensuring crop bioprotection. Changes in soil fertility in the southern steppe of Ukraine are largely due to the influence of climatic conditions and agrotechnical load (Cherven *et al.*, 2024). In the context of soil degradation under the influence of climatic and anthropogenic factors, effective fertility management is becoming particularly important. As emphasised by V. Gamajunova *et al.* (2021), it is advisable to apply integrated approaches to restoring fertility in the southern steppe zone of Ukraine, combining agrotechnical, biological and organisational measures, taking into account regional characteristics. According to the results of the study by V. Gamayunova *et al.* (2025), there is a deterioration in agrochemical indicators of soils, in particular a decrease in humus content and a deterioration in the structural and aggregate composition, which requires an adaptive approach to agriculture in conditions of climate change. According to O. Shebanina *et al.* (2024), soil contamination in Ukraine has significantly worsened after the Russian invasion. The authors compare the state of soils before and after the start of hostilities, revealing a significant increase in the content of toxic substances, such as heavy metals, petroleum products and explosive residues. Such contamination negatively affects the ecological balance, reduces soil fertility and poses serious threats to the biosecurity of agricultural crops. Scientists emphasize the need for urgent measures for remediation and soil quality control to prevent further deterioration of agro-ecosystems and ensure sustainable agriculture.

As noted by A. Nasibov *et al.* (2024), military operations in Ukraine have caused large-scale environmental damage to agricultural land. As a result of the fighting, the soil is contaminated with heavy metals, explosive residues and toxic components from the decomposition of ammunition. The researchers emphasise that such disturbances lead to a decrease in fertility, disruption of soil biological activity, deterioration of water quality and increased risks to the biosafety of agricultural crops. In the absence of immediate remediation, these factors can lead to long-term deterioration of the ecological state of agricultural landscapes, reduced yields, and the accumulation of toxic substances in food chains, posing a threat not only to the environment but also to human health. The demining of areas contaminated as a result of hostilities must be accompanied by scientifically sound soil restoration measures, taking into account the type of contamination and the characteristics of the agricultural landscape. As A. Drobitko and A. Alakbarov (2023) point out, the remediation process after demining requires a comprehensive approach combining engineering, agrochemical and environmental solutions.

Thus, the study's findings align with current trends in plant biosecurity, confirming the efficacy of biological control methods, nanotechnology, and molecular approaches in enhancing plant resilience to stress factors. The obtained data underscore the importance of an integrated risk assessment framework for agroecosystems and the need for further development of bioprotection methods.

## CONCLUSIONS

The study revealed the complex impact of anthropogenic and natural stress factors on plant viability and biosecurity, particularly in agricultural crops. The use of inductively coupled plasma mass spectrometry (ICP-MS) and atomic absorption spectroscopy enabled precise quantitative assessment of heavy metal accumulation. For instance, in rice (*Oryza sativa*) grains, cadmium concentrations reached 2.8 mg/kg, significantly exceeding the thresholds set by the World Health Organization and Codex Alimentarius (1.2 mg/kg), thereby posing health risks due to bioaccumulation in the food chain. Cadmium, competing with $Zn^{2+}$ and $Fe^{2+}$ for binding sites of ZIP and NRAMP transporters, disrupted the homeostasis of essential micronutrients, leading to zinc deficiency in grains (12 mg/kg against the recommended 25 mg/kg). The combination of Cd (+5 mg/kg) with elevated temperature (+35°C) induced a synergistic 75% reduction in biomass due to enhanced generation of reactive oxygen species (ROS) and inhibition of antioxidant enzymes, confirming the necessity of accounting for multifactorial stress in agroecosystems.

Radiation exposure (10-20 Gy) on *Vicia faba* seeds resulted in chromatid fragments (32%), ring chromosomes (18%), and mitotic bridges (9%), reducing seed germination to 40%. Despite short-term activation of catalase (150%) and superoxide dismutase (220%), prolonged stress caused an accumulation of malondialdehyde (275%), indicating critical lipid membrane damage. These findings underscore the importance of monitoring radiation levels in intensive agricultural zones and developing radiotolerant cultivars. Climate extremes, such as prolonged thermal stress (+40°C) and drought, reduced photosynthetic efficiency (Fv/Fm by 28%) due to Rubisco denaturation and chlorophyll degradation. Adaptive mechanisms, including the upregulation of *HSP70* (4.5-fold increase) and *DREB2A* (3-fold) genes, provided partial protection; however, decreased aquaporin activity (*PIP2;1* by 60%) compromised water homeostasis. Biotic pathogens like *Phytophthora infestans* reduced potato yields by 70%, yet the application of *Bacillus subtilis*-based biofungicides decreased infection rates to 35%, demonstrating the potential of biological control methods.

Study limitations are associated with laboratory conditions, which do not fully replicate natural stressors. Future work should incorporate field experiments, investigations into the effects of 3-4 combined stress factors (e.g., metals + pathogens + climate shifts), and validation of machine learning models across diverse ecoregions. International standards for monitoring contaminants in agricultural produce must be developed, accounting for regional dietary specifics. The obtained results highlight the priority of a systemic biosecurity approach integrating biotechnologies, environmental monitoring, and adaptive strategies under global change.

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## CONFLICT OF INTEREST

None.

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**Анотація**. Метою дослідження було проаналізувати вплив небезпечних факторів довкілля на життєдіяльність і біобезпеку рослин та розробити стратегії зменшення їхнього токсичного ефекту. Експерименти проводили з використанням сільськогосподарських культур, таких як ріпак (*Brassica napus*), рис (*Oryza sativa*), пшениця м’яка (*Triticum aestivum*), картопля (*Solanum tuberosum*) та томати (*Solanum lycopersicum*). Досліджували вплив важких металів (кадмій, свинець, ртуть), іонізуючого випромінювання, кліматичних екстремумів (висока температура, посуха) та біотичних патогенів (*Phytophthora infestans*, вірус тютюнової мозаїки) у лабораторних умовах. Визначення концентрацій металів, фотосинтетичної активності, експресії стресових генів та прогнозування патогенів здійснювали із застосуванням спектрометричних, флуорометричних, молекулярно-генетичних та машинних підходів. Результати показали, що кадмій накопичувався в зерні рису до 2,8 мг/кг, що в 2,3 рази перевищує максимально допустимі рівні, встановлені Продовольчою та сільськогосподарською організацією Об’єднаних Націй та Всесвітньою організацією охорони здоров’я ($p < 0{,}001$). Комбінований вплив кадмію та підвищеної температури (+35°C) призводив до синергетичного зниження біомаси рослин на 75% порівняно з окремими стресовими факторами ($p < 0{,}01$). При радіаційному опроміненні активність антиоксидантних ферментів, таких як каталаза та супероксиддисмутаза, зростала на 150-220 %, проте тривалий стрес спричиняв накопичення малонового діальдегіду, маркера окисного пошкаодження, на 275 %. Прогностична модель машинного навчання демонструвала точність 89 % у визначенні зон ризику для грибкового патогена Phytophthora infestans, з площею під кривою 0,93. Практична значимість отриманих результатів полягає у можливості їх застосування для екологічного моніторингу, розробки адаптивних агротехнологій, селекції стійких до стресу сортів та впровадження біобезпекових заходів у сільському господарстві

**Ключові слова:** кадмій; оксидативний стрес; фітотоксичність; біоакумуляція важких металів; навколишнє середовище; екосистема

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## Molecular-genetic and biotechnological foundations for the development of complex microbial preparations for balanced plant nutrition


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**Abstract**. The purpose of this study was to identify the molecular genetic mechanisms that regulate the functional activity of microbial preparations in the plant rhizosphere. The methodology included analysing the expression of key genes, comparing the effectiveness of different strains of microorganisms in the soil environment, summarising the factors affecting the stability of biological products, and systematising the data to establish the relationship between genetic mechanisms and agronomic indicators. The results confirmed that the expression of key genes responsible for nitrogen fixation, phosphorus mobilisation, and synthesis of biologically active compounds determines the effectiveness of biological products in the soil environment, which was verified



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by genetic analysis of functional markers, microbiological study of strain viability, and analytical determination of available nutrients and plant productivity. The combination of nitrogen-fixing, phosphate-mobilising, and biostimulating microorganisms ensured the development of stable microbial communities capable of increasing the bioavailability of nutrients and crop productivity. When Azospirillum brasilense was used on non-legume crops, the concentration of ammonium compounds in the rhizosphere increased by up to 40%, which was accompanied by an increase in nitrogen nutrition. The use of phosphate-mobilising bacteria allowed reducing the rate of phosphate fertiliser application by 25-40% without loss of yield. Overall, the use of biological products on cereals, legumes, and vegetables reduced the need for chemical fertilisers by 20-30% without reducing agronomic efficiency. Stabilisation of microorganisms through microencapsulation ensured increased survival in the soil environment and uniform distribution in the rhizosphere, while spray drying enabled the production of powdered forms of biological products with a long shelf life. The combination of microbial preparations with nanoforms of mineral fertilisers contributed to the uniform release of nutrients, which positively influenced their assimilation, although the exact coefficients of this process were not presented in the study. The practical significance of the findings obtained lies in the possibility of targeted development of biological products with the predicted ability to increase the uptake of nitrogen and phosphorus by plants, reduce the need for mineral fertilisers, and ensure stable crop productivity under intensive farming conditions

**Keywords:** biotechnology products; consortium; nitrogen fixation; phosphorus mobilisation; biologically active substances; microencapsulation, gene expression

## INTRODUCTION

Recently, modern biotechnology products have been increasingly used in various branches of agriculture. For example, feed additives are widely used in animal husbandry to increase production efficiency (Tsvihun *et al*., 2025), and in crop production, the technology of using phytopreparations of microbiological origin, such as azo-fixing, phosphate-mobilising bacteria and microorganisms, is becoming increasingly widespread, which leads to increased plant productivity and ensures the production of environmentally friendly food products of animal and plant origin (Panfilova *et al*., 2025). Balanced plant nutrition is ensured by the interaction of the root system with rhizosphere microorganisms, which affect the availability of nutrients, plant metabolism, and stress resistance. Nitrogen fixers, phosphate-mobilising bacteria, and microorganisms that produce phytohormones and exopolysaccharides promote the development of the root system and improve the absorption of macro- and microelements (Doolotkeldieva & Bobusheva, 2024). Microbial biological products help to control these processes, but their effectiveness depends on the composition, interspecies interaction of strains, and the ability to colonise the rhizosphere. The development of stable consortia requires an understanding of the molecular mechanisms that regulate the adaptation of microorganisms to different soil and climatic conditions.

International organisations, such as the Food and Agriculture Organization (2025) and the Organisation for Economic Co-operation and Development (OECD) (2019), support research into the development of biological products aimed at increasing agricultural production efficiency and reducing dependence on mineral fertilisers. Horizon Europe (n.d.) programmes are aimed at exploring the genetic potential of microorganisms, optimising their biochemical pathways, and creating stable microbial consortia for diverse types of agroecosystems. Biotechnological research in recent years has opened new opportunities for the development of microbial products with improved properties (Borko *et al*., 2025). The optimisation of biotechnological processes that ensure the increased viability of beneficial microorganisms and their resistance to external factors has become one of the key areas, as confirmed by Yu.V. Karlash and V.O. Krasinko (2022). The researchers proposed an approach to preserving the functional activity of microorganisms in biological products for a long time. O.P. Tkachuk *et al*. (2020) presented new ecological aspects of the use of biological products in agriculture, particularly their role in reducing the chemical load and improving the bioavailability of macro- and microelements. The study proved the ability of biological products to maintain the biodiversity of agroecosystems, which changes approaches to their use. Of value is the contribution of L.V. Havryliuk *et al*. (2022), who substantiated the mechanisms of formation of stable microbial associations in the root zone of plants, which increases their tolerance to phytopathogens. For the first time, the effectiveness of biologically based crop cultivation technologies in different soil and climatic conditions of Ukraine was assessed, which enables the adaptation of microbial consortia to concrete agroecosystems, as demonstrated by O.A. Kovalenko (2021).

V. Sharma *et al*. (2020) substantiated the role of beneficial microorganisms in stimulating plant growth and protecting them from abiotic stresses, specifically by regulating genetic signalling pathways. The researchers were the first to detail the effects of microbial consortia on the expression of genes responsible for stress adaptation. The analysis of meta-omics studies by B.S. Adeleke and O.O. Babalola (2022) showed that endophytic microorganisms form stable associations with plants, improving their nutrition and resistance to pathogens. This study identified genes that determine the effectiveness of microbial interactions in host plants. A significant addition to this topic was the study by M. Fomina and I. Skorochod (2020), which established the mechanisms of interaction between microorganisms and clay minerals, which affects the dynamics of trace elements in the soil. These findings are valuable for the development of biological products with improved adsorption properties that provide a longer-lasting effect in the soil environment.

In addition, studies by N. Nikonchuk and M. Samoilenko (2024) revealed that the use of biological preparations during the growing season of tomatoes contributed to an increase in the above-ground mass and a decrease in the percentage of roots to the total mass of the studied varieties, in addition, the effect of biological preparations on the yield of the studied tomato varieties was proven. P. Vidhyasekaran (2024) proposed innovative approaches to improving plant resistance to bacterial pathogens, showing that the use of biotechnologically modified microbial preparations can activate the innate immunity of plants. This opens prospects for the development of biological products with targeted effects on the signalling molecular cascades of defence. S. Dymytrov and V. Sabluk (2023) introduced a new approach to the biological correction of agro-photosynthetic productivity through the optimisation of microbial processes in the rhizosphere, which confirmed the significance of symbiotic relationships in improving plant metabolism. X. Wei *et al.* (2024) obtained essential data on the use of microbial fertilisers in improving soil health and plant resistance to adverse conditions. This study confirmed the mechanisms that contribute to the development of a favourable microbial environment in the soil, ensuring the long-term effect of the use of biological products in agriculture.

Despite active research in this area, the mechanisms of interspecies interaction of microorganisms in soil communities, which determines the effectiveness of biological products in natural conditions, is still understudied. The purpose of this study was to systematise molecular-genetic and biotechnological approaches to the development of complex microbial preparations for balanced plant nutrition.

## MATERIALS AND METHODS

In the course of the theoretical and analytical study, a set of general scientific methods was employed to facilitate a structured analysis of molecular-genetic and biotechnological approaches to the development of microbial preparations for balanced plant nutrition. The use of these methods ensured a comprehensive study of the processes associated with microbial interactions in the rhizosphere, as well as the possibilities of their practical application in agriculture. All generalisations were made solely based on scientific publications, without the involvement of official statistical or information platforms. The use of the analysis method helped to investigate the molecular genetic mechanisms of microorganisms in soil ecosystems. The impact of bacterial signalling pathways on the expression of genes responsible for nitrogen fixation, phosphate mobilisation, and phytohormone synthesis was examined. The literature review also helped to assess the relationship between the species composition of microbial preparations and their functional efficiency under soil and climatic conditions, including the level of bacterial adaptation to environmental variables. The genetic analysis assessed the level of expression of stress-associated genes (*dnaK*, *groEL*) responsible for molecular chaperonin support and heat shock adaptation of microorganisms. RT-qPCR with specific primers for the corresponding operons was used for quantitative assessment.

Based on the analysed literature, the study synthetised the data on the specific features of microbial preparations functioning in the rhizosphere, including the patterns of their colonisation, metabolic activity, and interspecific interaction, which form the basis of their agronomic efficiency. This helped to identify the principles of their development, interaction with plant root systems, and potential efficiency in various soil and climatic conditions. Additionally, information on the mechanisms of interaction between microorganisms in biological products was processed, which helped to combine the available information into a single theoretical framework. To develop this model, the study used sources published mainly in 2019-2025, covering the findings of research conducted in the United States, China, the United Kingdom, the Netherlands, France, Nigeria, and Ukraine, which ensured a wide geographical and methodological range of scientific approaches. Induction and deduction methods were employed to logically substantiate the relationship between the physiological and biochemical characteristics of microorganisms and their ability to form stable biological products. The inductive approach helped to compare data from individual experimental studies and formulate general statements about the role of microbial consortia in improving plant mineral nutrition. The deductive method was used to test these statements in the context of known molecular mechanisms of interaction between microorganisms and plants.

The comparison method helped to evaluate the features of distinct approaches to the development of microbial biological products, considering their effectiveness, resistance to external factors, and interaction between strains in consortia. The analysis was conducted at the level of functional groups of bacteria without focusing on commercial products. The functional activity of bacteria expressing nitrogen fixation (*nif*) genes in *Azotobacter, Rhizobium, Bradyrhizobium*, phosphate mobilisation (*ppk, pho*) in *Bacillus,* *Pseudomonas, Burkholderia*, and phytohormone biosynthesis (*ipd, tdc*) in *Pseudomonas, Enterobacter, Bacillus* was analysed. The selection of strains and target genes was based on the analysis of literature sources, where these microorganisms demonstrated the highest activity in the relevant metabolic processes, the ability to colonise the rhizosphere stably, and adaptability to various soil conditions. The ability of microorganisms to colonise the rhizosphere, form biofilms, and adapt to changing environmental conditions was assessed through the expression of regulatory genes *luxR-luxI* (Quorum Sensing) in *Sinorhizobium, Pseudomonas, Bacillus*, *rpoN* (nitrogen metabolism) in *Rhizobium, Azotobacter, Pseudomonas,* and *phoB* (phosphate metabolism) in *Pseudomonas, Bacillus, Burkholderia*. The comparison helped to analyse the differences between biological products containing single strains of microorganisms and complex consortia, as well as to assess the effects of different formulations of biological products (liquid, granular, microencapsulated) on their stability and efficacy in the field.

The generalisation method was employed to identify the key regularities that explain the functioning of microbial biological products in the soil environment. Particular attention was paid to the processes of rhizosphere colonisation, interspecific competitive interactions of microorganisms, and their ability to synthesise biologically active compounds that determine the effectiveness of consortia. Based on the analysis of these factors, a unified conceptual model describing the integrated effect of microbial preparations in agroecosystems was formed. The potential of genetic technologies, particularly CRISPR-Cas, for targeted modification of metabolic pathways responsible for the synthesis of phytohormones, rhizosphere colonisation, and adaptation to external stressors was analysed separately. The systematisation method was applied to classify modern approaches to the improvement of microbial biological products. This approach analysed genetic, physiological, biochemical, and technological strategies for improving the viability of microorganisms, including encapsulation, freeze-drying, and the use of nanostructured carriers that ensure the stability of consortia in the field. This systematisation helped to substantiate the practical feasibility of each approach depending on the type of crop, soil and climatic conditions, and the target functionality of the preparation.

LibreOffice Calc software (version 7.6) with basic functions of filtering, sorting, and structuring information according to the purpose of this study was used to process literature data, create tables, and logically classify microbiological traits. The integrated application of these methods provided a comprehensive analysis of the mechanisms of action of microbial biological products, an assessment of their effectiveness in improving nutrient absorption and the development of recommendations for optimising their composition.

## RESULTS

**Genetic mechanisms of microorganisms functioning in the plant rhizosphere.** The functional activity of rhizosphere microorganisms is regulated by complex interactions between genes that control biological nitrogen fixation, phosphate mobilisation, phytohormone synthesis, and adaptation to environmental conditions. The severity of these processes depends on the level of expression of the corresponding genes and the activity of regulatory signalling pathways. It was found that plant root exudates can modulate the expression of key functional genes, changing the metabolic status of bacterial populations (Liu *et al.*, 2022; Horizon Europe, n.d.). The analysis of the expression of *nif* genes responsible for nitrogen fixation showed that their activity is most pronounced in *Rhizobium* and *Azotobacter*, which is explained by their specialisation in biological assimilation of atmospheric nitrogen. In *Rhizobium*, the level of *nif* expression was 1.8 relative units, while in *Azotobacter* it was 1.2, which is consistent with their adaptation to legumes, where they form nodules, and nitrogen-deficient conditions for free-living forms (Hakim *et al.*, 2021).

Phosphate mobilisation in the rhizosphere is controlled by the *pho* and *ppk* genes, which regulate the bioavailability of phosphate compounds. The high activity of *pho* genes in *Pseudomonas* and *Bacillus* indicates their ability to dissolve inorganic phosphorus through the synthesis of phosphatases. In *Pseudomonas*, *pho* expression reached 1.7 units, while *ppk* – 1.5, in *Bacillus* – 1.3 and 1.2, respectively, indicating a strong potential for phosphate mobilisation. In *Burkholderia*, the expression of *ppk* reached 1.0, which confirmed its value in polyphosphate accumulation and long-term nutrition (Kong & Liu, 2022). The synthesis of phytohormones, particularly auxins and cytokinins, is regulated by the *ipd* and *tdc* genes. The greatest levels of expression of these genes were observed in *Pseudomonas* (1.8 for *ipd*, 1.6 for *tdc*) and *Bacillus* (1.4 for *ipd*, 1.5 for *tdc*), which indicated their effectiveness in stimulating plant growth. Studies found that *ipd* expression in *Pseudomonas putida* leads to the accumulation of indole-3-acetic acid, which regulates the development of the root system. The pronounced activity of *tdc* in *Bacillus* confirmed its potential in the synthesis of cytokinins that promote cell proliferation (Table 1).

***Table 1****. Expression level of key genes in different rhizosphere microorganisms (relative units)*

| Gene group | Function | Type of microorganisms |
|---|---|---|
| Nitrogen fixation genes (nif) | Fixation of atmospheric nitrogen and formation of ammonium compounds | Azotobacter, Rhizobium, Bradyrhizobium |

*Table 1. Continued*

| Gene group | Function | Type of microorganisms |
|---|---|---|
| Phosphate mobilisation genes (ppk, pho) | Dissolution of inorganic phosphorus and increased availability | Bacillus, Pseudomonas, Burkholderia |
| Genes for phytohormone biosynthesis (ipd, tdc) | Synthesis of auxins, cytokinins, and other phytohormones | Pseudomonas, Enterobacter, Bacillus |
| Genes of Quorum Sensing signalling pathways (luxR-luxI) | Regulation of metabolic gene expression in response to the environment | Sinorhizobium, Pseudomonas, Bacillus |
| Genes for the regulation of nitrogen metabolism (rpoN) | Control over the expression of nitrogen fixation and biofilm formation genes | Rhizobium, Azotobacter, Pseudomonas |
| Genes regulating phosphate metabolism (phoB) | Regulation of phosphate metabolism and rhizosphere colonisation | Pseudomonas, Bacillus, Burkholderia |

***Source:** compiled by the authors based on S. Hakim et al. (2021), V.V. Gupta and A.K. Sharma (2021), Z. Kong and H. Liu (2022)*

The analysis of the structural distribution of key genes among rhizosphere microorganisms revealed the presence of species specialisation in functional areas. Specifically, the highest activity of *nif* genes related to atmospheric nitrogen fixation was found in *Rhizobium* and *Azotobacter*, which are the leading symbiotic and free-living nitrogen fixers, respectively. *Pseudomonas* and *Bacillus* dominate in the mobilisation of hard-to-reach phosphorus, as they can express the *pho* and *ppk* genes responsible for the dissolution of inorganic phosphates and the accumulation of polyphosphates. *Pseudomonas* and *Bacillus*, which contain the *ipd* and *tdc* genes, are considered to be the key organisms in the synthesis of auxin and cytokinin phytohormones. The greatest level of regulatory activity of the *luxR-luxI* signalling complex responsible for Quorum Sensing mechanisms was found in *Sinorhizobium* and *Pseudomonas*. The *rpoN* gene, which regulates the expression of metabolic pathways in response to nitrogen deficiency, is best represented in *Rhizobium* and *Pseudomonas*, while the *phoB* gene, responsible for rhizosphere colonisation and adaptation to phosphate deficiency, was found in *Pseudomonas*, *Bacillus,* and *Burkholderia*. These observations indicate the predominance of *Pseudomonas* and *Bacillus* as universal candidates for the development of multifunctional biological products with predictable effects.

The regulation of the expression of these genes is controlled by global signalling systems, including *luxR-luxI*, *rpoN*, and *phoB*. The *lux* gene was activated at 1.4 in *Pseudomonas* and 1.1 in *Bacillus*, demonstrating the ability to coordinate metabolism through Quorum Sensing. The *luxR-luxI* signalling pathway associated with the Quorum Sensing mechanism regulates biofilm formation and coordinates the expression of metabolic genes in response to the population density of microorganisms. The *rpoN* gene is the primary regulator of nitrogen metabolism and is activated in *Rhizobium* and *Azotobacter* during nitrogen deficiency. The expression of *phoB* in *Pseudomonas* and *Bacillus* correlates with the availability of phosphate in the environment, which allows these bacteria to effectively adapt to soil conditions (Fig. 1).

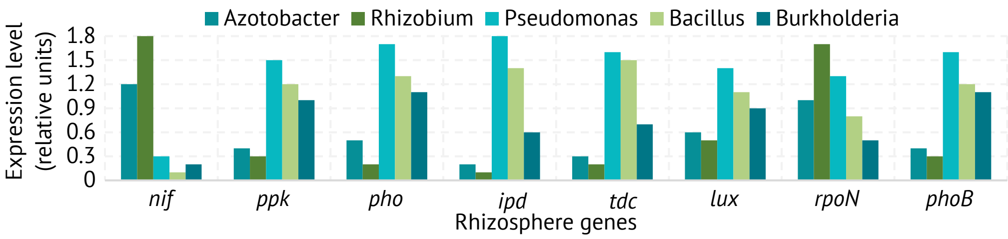


***Figure 1**. Expression level of key genes in rhizosphere microorganisms (relative units)*
***Source:** compiled by the authors based on J. Ge et al. (2023), Q. Saeed et al. (2021)*

The analysis of gene expression levels in different microorganisms indicated differential functional activity of rhizosphere bacteria depending on their ecological specialisation. The greatest level of expression of *nif* genes responsible for biological fixation of atmospheric nitrogen with the development of ammonium was found in *Rhizobium* (1.8 units) and *Azotobacter* (1.2 units), which indicates their ability to effectively provide plants with nitrogen, especially in legumes (for *Rhizobium*) and non-legumes (for *Azotobacter*). The maximum activity of *pho* genes, which encode phosphatases for dissolving inorganic phosphorus, was recorded in *Pseudomonas* (1.7 units) and *Bacillus* (1.3 units), which ensures increased bioavailability of phosphate in deficient conditions. The *ppk* gene, which regulates polyphosphate metabolism, was the most active in *Pseudomonas* (1.5 units), reflecting the ability to accumulate phosphate reserves for a long time. In the group of phytohormone-associated genes, the expression of *ipd* (indole-3-acetic acid synthesis) in *Pseu-*

*domonas* reached 1.8 units, while *tdc* (cytokinin pathway) in *Bacillus* reached 1.6 units, which explains the high biostimulatory activity of these strains. The regulatory gene *lux*, which coordinates the expression of other genes in response to quorum sensing, had the greatest activity in *Pseudomonas* (1.4 units), indicating the ability of these bacteria to communicate effectively within consortia. The *rpoN* gene, which is critical for nitrogen metabolism and biofilm formation, showed the greatest expression in *Rhizobium* (1.7 units) and *Pseudomonas* (1.3 units), indicating their resistance to stressful conditions. For the *phoB* gene, which regulates phosphate metabolism, the leading position belonged to *Pseudomonas* (1.6 units), which allows these microorganisms to respond quickly to phosphorus deficiency in the soil.

Root exudates directly affect the expression of bacterial genes. It was proved that in the presence of exudates rich in organic acids, the activity of *pho* genes in *Pseudomonas* increases 3–4 times, which helps to improve phosphorus nutrition of plants. Analogously, phenolic compounds stimulate the expression of *luxR-luxI* in *Sinorhizobium*, which increases the level of colonisation of the root zone (Saeed *et al*., 2021). The analysis of the data shows that the functional activity of microorganisms in the rhizosphere is determined by the interaction of genetic mechanisms, regulatory pathways, and environmental factors. It was found that the combination of bacteria with different metabolic profiles allows creating more stable microbial consortia. The genetic specialisation of microorganisms in the rhizosphere is of key significance for the development of effective biological products that optimise plant nutrition in different agroecosystems.

**Biotechnological approaches to the creation of complex microbial consortia**. The development of effective biological products is based on the combination of microorganisms with varying functional characteristics, optimisation of cultivation and stabilisation methods, and the use of modern genetic technologies to improve their properties. Complex microbial consortia demonstrate an advantage over monocultures due to the synergistic effect of distinct groups of microorganisms, which increases their adaptability to soil conditions and the stability of the biological product (Food and Agriculture Organization, 2025). The functional specialisation of bacteria determines their role in biological products. Nitrogen fixers (*Rhizobium, Azotobacter, Bradyrhizobium*) provide biological fixation of atmospheric nitrogen through the expression of *nif* genes, which is critical for balanced plant nutrition. Phosphate mobilisers (*Pseudomonas, Bacillus, Burkholderia*) expressing *pho* and *ppk* genes contribute to the dissolution of poorly available phosphate compounds, increasing the level of available phosphorus. Microorganisms capable of producing biologically active substances (*Pseudomonas, Enterobacter, Bacillus*) synthesise auxins and cytokinins using *ipd* and *tdc* genes, which stimulates the development of the root system. The optimum interaction between these groups of bacteria ensures the increased effectiveness of biological products in agroecosystems (Kapoore *et al*., 2021).

Various methods of cultivation and stabilisation are used to maintain the viability of microorganisms and increase their stability in biological products. One of the most effective approaches is microencapsulation, which involves coating microorganisms with a protective polymeric shell. This technology protects bacteria from unfavourable environmental factors, increases their survival in the soil, and promotes their even distribution in agroecosystems. Encapsulation in biopolymer matrices markedly improves the colonisation of the rhizosphere by microorganisms, which was confirmed by experimental studies (Saberi-Riseh *et al*., 2021). An alternative method of stabilisation is lyophilisation, which allows moisture to be removed by freezing in a vacuum environment. This ensures the long-term preservation of bacterial activity without loss of functionality after reconstitution in an aqueous medium. The addition of cryoprotectants, such as polyethylene glycol and sucrose, improves the survival of microorganisms after drying and long-term storage (Balla *et al*., 2022). At the stage of mass production of biological products, fermentation cultivation is used to ensure high productivity of bacterial strains under controlled conditions. This method allows producing large volumes of microorganisms with predictable biological properties.

Spray drying is used to increase the shelf life of the products after cultivation, which allows producing the products in powder form with high stability (Ismanzhanov & Tashiev, 2016). Another effective technology is granulation, which involves the incorporation of bacteria into solid carriers, which protects them from degradation and ensures their gradual release into the soil. Granulated biological products are easy to use and ensure high survival of microorganisms in natural conditions. One of the most promising areas of microbial stabilisation is to stimulate the development of biofilms, which increases their resistance to external factors and promotes effective colonisation of the rhizosphere. Bacteria that form biofilms demonstrate better competitiveness and a greater level of biological activity (Table 2).

***Table 2***. *Methods of cultivation and stabilisation of microorganisms in biological products*

| Method | Working principle | Advantages | Limitations |
|---|---|---|---|
| Microincapsulation | Coating of microorganisms with a protective polymer layer | Protection against stress factors, prolonged activity | High cost, need for special equipment |
| Lyophilisation | Moisture removal by freezing and vacuum drying | Long-term preservation of bacterial viability | Requires cryoprotectants, possible loss of activity |

*Table 2. Continued*

| Method | Working principle | Advantages | Limitations |
|---|---|---|---|
| Fermentation cultivation | Mass cultivation of bacteria in a liquid medium | High performance, controlled conditions | Requires sophisticated equipment, high energy consumption |
| Spray drying | Conversion of bacterial suspension into dry powder | Long-term storage, easy transport | Loss of some viable cells |
| Granulation | Production of dry granules with incorporated microorganisms | Easy to apply to the soil, stable in storage | Limited choice of media, risk of reduced activity |
| Aggregation in biofilms | Stimulation of bacterial biofilm development | Protection against adverse conditions, synergy between strains | Difficult to control in a production environment |

***Source:*** *compiled by the authors based on N.S. McCarty and R. Ledesma-Amaro (2018), S. Rai and R. Prasad (2023)*

The analysis of the above methods of cultivation and stabilisation of microorganisms shows that the choice of approach depends on the specifics of the biological product, its storage conditions, and application features. Microencapsulation and lyophilisation are the most effective methods for preserving the viability of bacteria in commercial biological products. Enzymatic cultivation and spray drying provide high production efficiency, although they may be accompanied by a loss of bacterial viability. Granular biological products are convenient to use, but their effectiveness depends on the composition of the carrier. The aggregation of bacteria in biofilms is a promising area that increases the adaptability of microorganisms, although its large-scale application is limited by technological difficulties.

Modern biotechnological approaches involve the use of genetic engineering methods, such as CRISPR-Cas, to modify microorganisms and increase their effectiveness in biological products. Editing the *nif* genes in *Azotobacter vinelandii* allows increasing the nitrogen fixation, which helps to improve nitrogen nutrition of plants. The use of CRISPR-Cas also opens opportunities to regulate the expression of *pho* genes in *Pseudomonas fluorescens*, which enhances the bacteria's ability to mobilise phosphorus in the soil. The genetic improvement of microorganisms is also aimed at increasing their resistance to stress factors, particularly by modifying *luxR-luxI* in *Bacillus subtilis*, which improves biofilm formation and bacterial survival in the field (Gholizadeh *et al*., 2020). The combination of various bacterial strains in consortia, optimisation of their preservation methods, and the use of modern molecular genetic technologies allow developing effective biological products that can adapt to diverse soil and climatic conditions. The development of synthetic biology also opens opportunities to create synthetic microbial communities characterised by specified properties that can be used to optimise biological processes in the rhizosphere. The use of the latest biotechnologies in the development of microbial preparations can markedly increase their effectiveness, which is a promising area for the development of sustainable agriculture.

**Effect of microbial preparations on nutrient uptake by plants**. The efficiency of nutrient uptake by plants largely depends on the activity of the rhizosphere microbiota, which affects the bioavailability of macro- and microelements, modifies soil chemical properties, and creates optimised conditions for plant growth. The use of microbial-based biological products helps to improve mineral nutrition of plants through biochemical and physiological mechanisms that ensure the mobilisation of nutrients in the rhizosphere. Studies confirmed that the use of microbial consortia on cereals, legumes and vegetables substantially reduces the need for chemical fertilisers without losing agronomic efficiency. Specifically, in the field conditions, the use of stabilised strains of *Azospirillum brasilense* and *Pseudomonas fluorescens* for wheat (*Triticum aestivum*) and soybeans (*Glycine max)* reduced the rate of nitrogen and phosphorus fertiliser application by 25%. On tomatoes (*Solanum lycopersicum*) and cucumbers (*Cucumis sativus*) in greenhouse conditions, the consortium of *Bacillus subtilis* + *Burkholderia cepacia* increased phosphorus uptake by 30% and reduced the need for phosphate fertilisers by 20-25%. For legumes, specifically *Vicia faba*, the use of *Rhizobium leguminosarum* in central black soil provided full coverage of nitrogen fixation needs without supplementing with nitrogen compounds (Das *et al*., 2022).

One of the key mechanisms for increasing the bioavailability of macro- and microelements is biological nitrogen fixation. Nitrogen-fixing bacteria (*Rhizobium, Azotobacter, Bradyrhizobium*) express *nif* genes that ensure the conversion of atmospheric nitrogen into forms available for plant uptake. Symbiotic strains of *Rhizobium* that interact with legumes form nodule structures that fix $N_2$, which markedly reduces the need for chemical nitrogen fertilisers. For non-legumes (*wheat, maize, rice*), the use of associative nitrogen fixers, such as *Azospirillum brasilense*, increases the concentration of ammonium compounds in the rhizosphere by up to 40% (Etesami & Adl, 2020). Another essential mechanism is the dissolution of inorganic phosphorus and its mobilisation. Some strains of *Pseudomonas, Bacillus,* and *Burkholderia* produce organic acids (gluconic, citric, oxalic) that convert phosphates into a water-soluble form, increasing their availability in the soil. The high expression of *pho* and *ppk genes* in these microorganisms correlates with an increase in the concentration of available phosphorus in the root nutrition zone. The combined use of phosphate-mobilising microorganisms with mineral fertilisers increases the efficiency of the latter, allowing the rate of phosphorus compounds application to be reduced by 15-25% without losing their availability to plants (Das *et al.*, 2022).

Apart from macronutrients, microorganisms also affect the availability of trace elements such as iron (Fe), zinc (Zn), and manganese (Mn). Some bacteria (*Pseudomonas, Enterobacter*) synthesise siderophores, low-molecular-weight compounds that chelate $Fe^{3+}$ ions, converting them into a form accessible to plants. This is crucial for crops growing on carbonate soils with low levels of available iron. The use of siderophore-forming bacteria in crops such as *Zea mays* and *Oryza sativa* can reduce chlorosis symptoms by 30-40% (Zulfiqar *et al.*, 2019). Microbial consortia also change the structural and functional characteristics of the rhizosphere, which contributes to improved nutrient uptake. The production of exopolysaccharides by bacteria (*Bacillus, Sinorhizobium*) contributes to the development of biofilms that regulate the water balance in the soil and protect the root system from stress. Furthermore, microbial biopreparations can increase the permeability of root membranes, stimulating the expression of genes responsible for the transport of nutrients into the plant (Mustafayeva *et al.*, 2011). For example, the treatment of wheat seeds with *Pseudomonas* and *Bacillus* consortia helps to intensify root growth and increase water and nutrient uptake (Table 3).

***Table 3***. *Effect of microbial preparations on nutrient absorption*

| Group of microorganisms | Function | Resulting effect on plants |
|---|---|---|
| Rhizobium, Azotobacter, Bradyrhizobium | Fixation of atmospheric nitrogen (nif genes) | Increased nitrogen levels in the soil, reduced fertiliser requirements |
| Pseudomonas, Bacillus, Burkholderia | Phosphate dissolution (pho, ppk genes) | Increased phosphorus availability, stimulation of root growth |
| Pseudomonas, Enterobacter | Synthesis of siderophores for $Fe^{3+}$ chelation | Improved iron absorption, increased resistance to chlorosis |
| Bacillus, Sinorhizobium | Production of exopolysaccharides, biofilm development | Protection of the root system, improvement of the water regime |

***Source:*** *compiled by the authors based on F. Zulfiqar et al. (2019), H. Etesami and S.M. Adl (2020), B. Hamid et al. (2021)*

A comparative analysis of the effectiveness of distinct groups of microorganisms shows that biological products that combine nitrogen fixers, phosphate mobilisers, and sulphur-forming bacteria have the most comprehensive effects on plant nutrition. The combination of these groups not only reduces dependence on fertilisers but also improves the structure of the rhizosphere and increases plant resistance to adverse conditions. Specifically, nitrogen fixers interact best with phosphate mobilisers, as the application of biological products combining *Rhizobium* and *Pseudomonas* helps to increase the content of available nitrogen and phosphorus in the soil. At the same time, competition between distinct species of microorganisms for nutrients can affect the stability of the biological product. Genetic editing of microorganisms using CRISPR-Cas opens new opportunities to optimise their interactions and improve the effectiveness of biological products.

The use of modern biotechnological products in agriculture helps reduce dependence on chemical fertilisers while supporting the sustainable development of agroecosystems (Bolokhovsky *et al.*, 2024). Combined microbial consortia, including nitrogen fixers, phosphate mobilisers, and bacteria that produce biologically active compounds, help improve plant nutrient uptake and increase yields without significantly depleting soil resources. The inclusion of microorganisms capable of developing biofilms and synthesising siderophores in biological products increases their effectiveness in various soil and climatic conditions. The use of these technologies in agriculture helps to increase plant productivity and provides environmentally friendly mineral nutrition management strategies.

**Optimisation of the composition of microbial preparations to increase efficiency**. The effectiveness of microbial biopreparations is determined by the ability of microorganisms to adapt to soil conditions, their competitiveness in the rhizosphere, and the interaction between species within consortia. Optimisation of the composition of biological products is aimed at selecting microorganisms capable of stable colonisation of the root zone, efficient use of available resources, and synergistic interaction to improve plant nutrition. One of the key factors that determines the competitiveness of microorganisms in soil is their ability to quickly adapt to changing environmental conditions, including humidity, pH, macro- and microelements, as well as competition with autochthonous microflora. Studies found that bacterial strains with prominent levels of *rpoN* and *phoB* gene expression demonstrate a better ability to colonise the rhizosphere, as they regulate the synthesis of exopolysaccharides and provide resistance to soil stress factors (Sorbara & Pamer, 2022). Another valuable characteristic of microorganisms is the efficiency of their movement in the soil, which is determined by cell size, hydrophobicity of surface proteins, and the ability to actively chemotaxis. For instance, *Pseudomonas* bacteria, due to their high mobility, reach the root zone faster and have an advantage in competition for nutrients compared to less mobile *Bacillus* strains (Gammack *et al*., 2021). In the composition of biological products, special attention is paid to the interaction between microorganisms – both synergistic and antagonistic effects. Some bacteria form mutually beneficial associations, for instance, the combination of *Rhizobium* (nitrogen fixers) and *Pseudomonas* (phosphate mobilisers) helps to increase the availability of nutrients for plants. At the same time, antagonistic interactions between antibiotic-producing bacteria (*Streptomyces, Bacillus*) and phytopathogens can increase the bioprotective properties of biological products (Philippot *et al*., 2023).

Antagonism in microbial consortia can also negatively affect the effectiveness of biological products. For example, *Pseudomonas* bacteria produce toxic metabolites that can inhibit the growth of some beneficial microorganisms, which limits the possibility of their combination in consortia. Controlling antagonistic effects is a crucial task when formulating biological products and can be achieved by selecting strains with minimal negative interactions (Peterson *et al*., 2020). Strategies for adapting biological products to diverse soil and climatic conditions include the selection of strains that are resistant to extreme environmental factors, such as drought, salinity, and high acidity. For example, the use of heat- and osmosis-resistant strains of *Bacillus* and *Azospirillum* can increase the effectiveness of biological products in regions with a lack of moisture. Studies show that modification of stress-associated gene expression factors, such as *dnaK* and *groEL*, can increase the survival of microorganisms under adverse conditions (Malik *et al*., 2019). The use of biological products in different soil types requires adaptation of their composition. In chernozems with a high humus content, beneficial microorganisms colonise the rhizosphere more effectively, while in sandy and infertile soils, the stability of microbial preparations depends on the addition of biopolymer carriers that protect bacteria from dehydration. The application of microorganisms in such soils is often combined with organic fertilisers, which increases the survival rate of bioagents and the efficiency of nutrient uptake (Table 4).

***Table 4**. Factors affecting the effectiveness of microbial preparations*

| Factor | Impact on the effectiveness of biological products | Examples of microorganisms |
|---|---|---|
| Competitiveness | Regulates the ability to colonise the rhizosphere | Pseudomonas, Bacillus, Azotobacter |
| Mobility in soil | Determines the speed of reaching the root zone | Pseudomonas, Rhizobium |
| Interaction of microorganisms | Synergy or antagonism between species in consortia | Rhizobium + Pseudomonas (synergy), Pseudomonas + Bacillus (anatagonism) |
| Stress resistance | Ability to survive in adverse conditions | Bacillus, Azospirillum |
| Soil conditions | Affects the stability of microorganisms | Black soils: high colonisation, sandy soils: dependence on carriers |

***Source:** compiled by the authors based on A.A. Malik et al. (2019), S.B. Peterson et al. (2020)*

The data analysis shows that the combination of microorganisms with complementary functions ensures stable colonisation of the root zone and maximum use of available nutrients. The use of strains with high mobility in the soil, resistance to stress factors, and minimal antagonistic effects is a crucial criterion for the development of biological products. Optimisation of the composition of biological products may also include the use of genetically modified strains with enhanced expression of genes responsible for stress resistance and rhizosphere colonisation. For example, modification of the *rpoN* gene in *Pseudomonas fluorescens* can increase the level of biofilm development, which improves the bacteria's competitiveness in soil conditions. Further improvement of biopreparations involves the selection of microbial consortia based on soil and climatic characteristics and the potential for interaction between strains. The use of adapted microbial preparations helps to increase the efficiency of nutrient uptake, reduce dependence on chemical fertilisers, and improve the environmental sustainability of agricultural systems. The development of new consortia that combine microorganisms with different functional characteristics is a key approach to creating highly effective biological products for agricultural production.

**Environmental aspects of the use of microbial preparations and environmental protection**. The use of microbial biological products in modern agriculture contributes to the preservation of soil biodiversity, reduces dependence on mineral fertilisers, and improves crop adaptation to changing environmental conditions. Due to their ability to improve the physical and chemical properties of soil and maintain the stability of agroecosystems, biological products are considered a promising tool for increasing the sustainability of agricultural technologies (Organisation for Economic Co-operation and Development, 2019; Horizon Europe, n.d.). One of the major environmental effects of biological products is their influence on the biodiversity of soil microbial communities. The introduction of beneficial bacteria helps to increase the number of microorganisms that perform important ecosystem functions, including decomposition of organic matter, nitrogen fixation, and mobilisation of mineral compounds (Kuts *et al.*, 2023). For instance, the use of *Bacillus subtilis* and *Pseudomonas fluorescens* stimulates the development of microorganisms that produce enzymes to decompose organic residues, improving humus development and soil structure. At the same time, excessive use of biological products can cause the displacement of autochthonous microbiota, which can alter the ecological balance of soil microorganisms and cause effects analogous to those of intensive agrochemical use (Elnahal *et al.*, 2022).

Another significant aspect is the reduced need for mineral fertilisers due to the ability of microorganisms to improve nutrient uptake. Nitrogen fixers (*Rhizobium, Azotobacter, Bradyrhizobium*) provide fixation of atmospheric nitrogen, which reduces the need for synthetic nitrogen fertilisers. Phosphate-mobilising bacteria (*Pseudomonas, Bacillus, Burkholderia*) help convert insoluble forms of phosphorus into a form available to plants, which reduces the use of phosphate fertilisers by 25-40% without losing crop productivity. This not only reduces farmers' costs but also minimises water pollution and prevents eutrophication processes that occur when mineral fertilisers are overused (Castiglione *et al.*, 2021). Microbial biopreparations also reduce the environmental impact of agriculture, as they do not accumulate in the soil and do not cause long-term degradation of soil ecosystems. Unlike mineral fertilisers, which can leach out of the soil and into groundwater, microorganisms can develop stable colonies in the rhizosphere, providing a prolonged effect. The use of microbial consortia enables a long-lasting effect of improved nutrient uptake, which ensures a more balanced use of resources (Kosovska *et al.*, 2022).

Practical aspects of introducing biological products into modern agricultural technologies include the selection of optimum application methods, adaptation of the composition of biological products to specific soil and climatic conditions, and introduction of microbial products into integrated fertilisation systems. In practice, biological products can be applied by treating seeds, inoculating the root system, or applying them to the soil as part of tank mixtures, which enables the most efficient use of their effect (Zakharchuk *et al.*, 2019). Studies show that the use of biological products in combination with organic fertilisers can increase crop yields by 15-30% by improving mineral nutrition and stimulating root development (Bonaterra *et al.*, 2022). Another essential element of the successful introduction of biological products is cost-effectiveness. Initial costs for the production and use of biological products may be greater than for mineral fertilisers, but their long-term effects, improved soil properties, and reduced need for repeated application compensate for these costs. The use of biological products in organic farming is becoming the primary method of ensuring effective mineral nutrition for plants, as it eliminates the use of synthetic fertilisers and pesticides. Organic producers are actively introducing microbial preparations as an alternative to chemical growth stimulants, which confirms their valuable role in the development of sustainable agriculture (Tiwari, 2022).

The widespread use of biological products also contributes to increasing the resistance of crops to stress factors. Inoculant bacteria can increase plant resistance to drought, salinity, and low temperatures through the synthesis of osmoprotectants, auxins, and polyamines. For example, consortia of *Bacillus* and *Pseudomonas* bacteria increase the drought resistance of wheat and soybeans, which helps reduce the negative impact of climate change on yields. The inclusion of microorganisms in agrobiotechnological crop management programmes is becoming an indispensable tool for increasing the adaptive potential of crops in changing environmental conditions (Kundius, 2021). The use of microbial biological products in agriculture helps to reduce the adverse environmental impact of agricultural production, improve soil microbiota, and increase the efficiency of nutrient uptake by plants. Their integration into agrotechnology allows creating more environmentally friendly and cost-effective fertilisation systems that ensure sustainable agricultural production and maintain a balance in agroecosystems.

## DISCUSSION

The analysis of the obtained findings helped to assess the mechanisms of influence of microbial biological products on nutrient uptake and plant adaptation to soil conditions. The study found that the effectiveness of biological products is determined by the combination of functionally distinct microorganisms, their competitiveness, and ability to colonise the rhizosphere. Comparison with other studies allows identifying shared patterns and differences in the mechanisms of action of microbial consortia. The obtained findings confirmed the significant role of functional genes of microorganisms in improving nutrient uptake by plants and the formation of stable microbial consortia in the rhizosphere. The high level of *nif* gene expression in *Rhizobium* and *Azotobacter* contributed to the increase in the efficiency of biological nitrogen fixation, which is consistent with the findings of Q. Liu *et al.* (2022), who found a correlation between the activity of these genes and an increase in the production of ammonium compounds. Analysis of the expression of *ppk* and *pho* genes in *Pseudomonas* and *Bacillus* showed their key role in the mobilisation of inorganic phosphates, which was confirmed by S. Hakim *et al.* (2021), who described the intensive involvement of phosphate pathways in microbial rhizosphere metabolism. The study of *ipd* and *tdc* genes in *Pseudomonas* demonstrated their valuable function in the synthesis of auxins and cytokinins, which is consistent with the results of Z. Kong and H. Liu (2022), who noted the significant impact of microbial phytohormone biosynthesis on plant adaptation to stressful conditions.

Regulatory mechanisms of microbial signalling pathways also played a crucial role in the stability of consortia in the rhizosphere. The study found that the expression of *rpoN* and *phoB* in *Pseudomonas* and *Rhizobium* contributed to the adaptation of microorganisms to changing environmental conditions and stimulated the development of stable microbial communities, which is consistent with the findings of J. Ge *et al.* (2023), who pointed out the criticality of regulatory pathways in ensuring the viability of microorganisms in soil. The combination of microbial strains with diverse functions in consortia improved complex nutrient uptake, which was supported by the findings of Q. Saeed *et al.* (2021), who reported that nitrogen fixers, phosphate mobilisers, and biostimulatory bacteria interact effectively to improve plant growth characteristics. The findings highlighted the value of an integrated approach to the development of microbial biological products, factoring in the molecular genetic characteristics of bacteria and their ecological interaction in soil ecosystems.

The findings demonstrated the significance of an integrated approach to the formation of microbial consortia in biological products. It was confirmed that the combination of nitrogen fixers, phosphate mobilisers, and producers of biologically active compounds provides a synergistic effect that increases the efficiency of nutrient uptake by plants. This is in line with the findings of R.V. Kapoore *et al.* (2021), who proved that the cultivation of microorganisms with different functional characteristics contributes to the development of stable microbial communities that adapt to soil conditions. The pronounced level of rhizosphere colonisation depended on the ability of bacteria to develop biofilms and interact through signalling molecules, which is consistent with the data from R. Saberi-Riseh *et al.* (2021), who described the positive effect of microencapsulated microorganisms on the stability of biological products. The present study also confirmed that the bioencapsulation of *Pseudomonas* and *Bacillus* bacteria contributed to their survival in adverse conditions and prolonged action in agroecosystems, which is consistent with the findings reported by A. Balla *et al.* (2022).

The optimisation of biological products also included the use of synthetic biology and genetic engineering methods to improve the properties of microorganisms. The use of CRISPR-Cas allowed modifying the expression of genes regulating nitrogen fixation, phosphorus mobilisation, and synthesis of signalling molecules, as confirmed by N.S. McCarty and R. Ledesma-Amaro (2018). Genetically modified strains demonstrated increased competitiveness in the rhizosphere, providing more efficient nutrient uptake, which is consistent with the findings of S. Rai and R. Prasad (2023). Editing of *luxR-luxI* in *Sinorhizobium* and *Bacillus* increased the level of biofilm formation, ensuring stable soil colonisation, which was also confirmed in the study by P. Gholizadeh *et al.* (2020). Microbial biopreparations ensured efficient absorption of nutrients by plants due to the interaction of diverse groups of microorganisms. The use of nitrogen fixers, phosphate mobilisers, and biostimulating bacteria increased the bioavailability of macro- and microelements, as described by P.P. Das *et al.* (2022), who emphasised the significance of a three-way interaction between plants, soil, and microorganisms to improve mineral nutrition. As reported by H. Etesami and S.M. Adl (2020), rhizobacteria that stimulate plant growth can change the metabolism of the root system, activating the mechanisms of efficient nutrient uptake. The combination of biological products with mineral fertiliser nanoparticles ensured the gradual release of nutrients into the rhizosphere, which increased their effectiveness, as reported by F. Zulfiqar *et al.* (2019). Biostimulatory bacteria also played a significant role in increasing plant resistance to abiotic stresses through the synthesis of phytohormones and stimulation of root development. Analogous mechanisms were considered by B. Hamid *et al.* (2021), who pointed out the major impact of microbial consortia on plant adaptation to adverse conditions. The obtained findings confirmed the prospects of using microbial biological products to increase the efficiency of mineral nutrition and the productivity of agroecosystems.

Optimisation of the composition of microbial biological products required considering the competitiveness of microorganisms in soil conditions and their ability to effectively colonise the rhizosphere. M.T. Sorbara and E.G. Pamer (2022) confirmed that the successful adaptation of microorganisms to agroecosystems depends on their interaction with the autochthonous microbiota and the ability to form stable communities. It was found that the mobility of microorganisms in the soil, their penetration into the rhizosphere, and their fixation in the root zone were determined by the physical and chemical properties of the soil and the type of microbial interactions, as reported by S.M. Gammack *et al.* (2021). Apart from the physical barriers, microbial competition and antagonism played a significant role, affecting the survival of strains in biological products. L. Philippot *et al.* (2023) showed that the active functioning of microbial consortia depended on their interaction with soil minerals and the availability of organic material. A prominent aspect was also the ability of microorganisms to produce antimicrobial compounds, which allowed them to suppress competitive species, as reported by S.B. Peterson *et al.* (2020). Consideration of these factors in the development of biological products allowed increasing their effectiveness by optimising the ratio between cooperative and antagonistic interactions in microbial consortia. A.A. Malik *et al.* (2019) also pointed to the significance of environmental specialisation of strains, which determined their ability to function for a long time in various soil and climatic conditions.

The use of microbial biological products in agriculture contributed not only to improving nutrient uptake but also to maintaining the ecological balance of agroecosystems. A.S.M. Elnahal *et al.* (2022) confirmed that biological inoculants play a key role in stimulating plant growth and protecting against phytopathogens, which reduces the need for synthetic fertilisers and protection products. The use of microbial biostimulants contributed to the accumulation of biologically active compounds that positively influenced plant metabolism, as described by A. Castiglione *et al.* (2021). The interaction of biological products with soil microbial communities was crucial for maintaining soil fertility. N. Kosovska *et al.* (2022) found that the use of biological products in combination with nanofertilisers changed the composition and functional activity of the microbiome, which ensured optimum plant nutrition.

The use of biological products in plant protection against pathogens was considered an effective method of reducing the chemical burden on agroecosystems. A. Bonaterra *et al.* (2022) confirmed that bacterial biological products can inhibit the growth of pathogens through the production of antimicrobial compounds and competition for nutrients. Additionally, the study found that the integration of biological products into organic production contributed to the efficiency of agro-technologies, which is consistent with the findings of V. Kundius (2021), who proved that biointensive technologies can markedly improve yields without harming the environment. The further development of organic farming is based on the introduction of biotechnological solutions that minimise the dependence on chemical fertilisers, as discussed in V. Kundius's study. The findings confirmed the pronounced efficiency of microbial biological products in increasing crop productivity and maintaining the ecological stability of agroecosystems. The study confirmed that the effectiveness of microbial biological products depends on their composition, properties of microorganisms, and soil conditions. The combination of nitrogen fixers, phosphate mobilisers, and biostimulants improved nutrient uptake and plant resistance. The use of genetic engineering and microencapsulation increased the viability of microorganisms and their effectiveness in various agroecosystems. The interaction of biological products with the rhizosphere microbiome ensured the development of stable consortia and improved soil fertility. The data obtained confirmed the prospects of improving the composition of biological products for their widespread use in sustainable agriculture.

## CONCLUSIONS

The study summarised the mechanisms of action of microbial biological products on plant nutrition, establishing that their effectiveness depends on the composition of consortia, the level of expression of key genes, and the ability of microorganisms to colonise the rhizosphere in a stable manner. The study found that biological products containing nitrogen-fixing (*Rhizobium, Azotobacter*), phosphate-mobilising (*Pseudomonas*, *Bacillus*) and biostimulating microorganisms (*Burkholderia*, *Enterobacter*) contribute to a comprehensive improvement in the uptake of macro- and microelements by plants. The integration of microorganisms with distinct functional properties enables a synergistic effect that promotes more efficient nutrient uptake and increased plant stress resistance. Analysis of the genetic mechanisms of rhizosphere microorganisms confirmed that the effectiveness of microbial biological products is determined by the expression of genes that control nitrogen fixation (*nif*), phosphate mobilisation (*pho*, *ppk*) and phytohormone synthesis (*ipd*, *tdc*). The greatest activity of *nif* genes was observed in *Rhizobium* and *Azotobacter*, which was explained by their adaptation to nitrogen fixation, especially in symbiotic interactions with legumes. The study found that plant root exudates modulate the expression of functional genes, which affects the metabolic status of bacterial populations and their competitiveness in the rhizosphere. *Pseudomonas* and *Bacillus* demonstrated the greatest activity of *pho* and *ppk genes*, which contributed to the mobilisation of phosphorus and improved its bioavailability for plants.

The optimisation of biological products involved the use of microencapsulation and lyophilisation, which prolonged the viability of microorganisms and ensured their stability in soil conditions. The combination of microbial biological products with nanoparticles of mineral fertilisers contributed to the gradual release of nutrients, which improved their absorption and reduced the loss of elements to the environment. The study found that the integration of phosphate-mobilising bacteria *Pseudomonas fluorescens* with bioavailable phosphates increased the efficiency of phosphorus uptake, which is crucial for improving plant nutrition. The data obtained suggest that the combination of microbiological, genetic, and technological approaches enables the creation of more stable and effective biological products for crops.

The present study was theoretical and based on the generalisation of scientific data, which may limit its application in practical agricultural technologies. The findings obtained require experimental confirmation in real field conditions to assess the effectiveness of the proposed biological products in various agroecosystems. Further research is recommended to focus on the experimental verification of theoretical conclusions through field trials in diverse soil and climatic conditions. A prominent area is to evaluate the effectiveness of microbial biological products in the long term, particularly their impact on the structure of the soil microbiome, plant productivity, and nutrient bioavailability. Additionally, it is advisable to investigate the genetic optimisation of microorganisms, specifically their competitiveness and resistance to stress factors, which will increase the stability and effectiveness of biological products in real agroecosystems.

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## CONFLICT OF INTEREST

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# Молекулярно-генетичні та біотехнологічні основи створення комплексних мікробних препаратів для збалансованого живлення рослин

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**Анотація**. Дослідження спрямоване на визначення молекулярно-генетичних механізмів, що регулюють функціональну активність мікробних препаратів у ризосфері рослин. Методологія включала аналіз експресії ключових генів, порівняння ефективності різних штамів мікроорганізмів у ґрунтовому середовищі, узагальнення факторів, що впливають на стабільність біопрепаратів, та систематизацію даних для встановлення взаємозв'язку між генетичними механізмами та агрономічними показниками. Отримані результати підтвердили, що експресія ключових генів, відповідальних за фіксацію азоту, мобілізацію фосфору та синтез біологічно активних сполук, визначає ефективність біологічних препаратів у ґрунтовому середовищі, що було верифіковано шляхом генетичного аналізу функціональних маркерів, мікробіологічного дослідження життєздатності штамів і аналітичного визначення вмісту доступних елементів живлення та продуктивності рослин. Комбінування азотфіксуючих, фосфатмобілізуючих і біостимулюючих мікроорганізмів забезпечувало формування стабільних мікробних спільнот, здатних підвищувати біодоступність елементів живлення та продуктивність культур. При застосуванні Azospirillum brasilense на небобових культурах концентрація амонійних сполук у ризосфері зростала до 40 %, що супроводжувалося посиленням азотного живлення. Використання фосфатмобілізуючих бактерій дозволяло зменшити норму внесення фосфорних добрив на 25-40 % без втрати врожайності. Загалом, застосування біопрепаратів на зернових, бобових та овочевих культурах забезпечувало скорочення потреби у хімічних добривах на 20-30 % без зниження агрономічної ефективності. Стабілізація мікроорганізмів шляхом мікроінкапсуляції забезпечувала підвищену виживаність у ґрунтовому середовищі та рівномірне поширення в зоні ризосфери, тоді як спрей-сушіння дозволяло отримати порошкові форми біопрепаратів із тривалим строком зберігання. Поєднання мікробних препаратів із наноформами мінеральних добрив сприяло рівномірному вивільненню елементів живлення, що позитивно впливало на їх засвоєння, хоча точні коефіцієнти цього процесу у роботі не наведені. Практичне значення отриманих результатів полягає у можливості цілеспрямованої розробки біологічних препаратів із прогнозованою здатністю підвищувати засвоєння азоту та фосфору рослинами, знижувати потребу в мінеральних добривах та забезпечувати стабільну продуктивність сільськогосподарських культур в умовах інтенсивного землеробства

**Ключові слова:** продукти біотехнології; консорціум; фіксація азоту; мобілізація фосфору; біологічно активні речовини; мікроінкапсуляціяж експресія генів

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# Forest inventory assessment of plus trees of hornbeam-oak and pine forests within the Malopolissia district

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**Abstract**. Improvement of the selection criteria and further testing of plus trees of Scots pine requires systematic updating of measurement data, their detailed analysis, and constant monitoring of the created sites. A significant task is to create new experimental plots to investigate the hereditary characteristics of trees, especially in the context of forestry adaptation to climate change. A forest inventory assessment of plus trees of hornbeam-oak and pine forests within the Malopolissia district was conducted to establish the relationship between the height-diameter ratio at 1.3 m and the age of trees in different forest types based on the results of their selection. Standard statistical methods were employed to establish reliable results of analytical and comparative conclusions. Two indices were calculated for the analysis: $I_1$ – an indicator of age-related changes in the A/H/DbH ratio, and $I_2$ – an indicator

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of changes in the H/DbH/A ratio depending on age. These indices were calculated for plus trees, considering their age (A), height (H), and trunk diameter at a height of 1.3 m (DbH). The dependencies of changes in the height and diameter of plus trees with age were studied, the speific features of these dependencies for the two most widespread forest types were determined, two types of indices of these dependencies were analysed, and the conclusions about their statistically reliable suitability for modelling the parameters of plus tree selection within the study area were summarised. The parametric dependencies of the plus trees of the Malopolissia district of pine forests of this region of origin established in the study can be used as additional criteria for selecting trees for productivity with a sufficient level of their biotic stability. The study summarised the possibility of establishing the regions of origin of forest reproductive material based on parametric assessment of stands and plus trees within the Malopolissia district based on their belonging to autochthonous forest stands by origin. The relevance of the study was enhanced by the introduction of approaches to regionalisation of forest reproductive material, which involves determining the boundaries of the regions of origin and the boundaries of the corresponding distribution of forest reproductive material from them. The proposed approach to regionalisation is a prerequisite for obtaining and putting into circulation forest reproductive material based on the implementation of Directive 1999/105/EC of 22 December 1999 on the marketing of forest reproductive material in the regulatory framework of forest seed production and nursery stock

**Keywords:** plus trees; forest reproductive material; regionalisation of forest reproductive material; regions of origin and distribution; taxonomic indicators; forest types; geobotanical zoning

## INTRODUCTION

The issue of identification of Scots pine plus trees by region of origin is of particular relevance in the context of intensified use of selectively improved plantation forest seeds for reforestation. Such studies of the dynamics of the parameters of plus trees with age, along with long-term tests of their offspring for selected traits, will allow further assessing the potential transfer of biotic resistance to offspring in the region of origin. The significance and relevance of updating the origin of plus trees is supported by the need to adapt existing regulatory documents on forest seed production and nursery farming to the level of European integration requirements in the field of forest reproductive resources. Plus trees form the basis of plantation forest seed production, and their offspring play an essential role in forest reproduction, influencing the development of genetically balanced populations. Determining the boundaries of regions of origin and distribution areas for the creation of mapping schemes of regionalisation of forest reproductive material (FRM) is currently an urgent and at the same time unresolved task. Clarification of the boundaries of the regions of origin and distribution based on available silvicultural data and materials obtained during forest management, as well as certification of permanent forest seed stock (PFS), their monitoring and inventory, if properly analysed, will help to solve the problem in the context of the urgent need to regulate the regionalisation of forest reproductive material (FRM) of the main forest-forming species using forest basic material (FBM) in Ukraine. This study was based on the analysis of the correlation between height (H) and diameter at breast height (DbH), which is commonly used in foreign scientific literature. No information on the above issues related to plus trees was found in the public domain, but in other areas of modelling tree and stand characteristics based on H/DbH relationships, this approach is widely used. V. Blystiv *et al.* (2021a) considered the dependencies of the ratios of growth in height to diameter and the growth of tree crown habitus to be organically related processes of tree development in a stand, which can be assessed by the potential for a tree to use living space. This opens the possibility of developing the area of plus selection to increase the biotic stability of individual trees and stands and its verification, which was also noted by H. Krynytskyy *et al.* (2023).

The ratio of H and DbH during tree growth is a complex consequence-dependent process, which is also regulated by edaphic and climatic stress factors, according to F. Sağlam and O. Sakici (2024). However, these issues of forestry motivation have not been sufficiently studied to date. J. Wang *et al.* (2023) covered the prediction of the H/DbH relationship in the future due to the influence of climate factors under conditions of multifactorial uncertainty and is often linked to projection size and crown diameter (CD). The findings indicated close links between the indicators in the dynamics and the possibility of their forecasting, provided that concrete parameters of the influencing factors in a particular region are chosen (mainly an increase in average temperatures and a decrease in precipitation). J. Wang *et al.* (2023) established the dependencies of the H/DbH/DC allometry for indigenous and derived stands under varying edaphic and climatic conditions. The results were mostly verified for the issues under study, however, the prospect of forecasting for derivative (secondary) forests is not entirely certain and unambiguous, especially against the background of increased stress factors. This raises the issue of forest reproduction using the potential of plus trees and the identification of their autochthonousness in the region of origin. On this issue, O. Danchuk *et al.* (2024) propose to establish the boundaries of the regions of origin based on geobotanical zoning, which is based on the distribution of autochthonous forest cover, considering the potential of forest typology. The basic area for identifying regions of origin by the main forest-forming species is to assess the growth characteristics of provenance in test (geographical) cultures. I. Neyko *et al.* (2020) presented the results of such long-term trials, indicating the comparative characteristics of representatives of different origins, which is significant for developing an algorithm for establishing the boundaries of local and geographical populations. An actual area under study is the secondary selection of plus trees in the tested progeny of plus Scots pine trees for the reasons of recognising elite characteristics. V. Voitiuk *et al.* (2020) indicates the age of 38-40 years at which the elite status of plus Scots pine trees can be verified in Polissia, and therefore if the ortet is preserved at this time, the dynamics of its H/DbH with age and the correspondence to the forest type are significant for modelling selection rates in the region of its origin. According to H. Krynytskyy *et al.* (2021), one of the promising areas for determining the vitality (biotic stability) of trees is the determination of their dielectric properties, which can be used for rapid assessment of plus trees. J. Brichta *et al.* (2023) emphasises that an essential form of conservation of the forest genetic fund is the archives of clones and families, the purpose of which is to concentrate in one place and preserve the characteristics of plus trees in the form of offspring. Prompt recognition of elite characteristics, considering the analysis of H/DbH dynamics with age, including autochthonousness and biotic stability, allows ensuring their preservation in the archives of the forest gene pool. One of the key tasks of modern selection of plus trees from defined plastic populations is to create new forest seed plantations capable of providing a yield of genetically improved seeds. According to J. Quegwer *et al.* (2024), this will contribute to the development of the adaptive potential of newly created forests to climate change through the use of suitable forest reproductive material.

When selecting and certifying plus trees, the key parameters are diameter, height, and age, and therefore it is their ratio and dynamics that are the object of assessment and analysis in this study. In this context, the study was aimed at establishing relationships between the above biometric characteristics of Scots pine plus trees selected and certified within the geobotanical region – Malopolissia district of hornbeam-oak and pine forests.

## MATERIALS AND METHODS

In this study, the assessment covers plus trees of Scots pine selected by phenotype according to the requirements of the Forest Seed Production Guidelines (Los *et al.*, 2017). The trees were selected in forestry enterprises located within the Malopolissia geobotanical district of hornbeam-oak and pine forests. Most of the sampling dates back to 1973, 1974, 1978, and the 2015 sampling in the eastern part of the district, where the trees of the 1980s sampling have not been preserved, was also considered. The taxation indicators of individual trees and the characteristics of forest stands at the time of selection were obtained from tree certification passports maintained by the Lviv Forestry and Seed Laboratory and verified by the state register of plus trees in Lviv and Ternopil regions. Data for the following periods were obtained from the results of inventory work reported in Yu. Debryniuk *et al.* (2021), simultaneous inventory of PFSBs in Ternopil (2022) and Lviv (2024) oblasts coordinated by the State Organisation "Ukrainian Forest Breeding Centre" (SO "UkrFSC") (n.d.). The data of the latest inventories were verified using auxiliary monitoring materials and the electronic accounting database of SO "UkrFSC", forest management materials of the branches of the State Enterprise "Forests of Ukraine", information data from the Relational Database (RDB) and Geographic Information and Analytical System (GIAS), Ukrainian State Forest Management Project and Production Association (PA "Ukrderzhisproekt") (n.d.), as well as a publicly available map of geobotanical zoning of Ukraine, adapted to the regionalisation of FRM. The location of the plus trees in the plots where they were sampled is presented in Table 1.

***Table 1**. Characteristics of the plots where plus trees were selected (as of 2020)*

| Branch of FHE, FE | Forestry | Quarter | Allotment | Area, ha | Trees, pcs. | Number of measurements, times |
|---|---|---|---|---|---|---|
| Radekhivske | Lopatynske | 64 | 13 | 19.0 | 10 | 3 |
| Radekhivske | Vytkivske | 15 | 1 | 4.9 | 6 | 3 |
| Buske | Hrabivske | 14 | 1 | 2.4* | 13 | 2 |
| Brodivske | Berlynske | 83 | 4 | 0.6* | 4 | 2 |
| Kremenetske | Stizhotske | 42 | 2 | 13.0 | 10 | 2 |

***Note:** *the areas of the allotments during tree selection were much larger and decreased due to the fragmentation of allotments during the next forest management*

***Source:** Ukrainian State Forest Management Project and Production Association (n.d.)*

Generally accepted forest inventory methods were employed to collect and process experimental, documentary materials, and electronic databases. For comparison and modelling, analytical and comparative methods were employed, based on the modal characteristics presented by A. Bilous *et al.* (2022), as well as age-appropriate inventory data. The basis of the comparative analysis included the inventory dependencies between the heights and diameters of tree trunks and their age, the correspondence between the site classes and forest vegetation conditions (forest types), the established dynamics of inventory indicators depending on age (stand growth), correlations, and the establishment of reliable differences between the indicators between plus trees and forest plots within the defined forest fund areas. The proposed research algorithm involves comparing the plus features with average and model indicators, as well as assessing their compliance within a certain territory – the region of origin.

The following algorithm for analysing data conformity was employed in the study: plots (allocations) were selected within the regions of origin (geobotanical districts), where on average about five plus trees were identified on an area of more than 5 ha. The number of trees and area were refined using the RDB and GIAS data of the SE "Ukrderzhlisproekt". At the next stage, the initial data for the plus trees were prepared at the time of their allocation (certification), including the height, diameter, age, site class, and average inventory indicators of the respective allocation. Subsequently, analogous inventory data were obtained for these trees in subsequent periods, with geolocation clarification and confirmation of parameters using photographic documentation. As a result, the estimated ratios for the model plots were established at the time of allocation of plus trees (according to the data of passports) and after a certain period (based on the results of measurements and taxation characteristics), with comparison of indicators for normal (full) stands. The key element of the analytical and comparative method was the calculation of the indices $I_1$ and $I_2$, which were calculated as the ratio of age (A), height (H), and diameter at 1.3 m (DbH) for the plus trees:

$$I_1 = A/H/DbH, \quad (1)$$

$$I_2 = H/DbH/A, \quad (2)$$

where $I_1$ is the index of age change by the H/DbH ratio; $I_2$ is the index of change in the H/DbH ratio by age; A is the age of the tree, years; H is the height of the tree, m; DbH is the diameter of the tree at a height of 1.3 m, cm.

Formulas 1 and 2 are considered basic. They are also used for average and model stand indices, to compare the respective indices. The indices are used to determine the indicators of compliance with the region of origin and its identification within the region of distribution. Specifically, the correlation of H/DbH values for plus trees by forest type was estimated. The proposed methodology of H/DbH index analysis was considered for comparing inventory data between plots of different local ecopopulations. This allows establishing reliable differences for their identification by the criteria of origin, forest types, and to ensure further selection of plus trees.

## RESULTS

The study was conducted for the plus trees of Scots pine according to the proposed sampling option within the Western Ukrainian forest-steppe region of broadleaf-mixed forests belonging to the Malopolissia district of hornbeam-oak and pine forests. The motives and necessity of its separation were presented in V. Blystiv *et al.* (2021b). Five model plots were identified in pure pine forests, where the number of plus trees was four or more per plot. The study established the allometry dependencies (A/H/DbH) for these plus trees in native and derivative stands under different edaphic and climatic conditions. The results were presented for two types of forest – $C_2$-h-oP and $C_3$-h-oP, which differ in the hygrotope of the habitat. The assessment results for the wet hornbeam-oak-pine forest ($C_3$-h-oP) are presented in Tables 2, 3, 4 and Figs. 1, 2, 3 – for plots in Radekhivse and Buske branches of the SE "Forests of Ukraine". The results for a fresh hornbeam-oak-pine forest ($C_2$-h-oP) are presented in Tables 4, 5 and Figs. 3, 4 for the plots in Brodivske and Kremenetske branches. For each site, in the sections of discussion and general conclusions on the dynamics of inventory indicators and the use of the A/H/DbH index, shared characteristics, specific features, and suggestions were noted.

**Evaluation results for a wet hornbeam-oak-pine forest (C3-h-oP).** The estimation of plus trees based on the results of measurements and analysis is presented in Table 2 and Fig. 1.

***Table 2**. Dynamics of morphometric parameters of plus trees of Scots pine (Pinus sylvestris L.) in the trial area of Lopatynske forestry of the Radekhivske forestry and hunting enterprise*

| Plus tree No. | Year of selection, examination, Condition | Age at the time of selection and examination | Height, m | Diameter, cm | Index A/H/DbH*100 | Model value of the index | Difference with the model |
|---|---|---|---|---|---|---|---|
| Model data, 1973 | | 85 | 33.9 | 39.2 | | 6.396 | 0 |
| 1 | Plus features | 84 | 34.0 | 46.0 | 5.371 | 6.396 | -1.025 |
| 2 | Plus features | 84 | 39.0 | 50.0 | 4.308 | 6.396 | -2.088 |
| 3 | Plus features | 84 | 34.0 | 48.0 | 5.147 | 6.396 | -1.249 |

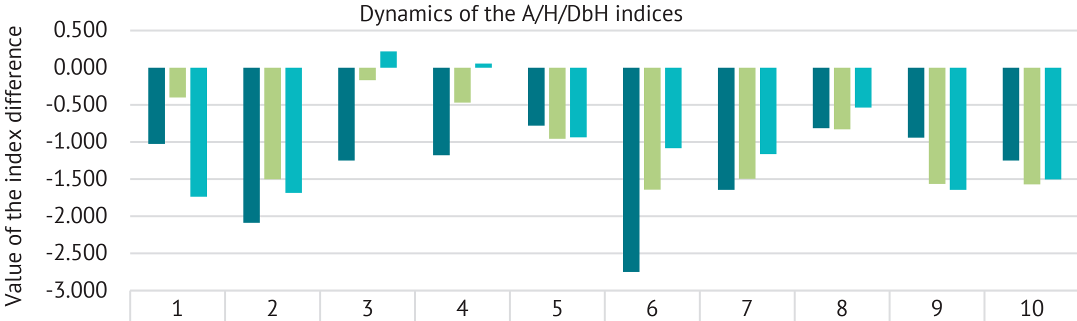
*Table 2. Continued*

| Plus tree No. | Year of selection, examination, Condition | Age at the time of selection and examination | Height, m | Diameter, cm | Index A/H/DbH*100 | Model value of the index | Difference with the model |
|---|---|---|---|---|---|---|---|
| 4 | Plus features | 84 | 35.0 | 46.0 | 5.217 | 6.396 | -1.179 |
| 5 | Plus features | 84 | 34.0 | 44.0 | 5.615 | 6.396 | -0.781 |
| 6 | Plus features | 84 | 36.0 | 64.0 | 3.646 | 6.396 | -2.750 |
| 7 | Plus features | 84 | 34.0 | 52.0 | 4.751 | 6.396 | -1.645 |
| 8 | Plus features | 84 | 35.0 | 43.0 | 5.581 | 6.396 | -0.815 |
| 9 | Plus features | 84 | 35.0 | 44.0 | 5.455 | 6.396 | -0.941 |
| 10 | Plus features | 84 | 34.0 | 48.0 | 5.147 | 6.396 | -1.249 |
| Mean | | | 35.0 | 48.5 | | | |
| Model data, 2004 | | 115 | 38.2 | 47.5 | | | |
| 1 | Satisfactory | 115 | 36.9 | 52.5 | 6.338 | 6.338 | 0.000 |
| 2 | Satisfactory | 115 | 41.0 | 58.0 | 5.936 | 6.338 | -0.402 |
| 3 | Satisfactory | 115 | 35.5 | 52.5 | 4.836 | 6.338 | -1.502 |
| 4 | Satisfactory | 115 | 40.0 | 49.0 | 6.170 | 6.338 | -0.168 |
| 5 | Satisfactory | 115 | 41.5 | 51.5 | 5.867 | 6.338 | -0.471 |
| 6 | Satisfactory | 115 | 39.5 | 62.0 | 5.381 | 6.338 | -0.957 |
| 7 | Satisfactory | 115 | 38.0 | 62.5 | 4.696 | 6.338 | -1.642 |
| 8 | Satisfactory | 115 | 36.0 | 58.0 | 4.842 | 6.338 | -1.496 |
| 9 | Satisfactory | 115 | 39.5 | 61.0 | 5.508 | 6.338 | -0.830 |
| 10 | Satisfactory | 115 | 36.0 | 67.0 | 4.773 | 6.338 | -1.565 |
| Mean | | | 38.4 | 57.4 | | | |
| Model data, 2024 | | 135 | 40.1 | 51.1 | 6.588 | 6.588 | 0.000 |
| 1 | Satisfactory | 135 | 37.0 | 75.2 | 4.852 | 6.588 | -1.736 |
| 2 | Satisfactory | 135 | 40.5 | 68.0 | 6.207 | 6.588 | -1.686 |
| 3 | Satisfactory | 135 | 33.5 | 59.2 | 6.807 | 6.588 | 0.219 |
| 4 | Satisfactory | 135 | 34.5 | 58.9 | 6.644 | 6.588 | 0.056 |
| 5 | Satisfactory | 135 | 37.5 | 63.7 | 5.651 | 6.588 | -0.937 |
| 6 | Dries out | 135 | 35.5 | 69.1 | 5.503 | 6.588 | -1.085 |
| 7 | Satisfactory | 135 | 34.0 | 73.2 | 5.424 | 6.588 | -1.164 |
| 8 | Satisfactory | 135 | 33.5 | 66.6 | 6.051 | 6.588 | -0.537 |
| 9 | Satisfactory | 135 | 36.5 | 74.8 | 4.945 | 6.588 | -1.643 |
| 10 | Satisfactory | 135 | 35.5 | 74.8 | 5.084 | 6.588 | -1.504 |
| Mean | | | 35.5 | 67.4 | | | |

***Source:*** *developed by the authors of this study*

The trees are located on a plot in the Lopatynske forestry, quarter 64, allotment 13. The inventory characteristics of the allotment according to the forest inventory as of 2010 were as follows: area – 19.0 ha; stand composition – 10Ps + Qr, Cb; age – 113 years; reserve – 400 $m^3 \cdot ha^{-1}$; relative density – 0.55; forest type – $C_3$-h-oP. According to the results of the last forest management, due to uneven density (reserve), the allotment was divided into several plots: quarter 64, allotment 19 – area of 4.1 ha; stand composition – 10Ps; age – 113 years; reserve – 200 $m^3 \cdot ha^{-1}$; relative density – 0.3. Quarter 64, allotment 24 – area 3.3 ha; stand composition – 10Ps; age – 113 years; reserve – 285 $m^3 \cdot ha^{-1}$; relative density – 0.4. Quarter 64, allotment 25 – area 3.3 ha; stand density – 10 Ps; age – 113 years; reserve – 260 $m^3 \cdot ha^{-1}$; relative density – 0.4. Quarter 64, allotment 26 – area 15.8 ha; stand composition – 10Ps + Qr, Cb; age – 113 years; reserve – 420 $m^3 \cdot ha^{-1}$; relative density – 0.55. The same forest type is preserved for all plots – $C_3$-h-oP. Over the past decade, there has been a decrease in reserves and a decrease in stand density.

The results of the analysis for the plot located in the Lopatynske forestry of the Radekhivske Forestry and Hunting Enterprise branch showed that the stands of Scots pine are original and of the same age. On the test site, in a 107-year-old stand, the stand had a stock of 481 $m^3 \cdot ha^{-1}$ with an average height of 34 m and a diameter of 44.3 cm, with a density of 0.55 and belonging to the $I^b$ class. The origin of the stand is not known, but it is likely to be autochthonous. The local population of Scots pine has been preserved due to the site's belonging to the permanent forest seed base and the nature reserve fund and is characterised by a small participation of common beech in the stand. Its distribution area is at altitudes of 220-230 m. The analysis of the dynamics of H/DbH indices showed that during growth, plus trees effectively use the forest-typological potential of the habitat conditions. At the same time, at the age of about 135 years, there is a certain lagging behind the modal indicators of the I[b] class, which is conditioned by a substantial decrease in the stand density. This trend is confirmed by the data for trees No. 1, 2, 5, 7, 8, 9, and 10, which have almost not lost their relative growth rates in height and diameter. The second group of trees – Nos. 3, 4, and 6 – demonstrates a deceleration in growth, with tree No. 6 weakened to the IV category of sanitary condition. Plus trees No. 1, 2, 5, 7, 8, 9, and 10 require urgent cloning to preserve and consolidate their genetic potential in archives or on forest seed plantations within the region of origin. It is also necessary to clarify the information on the participation of the descendants of these trees in existing forest-seed plantations and test crops within the distribution area.




| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| 84 years | -1.025 | -2.088 | -1.249 | -1.179 | -0.781 | -2.750 | -1.645 | -0.815 | -0.941 | -1.249 |
| 115 years | -0.4018 | -1.502 | -0.1676 | -0.4707 | -0.9573 | -1.6422 | -1.4959 | -0.8303 | -1.5652 | -1.57 |
| 135 years | -1.7361 | -1.686 | 0.21918 | 0.05554 | -0.9365 | -1.0846 | -1.1637 | -0.5372 | -1.6433 | -1.504 |

***Figure 1**. Difference in the values of the plus tree indices compared to the modal values (according to Table 2)*
***Source:** developed by the authors of this study*

Overall, the biotic stability of plus trees is not always determined by the initial phenotypic traits but is largely determined by hereditary factors and the effect of interactions within the forest ecosystem. Thus, the native stands of Scots pine in $C_3$-h-oP conditions of Small Polissia may be of autochthonous origin and, accordingly, have the potential for high biotic stability, provided that complex multi-age stands with an admixture of oak, beech, and hornbeam are formed. The assessment of plus trees based on the results of measurements and analysis (Table 3) is presented in Fig. 2. The studied trees are located on the plot of Vytkivske forestry, quarter 15, allotment 1. The inventory characteristics of the plot according to the forest management data as of 2010 were as follows: area – 4.9 ha; stand composition – 10 Ps + Bp, Cb; age – 113 years; reserve – 391 $m^3 \cdot ha^{-1}$; relative density – 0.55; forest type – $C_3$-h-oP. The stand is native, single-aged, of unknown origin, with a low probability of autochthonousness. The analysis of the dynamics of H/DbH indices revealed that during growth, plus trees effectively used the forest-typological potential of forest-plant conditions up to the age of 105 years, reaching values close to the modal indicators of the I[a] class.

***Table 3**. Dynamics of morphometric parameters of plus trees of Scots pine (Pinus sylvestris L.) in the trial area of Vytkivske forestry of the Radekhivske Forestry and Hunting Enterprise branch*

| Plus tree No. | Year of selection, examination, Condition | Age at the time of selection and examination | Height, m | Diameter, cm | Index A/H/DbH*100 | Model value of the index | Difference with the model |
|---|---|---|---|---|---|---|---|
| Model data, 1973 | | 75 | 31.8 | 35.8 | | 6.588 | |
| 1 | Plus features | 75 | 31.0 | 41.0 | 5.901 | 6.588 | -0.687 |
| 2 | Plus features | 75 | 31.0 | 43.0 | 5.626 | 6.588 | -0.962 |
| 3 | Plus features | 75 | 31.0 | 48.0 | 5.040 | 6.588 | -1.548 |
| 4 | Plus features | 75 | 31.0 | 48.0 | 5.040 | 6.588 | -1.548 |
| 5 | Plus features | 75 | 32.0 | 43.0 | 5.451 | 6.588 | -1.137 |
| 6 | Plus features | 75 | 34.0 | 44.0 | 5.013 | 6.588 | -1.575 |
| Mean | | | 31.6 | 44.5 | | | |
| Model data, 2004 | | 105 | 33.3 | 40.0 | | | |
| 1 | Satisfactory | 106 | 38.0 | 51.0 | 5.470 | 7.783 | -2.313 |
| 2 | Satisfactory | 106 | 31.5 | 54.5 | 6.174 | 7.783 | -1.609 |
| 3 | Satisfactory | 106 | 36.0 | 60.5 | 4.867 | 7.783 | -2.916 |
| 4 | Satisfactory | 106 | 38.5 | 61.5 | 4.477 | 7.783 | -3.306 |

*Table 3. Continued*

| Plus tree No. | Year of selection, examination, Condition | Age at the time of selection and examination | Height, m | Diameter, cm | Index A/H/DbH*100 | Model value of the index | Difference with the model |
|---|---|---|---|---|---|---|---|
| 5 | Satisfactory | 106 | 34.5 | 51.5 | 5.966 | 7.783 | -1.817 |
| 6 | Satisfactory | 106 | 34.5 | 54.0 | 5.690 | 7.783 | -2.093 |
| Mean | | | 35.5 | 36.5 | | | |
| Model data, 2024 | | 125 | 35.3 | 44.3 | | | |
| 1 | Dries out | 126 | 38.1 | 54.6 | 6.057 | 7.993 | -1.936 |
| 2 | Dries out | 126 | 33.8 | 58.2 | 6.405 | 7.993 | -1.588 |
| 3 | Dries out | 126 | 36.6 | 66.5 | 5.177 | 7.993 | -2.816 |
| 4 | Satisfactory | 126 | 39.0 | 70.4 | 4.589 | 7.993 | -3.404 |
| 5 | Dries out | 126 | 34.6 | 62.4 | 5.836 | 7.993 | -2.157 |
| 6 | Dries out | 126 | 35.0 | 56.5 | 6.372 | 7.993 | -1.621 |
| Mean | | | 36.2 | 61.4 | | | |

***Source:*** *developed by the authors of this study*

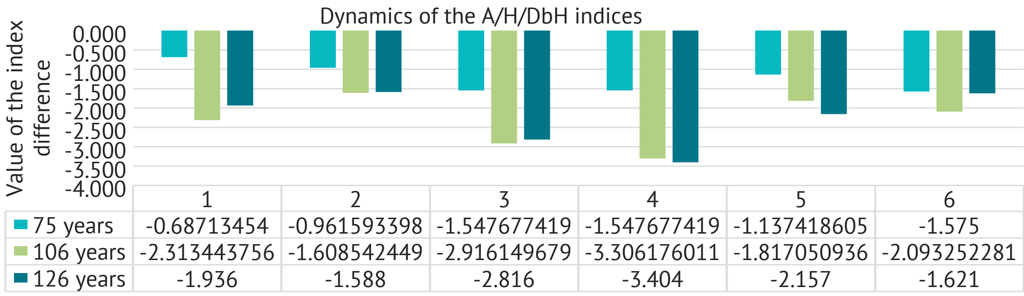



***Figure 2****. Difference in the values of the plus tree indices compared to the modal values (according to Table 3)*
***Source:*** *developed by the authors of this study*

At the age of 126 years, with a slight decrease in stand completeness, the best (plus) trees are observed to be weakened and dry out, indicating that the growth potential in these forest vegetation conditions is at its maximum. For plus trees, information on the participation of their breeding heritage in forest seed plantations and test crops within the region of distribution needs to be clarified. The increased biotic stability of these plus trees cannot be considered a confirmed stable plus hereditary trait. Thus, highly productive indigenous stands of Scots pine in the edaphic climatic conditions of Malopolissia may not be of autochthonous origin. It is advisable to use their offspring to create plantation-type forest crops to accelerate the production of business varieties. However, to substantiate this conclusion, it is necessary to conduct a detailed study of the factual state of the stand in this area, as well as its forest vegetation conditions.

The assessment of plus trees based on the results of measurements and analysis presented in Table 4 and Fig. 3. The trees are located on the plot in the Berlynske forestry, quarter 83, allotment 4, and were collected in 1974 at the age of 80 years. Inventory characteristics of the stand according to forest management materials as of 2010 were as follows: area – 0.7 ha (at the time of selection, the area was larger); stand composition – 10 Ps + Fs, Qr; age – 117 years; reserve – 390 $m^3 \cdot ha^{-1}$; relative density – 0.6; forest type – $C_3$-h-oP. As of 2024, new plus trees were selected on the adjacent allotment (quarter 83, allotment 4) with an area of 3.8 ha, with a stand composition of 10Ps + Qr + Cb, age 113 years, reserve of 391 $m^3 \cdot ha^{-1}$, relative density 0.55 and forest type $B_3$-oP.

***Table 4****. Dynamics of morphometric parameters of plus trees of Scots pine (Pinus sylvestris L.) on the trial area of the Berlynske forestry of the Brodivske Forestry Enterprise branch*

| Plus tree No. | Year of selection, examination, Condition | Age at the time of selection and examination | Height, m | Diameter, cm | Index A/H/DbH*100 | Model value of the index | Difference with the model |
|---|---|---|---|---|---|---|---|
| Model data, 1974 | | 80 | 29.4 | 33.4 | 8.147 | | |
| 1 | plus features | 80 | 29.0 | 44.0 | 6.270 | 8.147 | -1.877 |
| 2 | plus features | 80 | 30.0 | 38.0 | 7.018 | 8.147 | -1.129 |
| 3 | plus features | 80 | 30.0 | 47.0 | 5.674 | 8.147 | -2.473 |
| 4 | plus features | 80 | 30.0 | 41.0 | 6.504 | 8.147 | -1.643 |

*Table 4. Continued*

| Plus tree No. | Year of selection, examination, Condition | Age at the time of selection and examination | Height, m | Diameter, cm | Index A/H/DbH*100 | Model value of the index | Difference with the model |
|---|---|---|---|---|---|---|---|
| Mean | | | 29.75 | 42.5 | | | |
| | Model data, 2004 | 110 | 32.6 | 38.9 | 8.674 | 8.674 | |
| 1 | satisfactory | 110 | 32.0 | 46.0 | 7.473 | 8.674 | -1.201 |
| 2 | satisfactory | 110 | 32.0 | 40.0 | 8.594 | 8.674 | -0.080 |
| 3 | satisfactory | 110 | 30.5 | 49.0 | 7.514 | 8.674 | -1.160 |
| 4 | satisfactory | 110 | 31.6 | 46.0 | 7.567 | 8.674 | -1.107 |
| Mean | | | 31.5 | 45 | | | |

***Source:*** *developed by the authors of this study*

The stand was native in composition, single-aged. The stand was of unknown origin, with a low probability of autochthonousness. The nature of the dependence of changes in the A/H/DbH indices suggests that during growth, the plus trees effectively used the forest-typological potential of forest-plant conditions up to 110 years of age, close to the modal indicators of the I[a] class. By the age of 130 years, with a relatively slight decrease in stand completeness, the best (plus) trees are significantly weakened and dry out, indicating that the growth potential under these conditions is at its limit. In 2020, trees were excluded from the register of plus trees due to their drying out in previous years.

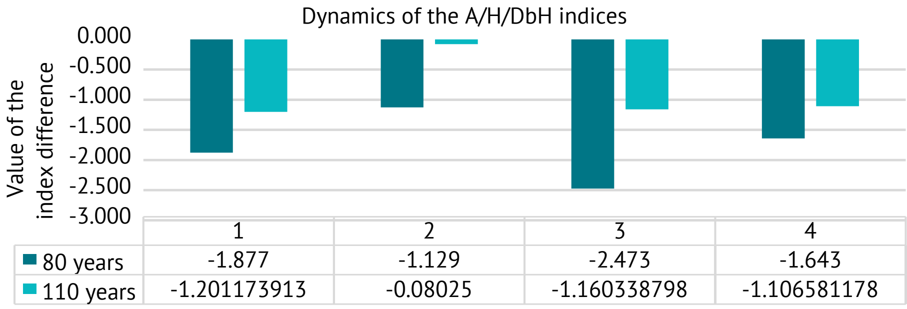



***Figure 3****. Difference in the values of the plus tree indices compared to the modal values (according to Table 4)*
***Source:*** *developed by the authors of this study*

For plus trees, information on the involvement of their heredity in forest-seed plantations and test crops in the region of distribution needs to be clarified, since these trees did not have pronounced plus traits in terms of productivity and proved to be biotically unstable. Overall, there is reason to believe that the biotic stability of plus trees within this area does not manifest itself as a hereditary trait that determines their plus value when they reach a height of 30 m or more. Such native stands of Scots pine in the edapho-climatic conditions of Malopolissia may not be of autochthonous origin. At the age of 120 years, they cannot withstand a sharp change in forest and vegetation conditions (e.g., the emergence and development of forest beech under the canopy) or changes in the functional properties of the forest environment. The targeted use of plantation offspring of such trees during reforestation requires further analysis of their evaluation in test cultures or more detailed specification of their origin.

**Evaluation results for a fresh hornbeam-oak-pine forest ($C_2$-h-oP).** The estimation of plus trees based on the results of measurements and analysis presented in Tables 5 and 6 and in Figs. 4 and 5. Data for one sample plot at the time of tree selection are presented in two tables with a five-year interval. The trees were located in the plot of Hrabivske forestry, quarter 18, allotment 3, and were collected in 1973 and 1978, which led to a separate analysis for each group. Inventory characteristics of the allotment according to forest management materials as of 2020 were as follows: area – 10.5 ha; stand composition – 10Ps; reserve – 400 $m^3 \cdot ha^{-1}$; density – 0.7; forest type – $C_2$-h-oP. The stand was of the native type, single-aged, and its composition corresponded to the forest and vegetation conditions of the site. The origin of the stand was not reliably established, with a low probability of autochthonousness. In 2018–2020, the trees were excluded from the registers due to the drying process. According to the data presented in Tables 4 and 5 and in Figs. 3 and 4, the nature of the dependence of changes in the A/H/DbH indices indicates that plus trees in the fresh forest are more vulnerable to prolonged periods of drought compared to trees growing in wet hygrotopes. For these plus trees, information on the involvement of their breeding heritage in forest seed plantations in the region of distribution needs to be clarified, as they have not been tested in test crops. The biotic stability of these plus trees is probably not a heritable plus trait in these forest conditions after they reach 100 years of age and a height of 30 m or more.

***Table 5**. Dynamics of morphometric parameters of plus trees of Scots pine (Pinus sylvestris L.) on the trial area of Hrabivske forestry of the Buske Forestry Enterprise branch (1973 selection)*

| Plus tree No. | Year of selection, examination, Condition | Age at the time of selection and examination | Height, m | Diameter, cm | Index A/H/ DbH*100 | Model value of the index | Difference with the model |
|---|---|---|---|---|---|---|---|
| Model data, 1973 | | 85 | 33.9 | 39.2 | | | |
| 1 | Plus features | 85 | 34.0 | 57.0 | 4.386 | 6.396 | -2.010 |
| 2 | Plus features | 85 | 35.0 | 54.0 | 4.497 | 6.396 | -1.899 |
| 3 | Plus features | 85 | 34.0 | 59.0 | 4.237 | 6.396 | -2.159 |
| 4 | Plus features | 85 | 33.0 | 55.0 | 4.683 | 6.396 | -1.713 |
| 5 | Plus features | 85 | 35.0 | 64.0 | 3.795 | 6.396 | -2.601 |
| 6 | Plus features | 85 | 33.0 | 56.0 | 4.600 | 6.396 | -1.796 |
| 7 | Plus features | 85 | 33.0 | 50.0 | 5.152 | 6.396 | -1.244 |
| 8 | Plus features | 85 | 34.0 | 65.0 | 3.846 | 6.396 | -2.550 |
| Mean | | | 33.9 | 55.5 | | | |
| Model data, 2004 | | 115 | 38.3 | 47.5 | | | |
| 1 | Satisfactory | 116 | 35.0 | 63.0 | 5.261 | 6.321 | -1.060 |
| 2 | Satisfactory | 116 | 35.0 | 60.5 | 5.478 | 6.321 | -0.843 |
| 3 | Satisfactory | 116 | 30.5 | 51.0 | 7.457 | 6.321 | 1.136 |
| 4 | Satisfactory | 116 | 38.5 | 62.5 | 4.821 | 6.321 | -1.500 |
| 5 | Satisfactory | 116 | 36.0 | 67.0 | 4.809 | 6.321 | -1.512 |
| 6 | Satisfactory | 116 | 37.0 | 67.5 | 4.645 | 6.321 | -1.676 |
| 7 | Satisfactory | 116 | 37.5 | 58.5 | 5.288 | 6.321 | -1.033 |
| 8 | Satisfactory | 116 | 35.0 | 73.5 | 4.509 | 6.321 | -1.812 |
| Mean | | | 35.9 | 61.2 | | | |

***Source:** developed by the authors of this study*

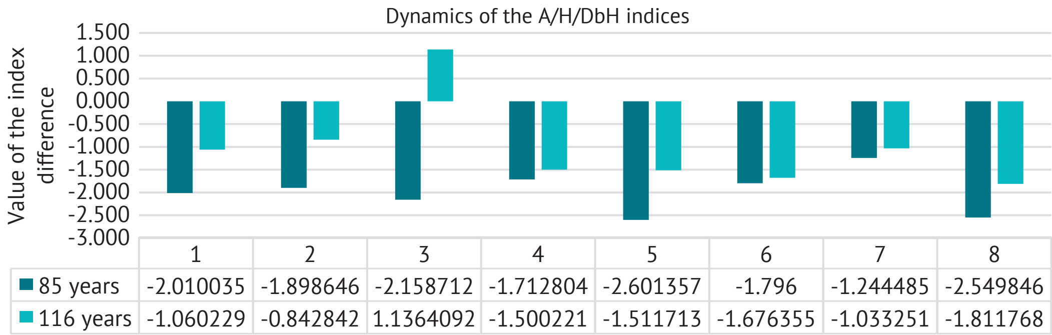



***Figure 4**. Difference in the values of the plus tree indices compared to the modal values (according to Table 5)*
***Source:** developed by the authors of this study*

Therefore, such native stands of Scots pine in the edapho-climatic conditions of Lesser Polissia may be of both autochthonous and, for the most part, non-autochthonous origin. Considering this, the selected new trees require more detailed monitoring of the state and dynamics of the parametric characteristics of the plus traits (Table 6, Fig. 5).

The results of the assessment of plus trees based on measurements and analysis are presented in Table 7 and Fig. 6. The trees are located on a plot of 13.0 ha belonging to the Kremenetske Forestry Enterprise branch, Stizhotske Forestry, quarter 42, allotment 2. The trees were selected in 2015 at the age of 88 years. Inventory characteristics of the stand according to forest management materials as of 2018 were as follows: stand composition – 10Ps + Cb; age – 90 years; reserve – 382 $m^3 \cdot ha^{-1}$; relative density – 0.62; forest type – $C_2$-h-oP. The stand of Scots pine is native in composition, single-aged, complex in structure, with a predominance of common hornbeam in the second tier. The origin of the stand has not been reliably established, but there is a high probability of its autochthonous nature.

***Table 6**. Dynamics of morphometric parameters of plus trees of Scots pine (Pinus sylvestris L.) in the trial area of Hrabivske forestry of the Buske Forestry Enterprise branch (1978 selection)*

| Plus tree No. | Year of selection, examination, Condition | Age at the time of selection and examination | Height, m | Diameter, cm | Index A/H/DbH*100 | Model value of the index | Difference with the model |
|---|---|---|---|---|---|---|---|
| Model data, 1978 | | 90 | 34.8 | 40.8 | | 6.339 | |
| 1 | Plus features | 90 | 33.0 | 56.0 | 4.870 | 6.339 | -1.469 |
| 2 | Plus features | 90 | 32.5 | 50.0 | 5.538 | 6.339 | -0.801 |
| 3 | Plus features | 92 | 32.0 | 48.0 | 5.990 | 6.339 | -0.349 |
| 4 | Plus features | 92 | 33.0 | 50.0 | 5.576 | 6.339 | -0.763 |
| 5 | Plus features | 92 | 32.5 | 48.0 | 5.897 | 6.339 | -0.442 |
| Mean | | | 32.6 | 50.4 | | | |
| Model data, 2004 | | 115 | 38.3 | 47.5 | | | |
| 1 | Satisfactory | 116 | 39.5 | 59.0 | 4.977 | 6.321 | -1.344 |
| 2 | Satisfactory | 116 | 38.0 | 60.0 | 5.088 | 6.321 | -1.233 |
| 3 | Satisfactory | 116 | 40.5 | 63.5 | 4.511 | 6.321 | -1.810 |
| 4 | Satisfactory | 116 | 44.0 | 59.0 | 4.468 | 6.321 | -1.853 |
| 5 | Satisfactory | 116 | 42.0 | 61.0 | 4.528 | 6.321 | -1.793 |
| Mean | | | 40.8 | 60.5 | | | |

***Source:** developed by the authors of this study*

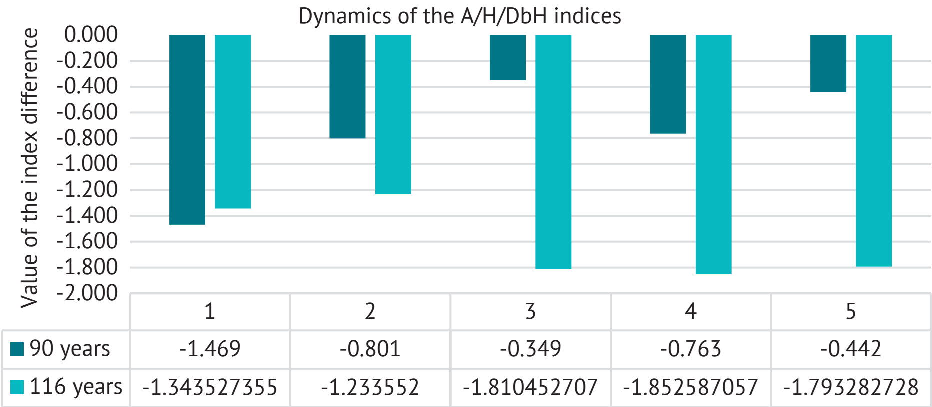



***Figure 5**. Difference in the values of the plus tree indices compared to the modal values (according to Table 6)*
***Source:** developed by the authors of this study*

***Table 7**. Dynamics of morphometric parameters of plus trees of Scots pine (Pinus sylvestris L.) on the trial area of Stizhotske forestry of the Kremenetske Forestry Enterprise branch*

| Plus tree No. | Year of selection, examination, Condition | Age at the time of selection and examination | Height, m | Diameter, cm | Index A/H/DbH*100 | Model value of the index | Difference with the model |
|---|---|---|---|---|---|---|---|
| Model data, 2015 | | 90 | 31.2 | 36.3 | | 7.947 | |
| 1 | Plus features | 88 | 32 | 52 | 5.288 | 7.947 | -2.659 |
| 2 | Plus features | 88 | 31 | 45 | 6.308 | 7.947 | -1.639 |
| 3 | Plus features | 88 | 32 | 52 | 5.288 | 7.947 | -2.659 |
| 4 | Plus features | 88 | 32 | 52 | 5.288 | 7.947 | -2.659 |
| 5 | Plus features | 88 | 31 | 52 | 5.459 | 7.947 | -2.488 |
| 6 | Plus features | 88 | 33 | 52 | 5.128 | 7.947 | -2.819 |
| 7 | Plus features | 88 | 32 | 44 | 6.250 | 7.947 | -1.697 |
| 8 | Plus features | 88 | 31 | 46 | 6.171 | 7.947 | -1.776 |
| 9 | Plus features | 88 | 30 | 44 | 6.667 | 7.947 | -1.280 |
| 10 | Plus features | 88 | 31 | 54 | 5.257 | 7.947 | -2.690 |

*Table 7. Continued*

| Plus tree No. | Year of selection, examination, Condition | Age at the time of selection and examination | Height, m | Diameter, cm | Index A/H/DbH*100 | Model value of the index | Difference with the model |
|---|---|---|---|---|---|---|---|
| Mean | | | 31.5 | 49.3 | | | |
| Model data, 2024 | | | | | | | |
| 1 | Satisfactory | 97 | 31 | 62 | 5.047 | 7.886 | -2.839 |
| 2 | Satisfactory | 97 | 33 | 52 | 5.653 | 7.886 | -2.233 |
| 3 | Satisfactory | 97 | 33 | 60 | 4.899 | 7.886 | -2.987 |
| 4 | Satisfactory | 97 | 32 | 62 | 4.889 | 7.886 | -2.997 |
| 5 | Satisfactory | 97 | 33 | 56 | 5.249 | 7.886 | -2.637 |
| 6 | Satisfactory | 97 | 33 | 64 | 4.593 | 7.886 | -3.293 |
| 7 | Satisfactory | 97 | 32 | 52 | 5.829 | 7.886 | -2.057 |
| 8 | Satisfactory | 97 | 31 | 52 | 6.017 | 7.886 | -1.869 |
| 9 | Satisfactory | 97 | 33 | 54 | 5.443 | 7.886 | -2.443 |
| 10 | Satisfactory | 97 | 33 | 62 | 4.741 | 7.886 | -3.145 |
| Mean | | | 32.4 | 57.6 | | | |

***Source:*** *developed by the authors of this study*

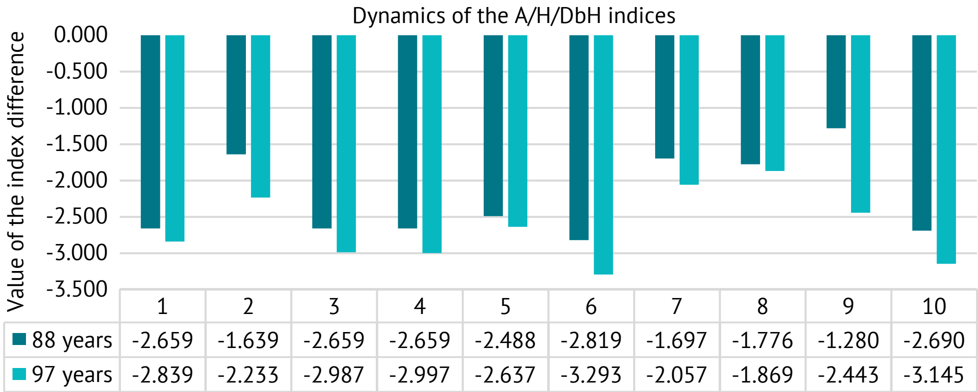



***Figure 6****. Difference in the values of the plus tree indices compared to the modal values (according to Table 7)*
***Source:*** *developed by the authors of this study*

The nature of the dependence of changes in the A/H/DbH indices suggests that plus trees during growth effectively use the forest-typological potential of forest-vegetation conditions in the age range of 90-100 years, approaching the modal values of the I[a] class. At the age of up to 98 years, under conditions of a relatively slight decrease in the relative completeness of the stand, there is a uniform dynamics of deviations of indicators compared to the modal values, with the exception of tree No. 9. For the plus trees listed in Table 7, information on their heredity in the formation of forest-seed plantations in the region of distribution needs to be clarified, since the progeny of these trees were not tested in test crops. The increased biotic stability of these plus trees is not a reliably established heritable plus trait after they reach 100 years of age and a height of more than 30 metres. However, further monitoring and testing of their offspring in more extreme conditions may confirm or refute this assumption. Thus, indigenous stands of Scots pine in the edaphic and climatic conditions of Malopolissia may be of both autochthonous and non-autochthonous origin, which necessitates further monitoring of their condition and parametric characteristics of plus traits.

The summary information of the assessment of the development of plus trees by plots allows discussing and identifying the key features of the results. The growth of a plus tree in height and thickness with age and its condition under certain parameters specific to each forest-forming tree species can serve as an assessment of the use of forest vegetation conditions and one of the indicators of confirmation of the assignment of forest stands to the established forest types and stand types, O. Danchuk *et al.* (2024). Another area of research is the analysis and comparison of the dynamics of the H/DbH ratio by dominant height, which can use both data on plus trees and average taxonomic characteristics of stands in model plots. Analogous relationships (regression models) were described by F. Sağlam *et al.* (2024) to predict timber reserves for certain time intervals in different forest-ecological conditions, which allows refining the data of growth tables. The ratio of tree heights and diameters in the dynamics with age is presented in Fig. 7. The results confirmed that the correlation between tree height and age is the lowest among the studied indicators. This indicates a substantial dependence of the height of Scots pine plus trees on habitat conditions and forest type, as well as a pronounced heterogeneity of stands of this species, where trees were selected based on their origin.

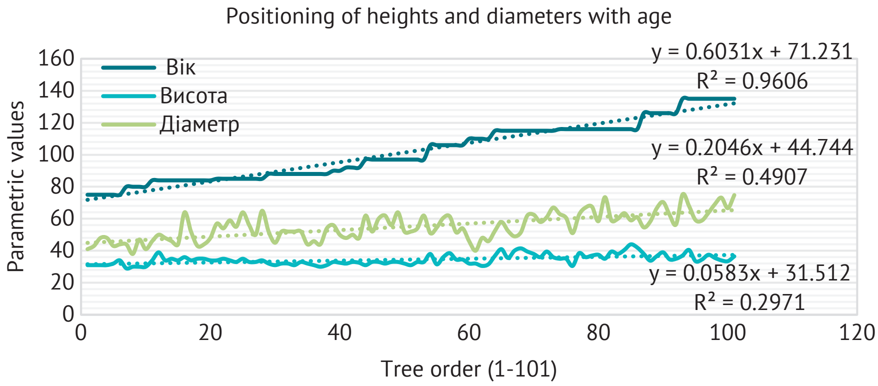



***Figure 7****. Ratio of heights and diameters of plus trees with increasing stand age*
***Source:*** *developed by the authors of this study*

Table 8 presents the correlation matrix between age, height, diameter and $I_1$ indices for the entire array of plus trees in the study area.

The correlation analysis shows that the $I_2$ index has a negative relationship with tree age, with a total correlation coefficient of -0.87, which indicates the presence of an inverse relationship of a significant level. For forest type $C_2$-h-oP, this indicator is -0.785, and for $C_3$-h-oP – -0.948, indicating that the $I_2$ index is more informative about the parametric characteristics of trees than the $I_1$ index. The $I_1$ index, on the other hand, proved to be more suitable for analysing the growth process (dynamics) of trees and was used to assess the biotic stability of plus trees. Figs. 8, 9, and 10 show the variance of height, diameter, $I_1$ index, and age of plus trees with the corresponding linear regression equations.

***Table 8****. Correlation matrix of parameters of plus trees and their indices* $I_1$

| Correlation matric | Age | Height | Diamere | Index $I_1$ |
|---|---|---|---|---|
| Age | 1 | 0.554 | 0.769 | 0.136 |
| Height | 0.554 | 1 | 0.506 | 0.330 |
| Diameter | 0.769 | 0.769 | 1 | 0.398 |
| Index | 0.136 | 0.330 | 0.398 | 1 |

***Source:*** *developed by the authors of this study*

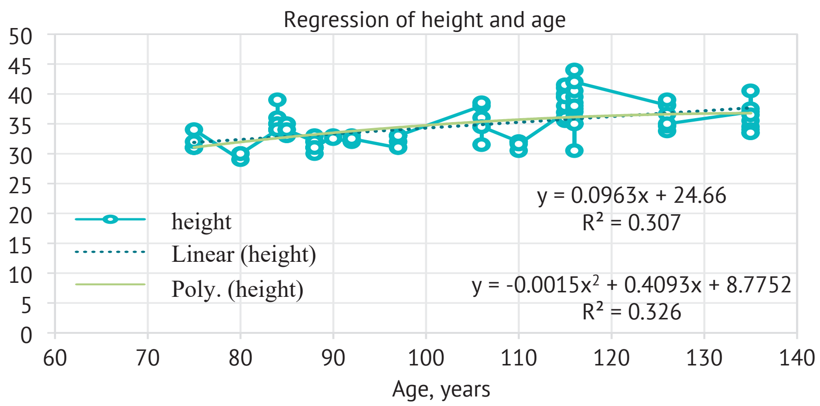



***Figure 8****. Positioning heights and ages of plus trees with stand age*
***Source:*** *developed by the authors of this study*

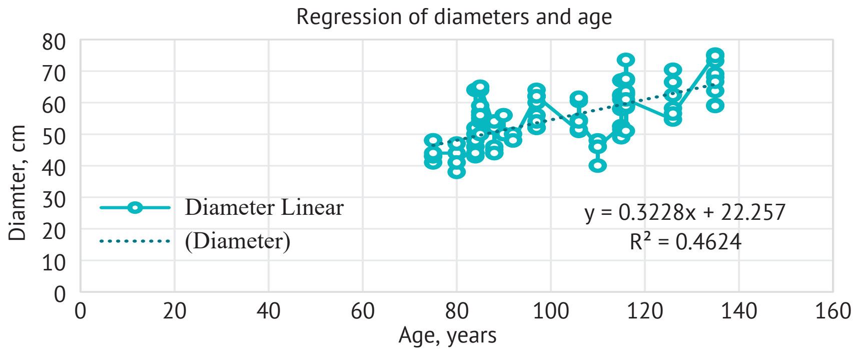



***Figure 9**. Positioning diameters and ages of plus trees with stand age*
***Source:** developed by the authors of this study*

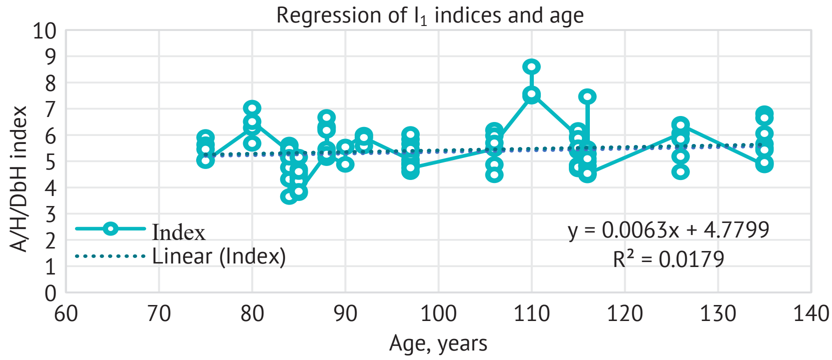



***Figure 10**. Positioning of indices and age of plus trees with stand age*
***Source:** developed by the authors of this study*

Figs. 8, 9, 10 show the trend lines of the one-factor linear regression with the corresponding coefficients of determination. Fig. 10 visualises the variance of indices and age, and Table 8 presents the statistical indicators of these series.

The analysis of variance based on Fisher's criterion and its significance ($F = 1.802083$; $p = 0.182531$) with a low correlation coefficient does not allow proposing an adequate model of the parametric indicators of plus trees according to the $I_1$ index. It also does not make practical sense due to the variability of forest-typological conditions of stand growth. Clearly, the use of indices is suitable only for modelling within specific forest types. In this regard, Table 10 shows the distribution of values of the $I_1$ index (A/H/DbH) by forest type, and adds the value of the $I_2$ index (H/DbH/A), which turned out to be more suitable for modelling tree parameters depending on age.

***Table 9**. Statistical information on age estimation and $I_1$ index for plus trees*

| Statistical indicators | Age, years | Index $I_1$ |
|---|---|---|
| Mean value | 102 | 5.425054 |
| Standard error | 1.8 | 0.084873 |
| Standard deviation | 18.0 | 0.852963 |
| The greatest value | 135 | 8.593750 |
| The smallest value | 75 | 3.645833 |

***Source:** developed by the authors of this study*

***Table 10**. Values of $I_1$ (A/H/DbH) and $I_2$ (H/DbH/A) indices in different forest types*

| Index | Forest type | Average value by forest type | Average value of all | The smallest of all | The greatest of all |
|---|---|---|---|---|---|
| $I_1$ | $C_3$-h-oP | 5.618 | 5.425 | 3.645 | 8.593 |
| | $C_2$-h-oP | 5.182 | 5.425 | 3.645 | 8.593 |
| $I_2$ | $C_3$-h-oP | 0.654 | 0.644 | 0.344 | 1.030 |
| | $C_2$-h-oP | 0.632 | 0.644 | 0.344 | 1.030 |

***Source:** developed by the authors of this study*

Figs. 11 and 12 show the regression dependencies of tree age on the $I_2$ index by forest type. The obtained relatively high coefficients of determination ($R^2 = 0.617$ and $R^2 = 0.897$) indicate a significant relationship, which allows proposing suitable models for predicting tree parameters depending on age.

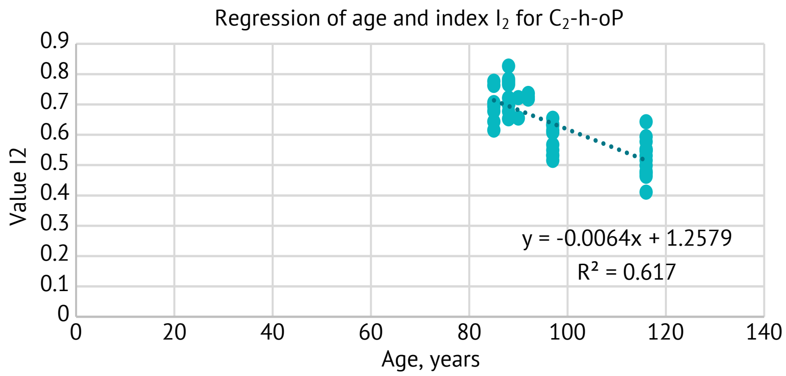



***Figure 11****. Positioning of indices and ages of plus trees with the age of stands (average age of trees – 98 years)*
***Source:*** *developed by the authors of this study*

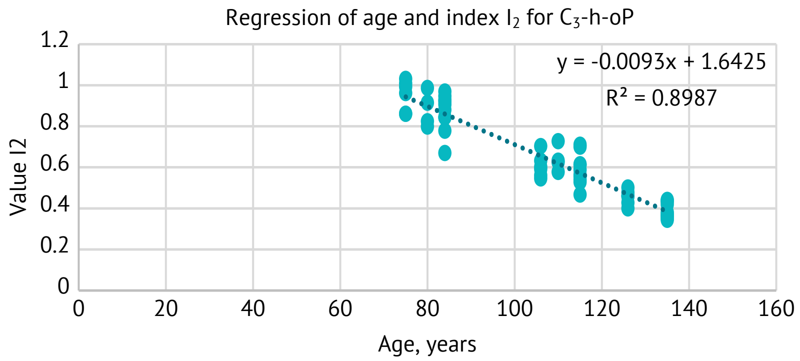



***Figure 12****. Positioning of indices and ages of plus trees with the age of stands (average age of trees – 98 years)*
***Source:*** *developed by the authors of this study*

The obtained equations can be used to form the parameters of plus trees by age through the value of the index $I_2$. Figs. 13 and 14 show the dynamics of the decrease in the values of the $I_2$ index as the age of the stands increases, separately for each forest type. The obtained equations of trend dependencies can be used to predict the parameters of plus trees by their age through the value of the $I_2$ index. The graph shows that as the age of trees in a given forest type increases, there is a gradual and stable decrease in the $I_2$ index, which illustrates the process of age-related deterioration of indicators related to tree growth or quality.

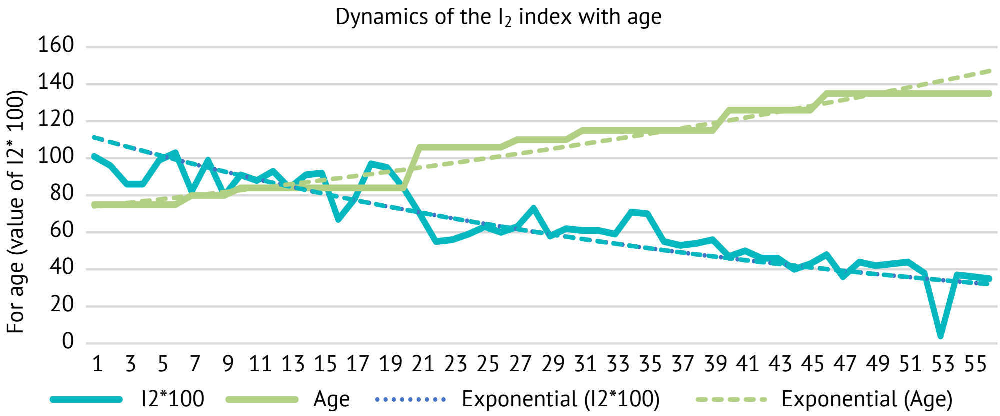



***Figure 13****. Positioning of index reduction with increasing age of plus trees (in conditions of forest type $C_3$-h-oP)*
***Source:*** *developed by the authors of this study*

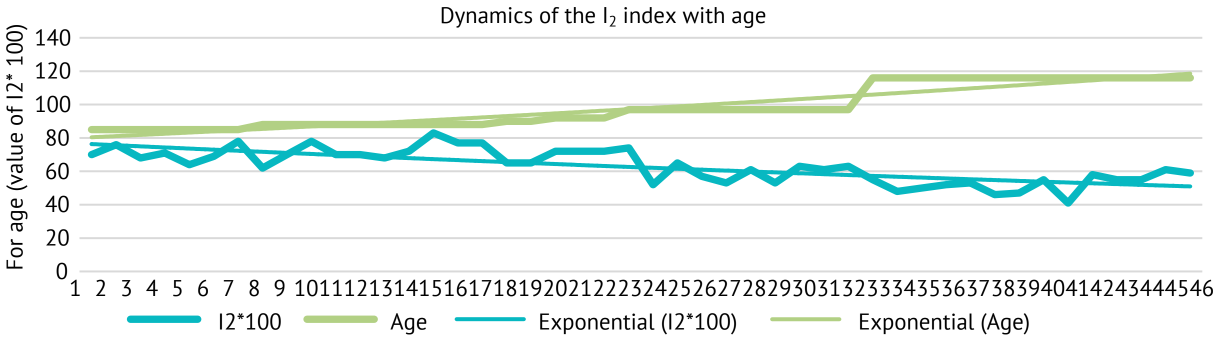



***Figure 14**. Positioning of index reduction with increasing age of plus trees (in conditions of forest type C2-h-oP)*
***Source:** developed by the authors of this study*

Analysing Figs. 13 and 14, it can be noted that for the $C_3$-h-oP conditions, the drop in the $I_2$ index is more dramatic than in the case of the $C_2$-h-oP forest type, where Scots pine grows in more optimal soil conditions. In both cases, the rate of index decline accelerates after the plantation reaches the age of about 100 years, when the risk of losing biotic stability increases sharply, especially under the influence of summer droughts. The increased sensitivity to a drop in the $I_2$ index in old-growth stands under fresher and drier conditions is explained by the development of a greater moisture deficit, which limits the adaptive capacity of trees at a later age. However, even in humid conditions, a sharp decline in the $I_2$ index is a negative factor, as it can lead to a weakening of old-growth stands and create prerequisites for the activation of stem pests. Thus, the graphical data confirm the need to consider the age threshold of about 100 years when planning silvicultural measures aimed at maintaining the biotic stability of pine stands in different types of forest vegetation conditions.

## DISCUSSION

Assessment of the impact of abrupt changes in edaphic and climatic conditions on the condition of plus trees and determination of their further growth by parametric indices calculated by age dynamics is not sufficiently informative without a comprehensive analysis, including the dynamics of the spatial and age structure of the forest stands in which the trees were selected. At the same time, the impact of stress factors on Scots pine stands is a relevant topic in silvicultural research, and the results presented here are of both silvicultural and breeding value. The analysis of the identified correspondences and differences in H/DbH of plus trees at different ages allowed proposing an auxiliary area for practical validation of regionalisation of forest reproductive material by taxonomic parameters of research sites. Analogous studies were presented in O. Danchuk *et al.* (2024), where the assessment was conducted using a complex indicator – the site class, which reflects the dynamics of average heights with age. The proposed approach using the H/DbH ratio complements such assessments, expanding the possibilities of determining the vital status and ecological plasticity of trees, which are valuable criteria for the autochthonous nature of forest stands.

Analogous studies on adaptability were presented in P. Przybylski *et al.* (2020), where the dependence of crown defoliation on taxonomic parameters was assessed based on five-year monitoring of mature pine stands (> 130 years). No correlation was found between tree height and diameter and the degree of defoliation, which the researchers explained by the lack of analysis of the relationship with tree age, which could reduce the accuracy of the regression model. At the same time, the researchers found a dependence of defoliation on site moisture, which is consistent with current data on the hygrotope, as the main stress factor of the study period was a two-year drought.

Another study by P. Przybylski *et al.* (2021) assessed crown defoliation depending on the level of biodiversity, specifically, considering genetic analysis, which concluded that genotype is closely related to adaptability to stress factors. An analogous relationship can be observed in the findings of R. Matisons *et al.* (2021), where, based on a combination of dendrochronological analysis of trees and genotypic assessment of local populations that differ in taxonomic indicators, a response to changes in meteorological conditions was identified. These studies confirmed the significance of phenotypic evaluation (selection of plus trees) in further studies of their hereditary properties to determine the level of autochthonous forest stands of the metapopulation of the (local) region of origin. Therefore, when discussing the results of using the dynamics of the A/H/DbH index in the edaphic and climatic conditions of Malopolissia from the standpoint of establishing the region of origin of forest reproductive material for Scots pine, the primary task is to determine the autochthonousness of the forest stands in which plus trees are selected.

Notably, in conditions of wet hornbeam-oak woodland, plus trees have greater heights while maintaining an average diameter: plus trees in plots in Buske and Radekhivske branches ($C_3$-h-oP) show greater values of average height with a lower DbH/H ratio compared to trees in plots in Kremenetske and Brodivske branches ($C_2$-h-oP). There are some discrepancies with the findings of P. Lakyda *et al.* (2020) and V. Lovynska *et al.* (2021) in terms of biomass estimation. Comparing Polissia as a whole with the Northern Steppe, the researchers noted the greater productivity of pine stands for the $C_2$ edatope in Polissia. This may also indicate the specific features of the conditions of forest stand development in the Malopolissia district for pine forests in relation to the conditions of Polissia as a whole. The question of the autochthonous nature of forest stands by origin often continues to be open. This applies even to native pine stands. Defining the boundaries of the region of origin is more challenging when it comes to local populations of forest-forming species. In Ukraine, there are currently no opportunities to assess the genomes of such populations using genetic and molecular markers. Analogously, genetic assessment of plus trees is not available. Marker and genomic selection methods are considered to be promising, especially in the context of climate change. Their potential and features were summarised in Ch. Li *et al.* (2025). According to by B.É.D. Borges da Silva *et al.* (2021), it is advisable to combine genomic testing, field trials, and selection. M. Lstiburek *et al.* (2023) proposed a new concept of breeding evaluation, which involves the gradual determination of a set of genetic traits in forest stands depending on their age. This will help to improve approaches to the dynamics of phenotypic evaluation in a wide range of environmental conditions. The analysis of growth dynamics, provenance in test cultures, population stands, and plus stands, especially those with high performance (e.g., Lopatynska pine), in combination with genetic methods, will enable faster assessment of the adaptability of offspring. This applies to both individual trees and populations overall.

The study also considered the possibility of modelling the parameters of plus trees and the sustainable forest stands from which they are selected to improve their functional purpose in forest reproduction. This approach complements the results of modern ecopopulation studies in Ukraine, namely: V. Voitiuk *et al.* (2020) – assessment of the adaptability of local pine populations from Volyn; I. Neyko *et al.* (2020) – assessment of the adaptability of northern populations of Scots pine in the southern limit of the range; V. Blystiv *et al.* (2021) – assessment of the sustainability of stand development. The selection of plus trees in stands of autochthonous origin, as well as in populations that are plastic to stress factors, increases the significance of plus selection in the development of sustainable forests of the future. The massive damage to Scots pine (*Pinus sylvestris* L.) stands by stem pests, particularly bark beetles, which has become widely publicised in recent years, has even affected plus trees, which were conventionally considered more resistant. This has exacerbated the scientific and practical problem of prompt preventive assessment of the physiological state of plantations and identification of the principal causes of their weakening before large-scale phytophage invasions. K. Davydenko *et al.* (2021) addressed these issues in detail, but it should be noted that the effectiveness of pest prediction and the development of a forest ecosystem protection system largely depends on the integrated impact of a complex of stress factors (climatic, edaphic, anthropogenic) and the adaptive response of trees to them.

It was found that the assessment of the state of stands by the difference in changes in the A/H/DbH indices (the ratio of absolute height, mean diameter, and other morphometric parameters), illustrated in Figs. 1–6, is an informative indicator of stability dynamics. Specifically, smaller values of the difference in these indices correlate with increased biotic stability of plantations, while their growth indicates a gradual loss of stability, accelerated drying out of even plus trees and, accordingly, more intensive colonisation by bark beetles. The results are in line with the findings of earlier studies, specifically O. Danchuk *et al.* (2024), who substantiated the need to allocate the Malopolissia region of origin for pine forests based on geobotanical zonation. This emphasises the significance of regionally oriented approaches to assessing the resilience and adaptive potential of forest ecosystems.

## CONCLUSIONS

The results of the regression analysis based on the measurements of 43 plus trees in five model plots (102 replications in total) showed that there are no direct statistically significant relationships between tree age (A), height (H), and diameter at 1.3 m (DbH) in the dynamics of age. Instead, a high inverse correlation was found between the $I_2$ index (H/DbH/A) and the $C_2$-h-oP forest type (correlation coefficient r = -0.785) and a very high inverse correlation for the $C_3$-h-oP forest type (r = -0.948). The coefficients of determination ($R^2$) for the respective linear regression models are 0.617 ($C_2$-h-oP) and 0.897 ($C_3$-h-oP). The equations of the obtained linear regressions are as follows: for forest type $C_2$-h-oP: $I_2 = -0.0064A + 1.2579$ ($R^2 = 0.617$, $P < 0.0001$); for forest type $C_3$-h-oP: $I_2 = -0.0093A + 1.6425$, ($R^2 = 0.899$, $P < 0.0001$). The proposed models can be used to predict the parameters of selection of plus trees within the Malopolissia district of hornbeam-oak and pine forests. The results of the analysis of growth dynamics and biotic stability (preservation) of plus trees showed that, despite greater taxonomic indicators in wet hygrotopes, they have lower adaptability to abrupt changes in humidity at the age of more than 100 years compared to fresh hygrotopes. In fresh hygrotopes, the critical effect is mainly manifested under conditions of prolonged moisture deficit. This difference is levelled out with the systematic increase in the dynamics and amplitude of climatic factors observed over the past decade. Notably, a significant part of the plus trees requires urgent cloning and preservation of their genetic potential in clone and family archives or on forest seed plantations.

The findings of the study of plus trees revealed high germination of stands in terms of productivity in the experimental plots. The productivity varies depending on the hygrotope in hornbeam-oak-pine stands, which is natural. At the same time, these forest stands can be attributed to the same region of origin of Scots pine forest reproductive material (FRM) – Malopolissia district, which covers autochthonous hornbeam-oak and pine forests. Clarification of the boundaries of this district is a necessary step for the development of a reliable forest seed base, particularly for the further selection of plus trees for plantations. The feasibility of such a designation is also confirmed by the fact that in the context of global climate change, intraspecific variability results in the development of local ecotypes of Scots pine, which should be considered as the most promising for reforestation. To effectively select plus trees that meet the forest vegetation potential of fresh and wet pine forest types in this region, it is necessary to clarify quantitative parameters. It is proposed to use regression models with the $I_2$ index (H/DbH/A).

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## CONFLICT OF INTEREST

The authors of this study declare no conflict of interest.

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# Лісівничо-таксаційна оцінка плюсових дерев у межах Малополіського округу грабово-дубових та соснових лісів

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**Анотація**. Удосконалення критеріїв добору та подальше випробування плюсових дерев сосни звичайної потребує систематичного оновлення даних обмірів, їх детального аналізу, а також постійного моніторингу створених об'єктів. Важливим завданням є формування нових експериментальних ділянок для дослідження спадкових характеристик дерев, особливо в умовах адаптації лісового господарства до кліматичних змін. Лісівничо-таксаційна оцінка плюсових дерев у межах Малополіського округу грабово-дубових та соснових лісів проведена з метою встановлення залежностей між співвідношенням висоти та діаметра на висоті 1,3 м і віком дерев у різних типах лісу за результатами їх добору. Для встановлення достовірних результатів аналітично-порівняльних висновків використовувалися стандартні статистичні методи. Для проведення аналізу було використано розрахунок двох індексів: $I_1$ – показник зміни віку у співвідношенні A/H/DbH, та $I_2$ – показник зміни співвідношення H/DbH/A залежно від віку. Обчислення цих індексів здійснювалося для плюсових дерев, з урахуванням їх віку (A), висоти (H) та діаметра стовбура на висоті 1,3 м (DbH). Досліджено залежності зміни висоти та діаметра плюсових дерев із віком, визначено особливості цих залежностей для двох найпоширеніших типів лісу, проаналізовано два види індексів зазначених залежностей та узагальнено висновки щодо їх статистично достовірної придатності для моделювання параметрів добору плюсових дерев у межах досліджуваної території. Встановлені у дослідженні параметричні залежності плюсових дерев Малополіського округу соснових лісів даного регіону походження можуть бути використані як додаткові критерії відбору дерев за продуктивністю із забезпеченням достатнього рівня їх біотичної стійкості. У статті узагальнена можливість встановлення регіонів походження лісового репродуктивного матеріалу на основі параметричної оцінки деревостанів і плюсових дерев в межах Малополіського округу на підставі їх приналежності до автохтонних лісостанів за походженням. Актуальність досліджень підсилюється впровадженням підходів до регіоналізації лісового репродуктивного матеріалу що, полягає у визначенні меж регіонів походження та меж відповідного поширення з них лісового репродуктивного матеріалу. Запропонований підхід до впровадження регіоналізації є передумовою отримання і введення в обіг лісового репродуктивного матеріалу на засадах імплементації у нормативну базу лісового насінництва і розсадництва Директиви 1999/105/ЄС від 22 грудня 1999 року «Про маркетинг лісового репродуктивного матеріалу»

**Ключові слова:** плюсові дерева; лісовий репродуктивний матеріал; регіоналізація лісового репродуктивного матеріалу; регіони походження та поширення; таксаційні показники; типи лісу; геоботанічне районування

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# Impact of tillage and fertilisation on organic matter accumulation and soil fertility

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**Abstract**. The study aimed to determine the effectiveness of traditional, minimum and no-till tillage in combination with organic fertilisation methods, such as green manure, organic compost and green manure. The study was based on field experiments conducted on plots with different soil types, which was used to assess the impact of the applied agricultural technologies in different agroecological conditions. The results showed that no-till with combined fertilisation (NPK + manure) provided the highest increase in soil organic matter (SOM) by 0.7% (up to 3.4% in 2024) and humus by 0.5% (up to 2.6% in the 0-10 cm layer) compared to the control (1.7% SOM for ploughing). The nitrogen (N) content increased by 0.04% (to 0.16%), phosphorus (P) by 7 mg/kg (to 57 mg/kg), and potassium (K) by 20 mg/kg (to 140 mg/kg). Deep ploughing resulted in a loss of SOM (from 2.3% to 1.7%), while minimum tillage-maintained

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SOM at 2.1-3.3%. Organic fertilisers (manure, green manure) stabilised humus with a correlation of $r = 0.85 - 0.90$ ($p < 0.05$), while no-till increased soil moisture by 10-15% and cation exchange by 510%. On fertile soils (Eutric Cambisol), no-till with combined fertiliser increased the SOM by 0.7%, while on sandy soils (Quartzipsamment) organic fertiliser added 0.4%. The ANOVA analysis confirmed the significant effect of tillage ($F = 45.2$, $p < 0.001$) and fertiliser ($F = 38.7$, $p < 0.001$) on the SOM. The seasonal dynamics showed a peak in the spring (3.6% in 2023 for no-till). No-till with combined fertiliser was found to be the most effective. The results obtained can be useful for improving agricultural technologies in the field of organic farming, developing ecological farming systems and formulating practical recommendations for farmers and agronomists on optimal soil resource management

**Keywords:** agrotechnology; environmental sustainability; the biological activity of agrocenoses; fertility conservation; biogeochemical processes

## INTRODUCTION

Soil is the main component of terrestrial ecosystems and maintains a balance between physical, chemical and biological properties. Soil organic matter is central to improving soil structure, water-holding capacity and fertility. Increasing the organic matter content is an important factor in sustainable soil management and maintaining the productivity of agricultural land. In this regard, organic farming is becoming increasingly widespread as an alternative to conventional farming methods aimed at the long-term preservation of soil fertility and environmental sustainability of agricultural landscapes. Organic farming is based on methods such as crop rotation, legume cover crops, green fertilisers and compost, which contribute to the accumulation of organic matter.

The annual growth of organic farming areas in the world is about 0.2 million hectares, which is 0.5% of the total area (Willer *et al.,* 2025). The main feature of organic farming is the exclusion of the use of synthetic fertilisers, pesticides, plant growth regulators and genetically modified organisms (Tanchyk *et al.*, 2024). This approach views the farm as a complete ecosystem in which minerals, organic matter, microorganisms, insects, plants, animals and people interact to form a balanced and stable environment. The impact of tillage systems and fertilisation methods on organic matter accumulation has been the subject of numerous studies. In the study by M. Allam *et al.* (2022), a meta-analysis showed that organic fertilisers increased the organic matter content (Soil organic carbon (SOC)) by 12.9% (up to 20.6% for No-Till (NT)), mineral-organic fertilisers increased yields by 13.4% (Conventional Tillage (CT)) and 12.7% (Reduced Tillage (RT)) in coarse soils, and the best effect of organic fertilisers was observed in legumes (15.2%), which emphasises the importance of adapting fertiliser to the conditions. A nine-year study of rice-wheat rotation by Z. Zhao *et al.* (2021) showed that organic fertilisers (conventional tillage with organic manure (CTOM), reduced tillage with organic manure (RTOM)) increased SOC, its active fractions and macroaggregates (250 μm), with the highest concentration recorded in CTOM, which confirms the effectiveness of organic fertilisation and reduced tillage.

J. Gerke (2022) showed that humus substances (50-80% SOC) improve the availability of phosphorus (P), iron (Fe) and copper (Cu), reduce the toxicity of aluminium (Al), bind heavy metals, and perennial plants and organic fertilisers effectively increase SOC and contribute to $CO_2$ regulation. According to a study by D. Martin-Lammerding *et al.* (2021), over ten years in a semi-arid agroecosystem, organic fertilisers (ORG) under minimal tillage (MT) increased wheat yields compared to mineral fertilisers (MIN) and provided stable nutrient levels. A. Feilinezhad *et al.* (2022) analysed that minimum tillage with manure application provided the highest maize yield and improved nutrient uptake compared to no-till. A. Morugán-Coronado *et al.* (2022) evaluated the effects of climate, soil and agronomic practices on crop yields under minimum tillage, and the results showed that a combination of organic and mineral fertilisers gave the best results for cereals, while organic fertilisers were sufficient for pulses.

H. Cui *et al.* (2023) studied the effect of different tillage methods and different levels of nitrogen fertilisation on soil fertility and wheat yield. The results showed that alternating deep and rotary tillage with corn straw and 225 kg of nitrogen per ha resulted in the highest level of fertility and yield at low costs. H. Zhang *et al.* (2020) evaluated the long-term (33 years) effects of different tillage methods and straw return on soil aggregate stability, organic carbon and nitrogen, and the results showed that no-till and straw incorporation increased the number of large macroaggregates, their organic carbon (OC) and nitrogen (N) content, improving soil fertility. J. Balik *et al.* (2020) studied changes in SOC content over 26 years of silage maize cultivation. The results showed that mineral nitrogen fertilisation accelerates the mineralisation of stable organic matter, straw application reduces organic matter losses, and manure increases carbon in stable organic matter (CSOM) by 16%. L. Lv *et al.* (2023) assessed the impact of conservation tillage technologies on soil chemical properties through a global meta-analysis, and the results showed that such methods, especially stubble cover, significantly increase the content of organic matter, carbon, and key nutrients, namely nitrogen (N), phosphorus (P), potassium (K), and cation exchange potential in the soil.

Despite numerous studies, there are gaps in understanding the long-term impact of different tillage systems and organic fertilisation on organic matter accumulation in different soil types and climatic conditions. In particular, the optimal combinations of fertilisation and tillage practices that maximise organic carbon storage and improve soil structure remain poorly understood. The study aimed to investigate the impact of different tillage methods (traditional, minimum, zero tillage) and organic fertilisation (green manure, compost, green manure) on the level of organic matter and agrochemical fertility indicators.

## MATERIALS AND METHODS

The study of the impact of tillage and fertilisation on the accumulation of organic matter and soil fertility was conducted during 2021-2024 years on the experimental field of the Agricultural Research Institute of Agriculture of Central Ukraine of the National Academy of Agrarian Sciences of Ukraine, in Kryvyi Rih. The study area is characterised by chornozem soils of medium humus content, which provides favourable conditions for the experiment and representative results. The climate of the region is temperate continental, with warm summers and mild winters, which also contributes to the successful cultivation of crops and field research. The study area is characterised by the following soil types: Typical Hapludult (acidic, well-drained soils with low organic matter content); Typical Xerorthent (dry, underdeveloped soils with low fertility); Typical Udipsamment (sandy soils with low humus content); Eutric Cambisol (fertile soils with high nutrient content); Typical Quartzipsamment (sandy soils with a predominance of quartz). The study focused on agronomic tillage systems, including conventional, minimum and no-till methods, as well as various fertilisation schemes, including both organic and mineral fertilisers. The assessment of the dynamics of changes in soil organic matter, humus content, physical and chemical properties of the soil profile, and the effectiveness of different fertilisation systems in a changing climate were emphasised.

The impact of deep ploughing, minimum tillage and no-till on the accumulation of organic matter in the topsoil (0-30 cm) was studied. The effect of mineral and organic fertilisers (nitrogen ammonium nitrate, phosphate superphosphate, potassium chloride) and organic fertilisers (rotted manure and green manure) on changes in humus, nitrogen, phosphorus and potassium content was studied. The experiment was laid out in a randomised quadruplicate design with three tillage options and three fertilisation schemes: deep ploughing (20-25 cm), minimum tillage (10-15 cm), no-till and mineral fertiliser (NPK), organic fertiliser (manure), and combined fertiliser (manure + NPK). Each variant included a control plot without fertilisation. Soil samples were collected in the spring and autumn of each year at depths of 0-10 cm, 10-20 cm and 20-30 cm.

The analyses were performed using a UV-1800 spectrophotometer (Shimadzu, Japan), an electronic balance Radwag AS 220.R2 (Radwag, Poland) and a pH meter pH-150MI (Institute of Electric Welding named after E.O. Paton Institute of Electric Welding, Ukraine), a drying cabinet and analytical chemicals, including potassium sulphate ($K_2SO_4$), hydrochloric acid (HCl), sulphuric acid ($H_2SO_4$), nitric acid ($HNO_3$), hydrogen peroxide ($H_2O_2$), sodium hydroxide (NaOH), phenolphthalein indicator, calcium chloride ($CaCl_2$), and standardised solutions for photometric determination of nitrogen, phosphorus and potassium. The data were processed using statistical methods of analysis of variation (ANOVA) to assess the significance of the influence of various factors. Plots with homogeneous soil types were selected for the study, which ensured a correct comparison of results between the variants. The sites were selected based on the criteria of equal exposure and the absence of factors that could affect the results, such as excessive moisture or erosion processes. The results of soil analysis were compared between the experimental variants to assess the effectiveness of each tillage and fertilisation system.

Before the experiment, a detailed characterisation of the initial soil properties was carried out. Fertilisers, namely nitrogen (ammonium nitrate), phosphorus (superphosphate) and potassium (potassium chloride), were applied following agronomic recommendations, considering the potential balance of nutrients in the soil. At the same time, the mass, moisture content and degree of decomposition of plant residues were recorded to analyse their impact on the level of organic matter, following the requirements of DSTU 4287:2004 (2005). Soil sampling was carried out three times a year: in April (spring), July (summer) and October (autumn) throughout the study period. This was used to trace the seasonal dynamics of changes in soil fertility indicators and avoid errors associated with weather fluctuations between years. Areas with anthropogenic pollution (industrial or urbanised areas) were excluded, as well as soils that had been severely depleted due to intensive previous use without appropriate fertilisation.

## RESULTS

This section presents the results of an experimental study of the impact of tillage systems (deep ploughing, minimum tillage, no-till) and fertiliser types (mineral, organic, combined) on the accumulation of organic matter, humus content and agrochemical soil fertility indicators over the period of 2021-2024 years. An experiment to study the impact of tillage systems on the accumulation of organic matter (SOM) and humus was conducted for 2021-2024 years based on an experimental field located in the agro-climatic conditions of Kryvyi Rih. The study aimed to determine how different tillage methods affect the accumulation and preservation of organic matter and humus, which are important indicators of soil fertility. Three tillage approaches were used in the study: deep ploughing at 20-25 cm, minimum tillage at 10-15 cm, and no-till. Soil samples for analysis were taken at depths of 0-10, 10-20 and 20-30 cm in spring, summer and autumn, which tracked the seasonal dynamics of indicators.

When comparing the organic matter content in the top 30 cm of soil, it was found that the tillage system significantly affects its concentration. Under deep ploughing, the content of POM was the lowest among all variants and tended to gradually decrease from 2.3% in 2021 (without fertiliser) to 1.7% in 2024. Under combined fertilisation (organic and mineral fertilisers), this figure was higher at 3.5% in 2021 but decreased to 2.9% in 2024. Minimal tillage provided slightly better results in terms of organic matter accumulation. In the variants without fertiliser, the content of SOM varied from 2.5% in 2021 to 2.1% in 2024. When applying the combined fertiliser, maximum values of 3.8% were observed in 2021 and 3.3% in 2024, indicating the effectiveness of the combination of reduced mechanical intervention and nutritional support. The soil was less compacted and easier to penetrate by the root system, which created favourable conditions for crop growth. In the no-till areas, there were also more earthworms, a bioindicator of soil health, which indirectly indicates an increase in biological activity and stabilisation of organic matter (Mirzavand *et al.,* 2022). In addition to quantitative indicators, there was also a noticeable decrease in erosion processes in areas with minimal and no-tillage. The stubble residues on the surface retained moisture reduced the spraying of soil particles during rain and lowered the temperature of the top layer on hot days.

The highest results were recorded with the no-till system. In the no-fertiliser variants, the organic matter content was 2.7% in 2021 and decreased to 2.3% in 2024. When combined fertilisers were used in the first year of the study, the SOM reached 4.0%, and in 2024 it remained at 3.4%. This confirms the positive impact of no-till on the organic part of the soil due to the preservation of plant residues on the surface and minimal disturbance of the soil structure. An analysis of the dynamics of humus content by year and depth showed a general downward trend. On average, the humus content of all variants decreased from 2.4% in 2021 to 2.0% in 2024. The highest concentrations of humus were observed in the topsoil (0-10 cm), especially in the no-till system, where the content varied from 2.8-3.0% in 2021 to 2.4-2.6% in 2024. Under deep ploughing, the same layer saw a decrease from 2.5% to 1.9%, indicating a loss of humus due to intensive mechanical intervention and accelerated mineralisation. At deeper depths (10-20 and 20-30 cm), there was a less pronounced difference between the tillage systems, but the general trend of decreasing humus content was noticeable in all variants.

SOM has become increasingly important scientifically and practically due to the problem of global warming and the potential of the soil environment as a natural reservoir for the accumulation of atmospheric carbon released as $CO_2$ due to anthropogenic activities (Harenda *et al.,* 2018; Navarro-Pedreño *et al.,* 2021). The most effective measures to increase soil carbon stocks include increasing the level of organic residues and reducing the intensity of organic matter mineralisation processes. The dynamics of organic carbon in the soil profile are determined by the balance between its supply with plant residues and organic fertilisers and its losses caused by the decomposition of SOM, $CO_2$ emissions, erosion processes and leaching of dissolved organic compounds (Rajpoot *et al.,* 2024). The main sources of organic material returned to the soil system are crop residues and organic fertilisers, including manure. These components contain a wide range of organic compounds that are involved in the formation of microbial structures (Brichi *et al.,* 2023). These structures perform two main functions: first, they contribute to the stabilisation of soil particles, improving the aggregate stability of the soil, and second, they participate in microbial respiration, which causes $CO_2$ emissions into the atmosphere. Thus, effective management of SOM transformation processes is crucial for maintaining ecological balance, increasing agricultural land productivity and minimising the negative effects of climate change.

Organic carbon (C) is one of the key elements in organic farming systems, as its increased accumulation has a positive impact on the soil carbon balance. A high level of SOM is directly correlated with the application of organic C, which contributes to a long-term increase in its concentration (Dittmar & Lennartz, 2024). The conversion of natural soil ecosystems into agricultural land, especially under active mechanical cultivation, leads to a decrease in soil organic matter content in 60-70% of cases. According to long-term observations, the SOM decreased by 25-40% in arable land compared to virgin meadows and pastures. This phenomenon is since traditional cultivation methods intensify the mineralisation of organic matter compared to natural conditions, and do not contribute to the effective accumulation of organic carbon in the soil profile (Fig. 1). In the transition period from natural vegetation to cultivated agrocenoses, mechanical impact on the soil leads to significant losses of SOM (Filipović *et al.,* 2024). Thus, the introduction of conservation tillage practices is an important measure to reduce the negative impact of agricultural activities on soil organic matter and ensure its long-term preservation and accumulation.

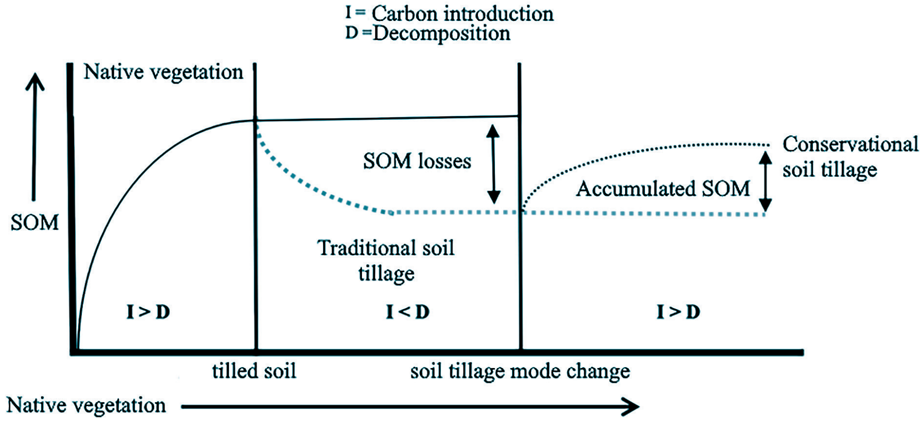


***Figure 1**. The tendency of soil organic matter accumulation from conventional tillage and conservation tillage compared to native soil*
***Source:** compiled by the authors based on A.S.F. de AraÃºjo et al. (2016)*

As organic matter is the main indicator of soil fertility, changes in its content over time can be used to conclude regarding the effectiveness of agricultural practices. In addition, variations in the content of elements such as nitrogen, phosphorus and potassium indicate changes in the physical and chemical properties of soils, which is relevant for the formation of sustainable and productive agroecosystems (Muraru *et al.,* 2021). Accounting for the factors, for further analysis, data characterising changes in soil organic matter and humus content in the period 2021-2024 are presented. These results are presented in Table 1, which shows how different tillage systems and fertilisation options affected the accumulation of organic matter in the soil profile. The dynamics of changes in organic matter content can be used to assess not only the effectiveness of individual agricultural practices but also their long-term impact on soil fertility. Various fertilisation options, including organic and mineral fertilisers, were applied to compare their impact on soil conditions, including humus levels and nutrient content, which are important for ensuring crop health and productivity.

***Table 1**. Organic matter (SOM) content in soil (0-30 cm) depending on tillage system and fertiliser type (2021-2024)*

| Year | Cultivation system | Without fertilizer, % | Mineral fertilisers, % | Organic fertilisers, % | Combined fertilisers, % |
|---|---|---|---|---|---|
| 2021 | Deep ploughing | 2.3 | 2.8 | 3.2 | 3.5 |
| 2021 | Minimum cultivation | 2.5 | 3.0 | 3.5 | 3.8 |
| 2021 | No cultivation | 2.7 | 3.2 | 3.8 | 4.0 |
| 2022 | Deep ploughing | 2.1 | 2.7 | 3.1 | 3.3 |
| 2022 | Minimum cultivation | 2.4 | 2.9 | 3.4 | 3.6 |
| 2022 | No cultivation | 2.6 | 3.1 | 3.7 | 3.9 |
| 2023 | Deep ploughing | 1.9 | 2.5 | 2.9 | 3.1 |
| 2023 | Minimum cultivation | 2.3 | 2.8 | 3.2 | 3.5 |
| 2023 | No cultivation | 2.5 | 2.9 | 3.5 | 3.7 |
| 2024 | Deep ploughing | 1.7 | 2.3 | 2.7 | 2.9 |
| 2024 | Minimum cultivation | 2.1 | 2.6 | 3.0 | 3.3 |
| 2024 | No cultivation | 2.3 | 2.8 | 3.2 | 3.4 |

***Source:** compiled by the authors*

According to the data, organic matter retention was best in the no-till variant, which confirms the high efficiency of this method for improving soil fertility in the long term. The use of no-till can significantly reduce soil erosion, increase soil moisture retention, and promote the accumulation of organic matter on the soil surface, which has a positive impact on the overall health of the ecosystem. The effectiveness of the fertilisation systems was studied in parallel with the impact of tillage and included four options: no fertilisation, mineral fertilisation (using a classic NPK scheme that included ammonium nitrate, superphosphate and potassium chloride), organic fertilisation (rotted manure and green manure), and a combined fertilisation that combined mineral and organic nutrient sources. The effectiveness of each system was assessed by the content of organic matter (OM) and the main nutrients nitrogen (N), phosphorus (P) and potassium (K) in the soil.

In the no-fertiliser variant, the organic matter content was the lowest regardless of the tillage system. In deep ploughing, it decreased from 2.3% in 2021 to 1.7% in 2024, while in the no-till system, this figure was 2.7%

in 2021 and decreased to 2.3% in 2024. The concentration of nutrients under these conditions remained at a minimum level: nitrogen content ranged from 0.10-0.12%, phosphorus from 45-50 mg/kg, and potassium from 110-120 mg/kg. This indicated a limited natural ability of the soil to maintain the nutrient balance without additional inputs. The use of mineral fertilisers provided some improvement. In deep ploughing, organic matter increased to 2.8% in 2021 but decreased to 2.3% in 2024. In the no-till system, the SOM content was 3.2% at the beginning of the study and 2.8% at the end of the period. The nitrogen content increased to 0.13-0.15%, phosphorus to 50-55 mg/kg, and potassium to 120-135 mg/kg. These results indicate a positive but moderate effect of mineral nutrition alone.

Organic fertilisation had a more noticeable effect. With deep ploughing, the SOM ranged from 3.2% in 2021 to 2.7% in 2024. In the no-till system, the figures were higher at 3.8% at the beginning of the period and 3.2% at the end. At the same time, there was an increase in the content of nutrients: nitrogen 0.12-0.14%, phosphorus 48-54 mg/kg, and potassium 115-130 mg/kg. The introduction of organic matter contributed to soil enrichment, improving its structure and water retention capacity. The highest rates were recorded in the variants with combined fertilisation. The content of organic matter reached a maximum: from 3.5% in 2021 to 2.9% in 2024 with deep ploughing, and from 4.0% to 3.4% with no-till. The nitrogen content reached 0.14-0.16%, phosphorus 52-57 mg/kg, and potassium 125-140 mg/kg. This confirms the synergistic effect of simultaneously applying both mineral and organic fertilisers, which efficiently maintains a high level of soil nutrition.

The overall analysis showed an upward trend in the concentration of available nitrogen, phosphorus and potassium during the study period. According to Table 2, the average soil nitrogen content increased from 0.12% in 2021 to 0.15% in 2024. Phosphorus content increased from 50 to 55 mg/kg, and potassium from 120 to 135 mg/kg. The highest concentrations of nutrients were observed in the no-till variants with combined fertilisation, which once again confirms the effectiveness of combining biological enrichment and chemical support for soil fertility. Furthermore, changes in the content of humus, nitrogen, phosphorus and potassium in the soil over the years are notable, as these indicators are critical for assessing soil fertility and crop productivity. Changes in the content of these elements in the soil directly affect the ability of soils to provide plants with the necessary nutrients, which is important for the efficiency of agricultural systems (Da Gama, 2023). Therefore, for a greater description of the dynamics of these changes, as well as for a clear comparison of different tillage and fertilisation options, Table 2 is presented. This table shows the results of analyses of the content of the main nutrients in the soil at different study sites, which demonstrates the effects of different agronomic measures over several years.

***Table 2**. Changes in the content of humus, nitrogen, phosphorus and potassium in the soil over the years (0-30 cm)*

| Parameter | 2021 | 2022 | 2023 | 2024 |
|---|---|---|---|---|
| Humus content, % | 2.4 | 2.2 | 2.1 | 2.0 |
| Nitrogen (N), % | 0.12 | 0.13 | 0.14 | 0.15 |
| Phosphorus (P), mg/kg | 50 | 52 | 54 | 55 |
| Potassium (K), mg/kg | 120 | 125 | 130 | 135 |

***Source:** compiled by the authors*

As the Table 2 shows, the increase in nitrogen, phosphorus and potassium in the soil was due to the use of both mineral and organic fertilisers. This demonstrates the significant efficiency of the combined use of these fertilisers to improve the chemical composition of the soil. Mineral fertilisers ensure fast and accurate application of the necessary nutrients, while organic fertilisers contribute not only to replenishing the elements but also to improving the soil structure, increasing its water retention capacity and activating microbiological activity (Varalakshmi *et al.,* 2024). Thus, the combined use of these two types of fertilisers can be used for a comprehensive improvement in soil fertility, which in turn ensures a stable increase in crop yields in the long term. However, the effectiveness of these methods is largely determined by natural and climatic conditions, soil typology and specific agronomic management. In particular, under conditions of high temperature and humidity, the intensity of organic matter decomposition increases significantly, which can complicate its accumulation.

The use of conservation tillage can reduce the loss of organic matter that is usually observed with conventional ploughing. Reduced or no-till tillage not only increases organic matter levels but also increases the biodiversity of soil microorganisms, which is substantial in transforming nutrients and maintaining agroecosystems. The study was conducted on six different soil types, reflecting a wide range of agronomic and geochemical conditions: Typical Hapludult, Typical Xerorthent, Typical Udipsamment, Eutric Cambisol, Haplic Calcisol and Typical Quartzipsamment. For each type, the key fertility indicators of macronutrients (nitrogen, phosphorus, potassium) and acidity (pH) were determined after applying different tillage systems (deep ploughing, minimum tillage, no-till) and fertilisation schemes (no fertiliser, mineral, organic, combined). Typical Hapludult, soil with an acidic environment and relatively low initial organic matter content, showed a pH in the range of 5.5 to 6.0. The concentrations of N (0.10-0.13%), P (40-50 mg/kg) and K (100-120 mg/kg) were almost unchanged by mineral fertilisers, while organic amendments significantly increased the SOM stock by about 0.5% over four years. This result demonstrates the importance of biological cover and humus amendment for acidic soils with low buffering capacity.

Eutric Cambisol, which is characterised by high fertility and neutral reaction (pH 6.5-7.0), had the highest initial nutritional values: nitrogen 0.14-0.16%, phosphorus 55-60 mg/kg, potassium 130-145 mg/kg. In this type of soil, the no-till system together with combined fertiliser provided a 0.7% increase in organic matter by the end of the experiment, confirming the synergistic effect of combining minimal intervention and balanced nutrient application. Typic Quartzipsamment, a typical sandy soil with a pH of 6.0-6.5, had the lowest N (0.08-0.11%), P (35-45 mg/kg) and K (90-110 mg/kg) values. This soil showed a weak response to mineral fertilisers, while organic fertilisers contributed to a significant increase in the SOM by 0.4% during the study period. Such dynamics are determined by the low water-holding capacity of sands and limited adsorption capacity. Typical Xerorthent, Typical Udipsamment and Haplic Calcisol soils showed intermediate characteristics: their performance varied depending on the combination of tillage and fertilisation, but Haplic Calcisol and Typical Xerorthent were more sensitive to no-till and combined fertilisation systems. This was particularly evident in the accumulation of SOM and improvement of pH, which creates favourable conditions for soil microflora. Thus, the results showed that for acidic and sandy soils (Typic Hapludult, Quartzipsamment), the most effective application of organic fertilisers is in systems with minimal or no mechanical intervention. On the other hand, fertile and neutral soils (Eutric Cambisol, Haplic Calcisol) respond best to a combination of no-till and compound fertiliser, which maximises the growth of organic matter and nutrients.

The no-till system provided a consistently higher level of moisture in the tilth layer, especially in the 010 cm interval, where a 1,015% increase in moisture was observed compared to traditional deep ploughing. This effect is due to the presence of plant residues on the surface, which reduce evaporation, suppress weed growth and create a natural mulch. This improves moisture retention even during periods of short-term drought. Organic fertilisers positively affected soil acidity, especially in acidic soil types such as Typic Hapludult. In this case, the pH increased from 5.5 to 6.0, which significantly improved the conditions for the growth of most crops. At the same time, mineral fertilisers, in particular phosphate and nitrogen fertilisers, tended to increase the pH to 5.8-6.2, which partially offset the acidity but did not reach a stable neutral level. The best results in terms of chemical stabilisation of the soil environment were obtained under conditions of combined fertilisation in the no-till system. This combination resulted in a 5-10% increase in cation exchange potential, which means better soil buffering, higher nutrient retention capacity, and increased resistance to pH fluctuations.

Crop residues that accumulated in the no-till system (within 23 t/ha) proved to be a key factor in enriching the soil with organic matter. A statistically significant correlation was found between the mass of residues and the content of SOM ($r = 0.75$, $p < 0.05$), which confirms their important role in the formation of the humus horizon. The accumulation of residues provided an increase in organic matter in the surface layer at the level of 0.3-0.5% over four years, which is a significant contribution to the process of humus formation. The most significant changes in the content of organic matter and humus were observed in the upper soil layer (0-10 cm) when using the no-till system. Here, the increase over four years ranged from 0.5 to 0.7%, indicating a high efficiency of preserving surface biomaterial. At a depth of 10-20 cm, the effect was less noticeable, with changes of 0.2-0.3%. Even lower, at a depth of 20-30 cm, the increase was minimal at only 0.1-0.2%. This indicates a clear localisation of the effects of tillage and fertilisation in the upper layers of the soil profile, which is of great practical importance for optimising agronomic practices.

The statistical analysis of the experimental results was carried out using the ANOVA method to determine the impact of tillage systems, fertiliser types and their interaction on agrochemical parameters, in particular, the content of organic matter, humus and major macroelements (nitrogen, phosphorus, potassium). The analysis demonstrated a high statistical significance of the effect of each factor on the SOM. In particular, the tillage system had an F-value of 45.2 at $p < 0.001$, indicating a very strong effect. The fertiliser type also showed a significant effect ($F = 38.7$, $p < 0.001$). The interaction between tillage and fertiliser was less pronounced, but still statistically significant ($F = 12.5$, $p < 0.01$), indicating that fertiliser effectiveness depends on tillage conditions. The humus content also significantly depended on the tillage ($F = 30.1$, $p < 0.001$) and fertiliser type ($F = 25.4$, $p < 0.001$), although the interaction effect was weaker and did not reach the level of high confidence. This means that the factors act independently, but powerfully, affecting the level of humus formation.

A similar pattern was observed for macronutrients. In the case of nitrogen (N), the effect of the tillage system was $F = 22.5$ ($p < 0.01$), and fertiliser $F = 20.3$ ($p < 0.01$). Similar values were obtained for phosphorus (P) and potassium (K), where the level of significance of the effect remained within $p < 0.05$, which also indicates significant changes in the content of elements depending on agronomic measures. The no-till system in combination with combined fertiliser was the most effective, providing a 0.7% increase in SOM and a 0.5% increase in humus in the top layer (0-10 cm) over four years. These results confirm previous research on the benefits of conservation tillage, in particular its ability to promote organic carbon accumulation and improve soil structure. The results underline the significant positive impact of this approach on the stabilisation and increase of organic matter, which is key to maintaining and improving soil fertility. The use of no-till with combined fertilisation improves agrophysical properties, including water retention capacity and aeration, and stimulates soil biological activity, including the growth of beneficial microflora. This ensures long-term resistance of the soil environment to degradation processes such as erosion or compaction. In addition, this approach helps to reduce the carbon footprint of agricultural production, which meets the current requirements of sustainable development. Thus, the introduction of no-till with combined fertilisation is an economically and environmentally viable solution for widespread use in agricultural production, especially in the face of climate challenges and the need to preserve soil resources.

## DISCUSSION

The study of the impact of tillage and fertilisation on the processes of organic matter accumulation and soil fertility showed that minimising mechanical intervention in combination with the rational use of organic fertilisers significantly improves the physical and chemical properties of the soil profile increasing the humus content, optimising the soil structure and improving its water-air regime. The study established that reducing the intensity of agronomic impact reduces the risk of degradation processes, such as erosion, compaction and disruption of microbiological balance, which, in turn, contributes to the activation of soil microbiota and the increase in microbiological activity of agrocenosis. The study confirmed that organic farming contributes to the increase of SOM reserves and soil biological activity. This is consistent with the findings of A.Y. Srour *et al.* (2020), proved that the long-term use of different tillage and fertilisation systems in corn-soybean rotations significantly changes the composition of microbial communities, which in turn affects the biological activity and fertility of the soil. Changes in the microbial composition are one of the important factors that determine the level of organic matter decomposition (Fedoniuk *et al.*, 2024), which was also confirmed in the study. This is consistent with the results obtained, which confirm that the application of organic fertilisers in a minimum tillage system contributes to the improvement of the soil microbial environment.

M. Githongo *et al.* (2023) highlighted that organic matter management is a key factor in maintaining soil fertility in the Central Highlands of Kenya. The study noted that organic fertilisation increases the stability of soil aggregates and the content of stable forms of humus. In addition, the study highlights the importance of soil biological activity as one of the determining factors in the stabilisation of humus compounds, which directly affects the long-term accumulation of organic carbon in organic farming systems. The study established that microbiological processes, in particular the activity of fungi and bacteria, play a crucial role in the transformation of organic matter and the formation of stable humus complexes. This confirms the importance of regulating the organic cycle through the use of biologically active organic materials, which not only improve the physical and chemical properties of the soil but also help to reduce degradation processes and maintain agroecosystem sustainability (Poliovyi *et al.*, 2023).

Similar results were obtained by S. Sahoo *et al.* (2022) in a study on the effects of tillage and nitrogen fertilisation in a maize and rice cropping system in the Terai zone of India. The results showed that minimal tillage combined with organic nitrogen sources improved soil enzyme activity and increased total organic matter levels. The results show that under minimum and no-till conditions, the humus content in the upper soil layer (0-30 cm) was higher than in the conventional ploughing variants. This indicates the potential positive value of conservation tillage for the preservation of organic matter in the soil, even without a dynamic comparison with the baseline. Furthermore, the study determined that the biological processes of nitrogen transformation significantly depend on the level of organic matter in the soil, which confirms the importance of organic fertilisation in maintaining soil fertility.

M. Voitovyk *et al.* (2023) showed that the use of organic fertilisers in typical chornozems contributes to a long-term increase in humus content. Comparison of the results with their data shows a general trend towards a positive effect of organic fertilisers on the content of humus compounds in the soil. Black soils have naturally high fertility, but conventional cultivation can lead to losses of organic matter (Aipova *et al.*, 2020). Instead, organic farming methods can reduce these losses and maintain high levels of humus. The study also showed that no-till increases levels of soil organic matter to be retained in the soil compared to conventional ploughing, confirming the results of this study. A study conducted by M. Luce *et al.* (2021) in Canada found that long-term exposure to tillage and nitrogen fertilisers affects carbon and nitrogen fractions. The results indicate that conventional ploughing accelerates the mineralisation of organic matter, which leads to its loss. This correlates with the findings that traditional tillage promotes the rapid decomposition of organic carbon, as evidenced by the decrease in humus content in the samples. This effect may be particularly significant in colder climates where humification processes are slower and the preservation of organic matter is critical to maintaining soil fertility (Boiko & Kovalenko, 2024). The data obtained in this study indicate that reducing the intensity of mechanical tillage reduces organic matter losses and contributes to its stabilisation, which is consistent with their findings.

Y. Li *et al.* (2024) highlighted the impact of conservation tillage on microbial communities in aeolian sandy soils. They confirmed that minimising mechanical intervention increases microbial activity and organic carbon accumulation. The results show a similar trend, in particular, increased activity of the microbial community under conditions of minimal and no-tillage. At the same time, the study shows that in sandy soils, the stability of organic matter depends not only on tillage but also on the level of moisture and availability of nutrients. E.C. Coonan *et al.* (2020) highlighted the microbiological aspect and analysis of microorganisms in the nutrient cycle in soil and found that tillage and fertilisation systems largely determine the humification processes. They note that the optimal ratio between nitrogen and carbon is a critical factor for maintaining stable organic matter. These results confirm these conclusions, as the use of organic fertilisers in combination with minimal tillage provided optimal conditions for humification and improved soil physicochemical characteristics.

Thus, the results of the study are consistent with the findings of other scientists, which confirms the high efficiency of organic fertilisation and conservation tillage technologies in maintaining and restoring soil fertility. The study established that minimising mechanical intervention, including the use of minimal and no-till tillage, combined with the application of organic fertilisers, contributes to the accumulation of organic carbon, activation of soil microbiota, improvement of soil structure and increase in its water-holding capacity. These agro-technological measures have a positive impact on the formation of sustainable agro-ecosystems, reduce the risks of degradation processes such as compaction and erosion, and contribute to the efficiency of nutrient use by plants (Novakovska *et al.*, 2025).

The findings are consistent with numerous scientific studies that confirm the key role of organic farming in preserving soil fertility and creating sustainable agroecosystems. The study proved that the systematic use of organic fertilisers contributes to the gradual accumulation of organic carbon in the soil profile, which has a positive impact on the carbon balance in agricultural landscapes and helps reduce greenhouse gas emissions. The introduction of sustainable tillage and organic fertilisation technologies ensures the long-term preservation of agronomically valuable soil properties, which is a prerequisite for maintaining soil productivity and the environmental sustainability of agricultural systems (Borko *et al.*, 2025). The results obtained indicate the expediency of further development and implementation of organic technologies in modern agriculture as an important tool for ensuring sustainable land use. Further research identifies the optimal combinations of agricultural technologies for different soil and climatic conditions, incorporating soil types, moisture levels and crop characteristics. It is especially important to conduct long-term studies aimed at assessing changes in biogeochemical processes under the influence of organic farming, which will ensure the development of scientifically sound strategies to improve the efficiency of agricultural systems and preserve ecological balance.

## CONCLUSIONS

Based on the study, the study determined that minimal tillage and no-till system in combination with organic fertilisation (manure, green manure) contributed to a higher accumulation of organic matter in the upper soil layer (0-30 cm), achieving an increase of 0.7% (up to 3.4% in 2024) compared to the control (1.7% with deep ploughing). The humus content increased by 0.5% (to 2.6% in the 0-10 cm layer), while deep ploughing led to a decrease in SOM from 2.3% to 1.7% due to accelerated decomposition. Minimal tillage maintained the SOM at 2.1-3.3%. No-till with combined fertiliser increased the concentration of nitrogen (N) by 0.04% (to 0.16%), phosphorus (P) by 7 mg/kg (to 57 mg/kg) and potassium (K) by 20 mg/kg (to 140 mg/kg). Organic fertilisers (manure, green manure) stabilised the humus with a correlation of $r = 0.85 - 0.90$ ($p < 0.05$), and no-till increased soil moisture by 10-15% and cation exchange potential by 5-10%. On fertile soils (Eutric Cambisol), no-till with combined fertiliser added 0.7% of the SOM, while on sandy soils (Quartzipsamment), organic fertiliser increased the SOM by 0.4%. The seasonal dynamics showed a peak in the spring (3.6% in 2023 for no-till). ANOVA analysis confirmed a significant effect of tillage ($F = 45.2$, $p < 0.001$) and fertiliser ($F = 38.7$, $p < 0.001$) on TPG and humus. These results indicate the benefits of no-till for reducing erosion, stabilising carbon balance and reducing $CO_2$ emissions.

The optimal combination of organic fertilisation with zero or minimal tillage ensures the restoration of the humus layer, activation of the soil microbiota and an increase in the cation exchange potential. The results obtained are of great practical importance, as they indicate the need to improve modern agricultural technologies to preserve and restore soil fertility. Further research could focus on the long-term impact of different tillage systems on the dynamics of organic matter in different climatic conditions, as well as on the development of combined approaches to agricultural production that consider environmental sustainability and resource efficiency. An important area for further research is to determine the optimal combinations of agronomic practices to maximise the effect of SOM accumulation. Therefore, further research should include an expanded range of environmental and agronomic conditions to obtain more generalised conclusions about the effectiveness of different tillage and fertilisation systems.

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## CONFLICT OF INTEREST

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# Вплив обробітку ґрунту та удобрення на накопичення органічної речовини та родючість ґрунту

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**Анотація**. Метою дослідження було визначення ефективності традиційного, мінімального та безорного обробітку ґрунту в поєднанні з методами органічного удобрення, такими як сидерати, органічний компост та зелене добриво. Дослідження базувалося на польових дослідах, проведених на ділянках з різними типами ґрунту, які були використані для оцінки впливу застосованих сільськогосподарських технологій у різних агроекологічних умовах. Результати показали, що безорний обробіток ґрунту з комбінованим удобренням (NPK + гній) забезпечив найбільше збільшення вмісту органічної речовини (ОРР) у ґрунті на 0,7 % (до 3,4 % у 2024 році) та гумусу на 0,5 % (до 2,6 % у шарі 0-10 см) порівняно з контролем (1,7 % ОРР під оранку). Вміст азоту (N) збільшився на 0,04 % (до 0,16 %), фосфору (P) на 7 мг/кг (до 57 мг/кг) та калію (K) на 20 мг/кг (до 140 мг/кг). Глибока оранка призвела до втрати поверхневого органічного складу (з 2,3 % до 1,7 %), тоді як мінімальний обробіток ґрунту підтримував рівень поверхневого органічного складу на рівні 2,1-3,3 %. Органічні добрива (гній, сидерати) стабілізували гумус з кореляцією $r = 0,85 - 0,90$ ($p < 0,05$), тоді як безорний обробіток збільшив вологість ґрунту на 10-15 %, а катіонний обмін – на 510 %. На родючих ґрунтах (евтричний камбісоль) безорний обробіток з комбінованими добривами збільшив рівень поверхневого органічного складу на 0,7%, тоді як на піщаних ґрунтах (кварцовий ґрунт) органічні добрива додали 0,4 %. Дисперсійний аналіз підтвердив значний вплив обробітку ґрунту ($F = 45,2$, $p < 0,001$) та добрив ($F = 38,7$, $p < 0,001$) на рівень поверхневого органічного складу. Сезонна динаміка показала пік навесні (3,6 % у 2023 році для безорного обробітку). Безорний обробіток з комбінованими добривами виявився найефективнішим. Отримані результати можуть бути корисними для вдосконалення сільськогосподарських технологій у сфері органічного землеробства, розробки екологічних систем землеробства та формулювання практичних рекомендацій для фермерів та агрономів щодо оптимального управління ґрунтовими ресурсами

**Ключові слова:** агротехнологія; екологічна стійкість; біологічна активність агроценозів; збереження родючості; біогеохімічні процеси

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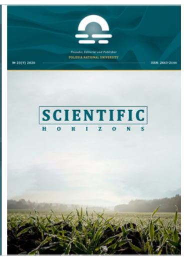

# The impact of renewable energy implementation on agricultural sustainability in the context of climate change

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**Abstract**. This study was aimed at exploring the potential of renewable energy sources (RES) in enhancing the resilience of agriculture to climate change, with a focus on the applicability of these technologies in the context of Kazakhstan. The study was based on qualitative and quantitative analysis of the current state of the sector, comparative analysis of renewable energy technologies, examination of practical cases, and assessment of the applicability in the Republic of Kazakhstan. As a result of the study, it was found that RES, such as solar, wind, and biogas energy, had significant potential for increasing the resilience of agriculture in the context of climate change. It was established that these technologies effectively reduced the dependence of the agricultural sector on fossil fuels, which was particularly valuable for remote regions.

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It was emphasised that a 2 kW system could generate 6-10 kWh per day in sunny regions such as Turkestan or Almaty regions, where insolation reached 1,500-1,800 kWh/m$^2$ per year, and 1-10 kW turbines could generate 2-20 kWh per day in windy areas such as Kostanay and Akmola, where wind speeds reached 5-7 m/s. The study also revealed that in Kazakhstan, from 2020 to 2024, electricity generation from RES increased from 3.24 billion kWh to 7.581 billion kWh. It was noted that such energy sources contributed to climate adaptation by ensuring reliable energy supply for irrigation in arid zones or heating during unstable seasons, while also reducing greenhouse gas emissions, thereby lowering the environmental burden on the agricultural sector. For Kazakhstan, this opened up prospects for increasing yields due to stable access to energy, reducing dependence on fuel imports, and improving environmental performance, especially considering that from 2020 to 2024, the cost of RES decreased from USD 0.057 to USD 0.04 for solar energy and from USD 0.039 to USD 0.03 for wind energy, while coal and gas ranged from USD 0.04-0.25 with a peak in 2022. Thus, RES addressed current problems and also created a foundation for the long-term sustainable development of agriculture, which was especially important in the context of climate risks

**Keywords:** solar energy; wind turbines; biogas plants; energy supply to remote areas; social and environmental benefits; economic efficiency

## INTRODUCTION

Agriculture worldwide faced growing challenges related to global processes such as climate change, depletion of traditional energy resources, and the need to ensure food security for the planet's rapidly growing population. Dependence on fossil fuels, including oil, gas, and diesel, made the agricultural sector vulnerable to economic factors such as price fluctuations and increased its contribution to environmental problems, which required the search for sustainable alternatives. For countries with developed agriculture, such as Kazakhstan, these issues gained particular importance since the agri-sector played a key role in the economy, ensuring employment, exports, and stability of the food market. Renewable energy sources (RES) in this context represented a promising strategic solution, capable of reducing the environmental footprint of the sector, improving the efficiency of natural resource use, and strengthening its ability to adapt to future challenges (Iurchenko *et al.*, 2024). The potential of RES to transform agriculture was especially important in the context of the global transition to a green economy, which made the study timely and relevant, while also underlining the importance of this topic for the sustainable development of the agricultural sector.

One of the key aspects in agriculture was the study of the possibilities of using RES to reduce dependence on fossil fuels and increase the resilience of agricultural production. In the work of S. Sydykov *et al.* (2023), the prospects for using renewable energy installations in the agricultural sector were considered, highlighting the accessibility and environmental advantages, but at the same time paying insufficient attention to the practical aspects of implementation, such as adapting technologies to various climatic conditions, which limited the practical value of the results obtained. The study by M. Laljebaev *et al.* (2022) noted the potential of wind energy, focusing on the prospect of turbine installation and scaling in the windy regions of Kazakhstan, but overlooked economic barriers and the issue of payback for small farms. In the study by D. Esenzhol *et al.* (2023), the role of biogas units in waste processing and energy production was analysed, emphasising the ability to create a closed-loop system in production, but without addressing the challenges associated with training farmers and ensuring stable system operation under limited resources, which restricted the applicability of the findings.

Another important area was the analysis of the social and economic effects of implementing RES in agriculture, which made it possible to assess the impact on the lives of farmers and the development of rural areas. In the work of A.T. Omarova *et al.* (2022), the economic benefits of solar systems in agriculture were analysed, highlighting the capacity to save fuel costs, but without considering the long-term consequences of such systems for small farms, including equipment wear and the need for component replacement. In the study by D. Streimikiene *et al.* (2021), the factors influencing the integration of RES in agriculture were examined, focusing on social aspects and the prospects of this direction, but at the same time paying insufficient attention to workforce qualification and the availability of training for local residents. The study by C.A. Pizarro-Loaiza *et al.* (2021) noted the potential of biogas technologies for improving living conditions in rural areas, particularly highlighting the role in providing heat and energy, but failed to address logistical and infrastructure challenges necessary for scaling such systems.

An additional aspect requiring attention was the provision of energy supply to remote rural areas using RES, which was particularly relevant for regions with limited access to centralised grids and traditional fuel types. In the study by G. Augustyn *et al.* (2021), the potential for using solar panels for autonomous farm power supply was explored, emphasising the independence from external power sources, but without paying due attention to seasonal fluctuations in solar activity and the impact on the stability of energy supply in rural regions. In the work of N. Ganjei *et al.* (2022), a hybrid solar-wind power station was designed for use in isolated rural areas, focusing on its adaptability to local farm conditions, but giving insufficient consideration to issues of transportation and installation of equipment in hard-to-reach locations. The study by Y. Chen *et al.* (2023) analysed the efficiency of a solar and biogas-based system for heating farms and communities, highlighting the advantages of such energy sources in the ability to use local resources, but overlooking the issue of limited technical support availability for such systems. Thus, this study proposed a broader approach, combining aspects of energy accessibility and social impact while considering the interconnection and practical applicability, which made its conclusions more comprehensive and oriented towards the real conditions typical for Kazakhstan.

The aim of this study was to examine the potential of RES – solar, wind, and biogas energy – in increasing the resilience of agriculture to climate change, reducing dependence on traditional energy sources, and improving energy supply to rural areas, with an emphasis on the applicability under the conditions of Kazakhstan. The research objectives included: identifying the economic, environmental, technical, and social benefits of RES for the agricultural sector to determine how these technologies could help reduce costs and improve farmers' living conditions; analysing the use of RES in various countries such as India, Germany, and Kenya, as well as adapting the collected data to the specific features of Kazakhstan, including its climatic diversity and agricultural needs; assessing the prospects of scaling RES in the agri-sector and the impact on food security in the long term.

## MATERIALS AND METHODS

The methodology of this study, conducted at Astana International University, was based on qualitative and quantitative analysis, which allowed for a comprehensive examination of how solar, wind, and biogas energy could reduce the agricultural sector's dependence on traditional energy sources, improve energy supply in remote areas, and enhance resilience to climate challenges. The main focus was placed on data collection, systematisation, and interpretation, taking into account global trends and the specific context of Kazakhstan as a region with diverse climatic and economic conditions. During the study, an analysis was carried out of existing publications by international organisations dedicated to the use of RES in agriculture, such as reports by the International Renewable Energy Agency (n.d.), the Food and Agriculture Organization (n.d.a), and the World Bank (2025), which provided data on the cost of RES, the environmental effects, and the impact on food security. For the Kazakhstani context, national reports were examined, including key documents such as the Concept for the Development of the Agro-Industrial Complex of the Republic of Kazakhstan for 2021-2030 (2021), which defined strategic directions for the development of agriculture and energy in the agrarian sector. In addition, materials on climate change were considered, such as a publication by M. Syzdykova (2024), which covered the impact of temperature and precipitation on the agricultural sector. This analysis made it possible to identify key trends and determine gaps in current knowledge that required further examination.

The study also included a comparative analysis of three types of RES – solar, wind, and biogas energy – in terms of the applicability to agriculture. The technical characteristics were examined, such as principles of operation, resource requirements, and integration potential into agricultural processes (e.g., irrigation, product processing, heating). The comparison was carried out based on qualitative criteria (reliability, autonomy) and generalised economic indicators (cost reduction, payback period) to understand how each technology could meet the needs of farmers in the context of climate change. To support the theoretical findings, practical case studies of RES implementation in agriculture were analysed. Cases from various regions and countries, such as India, Germany, and Kenya, were considered in terms of implementation conditions, achieved results, and lessons that could be adapted for other countries, including Kazakhstan. Special attention was paid to how these technologies addressed problems similar to those in Kazakhstan – droughts in the south, power supply interruptions in the north, and waste management in livestock regions. This stage enabled the linking of general principles with real outcomes, providing a practical basis for projections.

A separate stage was dedicated to assessing the applicability of RES in Kazakhstan, taking into account its geographical, climatic, and socio-economic characteristics. Data on solar activity, wind potential, and volumes of organic waste were used to determine which technologies were best suited for different regions. In particular, solar activity data included information on the number of sunshine hours and radiation levels taken from a report by the Eurasian Research Institute (Akhmetkaliyeva, 2025). Information on wind potential was based on assessments from the same source. Data on organic waste volumes were obtained from materials by the Bureau of National Statistics Agency for Strategic Planning and Reforms of the Republic of Kazakhstan (n.d.), which presented statistical indicators of waste generation, including organic waste. The current problems of the agricultural sector were analysed, and modelling was carried out on how RES could address these challenges. This analysis was based on generalised assessments of costs and benefits, as well as potential social effects such as job creation and improved living conditions. Based on the conducted analysis, the findings were synthesised to form projections and prospects for Kazakhstan. The forecasting included an assessment of RES scaling in agriculture over the coming decades, the impact on food security, and the role of international cooperation. The forecasts were built considering global trends such as declining technology costs and growing investments in green energy, as well as adapting to the conditions of Kazakhstan, including its climatic diversity and economic priorities.

## RESULTS

Agriculture required significant energy resources to carry out daily tasks, from fieldwork to product processing. The main types of energy were electricity, diesel fuel, and natural gas, which were used in a wide range of processes. Processes such as irrigation depended on pumps powered by electricity or diesel to deliver water to fields, which was especially important in arid areas where crop cultivation was impossible without artificial irrigation. Greenhouses that provided year-round harvests of vegetables and herbs consumed energy for heating and lighting, most often relying on gas or electricity. Agricultural machinery such as tractors, combines, and trucks operated primarily on diesel fuel, which made up a significant portion of farmers' operating expenses. Finally, product processing, such as grain drying, milk cooling, or oil production, required stable power supply to operate the equipment. In Kazakhstan, energy consumption in the agricultural sector reflected the country's geographical diversity: in the south, in regions such as Almaty Region, a significant share of energy was used for orchard and field irrigation, while in the north, in grain belt regions such as Kostanay Region, the focus was on fuel for machinery used in vast wheat fields.

The high dependence of agriculture on fossil fuels created a range of vulnerabilities that became increasingly evident amid global instability. Rising oil and gas prices directly increased farmers' costs, reducing the incomes and competitiveness in the market. This issue was particularly acute in countries dependent on imported energy resources, such as Kazakhstan, where any disruption in supply could halt key processes, such as sowing or harvesting, especially in steppe regions like Akmola and Kostanay, where agriculture relied heavily on machinery. Moreover, in remote rural areas such as the mountainous zones of Almaty Region or the desert territories of Kyzylorda Region, energy supply was often unstable due to outdated infrastructure or its complete absence. Power or fuel outages led to machinery downtime, spoilage of perishable products, and a general decline in productivity. Such dependence on traditional energy sources made the agricultural sector fragile in the face of external shocks – whether economic crises or geopolitical constraints – highlighting the need for alternative solutions. Climate change further complicated matters by increasing the vulnerability of agriculture and hindering long-term planning. Droughts, which became more frequent and severe, limited access to water needed for irrigation – a critical issue for regions with low precipitation such as Turkestan and Mangystau. Conversely, floods destroyed crops, washed away fertile topsoil, and damaged infrastructure, disrupting supply chains and harming farmers' economies, as observed in flood-prone areas of East Kazakhstan Region. Changes in temperature patterns shifted traditional growing seasons, increased pest and disease activity, and worsened conditions for conventional crops such as wheat or barley, resulting in reduced yields and rising food prices.

Remote rural areas in many regions around the world faced chronic energy supply issues, which significantly limited the economic development and quality of life (Karlilar Pata *et al.*, 2025). The main challenge lay in the shortcomings of existing infrastructure: centralised energy grids were focused on cities and large settlements, leaving remote areas either without electricity or with unstable access. The length of power lines required to reach such zones demanded significant investment, and the maintenance was complicated by equipment wear, rugged terrain, and harsh climatic conditions. According to the International Energy Agency (2023a), as of 2023 around 9% of the world's population (approximately 685 million people) lacked access to electricity, primarily in rural areas. In Kazakhstan, this issue was evident in vast steppe and mountain territories such as parts of Almaty, East Kazakhstan, and Kyzylorda regions, which often remained beyond the reach of reliable energy grid coverage. According to the Ministry of Energy of the Republic of Kazakhstan, the electrification level of rural areas in Almaty Region reached 87% (as of 2023), but in mountainous districts such as Alakol or Raiymbek, outages occurred up to 12-15 times a year, lasting from 4 to 24 hours due to snowstorms and line breakages (Draft Law of the Republic of Kazakhstan, 2025). In East Kazakhstan Region, around 25% of the rural population (approximately 180,000 people) lived in remote villages where in 2023 power outages lasted up to 25 hours per month in winter due to network wear (the average equipment age was 40-50 years). In Kyzylorda Region, rural electrification was at 72%, and in remote Aral villages, outages lasted 3-5 days annually due to sandstorms and the absence of backup sources. The worn-out infrastructure built during the Soviet era led to frequent blackouts – in some villages, outages lasted from several hours to days, especially in winter when snowstorms damaged the lines (Samruk-Energy, 2025). Connecting small farms to centralised systems was economically unfeasible: the cost of laying lines (around 2-3 million tenge per kilometre) and energy losses over long distances made this option unaffordable for local budgets. Reliance on diesel generators as an alternative was also problematic, as deliveries were unreliable due to poor roads and weather. As a result, farmers faced equipment downtime, product spoilage, and limitations in using modern technologies, which reduced the competitiveness and worsened living conditions. These issues highlighted the need for alternative solutions for autonomous and sustainable energy supply where traditional infrastructure was ineffective.

At the same time, according to reports and updates from the Ministry of Energy of the Republic of Kazakhstan (n.d.), the implementation of RES already demonstrated potential in these regions. For example, in Almaty Region in 2023, 15 solar systems with a capacity of 2-5 kW were installed in mountainous districts (e.g., Yenbekshikazakh), generating 6-10 kWh per day and supplying energy to 70 farms due to high insolation. In East Kazakhstan Region, a biogas plant with a capacity of 8 kW had operated in Ulan District since 2022, processing 400 tonnes of manure annually and producing 2-4 kWh per day, covering the needs of a farm with 15 head of cattle and reducing emissions by 250-300 tonnes of $CO_2$-equivalent annually. In Kyzylorda Region, in Kazaly District, wind turbines with a capacity of 3 kW had been tested since 2021, generating 5-12 kWh per day at wind speeds of 5-6 m/s, supplying energy to pumps for farms up to 5 hectares. RES became an increasingly significant solution for overcoming the energy and environmental challenges faced by modern agriculture (Bialkovska *et al.*, 2025). These sources of energy offered an alternative to traditional energy sources, reducing dependence on fossil fuels and contributing to the resilience of the agricultural sector under climate change. Among the key types of RES were solar, wind, and biogas energy, each of which had unique characteristics and application potential in agriculture (Table 1).

***Table 1**. Advantages of renewable energy sources in electricity production in agriculture*

| Category | Advantage | Solar energy | Wind energy | Biogas energy |
|---|---|---|---|---|
| Economic | Reducing operating costs | No fuel costs: diesel expenses reduced by up to 100% Payback period of 5-7 years at a panel cost of USD 0.6/W | Payback of turbines (1-10 kW) in 5-10 years with 30% cost reduction over 10 years | Waste management: reduces manure management costs by 50-70% |
| | Cost stability | Protection against fuel price increases, such as diesel, which often fluctuates in remote areas | Independence from unstable gas prices subject to market changes | Local energy production from waste, eliminating fuel delivery costs |
| Ecological | Reducing $CO_2$ emissions | Diesel replacement: emissions reduced by 1.5-2 tonnes of $CO_2$/year per 5 kW system (2.7 tonnes of $CO_2$/year from diesel) | No emissions: reduction of carbon footprint by 0. 9-1 t $CO_2$/kW installed capacity/year | Methane capture: e.g., 250-400 t $CO_2$-eq/year reduction from a farm with 20 cattle |
| | Reducing pollution | No exhaust gases, protecting soil and air from diesel pollution | Clean operation: elimination of 20-30 t ash/year compared to coal plants | Waste treatment that prevents pollution of water bodies and soil from manure |
| Technical | Autonomy and reliability | Generation of 6-10 kWh/day (2 kW system) with 5-10 kWh batteries | Generation of 2-20 kWh/day (1-10 kW turbine), stability in 5-7 m/s wind | 2-5 kWh/day from an 8-10 kW unit, stable regardless of weather |
| | Flexibility and scalability | Easy adaptation from small farms to large settlements | Installation options from small turbines to large parks | Suitable for different scales, from small farms to enterprises |
| | Ease of maintenance | Panel cleaning 2-4 times/year, service life 20-25 years, battery replacement every 5-10 years | Inspection every 2-3 years, turbine life 20-30 years | Waste loading daily, repair every 5-7 years, service life 15-20 years |
| Social | Improving living conditions | Electricity access in homes, schools, and hospitals in remote areas | Providing light and heat in isolated communities | Possibility to produce energy and heat for rural settlements |
| | Job creation | Up to 500-700 jobs/GW (installation, maintenance) | Up to 300-500 locations/GW. | Up to 50-100 places/MW |
| | Availability of technology for farmers | Mastered within 2-4 weeks | Preparation in 2-3 weeks | Basic training in 1 month |

***Source:** developed by the authors based on Food and Agriculture Organization (2021), International Energy Agency (2022), International Renewable Energy Agency (2023a), World Bank (2025), Korem (n.d.)*

Solar energy was based on converting sunlight into electricity using photovoltaic panels, making it one of the most accessible and versatile renewable energy technologies. Its operating principle was simple: solar panels captured photons and converted photons into electric current, which was then used directly or stored in batteries for later use. The availability of solar energy depended on geographical location and climatic conditions, but even in regions with moderate insolation, it remained effective due to modern technologies (Moore *et al.*, 2022). In agriculture, solar panels were widely used: the solar panels powered irrigation pumps, providing water to fields without the cost of diesel fuel, which was especially valuable in arid areas. Solar energy was also used to light farms and greenhouses, extending daylight hours and improving growing conditions. Moreover, small-scale solar systems could support product processing operations such as fruit drying or milk cooling, reducing farmers' energy costs. This energy source was particularly promising for countries with high solar activity levels, where various regions could effectively use solar panels for agricultural needs (Agbor *et al.*, 2023).

Wind energy, in turn, utilised the kinetic energy of wind, converting it into electricity using turbines, and represented another powerful tool for agriculture. Its main feature was its dependence on wind strength and consistency, requiring careful selection of installation sites (Shorabeh *et al.*, 2022). Wind turbines came in various sizes: from large wind farms capable of supplying whole regions to small units suitable for individual farms. This scalability made the technology flexible and suitable for both large agricultural enterprises and smallholdings. In rural areas, wind energy was especially valuable thanks to open spaces with fewer obstacles, increasing turbine efficiency. For Kazakhstan, with its steppe expanses and strong winds in the north and west, wind energy could have become an important addition to the energy supply in rural areas, particularly where solar activity was lower. Applications included powering electrical systems for irrigation, mills, or lighting, as well as charging batteries for autonomous use. Wind as a resource was free and inexhaustible, reducing operational costs after initial investments.

Another promising area was biogas energy, based on the anaerobic decomposition of organic waste such as manure, plant residues, and food waste, releasing biogas, which was then used to generate electricity or heat. This process was carried out in special biogas plants, where microorganisms digested biomass to produce methane – the main component of biogas. In agriculture, this technology was particularly valuable as farms generated the raw material for energy production, creating a closed loop: livestock or crop waste turned into fuel, and the by-product (organic fertilisers) was returned to fields to improve soil quality (Singh & Kalamdhad, 2022). Biogas could power generators for electricity, heat greenhouses, or be used for household needs on farms. In the context of the Republic of Kazakhstan, where livestock farming was developed in regions such as Akmola and East Kazakhstan, biogas plants could not only provide energy for farms but also improve the environmental sustainability by minimising waste and improving land fertility. The advantage of biogas energy lay in its independence from weather conditions, unlike solar and wind energy, and in its environmental benefits, as it reduced methane emissions and solved the waste disposal problem.

The economic efficiency of RES became increasingly apparent against the background of rising fossil fuel prices and the development of renewable energy technologies. Although initial installation costs for solar panels, wind turbines, or biogas systems could be high, long-term operational expenses were significantly lower than those of conventional energy sources (Karlilar Pata & Pata, 2025). One example was diesel fuel for agricultural machinery or pumps, which required regular purchasing and was subject to price volatility. In contrast, solar panels, once installed, used free sunlight and had a lifespan of 20-25 years with minimal maintenance. Similarly, wind turbines, despite initial investment, paid for the turbines' operation within 5-10 years in regions with sufficient wind activity. Biogas plants were especially cost-effective for farms, due to using existing waste, converting it into energy without additional fuel costs. Research by the International Renewable Energy Agency (2024a) showed that in 2024, the cost of electricity from RES (Fig. 1) was on average 20-40% lower than from coal or gas plants (Fig. 2).

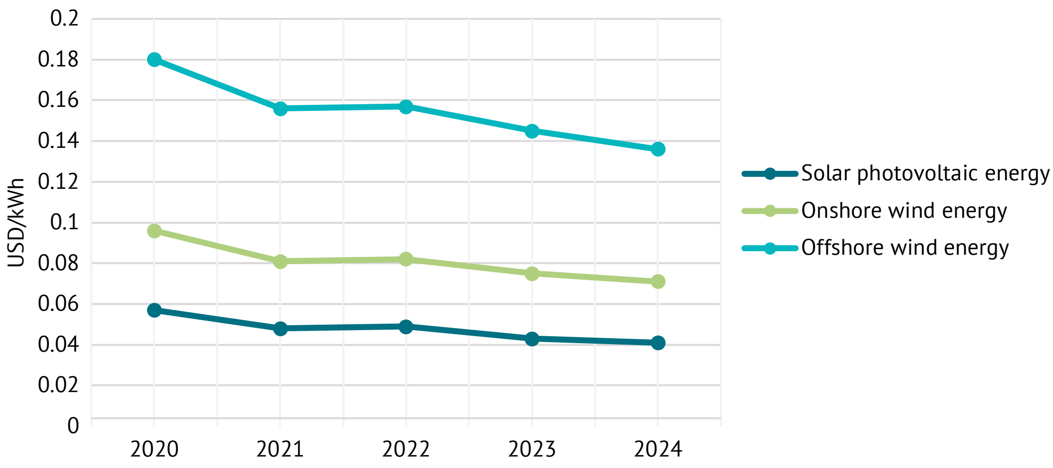


***Figure 1**. Dynamics of the cost of electricity production from renewable energy sources*
***Source:** developed by the authors based on International Renewable Energy Agency (2024a)*

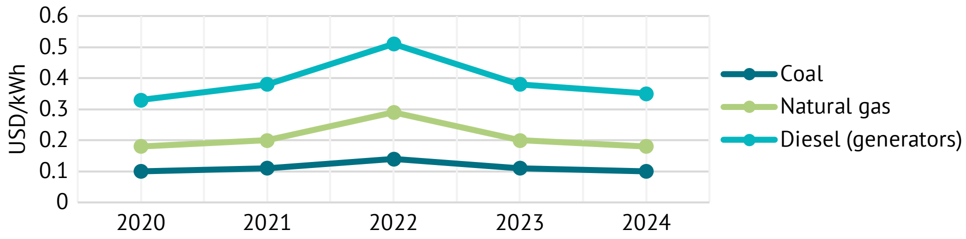


***Figure 2**. Dynamics of the cost of electricity production from traditional energy sources (average indicators)*
***Source:** developed by the authors based on International Energy Agency (2023b)*

Thus, during the period from 2020 to 2024, the cost of RES fell from USD 0.057 to USD 0.04 for solar energy and from USD 0.039 to USD 0.03 for wind energy, whereas coal and gas ranged from USD 0.04 to 0.25, peaking in 2022. Accordingly, in 2024, electricity production from RES was already 20-40% cheaper than from average coal and gas plants, as well as diesel generators. It should also be noted that diversifying energy supply through RES allowed agriculture to reduce risks associated with fossil fuel market instability. Oil and gas prices were influenced by multiple factors: from international sanctions and conflicts to changes in global trade. Such fluctuations created uncertainty for farmers, who found it difficult to plan fuel budgets for machinery or heating. RES, in contrast, provided stability: solar and wind energy were not subject to market prices, and biogas was produced locally from waste, eliminating the need for imports. This was especially important for countries dependent on external supplies, such as Kazakhstan, where a significant share of fuel was imported. The use of RES enabled the creation of autonomous energy supply systems, reducing vulnerability to supply disruptions and increasing cost predictability. Moreover, combining different types of RES – for example, solar panels and biogas plants – improved reliability, due to compensating for each other's weather- or season-related limitations.

Real-world examples demonstrated how RES already reduced agriculture's dependence on traditional energy sources across the globe (Aliyev *et al.*, 2024; Syzdykova, 2024). In India, where more than 60% of the population was employed in agriculture, a programme to install solar irrigation pumps reached millions of farmers. By 2023, over 300,000 such pumps replaced diesel counterparts, cutting annual fuel costs by USD 500-700 per farm and increasing yields due to stable water supply (Ministry of New and Renewable Energy of India, 2024). In Germany, farms actively used biogas plants: by 2022, more than 9,000 farms produced biogas from manure and plant waste, covering the own needs and selling energy surpluses to the grid, generating an additional income of EUR 4 billion (Concept for the Development of the Agro-Industrial Complex of the Republic of Kazakhstan for 2021-2030 2021; Statista, 2025). In Kenya, small-scale wind and solar systems became a lifeline for remote farms: a project in the Turkana region enabled local communities to abandon costly diesel fuel, reducing milk and fish processing costs by 40% (International Renewable Energy Agency, 2021). These cases highlighted the practical benefits of RES and the adaptability to various conditions.

Climate change posed a serious threat to agriculture by increasing the frequency and intensity of extreme weather events such as droughts, floods, and temperature anomalies. These factors disrupted production cycles, reduced yields, and jeopardised food security. RES became an important tool for increasing the agricultural sector's resilience, providing reliable energy supply, reducing environmental harm, and supporting key processes such as irrigation (Shahini *et al.*, 2025). Extreme weather events such as hurricanes, floods, or prolonged droughts often disrupted centralised power supply, leaving farmers without electricity and fuel at critical times. RES offered a decentralised solution that increased farm resilience to such crises (Tanchyk *et al.*, 2024; Akhmetkaliyeva, 2025). It was important to emphasise that agriculture not only suffered from climate change but also significantly contributed to global greenhouse gas emissions, exacerbating the climate crisis. According to the Food and Agriculture Organization (2024; n.d.b), the agricultural sector was responsible for 10-12% of all anthropogenic emissions, including carbon dioxide ($CO_2$) from fossil fuel combustion, methane ($CH_4$) from livestock, and nitrous oxide ($N_2O$) from fertiliser application. These emissions created a vicious cycle: agriculture accelerated climate change, and climate shifts, in turn, reduced its productivity. The transition to RES – solar, wind, and biogas – offered a real way to break this cycle by significantly reducing the industry's carbon footprint. Agriculture generated greenhouse gases through numerous processes. For instance, the use of diesel tractors, generators, and irrigation pumps emitted millions of tonnes of $CO_2$ annually. According to the International Energy Agency (2024), diesel combustion in agricultural machinery alone produced around 500 million tonnes of $CO_2$ per year globally. Livestock contributed even more: manure decomposition and ruminant digestion (cows, sheep) released methane, which was 25 times more potent than $CO_2$ in terms of greenhouse effect. The Food and Agriculture Organization (n.d.b) estimated that in 2022, livestock farming produced about 3.1 billion tonnes of $CO_2$ equivalent, of which 44% was methane. The use of nitrogen fertilisers in fields, especially in grain production, released nitrous oxide, whose global warming potential was 298 times higher than that of $CO_2$. Collectively, these sources made the agricultural sector one of the main polluters, and without changes, this share would increase with rising demand for food. RES could radically reduce these figures by replacing dirty technologies with clean ones and converting waste into useful resources.

One of the most effective ways to reduce emissions was the replacement of diesel systems with solar ones. Diesel pumps used for irrigation consumed millions of litres of fuel annually: on average, one 5 kW pump burned around 1,000 litres of diesel per year, emitting 2.7 tonnes of $CO_2$ (given that 1 litre of diesel emitted 2.68 kg of $CO_2$) (Environmental Protection Agency, 2025; Bureau of National Statistics Agency for Strategic Planning and Reforms of the Republic of Kazakhstan, n.d.). Solar pumps completely eliminated these emissions by operating on free solar energy. According to the International Renewable Energy Agency (2016), switching to medium-power solar pumps reduced emissions by 1.5-2 tonnes of $CO_2$ per unit per year. Solar panels also powered greenhouses and refrigeration units, replacing gas and coal systems. In Kazakhstan, irrigation played a key role in the southern regions, so replacing diesel pumps with solar ones could prevent a large amount of $CO_2$ emissions annually, reducing the carbon footprint. Wind energy also contributed to decarbonisation, especially for large agricultural enterprises with higher energy demand. Unlike fossil fuels, wind turbines did not produce emissions during operation, and the carbon footprint was limited to the production and transport stage (about 10-20 g $CO_2$ per kWh over the life cycle, compared to 800-1,000 g for coal). A 10 kW turbine installed on a farm could generate 20,000-30,000 kWh annually in windy regions, replacing diesel generators and reducing emissions by 15-20 tonnes of $CO_2$ per year. Biogas plants, in turn, had a unique dual effect: such plants not only replaced fossil fuels but also prevented methane emissions from organic waste. Manure decomposition in open lagoons released about 1 kg of $CH_4$ per tonne of waste per day, equivalent to 25 kg of $CO_2$ in greenhouse terms. Biogas systems captured this methane, burning it to produce energy and converting it into $CO_2$, which was 25 times less harmful.

Droughts also became one of the most destructive consequences of climate change threatening agriculture by limiting access to irrigation water. According to the World Meteorological Organization (2024), the frequency of droughts increased by 29% since 2000, and the duration and intensity continued to rise, reducing water availability for irrigation and land productivity. This was particularly critical for countries with hot climates or dependence on irrigated farming, where crop cultivation became impossible without artificial watering. Solar pumps became an effective solution, ensuring reliable water supply even in the most arid regions. These devices used solar energy to power water pumps that delivered water from wells, rivers, or reservoirs to fields, requiring no fuel, making this solution cost-effective in the long run. Unlike diesel pumps, solar systems operated silently, did not pollute the air, and could be equipped with batteries for night-time use. The mobility and ease of installation made solar systems ideal for remote farms with limited fuel access. From a technical perspective, solar pumps offered several advantages that enhanced the effectiveness in combating droughts. These pumps operated silently, unlike noisy diesel engines, improving working conditions for farmers and avoiding disturbance to livestock. The absence of exhaust gases prevented air and soil pollution, which was especially important near bodies of water used for irrigation. Another major advantage of such systems was mobility, as small solar pumps were easy to relocate from one plot to another, and installation took only a few hours and required no complex infrastructure.

RES also offered an effective solution for improving energy supply in remote rural areas where traditional infrastructure was either absent or unreliable. Decentralised systems based on solar, wind, and biogas energy allowed farmers and residents in such regions to become independent from centralised grids, eliminating issues related to high connection costs and supply disruptions (Sadovoy *et al.*, 2025). These technologies were adapted for local use, required minimal infrastructure, and could be scaled depending on needs – from a small farm to an entire village. Solar installations were among the most accessible and easiest solutions to implement in remote areas. Photovoltaic panels converted sunlight into electricity that could be used to power pumps, lighting, refrigeration, or household appliances. A 2 kW system, for example, could generate 6-10 kWh per day in sunny regions of Kazakhstan such as Turkestan or Almaty, where average insolation reached 1,500-1,800 kWh/m$^2$ per year. Equipped with batteries (5-10 kWh capacity), such systems provided energy even at night or on cloudy days, which was critical for uninterrupted farm operations. Unlike diesel generators, solar panels required no fuel, and maintenance was limited to periodic cleaning and battery replacement every 5-10 years. Thus, installing 1,000 such systems could supply up to 5,000 households or farms, eliminating the need for costly power lines. Wind turbines, in turn, complemented solar systems, especially in high-wind regions such as the steppe zones of northern Kazakhstan (Kostanay, Akmola regions), where average wind speeds reached 5-7 m/s. Small turbines with a capacity of 1-10 kW could generate 2-20 kWh per day, sufficient to power equipment, lighting, or charge batteries. The advantage was that wind was often available when solar energy was limited – for example, in winter or during storms. Combining solar and wind systems (hybrid systems) increased reliability, covering up to 80-90% of a farm's energy needs even in variable weather conditions. Biogas plants were particularly relevant for rural areas with developed livestock farming, such as East Kazakhstan or Akmola regions of Kazakhstan, where millions of tonnes of manure were generated annually. These plants converted organic waste into biogas (methane), which was used to produce electricity and heat. A small 10 kW station could process 1-2 tonnes of manure per day, producing 20-30 kWh of electricity and up to 50 kWh of heat – enough for a farm with 50-100 head of livestock. The by-product – biofertilizer – improved soil and boosted yields. Given that livestock farming produced about 10 million tonnes of manure per year, installing 500 biogas plants could generate up to 5 million kWh of electricity annually, powering thousands of farms and reducing heating and lighting costs. The electricity generation indicators of RES in Kazakhstan from 2020 to 2024 are presented in Figure 3.

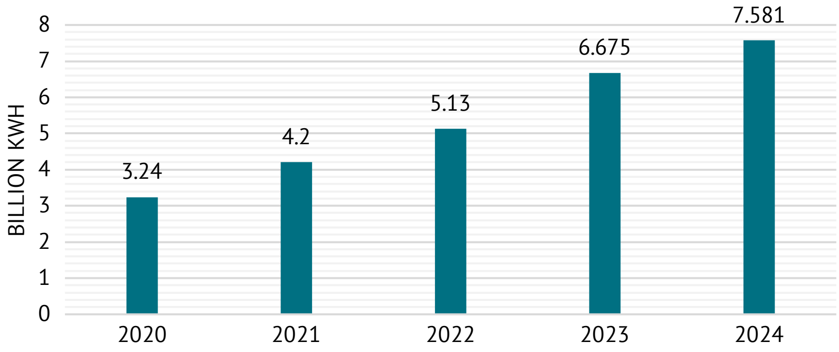


***Figure 3**. Dynamics of renewable energy production in Kazakhstan (2020-2024)*
***Source:** developed by the authors based on the sources RES power generation in Kazakhstan increased by 15% over the year (2022), United Nations Development Programme (2022), The share of RES in electricity generation in the Republic of Kazakhstan amounted to 5.92% by the end of 2023 (2024), Share of green electricity in Kazakhstan for 2024 amounted to 6.43% (2025)*

In 2020, electricity generation from RES facilities in Kazakhstan amounted to 3.24 billion kWh. The share of RES in total electricity production reached 3%. The installed capacity of RES facilities by the end of the year was approximately 1,970 MW. The main contribution was made by wind and solar power plants. In 2021, electricity generation from RES increased to 4.2 billion kWh, accounting for around 4% of total electricity production. Installed capacity reached 2,010 MW, due to the commissioning of new facilities, including wind farms in Zhambyl Region and solar farms in Almaty Region. Generation growth amounted to approximately 30% compared to the previous year. In 2022, electricity generation from RES grew to 5.13 billion kWh, with a share of 4.5% of the total volume. Installed capacity rose to 2,405 MW owing to new projects such as solar plants in the south and wind farms in the northern regions. The generation increase was about 22% compared to 2021. In 2023, RES electricity generation reached 6,675.5 million kWh (6.675 billion kWh), with a share of 5.92% of total production. Installed capacity rose to 2,868.6 MW, of which wind farms provided 1,394.6 MW, solar – 1,202.61 MW, small HPPs – 269.605 MW, and bio-power plants – 1.77 MW. The increase in generation compared to 2022 was 30%. Finally, in 2024, RES electricity generation amounted to 7,581.33 million kWh, which was significantly more than in the same period of 2023. Installed capacity in 2024 amounted to 3,032.12 MW, and growth continued through new projects.

It should be emphasised that the scaling potential of RES in agriculture was immense, given the declining cost of technologies and rising demand for sustainable solutions. According to the International Renewable Energy Agency (2019; 2023b; 2024b), in 2020 RES covered only about 5% of the global agricultural sector's energy needs, but by 2050 this figure could reach 30-40% with active policy and investment. Solar energy led this process: the cost of photovoltaic panels fell by 85% from 2010 to 2024 (from USD 4 to USD 0.6 per watt), and the efficiency improved due to new materials such as perovskites. By 2035, according to BloombergNEF (2025), solar systems were projected to become the cheapest energy source in 80% of the world's countries, making these systems accessible even for small farms. Wind energy also gained momentum: small turbines (1-10 kW) became popular in rural areas, and the cost dropped by 30% over the last 10 years. Biogas plants, although requiring initial investment, paid off in 3-5 years due to fuel savings and excess energy sales. On a global scale, the International Renewable Energy Agency predicted that by 2040 up to 50 million farms could use RES, generating up to 1,500 terawatt-hours of energy annually – equivalent to India's current consumption. In Kazakhstan, RES scaling could have started with the southern regions (solar irrigation pumps), steppe zones in the north (wind turbines for grain farms), and livestock areas (biogas). If by 2035, 10% of Kazakh farms transitioned to RES, this would provide up to 500 MW of capacity, covering the needs of 50-70 thousand farms. Scaling up RES in agriculture directly impacted food security and global sustainability by addressing key 21st-century challenges. According to the Food and Agriculture Organization (2018), by 2050, global food production would need to increase by 50% to meet the needs of 10 billion people, but climate change already reduced yields: since 2000, droughts and heatwaves had lowered global wheat and maize harvests by 5-10%. RES mitigated these risks by ensuring stable energy supply for irrigation, processing, and storage.

The implementation of RES potential in agriculture was impossible without international cooperation and technology exchange, especially for countries with limited resources. For the Republic of Kazakhstan, international cooperation played a key role in accelerating the transition to RES, especially through partnerships with leaders in this field, such as the European Union and India. The EU, as a global leader in wind energy, possessed advanced technologies and experience: in 2022, wind installations supplied 17% of the region's electricity (WindEurope, 2023), and companies like Vestas (n.d.) and Siemens (n.d.) developed efficient turbines for agricultural needs. Cooperation with the EU could have provided Kazakhstan access to these technologies, adapted for the steppe regions in the north. India, in turn, was an expert in solar pump usage: its PM-KUSUM programme had installed 300,000 such systems by 2023, increasing irrigated land by 1.5 million hectares (Ministry of New and Renewable Energy of India, n.d.). Partnership with these countries could have attracted investments through grants, loans, and foreign direct investment, accelerating RES deployment several times compared to the current pace, while scale effects and competition among suppliers reduced equipment costs. This would have made RES more affordable for Kazakh farmers and strengthened the country's position in global climate initiatives by demonstrating commitment to emission reduction.

RES also brought significant social benefits, among which were job creation and technology accessibility for farmers, contributing to stronger rural communities and the economic stability. The deployment of RES stimulated employment, covering a wide range of activities – from equipment manufacturing, installation, and configuration to routine maintenance and user training. This process created jobs for local residents, including technicians, installers, and system operators, which was especially valuable in rural areas where employment opportunities were often limited to traditional sectors such as livestock or crop production. In the Republic of Kazakhstan, RES development could have provided jobs for thousands of people involved in solar panel manufacturing, wind turbine assembly, or biogas plant management, helping reduce unemployment and retain population in rural areas. Employment in this sector did not require high qualifications at all stages: while design and complex repairs were handled by specialists, installation, basic maintenance, and training were accessible to people with minimal preparation, expanding the pool of potential workers. Moreover, RES development supported the local economy, as part of the equipment could be produced locally, and services for its installation and operation remained within the region, boosting economic activity and improving living standards.

An equally important social benefit was the accessibility of RES technologies for farmers, making such technologies a practical solution even for small holdings. These systems were designed to be easy to learn and operate, allowing farmers to quickly adapt to the use without the need for deep technical knowledge or lengthy training. Solar panels, for example, required only basic understanding for installation and connection, and the management was intuitive even for beginners. Wind turbines, despite more complex design, came with simple instructions, making these turbines suitable for independent use after minimal training. Biogas plants running on farm waste were also accessible to operate: farmers could learn to load raw material and keep the system functional in a short period, relying on basic skills. In Kazakhstan, this was especially relevant, as the rural population often had practical experience with machinery, easing the transition to RES. Such accessibility increased farmers' independence, enabling farmers to implement technologies without external experts, thereby reducing costs and accelerating integration. As a result, farmers gained the opportunity to improve the efficiency of the farms – whether irrigating fields with solar pumps or heating premises using biogas – enhancing the confidence in the future.

## DISCUSSION

In the course of this work, it was established that the introduction of RES has a significant impact on the resilience of agriculture in the context of climate change, opening new opportunities for adaptation and development of the sector. These findings highlight that the transition to RES can transform traditional approaches to energy supply in the agricultural sector, making it less dependent on external factors and more flexible in responding to global challenges. The significance of this discovery lies in the fact that RES address current issues such as fuel supply instability or vulnerability to climate shifts, while also laying the foundation for long-term stability, allowing agriculture to evolve under growing environmental and resource constraints. This approach may become key for countries seeking to maintain the food security and competitiveness in the global market, especially where natural conditions and infrastructural specifics require innovative solutions. However, the results also indicate the need for further research into how these technologies can be scaled to make the benefits accessible to a wide range of farmers, including small farms, which often face resource and knowledge constraints. The study by A. Bathaei and D. Štreimikienė (2023) also highlighted the role of RES in enhancing agricultural resilience with an emphasis on the adaptability to climate change, which confirms the findings of this work, although focusing more on theoretical aspects, whereas this study includes practical examples and the regional context of Kazakhstan. In the study by M. Rahman *et al.* (2022), the impact of RES on the resilience of agricultural systems was examined with an emphasis on improving production and process efficiency, which aligns with the results of this research, but with less focus on the social and environmental benefits, which are more thoroughly analysed here. The study by A.S. Pascaris *et al.* (2021) analysed the integration of RES into agricultural enterprises, emphasising the strategic importance, which echoes the conclusions of this study, although this research covers the potential for scaling and adaptation to the specific conditions of remote regions more broadly.

This work also established that the introduction of RES in agriculture has a profound impact on its economic and environmental dimensions, highlighting the potential as a tool for sustainable development. From an economic perspective, the results show that the transition to RES can reduce farmers' dependence on fossil fuels, stabilise costs, and improve the profitability of farms through the use of free natural resources and the minimisation of operational expenses. This means that the agricultural sector can become more resilient to market fluctuations and external economic shocks, which is especially important for countries with developed agriculture, such as Kazakhstan, where the stability of agricultural production directly affects the national economy. In addition, it was noted that environmentally, RES contributes to reducing the carbon footprint of the sector by lowering greenhouse gas emissions and easing the burden on the environment, which in the long term slows the degradation of natural resources necessary for agriculture. These findings underline the dual benefit of RES, as these energy sources not only improve the financial resilience of farmers but also contribute to global efforts to combat climate change, creating a more balanced production model. The study by Y. Majeed *et al.* (2023) highlighted the economic benefits of RES for agriculture with a focus on cost reduction and efficiency improvement, which aligns with the conclusions of this research, although the mentioned study concentrates more on general economic aspects, whereas this research additionally analysed the applicability under the conditions of Kazakhstan. The study by D. Maradin *et al.* (2021) conducted an analysis of the economic efficiency of wind installations, focusing on the long-term profitability, which resonates with the findings of this study, although the current study more extensively addressed environmental and regional aspects adapted to the steppe conditions of Kazakhstan. The study by A. Alengebawy *et al.* (2024) emphasised the environmental effects of biogas systems and the role in waste processing, which supports the results of this work, but places less emphasis on the economic and social benefits, which in this study were considered an important part of agricultural sustainability.

An important result of this research was the study of the impact of RES implementation on electricity availability for remote rural areas, noting that RES can significantly improve and have a notable impact on the social aspects of life in farming communities. Regarding electricity accessibility, it was noted that solar panels, wind turbines, and biogas units provide autonomous solutions, enabling stable energy supply where traditional grids are either absent or unstable, which is especially important for isolated regions. This means that farmers gain the ability to support key production processes without dependence on costly fuel delivery or vulnerable infrastructure, which increases the independence and productivity (Nakashydze *et al.*, 2021). At the same time, from a social perspective, the results emphasise that RES contribute to improving living conditions, creating jobs, and simplifying access to technologies, which strengthens rural communities and reduces social inequality. These findings indicate that the implementation of RES can become a catalyst not only for energy but also for social transformation, allowing remote areas to integrate into modern economic processes and improve the quality of life of the population. This, in turn, opens up opportunities for reducing the gap between urban and rural areas, increasing the resilience of local communities, and supporting the development amid global changes, which is especially relevant for countries with extensive rural regions, such as the Republic of Kazakhstan. The study by C. Roldán-Porta *et al.* (2023) focused on optimising solar and biogas-based systems for sustainable energy supply in farms, highlighting the autonomy, which aligns with the conclusions of this study, but places greater emphasis on technical optimisation, without considering the social effects and regional specifics of Kazakhstan. The study by N. Lefore *et al.* (2021) analysed the social and economic benefits of solar systems, emphasising the accessibility for farmers and small enterprises, which confirms the findings of this research, although focusing more on economic aspects, whereas this study covers the impact on quality of life and community resilience. The study by O. Saidmamatov *et al.* (2021) examined the use of biogas systems for rural areas with an emphasis on the potential for isolated regions, which confirms the conclusions of this work, but pays less attention to the integration of different types of RES. The study by R.F. Tagne *et al.* (2021) analysed the social effects of biogas installations in agriculture, highlighting the role in improving daily life and efficiency, which supports the conclusions of this study, but without analysing the accessibility of technologies and the impact on employment adapted to the conditions of Kazakhstan.

In general, the results of this research, combined with the findings of the mentioned works, underline the importance of RES for agriculture, demonstrating the capacity to support the sector under modern challenges. This reveals the potential of RES as a tool capable of improving various aspects of the agricultural sector, including its resilience, efficiency, and social well-being, which makes RES a vital direction for rural development. The results also confirm that RES can play a key role in adapting agriculture to global changes, offering solutions that resonate across various contexts, especially for regions with significant agricultural potential, where RES can become the foundation for strengthening the sector and its future progress.

## CONCLUSIONS

As a result of this work, it was established that the introduction of renewable energy sources (RES), such as solar, wind, and biogas energy, possesses significant potential for transforming agriculture, making it more sustainable under climate change conditions. The study revealed that these technologies are capable of reducing the agricultural sector's dependence on traditional energy sources such as oil, gas, and diesel fuel, improving energy supply in remote rural areas, and strengthening agriculture's capacity to withstand climate challenges such as droughts, floods, and unstable temperatures.

It was noted that in Kazakhstan, RES production increased from 3.24 billion kWh in 2020 to 7.581 billion kWh in 2024, demonstrating the growing contribution. The study also emphasised that decentralised systems, such as solar panels, wind turbines, and biogas plants, provide autonomy, allowing farmers to maintain the operation of irrigation systems, lighting, refrigeration units, and other equipment without reliance on external sources. In addition, the transition to RES reduces the environmental impact of the agricultural sector by cutting greenhouse gas emissions, as the agricultural sector globally produces 10-12% of anthropogenic emissions, including 500 million tonnes of $CO_2$ from diesel and 3.1 billion tonnes of $CO_2$-equivalent from livestock farming in 2022, which contributes to slowing climate change. As a result of analysing the experience of other countries such as India, Germany, and Kenya, it was noted that RES not only enhance the resilience of agriculture but also contribute to significant economic and environmental progress. The social and technical aspects of RES also highlighted the value, as the introduction of these technologies can stimulate job creation, and the use is characterised by ease of learning and operation, making these technologies accessible even to small farms without specialised training. Thus, globally, RES strengthens food security by increasing yields through stable energy supply and reducing costs, which opens up prospects for strengthening Kazakhstan's position in the grain and livestock markets, making production more competitive and sustainable, especially considering the decrease in the cost of RES-based electricity production from USD 0.057 to USD 0.04 for solar energy and from USD 0.039 to USD 0.03 for wind energy from 2010 to 2024.

It should be noted that this work has certain limitations in the form of predominantly considering the use of RES in the context of Kazakhstan, which may reduce the generalisability of the conclusions for other regions with different climatic and economic conditions. For further research in this field, a promising direction is the study of the economic feasibility of RES for small and medium-sized farms in various climatic conditions, which will allow for the adaptation of technologies to diverse natural and economic realities and expand the applicability on a global scale.

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## CONFLICT OF INTEREST

None.

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# Вплив впровадження відновлюваних джерел енергії на стійкість сільського господарства в умовах зміни клімату

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**Анотація**. Метою цього дослідження було вивчення потенціалу відновлюваних джерел енергії (ВДЕ) у підвищенні стійкості сільського господарства до зміни клімату, з акцентом на застосовності цих технологій в контексті Казахстану. Дослідження базувалося на якісному та кількісному аналізі поточного стану сектору, порівняльному аналізі технологій відновлюваної енергетики, розгляді практичних випадків та оцінці застосовності в Республіці Казахстан. В результаті дослідження було виявлено, що ВДЕ, такі як сонячна, вітрова та біогазова енергія, мають значний потенціал для підвищення стійкості сільського господарства в контексті зміни клімату. Було встановлено, що ці технології ефективно зменшили залежність сільськогосподарського сектору від викопного палива, що було особливо цінним для віддалених регіонів. Було наголошено, що система потужністю 2 кВт може генерувати 6-10 кВт·год на день у сонячних регіонах, таких як Туркестанська або Алматинська області, де інсоляція сягає 1500-1800 кВт·год/м² на рік, а турбіни потужністю 1-10 кВт можуть генерувати 2-20 кВт·год на день у вітряних районах, таких як Костанай та Акмола, де швидкість вітру сягає 5-7 м/с. Дослідження також показало, що в Казахстані з 2020 по 2024 рік виробництво електроенергії з ВДЕ зросло з 3,24 млрд кВт·год до 7,581 млрд кВт·год. Було зазначено, що такі джерела енергії сприяють адаптації до зміни клімату, забезпечуючи надійне енергопостачання для зрошення в посушливих зонах або опалення в нестабільні сезони, а також зменшуючи викиди парникових газів, тим самим знижуючи екологічне навантаження на сільськогосподарський сектор. Для Казахстану це відкрило перспективи збільшення врожайності завдяки стабільному доступу до енергії, зменшенню залежності від імпорту палива та покращенню екологічних показників, особливо враховуючи, що з 2020 по 2024 рік вартість ВДЕ знизилася з 0,057 до 0,04 доларів США для сонячної енергії та з 0,039 до 0,03 доларів США для вітрової енергії, тоді як вугілля та газ коливалися в межах 0,04-0,25 доларів США з піком у 2022 році. Таким чином, ВДЕ вирішували поточні проблеми, а також створювали основу для довгострокового сталого розвитку сільського господарства, що було особливо важливим в контексті кліматичних ризиків

**Ключові слова:** сонячна енергетика; вітрові турбіни; біогазові установки; енергопостачання віддалених територій; соціальні та екологічні вигоди; економічна ефективність

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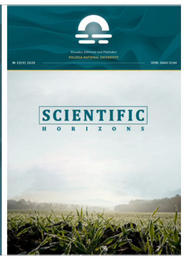


# Machine learning-based prediction of rice yield from rain feeding in monsoonal tropical area of Java, Indonesia

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**Abstract**. Rainfed rice cultivation in monsoonal tropical areas of Java, Indonesia, is challenged by nutrient deficiencies, unpredictable rainfall amounts, and limited agricultural investment, leading to fluctuating yields. The purpose of this study was to develop a precise rice yield prediction model using machine learning tailored to specific toposequences in Central Java. A combination of survey-based field and laboratory methods was employed, integrating climate, soil, socio-economic, and

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land management variables from 87 targeted sampling points. Machine learning analysis using Bayesian Neural Networks (BNN) demonstrated moderate accuracy with $R2 = 0.840$ and RMSE = 0.442 overall, but accuracy improved significantly when models were adjusted to elevation-specific categories, achieving R2 values up to 0.999. Lowland paddy field predictions were most influenced by available phosphorus (P), while rainfall, gender, education, and seed variety were key factors in medium-altitude zones; slope, available P, gender, and cropping patterns were dominant in highland areas. Pareto analysis supported the identification of these key yield determinants in each toposequence. The integration of BNN and Pareto approaches enabled the creation of a high-precision, location-specific yield prediction model. This work demonstrated that tailoring machine learning models to elevation-based agroecological zones enhances their performance and practical application. The findings are particularly valuable for agricultural stakeholders including policymakers, extension services, and farmers, who can leverage these predictive insights to optimise rainfed rice management practices and improve productivity under variable climatic conditions

**Keywords:** agricultural sustainability; Bayesian Neural Network (BNN); food security; Pareto analysis; precision agriculture

## INTRODUCTION

Rainfed rice production in monsoonal tropical environments continues to be a crucial element of food systems in many developing regions. These systems are typified by seasonal rainfall patterns, limited irrigation infrastructure, nutrient-depleted soils, and socio-economic challenges. The productivity of rainfed rice fields is constrained by environmental variability, soil degradation, and the inability of smallholder farmers to adopt efficient management strategies. These challenges result in fluctuating and often suboptimal yields, which limit long-term food security and economic resilience in rural areas. As global demand for rice continues to rise and climate patterns shift unpredictably, the development of accurate, site-specific predictive models for rice yields is vital to inform agricultural planning and optimise input allocation in such high-risk production systems.

Machine learning (ML) has gained popularity in agricultural modelling due to its ability to manage complex nonlinear interactions between diverse input data. J. Zhang *et al*. (2023) developed a hybrid ML model combining climatic and soil factors for smallholder farms in Southeast Asia and reported a significant increase in prediction accuracy when socio-environmental interactions were considered. Analogously, N. Din *et al*. (2024) employed convolutional neural networks for rice yield forecasting across South Asia, demonstrating that spatial-temporal data integration markedly enhanced model robustness. P. Velmurugan *et al*. (2023) introduced a fuzzy enumeration technique in big data environments for agricultural forecasting and emphasised the predictive advantage of including farmer demographics and cropping patterns. D. Paudel *et al*. (2021) proposed large-scale ML frameworks for European agricultural systems, highlighting the scalability of data-driven models while cautioning that prediction accuracy declines without site-specific calibration. S. Liu *et al*. (2017) compared various ML methods and concluded that Bayesian Neural Networks (BNN) outperform conventional regression models in handling uncertainty and sparse datasets widespread in rainfed regions.

J. Qian *et al.* (2025) examined the effects of declining rainfall suitability on paddy production in tropical monsoon climates, asserting the need for adaptive modelling that accounts for regional climate vulnerabilities. In a pixel-scale study, S. Jeong *et al.* (2022) applied deep learning models to rice yield prediction using satellite imagery, concluding that predictive power increased substantially when incorporating slope, elevation, and localised agroecological variables. J. Pant *et al*. (2021) emphasised the significance of non-agronomic inputs, such as education level and access to improved seed varieties, in boosting prediction accuracy in rainfed rice fields. T. Tu *et al*. (2023) further demonstrated that in highland paddy systems, land slope and contour significantly influenced yields, and concluded that predictive models must be tailored to local topographic and management realities. Although these studies have contributed to advancements in ML-based crop yield forecasting, gaps persist in understanding how location-specific topographical characteristics, particularly in tropical rainfed zones such as Central Java interact with agronomic and socio-economic variables to affect model performance. Prior research has tended to focus either on climate or soil characteristics alone, without a holistic integration of diverse influencing factors within a localised, elevation-specific context.

The purpose of this study was to construct a rice yield prediction model for rainfed agricultural systems in Central Java using a Bayesian Neural Network (BNN) integrated with Pareto analysis, with the aim of identifying key determinants of yield across multiple mountain toposequences classified by elevation.

## MATERIALS AND METHODS

**Study area.** Six mountain toposequences were the subject of the study in Central Java, Indonesia, including the eastern slopes of Mount Slamet (7°12’19.0”S – 7°17’07.1”S & 109°17’37.2”E – 109°26’11.4”E), southwest of Mount Sindoro (7°22’25.8”S – 7°29’13.1”S & 109°47’21.4”E – 109°53’34.3”E), south of Mount Sumbing (7°26’09.6”S – 7°37’16.0”S & 110°02’59.0”E – 110°08’04.3”E), northwest of Mount Merbabu (7°20’48.0”S – 7°26’05.2”S & 110°13’02.5”E – 110°20’38.7”E), southwest of Mount Merapi (7°37’08.0”S – 7°39’34.7”S & 110°18’12.0”E – 110°20’08.0”E), and south of Mount Lawu (7°46’6.3”S – 7°53’19.2”S & 111°9’27.3”E – 111°13’34.9”E) (Fig. 1). The gradient of the land topography was considered during the sampling procedure. A total of 57 locations were sampled with a gradient of 0-8%, 21 locations with a gradient of 8-15%, and 9 locations with a gradient of 15-25%. Additionally, topography was also considered for the analysis. There are 34 locations with an altitude of under 400 m above sea level, 33 locations with an altitude of 400-700 m above sea level, and 20 locations with an altitude of over 700 m above sea level. The survey used soil samples to identify their composition, as well as interviews to learn about land management practices and farmers’ socio-economic circumstances. The average yield from rainfed rice crops at the research site was 2.12 tonnes ha$^{-1}$.

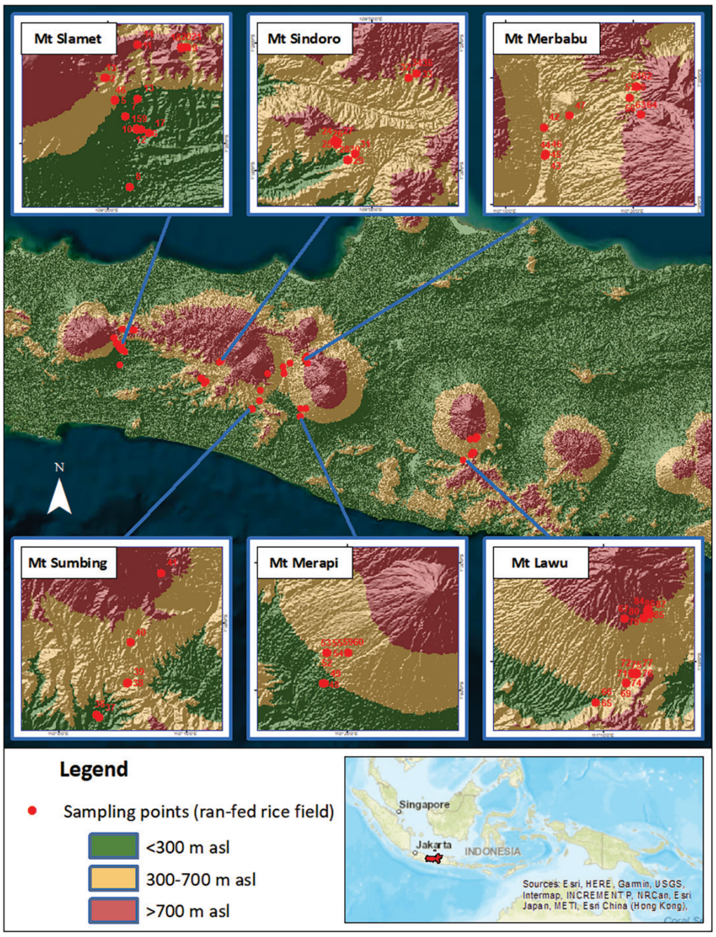



***Figure 1**. Research Site Sampling*

***Source:** ESRI (2022)*

**Characteristics of the soil.** The growth of plants depends on the characteristics of the soil, which also significantly influence agricultural yields. According to field observations and the outcomes of laboratory analyses of soil properties, this study employed data on soil characteristics in the field (Table 1). Soil types in the study area ranged between entisols, inceptisols, alfisols, ultisols, and andisols, with a slope of 0-25%. Generally, the soil pH at the study site ranged within 5-6, with organic C ranging within 0.39-7.74%. Soil sampling points were determined according to the toposequence, with 15-33 sampling points at each toposequence. Soil pH was measured with a pH stick (Jackson, 1973), and organic C was measured according to the Walkley and Black method (Walkley & Black, 1934). Total N was determined using the Kjeldahl method (Bremner & Mulvaney, 1982), and available P was determined using the Olsen method (soil pH > 5.5) (Olsen *et al.*, 1954). The method of ammonium acetate extraction was employed to analyse available K in soil (Thomas, 1982).

***Table 1**. Research parameters of soil characteristics at rainfed rice fields on location-specific toposequences*

| Parameters (units) | Lowland paddy fields | Midland paddy fields | Highland paddy fields | Total |
|---|---|---|---|---|
| pH | 5–6 | 5–6 | 5–6 | 5–6 |
| Organic C (%) | 0.39–6.03 | 0.67–4.02 | 0.96–7.74 | 0.39–7.74 |
| Total N (%) | 0.08–1.63 | 0.17–1.21 | 0.17–1.40 | 0.08–1.63 |
| Available P (ppm) | 0.29–23.74 | 1.04–18.16 | 1.59–25.78 | 1.04–25.78 |
| Available K (meq $100g^{-1}$) | 0.25–0.66 | 0.20–0.77 | 0.23–0.81 | 0.20–0.81 |
| Type of soil | Entisols, Inceptisols, Alfisols, Ultisols and Andisols | Entisols, Inceptisols, Alfisols and Andisols | Entisols, Inceptisols, and Andisols | Entisols, Inceptisols, Alfisols, Ultisols and Andisols |
| Slope (%) | 0-25 | 0-25 | 0-25 | 0-25 |

***Source:** compiled based on the analysis of soil characteristics based on (BRMP) Indonesian Agricultural Assembly and Modernization Agency (2005) and data on plant yield recap based on a social survey carried out by researchers at the research site*

**Climate characteristics.** The model incorporated climate data to estimate the amount of water available and usable by plants. In the yield prediction model, climate factors significantly affect crop productivity, with rainfall and air temperature as the primary determinants of yield outcomes. This predictive model considered two key variables: rainfall and evapotranspiration. The analysis relied on rainfall and air temperature data collected from climatology stations at the research site between 2014 and 2023, as presented in Table 2. Evapotranspiration was calculated using the Thornthwaite method (Moeletsi *et al.*, 2013).

***Table 2**. Climate characteristics at the research site*

| Parameters (units) | Lowland paddy fields | Midland paddy fields | Highland paddy fields | Total |
|---|---|---|---|---|
| Rainfall (mm $year^{-1}$) | 1,845-4,874 | 1,845-4,000 | 2,246-3,954 | 1,845-4,874 |
| Evapotranspiration (mm $day^{-1}$) | 1.90-5.81 | 1.90-5.81 | 1.90-5.27 | 1.90-5.81 |

***Source:** compiled based on the analysis of climate characteristics based on climatology stations at the research site (2014-2023)*

**Farmers' socio-economic land management.** Data were gathered through firsthand interviews with farmers who manage their land. A total of 87 respondents were included in the study, of whom 75 were male and 12 were female. The majority of respondents were aged between 51 and 60 years. The distribution of farmers' education was very diverse, ranging from uneducated to a master's degree. Most of the farmers still use conventional methods of cultivation. The farmer's tools and techniques affect the yields. To create a site-specific prediction model, this data presented an overview of farmers' socio-economic circumstances and unique land management techniques. However, this study used specific data on farmers' socio-economic circumstances (Table 3) and land management practices (Table 4) to obtain a site-specific prediction model that can support precision farming. Typically, the development of predictive models only uses climate and soil data as the input model.

***Table 3**. Research parameters of farmers' socio-economic characteristics at rainfed rice fields on location-specific toposequence*

| Parameters (units) | Lowland paddy fields | Midland paddy fields | Highland paddy fields | Total |
|---|---|---|---|---|
| Farmer's age (year) | 20-70 | 31-70 | 31-70 | 20-70 |
| Gender | Male and female | Male and female | Male and female | Male and female |
| Farming experience (years) | 1-30 | 6-30 | 6-30 | 1-30 |
| Education | No education–bachelor | No education – senior high school | No education–junior high school | No education–bachelor |
| Farmer's side job | No side job, labourer, civil servant | No side job, labourer, civil servant, trader | No side job, labourer, civil servant | No side job, labourer, civil servant, trader |
| Family involvement | Only the head of the family, several family members, and all family members | Only the head of the family, several family members, and all family members | Only the head of the family, several family members | Only the head of the family, several family members, and all family members |

***Source:** developed by the authors of this study based on questionnaire data collection*

*Table 4. Research parameters on farmers' land management characteristics at rainfed rice fields on location-specific toposequence*

| Parameters (units) | Lowland paddy fields | Midland paddy fields | Highland paddy fields | Total |
|---|---|---|---|---|
| Seed variety | IR64, IR32, ciherang, Brengkele, Liwu, Mlati, Inpari 43, Mapan, Mekong and Sugal | IR64, IR32, ciherang, bengawan, mentik wangi, umbul-umbul, and barito | IR64, ciherang, bengawan and cepogo | IR64, IR32, ciherang, Brengkele, bengawan, Liwu, Mlati, Inpari 43, HT, umbul-umbul, Mapan, Mekong, Sugal, barito and cepogo |
| Cropping method | Jajar legowo and conventional | Jajar legowo and conventional | Jajar legowo and conventional | Jajar legowo and conventional |
| Cropping technique | Semi-organic and conventional | Semi-organic and conventional | Semi-organic and conventional | Semi-organic and conventional |
| Cropping pattern | Paddy-paddy-paddy, paddy-paddy-secondary crops, paddy-secondary crops-secondary crops, paddy-paddy-fallow, paddy-paddy-horticulture, | Paddy-paddy-paddy, paddy-paddy-secondary crops, paddy-secondary crops-secondary crops, paddy-paddy-fallow, paddy-secondary crops-fallow, paddy-paddy-horticulture, paddy- horticulture-horticulture | Paddy-paddy-paddy, Paddy-paddy-secondary crops, paddy-secondary crops-secondary crops, paddy-paddy-fallow, paddy-secondary crops-fallow | Paddy-paddy-paddy, paddy-paddy-secondary crops, paddy-secondary crops-secondary crops, paddy-paddy-fallow, paddy-secondary crops-fallow, paddy-paddy-horticulture, paddy- horticulture-horticulture |
| Fertiliser type | Compost, manure, urea, ZA, TS, Phonska, SP36, TSP, KCl, NPK, dolomite, TSP | Compost, manure, urea, ZA, TS, Phonska, SP36, TSP, KCl, NPK | Compost, manure, urea, ZA, TS, Phonska, SP36, TSP, KCl, NPK | Compost, manure, urea, ZA, TS, Phonska, SP36, TSP, KCl, NPK, dolomite, TSP |
| Pest disease control | No pest disease control, preventive, chemical, mechanical | No pest disease control, chemical, mechanical | No pest disease control, preventive, chemical, mechanical | No pest disease control, preventive, chemical, mechanical |
| Yield (tonnes/ha) | 0.62-4.34 | 0.8-4 | 0.91-3.57 | 0.62-4.34 |

***Source:*** *developed by the authors of this study based on questionnaire data collection*

**Machine learning.** The BNN analysis results used 87 data samples, with 22 input parameters including 4 indicators: soil, climate, farmers' socio-economic data, and land management. The data was operated and tested using different numbers of neurons to get the most effective results and approach the factual results. The framework of the analysis conducted in this study is presented in Figure 2. The data was subsequently analysed using Pareto to optimise and identify the model determinants.

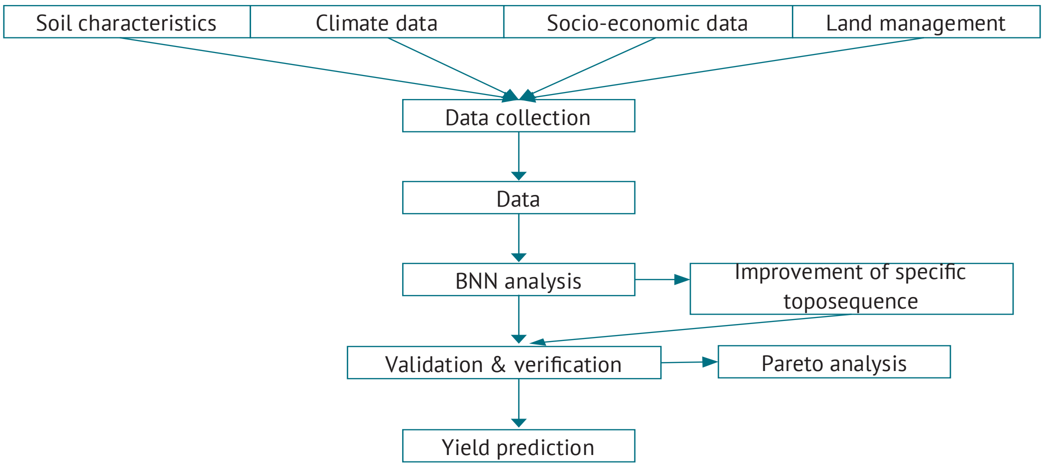



***Figure 2****. Framework of yield estimation using machine learning (Bayesian and Pareto approach)*
***Source:*** *developed by the authors of this study*

Six mountainous toposequences were used to create the forecast model for rainfed rice yields, using local and field data to produce a precise model. The BNN prediction model was made based on specific altitude characteristics to increase accuracy at particular places.

**Model accuracy analysis**. The capability and reliability of the predictive model were assessed using accuracy analysis, which was also employed to evaluate the model's performance. During the study, the coefficient of determination ($R^2$), root mean square error (RMSE), and mean absolute percentage error (MAPE) were calculated. The following equation was used for this evaluation:

$$\mathrm{RMSE} = \sqrt{\frac{\sum_{i=1}^{n}(y_i - y)^2}{n}}, \tag{1}$$

$$\mathrm{MAPE} = \sum_{i=1}^{n}\left|\frac{y_i - y}{y_i}\right| \times 100\%, \tag{2}$$

where $y_i$ is the prediction result; y is the average factual yield; *n* is the amount of data sample.

Measurement with root mean square error (RMSE) involved the analysis to measure the magnitude of error in the prediction results. The smaller the RMSE value, the more accurate the prediction result. Meanwhile, measurement with mean absolute percentage error (MAPE) involved statistical measurement to see the accuracy of estimates in forecasting methods.

## RESULTS

**Machine learning.** The trial-and-error method frequently starts with choosing the ideal weights, the number of hidden layers, and the neurons in the hidden layers (Fig. 3). The backpropagation algorithm's control settings often depend on the procedure of trial and error. The operation is analogous to the human brain. Numerous linked neurons translate input into new, useful information. Regardless of the magnitude of the input data being processed, NN systems can be trained on massive volumes of data rapidly and effectively. Additionally, NN creates patterns and trends for analysis that are unachievable for humans and ensures the extraction of pertinent data from extremely complicated data sets.

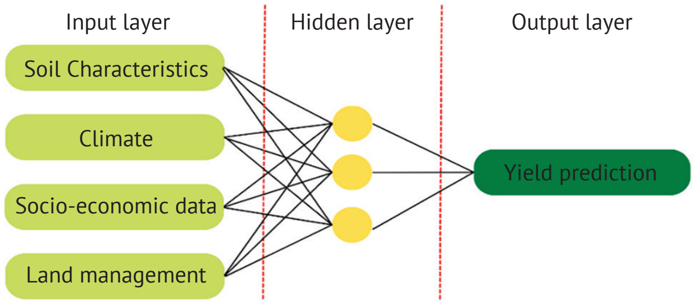



***Figure 3***. *Schematic of prediction yield using BNN*
***Source:*** *developed by the authors of this study*

The study employed an efficient model comprising three neurons, based on the findings of the test. The test involved ten different input neurons (Fig. 3). Table 5 presents trial and error testing using ten different neurons, with the lowest RMSE of 0.442 and the most outstanding $R^2$ score of 0.840.

***Table 5***. *Comparison of model accuracy and the number of neurons employed*

| Toposequence | Parameters | Number of neurons | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Total | RMSE | 0.701 | 0.607 | 0.442* | 0.696 | 0.704 | 0.704 | 0.704 | 0.705 | 0.704 | 0.704 |
| | $R^2$ | 0.519 | 0.638 | 0.840* | 0.525 | 0.513 | 0.512 | 0.512 | 0.511 | 0.512 | 0.512 |

***Note:*** *RMSE – smaller values are better; $R^2$ – greater values are better; * – best values*
***Source:*** *developed by the authors of this study*

**Step 1: BNN analysis.** The accuracy of yield projections at certain places was greater than the overall yield. The findings of this study revealed that predictions made using location-specific altitude data produced satisfactory outcomes, with the three models' coefficient of determination over 0.9. The ideal number of neurons for the models at the three toposequences varied, with highland paddy fields requiring 8 neurons, midland paddy fields – 1 neuron, and lowland paddy fields – 3 neurons. The results of the model accuracy comparison are presented in Figure 4.

As demonstrated in Figure 4, the three revisions of the toposequence-based model demonstrated an increase in performance, with the RMSE and $R^2$ values showing extremely satisfactory results when compared to when the model was built using total. Pearson values in all four models were more significant than 0.9, with the midland paddy fields prediction model having the greatest value. The total model value was 0.8396 based on the coefficient of determination, whereas the value of the three model modifications was above 0.99 or remarkably close to the factual value. Analogously to the RMSE and MAPE values, the evaluation value of the error of this model was low in the lowland paddy fields, midland paddy fields, and highland paddy fields models when compared to the total model with RMSE (0.4421) and MAPE (21.95%). This demonstrates that when the prediction model was modified based on the accuracy test value, the Pearson and $R^2$ values were close to 1, while the RMSE and MAPE values were close to 0.

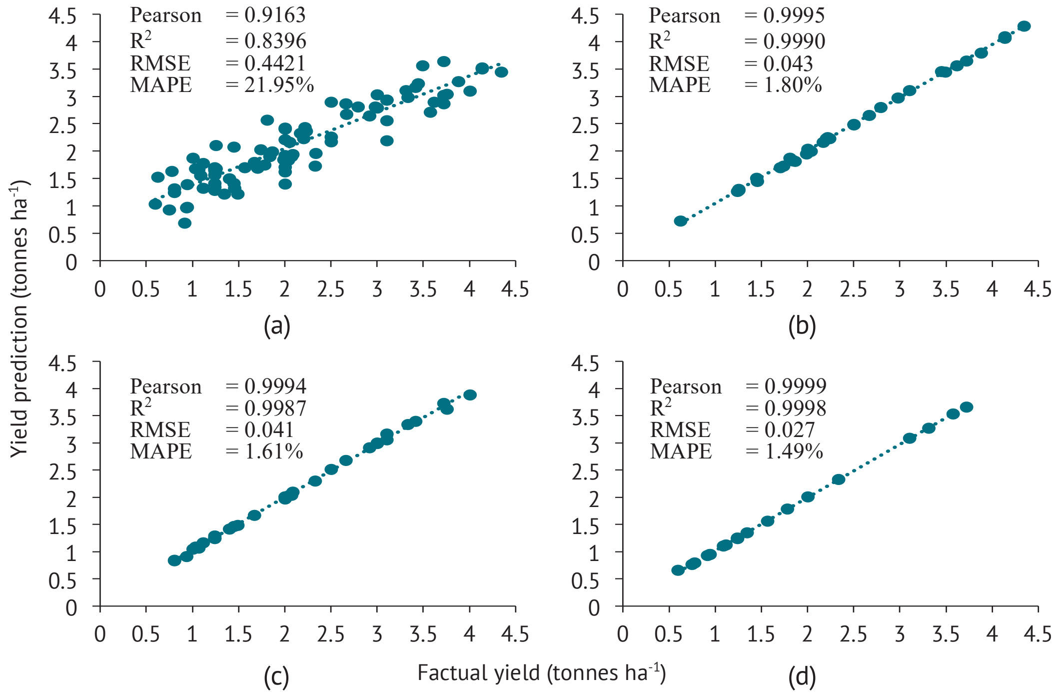



***Figure 4**. Location-specific crop yield prediction accuracy (a) Total; (b) Lowland paddy fields; (c) Midland paddy fields; (d) Highland paddy fields*

***Source:** developed by the authors of this study*

Table 6 depicts trial-and-error testing with ten different neurons, with the variation in toposequence indicating a different number of neurons used to construct a prediction model. According to M.K.A. Kadir *et al.* (2015), testing neurons using the trial-and-error approach is critical since this method can identify a model with the ideal composition of neurons and parameters. According to O. Bazrafshan *et al.* (2022), in machine learning models, the conditions in the hidden layer heavily influence the outcomes of the prediction models formed; the results of modifications and tests in Tables 5 and 6 demonstrate that the number of neurons in a different hidden layer produces different performance.

***Table 6**. Comparison of the number of effective neurons at each toposequence*

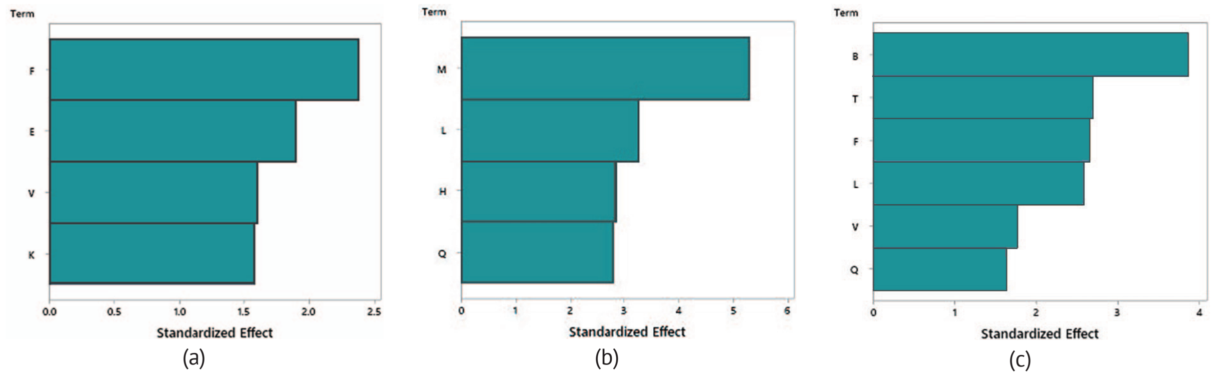
| Toposequence | Parameters | Number of neurons | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| lowland paddy fields | RMSE | 0.946 | 0.946 | 0.043* | 0.946 | 0.946 | 0.946 | 0.946 | 0.946 | 0.946 | 0.946 |
| | $R^2$ | 0.580 | 0.587 | 0.999* | 0.580 | 0.585 | 0.588 | 0.588 | 0.588 | 0.576 | 0.588 |
| midland paddy fields | RMSE | 0.041* | 0.089 | 0.093 | 0.090 | 0.095 | 0.105 | 0.104 | 0.098 | 0.104 | 0.107 |
| | $R^2$ | 0.999* | 0.994 | 0.993 | 0.993 | 0.992 | 0.990 | 0.991 | 0.992 | 0.991 | 0.990 |
| highland paddy fields | RMSE | 0.032 | 0.034 | 0.028 | 0.034 | 0.034 | 0.027 | 0.033 | 0.026* | 0.029 | 0.034 |
| | $R^2$ | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000* | 1.000 | 1.000 |

***Note:** RMSE – smaller values are better; $R^2$ greater values are better; * – best values*

***Source:** developed by the author of this study*

The results of the analysis by location classification are presented in Table 6 and Figure 4, demonstrating that the correlation values were highly significant and closely related to the analysis of input data in specific locations. This proves that presenting data based on specific altitude zones simplifies decision-making for users on issues related to agriculture. They show the same significance of correlation between lowland, midland, and highland rice fields. In this case, this was factored in when calculating the amount of input data that needed to be provided during the cultivation phase to obtain the best yields.

**Step 2: Pareto analysis.** According to the findings of this study, the rice harvest prediction model based on particular heights produces greater accuracy. According to the Pareto analysis's findings, each elevation criterion had a unique effect standard, as presented in Figure 5. The available P parameter was the factor that had the most significant influence according to the Pareto analysis results at a lowland paddy field. The farmer's education determines the prediction results for midland paddy fields, whereas the slope factor is the critical element for highland paddy fields.




| | | | | | | | |
|---|---|---|---|---|---|---|---|
| A | Type of soil | G | Available K | M | Education | R | Cropping method |
| B | Slope | H | Rainfall | N | Farming experience | S | Cropping technique |
| C | pH | I | Elevation | O | Farmer side job | T | Cropping pattern |
| D | Organic C | J | Evapotranspiration | P | Family involvement | U | Fertiliser type |
| E | Total N | K | Farmer's age | Q | Seed variety | V | Pest disease control |
| F | Available P | L | Gender | | | | |

***Figure 5**. Pareto analysis at each toposequence: (a) Lowland paddy fields; (b) Midland paddy fields; (c) Highland paddy fields*

***Source:** developed by the authors of this study*

The essential parameters – Available P, Total N, Pest disease control, and Farmer age, – presented in Figure 5, are what the lowland paddy fields prediction model depended on. However, only Available P had a p-value < 0.05 (Table 7). In the lowland paddy fields, primarily Available P, the data distribution varied, dominated by levels of Available P (< 4 ppm) with 41.2% (Table 8). Available P in lowland paddy fields, which had the greatest average yields, was at levels of > 15 ppm with 2.97 tonnes ha$^{-1}$. This shows that elevated levels of Available P can increase rice yields in rainfed rice fields. Based on research by S. Supriyadi *et al.* (2022), available P is closely related to the soil quality index, especially in paddy fields. This shows that Available P in lowland paddy fields is one of the determining factors for this toposequence.

***Table 7**. The p-value of each parameter classified by a specific toposequence*

| Toposequence | Parameter | p-value |
|---|---|---|
| Lowland paddy fields | Available P | 0.024 |
| Midland paddy fields | Rainfall | 0.008 |
| | Gender | 0.003 |
| | Education | 0.000 |
| | Seed variety | 0.009 |
| Highland paddy fields | Slope | 0.002 |
| | Available P | 0.020 |
| | Gender | 0.023 |
| | Cropping pattern | 0.018 |

***Source:** developed by the authors of this study*

*Table 8*. Prediction determinants of the rainfed lowland rice harvest for each toposesequence

| Toposequence | Parameter | Parameter class | Percentage (%) | Yield (ton ha$^{-1}$) |
|---|---|---|---|---|
| Lowland paddy fields | Available P (ppm) | < 4 | 41.2 | 2.23 |
| | | 4-8 | 14.7 | 2.17 |
| | | 8-10 | 14.7 | 2.36 |
| | | 10-15 | 5.9 | 2.33 |
| | | > 15 | 23.5 | 2.97 |
| Midland paddy fields | Rainfall (mm year$^{-1}$) | <1500 | 0.0 | 0.00 |
| | | 1,500-2,000 | 15.2 | 2.79 |
| | | 2,000-2,500 | 39.4 | 2.44 |
| | | 2,500-3,000 | 9.1 | 1.39 |
| | | >3,000 | 36.4 | 1.64 |
| | Farmers' gender | Male | 84.8 | 2.19 |
| | | Female | 15.2 | 1.65 |
| | Farmers' education | No education | 3.0 | 1.24 |
| | | Elementary school | 66.7 | 2.39 |
| | | Junior high school | 24.2 | 1.36 |
| | | Senior high school | 6.1 | 2.36 |
| | | Bachelor's degree | 0.0 | 0.00 |
| | Seed variety | Barito | 3.0 | 3.10 |
| | | Ciherang | 27.3 | 2.38 |
| | | IR32 | 6.1 | 1.32 |
| | | IR64 | 57.6 | 1.96 |
| | | Mentik wangi | 3.0 | 3.00 |
| | | Umbul-umbul | 3.0 | 2.05 |
| Highland paddy fields | Slope (%) | 0–8 | 60.0 | 3.57 |
| | | 8–15 | 35.0 | 1.93 |
| | | 15–25 | 5.0 | 1.37 |
| | | 25–40 | 0.0 | 0.00 |
| | | >40 | 0.0 | 0.00 |
| | Available-P (ppm) | <4 | 55.0 | 1.29 |
| | | 4–8 | 10.0 | 2.11 |
| | | 8–10 | 20.0 | 2.02 |
| | | 10–15 | 5.0 | 2.00 |
| | | >15 | 10.0 | 2.54 |
| | Farmers' gender | Male | 90.0 | 2.31 |
| | | Female | 10.0 | 1.61 |
| | Cropping pattern | paddy-paddy-paddy | 10.0 | 2.33 |
| | | paddy-paddy-fallow | 15.0 | 2.68 |
| | | paddy-paddy-secondary crop | 35.0 | 1.63 |
| | | paddy-secondary crop-secondary crop | 35.0 | 1.17 |
| | | Paddy-secondary crop-fallow | 5.0 | 1.24 |

***Source:*** *developed by the authors of this study*

In contrast, the midland paddy fields model used four parameters: rainfall, education level, gender, and seed type (Fig. 5b), with all parameters having a p-value < 0.05 (Table 7). Based on climatic characteristics, rainfall is one of the determining factors in rice cultivation in midland paddy fields. Based on the land suitability guidelines for food crop cultivation, rainfed rice is optimally cultivated with rainfall conditions of 1,500-2,000 mm year$^{-1}$ (Wahyunto *et al.*, 2016). This condition is under the distribution of analytical data in this study, where land with rainfall of 1,500-2,000 mm year$^{-1}$ had the greatest average yield of 2.79 tonnes ha$^{-1}$. Meanwhile, land with rainfall above 2,500 mm year$^{-1}$ had an average yield of 1.64 tonnes ha$^{-1}$. The use of seed varieties in the paddy fields medium also varied. In total, 6 types of varieties were cultivated in the medium of paddy fields. IR64 dominated the existing seed varieties by as much as 57.6%, while the greatest average yield was for the Barito variety, which had 3.10 tonnes ha$^{-1}$.

Apart from climatic factors and seed varieties, the midland paddy fields also had a significant correlation with the socio-economic aspects of farmers in the midland paddy fields, especially gender and education. Farmers managing rainfed lowland rice fields were dominated by men, reaching 84.8%, while women accounted for 15.2%. Additionally, the yields on land managed by men had greater yields, with 2.19 tonnes ha$^{-1}$, against those managed by women, with 1.65 tonnes ha$^{-1}$. Whereas in education, the distribution of data on midland paddy fields varied widely, starting from no education (3%), elementary school (66.7%), junior high school (24.2%), and senior high school (6.1%). The average yield based on the highest education classification in the elementary school education class was 2.39 tonnes ha$^{-1}$ and senior high school 2.36 tonnes ha$^{-1}$. In comparison, no education parameter resulted in the lowest indicators, with an average yield of 1.24 tonnes ha$^{-1}$.

The highland paddy fields based on Pareto analysis had six key parameters, namely Slope, cropping pattern, Available P, Gender, Pest disease control, and Seed variables that influenced the prediction model, with the four parameters having a p-value $< 0.05$. According to the findings of the p-value test in Table 7, like lowland paddy fields, Available P in highland paddy fields had a significant correlation with crop yields. Available P yields are directly proportional to yields, as presented in Table 8. Available P levels $< 4$ ppm had the lowest yields with 1.29 tonnes ha$^{-1}$, while Available P levels $> 15$ ppm were the greatest with 2.54 tonnes ha$^{-1}$. Gender also had a significant correlation with rice yields, as established for midland paddy fields. In the highland paddy fields, male farmers accounted for 90%, with an average harvest of 2.31 tonnes ha$^{-1}$, while only 10% were women with an average harvest of 1.61 tonnes ha$^{-1}$.

Slope was one of the predictive determinants with the most striking difference in each class on highland paddy fields (Table 8). The analysis results revealed that a slope of 0-8% dominated the area with 60% and had the greatest average yield, which reached 3.57 tonnes ha$^{-1}$, while the distribution of data which had a slope class of 8-15% with an average harvest of 1.93 tonnes ha$^{-1}$, and 15-25% with an average harvest of only 1.37 tonnes ha$^{-1}$. Slope is one of the critical points in every crop cultivation, including rice. According to N. Takeda *et al.* (2019), cultivation of rice on steep slopes poses significant challenges in the management of irrigation and groundwater resources. At the same time, according to T. Tu *et al.* (2023), rice cultivation in the highlands with steep slopes has many obstacles, such as management, difficulty in mechanisation, and distribution of fertiliser inputs.

## DISCUSSION

The tests conducted in this study, which applied four principal input parameters and three neurons in the hidden layer, yielded the most effective results among all the network configurations assessed. As demonstrated by L. Mou *et al.* (2022), Bayesian Neural Networks (BNN) were capable of simultaneously producing predicted outcomes and measurement uncertainties, thereby indicating the deviation of input data from the training data distribution. This approach, as highlighted by S.K. Bal *et al.* (2022), effectively mitigated the negative effects caused by out-of-distribution (OOD) data and prevented erroneous predictions, even with a single model parameter. The development of the BNN model in the present study was based on the specific toposequence of the farmer's land to enhance the accuracy of machine learning analysis. According to D. Paudel *et al.* (2021) regional and location-specific input data were shown to support the construction of reliable predictive models. The model's accuracy along defined toposequences corresponded closely with observed values, illustrating the high effectiveness of the predictive modelling approach. T. Hengl *et al.* (2018) recognised modern machine learning methods as powerful, data-driven tools for predicting soil properties and landscape variability. Moreover, A.M. J.-C. Wadoux *et al.* (2023) found that the integration of high-resolution, site-specific data – including environmental and management variables – substantially reduced prediction uncertainty and enhanced model robustness. This finding underscored the significance of tailoring modelling strategies to various elevation zones within a toposequence, as each zone was influenced by distinct factors.

It was therefore clear that individual elevation categories required separate modelling frameworks to account for their specific determinants. T. Van Klompenburg *et al.* (2020) demonstrated that yield prediction models incorporating site-specific factors markedly reduced errors. Furthermore, according to D. Paudel *et al.* (2022), such models provided stakeholders with improved insights and decision-making tools for policy development and strategic planning in agriculture. The prediction model developed using elevation categories followed an analogous structure, beginning with yield values and identifying key production determinants. According to Z.C. He *et al.* (2022), yield prediction methods generally required site-specific measurements due to diverse local conditions such as soil composition, climate variability, socio-cultural norms, and farmers' management practices. S. Jeong *et al.* (2022) observed that large-scale forecasts at the district or city level tended to be less sensitive to rice yield variations, owing to the compounding effects of climate change, soil heterogeneity, and inconsistent land management. Conversely, X. Yue *et al.* (2022) highlighted the significance of landscape-level predictions, as they provided deeper insights into crop responses under specific environmental and agronomic practices.

The Bayesian estimation approach employed in the present study contributed to the broader modelling efforts aimed at explaining long-term trends in Indonesia's agricultural productivity, as previously conceptualised in the literature. Specifically, it added nuance to existing research stock models by incorporating the complexity of Java's toposequences. Moreover, the current study extended this framework by introducing uncertainty analysis into the process of forming new research capital stocks from agricultural research investments. Although the unpredictable nature of individual research outcomes was already recognised within Indonesia's agricultural science system, this study offered empirical evidence to substantiate that claim. This uncertainty, as demonstrated in the findings, underscored the necessity of maintaining a diverse an d adaptable research portfolio. Supporting varied productivity-enhancing innovations and promoting flexible, incentive-based policies in agricultural science and technology were recommended strategies to address this challenge effectively. Understanding how neural networks learned was also considered essential. According to standard practice, several visualisation techniques were employed, including intermediate activation visualisation, convolution filter mapping, heat maps, and saliency diagrams. These methods, as shown in the study, illustrated how the model internally represented visual environments and how its feature extraction evolved across neural layers – enhancing compositional awareness, class discrimination, and yield prediction performance. Furthermore, visualisation tools helped in identifying model weaknesses and guiding model refinement.

The Bayesian approach also enabled the identification of critical limiting factors, which could inform policy recommendations for agricultural communities and governmental bodies aiming to strengthen and sustain the agrarian sector in pursuit of food security. Finally, W.S. Dewi *et al*. (2022) highlighted that agriculture is a cornerstone of global resource utilisation, consuming approximately 70% of surface and groundwater and 30% of global energy output, emphasising its centrality in food and energy supply systems. The creation of this framework for evaluating the performance of farming systems could make a critical contribution to the current study by presenting a systematic performance appraisal model for the distribution system using a Bayesian neural network. Previously, the development of such frameworks focused on increasing demand-oriented management of on-farm agricultural systems. Notably, the paradigm is limited to agricultural distribution systems as a critical element of off-farm agrarian management. The presented framework can be used as a decision-support tool to prioritise modernisation solutions for the farming distribution system. It also aids the yielding process and governance in determining the technical implications of building these systems and the environmental consequences.

## CONCLUSIONS

The presented study achieved high accuracy in predicting rainfed rice yields in the tropical monsoon climate of central Java, Indonesia, by combining machine learning methods, specifically Bayesian neural networks (BNN), and Pareto analysis. Models adapted to elevation toposequences showed a significant advantage over general approaches, demonstrating a coefficient of determination ($R^2$) of up to 0.999, while the general model only reached 0.8396. The error indicators (RMSE = 0.4421; MAPE = 21.95%) were low, while Pearson's correlation coefficients exceeded 0.90, confirming the high reliability of the models. The study identified specific factors that determine yield in different agroecological conditions. In lowland and mountainous areas, the most significant factor was the content of available phosphorus in the soil: at P levels > 15 ppm, the yield reached 2.97 tonnes $ha^{-1}$ in lowlands and 2.54 tonnes $ha^{-1}$ in highlands. In mid-mountain conditions, climatic (precipitation 1,500-2,000 mm/year – 2.79 tonnes $ha^{-1}$) and socio-economic factors played a decisive role: level of education, gender of the farmer, and choice of seed variety. It was found that male farmers, as well as those with at least a basic education, achieved greater yields.

Furthermore, the slope indicator proved to be critical for mountainous areas: plots with a slope of 0-8% had the greatest average yield (3.57 tonnes $ha^{-1}$), while on slopes of 15-25% it decreased to 1.37 tonnes $ha^{-1}$. This confirmed scientific observations regarding the limitations in mechanisation, irrigation, and fertiliser application on steep slopes. The findings confirmed the effectiveness of integrating environmental, socio-economic, and technological parameters into precision agriculture models. The high sensitivity of models to local conditions allows optimising the agrotechnical solutions, increasing resource efficiency, and supporting for sustainable agricultural development in conditions of climate instability. Prospects for further research include conducting long-term field observations involving dynamic agroclimatic changes, expanding the socio-economic database of farms, developing integrated resource management models based on remote sensing of the Earth, and adapting artificial intelligence to decision-making systems in precision farming.

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## CONFLICT OF INTEREST

The authors of this study declare no conflict of interest.

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# Прогнозування урожайності рису на богарних землях у мусонній тропічній зоні Яви, Індонезія з використанням машинного навчання

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**Анотація**. Вирощування рису на богарних землях у тропічних районах Яви, Індонезія, що знаходяться під впливом мусонних дощів, ускладнюється через нестачу поживних речовин, непередбачуваність опадів та обмежені інвестиції в сільське господарство, що призводить до коливань врожайності. Метою цього дослідження було розроблення точної моделі прогнозування врожайності рису з використанням машинного навчання, адаптованого до конкретних топологічних послідовностей у Центральній Яві. Застосовано комплекс польових та лабораторних методів, заснованих на опитуваннях, з урахуванням кліматичних, ґрунтових, соціально-економічних та агроменеджментних змінних із 87 цільових точок відбору проб. Аналіз за допомогою баєсівських нейронних мереж (BNN) показав помірну точність ($R^2$ = 0,840; RMSE = 0,442), однак точність суттєво підвищувалась при адаптації моделей до категорій за висотою, досягаючи $R^2$ до 0,999. Для низинних полів найбільший вплив мала наявність доступного фосфору (P); у зонах середньої висоти ключовими чинниками були кількість опадів, стать, освіта та сорт насіння; для високогірних районів – ухил, доступний фосфор, стать і сівозміна. Аналіз Парето підтвердив ідентифікацію цих основних детермінант урожайності у кожному топосліді. Інтеграція методів BNN і Парето дозволила створити високоточну, локалізовану модель прогнозування. Робота довела, що адаптація моделей машинного навчання до агроекологічних зон за висотою покращує їхню ефективність і практичне застосування. Результати є особливо цінними для аграрних стейкґолдерів – зокрема для політиків, дорадчих служб і фермерів, які можуть використати прогностичні дані для оптимізації управління богарним рисівництвом в умовах змінного клімату

**Ключові слова:** аграрна стійкість; баєсівська нейронна мережа (BNN); продовольча безпека; аналіз Парето; точне землеробство

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# Improvement of the mechanisms of taxation of forest resources of Ukraine to ensure sustainable forest management

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**Abstract**. The purpose of the study was to substantiate ways to optimise fiscal mechanisms in the forest sector to increase its economic efficiency and sustainability. A quantitative analysis was carried out based on the reporting data of six enterprises of various forms of ownership (state, municipal, and private), which allowed identifying imbalances in tax pressure. In the public sector, this figure was 31.1%, and in the municipal sector – 26.5%. In the private sector, the tax burden reached 40.1% in the Forest enterprise and 35.2% in the Karpatlis enterprise, which significantly exceeded the indicators of the public sector – 29.3% in Chernihivlishosp, 32.9% in Sumylishosp, and 40.4% in the Forests of Ukraine enterprise. In the municipal enterprise Kharkivlis,

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the tax burden was 26.5%. The level of profitability in private enterprises was 4.7% in the Forest and 6.0% in Karpatlis, while state-owned enterprises showed stable indicators: 9.1% in Chernihivlishosp, 7.4% in Sumylishosp, and 5.0% in the Forests of Ukraine. Municipal enterprise Kharkivlis showed the highest profitability – 9.7%. The structure of indirect fiscal expenditures was analysed separately, among which the main ones were expenditures on environmental monitoring, forest protection, certification, and reporting. It was established that the share of indirect expenses in the total expenses of enterprises ranged from 5.4% to 11.8%. The risk assessment of tax reforms was carried out on a point scale (from 1 to 10) according to five criteria: legal framework, personnel readiness, business resistance, digitalisation, and fiscal losses. The highest risk profile was recorded in the private sector (average level – 7.4 points), the lowest – in the public sector (4.8 points). The projected effects of the introduction of the adaptive tax model included a reduction in the tax burden by 5.5%, an increase in profitability by 3.2%, investment in reforestation – by 18%, and a decrease in the level of shading by 12.5%. The results of the study can be used to develop a tax policy and support sustainable forest management at the state and enterprise levels

**Keywords:** economic efficiency; indirect costs; digitalisation of reporting; fiscal regulation; reform risks

## INTRODUCTION

The relevance is conditioned by the need to reform the tax policy in the field of forestry in Ukraine, which is conditioned by the strategic role of forests in ensuring ecological balance, economic stability, and social employment in the regions. The forest sector is a source of public revenue, but in the current context of global changes, increased environmental requirements and integration with European standards, there is a need to create a fair, stimulating, and environmentally oriented tax model. Fiscal instruments should ensure both efficient use of resources and their recovery. The problem is caused by the fact that the current system of taxation of forest users is outdated, undifferentiated and does not consider the environmental responsibility of enterprises. The high tax burden, duplication of obligations, lack of incentives to invest in reforestation, and inconsistency of interdepartmental mechanisms cause economic losses and reduce the transparency of the industry, which requires an urgent reconsideration of the fiscal approach (Destek *et al.*, 2024; Shahini & Shahini, 2025).

As established by N. Tsehelnyk (2021), the level of tax burden without considering environmental specifics negatively affects the financial stability of forestry enterprises. It was noted that the fiscal system hinders the renewal of forest infrastructure. According to N. Pravdiuk (2024), information support in terms of taxation remains fragmented. It was emphasised that duplication of reporting leads to an increase in administrative costs. As noted by S.O. Proyava (2024), investors avoid investing in the forest industry due to non-transparent tax policies. It was also emphasised that the instability of fiscal conditions reduces interest in long-term environmental projects. Based on the conclusions of L. Sofitri (2025), a possible combination of a carbon tax with quota trading mechanisms in the forest sector. It was noted that the tax system can become an effective tool for climate policy. According to research by J. Stubenrauch *et al.* (2022), in the European Union (EU), fiscal mechanisms are being integrated into the climate strategy. It was noted that tax instruments support the sustainable forest development goals.

As highlighted in the study by D. Heine *et al.* (2021), it is advisable to set tax rates that vary depending on the sustainability of production. It has been proven that such a model can encourage environmentally responsible forest users and simultaneously ensure tax fairness. According to X. Shen *et al.* (2023), the effectiveness of market instruments in the forest sector depends on the quality of the institutional environment. It was clarified that it is stable legal mechanisms that strengthen the role of fiscal policy in ensuring sustainable forest management. As noted by S. Anane *et al.* (2023), small and medium-sized forest enterprises are essential to support the local economy, but they face tax pressures that do not consider the scale of their activities. It was found that it is necessary to introduce simplified fiscal models for small producers. C. Besacier *et al.* (2021) highlighted the importance of local financial mechanisms for forest restoration. The researchers concluded that local tools, in particular tax incentives, can activate environmental initiatives at the community level. As shown in the study by D. Deng *et al.* (2023), the assessment of sustainable forest management requires an integrated approach using multi-criteria models. It was determined that the integration of financial and environmental indicators should be part of tax analysis. According to S. Aggarwal *et al.* (2020), reforms in the area of forest use rights remain incomplete, which affects the effectiveness of tax administration. It was noted that without securing property rights, it is difficult to implement effective fiscal strategies. The impact of tax incentives on reforestation, differentiation of rates depending on the form of ownership, and the effectiveness of digital tools in the tax administration of the forest sector remain insufficiently investigated.

The purpose of the study was to substantiate the areas of improving the tax policy in the field of forestry in Ukraine to ensure its efficiency, fairness, and promotion of sustainable forest management.

## MATERIALS AND METHODS

The time limits of the study covered the period from 2020 to 2024, which allowed assessing the transformation processes in the forest taxation system against the background of legislative, economic, and environmental changes. The research methodology combined quantitative and qualitative analysis methods, including comparative statistics, structural analysis, expert evaluation, and predictive modelling of tax policy scenarios. Data processing was performed using the Python programming language (Pandas library, Matplotlib, Seaborn), the Microsoft Excel table editor, and IBM SPSS Statistics statistical analysis software. The main sources of data were financial reports of enterprises, official statistics of the State Statistics Service of Ukraine (2024), materials of the State Agency of Forest Resources (2024).

To analyse the economic indicators of the tax burden, six enterprises were selected that represent different organisational and legal forms, business scale, and regional affiliation. The selection included three state-owned enterprises (State Enterprise Chernihivlishosp (2022), State Enterprise Sumylishosp (n.d.), State Enterprise Forests of Ukraine (2024)), one utility company (Public Limited Liability Company Kharkivlis (n.d.)) and two private limited liability companies (LLC Karpatlis (n.d.), LLC Forest (n.d.)). This approach provided a typical, non-random sample based on different forms of ownership, availability of complete financial statements, and ongoing forestry activities. The sample included enterprises that carry out forestry activities on a permanent basis, have a registered legal address in different administrative regions of Ukraine and cover standard organisational management models. Enterprises with bankruptcy status, incomplete or unreliable reporting data, and those with a special tax regime (for example, experimental farms) were excluded. This sample composition made it possible to ensure comparability of indicators and adequacy of modelling the consequences of tax reform in the context of different types of property, which, according to Law of Ukraine No. 185-V (2006), directly affects the structure of tax liabilities and financial discipline. The tax burden was calculated using the equation (1):

$$\text{Tax}_{\text{exp}} = \left(\frac{\text{Total taxes paid}}{\text{Total operating expenses}}\right) \times 100, \quad (1)$$

where $\text{Tax}_{\text{exp}}$ – tax burden.

In addition, taxes were considered as a share of income (2):

$$\text{Tax}_{\text{rev}} = \left(\frac{\text{Total taxes paid}}{\text{Revenue}}\right) \times 100, \quad (2)$$

where $\text{Tax}_{\text{rev}}$ – revenue tax.

The structure of indirect fiscal expenditures was determined by collecting information on five groups of expenditures: forest protection, environmental monitoring, certification according to international standards of the Forest Stewardship Council (2024) and the Programme for the Endorsement of Forest Certification (2024)), administrative reporting (tax, environmental and forest reporting), and other mandatory payments related to obtaining permits, conducting examinations and audits. The volume of such expenses was recorded either in the structure of operating expenses of enterprises, or in separate environmental safety budgets. Total indirect fiscal expenses were calculated using the equation (3):

$$\text{Total indirect expenses} = \sum_{i=1}^{n} C_i, \quad (3)$$

where $C_i$ – expenses for each category: forest protection, certification, monitoring, reporting, permits, $i$ – cost group, $\sum$ – total of all expenses, $n$ – total number of expense groups included in the calculation.

The assessment of the risks of tax reform was carried out on a multi-factor point scale, which included five main risk groups: business resistance, legal barriers, personnel and technical readiness, fiscal budget losses, and interdepartmental coordination (Ministry of Finance of Ukraine, 2022). The risk assessment of tax reform was carried out in the range from 1 to 10 points based on a combination of quantitative models, scenario analysis, data extrapolation, and simulation modelling. An individual risk profile was formed for each enterprise, considering five key factors: business resistance, legal barriers, personnel and technical readiness, fiscal budget losses, and interdepartmental coordination. Conditional assessment scales were unified: scores of 8-10 corresponded to a high level of risk, 5-7 – moderate, and 1-4 – low. Final scores were calculated as the average values of forecast estimates for each factor, considering the weight coefficients determined based on the influence of the factor on the effectiveness of the reform.

In the block of forecasting the effects of the implementation of the tax reform, an alternative scenario was modelled, which provided for the introduction of such elements as a differentiated tax rate depending on the level of profitability of enterprises, an environmental tax discount of up to 10% subject to investment in reforestation, tax benefits for enterprises implementing electronic declaration, and setting the maximum level of tax burden at a level not higher than 32% of total expenses. Special attention was paid to the creation of a centralised digital tax reporting system that would simplify accounting and control the fulfilment of obligations. Based on this scenario, the projected changes in such key financial and economic indicators as the level of tax burden reduction, profitability growth, dynamics of investment in reforestation, reduction in the volume of shadowing of the sector and the amount of administrative savings due to process automation were calculated. These indicators were calculated for each enterprise separately using basic relative growth equations (4):

$$\Delta P = \left(\frac{P_{\text{after}} - P_{\text{before}}}{P_{\text{before}}}\right) \times 100, \quad (4)$$

where $\Delta P$ – relative change in the indicator, $P_{after}$ – projected value after the implementation of the reform, $P_{before}$ – current value.

Elements of scenario analysis and modelling of the impact of the proposed reforms on the economic behaviour of enterprises were used to verify the validity of the results obtained.

## RESULTS

**Economic indicators of the tax burden on forest users in Ukraine.** For state-owned enterprises, taxes in the cost structure ranged from 29.3% to 32.9%, while for private enterprises – from 35.2% to 40.4%. The utility company showed the lowest level of this indicator – 27.0%. These values were calculated based on equation (1), where the tax burden was defined as the share of the amount of taxes paid in total operating expenses. The highest absolute amounts of taxes were recorded in state-owned enterprises, but using equation (2), which reflects taxes as a share of income, it was found that it was private enterprises that experienced the greatest fiscal burden – 40.1% and 40.4%. The level of profitability also varied depending on the form of ownership. State-owned enterprises achieved profitability in the range of 7.4%-9.1%, which indicated the relative stability of their financial model. The utility showed the highest profitability among the entire sample – 9.7%, which was partly due to a lower level of tax liabilities. Private enterprises showed lower returns – from 4.7% to 6.0%, which directly correlated with an increased tax burden.

Reforestation costs, as an important element of environmental responsibility, were significantly lower in the private sector (Naumenkova *et al.*, 2023). State-owned enterprises spent an average of 12.1% of their profits for this purpose, while private enterprises spent only 6.9%. The utility company demonstrated an intermediate level of 9.4%. The estimate of the share of reforestation expenditures in total expenditures also confirmed the higher environmental orientation of the public sector: on average, 3.15%, while in the private sector this figure did not exceed 1.7%. The lowest values were recorded for enterprises with the highest tax liabilities – LLC Forest (1.3%) and State Enterprise Forests of Ukraine (1.7%). This led to the conclusion that the existing tax system did not provide adequate incentives for sustainable forest management, particularly in the private sector.

The average monthly amount of taxes paid ranged from UAH 0.47 million (Public Limited Liability Company Kharkivlis) to UAH 1.59 million (State Enterprise Chernihivlishosp), which indicated significant constant cash outflows even for enterprises with small incomes. This situation could create liquidity risks, especially for businesses with seasonal revenue. In addition, the tax burden was estimated as a share of total income. This indicator was the highest in the private sector: in LLC Forest – 40.1%, in State Enterprise Forests of Ukraine – 40.4%. This meant that more than 40 kopiykas from each hryvnia of income were sent to pay taxes. In the public sector, this figure was lower — on average 31.1%, in the municipal enterprise – 26.5% (Table 1).

***Table 1**. Economic indicators of the tax burden on forest users in Ukraine*

| Enterprise | Form of ownership | Income, mln UAH | Taxes, mln UAH | Taxes in expenses, % | Income taxes, % | Forest restoration, % of profit | Profitability, % | Reforestation in costs, % | Average monthly taxes, mln UAH |
|---|---|---|---|---|---|---|---|---|---|
| Chernihivlishosp | State | 65.1 | 19.1 | 29.3 | 29.3 | 12.8 | 9.1 | 3.3 | 1.59 |
| Karpatlis | Private | 44.3 | 15.6 | 35.2 | 35.2 | 8.3 | 6.0 | 2.1 | 1.30 |
| Forest | Private | 30.7 | 12.6 | 40.1 | 40.1 | 5.9 | 4.7 | 1.3 | 1.05 |
| Kharkivlis | Municipal | 21.1 | 5.6 | 27.0 | 26.5 | 9.4 | 9.7 | 2.6 | 0.47 |
| Sumylishosp | State | 56.2 | 18.5 | 32.9 | 32.9 | 11.4 | 7.4 | 3.0 | 1.54 |
| Forests of Ukraine | State | 25.5 | 10.3 | 40.4 | 40.4 | 6.6 | 5.0 | 1.7 | 0.86 |

***Source:** compiled by the authors*

Summarising, it can be noted that the financial efficiency of forestry enterprises in Ukraine in 2024 significantly depended on the level of tax burden. A direct relationship between the form of ownership and tax pressure was revealed, which, in turn, affected profitability and the volume of environmental expenditures. Private enterprises were in the most vulnerable position – they showed the highest share of taxes in expenses and income, the lowest profitability and insufficient investment in reforestation.

**Structure of indirect fiscal expenditures for forest sector enterprises**. The average amount of indirect fiscal expenses per enterprise was UAH 1.90 million. Most indirect costs were incurred SE Chernihivlishosp – UAH 2.60 million, which accounted for 4.0% of its total operating expenses. SE Sumylishosp incurred expenses in the amount of UAH 2.40 million, or 4.5%. Private enterprises had lower absolute indicators, but their share in total expenses was higher. Thus, in LLC Forest, indirect fiscal expenses amounted to UAH 1.55 million, or 5.1%, and in SE Forests of Ukraine UAH 1.59 million, which was the highest relative indicator in the sample – 5.3%.

Forest protection costs ranged from UAH 0.60 million (PLLC Kharkivlis) up to UAH 1.20 million (SE Chernihivlishosp). The average value for the sample was UAH 0.85 million, which corresponded to 1.33% of total expenses calculated according to equation (3). The largest share of such expenditures in the structure of indirect liabilities was found in state-owned enterprises, where they accounted for 46.2% of indirect expenditures. For private enterprises, this figure did not exceed 45.5%. Certification and reporting costs averaged UAH 0.42 million per enterprise. The highest indicator was SE Chernihivlishosp – UAH 0.65 million, the lowest – PLLC Kharkivlis (UAH 0.28 million). The share of such expenses in total fiscal encumbrances was 1.3% in the sample, but for some private enterprises (for example, LLC Forest), reporting expenses reached UAH 0.30 million, or 19.4% of all indirect liabilities.

Environmental monitoring required an average of UAH 0.37 million annually. The maximum expenses were in SE Chernihivlishosp (UAH 0.45 million), the minimum – in PLLC Kharkivlis (UAH 0.30 million). The share of these costs in the structure of indirect encumbrances ranged from 21.1% to 24.5%. This showed a constant nature of costs regardless of the form of ownership, but the share in relation to profit was more burdensome for the private sector. Other obligations, in particular, payment for licensing procedures, expert examinations, and internal accounting, ranged from UAH 0.18-0.35 million. The highest such expenses were recorded in State Enterprise Sumylishosp (UAH 0.35 million), which accounted for 14.6% of its indirect fiscal spending. On average, for the sample, expenses for other mandatory obligations (permits, examinations, audits) amounted to UAH 0.26 million.

Generalised data showed that indirect fiscal expenditures further increased the tax burden by another 4.65%. As a result, considering both direct and indirect obligations, the total fiscal burden on forest industry enterprises reached more than 38.8% of total expenses on average, and in some cases – exceeded 45% (in particular, in SE Forests of Ukraine – 45.7%). The imbalance also manifested itself in the disproportionate distribution of mandatory expenses between entities of various organisational and legal forms. State-owned enterprises, having a higher level of spending, provided a greater contribution to environmental protection, but did not have any advantages in fiscal regulation. However, private enterprises, despite the lower level of investment in environmental measures, experienced a comparable or even higher tax burden relative to their income (Table 2).

***Table 2**. Structure of indirect fiscal expenditures for forest sector enterprises*

| Enterprise | Forest protection expenses, mln UAH | Certification and reporting, mln UAH | Environmental monitoring, mln UAH | Other obligations (licenses, expertise in mln UAH) | Total amount of indirect expenses, mln UAH | Share in total expenses, % |
|---|---|---|---|---|---|---|
| Chernihivlishosp | 1.20 | 0.65 | 0.45 | 0.30 | 2.60 | 4.0 |
| Karpatlis | 0.85 | 0.40 | 0.35 | 0.28 | 1.88 | 4.7 |
| Forest | 0.70 | 0.30 | 0.33 | 0.22 | 1.55 | 5.1 |
| Kharkivlis | 0.60 | 0.28 | 0.30 | 0.18 | 1.36 | 4.3 |
| Sumylishosp | 1.10 | 0.55 | 0.40 | 0.35 | 2.40 | 4.5 |
| Forests of Ukraine | 0.66 | 0.32 | 0.36 | 0.25 | 1.59 | 5.3 |

***Source:** compiled by the authors*

Thus, the results of the study showed the presence of deep systemic inefficiencies in the tax model of forest management in Ukraine. The lack of transparency of some mandatory payments, non-recognition of environmental costs in tax accounting, the lack of incentive mechanisms for environmentally responsible behaviour, and unified approaches to taxation of enterprises with different economic and resource opportunities led to the need for a comprehensive revision of fiscal policy in the field of sustainable forest management.

**Assessment of risks of tax reform implementation in forest sector enterprises**. The highest level of risk was recorded in the sphere of resistance from enterprises, especially private ownership. Thus, LLC Forest and State Enterprise Forests of Ukraine, which had the highest share of tax expenditures in the cost structure (40.1% and 40.4%, respectively) and the lowest profitability (4.7% and 5.0%), declared a limited ability to adapt to changes in the tax approach without losing profitability. The overall risk score “business resistance” was estimated at 8.5 out of 10. Of the 152 regulations in force in the field of forestry, only 14 (9.2%) were directly or indirectly related to differentiated taxation or environmental benefits. SE Chernihivlishosp in its financial report for 2024 pointed out the need to adapt the legislation to account for the costs of certification (UAH 0.55 million) and environmental monitoring (UAH 0.40 million) in the form of a tax credit, but the current legislation did not provide for such a possibility. This structural flaw was rated at 7.8 points. The next challenge was the lack of specialists in the field of tax administration and environmental audit at the level of district tax services. In particular, in the Rokytne district of the Rivne Oblast, where LLC Karpatlis is located (taxes – UAH 15.6 million, certification – UAH 0.40 million), the tax inspectorate had only two certified employees with experience in environmental management. At the end of 2024, only 42% of territorial inspections had access to training programmes on environmental tax law. According to calculations, the risk of inefficient administration of the reform was 6.9 points.

The financial risk of reduced budget revenues was estimated at 7.5 points. In the case of the introduction of an environmental tax credit (discounts of up to 10% for enterprises that invest more than 5% of profits in reforestation), revenues from the Chernihivlishosp forestry enterprise could decrease by approximately UAH 2.1 million annually according to the results of modelling the financial impact of tax incentives. This is despite the fact that the company spent 12.8% of its profit on restoring (the highest figure in the sample), which is equivalent to UAH 2.3 million. In the case of LLC Karpatlis, the tax burden of 35.2% was estimated as marginal, and according to the company's management, a further increase in rates or a change in the system of accounting for reforestation costs could lead to a revision of production plans. Technical limitations have become an additional barrier (Khan *et al.*, 2025). Thus, in SE Chernihivlishosp, the lack of an integrated electronic record-keeping system (ERP) made it impossible to automatically submit reports on reforestation costs, and to ensure compliance with the new model, up to UAH 600 thousand one-time investments in digital infrastructure were required. This created unequal starting conditions for market participants. This risk was estimated at 6.2 points. Institutional inertia, that is, internal resistance to change at the level of state structures, was noticeable in the work of territorial branches of the state agency for forest resources of Ukraine. For example, the management of PLLC Kharkivlis, which demonstrated the highest profitability (9.7%) and the lowest tax burden (26.5%), noted the lack of centralised methodological recommendations for calculating the environmental tax credit. A request to the regional department was answered that "such tools are not yet regulated". The risk of inertial braking was estimated at 7.0 points.

There was also a low level of coordination between the State Tax Service of Ukraine, the State Environmental Inspectorate, the State Agency of Forest Resources of Ukraine and the Ministry of Environmental Protection and Natural Resources of Ukraine. Thus, LLC Forest in 2024 was forced to prepare three separate reports on the costs of environmental measures – for the tax service, the forest agency, and the state environmental inspectorate. These reports were not unified and were submitted in different formats, which increased the administrative burden on the enterprise. Total accounting and reporting costs reached UAH 0.30 million. This risk was estimated at 6.8 points. Political instability, in particular, frequent changes in the leadership of relevant ministries, created the risk of delaying reforms or changes in priorities. In 2024, three deputy ministers of environmental protection were replaced, which led to the suspension of approval of the Project "On Taxation of Rent for the Special Use of Forest Resources" (2024) for more than six months. This factor received a score of 7.9 points.

In general, according to the results of threat scaling, the average risk of implementing the reform was 7.2 points. The most vulnerable to the reforms were private enterprises, which combined high fiscal pressure, low profitability, and limited modernisation opportunities (LLC Forest, LLC Karpatlis). Public sector enterprises, despite the high level of environmental costs (SE Chernihivlishosp, SE Sumylishosp, SE Forests of Ukraine), showed greater adaptability to model changes, but faced regulatory and organisational barriers (Fig. 1).

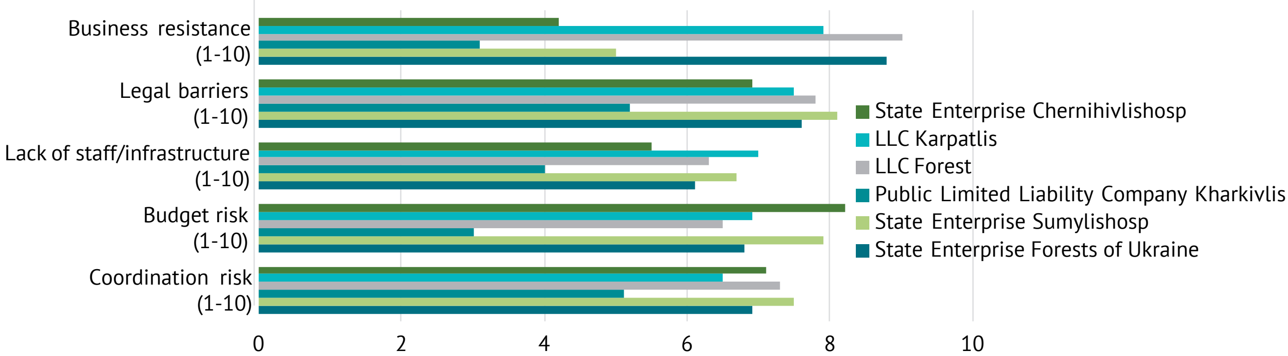


***Figure 1**. Assessment of risks of tax reform implementation in forest sector enterprises*
***Source:** compiled by the authors*

Thus, the practical implementation of the tax reform in the field of forest management required considering the regional, organisational and financial specifics of enterprises. The implementation strategy was to be based on an adaptive approach with step-by-step deployment, institutional support, tax compensators, and the creation of a single digital reporting platform.

**Projected effects of tax reform implementation in forest sector enterprises**. The basic proposal is to introduce a mechanism for differentiated tax burden depending on three key indicators: the share of investment in reforestation, the level of profitability of the enterprise, and the form of ownership. To estimate the share of investment in reforestation, equation (4) is used, which helps to determine the share of environmental costs in the company's profit. According to the simulation results, businesses that invest more than 10% of their profits in reforestation can qualify for a discount of up to 8% of their total tax liabilities. This will allow, in particular, LLC Karpatlis (which in 2024 invested 8.3% of its profit in environmental purposes) to reduce annual tax expenses from UAH 15.6 million to UAH 14.3 million, that is, by UAH 1.3 million.

It is also proposed to introduce an environmental tax credit – a mechanism for transferring part of tax liabilities to subsequent periods, subject to the targeted allocation of funds for environmental protection measures. The calculations provide that SE Chernihivlishosp, which in 2024 spent 12.8% of its profit (UAH 2.3 million) on reforestation, could receive an environmental loan in the amount of UAH 1.1 million with a two-year delay in paying taxes to the budget. This approach will reduce the burden on the company's working capital and stabilise capital expenditure planning. The next step is to introduce a unified tax reporting system that will combine fiscal, environmental and forestry components in a single digital form. It was estimated that enterprises Forest and Forests of Ukraine annually spend UAH 280-320 thousand only on the development, processing, and submission of reports to various authorities. The introduction of a centralised portal will reduce these costs to UAH 120-140 thousand, which will reduce the administrative burden by 55-60%. Estimated savings for the industry will reach up to UAH 72 million annually.

Attention is also paid to the problem of shading. According to an independent assessment, approximately 28% of the turnover of unaccounted timber is accounted for by small private enterprises, which avoid taxation due to the complexity of procedures and lack of incentives for transparency. The introduction of a tax break for voluntary electronic declaration of sales volumes can reduce the shadowing of the sector by 12.5% during the first two years (Tynaliev *et al.*, 2024). Another strategic component is the introduction of a marginal fiscal coefficient, which will limit the tax burden to no more than 32% of the company's total expenses. This is especially true for enterprises with excessive pressure, such as timber (40.1%). The introduction of such a restriction will reduce tax costs by UAH 2.42 million and UAH 2.17 million, respectively, which will create an additional financial resource for modernisation and certification (which currently requires UAH 300-350 thousand). The assessment of the total effect is based on a comparison of key financial and operational indicators in the forecast scenario. It is expected that the average level of tax burden may decrease from 34.15% to 28.65% (by 5.5%), the profitability of enterprises will increase by an average of 3.2%, the volume of investment in reforestation will increase by 18%, and the level of shading will decrease by 12.5%. The total savings in administrative costs for the industry will amount to about UAH 72 million per year. Consequently, the most tangible benefits of the reform may be an increase in environmental investment (+18%), a reduction in shadow timber turnover (-12.5%), and a reduction in tax pressure (-5.5%). These results show the potential of the proposed changes both to improve the financial stability of enterprises and to meet their environmental obligations (Table 3).

***Table 3***. *Projected effects of tax reform implementation in forest sector enterprises (forecast for 2025-2027)*

| Enterprise | Reduction in the tax burden, % | Profitability growth, % | Increased investment in reforestation, % | Reduction in shadow economy, % | Administrative savings, mln UAH |
|---|---|---|---|---|---|
| Chernihivlishosp | 4.5 | 2.4 | 12 | 7.0 | 1.1. |
| Karpatlis | 5.8 | 3.1 | 15 | 10.0 | 1.5 |
| Forest | 7.2 | 3.9 | 19 | 14.0 | 2.2 |
| Kharkivlis | 2.9 | 1.5 | 9 | 4.5 | 0.9 |
| Sumylishosp | 5.0 | 2.8 | 14 | 8.0 | 1.3 |
| Forests of Ukraine | 6.7 | 3.7 | 18 | 13.5 | 2.0 |

***Source:*** *compiled by the authors*

Thus, the proposed changes to the tax policy in the field of forestry had not only a fiscal, but also an environmental effect. They allowed creating an adaptive, socially fair tax environment that could stimulate the sustainable development of the forest sector. Their implementation required appropriate regulatory adaptation, technical re-equipment of the accounting system and interdepartmental coordination, but the projected results exceeded the cost of implementation, which made the reform justified both from economic and managerial standpoints.

## DISCUSSION

The results obtained confirm the existence of a high tax burden on forestry entities, especially in the private sector, where the share of taxes in the cost structure exceeded 40%. This burden complicates long-term planning, limits investment in reforestation, and increases the risks of shadowing activities. However, it was established that indirect fiscal expenditures related to forest protection, certification, reporting and environmental monitoring remain significant, but are not legally considered as a component of tax policy. The analysis of the risks of implementing reforms showed a high level of institutional and informational unpreparedness for changes, and the expected resistance from business in the absence of compensatory mechanisms. The projected effects of the proposed changes indicate a potential reduction in the tax burden, an increase in the profitability of enterprises and an increase in environmental investment. This indicates the feasibility of switching to a model of adaptive fiscal regulation based on environmental criteria and digital integration of accounting (Gavkalova *et al.*, 2024).

As shown in the paper by S.N. Chisika and C. Yeom (2021) and D. Huang *et al.* (2024), focused on integrating forest resources into broader environmental and economic programmes. D. Huang *et al.* emphasised the role of green investment and social factors in achieving carbon neutrality, but did not consider the tax system as a separate regulatory tool. S.N. Chisika and C. Yeom analysed public-private partnerships as the basis for sustainable management of public forests, but the issue of fiscal policy was ignored. In this context, the study supplemented existing approaches with a quantitative assessment of the tax burden, reform risks, and the effectiveness of digital solutions at the enterprise level. The use of a risk scale and scenario forecasting allowed considering both internal financial and external institutional factors that were not reflected in previous works.

As noted by E. Barbier *et al.* (2023) and S. Wang *et al.* (2024), the impact of global economic processes on forest policy was viewed through the prism of international trade and resource asset management. E. Barbier *et al.* focused on the tropical timber trade and the effects of deforestation, but did not explore domestic tax models as a regulatory mechanism. S. Wang *et al.* used the Data Envelopment Analysis toolkit to assess the effectiveness of natural resource management in China, but without detailing fiscal mechanisms at the level of individual business entities. The study allowed filling this analytical gap by highlighting indirect costs, fiscal asymmetries between enterprises of different forms of ownership, and modelling the effects of reforms. This provided a deeper link between tax policy and the economic behaviour of forest users, which was not reflected in the above papers.

As demonstrated by P. Kadam *et al.* (2021) and J. Yan *et al.* (2024), the focus was on the structural and organisational aspects of sustainable forest management. J. Yan *et al.* proposed a model for analysing the green productivity of the Chinese forest industry, which considered technological and environmental factors, but did not include tax incentives or risks. P. Kadam *et al.* compared certification and management systems for sustainable forest management, without paying attention to the tax burden or the effectiveness of fiscal administration. The analysis supplemented these approaches with an assessment of indirect fiscal expenditures, detailed calculations of tax pressure, and forecasting the impact of reforms on profitability and investment activity. A special feature of the study was the combination of quantitative modelling with the analysis of real reporting data, which provided high applied value and analytical accuracy. As emphasised by Y. Yang *et al.* (2021), along with C.R. Franco and J. Comje (2022), policy dialogue, paradigm formation, and forest ownership regimes were recognised as key determinants of sustainable forest management. In the case of C.R. Franco and J. Comje, the historical transformation of forest policy in the United States was traced, but financial instruments, in particular tax instruments, were not singled out as the basis for regulation. Y. Yang *et al.* focused on the impact of ownership rights on the forest fund of southern China, without highlighting the relationship between taxation and the level of responsible use of resources. The conducted research differed in that it allowed to quantify the fiscal burden, correlate it with the forms of ownership, and identify structural irregularities in the taxation of forest sector enterprises, which was not presented in the mentioned studies.

According to the findings of A. Raihan *et al.* (2022) and S.N. Chisika and C. Yeom (2024), special attention was paid to institutional restructuring of management models and econometric analysis of climate factors. S.N. Chisika and C. Yeom described a shift in forest management paradigms in Kenya, but specific tools for fiscal impact on sustainable enterprise behaviour remained out of the field of research. A. Raihan *et al.* proposed an econometric analysis of the potential for reducing emissions in Indonesia, without considering the direct impact of the tax system. In contrast to these approaches, the analysis included both an assessment of tax expenditures of enterprises and scenario forecasting of the effects of reforms, considering the form of ownership, the level of investment, and the scale of activities. W. Yang *et al.* (2022) and V. Imbrenda *et al.* (2023) showed that socio-economic and climate factors significantly influence the rate of adaptation of the forest sector to the requirements of sustainable development. W. Yang *et al.* analysed the challenges in the context of carbon neutrality, paying considerable attention to the regulatory framework, but without the financial aspect. V. Imbrenda *et al.* highlighted the relationship between employment, environmental efficiency, and economic stability in the European forest sector, but without a specific analysis of tax mechanisms. But in the current study, the tax burden was considered as an integral indicator that affects investment behaviour, the level of shadow economy, and the sustainable financial viability of enterprises, which allowed combining fiscal and environmental dimensions.

As revealed in studies by A. Saxena *et al.* (2022) and P.K.R. Chowdhury and D.G. Brown (2023), forest policies are increasingly combined with financial incentive and strategic planning mechanisms in the regional dimension. P.K.R. Chowdhury and D.G. Brown modelled the impact of carbon payments and cooperative management on carbon storage and income of forest owners in the United States, without considering the classical tax system as an integrated factor. A. Saxena *et al.* explored the barriers and potential of wooden infrastructure in the Himalayas, focusing on political and environmental contexts, without detailing the fiscal environment. In contrast to these approaches, the study covered not only general incentives or strategic frameworks, but also provided a complete structural analysis of fiscal pressures on forest users, considering tax indicators, the form of ownership of enterprises, the level of profitability, and the amount of indirect costs. This provided the applied value of the study for the development of specific tax decisions at the level of legislative initiative.

As demonstrated by L. Liagre *et al.* (2020) and A. Raihan *et al.* (2024), key attention is paid to the impact of specialised financial mechanisms and macroeconomic factors on ecosystem services and emissions. L. Liagre *et al.* analysed the role of national forest funds in Costa Rica, Vietnam, and Morocco, highlighting the effectiveness of funds as catalysts, but did not consider standard tax models as a source of funding. A. Raihan *et al.* applied a macroeconomic approach to investigating the impact of energy consumption, forest area, and globalisation on climate indicators in Poland, but without a detailed analysis of the relationship with tax systems in the forest industry. The study was distinguished by the fact that it was based on real data of forest sector enterprises, including an assessment of administrative costs, indirect fiscal obligations, imbalances between public and private structures, which allowed identifying the systemic limitations of the current tax model and substantiating the areas for its improvement.

As stated by B. Danley *et al.* (2021) and A. Blanton *et al.* (2024), the focus was on carbon markets and private owners' involvement in improving biodiversity. A. Blanton *et al.* investigated the state of the carbon certificate market in Latin America, where tax policies were mentioned only indirectly through regulatory conditions for trading. B. Danley *et al.* analysed the limits of voluntary participation of family forest owners in Sweden in biodiversity conservation, but fiscal instruments were presented as a general policy background without quantitative details. The analysis significantly expanded the existing problem, since for the first time a comparative tax diagnosis of various types of enterprises was carried out with modelling the effects of reforms and quantitative assessment of the potential impact on sustainable development, which significantly increases the practical suitability of the obtained conclusions for the development of a national tax policy in the field of forest management. As a result, the study differed from previous research in its practical direction, detailed tax analysis, and modelling of the effects of reforms, which helped to identify systemic problems and form applied recommendations for sustainable development of the forest sector.

## CONCLUSIONS

As part of the study, a comprehensive assessment of the tax policy in the field of forestry of Ukraine was carried out to form fiscal mechanisms that will contribute to sustainable forest management. The analysis covered key aspects of the tax burden, the structure of indirect fiscal expenditures, the risks of implementing reforms, and the projected effects of changes in the fiscal model at the enterprise level. At the first stage, the tax burden was calculated at six enterprises of different forms of ownership: SE Chernihivlishosp, SE Sumylishosp, SE Forests of Ukraine (state-owned), PLLC Kharkivlis (municipal), LLC Forest and LLC Karpatlis (private). It was found that the average level of taxes in the structure of total expenditures was 34.15%. The highest values of this indicator were recorded in private enterprises: 40.1% in LLC Forest and 40.4% in SE Forests of Ukraine, while the average value in the public sector was 31.1%, and in the municipal sector – 26.5%. A similar trend was observed in calculating the tax burden on income: 40.1% and 40.4% in the private sector, compared to 31.1% in the public sector and 26.5% in the municipal sector. This indicated an asymmetric fiscal burden and a lack of incentive mechanisms for private property entities. At the second stage of the study, the structure of indirect fiscal costs was analysed – expenses that are not classified as taxes, but are mandatory and include forest protection, certification, environmental monitoring, reporting and licensing costs. Their share in total expenses ranged from 4.3% to 5.3%, and in individual enterprises – exceeded 5% (in particular, in SE Forests of Ukraine – 5.3%, in LLC Forest – 5.1%). Such a hidden fiscal burden is not reflected in tax statistics, but it significantly affects the financial stability of enterprises. At the third stage of the study, the risks of tax reform implementation were assessed on a scale from 1 to 10 points, based on five groups of criteria: regulatory uncertainty, staffing, institutional readiness of business, the level of digitalisation, and fiscal losses. Private enterprises were the most vulnerable – the average risk level was 7.4 points, which is conditioned by the lack of centralised digital solutions and limited ability to adapt. In the public sector, the risk was assessed at 4.8 points, in the municipal sector – 5.6 points. At the final stage, the projected effects of implementing an adaptive tax model for the period 2025-2027 were modelled. It is expected that the tax burden can be reduced by an average of 5.5%, profitability will increase by 3.2%, investment in reforestation will increase by 18%, and the level of shading will decrease by 12.5%. In addition, the implementation of a digital reporting system and simplified accounting will reduce the administrative burden on enterprises, which will provide projected cost savings of UAH 72 million per year across the industry. In general, the results of the study proved the feasibility of switching to a differentiated, environmentally oriented tax policy that considers the economic viability of enterprises, their form of ownership, and contribution to the preservation of forest ecosystems. Further research should be aimed at developing models of tax incentives for reforestation and evaluating the effectiveness of their implementation in the regional context.

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## CONFLICT OF INTEREST

None.

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# Удосконалення механізмів оподаткування лісових ресурсів України для забезпечення сталого лісокористування

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**Анотація**. Метою дослідження було обґрунтування шляхів оптимізації фіскальних механізмів у лісовому секторі для підвищення його економічної ефективності та сталості. Проведено кількісний аналіз на основі звітних даних шести підприємств різних форм власності (державних, комунальних і приватних), що дозволило виявити диспропорції в податковому тиску. У державному секторі цей показник становив 31,1 %, а в комунальному – 26,5 %. У приватному секторі податкове навантаження сягало 40,1 % у підприємстві «Ліс» та 35,2 % у підприємстві «Карпатліс», що значно перевищувало показники державного сектору – 29,3 % у «Чернігівлісгоспі», 32,9 % у «Сумилісгоспі» та 40,4 % у «Лісах України». У комунальному підприємстві «Харківліс» податкове навантаження становило 26,5 %. Рівень рентабельності у приватних підприємствах становив 4,7 % у «Ліс» і 6,0 % у «Карпатліс», тоді як державні демонстрували стабільні показники: 9,1 % у «Чернігівлісгоспі», 7,4 % у «Сумилісгоспі» та 5,0 % у «Лісах України». Комунальне підприємство «Харківліс» показало найвищу рентабельність – 9,7 %. Окремо проаналізовано структуру непрямих фіскальних витрат, серед яких основними були витрати на екологічний моніторинг, охорону лісу, сертифікацію та звітність. Встановлено, що частка непрямих витрат у загальних витратах підприємств коливалась у межах від 5,4 % до 11,8 %. Оцінка ризиків реалізації податкових реформ проводилась за бальною шкалою (від 1 до 10) за п'ятьма критеріями: правова база, кадрова готовність, бізнес-опір, цифровізація та фіскальні втрати. Найвищий ризиковий профіль зафіксовано у приватному секторі (середній рівень – 7,4 бала), найнижчий – у державному (4,8 бала). Прогнозовані ефекти від впровадження адаптивної податкової моделі включають зниження податкового навантаження на 5,5 %, зростання рентабельності на 3,2 %, інвестицій у лісовідновлення – на 18 % і зменшення рівня тінізації на 12,5 %. Результати дослідження можуть бути використані для формування податкової політики та підтримки сталого лісокористування на рівні держави і підприємств

**Ключові слова:** економічна ефективність; непрямі витрати; цифровізація звітності; фіскальне регулювання; ризики реформ

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# Economic efficiency of preservation and utilisation of livestock products in the agrarian sector of the economy

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**Abstract.** The purpose of this study was to investigate the ways of increasing the economic efficiency of agricultural production by improving the systems of preservation and rational use of livestock products. The methodological framework of the study included a comprehensive approach, as well as the analysis of statistical data, structural-dynamic and comparative analysis, and elements of system and factor analysis. Gross output of livestock production in Kazakhstan increased from KZT 2,066.4 bn in 2015 to KZT 3,012.5 bn in 2023. Therewith, the number of cattle increased from 6.18 mn heads in 2015 to 7.98 mn heads in 2024, while the number of horses more than doubled from 2.07 mn heads to 4.35 mn heads over the same period. The analysis results showed that despite the steady growth in herd numbers and gross output, the efficiency of conservation is still at an average level. This is related to the deterioration of refrigeration infrastructure, poor development of the cold chain, lack of qualified personnel, insufficient digitalisation of logistics processes, and specific climatic features of the regions. Milk production volumes have almost halved. Losses at the storage and transport stages are also reflected in the decline in egg production from 5.59 bn eggs in 2018 to 4.48 bn in 2024. The study substantiated those investments in the modernisation of storage infrastructure, digitalisation of logistics, and development of deep processing are not excessive costs, but a tool to optimise the production process and increase the sustainability of agribusiness. The following strategic development areas were also proposed: development of agro-industrial clusters, development of cooperation, improvement of personnel skills, expansion of state support, and introduction of environmentally sustainable technologies. It was concluded that increasing the level of safety of livestock production in Kazakhstan can not only reduce losses, but also increase the profitability of farms, strengthen the food security of the country, and its export potential

**Keywords:** storage infrastructure; logistical constraints; digitalisation; climatic conditions; quality control

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## INTRODUCTION

The agricultural sector of the economy plays a key role in ensuring food security, providing jobs and sustainable development of rural areas. One of its crucial elements is animal husbandry, which is a source of high-quality protein, raw materials for processing, and the key element in the structure of agricultural production. However, despite major efforts aimed at the development of this sector, the problems of preservation and utilisation of livestock products are still topical and require a comprehensive approach. A prominent aspect is that a considerable part of products is lost at various stages – from collection to processing and transport. This is related to both insufficiently developed storage technologies and inefficient logistics, which leads to quality losses and increased costs. All these factors directly affect the economic efficiency of livestock production and constrain its potential for growth. Thus, effective management of the preservation and utilisation of livestock products becomes a prerequisite for increasing the competitiveness of agriculture, reducing losses, and increasing the profitability of agricultural enterprises.

In the scientific environment, there has been a steady growth of interest in the issues of increasing efficiency in the agricultural sector through improving the processes of storage and utilisation of livestock products. L. Zhang *et al.* (2021) focused their research on meat deep freezing technologies and found that the introduction of low-temperature controlled atmosphere chambers reduced specific product losses to a minimum. The researchers emphasised that such investments pay off not only by stabilising product quality, but also by reducing returns from retailers and expanding geographical distribution. T. Espolov *et al.* (2020) complemented these findings by focusing on weak links in supply chains in agricultural regions, especially in remote and hard-to-reach farms. The researchers proved that improving transport infrastructure and reducing the delivery time from processing to the consumer can improve the profitability of dairy production and increase the realisation period of products. G. Kandeepan and A. Tahseen (2022) investigated active packaging methods and concluded that the use of oxygen-isolating and modified gas media in packaging of meat products helps to maintain organoleptic properties, nutritional value, and microbiological stability over a sustained period. M.A.F.M. Fadzli and S.W.B. Nawawi (2024) proposed the design and implementation of automated systems for monitoring storage parameters such as temperature, humidity, and ventilation, showing that such solutions markedly reduce storage losses, especially in large agro-industrial complexes with large volumes of products.

D. Wang *et al.* (2022) considered the influence of climatic and seasonal factors on product preservation and proposed adaptive storage strategies depending on climatic zone and seasonal variations. This ensured more efficient utilisation of storage facilities, reduced energy costs, and reduced risks of product spoilage during periods of hot temperature or high humidity. J. García-Díez *et al.* (2023) showed the high significance of veterinary and sanitary control as a factor that indirectly but substantially affects economic losses: prompt detection of diseases in animals reduces the risks of rejects at the slaughter and processing stage and prevents the spread of infections that threaten the integrity of the entire lot. M. Taifouris and M. Martín (2022) investigated economic models for integrating livestock farms with processors and argued that such a system allows logistics and storage to be organised without the losses and costs that occur at the interface of the value chain. The researchers emphasised that vertically integrated structures are more resilient to external shocks and reallocate resources more efficiently. R. Gabdualiyeva *et al.* (2024) focused on digitalisation of processes – their modelling showed that farms that implemented digital residue management, predictive analytics, and real-time quality control had fewer losses due to product spoilage and more accurate forecasts of production and sales volumes.

A.S. George and A.S.H. George (2023) investigated automated climate systems in storage warehouses and found that their use had a significant effect on the quality stability of meat products during long-term storage. The researchers emphasised that precise microclimate control avoids microbiological risks, preserves the marketability of products, and avoids added costs for sorting and processing of spoiled batches. R.S. Alibekov *et al.* (2024) supported an analogous view, focusing on technologies for deep processing and recycling of meat residues. The researchers concluded that the processing of by-products and by-product components into semi-finished products and feed not only reduces losses, but also allows obtaining products with high added value, thereby increasing the profitability of production.

Thus, the accumulated scientific experience demonstrates a steady tendency to recognise the value of preservation and rational use of products as key factors in increasing the overall efficiency of agricultural production. Despite great strides in the field of increasing the efficiency of preservation and utilisation of livestock products, several key aspects are still understudied. There is a lack of comprehensive research that would integrate innovative technologies of storage and processing of products considering the specifics of agrarian enterprises. Little attention is paid to the economic consequences of introducing environmentally friendly and energy efficient preservation methods, which could reduce costs and increase competitiveness. Thus, the purpose of the present study was to analyse the methods of increasing the economic efficiency of preservation and use of livestock products in the agricultural sector.

## MATERIALS AND METHODS

The study employed a comprehensive methodological approach that combines quantitative and qualitative analyses of the livestock production safety system in the agrarian sector of the Republic of Kazakhstan. Official statistical materials of the Bureau of National Statistics (n.d.) for 2015-2023, as well as for 2024 – for certain types of indicators, for which updated data were available at the time of the study, were used as the primary source. Indicators of gross output of agricultural production overall and livestock production specifically, as well as the number of farm animals were analysed up to 2023 inclusive. This is because the data on these categories require further processing, reconciliation with administrative sources, and are often published with a time lag. At the same time, such indicators as volumes of production of the key types of livestock products – meat (in slaughter weight), milk, eggs, and wool – were considered up to 2024 inclusive. This is conditioned by the fact that data on these categories are published earlier and have a lower degree of detail. Additionally, the analysis included regulatory documents of the Ministry of Agriculture of the Republic of Kazakhstan (n.d.), including Order of the Minister of Agriculture of the Republic of Kazakhstan No. 3-4/617 (2014). This helped to compare actual and normative levels of losses, identify potential deviations, and assess the level of technological support of the industry.

The method of factor analysis was employed to assess the impact of individual elements of storage infrastructure on the overall level of safety of livestock products. Within the framework of the analysis, the study used data from reports of the Ministry of Agriculture of the Republic of Kazakhstan (n.d.). Storage conditions in different climatic zones of the country were analysed, considering the high continental nature of the climate, seasonal temperature fluctuations, and the length of transport routes. Qualitative analysis was aimed at identifying organisational, managerial, and institutional constraints affecting the efficiency of livestock products use. The mechanisms of interaction between producers, transport operators, and processors, the presence or absence of cooperation, problems of planning logistics routes, and compliance with sanitary norms were considered. State support measures were also analysed: subsidies for modernisation of storage facilities, the programme "Auyl – El besigi" (Improving the quality..., 2019), soft loans, investment subsidies, and digitalisation of agricultural product management.

Practices demonstrating the influence of climatic and infrastructural factors on the safety of livestock products, as well as the effectiveness of digital solutions and development programmes in the agricultural sector were investigated. Case studies were used as a basis for the study, including the ColdHubs project in Nigeria (How can sustainable..., 2022), as well as the Sustainable Livestock Development Programme in Kazakhstan for 2022-2026 (Kazakhstan: Sustainable livestock..., 2021) and the implementation of SAP S/4HANA in Eurasia Group Kazakhstan to optimise logistics and inventory management (SAP S/4HANA migration..., n.d.). Furthermore, initiatives on advanced processing of products were considered: Kraft cheese making in Zhambyl region (42 farms of Zhambyl..., n.d..) and processing of meat products in East Kazakhstan (Construction of a deep..., 2025). The study also analysed examples of vertical integration – the structure of Zhaiyk Et (n.d.) and the closed production cycle at Aknar PF (n.d.). Additionally, data from a Carrier Global Corporation report indicating meat losses in countries with poor cold infrastructure were used (BIO Intelligence Service for the Global Food Cold, 2015). As a result, the combination of statistical, regulatory, and expert-analytical sources helped to reasonably identify key constraints and growth points in the sphere of ensuring the safety of livestock products in Kazakhstan.

## RESULTS

Product safety in the agricultural sector is a multidimensional economic and technological phenomenon characterised by the ability of economic entities to minimise both quantitative and qualitative losses at all stages of the agro-production chain – from the moment of production to final consumption. This problem is especially relevant for livestock products, which have a limited shelf life, are sensitive to external influences and subject to rapid biochemical decomposition (Casino *et al.,* 2021). That is why the issues of preservation require a comprehensive, systematic, and scientifically based approach, including both technological solutions and organisational and economic measures. From the economic standpoint, product safety is the crucial factor determining the level of efficiency of agricultural production. Losses resulting from violations of temperature conditions, sanitary norms, technological failures, or deficiencies in logistical organisation led to increased production costs, reduced marketable yields and, as a consequence, lower farm profits (Focker & van der Fels-Klerx, 2020). Thus, investments in increasing the level of preservation are not considered as excessive costs, but as an effective mechanism to optimise the production process and strengthen the financial sustainability of agricultural enterprises.

Furthermore, a prominent level of product preservation provides agricultural producers with more opportunities within the framework of implementing a flexible marketing strategy. Modern storage infrastructure allows not only reducing the share of losses, but also regulating the volumes and terms of deliveries, adapting to market conditions. This allows building inventories for sales during periods of peak demand, expand sales channels, and enter distant markets. In an environment of price volatility and high competition, such flexibility becomes a valuable tool for profitability management (Nayak & Bagchi, 2022). Hence, preservation acts not only as a means of reducing losses but also as a factor for sustainable agribusiness development. From a macroeconomic standpoint, the conservation of livestock products contributes to sustainable resource utilisation and food security. Each unit of lost production means not only economic loss, but also inefficient use of invested resources – labour, feed, water, energy, and capital. In this context, losses in the agricultural sphere become an indicator of systemic imbalances that hinder the transition to sustainable and resource-efficient agriculture. Increasing the level of conservation, on the contrary, forms the prerequisites for greening production, improving the food balance and increasing the competitiveness of products in internal and external markets (Qian *et al.,* 2022).

The factors that determine the level of losses of livestock products have both biological and technical-economic nature. One of the most significant is the microbiological instability of products of animal origin, which causes their high sensitivity to even minor deviations from the recommended temperature, humidity, and sanitary storage conditions. In the absence of effective cooling, freezing, and hermetic packaging systems, the risk of biochemical decay, deterioration of organoleptic characteristics and, as a consequence, commercial unsuitability of the product increases significantly (Neethirajan, 2020). This is especially critical for dairy and meat products, which require strict adherence to temperature standards at all stages of transport and storage (Sattarov *et al.*, 2025).

Apart from biological constraints, technical and infrastructural constraints play a major role. In many countries, especially in developing regions, small and medium-sized farms face a shortage of modern refrigeration facilities with automatic climate control. This constraint substantially reduces the resilience of agricultural supply chains, forcing farmers to sell products at shorter lead times and at less favourable prices, which, in a volatile market environment, reduces their profitability and increases losses. One example is the ColdHubs initiative in Nigeria, where farmers rent solar-powered cold rooms at around USD 0.50 per day per plastic crate. This has saved over 42,000 tonnes of agricultural produce, minimising spoilage losses and increased the income of 5,240 smallholder farmers, retailers, and wholesalers by 50% (How can sustainable..., 2022). A comparable situation was observed in Rwanda, where the lack of infrastructure for cold storage resulted in high post-spoilage losses. In response, the government and international organisations started to actively adopt energy efficient technologies, including solar-powered refrigeration, to improve food security and income stability for farmers (Twilley, 2022).

Organisational and management aspects also considerably influence product safety. Inadequate staff qualifications, lack of internal quality control standards, inconsistent logistics processes, and planning errors lead to the accumulation of losses at production and marketing interfaces. Furthermore, poorly developed inter-linkage mechanisms between producers, transport operators, and processing organisations substantially hinder the prompt marketing and processing of products, reducing their commercial value (Shepherd *et al.,* 2018). Natural and climatic conditions drastically affect the safety of livestock products, as extreme temperature fluctuations create stressful conditions for animals and complicate the maintenance of optimal microclimate in the places where they are kept. In Kazakhstan, sharp seasonal temperature fluctuations, when summer heat can reach 41°C and winter frosts can drop to -48°C, cause temperature imbalance, which leads to poor health and reduced productivity of animals. In southern regions with hot summer temperatures above 30°C and relatively mild winters down to -3°C, farmers face the need for constant cooling and ventilation to prevent overheating or overcooling of produce (Syzdykova, 2024). Despite the use of shade canopies, fans, and water spray systems, these methods may not be sufficiently effective and may require extensive energy inputs, which hinders stable climate control and increases the risk of spoilage, reducing product quality and shelf life.

Modern approaches to the rational use of livestock products, which include the introduction of smart storage technologies with automatic microclimate control, the use of biotechnology to improve product quality and shelf life, the use of digital systems for monitoring and controlling production processes, and the development of integrated processing, imply not only minimising losses but also increasing economic returns through deeper processing, technological innovation, and digitalisation of management. In an increasingly competitive and resource-constrained environment, the transformation of production models from raw material-oriented to value-added production is of particular relevance (Leah *et al.,* 2023). Deep processing of meat and dairy raw materials produces a wide range of finished products, from semi-finished products to specialised food and feed products.

Modern software solutions, including Enterprise Resource Planning (ER) systems, implemented in agricultural enterprises of Kazakhstan, contribute to a marked increase in the efficiency of inventory management and reduction of product losses. For instance, Eurasia Group Kazakhstan, an official dealer of agricultural machinery, successfully implemented a project to migrate to SAP S/4HANA platform, which allowed it to upgrade its IT infrastructure and stabilise critical business processes. According to the company, it is expected to increase data accuracy by up to 15% and reduce order processing and warehouse transaction times. This transition enabled the integration of new SAP functions and tools, improving resource management and data processing agility (SAP S/4HANA migration…, n.d.). Additionally, the Sustainable Livestock Development Programme for Kazakhstan 2022-2026, supported by the World Bank, makes provision for the introduction of an improved animal recording and tracking system. This will improve the efficiency of veterinary services and livestock resource management, which helps to reduce losses and improve product quality (Kazakhstan: Sustainable livestock…, 2021)

In Kazakhstan, where many farms are located in remote or sparsely populated areas, advanced processing helps to significantly increase profitability by creating long-life products such as cheese, dried meat, and milk concentrates, which reduces dependence on perishables (Bogoyavlenskiy *et al.*, 2022). For example, farmer cooperatives in Zhambyl region are introducing the production of kraft cheeses, which not only allows them to preserve products longer, but also to enter new markets with higher added value (42 farms of Zhambyl…, n.d.). A large deep meat processing project is underway in East Kazakhstan, which helps to reduce logistics costs and minimise losses during long-distance transport. Construction of a KZT 7.7 bn industrial complex has begun in the village of Chekoman, Zhanasemey district, Abay region. The project envisages processing up to 35 tonnes of meat products per shift, including beef, mutton, and horse meat, with the production of high-quality sausages, canned goods, semi-finished products, and meat delicacies. The complex will be equipped with modern Australian and European technologies ensuring zero-waste production and compliance with environmental standards (Construction of a deep…, 2025).

The development of biotechnologies and waste-free processing should be emphasised among the promising solutions. The use of enzymatic and microbiological methods allows using animal waste – blood, fat, bones – to produce fodder, biologically active additives, and fertilisers. These approaches not only reduce the environmental load but also form closed production cycles that meet the requirements of sustainable agricultural development (Lainawa *et al.,* 2024). The digitalisation of storage and marketing processes is also becoming a key element of product stewardship. The implementation of automated systems for monitoring shelf life, temperature conditions, and logistics flows in agricultural enterprises can markedly improve the accuracy of management decisions. For example, the use of big data-based platforms such as IBM Food Trust or SAP Business Network for Logistics enables real-time monitoring of product condition and storage conditions, which reduces losses and optimises the distribution of goods. Thanks to analytical tools, companies can predict demand fluctuations and adapt production and logistics, which reduces costs and increases efficiency.

Organisational forms of cooperation, such as agro-industrial clusters, logistics centres, and agricultural cooperatives, ensure a more efficient allocation of resources and bring producers and processors together. There are already successful examples of vertical integration in Kazakhstan, where unified value chain management ensures quality control at all stages, from farm to consumer. One such example is Zhaiyk Et (n.d.), which covers all regions of the country and demonstrates the effectiveness of vertical integration, ensuring economic sustainability, and quality control of products. Another example is Aknar PF Group (n.d.), which rehabilitated and modernised a poultry farm in the Karaganda region, introducing the concept of a closed production cycle 'from field to fork'. In regions with extreme climatic conditions, the development of cold chain infrastructure is critical to ensure the safety of livestock products. Key elements that must be prioritised included refrigerated warehouses, refrigerated transport, and energy-independent plants that can maintain a stable microclimate during transport and storage. A study by Carrier Global Corporation indicates that in developing countries, including Kazakhstan, meat losses due to inefficient or non-existent cold chains are around 10%. This results not only in economic losses for producers, but also in lower quality products, which affects their market value and safety for consumers (BIO Intelligence Service for the Global Food Cold, 2015). The development of cold infrastructure, including the construction of modern refrigerated warehouses and the introduction of refrigerated transport, will significantly reduce product losses, improve product quality, and increase shelf life.

Greening of the agricultural sector is also becoming a vital area. Sustainable use of resources, waste minimisation, optimisation of logistics, reduction of water, and energy consumption – all this is becoming part of the modern paradigm of agricultural production (Mamenko *et al.*, 2024). The implementation of international environmental standards and certifications not only improves internal efficiency but also opens access to external markets where demands for sustainable products are increasing (Bhatti *et al.,* 2024). The livestock industry in the Republic of Kazakhstan occupies a prominent place in the agrarian sector of the country's economy. It has historically been one of the key branches of agriculture, providing a sizeable share in agricultural production (40%) and being of great significance for food security and sustainable economic growth. Table 1 presents the gross output of agricultural products (services).

Analysis of the data in Table 1 shows a stable trend of growth in gross output of agricultural products in the Republic of Kazakhstan in the period from 2015 to 2022, with an increase in volumes from KZT 3,307 bn to KZT 8,367.7 bn. However, in 2023, a decrease in the indicator to KZT 7,576.5 bn was recorded, which may suggest the influence of external economic or climatic factors. The dynamics of livestock production shows a positive trend throughout most of the period, with particularly notable growth observed in 2020-2021. However, there is a decline in volumes in 2022, followed by a recovery in 2023 to KZT 3,012.5 bn. This underscores the vulnerability of the industry to organisational, economic, and natural conditions. Against the background of considerable growth in crop production, livestock farming demonstrates less sustainable rates of development, which necessitates the need to improve the efficiency of storage, logistics, and processing of livestock products as a reserve for stabilisation and strengthening of the sector. Table 2 presents the gross output of livestock products by type.

***Table 1**. Gross output of agricultural products (services) in Kazakhstan for 2015-2023, KZT bn*

| | 2015 | 2016 | 2017 | 2018 | 2019 | 2020 | 2021 | 2022 | 2023 |
|---|---|---|---|---|---|---|---|---|---|
| Agriculture | 3,307 | 3,684.4 | 4,070.9 | 4,474.1 | 5,151.2 | 6,334.7 | 7,515.4 | 8,367.7 | 7,576.5 |
| Horticulture | 1,825.2 | 2,047.6 | 2,249.2 | 2,411.5 | 2,817.7 | 3,687.3 | 4,387.2 | 5,808.3 | 4,552.4 |
| Animal husbandry | 1,469.9 | 1,621.5 | 1,810.9 | 2,050.5 | 2,319.5 | 2,637.5 | 3,117.0 | 2,545.3 | 3,012.5 |
| Agricultural services | 11.8 | 15.3 | 10.8 | 12.1 | 14.0 | 9.9 | 11.2 | 14.2 | 11.6 |

***Source:** compiled by the author of this study based on Bureau of National Statistics (n.d.)*

***Table 2**. Gross output of livestock products in Kazakhstan for 2015–2023, KZT bn*

| | 2015 | 2016 | 2017 | 2018 | 2019 | 2020 | 2021 | 2022 | 2023 |
|---|---|---|---|---|---|---|---|---|---|
| Livestock products | 1,469.9 | 1,621.5 | 1,810.9 | 2,050.5 | 2,319.5 | 2,637.5 | 3,117 | 2,545.3 | 3,012.5 |
| Dairy cattle breeding | 597.3 | 661.1 | 729.3 | 822.4 | 928.9 | 1,069.5 | 1,283 | 812 | 1,000.6 |
| Breeding of other cattle and buffalo breeds | 386.7 | 412.7 | 464.8 | 532.1 | 627.9 | 705.9 | 825 | 649.9 | 724.5 |
| Breeding of horses and other ungulate breeds | 121.5 | 131.5 | 154.1 | 187.9 | 211.1 | 241.6 | 279.2 | 326.2 | 385.3 |
| Breeding of camels and camelids | 12.2 | 14.4 | 14.4 | 16.8 | 18.7 | 21.1 | 25.5 | 29 | 31.7 |
| Sheep and goat breeding | 163.5 | 167.5 | 175.4 | 198.2 | 217.4 | 245.2 | 269.7 | 257.3 | 285.5 |
| Pig and piglet breeding | 55.8 | 65.7 | 75.3 | 72.4 | 64.9 | 69.5 | 76.5 | 61.4 | 65 |
| Poultry breeding | 123.6 | 157.6 | 183.7 | 206.3 | 238.5 | 271.4 | 344 | 396.2 | 505.4 |
| Breeding of other animal species | 597.3 | 661.1 | 729.3 | 822.4 | 928.9 | 1,069.5 | 1.283 | 812 | 1,000.6 |

***Source:** compiled by the author of this study based on Bureau of National Statistics (n.d.)*

Among all areas, the contribution of dairy cattle breeding is particularly notable, which also showed growth to KZT 1,283.0 bn in 2021, but recorded a sharp decline to KZT 812.0 bn in 2022, followed by a partial recovery in 2023. The "other cattle and buffalo breeds" category showed comparable dynamics, with fluctuations in the last two years. Poultry is the most stable and fastest growing, rising from KZT 123.6 bn in 2015 to KZT 505.4 bn in 2023, indicating that this sub-sector is highly attractive for investment and technologically advanced. The growth in the production of sheep and goats, horses and camels is also positive, although with smaller absolute values. At the same time, pig breeding demonstrates stagnation with insignificant changes, which may be associated with both consumer preferences and epizootic restrictions. Overall, the structure of livestock output continues to be diversified, but requires attention to fluctuations in key areas, especially in dairy farming, in terms of sustainability and effective resource management. Table 3 summarises the production of the key livestock products.

***Table 3**. Volume of production of the main types of livestock products in Kazakhstan for 2015-2024*

| | 2015 | 2016 | 2017 | 2018 | 2019 | 2020 | 2021 | 2022 | 2023 | 2024 |
|---|---|---|---|---|---|---|---|---|---|---|
| Meat (live weight), thsd t | 1,651.1 | 1,702 | 1,794.4 | 1,871.6 | 1,975 | 2,058.5 | 2,162.2 | 1,799.1 | 1,920.3 | 1,994.9 |
| Meat (in slaughter weight), thsd t | 931 | 960.7 | 1,017.6 | 1,059.4 | 1,120.6 | 1,168.6 | 1,231.1 | 1,044.7 | 1,120 | 1,168.7 |
| Milk, thsd t | 5,182.4 | 5,341.6 | 5,503.4 | 5,686.2 | 5,864.9 | 6,051.4 | 6,247.2 | 3,354.6 | 3,472.9 | 3,628.5 |
| Eggs, mn pcs | 4,737 | 4,757.2 | 5,103 | 5,591.4 | 5,531.4 | 5,065.8 | 4,838.1 | 4,526.7 | 4,420.6 | 4,478.1 |
| Wool, thsd t | 38 | 38.5 | 39 | 39.2 | 39.5 | 40.2 | 41.2 | 35.6 | 36.6 | 36.3 |
| Karakul, thsd pcs | 7.1 | 4.3 | 8.1 | 3 | 1.4 | 1.3 | 2.1 | 0.5 | 0.3 | 0.2 |

***Source:** compiled by the author of this study based on Bureau of National Statistics (n.d.)*

Analysis of Table 3 suggests that the country's livestock industry has developed unevenly: there was a general increase in meat and milk production until 2021, but in 2022 there was a sharp decline in all major items, especially milk (almost doubled), which may reflect systemic problems in the industry, ranging from logistical failures to feed shortages or declining productivity. Volumes subsequently started to recover but have not reached previous highs. Egg production showed a steady decline after 2018, from 5,591.4 mn eggs to 4,478.1 mn eggs in 2024, while wool production stayed relatively stable. Particularly notable was the almost complete phasing out of Karakul breeding, reflecting a structural transformation of livestock production and a shift in focus towards more profitable or prioritised areas. Table 4 presents the number of the key types of farm animals.

***Table 4**. Number of livestock and poultry in Kazakhstan for 2015-2024, thsd heads*

| | 2015 | 2016 | 2017 | 2018 | 2019 | 2020 | 2021 | 2022 | 2023 | 2024 |
|---|---|---|---|---|---|---|---|---|---|---|
| Cattle | 6,183.9 | 6,413.2 | 6,764.2 | 7,150.9 | 7,436.4 | 7,850 | 8,192.4 | 6,536.3 | 6,616.8 | 7,976.7 |
| Sheep and goats | 18,015.5 | 18,184.2 | 18,329 | 18,699.1 | 19,155.7 | 20,057.6 | 20,876.8 | 18,843 | 18,667.4 | 20,224.4 |
| Pigs | 887.6 | 834.2 | 815.1 | 798.7 | 813.3 | 816.7 | 776.1 | 509.8 | 483.3 | 479.7 |
| Horses | 2,070.3 | 2,259.2 | 2,415.7 | 2,646.5 | 2,852.3 | 3,139.8 | 3,489.8 | 3,790.2 | 3,851.2 | 4,353 |
| Camels | 170.5 | 180.1 | 193.1 | 207.6 | 216.4 | 227.7 | 243.4 | 253.7 | 264.9 | 280.5 |
| Poultry, mn heads | 35.6 | 36.9 | 39.9 | 44.3 | 45 | 43.3 | 47.9 | 45.7 | 44.2 | 45.7 |

***Source:** compiled by the author based on Bureau of National Statistics (n.d.)*

The data in the table show stable positive dynamics in the number of most types of farm animals in Kazakhstan, reflecting the overall expansion of the livestock sector. Cattle and horses show the most significant growth, with cattle numbers increasing from 6,183.9 thsd heads in 2015 to 7,976.7 thsd heads in 2024, and horses more than doubling, which can be attributed to increased demand for meat and dairy products, as well as expanding exports. The sheep and goat population also increased despite a slight decline in 2022-2023, which is likely related to unfavourable weather or economic conditions. At the same time, a steady decline in pig numbers was observed, from 887.6 thsd heads in 2015 to 479.7 thsd heads in 2024, reflecting a structural shift in consumer preferences, religious and cultural factors, and low profitability of pig production. Camel numbers are gradually increasing, continuing to be a niche but stable growth area. Poultry numbers show volatility with a peak in 2021 and a subsequent decline, possibly caused by epizootics or changes in housing and feeding conditions.

Conservation efficiency of livestock production in Kazakhstan continues to be at an average level, demonstrating contradictory results due to a complex of structural, infrastructural, technological, organisational, and management factors. On the one hand, there is a stable growth in gross output of livestock products and an increase in the number of cattle, sheep, goats, and horses. This may suggest an improvement in the basic conditions of livestock breeding, growth of production potential, and certain investments in the agricultural sector. Furthermore, there is a positive dynamic in the volume of meat production (in slaughter weight), which also suggests partial modernisation of the processing and storage infrastructure. On the other hand, against the backdrop of these achievements, significant systemic constraints persist, which hinder further growth in preservation efficiency. The deterioration of the material and technical base, especially in the segment of small and medium-sized farms, limits the possibilities of long-term storage and compliance with temperature regimes. Problems with the 'cold chain' – lack of modern refrigeration facilities, automated control systems, and logistics centres – lead to product losses at the stages of shipment, transportation, and storage. Insufficient digitalisation and poor integration of information technology into inventory management and logistics processes limit the ability to react quickly to changes in demand, which is especially critical for perishable products.

Furthermore, fluctuations in milk, egg, and wool production with an overall increase in animal numbers indicate an inefficient post-production handling system. Such volatility may be the result of underestimation of losses due to storage irregularities, lack of staff, poor veterinary and sanitary controls, and poor coordination between production and logistics links. This is especially true during periods of seasonal temperature fluctuations, when there is an increased risk of product spoilage without proper climatic adaptation of warehouses and transport vehicles. According to the regulations of the Ministry of Agriculture of the Republic of Kazakhstan, the natural loss of meat products during storage and transport can vary: for instance, when transporting chilled meat for a distance of up to 100 km, losses of up to 0.05-0.08% are allowed depending on the season, and when transporting frozen meat for a distance of over 1,000 km – up to 0.35% of the cargo weight (Order of the Minister of..., 2014). These standards factor in shrinkage, shaking and spoilage of products, but in real conditions losses may be higher due to the wear and tear of refrigeration equipment, lack of modern storage facilities, and non-compliance with sanitary norms.

To improve the efficiency of the agricultural sector and, specifically, to ensure the safety of livestock products, the Republic of Kazakhstan is implementing a number of government support measures aimed at stimulating the modernisation of production and logistics infrastructure, improving the technological equipment of farms and reducing product losses at all stages of the agro-production chain. One of the key instruments is state subsidies. In the livestock sector, they cover support for fodder production, pedigree cattle breeding, construction, and modernisation of storage facilities, including refrigeration and processing complexes. The current state programme "Auyl – El besigi" and other initiatives make provision for preferential credit and leasing mechanisms (Improving the quality..., 2019). This allows agricultural producers to purchase modern equipment for storage and processing of livestock products with deferred payments and at reduced interest rates.

The state is also actively promoting investment subsidies aimed at reimbursing part of the costs of building cold storage facilities, logistics centres, and processing facilities. Additionally, work is underway to form agro-industrial clusters and cooperatives, which ensures more efficient allocation of resources, sharing of infrastructure and access to larger markets. Special attention is paid to the digitalisation of the agricultural sector. Systems of monitoring and electronic accounting of agricultural products are being introduced, which allows tracking their movement, condition, and storage periods, thereby helping to reduce losses. Specifically, the Unified State Subsidy System (USSS), the Farm Animal Identification System (FAIS), and the Unified Automated Management System for the Agro-Industrial Sector (e-Agriculture) were introduced. These systems allow tracking the movement, condition, and storage periods of agricultural products, which helps to reduce losses (Kazakhstan intensively introducing..., 2025). The development of transport logistics and export infrastructure are also among the priority areas of state policy. The creation of veterinary and sanitary checkpoints, certification laboratories and specialised transport routes helps to ensure compliance with international quality standards in the transportation and storage of products.

To increase economic efficiency in the sphere of livestock products safety in the Republic of Kazakhstan, it is necessary to implement a targeted and multidimensional strategy focused on both technological modernisation and institutional transformation. One of the key areas is the development of modern infrastructure for storage and processing of products. A major part of losses in livestock production is associated with the lack of proper storage conditions, especially in rural areas and remote regions. In this regard, it is advisable to invest in the construction and modernisation of refrigerated warehouses, dairy, and meat processing complexes, as well as the introduction of mobile cooling systems adapted to the climatic features of Kazakhstan. The use of energy-efficient solutions and equipment providing automatic microclimate regulation will help to increase shelf life and preserve the nutritional properties of products.

An equally significant aspect is the digitalisation of logistics, accounting, and product quality control processes. Implementing real-time monitoring systems – such as temperature sensors, radio frequency identification (RFID) tags, and automated batch tracking platforms throughout the supply chain – will not only minimise losses but also increase the transparency of production processes. Modern software solutions, including ERP-systems for agricultural enterprises, allow forecasting demand, managing stocks more efficiently, and promptly responding to the risks associated with the violation of storage conditions. These measures are especially relevant in the context of growing volumes of livestock products and increasing complexity of logistics routes. Special attention should be paid to deepening the processing of raw materials. Animal products should be used as much as possible, including by-products, bones, blood, and fat, which can be converted into meat concentrates, feed additives, gelatine, collagen, and organic fertilisers. Such approaches reduce waste, add value, and open new markets. This is particularly relevant in Kazakhstan, where there is often a disconnect between production and processing. Government subsidies for the purchase of equipment, tax breaks for processors, and support in accessing export markets would be a major incentive for the development of this industry.

It is also necessary to develop human resources. It is essential to expand professional education and training programmes, including courses for farmers and refrigeration system operators, training in modern methods of inventory and handling of animal products. Educational institutions should interact more actively with business and the state, forming applied programmes focused on the real needs of the agricultural sector. The next area is the improvement of logistics. To reduce losses during transportation, it is necessary to organise logistics centres that follow all sanitary and temperature requirements. In regions with extreme climatic conditions, especially in the southern and western regions of Kazakhstan, it is vital to develop "cold chain" infrastructure. This includes not only the purchase of specialised transport with refrigerated trucks, but also the establishment of transshipment points where short-term storage and transshipment can be provided without risk of product spoilage.

Finally, institutional development – expanding opportunities for cooperation between small and medium-sized producers – is of great significance. The creation of agro-industrial clusters that bring together farmers, processors, and logisticians will increase economic sustainability and reduce costs through economies of scale. It is also vital to ensure a favourable regulatory framework that establishes clear requirements for the conditions of storage, transportation, and sale of products. Strengthening state control and incentives (including grants and tax breaks for compliance with standards) will create incentives for investment in product preservation. Overall, the improvement of economic efficiency in the sphere of livestock product safety should be based on the integration of innovative solutions, support from the state, and active involvement of all participants in the agro-production chain. Such a model will ensure not only reduction of losses and growth of profitability but also strengthening of Kazakhstan's position as an export-oriented agrarian power.

## DISCUSSION

The analysis revealed that the current infrastructure for storing livestock products is still not adapted to modern requirements. Despite the availability of production facilities, the obsolescence of storage equipment and non-compliance with sanitary and technical standards limit the possibilities of effective storage. The identified correlation between the level of preservation and the state of infrastructure suggests that problems are stored not so much in production as in the post-production cycle. This means that further development of the sector should be focused not only on increasing output, but also on ensuring post-production quality through the renewal of the logistics and warehousing base. O.A. Chukwu and M. Adibe (2022) emphasised that even with limited technical capacity, acceptable product safety can be achieved through accurate adherence to sanitation and storage standards. They emphasised operational discipline as a major factor of efficiency. In contrast, the current findings showed that without renewing the infrastructure itself and eliminating wear and tear, organisational measures have only a temporary effect. Thus, the present study expressed greater confidence in the need for physical modernisation, whereas the researchers proposed a strategy of adaptation to existing conditions.

Regional differences in climatic conditions such as extreme temperatures, sharp seasonal variations, and irregular rainfall create a challenging environment for the storage and transport of products (Vovkotrub *et al.*, 2024). These factors particularly influence products with a high degree of sensitivity to external conditions. The analysis results emphasised the need for flexible logistics models adapted to concrete climatic zones. The presence of remote farms and long delivery routes exacerbate the problem of cold conditions, necessitating the development of regionally orientated storage and transport standards. M. Wróbel-Jędrzejewska and E. Polak (2021) emphasised climate as a background, but not a determining condition. In their opinion, with proper technological support (use of mobile refrigerators, modular storage facilities) the influence of climatic conditions is minimised. Current data, on the contrary, reflect a substantial influence of continental climate and geographical remoteness on the level of losses, especially during transportation. The difference here lies in the scale of the assessment: the researchers analysed individual pilot projects in limited regions rather than a nationwide system.

The high level of physical deterioration of storage equipment and the low level of technological equipment of storage facilities reflect chronic underinvestment in this part of the agro-industrial complex (Kazhymurat *et al.*, 2021). Additionally, the lack of qualified personnel capable of operating modern refrigeration and logistics equipment exacerbates the problem. Without a systematic training programme focused on logistics and storage, even extensive capital investments will have limited effect. Thus, modernisation must be accompanied by a parallel digital and educational transformation. D. Perkumienė *et al.* (2022) argued that the shortage of qualified personnel had a more devastating effect on the storage system than the technical ageing of facilities. The researchers emphasised that even modern equipment would not be effective without competent operators. D.E. Ufua *et al.* (2022) concluded that improved staffing yields results faster and cheaper than comprehensive technical modernisation. The present study agrees with this, but there is a problem in the complex: not only staffing shortages, but also the deterioration of the base itself limits efficiency.

Poor integration between actors in the logistics chain, from producer to processor, continues to be a systemic problem. Poor coordination leads to irrational use of transport resources, disruption of delivery times and, as a result, increased losses (Danchuk *et al.*, 2023). The problem also lies in the lack of transparent contractual relations and uniform logistics standards. Addressing these challenges requires institutionalisation of interaction between supply chain participants, creation of cooperation platforms, and logistics consolidation centres. T. Perdana *et al.* (2023) investigated horizontal linkages between farmers, processors and logisticians within localised clusters. Their findings demonstrated that losses are almost halved at high cooperative densities. A.-R. Alhassan and M.A. Akudugu (2020) also found that in the absence of centralised infrastructure, cooperation replaces government or corporate logistics solutions. This echoes the current findings on the significance of establishing cooperative chains. However, the present study focused on systemic failures (lack of centralised planning and digital platforms), while the cited study relied on self-regulating cooperative models.

Government support measures aimed at modernising infrastructure and logistics have a positive but limited impact due to their uneven coverage and weak targeting. The results showed that subsidy and concessional financing programmes often do not account for the specificity of regional logistics needs. Furthermore, administrative barriers and lack of transparency in resource allocation reduce the motivation of sector players to modernise. This points to the need not only for financial injections, but also for a revision of support implementation mechanisms towards greater flexibility and efficiency. U. Sekaran *et al.* (2021) argued that current forms of state support are working effectively, especially investment subsidy programmes. Their analysis was based on data from regions with active implementation of such programmes. The current study, on the other hand, criticised the support for being fragmented and poorly adapted to regional needs.

Despite declarations about the need for digitalisation, in practice, technologies are applied point-by-point and unsystematically. Temperature monitoring, route tracking, and metering automation are in most cases either absent or used in a fragmented manner (Uazhanova *et al.*, 2024). This limits opportunities to manage risks and improve the efficiency of logistics processes. Digitalisation should be a key element of the development strategy: from cargo tracking systems to platforms for coordinating the actions of all supply chain participants. This is the only way to ensure the accuracy, transparency, and sustainability of logistics infrastructure. M. Khanna (2021) explored the digital transformation of the agricultural sector. The researcher analysed the implementation of GPS tracking of trucking, automated temperature and humidity control systems in storage facilities, and digital supply management platforms. M. Khanna highlighted the growing interest of agribusiness in digitalisation as a way to optimise costs and improve product safety. According to M.A. Dayıoğlu and U. Turker (2021), the level of digitalisation is increasing faster than expected and forms the prerequisites for integrated logistics platforms. The current study, on the contrary, indicated the fragmentation and sporadic application of digital solutions. The present study indicated that digital solutions are implemented in a fragmented manner, most often in pilot projects or at the level of large farms, while the vast majority of small and medium-sized agribusinesses do not have access to such technologies.

A cumulative analysis of all factors confirmed that the safety of livestock production cannot be ensured by efforts in one direction, whether it is machinery, organisation, or legislation. A comprehensive transformation is needed, combining investments in infrastructure, human resources, digital technologies, institutional change, and adaptation to natural conditions. Such an approach will not only reduce losses but also improve the overall competitiveness of the sector, creating the conditions for sustainable development of agricultural logistics. W. Munro and R. Schurman (2022) considered that a systems approach is not possible without changes in the institutional architecture of the agribusiness sector – first of all, reforms in storage ownership, responsibility allocation, and inter-institutional interaction. The present study supported the idea of systematicity but proposed it as a result of the integration of infrastructural, managerial, and human resource solutions. The analysis of the results obtained in comparison with alternative approaches revealed that the efficiency of the system of storage of livestock products in the agricultural sector depends on a combination of institutional, technical, and human factors. The integrated approach continues to be the key condition for increasing product safety and sustainability of logistics chains.

## CONCLUSIONS

Based on the analysis, the study found that extensive product losses at various stages – from production to consumption – are conditioned by both biological properties of animal products and technical and infrastructural limitations. Problems in the area of cooling, packaging, and transport are particularly critical. The lack of modern storage facilities with automatic climate control results in the need to sell products quickly, limiting flexibility in supply management and increasing the share of write-offs. From 2015 to 2023, there was an increase in gross livestock production from KZT 1,469.9 bn to KZT 3,012.5 bn with marginal fluctuations that suggest the sector is vulnerable to logistical and climatic risks. Despite an increase in cattle (from 6.18 mn heads in 2015 to 7.98 mn heads in 2024) and horses (more than doubling), milk and egg production figures have been declining – especially in 2022, when milk production almost halved. This reflects inefficient storage and post-production processing, especially against the backdrop of seasonal temperature variations and limited access to modern infrastructure in rural areas.

Modern technologies allow agricultural enterprises to improve efficiency and reduce losses of livestock products substantially. In Kazakhstan, such measures are particularly relevant due to the extreme climate. The use of ERP systems (e.g., SAP S/4HANA in Eurasia Group), digital platforms, and advanced processing (kraft cheeses, dried meat) increases profitability and sustainability of farms. Vertical integration (Zhaiyk Et, Aknar PF) ensures control at all stages of production. Development of the cold chain and ecologisation of the agro-sector allow improving product quality, reducing losses by up to 10% and increasing competitiveness in foreign markets. Overall, the development of deep processing of raw materials, digitalisation of logistics processes, modernisation of refrigeration infrastructure, and development of agro-cooperatives are promising areas for improving preservation efficiency. In the future, it would be advisable to conduct farm-level studies to identify concrete loss factors and assess the effectiveness of implemented storage technologies.

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# Економічна ефективність збереження та використання тваринницької продукції в аграрному секторі економіки

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**Анотація**. Робота була спрямована на вивчення шляхів підвищення економічної ефективності аграрного виробництва за рахунок вдосконалення систем безпеки та раціонального використання продукції тваринництва. Методологічною основою дослідження виступав комплексний підхід, що включає аналіз статистичних даних, структурно-динамічний та порівняльний аналіз, а також елементи системного та факторного аналізу. Валовий випуск продукції тваринництва у Казахстані збільшився з 2066,4 млрд тенге у 2015 році до 3012,5 млрд тенге у 2023 році. При цьому чисельність великої рогатої худоби зросла з 6,18 млн. голів у 2015 році до 7,98 млн. у 2024 році, а кількість коней більш ніж подвоїлася – з 2,07 до 4,35 млн. голів за той же період. Результати аналізу показали, що незважаючи на стійке зростання поголів'я та валової продукції, ефективність безпеки залишається на середньому рівні. Це зі зносом холодильної інфраструктури, слабким розвитком "холодової ланцюга", дефіцитом кваліфікованих кадрів, недостатньою цифровізацією логістичних процесів і кліматичними особливостями регіонів. Обсяги виробництва молока знизилися майже вдвічі. Втрати на етапах зберігання та транспортування також відображені у зниженні виробництва яєць з 5,59 млрд штук у 2018 році до 4,48 млрд у 2024 році. У роботі обґрунтовано, що інвестиції у модернізацію інфраструктури зберігання, цифровізацію логістики та розвиток глибокої переробки є не надмірними витратами, а інструментом оптимізації виробничого процесу та підвищення стійкості агробізнесу. Також запропоновано напрями стратегічного розвитку: формування агропромислових кластерів, розвиток кооперації, підвищення кваліфікації кадрів, розширення державної підтримки та впровадження екологічно стійких технологій. Зроблено висновок, що підвищення рівня безпеки продукції тваринництва в Казахстані здатне не лише знизити втрати, а й підвищити рентабельність господарств, зміцнити продовольчу безпеку країни та її експортний потенціал

**Ключові слова:** інфраструктура схову; логістичні обмеження; цифровізація; кліматичні умови; контроль якості

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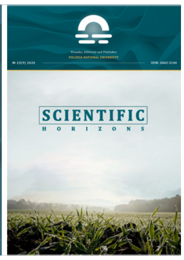


# The economic efficiency of the introduction of resource-saving technologies in crop production in Kazakhstan

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**Abstract**. The purpose of this study was to determine whether the implementation of resource-saving technologies in crop production in Kazakhstan during 2021-2024 led to statistically significant improvements in economic efficiency at the level of farming enterprises. The main focus was on the practical results of the application of innovative technologies, such as precision agriculture, Global Positioning System-monitoring, digital crop management, drip irrigation, remote sensing and the use of drones. The

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study found that the implementation of these technologies allowed to reduce fuel costs by up to 60%, fertilisers by 18-22%, and labour costs by 15-20%. At the same time, wheat yield increased by 50% compared to 2023 and amounted to 1.38 t/ha. The introduction of drip and sprinkler irrigation on an area of about 84 thousand ha allowed to reduce water consumption by 20-40% and increase crop stability in drought conditions. Economic efficiency was confirmed by a high level of payback: typical terms of return on investment in these technologies were 3-5 years with state co-financing of up to 80%. The regression model showed high explanatory power ($R^2 = 0.948$), with the most significant influencing factors being subsidies for drones (+106.51) and digital platforms (+7.29), while direct funding per 1 ha was not statistically significant (coefficient -0.0004; $p = 0.685$). The results confirmed the economic feasibility of innovative approaches to agriculture in Kazakhstan and proved that the effectiveness of state support depends on its targeting and integration into the institutional environment. The results of this study can be practically applied by government agencies to optimise subsidy policies, by agricultural enterprises to justify investments in digital technologies, and by international donors to support scalable, cost-effective solutions for sustainable agriculture

**Keywords:** innovation; sustainability; yield; precision farming; profitability

## INTRODUCTION

Increasing the efficiency of the agricultural sector of Kazakhstan in the face of climate change, depletion of natural resources and rising costs of material and technical resources is one of the key challenges of modern agriculture. Crop production is increasingly faced with problems of reduced soil fertility, water shortages, high energy intensity of technological processes and dependence on imported fertilisers and fuel. In such conditions, the introduction of resource-saving technologies becomes an effective means of ensuring the stability of production, reducing costs and increasing the competitiveness of products. Inefficient use of agricultural land in Kazakhstan, limited implementation of innovations and the lack of a unified approach to the organisation of agricultural enterprise territories make it difficult to achieve sustainable results. This was investigated by Y. Anarbayev *et al.* (2024), who proved that the transition to internal land management, taking into account the experience of the USA, China, Japan and the EU, contributes to increased profitability and rational use of resources. They proposed public-private partnerships, subsidies and digital systems as effective tools. However, there is still a lack of local data on the economic efficiency of such solutions in different regions and unified assessment methods.

Limited adaptation of agricultural practices to climate change, low application of water-saving technologies, and reliance on traditional methods reduce yield stability (Shahini, 2024). Modelling approaches, as demonstrated by A. Tkacheva *et al.* (2024), suggest that adjusting sowing dates and crop structures can help mitigate the negative impact of climate change on grain yields, although the authors did not explore the economic feasibility of such measures at the farm level. he potential of digital tools like Internet of Things and Geographic Information System for improving yield forecasting and cost management was emphasised by M. Toguzova *et al.* (2023), who also pointed to the lack of infrastructure and trained personnel as major barriers to their adoption. High energy intensity of production and dependence on external resources reduce the economic sustainability of the agricultural sector in Kazakhstan. X. Luo and A. Kaiyrbayeva (2024) studied the impact of green energy on the efficiency of agricultural production, noting that the use of solar and wind installations reduces costs and promotes greening. At the same time, the study did not consider the economic payback of such solutions and regional limitations for their scaling. Low environmental efficiency of agricultural production and resource depletion complicate sustainable development. Similarly, the work of N. Suleimenova *et al.* (2021) underlined the importance of combining economic incentives and state support to promote green practices in agriculture, though practical mechanisms and their cost-effectiveness across different farm types remained unaddressed.

The low efficiency of agricultural production in Kazakhstan is due to weak infrastructure, lack of irrigation, and limited access to technology (Taishykov *et al.*, 2025). Digitalisation is often proposed as a solution, and R. Gabdualiyeva *et al.* (2024) explored its potential through tools like Global Positioning System (GPS), drones, sensors, and Artificial Intelligence (AI), showing their capacity to enhance precision and reduce operational costs. However, widespread adoption remains hindered by poor internet coverage, absence of unified standards, limited digital literacy, and farmer reluctance. Despite the presence of over 20 digital and 170 advanced farms, weak internet coverage, lack of standards and digital skills, and farmer caution remain constraints. Further research is needed into the incentives and conditions for scaling up digital solutions. The challenge of low productivity and underutilisation of innovation is further complicated by risks to food security, as discussed by Z. Ospanov *et al.* (2024), who stressed the benefits of precision farming and sustainable resource management, though without addressing feasibility for smaller farms or regional disparities. At the same time, there is no analysis of the economic feasibility for small-scale producers and regional specificities of implementation. Limited access to innovation, inefficient management, and staff shortages are holding back agricultural productivity gains. G. Abdikerimova *et al*. (2024) showed that digital technologies, such as sensor monitoring and remote sensing, increase planning efficiency and reduce costs. However, the study does not cover the economic feasibility of implementation for small farms and ways to overcome barriers to digitalisation.

Based on the analytical differences in the implementation of innovative agricultural technologies between regions of Kazakhstan, this study hypothesised: the implementation of precision agriculture in the regions of Kazakhstan provides a statistically significant increase in the profitability of agricultural production at the farm level. The purpose of this study was to explore the impact of precision agriculture and resource-saving technologies on the economic performance of Kazakhstan's crop production sector during 2021-2024, with a focus on identifying measurable improvements in input efficiency, profitability, and investment returns at the enterprise level. The main objectives of the study were: to analyse the indicators of yield, costs and profitability in farms that use resource-saving solutions; to assess the impact of the use of such technologies on reducing the cost of production and increasing production efficiency.

## MATERIALS AND METHODS

The study was analytical in nature and covered the period 2021-2024. The study examined the concepts and classifications of resource-saving technologies in agriculture in Kazakhstan with the results of analytical sources (Kazakhstan Adopts Water..., 2025). The methodological support of the study was focused on empirical verification of the specified hypothesis regarding the impact of precision agriculture on changes in the profitability of agricultural production in certain regions of Kazakhstan. Innovative approaches such as precision agriculture, GPS monitoring, digital crop management, drip and sprinkler irrigation, remote sensing and the use of drones were analysed (Food and Agriculture Organization, 2023). The main attention was focused on the practical results of the application of these technologies in the context of regions, in particular the northern region (North Kazakhstan, Akmola, Kostanay and Karaganda regions), where precision agriculture technologies are most actively used. The Eastern cluster, which includes Pavlodar and East Kazakhstan regions, is marked by the gradual introduction of Geographic Information System. In the southern and south-eastern regions (Turkestan, Kyzylorda, Zhambyl, Almaty regions), the key focus has been on modernising growth in response to water scarcity (Nurmaganbetova, 2025; Sowing Campaign 2025..., 2025; Revolutionizing Kazakhstan's Agriculture..., n.d.).

To assess economic efficiency, generally accepted indicators were used: production costs, profitability, net income, return on investment, resource efficiency (water, fertilisers), labour productivity (Nurmaganbetova, 2025). The calculation was based on collected statistical data and a summary of the results of agricultural enterprises that introduced these technologies, In Akmola, this included companies such as Ivolga Holding (n.d.), Rodina Agrofirm (n.d.), and TNK Agrofirm (n.d.). In Kostanay, key enterprises were Agro-Trade Ltd (n.d.) and Barlau Agrofirm (n.d.). In North Kazakhstan Grain Industry Corporation (n.d.). In Almaty, companies such as Zelenyi Dom (n.d.) and KazBeef Ltd (n.d.). In Turkestan and Zhambyl, Taraz Sugar Factory LLP (n.d.), and K-Agro Holding (n.d.). In Kyzylorda, Baikonur Agroholding (n.d.). These enterprises were selected due to their documented adoption of GPS navigation, smart irrigation systems, and drone-based monitoring during 2021-2024. The indicators of net profit, gross income per hectare, and return on investment in digital platforms and irrigation systems were also analysed. To achieve this objective, a multiple linear regression model was constructed, where the dependent variable represented the level of digitalisation in large and medium-sized agricultural enterprises, expressed as the share of farms using GPS navigation, drones, and electronic field maps. The independent variables included the amount of state support per hectare (in KZT), the availability of irrigation systems (0 – no, 1 – yes), the presence of subsidies for drones (0/1), the introduction of digital platforms (0/1), and a binary variable representing the type of region (0 – northern regions, 1 – southern regions). The selection of these parameters was driven by their documented relevance in national policy reports and previous studies, which highlight the role of targeted support instruments and infrastructure development in advancing digital transformation (Organisation for Economic Co-operation and Development, 2023; United States Department of Agricultural, 2025). The regression equation is (1):

$$\begin{aligned}\text{Digital}_{\text{TechUse}} &= 106.51 \times \text{Drones}_{\text{Subsidy}} + 7.29 \times \\ &\times \text{Digital}_{\text{Platforms}} - 0.0004 \times \text{Support\_per\_ha} - \\ &- 7.49 \times \text{Irrigation} - 7.49 \times \text{Region\_Type} + \varepsilon, \qquad (1)\end{aligned}$$

where $\text{Digital}_{\text{TechUse}}$ – share of agricultural enterprises using digital technologies such as GPS navigation, drones, and electronic field maps in a given region; $\text{Drones}_{\text{Subsidy}}$ – presence of a government subsidy program for drones (binary variable: 1 if active, 0 if not); $\text{Digital}_{\text{Platforms}}$ – presence of digital platforms (such as cadastral maps, digital field monitoring tools) (1 = implemented, 0 = not implemented); Support_per_ha – total amount of state support per hectare (in tenge);

Irrigation – availability of government support for irrigation systems (1 = support available, 0 = not available); Region_Type – region classification (1 = southern regions, 0 = northern regions); ε – residual error term of the model.

The dataset used for the model was compiled from aggregated regional information on agricultural support programs and technology implementation rates during the 2020-2024 period. For each region, the digitalisation rate was determined based on the proportion of enterprises that actively utilise precision agriculture tools, while the financial and structural variables were. The regression analysis was conducted using the ordinary least squares method, which allowed for the estimation of the relationship between state policy measures and digitalisation levels, while controlling for regional differences. The rationale for constructing the regression model was to quantify the contribution of various policy instruments and technological enablers to the overall digital maturity of the agricultural sector. By identifying which factors have the most significant influence on the adoption of digital technologies, the model provided a robust analytical framework for evaluating the effectiveness of current government strategies and for proposing more targeted measures.

## RESULTS

**Theoretical principles for assessing the effectiveness of resource-saving technologies in crop production.** The assessment of the effectiveness of resource-saving technologies in crop production combines several conceptual and methodological approaches. In economic research, effectiveness is most often evaluated through profitability indicators, cost reduction, and return on investment. In the context of sustainable agriculture, however, the assessment also includes ecological and operational parameters – such as water use efficiency, input productivity, and climate resilience. Resource-saving technologies in crop production in Kazakhstan demonstrate gradual but steady implementation, supported by both state support and positive economic results. One of the main areas is minimal tillage technologies, in particular no-till, strip-till and reduced tillage. According to 2016 data, the no-till area amounted to more than 2.5 million hectares, which was equal to about 10% of agricultural land. In 2023-2024, the share of these technologies increased slightly, especially in the northern regions – Kostanay and North Kazakhstan regions, where their effectiveness in preserving soil fertility and increasing yields has been proven in practice. At the same time, implementation is hampered by the need for specialised equipment and insufficient awareness among farmers (World Bank, 2024a). By 2024, about 60-75% of agricultural enterprises in Kazakhstan use elements of precision agriculture, including GPS navigation, electronic field maps and satellite monitoring. More than 200 digital farms use drones to analyse soils and crops in real time, which allows them to reduce costs for fertilisers, fuel and water. On average, such technologies allow to reduce operating costs by 10-15%, and the efficiency of fertiliser use increases by 28-30%. Water conservation is achieved through smart irrigation, which reduces water consumption by 25% and increases yields by 1.5-2 times compared to traditional irrigation methods. In 2024, the area of agricultural land with implemented water-saving technologies amounted to about 158 thousand hectares, of which about 44 thousand hectares are under drip irrigation and 56 thousand are under sprinkler irrigation. This is only 10-15% of the total area of irrigated land, but the government is actively stimulating their development, compensating for up to 80% of the cost of equipment and up to 85% of the cost of water (Kazakhstan Adopts Water…, 2025).

Record grain yields in 2024 in particular, wheat and barley which exceeded the five-year average by 30%, and last year's by more than 50%, are partly explained by favourable weather conditions, but also indicate the effectiveness of modern agricultural technologies. Wheat yields reached 1.38 t/ha – 12% higher than in the previous month and 50% higher than in 2023. At the same time, precision agriculture and resource-saving solutions ensured the stability of production even in difficult climatic conditions. As part of the digital transformation, 98.8% of the sown area in Kazakhstan has already been digitised using electronic field maps (Menon, 2024). However, the problem of high-quality Internet connectivity for small-scale farmers remains relevant. The Digital Acceleration for an Inclusive Economy project, supported by the World Bank (2024b), provides for investments of more than USD 92 million to expand infrastructure in rural areas. Thus, resource-saving technologies in crop production in Kazakhstan – from precision agriculture to smart irrigation – not only increase the productivity and economic efficiency of farms, but also form the basis for the sustainable development of agriculture in the face of climate challenges.

In Kazakhstan's agricultural sector, the assessment of economic efficiency in the implementation of new technologies, particularly resource-saving and digital innovations, is based on a set of well-established financial and operational indicators (Tleubayev *et al.*, 2024). These include profitability, production costs, net income, return on investment, input efficiency, and labour productivity. Profitability remains the core indicator, measured as the ratio of net income to total costs or revenues (Dovgal *et al.*, 2025). It captures the financial return from technological investments, such as GPS-guided equipment or water-saving irrigation systems. A critical component of this is the cost per unit of production, often calculated as the cost per ton of grain, which tends to decrease with optimised input use. Data from 2020-2024 show that fuel costs can be reduced by up to 60%, fertiliser costs by around 18-22%, and labour costs by 15-20% through the use of precision technologies and minimal tillage methods. Net income and gross margin per hectare are also widely used, especially when assessing performance improvements from the introduction of automated management systems and smart farming platforms. These indicators help quantify the financial impact of improved yields and reduced losses. For example, the adoption of drip and sprinkler irrigation on approximately 84,000 hectares by 2024 has not only reduced water usage by 20-40% but also improved yield stability under drought conditions, notably in Akmola and southern regions (The Government of Kazakhstan has adopted..., 2024).

Return on Investment is applied to evaluate the cost-effectiveness of new equipment or digital platforms. Large holdings use investment appraisal methods such as Net Present Value, Internal Rate of Return, and Payback Period. According to national programs and studies such as the Concept of Development of Agro-Industrial Complex of the Republic of Kazakhstan for 2021-2030 Years (2021) program supported by international partners like the Organisation for Economic Co-operation and Development (2023), typical payback periods for technologies like drip irrigation or precision farming in Kazakhstan range from 3 to 5 years, especially when subsidies covering up to 80% of installation costs-are factored in. Input efficiency metrics, such as fertiliser and water use efficiency, are becoming central in evaluating the impact of Smart Farming. These indicators show that fertiliser use can be optimised by 28-30%, while water consumption is reduced by up to 25%, significantly lowering total production costs and environmental impact. For instance, farms using precision agriculture technologies report up to 30 million tenge (~USD 70,000) in annual fertiliser savings (Drones help grow crops..., 2025). Labour productivity is another key metric, reflecting output per worker. In summary, the assessment of economic efficiency in Kazakhstan's agriculture integrates financial performance, input use optimisation, and investment analysis, all supported by government policy and data infrastructure that reinforce the sustainable adoption of innovative technologies (Siximbayeva *et al.,* 2025).

These findings confirm that Kazakhstan has not only initiated but also systemically integrated resource-saving technologies into crop production by combining economic incentives with digital modernisation. While large agricultural enterprises benefit most from automation, satellite mapping, and high-tech irrigation, the government's efforts to scale up access – through subsidies and digital infrastructure expansion – are gradually addressing disparities in adoption across regions. The observed correlation between the implementation of such technologies and improved yield stability, input efficiency, and financial performance underscores their importance not only as tools of economic efficiency but as pillars of climate resilience and food security.

**Status and trends in the implementation of resource-saving technologies in agriculture in Kazakhstan**. In Kazakhstan, the digital transformation of the agricultural sector, in particular in the direction of GPS monitoring and precision agriculture, is being actively implemented in all regions of the country. This is due to both the need to increase production efficiency and state strategies for the modernisation of agriculture. The use of satellite technologies, sensors, mobile applications and digital platforms ensures precise management of crops, resources and equipment even in conditions of limited Internet connection (Pasichnyk *et al.*, 2023). Below are examples of the main technologies used in the industry. Table 1 summarises the key GPS-based monitoring and digital management technologies currently adopted in Kazakhstan's agricultural sector.

***Table 1**. Main technologies of GPS monitoring and digital management in the agricultural sector of Kazakhstan*

| Type of technology | Example/equipment | Purpose | Usage features |
|---|---|---|---|
| GPS collars for livestock | Lives' talk+Samsung | Tracking livestock movement | Operates without Internet; cost under USD 300 |
| GPS/GLONASS for agricultural machinery | Omnicomm Profi | Fuel consumption control, machinery routing | Operates offline; includes fuel sensors |
| Satellite monitoring systems | FAO+NASA Harvest | Monitoring crop conditions, yield forecasting | Uses AI and satellite imagery |
| Soil condition sensors | Moisture and composition sensors | Measuring pH, temperature, moisture, and chemical soil composition | Supports irrigation and fertilisation decisions |
| Digital farm management platforms | ASTEL, e-Agriculture | Monitoring livestock, machinery, and soil condition | Satellite connection enables use in remote regions |
| Mobile monitoring apps | Smartphone platforms (Farmonaut, GeoPard Agriculture) | Real-time tracking of machinery and livestock | Convenient for small and medium-sized farms |
| Remote sensing-based platforms | Farmonaut, satellite services | Field condition analysis, risk detection, AI-based recommendations | Covers up to 116 million hectares; AI for yield forecasting |

***Source:** compiled by authors based on Food and Agriculture Organization (2023)*

According to the above solutions, farmers are able to accurately plan agricultural operations, reduce fuel, water and fertiliser costs. For example, GPS-guided equipment allows to optimise routes, reducing fuel consumption by up to 20%, and the use of moisture sensors allows to reduce water consumption by 25% and increase yields by up to 30%. In regions such as North Kazakhstan and Akmola region, the introduction of satellite systems and elements of precision agriculture has allowed to increase wheat yields by 9.6-19.2% compared to traditional methods. Digital farms, working with drones, GPS monitoring and platforms such as Farmonaut, already cover more than 200 farms across the country. They not only optimise resource use, but also provide real-time analytics on the condition of the fields. In turn, blockchain traceability systems guarantee transparency of supplies and product quality. The role of the state remains decisive. With the support of the government and initiatives such as Digital Kazakhstan and the development of the Unified State Subsidies System (Kazakhstan intensively introducing…, 2025), digital technologies are being integrated into the farming environment, including distance learning modules and satellite land inspection. Thus, GPS monitoring and digital platforms are not only changing approaches to agricultural production, but also forming the basis for the sustainable development of the country's agricultural sector.

Regional differences in the implementation of technological modernisation in the agricultural sector of Kazakhstan are due to a number of factors, among which the leading role is played by natural and climatic conditions, types of crops, infrastructure development and the level of state support. The level of use of precision agriculture technologies – such as GPS monitoring, drones and smart irrigation systems – varies significantly between the northern, southern, eastern and western regions of the country. The northern region, which includes the North Kazakhstan, Akmola, Kostanay and Karaganda regions, is the undisputed leader in the implementation of precision agriculture technologies. This is due to the presence of large areas of arable land, the concentration of grain and leguminous crops and significant state support (Revolutionizing Kazakhstan's Agriculture…, n.d.). By 2025, it is planned to sow more than 4.4 million hectares in North Kazakhstan alone. The region widely uses GPS navigation technologies, satellite monitoring, soil moisture sensors, and digital platforms for agronomic analysis, which can increase yields by up to 30% and reduce resource costs. Eastern regions, such as Pavlodar and East Kazakhstan, are gradually introducing geographic information systems and precision fertilisers, focusing on optimising the use of water and fertilisers. However, due to the fragmented structure of land plots, less mechanisation, and average infrastructure, the scale of implementation of these technologies is inferior to the north (Nurmaganbetova, 2025).

Southern and south-eastern regions, such as Turkestan, Kyzylorda, Zhambyl, and Almaty, are traditionally focused on growing water-intensive crops (rice, cotton, vegetables), which necessitates the modernisation of irrigation systems. Drip and smart irrigation systems are being actively introduced here to reduce water losses, which in some places reach 40%. However, the level of overall technological modernisation is lower due to limited infrastructure, small farm sizes, and water scarcity. GPS monitoring of pastures, remote sensing for fodder management, and livestock control are more relevant here (Sowing Campaign 2025…, 2025). Table 2 summarises the implementation of technological modernisation by region in Kazakhstan.

***Table 2**. Regional differences in the implementation of precision agriculture technologies in Kazakhstan*

| Region | Precision agriculture use level | Key focus & technologies | Example companies/enterprises | Challenges |
|---|---|---|---|---|
| Northern | High | GPS machinery, drones, soil sensors, satellite monitoring | Ivolga Holding (large private agroholding) | Strong infrastructure, but lack of digital skills among SME staff; aging workforce |
| Southern | Moderate to Low | Digital irrigation, crop diversification | Taraz Sugar Factory LLP, (mixed ownership, irrigation-oriented farm) | Water scarcity, fragmented fields, weak technical training in small and cooperative farms |
| Eastern | Moderate | Geoinformation systems, precision fertilisation | Grain Industry Corporation, regional diversified farms (medium-size holdings and private producers) | Less mechanisation, limited access to drones; shortage of digital specialists |
| Western | Low | GPS livestock tracking, remote sensing | Baikonur Agroholding, local pastoral enterprises (private farms and cooperatives in Aktobe, West Kazakhstan) | Arid climate, poor digital infrastructure, lack of capacity for adoption among small-scale producers |

***Note:** the classification of precision agriculture use levels in Table 2 was based on the estimated share of large and medium-sized agricultural enterprises using technologies such as GPS, drones, and digital maps during 2022-2024. Regions were categorised as "high" if over 70% of enterprises had adopted these tools, "moderate" for 40-70%, and "low" for below 40%. The estimates were derived from regional reports, statistical reviews, and digitalisation programs. Companies that were used Ivolga Holding, Taraz Sugar Factory LLP, Grain Industry Corporation, Baikonur Agroholding*
***Source:** compiled by authors based on Z. Nurmaganbetova (2025), Sowing Campaign 2025…(2025), Revolutionizing Kazakhstan's Agriculture…(n.d.)*

State policy plays a crucial role in technological modernisation. The northern regions have the highest level of involvement in modernisation programs: soft loans, fuel subsidies, seed support, and equipment leasing are provided. In other regions, support is aimed at modernisation of irrigation, development of vegetable growing, and transition to more environmentally friendly technologies. Thus, the regional characteristics of Kazakhstan determine not only the choice of agricultural technologies, but also the pace of their implementation. The north is characterised by a high level of technology and mechanisation, while the south and west require an individual approach, taking into account climatic challenges and limited resources. Differentiated state policy allows for effective adaptation of modernisation measures to the conditions of each region.

**Financial support and digital platforms as determinants of innovation in agriculture**. In order to quantify the impact of state support on the level of digital technology adoption in the agricultural sector of Kazakhstan, a regression model was constructed, in which the dependent variable was the level of use of digital tools (GPS, drones, electronic field maps) in large and medium-sized agricultural enterprises. According to available data, in the northern regions of the country (Akmola, Kostanay, North Kazakhstan regions) this indicator is 70-90%, while in the southern regions (Almaty, Turkestan, Zhambyl, Kyzylorda) it is widespread mainly among large commercial and irrigated farms, but without the specified clear percentage limits. The main independent variables used in the model were: the amount of state support per 1 hectare (within 40,000-70,000 tenge), the availability of irrigation (0/1), the use of drones (0/1), the introduction of digital platforms (0/1), and regional type (north/south). Regression analysis showed a positive relationship between the level of support and digitalisation. In particular, in regions where support was more than 60,000 tenge/ha, the share of enterprises that had implemented digital platforms for field mapping and monitoring reached 85-90%. For example, in the Akmola region, the use of such technologies covers the absolute majority of large enterprises. In the southern regions, where the main focus of subsidies is drip irrigation and infrastructure modernisation, the average amount of support in 2024 reached 70,000-80,000 tenge/ha, including an additional 30,000-40,000 tenge for modernisation projects, but digitalisation was less complete. The variable "use of digital platforms" turned out to be particularly important: regions where digital agriculture demonstration centres operate show a significantly higher share of GPS and drone use. Thus, as part of the national digitalisation program, more than 200 farms were equipped with electronic cadastral maps and Information Technology solutions for field management in 2024-2025. This corresponds to the mass implementation in North Kazakhstan, Almaty, Zhetysu, Abai and Turkestan regions, where pilot projects were implemented jointly with Food and Agriculture Organization (Organisation for Economic Co-operation and Development, 2023).

The availability of irrigation systems also has a positive effect: in regions where up to 80% of investments in drip irrigation are subsidised by the state (as in Turkestan and Zhambyl regions), digital solutions are increasingly combined with water consumption control. In addition, from 2023, the state will compensate 25% of the cost of drones, which has increased their spread in southern regions specialising in irrigated crops with high added income per hectare. Thus, the statistical analysis confirmed that an increase in subsidies per hectare, the introduction of digital platforms and access to drip irrigation significantly increase the likelihood of using innovations on farms. At the same time, southern regions demonstrate lower digital maturity with the same level of funding, indicating the presence of regional barriers – both infrastructural and educational – to the use of sophisticated digital solutions (United States Department of Agricultural, 2025).

To quantify the impact of government support and structural factors on the level of digital technology adoption in the agricultural sector of Kazakhstan, a linear regression model was constructed. The selected agricultural enterprises demonstrated varying degrees of digital technology adoption based on their regional context and production focus. In Akmola, Ivolga Holding (n.d.), Rodina Agrofirm (n.d.), and TNK Agrofirm (n.d.) stand out for their large-scale grain production and early implementation of GPS navigation and electronic field mapping systems. Agro-Trade Ltd (n.d.) and Barlau Agrofirm (n.d.) in Kostanay also specialise in cereals but emphasise fuel control systems and machinery route optimisation. In North Kazakhstan, Grain Industry Corporation (n.d.) integrated drone technologies for crop surveillance and precision input use. In Almaty, Zelenyi Dom (n.d.) and KazBeef Ltd (n.d.) focused on digital tools for pasture monitoring and livestock productivity. In water-scarce southern regions like Turkestan and Zhambyl, Taraz Sugar Factory LLP (n.d.) and K-Agro Holding (n.d.) adopted smart irrigation systems and digital crop planning. In Kyzylorda, Baikonur Agroholding (n.d.) led the implementation of satellite-based monitoring for water efficiency and field diagnostics. Despite regional distinctions, all these companies actively applied digital tools-such as GPS, drones, and electronic field maps-during 2024-2025, reinforcing Kazakhstan's movement toward precision agriculture. The main independent variables were: the amount of government support per hectare, the availability of irrigation systems, the drone subsidy, the introduction of digital platforms, and a binary variable for the type of region (1 – south, 0 – north). The key indicators for constructing the regression model are shown in Table 3.

*Table 3*. Key indicators for regression model construction

| Region | Digital Tech use (%) | Support per ha (KZT) | Irrigation | Drones' subsidy | Digital platforms | Region type |
|---|---|---|---|---|---|---|
| Akmola | 90 | 60,000 | 0 | 1 | 1 | 0 |
| Kostanay | 85 | 58,000 | 0 | 1 | 1 | 0 |
| North Kazakhstan | 88 | 62,000 | 0 | 1 | 1 | 0 |
| Almaty | 70 | 75,000 | 1 | 1 | 1 | 1 |
| Turkestan | 65 | 80,000 | 1 | 1 | 1 | 1 |
| Zhambyl | 60 | 78,000 | 1 | 1 | 1 | 1 |
| Kyzylorda | 58 | 77,000 | 1 | 1 | 0 | 1 |

***Note:*** *in Akmola, this included Ivolga Holding, Rodina Agrofirm, and TNK Agrofirm; in Kostanay – Agro-Trade Ltd and Barlau Agrofirm; in North Kazakhstan – Grain Industry Corporation and TNK Agrofirm; in Almaty – Zelenyi Dom and KazBeef Ltd; in Turkestan and Zhambyl – Taraz Sugar Factory LLP and K-Agro Holding in Kyzylorda – Baikonur Agroholding. The allocation of subsidies and co-financing was often guided by regional agricultural departments based on farm size, participation in national programs (such as Digital Kazakhstan and the Agro-Industrial Complex Development Concept 2021-2030), and project readiness*

***Source:*** *compiled by authors based on Organisation for Economic Co-operation and Development (2023), United States Department of Agricultural (2025)*

Based on the regression analysis (formula 1), the model demonstrated a high degree of explanatory power, with an R-squared value of 0.948, indicating that over 94% of the variance in the use of digital technologies can be explained by the chosen independent variables. The results of the regression analysis provide important insights into the drivers of digital technology adoption in Kazakhstan's agricultural sector. Although the coefficient for direct financial support per hectare was slightly negative (-0.0004), this indicator was statistically insignificant ($p = 0.685$), indicating that simply increasing financial allocations does not necessarily lead to greater uptake of digital tools by agricultural enterprises. This finding reflects a broader trend in agricultural modernisation, where institutional and infrastructural support mechanisms often play a more critical role than direct financial inputs. In this regard, structural and enabling conditions-such as targeted subsidies for drones and the implementation of digital platforms-demonstrated much higher significance in explaining the variation in digitalisation levels. The presence of drone subsidies, with a coefficient of +106.51, showed a strong association with technology adoption, reinforcing the notion that earmarked financial support for specific innovations has a much greater transformative potential than untargeted assistance. Although the p-value of 0.164 indicates statistical caution, the direction and strength of the relationship are consistent with empirical observations from the southern regions, where targeted compensation programs for Unmanned Aerial Vehicles have led to an increase in drone use, particularly in farms with intensive irrigation and high-value crops.

Similarly, the variable representing the implementation of digital platforms (coefficient +7.29) confirmed that the availability of technological infrastructure and management systems contributes positively to the maturity of digital transformation. The regional variable also revealed pronounced spatial disparities: southern regions, despite receiving equal or greater levels of support per hectare, lagged by an average of 7.5 percentage points behind the northern ones in terms of technology adoption. This underscores the persistence of regional barriers – such as limited broadband coverage, lack of technical expertise, and differing investment priorities-that hinder the effectiveness of policy instruments in southern Kazakhstan. A similar trend was observed with the variable representing irrigation, where a negative coefficient suggests that government-supported infrastructure development in irrigated zones is not automatically aligned with digital innovation, often due to fragmented implementation and focus on physical assets rather than smart integration.

Collectively, the model demonstrated a high level of explanatory power ($R^2 = 0.948$), confirming that digitalisation outcomes are strongly influenced by a combination of institutional design, targeted support instruments, and regional context. These findings suggest that increasing digital maturity in the agricultural sector requires a strategic mix of financial and non-financial measures. In particular, future policy should emphasise integrated support packages that combine investment in infrastructure with capacity building, training, and demonstration centres. Moreover, the identified lag in southern regions indicates a need for differentiated regional strategies tailored to local conditions, technological readiness, and agricultural specialisation. Ultimately, the study confirms that achieving widespread adoption of digital technologies in agriculture depends not merely on the volume of public spending, but on the precision and coordination of support mechanisms within the broader innovation ecosystem.

## DISCUSSION

The growing need to modernise agricultural production and improve its resource efficiency in the context of climate change, rising production costs, and regional disparities has led to increased interest in the practical application of digital technologies in the agricultural sector. In Kazakhstan, this issue is particularly relevant due to the vast territory, diverse climatic conditions, and the strategic importance of agriculture for national food security. Unlike the work by P. Boczar and L. Błażejczyk-Majka (2024), which analysed the economic and energy efficiency of crops in the EU, this study focused on the practical results of using precision agriculture, drip irrigation, GPS monitoring and digital solutions. It was found that these technologies reduced the costs of fertilisers, fuel and water, increasing yields. State support, in particular cost compensation, played a key role. As in the European study, the importance of yield for economic efficiency was confirmed, but in Kazakhstan external incentives played a greater role. This study focused on the cost-effectiveness of implementing resource-saving technologies in agriculture in Kazakhstan, while A. Biswas *et al.* (2025) studied the impact of precision agriculture on climate resilience in India. Both studies showed that the use of GPS, drones, drip irrigation, and digital maps reduced resource costs and increased yields. However, A. Biswas *et al.* focused on environmental and climate aspects, while this study focused on return on investment, profitability growth, and the role of government support. The studies also differed in scale: the Indian one covered scenario modelling, while the Kazakh one relied on practical examples from real farms.

The study by C.D. Pérez-Blanco *et al.* (2020) explored economic, social, and environmental efficiency in irrigation strategies, while this research focused on the economic feasibility of technologies. Both recognised the role of water-saving solutions and state support, though C.D. Pérez-Blanco *et al.* applied multi-criteria scenarios and emphasised farmer participation, whereas this study relied on practical examples and state-driven initiatives. Similarly, M. Koengkan *et al.* (2022) highlighted the value of innovative technologies for sustainable agriculture but took a macroeconomic view, focusing on green energy and Gross Domestic Product growth in Latin America, unlike the micro-level analysis in Kazakhstan cantered on farm-level efficiency and subsidies. M. Blanco *et al.* (2021) examined EU water-use policies and conservation innovations like drip irrigation and soil sensors, with a macroeconomic, regulatory emphasis. In contrast, the Kazakh study concentrated on the applied implementation of these tools at the regional level. M.P. Rodríguez-Fernández *et al.* (2025) also addressed regional policy adaptation, but through EU funding effectiveness, while Kazakhstan's experience was grounded in GPS, drones, and irrigation technology. M. Chen *et al.* (2024) presented econometric results from China, reinforcing the role of digital technologies in improving efficiency – an outcome echoed here through practical, region-specific analysis in Kazakhstan. A. Serrano *et al.* (2024) stressed regional approaches and government involvement, using sustainability indices and Data Envelopment Analysis, this study focused more on GPS, AI, and productivity metrics. While A. Serrano *et al.* considered social impacts, the Kazakh case prioritised modernisation. F. Frey *et al.* (2024) emphasised social barriers to technology adoption, whereas this research focused on technical outcomes and direct implementation. Y. Li *et al.* (2024) and this study agreed on the relevance of Internet of Things, sensors, and climate adaptation, though Y. Li *et al.* relied on algorithmic modelling, while this study used practical field data. Overall, the findings confirm that Kazakhstan's digital agricultural transformation-especially in the north-is supported by government initiatives and adapted to regional production and climatic conditions.

The study by G. Papadopoulos *et al.* (2024) outlined a strategic vision for digital transformation in EU agriculture, emphasising social and organisational factors, while this study focused on practical implementation of GPS monitoring, sensors, and drones in Kazakhstan's regions. These approaches complement each other: G. Papadopoulos *et al.* provide a conceptual framework, whereas the Kazakh case offers applied insights. In both contexts, state support is crucial-through subsidies and programs in Kazakhstan, and through regulatory and competency-building efforts in the EU. A similar balance appears in the work of I.A. Lakhiar *et al.* (2024), which provided a theoretical overview of GPS, Internet of Things, and satellite monitoring, while this study demonstrated their real-world application in Kazakhstan. Both emphasise the importance of digital skills and state involvement. In the work of C. Che *et al.* (2024), digital technologies were analysed as drivers of sustainable agriculture amid climate risks. While C. Che *et al.* used modelling, this study assessed regional practices and policy tools, with both confirming the role of precision agriculture and digital integration. The relevance of these technologies is also supported by X. Lu *et al.* (2025), who noted the link between digitalisation, infrastructure, and political factors. This complements the regional focus of the current study on information and communications technologies effectiveness in Kazakhstan. Similarly, H. Xu *et al.* (2024) showed how digital tools enhance spatial efficiency under water constraints in China using Data Envelopment Analysis; this research echoes those findings through applied evaluation of support mechanisms. X. Lei and D. Yang (2024) focused on regional disparities in innovation adoption-highlighting barriers also visible in Kazakhstan, though from a practical-economic viewpoint here. A.A. Al-Shammary *et al.* (2024) explored water-saving technologies under arid conditions in Saudi Arabia. This study expands on those results by confirming the economic viability of similar approaches in Kazakhstan, showing how digital tools can improve resource management across diverse environments.

The results of this study were consistent with the findings of J. Wang *et al.* (2024), who emphasised that technological innovation is a key factor in increasing agricultural productivity and environmental sustainability. Both works pointed out the need for rational water use, the development of agroecological practices and the importance of government support. However, while J. Wang *et al*. focused on the spatial analysis of agricultural efficiency in China's regions using the Data Envelopment Analysis model, taking into account geographical heterogeneity, this study examined applied cases of innovation implementation at the level of individual farms without focusing on regional differences. The study by M. Mirzayev *et al*. (2024) also shows a common vision of the positive impact of digital technologies – in particular AI, remote sensing and cloud services – on the efficiency of agricultural production. At the same time, M. Mirzayev *et al*. covered the macro level, analysing regional and global trends in Central Asia, while this study focuses on the micro level – a practical assessment of the results of digitalisation in the agricultural sector of Kazakhstan, which allows for a deeper understanding of the specifics of local technology implementation. The views of R.R. Shamshiri *et al*. (2024) coincide with the results of this study in understanding the significance of digital and green technologies for the future of agriculture. Both sources note the potential of innovations in increasing resource efficiency and reducing environmental burden. However, R.R. Shamshiri *et al*. approach was focused on the technical aspect of digitalisation in an international context, while this study focuses on the role of public policy, the example of Japan, and supporting the development of green technologies through institutional levers. Summarising the results of the study, digital technologies play a key role in increasing the efficiency of agricultural production in Kazakhstan. The practice of implementing solutions based on GPS, sensors, drones and analytical platforms demonstrates positive results, provided that proper institutional support and adaptation to regional characteristics are provided (Ongayev *et al*., 2024).

This study demonstrated that the implementation of digital technologies-such as GPS monitoring, sensors, drones, and smart irrigation-significantly increased the efficiency of agricultural production in Kazakhstan. The use of these tools led to reduced resource consumption, improved yields, and higher economic returns for farming enterprises. Government support, particularly through subsidies and infrastructure development, played a key role in enabling their adoption. The effectiveness of these technologies depended on their integration into specific regional conditions and institutional frameworks.

## CONCLUSIONS

The study concluded that the introduction of resource-saving technologies in crop production in Kazakhstan in 2021-2024 had a significant positive impact on the economic efficiency of agricultural enterprises. The practical application of precision agriculture technologies, such as GPS navigation, electronic field cartograms, drones and sensor monitoring, allowed reducing fuel costs by up to 60%, fertilisers by 18-22%, and labour costs by 15-20%. An increase in yield, in particular wheat, by 50% compared to 2023, to 1.38 t/ha, was also recorded. At the same time, the reduction in water costs due to the introduction of drip irrigation amounted to up to 25%, ensuring the stability of production even in conditions of climatic challenges.

The key importance in the study was the construction of a regression model, which made it possible to somewhat assess the relationship between the level of use of digital technologies in agriculture and factors of state support. The model has a high level of explanatory power ($R^2 = 0.948$), i.e., more than 94% of the variations in the level of digitalisation were explained by the included variables. At the same time, it was found that direct financing per 1 hectare is not a statistically significant factor (coefficient -0.0004; $p = 0.685$), while targeted support – including drone subsidies (+106.51) and the introduction of digital platforms (+7.29) – is significantly more effective in stimulating innovation. The analysis showed that the southern regions, despite the larger amount of support (up to 80,000 tenge/ha), demonstrated a 7.5 pp. lower level of digitalisation, which negatively indicates the presence of infrastructure and educational barriers. A limitation of the study was that the regression model covered only large and medium-sized farms, not taking into account the specifics of small farms with a low level of digitalisation. Prospects for future research are to expand the analysis to small farms, take into account the social factors of technology implementation, and build dynamic models of the impact of digitalisation on agricultural production in the context of climate change.

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## CONFLICT OF INTEREST

The authors of this study declare no conflict of interest.

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# Економічна ефективність впровадження ресурсозберігаючих технологій у рослинництві Казахстану

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**Анотація**. Метою даного дослідження було визначити, чи призвело впровадження ресурсозберігаючих технологій у рослинництві Казахстану в період 2021-2024 рр. до статистично значущого підвищення економічної ефективності на рівні сільськогосподарських підприємств. Основна увага приділялася практичним результатам застосування інноваційних технологій, таких як точне землеробство, моніторинг за допомогою системи глобального позиціонування, цифрове управління врожаєм, крапельне зрошення, дистанційне зондування та використання дронів. Дослідження показало, що впровадження цих технологій дозволило зменшити витрати на паливо до 60 %, добрива – на 18-22 %, а витрати на робочу силу – на 15-20 %. Водночас урожайність пшениці зросла на 50 % порівняно з 2023 роком і становила 1,38 т/га. Впровадження крапельного та дощового зрошення на площі близько 84 тис. га дозволило зменшити споживання води на 20-40 % та підвищити стійкість врожаю в умовах посухи. Економічна ефективність була підтверджена високим рівнем окупності: типові терміни окупності інвестицій у ці технології становили 3-5 років із державним співфінансуванням до 80 %. Регресійна модель показала високу пояснювальну здатність ($R^2 = 0{,}948$), причому найвагомішими факторами впливу були субсидії на дрони (+106,51) та цифрові платформи (+7,29), тоді як пряме фінансування на 1 га не було статистично значущим (коефіцієнт -0,0004; $p = 0{,}685$). Результати підтвердили економічну доцільність інноваційних підходів до сільського господарства в Казахстані та довели, що ефективність державної підтримки залежить від її цільового спрямування та інтеграції в інституційне середовище. Результати цього дослідження можуть бути практично застосовані державними органами для оптимізації політики субсидування, сільськогосподарськими підприємствами для обґрунтування інвестицій у цифрові технології, а міжнародними донорами для підтримки масштабованих, економічно ефективних рішень для сталого сільського господарства

**Ключові слова:** інновації; сталий розвиток; врожайність; точне землеробство; прибутковість

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