



Experimental investigation of the antimicrobial activity of the preparation and its influence on the viability of honey bees (*Apis mellifera*)

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Abstract. The epizootic spread of bacterial infections in apiculture, intensified by the phenomenon of colony collapse disorder, necessitates the development of new and safe preventive agents. Due to legislative restrictions on the use of antibiotics, priority is given to the study of antiseptics with immunomodulatory properties. The aim of this work was a comprehensive assessment of the effectiveness of the preparation *Kombiyod*, namely the determination of its antimicrobial activity *in vitro* and the establishment of optimal safe concentrations to enhance the viability of honeybees *in vivo*. The study included the disc diffusion method using pure strains of enterobacteria and mixed microbial cultures isolated from diseased bees *in vitro*, as well as a cage experiment evaluating the viability and physiological state of bees when the preparation was added to sugar syrup (0.01-2.05%) *in vivo* under laboratory conditions. The results demonstrated the bacteriostatic efficacy of the preparation *in vitro* at concentrations of

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0.1-0.5%. A dose-dependent effect was observed in bees under laboratory conditions: concentrations of 0.5-2.05% caused acute toxicity with a mortality peak on the 7th day, whereas optimal concentrations of 0.05-0.1% exhibited a stimulating effect – maximum lifespan of 17-18 days, a 25% increase in viability compared to the control, and a probable immunomodulatory effect associated with iodine deficiency correction. Based on the obtained results, a 0.1% solution of the preparation in sugar syrup is recommended for oral administration and a 0.2% aqueous solution for hive sanitation in the presence of bees. The therapeutic concentration range has been established for further research. The findings may serve as a foundation for developing new prevention schemes of bee bacterioses based on iodine-containing preparations, considering the identified dose-dependent effects

Keywords: honeybees (*Apis mellifera*); povidone-iodine; antimicrobial activity; enterobacteria; microbiological studies; bee viability; immunomodulatory effect

INTRODUCTION

The decline in the population of honeybees (*Apis mellifera*) represents one of the most acute challenges in modern agricultural production, as the stability of pollination and global food security depend directly on the health of these insects. The mass losses of bee colonies, known as Colony Collapse Disorder (CCD), are of a multifactorial nature, combining biological, toxicological and microbiological factors. According to the findings of B. Mohammadian *et al.* (2024), the primary causes of this phenomenon include dysbiosis of the gut microbiota, pesticide burden and a decline in immune resistance, which together lead to the progressive weakening of colonies. Similar conclusions were drawn by G. Singh and A. Rana (2025), who emphasised that the economic losses caused by colony collapse reach hundreds of millions of dollars, with global ecological consequences.

Bacterial infections remain among the key factors contributing to brood mortality and reduced productivity in honeybees. M. Alburaki *et al.* (2024) reported that American and European foulbroods were detected in more than half of the apiaries examined across the USA, and their pathogens exhibited antibiotic resistance. Comparable results were obtained by S. Yakan *et al.* (2025) in Turkey, where the combination of bacterial infections and pesticide exposure led to increased colony mortality. Ukrainian researchers O. Galatyuk *et al.* (2020) also noted the high resistance of enterobacteria isolated from diseased bees to conventional disinfectants, which complicates the effective prevention of bacterial diseases in apiaries.

Due to legislative restrictions on the use of antibiotics in apiculture, the development of alternative antimicrobial agents has become a particularly relevant direction. According to R. Dinata *et al.* (2023), bee-derived bioactive peptides demonstrate a broad spectrum of antibacterial activity; however, their industrial application is limited by structural instability. Meanwhile, A. Truong *et al.* (2023) demonstrated that the use of probiotic *Lactobacillus* strains reduces larval mortality during *Paenibacillus larvae* infection, although the effect depends on the specific strain combination and environmental temperature.

M. Damico *et al.* (2025) found that commercial probiotic formulations do not always ensure consistent outcomes, highlighting the need for compounds with prolonged antimicrobial effects.

One of the most promising approaches involves the use of iodine-containing antiseptics, which combine bactericidal and immunomodulatory properties. G. Kampf (2024) notes that povidone-iodine provides effective disinfection through the controlled release of active iodine, acting against a wide range of pathogens without inducing resistance. According to J. Meehan (2025), povidone-iodine solutions exhibit potent antimicrobial activity even at low concentrations and do not cause damage to animal cellular structures. At the same time, M. Karbownik-Lewińska *et al.* (2022) stress the importance of maintaining optimal dosages, as excessive iodine intake can affect the endocrine system through the activation of oxidative stress.

Ukrainian researchers have made a significant contribution to the study of iodine-containing preparations in veterinary medicine. D. Demyanenko *et al.* (2021) demonstrated the efficacy of the preparation *Kombiyod* in disinfecting poultry farm water systems without signs of toxicity and with the preservation of high bactericidal activity even at low concentrations. The advantages of such preparations lie in their broad-spectrum activity: they act against Gram-positive and Gram-negative bacteria, fungi, spores and viruses, without promoting pathogen habituation. As noted by I. Tlak Gajger *et al.* (2024), iodine-containing antiseptics effectively suppress *P. larvae* under laboratory conditions, indicating the feasibility of their application for hive sanitation. Given the proven effectiveness of *Kombiyod* in poultry and aquaculture, investigating its impact on the microflora and viability of honeybees represents a logical continuation of scientific inquiry in the field of safe antimicrobial technologies.

Thus, scientific interest in the study of the antiseptic properties of povidone-iodine and in determining its safe concentrations in apiculture is driven by the need to identify effective antibiotic alternatives capable not only of controlling pathogenic microflora but also of supporting the physiological state of bees. At

present, there is a lack of comprehensive studies that simultaneously evaluate the antimicrobial activity of preparations *in vitro* and their effects on bee viability *in vivo*. Therefore, the aim of this study was to investigate the effects of various concentrations of *Kombiyod* on pathogenic enterobacteria *in vitro* and on the viability of honeybees *in vivo*.

MATERIALS AND METHODS

A mixed microbial association isolated from hives where bees exhibited signs of dysbiosis, along with pathogenic enterobacterial cultures of *Klebsiella pneumoniae* and *Klebsiella (Enterobacter) aerogenes*, was used for the study. The cultures were obtained from diseased colonies originating from various apiaries in the north-western region of Ukraine. Pure cultures of enterobacteria (*Klebsiella pneumoniae*, *Klebsiella (Enterobacter) aerogenes*) were identified at the Research Laboratory of the Department of Veterinary Epidemiology, Faculty of Veterinary Medicine, Polissia University, and the State Institution “Zhytomyr Regional Laboratory Centre of the Ministry of Health of Ukraine”. The studied cultures are maintained at a temperature of 5-7°C at the Department of Veterinary Epidemiology, Faculty of Veterinary Medicine, Polissia University.

At the first stage of the study (*in vitro*), the effect of *Kombiyod* on pathogenic enterobacteria of bees was examined using the disc diffusion method in Petri dishes. The preparation was tested at the following concentrations: 0.01%, 0.05%, 0.1%, 0.2%, 0.3% and 0.5%. The inoculation of test cultures was carried out on Mueller-Hinton Agar (MHA). Distilled water was used for dilution. The disc diffusion test was conducted using sterile absorbent paper discs, which were impregnated with the respective concentrations of the preparation for 30 minutes. Following deep inoculation, the impregnated discs were placed on the surface of solidified MHA media containing the test cultures (Balázs *et al.*, 2021). The study was performed in five Petri dishes per culture, with observations made over three consecutive days.

At the second stage of the study (*in vivo*), the effect of *Kombiyod* on mortality dynamics and lifespan of worker bees of the Ukrainian steppe breed (summer generation) was assessed under laboratory conditions. The experiment was conducted on worker bees aged 10-15 days. The influence of the preparation on bee longevity was evaluated in entomological cages by daily monitoring of the insects' physiological state and recording the number of dead bees in each cage (Fig. 1). Each cage was populated with 40 bees, with a total of 10 cages (400 bees). Bees in the control groups were fed native 50% sugar syrup simultaneously with those in the experimental groups. The cages were kept in a thermostat at a temperature of +22 to +24°C and a relative humidity of 50-70%. The experiment continued until the death of the last bee in each cage.

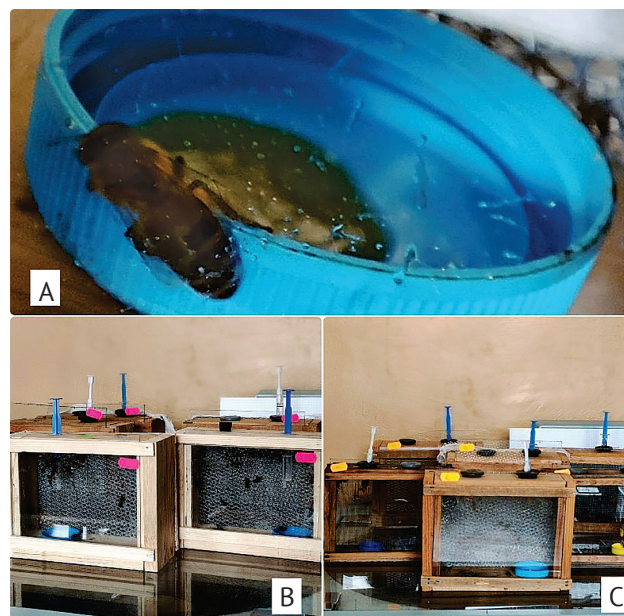


Figure 1. Determination of the effect of preparations on the lifespan of honeybees under laboratory conditions (in entomological cages)

Note: A – consumption of the *Kombiyod* solution diluted with sugar syrup by bees per os; B, C – appearance of entomological cages populated with experimental bees

Source: developed by the authors

The preliminary stage involved assessing the impact of “high” concentrations of *Kombiyod*: 2.05% (cage 3), 1.7% (cage 4), 1.35% (cage 5), and 1.0% (cage 6), each diluted with sugar syrup (1 part sugar: 1 part water). Cage 1 served as the control for testing high concentrations. During the experiment, these doses demonstrated high toxicity for bees; therefore, further observations were conducted using lower concentrations. The subsequent concentrations tested were 0.5% (cage 7), 0.1% (cage 8), 0.05% (cage 9), and 0.01% (cage 10), also diluted with sugar syrup (1 part sugar : 1 part water). Cage 2 served as the control for the assessment of low concentrations. The solutions were freshly prepared each day and administered in a volume of 4-5 cm³ per feeding into the feeders of each cage three times daily. The experimental studies involving live honeybees (*Apis mellifera*) were conducted in accordance with the ethical principles of animal research. All procedures were carried out with minimal stress and harm to the bees, in compliance with the Law of Ukraine No. 3447-IV (2006) and international standards and recommendations, including those of the OECD (n.d.).

RESULTS AND DISCUSSION

Honeybees (*Apis mellifera*) are highly sensitive to pathogenic bacteria that can significantly affect their viability and productivity (Motta & Moran, 2024). The present *in vitro* studies indicate the antimicrobial activity of the preparation *Kombiyod* against enterobacteria and

demonstrate its potential for protecting bees from bacterial infections in apiculture. The effect of *Kombiyod* on enterobacterial cultures isolated from bees, specifically *Klebsiella pneumoniae* and *Klebsiella (Enterobacter) aerogenes*, as well as on a mixed microbial association, was examined using the disc diffusion method (DDM) on Mueller-Hinton Agar (MHA). The aqueous solutions of *Kombiyod* were tested at concentrations of 0.01%, 0.05%, 0.1%, 0.2%, 0.3%, and 0.5%. Visual changes observed at

different concentrations and the manifestation of the preparation's activity on the tested bacterial cultures are presented in Figures 2 and 3. As can be seen from the results displayed in Figures 2 and 3, no bactericidal effect of the preparation was detected against the tested bacterial cultures. A negative bactericidal effect was recorded when the preparation diluted with distilled water was applied at the studied concentrations, both on the first and the third day of *in vitro* observations.

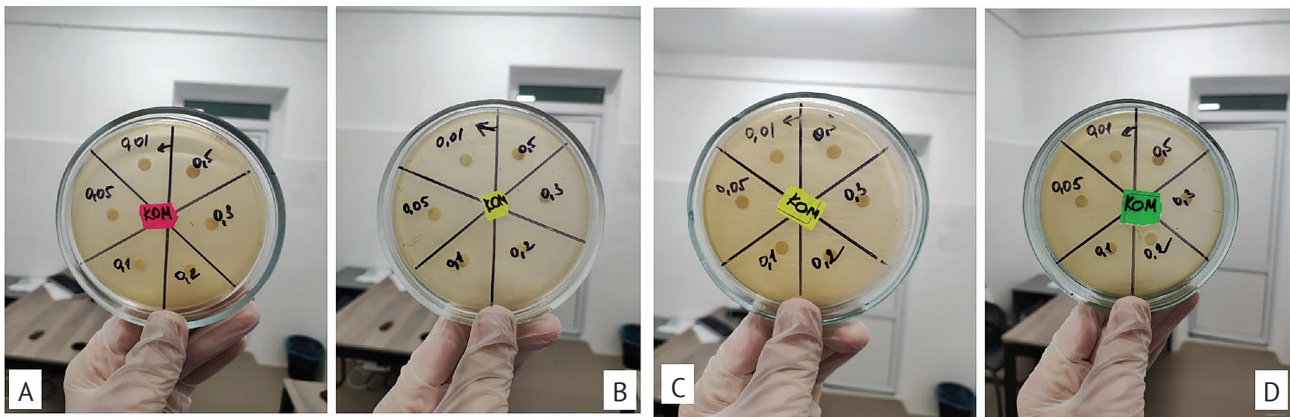


Figure 2. Visual changes observed during bacteriological studies of the preparation's activity on the tested bacterial cultures

Note: medium – Mueller-Hinton Agar (MHA); tested concentrations: 0.01%, 0.05%, 0.1%, 0.2%, 0.3%, 0.5%; A – *Klebsiella pneumoniae* (1 day); B – *Klebsiella (Enterobacter) aerogenes* (1 day); C, D – mixed microbial association (3 days)
Source: developed by the authors

At the same time, a bacteriostatic effect of *Kombiyod* was recorded on the first day of the experiment at concentrations of 0.1%, 0.2%, 0.3%, and 0.5%, where growth inhibition of the mixed bacterial culture was observed, indicating the development of bacteriostasis in the pathogenic microorganisms (Fig. 3). These concentrations of the preparation affect pathogenic microorganisms by altering their metabolism, energy exchange, and cellular structure. Such pathogens are no longer capable of exhibiting a high degree of

pathogenicity within the host organism; however, they may persist latently and be excreted into the external environment. In this state, the infected organism becomes a bacterial carrier, and such bee colonies continue to serve as a source of infection within the apiary. Therefore, it can be assumed that the treatment of dysbiotic conditions in bees from an affected apiary, following a course of *Kombiyod*, should be continued with the administration of probiotics until complete recovery is achieved.

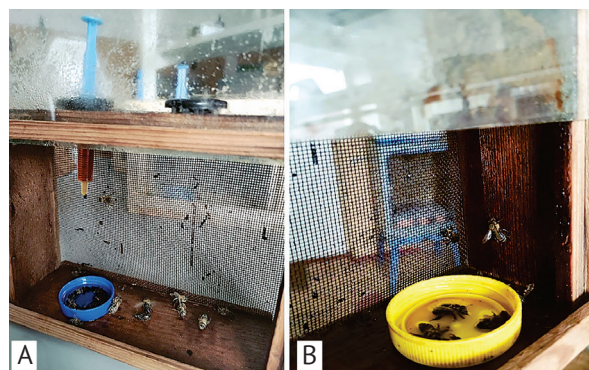


Figure 3. Visual changes observed during bacteriological studies of *Kombiyod* activity against the mixed bacterial culture on Mueller-Hinton Agar (MHA) on the first day of the experiment

Note: concentrations – 0.01%, 0.05%, 0.1%, 0.2%, 0.3%, 0.5%
Source: developed by the authors

Thus, *Kombiyod* at concentrations of 0.1%, 0.2%, 0.3%, and 0.5% exhibits a bacteriostatic effect against the mixed bacterial culture isolated from hives *in vitro*, where bees showed clinical signs of dysbiosis. Therefore, the preparation represents an environmentally safe therapeutic agent of potential interest for apiculture. The active substance of the preparation is povidone-iodine (polyvinylpyrrolidone-iodine complex) – an iodine-containing polymer compound characterised by pronounced bactericidal properties combined with lower toxicity compared to preparations containing free iodine (Boisson *et al.*, 2019; Makhayeva *et al.*, 2023). This effect is due to the gradual release of free iodine, which binds to proteins and penetrates through cellular membranes (Eggers, 2019). In the present *in vitro* experiment, at low concentrations (0.01-0.05%), the bacterial test cultures multiplied faster than free iodine was released during the dissociation of povidone-iodine, which explains the absence of both bactericidal and bacteriostatic effects.

At the same time, *Kombiyod* at concentrations of 0.1%, 0.2%, 0.3%, and 0.5% exhibited a bacteriostatic effect against the mixed bacterial culture isolated from hives *in vitro*, where bees presented clinical manifestations of dysbiosis, confirming its potential as an environmentally safe therapeutic preparation. Subsequently, the effect of higher concentrations (1.0%, 1.35%, 1.7%, and 2.05%) of *Kombiyod* on the lifespan dynamics of worker bees of the summer generation was studied in cage experiments under laboratory conditions. The results of the influence of high concentrations of the preparation on caged bees are presented in Table 1. According to the data in Table 1, bees in the control group, which received native sugar syrup, showed mortality as follows: on day 1 – 2 bees (5%), on day 6 – 4 bees (10%), and on day 12 – 7 bees (17.5%). In the subsequent days of the experiment, bees in the control group continued to die daily, with all individuals perishing between the 13th and 17th days of the experiment. Therefore, bees in the first control group survived for a total of 17 days.

Table 1. Dynamics of bee mortality in cages at different concentrations of *Kombiyod* in sugar syrup

Day of experiment	Number of dead bees									
	Control group (1)		Group 3 2.05% <i>Kombiyod</i> in sugar syrup		Group 4 1.7% <i>Kombiyod</i> in sugar syrup		Group 5 1.35% <i>Kombiyod</i> in sugar syrup		Group 6 1.0% <i>Kombiyod</i> in sugar syrup	
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
1	2	5	0	0	0	0	0	0	0	0
2	0	0	5	12.5	3	7.5	0	0	2	5
3	0	0	2	5	5	12.5	1	2.5	8	20
4	0	0	0	0	0	0	0	0	3	7.5
5	0	0	5	12.5	5	12.5	4	10	9	22.5
6	4	10	0	0	1	2.5	1	2.5	8	20
7	0	0	13	32.5	9	22.5	3	7.5	3	7.5
8	0	0	5	12.5	3	7.5	1	2.5	0	0
9	0	0	2	5	4	10	4	10	2	5
10	0	0	2	5	6	15	5	12.5	5	12.5
11	0	0	6	15	1	2.5	0	0		
12	7	17.5			3	7.5	6	15		
13	2	5					15	37.5		
14	8	20								
15	11	27.5								
16	4	10								
17	2	5								
Total	40	100	40	100	40	100	40	100	40	100

Note: concentrations – 1.0%, 1.35%, 1.7%, 2.05%

Source: developed by the authors

Studies of bee mortality dynamics at high concentrations of the preparation *Kombiyod* demonstrated that insect mortality was higher compared with the first control group. At concentrations of 2.05% (Fig. 4A) and 1.7%, the maximum lifespan of bees was 11-12 days, accompanied by diarrhoea and subsequent death of the individuals. The highest mortality rate was

recorded on the 7th day of the experiment. It is likely that the gradual accumulation of the preparation in the bodies of experimental bees reached critical levels for most individuals during this period. A reduction in concentration to 1.35% did not alter the overall trend in mortality dynamics (Fig. 4B). When *Kombiyod* was administered at a concentration of 1%, a reduction

in bee lifespan and a high mortality rate within 3-5 days were observed. This concentration is likely toxic to the experimental insects (cage 6). During observation of the physiological condition of bees in this cage, clustering behaviour was noted, with bees forming a tight cluster on the wall of the cage starting from the third day of the experiment, indicating hyperthermia –

a sign of intoxication. In some individuals from this cage, diarrhoea was also observed, visible on the mesh surface of the cage, further confirming the presence of intoxication (Fig. 5A). In contrast, no signs of hyperthermia or diarrhoea were observed in bees from the control cage; the bees remained active and consumed all the syrup provided (Fig. 5B).

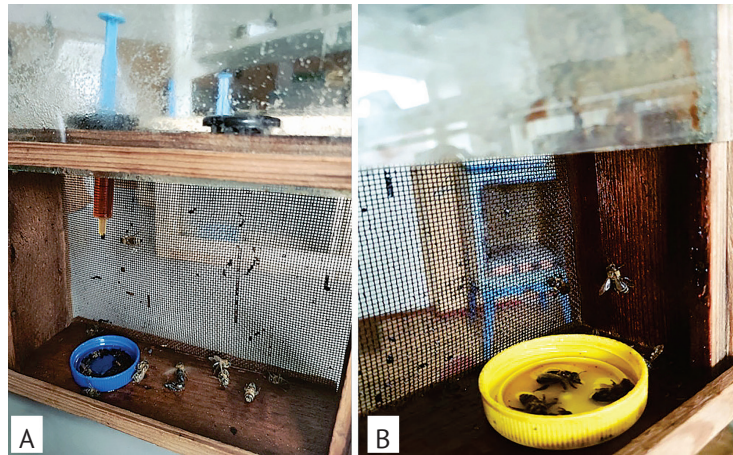


Figure 4. Physiological condition of experimental insects from cages 3 and 5

Note: A – Appearance of bees fed with 2.05% Kombiyod solution diluted with sugar syrup (cage 3, day 7 of the experiment); B – Appearance of experimental bees fed with 1.35% Kombiyod solution diluted with sugar syrup (cage 5, day 7 of the experiment)

Source: developed by the authors



Figure 5. Physiological condition of insects from the experimental and control groups

Note: A – Clustering of experimental bees following administration of a 1% Kombiyod solution diluted with sugar syrup (cage 6, day 3 of the experiment); B – Appearance of bees from the control group (day 3 of the experiment)

Source: developed by the authors

Thus, the consumption of sugar syrup containing *Kombiyod* at concentrations ranging from 1% to 2.05% proved to be acutely toxic, causing symptoms of toxicosis and bee mortality by days 10-12. The subsequent stage of experimental research focused on analysing the effects of lower *Kombiyod* concentrations (0.01%, 0.05%, 0.1%, and 0.5%) on the lifespan of summer-generation worker bees under laboratory cage conditions. It was established that the use of low-concentration *Kombiyod* solutions resulted in an extension of bee

lifespan compared with administration of the preparation at higher concentrations. Moreover, the viability of worker bees exposed to low concentrations of *Kombiyod* was longer (at 0.1% and 0.05%) or comparable (at 0.01%) to that of insects from the second control group (Table 2). The physiological state of bees in entomological cages 8 and 9 was characterised by pronounced activity: constant buzzing, absence of anorexia and thirst, rapid movement of bees along the cage walls, and the absence of diarrhoea or depression (Fig. 6).

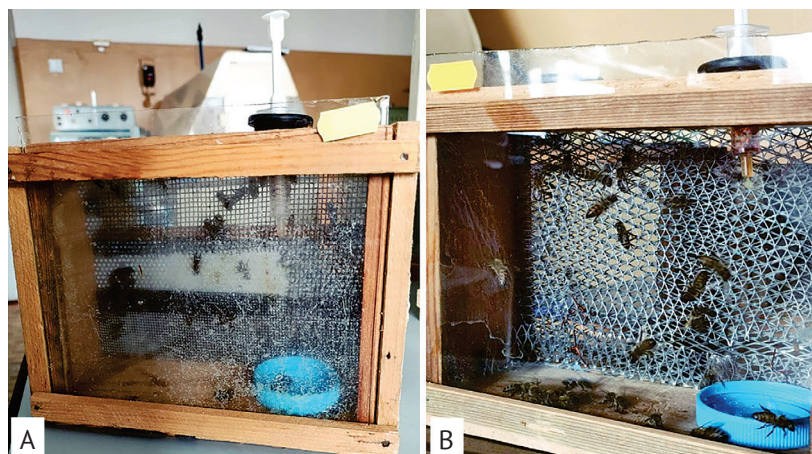


Figure 6. Physiological condition of insects from experimental cages 8 and 9

Note: A – Appearance of experimental bees fed with 0.1% Kombiyod solution diluted with sugar syrup (cage 8, day 10 of the experiment); B – Appearance of experimental bees fed with 0.05% Kombiyod solution diluted with sugar syrup (cage 9, day 10 of the experiment)

Source: developed by the authors

Table 2. Dynamics of bee mortality in cages with Kombiyod diluted in sugar syrup (0.01%, 0.05%, 0.1%, 0.5%) under laboratory conditions

Day of experiment	Number of dead bees									
	Control group (2)		Group 7		Group 8		Group 9		Group 10	
	<i>n</i>	%	 0.5% Kombiyod in sugar syrup		 0.1% Kombiyod in sugar syrup		 0.05% Kombiyod in sugar syrup		 0.01% Kombiyod in sugar syrup	
1	0	0	0	0	0	0	0	0	0	0
2	0	0	2	5	2	5	0	0	0	0
3	1	2.5	5	12.5	4	10	2	5	1	2.5
4	1	2.5	1	2.5	1	2.5	0	0	0	0
5	0	0	3	7.5	2	5	0	0	0	0
6	0	0	4	10	2	5	2	5	2	5
7	3	7.5	9	22.5	2	5	1	2.5	1	2.5
8	6	15	8	20	2	5	1	2.5	8	20
9	5	12.5	8	20	2	5	1	2.5	4	10
10	10	25			2	5	1	2.5	7	17.5
11	8	20			7	17.5	4	10	10	25
12	3	7.5			3	7.5	11	27.5	7	17.5
13	3	7.5			2	5	4	10		
14					2	5	6	15		
15					2	5	4	10		
16					2	5	2	5		
17					3	7.5	1	2.5		
18					2	5				
Total	40	100	40	100	40	100	40	100	40	100

Source: developed by the authors

Changes in the trends of bee mortality dynamics and lifespan may be associated with the kinetics of iodine release from the complex compound polyvinylpyrrolidone-iodine (Meehan, 2025). From a chemical perspective, the release dynamics of iodine in a 0.1% solution of the preparation reach an equilibrium state, meaning that a stable ratio between chemically bound and free iodine is maintained. Disruption of this

equilibrium occurs depending on the depletion of one of the compound's components. The release of iodine therefore proceeds gradually, as the available free iodine is consumed (Turganbay *et al.*, 2025). Consequently, no abrupt imbalance in concentration occurs, which creates favourable conditions for the preparation's physiological effect on the organism of experimental bees under laboratory conditions.

The optimal disinfectant concentration providing the longest lifespan and best physiological condition of bees was determined to be 0.1%, as confirmed by the obtained experimental data (Fig. 6A). Thus, the most “favourable” concentrations for the insects were 0.1% and 0.05%, at which the preparation exhibited a stimulating effect, improving the viability of experimental bees under laboratory conditions. The lifespan of bees in these cages was maximal, lasting 17-18 days. Further reduction of the solution concentration led to mortality

and lifespan indicators approaching those of the control group, suggesting a weak physiological impact of the preparation on the experimental insects. Conversely, the use of higher concentrations ranging from 0.5% to 2.05% resulted in rapid death due to the toxic effects of the preparation. A relatively high concentration of 0.5% at this stage of the experiment demonstrated a clear tendency towards toxicity: bees in group seven survived for only nine days – four days less than the bees in the control group (Fig. 7).



Figure 7. Physiological condition of insects from experimental cages 7 and 10

Note: A – Appearance of experimental bees fed with 0.5% *Kombiyod* solution diluted with sugar syrup (cage 7, day 7 of the experiment); B – Appearance of experimental bees fed with 0.01% *Kombiyod* solution diluted with sugar syrup (cage 10, day 8 of the experiment)

Source: developed by the authors

The active substance of *Kombiyod* is povidone-iodine (polyvinylpyrrolidone-iodine complex), an iodine-containing polymer compound possessing bactericidal properties while exhibiting lower toxicity compared with free iodine formulations. This is attributed to the controlled release of free iodine, which binds to proteins and penetrates cellular membranes. The molecular structure of the polymer forms steric barriers that restrict substance transport through biological membranes, thereby reducing systemic toxicity (Verwold *et al.*, 2021). The toxicity of povidone-iodine is likely associated with several mechanisms of action: poisoning by free iodine, leading to inactivation or degradation of structural and enzymatic proteins, disruption of protein and nucleic acid synthesis, impairment of excretory system function, induction of epithelial cell apoptosis, and disturbances in the intestinal microbiota balance of bees (Lepelletier *et al.*, 2020). On a molecular level, toxicity involves oxidation of sulfhydryl groups in enzymes and structural proteins by free iodine, resulting in loss of their functional activity (Shi *et al.*, 2024). The inhibition of macromolecular synthesis is related to oxidative stress induction and nucleic acid damage (Karbownik-Lewińska *et al.*, 2022). Epithelial toxicity manifests through activation of the

caspase cascade (Wang *et al.*, 2022), while microbiome disturbances arise due to the non-selective antiseptic action of the compound (Chen *et al.*, 2024). The bactericidal activity of *Kombiyod* ensures the prevention of bacterial, helminthic, viral, and protozoal infections. In addition, its application contributes to the correction of iodine deficiency in bees, an important factor for maintaining metabolic homeostasis (Morthorst *et al.*, 2023). The obtained results suggest that the preparation also exhibits immunomodulatory properties, likely realised through two interrelated mechanisms: enhancement of bacterial toxin neutralisation followed by the induction of anatoxin formation (Yu *et al.*, 2022).

Modern research into infectious diseases of honeybees demonstrates an integration of microbiological, toxicological, and immunological approaches to assessing colony health. G. Peña-Chora *et al.* (2023) emphasise that American and European foulbroods remain the most widespread bacterial infections globally, while the absence of safe antimicrobial agents limits the possibilities for effective prevention. These conclusions confirm the relevance of exploring antiseptic preparations, particularly those based on iodine-containing compounds. B.A. Daisley *et al.* (2023) demonstrated that the development of bacterial infections

in bees is closely associated with intestinal dysbiosis, which reduces the colonisation resistance of the organism. S.M. Killam *et al.* (2024) argue that maintaining a stable microbiota is key to sustaining colony health and therefore propose microbial therapeutics as alternatives to antibiotics. The obtained antimicrobial test results confirm the potential for using preparations with selective bacteriostatic effects that inhibit pathogenic microorganisms without destroying the natural microflora. A similar perspective is expressed by A. Smriti Rana *et al.* (2024), who highlight the importance of combining probiotics with antiseptic agents to achieve long-term remission in cases of bee dysbiosis.

N.S. DesJardins *et al.* (2023) reported that sublethal doses of pesticides can cause cognitive impairments in bees, including reduced learning and orientation abilities. The absence of such effects in laboratory observations when using low concentrations of the iodine-containing preparation indicates its relative safety. R. Hussain *et al.* (2023) proposed silver and zinc nanoparticles as antibiotic alternatives but noted the risk of metal accumulation and toxicity with prolonged exposure. Z.S. Lamas *et al.* (2024) stressed that mass colony losses result not only from infections but also from the combined influence of environmental stressors. A. Brhich *et al.* (2025) similarly noted the synergistic interaction between pesticides and bacterial agents, which increases the risk of colony collapse. In this context, the toxic effect of high concentrations of iodine-containing compounds may be regarded as a model for studying the combined impact of chemical stress on bee physiology. Meanwhile, S. Nekoei *et al.* (2023), in a systematic review, questioned the efficacy of chemical disinfectants and recommended focusing on biological approaches. However, the present experimental data demonstrate that, within the therapeutic concentration range, the antiseptic can be both safe and effective.

Several researchers adopt contrasting positions. L. Silva Morais *et al.* (2023) consider genetic selection of resistant bee lines the primary means of preventing diseases, while M.A. Aziz and S. Alam (2024) suggest prioritising the enhancement of natural resistance rather than relying on chemical agents. Although these approaches possess undeniable scientific value, they do not provide a rapid reduction in pathogenic load under practical apiary conditions. Experimental data on the dose-dependent activity of the iodine-containing preparation confirm the feasibility of a combined approach that integrates antiseptic action with the maintenance of physiological balance in the bee organism. M. Zhyla *et al.* (2024) reported that *Kombiyod* in low doses improves morphofunctional parameters in

poultry, indicating a potentially universal mechanism of polymeric iodine action. These findings support the hypothesis that the preparation may exert immunomodulatory properties and enhance resistance to bacterial infections across various animal species.

The synthesis of literature data allows the conclusion that polymeric iodine-based antiseptics can combine bactericidal, anti-inflammatory, and immunocorrective properties. The demonstrated effectiveness of *Kombiyod* at low concentrations confirms its potential for preventive use in apiculture as a safe alternative to antibiotics in the control of bacterial infections.

CONCLUSIONS

Bacteriostatic effects were established when *Kombiyod*, diluted with distilled water, was applied *in vitro* at concentrations of 0.1%, 0.2%, 0.3%, and 0.5% against a mixed bacterial culture isolated from hives where bees exhibited clinical signs of dysbiosis. High concentrations of *Kombiyod* ranging from 0.5% to 2.05%, when administered to bees with sugar syrup, caused acute toxicosis, with the highest mortality rate observed on the seventh day. The lifespan of bees in these groups was 11-12 days. Such concentrations of *Kombiyod* are classified as toxic and unsuitable for use in the presence of bees. It was determined that the most acceptable concentrations for bees are 0.05% and 0.1%. The administration of the preparation in these doses with sugar syrup exhibited a stimulating effect, resulting in the maximum mean lifespan of 17-18 days – 4-5 days (approximately 25%) longer than that of the control group – demonstrating a significant improvement in bee viability. For the prevention and treatment of infectious diseases in bees, it is recommended to administer a 0.1% solution of *Kombiyod* in 50% sugar syrup, or to apply a 0.2% aqueous solution for spraying bees and combs as stimulating doses of the preparation. Further experimental studies are planned to assess the therapeutic efficacy of 0.1% and 0.2% *Kombiyod* solutions in apiaries affected by infectious bee diseases.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

- [1] Alburaki, M., Abban, S.K., Evans, J.D., & Chen, Y.P. (2024). Occurrence and distribution of two bacterial brood diseases (American and European foulbrood) in US honey bee colonies and resistance to antibiotics from 2015 to 2022. *Journal of Apicultural Research*, 63(4), 701-710. doi: [10.1080/00218839.2024.2329854](https://doi.org/10.1080/00218839.2024.2329854).

- [2] Aziz, M.A., & Alam, S. (2024). Diseases of honeybee (*Apis mellifera*). *IntechOpen*. doi:10.5772/intechopen.1003947.
- [3] Balázs, V.L., Nagy-Radványi, L., Filep, R., Kerekes, E., Kocsis, B., Kocsis, M., & Farkas, Á. (2021). In vitro antibacterial and antibiofilm activity of Hungarian honeys against respiratory tract bacteria. *Foods*, 10(7), article number 1632. doi: 10.3390/foods10071632.
- [4] Boisson, M., et al. (2019). Multicentre, open-label, randomised, controlled clinical trial comparing 2% chlorhexidine-70% isopropanol and 5% povidone iodine-69% ethanol for skin antisepsis in reducing surgical-site infection after cardiac surgery: The CLEAN 2 study protocol. *BMJ Open*, 9(6), article number 026929. doi: 10.1136/bmjopen-2018-026929.
- [5] Brhich, A., Hachimi, T., Chatoui, H., Ait Sidi Brahim, M., Hnini, R., Chatoui, R., Merzouki, H., & Merzouki, M. (2025). Evaluation of pesticide effects on honeybee health and colony collapse: Findings from a beekeeper survey in the Beni Mellal-Khenifra Region, Morocco. *Journal of Environmental & Earth Sciences*, 7(2), 89-100. doi: 10.30564/jees.v7i2.7645.
- [6] Chen, Z., Chen, S., Huang, Y., Qin, Z., Shi, F., & Lin, L. (2024). Effects of enrofloxacin and povidone-iodine on immunity, intestinal microbes, and transcription pathways of grass carp. *Intestinal Microbes, and Transcription Pathways of Grass Carp*. doi: 10.2139/ssrn.4648779.
- [7] Daisley, B.A., Pitek, A.P., Mallory, E., Chernyshova, A.M., Allen-Vercoe, E., Reid, G., & Thompson, G.J. (2023). Disentangling the microbial ecological factors impacting honey bee susceptibility to *Paenibacillus* larvae infection. *Trends in Microbiology*, 31(5), 521-534. doi: 10.1016/j.tim.2022.11.012.
- [8] Damico, M.E., Beasley, B., Greenstein, D., & Raymann, K. (2025). Testing the effectiveness of a commercially sold probiotic on restoring the gut microbiota of honey bees: A field study. *Probiotics and Antimicrobial Proteins*, 17, 991-1000. doi: 10.1007/s12602-023-10203-1.
- [9] Demyanenko, D.V., Berezovsky, A.V., Vashchuk, Y.V., Fotina, T.I., & Nazarenko, S.M. (2021). Efficiency of the "Combiiod" means for disinfection of water supply system in the conditions of a poultry factory. *Scientific and Technical Bulletin of State Scientific Research Control Institute of Veterinary Medical Products and Fodder Additives and Institute of Animal Biology*, 22(2), 118-123. doi: 10.36359/scivp.2021-22-2.13.
- [10] DesJardins, N.S., Harrison, J.F., & Smith, B.H. (2023). The effects of anthropogenic toxins on honey bee learning: Research trends and significance. *Apidologie*, 54, article number 59. doi: 10.1007/s13592-023-01040-w.
- [11] Dinata, R., et al. (2023). Pharmacological and therapeutic potential of honey bee antimicrobial peptides: Bee antimicrobial peptides. *Indian Journal of Biochemistry and Biophysics (IJBB)*, 60(5), 365-384. doi: 10.56042/ijbb.v60i5.552.
- [12] Eggers, M. (2019). Infectious disease management and control with povidone iodine. *Infectious Diseases and Therapy*, 8, 581-593. doi: 10.1007/s40121-019-00260-x.
- [13] Galatyuk, O., Romanyshyna, T., Lakhman, A., Lysenko, O., & Shimanska, V. (2020). The pathogenic bee enterobacteria resistance to the experimental iodine-containing disinfectant "Jodis des no. 2". *Scientific Horizons*, 23(1), 71-78. doi: 10.33249/2663-2144-2020-86-1-71-78.
- [14] Hussain, R., Hasan, M., Iqbal, K.J., Zafar, A., Tariq, T., Saif, M.S., Gul Hassan, Sh., Shu, X., Caprioli, G., & Anjum, S.I. (2023). Nano-managing silver and zinc as bio-conservational approach against pathogens of the honey bee. *Journal of Biotechnology*, 365, 1-10. doi: 10.1016/j.jbiotec.2023.01.009.
- [15] Kampf, G. (2024). Povidone iodine. In *Antiseptic stewardship: Biocide resistance and clinical implications* (pp. 915-964). Cham: Springer International Publishing. doi: 10.1007/978-3-031-66074-0_18.
- [16] Karbownik-Lewińska, M., Stępnik, J., Iwan, P., & Lewiński, A. (2022). Iodine as a potential endocrine disruptor – a role of oxidative stress. *Endocrine*, 78, 219-240. doi: 10.1007/s12020-022-03107-7.
- [17] Killam, S.M., Daisley, B.A., Kleiber, M.L., Lacika, J.F., & Thompson, G.J. (2024). A case for microbial therapeutics to bolster colony health and performance of honey bees. *Frontiers in Bee Science*, 2, article number 1422265. doi: 10.3389/frbee.2024.1422265.
- [18] Lamas, Z.S., Chen, Y., & Evans, J.D. (2024). Case report: Emerging losses of managed honey bee colonies. *Biology*, 13(2), article number 117. doi: 10.3390/biology13020117.
- [19] Law of Ukraine No. 3447-IV "On Protection of Animals from Cruel Treatment". (2006, February). Retrieved from <https://zakon.rada.gov.ua/laws/show/3447-15#Text>.
- [20] Lepelletier, D., Maillard, J.Y., Pozzetto, B., & Simon, A. (2020). Povidone iodine: Properties, mechanisms of action, and role in infection control and *Staphylococcus aureus* decolonization. *Antimicrobial Agents and Chemotherapy*, 64(9), article number 10.1128/aac.00682-20. doi: 10.1128/aac.00682-20.
- [21] Makhayeva, D.N., Irmukhametova, G.S., & Khutoryanskiy, V.V. (2023). Advances in antimicrobial polymeric iodophors. *European Polymer Journal*, 201, article number 112573. doi: 10.1016/j.eurpolymj.2023.112573.
- [22] Meehan, J.P. (2025). Dilute povidone-iodine irrigation: the science of molecular iodine (I₂) kinetics and its antimicrobial activity. *JAAOS-Journal of the American Academy of Orthopaedic Surgeons*, 33(2), 65-73. doi: 10.5435/JAAOS-D-24-00471.

- [23] Mohammadian, B., Toopchi, B., Moharrami, M., & Rahimi, A. (2024). Determination of factors influencing honeybee colony collapse disorder phenomenon using analytic hierarchy process. *Honeybee Science Journal*, 14(27), 60-70. doi: [10.22034/hbsj.2024.364586.1153](https://doi.org/10.22034/hbsj.2024.364586.1153).
- [24] Morthorst, J.E., Holbech, H., De Croz , N., Matthiessen, P., & LeBlanc, G.A. (2023). Thyroid-like hormone signaling in invertebrates and its potential role in initial screening of thyroid hormone system disrupting chemicals. *Integrated Environmental Assessment and Management*, 19(1), 63-82. doi: [10.1002/ieam.4632](https://doi.org/10.1002/ieam.4632).
- [25] Motta, E.V., & Moran, N.A. (2024). The honeybee microbiota and its impact on health and disease. *Nature Reviews Microbiology*, 22, 122-137. doi: [10.1038/s41579-023-00990-3](https://doi.org/10.1038/s41579-023-00990-3).
- [26] Nekoei, S., Rezvan, M., Khamesipour, F., Mayack, C., Molento, M.B., & Revainera, P.D. (2023). A systematic review of honey bee (*Apis mellifera*, Linnaeus, 1758) infections and available treatment options. *Veterinary Medicine and Science*, 9(4), 1848-1860. doi: [10.1002/vms3.1194](https://doi.org/10.1002/vms3.1194).
- [27] OECD. (n.d.). *Guidelines for the testing of chemicals*. Retrieved from <https://www.oecd.org/en/topics/sub-issues/testing-of-chemicals/test-guidelines.html>.
- [28] Pe a-Chora, G., Toledo-Hern andez, E., Sotelo-Leyva, C., Damian-Blanco, P., Villanueva-Flores, A.G., Alvarez-Fitz, P., Palem n-Alberto, F., & Ortega-Acosta, S.A. (2023). Presence and distribution of pests and diseases of *Apis mellifera* (Hymenoptera: Apidae) in Mexico: A review. *The European Zoological Journal*, 90(1), 224-236. doi: [10.1080/24750263.2023.2182920](https://doi.org/10.1080/24750263.2023.2182920).
- [29] Shi, F., Chen, Z., Yao, M., Huang, Y., Xiao, J., Ma, L., Li, L., & Qin, Z. (2024). Effects of glutaraldehyde and povidone-iodine on apoptosis of grass carp liver and hepatocytes. *Ecotoxicology and Environmental Safety*, 272, article number 116078. doi: [10.1016/j.ecoenv.2024.116078](https://doi.org/10.1016/j.ecoenv.2024.116078).
- [30] Silva Morais, L., et al. (2023). Africanized honeybee (*Apis mellifera*) semen freezing using Tris-based and Collins extenders. *Tropical Animal Health and Production*, 55, article number 329. doi: [10.1007/s11250-023-03762-6](https://doi.org/10.1007/s11250-023-03762-6).
- [31] Singh, G., & Rana, A. (2025). Honeybees and colony collapse disorder: Understanding key drivers and economic implications. *Proceedings of the Indian National Science Academy*, 91, 750-766. doi: [10.1007/s43538-025-00399-x](https://doi.org/10.1007/s43538-025-00399-x).
- [32] Smriti Rana, A., Singh, G., & Gupta, G. (2024). Prospects of probiotics in beekeeping: A review for sustainable approach to boost honeybee health. *Archives of Microbiology*, 206, article number 205. doi: [10.1007/s00203-024-03926-4](https://doi.org/10.1007/s00203-024-03926-4).
- [33] Tlak Gajger, I., Tomljanovi , Z., Mutinelli, F., Granato, A., & Vlaini , J. (2024). Effects of disinfectants on bacterium *Paenibacillus larvae* in laboratory conditions. *Insects*, 15(4), article number 268. doi: [10.3390/insects15040268](https://doi.org/10.3390/insects15040268).
- [34] Truong, A.T., Kang, J.E., Yoo, M.S., Nguyen, T.T., Youn, S.Y., Yoon, S.S., & Cho, Y.S. (2023). Probiotic candidates for controlling *Paenibacillus larvae*, a causative agent of American foulbrood disease in honey bee. *BMC Microbiology*, 23, article number 150. doi: [10.1186/s12866-023-02902-0](https://doi.org/10.1186/s12866-023-02902-0).
- [35] Turganbay, S., Sabitov, A., Askarova, D., Jumagaziyeva, A., Iskakbayeva, Z., Seisembekova, A., Bukeyeva, T., & Azembayev, A. (2025). Dextrin/polyvinyl alcohol/iodine complexes: Preparation, characterization, and antibacterial, cytotoxic activity. *Engineered Science*, 35, article number 1595. doi: [10.30919/es1595](https://doi.org/10.30919/es1595).
- [36] Verwold, C., Ortega-Hernandez, A., Murakami, J., Patterson-Fortin, L., Boutros, J., Smith, R., & Kimura, S.Y. (2021). New iodine-based electrochemical advanced oxidation system for water disinfection: Are disinfection by-products a concern? *Water Research*, 201, article number 117340. doi: [10.1016/j.watres.2021.117340](https://doi.org/10.1016/j.watres.2021.117340).
- [37] Wang, D., Huang, X., Lv, W., & Zhou, J. (2022). The toxicity and antibacterial effects of povidone-iodine irrigation in fracture surgery. *Orthopaedic Surgery*, 14(9), 2286-2297. doi: [10.1111/os.13422](https://doi.org/10.1111/os.13422).
- [38] Yakan, S., Akpınar, R., Geni el, M.,  elik, S., & Bozdeveci, A. (2025). Honey bee pathogen prevalence and interactions in the a rı province of T rkiye. *Sociobiology*, 72(3), article number 11603. doi: [10.13102/sociobiology.v72i3.11603](https://doi.org/10.13102/sociobiology.v72i3.11603).
- [39] Yu, X., Sun, N., Cheng, Y., & Yang, X. (2022). Povidone-iodine modulates the antioxidant capacity, immunity, and resistance to *Aeromonas hydrophila* of the Chinese mitten crab, *Eriocheir sinensis*. *Aquaculture International*, 30(6), 2953-2967. doi: [10.1007/s10499-022-00942-3](https://doi.org/10.1007/s10499-022-00942-3).
- [40] Zhyla, M., Pyatnychko, O., Kotsyumbas, H., Shkodyak, N., Stronskyi, Y., & Palonko, R. (2024). Evaluation morphofunctional state of broiler chickens' body under the application of various doses of Combiiod. *Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies. Series: Veterinary Sciences*, 26(113), 156-164. doi: [10.32718/nvlvet11324](https://doi.org/10.32718/nvlvet11324).

Експериментальні дослідження антимікробної активності препарату та його впливу на життєздатність медоносних бджіл

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Анотація. Епізоотія бактеріальних інфекцій у бджільництві, посилена явищем колапсу бджолиних сімей, вимагає розробки нових та безпечних профілактичних засобів. Через законодавчі обмеження застосування антибіотиків пріоритетним напрямком є дослідження антисептиків з імуномодулюючими властивостями. Метою роботи стала комплексна оцінка ефективності препарату «Комбійод», а саме – визначення антимікробної активності *in vitro* та встановлення оптимальних безпечних концентрацій для підвищення життєздатності бджіл *in vivo*. Дослідження включало диско-дифузійний метод з використанням чистих штамів ентеробактерій та змішаної культури мікрофлори від хворих бджіл *in vitro*, а також садковий експеримент з оцінки життєздатності та фізіологічного стану бджіл при додаванні препарату до цукрового сиропу (0,01-2,05 %) *in vivo* у лабораторних умовах. Встановлено бактеріостатичну ефективність препарату *in vitro* при концентраціях 0,1-0,5 %. Виявлено дозозалежний ефект у бджіл в умовах лабораторії: концентрації 0,5-2,0 5 % викликають гостру токсичність з піком смертності на 7 добу, тоді як оптимальні концентрації 0,05-0,1 % демонструють стимулюючу дію – максимальну тривалість життя 17-18 діб, підвищення життєздатності на 25 % відносно контролю та ймовірний імуномодулюючий ефект із корекцією йодного дефіциту. На підставі отриманих результатів рекомендовано 0,1 % розчин засобу в цукровому сиропі для перорального застосування та 0,2 % водний розчин для санації гнізда в присутності бджіл. Встановлено терапевтичний діапазон концентрацій для подальших досліджень. Отримані результати можуть бути основою для розробки нових схем профілактики бактеріозів бджіл на основі йодовмісних препаратів з урахуванням виявленої дозозалежності їхньої дії

Ключові слова: медоносні бджоли (*Apis mellifera*); повідон-йод; антимікробна активність; ентеробактерії; мікробіологічні дослідження; життєздатність бджіл; імуномодулюючий ефект
