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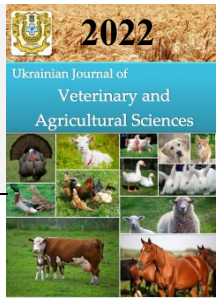

Study of the activity of sunflower honey against a mixed microbial association isolated from bees with signs of dyspepsia in laboratory conditions

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Contents

Abstract

Beekeeping is one of the essential branches of agriculture in Ukraine, the main activity of which is aimed at breeding bees and obtaining honey and other products beneficial for human health. Maintaining the physiological state of bee colonies at the proper level is one of the essential tasks of veterinary medicine doctors and beekeepers. Compliance with veterinary and sanitary requirements at bee farms is the basis of preventing infectious pathologies in bee colonies. Since the body of insects has a fast metabolism, any infectious pathology quickly acquires a massive manifestation. One indicative symptom of infectious damage to the "gut" of bees is dyspepsia. Diarrhea, anorexia, and loss of productivity are observed in bees in this condition. The market of means to prevent such pathological manifestations in beekeeping is relatively narrow. The healing and favorable properties of honey, manifested in pain-relieving, antimicrobial and anti-inflammatory effects, have been known since ancient times. Sunflower honey differs from other types in a wide range of components, particularly phytoncides, which inhibit the growth of pathogenic microorganisms. Therefore, the main goal of the experiment was a laboratory study of the effect of different concentrations of sunflower honey sieve on the mixed microbial association isolated from bees with signs of dyspepsia. The activity of sunflower honey in laboratory conditions was studied by the disco-diffusion method in Petri dishes on MPA medium (meat-peptone agar). To obtain syrup from sunflower honey, sunflower honey was diluted with distilled water in a ratio of 1:1, working solutions were prepared from sunflower honey syrup in dilutions of 1:2, 1:4, 1:10, 1:100. The data analysis indicates the bacteriostatic activity of the syrup from sunflower honey in all studied concentrations concerning the mixed microbial association. Moreover, the largest diameter of the growth inhibition zone of the studied microorganisms was noted when the syrup from sunflower honey was diluted in a ratio of 1:10 (19.2 ± 0.42 mm). In turn, the antagonistic effect of the syrup from sunflower honey was observed in all dilutions concerning the mixed microbial association, where the largest diameter of the antagonism was 19.6 ± 0.27 mm when the disc was impregnated with the native working solution. Thus, it is promising to accumulate and identify a pure culture antagonist concerning a mixed culture of bacteria isolated during bee diarrhea to further create a pharmacological agent for preventing infectious pathologies of bees in unhealthy apiaries.

Keywords: honey, bee dyspepsia, mixed microbial association, antagonistic action.

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1. Introduction

Scientists and practitioners have recorded fewer bee colonies worldwide since 2015 (Becsi et al., 2021; Qu et al., 2022). Non-observance of veterinary and sanitary conditions in apiaries is one of the etiological factors of dysbiosis. After all, the body of bees, like all insects in general, has rapid metabolic processes (Kovalchuk et al., 2019; Kovalskyi et al., 2021; Saranchuk et al., 2021). Therefore, diseases of infectious etiology almost immediately acquire a mass character, first in the hive and then in the entire apiary. Dyspepsia in bees is one of the symptoms of many contagious, including invasive, bee diseases (Becsi et al., 2021; Braglia et al., 2021). For example, with nosematosis in bees, intestinal dysfunction is detected, manifested by diarrhea, abdominal distension, and mass death of insects in the first months after wintering (Braglia et al., 2021; Galajda et al., 2021).

Moreover, this invasive pathology occurs when veterinary and sanitary measures regarding disinfection in the apiary are not observed. For the same reason, amoebiasis occurs – a disease of bee colonies with damage to the Malpighian vessels (Schäfer et al., 2022). Due to amebiasis, bees quickly die and lose the strength of families, leading to significant economic losses for beekeepers, primarily where beekeeping is aimed at selling honey and beekeeping prod-

ucts. That is why compliance with veterinary and sanitary regulations in apiaries is not only a norm but also warns against the occurrence of those mentioned above and other contagious diseases of bees.

The market for means for preventing bee dyspepsia pathology caused by infectious agents is relatively narrow. Since high concentrations of disinfectants can lead to toxic poisoning of bee colonies, antibiotic therapy is undesirable due to the transfer of active ingredients to beekeeping products. In addition, using unknown, for example, pro- or prebiotics can disrupt some systems' functioning in the bee body (Mustar & Ibrahim, 2022). Since ancient times, honey has been known to have healing properties, manifested in antimicrobial, anti-inflammatory, and antiseptic effects (Nemo & Bacha, 2021). However, in terms of composition and quality, there are many types of this product, which distinguishes its quality (Zhang et al., 2021). Such differences are due to the combination of the country of the honey producer, the plants from which the pollen was obtained, and the method of its production (Nemo & Bacha, 2021). For example, buckwheat honey can have a bactericidal effect on gram-positive and gram-negative microorganisms due to its low pH. Rapeseed honey has antibacterial activity against Proteus Vulgaris (Subrahmanyam et al., 2001; Mulu et al., 2017; Chirsanova et al., 2021), and forest honey has anti-inflammatory and analgesic effects (Sajtos et al., 2022). In turn, sunflower honey is characterized by its ability to be easily digested due to mono sugars and other nutrients (Chirsanova et al., 2021; Sajtos et al., 2022). Phytoncides of this type of honey suppress the growth of pathogenic microflora (Nemo et al., 2021). The antimicrobial activity of honey is of interest to practitioners and researchers, as reported by several workers (Nemo & Bacha, 2021; Zhang et al., 2021; Hossain et al., 2022; Mustar & Ibrahim, 2022; Sajtos et al., 2022), but most of these reports are pretty contradictory. Therefore, our experiment aimed to study the activity of sunflower honey against a mixed microbial association isolated from bees with signs of dyspepsia in laboratory conditions.

2. Materials and methods

Experimental studies were carried out by the disco-diffusion method (Balázs et al., 2021) in the conditions of the research laboratory of the Department of Microbiology, Pharmacology and Veterinary Epidemiology, Faculty of Veterinary Medicine of the Polissia National University of Zhytomyr in September 2022. The object for determining the antagonistic activity of honey served as a mixed microbial association isolated from bees with signs of dyspepsia. It is stored at a temperature of +8 ºC on MPA medium (meat-peptone agar) in Petri dishes and is reseeded by the deep method every 14 days. A troubled private apiary is located in the Khmelnytskyi region. To set up the experiment:

1. Syrup from sunflower honey (1) was prepared by diluting it with sterile distilled water in a ratio of 1:1.

2. Working solutions (2, 3, 4, 5) were prepared from syrup from sunflower honey (1) and distilled water in dilutions of 1:2, 1:4, 1:10, and 1:100, respectively.

The studied concentrations of syrup from sunflower honey were soaked in discs for 40 minutes, and they were placed on the agar surface of Petri dishes (n = 5) with 1 ml of inoculum of microorganisms (washed from the surface of a 24-hour mixed culture of the affected hive) previously introduced by a deep method. Cultivation continued at a temperature of +38 ºC for five days.

Processing of the results, namely, the diameters of the zones of growth retardation and antagonism of honey bacteria ((M ± m), mean square deviation (δ)), were calculated in the Statistica program and characterized according to the Student's test.

3. Results and discussion

Carrying out veterinary and sanitary measures is preventive for every infectious pathology in higher animals and bees. Therefore, special attention should be paid to preventive measures in bee farms. The antimicrobial activity of honey and its solutions is known (Chirsanova et al., 2021; Sajtos et al., 2022). After all, by taking such mixtures, the human body acquires a strong non-specific immunity (Carnwath et al., 2014; Mustar & Ibrahim, 2022). At the same time, “wild” bee colonies, consuming honey, form a high level of population resistance.

Pathologies associated with damage to the gastrointestinal tract of bees are often accompanied by dyspeptic syndrome, characterized by diarrhea and loss of strength of bee colonies. Such diseases of bees are most often caused by biological infectious agents and processes associated with their reproduction, not only in the body of insects but also in the hive with subsequent disruption of its microflora. Sunflower honey serves as a building material for the bee's body, as it contains a wide range of amino acids; also, the honey of this species is a source of energy due to the high concentration of monosaccharides (Chirsanova et al., 2021; Sajtos et al., 2022).

We established that all working concentrations of honey syrup showed bacteriostatic activity against the studied microbial association in laboratory conditions (Fig. 1-a).

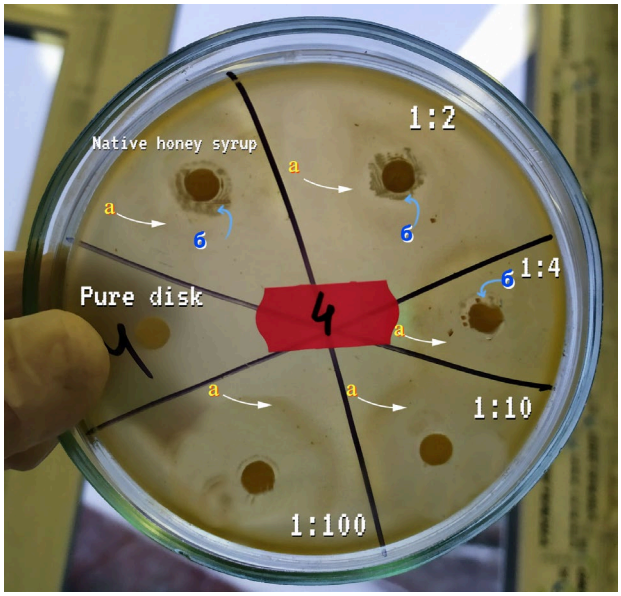

Fig. 1. The action of a syrup from sunflower honey on a mixed culture of bacteria isolated during bee diarrhea for one day of cultivation, MPA medium: a – a manifestation of bacteriostatic action; b – a manifestation of antagonistic action

At 24 hours, the interaction of honey bacteria and test cultures was characterized by the largest diameter of the

zone of inhibition of the growth of the investigated microorganisms under the influence of the syrup from sunflower honey dilution 1:10 at the level of 19.2 ± 0.42 mm, and the little indicator under the power of the native solution of the syrup from sunflower honey – 14.8 ± 0.42 mm (table 1). The minimum diameter of the bacteriostatic effect around the disc impregnated with the native syrup from sunflower honey solution is interpreted as the passive movement of bacteria and honey substances in the thickness of the nutrient agar. In addition, the components of honey are a nutrient medium for a mixed test culture, so part of the biofilm bacteria is destroyed under the influence of lysing substances of honey (Hossain et al., 2022; Yupanqui Mieles et al., 2022), while the vast majority of microorganisms multiply intensively, consuming monosaccharides (glucose) of syrup from sunflower honey (Sajtos et al., 2022).

The inhibitory effect of honey is characterized by the activity of H_2O_2, which is a component of various types of honey. Stores of metals (cations$^+$) from the cytoplasm of test cultures are probably transformed, as a result of catalysis of the latter, into hydroxyl free radicals, which causes the bacteriostatic effect (Chirsanova et al., 2021).

It should be noted that 1:2 and 1:100 syrup from sunflower honey dilutions caused the same bacteriostatic effect at 17.8 ± 0.55 mm and 16.2 ± 0.42 mm, respectively (table 1). Such results are explained by the ability of the components of the syrup from sunflower honey to permeate agar in a diluted state since sunflower honey is rich in carbohydrates, which prevents its diffusion into agar in a concentrated state.

Table 1
The activity of sunflower honey sieve against mixed bacterial culture isolated during bee diarrhea (n = 5)

Types of the interaction of microorganisms	The time of recording the results was hanging	Dilution of sunflower honey sieve				
		Negative	1:2	1:4	1:10	1:100
The bacteriostatic effect, mm (M ± m)	24 hrs	14.8 ± 0.42	17.8 ± 0.55*	19.2 ± 0.42***	24.4 ± 0.27**	16.2 ± 0.42*
Antagonistic action, mm (M ± m)	72 hrs	19,6 ± 0.27	16.2 ± 0.42*	12.8 ± 0.42**	-	-

Note: * – P < 0.05, ** – P < 0.01, *** – P < 0.001, respectively, according to parameters of the diameter of the action caused by the native honey sieve solution

Thus, an inversely proportional dependence of the bacteriostatic action on syrup from sunflower honey dilutions from 1:10 to the native and a directly proportional relationship of the antagonistic effect on syrup from sunflower honey solutions from 1:4 to the native was registered. The largest zone of the antagonism (19.6 ± 0.27 mm) was formed around the disc impregnated with the native solution of syrup from sunflower honey. At the 72nd hour, when the sieve was diluted 1:4, the growth of bacillary colonies with a size of 12.8 ± 0.42 mm was observed (Fig. 1-b). According to cultural characteristics, this microorganism probably belongs to saprophytic bacteria of the taxonomic group *Bacillus* spp. (Irkitova et al., 2018; Zulkhairi et al., 2021). Since bacteria of this genus contain polymyxin and definidine – substances that inhibit the reproduction of prokaryotes with different contents of murein in the cell wall (Kumar et al., 2012; Moore et al., 2013). A scientifically proven fact is the manifestation of the antagonism of *Bacillus* spp. against salmonella, pseudomonads, staphylococci, and Shigella (Irkitova et al., 2018).

The lack of growth of the sunflower mentioned above honey antagonist bacteria at high syrup from sunflower honey dilutions (1:10 and higher) is interpreted as their low concentration in working solutions and a decrease in the concentration of easily digestible energy resources of honey (Bodó et al., 2021).

4. Conclusions

1. The most pronounced bacteriostatic effect concerning the mixed microbial association isolated from bee dyspepsia was recorded when diluting syrup from sunflower honey 1:10 with a diameter of the zone of inhibition of biofilm growth 24.4 ± 0.27 mm.

2. The maximum antagonism of the microflora of honey was detected during the interaction of the native syrup from sunflower honey with the bacteria of the test culture at the 72nd hour of the experiment at the level of 19.6 ± 0.27 mm.

3. It is promising to isolate a pure antagonistic culture from sunflower honey and its subsequent identification for creating a pharmacological agent for the organization of preventive measures in dysfunctional apiaries for bee dyspepsia.

Conflict of interest

The authors declare that there is no conflict of interest.

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