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ANTIBACTERIAL EFFECT OF FLOWER EXTRACTS ON MICROORGANISMS ISOLATED FROM HONEYCOMBS WITH AFFECTED BEE BROOD

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Aim. To investigate the antibacterial properties of the flowers of melliferous plants on the cultures isolated from honeycombs affected by foulbrood. **Methods**. Microbiological, cultural-morphological, biochemical, electron-microscopic, statistical. **Results**. Antibacterial effect on the *Melissococcus pluton* 8.1 strain was demonstrated by the flower extracts of 14 plant species and that on *Bacillus*aspecies – by the flower extracts of 27 plant species blossoming in the early spring. **Conclusions**. To study bee colonies affected by foulbrood, it is possible to use the properties of such nectariferous plants as *Lamium album, Acer campestre, Prunus tomentosa, Allium cepa, Tagetes patula, Spiraea japonica, Achillea millefolium, Calluna vulgaris, Mentha piperita, Tilia cordata, Centaurea jacea, Lysimachia nummularia.* The results of the experiments on the culture of microorganisms, isolated from the honeycombs of the bee colonies affected by foulbrood, demonstrated that these plants could be effective for the prevention and treatment of bacterial bee diseases.

Key words: melliferous plant, bacterial bee diseases, microbiota of honeycombs with affected bee brood, antibacterial activity, *Bacillus, Melissococcus pluton.*

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INTRODUCTION

It is known that in case of some bacterial diseases, bee colonies are capable of "self-healing" and this process coincides with the start of massive blossoming of melliferous plants. Thus, this "self-healing" phenomenon may be related to medicinal and health-improving properties of melliferous flora. In addition to sucrose, fructose, and glucose, the nectar of flowers also contains dextrins, organic acids, mineral salts and enzymes, glycosides, essential oils, tannins, saponins, derivatives of phenol and flavones [1]. The available microelements are K, Ca, Mn, Fe, Mg, Cu, Zn, Co, Cr, Al, V, Se, Ni, Sr, Pb, B. It is recommended to place the beehives with bee colonies in the places with the variety of blossoming plants to ensure prevention and treatment of diseases [2, 3, 4]. nthe impact of plant extracts on the agents of bacterial, fungal and microsporidial diseases of bees is well known. Therefore, anti-ascospherosis properties of over 500 plant species, presented in Ukraine's flora, were investigated using the culture of *Ascosphaera apis* (*in vitro*) in the Laboratory of technological and special measures of preventing bee diseases, NSC "Institute of Beekeeping named after P.I. Prokopovych". Further on, the bees (*in vivo*), intentionally infected with spores of *Nosema apis*, were used to investigate anti-nosematosis properties of the extracts, obtained from the plants, which demonstrated anti-askospherosis effect. The results were used to select the plants, extracts of which hed complex anti-nosematosis-ascospherosis properties and which could

be used as a basis for ecologically safe plant preparations for the treatment of bees from nosematosis and ascospherosis [5, 6]. Many studies have been dedicated to the investigation of phytoncides of plants, in particular, garlic and onions, on the agents of bacterial diseases of bees. During thehstudy, conducted by a group of American scientists, garlic was used to extract the antibiotic substance – allicin, formed in the damaged clove with sulfur-containing aminoacid (+)-S-alyl-*l*-cysteine sulfoxide. It was demonstrated that allicin inhibited the growth of *Paenibacillus larvae* — the agent of American foulbrood, the areas of growth delay were 45–50 mm [2]. V.I. Polteev studied the antibacterial effect of pine phytoncides and recommended their use in treatment of infectious diseases of bees [7]. However, the data on the impact of flower extracts of melliferous plants on the agents of bacterial diseases of bees are still unavailable in scientific literature.

We havs previously determined that the flower extracts of 38 plant species showed antibacterial properties against the museum culture of *Bacillus subtilis* UKM B-901, the areas of growth delay for all the investigated extracts were 7–21 mm. The highest bactericide effect was recorded foy the flower extracts of onion (*Allium cepa*) – the diameter of the area of growth delay was 21 mm, treacle mustard (*Erýsimum cheiranthoídes*) – 17 mm, purple coneflower (*Ehinacea purpurea*) and tufted vetch (*Vicia cracca*) – 16 mm [8].

This study was aimed at investigating the antibacterial properties of flowers of melliferous plants regarding the cultures, isolated from honeycombs of bee brood, affected by foulbrood.

MATERIALS AND METHODS

The study was conductedein cooperation between the Laboratory of technological and special measures of preventing bee diseases, NSC "Institute of Beekeeping named after P.I. Prokopovych" and NSL of Microbiology and Immunology Issues of Biotechnology, NSC "Institute of Biology" of the Taras Shevchenko National University of Kyiv.

The objects of our study were 10 strains of bacteria, isolated from 10 samples of honeycombs with sealed bee brood, selected in May-August of 2012–2014 in Kyiv and Zhytomyr regions [9]. The cultures were kept at the temperature of +4 °C in the medium of meat-and-peptone agar (MPA) under the layer of paraffinic oil. The daily culture of microorganisms was used for the study.

The ethanol flower extracts of 153 plant species, collected in 2012–2015 in different regions of Ukraine, were used in thekstudy. The antibiotic activity of flower extracts was analyzed using the disk-diffusion method [10]. The ethanol flower extracts were prepared using the following method: 1 g of flowers was added to 2 ml of 90 % ethyl alcohol with subsequent careful homogenization. The obtained ethanol extracts were applied to paper discs with the diameter of 6 mm. Paper discs, treated with the mixture of physiological solution and alcohol (1:2), were used as the control. The experimental and control discs were dried in the laminar box at room temperature. The suspension of the daily culture of investigated microorganisms was prepared using the sterile physiological solution until obtaining the concentration of $1^{x}10^{9}$ CFU/ml. 0.1 ml of the prepared suspension was inoculated in a lawn on Petri dish with the Mueller-Hinton medium. The discs with the extracts were placed on the surface of the medium with inoculated microorganisms, cultivated for 24 h in the thermostat at 37 °C, with subsequent registration of the areas of microorganism growth delay.

The results of statistical processing were presented in the form of confidence intervals, built using Statistica program.

RESULTS AND DISCUSSION

The ethanol flower extracts of 125 plant species, including 11 garden plants, 44 – ornamental plants, 70 – wild plants were studied for the antibacterial activity of the ethanol flower extracts regarding the strain of *Melissococcus pluton* 8.1. It was demonstrated that only 14 of all the investigated extracts had antibacterial activity against *Melissococcus pluton* 8.1 – the agent of European foulbrood (Fig.1): one of garden plant, five of ornamental plants and eight of wild plants.

The highest activity was demonstrated by the following flower extracts: onion (17 mm), French marigold (15 mm), Japanese meadowsweet (15 mm), yarrow (15 mm).

The *Bacillus* sspecies, isolated from the honeycombs with infected bee brood [11], were used as test-cultures, because the representatives of this genus are frequent agents of bee diseases: American foulbrood (*Paenibacillus larvae* (*Bacillus larvae*)), European foulbrood (*Paenibacillus alvei* (*Bacillus alvei)*; *Brevibacillus laterosporus* (*Bacillus laterosporus = Bacillus orpheus)*; *Paenibacillus apiarius* (*Bacillus apiarius*);

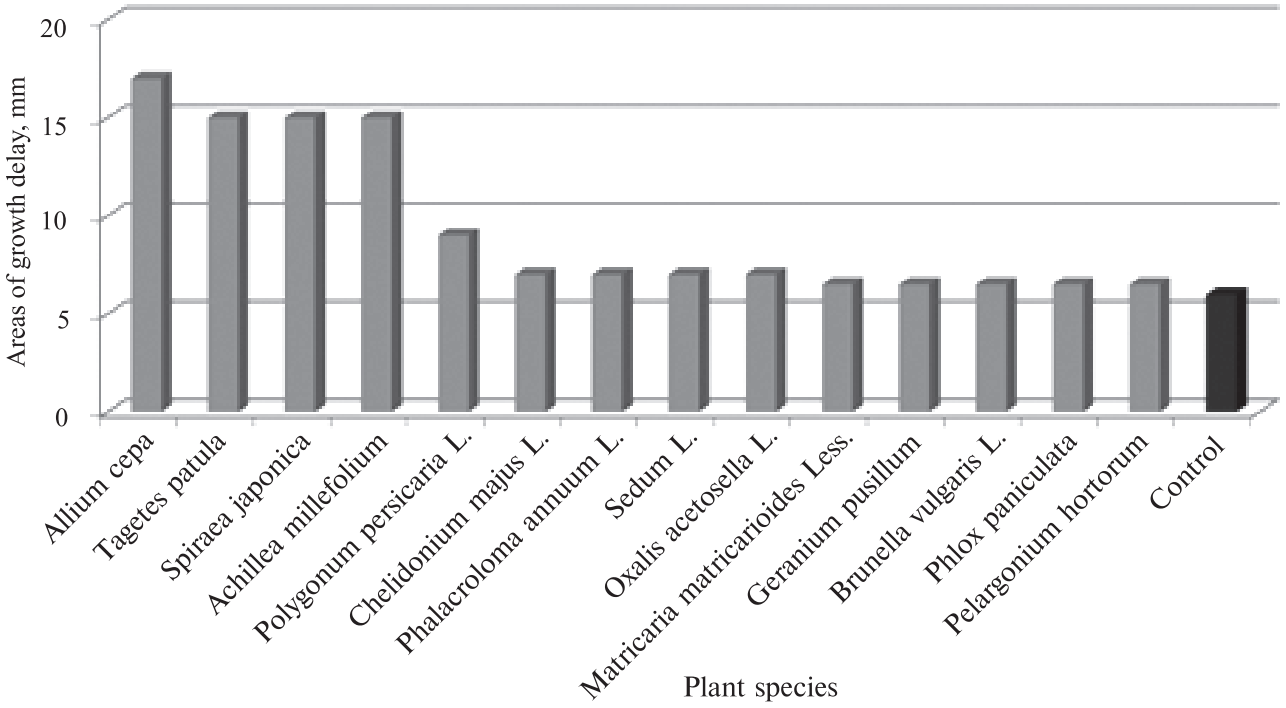

Fig. 1. Areas of growth delay for the ethanol flower extracts on the strain *Melissococcus pluton* 8.1

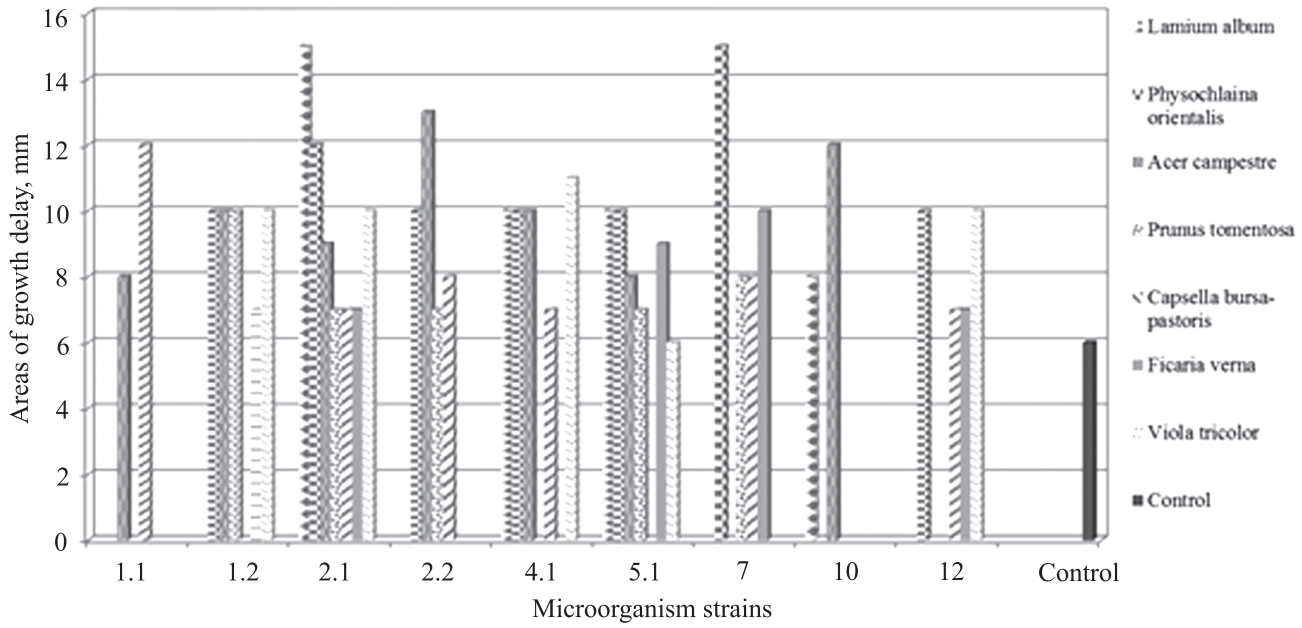

Fig. 2. Antibacterial activity of the ethanol flower extracts of *Acer campestre*, *Prunus tomentosa*, *Lamium album* L., *Physochlaina orientalis*, *Capsella bursa-pastoris* L., *Ficaria verna*, *Viola tricolor* on microorganisms, isolated from the honeycombs with bee brood affected by foulbrood

parafoulbrood (Bacillus paraalvei), powder-like brood (*Paenibacillus larvae pulvifaciens*) [12, 13].

The of ethanol flower extracts of 28 species of wild, ornamental plants, trees and bushes, collected in March-May 2015 in the city of Kyiv and Kyiv region, were used to study antibacterial activity against *Bacillus* species.

It was demonstrated that the extracts of 27 plant species revealed their antibacterial activity against the investigated microorganisms, the areas of growth delay were 7–15 mm. The highest activity against all investigated strains was demonstrated by the flower extracts of field maple (*Acer campestre*) (89 %),

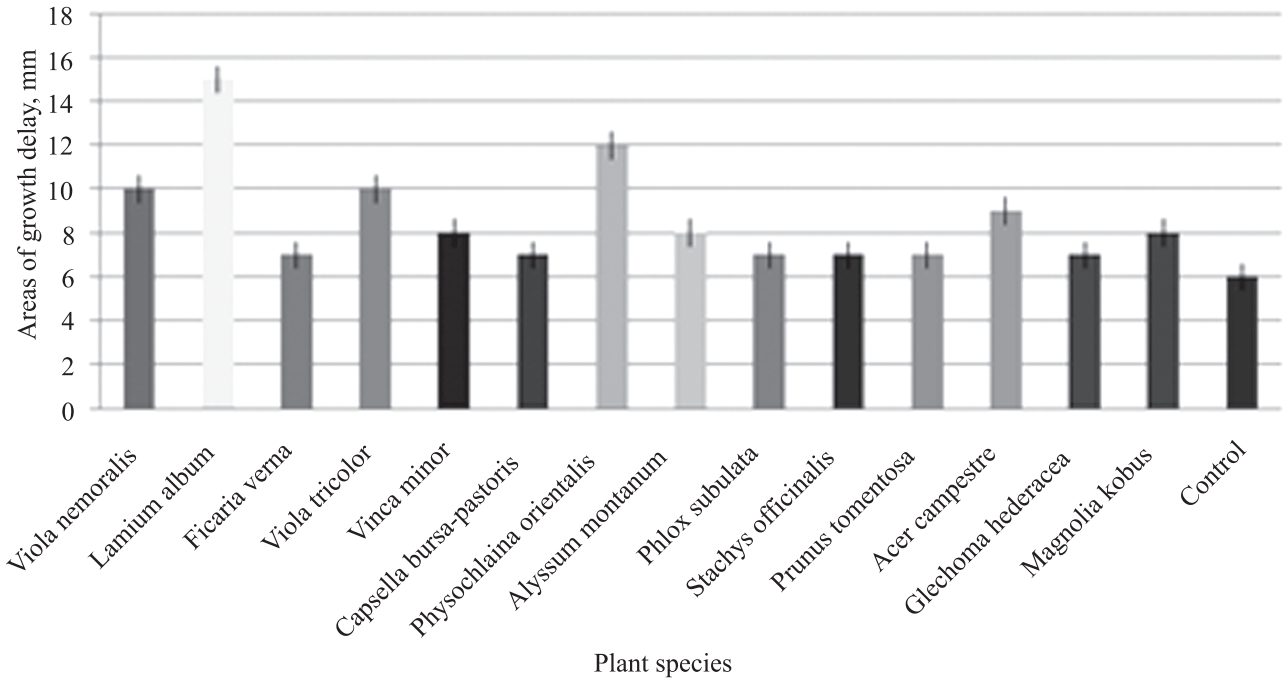

Fig. 3. Antibacterial activity of the ethanol extracts of spring flowers on the strain N 2.1

Nanking cherry (*Prunus tomentosa*), Hyoscyamus physaloides (*Physochlaina orientalis*), shepherd's purse (*Capsella bursa-pastoris*) (67 %). The flower extracts of lesser celandine *(Ficaria verna*) delayed the growth of 56 % of all investigated strains, those of heartsease (*Viola tricolor*), ground-ivy (*Glechoma hederacea*), forsythia (*Forsythia europaea*) – 44 %, respectively (Fig. 2). tThe antibacterial activity was also demonstrated by the flower extracts of all investigated ornamental plants, bushes and trees, and 95 % of all studied wild plants. The average value for the area of growth delay for wild species, ornamental plants, trees and bushes was 9.2 mm, 8 mm, and 8.5 mm, respectively.

It is noteworthy that some plants, namely, white dead-nettle (*Lamium Album*), field maple (*Acer Campestre*), Nanking cherry (*Prunus Tomentosa*), Scotch heather (*Calluna Vulgaris*), lungwort (*Pulmonaria Officinalis*), alpine squill (*Scilla Bifolia*), hedge woundwort (*Stachys Sylvatica*), spring speedwell (*Veronica Verna*), the flower extracts of which demonstrated the antibacterial activity against test-cultures, were melliferous plants.

The highest sensitivity was revealed for strain No. 2.1, which was influenced by 50 % of all the investigated flower extracts (Fig. 3). High sensitivity was also demonstrated by strain No. 7 (the areas of growth inhibition were found at the impact of 43 % of the investigated flower extracts), No. 1.2 and 4.1 (39 %), No. 2.2 and 5.1 (36 %).

Therefore, the antibacterial effect against the strain of *Melissococcus pluton* 8.1 was demonstrated by the flower extracts of 14 plant species, the effect against the bacteria of *Bacillus* genus, isolated from the pathological material – by the flower extracts of 27 species of early-blossoming plants, 10 of which ware ornamental plants and 17 – wild ones. It is possible to assume that the nectar of spring flowers, especially trees and bushes, is the most healing for bees.

CONCLUSIONS

The obtained data may be used to study the antibacterial effect of such melliferous plants as white dead-nettle (*Lamium album*), field maple (*Acer campestre*), Nanking cherry (*Prunus tomentosa*), onion (*Allium cepa*), French marigold (*Tagetes patula*), Japanese meadowsweet (*Spiraea japonica*), yarrow *(Achillea millefolium*), Scotch heather (*Calluna vulgaris*), peppermint (*Mentha piperita*), small-leaved linden (*Tilia cordata*), brown knapweed (*Centaurea jacea*), golden creeping Jenny (*Lysimachia nummularia*) on bee colonies, affected with foulbrood. The results of experiments on the culture of microorganisms, isolated from honeycombs, affected with foulbrood, testify to the fact that these plants may be efficiently used for prevention and treatment of bacterial diseases of bees.

Антибактеріальна активність екстрактів квітів щодо мікроорганізмів, виділених зі стільників з ураженим гнильцями розплодом бджіл

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Мета. Дослідити антибактеріальні властивості квітів рослин-нектароносів щодо культур, виділених зі стільників з ураженим гнильцями розплодом бджіл. **Методи.** Мікробіологічні, культурально-морфологічні, біохімічні, електронно-мікроскопічні, статистичні. **Результати**. Антибактеріальний ефект до штаму *Melissococcus pluton* 8.1 проявили екстракти квітів 14 видів рослин, до бактерій роду *Bacillus* – екстракти квітів 27 видів ранньоквітучих рослин. **Висновки**. Отримані дані дозволяють рекомендувати для дослідження на бджолиних сім'ях, уражених гнильцем, протигнильцеві властивості таких рослин-нектароносів, як глуха кропива біла, клен польовий, вишня войлочна, цибуля ріпчаста, чорнобривець розлогий, спірея японська, деревій звичайний, вереск звичайний, м'ята перцева, липа дрібнолиста, волошка лучна, вербозілля лучне. Результати експериментів на культурі мікроорганізмів, виділених зі стільників хворих на гнильці сімей, свідчать про те, що вказані рос-лини можуть бути ефективними для профілактики та лікування бактеріальних хвороб бджіл.

Ключові слова: рослини-нектароноси, бактеріальні хвороби бджіл, мікробіота стільників з ураженим розпло-дом бджіл, антибактеріальна активність, *Bacillus, Me-lissococcus pluton*.

Антибактериальная активность экстрактов цветов относительно микроорганизмов, выделенных из сотов с пораженным гнильцами расплодом пчел

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Цель. Исследовать антибактериальные свойства цветков растений-нектароносов относительно культур, выделенных из сотов с пораженным гнильцами расплодом пчел. **Методы.** Микробиологические, культурально-морфологические, биохимические, электронно-микроскопические, статистические. **Результаты**. Антибактериальный эффект по отношению к штамму *Melissococcus pluton* 8.1 проявили экстракты цветков 14 видов растений, относительно бактерий рода *Bacillus* – экстракты цветков 27 видов растений, цветение которых приходится на раннюю весну. **Выводы**. Для исследований на пчелиных семьях, пораженных гнильцом, можно использовать противогнильцовые свойства таких растений-нектароносов, как глухая крапива белая, клен полевой, вишня войлочная, лук репчатый, бархатцы мелкоцветные, спирея японская, тысячелистник обыкновенный, вереск обыкновенный, мята перечная, липа мелколистная, василек луговой, вербейник монетчатый. Результаты экспериментов на культуре микроорганизмов, выделенных из сотов больных гнильцами семей, свидетельствуют о том, что указанные растения могут быть эффективными для профилактики и лечения бактериальных болезней пчел.

Ключевые слова: растения-нектароносы, бактериальные болезни пчел, микробиота сотов с пораженным расплодом пчел, антибактериальная активность, *Bacillus*, *Melissococcus pluton*.

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