ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at: www.jgpt.co.in

RESEARCH ARTICLE

Determination of the Fungicidal Properties of Plants and Herbal Combinations against *Ascosphaera Apis* l.

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Abstract

The urgency of the problem under investigation is due to the need to develop plant means for the prevention and treatment of fungal diseases in bee colonies. The aim of the article is: * to search for medicinal plants with fungistatic activity in relation to the causative agent of chalkbrood; * to create herbal combinations and to comparatively evaluate their activity in laboratory and production conditions with the chemical compounds recommended for chalkbrood treatment. The main laboratory method for assessing the fungistatic activity of plants and herbal combinations is comparing the zones of delayed growth of the chalkbrood causative agent in experimental and control media. In the production environment, the influence of herbal combinations was assessed by comparing the quality of wintering of bee colonies, the rate of their spring development, the reduction of bee larvae infected with chalkbrood and the amount of collected honey. Laboratory conditions revealed that alcohol extracts of celandine (*Chelidonium majus*), fir needles (*Abietis sibiricae*) and pot marigold, or calendula (*Calendula officinalis*), had a pronounced fungistatic effect (comparable to that of the chemical compounds) on the growth of the mycelium of *Ascosphaera apis*. Several herbal combinations were prepared which, in vitro, also had a pronounced fungistatic effect. Under their influence, the preservation of bee colonies during wintering was improved, the incidence decreased, and honey production increased. The materials of the article can be of use for scientists working on developing drugs for the prevention and treatment of fungal diseases in bee colonies, as well as for beekeepers who do not use chemical compounds in their produce.

Keywords: *Medicinal plants, Wintering of bee colonies, Catalase, Spring development, Chalkbrood.*

Introduction

Chalkbrood is an infectious disease of bee colonies caused by the parasitic fungus *Ascosphaera apis*, which affects bee larvae [1, 2]. The spores get into the bee colonies mainly via pollen and nectar [3, 2]. As a result, larvae die, and there is no generation change in the bee family, which leads to a critical population reduction and then to the death of the whole family [4].The spread of chalkbrood in the apiaries of Russia is of a menacing nature [5]. Developing strategies for the prevention and control of bee colonies diseases is possible through an integrated prevention system using means that affect several pests in the hive simultaneously. In Russia, as in many other countries, traditional treatment of diseases is carried out with the help of antibiotics and other synthetic molecules [5]. However, long-term chemotherapy in bee colonies adversely affects the health of bees and can accumulate both in their body and in beekeeping products, and this hinders their marketing [6]. In addition, chemotherapy causes resistance of pathogenic microorganisms; besides, multiple effects of agricultural pesticides can reduce the immunity of honey bees [6, 7, 9]. Another reason for the spread of chalkbrood is the uncontrolled use of chemical acaricides against the parasitic mite *Varroa jacobsoni*, and this often contributes to the spread of infectious diseases of the bee

family (chalkbrood, American and European foulbrood, viral diseases) [1, 10].Therefore, in many countries, these substances were banned, or their usage was restricted. An example of creating alternative means for combating diseases with natural substances are extracts of medicinal plants in the form of herbal combinations with a certain ratio of components [11, 15].

The development and application of plant extracts in the beekeeping (as medical and preventive measures) is an actual alternative to the use of chemical compounds. Previously, the authors developed the herbal combination which had a certain fungicidal activity, yet inferior in effectiveness to chemical compounds [16, 17]. The task of the study was to compile a specific combination of medicinal plants, the effectiveness of which would be close to the action of modern chemicals. The authors believe this possible due to the synergistic effect of several extracts of medicinal plants.

Materials and Methods

The object of the study was honey bees of the Middle Russian breed *Apis millifera millifera* L. The bee colonies were contained, in all variants of the experiments, in typical 12-frame hives and in the same feeding and maintenance conditions. Collection of pathological material for laboratory studies was carried out in the bee colonies of Bashkiria under conditions of natural infection. Studies were conducted in the bee families divided into groups of 5 families each. Groups were selected by the method of analogue pairs taking into account the following indicators: strength of bee colonies and number of sealed brood and feed.

Wintering of bee colonies was carried out in nests corresponding to zoo technical and veterinary requirements. Extracts of plants and herbal combinations were obtained by infusion in 40% ethyl alcohol at room temperature for 14 days, and aqueous extracts by infusing the herbal combination in water in a heated bath for 30 minutes. For extraction, dry finely ground (5mm) materials were used. For a microscopic study of the presence of the causative agent *Ascosphaera apis*, scrapings were made from the body surface of the affected bee larvae. A small amount of the obtained material was placed on a slide in a 50% aqueous solution of glycerol and examined using a Lomo 20x/0.45 objective microscope 'Mikmed 5' to detect and confirm the presence of mycelium and fruiting bodies of *Ascosphaera apis*. If multicellular septate mycelium with multinucleated cells, branching hyphae and/or globular cysts were detected, the conclusion of a positive result was made. To confirm the microscopic examination results, pure fungus culture was isolated from the pathological material.

For this, the dead larvae were removed from the cells, placed in a sterile tube with 2 ml of saline with 1000 units of penicillin and streptomycin added, were thoroughly ground and the material was applied on wort-agar or Sabouraud medium in test tubes, and cultured for 10 days at a temperature of 28-32 °C. On Day 3-5, white fluffy colonies appeared on the surface of the medium, and in 8-10 days they produced a greenish-gray coating on the bottom and at the edges of the colony (formed by fruiting bodies). The pure culture was obtained by planting the periphery colonies characteristic of this fungus.

The determination of fungi static and fungicidal properties of various herbal extracts was carried out on Sabouraud agar with the addition of an alcohol extract (containing 10% of dry matter), added before autoclaving the medium. Then, *A. apis* was inoculated. Observations were carried out for 5 days, and the control ingredients of the agar were Nystatin (a.i. Nystatinum, 400 µg/ml, included in many drugs for chalkbrood treatment in bees) and 40% ethyl alcohol. On the basis of a comparison of delayed growth zones of chalkbrood causative agent in experimental and control media, the conclusion was made about the fungicidal efficiency of alcohol extracts of individual plants and herbal combinations.

Prior to the therapeutic feeds with plant extracts, all the bee families including the control group underwent the necessary veterinary and zootechnical measures: disinfection of hives, burning dead bees and beehive sweepings. In the spring, 15 bee colonies with signs of chalkbrood were divided into 3 groups of 5 families each, taking into account the following indicators: the strength of bee colonies, the number of sealed brood and the extent to which the brood was affected. Counting of affected larvae in the frames and the number of

sealed broods was determined using a net frame (10 x 10 cm). The broods were photographed, and the pictures then analyzed in laboratory conditions. To confirm the epizootiological diagnosis, samples analysis of mummified larvae was performed. In Group 1, bee colonies received sugar syrup with Nystatin, previously dissolved in 40% alcohol at a rate of 500 thousand units (0.5 g) per liter. Feeds were given at the rate of 1 liter three times with an interval of 5 days.

In Group 2, bee colonies received syrup with the herbal combination extract in the amount of 1 liter per family three times with an interval of 5 days. This syrup was prepared as follows: 40% alcohol extract with 5% of solid content was mixed with sugar syrup at the ratio of 1:6. In Group 3 (control), bee colonies were given sugar syrup with 1/6 of 40% pure ethyl alcohol (added due to its presence in the experimental groups).

Samples of bees for the analysis of enzyme activity were selected by 20-25 pieces. In each group, 20 bee colonies were examined. The enzyme activity was analyzed by permanganometric method; the data were expressed in µmol/min/mg. Fecal dynamics was determined by the mass of the lower part of the intestine, by weighing it on the torsion balance VT-1000 (Russia) [18]. In each experimental group, 30 samples of bees were regarded; the weight of the rectum depended directly on fecal masses.

In the spring-summer period, a comparative application of Ascocin (a.i., propoxonazole-4-propyl-1-[2-(2, 4-dichlorophenyl)-1, 3-dioxolan-2-ylmethyl]-1H-1, 2, 4-triazole) and plant extracts was made. 0,006% Ascocin (according to the instruction of the manufacturer-NPO Eltos, Russia) is recommended for therapeutic use in apiaries endangered in terms of chalkbrood during the spring and summer periods by feeding it with sugar syrup.

Bee families were given therapeutic syrup-1 liter three times every 5 days (dosage based on herbal combinations as indicated before).The effectiveness of therapeutic treatments of the bee family was determined by comparing the number of larvae affected by chalkbrood. The number of infected larvae was counted every ten days for 2 months. Statistical analysis of the obtained data was carried out using the arithmetic mean, standard error, Student's t-test using MS Excel 2007 software (Microsoft). Differences were considered valid for $p < 0.05$.

Results and Discussion

Determined Fungicidal activity of Medicinal Plants Extracts

The authors previously found out that alcohol extracts of herbal combinations are more active against *A. apis* than water extracts, which is apparently related to a certain ratio of substances in the former [17]. Determination of the antifungal effect of extracts from individual plants in the combination showed that all plants had a certain fungistatic activity which varied (Table 1).

This is explained by the fact that, in addition to fungicidal plants, the analysis included plants with properties stimulating immunity and productivity of bee families [4]. The most effective in its fungistatic action against *A. apis* on Day 5 were extracts (in descending order) of celandine, fir needles, calendula flowers, aspen bark, eucalyptus leaves, birch leaves, filipendula flowers and thyme. On Day 9 substantial fungistatic action was revealed in extracts of celandine, aspen bark, calendula flowers, fir needles and field horsetail.

On Day 16, the impact of medicinal plants extracts on the growth of *A. apis* changed. As before, the most effective extract was that of celandine and fir needles, their fungistatic efficacy comparable to the chemical drug Nystatin. The group with less efficiency (28-44%) included calendula flowers, thyme, birch leaves and cetraria (Iceland moss) thalli (which previously showed no pronounced fungi-static activity).Extracts of veronica and coneflower were less effective (approximately 60-80%).

Aspen bark extract reduced its fungistatic activity against *A. apis* in 16 days. As can be seen from the table, only three extracts (celandine, fir needles and calendula flowers) retained their fungistatic activity from 5 to 16 days. Quite interestingly, on Day 16 the fungus growth on the agar containing extracts of cetraria thalli and coneflower was actively suppressed, while at the earlier stages of the experiment said extracts were not active.

Table 1: Determined fungicidal activity of medicinal plants extracts against *Ascosphaera apis*

	Extracts	*Ascosphaera apis* colony area, cm²		
		5 days	9 days	16 days
1.	Immortelle flowers *(flores Helichrysi arenarii)*	1,53±0,12	4,28±0,51	16,53±1,81
2.	Marigold flowers *(flores Calendulae)*	0,6±0,03*	1,83±0,21*	10,81±1,31*
3.	Filipendula flowers *(flores Filipendula ulmaria)*	0,91±0,08*	3,51±0,27	18,34±1,33
4.	Fur needles *(folia Abietis sibiricae)*	0,49±0,03*	2,18±0,24*	9,83±0,87*
5.	Birch leaves *(folia Betulae)*	0,89±0,09*	3,22±0,41	11,19±1,22*
6.	Eucalyptus leaves *(folia Eucalypti viminalis)*	0,78±0,09*	3,56±0,46	11,5±1,24
7.	Aspen bark *(cortex Populiae tremulae)*	0,77±0,08*	1,22±0,15*	17,92±1,88
8.	Coneflower *(herba Echinaceae purpureae)*	1,73±0,2	2,62±0,21	15,2±1,57
9.	Horsetail *(herba Equiseti arvensis)*	1,07±0,2	2,31±0,19*	17,66±1,67
10.	Celandine *(herba Chelidonii majoris)*	0,49±0,05*	1,16±0,17*	9,52±0,99*
11.	Veronica *(Veronicae chamaedris)*	1,15±0,2	3,27±0,29	14,25±1,67
12.	Thyme *(herba Thymi serpylli)*	0,9±0,1*	2,63±0,3	11,04±1,56*
13.	Cetraria *(thalli Cetrariae islandicae)*	1,05±0,1	2,56±0,22	12,17±1,02*
14.	Nystatin	0,49±0,03	1,22±0,1	8,44±0,78
15.	Control	4,3±0,5	16,41±1,8	60,13±6,78

* The difference in the results between Nystatin and plant extract is not more than 100%

The results presented in Table 1 show that there are medicinal plants with a fungistatic effect similar to that of the chemical compound. Thus, it seemed important to create an herbal combination which would combine fungistatic (against *Ascosphaera apis*) and stimulating impact on honey bees. The authors attempted to create a herbal combination earlier [17], but is prove less active than chemical preparations [16]. In connection with this, several combinations of medicinal plants were created, different in composition and proportions (Table 2). Number 1 was the combination previously developed [17]. In total, 23 variants of a herbal combinations were tested in the experiment. Table 2 presents the most illustrative results.

Table 2: Herbal combinations under study

	Medicinal plants	Variants and part of the combination				
		1	2	3	4	5
1.	Veronica (*Veronica longifolia)*	7				
2.	Birch leaves (*Betulla pendula)*	3	3	3		
3.	Filipendula (*Filipendula ulmariae)*	2	1			
4.	Calendula flowers (*Caléndula officinális)*	2	5	5	5	5
5.	Fir needles (*folia Abietis sibiricae)*	2	7	7	7	7
6.	Coneflower (*Echinácea purpúrea)*	1	1			
7.	Eucalyptus leaves (*Eucalupti viminalis)*	1	4	4	4	
8.	Horsetail (*Equiseti Arvensis)*	1	1			
9.	Immortelle flowers (*Helichrýsum arenárium)*	0,5				
10.	Thyme *(Thýmus serpýllum)*	0,5	2			
11.	Aspen bark (*Pópulus trémula)*	0,3	3	3	3	3
12.	Celandine (*Chelidónium május)*	0,2	1	1	1	
13.	Cetraria thalli (*Cetraria islandica*)	0,1				

The extracts obtained from the herbal combinations were tested in the same way as individual extracts of medicinal plants, comparing them with each other and with Nystatin. The increase in the proportion of plants in the herbal combinations leads, as a rule, to an increase in the concentration of certain biologically active substances in the extract and to the enhancement of a certain pharmacological action [19]. As can be seen from Table 3, fungistatic activity at Day 5 of herbal combinations 1 and 2 was comparable with Nystatin. Removal of veronica, cetraria and immortelle from Herbal combination 2 with simultaneous increase in the proportion of calendula, fir needles, eucalyptus, thyme and aspen bark as a whole did not change the fungistatic activity. Removing filipendula, coneflower, horsetail and thyme from Combination 3 on Day 5 showed a lower

fungistatic activity than that of Nystatin and samples 1 and 2. However, on Day 16 and 30 the growth of *A. apis* was suppressed more actively than in the case of Nystatin. From Combination 4, birch leaves were excluded, which reduced the fungistatic activity. Combination 5 consisted of 3 components - calendula, fir and aspen; its extract on Day 5 was more active against the colonies of *Ascosphaera apis* compared to Nystatin and other combinations of medicinal plants.

Table 3: Comparative analysis of fungistatic activity of different herbal combinations (Table 2) against *Ascosphaera apis*

Variants	*Ascosphaera apis* colony area, cm^2		
	5 days	16 days	30 days
1	0,68±0,07	14,36±1,47	44,28±3,92
2	0,66±0,06	14,62±0,98	46,40±4,22
3	1,02±0,14	8,06±1,02	38,75±5,06
4	1,05±0,12	15,2±1,46	41,92±4,45
5	0,49±0,07	5,81±0,64	26,54±2,33
Nystatin	0,63±0,05	14,86±1,23	44,81±4,43
Control	1,41±0,17	39,13±4,3	77,50±7,8

Thus, in the model systems, Combinations 3 and 5 proved to be the most active. The authors continued to study Combinations 3 and 5 in the field, by feeding bee colonies with the extracts in the form of syrups. For this, the effect of herbal combinations on catalase enzyme activity was evaluated, the overall winter resistance and productivity of bee colonies was estimated, and a comparative assessment of the effect of herbal combinations on chalkbrood incidence during spring development.

Effect of the Extract of Herbal Combinations on Catalase Enzymes activity during the Wintering of Bees

Successful wintering depends on many factors: winter resistance of bees, winter nest formation, feed quantity and quality, time of the last flight before wintering, preparation of bees for wintering, bee care, health and number of wintering bees, etc [20, 18].A study of catalase enzyme activity during wintering is associated with the fact that this enzyme protects the bees during wintering from the toxic effect of hydrogen peroxide and is the source of molecular oxygen in tissues [21, 23].

Therefore, the higher the catalase enzyme activity level in the feces, the less the incest will be negatively affected by hydrogen peroxide, and the tissue cells will not suffer oxygen deficiency in [24, 23]. The winter activity of catalase in bees has the maximum level [25, 26, 27].The authors established the positive effect of herbal extract syrup on the increase in the activity of catalase enzymes as an index of winter resistance of bees (Table 4). In November, two months after the 2-time syrup feeds containing 5% of the herbal combination extract, the authors determined catalase enzymes activity in the intestines of bees. They managed to establish that using herbal combination in sugar feeds in all cases leads to an increase in the enzyme activity. This activity during the wintering period reached its maximum in April and its minimum in February (Table 4). The authors established differences in the enzyme activity between groups that received extracts from different herbal combination. Thus, catalase activity in November was 74% higher in the group of bee colonies receiving Combination 1 extract (Table 2) in comparison with the control group.

However, the differences between the experimental groups in November were not so obvious. In February, there was a decrease in catalase activity in all study groups, a minimal decrease in enzyme activity compared to November (about 10%) was observed in the group receiving Combination 3 (Table 4).Differences in the enzyme activity in Group 3 compared with the control was 48%. Analysis of enzyme activity in April showed an increase in all the experimental groups (Table 4). In April, as in February, the maximum level of enzyme activity in the intestines of bees receiving Combination 3 extract was observed, the differences with the control reaching 22%.

For Group 1, the difference was 17%, and for Group 5 it reached 10%. In general, the

difference in the values of catalase enzyme activity in the experimental groups was almost leveled by April. In April, before the return of bee colonies from wintering, the authors determined the mass of the rectum and found that the use of drugs reduced the amount of feces in the intestines by about 17-22%. The difference between the experimental groups was not reliable (Table 4).The general dynamics of changes in catalase enzyme activity during wintering is generally similar to that described in the literature [25, 26].

Table 4: The effect of herbal combinations on the catalase activity in the rectal glands of the honey bee and on the mass of the rectum, n=20

Group	Catalase activity, µmol/min/mg			Rectum mass, mg
	Collection time, month			
	November	February	April	April
1	12.72±1.2	9.27±1.1	15.83±1.3	30.4±2.2
3	11.22±0.9	10.11±1.9	16.92±1.7	29,4±1.6
5	10.96±1.1	8.72±0.8	14.6±1.3	28.7±1.9
Control	7.3±0.5	5.27±0.4	13.2±0.9	36,6±1.7

In spring, the number of sealed broods, the amount of food left and the number of bees dead during the wintering were compared (Table 5). The number of sealed brood in Group 1 was 25% higher, in Group 2 it was 40% higher and in Group 3 it was 14% higher compared with the control group. Table 5 provides data on the weight of dry bees dead during wintering. This indicator is important for beekeepers, as the number of dead bees serves as a criterion of wintering quality. As seen from the table, due to the use of herbal combinations the amount of dead bees decreased by 40% (sample 1), 47% (sample 3) and 49% (sample 5). Families from Groups 1, 3 and 5 which received stimulating feeds before wintering consumed less honey than the control group-by 20%, 27% and 23%, respectively. Thus, estimating the zootechnical parameters of wintering, it is possible to come to the conclusion that using Combination 3 is the most rational choice, first of all, due to the large number of broods obtained in this variant (Table 5). In further studies, the authors evaluated the effect of the studied combinations on the incidence of chalkbrood.

Table 5: Effect of herbal combinations on the bee family during wintering, n=15 (April 15, 2017)

Group	Amount of food consumed per bee family, kg	Number of sealed brood, hundreds of cells	Number of bees dead during wintering, g
1	8.5±0.4	167±4.7	570±21
3	7.8±0.6	187±9.6	510±34
5	8.2±0.3	152±9.6	490±23
Control	10.6±0.4	133±3.6	960±47

Evaluation of the effectiveness of the fungicidal action of plant extracts during spring development and the productivity of bee colonies Studies conducted in the spring-summer period in bee colonies were interesting from the point of view of the potential application of herbal combinations in practical beekeeping. The bee colonies which did not receive syrup with medicinal feeds before wintering were selected.

The incidence of chalkbrood in the apiary in the spring (during 5 years of observation) was 50-70% of bee colonies. We analyzed the dynamics changes in the brood affected by the disease in the course of treatment with herbal combinations and with Ascocin (a.i. propiconazole-4-propyl-1-[2-(2, 4-dichlorophenyl)-1, 3-dioxolane-2-ylmethyl]-1H-1, 2, 4-triazole). The use of Ascocin instead of Nystatin which was used in preliminary experiments (Tables 1 and 3) is due to the wish to compare the fungicidal activity of herbal combinations extracts with another chemical active substance, also used in beekeeping.

As seen from Table 6, the initial differences in the number of infected larvae were insignificant; after 10 days it turned out that in Group 1 their number decreased by 14%, in Group 3 - by 24%, in Group 5 and in the

Ascocin group by 36%, and in the control group it increased by 16% (i.e. in the control group where bees did not receive treatment, the disease progressed) (Table 6).

After 20 days in comparison with the initial value the number of infected larvae in Group 1 significantly decreased by 44%, in Group 3 by 48%, in Groups 5 and Ascocin the incidence decreased by approximately 56%. In the control group, the number of infected larvae increased by 32%. After 30 days, single infected larvae were found in the experimental groups, and in the control group self-healing of the bee colonies began, the number of infected larvae was 18% lower than the initial value obtained a month before.

Table 6: Differences in the efficacy of fungistatic action of different herbal combinations on the course of chalkbrood and on honey production of bee colonies (2017 r.), n=5

Group	Number of infected larvae, pcs. per 10 cm ²				Marketable honey obtained, kg
	before treatment	10 days	20 days	30 days	
1	39±4	36±5	22±3	9±2	23±1
3	34±6	32±3	18±3	5±1	25±2
5	36±5	27±5	16±2	3±1	21±1
Ascocin	36±4	28±4	15±2	4±1	20±2
Control	37±5	42±4	49±5	44±3	12±3

A complete recovery after chalkbrood (absence in the honeycomb and on the fly-board) occurred in the experimental groups after about 1.5 months and in the control group in 2.5-3 months. Group 5 and the group receiving Ascocin were the most effective in recovery rate, especially in the first decade of treatment, yet then by the second decade Group 3 also had similar data on the effectiveness of treatment. Bee colonies from Group 1 did not show any obvious signs of illness after 30 days of treatment.

The effect of herbal combinations on the productivity of bee colonies at the end of the beekeeping season was also studied.As seen from Table 6, in all groups of bee colonies that received treatment more honey was collected than in the control group (without treatment). The largest honey crop was obtained in the group receiving Combination 3. Quite interestingly, the most effective herbal combination in terms of fungistatic activity had similar levels of honey production to the group of bee colonies receiving Ascocin (Table 6).

Conclusion

Thus, it was established that the extracts of medicinal plants and their combinations in vitro and in the apiary had a fungistatic action against *Ascosphaera apis*. When feeding bees with syrup and plant extracts, the winter resistance increased due to reduced fecal mount, feed intake and the number of dead bees decreased, and more honey was retained.

In the spring-summer period there was an increase in egg production, improvement of the brood and increase of honey production. Estimating the fungistatic efficacy, the safety of bees during wintering and the amount of honey collected, the authors came to the conclusion that using Herbal Combination 3 is most rational in practical beekeeping.

Review

Fugistatic properties of alcohol extracts of 13 species of medicinal plants were established in vitro against *Ascosphaera apis*. It was found that alcohol extracts of celandine (Chelidonium majus), fir needles (*Abietis sibiricae*) and calendula flowers (*Calendula officinalis*) had a pronounced fungistatic effect comparable to the effect of Nystatin (active ingredient 19-mycosaminilistinomide) on *Ascosphaera apis*. Various combinations of the analyzed herbs were compiled, some of which in vitro delayed the growth of the fungal infection on the nutrient medium.

The determination of the fungistatic activity of herbal combinations was carried out on the basis of comparing the zones of delayed growth of chalkbrood causative agent in vitro between the alcohol extracts of herbal combinations and Nystatin. With herbal combination extracts in the form of syrup during the autumn feeding of bees catalase enzymes activity in the intestines increased throughout the entire wintering period, which is an indicator of an increase in the winter resistance of bee colonies.

In the experimental groups during wintering a decrease in the fecal amount in the lower part of the intestine was observed, together with a decrease in the consumption of honey and in the number of dead bees. It was established that said extracts stimulate the spring development of the bee family (increase in the number of sealed brood). The increase in broods exceeded the control group by 14-40%, depending on the herbal combination. The spring feeding of bee colonies (which showed signs of chalkbrood) with syrup and Ascocin (active ingredient is propiconazole- 4- propyl-1- [2- (2, 4- dichlorophenyl)- 1, 3-dioxolane-2-ylmethyl]- 1H- 1, 2, 4-triazole) allowed finding out that the therapeutic effect of the chemical and herbal combinations was comparable, and a full recovery of the bee colonies was observed 30 days after the beginning of treatment. In the control group of bee colonies, a natural recovery occurred in 2.5 months. Groups that received plant extracts produced more marketable honey. The possibility of using herbal combinations for the prevention and treatment of chalkbrood and other infectious diseases of bee colonies is discussed.

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