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ACARICIDAL ACTIVITY OF THE FUNGUS FOMITOPSIS OFFICIONALIS IN VARROATOSIS OF THE HONEY BEES

ABSTRACT. Evaluation of the acaricidal activity of the fungus Fomitopsis officionalis was carried out on bees of Central Russian breed. In the laboratory, the effect of four doses of the fungus Fomitopsis officionalis on the mite Varroa destructor and worker bees was studied. Each dose was studied in triplicate. The control groups of bees were infested with Varroa destructor, though not exposed to the tested acaricide. We established a relatively rapid effect of the aerosols of the fungus Fomitopsis officionalis on the population of the mite Varroa destructor, parasitizing on adult bees. Within four hours after the application of the acaricide a significant reduction in the number of ectoparasites on the bees was noted at doses of 3 grams/ per application, 4 grams/per application ($P \leq 0,05$). An increased dose of 5 grams of acaricide/ per application has significantly reduced the number of mites on the bees within one hour after the application ($P \leq 0,01$). When using the fungus Fomitopsis officionalis in the bee families it is necessary to keep the bees in the beehives with removable bottoms and under-frame mite-nets

KEY WORDS. Honeybees, ectoparasite, acaricide, varroatosis.

Varroatosis is one of the widespread and dangerous diseases in contemporary bee-keeping. The causative agent of the disease mite Varroa destructor parasitizes in the brood and bees. Infestation of the brood with the mite Varroa destructor causes various disorders and leads to appearance of sick bees. In the case of serious infestation of the brood the larva and pupa die. The bee families which are infected with the mite in question develop slowly, become poorly productive and weaken or die in winter time.

Infestation of bee families with the mite Varroa destructor affects their immune status which causes various viral, bacterial and fungus diseases [1], [2].

There are a number of works devoted to the study of causes of Varroa destructor outspread and ways to cure the disease [3], [4], [5], [6], [7]. One of the efficient means of dealing with it is the use of biologically active materials for sanitation of the bee families.

The aim of this research was to study the medicinal properties of the fungus Fomitopsis officionalis and its influence on the mite Varroa destructor and honey bees in the laboratory conditions.

The tasks of the research.

1. To evaluate the attack rate of the mite Varroa destructor on the control and test groups of bees.

2. To define an efficient way of usage of the fungus Fomitopsis officionalis against the mite Varroa destructor.

3. To evaluate the efficiency of different doses of the fungus Fomitopsis officionalis when applied on adult females of the mite Varroa destructor in laboratory experiments.

4. To define the influence of different doses of the fungus Fomitopsis officionalis on worker-bees.

Material and methodology of the research. The research was carried out in the laboratory for the study of bee diseases of the Central Russian breed in the All-Russian Research Institute for Veterinarian Entomology and Arachnology (ARRIVEA) 04.09.12. On the bee farm of the Tyumen State Institution a frame with sealed brood was taken from bee family No. 11 infested with mites Varroa destructor. Within two hours the brood frame was brought to the laboratory for the study of bee diseases of the ARRIVEA and placed into an isolated chamber with a thermostat (temperature range 34-36°C, relative humidity range 60-80%). From 04.09.2012 to 12.09.2012 there appeared worker bees both infested and non-infested with the mite.

We divided the hatched 8-15-day-old bees into groups (each group of 100 bees was kept in a rearing cage):

— control (6 cages);
— test-1 (3 cages) to evaluate the effect of a dose of 1 gram/ per 100 bees;
— test-3 (3 cages) to evaluate the effect of a dose of 3 grams/ per 100 bees;
— test-4 (3 cages) to evaluate the effect of a dose of 4 grams/ per 100 bees;
— test-5 (3 cages) to evaluate the effect of a dose of 5 grams/ per 100 bees.

We studied the influence of the four doses (concentrations) of the fungus Fomitopsis officionalis on the mites and bees in the rearing cages. Each dose was studied in triplicate. The control groups (six repetitions) were analogous groups of infested bees without the fungus Fomitopsis officionalis treatment.

The bees in the groups were provided with sugar syrup according to the accepted methodology [8]. Before the fungus Fomitopsis officionalis treatment the bees (test and control groups) were kept under the conditions of the thermostat at temperature 34-36°C and relative humidity 60-80%. There was no death of bees, detachment or death of mites before the fungus treatment.

We prepared appropriate doses of the dried out and crushed fungus Fomitopsis officionalis in filter paper. Fungus treatment of each group of bees was performed in a special tight chamber made from transparent material, volume of which was 0.06m^3. Exposure time equaled to 30 min. At the end of the exposure time the rearing cages were taken out and placed in the thermostat. Before the work with the test groups the control groups were also kept in the tight chambers however without the fungus Fomitopsis officionalis treatment. Destructive distillation of the fungus Fomitopsis officionalis was carried out in the weighing bottle. After burning of the filter paper the bottle with the moldering weighed portions were put in the tight chamber with the test groups. After 30 min. the cages were taken out of the tight chamber and put in the thermostat. Prior to treatment of each group the chambers were cleaned from the remnants of the tested product.

We evaluated the influence of the fungus under study on the worker bees in 1, 2, 3, 12 and 96 hours by assessing the health of the bees and defining the number of the dead bees.

Evaluation of the influence of the fungus Fomitopsis officionalis on the mite Varroa destructor was done in 1, 2, 3, 12 and 96 hours by counting the number of the detached mites and assessing their viability. For this purpose we put the detached mites on the objet-plate and assessed their limb mobility by touching them with a heated microscopic needle. The mites with no signs of life we considered dead.

Dynamics of the bees' infestation with the mite Varroa destructor was traced on the basis of the calculation of the abundance index [9].

Statistical processing of the data was performed according to standard methodologies [10].

Research findings and discussion. In order to compare the calculated indices of abundance depending on the dynamics of the disease we divided the control groups into control-I (medium degree of ectoparasitic bees' infestation) and control-II (high degree of bees' ectoparasitic infestation). Medium degree of infestation was recorded for test groups-I. We evaluated their abundance index dynamics relatively to control groups-I. Test groups-II-3, II-4 and II-5 were compared in relation to control groups-II. The highest degree of ectoparasitic bees' infestation was recorded in test groups-II-3 (table 1).

Proved decrease of the mites' abundance index occurred within four hours after the application of the fungus Fomitopsis officionalis in test groups-I, II-3 and II-4 ($P \leq 0.05$).

In test groups-II-5 proved decrease of the mites' abundance index occurred within one hour after the application of the fungus Fomitopsis officionalis ($P \leq 0.01$). Increase of the fungus dose contributed to a faster response of the mite Varroa destructor population.

There was no detachment of mites in the control groups one hour after the beginning of the experiment (fig. 1). Four hours after the beginning of the experiment 89.36% of the mites were still found on the bees from the control groups. We recorded a significant decrease of the mite population in the control groups only 2-4 days later.

Table 2

Antimite efficiency of the fungus Fomitopsis officionalis

Groups	Abundance index of the mite Varroa destructor, $\overline{X} \pm S_{\overline{x}}$				
	At the beginning of the experiment	1 hour later	3 hours later	12 hours later	96 hours later
Control-I	0,0343 ±0,0047	0,0343 ±0,0047	0,0302 ±0,0062	0,0302 ±0,0063	0,0124 ±0,0011
Test-I	0,0422 ±0,0052	0,0394 ±0,0032	0,0254^{x} ±0,0057	0,0197 ±0,0081	0,0113 ±0,0083
Control-II	0,0747 ±0,0083	0,0747 ±0,0083	0,0718 ±0,0082	0,0567 ±0,0069	0,0261 ±0,0075
Test-II-3	0,1458 ±0,01692	0,1180 ±0,0151	0,0684 ±0,0133	0,0443 ±0,0118	0,0104 ±0,0054
Test-II-4	0,1189 ±0,07403	0,0868 ±0,00251	0,0394^{xx} ±0,00698	0,0327 ±0,00456	0,0068 ±0,00349
Test-II-5	0,1094 ±0,01236	0,0884 ±0,01289	0,0466^{x} ±0,00981	0,0409 ±0,01134	0,0156 ±0,00421

x - $P \leq 0,05$; xx - $P \leq 0,01$

12 hours later percentage of detachment in test groups-II was 20.58. In the test groups with a high degree of infestation such value of detachment was registered an hour after the fungus Fomitopsis officialis application. In general the mite detachment percentage in the test groups with a high degree of infestation during four hours was about 52.8-67.5%.

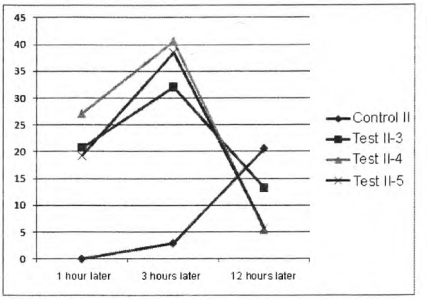

Fig. 1. Mite detachment dynamics in the groups with a high degree of infestation

Detachment of mites in test groups II-3 was 17.9 times more often than in control groups I – II; and 22.9 and 19.6 times more often in test groups II-4 and II-5 respectively. Percentage of the detached mites was evaluated in relation to the total number of the mites during the observations in the test and control groups. Death of the mites was evaluated in relation to the total number of the detached mites. We registered death of the mites in the test groups II-4 and II-5 an hour later after the fungus Fomitopsis officialis application (fig. 2). Cumulative percentage of the dead mites is less than the percentage of the mites which stayed alive in all the observed groups. In connection with this we claim that while fungus treatment it is necessary to keep the bee families in the hives with a removable bottom and a netted subframe for collecting mites, which will allow to remove detached mites from the family. The use of such subframe will lead to 5.23 ($P \leq 0.05$), 6.35 ($P \leq 0.01$) and 5.42 ($P \leq 0.01$) decrease of the mite population three hours after the application of the doses of 3, 4, and 5 grams of the fungus Fomitopsis officialis.

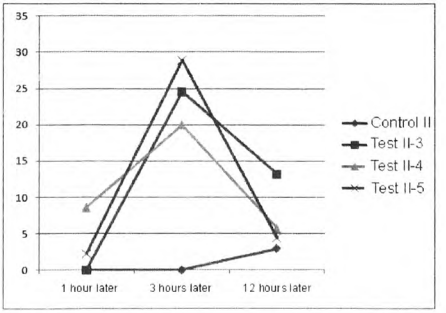

Fig. 2. Mite death dynamics in the groups with a high degree of infestation

The Varroa destructor can be a transmitter of fungal, bacterial and viral diseases of bees. The mixed infections complicate the pathological process and accelerate the death of bees [9].

After we took out the rearing cages from the tight chamber 86-90% of the bees in test groups-1 were found on the walls of the cage and 10-14% were found on its bottom. On the bottom of the cage there was insignificant regurgitation of the content of the honey bag.

2-3% of the bees in test groups-3-4 were found on the walls of the cage and 97-98% were found on its bottom with the signs of regurgitation of the content of the honey bag.

All the bees in test groups-5 were found on the bottom of the cage with the signs of regurgitation of the content of the honey bag.

The bees that were located on the bottom of the rearing cage after the fungus treatment recovered their vitality three hours later and moved up to the walls. The bottom of the cage contained only the dead bees.

In the first three hours after the fungus Fomitopsis officionalis aerosol treatment we recorded death of adult bees in test groups 3-4-5 with a high level of mite infestation. Later we registered death of bees in the control group that were not subject to the fungus treatment. This outcome is connected with poor aeration of the cages and absence of defecation of the bees. These factors may also contribute to the difference in the laboratory and field results.

Table 2

Influence of different doses of the fungus Fomitopsis officionalis on adult bees

Groups	Death of adult bees, %				
	Within 0-3 hours	Within 4-12 hours	Within 13-96 hours		
Control-I	0,00	0,00	0,36±0,006	0,00	0,00
Control-II	0,00	0,00	0,98±0,006	0,00	0,00
Test-I	0,00	0,00	0,00	0,00	0,00
Test-II-3	1,32±0,35	1,61±0,42	4,63±0,37	1,32±0,35	1,61±0,42
Test-II-4	1,28±0,63	0,97±0,56	4,24±1,14	1,28±0,63	0,97±0,56
Test-II-5	0,63±0,36	1,91±0,62	3,88±0,89	0,63±0,36	1,91±0,62

The work experience of the ARRIVEA laboratory for the study of bee diseases indicates that sometimes it is impossible to use aerosols, gases and powders in the hives with bee brood due to the brood's high sensitivity to the medicinal products. Therefore in order to avoid the negative effect on the brood field tests should always begin with an acute experiment on adult bees. Taking into account the results of the acute experiment a series of tests on bee families should be carried out to correct the dose/repetition of the medicinal application and assess its effect on the development of the bee families.

Conclusions:

1. We stated a relatively fast influence of the fungus Fomitopsis officialis on the mite Varroa destructor population found on adult bees. Proved decrease of the mites' abundance index occurred within four hours after the application of the fungus Fomitopsis officionalis in test groups-I, II-3 and II-4 ($P \leq 0.05$); in test groups-II-5

proved decrease of the mites' abundance index occurred within one hour after the application of the fungus Fomitopsis officionalis ($P \leq 0.01$). Increase of the fungus dose contributed to a faster response of the mite Varroa destructor population.

2. The amount of the detached mites Varroa destructor when applying fungus Fomitopsis officialis is higher than the amount of the dead mites. While implementing fungus treatment it is necessary to keep bee families in the hives with a removable bottom and a netted subframe for collecting mites, which will allow to remove detached mites from the family. The use of such subframe will lead to 5.23 ($P \leq 0.05$), 6.35 ($P \leq 0.01$) and 5.42 ($P \leq 0.01$) decrease of the mite population three hours after the application of the doses of 3, 4, and 5 grams of the fungus Fomitopsis officialis per each treatment.

In the first three hours after the fungus Fomitopsis officionalis aerosol treatment we recorded death of adult bees in test groups 3-4-5 with a high level of mite infestation. We registered death of bees in the control groups which were not subject to the fungus treatment. This outcome is connected with poor aeration of the cages and absence of defecation of the bees. These factors may also contribute to the difference in the laboratory and field results.

4. In order to avoid the negative effect of the fungus Fomitopsis officialis aerosol on the brood, field tests should begin with an acute experiment on adult bees and continue with a series of tests on bee families for correction of the dose/repetition of the medicinal application and assessment of its effect on the development of the bee families.

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