THE ENTOMOPATHOGENIC FUNGI, *BEAUVERIA BASSIANA* (BALS.) VUILL. AND *METARHIZIUM ANISOPLIAE* (METS.) SOR. IN CONTROLLING *VARROA DESTRUCTOR* IN HONEYBEE (*APIS MELLIFERA* L.) COLONIES

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ABSTRACT

The present study was carried out at the apiary of Sakha Agricultural Research Station, ARC, Kafr El-Sheikh Governorate during March-June 2006 season to evaluate the effectiveness of the entomopathogenic fungi; *Beauveria bassiana* and *Metarhizium anisopliae* as microbial control agents against *Varroa* mite infesting honeybee colonies and their effects on honeybee activities. Using the fungi *B. bassiana* and *M. anisopliae* at concentrations of 5 x 10^7 conidia/ml at the rate of 10 ml/colony spraying between combs; formic acid (60%) 15 ml/colony absorbed in cardboard plates (5 x 20 cm) placed on top bar of brood combs at dusk and lactic acid (5%) 10 ml/colony spraying between combs caused high decline in *Varroa* mite infestation recording 93.2%, 90.3%, 88.8% and 73.8% reduction in the rate of infestation, respectively. The respective mean numbers of fallen *Varroa* mite after 48 h of using formic acid (60%) were less effective, however the number of dead workers reached 35.33 worker/colony, while it recorded 13.33, 10.40 and 12.00 workers/colony for *B. bassiana, M. anisopliae* and lactic acid, respectively.

Applying the fungi *B. bassiana* and *M. anisopliae* recorded highly increasing number of forager bees, stored pollen, brood rearing and honey yield in comparison with the control (untreated colonies).

INTRODUCTION

The honeybee is of a great economic importance not only for honey production, but also for crop pollination. Each year, the bee industry in Egypt contributes high value of honeybee and Agricultural production. The mite *Varroa destructor* is an ecto-parasitoid of honeybees, being widely spread causing great damages allover the world (Bradbear, 1988). *Varroa* mite has become one of the

most serious enemies to honeybee in Egypt. This pest has caused extensive damage to the Western honeybee (*Apis mellifera* L.). The adult females and nymphs of the mite feed on heamolymph of immature and adult bees throughout the abdomen intersegmental membrane (Ritter, 1981) causing changes in heamolymph proteins and weakening the colonies ability to pollinate plants and reduce the other honeybee products (DeJong *et al.*, 1982). The danger of *Varroa* infestation depends upon the degree of infestation in honeybee colonies; lower infestation apparently causes little damage, while higher one leads to colony death (Cavalloro, 1988).

Varroa is controlled chemically (acaricides), but this technique results in the contamination of hive products with their residues (Peter, 1999 and Sanunataro *et al.*, 2004). In addition, these chemicals lead to occurrence of developed *Varroa* resistant strains (El-Zen *et al.*, 1999). Therefore, the researchers and beekeepers are requested to find out alternative softer measurements such as microbial control and natural materials, *i.e.* organic acids. From this stand point, the present work was designated and performed to evaluate the efficacy of the entomopathogenic fungi *Beauveria bassiana* (Bals.) Vuill. and *Metarhizium anisopliae* (Mets.) Sor. as biological control agents against *Varroa destructor* on honeybees compared with formic and lactic acids.

MATERIAL AND METHODS

This study was carried out at the apiary of Beekeeping Research Section at Sakha Agricultural Research Station, ARC, Kafr El-Sheikh Governorate during March-June 2006.

Fifteen Caraniolan hybrid honeybee colonies (5 combs each) headed by young sister queens, similar in strength (stored food, brood and bees) and heavily infested by the *Varroa* mite were assigned for this study. The colonies were divided into five groups of three colonies each. The percentage of *Varroa* mite infestation was estimated on adult bees 5 times, before treatment and each respective 4th day after each treatment.

Treatments

1. **The fungi, *Beauveria bassiana* and *Metarhizium anisopliae* (5×10^7 conidia/ml**

Suspensions of *B. bassiana* and *M. anisopliae* at a concentration of 5×10^7 conidia/ml water containing 0.1% Tween 80 (as spreader) were prepared according

to Mansour (1999). The suspensions were sprayed precisely to cover bees on both sides of each comb at a rate of 10 ml/colony. The spray was conducted, using an automizer, four times at 4-days intervals, repeated once again after three months.

2. Formic acid

Formic acid at 60% concentration was applied on absorbent cardboard strips (5x 10 cm) to absorb 15 ml/colony of formic acid. The treated cardboard plates were placed over the top bars of the frames at evening time four times at 4-day intervals twice during three months.

3. Lactic acid

Lactic acid at 5% concentration was sprayed on combs covered with bees at the rate of 10 ml/colony four times at 4-day intervals twice during the months.

4. Check colonies

Three colonies were smoked with classic fumes for two minutes as control.

Estimation of *Varroa* infestation

Hundred bees were randomly picked up form the experimental colonies, and anaesthetized using chloroform. Then, the bees were introduced into a beaker containing boiling water. This process ensures bee death and detachment of *Varroa*. The filtration process was practiced in two steps. The first step was used to separate bees (B) from mites using a wire screen net. The second step was used to separate *Varroa* (V) from water using filter paper. The infestation percentage with *Varroa* was calculated before treatments, and four times after each treatment at 4-day intervals using the following formula:

$$\textit{Varroa}\ \text{infestation}\ (\%) = \frac{V}{B} \times 100$$

Reduction in the percentage of infestation was calculated according to the formula of Henderson and Tilton (1955).

Number of fallen *Varroa* mites and dead bees

To evaluate the efficacy of the different treatments on the mortality of the ecto-parasitoid mite, *Varroa destructor* and their side effects on the bees, a paper board covered with vaseline was placed on the bottom board of the hives, to receive the dropping mites and bees. Dead individuals of mites and bees stuck to vaseline were counted after 48 h from each treatment. *Varroa* infestation percentage was estimated as previously mentioned. The accumulated honeybee mortality was

estimated for workers (larvae/100 inches^2, pupae/100 inches^2 and adults) and queens at 48 h after treatments.

Influence of treatments on honeybee activities

1. Number of foraging workers/colony/min was counted once a week. The foraging activity was recorded three times at 9.00 am, mid-day and 3.00 pm. The number of foragers was calculated as an average of the three times (Serag El-Dein, 1991).
2. Three colonies of each group were used to measure stored pollen and sealed brood areas at 12 days intervals using a standard frame divided into square inches by nylon threads according to Fresnay (1962).
3. Weight of the harvested honey yield (kg) was recorded from the difference between the weight of honey combs before and after honey extraction.

RESULTS AND DISCUSSION

I. Reduction Percentage in the *Varroa* infestation

Data presented in Table (1) reveal that reduction percentage in the *Varroa* mite infestation on adult of honeybees in the colonies treated with *B. bassiana* (10.1, 48.8, 55.4 and 66.9%), *M. anisopliae* (10.0, 47.4, 53.3 and 56.2%), formic acid (22.4, 38.9, 42.6 and 58.7%) and lactic acid (10.3, 33.0, 30.7 and 37.2%) after the first, second, third and fourth treatment, respectively. The respective overall percentage reductions in *Varroa* mites were 93.2, 90.3, 88.8 and 73.8%. On the contrary, the percentage of mite infestation in the control colonies during the period of treatments increased from 13.3% to 26.6%.

II. Number of fallen *Varroa* mites

The mean number of fallen mites (Table 1) in the honeybee colonies treated with the fungus *B. bassiana* (5 x 10^7 conidia/ml), *M. anisopliae* (5 x 10^7 conidia/ml), formic acid (60%) and lactic acid (5%) after 48 h of the application were 181, 173, 212 and 185 mites/colony after the first; 190, 185, 160 and 151 mites/colony after the second; 199, 171, 163 and 125 mites/colony after the third and 160, 167, 125 and 115 mites/colony after the fourth treatment, respectively.

The total recorded numbers of fallen *Varroa* mites were 730, 696, 660 and 576 mites/colony after treatments with *B. bassiana*, *M. anisopliae*, formic acid and lactic acid, respectively.

TABLE (I)

Efficacy of the entomopathogenic fungi, *B. bassiana, M. anisopliae*, formic and lactic acids as control agents against *Varroa* mite infesting honeybee colonies.

Treatments	% inf. before tr.	Applications												General mean			
		1			2			3			4						
		No. fallen mite after 48 h.	% inf. after 96 h.	% Reduction	No. fallen mite after 48 h.	% inf. after 96 h.	% Reduction	No. fallen mite after 48 h.	% inf. after 96 h.	% Reduction	No. fallen mite after 48 h.	% inf. after 96 h.	% Reduction	% infe. before tr.	No. fallen mite after 48 h.	% inf. after 96 h.	% Reduction
B. bassiana 5 x 10^7 conidia/ml	16.9	181	16.0	10.1	190	11.3	48.8	199	6.6	55.4	160	2.3	66.9	16.9	730	2.3	93.2
M. anisopliae 5 x 10^7 conidia/ml	15.0	173	14.2	10.1	185	10.3	47.4	171	6.3	53.3	167	2.9	56.2	15.0	696	3.1	90.3
Formic acid 60%	14.7	212	12.0	22.4	160	10.1	38.9	163	7.6	42.6	125	3.3	58.7	14.7	660	3.3	88.8
Lactic acid 5%	12.6	185	11.9	10.3	151	11.0	33.0	125	10.0	30.7	115	6.6	37.2	12.6	576	6.6	73.8
Control	13.3	6	14.0	-	5	19.3	-	7	25.3	-	6	26.6	-	13.3	24	26.6	-

The current investigation shows that the fungi, *B. bassiana* and *M. anisopliae* were the most effective in reducing the percentage of *Varroa* mite infestation. In this respect, Shaw *et al.* (2002) reported that one isolate of *B. bassiana* killed 100% of *Varroa destructor* within 7 days at a conidial concentration of 1 x 10^8 conidia/ml. *M. anisopliae* also killed 97% of *Varroa* mite within 7 days at the same concentration. These results indicated that entomopathogenic fungi are potential as microbial control agents of *V. destructor* in honeybee colonies. Also, these results are in agreement with those obtained by Chernov (1981); Kanga *et al.* (2002); Calderon *et al.* (2004) and Caroline *et al.* (2004).

Also, formic acid was effective and reduced the percentage of *Varroa* mite infestation. In this respect, Mutinelli *et al.* (1997) found that the efficacy of formic acid ranged between 89.6 to 94.3% in the colonies treated once a week for 4 weeks and 98.8% in the colonies treated every 3 days for 2.5-3 weeks. Similar results were obtained by Infantidis (2003). Luganskii *et al.* (1987), Okada & Nakan (1998) and Mansour (2003) used formic acid, lactic acid and oxalic acid to spray or fumigate colonies infested by *Varroa*. They observed that the mite died within 10 h after treatments with no adverse effects on both workers and queens.

III. Accumulative honeybee mortalities

The subsequent effect of treating *Varroa* mite infesting honeybee colonies with *B. bassiana, M. anisopliae,* formic and lactic acids on honeybee worker stages (larvae, pupae and adults) was investigated.

Data presented in Table (2) reveal that the accumulative honeybee mortalities in honeybee colonies treated with *B. bassiana, M. anisopliae,* formic and lactic acids and untreated colonies (control) were 13.33, 10.40, 35.33, 12.00 and 8.40 dead workers/colony; 4.33, 5.66, 10.33, 6.67 and 4.30 dead larvae/100 inches^2; 4.33, 4.90, 7.67, 4.00 and 3.40 dead pupae/100 inches^2; respectively. Except formic acid that killed 0.13% of the queens, no mortality was recorded for other treatments.

Sammataro (2004) identified isolates of fungi *B. bassiana* and *M. anisopliae* as pathogenic to *Varroa destructor* in the honeybee colonies, and each had low impact on bees, brood and other beneficials. Brownbridge (2002) evaluated the potential of the fungus *M. anisopliae* in two field trails as a microbial control agent for *Varroa* mite. He found a significant increase in the number of mites killed in hives, but it did not affect honeybee brood survival or honey production. The fungus was found to be most effective against the mites when applied as a powder to plastic strips.

Moosbeckhofer & Derakhshifar (2001) and Shawer *et al.* (1993) referred to formic acid and lactic acid as efficient chemicals for controlling *Varroa* mite in brood. They found that formic and lactic acids had no direct or side adverse effects on honeybee workers, drone or brood. Similar observations were detected by Marchetti *et al.* (1984); Ruttner *et al.* (1984) and Khater *et al.* (2005).

TABLE (II)

Effect of *B. bassiana*, *M. anisoplia*, formic acid and lactic on accumulative honeybees mortality.

Treatmentss	Accumulative honeybees mortality			
	Workers	Larvae/ 100 inches^2	Pupae/ 100 inches^2	Queen
B. bassiana 5 x 10^7 conidia/ml	13.33	4.33	4.33	0.00
M. anisopliae 5 x 10^7 conidia/ml	10.40	5.66	4.90	0.00
Formic acid 60%	35.33	10.33	7.67	0.13
Lactic acid 5%	12.00	6.67	4.00	0.00
Control	8.40	4.30	3.40	0.00

IV. Honeybee activities

Data in Table (3) show that the numbers of forager bees/colony/min, stored pollen and sealed brood areas, and honey yield in all treated colonies were better than control. The highest numbers of forager bees/colony/min, stored pollen area, sealed brood area and honey yield (63.36 bees, 300.00 inches^2, 639.33 inches^2 and 4.70 kg/colony, respectively) were obtained in colonies treated with *B. bassiana*, followed by 55.36 bees, 289.33 inches^2, 631.66 inches^2 and 4.50 kg/colony, respectively in colonies treated with *M. anisopliae*.

These results may be due to the weakness of the honeybee colonies resulted from *Varroa* infestation. Similar results were obtained by Komneli (1988) who found that *Varroa* infestation weakened the honeybee colonies, hence, decreased honey production. Serag El-Dien (1999) studied the influence of infestation by *Varroa* mite on certain honeybee activities. He found that areas of worker sealed brood measured 424.30 inches^2 in each healthy colony compared to 207.70 inches^2 in the unhealthy ones. Ramirez & Navarro (1999) found that *Varroa* mite infestation reduced areas of stored pollen from 293.83 to 151.77 inches^2/colony. In the current investigation, the extracted honey in colonies treated with formic acid, lactic acid and untreated (control) weighed 4.0, 3.6 and 1.5 kg/colony, respectively.

Table (III)

Effect of *B. bassiana, M. anisoplia,* formic and lactic acid on certain activities of honeybee colonies.

Treatments	Honeybee activities/colony			
	No. forager bees/min	Stored pollen (inches^2)	Sealed brood (inches^2)	Honey yield (kg)
B. bassiana 5 x 10^7 conidia/ml	63.36	300.00	639.33	4.7
M. anisopliae 5 x 10^7 conidia/ml	55.36	289.33	631.66	4.5
Formic acid 60%	53.60	299.32	601.67	4.0
Lactic acid 5%	51.65	271.67	552.00	3.3
Control	44.00	200.31	303.33	2.0

On the light of the previous results, it is advisable to use the entomopathogenic fungi *B. bassiana* and *M. anisopliae*, and natural materials like formic and lactic acids to control *Varroa* mite infesting honeybee colonies to avoid the appearance of mite resistant strains and products pollution.

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