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Beauveria bassiana (Balsamo), a Potential Mycopesticide for Efficient Control of the Honey Bee Ectoparasitic Mite, *Varroa destructor* Anderson and Trueman

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ABSTRACT

The ectoparasitic mite *Varroa destructor*is Anderson and Trueman is considered a destructive pest of honey bee *Apis mellifera*. Potentiality of using the entomopathogenic fungi *Beauveria bassiana* (Balsamo) as mycoacaricide against *V. destructor* was evaluated**.** Considerable effect of *B. bassiana* on Varroa mite numbers was recorded in treated colonies compared to the control. There were significant differences in mean numbers of mite mortality rates between treated and untreated colonies. Results indicated that conidial concentration of $5x10^6$ spores/g caused higher mortality rate compared to the other tested concentrations. Results showed that *B. bassiana* had a good potential on *V. destructor* when was dusted more than one time (0, 6 and 10day)**.** Scanning electronic microscopy examination of infected mite showed abundant hyphal growth and sporulation on treated mite cuticle. The results indicated that dusting of colonies with fungal conidiospores caused a reduction in the mite population reached 47.68, 33.44, 70.00 and 46.38%, 13 days after applications at concentrations of 1×10^6 conidia/g, 2.5×10^6 conidia/g, 5×10^6conidia/g, and 7.5×10^6conidia/g, respectively. The results showed also that the impact of fungus on workers bees was very low. Obtained results can suggest using *B. bassiana* as a potential effective bioacaricide against *V. destructor* in honey bee colonies.

Key words: Entomopathogenic fungi, *Beauveria bassiana*, *Varroa destructor*, *Apis mellifera.*

INTRODUCTION

Ectoparasitic mite *Varroa destructor* Anderson and Trueman is considered as dangerous pest of Western honey bee (*Apis mellifera* L.) (Hymenoptera: Apidae) (Anderson and Truemann, 2000). A great damage of honey bee colonies were caused by *V. destructor* whereas it feeds on the haemolymph of developing honey bee larvae and adult bees as well it reproduces in bee colonies (Chandler *et al*. 2001 and Martin 2001). Further, it transmits viral diseases such as Kashmir Bee Virus (KBV) (Shen *et al*., 2005) and Deformed Wing Virus (DWV) (Highfield *et al*., 2009) that causes severe damage to colonies and reduces the longevity of queen bees.

Chemical compounds such as Apistan, Coumaphos, Amitraz, Folbex, and Apitol were used extensively to control the Varroa mite population inside beehives (Fouly and Al-Dehhairi, 2009). Environmental impact of pesticides consists of increase in resistance of pests, pollution of the ecosystem and other deleterious side effects on non-target organisms. It is urgent requests to produce food safety products free from insecticide to avoid human being health problems. Alternative treatment methods for control the Varroa mite, without using chemicals to circumvent the problems of acaricides resistance, should include fast acting microbial control agents. Therefore, studies have focused on the fungal pathogens that infect arthropod hosts directly through the exoskeleton and do not have to be ingested (Chandler *et al*., 2001 and Kanga *et al*., 2002). The entomopathogenic fungus (EPF), *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycota: Hyphomycetes) was tested as biological control agent against *V. destructor* (Kanga *et al*., 2003, Meikle *et al*., 2006 and Ahmed and Abd-Elhady, 2013). Biological pesticides based on EPF as mycoinsecticides and mycoacaricides are well suited and promised control tools. There are 12 species or subspecies (varieties) of EPF have been used as active ingredients of mycoinsecticides and mycoacaricides for field applications against arthropod pests. A total of 28 products are claimed to control acarines (mites and ticks) in at least 4 families, although only three products (all based on *Hirsutella thompsonii*) were exclusively developed as acaricides (Faria and Wraight, 2007).

The present study aimed to evaluate the potentiality of using the entomopathogenic fungi *B. bassiana* as mycoacaricides against *V. destructor.*

MATERIALS AND METHODS

This study was carried out at the apiary of Agriculture Experimental Station, Faculty of Agriculture, Cairo University, Giza, Egypt in September 2013. Fifteen carniolan hybrid honeybee colonies headed by sister open mated queens equal in strength and infested by *Varroa* mite were assigned for this study. The colonies were divided into five groups of three colonies each.

Fungal inoculum

EPF *B. bassiana,* used in the experiments, was originally isolated from red palm weevil *R. ferrugineus* found at Ismailia Governorate, Egypt. The fungus was grown on autoclaved Sabouraud and dextrose yeast agar (SDAY), containing 1% peptone, 0.2% yeast extract, 4% dextrose and 1.5% agar in distilled water and incubated for two weeks at 26+1 °C.

Conidiospores production

B. bassiana erial conidia were produced using biphasic culture system (Bradley *et al*., 2002, Leland *et al*., 2005 and Sewify *et al*., 2014). Flasks (100 ml) of liquid biomalt medium (25 g biomalt and 2 g yeast extract) were incubated for 3-4 days at 25°C. The liquid cultures were then used to inoculate autoclaved white rice in sterilized plastic bags. The rice was first soaked in water for 12 hours and autoclaved for 30 min in autoclaveable plastic bags (60 x 80 cm) at the ratio of 2 kg rice/ bag at 103 k pa for 20 min. After cooling, three to four day old liquid culture of *B. bassiana* was mixed by hand with the substrate, under aseptic conditions, at the ratio of 50ml /bag. An absorbent cotton plug rolled around tube (15cm long) was used to plug each bag. The bags contained the cooled solid substrates were connected with the source of filtered air through air valves. Solid substrate fermentation was kept for 11 days at 26°C. The culture was observed daily and crumbled by hand within bags to prevent binding of the substrate. Whole culture was then transferred to wooden screen shelves where it was dried for 7 days at 28°C. Conidia were harvested by mechanical sieving.

Field application

Harvested conidiospoeres were mixed with talc powder to prepare the tested concentrations of $1x10^6$ conidia/g, $2.5x10^6$ conidia/g, $5x10^6$ conidia/g, and 7.5×10^6 conidia/g. Twelve colonies were identified as being infested with *V. destructor* and divided into four treatments. Each treatment was dusted with one concentration of each by using 600w insecticide dust blower**.** Dusted conidiospores were directed to spaces between wood frames. Formulated fungal conidiospores were applied three times at three day interval. Three colonies were dusted by talc powder as control treatment. Applications were carried out just before sunset, when all honeybees had returned to the hives**.**

Effect of fungal treatment on mite mortality

A strong white sheet, coated with vaseline, was inserted on hive' s floor. The sheet was removed daily and the fallen mites were counted (Calderone and Turcotte.1998). The mites were transferred using a camel-hair brush into new Petri-dish with moistened filter paper and incubated at 25ºC to observe the outgrowth of fungus and the numbers of dead mites with external hyphae. Data were recorded daily. Only mites showed hyphal growth were considered to have acquired and died by fungal infection (Kanga *et al*., 2003).

Effect of fungal treatment on mite infested adult bees

A wide mouthed jar with a lid of which the centre part is replaced by a 2mm hardware cloth or . A count of 100 bees were collected in the jar that covered with the lid..Table spoon (at least 7g) of powdered sugar was poured through the mesh then, the jar was rolled to cover all the bees with sugar and let stand for 1 min and was turned upside down over a white surface. The number of mites fallen out of the jar and bees in the washed sample was counted. One hundred of worker bees were examined. The counted number of mites divided by the number of bees in the sample and multiply by 100 determined the number of mites per 100 bees. Reduction percentage of mite population was calculated according to Henderson &Tilton's equation (1955).

Effect of fungal treatment on worker bees' mortality

The fallen dead bee workers on the white sheet were counted. Then they were transferred into new Petri-dish with moistened filter paper and incubated at 25ºC to observe the outgrowth of fungus.

Scanning electron microscopy examination

Dead mites resulted from fungal treatment in colonies three days after application at concentration of $5x10^6$ conidia/g. were used for SEM examination. Direct osmium tetroxide vapor fixation was used according to Brey *et al.* (1985). Inoculated Varroa specimens were transferred to watch glass, which was placed in a closed Petri dish overnight. After fixation in OSO4 vapor, the Varroa was directly mounted, coated with 15 nm of gold and examined by a JEN-JSM- 5200 scanning electron microscope.

RESULTS AND DISCUSSION

Effect of fungal treatment on mite mortality

Considerable effect of *B. bassiana* on fungal infected Varroa mites' numbers was noticed in treated colonies compared with control. Fungal infected mites were varied according to fungal conidiospores concentrations. Effect of fungal application on fungal infected mites was illustrated in figs. (1&2). There were significant differences in mean numbers of dead mites between treated and untreated colonies. Results indicated that the fungal concentration at $5x10^6$ spores/g caused high mortality rates compared with the other concentrations and control whereas the, total mean numbers of dead mites were 1.44, 6.37,

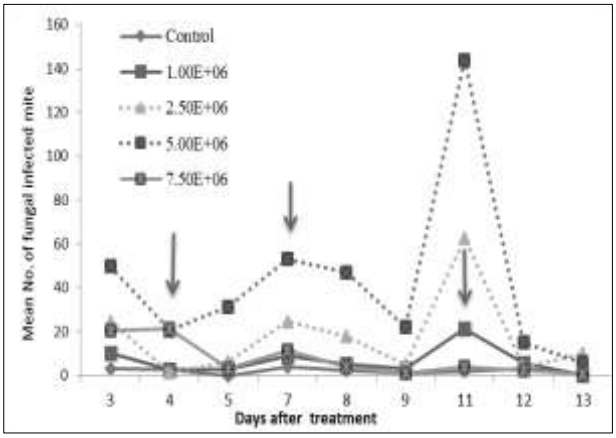

Fig. (1): Effect of the fungus *B. bassiana* on mean numbers of fungal infected the mite *V. destructor* (Arrows indicate times of treatment)

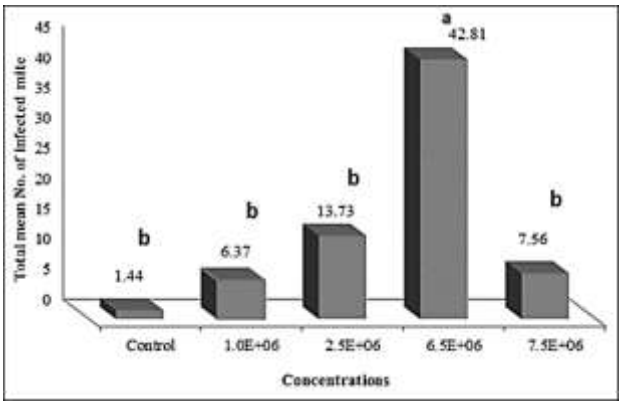

Fig. (2): Effect of the fungus *B. bassiana* on mortality rate of the mite *V. destructor*. Means with the same superscripted letters within the same column are not significantly different (P≥0.05).

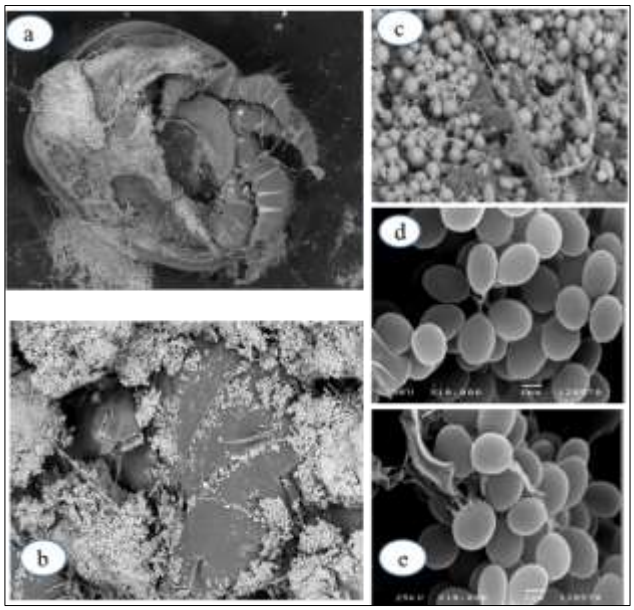

Fig. (3): SEM of *V. destructor* cuticle infected with *B. bassiana*. (a) Abundant hyphae on cuticle surface 2 days after treatment. (b, c) Extensive sporulation on Varroa mite cuticle 3days after treatment, and (d, e) typical sympodial (zig-zig) *B. bassiana* conidiophores.

13.73, 42.81, and 7.56 for control, $1x10^6$, $2.5x10^6$, $5x10^6$, and $7.5x10^6$ conidia/g, respectively. *V. destructor* did not well controlled when exposed to only one dusting application. The results suggest that *B. bassiana* has a good potential against *V. destructor* when it was dusted more than one time (0, 6 and 10 days). EPF have been used successfully against different arthropod pests (Abolins *et al*., 2007). Several studies have investigated the use of *B. bassiana* against the ectoparasitic mite *V. destructor*. Results revealed that dusting application of dry conidiospores of *B. bassiana* to beehives caused substantial effect the mortality of Varroa mite. This is in line with the previous study of Ahmed and Abd-Elhady, (2013). The two commercial preparations; Biovar (*B. bassiana*) and Bioranza (*M. anisopliae*), through their applications into the beehives against Varroa mite were evaluated. Significant effect for mortality of both fungi on Varroa mite was found. The fungal formulated spores provided successful control when it was applied twice (day 0 and day10). The results showed that the mean numbers of dead mites was decreased with increasing the conidial concentration to $7.5x10^6$ spores/g. Goettel *et al*. (1993) reported a negative correlation between dose and mortality at concentrations greater than 10^4 ascospores of *Ascosphaera aggregate* per leaf-cutting bee *Megacbilero tundata*, larva. Efficacy of EPF against *Varroa* is not constant (Meikle *et al*. 2006). Gerritsen and Cornelissen (2006) found that *M. anisopliae* and *Lecanicillium lecanii* were not effective against *Varroa* within beehives while Hamiduzzaman *et al*. (2012) found a good ability of the EPF to reduce *Varroa* damages to honey bees. In Rodríguez *et al*. (2009) study, *M. anisopliae* had high pathogenic capacity against *Varroa* with a mortality rate of 85% and with good ability to tolerate beehive temperature.

Scanning electron microscopy examination

SEM examination was carried out to describe the infection and fungal *B. bassiana* growth on Varroa mite cuticle after fungal treatment in beehives. The Varroa mite which was treated by dry conidia of the fungus showed signs of infection within 2-3 days. The examinations showed abundant hyphae on cuticle surface 2 days post treatment (Fig. 3a). Extensive sporulation was noticed on Varroa mite cuticle 3 days after treatment (Fig. 3 b, c). Formation of typical sympodial (zig-zig) *B. bassiana* conidiophores were found on mite cuticle (Fig. 3d, e). Ahmed and Abd-Elhady (2013) described external development of *M. anisopliae* and *B. bassiana* on Varroa mite cuticle. They explained the sequence of events in the infection of *V. destructor*.

Table (1): Effect of the fungus *B. bassiana* on mite infestation living on workers bees and reduction % at different concentrations

Fungal concentrations (spores/g.)	% mite infestation at indicated days				% reduction of infestation
	0	5	9	13	
control	18.00	16.00	15.30	12.00	
10^6	41.00	40.30	23.00	14.30	47.68
2.5x 10^6	48.00	46.00	23.00	21.30	33.44
5 x 10^6	25.00	24.30	7.30	5.00	70.00
7.5x 10^6	40.00	35.30	27.30	14.30	46.38

Table (2): Effect of *B. bassiana* on workers bees at different concentrations

Days after treatment	Control	Mean numbers of infected worker bees at indicated concentrations (spores/g.)			
		10^6	2.5 X 10^6	5 X 10^6	7.5 X 10^6
1	0.67	6.00	4.67	7.00	0.00
2	0.33	0.33	0.00	2.33	0.00
3	0.00	0.00	0.00	1.33	0.00
5	2.33	0.67	11.00	10.67	13.33
6	0.67	0.00	0.00	0.67	0.00
7	0.00	0.00	0.33	3.00	0.00
9	3.33	9.33	7.33	11.00	13.33
10	1.67	0.00	0.00	2.00	0.33
11	1.00	0.00	1.00	0.00	0.67
Mean	1.11^a	1.81^a	2.70^a	4.22^a	3.07^a

Means with the same superscripted letters within the same column are not significantly different (P≥0.05).

Effect of fungal treatment on mite infested adult bees

Infestation levels of mite living on workers bees were affected as the result of fungal application. The population of *V. destructor* decreased slightly 5 days after first fungal application at all tested concentrations and then much decrease was occurred after the second and third applications (Table, 1). For 13 days after first application, the percentage of mite infestation reached 12, 14.30, 21.30, 5 and 14.30% compared to 18, 41, 48, 25 and 40% in zero time, at concentrations of control, 1 $\times 10^6$, 2.5 $\times 10^6$, 5 $\times 10^6$ and 7.5$\times 10^6$ conidia/g., respectively. Results in table (1) indicated that the dusting of colonies with fungal conidiospores caused a reduction in the mite population reached to 47.68, 33.44, 70.00 and 46.38%, 13 days after applications at the tested concentrations of 1x10^6, 2.5x10^6, 5x10^6 and 7.5$\times 10^6$ conidia/g, respectively. Greatest reduction (70%) occurred at the concentration of 5x10^6 conidia/g compared to other tested concentration. The results shown in table (1) indicated that dusting of colonies with fungal conidiospores caused a reduction in the mite population reached 47.68, 33.44, 70.00 and 46.38%, 13 days after applications at the tested concentrations, respectively. This result coincides with previous study of Kanga *et al.* (2003) who reported that the mite infestation levels on adult were significantly reduced after application with fungus *M. anisopliae*.

Obtained results in table (2) showed that the impact of the fungus on workers bees was very low. Insignificant differences in worker bees' mortality rates were found between each of the different treated concentrations and control. Results showed that number of infected worker bees varied according to fungal conidiospores concentration, whereas the mean numbers were 1.11, 1.81, 2.70, 4.22 and 3.07 for control, 1 $\times 10^6$, 2.5 $\times 10^6$,5 $\times 10^6$, and 7.5$\times 10^6$conidia/g, respectively. Highest infected numbers of worker bees occurred at the concentration of 5$\times 10^6$ conidia/g. These findings coincide with the previous study of Alves *et al*., (1996). They reported that *B. bassiana* and *M. anisopliae* caused low mortality for infected bees in treated hives but without noticeable effect on bee behavior, larval development or colony. Almazara'awi (2007) found that, three isolates of *B. bassiana* caused high mortalities to the caged bees when dusted with a formulation at high concentration. However, exposure of honey bee hives to high densities of *B. bassiana* resulted in very low mortality. The recent study suggests that *B. bassiana* can be used for controlling *V. destructor* and most likely does not cause bee mortality following application.

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