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Susceptibility of *Bryobia cristata* (Acari: Tetranychidae) adults to infection by *Metarhizium anisopliae* and *Beauveria bassiana*

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ABSTRACT

Laboratory bioassay of the two entomopathogenic fungi *Metarhizium anisopliae* (Metschnikoff) Sorokin and *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota : Hypocreales) against *Bryobia cristata* (Dugès) (Acari: Tetranychidae) adults was evaluated. *M. anisopliae* had higher activity and rapid mortality against mite adults. The correspondent LC_{50} for *M. anisopliae* and *B. bassiana* was $1.82x10^6$ and $2.98x10^6$ spores/ml, respectively. The LT_{50} at dosage 10^8 spores/ml of *M. anisopliae* and *B. bassiana* was 2.2 and 4.08 days, respectively.

Key Words: *Bryobia cristata*, Entomopathogenic fungi, *Metarhizium anisopliae, Beauveria bassiana,* bioassays.

INTRODUCTION

Bryobia cristata (Tetranychidae) is a plant feeder with a long list of known hosts. This list includes legumes (such as alfalfa, peas, and clovers), various weed species found in lawns, lawn grasses, vegetables, fruit trees (e.g., plume, apple, orcherry trees), certain ornamental plant shrubs and trees. *B. cristata* was recorded in Egypt on wormseed grass *Chenopodium ambrosioides* L. at Dakahlia Governorate (Zaher 1984). The wide use of acaricides caused many problem's such as environmental pollution, distruction in the natural balance between the pest and natural enemy. Fungal pathogens as biocontrol agents now are accepted and have become important as one of the biological control components in the IPM programs (Nugroho and Bin Ibrahim 2004). Entomopathogenic fungi like *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin (Ascomycota: Hypocreales) are density-dependent mortality factors in susceptible host populations and successful infection depends on the host receiving a threshold of infective conidia (Vandenburg *et al.*, 1998; Hughes *et al.*, 2004). This study was conducted to investigate the potential importance of the two entomopathogenic fungi *B. bassiana* and *M. anisopliae* against *B. cristata* adults.

MATERIALS AND METHODS

Experimental mite cultures

B. cristata was collected from infested malva plants (*Malva sylvestris*) from the Faculty of Agriculture, Cairo University, Giza, Egypt. *B. cristata* was maintained on malva leaves upside down on moisten cotton pads in fiber-dishes (20 cm in diameter) at 25±1 °C. The cotton pads were moistened daily and all the ends of the leaves were covered with wet cotton to avoid disc dryness and to prevent mite escape. Mites were transferred on fresh malva leaves every 3 days. Newly deposited eggs were singly transferred using a fine brush to the aforementioned prepared leaves.

Entomopathogenic fungi isolates

Two isolates of entomopathogenic fungi were used in this study. *B. bassiana* was originally isolated from *Aphis gossypii* and *M. anisopliae* from soil at Giza Governorate , Egypt. The fungus was grown on autoclaved Sabourad dexstrose yeast agar (SDAY), containing 1% peptone, 0.2% yeast extract, 4% dextrose and 1.5% agar dissiloved in 1L distilled water and incubated for two weeks at 25±1 °C (Nada 2006).

Bioassay procedure

Spores were harvested by rinsing with sterilized aqueous solution of 0.02% Tween 80, then filtered through cheesecloth to reduce mycelium clumping. The spores were counted in the suspension using a haemocytometer (Neubauer Improved HBG, Germany 0.100 mm x 0.0025 mm^2). Five concentrations: 10^6, $5x10^6$, 10^7, $5x10^7$ and 10^8 spores/ml of each isolate were prepared. Aqueous solution of 0.02% Tween 80 was used as control.

Fresh uninfested malva leaves were transferred to the laboratory and cleaned by tab water. Leaf discs 1.5 cm in diameter were submerged into a suspension of *B. bassiana* and *M. anisopliae* concentrations for 10 second as well as aqueous solution of 0.02% Tween 80 for control. The leaf discs were left to air dry on tissue paper and then transferred upside down to Petri dishs prepared with moistened cotton pads. Each four discs were placed in one Petri dish. Eight replicates for each treatment were used. Five *B. cristata* adults were placed on

each treated leaf disc and kept at 25±1°C. The cotton pads were moistened daily and all the ends of the leaf discs were covered with wet cotton to avoid disc dryness and to prevent mite from escaping. The leaf discs were changed by fresh clear ones from time to time when necessary. Percentage mortality was assessed daily from 3 to 6 days after inoculation. All dead mites were removed from the Petri dishes and kept in an incubator and the cadavers showing mycosis were considered to be dead as a result of fungus infection.

Statistical analysis

The concentration and time mortality responses were subjected to probit analysis (Finney, 1971). Susctiplity index according to the methods described by Khidr *et al.* (2004) were estimated.

Susceptibility index =

$$\frac{LC_{25},\ LC_{50} \text{ and } LC_{90} \text{ of } M.\ anisopliae}{LC_{25},\ LC_{50} \text{ and } LC_{90} \text{ of } B.\ bassiana} \times 100$$

RESULTS AND DISCUSSION

The efficiency of the two isolated entomopathogenic fungi, *B. bassiana* and *M. anisopliae* against adult *B. cristata* was presented in Tables (1 & 2). The obtained results revealed that the mite was highly susceptible to both *B. bassiana* and *M. anisopliae*. Fungi sporulated outside the body of mites. This mite entered the state of moribund two days after treatment in the high concentrations (i.e. $5x10^7$ and 10^8 spores/ml). The fungal sporulation was observed over the surface of the cadaver 4-5 days after treatment Fig (1). Alves *et al,*. (2005) reported that *B. bassiana* was considered a pathogen of citrus rust mite *Phyllocoptruta oleivora* (Ashmead). Spores of *B. bassiana* were found to adhere all over the mite body surface, especially at the anal region, where vegetative mycelium was found entering the mite body. The formation of small crystals was noticed inside the mite bodies that were produced during colonization on the mite body cavity by the fungus.

Efficiency based on slope and LC_{25}, LC_{50} and LC_{90} values

Results of the slope, LC_{25}, LC_{50} and LC_{90} values represented in Table (1) proved that *M. anisopliae* had higher activity against *B. cristata* adults than *B. bassiana*. The correspondent LC_{25}, LC_{50} and LC_{90} were $2.31x10^5$, $1.82x10^6$ and $8.8x10^7$ spores/ml, respectively for *M. anisopliae* and $3.74x10^6$, $2.98x10^6$ and $1.6x10^8$ spores/ml for *B. bassiana*, respectively. Based on the slope values, the slope of *M. anisopliae* was steeper than that of *B. bassiana* (i.e. 0.77 and 2.7, respectively).

Efficiency based on slope and LT_{25}, LT_{50} and LT_{90} values

The data reported in Table (2) showed that *B. cristata* adult treated with *M. anisopliae* had higher susceptibility as well as a rapid mortality at concentration of 10^8 spores/ml. The LT_{25}, LT_{50} and LT_{90} were 1.34, 2.2 and 5.85 days, respectively. *B. bassiana* corresponding values were 3.18, 4.08 and 6.6 days respectively. Based on the slope values, the slope of *M. anisopliae* was steeper than that of *B. bassiana* (i.e. 3 and 6, respectively).

Susceptibility index was used to find out the relative of susceptibility of *B. cristata* adults to tested entomopathogenic fungi isolates.

Results summarized in Tables (1&2) showed that the susceptibility index of *B. bassiana* based on the LC_{25}, LC_{50} and LC_{90} were 61.75, 61.07 and 55%, respectively as effective as *M. anisopliae* against *B. cristata* adults. On the other hand efficacy time of *B. bassiana* at LT_{25}, LT_{50} and LT_{90} were 50, 53.92 and 88.64% , respectively as effective as the effectiveness of *M. anisopliae* against *B. cristata* adults.

Table (1) Estimated LC values and susceptibility index for the effect of *Metarhizium anisopliae* and *Beauveria bassiana* against, *Bryobia cristata*

	slope	LC_{25}	LC_{50}	LC_{90}	Susceptibility index relative to		
					LC_{25}	LC_{50}	LC_{90}
M. anisopliae	0.77	$2.31x10^5$	$1.82x10^6$	$8.8x10^7$	100	100	100
B. bassiana.	2.7	$3.74x10^5$	$2.98x10^6$	$1.6x10^8$	61.76	61.07	55

Table (2) Estimated LT values and susceptibility index for the effect of *Metarhizium anisopliae* and *Beauveria bassiana* against, *Bryobia cristata* at 10^8 spores/ml

	slope	LT_{25}	LT_{50}	LT_{90}	Susceptibility index relative to		
					LT_{25}	LT_{50}	LT_{90}
M. anisopliae	3	1.34	2.2	5.85	100	100	100
B. bassiana.	6	3.18	4.08	6.6	50	53.92	88.64

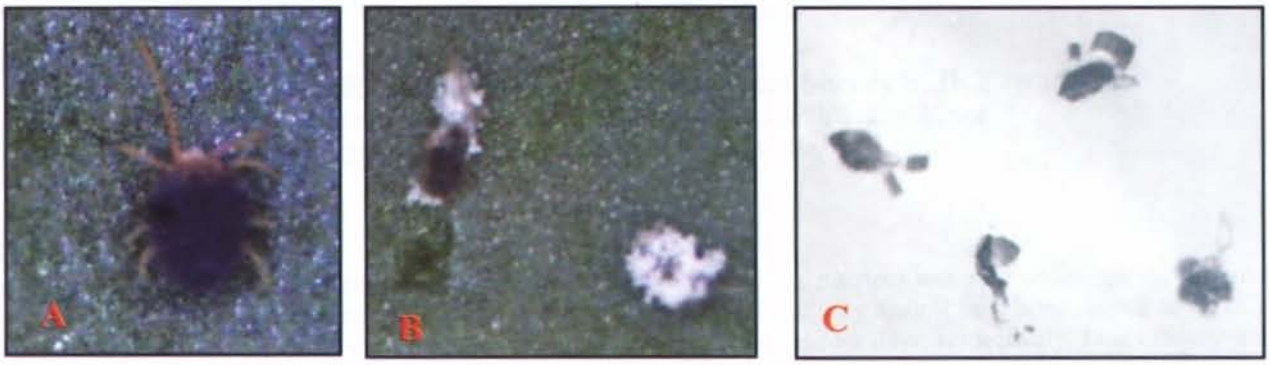

Fig. (1): (A) Healthy adult, *B. cristata* -(B) Sporulation cadavers infected with *B. bassiana* showing white mycelial growth- (C) Sporulation cadavers infected by *M. anisopliae* showing laterally adherent conidia chains .

B. bassiana and *M. anisopliae* had high pathogenic activity against *Tetranychus urticae* (Koch) (Hassan 2008). Similar susceptibility of the broad mite *Polyphagotarsonemus latus* Bank to the isolated entomopathogenic fungi *Paecilomyces fumosoroseus, B. bassiana* and *M. anisopliae* was obtained by Nugroho and Ibrahim 2004. Our results represent the first report on the pathogenic effect of the two entomopathogenic fungi *M. anisopliae* and *B. bassiana* against *B. cristata* adults.

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