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Efficacy of the Entomopathogenic Fungi; *Beauverai bassiana* and *Metarhizium anisopliae* on Some Insect Pests under Laboratory Conditions

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ABSTRACT

Pathogencity of two entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae* isolates was evaluated against the beet fly, *Pegomyia mixta* Vill., the sugar beet moth, *Scrobipalpa ocellatella* Boyd. and the cowpea aphid, *Aphis craccivora* Koch. under laboratory conditions. Obtained results showed that the third instar larvae of *P. mixta* and *S. ocellatella* were affected by both isolates than the pupae after the tenth day post treatment. Mortality occurred in larvae from the third day of treatment, while it occurred in pupae after the fourth day. *A. craccivora* was slightly affected by both isolates at all concentrations. Maximum mortality percent (32.6%) took place with the third concentration (2 x 10^5 conidia /ml) of *M. anisopliae* isolate. *M. anisopliae* isolate was more effective against the three pests than *B. bassiana* isolates. These results suggested that both *B. bassiana* and *M. anisopliae* isolates were virulent against the larvae and pupae of *P. mixta* and *S. ocellatella* but less virulent to *A. craccivora*.

Key words: *Pegomyia mixta, Scrobipalpa ocellatella, Aphis craccivora,* Entomopathogenic fungi.

INTRODUCTION

In recent years, development of resistance to pesticides has prompted researches into novel biological control. One such agent is entomopathogenic fungi. Moreover, the over use of pesticides resulted in environmental pollution as well as adverse effects on the health of human and other organisms (Van *et al.*, 2007). The sugar-beet fly, *Pegomyia mixta* Vill. and the sugar beet moth, *Scrobipalpa ocellatella* Boyd. are considered the most serious pests attacking sugar-beet in Egypt (Hafez *et al.*, 1970). As well is the cowpea aphid, *Aphis craccivora* Koch. *Metarhizium anisopliae* is widely distributed insect pathogen with adverse host range (Tanada and Kaya, 1993). Success of *M. anisopliae* infection has been achieved with adults and immature stages of insects (Ferron, 1978). Campbell *et al.* (2000) used *M. anisopliae* isolate to protect sugar beet plants from sugar beet maggot and found that control by *M. anisopliae* was equal to that obtained from chlorpyrifos (organophosphors insecticides). There are various strains of entomopathogenic fungi used for the control of aphids and other pests such as; *Verticillium* sp. (Jackson *et al.*, 1985), *Beauveria bassiana* (Quesada *et al.*, 2006), *M. anisopliae*, *Paecilomyces* sp. (Shia and Feng, 2004) and *Nomuraea rileyi* (Devi *et al.* 2003). Mesbah *et al.* (2004) found that *B. bassiana* reduced the percent of infestation with *P. mixta* to 6.53 and 35.53% and *S. ocellatella* to 39.42 & 26.43% in 1999 and 2000 seasons, respectively. Abubakar *et al.* (2000) evaluated four isolates from the pathogenic fungi *B. bassiana* and *M. anisopliae* against *A. craccivora* under laboratory conditions. The results indicated that these isolates are promising candidates for the control of the aphid species. Nyle *et al.* (2005) compared the efficacy of *B. bassiana* and *M. anisopliae* against sugar beet maggot. Comparison indicated that *M. anisopliae* had greater potential for the control of the sugar beet maggot larvae than *B. bassiana*.

The present work aims to evaluate the efficacy of *B. bassiana* and *M. anisopliae* isolates against the third instar larvae and pupa of beet fly, *P. mixta* and sugar beet moth, *S. ocellatella*, as well as the aphid species, *A. craccivora* individuals under laboratory conditions.

MATERIALS AND METHODS

Fungi cultures

The entomopathogenic fungi, *B. bassiana* and *M. anisopliae* were isolated from the soil in El-Behira Governorate, and grown on peptone media (10g Peptone, 40g Dextrose, 2gm Yeast extract, 15gm Agar and 500 ml. Chloramphenicol and completed to one liter by distilled water). The medium was autoclaved at 120 °C for 20 minutes, poured in Petri-dishes (12 cm diameter) then inoculated with the fungus isolate and kept at 25 ± 2°C and 85 ± 5% R.H. Fungal isolates were re-cultured every 14-30 days and kept at 4 °C.

To obtain sufficient quantity of conidia, both *B. bassiana* and *M. anisopliae* isolates were propagated on wetted rice. Two kilograms wetted rice were washed in boiled water for 10 min. and packed in thermal bags. These bags were autoclaved at 120 °C for 20 min., then inoculated by both isolates and incubated at 26 ± 1 °C for 15 days. The conidia were harvested by distilled water and filtered through cheese cloth to reduce mycelium clumps and Tween 80% was added (Lacey 1997).

Preparing of the concentrations

Conidia of fungal isolates harvested by rinsing with sterilized water and 0.5% Tween 80 from 14 days old culture rice media. The suspensions were filtered through cheese cloth to reduce mycelium clumps. Conidia were counted in the suspension using a haemocytometer (Hirscmann 0.1 mm x 0.0025 mm 2). The suspension was put in plastic bottles (2 liter). To maintain the virulence of the isolates, the isolates were passed through insect host, wax moth larvae *Galleria mellonella* L. Three concentrations were prepared, (C_1) $2x10^3$, (C_2) $2x10^4$ and (C_3) $2x10^5$ conidia/ml for the two isolates.

Rearing the insects

P. mixta and *S. ocellatella* were reared in the laboratory by collecting the larvae from infested leaves of sugar beet and kept them in glass jars. The larvae were fed on fresh clean sugar beet leaves until pupated. The cowpea aphid was reared on broad bean plants (10 day old). These plants were cultivated in small pots (8 cm diameter, one plant /pot) under laboratory conditions (20±1 °C, 70±5 % RH and photoperiod 16 L: 8 D for several generations). The pots were individually enclosed in glass cylinders (10 cm diameter, 22 cm long). The top of each cylinder was covered with muslin held in place with rubber bands.

Bioassay procedure

1- Effect of *B. bassiana* and *M. anisopliae* on the larvae of *P. mixta* and *S. ocellatella*

Third instar larvae of *P. mixta* and *S. ocellatella* were fed on sugar beet leaves treated with different fungus concentrations of $2x10^3$, $2x10^4$ and $2x10^5$ conidia/ ml. and each concentration included three replicates. Each replicate contained five larvae. Another three replicates were treated with water as a control.

2- Effect of *B. bassiana* and *M. anisopliae* on the pupae of *P. mixta* and *S. ocellatella*

Pupae of *P. mixta* and *S. ocellatella* were placed in Petri dishes (12 cm diameter) on a wetted filter paper and sprayed with the tested fungal concentrations. Each concentration included three replicates; each replicate contained five pupae. Another three replicates were treated with water as a control.

3- Effect of *B. bassiana* and *M. anisopliae* on *A. craccivora* individuals

The broad bean plants infested with *A. craccivora* (adults and nymphs) were sprayed by tested concentrations and the percent of mortalities was calculated by Abbot's formula (1925) as follow:

$$\frac{\text{Survival \% in control} - \text{survival \% in treatment}}{\text{Survival \% in control}} \text{ X } 100$$

Treatments incubated at 25±2°C and 70±5% R.H. and inspected daily. Control was treated with distilled water. Manual spryer was used for spraying.

Statistical analysis

Data were analyzed by analysis of variance (one ways classification ANOVA) followed by a least significant difference, L.S.D at 5% (Costat Statistical Software, 1990).

RESULTS AND DISCUSSION

1- Effect of *B. bassiana* and *M. anisopliae* isolates on *P. mixta* larvae

Mortality among third instar larvae occurred in the fourth day of treatment and gradually increased to reach 100% in the ninth day (Table 1). Highest mortality was observed in the ninth day (the third concentration in *M. anisopliae* treatment). The percent of mortality was 100% in all concentrations of *M. anisopliae* and the third concentration only in *B. bassiana* in the tenth day post treatment. This

Table (1): Mortality percents of *P. mixta* larvae treated with *B. bassiana* and *M. anisopliae* under laboratory conditions

Days after treatment	control	Percent of mortality						L.S.D
		Beauveria bassiana			*Metarhizium anisopliae*			
		C_1	C_2	C_3	C_1	C_2	C_3	
2^{nd}	0	0	0	0	0	0	0	----
3^{rd}	0	0	0	0	0	0	0	----
4^{th}	0^a	2.0^a	3.0^a	15.3^b	25.3^c	33.3^d	37.3^d	7.28
5^{th}	0^a	4.3^a	5.0^a	20.0^b	30.3^c	38.3^d	42.3^d	8.19
6^{th}	0^a	6.6^a	6.6^a	30.2^b	35.2^{bc}	40.2^{cd}	45.6^d	6.6
7^{th}	0^a	6.6^b	13.3^c	54.6^d	54.9^d	65.7^c	76.3^f	6.3
8^{th}	0^a	13.3^b	26.7^c	73.3^d	78.8^d	80.9^d	95.5^c	6.9
9^{th}	6^a	26.6^b	35.2^b	85.5^c	90.3^{cd}	95.9^{cd}	100^d	10.4
10^{th}	14^a	46.6^b	75.9^c	100^d	100^d	100^d	100^d	10.5

*Means under each variety sharing the same letter in a column are not significantly different at P<0.05.

Table (2): Mortality percents of *P. mixta* pupae treated with *B. bassiana* and *M. anisopliae* isolates under laboratory conditions

Days after treatment	Percent of mortality							L.S.D
	control	*Beauveria bassiana*			*Metarhizium anisopliae*			
		C_1	C_2	C_3	C_1	C_2	C_3	
2nd	0	0	0	0	0	0	0	---
3rd	0	0	0	0	0	0	0	----
4th	0	0	0	0	0	0	0	---
5th	0	0	0	0	0	0	0	----
6th	0^{a}	0^{a}	0^{a}	7.0^{b}	20.0^{d}	13.3^{c}	33.3^{e}	4.6
7th	0^{a}	7^{b}	13.3^{b}	33.0^{c}	20.0^{bc}	13.3^{c}	33.3^{d}	6.1
8th	0^{a}	13.3^{b}	47.0^{c}	40.0^{c}	27.0^{b}	53.3^{c}	53.3^{c}	9.9
9th	0^{a}	26.7^{b}	47.0^{c}	47.0^{c}	33.0^{b}	80.0^{d}	93.3^{e}	7.4
10th	6^{a}	46.7^{b}	67.0^{c}	100^{e}	67.0^{c}	86.7^{d}	100^{e}	5.6

*Means under each variety sharing the same letter in a column are not significantly different at P<0.05

Table (3): Mortality percents of *Scrobipalpa ocellatella* larvae treated with *M. anisopliae* and *B.bassiana* under laboratory conditions

Days after treatment	Percent of mortality							L.S.D
	control	*Beauveria bassiana*			*Metarhizium anisopliae*			
		C_1	C_2	C_3	C_1	C_2	C_3	
2nd	0	0	0	0	0	0	0	--
3rd	0	0	0	0	0	0	0	--
4th	0	0	0	0	0	0	0	---
5th	0^{a}	34^{b}	34^{b}	48^{bc}	46^{bc}	60^{c}	74^{d}	11.7
6th	0^{a}	34^{b}	40^{b}	60^{c}	54^{c}	60^{c}	80^{d}	6.7
7th	0^{a}	40^{b}	60^{c}	60^{c}	60^{c}	66^{c}	94^{d}	9.7
8th	0^{a}	54^{b}	68^{c}	74^{c}	66^{c}	86^{d}	100^{e}	8.7
9th	0^{a}	66^{b}	68^{b}	80^{c}	80^{c}	86^{c}	100^{d}	9.3
10th	6^{a}	74^{b}	87^{b}	94^{cd}	86^{c}	94^{cd}	100^{d}	7.7

*Means under each variety sharing the same letter in a column are not significantly different at P<0.05

Table (4): Mortality percents of *S. ocellatella* pupae treated with *B. bassiana* and *M. anisopliae* isolates under laboratory conditions.

Days after treatment	Percent of mortality							L.S.D
	control	*Beauveria bassiana*			*Metarhizium anisopliae*			
		C_1	C_2	C_3	C_1	C_2	C_3	
2nd	0	0	0	0	0	0	0	--
3rd	0	0	0	0	0	0	0	--
4th	0	0	0	0	0	0	0	--
5th	0	0	0	0	0	0	0	--
6th	0^{a}	6^{b}	6^{b}	6^{b}	0^{a}	6^{b}	14^{c}	3.6
7th	0^{a}	20^{b}	34^{c}	46^{d}	34^{c}	46^{d}	٦٤^{e}	8.1
8th	0^{a}	26^{b}	40^{c}	54^{d}	40^{c}	60^{de}	٦٦^{e}	8.7
9th	0^{a}	40^{b}	60^{c}	66^{c}	66^{c}	80^{d}	86^{d}	9.2
10th	0^{a}	54^{b}	66^{c}	74^{cd}	80^{d}	86^{d}	100^{e}	9.5

*Means under each variety sharing the same letter in a column are not significantly different at P<0.05

Table (5): Mortality percents of *Aphis craccivora* individuals treated with *M. anisopliae* and *B. bassiana* under laboratory conditions

Days after treatment	Percent of mortality							L.S.D
	Control	*Beauveria bassiana*			*Metarhizium anisopliae*			
		C_1	C_2	C_3	C_1	C_2	C_3	
2nd	0	0	0	0	0	0	0	--
3rd	1^{a}	1.3^{b}	4^{c}	6.9^{d}	2.4^{b}	2.3^{b}	7.2^{d}	1.02
4th	2.1^{a}	2.2^{a}	4.5^{b}	9.6^{c}	4.4^{b}	4.6^{b}	14.5^{d}	8.1
5th	2.1^{a}	2.2^{a}	6.8^{b}	14.9^{d}	6.4^{b}	10.4^{c}	23.1^{e}	8.7
6th	3^{a}	4^{a}	8.6^{b}	20.2^{d}	8.3^{b}	17.3^{c}	26.2^{e}	1.2
7th	4^{a}	6.2^{b}	11.4^{c}	29.8^{e}	10.4^{c}	19.7^{d}	32.6^{f}	1.13

*Means under each variety sharing the same letter in a column are not significantly different at P<0.05.

means that the third instar larvae of *P. mixta* were affected by *M. anisopliae* than *B. bassiana*.

Statistical analysis showed that, there was a significant difference between the third concentration in *M. anisopliae* isolate and other concentrations in the eighth day. The less significant difference (L.S.D) was 6.9.

2- Effect of *B. bassiana* and *M. anisopliae* on *P. mixta* pupae

As mentioned in table (2), mortalities in pupae occurred in the sixth day for the third concentration ($2x10^5$conidia/ml) of *B. bassiana* treatments and all concentrations of *M. anisopliae* treatments. Mortality occurred in all concentrations in the seventh day post treatment and gradually increased to reach 100% in the tenth day by the third concentration of *B. bassiana* and *M. anisopliae* only.

Statistical analysis showed significant difference between the third concentration of both fungi and the other concentrations.

Data in tables (1 and 2) and figures (1 and 3) showed that the third instar larvae were affected than the pupae. Mortality occurred in the fourth day post treatment in larvae, while it appeared in the sixth day in pupae. Percent of larval mortality reached 100% (the third concentration in *M. anisopliae*) in the ninth day post treatment.

These results mean that larvae were more susceptible to the entomopathogenic fungi, *B. bassiana* and *M. anisopliae* than their pupae.

3- Effect of *B. bassiana* and *M. anisopliae* on *S. ocellatella* larvae

Data in table (3) and figure (2) showed that the percent of mortality increased sharply in the fifth day post treatment. The percent of mortality was 74% in the third concentration of *M. anisopliae* isolates. and reached 100% in the eighth day. Data cleared that the larvae of *S. ocellatella* were affected by *M. anisopliae* more than *B. bassiana*.

The percents of mortality in the tenth day of *B. bassiana* treatment were 74, 87, and 94% in C_1, C_2 and C_3, respectively. Corresponding results in *M. anisopliae* treatments were 86, 94 and 100%.

4- Effect of *B. bassiana* and *M. anisopliae* isolates on *S. ocellatella* pupae

As mentioned in table (4) and figure (4), the pupae of *S. ocellatella* were affected by both isolates. Mortality was recorded in the sixth day and increased sharply from 14 to 64% in the third

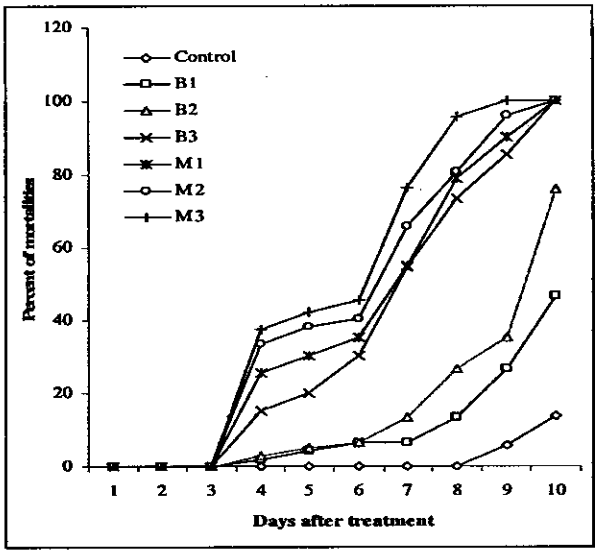

Fig. (1): Effect of *B. bassiana* and *M. anisopliae* on the third instar larvae of *P. mixta*.

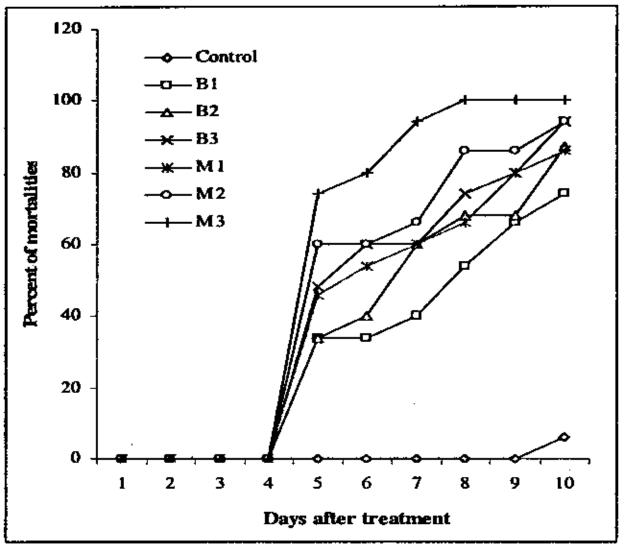

Fig. (2): Effect of *B. bassiana* and *M. anisopliae* on the third instar larvea of *S. ocellatella*.

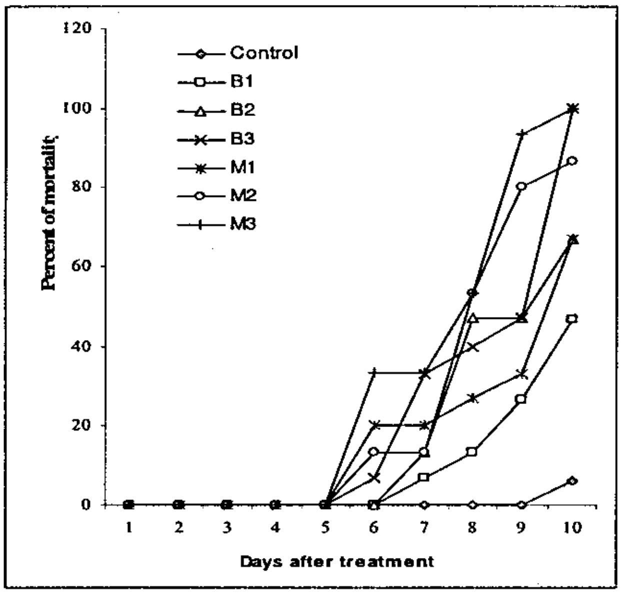

Fig. (3): Effect of *B. bassiana* and *M. anisopliae* on the pupae of *P. mixta*.

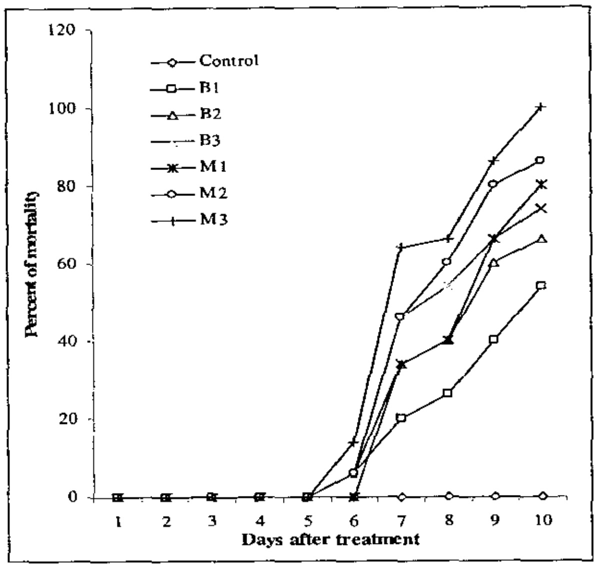

Fig. (4): Effect of *B. bassiana* and *M. anisopliae* on the pupae of *S. ocellatella.*

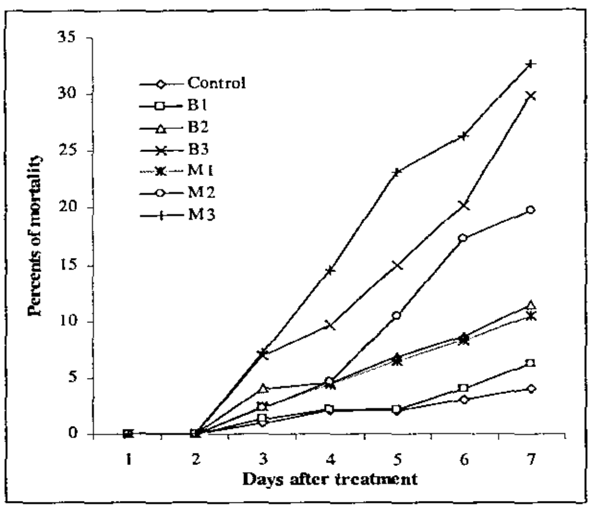

Fig. (5): Effect of *B. bassiana* and *M. anisopliae* on *A. craccevora*

concentration of *M. anisopliae* treatment in the sixth and seventh days, respectively. After that the percents of mortality increased gradually and reached 100% in the tenth day.

Statistical analysis showed that there were great significant differences between the third concentration in *M. anisopliae* isolate and other concentrations in the tenth day post treatment. The least significant difference (L S D) was 9.5.

The pupae of *S. ocellatella* were affected by *M. anisopliae* rather than *B. bassiana*. Highest mortality percent was 100% in *M. anisopliae* treatment (with the third concentration), but it was (74%) in *B. bassiana* treatment in the tenth day of treatment.

5- Effect of *B. bassiana* and *M. anisopliae* on *A. craccivora*

As shown in table (5) and figure (5), *A. craccivora* individuals were slightly affected by both fungi. Maximum mortality percent (32.6%) was found in the third concentration of *M. anisopliae* in the seventh day post treatment.

Mortality percents increased gradually after the third day post treatment. *A. craccivora* were relatively affected by *M. anisopliae* than by *B. bassiana.* The percents in the seventh day reached 6.2, 11.4 and 29.8%, and 10.4, 19.7 and 32.6% in C_1, C_2 and C_3 of *B. bassiana* and *M. anisopliae,* respectively. The results showed that both *P. mixta* and *S. ocellatella* larvae were affected by *B. bassiana* and *M. anisopliae* treatments. These results are in agreement with those of Mesbah *et al.* (2004) who recorded that Biofly (*B. bassiana*) suppressed 6.53 and 35.53% of *P. mixta* population and 39.42 and 26.43% of *S. ocellatella* in 1998/99 and 1999/2000 seasons, respectively. The third concentration of *M. anisopliae* (2X 10^5 conidia /ml) was the best concentration in both fungi against the third instar larvae and pupae of *P. mixta* and *S. ocellatella,* and also *A. craccivora.* The third instar larvae of *P. mixta* and *S. ocellatella* were affected by both *B. bassiana* and *M. anisopliae* more than pupae. This may be due to the rigid skeleton in pupae which make them relatively resistant to entomopathogenic fungi than larvae. The third instar larvae of *P. mixta* and *S. ocellatella* were affected by *M. anisopliae* more than by *B. bassiana* after the tenth day from treatment. This means that *M. anisopliae* isolate was more effective than *B. bassiana.* The same result was found by Nyle *et al.* (2005) when compared the efficacy of *B. bassiana* and *M. anisopliae* against sugar beet maggot (which has a similar behavior for *P. mixta*). Comparison also indicated that *M. anisopliae* showed greater potential for control of sugar beet maggot larvae than *B. bassiana.* Mortality percent was 100% at all concentrations in the tenth day of *M. anisopliae* treatment, while it was 86, 94 and 100 % with the first, second and third concentrations, respectively. The pupae of *P. mixta* and *S. ocellatella* were approximately equal in susceptibility to both fungi.

On the other hand, *A. craccivora* was slightly affected by both fungi with all concentrations. Saranya *et al.* (2010) found that the concentration 1x 10^5 conidia /ml of *B. bassiana* and *M. anisopliae* caused 50 and 38.5 % mortality, respectively, in the seventh day of treatment. The percents reached 96.7 and 80.8%, respectively when 1 x 10^8 conidia /ml was used. Results showed that both *B. bassiana* and *M. anisopliae* isolates were not virulent against *A. craccivora* when low concentrations were used but it showed high virulent when concentrations were increased to 1 x 10^8 conidia /ml. The same result was found by Abubakar *et al.* (2000) who found that 100% mortality in *A. craccivora* was recorded when the concentrations were increased to $1x10^8$ conidia/ml with *M. anisopliae* and *B. bassiana* in the seventh day of treatment.

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