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Microbial Control of Maize Army Worm, *Mythimna separata* (Lepidoptera : Noctuidae) by Entomopathogenic Fungi

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Abstract.- *Mythimna separata* is a destructive polyphagous insect of almost 33 plant species including maize and wheat. To evaluate the pathogenecity of entomopathogenic fungi, *Beauveria bassiana* and *Isaria fumosorosea*, a bioassay was conducted under laboratory conditions in which fungi were applied at different dose rates on different larval instars of *M. separata*. The results showed a dose and time dependent response on the mortality. Both fungi were found to be effective at all concentrations, but the uppermost concentration (6×10^8 spores/ml) provided maximum control within short period of time ($P<0.001$). *B. bassiana* showed its effectiveness after 4th day of application, while the 50% mortality of test population treated by *I. fumosorosea* was recorded between 5-6 days of treatment. Results are evident that entomopathogenic fungi have potential to be used for the management of *M. separata*.

Keywords: Maize army worm, maize, pathogenecity, polyphagous, entomopathogenic fungi, *Zea mays.*

INTRODUCTION

Maize (*Zea mays* L.) is an important feed and food crop of the world. In Pakistan, area under maize cultivation (896 thousand hectares) is after rice and wheat (mainly in KPK and Punjab), with an annual production of 2775 thousand tons of grain with average grain yield of about 3097 $kgha^{-1}$ (GOP, 2005). Maize has high nutritional value as it contains 10% protein, 72% starch, 4.8% oil, 8.5% fiber, 3% sugar and 1% ash (Chaudhary, 1983). A number of insect pest species attack maize crop, which reduce the production by 75% (Songa *et al*., 2002). *Mythimna separata* (Noctuidae: Lepidoptera) is very important polyphagous insect of erratic occurrence. Its unimpeded multiplication results in epidemics, which causes loss of foliage (Bai *et al*., 1990).The damage of leaves (44%) due to its attack in maize along with many other plants, i.e. sorghum, sugarcane, oats, wheat etc. has been reported (Hill and Atkins, 1983; Ali, 1993).

Injury to maize is caused by a single generation of *M. separata* larvae, usually during the time of grain filling (January-March). Larval development completes in one month, while the last two weeks of development are very crucial in which most of the leaves are consumed (Hill, 1986). For the management of insect pests on different crops, insecticides are being used, which cause serious problems like resistance, environmental pollution and toxicity to beneficial organisms. To overcome the harmful effects of chemical residues to animal and human health, several studies have been conducted to explore the most efficient control methods without using pesticides. One of the most recent ~~discovered~~ method is to exploit the entomopathogens such as bacteria, virus and fungi (Quintela and McCoy, 1997; McCoy *et al.,* 2000; Sabbour and Sahab, 2005).The insect pathogenic fungi have enormous potential as biological control agent against various insect pests including lepidopterans and are being developed on commercial scale for the control of many agricultural pests (Ferron, 1985; Thungrabeab and Tongma, 2007; Razek *et al.,* 2006).

Environment friendly microbial based pesticides can be applied for organic agriculture produce and in integrated pest management programs (Bhattacharya *et al.*, 2003; Carvalho,

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2006). Various entomopathogenic fungi such as *Beauveria bassiana* (Quesada-Moraga *et al.,* 2006; Sivasundaram *et al.,* 2007; Vega *et al.,* 2010; Freed *et al.,* 2012a, b; Akmal *et al.,* 2013), *Lecanicillium* sp. (Jung *et al.,* 2006; Ownley *et al.,* 2010) and *Metarhizium anisopliae* (Chandler and Davidson, 2005; Ansari *et al.,* 2007; Dong *et al.,* 2007; Suganya and Selvanarayanan, 2009; Bukhari *et al.,* 2010) have been used to control lepidopteran larvae, aphids and other pests. The present study elucidates the efficacy and virulence of *B. bassiana* and *I. fumosorosea* on *M. separata* for finding an alternate management tool for this pest.

MATERIALS AND METHODS

Insects

Mythimna separata larvae were collected from infested maize field from Bahauddin Zakariya University, Multan. These larvae were kept in plastic jars (6×10 cm) under laboratory conditions at 28±5°C and 70±5% relative humidity. Fresh maize leaves were provided as feed to the larvae, which were changed daily and new clean jars were provided to the larvae.

Entomopathogenic fungi

The entomopathogenic fungi, *B. bassiana* and *I. fumosorosea*, used in the study were isolated from the soil samples collected from the Northern areas of Pakistan. These fungi were cultured on Potato Dextrose Agar (PDA) medium for 12-15 days and later on the spores were collected from two weeks old fungus culture for the preparation of different concentrations.

Bioassay

The effect of *B. bassiana* and *I. fumosorosea* on different larval instars was checked by immersion method. Four replications were maintained and each replicate consisted of ten larvae. Each instar larvae were immersed in the suspension of nine concentrations *i.e.,* 1×10^5, 1×10^6, 1×10^7, 1×10^8, 2×10^8, 3×10^8, 4×10^8, 5×10^8 and 6×10^8 spores/ml and then placed on filter paper to soak the additional liquid. The treated larvae were shifted into separate petri plates, ten minutes after air dry. The larvae of control groups were treated with sterilized Tween 80 solution. Data was taken on daily basis for consecutive seven days

Data analysis

Completely Randomized Design was used for the experiments of all fungi with all treatments including control and each treatment was replicated four times. Duncan's Multiple Range Test was used to separate the means for the corrected mortality and the comparison of different concentrations was done on different days, respectively. All statistical analysis was performed by using SAS (SAS, 2002), while to calculate the LC_{50} and LT_{50} values of *B. bassiana* and *I. fumosorosea*, probit analysis was used (Finney, 1971).

RESULTS

Efficacy of B. bassiana

B. bassiana used in the study showed effective results against all instars of *M. separata.* The data showed that the mortality of the insects was dose and time dependent, as the concentration increased the time required to kill 50% population of the test insect decreased. The lower concentration 1×10^8 spores/ml of *B. bassiana* showed 92.5% mortality of first instar larvae of *M. separata*, while highest concentration *i.e.,* 6×10^8 spores/ml exhibited 90% mortality of 5th instar larvae (Figs. 1-5). The results showed that the LC_{50} values of *B. bassiana* for different instars of *M. separata* increased with the age of the insects being minimum for the younger insects and maximum for the mature insects on different days, respectively (Table I) and the LT_{50} values decreased with the increase in concentrations of *B. bassiana* for the same instar (Table II).

Efficacy of I. fumosorosea

I. fumosorosea used on all instars of *M. separata* showed significant and somewhat similar results to that of *B. bassiana*. After the application of the fungi, 87.5% mortality was recorded on first instar at the concentration of 1×10^8 spores/ml, while 80% mortality was recorded for 5th instar larvae after applying the concentration of 6×10^8 spores/ml (Figs. 1-5) Different concentrations of *I. fumosorosea* used against 1st instar of *M. separata* showed a linear relation between concentrations and percent mortality. The results showed that the LC_{50}

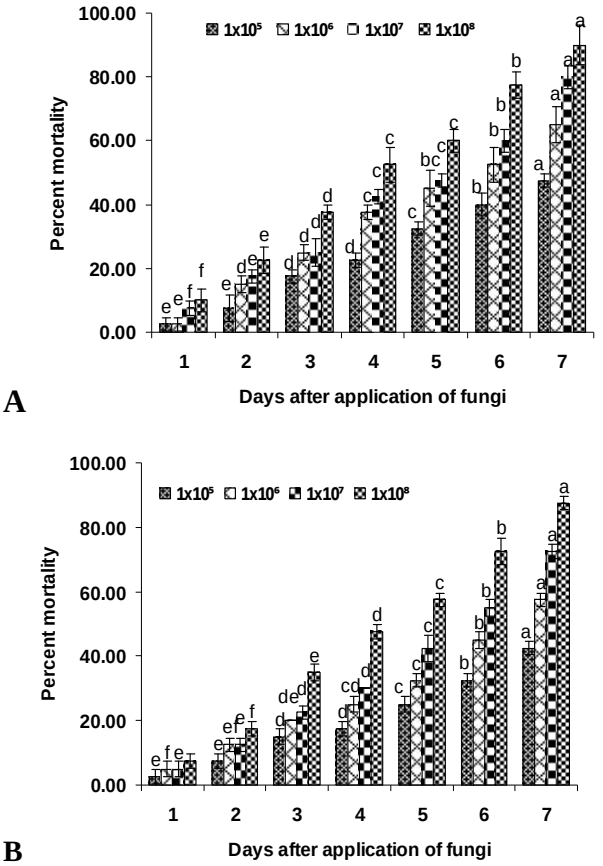


Fig. 1. Toxicity of *B. bassiana* (A) and *I. fumosorosea* (B) against 1st instar larvae of *M. separata*. For each day the same letters are not significantly different ($P < 0.001$) according to Duncan's Multiple Range Test (DMRT).

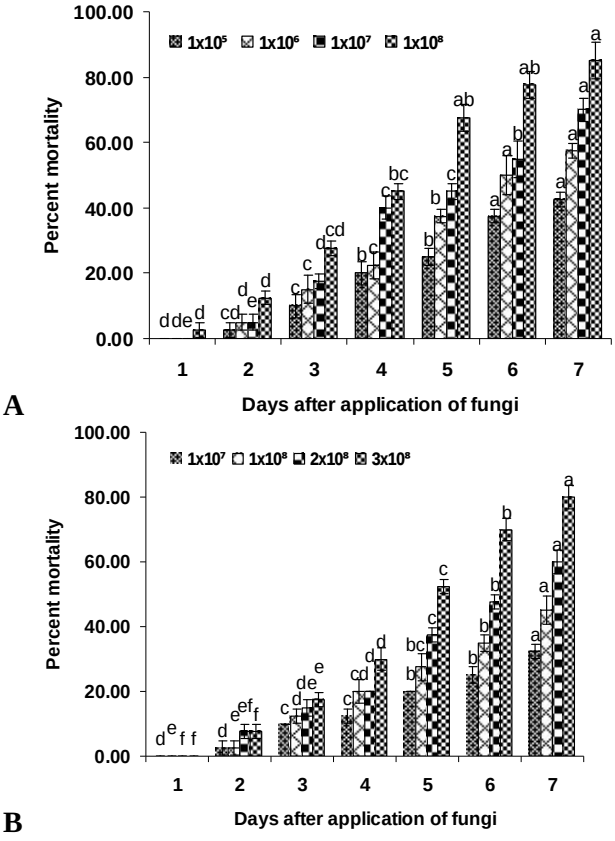


Fig. 2. Toxicity of *B. bassiana* (A) and *I. fumosorosea* (B) against 2nd instar larvae of *M. separata*. For each day the same letters are not significantly different ($P < 0.001$) according to Duncan's Multiple Range Test (DMRT).

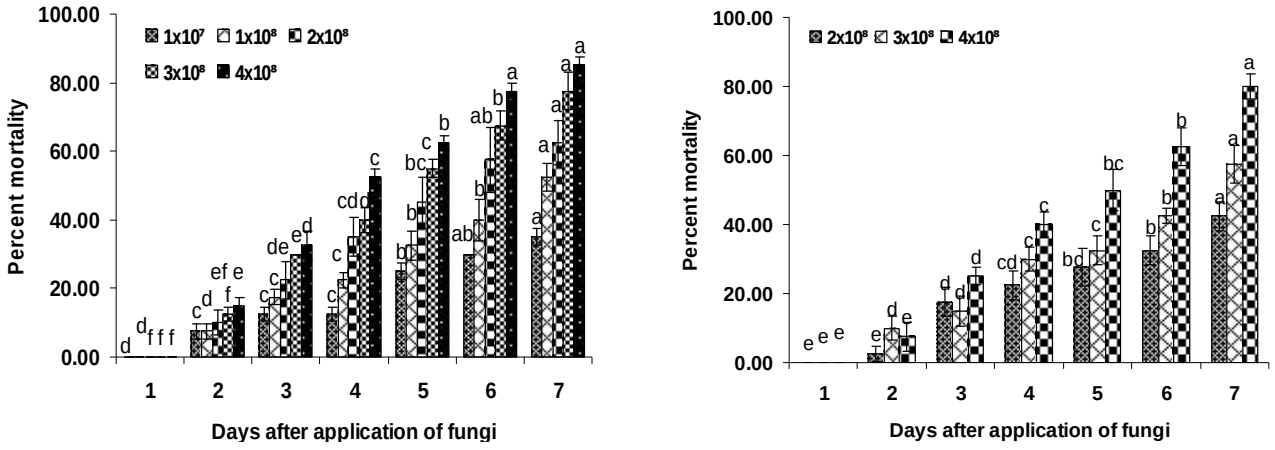
values of *I. fumosorosea* on different instars of *M. separata* increased with the age of the insects being minimum for the younger instars and maximum for the mature insects after different days of application, respectively (Table I) and the LT_{50} values decreased with the increase in concentrations of *I. fumosorosea* for the same instar (Table II).

DISCUSSION

M. separata is a destructive polyphagous insect of sporadic occurrence. Its restrained multiplication results in outbreak amounting to a total loss of vegetation over a given vast area (Hill and Atkins, 1983). The efficacy of entomo-pathogenic fungi was evaluated against different instars of *M. separata*, which showed mortality to be dose dependent, mortality increased and time required for causing 50% mortality reduced with the increase in concentration. Higher mortalities were obtained by the application of maximum concentration of *B. bassiana* and *I. fumosorosea*. Quite a number of researchers have observed high efficacy of fungus against many important pests. EI-Khawaas *et al*. (2002) reported that the LC_{50} of *Spodoptera littoralis* was 3.065×10^5 conidia/cm^3 and it was also suggested that the treatments with the fungi caused an elongation to the larval and pupal duration and mortality reached to 59 -92 percent. The findings of EI-sufty *et al*. (1982) emphasized for the use of insect pathogenic fungi as biological control agents against the leaf worm and beet armyworm, while their finding elaborated

Table I.- LC_{50} (spores/ml) of *B. bassiana* and *I. fumosorosea* against different instars of *M. separate.*

Fungi	Instar	Days	LC_{50}	F.D Limit	Slope	D.F
B. bassiana	1st	4th	4.66×10^{7}	3.63×10^{6}-5.97×10^{8}	0.25±0.09	2
		5th	8.81×10^{6}	9.50×10^{5}-8.17×10^{7}	0.22±0.09	2
		6th	7.33×10^{5}	1.43×10^{5}-3.76×10^{6}	0.32±0.09	2
		7th	1.49×10^{5}	3.48×10^{4}-6.41×10^{5}	0.49±0.11	2
	2nd	5th	1.98×10^{8}	7.95×10^{7}-4.93×10^{8}	0.58±0.19	2
		6th	5.67×10^{7}	2.17×10^{7}-1.47×10^{8}	0.56±0.18	2
		7th	2.51×10^{7}	9.56×10^{6}-6.60×10^{7}	0.65±0.18	2
	3rd	4th	6.48×10^{8}	2.24×10^{8}-1.87×10^{9}	0.65±0.19	3
		5th	2.29×10^{8}	1.21×10^{8}-4.35×10^{8}	0.70±0.17	3
		6th	8.91×10^{7}	5.12×10^{7}-1.55×10^{8}	0.78±0.17	3
		7th	4.38×10^{7}	2.24×10^{7}-8.57×10^{7}	0.78±0.16	3
	4th	5th	5.02×10^{8}	3.33×10^{8}-7.58×10^{8}	1.97±1.28	1
		6th	3.68×10^{8}	3.14×10^{8}-4.32×10^{8}	3.41±1.29	1
		7th	3.21×10^{8}	2.71×10^{8}-3.79×10^{8}	4.28±1,34	1
	5th	6th	4.67×10^{8}	4.14×10^{8}-5.26×10^{8}	3.99±0.95	2
		7th	3.82×10^{8}	3.45×10^{8}-4.22×10^{8}	5.23±0.98	2
I. fumosorosea	1st	5th	3.06×10^{7}	3.79×10^{6}-2.47×10^{8}	0.29±0.09	2
		6th	2.50×10^{6}	6.45×10^{5}-9.74×10^{6}	0.34±0.09	2
		7th	3.22×10^{5}	8.04×10^{4}-1.29×10^{6}	0.44±0.10	2
	2nd	5th	5.36×10^{8}	1.25×10^{8}-2.29×10^{9}	0.54±0.19	2
		6th	1.50×10^{8}	7.32×10^{7}-3.09×10^{8}	0.68±0.19	2
		7th	6.14×10^{7}	3.13×107-1.20×10^{8}	0.72±0.18	2
	3rd	6th	3.19×10^{8}	2.55×10^{8}-3.98×10^{8}	2.50±0.96	1
		7th	2.34×10^{8}	1.93×10^{8}-2.85×10^{8}	3.32±0.98	1
	4th	6th	5.0×10^{8}	4.32×10^{8}-5.78×10^{8}	3.59±1.62	1
		7th	4.18×10^{8}	3.33×10^{8}-4.78×10^{8}	5.49±1.70	1
	5th	6th	4.82×10^{8}	3.78×10^{8}-6.14×10^{8}	2.17±1.60	1
		7th	4.20×10^{8}	3.59×10^{8}-4.93×10^{8}	4.54±1.66	1



A **B**

Fig. 3. Toxicity of *B. bassiana* (A) and *I. fumosorosea* (B) against 3rd instar larvae of *M. separata*. For each day the same letters are not significantly different ($P < 0.001$) according to Duncan's Multiple Range Test (DMRT).

Table II.- LT_{50} (days) of *B. bassiana* and *I. fumosorosea* against different instars of *M. separate.*

Fungi	Instar	Concentration (spores/ml)	LT_{50} (Days)	F.D Limit	Slope	D.F
B. bassiana	1st	1×10^{6}	5.36	4.55-6.32	2.60±0.39	5
		1×10^{7}	4.76	4.10-5.54	2.60±0.37	5
		1×10^{8}	3.47	3.05-3.94	2.95±0.36	5
	2nd	1×10^{8}	6.16	5.33-7.12	3.50±0.54	5
		2×10^{8}	5.25	4.69-5.87	3.82±0.53	5
		3×10^{8}	4.14	3.72-4.60	3.64±0.44	5
	3rd	1×10^{8}	7.02	5.71-8.64	2.82±0.48	5
		2×10^{8}	5.36	4.67-6.14	3.16±0.45	5
		3×10^{8}	4.46	3.99-4.97	3.58±0.45	5
		4×10^{8}	3.19	3.53-4.32	3.95±0.46	5
	4th	4×10^{8}	6.00	5.16-6.98	3.23±0.49	5
		5×10^{8}	4.47	4.06-4.93	4.16±0.51	5
	5th	5×10^{8}	5.69	5.14-6.30	4.62±0.66	5
		6×10^{8}	4.64	4.28-5.03	5.16±0.63	5
I. fumosorosea	1st	1×106	7.01	5.43-9.06	2.17±0.39	5
		1×107	5.35	4.56-6.26	2.70±0.40	5
		1×10^{8}	3.84	3.39-4.35	3.00±0.38	5
	2nd	2×10^{8}	6.23	5.34-7.27	3.33±0.52	5
		3×10^{8}	4.76	4.34-5.22	4.47±0.57	5
	3rd	3×10^{8}	6.42	5.37-7.68	2.93±0.47	5
		4×10^{8}	4.69	4.23-5.20	3.93±0.50	5
	4th	5×10^{8}	6.14	5.44-6.92	4.99±0.66	5
		6×10^{8}	5.00	4.60-5.44	5.17±0.66	5
	5th	5×10^{8}	5.85	5.26-6.51	4.56±0.67	5
		6×10^{8}	5.25	4.82-5.71	5.21±0.69	5

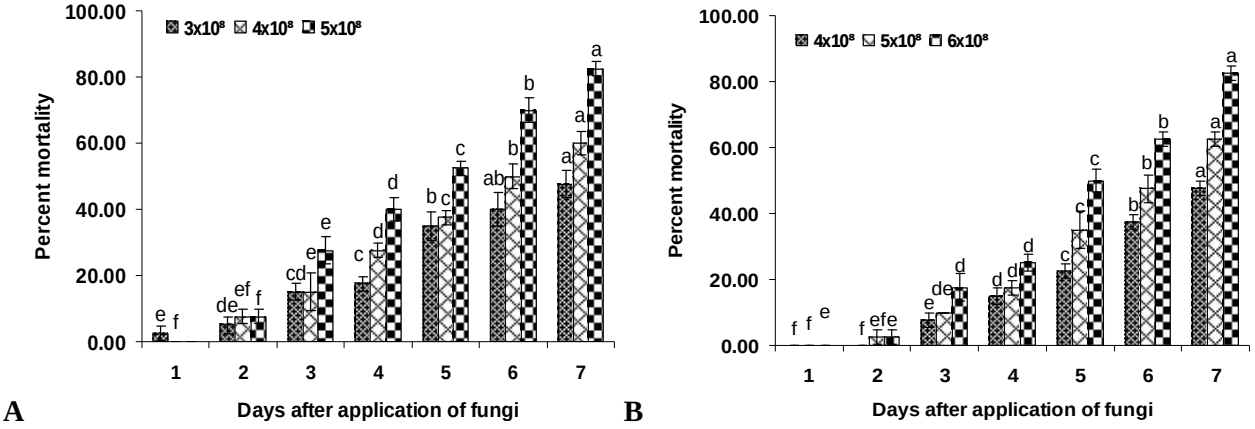


Fig. 4. Toxicity of *B. bassiana* (A) and *I. fumosorosea* (B) against 4th instar larvae of *M. separata*. For each day the same letters are not significantly different (P < 0.001) according to Duncan's Multiple Range Test (DMRT).

that *B. bassiana* causes higher mortality to the insect pests, which confirms our results that this fungus causes mortalities in *M. separata* treated at different concentrations.

The current findings are also verified by the results of Sabbour and Sahib (2005) and Lozano *et al.* (2008) who observed *B. bassiana* to be highly effective against *Plutellla xylostella, Pieris rapae,*

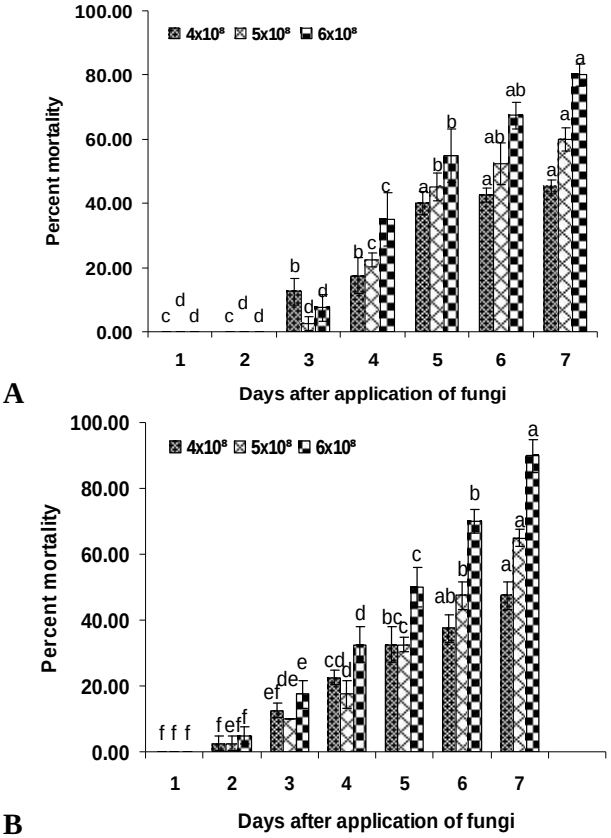


Fig. 5. Toxicity of *B. bassiana* (A) and *I. fumosorosea* (B) against 5th instar larvae of *M. separata*. For each day the same letters are not significantly different (P < 0.001) according to Duncan's Multiple Range Test (DMRT).

white grubs and *S. exigua* and percentage infestation reached to 20 and 21 after 9 days of treatment. From our experiment, both fungi proved pathogenic against *M. separata* and percent mortalities obtained were 92.5, 82.5, 85, 82.5 and 90 by using 1×10^8-6×10^8 spores/ml of *B. bassiana* on 1st- 5th instars. On the other hand, 87.5, 80, 80, 82.5 and 80% mortality was recorded on 1st -5th instar with the concentrations of 1×10^8-6×10^8 spores/ml of *I. fumosorosea.*

The results of Quesada-Maroga *et al.* (2006) indicated that the death of *S. littoralies* larvae by *B. bassiana* and *M. anisopliae* was due to the crude toxic protein produced by fungi and that was dose dependent because in high doses, mortality may reach 100% of treated larvae. This showed that these entomopathogenic fungi have the potential to be used as biological control agents against many insect pests. The findings of Quintela and McCoy (1997) also support our findings in which they used fungal concentrations of 10^6 and 10^7 conidia /ml of *B. bassiana* and *M. anisopliae* which affected larval development, movements, mobility and caused about 90-100 percent larval mortality, while in our experiment, 6×10^8 spores/ml of *I. fumosorosea* and *B. bassiana* caused 80 and 90% mortality, respectively.

It has also been reported by McCoy *et al.* (2000), that the conidia of *B. bassiana* and mycelia of *M. anisopliae,* suppressed root weevil larval populations when applied at high inoculumn rates. The findings of Prasad and Syed (2010) showed that *H. armigera* treated with *B. bassiana* showed 86.25 percent mortality, which also confirms our results that maximum 80 and 90 percent mortalities can be obtained on 5th instar *M. separata* by using 6×10^8 spores/ml of *B. bassiana* and *I. fumosorosea*, respectively. From the current findings it is concluded that the isolates of *B. bassiana* and *I. fumosorosea* have potential to be used against *M. separata* and further their inclusion in the integrated pest management program of maize insect pests.

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