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Pathogenicity of *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes) Isolates to the *Ailanthus* Webworm, *Atteva fabriciella* (Lepidoptera: Yponomeutidae) Under Laboratory and Field Conditions

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The virulence of 25 Metarhizium anisopliae *isolates was tested under laboratory conditions and the two most effective isolates were evaluated in the field for control of the* Ailanthus *defoliator,* Atteva fabriciella. *A bioassay was carried out to determine the dose and time mortality responses. The* LC_{50} *of the isolates ranged from 3.16 to 647.81* $\times$ 10^5 *conidia* mL^{-1}. *Toxicity tests of the isolates MIS7 and MIS13 and 0.5%* Pongamia pinnata *seed oil, individually and in different combinations, indicated improved efficacy of the isolates when used in combination and also when combined with seed oil. Evaluation of these formulations in the field showed 66.36% reduction of infestation with MIS7 + MIS13 + 0.5%* P. pinnata *seed oil and 61.15% reduction with MIS7 + MIS13. The study indicated a possibility of employing combined formulations of* M. anisopliae *and also combination with* P. pinnata *seed oil for augmenting the effectiveness of the fungus.*

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KEYWORDS Ailanthus, Atteva fabriciella, Metarhizium anisopliae, *biocontrol*

INTRODUCTION

Ailanthus excelsa Roxb., commonly known as Maharukh or Mahaneem, is a large lofty deciduous fast-growing multipurpose tree species in India (Tewari, 1992). *A. fabriciella* Swed. is a major insect pest of *Ailanthus* spp. causing large-scale defoliation in nurseries and plantations. This insect is commonly known as *Ailanthus* webworm because of the webbing of leaves by the larvae and feeding from within (Nair, 2007). It is reported throughout the year, signifying continuous breeding with overlapping generations (Varma, 1986). As a result of repeated defoliation, the young plants are weakened badly or killed completely and the growth of mature trees are severely retarded, leaders and laterals die back, seed formation is drastically reduced, and tender fruits are damaged (Varma, 1992, 1994). A common practice for control of this larva is heavily dependent on chemical methods. Heavy reliance on pesticides and their indiscriminate use usually results in severe negative impacts on the environment. The development of alternate control strategies assumes significance in this context.

Several species of entomopathogenic fungi are exploited for development of biopesticides for control of insect pests, especially in agriculture. *Metarhizium anisopliae* and *Beauveria bassiana* are the two most extensively studied groups of entomopathogenic fungi in various parts of the world (Tanada, 1959). There are many different strains of *M. anisopliae* and they vary in their host range and abilities to kill insects. The high virulence and broad spectrum of pathogenicity exhibited by many isolates of *M. anisopliae* have prompted workers to exploit this organism as an important biocontrol agent. Numerous reports on the pathogenicity of *M. anisopliae* have been published by various researchers (Ferron, 1981; McCoy, Samson, & Boucias, 1988; Goettel, 1992). In this study we have tried to assess the potential of the entomopathogenic fungus, *M. anisopliae*, for control of the *Ailanthus* webworm, *A. fabriciella*. The virulence of 25 *M. anisopliae* isolates was assessed under laboratory conditions of which two potent isolates were subsequently evaluated for control of webworm infestation in the field.

MATERIALS AND METHODS

Insects

Healthy larvae of *A. fabriciella* (Figure 1) collected from the field were reared in the laboratory and allowed to pupate and eclose. Male and female moths were released into glass bottles covered with muslin cloth for mating and

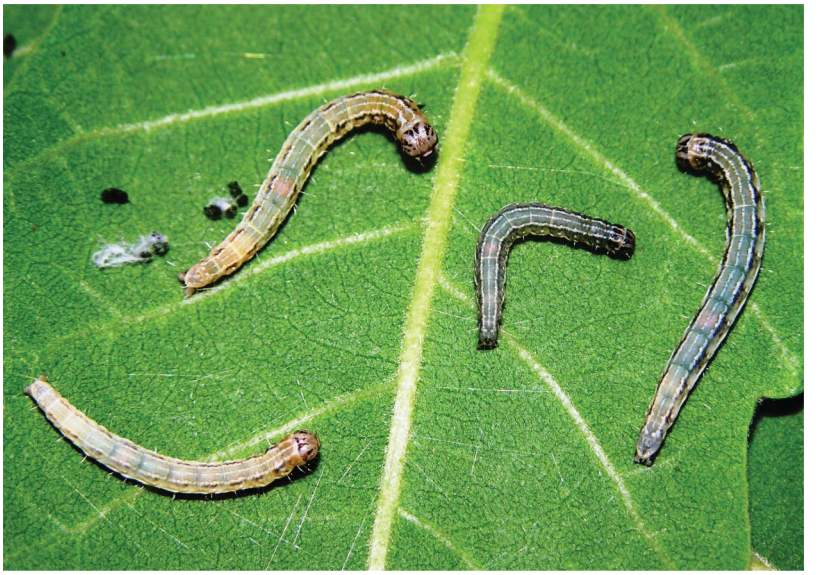

FIGURE 1 Healthy larvae of *Atteva fabriciella* (color figure available online).

egg laying. Dilute sucrose solution (10%) was provided on cotton balls as food. The muslin cloths with eggs were surface sterilized with 1% sodium hypochlorite for 15 min and dipped in sterile distilled water for 10 min and placed over a blotting paper for drying. It was then covered with tender leaves and transferred to glass bottles for hatching. Larvae initially established on tender leaves were transferred with fine camel hairbrush to plastic boxes (14 cm in diameter, 6 cm in height) with fresh leaves. The petioles of the leaves were wrapped in a layer of moist tissue paper and sealed with parafilm to prevent wilting. Fresh leaves were provided once every 2 days.

Fungus

Among the 25 fungal isolates (MIS1 to MIS25) used in this study, 16 were isolated either from soil or from infected insects and nine procured from different institutions. Soil samples were collected from a depth of 30 cm from different study areas. The Galleria bait method was used to isolate the fungi from soil samples. After removing roots and gravel, soil samples were sifted through a 5-mm sieve. Thereafter, plastic boxes (10 cm in height, 8 cm in diameter) were filled with 100 g of soil and 10 late-instar larvae of *Galleria mellonella* were introduced. The lids were punched for making air holes. The larvae were incubated at 20°C in dark conditions. During the first 5 days, the boxes were turned once daily to make bait insects penetrate as much soil as possible. After 7–10 days, boxes were examined every day and dead larvae were collected. Cadavers thus obtained as well as those

collected from field were surface-sterilized by dipping consecutively in 70% ethyl alcohol, 1% sodium hypochlorite, and sterile distilled water, each for 3 min. The larvae were dissected and placed on Veen's medium and incubated at 28 ± 1°C and 90% relative humidity (RH) to facilitate growth and sporulation of the fungus. Slant cultures were prepared from a single colony and stored at −20°C until used.

Bioassay

Culture plate of each isolate was prepared by spreading 200 μL of conidial suspension (10^7 conidia mL^{-1}) onto PDAY medium. Plates were incubated in dark at 28 ± 1°C for 14 days to maximize spore production. Spores were harvested by flooding each plate with 10 mL of 0.05% Tween 80 in sterile distilled water and dislodging the conidia into suspension with a glass rod. The suspension was filtered through a double layer sterile cheese cloth and centrifuged at 1,700 rpm for 15 min. The supernatant was discarded and the conidia were resuspended in 5 mL sterile distilled water. This stock spore suspension was stored at 4°C for 24 hr until spore viability was determined. Only cultures with >90% viability were used. Counts of conidia were made from the stock suspension using an improved Neubauer haemocytometer (Hausser Scientific, USA). Spore suspensions containing 10^3, 10^4, 10^5, 10^6, 10^7, and 10^8 conidia mL^{-1} sterile distilled water with 0.05% Tween 80 were prepared from the stock for bioassay.

A bioassay of all the 25 isolates was carried out against *A. fabriciella* using inoculum concentrations ranging from 10^3–10^8 conidia mL^{-1} to determine the multiple dose-mortality (LC_{50}) and time-dose-mortality (LT_{50}) responses. Twenty-two second-instar larvae of *A. fabriciella* were placed separately in sterile 20-mL vials containing 10 mL of fungal suspension. The vial was capped and inverted five times over a 5-s period, to ensure that the insects were completely drenched with the fungal suspension. The suspension with insects was filtered through a tea strainer (6 cm in diameter). For the controls, insects were treated with 0.05% Tween 80. Treated and untreated (control) larvae were transferred with fine camel hair brush to separate plastic boxes (14 cm in diameter, 6 cm in height) containing fresh leaves as food. The petiole of the leaves were wrapped in a layer of moist tissue paper and sealed with parafilm to prevent wilting. A vented lid with mesh screen was used to close the plastic boxes. The boxes were incubated at 26 ± 1°C, 90% RH, 12:12 (L:D). Fresh leaves were provided after every 2 days. Four replications were maintained for each concentration of a single isolate. The mortality of larvae was recorded every 24 h for 8 days after exposure. Dead larvae were counted and removed each day to prevent horizontal contamination. The dead larvae from each treatment were incubated in moist conditions to determine if death resulted from mycosis (Figure 2).

FIGURE 2 Mycosed cadavers of *Atteva fabriciella* (color figure available online).

The toxicity of two promising isolates, MIS7 (10^7 conidia/mL) and MIS13 (10^7 conidia/mL), and 0.5% *P. pinnata* oil was further evaluated individually and in different combinations as per the above method to determine the synergistic effect of combinations on the mortality of *A. fabriciella*.

Field Study

Two formulations of each of the isolates, MIS7 and MIS13, that proved promising in the laboratory were evaluated in *Ailanthus* plantations at two locations of Odagathur forest division in the Vellore district of Tamil Nadu where peak pest attack was observed. The different treatments—viz. T1 (water formulation of MIS7 and MIS13 at 10^{14} conidia mL^{-1} + 0.08% Tween 80), T2 (oil formulation of MIS7 and MIS13 at 10^{14} conidia mL^{-1} + 0.08% Tween 80 + 0.5% *P. pinnata* oil) and T3 (control—0.08% Tween 80) were evaluated in a 4-yr-old *Ailanthus* plantation infested by *A. fabriciella*. Germination test of the formulations was done 1 day prior to application and was found to be over 80%. The experimental layout was made in a randomized block design (RBD). Each treatment including control was replicated 4 times. The subplots in each replication had seven rows of 10 plants each, 2 m apart (five main rows and two skip rows, one on either side of the main rows). Each subplot was separated from the others by two

skip rows, 2 m apart (one row from each subplot). The population counts of *A. fabriciella* larvae were recorded a day before the imposition of treatments. The total number of larvae on all the leaves of 10 randomly selected tagged plants in each treatment plot was recorded. The treatments were done using a power sprayer. The number of surviving larvae was recorded after 7 and 15 days of treatment. For each treatment, the average of all the observations from two locations were used to determine the average percent reduction of pest population calculated using the Henderson and Tilton (1955) equation.

Statistical Analysis

Median lethal concentration (LC_{50}) and median lethal time (LT_{50}) values were calculated using probit analysis (Finney, 1971). Field trial data were subjected to analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Bioassay

Among the 25 isolates, MIS13, MIS2, MIS7, and MIS20 were found to be more effective with lower LC_{50} values. MIS13 was the most effective isolate with the lowest LC_{50} value (3.16×10^5 conidia mL^{-1}) followed by MIS2 and MIS7. MIS14 was the least effective isolate with the highest LC_{50} value (Table 1). The lowest LT_{50} of 4.8 days was recorded at an inoculum load of 10^7 conidia mL^{-1} for MIS13 and MIS2 followed by MIS7 and MIS20. MIS13 showed LT_{50} value of 5.3 and 6.6 days while MIS2 recorded 6.1 and 6.8 days at 10^6 and 10^5 conidia mL^{-1}, respectively. LT_{50} of 6.8 days was shown by MIS13 and MIS2 when inoculated with 10^4conidia mL^{-1}. MIS7 needed 5.3, 6.5, 6.6 and 7.2 days to kill 50% population at 10^7, 10^6, 10^5, and 10^4 dosage of conidia mL^{-1} respectively. Highest LT_{50} of 9 days was recorded for MIS17 at spore load of 10^4 conidia mL^{-1} (Table 2). With respect to LC_{50} and LT_{50}, MIS13 proved to be superior than other isolates against *A. fabriciella*. Hitherto there has been no report of any study on the efficacy of *Metarhizium* fungus against *A. fabriciella* in India. Mohanan and Varma (1988) reported the pathogenicity of the fungus, *Paceliomyces farinosus*, to *A. fabriciella*. Inoculation of larvae of *A. fabriciella* with this fungus caused mortality within 48–72 h of incubation. Singh and Misra (1978) screened a *Bt*-based microbial insecticide, Thuricide, against *A. fabriciella* and found it to be moderately effective. Evaluation of the insecticidal activity of Ivermectin produced by a soil actinomycete, *Streptomyces avermitilis*, against the larvae of *A. fabriciella* in laboratory revealed that Ivermectin was highly toxic and induced larval mortality with LC_{50} value of 0.023903% against *A. fabriciella* (Roychoudhury & Joshi, 2009). Various insecticides used for the control of *A. fabriciella* in nurseries include

TABLE 1 Dose-Mortality Response (LC_{50}) of *Metarhizium* Isolates to *A. fabriciella*

Rank	Isolates	LC_{50} ($\times 10^5$)	Fiducial limits		Slope ± *SE*	χ^2	*p*
			Lower ($\times 10^5$)	Upper ($\times 10^5$)			
1	MIS13	3.16	0.53048	18.85051	2.5 ± 0.7	0.108	.991
2	MIS2	4.05	0.11150	558.80469	1.6 ± 0.7	0.483	.785
3	MIS7	15.14	2.96968	530.38795	2.5 ± 0.7	0.109	.947
4	MIS20	24.87	4.53853	2,866.49341	2.5 ± 0.7	0.552	.759
5	MIS24	63.16	15.63211	1,917.13520	3.7 ± 0.9	0.664	.717
6	MIS10	77.64	18.22976	3,334.50181	3.7 ± 0.9	0.189	.910
6	MIS23	77.64	18.22976	3,334.50181	3.7 ± 0.9	0.189	.910
7	MIS19	78.62	10.31921	2,380,789.15492	2.4 ± 0.7	0.068	.967
8	MIS1	91.64	15.47699	48,319.17607	2.9 ± 0.8	0.013	.993
9	MIS9	117.62	25.02286	10,278.98133	3.8 ± 0.9	0.736	.692
10	MIS22	123.14	33.27831	15,707.65130	4.8 ± 1.2	0.612	.736
10	MIS25	123.14	33.27831	15,707.65130	4.8 ± 1.2	0.612	.736
11	MIS11	144.39	81.74336	2.251704E+20	3.1 ± 0.9	0.380	.827
12	MIS17	149.68	26.81306	4,790.46971	3.5 ± 0.9	0.093	.955
13	MIS18	159.82	22.38051	656,584.13095	2.7 ± 0.8	0.320	.852
14	MIS8	217.13	26.95383	3,925,480.88407	2.9 ± 0.8	0.107	.948
15	MIS3	231.18	14.87603	7.235317E+13	2.0 ± 0.7	0.457	.796
16	MIS4	300.48	32.58871	35,029,799.27609	2.9 ± 0.8	0.226	.893
16	MIS15	300.48	32.58871	35,029,799.27609	2.9 ± 0.8	0.226	.893
17	MIS5	314.67	44.20990	959,879.03055	3.6 ± 0.7	0.075	.963
17	MIS21	314.67	44.20990	959,879.03055	3.6 ± 0.7	0.075	.963
18	MIS6	344.54	41.35598	8,152,760.21599	3.3 ± 0.9	0.093	.955
19	MIS16	346.61	24.31127	8.471442E+21	2.3 ± 0.8	0.108	.947
20	MIS12	424.41	39.57977	5.414953E+13	2.9 ± 0.8	0.712	.700
21	MIS14	647.81	44.49018	1.189121E+18	2.7 ± 0.8	0.071	.965

application of 0.1% of endosulfan and malathion, 0.01 to 0.02% formothion and fenvalerate; and also by DDT, BHC, aldrin, and dieldrin (Singh & Gupta, 1978). Varma (1986) suggested the use of the insecticides monocrotophos, quinalphos, or methylparathion in controlling the pest based on the evaluation of the insecticides in the laboratory and field. Synthetic pyrethroids, BHC (5% dust) and endrin (1% dust), were effective and highly residual in action against *A. fabriciella* (Nair, 2007). At present, of the above mentioned insecticides, use of aldrin, dieldrin, and endrin dust have been banned in India and BHC is not permitted for crop protection.

A significant difference in mortality was observed between the seven combinations tested. Increased mortality was recorded with the combination treatments compared to individual treatments. The MIS7 + MIS13 + 0.5% *P. pinnata* oil formulation proved to be superior over other formulations, which recorded 81.13% mortality followed by the MIS7 + MIS13 formulation with mortality of 78.86%. The MIS7 + 0.5% *P. pinnata* oil formulation recorded mortality of 68.05% and the MIS13 + 0.5% *P. pinnata* oil formulation resulted in 77.87% mortality. MIS7 caused

TABLE 2 Time-Dose-Mortality Response (LT_{50}) of *Metarhizium* Isolates to *A. fabriciella*

Isolates	Conc.	LT_{50}	Fiducial limits		Slope ± *SE*	χ^2	*p*
			Lower	Upper			
MIS1	1×10^4	7.0	—	—	15.7 ± 13.9	0.06	.99
	1×10^5	6.8	5.7	13.1	5.1 ± 1.4	0.48	.97
	1×10^6	6.6	—	—	15.6 ± 9.2	0.19	.99
	1×10^7	6.1	5.1	9.1	3.7 ± 0.8	0.62	.96
MIS2	1×10^4	6.8	5.7	13.1	5.1 ± 1.4	0.48	.97
	1×10^5	6.8	5.8	14.0	5.9 ± 1.8	0.14	.99
	1×10^6	6.1	5.3	8.6	4.6 ± 1.1	0.47	.97
	1×10^7	4.8	4.3	5.7	3.9 ± 0.7	0.60	.96
MIS3	1×10^4	8.7	6.5	36.5	5.2 ± 1.9	0.82	.93
	1×10^5	6.7	5.8	12.5	5.8 ± 1.7	0.20	.99
	1×10^6	6.6	—	—	15.6 ± 9.2	0.19	.99
	1×10^7	5.9	5.3	7.4	6.2 ± 1.5	0.30	.98
MIS4	1×10^4	7.4	6.2	40.0	7.3 ± 3.0	0.53	.97
	1×10^5	7.0	—	—	15.7 ± 13.9	0.06	.99
	1×10^6	6.7	—	—	15.7 ± 10.9	0.11	.99
	1×10^7	6.5	5.5	10.5	4.6 ± 1.7	1.31	.85
MIS 5	1×10^4	7.5	—	—	14.3 ± 10.7	0.04	1.00
	1×10^5	7.2	6.1	72.7	7.1 ± 2.7	0.35	.98
	1×10^6	7.0	—	—	15.7 ± 13.9	0.06	.99
	1×10^7	6.5	5.6	11.0	5.3 ± 1.4	0.43	.98
MIS6	1×10^4	7.5	—	—	14.3 ± 10.7	0.04	1.00
	1×10^5	7.2	6.1	72.7	7.1 ± 2.7	0.35	.98
	1×10^6	6.7	—	—	15.7 ± 10.9	0.11	.99
	1×10^7	6.5	5.4	11.3	3.5 ± 0.8	0.90	.92
MIS7	1×10^4	7.2	5.9	18.5	4.8 ± 1.4	0.61	.96
	1×10^5	6.6	—	—	15.6 ± 9.2	0.19	.99
	1×10^6	6.5	5.5	10.6	4.3 ± 1.0	0.41	.98
	1×10^7	5.3	4.7	6.3	4.9 ± 1.0	0.11	.99
MIS8	1×10^4	8.1	—	—	7.2 ± 3.5	0.15	.99
	1×10^5	7.0	—	—	15.7 ± 13.9	0.06	.99
	1×10^6	7.0	6.0	27.3	7.0 ± 2.5	0.50	.97
	1×10^7	6.2	5.5	8.8	6.0 ± 1.6	0.31	.98
MIS9	1×10^4	7.5	—	—	14.3 ± 10.7	0.04	1.00
	1×10^5	7.0	—	—	15.7 ± 13.9	0.06	.99
	1×10^6	6.7	5.8	12.5	5.8 ± 1.7	0.20	.99
	1×10^7	6.3	5.3	9.8	3.9 ± 0.9	0.34	.98
MIS10	1×10^4	8.1	—	—	7.2 ± 3.5	0.15	.99
	1×10^5	7.5	—	—	14.3 ± 10.7	0.04	1.00
	1×10^6	6.8	5.8	14.0	5.9 ± 1.8	0.14	.99
	1×10^7	6.0	5.2	8.3	4.5 ± 1.0	0.60	.96
MIS11	1×10^4	7.5	—	—	14.3 ± 10.7	0.04	1.00
	1×10^5	7.0	—	—	15.7 ± 13.9	0.06	.99
	1×10^6	6.9	5.9	15.8	5.6 ± 1.7	0.39	.98
	1×10^7	6.4	—	—	16.3 ± 8.7	0.22	.99
MIS12	1×10^4	7.0	—	—	15.7 ± 13.9	0.06	.99
	1×10^5	6.7	—	—	15.7 ± 10.9	0.11	.99
	1×10^6	6.6	—	—	15.6 ± 9.2	0.19	.99

(*Continued*)

TABLE 2 (Continued)

Isolates	Conc.	LT_{50}	Fiducial limits		Slope ± *SE*	χ^2	*p*
			Lower	Upper			
	1×10^7	6.3	5.6	9.3	6.1 ± 1.7	0.23	.99
MIS13	1×10^4	6.8	6.2	40.0	7.3 ± 3.0	0.53	.97
	1×10^5	6.6	5.8	11.7	6.5 ± 2.0	0.81	.93
	1×10^6	5.3	4.8	6.3	5.3 ± 1.2	1.60	.80
	1×10^7	4.8	4.2	5.6	3.8 ± 0.7	2.25	.69
MIS14	1×10^4	7.4	6.2	40.0	7.3 ± 3.0	0.53	.97
	1×10^5	7.0	—	—	15.7 ± 13.9	0.06	.99
	1×10^6	6.7	—	—	15.7 ± 10.9	0.11	.99
	1×10^7	6.4	5.6	9.8	5.8 ± 1.6	0.42	.98
MIS15	1×10^4	8.1	—	—	7.2 ± 3.5	0.15	.99
	1×10^5	7.0	—	—	15.7 ± 13.9	0.06	.99
	1×10^6	7.0	6.0	27.3	7.0 ± 2.5	0.50	.97
	1×10^7	6.5	5.5	10.6	4.3 ± 1.0	0.41	.98
MIS16	1×10^4	8.5	6.3	58.2	4.1 ± 1.1	1.59	.81
	1×10^5	8.1	—	—	7.2 ± 3.5	0.15	.99
	1×10^6	6.7	—	—	15.7 ± 10.9	0.11	.99
	1×10^7	6.1	5.4	8.4	5.5 ± 1.4	0.46	.97
MIS17	1×10^4	9.0	—	—	6.6 ± 3.5	0.25	.99
	1×10^5	7.5	—	—	14.3 ± 10.7	0.04	1.00
	1×10^6	7.0	6.0	27.3	7.0 ± 2.5	0.50	.97
	1×10^7	6.2	5.4	9.2	5.0 ± 1.3	0.83	.93
MIS18	1×10^4	7.6	6.0	22.1	4.0 ± 1.0	0.71	.95
	1×10^5	7.0	—	—	15.7 ± 13.9	0.06	.99
	1×10^6	6.7	—	—	15.7 ± 10.9	0.11	.99
	1×10^7	6.2	5.4	8.8	5.6 ± 1.5	0.30	.99
MIS19	1×10^4	7.8	6.2	119.8	5.9 ± 2.1	1.09	.89
	1×10^5	6.7	—	—	15.7 ± 10.9	0.11	.99
	1×10^6	6.4	5.6	9.8	5.8 ± 1.6	0.42	.98
	1×10^7	6.1	5.3	8.6	4.6 ± 1.1	0.47	.97
MIS20	1×10^4	8.2	6.2	38.6	3.7 ± 1.0	0.44	.97
	1×10^5	6.7	—	—	15.7 ± 10.9	0.11	.99
	1×10^6	6.1	5.2	8.7	4.3 ± 1.0	0.14	.99
	1×10^7	5.7	5.0	7.3	4.7 ± 1.0	0.13	.99
MIS21	1×10^4	7.5	—	—	14.3 ± 10.7	0.04	1.00
	1×10^5	7.0	—	—	15.7 ± 13.9	0.06	.99
	1×10^6	6.9	5.7	13.1	4.6 ± 1.2	1.29	.86
	1×10^7	6.8	5.8	14.0	5.9 ± 1.8	0.14	.99
MIS22	1×10^4	—	—	—	—	—	—
	1×10^5	7.4	6.2	40.0	7.3 ± 3.0	0.53	.97
	1×10^6	7.0	—	—	15.7 ± 13.9	0.06	.99
	1×10^7	6.5	5.5	10.6	4.3 ± 1.0	0.41	.98
MIS23	1×10^4	7.5	—	—	14.3 ± 10.7	0.04	1.00
	1×10^5	7.5	6.0	21.1	4.3 ± 1.2	1.33	.85
	1×10^6	6.7	—	—	15.7 ± 10.9	0.11	.99
	1×10^7	6.1	5.2	8.7	4.3 ± 1.0	0.14	.99
MIS24	1×10^4	7.5	—	—	14.3 ± 10.7	0.04	1.00
	1×10^5	7.0	—	—	15.7 ± 13.9	0.06	.99

(*Continued*)

TABLE 2 (Continued)

Isolates	Conc.	LT_{50}	Fiducial limits		Slope ± *SE*	χ^2	*p*
			Lower	Upper			
	1×10^6	6.4	5.6	10.0	6.3 ± 1.8	0.10	.99
	1×10^7	6.1	5.3	8.6	4.6 ± 1.1	0.47	.97
MIS25	1×10^4	—	—	—	—	—	—
	1×10^5	7.8	6.2	119.8	5.9 ± 2.1	1.09	.89
	1×10^6	7.0	—	—	15.7 ± 13.9	0.06	.99
	1×10^7	6.4	5.4	10.2	4.2 ± 1.0	0.25	.99

65.45% mortality when used independently while MIS13 and 0.5% *P. pinnata* oil showed 75.05 and 59.23% mortality when used separately (Table 3). Mahmoud (2009) studied the effect of interaction among different species of entomopathogenic fungi with respect to synergistic and antagonistic responses based on a comparison of mortality of adults by the fungi when used alone or in combination and reported a synergistic effect with combination of *B. bassiana* + *M. anisopilae*. In the present study we observed a synergistic effect with respect to mortality when *Metarhizium* isolates were used in combinations which are perhaps like the response observed between distinct species by Mahmoud (2009). The possibility of using mixtures of different species of entomopathogenic fungi for the control of western flower thrips, *Frankliniella occidentalis*, was reported by Gouli et al. (2008). Oil in formulations enhances the virulence, desiccation tolerance, thermal tolerance, speed of germination and infection, environmental stability, and reproduction of fungal biopesticides (Jackson, Dunlap, & Jaronski, 2010). In the present study, usage of *P. pinnata* seed oil would provide these advantages in addition to its insecticidal activity.

TABLE 3 Evaluation of Different Combinations of *M. anisopliae* Isolates and *P. pinnata* Seed Oil Against *A. fabriciella*

Treatments	Mean mortality of *A. fabriciella*
MIS7	65.45 ± 0.66
MIS13	75.05 ± 0.25
0.5% *P. pinnata* seed oil	59.23 ± 1.23
MIS7 + MIS13	78.86 ± 0.56
MIS7 + 0.5% *P. pinnata* seed oil	68.05 ± 0.13
MIS13 + 0.5% *P. pinnata* seed oil	77.87 ± 0.81
MIS7 + MIS13 + 0.5% *P. pinnata* seed oil	81.13 ± 0.80
SED CD (0.05) CD (0.01)	
0.2691 0.5459 0.7320	

Note. SED = standard error of the difference between means; CD = critical difference.

Pathogenicity in the Field

Pretreatment larval count in the field ranged from 15.45 to 16.01 per plant in Location I and 18.97 to 19.27 in Location II. After imposing treatment T2, 8.29 and 6.94 larvae were recorded per plant in Locations I and II, respectively, whereas Treatment T1 recorded 8.15 and 10.72 larvae. Both T2 and T1 differed significantly from the control (T3), which recorded 16.29 and 18.86 larvae per plant in Locations I and II after 7 days of treatment. After 15 days of treatment, significant difference in reduction of infestation was recorded between T2 and T1 in both locations. The Treatment T2 recorded 3.21 and 5.00 larvae while T1 recorded a larval count of 4.35 and 4.10 in Locations I and II, respectively. In Locations I and II, 16.99 and 19.06 larvae per plant was recorded in the untreated control (T3) plot. The average data on larval number per plant based on observations from both the locations showed significant difference in the reduction of infestation between treatments. The Treatment T2 recorded 5.86 larvae per plant amounting to 66.36% reduction of infestation and 61.15% reduction of infestation was observed in T1 with 6.83 larvae per plant (Table 4).

Field studies using fungal formulations for the management of *Ailanthus* defoliators have been very scanty. Most of the control methods reported were based on bacterial pathogens, plant extracts, and insecticides. Field testing of various plant products and three *B. thuringiensis* toxins against *A. fabriciella* was made in the past by Meshram (2010). Meshram and Jamaluddin (1989) carried out a field trial to determine the effect of monocrotophos in controlling *A. fabriciella* and reported that control may be achieved by applying 0.02% monocrotophos. Kulkarni and Joshi (1998) reported the antifeedant property of methanolic extracts of the leaves of four plant species and the seeds of *Azadirachta indica* (neem) against *A. fabriciella*. The seed extract of *A. indica* as well as leaf extracts of *Annona squamosa* and *Lantana camara* were very effective in protecting foliage from webworm infestation. Spraying of synthetic pyrethroids, Fenvalerate and Carbaryl at 0.01 and 0.2% concentration, respectively, was claimed to provide good control of *A. fabriciella* (Misra, Prasad, & Rawat, 1987).

Virulent isolates of *M. anisopliae*, which could serve as good candidates for development as mycoinsecticides against *A. fabriciella*, were identified from the present study. Since the virulence of the isolate impinges on the environmental factors, the growth requirements of the formulated pathogen must be in tune with the habitat of the target insect. Further works on strain improvement through physiological manipulations, by modification of culture conditions and genetic manipulation via selection of mutant or recombinant strains are of much significance in the development of an effective pest control strategy for *A. fabriciella* by biological means.

TABLE 4 Reduction of *A. fabriciella* Infestation in the Field After Treatment With *Metarhizium* Formulations

	Average number of larvae/plant										
	Location-I				Location-II				Location mean		
Treatments	1 DBT	7 DAT	15 DAT	Mean	1 DBT	7 DAT	15 DAT	Mean	DBT	DAT	R I (%)
T1: MIS7 + MIS13	15.73	8.15	4.35	6.25	19.01	10.72	4.10	7.41	17.37	6.83	61.15
T2: MIS7 + MIS13 + *P. pinnata* seed oil (0.5%)	15.45	8.29	3.21	5.75	18.97	6.94	5.00	5.97	17.21	5.86	66.36
T3: 0.08% Tween 80 (control)	16.01	16.29	16.99	16.64	19.27	18.86	19.26	19.06	17.64	17.85	

	SED	CD (0.05)	CD (0.01)
l-location	0.05156	0.10482	0.14067
t-treatment	0.06314	0.12838	0.17228
d-days	0.06314	0.12838	0.17228
l t	0.08930	0.18156	0.24365
t d	0.10936	0.22236	0.29840
l d	0.08930	0.18156	0.24365
l t d	0.15467	0.31447	0.42201

Note. DBT = day before treatment; DAT = days after treatment; RI = reduction of infestation; SED = standard error of the difference between means; CD = critical difference.

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