Biocontrol Potential of *Metarhizium anisopliae* (Metsch.) Sorokin (Deuteromycotina: Hyphomycetes) against *Ailanthus* Defoliator, *Eligma narcissus* (Cram.)

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Abstract

Eligma narcissus is recognised as a serious pest of *Ailanthus* in Southern India and defoliation of *Ailanthus* by this pest causes apparent loss of growth increment. The common control methods for this pest is mostly insecticides and the concern about the environmental effects of chemical insecticides, has emphasised the use of environmentally more benign microbial agents. Among entomopathogens, Fungi are the most explored and often act as important natural control agents that limit insect populations. On this point of view, Bio efficacy of 25 isolates of *Metarhizium anisopliae* was assessed to establish their virulence against *E. narcissus* in the laboratory and effective formulations of two potent isolates were subsequently evaluated in the field. MIS7 and MIS13 were more effective among the different isolates evaluated against *E. narcissus*. The median lethal concentration (LC_{50}) of all the isolates ranged from 6.46×10^5 conidia/ml to 628.92×10^5 conidia/ml. Median lethal concentration of (LT_{50}) of 4.9 and 5.4 days were recorded for MIS7 and MIS13 respectively at a concentration of 1×10^7 conidia/ml. Virulence 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. Formulations composed of "MIS7+MIS13+0.5% *Pongamia pinnata* seed oil" and "MIS7+MIS13" proved to be superior against *E. narcissus*, causing 76.30% and 93.93% mortality, respectively. Field evaluation of the formulation MIS7+MIS13+0.5% *Pongamia pinnata* seed oil recorded 5.79 larvae per plant resulting in 60.53% reduction of infestation while the formulation, MIS7+MIS13 showed 53.76% reduction of infestation with 6.56 larvae per plant. The observations from this study suggest the prospects of using the entomopathogenic fungus, *M. anisopliae* for the control of *E. narcissus*.

Keywords: Ailanthus, defoliation, Eligma narcissus, Metarhizium anisopliae, biocontrol

1. Introduction

Ailanthus excelsa Roxb. is a genus of trees belonging to the family Simaroubaceae. It is one of the promising fast growing multipurpose tree species in India (Tewari, 1992). Like any other forest tree,

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Ailanthus is also submitted to the attacks of insect pests. Bhasin and Roonwal (1954) reported 17 insects associated with *Ailanthus* belonging to 5 orders such as Coleoptera, Lepidoptera, Hemiptera, Thysanoptera and Isoptera. Among the various pest species, *Eligma narcissus* is recognised as a serious pest of *Ailanthus* in Southern India (Chatterjee and Sen Sarma, 1968). It is distributed all over India and feeds on almost all species of *Ailanthus*. Several species have been recognised and the one that occurs in India has been identified as *E. narcissus* (Roonwal, 1982). It does not show any clear seasonal trend in occurrence. Pest build up is generally on increase during September-January (Varma, 1986, 1991). Jha and Sen Sarma, (2008) opined that the occurrence of the pest is unpredictable and generally no association is observed with rainfall and the incidence followed a clustered pattern each time the density of the population increased. They feed on young as well as mature leaves. About 20-40 larvae feed voraciously on each leaf at times of heavy defoliation. Pest incidence in older plantations is rare compared to young plantations (David and Ananthakrishnan, 2004). Usually saplings up to five years old are infected and mature trees are free from attack. Larvae are reported to feed on green parts of the stem when all the leaves are consumed. Defoliation by this pest causes apparent loss of growth increment (Varma, 1986; Nair, 2007).The common control methods reported for this pest is by using chemicals, mostly insecticides although a few bacterial pathogens and plant extracts are tried. In recent times there has been growing public concern about the environmental effects of synthetic chemical insecticides, which has led to increased use of specific, environmentally more benign microbial agents (Cunningham and Frankenhuyzen, 1991).

Biological control involves employment of natural enemies such as predators, parasitoids, pathogens or competitors of a pest to help keep its numbers in check. In general the pathogens function naturally in the environment as population suppressors (Saxena, 2008). Fungi are the maximum explored organisms among entomopathogens and often act as important natural control agents that limit insect populations (Weinzierl and Henn, 1991). *M. anisopliae* is a recognised pathogen of more than 200 insect species, including several major pests. *Metarhizium* has been developed into commercial products for use in several countries (Kabuluk et al., 2001). While the products of this fungus have found major use in many developed countries, the efforts on these lines are yet to get popularized in India. Hitherto, there are no reports of any study on efficacy of *Metarhizium* fungi against *E. narcissus* in India. In view of this, the present investigation was undertaken to evaluate the potential of various isolates of *M. anisopliae* for controlling *E. narcissus*. Bioefficacy of 25 isolates of *M. anisopliae* was assessed for their virulence against *E. narcissus* with the objective of identifying potential strains. Two most promising isolates and *Pongamia pinnata* seed oil were evaluated individually and in different combinations in the laboratory and the effective formulations were tested in the field.

2. Methodology

2.1 Insect culture

Healthy larvae of *E. narcissus* (Figure 1) collected from field were reared in the laboratory and allowed to pupate and develop into adults. Male and female moths were released into glass bottles covered with muslin cloth. Diluted sucrose (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 *Ailanthus* leaves and transferred to glass bottles for hatching. The larvae initially established on tender leaves were transferred with fine camel hair brush to plastic boxes (14 cm diameter, 6 cm height) with fresh *Ailanthus* leaves. The petiole of the leaves were wrapped in a layer of moist tissue paper and sealed with parafilm to prevent wilting. Every two days, fresh leaves were provided. The pupae were

removed from the rearing containers within 24 hours of pupation and transferred to rearing cages for emergence.

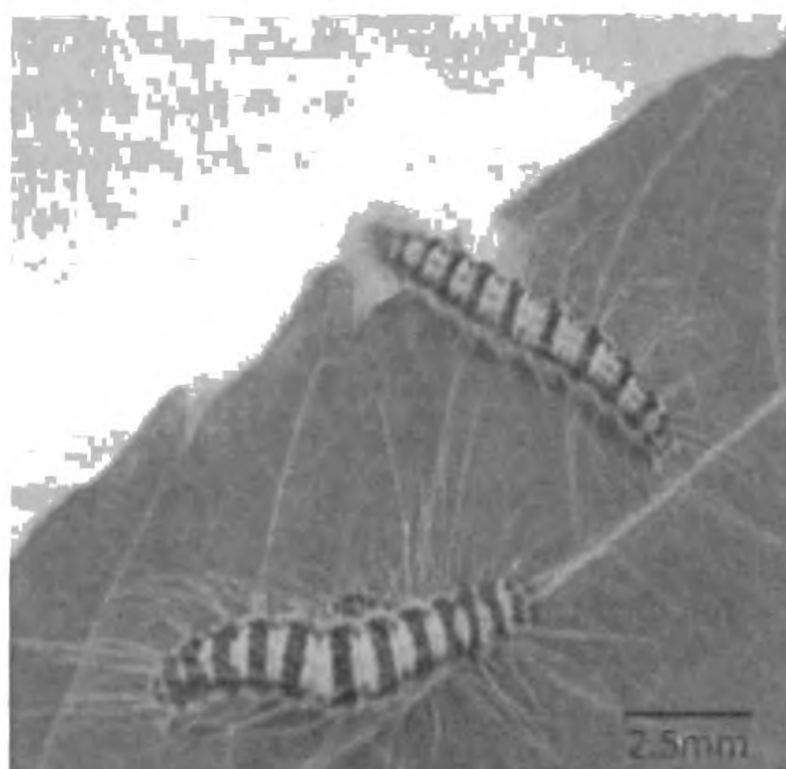

Figure 1: Healthy larva of *E. Narcissus.*

2.2 Fungus

Among the 25 fungal isolates used in this study, 16 were collected from field and nine received from different institutions. The isolates from field were recovered and purified from soil and insects. Soil samples were collected up to a depth of 30 cm from different study areas. Galleria bait method was used to isolate the fungus from soil samples. After removing roots and gravel, soil samples were sifted through a 5 mm sieve. Thereafter, plastic boxes (10 cm high, 8 cm diameter) were filled with 100 g of soil and ten *Galleria mellonella* late instar larvae were introduced. The lids were punched for making air holes. The larvae were incubated at 20°C in dark conditions.

During the first five 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 other day and dead larvae were collected. Cadavers thus obtained and those collected from field were surface-sterilised by dipping sequentially in 70% ethyl alcohol, 1% sodium hypochlorite, and sterile distilled water; each for 3 min. The larvae were dissected and placed on PDA/Veen's medium and incubated at 28±1°C and 90% RH to facilitate growth and sporulation of the fungus. Slant cultures were prepared from a single colony and stored at -20°C. The viability and virulence of the cultures were maintained by sub culturing and passage through the host at regular intervals (Zimmermann, 1986; Zayed, 2003).

2.3 Pathogenicity studies

Inoculum preparation

Metarhizium spores were harvested from PDAY plate culture by flooding 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 layered sterile cheese cloth and centrifuged at 1,700 rpm for 15 min. The supernatant was discarded and the conidia re-suspended in 5 ml sterile distilled water. This stock spore suspension was stored at 4°C for 24 h 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. Spore suspensions containing 1×10^3, 1×10^4, $1x10^5$, 1×10^6,

1×10^7 and 1×10^8 conidia/ml sterile distilled water with 0.05% Tween 80 were prepared from the stock for bioassay.

Bioassay

Bioassay of all the 25 isolates was carried out against the selected pests using inoculum concentrations ranging from 10^3-10^8 conidia/ml to determine the multiple dose-mortality (LC_{50}) and Time-dose-mortality (LT_{50}) responses. Ten second instar pest larvae were placed separately in sterile 20 ml vials and 10 ml of fungal suspension was added. The vials were capped and inverted five times over a 5 s period, to ensure that the insects were completely drenched. For the controls, insects were treated with 0.05% Tween 80 in the same manner. Treated and untreated (control) *E. narcissus* were transferred with fine camel hair brush into separate plastic boxes (14 cm diameter, 6 cm height) with fresh *Ailanthus* leaves as food source. The petiole of the leaves were wrapped in a layer of moist tissue paper and sealed with parafilm to prevent wilting. The boxes were incubated at 26±1°C, 90% RH, 12:12 (L:D). Once in two days, the leaves were replaced with fresh leaves. Each concentration of a single isolate was replicated four times. Mortality of larvae was recorded every 24 h for eight 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. Symptoms of mycosis in cadavers included distension and rigidity, a mottled rusty brown coloring, and development of *M. anisopliae* hyphae on the exterior of the integument (Figure 2).

Figure 2: Mycosed cadavers of *E. Narcissus*.

The efficacy of two most promising isolates, MIS7 (10^7 conidia/ml) and MIS13 (10^7 conidia/ml), and 0.5% *P. pinnata* seed 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 *E. narcissus*.

Field trial

Two formulations of each of the isolates, MIS7 and MIS13, which 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. Different treatments *viz.,* water formulation of conidia: T1 (MIS7 and MIS13 at a concentration of 10^{14} conidia/ml 0.08% Tween 80), Oil formulation of conidia:T2 (MIS7 and MIS13 at 10^{14} conidia/ml of 0.08% Tween 80 with 0.5% *P.*

pinnata seed oil) along with control T3 (0.08% Tween 80) were field evaluated in four year old *Ailanthus* plantation infested by *E. narcissus*. Germination test of the formulations was done one day prior to application and was found to be over 80%. The field layout was in a Randomised Block Design (RBD) consisting of three treatments (two treatments and control) in a plot of size 82×46 m^2 with each treatment replicated four times. The subplots measuring 22×12 m^2 in each replication had seven rows (ten plants each), 2 m apart (five main rows and two skip rows, one on either side of main rows). Each subplot was separated from the other by two skip rows 2 m apart (one row from each subplot). The population counts of *E. narcissus* were recorded a day before the imposition of treatments.

The total numbers of larvae on all the leaves of ten randomly selected tagged plants in each plot were recorded. The treatments were imposed using power sprayer. Post treatment observations on the number of larvae were recorded at seven and fifteen days after the spray. For each treatment, the averages of all the observations from two locations were used to determine the average percent reduction of pest population calculated using Henderson and Tilton equation (Henderson and Tilton, 1955).

2.4 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).

3. Results

3.1 Bioassay

MIS7, MIS13, MIS2 and MIS20 were more effective among the different isolates evaluated against *E. narcissus*. The LC_{50} values of all the isolates ranged from 6.46×10^5 conidia/ml to 628.92×10^5 conidia/ml. The least LC_{50} value of 6.46×10^5 conidia/ml was exhibited by isolate MIS7, followed by MIS13 (14.48×10^5 conidia/ml) (Table 1). The LT_{50} values were 4.9, 5.7, 6.8 and 7 days for MIS7 at 1×10^7, 1×10^6, 1×10^5 and 1×10^4 conidia/ml respectively and 5.4, 6.1, 6.8 and 7.1 days for MIS13 (Table 2). The least LC_{50} and LT_{50} values of MIS7 proved it to be the most effective isolate against *E. narcissus* followed by MIS13. Hitherto there are no reports of any study on efficacy of *Metarhizium* fungi against *E. narcissus* in India. The present study assumes significance in this context. Chatterjee et al. (1969) reported the ability of entomopathogenic fungi, *Beauveria bassiana* to cause white muscardine disease on *E. narcissus*. *Paecilomyces farinosus* was isolated from naturally infected pupae of *E. narcissus*. Mortality of *E. narcissus* larvae within 48-72 h of incubation and 40% pupal mortality were reported due to *P. farinosus* (Mohanan and Varma, 1988).

P. fumosoroseus was also recognised to be effective in controlling larvae and pupae of *E. narcissus* (David and Ananthakrishnan, 2004). Laboratory studies by releasing *E. narcissus* larvae on host plant leaves treated with *P. farinosus* spores showed mortality within 72 h which ranged from 77% for late instar larvae to 90% for early instars. Some of the inoculated larvae which could pupate, failed to emerge and even when emerged died in few hours (Mohammed Ali and Varma, 1992; Mohammed Ali et al., 1991). Varma and Mohammed Ali (1986) isolated a bacterial pathogen, *Bacillus firmus* from field population of *E. narcissus* and confirmed their pathogenicity with 80-100% mortality in larvae within 18-24 h under laboratory conditions. The antifeedant and growth inhibitory effects of methanolic extract of neem seed kernel (NSKE) was evaluated against final instar larvae of *E. narcissus* which pointed out the feeding deterrence and growth inhibition of the treated larva in a dose-dependent manner (Joseph, 2000).

Table 1: Dose-mortality response (LC_{50}) of *Metarhizium* isolates to *E. narcissus*.

Rank	Isolates	$LC_{50}(\times 10^5)$	Fiducial Limits		Slope±SE	χ^2	P
			Lower($\times 10^5$)	Upper($\times 10^5$)			
1	MIS7	6.46	1.37450	57.47463	2.6±0.7	0.027	0.986
2	MIS13	14.48	2.29119	1562.76166	2.2±0.7	0.124	0.940
3	MIS2	17.87	4.41148	267.80663	3.0±0.8	0.082	0.960
4	MIS20	37.35	8.76363	1164.59796	3.2±0.8	0.448	0.799
5	MIS1	62.61	13.85899	3275.18707	3.3±0.8	0.264	0.876
6	MIS3	110.02	12.70988	56424919.74126	2.3±0.7	0.018	0.991
7	MIS19	116.42	14.60324	4672517.23670	2.5±0.8	0.139	0.933
8	MIS15	123.14	33.27831	3809.16306	4.8±1.2	0.612	0.736
9	MIS23	156.12	22.38051	656584.13095	2.9±0.8	0.320	0.852
10	MIS18	157.60	20.04840	3631025.35580	2.7±0.8	0.118	0.943
11	MIS4	178.96	63.58669	2.458914E+49	2.5±0.8	0.019	0.990
12	MIS10	192.66	31.65834	127503.57566	3.5±0.9	0.087	0.958
13	MIS14	201.92	38.73739	53822.93829	4.0±1.0	1.366	0.505
14	MIS9	217.13	26.95383	3925480.88407	2.9±0.8	0.107	0.948
15	MIS16	256.41	34.62919	1531082.54686	3.3±0.9	0.268	0.874
16	MIS8	300.48	32.58871	35029799.27609	2.9±0.8	0.226	0.893
17	MIS17	314.67	44.20990	959879.03055	3.6±0.9	0.075	0.963
18	MIS6	344.54	41.35598	8152760.21599	3.3±0.9	0.093	0.955
19	MIS11	362.13	59.99479	475700.31977	4.3±1.1	0.762	0.683
19	MIS21	362.13	59.99479	475700.31977	4.3±1.1	0.762	0.683
19	MIS22	362.13	59.99479	475700.31977	4.3±1.1	0.762	0.683
20	MIS5	414.59	52.44195	3709702.67431	3.6±1.0	0.106	0.948
21	MIS25	416.82	39.57977	5.414953E+21	2.8±0.7	0.187	0.915
22	MIS12	424.41	39.57977	5.414953E+13	2.9±0.8	0.712	0.700
23	MIS24	628.92	41.49018	1.1956121E+30	2.7±0.8	0.065	0.971

E=exponent

Table 2: Time-dose-mortality response (LT_{50}) of *Metarhizium* isolates to *E. narcissus*.

Isolates	Conc.	LT_{50}	Fiducial Limits		Slope±SE	χ^2	P
			Lower	Upper			
MIS 1	1×10^4	8.1	-	-	7.2±3.50	0.15	0.99
	1×10^5	7.0	-	-	15.7±13.90	0.06	0.99
	1×10^6	6.7	5.8	12.5	5.8±1.70	0.20	0.99
	1×10^7	5.9	5.1	8.0	4.1±0.90	1.81	0.77
MIS 2	1×10^4	8.1	-	-	7.2±3.50	0.15	0.99
	1×10^5	7.0	6.0	29.8	7.0±2.50	0.91	0.92
	1×10^6	6.5	5.6	11.0	5.3±1.40	0.43	0.98
	1×10^7	5.2	4.6	6.1	4.6±0.90	0.16	0.99
MIS 3	1×10^4	8.1	-	-	7.2±3.50	0.15	0.99
	1×10^5	7.2	6.1	72.7	7.1±2.70	0.35	0.98
	1×10^6	7.1	5.9	18.8	5.8±1.80	0.12	0.99
	1×10^7	5.8	5.3	7.2	7.1±1.80	0.38	0.98
MIS 4	1×10^4	9.0	-	-	6.6±3.50	0.25	0.99
	1×10^5	8.1	-	-	7.2±3.50	0.15	0.99
	1×10^6	7.7	6.2	64.9	5.5±1.80	0.26	0.99
	1×10^7	6.8	5.6	12.8	4.5±1.20	1.13	0.88
MIS 5	1×10^4	9.0	-	-	6.6±3.50	0.25	0.99
	1×10^5	8.7	6.5	36.5	5.2±1.90	0.82	0.93
	1×10^6	7.5	-	-	14.3±10.70	0.04	1.00
	1×10^7	6.5	5.7	11.0	6.4±1.90	1.12	0.89
MIS 6	1×10^4	8.1	-	-	7.2±3.50	0.15	0.99
	1×10^5	7.6	-	-	7.4±3.30	0.23	0.99
	1×10^6	7.5	-	-	14.3±10.70	0.04	1.00
	1×10^7	6.5	5.6	11.0	5.3±1.40	0.43	0.98

MIS 7	$1x10^{4}$	7.0	-	-	7.4±3.30	0.23	0.99
	$1x10^{5}$	6.8	5.7	13.1	5.1±1.40	0.48	0.97
	$1x10^{6}$	5.7	4.9	7.9	3.8±0.80	0.46	0.97
	$1x10^{7}$	4.9	4.4	5.9	3.8±0.70	0.90	0.92
MIS 8	$1x10^{4}$	9.0	-	-	6.6±3.50	0.25	0.99
	$1x10^{5}$	8.1	-	-	6.5±2.80	0.76	0.94
	$1x10^{6}$	7.0	6.0	29.8	7.0±2.50	0.91	0.92
	$1x10^{7}$	6.2	5.4	8.8	5.6±1.50	0.30	0.99
MIS 9	$1x10^{4}$	9.0	-	-	6.6±3.50	0.25	0.99
	$1x10^{5}$	8.1	-	-	7.2±3.50	0.15	0.99
	$1x10^{6}$	7.1	5.9	18.8	5.8±1.80	0.12	0.99
	$1x10^{7}$	6.2	5.4	8.8	5.6±1.50	0.30	0.99
MIS 10	$1x10^{4}$	11.5	-	-	5.6±3.40	0.66	0.95
	$1x10^{5}$	8.1	-	-	7.2±3.50	0.15	0.99
	$1x10^{6}$	7.2	6.1	72.7	7.1±2.70	0.35	0.98
	$1x10^{7}$	6.2	5.4	8.8	5.6±1.50	0.30	0.99
MIS 11	$1x10^{4}$	9.0	-	-	6.6±3.50	0.25	0.99
	$1x10^{5}$	7.6	-	-	7.4±3.30	0.23	0.99
	$1x10^{6}$	7.5	-	-	14.3±10.70	0.04	1.00
	$1x10^{7}$	7.0	6.0	27.3	7.0±2.50	0.50	0.97
MIS 12	$1x10^{4}$	9.5	-	-	4.6±1.70	0.37	0.98
	$1x10^{5}$	9.0	-	-	6.6±3.50	0.25	0.99
	$1x10^{6}$	7.5	6.2	45.2	7.0±2.80	0.47	0.97
	$1x10^{7}$	6.4	5.6	10.3	5.2±1.40	0.40	0.98
MIS 13	$1x10^{4}$	7.1	5.9	18.8	5.8±1.80	0.12	0.99
	$1x10^{5}$	6.8	5.7	13.1	5.1±1.40	0.48	0.97
	$1x10^{6}$	6.1	5.3	8.6	4.6±1.10	0.47	0.97
	$1x10^{7}$	5.4	4.8	6.6	4.7±1.00	0.06	1.00
MIS 14	$1x10^{4}$	9.0	-	-	6.6±3.50	0.25	0.99
	$1x10^{5}$	8.1	-	-	7.2±3.50	0.15	0.99
	$1x10^{6}$	7.2	6.1	72.7	7.1±2.70	0.35	0.98
	$1x10^{7}$	6.7	5.6	12.3	4.2±1.00	0.27	0.99
MIS 15	$1x10^{4}$	9.0	-	-	6.6±3.50	0.25	0.99
	$1x10^{5}$	8.2	6.3	11.1	5.7±2.30	1.85	0.76
	$1x10^{6}$	7.7	6.2	64.9	5.5±1.80	0.26	0.99
	$1x10^{7}$	6.5	5.5	10.6	4.3±1.00	0.41	0.98
MIS 16	$1x10^{4}$	8.1	-	-	7.2±3.50	0.15	0.99
	$1x10^{5}$	7.5	-	-	14.3±10.70	0.04	1.00
	$1x10^{6}$	7.2	6.1	72.7	7.1±2.70	0.35	0.98
	$1x10^{7}$	6.5	5.5	10.6	4.3±1.00	0.41	0.98
MIS 17	$1x10^{4}$	9.0	-	-	6.6±3.50	0.25	0.99
	$1x10^{5}$	7.5	-	-	14.3±10.70	0.04	1.00
	$1x10^{6}$	7.2	6.1	72.7	7.1±2.70	0.35	0.98
	$1x10^{7}$	6.5	5.6	11.0	5.3±1.40	0.43	0.98
MIS 18	$1x10^{4}$	9.0	-	-	6.6±3.50	0.25	0.99
	$1x10^{5}$	7.5	6.2	45.2	7.0±2.80	0.47	0.97
	$1x10^{6}$	7.1	5.9	18.8	5.8±1.80	0.12	0.99
	$1x10^{7}$	6.0	5.3	8.4	4.8±1.20	1.02	0.90
MIS 19	$1x10^{4}$	8.1	-	-	7.2±3.50	0.15	0.99
	$1x10^{5}$	8.1	6.3	167.1	5.0±1.70	0.46	0.97
	$1x10^{6}$	6.7	5.8	12.5	5.8±1.70	0.20	0.99
	$1x10^{7}$	5.9	5.3	7.4	6.2±1.50	0.30	0.98
MIS 20	$1x10^{4}$	8.1	-	-	7.2±3.50	0.15	0.99
	$1x10^{5}$	8.0	6.3	167.1	5.0±1.70	0.46	0.97
	$1x10^{6}$	6.2	5.4	8.8	5.6±1.50	0.30	0.99
	$1x10^{7}$	5.7	5.0	7.3	4.7±1.00	0.13	0.99

MIS 21	1×10^{4}	-	-	-	-	-	-
	1×10^{5}	9.0	-	-	6.6±3.50	0.25	0.99
	1×10^{6}	7.5	6.2	45.2	7.0±2.80	0.47	0.97
	1×10^{7}	6.9	5.8	14.6	5.2±1.50	0.77	0.94
MIS 22	1×10^{4}	-	-	-	-	-	-
	1×10^{5}	7.5	6.1	37.0	5.3±1.70	0.22	0.99
	1×10^{6}	7.0	-	-	15.7±13.90	0.06	0.99
	1×10^{7}	6.5	5.5	10.6	4.3±1.00	0.41	0.98
MIS 23	1×10^{4}	-	-	-	-	-	-
	1×10^{5}	8.1	-	-	7.2±3.50	0.15	0.99
	1×10^{6}	7.2	6.0	24.0	5.9±1.90	0.38	0.98
	1×10^{7}	6.2	5.4	8.8	5.6±1.50	0.30	0.99
MIS 24	1×10^{4}	9.3	-	-	5.6±2.60	1.26	0.86
	1×10^{5}	7.5	-	-	14.3±10.70	0.04	1.00
	1×10^{6}	7.5	6.1	37.0	5.3±1.70	0.22	0.99
	1×10^{7}	6.4	5.4	10.2	4.2±1.00	0.25	0.99
MIS 25	1×10^{4}	-	-	-	-	-	-
	1×10^{5}	8.1	-	-	7.2±3.50	0.15	0.99
	1×10^{6}	7.1	5.9	18.8	5.8±1.80	0.12	0.99
	1×10^{7}	5.9	5.1	8.0	4.4±1.00	0.13	0.99

Significant difference in mortality was observed between the seven combinations tested. Increased mortality was recorded with the combination treatments compared to individual treatments. Evaluation of various formulations revealed the combination MIS7+MIS13+0.5% *P. pinnata* seed oil to be superior over the other treatments which resulted in 76.30% mortality. MIS7+MIS13 showed 74.82% mortality (Table 3). About 6-18% increase in mortality was reported with formulations when used in combinations.

Synergistic effects of different isolates of a single fungal species, especially *Metarhizium* on insect mortality have not been reported. However, the effect of interaction of *B. bassiana*, *M. anisopilae* and *L. lecanii* was tested by Mahmoud (2009). These authors analysed the synergistic and antagonistic interactions based on a comparison of mortality of adults by these fungi when used alone and in combination.

The combination of *B. bassiana+M. anisopilae* gave a synergistic response while the combination of *B. Bassiana+L. lecanii* and *M. Anisopliae+L. lecanii* gave an antagonistic response. 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).

Interaction between the fungi *B. bassiana*, *M. anisopilae* and the diatomaceous earth dusts with negligible effect on the viability of conidia was observed by Batta (2008). Oil based formulations have shown better tolerance to temperature and desiccation, enhanced speed of germination of conidia, improved environmental stability and overall performance as fungal biopesticides (Jackson et al., 2010). In the present study, usage of *P. pinnata* seed oil would have provided these advantages in addition to its insecticidal activity.

Table 3: Evaluation of different combinations of *M. anisopliae* isolates and *P. pinnata* seed oil against *E. narcissus.*

Treatments	Mean mortality of *E. narcissus*
MIS7	70.33
MIS13	64.80
0.5% Pongam oil	58.86
MIS7 + MIS13	74.82
MIS7+0.5% Pongam oil	73.18
MIS13+0.5% Pongam oil	68.25
MIS7 + MIS13+0.5% Pongam oil	76.30
SED	0.43
CD(.05)	0.87
CD(.01)	1.17

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

3.2 Field Trial

Mean number of pest larvae in the experimental plots prior to treatment ranged from 13.07 to 14.13 per plant in Location I and 13.99 to 14.27 in Location II. After seven days of treatment, the treatment T2 (MIS7+MIS13+0.5% *P. pinnata* seed oil) proved to be superior over other treatments as indicated by the high reduction of infestation in both locations. The treatment T2 recorded 5.01 larvae per plant in location I and 6.59 larvae per plant in location II which differed significantly from the treatment T1. Mean number of 14.12 larvae/plant in location I and 15.03 larvae/plant in location II were recorded in Treatment T3 (untreated control). Observations recorded after fifteen days of imposition of treatments revealed significant differences between the treatments. Treatment T2 recorded a mean number of 6.13 larvae/plant in location I while T1 recorded 5.57 larvae. In Location II also, T2 was found promising with 5.43 larvae per plant. The control (T3) recorded 14.38 larvae/plant and 15.91 larvae/plant in location I and II respectively. Overall reduction of infestation for different treatments was calculated by combining the data from both the locations. The treatment T2 recorded a mean number of 5.79 larvae/plant which works out to 60.53% reduction of infestation. 53.76% reduction of infestation was observed in T1 (Table 4). Augmenting the formulation with *P. pinnata* seed oil increased the overall reduction in infestation of *E. narcissus* by about 7%. There has been no field trial studies reported using *Metarhizium* isolates against *E. narcissus.*

In one reported case of natural infection by an entomopathogenic fungus *P. farinosus*, 40% pupal mortality of *E. narcissus* was reported by Mohanan and Varma (1988) indicating the prospects of using entomopathogenic fungi for biological control of this pest. Control of *E. narcissusin* nurseries and young plantations using insecticides, Fenvalerate and Quinalphos was reported by Varma (1986) and Roonwal (1990). The observations from this study suggest the prospects of using the entomopathogenic fungus, *M. anisopliae* for the control of *E. narcissus*. The death of the host insect results from the invasion and colonization of the host body by the fungus and/or due to the toxins produced by the fungus. Many chemical pesticides are now being phased out because of their wider impact on ecosystems and this study reaffirms the fact that entomopathogenic fungi can be an important alternative to chemicals for pest management.

Table 4: Reduction of *E. narcissus* infestation on *A. excelsa.*

Treatments	Average number of larvae/Plant										RI (%)
	Location-I				Location-II				Location Mean		
	1 DBT	7 DAT	15 DAT	Mean	1 DBT	7 DAT	15 DAT	Mean	DBT	DAT	
T1	13.07	6.87	5.57	6.22	13.99	7.38	6.42	6.90	13.53	6.56	53.76
T2	13.86	5.01	6.13	5.57	14.18	6.59	5.43	6.01	14.02	5.79	60.53
T3	14.13	14.12	14.38	14.25	14.27	15.03	15.94	15.47	14.20	14.86	

	SED	CD (0.05)	CD (0.01)
l-location	0.02270	0.04615	0.06193
t-treatment	0.02780	0.05652	0.07585
d-days	0.02780	0.05652	0.07585
l t	0.03931	0.07993	0.10727
t d	0.04815	0.09790	0.13137
l d	0.03931	0.07993	0.10727
l t d	0.06809	0.13845	0.18579

DBT= Day before treatment; DAT= Days after treatment; RI: Reduction of infestation,
T1- MIS7+MIS13; T2- MIS7+MIS13+pongam oil (0.5%); T3-0.08% tween 80 (control)

The key question that arises with the use of fungi in field is the long term storage and viability as the conservation of viability and efficacy after long term storage and field persistence are very important for the successful application of the formulation under field conditions. Further studies to ascertain the efficacy of the isolates in the field even after storage and methods to enhance the field persistence will pave way for successful application of these formulations as a biocontrol agent for *Ailanthus* defoliator, *E. narcissus*. .

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