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Research Note

Laboratory evaluation of 7 isolates of fungi against army worm, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) and Castor hairy caterpillar, *Euproctis fraterna* (Moore) (Lepidoptera: Lymantridae)

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ABSTRACT: In this report, the insecticidal activity of *Metarhizium anisopliae* isolated from different geographical regions of Tamil Nadu was evaluated against *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) and *Euproctis fraterna* (Moore) (Lepidoptera: Lymantridae) under laboratory conditions. Among the tested isolates, Ma2 isolate at 10^8 spore/mL was found more effective against *S. litura* and *E. fraterna* than Ma1 than and Ma3 isolates.

KEY WORDS: Bioassay, LC_{50}, LC_{90}, Lepidoptera, entomopathogenic fungi

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The fungus *Metarhizium anisopliae* was extensively studied and is one of the best-characterized entomopathogens used for the biological control of insects and acarines (Scholte *et al*., 2007). *M. anisopliae* infects arthropods by a combination of the secretion of hydrolytic enzymes, commonly referred to as cuticle degrading enzymes, and mechanical pressure exerted by the appressorium (Wang and St Leger, 2005). Studies demonstrate that *M. anisopliae* recognizes specific host signals, like lipids present in the epicuticle and the surface structure, which could define the spore attachment and induce the secretion of different host specific proteins (Pedrini *et al*., 2007). Currently, *M. anisopliae* var. *acridum* is being used in the field as a registered mycopesticide or is under commercial development for microbial control of different insect pests in Australia, Brazil and South Africa (Zimmermann, 1993; Wraight *et al*., 2001). The present investigation evaluated the pathogenic role of *M. anisopliae* isolated from different geographical regions of Tamil Nadu against the lepidopteran insect pests such as army worm, *Spodoptera litura* (Fabricius) and castor hairy caterpillar, *Euproctis fraterna* (Moore).

Three *Metarhizium anisopliae* strains were isolated from different geographical regions of Southern Tamil Nadu, India. Details of the isolates along with their host insect and place of origin are listed in Table 1. All the three fungal isolates used in this study were isolated from different sources as per the methodology developed by Haraprasad *et al*. (2001). The isolates were identified by microscopic observation using lactophenol cotton blue staining and cultured in potato dextrose agar (PDA) medium. Plates were incubated at 26°C for 10 days and after sporulation, aerial conidia were harvested by flooding the plate with sterile deionized water (dH_2O). Conidial spore concentrations were determined by direct count using a haemocytometer. All the cultures were adjusted to 1 x 10^8 conidia mL^{-1} from which the lower concentrations

Table 1. Details of *Metarhizium anisopliae* isolates source and host

Fungus	Strain	Original Host	Origin
M. anisopliae	*Ma1*	*Hypothenemus hampei* (Ferari)	Thandikkudi, Dindugul, Tamil Nadu, India
M. anisopliae	*Ma2*	Soil (Rhizosphere)	Alagar Hills, Madurai, Tamil Nadu, India
M. anisopliae	*Ma3*	Soil (Rhizosphere)	Alwarkurichi, Tirunelveli, Tamil Nadu, India

(1×10^5, 1×10^6 1×10^7 and 1×10^8) were prepared using Tween 80 by serial dilution technique for bioassay studies.

Three isolates were selected based on the spore germination (65-91%) potential for bioassays against *S. litura* & *E. fraterna* and *M. anisopliae* spore suspensions were sprayed using hand sprayer (500mL) onto castor leaves with their stalks dipped in water. Number of spores treated over the leaves were counted using a haemocytometer. Ten to fifteen third instar field collected larvae of *S. litura* and *E. fraterna* were released onto the *M. anisopliae* sprayed leaves (when still wet) kept in a glass jar. Three replicates were maintained for each treatment. Fresh untreated leaves were fed daily to the larvae, two days after exposure. Control was maintained by releasing the larvae on the leaves sprayed with 0.02% Tween-80 solution. Observations of larval mortality were recorded everyday for 10 days. Death due to mycosis was inferred by observing for mummification of cadavers. The cadavers were washed in sterile water and placed on moist tissue paper in petri plates and observed for mycelial growth and sporulation in the next 48 h to confirm mortality due to infection by *M. anisopliae* isolates.

Percent mortality data were transformed to arc sine root values and subjected to analysis of variance (ANOVA) and the Tukey least significant difference test at P<0.05 using PAST software. Lethal concentration for 50% and 90% mortality (LC_{50} and LC_{90} respectively) was estimated using Probit Regression analysis using Statistical Packages for Social Sciences (SPSS 10).

The study suggested that Ma2 isolate infected the larvae of *S. litura* and *E. fraterna* in a dose – dependent manner. Mortality in control (0.02% Tween 80) was 1.33% and 3.21% with respect to *S. litura* and *E. fraterna* respectively ten days after treatment (Table 2 and 3). The tested fungal isolates of *M. anisopliae* were pathogenic to both *S. litura* and *E. fraterna* with significant difference among the isolates. Frazzon *et al*. (2000) studied 12 strains of *M. anisopliae* and observed that 4 strains killed 50% of engorged females after a single fungal immersion. During a second immersion (1×10^7 conidia/ml), nine strains killed 100% of ticks. Evidently, Mochi *et al*. (2006) also observed a reduction in the emergence of adults after treating *Ceratitis capitata* (Wied.) larvae with the E9 *M. anisopliae* isolate at a concentration of 10^8 conidia ml^{-1}. Recently, Joseph *et al*. (2010) in their laboratory studies, reported that *B. bassiana* at spore density of 10^9 spores/ml caused 100% larval mortality while, LC_{50} value was found to be 0.5×10^6 spores / ml, whereas, in the present study, 10^8spore/mL of Ma2 performed a potential active substance. Similarly, Ma2 isolate at 1×10^8 conidia/ml caused 100% mortality of *E. fraterna.* Substantially, Ma2 ($F_{3,16}$ = 3.408, P <0.05%) isolate had higher population reduction potential followed by Ma1 ($F_{3,16}$ = 4.162, $P < 0.05\%$) and Ma3 ($F_{3,16}$ = 2.106, $P < 0.05\%$). Maximum of 100% suppression of *E. fraterna* was obtained after ten days treatment by Ma2 ($F_{3,16}$ = 6.214, $P <$ 0.05%) isolate followed by Ma1 ($F_{3,16}$ = 6.481, P <0.05%) and Ma3 ($F_{3,16}$ = 2.7, P <0.05%). However, the conidial concentrations of *M. anisopliae* are directly proportional to the mortality of the tested insects. Lowest lethal concentration value (LC_{50} and LC_{90}) for *S. litura* were observed with Ma2 isolated from rhizosphere soil of Azhagar hill (2.0×10^8spore/mL) followed by Ma1 isolated from *H. hampei* (2.6×10^8) and Ma3 isolated from rhizosphere soil of Alwarkurichi Thandigudi (4.9×10^8) isolates whereas the values (LC_{50} and LC_{90}) of *E. fraterna* were Ma2 isolate (1.0×10^8spore/mL)

Table 2. Percent mortality, LC_{50}, LC_{90} and intercept of different geographical isolates of *Metarhizium anisopliae* against 3rd instar larvae of *Euproctis fraterna* after 10 days of inoculation

Treatment	% Mortality ± SD*	LC_{50}	LC_{90}	Intercept± SE	Chi - Square
Ma1	93.33 ± 0.08[b]	6.3×10^8 (2.5×10^8 – 4.4×10^9)	2.13×10^8 (1.21×10^8 – 2.10×10^9)	–1.13 ± 0.30	0.911**
Ma2	100 ± 0.08[a]	1.0×10^8 (6.1×10^7 – 6.4×10^9)	2.4×10^8 (1.4×10^8 – 1.8×10^9)	–1.19 ± 0.30	0.831
Ma3	73.33 ± 0.08[c]	4.91×10^8 (4.1×10^8 – 5.0×10^9)	4.8×10^8 (2.9×10^8 – 3.1×10^9)	–1.36 ± 0.33	0.416
Control	3.21 ± 0.08[d]				

* Means with different lowercase letters in each column differed significantly (Tukey's HSD, $P \leq 0.05$)

**Significant at 5% level

Table 3. Percent mortality, LC_{50}, LC_{90} and intercept of different geographical isolates of *Metarhizium anisopliae* against 3rd instar larvae of *Spodoptera litura* after 10 days of inoculation

Treatment	% Mortality ± SD*	LC_{50}	LC_{90}	Intercept ± SE	Chi – Square
Ma1	57 ± 0.81b	$2.6 \times 10_8$ ($1.8 \times 10_9 – 2.2 \times 10_{10}$)	$5.5 \times 10_9$ ($2.8 \times 10_9 – 1.3 \times 10_{10}$)	1.14 ± 0.24	0.685**
Ma2	87 ± 0.81a	$2.0 \times 10_8$ ($1.1 \times 10_9 – 1.4 \times 10_{10}$)	$4.2 \times 10_9$ ($1.5 \times 10_9 – 1.0 \times 10_{10}$)	0.96 ± 0.22	0.401
Ma3	40 ± 0.81c	$4.9 \times 10_8$ ($2.3 \times 10_9 – 1.4 \times 10_{10}$)	$9.5 \times 10_9$ ($6.1 \times 10_9 – 2.5 \times 10_{10}$)	1.37 ± 0.27	0.607
Control	1.33 ± 0.08d				

* Means with different lowercase letters in each column differed significantly (Tukey's HSD, $P \leq 0.05$)

**Significant at 5% level

followed by Ma1 (6.3×10^8) and Ma3 (4.91×10^8) isolates (Table 2 and 3) and suggesting that Ma2 from rhisosphere soil of Azhagar hill was found to be more virulent fungal isolate followed by Ma1. Whereas, Ma3 was the least virulent amongst the three isolates tested in the bioassay. Many investigators reported that the pathogenicity was not always related to the original host or geographic origin (Moorehouse *et al*., 1993). Differences in pathogenicity between fungal species and isolates have been observed for other insects species (Moorehouse *et al.*, 1993). Feng and Johnson (1990) noted that the original host has no significant influence on the virulence. Moreover, they found that entomopathogenic fungi isolated from soil showed a high pathogenicity to insect. Ekesi *et al*. (1999) found that one isolate of *B. bassiana* and two isolates of *M. anisopliae* from soil were highly virulent to the legume flower thrips, *Megalurothrips sjostedti* (Trybom) and Maniania (1992) reported that they were highly pathogenic to the stem borers *Chilo partellus* (Swinhoe) and *Busseola fusca* (Fuller). Furthermore, Ekesi *et al.,* (1999) noted that *M. anisopliae* isolated from the soil showed high level of pathogenicity to pod bug, *Clavigralla tomentosicollis* (Stal). In the present study, less virulence of the insect isolate may be attributed either as it was originally isolated from the coleopteran insect or due to continuous subculturing of the isolate in the artificial medium without passing through the host insect.

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