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VIRULENCE OF ENTOMOPATHOGENIC FUNGUS *METARHIZIUM ANISOPLIAE* (METSCH.) SOROKIN ON SEVEN INSECT PESTS

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ABSTRACT

In-vitro bioassay was conducted to evaluate the bioefficacy of *Metarhizium anisopliae* (Metsch.) Sorokin (Deuteromycotina: Hyphomycetes) against seven common insect pests of Tamil Nadu, India *viz., Dysdercus cingulatus* (Fab.) (Hemiptera: Pyrrhocoridae), *Oxycarenus hyalinipennis* (Costa) (Hemiptera: Lygaeidae), *Aphis craccivora* (Koch) (Homoptera: Aphididae), *Mylabris pustulata* (Thunb.) (Coleoptera: Meloidae), *Pericallia ricini* Fab. (Lepidoptera: Arctiidae), *Spodoptera litura* (Fab.) and *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) by dermal toxicity tests. LC_{50} value was ranged from 1.62 x 10^4 to 1.75 x 10^6 spores/ml. Results revealed that among the young ones tested, the lowest and highest LC_{50} values were recorded for *P. ricini* II instar (1.62x10^4) and *H. armigera* III instar (1.75 x10^6), respectively. Among the adults, *A. craccivora* recorded lowest LC_{50} (1.84 x 10^4), followed by *O. hyalinipennis, D. cingulatus* and *M. pustulata.* Thus the results show that *M. anisopliae* can be used for the control of *H. armigera.*

Keywords: Biological Control potential, Cotton pests, *Metarhizium anisopliae*

INTRODUCTION

Several control campaigns have been conducted in India against insect pests using chemical pesticides still today; however, the pest problem remains unchanged. Due to the ineffectiveness, inherent health and environmental hazards of chemical pesticides. One possible alternative pest control option is biological control. The entomopathogenic fungi (Sahayaraj and Borgio, 2009) place an important role and have higher potential for biological control of sucking and defoliator insect pests.

Dysdercus cingulatus (Fab.) (Hemiptera: Pyrrhocoridae) is a serious sucking pest of cotton (David and Kumaraswami, 1978; Sahayaraj, 2007), which infests cotton in all the cotton and Pericallia *ricini* Fab. (Lepidoptera: Arctiidae) commonly known as hairy caterpillar is the major pest of castor, gingelly, cotton, country bean, brinjal, drum stick, coccina, banana, calotropis, sunflower, oleander, tea, sweat potato and pumpkin (David and Ananthakrishnan, 2004). Tobacco caterpillar, *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae) is a polyphagous pest affecting several crops worldwide causing extensive loss in agricultural production (Guo *et al.,* 2007). *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae is commonly known as the gram pod borer as it is a serious pest on pulses (Nahar *et al.,* 2004; David and Ananthakrishnan, 2004). *Mylabris pustulata* (Thunb.) (Coleoptera: Meloidae) is a common insect pest of many field crops in India (Khan *et al.,* 2005).

Metarhizium anisopliae (Metsch.) Sorokin (Deuteromycotina: Hyphomycetes) is a filamentous entomopathogenic fungi, which is used commercially as a biological control agent against

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many insect pests. *M. anisopliae* has a wide host range and individual isolates can be considerably host-specific. No previous reports available about the virulence of *M. anisopliae* against *M. pustulata, P. ricini* and *O. hyalinipennis*. Even though few studies (Nahar *et al.,* 2004; Anonymous, 2004) about the virulence of *M. anisopliae* against *S. litura, D. cingulatus* and *H. armigera* were conducted separately. No report was available for the impact of single fungal isolate against all the above-mentioned pests. The present study was undertaken to evaluate the virulence of single *M. anisopliae* isolate against the insect pests tested under laboratory conditions.

MATERIAL AND METHODS

M. anisopliae was obtained from our pure stock culture. Spores were removed from the sub-culture of the isolate that had been purified using Potato Dextrose Agar (PDA) (HiMedia, Mumbai, India). Inoculated test tubes were maintained at 26°C ± 2°C in an incubator till sporulation and the slope cultures were then maintained at 4°C. More than 15 days old working cultures have not been utilized for the study. The fungal conidia were collected from 7 days old cultures, $1.6x10^7$ conidia/ml were prepared and treated as stock. Four spore concentrations such as 1.7×10^4, 2.6×10^5, 1.9×10^6 and 1.6×10^7 spores/ml were prepared from the stock. Conidial concentrations of the suspensions were determined using an improved Neubauer Haemocytometer (Pohem, Mumbai, India). Water was used as control. A homogeneous conidial suspension was prepared in sterile distilled water by adding few drops of castor oil (0.1 %) as surfactant.

Life stages of *P. ricini*, *H. armigera* and *A. craccivora* were collected from castor, cotton and groundnut (fields) respectively and. *S. litura* from groundnut field. Whereas adults of *O. hyalinipennis*, *M. pustulata* and *D. cingulatus* were also collected from the cotton fields from different parts of Tamil Nadu, India and were maintained on their respective hosts at room temperature (29°±2C), RH (70-80 %) and 11L and 13D photoperiod in plastic containers (1L capacity). Laboratory emerged lepidopteran adults (>1 day) (five males and five females) were separately were introduced into the oviposition cages and fed with 10% sucrose solution fortified with a few drops of vitamin mixture to enhance the oviposition. The egg batches were removed and kept in Petri dishes for hatching.

The tested insects were released separately into the plastic containers (500 ml capacity). 1.7×10^4 spore/ml of fungal conidial suspension was sprayed on the pests by hand sprayer. After 10 minutes they were transferred and released into a clean plastic container (500 ml) and provided with their respective host plants. Similar procedure was adapted for the remaining concentrations such as 2.6×10^5, 1.9×10^6 and 1.6×10^7, replicated six times for each pest. The mycelial growth on the pests and also mortality were recorded for every 24 hours till 96 hours. Finney's formula was used to calculate corrected mortality (Finney, 1971). From the corrected mortality data, the probability integral of the chi-square distribution and LC_{50} were calculated in order to find out the efficacy of *M. anisopliae* on pests. Mortality data of the individual pest was correlated with all other pests using STATISCA-2004 software and their correlation coefficient (r^2) was recorded in the Table 2.

RESULTS AND DISCUSSION

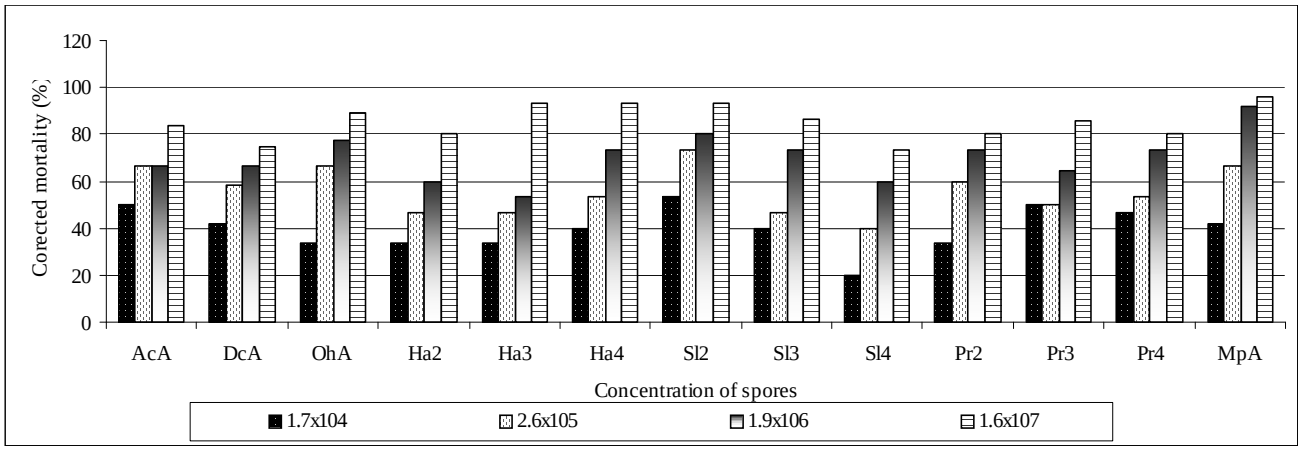
Irrespective of the pests tested, the corrected mortality varied from pest to pest. Dose dependent corrected mortality was observed for 1.7×10^4, 2.6×10^5, 1.9×10^6 and 1.6×10^7 and control to *O. hyalinipennis*, *A. craccivora*, *M. pustulata*, *D. cingulatus, M. pustulata* and *D. cingulatus* adults, respectively. Similar observation was also observed for II, III and IV instars of *P. ricini*, *S. litura,* and *H. armigera* under laboratory conditions (Figure 1).

The LC_{50} values under *invitro* conditions (Table1) showed that it ranged from $1.62x10^4$ to $1.75x10^6$ spores/ml. Lowest spore concentration (1.7×10^4 spore/ml) had highest virulence on *A. craccivora* (50.00%) followed by *D. cingulatus*

(41.67%), *M. pustulata* (41.67%) and *O. hyalinipennis* (33.37%) adults. Previously it was reported that this fungi caused up to 92.30 % mortality to *D. cingulatus* adults (Sahayaraj and Borgio, 2008). Highest and lowest slope and intercept were observed for *M. pustulata* and *O. hyalinipennis* adults, respectively. Lowest (1.84 x 10^4) and highest (2.38 x 10^5) LC_{50} values were recorded on *A. craccivora* and *M. pustulata,* respectively. Spore concentration and mortality effect were directly proportional to each other in all the pests.

Even though all the adults were positively correlated among themselves, 100% correlations coefficient (1.00) was observed on *O. hyalinipennis* adult with *S. litura* III instar, *A. craccivora* adult with *P. ricini* II instar, *M. pustulata* adult with *S. litura* III instar, *P. ricini* II instar with *D. cingulatus* adult, *P. ricini* III instar with *H. armigera* IV instar and *H. armigera* II with *H. armigera* IV. Lowest (1.62 x 10^4) and highest (6.79 x 10^5) LC_{50} values were recorded for II and IV instar of *P. ricini,* respectively. An opposite trend was observed for *S. litura.* LC_{50} value was highest in II and lowest in IV instars of *H. armigera.* Between these two pests, the highest (18.09696) and lowest (15.46048) slop were observed for IV instars *H. armigera* and *S. litura,* respectively (Table 1). Even though all the instars are positively correlated among them, 0.99 correlations were noted on more 8 comparisons. Highest correlation coefficient value was noted in IV with III *H. armigera* and also between IV *H. armigera* with III instar *P. ricini* (Table 2). Cumulatively the pathogenicity was lowest in II instar of *P. ricini* and the highest on *H. armigera* III instar. Correlations coefficient value of various pests (Table 2) showed that all the comparisons were positively correlated among themselves. Among all the tested pests, the mycelial growth was observed only on *A. craccivora* treated with 2.6 x 10^5, 1.9 x 10^6 and 1.6 x 10^7 conidia/ml on 3, 4 and 7 adults, respectively after 4 days of *M. anisopliae* treatment.

It was observed that 93.33% of mortality against III and IV instars of *H. armigera* in 96 h after the treatment of *M. anisopliae.* Kencharaddi and Jayaramaiah (1997) reported the pathogenicity of *M. anisopliae* against *H. armigera.* Furthermore Nahar *et al.* (2004) observed 66.74% of mortality against III instar of *H. armigera* with *M. anisopliae.* The present observation reveals that *M. anisopliae*



AcA = *Aphis craccivora* Adult; DcA = *Dysdercus cingulatus* Adult; OhA = *Oxycarenus hyalinipennis* Adult
Ha2 = *Helicoverpa armigera* II Instar; Ha3 = *H. armigera* III Instar; Ha4 = *H. armigera* IV Instar;
Sl2 = *Spodoptera litura* II Instar; Sl3 = *S. litura* III Instar; Sl4 = *S. litura* IV Instar;
Pr2= *Pericallia ricini* II Instar; Pr3 = *P. ricini* III Instar; Pr4 = *P. ricini* IV Instar;
MpA = *Mylabris pustulata* Adult.

Figure 1: Corrected mortality (%) due to *Metarhizium anisopliae* against chosen insect pests

caused higher mortality than the previous reports. Anonymous (2004) reported the disease causing ability of *M. anisopliae* to *S. litura*. In dermal toxicity entomopathogenic fungi enter into the hosts by direct penetration of the cuticle that functions as a barrier against most microbial attack. Present study strengthened the pathogenicity reports of *M. anisopliae* against aphid a sucking pest of many agricultural crops (Miranpuri and Khachatourians, 1996). Our study revealed that *M. anisopliae* caused 75 % mortality with in 96 hours after exposure on *D. cingulatus* adult. Hence this fungus can be used for managing this devastating pest of cotton.

In cotton *M. pustulata,* and *P. ricini* and *O. hyalinipennis* are the minor and major pests in India. Gloviana *et al.* (2004) reported the pathogenicity of *Beauveria bassiana* to the larvae of *P. ricin* and Khan *et al.* (2005) reported against *M. pustulata*. However, informations about the impact of *M. anisopliae* spores on *M. pustulata, P. ricini* and *O. hyalinipennis* were not available in the literature and hence, the present study is considered the first report. Among the three lepidopteran pests, *P. ricini* need more spore concentrations of *M. anisopliae* than *H. armigerad* and *S. litura*. However, our observation shows that 1.6×10^7 was enough to cause the highest (80.00%) mortality to *P. ricini.* Recent reports of Elumalai *et al.* (2006) on *P. ricini* reveal that fractions (2000ppm) of *Hyptis suaveolens* (Lamiaceae) and *Melochia chorcorifolia* (Sterculiaceae) caused 77.2 and 63.51% mortality, respectively. Presence of tuft of hairs all over the body of this pest may prevent the easy enter of the pathogen into the body.

Recently, destruxin (dtx) A, B, D, E, and E-diol were identified from this fungi (Seger *et al.,* 2006) – the *M. anisopliae* and might depress the cellular immune reaction. Many researchers suspected that the rapid killing ability of *M. anisopliae* on its host could be caused not only through direct physical invasion of the hyphae, but also possible due to some enzymatic mechanisms of glucosidase and acid trehalase which extracellular hydrolysis of trehalose occurs in host haemolymph during fungal infection (Xia *et al.,* 2002) and also cuticle degrading enzymes. In addition immune system of the host and the fungal response was likely to be another important factor governing the pathogenicity of *M. anisopliae* (Moorhouse, 1993).

The present laboratory bioassay of *M. anisopliae* against seven important insect pests revealed that the *M. anisopliae* was more pathogenic to *H. armigera* than the remaining pests. More than 50% mortality was observed in *A. craccivora*, *D.*

Table 1: Efficacy of *Metarhizium anisopliae* against seven insect pests

Pest name	Life Stage	LC_{50}	Fiducial limits		Regression Slope	Intercept	Chi ^{2}
			Lower	Higher			
Aphis craccivora	Adult	1.84×10^4	1.23×10^4	2.63×10^5	20.76167	10.76481	14.75082
Dysdercus cingulatus	Adult	2.25×10^5	2.38×10^4	2.73×10^6	20.13633	10.43938	11.0791
Oxycarenus hyalinipennis	Adult	1.93×10^5	1.64×10^4	2.28×10^6	7.49608	5.46732	2.27029
Helicoverpa armigera	II Instar	1.25×10^6	2.63×10^4	2.78×10^7	15.55486	8.53188	2.02876
	III Instar	1.75×10^6	9.45×10^4	2.72×10^7	17.09368	9.19614	5.13606
	IV Instar	2.40×10^5	1.22×10^4	5.86×10^6	18.09696	9.69856	2.51256
Pericallia ricini	II Instar	1.62×10^4	1.08×10^3	2.43×10^5	17.03507	9.36786	7.50341
	III Instar	4.25×10^5	2.27×10^4	6.24×10^6	17.00799	9.20280	2.52812
	IV Instar	6.79×10^5	2.45×10^4	9.24×10^6	15.64328	8.50187	0.20951
Spodoptera litura	II Instar	2.45×10^5	1.22×10^4	6.55×10^6	16.70294	9.07809	1.45293
	III Instar	2.47×10^4	2.28×10^3	2.66×10^6	16.88116	9.14469	7.70020
	IV Instar	2.43×10^5	2.24×10^4	2.64×10^6	15.46048	8.57438	3.91314
Mylabris pustulata	Adult	2.38×10^5	1.26×10^4	2.91×10^6	25.32597	12.75219	6.99095

Table 2: Correlation coefficient value (r^2) of mortality due to *Metarhizium anisopliae* against seven insect pests

	AcA	DcA	OhA	HaII	HaIII	HaIV	SlII	SlIII	SlIV	PrII	PrIII	PrIV	MpA
AcA	1.00	.99	.96	.96	.93	.96	.96	.97	.97	1.00	.95	.91	.96
DcA	.99	1.00	.98	.97	.92	.97	.99	.97	.99	1.00	.97	.95	.98
OhA	.96	.98	1.00	.98	.93	.98	1.00	.93	.96	.98	.96	.98	.99
HaII	.96	.97	.98	1.00	.98	1.00	.98	.98	.98	.97	.99	.98	.98
HaIII	.93	.92	.93	.98	1.00	.97	.92	.96	.93	.93	.95	.95	.93
HaIV	.96	.97	.98	1.00	.97	1.00	.98	.98	.98	.97	1.00	.98	.99
SlII	.96	.99	1.00	.98	.92	.98	1.00	.94	.98	.98	.97	.98	1.00
SlIII	.97	.97	.93	.98	.96	.98	.94	1.00	.98	.97	.98	.93	.95
SlIV	.97	.99	.96	.98	.93	.98	.98	.98	1.00	.99	.99	.95	.98
PrII	1.00	1.00	.98	.97	.93	.97	.98	.97	.99	1.00	.96	.94	.98
PrIII	.95	.97	.96	.99	.95	1.00	.97	.98	.99	.96	1.00	.98	.99
PrIV	.91	.95	.98	.98	.95	.98	.98	.93	.95	.94	.98	1.00	.99
MpA	.96	.98	.99	.98	.93	.99	1.00	.95	.98	.98	.99	.99	1.00

AcA = *Aphis craccivora* Adult; DcA = *Dysdercus cingulatus* Adult; OhA = *Oxycarenus hyalinipennis* Adult
HaII = *Helicoverpa armigera* II Instar; HaIII = *H. armigera* III Instar HaIV = *H. armigera* IV Instar;
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PrII= *Pericallia ricini* II Instar; PrIII = *P. ricini* III Instar; PrIV = *P. ricini* IV Instar;
MpA = *Mylabris pustulata* Adult.

cingulatus, *O. hyalinipennis, P. ricini, S. litura* and *H. armigera* when 1.6 x 10^7 spores/ml of *M. anisopliae* after 48 hours of treatment. It can be concluded that *M. anisopliae* is a suitable fungal insecticide against both defoliators and sucking pests.

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