Bioefficacy of entomopathogenic fungi *Metarhizium anisopliae* (Metschn.) Sorokin against the cotton stainer, *Dysdercus cingulatus* (Fab.) (Hemiptera: Pyrrhocoridae)

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ABSTRACT

Entomopathogenic fungi play an important role in the biological control of the insect pest population in an agroecosystem. *Metarhizium anisopliae* is one such entomopathogenic fungus, proved to be an effective biocontrol agent against different insect pests. However, different strains of entomopathogens show variations in their pathogenicity and host specificity. Hence the present study is aimed to determine the bioefficacy of local isolates of the entomopathogenic fungus *M. anisopliae* to control *Dysdercus cingulatus*. Fungal strains were isolated from cotton fields in Tirunelveli, Thoothukudi, Thenkasi, and Viruthunagar districts of Tamil Nadu following standard protocols. Four different isolates were identified and used for the bioassay. Bioefficacy trials were carried out in all the five nymphal instars and the adults of *D. cingulatus* and were treated with eight different concentrations of *M. anisopliae* (10^1,10^2,10^3,10^4,10^5,10^6,10^7 and 10^8 spores/mL). The formulations were evaluated for their pathogenicity and efficiency against *D. cingulatus* nymphal instars and adults which resulted in 70% to 100% mortality. A 100% mortality rate was observed in four isolates of *M. anisopliae* at higher concentrations (120 hrs) after treatment. Lethal concentration (LC_{50}) values of *M. anisopliae* isolates against *D. cingulatus* were calculated as 5.94×10^7 (ERUM1), 6.09×10^7 (ERUM2), 2.62×10^7 (ERUM3), 2.69×10^7 (ERUM4). Approaching biocontrol agents instead of chemical pesticides seems to be very promising in the march towards more sustainable, eco-friendly agricultural pest management practices and protecting the environment.
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Key words: Local strain, Biological control, *Metarhizium anisopliae*, *Dysdercus cingulatus.*

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INTRODUCTION

Indiscriminate application of conventional synthetic chemical insecticides heavily damaged the health of the environment, livestock, and man which emphasized the paramount of insecticide management programs to preserve the natural balance of biodiversity, biosafety of non-target organisms, and ensure human health (Ambethgar, 2009; Umaru and Simarani, 2022). Biological control programs using natural enemies have been employed globally for insect pest management in agriculture and forestry using predatory and parasitic insects and pathogenic microbes (Idrees *et al*., 2022).

Cotton, *Gossypium hirsutum* (Linn.) "The king of fibers" is the most economically important natural fiber. The economy of many developing countries depends upon cotton production of which India accounts for nearly 24% of the total cotton production. However, cotton production is declining due to the infestation of insect pests and diseases (Vinayaga Moorthi *et al.,* 2012).

Dysdercus cingulatus (Fab.) (Hemiptera: Pyrrhocoridae) is one of the notorious pests of cotton in Southeast Asia (Kohno and Thi 2004). Both nymphs and adults feed on immature seeds accounting for heavy losses in the cotton yield, seed weight, and oil content (Sontakke Harshalata *et al.*, 2013).

Entomopathogenic fungi (EPFs) cause lethal infections to the host insects and they help to maintain the natural balance of the insect pest population, including those living in soil by epizootics (Vega *et al.*, 2005; Erper *et al.*, 2022). These microorganisms have attracted remarkable attention for their usage in biological control programs for insect pests (Lacey *et al.*, 2015). *M. anisopliae* (Metschnikoff) Sorokin (Hypocreales: Ascomycota) is a cosmopolitan entomopathogen that causes natural infection to a wide range of insects (Biryol *et al.*, 2021). Hence in the present study, we attempted to explore the native isolates of *M. anisopliae* with high efficacy (biocontrol potential) and environmental adaptability to control *D. cingulatus* in its natural ecosystem.

MATERIALS AND METHODS

Collection of soil samples

Entomopathogenic fungi, *M. anisopliae* isolates used in the investigation were isolated from soil samples collected at different locations of the Tirunelveli, Thoothukudi, Thenkasi, and Viruthunagar districts of Tamil Nadu, using sterilized stainless-steel spatula and sterile plastic bags. About 100 grams of soil samples were taken from each site at a depth of 15 cm (Sahayaraj and Borgio, 2009).

Media Preparation

A selective media containing 1% Dodine (N-dodecylguanidine monoacetate) aqueous solution, was autoclaved separately and then thoroughly mixed with autoclaved Potato Dextrose Agar (PDA) in appropriate quantities to obtain the designated concentration. It consists of PDA supplemented with yeast extract, gentamicin, and 1% dodine "Dodine medium" (Everton *et al*., 2010).

Preparation of fungal spore concentration

The fungal isolates were cultured in Potato Dextrose Agar (PDA) supplemented with Dodine medium and were incubated at 26˚C ± 2˚C for 10-14 days. After sporulation, conidia were harvested by flooding the plate with sterile deionized water (dH_2O) containing 0.02% Tween-80. Then the experimental concentrations were prepared by serial dilution technique for bioassay studies.

Laboratory bioassay

Suspensions of *M. anisopliae* isolates at different concentrations *viz.,* 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4},10^{-3},$10^{-2,}$ and 10^{-1} spores/mL were prepared by following a serial dilution procedure. Two to three drops of 0.02% Tween-80 (adjuvant) were added to suspensions in different concentrations and transferred to 20 mL spray bottles and mixed thoroughly. The assay was carried out in standard (insect culture) aerated plastic containers (30 × 15 cm) and fed with water-soaked cotton seeds. Ten insects each of different life stages of *D. cingulatus* (1st, 2nd, 3rd, 4th, 5th instars, and adults) were introduced in each container. These experimental solutions were sprayed over the insects in the respective experimental containers. Distilled water with 0.02% of Tween-80 was used to treat insects in the control. Six replicates each was maintained for both treatment and control. Mortality counts were recorded every 24 hrs up to 120 hrs.

Statistical analysis

The LC_{50} values and their fiducial limits were estimated by Probit analysis at 0.05 level was used to determine significant differences between treatments. The data obtained were analyzed using SPSS software version 25.

RESULT

Bioassay was performed with four isolates of *M. anisolpliae* against *D. cingulatus*. The pathogenicity of entomopathogenic fungi differed from each other. The *D. cingulatus* infected by the fungal isolates were mummified and hard to touch and mycelial growth developed after 24 to 48 hours of death. Initially, the growth of the fungi was uneven in the intermembrane of the abdomen, and eventually, the entire cadaver was covered by the growth of the fungi. The results show that mortality increases with an increase in concentrations. The four isolates showed a significant mortality rate against cotton seed bug *D. cingulatus* (Figures 1, 2).

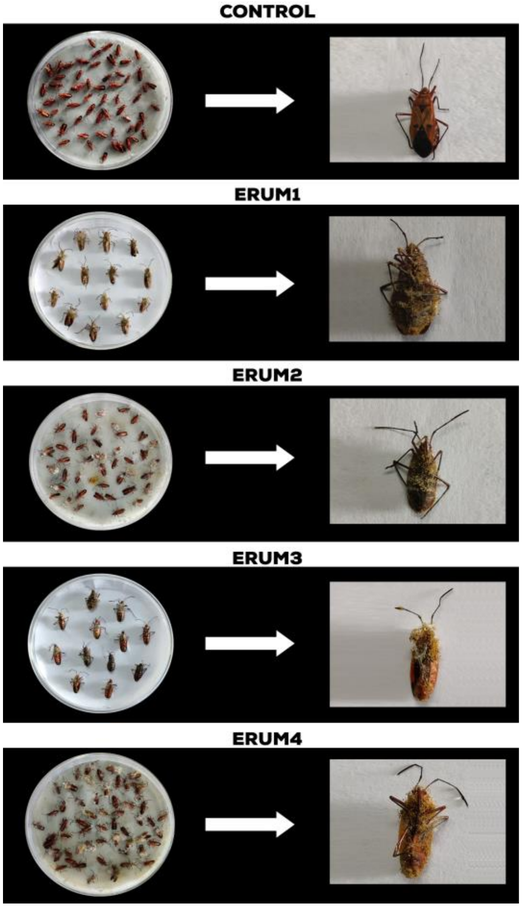

Figure 1. The growth of *M. anisopliae* isolates on *D. cingulatus* adult.

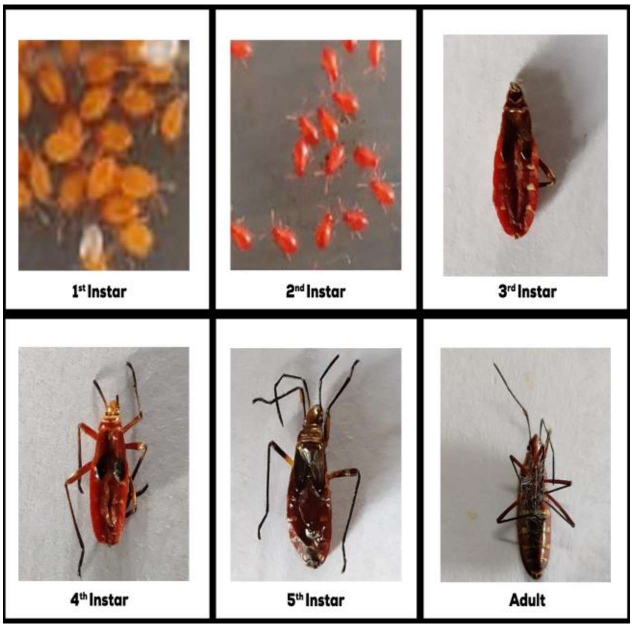

Figure 2. The *D. cingulatus* after treatment of *M. anisopliae*

For each fungal product, mortality rates of young and older instars of the insect were significantly different at different conidial concentrations and elapsed time up to 120 hrs after application. The mortality rates of adults and instars of *D. cingulatus* are listed in tables 1-6.

In 1st instar, the highest mortality was recorded in 2.3 $\times 10^8$ spores/mL concentration of ERUM2 and the minimum mortality in 1.3×10^1 spores/mL concentration of ERUM3 at 24 hrs after treatment. At the 48 hrs, the highest mortality was recorded in 1.1×10^8 spores/mL concentration of ERUM1 and the minimum mortality rate was observed in 1.3×10^1 spores/mL concentration of ERUM3. At 72 hrs the highest mortality rate of 100 % was recorded in *viz,* 10^3, 10^4, 10^5, 10^6, 10^7, and 10^8 of all the experimental isolates, and the minimum mortality rate was observed in 1.3×10^1 spores/mL concentration of ERUM3. At 96 hrs the highest mortality rate of 100 % was recorded in *viz.,* 10^1, 10^2 of all the experimental isolates. In 1st instar ERUM1is more significant than ERUM2, ERUM3 and ERUM4 (p=0.015).

In the 2nd instar, at 24 hrs the highest mortality was recorded in 1.3×10^8 spores/mL concentration of ERUM3 and the minimum mortality was observed in 3.1×10^1 spores/mL concentration of ERUM4. At the 48 hrs, the highest mortality was recorded in 2.3×10^8 spores/mL concentration of ERUM2 and the minimum mortality was observed in 1.3×10^1 spores/mL concentration of ERUM3. At 72 hrs the highest mortality rate of 100 % was recorded in *viz.*, 10^3, 10^4, 10^5, 10^6, 10^7, and 10^8 of all the experimental isolates, and the minimum mortality was observed in 6.6 $\times10^1$ spores/mL concentration of ERUM1. At 96 hrs the highest mortality rate of 100 % was recorded in *viz.*, 10^1, 10^2, 10^3, and 10^4 of all the experimental isolates. In 2nd instar ERUM2 is more significant than ERUM1, ERUM3 and ERUM4 (p = 0.008).

In 3rd Instars, the highest mortality was recorded in 1.1×10^8 spores/mL concentration of ERUM1, followed by 4.3×10^8 spores/mL concentration of

ERUM2, and no mortality rate was observed in the lower concentration *viz.,* 10^1, 10^2, 10^3 of all the experimental isolates at 24 hrs after treatment. At the 48 hrs, the highest mortality rate was recorded in 2.4×10^8 spores/mL concentration of ERUM4 and the minimum mortality rate was observed in 3.1×10^1 spores/mL concentration of ERUM4. At the 72 hrs, the highest mortality was recorded in 2.4×10^8 spores/mL concentration of ERUM4 and the minimum mortality was observed in 1.3 $\times10^1$ spores/mL concentration of ERUM3. At 96 hrs, the highest mortality was recorded in 1.3×10^8 spores/mL concentration of ERUM3 and the minimum mortality was observed in 1.3 $\times10^1$ spores/mL concentration of ERUM3. At 120 hrs the highest mortality rate of 100 % was recorded in *viz.,* 10^4, 10^5, 10^6, 10^7, and 10^8 of all the experimental isolates, and the minimum mortality was observed in 1.3 $\times10^1$ spores/mL concentration of ERUM3.In 3rd instar ERUM4 is more significant than ERUM1, ERUM2 and ERUM3($p = 0.005$).

In the 4th instar, the highest mortality was recorded in 10^8 spores/mL concentration of all the experimental isolates, and no mortality rate was observed in the lower concentration *viz.,* 10^1, 10^2, and 10^3 of all the experimental isolates at 24 hrs after treatment. At the 48 hrs, the highest mortality was recorded in 2.3×10^8 spores/mL concentration of ERUM2 and the minimum mortality was observed in 10^1 spores /mL concentration of all the experimental isolates. At the 72 hrs, the highest mortality was recorded in 2.3×10^8 spores/mL concentration of ERUM2, and the minimum mortality was observed in 4.3 $\times10^1$ spores/mL concentration of ERUM2. At 96 hrs, the highest mortality rate was recorded in 2.3×10^8 spores/mL concentration of ERUM2, and 4.3×10^1 spores/mL concentration of ERUM2. At 120 hrs, the highest mortality rate of 100 % was recorded in *viz.,* 10^5, 10^6, 10^7, and 10^8 of all the experimental isolates, and the minimum mortality rate was observed in 4.3×10^1 spores/mL concentration of ERUM2. In 4th instar ERUM3 is more significant than ERUM1, ERUM2 and ERUM4 ($p = 0.004$).

In the 5th instar, the highest mortality was recorded in 2.3×10^8 spores/mL concentration of ERUM2 and no mortality rate was observed in the lower concentrations in *viz.,* 10^1, 10^2, and 10^3 of all the experimental isolates at 24 hrs after treatment. At the 48 hrs, the highest mortality was recorded in 2.4×10^8 spores/m concentration of ERUM4 and no mortality rate was observed in the lower concentration of 10^1 of all the experimental isolates. At the 72 hrs, the highest mortality was recorded in 1.1×10^8 spores/mL concentration of ERUM1 and 2.3×10^8 spores/mL concentration of ERUM2 and in minimum mortality was observed in 6.6 $\times10^1$ spores/mL concentration of ERUM1, 1.3 $\times10^1$ spores/mL concentration of ERUM2, and 3.1 $\times10^1$ spores/mL concentration of ERUM3. At 96 hrs, the highest mortality was recorded in 2.4×10^8 spores/mL concentration of ERUM4 and the minimum mortality was observed in 1.3 $\times10^1$ spores/mL concentration of ERUM3. At 120 hrs the highest mortality rate of 100 % was recorded in viz, 10^5, 10^6, 10^7, and 10^8 of all the experimental isolates, and the minimum mortality was observed in 1.3×10^1 spores/mL concentration of ERUM3.In 5th instar ERUM1 is more significant than ERUM2, ERUM3 and ERUM4 ($p = 0.020$).

In the adult, the highest mortality was recorded in 1.3×10^8 spores/mL concentration of ERUM3 and 2.4×10^8 spores/mL concentration of ERUM4 and no mortality rate was observed in the lower concentration of 10^1, 10^2, 10^3, and 104 of all the experimental isolates at 24 hrs after treatment. At the 48 hrs, the highest mortality was recorded in 2.3×10^8 spores/mL in the isolate ERUM2 and no mortality rate was observed in the lower concentration of 10^1, 10^2, 10^3, 10^4 of all the experimental isolates. At 72 hrs, the highest mortality was recorded in 2.3×10^8 spores/mL concentration of ERUM2 and no mortality rate was observed in 1.3 $\times10^1$ spores/mL concentration of ERUM3. At 96 hrs, the highest mortality rate was recorded in 2.3×10^8 spores/mL in ERUM2, and the minimum mortality was observed concentration of 1.3×10^1 spores/mL concentration of ERUM3.

Table 1. Efficacy of *Metarhizium anisopliae* isolates against *Dysdercus cingulatus* first instar

Isolate	Spores/mL	Mortality in hours (%)				
		24	**48**	**72**	**96**	**120**
ERUM1	Control	0.00 ± 0.00^{f}	0.00 ± 0.00^{f}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{a}
	6.6 x10^{1}	6.66 ± 5.16^{e}	43.33 ± 5.16^{d}	76.33 ± 10.32^{b}	100.00 ± 0.00^{a}	-
	5.2 x10^{2}	10.00 ± 0.00^{d}	43.33 ± 5.16^{d}	78.33 ± 7.52^{b}	100.00 ± 0.00^{a}	-
	4.8 x10^{3}	10.00 ± 6.32^{d}	51.33 ± 5.16^{c}	100.00 ± 0.00^{a}	-	-
	4.2 x10^{4}	15.33 ± 5.16^{bc}	53.00 ± 5.47^{b}	100.00 ± 0.00^{a}	-	-
	2.8 x10^{5}	18.66 ± 4.08^{b}	53.00 ± 5.47^{b}	100.00 ± 0.00^{a}	-	-
	1.9 x10^{6}	20.00 ± 6.32^{b}	54.00 ± 6.32^{b}	100.00 ± 0.00^{a}	-	-
	1.4 x10^{7}	21.66 ± 7.52^{a}	56.66 ± 5.16^{a}	100.00 ± 0.00^{a}	-	-
	1.1 x10^{8}	25.00 ± 5.47^{a}	58.88 ± 7.52^{a}	100.00 ± 0.00^{a}	-	-
Mean		15.91	51.69	94.33	100.00	
ERUM2	Control	0.00 ± 0.00^{e}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{b}	0.00 ± 0.00^{a}
	4.3 x10^{1}	6.60 ± 5.16^{d}	40.00 ± 6.32^{e}	68.33 ± 4.08^{c}	100.00 ± 0.00^{a}	-
	4.4 x10^{2}	6.66 ± 5.16^{d}	43.33 ± 12.11^{d}	72.33 ± 14.70^{b}	100.00 ± 0.00^{a}	-
	2.3 x10^{3}	12.66 ± 5.16^{c}	50.66 ± 4.08^{bc}	100.00 ± 0.00^{a}	-	-
	1.2 x10^{4}	18.33 ± 7.52^{b}	51.66 ± 7.52^{b}	100.00 ± 0.00^{a}	-	-
	2.8 x10^{5}	23.33 ± 5.16^{a}	52.66 ± 11.69^{b}	100.00 ± 0.00^{a}	-	-
	3.1 x10^{6}	24.33 ± 7.52^{a}	53.66 ± 11.69^{b}	100.00 ± 0.00^{a}	-	-
	1.9 x10^{7}	25.66 ± 7.52^{a}	56.99 ± 8.16^{a}	100.00 ± 0.00^{a}	-	-
	2.3 x10^{8}	26.33 ± 8.16^{a}	58.66 ± 5.16^{a}	100.00 ± 0.00^{a}	-	-
Mean		17.99	50.95	92.58	100.00	
ERUM3	Control	0.00 ± 0.00^{e}	0.00 ± 0.00^{g}	0.00 ± 0.00^{e}	0.00 ± 0.00^{b}	0.00 ± 0.00^{a}
	1.3 x10^{1}	0.05 ± 0.54^{d}	28.33 ± 7.52^{f}	61.06 ± 16.02^{d}	100.00 ± 0.00^{a}	-
	1.5 x10^{2}	6.66 ± 5.16^{c}	38.33 ± 7.52^{e}	73.33 ± 5.16^{c}	100.00 ± 0.00^{a}	-
	6.0 x10^{3}	8.33 ± 4.08^{c}	43.33 ± 8.16^{d}	90.33 ± 8.16^{ab}	100.00 ± 0.00^{a}	-
	4.5 x10^{4}	20.00 ± 6.32^{b}	48.33 ± 11.69^{c}	94.33 ± 7.52^{a}	100.00 ± 0.00^{a}	-
	7.0 x10^{5}	20.00 ± 6.32^{b}	53.33 ± 8.16^{b}	100.00 ± 0.00^{a}	-	-
	1.4 x10^{6}	21.66 ± 7.52^{a}	56.66 ± 5.16^{a}	100.00 ± 0.00^{a}	-	-
	2.3 x10^{7}	23.33 ± 5.16^{a}	58.33 ± 9.83^{a}	100.00 ± 0.00^{a}	-	-
	1.3 x10^{8}	26.66 ± 5.16^{a}	58.33 ± 9.83^{a}	100.00 ± 0.00^{a}	-	-
Mean		15.84	48.12	79.76	100.00	
ERUM4	Control	0.00 ± 0.00^{e}	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{a}
	3.1 x10^{1}	1.66 ± 4.08^{d}	40.00 ± 6.32^{c}	75.00 ± 10.48^{b}	100.00 ± 0.00^{a}	-
	2.8 x10^{2}	10.00 ± 0.00^{c}	45.00 ± 5.47^{c}	78.33 ± 7.52^{b}	100.00 ± 0.00^{a}	-
	4.3 x10^{3}	11.60 ± 4.08^{c}	53.33 ± 5.16^{b}	100.00 ± 0.00^{a}	-	-
	4.7 x10^{4}	20.00 ± 6.32^{b}	53.33 ± 5.16^{b}	100.00 ± 0.00^{a}	-	-
	1.8 x10^{5}	21.66 ± 7.52^{a}	55.00 ± 5.47^{a}	100.00 ± 0.00^{a}	-	-
	3.0 x10^{6}	21.66 ± 4.08^{a}	55.00 ± 5.47^{a}	100.00 ± 0.00^{a}	-	-
	2.9 x10^{7}	21.66 ± 9.83^{a}	55.00 ± 10.49^{a}	100.00 ± 0.00^{a}	-	-
	2.4 x10^{8}	23.33 ± 5.16^{a}	56.66 ± 5.16^{a}	100.00 ± 0.00^{a}	-	-
Mean		s16.45	51.67	94.17	100.00	

Within each treatment, values followed by the same letter(s) are not significantly different (P ≤0.05)

Table 2. Efficacy of *Metarhizium anisopliae* isolates against *Dysdercus cingulatus* second instar

Isolate	Spores/mL	Mortality in hours (%)				
		24	**48**	**72**	**96**	**120**
ERUM1	Control	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{a}
	$6.6\ x10^{1}$	6.66 ± 5.16^{d}	30.00 ± 8.94^{d}	51.66 ± 9.83^{b}	100.00 ± 0.00^{a}	-
	$5.2\ x10^{2}$	10.00 ± 0.00^{c}	30.00 ± 6.32^{d}	55.00 ± 8.36^{b}	100.00 ± 0.00^{a}	-
	$4.8\ x10^{3}$	10.00 ± 0.00^{c}	40.33 ± 5.16^{bc}	100.00 ± 0.00^{a}	-	-
	$4.2\ x10^{4}$	20.00 ± 0.00^{b}	45.00 ± 5.47^{b}	100.00 ± 0.00^{a}	-	-
	$2.8\ x10^{5}$	22.33 ± 5.16^{a}	50.33 ± 10.32^{ab}	100.00 ± 0.00^{a}	-	-
	$1.9\ x10^{6}$	23.00 ± 5.47^{a}	52.00 ± 10.95^{a}	100.00 ± 0.00^{a}	-	-
	$1.4\ x10^{7}$	24.33 ± 5.16^{a}	54.66 ± 4.08^{a}	100.00 ± 0.00^{a}	-	-
	$1.1\ x10^{8}$	25.00 ± 5.70^{a}	55.00 ± 5.47^{a}	100.00 ± 0.00^{a}	-	-
Mean		17.67	44.67	88.33	100.00	
ERUM2	Control	0.00 ± 0.00^{f}	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{a}
	$4.3\ x10^{1}$	6.66 ± 5.16^{e}	30.00 ± 8.94^{c}	53.66 ± 18.61^{b}	100.00 ± 0.00^{a}	-
	$4.4x10^{2}$	7.00 ± 5.47^{e}	32.33 ± 7.52^{c}	56.33 ± 12.11^{b}	100.00 ± 0.00^{a}	-
	$2.3x10^{3}$	10.00 ± 0.00^{d}	53.33 ± 5.16^{b}	100.00 ± 0.00^{a}	-	-
	$1.2x10^{4}$	18.33 ± 4.08^{c}	54.33 ± 7.52^{b}	100.00 ± 0.00^{a}	-	-
	$2.8x10^{5}$	23.33 ± 5.16^{b}	55.66 ± 7.52^{a}	100.00 ± 0.00^{a}	-	-
	$3.1x10^{6}$	26.66 ± 5.16^{a}	58.66 ± 5.16^{a}	100.00 ± 0.00^{a}	-	-
	$1.9x10^{7}$	27.00 ± 5.16^{a}	59.00 ± 6.32^{a}	100.00 ± 0.00^{a}	-	-
	$2.3x10^{8}$	28.00 ± 8.94^{a}	60.33 ± 13.66^{a}	100.00 ± 0.00^{a}	-	-
Mean		18.37	50.46	88.75	100.00	
ERUM3	Control	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{d}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
	$1.3\ x10^{1}$	0.66 ± 0.51^{d}	21.66 ± 4.08^{d}	55.00 ± 8.36^{c}	100.00 ± 0.00^{a}	-
	$1.5\ x10^{2}$	3.33 ± 5.16^{c}	31.66 ± 9.83^{c}	56.66 ± 8.16^{c}	100.00 ± 0.00^{a}	-
	$6.0\ x10^{3}$	10.00 ± 0.00^{c}	43.33 ± 12.11^{b}	85.00 ± 5.47^{b}	100.00 ± 0.00^{a}	-
	$4.5\ x10^{4}$	21.66 ± 4.08^{b}	45.00 ± 8.36^{b}	90.00 ± 8.94^{a}	100.00 ± 0.00^{a}	-
	$7.0\ x10^{5}$	23.33 ± 5.16^{b}	50.00 ± 6.32^{a}	100.00 ± 0.00^{a}	-	-
	$1.4\ x10^{6}$	23.33 ± 5.16^{b}	55.00 ± 5.47^{a}	100.00 ± 0.00^{a}	-	-
	$2.3\ x10^{7}$	25.00 ± 5.47^{a}	56.66 ± 5.16^{a}	100.00 ± 0.00^{a}	-	-
	$1.3\ x10^{8}$	28.33 ± 7.52^{a}	56.66 ± 5.16^{a}	100.00 ± 0.00^{a}	-	-
Mean		16.96	45.00	85.83	100.00	
ERUM4	Control	0.00 ± 0.00^{d}	0.00 ± 0.00^{f}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{a}
	$3.1\ x10^{1}$	5.00 ± 5.47^{c}	28.33 ± 7.52^{e}	53.33 ± 8.16^{b}	100.00 ± 0.00^{a}	-
	$2.8\ x10^{2}$	6.66 ± 5.16^{c}	30.00 ± 8.94^{d}	55.33 ± 12.10^{b}	100.00 ± 0.00^{a}	-
	$4.3\ x10^{3}$	10.00 ± 0.00^{b}	48.33 ± 7.52^{c}	100.00 ± 0.00^{a}	-	-
	$4.7\ x10^{4}$	20.00 ± 0.00^{a}	48.33 ± 7.52^{c}	100.00 ± 0.00^{a}	-	-
	$1.8\ x10^{5}$	23.33 ± 5.16^{a}	51.66 ± 7.52^{b}	100.00 ± 0.00^{a}	-	-
	$3.0\ x10^{6}$	23.33 ± 5.16^{a}	53.33 ± 5.16^{b}	100.00 ± 0.00^{a}	-	-
	$2.9\ x10^{7}$	25.00 ± 5.47^{a}	55.00 ± 5.47^{a}	100.00 ± 0.00^{a}	-	-
	$2.4\ x10^{8}$	25.00 ± 5.47^{a}	60.00 ± 10.95^{a}	100.00 ± 0.00^{a}	-	-
Mean		17.29	46.87	88.58	100.00	

Within each treatment, values followed by the same letter(s) are not significantly different ($P \leq 0.05$)

Table 3. Efficacy of *Metarhizium anisopliae* isolates against *Dysdercus cingulatus* third instar

Isolate	**Spores/mL**	**Mortality in hours (%)**				
		24	**48**	**72**	**96**	**120**
ERUM1	Control	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}	0.00 ± 0.00^{d}	0.00 ± 0.00^{f}	0.00 ± 0.00^{e}
	6.6×10^{1}	0.00 ± 0.00^{c}	6.66 ± 10.32^{d}	26.66 ± 5.16^{c}	46.66 ± 5.16^{e}	68.33 ± 7.52^{c}
	5.2×10^{2}	0.00 ± 0.00^{c}	11.66 ± 13.29^{c}	30.00 ± 15.49^{c}	51.66 ± 17.22^{d}	80.00 ± 12.64^{b}
	4.8×10^{3}	0.00 ± 0.00^{c}	26.66 ± 5.16^{b}	45.00 ± 8.36^{b}	65.00 ± 8.33^{c}	88.33 ± 11.69^{b}
	4.2×10^{4}	11.66 ± 9.83^{b}	27.00 ± 10.48^{b}	50.33 ± 8.16^{b}	70.00 ± 5.47^{c}	100.00 ± 0.00^{a}
	2.8×10^{5}	15.66 ± 5.16^{a}	29.33 ± 9.23^{ab}	50.33 ± 7.52^{b}	70.66 ± 5.16^{c}	100.00 ± 0.00^{a}
	1.9×10^{6}	16.33 ± 4.08^{a}	30.33 ± 8.16^{a}	51.33 ± 8.16^{a}	71.66 ± 7.52^{b}	100.00 ± 0.00^{a}
	1.4×10^{7}	17.66 ± 5.16^{a}	33.33 ± 5.16^{a}	52.33 ± 5.16^{a}	73.33 ± 5.16^{b}	100.00 ± 0.00^{a}
	1.1×10^{8}	18.33 ± 4.08^{a}	33.33 ± 5.16^{a}	53.33 ± 5.16^{a}	76.66 ± 8.16^{a}	100.00 ± 0.00^{a}
Mean		9.96	24.79	41.4	65.70	92.08
ERUM2	Control	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}
	4.3×10^{1}	0.00 ± 0.00^{c}	6.66 ± 10.32^{d}	26.66 ± 5.16^{d}	46.66 ± 5.16^{d}	68.33 ± 4.08^{d}
	4.4×10^{2}	0.00 ± 0.00^{c}	11.66 ± 13.29^{c}	30.00 ± 15.49^{c}	51.66 ± 17.22^{c}	70.00 ± 14.14^{c}
	2.3×10^{3}	0.00 ± 0.00^{c}	26.66 ± 5.16^{b}	45.00 ± 8.36^{b}	52.00 ± 8.36^{c}	85.00 ± 8.36^{b}
	1.2×10^{4}	12.00 ± 0.00^{b}	28.33 ± 7.52^{b}	46.66 ± 8.16^{b}	54.33 ± 7.52^{c}	100.00 ± 0.00^{a}
	2.8×10^{5}	16.66 ± 5.16^{a}	32.33 ± 9.83^{b}	51.33 ± 7.52^{a}	65.66 ± 5.16^{b}	100.00 ± 0.00^{a}
	3.1×10^{6}	17.00 ± 5.47^{a}	33.00 ± 5.47^{b}	52.66 ± 5.16^{a}	75.66 ± 5.16^{a}	100.00 ± 0.00^{a}
	1.9×10^{7}	17.00 ± 5.47^{a}	35.33 ± 5.16^{a}	53.33 ± 5.16^{a}	76.33 ± 5.16^{a}	100.00 ± 0.00^{a}
	2.3×10^{8}	18.88 ± 4.08^{a}	35.33 ± 5.47^{a}	55.00 ± 5.47^{a}	78.33 ± 7.52^{a}	100.00 ± 0.00^{a}
Mean		10.19	26.16	45.08	62.58	90.2
ERUM3	Control	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{g}	0.00 ± 0.00^{e}	0.00 ± 0.00^{f}
	1.3×10^{1}	0.00 ± 0.00^{d}	5.00 ± 5.47^{d}	20.00 ± 6.32^{f}	40.00 ± 6.32^{d}	56.66 ± 16.32^{e}
	1.5×10^{2}	0.00 ± 0.00^{d}	5.00 ± 8.36^{d}	25.00 ± 8.36^{e}	43.33 ± 10.32^{d}	61.66 ± 7.52^{d}
	6.0×10^{3}	0.00 ± 0.00^{d}	15.00 ± 5.47^{c}	31.66 ± 13.29^{d}	50.00 ± 16.73^{c}	70.00 ± 14.14^{c}
	4.5×10^{4}	5.00 ± 5.47^{c}	25.00 ± 5.47^{b}	48.33 ± 7.52^{c}	53.33 ± 8.16^{c}	86.66 ± 10.32^{b}
	7.0×10^{5}	8.33 ± 4.08^{c}	28.33 ± 4.08^{b}	51.66 ± 9.83^{b}	70.00 ± 8.94^{b}	100.00 ± 0.00^{a}
	1.4×10^{6}	13.33 ± 5.16^{b}	30.00 ± 8.94^{a}	53.33 ± 8.16^{b}	76.00 ± 8.16^{a}	100.00 ± 0.00^{a}
	2.3×10^{7}	16.66 ± 5.16^{a}	31.66 ± 7.52^{a}	53.33 ± 8.16^{b}	78.33 ± 9.83^{a}	100.00 ± 0.00^{a}
	1.3×10^{8}	16.66 ± 7.7^{a}	35.00 ± 8.36^{a}	58.33 ± 7.52^{a}	78.33 ± 9.33^{a}	100.00 ± 0.00^{a}
Mean		7.50	21.87	42.1	61.17	84.7
ERUM4	Control	0.00 ± 0.00^{d}	0.00 ± 0.00^{f}	0.00 ± 0.00^{f}	0.00 ± 0.00^{f}	0.00 ± 0.00^{e}
	3.1×10^{1}	0.00 ± 0.00^{c}	3.33 ± 5.16^{e}	23.33 ± 5.16^{e}	43.33 ± 5.16^{e}	65.00 ± 8.36^{d}
	2.8×10^{2}	0.00 ± 0.00^{c}	11.66 ± 13.29^{d}	30.00 ± 15.49^{d}	51.66 ± 17.20^{d}	73.33 ± 16.32^{c}
	4.3×10^{3}	0.00 ± 0.00^{c}	26.66 ± 5.16^{c}	45.00 ± 8.36^{c}	63.33 ± 12.11^{c}	86.66 ± 15.05^{b}
	4.7×10^{4}	11.66 ± 9.83^{b}	33.33 ± 8.16^{b}	53.33 ± 8.16^{b}	73.33 ± 5.16^{b}	100.00 ± 0.00^{a}
	1.8×10^{5}	16.66 ± 5.16^{a}	33.33 ± 5.16^{b}	53.33 ± 8.16^{b}	73.33 ± 5.16^{b}	100.00 ± 0.00^{a}
	3.0×10^{6}	16.66 ± 5.16^{a}	33.33 ± 8.16^{b}	53.33 ± 5.16^{b}	73.33 ± 5.16^{b}	100.00 ± 0.00^{a}
	2.9×10^{7}	18.33 ± 4.08^{a}	35.00 ± 10.48^{a}	56.66 ± 5.16^{a}	75.00 ± 5.47^{a}	100.00 ± 0.00^{a}
	2.4×10^{8}	18.33 ± 4.08^{a}	38.33 ± 9.83^{a}	58.33 ± 8.16^{a}	76.66 ± 5.16^{a}	100.00 ± 0.00^{a}
Mean		10.21	26.87	46.66	66.25	90.62

Within each treatment, values followed by the same letter(s) are not significantly different ($P \leq 0.05$)

Table 4. Efficacy of *Metarhizium anisopliae* isolates against *Dysdercus cingulatus* fourth instar

Isolate	Spores/mL	Mortality in hours (%)				
		24	48	72	96	120
ERUM1	Control	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{f}	0.00 ± 0.00^{d}
	6.6×10^{1}	0.00 ± 0.00^{c}	1.66 ± 4.08^{d}	20.00 ± 6.32^{d}	43.33 ± 8.16^{e}	65.00±5.47^{c}
	5.2×10^{2}	0.00 ± 0.00^{c}	8.33 ± 4.08^{c}	22.00 ± 6.32^{d}	55.00 ± 10.48^{d}	78.33±9.83^{b}
	4.8×10^{3}	0.00 ± 0.00^{c}	8.33 ± 9.83^{c}	28.33 ± 9.83^{d}	55.00 ± 5.47^{d}	78.00±9.83^{b}
	4.2×10^{4}	5.00 ± 5.47^{b}	25.00 ± 10.40^{b}	45.00 ± 16.43^{c}	65.00 ± 13.78^{c}	90.00±12.61^{a}
	2.8×10^{5}	5.00 ± 5.47^{b}	26.66 ± 5.16^{b}	48.33 ± 7.52^{c}	71.66±4.08^{b}	100.00 ± 0.00^{a}
	1.9×10^{6}	6.66 ± 5.16^{b}	31.66 ± 4.08^{a}	53.33 ± 5.16^{b}	75.00±5.47^{a}	100.00 ± 0.00^{a}
	1.4×10^{7}	10.00 ± 0.00^{a}	35.00 ± 5.47^{a}	56.66 ± 5.16^{b}	75.00±5.47^{a}	100.00 ± 0.00^{a}
	1.1×10^{8}	10.00 ± 0.00^{a}	35.00 ± 5.47^{a}	76.66 ± 5.16^{a}	76.66±5.16^{a}	100.00 ± 0.00^{a}
Mean		4.58	21.46	43.79	64.58	88.92
ERUM2	Control	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{g}	0.00 ± 0.00^{e}
	4.3×10^{1}	0.00 ± 0.00^{c}	1.66±4.08^{d}	15.00±0.00^{d}	38.33±7.52^{f}	60.00±10.95^{d}
	4.4×10^{2}	0.00 ± 0.00^{c}	8.33±4.08^{c}	16.00±0.00^{d}	45.00±10.48^{e}	75.00±15.16^{c}
	2.3×10^{3}	0.00 ± 0.00^{c}	9.66±8.16^{c}	28.33±9.83^{c}	55.00±13.78^{d}	82.66±8.16^{b}
	1.2×10^{4}	3.30 ± 5.16^{b}	25.00±5.47^{b}	28.33±9.83^{c}	72.33±8.16^{c}	100.00 ± 0.00^{a}
	2.8×10^{5}	5.00 ± 5.47^{a}	25.00±8.36^{b}	51.66±9.83^{b}	74.33±13.29^{c}	100.00 ± 0.00^{a}
	3.1×10^{6}	6.66 ± 5.16^{a}	28.33±4.08^{b}	53.33±5.16^{b}	75.66±8.16^{b}	100.00 ± 0.00^{a}
	1.9×10^{7}	8.33 ± 4.08^{a}	30.00±6.32^{a}	53.33±10.32^{b}	79.66±13.66^{b}	100.00 ± 0.00^{a}
	2.3×10^{8}	10.00 ± 0.00^{a}	36.33±5.16^{a}	56.66±8.16^{a}	81.66±7.52^{a}	100.00 ± 0.00^{a}
Mean		4.16	20.54	37.83	65.25	89.71
ERUM3	Control	0.00 ± 0.00^{e}	0.00 ± 0.00^{f}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}
	1.3×10^{1}	0.00 ± 0.00^{d}	1.66 ± 4.08^{e}	16.66 ± 8.16^{d}	40.00 ± 8.94^{d}	60.00 ± 8.94^{d}
	1.5×10^{2}	0.00±0.00^{d}	5.00±5.47^{d}	26.66±5.16^{c}	48.33±7.52^{d}	71.66±7.52^{c}
	6.0×10^{3}	0.00±0.00^{d}	8.33±9.83^{d}	28.33±9.83^{c}	51.66±9.83^{c}	76.66±12.11^{c}
	4.5×10^{4}	3.33±5.16^{c}	18.33±9.83^{c}	43.33±5.16^{b}	60.00±8.94^{c}	86.66±15.05^{b}
	7.0×10^{5}	6.66 ± 5.16^{b}	26.66 ± 5.16^{b}	48.33 ± 7.52^{b}	70.00 ± 6.32^{b}	100.00 ± 0.00^{a}
	1.4×10^{6}	8.33 ± 4.08^{b}	30.00 ± 6.32^{b}	50.00 ± 6.32^{b}	75.00 ± 8.36^{a}	100.00 ± 0.00^{a}
	2.3×10^{7}	10.00 ± 0.00^{a}	31.66 ± 4.08^{a}	51.66 ± 7.52^{a}	75.00 ± 8.36^{a}	100.00 ± 0.00^{a}
	1.3×10^{8}	10.00 ± 0.00^{a}	31.66 ± 4.08^{a}	55.00 ± 5.47^{a}	76.66 ± 10.36^{a}	100.00 ± 0.00^{a}
Mean		4.79	19.16	40.00	62.08	86.87
ERUM4	Control	0.00 ± 0.00^{e}	0.00 ± 0.00^{f}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{d}
	3.1×10^{1}	0.00 ± 0.00^{d}	1.66 ± 4.08^{e}	20.00 ± 6.32^{d}	43.33 ± 8.16^{d}	65.00 ± 5.47^{c}
	2.8×10^{2}	0.00 ± 0.00^{d}	8.33 ± 4.08^{d}	28.33 ± 9.83^{c}	55.00 ± 10.48^{c}	78.33 ± 9.83^{b}
	4.3×10^{3}	0.00 ± 0.00^{d}	8.33 ± 9.83^{d}	30.00 ± 6.32^{c}	55.00 ± 5.47^{c}	78.33 ± 9.83^{b}
	4.7×10^{4}	5.00 ± 5.47^{c}	23.33 ± 5.16^{c}	45.00 ± 1.43^{b}	65.00 ± 13.78^{b}	90.00 ± 12.64^{a}
	1.8×10^{5}	6.66 ± 5.16^{b}	25.00±10.48^{c}	45.00±5.16^{b}	68.33±7.52^{b}	100.00 ± 0.00^{a}
	3.0×10^{6}	7.00±0.00^{b}	31.66±4.08^{b}	53.33±5.16^{a}	75.00±5.47^{a}	100.00 ± 0.00^{a}
	2.9×10^{7}	10.00±0.00^{a}	35.00±5.47^{a}	56.66±5.16^{a}	76.66±5.16^{a}	100.00±0.00^{a}
	2.4×10^{8}	10.00±0.00^{a}	35.00±5.47^{a}	56.66±5.16^{a}	76.66±5.16^{a}	100.00±0.00^{a}
Mean		4.83	21.4	41.87	64.37	85.28

Within each treatment, values followed by the same letter(s) are not significantly different ($P \leq 0.05$)

Table 5. Efficacy of *Metarhizium anisopliae* isolates against *Dysdercus cingulatus* fifth instar **67**

Isolate	Spores/mL	Mortality in hours (%)				
		24	**48**	**72**	**96**	**120**
ERUM1	Control	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}	0.00 ± 0.00^{f}	0.00 ± 0.00^{f}	0.00 ± 0.00^{e}
	6.6 x10^{1}	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	10.00 ± 0.00^{e}	40.00 ± 0.00^{e}	63.33±5.16^{d}
	5.2 x10^{2}	0.00 ± 0.00^{c}	3.33 ± 5.16^{c}	13.33 ± 5.16^{d}	43.33 ± 5.16^{d}	76.66±8.16^{c}
	4.8 x10^{3}	0.00 ± 0.00^{c}	3.33 ± 5.16^{c}	23.33 ± 5.16^{c}	56.66 ± 8.16^{c}	83.33±8.16^{b}
	4.2 x10^{4}	3.33 ± 5.16^{b}	13.33 ± 5.16^{b}	33.33 ± 5.16^{b}	60.00 ± 6.32^{b}	93.33±5.16^{a}
	2.8 x10^{5}	6.00 ± 0.00^{a}	14.00 ± 0.00^{b}	41.33 ± 5.16^{b}	61.66 ± 5.16^{b}	100.00 ± 0.00^{a}
	1.9 x10^{6}	8.33 ± 4.08^{a}	16.66 ± 5.16^{a}	43.66 ± 7.52^{b}	65.00 ± 9.36^{b}	100.00 ± 0.00^{a}
	1.4 x10^{7}	9.66 ± 5.16^{a}	16.66 ± 5.16^{a}	45.00 ± 6.32^{b}	66.66 ± 0.00^{a}	100.00 ± 0.00^{a}
	1.1 x10^{8}	10.00 ± 0.00^{a}	20.00 ± 0.00^{a}	46.66 ± 5.16^{a}	70.00 ± 0.00^{a}	100.00 ± 0.00^{a}
Mean		4.67	10.91	32.08	57.91	89.58
ERUM2	Control	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}	0.00 ± 0.00^{f}	0.00 ± 0.00^{f}	0.00 ± 0.00^{d}
	4.3 x10^{1}	0.00 ± 0.00^{c}	0.00±0.00^{d}	13.33±5.16^{e}	41.66±7.52^{e}	73.33±12.11^{c}
	4.4 x10^{2}	0.00 ± 0.00^{c}	3.33±5.16^{c}	23.33±5.16^{d}	53.33±10.32^{d}	83.33±8.16^{b}
	2.3 x10^{3}	0.00 ± 0.00^{c}	3.33±5.16^{c}	25.00±5.47^{d}	56.66±8.16^{d}	83.33±8.16^{b}
	1.2 x10^{4}	6.66 ± 5.16^{b}	15.00±5.47^{b}	25.00±5.47^{d}	61.66±4.08^{c}	96.66±5.16^{a}
	2.8 x10^{5}	7.00 ± 5.47^{b}	16.66±5.16^{b}	35.00±5.47^{c}	66.66±12.11^{b}	100.00 ± 0.00^{a}
	3.1 x10^{6}	8.33 ± 4.08^{b}	16.66±5.16^{b}	43.33±10.32^{b}	68.33±7.52^{b}	100.00 ± 0.00^{a}
	1.9 x10^{7}	9.66 ± 5.16^{b}	18.33±4.08^{b}	45.00±10.32^{a}	70.00±0.00^{a}	100.00 ± 0.00^{a}
	2.3 x10^{8}	10.00 ± 0.00^{a}	20.00±0.00^{a}	46.66±5.16^{a}	70.00±0.00^{a}	100.00 ± 0.00^{a}
Mean		5.21	11.66	32.08	61.04	92.08
ERUM3	Control	0.00 ± 0.00^{e}	0.00 ± 0.00^{g}	0.00 ± 0.00^{g}	0.00 ± 0.00^{f}	0.00 ± 0.00^{d}
	1.3 x10^{1}	0.00 ± 0.00^{d}	0.00±0.00^{f}	10.00±0.00^{f}	35.00±5.47^{e}	58.33±7.52^{c}
	1.5 x10^{2}	0.00 ± 0.00^{d}	1.66±4.08^{e}	11.66±4.08^{e}	36.00±8.16^{e}	70.00±8.94^{b}
	6.0 x10^{3}	0.00 ±0.00^{d}	1.66±4.08^{e}	18.33±11.69^{d}	43.33±5.16^{d}	72.38±16.02^{b}
	4.5 x10^{4}	3.33±5.16^{c}	13.33±5.16^{d}	33.33±5.16^{c}	45.00±1.16^{d}	93.33±5.16^{a}
	7.0 x10^{5}	6.66±5.16^{b}	16.66±5.16^{c}	40.00±6.32^{b}	60.00±6.32^{c}	100.00±0.00^{a}
	1.4 x10^{6}	6.66±5.16^{b}	18.33±4.08^{b}	41.66±7.52^{a}	61.66±7.52^{b}	100.00±0.00^{a}
	2.3 x10^{7}	6.66±5.16^{b}	20.00±0.00^{a}	43.33±5.16^{a}	65.00±5.47^{b}	100.00±0.00^{a}
	1.3 x10^{8}	10.00±0.00^{a}	20.00±0.00^{a}	43.33±5.16^{a}	68.33±11.69^{a}	100.00±0.00^{a}
Mean		4.16	11.46	30.21	51.79	86.76
ERUM4	Control	0.00 ± 0.00^{e}	0.00 ± 0.00^{f}	0.00 ± 0.00^{f}	0.00 ± 0.00^{f}	0.00 ± 0.00^{d}
	3.1 x10^{1}	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}	10.00±0.00^{e}	40.00 ± 0.00^{e}	63.33±8.16^{c}
	2.8 x10^{2}	0.00 ± 0.00^{d}	1.66 ± 4.08^{d}	21.66±7.52^{d}	41.66 ± 4.08^{e}	70.00±6.32^{b}
	4.3 x10^{3}	0.00 ± 0.00^{d}	3.33 ± 5.16^{d}	30.00±15.49^{c}	46.66 ± 8.16^{d}	70.00±6.32^{b}
	4.7 x10^{4}	3.33 ± 5.10^{c}	13.33 ± 5.16^{c}	33.33±5.16^{c}	58.33 ± 4.08^{c}	95.00±5.47^{a}
	1.8x10^{5}	3.33± 5.16^{c}	13.33± 5.16^{c}	36.66±10.32^{b}	60.00± 9.84^{b}	100.00±0.00^{a}
	3.0 x10^{6}	5.00 ± 5.47^{b}	15.00 ± 5.47^{b}	40.00±6.32^{a}	63.33 ± 8.16^{b}	100.00±0.00^{a}
	2.9 x10^{7}	6.66 ± 5.16^{b}	16.66 ± 5.16^{b}	40.00±6.32^{a}	65.00 ± 8.36^{b}	100.00±0.00^{a}
	2.4 x10^{8}	10.00 ± 0.00^{a}	20.00 ± 5.16^{a}	45.00±5.47^{a}	71.66 ± 7.52^{a}	100.00±0.00^{a}
Mean		3.54	10.41	32.08	55.83	87.29

Within each treatment, values followed by the same letter(s) are not significantly different ($P \leq 0.05$)

Table 6. Efficacy of *Metarhizium anisopliae* isolates against *Dysdercus cingulatus* adult

Isolate	**Spores/mL**	**Mortality in hours (%)**				
		24	**48**	**72**	**96**	**120**
ERUM1	Control	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}
	6.6 x10^{1}	0.00 ± 0.00^{c}	0.00±0.00^{d}	10.00±0.00^{d}	45.66±5.16^{c}	73.33±8.16^{d}
	5.2 x10^{2}	0.00 ± 0.00^{c}	0.00±0.00^{d}	10.00±0.00^{d}	46.33±5.16^{b}	73.33±10.32^{d}
	4.8 x10^{3}	0.00 ± 0.00^{c}	0.00±0.00^{d}	10.00±0.00^{d}	46.33±5.16^{b}	80.00±8.94^{c}
	4.2 x10^{4}	0.00 ± 0.00^{c}	0.00±0.00^{d}	15.00±5.47^{c}	48.33±4.08^{b}	93.33±8.16^{b}
	2.8 x10^{5}	3.33 ± 5.16^{b}	5.00±8.94^{c}	31.66 ±11.69^{b}	61.66±7.52^{a}	96.66±8.16^{a}
	1.9 x10^{6}	4.66 ± 4.08^{b}	6.66±8.16^{b}	31.66±7.53^{b}	62.00±8.94^{a}	100.00±0.00^{a}
	1.4 x10^{7}	5.33 ± 5.16^{a}	10.00±8.94^{b}	36.66±10.32^{a}	63.33±5.16^{a}	100.00±0.00^{a}
	1.1 x10^{8}	6.66 ± 5.16^{a}	15.00±5.47^{a}	40.00±8.94^{a}	63.33±5.16^{a}	100.00±0.00^{a}
Mean		2.50	4.58	23.12	54.62	89.58
ERUM2	Control	0.00 ± 0.00^{d}	0.00 ± 0.00^{f}	0.00 ± 0.00^{g}	0.00 ± 0.00^{f}	0.00 ± 0.00^{e}
	4.3 x10^{1}	0.00 ± 0.00^{c}	0.00 ± 0.00^{e}	6.66±5.16^{f}	31.66±7.52^{e}	78.00±18.97^{d}
	4.4 x10^{2}	0.00 ± 0.00^{c}	0.00 ± 0.00^{e}	6.66±5.16^{f}	33.33±8.16^{e}	81.66±17.22^{c}
	2.3 x10^{3}	0.00 ± 0.00^{c}	0.00 ± 0.00^{e}	6.66±5.16^{f}	35.00±73.78^{e}	91.66±11.69^{b}
	1.2 x10^{4}	0.00 ± 0.00^{c}	0.00 ± 0.00^{e}	10.00±8.94^{e}	38.33±14.71^{d}	93.00±0.00^{b}
	2.8 x10^{5}	3.33 ± 5.16^{b}	6.66 ± 8.16^{d}	28.33±7.52^{d}	56.66±5.16^{c}	95.67±8.16^{b}
	3.1 x10^{6}	5.00 ± 5.47^{b}	10.00 ± 8.94^{c}	38.33±9.83^{c}	65.00±1.24^{b}	100.00±0.00^{a}
	1.9 x10^{7}	5.00 ± 5.47^{b}	15.00 ± 13.78^{b}	40.00±16.73^{b}	65.00±5.47^{b}	100.00±0.00^{a}
	2.3 x10^{8}	6.66 ± 5.16^{a}	18.33 ± 4.08^{a}	48.33±7.52^{a}	71.66±4.08^{a}	100.00±0.00^{a}
Mean		2.50	6.25	23.12	49.58	92.50
ERUM3	Control	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{f}	0.00 ± 0.00^{e}
	1.3x10^{1}	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{d}	6.66±8.16^{e}	66.66±8.16^{d}
	1.5x10^{2}	0.00±0.00^{c}	0.00±0.00^{c}	6.66±5.16^{c}	33.33±16.00^{d}	70.00±26.07^{c}
	6.0x10^{3}	0.00±0.00^{c}	0.00±0.00^{c}	6.66±6.66^{c}	36.66±10.32^{c}	74.33±11.69^{c}
	4.5x10^{4}	0.00±0.00^{c}	0.00±0.00^{c}	10.00±6.32^{c}	38.33±4.08^{c}	76.66±8.16^{b}
	7.0x10^{5}	1.66±4.08^{b}	6.66±8.16^{b}	31.66±7.52^{b}	56.66±8.16^{b}	95.00±5.47^{a}
	1.4x10^{6}	3.33±5.16^{b}	6.66±8.16^{b}	33.33±8.16^{b}	56.66±8.16^{b}	100.00±0.00^{a}
	2.3x10^{7}	3.33±5.16^{b}	10.00±8.94^{a}	33.33±12.11^{b}	58.33±11.36^{b}	100.00±0.00^{a}
	1.3x10^{8}	10.00±0.00^{a}	12.00±8.94^{a}	36.66±10.32^{a}	61.66±11.69^{a}	100.00±0.00^{a}
Mean		2.29	4.42	19.79	43.54	85.33
ERUM4	Control	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{f}	0.00 ± 0.00^{f}	0.00 ± 0.00^{e}
	3.1 x10^{1}	0.00 ± 0.00^{c}	0.00±0.00^{c}	3.33±5.16^{e}	35.00±10.48^{e}	60.00±12.64^{d}
	2.8 x10^{2}	0.00 ± 0.00^{c}	0.00±0.00^{c}	5.00±5.47^{e}	38.33±7.52^{d}	66.66±13.66^{d}
	4.3 x10^{3}	0.00 ± 0.00^{c}	0.00±0.00^{c}	10.00±0.00^{d}	40.00±6.32^{c}	73.33±5.16^{c}
	4.7 x10^{4}	0.00 ± 0.00^{c}	0.00±0.00^{c}	16.66±8.16^{c}	46.66±8.16^{c}	80.00±6.32^{b}
	1.8 x10^{5}	3.00 ± 0.00^{b}	6.66±8.16^{b}	31.66±7.52^{b}	56.66±8.16^{b}	100.00±0.00^{a}
	3.0 x10^{6}	3.33 ± 5.16^{b}	10.00±8.94^{b}	35.00±10.48^{b}	56.66±8.16^{b}	100.00±0.00^{a}
	2.9 x10^{7}	3.33 ± 5.16^{b}	13.33±8.16^{a}	36.66±10.30^{a}	60.00±10.95^{b}	100.00±0.00^{a}
	2.4 x10^{8}	10.00 ± 0.00^{a}	13.33±8.94^{a}	36.66±10.32^{a}	65.00±5.47^{a}	100.00±0.00^{a}
Mean		2.46	5.42	21.87	49.79	85.00

Within each treatment, values followed by the same letter(s) are not significantly different ($P \leq 0.05$)

Table 7. LC_{50} Mortality rate caused by *Metarhizium anisopliae* isolates against *Dysdercus cingulatus*

Isolate	Instar	LC_{50}	Fiducial limit		Chi^2	p
			lower	higher		
ERUM1	1st instar	1.47×10^6	1.61×10^6	4.71×10^6	7.087	0.015
	2nd instar	1.07×10^5	7.70×10^5	3.25×10^5	18.964	0.032
	3rd instar	2.13×10^6	3.51×10^6	5.96×10^6	4.206	0.027
	4th instar	1.61×10^6	1.37×10^6	6.05×10^6	8.568	0.061
	5th instar	2.08×10^6	3.07×10^6	6.80×10^6	3.861	0.020
	Adult	5.94×10^7	1.46×10^7	3.43×10^6	13.148	0.159
ERUM2	1st instar	2.76×10^6	1.52×10^6	2.77×10^6	32.155	0.178
	2nd instar	6.20×10^6	1.68×10^6	2.66×10^6	30.439	0.008
	3rd instar	1.24×10^5	1.50×10^5	4.29×10^5	5.177	0.129
	4th instar	3.03×10^6	4.23×10^6	4.75×10^6	13.387	0.007
	5th instar	1.75×10^6	2.52×10^6	3.59×10^6	13.841	0.107
	Adult	6.09×10^7	1.31×10^7	1.78×10^7	10.724	0.023
ERUM3	1st instar	3.45×10^6	3.67×10^6	1.46×10^6	9.002	0.033
	2nd instar	2.09×10^6	4.93×10^6	5.98×10^6	8.948	0.432
	3rd instar	1.91×10^5	0.75×10^5	2.11×10^5	19.748	0.267
	4th instar	6.66×10^6	3.18×10^6	4.74×10^6	12.438	0.004
	5th instar	1.11×10^6	1.40×10^6	1.41×10^6	20.824	0.021
	Adult	2.62×10^7	1.01×10^7	7.93×10^7	18.069	0.001
ERUM4	1st instar	6.24×10^6	3.07×10^6	4.92×10^6	7.563	0.029
	2nd instar	6.40×10^6	3.61×10^6	4.94×10^6	16.720	0.111
	3rd instar	1.20×10^5	2.01×10^5	3.59×10^5	6.175	0.005
	4th instar	5.90×10^6	5.08×10^6	5.66×10^6	10.075	0.047
	5th instar	1.71×10^6	1.48×10^6	1.53×10^6	17.076	0.023
	Adult	2.69×10^7	1.28×10^7	2.50×10^7	17.148	0.008

At 120 hrs the highest mortality rate of 100% was recorded in *viz.*, 10^6, 10^7, and 10^8 of all the experimental isolates, and the minimum mortality was observed in 3.1×10^1 spores/mL concentration of ERUM4. And also, zero mortality (%) was recorded in all the instars of control experiments. In adult ERUM3is more significant than ERUM1, ERUM2 and ERUM4 (p = 0.001).

DISCUSSION

Entomopathogenic fungi are ecologically considered as fungi that grow either inside the insect bodies or on the surface of their exoskeleton, which eventually causes the death of the host insect (Hallouti *et al.*, 2021). Entomopathogenic fungi enter the hosts by direct penetration of the cuticle, which functions as a barrier against most microbial infections. Similarly, our experiment demonstrated that the *M. anisopliae* isolates penetrated through the cuticle from the suspension grew inside the body, and caused death (Mathulwe *et al*., 2021). In the present experiment, higher conidial concentration caused significantly higher mortalities than lower concentrations. The mortality rate increases with an increase in the number of conidial concentrations used. It could be concluded that *M. anisopliae* varied inability to infect *D. cingulatus*, based on the conidial concentration used. And among the eight concentrations, 10^8,10^7 and 10^6 spores/mL showed the highest efficacy of 100% against *D. cingulatus* at the end of the 120 hrs after treatment. LC_{50} values (Table 7) were calculated after converting the percentage into probit values and the relative potency of different isolates was worked out using probit regression analysis (Finney, 1971). The result of the pathogenicity tests showed that entomopathogenic fungi have the potential on controlling the sucking pests *D. cingulatus.* All the *M. anisopliae* isolates tested were pathogenic to *D. cingulatus* adults.

Compared to chemical insecticides, entomopathogenic fungi are promising biological control agents for many insect pests and show efficient potential for insecticide-resistant pests with less environmental risk (Ramteke *et al*., 2022). Our results found that *M. anisopliae* isolates could effectively infect the adults and instars of the *D. cingulatus* and it suggesting the potential of this fungus for pest control. Sahayaraj and Borgio (2010) also observed 92.30% mortality of the *D. cingulatus* treated with green muscardine fungus, *M. anisopliae*. Sahayaraj and Tomson (2010) reported the efficiency of the crude metabolites of the *M. anisopliae* capable of causing 45% mortality against *D. cingulatus*. Besides, this entomopathogen is also reported to the highly virulent against the caterpillar of *S. litura* (Kawpet *et al*., 2022). Similarly in our study,a 100 % mortality rate was recorded in all the isolates, after 120 hrs of treatment. And the lethal concentration (LC_{50}) values of *M. anisopliae* isolates against *D. cingulatus* showed the mortality rate in ERUM1 5.94×10^{7}, ERUM2 6.09×10^{7}, ERUM3 2.62×10^{7}, ERUM4 2.69×10^{7}. Baja *et al*. (2020) suggested that *M. anisoplae* had the potential for biological control of *Tutaabsoluta*. Sublethal concentrations of *T. absoluta* arose from parental generations and its third instar larvae treated by the fungus resulted in a reduction in fitness by both decreasing longevity and fecundity (Kushiyev *et al*., 2022).

Jiang *et al.* (2010) reported that Entomopathogenic fungi infect insect pests directly via the host cuticle, while the chemical insecticide thiamethoxam has different routes, including physical contact, stomach action, or systemic poison. In addition, entomopathogenic fungi affect gut bacterial genera, which is one of the major factors leading to host death (Idrees *et al*., 2022). However, it is unknown whether the chemicals cause the death of the host due to changes in bacterial genera. In this study, we challenged *D. cingulatus* with *M. anisopliae* to evaluate the immune responses of hosts. Similarly, in our study, Insect pest management using entomopathogenic fungi is an efficient and promising alternative strategy. Approximately 170 commercial products have been developed based on different EPF species (Hallouti *et al.*, 2021).

The present study exemplifies the excellent biocontrol potential of the soil isolate *M. anisopliae* towards red cotton stainer *D. cingulatus* and this fungus is one of the most promising microbial control agents combating different insect pests. Compared to chemical insecticides, EPF are ensuring biological control agents for many insect pests and show efficient potential for insecticide-resistant pests with less environmental risk.

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