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Pathogenicity of native *Beauveria bassiana* (Balsamo-Crivelli) vuillemin isolate on *Dysdercus cingulatus* (Hemiptera: Pyrrhocoridae)

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ABSTRACT

Entomopathogenic fungi (EPF) are the microorganisms that specifically infect and often kill insects and other arthropods. EPF is the most effective biocontrol agent against insects in the natural ecosystem which could be an effective alternative to chemical insecticides in bio-intensive pest management. *Beauveria bassiana*, one of the most prevalent soil-borne entomopathogens, has virulence on insect pests. The present study is aimed to evaluate the pathogenicity of a native isolate of the entomopathogenic fungus *B. bassiana* isolated from the soil samples of a cotton field (Kuthukkal) in the Tirunelveli district of Tamil Nadu against *Dysdercus cingulatus*. Bio-efficacy trials were carried out with six different concentrations *viz.,* 4.6×10^3,1.5×10^4, 5.0×10^5, 2.7×10^6, 3.2×10^7, and 2.8×10^8 (spores/mL) in all the five nymphal instars and the adults of *D. cingulatus*. A 100% mortality was observed in higher concentrations 2.8×10^8 (spores/mL) at 120hrs after treatment. The results of the present study show that the isolate seems to be highly promising in the pest management of *D. cingulatus*.

Keywords: Entomopathogenic fungi, *Beauveria bassiana*, *Dysdercus cingulatus*, biocontrol

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INTRODUCTION

Insect pest infestation is one of the most important impediments hindering the cultivation of Cotton, the most economically important natural fiber (Moorthi *et al*., 2012). The cotton stainer *Dysdercus cingulatus* (Fab.) (Hemiptera: Pyrrhocoridae) causes serious damage by feeding on developing cotton bolls and ripe cotton seeds and transmits fungal spores of the pathogen into the boll and the developing lint (Sahayaraj and Ilyaraja, 2008). The insect feed mainly on the milky contents of seed kernels. Little damage is done to very young fruits but the perforation may cause premature fall of the bolls (Rafiq *et al*., 2014; Ranilalitha *et al*., 2015). Numerous synthetic pesticides have been used to control this pest but were unsuccessful because of the swift movement of the nymphs and adults from one location to another. Finding a different approach to manage this economically significant pest is therefore imperative. Hence pest management specialists have been employing fungal pathogens over the past 20 years (Sahayaraj and Tomson, 2010). Recently, three entomotoxic proteins of *B. bassiana* were isolated, fractionated using HPLC (BBI, BBII, and BBIII), and tested against two hemipteran insect pests, *Dysdercus cingulatus* Fab., and *Phenacoccus solenopsis* (Hemiptera: Pseudococcidae). The results indicated that the protein content was higher in fraction BBII than in BBI and BBIII (Tomson *et al.,* 2021). Entomopathogenic fungi (EPF) are bioinsecticides

with the ability to infect and kill insects (Barelli *et al*., 2016 and Tomson *et al*., 2021). These fungi are categorized into six classes namely: Oomycetes, Chytridiomycota, Microspia, Entomophtoromycota, Basidiomycota and the most common Ascomycota. Several entomopathogenic fungi have been used to control insect pests from different orders such as Diptera (Shoukat *et al*., 2016; Shoukat *et al*., 2018), Hemiptera (Zafar *et al*., 2016), Coleoptera (Khan *et al*., 2016; Amrit Sharma *et al*., 2023), Homoptera (Khan *et al*., 2014), and Lepidoptera (Duarte *et al*., 2016). EPF helps in maintaining a natural balance of the insect population (Litwin *et al*., 2020), especially the sucking pests where synthetic insecticides fail (Xiaoyan *et al*., 2019). As a result, entomopathogenic fungi are utilized in organic farming as an eco-friendly alternative to hazardous insecticides (Anna Litwin *et al*., 2020). The white muscardine fungus *B. bassiana* is one of the most promising fungal entomopathogens reported to infect 707 species of insect hosts (Sain *et al*., 2019), which could play a vital role in the control of sucking and chewing insect pests (Malekan *et al*., 2015). The environmental adaptability of *B. bassiana* makes them suitable to control *D. cingulatus* in its natural ecosystem. Hence in the present study, we have evaluated the bio-efficacy of a native isolate of *B. bassiana* (ERUB001) against a notorious sucking insect pest*, D. cingulatus.*

MATERIALS AND METHODS

Collection of soil samples

Soil samples were collected from the cotton fields of Kuthukkal, Tirunelveli district of Tamil Nadu. About 100 grams of soil samples were taken from each site at a depth of 15 cm using a sterilized stainless-steel spatula and sterile plastic bags (Sahayaraj and Borgio, 2009).

Soil sample preparation

One gram of the soil sample was taken in a test tube containing 10 mL of sterile distilled water under aseptic conditions. The test tube was agitated using a vortex mixture for 15 seconds. The suspension was serially diluted according to the tenfold series (10-1 to 10-10) (Sahayaraj and Borgio, 2009 and Velankanny *et al*., 2022).

Isolation of EPF

A selective media containing 1% Dodine (N-dodecylguanidine monoacetate) aqueous solution was autoclaved separately and then thoroughly mixed with autoclaved Potato Dextrose Agar (PDA supplemented with yeast extract and gentamicin) in appropriate quantities to obtain the designated concentration (Everton *et al*., 2010). From each dilution (10^{-1} to 10^{-10}), 0.1 mL of the sample was transferred into separate PDA containing petriplates and spread using a sterile L-shaped glass rod. The seeded plates were incubated at 26º C ± 2º C for 14 days. Based on the cultural characteristics, the fungi suspected to be *B. bassiana* were sub-cultured to obtain a pure culture (Sahayaraj and Borgio, 2009; Velankanny *et al*., 2022).

Laboratory bioassay

Different concentrations of *B. bassiana* (ERUB001) isolate were prepared using serial dilutions and the spore count was determined using Neubauer Haemocytometer. Six different concentrations were chosen for the study *viz.*, 4.6×10^3, 1.5×10^4, 5.0×10^5, 2.9×10^6, 3.2×10^7, and 2.8×10^8 (spores/mL). And 0.5 mL of 0.02% Tween-80 (adjuvant) was added to each concentration, transferred to 20 mL spray bottles, and mixed thoroughly. The assay was carried out in standard aerated plastic containers. Ten insects each from the life stages of *D. cingulatus* (first, second, third, fourth, and fifth instars and adults) were introduced in each container and fed with water-soaked cotton seeds. The experimental solution of 1 mL was sprayed over the insects in the respective experimental containers. Distilled water with 0.5 mL of 0.02% Tween-80 was used to treat insects in the control. Six replicates each were maintained for both treatment and control. Mortality counts were recorded every 24hrs up to 120hrs (Sahayaraj and Borgio, 2009 and Velankanny *et al*., 2022).

Statistical analysis

The LC_{50} values and their fiducial limits were calculated by Probit analysis at 0.05 level and were used to determine significant differences

between treatments. The data obtained were analyzed using SPSS version 25.

RESULTS

Insecticidal bioassay (contact toxicity) was performed with a native *B. bassiana* (ERUB001) isolate against *D. cingulatus*. The *D. cingulatus* infected by the fungal isolate were mummified and hard to touch, mycelial growth was observed at 24hrs to 48hrs after death. Initially, the growth of the fungi was uneven in the intermembrane of the abdomen and eventually, the entire cadaver was covered by a fungal mat. The results show that the mortality increases with an increase in concentration. The isolate ERUB001 showed a significant mortality rate against *D. cingulatus*. For each fungal concentration, the mortality rates of young and older instars of the insect were significantly different at different conidial concentrations and elapsed time up to 120hrs after application. The mortality rates of adults and nymphal instars of *D. cingulatus* are listed in Table 1.

The highest mortality 40.00 ± 8.94 % was recorded in 2.8×10^8 spores/mL concentration in the second instars and zero mortality was observed with lower concentrations of 4.6×10^3 and 1.5×10^4 spores/mL in the third, fourth, and fifth instars and the adult respectively after 24hrs of treatment. A maximum of 70% mortality was recorded at 2.8×10^8 spores/mL concentration in second instars and zero mortality was observed with lower concentrations of 4.6×10^3, 1.5×10^4 spores/mL in the adult at 48hrs of treatment. The highest mortality of 100 % was recorded at 1.5 ± 10^4, 5.0×10^5, 2.7×10^6, 3.2×10^7, 2.8×10^8 spores/ mL concentration in the first and second instar and a minimum of 10.00 % mortality was observed with the lower concentration (4.6×10^3 spores/mL) in the adults after 72hrs of treatment. The highest mortality of 100 % was recorded at 4.6×10^3 spores/ mL concentration in the first, and second instar, and 38.33 % mortality was observed in the lower concentration (4.6×10^3 spores/mL) at 96hrs of treatment in adults. The highest mortality of 100 % was recorded at 5.0×10^5, 2.7×10^6, 3.2×10^7, 2.8×10^8 spores/ mL concentration in the third, fourth, and fifth instars, and in the adult, 68.33 % mortality was observed at the lowest concentration 4.6×10^3 spores/mL at 120hrs of treatment in adult. It was evident from the LC_{50} value, that the native *B. bassiana* isolate is highly virulent against the different life stages of *D. cingulatus.* The highest mortality was observed in adults (5.94×10^7; Y=48.22 + 1.21E-9X) and was statistically significant ($p < 0.0159$) and the lowest LC_{50} was recorded in first instar (2.83×10^4; Y=53.74 + 6.43E-9X) and statistically significant ($p < 0.0046$) (Table 2).

DISCUSSION

Entomopathogenic fungi are potential biocontrol agents that can play an important role in integrated pest management. The results of the present investigation showed that the native isolate of the EPF *B. bassiana* (ERUB001) has great potential to control *D. cingulatus*. Irrespective of the life stages, the mortality rate increases with an increase in the conidial concentrations and it resulted in 100 % mortality at the higher conidial concentration (2.8×10^8 spores/mL). The insect cuticle is an important structure in the infection process of EPF as it is the main route for fungus penetration (Tahira *et al*., 2014). The fungus must first adhere to and interact with the epicuticular layer of the host by developing physical or enzymatic activities upon penetration into the insect cuticle (Ortiz-Urquiza *et al*., 2013). The results of the present investigation demonstrate the sequence of infection and the final mat formation on the cadavers (Figure 1).

However, some insects have a substance that can inhibit or promote conidia attachment or germination (Abdelghany, 2015). Attachment and germination of fungal spores start once they have landed on the insect cuticle. The EPF and the insect's pathogenic interaction is established by forming an infective structure called the appressorium (Sandhu *et al*., 2012), which penetrates the insect cuticle using mechanical pressure and cuticle-degrading enzymes (Vega *et al.,* 2012).

Table 1. Efficacy of a native *Beauveria bassiana* isolate (ERUB001) on *Dysdercus cingulatus* Within the experimental concentrations, the mean mortality (%) mean ± SD values followed by the

Instars	Spores/mL	MORTALITY % (Mean ± S.D.)				
		24hrs	**48hrs**	**72hrs**	**96hrs**	**120hrs**
I	Control	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00±0.00
	4.6×10^3	16.66 ± 5.16d	55.00± 5.47d	81.66 ± 4.08b	100.00 ± 0.00a	-
	1.5×10^4	25.00± 5.47c	58.33 ± 4.08c	100.00 ± 0.00a	-	-
	5.0 × 105	28.33± 7.52b	63.33 ± 8.16c	100.00 ± 0.00a	-	-
	2.7 × 106	31.66 ± 7.52ab	65.00±5.47ab	100.00 ± 0.00a	-	-
	3.2 × 107	33.33 ± 5.16a	66.66 ± 8.16a	100.00 ± 0.00a	-	-
	2.8 × 108	36.00 ± 5.47a	68.00 ± 4.47a	100.00 ± 0.00a	-	-
	Mean	28.33 ± 8.45	62.77 ± 7.41	96.94 ± 7.09	100.00 ± 0.00	-
II	Control	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00±0.00
	4.6 × 103	30.00 ± 0.00b	56.66 ± 5.16d	76.66 ± 12.11b	100.00 ± 0.00a	-
	1.5 × 104	31.66 ± 7.52b	63.33 ± 8.16c	100.00 ± 0.00a	-	-
	5.0 × 105	33.33 ±5.16 b	65.00 ± 8.36b	100.00 ± 0.00a	-	-
	2.7× 106	35.00 ± 5.47ab	66.66 ± 5.16ab	100.00 ± 0.00a	-	-
	3.2 × 107	36.66 ± 5.16a	68.33 ± 7.52ab	100.00 ± 0.00a	-	-
	2.8 × 108	40.00 ± 8.94a	70.00 ± 12.64a	100.00 ± 0.00a	-	-
	Mean	34.44 ± 6.52	65.00 ± 8.78	96.11 ± 9.93	100.00 ± 0.00	-
III	Control	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00
	4.6 × 103	0.00 ± 0.00	15.00 ± 5.47d	35.00 ± 8.36c	50.00 ± 6.32d	70.00 ± 6.32b
	1.5 × 104	5.00 ± 5.47c	23.33 ± 5.16c	46.66 ± 8.16b	68.33 ± 7.52c	70.00 ± 29.66b
	5.0 × 105	10.00 ± 0.00ab	28.33 ± 4.08bc	53.33 ± 8.16b	73.33 ± 8.16bc	100.00 ± 0.00a
	2.7 × 106	13.00 ± 5.16ab	30.00 ± 6.32b	55.33 ± 10.32ab	76.66 ± 5.16ab	100.00 ± 0.00a
	3.2 × 107	16.66 ± 5.16a	40.00 ± 6.32a	58.33 ± 7.52a	80.00 ± 6.32a	100.00 ± 0.00a
	2.8 × 108	16.66 ± 5.16a	41.66 ± 4.08a	65.00 ± 5.47a	83.33 ± 5.16a	100.00 ± 0.00a
	Mean	10.27 ± 7.36	29.72 ± 10.55	52.22 ± 12.21	71.94 ± 12.60	90.00 ± 18.36
IV	Control	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00
	4.6 × 103	0.00 ± 0.00	6.66 ± 5.16c	28.33 ± 7.52cd	51.66 ± 4.08c	78.33 ± 4.08c
	1.5 × 104	5.00 ± 5.47ab	26.66 ± 5.16b	46.66 ± 12.11c	70.00 ± 10.95ab	96.66 ± 5.16b
	5.0 × 105	5.00 ± 5.47b	28.33 ± 4.08ab	53.33 ± 10.32ab	78.33 ± 13.29b	100.00 ± 0.00a
	2.7 × 106	8.33 ± 4.08b	31.66 ± 5.16ab	53.33 ± 10.32ab	80.00 ± 0.00a	100.00 ± 0.00a
	3.2 × 107	10.00 ± 0.00a	33.33 ± 8.16a	55.00 ± 8.36a	81.66 ± 4.08a	100.00 ± 0.00a
	2.8 × 108	10.00 ± 0.00a	35.00 ± 5.47a	58.33 ± 4.08a	81.66 ± 7.52a	100.00 ± 0.00a
	Mean	6.38 ± 4.87	26.94 ± 11.41	49.16 ± 13.17	73.88 ± 13.15	95.83 ± 8.40
V	Control	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00
	4.6 × 103	0.00 ± 0.00	3.33 ± 5.16d	21.66 ± 4.08d	51.66 ± 11.69c	78.33 ± 11.69c
	1.5 × 104	3.33 ± 5.16c	13.33 ± 5.16bc	35.00 ± 5.47c	61.66 ± 5.16b	95.00 ± 5.47b
	5.0 × 105	10.00 ± 0.00b	20.00 ± 0.00ab	43.33 ± 5.16b	66.66 ± 5.16ab	100.00 ± 0.00a
	2.7 × 106	11.66 ± 4.08a	21.66 ± 4.08a	45.00 ± 8.36ab	68.33 ± 9.83ab	100.00 ± 0.00a
	3.2 × 107	13.33 ± 5.16a	21.66 ± 7.52a	46.66 ± 8.16a	71.66 ± 4.08a	100.00 ± 0.00a
	2.8 × 108	15.00 ± 5.47a	26.66 ± 8.16a	53.33 ± 12.11a	76.66 ± 9.83a	100.00 ± 0.00a
	Mean	8.88 ± 6.66	17.77 ± 9.29	40.83 ± 12.50	66.11 ± 11.02	95.55 ± 9.39
Adult	Control	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00
	4.6 × 103	0.00 ± 0.00	0.00 ± 0.00	10.00 ± 0.00	38.33 ± 4.08d	68.33 ± 9.83c
	1.5 × 104	0.00 ± 0.00	0.00 ± 0.00	13.33 ± 8.16bc	43.33 ± 12.11c	78.33 ± 11.69b
	5.0 × 105	3.33 ± 5.16c	13.33 ± 8.16bc	35.00 ± 10.48b	65.00 ± 5.47ab	100.00 ± 0.00a
	2.7 × 106	5.00 ± 8.36ab	15.00 ± 10.48ab	40.00 ± 8.94ab	68.33 ± 7.52ab	100.00 ± 0.00a
	3.2 × 107	6.66 ± 5.16a	16.66 ± 8.16a	43.33 ± 5.16a	70.00 ± 0.00a	100.00 ± 0.00a
	2.8 × 108	6.66 ± 5.16a	18.33 ± 7.52a	45.00 ± 12.24a	71.66 ± 9.83a	100.00 ± 0.00a
	Mean	3.61 ± 5.42	10.55 ± 10.12	31.11 ± 16.34	59.44 ± 15.29	91.11 ± 14.29

alphabet(s) show significant difference at $P \leq 0.05$

Table 2. LC_{50} Mortality rate caused by *Beauveria bassiana* isolate against *Dysdercus cingulatus*

Isolate	Instars	LC_{50}	Fiducial limit		Chi^2	p	Regression equation
			lower	Higher			
ERUB001	1st instar	2.83×10^4	2.25×10^5	2.52×10^7	8.0289	0.0046	Y=53.74 + 6.43E-9X
	2nd instar	2.27×10^5	2.16×10^4	2.04×10^6	19.3413	0.00001	Y=53.29 + 6.89E-9X
	3rdinstar	2.40×10^5	2.26×10^4	2.55×10^6	12.4377	0.00421	Y=49.41 + 1.09E-9X
	4th instar	1.09×10^5	8.97×10^5	9.93×10^5	8.5681	0.0158	Y=51.5 + 8.72E-9X
	5th instar	2.08×10^6	3.07×10^5	6.80×10^6	3.0261	0.0201	Y=52.39 + 7.8E-9X
	Adult	5.94×10^7	1.46×10^5	3.43×10^6	13.148	0.0159	Y=48.22 + 1.21E-9X

Muthukumar (2005) and Seiedy *et al*. (2010) also reported similar results with *B. bassiana* against *T. urticae* with the LC_{50} values of 1.46 x 10^5 spores mL^{-1} and 3.7 × 10^5 conidia mL^{-1}, respectively. Gatarahiya *et al*. (2012) also showed that *B. bassiana* strain PPRI 7315 had a median lethal concentration of 1.13 x 10^6 conidia mL-1 against *T. urticae*. Sain *et al.* (2019) reported the LC_{50} values of 0.7 x 10^7 and 2.5x 10^7 conidia mL-1 for *B. bassiana* and *M. anisopliae*, respectively against *T. urticae*. The interaction of *B. bassiana* with other biological control agents, such as *Bacillus thuringiensis* for the biological control of *Bemisia tabaci*, was shown to have an antagonistic effect, and mortality greater than 50% was observed over a period of 7 days. The *B. bassiana* isolate is virulent and could be promising in future mycoinsecticidal development. However, its field efficacy, especially in cotton, needs to be evaluated. In conclusion, this study exemplifies the excellent biocontrol potential of the soil isolate *B. bassiana* towards red cotton stainer *D. cingulatus.*

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