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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 x 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 (1.75×10^7; Y=48.22 + 1.21E-9X) and was statistically significant ($p < 0.028$) and the lowest LC_{50} was recorded in first instar (2.51×10^4; Y=53.74 + 6.43E-9X) and statistically significant ($p < 0.013$) (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.

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*

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 ± 5.16^{d}	55.00± 5.47^{d}	81.66 ± 4.08^{b}	100.00 ± 0.00^{a}	-
	1.5×10^4	25.00± 5.47^{c}	58.33 ± 4.08^{c}	100.00 ± 0.00^{a}	-	-
	5.0×10^5	28.33± 7.52^{b}	63.33 ± 8.16^{c}	100.00 ± 0.00^{a}	-	-
	2.7×10^6	31.66 ± 7.52^{ab}	65.00±5.47^{ab}	100.00 ± 0.00^{a}	-	-
	3.2×10^7	33.33 ± 5.16^{a}	66.66 ± 8.16^{a}	100.00 ± 0.00^{a}	-	-
	2.8×10^8	36.00 ± 5.47^{a}	68.00 ± 4.47^{a}	100.00 ± 0.00^{a}	-	-
	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
	4.6×10^3	30.00 ± 0.00^{b}	56.66 ± 5.16^{d}	76.66 ± 12.11^{b}	100.00 ± 0.00^{a}	-
	1.5×10^4	31.66 ± 7.52^{b}	63.33 ± 8.16^{c}	100.00 ± 0.00^{a}	-	-
	5.0×10^5	33.33 ±5.16 ^{b}	65.00 ± 8.36^{b}	100.00 ± 0.00^{a}	-	-
	2.7×10^6	35.00 ± 5.47^{ab}	66.66 ± 5.16^{ab}	100.00 ± 0.00^{a}	-	-
	3.2×10^7	36.66 ± 5.16^{a}	68.33 ± 7.52^{ab}	100.00 ± 0.00^{a}	-	-
	2.8×10^8	40.00 ± 8.94^{a}	70.00 ± 12.64^{a}	100.00 ± 0.00^{a}	-	-
	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
	4.6×10^3	0.00 ± 0.00	15.00 ± 5.47^{d}	35.00 ± 8.36^{c}	50.00 ± 6.32^{d}	70.00 ± 6.32^{b}
	1.5×10^4	5.00 ± 5.47^{c}	23.33 ± 5.16^{c}	46.66 ± 8.16^{b}	68.33 ± 7.52^{c}	70.00 ± 29.66^{b}
	5.0×10^5	10.00 ± 0.00^{ab}	28.33 ± 4.08^{bc}	53.33 ± 8.16^{b}	73.33 ± 8.16^{bc}	100.00 ± 0.00^{a}
	2.7×10^6	13.00 ± 5.16^{ab}	30.00 ± 6.32^{b}	55.33 ± 10.32^{ab}	76.66 ± 5.16^{ab}	100.00 ± 0.00^{a}
	3.2×10^7	16.66 ± 5.16^{a}	40.00 ± 6.32^{a}	58.33 ± 7.52^{a}	80.00 ± 6.32^{a}	100.00 ± 0.00^{a}
	2.8×10^8	16.66 ± 5.16^{a}	41.66 ± 4.08^{a}	65.00 ± 5.47^{a}	83.33 ± 5.16^{a}	100.00 ± 0.00^{a}
	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
	4.6×10^3	0.00 ± 0.00	6.66 ± 5.16^{c}	28.33 ± 7.52^{cd}	51.66 ± 4.08^{c}	78.33 ± 4.08^{c}
	1.5×10^4	5.00 ± 5.47^{ab}	26.66 ± 5.16^{b}	46.66 ± 12.11^{c}	70.00 ± 10.95^{ab}	96.66 ± 5.16^{b}
	5.0×10^5	5.00 ± 5.47^{b}	28.33 ± 4.08^{ab}	53.33 ± 10.32^{ab}	78.33 ± 13.29^{b}	100.00 ± 0.00^{a}
	2.7×10^6	8.33 ± 4.08^{b}	31.66 ± 5.16^{ab}	53.33 ± 10.32^{ab}	80.00 ± 0.00^{a}	100.00 ± 0.00^{a}
	3.2×10^7	10.00 ± 0.00^{a}	33.33 ± 8.16^{a}	55.00 ± 8.36^{a}	81.66 ± 4.08^{a}	100.00 ± 0.00^{a}
	2.8×10^8	10.00 ± 0.00^{a}	35.00 ± 5.47^{a}	58.33 ± 4.08^{a}	81.66 ± 7.52^{a}	100.00 ± 0.00^{a}
	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
	4.6×10^3	0.00 ± 0.00	3.33 ± 5.16^{d}	21.66 ± 4.08^{d}	51.66 ± 11.69^{c}	78.33 ± 11.69^{c}
	1.5×10^4	3.33 ± 5.16^{c}	13.33 ± 5.16^{bc}	35.00 ± 5.47^{c}	61.66 ± 5.16^{b}	95.00 ± 5.47^{b}
	5.0×10^5	10.00 ± 0.00^{b}	20.00 ± 0.00^{ab}	43.33 ± 5.16^{b}	66.66 ± 5.16^{ab}	100.00 ± 0.00^{a}
	2.7×10^6	11.66 ± 4.08^{a}	21.66 ± 4.08^{a}	45.00 ± 8.36^{ab}	68.33 ± 9.83^{ab}	100.00 ± 0.00^{a}
	3.2×10^7	13.33 ± 5.16^{a}	21.66 ± 7.52^{a}	46.66 ± 8.16^{a}	71.66 ± 4.08^{a}	100.00 ± 0.00^{a}
	2.8×10^8	15.00 ± 5.47^{a}	26.66 ± 8.16^{a}	53.33 ± 12.11^{a}	76.66 ± 9.83^{a}	100.00 ± 0.00^{a}
	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
	4.6×10^3	0.00 ± 0.00	0.00 ± 0.00	10.00 ± 0.00	38.33 ± 4.08^{d}	68.33 ± 9.83^{c}
	1.5×10^4	0.00 ± 0.00	0.00 ± 0.00	13.33 ± 8.16^{bc}	43.33 ± 12.11^{c}	78.33 ± 11.69^{b}
	5.0×10^5	3.33 ± 5.16^{c}	13.33 ± 8.16^{bc}	35.00 ± 10.48^{b}	65.00 ± 5.47^{ab}	100.00 ± 0.00^{a}
	2.7×10^6	5.00 ± 8.36^{ab}	15.00 ± 10.48^{ab}	40.00 ± 8.94^{ab}	68.33 ± 7.52^{ab}	100.00 ± 0.00^{a}
	3.2×10^7	6.66 ± 5.16^{a}	16.66 ± 8.16^{a}	43.33 ± 5.16^{a}	70.00 ± 0.00^{a}	100.00 ± 0.00^{a}
	2.8×10^8	6.66 ± 5.16^{a}	18.33 ± 7.52^{a}	45.00 ± 12.24^{a}	71.66 ± 9.83^{a}	100.00 ± 0.00^{a}
	Mean	**3.61 ± 5.42**	**10.55 ± 10.12**	**31.11 ± 16.34**	**59.44 ± 15.29**	**91.11 ± 14.29**

Within the experimental concentrations, the mean mortality (%) mean ± SD values followed by the 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	2.51×10^4	2.19×10^6	2.71×10^6	25.172	0.013	Y=53.74 + 6.43E-9X
	2nd instar	2.64×10^6	2.34×10^7	2.52×10^7	5.469	0.041	Y=53.29 + 6.89E-9X
	3rdinstar	1.87×10^5	2.55×10^6	2.69×10^7	2.696	0.191	Y=49.41 + 1.09E-9X
	4th instar	2.77×10^5	1.15×10^5	3.92×10^6	25.952	0.042	Y=51.5 + 8.72E-9X
	5th instar	2.47×10^6	4.22×10^6	4.25×10^6	13.683	0.001	Y=52.39 + 7.8E-9X
	Adult	1.75×10^7	3.05×10^7	2.75×10^7	76.264	0.028	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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REFERENCES

Abdelghany, T. M. 2015. Entomopathogenic Fungi and Their Role in Biological Control. El-Ghany, T.M.A., Ed. OMICS Group eBooks: Foster City, CA, USA, P. 1–42.

Anna Litwin, Monika Nowak, and Sylwia Rozalska. 2020. Entomopathogenic fungi: unconventional applications. *Reviews in Environmental Science and Biotechnology*, **19**: 23-42.

Amrit, S., Shaurav, S and Pankaj K. Y. 2023. Entomopathogenic fungi and their relevance in sustainable agriculture: A review, Cogent Food & Agriculture, *Pakistan Journal of Zoology*, **48**, 91–99.

Barelli, L., Moonjely, S., Behie, S.W and Bidochka, M.. J. 2016. Fungi with multifunctional lifestyles: endophytic insect pathogenic fungi. *Plant Molecular Biology*, **90**(6), PP. 657-664.

Duarte, R., Goncalves, K., Espinosa, D., Moreira, L., De Bortoli, S., Humber, R and Polanczyk, R. 2016. The potential of entomopathogenic fungi as biological control agents of diamondback moth (Lepidoptera: Plutellidae) and compatibility with chemical insecticides. *Journal of Economic Entomology*, **109**, 594–601.

Everton, K. K., Fernandes, Chad Keyser, A., Drauzio, EN., Rangel, R., Nelson Foster and Donald Roberts, W. 2010. CTC Medium: A

novel dodine –free selective medium for isolating entomopathogenic fungi, especially *Metarhizium acridum*, from the soil. *Biological control*, **54**: 197-205.

Gatarayiha, M. C., Laing, M. D and Miller, R. M. 2012. Selection of *Beauveria bassiana* strains against the two-spotted spider mite, *Tetranychus urticae* Koch in laboratory and greenhouse trials. *African Journal of Microbiology*, Res. **6** (11): 2694-2703.

Khan, B. A., Freed, S., Zafar, J and Farooq, M. 2016. Evaluation of three different insect pathogenic fungi for the control of *Dysdercus koenigii* and *Oxycarenus hyalinipennis*. *Pakistan Journal of Zoology*, **46**, 1759–1766.

Litwin, A., Nowak, M and Rozalska, S. 2020. Entomopathogenic fungi: unconventional applications. *Reviews in Environmental Science and Biotechnology*, **19**(1), PP. 23-42.

Malekan, A., Hatami, B., Ebadi, R., Akhavan, A and Rajab, R. 2015. Evaluation of entomopathogenic fungi *Beauveria bassiana* and *Lecanicillium muscarium* on different nymphal stages of greenhouse whitefly *Trialeurodes vaporariorum* in greenhouse conditions. *Biharean Biology*, **9**:108–112.

Moorthi, P. V., Balasubramanian, C., Avery, P. B., Banu, A.N. and Kubendran, T. 2012. Efficacy of fungal entomopathogens against red cotton stainer, *Dysdercus cingulatus* Fabricius (Hemiptera: Pyrrhocoridae). *Journal of Biopesticides*, **5**(2), P. 140.

Muthukumar, 2005. Using fungal pathogens against two-spotted *tetranychus urticae* Koch on okra is possible. M.Sc. (Ag.) Thesis, Tamil Nadu Agricultural University, Coimbatore, India.

Ortiz-Urquiza, A and Keyhani, N. O. 2013. Action on the surface: Entomopathogenic fungi versus the insect cuticle. *Insects*, **4**, 357–374.

Rafiq, M., Shah, S. I. A., Jan, M.T., Khan, I. R., Shah, S. A. S and Hussain, Z. 2014. Efficacy of different groups of insecticides against cotton stainer (*Dysdercus koenigii*) in field conditions. *Pakistan Journal of Zoology*, **36**(2), PP.105-110.

Ranilalitha, P. M., Sukumaran, S., Raveendran, M. A and Amirthanayagi, A. K. 2015. Evaluation of nymphicidal effect of two indigenous plant extracts on cotton pest, Dysdercus cingulatus (Fab.). *International Journal of Pure and Applied Zoology*, **3**(1), PP. 24-30.

Sahayaraj, K and Francis Borgio, J. 2009. Distribution of *Metarhizum anisopliae* (Metsch) Sorokin (Deuteromycutina: hyphomycetes in TamilNadu, India, its biocontrol potential on *Dysdercus cingulatus* (Fab.) (Hemiptera: Pyrrhocoridae). *Archives of phytopathology and plant protection*, **42**(5): 424-435.

Sahayaraj, K. and Ilyaraja, R. 2008. Ecology of *Dysdercus cingulatus* morphs. *Egyptian Journal of Biological Pest Control*, **10**, PP.122-125.

Sahayaraj, K and Tomson, M. 2010. Impact of two pathogenic fungal crude metabolites on mortality, biology, and enzymes of *Dysdercus cingulatus* (Fab.) (Hemiptera: Pyrrhocoridae). *Journal of Biopesticides*, **3** (Special Issue), P.163.

Sain, S. K., Monga, D., Kumar, R., Nagrale, D. T., Kranthi, S and Kranthi, K. R. 2019. Comparative effectiveness of bioassay methods in identifying the most virulent entomopathogenic fungal strains to control *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae). *Egyptian Journal of Biological Pest Control*, **29**(1), PP.1-11.

Sandhu, S. S., Sharma, A. K., Beniwal, V., Goel, G., Batra, P., Kumar, A., Jaglan, S and Sharma, A. K. 2012. Myco-Biocontrol of insect pests: Factors involved, mechanism, and regulation. *Journal of Pathogens*, P.126.

Seiedy, M., Saboori, A., Allahyari, H., Hassanloui, R. T and Tork, M. 2010. Laboratory investigation on the virulence of two entomopathogenic fungi *Beauveria bassiana* isolates against the two-spotted spider mite *Tetranychus urticae* (Acari: Tetranychidae).

International Journal of Acarology, **36**(6): 527-532.

Shoukat, R. F., Freed, S and Ahmad, K. W. 2016. Evaluation of binary mixtures of entomogenous fungi and botanicals on biological parameters of *Culex pipiens* (Diptera: Culicidae) under laboratory and field conditions. *International Journal of Mosquito Research*, Res. **3**, 17–24.

Shoukat, R. F., Freed, S., Ahmad, K. W. and Rehman, A. U. 2018. Assessment of Binary Mixtures of Entomopathogenic Fungi and Chemical Insecticides on Biological Parameters of *Culex Pipiens* (Diptera: Culicidae) under laboratory and field conditions. *Pakistan Journal of Zoology*, **50**, 299–309.

Tomson, M., Sahayaraj, K., Sayed, S., Elarnaouty, S. A and Petchidurai, G. 2021. Entomotoxic proteins of *Beauveria bassiana* Bals. (Vuil.) and their virulence against two cotton insect pests. *Journal of King Saud University-Science*, **33**(8), P. 101595.

Tahira, H. G., Saeed, S and Khan, F.Z.A. 2014. Entomopathogenic fungi as effective insect pest management tactic: *A Review. International Journal of Acarology*, **1**, 10–18.

Velankanny, M., Selvaraj, P., Francis Borgio, J., Sherlin John, J and Princy Rathnamala Jayaseeli, J. 2022. Bioefficacy of entomopathogenic fungi *Metarhizium anisopliae* (Metschn.) Sorokin against the cotton stainer, *Dysdercus cingulatus* (Fab.) (Hemiptera: Pyrrhocoridae). *Journal of Biopesticides*, **15(**1): 59-72.

Vega, F. E., Meyling, N. V., Luangsa-Ard, J. J and Blackwell, M. 2012. Fungal entomopathogens in Insect Pathology. *Journal of Biological Control*, The Netherlands, p. 171–220.

Xiaoyan Niu Weiwen Xie, Jing Zhang and Qiongbo, H. U. 2019. Biodiversity of entomopathogenic fungi in the soils of south China. *Microorganisms*, **7**, 311.

Zafar, J., Freed, S., Khan, B. A and Farooq, M. 2016. Effectiveness of *Beauveria bassiana* Against Cotton Whitefly, *Bemisia tabaci* (Gennadius) (Aleyrodidae: Homoptera) on Different Host. *Pakistan Journal of Zoology*, **48**(1).

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