

Pathogenicity of *Beauveria bassiana* (Balsamo) Vuillemin isolate (TNAU ENT BB1) against rice leaf folder, *Cnaphalocrocis medinalis* (Guenee) in *in-vitro*(laboratory) conditions

ABSTRACT

Rice productivity is impaired by sucking, leaf feeding and borer insect pests. Among the leaf feeders, rice leaf folder *Cnaphalocrocis medinalis* (Guenee) cause significant yield loss. The non-chemical insect pest management is gaining momentum among farmers, and biocontrol management is one of the essential components. The efficacy of entomopathogenic fungi *Beauveria bassiana* (Balsamo) Vuillemin isolate against *C. medinalis* was studied using the laboratory culture maintained in rice leaves. Different concentrations of *B. bassiana* were applied topically on the second instar *C. medinalis* larvae. The mortality observations were recorded at 48 hrs intervals from the third day and continued up to 11 days. The highest mortality of 78.33% was observed at concentration 1×10^8 conidia/ml and the lowest mortality of 36.67% was observed at concentration 1×10^3 conidia/ml. The determined median lethal concentration (LC50) of *B. bassiana* (Balsamo) Vuillemin isolate against *C. medinalis* is 5.44×10^5 conidia/ml.

Keywords: *Beauveria bassiana*, *Cnaphalocrocis medinalis*, mortality, median lethal concentration

1. INTRODUCTION

Rice leaf folder (*Cnaphalocrocis medinalis* (Guenee)) is a significant pest in rice which is grown all over the world and is epidemic in several nations, including India [9][20][7]. Out of the eight species of leaf folders, *C. medinalis* is the most prevalent and significant one and is responsible for considerable yield loss in rice [4]. The Kasturi Basmati variety showed higher leaf infestation of 77.2%, causing a 37.9% yield loss compared to the HPR 2143 variety, which showed leaf infestation of 57.7% with a yield loss of 11.9% [22]. The second instar larvae scrape on the leaves [8]. A single larva can damage several rice leaves, disrupting photosynthesis and lowering rice output [1]. Many insecticides are used to control rice leaf folders, but usage of insecticides can cause resurgence, residue and resistance. For the effective management of rice leaf folders, numerous biological control techniques based on botanical insecticides [12][14][15], pheromone traps [17] and microbial pesticides have been developed over the past [11][13]. Among these, entomopathogenic fungi are also an important candidate for managing insect infestations [16][5]. For the biological management of insect pests, *Beauveria bassiana* and *Metarhizium anisopliae* are the two most commonly used entomopathogenic fungus species [18]. *B. bassiana* causes mortality in the rice leaf folder, and mortality increases with increased concentration [10]. The present investigation was carried out to determine the toxicity level of *B. bassiana* isolated from the diseased leaf folder cadaver collected from the rice ecosystem.

2. MATERIALS AND METHODS

2.1 Laboratory cultures of rice leaf folder, *C. medinalis*

The leaf folder laboratory cultures were established from the field-collected population. The leaf folders were collected from the Paddy Breeding Station, Tamil Nadu Agricultural University,

Coimbatore and Wetland, TNAU, Coimbatore. The field-collected populations were maintained in TN 1 rice seedlings grown in cages. Continuous and uniform culture of rice leaf folder was maintained on 60-day-old rice seedlings. Moths collected from rice fields were released into an oviposition cage (50 x 65 x 90 cm) with rice seedlings. A honey solution containing 10% vitamin E was kept inside the cages as feed for adults to improve fecundity [6]. The eggs hatched 7 -10 days after oviposition. The larvae inside the folded leaves were clipped off and transferred to healthy rice seedlings with a brush. From these, the second instar larvae were used for the laboratory experiments.

2.2 Fungal culture

The fungal-infected leaf folder larvae were collected from the field population through regular field surveys in the rice ecosystem. The field-infected cadavers collected from Paddy Breeding Station, TNAU, Coimbatore were used to isolate *B. bassiana*. The infected cadavers were sterilized with 70 per cent ethanol for a minute, and then it was followed by 0.1 per cent sodium hypochlorite and rinsed with sterile distilled water. Fungi were isolated from the infected cadavers, which have an external mycelial growth. The conidial mass is transferred to a 90 mm Petri plate which contains Potato Dextrose Agar (PDA) medium, and incubated at $25 \pm 2^\circ\text{C}$ for a week; the colonies obtained were subcultured and transferred to a PDA slant for preservation.

2.3 Preparation of fungal suspension

Discs of mycelium were inoculated in Potato Dextrose Agar broth. The conidial suspension was harvested from a 15-day-old culture of *B. bassiana*. The fungal spores were harvested in 10-15ml of sterile distilled water (SDW) containing 0.01% Tween 80 (Polyoxyethylene sorbitan monolaurate). The conidial suspension was vortexed to obtain a homogenous suspension, and the spore count of this stock suspension was estimated with an improved Neubauer haemocytometer. The spore concentration of the isolate was adjusted to 1×10^8 to 1×10^3 conidia/ml to conduct the leaf folder bioassay.

2.4 Pathogenicity on leaf folder

Rice leaf folder larvae (second instar) were treated with *B. bassiana* suspension ranging from 1×10^8 to 1×10^3 conidia/ml by spraying the suspension directly on the larvae. Larvae treated with 0.01% tween 80 served as control. Later the rice leaves were placed in a Petri plate with moist filter paper to maintain the moisture, and ten larvae were released to each Petri plate. Each treatment was replicated six times. The mortality of the rice leaf folder was calculated at 48 hrs intervals from the third day of treatment to the eleventh day.

3. STATISTICAL ANALYSIS

The mortality of second-instar larvae was recorded, and percentage mortality was calculated. The mortality difference between the fungal isolates with different concentrations and the control was estimated using analysis of variance using statistical software SPSS 21.0 and

4. RESULT AND DISCUSSION

4.1 Pathogenicity of the isolate

The mortality of rice leaf folder larvae was recorded after 72 days after inoculation. In this study, mortality was increasing with an increase in concentration. The highest spore concentration was 1×10^8 conidia/ml 78.33% mortality after 11 days. The least larval mortality of 36.6% was observed at lower conidial concentration of 1×10^3 conidia/ml after 11 days of inoculation (Table 1). A difference of 41.67% in mortality was recorded between the highest and lowest conidial concentrations in the present investigation. The present study's median lethal concentration (LC 50) of *B. bassiana* isolate was 5.44×10^5 conidia/ml which was calculated by using SPSS 21.0 software (Tab 2 & Fig 2). The median lethal time (LT50) was found to be 154.92 hours (Fig 1).

Table 1. Pathogenicity of *B. bassiana* isolates against *C. medinalis*

Treatment details	Per cent mortality (%)				
	3 DAI	5 DAI	7 DAI	9 DAI	11 DAI
T1 (1×10^3 conidia/ml)	1.67(7.41) ^{cd}	8.33(16.77) ^c	13.33(21.41) ^d	23.33(28.88) ^d	36.67(37.26) ^d
T2 (1×10^4 conidia/ml)	6.67(14.96) ^{bcd}	11.67(19.97) ^{bc}	16.67(24.09) ^d	28.33(32.16) ^d	41.67(40.20) ^{cd}
T3 (1×10^5 conidia/ml)	8.33(16.77) ^{bc}	18.33(25.35) ^b	28.33(32.16) ^c	40.00(39.23) ^c	48.33(44.04) ^c
T4 (1×10^6 conidia/ml)	11.67(19.97) ^{ab}	26.67(31.09) ^a	41.67(40.20) ^b	51.67(45.95) ^b	63.33(52.73) ^b
T5 (1×10^7 conidia/ml)	13.33(21.41) ^{ab}	31.67(34.34) ^a	46.67(43.08) ^b	58.33(49.79) ^b	71.67(57.83) ^a
T6 (1×10^8 conidia/ml)	16.67(24.09) ^a	35.00(36.27) ^a	53.33(46.91) ^a	68.33(55.75) ^a	78.33(62.25) ^a
T8 (Control)	0.00(2.87) ^d	0.00(2.87) ^d	0.00(2.87) ^e	0.00(2.87) ^e	0.00(2.87) ^e
CD(0.05)	2.77	2.04	1.22	1.04	1.07
S.Ed	1.36	1.00	0.60	0.51	0.52

* No. of insects per replication: 10

*Values in parenthesis are subjected to arc sign transformation.

*Values sharing the same alphabets in superscript are statistically on par based on ANOVA

Table 2. Concentration and mortality response of *B. bassiana* isolate against *C. medinalis*

Heterogeneity	Regression equation	LC50	LT50	Upper limit	Lower limit
0.73	$y = 0.2387x + 3.8713$	5.44×10^4 conidia/ml	-	2.21×10^5	1.33×10^4
0.25	$y = 3.1124x - 1.7979$		154.92	176.92	135.66

Field tests were carried out in 2013 and 2014 at the research farm of the Institute of Pesticide Formulation Technology in Gurgaon, Haryana, to assess the potential of *B. bassiana* and its safety against natural enemies. The results revealed that after two sprays, *B. bassiana* 1.15%WP@3000 and 22500 ha⁻¹ was successful.[3]. The *B. bassiana* isolate from Arachalore recorded 76.7% mortality against rice leaf folder [21]. The application of *B. bassiana*, potassium silicate, and imidacloprid resulted in 61.91% mortality of *C. medinalis*[19]. In contrast, in the current study on *B. bassiana* isolate, test mortality of 78.33% was observed at a spore concentration of 1×10^8 conidia/ml.

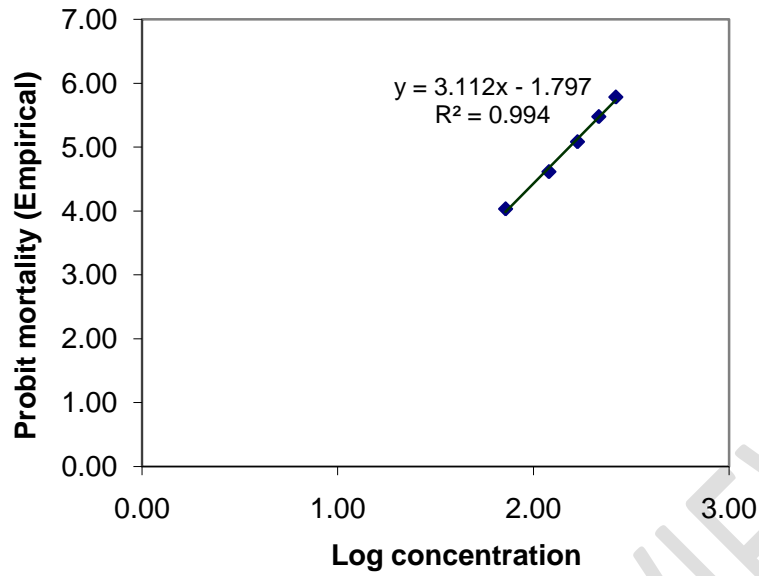


Fig.1. Time mortality response

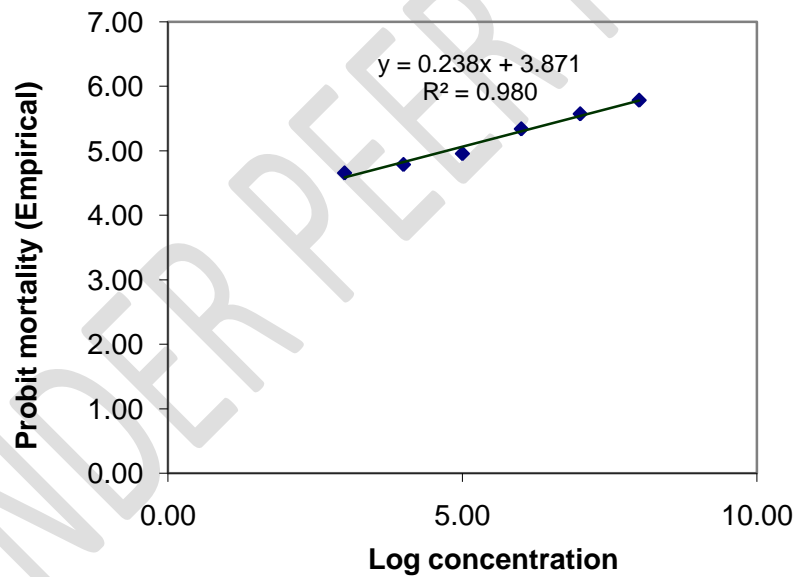


Fig. 2. Dose mortality response

The median lethal concentration of *B. bassiana* isolate MTCC7690 against *C. medinalis* was 9.09×10^4 conidia/ml [10]. [2] Ambethgar *et al.* reported the LC50 of *B. bassiana* isolate BbCm KKL 1100 against *C. medinalis* was 2.8×10^3 conidia/ml. The median lethal concentration in the present investigation was 5.44×10^5 conidia/ml, slightly higher than the previous reports. The median lethal time (LT50) was found to be 154.92 hours. The present study proves the ability of *B. bassiana* against rice leaf folders under laboratory conditions. Field studies at different agro ecological zones are required to study its potential.

4. CONCLUSION

The *B. bassiana* isolates collected from the rice fields caused 78 per cent mortality in 11 days under laboratory conditions in the present investigation. The field evaluation in different seasons will help study its performance.

AUTHORS CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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