International Journal of Environment and Climate Change

Volume 13, Issue 10, Page 472-477, 2023; Article no.IJECC.105215
ISSN: 2581-8627
(Past name: British Journal of Environment & Climate Change, Past ISSN: 2231–4784)

Pathogenicity of *Beauveria bassiana* (Balsamo) Vuillemin Isolate (TNAU ENT BB1) Against Rice Leaf Folder, *Cnaphalocrocis medinalis* (Guenee) in *In-vitro* (Laboratory) Conditions

R. Sharmila [a], V. Radhakrishnan [b*], P. S. Shanmugam [a], V. Sendhilvel [c] and S. Harish [c]

[a] Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore-641 003, India.
[b] ICAR - Krishi Vigyan Kendra, Tamil Nadu Agricultural University, Needamangalam-614 404, India.
[c] Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore-641 003, India.

Authors' contributions

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

Article Information

DOI: 10.9734/IJECC/2023/v13i102669

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/105215

Original Research Article

Received: 12/06/2023
Accepted: 16/08/2023
Published: 17/08/2023

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

**Corresponding author: E-mail: drvradhakrishnan@tnau.ac.in;*

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 x 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 [1,2,3]. 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% [5]. The second instar larvae scrape on the leaves [6]. A single larva can damage several rice leaves, disrupting photosynthesis and lowering rice output [7]. 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 [8,9,10], pheromone traps [11] and microbial pesticides have been developed over the past [12,13]. Among these, entomopathogenic fungi are also an important candidate for managing insect infestations [14,15]. For the biological management of insect pests, *Beauveria bassiana* and *Metarhizium anisopliae* are the two most commonly used entomopathogenic fungus species [16]. *B. bassiana* causes mortality in the rice leaf folder, and mortality increases with increased concentration [17]. 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 [8]. 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 ± 2ºC 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 x

10^8 to 1 x 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 x 10^8 to 1 x 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.

2.5 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.

3. RESULTS AND DISCUSSION

3.1 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 1x10^8 conidia/ml 78.33% mortality after 11 days. The least larval mortality of 36.6% was observed at lower conidial concentration of 1x10^3 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 x10^5 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).

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 tha after two sprays, *B. bassiana* 1.15%WP@3000 and 22500 ha-1 was successful [19]. The *B. bassiana* isolate from Arachalore recorded 76.7% mortality against rice leaf folder [20]. The application of *B bassiana*, potassium silicate, and imidacloprid resulted in 61.91% mortality of *C. medinalis* [21]. In contrast, in the current study on *B. bassiana* isolate, test mortality of 78.33% was observed at a spore concentration of 1 x 10^8 conidia/ml.

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 x 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 x 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 x 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 x 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 x 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 x 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 x10^4 conidia/ml	-	2.21x10^5	1.33x10^4
0.25	y = 3.1124x - 1.7979		154.92	176.92	135.66

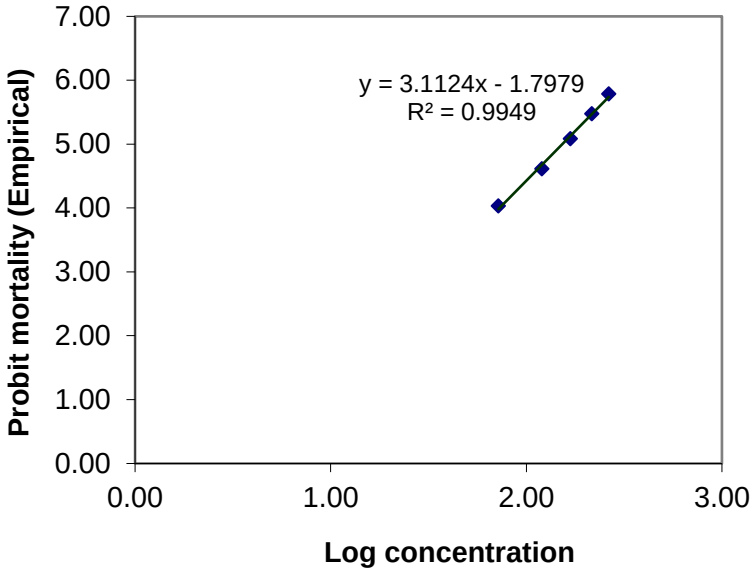

Fig. 1. Time mortality response

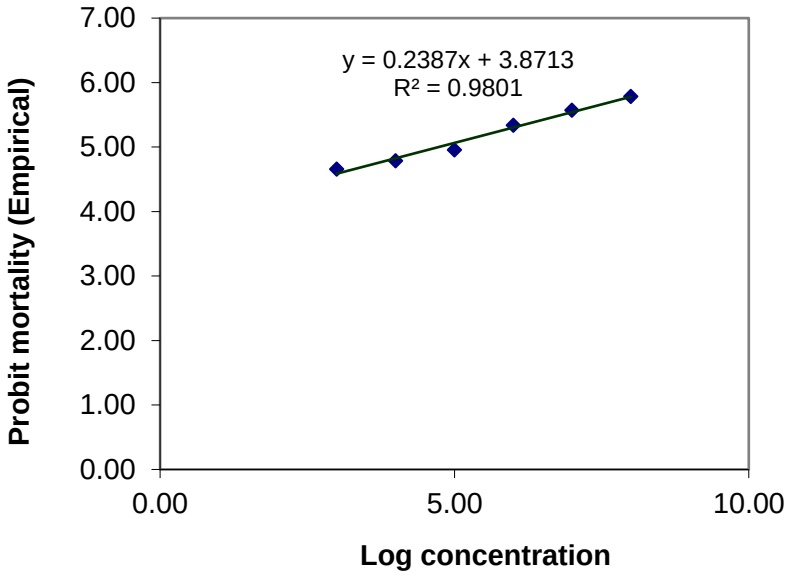

Fig. 2. Dose mortality response

The median lethal concentration of *B. bassiana* isolate MTCC7690 against *C. medinalis* was 9.09 x 10^4 conidia/ml [17,22]. Ambethgar *et al.* reported the LC50 of *B. bassiana* isolate BbCm KKL 1100 against *C. medinalis* was 2.8x10^3 conidia/ml. The median lethal concentration in the present investigation was 5.44 x 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.

ACKNOWLEDGEMENT

The authors thank the Department of Agricultural Entomology, TNAU, Coimbatore and the Department of Rice, TNAU, Coimbatore for providing research facilities.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Khan ZR, Barrion AT, Litsinger JA, Castilla NP, Joshi RC. A bibliography of rice leaf folders (Lepidoptera: Pyralidae). International Journal of Tropical Insect Science. 1988;9(2):129-74.
2. Shanmugam T.R., Sendhil, Thirumalvalavan V. Quantification and prioritization of constraints causing yield loss in rice (*Oryza sativa*) in India. Agricultura Tropica et Subtropica. 2006;39(3):194-201.
3. Kaushik C. Extent of damage by leaf folder, C. medinalis (Guenee) in paddy cultivars at Raiganj, Uttar Dinajpur, West Bengal. Current Biotica. 2010;4(3): 365-7.
4. Bhatti MN. Rice leaf folder (*Cnaphalocrosis medinalis*): A review. Pak. Entomol. 1995;17:126-31.
5. Srivastava A, Sherma K. Assesment of yield losses of rice caused by paddy leaf folder, C. medinalis Guenee. Agricultural Science Digest-A Research Journal. 2017; 37(1):72-4.
6. Khan MR, Ahmad M, Ahmad S. Some studies on biology, chemical control and varietal preference of rice leaf folder, C. medinalis. Pak. J. Agric. Sci. 1989;26: 253-63.
7. Alvi SM, Ali MA Chaudhar SA, Iqbal SH. Population trends and chemical control of rice leaf folder, C. medinalis on rice crop. Int. J. Agric. Biol. 2003;5(4):615-6
8. Nathan SS Kalaivani K, Murugan K, Chung PG. The toxicity and physiological effect of neem limonoids on *C. medinalis* (Guenée), the rice leaf folder. Pesticide Biochemistry and Physiology. 2005 Feb 1;81(2):113-22.
9. Nathan SS, Kalaivani , Murugan K, Chung PG. Efficacy of neem limonoids on C. medinalis (Guenée) (Lepidoptera: Pyralidae) the rice leaf folder. Crop Protection. 2005;24(8):760-3
10. Nathan SS, Kalaivani K, Sehoon K, Murugan K. The toxicity and behavioural effects of neem limonoids on C. medinalis (Guenée), the rice leaf folder. Chemosphere. 2006;62(8):1381-7
11. Parker BL Skinner M, Costa SD, Gouli S, Reid W, El Bouhssini M. Entomopathogenic fungi of Eurygaster integriceps Puton (Hemiptera: Scutelleridae): collection and characterization for development. Biological Control. 2003;27(3):260-72.
12. Nathan SS, Chung PG, Murugan K. Effect of botanical insecticides and bacterial toxins on the gut enzyme of the rice leaf folder C. medinalis. Phytoparasitica. 2004;32:433-43.
13. Nathan SS Chung PG, Murugan K. Effect of biopesticides applied separately or together on nutritional indices of the rice leaf folder C. medinalis. Phytoparasitica. 2005;33:187-95.
14. Parker BL Skinner M, Costa SD, Gouli S, Reid W, El Bouhssini M. Entomopathogenic fungi of Eurygaster integriceps Puton (Hemiptera: Scutelleridae): collection and characterization for development. Biological Control. 2003;27(3):260-72.
15. De Muro MA, Elliott S Moore D, Parker BL, Skinner M, William RE, El Bouhssini M. Molecular characterization of B. bassiana isolates obtained from overwintering sites

of Sunn Pests (Eurygaster and Aelia species). Mycological Research. 2005;109(3):294-306.

16. Sandhu SS, Unkles SE, Rajak RC, Kinghorn JR. Generation of benomyl-resistant *Beauveria bassiana* strains and their infectivity against *Helicoverpa armigera*. Biocontrol Science and Technology. 2001;1(2):245-50.
17. Kirubakaran SA, Sathish-Narayanan S, Revathi K, Chandrasekaran R, Senthil-Nathan S. Effect of oil-formulated *Metarhizium anisopliae* and *B. bassiana* against the rice leaffolder *C. medinalis* Guenée (Lepidoptera: Pyralidae). Archives of Phytopathology and Plant Protection. 2014;47(8):977-92.
18. Fujiyoshi N, Noda M, Akai H. Simple mass-rearing method of the grass leaf roller, *Cnaphalocrocis medinalis* Guenee, on young rice seedlings [*Oryza sativa*]. Japanese Journal of Applied Entomology and Zoology; 1980.
19. Bajya DR, Ranjith M. Field efficacy of B. bassiana against rice leaf folder and its safety to spiders and coccinellids. Indian Journal of Entomology. 2018;80(1):68-72.
20. Sivasundaram V, Rajendran L, Muthumeena, Suresh S, Raguchander T, Samiyappan R. Effect of talc-formulated entomopathogenic fungus B. against leaffolder (*Cnaphalocrosis medinalis*) in rice. World Journal of Microbiology and Biotechnology. 2008;24:1123-32.
21. Shakir HU, Saeed M, Anjum N, Farid A, Khan IA, Liaquat M, Badshah T. Combined effect of entomopathogenic fungus (*B. bassiana*, imidacloprid and potassium silicate against C. Medinalis Guenée (Lepidoptera: Pyralidae) in Rice Crop. J. Entomol. Zool. Stud. 2015;3:173-7.
22. Ambethgar V, Swamiappan M, Rabindra RJ, Rabindran R. Pathogenicity of certain indigenous isolates of entomopathogenic fungi against rice leaf folder, *C. medinalis* (Guenee). Journal of Biological Control. 2007;21(2):223-34.

© 2023 Sharmila et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/105215