Journal of Ecobiotechnology 2/2: 65-68, 2010
ISSN 2077-0464

Journal of Ecobiotechnology
http://journal-ecobiotechnology.com/

Efficacy of White Muscardine Fungus *Beauveria bassiana* (Balsamo) Vuillemin against Fruit borer *Earias vitella* (Fabricius) in Okra (*Abelmosculus esculentus*)

N. Manimekalai[1]*, N. Vijayakumar[1], K. Balaraman[2], M. Deiveekasundaram[3] and A. Ramamourthi[1]
[1]Perunthalaivar Kamaraj Krishi Vigyan Kendra, Puducherry, Tamilnadu, India
[2]Retd. Senior Grade Deputy Director, Vector Control Research Centre, Puducherry, Tamilnadu, India
[3]Department of Microbiology, Faculty of Agriculture, Annamalai University, Annamalai Nagar, Tamilnadu, India

**Corresponding Author, Email: breeze_rpgm@yahoo.co.in*

Keywords

Beauveria bassiana
Earias vitella
Entomopathogens
Bhendi
Fruit Borer

Abstract

The White Muscardine fungus *Beauveria bassiana* was tested against second, third and fourth instar larvae of Bhendi fruit borer *Earias vitella* with conidial suspension mass multiplied in Sabouraud's Dextrose Broth under *invitro* conditions. Of which the second instar larvae showed the maximum mortality with a LC, LD and LT 50 of 2.1 x 10^7, 4 g/lit of water and 72 h. The Bioassay of the third and fourth instar larvae of *Earias vitella* for their susceptibility to *Beauveria bassiana* showed that susceptibility decreased with increase in age of the larvae and required higher concentrations to kill the larvae which indicated that susceptibility to culture depends on the age of the larvae.

1. Introduction

Okra, a malvacean crop severely affected by Fruit Borer *Earias vitella* can be controlled by the entomopathogenic microbes like *Bacillus thuringiensis and Beauveria bassiana* (Mandal *et al.,* 2007, Singh Hem *et al.,* 2009**)**. The White Muscardine Fungus *Beauveria bassiana* is a promising and extensively researched biocontrol agent that can suppress a variety of economically important insect pests (Ramaraj Urs *et al*., 1965, Gowda and Ravi Prasad, 1992, Sandhu *et al.,* 2001). This Entomopathogenic fungi *Beauveria bassiana* is found to control many lepidopteran pests like *Cnaphalocrocis medinalis, Earias vitella, Spodoptera litura, Helicoverpa armigera, Nomuraea reliyi* and *Leucinodes orbonalis* (Devaprasad *et al.,* 1989*;* Ajaykumar Pandey and Kanaujia, 2005, Devaraj and Nandihalli,2003). The fungus infects the insect cuticle by producing enzymes like Chitinases, Proteases, Amylases and toxins like Beauvericin, Bassionolides and Osporein.

The conidiospores of the fungi germinate on the surface of the cuticle and the germinated hyphal tubes penetrate the insect's integument directly and produces hyphal bodies, which circulate in the Haemolymph and proliferates by budding (Clarkson *et al.,* 1998). The death of the insect results from a combination of factors: mechanical damage resulting from tissue invasion, depletion of nutrient resources and toxicosis.

In the present study, the fungus *Beauveria bassiana* was bioassayed against second, third and fourth instar larvae of *Earias vitella* with a view to find out LC 50, LD 50 and LT 50 and the susceptibility of the pest to the pathogenic fungal species *Beauveria bassiana.*

2. Materials and Methods

Larvae of Bhendi Fruit Borer *Earias vitella* (Fabricius) were collected from the Fruit Borer infested Okra fields. The culture of *Earias vitella* was raised from field collected larvae on the natural Bhendi fruits. The collected larvae were reared in plastic container (3.5cm length x 3.5 cm breadth) providing small fresh pieces of circular bhendi as natural diet and they were allowed to grow upto sixth instar and pupal emergence. Routine surface sterilization of eggs and rearing containers with 10 percent formaldehyde was carried out to prevent bacterial and fungal contamination. The pupae were then transferred to the petriplates (9 cm dia) for adult emergence. Number of larvae, pupae, adult, egg and Percent adult emergence were observed. Rearing was repeated thrice.

Isolate of *Beauveria bassiana* obtained from National Bureau of Agriculturally Important Insects, Hebbal, Bangalore (NBAII) was used in the study. The isolate was maintained by subculturing in Sabouraud's Dextrose Agar medium enriched with yeast. The conidia for the tests were harvested from 10 day old cultures and it was scrapped by washing with 100 ml of distilled water containing 0.02 percent Tween 80. Conidial suspensions of different concentrations ranging from 10^4 to 10^9 conidia ml^{-1} were standardized by assessing the number of conidia in the suspension using Neubauer Haemocytometer. The larvae were first

inoculated with the fungus and reisolated in pure form using Ascending conidial isolation. The isolation was carried out by discharging the conidia from the mycelium of the infected cadaver showing typical mycosis using sterile distilled water. After reisolation from the cadavers, the isolates were purified by subculturing on Sabouraud's Dextrose Agar enriched with yeast (SDAY).

To check the effectiveness and LC 50 of the virulent fungal *Beauveria bassiana* against Bhendi Fruit Borer *Earias vitella* under laboratory conditions, the fungal broth was taken at different concentrations ranging from 10^4 to 10^9 conidia / ml. Then 2 ml of the conidial suspension was sprayed over the surface of the six hour's pre-starved of 2nd, 3rd and 4th instar larvae using hand atomizer. Ten larvae was taken for each treatment. Three replications were maintained for each treatment. Control insects received a spray of only 0.02 per cent Tween 80 in sterile distilled water. After air drying the treated larvae were kept in individual plastic container (3.5 x 3.5 cm) containing fresh bhendi fruit piece and incubated at 27 $\pm$ 2 ^{0}c Symptoms and mortality of the larvae due to molecular mechanism of *Beauveria bassiana* were recorded for every 24hrs.

The virulent strain was mass multiplied in Molasses yeast extract broth and formulated using Talc as Carrier material. Thirty millilitre of *Beauveria bassiana* broth containing conidiospores was taken and mixed with 60 g of talc as carrier material, 2g clay material silica powder, 1g of fortifying agent calcified sorghum, 1g of fortifying agent cassamino acid, 2g of invasive agent crustaceous chitin, 1.5g of suspending agent Dodamol F, 1.5g of binding agent carboxy methyl cellulose, 1g of gluming agent guargum were added and the contents were manually mixed to obtain powder formulation. It was shade dried for 24 hours to bring down the moisture condition up to 8 % and sieved using the sieve to obtain fine powder of 38µ. The powdered mycoinsecticide was packaged in pre-sterilized polythene cover (20 cm L × 150cm B) and stored at 30ºC. Then the mycoinsecticide was checked for their spore load.

To check the effectiveness of the fungal biopesticide *Beauveria bassiana* against Fruit borer *Earias vitella* under laboratory conditions, the mycoinsecticide formulation was taken at different dosages like 1.0 g, 2.0 g and 4.0g / litre of sterile distilled water in a 2 litre conical flask separately. Then it was sprayed over the surface of the six hour's pre-starved 25 larvae of 2nd, 3rd and 4th instar stages of larvae and kept in the individual plastic container (3.5 x 3.5 cm) by using hand atomizer. Then, the larvae were allowed into each container containing fresh bhendi fruit piece and incubated at 27 $\pm$ 2 ^{0}c Symptoms and mortality of the larvae due to molecular mechanism of *Beauveria bassiana* were recorded for every 24h. until the larvae in the control grew into pupae Three replications were maintained for each treatment.

To assess the LT 50 of the fungal biopesticide *Beauveria bassiana* against Fruit borer *Earias vitella* under laboratory conditions, the highest concentration of about 4g / litre of fungal biopesticide was tested against the Fruit borer *Earias vitella*. The mortality rate of the larvae due to the mechanism of *Beauveria bassiana* were recorded for every 6 hr up to 168 h. and the cumulative mortality were subjected to Probit analysis (Finney 1964).

Table 1. Efficacy of White Muscardine Fungus *Beauveria bassiana* to various instars of Bhendi fruit borer *Earias vitella.*

Instar	Chi^2	Probit Analysis of Dosage mortality response		
		Regression equation	LC_{50} (Conidia ml^{-1}) x 10^6	Fiducial limits x 10^6
II	0.46	Y= 0.23456x +2.3425	2.17	0.8 – 31.2
III	0.68	Y = 0.3614x + 3.2458	32.6	2.4 – 92.6
IV	0.62	Y = 0.5218x + 4.1296	113.2	96.3 – 496.2

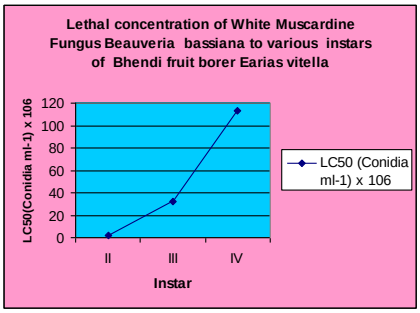


Table 2. Probit analysis of time – mortality responses of various instars of Bhendi fruit borer *Earias vitella* to White Muscardine Fungus *Beauveria bassiana*

Instar	Chi²	Probit Analysis of Time mortality response		
		Regression equation	LT^*_{50} (h)	Fiducial limits (h.)
II	0.56	Y = 0.2565x+ 3.6245	98.23	89.3 – 112.6
III	0.48	Y = 0.3625x+ 4.9564	113.11	100.32-120.35
IV	0.92	Y= 0.4216x+ 3.2512	120.32	116.32 – 128.36

* at 10^8 conidia / ml

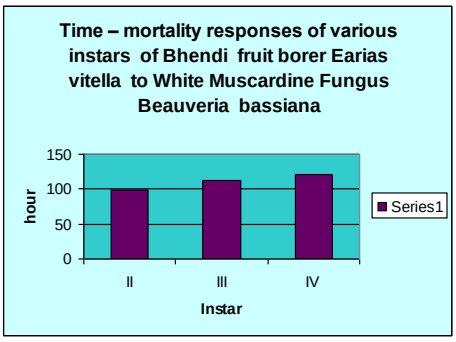
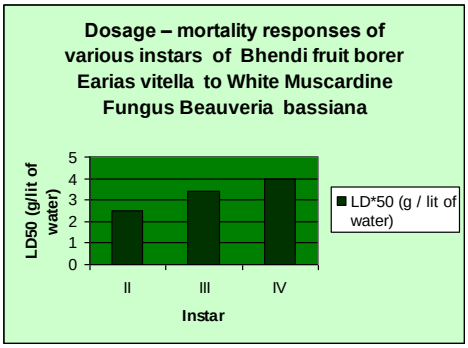


Table 3. Probit analysis of dosage – mortality responses of various instars of Bhendi fruit borer *Earias vitella* to White Muscardine Fungus *Beauveria bassiana*

Instar	Chi²	Probit Analysis of Dosage mortality response		
		Regression equation	LD^*_{50} (g / lit of water)	Fiducial limits
II	1.11	Y= 0.28265x+3.2861	2.5	1.8- 2.3
III	0.86	Y = 0.3561x+4.2315	3.4	3.0- 4.2
IV	0.23	Y = 0.2336x+3.2101	4.0	3.8- 4.9

*at 10^8 conidia / ml



3. Results and Discussion

The Bioassay of the insect based on probit analysis, and the chi – square test showed homogeneity of the test population. The comparison of LC_{50} against various instars indicated that the LC_{50} increased with increase in instar of the larvae i.e., Lowest LC_{50} of 2.17 x 10^6 for the II Instar larvae and 32.6 x 10^6 and 213.2 x 10^6 for the III and IV Instar larvae. Shallow dose-mortality responses seem to be typical for fungus – Insect interactions according to Hall (1954), Ignoffo *et al.,* 1982 and Rombach *et al.,*1986a. The dose mortality for the II Instar larvae was 2.5 g / lit of water when compared to III and IV Instar larvae which is 3.4 and 4.0 g / lit of larvae. Similarly, the time mortality was minimum with II instar larvae which is 98.23 h. where as the IV Instar larvae was controlled only after 120 h. This was in correlation with the study of Deva Prasad *et al.,* 1990 who studied on the susceptibility of *Heliothis* to certain entomogenous fungi.

Reference

Ajaykumar Pandey and R. Kanaujia, 2005. Effect of different grain media on sporulation, germination and virulence of *Beauveria bassiana* (Balsam0) Vuillemin against *Spodoptera litura* Fabricius larvae, *J. Biol. Control*, **19** (2); 129 -133.

Clarkson. J, S. Screen, B. Cobb and K. Chrnley. 1998. Fungal pathogenesis in insect. *In:* Molecular variability of Fungal Pathogens. (Eds.) P. Bridge, Y. Couteaudier and J. Clarkson. CAB International, U. K. P_p 191 - 199.

Deva Prasad. V, S . Jayaraj. and R. J. Rabindra, 1990.Susceptibitility of Gram Caterpillar, *Heliothis armigera* Hbn. (Noctuidae : Lepidoptera) to certain Entomogenous Fungi. *J. Biol. Control,* 4 (1), 44 – 47, 1990.

Devaraj, K and B. S. Nandihalli.2003. Bioefficacy of *Beauveria bassiana* (Balsamo) Vuillemin and Nomuraea rileyi (Farlow) Samson in the management of *Helicoverpa armigera* (Hubner) on Chickpea. Natl. Symp. on Biomanagement of Insect Pests, 29 to 31st March, 2003. Annamalainagar, Abstract: 83.Finney D. J. 1964. Probit Analysis. A statistical treatment of the sigmoid response curve, 2nd ed. Cambridge University Press, London. $318_{pp.}$

Finney D. J. 1964. Probit Analysis. A statistical treatment of the sigmoid response curve, 2nd ed. Cambridge University Press, London. 318pp.

Gowda Javare and Raviprasad, T. N.1992. Occurrence of *Beauveria bassiana* (Balsamo)Vuillemin on the red gram pod borer *Heliothis armigera* (Huebner) *J. Biol. Control,* **6** (1) : 48 – 49.

Hall, I. M, 1954. Studies on micro organism pathogenic to the sod webworm. *Parasitol.,* **84** : 205 – 240.

Ignoffo, C. M, Garcia, C, M. Kroha and. T. L. Couch. 1982. Use of larvae of *Trichoplusia ni* to bioassay conidia of *Beauveria bassiana. J. econ. Ent.,* 75: 275 – 276.

Mandal. S. K, S. B. Sah and S. C. Gupta, 2007. Management of Insect pests on Okra with biopesticides and chemicals, Annuals of Plant Protection Sciences, **15**, (1), ISSN : 0971-3573.

Ramaraj, Urs, M, V. Govindu. H. C and Shivansankara Sastry, K. S.1965. A preliminary study of White Muscardine fungus on cabbage semilopper from mysore, *Current Sciences,* **34:** 382 – 383.

Rombach, M. C, Aguda, R. M, Shepherd, B. M and Roberts, D. W. 1986a. Entomopathogenic fungi (Deuteromycotina) in the control of the black bug of rice, Scotinophara coarctata (Hemiptera : Pentatomidae) *J. Invertbr, Pathol.,* **48** : 174 – 179.

Sandhu, S. S., Unkles, S. E., Rajak, R. C. and Kingdhorn, J. R. 2001. Generation of Benomyl resistant *Beauveria bassiana* strains and their infectivity against *Helicoverpa armigera. Biocontrol Science and Technology,* 11 : 245 -250.

Singh Hem , Kumar Arvind, Yadav, R. N, Yadav, K. G, Yadav Abbhut, Yadav, J.L, Khilari, K, 2009. Efficacy and economics of Biopesticides for management of Fruit borers in Okra.Annuals of Plant Protection Sciences, Vol: 17(2), Print ISSN : 0971-3573.