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Research Note

Bioefficacy of white muscardine fungus, *Beauveria bassiana* (Balsamo) Vuillemin and entomopathogenic nematode, *Heterorhabditis indica* (Poinar) against rice blue beetle, *Leptispa pygmaea* Baly

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ABSTRACT: Laboratory studies were conducted during the year 2005-2006 to find out the efficacy of the white muscardine fungus, *Beauveria bassiana* (Balsamo) Vuillemin and the entomopathogenic nematode, *Heterorhabditis indica* (Poinar) against adult and grubs of rice blue beetle, *Leptispa pygmaea* Baly. *B. bassiana* caused a cumulative adult mortality of 56.67 – 80.00 per cent at 10^5 – 10^9 spores ml^{-1} and the LC_{50} value was 2.26 x 10^4 spores ml^{-1}. The entomopathogenic nematode, *H. indica* caused a cumulative mortality of 66.67 – 91.67 per cent at concentrations of 5IJs to 9IJs in the grubs of *L. pygmaea*. The cumulative LC_{50} value was 3.83IJs. The mortality of *L. pygmaea* was found to increase with reducing dose and increasing exposure period of both *B. bassiana and H. indica*. The effective pathogenicity of *B. bassiana* and *H. indica* against *L. pygmaea* is reported for the first time.

KEY WORDS: *Beauveria bassiana*, *Heterorhabditis indica*, rice blue beetle.

The utilization of entomopathogens as biocontrol agents for insect pests is gaining much importance recently. The scenario of insect pest complex in rice has undergone a tremendous change during the past few years. The rice blue beetle, *Leptispa pygmaea* Baly, hitherto reported as a minor pest (Trehan, 1946) has recently assumed a serious status as an emerging problem by causing much concern to the rice cultivation in the northern districts of Kerala, India. The beetle attacks the early tillering stage of rice crop and causes severe damage during *Kharif* and *Rabi* seasons. It is difficult to manage the pest by spraying insecticides especially during the first crop season owing to the incidence of continuous heavy showers. The utilization of pathogenic microorganisms holds a high possibility for the suppression of rice insect pests (Chatterjee *et al.* 1983). Rice provides a favourable environment for the exploitation of entomopathogenic fungi (EPF) as mycoinsecticides. The white muscardine fungus, *Beauveria bassiana* (Bals.) Vuill. occurs naturally and has been utilized for the control of many insect pests of rice. Beetle pests have been reported to be more vulnerable to infection by fungal pathogens (Carruthers and Soper, 1987). Entomopathogenic nematode (EPN) as a biopesticide has received worldwide attention as a safer alternative for the use of insecticides in different crops including rice. The present preliminary study was, therefore, undertaken to assess the efficacy of the *B. bassiana* and the EPN, *Heterorhabditis indica* Poinar and explore the feasibility of their utilization for the management of *L. pygmaea.*

A laboratory study was carried out to study the pathogencity and effectiveness of *B. bassiana* against *L. pygmaea* during the year 2005-2006. *B. bassiana* was grown in a culture medium consisting of sterile 200gm boiled sorghum grains mixed with 12.5gm lime. The medium was sterilized in a polypropylene bag in an autoclave for 30 minutes. The pathogencity of *B. bassiana* on *L. pygmaea* was tested initially. Different treatment concentrations of *B. bassiana* spores were prepared with 1% teepol (spreading agent) in distilled water for the experiment. The spore counting was done using a haemocytometer. The experiment consisted of six treatments, *viz.,* 10^5, 10^6, 10^7, 10^8 and 10^9 spores ml^{-1} along with an untreated control (water and teepol only). Each treatment was replicated five times. Fifteen days old rice seedlings (variety Jyothi) were dipped in the different treatments of fungal spore suspension and air dried for 15 minutes and then kept in petri dishes. Three treated seedlings were kept in each replication. Ten days old adult rice blue beetles @ 5 / treatment were released on the treated seedlings. Observations on per cent mortality of beetles were recorded at 24, 48 and 72 hours after treatment.

Another experiment was carried out in the laboratory to test the effectiveness of the EPN, *H. indica* at different concentrations against the grub of *L. pygmaea*. The EPN was reared on the larvae of wax moth, *Galleria melonella* in the laboratory and freshly emerged infective juveniles were used for the study. The counts of nematodes

Table 1. Efficacy of *B. bassiana* against adult beetles of *L. pygmaea*

Treatments	Mortality (per cent)			
	Hours after treatment			
	24	48	72	Cumulative mortality
B. bassiana @ 10^5 spores ml^{-1}	45	55 (52.63)	70 (66.67)	56.67 (54.39)
B. bassiana @ 10^6 spores ml^{-1}	45	65 (63.16)	80 (77.78)	63.33 (61.40)
B. bassiana @ 10^7 spores ml^{-1}	50	60 (57.90)	90 (88.89)	66.67 (64.92)
B. bassiana @ 10^8 spores ml^{-1}	50	70 (68.42)	90 (88.89)	70.00 (68.42)
B. bassiana @ 10^9 spores ml^{-1}	60	80 (78.98)	100 (100)	80.00 (78.95)
Control	0	5	10	5.00
LC_{50}	9.97 x 10^6	4.44 x 10^4	8.84 x 10^3	2.26 x 10^4

* Figures in parentheses in right side of original value are corrected mortality by Abbott's formula

(infective juveniles) were made using a haemocytometer. After confirmation of the initial pathogencity, different concentrations, *viz.,* 5, 6, 7, 8 and 9 infective juveniles (IJs) per ml were prepared with teepol as a spreading agent. Five replications were maintained for each treatment. Different concentrations of IJs were sprayed on 15 days old rice (variety Jyothi) seedlings kept in petri dishes. An untreated check sprayed with 1% teepol alone was also maintained. Five grubs (third instar) of *L. pygmaea* were released in each treatment. Mortality of grubs was recorded at 24, 48 and 72 hours after treatment.

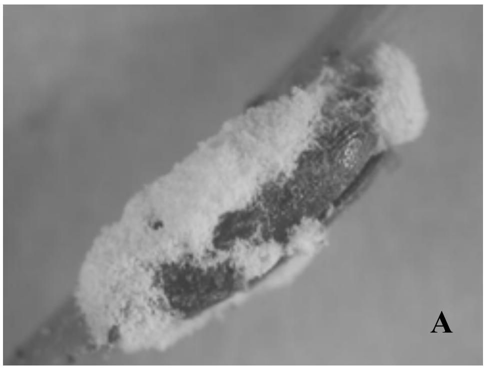
Both experiments were conducted under laboratory conditions of maximum temperature (30.1°C ±1.40°C), minimum temperature (23.1°C ± 0.69°C) and relative humidity (94.33% ± 2.11%). Mortality data were corrected by Abbott's formula and LC_{50} values were worked out by probit analysis (Finney, 1977).

Bioassay studies with *B. bassiana* on blue beetle indicated that the mortality of adults was dependent on the fungal spore concentration. The spore concentrations ranging from 10^7 – 10^9 spores ml^{-1} resulted in 50 – 60 per cent mortality at 24 hours after treatment (Table 1). The body of infected dead rice blue beetle was found to be covered with white mycelial growth (Plate 1A). The concentration of 10^8 – 10^9 spores ml^{-1} caused 70 to 80 per cent mortality at 48 hours after treatment while the mortality was increased by 90 to 100 per cent at 72 hours after treatment in the lower concentration of 10^7 –10^9 spores ml^{-1}. The highest concentration of 10^9 spores ml^{-1} caused a maximum of 60 per cent mortality in adult beetles at 24 hrs after treatment. But the same mortality was produced with a lower concentration of 10^7 spores ml^{-1} at a longer period of 72 hours after treatment. The lowest concentration of 10^5 spores ml^{-1} caused a maximum of 70 per cent mortality of beetles at 72 hours after treatment. It is thus indicated that *B. bassiana* was more effective in the concentration of 10^7 – 10^9 spores ml^{-1} against *L. pygmaea*. The cumulative mortality of rice blue beetle ranged from 56.67 – 80.00 per cent at 10^5 – 10^9 spores ml^{-1} of *B. bassiana.* The LC_{50} values *B. bassiana* were found to be 9.97 x 10^6, 4.44 x 10^4, 8.84 x 10^3 spores ml^{-1} at 24, 48 and 72 hours respectively. The LC_{50} value for cumulative mortality was 2.26 x 10^4 spores ml^{-1}. The present finding showed that the lower dosages with a longer period of exposure with *B. bassiana* cause a higher mortality in rice blue beetle and corroborates the report on rice hispa, *Dicladispa armigera* (Agarwal,1990). The bioefficacy of *B. bassiana* against *L. pygmaea* is reported for the first time and it has to be further tested in the field. The field efficacy of *B. bassiana*

Table 2. Evaluation of *H. indica* against grubs of *L. pygmaea*

Treatments^{-ml}	Mortality (%)			
	Hours after treatment			
	24	48	72	Cumulative mortality
H. indica @ 5IJs	40	65 (63.16)	90 (88.89)	66.67 (64.92)
H. indica @ 6IJs	65	80 (78.95)	95 (94.44)	80.00 (78.95)
H. indica @ 7IJs	65	85 (84.21)	100 (100)	83.33 (82.45)
H. indica @ 8IJs	80	85 (84.21)	100 (100)	88.33 (87.72)
H. indica @ 9IJs	85	90 (89.47)	100 (100)	91.67 (91.23)
Control	0	5	10	5.00
LC_{50}	5.45IJs	3.74IJs	3.72IJs	3.83IJs

* IJs: Infective juveniles; figures in parentheses in right side of original value are corrected mortality by Abbott's formula



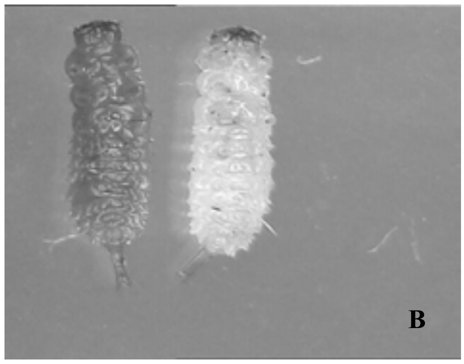


Plate 1. Dead beetle covered by white mycelial growth of *Beauveria bassiana* (A) and *Heterorhabditis indica* affected and healthy grub (B)

against *D. armigera* was reported earlier by Hazarika and Puzari (1995, 1997).

The infection of EPN, *H. indica* on the grub of rice blue beetle resulted in its change of colour to pink (Plate 1B). All the concentrations of 5IJs, 6IJs, 7IJs, 8IJs and 9IJs ml^{-1} of *H. indica* tested in the laboratory produced 40 to 85 per cent mortality at 24 hours after treatment with grubs of *L. pygmaea* (Table 2). The highest concentration of 9IJs of *H. indica* per ml was superior to other concentrations by causing 90 per cent mortality after 48 hours of treatment. After 72 hours of treatment, all the three higher concentrations of 7IJs, 8IJs and 9IJs ml^{-1} produced 100 per cent mortality of blue beetle grubs. The lower concentrations of 5IJs and 6IJs caused 90 – 95 per cent mortality after 72 hours of treatment. The LC_{50} values at 24, 48 and 72 hours after treatment were 5.45, 3.74 and 3.72IJs, respectively. The various tested concentrations caused cumulative mortalities of 66.67 – 91.67 per cent with a cumulative LC_{50} value of 3.83IJs. The present finding on the efficacy of *H. indica* against *L. pygmaea* is reported for the first time. The mortality was found to increase with reducing dose and increasing exposure period thus indicating an inverse proportion for the dose and period of exposure. Earlier, rice blue beetle was reported to be parasitized by *Hexamermis* (Patel and Shah, 1988), while Prasad (2006) reported that *H. indica* caused 100 per cent mortality of rice leaf folder, *C. medinalis* within 18 to 20 hours of exposure.

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