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Studies on the Dose-Mortality Relationship Between the Entomofungal Pathogen *Beauveria bassiana* (Bals.) Vuillemin and *Heliothis armigera* Hubner (Lepidoptera : Noctuidae)

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ABSTRACT

***Beauveria bassiana* (Bals.) Vuillemin was pathogenic to all the stages of *Heliothis armigera* Hubner inflicting 60-100 and 100% mortality respectively, to the larval instars I-V and the eggs at 1.0 x 10^7 conidia/ml. Pupal mortality was 60% at 1.0 x 10^9 conidia/ml and 80% mortality was observed in the pre-pupae and the adults at 1.0 x 10^{10} conidia/ml with an incubation period ranging from 2-14 days.**

Key Words : *Beauveria bassiana, Heliothis armigera,* dose-mortality relationship

Though the white muscardine fungus *Beauveria bassiana* (Bals.) has been reported from India on several pests like the cabbage semilooper (Ramaraj Urs *et al.*, 1965) rice pests (Rao, 1975; Srivastava and Nayak, 1978), banana leaf beetle (Roy and Pujari, 1979), gram pod borer (Agarwal and Rajak, 1985) and brinjal ash weevil (Gopalakrishnan and Narayanan, 1988), no attempt has been made so far to study the host-pathogen relationship of the fungus against its host insects. Hence, an attempt was made to study the dose-mortality relationship of the fungus against the gram pod borer *Heliothis armigera* Hbn, in the laboratory.

MATERIALS AND METHODS

Preparation of conidial suspension

The conidial suspension used in this study was obtained from *B. bassiana* cultures derived originally from diseased *H. armigera* larvae collected from tomato at the Indian Institute of Horticultural Research experimental station at Hessaraghatta, Bangalore. The conidia were inoculated on sorghum grains which were previously soaked in water for 24 h and autoclaved at 15 1b/cm^2 pressure for half-an-hour. The fungus was cultured on sorghum grains on which it sporulated profusely on the eighth day of inoculation. The fungus culture was maintained on sorghum grains at 27° ± 2°C. The conidia were harvested using sterile distilled water containing 0.01% Triton x-100 and passed through a double layered muslin cloth. Serial dilutions were made to obtain four different concentrations, *Viz.*, 10^{10}, 10^9, 10^8 and 10^7 conidia/ml. The counting of conidia was done with the help of an improved Neubauer double ruled haemocytometer and a phase contrast microscope at a magnification of 600x. Conidia harvested within 24 h were used for the experiment.

Source of host insect

The different stages of *H. armigera* were obtained from the laboratory culture reared on a semisynthetic diet (Nagarkatti and Sathyaprakash, 1974). The larval instars were selected immediately after moulting, since the newly formed integument is highly susceptible

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for conidial germination and infection (Fargues and Vey, 1974).

Method of inoculation

About twenty larvae, pre-pupae, pupae and adults were inoculated with different doses of the fungus. About 100 freshly laid fertile eggs were treated per dose. Each dose was replicated three times. Diet surface treatment (Garcia and Ignoffo, 1978) and insect body surface contamination technique were adopted for inoculation.

The semi synthetic diet for *H. armigera* was prepared, excluding formalin, in plastic containers (about one third) of size 4.0 x 3.5 cm. The containers were provided with brass wire mesh lids to provide aeration. About 0.1 ml conidial suspension was added to the diet containers and the surface was well contaminated with the help of glass rod. The larvae were just dipped in conidial suspension and placed on the contaminated diet. This was done to ensure contact of the conidia with the larval body, as this fungus grows only when it comes in contact with the integument. The larvae were transferred to fresh diet 48 h after inoculation, since the entomogenous fungi, generally, penetrate the integument within the first 24 h of inoculation (yen, 1962; Yendol and Paschke, 1965). As the larvae were cannibalistic, they were kept individually in plastic containers.

The pre-pupae and pupae were dipped in the conidial suspension and placed on moist filter paper in Petri dishes (100 x 17 cm), five in each Petri dish. Adults were allowed to swim in conidial suspension for a few seconds and then placed on a moist filter paper in sterile plastic containers of size 12.5 x 10.0 cm at the rate of five adults per container. Cotton swabs dipped in 50% honey was placed inside. In case of eggs, the cloth bits containing 100 eggs each were directly dipped in the conidial suspensions, shade dried and placed in plastic containers (12.5 x 10.0 cm) containing moist filter paper. Sterile distilled water with 0.01% Tritonx-100 served as control in all the cases. The whole experiment was carried out under laboratory condition at 27° ± 2°C and observations recorded daily.

RESULTS AND DISCUSSION

The fungus at 10^8 conidia/ml caused 60-100% mortality to the larval instars I-V (Table 1). The mean mortality of 82% obtained at this concentration was on par with the highest concentration of 10^{10} conidia/ml tested (88%). Younger instars I-III, were more susceptible to the fungus (Table 1).

Generally, the infected caterpillars became lethargic and died on the sixth day. The body became hard and mummified and on the ninth day, white mycelial growth was noticed all over the body. About 30% of the fifth instar larvae treated with the fungus did not die at larval stage but died at pupal stage fourteen days after treatment. The dead pupae became tough and on the fifteenth day white mycelial growth was noticed through all the six spiracles on either side. The larvae that escaped infection pupated normally and the adults that emerged from such pupae were normal.

Mortality of pre-pupae and pupae varied from 20 to 80% (Table 2). Among the two pre-pupae were found to be more susceptible to the fungus, recording 80% mortality when compared to 60% mortality recorded in the case of pupae. Pupal death was noticed within 48h, whereas in the case of pre-pupae, the incubation period ranged from 2-14 days. The pre-pupae (just formed) treated with conidial suspension showed infection only at pupal stage. The pre-pupae which escaped death pupated normally and the adult emergence in such pupae was also normal.

The pupae treated with the fungus died on the second day of treatment. Mycelial growth through spiracles occurred on the fifteenth day; later, the growth was prominent on the lateral segments of the wing pad. All the diseased pupae showed fungal ramifications through spiracles and the lateral segments in the region of wing pad. Thus, pupal death within 48 h after

Table 1. Effect of *Beauveria bassiana* on different instars of *Heliothis armigera*

Treatments	% Mortality					Mean
	I instar	II instar	III instar	IV instar	V instar	
1.0 x 10^{10}	100.0 (89.9)	90.0 (74.9)	80.0 (63.5)	80.0 (63.4)	90.0 (71.9)	88.0 (72.7)*
1.0 x 10^{9}	100.0 (89.9)	90.0 (71.9)	90.0 (71.9)	70.0 (56.7)	70.0 (56.9)	84.0 (69.4)
1.0 x 10^{8}	100.0 (89.9)	90.0 (74.9)	90.0 (74.9)	70.0 (56.8)	60.0 (50.7)	82.0 (69.4)
1.0 x 10^{7}	95.0 (77.0)	80.0 (63.5)	100.0 (89.9)	70.0 (56.9)	60.0 (50.7)	81.0 (67.6)
Control	00.0 (00.0)	00.0 (00.0)	00.0 (00.0)	00.0 (00.0)	00.0 (00.0)	00.0 (00.0)
Mean	79.0 (69.3)	70.0 (57.0)	72.0 (60.0)	58.0 (46.7)	56.0 (46.0)	

C.D. (P = 0.05)

Treatment	4.09
Instar	4.09
Interaction	9.15

* Figures in parenthesis are transformed values

inoculation may be due to the physiological changes brought by the fungal metabolites during the course of metamorphosis.

Table 2. Effect of *Beauveria bassiana* on pre-pupa and pupa of *Heliothis armigera*

Treatments (Condia/ml)	% mortality [a]	
	Pre-pupa	Pupa
1.0 x 10^{10}	80 (2-5)	60 (2)*
1.0 x 10^{9}	40 (2-12)	60 (2)
1.0 x 10^{8}	40 (2-14)	40 (2)
1.0 x 10^{7}	20 (2)	40 (2)
Control	-	-

Note : *Figures in parentheses are incubation period in days
a = Mean of three replicates

Adults treated with higher concentrations of 1.0 x 10^{10} and 1.0 x 10^{9} conidia/ml showed 80 and 50% mortality respectively. Whereas, at lower concentrations none of the adults tested showed any mortality. The infected adults became less active and died on the sixth day of treatment. The body became tough and mummified and on the ninth day white mycelial growth was noticed on the abdomen, antennae and on appendages. Similar results obtained by Bajan *et al.* (1976, 1977) Muller-Kogler and Stein (1970) Keller (1978) Lappa and Goral (1980) as quoted by Roberts and Humber (1980) support our findings.

The eggs treated with all the four concentrations failed to hatch. Complete shrinking and browning of eggs were noticed 24 h after treatment. However, none of the diseased eggs developed mycelial growth. The observation made by Caroll (1988) that *B. bassiana* was pathogenic to eggs supports our observations. Thus, it is clear from the result (Table 1 and 2)

that there is an increase in percentage mortality due to fungal infection when there is an increase in conidial concentration. These results indicate the possibility of using *B. bassiana* in the management of *H. armigera*.

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