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Susceptibility of Gram Caterpillar, *Heliothis armigera* Hbn. (Noctuidae : Lepidoptera) to Certain Entomogenous Fungi

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ABSTRACT

Five entomogenous fungi were bioassayed for their infectivity to second instar larvae of *Heliothis armigera* Hbn. by spraying them with the conidial suspension in the laboratory. Of them, *Beauveria bassiana* (Bals.) Vuill. (Bapatla isolate) was found to be the most virulent recording the lowest LC_{50} of 2.17×10^5 conidia ml^{-1}. The LC_{50} values of the Bangalore and New Delhi isolates of *B. bassiana*, *Paecilomyces fumosoroseus* (Wize) Brown and Smith and *P. farinosus* (Holm ex Gray) Brown and Smith ranged from 4.19×10^8 to 5.22×10^9 conidia ml^{-1}. Bioassay of second, third and fourth instar larvae of *H. armigera* for their susceptibility to Bapatla isolate of *B. bassiana* showed that susceptibility decreased with age of the larvae in terms of both LC_{50} and LT_{50}.

Key Words : *Beauveria bassiana*, *Paecilomyces farinosus*, *P fumosoroseus*, *Heliothis armigera*, susceptibility

Species of *Heliothis* are known to be susceptible to almost all the groups of entomopathogens. Considerable studies have been made in India only with the nuclear polyhedrosis viruses (NPV) which effectively controlled *Heliothis armigera* Hbn. on chickpea, pigeonpea, lablab, sunflower and tomato (Jayaraj, 1986). Recently Gopalakrishnan and Narayanan (1988) reported natural occurrence of *Metarhizium anisopliae* (Metschn.) Sorok. and *Nomuraea rileyi* (Farlow) Samson on *H. armigera* larvae on tomato and field beans in Karnataka, India. Despite several reports on seasonal outbreaks of fungal diseases on this polyphagous pest in India and other countries (Urs and Govindu, 1971; Alma, 1975), no detailed studies have been made so far to find out the scope of utilizing them in IPM strategies.

In the present investigation, three isolates of *Beauveria bassiana* (Bals.) Vuill., one isolate each of *Paecilomyces fumosoroseus* (Wize) Brown and Smith and *P. farinosus* (Holm ex Gray) Brown and Smith were bioassayed against second instar larvae of *H.armigera* with a view to quantify entomopathogenic fungal spore load to achieve 50 per cent mortality of the host larvae for a clear understanding of the relative susceptibility of the pest to the pathogenic fungal species/ isolates. Susceptibility of three larval instars of *H. armigera* to a highly virulent strain of *B. bassiana* was also studied.

MATERIALS AND METHODS

The culture of *H. armigera* was raised from field-collected larvae on a modified semisynthetic diet (Shorey and Hale, 1965). The larvae were reared in individual glass vials and routine surface sterilization of eggs and rearing containers with 10 per cent formaldehyde was carried out to prevent viral and fungal contaminations of the healthy stock. Neonate larvae were transferred to potted plants of chickpea (*Cicer arietinum* L.) and reared through the first instar.

Isolates of white muscardine fungus, *B. bassiana* were obtained from Indian Agricultural Research Institute, New Delhi (NDL), Indian Institute of Horicultural Research, Bangalore (BNG) and Agricultural Research Station, Bapatla (BPT). Isolates of

Table 1. Susceptibility of second instar larvae* of *H. armigera* to the fungal isolates

Fungus	Chi^2	Probit analysis of dosage-mortality response		
		Regression equation	LC_{50} (Conidia ml^{-1}) x 10^5	Fiducial limits x 10^5
B. bassiana (NDL)	0.42	Y = 0.28246X + 2.54274	5007.01	115.25 - 21753.46
B. bassiana (BNG)	0.28	Y = 0.25389X + 2.81100	4187.71	76.17 - 23022.58
B. bassiana (BPT)	0.84	Y = 0.33275X + 3.22442	2.17	0.45 - 0.40
P. fumosoroseus	0.16	Y = 0.30665X + 2.29370	6691.25	172.70 - 25924.91
P. farinosus	0.04	Y = 0.26306X + 2.44363	52207.79	192.84 - 141339.38

* @180/assay

P.fumosoroseus and *P. farinosus* were obtained from Kerala Forest Research Institute, Peechi and Indian Agricultural Research Institute, New Delhi respectively. Larvae were first inoculated with the different isolates and the fungi reisolated in pure form from the diseased cadavers showing typical mycosis. After reisolation from the cadavers, the isolates were purified by sub-culturing on Sabouraud dextrose agar enriched with yeast (SDAY) for *B. bassiana* and potato dextrose agar (PDA) for *Paecilomyces* spp. All the fungal isolates were maintained at 25°C.

The conidia for the bioassay were harvested from 10-day-old cultures just before use by washing from the surface of the plates using 75-100 ml of sterile distilled water containing 0.02 per cent Tween 80. The viability of the conidia was determined just prior to application as suggested by Gillespie (1986). Conidial suspensions of different concentrations ranging from 10^4 through 10^9 conidia ml^{-1} were standardised for each isolate after assessing the number of conidia in the suspension with an improved Neubauer haemocytometer. Newly moulted second instar larvae of *H. armigera* were bioassayed for their susceptibility to the different fungal isolates. Ten larvae taken in a Petri plate lined by a filter paper were directly sprayed with 2 ml conidial suspension using a hand atomizer. Three such replicates were maintained for each concentration. Control insects received a spray of only 0.02 per cent Tween 80 in sterile distilled water. After air-drying, the treated larvae were carefully transferred to individual glass vials containing freshly prepared semisynthetic diet (without formalin) and incubated at 25 ± 2°C. During the incubation period, the relative humidity was maintained at more than 95 per cent by placing the vials in plastic trays containing moist absorbent cotton and covering with a glass plate. The larval mortality was recorded at 6h intervals until eighth day of treatment. From the eighth day data, percentage larval mortality due to observable mycosis was calculated.

In the subsequent study, the highly virulent isolate of *B. bassiana* originally obtained from the Agricultural Research Station, Bapatla was used to study the relative susceptibility of second, third and fourth instar larvae to the fungal pathogen following the bioassay procedure already described.

RESULTS AND DISCUSSION

Among the various estimates of the regression based on probit analysis, the chi-square test in all the bioassays showed homogeniety of the test population which is a reflection of a good fit of the observed and expected responses (Table 1). The slopes (regression coefficients) in general, were very low in all the bioassays and were found to be more or less the same indicating that the dose-dependent responses were not pronounced. The comparison of LC_{50} indicated that BPT isolate of *B. bassiana* was the most

Table 2. Probit analysis of dosage-mortality responses of *H. armigera* to *B. Bassiana*

Host	Instar*	Chi^2	Probit analysis of dosage-mortality response		
			Regression equation	LC_{50} (Conidia ml^{-1}) x 10^5	Fiducial limits (95%) x 10^5
H. armigera	II	1.11	Y = 0.33587X + 3.25073	1.61	0.32 - 8.08
	III	0.62	Y = 0.29214X + 3.30286	6.45	1.25 - 33.23
	IV	0.09	Y = 0.33584X + 2.96180	11.72	2.78 - 49.36

* @180/assay

virulent with the lowest LC_{50} value. There was a sharp increase in the LC_{50} value in other isolates among which the order of virulence was BNG followed by NDL isolates of *B.bassiana*, *P. fumosoroseus* and *P. farinosus*.

The results of the bioassay using BPT isolate of *B. bassiana* against second, third and fourth instar larvae of the test insect showed that the susceptibility to infection decreased with the age of the larvae. The LC_{50} of the fungus was lowest in the second instar larvae which increased as the stage of the larvae advanced (Table 2). The LT_{50} (at 10^7 conidia ml^{-1}) also increased with the age of the larvae indicating that older larvae were relatively more tolerant to the infection (Table 3).

Shallow dose-mortality responses seem to be typical for fungus- insect interactions according to Hall (1980), Ignoffo *et al.* (1982) and Rombach and Gillespie (1988). Earlier, Maniania and Fargues (1984) observed wide variations in susceptibility of some noctuids to isolates of different entomogenous hyphomycetes. The differential susceptibility of the test larvae to the fungal isloates used in the present study may be due to the inherent variations in the susceptibility of the host insect to a particular fungal pathogen. The biochemical interactions in the infection process which may be specific to a host-pathogen interaction might have contributed to the differential susceptibility. Variations in susceptibility of certain noctuid larvae including *Heliothis* spp. to different geographical isolates of *Nomuraea rileyi* (Farlow) Samson have been observed by Ignoffo *et al.* (1976) and Ignoffo and Garcia (1985).

In another study using BPT isolate of *B. bassiana*, it was observed that susceptibility to infection decreased as the larvae aged. Earlier, in the case of larval instars of certain noctuids, a similar phenomenon was observed in their responses to *B. bassiana*, *N. rileyi* and *P. fumosoroseus* (Gardner and Noblet, 1978; Ignoffo *et al.*, 1978; Fargues and Rodriguez-Rueda, 1980). Host-pathogen interactions occur not only at the host integument but also in the haemocoel where many intrinsic factors operate. Incidentally, chemical constituents

Table 3. Probit analysis of time-mortality responses of different larval instars of *H. armigera* to *B.bassiana*

Host	Instar*	$Chi^2_{(5)}$	Regression equation	LC^*_{50} (h)	Fiducial limits (95%)
H. armigera	II	0.35	Y = 5.28410X - 5.59466	101.16	92.18-111.01
	III	0.97	Y = 4.68838X - 4.59167	111.13	100.14-123.33
	IV	0.91	Y = 7.67575X -11.18362	128.35	116.73-141.14

*at 10^7 conidia ml^{-1}

vary as the larvae advance in age resulting in progressive hardening of the cuticle and increased humoral defence mechanisms to the microbial infections (Boman, 1981). Higher susceptibility of younger instars to the fungal infection as observed in the present study is advantageous, as pests controlled in the early stages are less likely to cause economic injury to the crop plants.

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