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BIOACTIVITY OF *BEAUVERIA BASSIANA* AGAINST *HELICOVERPA* (=*HELIOTHIS*) *ARMIGERA* : EFFECT OF INSTAR, DOSAGE AND TEMPERATURE

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Beauveria bassiana **when tested against larvae of *Helicoverpa* (=*Heliothis*) *armigera* caused maximum mortality ca. 100 per cent in I and II instars at 1x10^5 conidia/ml with LT 50 as 72 hr. Mortality dose response showed LT 50 as 216 hr at 1.2 x 10^4 conidia/ml, decreasing to 98.8 hr when concentration was increased to 1.2 x 10^5 conidia/ml. LD 50 value was calculated to be 96358.94 conidia/ml. The pathogen was infective between 20°-30°C temperature, optimum being 25°C.**

Key Words : ***Beauveria bassiana, Helicoverpa armigera,*** **microbial control, bioactivity; instar, dosage, temperature.**

Helicoverpa (=*Heliothis*) *armigera* (Lepidoptera : Noctuidae) is a cosmopolitan and polyphagous pest recorded as damaging 60 cultivated plant species and atleast 67 other plant species in 37 families (Reed & Pawar, 1981). In India only the nuclear polyhedrosis virus (NPV) has been used for control of *Heliothis armigera* on chickpea, lablab, sunflower and tomato (Jayaraj, 1986). Recently Gopalakrishna and Narayanan (1988) reported the natural occurrence of *Metarhizium anisopliae* and *Nomuraea rileyi* on *H. armigera* larvae infesting tomato and field beans in India. Despite several reports on seasonal outbreaks of fungal diseases of this pest (Urs & Govindu, 1971; Agrawal & Rajak, 1985; Alma, 1975), no detailed studies have been made so far to find out the scope of utilizing fungi in the management of chickpea borer. Herein, we report the effect of different larval instars, dose levels and temperature on the susceptibility of *H. armigera* to *Beauveria bassiana.*

MATERIALS AND METHODS

Larvae : The culture of *H. armigera* was raised from moths collected from field with the help of light trap. Rearingof larvae was done individually in plastic cups on chickpea leaves disinfected for 10 min with 0.5% sodium hypochlorite (Ignoffo *et al.*, 1975). Routine surface sterilization of eggs with 0.5% sodium hypochlorite and plastic cups with 10% formaldehyde was followed to prevent viral and fungal contamination of the healthy culture. Neonate larvae emerging from eggs were transferred to plastic cups and reared on natural diet through the first instar.

Pathoghen : First subculture of *B. bassiana* was grown on Sabouraud's maltose yeast extract medium (Peptone, 10.0 g; Maltose, 40.0 g; Yeast extract, 10.0 g; Agar, 20.0 g; Glass distilled water, 1.0 lat 28° ± 1°C. The conidia for bioassay were harvested from 10 day old cultures by washing from the surface using 10 ml of sterile distilled water containing 0.5 per cent Tween -20. The spore suspension was taken in 45 ml glass bottles containing 36 glass beads (3 mm), stoppered and agitated on a mechanical shaker for 5 min to get homogenous suspension. The viability of the conidia was determined prior to application as suggested by Gillespie (1986). Different concentrations of conidia were prepared after assessing the conidial count with haemocytometer.

Bioassay : Instar susceptibility was evaluated by directly spraying 10 ml of conidial suspension (1 x 10^5 conidia/ml) on to larvae of first to six instars of *H.*

Table 1: Susceptibility of different larval instars of *Helicoverpa armigera* to *Beauveria bassiana.*

Temperature - 28° ± 1 °C
Relative humidity - 95% ± 5%
Dose - ca 1 x 10^5 conidia/ml

Larval Instar	Mortality AM ± SD[a]	Mean % mortality in control	LT 50 (in hours)	Correct Percent mortality[b]
I	100 ± 0.0	09	72	100
II	100 ± 0.0	02	72	100
III	88 ± 6.5192	00	96	88
IV	63 ± 5.244	00	120	63
V	52 ± 5.7879	00	120	52
VI	41 ± 4.3011	00	-	41

a - Arithmatic Mean ± Standard deviation.
b - Calculated as per Abott's Formula.

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Table 2: Dosage mortality response of third instar larvae of *H. armigera* to *B. bassiana*.

No.	Inoculum concentration (Conidia/ml)	Corrected Percentage Mortality[a]	Emperical probit	LT 50 (in hours)
1.	1.2 x 10^4	53.3	5.0753	216
2.	2.4 x 10^4	56.6	5.1764	192
3.	3.6 x 10^4	61.6	5.3055	180
4.	4.8 x 10^4	70.0	5.5244	168
5.	6 x 10^4	73.3	5.6128	156
6.	7.2 x 10^4	76.6	5.7388	144
7.	8.4 x 10^4	80.0	5.8416	125
8.	9.6 x 10^4	93.3	6.4758	118.8
9.	1.08 x 10^4	98.3	7.0537	117.6
10.	1.2 x 10^5	99.9	8.0900	098.8

a - Calculated as per Abott's Formula.

armigera using an atomizer. Five replicates of 10 larvae were used in each case. Two lots of 10 larvae sprayed with 5 ml of sterile distilled water with 0.05 % Tween-20 served as control . For dose mortality response ten different concentrations of conidia ranging from 1.2 x 10^4 to 1.2 x 10^5 conidia/ml were tested against third instar larvae of *H. armigera.*

After treatment larvae were air dried by keeping them in laminar air flow hood (Klenzaids, India) for 20-30 min. Each replicate lot of 10 larvae was placed in transparent disposable sterile Petridishes (85 mm) lined with moist cotton pad and were allowed to fed *ab libitum* on disinfected chickpea leaves. Petridishes were incubated at 28° ± 1°C in B.O.D. Incubator (SEW, India), having a 16 h photoperiod. The observations started after 24 h and larval lots were checked for mortality at 6 hr intervals till mortality or pupation. Dead larvae were kept in sterile saturated atmosphere at test temperature and cause of mortality was checked in all the cases. The LC 50 values were calculated after converting percent mortality into probits by probit regression analysis (Finney, 1971).

Effect of Temperature : The effect of temperature on bioactivity of *B. bassiana* was evaluated by spraying 10 ml of conidial suspension (ca. 9.6 x 10^4 conidia/ml) on to III instar larvae of *H. armigera,* and incubated at five different temperatures ranging from 15°-35° ± 1°C. Methods were similar as described earlier. Mortality was recorded daily and corrected by Abott's formula (Abott, 1925).

RESULTS AND DISCUSSION

Maximum mortality ca 100 percent occurred in I and II instar larvae of *H. armigera* with LT 50 as 72 hr. In III instar larvae LT 50 was 96 h and maximum mortality was 88 per cent after 10 days. Larvae of IV to VI instar were found to be relatively more tolerant (Table 1.) Similar observations were recorded earlier in larval instars of certain noctuids bioassayed for their susceptibility to *B. bassiana, Nomuraea rileyi* and *Paecilomyces fumosoroseus* (Gardner & Noblet, 1978; Devaprasad *et al.*, 1989; Ignoffo *et al.*, 1978; Fargues & Rodrigue-Rueda, 1980).Chemical constituent vary as the larvae advance in age resulting in progressive harden- ing 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, because control in the early stages is less likely to cause economic injury to the crop plants.

Table 3: Effect of temperature on cohorts of control and *B. bassiana* treated third instar larvae of *H. armigera.*

Relative humidity = 95% ± 5%

Temperature (In °C)	Control		*B. bassiana* treated[a]		*B. basiana* caused mortality (AM ± SD)[b]
	(N)[c]	Total % mortality	(N)	Total % mortality	
10	20	10	50	10.0	00 ± 00
15	20	10	50	10.0	00 ± 00
20	20	00	50	65.0	65 ± 9.514
25	20	00	50	91.6	91.6 ± 6.8
30	20	00	50	90.0	90.0 ± 1.0
35	20	50	50	51.0	00 ± 00

a - Dose 9.6 x 10^4 conidia/ml.
b - Arithmetic mean ± Standard deviation.
c - No. of larvae used.

Larval mortality was rapid with higher conidial concentrations. LT 50 at 1.2 x 10^4 conidia/ml was 216 h decreasing to 98.8 h when concentration was increased to 1.2 x 10^5 conidia/ml (Table 2). The percentage mortality ranged from 53.3 to 99.9 in all the ten concentrations tested. Chi square value tabulated at 5% level of significance was 2.082 suggesting that there is an indication of homogenity in the data. The regression equation was found to be Y = 1.3718 x -0.7894. Data on dose mortality

Table 4: Probit Analysis of dose mortality responses of third instar larvae of *H. armigera* to *B. bassiana.*

1.	Chi^2	- 2.082
2.	Regression equation	- Y = 1.3718 x - 7894
3.	LD 50	- 9635.94 Conidia/ml
4.	Upper Limit (UL)	- 115252.6 Conidia/ml
5.	Lower Limit (LL)	- 80559.1 Conidia/ml

response of the test larvae to the conidial suspension of *B. bassiana* indicate a good fit of the observed and expected responses based on chisquare (Table 4). LD 50 value was found to be ca 96356.94 conidia/ml after 118 h. Dose dependent response in III instar larvae of *H. armigera* at higher concentrations, 9.6×10^4 to 1.2×10^5 conidia/ml was not much pronounced. Similar observations seem to be typical for fungus insect interactions according to earlier workers (Hall, 1980; Ignoffo *et al.*, 1982).

Results with temperature showed that the ambient temperature had a direct influence on the bioactivity of *B. bassiana* (Table 3). Infection and host death occurred only between 20°-30°C, optimum being 25°C. showing 91 percent mortality in larvae of III instar. Significant mortality also occurred at 30°C. The temperature range is particularly important if *B. bassina* is to be used against *H. armigera* as the temperature in chickpea agroecosystem persist between 18°C to 30°C in Central India.

Observations presented herein indicate that the present isolate of *Beauveria bassiana* was quite effective against young larve of *H. armigera* and offers great potential for its use in the management of *H. armigera*. Considering these facts, attempts are being made at this institute to develop *B. bassiana* as a mycoinsecticide for the management of chickpea borer.

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