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Influence of temperature and relative humidity on the growth, sporulation and pathogenicity of *Beauveria* nr. *bassiana*

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ABSTRACT: Among the five different temperature conditions tested; radial growth, biomass production and sporulation were greater at 25°C followed by 30 and 20°C. Similarly susceptibility of third instar shoot borer (*Chilo infuscatellus* Snellen) larvae to fungal infection was higher when the larvae were held at 25°C after treatment. Among the 5 relative humidity (RH) levels tested at 25°C, the radial growth of the fungus was maximum at 90 per cent RH, whereas biomass production and sporulation were greater at 100 per cent RH. Higher mortality of borer larvae occurred at 100 and 90 per cent RH levels and the susceptibility decreased as the RH increased.

KEY WORDS: *Beauveria* nr. *bassiana*, *Chilo infuscatellus*, relative humidity, temperature

In sugarcane, the shoot borer, *Chilo infuscatellus* Snellen is an important and destructive pest (Avasthy and Tiwari, 1986) with cosmopolitan distribution. A fungal pathogen *Beauveria* nr. *bassiana* was reported to infect the larvae of shoot borer (Easwaramoorthy and Santhalakshmi, 1987). Studies carried out in the laboratory indicated that all the larval instars were susceptible to the fungal infection (Sivasankaran *et al.*, 1990). In pot culture studies, the fungus was found to be effective at 10^8 spores/ml in reducing the deadheart incidence (Sivasankaran, 1988). Before taking up evaluation of the fungus under field conditions, it is essential to know the influence of temperature and relative humidity that affect the growth, sporulation and pathogenicity of fungal pathogens. The details of studies conducted on these aspects are presented in this paper.

The radial growth, biomass production, sporulation and pathogenicity of *B.* nr. *bassiana* were studied incubating the fungus at 15, 20, 25, 30, 35 ± 1°C in BOD incubators. For studies on radial growth, petri dishes containing carrot agar medium

were inoculated with one cm mycelial discs obtained from an actively growing colony and incubated at different temperatures. Each treatment was replicated four times. The maximum diameter of the growing mycelium was measured on the 10th day. The biomass production was studied on carrot broth. Conical flasks (250 ml) containing 100 ml of the broth were inoculated with one cm mycelial discs of the fungus and incubated at different temperatures. Each treatment was replicated four times. After 20 days, the mycelial mat was filtered through Whatman No. 1 filter paper and dried at 80°C to a constant weight. Chopped carrots were taken at 100g in 250 ml conical flasks and sterilized for 20 minutes at 1.055 kg/cm^2. It was inoculated with one cm mycelial disc and kept at different temperatures for 10 days. The treatments were replicated thrice. After the incubation period, the fungus was blended with addition of 80 ml water and filtered through a muslin. The final volume was made 100 ml and spore counts were recorded using ruled Neubaur haemocytometer.

For pathogenicity studies, suspension of fungus having concentrations of 10^5 and 10^7 spores/ml were prepared from fungus grown on pieces of moist sterile carrot. Third instar shoot borer larvae were sprayed with the fungal spores and transferred to plastic boxes (7.0 cm diam x 7.5 cm ht) provided with filter paper discs at the bottom and two bits of sugarcane shoot, split open at one end. Two larvae weremaintained in a box. Each treatment was replicated thrice with 10 larvae per replication. The filter paperand shoot bits were changed daily, after recording mortality due to fungal infection and other causes. The per cent mortality due to fungal infection and time taken to kill were calculated.

Five relative humidity levels *viz.*, 60, 70, 80, 90 and 100 per cent were maintained in individual dessicators containing salt solutions (Jones, 1977). The dessicators were kept inside a BOD incubator at 25 ± 1°C. Studies on radial growth, biomass production, sporulation and pathogenicity were conducted as described earlier.

The radial growth observed on 10th day

Table 1. Effect of temperature on radial growth, biomass production and sporulation

Temperature (°C)	Growth circle (in cm) on 10th days	Biomass (in mg/ 100 ml) on 20th day	No. of spores (x 10^4/100 g) on 20th day
15	2.80	15.25	90.00
20	3.40	20.00	108.00
25	4.20	24.25	122.00
30	3.80	21.00	106.00
35	1.33	2.00	15.00
CD (P=0.05)	0.25	2.19	18.42

was significantly greater (4.2 cm) at 25°C followed by incubation at 30°C (3.8 cm). Least fungal growth (1.4 cm) was observed at 35°C (Table 1). A similar trend was noticed in biomass and spore production.

and the present study confirmed the results of earlier studies.

The fungal growth observed on 10th day was significantly greater at 90 per cent

Table 2. Influence of incubation temperature on the pathogenicity of the fungus

Temperature (°C)	Per cent corrected mortality at			Time taken for kill (in days) at		
	10^5 spores/ml	10^7 spores/ml	Mean	10^5 spores/ml	10^7 spores/ml	Mean
15	37.4 (37.7)	44.8 (42.0)	41.1 (39.9)	7.0	6.1	6.6
20	53.3 (46.9)	63.3 (52.7)	58.3 (49.8)	6.3	5.3	5.8
25	60.0 (50.8)	73.3 (58.9)	66.7 (54.7)	5.0	4.4	4.7
30	53.3 (49.6)	60.0 (50.8)	56.7 (48.8)	6.0	5.2	5.6
35	0.0 (0.6)	0.0 (0.6)	0.0 (0.6)	-	-	-
Mean	51.0 (45.6)	60.4 (50.9)	55.7 (48.2)	6.1	5.3	5.7

* Per cent mortality was corrected using Abbott's formula

	Mortality	Time taken for kill
CD (P=0.01) : Temperature	12.7	0.4
Dose	8.0	0.3
Temperature x Dose	NS	NS

At 25°C the biomass and spores produced were 24.25 mg/100 ml and 122 x 10^4 spores/100g, respectively. Larval mortality was higher at 25°C (60.0% at 10^5 and73.3% at 10^7 spores/ml) and it decreased both with increasing or decreasing levels of temperature (Table 2). The mean optimum temperature for *B. bassiana* to cause greater mortality is between 20 and 25°C (Zimmermann, 1982)

relative humidity (4.6 cm) which was closely followed by the growth at 100 per cent (4.5 cm) (Table 3). Poor growth (1.2 cm) was observed at 60 per cent relative humidity. Highest biomass production (27.58 mg/100 ml) and spore production (135 x 104 spores/100g) was observed at 100 per cent relative humidity. Biomass as well as spore production were significantly low at 60 per cent relative humidity.

Table 3. Effect of relative humidity on radial growth, biomass production and sporulation

Relative humidity (%)	Growth circle (in cm) on 10th day on 15th day	Biomass (in 100 ml) on 15th day	No. of spores (x 10^4/100 g)
60	1.23	3.00	16.50
70	2.20	9.75	72.00
80	3.50	17.75	105.00
90	4.60	23.75	126.00
100	4.50	25.75	135.00
CD (P= 0.05)	0.26	2.90	19.62

Table 4. Influence of incubation, relative humidity on the pathogenicity of the fungus

Relative humidity (%)	Per cent corrected mortality at			Time taken for kill (days at)		
	10^5 spores/ml	10^7 spores/ml	Mean	10^5 spores/ml	10^7 spores/ml	Mean
60	14.8 (22.6)	25.9 (30.6)	20.4 (26.8)	8.0	6.9	7.5
70	40.0 (39.2)	53.3 (46.9)	46.7 (43.1)	6.8	5.9	6.4
80	53.3 (46.9)	63.3 (52.7)	58.3 (49.8)	5.8	5.1	5.5
90	63.3 (52.7)	76.7 (61.1)	70.0 (56.8)	5.3	4.5	4.9
100	66.7 (54.7)	80.0 (63.4)	73.3 (59.1)	4.5	4.0	4.3
Mean	47.6 (43.6)	59.9 (50.7)	53.7 (47.1)	6.1	5.3	5.7

* Per cent mortality was corrected using Abbott's formula

		Mortality	Time taken for kill
CD (P=0.01) :	Temperature	14.5	0.3
	Dose	9.1	0.2
	Temperature x Dose	NS	NS

Cent per cent relative humidity was more congenial for the fungus to inflict high mortality of third instar (66.67 and 80.0 % at 10^5 and 10^7 spores/ml) shoot borer larvae followed by 90 per cent relative humidity and the two treatments were statistically on par with each other (Table 4). Lowest mortality was observed at 60 per cent relative humidity. The fungus at 10^7 spores/ml caused greater mortality of larvae at all the humidity levels compared to 10^5 spores/ml. The time taken to kill was less at 100 per cent and it increased progressively with decrease in relative humidity levels. Earlier studies have shown that infections of various host insects by *B. bassiana* were possible in wide range of ambient relative humidity levels. For instance infection of

Scolytus scolytus F. were possible at a relative humidity as low as 51 per cent (Doberski, 1980). Ferron (1977) demonstrated that while 92 per cent relative humidity is required for germination of *B. bassiana*, the fungus would infect *Acanthoscelides obtectus* Say., regardless of the ambient relative humidity. However, observations of McLaughlin (1962) showed that the susceptibility of larvae, pupae and adults of *Anthonomus grandis* Boh. to *B. bassiana* was found under conditions of high moisture. Lappa and Goral (1976) found that maximum pathogenicity occurred at 100 per cent relative humidity in *Melolontha melolontha* L. and *Cydia pomonella* (L.). This clearly shows that a relative humidity of 90-100 per cent is essential for the fungus to cause high mortality of the host, though reduced levels of infection can occur at lower relative humidity levels.

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