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Occurrence of *Metarhizium anisopliae* var. *anisopliae* on sugarcane internode borer, *Chilo sacchariphagus indicus* (Kapur)

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ABSTRACT: ***Metarhizium anisopliae* var. *anisopliae* is found on the larvae of sugarcane internode borer, *Chilo sacchariphagus indicus* (Kapur) under field conditions at Coimbatore. In laboratory tests, the fungus caused 20.0 to 83.3 per cent mortality in third instar larvae and 10.0 to 90.0 per cent mortality in fourth instar when treated with different doses ranging from 10^4 to 10^9 spores/ml. The time taken to kill the larvae varied from 5.6 to 13.1 days in third instar and 5.9 to 9.9 days in fourth instar. The mean number of spores produced per dead larva varied from 0.17 x 10^9 to 0.48 x 10^9 in third instar and 0.92 x 10^9 to 1.52 x 10^9 in fourth instar.**

KEY WORDS: *Chilo sacchariphagus indicus*, first report, *Metarhizium anisopliae*, pathogenicity

The internode borer, *Chilo sacchariphagus indicus* (Kapur) (Crambidae, Lepidoptera) is a major pest of sugarcane in peninsular India (Gupta, 1957). It damages the crop soon after internode formation and its activity continues till harvest (Ananthanarayana and Balasubramanian, 1980). Due to its infestation, considerable reduction in weight of cane occurs resulting in yield loss (David *et al.*, 1979). The infested canes also suffer a significant deterioration in juice quality with reduced sucrose content (David and Ranganathan, 1960).

A number of parasitoids, predators and some pathogenic microorganisms are reported to occur on various life stages of internode borer (David, 1986). During the routine collection of larvae made at Coimbatore for checking up the activity of larval parasites, some of the field-collected larvae were found infected and mummified by a fungal pathogen. Results of the studies carried out on this fungal pathogen and its pathogenicity to internode borer larvae are presented in this paper.

MATERIALS AND METHODS

The fungus was isolated from freshly mummified larvae on Emerson YPSS medium and brought into pure culture. Stock cultures were maintained on slants of the same medium and sub-cultured as and when required. Identification of the fungus was done based on the morphological characters using the key given by Samson (1981).

Pathogenicity tests were conducted on field collected internode borer larvae. The field collected larvae were surface sterilised with 0.1 per cent sodium hypochlorite and separated instar-wise. Only third and fourth instar larvae were used for the study. The larvae were treated with the fungal

suspension containing 10^4, 10^5, 10^6, 10^7, 10^8 and 10^9 spores/ml along with a surfactant (Teepol).

Quantification of the fungal spores was done using a Neubauer double ruled haemocytometer. Each treatment was replicated thrice with 10 larvae per replication. A suitable untreated control was also maintained. The treated larvae were reared on sugarcane pieces at 27 ± 2°C and 75 ± 10 per cent relative humidity. The sugarcane pieces were changed once in a day after recording data on mortality of larvae. Upon death, the cadavers were placed on a moist filter paper and observed for mycosis. Those larvae showing fungal develop ment and greenish colouration after sporulation were separated and mortality data computed. The time taken for kill was calculated on individual larval basis and the mean was worked out. The fungus was allowed to grow and sporulate on the cadaver placed on moist filter paper and when the sporulation was over the spores were extracted in known quantity of water using an anionic detergent. The average number of spores produced on each larva was worked out by counting the spores in a haemocytometer. The data were analysed in a randomised block design.

RESULTS AND DISCUSSION

The fungus was identified as *Metarhizium anisopliae* var. *anisopliae* based on the cultural and morphological characteristics. The dead cadavers, when kept on moist filter paper, white mycelial growth appeared within 24 hours after death on the inter-segmental regions followed by segmented region except on brown spots. In the next 24 hours, the cadavers were fully covered with dense, white mat and the fungus started sporulation, simultaneously. Sporulation characterised by green powdery mass was completed in 3-4 days after the death of the larvae.

This is the first report of *M. anisopliae* on sugarcane internode borer. This pathogen has been recorded as the fourth fungal pathogen on this pest, the earlier reports being *Aspergillus flavus* (David, 1964), *Isaria* sp. (David and Kalra, 1965) and *Hirsutella nodulosa* (Easwaramoorthy *et al.*, 1997).

In pathogenicity tests, the fungus caused 20 per cent mortality of third instar larvae at 10^5 spores/ml and the mortality increased to 83.3 per cent at 10^9 spores/ml (Table 1). In the case of fourth instar larvae, the mortality ranged from 10.0 per cent at 10^4 spores/ml to 90.0 per cent at 10^9 spores/ml. In both the larval stages, there is an increase in the mortality rate with increase in the spore concentration. Walstad and Anderson (1971) also found that the mortality was a function of the quantity of inoculum applied. Positive correlation between the number of infective spores and mortality due to mycosis has been established by many authors as reviewed by Ferron (1978).

The time taken for kill varied from 6 days at the highest concentration of 10^9 spores/ml in third instar larvae and it increased with decrease in the dosage level (Table 2). The mean time taken at 10^4 spores/ml was 13.1 days. A similar trend was noticed in the fourth instar larvae. It varied from 5.9 to 9.9 days in fourth instar. The study revealed that the time taken to kill decreased with increase in the dosage of the fungus. Similar results were

Table 1. Mortality of internode borer larvae due to *M. anisopliae*

Dose (Spores/ml)	Mortality (%)	
	III instar	IV instar
10^4	23.3(27.27)	10.0(15.19)
10^5	20.0(26.07)	13.3(21.13)
10^6	66.6(54.80)	50.0(45.27)
10^7	66.6(62.73)	69.3(57.27)
10^8	50.0(44.73)	76.6(66.93)
10^9	83.3(66.13)	90.0(77.73)
Control	0.0 (0.57)	0.0 (0.57)
CD (P=0.05)	26.84**	24.75**

Figures in parentheses are arcsine √ percentage values.

** Significant at 1 per cent level.

reported in sugarcane shoot borer, *Chilo infuscatellus* Snellen infected with *Beauveria bassiana* (Sivasankaran *et al.*, 1990).

The average number of spores produced on individual larva varied from 0.17 x 10^9 to 0.48 x 10^9 spores in third instar. In fourth instar larvae a higher amount of spore production was noticed and it varied from 0.92 x 10^9 to 1.52 x 10^9 spores/ larva. The quantity of spore production is directly

Table 2. Time taken to kill internode borer larvae by *M. anisopliae*

Dose (Spores/ml)	Time taken to kill in days	
	III instar	IV instar
10^4	13.1	9.3
10^5	8.0	8.2
10^6	8.6	9.9
10^7	5.6	6.6
10^8	8.2	7.9
10^9	6.1	5.9
Control	-	-
CD (P=0.05)	NS	4.7*

N S: Non-significant

* Significant at 5 per cent level

related to the amount of nutrients available and as more amounts of nutrients are available in fourth instar larvae compared to third instar, higher spore production is noticed.

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