Identification of virulent isolate of *Metarhizium anisopliae* (Metschin) Sorokin (Deuteromycotina: Hyphomycetes) for the management of *Helicoverpa armigera* (Hubner)

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ABSTRACT

Conidia of the entomopathogenic fungi *Metarhizium anisopliae* (SBT # 27 and SBT # 28) were produced under standard condition and then examined for influences on vitro conidial germination speed and virulence to an insect host, *Helicoverpa armigera.* Conidia were most virulent (based on mortality at 6 d) and had the fastest germination rates when produced on SMB (Sabouraud maltose broth) and the media pH is 6.0 - 6.2. The second instar larvae were exposed to the fungus and the concentration is of 1 x 10^7 and 1 x10^8 conidia/ml were tested against host insects at 28^0C, SBT # 27 isolates showing 98 - 100% mortality in 8days against *H. armigera* and SBT # 28 showing 90 - 92% in 8 days. Among the two isolates SBT # 27 is superior in terms of high percent kill as well as 100% germination of conidia within 48 hours. However, SBT # 27 isolate showing greater pathogenicity against insect pest.

Key words : Conidia, *Helicoverpa armigera*, media, *Metarhizium anisopliae*, sporulation, virulence.

INTRODUCTION

Metarhizium anisopliae (Metchn.) Sorokin (Deuteromycetes : Hyphomycetes) belonging to the order Moniliales is of cosmopolitan occurrence infecting mainly Lepidoptera. The fungus has potential for the control of several economically important insect pest of global importance *viz.*, *Helicoverpa armigera*, *Spodoptera litura*, that attack crops such as groundnut, soyabean, sunflower, cotton and tomato (Sahayaraj and Borgio, 2010). Ferron (1981) reported that susceptibility of most insects depends on spore dosage and that there is a positive correlation between the number of infective spores and mortality by mycosis.

The production and formulation of entomopathogens, and of *Metarhizium anisopliae* and *Beauveria bassiana* specifically, has been discussed by several authors, but little is known about the subject. Entomopathogenic hyphomycetes, such as *Beauveria bassiana* (Balsamo) Vuellemin and *Metarhizium anisoplae* (Metschnikoff) Sorokin, are of high potential for control of sucking insect pests (Faria and Wraight, 2001; Vandenberg *et al.*, 2001; Feng *et al.*, 2004a, 2004b). Studies on nutrition of entomogenous fungi have generally been concerned with the effect of nutritional factors on the growth and sporulation of fungi rather than on virulence (Campbell *et al.*, 1978; Barnes *et al.*, 1975; Lihnell, 1944). Goral (1978), however, showed that the virulence of *M. anisopliae* conidia against insect species was increased when spores were produced on certain complete media. Recently, Vimala and Suriachandrasekaran (2008), Yankanchi and patil (2009) and Mehta *et al.* (2010) proposed ecofriently management options for this pest This study reports on the assessment of bioassay and germination of indigenous fungi *M.anisopliae* in the control of *H. armigera* was studied.

MATERIALS AND METHODS

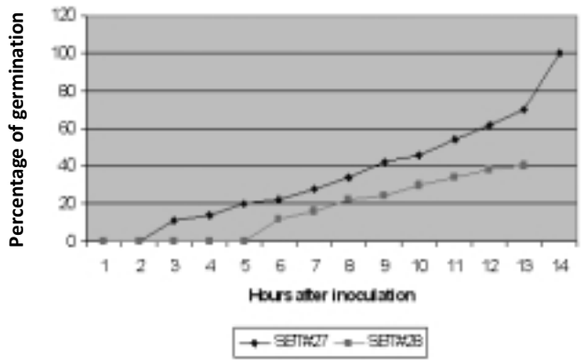
The fungal isolates used in this study were isolated form M. *anisopliae* infected *H.armigera* larvae collected from Nandigama village (SBT # 27) (Patancheruvu, Hyderabad) and Medchal, Hyderabad (SBT # 28). The isolates were maintained on PDA and the isolates were passed through the host insect at Bi-monthly intervals to maintain the virulence. The conidia of *Metarhizium anisopliae* were produced by growing the fungus on SMB (Saboraud's maltose broth). The fungus broth is incubated at room temperature (28 ± 2^0C) for 7 days. Tween-80(0.01%) added to the spore suspension and the spore concentration was determined using an improved Haemocytometer and adjusted the concentration 1 x 10^7 and 1 x 10^8 conidia/ml.

Before going to bioassay, spore concentration was adjusted to 1 x 10^7 and 1 x 10^8 conidia/ml under a phase contrast microscope and concentrations was pipetted and applied on target insects. Four replication of 20 insects of *H. armigera* were used for conidial concentration of *M. anisopliae*. Control was treated with distilled water. The insects' plates were kept in a 28±2^{0}C and an average ambient relative humidity range of 80 ± 2^{0}C RH. Observation of larval mortality was recorded every day till 8th day. The cadavers were washed in sterile water and placed on moist tissue paper in Petri plates and observed for mycelial growth and sporulation in the next 48 h to confirm death due to infection by *M.anisopliae*. Isolates of *Metarhizium anisopliae* conidia were inoculated separately into 50ml of Saboraud's maltose broth in 250ml flasks. The flasks were then incubated in an incubator shaker at 25^{0}C. Three flasks were inoculated for each isolate treating each flask as a replicate. Samples were drawn at different intervals from the broth and counts of germinated conidia were taken under a phase contrast microscope (Olympus C X 31) using a Neubauer's Haemocytometer. Germination was assessed by examining each time 100 conidia in three different fields for germ tube formation and elongation. Counts were averaged and percentage germination was worked out. The percentage larval mortality of *H.armigera* were angularly transformed prior to the analysis of variance (ANOVA) and the means were separated by Duncan's multiple range test (DMRT) at P = 0.05.

RESULTS AND DISCUSSION

At the recommended dose of 1 x 10^7 and 1x10^8 spore/ml, bioassay of SBT # 27 and SBT # 28 isolates against *H .armigera* showed optimum larval mortality within 8 days after spraying. Initial mortality was observed within 6 days itself after treatment. The percentage of cumulative mortality of *H. armigera* larvae treated with $10^7$and 10^8 conidia / ml of *M. anisopliae* formulation (SMB). Among the two isolates, BST # 27 strain caused 95% larval mortality, which was significant (P < 0.01) than the control. Isolates of a fungus from different host insects have varying degrees of virulence as measured by percent mortality in Bioassays (Ignoffo *et al.*, 1976), broader host range and time taken for spore germination (Altre *et al*., 1999).

Figure 1. Germination times for conidia of *M. anisopliae* isolates



In order to find out if germination as well as amenability for multiplication was correlated to the pathogenicity, germination studies were undertaken with these promising isolates on Saboraud maltose broth (SMB). Only SBT # 27 isolate showed 100% germination 48 h (Figure 1). The fastest germinating *M. anisopliae* (SBT # 27) isolate spores (concentration of 2 x 10^8 spores/ml) in both were the most virulent against *H. armigera* larvae followed by the isolate of SBT # 28 (Table 1). Virulence against the target insect pests is the most

Table 1. Bioassay of *M. anisopliae* isolates against *Helicoverpa armigera*

Strain	Cumulative mortality (%)		
	6Days	7Days	8 Days
Control	18.0±3.7^a	26.0 ± 5.1^a	32.0 ± 3.7^a
SBT # 27(2 X 10^7 conidia / ml)	82.0 ± 6.6^b	94.0 ± 4.0^b	98.0 ± 2.0^c
SBT # 27(2 X 10^8 conidia / ml)	90.0 ± 5.5^b	98.0 ± 2.0^b	100.0 ± 0.0^c
SBT # 28(2 X 10^7 conidia / ml)	78.0 ± 3.7^b	90.0 ± 5.8^b	91.0 ± 2.4^b
SBT # 28(2 X 10^8 conidia / ml)	76.0 ± 5.1^b	90.0 ± 3.7^b	92.0 ± 2.0^b

Means within the same column followed by the same letter are not significantly different (p > 0.05).

important criterion for commercial exploitation of the fungal pathogen. It can be inferred from this study that virulent isolates of *M. anisopliae* are necessarily fast in germination and showing high infectivity against the insects. These parameters can be successfully employed to identify virulent isolates. It can be concluded that the isolate isolated in Nandigama village is a virulent isolate and can be effectively used against *H. armigera*.

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