ABSTRACT

Sugarcane is the most ancient source of sugar used by man and forms one of the important cash crops in the world economy. Sugarcane is the native of India and at present it is being grown over 4.202 million hectares of land with 2.77 million tones of production. In Tamil Nadu sugarcane is cultivated in area of 324 thousand hectares with production of 34020 thousand tones Saccharum officinarum L. is the noble cane which produces 30 metric tons per annum with value of Rs 60,000. For the past two decades, there is a considerable loss in sugar production due to insect pests. The important subterranean pests infesting sugarcane are white grubs and termites. For the past three years, the pest infestation is very high in Salem, Dharmapuri, Subramaniya Siva and Vellore Co-operative sugar mills areas of Tamil Nadu resulting in serious damage and yield losses in sugarcane. Among the several species of white grub, Holotrichia serrata Blanch occur most abundantly during August to September and pose a serious threat to sugarcane. The yield loss due to white grub attack alone on sugarcane is reported to be as high as 80-100 per cent in affected clumps.

The entomopathogenic fungi is the best alternative biological control measures to control white grubs. The main advantages of entomopathogenic fungi over chemical pesticides are their significant higher host specificity and the reduction of toxicity. Most entomopathogenic-fungus research is directed toward developing a product for controlling pest insects. The present study focuses on the six Metarhizium anisopliae isolates and its mass production on different solid substrates and assesses the virulence of isolates in laboratory and field trials against H. serrata. Soil samples were collected from sugarcane fields from different sugarcane growing areas of Tamil Nadu viz., Rasipuram, Sathyamangalam, Thimiri, Madurai, Dharmapuri and Coimbatore to isolate the effective strain of the fungus M. anisopliae against white grub. Out of 26 isolates isolated, six strains were identified as M. anisopliae. The sequencing data of the six isolates were deposited in NCBI for getting accession numbers and their accession numbers obtained as JQ013738, JQ013739, JQ013740, JQ013741, JQ013742 and JQ031714.
The phylogenic analysis of all six isolates were done with MEGA 4 software. Effect of different solid substrate on the sporulation, radial growth and biomass of six isolates of *M. anisopliae* were assessed. Among the seven solid substrates *ie.* pearl millet, maize, cowpea, rice bran, wheat bran, pressmud +1 per cent yeast extract and bagasse +1 per cent yeast extract tested for the conidia production, radial growth and biomass yield of *M. anisopliae*, it was found that wheat bran produced the highest spore, radial growth and biomass for the strain JQ013739. The strain JQ031714 was found to be the lowest in spore production. The effects of different temperature, relative humidity and pH on the spore production of two efficient isolates of *M. anisopliae* on wheat bran substrate were studied. The optimum temperature for maximum spore production was found to be between 25°C and 30°C for both the strains of *M. anisopliae*. Maximum spore production was recorded between 90 and 100 per cent relative humidity while lowest was at 60 per cent relative humidity. At pH 6.0 the spore production was the highest (7.20 x10^8 per g of substrate) and it was followed by pH 7 with 6.30 x10^8 per g of substrate.

Three entomopathogenic fungi *Beauveria bassiana*, *Beauveria brongniarti* and *M. anisopliae* were tested against third instar larvae of *H. serrata* under laboratory condition during 2008. Under laboratory condition, the green muscardine fungus *M. anisopliae* caused highest larval mortality after 15 days of treatment. The field efficacy of the talc based formulation of the three entomopathogenic fungi was tested against *H. serrata* separately and in combination. Among the talc formulation tested, the combination of three entomopathogenic fungi *B. bassiana*, *B. brongniarti* and *M. anisopliae* was most virulent causing 84 per cent mortality at 20 days after second application. Highest cane yield of 98.25 mt per ha was recorded in the treatment where the three entomopathogenic fungi *B. bassiana*, *B. brongniarti* and *M. anisopliae* were applied in combination and it significantly differed from other treatments tested. The next best treatment was the application of *M. anisopliae* alone which gave the cane yield of 93.50 mt. Field efficacy of the different doses of *M. anisopliae* against *H. serrata* was assessed. The per cent reduction in the grub population over the control was maximum at the dosage of 8x10^9 conidia. All the six strains of *M. anisopliae* were tested in oil formulation and conidial suspension against
third instar grubs of *H. serrata* under laboratory condition. The strain JQ013739 in oil formulation caused maximum mortality at 10 days after treatment.

All the six strains of *M. anisopliae* in oil formulations as well as aqueous conidial suspension tested under field condition showed higher efficacy in controlling the *H. serrata* grubs with high mortality levels when compared with water and oil controls. In the field experiment, *M. anisopliae* strain JQ013749 when applied to white grubs at the rate of $3 \times 10^{12}$ conidia per ha in an oil emulsion caused maximum mortality at 10 days after application. When applied as an aqueous suspension, the same concentration of spores caused only 81.50 per cent mortality. Cane yield and quality attributes of sugarcane recorded in different treatments are assessed. The data revealed that treatment with oil formulation of *M. anisopliae* strain JQ013749 recorded significantly higher cane yield of 120 mt per ha and significantly differed from all other strains. Higher quality parameters were recorded in plots treated with aqueous and oil formulation of *M. anisopliae* strains compared to control plots. The field efficacy of the different formulations of *M. anisopliae* strains JQ013738 and JQ013739 against sugarcane white grub *H. serrata* were assessed. Apparently, successful use of entomopathogenic fungi as microbial control agents would ultimately depend on the use of the right propagate formulated in an optimal manner and applied at an appropriate time and dosages. From our study, it was evident that the *M. anisopliae* spores formulated in an oil formulation significantly caused higher mortality than conidial suspension in both laboratory and field experiments.

The results obtained in field experiments have shown that without chemical pesticides the pest can be controlled by *M. anisopliae* in oil and liquid formulations. Remarkable control of the pest observed with high yield and quality parameters recorded in oil and liquid formulation of the isolate JQ013739 indicated that this isolate need to be popularized for the control of white grubs under field conditions in sugarcane growing areas.