Field efficacy of formulations of microbial insecticide *Metarhizium anisopliae* (Hyphocreales: Clavicipitaceae) for the control of sugarcane white grub *Holotrichia serrata* F(Coleoptera :Scarabidae)

C. Thamarai Chelvi[1], W. Richard Thilagaraj[2] and R. Nalini [3]

ABSTRACT

An attempt is made to control the white grub *Holotrichia serrata* using entomopathogenic fungi at field level for the first time in India in sugarcane crop using different formulations of the microbial insecticide *Metarhizium anisopliae*. Three formulations of the microbial insecticide *M. anisopliae* via Talc, Lignite and Liquid formulations were used. Field trials were conducted in Vellore Co-operative Sugar mill with five treatments and three replications in Randomized Block Design. Pretreatment count was taken in 1 m^2 area. The treatments of the formulations i.e. talc, lignite and liquid were mixed well with decomposed Farm Yard Manure and applied near the root zone of the cane at 5-10 cm depth and irrigated immediately after application. Two applications were made at 15 days interval .Grub mortality was assessed after 15 days after each application .The data were subjected to analysis of variance. Results on growth and yield parameters were recorded. The liquid formulation of the biopesticide *M.anisopliae* was found to be efficient for the control of sugarcane white grub *H.serrata*.

Key words: Sugarcane, Microbial Insecticide, *Metarhizium anisopliae* ,*Holotrichia serrata*

INTRODUCTION

Sugarcane is an important commercial crop grown in an area of 4.2 lakh ha with an average production of 66 tonnes per ha. The average production is less than that in other sugar producing countries of the world due to incidence of pests and diseases .Sugarcane crop is infested with more than 200 species of pests. Among the pests, the subterranean white grub has potential to cause 80-100% damage to sugarcane crop. White grubs (Coleoptera: Scarabaeidae) are soil inhabiting and root feeding immature stages of scarab beetles. The white grub family, Scarabaeidae is the second largest and omnipresent family within the order Coleoptera (Mishra and Singh, 1999). White grubs have become serious pests of most agricultural crops, fruits, vegetables, ornamental plants, plantation crops, pastures, turf and meadow grasses, lawns, golf courses and forest trees in different part of the world (Potter *et al.* 1992). Chemical control measures are ineffective since the pests are subterranean.

Metchnikoff was the first to describe *Metarhizium anisopliae* "green muscardine " infections on the cereal cockchafer and to suggest the use of the microorganism as a biological control agent for insects(Zimmerman *et al*., 1995). The insect pathogenic fungi *M. anisopliae* and *Beauveri brongniartii* (Keller, 2000) have been reported throughout the world. Fungus based natural enemies have successfully been applied in countries like Switzerland, Austria, New Zealand and Australia (Keller, 2000). After application, the fungi persisted in the soil due to their capacity to multiply in the host (Fox, 1949). They are also easily isolated from the soils (Zimmermann, 1993). In USA, UK and Germany, both *Beauveria* and *Metarhizium* are being used to control several insect pests of Agricultural importance (Munnan and Wikardi, 1986).The entomopathogenic fungus *M.anisopliae* has been reported to infect more than 100 insects including a number of soil dwelling insects. Commercially important targets include root weevils, soil grubs, rootworms, wireworms, fruit flies and root maggots (Bruck, 2005). Our study focused on evaluating formulations of the microbial insecticide *M.anisopliae* based on their ability to infect and kill grubs in soil.

MATERIALS AND METHODS

Maintenance of fungal cultures

Metarhizium anisopliae was maintained in Emerson YPSS agar medium. The stock cultures were maintained at 4^0C until used. Sub culturing was done on *Holotrichia serrata* every year and the passaged culture was reisolated and maintained on SDA slopes and preserved by lyophilization. The passaged stock culture of entomopathogenic fungi was used for all the experiments.

Preparation of formulations

Talc based formulations were prepared as per procedure adopted by Samiyappan *et al*.,(2003). Liquid formulations were prepared as per modified procedure of Batta (2000).

Field efficacy of different formulation of Entomopathogenic fungi *M. anisopliae*

White grub infested sugarcane field in Thimiri village of Vellore Co-operative Sugar Mills, Tamil Nadu, India was selected as the test site for the conduct of field experiments with *M. anisopliae* wherein the grubs were found to occur at a density of 20-25 grubs per m^2 .Size of the trial plot was 4000 M^2. Liquid Formulations, talc and lignite formulations were applied at the rate of $3x10^{12}$conidia /ha and application in 5cm wide by 5cm deep furrow extending front to back .The raised soil beside the furrow was pushed back to cover the formulations. The field was irrigated immediately after application. Control plots were treated with sterile distilled water with 0.01 % Tween 80 alone .The treatments were randomized and five true replicates were maintained. On 10 and 15 days after treatment, the grub population per square meter was taken and observed for mortality by the *M. anisopliae*. The mortality percentage due to *M. anisopliae* was calculated for each treatment and compared. Data were subjected to analysis of variance.

RESULTS AND DISCUSSION

The results are presented in table 1. All the three treatments were effective in checking the population of *H.serrata* which ranged between 76.87-81 per cent on 15 days after treatment (Table 1) compared to control. Among the formulation liquid formulation was more virulent causing 81 % mortality. Highest yield and quality parameters recorded in liquid formulation treated plot. Control mortality was 0%. Lowest quality and yield parameters were recorded in control plot.

The results of the present study showed that the liquid formulation of the microbial insecticide is effective in controlling sugarcane white grub. The combination of formulation, application and selection of the strain is one of the key steps for field trials. It has been suggested that oil formulation can prevent conidial dessication and improve adhesion of conidia to the hydrophobic surface of insect cuticle (Inyang *et al*, 2000, Vimala devi and Hari,1999). Keller (1998) suggested that repeated application of the entomopathogenic fungal formulations enhance the pest control process and white grubs could be controlled in field situations in various crops, like *H. consanguinea* infesting potatoes were controlled by *M. anisopliae* (Kulye and Pokharkhar, 2009) or high virulence has been reported against *H. serrata* using *B. brongniarti* as lignite or press mud formulations (Eswaramoorthy *et al*., 2005). Glare and Milner, 1991 reported that high dosages of 10^8 to 10^9 conidia per ml causes normally the higher mortality of white grub larvae. Sharma *et al.,* (1999) reported that the mass multiplication and formulation of entomopathogenic fungi *B.brongniarti* and *M. anisopliae. M.anisopliae* and *B.brongniarti* showed high virulence against both the target insects .*H. consanguinea* with LT_{50} of 7.95 .When third instar larvae

Table1. Field efficacy of formulations of *M.anisopliae* and Quality parameters recorded in different treatments

Treatments	Mean mortality % (15 DAT)	Pol%	Purity %	CCS%	Sucrose
T1 Talc formulation	78.58(96.08)^b	19.27^b	90.22^b	12.34^b	17.24^b
T2 Lignite formulation	76.87(95.58)^c	19.25^c	90.23^c	12.32^c	17.22^c
T3 Liquid formulation	81(87.49)^a	19.30^a	90.28^a	12.40^a	17.28^a
T4 Control	0(0.78)^d	16.42^d	85.64^d	11.62^d	16.32^d
CD	3.20	0.08	0.06	0.03	0.09

Figures in parenthesis are angular transformed values. In the columns means followed by same letters are significantly different at ($p<0.05$) by DMRT.

exposed to their highest dose of inoculum (4 x 10^8 conidia g-l). Mohoiddein *et al.* (2006) tested the pathogenicity of nine fungi in the laboratory against *Holotrichia* spp. All the fungi proved to be pathogenic at a spore concentration 1 x 10^8 spore / ml to grub with varying mortality. *B. bassiana* (local), *B. bassiana* (commercial), *B. brongniarti* and *M. anisopliae* were found to be the most effective.

Thamarai Chelvi *et al.* (2010) reported that the biopesticide *M. anisopliae* at the concentration of $8x10^9$ conidia per ml found to be effective in controlling the population of white grub and also reported that yield and quality parameters recorded were higher in treated plots compared to control plots. Thamarai Chelvi *et al*. (2010a) reported that the combination of the three entomopathogenic talc based fungal formulation of *B .bassiana* ,*B* ,*brongniarti* and *M .anisopliae* showed relatively higher virulence and proved to be suitable candidates for controlling larvae of sugarcane white grub *H. serrata*. The higher colony-forming unit counts of *M.anisopliae* found in association with plant roots and root exudates suggest these fungi may be capable of survival in soils without an insect host (Hu and St Leger,2002). In addition to strain selection and genetic modification formulation can have a considerable impact on improving the efficacy of biopesticides. An ideal formulation aids the handling and application of the biopesticide as well as increases its efficacy by improving contact with the host and protecting the active agent from environment factors(Goettel, 2005).

Entomogenous fungi have great promise for use as biological control agents against different insects. However, their infectivity is quite different depending on fungus species and developmental stage of the target insects (Samson, 1981). Therefore when a particular insect pest control programme is considered using these fungi, the particular species or strains which are most suitable have to be taken into account. Similarly, dose and time of exposure of the host to the insect pathogenic fungus and the time taken to kill the host are also important parameters for evaluating the suitability of insect pathogenic fungi. The results of our field experiments to control sugarcane white grub have demonstrated the utility of using microbial insecticide *M.anisopliae* as a control tool for this insect pest without any hazardous chemical pesticides.

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C. Thamarai chelvi[1] , W. Richard Thilagaraj[2] and R. Nalini [3]

[1]C.Thamarai chelvi, Main Biocontrol Research Laboratory, Venpakkam, Chengalpattu - 603111.
Email:chelvithamarai1@gmail.com
Phone: 04427431393,9003611419

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