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Field Evaluation of *Metarhizium anisopliae* (Metschnikoff) Sorokin against *Holotrichia serrata* (Blanch) in sugarcane

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ABSTRACT

Field experiments were conducted on sugarcane for white grub management using *Metarhizium anisopliae* (Metschnikoff) Sorokin at Agricultural College and Research Institute, Madurai District and Kuchanoor village in Theni District during 2010-2011. Application of *M. anisopliae* against sugarcane white grub *Holotrichia serrata* (Blanch) at 4 x1 0^9 conidia ha^{-1} was found effective next to chlorpyriphos and registered 92% reduction in grub population on 60^{th} DAT. The highest cane yield was recorded when chlorpyriphos was applied @ 3lit ha^{-1} (110.5t ha^{-1}), followed by *M. anisopliae* @ $4x10^9$ conidia ha^{-1} (100.6t ha^{-1}). However, incremental benefit cost ratio (IBCR) was high with higher doses of *M. anisopliae* (7.58) followed by drenching of chlorpyriphos (6.09).

Key words: Sugarcane, *Holotrichia serrata, Metarhizium anisopliae,* chlorpyriphos, field evaluation

INTRODUCTION

White grubs have become increasingly difficult pests in Tamil Nadu during the last few years. Their infestation has been reported throughout the country and the magnitude of the problem has been widespread over the past years. Nearly 20 species of white grubs are reported to attack sugarcane in India. Of these, *Holotrichia* sp, *Anomala varicolor* (Gryll), *A.viridis* (F,) *Apogonia destructor* (Bos.) *Cyclocephala parallela* (Casey) *Dermolepidia pica* (Arrow) *Lepidiota stigma Ligyrous subtropicius* (Blanch) *Leucopholis* sp (F.), *Phyllophaga helleri* (Brsk), and *Schizonycha* sp. have been reported to assume pest status in sugarcane-growing regions.(Yubak Dhoj, 2006) Besides sugarcane other cultivated crops such as groundnut, cereals, millets, pluses, vegetables and plantation crops were also attacked by white grub (David *et al.*, 1986). The yield loss due to white grubs was reported to be as high as 100 per cent in Tamil Nadu (Thamarai Selvi *et al.,* 2010). In a majority of the farming situation, control of these pests are have become increasingly difficult because of the lack of control over the damages they cause. In general, the management strategy depends primarily on the use of highly poisonous poor graded chemical pesticides.

Several tactics have been adopted for the management of white grubs including cultural, mechanical, biological, chemical and integrated methods suggested by various workers (Sahayaraj and Borgio, 2009; Srikanth and Singaravelu, 2011). Application of chemical is practically uneconomical, difficult and associated with high cost, environmental pollution and other problems. Hence, there is a strong need for the development of alternative strategies for the control of white grubs, which are ecofriendly and economically feasible. The use of bio-control agents in general and fungal based myco-insecticides in particular are lacking in the country. About 90 genera and 700 species of fungi representing a large group of entomophthroals (*Metarhizium* spp., *Beauveria* spp., and *Verticillium* spp.) which are entomopathogenic have been reported. Among these, *Metarhizium* is of greater importance in the management of white grubs. *Metarhizium anisopliae* (Metschnikof) Sorokin can be effectively utilized as one of the components in the management of white grubs (Mohi-ud-din *et al*., 2006; Chroton, 2007). The fungus is eco-friendly, cost effective, highly persistent and also self-perpetuating in nature. Moreover the microclimate of sugarcane eco-system is ideal for the fungus to multiply. Further, rainfall, high humidity and soil with high organic content also help the fungus to perpetuate itself in nature.

MATERIALS AND METHODS

Field evaluation of *M. anisopliae*

Field experiments were carried out at Agricultural College and Research Institute, Madurai District and Kuchanoor village in Theni District. The sugarcane variety Co86032 was planted during January with a spacing of 0.9 m between rows with a plot size of 6 x 5 m^2 and all the recommended package of practices was adopted except for white grub management. There were seven treatments as listed in table 1 and 2 which were replicated thrice in a randomized block design. Twenty day old fungal culutre was blended with addition of 100ml of stérile water and filtered through muslin cloth. The final volume was made up to 1000ml and spore counts were recorded under a phase contrast microscope with a double ruled Neubaur haemocytometer. Spore concentration was estimated using the formula and the optimum dose was arrived at by probit analysis

$$X = \frac{1}{400} \times \frac{1}{10} \times \frac{1}{10} \times \frac{1}{10} \times 10 \times \text{dilution factor}$$

The treatments were imposed in the third week of July. *M. anisopliae* @ 4 x 10^9 conidia ha^{-1} and @ 1 x 10^9 conidia ha^{-1} were applied to the root zone by mixing with well decomposed Farm Yard Manure. Chlorpyriphos was soil drenched @ 2 and 3 lit. ha^{-1} through the hole made by using a crowbar, while carbofuran and neem cake were applied to soil near root zone and irrigated. Observations on the number of white grubs per meter row in the root zone were recorded a day before and 15, 30, 45 and 60 days after treatment (DAT). Millable cane and yield was also recorded at harvest.

Statistical analysis

The data on the number of grubs were subjected to x+ 1 square root transformation. These transformed data were subjected to analysis of variance (Panse and Sukhatme, 1967) and Duncan's Multiple Range Test (Gomez and Gomez, 1984) was used to determine the significance in different treatments.

RESULTS AND DISCUSSION

Regardless of the periods *viz.,* 15, 30, 45 and 60 DAT, application of chlorpyriphos was significantly effective in reducing the grub population (Table 1). Particularly, chlorpyriphos at its higher dose of 3 lit. ha^{-1} showed cent per cent reduction in grub population at 45 and 60DAT whereas at 15 DAT, application of *M. anisopliae* @ 4 x 10^9 conidia ha^{-1} and @ 1 x 10^9 conidia ha^{-1} failed to reduce the grub population and was found on a par with untreated check. It is due to the fact that though grubs were already infected by the fungus, they require time to produce external symptom and cause death of white grubs. On 30 DAT, *M. anisopliae* @ 4x10^9 proved significantly superior to neem cake @2.5q ha^{-1}, *M. anisopliae* @ 1x10^9 conidia ha^{-1} and untreated control. It has reduced the grub population to the extent of 4.56 grubs / m row. As the day after treatment advanced, the effect of *M. anisopliae* also increased and it was on a par with carbofuran @ 33 kg. ha^{-1} at 45 DAT, while at 60 DAT the effect of *M. anisopliae* at its higher dose (0.75) was on a par with chlorpyriphos @ 2 litres ha^{-1}.

Table1. Efficacy of *M. anisopliae* against sugarcane white grub, *Holotrichia serrata*

Treatments	No. of white grubs per m row					
	1 DBT	15 DAT	30 DAT	45 DAT	60 DAT	% decrease over untreated check at 60 DAS
M. anisopliae @ 4x10^9 conidia ha^{-1}	9.22^a (4.03)	8.89^c (3.98)	4.56^c (3.13)	2.56^{bc} (2.60)	0.75^b (1.86)	91.70
M. anisopliae @ 1x10^9 conidia ha^{-1}	8.88^a (3.98)	8.88^c (3.98)	7.88^d (3.80)	5.88^d (3.42)	5.22^e (3.28)	42.25
Chlorpyriphos 20 EC @ 2 lit. ha^{-1}	8.22^a (3.87)	3.22^b (2.79)	2.56^b (2.60)	1.89^b (2.37)	1.35^b (2.26)	85.07
Chlorpyriphos 20 EC @ 3 lit. ha^{-1}	8.55^a (3.92)	2.56^a (2.60)	1.56^a (2.25)	0.00^a (1.00)	0.00^a (1.00)	100.00
Carbofuran 3G @ 33 kg ha^{-1}	8.22^a (3.87)	3.56^b (2.88)	2.89^b (2.70)	2.42^{bc} (2.55)	2.14^c (2.46)	76.32
Neem cake @ 2.5 q ha^{-1}	9.22^a (4.03)	8.88^c (3.98)	7.56^d (3.75)	5.22^d (3.28)	4.89^d	45.90
Untreated check	9.55^a	9.55^d	9.39^e	9.24^e	9.04^f	

DBT: Days before treatment; DAT: Days after treatment,
Means followed by the same alphabets in columns did not differ significantly [p=0.05] by DMRT

From Table 1 it is evident that *M. anisopliae* @ 4x10^9 conidia at 60 DAT recorded 92% mortality in grubs and was next to drenching of chlorpyriphos which was found to be most effective by recording cent per cent reduction in grub population.

From Table 2, it is evident that the millable cane and sugarcane yield varied significantly among the treatments and were significantly superior to untreated check. The treatment with chlorpyriphos @ 3lit. ha^{-1} and *M. anisopliae* at higher dosage resulted in higher cane yield. The yields of cane were less in rest of the treatments with the lowest in the untreated check. Though chlorpyriphos @ 3lit ha^{-1} succeeded in recording the highest yield, the incremental benefit was less when compared with *M. anisopliae* at higher dose.

The highest per cent increase in yield over control was noticed in the treatment with chlorpyriphos @ 3lit ha^{-1} followed by *M. anisopliae* @ 4 x 10^9 conidia ha^{-1} whereas chlorpyriphos @ 2lit ha^{-1}, *Metarhizium* at lower dosage, carbofuran @ 33kg.ha^{-1} and neem cake @ 2.5q ha^{-1} recorded 36.15, 26.75, 25.78 and 22.81 per cent increase over untreated check, respectively. The economic analysis revealed that additional returns from chlorpyriphos @ 3lit ha^{-1} were found to be the highest followed by higher dosage of fungus whereas chlorpyriphos @ 2lit ha^{-1}, lower dosage of fungus, carbofuran @ 33kg. ha^{-1} and neem cake @ 2.5q ha^{-1} provided additional returns of Rs. 18,474/-Rs. 13,116/- Rs. 11,666/- and Rs. 9,815/- respectively.

The incremental benefit for every rupee investment was the highest in higher dosage of *M. anisopliae* (7.58) followed by chlorpyriphos @ 3lit ha^{-1} (6.09), whereas *M. anisopliae* at lower dosage, chlorpyriphos @ 2lit ha^{-1}, carbofuran @ 33kg ha^{-1} and neem cake @ 2.5q ha^{-1} recorded 5.72, 5.69, 3.78 and 3.33 incremental benefit on root grub management respectively.

The insect pathogenic fungi *M. anisopliae* and *B. 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). Use of fungal pathogens (Keller, 2000; Schweigkofler and Zelger, 2002) with different formulations such as fungus colonized grain or spore suspension (Keller *et al.*, 1997) are in use. New methods like application of spore powder during ploughing are yet to develop. The present findings are in line with observation on large scale field application of *M. anisopliae* @ 3.3 x 10^{13} conidia ha^{-1} against gray back cane grub in Australia. They have recorded 50-60 and 70 -90 per cent reduction in grub population in plant cane and next ratoon crop respectively (Samson *et al*., 1999). Further, application of *M. anisopliae* at higher dosage was as good as Fenthion in reducing root damage by *Lepidiota negatoria* in sugarcane as observed by Samson *et al*. (1999). *M. anisopliae* and *B. bassiana* @ 5 x 10^8conidia ha^{-1} along with chlorpyriphos @ 2lit. ha^{-1} was found effective in reducing grub population (Bhagat *et al*., 2003). Samuels *et al. (*1990) obtained higher cane yield by the application of *M. anisopliae*@1x10^9

Table 2. Cost effectiveness of *M. anisopliae* in the control of sugarcane white grub, *H. serrata*

Treatments	Millable canes at Harvest [000' ha]	Yield [t ha^{-1}]	% decrease over control	Gross Income Rs.	Economics		
					Additional Income Rs.	Cost Rs.	IBCR
M. anisopliae @ 4x10^9 conidia ha^{-1}	127.24	100.64[b]	42.38	95608	22890	3018	1: 7.58
M. anisopliae @ 1x10^9 conidia ha^{-1}	123.70	89.59[d]	26.75	85111	13116	2295	1: 5.72
Chlorpyriphos20EC@ 2 lit. ha^{-1}	122.00	96.23[c]	36.15	91419	18474	3245	1: 5.69
Chlorpyriphos20 EC @ 3 lit. ha^{-1}	135.80	110.53[a]	56.40	105004	30323	4981	1: 6.09
Carbofuran 3 G @ 33 kg ha^{-1}	119.70	88.90[d]	25.78	84455	11666	3089	1: 3.78
Neem cake @ 2.5 q ha^{-1}	112.30	86.80[e]	22.81	82460	9815	2945	1: 3.33
Untreated check	97.00	70.68[f]	--	--	--	---	---

In the present investigations, application of *M. anisopliae* at higher dosage was as effective as insecticides in reducing the grub population. As myco-pathogens persist in the soil for a longer period than chemicals, *M. anisopliae* can be an ideal choicee for the management of white grubs in endemic areas.

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