Journal of Biological Control, 24 (4): 317–321, 2010

Research Article

Field evaluation of *Metarhizium anisopliae* (Metschnikoff) Sorokin against white grubs in sugarcane and arecanut

U. H. KESARASING, R. K. PATIL and Y. H. SUJAY*
Department of Agricultural Entomology, College of Agriculture, University of Agricultural Sciences, Dharwad 580 005, Karnataka, India.
**Corresponding author E-mail*: morphosis77@gmail.com

ABSTRACT: Field experiments against root grubs in sugarcane and arecanut were laid out at Agricultural Research Station, Sankeshwar, Belgaum district and Sugavi village in Sirsi taluk of Uttar Kannada district of Karnataka, India, respectively. Application of *Metarhizium anisopliae* (Metschnikoff) Sorokin (Ma-1) against sugarcane white grub, *Holotrichia serrata* (Hope) at $1x10^{13}$ conidia ha^{-1} was found next best to chlorpyriphos and registered 91.95% reduction in grub population (60 DAT). The highest cane yield was recorded when *M. anisopliae* was applied @ $1x10^{13}$ conidia ha^{-1} (94.21t ha^{-1}) and it was on par with chlorpyriphos @ 3lit a.i. ha^{-1} (93.76t ha^{-1}). However, incremental benefit-cost ratio (IBCR) was high with higher dose of mycopathogen (7.83) followed by drenching of chlorpyriphos @ 2lit a.i. ha^{-1} (6.09). Application of *M. anisopliae* (Ma-1) against arecanut white grub, *Leucopholis lepidophora* (Blanchard) @ $2x10^{13}$ conidia ha^{-1} recorded 77.10% reduction in grub population and was next best to chlorpyriphos drenching @ 1 lit. a.i. ha^{-1} (96.80%).

KEY WORDS: Sugarcane, arecanut, *Holotrichia serrata, Leucopholis lepidophora, Metarhizium anisopliae,* field evaluation

(Article chronicle: Received: 20.03.2010; Sent for revision: 14.05.2010; Accepted: 19.07.2010)

INTRODUCTION

The grubs of scarabaeid beetles are known as 'white grubs' and are among the most serious pests of almost all cultivated crops (Veeresh, 1983). They are recognized as the most serious pests on sugarcane, groundnut, cereals, millets, pulses, vegetables (David *et al.,* 1986) and plantation crops. The yield loss due to white grubs was reported to be as high as 100 per cent in Karnataka (Veeresh, 1974) and 80 per cent in Maharashtra (Patil *et al.,* 1988). Among various species of white grubs, members of *Holotrichia* (Hope) are the most important and are known to attack a variety of crops mostly in the plains. Apart from *Holotrichia, Leucopholis* also contains few economically important species such as *Leucopholis lepidophora* (Blanchard), which causes damage to arecanut, coconut, sugarcane, paddy and groundnut in hilly areas of Western Ghats, Karnataka and Maharashtra.

Several tactics have been adopted for the management of white grubs including cultural, mechanical, biological, chemical and integrated methods as suggested by various workers from time to time (David *et al.,* 1986; Veeresh, 1977, 1984; Yadav, 1981; Yadav and Sharma, 1995). Application of chemicals is practically uneconomical, difficult and is associated with large number of problems. Hence, there is a strong impetus for the development of alternative strategies for the control of white grubs, which are ecofriendly and economically feasible. Rabindra *et al.* (2001) reported some 90 genera and 700 species of fungi, representing a large group of Entomophthorales (*Beauveria* spp., *Metarhizium* spp. and *Verticillium* spp.), which are entomopathogenic. Among these, *Metarhizium* is of greater importance in the management of white grubs. *Metarhizium anisopliae* (Metschnikoff) Sorokin can be effectively utilized as one of the components in the management of white grubs under integrated approach and in organic farming. *Beauveria bassiana* (Local), *B. bassiana* (commercial), *B. brongniartii* and *Metarhizium anisopliae* were found most effective against *Holotrichia* sp. at a spore concentration of $1x10^{8}$ spore ml^{-1} (Mohi–ud-din *et al.,* 2006). The application of *B. bassiana* and *M. anisopliae* at $5x10^{13}$ conidia ml^{-1} in combination with imidacloprid 200 SL at 48g a.i. ha^{-1} was found most effective against white grubs in Assam.

The fungus is not only eco-friendly and cost-effective, but also highly persistent and self-perpetuating in nature. In addition, sugarcane and arecanut ecosystems have the ideal microclimate for the fungus to multiply and heavy rainfall, high humidity and soil containing high organic matter helps the fungus to perpetuate itself in nature.

MATERIALS AND METHODS

Mass culturing of fungal cultures

Crushed rice grains (75 g) were taken in 500 ml saline bottle, 75 ml of distilled water containing yeast (1%) was added to the flask, thoroughly mixed and plugged with cotton. After soaking for 6 hrs, the bottles were sterilized in an autoclave at 15 psi pressure and 121°C for 20 minutes. After cooling, 3 ml of conidial suspension of *M. anisopliae* ($1x10^{8}$ conidia ml^{-1}) was added under aseptic condition using laminar air flow. Then the bottles were incubated at room temperature for 20 days at 26±1°C at 80% relative humidity. After 20 days digested material was harvested and dried under laminar air flow for 2 days. Then it was ground to powder and the conidia were further air dried to bring moisture level below 8 per cent. After sieving in 355 mesh sieve it was packed in polythene bags and stored in a refrigerator at 4°C for further use.

Field evaluation of M. anisopliae *against root grubs in sugarcane ecosystem*

A field experiment against sugarcane root grub was laid out at Agricultural Research Station, Sankeshwar, Belgaum district. The sugarcane variety Co-92020 was planted during January with a spacing of 0.9 m between rows with a plot size of 6x5.4 m^2 following all the recommended package of practices except for root grub management. There were seven treatments laid out in a randomized block design with three replications. The treatments were imposed in the first week of August. *M. anisopliae* ($5x10^{12}$ and $1x10^{13}$) was applied to the root zone of cane by mixing with vermicompost. Chlorpyriphos was soil drenched @ 2lit a.i. ha^{-1} by using a crowbar and phorate and neem cake were applied to soil near root zone. Observations were made separately on number of grubs per meter row in the root zone a day before and 15, 30, 45 and 60 days after imposition of treatments. Plant height (cm), number of millable canes and cane yield (t ha^{-1}) were recorded at harvest. The data obtained on different parameters were subjected to suitable statistical analysis.

Field evaluation of M. anisopliae *against root grub in arecanut ecosystem*

A field experiment in arecanut was laid out in a farmer's field at Sugavi village in Sirsi taluk of Uttar Kannada district. There were seven treatments laid out in randomized block design with three replications (three palms per replication). The treatments were imposed during August. *M. anisopliae* and *B. bassiana* were applied at the root zone of arecanut palm along with vermicompost and chlorpyriphos was applied by soil drenching. Observations were made on number of grubs per plant in the root zone a day before and 15, 30, 45 and 60 days after treatment. The data were subjected to suitable statistical analysis using DMRT.

RESULTS AND DISCUSSION

On sugarcane, chlorpyriphos @ 151 ha^{-1} was the most effective treatment at all the intervals of observation and registered cent per cent reduction in grub population at 45 and 60 days after treatment (DAT) (Table 1). At 15

Table 1. Efficacy of *M. anisopliae* against sugarcane white grub

Treatments	Root grubs/m row					
	1 DBT	15 DAT	30 DAT	45 DAT	60 DAT	Per cent decrease
M. anisopliae @ $5x10^{12}$ conidia ha^{-1}	8.00^{a} (3.82)	8.00^{d} (3.82)	7.00^{e} (3.64)	5.00^{e} (3.23)	4.33^{d} (3.09)	45.87 45.87
M. anisopliae @ $1x10^{13}$ conidia ha^{-1}	8.33^{a} (3.88)	8.00^{d} (3.82)	3.67^{d} (2.89)	1.67^{bcd} 1.67^{bcd}	0.67^{b} 0.67^{b}	91.95 91.95
Phorate @ 2.5kg a.i ha^{-1}	7.33^{a} (3.70)	2.67^{bc} (2.62)	2.00^{bc} (2.41)	1.33^{bc} (2.14)	0.67^{b} (1.67)	90.85 90.85
Neem cake 5q ha^{-1}	8.33^{a} (3.88)	8.00^{d} (3.82)	6.67^{e} (3.57)	4.33^{e} (3.07)	4.00^{c} (3.00)	51.98 51.98
Chlorpyriphos 3lit a.i ha^{-1}	7.66^{a} (3.76)	1.67^{a} (2.27)	0.67^{a} (1.66)	0.00^{a} (1.00)	0.00^{a} (1.00)	100.00 100.00
Chlorpyriphos 2lit a.i ha^{-1}	7.33^{a} (5.69)	2.33^{ab} (2.52)	1.67^{b} (2.29)	1.00^{b} (2.00)	0.67^{b} (1.67)	90.85 90.85
Control	8.66^{a} (3.94)	8.66^{d} (3.94)	8.50^{e} (3.91)	8.35^{f} (3.88)	8.15^{f} (3.85)	5.80 5.80

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

DAT, *M. anisopliae* @ 5x10^{12} conidia and 1x10^{13} conidia ha^{-1} failed to reduce the grub population significantly and found on par with untreated check. It is due to the fact that mycopathogens require more time to invade, establish themselves within their host and cause death of the host. Though the grubs were already infected by the fungus, they require time to produce external symptoms. On the day of second observation (30 DAT), *M. anisopliae* started to show its effect on the grubs and the fungus @ 1x10^{13} conidia ha^{-1} proved significantly superior to neem cake (@ 5g ha^{-1}), *M. anisopliae* @ 5x10^{12} conidia ha^{-1} and untreated control. It reduced the grub population to the extent of 3.67 grubs/m row. As days after treatment advanced, the effect of *M. anisopliae* (1.67 grubs/m row) also increased and it was on par with phorate @ 25kg ha^{-1} and chlorpyriphos @ 101 ha^{-1} (45 DAT). A similar trend was noticed at the final observation interval (60 DAT).

Application of *M. anisopliae* at higher dosage was as good as chemical insecticides (Fenthion) in reducing root damage by *Lepidiota negatoria* and recording higher sugarcane yield (Samson *et al.,* 1999). Large scale field trials to control gray back cane grub in sugarcane using *M. anisopliae* @ 3.3x10^{13} conidia ha^{-1} in Australia revealed 50-60 and 70-90 per cent reduction of pest population in plant cane and next ratoon crop, respectively (Robertson *et al.,* 1996; Logan *et al.,* 1999; Samson *et al.,* 1999). Application of talc based conidial formulations of *M. anisopliae* and *B. bassiana* at 5x10^{13} conidia ha^{-1} along with chlorpyriphos 20EC at 200g a.i. ha^{-1} was found effective exhibiting maximum reduction in plant mortality (75-80%) and tuber damage (63.7%) by way of controlling the grub population (Bhagat *et al.,* 2003). All these findings support the present investigation.

Cane yield varied significantly among the treatments, but all the treatments were significantly superior to control. The treatment with chlorpyriphos @ 3lit a.i. ha^{-1} (94.21t ha^{-1}) and *M. anisopliae* (93.76t ha^{-1}) at higher dosage resulted in higher cane yield. However, these two treatments did not differ significantly from phorate @ 2.5kg a.i. ha^{-1} (88.90t ha^{-1}) and chlorpyriphos @ 2lit a.i. ha^{-1} (88.65t ha^{-1}) but significantly superior to neem cake @ 5q ha^{-1} (79.18t ha^{-1}) and lower dosage of *Metarhizium* (78.75t ha^{-1}), which were on par (Table 2). The lowest cane yield (70.12t ha^{-1}) was recorded in the untreated check. Chlorpyriphos @ 3lit a.i. ha^{-1} and *M. anisopliae* @ 1x10^{13} conidia ha^{-1} recorded considerably higher cane yield (94.21 and 93.76t ha^{-1}, respectively). These treatments did not differ statistically from phorate @ 25kg ha^{-1} (88.90t ha^{-1}) and chlorpyriphos @ 2lit a.i. ha^{-1} (88.85t ha^{-1}). Though chlorpyriphos @ 3lit a.i. ha^{-1} succeeded in recording the highest yield, the incremental benefit was less when compared with the fungus.

The highest per cent increase in yield over control was noticed in the treatment with chlorpyriphos @ 3lit a.i. ha^{-1} (23.94%), followed by *M. anisopliae* @ 1x10^{13} conidia ha^{-1} (23.58%), whereas phorate @ 2.5kg a.i. ha^{-1}, chlorpyriphos @ 2lit a.i. ha^{-1}, neem cake @ 5q ha^{-1} and *Metarhizium* at lower dosage recorded 19.40, 19.35, 9.43 and 9.01 per cent increase over untreated control, respectively.

On economic analysis of the treatments in the management of root grubs in sugarcane, additional returns from chlorpyriphos @ 3lit a.i. ha^{-1} were found to be the as highest (Rs. 17,596/-) followed by higher dosage of fungus (Rs. 17,245/-), whereas phorate @ 2.5kg a.i. ha^{-1}, chlorpyriphos @ 2lit a.i. ha^{-1}, neem cake @ 5q ha^{-1} and lower dosage of fungus provided additional returns of

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

Treatments	Plant height (cm)	Milleble canes at harvest (000'ha)	Yield (t ha^{-1})	Per cent increase over control	Gross returns (Rs.)	Additional income		IBCR
						Returns (Rs.)	Cost (Rs.)	
M. anisopliae @ 5x10^{12} conidia ha^{-1}	169.00	99.56^{b}	78.75^{ab}	9.01	61425	5538	1200	4.61
M. anisopliae @ 1x10^{13} conidia ha^{-1}	171.83	114.06^{d}	93.76^{de}	23.58	73132	17245	2200	7.89
Phorate @ 2.5kg a.i. ha^{-1}	171.58	110.56^{d}	88.90^{bcd}	19.40	69342	13455	2450	5.49
Neem cake 5q ha^{-1}	167.50	110.05^{bc}	79.18^{abc}	9.43	61760	5873	1700	3.45
Chlorpyriphos 3lit a.i. ha^{-1}	172.41	115.75^{d}	94.21^{de}	23.94	73483	17596	3200	5.49
Chlorpyriphos 2lit a.i. ha^{-1}	169.41	109.78^{cd}	88.85^{bcd}	19.35	69303	13416	2200	6.09
Control	166.48	96.23^{a}	70.12^{a}	–	–	–	–	–

Rs. 13,455/-, Rs. 13,416/- Rs. 5,873/- and Rs. 5,538/-, respectively.

The incremental benefit for every rupee investment was highest in higher dosage of *M. anisopliae* (7.89), followed by chlorpyriphos @ 2lit a.i. ha^{-1} (6.09). Phorate @ 25kg ha^{-1}, chlorpyriphos @ 3lit a.i. ha^{-1}, *M. anisopliae* at lower dosage and neem cake @ 5q ha^{-1} recorded 5.49, 5.49, 4.61 and 3.45 incremental benefit on root grub management, respectively.

The present findings are in line with Samuels *et al.* (1990), who obtained significantly higher yield by the application of *M. anisopliae* @ $1x10^{15}$ spores ha^{-1}. In the present investigations, application of *M. anisopliae* @ $1x10^{13}$ conidia ha^{-1} was as effective as insecticides in reducing the grub population. As mycopathogens persist for a longer period than chemicals, *M. anisopliae* can be an ideal candidate for control of such pests which are endemic in nature.

Chlorpyriphos @ 5l ha^{-1} proved to be the best treatment against *L. lepidophora* in arecanut at all observation intervals (15, 30, 45 and 60 DAT) whereas at 15 DAT *M. anisopliae* at four different dosages and *B. bassiana* did not cause any significant reduction in grub population and were found on par with control (Table 3). As time after imposition of treatment was prolonged the effect of mycopathogens more pronounced and significantly superior to untreated control at its lower doses. At the final observation interval (60 DAT), *M. anisopliae* @ $2x10^{13}$ conidia ha^{-1} proved next best to chlorpyriphos and was significantly superior to all other treatments. Superiority of chlorpyriphos in the present study is in agreement with Kumar (1997) with respect to *L. lepidophora* in arecanut ecosystem. Studies related to white grub management using mycopathogens are lacking in arecanut in India and elsewhere. However, contradictory to the present investigation, studies conducted on sugarcane root grubs (Samson *et al.,* 1999) reported that application of *M. anisopliae* was as good as chemical insecticides in reducing root damage by *L. negatoria.* It might be due to very the meager precipitation received during the experimental period and no irrigation was provided, as a result of which the grubs remained deep in soil for a long period, which made them to escape from the fungus.

At 60 DAT, *M. anisopliae* @ $2x10^{13}$ conidia ha^{-1} caused 86.2 per cent mortality of early instar grubs, 81.74 per cent second instar and 60.06 per cent of third instar grubs. As there is overlapping of generations of *L. lepidophora* in the field, it is advisable to incorporate the fungus culture with the onset of monsoon when the beetles emerge in large number and lay eggs. As the early instar feeds on decaying matter and is more susceptible to the pathogen, it will be easily infected. Further the pathogen will be multiplied in the soil because of early infection, helping to build up the inoculum in the soil. This will help to take care of older larvae which require higher inoculum than the younger larvae.

Of two dosages of *M. anisopliae* ($5x10^{12}$ and $1x10^{13}$ conidia ha^{-1}) evaluated against sugarcane root grub, higher dosage of *M. anisopliae* ($1x10^{13}$ conidia ha^{-1}) was found

Table 3. Management of arecanut white grub, *Leucopholis lepidophora* using *M. anisopliae*

Treatments	Root grubs/m row					
	1 DBT	15 DAT	30 DAT	45 DAT	60 DAT	Per cent decrease
M. anisopliae @ $5x10^{12}$ conidia ha^{-1}	10.34 (4.21)	10.34 b (4.21)	9.83 bcd (4.13)	9.33 cde (4.05)	8.47 cd (3.91)	18.08 18.08
M. anisopliae @ $1x10^{13}$ conidia ha^{-1}	11.00 (4.31)	11.00 b (4.31)	9.66 bcd (4.10)	9.00 cd (4.00)	7.33 cd (3.70)	33.36 33.36
M. anisopliae @ $1.5x10^{13}$ conidia ha^{-1}	10.67 (4.26)	10.34 b (4.21)	8.67 bc (3.94)	7.33 c (3.70)	6.00 c (3.44)	43.76 43.76
M. anisopliae @ $2x10^{13}$ conidia ha^{-1}	11.66 (4.41)	10.33 b (4.21)	7.33 b (9.70)	4.67 b (3.16)	2.67 b (2.63)	77.10 77.10
B. bassiana @ $1x10^{13}$ conidia ha^{-1}	10.01 (4.16)	10.01 b (4.16)	9.50 bcd (4.08)	9.00 cd (4.00)	8.17 cd (3.85)	18.38 18.38
Chlorpyriphos @ 1lit a.i. ha^{-1}	10.34 (4.21)	1.33 a (2.15)	0.33 a (1.57)	0.33 a (1.57)	0.00 a (1.00)	100.00 100.00
Control	11.99 (4.46)	11.99 b (4.46)	11.91 d (4.45)	11.91 de (4.45)	11.58 f (4.40)	3.41 3.41

DBT: Days before treatment; DAT: days after treatment; means followed by the same alphabets in vertical columns did not differ significantly ($P = 0.05$) by DMRT next best to chlorpyriphos treatment and registered 91.95 per cent reduction in grub population (60 days after treatment). The highest cane yield was obtained in chlorpyriphos @ 3lit a.i. ha^{-1} and *M. anisopliae* @ $1x10^{13}$ conidia ha^{-1} (94.21 and 93.76 t ha^{-1}, respectively) with the maximum gross return of Rs. 73,480/- and Rs. 73,132/-, respectively. However, incremental benefit to every rupee investment was high with higher dosage of fungus (7.83) followed by chlorpyriphos @ 10l ha^{-1} (6.09).

Field evaluation of *M. anisopliae* against root grubs in arecanut revealed that at 60 days after application of *M. anisopliae* @ $2x10^{13}$ conidia ha^{-1} recorded 86.52, 81.74 and 60.06 per cent mortality of first, second and third instar grubs, respectively. It has registered 77.10 per cent reduction in grub population and was next best to drenching of chlorpyriphos 1lit a.i. ha^{-1}, which was found most effective by recording per cent reduction in the grub population.

ACKNOWLEDGEMENT

The authors are thankful to the University of Agricultural Sciences, Dharwad for providing necessary facilities for the investigations.

REFERENCES

Bhagat, R. M., Gupta, R. B. L. and Yadav, C. P. S. 2003. Field efficacy of two entomopathogenic fungal formulations against whitegrubs in Himachal Pradesh. *Indian Journal of Entomology*, **65**: 76–81.

Bhattacharyya, B., Baruah, A. A. L. H., Das, P. and Bhuyan, U. 2008. Field efficacy of *Beauveria bassiana* (Bals.) Vuill. and *Metarhizium anisopliae* (Metch.) Sorok. against white grub in Assam. *Journal of Biological Control*, **22**: 81–84.

David, H., Nandagopal, V. and Anantha Narayana, K. 1986. Recent studies on the control of white grub, *Holotrichia serrata* infesting sugarcane. *Journal of Soil Biology and Ecology,* **6**: 117–127.

Kumar, A. R. V. 1997. Bioecology and management of arecanut whitegrubs, *Leucopholis* spp. (Coleoptera: Scarabaeidae) in Karnataka. Ph.D. Thesis, University of Agricultural Sciences, Bangalore, India.

Lingappa, S. and Patil, R. K. 2002. *Nomuraea rileyi – A Potential Mycoinsecticide.* University of Agricultural Sciences, Dharwad, India, 30 pp.

Logan, D. P., Roberson, L. N. and Milner, R. J. 1999. Review of the development of *Metarhizium anisopliae* as a microbial insecticide. *Proceedings of the 2nd IOBC/WPRS Meeting on Melolantha,* **23**: 131–137.

Mohi-ud-din, S., Zaki, F.A., Munshi, N. A., Jan, A. and Sultan, P. 2006. Evaluation of some entomopathogenic fungal isolates from Kashmir for the biocontrol of white grub infesting turf in golf course. *Journal of Biological Control*, **20**: 45–49.

Patil, A. S., Hapase, D. G., Kangane and Jaiswa, P. M. 1988. Insecticidal control of root borer in sugarcane, pp. 129–153. In: *Proceedings of 51 Annual Convention,* STAI.

Robertson, L. N., Dall, D. J., Lai-Fook, J. and Walker, P. W. 1996. Disease and micro-organism incidence in grey back cane grub in Australian sugarcane, pp. 209–215. In: Granados, R. R. and Jockson, T. A. (Eds.). *Use of Pathogens in Scaraba Pest Management*, Intercept, Andover, Australia.

Samson, P. R., Milner, R. J., Bullard, G. K. and Hogorth, D. M. 1999. Development of *Metarhizium* based biopesticides for sugarcane pest management, current progress and future prospects, pp. 156-163. In: *Proceedings of the 1999 conference of the Australian Society of Sugarcane Technologists,* Town Ville, Queensland, Australia.

Samuels, K. D. Z., Dinnock, D. E. and Bull, R. M. 1990. Scarabaeid larvae control in sugarcane using *Metarhizium anisopliae* (Met.) Sorokin. *Journal of Invertebrate Pathology,* **55**: 135–137.

Veeresh, G. K. 1974. War against white grubs. *Current Research,* **3**: 87–88.

Veeresh, G. K. 1977. Root grub control campaign in Karnataka. *White Grub Newsletter,* **1**: 28–33.

Veeresh, G. K. 1983. White grubs. In: Veeresh, G. K. and Rajagopal, D. (Eds.). *Applied Soil Biology and Ecology* Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, India.

Veeresh, G. K. 1984. Management of white grubs in sugarcane cropping system. *Journal of Soil Biology and Ecology,* **4**: 124–131.

Yadav, C. P. S. 1981. Final Research Report of ICAR scheme. Integrated control of white grubs. Department of Entomology, University, Udaipur, Rajasthan, India, 219 pp.

Yadav, C. P. S. and Sharma, G. K. 1995. *Indian white grubs and their management.* Indian Council of Agricultural Research, New Delhi, India, *Technical Bulletin No.2*, 26 pp.