Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2021; 9(1): 1207-1209

Received: 10-11-2020
Accepted: 12-12-2020

Rinni Saharwat
Foundation for Agricultural Resources Management and Environmental Remediation (FARMER) SJ 14, B Block, Shastri Nagar, Ghaziabad, Uttar Pradesh, India

Riazuddin
Foundation for Agricultural Resources Management and Environmental Remediation (FARMER) SJ 14, B Block, Shastri Nagar, Ghaziabad, Uttar Pradesh, India

Seema Rani
Foundation for Agricultural Resources Management and Environmental Remediation (FARMER) SJ 14, B Block, Shastri Nagar, Ghaziabad, Uttar Pradesh, India

Jagpal Singh
Foundation for Agricultural Resources Management and Environmental Remediation (FARMER) SJ 14, B Block, Shastri Nagar, Ghaziabad, Uttar Pradesh, India

Corresponding Author:
Jagpal Singh
Foundation for Agricultural Resources Management and Environmental Remediation (FARMER) SJ 14, B Block, Shastri Nagar, Ghaziabad, Uttar Pradesh, India

Pathogenicity of entomopathogenic fungi *Metarhizium anisopliae* to white grubs *Holotrichia serrata*

Rinni Saharwat, Riazuddin, Seema Rani and Jagpal Singh

DOI: https://doi.org/10.22271/j.ento.2021.v9.i1q.8303

Abstract
Holotrichia serrata species of white grub is one of the major pests in sugarcane causing severe damage to kharif crops by its larval stages which lives inside the soil and causes quantifiable losses in the crops. Their infestation has been reported across the country and incidence is increasing every year. Current suppression strategy is mainly strategized with the use of chemical pesticides; however, none of them are found effective by farmers in lowering down their population below ETL. The biological factors that influence populations of white grubs' complex are relevant to the potentiality of the biological control with soil fungi. *Metarhizium anisopliae* is an entomopathogenic fungus that occurs naturally in soils and causes disease in various insect pests. Laboratory assays were done to measure the pathogenicity of *M. anisopliae* against *H. serrata*. *M. anisopliae* identified as pathogenic for eggs to adult stage of test insect pest at higher dose 1010 spores/ml. The result showed that higher doses achieving greater killing in eggs and much effective for 1st and 2nd instar grub, pupal and adult stage after 20 days of treatment; however it is not recorded to be effective against 3rd instar of grub at this doze.

Keywords: white grubs, *Holotrichia serrata*, entomopathogenic fungi, *Metarhizium anisopliae*, bioassay

Introduction
Metarhizium anisopliae is an entomopathogenic fungus that grows naturally in soils throughout the world and causes disease in various insects pests like root grubs, termites, and locusts by acting as a parasitoid. The disease caused by the fungus is sometimes called green muscardine disease because of the green colour of its spores. When these asexual spores (conidia) of the fungus come into contact with the body of an insect host, they germinate and the hyphae that emerge penetrate the cuticle. The fungus then develops inside the body, eventually killing the insect, after a few days the cuticle of the cadaver often becomes red. If the ambient humidity is high enough, a white mould then grows on the cadaver that soon turns green as spores are produced. Control is achieved through the induction of a fungal epizootic where new spores and vegetative cells produced in infected insects are spread to healthy members of the population.

In India, the white grubs are pests of national importance and *H. serrata* is one of the main white grub species involved in various crops especially in sugarcane. Larval stages live inside the soil and causes poorly quantifiable losses in the crops. Their infestation has been reported across the country and incidence is increasing every year. Control is mainly targeted with the use of chemical pesticides; however, none of them are effective in lowering down their population. The biological factors that influence populations of white grubs' complex are relevant to the potentiality of the biological control with soil fungi. The present study deal with isolation, identification of entomopathogenic fungus and its effectiveness against various developmental stages of *H. serrata* under controlled conditions and field tests on sugarcane based cropping areas so as to reduce the dependence on chemical pesticides and, therefore, lower cost of production in the long run.

Materials and Methods
The nucleus culture of *M. anisopliae* collected from National Center for Integrated Pest Management, New Delhi. Further mass multiplication was done in laboratory in low cost fermenter designed by FARMER.

Rearing of *H. serrata*

The field collected adult beetles of *H. serrata* were placed in desiccators containing moist soil for oviposition and monitored eggs population laid by beetles on alternate days. The eggs were collected by sieving with 5 meshes on trays and then transferred to Petri-plates containing moist soil. After hatching the neonates were transferred in to individual plant pots on live roots of maize up to pupation. The rearing was carried out at temperature 25 ± 2 0C and humidity 65 ± 5% RH.

Bioassay with M. anisopliae

Laboratory bioassays were performed to assess the infective potential of *M. anisopliae* on *H. serrata* species of white grubs prevalent in the study sites. The insect bioassay was performed using the dipping method, with eggs, 1st, 2nd and 3rd instars larvae of *H. serrata*. The white grubs larvae used in the experiment were reared in laboratory and observations were taken after five days intervals up to 20 days. The fungus culture was used as fresh. The observation units, treated white grubs were replicated each with 10 white grubs per replication and single larvae in each pot were treated with a single dose and positive check with water treatment.

Two types of bioassays, the time and dose mortality bioassay were performed under laboratory conditions. Dipping (5 seconds) method was followed for each individual concentration of the

M. anisopliae. Excess liquid was dropped off the grubs and they were placed individually in petridish and incubated at 270 C until recording time. The experiment was conducted up to 20 days and the fungus invading in each treatment was determined under the Leica S9i stereomicroscope where fungal infection was checked daily. In dose mortality experiment, three different doses of *M. anisopliae* such as 104 spores/ ml, 106 spores/ ml, 1010 spores/ ml and one untreated treatments were tested. Mortality due to the original fungus was recorded.

Statistical analyses

Cumulative mortality was corrected for natural mortality using Abbott's formula [1]. Excel software was used to draw graph.

Mass production of the insect pathogenic fungi

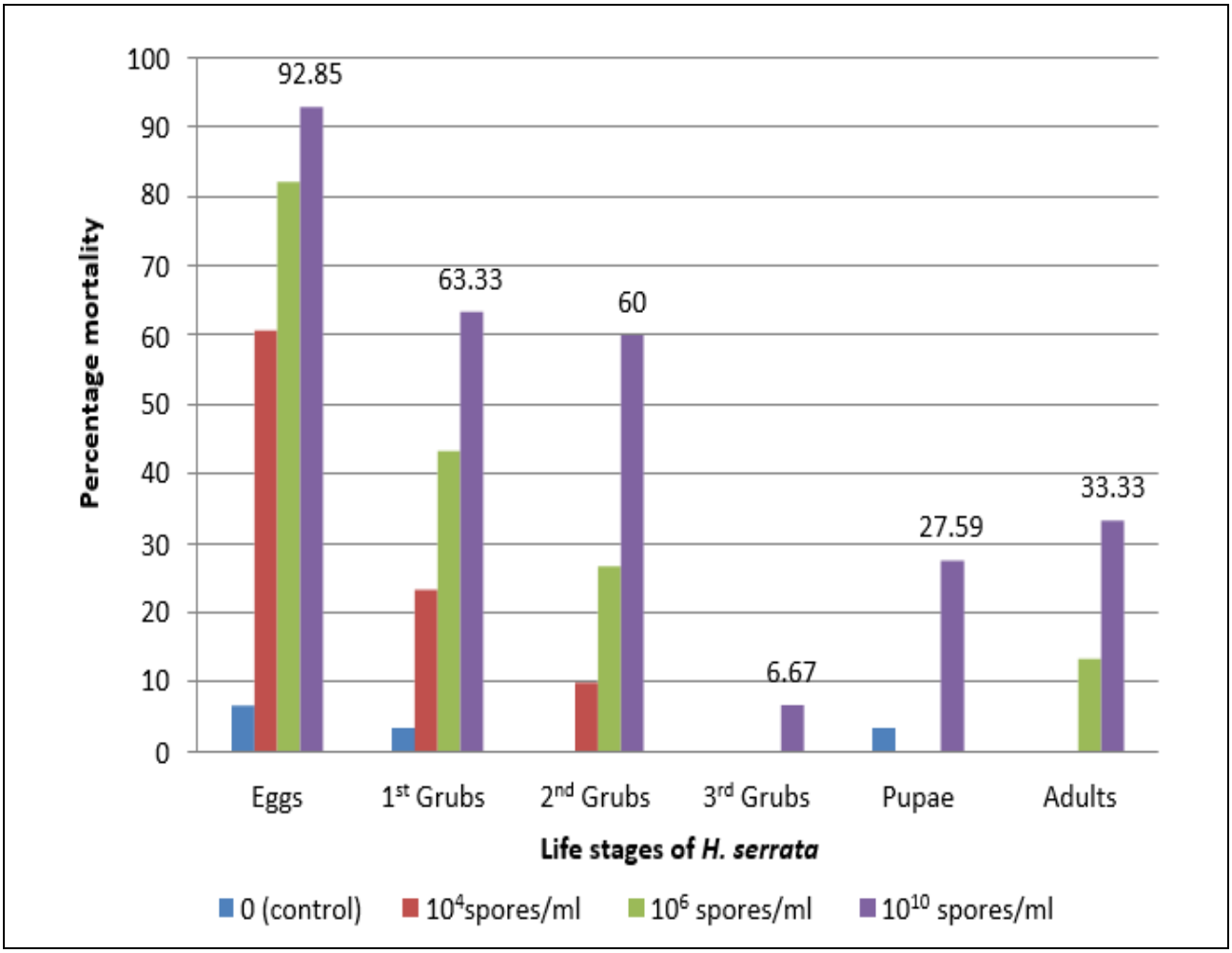
A mass production system for the development of an entomopathogenic fungus as a microbial control agent was set up for rapid multiplication of promising isolates for field application as and when required.

Results and Discussion

The results obtained from the present investigation are summarized below in table and graph. The *M. anisopliae* at higher dose (1010 spores/ml) was found effective on eggs, 1st, 2nd instar grub, pupae and adults, however not found effective against 3rd instars grubs of *H. serrata.* The test fungicide find most effective against eggs of *H. serrata* as achieving highest mortality 60.71%, 82.14% and 92.85% at 104, 106 and 1010 spores/ml inoculation respectively. The cumulative mortality after 20 days of inoculation was recorded as 23.33%, 43.33% and 63.33% in case of 1st instar grub and 10.0%, 26.67% & 60.0% in 2nd instar grub at 104, 106 and 1010 spores/ml inoculation, respectively; however not found effective against 3rd instar grub of *H. serrata* up to 20 days after treatment. The results are similar with the earlier studied [2]; *M. anisopliae* was evaluated against 3rd instar larvae of *H. serrata* under laboratory and field conditions and found that the test fungi was pathogenic to the test pest at varying degrees. Pupae (27.59%) and adults mortality (33.33%) also recorded at higher dose 1010 spores/ml. These findings are in tune with studies 8 local isolates of *M. anisopliae* against white grub and recorded more than 80% mortality at 107 conidia/ml concentration however they not mentioned the grub instars in their studies [3]. Therefore, the effective dose in laboratory killing of the grubs could be targeted from 1010 spores/ml against each stage of *H. serrata* except 3rd instar grub. Earlier studies also reported 55% mortality in *Leucopholis lepidophora* (white grub) at 15 days after treatment of *M. anisopliae* [4]. Application of *M. anisopliae* at higher dosage was as good as synthetic pesticide Fention in reducing root damage by *Lepidiota negatoria* in sugarcane as reported [5]. Earlier studied recommended *M. anisopliae* as one of the major components which can effectively utilized in the management of white grub [6]. Entomopathogenic funguses which need shorter exposure period and kill the host quickly are very important in the practical application and *M. anisopliae* is found most effective on earlier stages viz., eggs and 1st & 2nd grubs of *H. serrata* in shortest period of 20 days.

Table 1: Effect of different doses of *M. anisopliae* against different life stages of *H. serrata*

Dose	% mortality in *Holotrichia serrata* grub								
	1st Grubs			2nd Grubs			3rd Grubs		
	Days after treatment			Days after treatment			Days after treatment		
	10	15	20	10	15	20	10	15	20
0 (control)	3.33	3.33	3.33	0	0	0	0	0	0
104spores/ml	0.00	13.33	23.33	6.67	10.00	10.00	0	0	0
106 spores/ml	3.45	16.67	43.33	10.00	13.33	26.67	0	0	0
1010 spores/ml	6.90	20.00	63.33	13.33	16.67	60.00	0	0	6.67
Dose									
	Eggs			Pupae			Adults		
	Days after treatment			Days after treatment			Days after treatment		
	10	15	20	10	15	20	10	15	20
0 (control)	3.33	3.33	6.67	0	3.33	3.33	0	0	0
104spores/ml	0.0	0.0	60.71	0	0	0	0	0	0.00
106 spores/ml	6.9	48.28	82.14	0	0	0	3.33	6.67	13.33
1010 spores/ml	17.24	58.62	92.85	0	0	27.59	16.67	26.67	33.33



Graph 1: Comparative effect of *M. anisopliae* in different life stages of *H. serrate* after 20 days of treatment

Conclusion

Entomopathogenic nematodes *Metarhizium anisopliae* has a great potential for the management of white grub on sugarcane and other crops as it is effective to kill different stages of *H. serrata*, a predominant species of white grub prevailing in sugarcane growing areas. The application of compost enriched with effective dose of Entomopathogenic fungi *M. anisopliae* will give promising results by controlling white grub pest at eggs and early growing stages of white grub and preventing damage to the crops. *M. anisopliae* can give promising results by using as biological control agents against different soil born insect pests however; their infectivity is quite different depending on its native strains, time of exposure and developmental stage of the insects. This entomopathogenic fungus has regenerative capability in the natural environment and there is no scope of resistance build up by the insect pest, as has been observed in chemical pesticides. The preliminary studies has indicated ample opportunity of proceeding ahead with the fungal based bio-pesticide in sugarcane growing areas of western Uttar Pradesh and other states in targeting white grubs in particular and soil-dwelling insect pests in general.

Acknowledgement

The authors are thankful to; Dr. A. S. Baloda, Coordinator, AINP-ICAR Project, RARI, Rajasthan for valuable scientific guidance, Indian Council of Agriculture Research, Government of India for financial support to conduct the research and Dr. T. P. Rajendran, former Asst DG (PP), for valuable guidance and review of the manuscript.

References

1. Abbott WS. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology 1925; 18:265-267.
2. Chelvi CT, Thilagaraj WR, Kandasamy R. Laboratory and field efficacy of entomopathogenic fungi *Beauveria brongniarti*, *Beauveria bassiana* and *Metarhizium anisopliae* in the control of sugarcane white grubs (*Holotrichia serrata*). Biopesticides International 2010;6(1):85-88.
3. Yubak DGC, Siegfried K, Peter N, Lekhnath K. Virulence of *Metarhizium anisopliae* and *Beauveria bassiana* Against Common White Grubs in Nepal. Formosan Entomol 2008;28:11-20.
4. Shewale CP, Mohite PB. Combined efficacy of entomopathogenes and insecticides against white grub, *Leucopholis lepidophora* (Blanchard) infesting sugarcane *in vitro*. Journal of Entomology and Zoology Studies. 2018;6(2):1824-1827.
5. Samson PR, Milner RJ, Ballard GK, Hogorth DM. Development of *Metarhizium* as biopesticides for sugarcane pest management, current progress and future prospects 1999, 63.
6. Chroton P. Insect pest control by entomopathogenic fungi. Mycologia 2007;16(2):23-27.