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Dose Optimization of Promising Entomopathogenic Fungi Against White Grub (*Holotrichia* sp.)

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Abstract: The three bio-control agents, viz. *Beauveria bassiana*, *B. brongniartii* and *Metarhizium anisopliae* proved pathogenic at each level of concentration (1×10^8 , 1×10^6 , 1×10^4 and 1×10^2 spore mL⁻¹) studied, but mortality in a shorter duration was observed at a spore concentration of 1×10^8 spore mL⁻¹. Enhanced mortality was observed with increase in inoculum dose.

Key words: *Beauveria bassiana*, *Holotrichia* sp., concentration, 1×10^8 spore mL⁻¹

INTRODUCTION

White grub is the immature stage of scarab beetles popularly known as cock chaffers, leaf chaffers, chaffer beetles, May or June beetles. The phytophagous chaffers are highly evolved as compared to scavengers. It is this grub which is causing concern to agriculturists throughout the world. The extent of damage caused by white grub's ranges from 40-80% (Mathur and Upadhyay, 1985). Considering the economic importance of the pest it has become necessary to develop viable control strategies against the pest, that is eco- friendly and sustainable method of pest control. Sustainable agriculture in the 21st century will rely increasingly on the alternative methods for pest management that are sustainable and environmentally friendly. One of the most promising bio control agent, entomopathogenic fungi are important as they are virulent, infect by contact, persist in environment for long time and have one of the largest host list (Santharam, 2001). Rabindra *et al.* (2001) reported some 90 genera and 700 species of fungi, representing a large group of Entomophorales (*Beauveria* sp., *Aspergillus* sp., *Fusarium* sp., *Metarhizium* sp. and *Verticillium* sp.) involved with entomopathogenicity. There is a continuous need to discover and develop new entomopathogens, if we are to meet the future challenges of sustainable food and fibre production with concomitant reduction in use of chemical pesticides. Considering the importance of the pest and loopholes in different methods of insect control, the present studies have been carried out.

MATERIALS AND METHODS

The studies on determination of minimum dose requirement of promising bio-control agents against white grubs for maximum mortality were carried out at Regional Research Laboratory (CSIR) Sanatnagar (Srinagar). The bio-control agents which were evaluated include *Beauveria bassiana*, *B. brongniartii* and *Metarhizium anisopliae*. Different concentrations viz., 1×10^8 , 1×10^6 , 1×10^4 and 1×10^2 spore mL⁻¹ were tested. The spore suspension was prepared from freshly grown cultures of the fungus cultured on Samsankova medium. Different concentrations were prepared with the help of hemocytometer. In all there were 13 treatments including a control and each treatment was replicated thrice in CRD. Third stage healthy grubs collected from Kashmir Golf course were used in this experiment, after surface sterilization with distilled sterile water.

In each replicate 10 grubs were allowed to move on 90 mm Petri plate and sprayed with 5 mL of spore suspension except in control where only sterilized water was sprayed. The inoculated grubs were then transferred to sterilized glass beakers containing sterilized soil and were allowed to feed on surface sterilized grass roots (*Cynodon dactylon*). All the beakers were sprayed with distilled sterilized water to keep the soil moist and kept in a BOD incubator at 27 ± 1 °C. The mortality of grubs was recorded after every 48 h and the percent mortality was computed on the basis of total grubs in each experiment.

The data in case analysed by the method of Analysis of Variance advocated by Snedcorn and

Cocran, (1967) after necessary transformations and means were compared by critical difference at $p = 0.05$.

RESULTS AND DISCUSSION

The results (Table 1) showed that *Beauveria bassiana* imposed an initial mortality of 50 and 46.66% on 6th day at a concentration of 1×10^8 and 1×10^6 spore mL, respectively. Initial mortality of 36.66, 30.00% and 33.33, 26.66% at 1×10^8 and 1×10^6 spore mL was observed in *B. brongniartii* and *Metarrhizium anisopliae*, respectively. These results showed that initial grub mortality at higher concentrations preceded to that in lower concentrations where it took some time more to cause initial mortality. Cent percent mortality occurred, within shorter time at higher concentrations as compared with lower concentrations. *B. bassiana* showed

complete mortality on 14th day followed by 18th day in *B. brongniartii* and *M. anisopliae* at 1×10^8 spore mL⁻¹. In their subsequent lower concentration it took some more time to cause cent percent mortality. The results showed that the mortality, in general in each treatment was recorded in the order of $1 \times 10^8 > 1 \times 10^6 > 1 \times 10^4 > 1 \times 10^2$ spore mL⁻¹. In control, some mortality was recorded on 10th day of experiment, which increased to a maximum of 10% on 14th day and remained unchanged till termination of the experiment. The mortality was due to natural death and may be due to repeated handling of experimental materials. The enhanced mortality with increase in dose of inoculum is in accordance with the findings of Vimala (1994) in case of *Nomuraea rileyi* tested against *Spodoptera litura* on castor and Shashi Sharma *et al.* (1999) against white grub *Holotrichia consanguinea* and *Maladera insanabilis*.

Table 1: Effect of different dose concentrations promising entomopathogenic fungi against white grub (*Holotrichia* sp.)

Treatments	Conc.	Days after inoculation				
		2	4	6	8	10
Mean mortality (%)						
<i>Beauveria bassiana</i> (local)	1×10^8	0.00	0.00	50.00 (45.00) ^a	66.66 (54.78) ^a	73.33 (59.00) ^a
	1×10^6	0.00	0.00	46.66 (43.05) ^a	63.33 (52.77) ^a	73.33 (59.00) ^a
	1×10^4	0.00	0.00	0.00 (0.90) ^d	33.33 (35.21) ^{cd}	53.00 (46.92) ^b
	1×10^2	0.00	0.00	0.00 (0.90) ^d	23.33 (28.78) ^{bd}	30.00 (33.00) ^{cd}
<i>Beauveria brongniartii</i>	1×10^8	0.00	0.00	36.66 (37.93) ^a	53.33 (46.92) ^{ab}	60.00 (52.77) ^{ab}
	1×10^6	0.00	0.00	30.00 (33.00) ^d	36.66 (37.14) ^c	46.66 (43.07) ^{bc}
	1×10^4	0.00	0.00	0.00 (0.90) ^d	23.33 (28.78) ^{bd}	36.66 (37.22) ^c
	1×10^2	0.00	0.00	0.00 (0.90) ^d	16.66 (23.85) ^e	23.33 (28.78) ^d
<i>Metarrhizium anisopliae</i>	1×10^8	0.00	0.00	33.33 (35.21) ^a	46.66 (43.07) ^a	53.33 (46.00) ^b
	1×10^6	0.00	0.00	26.66 (31.09) ^{ab}	33.33 (35.21) ^{cd}	46.66 (43.07) ^{bc}
	1×10^4	0.00	0.00	20.00 (26.07) ^{bc}	30.00 (33.00) ^d	36.66 (37.14) ^c
	1×10^2	0.00	0.00	13.33 (12.14) ^{cd}	23.33 (28.78) ^d	33.33 (35.21) ^a
Control		0.00	0.00	0.00 (0.90) ^d	0.00 (0.90) ^f	6.67 (14.97) ^e
SE±CD at 5%				6.91 14.21	3.85 7.92	4.72 9.80

Table 1: Continued

Treatments	Conc.	Days after inoculation				
		12	14	16	18	20
Mean mortality (%)						
<i>Beauveria bassiana</i> (local)	1×10^8	89.99 (71.55) ^a	100.00 (89.04) ^a	100.00 (89.04) ^a	100.00 (89.04) ^a	100.00 (89.04) ^a
	1×10^6	86.65 (68.5) ^a	93.31 (75.01) ^a	100.00 (98.04) ^a	100.00 (89.04) ^a	00.00 (89.04) ^a
	1×10^4	63.33 (65.77) ^c	70.00 (56.99) ^c	83.32 (65.77) ^b	89.99 (71.55) ^b	100.00 (89.04) ^a
	1×10^2	40.00 (39.14) ^e	46.66 (43.07) ^d	53.33 (46.92) ^d	56.66 (48.84) ^c	63.33 (52.77) ^b
<i>Beauveria brongniartii</i>	1×10^8	73.33 (59.00) ^{ab}	86.65 (68.55) ^b	93.31 (75.01) ^{ab}	100.00 (89.09) ^a	100.00 (89.09)
	1×10^6	63.33 (52.77) ^b	73.33 (59.00) ^c	83.32 (65.77) ^b	93.31 (75.01) ^{ab}	100.00 (89.09) ^a
	1×10^4	46.66 (43.07) ^a	60.00 (50.85) ^{cd}	70.00 (56.99) ^c	86.65 (68.37) ^b	100.00 (89.04) ^a
	1×10^2	33.33 (35.21) ^e	40.00 (39.14) ^d	46.66 (43.07) ^d	56.66 (58.84) ^c	60.00 (50.85) ^{cd}
<i>Metarrhizium anisopliae</i>	1×10^8	66.66 (54.99) ^b	80.00 (63.93) ^{bc}	93.32 (75.40) ^a	100.00 (89.04) ^a	100.00 (89.04) ^a
	1×10^6	56.66 (48.84) ^{bc}	66.66 (54.78)	80.00 (63.93) ^{bc}	89.99 (71.55) ^a	100.00 (89.04) ^a
	1×10^4	50.00 (45.00) ^e	60.00 (50.85) ^{cd}	66.66 (54.78) ^{cd}	83.33 (66.14) ^b	100.00 (89.04) ^a
	1×10^2	40.00 (39.14) ^e	46.66 (43.07) ^a	53.66 (46.93) ^d	56.66 (48.84) ^c	60.00 (50.85) ^{cd}
Control		6.67 (14.97) ^d	10.00 (18.43) ^e	10.00 (18.43) ^e	10.00 (18.43) ^d	10.00 (18.43) ^d
SE±CD at 5%		6.72 13.81	6.97 14.33	7.55 15.52	6.82 14.03	2.04 4.21

Each figure is the mean of three replicates with 10 grubs each, Data in parenthesis is the arcsine transformed value, The value in individual columns superscripted by similar letter(s) do not differ significantly

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REFERENCES

- Mathur, Y.K and K.D. Upadhyay, 1985. Life history and control measures of white grubs. In: Textbook of Entomology. Goel Printing Press, Meerut, pp: 133-136
- Rabindra, R.J., P. Muthulakashmi, G. Ramakrishnan and H.D. Sabi, 2001. Isolation and Purification of Entomopathogenic Fungi. In: Microbial Control of Crop Pests. Rabindra, R.J., J.S Kennedy, N. Sathiah B. Rajasekaran and M.R. Srinivasan, (Eds.), Dept. of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore, pp: 66-79.
- Santharam, G., 2001. Prospects of using *Metarhizium anisopliae*, *B. bassiana* and *Paecilomyces fumosoroseus* for pest management. In Cabore book.
- Sharma, S., R.B.L. Gupta and C.P.S. Yadav, 1999. Mass multiplication and formulation of Entomopathogenic fungi and their efficacy against white grubs. *J. Mycol. Plant Pathol.*, 29: 299-305..
- Snedcorn, G. and W. Corcran, 1967. Statistical Methods. LOWA, State University Press.
- Vimala, D.P.S., 1994. Conidia production of the entomopathogenic fungus *N. releyi* and its evaluation for control of *Spodoptera litura*., *J. Invertebr. Pathol.*, 63: 145-150.