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ANTAGONISTIC POTENTIAL OF VARIOUS ENTOMOPATHOGENS AGAINST WHITE GRUB, *HOLOTRICHIA CONSANGUINEA*

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ABSTRACT : An experiment was conducted at the Division of Entomology, Rajasthan Agricultural Institute, Durgapura, Jaipur (SKNAU, Jobner) during 2018 to find out the antagonistic potential of various entomopathogens against white grub, *Holotrichia consanguinea.* Four entomopathogens, *viz. Metarhizium anisopliae, Beauveria bassiana* in powder form, *Heterorhabditis indica* and *Steinernema glaseri* as infected gallerias as well as in powder form were tested in the laboratory for their antagonistic effect against all the three instar grubs of *H. consanguinea.* The data revealed that the treatment *B. bassiana* was significantly superior over other treatments and recorded maximum mean per cent mortality, *i.e.* 75.56, 65.56 and 57.50 in the 1st, 2nd and 3rd instar grubs of *H. consanguinea,* respectively. The treatment *M. anisopliae* recorded mean per cent mortality of 71.11, 60.00 and 53.33 in 1st, 2nd and 3rd instar grubs, respectively and was found next in order to efficiency against *H. consanguinea.*

Key words : Antagonistic potential, bioagents, entomopathogens, mortality, white grub.

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INTRODUCTION

White grub or May-June beetle is a devastating and annoying insect pest all over creation (Theurkar *et al*, 2013). The root-feeding larvae cause economic devastation to diverse agricultural and horticultural crops (Koppenhofer and Fuzy, 2003). Nearly 300 species of white grub have been listed from India (Bhawane *et al*, 2012). In India, mostly the white grubs from genera *Holotrichia*, *Brahmina*, *Leucopholis* and *Lepidiota* are frequently recorded to be the key pests of crops (Kumar, 2015). *Holotrichia consanguinea* is the greatest important species in Rajasthan, Gujarat, Haryana, Punjab, Bihar and Uttar Pradesh followed by *Maladera insanabilis*.

The scarabaeids have become difficult insects to control as both the adult and immature grubs have different habitats (Khagta, 2006). To combat this pest, usually insecticides are recommended, but the management of the grubs is often ineffective because of the difficulty of insecticides to move into the root zone. Due to ineffective control and deleterious effects on the environment, there is a need for the enlargement of alternate eco-friendly and economically realistic strategies for the control of white grub, for this biological control may be promising in the management of white grub. Several isolates of fungi, *Beauveria bassiana*, *B. brongniartii*, *Metarhizium anisopliae*, *Paecilomyces fumusoroseus* and *Verticillium lecanii* have been applied in a number of countries (Rath *et al*, 1995). The use of nematodes as biological pest control agents has increased exponentially over the past few decades. Nematodes that parasitize insects have been described from 27 nematode families but the members of the families, Heterorhabditidae and Steinernematidae have received the most attention because of their potential as inundatively applied biological control agents (Kaya and Gaugler, 1993; Grewal *et al*, 2005; Koppenhofer, 2007).

MATERIALS AND METHODS

Details of treatments are as follows : 7 treatments

T_1 : *Metarhizium anisopliae* @ 0.5 g/jar (1×10^{12} cfu/g)

T_2 : *Beauveria bassiana* @ 0.5 g/jar (1×10^{12} cfu/g)

T_3 : *Heterorhabditis indica* @ 1 galleria/jar

T_4 : *Heterorhabditis indica* powder @ 0.5 g/jar

T_5 : *Steinernema glaseri* @ 1 galleria/jar

T_6 : *Steinernema glaseri* powder @ 0.5g/jar

T_7 : Control

Techniques used during investigation

For rearing of test insect white grub (*H. consanguinea*), Greater Wax Moth (*Galleria mellonella*) and mass culturing of test nematodes or fungi, various techniques used are described in detail, which includes the method used for rearing of *H. consanguinea*, mass culturing of nematodes on *Galleria mellonella* larvae, culturing of fungi, inoculations of bio-agents (testing nematodes or fungi) against *H. consanguinea*.

Mass rearing of white grub, *Holotrichia consanguinea*

To ensure regular supply of various stages of *H. consanguinea* for different tests, the adult beetles of *H. consanguinea* were collected from the host trees for 3 to 4 days, immediately with the onset of monsoon. The collected beetles were released in rectangular wire gauge cages (100 cm × 100 cm × 60 cm), containing a 10 cm thick layer of moist soil, spread on the bottom.

Small branches of neem (*Azadirachta indica*) tree were placed daily in the cages, whose leaves provided food for the beetles. The eggs laid by gravid females were collected every day in the morning from the soil in the cages by screening the soil through seven mesh sieves. Those eggs were then placed in earthen crucibles (diyas, 10 cm dia.) containing moistened soil. Twenty-five eggs were placed in each crucible and were covered with an inverted crucible of the same size. This is ensured that the soil in the crucible remains moist till the eggs hatch. The egg contained in the earthen crucibles were examined daily and freshly hatched first instar grubs were transferred to earthen pots (50 cm dia, 11 cm height) containing moist sandy loam soil mixed with organic manure.

For rearing grown-up grubs (more than one week old), the pearl millet seedlings were raised in these earthen pots to provide food for the grubs. In each pot 5 (third instar) to 10 (early second instar) grubs were reared on the consumption of food *i.e.* pearl millet seedlings. The different stages of the test insect *i.e.* I, II and III instar grubs were procured from these earthen pots to carry out different tests during the study.

Mass production of nematode on *Galleria mellonella*

The entomopathogenic nematode species, *Heterohabitidis indica* and *Steinernema glaseri* were tested against white grub, *H. consanguinea.* The nucleus culture of *H. indica* and *S. glaseri* were obtained from the "Foundation for Agricultural Resources Management and Environmental Remediation" (FARMER), Ghaziabad.

The test nematodes *H. indica* and *S. glaseri* were multiplied on the larvae of Greater Wax Moths (GWM). The GWM were reared on the diet described by Haydak (1936) with the following composition: wheat flour 91 g; wheat bran 91 g; cornflour 182 g; milk powder 91 g; glycerol 226 g; honey 273 g; and brewer's yeast 45 g. For GWM rearing, 1 and 2 litre capacity polyethylene tetrapthalate jars with screw caps (lids) were used. In each jar lid, 8 to 10 cm diameter circular windows were cut, over which a hundred mesh brasses net was fixed using the adhesive solution. The mating and oviposition chambers consisted of three-litre jars. The jar lids were lined internally with wax paper. In each jar, accordion-pleated wax paper strips 5×15 cm^2 were placed which served as oviposition sites. In each jar, 5 pairs of the moth were released and food was provided. The eggs were laid on wax paper strips and lining on the inner side of the lid. Most of the eggs were laid within 3-4 days. On the fifth day, the wax paper strips were taken out from the oviposition chambers and cut into small pieces containing eggs. In each rearing jar, larval diet (ca. 500 g/jar) was placed and about 300 eggs on wax paper pieces were placed over the diet. The larvae upon hatching entered into the diet. In about 5 to 6 weeks, the larvae reached the final instar, which were then used as the host for the multiplication of nematodes.

Nematode production was done using the White trap method (White, 1927). Petri plates (9 cm dia) were lined by a single layer of Whatman No. 1 filter paper. The concentration of infective juveniles was adjusted to 200 IJs/ml and 2 ml of the infective juvenile suspension was evenly distributed on filter paper. The full-grown larvae of the wax moth were anaesthetized and placed in a Petri dish.

After 5-7 days, the cadavers were placed in a modified White trap. The trap consisted of an inverted Petri plate surrounded by 0.1 per cent formalin solution in a larger Petri plate. The cadavers of wax moth were placed on the filter paper of the smaller inverted dish by taking precautions that cadavers do not touch each other. These larvae were arranged on the edge of the smaller Petri plate. The central dish containing the cadavers provided moist surroundings for the emergence of entomopathogenic nematodes from cadavers. New progeny of infective juveniles that emerged from cadavers migrated to the surrounding water where they were trapped and subsequently harvested. The infective juveniles migrated away from the host cadavers on the emergence and continued to do so until the body contents of the host are consumed.

For harvesting, nematode suspension from the trap was poured onto a beaker and formalin solution (0.1%) was added to the large Petri dish. The harvesting was done daily for up to 4-5 days or till the production continued. After harvesting, the nematodes were washed several times with 0.1 per cent formalin solution at 5-10^{0}C before their use in various experiments.

Preparation of entomopathogenic fungi culture

The pure culture of *Beauveria bassiana* and *Metarhizium anisopliae* maintained in the Division of Entomology, RARI, Durgapura, Jaipur were used in the experiments. Potato dextrose agar medium was used for culturing these entomopathogenic fungi. The medium consisted of the following ingredients; Potato: 250 g, Dextrose: 20 g, Agar agar: 20 g and Distilled water: 1 litre.

The harvesting of the conidia from the culture tubes containing 15 days old sporulated cultures was done by pouring 10 ml of sterilized emulsified (0.5% Tween 80) distilled water in each tube. The concentration of the conidia in the suspension was determined by Neubauer haemocytometer and further adjusted to make conidial suspension.

Inoculation of bioagents

For testing of various treatments against different stages of *H. consanguinea* in the laboratory, plastic jars were used which had 1 liter capacity. Each jar was filled with 100 g well-sieved air-dried sandy loam soil to which 8 ml water was added and thoroughly mixed. The soil was then inoculated with treatments required as an infested galleria of nematodes or WP of nematodes. The virulent strains of *M. anisopliae* and *B. bassiana* @ 1×10^{10} cfu per ml were assessed against I, II and III instars of *H. consanguinea*. Efficacy of fungi were conducted by dipping the first instar, second instar and third instar larvae in the fungal suspension for five seconds. After allowing the excess liquid to drip off, the larvae were placed individually in plastic jars filled with 100 g of sterile soil. Sliced potatoes serving as the food was added to the container. The larvae were checked for fungal infections leading to mortality. In control only sterilized distilled water was added. There were one grub per jar and 10 jars were used per replication of each treatment.

Observation recorded

Observation on grub mortality was recorded 3, 7, 10 days after treatment (DAT). To workout percent grub mortality, the dead larvae were counted and transferred to a new Petri plate containing moist filter paper and observing the symptoms. The data on percent mortality were corrected by Abbott's formula (Abbott, 1925) as follows.

$$\%\ \text{Corrected mortality} = 1 - \frac{\text{n in T after treatment}}{\text{n in C after treatment}} \times 100$$

Where,

n = Insect population

T = Treated

C = Control

Data on infected grubs in laboratory experiments were subjected to arcsine transformations; these transformed data were subjected to analysis of variance.

RESULTS AND DISCUSSION

Efficacy of entomopathogens against first grub instar of *Holotrichia consanguinea*

The data recorded at 3 days after treatment revealed that the mortality of grubs varied from 30.00 to 56.67 per cent. The treatment *B. bassiana* was significantly superior over the other treatments and recorded a maximum of 56.67 per cent grub mortality followed by *M. anisopliae* with 53.33 per cent grub mortality. While the treatment *S. glaseri* powder recorded the lowest per cent grub mortality (30.0%) (Table 1).

The treatment *B. bassiana* was the most effective treatment recording 80.00 per cent grub mortality and it was found to be significantly superior over all other treatments when the observations were recorded at 7 DAT. The treatment *M. anisopliae* recorded 73.33 per cent grub mortality and was found next in the order of efficiency. The least of 50.00 per cent grub mortality was observed in treatment *S. glaseri* powder formulation.

The 90.00 per cent grub mortality was observed in treatment *B. bassiana* when observations were recorded at 10 DAT, which was superior to the rest of the treatment under test where the treatment *M. anisopliae* was found the next in order of grub mortality with 86.67 per cent followed by *H. indica* infected galleria, *H. indica* powder,

Table 1 : *In vitro* evaluation of different bioagents against 1st instar grub of *Holotrichia consanguinea.*

S. no.	Treatments	Dosage	Percent grub mortality			
			3 DAT	7 DAT	10 DAT	Mean
1.	*M. anisopliae*	0.5 g/jar	53.33(46.92)*	73.33(59.00)	86.67(68.86)	71.11
2.	*B. bassiana*	0.5 g/jar	56.67(48.85)	80.00(63.43)	90.00(71.57)	75.56
3.	*H. indica* galleria	1 galleria/jar	50.00(45.00)	66.67(54.78)	80.00(63.43)	65.56
4.	*H. indica* powder	0.5 g/jar	40.00(39.23)	60.00(50.77)	70.00(56.79)	56.67
5.	*S. glaseri* galleria	1 galleria/jar	40.00(39.23)	60.00(50.77)	76.67(61.22)	58.89
6.	*S. glaseri* powder	0.5 g/jar	30.00(33.21)	50.00(45.00)	70.00(56.79)	50.00
7.	Control	-	0.00(0.00)	0.00(0.00)	3.33(6.14)	1.11
	S.Em.±	-	1.03	1.13	2.67	-
	C.D. at 5%	-	2.20	2.42	5.73	
	C.V. (%)	-	4.93	4.23	4.42	

* Figures in parentheses are angular transformed values, DAT: Days after treatment.

S. glaseri infected galleria and *S. glaseri* powder where 80, 70, 76.67 and 70% grub mortality, respectively. No grub mortality was observed in the untreated control.

Efficacy of entomopathogens against second grub instar of *Holotrichia consanguinea*

The data recorded at 3 DAT revealed that the mortality of grubs varied from 20.00 to 50.00 per cent. The treatment *B. bassiana* was found to be significantly superior over the rest of the treatments and recorded a maximum of 50.00 per cent grub mortality. The treatment *M. anisopliae*, which recorded 40.00 per cent grub mortality was found next in the order of efficacy and the least of 20.00 per cent grub mortality was recorded in treatment *S. glaseri* powder (Table 2).

In the observation recorded at 7 DAT, the treatment *B. bassiana* recorded 63.33 per cent grub mortality which was significantly superior to the rest of the treatments and the least grub mortality (40.00%) was observed in treatment *S. glaseri* powder.

Similar trends of observations were recorded at 10 DAT with the highest (83.33%) grub mortality treatment *B. bassiana* recorded, which was significantly superior to the rest of treatments and minimum (60.00%) grub mortality was recorded in treatment *S. glaseri* powder. No grub mortality was observed in the untreated control.

Efficacy of entomopathogens against third gurb instar of *Holotrichia consanguinea*

The data recorded at 3 DAT revealed that the mortality of grubs varied from 10.00 to 30.00 per cent. The treatment *B. bassiana* was significantly superior over the other treatments and recorded a maximum of 30.00 per cent grub mortality. While the treatment, *S. glaseri* powder recorded the lowest (10 %) grub mortality (Table 3).

The treatment *B. bassiana* as well as *M. anisopliae* recording similar (50.00%) grub mortality and these were found to be significantly superior over all other treatments when the observations were recorded at 7 DAT. The treatment *H. indica* infected galleria recorded 43.33 per cent grub mortality and was found next in the order of efficiency. The least of 30.00 per cent grub mortality was observed in both treatments *H. indica* powder and *S. glaseri* powder.

The 70.00 per cent mortality was observed in treatment *B. bassiana* when observations were recorded at 10 DAT which was superior to the rest of the treatment under test. The least (40.00%) grub mortality was observed in treatment, *S. glaseri* powder formulation.

Similar trends of observations were recorded at 15 DAT with the highest (80.00%) grub mortality in treatment *B. bassiana*, which was significantly superior to over the rest of treatments and least (56.67%) grub mortality was recorded in treatment *S. glaseri* powder formulation. Thus, the treatment *B. bassiana* proved to be consistently superior to other treatments. Whereas no grub mortality was observed in control. The present results are in line with Yadav *et al* (2000), who studied mortality rates (MR) of first, second and third instar grubs of *H. consanguinea* with *M. anisopliae* inoculated soil. The highest MR (70%) for first instar grubs was recorded upon treatment with 1×10^{11} spores for 16 days. Second instar grubs exhibited the highest MR (60%) when treated with 1×10^{11} and 5×10^{10} spores/ml soil for 30 days. Third instar grubs showed the highest MR (50%) after treatment with 1×10^{11} and 5×10^{10} for 25-30 days, and with 1×10^{10} for 30 days. Similar resuts were also reported by Sharma and Gupta (2003), Mohi-ud-din *et al* (2007),

Table 2 : *In vitro* evaluation of different bioagents against 2nd instar grub of *Holotrichia consanguinea*.

S. no.	Treatments	Dose	Percent grub mortality			
			3 DAT	7 DAT	10 DAT	Mean
1.	*M. anisopliae*	0.5 g/jar	40.00(39.23)*	60.00(50.77)	80.00(63.43)	60.00
2.	*B. bassiana*	0.5 g/jar	50.00(45.00)	63.33(52.78)	83.33(66.14)	65.56
3.	*H. indica galleria*	1 galleria/jar	36.67(37.22)	50.00(45.00)	76.67(61.22)	54.44
4.	*H. indica powder*	0.5 g/jar	30.00(33.21)	43.33(41.15)	70.00(56.79)	47.78
5.	*S. glaseri galleria*	1 galleria/jar	30.00(33.21)	50.00(45.00)	70.00(56.79)	50.00
6.	*S. glaseri powder*	0.5 g/jar	20.00(26.57)	40.00(39.23)	60.00(50.77)	40.00
7.	Control	-	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00
	S.Em.±	-	0.75	1.05	1.32	-
	C.D. at 5%	-	1.62	2.25	2.83	
	C.V. (%)	-	4.28	4.65	4.51	

* Figures in parentheses are angular transformed values, DAT: Days after treatment.

Table 3 : *In vitro* evaluation of different bioagents against 3rd instar grub of *Holotrichia consanguinea*.

S. no.	Treatments	Dosage	Percent grub mortality				
			3 DAT	7 DAT	10 DAT	15 DAT	Mean
1.	*M. anisopliae*	0.5 g/jar	20.00(26.57)*	50.00(45.00)	66.67(54.78)	76.67(61.22)	53.33
2.	*B. bassiana*	0.5 g/jar	30.00(33.21)	50.00(45.00)	70.00(56.79)	80.00(63.43)	57.50
3.	*H. indica galleria*	1 galleria/jar	20.00(26.57)	43.33(41.15)	60.00(50.77)	70.00(56.79)	48.33
4.	*H. indica powder*	0.5 g/jar	10.00(18.43)	30.00(33.21)	50.00(45.00)	60.00(50.77)	37.50
5.	*S. glaseri galleria*	1 galleria/jar	16.67(23.86)	40.00(39.23)	56.67(48.85)	63.33(52.78)	44.17
6.	*S. glaseri powder*	0.5 g/jar	10.00(18.43)	30.00(33.21)	40.00(39.23)	56.67(48.85)	34.17
7.	Control	-	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00
	S.Em.±		1.18	0.72	1.05	1.34	
	C.D. at 5%		2.54	1.55	2.25	2.88	
	C.V. (%)		3.74	3.72	4.31	4.87	

* Figures in parentheses are angular transformed values, DAT: Days after treatment.

Kulye and Pokharkar (2009), Rakesha *et al* (2012), Mane and Mohite (2014), Mane and Mohite (2015) Singh *et al* (2001), Bhatnagar *et al* (2004), Singh and Gupta (2006), Sharma *et al* (2009), Shapiro-Ilan *et al* (2010) and Bharathi and Mohite (2015). Kamaliya *et al* (2019) carried out experiments on bio-efficacy of *H. indica* against groundnut white grub in pot culture and revealed that mortality of white grub reached up to 73.34 per cent at higher inoculums level (100 IJs/grub) after 120 hours of application. Per cent mortality of white grub increased with rise in inoculums levels and exposure time.

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