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Comparative Susceptibility of Different Grub Stages to the Local Strain of *Beauveria bassiana*

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Abstract: *Beauveria bassiana* isolated locally from the larvae of *Bombyx mori* was evaluated at different concentrations viz. 1×10^8, 1×10^6, 1×10^4 and 1×10^2 spore mL^{-1} against white grub (*Holotrichia* sp.) for dose optimization of this fungus. It was observed that among these concentration 1×10^8 spore mL^{-1} was the most promising one, showing initial mortality very early and cent per cent in a shorter period of time. The mortality in general in each treatment was recorded in the order of 1×10^8, 1×10^6, 1×10^4 and 1×10^2 spore mL^{-1}. This spore concentration viz. 1×10^8 of *B. bassiana* was tested against different stages of white grub (*Holotrichia* sp.) to determine the comparative susceptibility of different stages to this fungus. It was observed that initial (56.66%) mortality occurred on 4th day and cent percent on the 10th day, respectively in case of 1st instar. The initial mortality (46.66 and 43.33%) in 2nd and 3rd instars was recorded on 4th and 6th day and 100% mortality on 12th and 16th day, respectively. These results showed that 1st stage grubs were more susceptible followed by 2nd and 3rd instar grubs.

Key words: *Beauveria bassiana*, susceptibility, *Holotrichia* sp., 1×10^8

Introduction

White grub or root grub is the immature stage of scarab beetles which are very serious pests feeding on roots. The plants whose roots are damaged by the grubs reflect a dull and yellowish appearance and ultimately die (Manjula and Solochanama, 2001). White grubs being more prevalent in lawns, tourist parks, golf courses, meadows, plantations and horticultural trees. The application of chemical control is practically uneconomical, difficult and is undesirable to treat large areas with chemicals. Biological control is one of the promising methods of insect control and constitutes an ecofriendly alternative strategy.

Biointensive IPM is the most relevant approach in the present day agricultural production system (Reinert and Read, 2001; Lacey and Goettel, 1995). Among entomopathogens fungi are important as they are virulent, infect insect by contact, persist in environment for long time and have one of the largest host list (Santharam, 2001). *Beauverai bassiana* causing white muscardine was the first disease in animals shown to be caused by fungus or any microorganism. Some of the major economic insect pests that are susceptible to this fungus are the European corn borer, Codling moth and *Helicoverpa armigera* (Santharam, 2001). During our studies to control white grubs, it was observed that chemical application even with potent pesticides does not yield impressive results when applied to 3rd stage grubs, though these were effective against neonates and 1st stage grubs. Therefore,

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alternative bioagents against 3rd stage grubs was imperative. *B. bassiana* was found the most effective as compared to other isolates in other experiments. In the present studies the comparative susceptibility of the different stages of white grubs with the local strains of *B. bassiana* was evaluated.

Materials and Methods

The present studies were carried out at Regional Research Laboratory (CSIR) Sanatnagar, Srinagar-India during the year 2002-2003. In this experiment different concentration viz 1×10^8, 1×10^6, 1×10^4 and 1×10^2 spore mL^{-1} of locally isolated *B. bassiana* were tested. The spore suspension was prepared from freshly grown cultures of the fungus cultured on Samsankoa medium. Different concentrations were prepared with the help of haemocytometer. In all there were 5 treatments in the first experiment including control and each treatment was replicated thrice in CRD. Third stage health grubs collected from Kashmir Golf Course were used in this experiment, after surface sterilization with double distilled water. In each replicate, 10 grubs were allowed to move on 90 mm petri plates and sprayed with 5 mL of spore suspension except in control where only sterilized water was sprayed. The inoculated grubs transferred to sterilized glass beakers containing sterilized soil 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 BOD incubator at 27±1°C. The most effective concentration of *B. bassiana* viz. 1×10^8 spore mL^{-1} was tested to determine the comparative susceptibility of different grub stages. All the three stages of white grubs were collected from Kashmir Golf Course Srinagar and assorted on the basis of head width and body length and divided into groups under each treatment. There were total nine replicates for each treatment The methodology regarding collection of grubs, preparation of culture, application remained same as in first experiment.. The observation with respect of mortality was recorded after every 48 h. The data in each case analyzed by the method of Analysis Of Variance advocated by Snedcorn and Corcrans (1967) after necessary transformation and means were compared by critical difference at $p = 0.05$.

Results and Discussion

Dose Optimization

The results (Table 1) showed that *B. 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^{-1}, respectively. The lowest concentration viz. 1×10^2 spore mL^{-1} showed a little mortality (23.33%) on 8th day. Cent percent mortality was observed only on 14th day at a concentration of 1×10^8 spore mL^{-1}, followed by 1×10^6 spore mL^{-1} which recorded it on 16th day. However, in the other two concentrations viz. 1×10^4 and 1×10^2 spore mL^{-1},

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

Treatment	Days after inoculation							
(Conc.)	2	4	6	8	10	12	14	16
1×10^8	0.00	0.00	50.00 (45.00)^a	66.66 (55.07)^a	73.33 (59.00)^a	89.99 (75.00)^a	100.00 (90.00)^a	100.00 (90.00)^a
1×10^6	0.00	0.00	46.66 (43.07)^b	63.33 (52.77)^{ab2}	73.33 (59.00)^a	86.65 (72.29)^a	93.31 (81.14)^a	100.00 (90.00)^a
1×10^4	0.00	0.00	0.00 (0.00)	33.33 (34.13)^b	53.00 (47.00)^b	63.33 (52.77)^b	70.00 (56.99)^b	83.32 (66.14)^b
1×10^2	0.00	0.00	0.00 (0.00)	23.33 (28.44)^b	30.00 (33.00)^c	40.00 (39.14)^c	46.66 (42.99)^c	53.33 (47.00)^c
Control	0.00	0.00	0.00 (0.00)	0.00 (0.00)	6.675 (12.29)^d	6.67 (12.29)^d	10.00 (18.43)^d	10.00 (18.43)^d
CD			1.89	10.99	10.41	12.18	8.36	5.11

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) does not differ significantly

Table 2: Comparative susceptibility of different grub stages to most promising fungal isolate, *Beauveria bassiana* (local)

Grub stage	Mean mortality (%) Days after inoculation							
Treatment	2	4	6	8	10	12	14	16
1st Instar	0.0	56.66	73.33	86.66	100.00	100.00	100.00	100.00
	0.0	(48.84)[a]	(59.00)[a]	(68.85)[a]	(89.04)[a]	(89.04)[a]	(89.04)[a]	(89.04)[a]
2nd Instar	0.0	46.66	63.33	76.66	93.32	100.00	100.00	100.00
	0.0	(43.07)[b]	(52.77)[b]	(61.22)[b]	(75.12)[b]	(89.04)[a]	(89.04)[a]	(89.04)[a]
3rd Instar	0.0	0.00	43.33	60.00	73.33	76.66	86.66	100.00
	0.0	(0.90)[c]	(41.5)[c]	(50.85)[c]	(59.00)[c]	(61.22)[b]	(68.85)[b]	(89.04)[b]
SE	0.0	1.40	1.83	2.52	3.22	1.40	0.00	0.00
CD	0.0	2.90	3.91	5.37	6.37	2.98	0.00	0.00

Each figure is the mean of nine replicates with 4 grubs each, Data in parenthesis is the arcsine transformed value, The value in individual columns superscripted by similar letter(s) does not differ significantly

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the complete mortality was not observed till the termination of the experiment. These results showed that initial grub mortality at higher concentration preceded to their lower concentration where it took some time more to cause initial mortality. These results showed that the mortality in general in each treatment was recorded in the order of $1\times10^{8} > 1\times10^{6} > 1\times10^{4} > 1\times10^{2}$ spore mL^{-1}. In control some mortality was recorded on 10th day of the experiment, which increases 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 experiment 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 Sharma *et al.* (1999) against white grub, *H. cosangunea* and *Maladera insanabilis*.

Grub Susceptibility

Inoculation of *B. bassiana* at the rate of 1×10^{8} spore mL^{-1} on white grub (*Holotrichia* sp.) at different stages revealed no mortality on 4th day in case of 3rd instar grubs (Table 2). In 1st instar grub mortality of 56.66% and in second grubs 46.66% mortality was recorded on 4th day after inoculation. On 6th day mortality in all three instars varied significantly. Highest mortality (73.33%) was observed in first instar grub which was followed by that in 2nd instar and 3rd instar grubs where in a mortality of 63.33 and 43.33% was observed. On 8th day 86.66% mortality was recorded in case of first instar grubs and minimum mortality (60%) recorded in 3rd instar grubs. The mortality in case of Ist instar grubs was followed by 2nd instar grubs. On 10th day first instar grub had cent per cent mortality followed by 2nd instar recording 93.32% mortality. Cent per cent mortality in 2nd and 3rd instar grubs was recorded on 12th and 16th day of inoculation, respectively. During evaluation of comparative susceptibility of different grub stages to *B. bassiana* at the concentration of 1×10^{8} spore mL^{-1}, the first mortality occurred in first and 2nd instar grubs on 4th day, where as in 3rd instar grubs first mortality was observed on 6th day. It was also observed that cent per cent mortality of 1st instar and 2nd instar grubs occurred on 10th and 12th day, respectively and that of 3rd instar grubs on 16th day. These results showed that 1st instar grubs were more susceptible to this fungus followed by 2nd and 3rd instar grubs or it can be inferred that time required for 100% mortality prolonged with the advancement of larval stages (Table 2).

These results are in conformity with the results obtained by Sharma *et al.* (1999) and Yadav *et al.* (2002).

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