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Evaluation of some entomopathogenic fungal isolates from Kashmir for the biocontrol of white grubs infesting turf grass in golf course

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ABSTRACT: The pathogenicity of nine fungi was tested in the laboratory against *Holotrichia* sp. All the fungi proved to be pathogenic at a spore concentration $1x10^8$ spore/ml to grub with varied mortality. *Beauveria bassiana* (Local), *B. bassiana* (commercial), *B. brongniartii* and *Metarhizium anisopliae* were found to be the most effective. *B. bassiana* @ $1x10^8$ spore/ ml concentration inflicted initial mortality on 6th day (46.6%) and cent per cent mortality was observed on 12th day. In *B. brongniartii* initial mortality started on 8th day (33.33%) and cent per cent mortality occurred on 20th day, while as in case of *M. anisopliae* mortality was initiated on 8th day (30%) and highest mortality was observed on 20th day (93.91%). In the field, all the three screened biocontrol agents caused heavy mortality at the highest concentration of $1x10^8$ spore/ml. However, *B bassiana* was found most effective as compared to the rest. In case of *B. bassiana*, the first mortality was observed on 10th day (36.66%) and cent per cent mortality occurred on 20th day, while in case of *B. brongniartii* and *M. anisopliae* the first mortality was observed on 10th and 12th day and complete mortality was observed on 22nd and 24th day, respectively.

KEY WORDS: *Beauveria bassiana*, *Holotrichia* sp., Indigenous fungal isolates

INTRODUCTION

'White grub' is the immature stage of scarab beetles popularly known as cock chaffers, leaf chaffers, chaffer beetles and 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 grubs ranges from 40-80 per cent (Mathur and Upadhayay, 1985) in different crops. White grubs being more prevalent in lawns, golf courses, horticultural trees, application of chemicals is practically uneconomical, difficult and is associated with large number of problems. Sustainable agriculture in the 21st century will rely increasingly on the alternative methods for pest management that are environmentally friendly. One of the most promising biocontrol agents is entomopathogenic fungi, which infect by contact, persist in environment for long time, and

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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* sps., *Aspergillus* sps., *Fusarium* sps., *Metarhizium* sps. *and Verticillium* sps.) involved with entomopathogenicity. There is a continuous need to discover and develop new entomopathogens if we are to meet the future challenges of microbial control with concomitant reduction in use of chemical pesticides.

MATERIALS AND METHODS

The studies were carried out at Division of Entomology, SKUAST-K Srinagar. Nine entomopathogens of which eight were locally isolated and one obtained from the Multiplex Agro Technology, Bangalore were evaluated for their efficacy against white grub (*Holotrichia* sp.). These included *B. bassiana* (Bals.) Vuil., *B. brongniartii* (Sacc) Petch., *Aspergillus flavus* Link Ex Gray, *A. terreus* Thom, *Fusarium palladoroseum* (Cooke) Sacc., *Metarhizium anisopliae* (Metschn.) Sorok, *Tricothecium roseum* (Pers.) Link Ex Gray, *Verticillium lecanii* (Zimm.)Viegas and the commercial product of *B. bassiana*. These local isolates were cultured on Samsonkova medium (Peptone =10g, Agar agar = 20g, Glucose =25g, Starch =25g, Yeast extract =10g, NaCl=1g and distilled water=1L) at a temperature of 27±1°C under laboratory conditions.

The pathogenicity of the test isolates on the white grub was studied under laboratory conditions. A spore suspension (15ml) of each isolate was adjusted to a concentration of $1x10^8$ spore/ml in sterilized distilled water by using a haemocytometer. Healthy grubs of uniform larval instar (3rd instar) collected from Kashmir Golf Course- Srinagar were surface sterilized by distilled sterile water (DSW). Each treatment was replicated thrice in a Complete Randomized Design. In each replicate 10 grubs were allowed to move on 90 mm Petri-plate and sprayed with 5ml of spore suspension except in control where only DSW was sprayed. The inoculated grubs were then carefully 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 DSW to keep the soil moist and then kept in incubator at 27±1°C temperature. The mortality of grubs was recorded after every two days. The dead grubs of each treatment were kept in moist chamber for re-isolation. The fungus obtained was compared with original culture to compare the re-isolated fungus with the original.

Three most effective locally isolated entomopathogenic fungi included *Beauveria bassiana* (Local), *B. brongniartii* and *Metarhizium anisopliae* were evaluated *in vivo*. Each treatment was replicated six times and each replicate was represented by plot size of 50'50cm, where 10 grubs of 3rd instar were released by hand. The beds were separated by plastic sheet (1mm thick) kept at a depth of 15cm to restrict the movement of grubs to adjacent plots. Freshly prepared spore suspension of each isolate was adjusted to a concentration of $1x10^8$ spore/ml and sprayed @1000ml/plot on the moistened experimental plots. In check plots, sterilized water was sprayed instead of the fungal spore suspension. The observation with respect of mortality was recorded after every 48 hours upto 20th day. The data were subjected to Analysis of Variance (Snedcor and Cochran, 1967) after necessary transformations, and means were compared by critical difference at p=0.05.

RESULTS AND DISCUSSION

Pathogenicity

Pathogenicity of nine entomopathogenic fungi was established against white grub (*Holotrichia* sp.). The data are presented in Table 1. The first mortality (46.66%) was recorded in *Beauveria bassiana* on 6th day, while as *B. brongniartii* and *Metarhizium anisopliae* recorded first mortality (33.33% and 30.00%) on 8th day. The mortality continued in all the treatments with the passage of time. Cent per cent mortality was observed in *B. bassiana* (local) treated grubs on 12th day, respectively. However, *B. bassiana* (commercial) and *B. brongniartii* recorded complete mortality on 18th and 20th day, respectively. *M. anisopliae* recorded maximum of 93.3 per cent

Table 1. ***In vitro*** **screening of indigenously isolated fungi for their pathogenicity against** ***Holotrichia*** **sps.**

Entomopathogenic fungi	Per cent mean mortality days after inoculation							
	6	8	10	12	14	16	18	20
Beauveria bassiana (local)	46.66 (43.1)a	80.0 (63.9)a	86.7 (72.0)a	100.0 (89.0)a	100.0 (89.0)a	100.0 (89.0)a	100.0 (89.0)[a]	100.0 (89.0)[a]
B. bassiana (commercial)	0.0 (0.9)b	43.3 (41.1)b	56.7 (48.9)b	60.0 (50.9)b	76.7 (61.71)b	90.0 (74.7)b	100.0 (89.0)a	100.0 (89.0)a
B. brongniartii	0.0 (0.9)b	33.3 (35.3)b	66.7 (55.1)b	73.3 (59.0)b	80.0 (63.9)b	80.0 (63.9)c	86.7 (72.0)b	100.0 (89.0)a
Metarrhizium anisopliae	0.0 (0.9)b	30.00 (33.0)b	46.66 (43.0)bc	60.0 (50.9)b	70.00 (57.0)bc	76.7 (61.7)c	83.3 (66.1)b	93.3 (75.0)b
Aspergillus flavus	0.0 (0.9)b	0.0 (0.9)c	33.3 (35.3)c	53.3 (47.0)c	63.3 (52.8)c	70.0 (57.0)c	80.0 (63.9)bc	83.3 (65.9)bc
A. terreus	0.0 (0.9)b	0.0 (0.9)c	30.0 (33.0)c	46.7 (43.10)c	56.7 (48.8)c	63.3 (52.8)cd	70.0 (57.0)c	80.0 (63.9)c
Verticillium lecanii	0.0 (0.9)b	0.0 (0.9)c	20.0 (20.6)d	56.7 (48.8)bc	63.3 (52.8)c	66.66 (54.8)c	73.3 (59.0)c	76.7 (61.2)c
Fusarium pallodoroseum	0.0 (0.9)b	0.0 (0.9)c	0.0 (0.9)e	46.7 (43.0)c	56.7 (48.8)c	60.0 (50.9)d	63.3 (52.8)c	70.0 (57.0)c
Trichothecium roseum	0.0 (0.9)b	0.0 (0.9)c	0.0 (0.9)e	10.0 (18.3)d	13.3 (21.1)d	13.3 (21.14)d	16.7 (23.9)d	16.7 (23.9)d
Control	0.0 (0.9)b	0.0 (0.9)c	0.0 (0.9)e	0.0 (0.9)e	3.5 (6.7)e	6.7 (12.6)e	6.67 (12.6)d	6.8 (12.6)e
SEM±	0.86	5.18	5.88	5.4	4.99	5.9	5.9	5.17
CD (P=0.05)	1.79	10.81	12.27	11.3	10.4	12.31	12.3	10.8

Data in parentheses are the arcsine transformed value.

The values in individual columns superscripted by similar letter(s) do not differ significantly.

mortality on 20[th] day till the termination of the experiment. Within 3 to 5 days after inoculation, the grubs appeared to have become less active or sluggish. One day before death oily specks appears on the surface of the integument. The dead cadavers are first rubbery but within 48 hours after death become hardened. Soon sporulation occurs on the surface of hardened bodies. All the isolated proved to be pathogenic though with varied degree and virulence to the white grub. On the basis of pathogenicity, locally isolated *B. bassiana* (local), *B. brongniartii* and *M. anisopliae* were considered most promising causing initial and cent per cent mortality in a shorter period of time.

B. bassiana has already been reported to be pathogenic to various white grub species by Jayaramaiah and Veeresh (1983a) and Sharma *et al.* (1998). *B. brongniartii* has been reported to be pathogenic to various grub species by Rangnathaiah *et al.* (1973) and Jayaramaiah and Veeresh (1983b). The results of *F. palladoroseum*,

Table 2. Evaluation of most promising entomopahtogenic fungi against the white grub

Treatment	Per cent mean mortality days after inoculation							
	10	12	14	16	18	20	22	24
B.bassiana (local)	36.7 (37.3)a	53.3 (46.9)a	66.7 (54.8)a	80.0 (63.9)a	93.3 (75.0)a	100.0 (89.0)a	100.0 (89.0)a	100.0 (89.0)a
B.brongniartii	30.0 (33.0)b	50.0 (45.0)a	63.3 (53.1)a	76.7 (61.1)a	86.7 (68.6)a	90.0 (75.6)b	100.0 (89.0)a	100.0 (89.0)a
Metarrhizium anisopliae	0.0 (0.9)b	30.0 (33.0)b	46.7 (43.0)b	60.0 (50.9)b	76.7 (61.1)b	90.0 (71.6)b	96.7 (79.5)	100.0 (89.0)
Control	0.0 (0.9)	3.4 (10.5)c	6.7 (15.0)c	6.7 (15.0)c	10.0 (18.4)c	10.0 (18.4)c	10.0 (18.4)	10.0 (18.4)
SEM±	1.83	3.53	4.35	4.2	4.7	4.8	2.62	0.00
C D (P=0.05)	3.88	7.36	9.06	8.75	9.88	9.98	5.45	0.00

Data in parentheses are the arcsine-transformed value.

The values in individual columns superscripted by similar letter(s) do not differ significantly.

V. lecanii, *A. flavus*, *A. terreus* used in our experiments though exhibited good mortality but was not on par with *Beauveria* and *Metarhizium* species. *M. anisopliae* has been reported to be pathogenic to different white grub species by Sharma *et al.* (1998) and Yadav *et al.* (2002).

Field Evaluation

The most effective isolates, *viz. B. bassiana* (local), *B. brongniartii* and *M. anisopliae* were evaluated against the white grub (*Holotrichia* sp.) at $1x10^8$ spore/ml under field conditions. The data are presented in Table 2. It is evident from the data that mortality was initiated on 10th day of *B. bassiana* (local) and *B. brongniartii* recording 36.66 and 30 per cent, respectively. *M. anisopliae* recorded first mortality (30%) on 12th day. Among these treatments, *B. bassiana* recorded cent per cent on 10th day, followed by *B. brongniartii* and *M. anisopliae* recording cent per cent mortality on 22th and 24th day, respectively. All the three entomopathogens performed well against white grub, but *B. bassiana* (local) proved to be the most effective. The efficacy of these entomopathogens and superiority *B. bassiana* is in accordance with the observations made by Verma *et al.* (1988). Sharma *et al.* (1999) and Gareria *et al.* (1990) reported *M. anisopliae* was superior over *B. bassiana*. However, our results showed *B. bassiana* was virulent than *B. brongniartii* and *M. anisopliae*. This may be due to high virulence of *B. bassiana* strain isolated locally. The variation in virulence among various fungi strains is of prime importance in determining their efficacy against an insect.

In both the experiments some mortality was observed in control, which started very late and was little as compared to treatment involving fungal isolates, where mortality started earlier and reached to maximum till termination of the experiment. The reason for this mortality may be natural death or due to mishandling of grubs during the record of observations

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