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Pathogenicity of entomopathogenic fungi against white grub, *Leucopholis lepidophora* (Blanchard) infesting sugarcane under pot culture experiment

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ABSTRACT

The pathogenicity of entomopathogenic fungi, *Metarhizium anisopliae* (Metsch.) Sorokin, *Beauveria brongniartii* (Sacc.) and *Beauveria bassiana* (Balsana) Vuillrmin, was done against white grub, *Leucopholis lepidophora* (Blanchard) infesting sugarcane under pot culture experiment. In this investigation the virulence of different entomopathogenic fungi were determined by FYM enriched techniques against third instar grubs of *Leucopholis lepidophora* (Blanch.).An overall concentration range of $2x10^4$-$2x10^8$ conidia ml^{-1} of entomopathogenic fungi were used but among these, $2x10^8$ conidia ml^{-1} concentration was the most promising for highest grub mortality in each entomopathogenic fungi. *M. anisopliae* was found to be most effective fungus as compared to other fungi. It was observed that 34.49-62.07 per cent grub mortality occurred on 45 DAT in *M. anisopliae* at different conidial concentrations. *B. brongniartii* recorded 31.03 to 58.62 per cent grub mortality while, *B. bassiana* caused 27.59 to 55.18 per cent grub mortality at different conidial concentration. The results showed that *M. anisopliae* was found to be more pathogenic than *B. brongniartii* and *B. bassiana.*

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INTRODUCTION

White grubs are serious pests of number of crops including sugarcane (*Saccharum officinarum* L.) in the states of Maharashtra, Gujarat, Punjab, Karnataka, Uttar Pradesh etc. The government of India has considered white grub as one of the five pests of national importance from 1975. Since, last five years the white grub menance has to the sugarcane crop in endemic pockets of Maharashtra. In India near about 300 species of white grubs are recorded (Raodeo and Deshpande, 1987). The white grub, *Leucopholis lepidophora* (Blanch) has recently been reported to be theat to sugarcane, paddy, groundnut cultivation in south Maharashtra particularly in Kolhapur area (Patil *et al.*, 1986). Due to complex life cycle and survival of pest in soil the control of this pest becomes difficult. The utilisation of entomopathogenic fungi as biocontrol agents for insect pests is gaining much importance recently due to hazards of indiscriminate use of chemical pesticides. Rabindra *et al.* (2001) reported some 90 genera and 700 species of fungi, representing a large group of entomophorales (*Beauveria* spp., *Asprgillus* sp., *Metarhizium* sp. and *Verticillium* sp.), involved with ntomopathogenicity.There is a continuous need to discover

and develop new entomopathogens for control of insect pests. Therefore, the present study was undertaken.

MATERIAL AND METHODS

Source of material :

Different fungus like *B. bassiana* and *M. anisopliae* (having 2×10^8 CFU ml^{-1}) were used in the present study obtained from M/s. Jay Biotech, Pune and *B. brongniartii* from NBAIP, Bangalore were used for present study.

Host culture :

White grubs of the same instar and same sizes particularly third instar grub stage were collected from infested sugarcane field from riverbank area. Immediately after the collection of grubs, they were placed in sterile plastic vials (4×3.5 cm) with soil from the same collection site for transporting them to the laboratory. Only one grub was put into each vial and roots of paddy and sugarcane which was disinfected for 10 min in 0.5 per cent sodium hypochloride solution were added to each vial as a diet and avoid cannibalism. The larval culture were maintained at 25 ± 2°C and 65 ± 5 per cent RH.

Method of testing :

In pot culture experiment, *M. anisopliae*, *B. brongniartii* and *B. bassiana* were evaluated against third instar at dosage equivalent of 2×10^4, 2×10^5, 2×10^6, 2×10^7, 2×10^8 conidia/ml prepared by serial dilution method. Soil and FYM were mixed at 2:1 proportion. Before addition of fungal formulations, the farm yard manure (FYM) was solarized. For solorization, the FYM was moistened and then spread into a 10 cm thick layer, which was covered with a polythene sheet, all sides of the sheet and were covered with soil to make if leak proof. The solorization was done for 3 weeks and the temperature was recorded daily using soil thermometer. For enrichment of FYM with the fungal formulations, a quantity of 5 kg well decomposed FYM was mixed thoroughly with one lt. each formulation of each fungal culture and sprayed as a layer (12.5 cm thick) under shade. It was covered with gunny bags and water was sprinkled on the top to maintain the humidity. It was incubated (25-32ºC) for 15 days. Sufficient turning and watering was given to the treated FYM at the interval of 6 days to improve the aeration and maintain moisture content. Grubs were kept in fungus enriched FYM with sugarcane settlings in a earthen pot.

Five treatments of each fungus was carried out whereas, in the sixth treatment, the pot was treated as control. The experiment was conducted with three replications and ten grubs were used for each treatment.

Observations :

The mortality was recorded after the treatment at an interval of 15, 30, 45 DAT. The exact time required to kill the test larvae was strictly recorded.

Statistical analysis :

Data on per cent grub mortality in pot culture experiment were subjected to square root or arcsin transformations. These transformed data were subjected to analysis of varience to determine the significance of different treatments.The data were corrected by the formula of Abott (1925).

RESULTS AND DISCUSSION

Response of five different concentrations of *M. anisopliae viz*., 2×10^4, 2×10^5, 2×10^6, 2×10^7 and 2×10^8 conidia ml^{-1} were tested for determining the bioefficacy of *M. anisopliae* on the third instar grub of *L. lepidophora* Blanch. and results are presented in Table 1.

The grub infected with *M. anisopliae* became sluggish and ceased feeding. After death, white mycelia spots were observed on the grub body. Later the grub were covered with turf of pure white mycelial growth which turned green covering the entire body of grub. The data recorded at 15DAT revealed that the treatments with concentration 2×10^8 conidia ml^{-1} was superior to rest of treatments indicated 10.00 per cent grub mortality. At 30 DAT, the mortality data indicated that treatment with concentration 2×10^8 conidia ml^{-1} and treatment with concentration 2×10^7 conidia ml^{-1} recorded highest (24.14 %)

Table 1: Evaluation of *Metarhizium anisopliae* against third instar grubs of *L. lepidophora*

Treatments	Dose (conidia ml^{-1})	Per cent grub mortality DAT		
		15 DAT	30 DAT	45 DAT
T_1	2×10^4	0.0 (0.0)	10.34 (15.98)	34.49 (33.11)
T_2	2×10^5	0.0 (0.0)	17.24 (21.75)	37.93 (35.16)
T_3	2×10^6	0.0 (0.0)	20.69 (24.10)	41.38 (37.29)
T_4	2×10^7	3.33 (6.15)	24.14 (26.47)	55.18 (45.49)
T_5	2×10^8	10.00 (18.44)	24.14 (26.47)	62.07 (49.68)
T_6	Untreated control	0.0 (0.0)	3.33 (6.15)	3.33 (6.15)
	S.E. ±	3.55	4.46	4.65
	C.D. (P = 0.05)	7.33	9.73	10.13

Figures in parentheses are arcsin transformation

mortality, indicating no significant difference among them. While in 45 DAT with *M. anisopliae* concentration 2×10^8 conidia ml^{-1} recorded 62.07 per cent reduction in grub population which was significantly superior over all other treatments and was on par with treatment 2×10^7 conidia ml^{-1} where 55.18 per cent mortality was recorded. In untreated control, 3.33 per cent grub mortality was observed (Table 1).

The tested conidial concentration of *B. brongniartii* and grub mortality data of at respective days interval are presented in Table 2. The data recorded at 15 DAT revealed that treatment with concentration 2×10^8 conidia ml^{-1} was recorded highest 10.00 per cent grub mortality and treatment with 2×10^7 conidia ml^{-1} was next in order of efficacy where 3.33 per cent mortality was recorded. At 30 DAT treatment with 2×10^8 conidia ml^{-1} concentration recorded highest 24.14 per cent grub mortality which was superior over other treatments. In untreated control 3.33 per cent grub mortality was observed. The treatment with concentration 2×10^8 conidia ml^{-1} recorded highest 58.62 per cent mortality at 45 DAT, which was superior to rest of the treatments.

Grubs infected with *B. bassiana* became sluggish and ceased feeding. The mortality was observed at 15 DAT, 30 DAT, 45 DAT. After death white mycelial growth was observed on the grub body. The tested conidial concentration and corrected percentage mortality data of *B. bassiana* at respective days interval are presented in Table 3. The data recorded at 15 DAT revealed that treatment with concentration 2×10^8 conidia ml^{-1} recorded 6.67 per cent grub mortality. At 30 DAT the treatment with concentration 2×10^8 conidia ml^{-1} was found most effective over the other treatments and recorded 24.14 per cent grub mortality. In untreated control 3.33 per cent grub mortality was observed. Significant differences did not exist among the rest of the treatments. The 55.18 per cent grub mortality was observed in treatment with 2.0×10^8 conidia ml^{-1} when observations were recorded at 45 DAT, which was superior to the rest of treatments under test.

Studies conducted under pot culture experiment revealed that treatment *M. anisopliae* @ 2×10^8 conidia ml^{-1} was most effective in controlling the third instar grubs of *L. lepidophora* Blanch. Treatment *M. anisopliae* recorded 34.49 to 62.07 per cent grub mortality at 45 DAT. However, 31.03 to 58.62 per cent grub mortality was recorded in *B. brongniartii* treatment. While, *B. bassiana* recorded 27.59 to 55.18 per cent grub mortality. The reason for mortality in untreated control may be natural death or repeated handlings of experiment material Fujjie and Yokoyama (1996) use *M. anisopliae* for controlling *Anomala cuprea*.

The present findings are in line with that of observed by Easwaramoorthy *et al*. (2005) who carried out pot culture experiments with *B. brongniartii* at a dosage equivalent of 10^{12}-10^{18} spores ha^{-1}, third instar grubs showed upto 68 per cent mortality.

Table 2 : Evaluation of *B. brongniartii* against third instar grubs of *L. lepidophora*

Treatments	Dose (Conidia ml^{-1})	Per cent grub mortality DAT		
		15DAT	30DAT	45DAT
T_1	2×10^4	0.0 (0.0)	10.34 (15.98)	31.03 (30.97)
T_2	2×10^5	0.0 (0.0)	13.79 (18.86)	34.49 (33.10)
T_3	2×10^6	0.0 (0.0)	17.24 (21.22)	37.93 (35.23)
T_4	2×10^7	3.33 (6.15)	20.69 (24.10)	48.27 (41.39)
T_5	2×10^8	10.00 (18.44)	24.14 (26.47)	58.62 (47.63)
T_6	Untreated control	0.0 (0.0)	3.33 (6.15)	3.33 (6.15)
	S.E. ±	3.55	5.18	4.37
	C.D. (P = 0.05)	7.33	11.28	9.53

Figures in parentheses are arcsin transformation

Table 3 : Evaluation of *B. bassiana* against third instar grubs of *L. lepidophora*

Treatments	Dose (Conidia ml^{-1})	Per cent grub mortality DAT		
		15DAT	30DAT	45DAT
T_1	2×10^4	0.0 (0.0)	10.34 (15.98)	27.59 (28.83)
T_2	2×10^5	0.0 (0.0)	13.80 (18.86)	31.03 (30.97)
T_3	2×10^6	0.0 (0.0)	13.80 (18.86)	37.93 (33.10)
T_4	2×10^7	0.0 (0.0)	20.69 (24.10)	44.83 (39.35)
T_5	2×10^8	6.67 (12.29)	24.14 (26.47)	55.18 (45.48)
T_6	Untreated control	0.0 (0.0)	3.33 (6.15)	3.33 (6.15)
	S.E. ±	3.55	4.82	4.05
	C.D. (P = 0.05)	7.33	10.49	8.82

Figures in parentheses are arcsin transformation

In contrast, *M. anisopliae* was found to be more virulent than *B. brongniartii* in present investigation and showed 62.07 per cent grub mortality. Further, *B. brongniartii* either alone or in combination with *M. anisopliae* effectively controlled *H. consanguinea* on groundnut in pot culture experiment as reported by Vyas *et al.* (1997) and Patel *et al.* (2013).

With the ever increasing awareness of the harmful effects of the chemical pesticides on man and environment, there is need of ecofriendly, sustainable pest management which has been felt very strongly providing an impetus to research and development of entomopathogenic fungi. Therefore, in present investigation an attempt was made to study the pathogenicity of entomopathogenic fungi under pot culture experiment.

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