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RESEARCH PAPER

Bioefficacy of different species of entomopathogenic fungi against white grub, *Leucopholis lepidophora* (Blanchard) infesting sugarcane in Maharashtra

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The bioefficacy of three species of entomopathogenic fungi *viz.*, *Metarhizium anisopliae* (Metsch.) Sorokin, *Beauveria brongniartii* (Sacc.) and *Beauveria bassiana* (Balsana) Vuillrmin, against white grub, *Leucopholis lepidophora* (Blanchard) infesting sugarcane crop was studied under laboratory conditions. In this bioassay *Metarhizium anisopliae* fungus caused higher rate of grub mortality at an overall concentration range of 4×10^5 to 2×10^6 conidia ml^{-1} as compared to *Beauveria brongniartii* and *Beauveria bassiana* The treatments with *Metarhizium anisopliae* fungus was found to be most effective and recorded 34.48-58.62 per cent grub mortality on 15 DAT at different conidial concentrations. The fungi *Beauveria brongniartii* recorded 31.03-55.18 per cent grub mortality, while as in case of fungi *Beauveria bassiana* 24.14-51.72 per cent grub mortality was observed on 15 DAT at different conidial concentrations. The estimated LC_{50} values for *M. anisopliae, B. brongniartii* and *B. bassiana* towards third instar grubs of *Leucopholis lepidophora* Blanch. were 9.05×10^5,10.44×10^5 and 11.78×10^5 conidia ml^{-1}, respectively.

Key words : *Metarhizium anisopliae, Beauveria brongniartii, Beauveria bassiana, Leucopholis lepidophora,* Sugarcane.

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INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is the most important cash crop and plays the main role in Indian economy. Uptill now 200 insect pests have been reported causing damage to sugarcane crop (David *et al.*, 1986). Among them, white grub has became the most important polyphagous pest causing serious threat to sugarcane crop since 1960 (Mohalkar *et al*., 1977). About 40 different species under these subfamilies have been recorded as important pests of various crops in different parts of country white grubs remain a problem in varied agroecosystem and soil types. White grubs (Coleoptera: Scarabaeidae) are soil inhabiting and root feeding immature stages of scarab beetles. The white grub family, Scarabaeidae is the second largest and omnipresent family within the order Coleoptera (Mishra and Singh, 1999). White grubs have become serious pests of most agricultural crops, fruits, vegetables, ornamental plants, plantation crops, pastures, turf and meadow grasses, lawns, golf courses and forest trees in different part of the world (Potter *et al.* 1992). Chemical control measures are ineffective since the pests are subterranean. This pest consider threat to sugarcane cultivation in parts of Kolhapur and Sangli districts (Adsule and Patil, 1990). However, its incidence was also recorded in maize, groundnut, paddy and vegetables. The success of control tactics is governed by the seasonality of adults and the susceptible stage of the grub. The chemical insecticides so far evaluated against the grub stage proved less effective (Patil *et al.*, 1986) and also there are lot of limitation to use higher dose of insecticides as the infestation is more pronounce on the banks of rivers. Therefore, sustained attempts in managing the white grub with alternative techniques that would eliminate these problems have not become forth. Among these, biological control especially using fungal pathogens holds promise.

The entomopathogenic fungi occupy the vital role in control of insect pests, some of the important entomopathogenic fungi genera are *Metarhizium anisopliae*

(Metschnikoff) Sorokin, *Beauveria bassiana* (Balsamo) Vuillrmin, *Nomuraea rileyi* (Farlow) Samson, *Entomophthora, Coelomomyces, Aschersonia, Hirsutella, Verticillium, Paecilomyces* etc. containing several species which are commonly used in microbial control (Agarwal and Rajak, 1985). The entomopathogenic fungi *M. anisopliae* and *B. bassiana* have been successfully utilized as potential biological control agents for many soil inhibiting insect pests (Milner *et al*., 1993; Robertson *et al*., 1997; Bhagat *et al*., 2003; Sharma and Gupta, 1998; Gupta *et al*., 2003). Therefore, it was thought desirable to explore the possibility of using *M. anisopliae, B.brongniartii* and *B. bassiana* for the management of white grubs and its efficacy under laboratory conditions.

RESEARCH METHODOLOGY

Source of material :

Different fungus like *B. bassiana and M. anisopliae* (having 2 $x10^8$ CFU ml^{-1}) used in the present study were obtained from M/s Jay Biotech, Pune and *B. brongniartii* from NBAIP Bangalore were used for present study.

Host culture :

A 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 cm x 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 were 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.

Determination of median lethal concentration :

To determine the median lethal concentration (LC_{50}) values of are *M. anisopliae, B. brongniartii* and *B. bassiana*, third instar grubs of *Leucopholis lepidophora* were employed. The susceptibility of third instar grub was determined by using larval dip method. Five concentration of each formulation *i.e.* 2, 4, 6, 8, 10 g lt^{-1} water, were tested to observe the possibility of defecting small changes in virulence and the spore count obtained in resulting suspension was 4 x 10^5, 8 x 10^5, 1.2 x 10^6, 1.6 x 10^6, 2.0 x 10^6 conidia ml^{-1}, respectively.

The grub were dipped in 30 ml of conidial suspensions for 5 seconds. A set of 10 larvae with three replications of each concentration of fungal formulation and a control treated with sterile distilled water was maintained treatment individual larvae was transferred separately in to earthen pots containing sterile soil with 30 per cent moisture at bottom and sugarcane settling were planted into earthen pot as a food. It was placed in a green shed to provide 25 $\pm$ 2^0C temperature and 65 $\pm$ 5 per cent relative humidity by regular watering and 16L : 8D till death. The dead larvae were then transferred to sterile Petriplates containing moist whatman's No. 1 filter paper and kept at 28^0C and 70 – 80 per cent relative humidity for at least 3-7 days to allow mycelia growth and conidia formation over the cadavers.

Observations :

The grub mortality was recorded after the treatment at an interval of 7, 10, 15 DAT. The exact time required to kill the test larvae was strictly recorded.

Statistical analysis :

Suspensions of *M. anisopliae, B. brongniartii* and *B. bassiana* were prepared with dilution of fungal formulation in sterile diluted water. The mortality data were subjected to probit analysis (Finney, 1964) method, the LC_{50} values for different conidial concentration of entomopathogenic fungi on the third instar grub of *L. lepidophora* were worked out in SPSS 7.5 software package.

RESEARCH FINDINGS AND ANALYSIS

Five different conidial concentration of *M. anisopliae*

Table 1 : Evaluation of *Metarhizium anisopliae* against third instar grub of *L. lepidophora* Blanch. in larval dip method

Treatments	Dose (Conidia ml^{-1})	Per cent grub mortality DAT		
		7DAT	10DAT	15DAT
T_1	4 x 10^5	0.0 (0.0)	16.67 (23.85)	34.48 (33.10)
T_2	8 x 10^5	0.0 (0.0)	20.00 (26.56)	37.93 (35.24)
T_3	1.2 x 10^6	0.0 (0.0)	23.33 (28.78)	41.38 (37.29)
T_4	1.6 x 10^6	6.67 (12.29)	30.00 (33.21)	48.28 (41.39)
T_5	2.0 x 10^6	6.67 (12.29)	33.33 (35.21)	58.62 (47.54)
T_6	Untreated control	0.0 (0.0)	0.0 (0.0)	3.33 (6.15)
	S.E. ±	5.02	2.33	3.89
	C.D. (P = 0.05)	10.93	5.07	8.68

Figures in parentheses are arcs n transformation

viz., 4 x 10^5, 8 x 10^5, 1.2 x 10^6, 1.6 x 10^6, 2 x 10^6 conidia ml^{-1} were tested for determining the bioefficacy of *M. anisopliae* on the third instar grub of *L. lepidophora* Blanch. and are presented in Table 1.

The initial mortality was observed at 7 DAT revealed that the treatment with concentration 1.6 x 10^6 conidia ml^{-1} and 2.0x10^6 conidia ml^{-1} were equally superior over other treatments and recorded 6.67 per cent grub mortality in both the treatments. The 58.62 per cent mortality was observed in treatment with 2.0 x 10^6 conidia ml^{-1} at 15 DAT which was significantly superior to the rest of the treatment under test. The treatments with conidial concentration, 1.6 x 10^6, 2.0 x 10^6 conidia ml^{-1} was at par recorded 48.28 per cent and 58.62 per cent mortality, respectively. The LC_{50} value recorded for *M. anisopliae* was 9.05 x 10^5 conidia ml^{-1}.

At 7 DAT, grub mortality was observed in 1.6 x 10^6, 2.0 x 10^6 conidia ml^{-1} concentration treatments which were significantly superior to untreated control the treatments with concentration 1.6 x 10^6 conidia ml^{-1} and 2.0 x 10^6 conidia ml^{-1} recorded 6.67 per cent mortality in both the treatments. The treatment with concentration 2.0 x 10^6 condia ml^{-1} recorded highest (55.18%) grub morality, which was at par with treatment with concentration 1.6 x 10^6 conidia ml^{-1} and recorded 48.28 per cent grub mortality of third instar grub of *L. lepidophora*. The mortality of grubs maximum (55.18%) in treatment with 2.0 x 10^6 conidia ml^{-1} to a minimum (31.03%) in treatment with 4 x 10^5 conidia ml^{-1} (Table 2). The LC_{50} value recorded for *B. brongniiartii* was 10.44 x 10^5 conidia ml^{-1}.

The tested conidial concentration and grub mortality data due to *B. bassiana* at respective days interval are presented in Table 3. The data recorded at 7 DAT revealed that the treatment with concentration 2.0 x 10^6 conidia ml^{-1} recorded 6.67 per cent mortality and found to be superior over all other treatment.The maximum grub mortality (51.72%) was recorded in treatment with concentration 2.0 x 10^6 conidia ml^{-1} at 15 DAT, which was superior over the rest of treatments. The LC_{50} value recorded for *B. bassiana* was

Table 2 : Evaluation of *Beauveria brongniiartii* against third instar grubs of *L. lepidophora* Blanch. in larval dip method

Treatments	Dose (Conidia ml^{-1})	Per cent grub mortality DAT		
		7DAT	10DAT	15DAT
T_1	4 x 10^5	0.0 (0.0)	13.33 (21.15)	31.03 (30.97)
T_2	8 x 10^5	0.0 (0.0)	16.67 (23.85)	34.48 (33.11)
T_3	1.2 x 10^6	0.0 (0.0)	20.00 (26.67)	37.93 (35.25)
T_4	1.6 x 10^6	6.67 (12.29)	26.67 (30.99)	48.28 (41.39)
T_5	2.0 x 10^6	6.67 (12.29)	30.00 (33.21)	55.18 (45.49)
T_6	Untreated control	0.0 (0.0)	0.0 (0.0)	3.33 (6.15)
	S.E. ±	5.02	3.55	4.06
	C.D. (P = 0.05)	10.93	7.73	8.85

(Figures in parentheses are arcs in transformation)

Table 3 : Evaluation of *Beauveria bassiana* against third instar grubs of *L. lepidophora* Blanch, in larval dip method

Treatments	Dose (Conidia ml^{-1})	Per cent grub mortality DAT		
		7DAT	10DAT	15DAT
T_1	4 x 10^5	0.0 (0.0)	10.00 (18.44)	24.14 (26.47)
T_2	8 x 10^5	0.0 (0.0)	13.33 (21.15)	31.03 (30.96)
T_3	1.2 x 10^6	0.0 (0.0)	16.67 (23.85)	35.06 (33.11)
T_4	1.6 x 10^6	0.0 (0.0)	20.00 (26.56)	44.83 (39.34)
T_5	2.0 x 10^6	6.67 (12.29)	26.67 (30.99)	51.72 (43.44)
T_6	Untreated control	0.0 (0.0)	0.0 (0.0)	3.33 (6.15)
	S.E. ±	3.55	2.55	4.40
	C.D. (P = 0.05)	7.73	5.56	9.59

(Figures in parentheses are arcs in transformation)

Table 4 : Evaluation of median lethal concentration of entomopathogenic fungi against third instar stage of *Leucopholis lepidophora* Blanch

Entomopathogenic fungus	LC50 (x10^5 conidia ml^{-1})	Fiducial limit	Regression equation	$\chi 2$ Value
M. anisopliae	9.05	6.312-21.173	Y=0.841x+4.194	2.838
B. brongniartii	10.44	7.304-24.599	Y=0.923x+4.059	2.650
B. bassiana	11.7	8.470-24.173	Y=1.120x+3.799	1.439

11.78 x 10^5conidia ml^{-1}. Thus, the treatment with concentration 2 x10^6 conidia ml^{-1} proved to be consistently superior to other treatment at all the intervals of observations.

These results showed that in each treatment was recorded in the order of 2.0 x 10^6 > 1.6 x 10^6 > 1.2 x 10^6> 8 x 10^5> 4x 10^5 conidia ml^{-1}. In control some mortality was recorded which may be due to natural death or repeated handlings of experimental materials. However, the results of present study biocontrol agent.

are similar to Ansari *et al.* (2004) and Kulye and Pokharkar (2009) who reported that *M. anisopliae* strains were more virulent than *B. bassiana* strains against june beetle, *H. philanthus* and *H.consanguinea*, respectively. Highest virulence of *M. anisopliae* against white grub was also reported by Dhoj and Keller (2005), Najera-Rincon *et al.* (2005) Samson *et al.* (2005). The results of the present study suggest that entomopathogenic fungi could be a potential

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