Short Communication

Laboratory and Field Efficacy of Entomopathogenic Fungi *Beauveria brongniarti, Beauveria bassiana* and *Metarhizium anisopliae* in the Control of Sugarcane White Grubs (*Holotrichia serrata*)

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ABSTRACT The pathogenicity of the three entomopathogenic fungi *Beauveria brongniarti* (Sach.), *Beauveria bassiana* (Bals) and *Metarhizium anisopliae* (Metc.) were evaluated against third instar larvae of sugarcane white grub (*Holotrichia serrata*) under laboratory and field conditions. All the three entomogenous fungi tested were pathogenic to the pest at varying degrees. Under laboratory conditions the spore suspension containing 3×10^9 conidia/ml all the three evaluated fungi were highly toxic to the grubs. In field experiments, talc based formulations of these fungi controlled the pest at the rate of 1 kg/acre of the formulation containing 7.8×10^9 conidia/ml. The combination treatments also gave a highest yield of 95.25 t/ha against 60.5 t/ha in control plots.

KEY WORDS : *Beauveria bassiana, B. brongniarti, Metarhizium anisopliae*, sugarcane white grub, *Holotrichia serrata*

INTRODUCTION

White grubs, *Holotrichia* spp. (Coleoptera: Scarabidae) are noxious subterranean pests damaging the root system of several crops and mostly *Holotrichia consanguinea* (Blanchard), *H. serrata* (Fabricius), *H. longipennis* (Blanchard) and *H. fugei* (Mittel) infest the crops. Of these *H. consanguinea* and *H. serrata* are abundant during August to September and pose serious threat to many crops. Sugarcane is an important commercial crop grown in an area of 5.4 million hectares with average production of 68 metric ton per hectare in India. The average production is less than other cane producing countries of the world due to high incidence of pests and *H. serrata* alone causes about 80% yield loss in the tropics (David and Ananthanarayanan, 1986).

In an effort to look for alternatives to chemical pesticides, in recent past substantial emphasis has been given to the use of microbial biopesticides, particularly the fungal pathogens, which can compete with the subterranean habit of grubs, long larval period and synchronization of larval activity with humid months (Eswaramoorthy *et al*., 2005). There are about 700 genera of entomopathogenic fungi reported all over the world. Unlike insect pathogenic bacteria and viruses, fungi invade hosts by directly penetrating cuticles thus they can infect non-feeding stages like eggs and pupae easily (Charnley, 2003; Heycle and St. leger, 1994). The three entomopathogenic fungi selected in the present study, which are environment friendly, are important as

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they are pathogenic to many agriculturally important insects belonging to Lepidoptera, Coleoptera and Hemiptera (Roberts and St Leger, 2004; Wang *et al* ., 2004; Thomas and Reed, 2007; Pandey and Kanaujia, 2008). The objective of the present study was to demonstrate the efficacy of *B. brongniarti, B. bassiana* and *Metarhizium anisopliae* against sugarcane white grub *Holotrichia serrata* and to determine the potential of these biopesticides under field conditions to control these grubs.

MATERIALS AND METHODS

Fungal Strains and the Formulation

Entomopathogenic fungal strains of *B. brongniarti, B. bassiana* and *Metarhizium anisopliae* were obtained from PDBC, Bangalore. All the three entomopathogenic fungi were maintained in Potato Dextrose Agar slants at 25 ± 2°C.

Insect Culture

The third instar larvae were collected from sugarcane fields of M/S Bannari Amman Sugars Ltd, Sathyamangalam, Tamil Nadu, which is an area endemic for white grubs. The larvae were maintained on sugar cane plants grown in the pots. These grubs were used for laboratory bioassay in assessing the virulence of three entomopathogenic fungi.

Laboratory Bioassay Studies

The homogenous conidial suspensions of the three entomopathogenic fungi *B. brongniarti, B. bassiana* and *M. anisopliae* were obtained from the cultures maintained on Potato Dextrose Agar media in Petri plates using sterile distilled water and conidial count enumerated using double ruled Neubauer haemocytometer. Serial dilutions of the stock solution were prepared to give concentration of 10^5 to 10^9 in sterile distilled water containing 0.01% Tween 80 as wetting agent. A spore suspension (30 ml) of each isolate was adjusted to concentration of 3×10^9 spores/ml using haemocytometer. Larvae were treated in 30 ml suspension for 1 min and transferred to moist soil placed in plastic rearing boxes and after treatment the remaining suspension was also poured into the plastic boxes. In each treatment 30 larvae were used and replicated thrice. The grubs were fed with sugarcane roots which were changed every week. Control set of larvae were treated with 0.01% Tween 80 alone. The mortality due to mycosis was recorded from 5th day onwards and continued up to 15 days.

Efficacy under Field Conditions

Field trials were conducted to test the efficacy of talc-based formulation of three entomopathogenic fungi against sugarcane white grub in Dharmapuri and Vellore Co-operative Sugar mills area of Tamil Nadu, India. The trials were conducted in randomized block design with five treatments and three replications. One kg talc based formulation with spore count of 7.8×10^9/ml was mixed with 50 kg of farmyard manure and applied near the root zone of the infected field. The field was irrigated after application. Pre-treatment count was taken in 1 m^2 area of the infected field two times at 15 days interval and post-treatment count taken after15 and 20 days.

Table 1. Laboratory bioassay of the three entomopathogenic fungi against sugarcane white grub *Holotrichia serrata*

Treatment	Mean mortality (%) days after treatment		
	5	10	15
Beauveria bassiana	33.6 (35.42)	56.0 (48.44)	65.3 (53.91)
Beauveria brongniarti	39.0 (38.64)	57.0 (49.02)	66.3 (54.51)
Metarhizium anisopliae	48.0 (43.85)	68.3 (55.73)	74.6 (59.73)
Control	(0.73)	(0.73)	(0.73)
SEM	1.83	3.53	4.35
CD (*P* = 0.05)	3.88	7.36	9.06

The figures in parenthesis are arcsine-transformed values

Table 2. Field efficacy of the three entomopathogenic fungal formulations against sugarcane white grub *Holotrichia serrata*

Treatment	Mean mortality (%) days after treatment	
	15	20
T1 Beauveria bassiana	59.0 (50.18)	62.0 (51.94)
T2 Beauveria brongniarti	60.0 (50.76)	65.0 (54.33)
T3 Metarhizium anisopliae	66.0 (56.37)	72.0 (58.05)
T4 B.bassiana+B.brongniarti +M.anisopliae	76.0 (58.65)	84.0 (63.50)
Control	0 (0.73)	10 (0.73)
SEM	4.2	4.3
CD (*P* = 0.05)	8.75	9.0

The figures in parenthesis are arcsine-transformed values

Data analysis

Mortality (%) data was transformed into arcsine-transformed values. The means were compared using Duncans Multiple Range Test (DMRT) (Gomez and Gomez, 1984).

RESULTS

Pathogenicity of Entomopathogenic Fungi in Laboratory Experiments

Under laboratory conditions the green muscardine fungus *Metarhizium anisopliae* induced highest mortality of 74.6% after 15 days of treatment and 65.3% mortality was obtained after white muscardine fungus *B. bassiana* treatment. The three treatments were not significantly different from each other (Table 1).

Field Efficacy

The pre-treatment count of grubs in all the five treatments in field conditions ranged from 10 to12/ m^2. All the four treatments were effective in checking the population of *H. serrata* (Table 2), which ranged between 62 to 72% in 20 days post-treatment. Among the formulations tested combination of the three-entomopathogenic fungi *B. bassiana, B. brongniarti and M. anisopliae* was most virulent causing 84% mortality (Table 2). The yield and quality parameters recorded in different treatments are presented in Table 3. The treatment T4 resulted in the highest yield of 98.25 t/ha and lowest yield was recorded in control plots (60.5 t/ha). The highest quality parameters were recorded in combination treatment (T4), i.e. POL (19.2%), CCS (13.5%), Brix (19.9%) and purity (89.76%). However, the lowest quality parameters POL (16.83%), CCS (11.89%), Brix (19.64%) and purity (84.93%) were recorded in control plots (T5). Individual treatments, i.e. T1, T2 and T3 were almost similar in quality but there was significant difference in yield ($P < 0.05$) (Table 3).

DISCUSSION

The results of the present study show that the

Table 3. Average yield and quality parameters recorded in different treatments of the three-entomopathogenic fungi

Treatment	Yield t/ha	CCS (%)	POL (%)	BRIX (%)	PURITY (%)
T1 Beauveria bassiana	78.55d	12.00 c	18.00 b	19.00 b	85.00 bc
T2 Beauveria brongniarti	83.50c	12.80 bc	18.40 b	19.40 ab	86.00 b
T3 Metarhizium anisopliae	93.50b	13.00 b	19.00 a	19.80 a	87.73 ab
T4 B.bassiana+B.brongniarti +M.anisopliae	98.25a	13.50 a	19.20 a	19.90 a	89.76 a
Control	60.50e	11.89 d	16.83 c	19.64 ab	84.93 c
CD (*P* = 0.05)	1.70	2.64	2.59	1.64	1.23

In the columns means followed by the same letter are not significantly different ($P < 0.05$, Duncans Multiple Range Test).

combined effect of the three-entomopathogenic fungi *B. bassiana*, *B. brongniarti and M. anisopliae* in controlling the sugarcane white grub larvae *H. serrata* was highly significant. The yield and quality parameters improved in all the treatments compared to control. The combination of the three-entomopathogenic talc based fungal formulation showed relatively higher virulence and proved to be suitable candidates for controlling larvae of sugarcane white grub *H. serrata*. These observations are similar to those provided by Keller *et al*. (2002), which suggest that repeated application of the entomopathogenic fungal formulations enhance the pest control process and white grubs could be controlled in field situations in various crops, like *H. consanguinea* infesting potatoes were controlled by *M. anisopliae* (Kulye and Pokharkar, 2009) or high virulence has been reported against *H. serrata* using *B. brongniarti* as lignite or press mud formulations (Eswaramoorthy *et al*., 2005).

The present studies suggest that combination of entomopathogenic fungi are more useful in field situations and there is significant effect on the grubs on one hand and on the other yield and quality is also enhanced. Apparently, successful use of entomopathogenic fungi as microbial control agents will ultimately depend on the use of the right propagule formulated in an optimal manner and applied at an appropriate time and dosage.

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REFERENCES

Charnley, A.K. (2003) Fungal pathogens of insect cuticle degrading enzymes and toxins. *Adv. Bot. Res*,, **40**, 241–321.

David , H. and Ananthanarayanan (1986) White grubs. In H. David , S. Eswaramoorthy and R. Jayanthi (eds.), *Sugarcane Entomology in India*, SBI, Coimbator, pp. 198–208.

Eswaramoorthy, S., Srikanth, J., Santhalakshmi, G. and Geetha, N. (2005) Laboratory and field studies on *Beauveria brongniarti* (Sacc) Petch against *Holotrichia serrata* Fab. (Coleoptera: Scarabidae) in sugarcane. *Cooperative Suga*., **36**, 493–502.

Heycle, A. and St. Leger, R.J. (1994) Interactions between fungal pathogens and insect hosts .*Annu. Rev. Entomol.*, **39**, 293–322.

Keller, S., Kessler, P., Jensen, D.B. and Schweizer, C. (2002) How many spores of *Beauveria brongniarti* are needed to control *Melolantha melolantha*? *Bull. IOLB SROP*, **25**, 59–63.

Kulye, M.S. and Pokharkar, D.S. (2009) Evaluation of two species of entomopathogenic fungi against white grub, *Holotrichia consanguinea* (Blanchard) infesting potato in Maharastra, India. *J. Biol. Contr.*, **23**, 1–4.

Pandey, A.K. and K.R. Kanaujia (2008) Effect of different grains as solid substrate on sporulation, viability and pathogenicity of *Metarhizium anisopliae* (Metschnikoff) sorokin. *J. Biol. Contr*., **22**, 369–374.

Roberts, D.W. and St Leger, R.J. (2004) *Metarhizium* spp. cosmopolitan insect-pathogenic fungi mycological aspects. *Adv. Appl. Microbiol.*, **54**, 1–70.

Thomas, M.B. and Reed, A.F. (2007) Can fungal biopesticide control malaria? *Nat. Rev. Microbiol.*, **5**, 377–383.

Wang, C., Fan, M., Liz, Butt, T.M. (2004) Molecular monitoring and evaluation of the application of the insect pathogenic fungus *Beauveria bassiana* in Southeast China. *J. Appl. Microbiol.*, **96**, 861–870.

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