RESEARCH ARTICLE

RHIZOSPHERE COMPETENCE OF THREE ENTOMOPATHOGENIC FUNGI IN RELATION TO HOST PLANT AND INTER-SPECIFIC INTERACTION

N. Geetha*, D. Nithya, K. Hari, M. Preseetha, K. Subadra Bai and G. Santhalakshmi

Abstract

Three commonly used entomopathogenic fungi (EPF) in sugarcane ecosystem, namely Beauveria bassiana (Balsamo) Vuillemin, Beauveria brongniartii (Saccardo) Petch and Metarhizium anisopliae (Metchnikoff) Sorokin were assessed for their ability to sustain themselves in the rhizosphere in the presence or absence of competition with other fungi, either native or inoculated. In the first of two studies, the inoculated EPFs applied singly were recovered from rhizosphere of five dicots and five monocot crops from sterilized soil as well as unsterilized soil. In the second study, the efficacy of the EPF to survive in the rhizosphere of sugarcane with or without competition from either of the other two EPF or two nematophilic fungi or two antagonistic fungi or two soil saprophytes or sugarcane wilt pathogen Fusarium sp. in sterilized or unsterilized soil was estimated. Results of the first study indicated that in sterilized soil medium, the rhizosphere of okra (82.2%) was the most favourable for survival of EPF which was comparable with most monocots tested. Beauveria bassiana (75.0%) and M. anisopliae (79.0%) were more efficient than B. brongniartii (66.3%) regardless of plant species. The lowest spore harvest was from larvae trapped in rhizosphere of brinjal (3.88 x 10^7/larva) followed by that from cotton rhizosphere (5.55x 10^7/larva). In the second study, in sugarcane rhizosphere in sterilized medium, the mortality of the larvae caused by B. bassiana was synergized by M. anisopliae (96.7%) and significantly higher than all other treatments. In case of B. brongniartii, mortality rates were most affected by fungal antagonists and Fusarium sp. (50.0-53.3%) while M. anisopliae was affected by Trichoderma harzianum (53.3%). Sporulation of Beauveria spp. was consistently affected by Penicillium sp./Aspergillus sp. while most species were competitive with M. anisopliae. In both the studies, only M. anisopliae spores could be recovered from unsterilized soil.

***Key words**: Beauveria bassiana, Beauveria brongniartii, Metarhizium anisopliae, rhizosphere competence, species interaction, sugarcane*

Introduction

In the microbial control of soil pests with entomopathogenic fungi (EPF), the aspect that is least understood and studied is the ability of the applied biocontrol fungal species to survive in the soil in the absence of the target host. To judge the usefulness of the introduced bioagent, it is vital to assess the interactive dynamics among the entomopathogenic organisms, host and environment (Inglis et al. 2001; Vega et al. 2009). The ecology of fungal entomopathogens is to be known in order to improve the chances of success in agricultural production systems (Vega et al. 2009). It is also further required to monitor the microbial control agents which are introduced in the environment by evaluating their establishment, persistence and virulence (Bidochka 2001; Hintz et al. 2001). The use of a rhizosphere competent fungal

N. Geetha, D. Nithya, K. Hari, M. Preseetha,
K. Subadra Bai and G.Santhalakshmi
Sugarcane Breeding Institute
Coimbatore 641007, Tamil Nadu, India
email : mvsbi@yahoo.com

entomopathogen incorporated into the soil during plant propagation would result in a 10-fold reduction in the amount of fungal inoculum required (Bruck 2010).

Among the commonly used fungi, *Metarhizium anisopliae* (Metchnikoff) Sorokin and *Beauveria bassiana* (Balsamo) Vuillemin are ubiquitous insect pathogens and possible plant symbionts as some strains are endophytic or colonize the rhizosphere (Ripoll et al. 2011); *Beauveria brongniartii* is used for the control of white grubs in sugarcane (Easwaramoorthy et al. 2004). The major factors that influence the survival of these pathogens are soil type, pH and moisture, plant species and root exudates, besides other introduced biocontrol fungi and microflora native to the soil. In the present study, we have attempted to assess the ability of three EPF species to remain active (a) when applied as individual species in the rhizosphere of different crops; (b) when applied in combination with either other entomopathogenic fungi or nematophilic fungi or fungal antagonists or a fungal pathogen or commonly found saprophytes and remain virulent enough to cause mortality of target larvae. Competition from the native species of fauna can often render the applied fungus less efficient in terms of establishment and thus persistence. Hence, the efficacy of the three EPF as influenced by the rhizosphere of sugarcane in the presence (unsterilized soil) or absence (sterilized soil) of the native species was also assessed.

Materials and methods

Maintenance of *Galleria mellonella* culture

Fifth instar larvae of *G. mellonella*, a laboratory host suitable as bait for isolation of fungi from soil samples (Zimmermann 1986), were obtained from a laboratory culture maintained on artificial diet at ambient temperature in the insectary of Section of Entomology, Sugarcane Breeding Institute, Coimbatore. The larvae were processed prior to fungus treatment as per Meyling (2007) to prevent webbing.

Rhizosphere competence of EPF

Two studies were taken up to assess the rhizosphere competence of EPF. In study 1, the persistence of the three EPF, namely *B. bassiana, B. brongniartii and M. anisopliae* was evaluated individually in the rhizosphere of different crops. In study 2, the persistence of the three different EPF in different combinations with other biocontrol fungi was examined in sugarcane rhizosphere. Two sets of experiments, *viz*. pots with autoclaved soil mixture and unsterilized soil mixture were maintained. Controls were maintained for each set without inoculation of the fungus.

Fungal species tested for species competence of EPF

Nematophilic fungi : *Paecilomyces lilacinus* (Thom) Samson and *Verticillium chlamydosporium* Goddard

Fungal antagonists:*Trichoderma harzianum* Rifai and *Trichoderma viridae* Pers.

Plant pathogenic fungus: *Fusarium* sp.

Soil saprophytic fungi: *Aspergillus* sp. and *Penicillium* sp.

Rhizosphere competence of EPF in different plant species

Five monocot plant species and five dicot plant species namely, sugarcane (*Saccharum officinarum* L.) maize (*Zea mays* L.), sorghum (*Sorghum vulgare* Pers.), pearl millet (*Pennisetum glaucum* L.), finger millet (*Eleucine coracana* (L.) Gaertn.), cotton (*Gossypium* sp.), okra (*Abelmoschus esculentus* Moench), tomato (*Lycopersicon esculentum* Mill.), chillies (*Capsicum frutescens* L.) and brinjal (*Solanum melongena* L.) were raised individually in sterilized soil mixture in pot culture. When the plants were 30 days old, spores from liquid cultures of each of the three EPF were inoculated in the rhizosphere at field recommended dose (10^{12}/ha) in the pots. Two replicates for each fungus in each plant species were maintained. Pots were maintained in ambient temperature and sunlight. Soil moisture in the pots was maintained by watering on alternate days. The rhizosphere soil was sampled one month after fungus inoculation in plastic containers in which 15 fifth instar larvae of *G. mellonella* were placed per replicate for each fungus. The dead larvae were transferred from the soil sample to petriplates lined with moist sterile filter papers for further incubation

and sporulation of fungus if any; percent mortality of larvae was recorded. The cadavers showing death due to fungus were macerated with a pestle in a mortar in proportionate quantity of sterilized water (0.5ml/larva) and the suspension was filtered through muslin cloth. Serial dilutions were made when necessary. The spore counts were done using haemocytometer.

Species interactions of EPF in sugarcane rhizosphere

Sugarcane plants were raised in pot culture and when the plants were 45 days old, spore suspensions of the three EPF, two fungal antagonists and the nematophilic fungi were applied in the rhizosphere at the field recommended dose. Each of the three EPF was evaluated in combinations with another fungus from the three groups. There were 27 combinations each with two replicates. Suitable controls without application of fungus were maintained. After 30 days of fungal inoculation, the rhizosphere soil sample was retrieved in plastic containers. The fungus was recovered using fifth instar *G. mellonella* larvae as baits. The dead larvae were transferred from the soil sample to petriplates lined with moist sterile filter paper for further incubation and sporulation of fungus; percent larval mortality was recorded. The cadavers showing death due to fungus were macerated with a pestle in a mortar in proportionate quantity of sterilized water (0.5ml/larva) and the suspension was filtered though muslin cloth. Serial dilutions were made when necessary. The spore counts were done using haemocytometer.

Data analysis

The data from the various experiments were subjected to log (x + 0.5) or arc sine transformation as needed and analysis of variance was done in SPSS version 11.5 and treatment means were separated by Duncan's Multiple Range Test (DMRT).

Results and discussion

Rhizosphere competence of the three EPF as influenced by different plant species

Sterilized soil

In the sterilized soil medium of the different plant rhizospheres, while the main effects of fungal species and rhizosphere effect of plant species were highly significant, the interaction effects were not significant in causing mortality of *G. mellonella* larvae used as bait (Table 1). Among the three EPF,

Table 1. Mortality of *Galleria mellonella* due to EPF as influenced by plant species in sterilized soil

Crop rhizosphere	Percent mortality of *G. mellonella* larvae *			Effect of crop alone$
	B. bassiana	*B. brongniartii*	*M. anisopliae*	
Tomato	70.0 a	56.7 ab	76.7 ab	67.8 ab
Cotton	70.0 a	60.0 abc	83.3 ab	71.1 abc
Brinjal	70.0 a	50.0 a	70.0 a	63.3 a
Okra	90.0 b	70.0 bcd	86.7 b	82.2 d
Chillies	73.3 a	70.0 bcd	76.7 ab	73.3 bc
Pearl millet	76.7 a	60.0 abc	76.7 ab	71.1 abc
Finger millet	70.0a	73.3 de	80.0 ab	74.5 bcd
Sorghum	76.7 a	76.7 e	80.0 ab	77.8 cd
Maize	70.0 a	70.0 bcd	83.4 ab	74.5 bcd
Sugarcane	83.3 ab	76.7 e	76.7 ab	78.9cd
Effect of species#	75.0 B	66.3 A	79.0 B	

*S.E. 4. 51; $ S.E. 2.61; # S.E. 1.43

Means separated by DMRT (α =0.05); Means followed by same alphabets in a column (lower case) and row (upper case) are not significantly different. No larval mortality or sporulation of any species of fungus was observed in control and thus not included in analysis.

Table 2. Spore harvest from *Galleria mellonella* cadavers recovered from different plant rhizospheres in sterilized soil

Crop tested	Sporulation in *G. mellonella* larvae x 10^7/larva *			Effect of crop alone$
	B. bassiana	*B. brongniartii*	*M. anisopliae*	
Tomato	6.6 b	4.4 bc	8.4 bc	6.5.bc
Cotton	6.2 b	3.7 b	6.8 b	5.6 b
Brinjal	4.3 a	2.6 a	4.9 a	3.9 a
Okra	9.2 cd	5.0 bcd	9.3 cde	7.8 c
Chillies	8.7 cd	4.9 bcd	9.0 cd	7.6 d
Pearl millet	7.6 bc	4.9 bcd	9.6 cde	7.3 cd
Finger millet	8.4 cd	4.6 bc	8.7 bc	7.2 cd
Sorghum	10.0 d	7.0 d	1.2 e	9.6 f
Maize	10.0 d	5.8 cd	1.1 de	9.0 ef
Sugarcane	8.9.cd	5.5 cd	10.1 cde	8.2 de
Species alone#	8.0 B	4.8 A	9.0 C	

*S.E. : 57.87; $ S.E. :33.41; # S.E.: 18.30; log (x+0.5).

Means separated by DMRT (α =0.05); Means followed by same alphabets in a column (lower case) and row (upper case) are not significantly different. No larval mortality or sporulation of any species of fungus was observed in control and thus not included in analysis.

B. bassiana and *M. anisopliae* were more efficient than *B. brongniartii* in causing larval mortality regardless of plant species. The overall effect of plant species was such that brinjal rhizosphere was the least favourable for the fungal efficacy (63.3%). However, it was on par with the effect of tomato rhizosphere (67.8%). The most encouraging rhizosphere was that of okra resulting in the highest efficacy of fungus across the species (82.2%) which was, however, not significantly different from those of all monocots (74.5-78.9%) barring pearl millet..

The spore recovery per cadaver among the different crop rhizospheres (Table 2) was the lowest from bait larvae in rhizosphere of brinjal (3.9 x 10^7/larva) followed by that from larvae used as baits in cotton rhizosphere (5.6 x 10^7/larva). Irrespective of the crop, among the three EPF tested *B. brongniartii* had the lowest spore recovery (4.8 x 10^7/larva) and *M. aniospliae* was the most abundant (9.0 x 10^7/larva).

There was no mortality of *G. mellonella* larvae or sporulation of any of the fungi when the soil was sterilized and not inoculated by any fungus.

Unsterilized soil

When the plants were raised in unsterilized soil the bait larvae died only due to *M. aniospliae* and the mortality rates ranged from 66.7- 80.6% irrespective of the species of inoculated fungus (Table 3). There were overlapping but significant differences in the mortality rates. The highest mortalities were from rhizosphere of cotton, brinjal, okra and chillies which were on par (80.6-86.1%) with that in sugarcane. There were no significant differences among the mortality rates caused due to *M. anisopliae* in soils infused with *B. bassiana* (77.1%), *B. brongniartii* (77.9%) and *M. anisopliae* (77.9%) indicating the fact that the natural population of *M. anisopliae* dominated over the artificially inoculated fungi. The spore harvest of *M. anisopliae* was overlappingly similar irrespective of either the plant species or fungus species inoculated (Table 4).

The mortality rates of larvae and sporulation of *M. anisopliae* were similar to the treatments (data not shown) in the control pots for each plant species with unsterilized soil medium which did not have inoculation of any fungus.

Table 3. Mortality of *Galleria mellonella* due to EPF as influenced by plant rhizosphere in unsterilized soil

Crop tested	Percent mortality of *G. mellonella* larvae*			Effect of crop alone$
	B. bassiana	*B. brongniartii*	*M. anisopliae*	
Tomato	66.7 a	62.5 ab	70.8 ab	66.7 a
Cotton	79.2 ab	83.3 bcd	79.2 ab	80.5 bc
Brinjal	79.2 ab	87.5 cd	83.3 b	83.3 c
Okra	83.3 b	91.7 d	83.3 b	86.1 c
Chillies	83.3 b	83.3 bcd	83.3 b	83.3 c
Pearl millet	79.2 ab	75.0 abc	58.3 a	70.8 ab
Finger millet	79.2 ab	83.3 bcd	79.2 ab	80.6 bc
Sorghum	70.8 ab	54.2 a	79.2 ab	68.1 a
Maize	83.3 b	75.0 abc	83.3 b	80.6 bc
Sugarcane	66.7 a	83.3 bcd	79.2 ab	76.4 abc
Effect of species alone#	77.1 A	77.9 A	77.9 A	

*S.E.: 5.54; $ S.E.: 3.20; # S.E.: 1.75

Means separated by DMRT (ά= 0.05); Means followed by same alphabets in a column (lower case) and row (upper case) are not significantly different. Recovery of *M. anisopliae* spores alone was observed from cadavers irrespective of the fungus species applied in the soil.

Table 4. Spore harvest from *Galleria mellonella* cadavers recovered from different plant rhizospheres in unsterilized soil

Crop tested	Sporulation in *G. mellonella* larvae x 10^7/larva*			Effect of crop alone$
	B. bassiana	*B. brongniartii*	*M. anisopliae*	
Tomato	7.6 ab	3.1 a	14.5 b	8.4 ab
Cotton	7.7 ab	4.4 ab	8.3 ab	6.8 ab
Brinjal	4.9 a	5.2 abc	4.5 a	4.9 a
Okra	11.6 b	2.3 a	14.0 b	9.3 ab
Chillies	6.8 ab	11.9 bcd	8.4 ab	9.0 b
Pearl millet	9.6 ab	12.6 bcd	6.7 ab	9.6 b
Finger millet	7.7 ab	10.6 bcd	7.7 ab	8.7 b
Sorghum	12.5 b	15.1 d	7.8 ab	11.8 b
Maize	10.8 ab	14.5 d	8.7 ab	11.3 b
Sugarcane	10.0 ab	3.1d	7.7 ab	10.1 b
Effect of species alone#	8.9 A	9.2 A	8.8 A	

S.E. 3.52 ; # S.E. : 1.75; $$ S.E. : 181.00; $$$:258.42; $$$$ S.E : 278.05

Log (x+0.5) transformed data. means separated by DMRT (α =0.05); Means in a column followed by same alphabets (lower case) and similar alphabet in a row (upper case) are not significantly different. Recovery of *M. anisopliae* spores alone was observed. The sporulation rates were similar to the controls kept (data not shown).

Table 5. *In vivo* efficacy of the entomopathogenic fungi as influenced by species interaction in sugarcane (sterilized soil)

Combination fungus tested	Target entomopathogenic fungi					
	Beauveria bassiana		*Beauveria brongniartii*		*Metarhizium anisopliae*	
	% Mortality	Spore harvest (x 10^7/larva)	% Mortality	Spore harvest (x 10^7/larva)	% Mortality	Spore harvest (x 10^7/larva)
B. bassiana	80.0[1] a	10.6[1] e	93.3 e	0.5 ab	96.7 c	3.8 bc
B. brongniartii	86.7 a	10.2 e	83.4[1] de	0.9[1] b	83.3 b	22.0 e
M. anisopliae	96.7 b	1.9 c	90.0 de	0.9 b	86.7[1] bc	11.0[1] d
P. lilacinus	83.3 a	9.2 e	76.6 cd	0.4 ab	83.3 b	2.0 a
V. chlamydosporium	80.0 a	1.5 c	70.0 bc	0.9 b	76.7 b	2.6.a
T. viride	76.7 a	8.5 e	53.3 bc	0.5 ab	73.3 ab	5.5 c
T. harzianum	80.0 a	5.1 d	50.0 a	0.3 a	53.3 a	2.7 ab
Fusarium sp	90.0 ab	9.5.b	53.3 ab	0.3 a	90.0 bc	10.3 d
Penicillium sp.	80.0 a	0.0 a	83.3 cde	0.3 a	83.3 b	3.6 abc
Aspergillus sp.	83.3 a	0.0 a	90.0 de	0.3 a	73.3 ab	3.5 abc
Mean	83.7	4.8	74.3	0.5	80.0	6.7

Means separated by DMRT (α =0.05); Means in a column followed by same alphabets are not significantly different. The sporulation rates were similar to the controls kept (data not shown)

[1] Target fungus alone tested

Rhizosphere competence and species competition influencing EPF efficacy in sugarcane

Sterilized soil

The mortality of the larvae caused by *B. bassiana* was similar in all treatments (80.0 to 90.0%) except the combination treatment with *M. anisopliae* which was 96.7% and significantly higher than in all other treatments (Table 5). Combination treatment of *B. bassiana* with *Fusarium* sp. was on par with *B. bassiana* + *M. anisopliae* as well as other treatments. Sporulation of *B. bassiana* on *G. mellonella* cadavers was the highest when it was either applied alone (1.06 x 10^8/ larva) or with *B. brongniartii* (1.02x 10^8/ larva). There was no sporulation of *B. bassiana* when it was combined with either *Aspergillus* sp. or *Penicillium* sp.

The effect of fungus combinations in causing larval mortality (*P*=0.00) and sporulation (*P*=0.03) was significant when *B. brongniartii* was applied alone or in combination in sugarcane rhizosphere in sterilized soil (Table 5). Lowest mortality rates were recorded in the combination treatments of *B. brongniartii* + *T. viride* (53.3%), *B. brongniartii* + *T. harzianum* (50.0%) as well as *B. brongniartii* + *Fusarium* sp. (53.3%). The efficacy of the fungus was unaffected when it was combined with either *B. bassiana* (93.3%) or *M. anisopliae* (90.0%) or *P. lilacinus* (76.6%) or *Penicillium* sp. (83.3%) or *Aspergillus* sp. (90.0%). Severe competitive effect was observed during sporulation of *B. brongniartii* when it was inoculated with either *T. harzianum* (3.0 x10^6/larva) or *Fusarium* sp. (2.8 x10^6/larva) or *Penicillium* sp. (3.0 x10^6/larva) or *Aspergillus* sp. (2.5 x10^6/larva) in sterile sugarcane rhizosphere. Neither the EPF nor nematophagous fungi nor *T. viride* was inhibitive to the *in vivo* sporulation of *B. brongniartii* in *G. mellonella* larvae used as bait in sterile sugarcane rhizosphere.

The mortality rates caused by *M. anisopliae* were not affected by *B. bassiana* (96.7%) and *Fusarium*

sp. (90.0%) whereas they were unfavourably influenced by all the other species of which both species of *Trichoderma* (73.4 and 53.3%, respectively) and *Aspergillus* sp. (73.3%) were the most harmful (Table 5). Except *Fusarium* sp. (1.03 x 10^8/larva) which was on par and *B. brongniartii* which was complimentary (2.2 x 10^8/larva), all other species of fungi significantly reduced the sporulation of *M. anisopliae*.

Unsterilized soil

The mortality of larvae was found to be statistically similar in all the treatments irrespective of the combinations in which *B. bassiana* was applied. Mortality of the larvae may have been caused by any of the EPF but sporulation of only *M. anisopliae* was observed in the cadavers (Table 6). Similarly, except when the inoculations had *B. bassiana* with one of the two fungi, namely *V. chlamydosporium* (4.0 x 10^7/larva) and *Aspergillus* sp. (4.2 x 10^7/larva), the spore harvest of native *M. anisopliae* was unaffected by any other fungal combinations inoculated.

No effect of species combination was observed in the larval mortality in the treatments involving *B. brongniartii* except the treatment that had *B. brongniartii* + *T. viride* (54.2%) but sporulation showed that only *M. anisopliae* was found on the cadavers irrespective of treatment combinations (Table 6). Similarly, no significant effect of treatment combinations was found on sporulation of *M. anisopliae*.

In the treatments involving *M. anisopliae*, mortality rates of *G. mellonella* were found be overlappingly significant among the various treatments with the lowest in the treatment combination of *M. anisopliae* with *T. viride* (Table 6). However, the sporulation on the cadavers indicated the presence of only *M. anisopliae*. The data on sporulation clearly demonstrated the dominance of *M. anisopliae* over the applied fungus irrespective of the combination of the fungus, as the sporulation rates of *M. anisopliae* in control (data not shown) were similar to the sporulation rates of the fungus in applied pots.

Table 6. *In vivo* efficacy of the entomopathogenic fungi as influenced by species interaction in sugarcane (unsterilized soil)

Combination fungus tested	Target entomopathogenic fungi					
	Beauveria bassiana		*Beauveria brongniartii*		*Metarhizium anisopliae*	
	% Mortality	Spore harvest of *M. anisopliae* (x 10^7/larva)	% Mortality	Spore harvest of *M. anisopliae* (x 10^7/larva)	% Mortality	Spore harvest of *M. anisopliae* (x 10^7/larva)
B. bassiana	83.3[1] a	3.2[1] a	83.3 ab	2.7 ab	70.8 abc	3.2 a
B. brongniartii	70.8 a	7.9 bc	79.2[1] ab	3.1[1] abc	79.2 bc	2.7 a
M. anisopliae	70.8 a	14.3 c	79.2 ab	9.5 c	83.3[1] cd	12.8[1] b
P. lilacinus	62.5 a	11.5 c	87.5 ab	4.4 abc	91.7 d	4.6 a
V. chlamydosporium	79.2 a	4.0 ab	66.7 ab	2.7 ab	83.3 cd	4.0 a
T. viride	79.2 a	6.8 bc	54.2 a	2.9 ab	62.5 a	8.4 b
T. harzianum	62.5 a	12.0 c	83.3 ab	1.4 a	70.8 abc	3.6 a
Fusarium sp	91.7 a	8.8 bc	91.7 b	4.4 abc	83.3 cd	3.4a
Penicillium sp.	91.7 a	12.4 c	79.2 ab	3.4 abc	66.7 ab	13.3 b
Aspergillus sp.	70.8 a	4.2 ab	91.7 b	7.7 bc	83.3 cd	2.4 a
Mean	67.1	8.5	79.6	4.2	77.5	5.8

Means separated by DMRT (α =0.05); Means in a column followed by same alphabets are not significantly different. Recovery of *M. anisopliae* spores alone was observed. The sporulation rates were similar to the controls kept (data not shown)

[1] Target fungus alone tested

It has been well documented that native species or strains of entomopathogens have been proven to be more virulent than the introduced or exotic stains of fungi at least in the case of *M. anisopliae* (Dhoj et al. 2008). In the present case too, it could well be seen that in unsterilized soil the native fauna of *M. anisopliae* superceded the other species of the fungi in causing mortality as well as in spore recovery in both the studies. It overtook the effect of rhizosphere as well as the interactive effect with other inoculated fungi. Part of the reasoning could be that the native isolates/species might have well adapted or evolved to utilize and survive in the root exudates of the plants they colonize. The germination rates of spores in the inoculum which were maintained in the laboratory in the present studies could have been affected partially by the root exudates causing lesser establishment and survival. Variability in the germination capacity of *M. anisopliae, B. bassiana* and the soil saprophytes *T. harzianum* and *Aspergillus niger* has been reported elsewhere (Ripoll et al. 2011).

Dicot rhizosphere has been shown to support greater EPF survival than monocot rhizosphere. *Metarhizium anisopliae* could be more frequently isolated from soils under vegetable crops as compared to soils under sugarcane or habitat with natural vegetation (Sookar et al. 2008). Positive influence of root exudates from Bengal gram on *T. harzianum* aiding it to colonize the rhizosphere better has been reported (Jash and Pan 2007). They also found that isolates with high biological activity were not always highly rhizosphere-competent. Hence, recovery does not always mean higher virulence of fungus, though in the present study it was proven true to be so in the case of *M. anisopliae*. Incidentally and indirectly, the positive influence of exudates on associate biocontrol agents could have a competition effect on the target bioagent which has to be taken into account in deciding higher inoculative dose of the latter. In the present study, specific distinction between dicot and monocot rhizosphere in favouring fungal survival was not observed despite individual crop variation. Despite certain differences in the prevalence of EPF in the soil samples taken from refugia compared to cultivated orchards, no significant differences in the recovery rates of fungal isolates from both was observed by Goble et al. (2010).

Rhizosphere competent microorganisms are those that show enhanced growth in response to developing roots (Schmidt 1979). *Metarhizium anisopliae* turned to be a better candidate in the present study in terms of rhizosphere competence and spore recovery in both the tests with different rhizosphere as well as species competition. The use of rhizosphere- competent fungal entomopathogens has been suggested in effective control of root-feeding insect pests without the added cost of treating the surrounding bulk soil with large numbers of fungal propagules. Great numbers of fungal entomopathogen propagules are applied or incorporated into soil for the control of root-feeding insects, most of which are not involved in control (Bruck 2010).

Synergism has been observed between fungal and bacterial antagonists for biocontrol of diseases earlier (Barua and Bora 2009; Dixon and Tilston 2010). Synergistic action between *Heterorhabditis bacteriophora* and *M. anisopliae* resulted in faster and higher mortality of larvae but reduced the number of infective juveniles of the nematodes harvested (Acevedo et al. 2007). When two agents share the same food source and niche it is to be expected that one of the lesser adapted one will be affected. Similar results have been observed in the present assessment wherein severe competition has been observed among different fungi resulting in the reduction of spore harvest of which *B. brongniartii* was the most sensitive.

Conclusions

EPFs are potential biocontrol agents that can be used for the control of soil pests in sugarcane. While much importance has been given to the influence of abiotic factors and standardization of dosages and formulations, adequate concentration on the effect of rhizosphere and the interaction with the other biotic elements has not been paid. In order to make effective use of these fungi, it is essential not only to choose/develop a pest- specific biologically virulent strain but also a rhizosphere-competent species or strain with the ability to withstand competition from native fauna. The studies using *G. mellonella* would serve as a model for testing the virulence and persistence of EPF under natural conditions. An allowance in the dosages for the species competition as well as the plant rhizosphere

effect would enable better control of target pests. Further, as seen in the tests with unsterilized soil with native fauna, *M. anisopliae* is naturally dominant over all other fungi irrespective of fungi inoculated or otherwise. Such findings would lead us to understand why some fungal species effective in the laboratory fail in the field.

Acknowledgements

The work forms part of the thesis work submitted for the M.Sc. dissertation by the second author. The authors thank the Director, Sugarcane Breeding Institute, for the facilities and support provided.

References

Acevedo JPM, Samuels RI, Machado IR, Dolinski C (2007) Interactions between isolates of the entomopathogenic fungus *Metarhizium anisopliae* and the entomopathogenic nematode *Heterorhabditis bacteriophora* JPM4 during infection of the sugar cane borer *Diatraea saccharalis* (Lepidoptera: Pyralidae) Journal of Invertebrate Pathology 96:187-192.

Barua L, Bora BC (2009) Compatibility of *Trichoderma harzianum* and *Pseudomonas fluorescens* against *Meloidogyne incognita* and *Ralstonia solanacearum* complex on brinjal. Indian Journal of Nematology 39(1): 29-34.

Bidochka MJ (2001) Monitoring of the fate of biocontrol fungi. In: Fungi as Biocontrol Agents: Progress, Problems and Potential (ed. T.M. Butt, C.W. Jackson, and N. Magan) Oxon: CABI Publishing: 193–218.

Bruck DJ (2010) Fungal entomopathogens in the rhizosphere. BioControl 55:103–112.

Dhoj YGC, Keller S, Nagel P (2008). Occurrence of insect pathogenic fungi in Nepal with especial reference to *Beauveria bassiana* and *Metarhizium anisopliae*. Journal of Plant Protection Society 1: 106-114.

Dixon GR, Tilston EL (2010) Soil microbiology and sustainable crop production, (Ed) Springer, Newyork: 340.

Easwaramoorthy S, Srikanth J, Santhalakshmi G, Geetha N (2004) Laboratory and field studies on *Beauveria brongniartii* (Sacc.) Petch. against *Holotrichia serrata* F. (Coleoptera: Scarabaeidae) in sugarcane. Proc A Conv Sug Technol Ass India 66:A3-A19

Goble T, Dames J, Hill PM, Moore S (2010) The effects of farming system, habitat type and bait type on the isolation of entomopathogenic fungi from citrus soils in the Eastern Cape Province, South Africa. BioControl 55:399-412.

Hintz WE, Becker EM, Shamoun SF (2001) Development of genetic markers for risk assessment of biological control agents. Canadian Journal of Plant Pathology 23:13-18.

Inglis GD, Goettel MS, Butt TM, Strasser H (2001) Use of *Hyphomycetous* fungi for managing insect pests. In: Fungi as Biocontrol Agents (ed. T.M Butt, C. Jackson, and N. Magan) Wallingford: CAB International 23–69.

Jash S, Pan S (2007) Variability in antagonistic activity and root colonizing behaviour of *Trichoderma* isolates Journal of Tropical Agriculture 45: 29–35.

Meyling, NV (2007) Methods for isolation of entomopathogenic fungi from the soil environment. Laboratory manual, Department of Ecology, Faculty of Life Sciences, University of Copenhagen,Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark: http://orgprints.org/11200/1/11200.pdf

Ripoll MP, Fang CA, Wang S, Posada FJ, St Leger R (2011) The rhizosphere-competent entomopathogen *Metarhizium anisopliae* expresses a specific subset of genes in plant root exudate *Microbiology* 157:47-55.

Sookar P, Bhagwant S, Ouna EA (2008) Isolation of entomopathogenic fungi from the soil and their pathogenicity to two fruit fly species (Diptera:Tephiritidae). Journal of Applied Entomology 132**:**778-788.

Schmidt EL (1979) Initiation of plant root-microbe interactions. Annual Review of Microbiology 33: 355–376.

Vega FE, Goettel MS, Blackwell M, Chandler D, Jackson MA, Keller S, Koike M, Maniania NK, Monzo´n A, Ownley BH, Pell JK, Rangel DEN, Roy HE (2009) Fungal entomopathogens: new insights on their ecology. Fungal Ecology 2:149–159.

Zimmermann G (1986) The *Galleria* bait method for detection of entomopathogenic fungi in soil. Journal of Applied Entomology 102**:** 213-215.