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EVALUATION OF *TRICHODERMA* SPP AND *PSEUDOMONAS FLUORESCENS* FOR SUPPRESSION OF *PHYTOPHTHORA CAPSICI* INFECTING BLACK PEPPER

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ABSTRACT

Foot rot caused by *Phytophthora capsici* is a serious disease of black pepper in India. A filed experiment was conducted at Thamarassery, Kozhikode district, Kerala, India to study the effect of eight *Trichoderma*, four *P. fluorescens* and four combinations of *Trichoderma* + *P. fluorescens* in order to establish their potential for disease suppression. These isolates were short-listed based on their antagonistic potential in *in-vitro* and *in-vivo* (pot culture) tests. Biocontrol inoculum was applied to black pepper vines twice a year in June (pre-monsoon) and September (during monsoon break) and disease incidence and growth parameters were recorded. After two years of experimentation *T. harzianum* IISR 1369 + *P. fluorescens* IISR 41 combination showed maximum plant height followed by *T. harzianum* IISR 1369 + *P.*

fluorescens IISR 6 and *T. virens* IISR 1370 + *P. fluorescens* IISR 11. External manifestation of symptoms was not noticed in any of the treatments. Since destructive sampling was not undertaken to score the root rot their exact role on the disease suppression could not be studied. However, population of *P. capsici* was monitored and detected in different treatments at periods of interval by soil baiting using *Albizzia* leaves. Pooled analysis of population of *Trichoderma* over the years showed that it was able to survive maintaining a population level of about 10^4 cfu / g of soil. A similar trend was observed in the case of *P. fluorescens* also. However the population of *P. fluorescens* was higher than *Trichoderma*. Since there was no natural incidence of disease in the field, treatments were repeated in pot culture taking large amount of soil (4 kg / pot). When challenge inoculated with *P. capsici*, treatments applied with *P. fluoreoscens* IISR 13 and *P. fluorescens* IISR 41 were highly disease suppressive showing only 10% disease incidence as compared to 90% in the control. Combination of *T. harzianum* IISR 1369 + *P. fluorescens* IISR 41, *T. harzianum* IISR 1369 + *P. fluorescens* IISR 6 and *T. virens* IISR 1370 + *P. fluorescens* IISR 13 were also on par with the above treatments. Hence it is inferred that *P. fluorescens* IISR 41 and *P. fluorescens* IISR 13 can be utilized for the suppression of *P. capsici*. Combination of *T. harzianum* IISR 1369 + *P. fluorescens* IISR 41 and *T. harzianum* IISR 1369 + *P. fluorescens* IISR 6 are disease suppressive and promote growth of black pepper.

INTRODUCTION

Foot rot of black pepper (*Piper nigrum* L.) caused by *Phytophthora capsici* is a serious disease of the crop in India (Sarma *et al.*, 1994). Several antagonists like *Trichoderma harzianum, T. virens* (Rajan, 1999; Rajan *et al.*, 2002) and VAM fungi (Sarma *et al.*, 1996) were isolated and evaluated for the biological disease suppression. The technology is being used in large scale during the past few years. In order to update the technology more potent strains of antagonists are needed. In this study we report on the performance of few *Trichoderma* spp and *Pseudomonas fluorescens* and some of their combinations under field conditions in comparison with existing potent ones.

MATERIALS AND METHODS

Biocontrol agents and mass multiplication

Biocontrol agents were taken based on their antagonistic potential in *in-vitro* and *in-vivo* (pot culture) studies (Saju *et al.*, 1999; Saju *et al.*, 2002). *Trichoderma* spp was multiplied on sorghum grains (Prakash *et al.*, 1999) and *P. fluorescens* in nutrient broth.

Field Experiment

Field trial was conducted at Thamarassery, Kozhikode district, Kerala to study the disease suppressive effect of *Trichoderma* spp and *Pseudomonas fluorescens* and some of their combinations in comparison with existing potent ones.

Experimental details:

Design:	RBD
Treatments	17

Replications	3
No. of plants per replication	20
Standard	seven years old Arecanut

The plants consisted of Karimunda, Panniyur 1, Panniyur 2, Panniyur 4, Panniyur 5, which were first raised in poly bags. The plants were cut into uniform length of 30 cm before planting July 2000. About 50 g of *Trichoderma* was added to each pit during planting of cuttings. About 1 litre of nutrient broth, 48 h old shake culture was diluted to 10 L with water and 50 ml was added to each pit. In treatments were combinations were used 25 g of *Trichoderma* and 25 ml of *P. fluorescens* was added. There were two applications per year in June and September. Periodically soil samples were collected from the base of five plants randomly in each replication. The samples were pooled and 100 g soil was used to detect the presence of *Phytophthora* by baiting. About 150 ml of water was added to it and stirred well and 10 leaves of *Albizzia* was added and after 48 hours the leaves were observed under the microscope for the presence of sporangia (Anandaraj and Sarma, 1990). Remaining soil was air dried for 24 hours and number of colony forming units of *Trichoderma*, *P. fluorescens* an other fungi were determined by SDPT in TSM, Kings' B agar and Rose Bengal Agar respectively. Plant height was measured in June 2002 and disease symptoms / incidence was recorded, if any.

Challenge inoculation

Since there was no natural infection of *Phytophthora* in the field planted black pepper the same set of treatments was carried out in glass house by taking large quantity of soil (4 kg / pot). Cuttings of black pepper var. Panniyur 1 were planted in polythene bags of size 17.5 X 9.5''. *Trichoderma* spp and *P. fluorescens* were multiplied and applied as above. There were 10 plants per treatments. The plants in each treatment were challenge inoculated with *P. capsici* isolate 99-101. Ten sporulating discs were added to each bag after removing the topsoil and replacing the same. The number of mortality / root rot affected plants was recorded. Results of mortality / root rot was converted to percentages for presentation.

Preparation of *P. capsici* inoculum

P. capsici was sub-cultured on carrot agar and grown for 4 days at room temperature (26-28^{0}C). Discs of 1 cm diameter were cut from the plates and put in another plate having 15 ml of sterile distilled water. The plates were incubated under continuous fluorescent light for 48 hours. The discs were observed under the microscope for presence of mature sporangia. Sample plates were given a cold shock for 10 minutes in a refrigerator and again observed under the microscope for the release of zoospores.

Statistical analysis

Increase in plant height was analyzed by analysis of variance followed by range test for mean comparison using MSTATC software. The number of cfu of *Trichoderma*, *P. fluorescens* and other fungi were converted to log cfu / g and pooled over the years. The pooled data was analyzed by analysis of variance.

RESULTS

In treatment with *T. harzianum* IISR 1369 + P. *fluorescens* IISR 41 recorded maximum plant height of 204.9 cm followed by *T. harzianum* IISR 1369 + *P. fluorescens* IISR 6 (199.0 cm) and *T. virens* IISR 1370 + *P. fluorescens* IISR 11 (192.6 cm) (Table 1). Single application of fungi like *T. harzianum* IISR 1369, *T. aureoviride* IISR 143, *T. pseudokoningii* IISR 187, *T. harzianum* IISR 167 were on par with *P. fluorescens* IISR 6 and combination of *T. virens* IISR 1370 + *P. fluorescens* IISR 13 (Table 1). In general, treatments applied with *Trichoderma* spp *P. fluorescens* were recorded increase in plant height than the control.

Disease incidence was not noticed in any of the treatments including control hence the exact role of these biocontrol agents in disease suppression could not be studied at field level. However *Phytophthora* was detected in all the various treatments at periods of interval by baiting. Population of *Trichoderma* in various treatments varied form $10^3 \ldots 10^6$ cfu / g of soil in dry and wet months. A decline in population in dry months and an increase in wet months was recorded. However, the pooled analysis of population over the years showed that *Trichoderma* is able to survive maintaining a population level of 10^4 cfu / g (Figure 1). A similar trend was observed in the case of *P. fluorescens* also (Figure 2). However the population of *P. fluorescens* was higher than *Trichoderma*. Pooled analysis of population of other fungi also showed variation over the period (Figure 3).

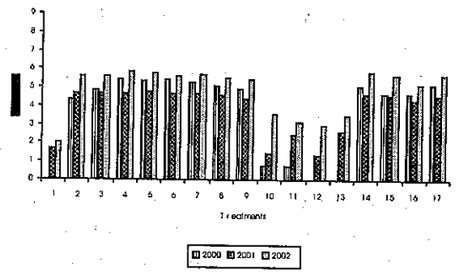

Fig 1. Population of *Trichoderma* applied to black pepper in the field.

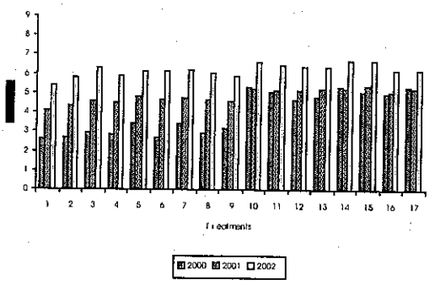

Fig. 2. Population of *P. fluorescens* applied to black pepper in the field

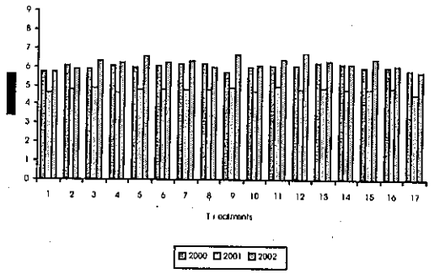

Fig. 3. Population of other fungi in black pepper field applied with *Trichoderma* and *P. fluorescens*

When challenge inoculated with *P. capsici* treatments applied with *P. fluoreoscens* IISR 13 and *P. fluorescens* IISR 41 were highly disease suppressive showing only 10% disease incidence as compared to 90% in the control. Combination of *T. harzianum* IISR 1369 + *P. fluorescens* IISR 41, *T. harzianum* IISR 1369 + *P. fluorescens* IISR 6 and *T. virens* IISR 1370 + *P. fluorescens* IISR 13 were also on par with the above treatments (Table 1).

DISCUSSION

For inundative biological control data on field performance of biocontrol agents is necessary. Data shown that combination of *Trichoderma* and *P. fluorescens* are superior over others in growth promotion. Out of the 16 treatments, three were able to promote plant growth significantly over the others. This is true with most of the biocontrol experiments where only 5% of the short-listed biocontrol agents from the glass house were able to perform well in the field (Powell and Faull, 1991). *T. harzianum* IISR 1369 + *P. fluorescens* IISR 41 combination showed maximum plant height followed by *T. harzianum* IISR 1369 + *P. fluorescens* IISR 6 and *T. virens* IISR 1370 + *P. fluorescens* IISR 11. Growth promotion is effected by production of hormones and suppression of minor pathogens. Therefore the isolates studied are capable of exhibiting this mechanism. In preliminary experiments *T. harzianum* IISR 1369 is compatible with *P. fluorescens* IISR 41 and *P. fluorescens* IISR 6 without inhibiting each other. Similarly *T. virens* IISR 1370 is not inhibiting *P. fluorescens* IISR 11.

Table 1. Effect of *Trichoderma* spp and *P. fluorescens* on the growth of black pepper and suppression of *P. capsici*

Sl No.	Treatment	Increase in plant height (cm)*	% incidence of root rot**
1	Control	149.0 $_{fg}$	90
2	*T. virens* IISR 112	157.8 $_{ef}$	30
3	*T. virens* IISR 18	144.8 $_{g}$	40
4	*T. harzianum* IISR 1369	175.1 $_{c}$	30
5	*T. virens* IISR 1370	171.7 $_{cd}$	30
6	*T. aureoviride* IISR 143	180.7 $_{c}$	20
7	*T. pseudokoningii* IISR 187	175.8 $_{c}$	50
8	*T. harzianum* IISR 167	175.9 $_{c}$	40
9	*T. harzianum* IISR 178	157.3 $_{ef}$	40
10	*P. fluorescens* IISR 11	150.5 $_{fg}$	20
11	*P. fluorescens* IISR 13	164.3 $_{de}$	10
12	*P. fluorescens* IISR 41	165.4 $_{de}$	10
13	*P. fluorescens* IISR 6	176.1 $_{c}$	30
14	*T. harzianum* IISR 1369 + *P. fluorescens* IISR 41	204.9 $_{a}$	10
15	*T. harzianum* IISR 1369 + *P. fluorescens* IISR 6	199.0 $_{ab}$	10
16	*T. virens* IISR 1370 + *P. fluorescens* IISR 13	179.3 $_{c}$	10
17	*T. virens* IISR 1370 + *P. fluorescens* IISR 11	192.6 $_{b}$	20
	CD at 5%	16.67	

Values followed by same letter(s) in a column do not differ significantly according to Duncan's multiple range test at 5% level. * Data from field experiment. ** Data from challenge inoculation of black pepper with *P. capsici* in the glass house (Number of plants showing root rot)

Eventhough *Phytophthora* was detected in soil by baiting no infection of vines was noticed. The presence of *Phytophthora* in various treatments varied during different months and their presence in various treatments is negligible in January, February and April 2001. However they have been detected in subsequent months.

Trichoderma species were distinguished and enumerated based on colony morphology. An increase in population was observed during wet months and a decline during dry months. This is common with many of the *Trichoderma* spp (Eastburn and Butler, 1991). Since markers were not available *P. fluorescens* was not enumerated specifically. Number of cfu on KB medium was considered as *P. fluorescens* even though other species also grow on this media. Population of *Trichoderma* spp, *P. fluorescens* and other fungi showed the coexistence of these species in the soil ecosystem and they were able to present in large numbers.

When challenge inoculated with *P. capsici* treatments applied with *P. fluoreoscens* IISR 13 and *P. fluorescens* IISR 41 were highly disease suppressive showing only 10% disease incidence as compared to 90% in the control. Combination of *T. harzianum* IISR 1369 + *P. fluorescens* IISR 41, *T. harzianum* IISR 1369 + *P. fluorescens* IISR 6 and *T. virens* IISR 1370 + *P. fluorescens* IISR 13 were also on par with the above treatments.

Hence it is inferred that *P. fluorescens* IISR 41 and *P. fluorescens* IISR 13 can be utilized for the suppression of *P. capsici.* Combination of *T. harzianum* IISR 1369 + *P. fluorescens* IISR 41 and *T. harzianum* IISR 1369 + *P. fluorescens* IISR 6 are disease suppressive and promote growth of black pepper. Since nature harbours a mixture of these agents for natural biocontrol combination or mixtures of compatible organisms would be an added advantage over single species application.

Acknowledgements

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BIOMANAGEMENT OF LESION NEMATODES IN BANANA

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ABSTRACT

The efficacy of biocontrol agents viz., *Pseudomonas fluorescens* @ 20g/plant, *Trichoderma viride* @ 20g/plant, *Bacillus subtilis* @ 20 g/plant, *Paecilomyces lilacinus*@ 20g infested sorghum grains/plant and VAM fungi @250 g/plant were evaluated against lesion nematodes viz., *Radopholus similis, Pratylenchus coffeae* and *Helicotylenchus multicinctus* in banana cv.Robusta under glasshouse conditions. The results revealed that, the biocontrol agents were found effective in enhancing the plant growth characters of banana compared to standard chemical carbofuran 3G. Among the biocontrol agents tested, soil application of *P.fluorescens* @ 20 g/plant was found superior over others in increasing the pseudostem height, pseudostem girth, number of leaves, root length and root weight. The per cent increase was 80.67, 56.63, 130.88, 33.65, 70.07 and 129.85 respectively over control. *T.viride* was found next best followed by VAM fungus, *B.subtilis* and *P.lilacinus*. Application of