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Management of foot rot disease of black pepper with *Trichoderma* spp.

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ABSTRACT: Phytophthora foot rot of black pepper is one of the most serious production constraints. As part of the disease management, a number of *Trichoderma* isolates were isolated and screened both *in vitro* and *in vivo*. Five efficient isolates tested in the field were monitored and statistically analyzed for three years. There was reduction in the disease incidence over the years of study, where these isolates were applied. Isolates (*T.virens*-12 & *T.harzianum*-26) were found more effective to control the disease and isolate *T.harzianum*-26 most adaptive to the rhizosphere of black pepper as compared to other isolates.

Key words: Biological control, Phytophthora foot rot, *Piper nigrum*, *Trichoderma*

The production of black pepper (*Piper nigrum* L.) is hampered by diseases not only in India but also in other black pepper growing countries. Among the serious diseases affecting black pepper, Phytophthora foot rot caused by *Phytophthora capsici* is very serious (Anandaraj *et al.*, 1996). On global scale, an annual crop loss of $4.5-7.5 million has been reported due to foot rot alone (De Waard, 1979). Crop loss due to foot rot in Kerala was estimated to range from 3.4-9.4% (Anadaraj *et al.*, 1989). The fungus is soil borne and all parts of black pepper are prone to infection. Infected plant debris in the soil and infected and dried up vines in the gardens appear to be the primary source of inoculum (Anandaraj, 1997).

Present study was undertaken to evaluate the efficacy of different *Trichoderma* isolates, isolated from rhizosphere areas of black pepper to suppress the foot rot incidences. Twenty-seven isolates of *Trichoderma* were isolated and screened against *P.capsici* both *in vitro* and *in vivo*. Five efficient isolates have been selected based on the *in vivo* studies and used in the field for evaluation. Field experiment was conducted for three consecutive years and disease incidence in the field was recorded every month for three years and analyzed statistically (MSTAT). From the studies, the effective isolates of *Trichoderma* on disease suppression as well as their proliferation in the rhizosphere were monitored and discussed.

MATERIALS AND METHODS

Rhizosphere soil samples along with feeder roots of pepper were collected from different pepper growing areas of South India. Feeder roots were cut into small pieces and washed thoroughly with sterile distilled water and plated on potato dextrose agar medium for isolation of *Trichoderma* spp. Isolation of *Trichoderma* spp. from rhizosphere soil samples was done by using *Trichoderma* Specific Medium (TSM - Elad and Chet, 1983).

One gram of air-dried soil samples were weighed and suspended in 9 ml of sterile distilled water and stirred well. Serial dilution technique was adopted for isolation of *Trichoderma* from rhizosphere soil samples. One ml of soil suspension at 10^3 dilution was added to each petriplate and TSM medium was incorporated and plates were incubated at room temperature (25-28^{0}C) for 72 h *Trichoderma* colonies were picked from the medium and subcultured onto PDA slants. *Trichoderma*

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isolates were identified to its species level by using the key of Rifai (1969).

In vitro screening

Dual culture plate technique was adopted for initial screening of *Trichoderma* isolates against *P. capsici*. Culture discs (0.5 cm) collected from 48-hr-old *P. capsici* grown in Carrot Agar medium, were placed at one side on the carrot agar plate (3.5 cm apart from center) and culture discs of 0.5 cm of 48-h-old test fungus (*Trichoderma*), grown on PDA were placed at 3.5 cm apart from center, the other side of the plate, so the distance between discs were 7 cm and incubated at 28°C for 72 hr. Radial growth of *P. capsici* in each Petriplate was measured after 72 h of incubation and inhibition percentage was calculated. Based on the initial screening, isolates were short-listed, and maintained in PDA slants at 20°C in BOD incubator for further study.

Greenhouse studies

Bioefficacy of antagonistic *Trichoderma* isolates against root rot pathogen was evaluated on black pepper cuttings raised in poly bags. Single noded rooted runner shoots of susceptible cultivar of black pepper, Subhakara were raised in polythene bags (6 × 10") filled with 1 kg of nursery mixture. When cuttings reached the stage of 5-6 leaves (six months old), they were used for inoculation.

Twenty-seven *Trichoderma* isolates were used for the bioefficacy study. All *Trichoderma* isolates stored in PDA slants were subcultured on PDA plates. Broken sorghum seeds were moistened with tap water (100 ml water/250 g seed) and filled in 8 × 12" polypropylene bags at 250g/bag. Bags were autoclaved for one hour at 121°C and 15 lb. pressure. Culture discs (0.5 cm.) were collected from 48hr old culture plates and were inoculated the bags at 5 discs/bag. Five bags were maintained for each isolate and bags were incubated at room temperature (25-28°C) for 20 days. For each cutting, 20 g of inoculum was added around the root system, after removing the upper layer of soil and it was replaced later. For each isolate, five cuttings were maintained. Irrigation was done on alternate days with tap water.

For mass multiplication of *P.capsici*, sand-carrot broth medium was used. Sieved sand (1-2 mm) 500 g was filled in polypropylene bags (12x 8")and moisturized with 150 ml carrot broth. For the preparation of carrot broth, 200 g of fresh carrot was cut into small pieces and juice was made with the help of a mixer. The carrot juice was passed through a double folded muslin cloth to obtain clear carrot broth. Total volume of the juice was adjusted into 1litre with distilled water. The sand - carrot broth mixture was autoclaved for one hour at 121°C. After cooling, each bag was inoculated with five culture discs (5.0mm) from 48-hr-old virulent culture of *P. capsici* and incubated at 28 ± 1°C for 20 days.

Pathogen inoculum was applied at the base of the pepper cuttings at 2% of the soil weight, 2 weeks after the biocontrol application. The plants watered daily to maintain the soil moisture. Three months after the application of the pathogen, the plants were uprooted and the root rot incidence, root and shoot weight, height of plants and number of leaves per vine were recorded.

Field application

Five *Trichoderma* isolates (2 isolates from *T. virens*, one each from *T. harzianum*, *T. hamatum* and *T. polysporum*) isolated from rhizosphere of black pepper were tested for their bioefficacy against the foot rot pathogen *P.capsici* under field conditions. A field experiment was conducted with the short-listed isolates to study their field efficacy on disease suppression and also their survival ability in the field. The field trial was set up at Pulpally in Wynad district of Kerala state in a farmer's field, where the foot rot incidence was very severe for the last many years. The plot was planted with the susceptible cultivar Karimunda and the vines showed different stages of foot rot. The vines were about 10 years old and were cultivated as rain-fed crop.

Mass multiplication of biocontrol agents (BCA) were carried out as mentioned earlier. The colony forming units (CFU) of each isolate was assessed by using by *Trichoderma* specific medium (TSM) before application and it was *Trichoderma virens* (T.v-12) = 5 × 10^5/g inoculum, *Trichoderma hamatum* (T.ham-3) = 5 × 10^5/g inoculum, *Trichoderma virens* (T.v-72) = 6 × 10^5/g inoculum,

Trichoderma harzianum (T.harz-26) = 6 x 10^5/g inoculum, and *Trichoderma polysporum* (T.p-1) = 5 x 10^5/g inoculum.

Different isolates of *Trichoderma* were included in the experiment. Individually (50 g/vine) as well as their combination (*T.virens* (Tv-12)+ *T.hamatum* (T.ham-3) + *T.virens* (Tv-72) + *T.harzianum* (T.harz-26) + *T.polysporum* (T.p-1) – 50 g each) were used to assess their efficacies in the control of foot rot infection. Between the treatments a border row of vines, without the biocontrol application was maintained. For each isolate, 48 vines were treated (4 plots at 12 vines per plot). Biocontrol inocula were applied before the onset of monsoon (during May), and application of the antagonists was carried out for three consecutive years (1994-1996). Soil application of BCA were done after mixing 50 g of BCA with 1 kg of neem cake/vine. Inocula were applied at the base of the vines and earthed up. Apart from this, one basket full (10 kg) of farm yard manure (FYM) was applied per vine as farmer's practice. All cultural practices such as shade regulations, pruning runner shoot and minimum tillage were carried out.

Table 1. Efficacy of different *Trichoderma* isolates in suppression of mycelial growth of *Phytophthora capsici* and disease incidence in black pepper cuttings

Isolates	Inhibition of mycelial growth (%)	Root rot (%)	Root wt. (mg)	Height (cm)	Leaves	Shoot wt. (g)
T.vir-1	49	46	520	33	3.8	1.0
2	33	80	30	8	1.0	0.6
3	69	06	540	46	6.2	2.5
4	53	20	620	35	2.8	1.5
5	42	18	630	30	8.0	1.5
6	40	30	260	40	7.2	2.0
7	73	23	520	17	3.8	0.4
8	37	100	00	00	0.0	0.0
9	49	20	580	24	8.0	0.5
10	54	00	1190	40	8.0	5.8
11	42	100	190	4	1.0	0.2
12	40	00	1540	22	3.0	2.8
13	45	50	170	51	11.2	2.7
14	54	40	280	43	2.4	2.2
15	55	26	370	78	8.8	2.2
16	38	100	00	00	0.0	0.0
17	54	100	20	16	0.4	0.6
18	41	46	70	13	1.2	1.2
19	54	00	50	48	7.0	1.8
20	42	100	00	00	0.0	0.0
21	45	100	00	00	0.0	0.0
72	57	97	70	14	2.4	0.0
T.aur-1	42	62	420	52	2.8	0.8
T.ham-3	44	30	540	46	6.0	0.9
T.harz-26	34	75	500	22	2.2	0.9
T.harz-27	59	97	70	14	2.4	2.1
T.poly-1	42	06	260	30	4.0	0.9
Control	00	100	00	00	0.0	0.0
LSD at 5%	04	37	400	27	2.9	1.1

Seasonal fluctuations of populations of *Trichoderma* (0-30 cm.depth) in the treated soils were monitored and compared with control at bimonthly intervals. Disease incidence and health of the vines were monitored by disease indexing. Disease indexing was done by visual observation on defoliation and yellowing of vines (Healthy =0, 1-25% yellowing and defoliation = 1, 26-50% yellowing and defoliation = 2, 51-75% yellowing and defoliation = 3, 76-100% yellowing and defoliation = 4) every alternate month and data were analyzed by using MSTAT software.

RESULTS AND DISCUSSION

All 27 *Trichoderma* isolates tested were found to inhibit growth of *P.capsici* (Table 1). Percentage of inhibition ranged from 33-73. Three isolates of *T.virens* (*T.virens* 10, 12 & 19) gave 100% root protection and increased root biomass (Table 1).

During the 3 years of field study, there was significant reduction in the disease incidence was observed where the individual isolates applied. *T.virens* isolates (Tv-12 and Tv-72), *T.hamatum* (T.ham-3) and *T.harzianum* (T.harz-26) effectively controlled the foot rot disease and *T.polysporum* was ineffective (Table 2). However, when all the four isolates applied in combination, there was no reduction in the disease incidence noticed.

During different seasons of the year, maximum BCA population was noticed during July-November.

Table 2. Efficacy of *Trichoderma* isolates in control of foot rot of black pepper

Treatments	Disease index*		
	1994	1995	1996
Trichoderma virens - (Tv-12)	10.5	01.0	01.6
Trichoderma hamatum - (T.ham-3)	06.1	00.9	03.2
Trichoderma virens - (Tv-72)	12.3	03.0	04.2
Trichoderma harzianum - (T.harz-26)	16.7	10.3	08.1
Trichoderma polysporum - (T.p-1)	34.1	31.7	07.2
Mixture (Tv12+T.ham3+Tv72+T.harz26)	26.1	45.9	11.2
Control	35.6	43.0	10.2
LSD at 5%	15.6	20.6	06.2

* Three years pooled data, scores from 0-4 were allocated for those vines having healthy (0), 1=1-25% yellowing/defoliation, 2=26-50%, 3= 51-75% and 4=76-100% respectively. For complete death, scored as 4

Table 3. Changes in population of *Trichoderma* spp. in different months of a year in the black pepper field (CFU x 10^3 /g soil)*

Isolates	Months					
	Jan	March	May	July	Sept	Nov
T.virens (Tv-12)	3.1	2.1	1.6	2.4	1.0	1.7
T.hamatum (T.ham-3)	2.8	3.0	2.1	3.0	1.9	2.9
T.virens (Tv-72)	2.3	10.2	9.8	2.2	2.7	3.7
T.harzianum (T.harz-26)	13.9	24.2	22.7	37.5	43.6	40.2
T.polysporum (Tp-1)	3.0	5.4	2.6	5.4	1.9	3.1
Mixture (Tv12+T.ham3+Tv72+T.harz26)	9.5	6.2	4.5	7.7	4.1	8.7
Control	0.4	0.7	0.8	0.5	0.4	0.6
LSD at 5%			1.7			

* Data are mean of 3 years (1994-1996)

The population of *Trichodrma harzianum*-26 was significantly higher and persistant in all the seasons compared to all other isolates used (Table 3).

As reported by Davet (1979, 1981), the BCA population was more during the wet season than the dry season, it might be the reason which prevent the pathogen multiplication around the root and protect the plant from infection. It was noticed that, *T.harzianum* has more adaptability than other isolates. The findings of Papavizas (1985) on survival of *T.harzianum* and persistance during the dry season were confirmed through this study. Survival ability of each isolate depends on their ability to degrade various organic substrates in the soil than metabolic versatility and their resistance to microbial inhibitors depending on prevailing conditions and species or strains involved (Papavizas, 1985).

From the study, it was confirmed that, *Trichoderma harzianum* (T.harz-26) and *T.virens* (Tv-12) is able to control the foot rot infection in black pepper and the isolate (T.harz-26) was more efficiently proliferating in the soil and can remain in the soil for long time as well as gave good protection to the root system against *P.capsici*. It was also noticed that, when the mixtures of different *Trichoderma* isolates were used, the infection was not reduced. This might be due to the competition among the isolates and which might have nullified their individual effects.

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