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Effect of organic amendments on the proliferation stability of *Trichoderma harzianum* and suppression of *Phytophthora meadii* in cardamom soils in relation to soil microflora

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ABSTRACT: An even economical and more frequently used method of attaining biological control of soil borne plant pathogens is incorporating plant residues and organic amendments to the soil, which support high level of microbial activity. In the present study farm yard manure (FYM), poultry manure (PM), coffee husk (CH) and neem cake (NC) were used as soil amendments with or without *Trichoderma harzianum* to evaluate their efficacy in suppressing *Phytophthora meadii* under varying moisture conditions. The survival of *Phytophthora* was found higher at 50% moisture level. Neem cake with *Trichoderma* appeared as a suitable amendment at all moisture level and has shown 69% reduction in *Phytophthora* infection over control. At 75% and 100% (field capacity), no detectable level of *Phytophthora* was observed in treatments where *T. harzianum* was fortified with CH, PM and NC. At field capacity, PM and FYM with *Trichoderma* also reduced the build up of *Phytophthora*. Native population of *Trichoderma* was found negligible in non-amended treatments. All the four amendments maintained the population and supported the growth of *Trichoderma* at all moisture levels. In general, organic manures with *T. harzianum* reduced the population level of other fungi, while crop residues supported the growth of fungi like *Penicillium*, *Rhizopus*, *Aspergillus*, *Mucor*, *etc*. besides T. *harzianum*.

KEY WORDS: Coffee husk, crop residue, *Elettaria cardamomum,* farm yard manure, neem cake, organic amendments, *Phytophthora meadii,* poultry manure, *Trichoderma harzianum*.

INTRODUCTION

Capsule rot of cardamom caused by *Phytophthora meadii* McRae of A2 mating type is a serious threat to cardamom cultivation in the high ranges of Kerala in India. An economical and ecofriendly disease management strategy developed for capsule rot is the use of biocontrol agent, *T. harzianum.* Fortification of organic substrates with *Trichoderma* was found as an important component of IPM, which fit well into the organic farming system (Suseela Bhai, 1998). It is known for its proliferation stability that can be enhanced by augmenting the soil with organic amendments which in turn promote biocontrol activity against soil borne pathogens (Baker and Cook, 1974). There are reports for the use of organic amendments in reducing the population level of *Phytophthora*. Tsao and Guy (1977) reported the effect of nitrogenous organic amendments in controlling *P. cinnamomi* and *P. parasitica* in avocado and citrus soils. They reported that chicken manure, alfalfa meal and processed sewage sludge rendered the soil suppressive ness as a result of increased microbial activity due to decomposing of amendments. The present work was aimed to study the proliferation and stability of *T. harzianum* with soil amendments such as farm yard manure, poultry manure, coffee husk and neem cake and also to study their effect on the survival /suppression of *Phytophthora* in the soil under varying conditions of moisture.

MATERIALS AND METHODS

The experiment was laid out under green house conditions in a split- plot design with moisture level as main plot and amendments with or without *T. harzianum* as sub plots. The sub -plots consisted of nine treatments, *viz*., T_1 - Farm yard manure (FYM) @ 5 kg clump^{-1} (11g / 250g soil), T_2 - Decomposed coffee husk (CH) @ 5kg clump^{-1}, T_3 - Poultry manure (PM) @ 3kg / clump (6.75g / 250g soil), T_4 - Neem cake (NC) @ 2 kg /clump (4.5g / 250g soil), T_5 - T_1 + *T. harzianum* , T_6 - T_2 + *T. harzianum,* T_7 - T_3 + *T. harzianum,* T_8 - T_4 +*T. harzianum* and T_9 - Control with NPK @ 40: 40: 75.

Soil was collected from the cardamom growing tracts and disinfected by solarization, sieved through a 2mm sieve

and dispensed into poly containers @ 250g / container. FYM, CH, PM and NC were used as amendments. The NPK values of these amendments were determined by standard analytical method and adjusted to 40: 40: 75 NPK level by the addition of inorganic N, P or K. in the form of urea, single super phosphate and muriate of potash in order to maintain uniformity in NPK level in all the treatments.

The moisture level of the soil was maintained at 50%, 75%, and 100% of field capacity following the method of Brady (Brady, 1990). The container was weighed at the time of adding water and the weight (moisture level) was maintained throughout the experimental period.

T. harzianum was grown in 50ml potato dextrose agar in 250ml EM flasks for seven days for sporulation. From this spore suspension (2 x 10^{10} spores mL^{-1}) was prepared in 100ml of sterile distilled water and used for inoculating the soil.

P. meadii was grown in carrot agar at 24 - 28°C in the dark for 72h. From these inoculum plugs of 4 mm size were inoculated to 20ml carrot broth in 100ml Erlenmeyer flask and incubated in the dark at 24 - 28°C for 15 days. The mycelial mat was harvested, washed in sterile distilled water, blended and made up to 30ml. This was applied @ 5ml/ container to all the treatments and thoroughly mixed using a glass rod. The spore suspension of *Trichoderma* was applied @ 5ml/container in treatments T5-T8 seven days after the application of *P. meadii*. Soil samples were collected at bi-monthly intervals and estimated the population of *Trichoderma*, total fungi, bacteria and actinomycetes on their respective medium. *P. meadii* population was estimated by soil baitng method (Tsao *et al.,* 1983) and the data was analysed using MSTATC package.

RESULTS AND DISCUSSION

Population level at 50% of field capacity (FC)

At 50% of FC *Phytophthora* infection (as indicated by bait infection) in the treatments varied from 18.43% to 90%. The lowest infection (18.43%) was noticed in NC amended with *T. harzianum,* while the highest infection (90%) was noticed in FYM amended with *T. harzianum* (Table 1). In amended treatments, the *Trichoderma* population ranged from 4.5-9.4 x 10^6CFUs g^{-1} while in other treatments, the population remained very low (Table 1). FYM and PM either alone or with *T. harzianum* were found least in maintaining the population of other fungal flora (Table 1)**.** Bacterial population was found high in coffee husk and control as compared to other treatments. Actinomycetes population was found negligible in all the treatments (Table 1).

Population level at 75% of field capacity

P. meadii infection was found comparatively low at 75% FC. PM with out *T. harzianum* showed the highest infection comparable to control (90%). CH, PM and NC with *T. harzianum* showed no detectable infection, while farm yard manure with *T. harzianum* showed comparatively higher infection.

Trichoderma with NC and PM showed higher proliferation (7.7 and 7.8 x 10^7 CFUs g^{-1}, respectively) compared to FYM and CH.

Total fungal population was more in CH followed by NC in both *Trichoderma* amended and non-amended treatments. It was interesting to note that there is a substantial reduction in fungal population when the amendments were fortified with *Trichoderma*. However, bacterial population was higher with NC in both the conditions. Actinomycetes population was least at 75% of FC irrespective of treatments. The maximum population was noticed in CH amended with *T. harzianum* (Table 1).

Population level at 100% FC

At 100% of FC, *Phytophthora* infection was 49.3%, 70.39%, 45% and 48% respectively in FYM CH, NC without *Trichoderma* and control, whereas PM showed no detectable infection (Table 1). All the amendments with *T. harzianum* showed no detectable bait infection at 100% FC. *T. harzianum* population was not in detectable level at 100% FC in treatments T1-T4. But in *Trichoderma* amended treatments the population ranged from 4.4 -8.3 x 10^6 CFUs g^{-1} and T. *harzianum* with PM showed comparatively the higher population (8.3 x 10^6 CFUs g^{-1}).

In general microbial population was low at 100% FC. Among the treatments CH, NC and NPK40:40:75 supported maximum population. Amending with *T. harzianum* showed reduced growth of other fungi, but CH amended with *T. harzianum* showed comparatively higher population. Bacterial population was found significantly high with PM followed by NC with *T. harzianum* when compared to other treatments (Table 1). Actinomycetes population showed no significant difference between treatments.

Thus the overall result showed that survival of *Phytophthora* was higher at 50% moisture level. Neem cake supplemented with *Trichoderma* appeared as a suitable amendment at all moisture level and has shown 69% reduction in *Phytophthora* infection over control. All the four amendments maintained the population and supported the growth of *Trichoderma* at all moisture levels. Among the amendments, organic manures with *T. harzianum* reduced the population level of other fungi, while crop residues with *T. harzianum* supported their growth and

Table 1. Comparative effect of moisture level and amendments on *P. meadi*i, *T. harzianum* and native soil microbial population

No.	Treatments	*P. meadii* (% bait infection	*T. harzianum* (CFUs g^{-1} x 10^5)	Total fungi CFUs x 10^6 g^{-1}	Bacteria CFUs x 10^6 g^{-1}	Actinomycetes CFUs x 10^6 g^{-1}
At 50% field capacity						
T_1	Farm yard manure (FYM)	67.50	0.00	1.6	4.3	0.50
T_2	Coffee husk (CH)	65.61	0.00	35.2	60.4	0.00
T_3	Poultry manure (PM)	64.17	0.50	2.8	55.4	0.00
T_4	Neem cake (NC)	45.00	0.00	29.7	4.5	0.00
T_5	FYM + *T. harzi*anum	90.00	94.00	0.0	3.2	0.00
T_6	CH + *T. harzianum*	67.50	51.00	14.8	5.65	0.50
T_7	PM + *T. harzianum*	45.00	45.00	1.0	6.8	0.00
T_8	NC + *T. harzianum*	18.43	84.00	3.7	7.7	1.50
T_9	Control (NPK 40: 40:80)	60.11	1.50	4.0	122.00	0.50
At 75% of field capacity						
T_1	Farm yard manure (FYM)	31.72	0.00	0.7	3.43	0.00
T_2	Coffee husk (CH)	13.29	0.00	24.8	21.0	1.50
T_3	Poultry manure (PM)	90.00	0.50	4.5	23.0	0.50
T_4	Neem cake (NC)	34.69	1.50	19.5	38.1	0.50
T_5	FYM + *T. harzi*anum	48.00	65.00	0.0	23.6	0.50
T_6	CH + *T. harzianum*	0.00	59.00	15.8	17.4	2.50
T_7	PM + *T. harzianum*	0.00	78.00	1.6	16.3	1.50
T_8	NC + *T. harzianum*	0.00	77.00	9.4	17.8	0.00
T_9	Control (NPK 40: 40: 80)	90.00	0.00	7.8	11.7	2.00
At 100% of field capacity						
T_1	Farm yard manure (FYM)	49.30	0.00	0.4	14.0	0.00
T_2	Coffee husk (CH)	70.39	0.00	18.2	22.8	1.50
T_3	Poultry manure (PM)	0.00	0.00	0.6	151.2	2.00
T_4	Neem cake (NC)	45.00	0.00	10.5	34.6	1.00
T_5	FYM + *T. harzi*anum	0.00	50.00	0.0	13.2	0.50
T_6	CH + *T. harzianum*	0.00	43.50	7.4	22.8	2.00
T_7	PM + *T. harzianum*	0.00	82.500	0.0	37.8	1.50
T_8	NC + *T. harzianum*	0.00	67.50	0.5	61.2	0.50
T_9	Control (NPK 40: 40:80)	48.00	0.50	10.5	16.5	1.00
	LSD at P = 0.05	**31.42	**10.11	**2.2	**11.1	NS

LSD value 31.42 ($S\text{-}_{X=}$ 10.83) at alpha 0.050 (*Phytophthora*), LSD value 10.11($S\text{-}_{X=}$ 3.484) at alpha 0.050 (*Trichoderma*), LSD value 2.23($S\text{-}_{X=}$ 7.592) at alpha 0.050 (total fungi), LSD value 11.1 ($S\text{-}_{X=}$ 38.22) at alpha 0.050 (Bacteria)

promoted fungi *like Penicillium*, *Rhizopus*, *Aspergillus, Mucor* etc. Proliferation of bacterial cells was more under high moisture levels irrespective of the application of amendments. Generally actinomycetes population had no significant difference between or with in treatments. PM showed the lowest population when compared to other amendments.

It is a known fact that nitrogenous fertilizers have a wide range of effects on *Phytophthora* disease severity (Malajezuk, 1983). Similarly variable effect of nitrate and ammoniacal fertilizers were also reported by many workers. These were reported to be toxic to *Phytophthora* (McIntosh 1972 and Tsao *et al.,* 1975) while in some other studies these were reported to stimulate sporulation of *Phytophthora* (Halsall, 1978) and increase disease severity (Apple, 1961). It was also reported that the effects varied with species of *Phytophthora.* Tsao and Zentmyer (1979) observed suppression of *P.cinnamomi* and *P. parasitica* in urea amended soil. Thus the quality of organic and inorganic amendments may strongly influence the pathogen. In the present study, the addition of organic amendments or plant residues are found effective at high moisture levels i.e., at 75% and 100% of field capacity in suppressing the *Phytophthora* population. With FYM and PM, microbial population was very low when compared to plant residues. But addition of *Trichoderma* along with these organic matter strongly influenced the proliferation and stability of *Trichoderma* as well as suppression of *Phytophthora.* Locke *et al.* (1984) reported that *Trichoderma* species were excellent biocontrol agents when applied to sterile soil or soilless mix in the green house, but not when applied to natural soil. Organic manures such as FYM and PM were found suitable for the proliferation of *T. harzianum* due to the low population of other microflora at all the moisture levels (Table 1).

It is a known fact that organic matter can affect the vigor of the host by improving the structure and moisture and nutrient holding capacity of the soil but its principal effect is to provide a more complex antagonistic soil microflora and fauna. Plant residues and chicken manure have already been used in Australia by avocado growers in copious amounts to build up the organic matter levels near to that of the surrounding undisturbed forests (Shea and Broadbent, 1983). Similarly decomposed chicken manure reduced foot rot of sweet pepper caused by *P. capsici* up to 30% when incorporated into the plant bed (Corrales *et al*., 1990). It was found that coffee husk and poultry manure in all combinations and at all moisture levels harboured more fungi other than *Trichoderma* spp. FYM in all combinations showed only very low population of other fungi and that may be the reason for the establishment of *Trichoderma* in FYM. Comparatively poor multiplication of *Trichoderma* in non-sterile coffee husk and poultry manure was found to be due to the high population of other fungal flora. Thus it was clear that coffee husk was effective for the multiplication of *Trichoderma* only under sterile conditions. Under non-sterile conditions it favoured the growth of other fungi. Studies on the effect of different organic soil amendments on the population density of *Phytophthora* revealed the above aspects which were found to be relevant for the selection of soil amendments. Plant residues were found to be poor in maintaining the *Trichoderma* population except under sterile conditions.

The results clearly indicated that the mere addition of a favourable substrate together with an antagonist to the soil is not sufficient to guarantee its survival. Other organisms will compete with it as decomposition progresses and overwhelm it. The present study leads to the conclusion that at field capacity the fungal flora will be on the upper hand and proper manuring of the soil with an organic litter is sufficient to enhance the population of *Trichoderma*. Other organic residues such as poultry, manure will also be as efficient as FYM, while the crop residue such as CH and NC enhance other saprophytic fungi like *Penicillium, Rhizopus*, *Aspergillus, Mucor*, *etc*. though these crop residues can be used for multiplying *Trichoderma* for field application under sterile conditions.

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