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Effect of Trichoderma fortified Vermicompost managing root rot diseases in Cowpea

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Abstract

The present work was carried out to isolate and test the efficacy of *Trichoderma harzianum* with biofortified vermicompost against root rot disease of cowpea and assess the mechanisms involved in disease suppression. Among the tested *Trichoderma* isolates, it was found that *Trichoderma harzianum* (Th_2),exhibiting strong inhibition against *M. phaseolina*. Five different types of compost were screened against the root rot of cowpea, where vermicompost showed maximum mycelia growth inhibition and smaller root rot index. The vermicompost enriched with selective biocontrol agents were analysed for its microbial population and found that vermicompost fortified with *Trichoderma harzianum*showed highest microbial population after 14 days of fortification. *Trichoderma* was able to fortify vermicompost and significantly reduce the occurrence and severity of disease in cowpea crop.

Keywords: *Trichoderma harzianum*; Vermicompost;*M.phaseolina;* Cowpea; Biocontrol

Introduction

Cowpea (*Vigna unguiculata* [L.] Walp) is an important legume crop grown in places with severe weather conditions both in tropical and sub -tropical regions of, India and semi arid tropics of Africa (Singh *et al.*, 2012). Among the different grown pulse crops, cowpea (southern pea) is found to be as one of the main crops grown in Tamil Nadu and Andhra Pradesh mostly as rainfed crop. The name of "Cowpea" is derived from livestock feed for cows in United States and Asian countries. It is also referred as "hungry-season crop" because it is the first crop to yield prior to cereal crops (Gomez, 2004).Cowpea is well known for its nutritional value in human diet ,for livestock feed and a source of income generation for farmers (Sheahan, 2012).The crop is affected with different diseases caused by fungi, bacteria and viruses . Among these pathogens, root/stem rot/charcoal rot diseases caused by *Macrophomina phaseolina* (Tassi) Goid is found to be the major disease, which is distributed worldwide and occurs during seedling stage onto the maturity *stage (Meena et al., 2018). Trichoderma harzianum and T. viride* is an efficient biocontrol agent that is commercially available to inhibit different types of soil pathogenic fungi.

These mycoparasitism inhibitory compounds are able to control soil borne pathogens (Bari, 2001). Disease suppressive properties of compost amendments are well documented and the wide spectrum of pathogens and diseases that can be effectively managed by compost amendments have been well studied (Hoitink *et al.,* 2001; Noble and Coventy, 2005). Composts prepared from residues of certain farm wastes have been found to decrease the incidence of charcoal rot besides promoting the yield of legume seeds (Lodha *et al.,* 2002) Compost and decomposing residues also served as a food substrate for the survival and multiplication of bio-agents (Singh *et al.*,2012).The current study was carried out to investigate the mutual relationship occurring between the vermicompost and cow pea along with microbial antagonist of *Trichoderma* spp inhibiting root rot fungi. The use of easily accessible and moderately economical compost / substrates can facilitate the *Trichoderma* spp. to have a better impact on cowpea disease.

Materials and Methods

Isolation of *Trichoderma* spp

Rhizosphere soil samples from cowpea field was collected fromdifferent regions of Tamil Nadu. Isolation of *Trichoderma* spp. was carried out using Trichoderma selective medium (TSM) (Elad and Chet, 1982). The pure culture was obtained using single hyphal tip method and stored until further use.

Antagonistic activity of native *Trichoderma* isolates against *M. phaseolina* using Dual culture techniques

The antagonistic activity of bio inoculants (Tv_1-Tv_5&Th_1-Th_5) against *M. phaseolina* was tested using Dual culture technique (Dennis and Webster 1971). A 9 mm mycelial disc obtained from seven days old culture of *M. phaseolina* was inoculated at one side of petriplate containing 15 ml of the solidified Potato dextrose agar (PDA) medium. Similarly, five days old culture of *T. viride* and *T. harzianum* were placed in opposite sides of the petri plate. These plates were incubated in room temperature at 28±2°c for five days along with the non-antagonist. The radial growth (mm) of pathogen , antagonists and the extent of inhibition zones were measured. The radial mycelia growth of the pathogen and percent reduction over control was calculated using the formula (Vincet, 1927).

Percent inhibition(I)= C-T/C X 100

Where,

C- Mycelial growth of pathogen in control (mm)
T- Mycelial growth of pathogen in dual plate(mm)

Estimation of root rot severity indices on cowpea plant grown on five different composts, challenge inoculated with *M. phaseolina* (Pot culture)

Different composts (composted cow manure, vermicompost, wood ash compost, Rice husk compost, Garden waste compost)were screened to determine their capability in inhibiting cowpea root rot. The composts were mixed with sand in the ratio of 20:80% (V: V) and the mixture was filled in pots. Cowpea seeds were surface sterilized with sodium hypochlorite 0.1% ,sown in pots and grown for 20 days. After growth, cowpea seedlings were treated with 3ml of *M. phaseolina* conidial suspension, planted in pots and maintained in growth chamber at 25˚C temperature under 8hrs of dark and 16 hrs of light conditions along with regular watering. Cowpea plants were observed on 10^{th} day to determine the root rot disease index, using the scale 0 to 4. (0 = Asymptomatic, 1= drying of leaves, 2 =Bark shredding, 3 = Rotting and 4 = plant dead).

Microbial population assessment in vermicompost fortified with Bio- inoculants

The vermicompost was subjected individually with different bio-inoculants individually. Vermicompost was measured (25 kg) ,taken in a tray and mixed with 1L of 2 days old culture of *T.viride* broth (CFU count approximately 1.8×10^6). Accordingly, 25 kg of vermicompost was taken in another separate tray and mixed with 1L of five days old culture of *T. harzianum* broth (CFU count approximately 3.2×10^6). Now, the trays were covered with polythene sheet and incubated under shady condition for 15 days. Microbial population was assessed from the fortified compost on 7^{th} and 14^{th} using serial dilution technique. 1g of sample was taken from each tray and diluted until 10^{-6} and transferred to PDA medium. The microbial population was assessed and designated as CFU/gm of dry weight.

Results and Discussion

The antagonistic activity of native *Trichoderma* isolates against *M .phaseolina* using dual culture technique is shown in **Table 1**. It is seen that the effective isolates of *T.viride* (Tv_3& Tv_2) and *T.harzinaum* (Th_2,Th_5,Th_3) tested significantly inhibited the mycelial growth of *M. phaseolina* whereas, the isolate Th_2 showed significant inhibition on the growth of *M. phaseolina* (18.00 mm), found to be 80 % reduction on the growth of pathogen against the growth of 90 mm in comparison to the control. Choudhary and Ashraf (2019) reported, *T. harzianum* (23 mm) and *T. viride* (24.33 mm)to be effective in inhibiting the fungal growth of the pathogen. Muhammed Taha Yassin (2021) reported, *T. viride* to show antagonistic activity against *F. proliferatum* and *F. verticillioides* with mycelial inhibition rates of 80.17% and 70.46% whereas *T. harzianum* showed inhibition rates of 68.38% and 60.64%.

Table. 1 Antagonistic activity of *Trichoderma* isolates against *M.phaseolina* using dual culture techniques

S.no	Isolates	Mycelial growth of *M.phaseolina*	Inhibition zone (mm)	% Inhibition over control
1.	Tv_3	18.59^b	0.75	79.34
2.	Tv_2	30.26^d	7.10	66.37
3.	**Th_2**	**18.00^a**	**0.50**	**80.00**
4.	Th_5	20.43^c	5.25	77.3
5.	Th_3	35.32^e	7.00	60.75
6.	control	90.00^f	-	-

Determination of root rot severity index on cowpea plant grown on different composts, inoculated with *M. phaseolina*

The cowpea seeds were grown in five different composts and mixed with the inoculants of *M. phaseolina*. After 15 days of sowing, the application rate of various compost . The root rot severity was observed on the 25 Day after sowing and results were recorded using scale (0-4). The root rot index recorded for vermicompost was (1.5), composted cow manure (1.8), rice husk compost (2.0), wood ash compost (2.5), and for garden waste compost it was found to be (3.2) as shown in Table. 2.

Lakhran and Ahir (2018) reported that organic manure tested reduced root rot incidence of chickpea significantly over check. It was observed that vermicompost (20.00 and 25.00%) and Farm yard manure (26.60 and 36.36%) was most effective against dry root rot of chickpea.

Table. 2 Determination of root rot severity index on cowpea plant grown on different composts, inoculated with *M.phaseolina*

Tr.no	Composts	Application rate of various compost (kg/ha)	Root rot index (0-4)scale
T_1	Composted cow manure	400	1.8^b
T_2	Garden waste compost	200	3.2^e
T_3	**Vermicompost**	**500**	**1.5^a**
T_4	Rice husk compost	300	2.0^c
T_5	Wood ash compost	250	2.5^d

Assessment of microbial population in vermicompost fortified with *Trichoderma harzianum* and *Trichoderma viride*

The total microbial dynamics of biocontrol agent in the soil after fortification with vermicompost is shown in **Table 3.** The fortified vermicompost was found to have more number of fungal pathogens. The fungal population of *Trichoderma harzianum* at 0 days after fortification was found to be (3.2×10^6 (cfu g^{-1}), 7 days after fortification (4.5×10^6 (cfu g^{-1}) and after 14 days of fortification it was (5.8×10^6 (cfu g^{-1}). Similarly, the population of *T. viride* at 0 days after fortification (1.8×10^6 (cfu g^{-1}), 7 days after fortification (2.8×10^6 (cfu g^{-1}) and 14 days after fortification it was observed to be (4.2×10^6 (cfu g^{-1}). *Trichoderma harzianum* was found to be more compatible in comparison to vermicompost. Nandini *et al.*, (2014) reported that the population of free- living nitrogen fixers (FNF) was highest on 14th day in the enriched vermicompost (12.24×10^3cfu/g) followed by enriched FYM and biogas spent slurry (7.9×10^3cfu/g and 7×10^3cfu/g,) in comparison to the population at zero day of enrichment in vermicompost, FYM and biogas spent slurry (0.17×10^3 , 0.11×10^3 and 0.022×10^3cfu/ g,).

Table. 3 Assessment of microbial population in vermicompost fortified with *Trichoderma harzianum* and *Trichoderma viride*

Microbial Population	Antagonistic organisms	
	Trichoderma harzianum	*Trichoderma viride*
0 day after fortification (CFU g^{-1})	3.2×10^6	1.8×10^6
7 thdays after fortification (CFU g-1)	4.5×10^6	2.8×10^6
14 th days after fortification (CFU g-1)	**5.8×10^6**	**4.2×10^6**

Conclusion

In conclusion, the results of the present study have proved that combined application of biocontrol agents and biofortified vermicompost exhibited a general trend towards greater suppression of cowpea root rot caused by *Macrophomina phaseolina.* This enhanced suppression exerted in combination of biocontrol agent's biofortified vermicompost is due to the combined action of different mechanisms and improved performance rate of antagonists in different microclimates and seasons.

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