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EFFECT OF COMPOSTED POULTRY MANURE AMENDMENT ON ROOT ROT DISEASE INCIDENCE OF SUNFLOWER

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Abstract

The present studies were undertaken to investigate the effect of Composted Poultry manure amendment against root rot disease on Sunflower. Pot culture trial conducted in *kharif* and *rabi* seasons using hybrid Jaya was laid in a randomized block design with nine treatments and five replications. The sunflower crop was established by using standard cultural practices and recommended rates of fertilizers followed by State Agricultural Department. Soil and composted poultry manure amendments at rates of 5 t/ha and 10 t/ha manure by weight of soil (w/w) were mixed thoroughly for 15 min in a cement mixer and placed in pots 28, 14 and 0 days before sowing. As a comparison fungicide, carbendazim was used as seed treatment (3 g/kg of seeds) and soil drenching (500 g/ha at 60 DAS). The results revealed that, in both seasons, charcoal rot disease incidence was decreased at 10 t/ha rate of manure when incorporated 14 and 28 days before sowing and at sowing. Also, Studies on induction of defense mechanisms revealed that positive changes of total and OD phenols was observed in plants amended with composted poultry manure followed by challenge inoculation with *Macrophomina phaseolina*.

Key words: Sclerotial population, root rot, animals manure, compost

Introduction

Sunflower (*Helianthus annuus* L.) is one of the important oilseed crops affected by a large number of insect pests and diseases. Among them charcoal rot disease caused by *Macrophomina phaseolina* (Tassi) Goidanich is becoming more serious under rainfed conditions inflicting estimated loss of 36.8-79.2 per cent seed yield (Theradimani and Hepziba, 2003) and reduction in seed weight of 30-46 percent. This disease was observed in higher proportions in the sunflower growing areas of Tamil Nadu leading to severe loss to farmers. Very unfortunately, the absence of suitable resistant or tolerant varieties or hybrids, chemical control is only alternative method. But in view of the high cost of chemicals required for soil application, development of fungicide resistance by target organism and the hazardous effects of chemicals on the eco-system have forced the scientists to develop alternate methods to control the disease. Alternative management practices utilizing antagonists and organic amendments are being investigated to replace or supplement existing management practices (Riegel and Noe, 2000).

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Animal manures have been used since the beginning of agricultural food production to improve soil fertility, recycle nutrients and improve biological and physical properties of soil and increase crop yield (Dick and McCoy, 1993). Composted poultry manure as an organic amendment has shown that application to soil to suppression of plant pathogenic fungi (Aryantha *et al.*, 2000). Suppression of pathogenic fungi by manure is probably enhanced microbial activity (Riegel and Noe, 2000). The Present studies were undertaken to investigate the effect of Composted Poultry manure against Charcoal rot of sunflower.

Materials and methods

Raw poultry manure and composting process

Fresh poultry manure was collected from the floor of the housing immediately after deposition to avoid any material contaminated by contact with the floor. Poultry manure was composted from March to June 2006 in windrows (height of 1.5 m) with temperatures within the range of 55-70ºC. Initially, the windrows were turned daily, if needed, to reduce the moisture content, avoid leachate formation and reduce odour generation.

Thereafter, they were turned weekly to maintain porosity and adjust windrow height due to compost shrinkage. Water was added when needed to maintain optimum process conditions for composting of poultry manure (Wang *et al*., 2004).

Fungicide

Systemic fungicide carbendazim (Bavistin 50WP) belonging to the benzimidazole group was used as test fungicide.

Evaluation of composted poultry manure against charcoal rot disease of sunflower under glasshouse condition

Glasshouse experiments were conducted at Department of Plant Pathology, Annamalai University, Annamalainagar *kharif* and *rabi* seasons. Pot culture trial using hybrid Jaya was laid in a randomized block design with nine treatments and five replications. Three seeds were sown in 30-cm-diameter earthen pots filled with 5 kg steam sterilized sandy loam soil incorporated with the fungal culture multiplied in sand-maize medium at a ratio of 1:19 (medium: soil). The sunflower crop was established by using standard cultural practices and recommended rates of fertilizers. Soil and composted poultry manure amendments at rates of 0.25% (5 t/ha) and 0.5% (10 t/ha) manure by weight of soil (w/w) were mixed thoroughly for 15 min in a cement mixer and placed in pots 28, 14 and 0 days before sowing. As a check, carbendazim was used as seed treatment (3 g/kg of seeds) and soil drenching (500 g/ha at 60 DAS). The crop was observed regularly and data were recorded on charcoal rot disease incidence (%) and seed yield (kg/ha). Sclerotial population of *M. phaseolina* (Shanmugam *et al*., 2003) per g of soil was also recorded.

Effect of composted poultry manure amendment on biochemical constituents and defense enzymes - Method of sampling

Sample of plant leaves in each treatment was collected at 45, 52, 59 and 66 DAS for the reason that sunflower plants were susceptible to charcoal rot at reproductive stage. Leaves were collected and washed in running tap water and homogenized with liquid nitrogen in a pre-chilled pestle and mortar. The homogenized leaf tissues were stored in deep freezer (-20ºC) until used for biochemical analysis.

Preparation of ethanol extracts (Mahadevan and Sridhar, 1996)

Samples were collected and four grams quantities were taken. They were chopped and then extracted in 80% ethanol and used for the estimation of total and *ortho*-dihydroxy phenols.

Quantitative estimation of phenols

Total phenol

Total phenol content was estimated by following the procedure proposed by Bray and Thorpe (1954). To 1.0 ml of ethanol extract, 5.0 ml of distilled water and 250 ml of Folin-Ciocalteau reagent were added. After incubation, the mixture was kept at 25^{o}C for three min. One ml of saturated sodium carbonate solution was added and the mixture was further incubated for 15 min. The intensity of blue color was read at 725 nm using spectrophotometer. A blank containing the entire reagent excluding the sample extract was used to adjust the absorbance of the spectrophotometer to zero. The total phenol content was expressed in catechol equivalents as mg/g of fresh weight basis.

***Ortho*-dihydroxy phenol**

Ortho-dihydroxy (OD) phenol was estimated by the method described by Johnson and Schaul (1957) employing Arnow's reagent (sodium nitrate 10 g, sodium molybdate 10 g and distilled water 100 ml) specific to *ortho*-groups. To 1.0 ml of ethanolic extract, 1.0 ml each of 0.5N hydrochloric acid and Arnow's reagent were added followed by 10.0 ml of distilled water and 2.0 ml of 1N sodium hydroxide. A reagent blank was maintained without the extract. Soon after addition of alkali, pinkish yellow color developed. The absorbance of the solution was read at 515 nm. The OD phenol content was expressed in mg/g of fresh weight basis in catechol equivalents.

Results and Discussion

Evaluation of composted poultry manure against charcoal rot of sunflower under glasshouse condition

In the both pot trials, charcoal rot disease incidence was decreased at 0.5% rate of manure when incorporated 14 and 28 days before sowing and at sowing (Table 1 and 2). In both trials, the 0.25% manure rate had lower disease incidence than the non amended controls. Seed yield in *M. phaseolina*-infested soil in the both trials had a positive response to increasing manure rates. Seed yield from the 0.5% manure level was greater than the non amended controls. In the both trials, sclerotial population recorded at 75 days after sowing decreased linearly with increasing rate of manure where manure was incorporated 28 days before sowing.

Compost is produced from the degradation of organic waste materials by diverse microbial populations (Hadar and Mandelbaum, 1992). Compost is of value to the agriculture and horticulture industries due to its chemical and physical properties that enhance soil fertility, structure, water-holding capacity and overall plant health (Stofella and Khan, 2000), and also for its biological properties, which may be utilized to suppress plant disease.

A wide variety of composts, generated from animal and plant waste materials, have been studied for suppression of plant diseases (Hadar and Mandelbaum, 1992). Biological suppression of pathogens in compost results from microbial activity developing during the composting process (Hadar and Mandelbaum, 1992). Suppression of *Phytophthora cinnnamomi* was observed in media amended with chicken manure compost containing high microbial activity and high populations of endospore-forming bacteria (Aryantha *et al.*, 2000).

Compost-amended soil is the potential for microbial-induced suppression of soil-borne pathogens (Hoitink *et al.*, 1991). Suppression of soil-borne plant pathogenic fungi in composted organic waste-amended potting mixes has been demonstrated (Hadar and Mandelbaum, 1992). Compost also can serve as a food base for endogenous microbe sustains suppression based on the activities of microbial communities (Hoitink and Boehm, 1999). Composts and manures harbour populations of

Table 1: Effect of composted poultry manure (CPM) amendment on disease incidence, sclerotial population of *M. phaseolina* and seed yield of sunflower (Pot trial-I; *kharif*).

Treatments	Charcoal rot incidence (%)	Sclerotia/g of soil	Seed yield (kg/ha)
Uninoculated control	0.00	0.0	562[b]
Inoculated control	72.96[g]	32.8[g]	125[a]
CPM (0.25%, 0 DBS*)	31.26[f]	21.1[f]	760[d]
CPM (0.25%, 14 DBS)	29.12[e]	18.7[e]	815[e]
CPM (0.25%, 28 DBS)	26.45[d]	16.5[d]	850[f]
CPM (0.5%, 0 DBS)	22.96[c]	14.5[c]	910[g]
CPM (0.5%, 14 DBS)	18.42[b]	10.4[b]	1025[h]
CPM (0.5%, 28 DBS)	12.96[a]	9.5[a]	1085[i]
Carbendazim (0.1%)	31.42[f]	22.0[f]	685[c]
LSD (0.05)2.53	1.27	26	

*DBS - days before sowing.
Values are mean of five replications.
In column means followed by same letter(s) are not significantly different (P=0.05) by DMRT.

Table 2: Effect of composted poultry manure (CPM) amendment on disease incidence, sclerotial population of *M. phaseolina* and seed yield of sunflower (Pot trial-II; *rabi*).

Treatments	Charcoal rot incidence (%)	Sclerotia/g of soil	Seed yield (kg/ha)
Uninoculated control	0.00	0.0	520[b]
Inoculated control	74.56[g]	35.6[h]	110[a]
CPM (0.25%, 0 DBS*)	33.72[f]	19.2[f]	700[d]
CPM (0.25%, 14 DBS)	30.92[e]	16.7[e]	775[e]
CPM (0.25%, 28 DBS)	29.97[d]	13.3[d]	830[f]
CPM (0.5%, 0 DBS)	24.67[c]	11.9[c]	915[g]
CPM (0.5%, 14 DBS)	20.73[b]	8.6[b]	1000[h]
CPM (0.5%, 28 DBS)	15.92[a]	7.8[a]	1010[h]
Carbendazim (0.1%)	34.56[f]	25.0[g]	674[c]
LSD (0.05)2.89	1.61	29	

*DBS - days before sowing.
Values are mean of five replications.
In column means followed by same letter(s) are not significantly different (P=0.05) by DMRT.

Table 3: Changes in ^total phenol as influenced by composted poultry manure (CPM) against challenge inoculation with *M. phaseolina*.

Treatments	Sampling periods (days after sowing)			
	45	52	59	66
Inoculated control	2.98[d]	3.57[c]	3.83[e]	3.95[d]
CPM (0.25%, 0 DBS*)	3.74[bcd]	4.23[b]	4.37[d]	4.63[c]
CPM (0.25%, 14 DBS)	3.87[bc]	4.03[bc]	4.59[c]	4.68[c]
CPM (0.25%, 28 DBS)	3.74[bcd]	4.08[bc]	4.59[c]	4.73[c]
CPM (0.5%, 0 DBS)	3.91[b]	4.08[bc]	4.91[b]	5.05[b]
CPM (0.5%, 14 DBS)	3.70[cd]	4.23[b]	4.93[b]	5.08[b]
CPM (0.5%, 28 DBS)	4.27[a]	4.93[a]	5.23[a]	5.36[a]

^mg/g of tissue.
*DBS - days before sowing.
Values are mean of three replications.
In column means followed by same letter(s) are not significantly different (P=0.05) by DMRT.

Table 4: Changes in ^*ortho*-dihydroxy phenol as influenced by composted poultry manure (CPM) against challenge inoculation with *M. phaseolina*.

Treatments	Sampling periods (days after sowing)			
	45	52	59	66
Inoculated control	1.17[c]	2.10[d]	3.15[d]	3.55[d]
CPM (0.25%, 0 DBS*)	3.15[b]	3.68[c]	4.73[c]	5.14[c]
CPM (0.25%, 14 DBS)	3.15[b]	3.68[c]	5.12[b]	5.23[c]
CPM (0.25%, 28 DBS)	3.99[a]	4.34[b]	4.99[c]	5.32[c]
CPM (0.5%, 0 DBS)	4.07[a]	4.83[a]	5.96[a]	6.04[b]
CPM (0.5%, 14 DBS)	3.94[a]	4.20[b]	6.04[a]	6.17[b]
CPM (0.5%, 28 DBS)	3.94[a]	4.20[b]	6.04[a]	6.44[c]

^mg/g of tissue.
*DBS - days before sowing.
Values are mean of three replications.
In column means followed by same letter(s) are not significantly different (P=0.05) by DMRT.

microorganisms, although microbial population structures may change during composting (Hoitink and Boehm, 1999). Disease suppression in these mixes has been attributed mainly to elevated levels of microbial activity (Mandelbaum and Hadar, 1990). Competition among microbial populations for available carbon or nitrogen has been proposed as the principal mechanism of disease suppression (Mandelbaum and Hadar, 1990). Other mechanisms proposed include mycoparasitism, iron competition, production of inhibitors or hydrolytic enzymes by soil microflora and interactions with some saprophytes (Diab *et al*., 2003).

Post infectional biochemical changes

Studies on induction of defense mechanisms revealed that higher accumulation of total and OD phenols was observed in plants amended with composted poultry manure followed by challenge inoculation with *M. phaseolina* (Table 3 and 4). Accumulation of total and OD phenols started 52nd day after challenge inoculation. The maximum accumulation was observed at 66th day after challenge inoculation. Gradual increase in phenolic content was observed throughout the sampling periods. Plants inoculated with pathogen alone also recorded less accumulation of phenolic content when compared to compost-amended plants challenged with pathogen.

Phenolic compounds enhance the mechanical strength of host cell wall and also inhibit the invading pathogenic organisms. Seed treatment with *Pseudomonas fluorescens* induced the accumulation of phenolics in tomato root tissue (M'Piga *et al*., 1997). The hyphae of the pathogen surrounded by phenolic substances exhibited considerable morphological changes including cytoplasmic disorganization and loss of protoplasmic content. Accumulation of phenolics by prior application of *P. fluorescens* in pea has been reported against *Pythium ultimum* and *Fusarium oxysporum* f.sp. *pisi* (Benhamou *et al*., 1996). Similar findings were reported in sugarcane against *Colletotrichum falcatum* (Viswanathan and Samiyappan, 1999) in tomato and hotpepper against *P. aphanidermatum* (Ramamoorthy *et al*., 2002) and in rice against *Rhizoctonia solani* (RadjaCommare *et al*., 2002). Benhamou *et al*., (2000) reported that an endophytic bacterium, *Serratia plymuthica* induced the accumulation of phenolics in cucumber roots against *P. ultimum*.

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