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STUDIES ON THE HOST DEFENSE ENZYMES AS INFLUENCED BY APPLICATION OF COMPOSTED POULTRY MANURE AGAINST CHALLENGE INOCULATION WITH ROOT ROT PATHOGEN IN SUNFLOWER

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Abstract

The present studies were undertaken to investigate the changes in host defense enzymes as influenced by application of composted poultry manure against challenge inoculation with root rot pathogen in 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. 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. 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. 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 for defense enzyme studies. The maximum defense enzymes activities were observed at 59th day after challenge inoculation and the activity was maintained at higher levels throughout the experimental period. Plants inoculated with pathogen alone were recorded comparatively less enzyme activity. Plants amended with composted poultry manure and challenged with the pathogen an additional increase in the accumulation of PAL was observed the activity started to increase at 52nd day thereafter remained at higher level throughout the experimental period. The plants inoculated with pathogen, increased activity of PAL was observed for a period of 45th -59th days thereafter declined drastically

Key words: root rot, Sunflower, plant defense enzymes, animals manure, compost

Introduction

Sunflower (*Helianthus annuus* L.) is originated in North America. Sunflower is grown primarily as an oilseed crop, with the greatest production in Russia, Ukraine, the European Union, Argentina, China and USA. Sunflower seed was the third largest source of vegetable oil worldwide, following soybean and palm. *Macrophomina phaseolina* (Tassi) Goidanich is one of the most important pathogen on sunflower in warmer countries (Theradimani and Hepziba, 2003; Bokor, 2007; Habib *et al*., 2007). The fungus causes charcoal rot disease on more than 500 plant species worldwide (Su *et al*., 2001). Uses of synthetic fungicides (Rettinassababady and Ramadoss, 2000) for the control of charcoal rot disease. But the use of chemicals may pose danger to the environment by polluting the eco-system. Further, the seed treatment with fungicides does not protect the crop for a longer period. Under rainfed conditions, soil drenching with fungicides is uneconomical. Alternative methods are needed to reduce the density of *M. phaseolina* in soil. The use of composts to suppress soil-borne plant pathogens has been extensively reviewed by several authors (Hoitink and Boehm, 1999; Hoitink *et al*., 2001; Ryckeboer *et al*., 2002). Composted animal manures have long been known to provide benefits to soil systems by improving soil structure and nutrient availability and are part of the foundation of organic fertility management (Dick and McCoy, 1993). Hence biological control using composted animal manures offers a practical management of the disease (Aryantha *et al*., 2000; Aldahmani *et al*., 2005). Therefore, the present studies were undertaken to

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investigate the changes of host defense enzymes as influenced by application of Composted animal manure along with root rot pathogen inoculation in sunflower.

Materials and methods

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).

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.

Table 1: Changes in ^peroxidase 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	1.12^{c}	3.72^{d}	21.23^{d}	10.69^{d}
CPM (0.25%, 0 DBS*)	1.73^{b}	9.27^{c}	85.38^{c}	56.43^{c}
CPM (0.25%, 14 DBS)	2.13^{b}	10.09^{c}	85.27^{b}	74.76^{c}
CPM (0.25%, 28 DBS)	1.53^{a}	9.99^{b}	114.39^{b}	77.00^{c}
CPM (0.5%, 0 DBS)	2.38^{a}	17.08^{b}	116.59^{a}	78.27^{b}
CPM (0.5%, 14 DBS)	2.38^{a}	18.23^{b}	117.32^{a}	78.38^{b}
CPM (0.5%, 28 DBS)	2.95^{a}	40.72^{a}	119.21^{a}	80.21^{a}

^min/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 2: Changes in ^polyphenol oxidase 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	0.16^{c}	1.60^{c}	17.6^{d}	12.34^{c}
CPM (0.25%, 0 DBS*)	0.29^{c}	1.83^{bc}	20.90^{c}	17.90^{bc}
CPM (0.25%, 14 DBS)	0.37^{c}	2.14^{abc}	22.65^{c}	17.75^{bc}
CPM (0.25%, 28 DBS)	0.29^{c}	2.04^{bc}	22.75^{c}	17.92^{bc}
CPM (0.5%, 0 DBS)	0.85^{abc}	2.21^{abc}	23.22^{bc}	18.41^{ab}
CPM (0.5%, 14 DBS)	1.09^{ab}	2.36^{ab}	23.61^{b}	18.61^{ab}
CPM (0.5%, 28 DBS)	1.52^{a}	2.86^{a}	24.37^{a}	19.07^{a}

^min/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

Defense enzymes assay - Enzymes extraction

Using pre-chilled pestle and mortar, one gram of samples was homogenized using 5.0 ml of 0.1M potassium phosphate buffer, pH 6.5 with a pinch of polyvinyl pyrollidone (PVP). The homogenate was centrifuged at 10,000 rpm for 10 min at 4ºC and the supernatant was used as enzyme source for the assay of defense enzymes activity.

Peroxidase

Peroxidase (PO) activity was assayed as per the method described by Hartee (1955). Reaction mixture consisted of 1.5 ml of 0.05M pyrogallol and 0.5 ml of the enzyme extract. The absorbance of the mixture was set to zero at 420 nm in a spectrophotometer and change in absorbance was recorded at 30 sec intervals for three min. Boiled enzyme extract served as control. PO activity was expressed as change in absorbance min/g of fresh weight basis (Hammerschmidt *et al*., 1982).

Polyphenol oxidase

Polyphenol oxidase (PPO) activity was assayed using the method described by Mayer *et al*., (1965). The reaction mixture consisted 1.5 ml of 0.1M sodium phosphate buffer (pH 6.5) and 200 ml of the enzyme extract. To start the reaction, 0.01M catechol was added and the absorbance was set to zero at 495 nm in a spectrophotometer and change in the absorbance was recorded at 30 sec intervals for three min. PPO activity was expressed as change in absorbance of the reaction mixture min/g of fresh weight basis.

Phenylalanine ammonia-lyase

Phenylalanine ammonia-lyase (PAL) activity was assayed using the method described by Southern and Deverall (1990) with minor modification by measuring

Table 3: Changes in ^phenylalanine ammonia-lyase 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	14.65^{c}	80.97^{d}	87.80^{e}	58.19^{d}
CPM (0.25%, 0 DBS*)	22.26^{bc}	97.26^{c}	98.42^{d}	74.22^{c}
CPM (0.25%, 14 DBS)	23.54^{abc}	100.37^{cd}	104.70^{c}	75.26^{bc}
CPM (0.25%, 28 DBS)	23.78^{ab}	101.83^{bc}	108.90^{bc}	78.31^{abc}
CPM (0.5%, 0 DBS)	24.51^{a}	102.20^{bc}	113.17^{a}	78.79^{ab}
CPM (0.5%, 14 DBS)	26.04^{a}	105.10^{ab}	116.83^{a}	80.17^{a}
CPM (0.5%, 28 DBS)	26.34^{a}	106.10^{a}	116.03^{a}	80.97^{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

the rate of formation of cinnamic acid from phenylalanine. PAL activity was assayed with 300 ml extract and 600 ml of 0.25M borate buffer, pH 8.8, containing 6mM L-phenylalanine. After two hours of incubation at 40^{o}C, the reaction was stopped with 100 ml of 6M hydrochloric acid. The product, cinnamic acid, was extracted into chloroform. The chloroform was evaporated by boiling for 1-2 min. The residue was dissolved in 1.0 ml of 0.1M borate buffer and the absorbance at 270 nm was recorded. PAL activity was calculated as mg cinnamic acid formed per hour for gram of fresh weight basis. Cinnamic acid dissolved in 0.1M borate buffer, pH 8.8 was used as a standard.

Results and Discussion

In the present study, PO activity also increased in plants amended with composted poultry manure followed by challenge inoculation with pathogen. The maximum PO activity was observed at 59th day after challenge inoculation and the activity was maintained at higher levels throughout the experimental period. Plants inoculated with pathogen alone were recorded comparatively less PO activity (Table 1). Similar pattern of increased activity of PPO was observed in composted poultry manure-amended plants challenged with pathogen (Table 2). Soil amended with composted poultry manure increased the PAL activity as compared with non amended control plants. Plants amended with composted poultry manure and challenged with the pathogen an additional increase in the accumulation of PAL was observed the activity started to increase at 52nd day thereafter remained at higher level throughout the experimental period. The plants inoculated with pathogen, increased activity of PAL was observed for a period of 45th-59th days thereafter declined drastically (Table 3).

Increased peroxidase activity has been shown in a number of resistant interactions involving plant pathogenic fungi (Kandan *et al*., 2002; Ramamoorthy *et al*., 2002). PO has been implicated in a number of physiological functions that contribute to resistance including exudation of hydroxyl cinnamyl alcohol into free radical intermediates (Gross, 1980), phenol oxidation, polysaccharide cross linking, cross linking of extensin monomers (Everdeen *et al*., 1988) and lignification (Walter, 1992) and also associated with deposition of phenolic compounds into plant cell walls during resistant interactions (Graham and Graham, 1991). Increased activity of cell wall bound PO has been elicited in different plants such as cucumber (Chen *et al*., 2000). Cow and horse manure compost increased the PO activity of treated cucumber plants (Liping *et al*., 1996). Peroxidase generated hydrogen peroxide which may function as an antifungal agent in disease resistance. Hydrogen peroxide inhibits directly and it may generate other reactive free radicals that are antimicrobial (Podile and Laxmi, 1998). Kenten (1956) found that rapid initial increase in the activity of polyphenol oxidase in plant tissue after infection results from the accumulation of latent phenolase or from solubilizing phenolase from cellular structure. Higher activity of PPO was correlated with resistance of host tissue to various pathogens (Constabel *et al*., 1995). This could be due to the conversion of phenols by the activity of this enzyme to fungitoxic compounds, quinones, which are more toxic to the pathogens than the phenolics (Retig, 1974).

PAL plays an important role in the biosynthesis of various defense chemicals in phenylpropanoid metabolism (Daayf *et al*., 1997). PAL activity was induced in plant-pathogen interactions and fungal elicitor treatment (Ramanathan *et al*., 2000). DeMeyer *et al*., (1999) reported that rhizosphere colonization of *P. aeruginosa* activated PAL in bean roots and increased the salicylic acid levels in leaves. In several host-pathogen interactions increased PAL levels have been shown to be correlated with incompatibility (Rathmell, 1973; Ratton *et al*., 1989). The product of PAL is *trans*-cinnamic acid which is an immediate precursor for the biosynthesis of salicylic acid, a signal molecule in systemic acquired resistance (Klessig and Malamy, 1994). These earlier reports lend support to the present findings.

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