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SUPPRESSION OF TOMATO EARLY BLIGHT BY SPRAYING OF ANIMAL MANURE BASED COMPOST WATER EXTRACTS

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ABSTRACT

Early blight incited by *Alternaria solani* is one of the most common and destructive foliar diseases of tomato in most of tomato growing areas. Currently management of tomato early blight relies principally on foliar spray of fungicides, which exert marked protective and curative efficacy against early blight disease. However the excessive use of fungicides raises a number of problems. Hence, in the present study, the possibility of induction of resistance in tomato against *A. solani* using foliar spraying of compost water extracts was investigated. Foliar spray of extracts of animal manure based composts and extracts of same composts amended with *Trichoderma viride* was tested for the suppression of tomato early blight each at two concentrations. Foliar spray of cattle manure based compost extracts were superior over control in suppression of early blight, whereas the percent disease reduction with poultry manure based compost extracts were statistically on par with the control. Disease control ability of compost extracts increased when compost was pre-amended with *T. viride*. Highest percent disease reduction of 61.76 was recorded with full strength extract of cattle manure based compost amended with *T. viride* followed by 49.02 with 1:1 dilution of same extract. However it is evident that compost extracts could be diluted up to 50 percent without affecting the efficacy against early blight of tomato.

KEYWORDS: *Alternaria solani*, Induced resistance, *Trichoderma viride*, Tomato.

INTRODUCTION

Early blight incited by *Alternaria solani* (Ellis and Martin) Jones and Grout is one of the most common and destructive foliar diseases of tomato in most of tomato growing areas and responsible for a large proportion of total monetary losses sustained by tomato producers in every growing season. Currently management of tomato early blight relies principally on foliar application of fungicides, which exert marked protective and curative efficacy against early blight. However the excessive and abusive use of fungicides raises a number of problems such as emergence of resistant pathogens, adverse effects on environment, non-target effects on other micro flora and the residuals, which are harmful to human beings. These disadvantages associated with fungicide application have forced scientists to develop more economical, environmentally sound and reliable methods of early blight disease management.

As an alternative to fungicide application, one of the potential disease management strategies would be the induction of resistance in the host against the pathogen. In general, induced resistance can be defined as an activation of mechanisms that allow plants to defend themselves against a broad spectrum of pathogens without any changes of the plant genome (Siegrist *et al.*, 2000). Induced resistance involves expression of a set of genes including those encoding pathogenesis related (PR) proteins and enzymes (Sticher *et al.*, 1997). These include β 1,3-glucanase, chitinase, thaumatin and PR-1 proteins (Tosi *et al.*, 1999) that have direct antimicrobial activity or are closely related to classes of antimicrobial proteins.

Compost teas, also known as compost watery extracts are gaining increased attention as a crop protection tool for the control of foliar diseases by inducing resistance in plants. Compost water extracts have been used for years as topical spray to control foliar diseases of plants (Zhang *et al.*, 1998). A significant reduction in early blight disease was observed in compost extract treated tomato plants (Tsror and Bieche, 1999). Further amendment of compost with specific antagonists is a valuable option for amplifying their beneficial properties in terms of plant disease suppression (Hoitink *et al.*, 1996).

In the light of above information, present study was undertaken to investigate the effect of extracts of animal manure based composts and extracts of same animal manure based composts pre-amended with *Trichoderma viride* in suppression of tomato early blight by inducing resistance in tomato plants.

MATERIALS AND METHODS

Seed and plant materials

Seeds of tomato (*Lycopersicon esculentum* Miller) cultivar, Pusa Early Dwarf (PED); Ankur Seeds Limited, Nagpur-18, India were sown in plastic crates and 25 days old seedlings were transplanted in earthen pots (20 cm X 20 cm) containing a non sterilized red soil and farm yard manure (4:1 V/V). Plants were grown in a glasshouse under natural conditions of temperature and light. Plants were irrigated daily and fertilized weekly with commercially available 14:14:14 NPK mixture.

Pathogen and preparation of inoculum

The pathogen was isolated from naturally infected tomato leaves showing typical early blight symptoms under aseptic conditions by tissue segment method (Aneja, 2000). The culture was purified by single spore isolation described by Tuite (1969) and identified as *A. solani* based on morphological characteristics described by Ellis (1971) and cultural characteristic given by Spletzer and Enyedi (1999).

Since *A. solani* seldom produces conidia under laboratory condition, standard-mycelial spore suspension was prepared as previously described by Dhiman *et al*., (1980) for mass inoculation of tomato plants. Seven day old culture of *A. solani* growing in three test tubes containing 5 ml of Czapex-Dox agar medium in each were collected and transferred to an motor operated electric blender containing 250 ml of 0.01% tween 20 solution prepared with distilled water. Then the blender was run in alternate cycles of high and low speed for 3-4 minutes. The slicing and blending action of the blades accompanied by a strong centrifugation reduced the coarse fungal mats into bits transverse through a fine nozzle of an atomizer. The concentration of infective propagules was adjusted to 10^4 per ml using haemocytometer.

Mass multiplication of *Trichoderma viride* in liquid molasses yeast medium

A biocontrol agent, *Trichoderma viride* was isolated from talc based commercial formulation Basderma$^{(R)}$ (Basarass Biocontrol Research Laboratory, Pennadam, India). Mass multiplication of *T. viride* was done on molasses yeast medium. 100 ml of molasses yeast medium in each of 250 ml volume conical flask was sterilized in an autoclave at 121.6^{o}C for 20 min. Then pure culture of *T. viride* was transferred into each conical flask with molasses yeast medium. Conical flasks inoculated with *T. viride* were incubated at 25$\pm$1^{o}C in shaker incubator at 100 rpm for one week. After one week, the amount of *T. viride* mycelia and spores produced was sufficient for mixing with compost.

Pre-amendment of composts with *Trichoderma viride*

Compost was amended with entire biomass of *T. viride* in molasses yeast medium at the rate of 100 ml (biomass) with 7.5 kg of compost. Then composts were incubated in pits dug in an open field. The pits were separately filled with cattle manure based compost, poultry manure based compost, cattle manure based compost amended with *T. viride and* poultry manure based compost amended witrh *T. viride*. These pits were covered with black polythene followed by watering and water was added every week to maintain sufficient moisture and left in the field for 60 days.

Preparation of compost water extracts (CEX)

Compost water extracts (CEX) were prepared according to the procedure previously adopted by Zhang *et al*., (1998) with slight modifications. Composts incubated in pits were mixed with tap water at the ratio of 1:1 (V/V) in separate plastic containers. These mixtures were stirred thoroughly and allowed to ferment indoors for one week after covering with polythene sheet. After soaking period, which was referred to as "extraction time", the solution was strained through double-layered muslin cloth to get compost water extract.

Treatment with compost water extracts and challenge inoculation

All compost water extracts were sprayed at full strength and in 1:1 dilution with water on 30-day-old tomato plants in a glasshouse. Plants treated with mancozeb 2000 ppm served as fungicide check, and those treated with water served as control. Five days after spraying with compost extracts, plants were challenge inoculated with standard mycelial spore suspension of *A. solani* (10^4 infective propagules per ml) followed by incubation in a moist chamber for 48 hours in the dark. Two days after challenge inoculation, plants were transferred to glasshouse bench from moist chamber. Plants were regularly observed for the disease development.

Assessment of induced resistance

Induced resistance was determined by the incubation period, number of lesions per leaf and area of lesions. The level of resistance was considered relative to symptoms on control plants treated with water instead of chemical salts. Seven days after challenge inoculation, the maximum disease development was usually evident in the controls and data was obtained at this time.

Disease severity of early blight was recorded using 0-4 scale (Rajagopal and Vidyasekaran, 1982; Devanathan and Ramanujam, 1995).

Numerical ratings	**Disease intensity**
0	Infection free or nearly so
1	Trace to 25% of leaf area blighted
2	26-50% of leaf area blighted
3	51-76% of leaf area blighted
4	76-100% of leaf area blighted

Percent Disease Index (PDI) was computed by using the formula suggested by Rajagopal and Vidyasekaran (1982).

$$\text{PDI} = \frac{\text{Sum of numerical ratings x100}}{\text{Total number of leaves assessed x Maximum rating}}$$

Experimental Design and Statistical analysis

The experiments arranged in simple CRD with 3 replications. Statistical analysis of variance (ANOVA) and mean separation were carried out using *Minitab,* statistical software. Whenever necessary, appropriate transformation of the values were performed to normalize data and stabilize the variance throughout the data range prior to analysis of variance. All data expressed as percentage were angular (arcsine) transformed before analysis.

RESULTS AND DISCUSSION

This experiment is planed to determine whether animal manure based compost extracts can induce Systemic Acquired Resistance (SAR) in tomato and amendment of composts with *T. viride* can increase the effectiveness of subsequent compost extract in inducing SAR in tomato against early blight. Topical sprays of compost extracts reduce the severity of foliar diseases such as powdery mildew, downy mildew of grape, gray mold of strawberries and late blight of potato (Zhang *et al.*, 1998).

Highest incubation period of 5.75 days were recorded in mancozeb treated plants followed by full strength extract of cattle manure based compost amended with *T. viride* (5.83 days), 1:1 dilution extract of cattle manure based compost amended with *T. viride* (4.83 days) and full strength extract of poultry manure amended with *T. viride* (4.67 days). Dilution of all kinds of compost extracts except poultry manure amended with *T. viride* showed significant reduction of incubation period. Further pre-amendment of composts with *T. viride* showed enhancement of disease suppression ability of compost extracts compared to non-amended compost extract by increasing of incubation period.

Least number of lesions per leaf (1.81) was recorded in plants treated with mancozeb 2000 ppm whereas maximum of 18.72 in control plants. Among the compost extract treated plants, minimum number of lesions (6.28) was recorded in plant treated with full strength extract of cattle manure amended with *T. viride* followed by 1:1 dilution extract of cattle manure amended with *T. viride* (8.81) and both concentrations of extracts of poultry manure amended with *T. viride* (10.14 and 9.11 respectively). The results indicate that declining of disease suppression ability of extracts upon dilution of all compost extracts except poultry manure amended with *T. viride*. Similar to the incubation period, preamendment of compost with *T. viride* showed the synergistic effect of disease control ability of compost extract compared to non-amended compost extract by reducing the lesion number per leaf.

Table 1. Effect of extracts of composts and extracts of composts amended with T*richoderma viride* against *Alternaria solani*.

S. No.	*Treatments*	*Incubation period (days)*	*Number of lesions per leaf*	*Per cent disease index*	*Per cent disease reduction over control*
1.	Cattle manure based CEX (Full strength)	3.50	10.42	49.31 (44.60)	30.39 (33.42)
2.	Cattle manure based CEX (dilution 1:1)	2.92	15.72	56.25 (48.62)	20.59 (26.38)
3.	Poultry manure based CEX (Full strength)	3.42	11.92	61.81 (51.85)	12.74 (20.36)
4.	Poultry manure based CEX (dilution 1:1)	2.83	17.78	65.28 (53.99)	8.82 (13.67)
5.	CEX of cattle manure amended with *T. viride* (Full strength)	5.33	6.28	27.08 (31.29)	61.76 (51.86)
6.	CEX of cattle manure amended with *T. viride* (dilution 1:1)	4.83	8.81	36.11 (36.92)	49.02 (44.44)
7.	CEX of poultry manure amended with *T. viride* (Full strength)	4.67	10.14	43.75 (41.39)	38.24 (38.14)
8.	CEX of poultry manure amended with *T. viride* (dilution 1:1)	4.25	9.11	52.78 (46.59)	25.51 (30.28)
9.	Mancozeb - 2000 ppm	5.75	1.81	7.64 (15.80)	89.22 (71.12)
10.	Control	2.25	18.72	70.83 (57.35)	--
	SEm$\pm$	0.144	0.542	1.890	3.531
	CD ($P \leq 0.05$)	0.425	1.599	5.578	10.418

Figures in parentheses are angular transformed values ; CEX: Compost extract

The results of this study revealed that foliar application of cattle manure based compost extract showed 20.59 to 30.39 per cent disease reduction over control whereas poultry manure based compost extract showed substantially lower efficacy. Tsror and Bieche (1999) sprayed tomato plants with 7 and 14-day-old compost extracts prepared in a ratio of 1:5 compost to water (V/V). They observed a significant reduction in early blight disease in compost extract water sprayed plants.

The present results are in agreement with those of Dittmer *et al*., (1990) who reported that several compost extracts with varying extraction periods controlled *Botrytis cinerea* in field and on detached *Phaseolus vulgaris* leaves. Similarly Winterscheidt *et al*., (1990) reported that watery extract of composted cattle manure, sea weed, grape mare and horse manure reduced infection of *Pseudoperonospora cubensis* in detached cucumber leaves, while extracts of green plant matter, pig manure and wheat straw did not. Zhang *et al*., (1998) reported that the activity of compost water extract against bacterial speck of *Arabidopsis* was almost as effective as chemical inducer salicylic acid 5 mM. Ma *et al*., (1999) demonstrated that weekly spray of macerated extracts of horse, cow and pig manure significantly reduced the incidence of cucumber powdery mildew (*Sphaerotheca fuliginea*) by 72.3 – 79.7 per cent compared to the control.

Compost extracts enable suppression of plant pathogens through their action on the phyllosphere. A wide range of mechanisms such as induced resistance, inhibition of spore germination, antagonism and competition with pathogens seem to contribute to suppressive effects.

Compost extracts contain biocontrol agents as well as unidentified factors that appear to play a role in efficacy (Yohalem *et al*., 1994). Cronin *et al*. (1996) demonstrated that a low molecular weight compound was critical for *in vitro* lyses of conidia of *Venturia inaequalis*. Dittmer *et al*., (1990) reported that reduction of incidence of *Botrytis cinerea* in *Phaseolus vulgaris* upon topical application of compost extract was thought to be due to the antagonistic activity of microorganisms in the extract. Similarly, direct inactivation of inhibitors in the extracts with the pathogen in the sprayed leaves could not however be excluded. Further compost extract can induce the host-mediated resistance in plants (Zhang *et al*., 1998 and Boulter *et al*., 2002). Zhang *et al*., (1998) demonstrated that β-D-gluconidase (GUS) activity was increased to a greater extent in cucumber plants by compost water extract treatment like SA treatment. This compost mediated induced resistance appeared to correlate with an increase in the activity of some enzymes such as peroxidases and β-1,3-glucanases, which are important components in the overall plant defense strategy (Pharand *et al*., 2002).

The results of the present investigation clearly indicate that disease control ability of the compost extract was significantly increased when compost was amended with *T. viride* nearly 2 months before extraction of the compost extract. Percent disease reduction treated with cattle manure compost extracts on plants ranged from 20.59 (dilution 1:1) to 30.39 (full strength). However cattle manure compost extracts prepared from the cattle manure compost amended with *T. viride* showed increased disease reduction and per cent disease reduction varied from 49.02 (dilution 1:1) to 61.76 (full strength) (table 1). Similar trend was observed for poultry manure based compost extracts and extracts from poultry manure amended with *T. viride*.

Amendment of composts with specific antagonists is a valuable option for amplifying their beneficial properties in terms of plant disease suppression (Kwok *et al*., 1987; Hoitink *et al*., 1996). Many workers have suggested amendment of compost to confer the increased resistance, and suppression of plant diseases. Zhang *et al*., (1998) provided evidence, that amendment of composts with a selected bio-control agent was a valuable option to confer increased resistance against anthracnose in cucumber.

It is evident that *Trichoderma* spp itself can induce resistance in crop plants against various pathogens. De Meyer *et al*., (1998) reported that *Trichoderma harzianum* strain T-39 induced resistance in tomato, pepper, bean, lettuce and tobacco against *Botrytis cinerea*. In addition, *Trichoderma* spp. exhibits antagonism against a large number of soil borne pathogens and massively colonize cellulose-containing composts (Pharand *et al*., 2002). This suggests that *Trichoderma viride* in the present investigation colonized more efficiently the cattle manure compost than poultry manure compost as cattle manure composts contain more cellulose.

Further mycelial extracts of *Trichoderma* spp. can induce systemic resistance and PR proteins in tobacco (Chen *et al*., 1994). There are evidences that *Trichoderma* can produce antibiotics, which contribute for disease suppression. Blakeman and Fokkema (1982) reported that *Trichoderma* though not considered phylloplane colonizer could produce chemicals such as trichodermin (a sesquiterpene antibiotic) active against fungi. Further they reported, it was possible to control naturally occurring dry eye rot on apple caused by *Botrytis cinerea* in the field by spraying with *Trichoderma harzianum* in 0.1% malt extract at similar frequencies as currently used fungicides.

CONCLUSIONS

Topical spray of animal manure based compost extracts can be used for the management of early blight of tomato either by inducing resistance in tomato or by bio-control associated attributes. Foliar spray of cattle manure based compost extracts is superior over that of poultry manure. However, disease reduction with poultry manure based compost (non amended with *T. viride*) extracts are statistically on par with control. Disease control ability of compost extracts increase when composts are amended and incubated with *T. viride* for two months period prior to the preparation of the extracts. Compost extracts can be diluted up to 50 percent without affecting their efficacy against early blight in tomato.

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