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Biological control of *Alternaria solani*, the causal agent of early blight of tomato

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ABSTRACT: **Plant extracts and saprophytic fungi isolated from tomato phylloplane were evaluated against *Alternaria solani*, the causal agent of early blight of tomato, under screen house and field conditions. Foliar spray of *Clerodendron* leaf extract (15%) immediately after appearance of symptoms or foliar spray of *Trichoderma viride* (10^7 CFUs ml^{-1}) 24h before challenge inoculation with the test fungus was found effective in reducing the disease severity under screen house conditions. Under field studies, effective control of the disease was recorded with three sprays of mancozeb (2000ppm). However, disease severity could be significantly reduced with initial spray of *Clerodendron* leaf extract (15%) or *T. viride* (10^7 CFUs ml^{-1}) followed by two sprays of mancozeb.**

KEY WORDS: *Alternaria solani*, antagonist, *Clerodendron*, plant extract, *Trichoderma viride*

INTRODUCTION

Early blight of tomato incited by *Alternaria solani* (Ellis and Martin) Jones and Grout is a serious disease attacking foliage, stem and fruits, particularly in arid and semi-arid regions. The losses due to this disease have been reported from different parts of India to the extent of 48-80% (Datar and Mayee, 1981). Since hybrids are more susceptible to early blight and constitute more than seventy per cent of total tomato cultivation losses can be enormous.

Although satisfactory control of the disease by using various chemicals has been documented (Choulwar and Datar, 1988; Maheshwari *et al.*, 1991; Abdul-Mallek *et al.*, 1995), continuous use of agrochemicals for controlling the disease may pose several problems like toxicity to non-target organisms, development of resistance in the populations of the pathogen and environmental pollution. Bioagents and plant extracts are considered as new rays of hope, because they are ecofriendly and can be used as an effective alternative measure to control plant diseases.

Several bioagents and plant extracts are reported to suppress many plant pathogens effectively. Bora (1977) demonstrated the antagonistic effect of *Myrothecium verrucaria* and

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Trichoderma viride against *Alternaria alternata* on eggplant seedlings under *in vitro* and screen house tests. Saprobic fungi inhabiting the tomato phylloplane, *viz.*, *Nigrospora* spp., *Penicillium* spp., *Chaetomium globosum*, *Cladosporium cladosporioides* and *T. polysporum* were found to be potential antagonists of *A. solani* (Monaco *et al.*, 1999). Further, spraying tomato cultivar PKM-1 with suspension of *P. fluorescens* strains 48 hours after inoculation with *A. solani* reduced leaf blight disease by 15-38 per cent compared to control (Babu *et al.*, 2000a).

Several plant extracts, *viz.*, *Allium cepa* L., *Allium sativum* L., *Ocimum sanctum* L. and Mont., *Mentha piperata* L. and *Beta vulgaris* L. were found inhibitory to *A. tenuis* (Shekawat and Prasad., 1971). Fresh rhizome extracts of ginger were found to be fungitoxic to *A. solani* (Singh *et al.*, 1983). Leaf extract of *Clerodendrum aculeatum* Gaertn. inhibited spore germination of *A. alternata* and development of disease on tomato fruits (Sharma and Sharma, 1992).

The present investigation was undertaken to study the effect of plant extracts and antagonists isolated from tomato phylloplane against *A. solani*. An attempt was made for integration of management practices for the management of early blight of tomato with minimal interference of biological equilibrium.

MATERIALS AND METHODS

Evaluation of plant extracts and antagonists under screen house conditions

The selected plant extracts and antagonists were evaluated for biological control of early blight of tomato under screen house conditions during *rabi* 2003-2004. The experiment was laid out in a completely randomized design (CRD). Three replications were maintained for each treatment. The cold aqueous extracts of the botanicals were sprayed at a concentration of 15 per cent immediately after first appearance of the disease symptoms. Foliar sprays of the respective fungal (10^7 CFUs ml^{-1}) and bacterial (10^8 CFUs ml^{-1}) antagonists were given to the tomato plants (cv. Selection7) 30 days after transplantation. The challenge inoculation of the test pathogen (10^4 conidial ml^{-1}) was done 24h after antagonist spray. Each treatment was replicated thrice. Disease severity was recorded at 15 and 30 days after inoculation of the pathogen based on 0-5 scale (Datar and Mayee, 1986) and per cent disease index (PDI) was calculated as per the formula of Wheeler (1969) and the data was analyzed statistically.

Integrated management of early blight of tomato

The field experiment was conducted to evaluate the combined effect of phyto-extract, antagonist and fungicide against early blight of tomato. The phyto-extract and antagonist found effective *in vitro* were tested under field conditions to evaluate their antifungal activity *in vivo* during *rabi* 2004-05. The following treatments were imposed.

1. Foliar spray with *T. viride* (10^7 CFUs ml^{-1}) 24h before inoculation with test fungus followed by 2 sprays of *T. viride* at 15 days interval (TV+TV+TV).
2. Foliar spray with *T. viride* 24h before inoculation with test fungus + foliar spray with *T. viride* at 15 days after inoculation (DAI) + foliar spray with mancozeb (0.2%) at 30 DAI (TV+TV+M).
3. Foliar spray with *T. viride* 24h before inoculation with test fungus + foliar spray with Clerodendron (15%) at 15 DAI + foliar spray with mancozeb at 30 DAI (TV+Cl+M).
4. Foliar spray with *T. viride* 24h before inoculation with the test fungus followed by 2 sprays of Clerodendron at 15 days interval (TV+Cl+Cl).
5. Foliar spray with Clerodendron on first appearance of symptoms + foliar spray with *T. viride* at 15 DAI + foliar spray with Clerodendron at 30 DAI (Cl+TV+Cl).
6. Foliar spray with Clerodendron on first appearance of symptoms followed by 2

sprays of Clerodendron at 15 days interval (Cl+Cl+Cl).

7. Foliar spray with Clerodendron on first appearance of symptoms + foliar spray with Clerodendron at 15 DAI + foliar spray with mancozeb at 30 DAI (Cl+Cl+M).

8. Foliar spray with mancozeb on first appearance of symptoms followed by two sprays of Clerodendron at 15 days interval (M+Cl+Cl).

9. Foliar spray with *T. viride* 24h before inoculation of the pathogen followed by two sprays of mancozeb at 15 days interval (TV+M+M).

10. Foliar spray with Clerodendron on first appearance of the symptoms followed by two sprays of mancozeb at 15 days interval (Cl+M+M).

11. Foliar spray with mancozeb on first appearance of the symptoms followed by two sprays of mancozeb at 15 days interval (M+M+M).

12. Inoculated control.

The experiment was laid out in a randomized block design (RBD). Water sprayed plots were considered as control. Treatment in each replication was applied randomly. There were 12 treatments with a plot size of 2.5x2.0m^2. Three replications were maintained for each treatment. The cultivar used was Selection 7. The usual cultivation practices were carried out as per the recommendations. The fungicides, phyto extracts and biocontrol agents were sprayed as per the treatments mentioned above. Observations on the disease severity were recorded 15 days after last spray. In each treatment, ten plants were selected at random, for scoring early blight based on 0-5

Table 1. Evaluation of plant extracts* against *Alternaria solani* under pot conditions

Treatment	Per cent disease index		Mean
	75 DAS	90 DAS	
Bougainvillea leaf extract	28.43 (32.20)	68.43 (55.79)	48.43 (44.00)
Clerodendron leaf extract	16.22 (23.71)	30.22 (33.33)	23.22 (28.52)
Garlic bulb extract	29.85 (33.09)	72.43 (58.31)	51.14 (45.70)
Lantana leaf extract	20.66 (27.01)	42.00 (40.38)	31.33 (33.69)
Neem leaf extract	20.69 (27.03)	44.00 (41.54)	32.35 (34.28)
Onion bulb extract	26.56 (30.99)	66.00 (54.32)	46.28 (42.66)
Inoculated control	30.33 (33.39)	74.16 (59.43)	52.25 46.41)
Mean	24.68 (29.63)	56.75 (49.02)	
Source	SEM ±		LSD (P = 0.01)
Time of observation (D)	(0.46)		(1.319)
Treatment (T)	(0.85)		(2.469)
D x T	(1.21)		(3.492)

Figures in parentheses indicate angular transformed values; *at 15% concentration

scale (Datar and Mayee, 1986). The per cent disease index was calculated and data were analyzed statistically.

RESULTS AND DISCUSSION

All treatments, except *Bougainvillea* leaf extract and garlic bulb extract, significantly reduced the per cent disease index compared to inoculated control (Table 1). The per cent disease index was minimum (23.22%) in pots sprayed with *Clerodendron* leaf extract, which was significantly less compared to all other treatments. This was followed by neem leaf extract (PDI of 32.35%), which is on par with lantana leaf extract (PDI of 31.33%).

Fungal and bacterial antagonists significantly reduced the per cent disease index compared to inoculated control (Table 2). The per cent disease index was minimum (27.52%) with foliar spray of *Trichoderma viride* (10^7 CFUs / ml^{-1}) which was significantly superior to all other treatments. This was followed by foliar spray of *Trichoderma harzianum* (PDI of 31.72%), which was on par with *Bacillus subtilis* (PDI of 33.35%). Babu *et al.* (2000b) reported that there was no significant difference between the effectiveness of *Trichoderma* species in pot culture studies. In the present study, *T. viride* was found to be superior over *T. harzianum* in inhibiting the disease under screen house conditions. This may be correlated with strain differences existing within the fungal species. Monaco *et al.* (1999) reported that foliar spray of *Trichoderma polysporum* (10^7 CFUs / ml^{-1}) one hour before inoculation with *A. solani* (10^6 CFUs/ml)

Table 2. Evaluation of antagonists* against *Alternaria solani* under pot conditions

Treatment	Per cent disease index		Mean
	75 DAS	90 DAS	
Aspergillus niger	26.56 (30.99)	66.00 (54.32)	46.28 (42.66)
Bacillus subtilis	22.69 (28.42)	48.00 (43.84)	35.35 (36.13)
Gliocladium virens	24.80 (29.84)	52.33 (46.32)	38.57 (38.08)
Paecilomyces variotii	24.00 (29.31)	48.00 (43.84)	36.00 (36.57)
Penicillium expansum	27.33 (31.49)	62.00 (51.93)	44.67 (41.71)
Pseudomonas spp.	26.00 (30.63)	62.00 (51.93)	44.00 (41.28)
Trichoderma harzianum	21.43 (27.55)	42.00 (40.38)	31.72 (33.96)
Trichoderma viride	18.53 (25.47)	36.50 (37.15)	27.52 (31.31)
Inoculated control	32.33 (34.63)	72.83 (58.57)	52.58 (46.60)
Mean	24.85 (29.82)	54.41 (47.58)	
Source	SEM ±		LSD (P = 0.01)
Time of observation (D)	(0.39)		(1.10)
Treatment (T)	(0.82)		(2.34)
D x T	(1.16)		(3.31)

Figures in parentheses indicate angular transformed values; *foliar applicatrion of antagonist (10^7 CFUs / ml^{-1}) 24h before inoculation of pathogen

Table 3. Integrated management of early blight of tomato

Treatment*	Per cent disease index			Mean
	75DAS	90DAS	105DAS	
TV+TV+TV	21.53 (27.21)	45.60 (42.45)	68.22 (55.84)	45.12 (41.83)
TV+TV+M	20.44 (26.71)	46.80 (43.14)	56.00 (48.44)	41.08 (39.43)
TV+Cl+M	22.69 (28.38)	40.22 (39.33)	46.66 (43.06)	36.53 (36.92)
TV+Cl+Cl	22.09 (27.91)	38.00 (37.94)	56.00 (48.43)	38.69 (38.09)
Cl+TV+Cl	15.55 (23.04)	33.88 (35.56)	54.84 (47.76)	34.76 (35.45)
Cl+Cl+Cl	17.67 (24.79)	26.62 (30.95)	47.00 (43.26)	30.43 (33.00)
Cl+Cl+M	16.46 (23.69)	27.33 (31.47)	35.00 (36.22)	26.26 (30.46)
M+Cl+Cl	12.00 (18.78)	23.33 (28.79)	33.50 (35.33)	22.94 (27.63)
TV+M+M	20.44 (26.79)	27.00 (31.27)	34.66 (36.03)	27.37 (31.37)
Cl+M+M	15.88 (23.37)	22.89 (28.52)	29.43 (32.79)	22.73 (28.22)
M+M+M	11.33 (19.47)	15.88 (23.37)	22.69 (28.38)	16.63 (23.74)
Inoculated control	26.67 (30.81)	52.22 (46.26)	78.00 (62.14)	52.29 (46.40)
Mean	18.56 (25.08)	33.31 (34.92)	46.83 (43.14)	
Source	SEM ±		CD (P = 0.05)	
Time of observation (D)	(0.66)		(1.87)	
Treatment (T)	(1.33)		(3.75)	
D x T	(2.29)		(6.49)	

Figures in parentheses indicate angular transformed values; *TV = *Trichoderma viride* (10^7 CFUs / ml^{-1}) sprayed 24h before inoculation of the pathogen, Cl = *Clerodendron* leaf extract (15%) sprayed after initial symptom development; M = Mancozeb (0.2%) sprayed after initial symptom development

reduced disease severity under greenhouse studies. Observations at 90 days after sowing revealed that there was no significant difference between foliar spray of *B. subtilis* and *Paecilomyces variotii*.

All treatments significantly reduced the percent disease index compared to inoculated control (Table 3). On 105th day of planting (15 days after the final spray) the least per cent disease index (22.69%) was recorded in plots which received three sprays of mancozeb (2000ppm). The results are in accordance with that of Prasad and Naik (2003) who reported that mancozeb (0.2%) and iprodione (0.2%) were effective in controlling early blight of tomato under field conditions.

Treatments Cl+M+M (foliar spray with *Clerodendron* leaf extract on initial appearance of symptoms followed by two sprays of mancozeb at 15 days interval), M+Cl+Cl (foliar spray with mancozeb on first appearance of symptoms followed by two sprays of *Clerodendron* leaf extract at 15 days interval), TV+M+M (foliar spray with *T. viride* 24h before inoculation with the test fungus followed by two sprays of mancozeb at 15 day interval) and Cl+Cl+M (foliar spray with *Clerodendron* leaf extract on first appearance of symptoms + foliar spray with *Clerodendron* leaf

extract at 15 days DAI + foliar spray with mancozeb at 30 DAI) were the next best treatments with PDI of 29.43 per cent, 33.50 per cent, 34.66 per cent and 35.00 per cent, respectively. Least control of the disease was recorded in the treatment, where foliar spray with *T. viride* 24h before challenge inoculation with test fungus was followed by two sprays of *T. viride* at 15 days interval. However, disease severity can be significantly reduced with initial spray of *Clerodendron* leaf extract (15%) or *T. viride* (10^7 CFUs ml^{-1}) followed by two sprays of mancozeb.

Foliar spray of *Clerodendron* leaf extract (15%) immediately after appearance of symptoms or foliar spray of *T. viride* (10^7 CFUs ml^{-1}) 24h before challenge inoculation with the test fungus was found effective in reducing the disease severity under screen house conditions. Under field studies, disease severity could be significantly reduced with initial spray of Clerodendron leaf extract (15%) or *T.viride* (10^7 CFUs ml^{-1}) followed by two sprays of mancozeb.

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