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Evaluate the efficacy of bio-control agents and botanicals against early blight of tomato caused by *Alternaria solani*

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Abstract
Tomato is one of the commercial vegetable crops, being affected with many diseases, among which early blight caused by *Alternaria solani* has become the constraint resulting in huge yield losses. Continuous usage of chemical methods leads to environment, soil and water pollution. Whereas biological control of diseases is long lasting, inexpensive, eco-friendly and harmless to target organisms. Apart from native and commercial bio-control agents, botanicals *viz.*, *Trichoderma viride*, *Trichoderma harzianum*, *Pseudomonas fluorescens*, neem oil and neem leaf extract were also screened against the pathogen. All treatment shows the antifungal activity against the pathogen. *Trichoderma harzianum* was most effective in disease severity (24.67%) followed by *P. fluorescens* (25.33%), *Trichoderma viride* (31.33%), Datura leaf extract (32.67%), Neem leaf extract (36.67%) compare to treated and untreated control (20.33% and 40.33%) respectively.

Keywords: bio-control agents, botanicals against early blight, potato, *Alternaria solani*

Introduction
Tomato [*Solanum lycopersicum L.*] is the second most important vegetable crop. It is used in preparation of soup, pickles, ketchup, purees, sauces etc. and also uses to add variety of colours and flavours to the food materials by various ways. Therefore, it is one of the most important vegetable crops in the world as well as in India considered as "protective food" both because of its special nutritive value. According to (Saini 2006) the nutritive value of tomato per 100 gm of edible portion are as moisture 93.19 per cent, protein 1.90 gm, potassium 144 mg, copper 0.19 mg, sulphur 24 mg, chlorine 38.00 mg, vitamin C 31.00 mg, thiamine 0.07 mg, riboflavin 0.01mg. nicotinic acid 0.40 mg, magnesium 15.00 mg, oxalic acid 2.00 mg, phosphorous 36.00 mg, Iron 1.80 and vitamin A 320.00 mg. I.U.

Tomato is found to suffer from a variety of disease caused by fungi, bacteria, viruses and nematodes. The important diseases are damping off, early blight, late blight, Fusarium wilt, *Verticillium* wilt, bacterial wilt and tomato mosaic virus. Among the diseases early blight caused by *Alternaria solani* is one of the most yield limiting factors in India. The survival of the pathogen mainly in the soil and penetrates into the plant through the root system. The early blight or fruit rot disease caused by *Alternaria solani* (Ellis and Martin) Jones and Grout is one of the most destructive diseases of tomato in the tropical and subtropical regions. The causal organism is air borne and soil inhabiting and is responsible for leaf blight, seedling collar rot and fruit rot of tomato (Datar and Mayee, 1981) [5]. This disease cause direct loss by the infection of fruits and indirect loss by reducing plant vigour. The early blight was the most catastrophic diseases incurring loss both at pre and post-harvest stages causing 35 to 78 per cent reduction in yield (Jones *et al.*, 1993) [6]. The effective management of the disease could be through cultural practices, chemical, biological control and use of resistant variety. Since the synthetic fungicides are being widely used by the farmers to eradicate pathogens but it results in environmental hazards and have harmful effects on human beings and animals. The chemical fungicides not only develop fungicidal resistant strains but also accumulate in food and ground water as residues. In order to overcome such hazardous control strategies, scientists, researchers from all over the world paid more attention toward the development of alternative methods which are, by definition, safe in the environment, non-toxic to humans and animals and are rapidly bio-degradable, one such strategy is use of bio-control agents (BCAs) to control fungal plant diseases.

Material and Methods

Symptoms caused by *Alternaria solani* on tomato plant

The first symptom of the early blight disease caused by *Alternaria solani* on tomato appeared as small brown water soaked lesions on the older leaf. The symptoms were oval or angular in shape from one to four mm diameter and there was a narrow chlorotic zone around the spot. These spots enlarged and covered the entire stem and petioles leading to withering of the plants. Symptoms also developed on calyx and flower buds in the form of minute brown to dark brown spots which enlarged later and spread to sepals and fruits resulting in pre-mature dropping of fruits.

Pathogenicity test

The pure culture of *Alternaria solani* was obtained by single spore isolation method and sub culture was used for pathogenicity test by following Koch's postulate. The pathogenicity test was carried out as described in material and methods by pre-inoculation with spore suspension and homogenized mycelial bits of *A. solani* on foliage of 30 days old Angoor Lata variety of tomato. After twenty days of inoculation, the symptoms appeared on inoculated leaves as brown, oval or angular necrotic spots with concentric rings and surrounded by a border of yellow host tissue. The fungus was re-isolated and purified culture from these artificially infected leaves was similar to that of original culture. The plants which were not inoculated with the fungal spore suspension did not show any symptoms of the disease. Thus pathogenicity on tomato was confirmed.

Preparation of plant based products

Fresh healthy plant parts of 100 gm (leaves/rhizome/bulb) were collected from field, then they were washed with distilled water, air dried and crushed in 100 mL of sterile water. The crushed product was filtered by using muslin cloth. The culture filtrate (100%) was further diluted to required concentrations of 5, 10 and 15 per cent. Potato dextrose agar was used as nutrient medium and required quantity of each botanical extract was added separately to get a required concentration of the plant extract. The botanical extract were thoroughly mixed with PDA medium and sterilized. About 20 ml of poisoned medium was poured to each of the 90 mm Petri dishes and allowed for solidification. The actively growing periphery of the twelve day old culture of *A. solani* was carefully cut using a cork borer and transferred aseptically to the centre of each Petri dish containing the poisoned solid medium. Suitable control was maintained by growing the cultures on PDA without the plant extract. The plates were incubated at 25±1 °C for twelve days and the colony diameter was recorded.

Trichoderma spp.

Trichoderma spp., are free-living fungi that are common in soil and root ecosystems. They are highly interactive in root, soil and foliar environments. They produce or release a variety of compounds that induce localized or systemic resistance responses in plants. This fungal biocontrol agent has long been recognized as biological agents, for the control of plant disease and for their ability to increase root growth and development, crop productivity, resistance to abiotic stresses and uptake and use of nutrients. It can be efficiently used as spores (especially, conidia), which are more tolerant to adverse environmental conditions during product formulation and field use, in contrast to their mycelial and chlamydospore forms as microbial propagules (Amsellem *et al*., 1999) [9]. However, the presence of a mycelial mass is also a key component for the production of antagonistic metabolites (Benhamou and Chet, 1993; Yedidia *et al*., 2000) [10, 11].

Pseudomonas spp.

Pseudomonas spp. are particularly suitable for application as agricultural bio-control agents since they can use many exudates compounds as a nutrient source (Lugtenberg *et al*., 1999) [12]; abundantly present in natural soils, particularly on plant root systems, high growth rate, possess diverse mechanisms of actions towards phytopathogens including the production of a wide range of antagonistic metabolites, easy to grow *in vitro* and subsequently can be reintroduced into the rhizosphere (Rhodes and Powell, 1994) [13] and capable of inducing a systemic resistance to pathogens (van Loon *et al*., 1998) [14].

Table 1: Treatments details

No.	Botanical name	Common name	Plant part used
1.	*Azadirachta indica Juss*	Neem	Leaf
2.	*Zingiber officinale*	Ginger	Rhizome
3.	*Allium sativum* L	Garlic	Bulb
4.	*Allium cepa*	Onion	Bulb
5.	*Dhatura stramonium* L	Datura	Leaf
6.	*Ociumum tenuiflorum*	Tulsi	Leaf

Table 2: Description of disease scale (0-5) (Mayee and Datar, 1986) [5]

Scale	Description
0	No symptoms on the leaf
1	0-5 per cent leaf area infected and covered by spot, no spot on petiole and branches
2	6-20 per cent leaf area infected and covered by spot, some spots on petiole
3	21-40 per cent leaf area infected and covered by spot, spots also seen on petiole, branches
4	41-70 per cent leaf area infected and covered by spot, spots also seen on petiole, braches, stem
5	>71 per cent leaf area infected and covered by spot, spots also seen on petiole, branch, stem and fruits

Per cent disease index (PDI) was calculated by using following formula proposed by Wheeler (1969) [2].

$$\text{PDI}= \frac{\text{Sum of the individual disease ratings}}{\text{Number of fruits/ leaves observed}} \times \frac{100}{\text{Maximum disease grade}}$$

The fruit yield in each plot was recorded. The per cent yield increase over untreated control was calculated as

$$\text{Yield over control} = \frac{\text{Yield in treatment} - \text{Yield in control}}{\text{Yield in control}}$$

Evaluation of botanicals, bio-agents and fungicides against *Alternaria solani* of tomato under Net house condition

Totally four sprays were given at an interval of 15 days starting from 15 days after transplantation. The per cent disease index (PDI), number of branches per plant and length of plant were recorded at 15 days after the final spray. Number of fruits, total fruit weight and fruit yield were recorded after each harvest. The results are presented in the table 3.

All the treated plots recorded significantly less per cent disease index over control at 90 days after sowing. Maximum per cent disease index with was recorded in the control (untreated). The treatment treated with mancozeb (PDI= 10.71%) was found the most effective on early blight.

Result and discussion

Effect of BAU-fungicides and botanicals on germination per cent of tomato

Among the bio-agents and botanicals used the maximum germination per cent was recorded in T_5-*Pseudomonas fluorescens* (75.20) as compared to treated and untreated control (88.04 and 51.22, respectively). The second best treatment was T_4- *Trichoderma harzianum* (72.57), which was followed by T_3- *T. viride* (68.27), T_1 – Dhatura leaf extract (65.45) and T_2 Neem leaf extract (63.02) as compared to T_7 - untreated control (51.22). Among the treatments most effective was T_5- *Pseudomonas fluorescens* (75.20) respectively.

Table 3: Effect of seed treatment with *T. harzianum & P. fluorescens (alone and in combination)* on the biometrics of tomato crop (Net house conditions)

Treatment symbols	Germination (%)	Shoot length (cm)	Root length (cm)	Vigour Index
T_1	65.45	72.87	18.00	5947.44
T_2	63.02	70.53	16.00	5453.12
T_3	68.27	77.70	22.33	6829.04
T_4	72.57	82.30	23.00	7641.62
T_5	75.20	89.40	23.67	8502.86
T_5	88.04	99.53	29.67	11372.13
T_7	51.22	57.23	15.00	3699.62
	0.82	5.56	3.00	-
	1.75	11.92	6.46	-

Effect of bio-fungicides and botanicals Shoot length and root length on tomato crop

The result presented in Table 3 revealed that all the treatments were statistically significant and increased plant height as compared to treated and untreated control. Among the bio-agents and botanicals used the maximum shoot length (cm) was recorded in T_5-*Pseudomonas fluorescens* (89.40) as compared to treated and untreated control (99.53 and 57.23, respectively). The second best treatment T_4- *Trichoderma harzianum* (82.30), which was followed by T_3- *T. viride* (77.70), T_1 – Dhatura leaf extract (72.87) and T_{12} – Neem leaf extract (70.53) as compared to T_7 - control (57.23).

The result presented in Table 2 revealed that all the treatments were statistically significant and increased root length as compared to treated and untreated control. Maximum root length (cm) was recorded in T_5-*Pseudomonas fluorescens* (23.67) as compared to treated and untreated control (29.67 and 15.00, respectively). The second best treatment T_4- *Trichoderma harzianum* (23.00), which was followed by T_3- *T. viride* (22.33), T_1 – Dhatura leaf extract (18.00) and T_2 – Neem leaf extract (18.00) respectively.

Table 4: Effect of different treatments on disease control of tomato in net house conditions

Tr. No.	Treatment details	Yield (g/pot)	Disease severity per cent
T_1	Dhatura (Leaf extract)	690.00	32.67
T_2	Neem (Leaf extract)	646.67	36.67
T_3	*T. viride* (2% Seedling treatment)	780.00	31.33
T_4	*T. harzianum* (2% Seedling treatment)	970.00	24.67
T_5	*P. fluorescens* (2% Seedling treatment)	860.00	25.33
T_5	Mancozeb (0.2% Spray)	1045.00	20.33
T_7	Control (Untreated)	565.00	40.33
	SEd	28.27	1.09
	CD@5%	61.59	2.15

The results showed that the minimum early blight incidence, per cent early infected leaves per plant and per cent leaf area diseased per plant were recorded in T5- *Trichoderma harzianum* (24.67) as compared to treated and untreated control (20.33 and 40.33 % respectively). The second best treatment T_4 *Pseudomonas fluorescens* - (25.33 %), which was followed by T_3- *T. viride* (31.33 %), T_1 – Dhatura leaf extract (32.67) and T_2 – Neem leaf extract (36.67 %) respectively (Table 4). Similar result was found Mahapatra and Swain (2013) reported (82.82 %) disease control by spraying with *T. viride* (78.24 %) and disease control by spraying with *P. fluoroscens* against pod rot of ground nut.

Among the bio-agents and botanicals used the maximum yield was recorded in (gm.) T_5- *Trichoderma harzianum* (970.00) as compared to treated and untreated control (1045.00 and 565.00, respectively). The second best treatment was T_4- *Pseudomonas fluorescens* (860.00), which was followed by T_3- *T. viride* (780.00), T_1 – Dhatura leaf extract (690.00) and T_2 Neem leaf extract (646.67) as compared to T_7 - untreated control (565.00). Among the treatments most effective was T_5-*Pseudomonas fluorescens* (970.00) respectively. Foliar spraying with *Trichoderma viride* and *T. harzianum* were also successfully demonstrated to manage *Alternaria porri*, and increase of yield causing purple blotch of onion by Mishra and Gupta (2008) [8].

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