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PLANT PATHOLOGY

# Evaluation of suitable antagonists in the management of early blight of tomato cultivar CO-3

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**ABSTRACT**

Early blight of tomato is one of the most destructive diseases caused by *Alternaria solani* causing considerable loss to quality and quantity of fruits. To avoid chemical fungicides in the management of this menace, soil borne rhizospheric organisms were isolated and evaluated against the pathogen. Different antagonists i.e *Aspergillus* sp. *Trichoderma* sp. *Pseudomonas* sp. and *Bacillus* sp. were used against most virulent isolates of *A. solani* under *in vitro* and *in vivo* conditions. Maximum *in vitro* inhibition of mycelial growth of *Alternaria solani* was observed in case of *Trichoderma* sp. (87.69%) followed by *Aspergillus* sp. (84.23%) as compared to control. Under glass house conditions *Trichoderma* sp. exhibited a similar efficacy with a percent disease control ranging from 82.6-91.3.

**Highlights**

- Six bio-antagonists T-8, T-17, F-4, T-2, PS-4 and BAS-2 were antagonistic to tomato early blight pathogen.
- T-8 recorded the highest inhibition under *in vitro* conditions as well as in glass house conditions

**Keywords:** *Alternaria solani*, screening, evaluation, antagonists

Tomato (*Solanum lycopersicum*) is one of the most important solanaceous vegetable crops grown worldwide (Maria *et al.,* 2014). The acid sweet taste and unique flavor account for its popularity and diverse usage. Due to its high per capita consumption, tomatoes are nutritionally valuable for their high pro vitamin A and vitamin C. Besides being an integral part of Indian cuisine, tomatoes hold a pivotal position in the food processing industries. "Lycopene" produced only by tomato is a natural antioxidant that works effectively to slow the growth of cancerous cells (Bhowmik *et al.,* 2012). The total production of tomato in the country is 168.2 lakhs tonnes with a productivity of 19.5MT/ Ha (Anon. 2011). Diseases are one of the most limiting factors for the production of tomato. The early blight disease caused by *Alternaria solani* causes yield loss of tomato to a great extent (Derbalah *et al.,* 2011). Under favourable conditions more than 80% disease severity of early blight was recorded in tomato (Pandey *et al.,* 2002). Irregular spots appear in scattered form with brown conspicuous concentric rings and often with

chlorotic halo on the lower leaves giving a typical target board appearance (Vloutoglou and Kalogerakis 2000. It occasionally attacks the fruit, producing large sunken black spots at the stem end,which drop prematurely leading to a severe yield loss. Constant use of chemical fungicides like mancozeb and chlorothalonil have given good control of the disease but indiscriminate use of the same have resulted in pesticide residue in the fruits and environmental hazards (López-Pérez *et al.,* 2006). Soil inhabiting microbes co-existing in the same niche as the pathogens, are potential tools for controlling the pathogens (Loganathan *et al.,* 2014) and they possess properties of plant growth promotion (Lahan *et al.*, 2012, Ratul *et al* 2013) as well. In the present investigation, several rhizospheric organisms have been isolated and their efficacy against early blight pathogen was studied both *in vitro* as well as in glasshouse conditions. *Trichoderma* sp, *Pseudomonas* sp., *Aspergillus* sp. and *Bacillus* sp proved to be antagonistic to the pathogen *Alternaria solani*. Based on this performance suitable formulations of these microbes, both singly and in consortium, will be developed which will drastically reduce the chemical load of the environment and will be a safe weapon in the arsenal of farmers to ward off this obnoxious pathogen in the near future.

## Materials and Methods

### Collection, isolation and purification of *A.solani* and bio control agents

Tomato growing areas of different agro-climatic zones and adjoining areas were surveyed and tomato plant parts showing typical symptoms of *Alternaria* blight were collected and isolated on potato dextrose agar medium (PDA) using standard procedure (Kumara 2006).

Several rhizosperic microorganisms and endophytes were isolated from the rhizospheric soil samples as well as phylloplane of solanaceous vegetables *viz*. tomato, chilli and brinjal (Vieira *et al.,* 2004) using dilution plate technique on PDA, nutrient agar (NA) and selective media for *Trichoderma, Pseudomonas* and *Bacillus* respectively (Elad *et al*., 1981).

### Pathogenicity test

All the collected isolates of *Alternaria solani* were tested for pathogenicity on susceptible tomato cultivar CO-3 by foliar spray method to assess their virulence under greenhouse conditions. Ten days old cultures of different isolates were taken and ground in 50 ml of distilled water using pestle and mortar and filtered through four layers of muslin cloth in conical flasks. Separate flasks were used for individual isolates and sterile condition was maintained throughout the experiment. The culture suspension maintained approximately at 125 cfu/ ml was sprayed on the leaves and stem of tomato plants. In control treatments the plants were sprayed only with distilled water. Five replications for each treatment were maintained. Inoculated plants were kept in manually fabricated moist polythene chamber in a glass house for 3 days. For maintaining the humidity, a humidifier was set up in the chamber, and the temperature was maintained at 30±1°C for the development of the symptoms. Observations were made at regular intervals.The pathogenicity test as above was repeated twice to confirm the results. Disease intensity was recorded as per 0-5 the scale (Pandey *et al.,* 2002). where, 0 = no infection,1= < 10%- surface area covering leaf, stem, and fruit infected by early blight, 2= 11-25%- foliage of plant covered with a few scattered spots, 3=26-50%- many spot coalesced on the leaves, covering surface area of plant, 4 = 51-75% area of the plants infected, fruit also infected at apical end, defoliation and blightening started. Sunken lesions with prominent concentric rings on stem, petioles, and fruit, 5= < 75% area of plant part blighted, severe lesion on stem, and fruit rotting on apical end.

Percent disease incidence was calculated following Wheeler (1969) where,

PDI= (Sum of numerical rating/Number of samples observed X maximum rating in scale) x 100 and based on the reaction i.e., PDI value (mean of five replications), the isolates can be placed into different categories (0-10%-Weakly virulent, 11-29%-Moderately virulent, 30-49%- Virulent, > 50%- Highly virulent).

### *In vitro* screening of rhizospheric microorganisms and endophytes against *Alternaria solani*

The rhizospheric microorganisms and endophytes isolated from the rhizospheric region and phylloplane respectively of infected plants were used against highly virulent isolate of *A. solani* selected through pathogenicity test. Fourteen days old *A. solani* isolate as well as the rhizospheric microorganisms and endophytes were considered for dual culture study following Sumana *et al.,* (2012). Inoculation was done by placing a disc of 5mm each of both, at the two opposite extremes on Petri plates (90 mm.) and a Petriplate comprising only of the pathogen (*A. solani*) was also maintained as a control. The plates were incubated at 28ºC±2ºc for six days. Three replications for each treatment were maintained. Percent inhibition of mycelial growth for *Alternaria* (figures in parenthesis in Table 2 indicates percent control) isolate against each antagonist as compared to pathogen control was calculated followingVincent (1947).

I=C-T/Cx100

Where, I= Per cent inhibition of the mycelial growth, C= mycelial growth in control, T= mycelial growth in treatment.

### Greenhouse evaluation of efficient antagonists against *A. solani*

Based on *in vitro* screening of rhizospheric microorganisms and endophytes against Alternaria solani, six (T-2, T-8, T-17, F-4, Ps-4 and BAS-2) efficient antagonists were evaluated for their efficacy as biocontrol against Alternaria blight under greenhouse conditions. Susceptible tomato variety CO-3 (obtained from the Seed Production Unit, Crop Improvement Division, IIVR, Varanasi) was transplanted in pots containing sterilized soil and sand mixture. Five replications for each treatment were maintained. Culture suspension of the selected antagonists was sprayed over the plants, each having a concentration of 125 cfu/ml. After 72 hours culture suspension of highly virulent isolate of A. solani (ALT-4), having concentration of approximately 125 cfu/ml was sprayed over the plants PDI was assessed 15, 30 and 45 days after, treatment following Wheeler (1969) and percent disease control was calculated using the following the formula of Kishore *et al.,* (2005)

C-T/C × 100, where C = disease developed in control, and T = disease developed in treatment

## Result and Discussion

### *Collection, isolation and purification of pathogen and bio control agents*

Fourteen isolates of *A.solani* were collected from different parts of India, isolated and purified and designated as ALT1, ALT2, ALT3, ALT4, ALT5, ALT6, ALT7, ALT8, ALT9, ALT10, ALT11, ALT12, ALT13, and ALT 14. Among the several rhizospheric microorganisms and endophytes collected, thirty nine were isolated from the rhizosphere and phyllosphere of solanaceous crops. Out of these, 28 were fungi and 11 were bacteria. Morphological identification of these microbes revealed that 18 were *Trichoderma* species (T1,T2,T3,T4,T5,T6,T7, T8,T9,T10,T11,T12,T13,T14,T15,T16,T17,T18), 10 were *Aspergillus* species (F1, F2, F3, F4, F5, F6, F7, F8, F9, F10), 6 were *Pseudomonas* species, (PS1, PS2, PS3, PS4, PS5, PS6), and 5 were *Bacillus* species (BAS1, BAS2, BAS3, BAS4 and BAS5).

### Pathogenicity test

On the basis of pathogenicity test and degree of virulence of isolates were assessed and it was observed that, out of 14 isolates of *Alternaria solani*, 6 isolates (ALT-2, ALT-4, ALT-7, ALT-8, ALT-12 and ALT-14) were found highly virulent, 7 isolates (ALT-1, ALT-3, ALT-5, ALT-6, ALT-10, ALT-11 and ALT-13) were found to be virulent and 1 isolate (ALT-9) was moderately virulent (Tab 1). Among the highly virulent isolates, ALT4 had the maximum PDI; hence, it was used in further experiments, *viz. in vitro* screening and green house experiments.

**Table 1. Effect of different *A.solani* isolates on pathogenicity reaction on tomato (cv. CO-3)**

| S. No. | Isolate code | Place of collection | Percent disease incidence | Reactions |
|---|---|---|---|---|
| 1 | ALT-1 | Narottampur, Varanasi-U.P. | 33.33 | V |
| 2 | ALT-2 | Lohata, Varanasi-U.P. | 53.28 | HV |
| 3 | ALT-3 | Bachchaon, Varanasi-U.P. | 46.63 | V |
| 4 | ALT-4 | IIVR, campus, Varanasi-U.P. | 76.67 | HV |
| 5 | ALT-5 | Kailhat, Mirzapur-U.P. | 33.33 | V |
| 6 | ALT-6 | Narayanpur,.Mirzapur-U.P. | 46.63 | V |
| 7 | ALT-7 | Dhaula, Solan-H.P. | 66.66 | HV |
| 8 | ALT-8 | Solan, H.P. | 53.28 | HV |
| 9 | ALT-9 | Hissar, Haryana | 13.33 | MV |
| 10 | ALT-10 | Patran, Patiala-Punjab | 33.33 | V |
| 11 | ALT-11 | Haidergharh, Ludhiana, Punjab | 46.63 | V |
| 12 | ALT-12 | IGKVV, Campus, Raipur- C.G. | 53.28 | HV |
| 13 | ALT-13 | UAS, Dharwadh-Karnataka | 46.63 | V |
| 14 | ALT-14 | UAS, Raichur-Karnataka | 66.66 | HV |

# V: Virulent; HV: Highly virulent; MV: Moderatly virulent

**Table 2 *In vitro* screening of bio-agents against *Alternaria solani***

| S. No. | Bio-agent | Radial growth of pathogen in mm |
|---|---|---|
| 1 | F-1 | 48.00 (29.06) |
| 2 | F-2 | 50.00 (26.11) |
| 3 | F-3 | 48.33 (28.57) |
| 4 | F-4 | 10.67 (84.23) |
| 5 | F-5 | 38.33 (43.35) |
| 6 | F-6 | 28.33 (58.13) |
| 7 | F-7 | 41.33 (38.92) |
| 8 | F-8 | 34.33 (49.26) |
| 9 | F-9 | 29.33 (56.65) |
| 10 | F-10 | 30.00 (55.66) |
| 11 | T-1 | 39.00 (42.36) |
| 12 | T-2 | 11.33 (83.25) |
| 13 | T-3 | 50.00 (26.11) |
| 14 | T-4 | 28.33 (58.13) |
| 15 | T-5 | 28.67 (57.63) |
| 16 | T-6 | 33.00 (51.23) |
| 17 | T-7 | 31.33 (53.70) |
| 18 | T-8 | 8.33 (87.69) |
| 19 | T-9 | 20.00 (70.44) |
| 20 | T-10 | 32.33 (52.22) |

| | | |
|---|---|---|
| 21 | T-11 | 28.67 (57.63) |
| 22 | T-12 | 40.00 (40.88) |
| 23 | T-13 | 30.33 (55.17) |
| 24 | T-14 | 20.67 (69.45) |
| 25 | T-15 | 33.67 (50.24) |
| 26 | T-16 | 30.67 (54.67) |
| 27 | T-17 | 10.67 (84.23) |
| 28 | T-18 | 44.33 (34.49) |
| 29 | PS-1 | 28.00 (58.62) |
| 30 | PS-2 | 30.00 (55.66) |
| 31 | PS-3 | 44.67 (33.98) |
| 32 | PS-4 | 14.33 (78.82) |
| 33 | PS-5 | 48.00 (29.06) |
| 34 | PS-6 | 32.33 (52.22) |
| 44 | BaS-1 | 28.00 (58.62) |
| 45 | BaS-2 | 20.00 (70.44) |
| 46 | BaS-3 | 29.67 (56.15) |
| 47 | BaS-4 | 39.33 (41.87) |
| 48 | BaS-5 | 28.33 (58.13) |
| 49 | Control | 67.67 (0.00) |
| | SEM± | 0.30 |
| | CD @ 5% | 0.85 |

Greenhouse evaluation of antagonists against A. solani

### *In vitro* screening of bio-agents against *Alternaria solani*

*In vitro* results indicated that that among 39 cultures used, 6 antagonists manifested vigorous reaction against *A. solani*. T-8 recorded the highest inhibition i.e. 87.69 % against ALT-4 followed by T-17 (84.23 %), F-4 (84.23), T-2 (83.25), PS-4 (78.82%) and BAS-2 (70.44 %) respectively. The inhibitory effect of isolates is possibly due to the production of cell wall degrading enzymes, antibiotics and competition (Schneider and Ullrich 1994, Sharma and Dureja 2004). This effectiveness may vary due to the nature, quality and quantity of the inhibitory substance secreted by the isolates (Skidmore and Dickinson 1976) (Tab 2).

The effective antagonists *viz.* T-8, T-17, F-4, T-2, PS-4 and BAS-2 were evaluated against *A. solani* under greenhouse conditions. No disease development was observed till 15$^{th}$ day after application except the control pot which exhibited 13.33 percent incidence (Tab 3). After 30$^{th}$ day of application, disease incidence ranged from 6.66 to 46.67% in different treatments but there was no disease development in T-8 treated pots. However, marginal disease development was observed on 45$^{th}$ day in T-8 treated pots which highlights its superiority than other treatments. Thus, T-8 (91.31) was best amongst all the selected antagonists on the basis of percent disease control, followed by T-17 (82.61), F-4 (56.53), T-2 (47.83), PS-4 (47.83) and BS-2 (30.44) when compared to pathogen control after 45 days of application.

Similar reports regarding the efficacy of *Trichoderma* sp. against *Alternaria* sp. commensurate the above findings (Varma et al., 2008, Mishra et al., 2011). However, for an unequivocal conclusion

regarding the efficacy of T-8 as a biocontrol agent, multilocational field trials need to be done and validated.

**Table 3** *In vivo* **evaluation of selected bio-agents against** *Alternaria solani* **(ALT 4)**

| S. N. | Treatment | PDI* | | | PDC# |
|---|---|---|---|---|---|
| | | 15 DAI | 30 DAI | 45 DAI | 45 DAI |
| 1 | ALT 4 x F-4 | 0.00 | 6.66 | 33.33 | 56.53 |
| 2 | ALT4 x T-2 | 0.00 | 13.33 | 40.00 | 47.83 |
| 3 | ALT4 x T-8 | 0.00 | 0.00 | 6.66 | 91.31 |
| 4 | ALT4 x T-17 | 0.00 | 6.66 | 13.33 | 82.61 |
| 5 | ALT4x PS-4 | 0.00 | 13.33 | 40.00 | 47.83 |
| 6 | ALT4 x BAS-2 | chlorotic | chlorotic | 53.33 | 30.44 |
| 7 | ALT4 | 13.33 | 46.67 | 76.67 | 0.00 |
| 8. | Untreated control | - | 13.33 | 32.69 | |
| | SEM± | - | - | 2.06 | - |
| | CD @ 5% | - | - | 6.24 | - |

## Conclusion

Antagonistic efficacy of different selected phylloplane and rhizospheric cultures from solanaceous crops were examined on different pathogenic isolates of *A. solani* and experimented their potential efficacy in controlling the pathogen under lab as well as greenhouse conditions. *Trichoderma* sp. gave the best control of the pathogen in both the conditions and forms the cornerstone for designing formulations of these antagonists to control the disease.

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## References

Anon. (2011) Indian Horticulture Database, Pub: National Horticultural Board Ministry of Agriculture, GOI, pp296.

Bhowmik D, Kumar KPS, Paswan S, Srivastava S (2012) Tomato-A Natural Medicine and Its Health Benefits. *Journal of Pharmacognosy and Phytochemistry* **1**(1) Pp. 33

Derbalah AS, El-Mahrouk MS, El-Sayed AB (2011) Efficacy and Safety of Some Plant Extracts against Tomato Early Blight Disease Caused by Alternaria solani. *Plant Pathology* **10**: 115-121.

Elad Y, Chet I, Henis Y (1981) A selective medium for improving quantitative isolation of Trichoderma spp. from soil. *Phytoparasitica* **9**(1):59-67.

Halfeld-Vieira BA, Romeiro RS, Mizubuti ESG (2004) Comparação entre diferentesmétodos de isolamento de bactérias do filoplano de tomateirovisandopopulaçõesespecíficas e implicações nasuaeficiênciacomoagentes de biocontrole. *FitopatologiaBrasileira, Fortaleza* **29**(6): 638-643.

Kishore GK, Pande S, Podile AR (2005) Biological control of collar rot disease with broad-spectrum antifungal bacteria associated with groundnut. *Canadian Journal of Microbiology* **51**: 123–132.

Kumara KT (2006) Studies on Alternaria solani (Ellis and Martin) Jones and Grout causing Early Blight of Tomato. Thesis Ph.D. University of Agricultural Sciences, Dharwad, Karanataka, India.

Loganathan M, Garg R, Venkataravanappa V, Saha S, Rai AB (2014) Plant growth promoting rhizobacteria (PGPR) induces resistance against Fusarium wilt and improves lycopene content and texture in tomato. *African Journal of Microbiology Research* **8**(11): 1105-1111.

Lahan JP, Kalita R, Sudipta BS, Barooah M (2012) Screening of Potential Oleaginous Microalgae from Dhemaji District of Assam, India. *International Journal of Agriculture, Environment and Biotechnology* **5**:141-144.

López-Pérez GC, Arias-Estévez M, López-Periago E, Soto-Gonzalez B, Cancho-Grande B, Simal-Gandara J (2006) Dynamics of pesticides in potato crops. *Journal of Agriculture Food Chemistry* **54**: 1797-1803.

Mishra BK, Mishra RK, Mishra RC, Tiwari AK, Yadav RS, Dishit A (2011) Biocontrol efficacy of Trichoderma viride isolates against fungal pathogens causing disease in Vigna radiata L. *Archives of Applied Science Research* **3**(2): 361-369.

Pandey KK, Pandey PK, Satpathy S (2002) Integrated management of disease and insects of tomato, chilli and cole crops. Technical bulletin -9.pub: IIVR and DBT, MoS&T, GOI Pp. 22.

Maria R, Guzman MGD, Amanda MW, Frusciante L, Barone A (2013) Production of Pharmaceutical Proteins in Solanaceae Food Crops. *International Journal of Molecular Science* **14**: 2753-2773.

Nath R, Sharma GD, Barooah M (2013) Screening of endophytic bacterial isolates of Tea (*Camellia sinensis* L.) roots for their multiple plant growth promoting activities. *International*

*Journal of Agriculture, Environment and Biotechnology* **6**(2): 211-215.

Schneider S, Ullrich WR (1994) Differential induction of resistance and enhanced enzyme activities in cucumber and tobacco caused by treatment with various abiotic and biotic inducers. *Physiology Molecular Plant Pathology* **45**: 291-304.

Sharma P and Dureja P (2004) Evaluation of *Trichoderma harzianum* and *Trichoderma viride* isolates at BCA pathogen crop interface. *Journal of Mycology and Plant Pathology* **34**: 47-55.

Skidmore AM and Dickinson CH (1976) Colony interactions and hyphal interference between Septorianodorum and phyloplane fungi. *Transactions of British Mycological Society* **66**: 57-64.

Sumana K, Radhakrisnan S, Srinivasan SS, Devaki NS (2012) Field evaluation of promising fungicides and bioagents against Fusarium wilt and root knot complex disease in FCV Tobacco crop. *Journal of Agricultural technology* **8**(3): 983-991.

Vincent JM (1947) Distortion of fungal hyphae in the presence of certain inhibitors. *Nature* **159**:850.

Varma PK, Gandhi SK, Singh S (2008) Biological control of Alternaria solani, the causal agent of early blight of tomato. *Journal of Biological Control* **22**(1): 67-72.

Vloutoglou I, Kalogerakis SN (2000) Effects of inoculum concentration, wetness duration and plant age on development of early blight (Alternaria solani) and on shedding of leaves in tomato plants. *Plant Pathology* **49**: 339-345.

Wheeler BEJ (1969) An Introduction to Plant Diseases. John Wiley and Sons. Ltd., London.