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Biocontrol agents against early blight (*Alternaria solani*) of tomato

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Abstract

Biological control agents are the best alternative to the hazardous chemical fungicides and other agro chemicals. Various biological control agents viz., *Trichoderma viride*, *Pseudomonas fluorescens* and *Bacillus subtilis* were evaluated against *Alternaria solani* (Ellis and Martin) Jones and Grout causing early blight of tomato resulting in severe yield loss. All treatments showed the varied antifungal activity against the pathogen. Among the *in vitro* screened biological control agents percent growth inhibition of pathogen was higher in *Trichoderma viride* (77%). Plant biometric observations viz., seed germination (89%) and seedling vigor (1994) was maximum with lower percent disease index (16%) was recorded in *Trichoderma viride* treated seeds and seedlings of tomato cultivar PKM 1.

Keywords: Biological control agents, *Trichoderma viride*, *Pseudomonas fluorescens*, *Bacillus subtilis*

Introduction

Tomato (*Solanum lycopersicum* Mill.) is one of the most important vegetables. The fungus *Alternaria solani* (Ellis and Martin) Jones and Grout, the causal agent of early blight disease, is a major pathogen of tomato causing considerable yield losses all over the world. Control of early blight disease using chemical fungicides has become increasingly difficult due to the limited number of effective fungicides, some of which were recently withdrawn from the market due to the environmental and human health concerns. Moreover, the use of fungicides during fruiting is discouraged due to their residual nature, Thus search for alternative control methods is quite important and necessary. Although the use of resistant varieties which may be considered as a good alternative but there is lack of suitable resistant tomato germplasm against *A. solani*. (Chowdappa *et al.*, 2013 and Moore *et al.*, 2006) ^[5 13]

The initial symptom of early blight is small dark brown spots on the lowest and oldest leaves. The tissue around the primary lesions may turn bright yellow and if lesions are numerous, the entire leaves may become necrotic and chlorotic. The spots get enlarged and develop into concentric rings which appears like bull's eye. In favourable weather conditions, disease develop, lesions can become numerous and plants defoliate, which damage the quantity and quality of tomato fruits (Kouyoumjian, 2007) ^[11]. The disease had resulted 78% loss in yield of fruit (Saad *et al.*, 2014) ^[15]. *A. solani* can infect every part of the plant (causing foliage blight, fruit lesions and stem collar rot) and can damage during all stages of plant development (Abada *et al.*, 2008) ^[1]. So management of this disease is very necessary. Use of resistant varieties is the ultimate control of this disease. Wide use of chemical fungicides often leads to serious environmental problems besides affecting the health of ecosystem. So, it is necessary to minimize the use of chemicals for controlling disease.

Hence, the attempt has been made to evaluate at both *in vitro* and *in vitro* conditions of some bio agents against *Alternaria solani*, as it will be use full in formulating eco-friendly management strategies for early blight of tomato.

Materials and Methods

The pathogen was isolated and pathogenicity was proved. Later the biocontrol agents efficacy at both *in vitro* and *in vitro* conditions in the seeds and seedlings of tomato cultivar PKM 1 was studied by following below methodologies.

Isolation of pathogen and 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 by pre-inoculation with spore suspension and homogenized mycelial bits of *A. solani* on foliage of 30 days old plants of PKM 1 cultivar of tomato. After 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.

In vitro efficacy of bio-control agents on mycelial growth of *Alternaria solani*

Bio-efficacy of biological control agents viz., *Trichoderma viride*, *Pseudomonas fluorescens* and *Bacillus subtilis* were evaluated *in vitro*, against *Alternaria solani*. The antagonistic activity of these bio-control agents were studied on *A. solani* by dual culture technique (Cherif and Benhamou, 1990) [4]. On Petri dishes with PDA and placing equidistantly a disk (5 mm in diameter) with mycelium of the pathogen and on the other side of petri dish, a disk of the mycelium of the same diameter of bio-control agents. The inoculated plates were incubated at 25± 1 °C until the growth of control treatment (with only plant pathogen disk) covered the Petri dish.

Table 1: Description of disease scale (Datar and Mayee, 1986) [6]

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

Germination (%)

Seeds were soaked in bio-control agents for 12 h and then shade dried. Four replicates of 100 seeds were uniformly placed on standard germination paper roll-towel medium and kept in germination room maintained at 25± 2 °C and 90 ±2 per cent relative humidity. After 14 days, the seedlings were evaluated as total number of normal seedlings and germination as percentage. (ISTA, 1993) [8]

Root length and Shoot length

On fourteenth day, ten normal seedlings per replication from

roll towel medium were carefully removed at random from each treatment. The root length was measured from the base to the top of the primary root and the shoot length was measured from the base of the shoot to tip of primary leaf and the mean value was calculated and expressed in cm. (ISTA, 1993) [8]

Vigour index

The Vigour index was calculated and compared by adopting the following formula and expressed as whole number. (Abdul-Baki and Anderson, 1973) [2]

$$\text{Percentage of inhibition} = \frac{A - B}{A} \times 100$$

where, A = Growth of the pathogen in the absence of the bio-control agent.

B = Growth of the pathogen in the presence of the bio-control agent.

In vitro efficacy of bio-control agents on *Alternaria solani*

Seeds were treated with bio-control agents for 12 h and dried back to original moisture content. Pin prick method of *Alternaria solani* inoculation was followed. The leaves were injured with sterilized pin and the mycelial disc of pathogen was placed over the injured leaf portion and covered with moist cotton and incubated in the moist chamber. The plants were sprayed frequently with water for 2 days. After 48 h, the plants were sprayed with different bio-control agents. The plant biometric observations viz., Germination%, Root length, Shoot length, Vigour index was recorded and the per cent disease index (PDI) was calculated by using following formula proposed by Wheeler (1969) [19].

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

Table 2: *In vitro* efficacy of bio-control agents on mycelial growth of *Alternaria solani*

Biocontrol Agent	Average Colony diameter (mm)	Per cent Growth Inhibition (%)
<i>Trichoderma viride</i>	20.32	77.42
<i>Pseudomonas fluorescens</i>	31.13	65.41
<i>Bacillus subtilis</i>	44.25	50.83
Control	90.00	—
Mean	46.43	64.56
SEd	0.94	1.21
C D (P = 0.05)	2.17	2.79

*Values based on five replications

Results and discussion

In vitro efficacy of bio-control agents on mycelial growth of *Alternaria solani*

All the bio-control agents viz., *Trichoderma viride*, *Pseudomonas fluorescens* and *Bacillus subtilis* showed significantly different anti-fungal activity against the

pathogen. Among the various bio-control agents, *Trichoderma viride* recorded the minimum mycelial growth of the pathogen (20.32 mm) and exhibited the maximum percent growth inhibition of pathogen (77.42%) and it was followed by *Pseudomonas fluorescens* and *Bacillus subtilis*. Bio-agents possess antagonistic effects against pathogen

(Muriungi *et al.*, 2013) [14]. Similar results were obtained by Ganeshan and Kumar, 2005 [7]; Kaur *et al.*, 2016 [9, 10]; Verma *et al.*, 2008 [18]. (Table 2)

Table 3: *In vitro* efficacy of bio-control agents on *Alternaria solani* on the biometrics of tomato cultivar PKM 1

Biocontrol Agent	Germination (%)	Shoot length (cm)	Root length (cm)	Vigour Index	Percent Disease Index (%)
<i>Trichoderma viride</i>	89	7.1	15.3	1994	15.87
<i>Pseudomonas fluorescens</i>	82	6.7	14.1	1706	21.55
<i>Bacillus subtilis</i>	76	6.5	13.7	1535	33.87
Control	61	6.1	12.9	1159	70.23
Mean	77	6.6	14.0	1598	35.38
SEd	1.41	0.11	0.23	31.61	0.92
C D (P = 0.05)	3.25	0.26	0.52	72.89	2.12

*Values based on five replications

In vitro* efficacy of bio-control agents on *Alternaria solani

In vitro efficacy of bio-control agents *viz.*, *Trichoderma viride*, *Pseudomonas fluorescens* and *Bacillus subtilis* showed significantly different anti-fungal activity against the pathogen on seeds and seedlings of tomato cultivar PKM 1. Among the various bio-control agents, *Trichoderma viride* recorded the maximum seed germination (89%), shoot length (7.1 cm), root length (15.3 cm), vigour index (1994) and also exhibited the lowest percent disease incidence (15.87%) and it was followed by *Pseudomonas fluorescens* and *Bacillus subtilis*. Control treatment recorded the minimum seed germination (61%) and other biometrics with highest percent disease incidence (70.23%). Bio-control agents seed treatment or foliar application induces systemic resistance in tomato against early blight (Babu *et al.*, 2000; Latha *et al.*, 2009; Vanitha *et al.*, 2009 and Kaur *et al.*, 2016) [3, 12, 17, 9, 10]. (Table 3)

Conclusion

The present investigation revealed that bio-control agents *viz.*, *Trichoderma viride*, *Pseudomonas fluorescens* and *Bacillus subtilis* had inhibitory effect against *Alternaria solani*. Hence these bio-control agents could be exploited as an alternate management strategy for chemical pesticides in the management of early blight of tomato.

References

- Abada KA, Mostafa SH, Mervat R. Effect of some chemical salts on suppressing the infection by early blight disease of tomato. *Egyptian J Appl. Sci* 2008;23:47-58.
- Abdul-Baki AA, Anderson JD. Vigour determination in soybean seed by multiple criteria. *Crop Sci* 1973;13:630-633.
- Babu S, Seetharaman K, Nandakumar R, Johnson I. Biocontrol efficacy of *Pseudomonas fluorescens* against *Alternaria solani* and tomato leaf blight disease. *Annals of Plant Protection Sciences* 2000;8(2):252-254.
- Cherif M, Benhamou N. Cytochemical aspects of chitin breakdown during the parasitic action of *Trichoderma* spp. on *Fusarium oxysporum* f. sp. radicles-lycopersici. *Phytopathology* 1990;80:1406-1412.
- Chowdappa P, Mohan Kumar SP, Jyothi Lakshmi M, Upreti KK. Growth stimulation and induction of systemic resistance in tomato against early and late blight by *Bacillus subtilis* OTPB1 or *Trichoderma harzianum* OTPB3. *Biological Control* 2013;65(1):109-117.
- Datar VV, Mayee CD. Assessment of loss in tomato yield due to early blight. *Indian Phytopath* 1981;3(4):191-195.
- Ganeshan G, Manoj Kumar A. *Pseudomonas fluorescens*, a potential bacterial antagonist to control plant diseases. *Journal of Plant Interactions* 2005;1(3):123-134.
- ISTA Proceedings of International Seed Test Association, International Rules for Seed Testing. *Seed Sci Technol* 1993;21:1152.
- Kaur R, Joshi N, Virk JS, Sharma S. Evaluation of *Pseudomonas fluorescens* for the management of tomato early blight disease and fruit borer. *Journal of environmental biology* 2016;37(5):869-872.
- Kaur R, Joshi N, Virk JS, Sharma S. Evaluation of *Pseudomonas fluorescens* for the management of tomato early blight disease and fruit borer. *Journal of environmental biology* 2016;37(5):869-872.
- Kouyoumjian RE. Comparison of compost tea and biological fungicides for control of early blight in organic heirloom tomato production. M.Sc. Thesis, Clemson University, South Carolina 2007, 16-37.
- Latha P, Anand T, Ragupathi N, Prakasam V, Samiyappan R. Antimicrobial activity of plant extracts and induction of systemic resistance in tomato plants by mixtures of PGPR strains and Zimmu leaf extract against *Alternaria solani*. *Biological Control* 2009;50(2):85-93.
- Moore ERB, Tindall BJ, Martins Dos Santos VAP, Pieper DH, Ramos JL, Palleroni NJ. Nonmedical: *Pseudomonas*. In M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer, & E. Stackebrandt (Eds.), *The Prokaryotes* Springer New York 2006, 646-703.
- Mueiungi JS, Mutitu EW, Show MG. Biocontrol of *Fusarium* root rot in beans by antagonistic *Trichoderma* fungi. *Int. J. Agri. Science* 2013;3(7):550-557.
- Saad ASA, Kadous EA, Tayeb EH, Massoud MA, Ahmed SM, Abou El- Ela ASA. The inhibitory effect of some antioxidants and fungicides on the growth of *Alternaria solani* and *Fusarium solani* in vitro. *Middle east J Agric. Res* 2014;3(2):123-134.
- Singh G, Saha S, Garg R, Sharma BK, Rai AB, Singh RP. Evaluation of suitable antagonists in the management of early blight of tomato cultivar CO-3. *International Journal of Agriculture, Environment and Biotechnology* 2014;8(1):127.
- Vanitha SC, Niranjana SR, Mortensen CN, Umesha S. Bacterial wilt of tomato in Karnataka and its management by *Pseudomonas fluorescens*. *Bio Control*, 2009;54(5):685-695.
- Verma PK, Gandhi SK, Surender S. Biological control of *Alternaria solani*, the causal agent of early blight of tomato. *Journal of Biological Control* 2008;22(1):67-72.
- Wheeler BEJ. An introduction to Plant disease. John Wiley and Sons Limited, London 1969, 301.