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Biradar Pratiksha D
Department of Plant Pathology, College of Agriculture, Latur, Maharashtra, India

Suryawanshi AP
Department of Plant Pathology, College of Agriculture, Latur, Maharashtra, India

Patait NN
Department of Plant Pathology, College of Agriculture, Latur, Maharashtra, India

Corresponding Author:
Biradar Pratiksha D
Department of Plant Pathology, College of Agriculture, Latur, Maharashtra, India

In vitro bioefficacy of bioagents against *Alternaria solani* and *Colletotrichum capsici*, causing tomato fruit rot

Biradar Pratiksha D, Suryawanshi AP and Patait NN

Abstract
Fruit rot of tomato caused by *Alternaria solani* and *Colletotrichum capsici* is an important diseases of tomato inflicting heavy losses. The present investigation was carried out to test the *in vitro* efficacy of bioagents. In *A. solani*, among the nine bioagents *T. harzianum* resulted with highest mycelial growth inhibition (95.56%), followed by *T. hamatum* (94.08%), *A. niger* (92.59%), *M. anisopliae* (90.00%). Whereas, least inhibition of test fungus was recorded in *B. subtilis* (73.70%). However, in *C. capsici*, *Trichoderma koningii* resulted with highest mycelial growth inhibition (92.48%), followed by *T. harzianum* and *T. hamatum* (92.41%). Whereas, least inhibition of test fungus was recorded in *B. subtilis* (65.03%).

Keywords: *Alternaria solani*, *Colletotrichum capsici*, Bioagents, inhibition

Introduction
Tomato (*Lycopersicon esculantum*) fruits are referred as "Poor Man's Apple", due to their diversified nutritional values and a wide range of processed products. India is second largest producer of tomato next to china. In India, area, production and productivity of tomato during 2018-19 were 781 Lakh ha, 19007 Lakh metric tons and 24.33 Lakh metric tons per ha, respectively. Whereas, in Maharashtra were 40.34 Lakh ha, 805.90 Lakh metric tons and 20.01 Lakh metric tons per ha, respectively (Anonymous, 2019) [1].

Among the fungal diseases, early blight also known as target spot disease incited by *Alternaria solani*, is one of the most catastrophic diseases of the crop in the world as well as in India. The disease becomes wide- spread and serious, causing defoliation, drying off of twigs and premature fruit drop causing 28-57% losses in fruit yield (Naik, 2020) [10] to the growers when the season begins with abundant moisture or frequent rains followed by warm and dry weather which are unfavorable for the host and help in rapid disease development.

Tomato crop is subjected to several diseases caused by fungi, bacteria, viruses, nematodes and abiotic factors (Balanchard, 1992) [4]. Tomato anthracnose was first reported in India on from the Coimbatore of Madras Presidency (Sydow, 1913) [11]. The disease has been identified in all the tomato producing regions of the world and has become a serious constraint to tomato production. Different species of *Colletotrichum*, namely *C. capsici, C. gloeosporioides, C. acutatum* are known to cause anthracnose in tomato in India. Anthracnose disease appears as small circular spots that coalesce to form large elliptical spots on fruits and leaves. Under severe conditions, defoliation of affected plants occurs. This disease is controlled mainly by the application of agrochemicals. Post-harvest decays of fruits and vegetables account for significant levels of post-harvest losses. It has been estimated that about 20-25% of the harvested fruits and vegetables are decayed by pathogens, during post-harvest handling even in developed countries (Droby *et al.*, 2009; Abano and San-Amaoh, 2012) [6].

However, the worldwide trend towards environmentally safe methods of plant disease control in sustainable agriculture calls for reducing the use of these synthetic chemical fungicides. In an attempt to modify this condition, some alternative methods of the management have been adopted. Recent efforts have focused on developing environmentally safe, long- lasting, and effective biocontrol methods for the management of plant diseases. Natural plant products are important sources of new agrochemicals for the control of plant diseases (Kagale *et al.*, 2004) [7].

It is now known that various natural plant products can reduce populations of foliar pathogens and control the disease development, and then these plant extracts have a potential as environmentally safe alternatives and as components in integrated disease management programs. Not much light has been shed on the biological control, use of botanicals and essential oils which are effective against *C. capsici*. Hence, an attempt has been made to test some of the commonly available bio-agents, botanicals and essential oils against the pathogen *in vitro* condition.

Materials and Methods

The experiment was conducted during winter, 2020 at Department of Plant Pathology, College of Agriculture, Latur, during present investigations on *in vitro* evaluation of bioagents against *A. solani* and *C. capsici*, causing tomato fruit rots.

Most potential fungal and bacterial biocontrol agents *viz*., *Trichoderma asperellum*, *T. koningii*, *T. hamatum*, *T. harzianum*, *Aspergillus niger*, *Metarhizium anisopliae*, *Verticillium lecanii*, *Bacillus subtilis* and *Pseudomonas fluorescens* were evaluated *in vitro* against the test fungi (*A. solani*), by applying Dual Culture Technique (Dennis and Webter, 1971) [5], by using PDA as basal culture media. Seven days old cultures of the test bioagents and test fungi grown on respective culture media were used for the study. One each 5 mm culture disc of the test fungus and the test fungal bioagents cut out with sterilized cork borer was placed at equidistance and exactly opposite to each other on autoclaved and solidified PDA medium in Petri plates. For bacterial biocontrol agents, a culture disc (5mm) for the test fungus was placed along periphery of the PDA plate and exactly opposite to it pure culture suspension of the test bacterial biocontrol agent was streaked with wire / inoculation needle loop. The PDA plates inoculated alone with pure culture disc of the test fungus were maintained as untreated control. Observations on linear colony growth (mm) of the test fungus and the test bio-agent were recorded at an interval of 24 hrs of incubation and continued upto seven days or till the untreated control plates were fully covered with mycelial growth of the test fungus. Based on cumulative data, per cent mycelial growth inhibition of the test fungus with the test bioagents, over untreated control was calculated by applying the following formula (Arora and Upadhay, 1978) [2].

$$\text{Growth Inhibition (\%)} = \frac{\text{Colony growth of the fungus in control plate} - \text{Colony growth of the fungus in intersecting plate}}{\text{Colony growth in control plate}} \times 100$$

Results and Discussion

In vitro efficacy of Bioagents against *A. solani,* causing tomato fruit rot

The results (Table 1, Plate 1) revealed that all the Bioagents evaluated *in vitro* exhibited antifungal activity against *A. solani* and significantly inhibited its growth, over untreated control.

The results (Table 1, Plate 1) revealed *Trichoderma harzianum* as most effective with significantly least mycelial growth (4.00 mm) and significantly highest mycelial growth inhibition (95.56%), which was on par with *T. hamatum* (5.33 mm and 94.08%), followed by *Aspergillus niger* (6.67 mm and 92.59%), *Metarhizium anisopliae* (9.00 mm and 90.00%), *T. asperellum* (11.33 mm and 87.41%), *T. koningii* (15.00mm and 83.33%), *Verticillium lacani* (20.00 mm and 77.78%), *Pseudomonas fluorescens* (20.67 mm and 77.03%) and *Bacillus subtilis* (23.67 mm and 73.70%), for colony diameter and mycelial growth inhibition, respectively.

These test bioagents found effective in present study against *A. solani* causing tomato fruit rot, were also reported as potential antagonists against *A. solani*, by several workers (Singh *et al.*, 2018a; Bais *et al.*, 2019; Naik *et al*., 2020) [3, 10].

Table 1: *In vitro* efficacy of bioagents against *A. solani,* causing tomato fruit rot

Tr. No.	Treatments	Col. Dia.* of test pathogen (mm)	% Inhibition*
T1	*Trichoderma asperellum*	11.33	87.41 (69.22)
T2	*T. harzianum*	4.00	95.56 (77.84)
T3	*T. hamatum*	5.33	94.08 (75.92)
T4	*T. koningii*	15.00	83.33 (65.90)
T5	*Aspergillus niger*	6.67	92.59 (74.20)
T6	*Metarhizium anisopliae*	9.00	90.00 (71.57)
T7	*Verticillium lacani*	20.00	77.78 (61.87)
T8	*Pseudomonas fluorescens*	20.67	77.03 (61.36)
T9	*Bacillus subtilis*	23.67	73.70 (59.15)
T10	Control (untreated)	90.00	0.00
	S.E. ±	0.741	0.791
	C.D. (P=0.01)	2.13	2.367

*: Mean of three replications, Dia.: Diameter, Figures in parentheses are arcsine transformed values.

In vitro efficacy of bioagents against *C. capsici,* causing tomato fruit rot

The results (Table 2, Plate 2) revealed that the bioagents evaluated *in vitro* exhibited antifungal activity against *Colletotrichum capsici* and significantly inhibited their mycelial growth, over untreated control.

Table 2: *In vitro* efficacy of bioagents against *C. capsici*, causing tomato fruit rot

Tr. No.	Treatments	Col. Dia.* of test pathogen (mm)	% Inhibition*
T1	*Trichoderma asperellum*	24.00	73.33 (58.91)
T2	*T. harzianum*	6.83	92.41 (74.01)
T3	*T. hamatum*	6.83	92.41 (74.01)
T4	*T. koningii*	6.77	92.48 (74.08)
T5	*Aspergillus niger*	14.33	84.08 (66.48)
T6	*Metarhizium anisopliae*	8.50	90.56 (72.11)

T7	*Verticillium lacani*	26.93	70.08 (56.84)
T8	*Pseudomonas fluorescens*	31.17	65.37 (53.95)
T9	*Bacillus subtilis*	31.47	65.03 (53.75)
T10	Control (untreated)	90.00	0.00
	S.E. ±	0.817	0.907
	C.D. (P=0.01)	2.446	2.715

*: Mean of three replications, Dia.: Diameter, Figures in parentheses are arcsine transformed values.

The results (Table 2, Plate 2) revealed *Trichoderma koningii* as most effective bioagent with significantly least mycelial growth (6.77 mm) and significantly highest mycelial growth inhibition (92.48%), which was on par with *T. harzianum* and *T. hamatum* (each 6.83mm and 92.41%) and *Metarhizium anisopliae* (8.50 mm and 90.56%), followed by *Aspergillus niger* (14.33 mm and 84.08%), *T. asperellum* (24.00 mm and 73.33%), *Verticillium lacani* (26.93 mm and 70.08%), *Pseudomonas fluorescens* (31.17 mm ad 65.37%) and *Bacillus subtilis* (31.47 mm and 65.03%), for colony diameter and mycelial growth inhibition, respectively.

These test bioagents found effective in present study against tomato fruit rot causing *C. capsici* and also other *Colletotrichum* app., infecting various crop hosts were reported as potential antagonists, by several earlier workers (Vani and Somashekhara 2018 and Lokhande *et al*. 2019) [12, 8].

Plate 1: Show the results revealed that all the Bioagents evaluated *in vitro* exhibited antifungal activity against *A. solani* and significantly inhibited its growth, over untreated control

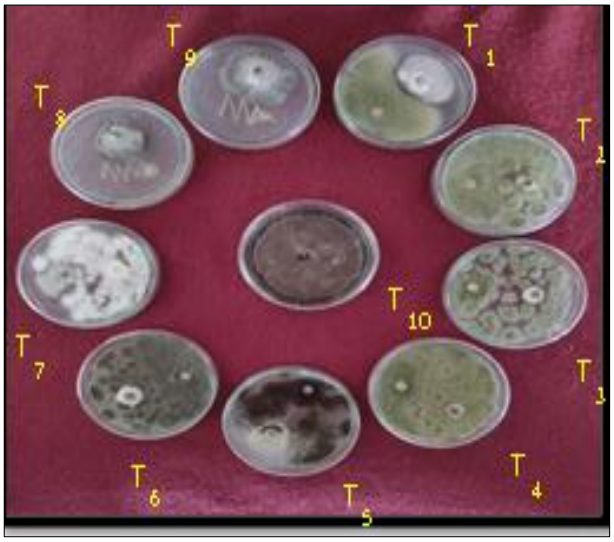

Plate 2: The bioagents evaluated *in vitro* exhibited antifungal activity against *Colletotrichum capsici* and significantly inhibited their mycelial growth, over untreated control

Conclusions

From the results obtained on various aspects during present investigations on "*In vitro* Bioefficacy of Bioagents against *A. solani* and *C. capsici*, Causing Tomato Fruit Rots" following conclusions are being drawn:

All nine test bioagents evaluated *in vitro* significantly inhibited mycelial growth over untreated control, of the test pathogenic fungi *A. solani* and *C. capsici*, causing tomato fruit rot. However, in *A. solani*, highest mycelial growth inhibition was resulted with *Trichoderma harzianum* (95.56%) and it was on par with *T. hamatum* (94.08%), *Aspergillus niger* (92.59%) and *Metarhizium anisopliae* (90.00%), followed by *T. asperellum* (87.41%) and *T. koningii* (83.33%). Rest of the bioagents also significantly inhibited mycelial growth inhibition of *A. solani*, over untreated control. Similarly, in

C. capsici, highest mycelial growth inhibition was resulted with *T. koningii* (92.48%) and it was on par with *T. harzianum* and *T. hamtum* (each 92.41%) and *Metarhizium anisopliae* (90.56%), followed by *A. niger* (84.08%), *T. asperellum* (73.33%), *Verticillium lacani* (70.08%), *P. fluorescens* (65.37%) and *B. subtilis* (65.03%).

Therefore, for the effective management of plant diseases, the farmers may be advised to take an integrated approach, which should be raised a profitable production without polluting the environment and adding toxins in the food chain.

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