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Assessment of different fungicides and bioagents against *Alternaria solani* (Ellis and Martin) Jones and Grout causing early blight of Tomato (*Lycopersicon esculentum* Mill) under laboratory conditions

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Abstract
Tomato (*Lycopersicon esculentum* Mill.), is one of the most popular fruit vegetable crops grown throughout the world. In India, tomato is grown in almost all states, and the major states are: Bihar which ranks first followed by Uttar Pradesh, Karnataka, Punjab, West Bengal, Assam, Orissa and Maharashtra. Among the fungal diseases infecting tomato crop, early blight caused by *Alternaria solani* (Ellis and Martin) Jones and Grout, is one of the most destructive diseases causing accountable qualitative and quantitative losses. The study investigated the efficacy of eleven fungicides, tested at concentrations of 1000 and 1500 parts per million (PPM), in inhibiting the mycelial growth of *A. solani*, a common pathogen. Results demonstrated significant inhibition of mycelial growth across all tested fungicides compared to the control. At 1000 PPM, systemic fungicides exhibited inhibition ranging from 56.88% to 94.44%, with Propiconazole showing the highest efficacy. At 1500 PPM, non-systemic fungicides ranged from 50.81% to 94.44% inhibition, with Propineb and Captan ranking highest. Combi-fungicides at 1500 PPM displayed inhibition ranging from 64.23% to 94.44%, with Azoxystrobin + Difenoconazole and Tebuconazole + Trifloxystrobin being the most effective. Among bioagents, *Trichoderma* spp. showed significant inhibition, with *T. harzianum* demonstrating the highest efficacy (81.36% inhibition).

Keywords: Tomato, *Alternaria solani*, early blight, fungicides, bioagents

Introduction
Tomato (*Lycopersicon esculentum* Mill.), is one of the most popular fruit vegetable crops grown throughout the world. It is the most remunerative vegetable crop, which ranks next to potato in world acreage and ranks first among the processing crops. In India, tomato is grown in almost all states, and the major states are: Bihar which ranks first followed by Uttar Pradesh, Karnataka, Punjab, West Bengal, Assam, Orissa and Maharashtra. Among the biotic causes, fungi are most important which cause the major diseases *viz.*, damping off (*Pythium aphanidermatum*), Late blight (*Phytophthora infestans*), Fruit rot (*Alternaria alternata*), Early blight (*Alternaria solani*), Fusarium wilt (*Fusarium oxysporium* f. sp. *lycopersici*) and Powdery mildew (*Leveillula taurica*). Among the fungal diseases infecting tomato crop, early blight caused by *Alternaria solani* (Ellis and Martin) Jones and Grout, is one of the most destructive diseases causing accountable qualitative and quantitative losses. The causal organism is air borne and soil inhabiting and responsible for early blight, it also infects fruits causing shedding of immature fruits up to 30 per cent (Walker, 1951) [17]. The characteristic symptoms of the disease are, initial dark brown to black spots which enlarge and develop raised concentric rings and depressed necrotic tissue on foliage. As disease progress, affected leaves turn yellow with senescence and either dry up or fall off. Symptoms also appear on stem and fruits. The yield losses in the range of 48-80 per cent due to early blight (*Alternaria solani*) damage in tomato were reported from India.

Materials and Methods
During the present investigations on early blight of tomato caused by *Alternaria solani* (Ell. and Mart.) Jones and Grout, various *in vitro* experiments were conducted at the Department of Plant Pathology, College of Agriculture, VNMKV, Parbhani.

Culture media
Potato Dextrose Agar (PDA) the common laboratory culture medium was used as basal medium for isolation, purification, multiplication and maintenance of the pure culture of *A. solani*.

Chemicals
Standard chemicals, reagents, fungicides, culture media etc. required for the experimentation were obtained from the Department of Plant Pathology, College of Agriculture, Parbhani.

Fungicides
The following ten fungicides (systemic, non-systemic and combi) were used for *in vitro* and *in vivo* experiments conducted during present studies.

Biocontrol agents
Carrier based (Talc powder) preparations of the bioagents and pure cultures of biocontrol agents *viz., T. viride, T. harzianum, T. hamatum, T. longibrachiatum, T. koningii, Gliocladium virens, Bacillus subtilis and Pseudomonas fluorescens* were obtained from the Spawn Production-cum-Biocontrol Laboratory, Department of Plant Pathology, College of Agriculture, VNMKV, Parbhani; maintained and multiplied on appropriate culture media and used for further studies.

Disease management strategies

***In vitro* evaluation of fungicides**
A total of 11 fungicides (systemic @ 1000 ppm conc, Non-systemic and Combi @ 1500 ppm) evaluated *in vitro* against *A. solani* exhibited a wide range of mycelial growth and inhibition of the test pathogen. The results obtained are presented in the Table 1 and Plate-I.

Mycelial growth
At 1000 ppm, systemic fungicides exibited (Table 1 and PLATE- I) radial mycelial growth of the test pathogen from 5.00 mm (Propiconazole) to 38.90 mm (Pyraclostrobin), as against 90.00 mm in untreated control. However, Propiconazole was found with least of the mycelial growth (5mm). This was followed by the fungicides *viz*., Azoxystrobin (30.38 mm).
At 1500 ppm, (Table 1 and PLATE- I) four non systemic fungicides showed, radial mycelial growth of the test pathogen was ranged from 5.00 mm (Propineb) to 42.26 mm (Copper oxychloride), as against 90.00 mm untreated control. This was followed by the fungicides Captan (30.43 mm) and Mancozeb (33.5mm). Copper oxychloride was found comparatively less effective with maximum mycelial growth of 42.26 mm. At 1500 ppm, Combi fungicides (Table 1 and PLATE- I) radial mycelial growth of the test pathogen ranged from 5.0 mm (Azoxystrobin18.2%W/W + Difenoconazole 11.4% W/W) and Tebuconazole 50% + Trifloxystrobin 25% WG to 32.26 mm (Mancozeb 64%w/w +Metalaxyl 4%w/w), as against 90.00 mm in untreated control.

Table 1: *In vitro* efficacy of Systemic, Non-systemic & Combi fungicides against *A. solani*

Treatment No.	Treatments	*Average Colony Dia. (mm)	#Average% Inhibition Over control
Systemic fungicides @1000ppm			
T_1	Azoxystrobin 23%SC	30.38(17.68)	66.24(41.47)
T_2	Pyraclostrobin 20%WG	38.90(22.88)	56.88(34.67)
T_3	Propiconazole 25% EC	5.0(28.65)	94.44(70.79)
Non-systemic fungicides @1500ppm			
T_4	Copper oxychloride50%WP	42.26(26.27)	50.81(29.75)
T_5	Captan 50%WP	30.43(17.75)	66.10(40.32)
T_6	Mancozeb 75%WP	33.5(19.57)	62.77(38.90)
T_7	Propineb 70% WP	5.0(28.65)	94.44(70.79)
Combi fungicides@1500ppm			
T_8	Azoxystrobin18.2%W/W+ Difenoconazole 11.4%W/W	5.0(28.65)	94.44(70.79)
T_9	Tebuconazole 50% + Trifloxystrobin 25%WG	5.0(28.65)	94.44(70.79)
T_{10}	Mancozeb 64%w/w + Metalaxyl 4%w/w	32.26(18.82)	64.23(39.97)
T_{11}	Control	90.00(64.15)	00.00
SE±		0.67	0.70
CD (P=0.05)		1.97	2.07

*Mean of three replications, Dia.=Diameter, #Figures in parenthesis are arc sine transformed value

Mycelial growth inhibition
Results (Table 1 and Plate-I) revealed that all the 11 fungicides tested (@1000, 1500 PPM) significantly inhibited mycelial growth *of A solani* over untreated control (00.00%). Further, the percentage mycelial growth inhibition increased with increase in concentrations of the fungicides tested.
At 1000 ppm, systemic fungicides (Table 1 and Plate-I) mycelial growth inhibition of the test pathogen ranged from 56.88% (Pyraclostrobin 20%WG) to 94.44 per cent (Propiconazole 25% EC). However, fungicide Propiconazole was found best, inhibited (94.44%) mycelial growth. The second and third best fungicides found were 56.88% (Pyraclostrobin 20% WG) and Azoxystrobin 23% SC (66.24%).
At 1500 ppm Non-systemic) (Table 1 and PLATE- I) mycelial growth inhibition was ranged from 50.81% (Copper oxychloride) to 94.44% (Propineb). The second best fungicide was found Captan (66.10%) followed by the fungicide Mancozeb (62.77%).
At 1500 ppm Combi-fungicides (Table 1 and Plate-I) tested mycelial growth inhibition was ranged from 64.23 (Mancozeb + Metalaxyl) to 94.44% (Azoxystrobin + Difenoconazole) and (Tebuconazole + Trifloxystrobin).
Thus, all the fungicides tested were found fungistatic against *A solani* and significantly inhibited its mycelial growth over untreated control.
Similar fungistatic effects of the test fungicides against *A solani* infecting tomato and many other crops were reported

earlier by several workers studied management of fruit rot of chilli caused by *A. alternata* with systemic and non-systemic fungicides *in vitro* as well as *in vivo* conditions. Systemic fungicide Hexaconazole gave cent per cent inhibition, followed by Tridemorph (93.65%) and Propiconzaole (91.42%), whereas in non-systemic fungicides Mancozeb and Zineb gave cent per cent inhibition of *A. alternata*.

Kumar *et al.* (2017) [14] was carried out similar *in vitro* management of early blight of tomato in year 2015-2016. Among the fungicides tested most effective was score with mycelium inhibition growth upto 78.61 percent followed by carbendazim (76.67 per cent).

In vitro evaluation of bioagents

The results obtained on mycelial growth and inhibition of *A. solani with* six fungal and two bacterial antagonists are presented in (Table 2 and PLATE II). Results revealed that all the bioagents evaluated exhibited fungistatic / antifungal activity against *A. solani* and significantly inhibited its growth over untreated control.

Table 2. *In vitro* efficacy of bio agents against *A. solani*

Treatment No.	Treatments	*Average Colony Dia. of test pathogen (mm)	#Average% Inhibition over control
T_1	*Trichoderma viride*	22.50	74.92(48.55)
T_2	*T. harzianum*	16.76	81.36(54.50)
T_3	*T. hamatum*	20.00	77.77(51.06)
T_4	*T. koningii*	27.36	69.59(44.10)
T_5	*T.Longibrachiatum*	44.90	50.18(30.11)
T_6	*Gliocladium virens*	42.33	53.03(32.12)
T_7	*Bacillus subtilis*	40.46	55.03(33.39)
T_8	*Psudomonas fluorescens*	50.26	44.14(26.20)
T_9	Control	90.00	00.00(00.00)
S.E. ±		1.34	0.82
C.D. (P=0.05)		3.98	2.44

* Mean of three replications, Dia.: Diameter

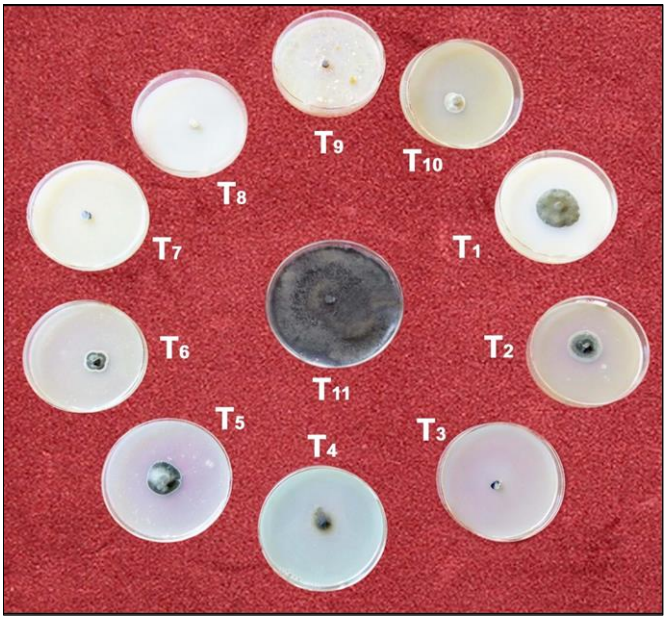
Figures in parenthesis are Arcsin transformed values

Among bioagents tested, *T. harzianum* was found most effective with significantly least mycelial growth and highest mycelial growth inhibition of the test pathogen (16.76 mm) followed by *T. hamatum* (20.00mm) which was at par with each other, and highest mycelial growth inhibition (81.36%) of the test pathogen followed by *T. hamatum* (77.77%). The third best antagonists found *T. viride* with mycelial growth of 22.56 mm and inhibition of 74.92 per cent. This was followed by *T. koningii* and *Bacillus subtilis* (27.36 and 40.46 mm) and (69.59 and 55.03%) of mycelium growth and its inhibition, respectively. *Psudomonas fluorescens* was found comparatively less effective with maximum mycelial growth (50.26mm) and minimum mycelial growth inhibition (44.14%).

Results of the present study on antifungal activity of the *T. viride, T. harzianum, T. hamatum T. koningii* and *G. virens* two bacterial antagonists *viz., P. fluorescens and Bacillus subtilis* against *A.solani* are in conformity with those reported earlier by several workers.

Naik *et al.* (2010 b) evaluated *in vitro* the bioagents *viz., Trichoderma harzianum, T. viride, T. koningii* and *G. virens* against *A. chlamydospora*, causing leaf blight of okra. They reported all the test bioagents as effective against the test pathogen. However, significantly highest mycelial growth inhibition (86.29%) was recorded with *G. virens*, followed by *T. viride* (85.18%), *T. harzianum* and *T. koningii* (84.44%).

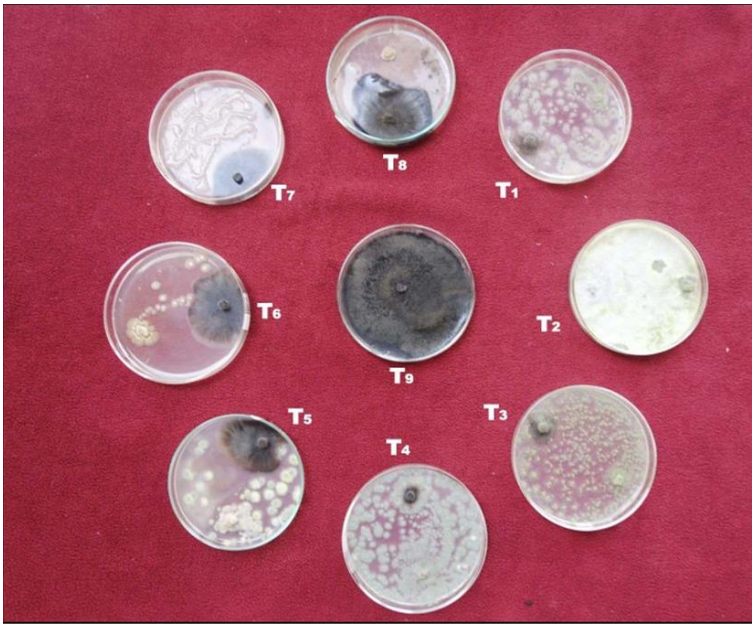
Plate-I



T_1 : Azoxystrobin 23%SC
T_2 : Pyraclostrobin 20%WG
T_3 : Propiconazole 25% EC
T_4 : Copper oxychloride50 %WP
T_5 : Captan 50%WP
T_6 : Mancozeb 75 %WP
T_7 : Propineb 70% WP
T_8 : Azoxystrobin18.2%W/W+ Difenoconazole 11.4%W/W
T_9 : Tebuconazole 50% + Trifloxystrobin 25%WG
T_{10} : Mancozeb 64%w/w + Metalaxyl 4%w/w
T_{11} : Control

In vitro **efficacy of Systemic, Non -systemic & Combi fungicides against** ***A. solani***

Plate II



T_1 : *Tricoderma viride*
T_2 : *T. harzianum*
T_3 : *T. hamatum*
T_4 : *T. koningii*
T_5 : *T. Longibrachiatum*
T_6 : *Gliocladium Virens*
T_7 : *Bacillus subtilis*
T_8 : *Psudomonas fluorescens*
T_9 : *Control*

In vitro **efficacy of bioagents against** ***A. solani***

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