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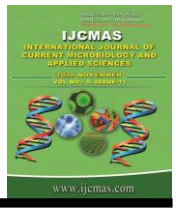

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Efficacy of Fungicides and Bioagents against Early Blight of Tomato caused by *Alternaria solani*

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A B S T R A C T

Tomato (*Lycopersicon esculentum* Mill.), is one of the most popular fruit vegetable crop grown throughout the world. However, during recent years the crop has severely been affected by the early blight of tomato (*Alternaria solani*), causing about 48-80 per cent fruit yield losses. Though, the disease can be managed with various conventional fungicides and bio-agents under laboratory condition but new generation chemical molecules needs to be assessed for their efficacy against the disease. Three Systemic fungicides (@1000 ppm), four non-systemic fungicides (1500 ppm) and three combi (1500 ppm) fungicides were evaluated. Similarly six fungal antagonists and two bacterial antagonist were evaluated against *M. phaseolina* by poison food technique and duel culture method respectively. Per cent Inhibition was ranged from 50.81 to 94.44 and bioagent tested per cent inhibition ranged from 44.14 to 74.92.

Introduction

Tomato (*Lycopersicon esculentum* Mill.), is one of the most popular fruit vegetable crop grown throughout the world. It is considered as "Protective Food", because of its special nutritive values and its wide spread cultivation. It is the most remunerative vegetable crop, which ranks next to potato in world acreage and ranks first among the processing crops.

Tomato is native of Central and South America from where it has spread to other parts of the world in sixteenth century. It was introduced in India by the Portuguese during 1700 (Kale and Kale, 1994).

In India's total production of 19696.9 thousand tones from an area of 808.5 thousand hectares with an average productivity of 24.40 tones ha-1 during 2016-17 (Anonymous, 2017) In Maharashtra area under tomato cultivation was 43.64 thousand hectares with production of 957.17 metric tonnes productivity of 21.93 metric tonnes per hectares during 2016-2017 (Anonymous, 2017) fiber (0.7 g) per 100 g of edible portion.

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*).

The important bacterial diseases include, bacterial wilt (*Ralstonia solanacearum*), bacterial canker (*Clavibacter michiganense* subsp. *michiganense*), bacterial leaf spot (*Xanthomonas campestris* pv. *vesicatoria*), nematode diseases like root knot nematode caused by *Meloidogyne* spp. and viral diseases like tomato leaf curl, tomato spotted wilt virus and tomato mosaic.

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 disease 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).

The disease is most severe in *Kharif* but it also appears throughout the year affecting the yield and quality of fruits (Wilson 1943, Basu, 1973 and Gleason *et al.*, 1993).

The yield losses in the range of 48-80 per cent due to early blight (*Alternaria solani*) damage in tomato were reported from India, Canada, USA and Nigeria (Basu, 1974; Datar and Mayee, 1984; Mathur and Shekhawat, 1986; Sherf and MacNab, 1986; Gwary and Nahunnarao, 1998 and Pandey and Pandey, 2002). The annual economic yield losses due to early blight have been estimated at 79% (Adhikari and Panthee, 2017).

Materials and Methods

In vitro evaluation of fungicides

Efficacy of three systemic fungicide *viz.*, Azoxystrobin 23%EC, Pyraclostrobin 20% WG, Propiconazole 25EC, and Four Non systemic fungicides *viz.*, Mancozeb 75 WP, Copper oxychloride 50 WP, Captan 50% WP, Propineb 70 WP, and three combi fungicide *viz* Azoxystrobin18.2 w/w+ Difenoconazole 11.4%w/w (Amister Top), Tebuconazole 50 % + Trifloxystrobin 25% WG(Nativo), Mancozeb + Metalaxyl (Redomil - MZ 72%WP) were evaluated at different concentrations (systemic each @ 1000 and Non systemic & combi each @ 1500) *in vitro* against *A. solani* (PBN-3 isolate) applying Poisoned Food Technique (Nene and Thapliyal, 1993) and using Potato dextrose agar (PDA) as basal culture medium. Based on active ingredient, requisite quantity of each test fungicide was calculated and mixed thoroughly with autoclaved and cooled (40^0C) PDA medium in conical flasks to obtain desired concentrations of the test fungicides. Fungicide amended PDA medium was then poured aseptically in Petri plates (90 mm dia.) and allowed to solidify at room temperature. For each of the test fungicide and its desired concentrations, three plates / treatment / replication were maintained. After solidification of the medium, all the plates were inoculated aseptically with 5 mm culture disc obtained from a week old actively growing pure culture of *A. solani*.

The disc was placed on PDA in the centre of the Petri plate and plates were incubated in inverted position at 26 $\pm$ 2^0C. Each of the test fungicide and its concentration were replicated thrice. Petri plates filled with plain PDA (without fungicide) and inoculated with the culture disc of *A. solani* were maintained as unpoisoned control.

Experimental details

Design : C.R.D.
Replications : Three
Treatments : Eleven

Systemic, Non-systemic and combi fungicides

Treatment No.	Treatments
Systemic fungicides @1000ppm	
T_1	Azoxystrobin 23%SC
T_2	Pyraclostrobin 20%WG
T_3	Propiconazole 25% EC
Non-systemic fungicides @1500ppm	
T_4	Copper oxychloride 50 %WP
T_5	Captan 50%WP
T_6	Mancozeb 75 %WP
T_7	Propineb 70% WP
Combi fungicides@1500ppm	
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

Observations on radial mycelial growth/ colony diameter were recorded at an interval of 24 hours and continued till untreated control plates were fully covered with mycelial growth of the test fungus. Per cent inhibition of the test fungus with the test fungicides over untreated control was calculated by applying following formula (Vincent, 1927).

$$\text{Per cent inhibition} = \frac{C - T}{C} \times 100$$

Where,

C= growth of the test fungus in untreated control plates
T= growth of the test fungus in treated plates

In vitro evaluation of bioagents

Six fungal antagonists *viz., Trichoderma viride, T. harzianum, T. hamatum, T. longibrachiatum, T. koningii, Glocladium virens,* and two bacterial antagonist *Pseudomonas fluorescens* & *Bacillus subtilis* were evaluated *in vitro* against *A. solani* (PBN3 isolate), applying Dual Culture Technique (Dennis and Webster, 1971). Seven days old cultures of the test bioagents and test fungus (*A. solani*) grown on (PDA) were used for the study. Discs (5 mm dia) of PDA along with culture growth of the test fungus and bioagents were cut out with sterilized cork borer. Then two culture discs, one each of the test fungus and bioagent were placed at equidistance and exactly opposite with each other on solidified PDA medium in Petri plates aseptically and plates were incubated at 26 $\pm$ 2^0C. PDA plates inoculated only with culture disc of the test fungus were maintained as untreated control.

Experimental details

Design : CRD
Replications : Three
Treatments : Nine
T_1 : *T. viride*
T_2 : *T. harzianum*
T_3 : *T. hamatum*
T_4 : *T. longibrachiatum*
T_5 : *T. koningii*
T_6 : *Gliocladium virens*
T_7 : *Bacillus subtilis*
T_8 : *P. fluorescens*
T_9 : Control (untreated)

Observations on linear mycelial growth of the test fungus and bioagent were recorded at an interval of 24 hours and continued till untreated control plates were fully covered with mycelial growth of the test fungus. Per cent inhibition of the test fungus by the bioagents over untreated control was

calculated by applying following formula (Arora and Upaddhyay, 1978).

$$\text{Per cent Growth Inhibition} = \frac{\text{Colony growth in control plate} - \text{Colony growth in Intersecting plate}}{\text{Colony growth in control plate}} \times 100$$

The data obtained in all the experiments were statistically analyzed (Panse and Sukhatme, 1978). The percentage values were transformed into arcsine values. The standard error (SE) and critical difference (C.D.) at level P = 0.05 were worked out and results obtained were compared statistically. All the statistical analysis was done using MAU-STAT statistical programme at Central Computer Laboratory, VNMKV, Parbhani.

Results and Discussion

In vitro efficacy of fungicides on growth of *Alternaria solani*

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.

Rajani and Rakholia (2012) studied management of fruit rot of chilli caused by *A. alternata* with systemic and non-systemic fungicides *in vitr*o 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) were carried out similar *in vitro* managment 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 (76.67per 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.1 *In vitro* efficacy of Systemic, Non-systemic & Combi fungicides against *A. solani*

Treatment	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

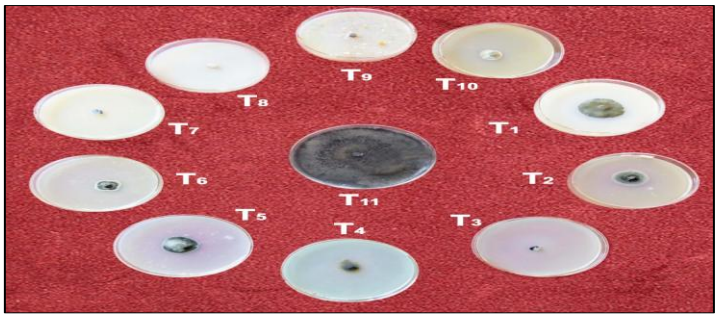
*Mean of three replications, Dia.=Diameter, #Figures in parenthesis are arc sine transformed value

Table.2 *In vitro* efficacy of bioagents against *A. solani*

Treatments	*Average Colony Dia. of test pathogen (mm)	#Average % Inhibition over control
Trichoderma viride	22.50	**74.92 (48.55)**
T. harzianum	16.76	**81.36 (54.50)**
T. hamatum	20.00	**77.77 (51.06)**
T. koningii	27.36	**69.59 (44.10)**
T.Longibrachiatum	44.90	**50.18 (30.11)**
Gliocladium virens	42.33	**53.03 (32.12)**
Bacillus subtilis	40.46	**55.03 (33.39)**
Psudomonas fluorescens	50.26	**44.14 (26.20)**
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 Arcs in transformed values

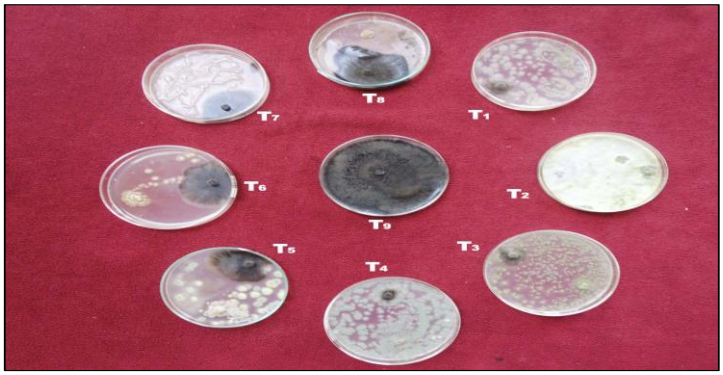
PLATE -I



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

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 %).

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