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In vitro management of *Alternaria solani* through fungicide, bioagents and botanicals

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Abstract
All the fungicides, botanicals and bioagents evaluated *in vitro* were exhibited fungistatic / antifungal activity against *A. solani*. Most effective fungicides with significantly highest i.e. cent per cent mycelial growth inhibition were recorded in Difenoconazole, Propiconazole, Tebuconazole and Propineb. Among different bio agents *T. viride* was found potential antagonist activity against *A. solani* in reducing the mean colony diameter of the fungus (79.78 per cent). Among the botanicals Garlic and Mint leaf extract were found most antifungal to *A. solani* with maximum mycelial growth inhibition of 78.15 and 73.33 per cent, respectively.

Keywords: Tomato, Alternaria, fungicide, bioagent, botanicals

Introduction
Tomato (*Lycopersicon esculentum* Mill.) is one of the most remunerative and widely grown Vegetable in the world. It is a small annual or short-lived perennial herb belonging to the family Solanaceae, probably native of Peru-Equador. It is a regular kitchen component of Indian diet. Tomato has many uses in food industry. It is used for both fresh consumption as well as for processing purpose as soups, salad, pickles, ketchup, puree, chutney, jam and many other products. Tomato ranks third in priority after Potato and Onion in India but ranks second after potato in the world. India ranks second in the area as well as in production of Tomato. Tomato is highly sensitive to abiotic stresses especially extreme temperature, salinity, drought, excessive moisture, environmental pollution and biotic stresses. Tomato plants suffer with large number of biotic stresses including insect pests and diseases from the time of emergence to till harvest. Among the fungal diseases, early blight caused by *Alternaria solani* is one of the most important and frequent occurring disease of the crop nation and worldwide (Jones and Grout, 1897) [6]. The present research was therefore conducted in order to identify effective steps for improved disease control through fungicide, bioagents and botanical treatment against early blight of tomato caused by *A. solani*.

Material and Methods
Lab experiments was carried out on *in vitro* management of *A. solani* was conducted in the laboratories under department of Plant Pathology, Pune during 2020- 2021. The material used and the methods followed are described in this chapter.
Early blight leaf samples of tomato were collected from field of College of Agriculture, Pune and the pathogen *Alternaria solani* was isolated from the naturally infected tomato plants showing typical symptoms of the disease. The infected portions of the leaves along with some healthy tissue were cut into small pieces. These pieces were surface sterilized with 0.1 per cent mercuric chloride solution for 30 seconds then washed thoroughly in sterile distilled water thrice to remove traces of mercuric chloride, if any, and then transferred aseptically to sterilized potato dextrose agar (PDA) plates. They were incubated at 27±1 °C and checked after every 24 hr for the growth of the fungus. The fungus was identified based on the morphological characteristics. Later, a bit of the fungal growth was transferred to PDA plates. The pure culture of the fungus was obtained by following hyphal tip culture under aseptic conditions.

1. *In vitro* evaluation of fungicides against *A. solani*

The poisoned food technique (Nene and Thapliyal, 1984) was followed to evaluate the efficacy of ten different fungicides against *A. solani* at its recommended dose of applications (concentrations). Both non-systemic and systemic fungicides were tested *in vitro* against *A. solani*. Fungicides were added to the sterilized potato dextrose agar medium as per treatment details. Five mm disc of *A. solani* was taken from seven days old culture and placed at center of petri dish. Simultaneously, a control was also maintained by growing the fungus on fungicide free PDA medium. The plates were incubated at 25 ± 2 ^{0}C. The efficacy of fungicides was recorded by measuring the colony diameter of *A. solani* in each treatment and compared with control. The per cent growth inhibition of the fungus in each treatment in comparison with control was calculated by the equation given by Vincent (1947).

$$I = \frac{100(C - T)}{C}$$

Where

I = Per cent inhibition of fungal growth.
C = Growth of fungus colony diameter (mm) in control.
T = Growth of fungus colony diameter (mm) in treatment.

Table 1: *In vitro* evaluation of fungicides treatment details is given below

Treatments	Chemical Name	Concentration
T_1	Azoxystrobin	0.1%
T_2	Azoxystrobin + Tebuconazole	0.1%
T_3	Tebuconazole + Trifloxystrobin	0.05%
T_4	Tebuconazole	0.1%
T_5	Propineb	0.25%
T_6	Carbendazim + Mancozeb	0.1%
T_7	Thiophanate methyl	0.05%
T_8	Difenoconazole	0.05%
T_9	Propiconazole	0.1%
T_{10}	Control	-

2. *In vitro* evaluation of botanicals against *A. solani*

Botanical extracts of Garlic, Onion, Ginger, Nilgiri, Ghaneri, Mint, Turmeric, Tulsi and Neem were evaluated against *A. solani*. The economical parts of each plant were cleanly washed in distilled water, air dried and were prepared by grinding known weight of fresh materials with distilled water in ratio of 1:1 (w/v). Aqueous botanicals were prepared by grinding with mortar and pestle. 100 g washed leaves of each plant species in 100 ml distilled water are grinded and filtered through double layered muslin cloth. Each of the filtrate obtained was further filtered separately through Whatman No. 1 filter paper. The final clear extracts obtained formed the standard leaf extracts of 100% concentration and these botanicals were evaluated at 10% concentration, *in vitro* against *A. solani* by applying Poisoned food technique similar as that of *in vitro* evaluation of fungicides against *A. solani*.

Table 2: List of botanicals used against *A. solani*

Sr. No.	Local name	Scientific name	Plant part used	Conc. (%)
1	Garlic	*Allium sativum*	Clove	5%
2	Onion	*Allium cepa*	Bulb	5%
3	Ginger	*Zingiber officinale*	Rhizome	5%
4	Nilgiri	*Eucalyptus globulus*	Leaf	10%
5	Ghaneri	*Lantana Camara*	Leaf	10%
6	Mint	*Mentha arvensis*	Leaf	10%
7	Turmeric	*Curcuma longa*	Rhizome	5%
8	Tulsi	*Osmium sanctum*	Leaf	10%
9	Neem	*Azadirachta indica*	Leaf	10%

3. *In vitro* evaluation of biocontrol agent against *A. solani*

Four fungal antagonists *viz*., *Trichoderma harzianum*, *Trichoderma viride*, *Trichoderma hamatum* and *Trichoderma koningii* isolates were tested *in-vitro* against *A. solani* by dual culture technique given by Dennis and Webster (1971). At equal distances, exactly opposite to each other on solidified PDA medium in plates two culture discs, one each of the test fungus and bioagents, were placed under aseptic conditions and plates were incubated at 27±1 °C. Plates inoculated with culture disc of test fungus were maintained as untreated control. The formula given by Vincent (1947) was used to determine the percent inhibition of the test fungus over the untreated control.

Result and Discussion

1. *In vitro* evaluation of fungicides against *A. solani*

The observations regarding mean colony diameter and per cent mycelium inhibition of *A. solani* presented in Table 3. The results revealed that all the nine fungicides tested significantly inhibited mycelial growth of *A. solani* over untreated control. Among the nine fungicides, most effective fungicides were Tebuconazole (0.1%), Propineb (0.25%), Difenoconazole (0.05%) and Propiconazole (0.1%) which exhibited cent per cent inhibition in mycelium growth of the test pathogen. The next best fungicide was Carbendazim + Mancozeb (0.1%) with 88.52 per cent inhibition in mycelium growth followed by Thiophanate methyl (0.05%) with 84.82 per cent inhibition in mycelium growth of the fungus. Fungicides Azoxystrobin (0.1%) and Tebuconazole + Trifloxystrobin (0.05%) recorded 81.11 and 78.52 per cent inhibition in mycelium growth of *A. solani.* Fungicide Azoxystrobin + Tebuconazole at 0.1 per cent showed the least inhibition in mycelium growth (76.30%) of the test pathogen.

The results are in conformity with several workers. In present investigation the fungicide Propiconazole (0.1%) was found highly effective with cent per cent mycelial inhibition of *A. solani* are in conformity with the earlier scientist Vasudha *et al*., (2018). Also the fungicide Tebuconazole and Difenconazole cent per cent mycelia inhibition of *Alternaria* is matching with the earlier findings of Sanjeev *et al*., (2017) where he reported cent per cent mycelia inhibition of *A. alternata.*

Table 3: *In vitro* evaluation of Fungicides against *A. solani*

Treatments	Treatment Name	Average colony diameter (mm)	Average inhibition Over Control (%)
T_1	Azoxystrobin @ 0.1%	17.00	81.11 (64.25)
T_2	Azoxystrobin + Tebuconazole @ 0.1%	21.33	76.30 (60.87)
T_3	Tebuconazole + Trifloxystrobin @ 0.05%	19.33	78.52 (62.40)
T_4	Tebuconazole @ 0.1%	0.00	100.00

			(90.00)
T_5	Propineb @ 0.25%	0.00	100.00 (90.00)
T_6	Carbendazim + Mancozeb @ 0.1%	10.33	88.52 (70.23)
T_7	Thiophanate methyl @ 0.05%	13.67	84.82 (67.07)
T_8	Difenoconazole @ 0.05%	0.00	100.00 (90.00)
T_9	Propiconazole @ 0.1%	0.00	100.00 (90.00)
T_{10}	Control	90.00	0.00 (0.00)
S.E (m) ±		0.53	0.44
CD at 1%		2.12	1.79
CV (%)		5.32	1.12

2. *In vitro* evaluation of bioagents against *A. solani*

The results obtained on mycelial growth and per cent growth inhibition of *A. solani* with four fungal antagonists are presented in Table 5. It revealed that, all the bioagents exhibited fungistatic / antifungal activity against *A. solani* and significantly inhibited its growth over untreated control.

Among the tested bio agents, *T. viride* was found most effective with significantly least mycelial growth (18.00 mm) and highest mycelial growth inhibition of the test pathogen (79.78%) followed by *T. hamatum* (22.25 mm) and *T. koningii* (22.5 mm) which showed mycelial growth inhibition 75.00 per cent and 74.72 per cent, respectively, both treatments are at par with each other. However, *T. harzianum* was found comparatively less effective with maximum mycelial growth (30.00 mm) with minimum mycelial growth inhibition (66.29%).

Table 5: *In vitro* evaluation of bio agents against *A. solani*

Treatments	Treatments	Average colony diameter of test pathogen (mm)	Average inhibition of test pathogen (%)
T_1	*Trichoderma viride*	18.00	79.78 (63.29)
T_2	*Trichoderma koningii*	22.50	74.72 (60.13)
T_3	*Trichoderma hamatum*	22.25	75.00 (60.69)
T_4	*Trichoderma harzianum*	30.00	66.29 (54.96)
T_5	Control	89.00	0.00 (0.00)
S.E (m) ±		0.77	0.57
CD at 1%		3.22	2.57
CV (%)		4.25	2.08

Results of the present study on antifungal activity of the *T. viride*, *T. koningii*, *T. hamatum* and *T. harzianum* against *A. solani*. *T. viride* was found best among all the bio agents against *A. solani* was completely matching with the findings of Ganie *et al*., (2013) and Naik, *et al*., (2020) where they reported the efficacy of *T. viride* against the *A. solani*. Efficacy of *T. viride* was also reported by Waghe *et al*., (2014) that *T. viride* up to (70.27 per cent) mycelial growth inhibition against *A. helianthi*.

3. *In vitro* evaluation of botanicals against *A. solani*

Results obtained on mycelial growth inhibition of test pathogen with the botanicals at different concentrations revealed that, all the botanicals significantly inhibited mycelial growth of the test fungus over untreated control (Table 6)

Among the nine botanicals, most effective botanicals were found Garlic (5%), Mint (10%), Neem (10%) and Ghaneri (10%) which exhibited 78.15, 73.33, 67.41 and 65.56 per cent inhibition in mycelium growth, respectively. This was followed by Ginger (5%), Turmeric (5%), Tulsi (10%) in which 60.00, 59.26 and 58.52 per cent inhibition in mycelium growth was recorded. Botanicals Onion (5%) and and Nilgiri (10%) per cent showed the least inhibition in mycelium growth about 51.11 and 41.11 per cent, respectively.

Thus, all the botanicals showed antifungal effect against *A. solani* and significantly inhibited its mycelial growth over untreated control.

The toxicity of garlic bulb extract had been reported earlier by Balai and Ahir (2011), Naik *et al*., (2010) and Deshmukh *et al*., (2020) that is in accordance with present findings. The findings are exactly matching with the report of Anamika and Sobita (2011) and Naik *et al*., (2010) who noticed that leaf extracts from neem (*A. indica*) was the most effective against *A. solani*.

Table 6: *In vitro* evaluation of botanicals against *A. solani*

Treatments	Treatment Name	Average colony diameter (mm)	Average inhibition Over Control (%)
T_1	Garlic @ 5%	19.67	78.15 (62.14)
T_2	Onion @ 5%	44.00	51.11 (45.64)
T_3	Ginger @ 5%	36.00	60.00 (50.77)
T_4	Nilgiri@ 10%	53.00	41.11 (39.88)
T_5	Ghaneri@ 10%	31.00	65.56 (54.07)
T_6	Mint@ 10%	24.00	73.33 (58.91)
T_7	Turmeric @ 5%	36.67	59.26 (50.34)
T_8	Tulsi@ 10%	37.33	58.51 (49.91)
T_9	Neem@ 10%	29.33	67.41 (55.19)
T_{10}	Control	90.00	0.00 (0.00)
S.E. (m) ±		0.80	0.54
CD at 1%		3.23	2.17
CV (%)		3.47	2.00

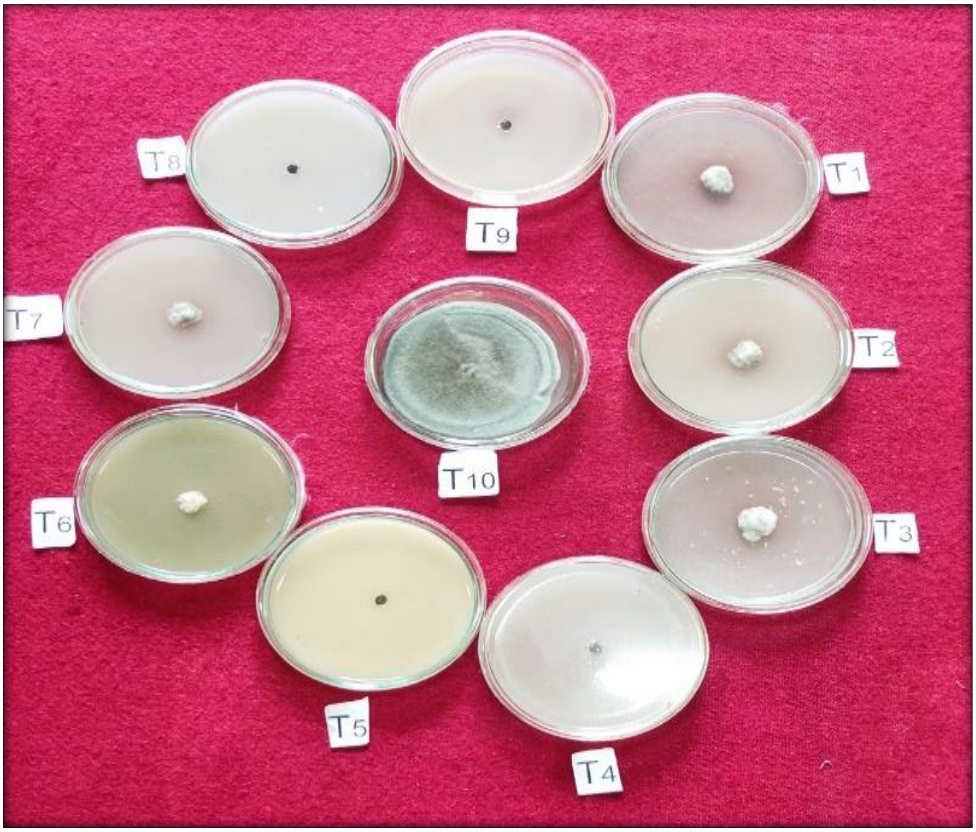

Fig 1: *In vitro* evaluation of different fungicides against *A. solani*

T_1 - Azoxystrobin @ 0.1%
T_2 - Azoxystrobin + Tebuconazole @ 0.1%
T_3 - Tebuconazole + Trifloxystrobin @ 0.05%
T_4 - Tebuconazole @ 0.1%
T_5 - Propineb @ 0.25%
T_6 - Carbendazim + Mancozeb @ 0.1%
T_7 - Thiophanate methyl @ 0.05%
T_8 - Difenoconazole @ 0.05%
T_9 - Propiconazole @ 0.1%

Fig 2: *In vitro* evaluation of different bio-agents against *A. solani*

T_1 - *Trichoderma viride*
T_2 - *Trichoderma koningii*
T_3 - *Trichoderma hamatum*
T_4 - *Trichoderma harzianum*

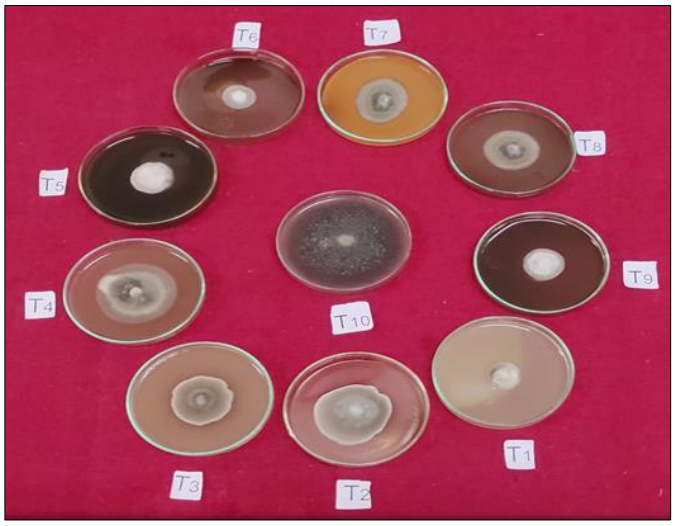

Fig 3: *In vitro* evaluation of different botanicals against A. solani

T_1 - Garlic @ 5%
T_2 - Onion @ 5%
T_3 - Ginger @ 5%
T_4 - Nilgiri @ 10%
T_5 - Ghaneri @ 10%
T_6 - Mint @ 10%
T_7 - Turmeric @ 5
T_8 - Tulsi @ 10%
T_9 - Neem @ 10%
T_{10} - Control

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