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The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(7): 589-594

www.thepharmajournal.com
Received: 05-05-2022
Accepted: 15-06-2022

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Evaluation of different plant extracts and fungicides against *Alternaria solani* initiating early blight of tomato (*Lycopersicon esculentum* Mill.)

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Abstract
Early blight disease of tomato caused by *Alternaria solani* (Ellis and Martin) Jones and Grout is an economically important disease causing massive losses throughout the country. Eleven plant extracts and 10 fungicides were tested *in-vitro* against *A. solani* in this study. At 10 percent concentration the most effective plant extract against *A. solani in-vitro* was leaf extract of *Ocimum sanctum*, inhibited mycelial growth by 65.00 percent. However leaf extract of *Tagetes erecta* inhibited mycelial growth by the minimum level (22.22%).The maximum inhibition of mycelial growth (68.33%) was observed with leaf extract of *Datura stramonium* at 15 percent concentration whereas least inhibition of mycelial growth observed in leaf extract of *Tagetes erecta* (38.14%). Contaf Plus, Folicur at 500 ppm, 750 ppm, 1000 ppm concentrations and Avtar at 1000 ppm, 1500 ppm, and 2000 ppm concentrations were the most effective fungicides.

Keywords: *Alternaria solani*, fungicides, *In-vitro*, plant extracts, tomato

Introduction
Tomato is a prominent vegetable crop in India and across the world. It is a self-pollinated crop that belongs to the Solanaceae family. Vitamins C and A are abundant in this fruit. Tomato contains lycopene, which has been linked to a variety of health advantages, including a lower risk of heart disease and cancer. It's also used as a table meal and in the preparation of ketchup, juice soup, and other items. Plant diseases such as bacterial, fungal and viral diseases, which reduce tomato production and quality, are quite common in the tomato crop. *Alternaria solani* (Ellis and Martin) Jones and Grout causes early blight, which is one of the most common and prominent fungal diseases of crops across the world (Jones *et al*., 1991) [5]. Early blight disease reduces fruit yield by 50 to 86 percent in the field and post-harvest phases (Mathur and Shekhawat, 1986) [10]. For every 1 percent increase in disease severity, the yield reduces by 1.36 percent. When the disease is at its worst, this leads in a complete crop loss. Saha and Das (2012) [15] reported yield losses ranging from 0.75 to 0.77 tonnes ha^{-1}. Each year, early blight is anticipated to inflict a 79 percent loss of crops (Adhikari *et al*., 2017) [1]. Fungicide treatments are the most effective control approaches, however they are not cost-effective in many regions of the world and may not be successful if the weather is favourable to outbreaks. The goal of this study was to record the effect of different plant extracts and fungicides on *Alternaria solani*, the causal agent of tomato early blight.

Materials and Methods
The experiment was conducted in the Department of Plant Pathology, Naini Agricultural Institute, Sam Higginbottom University of Agriculture, Technology & Sciences, Prayagraj, UP, during Rabi, 2020-21 in order to evaluate the efficacy of different plants extract and fungicides against *Alternaria solani*.

Isolation of pathogen
Early blight-infected tomato leaves were taken from the field and washed thoroughly with water before being put in between blotting paper to remove excess moisture. The infected portion of the leaf along with some healthy tissue were cut into small pieces (2-5mm) and surface sterilised with mercuric chloride (0.1%) for 30 seconds then washed three times with sterilised distilled water and then placed aseptically on solidified potato dextrose agar (PDA)

in petri plates. Inoculated petri plates were incubated at 25±2° C in a BOD incubator for pathogen mycelial development. The pure culture of the fungus was obtained by following hyphal tip culture under aseptic condition (Rangaswamy, 1972) [14].

Evaluation of plants extracts

The leaf extracts of plants viz., Neem (*Azadirachta indica* A. Juss.), Lantana (*Lantana camara* L.), Datura (*Datura stramonium* L.), Ashoka (*Polyalthia longifolia* (Sonn.), Yellow Kaner (*Cascabela thevetia* (L.) Lippold), Tulsi (*Ocimum sanctum* L.**),** Periwinkle (*Vinca rosea* L.), Marigold (*Tagetes erecta*), Congress grass (*Parthenium hysterophorus* L.), Bulb extract of Onion (*Allium cepa* L.**)** and Rhizome extract of Ginger (*Zingiber officinale* Roscoe) were evaluated against *Alternaria solani* using poison food technique *in- vitro* (Nene and Thapliyal, 1993) [12]. Fresh, healthy plant parts weighing 100 grams (leaf, bulb, and rhizome) were taken from the field, washed with tap water, air dried, and crushed in 100 ml sterile water (1:1 w/v). The macerate was first filtered through double-layered muslin cloth, and then centrifuged at 4000 rpm for 30 minutes. The supernatant was filtered through Whatman No. 1 filter paper. The resulting solution had a concentration of 100 percent (stock solution) which was subsequently diluted to the required concentrations of 10 percent and 15 percent. The nutritional medium was potato dextrose agar, and the required amount of each plant extract was added individually to get the required concentration of the plant extract. The plant extract was properly mixed with PDA medium before being sterilised for 20 minutes at 15 lbs pressure. Twenty millilitre of poisoned medium was poured to each of the 90 mm petri plate and allowed for solidification. PDA without plant extract was used as a control petri plates at the same time. Using a cork borer, one actively growing 7-days-old *A. solani* culture was carefully cut (5 mm) from the edge and transferred aseptically to the centre of each petri plate containing the poisoned/non-poison solid media and incubated at 25±2°C in BOD and radial growth was measured when fungus attained full growth in control plate. The experiment was conducted in completely randomized design (CRD) with three replications in each treatment. The significant difference of the treatments mean were compared using the Duncan's Multiple Range Test (DMRT) at P=0.05.

Evaluation of fungicides

The poison food technique was used to assess the efficacy of four systemic fungicides viz. Hexaconazole 5% SC (Contaf Plus), Tebuconazole 25.9% EC (Folicur), Propiconazole 25%EC (Tilt), Carbendazim 50% WP (Bavistin) at 500 ppm 750 ppm and 1000 ppm concentration each and six non systemic fungicides viz. Zineb 68% + Hexaconazole 4% WP (Avtar), Metalaxyl 4%+ Mancozeb 64% WP (Ridomil Gold), Carbendazim 12% +Mancozeb 63% (Saaf), Mancozeb 75% WP (Indofil M-45), Chlorothalonil 75% WP (Kavach), Copper oxychloride 50% WP (Blitox-50) at 1000 ppm, 1500 ppm and 2000 ppm concentration against *Alternaria solani*. Fungicides were added to the potato dextrose agar medium before sterilization as per treatment details (Table 2, 3&4) simultaneously, PDA without fungicides was poured in petri plate as control. Five mm disc of *A. solani* isolate was taken from seven days old culture and placed at centre of petri plate and incubated at 25±2 °C in BOD. The experiment was conducted in completely randomized design (CRD) with three replications in each treatment. The significant difference of the treatments mean were compared using the Duncan's Multiple Range Test (DMRT) at P=0.05.

Observations recorded

The colony diameter of the fungus on the poisoned medium was recorded when mycelial growth in control plate was full. Per cent inhibition of mycelium growth of the fungus was calculated by using the formula described by Vincent (1927) [18].

Results and Discussion

Eleven plant extracts were collected and evaluated *in-vitro* for antifungal activity against *A. solani*, the causal agent of tomato early blight at two concentrations (10% and 15%). The results presented in Table 1 indicated that all the tested plant extracts caused a significant reduction in the mycelial growth of *Alternaria solani* at 10 and 15 percent concentrations as compared to control. Maximum mycelial growth inhibition i.e. 65.00% was recorded by leaf extract of *Ocimum sanctum* (T_6) this treatment was at par with leaf extract of *Datura stramonium* (T_3) recorded 63.33 percent mycelial inhibition followed by leaf extract of *Azadirachta indica* (T_1) and rhizome extracts of *Zingiber officinale* (T_{11}) which inhibited mycelial growth of 55.00 percent and 54.44 percent respectively. However, leaf extract of *Tagetes erecta* (T_8) recorded minimum mycelial growth inhibition (22.22%) at 10 percent concentration. Treatments (T_2, T_4, T_5, T_7, T_9), (T_1, T_{11}) and (T_3, T_6) were non-significant among each other (Table 1, Plate I).

At 15 percent concentration leaf extract of *Datura stramonium* (T_3) recorded maximum mycelial growth inhibition (68.33%) followed by leaf extract of *Ocimum sanctum* (T_3) and bulb extract of *Allium cepa* (T_{10}) recorded 65.73 percent and 64.63 percent mycelial growth inhibition respectively. Minimum mycelial growth inhibition was recorded with leaf extract of *Tagetes erecta* (T_8) i.e. 38.14 percent. Treatments (T_7, T_2, T_9), (T_4, T_1) and (T_{10}, T_6) were non-significant among each other (Table 2, Plate II). It was observed that with the increase in the concentration of the extract, there was a corresponding increase in the inhibition of the pathogen. These observations are in confirmation with Verma *et al.* (2020)[17] who reported that under *in-vitro* condition leaf extracts of *D. stramonium* at 150 ppm concentration caused highest (68.60%) reduction of mycelia growth of *Alternaria solani*. Koley *et al.* (2015)[6] reported that *D. stramonium* aqueous leaf extract was the most effective, followed by *A. indica* oil and *L. camara* leaf extract, which inhibited fungal growth by 57.03, 51.35, and 48.02 percent, respectively. Nashwa (2011)[11] who reported that under *in-vitro* condition leaf extracts of *D. stramonium*, *A. indica*, and *A. sativum* at 5 percent concentration caused highest reduction of mycelia growth of *Alternaria solani*. These results also lined up with previous work on the role of plant extracts in the fungal disease control done by several workers including (Curtis *et al*., 2004, Krebs *et al*., 2006 and Latha *et al*., 2009) [3, 7, 8]. Certain plants containing products such as alkaloids, tannins, quinines, coumarins, phenolic compounds, phytoalexins, Ipomeamarone in the extracts and exudates are known for antifungal activities (Dattar,1999) [4].

Table 1: *In-vitro* evaluation of different plant extracts on the inhibition of mycelial growth of *Alternaria solani*

Treatments details	Average colony diameter of *A. solani* (mm)*		Percent inhibition of mycelial growth	
	At 10% concentration	At 15% concentration	At 10% concentration	At 15% concentration
T_1 - Neem leaf extract (*Azadirachta indica*)	40.50[b]	39.50[b]	55.00	56.11
T_2 -Lantana leaf extract (*Lantana camara*)	50.33[a]	48.17[a]	44.08	46.48
T_3 - Datura leaf extract (*Datura stramonium*)	33.00[c]	28.50	63.33	68.33
T_4 - Ashoka leaf extract (*Polyalthia longifolia*)	50.50[a]	40.33[b]	43.89	55.19
T_5 -Yellow Kaner leaf extract (*Cascabela thevetia*)	51.00[a]	44.50	43.33	50.56
T_6 -Tulsi leaf extract (*Ocimum sanctum*)	31.50[c]	31.17[c]	65.00	65.37
T_7 -Periwinkle leaf extract (*Vinca rosea*)	50.50[a]	48.50[a]	43.89	46.11
T_8 - Marigold leaf extract (*Tagetes erecta*)	70.00	55.67	22.22	38.14
T_9-Congress grass leaf extract (*Partheniu hysterophorus*)	51.50[a]	47.00[a]	42.78	47.78
T_{10} - Onion bulb extract (*Allium cepa*)	55.00	31.83[c]	38.89	64.63
T_{11} -Ginger rhizome extract (*Zingiber officinale*)	41.00[b]	37.00	54.44	58.89
T_0 -Control	90.00	90.00		
S.Em (±)	0.61	0.62		
CD (p=0.05)	1.80	1.83		

* Mean of three replications

Plate I & II: *In-vitro* efficacy of plant extracts against *Alternaria solani* at 10 and 15 percent concentration

Plate I: 10% concentration | **Plate II:** 15% concentration

T_1- Neem extract, T_2-Lantana extract, T_3- Datura extract, T_4- Ashoka extract, T_5-Yellow Kaner extract, T_6-Tulsi extract, T_7-Periwinkle extract, T_8- Marigold extract, T_9-Congress grass extract, T_{10}- Onion extract, T_{11}-Ginger extract, T_0- Control

Efficacy of different fungicides against *Alternaria solani*

Efficacy of four systemic and six non systemic fungicides were evaluated at different concentrations by poisoned food technique. The per cent mycelial growth inhibition over control was worked out based on the fungal growth in control plate. The results have been presented in table 2, 3, 4 and Plate III, IV & V. The results revealed that all the fungicides were inhibited the mycelial growth significantly over the control. Among the systemic fungicides at 500 ppm the most effective treatment was Contaf Plus (T_1) and Tilt (T_3) which inhibited 100 percent mycelial growth of the test fungus whereas among the non-systemic fungicides at 1000 ppm the most effective treatment was Avtar (T_5) which also inhibited 100 percent of mycelial growth of *A. solani* followed by Saaf (T_7) which inhibited mycelial growth to the extent of 88.33 percent. Treatments (T_6, T_9, T_{10}), (T_2, T_7), (T_1, T_3, T_5) were non-significant among each other.
(Table 2, Plate III).

At 750 ppm concentration among the systemic fungicides the most effective treatment was Contaf Plus (T_1) and Tilt (T_3) which inhibited 100 percent mycelial growth of *A.solani* whereas among the non-systemic fungicides at 1500 ppm the most effective treatment was Avtar (T_5) which also inhibited 100 percent of mycelial growth of *A. solani* followed by Saaf (T_7) which inhibited mycelial growth to the extent of 88.33 percent. Treatments (T_4,T_8, T_9), (T_2, T_7), (T_1, T_3,T_5) were non-significant among each other (Table 3, Plate IV).

At 1000 ppm concentration among the systemic fungicides, the

most effective treatment was Contaf Plus (T_1) and Tilt (T_3) which inhibited 100 percent mycelial growth of *A.solani* whereas among the non-systemic fungicides at 2000 ppm the most effective treatment was Avtar (T_5) which also inhibited 100 percent of mycelial growth of *A. solani* followed by Indofil M-45 (T_8) which inhibited mycelial growth to the extent of 88.89 percent. Treatments (T_4, T_6), (T_2, T_7, T_8) and (T_1, T_3, T_5) were non-significant among each other (Table 4, Plate V). Similar observations have also been reported by Patel *et al.* (2005) [13]. Chapei (2019) [2] reported that Hexaconazole 5EC at 0.1 percent, 0.2 percent and 0.3 percent concentration, Mancozeb 75WP at 0.2% concentration inhibited mycelial growth up to 94.44 and 93.33 percent respectively at all concentrations. Mahantesh *et al*. (2017) [9] reported that Mancozeb at 1000 ppm, Hexaconazole at 1000 ppm, and Carbendazim 12 percent + Mancozeb 63 percent at 0.2 percent recorded the maximum mycelial growth inhibition i.e. 88.42, 90.58, 88.07 percent respectively. Singh *et al*. (2018) [16] reported that Propiconazole at 50, 100, 150, and 200 ppm concentration was the most effective treatment at all concentrations tested against *A. solani*

Table 2: *In-vitro* evaluation of different fungicides on the inhibition of mycelia growth of *Alternaria solani*

Treatments	Concentration (ppm)	Average colony diameter(mm)*	Percent inhibition of mycelial growth
T_1 - Contaf Plus	500	0.00^c	100.00
T_2 - Folicur	500	10.50^b	88.33
T_3 -Tilt	500	0.00^c	100.00
T_4 - Bavistin	500	20.50	77.22
T_5 - Avtar	1000	0.00^c	100.00
T_6 - Ridomil Gold	1000	30.17^a	66.48
T_7 - Saaf	1000	10.50^b	88.33
T_8 - Indofil M-45	1000	25.50	71.67
T_9 - Kavach	1000	30.33^a	66.30
T_{10} -Blitox -50	1000	31.00^a	65.56
T_0- Control		90.00	
S.Em(±)		0.42	
CD (p=0.05)		1.25	

* Mean of three replications

Plate III & IV: *In-vitro* efficacy of different fungicides against *Alternaria solani*

Plate III: T_1- Contaf Plus, T_2 - Folicur. T_3 -Tilt, T_4- Bavistin (at 500 ppm) T_5 -Avtar, T_6 -Ridomil Gold, T_7- Saaf, T_8 - Indofil M-45, T_9- Kavach, T_{10} -Blitox -50 (at 1000ppm), T_0- Control

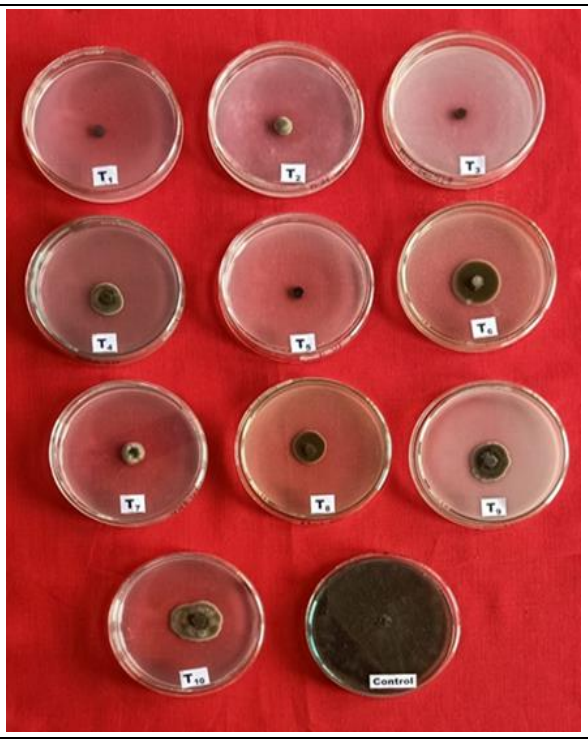

Plate IV: T_1- Contaf Plus, T_2 - Folicur. T_3 -Tilt, T_4- Bavistin (at 750 ppm) T_5 -Avtar, T_6 -Ridomil Gold, T_7- Saaf, T_8 - Indofil M-45, T_9- Kavach, T_{10} -Blitox -50 (at 1500ppm),T_0- Control

Table 3: *In-vitro* evaluation of different fungicides on the inhibition of mycelia growth of *Alternaria solani*

Treatments	Concentration (ppm)	Average colony diameter(mm)*	Percent inhibition of mycelial growth
T_1 - Contaf Plus	750	0.00[c]	100.00
T_2 - Folicur	750	10.67[b]	88.15
T_3 -Tilt	750	0.00[c]	100.00
T_4 - Bavistin	750	20.17[a]	77.59
T_5 - Avtar	1500	0.00[c]	100.00
T_6 - Ridomil Gold	1500	30.17	66.48
T_7 - Saaf	1500	10.50[b]	88.33
T_8 - Indofil M-45	1500	20.50[a]	77.22
T_9 - Kavach	1500	21.00[a]	76.67
T_{10} -Blitox -50	1500	25.33	71.85
T_0- Control		90.00	
S.Em (±)		0.57	
CD (p=0.05)		1.67	

*Mean of three replications

Table 4: *In-vitro* evaluation of different fungicides on the inhibition of mycelia growth of *Alternaria solani*

Treatments	Concentration (ppm)	Average colony diameter(mm)*	Percent inhibition of mycelial growth
T_1 - Contaf Plus	1000	0.00[c]	100.00
T_2 - Folicur	1000	10.00[b]	88.89
T_3 -Tilt	1000	0.00[c]	100.00
T_4 - Bavistin	1000	19.83[a]	77.97
T_5 - Avtar	2000	0.00[c]	100.00
T_6 - Ridomil Gold	2000	20.50[a]	77.22
T_7 - Saaf	2000	10.50[b]	88.33
T_8 - Indofil M-45	2000	10.00[b]	88.89
T_9 - Kavach	2000	12.33	86.30
T_{10} -Blitox -50	2000	25.00	72.22
T_0- Control		90.00	
S.Em (±)		0.39	
CD (p=0.05)		1.14	

Plate V: *In-vitro* efficacy of different fungicides against *Alternaria solani*

Conclusion

It is concluded that the fungicides were found more effective as compared to plant extracts for the management of *Alternaria solani* initiating early blight disease in tomato. At all concentrations fungicides Contaf Plus, Folicur (500 ppm, 750 ppm & 1000 ppm), Avtar (1000 ppm, 1500 ppm & 2000 ppm) and Leaf extracts of *Datura stramonium* at 15 percent concentration were found most significant in checking the mycelial growth of *A.solani*.

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