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Management of tomato early blight [*Alternaria solani* (Ellis & Martin) Sorauer] through botanicals and fungicides under *in-vitro* and *in-vivo* conditions

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ABSTRACT

Early blight is the most harmful and dangerous disease of tomato worldwide. In the present investigation, five plant extracts and five fungicides were evaluated against *Alternaria solani* under *in-vitro* condition and effective botanicals and fungicides were evaluated under field condition. Among all the botanicals, *Azadirachta indica* leaf was found most effective botanicals under *in-vitro* evaluation followed by *Datura fastuosa* leaf and *Allium cepa* leaf. Among the fungicides, minimum mycelial growth of *Alternaria solani* was found in Fluciazole 40% followed by Carbendazim 50% WP, Difenoconazole 25% EC, Azoxystrobin 23% SC and Mancozeb 75% WP. In field experiment, Fluciazole 40% EC and Carbendazim 50% WP found most effective among all the treatments.

Key words: Early blight of tomato, Alternaria solani, Botanicals, Fungicides, In-vitro, in-vivo

Introduction

The tomato is one of India's most vital and wellliked vegetables and a member of the Solanaceae family. It originated in Mexico and spread progressively throughout the world. Nowadays, a vast variety of crops are grown both outside and within greenhouses. Next to potato and sweet potato, which lead the list of canned vegetables, it is the third most consumed vegetable crop in the world (Chowdhury, 1979). Vitamin A, Vitamin C, iron, calcium, and minerals are all present in tomatoes in good amounts (Matin et al., 1996). Across the world, including India, it is one of the most well-liked and nutrient-dense vegetables. Due to the attack of numerous diseases incited by fungi, viruses, bacteria, and nematodes, tomato production of India is low in comparison to other developed nations. One of the most damaging fungal diseases to affect tomato in tropical and subtropical areas is early blight disease, which is caused by *Alternaria solani*. The airborne and soil-dwelling causative fungi that causes tomato leaf spot blight, seedling collar rot, and fruit rot (Datar and Mayee, 1981). The early blight was the most devastating disease, causing losses both before and after harvest and a production decrease of 35 to 78%. (Jones et al., 1993). At all phases of plant development, early blight manifests a wide variety of symptoms. Under favourable conditions, the disease manifests itself on leaves, petioles, stems, twigs, and fruits, which defoliates, dries up the twigs, and prematurely drops its fruit (Mathur and Shekhawat, 1986). It results in leaf blight, seedling collar rot, fruit rot, damping-off, stem canker, and other plant diseases. The fungus can also spread to tomato fruit that is both green and ripe. The calyx end is typically where infection starts. At infection locations, brown leathery patches SINGH ET AL S399

are S developed. Usually, an infection starts at the calyx end. The infected sites developed brown leathery patches.

The disease is controlled by the application of several conventional fungicides, but the development of resistance in the majority of pathogen against fungicides as well as the risk of exposure, fungicide residues, and human health hazards have pushed for the development of alternatives to control A. solani (Patil et al., 2002). Adopting such ecologically smart and environmentally safe management mechanisms is important. Due to their lower impact on the environment, natural products are thought to be the ideal substitute for synthetic chemicals in this regard. For preventing the spread of disease and lowering the formation of airborne spores, many botanicals are utilised (Sharma and Kumar, 2009; Bowers and Locke, 2004). For both in vitro and in vivo management of early blight and other plant diseases, plant extracts also have antifungal action (Wszelaki and Miller, 2005; Kagale et al., 2004). The current study's goal is to assess the antifungal efficacy of botanicals and fungicides plant extracts against A. solani in vitro using the food poisoning technique and under field condition.

Materials and Methods

The present investigation was carried out of evaluation of botanicals and plant extract against Alternaria solani causing early blight of tomato under *invitro* and *in-vivo* conditions. In-vitro experiments were conducted in laboratory of School of Agriculture, ITM university, Gwalior and field experiment was conducted at Crop Research Center, Farm Turari, ITM University, Gwalior, during 2021-22.

In-vitro evaluation of botanicals

Five botanicals, including Azadirachta indica leaf, Allium cepa leaf, Citrus limon leaf, Datura fastuosa leaf, and Momordica charantia leaf, were assessed in a lab experiment to see how well they inhibited the growth of Alternaria solani under in vitro conditions using the technique of poisoned food (Nene and Thapliyal, 1979). The experiment was replicated four times and set up using a Completely Random Design. The leaves were picked while they were healthy and fresh, completely cleaned with fresh water, and then rinsed with sanitised distilled water before being air dried on newspaper. 50 grammes of fresh leaves were split into smaller pieces and

minced using a grinder with the addition of 50 ml of distilled water that had been sterilised. In 150 ml conical flasks, the phyto extracts were filtered through double-layered muslin fabric and the flask was plough with non-absorbent cotton. These filtered extracts then sterilized in autoclave for 15-20 minute at 15 psi. The autoclaved phytoextract were mixed individually in Potato Dextrose Agar medium @ 10 % at the time of pouring. After pouring all the plates were allowed for 15-20 minute for solidification. After solidification, all the plates were inoculated with 5 days old cure culture of Alternaria solani isolated from early blight infected leafAs a control, a plate without phyto extract was used. Details of treatment were mentioned on the petri plate with the help of marker and all the plates were placed in BOD incubator at 25±1 °C. The data on mycelial growth in all the botanicals and control was recorded at four and six days after inoculation (DAI) and mycelial inhibition per cent was calculated by formula given below (Vincent, 1947).

$$PGI = \frac{C-T}{C} \times 100$$

where,

PGI = Per cent growth inhibition

C = Growth in control

T = Growth in treatment

In-vitro evaluation of fungicides

fungicides, including Mancozeb, Carbendazim, Difenoconazole, Fluciazole and Azoxystrobin were assessed 500 ppm concentration in a lab experiment to find out the efficacy against the growth of Alternaria solani under in vitro conditions using the technique of poisoned food (Nene and Thapliyal, 1979). The experiment was replicated four times and set up using a CompletelyRandom Design. To achieve the appropriate fungicide concentration, the necessary amount of each fungicide was added separately to molten, sterilised Potato dextrose agar before it cooled. The poisoned medium was then put 20 ml at a time into sterile Petriplates. Without fungicide being added to the medium, control was preserved. All the plates were inoculated with 5 days old pure culture of Alternaria solani isolated from early blight infected leaf. Details of treatment were mentioned on the petri plate with the help of marker and all the plates were placed in BOD incubator at 25±1 °C. The data on mycelial growth in all the fungicides and control was recorded at four and six days after inoculation (DAI) and mycelial inhibition per cent was calculated by above formula (Vincent, 1947).

Field evaluation of botanicals and fungicides

After In vitro evaluation of botanicals and fungicides, effective botanicals and fungicides were selected for field experiment. In the present investigation, the efficacy of three botanicals viz., Azadirachta indica leaf, Allium cepa leaf and Datura fastuosa leaf and five fungicides viz., Mancozeb, Carbendazim, Difenoconazole, Fluciazole and Azoxystrobin were tested against early blight of tomato. The experiment was conducted in Randomised Block Design with three replications. With a row to row distance of 60 cm and a plant to plant distance of 45 cm, the plot measured 5 x 2.25 metres. In order to apply the appropriate amount of fertilisers (N: P: K-120:50:50kg/ha), urea, single super phosphate (SSP), and muriate of potash (MOP) were used. As a basal dressing, full doses of phosphorus P₂O₅ and K₂O were placed in the furrows at the time of sowing, half doses of N were applied at the time of transplanting, and half doses were applied 30 and 60 days later. At 30 days following seeding, plants were artificially inoculated by spraying A. solani mycelial suspension on plants. 15 days following the onset of the disease, two sprays of each fungicide and botanical were used. Each fungicide was evaluated at 0.1 % concentration and all botanicals were evaluated at 10 % concentration. Fiveplants were chosen randomlyfrom each plot and three leaves (top, middle, and bottom) were selected from each plant which were used to measure the severity of the tomato early blight disease using a 0 to 5 scale (Table 1). Data on early blight severity was recorded prior to spray, 10 days after first and second sprays. Per cent disease reduction of all the treatments were calculated by following formula.

$$A = \frac{B-C}{B} \times 100$$

Where,

A=Per cent disease reduction

B=Disease severity in control

C=Disease severity in treatment

Results and Discussion

In-vitro evaluation of botanicals

In the present investigation, the efficacy of five bo-

Table 1. Standard scale for assessment of disease intensity of tomato early blight

Leaf / fruit area affected	Grade
No infection	0
Up to 5 percent	1
6 – 10 percent	2
11 – 20 percent	3
21 – 50 percent	4
More than 50 percent	5

tanicals were tested against Alternaria solani under in-vitro evaluation and data on mycelial growth of pathogen was recorded at four and six DAI (Table 2). At four days after inoculation, A. indica leaf (30.25 mm) had the lowest mycelial growth of A. solani, followed by D.fastuosa leaf (33.75 mm), A. cepa leaf (35.00 mm) M.charantia (41.75 mm), C. limon Leaf (43.25 mm), whereas the maximum mycelial growth of pathogen was recorded in control (49.75 mm). At six days after inoculation, A. indica leaf had the lowest mycelial growth of A. solani (43.00 mm), which was shown to be significantly better than other botanicals. The next-best botanical was *D.fastuosa* leaf (49.75 mm), followed by A. cepa leaf (51.25 mm), M.charantia (64.75 mm), and C. Limon Leaf (66.75 mm), while the control saw the highest mycelial growth (75.00 mm). Allium sativum (garlic) was reported to be the most effective botanical (plant extract), while A. indica (neem) was found least effective against A. solani by Deshmukh et al. (2020). Naik et al. (2020) evaluated five plants against A. solani and reported that maximum inhibition of pathogen was offered by A. indica. The poisoned food technique was employed by Kumar et al. (2021) to test five plant extracts against A. solani and reported that A. sativum had a lowest mycelium of the pathogen followed by *D. stramonium* and *A. indica*. In this study, it was revealed that A. indica had the highest amount of mycelium inhibition. The findings were supported by Ganie et al. (2013). Balai and Ahir (2011) obtained findings that were similar to those that were shown above.

In-vitro evaluation of fungicides

Efficacy of five fungicides were tested in the present investigation reveals data (Table 3) all the fungicides significantly reduced the radial growth of pathogen as compared to control. At four days after inoculation, Fluciazole 40 % (9.50 mm) had the least mycelial growth of pathogen, and it was on par with carbendazim 50 % (10.75 mm) and difenoconazole

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25 per cent (12.25 mm). Highest mycelial growth 48.75 mm was observed in the control, followed by Mancozeb 75 % (17.75 mm) and Azoxystrobin 23 % (14.50 mm). at six days after inoculation, all the fungicides considerably suppressed the mycelial growth of A. solani. The most effective fungicide was Fluciazole 40 % (12.25 mm), which was shown to be comparable to Carbendazim 50 % WP (15.00 mm). The following effective fungicides were Difenoconazole 25 % EC (18.50 mm), Azoxystrobin 23 % SC (23.75 mm), and Mancozeb 75 % WP (28.00 mm), while the control had the highest mycelial growth (75.00 mm). Osowski (2003) claimed that vitavex completely stopped the mycelial development of A. solani. According to Patel and Chaudhury (2010), mycelium of A. solani was fully suppressed by difenconazole, mancozeb, and metalaxyl + Mancozeb. According to Mohan Venkata Siva Prasad et al. (2018), A. macrospora was inhibited from growing by mancozeb, carbendazim, hexaconazole, propiconazole, and carbendazim + mancozeb 1%. The most successful treatment against A. solani was pyroclostrobin (Farooq et al., 2019; Husain et al., 2020). According to Singh and Singh (2006) and Mesta *et al.* (2009), hexaconazole was found to have a 100% inhibition rate, making it the most effective treatment. Fusilazole 40 percent EC 100 reportedly prevented the mycelial growth of *A. solani*, according to Tiwari *et al.* (2019).

Field evaluation of botanicals and fungicides

Three botanicals and five fungicides were selected for field experiment which were found effective in invitro evaluation (Table 4). Data on severity of early blight was recorded before spray showed that there is no significant difference between any treatment. At 10 days after first spray, No variation found in any treated before spray. Data on disease severity before spray was showed Fluciazole 40 % EC (16.56 %) had the lowest disease severity, and it was determined to be comparable to Carbendazim 50% WP, Difenoconazole 25% EC and Azoxystrobin 23 % SC with 17.44, 19.11 and 19.78 per cent severity respectively. Mancozeb 75 % was the next best treatment with 20.81 per cent disease severity followed by *A*. indica leaf (23.33 %), D.fastuosa leaf (25.77) and A. cepa leaf (27.22 %). Untreated contract had highest severity (29.33 %) of early blight. In terms of statis-

Table 2. Efficacy of botanicals against Alternaria solani under in-vitro conditions

Treatment	4 DAI		6 DAI	
	Mycelial growth	Per cent inhibition	Mycelial growth	Per cent inhibition
Azadirachtaindica leaf	30.25	39.20	43.00	42.67
Allium cepa leaf	35.00	29.65	51.25	31.67
Citrus limon Leaf	43.25	13.07	66.75	11.00
Datura fastuosa leaf	33.75	32.16	49.75	33.67
Momordica charantia leaf	41.75	16.08	64.75	13.67
Control	49.75	0.00	75.00	0.00
SE.m.±	1.33	-	1.74	-
C.D. at 1 %	3.96	-	5.16	-

Table 3. Efficacy of fungicides against Alternaria solani under in-vitro conditions

Treatment	4 DAI		6 DAI	
	Mycelial growth	Per cent inhibition	Mycelial growth	Per cent inhibition
Mancozeb 75% WP	17.75	63.59	28.00	62.67
Carbendazim 50% WP	10.75	77.95	15.00	80.00
Difenoconazole 25% EC	12.25	74.87	18.50	75.33
Fluciazole - 40% EC	9.50	80.51	12.25	83.67
Azoxystrobin 23% SC	14.50	70.26	23.75	68.33
Control	48.75	0.00	75.00	0.00
SE.m.±	0.95	-	1.43	-
C.D. at 1 %	2.82	-	4.24	-

Table 4. Evaluation of botanicals and	d fungicides against	early blight of tomato
	0	9

Treatments	Dose	Disease severity		
		Before Spray	After Istspray	after II nd spray
Mancozeb 75% WP	0.1%	12.78 (20.88)	20.78 (27.09)	17.89 (24.96)
Carbendazim 50% WP	0.1%	12.44 (20.63)	17.44 (24.66)	15.44 (23.04)
Difenoconazole 25% EC	0.1%	13.89 (21.87)	19.11 (25.92)	16.56 (23.96)
Fluciazole - 40% EC	0.1%	12.55 (20.72)	16.56 (23.98)	12.78 (20.93)
Azoxystrobin 23% SC	0.1%	11.44 (19.65)	19.78 (26.38)	17.33 (24.53)
Azadirachta indica leaf	10%	13.00 (21.12)	23.33 (28.88)	22.00 (27.97)
Allium cepa leaf	10%	11.11 (19.43)	27.22 (31.43)	25.78 (30.51)
Datura fastuosa leaf	10%	11.00 (19.36)	25.78 (30.47)	25.11 (30.05)
SE.m.±		0.88	1.00	1.19
C.D. at 5 %		2.63	3.01	3.56

Figures in parentheses are angular transformed value

tics, there was no significant variance between the control, A. cepa leaf, and D.fastuosa leaf. All the fungicides and botanicals considerably minimized the disease severity at 10 days after the second spray. Spray with Fluciazole 40% EC was recorded least disease severity (12.78 %) which was on par with Carbendazim 50% WP and Difenoconazole 25% EC with 15.44 and 16.86 per cent disease severity respectively. Azoxystrobin 23% SC was the next best treatment with 17.33 per cent disease severity followed by Mancozeb 75% WP, A. indica leaf D.fastuosa leaf A. cepa leaf with 17.89, 22.00, 25.11 and 25.78 per cent disease severity respectively. Neem extract decreased disease under in vivo condition, according to Murmu et al. (2015). Hexaconazole was reported by Sudarshana et al. (2012) and Yadav et al. (2018) to minimize disease severity and boost yield. According to Sharma et al. (2018) and Raavi Sreenivasulu et al. (2019), the application of Propiconazole decreased disease and boosted tomato yield. Additionally, many different substances have been used to treat this disease, such as Thiram 75 percent, which was revealed to be very beneficial in field conditions (Nayyar et al., 2014). Chlorthalonil, Mancozeb, Hexaconazole, and Azoxystribin are some additional fungicides that have shown to be quite efficient against this disease and have assisted in reducing its severity in a variety of crops (Prasad and Naik, 2003; Singh and Singh, 2006). In one of the investigations, topsin-M was found to be highly effective against this pathogen when used at a rate of 0.2% under field conditions (Katiyar et al., 2001). Another investigation discovered that certain substances, including Indofil M-45, Indofil Z-78, and carboxin, were particularly effective against this fungus (Singh and Rai, 2003).

Conclusion

In the laboratory experiment on evaluation of botanicals against Alternaria solani, *A. indica* leaf was found most effective botanical. In another in vitro experiment, Fluciazole 40 % and Carbendazim 50 % WP were found effective against the pathogen. In field experiment, all the treatment including fungicides and botanicals significantly reduced the severity of early blight, but comparatively fungicides were found more efficient to minimize the severity of early blight compared to botanicals.

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