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Evaluation of different fungicides against *Alternaria solani* (Ellis & Martin) Sorauer cause of early blight of tomato under laboratory conditions

[1] Muhammad Usman Ghazanfar, [2] Waqas Raza, [*3] Kanwer Shahzad Ahmed, [4] Jamshaid Qamar, [5] Naveed Haider, [6] Muhammad Hamid Rasheed

[1, 2] Department of Plant Pathology, University College of Agriculture, University of Sargodha, Sargodha, Punjab, Pakistan
[3, 6] Department of Entomology, University College of Agriculture, University of Sargodha, Sargodha, Punjab, Pakistan
[4] Department of Agronomy, University College of Agriculture, University of Sargodha, Sargodha, Punjab, Pakistan
[5] Department of Entomology, University of Agriculture, Faisalabad, Punjab, Pakistan

Abstract
Early blight of tomato caused by *Alternaria solani* (Ellis & Martin) Sorauer is the most destructive disease that hampered its successful production all over the world. In the present study, fungi toxic activity of fungicides, namely Propineb (Antracol® 70 WP), Thiophanate-methyl (Topsin-M® 70 WP), Chlorothalonil (Kavach® 75 WP), Mancozeb (Dithane® M-45 80 WP) and Copper oxychloride (Blitox® 50 WP) was evaluated by poisoned food technique at laboratory of Plant Pathology, University college of Agriculture, University of Sargodha, Pakistan during 2013-14 for the management of early blight of tomato caused by *Alternaria solani*. All the tested chemicals significantly ($P \leq 0.01$) inhibited the mycelial growth of a pathogen when compared with control. However, among all five tested fungicides, Dithane® M-45 80 WP (89.83%) was significantly superior over other treatments followed by Antracol® 70 WP (87.40%), Topsin-M® 70 WP (87.10%), and Blitox® 50 WP (79.21%). This reduction was gradually increased by increasing the incorporated concentration. Least inhibition was observed in Kavach® 75 WP (70.40%). Overall results demonstrated that all tested concentrations of Dithane® M-45 80 WP were found significantly effective for controlling early blight of tomato.

Keywords: *Alternaria* solani, early blight, Fungicides, *Lycopersicon esculentum* Mill, Inhibition percentage

1. Introduction
Tomato (*Lycopersicon esculentum* Mill.) is most important solanaceous vegetable and fruit crop, ranked first by sharing it 14% of the total fruit and vegetable production worldwide [1, 2]. The world annual production of tomato was 100 million tons covered an area of 4 million hectares with financial benefit of 5 to 6 billion US$ [3]. In the world acreage, tomato crop ranks first among processing crops [1]. Tomatoes are being grown in all four provinces of Pakistan, over an area of about 52,300 hectors with annual production of 530,000 tons in 2011-12 [4].
Tomato is consumed in our daily life because it contains good source of antioxidants and also have balanced source of vitamins i.e. A, C and E, which is necessary for metabolic activities for maintaining good human health [5]. Adaptability in relation to different habitats and high nutritive value made tomato more popular in recent years. Various factors are responsible for low yield and among them diseases are of most concern. Tomato crop is prone to different fungal, bacterial, nematode and viral diseases. Among the fungal diseases, early blight of tomato caused by *Alternaria solani* Sorauer is the most worst and caused large destruction in terms of both quality and quantity [6, 7]. It is soil inhibiting and air borne pathogen found mostly in tropical and sub-tropical regions. It caused bull eye pattern in which concentric rings with brown to black spots appeared on lower side of infected leaves (Fig. 1) [8]. Leaves of infected plant become pale yellow and dry afterward [9]. During humid weather conditions, these lesions become unite and leading to premature defoliation. Fruit drop and blossom blight symptoms are also due to this pathogen depending on the plant parts affected. During the fruiting period, tomato plant is more vulnerable to early blight infection [10, 11].
During the last few years, it has been noticed that early blight found most openly due to favorable environmental conditions and currently its management is done excellently with the use of different fungicides [12, 13]. Inappropriate use of chemicals lead to serious human health hazards so proper concentration of fungicides at proper intervals should be mandatory [14-16]. The main objective of this study was to evaluate the *in-situ* efficacy of different fungicides with different concentrations against early blight of tomato.

2. Materials and Methods
This present study was conducted in Laboratory of Plant Pathology, University College of Agriculture, University of Sargodha (Sargodha, Pakistan) during 2013-2014, in order to evaluate the efficacy of different fungicides against early blight of tomato. The pathogen was isolated from infected tomato leaves and then these leaves cut into small pieces along with growing margins of about 1.5-2cm. Surface of these leave cuttings sterilized with 0.1% bleach for approximately 2 minutes then washed three times with distilled water and placed on petri plates containing potato dextrose agar (PDA). These petri plates were incubated at 26 ±1°C for one week to check the sporulation for further studies. Pure culture was obtained with the help of single spore technique by incubating at about 28°C for seven days and observed it daily to get rid of contamination such as bacteria and other pathogens [17].

Fig 1: Symptoms of early blight on leaves of tomato

Fig 2: Conidia of *A. solani* (40x)

On the cause of symptomology and conidial characteristics of the fungus, was identified as *A. solani*, causative agent of early blight of tomato (Fig 2) [18].

2.1 Evaluation of fungicides

Five different fungicides at different tested concentrations (Table 1) i.e. 0.1, 0.2 and 0.3% and each concentration replicated thrice through use of poisoned food technique [19] against *A. solani*, causative agent of early blight of tomato. Insecticides were obtained from registered pesticide dealers located in the local grain market of district Faisalabad (Punjab, Pakistan).

Table 1: Fungicides description

Trade names	Common names	Formulation
Antracol®	Propineb	70 WP
Topsin-M®	Thiophanate-methyl	70 WP
Kavach®	Chlorothalonil	75 WP
Dithane® M-45	Mancozeb	80 WP
Blitox®	Copper oxychloride	50 WP

2.2 Preparation of fungicides concentration

Molten pure PDA was used and required concentration of each fungicide was added separately so as to get a requisite concentration of that chemical used. The fungicides were thoroughly mixed by rousing and about 15 ml poisoned medium was poured to each of the 90 mm petri dishes and permitted for solidification. The actively growing periphery of the seven days old culture of the pathogen, *A. solani* was carefully cut using cork borer and transferred aseptically to the center of each solid petri dish containing the poisoned medium. Appropriate control was maintained by growing the culture on PDA having no fungicide. The plates were incubated at 26 ± 1°C for seven days and the colony diameter was recorded after seven days growth. After the inoculation, data was taken starting from 24 hours after application for period of 7 days and measured the mycelial mean growth (millimeter, mm) per plate according to the description [20].

2.3 Data analysis

Recorded data was subjected to statistical analysis by using R-software. Lease significant test was used to determine the most significant treatment [21].

3. Results

Five fungicides were evaluated *in vitro* at three concentrations for knowing their efficacy against the pathogen, *A. solani* causing early blight of tomato through poisoned food technique. The results showed that all tested fungicides with all concentrations significantly ($P \leq 0.01$) inhibit the mycelial growth of the pathogen compared with control (Table 2, 3 and 4).

The fungicides evaluated after seven days of colony growth by taken average mycelial growth and inhibition percentage of all tested concentrations. Data regarding mycelial growth (Table 4) revealed that Dithane® M-45 was the most effective fungicide on reducing the average linear mycelial growth of *A. solani* (27.61 mm) followed by Antracol® (31.80 mm), Topsin-M® (38.13 mm), Blitox® (42.02 mm) while Kavach® was the least effective (44.22 mm). Results also indicated that the inhibition percentage was increased by increasing the concentrations of fungicides tested. Dithane® M-45 fungicide inhibited the mycelial growth of *A. solani* (62.29%) followed by Antracol® (56.56%), Topsin-M® (47.91%), Blitox® (42.59%) while Kavach® was the lowest effective one (39.6%).

Table 2: Efficacy of different fungicides *in vitro* against the growth of *A. solani*

Fungicides	Concentrations %					
	Linear area	Control	0.1%	0.2%	0.3%	Mean
Dithane® M-45	CG.	29.67±0.50[a]	1.22±0.44[b]	1.22±0.44[c]	11.56±0.52[d]	10.91
	IP.	00	95.88	95.88	61.03	63.19
Antracol®	CG.	29.67±0.50[a]	3.78±0.44[b]	1.22±0.44[c]	1.22± 0.44[c]	8.97
	IP.	00	87.25	95.88	95.88	9.75
Topsin-M®	CG.	29.67±0.50[a]	11.44±0.5[b]	1.33±0.50[c]	0.00±0.0[d]	10.61
	IP.	00	61.44	95.51	100	64.23
Blitox®	CG.	29.67±0.50[a]	11.56±0.52[b]	6.33±0.50[c]	2.22 ±0.44[d]	12.44
	IP.	00	61.03	78.66	92.51	58.05
Kavach®	CG.	29.67 ±0.5[a]	7.33± 0.5[b]	5.67± 0.50[c]	2.67± 0.5[d]	11.33
	IP.	00	75.29	80.89	91.00	61.79

CG= Colony growth IP=Inhibition percentage
Means within the column with same letters are statistically non-significant

Table 3: Mycelium growth (mm) and inhibition (%) after 5 days colony of *A. solani* on PDA influenced by different concentrations of different fungicides

Fungicides	Concentrations %					
	Linear area	Control	0.1%	0.2%	0.3%	Mean
Dithane® M-45	CG.	54.00±0.70[a]	7.78±0.44[b]	4.78± 0.44[c]	2.22± 0.44[d]	17.9
	IP.	00	85.59	91.14	95.88	68.15
Antracol®	CG.	54.00±0.70[a]	11.67±0.5[b]	4.44±0.52[c]	3.89 ±0.33[d]	18.5
	IP.	00	78.38	91.77	92.79	65.73
Topsin-M®	CG.	54.00±0.70[a]	28.67±0.5[b]	7.33±0.50[c]	2.78± 0.44[d]	23.19
	IP.	00	46.90	86.42	94.85	57.05
Blitox®	CG.	54.00±0.70[a]	27.22±0.6[b]	13.33±0.5[c]	7.56± 0.52[d]	25.52
	IP.	00	49.59	75.31	86.00	52.72
Kavach®	CG.	54.00±0.70[a]	22.41±0.5[b]	17.67±0.5[c]	10.56±0.52[d]	26.16
	IP.	00	58.05	67.27	80.48	51.45

CG= Colony growth IP=Inhibition percentage
Means within the column with same letters are statistically non-significant

Table 4: Mycelium growth (mm) and inhibition (%) after 7 days colony of *A. solani* on PDA influenced by different concentrations of different fungicides

Fungicides	Concentrations %					
	Linear area	Control	0.1%	0.2%	0.3%	Mean
Dithane® M-45	CG.	73.22±0.83[a]	16.33±0.5[a]	13.44±0.7[b]	7.44± 0.52[d]	27.61
	IP.	00	77.69	81.64	89.83	62.29
Antracol®	CG.	73.22±0.83[a]	27.44±0.5[b]	17.33±0.5[c]	9.22± 0.44[d]	31.80
	IP.	00	62.52	76.33	87.40	56.56
Topsin-M®	CG.	73.22±0.83[a]	50.33±0.5[b]	19.56±0.5[c]	9.44± 0.52[d]	38.13
	IP.	00	31.26	73.28	87.10	47.91
Blitox®	CG.	73.22±0.83[a]	49.67±1.3[b]	30.00±0.8[c]	15.22±0.83[d]	42.02
	IP.	00	32.16	59.02	79.21	42.59
Kavach®	CG.	73.22±0.83[a]	44.22±0.6[b]	37.78±0.4[c]	21.67±0.50[d]	44.22
	IP.	00	39.60	48.40	70.40	39.6

CG= Colony growth IP=Inhibition percentage
Means within the column with same letters are statistically non-significant

The mycelial growth of *A. solani* showed a different trend in response to different tested fungicides. The mycelial growth of pathogen was significantly different within the 3 concentrations of each fungicide. Data (Table 4) regarding mycelial growth of the tested pathogen revealed that at 0.1% concentration the lowest growth and highest inhibition percentage was observed in the treatment Dithane® M-45 (16.33 mm and 77.69%) followed by Antracol® (27.44 mm and 62.52%), Kavach® (44.22 mm and 39.60%), Blitox® (49.67 mm and 32.16%) and the highest in the control (73.22 mm).While fungicide Topsin-M® found to be least effective (50.33 mm) with 31.26 inhibition percentage. Fungicides treatment at 0.2% concentration differed significantly ($P \leq 0.01$) from one another in which the lowest growth and highest inhibition percentage were showed in treatment Dithane® M-45 (13.44 mm and 81.64%) followed by Antracol® (17.33 mm and 76.33%), Topsin-M® (19.56 mm and 73.28%) and Blitox® (30.00 mm and 59.02%). Kavach® showed less effectiveness in terms of mycelial growth (37.78 mm) and inhibition percentage (48.40%). Growth of pathogen (*A. solani*) varied significantly ($P \leq 0.01$) at 0.3% concentration of different

fungicides tested. Highest growth was observed in control (73.22 mm) while lowest in the treatment Dithane® M-45 (7.44 mm). These two treatments were significant difference to one another and also from other treatments followed by Antracol®, Topsin-M®, Blitox® and Kavach® (9.22mm, 9.44mm, 15.22mm and 21.67mm) and (87.40%, 87.10%, 79.21% and 70.40%) respectively.

4. Discussion

The effect of five fungicides, i.e. Dithane® M-45, Antracol®, Topsin-M®, Blitox® and Kavach® were tested *in vitro* for their inhibitory effect on the linear growth of *A. solani*, the causal of tomato early blight. Obtained data revealed that all tested chemicals caused significant reduction in linear growth of *A. solani* but among them Dithane® M-45 and Topsin-M® were found most capable of inhibiting infection. The inhibitory effect of chemicals on fungus reported by many researchers, earlier [7, 22-24]. All tested concentrations of mancozeb were the most effective against early blight of tomato, resulted in the inhibition of disease producing activity of the pathogen by inducing resistant in plant. Similar findings were reported by several workers who found mancozeb was the most effective fungicide for the controlling of *A. solani* [25-29]**.** Baraka *et al*. [30] reported that Topsin-M® found most effective against many plant pathogenic fungi. Catao *et al*. [31] observed that mancozeb gave satisfactory results by totally inhibiting the mycelial growth followed by Copper oxychloride used at 100% of the active ingredient inhibited 83% growth of the pathogen. Hawamdeh [32] reported that mancozeb was the best fungicide when used at 1000ppm concentration. The results of present study lined with all above mentioned reports that the tested chemicals caused mycelial inhibition of *A. solani.*

5. Conclusion

Conclusively, it is urged that fungicide application is one of a sharp tool against disease control in plants if use in integrated manner. Therefore, results of present study demonstrated that if timely application of fungicides with regular intervals during peak infection levels of pathogens, could be effective to control and manage early blight of tomato caused by *A. solani* in field or in green house.

6. References

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