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EFFICACY OF DIFFERENT CHEMICALS FOR THE *IN VITRO* CONTROL OF *ALTERNARIA SOLANI* ISOLATES COLLECTED FROM VARIOUS AREAS OF PESHAWAR

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ABSTRACT

A research was conducted at the department of Plant Pathology Kyber Pakhtunkhwa agricultural university Peshawar to evaluate different fungicides *in vitro* for the control of *Alternaria solani* isolated from infected tomato leaves. Three isolates of *A.solani* were obtained from the department of plant pathology Khyber Pakhtunkhwa agricultural university peshawer. For the *In vitro* control of *A.solani*, three fungicides i.e., Dithane M-45, Topsin-M and Cupravit were tested by Poison food technique at concentration of 500mg/L. In case of Drench method, a fungicide suspension was prepared by dissolving 0.05g of each fungicide in sterile distilled water to give a concentration of 500mg/L whereas control treatment had no fungicide. The pure culture of three isolates were cut with 5 mm diameter sterile cork borer. These inoculm discs were treated with fungicides by dipping in the fungicides suspension for 1-2 minutes and were seeded in the centre of each petri dish. The petri dishes were incubated at 25^0C for 10 days. In case of poison food technique, the lowest colony diameter (32.53mm) was recorded in treatment with Dithane M-45 followed by Cupravit (46.26mm) and Topsin M (55.93mm). Dithane m-45 also inhibited spore concentration significantly (42.45%) where the mean highest colony growth (62.76mm) was in control.
Keywords: *Alternaria solani,* fungicides, Poison Food Technique, Drench Method

INTRODUCTION

Tomato (*Lycopersicon esculentum* L.) is a rich source of vitamin A and C and a popular vegetable due to its color, taste and number of uses such as salad and sauce preparation. During 2008-2009, the area and production of the crop in Pakistan was 53400 ha and 561900 tonnes respectively. The share of the Khyber Pukhtunkhwa of Pakistan in area and production during this period was 16500 ha and 161800 tonnes respectively (Anonymous).

In Pakistan, per hectare yield of tomato is very low (10.5 tons per ha) due to several production constraints including diseases. One of the diseases is the early blight caused by a fungus *Alternaria solani* (Elliss). Leaf spot symptoms are characteristic for early blight and are circular up to ½ inch in diameter, brown and contain dark concentric ring like target board. The spot occur singly or in large number on each leaf. Yellowish areas may develop on affected leaves and eventually they turn brown and usually drop from the plant. In the absence of fungicide treatment, maximum fruit infection for susceptible varieties was about 30%, while fruit size reduced to 10% (Sherf and Macnab, 1986).

The attack of early blight has been observed on tomato crop in several parts of the Khyber Pakhtunkhwa. Losses from this disease may increase if no protective measures are adopted well ahead of time. However, very little work has been done in this province on the ecology and control of this disease. Therefore, a research was conducted at the department of Plant Pathology for the evaluation of different fungicides *in vitro* to control *A. solani* comparing two methods of fungicides application.

MATERIALS AND METHODS

Source of *Alternaria solani* isolates

Three isolates of *A. solani* were obtained from department of Plant Pathology, The University of Agriculture, Peshawar-Pakistan.

In vitro control of *Alternaria solani*

Two techniques were used for the in *vitro control* of *A.solani*

A. Poison food technique

PDA medium was prepared as per standard procedure. Fungicides Dithane m-45, Cupravit and Topsin M were mixed with PDA medium

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at a concentration of 500 mg per liter (PDA medium) before pouring, control petri dishes did not contain any fungicides. After mixing fungicides and solidification of medium, three different isolates of *A. solani* were seeded in the centre of separate petri dishes using sterile needle. Each treatment was replicated 5 times using a completely randomized (CR) design. The plates were incubated at 25°C for 10 days.

B. Drench method

In case of drench method, fungicides suspensions were made by dissolving 0.05g of each fungicide in 100 ml sterile distilled water to give a concentration of 500mg/litre whereas control treatment had no fungicide. The pure cultures of 3 isolates were cut with 5 mm diameter sterile cork borer. These inoculum discs were treated with fungicides by dipping in the fungicides suspension or sterile distilled water for 1-2 minutes and were seeded in the center of each Petri dish. The petri dishes were incubated at 25 °C for 10 days.

Data analysis

Colony growth was measured periodically along two perpendicular lines. To determine spore concentration in each treatment 10 ml of sterile distilled water was added to each Petri dish. The surface was scratched gently to release spores in the suspension. Spore concentration was then determined with the help of a haemocytometer. The data were analyzed statistically using ANOVA and LSD test at 5% probability level.

***Alternaria solani* isolated collected from various regions of Peshawar**

Isolate number	Source
1	Isolated from tomato leaves collected from Malkandhere.
2	Isolated from tomato leaves collected from Regi Lalma.
3	Isolated from tomato leaves collected from Nasir Bagh.

RESULTS AND DISCUSSION

Colony diameter (mm) of *Alternaria solani* after 3 and 10 days of incubation (Poison Food Technique)

In case of Poison food technique, after three days of incubation, all the three fungicides reduced the colony diameter of *A. soloni* isolates significantly ($P<0.05$) as compared to the control (Table 1). However, the three fungicides differ significantly from one another.

After 10 days of incubation, all the three fungicides reduced the colony growth of *A.sloni* isolates significantly ($P<0.05$) as compared to the control (Table 1). However three fungicides differed significantly from one another. The three isolates also differed signicantly from one another. The interaction between fungicides and isolates was also found to be significant.

Colony diameter (mm) of *Alternaria solani* after 3 and 10 days of incubation (Drench Method)

In case of drench method the different treatments showed significant differences ($P<0.05$) among themselves (Table 1). After 3 days of incubation, the lowest colony diameter (20.46 mm) was recorded in treatment with Dithane M-45, which reduced colony diameter by 25.46 % as compared to the control. However Cupravit and Topsin M were equally effective in reducing colony diameters of *A.solani*.

After 10 days of incubation, the lowest colony diameter (55.58 mm) was recorded in treatment with Dithane M-45 (Table 1) it caused maximum reduction in colony diameter (27.34%.). Both Cupravit and Topsin M were less effective than Dithan M-45, but reduced the growth of the fungus significantly ($P< 0.05$).

Table 1. Colony diameter (mm) of *A. solani* after 3 and 10 days of incubation (Poison Food Technique)

Fungicides	Colony diameter after 3 days		Colony diameter after 10 days		Colony diameter after 3 days		Colony diameter after 10 days	
Control	35.43 (---)	A	62.76 (---)	A	27.54 (---)	A	76.50 (---)	A
Dithane	11.33 (68.02)	D	32.53 (48.21)	C	20.46 (25.46)	B	55.58 (27.34)	C
Cupravit	29.90 (24.04)	C	46.26 26.29	B	22.0 (19.85)	B	66.08 (13.62)	B
Topsin M	30.36 (14.30)	B	55.93 (10.88)	A	23.08 (15.91)	B	72.50 (5.22)	

Comparison of colony diameter (mm) of *A. solani* isolates after 3 and 10 days of incubation (Poison Food Technique)

After 3 days of incubation; among the three isolates tested, isolate 2 was found to be the most vigorous with a colony diameter of 27.85 mm (Table 2). Isolate 1 and 3 were statistically at par with each other. The interaction between fungicides and isolates was found to be non-significant.

After 10 days of incubation, isolate 2 was found to be the least vigorous with a colony diameter of 48 mm (Table 2). Isolate 1 and 3 were statistically at par with each other. The interaction between fungicides and isolates was found to be non-significant.

Comparison of colony diameter (mm) of *A. solani* isolates after 3 and 10 days of incubation (Drench Method)

After 3 days of incubation; among the three isolates, isolate 2 was found to be the most vigorous, while isolate 1 and 3 were both less vigorous than isolate 2 and comparable with each other (Table 2). When treated with Cupravit isolate-1 was affected more adversely than isolate 2 and 3.On the other hand Isolate 1 and 3 responded with greater reduction in growth as compared to isolate 2 when treated with Topsin M. However, Dithane M-45 affected the growth of all the three isolates in a similar way.

After 10 days of incubation, isolate 3 was found to be the most vigorous with a colony diameter of 73.63 mm (Table 2). Interaction between isolates and fungicides was found to be significant. Dithane M-45 supressed the growth of isolate 2 significantly more than isolate 1 and 3. On the other hand, Topsin M had more adverse effect on the growth of isolate 1 as compared to isolates 2 and 3.

Table 2. Comparison of colony diameter (mm) of *A. solani* after 3 and 10 days of incubation (Poison Food Technique)

	Poison Food Technique		**Drench Method**	
Isolates	Colony diameter after 3 days	Colony diameter after 10 days	Colony diameter after 3 days	Colony diameter after 10 days
1	24.80 B	50.7	24.80 B	62.75 B
2	27.85 A	48	27.85 A	66.63 AB
3	25.37 B	49.42	25.38 B	73.63 A

Spore concentration of *Alternaria solani* as affected by fungicides through Poison Food Technique.

Application of fungicides reduced spore concentration of *A.solani* significantly (Table 3). The lowest concentration (15355.55spores/ml) was counted in treatment of *A.solani* with Dithane

M-45, which reduced the concentration by 42.45% as compared with that of control. That was followed by Cupravit and Topsin M respectively.

Spore concentration of *Alternaria solani* as affected by fungicides applied through Drench Method.

The fungicides drench application reduced spore concentration of *A.solani* (Table 3). Dithane M -45 was the most effective fungicide reducing spore concentration by 37.10% to 12572.22 spores/ml. This was followed by Cupravit and then Topsin M.

Table 3 Spore concentration of *Alternaria solani* as affected by fungicides through Poison Food Technique and Drench Method

Fungicides	Spore concentration as affected by Poison Food Technique		Spore concentration as affected by Drench Method
Control	26685.33 (---)	A	19988.89 (--)
Dithane	15355.55 (42.45)	D	12572.22 (37.10)
Cupravit	17388.88 (34.38)	C	17766.6 (11.11)
Topsin M	20216.66 (24.90)	B	19616.6 (1.86)

Comparison of Spore concentration of *Alternaria solani* isolates as affected by fungicides through Poison Food Technique and Drench Method

Poison Food Technique

Among the three isolates, the lowest concentration was counted in isolate 2, while isolate 1 and 3 were comparable with each other (Table 4). The interaction between isolates and fungicides was found to be significant. While Dithane M-45 and Topsin – M suppressed spore concentration of isolate 2 significantly more than isolate 1 and 3,cupravit had more inhibitory effect on isolate 1.

Drench Method

Isolates of *A.solani* did not differ significantly in spore concentration (Table 4). The interaction of isolates and fungicides was also non-significant.

Table 4 Comparison of spore concentration of *Alternaria solani* as affected by fungicides through Poison Food Technique and Drench Method

Isolates	Spore concentration as affected by Poison Food Technique	Spore concentration as affected by Drench Method
1	22654.16 A	14462.5
2	16645.83 C	14979.16
3	20808.33 B	29883.33

Tomato is an important vegetable crop of KPK, grown under different agro climatic conditions of the province. The crop is susceptible to various diseases caused by viruses, nematodes and fungi. One of such diseases is the early blight of tomato caused by a fungus *Alternaria solani*. Early blight is known to attack tomato from a very long time Ellis (1976), but no specific work has been conducted in this province on the disease.

No significant interaction was found for colony diameter between fungicides and isolates of *A.solan*i in poison food technique, whereas interaction was significant in drench method, this might be due to less exposure time of the pathogen to the fungicides in the drench application because inoculum discs were dipped in fungicides for only 1-2 minutes. Among the three isolates tested, their response was almost similar in poison food technique. Isolate 3 was found to be the most vigorous in drench method with a colony diameter 73.63 mm. This could also be due to less exposure time.

Among several methods for early blight control in tomato, the use of fungicides is noteworthy. In treatments where fungicides were used, the colony growth of *A.Solani* was reduced substantially as compared to the treatment where no fungicide was used. The comparison of different fungicides showed that Dithane was better than cupravit and Topsin M. Dithane is a complex of Zn and Manneb, containing 20 % Maneb and 2.55 % Zn with active ingredient 87.55 %. Dithane compete with the fungus in soil. The availability of this fungicide at reasonable price is an additional advantage of this over other fungicides for the effective control of early blight in tomato.

CONLUSIONS AND RECOMMENDATION

The comparison among three fungicides indicated the superiority of Dithane M-45 over Cupravit and Topsin -M in reducing colony growth of the fungus. Isolate 3 was found to be the most vigorous among the three isolates tested. Interaction between fungicides and isolates was found to be significant in drench method and non-significant in poison food technique. These fungicides should be used as a part of an integrated control strategy, incorporating resistant varieties and proper cultural practices. Detailed studies are needed under field conditions to elaborate the result of this research.

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