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Research Article

In vitro efficacy of fungicides on mycelial growth and spore germination of Alternaria alternata and Mucor plumbeus

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Abstract

Tomato fruits were infected by various pathogens especially fungal pathogens under storage conditions as well as in field conditions. It was revealed that tomato fruits are attacked by *Alternaria alternata* causing *Alternaria* rot of tomato and *Mucor plumbeus* causing *Mucor* rot of tomato. Antifungal activity of some fungicides was undertaken during the present study. Different concentrations of fungicides brought about significant reduction in the mycelial growth and spore germination of *Alternaria alternata* and *Mucor plumbeus* under *in vitro* conditions. Hexaconozole proved highly effective in inhibiting the mycelial growth and spore germination of *Alternaria alternata* followed by carbendazim and copper oxychloride respectively. However, the most effective fungicide in inhibiting the mycelial growth and spore germination of *Mucor plumbeus* was hexaconozole followed by carbendazim and copper oxychloride respectively.

Keywords: In vitro, fungicides, Alternaria alternata and Mucor plumbeus, mycelial growth, spore germination

1. INTRODUCTION

Tomato (Lycopersicon esculentum Mill.) is one of the most important vegetable crops in the world. It is considered as an important cash and industrial crop in many parts of the world1. Fungal rot is common, destructive and wide spread disease of all fruits and vegetables 2. Several species of fungi, viz. Rhizopus sp., Mucor sp., Penicillium sp., Aspergillus sp., Colletotrichum sp., Botrytis sp., Monilinia sp., Alternaria sp., Phytophthora sp., have been reported to cause fungal rot diseases3, 4. Various chemical control measures have been tested and found effective in controlling plant diseases. ⁵highlighted some protective fungicides that have been found effective in controlling some plant diseases. The present study was carried out with the main objectives of identifying the fungal rot pathogens that cause decaying in tomatoes under storage conditions in Kashmir valley. The study was also undertaken for the management of identified fungal pathogens with some selected fungicides.

2. MATERIALS & METHODS

To investigate the fungi which cause rotting of tomato fruits in Kashmir Valley, diseased fruits were collected from markets, godowns and storage houses of Kashmir Valley. These samples were either used immediately or stored at 10°C in the laboratory for different pathological studies. Small portions of rotted tissues were taken aseptically from the tomato fruits and transferred to potato dextrose agar (PDA) medium. Pure colony cultures were obtained by sub-

culturing the fungal growth in separate Petri plates containing the same medium. The pathogens were identified by their morphological, reproductive and cultural characteristics ^{6,7,8,9}. For pathogenicity tests, pathogens were re-inoculated after isolation onto healthy tomato fruits ¹⁰ and incubated at 25+2°C for 10 days. Identification of the disease and the pathogen was done following Koch's postulates. Different parameters such as symptoms caused by these fungi on the healthy tomato fruits, cultural characteristics of the pathogens and microscopic studies of the pathogens were studied.

In the present study an attempt was made to study the effect of some selected fungicides under *in vitro* conditions for the control of *Alternaria* rot of tomato caused by *Alternaria alternata* and *Mucor* rot caused by *Mucor plumbeus*.

2.1 Preparation and evaluation of different fungicide concentrations

Different concentrations (1000 ppm, 500 ppm, 250 ppm and 125 ppm) of the fungicides copper oxychloride, carbendazim and hexaconozole were prepared in sterilized distilled water and evaluated for their effect on the mycelial growth of rot causing fungus, *Alternaria alternata* and *Mucor plumbeus* by food poisoning technique¹¹. Appropriate concentration (1 ml) of fungicide solution was mixed with autoclaved and cooled PDA just before pouring into Petri plates. The medium was then dispensed uniformly into 90 mm diameter Petri plates and inoculated with 5 mm mycelial disc of the

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pathogen from 10-days-old fungal culture. Three replicates were maintained for each concentration including control without any treatment. The Petri plates were incubated at $25\pm2^{\circ}\text{C}$ and observations of the mycelial growth of test fungus were recorded after 7 days of incubation. The percent inhibition in mycelial growth due to various fungicidal treatments at different concentrations was computed as formula

Mycelial growth inhibition (%) = $\{(dc - dt) / dc\} \times 100$

Where dc = average diameter of fungal colony in control, and dt= average diameter of fungal colony in treatment group.

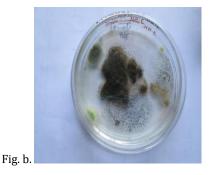
For evaluating the effect of fungicides on spore germination, a spore suspension was prepared in sterilized distilled water. Spore suspension (0.5 ml) was mixed with 0.5 ml of the fungicides of different concentrations in a test tube and then shaken. In case of control 0.5 ml of spore suspension was mixed with equal volume of distilled water. A drop of the mixture (about 0.1 ml) was then placed in a cavity slide and these were incubated for $25\pm2^{\circ}\text{C}$ in a moist chamber created in 100 mm Petri plates by covering both sides of the Petri plate with moist filter paper to maintain humidity. Three replicates were maintained for each treatment including the control. The slides were examined after 24 hours by hand tally counts of different microscopic fields. Percent spore germination of each treatment was calculated by the formula given by 12 .

Percent spore germination = (No. of spores germinated/ Total no. of spores examined) ×100

3. RESULTS

In the present study the casual pathogens infecting tomato fruits were identified as Alternaria alternata (Fr) Keissler and Mucor plumbeus Fisher resulting in Alternaria rot of tomato and Mucor rot of tomato. These two fungi were identified on the basis of symptoms caused by the fungus on tomato fruits and on the basis of cultural and microscopic characteristics. The symptoms of disease on tomato fruits are manifested by having dark, brown to black lesions which were upto 8-8.7 mm in diameter with white mycelium around the boundary. The lesion becomes darker and broadened in size (12.5-13.2 mm) with marked depression in the centre and elevated portion around the diameter. The lesions became slightly sunken after 7 days, which were of firm texture and 24.0-25.4 mm in diameter. The rot extended deep into the fruit rendering the tissues black, soft and watery. (Fig. a). The colony of Alternaria alternata appeared dark olive gray and finally black after ten days of inoculation on Potato Dextrose Agar (PDA) medium (Fig b). Microscopic studies revealed that the fungus is septate, hyaline with brown mycelia. The conidia are produced in chains of 2-20 at the tip of conidiophores which are branched or unbranched. The conidiophores are macronematous, mononematous, golden brown, septate, branched, walls smooth or slightly rough measuring 22.5-30.0 x 4.8-8.0 μm in diameter, flexous, geniculate; conidia are brown, variously shaped, usually obclavate, in long branched chains with short cylindrical beak upto 5 transverse septa, longitudinal and oblique septa also conspicuous, $19.2-28.8 \times 8.0-14.4 \mu m$ in diameter (Fig c).





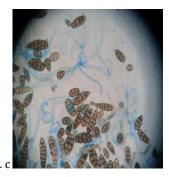


Figure a: Infected tomato fruit; **Figure b:** Culture of *Alternaria alternata* on PDA medium. **Figure c:** *Alternaria alternata*; Mycelium with conidia (400x)

The infected fruits are covered by gray, coarse and hairy mycelium that forms a mass of black sporangia at the tips. The rotten fruits have sour odor. The rot spreads by infection through wounds. The mycelium is white at first and then turns gray to black depending on sporulation (Fig. d). The fungus on Potato Dextrose Agar medium (PDA) produced colonies which are white at first and then turns black due to

sporulation (Fig. e). Microscopic characteristics of the fungus revealed that the mycelium is non-septate and branched. Sporangiophore arises singly from the mycelium that terminate into sporangia. Sporangia are ovate or globose measuring about 80 μm - 200 μm in diameter. Collumela is colourless and pear shaped. Spores are hyaline, ellipsoidal, 4 μm – 6 μm × 7 μm -11 μm in diameter (Fig. f).





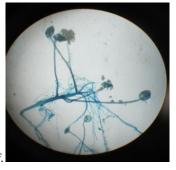


Figure d: Infected tomato fruit; **Figure e:** Culture of *Mucor plumbeus* on PDA medium. **Figure f:** *M. plumbeus*; Sporangiophores and sporangia (400x)

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3.1 Effect of different concentration of fungicides on the mycelial growth of *Alternaria alternata*

It was found from results (Table 1, Fig. 1) that all the fungicides, viz. copper oxychloride, carbendazim and hexaconozole at different concentrations (1000ppm, 500 ppm, 250ppm, 125ppm) brought about significant inhibition in the mycelial growth of Alternaria alternata as compared to control. However, the most effective fungicide in inhibiting the mycelial growth of Alternaria alternata was hexaconozole followed by carbendazim and copper oxychloride respectively. Hexaconozole at highest concentrations brought about maximum inhibition in mycelial growth followed by carbendazim and copper oxychloride at the same concentration. Other concentrations also caused significant inhibition in mycelial growth but to a lesser extent. In different concentrations of hexaconozole, the inhibition in mycelial growth varies from 100% -50.29% and in carbendazim it varies from 84.65%-48.31%. Likewise, the inhibition in mycelial growth in copper oxychloride varies from 72.84% -39.98% in different concentrations of the fungicides respectively.

Effect of different concentrations of fungicides on the spore germination of *Alternaria alternata*

It was observed from results (Table 2, Fig. 2) that all the fungicides, viz. copper oxychloride, carbendazim and hexaconozole at different concentrations (1000ppm, 500 ppm, 250ppm, 125ppm) brought about considerable inhibition in the spore germination of Alternaria alternata as compared to control. Amongst the fungicides, the hexaconozole at highest concentration (1000ppm) was found most effective in inhibiting the germination of spores followed by carbendazim and copper oxychloride at same concentration. Other concentrations also brought about significant reduction in spore germination but to lesser extent. In hexaconozole the inhibition in spore germination varies from 63.36%-13.09% in different concentrations. In carbendazim, the reduction in spore germination varies from 69.71%- 16.55% and in copper oxychloride it varies from 71.60% - 18.10% respectively in different concentrations.

Table 1: Effect of different concentrations of fungicides on the mycelial growth of Alternaria alternata

Conc.	Mycelial growth (mm)					
Treatment	125ppm	250ppm	500ppm	1000ppm	Control	
Copper oxychloride	34.81±0.21b	21.77±0.65 ^c	18.24±0.66d	15.75±0.37e	58.00±0.55a	
	(39.98)	(62.46)	(68.55)	(72.84)		
Carbendazim	29.98±0.08 ^b	18.18±0.45¢	12.33±0.90d	8.90±0.60e	58.00±0.55ª	
	(48.31)	(68.65)	(78.74)	(84.65)		
Hexaconozole	28.83±0.39b	12.62±0.50 ^c	6.44±0.74d	0.00 ±0.00e	58.00±0.55a	
	(50.29)	(78.24)	(88.89)	(100)		

Each value is mean of 3 replicates ± SD Figures in parenthesis is the mycelial growth inhibition (%)

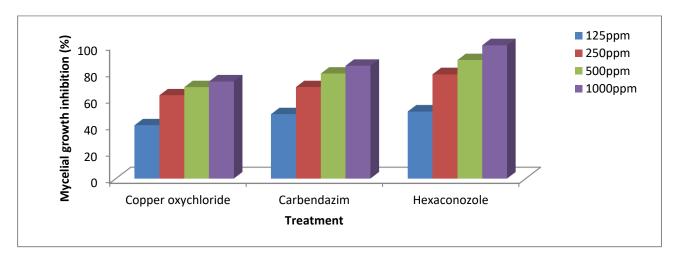


Figure 1: Effect of different concentrations of fungicides on the mycelial growth of *Alternaria alternata* Table 2: Effect of different concentration of fungicides on the spore germination of *Alternaria alternata*

Conc.	Spore germination (%)				
	125ppm	250ppm	500ppm	1000ppm	Control
Treatment					
Copper oxychloride	71.60±0.20 ^b	57.83±0.61 ^c	37.15±0.30 ^d	18.10±0.6e	94.73±0.24 ^a
Carbendazim	69.71±0.38b	56.73±0.68 ^c	34.41±0.40d	16.15±0.58e	94.56±0.55ª
Hexaconozole	63.36±0.12 ^b	51.46±0.12 ^c	33.26±0.90d	13.09±0.48e	94.56±0.55a

^{*} Each value is mean of spore germination %age of 3 replicates ± SD

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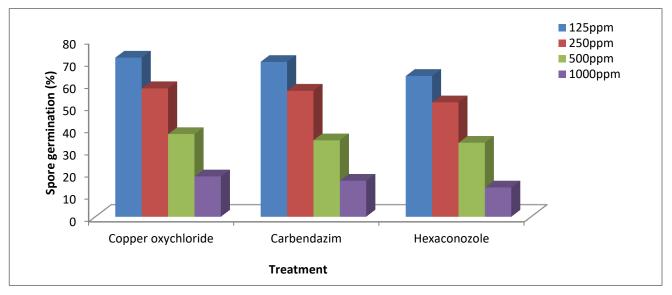


Figure 2: Effect of different concentration of fungicides on the spore germination of Alternaria alternata

3.2 Effect of different concentration of fungicides on the mycelial growth of *Mucor plumbeus*

It was revealed from the results (Table 3, Fig 3) that all the fungicides, viz. copper oxychloride, carbendazim and hexaconozole at different concentrations (1000ppm, 500 ppm, 250 ppm, 125 ppm) brought about significant inhibition in the mycelial growth of Mucor plumbeus. However, the most effective fungicide in inhibiting the mycelial growth of Mucor plumbeus was hexaconozole (71.92%) at highest concentration followed by carbendazim (71.19%) and copper oxychloride (60.04%) at the same concentration. Other concentrations also caused significant inhibition in mycelial growth but to a lesser extent. In different concentrations of hexaconozole, the inhibition in mycelial growth varies from 71.92% -50.82% and in different concentration of carbendazim it varies from 71.19%-47.01%. Likewise, the inhibition in mycelial growth in copper oxychloride ranges from 60.04% -42.93% in different concentrations respectively of the fungicides.

Effect of different concentrations of fungicides on the spore germination of *Mucor plumbeus*

It was found from the results (Table 4, Fig 4) that all the fungicides, viz. copper oxychloride, carbendazim and hexaconozole at different concentrations (1000 ppm, 500 ppm, 250ppm, 125ppm) brought about significant inhibition in the spore germination of Mucor plumbeus as compared to control. Maximum inhibition in spore germination was found in the highest concentration of the fungicide followed by lower concentration of fungicides. Amongst the fungicides, hexaconozole at highest concentration (1000ppm) was found most effective in reducing the germination of spores followed by carbendazim and copper oxychloride at same concentration. Other concentrations also brought about significant reduction in spore germination but to lesser extent. In hexaconozole the inhibition in spore germination varies from 43.51%-16.40% in different concentrations. In carbendazim, the reduction in spore germination varies from 69.75%- 20.23% and in copper oxychloride it varies from 74.30% - 23.53% respectively in different concentrations.

Table 3: Effect of different concentrations of fungicides on the mycelial growth of Mucor plumbeus

Conc.	Mycelial growth (mm)				
Treatment	125ppm	250ppm	500ppm	1000ppm	Control
Copper oxychloride	39.80 ±0.60 ^b (42.93)	35.14±0.05¢ (49.62)	31.99±0.08 ^d (54.13)	27.87±0.61e (60.04)	69.75±0.36a
Carbendazim	36.96 ±0.05 ^b (47.01)	33.23±0.30 ^c (52.35)	29.60±0.30 ^d (57.56)	20.09±0.36e (71.19)	69.75 ±0.36 ^a
Hexaconozole	34.30 ±0.35 ^b (50.82)	32.41±0.59¢ (53.53)	25.70±0.7 ^d (63.15)	19.58 ±0.9e (71.92)	69.75 ±0.36a

Each value is mean of 3 replicates ± SD Figures in parenthesis is the mycelial growth inhibition (%)

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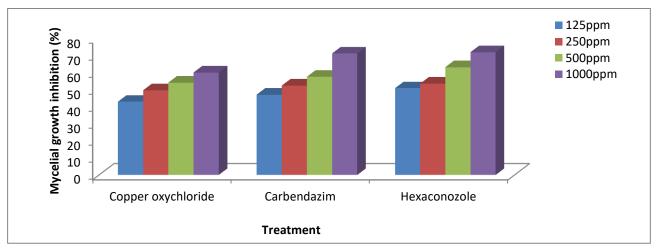


Figure 3: Effect of different concentrations of fungicides on the mycelial growth of *Mucor plumbeus*.

Table 4: Effect of different concentration of fungicides on the spore germination of *Mucor plumbeus*

Conc.	Spore germination (%)				
	125ppm	250ppm	500ppm	1000ppm	Control
Treatment					
Copper oxychloride	74.30±0.20b	56.53±0.30 ^c	36.75±0.22d	23.53±0.15e	91.31±0.23a
Carbendazim	69.75±0.27 ^b	51.41±0.17 ^c	33.39±0.37d	20.23±0.37e	91.38±0.29a
Hexaconozole	43.51±0.17 ^b	32.63±0.45 ^c	23.51±0.28d	16.40±0.20e	91.31±0.23 ^a

^{*} Each value represents the mean spore germination %age of 3 replicates ± SD

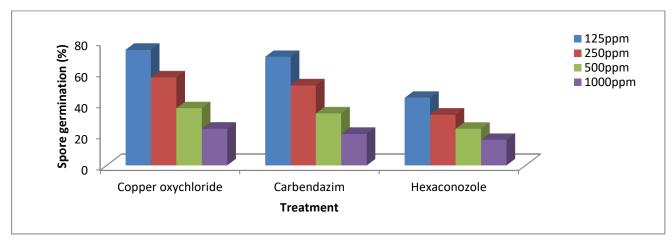


Figure 4: Effect of different concentration of fungicides on the spore germination of *Mucor plumbeus*

4. DISCUSSION

It was clear from above results that the fungus *Alternaria alternata* and *Mucor plumbeus* attacks tomato fruits in storage and causes *Alternaria* rot and *Mucor* rot of tomato fruits. Such studies on fungal rot of tomato have been carried out for the first time in Kashmir Valley. In the present study, *Alternaria alternata* and *Mucor plumbeus* were identified on the basis of symptoms caused on the infected fruits, cultural and microscopic characteristics of the fungus. Some workers also used symptomological studies, cultural and morphological and reproductive characteristics for the identification of fungus ^{13,14,15,16}.

In the present study some fungicides were evaluated for their antifungal activity against the fungus *Alternaria alternata* and *Mucor plumbeus*. All the concentrations of fungicides proved highly effective in reducing the mycelial growth and caused significant inhibition in the spore germination of *Alternaria alternata* and *Mucor plumbeus*. The highest concentrations of the fungicides proved more effective than lower concentrations. The efficacy of different fungicides in inhibiting the growth of rot causing fungi have been reported earlier by^{17,18}. ¹⁹screened seven fungicides against *Alternaria solani* through poison food techniques and reported that Dithane M-45 is the most effective fungicide at higher concentration. ²⁰reported that difenoconazole had a better effectiveness *in vitro* than Chlorothalonil in inhibition

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of mycellial growth and conidial germination of *Alternaria solani* and *A. alternata*. ²¹tested various chemical fungicides, systemic and non-systemic, against the mycelial growth of two fruit rot pathogens, viz. *Alternaria alternata* and *Mucor piriformis* and observed hexaconozole and carbendazim as effective. ²²tested various fungicides against many vegetable rot causing fungi including *R. stolonifer* and observed carbendazim as the most effective fungicide for reducing *Rhizopus* rot. Some workers have studied the effect of different fungicides against different rot causing fungi of different plants ^{23,24,25,26,27,28}.

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