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Management of postharvest fungal rot of tomato (*Lycopersicon esculentum*) in Kashmir Valley, India

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ABSTRACT

Tomato fruits undergo decay due to postharvest infestation by various pathogens especially fungal pathogens. Therefore, the fungal rot of tomato under storage conditions was studied. It was revealed that tomato fruits are attacked by Penicillium expansum, causing blue mold or Penicillium rot of tomato and Aspergillus niger causing black mold or Aspergillus rot of tomato. Study was also undertaken to observe the antifungal activity of some fungicides. Different concentrations of fungicides brought about significant reduction in the mycelial growth and spore germination of Penicillium expansum and Aspergillus niger under in vitro conditions. Carbendazim proved highly effective in inhibiting the mycelial growth and spore germination of P. expansum followed by hexaconozole and copper oxychloride respectively. However, the most effective fungicide in inhibiting the mycelial growth and spore germination of Aspergillus niger was hexaconozole followed by carbendazim and copper oxychloride respectively.

Key Words: Fungicide, Inhibition, Mycelial Growth, Tomato, Spore Germination, Storage

I.INTRODUCTION

Rot diseases cause heavy losses to the vegetables in storage as well as in fields [1-2]. In India, postharvest diseases of fruits due to fungi are responsible for about 30 percent losses during harvest and consumption [3-4]. Tomato (*Lycopersicon esculentum* Mill.) belongs to family Solanaceae and is widely grown vegetable in the world. The leading producer of tomato in the world is USA followed by China, Italy, Turkey, Egypt, Spain, Romania, Brazil and Greece. In Kashmir Valley (India), the crop is grown over an area of 1200 hectares with an average yield of 250-300 quintal per hectare [5]. Tomato (*Lycopersicon esculentum* Mill.) is a short-lived perennial herbaceous plant. It is one of the most popular vegetable crops widely grow for its edible fruits, high nutritive values and also for its diversified uses [6-7]. They are important source of vitamins and important cash crop for both small landholders and medium scale commercial farmers [8]. Tomato (*Lycopersicon esculentum* Mill.) represents a valuable source for improving the antioxidants (lycopene, ascorbic acid and phenols) in our diet [9], and is famous for its application in drug, fruit, food products, flowering, ornamental and horticulture sectors [10]. Therefore, the present study was carried out with the main objective of identifying the fungal rot

pathogen that causes decaying in tomato under storage conditions in Kashmir Valley. The study was also undertaken for the management of identified fungal pathogen with some fungicides.

II.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^{o}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 [11-14]. For pathogenicity tests, pathogens were re-inoculated after isolation onto healthy tomato fruits [15] and incubated at 25+2^{o}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 *Penicillium* rot of tomato caused by *Penicillium expansum* and *Aspergillus* rot caused by *Aspergillus niger.*

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, *Penicillium expansum* and *Aspergillus niger* by food poisoning technique [16]. 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 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±2°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

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

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±2oC 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 [17].

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

III.RESULTS

In the present study the casual pathogens infecting tomato fruits were identified as *Penicillium expansum* Link ex Thorn and *Aspergillus niger* Van Tiegh resulting in blue mold or *Penicillium* rot of tomato and black mold or *Aspergillus* 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 on tomato fruits starts with a circular lesion which is soft, pale brown and has a sharp margin between diseased and healthy tissue. The white mold soon turns bluish green with the production of bluish green mass of spores (Fig. a). The colony of *Penicillium expansum* appeared white, velvety at first and then turns bluish green due to formation of conidiophores and conidia on Potato Dextrose Agar (PDA) medium (Fig b). Microscopic observation revealed that mycelium is septate with branched conidiophores 48 µm -120µm long, terverticilate (two stage branching) with three types of branches, viz. stipe, metula and phialides. Metulae are 3.7µm-7.8 µm long. Metulae forms conidiogenous cells called sterigmata or phialides. Phialides are 3.5µm-7.5µm long and gives rise to conidia that are ellipsoid or globose and 1.48µm-3.7µm in diameter (Fig c).

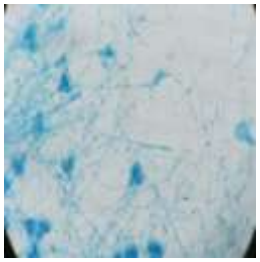
Fig a.  **Fig. b.**  **Fig. c** 

Fig. a Infected tomato fruit; Fig. b Culture of *Penicillium expansum* on PDA medium.

Fig. c. *P. expansum*; Mycelium with conidiophores and conidia (100x)

The infected fruits produce soft watery rot. The surface of the fruits becomes covered with black conidial heads of the pathogen. The infection is characterized by pale and water soaked lesions. The pathogen causes brownish discolorations which turn slimy. The epidermis showed cracks through which fungus comes out in small white tufts that later on due to formation of spore's turns black (Fig. d). The causal agents were isolated from the diseased fruits and are cultured on PDA medium. After 48 hours of inoculation at 24 ± 2^{o}C, the fungus produces white colonies and then due to formation of conidia turns black in colour (Fig. e). Microscopic studies revealed that mycelium is septate, hyaline and branched. Conidiophores arising from the mycelium are 350µm-600µm × 5.0µm-14.0µm in diameter, swollen at the tips giving rise to vesicles which is globose and flask shaped over which phialides or sterigmata are present that produce chains of conidia. Conidia are colourless to yellowish green, measuring 4.5µm-6.0µm in diameter (Fig. f).

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Fig d.  **Fig e.**  **Fig f.** 

Fig. d. Infected tomato fruit; Fig e. Culture of *Aspergillus niger* on PDA medium. Fig. f. *A. niger*; Mycelium with conidia (400x)

3.1 Control of fungi causing rot of tomato with fungicides

The present study was carried out to evaluate the effect of some fungicides on *Penicillium expansum* causing *Penicillium* rot and *Aspergillus niger* causing *Aspergillus* rot of tomato. Different concentrations of fungicides were evaluated for their effect on the mycelial growth and spore germination of the test fungi

3.1.1 Effect of different concentration of fungicides on the mycelial growth of *Penicillium expansum*

It was revealed from the results (Table 1, Fig g) 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 *Penicillium expansum* as compared to control. However, the most effective fungicide in inhibiting the mycelial growth of *Penicillium expansum* was carbendazim followed by hexaconozole and copper oxychloride respectively. Carbendazim and hexaconozole at highest concentrations brought about significant inhibition in mycelial growth followed by copper oxychloride at the same concentration. Other concentrations also caused significant inhibition in mycelial growth but to a lesser extent. In different concentrations of carbendazim the inhibition in mycelial growth varies from 100% -89.80% and in hexaconozole it varies from 100%-86.47%. Likewise, the inhibition in mycelial growth by copper oxychloride varied from 100% -84.36% at different concentrations of the fungicides.

Table 1 Effect of different concentrations of fungicides on the mycelial growth of *Penicillium expansum*

Conc. / **Treatment**	**Mycelial growth (mm)**				
	125ppm	**250ppm**	**500ppm**	**1000ppm**	**Control**
Copper oxychloride	6.76 ±0.49 (84.36)	4.12±0.82 (90.47)	2.00±1.00 (95.37)	0.00±0.00 (100)	43.25±1.05
Carbendazim	4.41 ±0.89 (89.80)	2.16±0.28 (95.00)	0.00±0.00 (100)	0.00±0.00 (100)	43.25+1.05
Hexaconozole	5.85 ±0.49 (86.47)	4.00±1.00 (90.75)	0.00±0.00 (100)	0.00 ±0.00 (100)	43.25 ±1.05

Each value is mean of 3 replicates ± SD

Figures in parenthesis is the mycelial growth inhibition (%)

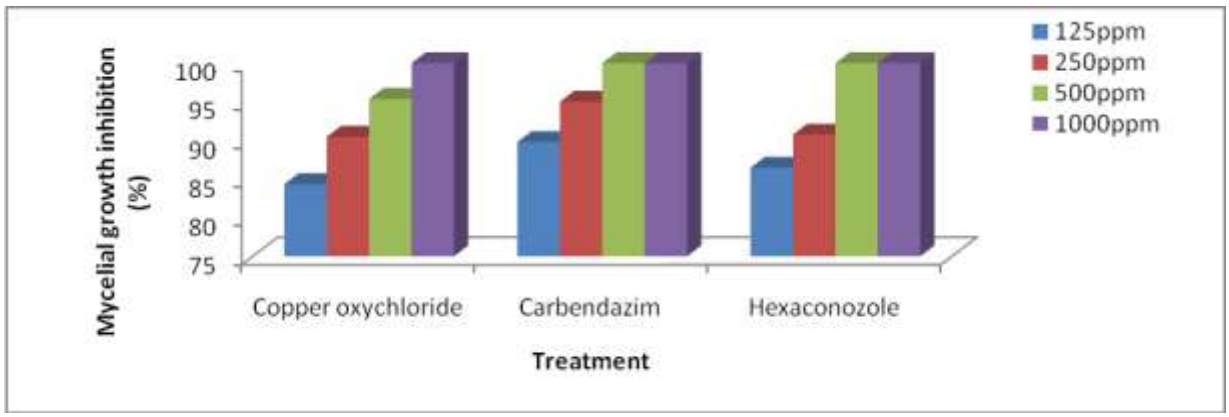

Fig g Effect of different concentrations of fungicides on the mycelial growth of *Penicillium expansum*

3.1.2 Effect of different concentrations of fungicides on the spore germination of *Penicillium expansum*

It was observed from the results (Table 2, Fig h) that all the fungicides, viz. copper oxychloride, carbendazim and hexaconozole at different concentrations (1000ppm, 500 ppm, 250ppm, 125ppm) brought about significant inhibition in the spore germination of *Penicillium expansum* as compared to control. Amongst the fungicides carbendazim and hexaconozole at highest concentration (1000ppm) was found most effective in reducing the germination of spores followed by copper oxychloride at the same concentration. The other concentrations also brought about significant reduction in spore germination but to lesser extent. In carbendazim, the inhibition in spore germination varies from 12.50%-0.00% in different concentrations. In hexaconozole, the reduction in spore germination varies from 25.50%- 0.00% and in copper oxychloride it varies from 33.36% - 12.36% respectively in different concentrations.

Table 2 Effect of different concentration of fungicides on the spore germination of *Penicillium expansum*

Conc. / **Treatment**	**Spore germination (%)**				
	125ppm	**250ppm**	**500ppm**	**1000ppm**	**Control**
Copper oxychloride	33.36±0.96	25.56±0.66	18.24±0.6	12.36±0.90	78.60±0.52
Carbendazim	12.50±1.05	5.26±1.00	0.00±0.00	0.00±0.00	78.60±0.52
Hexaconozole	25.50±0.62	12.26±0.90	0.00±0.00	0.00±0.00	78.60±0.52

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

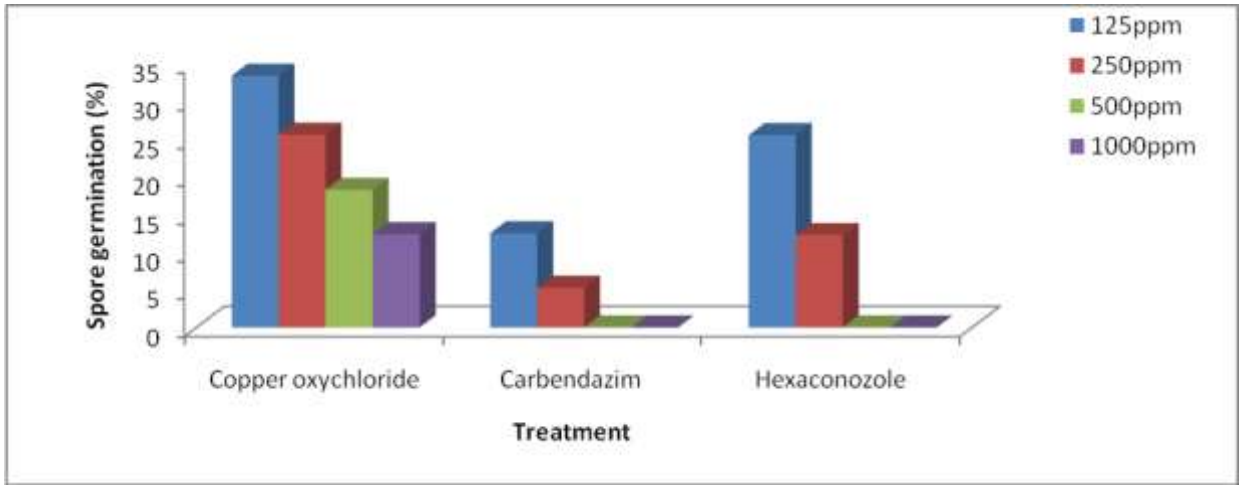

Fig h Effect of different concentration of fungicides on the spore germination of *Penicillium expansum*

3.1.3 Effect of different concentration of fungicides on the mycelial growth of *Aspergillus niger*

It was observed from the results (Table 3, Fig i) 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 *Aspergillus niger* as compared to control. However, the most effective fungicide in inhibiting the mycelial growth of *Aspergillus niger* was hexaconozole followed by carbendazim and copper oxychloride respectively. Hexaconozole at highest concentrations brought about maximum inhibition in mycelial growth (51.88%) followed by carbendazim (38.88%) and copper oxychloride (37.71%) 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 5.88% -22.22% and in carbendazim it varies from 38.88%-16.66% respectively. Likewise, the inhibition in mycelial growth in copper oxychloride varies from 37.71% -5.66% respectively in different concentrations of the fungicides.

Table 3 Effect of different concentrations of fungicides on the mycelial growth of *Aspergillus niger*

Conc. / **Treatment**	**Mycelial growth (mm)**				
	125ppm	**250ppm**	**500ppm**	**1000ppm**	**Control**
Copper oxychloride	16.66 ±1.52 (5.66)	15.00±1.0 (15.06)	12.66±1.52 (28.31)	11.00±1.00 (37.71)	17.66±1.52
Carbendazim	15.00 ±1.00 (16.66)	14.66±1.15 (18.55)	12.66±0.57 (29.66)	11.00±1.00 (38.88)	18.00 ±1.00
Hexaconozole	14.00 ±1.00 (22.22)	12.66±0.57 (29.66)	11.00±1.00 (38.88)	8.66 ±0.57 (51.88)	18.00 ±1.00

Each value is mean of 3 replicates ± SD

Figures in parenthesis is the mycelial growth inhibition (%)

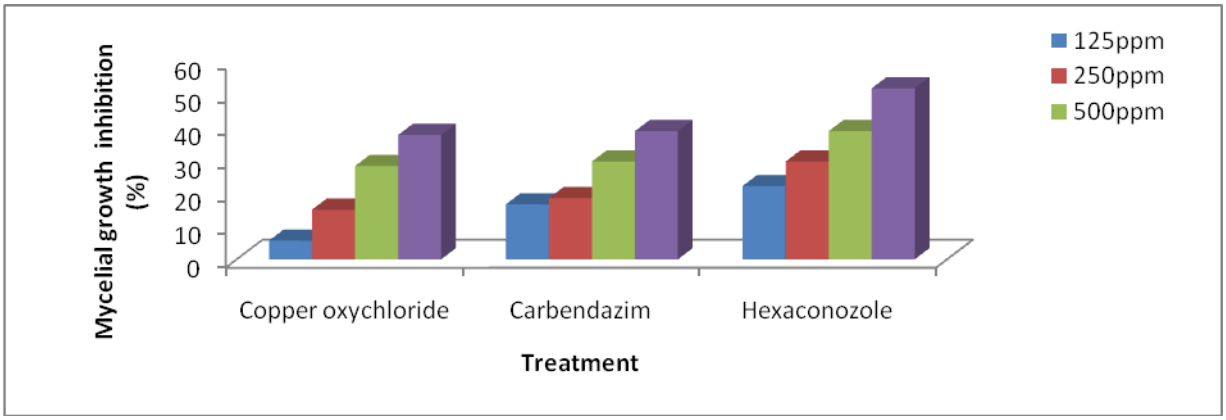

Fig i Effect of different concentrations of fungicides on the mycelial growth of *Aspergillus niger*

3.1.4 Effect of different concentrations of fungicides on the spore germination of *Aspergillus niger*

It was observed from the results (Table 4, Fig j) that all the fungicides, viz. copper oxychloride, carbendazim and hexaconozole at different concentrations (1000ppm, 500 ppm, 250ppm, 125ppm) brought about significant inhibition in the spore germination of *Aspergillus niger* as compared to control. However, the maximum inhibition in spore germination was brought about by hexaconozole at highest concentration (1000ppm) followed by carbendazim and copper oxychloride at same concentration. The other concentrations also brought about significant reduction in spore germination but to lesser extent. In hexaconozole the inhibition in spore germination varies from 37.56% - 18.18% in different concentrations respectively. In copper oxychloride, the reduction in spore germination varies from 48.88%- 31.62% and in carbendazim it varies from 42.73% - 25.22% respectively in different concentrations.

Table 4 Effect of different concentration of fungicides on the spore germination of *Aspergillus niger*

Conc. / Treatment	Spore germination (%)				
	125ppm	**250ppm**	**500ppm**	**1000ppm**	**Control**
Copper oxychloride	48.88±1.90	47.60±2.09	39.20±2.170	31.61±1.54	48.93±0.52
Carbendazim	42.73±2.38	40.43±2.22	31.78±1.36	25.22±1.34	46.05±2.00
Hexaconozole	37.56±1.29	33.93±1.56	24.33±1.11	18.18±1.90	46.05±2.00

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

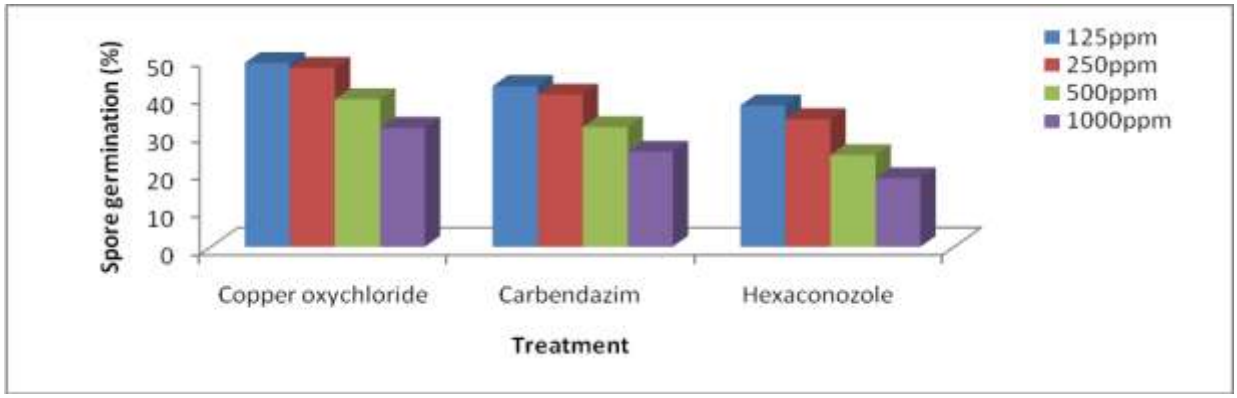

Fig j Effect of different concentration of fungicides on the spore germination of *Aspergillus niger*

IV.DISCUSSION

It was clear from the above observation that the fungus *Penicillium expansum* and *Aspergillus niger* attacks tomato fruits in storage and causes blue mold or *Penicillium* rot and black mold or *Aspergillus* rot of tomato fruits. Such studies on fungal rot of tomato have been carried out for the first time in Kashmir Valley. Earlier reports also indicate that species of both fungi are responsible for causing *Penicillium* and *Aspergillus* rot of fruits and vegetables in storage [18-19]. In the present study, *Penicillium expansum* and *A. niger* was 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 the fungus [20-21].

In the present study some fungicides were evaluated for their antimycotic activity against the fungus *P. expansum* and *A. niger*. All of the tested fungicides proved highly effective in reducing the mycelial growth and caused significant inhibition in the spore germination of *P. expansum* and *A. niger*. The highest concentrations of the fungicides proved more effective than lower concentrations. Similar findings were reported with respect to antifungal activity of other fungicides by [22-28]. [29] tested various fungicides against many vegetable rot causing fungi including *R. stolonifer* and observed carbendazim as the most effective fungicide for reducing *Rhizopus* rot. [30] tested 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.

V.CONCLUSION

It was concluded from the results that fungal rot of tomato is caused by several pathogenic fungi under storage conditions. Many studies have been carried out with respect to occurrence, causal organisms and disease control with fungicides. This research article may help the future researchers to devise a concrete strategy to evaluate the management of the post harvest fungal diseases of tomato. However, further study is needed to reveal all the other recent reports about management strategies opted for post harvest diseases of tomato.

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