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IN VITRO INHIBITORY EFFECT OF FUNGICIDES ON MYCELIAL GROWTH AND SPORE GERMINATION OF *PENICILLIUM CHRYSOGENUM*

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ABSTRACT

Brinjal fruits was infected by different pathogens especially fungal pathogens under storage conditions as well as in field conditions. It was observed that brinjal fruit was attacked by *Penicillium chrysogenum*, causing *Penicillium* rot of brinjal. In the present study antimycotic activity of some fungicides was undertaken against *Penicillium* rot of brinjal. Different concentrations of fungicides brought about significant reduction in the mycelial growth and spore germination of *Penicillium chrysogenum* under *in vitro* conditions. Hexaconozole proved highly effective in inhibiting the mycelial growth and spore germination of *P. chrysogenum* followed by carbendazim and copper oxychloride respectively. However, other concentrations also proved effective but to lesser extent.

KEYWORDS: *In vitro*, fungicides, *Penicillium chrysogenum,* mycelial growth, spore germination.

1. INTRODUCTION

Brinjal (*Solanum melongena* L.) belongs to family Solanaceae and is an indigenous vegetable crop of India. It contributes 9% of the total vegetable production of the country. China is the largest producer of brinjal followed by India. It is grown in India over 0.4 million hectors of land with an annual production of 7.8 million tones (Datar, 1999). Brinjal is attacked by fungal diseases caused by pathogenic fungi resulting in huge losses to its production (Kumar *et al.* 1986). Fungal rots are world-wide in occurrence and have been reported from all parts of the world (Janisiewicz and Korsten, 2002). In Kashmir valley, vegetables are attacked by variety of pathogens; predominant being the fungal rots (Taskeen-un-Nisa *et al*. 2009, 2011; Koka *et al* 2018). These fungal rots are responsible for causing serious production problems and have become menace for successful cultivation of vegetables in the Valley. 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 AND METHODS

To investigate the fungi which cause rotting of brinjal 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 brinjal 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 (Ellis, M.B. (1971); Barnett, H.L. and Hunter, B.B. (1972); Watanabe, T. (2002); Gilman, J.C. (2008). For pathogenicity tests, pathogens were re-inoculated after isolation onto healthy brinjal fruits (Tomkin, R.G. and Trout, S.A. (1989) 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 brinjal 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 brinjal caused by *Penicillium chrysogenum*.

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 chrysogenum* by food poisoning technique (Adams, P.B and Wong, J.A.L. (1991). 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

Mycelial growth inhibition (%) = {(dc – dt) / dc} ×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±2^{o}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 Kiraly *et al.* (1974).

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

3. RESULTS

In the present study the casual pathogen infecting brinjal fruit was identified as *Penicillium chrysogenum* Thorn resulting in *Penicillium* rot of brinjal. The fungi was identified on the basis of symptoms caused by the fungus on brinjal fruit and on the basis of cultural and microscopic characteristics. The symptoms on brinjal start with a green–blue velvety colonies with a heap of white mycelium at the centre. The reverse is yellow-white. Disease is characterized by small water soaked spot mainly associated with an injury and tissues became soft. The penicillia are asymmetrical and often complex with diverging branching patterns (Fig. a). The colony of *Penicillium chrysogenum* appeared green-blue velvety at first and then turns yellow white due to formation of conidiophores and conidia on Potato Dextrose Agar (PDA) medium (Fig b). Microscopic observation revealed that mycelium is septate with smooth walled branched conidiophores 200-1000 µm long, terverticilate (two stage branching) with three types of branches, viz. stipe, metula and phialides. Metulae are 8-12 x 2-4 µm long. Metulae forms conidiogenous cells called sterigmata or phialides. Phialides are 7-10 x 2 µm long and gives rise to conidia that are sub globose to elliptical and 2-4 x 2-3.5 µm in diameter (Fig c).

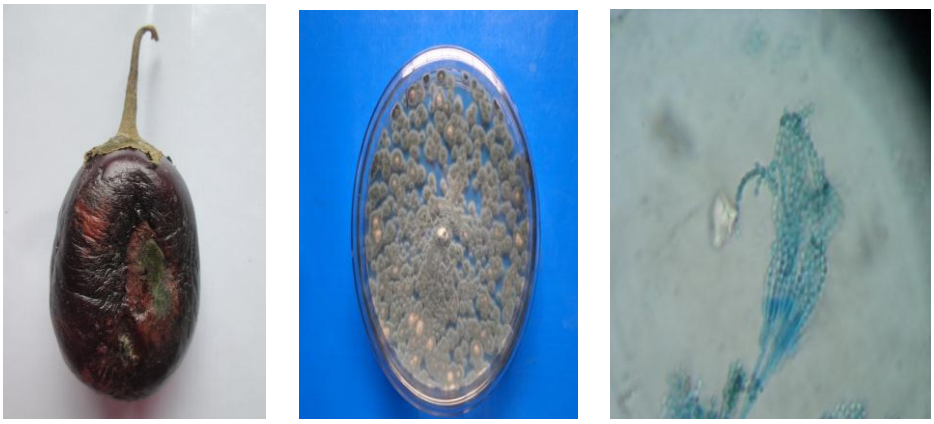

Fig a. **Fig. b.** **Fig. c**

Fig. a Infected brinjal fruit; Fig. b Culture of *Penicillium chrysogenum* on PDA medium.
Fig. c. *P. chrysogenum*; Mycelium with conidiophores and conidia (400x)

3.1 (a). Effect of different concentration of fungicides on the mycelial growth of *Penicillium chrysogenum*

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 *Penicillium chrysogenum* compared to control. The maximum inhibition in the mycelial growth was observed at the highest concentration (1000ppm) of fungicide followed by their lower concentrations (500ppm, 250ppm, 125ppm). However, hexaconozole proved most effective fungicide in inhibiting the mycelial growth of *Penicillium chrysogenum* followed by other fungicides. The hexaconozole at highest concentration brought about maximum inhibition in mycelial growth (54.94%) followed by carbendazim (44.88%) and copper oxychloride (27.77%) respectively 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 ranges from 54.94% -

19.64% and in different concentration of carbendazim it ranges from 44.88% - 2.02%. Likewise, the inhibition in mycelial growth in copper oxychloride ranges from 27.77% - 3.72% in different concentrations of the fungicides respectively.

b. Effect of different concentrations of fungicides on the spore germination of *Penicillium chrysogenum*

It was observed from the results (Table 2, Fig. 2) that fungicides, viz. copper oxychloride, carbendazim and hexaconozole at different concentrations (1000ppm, 500 ppm, 250ppm, 125ppm) caused significant inhibition in the spore germination of *Penicillium chrysogenum* compared to control. The maximum inhibition in spore germination was observed at highest concentrations (1000ppm) of fungicides followed by their lower concentrations (500ppm, 250ppm, 125ppm). Amongst the fungicides, the 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. The percentage reduction of spore germination in hexaconozole varies from 34.77% - 11.49% in different concentrations. In carbendazim, the reduction in spore germination varies from 40.79% - 20.60% and in copper oxychloride it varies from 47.93% - 26.39% respectively in different concentrations.

Table 1: Effect of different concentrations of fungicides on the mycelial growth of *Penicillium chrysogenum*.

Conc. / Treatment	Mycelial growth (mm)				
	125ppm	**250ppm**	**500ppm**	**1000ppm**	**Control**
Copper oxychloride	17.33±0.57[ab] (3.72)	16.00±1.00[b] (11.11)	14.33±0.57[c] (20.00)	13.00±1.00[c] (27.77)	18.00±1.00[a]
Carbendazim	16.00 ±1.00[a] (2.02)	13.33±1.52[b] (18.37)	12.00±1.00[b] (26.51)	9.00±1.00[c] (44.88)	16.33±1.15[a]
Hexaconozole	13.66 ±1.15[b] (19.64)	12.00±1.00[b] (29.41)	10.00±1.00[c] (41.17)	7.66 ±0.57[d] (54.94)	17.00±1.00[a]

Each value is mean of 3 replicates ± SD

Figures in parenthesis is the mycelial growth inhibition (%)

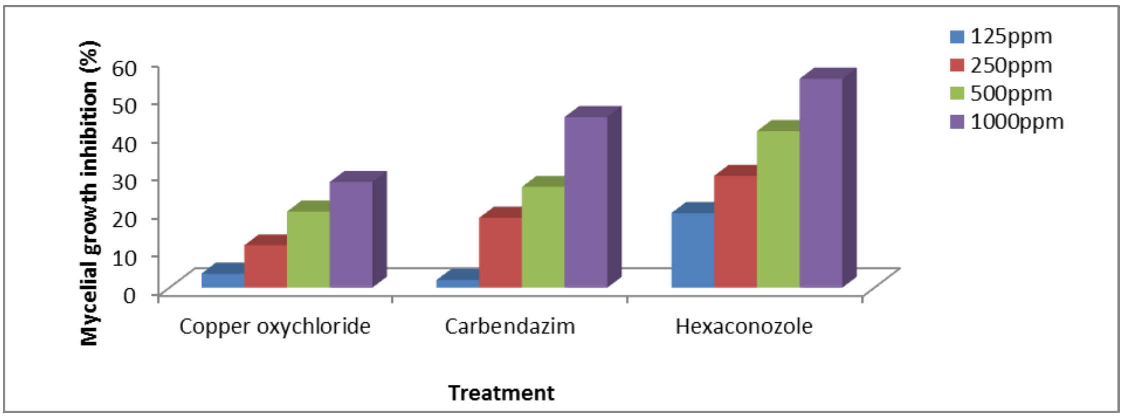

Fig 1. Effect of different concentrations of fungicides on the mycelial growth of *Penicillium chrysogenum*.

Table 2: Effect of different concentration of fungicides on the spore germination of *Penicillium chrysogenum*.

Conc. / Treatment	Spore germination (%)				
	125ppm	**250ppm**	**500ppm**	**1000ppm**	**Control**
Copper oxychloride	47.93±1.93[a]	41.16±1.12[b]	34.34±1.74[c]	26.39±1.03[d]	48.06±0.93[a]
Carbendazim	40.79±0.68[a]	35.83±2.56[b]	26.31±1.17[c]	20.60±2.13[d]	43.39±1.25[a]
Hexaconozole	34.77±1.27[b]	28.11±2.90[c]	18.11±1.67[d]	11.49±2.61[e]	43.68±0.79[a]

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

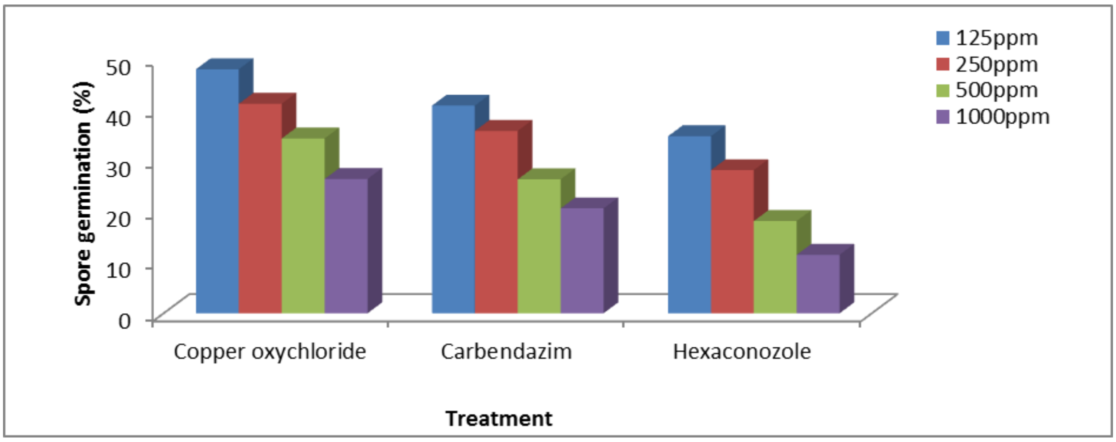

Fig 2. Effect of different concentration of fungicides on the spore germination of *Penicillium chrysogenum.*

4. DISCUSSION

It was clear from the above results that the fungus *Penicillium chrysogenum* attacks brinjal fruits in storage and causes *Penicillium* rot of brinjal 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 *Penicillium* fungi are responsible for causing rot diseases of fruits and vegetables in storage (Schmidt-Heydt *et al* (2013). In the present study, *Penicillium chrysogenum* 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 [Snowdon, (1990); Hassan, (1996)].

In the present study some fungicides were evaluated for their antimycotic activity against the fungus *Penicillium chrysogenum.* Concentations of the tested fungicides proved highly effective in reducing the mycelial growth and caused significant inhibition in the spore germination of *P. chrysogenum*. The highest concentrations of the fungicides proved more effective than lower concentrations. Shazia et al (2013) 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. In the previous work, similar findings were reported Patel et al. (2005); Banyal et al. (2008); Begum et al. (2010), Taskeen-Un-Nisa et al. (2011), Koka *et al* (2018).

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