



## Management of postharvest fungal rot of peach (*Prunus persica*) caused by *Rhizopus stolonifer* in Kashmir Valley, India

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### Abstract

A large proportion of peach fruit production is lost annually due to the prevalence of fungal rot disease in many parts of India including Kashmir. Therefore, the fungal rot of peaches under storage conditions was studied. It was revealed that peach fruits are attacked by *Rhizopus stolonifer*, causing *Rhizopus* soft rot of peaches. Study was also undertaken for the management of *Rhizopus* soft rot of peach with some fungicides and plant extracts. Different concentrations of fungicides brought about significant reduction in the mycelial growth and spore germination of *Rhizopus stolonifer* under *in vitro* conditions. Carbendazim proved highly effective in inhibiting the mycelial growth and spore germination of *R. stolonifer* followed by hexaconazole, bitertanol and myclobutanil. Amongst the plant extracts, *Artemisia absinthium* at highest concentration was found most effective against *R. stolonifer* and caused highest inhibition in the mycelial growth and spore germination, followed by extracts of *Rumex obtusifolius*, *Malva sylvestris*, *Plantago lanceolata* and *Taraxacum officinale* at the same concentration.

**Key words** – fungicide – inhibition – mycelial growth – peach – plant extract – spore germination – storage

### Introduction

Rot diseases caused by fungal pathogens provoke severe losses of agricultural and horticultural crops every year (Salman 2005). Due to their low pH, higher moisture content and nutrient composition fruits are susceptible to attack by pathogenic fungi, which cause decaying or rotting and make them unfit for consumption by producing mycotoxins (Phillips 1984, Moss 2002). In India, postharvest diseases of fruits due to fungi are responsible for about 30 percent losses during harvest and consumption (Parpia 1976, Bashar et al. 2012). Peach (*Prunus persica* (L.) Batsch) belongs to the family Rosaceae and is grown commercially in regions between 25° and 45° latitude above and below the equator. It is the third most important temperate fruit cultivated in India. Presently this crop is being cultivated in Jammu and Kashmir, Punjab, Haryana, Uttaranchal, Himachal Pradesh and to a

lesser extent in hilly areas of Tamil Nadu (FAO 2006). The storage period and market life of peaches is limited due to various postharvest rot diseases (Karabulut & Baykal 2004). Various plant pathogenic fungi have been identified all over the world that cause rotting in peach fruits and reduce their nutritional value, medicinal value and storage period (Gobayashi et al. 1992, Fan & Tian 2000, Karabulut et al. 2002, Karabulut & Baykal 2004, De Cal et al. 2009). Therefore, the present study was carried out with the main objective of identifying the fungal rot pathogen that causes decaying in peaches under storage conditions in Kashmir Valley. The study was also undertaken for the management of identified fungal pathogen with some fungicides and plant extracts.

## Materials & Methods

To investigate the fungi which cause rotting of peach 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 peach 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 1971, Barnett & Hunter 1972, Watanabe 2002, Gilman 2008). For pathogenicity tests, pathogens were re-inoculated after isolation onto healthy peach fruits (Tomkin & Trout 1931) 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 peach 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 and plant extracts under *in vitro* conditions for the control of *Rhizopus* rot of peaches caused by *Rhizopus stolonifer*.

## Preparation and evaluation of different fungicide concentrations

Different concentrations (1000 ppm, 800 ppm, 600 ppm, 400 ppm and 200 ppm) of the fungicides carbendazim, hexaconazole, bitertanol and myclobutanil were prepared in sterilized distilled water and evaluated for their effect on the mycelial growth of rot causing fungus, *Rhizopus stolonifer* by food poisoning technique (Adams & Wong 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 follows:

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

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

For studying 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°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).

$$\text{Percent spore germination} = \frac{\text{No. of spores germinated}}{\text{Total no. of spores examined}} \times 100$$

### Preparation and evaluation of different concentrations of plant extract

Different concentrations of aqueous extracts of leaves of *Artemisia absinthium* L., *Rumex obtusifolius* L., *Taraxacum officinale* Weber ex Wiggers, *Plantago lanceolata* L. and *Malva sylvestris* L. were evaluated for their effect on the mycelial growth of *Rhizopus stolonifer* isolated from decayed peach fruits. For the preparation of different concentrations of plant extracts, 200 g of leaves were washed with sterilized distilled water, ground in a mortar and pestle using 200 ml of sterilized distilled water (Bhat & Sivaprakasan 1994). The material was homogenized for 5 min. and filtered through double layered muslin cloth followed by Whatman's filter paper No. 1. The filtrate was then centrifuged at 5000 rpm for 10 min. and was considered as a standard solution (S). Other concentrations (S/2, S/10, and S/100) were obtained by adding appropriate amount of sterilized distilled water to the standard concentration. These concentrations were evaluated for their effect on the mycelial growth by food poisoning technique (Adams & Wong 1991). One ml from each concentration of the plant extract was mixed with 9 ml of autoclaved and cooled PDA just before pouring into Petri plates. The medium was then dispensed uniformly into 90 mm sterile 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 the 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 growth was computed as outlined above.

These plant extracts were also evaluated for their effect on spore germination of *R. stolonifer*. Spore suspension was prepared from 10-days-old fungal culture. A drop of spore suspension was then placed in a cavity glass slide containing a drop (about 0.1 ml) of different concentration of plant extract and then incubated at 25±2°C for 24 hours in a moist chamber created in 100 mm Petri plates by covering both sides of the Petri plates with moist filter paper to maintain enough humidity. Three replicates were maintained for each treatment including the control. The slides were examined after 24 hours and percent spore germination calculated (Kiraly et al. 1974).

### Results

The casual pathogen was identified as *Rhizopus stolonifer* (Ehrenb.:Fr.) Vuill. on the basis of symptoms caused by the fungus on peach fruits, cultural and microscopic characteristics. The symptoms on peach fruits appeared as small water soaked areas that become soft and rotten. The infected fruits were covered by white fluffy mycelium that later turned black due to sporulation. The infected tissue became a soft, watery rot (Fig. 1). The symptoms of *Rhizopus* rot rarely occurs in orchards as it is generally a problem of mature and fully ripe peach fruits and occurs mostly in storage. The pathogen enters the peach fruits only through injuries caused during harvesting. On PDA medium *R. stolonifer* was fast growing and produced luxuriant growth by means of stolons. The colonies were cottony, white at first and then became black due to sporulation (Fig. 2). Microscopic study of the fungus revealed that the mycelium is profusely branched. Three types of hyphae are recognized in the mycelium: rhizoids, stolons and sporangiophores. The stolons grow horizontally above the substrate and form tufts of rhizoids and sporangiophores. The rhizoids are formed at certain points towards the substrate. The sporangiophores are erect, aerial and unbranched and bear terminal sporangia measuring

90–185  $\mu\text{m}$  in diameter. The sporangiophores are 500–800  $\mu\text{m}$  in length. The sporangia produce many sporangiospores that are irregular, round or oval measuring 6–12  $\times$  5–9  $\mu\text{m}$  (Fig. 3).



**Figs 1–3** – 1, Infected peach fruit. 2, Culture of *Rhizopus stolonifer* on PDA medium. 3, *R. stolonifer*: Mycelium with sporangiophores, rhizoids and sporangia (100  $\times$ )

### Control of *Rhizopus stolonifer* causing *Rhizopus* rot of peach with fungicides and plant extracts

Different concentrations of fungicides and plant extracts were evaluated under *in vitro* conditions for their effect on the mycelial growth and spore germination of the test fungi.

#### Effect of different concentrations of fungicides on the mycelial growth of *Rhizopus stolonifer*

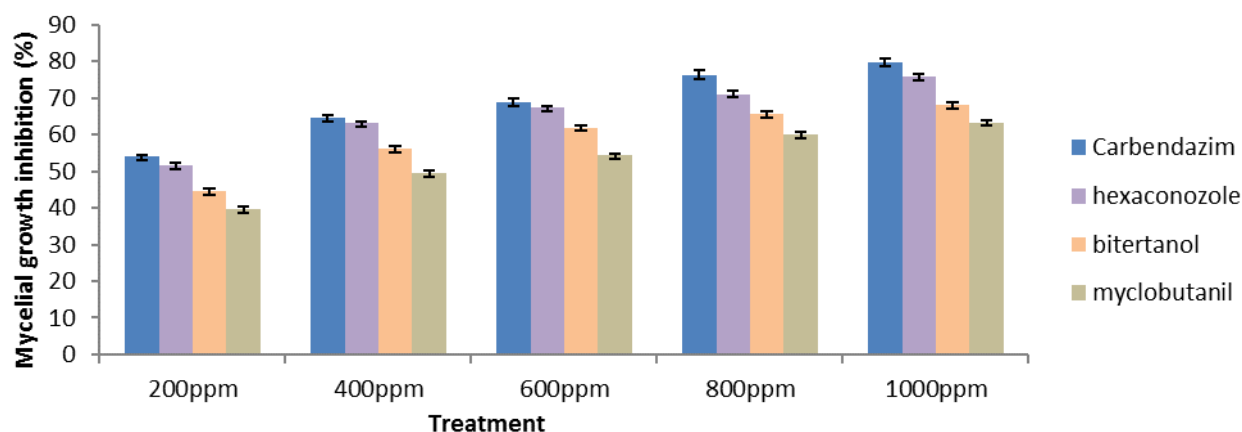
The fungicides brought about significant reduction in mycelial growth of *R. stolonifer*, being most effective at higher concentrations (Table 1, Fig. 4). Carbendazim at 1000 ppm was most effective reducing mycelial growth by 80% followed by hexaconazole (76%), bitertanol (68%) and myclobutanil (63%). Lower concentrations of the fungicides also brought about significant inhibition in the mycelial growth but to a lesser extent.

**Table 1** Effect of different concentrations of fungicides on the mycelial growth of *Rhizopus stolonifer*

Treatment	Treatment Concentration					Control
	200 ppm	400 ppm	600 ppm	800 ppm	1000 ppm	
<b>Carbendazim</b>	31.83 $\pm$ 0.29* (54.12)	24.53 $\pm$ 0.50 (64.64)	21.67 $\pm$ 1.04 (68.77)	16.50 $\pm$ 1.50 (76.22)	14.03 $\pm$ 1.05 (79.78)	69.38 $\pm$ 0.50
<b>Hexaconazole</b>	33.50 $\pm$ 0.50 (51.71)	25.60 $\pm$ 0.53 (63.10)	22.70 $\pm$ 0.61 (67.28)	20.07 $\pm$ 0.90 (71.07)	16.83 $\pm$ 0.76 (75.74)	69.38 $\pm$ 0.50
<b>Bitertanol</b>	38.37 $\pm$ 0.55 (44.69)	30.37 $\pm$ 0.55 (56.23)	26.40 $\pm$ 0.53 (61.95)	23.93 $\pm$ 0.90 (65.51)	22.17 $\pm$ 0.76 (68.04)	69.38 $\pm$ 0.50
<b>Myclobutanil</b>	41.83 $\pm$ 0.76 (39.71)	35.13 $\pm$ 0.81 (49.36)	31.73 $\pm$ 0.64 (54.27)	27.73 $\pm$ 0.64 (60.03)	25.43 $\pm$ 0.51 (63.35)	69.38 $\pm$ 0.50

\*Each value is mean of 3 replicates  $\pm$  SD

Figures in parenthesis is the mycelial growth inhibition (%)



**Fig. 4** Effect of different concentrations of fungicides on the mycelial growth of *Rhizopus stolonifer*

**Effect of different concentrations of fungicides on spore germination of *Rhizopus stolonifer***

The fungicides caused significant reduction in the percent spore germination of *Rhizopus stolonifer* compared to control (Table 2, Fig. 5). Carbendazim at highest concentration brought about highest reduction in spore germination of *R. stolonifer* followed by hexaconozole, bitertanol and myclobutanil at the same concentration. The other concentrations also brought about significant reduction in spore germination but to a lesser extent

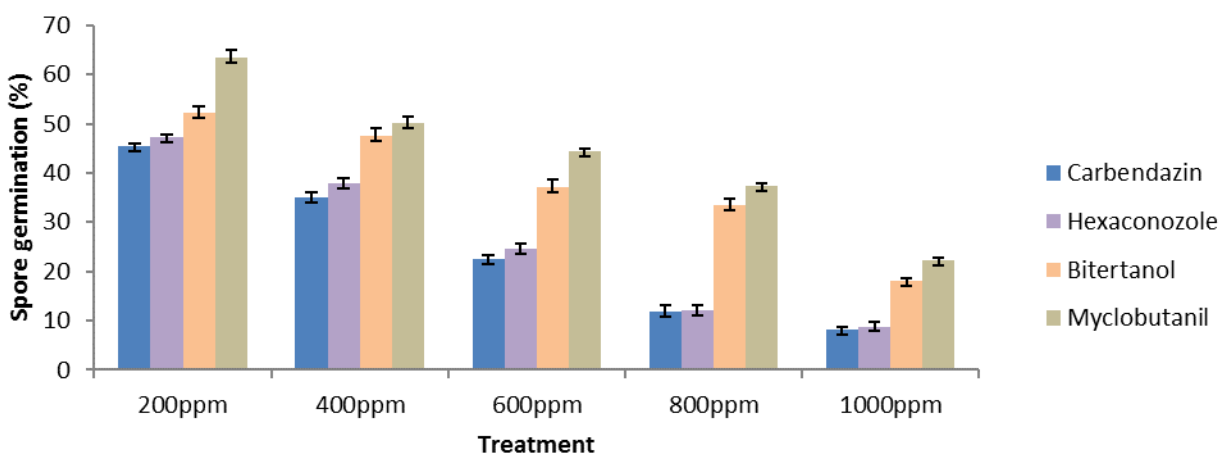
**Table 2** Effect of different concentrations of fungicides on the spore germination of *Rhizopus stolonifer*

Treatment	Treatment Concentration					
	200 ppm	400 ppm	600 ppm	800 ppm	1000 ppm	
<b>Carbendazim</b>	45.33±0.57*	34.92 ±1.15	22.24±0.58	13.13±1.15	8.21±0.58	81.54±0.58
<b>Hexaconozole</b>	47.21±0.58	37.88±1.15	24.59±1.00	14.28±1.00	8.82±1.00	81.54±0.58
<b>Bitertanol</b>	52.30±1.15	47.56±1.53	37.11±1.53	31.76±1.53	18.05±0.58	79.97±0.58
<b>Myclobutanil</b>	63.32±1.53	50.02±1.53	44.44±0.58	36.05±0.58	22.23±0.58	85.24±1.15

\*Each value represents the mean spore germination percentage of 3 replicates ± SD

**Effect of different concentrations of plant extracts on mycelial growth of *Rhizopus stolonifer***

The plant extracts caused significant inhibition in the mycelial growth of *R. stolonifer* (Table 3, Fig. 6). The maximum inhibition in mycelial growth was found at highest concentration ‘S’ of the plant extracts. *Artemisia absinthium* extract was most effective and caused highest inhibition in the mycelial growth (59%) followed by *Rumex obtusifolius* (56%), *Malus sylvestris* (53%), *Plantago lanceolata* (46%) and *Taraxacum officinale* (34%).



**Fig. 5** Effect of different concentrations of fungicides on spore germination of *Rhizopus stolonifer*

**Table 3** Effect of different concentrations of plant extracts on the mycelial growth of *Rhizopus stolonifer*

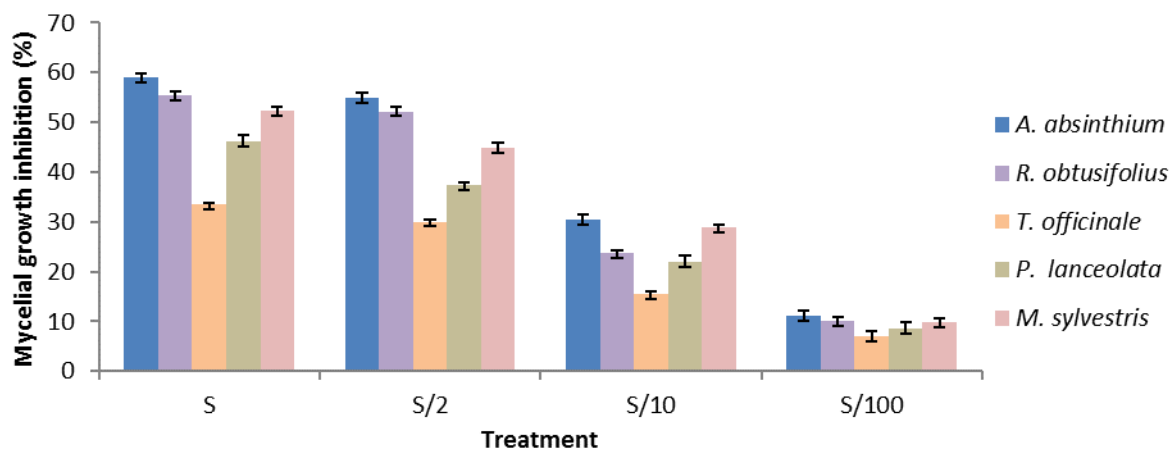
Treatment	Treatment Concentration				Control
	S	S/2	S/10	S/100	
<i>A. absinthium</i>	28.57±0.81* (58.94)	31.17±0.97 (54.94)	48.07±1.10 (30.50)	61.47±1.14 (11.13)	69.17±2.75
<i>R. obtusifolius</i>	30.67±0.76 (55.51)	33.00±1.00 (52.19)	52.70±0.56 (23.81)	62.93±0.91 (10.03)	69.17±2.75
<i>T. officinale</i>	45.97±0.25 (33.54)	48.40±0.51 (30.03)	58.53±0.74 (15.38)	64.27±1.18 (7.08)	69.17±2.75
<i>P. lanceolata</i>	37.20±1.11 (46.22)	43.37±0.67 (37.31)	53.93±1.10 (22.03)	63.30±1.41 (8.49)	69.17±2.75
<i>M. sylvestris</i>	32.93±0.86 (52.29)	38.10±1.04 (44.82)	49.30±0.53 (28.91)	62.30±0.79 (9.93)	69.17±2.75

\*Each value is mean of 3 replicates ± SD

Figures in parenthesis is the mycelial growth inhibition (%)

#### Effect of different concentrations of plant extracts on spore germination of *Rhizopus stolonifer*

The maximum reduction in spore germination was found at highest concentration ‘S’ of the plant extracts (Table 4, Fig. 7). The *A. absinthium* extract at highest concentration ‘S’ was most effective and caused highest reduction in spore germination followed by extracts from *M. sylvestris*, *R. obtusifolius*, *P. lanceolata* and *T. officinale*.

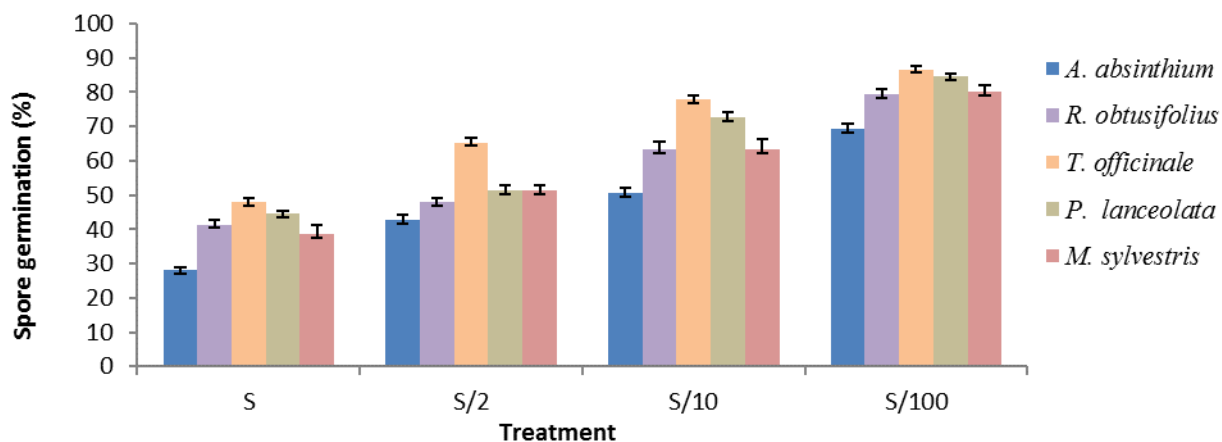


**Fig. 6** Effect of different concentrations of plant extract on the mycelial growth of *Rhizopus stolonifer*

**Table 4** Effect of different concentrations of plant extracts on spore germination of *Rhizopus stolonifer*

Treatment	Treatment Concentration				Control
	S	S/2	S/10	S/100	
<i>A. absinthium</i>	28.00±1.00*	42.66±1.53	50.66±1.53	69.34±1.53	91.34±2.08
<i>R. obtusifolius</i>	41.34±1.53	48.00±1.00	63.34±2.08	79.34±1.53	88.00±3.60
<i>T. officinale</i>	48.00±1.00	65.34±1.53	78.00±1.00	86.66±1.15	89.34±0.58
<i>P. lanceolata</i>	44.66±0.57	51.34±1.53	72.66±1.53	84.66±0.58	91.34±2.08
<i>M. sylvestris</i>	38.66±2.52	51.34±1.53	63.34±3.05	80.00±2.00	91.34±2.08

\*Each value represents the mean spore germination percentage of 3 replicates ± SD



**Fig. 7** Effect of different concentrations of plant extracts on spore germination of *Rhizopus stolonifer*

## Discussion

It was clear from the results that the fungus *Rhizopus stolonifer* attacks peach fruits in storage and causes *Rhizopus* soft rot of peach fruits. Such studies on fungal rot of peach have been carried out for the first time in Kashmir Valley. Earlier reports also indicate that species of *Rhizopus* are

responsible for causing *Rhizopus* rot of fruits and vegetables in storage (Ogawa et al. 1963, Heyns 1968, Leupschen et al. 1971, Gobayashi et al. 1992, Yasuda et al. 1999, Northover & Zhou 2002, Fan & Tian 2000, Janisiewicz & Korsten 2002, Karabulut & Baykal 2004, Al-Hindi et al. 2011). In the present study, *R. stolonifer* 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 (Hernández-Lauzardo et al. 2006, Kwon & Lee 2006). In India *Rhizopus* rot of peaches was first reported by Bhargava & Gupta (1957).

In the present study some fungicides and plant extracts were evaluated for their antimycotic activity against the fungus *R. stolonifer*. All of the tested fungicides proved highly effective in reducing the mycelial growth and caused significant inhibition in the spore germination of *R. stolonifer*. The highest concentrations of the fungicides and plant extracts proved more effective than lower concentrations. Similar findings were reported with respect to antifungal activity of other fungicides by Singh et al. (1997), Patel et al. (2005), Imtiaj et al. (2005), Kopacki & Wagner (2006), Banyal et al. (2008), Begum et al. (2010), Taskeen-Un-Nisa et al. (2011), Wani & Taskeen-Un-Nisa (2010) and Schmidt-Heydt et al. (2013). Taskeen-Un-Nisa (2009) tested various fungicides against many vegetable rot causing fungi including *R. stolonifer* and observed carbendazim as the most effective fungicide for reducing *Rhizopus* rot. Parveen 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 hexaconazole and carbendazim as effective. Plant extracts of all the tested plants also proved effective against *R. stolonifer*. In similar studies, several reports stated that the extracts of medicinal plants play an important role in controlling many phytopathogenic fungi (Okemo et al. 2003, Imtiaj et al. 2005, Khalil et al. 2005, Lee et al. 2007, Ogbebor et al. 2007, Baka 2010, Taskeen-Un-Nisa et al. 2010, 2011; Raji & Raveendran 2013, Znini et al. 2013). Mondall et al. (2009) reported the antifungal activity of aqueous and alcoholic neem leaf extracts on some fungi viz. *Aspergillus*, *Rhizopus* and reported that the alcoholic extracts of neem leaf was most effective in comparison to aqueous extract for reducing the growth of *Rhizopus* and *Aspergillus*. The crude aqueous and alcoholic leaf extracts of neem was more effective in inhibitions of growth of *Aspergillus* in comparison to inhibitory effects on *Rhizopus* growth in culture medium. Taskeen-Un-Nisa et al. (2010) tested plant extracts of three plants, onion (*Allium cepa*), garlic (*Allium sativum*) and mint (*Mentha arvensis*) for their antifungal activity against *Alternaria alternata* and *R. stolonifer*. They observed that the extract of *A. sativum* at highest concentration proved highly effective in reducing spore germination of *R. stolonifer* and *Alternaria alternata* followed by extract of *A. cepa* and *M. arvensis*. Jeyaseelan et al. (2012) reported antifungal activity of organic extracts of leaf, flower and fruit of *Lawsonia inermis* L. against *Aspergillus niger*, *Penicillium notatum*, *Fusarium oxysporum*, *Colletotrichum gloeosporioides* and *Rhizopus stolonifer*. Parveen et al. (2014) reported the inhibitory activity of five plant extracts, viz. *Artemisia absinthium*, *Rumex obtusifolius*, *Taraxacum officinale*, *Plantago lanceolata* and *Malva sylvestris* against the mycelial growth of three rot fungi, *Alternaria alternata*, *Penicillium expansum* and *Mucor piriformis*, and observed that all concentrations brought about significant reduction in the mycelial growth of these pathogenic fungi. However, the highest concentration caused maximum inhibition in the mycelial growth. The extract of *A. absinthium* leaves at highest concentration (S) proved highly effective in inhibiting the mycelial growth of all these pathogenic fungi followed by other plant extracts.

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