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Evaluation of different plant extracts for effective management of fungal rot of tomato and brinjal in Kashmir Valley

Jahangir Abdullah Koka*, Abdul Hamid Wani and Mohd Yaqub Bhat

University of Kashmir. Department of Botany. Section of Mycology and Plant Pathology. Srinagar. India. *Email: kokajahangir76@gmail.com.

Abstract. The aim of present research was focused on the antifungal activities of *Prunella vulgaris* L and *Paeonia suffruticosa* Andrews via *in vitro* approach through agar well diffusion assay at three concentrations (25 µL, 50 µL and 75 µL) against fungi causing diseases in tomato and brinjal. All the concentration of plant extracts showed antimycotic activity against tested pathogenic fungi. Antimycotic activity increased with the increased concentrations of plant extracts. However, higher concentrations proved more effective than lower concentrations. It was revealed from the present study that the ethanolic and aqueous extracts of *Prunella vulgaris* L showed maximum antimycotic activity against *Rhizoctonia solani* and least inhibitory effect against *Penicillium chrysogenum*. It was further revealed from the present study that the ethanolic extract of *Paeonia suffruticosa* Andrews showed maximum antimycotic activity against *Penicillium expansum* and least activity against *Mucor plumbeus*. Whereas the aqueous extract of *Paeonia suffruticosa* Andrews showed maximum antimycotic activity against *Rhizoctonia solani* and *Penicllium expansum* and least inhibitory effect against *Mucor plumbeus*.

Keywords: Antifungal activity; Rot causing fungi; Concentration; Plant extracts; Agar Well Diffusion.

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ORCID
0000-0003-0040-1624
Jahangir Abdullah Koka

0000-0002-6157-9656
Abdul Hamid Wani

0000-0002-0582-4813
Mohd Yaqub Bhat

Introduction

Almost, all plants are attacked by a number of plant pathogenic fungi resulting in many plant diseases which reduce their yield and quality of the products. Fungal rots are world-wide in occurrence and have been reported from all parts of the world (Janisiewicz and Korsten, 2002).The destructive pathogen causing rots on tomato is present in parts of

the country where moisture is plentiful and temperatures are moderate to favour their development (Sokhi and Sohi, 1982). Various workers have isolated and identified a diverse range of fungal pathogens belonging to the genera *Alternaria*, *Aspergillus*, *Rhizopus*, *Mucor*, *Penicillium*, *Phoma*, *Fusarium*, causing rot diseases of various fruits and vegetables (Snowdon, 1990; Jones, 1991; Iqbal et al., 2003; Mari et al., 2003; Patel et al., 2005; Ali et al., 2005; Ebele, 2011; Taskeen-un-Nisa et al., 2011; Abata et al., 2016; Wennekar et al., 2017).

Various biocontrol fungi and extracts obtained from many medicinal plants have gained much popularity and scientific interest for their antifungal and antibacterial activities (Santas et al., 2010; Parveen et al., 2016a; Koka et al., 2017). Plant extracts are believed to be more acceptable and less hazardous than synthetic compounds and can be therefore used as an alternative to synthetic antifungal chemicals (Nazzaro et al., 2000).

Several control strategies have been employed by agricultural scientists to minimize the losses caused by pathogenic fungi. In this study, the important medicinal plants *Prunella vulgaris* and *Paeonia suffruticosa* have been evaluated for their antifungal activity. Medicinal plants are the only affordable and accessible source of primary health care for many people in Asia, especially in absence of access to modern medical facilities. Most of the compounds extracted from these plants, used as pharmaceuticals, agrochemicals, flavour and fragrance ingredients, food additives and pesticides. Amin et al. (2013) studied the medicinal importance of variety of pharmacologically active compounds including quinones, triterpenoids, flavonoids, isoflavonoids and stilbene glycosides.

The aim of this present study is to investigate the antifungal activities of ethanolic and aqueous extracts of leaves of *Prunella vulgaris* L and *Paeonia suffruticosa* Andrews.

Materials and methods

Plant collection and identification

The fresh plant material of *Prunella vulgaris* L and *Paeonia suffruticosa* Andrews were collected from District Baramulla and Kashmir University Botanical Garden (KUBG), Srinagar. The authenticity of the plant was confirmed in Plant Taxonomy Department of Botany University of Kashmir. Adequate amount of the leaves of these plants were collected in polythene bags, brought to laboratory for evaluating their antimycotic activity under *in vitro* conditions.

Preparation of plant extracts

These plant leaves in a required quantity were sundried for 24 hours and then milled into powder using morter and pestle. About 20g of coarsely powdered leaves (20 g/100 mL) were extracted separately in a soxhlet extractor for 8 to 10 hours (30 °C-50 °C) sequentially with ethanol and water separately in order to extract non-polar and polar compounds (Elgorashi et al., 2004).

Preparation of inoculums of fungi

Pure fungal cultures of *Penicillium expansum*, *Aspergillus niger*, *Alternaria alternata*, *Mucor plumbeus*, *Penicillium chrysogenum*, *Trichothecium roseum* and *Rhizoctonia solani* were obtained from Plant Pathology and Mycology Laboratory, Department of Botany University of Kashmir. These pure cultures were grown on Potato dextrose agar (PDA) medium at 27 °C ± 1 °C in Petri plates. Spores of the each fungal species were collected from these cultures after 7 days (Broekaert et al., 1990).

Antifungal activity

The antifungal activity of the plant extracts was determined by agar well diffusion method as adopted by Perez et al. (1990), Alzoreky et al. (2003) and Ahmad et al. (2012). Seven day old fungal cultures grown on PDA medium were used to assess the antifungal activity of selected plant extracts. An aliquot of 100 µL inoculum from each fungal species was inoculated in 20 mL of molten SDA medium in culture tubes. The culture tubes were then homogenised manually and poured into 90 mm Petri plate. The culture plates were allowed to solidify inside the laminar airflow chamber and three wells at periphery of each Petri plate were made using sterile cork borers of 5 mm in diameter. A 2 mg/mL stock solution was made from the plant extract and then different volumes (25 µL, 50 µL and 75 µL) from that stock solution were loaded to respective wells. Hexaconazole solution (20 µL/well) was used as control in the separate well in the same petri plate. The effect of plant extracts on different rot causing fungi were evaluated and the plates were then sealed and incubated at 25 °C ± 2 °C for 4-5 days. Three replicates were made for each treatment. Antifungal potential was calculated by measuring inhibition zone diameters in millimeters (mm) with the help of standard measuring scale (Norrel and Messley 1997).

Statistical analysis

The data collected during these investigations were subjected to appropriate statistical analysis using SPSS statistical software (version 16.0). The data was statistically analyzed by one way analysis of variance (ANOVA) and comparison of the means was done by Duncan multiple comparison tests at $P \leq 0.05$.Standard deviation was calculated as $\delta = \sqrt{\frac{\sum x^2}{N-1}}$.

Results

Effect of leaf extracts of *Prunellla vulgaris* L on the zone of mycelial inhibition of some rot causing fungi

It was found from the results (Table 1, Figure 1) that the ethanolic leaf extract of *Prunella vulgaris* L. caused maximum inhibitory activity of mycelial growth at 25 µL, 50 µL and 75 µL concentrations with zone of inhibition of 22.66 mm, 24.33 mm and 26.00 mm against *Rhizoctonia solani*, respectively. The inhibition in zone of mycelial growth of *Aspergillus niger* and *Alternaria alternata* was 20.00 mm, 22.00 mm, 23.66 mm and 16.33 mm, 19.00 mm, 23.33 mm due to leaf extracts of *P. vulgaris* at 25 µL, 50 µL and 75 µL concentrations, respectively. The moderate inhibitory activity of ethanolic extract was found against *Penicillium expansum* with zone of mycelial inhibition of 16.00 mm, 19.00 mm, 22.00 mm, respectively, and against *Trichothecium roseum* with zone of mycelial inhibition of 15.66 mm, 17.00 mm, 21.00 mm at 25 µL, 50 µL and 75 µL, respectively. However, the inhibition in mycelial growth of *Mucor plumbeus* was 14.33 mm, 16.00 mm, 19.00 mm at 25 µL, 50 µL and 75 µL concentrations of plant extracts of *P. vulgaris*. Whereas least inhibitory activity was shown against *Penicillium chrysogenum* with zone of inhibition as 13.00 mm, 16.33 mm and 18.33 mm at 25 µL, 50 µL and 75 µL of ethanolic leaf extracts.

Further, it was observed from results (Table 2, Figure 2) that the aqueous extract of *Prunella vulgaris* L. showed maximum inhibitory activity in mycelial growth against *Rhizoctonia solani* at 25 µL, 50 µL and 75 µL concentrations with zone of inhibition of 20.33 mm, 22.66 mm and 24.66 mm, respectively. Moderate antifungal activity of leaf extract was recorded against *Aspergillus niger* with zone of mycelial inhibition of 18.00 mm, 20.00 mm, 21.66 mm, against *Alternaria alternata* with zone of mycelial inhibition of 14.33 mm, 17.33 mm, 20.66 mm and against *Penicillium expansum* with zone of mycelial inhibition of 13.00 mm, 16.00 mm, 19.33 mm at 25 µL, 50 µL and 75 µL

concentrations of leaf extracts, respectively. The inhibition in mycelial growth of *Mucor plumbeus* and *Trichothecium roseum* was observed as 12.33 mm, 15.33 mm, 17.00 mm and 11.66 mm, 15.00 mm, 19.00 mm at 25 µL, 50 µL and 75 µL concentrations of leaf extracts of *P. vulgaris*, respectively. The least mycelial inhibition found in *Penicillium chrysogenum* was as 11.00 mm, 13.33 mm and 16.00 mm at 25 µL, 50 µL and 75 µL concentrations, respectively.

Table 1. Effect of ethanolic leaf extracts of *Prunella vulgaris* L.at different concentrations on the zone of mycelial inhibition of some rot causing fungi.

Concentration / Fungal Pathogens	Zone of mycelial inhibition (mm)			
	25 µL	50 µL	75 µL	Control
Penicillium expansum	16.00±1.00^{d}	19.00±1.00^{c}	22.00±1.00^{b}	24.33 ±0.57^{a}
Aspergillus niger	20.00±1.00^{c}	22.00±1.00^{b}	23.66±1.15^{b}	27.00 ±1.00^{a}
Alternaria alternata	16.33±0.57^{d}	19.00±1.00^{c}	23.33±0.57^{b}	25.00 ±1.00^{a}
Mucor plumbeus	14.33±0.57^{c}	16.00±1.00^{c}	19.00±1.00^{b}	22.00 ±1.00^{a}
Penicillium chrysogenum	13.00±1.00^{d}	16.33±0.57^{c}	18.33±0.57^{b}	21.33 ±0.57^{a}
Trichothecium roseum	15.66±0.57^{c}	17.00±1.00^{c}	21.00±1.00^{b}	24.00 ±1.00^{a}
Rhizoctonia solani	22.66±0.57^{c}	24.33±0.57^{b}	26.00±1.00 ^{a}	27.00 ±1.00^{a}

Each value is mean of 3 replicates ± SD. Mean values followed by different superscript in a column are significantly different ($p \leq 0.05$).

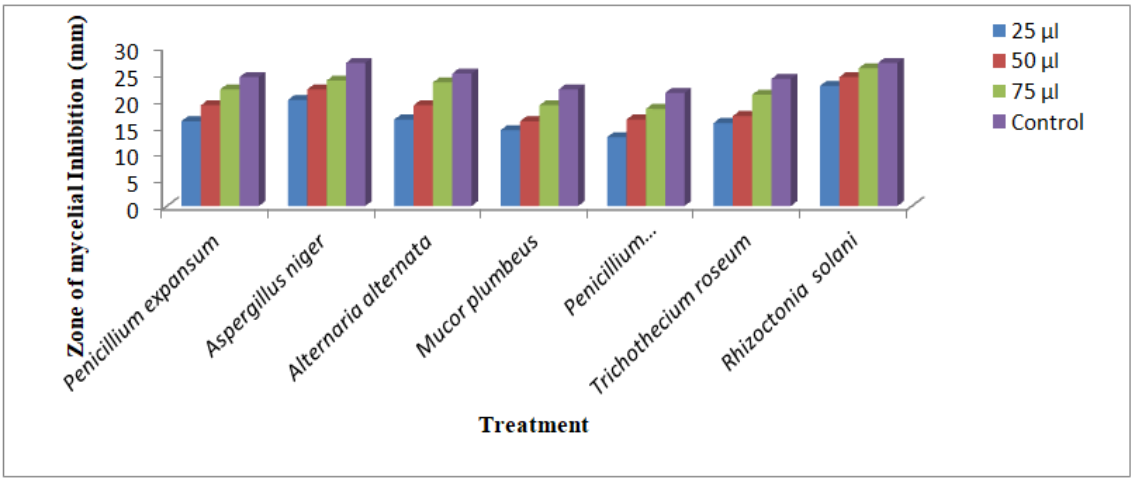

Figure 1. Effect of ethanolic leaf extracts of *Prunella vulgaris* L. at different concentrations on the zone of mycelial inhibition of some rot causing fungi.

Table 2. Effect of aqueous leaf extracts of *Prunella vulgaris* L. at different concentrations on the zone of mycelial inhibition of some rot causing fungi.

Concentration / Fungal Pathogens	Zone of mycelial inhibition (mm)			
	25 µL	50 µL	75 µL	Control
Penicillium expansum	13.00±1.00^{d}	16.00±1.00^{c}	19.33±0.57^{b}	22.00±1.00^{a}
Aspergillus niger	18.00±1.00 ^{c}	20.00±1.00^{b}	21.66±1.15^{ab}	23.00±1.00^{a}
Alternaria alternata	14.33±0.57^{d}	17.33±0.57^{c}	20.66±0.57^{b}	22.00±1.00^{a}
Mucor plumbeus	12.33±0.57^{d}	15.33±0.57^{c}	17.00±1.00^{b}	19.33±0.57^{a}
Penicillium chrysogenum	11.00±1.00^{d}	13.33±0.57^{c}	16.00±1.00^{b}	18.00±1.00^{a}
Trichothecium roseum	11.66±0.57^{d}	15.00±1.00^{c}	19.00±1.00^{b}	22.00±1.00^{a}
Rhizoctonia solani	20.33±0.57^{d}	22.66±0.57^{c}	24.66±0.57^{b}	26.00±1.00^{a}

Each value is mean of 3 replicates ± SD. Mean values followed by different superscript in a column are significantly different (p≤ 0.05)

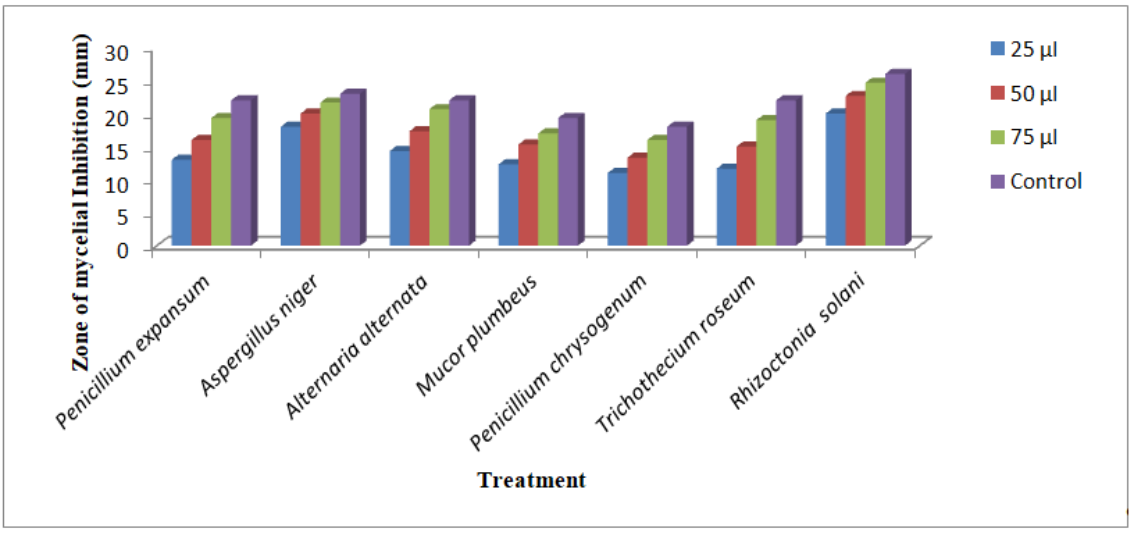

Figure 2. Effect of aqueous leaf extracts of *Prunella vulgaris* L. at different concentrations on the zone of mycelial inhibition of some rot causing fungi.

Effect of leaf extracts of *Paeonia suffruticosa* Andrews at different concentrations on the zone of mycelial inhibition of some rot causing fungi.

It was revealed from results (Table 3, Figure 3) that the ethanolic leaf extract of *Paeonia suffruticosa* Andrews showed maximum inhibitory activity at 25 µL, 50 µL and 75 µL concentrations with zone of mycelial inhibition of 23.00 mm, 25.00 mm and 26.66 mm against *Penicillium expansum*, respectively. The inhibition in mycelial growth of *Rhizoctonia solani* and *Alternaria alternata* was 22.33 mm, 25.00 mm, 28.33 mm and 21.00 mm, 24.00 mm, 26.00 mm at 25 µL, 50 µL and 75 µL concentrations of ethanolic leaf extracts of *P. suffruticosa*, respectively. The inhibition in mycelial growth of *Aspergillus niger* was 21.33 mm, 23.00 mm, 25.00 mm at 25 µL, 50 µL and 75 µL concentrations of ethanolic leaf extracts of *P. suffruticosa*, respectively. The moderate antifungal activity of ethanolic extract was shown against *Penicillium chrysogenum* and *Trichothecium roseum* with zone of mycelial inhibition as20.00 mm, 21.66 mm, 24.00 mm and 19.33 mm, 22.00 mm, 25.00 mm at 25 µL, 50 µL and 75 µL concentrations of leaf extracts, respectively. The least inhibition in mycelial growth was found in case of *Mucor plumbeus*

with zone of inhibition as19.66 mm, 22.00 mm and 24.33 mm at 25 µL, 50 µL and 75 µL concentrations respectively of ethanolic leaf extracts of *P. suffruticosa*.

Further it was found from the results (Table 4, Figure 4) that the aqueous extract of *Paeonia suffruticosa* Andrews showed maximum inhibitory activity at 25 µL, 50 µL and 75 µL concentrations with zone of mycelial inhibition of 20.66 mm, 23.33 mm, 25.33 mm and 19.33 mm, 21.33 mm, 25.00 mm against *Rhizoctonia solani* and *Penicillium expansum* respectively. The aqueous leaf extract of *Paeonia suffruticosa* showed moderate antifungal activity against *Alternaria alternata* and *Aspergillus niger* with the zone of mycelial inhibition as 19.00 mm, 22.00 mm, 24.33 and 19.00 mm, 21.00 mm, 23.33 mm at 25 µL, 50 µL and 75 µL concentrations respectively. However, the zone of inhibition in mycelial growth of *Trichothecium roseum* and *Penicillium chrysogenum* was 19.00 mm, 20.66 mm, 23.33 mm and 18.00 mm, 19.66 mm, 22.33 mm at 25 µL, 50 µL and 75 µL concentrations of leaf extracts respectively. Whereas the least mycelial inhibition was shown in *Mucor plumbeus* with the zone of inhibition as 17.66 mm, 20.00 mm and 23.33 mm at 25 µL, 50 µL and 75 µL, respectively of leaf extracts.

Table 3. Effect of ethanolic leaf extracts of *Paeonia suffruticosa* Andrews at different concentrations on the zone of mycelial inhibition of some rot causing fungi.

Concentration / Fungal Pathogens	Zone of mycelial inhibition (mm)			
	25 µL	50 µL	75 µL	Control
Penicillium expansum	23.00±1.00^{c}	25.00±1.00^{b}	26.66 ± 1.15^{b}	30.00±1.00^{a}
Aspergillus niger	21.33±0.57^{c}	23.00±1.00^{c}	25.00 ± 1.00^{b}	29.00±1.00^{a}
Alternaria alternata	21.00±1.00^{d}	24.00±1.00^{c}	26.00 ± 1.00^{b}	28.33±0.57^{a}
Mucor plumbeus	19.66±1.15^{d}	22.00±1.00^{c}	24.33 ± 1.00^{b}	27.00±1.00 ^{a}
Penicillium chrysogenum	20.00±1.00^{c}	21.66±1.15^{c}	24.00 ± 1.00^{b}	27.00±1.00^{a}
Trichothecium roseum	19.33±0.57^{d}	22.00±1.00^{c}	25.00 ± 1.00^{b}	30.00±1.00^{a}
Rhizoctonia solani	22.33±0.57^{d}	25.00±1.00^{c}	28.33 ± 0.57^{b}	30.33±1.00^{a}

Each value is mean of 3 replicates ± SD. Mean values followed by different superscript in a column are significantly different ($p \leq 0.05$).

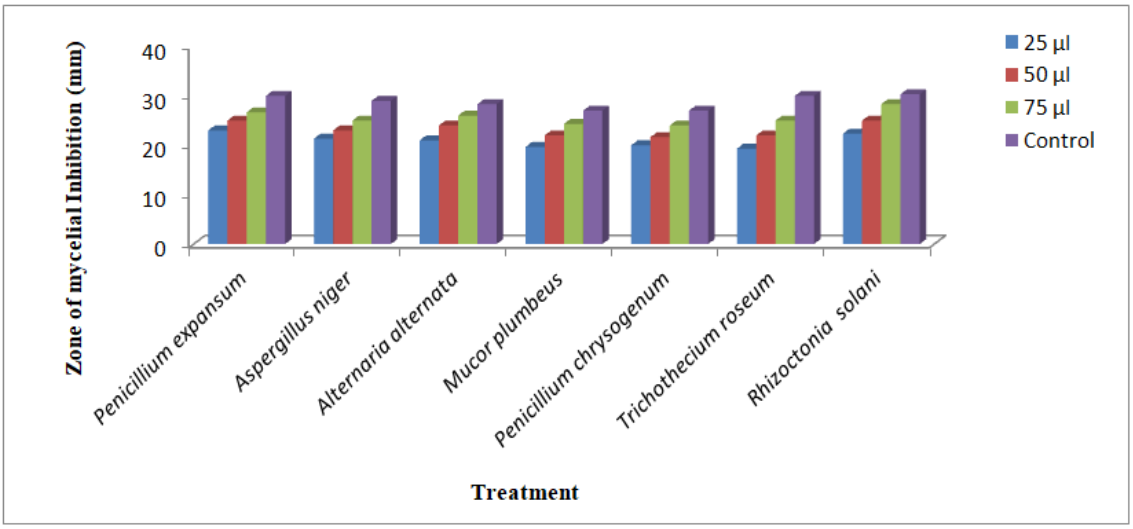

Figure 3. Effect of ethanolic leaf extracts of *Paeonia suffruticosa* Andrews at different concentration on the zone of mycelial inhibition of some rot causing fungi.

Table 4. Effect of aqueous leaf extracts of *Paeonia suffruticosa* Andrews at different concentration on the zone of mycelial inhibition of some rot causing fungi.

Concentration / Fungal Pathogens	Zone of mycelial inhibition (mm)			
	25 µL	50 µL	75 µL	Control
Penicillium expansum	19.33±0.57^{d}	21.33±0.57^{c}	25.00±1.00^{b}	27.00±1.00^{a}
Aspergillus niger	19.00±1.00^{d}	21.00±1.00^{c}	23.33±0.57^{b}	26.00±1.00^{a}
Alternaria alternata	19.00±1.00^{d}	22.00±1.00^{c}	24.33±0.57^{b}	26.33 ± 0.57^{a}
Mucor plumbeus	17.66±1.15^{c}	20.00±1.00^{b}	23.33±1.15^{a}	25.00 ± 1.00^{a}
Penicillium chrysogenum	18.00±1.00^{c}	19.66±1.15^{c}	22.33±0.57^{b}	25.00 ± 1.00^{a}
Trichothecium roseum	19.00±1.00^{c}	20.66±1.15^{c}	23.33±0.57^{b}	26.00 ± 1.00^{a}
Rhizoctonia solani	20.66±1.52^{d}	23.33±0.57^{c}	25.33±0.57^{b}	27.66 ± 0.57^{a}

Each value is mean of 3 replicates ± SD. Mean values followed by different superscript in a column are significantly different ($p \leq 0.05$).

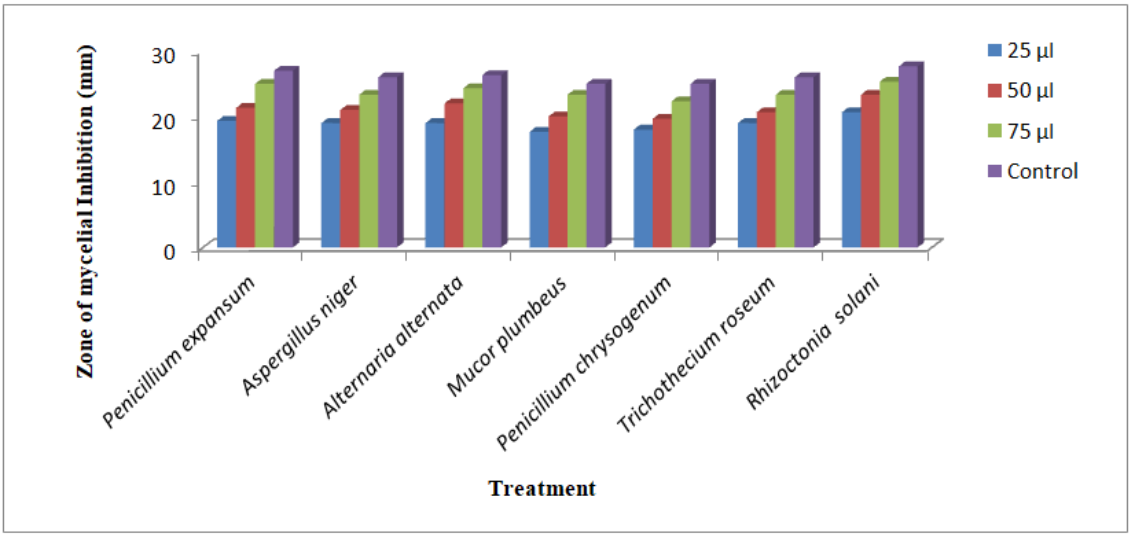

Figure 4. Effect of aqueous leaf extracts of *Paeonia suffruticosa* Andrews at different concentrations on the zone of mycelial inhibition of some rot causing fungi.

Discussion

It was clear from the results that extracts of two medicinal plants *Prunella vulgaris* and *Paeonia suffruticosa* brought about significant inhibition in the mycelial growth at their different concentration. Higher concentration proved effective than lower concentration. In the present study some plant extracts were evaluated for their antimycotic activity against the fungus causing rot of tomato and brinjal. These two test plant species proved highly effective in reducing the mycelial growth of fungi causing rot diseases of tomato and brinjal fruits. Such study has been carried for the first time on the extracts of *Prunella vulgaris* and *Paeonia suffruticosa*. However, extracts of other plants have been evaluated for their antimycotic activity in a similar way. In a similar study, efficacy of plant extract of *Maesa lanceolata,* var. goulungensis against many fungal plant pathogens such as *Phytophthora cryptogea*, *Trichoderma virens*, *Aspergillus niger*, *Phoma* sp., *Fusarium oxysporium*, *Pythium ultimum*, *Cochliobolus heterostrophus*, *Rhizoctonia solani*, *Sclerotium rolfsii* and *Pyrenophora teres* under *in vitro* conditions was reported by

Okemo *et al*. (2003). Abass (2007) studied the effect of leaf extracts of Henna *Lawsonia inermis* at different concenterations on some plant pathogenic fungi namely *Rhizoctonia solani*, *Thielaviopsis paradoxa*, *Fusarium oxysporum* f. sp. *melonis* and *Mauginiella scaettae*. Several workers tested the antifungal activity of aqueous, ethyl alcohol and acetone extracts of Garlic *Allium sativum* L., onion *Allium cepa* L., leek *Allium porrum* L., *Ocimum basilicum* L. and *Allium sativum* L. against *Aspergillus niger*, *Colletotrichum gloeosporioides* and other different fungi (Misra and Dixit, 1976; Irkin and Korukluoglu, 2007; Ogbebor et al., 2007). Webster et al. (2008) screened 14 plants for their antifungal activity against various pathogenic fungi and concluded that *Fragaria virginiana*, *Epilobium angustifolium* and *Potentilla simplex* show a promising antifungal potential. Baka (2010) reported the antifungal activity of six medicinal plants *Amaranthus spinosus*, *Barbeya oleoides*, *Clutia lanceolata*, *Lavandula pubescens*, *Maerua oblongifolia* and *Withania somnifera* against five plant pathogenic fungi *A. brassicae*, *A. solani*, *Botrytis fabae*, *Fusarium solani* and *Phytophthora infestans.* Activity of extracts obtained from nine herbaceous species, viz. *Borago officinalis*, *Orobanche crenata*, *Plantago lanceolata*, *Plantago coronopus*, *Sanguisorba minor*, *Silene vulgaris*, *Sonchus asper*, *Sonchus oleraceus* and *Taraxacum officinale*, were tested against some postharvest fungal rot causing pathogens *Monilinia laxa*, *Botrytis cinerea*, *Penicillium expansum*, *Penicillium digitatum*, *Penicillium italicum*, *Aspergillus carbonarius* and *Aspergillus niger* under *in vitro* and *in vivo* conditions (Gatto et al., 2011).

Conclusion

The present study indicates that the inhibitory effect of plant extracts which have been evaluated for the first time on these rot causing fungi in Kashmir may be attributed to the presence of some partially effective antifungal ingredients, in the plant extracts of all the test plants and thus may have potential to be used as the new natural fungicide for the management of fungal rot diseases.

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Conflicts of interest

Authors declare that they do not own any conflicts of interest.

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