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Allelopathic effect of leaf extracts of *Punica granatum* and *Spiraea prunifolia* against post-harvest rot of tomato and brinjal

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ABSTRACT

Antimycotic activities of different solvent extracts of *Punica granatum* L. and *Spiraea prunifolia* Siebold and Zucc. were carried out through agar well diffusion assay at three concentrations (25µl, 50µl and 75µl) against seven fungi causing rot diseases of tomato and brinjal. All the concentration of plant extracts showed antifungal 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 extract of *Punica granatum* L. showed maximum antifungal activity against *Rhizoctonia solani* and *Penicillium expansum* and least inhibitory activity against *Aspergillus niger*. However, the aqueous extract of *Punica granatum* L. showed maximum antimycotic activity against *Rhizoctonia solani* and *Alternaria alternata* and least inhibitory effect against *Penicillium expansum*. It was further observed from the present study that the ethanolic extract of *Spiraea prunifolia* Siebold and Zucc. showed maximum antimycotic activity against *Rhizoctonia solani* and least inhibitory effect against *Alternaria alternata*. Whereas the aqueous extract of *Spiraea prunifolia* Siebold and Zucc showed maximum antifungal activity against *Aspergillus niger* and least inhibitory activity against *Alternaria alternata*.

Keywords: Plant extracts, Concentration, Antimycotic effectiveness, tomato and brinjal, fungal rot pathogens, Inhibition zone.

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1. INTRODUCTION

Vegetables are attacked by several pathogens causing many fruit rot diseases under storage and on standing crop conditions[1,2]. Among all diseases of vegetables, postharvest decays of vegetables and fruits account for significant losses. It is estimated that harvested vegetables and fruits are decayed by pathogens during postharvest handling to an extent of 20–25% even in developed countries [3,4,5] . Several control strategies have been employed by agricultural scientists to reduce the losses caused by pathogenic fungi. In this study, the important medicinal plants *Punica granatum* L. and *Spiraea prunifolia* Siebold and Zucc. have been evaluated for their antimycotic activity. *Punica granatum* (Pomegranate) is a small tree, five to eight meters tall and mainly found in Iran, Northern Himalayas of India, China, USA and throughout the Mediterranean region. Many diseases like digestive disorders, urinary infections, arthritis, skin disorders, sore throats, coughs, tapeworm expulsion were treated by natural and holistic medicine, pomegranate as one of the constituent. However, as such the modern research is concerned, pomegranates are used in treatments of skin cancer, prostate cancer, diabetes and osteoarthritis [6,7].

Considering their medicinal importance, perishable nature and extent of losses caused by fungal rots of tomato and brinjal, the present study was undertaken to check the efficacy of *Punica granatum* L. and *Spiraea prunifolia* Siebold and Zucc. against rot causing fungi.

2. MATERIALS AND METHODS

2.1. Plant collection and identification

Fresh samples of local medicinal plants *Punica granatum* and *Spiraea prunifolia* were collected from Kashmir valley and identified at Kashmir University Herbarium (KASH), Centre of Plant Taxonomy, Department of Botany, University of Kashmir, Srinagar. 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.

2.2. 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 (20g/100mL) were extracted with ethanol and water separately in order to extract non-polar and polar compounds in a soxhlet extractor for 8 to 10 hours (30-50°C) [8] .

2.3. Preparation of inoculums of fungi

The fungal test organisms, viz, *Penicillium expansum*, *Mucor plumbeus*, *Alternaria alternata*, *Aspergillus niger*, *Trichothecium roseum Penicillium chrysogenum*, and *Rhizoctonia solani* used in present study were isolated and identified from rotten fruits. The tested strains are stored at section of Mycology and Plant Pathology, Department of Botany University of Kashmir. Fungal spores were collected from these cultures after 7 days and density of spore suspension was adjusted to $2x10^5$ (CFU/mL) spores [9,10].

2.4. Antifungal activity

The antifungal activity of the plant extracts were determined by agar well diffusion method as adopted by [11,12,13]. 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 20ml of molten SDA medium in culture tubes. The culture tubes were then homogenised manually and poured into 90mm 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 2mg/ml stock solution was made from the plant extract and then different volumes (25µl, 50µl and 75µl) from that stock solution were loaded to respective wells. Hexaconazole solution (20µl/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 ± 2 ºC 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 [14] .

2.5. 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 $\delta = \sqrt{\frac{\sum x^2}{N-1}}$.

3. RESULTS

3.1. Effect of leaf extracts of *Punica granatum* L on the zone of mycelial inhibition of some rot causing fungi.

It was observed from results (Table 1, Fig 1) that the ethanolic extract of *Punica granatum* L. showed maximum mycelial inhibition against *Rhizoctonia solani* and *Penicillium expansum* at 25 µl, 50 µl and 75µl concentrations (2mg/ml, 5mg/ml and 7mg/ml) with zone of inhibition of 23.33 mm, 25.00 mm, 27.33 mm and 21.00 mm, 24.00 mm, 27.00 mm respectively. Whereas moderate inhibitory fungal activity was shown against *Mucor plumbeus* and *Alternaria alternata* with zone of inhibition as 19.33 mm, 26.00 mm, 26.33 mm and 19.33 mm, 22.33 mm, 26.00 mm respectively. The zone of inhibition of mycelial growth of *Trichothecium roseum* and *Penicillium chrysogenum* was 18.00 mm, 21.33 mm, 23.66 mm and 16.33 mm, 19.66 mm, 23.00 mm by leaf extracts of *P. granatum* L respectively. The zone of inhibition of mycelial growth of *Aspergillus niger* was least with zone of inhibition as 16.33 mm, 18.33 mm and 20.66 mm at 25 µl, 50 µl and 75 µl concentrations of leaf extracts respectively.

It was also revealed from results (Table 2, Fig. 2) that the aqueous leaf extract of *Punica granatum* L. caused maximum inhibition in mycelial growth at 25 µl, 50 µl and 75 µl concentrations with zone of inhibition of 20.33 mm, 22.33 mm, 24.00 mm and 17.66 mm, 20.33 mm, 24.33 mm against *Rhizoctonia solani* and *Alternaria alternata* respectively. Moderate antifungal activity was recorded against *Mucor plumbeus* and *Trichothecium roseum* with zone of inhibition of 17.33 mm, 20.00 mm, 23.33 mm and 16.00 mm, 19.00 mm, 21.33 mm due to aqueous leaf extract of *P. granatum* L. respectively. The zone of inhibition in mycelial growth of *Aspergillus niger* and *Penicillium chrysogenum* was 14.66 mm, 16.00 mm, 18.66 mm and 13.00 mm, 15.66 mm, 21.00 mm by leaf extracts of *P. granatum* respectively. The zone of inhibition in mycelial growth of *Penicillium expansum* was least with zone of inhibition of 12.33 mm, 14.33 mm and 17.66 mm at 25 µl, 50 µl and 75 µl concentrations of aqueous leaf extracts of *P. granatum* respectively.

Table 1 Effect of ethanolic leaf extracts of *Punica granatum* L. at different concentration on the zone of mycelial inhibition of some rot causing fungi.

Concentration / Fungal Pathogens	Zone of mycelial Inhibition (mm)			
	25µl	50 µl	75 µl	Control
Penicillium expansum	21.00±1.00^{d}	24.00± 1.00^{c}	27.00±1.00^{b}	31.33±0.57^{a}
Aspergillus niger	16.33±0.57^{d}	18.33 ±0.57^{c}	20.66±0.57^{b}	22.66±0.57^{a}
Alternaria alternate	19.33±0.57^{d}	22.33± 0.57^{c}	26.00±1.00^{b}	29.33±0.57^{a}
Mucor plumbeus	19.33±0.57^{c}	26.00 ± 0.57^{b}	26.33±1.52^{a}	27.33±0.57^{a}
Penicillium chrysogenum	16.33±0.57^{d}	19.66± 0.57^{c}	23.00±1.00^{b}	26.33±0.57^{a}
Trichothecium roseum	18.00±1.00^{d}	21.33± 0.57^{c}	23.66±0.57^{b}	25.66±0.57^{a}
Rhizoctonia solani	23.33±0.57^{d}	25.00± 1.00^{c}	27.33±0.57^{b}	29.33±0.57^{a}

Each value is mean of 3 replicates ± SD

Mean values followed by different superscript in a column are significantly different ($p \leq 0.05$)

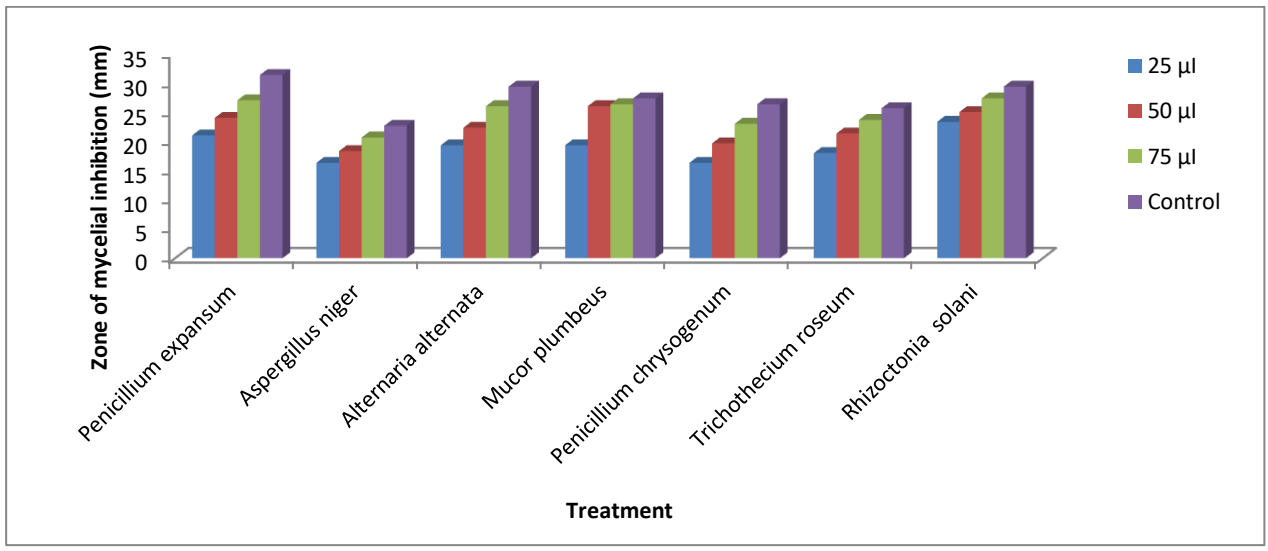

Fig 1. Effect of ethanolic leaf extracts of *Punica granatum* L. at different concentrations on the zone of mycelial inhibition of some rot causing fungi.

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

Concentration / Fungal Pathogens	Zone of mycelial Inhibition (mm)			
	25µl	50 µl	75 µl	Control
Penicillium expansum	12.33±0.57^{d}	14.33±0.57^{c}	17.66±1.00^{b}	25.00± 1.00^{a}
Aspergillus niger	14.66±0.57^{c}	16.00±1.00^{b}	18.66± 0.57^{a}	19.33± 0.57^{a}
Alternaria alternata	17.66±0.57^{d}	20.33±0.57^{c}	24.33±0.57^{b}	27.33±0.57^{a}
Mucor plumbeus	17.33±0.57^{d}	20.00±1.00^{c}	23.33±1.52^{b}	26.66±0.57^{a}
Penicillium chrysogenum	13.00±1.00 ^{d}	15.66±0.57^{c}	21.00±1.00^{b}	24.33±0.57^{a}
Trichothecium roseum	16.00±1.00^{d}	19.00±1.00^{c}	21.33±0.57^{b}	23.33±0.57^{a}
Rhizoctonia solani	20.33±0.57^{d}	22.33±0.57^{c}	24.00 ± 1.00 ^{b}	26.33±0.57^{a}

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

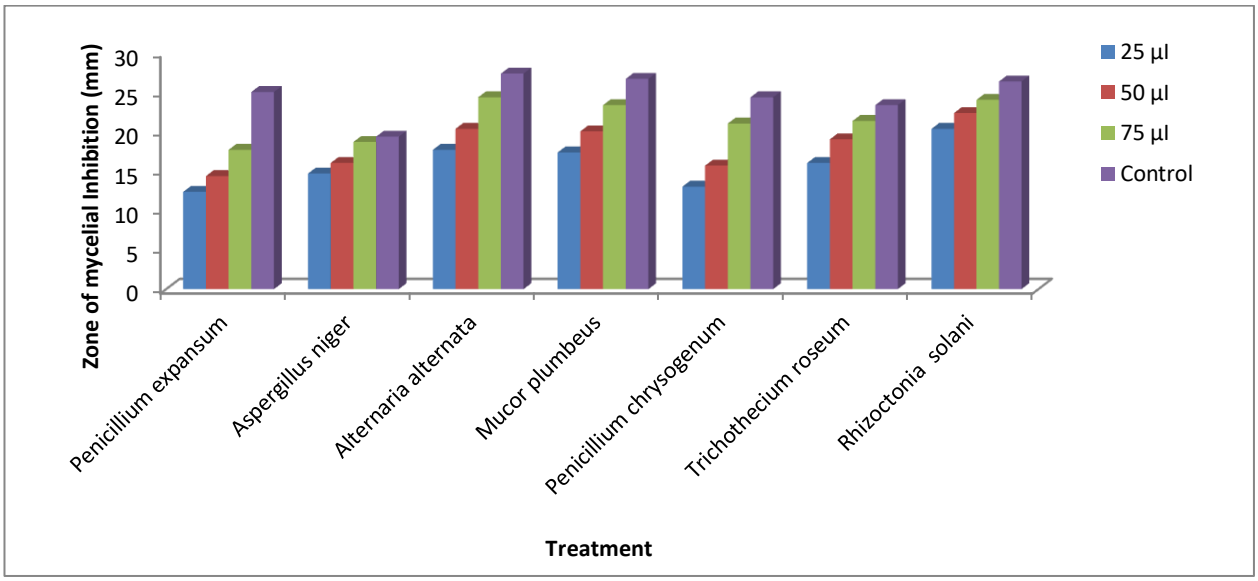

Fig 2 Effect of aqueous leaf extracts of *Punica granatum* L. at different concentrations on the zone of mycelial inhibition of some rot causing fungi.

3.2. Effect of leaf extracts of *Spiraea prunifolia* Siebold and Zucc. on the zone of mycelial inhibition of some rot causing fungi.

It was observed from results (Table 3, Fig. 3) that the ethanolic leaf extract of *Spiraea prunifolia* Siebold and Zucc. showed maximum inhibitory activity on mycelial growth at 25 µl, 50 µl and 75 µl concentrations with zone of inhibition of 20.00 mm, 20.66 mm and 23.00 mm against *Rhizoctonia solani* respectively. Whereas least inhibitory activity was shown against *Alternaria alternata* with zone of mycelial inhibition of 12.33 mm, 14.66 mm and 17.33 mm at 25 µl, 50 µl and 75 µl concentrations respectively. The moderate inhibition in mycelial growth was observed in ethanolic leaf extract at different concentrations against *Penicillium chrysogenum* with zone of inhibition of 19.00 mm, 21.00 mm and 23.33 mm and against *Aspergillus niger* with zone of inhibition of 18.00 mm, 20.00 mm and 22.33 mm at 25 µl, 50 µl and 75 µl concentrations of ethanolic leaf extracts respectively. The inhibition in mycelial growth of *Mucor plumbeus* and *Trichothecium roseum* was 16.33 mm, 19.00 mm, 21.00 mm and 14.00 mm, 16.00 mm, 18.00 mm at 25 µl, 50 µl and 75 µl concentration of ethanolic leaf extracts of *S. prunifolia* respectively. However, the ethanolic extract of *Spiraea prunifolia* showed antifungal activity against *Penicillium expansum* with zone of mycelial inhibition of 13.00 mm, 15.00 mm and 17.00 mm at 25 µl, 50 µl and 75 µl concentrations respectively.

Similarly, it was found from results (Table 4, Fig. 4) that the aqueous extract of *Spiraea prunifolia* Siebold and Zucc. showed maximum inhibitory activity in mycelial inhibition against *Aspergillus niger* at 25 µl, 50 µl and 75 µl concentrations with zone of inhibition of 16.00 mm, 18.00 mm and 20.00 mm respectively. Moderate inhibition in mycelial growth was found in aqueous plant extracts against *Penicillium chrysogenum*, *Mucor plumbeus* and *Rhizoctonia solani* with zone of inhibition of 14.33 mm, 17.00 mm, 20.00 mm., 14.00 mm, 16.66 mm, 20.00 mm and 14.00 mm, 16.33 mm, 20.00 mm at 25 µl, 50 µl and 75 µl concentrations respectively. The zone of inhibition of mycelial growth of *Trichothecium roseum* and *Penicillium expansum* was 11.00 mm, 13.33 mm, 16.00 mm and 10.33 mm, 12.33 mm, 14.00 mm at 25 µl, 50 µl and 75 µl concentrations of aqueous extract of *S. prunifolia* respectively. The least mycelial inhibition was found against *Alternaria alternata* with zone of inhibition as10.00 mm, 11.66 mm and 15.00 mm at 25 µl, 50 µl and 75 µl concentrations of aqueous leaf extracts of *S. prunifolia* respectively.

Table 3 Effect of ethanolic leaf extracts of *Spiraea prunifolia* Siebold and Zucc. at different concentrations on the zone of mycelial inhibition of some rot causing fungi.

Concentration / Fungal Pathogens	Zone of mycelial Inhibition (mm)			
	25µl	50 µl	75 µl	Control
Penicillium expansum	13.00±1.00^{d}	15.00±1.00^{c}	17.00±1.00^{b}	19.66 ± 0.57^{a}
Aspergillus niger	18.00±1.00^{d}	20.00±1.00^{c}	22.33±0.57^{b}	25.00 ± 1.00^{a}
Alternaria alternata	12.33±0.57^{c}	14.66±0.57^{b}	17.33±0.57^{a}	18.00 ± 1.00^{a}
Mucor plumbeus	16.33±0.57^{d}	19.00±1.00^{c}	21.00±1.00^{b}	25.00± 1.00^{a}
Penicillium chrysogenum	19.00±1.00^{d}	21.00±1.00^{c}	23.33±0.57^{b}	26.33 ± 0.57^{a}
Trichothecium roseum	14.00±1.00^{d}	16.00±1.00^{c}	18.00±1.00^{b}	24.66 ± 0.57^{a}
Rhizoctonia solani	20.00±1.00^{c}	20.66±1.52^{c}	23.00±1.00^{b}	25.66 ± 0.57^{a}

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

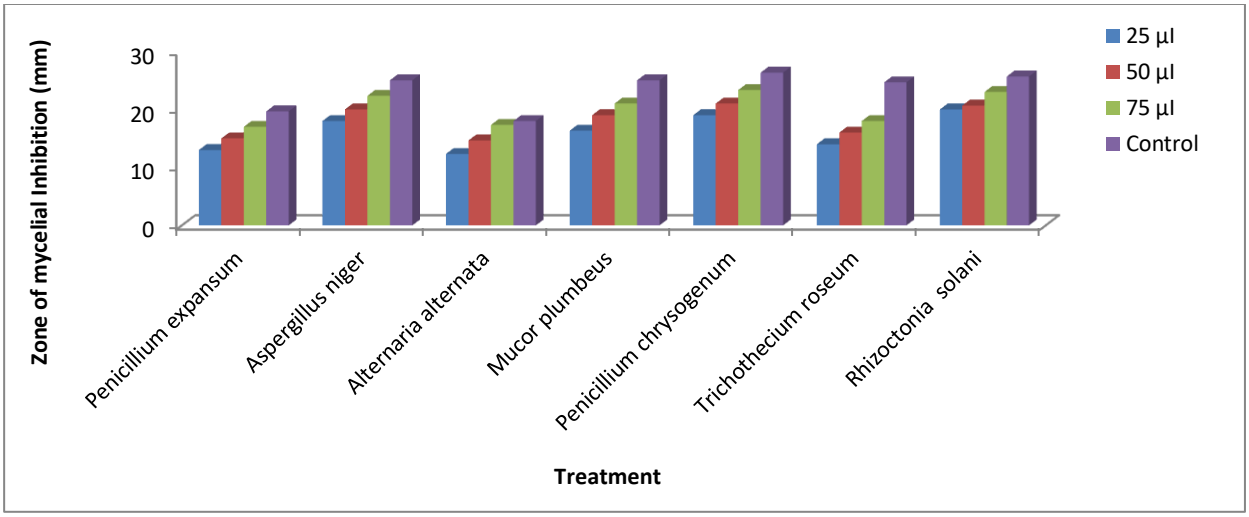

Fig 3 Effect of ethanolic leaf extracts of *Spiraea prunifolia* Siebold and Zucc. at different concentrations on the zone of mycelial inhibition of some rot causing fungi.

Table 4 Effect of aqueous leaf extracts of *Spiraea prunifolia* Siebold and Zucc.at different concentrations on the zone of mycelial inhibition of some rot causing fungi.

Concentration / Fungal Pathogens	Zone of mycelial Inhibition (mm)			
	25µl	50 µl	75 µl	Control
Penicillium expansum	10.33±0.57^{d}	12.33±0.57^{c}	14.00±1.00^{b}	17.33±1.00^{a}
Aspergillus niger	16.00±1.00 ^{d}	18.00±1.00^{c}	20.00±1.00^{b}	22.33±0.57^{a}
Alternaria alternata	10.00±1.00^{c}	11.66±1.52^{c}	15.00±1.00^{b}	17.33±0.57^{a}
Mucor plumbeus	14.00±1.00^{d}	16.66±0.57^{c}	20.00±1.00^{b}	21.66±0.57^{a}
Penicillium chrysogenum	14.33±0.57^{d}	17.00±1.00^{c}	20.00±1.00^{b}	21.66±0.57^{a}
Trichothecium roseum	11.00±1.00^{d}	13.33±0.57^{c}	16.00±1.00^{b}	20.33±0.57^{a}
Rhizoctonia solani	14.00±1.00^{c}	16.33±1.52^{b}	20.00± 1.00^{a}	21.33±0.57^{a}

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

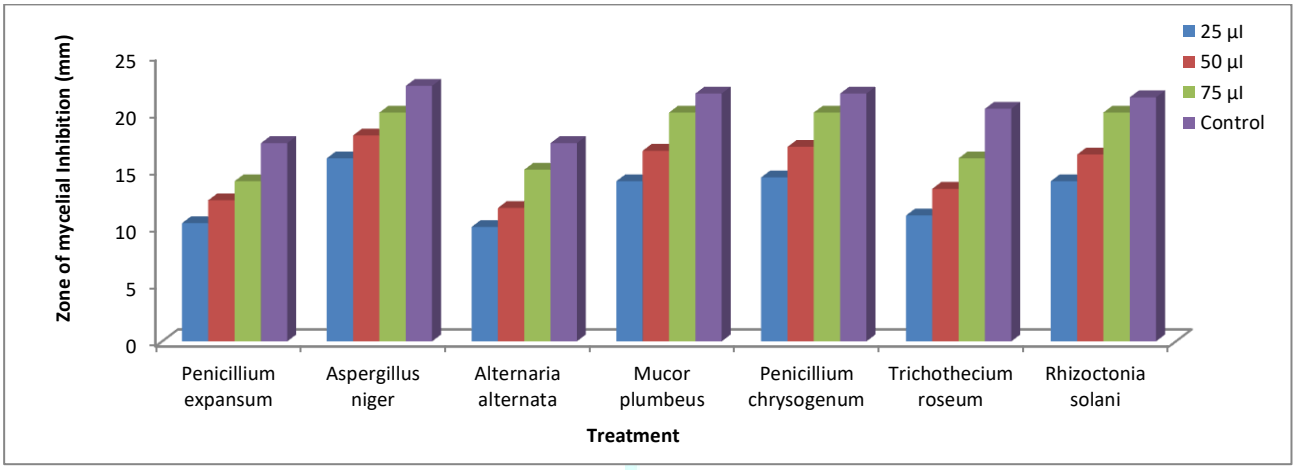

Fig 4 Effect of aqueous leaf extracts of *Spiraea prunifolia* Siebold and Zucc. at different concentrations on the zone of mycelial inhibition of some rot causing fungi.

4. DISCUSSION

The results clearly indicates that extracts of two medicinal plants *Punica granatum* L. and *Spiraea prunifolia* Siebold and Zucc. 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 antifungal activity against the fungus causing rot of tomato and brinjal. These two test plant species proved efficient 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 *Punica granatum* L. and *Spiraea prunifolia* Siebold and Zucc. However, extracts of other plants have been evaluated for their antimycotic activity in a similar way. In a similar study, efficacy of five plant extracts, viz. *Artimesia absinthium* L., *Datura stramonium* L., *Urtica dioica* L., *Juglans regia* L. and *Mentha arvensis* L. was studied against *Alternaria solani* causing disease on potato and found that *D. stramonium* proved effective botanical, followed by *A. absinthium* and least inhibitory effect shown by *Urtica dioica.* [15]. [16] tested different plant parts and found that root extract exhibits more results causing 81-92% reduction in biomass. Several workers studied the inhibitory effect of different plant extracts against mycelial growth of *Collectotrichum graminicola* and *Rhizopus stolonifer* [17,18] . [19] reported the efficiency of ethanolic extract of *C. procera* latex against *Candida albicans*. Increase in *Calotropis procera* extract concentration decreases the mycelia growth, percentage spores germination and germ tube extension in *Fusarium oxysporum* and *Aspergillus carbonaris,* whereas growth of *Humicola brevis* and *Penicillium lanosum* were not affected [20]. A large diameter of clearance of *C. albicans* than that of other fungal strains was shown due to *C. gigantea* latex extract [21]. Inhibitory effect of plant extract of onion, ginger and other plants on the spore germination and mycelial growth of several pathogenic fungi was studied by different workers as [22,23,24,25]. Leaf extracts of *O. sanctum* was found most effective against *A. brassicae* as compared to other tested extracts [26]. [27] reported the antimycotic 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. [28] studied the antimycotic activity of various extracts like aqueous, alcoholic and ethyl acetate extracts of leaves of five *Terminalia* species against five plant pathogenic fungi like *A. flavus*, *A. niger*, *Alternaria brassicicola*, *A. alternata* and *Helminthosporium tetramera* and found that the ethyl acetate extract showed most inhibitory effect against all the fungi tested. Antimycotic activity of chloroform and methanol leaf extracts against seven fungi viz., *Mucor* sp., *Trichoderma viride*, *Verticillium lecanii*, *Candida albicans*, *Penicillium* sp., *A. fumigates* and *A. niger* and found that methanol extract showed maximum inhibition in *Fusarium* sp. [29]. Similarly, other studies also confirmed the effect of different concentration of plant extracts against fungi causing rotting of tomato, brinjal and other fruits [30, 31, 32, 33, 34, 35]. The antifungal activities of these plant extracts are attributed to

different chemical compounds like phenols, flavonoids, isoflavonoids, coumarins, pyrones, alkaloids, etc. present in these plants which effect the growth of pathogenic fungi [36]. Hence these plant extracts may have potential as a new natural fungicide for management of fungal rot pathogens. However, further study is needed to explore the possibility of using plant extracts against other pathogenic fungi responsible for causing decaying of fruits and vegetables under storage and on standing plants.

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