

## ANTIFUNGAL ACTIVITIES OF *ZINNIA ELEGANS* AND *PLECTRANTHUS RUGOSUS* LEAVES AGAINST ROT CAUSING FUNGI

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### ABSTRACT

The ethanolic and aqueous leaf extracts of *Zinnia elegans* L. and *Plectranthus rugosus* Wall ex Benth were evaluated for their antifungal activities on rot causing fungi, viz. *Penicillium expansum*, *Aspergillus niger*, *Mucor plumbeus*, *Alternaria alternata*, *Penicillium chrysogenum*, *Trichothecium roseum* and *Rhizoctonia solani* isolated from rotten tomatoes and brinjal using the agar well diffusion method. 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 extract of *Zinnia elegans* showed maximum antimycotic activity against *Trichothecium roseum* and least activity against *Alternaria alternata*. However, the aqueous extract of *Zinnia elegans* showed maximum antifungal activity against *Trichothecium roseum* and least activity against *Rhizoctonia solani* and *Alternaria alternata*. It was further revealed from the present study that the ethanolic extract of *Plectranthus rugosus* showed maximum antimycotic activity against *Aspergillus niger* and least activity against *Mucor plumbeus*. Whereas the aqueous extract of *Plectranthus rugosus* showed maximum antimycotic activity against *Aspergillus niger* and least activity against *Penicillium expansum*.

**KEYWORDS:** Antifungal activity, Rot causing fungi, Concentration, Plant extracts, Agar Well Diffusion.

### 1. INTRODUCTION

Fruits and vegetables are attacked by wide range of fungi which are believed to cause rot diseases (Snowdon 1990; Ali *et al.* 2005). It is estimated that about 20–25% of the harvested fruits are decayed by pathogens during postharvest handling even in developed countries (Droby 2006; Zhu 2006). Many pathogens including *Aspergillus niger*, *Penicillium expansum* and *Mucor plumbeus* deteriorate the quality of fruits, reduce the market values and make them unfit for human consumption. Taskeen-Un-Nisa *et al.* (2010, 2011) reported the inhibitory activity of three plant extracts, viz. onion (*Allium cepa*), garlic (*Allium sativum*) and mint (*Mentha arvensis*) against *Alternaria alternata*, *Rhizopus stolonifer* and *Fusarium oxysporum* and observed that all concentrations brought about significant reduction in the mycelial growth of rot causing fungi. However, the highest concentration caused maximum inhibition in the mycelial growth. Extracts of many higher plants have been reported to exhibit antifungal properties under laboratory trails (Lee *et al.* 2007; Santas *et al.* 2010; Koka *et al.* 2020). Therefore, the 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 (Jobling

2000). In an approach towards development of eco-friendly antifungal control strategy, different concentrations of two plant extracts viz. *Zinnia elegans* L. and *Plectranthus rugosus* Wall ex Benth were evaluated for their antifungal activity in the present study.

### 2. MATERIALS AND METHODS

#### 2.1. Plant collection and identification

The fresh plant material of *Zinnia elegans* L. and *Plectranthus rugosus* Wall ex Benth were collected from Botanical Garden of University of Kashmir, Hazratbal, 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.

#### 2.2. Preparation of plant extracts

These plant leaves in a required quantity were sundried for 24 hours and then milled into powder using mortar and pestle. About 20g of coarsely powdered leaves (20g/100mL) were extracted separately in a soxhlet extractor for 8 to 10 hours (30-50°C) sequentially with

ethanol and water separately in order to extract non-polar and polar compounds (Elgorashi *et al.*, 2004).

### 2.3. 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  $27 \pm 1^\circ\text{C}$  in Petri plates. Spores of the each fungal species were collected from these cultures after 7 days (Broekaert *et al.*, 1990).

### 2.4. Antifungal activity

Antifungal activity of ethanolic and aqueous extracts was performed by agar well diffusion method (Perez *et al.* 1990; Alzoreky *et al.* 2003; Ahmad *et al.* 2012). 100  $\mu\text{l}$  of standardized inoculum of each test fungi were inoculated onto sterile molten Sabouraud Dextrose Agar homogenised and poured into a sterile Petri plate to yield a uniform depth of 5 mm. The Petri plates were allowed to solidify inside the laminar airflow chamber. Sterile cork borers of 5 mm in diameter were used to make three wells at periphery of each Petri plate. Different concentrations (25 $\mu\text{l}$ , 50 $\mu\text{l}$  and 75 $\mu\text{l}$ ) of each plant extract, prepared in respective solvents were loaded into three different peripheral wells. Hexaconazole solution (20 $\mu\text{l}$ /well) was used as control in the separate well in the same petri plate. The plates were then incubated at  $26 \pm 2^\circ\text{C}$  for 24 to 36 hours. After incubation period, the plates were observed for the zones of inhibition. Antifungal potential was evaluated by measuring inhibition zone diameters in millimeters (mm) with the help of standard measuring scale (Norrel and Messley, 1997).

### 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 *Zinnia elegans* L on the zone of mycelial inhibition of some rot causing fungi.

It was observed from results (Table 1, Fig 1) that the ethanolic leaf extract of *Zinnia elegans* L. caused maximum inhibition in mycelial growth at 25  $\mu\text{l}$ , 50  $\mu\text{l}$  and 75  $\mu\text{l}$  concentrations with zone of inhibition of 21.33 mm, 25.00 mm and 28.00 mm against *Trichothecium roseum* respectively. The inhibition in mycelial growth of *Mucor plumbeus* and *Penicillium*

*expansum* was 15.33 mm, 18.00 mm, 19.33 mm and 14.00 mm, 16.66 mm, 22.33mm at 25  $\mu\text{l}$ , 50  $\mu\text{l}$  and 75  $\mu\text{l}$  concentrations of ethanolic extracts of *Z. elegans* respectively. The moderate antifungal activity of ethanolic leaf extract was found against *Penicillium chrysogenum* with zone of mycelial inhibition of 13.00 mm, 16.66 mm, 21.00 mm and against *Rhizoctonia solani* with zone of inhibition of 13.33 mm, 16.00 mm and 19.33 mm at 25  $\mu\text{l}$ , 50  $\mu\text{l}$  and 75  $\mu\text{l}$  concentrations of leaf extracts of *Z. elegans* respectively. Likewise, the ethanolic leaf extract of *Zinnia* showed antifungal activity against *Aspergillus niger* with zone of inhibition of 13.00 mm, 15.00 mm and 18.66 mm at 25  $\mu\text{l}$ , 50  $\mu\text{l}$  and 75  $\mu\text{l}$  concentrations respectively. Whereas the least mycelial inhibition was found against *Alternaria alternata* with zone of mycelial inhibition as 11.66 mm, 15.00 mm and 18.33 mm at 25  $\mu\text{l}$ , 50  $\mu\text{l}$  and 75  $\mu\text{l}$  concentrations of extracts of *Z. elegans* respectively.

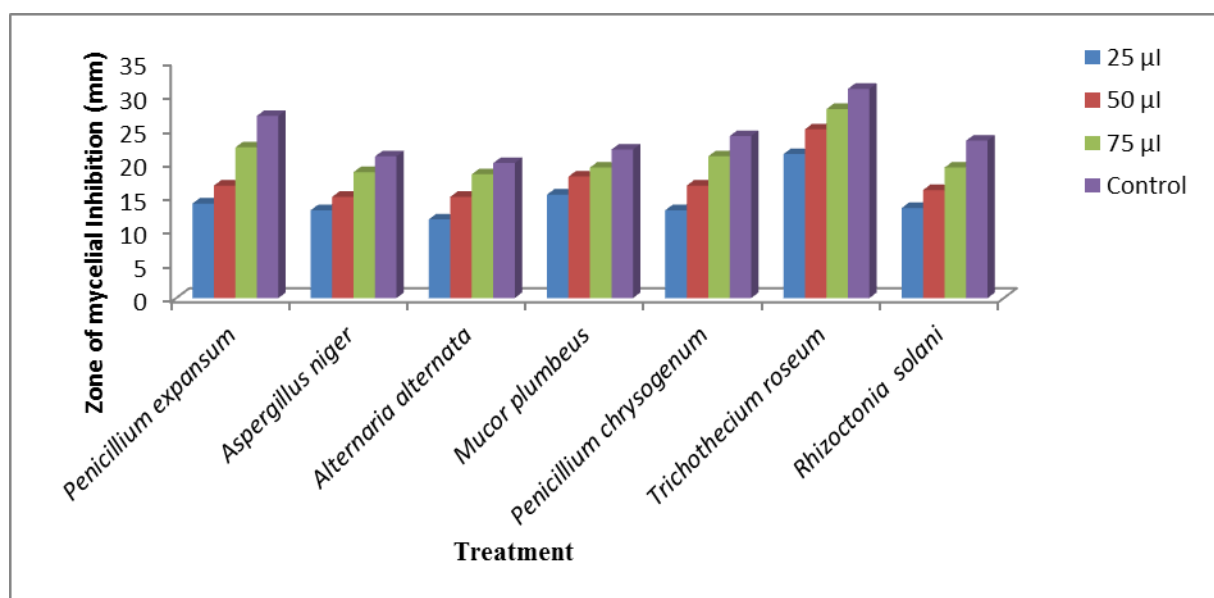
The results (Table 2, Fig. 2) further revealed that the aqueous leaf extract of *Zinnia elegans* L. showed maximum inhibitory activity in mycelial growth against *Trichothecium roseum* at 25  $\mu\text{l}$ , 50  $\mu\text{l}$  and 75  $\mu\text{l}$  concentrations with zone of inhibition of 18.33 mm, 23.33 mm and 26.00 mm respectively. Moderate antifungal activity was recorded against *Penicillium chrysogenum* with zone of mycelial inhibition of 13.00 mm, 15.00 mm, 17.00 mm and against *Mucor plumbeus* with zone of mycelial inhibition of 12.66 mm, 14.33 mm, 16.00 mm and against *Penicillium expansum* with zone of mycelial inhibition as 12.00 mm, 14.66 mm, 15.66 mm at 25  $\mu\text{l}$ , 50  $\mu\text{l}$  and 75  $\mu\text{l}$  concentrations of leaf extracts respectively. The zone of inhibition in mycelial growth of *Aspergillus niger* was 11.00mm, 12.66 mm, 14.66 mm at different concentrations. The least mycelial inhibition was observed against *Alternaria alternata* and *Rhizoctonia solani* with zone of mycelial inhibition of 10.66 mm, 12.66 mm and 15.00 mm and 10.66 mm, 12.33 mm, 15.00 mm at 25  $\mu\text{l}$ , 50  $\mu\text{l}$  and 75  $\mu\text{l}$  concentrations of aqueous leaf extracts of *Z. elegans* respectively.

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

Fungal Pathogens	Concentration	Zone of mycelial Inhibition (mm)			
		25µl	50 µl	75 µl	Control
<i>Penicillium expansum</i>		14.00±1.00 <sup>d</sup>	16.66±1.52 <sup>c</sup>	22.33±0.57 <sup>b</sup>	27.00±1.00 <sup>a</sup>
<i>Aspergillus niger</i>		13.00±1.00 <sup>d</sup>	15.00±1.00 <sup>c</sup>	18.66±0.57 <sup>b</sup>	21.00±1.00 <sup>a</sup>
<i>Alternaria alternata</i>		11.66±0.57 <sup>d</sup>	15.00±1.00 <sup>c</sup>	18.33±0.57 <sup>b</sup>	20.00±1.00 <sup>a</sup>
<i>Mucor plumbeus</i>		15.33±1.52 <sup>c</sup>	18.00±1.00 <sup>b</sup>	19.33±0.57 <sup>b</sup>	22.00±1.00 <sup>a</sup>
<i>Penicillium chrysogenum</i>		13.00±1.00 <sup>d</sup>	16.66±1.52 <sup>c</sup>	21.00±1.00 <sup>b</sup>	24.00±1.00 <sup>a</sup>
<i>Trichothecium roseum</i>		21.33±1.52 <sup>d</sup>	25.00±1.00 <sup>c</sup>	28.00±1.00 <sup>b</sup>	31.00±1.00 <sup>a</sup>
<i>Rhizoctonia solani</i>		13.33±1.52 <sup>d</sup>	16.00±1.00 <sup>c</sup>	19.33±1.52 <sup>b</sup>	23.33±1.52 <sup>a</sup>

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

Fungal Pathogens	Concentration	Zone of mycelial Inhibition (mm)			
		25µl	50 µl	75 µl	Control
<i>Penicillium expansum</i>		12.00±1.00 <sup>c</sup>	14.66±1.52 <sup>b</sup>	15.66±1.52 <sup>b</sup>	24.33±1.52 <sup>a</sup>
<i>Aspergillus niger</i>		11.00±1.00 <sup>c</sup>	12.66±1.52 <sup>bc</sup>	14.66±1.52 <sup>ab</sup>	17.00±1.00 <sup>a</sup>
<i>Alternaria alternata</i>		10.66±0.57 <sup>d</sup>	12.66±0.57 <sup>c</sup>	15.00±1.00 <sup>b</sup>	17.66±1.52 <sup>a</sup>
<i>Mucor plumbeus</i>		12.66±1.15 <sup>c</sup>	14.33±0.57 <sup>bc</sup>	16.00±1.00 <sup>b</sup>	18.00±1.00 <sup>a</sup>
<i>Penicillium chrysogenum</i>		13.00±1.00 <sup>c</sup>	15.00±1.00 <sup>bc</sup>	17.00±1.00 <sup>ab</sup>	17.66±1.52 <sup>a</sup>
<i>Trichothecium roseum</i>		18.33±0.57 <sup>c</sup>	23.33±1.52 <sup>b</sup>	26.00±1.00 <sup>a</sup>	27.00±1.00 <sup>a</sup>
<i>Rhizoctonia solani</i>		10.66±1.15 <sup>c</sup>	12.33±1.52 <sup>c</sup>	15.00±1.00 <sup>b</sup>	18.33±1.52 <sup>a</sup>

Each value is mean of 3 replicates ± SD

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

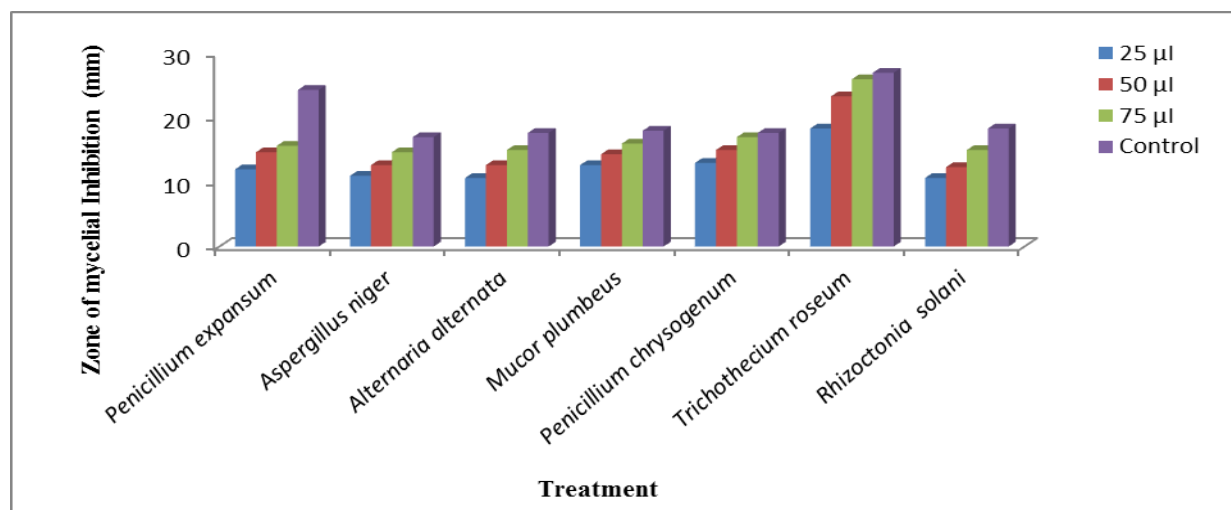


Fig. 2: Effect of aqueous leaf extracts of *Zinnia elegans* L. at different concentrations on the zone of mycelial inhibition of some rot causing fungi.

**3.2. Effect of leaf extracts of *Plectranthus rugosus* Wall ex Benth at different concentrations on the zone of mycelial inhibition of some rot causing fungi.**

It was found from the results (Table 3, Fig 3) that the ethanolic leaf extract of *Plectranthus rugosus* Wall ex Benth caused maximum mycelial inhibition at 25 µl, 50 µl and 75 µl concentrations with zone of inhibition of 24.66 mm, 27.33 mm and 29.00 mm against *Aspergillus niger* respectively. The inhibition in mycelial growth of *Penicillium chrysogenum* and *Rhizoctonia solani* was 22.66 mm, 25.00 mm, 27.66 mm and 20.33 mm, 22.66 mm, 24.00 mm at 25 µl, 50 µl and 75 µl concentrations of leaf extracts respectively. The moderate antifungal activity of ethanolic leaf extracts was observed against *Alternaria alternata* with zone of inhibition of 19.66 mm, 21.33 mm, 23.66 mm and against *Trichothecium roseum* with zone of inhibition of 18.33 mm, 20.00 mm and 21.33 mm at 25 µl, 50 µl and 75 µl concentrations of leaf extracts respectively. Likewise, the zone of inhibition in mycelial growth of *Penicillium expansum* was 18.00 mm, 20.00 mm, 22.66 mm at 25 µl, 50 µl and 75 µl concentrations of leaf extract of *P. rugosus*. The least mycelial inhibition was found in *Mucor plumbeus* with the zone of inhibition as 17.00 mm, 19.33 mm and

23.33 mm at 25 µl, 50 µl and 75 µl of ethanolic leaf extracts of *P. rugosus*.

Similarly, results (Table 4, Fig. 4) revealed that the aqueous leaf extract of *Plectranthus rugosus* Wall ex Benth showed maximum inhibitory activity at 25 µl, 50 µl and 75 µl concentrations with zone of mycelial inhibition of 22.66 mm, 25.33 mm and 27.66 mm against *Aspergillus niger*. Moderate antifungal activity was also recorded against *Penicillium chrysogenum* with zone of inhibition of 20.00 mm, 22.00 mm, 23.33 mm, against *Alternaria alternata* with zone of inhibition of 16.66 mm, 18.33 mm, 20.00 mm., and against *Trichothecium roseum* with zone of inhibition of 16.33 mm, 18.00 mm, 19.33 mm at 25 µl, 50 µl and 75 µl concentrations of plant extracts respectively. The zone of mycelial inhibition of *Rhizoctonia solani* and *Mucor plumbeus* was 16.00 mm, 18.33 mm, 20.00 mm and 14.66 mm, 17.66 mm, 19.33 mm at 25 µl, 50 µl and 75 µl concentrations of leaf extracts respectively. The least mycelial inhibition was observed in *Penicillium expansum* with the zone of mycelial inhibition as 14.00 mm, 17.66 mm and 20.66 mm at 25 µl, 50 µl and 75 µl concentrations of aqueous leaf extracts of *P. rugosus* respectively.

Table 3: Effect of ethanolic leaf extracts of *Plectranthus rugosus* Wall ex Benth at different concentrations on the zone of mycelial inhibition of some rot causing fungi.

Fungal Pathogens	Concentration	Zone of mycelial Inhibition (mm)			
		25µl	50 µl	75 µl	Control
<i>Penicillium expansum</i>		18.00±1.00 <sup>d</sup>	20.00±1.00 <sup>c</sup>	22.66±0.57 <sup>b</sup>	25.33±0.57 <sup>a</sup>
<i>Aspergillus niger</i>		24.66±0.57 <sup>d</sup>	27.33±0.57 <sup>c</sup>	29.00±1.00 <sup>b</sup>	31.33±0.57 <sup>a</sup>
<i>Alternaria alternata</i>		19.66±0.57 <sup>d</sup>	21.33±1.15 <sup>c</sup>	23.66±0.57 <sup>b</sup>	25.66±0.57 <sup>a</sup>
<i>Mucor plumbeus</i>		17.00±1.00 <sup>d</sup>	19.33±0.57 <sup>c</sup>	23.33±1.15 <sup>b</sup>	25.33±0.57 <sup>a</sup>
<i>Penicillium chrysogenum</i>		22.66±1.15 <sup>c</sup>	25.00±1.00 <sup>b</sup>	27.66±0.57 <sup>ab</sup>	29.33±0.57 <sup>a</sup>
<i>Trichothecium roseum</i>		18.33±0.57 <sup>d</sup>	20.00±1.00 <sup>c</sup>	21.33±0.57 <sup>b</sup>	27.66±0.57 <sup>a</sup>
<i>Rhizoctonia solani</i>		20.33±0.57 <sup>d</sup>	22.66±0.57 <sup>c</sup>	24.00±1.00 <sup>b</sup>	26.66±0.57 <sup>a</sup>

Each value is mean of 3 replicates ± SD

Mean values followed by different superscript in a column are significantly different (p≤ 0.05)

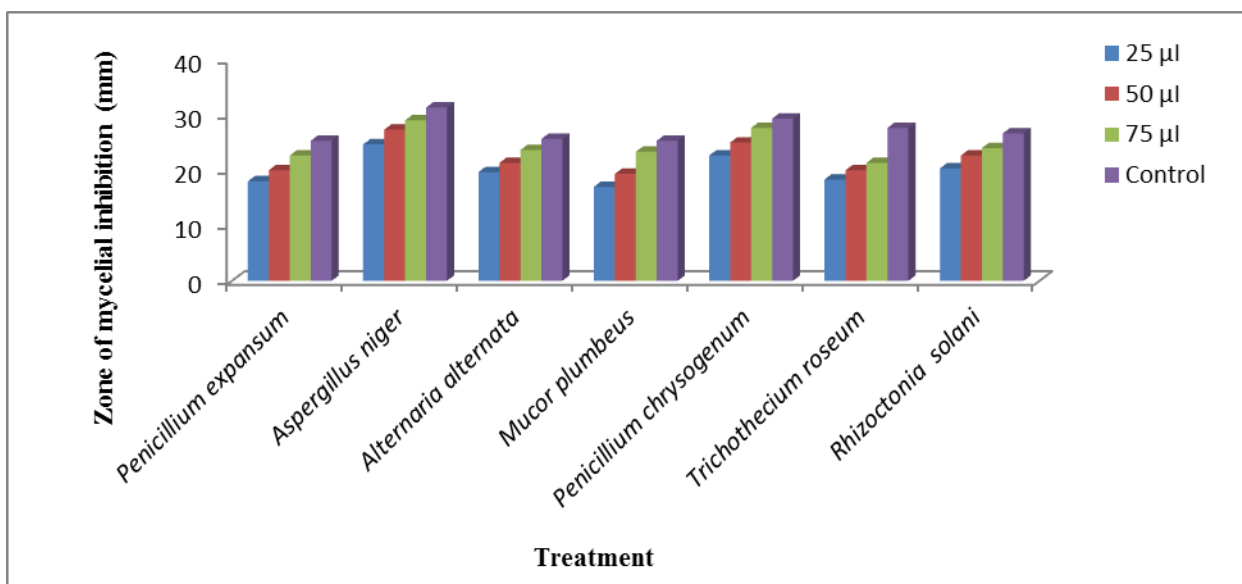


Fig 3: Effect of ethanolic leaf extracts of *Plectranthus rugosus* Wall ex Benth at different concentrations on the zone of mycelial inhibition of some rot causing fungi.

Table 4: Effect of aqueous leaf extracts of *Plectranthus rugosus* Wall ex Benth at different concentrations on the zone of mycelial inhibition of some rot causing fungi.

Fungal pathogens	Concentration	Zone of mycelial Inhibition (mm)			
		25µl	50 µl	75 µl	Control
<i>Penicillium expansum</i>		14.00±1.00 <sup>d</sup>	17.66±0.57 <sup>c</sup>	20.66±0.57 <sup>b</sup>	23.33±0.57 <sup>a</sup>
<i>Aspergillus niger</i>		22.66±0.57 <sup>c</sup>	25.33±1.52 <sup>b</sup>	27.66±1.15 <sup>ab</sup>	29.33±1.52 <sup>a</sup>
<i>Alternaria alternata</i>		16.66±0.57 <sup>d</sup>	18.33±0.57 <sup>c</sup>	20.00±1.00 <sup>b</sup>	22.66±0.57 <sup>a</sup>
<i>Mucor plumbeus</i>		14.66±1.15 <sup>d</sup>	17.66±0.57 <sup>c</sup>	19.33±0.57 <sup>b</sup>	22.33±0.57 <sup>a</sup>
<i>Penicillium chrysogenum</i>		20.00±1.00 <sup>d</sup>	22.00±1.00 <sup>c</sup>	23.33±0.57 <sup>b</sup>	25.00±1.00 <sup>a</sup>
<i>Trichothecium roseum</i>		16.33±0.57 <sup>c</sup>	18.00±1.00 <sup>b</sup>	19.33±0.57 <sup>b</sup>	25.00±1.00 <sup>a</sup>
<i>Rhizoctonia solani</i>		16.00±1.00 <sup>d</sup>	18.33±0.57 <sup>c</sup>	20.00±1.00 <sup>b</sup>	22.66±0.57 <sup>a</sup>

Each value is mean of 3 replicates ± SD

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

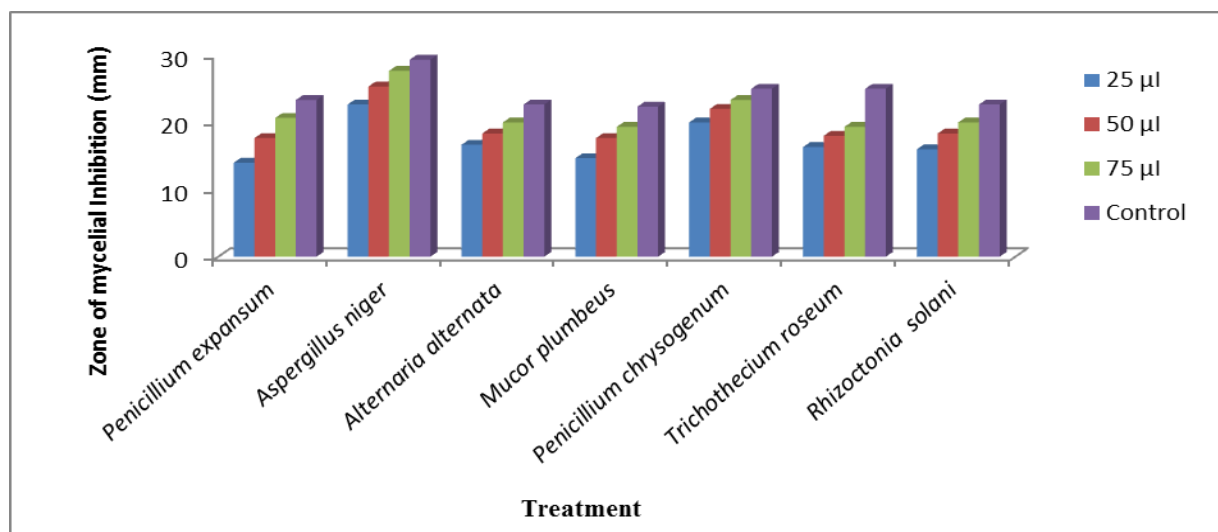


Fig. 4: Effect of aqueous leaf extracts of *Plectranthus rugosus* Wall ex Benth at different concentrations on the zone of mycelial inhibition of some rot causing fungi.

## DISCUSSION

The results clearly indicates that extracts of *Zinnia elegans* L. and *Plectranthus rugosus* Wall ex Benth brought about significant inhibition in the mycelial growth at their different concentration. Higher concentration proved effective than lower concentration. In the present study *Zinnia elegans* and *Plectranthus rugosus* plant extracts were evaluated for their antifungal 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 *Zinnia elegans* L. and *Plectranthus rugosus* Wall ex Benth. However, extracts of other plants have been evaluated for their antimycotic activity in a similar way. In a similar study, Al-Askar and Rashad (2010) tested antifungal activity of ethanol-water extracts of four medicinal plants viz., cinnamon (*Cinnamomum verum* Presl.), anise (*Pimpinella anisum* L.), black seed (*Nigella sativa* L.) and clove (*Syzygium aromaticum* L. Merr. & Perry) against pea (*Pisum sativum* L.) root-rot fungus *Rhizoctonia solani*. Olusanmi and Amadi (2010) evaluated the antimicrobial properties of garlic (*Allium sativum*) extracts on three fungi namely *Aspergillus flavus*, *Curvularia lunata* and *Fusarium moniliforme* using the pour plate method. Suleiman (2010) tested fungitoxic activity of crude extracts of neem (*Azadirachta indica* (A.) Juss and pawpaw (*Carica papaya*) (L.) against *Alternaria solani*, causing yam rot under *in vitro* condition and found that the methanolic extracts showed reduction in mycelia growth and disease incidence due to fungitoxic components. The inhibitory action of the extracts on mycelia growth increased with increase in concentration. Bobbarala *et al.* (2009) reported the antifungal activity of 49 different plant extracts against *Aspergillus niger*. Among the 49 plants used, 89% showed antifungal activity, while 11% were not effective. Znini *et al.* (2013) extracted an essential oil from the plant *Warionia saharae* and reported its antifungal activity against three apple phytopathogenic fungi, viz. *Alternaria* species (*Alternaria* rot), *Penicillium expansum* (blue mould), and *Rhizopus stolonifer* (*Rhizopus* rot). Dellavalle *et al.* (2011) evaluated the antifungal activity of extracts of 10 plant species against the phytopathogenic fungus *Alternaria* spp and reported that extracts of *Salvia sclarea*, *S. officinalis* and *R. officinalis* serve as potential sources of antifungal compounds for management of plant diseases. Hadi *et al.* (2013) studied the antifungal activity of *Mentha piperita* L., *Cinnamomum zeylanicum* Blume, *Allium hirtifolium* Boiss and *Allium sativum* L. against *Fusarium oxysporum* schlecht. Raji and Raveendran, (2013) reported antifungal activity of selected plant extracts against phytopathogenic fungi *Aspergillus niger*. Similarly, other studies also confirmed the effect of different concentration of plant extracts against fungi causing rotting of tomato, brinjal and other fruits ( Shafique *et al.* 2015; Rawal *et al.* 2015; Kalidindi *et al.* 2015; Koka *et al.*, 2017; Satpute *et al.*, 2017; Koka *et al.*,

2018). Thus, extracts of these plants used by different researchers against pathogenic fungi show promising antifungal activity which indicates that these plants can act as a good biological resource for producing safe biofungicides.

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