Antimycotic potential of some phytoextracts on some pathogenic fungi

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ABSTRACT

The present study was conducted to determine the inhibitory effects of five phytoextracts, *viz. Artemisia absinthium, Malva sylvestris, Plantago lanceolata, Rumex obtusifolius* and *Taraxicum officinale* on the mycelial growth and spore germination of *Drechslera* sp., *Penicillium expansum, Aspergillus niger* and *Aspergillus flavus*. The results revealed that all the concentrations of plant extracts caused significant inhibition in the mycelial growth and spore germination of the tested fungi as compared to control. However, the maximum inhibition in mycelial growth and spore germination was found at the highest concentration 'S' followed by lower concentrations of the plant extracts. Maximum inhibition in mycelial growth and spore germination was caused by *A. absinthium* plant extract followed by *R. obtusifolius, M. sylvertris, P. lanceolata* and least inhibition was found by *T. officinale*. The highest inhibitory activity of *A. absinthium* extract was shown against *P. expansum* (75.42%) at standard concentration 'S' followed by *A. flavus* (74.74%), *A. niger* (61.83%) and *Drechslera* sp. (61.64%) at same concentration.

Keywords: Plant extracts, concentrations, mycelial growth, phytoextracts, rot fungi.

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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 rot is a common, destructive and wide spread disease in all fruits and vegetables (Snowdon, 1990). Several species of fungi, *viz. Rhizopus* sp., *Mucor* sp., *Penicillium* sp., *Aspergillus* sp., *Colletotrichum* sp., *Botrytis* sp., *Monilinia* sp., *Alternaria* sp., *Phytophthora* sp., have been reported to cause fungal rot diseases (Hema Moorthy and Prakasam, 2013; Parveen and Wani, 2015). Various chemical fungicides have been used to control these fungal rot diseases, but these fungicides cause hazardous effect on humans and environment. Hence strong regulatory actions have been imposed on their use. So there is a strong need to control these diseases in an ecofriendly way. 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). They are believed to be less hazardous than chemical fungicides and can therefore be used as an alternative to control fungal rot diseases (Jobling, 2000). The use of these plant extracts for inhibition of fungal diseases is an important step towards the assessment of the degree of variability among the diverse natural flora (Khandare and Vasait, 2017). So in an approach towards ecofriendly management strategy, extracts of five different medicinal plants, viz. *Artemisia absinthium, Malva sylvestris, Plantago lanceolata, Rumex obtusifolius* and *Taraxicum officinale* were screened for their antifungal activity against some rot causing fungi.

MATERIALS AND METHODS

Different concentrations of aqueous leaf extracts 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 and spore germination of some rot causing fungal pathogens isolated from diseased samples of pear and peach fruits. For the preparation of plant extracts, 200g leaves of all the plants were washed with sterilized distilled water, grinded in mortar and pestle using 200ml of sterilized distilled water (Bhat and Sivaprakasan, 1994). The material was homogenized for 5 minutes and filtered through double layered muslin cloth followed by Whattman's filter paper No. 1. The filtrate was then centrifuged at 5000 rpm for 10 minutes and was considered as standard solutions (S). Then other concentrations such as 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 of fungi by food poisoning technique (Adams and Wong, 1991). 1ml from each concentration of the plant extract was mixed with 9ml of autoclaved and cooled PDA just before pouring into Petri plates. The medium was then dispensed uniformly into 90 mm sterile Petri plates and then inoculated with 5 mm mycelial disc of the pathogen from 10 day 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 seven days of incubation. The percent inhibition in 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.

To evaluate the effect of plant extracts on the spore germination, spore suspension of each selected fungus was prepared in sterilized distilled water. The concentration of the conidial suspension of each fungal isolate used during the present study was 2 x 10^5 conidia/ml (adjusted by haemocytometer). 0.5ml of spore suspension was mixed with 0.5ml of different concentrations of plant extract in a test tube and then shaken. In case of control 0.5ml of spore suspension was mixed with equal volume of distilled water. A drop of the mixture (about 0.1ml) was then placed in the cavity slide and these were incubated for 25±2°C in a moist chamber created in 100mm Petri plates by covering both sides of the Petri plate with moist filter paper to maintain enough humidity. Three replicates were maintained for each treatment including the control. The slides were examined after 24h by hand tally counts at different microscopic fields containing at least 30-50 spores per microscopic field. Percent spore germination of each treatment was calculated by the formula given by Kiraly *et al.* (1974).

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

Statistical analysis

Statistical analysis was carried out using SPSS statistical software (version 16.0). Data was analyzed by one way analysis of variance (ANOVA) and comparison of the means was done by Tukey's multiple comparison tests at $P \leq 0.05$.

RESULTS AND DISCUSSION

It was revealed from the results that all the plant extracts, *viz., Artemisia absinthium, Malva sylvestris, Plantago lanceolata, Rumex obtusifolius* and *Taraxicum officinale* in different concentrations (S, S/2, S/10 and S/100) were effective in inhibiting the mycelial growth and spore germination of some fungal pathogens, *viz*., *Drechslera* sp., *Aspergillus niger, Aspergillus flavus* and *Penicillium expansum.*

Effect of different plant extracts on the mycelial growth of some rot fungi

It was revealed from the results (Table 1) that all the plant extracts used at different concentrations brought about significant inhibition in the mycelial growth of all the fungal pathogens. *A. absinthium* was found most effective against all the fungal pathogens followed by *R. obtusifolius, T. officinale, M. sylvestris* and least effective was *P. lanceolata*. Highest inhibitory activity of *A. absinthium* extract was shown against *P. expansum* at standard concentration S followed by *A. flavus*, *A. niger* and *Drechslera* sp. at same concentration. Other plant extracts also caused significant inhibition in mycelial growth of all the tested fungi but to a lesser extent.

Table 1. Effect of different concentrations of plant extracts on mycelial growth of some selected rot fungi.

Plant extract	Phytopathogenic fungi	Mycelial growth (mm)					*F-value*	*P-value*
		S	**S/2**	**S/10**	**S/100**	**Control**		
Artemisia absinthium	*Drechslera* sp.	8.63±0.57a	11.00±0.87a	14.90±0.65b	17.77±0.58b	22.50±2.01c	77.118	0.0005
	Aspergillus flavus	10.07±0.90a	14.43±0.51b	21.27±1.10c	25.30±0.62d	39.87±0.70e	626.467	0.0005
	Aspergillus niger	16.67±0.42a	22.43±0.55b	28.00±0.20c	33.67±0.61d	43.67±1.60e	468.902	0.0005
	Penicillium expansum	4.67±0.58a	7.33±1.53ab	10.67±1.53b	14.67±1.53c	19.00±1.0d	58.840	0.0005
Malva sylvestris	*Drechslera* sp.	11.47±0.30a	12.93±0.30a	16.83±0.30b	19.90±0.36c	22.50±2.01d	72.187	0.0005
	Aspergillus flavus	13.33±0.61	19.93±0.85b	29.27±1.10c	35.00±0.80d	39.87±0.70e	518.848	0.0005
	Aspergillus niger	19.33±1.16a	25.47±0.70b	31.90±1.95c	38.77±0.25d	43.67±1.60e	174.245	0.0005
	Penicillium expansum	6.00±1.00a	9.33±1.53a	14.00±2.00b	16.00±2.00bc	19.00±1.00c	32.946	0.0005
Plantago lanceolata	*Drechslera* sp.	13.43±0.45a	14.70±0.65a	17.50±0.43b	20.63±0.47c	22.50±2.01c	43.489	0.0005
	Aspergillus flavus	13.37±0.60a	19.80±1.11b	29.67±0.61c	32.67±0.64d	39.87±0.70e	641.308	0.0005
	Aspergillus niger	22.07±0.66a	28.37±0.66b	36.60±0.56c	39.97±0.95d	43.67±1.60e	248.636	0.0005
	Penicillium expansum	7.00±1.00a	11.69±1.53b	15.00±2.00b	17.00±2.0c	19.00±1.0c	27.189	0.0005
Rumex obtusifolius	*Drechslera* sp.	10.00±0.80a	12.47±0.50a	15.67±0.70b	18.97±0.60c	22.50±2.01d	64.629	0.0005
	Aspergillus flavus	12.43±0.49a	19.03±1.06b	25.27±0.64c	29.37±0.55d	39.87±0.70e	630.891	0.0005
	Aspergillus niger	18.30±1.04a	25.03±0.86b	30.23±0.58c	37.13±1.10d	43.67±1.60e	248.683	0.0005
	Penicillium expansum	5.00±1.00a	10.33±1.53b	13.33±2.08bc	16.00±2.00c	19.00±1.00c	34.303	0.0005
Taraxicum officinale	*Drechslera* sp.	14.10±0.65a	15.37±0.40a	19.17±0.85bc	20.97±0.47bc	22.50±2.01c	34.790	0.0005
	Aspergillus flavus	15.20±0.60a	20.90±1.05b	32.73±0.64c	38.00±0.92d	39.87±0.70d	546.172	0.0005
	Aspergillus niger	24.03±0.85a	31.83±1.62b	38.40±1.06c	42.33±0.95d	43.67±1.60d	123.795	0.0005
	Penicillium expansum	6.33±0.58a	11.33±1.53b	16.33±1.53c	17.33±0.58c	19.00±1.00c	63.605	0.0005

*Mean ± S.D of three replicates. Mean values were compared using Tukey's multiple comparison test ($P \leq 0.05$). The numbers followed by same alphabets are not statistically different.

It was observed from the results (Table 2) that all the plant extracts at different concentrations caused significant reduction in spore germination of all the tested fungi. Maximum inhibition in spore germination was brought about by the highest concentration of the plant extract followed by lower concentrations. *A. absinthium* was found most effective in inhibiting the spore germination followed by *R. obtusifolius, M. sylvestris, P. lanceolata* and least effective was *T. officinale*. *A. absinthium, M. sylvestris, P. lanceolata* caused maximum reduction in the spore germination of *A. flavus* followed by *P. expansum*, while *R. obtusifolius* and *T. officinale* were found effective against *P. expansum* followed by *A. flavus, A. niger* and *Drechslera* sp.

Table 2. Effect of different concentrations of plant extracts on spore germination of some selected rot fungi

Plant extract	Fungal pathogen	Spore germination (%)						
		S	**S/2**	**S/10**	**S/100**	**Control**	***F-Value***	***P- Value***
Artemisia absinthium	*Drechslera* sp.	27.34±1.53a	36.66±0.58b	52.66±0.58c	70.66±1.53d	94.00±1.00e	457.647	0.0005
	Aspergillus flavus	10.66±1.53a	20.66±0.58b	26.66±1.53b	40.66±1.53c	93.34±2.52d	291.134	0.0005
	Aspergillus niger	30.00±1.00a	43.34±1.53b	55.34±1.53c	70.66±1.53d	90.00±3.00e	120.804	0.0005
	Penicillium expansum	12.00±1.00a	22.00±1.00b	28.66±1.15b	43.34±1.53c	94.66±1.53d	496.396	0.0005
Malva sylvestris	*Drechslera* sp.	34.66±0.57a	44.66±1.15b	56.66±1.53c	80.00±1.00d	92.00±1.00e	411.344	0.0005
	Aspergillus flavus	20.66±1.53a	27.34±1.53a	40.66±1.53b	56.66±1.53c	89.34±3.05d	200.936	0.0005
	Aspergillus niger	43.34±1.53a	51.34±0.58a	67.34±0.58b	80.66±1.53c	91.34±3.05d	99.442	0.0005
	Penicillium expansum	21.34±1.54a	28.66±0.58a	43.34±1.53b	56.00±2.00c	92.00±3.46d	303.500	0.0005
Plantago lanceolata	*Drechslera* sp.	39.34±1.53a	48.66±0.58b	56.66±1.53c	83.34±1.53d	93.34±0.58e	255.913	0.0005
	Aspergillus flavus	26.00±1.00a	33.34±1.53a	45.34±1.53b	63.34±1.53c	90.66±2.08d	190.035	0.0005
	Aspergillus niger	47.34±2.08a	54.66±0.58a	75.34±1.53b	85.34±0.58c	96.66±1.53c	80.990	0.0005
	Penicillium expansum	38.66±1.53a	47.34±1.53b	60.66±1.53c	75.34±1.53d	92.66±2.52e	160.500	0.0005
Rumex obtusifolius	*Drechslera* sp.	29.34±0.58a	38.00±1.00b	56.00±1.00c	68.66±1.15d	92.66±0.58e	634.042	0.0005
	Aspergillus flavus	18.66±0.58a	25.34±1.53a	36.00±1.00b	53.34±1.53c	92.66±2.52d	271.081	0.0005
	Aspergillus niger	39.34±1.53a	47.34±1.53a	63.34±1.53b	77.34±1.53c	90.00±2.00d	88.927	0.0005
	Penicillium expansum	17.34±1.53a	26.00±1.00b	38.00±1.00c	53.34±2.08d	93.34±1.15e	316.015	0.0005
Taraxicum officinale	*Drechslera* sp.	46.66±0.58a	56.00±1.00b	63.34±0.58c	90.66±0.58d	96.00±1.00d	693.357	0.0005
	Aspergillus flavus	32.66±1.53a	42.66±1.53b	61.34±1.53c	72.66±1.53d	94.00±1.00e	138.798	0.0005
	Aspergillus niger	50.66±1.53a	66.66±1.53b	82.00±1.00c	88.66±0.58c	90.66±0.58c	70.033	0.0005
	Penicillium expansum	29.34±1.53a	34.00±1.00a	52.66±1.53b	64.66±1.53c	94.66±2.08d	251.500	0.0005

*Mean ± S.D of three replicates. Mean values were compared using Tukey's multiple comparison test ($P \leq 0.05$). The numbers followed by same alphabets are not statistically different.

Thus it is clear from the above results that the extract of the plants used during the present study were found effective against all the tested rot fungi. The efficacy of different plant extracts in inhibiting the growth of different pathogenic fungi have been reported earlier (Taskeen-Un-Nisa *et al.,* 2011; Raji and Raveendran, 2013; Znini *et al.,* 2013; Ounchokdee *et al.,* 2016; Parveen *et al.,* 2016b; Zatla *et al.,* 2017). Gujar and Talwankar (2012) screened six different plants, *viz., Azadirachta indica, Aloe vera,*

Ocimum sanctum, Ocimum basilicum, Lantana camara and *Asparagus* sp. and evaluated them for their antifungal activity against the *Aspergillus niger, Aspergillus flavus, Rhizoctonia solani* and *Rhizoctonia bataticola.* Concluding that *Azadirachta indica* and *Aloe vera* can be utilized for the management of fungal disease caused by the *Aspergillus niger, Aspergillus flavus, Rhizoctonia solani* and *Rhizoctonia bataticola.* Parveen and Wani (2015) reported the antifungal activity of plant extracts, *viz. Artemisia absinthium, Rumex obtusifolius, Plantago lanceolata, Taraxicum officinale* and *Malva sylvestris* against *Mucor piriformis* and reported *A. absinthium* at highest concentration most effective followed by *P. lanceolata, T. officinale*, *R. obtusifolius* and *M. sylvestris*. Jantasorn *et al.* (2016) evaluated the antifungal activity of five plant extracts, *viz., Hydnocarpus anthelminthicus, Crateva magna, Caesalpinia sappan, Xanthophyllum lanceatum* and *Carallia branchiata* against five pathogenic fungi, *Pyricularia oryzae, Rhizoctonia solani, Phytophthora palmivora, Sclerotium rolfsii* and *Colletotrichum gloeosporiodes* causing economic crop diseases. 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 (Jantasorn *et al.,* 2016). 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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