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Antifungal Activity of *Polyalthia longifolia* (Sonn.) Thw. against Seed Borne Fungi of Paddy (*Oryza sativa*. L).

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Summary

Antifungal activity of aqueous (10-50% concentration) and solvent extract (500µl and 1000µl concentration) of *Polyalthia longifolia* were tested against ten seed borne fungi of paddy (*Oryza sativa.* L) *in vitro* condition. In aqueous extract, *A. alternata* recorded a maximum inhibition of 92.88% followed by *F. solani* (87.10%), *F. moniliforme* (86.40%), *D. halodes* (86.07%), *F. oxysporum* (85.14%), *C. lunata* (83.33%) *and D. tetramera* (83.02%) at 50% concentration compared to synthetic fungicide, Dithane M-45, Captan, Benlate, Thiram and Bavistin at 2% recommended dosage. In solvent extract petroleum ether extract recorded a complete and maximum inhibition in all the test fungi at 1000 µl concentration. Petroleum ether is followed by Benzene, ethanol, Methanol and Chloroform.

Key Words: *Polyalthia longifolia*, Paddy, Antifungal, Aqueous extract, Solvent extract

Introduction

Agriculture is the backbone of the nation's economy, growth and development (1). Despite the significant achievements in food grain production, since independence, Indian agriculture continues to face serious challenges from ever increasing population. From the earliest times, man has struggled against famine of disease both in field and storage condition i.e., pre and post harvest loss (2). Modern agriculture has been supplying the required food for the world's ever increasing population. However modern agriculture is increasingly dependent upon the use of large quantities of chemical fertilizers, chemical growth regulators and chemical pesticides. Synthetic chemical fungicides form a major part of the chemical pesticides used in modern agriculture to manage diseases both in field and during storage. The ill effects associated with the use of chemical fungicides like carcinogenicity, teratogenicity a health assaurds necessitated the search for alternative strategies for the management of pre and post harvest crop diseases. Further extensive use of chemicals leads to biohazordous effects on ecosystem, and their persistent applications lead to resistance in pathogens against these fungicides (3). Thus alternative approaches are preferred which are ecofriendly. To avoid the use of synthetic pesticides, *In vitro* evaluation for antifungal potency of plants against phytopathogenic fungi in general and biodeterioration causing fungi in particular is the first step towards developing plant based fungicides. Natural products perform various functions, and many of them have interesting and useful biological activities. Approximately 25 to 50 % of current pharmaceuticals are derived from plants (4). There are more than 35,000 plant species being used in various human cultures around the world for medicinal purpose (5). The use of medicinal plants to treat plant and human diseases has its roots in pre-historical times. Medicinal plants are used by 80% of the world population as the only available medicines especially in developing countries (6). Following the advent of modern medicine, herbal medicine suffered a setback, but during last two or three decades advances in phytochemistry and in identification of plant compounds effective against certain diseases have renewed the interest in herbal medicines (7). Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs. Plant products still remain the principal source of pharmaceutical agents used in traditional medicine (8). Hence, in the present study, *Polyalthia longifolia* (Sonn.) Thw leaves belongs to family Annonaceae were investígated to test the potency of antifungal activity aganist seed borne fungi of paddy.

Materials and Methods

Plant material

Fresh leaves of *P. longifolia* free from diseases were collected from Manasagangotri, University of Mysore, Mysore. The leaves were washed thoroughly 2-3 times with running water and once with sterile distilled water. Leaf material was then air dried on a sterile blotter under shade and used for extraction.

Extraction

Aqueous extraction

Fifty grams of thoroughly washed leaves of *P. longifolia* were macerated with 50ml of sterile distilled water in a waring blender (Waring International, New Hartford, CT, USA) for 10minutes. The macerate was first filtered through double-

layered muslin cloth, and then centrifuged at 4000g for 30 minutes. The supernatant was filtered through Whatman No.1 filter paper and sterilized at 120°C for 30 minutes. The extract was preserved aseptically in a brown bottle at 5°C until further use (9).

Solvent extraction

Thoroughly washed leaves of *P. longifolia* were dried in shade for five days, and then powdered with the help of Waring blender. 25 grams of shade dried powder was filled in the thimble and extracted successively with petroleum ether, benzene, chloroform, methanol and ethanol in a Soxhlet extractor for 48 hours. Solvent extracts were concentrated under reduced pressure. The extracts were preserved in airtight bottle until further use (9).

Test fungi

Paddy (*Oryza sativa*. L) seeds were collected from the farmer's field, regulated market and retail shops to isolate the important seed borne pathogenic fungi associated with the seeds. The collected seed samples were subjected to standard blotter method (10). Twenty five seeds per plate were plated on three layer moistened blotter discs in petriplates. These plates were incubated at 22±2°C under alternating cycle of 12/12 hours of near ultraviolet (NUV) light and darkness for seven days. Later the samples were screened for seed mycoflora with the help of stereo binocular microscope and also when required with the help of a compound microscope. Associated fungi were identified based on growth habits, morphology and spore morphological characters using standard manuals. Important and frequently occurring seed borne pathogens of paddy viz., *Pyricularia oryzae, Bipolaris oryzae, Alternaria alternata, Tricoconis padwickii, Drechslera tetramera, D. halodes, Curvularia lunata, Fusarium moniliforme, F. oxysporum* and *F. solani* were isolated and pure cultures maintained in the laboratory for further studies.

Antifungal activity assay

Poisoned Food Technique

Aqueous extract*:* Czapek Dox Agar (CDA) medium with 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50% aqueous extract of *P. longifolia* were prepared. 15ml of the medium was poured into petriplates, allowed to cool and solidify. 5mm disc of 7 day old culture of the test fungi were placed at the center of the petriplates and incubated at 22 ± 2°C for seven days. After incubation, the colony diameter was measured in millimeter. For each treatment three replicates were maintained. Czapek dox agar medium without aqueous extract served as control (11). The percent inhibition of mycelial growth was calculated using the formula PI= C-T/Cx100, where C= Diameter of control colony and T= Diameter of treated colony (12). The results were subjected to statistical analysis by ANOVA and DMRT.

Solvent extract: One gram of all the different solvent residue was dissolved in 10ml of Methanol. 500µl and 1000µl of each of the solvent extracts was amended with 15ml of Czapek Dox agar medium per plate before solidification of the medium. Methanol (500µl and 1000µl) amended with the medium served as control. 5mm discs of 7 day old culture of the test fungi were placed at the center of the petriplates and incubated at 22 ± 2^0 C for 7 days. The diameter of the colony was measured and percent inhibition of mycelial growth was calculated using the formula PI= C-T/Cx100, where C= Diameter of control colony and T= Diameter of treated colony (12).

Chemical fungicides: Five chemical fungicides viz., Bavistin, Benlate, Captan, Dithane M-45 and Thiram were evaluated for antifungal activity by poisoned food technique for comparison.

Results

Aqueous extract: Among the ten fungi tested , *A.alternata* recorded maximum inhibition of 92.88% at 50% concentration of the extract and at 45% concentration, it recorded 86.80% inhibition. Significant activity was also observed in 5% to 40% concentration which is in the range of 68.05% to 86.80% inhibition. *A. alternata* is followed by *F.solani* and recorded 87.10% inhibition at 50% concentration. *F.moniliforme* recorded 86.40%, *D. halodes* (86.07%), *F. oxysporum* (85.14%), *C. lunata* (83.33%) *and D. tetramera* (83.02%). Least inhibition was observed in *P. oryzae* (35.23%), *B. oryzae* (37.94%) and *T. padwickii* (35.18%) (Table-1).

Table-1: Antifungal activity of aqueous extract of *Polyalthia longifolia* on important seed borne pathogens of paddy

	Concentration (Percent inhibition)									
Test fungi	5%	10%	15%	20%	25%	30%	35%	40%	45%	50%
P. oryzae	9.52±0.95^{a}	9.52±0.95^{a}	12.37±0.95^{a}	23.80±0.95^{a}	35.23±0.95^{a}	35.23±0.95^{b}	35.23±0.95^{b}	35.23±0.95^{ab}	35.23±0.95^{a}	35.23±0.95^{a}
B. oryzae	35.89±0.51^{b}	35.89±0.51^{c}	35.89±0.51^{c}	35.89±0.51^{b}	35.89±0.51^{b}	35.89±0.51^{b}	35.89±0.51^{b}	37.94±0.51^{b}	37.94±0.51^{a}	37.94±0.51^{a}
A. alternata	68.05±0.69^{f}	74.30±0.69^{g}	84.02±0.69^{g}	84.02±0.69^{f}	84.02±0.69^{f}	86.80±0.69^{f}	86.80±0.69^{f}	86.80±0.69^{f}	86.80±0.69^{e}	92.88±0.69^{h}
T. padwickii	12.03±0.92^{b}	15.73±0.92^{b}	17.58±0.92^{b}	25.92±0.92^{a}	31.47±0.92^{a}	31.47±0.92^{a}	31.47±0.92^{a}	32.40±0.92^{a}	35.18±0.92^{a}	35.18±0.92^{a}
D.tetramera	51.50±0.60^{c}	60.60±0.60^{e}	69.59±0.60^{f}	69.69±0.60^{d}	71.50±0.60^{d}	71.50±0.60^{d}	71.50±0.60^{d}	71.50±0.60^{d}	72.11±0.60^{cd}	83.02±0.75^{g}
D. halodes	64.30±0.39^{e}	68.62±0.39^{f}	70.19±0.39^{f}	76.86±0.39^{e}	76.86±0.39^{e}	76.07±0.39^{e}	76.07±0.39^{e}	76.07±0.39^{e}	76.07±0.39^{d}	86.07±0.39^{g}
C. lunata	49.16±0.83^{c}	59.16±0.83^{e}	68.33±0.83^{ef}	68.33±0.83^{d}	68.33±0.83^{d}	69.16±0.83^{d}	69.16±0.83^{d}	69.16±0.83^{d}	69.16±0.83^{c}	83.33±0.83^{g}
F.moniliforme	60.51±0.51^{d}	68.71±0.51^{f}	68.71±0.51^{ef}	69.74±0.51^{d}	71.27±0.51^{d}	76.40±0.51^{e}	76.40±0.51^{e}	76.40±0.51^{e}	76.40±0.51^{d}	86.40±0.51^{g}
F. oxysporum	48.48±0.60^{c}	55.75±0.60^{d}	66.05±0.60^{e}	67.87±0.60^{d}	69.69±0.60^{d}	68.48±0.60^{d}	68.48±0.60^{d}	71.50±0.60^{d}	72.11±0.60^{cd}	85.14±0.60^{f}
F. solani	36.88±0.44^{b}	39.10±0.44^{c}	60.44±0.44^{d}	60.44±0.44^{c}	60.44±0.44^{c}	62.55±0.44^{c}	62.55±0.44^{c}	62.55±0.44^{c}	62.55±0.44^{c}	87.10±0.44^{e}
F-value	902.51	1130.07	1315.51	1028.71	803.42	887.06	887.06	834.63	336.660	829.74

- Values are means of three replicates ± standard error
- Analysis of Variance (ANOVA); d.h =13,28; P < 0.001
- In column a-h means with different letters are significantly different each others

Table-2: Antifungal activity of solvent extract of *Polyalthia longifolia* on important seed borne pathogens of paddy

Percent inhibition										
Test fungi	Petroleum ether extract		Benzene extract		Chloroform extract		Methanol extract		Ethanol extract	
	500µlt	1000µlt	500µlt	1000µlt	500µlt	1000µlt	500µlt	1000µlt	500µlt	1000µlt
P. oryzae	100.00±0.00^{h}	100.00±0.00^{h}	70.28±0.11^{c}	70.28±0.11^{a}	30.47±0.47^{a}	47.61±0.47^{a}	80.45±0.11^{f}	100.00±0.00^{f}	49.42±0.15^{d}	52.87±0.11^{ab}
B. oryzae	53.32±0.47^{a}	78.09±0.47^{b}	100.00±0.00^{h}	100.00±0.00^{e}	66.66±0.11^{e}	100.00±0.00^{h}	36.18±0.47^{b}	73.32±0.47^{d}	39.04±0.47^{b}	90.47±0.47^{f}
A. alternata	62.85±0.00^{b}	83.80±0.95^{cd}	46.66±0.95^{a}	80.95±0.95^{b}	43.80±0.95^{b}	49.52±0.95^{ab}	35.23±0.95^{b}	57.55±1.22^{b}	43.80±0.95^{c}	72.37±0.95^{cd}
T. padwickii	51.11±0.11^{a}	81.11±0.11^{c}	51.11±0.11^{b}	84.44±0.11^{b}	41.11±0.11^{b}	51.11±0.11^{b}	17.77±0.11^{a}	51.11±0.11^{a}	17.77±0.11^{a}	51.11±0.11^{a}
D. tetramera	89.77±0.44^{g}	89.77±0.44^{e}	84.44±0.44^{f}	87.10±0.44^{c}	60.44±0.44^{d}	67.10±0.44^{d}	47.10±0.44^{c}	73.77±0.44^{d}	44.44±0.44^{c}	76.44±0.44^{cde}
D. halodes	70.97±0.39^{c}	73.72±0.39^{a}	76.86±0.39^{d}	79.21±0.39^{b}	56.86±0.39^{c}	56.86±0.39^{c}	35.68±0.39^{b}	62.74±0.39^{c}	38.03±0.39^{b}	56.86±0.39^{ab}
C. lunata	79.21±0.39^{d}	86.27±0.39^{d}	85.09±0.39^{f}	89.01±0.15^{c}	67.44±0.39^{e}	70.97±0.39^{e}	59.29±0.39^{e}	79.21±0.39^{e}	48.62±0.39^{d}	77.62±0.93^{cde}
F. moniliforme	85.09±0.39^{f}	96.86±0.39^{g}	88.62±0.39^{g}	65.48±0.26^{a}	75.68±0.39^{f}	88.62±0.39^{g}	60.39±0.39^{e}	70.97±0.39^{d}	81.56±0.39^{f}	86.27±0.39^{df}
F. oxysporum	89.63±0.45^{g}	93.24±0.00^{f}	89.63±0.45^{g}	92.34±0.45^{d}	59.90±0.45^{cd}	81.53±0.45^{f}	53.15±0.45^{d}	53.15±0.45^{a}	46.39±0.45^{cd}	66.66±0.45^{bc}
F. solani	82.35±0.00^{c}	93.33±0.39^{f}	80.39±0.39^{e}	90.97±0.39^{d}	59.21±0.39^{cd}	68.62±0.39^{de}	53.54±0.60^{d}	60.39±0.39^{c}	57.70±0.56^{e}	75.68±0.39^{cde}
F-Value	1153.78	227.67	646.01	1.55	401.37	923.189	622.71	537.64	531.42	19.77

- Values are means of three replicates ± standard error
- Analysis of Variance (ANOVA); d.g =9,20; P < 0.001
- In column a-h means with different letters are significantly different each others

Solvent extract: In petroleum ether extract, at 500µl concentration, *P. oryzae* was completely inhibited. At 1000 µl concentration, *F.moniliforme* recorded maximum inhibition of 96.86%, *F. solani* (93.33%), *F. oxysporum* (93.24%), *D. tetramera* (89.77%), *A. alternata* (83.30%), *T. padwickii* (81.11%), *D. halodes* (73.72%) *and B. oryzae* (78.09%). Petroleum ether extract is followed by benzene and *B. oryzae* were completely inhibited at 500 µl concentration. In 1000 µl concentration, significant activity was observed in *F.solani* (90.97%), *F. oxysporum* (92.34%), *C. lunata* (89.01%) and *D. tetramera* (87.10%). Rest of the fungi showed inhibition in the range of 65.48% to 84.44%. Moderate activity was also observed in ethanol extract against all the test fungi and recorded the inhibition percentage in between the range of 51.11% to 90.47%. In methanol extract, the inhibition percentage is in the range of 51.11% to 79.21% in nine fungi among ten tested. *P. oryzae* was completely inhibited in 1000 µl concentration. In chloroform extract, *B. oryzae* was completely inhibited in 1000 µl concentration. Moderate and significant activity was also observed in nine fungi among ten and the inhibition percentage is in the range of 47.61% to 88.62% in 1000 µl concentration (Table- 2).

Chemical fungicides: Among the five fungicides tested for antifungal activity assay it was observed that *P. oryzae* was completely inhibited by all the fungicides at the recommended dosage (2 grams/liter). *F. moniliforme* was completely inhibited by Dithane M-45, Benlate and Bavistin. *F. oxysporum* was completely inhibited by Benlate, Bavistin and *T. padwickii* was completely inhibited by Thiram and Bavistin. Among the fungicides tested Bavistin showed higher degree of activity followed by benlate and Dithane M-45 against a wide range of test fungi (Table- 3).

Table-3: Comparative efficacy of different synthetic fungicides against important seed borne pathogens of paddy at recommended concentration

Test fungi	Percent inhibition(%)				
	Dithane-M-45	Captan	Benlate	Thiram	Bavistin
P. oryzae	100.00±0.00^{h}	100.00±0.00^{g}	100.00±0.00^{h}	100.00±0.00^{g}	100.00±0.00^{g}
B. oryzae	88.62±0.39^{g}	47.05±0.00^{b}	82.74±0.39^{d}	68.62±0.39^{e}	92.05±0.39^{e}
A. alternata	64.87±0.59^{d}	55.94±0.59^{d}	68.44±0.67^{b}	55.35±0.00^{b}	72.01±0.59^{b}
T. padwickii	69.53±0.57^{e}	81.60±0.57^{f}	96.55±0.00^{g}	100.00±0.00^{g}	100.00±0.00^{b}
D. tetramera	48.25±0.49^{b}	48.25±0.49^{b}	57.20±0.49^{a}	59.20±0.50^{c}	69.14±0.49^{a}
D. halodes	85.09±0.39^{f}	65.09±0.39^{e}	93.33±0.39^{f}	63.13±0.39^{d}	98.03±0.39^{f}
C. lunata	69.74±0.51^{e}	82.04±0.51^{f}	85.12±0.51^{e}	68.71±0.51^{e}	83.58±0.51^{d}
F. moniliforme	100.00±0.00^{h}	51.17±0.47^{c}	100.00±0.00^{h}	81.22±0.47^{f}	100.00±0.00^{g}
F. oxysporum	37.27±0.43^{a}	34.64±0.43^{a}	100.00±0.00^{h}	53.06±0.43^{a}	100.00±0.00^{g}
F. solani	59.21±0.39^{c}	47.44±0.39^{b}	72.15±0.39^{c}	59.60±0.39^{c}	76.86±0.39^{c}

- Values are means of three replicates ± standard error
- Analysis of Variance (ANOVA); d.f =13,28; P < 0.001
- In column a-h means with different letters are significantly different each others

Discussion

Biological control of plant pathogen is an approach to healthy environment and a natural pesticide for sustainable agriculture. Increasing resistance of many pathogens to currently available synthetic pesticides has become a serious problem around the globe. Because of their strict requirements of their efficacy, selectivity, toxicology and general impact on the environment (13-16).

Paddy seeds are known to harbor a wide variety of pathogen belonging to different groups of microorganisms. Among them fungi are of significant importance as many fungal diseases of paddy are transmitted through seeds (17-20). Among the diseases of paddy, blast caused by *P. oryzae*, stackburn caused by *T. padwickii*, grain discolouration due to *A. alternata* and leaf spot caused by *Drechslera* sps. are

important in Karnataka as they are known to cause significant loss in yield and quality of the grains (21-24).

Management of these diseases is achieved mainly due to the application of synthetic chemical fungicides. More than 13.5 million hectares of land in Karnataka is under paddy cultivation, consequently the amount of synthetic fungicide used for the management of these diseases is also very large. Thus there is a need to search for alternate, ecofriendly approaches for the management of diseases caused by fungi in paddy seeds, in order to significantly reduce the amount of pesticides used in paddy cultivation in general and fungicides in particular.

The earlier workers have evaluated the antifungal potential of *P. longifolia* against *C. lunata, A. alternata* and *Rhizoctonia solani* (25-27), where as they have not evaluated the antifungal potential of this plant against important phytopathogenic seed borne fungi of paddy which are known to cause severe loss in yield and quality in paddy production in the state. Thus the result of the present investigation is in conformity with the observations of the earlier workers.

In the present investigation the ability of *P. longifolia* to significantly inhibit the germination and growth of important phytopathogenic seed borne fungi of paddy has been demonstrated for the first time. It is evident from the results of the present investigation that the antifungal activity is concentration dependent. More than 70% inhibition was observed against *D. halodes* at 15% concentration, *D. tetramera* and *F. moniliforme* at 25% concentration, *F. oxysporum* at 40% concentration and *A. alternata* at 45% concentration.

Thus, considering the earlier observations this plant were subjected to solvent extraction. The results have revealed that petroleum ether extract is the most potent one in significantly inhibiting all the test pathogens. Thus it is evident, that the antifungal principle is in the petroleum ether extract and that it can be extracted and purified from petroleum ether extract.

A comparative evaluation of the antifungal potency of the aqueous and solvent extracts of this plant with that of the commonly used synthetic fungicides at their recommended dosage (2000ppm) has revealed that a concentration far below, the concentration of the synthetic fungicide is enough to bring about the required mycelial growth inhibition of the test pathogens *in vitro*.

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