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In vitro evaluation of the antifungal potentiality of *Polyalthia longifolia* against some sorghum grain moulds

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Anti-fungal activity assay of aqueous and different successive solvent extracts viz., Petroleum ether, benzene, chloroform, methanol and ethanol extracts of *Polyalthia longifolia* against important phytopathogenic fungi known to cause diseases in many agricultural crop, were carried out by poisoned food technique. Aqueous extract recorded significant activity. Among the five solvent extracts tested, petroleum ether extract was active against all the test fungi. Comparative evaluation of the aqueous extract and petroleum ether extract with the synthetic fungicides viz., Blitox, Captan, Dithane M-45 and Thiram, which are generally used in the management of these phytopathogenic fungi at their respective recommended dosage (2 g/l) revealed that the antifungal activity of the aqueous and petroleum ether extracts obtained from the plant was better than that of synthetic fungicide tested. Phytochemical analysis of leaf material revealed that antifungal activity is due to the presence of phenolic compounds. The results suggest that *P. longifolia* is a potential candidate plant for the management of phytopathogenic fungi associated with many agricultural crops.

Key words: *Polyalthia longifolia*, antifungal activity

Introduction

Microbial bio-deterioration of food grains during pre and post harvest are a well known phenomenon causing significant loss up to 30% (Agrios, 2006). Fungi are significant destroyers of foodstuffs and grains during storage, rendering them unfit for human consumption by retarding their nutritive value and often by producing mycotoxins. A significant portion of the cereals produced in the world are reported to contaminate with mycotoxins and other fungal metabolites which are reported to be toxic to human and animals (Galvano *et al.*, 2001). A sizable portion of the world population living below poverty line in the developing and undeveloped countries of Asia and Africa

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have been suffered from health problems associated with consuming mycotoxin contaminated grains and cereals (Miller, 1995).

Synthetic pesticides are an essential input for preventing pre and post harvest crop losses caused by phytopathogenic microorganisms (Wheeler, 2002; Agrios, 2006). Incessant and extensive use of these synthetic pesticides are posing serious problem to the life supporting systems due to their residual toxicity (Andrea *et al*., 2000; Harris *et al*., 2001; Campos *et al*., 2005). It is estimated that hardly 0.1% of the agro-chemicals used in crop protection reaches the target pest, leaving the remaining 99.9% to the environment to cause hazards to non target organisms including humans (Pimentel and Levitan, 1986). The large numbers of synthetic pesticides have been banned in the western world because of their undesirable attributes such as high and acute toxicity, long degradation periods, accumulation in the food chain and an extension of their power to destroy both useful and harmful pests (Barnard *et al*., 1997; Wodageneh and Wulp, 1997; Ortelli *et al*., 2005). The toxic effect of synthetic chemical can be overcome only by persistent search for new and safer pesticides accompanied by wide use of non-chemical pest control, which are eco-friendly and effective (Kumar and Berwal, 1998).

Many reports revealed that plant metabolites and plant-based pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to synthetic pesticides (Sing *et al*., 2002; Patni *et al*., 2005). Hence several plants selected randomly were screened for antifungal activity in our laboratory. During regular screening *P. longifolia* recorded significant antifungal activity. *P. longifolia* is a wild plant distributed in tropical and subtropical region belonging to the family solanaceae. Earlier report revealed that the fruits are used in chronic dysentery, constipation. Flowers used in snuff to relive headache, lotion for wounds and ulcers. Barks are used to increase fertility in women. They have rich source of tannin, saponin, alkaloids, glucoside, ursolic acid (Anonymous, 1985). A scientific and systematic investigation with regard to the various biological activities of this plant is lacking. Thus considering the vast potentiality of plant as a source of new fungicidal agents, detailed investigations was conducted to test the efficacy of *P. longifolia* against important seed borne phytopathogenic fungi.

Materials and methods

Collection of plant materials

Fresh leaves of *P. longifolia* free from disease were collected from Mysore (India), washed thoroughly 2-3 times with running tap water and once

with sterile water, shade-dried, powdered and used for extraction. A voucher specimen of the plant is deposited in the herbarium of Department of Studies in Botany, University of Mysore, Mysore, India.

Preparation of the aqueous extracts

Samples (50 gm) of shade dried, powder of leaves of *P. longifolia* was macerated with 100 ml of sterile distilled water in a Waring blender (Waring International, new Hartford, CT, USA) for 10 min. The macerate was first filtered through double layer muslin cloth and then centrifuged at 4000 *g* for 30 min. The supernatant was filtered through Whatman No. 1 filter paper and heat sterilized at 120 ºC for 30 min. The extract was preserved aseptically in a brown bottle at 5ºC until further use.

Preparation of solvent extractions

Fifty gram of shade dried, powder of *P. longifolia* was filled in the thimble and extracted successively with 200 ml each of Petroleum ether, Benzene, Chloroform, Methanol and Ethanol using a Soxhlet extractor for 48 h. All the extracts were concentrated using rotary flash evaporator and preserved at 5 ºC in airtight bottles until further use. All the extracts were subjected to antifungal activity assay.

Phytochemical analysis

Phytochemical analysis of the evaporated petroleum ether extract was conducted following the procedure of Anonymous (1985) and Harborne (1998). petroleum ether extract of *P. longifolia* was separated into different fractions as Acidic (fraction 1), Basic (fraction 2), Phenolic (fraction 3) and Neutral (fraction 4) following the procedure of Roberts *et al*. (1981). All the fractions were again subjected to antifungal activity.

Test fungi

Seed samples (sorghum) were plated on Czapeck-Dox-Agar (CDA), Malt extract-Salt-Agar (MESA) and Standard Blotter Method (SBM) to isolate the frequently occurring important seed-borne phytopathogenic field and storage fungi associated with these seeds. Eight species of *Fusarium* viz., *Fusarium equiseti, F. graminearum, F. lateritium, F. moniliforme, F. oxysporum, F. proliferatum, F. semitectum,* and *F. solani,* ten species of *Aspergillus* viz., *Aspergillus candidus, A. columnaris, A. flavipes, A. flavus, A. fumigatus, A. niger, A. ochraceus, A. tamari, A. terreus,* and *A. versicolor,* three species of

Penicillium viz., *Penicillium chrysogenum, P. griseofulvum* and *P. oxalicum,* two species of *Drechslera* viz., *Drechslera halodes* and *D. tetramera* and *Alternaria alternata* were isolated and served as test fungi. The cultures of *Aspergillus* and *Penicillium* were maintained on MESA, whereas cultures of *Fusarium, Drechslera* and *Alternaria* were maintained on CDA medium.

Anti-fungal activity assay

The aqueous extract, all the solvent extracts and different fractions of methanol extract of *P. longifolia* were subjected to antifungal activity assay by poisoned food technique. The extracts were added to the medium to achieve the desired concentrations in the medium, autoclaved and poured into Petri dishes (20 ml each) and allowed to cool. After complete solidification of the medium, 5 mm disc of 7-day-old culture of the test fungi were inoculated. Four replicates were maintained for each concentration. The Petri dishes containing media devoid of the extract served as control. The plates were incubated at 26±1ºC for seven days. The fungitoxicity of the extract in terms of percentage inhibition of mycelial growth was calculated by using the formula: %inhibition = dc – dt x 100/dc.

Where dc=Average increase in mycelial growth in control, dt=Average increase in mycelial growth in treatment (Singh & Tripathi 1999).Synthetic fungicides, viz., Blitox, Captan, Dithane M-45 and Thiram were also tested at their recommended dosage (2g/L) for antifungal activity by poisoned food technique for comparison (Zehavi *et al*., 1986).

Results

Antifungal activity

Aqueous extract: Antifungal activity was observed against all the test fungi and the extent of mycelial growth inhibition was varied for each fungi (Table 1). More than 50% inhibition was observed in all the fungi except *Fusarium equiseti, F. lateritium, F. semitectum, Aspergillus columnaris, A. flavipes* and *A. tamari*.

The antifungal activity of aqueous extract of *P. longifolia* at different concentrations as determined by poisoned food technique on CDA media and MESA media are presented in Table 1. Percent inhibition of the important field fungi on CDA media revealed that *D. halodes* is highly susceptible and *F. lateritium* is least susceptible. The maximum inhibition of *D. halodes* was observed at 50% concentration.

The percent inhibition of different storage fungi on MESA media revealed that *P. chrysogenum* was highly susceptible and *A. tamari* was least

susceptible. The statistical analysis of the data clearly reveals that inhibitory activity increases with increase in concentration.

Solvent extraction: After solvent evaporation the yield of different extracts were 1.9 g, 0.98 g, 0.50 g, 12.8 g and 10.8 g respectively in petroleum ether, benzene, chloroform, methanol and ethanol solvents. The percent mycelial growth inhibition of different solvent extracts on different phytopathogenic fungi was presented in Table 2.

Highly significant activity of field fungi was observed in petroleum ether extract. Benzene extract also showed moderate activity against test fungi. Antifungal activity varied among both field and storage fungi. Among field fungi *F. moniliforme* showed highest susceptibility, where as *Drechslera halodes* was least susceptible. *Aspergillus candidus* was highly susceptible and *A. tamari* was least susceptible among storage fungi.

Phytochemical analysis

Phytochemical analysis of petroleum ether extract which recorded significant antifungal activity revealed the presence of carbohydrates and glycosides, protein and amino acids, alkaloids, phenolic and tannin compounds, whereas other constituents such as phytosterols, saponins, oils and gum and mucilage (Table 3) were found absent.

Fractionation of petroleum ether extract

Out of four fractions viz., Acidic (fraction 1), Basic (fraction 2), Phenolic (fraction 3) and Neutral (fraction 4), phenolic fraction recorded highly significant antifungal activity against all the test fungi (Table 4) which was compared to aqueous and petroleum ether extract. No activity was observed in other fractions. More than 90% of mycelial growth inhibition was observed against *Drechslera halodes*, *Fusarium graminearum* and *F. moniliforme*. Among field fungi *D. halodes* was highly susceptible and *F. lateritium* was least susceptible. Mycelial growth of *Aspergillus fumigatus*, *A. ochraceous*, *A. versicolor* and *Penicillium chrysogenum* were inhibited more than 90% among storage fungi. *A. fumigatus* is highly susceptible and *A. tamari* was least susceptible.

Comparative efficacy with synthetic fungicides

Among four fungicides, thiram was recorded to complete inhibition of mycelial growth, whereas antifungal activity of blitox, captan and dithane M-45 varied among the test fungi (Table 4).

Comparative efficacy of phenolic fraction with synthetic fungicides revealed that significance of antifungal activity of phenolic fraction varied

among the test fungi. It was observed that antifungal activity of phenolic fraction was highly significant compared to blitox, captan and dithane M-45 against *Drechslera tetramera*, *Fusarium graminearum*, *F. proliferatum* among field fungi, *Aspergillus ochraceous* and *A. versicolor* among storage fungi. Phenolic fraction recorded highly significant antifungal activity against all the test fungi compared to dithane M-45 except *F. equiseti*, *F. semitectum* and *A. niger*.

Table 1. Antifungal activity (Percent mycelial inhibition) of aqueous extract of *P. longifolia* at different concentrations against phytopathogenic fungi.

Phytopathogenic field fungi	**Percent mycelial inhibition**				
	Concentrations (%)				
	10	**20**	**30**	**40**	**50**
Alternaria alternata	12.40±0.69	28.50±0.49	39.42±0.44	57.50±0.49	64.50±0.49
Drechslera halodes	34.40±0.49	52.40±0.49	85.48±0.49	93.58±0.34	99.60±0.00
D. tetramera	39.91±0.43	52.61±0.43	63.73±0.43	88.31±0.43	98.91±0.43
Fusarium equiseti	9.57±0.64	14.87±0.64	22.58±0.49	35.77±0.64	44.87±0.64
F. graminearum	37.73±0.48	64.65±0.48	75.01±0.59	88.75±0.46	90.75±0.48
F. lateritium	2.52±0.42	9.42±0.42	10.27±0.46	15.87±0.42	27.82±0.42
F. moniliforme	39.75±0.34	52.79±0.34	64.03±0.34	72.76±0.34	86.79±0.34
F. oxysporum	23.28±0.60	37.27±0.60	42.68±0.48	57.28±0.60	67.28±0.60
F. proliferatum	42.89±0.47	48.89±0.77	53.71±0.45	64.87±0.37	72.89±0.47
F. semitectum	9.10±0.39	17.10±0.39	22.89±0.44	33.10±0.79	44.10±0.39
F. solani	7.20±0.52	15.60±0.32	32.20±0.62	47.20±0.62	65.20±0.62
Aspergillus candidus	32.73±0.60	46.67±0.60	52.78±0.30	73.56±1.00	83.56±1.00
A. columnaris	8.20±0.43	14.30±0.43	26.50±0.43	34.63±0.39	42.65±0.39
A. flavipes	10.80±0.72	16.90±0.72	22.60±0.72	32.18±0.7	44.18±0.7
A. flavus	38.24±0.41	52.24±0.41	44.24±0.41	72.02±0.53	82.02±0.53
A. fumigatus	40.26±0.52	56.56±0.58	64.96±0.55	78.56±1.00	95.00±0.00
A. niger	16.00±0.52	21.04±0.52	32.00±0.52	41.21±0.42	52.21±0.42
A. ochraceus	23.42±0.60	44.82±0.60	55.82±0.60	76.75±0.56	92.75±0.56
A. tamari	9.40±0.21	14.70±0.61	19.60±0.61	26.35±0.30	39.32±0.30
A. terreus	8.65±0.34	12.62±0.04	25.67±0.0.45	37.88±0.34	53.48±1.0
A. versicolor	23.47±0.40	46.74±0.86	67.24±0.40	84.12±0.55	92.11±0.70
Penicillium chrysogenum	32.54±0.56	43.24±0.32	66.64±0.78	83.16±0.65	99.06±0.20
P. griseofulvum	18.67±0.56	28.45±0.56	46.34 ±0.67	64.89±0.72	72.12±0.45
P. oxalicum	18.06±0.67	28.67±0.32	37.45±0.25	54.63±0.78	71.63±0.9

The value means of four replicates ± standard error.

Table 3. Preliminary phytochemical analysis of petroleum ether extract of *Polylanthia longifolia.*

Tests	**Petroleum ether extract**
Carbohydrates/Glycosides	+ +
Proteins/aminoacids	+ +
Alkaloids	+ +
Phytosterols	- -
Phenolic compounds	+ +
Saponin	--
Tannin	++
Oils	--
Gums and mucilage	--

++ Present, -- Absent**.**

Discussion

The plant world is a rich storehouse of biochemical's that could be exploited for use as pesticides. The total number of plant chemicals may exceed 400,000 and of these 10,000 are secondary metabolites whose major role in the plants is reportedly defensive (Grayer and Harborne, 1994). Higher plants are much more important in the production of economically important organic compounds, pharmaceuticals and pesticides (Hostettman and Wolfender, 1997). Many species of higher plants have not been described, much less surveyed for chemical or biologically active constituent and new sources of commercially valuable pesticides (Varma and Dubey, 1999). This is mainly due to lack of information on the screening/evaluation of diverse plants for their antibacterial potential. Biologically active plant derived pesticides are expected to play an increasingly significant role in crop protection strategies. Exploitation of naturally available chemicals from plants, which retards the reproduction of undesirable microorganisms, would be shown a more realistic and ecologically sound method for plant protection and will have a prominent role in the development of future commercial pesticides for crop protection strategies, with special reference to the management of plant diseases (Gottlieb *et al.*, 2002).

Considering these as a first step, in the present investigation fiftyfour plants were screened in vitro for antifungal activity against important phytopathogenic fungi. These plants were selected based on traditional medicine knowledge and random choosing from the local flora. The screening revealed that aqueous extract of *P. longifolia* was showed highly significant antifungal activity. The antifungal activity assay of the five solvent extracts of *P. longifolia* revealed that petroleum ether extract has highest antifungal activity, suggesting that the bioactive compound responsible for antifungal activity is better extracted with petroleum ether than the other solvents. The finding of the present investigation is an important step towards isolation and characterization of the antifungal agent and its further evaluation for crop protection strategies.

Antifungal activity of phenolic fraction was highly pronounced to aqueous and petroleum ether extract tested during antifungal activity guided fractionation. Antifungal activity of *P. longifolia* is almost equal to blitox. This plant needs to be further investigated for exploitation in the management of phytopathogenic fungi. Aqueous extract of *P. longifolia* is known to effectively control fungal growth in vitro (Sobti *et al.*, 1995). Earlier workers have not screened the potential of different solvent extracts and phenolic fraction of this plant to inhibit the fungi, hence the present study. In the present investigation the efficacy of this plant to control a wide variety of seed borne fungi has been demonstrated for the first time. This study is also successful in identifying a candidate plant which recorded very interesting antifungal activity against both field and storage fungi.

The number of fungi which recorded susceptibility to phenolic fraction of *P. longifolia* is quiet interesting and indicates the broad spectrum antifungal activity of the phenolic compound/active principle. Further, it was observed that the antifungal activity is fungicidal and the compound is heat stable because all the fractions/extracts were incorporated to the media and sterilized before pouring to the sterile Petri plate.

In the present investigation, *P. longifolia* was screened for antifungal potential against different species of fungi, which are known to cause significant damage to a wide variety of crop in the field and cause bio-deterioration of grains and other agricultural products during storage. Further investigation is also needed for determining the mode of action of the bioactive principle present in this plant.

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Table 2. Antifungal activity (Percent mycelial inhibition) of different solvent extract of *Polyanthia longifolia* at 2000 µg/ml concentration against phytopathogenic fungi.

Phytopathogenic field fungi	Percent mycelial inhibition					
	Methanol control	Petroleum ether extract	Benzene extract	Chloroform extract	Methanol extract	Ethanol extract
Alternaria alternata	0.46± 0.29	32.87±0.48	24.54± 0.50	2.46± 0.25	0.00±0.00	0.00±0.00
Drechslera halodes	0.80± 0.38	26.27±0.56	12.45± 0.56	4.32± 0.23	0.00±0.00	0.00±0.00
D. tetramera	0.00±0.00	41.63±0.38	19.67 ±0.33	6.23± 0.23	0.00±0.00	0.00±0.00
Fusarium equiseti	0.20±0.18	42.73±0.19	28.00± 0.56	3.51± 0.23	0.00±0.00	0.00±0.00
F. graminearum	0.36± 0.23	57.57±0.29	28.00± 0.36	4.40± 0.26	0.00±0.00	0.00±0.00
F. lateritium	0.00±0.00	31.86±0.46	23.57± 0.67	3.68± 0.33	0.00±0.00	0.00±0.00
F. moniliforme	0.36± 0.28	84.90±0.25	44.68± 0.56	10.63± 0.29	0.00±0.00	0.00±0.00
F. oxysporum	0.00±0.00	35.08±0.40	26.00± 0.45	3.41± 0.27	0.00±0.00	0.00±0.00
F. proliferatum	0.46± 0.29	50.43±0.49	27.00± 0.56	6.39± 0.48	0.00±0.00	0.00±0.00
F. semitectum	0.80± 0.38	44.92±0.99	22.33± 0.57	7.74± 0.37	0.00±0.00	0.00±0.00
F. solani	0.39± 0.24	40.04±0.29	26.45 ±0.72	8.37± 0.23	0.00±0.00	0.00±0.00

Table continued

Table 2. continued.

Phytopathogenic storage fungi	Percent mycelial inhibition					
	Methanol control	**Petroleum ether extract**	**Benzene extract**	**Chloroform extract**	**Methanol extract**	**Ethanol extract**
Aspergillus candidus	0.00±0.00	56.07±0.5	32.45±0.34	6.25±0.16	0.00±0.00	0.00±0.00
A. columnaris	0.00±0.00	34.96±0.48	22.356± 0.45	0.00±0.00	0.00±0.00	0.00±0.00
A. flavipes	0.00±0.00	43.85±0.44	25.34±0. 20	9.00±0.520	0.00±0.00	0.00±0.00
A. flavus	0.00±0.00	39.67±0.46	27.36± 0.55	10.36± 0.23	0.00±0.00	0.00±0.00
A. fumigatus	0.00±0.00	52.09±0.76	36.58± 0.30	11.66± 0.30	0.00±0.00	0.00±0.00
A. niger	0.00±0.00	32.46±0.58	19.46± 0.27	8.46± 0.27	0.00±0.00	0.00±0.00
A. ochraceus	0.00±0.00	33.49±0.35	12.70± 0.89	6.80± 0.89	0.00±0.00	0.00±0.00
A. tamari	0.00±0.00	28.66±0.53	10.39± 0.20	0.00±0.00	0.00±0.00	0.00±0.00
A. terreus	0.00±0.00	44.49±0.28	17.46± 0.29	6.46± 0.29	0.00±0.00	0.00±0.00
A. versicolor	0.00±0.00	42.81±0.59	31.80± 0.38	4.80± 0.38	0.00±0.00	0.00±0.00
Penicillium chrysogenum	0.00±0.00	51.79±0.92	24.36± 0.67	7.39± 0.20	0.00±0.00	0.00±0.00
P. griseofulvum	0.00±0.00	28.29±0.36	18.56±0.34	0.00±0.00	0.00±0.00	0.00±0.00
P. oxalicum	0.00±0.00	38.76±0.67	18.32±0.34	6.34±0.34	0.00±0.00	0.00±0.00

Data given are mean of four replicates ± standard error.

Table 4. Antifungal activity assay of different fractions of *Polylanthia longifolia* and some synthetic fungicides against seedborne phytopathogenic fungi.

Phytopathogenic field fungi	Percent mycelial inhibition						
	Active fractions of *Polylanthia longifolia*				Synthetic fungicides		
	Acidic	Phenolic	Basic	Neutral	Blitox	Captan	Dithane M-45
Alternaria alternata	0.00	86.38±0.75	0.00	0.00	72.46±0.58	91.48±0.35	77.19±0.21
Drechslera halodes	0.00	99.45±0.25	0.00	0.00	83.25±0.46	100±0.00	97.15±0.39
D. tetramera	0.00	99.43±0.56	0.00	0.00	84.37±0.33	98.63±0.42	97.76±0.60
Fusarium equiseti	0.00	73.31±0.35	0.00	0.00	95.55±0.36	81.85±0.32	76.07±0.61
F. graminearum	0.00	98.145±0.67	0.00	0.00	88.66±0.26	73.35±0.26	73.91±0.74
F. lateritium	0.00	58.25±0.67	0.00	0.00	42.88±0.25	63.48±0.27	48.90±0.37
F. moniliforme	0.00	91.35±0.56	0.00	0.00	97.24±0.45	78.45±0.72	65.84±0.85
F. oxysporum	0.00	76.33±0.78	0.00	0.00	85.69±0.64	68.68±0.48	68.18±0.18
F. proliferatum	0.00	81.65±0.56	0.00	0.00	74.38±0.27	68.66±0.25	76.09±0.45
F. semitectum	0.00	83.57±0.45	0.00	0.00	78.38±0.12	95.59±0.03	91.47±0.18
F. solani	0.00	67.30±0.45	0.00	0.00	72.80±0.25	72.70±0.26	65.32±0.30

Table continued

Table 4. continued.

Phytopathogenic storage fungi	Percent mycelial inhibition						
	Active fractions of *P. longifolia*				Synthetic fungicides		
	Acidic	Phenolic	Basic	Neutral	Blitox	Captan	Dithane M-45
Aspergillus candidus	0.00	84.36±0.76	0.00	0.00	100±0.00	100±0.00	47.89±0.31
A. columnaris	0.00	75.25±0.65	0.00	0.00	85.90±0.22	87.67±0.31	44.90±0.40
A. flavipes	0.00	72.85±0.34	0.00	0.00	91.35±0.30	88.87±0.19	24.68±0.13
A. flavus	0.00	84.78±0.43	0.00	0.00	96.03±0.37	91.98±0.14	54.17±0.15
A. fumigatus	0.00	99.48±0.45	0.00	0.00	100±0.00	92.98±0.37	16.52±0.40
A. niger	0.00	84.38±0.45	0.00	0.00	100±0.00	96.45±0.14	92.75±0.31
A. ochraceus	0.00	96.85±0.26	0.00	0.00	93.64±0.14	93.85±0.66	86.82±0.44
A. tamari	0.00	56.45±0.56	0.00	0.00	73.53±0.28	87.69±0.13	22.45±0.50
A. terreus	0.00	63.45±0.56	0.00	0.00	95.73±0.27	87.16±0.51	45.87±0.35
A. versicolor	0.00	97.23±0.45	0.00	0.00	93.40±0.20	83.86±0.43	13.41±0.36
Penicillium chrysogenum	0.00	99.23±0.34	0.00	0.00	100±0.00	100±0.00	53.45±0.39
P. griseofulvum	0.00	84.35±0.89	0.00	0.00	92.91±0.38	92.25±0.15	51.98±0.38
P. oxalicum	0.00	82.37±0.67	0.00	0.00	65.94±0.32	82.60±0.85[d]	53.85±0.20

Data given are mean of four replicates ± standard error