Anti-fungal evaluation of some plant extracts against some plant pathogenic field and storage fungi

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Mohana, D. C. and Raveesha, K. A. (2007). Anti-fungal evaluation of some plant extracts against some plant pathogenic field and storage fungi. Journal of Agricultural Technology 4(1): 119-137.

Aqueous extract of eight plants were screened for antifungal activity against *Fusarium solani* and *Aspergillus flavus* at 10% concentration by dry mycelial weight, spore germination and poisoned food techniques. The results revealed that *Decalepis hamiltonii* Wight & Arn. (Asclepiadaceae) showed significant antifungal activity. The antifungal activity of aqueous extract of *D. hamiltonii* an edible plant, was further evaluated at different concentrations by poisoned food technique against eight species of *Fusarium*, ten species of *Aspergillus*, three species of *Penicillium*, two species of *Drechslera* and *Alternaria alternata*. These phytopathogenic fungi were isolated from Sorghum, Maize, and Paddy seeds. It was observed that aqueous extract showed significant antifungal activity against all the test pathogens. Species of *P. chrysogenum* was completely inhibited at 10% concentration. *D. halodes* and *A. fumigatus* were inhibited at 20% concentration, whereas *F. lateritium* and *F. moniliforme*, were inhibited at a higher concentration of 50%. *D. hamiltonii* was further subjected to different solvent extraction using petroleum ether, benzene, chloroform, methanol and ethanol to identify the solvent extract having high activity. It was observed that petroleum ether extract showed highly significant antifungal activity followed by benzene and chloroform extracts, whereas no activity was observed in methanol and ethanol extracts at 2000 µg/ml. This plant being an edible one can possible be exploited in the management of seed borne pathogenic fungi and prevention of biodeterioration of grains and mycotoxin elaboration during storage.

Key words: Plant extracts, anti-fungal activity, phytopathogenic fungi

Introduction

Microbial bio-deterioration of food grains during storage is a well known phenomenon causing significant loss upto 30% (Chhokar, 2001). The annual crop losses of world as a result of diseases have been estimated at 25,000 million US dollars, of this a major part is due to fungal pathogens carried through seed (Agrios, 1997; Chandler, 2005). Fungi are significant destroyers

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of foodstuffs and grains during storage, rendering them unfit for human consumption by retarding their nutritive value and often by producing mycotoxins (Park *et al*., 2004; Koirala *et al*., 2005; Domijan *et al*., 2005). A significant portion of the agricultural product in the country and the world over become unfit for human consumption due to mycotoxins contamination of grains, especially those produced by species of *Aspergillus*, *Fusarium* and *Penicillium*. More than 25% of the world cereals are contaminated with known mycotoxins and more than 300 fungal metabolites are reported to be toxic to man and animals (Galvano *et al*., 2001). The main toxic effects of these metabolites are carcinogenicity, genotoxicity, terratogenicity, nephrotoxicity, hepatotoxicity, reproductive disorders and immunosuppression (Rocha *et al*., 2005). A sizable portion of the world population living below poverty line in the developing and underdeveloped countries of Asia and Africa are suffering from health problems associated with consuming mycotoxin contaminated grains and cereals. Eventhough effective and efficient control of seed borne pathogenic fungi can be achieved by the use of synthetic chemical fungicides, the same can not be applied to grains for reasons of pesticide toxicity (Gupta, 2001; Harris *et al*., 2001). Thus there is a need to search for alternative approaches to store grains/cereals for human consumption without toxicity problems that are ecofriendly and not capital intensive.

Extracts of many higher plants have been reported to exhibit antibacterial, antifungal and insecticidal properties under laboratory trails. 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 (Varma and Dubey 1999). In view of these, the present investigation was undertaken to screen for the efficacy of antifungal potency of certain plant extracts against important phytopathogenic seed borne fungi viz., ten species of *Aspergillus*, eight species of *Fusarium*, three species of *Penicillium*, two species of *Drechslera* and *Alternaria alternata* known to cause significant crop loss in the fields and during storage.

Materials and methods

Plant materials

Eight different plant species, known for their medicinal value in traditional medicine and generally available in wild in Mysore region were selected for the study. The apparently healthy plant parts of these plants were washed thoroughly 2-3 times with running tap water and once with sterile distilled water, and used

for extraction. The voucher plant specimens have been deposited in the herbarium of Department of Studies in Botany, University of Mysore, Mysore. The list of plants tested for antifungal activity, the families to which they belong and parts used for antifungal activity is presented in Table 1.

Preparation of aqueous extracts

Thoroughly washed fresh plant material (50 g) was macerated with 50 ml sterile distilled water in a waring blender (Waring International, New Hartford, CT, USA) for 10 min. The macerate was first filtered through double-layered muslin cloth, and then centrifuged at 4000 *g* for 30 min; the supernatant was filtered through Whatman No. 1 filter paper and sterilized at 120 ^{o}C for 30 min. The extract was preserved aseptically in a brown bottle at 5 ^{o}C until further use (Satish *et al*., 1999). The obtained extracts served as the crude extract (100 % concentration). The plants that showed antifungal activity were only selected for further work in solvent extraction.

Preparation of solvent extracts

Rhizome of *Decalepis hamiltonii* that recorded antifungal activity in aqueous extract that selected for the study. Thoroughly washed rhizome of *D. hamiltonii* was shade dried for 15 days and then ground into powder with Waring blender (Waring International, New Hartford, CT, USA). 50 g of the powder was filled in the thimble and extracted in a soxhlet extractor with 200 ml each of petroleum ether, benzene, chloroform, methanol and ethanol successively till colorless extract was obtained on the top of the extractor. Each of the solvent extract was concentrated separately under reduced pressure. After complete solvent evaporation, each of these solvent extract were weighed and preserved in brown airtight bottle until further use at 5 ^{o}C (Harborne, 1998).

Isolation of important phytopathogenic fungi from sorghum, maize and rice seeds

Four seed samples each of sorghum [*Sorghum bicolar* L. (Moench.)], maize (*Zea mays* L.) and rice *Oryzae sativa* (L.) were collected directly from farmers field (sample 1), regulated market (sample 2), warehouse (sample 3) and retail shops (sample 4) to isolate the important pathogenic fungi associated with seeds. The collected seed samples were subjected to SBM (ISTA, 1996). On the seventh day of incubation, samples were screened for seed mycoflora with the help of stereobinocular and compound microscopes. Associated fungi

were identified based on growth characteristic, mycelial morphology, spore morphology and other important characters using standard manuals. The fungi were frequently associated in higher percentage in sorghum, maize and rice which served as test fungi.

The selected ten species of *Aspergillus* and three species of *Penicillium* were subcultured using Malt extract-Salt- Agar (MESA) medium and eight species of *Fusarium*, two species of *Drechslera* and a species of *Alternaria* were subcultured using Czapek-Dox-Agar (CDA) medium. The selection of media is based on the standard recommendation for culturing these fungi.

Anti-fungal activity assay

Determination of percent mycelial inhibition by dry mycelial weight technique

The aqueous extracts of 8 plant species were amended to Richard's solution to achieve 10% concentration of the plant extract in the liquid medium. 50 ml of extract amended media which was taken in a 100 ml Erlenmeyer conical flask and sterilized at 121°C, 15 lb/inch^2 pressure for 15 minutes and allowed to cool. Richard's solution without any aqueous extract of test plants served as control. The flasks were inoculated with 5 mm diameter mycelia disc of *Fusarium solani* and *Aspergillus flavus* taken from 7 days old culture and incubated for 7 days at 22 ± 1 ^{o}C temperature under alternate cycles 12 h. light and 12 h. darkness. After incubation the content of the each flask were poured into a preweighed Whatman No. 1 filter paper. The filter paper with the mycelial mat was dried in an oven at 60 ^{o}C until a constant weight was reached. The dry weight of the mycelia was determined by subtracting the weight of the filter paper from the total weight of the filter paper with mycelia. Three replicates were maintained for each treatment (Kumar and Prasad, 1992). The percent inhibition of mycelial growth was calculated using the formula:- Percent inhibition = C – T / C X 100 where C = Mycelial weight in control and T = Mycelial weight in treatment. Data were subjected to statistical analysis and compared with Turkey HSD at 0.5 subset.

Determination of percent inhibition of spore germination by cavity slide method

Conidial suspension of *F. solani* and *A. flavus* were prepared in sterile distilled water and spore concentrations which adjusted to 1.0 X 10^4 spores/ml. 90 µl of conidial suspension, then placed in a cavity slide. The setup was

placed in large Petri dishes laden with moist blotter paper to maintain moisture level. 10 µl each of eight plant aqueous extracts were placed in separate conidial suspended cavity slide and mixed well. 10µl of distilled water placed in 90 ml of conidia suspended media served as control. The setup was incubated for 12 hours at 22 ± 1 ^{o}C. Germination of spore was counted under compound microscope (Singh and Tripathi, 1999). The percent of inhibition was calculated using the formula:- Percent inhibition = C – T / C X 100 where C = Number of spores germinated in control (Average of 10 microscopic field) T= Number of spores germinated in treated (Average of 10 microscopic field). Data were subjected to statistical analysis and compared with Turkey HSD at 0.5 subset.

Determination of mycelial inhibition by Poisoned food technique

Aqueous extract

All the tested plants (Table 1) were subjected to antifungal activity assay. CDA and MESA medium with 10 % aqueous extract of all eight plants were prepared and sterilized at 121°C, 15 lb/inch^2 pressure for 15 minutes. 15 ml of each media was separately poured into petriplates, allowed to cool and solidify. After complete solidification of the medium, 5mm disc of seven day old culture of the *F. solani* was inoculated in to CDA and *A. flavus* was inoculated in to MESA at the centre of the Petri dishes. The plates were incubated at 22 ± 1 ^{o}C for seven days. The Petri dishes containing media devoid of the extract but with same amount of distilled water served as control. After incubation the colony diameter was measured in mm (Singh and Tripathi, 1999). For each treatment was repeated four times. The fungitoxicity of the extract in terms of percentage inhibition of mycelial growth was calculated using the formula:- Percent inhibition = C - T / C X 100, where C = Average increase in mycelial growth in control plate and T = Average increase in mycelial growth in treatment plate.

The aqueous extract (10%) of *D. hamiltonii* which tested antifungal activity against *F. solani* and *A. flavus* was further subjected to poisoned food technique using aqueous extract of rhizome of young (Fresh and dry) and old (Fresh and dry) at 10% concentration to know the antifungal efficacy.

The aqueous extract of young rhizome of *D. hamiltonii* which tested the inhibition on mycelial growth of *F. solani* and *A. flavus* were further subjected to poisoned food techniques at 10, 20, 30, 40 and 50% concentrations against 24 phytopathogenic tested fungi. This selection was based on the significant antifungal activity of young rhizome of *D. hamiltonii* at 10% concentration.

Table 1. Test plants used for antifungal activity assay.

Name	Family	Plant part used
Argemone mexicana L.	Papaveraceae	Leaf
Caesalpinia coriaria (Jacq.) Willd.	Caesalpinaceae	Leaf
Decalepis hamiltonii Wight & Arn.	Asclepiadaceae	Rhizome
Euphorbia tirucalli L.	Euphorbiaceae	Leaf
Leucas aspera Spr.	Lamiaceae	Leaf
Phyllanthus amarus Schum and Thonn	Euphorbiaceae	Leaf
Tinospora cordifolia Miers.	Menispermaceae	Leaf
Tribulus terrestris L.	Zygophyllaceae	Leaf

Solvent extract

Five solvent extracts viz., petroleum ether, benzene, chloroform, methanol and ethanol which obtained from root of *D. hamiltonii* was subjected to antifungal activity assay by poisoned food technique. 2 g each of all solvent extract was dissolved in 2 ml of methanol and poured in to 1000 ml medium to achieve 2000 μg/ml concentration of the extract 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 placed at the center of the Petri dishes. Four replicates were maintained for each concentration. The 1000 ml media containing two ml of methanol served as control. The plates were incubated at 22 ± 1 ^{0}C for seven days (Singh and Tripathi, 1999). The diameter of the colony was measured and percent inhibition was calculated as follows:- Percent inhibition = C - T / C X 100, where C = average increase in mycelial growth in control plate and T = average increase in mycelial growth in treatment plate. The synthetic fungicides Blitox, Captan and Dithane M-45 at recommended dosage were tested for its antifungal activity by poisoned food technique, for comparison.

Results

Isolation of phytopathogenic fungi from sorghum, maize and rice seeds

The percent incidence of seed borne fungi associated with the four seed samples each of sorghum, maize and rice has been presented in Table 2. The data revealed that in sorghum seeds, species of *Aspergillus* (35-100%), *Curvularia* (27-40%), *Drechslera* (29-51%), *Fusarium* (32-82%), *Penicillium* (13-72%) and *Rhizopus* (32-40%) were presented in high percentage. In maize

seed samples, species of *Aspergillus* (62-100%), *Fusarium* (44-95%) and *Penicillium* (38-66%) were presented in higher percentage and in rice seed samples, species of *Alternaria* (42-58%) and *Drechslera* (12-40%) were presented at higher percentage. The highest incidence of *A. alternata* was observed in rice seed samples and higher incidence of *C. lunata*, *D. halodes* and *A. flavus* were observed in sorghum seed samples, while in maize seeds samples, *F. moniliforme*, *A. niger* and *A. flavus* were the predominant fungi. The incidence data revealed that percent incidence was more in sorghum seeds than maize and rice, respectively. The fungi were frequently associated in higher percentage in sorghum, maize and rice served as the tested fungi for further investigation on antifungal activity (Table 3).

Antifungal activity assay

Determination of mycelial inhibition by dry mycelial weight method

Result revealed that dry mycelial weight of the tested fungi grown in Richard's medium varied with different plant extracts. The percent inhibition of *F. solani* in *Caesalpinia coriaria, Decalepis hamiltonii, Euphorbia tirucalli* and *Leucas aspera* were 9.0%, 55.3%, 12.6% and 3.0% respectively, whereas other aqueous plant extracts did not show any antifungal activities (Table 4). The inhibition of *A. flavus* in *D. hamiltonii* and *E. tirucalli* were 73.0% and 2.3%, respectively. None of the other plant extracts showed any inhibitory activities against *A. flavus*.

Table 3. Seed borne phytopathegenic field and storage fungi isolated from grains which served as test fungi.

Name of fungi	Source
Fusarium equiseti	Sorghum seeds
F.graminearum	Maize seeds
F.lateritium	Paddy rice seeds
F.moniliforme	Sorghum seeds
F.oxysporum	Maize seeds
F.proliferatum	Maize seeds
F.semitectum	Sorghum seeds
F.solani	Maize seeds
Alternaria alternata	Paddy rice seeds
Drechslera halodes	Sorghum seeds
Drechslera tetramera	Paddy rice seeds
Aspergillus candidus	Maize seeds
A.columnaris	Maize seeds
A. flavipes	Sorghum seeds
A. flavus	Sorghum seeds
A. fumigatus	Sorghum seeds
A.niger	Paddy rice seeds
A.ochraceus	Sorghum seeds
A.tamari	Sorghum seeds
A.terreus	Sorghum seeds
A. versicolor	Sorghum seeds
Penicillium crysoginum	Sorghum seeds
P. griseofulvum	Maize seeds
P. oxalicum	Sorghum seeds

Determination of percent inhibition of spore germination by cavity slide method

Result revealed that highly significant percent inhibition of spore germination of *F. solani* and *A. flavus* was observed in *D. hamiltonii* (95.3 and 38.3%). The aqueous extracts of *C. coriaria*, *E. tirucalli* and *L. aspera* did not show significant activities, whereas aqueous extracts of *A. mexicana, P. amarus, T. cordifolia* and *T. terrestris* did not show any inhibitory activities (Table 4).

Table 4. Mycelial inhibition of *Fusarium solani* and *Aspergillus flavus* by aqueous extract of different plant species at 10% concentration.

Test plants	Percent mycelial inhibition					
	Dry Mycelial Weight method		Cavity slide method		Poison Food Technique	
	F. solani	***A. flavus***	***F. solani***	***A. flavus***	***F. solani***	***A. flavus***
Argemone mexicana	00.0±0.0 a	00.0±0.0 a	00.0±0.0 a	00.0±0.0 a	00.0±0.0a	00.0±0.0 a
Caesalpinia coriaria	9.0±0.5c	00.0±0.57b	10.0±0.57c	00.0±0.0 a	12.0±0.5c	03.6±0.5b
Decalepis hamiltonii	55.3±0.8e	73.0±0.57d	95.3±0.88e	38.3±0.88b	48.0±0.5e	42.5±0.8c
Euphorbia tirucalli	12.6±0.3d	2.3±0.8c	12.0±0.57d	00.0±0.0 a	15.3±0.3d	4.3±0.8b
Leucas aspera	03.0±0.0b	00.0±0.0 a	08.0±0.0b	00.0±0.0 a	05.0±0.4b	00.0±0.0 a
Phyllanthus amarus	00.0±0.0 a	00.0±0.0 a	00.0±0.0 a	00.0±0.0 a	00.0±0.0 a	00.0±0.0 a
Tinospora cordifolia	00.0±0.0 a	00.0±0.0 a	00.0±0.0 a	00.0±0.0 a	00.0±0.0 a	00.0±0.0 a
Tribulus terrestris	00.0±0.0 a	00.0±0.0 a	00.0±0.0 a	00.0±0.0 a	00.0±0.0 a	00.0±0.0 a

The value means of four replicates ± standard error. The values followed by different alphabets differ significantly when subjected to Tukey HSD at 0.5 subset.

Determination of mycelial inhibition by Poisoned food technique

Aqueous extract

The result revealed that highly significant percent inhibition (48%) of mycelial growth of *F. solani* was observed in CDA media amended with the extract of *D. hamiltonii* and moderate or low activity was observed in extract of *C. coriaria* (12%), *E. tirucalli* (15.3%) and *L. aspera* (5%). Highly significant mycelial growth inhibition of *A. flavus* was observed in MESA media amended with the extract of *D. hamiltonii* (42.5%). *E. tirucalli* (4.3%) and *C. coriaria* (3.6%) showed least activity (Table 4). The Tukey HSD analysis of the data revealed that aqueous extract of *D. hamiltonii* significantly inhibited the mycelial growth and spore germination of *F. solani* and *A. flavus*.

The antifungal activity of fresh and dried rhizome extracts of *D. hamiltonii* against *F. solani* and *A. flavus* are presented in Table 5. The results revealed that highly significant antifungal activity was observed in young fresh rhizome extract. Dried rhizome extract showed the least activity against both of tested fungi. The antifungal activity of aqueous extract of *D. hamiltonii* at different concentrations as determined by poisoned food technique on CDA media and MESA media are presented in Table 6 and 7. Percent inhibition of the tested fungi on CDA media revealed that *D. halodes* was highly susceptible and *F. lateritium* showed the least susceptible. The total inhibition of *D. halodes* was observed at 20% and that of *F. graminearum* was observed at 30% concentration. At 40% concentration, all the tested fungi except *F.*

lateritium and *F. moniliforme* were totally inhibited. 50% concentration inhibits all the test fungi.

Table 5. Inhibition of aqueous extract of fresh and dried rhizome of *Decalepis hamiltonii* at 10% concentration.

***D. hamiltonii* extract (10%)**	**Percent mycelial inhibition**	
	F. solani	***A. flavus***
Fresh young root extract	49.66±0.4d	42.86±0.32d
Fresh old root extract	37.76±0.21c	34.45±0.27c
Dried young root extract	09.26±0.54b	04.25±0.32b
Dried old root extract	03.43±0.27a	01.50±0.50a

The value means of four replicates ± standard error. The values followed by different alphabets differ significantly when subjected to Tukey HSD (colum by colum analysis) at 0.5 subset.

Table 6. Antifungal activity of aqueous extract from *Decalepis hamiltonii* at different concentrations against phytopathogenic field fungi.

Phytopathogenic field fungi	**Percent mycelial inhibition**					**F-value**
	Concentrations (%)					
	10	**20**	**30**	**40**	**50**	
Alternaria alternata	29.92±0.44 a	63.50±0.49 b	84.83±0.77 c	100.00±0.00 d	100.00±0.00 d	4226.95
Drechslera halodes	87.48±0.49 a	100.00±0.00 b	100.00±0.00b	100.00±0.00 b	100.00±0.00 b	633.79
D. tetramera	49.43±0.43 a	97.91±0.43 b	100.00±0.00 c	100.00±0.00c	100.00±0.00c	335.73
Fusarium equiseti	4.58±0.49a	45.87±0.64 b	91.12±0.75 c	100.00±0.00d	100.00±0.00 d	7368.74
F. graminearum	87.01±0.59 a	92.75±0.48 b	100.00±0.00 c	100.00±0.00c	100.00±0.00c	294.49
F. lateritium	4.67±0.46 a	25.82±0.42 b	40.93±0.52 c	95.34±0.53d	100.00±0.00e	9343.30
F. moniliforme	83.03±0.34 a	87.79±0.34 b	93.80±0.48 c	98.91±0.59 d	100.00±0.00 d	318.62
F. oxysporum	44.68±0.48 a	62.28±0.60 b	75.53±0.50 c	100.00±0.00 d	100.00±0.00 d	3381.56
F. proliferatum	61.41±0.45 a	74.89±0.47 b	85.92±0.72 c	100.00±0.00 d	100.00±0.00 d	1415.69
F. semitectum	11.89±0.44 a	42.10±0.39 b	55.39±0.48 c	100.00±0.00 d	100.00±0.00 d	12554.85
F. solani	48.20±0.52 a	66.20±0.62 b	96.41±1.00 c	100.00±0.00 d	100.00±0.00 d	1674.95

The value means of four replicates ± standard error. The values followed by different alphabets differ significantly when subjected to Tukey HSD (row by row analysis) at 0.5 subset.

The percent inhibition of different storage fungi on MESA media revealed that *P. chrysogenum* was highly susceptible and *A. tamari* was least susceptible. At 40% concentration, all tested fungi were totally inhibited.

Table 7. Antifungal activity of aqueous extract of *Decalepis hamiltonii* at different concentrations against phytopathogenic storage fungi.

Phytopathogenic storage fungi	Percent mycelial inhibition					F-value
	Concentrations (%)					
	10	20	30	40	50	
Aspergillus candidus	57.77±0.90a	85.56±1.00b	100.00±0.00c	100.00±0.00c	100.00±0.00c	335.73
A. columnaris	33.20±0.43a	44.63±0.39b	100.00±0.00c	100.00±0.00 c	100.00±0.00c	16579.94
A. flavipes	10.90±0.72a	42.18±0.7 b	95.19±0.41c	100.00±0.00d	100.00±0.00d	8690.42
A. flavus	42.24±0.41a	86.02±0.53b	92.85±0.58c	100.00±0.00d	100.00±0.00d	3624.30
A. fumigatus	64.96±0.55a	100.00±0.00b	100.00±0.00b	100.00±0.00b	100.00±0.00b	3929.95
A. niger	32.00±0.52a	57.21±0.42b	74.83±0.80c	100.00±0.00d	100.00±0.00d	3687.35
A. ochraceus	22.82±0.60a	96.75±0.56b	100.00±0.00c	100.00±0.00c	100.00±0.00c	8659.64
A. tamari	24.70±0.61a	38.32±0.30b	60.81±0.56c	100.00±0.00d	100.00±0.00d	7528.94
A. terreus	30.65±0.04a	55.48±1.0 b	100.00±0.00c	100.00±0.00c	100.00±0.00c	1888.93
A. versicolor	62.24±0.40a	95.11±0.70 b	100.00±0.00c	100.00±0.00c	100.00±0.00c	2034.35
Penicillium chrysogenum	100.00±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a	620.68
P. griseofulvum	34.02±0.72a	75.12±0.75b	100.00±0.00c	100.00±0.00c	100.00±0.00c	3819.62
P. oxalicum	31.65±0.16a	72.63±0.9 b	100.00±0.00c	100.00±0.00c	100.00±0.00c	2239.56

The value means of four replicates ± standard error. The values followed by different alphabets differ significantly when subjected to Tukey HSD (row by row analysis) at 0.5 subset.

Solvent extraction

After solvent evaporation, the yield of different extracts from petroleum ether, benzene, chloroform, methanol and ethanol solvents were 1.29 g, 0.80 g, 0.30 g, 2.80 g and 1.80 g, respectively. The percent mycelial growth inhibition of different solvent extracts on tested fungi are presented in Table 8 and 9, respectively. Highly significant activity of field fungi was observed in petroleum ether extract. Benzene and chloroform extract also showed moderate and least activity respectively against field fungi, whereas no activity was

observed in methanol and ethanol extracts. Among the field fungi *F. moniliforme* was highly susceptible in petroleum ether extract and *D. halodes* was least susceptible at 2000 µg/ml concentration. Moreover, the highly significant activity of storage fungi was observed in petroleum ether extract, whereas no activity was observed against storage fungi in chloroform, methanol and ethanol extracts. Slight activity against storage fungi was observed in benzene extract. Among the storage fungi *P. chrysogenum* was highly susceptible in petroleum ether extract and *P. griseofulvum* was least susceptible at 2000 µg/ml concentration. Among the three synthetic fungicides Dithane M-45 showed least activity against both field and storage fungi (Table 10 and 11). The antifungal activity of aqueous and petroleum ether extract of *D. hamiltonii* showed better activity than tested synthetic fungicides tested.

Table 10. Antifungal activity assay of some synthetic fungicides against seed borne phytopathogenic field fungi.

Phytopathogenic field fungi	Percent mycelial inhibition		
	Blitox	**Captan**	**Dithane M-45**
Alternaria alternata	72.46±0.58	91.48±0.35	77.19±0.21
Drechslera halodes	83.25±0.46	100±0.00	97.15±0.39
D. tetramera	84.37±0.33	98.63±0.42	97.76±0.60
Fusarium equiseti	95.55±0.36	81.85±0.32	76.07±0.61
F. graminearum	88.66±0.26	73.35±0.26	73.91±0.74
F. lateritium	42.88±0.25	63.48±0.27	48.90±0.37
F. moniliforme	97.24±0.45	78.45±0.72	65.84±0.85
F. oxysporum	85.69±0.64	68.68±0.48	68.18±0.18
F. proliferatum	74.38±0.27	68.66±0.25	76.09±0.45
F. semitectum	78.38±0.12	95.59±0.03	91.47±0.18
F. solani	72.80±0.25	72.70±0.26	65.32±0.30

Date given are mean of four replicates ± standard error.

Discussion

The plants world is a rich storehouse of natural chemicals that could be exploited for use as pesticides. The total number of plant chemicals may exceed 4,000,000 and of these 10,000 are reported to be found secondary metabolites whose play a 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 constituents 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 activity. 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 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 (Varma and Dubey, 1999; Gottlieb *et al*., 2002).

Table 11. Antifungal activity assay of some synthetic fungicides against seed borne phytopathogenic storage fungi.

Phytopathogenic storage fungi	Percent mycelial inhibition		
	Blitox	**Captan**	**Dithane M-45**
Aspergillus candidus	100±0.00	100±0.00	47.89±0.31
A. columnaris	86.90±0.22	87.67±0.31	44.90±0.40
A. flavipes	92.35±0.30	88.87±0.19	24.68±0.13
A. flavus	97.03±0.37	91.98±0.14	54.17±0.15
A. fumigatus	100±0.00	92.98±0.37	16.52±0.40
A. niger	100±0.00	96.45±0.14	92.75±0.31
A. ochraceus	94.64±0.14	93.85±0.66	86.82±0.44
A. tamari	73.53±0.28	87.69±0.13	22.45±0.50
A. terreus	95.73±0.27	87.16±0.51	45.87±0.35
A. versicolor	93.40±0.20	83.86±0.43	13.41±0.36
Penicillium chrysogenum	100±0.00	100±0.00	53.45±0.39
P. griseofulvum	92.91±0.38	92.25±0.15	51.98±0.38
P. oxalicum	65.94±0.32	82.60±0.85	53.85±0.20

Date given are mean of four replicates ± standard error.

Considering these as a first step in the present investigation, eight plants were screened in vitro for antifungal activities against two important phytopathogenic fungi *F. solani* and *A. flavus*. These plants were selected based on traditional medicine knowledge and random choosing from the local flora. The screening revealed that only *D. hamiltonii* was effective for inhibition of mycelial growth and fungal spore germination as demonstrated by dry mycelial weight, spore germination and poisoned food technique at 10% concentration. The antifungal activity of young and old (fresh and dry) rhizome extract of *D. hamiltonii* revealed that fresh young rhizome aqueous extract

showed more activity suggesting that the active compound is in higher concentration in young fresh rhizome and that the active compound is soluble in water.

The *in-vitro* evaluation for antifungal activity of aqueous extracts of *D. hamiltonii* at different concentrations revealed that 50 % concentration completely inhibited all tested phytopathogenic fungi. The antifungal activity assay of the five solvent extracts of *D. hamiltonii* revealed that petroleum ether extract showed the highest antifungal activity, suggesting that the active compound is better extracted with petroleum ether than the other solvents and that it is one of potent solvent for isolation of the bioactive compound responsible for antifungal activity. Hence in all the further investigations, petroleum ether is exploited for isolation of the active compound. The analysis of antifungal activity of aqueous and petroleum ether extract of *D. hamiltonii* suggests that it shows broad-spectrum anti-fungal activity. Therefore aqueous extract of *D. hamiltonii* was only used to test the efficacy in controlling seed mycoflora viz., sorghum, maize and rice.

D. hamiltonii is an edible plant largely used in South India for pickling along with curds or lime juice (CSIR, 1952). The antimicrobial activity of *D. hamiltonii* against human pathogenic bacteria and food borne fungi has been demonstrated by Thangadurai *et al*. (2002), Elizabeth *et al*. (2005). Thangadurai *et al*., 2002 are mainly evaluated the essential oil constituent of *D. hamiltonii* against food borne pathogens. Even though Elizabeth *et al*. (2005) have demonstrated the antimicrobial activity against human pathogens the solvent used for the preparation of the extract is not known. In the present investigation the antifungal potential of both aqueous extract and petroleum ether solvent extract has been demonstrated that 50% concentration of aqueous extract totally inhibited both storage and field fungi. The broad spectrum antifungal activity of the petroleum ether extract of *D. hamiltonii* against phytopathogenic fungi has been demonstrated for the first time in the present investigation. None of the earlier reports (Phadke *et al*., 1994; George *et al*., 1999a; George *et al*., 1999b; Thangadurai *et al*., 2002; Elizabeth *et al*., 2005) have demonstrated the antifungal activity against the wide range of phytopathogenic field and storage known to cause a variety of diseases in sorghum, maize and rice which are important food and fodder crops of the country.

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. *D. hamiltonii* being an edible plant (non toxic to human and easily biodegradable) possessing significant broad spectrum antifungal activity against important field and storage fungi would probably be

an important candidate plant for prevention of biodeterioration of grains during storage and prevention of spoilage of processed food product.

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(Received 31 May 2007; accepted 20 May 2008)

Table 2. Percent incidence of fungi associated in sorghum, maize and paddy seed samples.

Fungi	Sorghum				Maize				Paddy rice			
	Sample I	Sample II	Sample III	Sample IV	Sample I	Sample II	Sample III	Sample IV	Sample I	Sample II	Sample III	Sample IV
Alternaria alternata	36	12	20	38	7	14	20	10	58	46	56	42
Aspergillus candidus	-	2	-	-	12	3	6	8	-	-	-	-
A. columnaris	-	3	5	-	8	6	5	6	-	2	-	3
A. flavipes	-	4	-	-	-	2	-	5	-	-	-	-
A. flavus	18	36	32	12	38	28	22	33	17	25	19	29
A. fumigatus	-	2	4	-	8	4	2	6	-	-	-	-
A. niger	14	22	18	12	39	29	16	34	10	14	9	18
A. ochraceus	12	18	17	10	8	-	-	-	-	-	-	4
A. tamari	8	4	2	1	18	4	3	12	2	6	5	8
A. terreus	-	2	-	-	5	6	3	4	-	-	-	-
A. versicolor	6	12	8	-	-	-	-	-	-	-	-	-
Chaetomium globosum	16	8	13	22	-	-	-	-	-	4	2	10
Cladosporium cladosporioides	18	8	4	15	-	8	12	4	-	3	-	2
Curvularia lunata	40	29	32	27	-	8	12	-	-	2	-	1
Drechslera halodes	48	32	29	40	-	-	-	-	11	8	15	5
D. tetramera	3	1	-	8	-	-	3	-	40	21	38	12
Fusarium equiseti	6	-	-	7	-	-	-	-	8	-	5	-
F. graminearum	8	1	3	14	5	15	22	8	-	-	-	-
F. lateritium	-	-	-	-	-	-	-	-	6	4	2	3
F. moniliforme	18	14	13	25	24	28	32	22	8	4	9	-
F. oxysporum	8	3	2	10	5	6	8	4	-	-	-	-
F. proliferatum	-	-	-	-	4	12	17	8	-	-	-	-
F. semitectum	12	6	12	14	-	-	-	-	-	-	-	-
F. solani	12	8	10	15	6	13	25	8	-	-	-	-
Penicillium crysoginum	8	26	19	4	8	-	-	5	-	-	-	-
Penicillium sp.	12	46	42	9	58	39	38	46	4	19	8	22
Nigrospora oryzae	-	2	1	0	4	-	-	-	-	-	-	-
Phoma sp.	5	8	7	3	-	-	-	-	-	-	-	-
Rhizopus sp.	32	40	36	37	18	38	38	27	12	18	16	22
Trichothecium roseum	4	3	-	-	5	3	2	1	5	3	1	-

Percent incidence is based on four replicates of 100 seeds each.

Table 8. Antifungal activity of different solvent extract from *Decalepis hamiltonii* at 2000 µg/ml concentration against phytopathogenic field fungi.

Phytopathogenic field fungi	Percent mycelial inhibition						F- value
	Methanol control	Petroleum ether extract	Benzene extract	Chloroform extract	Methanol extract	Ethanol extract	
Alternaria alternata	0.46± 0.29b	36.87±0.48e	12.00± 0.57d	0.46± 0.25c	0.00±0.00a	0.00±0.00a	1769.58
Drechslera halodes	0.80± 0.38b	25.27±0.56e	9.33± 0.57d	1.32± 0.23c	0.00±0.00a	0.00±0.00a	5789.43
D. tetramera	0.00±0.00a	42.53±0.38d	15.66 ±0.33c	0.23± 0.23b	0.00±0.00a	0.00±0.00a	5342.30
Fusarium equiseti	0.20±0.18b	44.74±0.19e	10.00± 0.57d	0.51± 0.23c	0.00±0.00a	0.00±0.00a	2114.16
F. graminearum	0.36± 0.23b	59.53±0.29e	11.00± 0.33d	0.40± 0.26c	0.00±0.00a	0.00±0.00a	1078.25
F. lateritium	0.00±0.00a	28.86±0.46d	10.57± 0.57c	0.68± 0.33b	0.00±0.00a	0.00±0.00a	4162.15
F. moniliforme	0.36± 0.28b	82.90±0.25e	9.66± 0.57d	0.63± 0.29c	0.00±0.00a	0.00±0.00a	1294.55
F. oxysporum	0.00±0.00a	35.08±0.40d	9.00± 0.57c	0.41± 0.27b	0.00±0.00a	0.00±0.00a	4650.43
F. proliferatum	0.46± 0.29b	50.43±0.49e	12.00± 0.57d	1.39± 0.48c	0.00±0.00a	0.00±0.00a	2435.25
F. semitectum	0.80± 0.38b	41.92±0.99e	12.33± 0.57d	0.74± 0.37c	0.00±0.00a	0.00±0.00a	3137.56
F. solani	0.39± 0.24b	41.04±0.29e	8.66 ±0.33d	0.37± 0.23c	0.00±0.00a	0.00±0.00a	4170.42

Date given are mean of four replicates ± standard error. The means with different alphabets are significantly different with each other as indicated by Tukey HSD (row by row analysis) at 0.5 subset.

Table 9. Antifungal activity of different solvent extract from *Decalepis hamiltonii* at 2000 µg/ml concentration against phytopathogenic storage fungi.

Phytopathogenic storage fungi	Percent mycelial inhibition						F- value
	Methanol control	Petroleum ether extract	Benzene extract	Chloroform extract	Methanol extract	Ethanol extract	
Aspergillus candidus	0.000±0.00a	58.07±0.5c	1.20±0.16b	0.00±0.00a	0.00±0.00a	0.00±0.00a	1123.51
A. columnaris	0.000±0.00a	32.96±0.48c	0.36± 0.30b	0.00±0.00a	0.00±0.00a	0.00±0.00a	945.23
A. flavipes	0.000±0.00a	44.85±0.44c	2.00±0.520b	0.00±0.00a	0.00±0.00a	0.00±0.00a	678.78
A. flavus	0.000±0.00a	37.67±0.46c	4.36± 0.23b	0.00±0.00a	0.00±0.00a	0.00±0.00a	1448.68
A. fumigatus	0.000±0.00a	49.09±0.76c	3.66± 0.30b	0.00±0.00a	0.00±0.00a	0.00±0.00a	1345.78
A. niger	0.000±0.00a	29.46±0.58c	0.46± 0.27b	0.00±0.00a	0.00±0.00a	0.00±0.00a	1423.00
A. ochraceus	0.000±0.00a	31.49±0.35c	5.80± 0.89b	0.00±0.00a	0.00±0.00a	0.00±0.00a	858.22
A. tamari	0.000±0.00a	26.66±0.53c	4.39± 0.20b	0.00±0.00a	0.00±0.00a	0.00±0.00a	1324.67
A. terreus	0.000±0.00a	40.49±0.28c	6.46± 0.29b	0.00±0.00a	0.00±0.00a	0.00±0.00a	1243.56
A. versicolor	0.000±0.00a	43.81±0.59c	4.80± 0.38b	0.00±0.00a	0.00±0.00a	0.00±0.00a	1152.12
Penicillium chrysogenum	0.000±0.00a	53.79±0.92c	7.39± 0.20b	0.00±0.00a	0.00±0.00a	0.00±0.00a	984.34
P. griseofulvum	0.000±0.00a	29.29±0.36b	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	1456.15
P. oxalicum	0.000±0.00a	40.76±0.67b	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	1045.56

Date given are mean of four replicates ± standard error. The means with different alphabets are significantly different with each other as indicated by Tukey HSD (row by row analysis) at 0.5 subset.