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Antimycotic, Antibiodeteriorative and Antiaflatoxigenic Potency of 2-hydroxy-4-methoxybenzaldehyde Isolated from *Decalepis hamiltonii* on Fungi Causing Biodeterioration of Maize and Sorghum Grains

D. C. Mohana[1&2] and K. A. Raveesha[2]

[1]Agricultural Microbiology Laboratory, Department of Microbiology, Bangalore University, Jnana Bharathi Campus, Bangalore 560 056 India; and [2]Agricultural Microbiology Laboratory, Department of Studies in Botany, University of Mysore, Manasagangotri, Mysore, India. E-mail: mohanadc@gmail.com

Abstract

We characterized the antimycotic, antibiodeteriorative and antiaflatoxigenic efficacy of the compound, 2-hydroxy-4-methoxybenzaldehyde isolated from *Decalepis hamiltonii* Wight & Arn, against different species of fungi that cause biodeterioration of maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L.) grains during storage. Fungal species that cause biodeterioration were isolated from maize and sorghum grains by agar plating method and standard blotter method (SBM). Poisoned food technique was adopted to assess fungitoxicity of the compound against fungal isolates. For different fungal species, the inhibitory concentration (IC_{50}), minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) of 2-hydroxy-4-methoxybenzaldehyde varied between 80 to 350µg/ml, 350 to 800µg/ml and 450 to 850µg/ml, respectively. Among the 14 fungal species tested, *Aspergillus niger*, *A. columnaris* and *A. tamari* were completely inhibited at higher concentrations, while *Fusarium oxysporum*, *F. proliferatum*, *Drechslera tetramera* and *Aspergillus ochraceus* were completely inhibited at low concentrations. The inhibitory effect of the compound was of broad-spectrum in activity and was concentration-dependent. Comparative evaluation of the active compound with the synthetic fungicides, Blitox and Thiram, at recommended dosages revealed that the antimycotic activity of 2-hydroxy-4-methoxybenzaldehyde was superior than that of synthetic fungicides. In vivo evaluation of the compound, 2-hydroxy-4-methoxybenzaldehyde at 0.5 g/kg as seed treatment on maize and sorghum revealed that carbohydrates, water soluble proteins, lipids, aflatoxin B_1 production and dry matter losses (DML) were significantly conserved in treated compared with control untreated grains up to 120 days storage. *D. hamiltonii* being an edible plant can be exploited in the management of seed-borne pathogenic fungi and in the prevention of biodeterioration of grains and mycotoxin production during storage in an eco-friendly way.

Key words: *Decalepis hamiltonii*, 2-hydroxy-4-methoxybenzaldehyde, antimycotic activity, seed treatment, biodeterioration, aflatoxin B_1.

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Fungi are significant destroyers of foodstuffs and cereal grains during pre- and post-harvest processing and storage (Agrios 1997). Most of the fungi are seed-borne, opportunistic and ubiquitous, causing significant deterioration; thus they are responsible for considerable economic loss (Marin et al 1999; Park et al 2004; Chandler 2005; Mohana et al 2008). Fungal infections alter the chemical and nutritional characteristics of grains and seeds and, more importantly, contaminate the remaining grains with mycotoxins (Miller 1995; Agrawal 1999; Reddy 2004; Mohana et al 2009). 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 (Galvão et al 2001; Domijan et al 2005). A sizable portion of the world population living below the poverty line in the developing and underdeveloped countries of Asia and Africa are suffering from health problems, associated with consuming mould and mycotoxin contaminated grains and cereals (Rocha et al 2005). Aflatoxin is one of the most common and dangerous mycotoxins produced by *Aspergillus flavus* during biodeterioration (Janardhana et al 1999).

Maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L.) are the two important cereal crops cultivated

as major rain fed crops in most of the semiarid regions. The susceptibility of maize and sorghum grains to various fungi has been well documented (Miller 1995; Janardhana et al 1998, 1999; Desjardins et al 2000; Reddy 2004; Domijan et al 2005; Mohana et al 2008). Seed treatment is the safest and the cheapest method to control seed-borne fungal diseases and to prevent biodeterioration of grains (Sagar and Sugha 2004; Chandler 2005; Bagga and Sharma 2006). Chemical fungicides are being used in the form of dusting, slurry and soaking treatment (Ghasolia and Jain 2004; Singh and Singh 2005). Even though the use synthetic chemical fungicides can achieve effective and efficient control of seed-borne fungi, but fungicides are not used to prevent biodeterioration of consumable grains for reasons of acute toxicity to the consumer (Harris et al 2001). Many reports have revealed that chemical fungicides cause serious environmental problems and are toxic to non-target organisms (Anonymous 2005). The toxic effect of synthetic chemicals can be overcome, only by persistent search for new and safer pesticides accompanied by wide use of pest control methods, which are eco-friendly and effective (Newman et al 2000; Raskin et al 2002).

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; Gottlieb et al 2002). Extracts of many higher plants have been reported to exhibit antifungal properties under laboratory trails (Parekh et al 2006; Aliero et al 2006; Buwa and Staden, 2006; Ergene et al 2006 and Shukla et al 2008). Exploitation of plant metabolites in crop protection and prevention of biodeterioration caused by fungi appear to be promising (Varma and Dubey 1999). In view of these, a large number of edible plants are routinely screened in our laboratory for antifungal properties. *D. hamiltonii* (Asclepiadaceae), an edible plant (Anonymous 1952), showed highly significant antifungal activity in vitro against many storage fungi. The active compound responsible for antifungal activity was 2- hydroxy-4-methoxybenzaldehyde (Mohana et al 2008). The present study evaluates antimycotic, antibiodeteriorative and antiaflatoxigenic potency of the compound, 2-hydroxy-4-methoxybenzaldehyde on mould induced biodeterioration of maize and sorghum grains in storage.

Materials and Methods

Isolation and identification of 2-hydroxy-4-methoxybenzaldehyde from rhizome of *Decalepis hamiltonii*. Fresh rhizome of *Decalepis hamiltonii* (Fig. 1) free from diseases were collected and washed thoroughly 2-3 times with running tap water and once with sterile water, shade dried, powdered and used for compound extraction. The bioactive compound was isolated by activity guided assay of phenolic fraction of petroleum ether extract of rhizome of *D. hamiltonii* by TLC (Mohana et al 2008). The pure active compound (band-5 with R_f value 0.77) was dissolved in chloroform and subjected to ^1H NMR (hydrogen nuclear magnetic resonance) at 300.1315MHz, ^{13}C NMR (carbon nuclear magnetic resonance) at 75.4734MDz and mass spectral analysis (MASPEC system [msw/9629]) to confirm the identity of the compound. The pure identified active compound was used for antimycotic, antibiodeteriorative and anti-aflatoxigenic assay.

Isolation and identification of seed-borne fungi from maize and sorghum samples. A total of 10 seed samples each of different cultivars of maize and sorghum seeds (approximately 1kg) were collected from markets, local stores, agricultural co-operatives and farm fields of different agro-climatic regions of Karnataka during the harvest seasons of 2005-2007. Samples were brought to the laboratory in sterile plastic bags and kept at 4C. All the samples were plated on blotter for testing by standard blotter method (SBM) and Czapek-Dox-agar (CDA) to isolate frequently occurring important seed-borne pathogenic fungi associated with these seeds (ISTA 1996). The fungal colonies expressed on maize and sorghum grains were identified up to the species level by using fungal keys (Booth 1977; Leslie and Summerell 2006; Nagamani et al 2006). In maize grains, *Aspergillus columnaris*, *A. niger*, *Fusarium moniliforme*, *F. oxysporum*, *F. proliferatum*, *F. verticillioides*, *Penicillium griseofulvum* were frequently associated at higher levels. In sorghum grains, *Alternaria alternata*, *Aspergillus flavus*, *A. fumigatus*, *A. ochraceus*, *A. tamari*, *A. versicolor* and *Drechslera tetramera* were frequently associated at higher levels. They were selected as test fungi for antimycotic activity assay.

Antimycotic activity assay of 2-hydroxy-4-methoxybenzaldehyde. The pure active compound, 2-hydroxy-4-methoxybenzaldehyde was subjected to antimycotic activity assay by poisoned food technique (Singh and Tripathi 1999). It was added to the medium to achieve the desired different concentrations in the medium, autoclaved, poured into Petri dishes (20 ml each) and allowed to cool. Five mm disc of 7-d-old culture of test fungi were inoculated. Four replicates were maintained for each concentration. Dishes containing media devoid of the compound served as control. The plates were incubated at 28±1C for 7 d. The fungitoxicity of the bioactive compound in terms of percentage inhibition of mycelial growth was calculated by using the formula: inhibition (%) = dc – dt X 100/dc, where dc= avg increase in mycelial growth in control, dt = avg increase in mycelial growth in treatment (Singh

and Tripathi 1999). Fungi-static or fungicidal concentration of the compound was determined by following the procedure of Mishra and Dubey (1994). The complete inhibited fungal discs were reinoculated in Petri dishes containing CDA media devoid of the compound. The plates were observed for fungal growth. The fungal growth if any on 6 d of incubation indicated fungistatic concentration while its absence denoted fungicidal concentration. The synthetic fungicides blitox and thiram, which are commonly used for seed treatment were obtained from Mysore agrochemical market. They were tested at their recommended dosage (2000 µg/ml) for antifungal activity by poisoned food technique for comparison.

In vivo effect of 2-hydroxy-4-methoxybenzaldehyde on fungi. Freshly harvested, popular and locally available maize (Deccon 105) and sorghum (CSH-5) seeds which recorded higher incidence of natural fungal infestation with diverse species of seed-borne fungi were selected for the study. Seed moisture content of the sample was maintained at 18%, above safe storage limit of 13% by the following formula: $W = A(b-a)/(100-b)$, where W is the volume of water required (ml), A is the initial weight of the sample (g), a is the initial moisture content (%) and b is the required moisture content (%).

The seeds were treated with three different concentrations (0.5, 1 and 1.5 g/kg) of the antifungal active compound by following the procedures of slurry treatment (Ghasolia and Jain 2004). Seeds without the active compound served as control. The treated and un-treated seeds were stored in polythene bags and conical flask at 20C for 120 d in separate sets of 500 g per each treatment in quadruplets. Sample (100g) was drawn at regular intervals of 30 d and subjected to mycological analysis (ISTA 1996). Total carbohydrates content was determined by phenol sulphuric acid method (Dubois et al 1956). The total proteins content was determined by Folin-phenol reagent method (Lowry et al 1951). The total crude lipid content was determined by following the procedure of Fabbri et al (1980), dry matter losses by hot air oven method (Reed 1987) and aflatoxin B_1 content was determined by following procedure of Kumar and Prasad (1992). The quantification of aflatoxin B_1 was done by visual comparison with standard. The authentic standard aflatoxin B_1 was obtained from National institute of public health and environment, post box 1, 3720BA, Bilthoven, Netherlands.

Phytotoxicity assay. Seed samples of maize (Deccon 105) and sorghum (CSH-5) which recorded higher incidence of natural fungal infestation with diverse species of seed-borne fungi were selected for the study. Three different concentrations viz., 500, 1000 and 1500 µg/ml of 2-hydroxy-4-methoxybenzaldehyde were prepared using sterile distilled water as dilutant. Sterile distilled water treated seeds served as control. The treatment involves soaking of maize and sorghum seeds in each of these concentrations for 4, 8 and 12 h periods. The seeds treated with water devoid of the compound served as control. The treated seeds were shade dried on blotter sheet for 12 h and later subjected to SBM for mycological analysis and also sown in plastic pot containing sterile sand soil mixture at the rate of six maize seeds/plate and 15 sorghum seeds/plate. After 7 d incubation, seed mycoflora and seedling vigour were determined by following procedure of Agrawal (1999).

Results

Isolation and identification of compound from rhizome of *D. hamiltonii*. The TLC separation of phenolic fraction showed seven bands (R_f values 0.05, 0.14, 0.23, 0.41, 0.77, 0.87 and 0.98). Band five at R_f 0.77 of phenolic fraction separated by chloroform as eluting solvent showed significant antimycotic activity. The ^{1}H NMR analysis of the compounds showed [d 3.85 (s,- OCH3), 6.52 (dd, J=2 HZ; 3-H), 6.55 (d, J=7 H_Z, 5-H), 7.40 (d, J=7 H_Z; 6-H), 9.70 (s, CHO),11.6 (s; -OH) functional groups. ^{13}C NMR analysis of the compounds showed eight carbon signals 135.6(1-CH), 108.7(3-CH), 167.2(C of carbonyl), 101.05(5-CH), 164.8(2-C), 115.5(C), 194.7(6-CH), and 56.09(CH_3) and its identity confirmed by the mass spectral analysis [m/z(% abundance): 57(48), 95(46), 108(24), 121(20), 151(100), 152(70). The strong molecular ion peak (m/z, 152) and stronger M-1 ion peak (m/z, 151) observed were characteristic of aromatic aldehyde. The melting point of this compound is 46C. These results revealed that the compound was 2-hydroxy-4-methoxybenzaldehyde reported in literature (Mohana et al 2008).

Antimycotic activity assay against seed-borne phytopathogenic fungi. The inhibitory activities of the active compound 2-hydroxy-4-methoxybenzaldehyde on mycelium of 14 phytopathogenic fungi were estimated (Table 1). The inhibitory concentration required to produce 50% growth inhibition (IC_{50}), minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) value of the compound 2-hydroxy-4-methoxybenzaldehyde were also estimated (Table 2). Tukey-HSD analysis of data revealed that, the inhibitory effect of the compound 2-hydroxy-4-methoxybenzaldehyde was broad-spectrum in its activity and was concentration-dependant. The highest inhibitory activity was observed in *Fusarium oxysporum*, followed by *Fusarium proliferatum*, *Drechslera tetramera* and *Aspergillus ochraceus*. The lowest inhibitory activity was observed in *Aspergillus niger*, *A. columnaris* and *A. tamari*.

Table 1. Antifungal activity of 2-hydroxy-4-methoxybenzaldehyde isolated from *D. hamiltonii* against phytopathogenic fungi isolated from maize and sorghum grains

Pathogenic fungi	100	300	600	800	B	T
Alternaria alternata	16	60	100	100	75	100
*Aspergillus flavus**	22	56	100	100	86	100
A. fumigatus	30	67	100	100	91	100
A. ochraceus	21	87	100	100	89	100
A. versicolor	27	65	100	100	95	100
A. columnaris	17	42	85	100	92	100
A. niger	11	27	84	100	77	100
A. tamari	38	56	94	100	86	100
Drechslera tetramera	25	86	100	100	86	100
Fusarium moniliforme	48	81	100	100	81	100
F. oxysporum	62	96	100	100	91	100
F. proliferatum	53	86	100	100	89	100
F. verticillioides#	49	82	100	100	82	100
Penicillium griseofulvum	16	70	100	100	93	69
CD (*P=0.05*)	33	40.8	11.7	0	12.5	17.2
CV (%)	1	1.5	1.2	0	1.4	1.1

.B = Blitox (2000µg/ml); T = Thiram (2000µg/ml); *Aflatoxin B1 producing strain, #Fumonisin producing strain.

The IC_{50}, MIC and MFC value of the compound, 2-hydroxy-4-methoxybenzaldehyde, against *Fusarium oxysporum* was 80, 350 and 450µg/ml, respectively. The IC_{50}, MIC and MFC values of 2-hydroxy-4-methoxybenzaldehyde against aflatoxin B_1 producing strain *Aspergillus flavus* were 200, 500 and 650 µg/ml, respectively.

The IC_{50}, MIC and MFC values of 2-hydroxy-4-methoxybenzaldehyde against *Aspergillus fumigatus* were 150, 450 and 500 µg/ ml, respectively. Similarly IC_{50}, MIC and MFC values of 2-hydroxy-4-methoxybenzaldehyde against fumonisin producing strain *F. verticillioides* were 100, 500 and 600 µg/ml, respectively. *A. niger* were totally inhibited at higher concentrations and IC_{50}, MIC and MFC values of the compound were 350, 800 and 950 µg/ml, respectively. *Penicillium griseofulvum* was not completely inhibited in any of the synthetic fungicides but 2-hydroxy-4-methoxybenzaldehyde totally inhibited its growth. The IC_{50}, MIC and MFC values of the compound 2-hydroxy-4-methoxybenzaldehyde against *P. griseofulvum* were 200, 550 and 650 µg/ml, respectively. The inhibitory activity on mycelium of, the two synthetic fungicides against 14 phytopathogenic fungi (Table 1) revealed that thiram completely inhibited the fungal growth of all test fungi except *P. griseofulvum*.

Effect of 2-hydroxy-4-methoxybenzaldehyde on maize and sorghum grains during storage. The percent incidence of different fungi in untreated, active compound 2-hydroxy-4-methoxybenzaldehyde (0.5, 1 and1.5 g/kg) treated maize and sorghum seeds were estimated (Table 3). In un-treated seeds, species of *Alternaria*, *Aspergillus*, *Chaetomium*, *Curvularia*, *Drechslera*, *Fusarium*, *Penicillium*, *Rhizopus* and *Trichothecium* which were present in higherlevels with increasing storage period. The fungal incidence gradually increased both in diversity and in the intensity of occurrence. The active compound treated maize and sorghum seeds, showed highly significant control of all these seed borne fungi up to 120 d of storage. Between the three different concentrations tested, 1g/kg treatment was highly effective in preventing fungi.

Table 2. IC_{50}, MIC and MFC value of 2-hydroxy-4-methoxybenzaldehyde isolated from *D. hamiltonii* against phytopathogenic fungal isolates from sorghum and maize grains

Pathogenic fungi	2-Hydroxy-4-methoxybenzaldehyde (µg/ml)		
	IC_{50}	MIC	MFC
Alternaria alternata	250	550	650
*Aspergillus flavus**	200	500	650
A. fumigatus	150	450	500
A. ochraceus	200	400	500
A. versicolor	200	450	600
A. columnaris	300	750	850
A. niger	350	800	950
A. tamari	250	700	800
Drechslera tetramera	200	400	500
Fusarium moniliforme	100	500	600
F. oxysporum	80	350	450
F. proliferatum	100	400	500
F. verticillioides#	100	500	600
Penicillium griseofulvum	200	550	650

IC_{50} = Inhibitory conc 50, MIC =minimal inhibitory conc, MFC = minimal fungicidal conc.

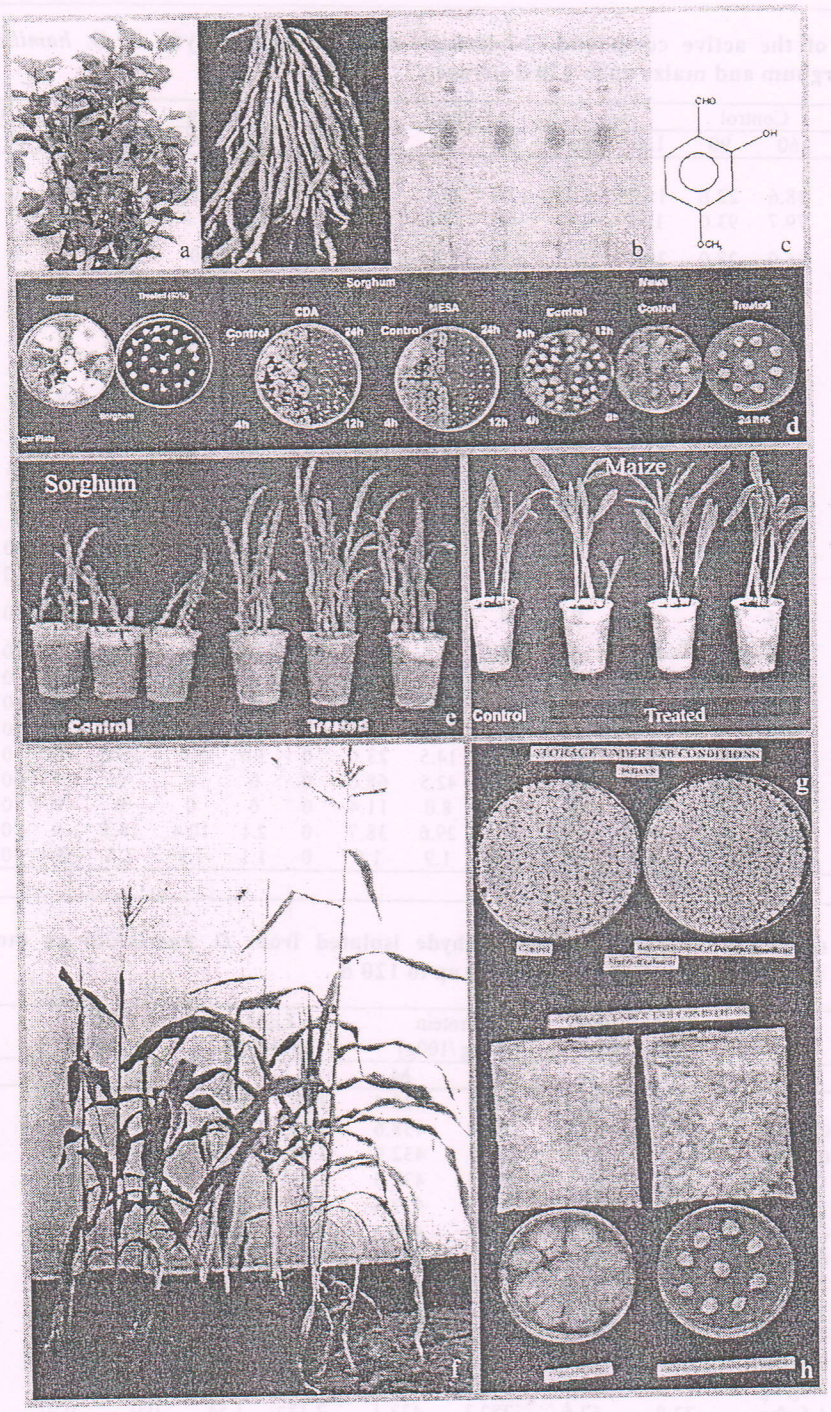

Figure 1: a = *Decalepis hamiltonii* plant and rhizome ; b = band 5 of phenolic fraction of *D.hamiltonii* showing antifungal activity; band 5 deduced as 2-hydroxy-4-methoxybenzaldehyde from1H, 13C and mass spectra; d = control and treated sorghum and maize seeds; e = effect on sorghum and maize seedlings; f = effect on maize plants; g = formulation of bioactive compound using maize grains; h = aflatoxin B1 evaluation

Table 3. Efficacy of the active compound, 2-hydroxy-4-methoxybenzaldehyde of *D. hamiltonii* on seed mycoflora (%) of sorghum and maize up to 120 d storage

	Control				0.5 g/kg				1 g/kg				1.5 g/kg			
Storage (days)	30	60	90	120	30	60	90	120	30	60	90	120	30	60	90	120
Sorghum																
Alternaria	32.4	38.6	22.6	15.0	5.0	12.6	20.4	32.0	0	0	13.0	15.4	0	0	0	5.0
Aspergillus	55.0	79.7	93.0	100	9.5	34.5	52.5	83.0	0	0	7.0	21.6	0	0	6.0	14.5
Chaetomium globosum	23.7	26.0	28.0	29.4	0	0	7.0	20.7	0	0	0	0	0	0	0	0
Curvularia	16.8	36.0	38.4	38.5	3.5	6.5	12.5	18.5	0	0	9	21.0	0	0	0	3.0
Drechslera	34.0	48.2	44.0	56.0	0	5.0	27.7	41.5	0	0	0	17.0	0	0	0	11.0
Fusarium spp.	58.0	68.0	54.0	32.6	0	21.5	25.0	34.0	0	0	5.5	20.5	0	0	0	15.5
Penicillium	47.3	53.4	84.5	97.5	5.0	32.6	54.0	79.0	0	8	14.5	31.0	0	0	10.5	21.0
Phoma	8.4	11.4	21.0	27.0	1.0	10.5	15.5	18.5	0	3	3.0	15.0	0	0	0	7.0
Rhizopus	18.5	34.3	48.5	89.6	0	2.4	29.0	38.5	0	0	0	6.0	0	0	0	6.0
Trichothecium	12.3	21.5	28.4	31.5	0	0	11.4	16.4	0	0	0	0	0	0	0	0
CD (*P=0.05*)	38.3	45.7	54.6	69.8	6.2	27.6	35.6	55.2	0	5.7	11.9	21.7	0	0	7.6	15.0
CV (%)	1.2	1.4	2.4	1.9	3.8	1.6	1.2	1.2	0	1.3	4.9	1.7	0	0	7.4	4.9
Maize																
Alternaria	34.0	38.5	28.0	25.0	0.0	18.0	26.5	28.5	0	0	10.5	9.0	0	0	0	0
Aspergillus	66.5	99.0	100	100	12.5	37.0	66.4	73.0	0	0	9.0	25.0	0	0	0	9
Chaetomium globosum	14.0	16.5	20.5	18.0	0	0	7.0	11.6	0	0	0	0	0	0	0	4.5
Curvularia	26.5	39.0	48.5	41.5	0	0	11.5	27.0	0	0	8.5	13.5	0	0	0	3.0
Drechslera	51.6	58.0	54.0	51.5	0	8.5	24.5	34.4	0	0	5.0	15.5	0	0	0	10.0
Fusarium spp.	44.7	49.5	44.0	32.6	0	16.0	25.0	32.5	0	0	7.0	18.0	0	0	0	11.5
Penicillium	33.0	56.6	92.0	100	2.0	25.6	66.0	82.0	0	6.5	20.5	32.0	0	0	5	21.0
Phoma	6.0	14.0	19.5	29.5	0	12.5	14.5	23.0	0	3.0	5.0	15.0	0	0	0	4.5
Rhizopus	22.0	38.5	68.5	98.0	0	4.0	42.5	68.0	0	0	0	0	0	0	0	6.0
Trichothecium	4.5	6.0	8.0	11.5	0	0	8.0	11.4	0	0	0	0	0	0	0	0
CD (*P=0.05*)	35.6	38.4	41.3	44.6	5.2	24.5	29.6	38.7	0	2.1	10.4	18.5	0	0	2.5	9.6
CV (%)	2.3	2.1	1.9	1.7	1.4	1.6	1.9	1.9	0	1.1	1.9	1.5	0	0	1.0	1.5

Table 4. Efficacy of 2-hydroxy-4-methoxybenzaldehyde isolated from *D. hamiltonii* on mould induced nutritional losses in sorghum and maize grains stored up to 120 d

Quality analysis		Carbohydrate (mg /100g)		Protein (mg /100g)		Lipid (g /100g)		DML (g /100g)		Aflatoxin B1 (µg/100g)	
Storage periods		S	M	S	M	S	M	S	M	S	M
Before storage		73.2	68.3	380.3	432.4	3.10	3.62	0.0	0.0	0.0	0.0
30 d	Control	68.8	65.4	394.8	438.6	2.13	2.98	1.2	0.9	8.2	2.4
	0.5g/kg	72.5	67.6	381.1	432.9	2.84	3.58	0.0	0.0	0.0	0.0
	1.0g/kg	73.2	68.3	380.3	432.5	3.08	3.62	0.0	0.0	0.0	0.0
	1.5g/kg	73.2	68.3	380.3	432.4	3.10	3.62	0.0	0.0	0.0	0.0
60 d	Control	61.5	60.8	426.4	429.3	1.15	2.37	2.9	1.6	22.5	10.3
	0.5g/kg	70.3	65.7	414.2	435.4	1.56	3.36	0.3	0.0	5.2	0.0
	1.0g/kg	72.8	67.8	381.5	432.9	2.86	3.58	0.0	0.0	0.0	0.0
	1.5g/kg	73.1	68.1	380.3	432.5	2.96	3.60	0.0	0.0	0.0	0.0
90 d	Control	49.7	57.2	412.3	412.4	0.55	1.68	3.9	2.8	48.5	22.5
	0.5g/kg	62.6	62.3	423.5	431.2	0.98	2.69	1.2	0.6	19.4	6.3
	1.0g/kg	71.4	65.7	385.4	434.3	1.95	3.46	0.2	0.0	0.0	0.0
	1.5g/kg	72.8	67.6	382.3	433.1	2.12	3.54	0.0	0.0	0.0	0.0
120 d	Control	38.8	51.6	362.5	397.3	0.08	1.02	5.6	3.9	67.7	43.5
	0.5g/kg	51.5	58.1	394.5	422.3	0.56	1.75	2.8	1.5	38.3	22.7
	1.0g/kg	70.6	63.6	397.4	435.6	1.45	2.84	0.9	0.2	5.8	2.3
	1.5g/kg	71.9	66.3	388.4	434.2	1.95	3.04	0.2	0.0	0.0	0.0
CD (*P=0.05*)		20.9	18.8	35.3	29.4	1.9	1.8	3.1	3.2	40.3	38.3
CV (%)		1.8	1.5	1.6	1.7	4.2	3.9	16.3	15.7	1.6	1.8

S = Sorghum seeds; M = maize seeds; A= active compound; T = treated

Changes in nutritional parameters, dry matter losses and aflatoxin B_1 production of both un-treated and treated seeds of maize and sorghum were analysed (Table 4). Among three concentrations tested 1g/kg appears to be the best. Carbohydrates, protein and lipid content were conserved in the 1g/kg treated seeds even after 120 d of storage in sorghum and maize. The DML was non-significant. A highly significant reduction in aflatoxin contamination was observed in both the seed systems at 1g/kg treatment. Total absence of aflatoxin was observed in sorghum and maize at 1.5g/kg treatment even after 120 days of storage,

Phytotoxicity assay. In maize and sorghum grains, with increase in period of exposure there was significant decrease in all the fungi but with significant increases in seed germination and seedling vigour (Table 5). Tukey HSD analysis of the data revealed that 8 and 12 h exposure at 1000 µg/ml and 8h exposure at 1500 µg/ml concentration were highly significant treatments in comparison with control. In case of maize and sorghum seeds, species of *Drechslera, Aspergillus*, *Fusarium, Penicillium, Curvularia*, and *Rhizopus* which were present in high levels were significantly decreased along with increases in germination and seedling vigour

Table 5. Efficacy of 2-hydroxy-4-methoxybenzaldehyde isolated from *D. hamiltonii* on seedling vigour of maize and sorghum seeds

Conc. (µg/ml)	Period of soaking	Maize	Sorghum
Control	4h	998	530
	8h	1012	560
	12h	1020	570
500	4h	1175	682
	8h	2188	1050
	12h	2081	1118
1000	4h	1304	913
	8h	1693	1150
	12h	1451	1023
1500	4h	1334	1100
	8h	1053	976
	12h	566	612
CD (P=0.05)		1140	597
CV (%)		8.2	4.3

Discussion

Southern part of India, with its varied agro-climatic conditions produces a variety of food crops throughout the year. Maize and sorghum are the two important cereals cultivated as major rainfed crops in most of the semiarid regions. Agricultural practices, poor storage facilities and unfavourable environmental conditions during pre- and post-harvest handling of crops is responsible for the severe contamination, infection and colonization by fungi (Janardhana et al 1999; Lineard et al 1993). Association of a variety of fungi, causing significant loss in seed quality and nutritional quality have been reported by Koirala et al (2005). 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 (Desjardins et al 2000). Most of the pathogens are disseminated predominantly by seeds (Miller 1995). Eventhough effective and efficient control of seed borne pathogenic fungi can be achieved by the use of synthetic chemical fungicides, the same cannot be applied to grains for reasons of pesticide toxicity (Wodageneh and Wulp 1997; Harris et al 2001).

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; Harborne 1998). 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 and biodeterioration (Varma and Dubey 1999; Gottlieb et al 2002).

Considering these as a first step, in the present investigation 25 plants were screened in vitro for antifungal activity against important phytopathogenic fungi isolated from maize and sorghum. Rhizome of *D. hamiltonii* is largely used in South India for pickling along with curds or limejuice (Anon 1952), showed significant antifungal activity. Antimicrobial properties of *D. hamiltonii* have been reported (Phadke et al 1994; George et al 1999a; Thangadurai et al 2002; Elizabeth et al 2005). In all these reports, the test organisms are human pathogenic microorganisms. The production process patent from the same plant was patented by Central Food Technological Research Institute, Mysore, India in 2005 (CFTRI (Patent no. WO 2005/120236 A1). However, Mohana et al (2008) standardized the new procedure for isolation of antifungal compounds (2-hydroxy-4-methoxybenzaldehyde) and also antifungal assay. The insecticidal property of this compound against important storage insects (*Sitophilus oryzae* L., *Rhyzopertha dominica* F. and *Tribolium castaneum* Hbst.) has also been demonstrated (George et al 2000, 1999b). Mohana et al (2008 and 2009) have

reported antifungal potency of *D. hamiltonii* against important phytopathogenic fungi. The efficacy of this compound for prevention of biodeterioration of grains during storage has not been worked out.

In the present investigation the antifungal active compound 2-hydroxy-4-methoxybenzaldehyde isolated from *D. hamiltonii* has been evaluated for the first time to improve grain quality and to prevent loss in nutritional quality of maize and sorghum. The present investigation demonstrates in vitro antifungal property of the active compound 2- hydroxy-4-methoxybenzaldehyde against 14 phytopathogenic fungi isolated from maize and sorghum grains. Similarly the in vivo efficacy of the active compound to prevent biodeterioration and aflatoxin production of maize and sorghum grains during storage has also been demonstrated. The result of the present investigations demonstrates highly significant antifungal activity of the active compound, 2-hydroxy-4-methoxybenzaldehyde isolated from rhizome of *D. hamiltonii*. The inhibitory activity was observed against all the test fungi which include both field and storage fungi known to cause significant crop loss in field and during storage. Synthetic fungicide blitox and thiram are generally used in the management of fungal pathogens in agriculture (Sagar and Sugha, 2004; Ghasolia and Jain, 2004; Bagga and Sharma, 2006). In vitro comparative evaluation of these synthetic fungicides with that of active compound, 2-hydroxy-4-methoxybenzaldehyde isolated from *D. hamiltonii* has revealed that less than 2/3 the concentration of the active compound is enough to bring about the desired effect in vitro.

The analysis of seed moulds, food reservoir, DML and aflatoxin B_1 production of maize and sorghum grains treated with the active compound (0.5, 1 and 1.5 g/kg) were significantly effective in controlling moulds, mould induced nutritional changes, dry matter losses and aflatoxin B_1 production. None of the earlier investigators have evaluated the efficacy of the compound 2- hydroxy- 4-methoxybenzaldehyde to prevent nutritional loss in seed during storage. For the first time, the bioactive compound has been evaluated and its efficacy to prevent nutritional loss during storage. The observations of the present investigations suggest that 1 g/kg treatment of the active compound is ideal to prevent nutritional loss during storage of maize and sorghum grain. Further investigations are necessary on the toxicological aspects of this treatment before it is finally recommended for the commercial exploitation.

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