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Effect of plant extracts on inhibition of *Fusarium verticillioides* growth and its toxin fumonisin B_1 production

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Abstract *Fusarium verticillioides* is one of the most prevalent and highly toxigenic fungi commonly associated with food grains. In the present investigation aqueous and solvent extracts of forty-eight plants belonging to twenty-four families were evaluated for their antifungal and antifumonisin efficacies. The test fungus *F. verticillioides* was isolated from maize and its fumonisin B_1 (FB_1) production was qualitatively estimated by comparison with standard FB_1. The antifungal activity was assessed by poisoned food technique and two fold broth microdilution methods. The results revealed that, petroleum ether extract of *Decalepis hamiltonii*, chloroform extract of *Albizia amara*, *Adenanthera pavonina*, *Breynia vitis-idaea*, *Cassia spectabilis* and *Solanum torvum*, and methanol extract of *Acacia catechu*, *Acacia ferruginea*, *Albizia odoratissima*, *Albizia saman*, *Anogeissus latifolia*, *Caesalpinia coriaria*, *Dodonaea viscosa*, *Prosopis juliflora* and *Salacia oblonga* showed promising antifungal activity with percent mycelial inhibition which ranged from 33.9% to 81.2% at 2mg/ml and the MIC ranged from 0.25 to 2.0 mg/ml. These extracts severely inhibited FB_1 production both *in vitro* (2.0 mg/ml) and *in vivo* (2.0 g/kg) conditions. The present findings indicate the possible exploitation of these plants for preserving food grains from post-harvest fungal deterioration and mycotoxin contamination.

Key words: *F. verticillioides*, fumonisin B_1, plant extracts, antifungal activity.

Introduction

Maize (*Zea mays* L.) is one of the most important cereals in human and animal diet as a source of food, forage and processed products (Garcia *et al.*, 2012). Several seed-borne fungi attack maize plants during its various growth stages and storage (Mohana *et al.*, 2010). *Fusarium verticillioides* is one of the most prevalent and highly toxigenic fungus commonly associated with maize worldwide (Glenn *et al.*, 2007; Alberts *et al.*, 1990). Its invasion may initiate at any stage from the standing crop to harvest and post harvest handling until they

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reach the consumers (Garcia *et al.,* 2012). During favourable conditions, it causes many diseases such as root, stalk, ear, kernel and seedling rots, damping off and wilt. Besides that, it is also responsible for decreased nutritive value, loss in germination, discoloration, increase in fatty acid and more importantly production of mycotoxins resulting to serious production losses in maize (Yates *et al.,* 2003; Machon *et al.,* 2006). Fumonisin B_1 is one of the most toxic mycotoxin of the fumonisin family produced by *F. verticillioides*. In animals, it causes nephrotoxic, hepatotoxic, leukoencephalomalacia, pulmonary edema and esophageal cancer (Domijan *et al.,* 2008; Reza *et al.,* 2010). Accumulation of FB_1 in maize based food and feedstuffs are increasing worldwide, possibly due to climate changes, use of different plant varieties of high yield, but are susceptible to moulds and mycotoxins contamination as well as improper agricultural practices (Quiroga *et al.,* 2009). The most important method of protecting maize against fungal attack is the use of synthetic fungicides, but residues of chemical fungicides in maize and its processed products cause damage to the health of animals and humans and also affect export (Deng *et al.,* 2011). Furthermore, development of resistance of *F. verticillioides* towards synthetic fungicides is of great concern (Mdee *et al.,* 2009). In an attempt to reduce the use of synthetic fungicides, extensive investigations towards the possible exploitation of plant extracts which are safe for human and the environment as alternative to synthetic chemical, have been undertaken over the past two decades worldwide (Quiroga *et al.,* 2009; Weerakkody *et al.,* 2010). Keeping these, the authors have screened large number of medicinal plants for their inhibitory activity against *F. verticillioides* and its toxin FB_1 production, with the ultimate aim to find out new sources of natural antifungal compounds and the scientific validation of their usage for management of the disease. In the present study, the antifungal activity of aqueous extract of 48 plants and antifungal and antifumonisin efficacies of successive solvent extract of 15 plants was evaluated.

Materials and methods

Chemicals and culture media

Sabouraud dextrose agar/broth (SDA/SDB) and dimethyl sulfoxide (DMSO) were purchased from Hi-Media, Mumbai (India). Mancozeb 75% WP (Dithane M-45) was purchased from Indofil chemicals, Mumbai (India). All solvents, reagents and iodo-nitro-tetrazolium (INT) were purchased from Sisco Research Laboratory, Mumbai (India). Microtiter plates (96 wells) and serological pipettes were purchased from Axiva, New Delhi (India). The standard fumonisin B_1 (FB_1) were obtained from Sigma, Germany and Silica

gel 60 F_{254} coated preparative aluminium Thin Layer Chromatography (TLC) plates (20 X 20 cm) were obtained from Merck, Darmstadt (Germany).

Plant materials

Fresh disease free leaves of 49 different medicinal plants (Table 1) were collected from southern part of Karnataka. The plant samples were authenticated by Dr. Seetharam, Professor, Department of Biological Sciences, Bangalore University, and the authenticated voucher specimens of these plants have been deposited at herbarium in the Department of Microbiology and Biotechnology, Bangalore University, Bangalore (Voucher numbers: BUB/MB-BT/DCM/JU10/01 to BUB/MB-BT/DCM/JU10/48.

Preparation of aqueous extracts

The aqueous extract of 48 medicinal plants (Table 1) was prepared following the procedure of Mohana *et al.* (2008a). Briefly, fifty grams of shade dried powder of each plant material was macerated separately with 250 ml of sterile distilled water and centrifuged at 4000*g* for 30 min. The supernatant was filtered and concentrated using rotary flash evaporator. After complete evaporation of the water, the dried crude plant extracts were re-suspended separately in DMSO and subjected to antifungal activity assay at 2 mg/ml.

Preparation of solvent extracts

The successive solvent extracts of selected plants (Table 2) were prepared following the procedure of Praveen *et al.* (2011). Briefly, fifty grams of shade dried powder of each selected plant material was extracted successively with 200 ml of petroleum ether, toluene, chloroform, methanol and ethanol using a soxhlet extractor. The residual solvent in the extract was removed using rotary flash evaporator. The dried organic plant extracts were re-suspended in DMSO and subjected to antifungal activity at desired different concentrations.

Antifungal activity assay

Isolation of FB_1 producing F. verticillioides from maize seed samples

Twenty-five isolates of *F. verticillioides* were isolated from 25 maize varieties by standard blotter method following the procedure of ISTA (1996) and were analysed for their FB_1 production using the standard procedures (Bailly *et al.,* 2005). Briefly, mycelial mat of *F. verticillioides* was extracted by mechanical agitation with acetonitrile : water (1:1, v/v) and filtered through

0.45µm membrane filter. The filtrate was spotted on TLC plates (10µl/spot) along with different concentrations of standard FB_1 and eluted by butanol-acetic acid-water (20:10:10 v/v/v) as a mobile phase. The air dried plates were sprayed with 0.5% p-anisaldehyde in methanol-acetic acid-H_2SO_4 (85:10:0.5 v/v/v) solution followed by heated at 110 ℃ for 10 min. The FB_1 concentration of each strain was determined by comparison with standard FB_1. The detection limit of FB_1 on TLC plates was 0.5 µg/spot. The strain *F. verticillioides* (R8) (the references in brackets are the code of maize variety from which the culture was isolated) was able to produce the highest concentrations of FB_1 was selected as a test organism to determine the antifungal and antifumonisin assay.

Poisoned food technique

Aqueous and successive solvent extracts were subjected to antifungal activity assay by poisoned food technique following the procedure of Mohana *et al.* (2010) with some minor modification. Briefly, 5 mm disc of 7 day old culture of *F. verticillioides* was placed on a SDA medium impregnated separately with desired different concentrations of extracts and incubated at 30 ℃ for 72 h. DMSO served as a negative control and dithane M-45 served as positive control. The fungitoxicity of the extract in terms of percentage inhibition (%) of mycelial growth was calculated by using the formula,

Percentage Inhibition (%I) = dc-dt/dc X 100

Where, dc - Average increase in mycelial growth in control.
dt - Average increase in mycelial growth in treatment

Determination of MIC by broth microdilution method

The broth microdilution method was used to determine the minimum inhibitory concentrations (MICs) of solvent extracts of selected plants following the procedure of Hajji *et al.* (2010) with some modifications. Briefly, 200 µl of two-fold serially diluted extracts (0.031 to 4 mg/ml) in SDB were added separately to the wells of a sterile 96-well microtiter plate and inoculated with 15 µl of *F. verticillioides* spore suspension containing 10^4 spores/ml and incubated at 30 ℃ for 72 h. DMSO served as a negative control and dithane M-45 was used as positive control. After incubation, the MIC values of the extracts were detected by the addition of 50 µl of iodo-nitro-tetrazolium (INT) (2 mg dissolved in 1ml of water). The colourless tetrazolium salt acts as an electron acceptor and is reduced to a red-coloured formazan product by biologically active organisms. Where microbial growth was inhibited, the solution in the well remained clear after incubation with INT. The colour

intensity was measured using microtiter plate reader (*EL$_X$800*, Bio-Tek Instruments, Vermont, US). The extracts impregnated SDB medium without inoculums served as blank. MIC was defined as the lowest concentration at which no visible fungal growth was observed.

In vitro and in vivo efficacy of extracts on FB$_1$ production by F. verticillioides

The *in vitro* efficacies of solvent extracts of some selected plants on FB_1 production were determined following the procedures of Bailly *et al.* (2005) with some modifications. Briefly, 100 µl of a spore suspension (10^4 spores/ml) of *F. verticillioides* was inoculated into SDA, containing the requisite amount of extracts and incubated at 28±2 ℃ for 10 d. The SDA along with the *F. verticillioides* culture was used to estimate FB_1. The efficacy of the extracts towards inhibiting FB_1 production was determined by TLC as mentioned above.

The *in vivo* efficacies of extracts on FB_1 production in maize seeds were determined following the procedures of Garcia *et al.* (2012) with some modifications. Briefly, freshly harvested maize samples were collected; surface sterilized under UV and the water activity (a_w) was adjusted to 0.95 by adding sterile distilled water to maize. The maize samples were treated with desired different concentrations of extracts separately and inoculated with 100 µl of a spore suspension (10^4 spores/ml) of *F. verticillioides*. All treatments were separately stored in plastic containers (200 g/pack) and incubated at 25 ℃ for up to 15 d. After incubation, the treated maize seeds were subjected to FB_1 extraction and quantification as well as determine the efficacy of extracts on the inhibition of *F. verticillioides* by SBM and seedling vigour index (SVI) was analysed using the formula.

SVI = (Mean of root length + Mean of shoot length) × percentage seed germination (Sparg *et al.*, 2005).

Results

The antifungal activity of aqueous extracts of 48 plants belonging to 24 families were evaluated against *F. verticillioides* by poisoned food technique and the percent mycelial inhibition was reported in Table 1. The aqueous extract of 26 plants *viz.*, *Acacia catechu*, *A. chundra*, *A. ferruginea*, *Adenanthera pavonina*, *Albizia amara*, *A. odoratissima*, *A. saman*, *Anogeissus latifolia*, *Abrus precatorius*, *Artabotrys odoratissimus*, *Breynia vitis-idaea*, *Caesalpinia coriaria*, *Carissa carandas*, *Cassia spectabilis*, *C. tora*, *Coleus amboinicus*, *Decalepis hamiltonii*, *Dodonaea viscosa*, *Holoptelea integrifolia*, *Lagerstroemia speciosa*, *Prosopis juliflora*, *Salacia oblonga*, *Solanum torvum*, *Spilanthes paniculata*, *Thespesia populnea* and *Tylophora indica* showed

antifungal activity with percent mycelial inhibition ranged from 11% to 75% at 2 mg/ml depending upon plant species. Among the 26 plants, 15 plants *viz*., *A. catechu*, *A. ferruginea*, *A. pavonina*, *A. amara*, *A. odoratissima*, *A. saman*, *A. latifolia*, *B. vitis-idaea*, *C. coriaria*, *C. spectabilis*, *D. hamiltonii*, *D. viscosa*, *P. juliflora*, *S. oblonga* and *S. torvum* showed promising antifungal activity (%I >30%) were selected for successive solvent extraction.

Table 1. Antifungal activity of aqueous extract of some medicinal plants against fumonisin B_1 producing *F. verticillioides* at 2 mg/ml

Name of the Plants	Family	Activity
Acacia catechu (L.f.) Willd.	Fabaceae	+++
Acacia chundra (Rottler) Willd.	Fabaceae	+
Acacia ferruginea DC.	Mimosaceae	++
Adenanthera pavonina L.	Mimosaceae	+++
Albizia amara (Roxb.) B.Boivin	Fabaceae	++++
Albizia odoratissima (L.f.) Benth.	Fabaceae	++
Albizia saman (Jacq.) Merr.	Fabaceae	++++
Anogeissus latifolia (Roxb. ex DC.) Wall.	Combretaceae	++
Abrus precatorius L.	Fabaceae	+
Argemone mexicana L.	Papaveraceae	-
Artabotrys odoratissimus Blume	Annonaceae	+
Asparagus racemosus Willd.	Liliaceae	-
Bauhinia acuminata L.	Caesalpiniaceae	-
Breynia vitis-idaea (Burm.f.) C.E.C.Fisch.	Euphorbiaceae	++
Caesalpinia coriaria (Jacq.) Willd.	Caesalpiniaceae	+++
Calotropis gigantea (L.) Dryand.	Apocyanceae	-
Caris sacarandas L.	Apocyanceae	+
Cassia alata L.	Fabaceae	-
Cassia siamea Lam.	Fabaceae	-
Cassia spectabilis DC.	Fabaceae	+++
Cassia tora L.	Fabaceae	+
Coleus amboinicus Lour.	Lamiaceae	+
Couroupita guianensis Aubl.	Lecythidaceae	-
Decalepis hamiltonii Wight &Arn.	Apocyanceae	+++
Delonix regia (Hook.) Raf.	Fabaceae	-
Dodonaea viscosa Jacq.	Sapindaceae	++
Ficus benghalensis L.	Moraceae	-
Ficus religiosa L.	Moraceae	-
Gliricidia sepium (Jacq.) Walp.	Fabaceae	-
Holoptelea integrifolia Planch.	Ulmaceae	+
Lagerstroemia speciosa (L.) Pers.	Lythraceae	+

Millingtonia hortensis L.f.	Bignoniaceae	-
Phyllanthus amarus Sch. &Thonn.	Phyllanthaceae	-
Phyllanthus polyphyllus Willd.	Phyllanthaceae	-
Peltophorum pterocarpum K.Heyne	Fabaceae	-
Prosopis juliflora (Sw.) DC.	Fabaceae	++
Ricinus communis L.	Euphorbiaceae	-
Saccharum spontaneum L.	Poaceae	-
Salacia oblonga Wall.	Celastraceae	++
Sesbania grandiflora (L.) Pers.	Fabaceae	-
Solanum torvum Sw.	Solanaceae	+++
Spathodea campanulata P.Beauv.	Bignoniaceae	-
Spilanthes paniculata Wall. ex DC.	Asteraceae	+
Tabebuia aurea Benth. &Hook.f. ex S.Moore	Bignoniaceae	-
Thespesia populnea (L.) Sol. ex Correa	Malvaceae	+
Tylophora indica (Burm. f.) Merr.	Asclepiadaceae	+
Vitex negundo L.	Lamiaceae	-
Ziziphus mucronata Willd.	Rhamnaceae	-

Data given are mean of four replicates; Leaves were used as test material; No activity was observed in DMSO impregnated control plates; The notations were used to estimate the percentage inhibition (PI) of mycelial growth of *F. verticillioides* as follows: - → no antifungal activity; + → scantly antifungal activity (PI 11% to 30%); ++ → moderate antifungal activity (PI 31% to 50%); +++ → strong antifungal activity (PI 51% to 70%); ++++ → very strong antifungal activity (PI ≥71%).

The antifungal activity of the successive solvent extracts of 15 plants was evaluated qualitatively and quantitatively by poisoned food technique and two fold broth microdilution methods. The percent mycelial inhibition and MIC values of the solvent extracts which are showed highest activity were presented in Table 2. Among the different solvent extracts tested, petroleum ether extract of *D. hamiltonii*, chloroform extract of *A. pavonina*, *A. amara*, *B. vitis-idaea*, *C. spectabilis* and *S. torvum*, and methanol extract of *A. catechu*, *A. ferruginea*, *A. odoratissima*, *A. saman*, *A. latifolia*, *C. coriaria*, *D. viscosa*, *P. juliflora* and *S. oblonga* showed highest activity with percent mycelial inhibition which ranged from 33.9% to 81.2% at 2mg/ml and MIC which ranged from 0.25 to 2.0 mg/ml which depends on plant species. The chloroform extract of *A. amara* showed highest percent mycelial inhibition with least MIC value, whereas chloroform extract of *B. vitis-idaea* showed least percent mycelial inhibition with highest MIC value. On comparative evaluation with synthetic antifungal compound dithane M-45, the activity of extracts of *A. amara*, *A. saman*, *D. hamiltonii*, *A. catechu*, *C. spectabilis*, *C. coriaria* and *S. torvum* was greater than the positive control.

The antifumonisin activity of petroleum ether extract of *D. hamiltonii*, chloroform extract of *A. pavonina, A. amara, B. vitis-idaea, C. spectabilis* and *S. torvum,* and methanol extract of *A. catechu, A. ferruginea, A. odoratissima, A. saman, A. latifolia, C. coriaria, D. viscosa, P. juliflora* and *S. oblonga* was determined by TLC method and quantitatively evaluated FB_1 inhibition by comparison with negative control and standard FB_1. The obtained results were presented in Table 2.

Table 2. Antifungal and antifumonisin efficacies of solvent extracts of some selected medicinal plants against fumonisin B_1 producing *F. verticillioides*

Plant names	**Extracts**	**% mycelial inhibition (2 mg/ml)**	**MIC (mg/ml)**	**FB_1 content***	
				In vitro (mg/l)	*In vivo* (mg/kg)
A. catechu	M	72.9 ±0.37	0.5	0.0	100 ±1.9
A. ferruginea	M	52.8 ±0.29	1.0	125 ±2.1	180 ±2.4
A. pavonina	C	65.1 ±0.23	0.5	0.0	100 ±0.8
A. amara	C	81.2 ±0.40	0.25	0.0	0.0
A. odoratissima	M	58.6 ±0.41	1.0	100 ±1.2	150 ±1.6
A. saman	M	78.8 ±0.26	0.25	0.0	0.0
A. latifolia	M	53.2 ±0.26	0.5	135 ±2.2	175 ±2.7
B. vitis-idaea	C	33.9 ±0.37	2.0	350 ±2.6	300 ±2.9
C. coriaria	M	68.7 ±0.31	0.5	0.0	100 ±0.6
C. spectabilis	C	69.8 ±0.14	0.25	0.0	0.0
D. hamiltonii	P	75.4 ±0.17	0.25	0.0	0.0
D. viscosa	M	36.1 ±0.21	1.0	350 ±2.9	250 ±1.8
S. torvum	C	67.2 ±0.21	0.25	0.0	0.0
P. juliflora	M	36.7 ±0.20	1.0	175 ±1.2	250 ±2.3
S. oblonga	M	49.1 ±0.29	0.5	100 ±0.7	225 ±1.9
Negative control	-	0.0	-	450 ±3.8	354 ±3.2
Positive control	-	65.6 ±0.17	0.5	100 ±0.4	150 ±0.8

Data given are mean of four replicates ± standard error; *- 2 mg/ml for *in vitro* treatment and 2g/kg for *in vivo* treatment; P-Petroleum ether extract; C-Chloroform extract; M-Methanol extract; dithane M-45 was used as positive control and DMSO served as negative control.

In the negative control, FB_1 production was 450 mg/l under *in vitro* and 354mg/kg under *in vivo*. The petroleum ether extract of *D. hamiltonii*, chloroform extract of *A. amara*, *C. spectabilis* and *S. torvum*, and methanol extract of *A. saman* were completely inhibited the FB_1 production both *in vitro* and *in vivo*, while the chloroform extract of *A. pavonina* and methanol extract of *A. catechu* and *C. coriaria* were completely inhibits FB_1 production only under *in vitro*. Whereas, the chloroform extract of *Breynia vitis-idaea* and

methanol extract of *A. ferruginea*, *A. odoratissima*, *A. latifolia*, *D. viscosa*, *P. juliflora*, *S. oblonga* were not inhibited FB_1 completely both under *in vitro* and *in vivo*. Similarly, the percent incidence of *F. verticillioides* in maize samples of the inoculated control was 98%. Whereas, the percent incidence of *F. verticillioides* was greatly decreased in petroleum ether extract of *D. hamiltonii*, chloroform extract of *A. amara*, *A. pavonina*, *C. spectabilis* and *S. torvum*, and methanol extract of *A. catechu*, *A. saman* and *C. coriaria* treated maize (Fig 1).

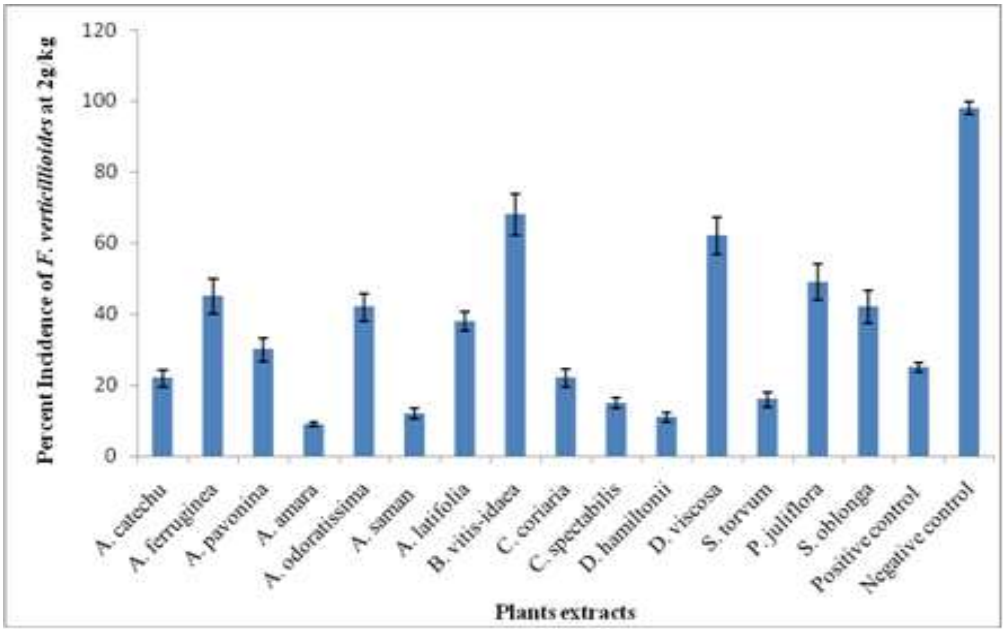

Fig. 1. *In vivo* efficacy of solvent extracts of some selected plants on percent incidence of *F. verticillioides* in maize model system

Data given are mean of four replicates ± standard error; dithane M-45 was used as positive control and DMSO served as negative control

The extracts of *A. amara*, *A. saman* and *D. hamiltonii* completely inhibited *F. verticillioides* growth at 2 g/kg. The present study confirms that the effectiveness of extract of petroleum ether extract of *D. hamiltonii*, chloroform extract of *A. amara*, *A. pavonina*, *C. spectabilis* and *S. torvum*, and methanol extract of *A. catechu*, *A. ferruginea*, *A. odoratissima*, *A. saman*, *A. latifolia*, *B. vitis-idaea*, *C. coriaria*, *D. viscosa*, *P. juliflora*, *S. oblonga* for the inhibition of *F. verticillioides* growth and FB_1 production.

Discussion

A perusal of the literature revealed antifungal activity of *A. catechu*, *A. amara*, *A. saman*, *A. pavonina*, *A. latifolia*, *C. spectabilis*, *S. torvum*, *D. hamiltonii*, *A. ferruginea*, *A. odoratissima*, *B. vitis-idaea*, *C. coriaria*, *D. viscosa* and *P. juliflora* against some storage moulds since the last decade (Joshi *et al.,* 2011; Praveen *et al.,* 2011; Thippeswamy *et al.,* 2011; Deepa *et al.,* 2012; Bari *et al.,* 2010; Mohana *et al.,* 2008; Gupta and Tripathi, 2011). However, no reports are available on inhibitory activity of these plants against *F. verticillioides* growth and its toxin fumonisin B_1 production. The present investigation reports the antifumonisin and anti- *F. verticillioides* activities of the plant samples in this study.

Moulds and mycotoxins contamination of stored maize grains is a chronic problem in the Indian storage system due to varied agro-climatic conditions, non-scientific methods of agricultural practices and poor storage facilities (Reddy *et al.,* 2009). *F. verticillioides* is one of the important phytopathogen which causes rot, damping off and wilt diseases in many crops, apart from it also produces health hazardous and carcinogenic fumonisins (Yates *et al.,* 2003; Machon *et al.,* 2006). FB_1 is one of the most toxic fumonisin which was common contaminant in agricultural products. The occurrences of FB_1 have been reported in maize and maize based food and feeds in worldwide (Garcia *et al.,* 2012). Eventhough effective and efficient control of seed borne fungi of seeds can be achieved by using the synthetic chemical fungicides; the same cannot be applied to grains for reasons of acute toxicity (Harris *et al.,* 2001). It is now realized that chemical fungicides cause serious environmental problems and are toxic to non-target organisms (Anon, 2005). The toxic effect of synthetic chemicals can be overcome only by persistent search for new and safer antifungal which are eco-friendly and effective. Considering these, the present investigation is an important step to develop plant based fungicide for enhancing shelf life of food commodities by controlling moulds and mycotoxins.

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References

Alberts, J.F., Gelderblom, W.C.A., Thiel, P.G., Arasas, W.F.O., Schalkwyk, J.V. and Behrend, Y. (1990). Effects of temperature and incubation period on production of Fumonisin B_1 by *Fusarium moniliforme.* Applied and Environmental Microbiology 56(6):1729-1733.

Anon. (2005). Pest control background. International Journal of Pest Control 45(2):232-233.

Bailly, J.D., Querin, A., Tardieu, D. and Guerre, P. (2005). Production and purification of fumonisins from a highly toxigenic *Fusarium verticillioides* strain. Revue de Medecine Veterinaire 156(11):547-554.

Bari, M.A., Islam, W., Khan, A.R. and Mandal, A. (2010). Antibacterial and antifungal activity of *Solanumtorvum*(solanaceae). International Journal of Agriculture and Biology 12:386-390.

Deepa, N., Nayaka, C.S., Shankar, A.C.U., Kumar, V.K., Niranjana, S.R., Prakash, H.S. and Raghavendra, M.P. (2012). Detection and Management of Seed-borne Toxigenic *Fusarium verticillioides* by Plant Alkaloids. Journal of Mycology and Plant Pathology 42(1):161-166.

Deng, Y., Yu, Y., Luo, H., Zhang, M., Qin, X. and Li, L. (2011). Antimicrobial activity of extract and two alkaloids from traditional Chinese medicinal plant *Stephaniadielsiana*. Food Chemistry 124:1556-1560.

Domijan, A.M., Zeljezic, D., Peraica, M., Kovacevic, G., Gregorovic, G., Krstanac, Z., Horvatin, K. and Kalafatic, M. (2008). Early toxic effects of fumonisin B_1 in rat liver. Human and Experimental Toxicology 27:895-900.

Garcia, D., Ramos, A.J., Sanchis, V. and Marin, S. (2012). Effect of *Equisetum arvense* and *Stevia rebaudiana* extracts on growth and mycotoxin production by *Aspergillus flavus* and *Fusarium verticillioides* in maize seeds as affected by water activity. International Journal of Food Microbiology 153:21-27.

Glenn, A.E. (2007). Mycotoxigenic *Fusarium* species in animal feed. Animal Feed Science and Technology 137:213-240.

Gupta, S.K. and Tripathi, S.C. (2011). Fungitoxic Activity of *Solanum torvum* against *Fusarium sacchari.* Plant Protection Science 47(3):83-91.

Hajji, M., Masmoudi, O., Souissi, N., Triki, Y., Kammoun, S. and Nasri, M. (2010). Chemical composition, angiotensin I-converting enzyme (ACE) inhibitory, antioxidant and antimicrobial activities of the essential oil from *Periplocalaevigata* root barks. Food Chemistry 121:724-731.

Harris, C.A., Renfrew, M.J. and Woolridge, M.W. (2001). Assessing the risk of pesticide residues to consumers: recent and future developments. Food Additives and Contaminants 18:1124-1129.

ISTA. (1996). International rules for seed testing. Seed Science and Techechnology 21(1):25-30.

Joshi, S., Subedi, Y.P. and Paudel, S.K. (2011). Antibacterial and Antifungal Activity of Heartwood of *Acacia catechu* of Nepal. Journal of Nepal Chemical Society 27:94-99.

Machon, P., Pajares, J.A., Diez, J.J. and Alves-Santos, F.M. (2006). Influence of the ectomycorrhizal fungus *Laccaria laccata*on pre-emergence, post-emergence and late damping-off by *Fusarium oxysporum* and *F. verticillioides* on Stone Pine Seedlings. Symbiosis 49:101-109.

Mdee, L.K., Masoko, P. and Eloff, J.N. (2009). The activity of extracts of seven common invasive plant species on fungal phytopathogens. South African Journal of Botany 75:375-379.

Mohana, D.C., Satish, S. and Raveesha, K.A. (2008a). Antibacterial activity of plant extracts against some human pathogenic bacteria. Advances in Biological Research 2:49-55.

Mohana, D.C., Raveesha, K.A. and LokanathRai, K.M. (2008). Herbal remedies for the management of seed-borne fungal pathogens by an edible plant *Decalepis hamiltonii* (Wight &Arn). Archives of Phytopathology and Plant Protection 41(1):38-49.

Mohana, D.C. and Raveesha, K.A. (2010). Antimycotic, antibiodeteriorative and antiaflatoxigenic potency of 2-hydroxy-4-methoxybenzaldehyde isolated from *Decalepis hamiltonii* on fungi causing biodeterioration of maize and sorghum grains. Journal of Mycology and Plant Pathology 40(2):197-206.

Praveen, P., Thippeswamy, S., Mohana, D.C. and Manjunath, K. (2011). Antimicrobial efficacy and phytochemical analysis of *Albizia amara* (Roxb.) Boiv. an indigenous medicinal plant against some human and plant pathogenic bacteria and fungi. Journal of Pharmacy Research 4(3):832-835.

Quiroga, E.N., Sampietro, D.A., Sgariglia, M.A., Soberon, J.R. and Vattuone, M.A. (2009). Antimycotic activity of 5′-prenylisoflavanones of the plant *Geoffroeadecorticans*, against *Aspergillus* species. International Journal of Food Microbiology 132:42-46.

Reddy, K.R.N., Reddy, C.S. and Muralidharan, K. (2009). Detection of *Aspergillus* spp. and aflatoxin B_1 in rice in India. Food Microbiology 26:27-31.

Reza, S.M.A., Rahman, A., Ahmed, Y. and Kang, S.C. (2010). Inhibition of plant pathogens *in-vitro* and *in-vivo* with essential oil and organic extracts of *Cestrum nocturnum* L. Pesticide Biochemistry and Physiology 96:86-92.

Sparg, S.G., Kulkarni, M.G., Light, M.E. and Staden, J.V. (2005). Improving seedling vigour of indigenous medicinal plants with smoke. Bioresource Technology 96:1323-1330.

Thippeswamy, S., Praveen, P., Mohana, D.C. and Manjunath, K. (2011). Antimicrobial evaluation and phytochemical analysis of known medicinal plant *Samanea saman* (Jacq.) Merr. against some human and plant pathogenic bacteria and fungi. International Journal of Pharma and Bio Sciences 2(2):443-452.

Yates, I.E., Arnold, J.W., Hinton, D.M., Basinger, W. and Walcott, R.R. (2003). *Fusarium verticillioides* induction of maize seed rot and its control. Canadian Journal of Botany 81(5):422-428.

Weerakkody, N.S., Caffin, N., Turner, M.S. and Dykes, G.A. (2010). *In vitro* antimicrobial activity of less-utilized spice and herb extracts against selected food-borne bacteria. Food Control 21:1408–1414.

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