Management of seed borne fungal pathogens of sorghum seeds by aqueous extract of *Lawsonia inermis* L.

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ABSRACT

The percent incidence of the seed borne fungi, seed germination and seedling vigor of sorghum seeds treated with 25, 50 and 100 % concentration of aqueous extract of *Lawsonia inermis* L. was evaluated at 1, 2, 4, 6, 12 and 24 h treatment duration. Untreated seeds were served as control. Significant decrease in seed mycoflora with significant increase in seed germination and seedling vigour was observed in treated seeds compared to control. Antifungal activity varied in different concentration at the different time interval tested. The complete inhibition of *Aspergillus flavus, A. ochraceous, F. moniliforme, Penicillium* spp. *Phoma* spp. was observed in seeds treated with 100% concentration at 24 h treatment. The incidence of *Fusarium solani, F. oxysporum, Drechslera halodes, Curvularia lunata, A. alternata, Trichothecium* spp. and *Rhizopus* spp. was significantly inhibited in treated seeds. The result of the present study is successful in identifying a candidate plant with significant antifungal activity which could be exploited as herbal remedy of the plant diseases.

Key words: *Lawsonia inermis*, antifungal activity, seed borne fungi

INTRODUCTION

Stored food commodities are severely damaged by different group of fungi including *Aspergillus* sp., *Fusarium* sp. and *Penicillium* sp. These fungi are associated with heavy loss of grains, fruits, vegetables and other plant products during picking, transit and storage rendering them unfit for human consumption even by producing mycotoxins and affecting their total nutritive value (Miller, 1995; Janardhana *et al*., 1999; Galvano *et al*., 2001). Many seed borne fungi, which cause severe damage to stored food commodities, were generally managed by synthetic chemicals, which were considered both efficient and effective. The continuous use of these synthetic fungicides started unraveling non biodegradability and known to have residual toxicity to cause pollution (Pimentel and Levitan, 1986). Pesticide pollution of soil and water bodies is well documented (Nostro *et al.*, 2000). Hence in recent time application of plant metabolites for plant disease management has become important viable component of Integrated Pest Management, as plant metabolites are eco-friendly where botanicals place an important role (Sahayaraj *et al*., 2009). Considering this several plants were screened in our laboratory for antifungal activity against several seed borne phytopathogenic fungi, during our regular screening *Lawsonia inermis* Linn recorded highly significant activity, Since the plant is already known to posses several biological activity, its capacity to inhibit seed borne fungi of sorghum seeds was evaluated in the present study.

Lawsonia inermis belongs to the family Lythraceae is known as henna in Arabic and mehndi in Hindi grows wild in abandoned areas (Muhammad and Mustafa, 1994). It is cultivated in tropical and warm temperate regions as a hedge plant. It is worldwide known as cosmetic agent used to stain hair, skin and nails (Hann *et al*., 1998) with antitumoural, antimicrobial and antituberculostatic effects (Malekzadeh, 1968; Kikuzaki and Nakatani 1993; Curreli *et al*., 2001; Dasgupta *et al*., 2003; Habbal *et al*., 2005; Singh and Pandey, 1989; Sharma, 1990; Kok *et al*., 2005; Saadabi, 2007). Also the serious oxidant effect of *L. inermis* has been recorded (Soker *et al*., 2000; Curreli *et al*., 2001; Dasgupta *et al*., 2003).

In addition, *Lawsonia inermis* can induce glucose-6-phosphate dehydrogenase (G6PD) enzyme deficiency in children (Zinkham and Oski 1996; Soker *et al*., 2000; Raupp *et al*., 2001; Lu *et al*., 2001). Syamsudim *et al*. (2008) proved that inai ethanol extract decreased the blood glucose and total cholesterol level. The leaves are rich in phenolic compounds which are found responsible for antioxidant activity (Khodaparast *et al*., 2007). Chloroform extract of henna displayed the cytotoxic effects (Endrini *et al*., 2002). The acetone soluble fraction of petroleum ether extract exhibited prominent nootropic activity. The fraction modified 5-HT and NA mediated behaviour. It is concluded that the leaves of *L. inermis* possess a potential for

exploring a nootropic principle (Iyer *et al*., 1998). All these reports indicates the potential of this plant in several human disease management, the present study is hence carried out to prove its efficacy as an antifungal component against phytopathogenic fungi associated with seeds. Seed treatment with already known medicinal plant is a better choice, which could display consumer and ecosafety.

MATERIALS AND METHODS

Collection and preparation of plant material

Fresh disease free leaves *Lawsonia inermis* L. were collected from Mysore, Karnataka, India. The leaves were washed thoroughly several times with running tap water and once with sterile distilled water. The leaf material was then air-dried on a sterile blotter under shade. A voucher specimen of the plant has been deposited in the herbarium of Department of Studies in Botany, University of Mysore, Mysore. Leaf samples (50g) of the plants were thoroughly washed, blot dried and macerated with 100 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 Whatmann No. 1 filter paper and heat sterilized at 120 ºC for 30 min. These extracts which served as a mother extract were allowed to cool to room tempera ture and their pH was determined just before subjecting it for antifungal activity assay. The mother extract was diluted to obtain 25% and 50% concentration. The extracts were preserved aseptically in sterile brown bottles at 5 ºC until further use.

In vivo antifungal activity

Sorghum seed sample belonging to the cultivar CSH-5 which recorded high degree of incidence of seed mycoflora with diverse species was selected for seed treatment studies. The seeds were soaked in different concentration of aqueous extract for 1, 2, 4, 6, 12 and 24 h. duration. After soaking seeds were dried on sterile blotter and subjected to further studies.

Control and treated seed samples were subjected to Standard Blotter Method (ISTA, 1996). Twenty-five seeds per plate were plated on three layer moistened blotter discs in petriplates. These plates were incubated at 22±2 ºC under alternating cycle of 12/12 h. of near ultraviolet (NUV) light and darkness for seven days. On the seventh day of incubation samples were screened for seed mycoflora with the help of stereo binocular microscope and also with the help of a compound microscope. Associated fungi were identified based on growth characteristics, colony and spore morphological characters using standard manuals. One hundred seeds from each treatment (Control and PAE treated) were subjected to germination and seedling vigour test by rolled paper towel method (ISTA, 1996). Four replicates were maintained for each treatment. Control and treated seeds were placed on the three layers of moist blotter sheets and rolled. These rolls were placed in trays containing sterile water at the bottom and covered by moist polyethylene covers and incubated for 8 days at 22±2 ºC.

Seed treatment with chemical fungicides

Sorghum seeds were also treated with chemical fungicides *viz.*, Blitox, Captan, Dithane M-45 and Thiram at the recommended dosage of 2g/l for 24 h. duration. Percent incidence of seed mycoflora and Seedling germination were calculated.

RESULTS

Seed germination was found increased in 1h. 2h. 4h. and 6h. at 100% concentration compared to control. The germination percentage was significant compared to Blitox, Dithane M-45 and Thiram, where as it is slightly less compa red to Captan (Table 1). Among four fungicides tested Thiram recorded highly significant antifungal activity against seed borne fungi. Complete inhibition of *Aspergi llus flavus, A. niger, A. ochraceous, Chaetomium globosum, Curvularia lunata, Drechslera halodes, F. moniliforme, F. solani, F. oxysporum, Penicillium* sp. *Phoma* sp. was observed at 24 h. treatment compared to control.

Even the plant extract completely inhibited the growth of *Aspergillus ochraceous, F. moniliforme, F. oxysporum, Phoma* sp. *Rhizopus stolonifer* and *Trichothecium roseum* at 100% concentration treated for 6h. with increase in germination. Even though, germination decreased after 6h. treatment with 100% concentration of the extract, *Aspergillus flavus, A. ochraceous, F. moniliforme, Fusarium* sp. *Penicillium* sp. and *Phoma* sp. were found completely inhibited. Where as the incidence of *Fusarium solani, F. oxysporum, Drechslera halodes, Curvularia lunata, A. alternata, Trichothecium* spp. and *Rhizopus* spp. was significantly inhibited in 24 h. treated seeds.The extract was found highly effective in inhibition of *Aspergillus* and *Fusarium* species in particular. Comparative evaluation of plant extracts with fungicides revealed that the *Lawsonia inermis* extract significantly inhibited the growth of several fungi compared to Blitox, Captan and Dithane M-45.

DISCUSSION

Many important diseases of plants caused by fungi are reported to be seed borne (Neergaard 1997). A seed borne pathogen present externally or internally or associated

Table 1 : Effect of plant extract of *Lawsonia inermis* L. and fungicides on seed mycoflora of Sorghum

Dosage	Period of soaking	Control	1 hour			2hour			4 hour			6 hour			12 hour			24 hour			Fungicides			
	Concentration		25%	50%	100%	25%	50%	100%	25%	50%	100%	25%	50%	100%	25%	50%	100%	25%	50%	100%	B	C	D	T
Sl. No.	Germination (%)	55.3 *ab*	57.0 *bcde*	57.6 *cdef*	58.6 *efgh*	59.6 *fgh*	59.7 *fgh*	60.2 *h*	58.7 *efgh*	60.0 *gh*	60.2 *h*	59.9 *gh*	60.0 *gh*	60.8 *hi*	55.6 *abc*	56.0 *abcd*	57.2 *bcde*	54.6 *a*	54.7 *a*	57.0 *bcde*	59.9 *gh*	62.8 *i*	57.9 *defg*	60.9 *hi*
	Fungi																							
1	**Alternaria alternata**	14.9 *h*	7.7 *cde*	6.8 *bcd*	6.9 *bcd*	7.8 *cde*	6.8 *bcd*	5.8 *bc*	9.8 *ef*	9.8 *ef*	6.8 *bcd*	8.6 *de*	6.0 *bc*	4.8 *ab*	8.7 *de*	6.7 *bcd*	5.8 *bc*	7.0 *bcd*	5.9 *bc*	4.7 *ab*	13.0 *gh*	6.0 *bc*	11.9 *fg*	3.0 *a*
2	*Aspergillus flavus*	5.8 *i*	5.8 *h*	5.6 *fgh*	3.9 *cdefg*	3.9 *cdefgh*	3.6 *cde*	2.6 *bc*	4.9 *defgh*	4.6 *defgh*	3.1 *bcd*	5.1 *efgh*	5.1 *defgh*	3.7 *cdef*	4.0 *cdefgh*	3.8 *cdefg*	0.0 *a*	4.7 *defgh*	1.5 *ab*	0.0 *a*	0.0 *a*	0.0 *a*	5.7 *gh*	0.0 *a*
3	*A. niger*	4.6 *c*	3.1 *bc*	3.0 *bc*	3.0 *bc*	2.8 *bc*	1.1 *ab*	0.0 *a*	3.8 *c*	2.8 *bc*	2.6 *bc*	3.8 *c*	2.7 *bc*	2.8 *bc*	2.9 *bc*	2.8 *bc*	0.0 *a*	2.7 *bc*	1.6 *ab*	1.2 *ab*	0.0 *a*	0.0 *a*	0.0 *a*	0.0 *a*
4	*A. ochraceus*	3.4 *c*	1.2 *b*	1.2 *b*	0.0 *a*	1.3 *b*	1.2 *b*	0.0 *a*	0.0 *a*	0.0 *a*	0.0 *a*	1.1 *b*	0.0 *a*	0.0 *a*	1.4 *b*	1.3 *b*	1.2 *b*	0.0 *a*	0.0 *a*	0.0 *a*	0.0 *a*	0.0 *a*	0.0 *a*	0.0 *a*
5	*Chaetomium globosum*	6.4 *ef*	6.2 *def*	4.9 *cdef*	3.0 *bc*	6.0 *def*	3.9 *bcde*	3.7 *bcd*	4.0 *bcde*	3.2 *bc*	1.6 *ab*	5.9 *def*	4.8 *cdef*	2.8 *bc*	5.1 *cdef*	2.9 *bc*	0.0 *a*	5.1 *cdef*	5.0 *cdef*	3.0 *bc*	6.7 *f*	5.2 *cdef*	4.2 *cdef*	0.0 *a*
6	*Curvularia lunata*	14.9 *j*	9.0 *def*	7.8 *cde*	0.0 *a*	10.9 *fgh*	9.7 *ef*	6.7 *c*	14.8 *j*	10.9 *fgh*	10.9 *fgh*	11.9 *ghi*	10.9 *fgh*	7.9 *cde*	8.8 *cdef*	7.8 *cde*	6.7 *c*	13.6 *ij*	9.9 *efg*	9.8 *ef*	12.9 *hij*	6.9 *bc*	2.6 *b*	0.0 *a*
7	*Drechslera halodes*	7.7 *g*	4.7 *cde*	3.6 *bcd*	2.9 *bc*	6.8 *fg*	6.0 *efg*	4.7 *cde*	5.6 *def*	4.8 *cde*	4.8 *cde*	5.6 *def*	5.3 *def*	5.4 *def*	4.0 *bcde*	3.7 *bcd*	0.0 *a*	3.1 *bc*	3.0 *bc*	2.6 *b*	0.0 *a*	0.0 *a*	0.0 *a*	0.0 *a*
8	*F.moniliforme*	4.8 *d*	2.7 *c*	1.2 *b*	1.8 *bc*	2.8 *c*	1.2 *b*	1.1 *b*	1.9 *bc*	1.3 *b*	1.2 *b*	0.0 *a*	0.0 *a*	0.0 *a*	2.6 *c*	1.4 *b*	0.0 *a*	1.6 *b*	0.0 *a*	0.0 *a*	1.0 *ab*	0.0 *a*	0.0 *a*	0.0 *a*
9	*F. solani*	6.6 *f*	3.8 *e*	3.7 *e*	1.5 *abc*	2.9 *bcde*	2.7 *bcde*	0.0 *a*	3.0 *cde*	2.8 *bcde*	1.5 *abcd*	3.4 *de*	2.7 *bcde*	1.1 *ab*	2.9 *bcde*	2.8 *bcde*	1.5 *abc*	2.8 *bcde*	2.8 *bcde*	1.4 *abc*	2.6 *bcde*	0.0 *a*	1.1 *ab*	0.0 *a*
10	*F. oxysporum*	4.7 *d*	3.0 *c*	2.9 *c*	0.0 *a*	3.1 *c*	1.5 *b*	1.4 *b*	1.5 *b*	1.0 *ab*	0.0 *a*	1.5 *b*	0.0 *a*	0.0 *a*	1.1 *ab*	0.0 *a*	0.0 *a*	3.4 *c*	2.9 *c*	1.4 *b*	2.8 *c*	1.0 *ab*	1.0 *ab*	0.0 *a*
11	*Fusarium* sp.	6.8 *g*	5.7 *fg*	4.7 *def*	3.0 *bcde*	3.7 *cde*	3.6 *cde*	0.0 *a*	4.8 *ef*	4.8 *ef*	3.6 *cde*	3.6 *cde*	3.0 *bcde*	1.4 *ab*	2.9 *bcde*	1.4 *ab*	0.0 *a*	3.7 *cde*	2.7 *bcd*	0.0 *a*	2.9 *bcde*	0.0 *a*	1.2 *ab*	2.7 *bc*
12	*Penicillium* sp.	6.8 *f*	4.6 *e*	3.8 *de*	3.7 *de*	3.7 *de*	2.9 *bcde*	1.5 *abc*	2.1 *bcd*	1.3 *ab*	0.0 *a*	3.9 *de*	3.1 *bcde*	3.1 *bcde*	3.0 *bcde*	2.6 *bcd*	0.0 *a*	4.0 *de*	3.0 *bcde*	0.0 *a*	0.0 *a*	0.0 *a*	2.9 *bcde*	0.0 *a*
13	*Phoma* sp.	6.5 *f*	3.8 *cd*	0.0 *a*	0.0 *a*	5.8 *ef*	5.8 *ef*	2.6 *bc*	5.6 *ef*	2.8 *bc*	0.0 *a*	4.8 *def*	3.7 *cd*	0.0 *a*	3.8 *cd*	2.7 *bc*	0.0 *a*	3.3 *cd*	2.6 *bc*	0.0 *a*	1.2 *ab*	2.8 *bc*	4.2 *cde*	0.0 *a*
14	*Rhizopus stolonifer*	7.8 *h*	5.6 *fg*	4.6 *defg*	0.0 *a*	5.0 *efg*	2.9 *bcd*	0.0 *a*	5.8 *g*	0.0 *a*	0.0 *a*	4.9 *efg*	4.7 *efg*	0.0 *a*	5.7 *g*	1.5 *ab*	0.0 *a*	5.6 *fg*	3.6 *cde*	2.8 *bc*	0.0 *a*	5.7 *g*	3.9 *cdefg*	3.8 *cdef*
15	*Sterile mycelia*	6.0 *g*	2.8 *defg*	1.2 *ab*	1.1 *a*	1.8 *efg*	4.0 *cdef*	1.1 *ab*	4.0 *cdef*	3.3 *cde*	2.9 *bcd*	2.7 *bcd*	2.7 *bc*	2.7 *bc*	5.7 *fg*	0.6 *a*	1.1 *ab*	3.7 *cde*	2.6 *bc*	1.3 *ab*	2.8 *bc*	0.0 *a*	3.0 *bcde*	0.0 *a*
16	*Trichothecium roseum*	4.8 *f*	3.6 *ef*	2.7 *ef*	1.1 *ab*	3.6 *def*	2.7 *bcd*	1.1 *ab*	2.8 *bcd*	2.7 *bcd*	0.0 *a*	3.0 *cde*	1.7 *abc*	0.0 *a*	3.0 *cde*	1.1 *ab*	1.4 *abc*	3.7 *def*	3.1 *cde*	2.6 *bcd*	1.2 *ab*	1.1 *ab*	1.3 *abc*	2.7 *bcd*

Incidence based 400 seed each on standard blotter method

The means followed by the same letter(s) are not significantly different at $P \leq 0.05$ when subjected to DMRT (row by row comparison)

B : Biltox C: Captan D : Dithane M-45 T – Thiram

with the seed as contaminant may cause seed abortion, seed rot, seed necrosis, reduction or elimination of germination capacity as well as seedling damage resulting in development of disease at later stages of plant growth by systemic or local infection (Khanzada *et al.*, 2002; Bateman and Kwasna, 1999). The screening of such mycoflora is a regular process and is important for developing seed treatment strategies. During regular screening fungi such as *Alternaria alternata, Aspergillus flavus, A. fumigatus, A. niger, Cladosporium* sp., *Fusarium moniliforme, F.oxysporum, F. pallidoroseum, Drechslera tetramera, Nigrospora* sp., *Phoma* sp., and *Rhizopus* sp., were reported to be common mycoflora present in the sorghum seeds (Abdullah and Kadhum, 1987). The present study also reports the incidence of 16 fungi including *Aspergillus* sp., *Fusarium* sp., *Alternaria alternata*, *Chaetomium globosum*, *Curvularia lunata*, *Drechslera halodes*, *Rhizopus stolonifer* and *Trichothecium roseum*. Recently from 27 samples of sorghum, 14 genera and 23 species of fungi, several fungi *viz., Aspergillus sulphureus, Nigrospora oryzae, Trichoderma hamatum, Fusarium subglutinans, Piptocephalis* sp., and *Syncephalastrum racemosum* were found not been reported earlier on sorghum seeds. The screening also identified *Alternaria alternata, Aspergillus* sp., *A. candidus, A. flavus, A. niger, A. sulphureus, Curvularia* sp., *C. lunata, Cladosporium* sp., *Drechslera* sp., *D. halodes, D. tetramera, D. hawaiiensis, Nigrospora oryzae, Trichoderma hamatum, Trichothecium roseum, Piptocephalis* sp., *Syncephalastrum racemosum, Fusarium moniliforme, F. subglutinans, Penicillium* spp., *and Rhizopus* sp.

All the reports indicates the contamination of seeds by several fungi which can be managed by proper seed treatment and hence in the present study aqueous extract of Lawsonia inermis was tested for its efficacy as a antifungal seed protectant. The results were highly promising as many of the seed mycoflora were found inhibited without affecting the seed germination compared to control. The results is highly significant even compared to fungicides such as Blitox, Captan, Dithane M-45 and Thiram indicating the possible application of this extract with already available fungicides to bring down the usage of synthetic fungicides and indirectly its side effects on several biological system.

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