

Protection of maize by storage fungi and aflatoxin production using botanicals

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In the present study the effect of alcoholic, hydroalcoholic and aqueous extracts of *Lawsonia inermis* Linn. (Henna), *Murraya paniculata* (Linn.) Jack (Orange jessmine) and *Psidium guajava* Linn. (Common guava) leaves on *Aspergillus flavus* and *A. parasiticus* growth and aflatoxin production and on growth in corn under storage conditions was studied. The extract that most effectively inhibited growth as well as aflatoxin production during *in vitro* experiments was found to be aqueous leaf extract of *L. inermis* and *M. paniculata*. The MIC obtained with *L. inermis* leaf aqueous extract was 5 mg/mL and 10 mg/mL of culture medium for *A. flavus* and *A. parasiticus*, respectively. Whereas it was 8 mg/mL and 9 mg/mL for *A. flavus* and *A. parasiticus*, respectively with *M. paniculata* leaf aqueous extract. A combination of both extracts was also assayed for the MIC, inhibition of aflatoxin production and applied to maize grains for prevention of infection and aflatoxin synthesis in storage conditions. The MIC obtained for combination of both extracts was 4 mg/mL for both test fungi. Aflatoxins were assayed qualitatively and quantitatively by TLC under 365 nm. The MIC of both extracts was found higher (>30 mg/mL) when examined in maize grains. However, concentrations lower than the MIC drastically inhibited the production of aflatoxins in culture medium and also in maize grains. Half of the MIC inhibited 99% of aflatoxins.

Keywords: Aflatoxins, *Aspergillus spp.*, Medicinal plants, Stored maize.

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Introduction

Approximately 25-40% raw agricultural products world wide are susceptible to invasion by the aflatoxigenic *Aspergillus flavus* and *Aspergillus parasiticus*. They destroy significantly foodstuffs and grains such as peanuts, corn, tree nuts, cottonseeds, etc. and cause economic loss^{1,2}. Synthesis of aflatoxins at some stage of postharvest processing, transportation, storage and grain development cause grain quality loss and renders them unfit for human consumption³. Aflatoxins are most dangerous secondary metabolites which are toxic to certain domesticated animals as well as humans⁴. They are hepatocarcinogenic, cause pulmonary interstitial fibrosis⁵, liver cirrhosis, depressed immune response, tumour induction and teratogenesis⁶. Production of aflatoxins in plants inhibit root elongation, chlorophyll and carotenoid synthesis, seedling growth, seed germination and retard synthesis of some enzymes⁷. So it is a challenge to store food products and grains safely from toxin producing moulds.

Synthetic chemical fungicides have greatly attributed to management of losses by aflatoxin producing fungi in stored products. But indiscriminate

application of synthetic fungicides has resulted in many problems such as long term ingestion of HCB (Hexachlorobenzene) treated grain lead to toxic porphyria or poisoning in humans^{8,9} and captan, captafol and folpet which are widely used to protect seed and stored products are responsible for irritation in skin, dermal sensitization and several respiratory problems^{10,11}. These all troubles attracted the attention of scientists towards alternative biological methods including use of botanicals as insecticides.

Plants contain antimicrobial secondary metabolites hence they are used for antifungal, antibacterial and nematocidal activities. Extracts of several plants have been screened for antifungal activity against *Aspergillus spp.*^{3,4,6,12}. These alternatives are biodegradable, renewable in nature, safe to human as well as plant health and simple in application. Hence present study reports *in vitro* and *in vivo* inhibitory effect of leaf extracts of *Lawsonia inermis* Linn. (Henna) (Plate 1), *Murraya paniculata* (Linn.) Jack (Orange jessmine) (Plate 2) and *Psidium guajava* Linn. (Common guava) (Plate 3) and their best combination for antifungal activity against *A. flavus* and *A. parasiticus* with ultimate aim of developing plant based formulation for safe storage of grains and to reduce or inhibit the aflatoxin production by these fungi.

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Plate 1—*Lawsonia inermis*Plate 3—*Psidium guajava*Plate 2—*Murraya paniculata*

Materials and Methods

Test Material

Pathogen: Toxicogenic strains of *Aspergillus flavus* (Navjot 4Nst) and *A. parasiticus* var. *globosus* (MTCC 411) were procured from Department of Botany, Banaras Hindu University, Varanasi and IMTECH, Chandigarh, India respectively. The fungi were grown on PDA (Hi Media) at 30°C for 7 days and the spore suspension was obtained by washing the surface of cultures with tween 80 (0.5 ml/l).

Preparation of crude extract: Fresh leaves of *L. inermis*, *M. paniculata* and *P. guajava* were collected from University College of Science, Udaipur campus. The plants were identified by Dr. Roop Singh Rathore, incharge of herbarium, Department of Botany, University of Rajasthan, Jaipur, India and were given voucher specimen numbers RUBL 20427 (*L. inermis*), RUBM 20223 (*M. paniculata*), RUBP

21526 (*P. guajava*), respectively. Crude extracts were prepared by grinding 20 g of fresh leaves with 100 ml absolute alcohol, 50% alcohol and sterile distilled water, respectively. The mixtures were allowed to stand for 24 h at room temperature and were subsequently filtered through Whatman filter paper No. 1. The filtrate was vacuum dried in rotary evaporator and stored at 4°C. These crude extracts were used for further experimental work.

Analysis of aflatoxins

Qualitative and quantitative analysis of aflatoxins was done using thin layer chromatography of aflatoxin extracted. A 5 mm fungal disc of 7 day old culture of test fungi was inoculated into 100 ml SMKY broth and incubated on a rotary shaker at 28 ± 2°C for 10 days. The culture medium was filtered through Whatman no. 1 filter paper and filtrate was extracted with 20 ml CHCl₃. Chloroform extract was evaporated to dryness and residue was then redissolved in 1 ml CHCl₃. 50 µl of the extract was spotted on the TLC plates. Plates were developed in toluene: isoamylalcohol: methanol (90:32:2). Qualitative estimation on TLC plate was done by visual examination of color of fluorescent spots under UV light (365 nm) and confirmation was done by R_f value obtained. Quantitative estimation of aflatoxin was done by spectrometric method¹³. The fluorescent spots were scrapped off from the plates, dissolved in

5 ml cold CH₃OH and centrifuged (3000 rpm, 5 min). Absorbance of supernatant was taken on 350 nm. The amount of AFB1, AFB2, AFG1 and AFG2 was calculated according to the following formula:

$$Af\ content\ (\mu g/mg) \times 10^2 = \frac{D \times M}{E \times L} \times 1000$$

Where,

D=Absorbance (nm), M=Molecular weight of aflatoxins (g/ml), E=Molar extinction coefficient of aflatoxins (m²/mol), L=Path Length (1 cm cell was used) (cm).

***In vitro* antifungal activity**

Requisite amount of various extracts was mixed with 20 ml of Czapek's Dox Agar (CDA) medium to get final concentration of 100, 200 and 1000 µg/ml. Antifungal screening was done by poison food technique¹⁴. Two types of control were used for experiment; 20 ml of CDA served as a negative control whereas 20 ml of CDA containing 100 mg of bavistin so as to get a concentration 100 µg /ml were used as positive control. 5 mm disc of 7 day old culture of each test fungus was used as inoculum and plates were incubated for 7 days at 30 ± 1°C. Diameter of fungal growth on each Petri dish was measured daily. Three replicates of the experiment were maintained. Percent inhibition of growth of test fungi was calculated by following formula:-

$$\% \text{ inhibition of growth of test fungi} = \frac{DC-DT}{DC} \times 100$$

Where, DC is average increase in mycelial growth in control and DT is average increase in mycelial growth in treatment.

Determination of minimum inhibitory concentration (MIC)

The MIC values of the selected extracts for *A. flavus* and *A. parasiticus* was determined according to broth dilution method¹⁵. Different concentration of aqueous extract (50-1000 µg/ml) was prepared in 10 ml of CD broth. Inoculation was done with fungal spore suspension (1×10⁶ spores/ml) in 0.1% Tween 80 and tubes were incubated as a static culture at 30° C for seven days. CD broth with 1 ml inoculum served as control. To determine that if the inhibition was reversible the tubes that did not show visible growth were sub-cultured on CDA plates. The MIC values of extract were compared with the MICs of commonly used fungicides *i.e.* bavistin.

***In vitro* inhibition of aflatoxin production**

Extracts gave best inhibitory activity against growth of test fungi were mixed and combination was used for study of aflatoxin inhibition. 50, 75, 100, 125, 150, 175, 200 µg/ml concentrations were prepared by mixing the appropriate amount of extract with SMKY medium. A fungal disc from 7 day old culture of test fungi was inoculated into each flask and incubated on a rotary shaker at 28 ± 2°C for 10 days. Rest of the method was similar as above. The inhibition of aflatoxin was estimated as difference in concentration of aflatoxin in extract treated broth as compared to control. Each experiment was repeated three times and three replicates were maintained.

***In vivo* antifungal activity**

To find out the practical applicability of the plants for protection of stored maize grains, combination of both plant extracts and dry leaf powder were separately used as antifungal formulation. Freshly harvested maize samples were used for the experiment. The experiment was designed according to method suggested by Kumar *et al* (2007)³. The treatment of maize grains with extracts dissolved in water¹⁶. Various concentrations of the combined extract of *L. inermis* and *M. paniculata* leaves was prepared so as to obtain final concentration of 10, 20, 30, 40, 50, and 100 mg/g maize grains. In experiment two types of treatments were used, uninoculated and inoculated treatment. In uninoculated treatment dry leaf powder (10 g powder/100 g grains) was introduced into separate plastic containers containing 500 g of maize. Similarly 500 g extract treated seeds were as such stored in separate plastic containers. In inoculated treatment maize samples, prior to treatment with extract or powder, was inoculated with a standard spore suspension of *A. flavus* and *A. parasiticus* in separate jars. After 3 days of inoculation, the samples, similar to the first set, were separately treated by the extract as well as dried leaf powder. The control set also contained two set, uninoculated and inoculated control. Uninoculated control contains maize grains as such stored in plastic containers. In inoculated control maize grains was inoculated with spore suspension of *A. flavus* and *A. parasiticus* and stored similarly.

The inoculation of maize seeds was done by preparing a standard spore suspension of both *A. flavus* and *A. parasiticus*. Spore suspensions of fungal strains were prepared in 0.1% tween 80 and the spore density was adjusted to 285×10⁴ spore/mL. The seeds

were dipped in normal spore suspension for 10 min and then dried in an oven at 45°C for 12 h. The moisture content of the dried maize was 11.13%. Three replicates were taken for each individual set.

After 6 months of storage at laboratory conditions (temperature 10-46°C and RH 30-80%) maize samples of all treatments and control sets were analyzed for fungal growth and aflatoxin production. Fungal species grown in 6 months were analyzed by standard blotter method. Aflatoxins were extracted from grains with chloroform and analyzed quantitatively and qualitatively by the method given above. The MIC value of extract and dry powder was determined in inoculated sets of treatment. After the analysis of fungi associated with maize samples of treatment and control sets, the percent occurrence of each fungus among the samples was analyzed by following formula:

$$\% \text{ occurrence of fungus} = \frac{\text{No of colonies of the fungus}}{\text{Total no of colonies of fungal species}} \times 100$$

Results and Discussion

Aflatoxin producing ability of test fungi

Qualitative analysis of TLC plates under 365 nm UV light suggests the presence of two types of aflatoxins i.e. B and G. Blue fluorescence was indicator of presence of B aflatoxins whereas green colored spots were aflatoxins G. Total four type of aflatoxins were recognized on the basis of R_f value obtained i.e. B1, B2, G1 and G2. All four types were extracted from *A. parasiticus* culture whereas *A. flavus* cultures were found with only B1 and B2. Presence of four aflatoxins, viz. B1, B2, G1 and G2 was already reported in *A. parasiticus* culture¹⁷ whereas in *A. flavus* only B1 and B2 were observed¹⁸. Quantitative estimation expressed that the amount of aflatoxin B1 was highest followed by B2. Aflatoxin G1 and G2 was present in minor quantity. The amount of aflatoxins B1 and B2 extracted from *A. flavus* was 407.66 µg/kg and 281.71 µg/kg, respectively whereas it was 381.16, 329.85, 228.89 and 252.65 µg/kg of B1, B2, G1 and G2, respectively from *A. parasiticus* cultures.

In vitro antifungal activity

Results of *in vitro* antifungal activity of all plant extracts are presented in Tables 1-3. Observations suggest that *L. inermis* and *M. paniculata* had significant antifungal activity. Aqueous extract of *L. inermis* had highest inhibitory effect on *A. flavus* as well as *A. parasiticus* followed by aqueous extract of

M. paniculata. Aqueous extract of *P. guajava* had less antifungal activity. As compared to individual extract combined extract of *L. inermis* and *M. paniculata* was more effective against both test fungi as presented in Table 3. Thus increase in activity demonstrates a possible synergistic activity between the chemical constituents of both extracts. The synergistic effect of various plant extracts, essential oils and fungicides is already reported by some authors¹⁹⁻²². Also, the biodeteriorating fungi can easily develop resistant traits against a single active component but the plant extract containing different antimicrobial ingredients may be exploited for fungitoxic potency owing to the synergistic effects of different components²³.

Minimum inhibitory concentration (MIC)

The MIC value of all three extracts and their combination are presented in Table 4. *L. inermis* crude aqueous extract had minimum MIC value i.e. 500 µg/mL and 1000 µg/mL against *A. flavus* and *A. parasiticus*, respectively. *M. paniculata* aqueous extract showed MIC on 1680 µg/ml and 1600 µg/ml against *A. flavus* and *A. parasiticus*, respectively. Combination of both leaf extracts showed minimum inhibition of both test fungi on 250 µg/mL. Bavistin, the commonly used synthetic fungicide had 165 µg/ml and 150 µg/ml MIC against *A. flavus* and *A. parasiticus*, respectively. All extracts tested showed only fungistatic activity even on higher concentrations also. Fungistatic properties do not indicate an ineffectiveness to control fungal deterioration and it is noteworthy that fungi-stats have also been found to be successful in preventing fungal development on stored products²⁴.

In vitro efficacy of combined extract in checking aflatoxins production

Prevention/inhibition of aflatoxin production in stored food commodities reduces the chances of hepatic cancer and various other diseases caused in humans and animals due to the consumption of aflatoxin contaminated food. Table 5 summarizes the effect of combined extract of *L. inermis* and *M. paniculata* on aflatoxins B1 production by *A. flavus* and *A. parasiticus*. The extract showed pronounce efficacy in arresting aflatoxin production by the toxigenic strains of *A. flavus* and *A. parasiticus*. Combined extract inhibited AFB1 synthesis completely at 125 µg/mL from both fungi despite the mycelial growth was inhibited on 250 µg/mL. The mycelium growth and aflatoxin inhibition was recorded to decrease on increasing the concentrations of

Table 1—Antifungal activity of crude extracts of *Lawsonia inermis*, *Psidium guajava* and *Murraya paniculata* against *Aspergillus flavus*

	Colony diameter (mm) after 7 days								
	<i>L. inermis</i>			<i>M. paniculata</i>			<i>P. guajava</i>		
	A	B	C	A	B	C	A	B	C
0	40±0.004	40±0.004	40±0.004	40±0.004	40±0.004	40±0.004	40±0.004	40±0.004	40±0.004
100	27±0.012	30±0.002	14±0.011	29±0.013	28±0.013	26±0.004	32±0.023	34±0.032	34±0.014
200	19±0.008	28±0.001	12±0.022	28±0.011	26±0.014	25±0.011	31±0.002	31±0.011	30±0.005
300	17±0.023	27±0.011	8±0.002	22±0.013	21±0.004	20±0.001	29±0.006	30±0.001	27±0.013
400	14±0.013	21±0.023	5±0.021	19±0.014	20±0.041	18±0.003	21±0.032	28±0.013	27±0.006
500	10±0.000	19±0.034	5±0.011	16±0.022	18±0.021	5±0.012	19±0.012	26±0.034	26±0.006
600	8±0.011	15±0.012	5±0.004	10±0.012	15±0.001	5±0.024	17±0.004	23±0.022	24±0.003
700	6±0.012	9±0.001	5±0.004	8±0.029	9±0.006	5±0.021	15±0.005	19±0.021	23±0.013
800	5±0.013	9±0.004	5±0.003	6±0.033	7±0.056	5±0.034	12±0.001	17±0.081	21±0.024
Concentration (µg/ml 900)	5±0.012	5±0.012	5±0.024	5±0.011	5±0.021	5±0.021	9±0.002	14±0.005	18±0.035
1000	5±0.012	5±0.003	5±0.012	5±0.021	5±0.002	5±0.003	7±0.071	12±0.002	16±0.048

Each data represents the average of three replications, A= 100% alcoholic, B = 50% alcoholic, C= 100% aqueous

Table 2—Antifungal activity of crude extracts of *Lawsonia inermis*, *Psidium guajava* and *Murraya paniculata* against *Aspergillus parasiticus*

Concentration (µg/ml)	Colony diameter (mm) after 7 days								
	<i>L. inermis</i>			<i>M. paniculata</i>			<i>P. guajava</i>		
	A	B	C	A	B	C	A	B	C
0	42±0.056	42±0.056	42±0.056	42±0.056	42±0.056	42±0.056	42±0.056	42±0.056	42±0.056
100	26±0.067	29±0.089	13±0.021	28±0.022	27±0.005	26±0.024	31±0.024	33±0.034	34±0.011
200	18±0.024	27±0.007	13±0.011	27±0.012	26±0.008	24±0.021	30±0.032	30±0.044	30±0.023
300	16±0.056	26±0.002	12±0.045	21±0.011	21±0.002	20±0.033	28±0.056	29±0.041	26±0.003
400	13±0.078	20±0.034	7±0.087	18±0.021	20±0.012	16±0.033	20±0.013	27±0.002	26±0.004
500	9±0.007	18±0.005	5±0.023	15±0.011	17±0.013	5±0.066	18±0.022	25±0.003	25±0.016
600	7±0.045	14±0.003	5±0.034	9±0.022	15±0.002	5±0.000	16±0.001	22±0.034	24±0.028
700	7±0.007	8±0.045	5±0.021	7±0.012	8±0.045	5±0.000	14±0.000	18±0.018	23±0.014
800	7±0.008	8±0.038	5±0.011	5±0.011	5±0.034	5±0.031	11±0.000	17±0.019	20±0.011
900	5±0.030	8±0.031	5±0.010	5±0.022	5±0.033	5±0.021	8±0.023	14±0.002	18±0.000
1000	5±0.000	6±0.034	5±0.023	5±0.032	5±0.066	5±0.012	6±0.003	11±0.003	15±0.022

Each data represents the average of three replications, A= 100% alcoholic, B = 50% alcoholic, C= 100% aqueous

the extract. It was found that reduction in mycelial biomass caused significant reduction in aflatoxin production. Lysis of the mycelia and spores of the toxigenic fungi is one of the characteristics of aflatoxin deactivation process which may proliferate under favourable conditions²⁵. Hence, in order to reduce the aflatoxin production, mycelial growth must be below the threshold limit so that aflatoxin cannot be produced. A significant observation was that for a slight increase in extract concentration there was an abrupt decline in aflatoxin synthesis in spite of a gradual decrease in mycelial biomass.

***In vivo* efficacy of combined aqueous extract of *L. inermis* and *M. paniculata* against fungal deterioration in stored maize**

The combined extract and dried powder of leaves showed its efficacy in control of fungal association of

Table 3—Antifungal activity of combined aqueous extract of *Lawsonia inermis* and *Murraya paniculata*

Concentration (µg/ml)	Colony diameter (mm) after 7 days	
	<i>A. flavus</i>	<i>A. parasiticus</i>
0	40±0.004	42±0.056
100	18±0.031	16±0.021
200	5±0.004	7±0.021
300	5±0.013	5±0.000
400	5±0.023	5±0.000
500	5±0.000	5±0.000
600	5±0.000	5±0.000
700	5±0.000	5±0.000
800	5±0.000	5±0.000
900	5±0.000	5±0.000
1000	2±0.002	2±0.011

Each data represents the average of three replications.

maize samples. The maize samples of uninoculated control set shows the presence of six fungi. *A. flavus* was found to be dominant fungus, followed by *A. parasiticus*, *Penicillium sp.*, *A. niger*. In extract and mixed dry leaf powder of *L. inermis* and *M. paniculata* treated grains, appearance of all the fungi was checked. Thus both dry leaf powder as well as extract significantly protected the maize samples from *A. flavus* and *A. parasiticus* as well as other storage fungi (Table 6).

MIC in maize grains under storage conditions

The MIC value of extract and dry powder were higher in storage conditions than culture media. In

terms of practical application the MIC value of combined extract was 33-40 mg/g for extract against *A. flavus* and 40-45 mg/g against *A. parasiticus*. Combination of dry leaf powder was less active against both fungi had MIC value ranging from 50-65 mg/g for *A. flavus* and *A. parasiticus* respectively.

Effect on aflatoxin synthesis under storage conditions

Production of aflatoxins in storage conditions was very low as compared to liquid media. Addition of extracts and dry powder to maize grains at concentrations lower than the MIC greatly inhibited the aflatoxin production by *A. flavus* and *A. parasiticus*. At 50% of the MIC value of the extract

Table 4—Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of selected leaf extracts against *A. flavus* and *A. parasiticus*

Type of extract	MIC (µg/ml)		MFC (µg/ml)	
	<i>A. flavus</i>	<i>A. parasiticus</i>	<i>A. flavus</i>	<i>A. parasiticus</i>
<i>L. inermis</i> aqueous	500	1000	Not up to 2500	Not up to 2500
<i>M. paniculata</i> aqueous	1680	1600	Not up to 2500	Not up to 2500
<i>P. guajava</i> aqueous	2500	Not up to 2500	Not up to 2500	Not up to 2500
Mixture of <i>L. inermis</i> and <i>M. paniculata</i>	250	250	Not up to 2500	Not up to 2500
<i>Bavistin</i>	165	150	Not up to 2500	Not up to 2500

Each data represents the average of three replications

Table 5—Effect of combined aqueous extract of *L. inermis* and *M. paniculata* on *A. flavus* and *A. parasiticus* biomass and aflatoxin B1 production

Extract (µg/ml)	<i>A. flavus</i>		<i>A. parasiticus</i>	
	Mycelial dry weight (g)	Mycotoxin content (µg/kg)	Mycelial dry weight (g)	Mycotoxin content (µg/kg)
0	0.444 ± 0.006	244.220 ± 20.19	0.498 ± 0.056	287.22 ± 27.19
50	0.300 ± 0.040	141.19 ± 16.63	0.430 ± 0.140	156.19 ± 13.83
75	0.214 ± 0.008	61.05 ± 13.75	0.319 ± 0.078	74.05 ± 12.56
100	0.146 ± 0.004	8.10 ± 4.58	0.286 ± 0.244	6.10 ± 4.54
125	0.107 ± 0.012	0.000 ± 0.000	0.149 ± 0.082	0.000 ± 0.000
150	0.096 ± 0.000	0.000 ± 0.000	0.084 ± 0.018	0.000 ± 0.000
175	0.084 ± 0.000	0.000 ± 0.000	0.072 ± 0.000	0.000 ± 0.000
200	0.073 ± 0.000	0.000 ± 0.000	0.069 ± 0.000	0.000 ± 0.000
225	0.062 ± 0.001	0.000 ± 0.000	0.051 ± 0.004	0.000 ± 0.000
250	-	-	-	-

Each data represents the average of three replications ± S.E.

Table 6—Percent occurrence of mycoflora on MIC in stored maize samples treated with combined aqueous extract as well as dry powder of *L. inermis* and *M. paniculata* after 6 month of storage

Fungi isolated	Aqueous extract				Dry powder			
	Uninoculated samples		Inoculated samples		Un-inoculated samples		Inoculated samples	
	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment
<i>A. flavus</i>	46.7 ± 0.56	0.00 ± 0.00	87.0 ± 0.45	9.3 ± 0.8	46.7 ± 0.56	0.00 ± 0.00	87.0 ± 0.45	10.35 ± 0.47
<i>A. parasiticus</i>	9.7 ± 0.3	0.00 ± 0.00	56.0 ± 0.23	0.45 ± 0.56	9.7 ± 0.3	0.00 ± 0.00	56.0 ± 0.23	0.89 ± 0.40
<i>A. terreus</i>	1.3 ± 0.3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.3 ± 0.3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
<i>A. candidus</i>	3.7 ± 0.98	0.00 ± 0.00	4.3 ± 0.45	0.00 ± 0.00	3.7 ± 0.98	0.00 ± 0.00	4.3 ± 0.45	0.00 ± 0.00
<i>Penicillium citrinum</i>	42.7 ± 1.05	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	42.7 ± 1.05	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
<i>A. niger</i>	14.3 ± 1.03	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	14.3 ± 1.03	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Each data represents the average of three replications ± S.E.

and dry powder >99% reduction in production of all types of aflatoxins in maize grains by *A. flavus* and *A. parasiticus* and 75% of MIC value inhibit the aflatoxin at undetectable level. Thus the results suggest that the extracts were significantly inhibited the aflatoxin synthesis from the *A. flavus* and *A. parasiticus*.

Hence, the important reduction of mycelial growth and mycotoxin production during *in vitro* as well as in storage conditions suggests that phytochemical compounds isolated from these extracts could be used alone or in conjunction with other substances or processes to control the presence of toxic metabolites in corn. The synergistic effect of the extracts was significant in both mycelial inhibition and toxin production.

Conclusion

On the basis of results obtained it can be concluded that *L. inermis* and *M. paniculata* leaf extracts possess significant antifungal activity against both strains. The extracts may be used as plant based ideal preservative for safe storage of food commodities after clinical trial studies.

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