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Efficacy of Botanicals against *Aspergillus flavus* and Aflatoxin Synthesis Inhibition in Peanut

M.A. Gud, S.A. Anarase, P.U. Bhosale and C.D. Deokar
Mahatma Phule Agriculture University, Rahuri, Maharashtra, India

Abstract

Peanut is frequently contaminated with aflatoxins which is the most toxic and is therefore listed as a group I carcinogen. Efficacy of botanicals were evaluated against *A. flavus* growth inhibition and aflatoxin synthesis in kernels of peanut cultivar SB-11. Methanolic extract of pomegranate fruit peel [3000 ppm concentration] was significantly found effective with minimum colony diameter of 30.67 mm without sporulation having 54.68 per cent growth inhibition of *A. flavus* over control and no aflatoxin was detected by qualitative method on PDA medium at *in-vitro* condition. Concentration of 3000 ppm was significantly found to be effective with minimum pod colonization severity of 26.67 per cent having 72.88 per cent growth inhibition. Also, kernel colonization severity of 26.67 per cent having 70.37 per cent growth inhibition of *A. flavus* over control was observed.

Introduction

Peanut (*Arachis hypogea* L.) is an important oilseed as well as legume which plays an essential role in terms of income for the rural people having marginal and sub-marginal land. The oil may be extracted and used for cooking, and the residual cake is used for production of food or more commonly, in animal feeds (1, 2). Peanut is frequently contaminated with aflatoxins which is the most toxic and is therefore listed as a group I carcinogen by the International Agency for Research on Cancer (3). *A. flavus* is one of the major fungal pathogen producing it. In India, many commercial peanut cake and seed samples tested contained aflatoxin, levels even reaching as high as 8000 g kg^{-1}. The permissible content of AFB is also regulated in china 20 ug/kg for corn, peanut core, peanut oil (4).

Harmful effects posed by aflatoxins are teratogenicity i.e. deformation of developing fetus (5), reduction in RBC, WBC and hemoglobin content in blood (6), delayed blood clotting (7) and suppression of immune system in case of chronic poisoning. Aflatoxins reach human beings through food chain as they were detected in milk, egg and meat when the animals were fed on contaminated diet. Aflatoxin formation in peanut pod or karnel, before or after crop harvest cannot be prevented in any known practical way, but it can sometimes be reduced by appropriate management practices (8). The spraying of fungicides due to its chemical and hazardous nature is less reliable. Many plant extracts and oils have been reported as safe and effective inhibitors of toxigenic fungi (9). Hence, during the present investigation efficacy of botanicals were evaluated against growth of *A. flavus* and inhibition of aflatoxin synthesis in kernels of peanut at laboratory condition.

Materials and Methods

The fungus *A. flavus* was isolated from the soil samples collected from peanut filed collected during flowering stage of peanut using AFPA medium by dilution plating method (10). Leaves of Neem (*Azadiracta indica* A. Juss), leaves of Pomegranate (*Punnica granatum*), flowers of Ghaneri (*Lantana camera*), bulb of onion (*Allium cepa* L.), cloves of garlic (*Allium sativum* L.) and fruit peel of Pomegranate (*Punnica granatum*) extracts were prepared and used during the investigation.

Antifungal activity of plant extract against *A. flavus* (PFT) : Antifungal activity of plant extract against *A. flavus* was carried out by poisoned food technique (Bora, 2008). Plant extracts were used as hundred per cent concentration. All the six plant extracts were tested against the *A. flavus* on the sterilized potato dextrose agar (PDA) in 1:2 propertions (11). The control set was runned using distilled water instead of plant extract. All the petri plates were incubated at room temperature (27$\pm$1^{o}C). The observation on colony diameter, sporulation of the test pathogen, aflatoxin detection were recorded for each treatment were recorded daily. The inhibition of mycelial growth of inoculated pathogen was worked out by using formula suggested by (12).

Antifungal activity of methanolic extract of pomegranate fruit peel against *A. flavus* (PFT) : The peel of fruits were manually removed, shade dried and powdered in grinder to 40 mesh. Dried powder of peels (15g) was extracted with 100 ml (80%) of methanol at room temperature for 1 hours. The extract was filtered through Whatman No. 42 filter paper to remove fine particles. The residue was re-extracted again with methanol. After extraction, the solvent was evaporated on a rotary evaporator under vacuum at 30^{0}C up to 20 ml and

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Table-1 : Antifungal activity of different plant extracts against *A. flavus* by poisoned food technique.

Tr. No.	Treatment details	*Mean colony diameter (mm)		Growth inhibition (%)		Degree of sporulation		Aflatoxin detection	
		5th Day	8th Day	5th Day	8th Day	5th Day	8th Day	5th Day	8th Day
T1	*A. indica* (Leaves)	41.33 (40.01)	42.00 (40.39)	38.92	53.33	Moderate	Moderate	+	+
T2	*A. cepa* L (Bulb)	42.00 (40.39)	43.00 (40.98)	37.93	52.22	Poor	Poor	-	+
T3	*L.camera* (Flowers)	52.67 (46.53)	53.00 (46.72)	22.17	41.11	Moderate	Moderate	+	+
T4	*P. granatum* (Leaves)	39.00 (38.64)	40.67 (39.62)	42.36	54.81	Moderate	Moderate	-	-
T5	*A.sativum* L (Cloves)	42.67 (40.78)	43.67 (41.36)	36.95	51.48	Moderate	Moderate	-	+
T6	*P.granatum* (Fruit peel)	32.00 (34.44)	33.00 (35.04)	52.71	63.33	Poor	Poor	-	-
T7	Control	67.67 (55.35)	90.00 (71.57)	-	-	Abundent	Abundant	++	++
	SE±	0.65	0.77						
	CD (0.05)	1.97	2.33						

*Figures in the parenthesis are arc sin transformed values.

Table-2 : Antifungal activity of methanolic extract of pomegranate fruit peel against *A. flavus* by poisoned food technique.

Tr. No.	Treatment details	*Mean colony diameter (mm)		Growth inhibition (%)		Degree of sporulation		Aflatoxin detection	
		5th Day	8th Day	5th Day	8th Day	5th Day	8th Day	5th Day	8th Day
T1	100 ppm	61.00 (51.36)	62.67 (52.34)	9.85	30.37	Abundant	Abundant	+	++
T2	500 ppm	49.33 (44.62)	51.00 (45.57)	27.09	43.33	Abundant	Abundant	+	++
T3	1000 ppm	39.67 (39.02)	41.33 (40.01)	41.38	54.07	Moderate	Moderate	-	+
T4	1500 ppm	38.00 (38.05)	39.67 (39.04)	43.84	55.93	Moderate	Moderate	-	+
T5	2000 ppm	37.33 (37.65)	39.00 (38.64)	44.83	56.67	Poor	Poor	-	-
T6	2500 ppm	33.67 (35.46)	35.33 (36.47)	50.25	60.74	Poor	Poor	-	-
T7	3000 ppm	30.67 (33.62)	32.33 (34.65)	54.68	64.07	No sporulation	No sporulation	-	-
T8	Control	67.67 (55.36)	90.00 (71.57)	-	-	Abundant	Abundant	++	+++
	SE±	0.94	0.45						
	CD (0.05)	2.82	1.34						

*Figures in the parenthesis are arc sin transformed values.

the concentrated extract was stored in a freezer. The antimicrobial activity of extract was tested against *A. flavus* by poison food technique (PFT) as mentioned above at different concentrations. The control set was runned using methanol instead of pomegranate peal extract. Observation on colony diameter, sporulation, aflatoxin detection were recorded daily. The inhibition of mycelial growth of inoculated pathogen was worked out by using formula suggested by Vincent (1947).

Inhibition of *A. flavus* colonization on peanut pods and kernels by methanolic extract of pomegranate fruit peel : Inhibitory effect of pomegranate peel extract was studied on pod and kernel of peanut cultivar SB-11 as suggested by (13). Pods and kernels were first washed under tap water, surface sterilized with 70 per cent aqueous solution of alcohol for 2 minutes and subsequently washed in two changes of distilled sterilized water to remove any traces of mercuric chloride. Five pods and five kernels each were placed separately on filter paper in sterile petri plate. Pods and kernels were inoculated with *A. flavus*, by gently putting conidial spore suspension (1×10^6 spore/ml) on pods and kernels so that

Table-3 : Antifungal activity of methanolic extract of pomegranate fruit peel against *A. flavus* on pods and kernels on 8th day.

Tr. No.	Treatment details	*Colonization severity (%)		Growth inhibition (%)		Degree of sporulation		Aflatoxin B1 (mg/kg)
		Pods	Kernels	Pods	Kernels	Pods	Kernels	Kernels
T1	100 ppm	81.67 (64.69)	75.00 (60.07)	16.95	16.67	Abundant	Abundant	2.960
T2	500 ppm	66.67 (54.75)	63.33 (52.74)	32.20	29.63	Abundant	Abundant	2.180
T3	1000 ppm	65.00 (53.76)	60.00 (50.79)	33.90	33.33	Moderate	Moderate	1.720
T4	1500 ppm	65.00 (53.76)	60.00 (50.79)	33.90	33.33	Moderate	Moderate	0.960
T5	2000 ppm	60.00 (50.79)	55.00 (47.88)	38.98	38.89	Poor	Poor	Not detected
T6	2500 ppm	36.67 (37.26)	31.67 (34.23)	62.71	64.81	Poor	Poor	Not detected
T7	3000 ppm	26.67 (31.07)	26.67 (31.07)	72.88	70.37	No sporuation	No sporulation	Not detected
T8	Control	98.33 (85.69)	90.00 (71.95)	-	-	Abundant	Abundant	-
	SE±	2.01	1.71					
	CD (0.05)	6.01	5.14					

*Figures in the parenthesis are arc sin transformed values.

inoculam get lodged on its surface. About 1-2 drops of Tween-80 was added in conidial spore suspension before inoculation for uniform dispersal of conidia. Petri plates were kept in incubation chamber at room temperature (27±1°C). Then after 24 hours, pomegranate peel extract was added gently at different concentration and incubated at room temperature (27±1°C) having relative humidity more than 95 per cent in dark. To maintain high humidity, sterile distilled water (1-2 ml) was added everyday during the first five days. Number of pods and kernels contaminated by isolate was counted daily. Observations on pod, kernel per cent colonization severity, per cent growth inhibition and sporulation of the test pathogen were recorded for each treatment on 8th day of incubation. Also, aflatoxin B_1 synthesis in kernels was quantified by chromatography technique.

Results and Discussion

Antifungal activity of plant extract against *A. flavus* [PFT] : The extract of pomegranate fruit peel was statistically found to be effective with minimum colony diameter of 32.00 mm having poor sporulation with 52.71 per cent growth inhibition over control and no aflatoxin was detected by qualitative method on 5th day of incubation (Table-1). The extract of pomegranate leaves shows 39.00 mm colony diameter with 42.36 per cent growth inhibition was at par with *A. indica* leaves showing 41.33 mm colony diameter with 38.92 per cent growth inhibition and bulb of *A. cepa* showing 42.00 mm colony diameter with 37.93 per cent growth inhibition. Observations on 8th day of incubation in respect of above study observed similar trend showing 33.00 mm colony diameter having poor sporulation with 63.33 percentage growth inhibition over control and no aflatoxin was detected in treatment of pomegranate fruit peel extract. However in control treatment colony diameter was 67.67 mm and 90.00 mm with abundant sporulation on 5th day and 8th day of incubation, respectively and aflatoxin was detected.

Antifungal activity of methanolic extract of pomegranate fruit peel against *A. flavus* [PFT] : All the treatments were significantly superior over control in respect of colony growth diameter and growth inhibition of *A. flavus* on 5th day of incubation. The treatment T7 having concentration 3000 ppm was statistically found to be effective with minimum colony diameter of 30.67 mm without sporulation with 54.68 per cent growth inhibition over control and no aflatoxin was detected. This treatment was followed with treatment T6, T5 and T4 which were at par showing colony diameter of 33.67mm, 37.33 mm and 38.00 mm, respectively with 50.25, 44.83 and 43.84 per cent growth inhibition, respectively over control (Table-2). On 8th day of incubation observed similar trend showing 32.33 mm colony diameter without sporulation with 64.07 percentage growth inhibition over control and no aflatoxin was detected in treatment T7 having concentration 3000 ppm followed by treatment T6 showing 35.33 mm colony diameter with 60.74 percentage growth inhibition over control. However in control treatment colony diameter was 67.67 mm and 90.00 mm with abundant sporulation on 5th day and 8th day of incubation, respectively and aflatoxin was detected. These findings were in confirmation with

(14) who studied laboratory experiments by using various plant extracts and found significant differences among them. Also (15) reported that extract of *Allium sativum* L was found to be effective in poison food technique against *A. flavus* isolated from peanut seed with the inhibition of 79.22 per cent followed by *Allium cepa* (78.71 %), *Azadirachta indica* (74.07 %). Also, (13) reported Methyleugenol 0.5 per cent inhibited *A. flavus* colonization completely on PDA which contain peanut substrate.

Antifungal activity of methanolic extract of pomegranate fruit peel on pods and kernels against *A. flavus* : All the treatments were significantly superior over control in respect of colonization severity and growth inhibition percentage of *A. flavus* on 8th day of incubation. The treatment T7 having concentration 3000 ppm was statistically found to be effective with minimum pod colonization severity 26.67 per cent with 72.88 per cent growth inhibition over control. All the treatments showed varied degree of sporluation except treatment T7, in which no sporulation was observed. Similarly, kernel colonization severity and growth inhibition percentage of *A. flavus* revealed that all the treatments were significantly superior over control on 8th day of incubation. The treatment T7 having concentration 3000 ppm was statistically found to be effective with minimum kernel colonization severity 26.67 per cent with 70.37 per cent growth inhibition over control (Table-3). These findings were in confirmation with several workers who demonstrated the antifungal activity of plant derived chemicals. (13) who reported the spray of methyleugenol at 0.5 per cent on peanut pod and kernels checked the colonization of *A. flavus* and aflatoxin synthesis. Also the potential of certain plant extracts for the reduction of AFB_1 in maize by (16) and in stored rice was investigated by (17). Thus the present study demonstrated the usage of extract of pomegranate fruit peel, a natural waste material is an ideal alternative to protect peanut pods and kernels from post- harvest infection of *A. flavus* and aflatoxin synthesis therein.

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