RESEARCH ARTICLE

Antifungal activity of extracts of weed biomass against *Aspergillus flavus* Link ex Fr., a causal agent of yellow mold of Groundnut

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ABSTRACT

During the present studies, weeds found in different crop fields were screened to observe their antifungal activity against yellow mold causing fungi of groundnut i.e. *Aspergillus flavus* Link ex Fr. To study the antifungal mechanism of weed extract, poisoned food technique was followed. The efficacy of weed extracts was expressed as percent inhibition of mycelial growth over control. Among these weeds, leaf and stem extracts of Parthenium hysterophorus L. and Commelina benghalensis L. were found to be most effective against yellow mold causing fungi of groundnut i.e. *Aspergillus flavus* Link ex Fr.

Key words: Weed, *Aspergillus flavus,* Yellow mold.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is considered as most important crop in the World (13th) and is most important source of edible oil (4th). Similarly Groundnut is ranked as 3rd most important source of vegetable protein in the world (Smith, 2002). It is recognized as a palatable "poor man's nut" and is eaten and relished by all classes of people.

In India, diseases are one of the major constraints responsible for low yield of Groundnut. Groundnut is attacked by over 55 pathogens including viruses. The important one among them responsible for huge losses in yield are early and late leaf spots, rusts, necrosis, bud blight, collar rot, stem rot, charcoal rot, wilt and yellow mold. These diseases are known to render Groundnut production highly unstable (Mishra and Ghewande, 1989). Approximately 5-15 % loss in the initial crop stand is due to seed-rot and seedling collapse (Pande and Narayana Rao, 2000). Out of these diseases, yellow mold caused by *Aspergillus* flavus Link ex Fr. produces aflatoxin which is carcinogenic to both human and animals. It is estimated that about 4% of the world's grains are lost due to biodeterioration caused by seed borne fungi (Clerke, 1966).

In recent years much attention has been given to non chemical systems for seed treatment to protect them against seed-borne pathogens. Plant extracts have played significant role in the inhibition of seed-borne pathogens and in the improvement of seed quality and field emergence of plant seeds (Patel et al., 2007).

In India many unwanted plants so called weeds (Plant biomass) are very common, dominant and wide spread in the crop fields. In India diversity of unwanted plant in crop fields is very common, dominant and easily available. Weeds also occupy almost all open spaces. They spread like wildfire and grow abundantly in the crop fields, forest and roadsides.

During botanical excursions to different parts of this phytogeographic region every year, it is observed fact that weeds produce a huge biomass. This fact promoted to work on the present research topic.

The present investigation was undertaken to study the efficacy of common, dominant and easily available weeds in the crop fields against the yellow mold causing fungi, *Aspergillus flavus* Link ex Fr. in a cost-effective and eco-friendly manner.

MATERIAL AND METHODS

I) Collection of seed samples

The method described by Neergaard (1977) has been adopted for the collection of seed samples. Accordingly, three random samples of seeds (half Kg each) were collected from oil mills, market place and Oil Seed Research Station (ORS), Latur. Groundnut cultivars used in the present study are TAG-24 and SB-XI.

II) Detection of seed mycoflora

The seed mycoflora was detected by using standard moist blotter paper method and agar plate methods as recommended by International Seed Testing Association (ISTA, 2003), Neergaard, (1977) and Agarwal, (1981).

III) Isolation and identification of test fungi

Infected groundnut seeds were selected for isolation of *A. flavus* by using the procedure published by ISTA (2003). Infected seeds were first disinfected with 0.1 % $HgCl_2$ for 2 to 3 minutes and then washed with sterile distilled water 2 to 3 times. Disinfected seeds were then aseptically transferred to the moist chamber plate. The plate was then incubated at room temperature for 7 day and also watered regularly with sterile distilled water. After 7 days incubation, fungus produced large green conidial heads with spores which was removed aseptically, transferred to PDA plates and slants. The fungus was identified by observing colony morphology and microscopic characteristic.

The identification was confirmed with the help of latest manuals, Subramanian, (1971), Neergaard and Mathur, (1980), Jha, (1993) and Mukadam, (1997) and with the help of Information Bulletin of International Crops Research Institute for the Semi-Arid tropics (ICRISAT), Pantancheru, Andhra Pradesh (India). Pure culture of the identified fungi was prepared and maintained on PDA (Potato Dextrose Agar) slants.

IV) Preparation of weed extracts

Fresh samples were washed in tap water and finally washed thrice using sterilized distilled water. They were crushed in a sterilized pestle and mortar by adding a little quantity of sterile distilled water just enough to crush the sample easily. The extracts were collected by filtering through the two layers of muslin cloth. Finally, filtrates thus obtained from the leaves were used as stock solution (Kuntal Das *et al.,* 2010).

V) Studies on antifungal activity of extracts of weed biomass on fungal growth (*in vitro*)

To study the antifungal mechanism of plant extract, poisoned food technique was followed as suggested by Nene and Thapliyal (1982).

Fresh weed extract stock solution were used at three concentrations (1 %, 5 % and 10 %) prepared by mixing aseptically 1 ml, 5 ml and 10 ml of stock solution in 100 ml semisolid sterilized potato dextrose agar medium (Tiwari *et al.,* 2005) at a temperature of 30ºC - 40ºC. Three replications were maintained for each treatment. Suitable control plates were maintained. Each plate was seeded with 5 mm mycelial discs aseptically taken from the periphery of 7 days old culture and incubated at 27 ºC till the growth of the colony touched the periphery in control plate. Mean colony diameter in each case was recorded (Dange, 2006). The efficacy of weed extracts was expressed as per cent inhibition of mycelial growth over control which was calculated by using the formula as given by (Verma and Kharwar, 2006).

RESULTS AND DISCUSSION

I) Detection of seed mycoflora

In order to study varietal variations in the seed mycoflora, seeds of different varieties of Groundnut i.e. TAG-24 and SB-XI were plated on moist blotters and the percent incidence of seed-borne fungi was recorded and presented in table-1. From the results it is clear that, there was variation in the degree of incidence of mycoflora in groundnut varieties.

Table- 1: Seed Mycoflora of Groundnut varieties TAG-24 and SB-XI (Moist Blotter Plate Method).

Sr. No.	Seed borne Fungi	Varieties of Groundnut	
		Per cent incidence of seed-borne fungi	
		TAG-24	SB-XI
1	*Alternaria alternata* (Fr.) Keissler	10	-
2	*Aspergillus candidus* Link	10	05
3	*Aspergillus flavus* Link ex Fr.	40	35
4	*Aspergillus fumigatus* Fresenius	10	-
5	*Aspergillus niger* van Tieghem	40	25
6	*Aspergillus terreus* Thom.	10	-
7	*Fusarium oxysporum* Schlechtend emend Sny. & Hans.	10	05
8	*Macrophomina phaseolina* (Tassi) Goldanich	10	-
9	*Rhizopus nigricans* Ehrenb.	20	20
10	*Sclerotium rolfsii* Sacc.	05	-

Table- 2: Antifungal activity of extracts of weed biomass against *Aspergillus flavus* Link ex Fr. by poisoned food technique.

Sr. No.	Name of Weeds	Part Used	Mean colony diameter (in mm)					
			Aqueous Extracts			Ethanolic Extracts		
			1%	5%	10%	1%	5%	10%
1	*Argemone maxicana* L.	Leaves	39	38	35	32	32	30
		Stem	38	38	38	35	32	30
2	*Commelina benghalensis* L.	Leaves	11	09	07	11	07	05
		Stem	25	24	20	26	25	25
3	*Cynodon dactylon* (L.) Pers.	Leaves	37	35	35	36	32	31
		Stem	39	36	35	38	35	35
4	*Cyperus rotundus* L.	Leaves	35	34	32	34	32	30
		Rhizome	39	36	35	38	35	35
5	*Euphorbia hirta* L.	Leaves	40	39	39	41	40	40
		Stem	42	42	40	40	40	40
6	*Parthenium hysterophorus* L.	Leaves	13	11	09	12	10	09
		Stem	08	07	05	08	06	06
7	*Phyllanthus amerus* Schumach.& Thonn	Leaves	41	41	41	40	36	35
		Stem	42	40	40	41	40	40
8	*Portulaca oleracea* L.	Leaves	28	28	27	25	22	22
		Stem	28	25	25	24	20	20
9	*Solanum nigrum* auct.	Leaves	37	35	35	35	32	30
		Stem	39	38	35	35	30	30
10	*Tridex procumbens* L.	Leaves	42	41	40	40	40	39
		Stem	45	43	42	45	40	40
11	**Control**			**100**			**100**	
	Leaves	S.E. ±	0.97	1.31	0.91	1.08	1.07	1.38
		C.D. at 0.05%	2.86	3.86	2.68	3.20	3.16	4.07
	Stem	S.E. ±	1.20	1.70	1.81	1.20	2.15	1.27
		C.D. at 0.05%	3.55	5.00	5.33	3.55	6.33	3.74

Table-3: Efficacy of extracts of weed biomass against mycelial growth of *Aspergillus flavus* Link ex Fr. by poisoned food technique.

Sr. No.	Name of Weeds	Part Used	Per cent mycelial inhibition					
			Aqueous Extracts			Ethanolic Extracts		
			1%	5%	10%	1%	5%	10%
1	*Argemone maxicana* L.	Leaves	61	62	65	68	68	70
		Stem	62	62	62	65	68	70
2	*Commelina benghalensis* L.	Leaves	89	91	93	89	91	95
		Stem	75	76	80	74	75	75
3	*Cynodon dactylon* (L.) Pers.	Leaves	63	65	65	66	68	69
		Stem	61	64	65	62	65	65
4	*Cyperus rotundus* L.	Leaves	65	66	68	66	68	70
		Rhizome	61	64	65	62	65	65
5	*Euphorbia hirta* L.	Leaves	60	61	61	59	60	60
		Stem	58	58	60	60	60	60
6	*Parthenium hysterophorus* L.	Leaves	87	89	91	88	90	91
		Stem	92	93	95	92	94	94
7	*Phyllanthus amerus* Schumach.& Thonn	Leaves	59	59	59	60	64	65
		Stem	58	60	60	59	60	60
8	*Portulaca oleracea* L.	Leaves	72	72	73	75	78	78
		Stem	72	75	75	76	80	80
9	*Solanum nigrum* auct.	Leaves	63	65	65	65	68	70
		Stem	61	62	65	65	70	70
10	*Tridex procumbens* L.	Leaves	58	59	60	60	60	61
		Stem	55	57	58	55	60	60
11	**Control**			**00**			**00**	
	Leaves	S.E. ±	1.93	2.1	2.49	2.54	2.04	2.30
		C.D. at 0.05%	5.68	6.20	7.33	7.47	6.00	6.77
	Stem	S.E. ±	2.40	1.86	2.25	2.11	2.30	2.28
		C.D. at 0.05%	7.08	5.48	6.64	6.22	6.77	6.71

Groundnut variety TAG-24 showed maximum seed mycoflora including ten fungi while variety SB-XI showed presence of five fungi. The seeds of both the varieties, TAG-24 and SB-XI dominantly showed presence of *Aspergillus flavus*.

II) Identification of yellow mold causing fungi

Microscopic characteristics like conidial head, conidia shape, roughness and vesicle serration, etc were taken into consideration for identification. *Aspergillus flavus* Link ex Fr.:-The mycelium is found to be submerged in the seed coat and forms a white to grey, tough mass. Conidiophores erect, simple, unbranched, hyaline, transparent and smooth. The apex of the conidiophores was inflated into a vesicle upon which radiating phialides are formed. Conidial heads were biseriate, globose to radiate often columnar, very light to deep yellow green, olive brown often brown. Conidia were found to be hyaline, single celled and produced in chains. They were globose to subglobose, often elliptical to pyriform and conspicuously echinulate.

III) Antifungal activity of extracts of weed biomass against *Aspergillus flavus* Link ex Fr. **by poisoned food technique.**

During the present investigation it was noticed that the yellow mold causing fungi, *Aspergillus flavus* was dominant causing heavy damage to Groundnut seeds. Hence antifungal activity of extracts of weed biomass was tested against *Aspergillus flavus* by Poisoned food technique.

From the results presented in table- 2 it is clear that maximum antifungal activity was observed in 10 % Aqueous and 10 % Ethanolic stem extracts of *Parthenium hysterophorus* L. (05 mm and 06 mm respectively) followed by 10 % Ethanolic leaf extracts of *Commelina benghalensis* L. (05 mm) as compared with control (100 mm).

Other test weeds do not show much antifungal activity as compared with *Parthenium hysterophorus* L. and *Commelina benghalensis* L.

IV) Efficacy of extracts of weed biomass against mycelial growth of *Aspergillus flavus* Link ex Fr **by poisoned food technique.**

The efficacy of weed extracts was expressed as per cent inhibition of mycelial growth over control which was calculated by using the formula as given by Verma and Kharwar (2006). From the results presented in table 3, it was observed that maximum per cent mycelial growth inhibition of *Aspergillus flavus* was done by 10% Aqueous stem extract of *Parthenium hysterophorus* L (95%) and 10% Ethanolic leaf extracts of *Commelina benghalensis* L (95 %) followed by 5% and 10 % Ethanolic stem extracts of *Parthenium hysterophorus* L. (94 %).

Among all test weeds, leaf and stem extracts of *Tridex procumbens* L. and *Euphorbia hirta* L.showed minimum per cent mycelial growth inhibition.

CONCLUSION

Variation in the composition of seed mycoflora in different varieties of Groundnut gives an idea about the degree of varietal resistance against the incidence of seed borne fungi. Similar type of variations in mycoflora in different varieties of Groundnut has been reported by various workers. Embaby et al. (2006), Hossain *et al.,* (2007), El-Wakil *et al.,* (2001) also studied the seed mycoflora of Groundnut and reported varietal variations in Groundnut.

Many workers reported the antifungal activity of higher plants is an important factor for disease resistance and control against a wide range of fungi that infect crops.

During present studies it was observed that literature regarding antifungal activity of *Commelina benghalensis* L. against *Aspergillus flavus* remain unreported till date hence the leaf extracts of *Commelina benghalensis* L. which were found effective against *Aspergillus flavus* after *Parthenium hysterophorus* L. is reported for the first time.

Conflicts of interest: The authors stated that no conflicts of interest.

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