ISSN 0975-6299 Vol 2/Issue 2/Apr-Jun 2011

International Journal of Pharma and Bio Sciences

RESEARCH ARTICLE MICROBIOLOGY

PLANT EXTRACTS IN THE MANAGEMENT OF AFLATOXIN PRODUCTION BY *ASPERGILLUS FLAVUS*

V. KRISHNA REDDY*, M. SRINIVAS, A. RAJENDER REDDY, G. SRUJANA, M. SUREKHA AND S. M. REDDY

Toxicology Laboratory, Department of Botany, Kakatiya University, Warangal – 506 009

V. KRISHNA REDDY

Toxicology Laboratory, Department of Botany, Kakatiya University, Warangal – 506 009

ABSTRACT

Efficacy of some local plants in the management of *Aspergillus flavus* and aflatoxin contamination was investigated. Aqueous extracts of rhizome of *Zingiber officinalis* and leaf extracts of *Trigonella foenum-graecum* inhibited aflatoxin production to a significant level. While solvent extracts of *Zingiber officinalis* and *Oxalis corniculata* could suppress both the growth and aflatoxin production by *A. flavus*. No positive correlation could be observed between mycelial growth and aflatoxin production by *A. flavus*.

KEY WORDS

Aspergillus flavus, aflatoxin, plant extracts, management.

INTRODUCTION

Though synthetic fungicides improved plant protection but proved to be health hazardous. Hence antimicrobial properties of some plant constituents are being exploited in protecting food from storage moulds. Antifungal action of plant extracts has great potential as they are easy to prepare and apply. Further, these are safe and effective in view of systemic in their action and lack residual effect, easily biodegradable and exhibit stimulating effect on plant metabolism. Besides large number of earlier workers[1-14] have reported antifungal properties of large number of plants. Bilgrami[15], Surekha[16] reported the efficacy of some plant extracts screened by them in the control of aflatoxin and penitrem production by *A. flavus* and *Penicillium crustosum* respectively. Giridhar[17] advocated the exploitation of some local plants in the management of citrinin contamination of spices and fruits. In the present investigation aqueous and solvent extracts of local plants in the management of aflatoxin production by *A. flavus* was studied and discussed in this communication.

MATERIALS AND METHODS

Monosporic cultures of *Aspergillus flavus* isolated from maize seeds was employed in the present study. The organism was grown in 250ml Erlenmeyer conical flasks containing 50ml Czepek's medium (sodium nitrate 2g, dipotassium hydrogen phosphate 1g, magnesium sulphate 0.5g, potassium chloride 0.5g, ferrous sulphate 0.01g, sucrose 30g and distilled water 1 litre). Aqueous extracts of different plants (Table 1) was prepared and added to the medium before the inoculation of the fungus. Further promising plants for fungitoxicity were investigated for the fungitoxic principle by extract them in different solvents (Table 2) which can inhibit aflatoxin production. The flasks thus inoculated were incubated under stationary conditions for 10 days at 27-29^{0}C. At the end of incubation period, flasks were harvested on preweighed Whatmann filter paper No.42 for determination of biomass. Dry weight of the mycelium was determined after drying to a constant weight in an oven at 70^{0}C for 48 hours. The culture filtrate was subjected to extraction and estimation of aflatoxin produced as described by Nabney and Nesbitt[18]. Fifty ml of culture filtrate and 25ml of chloroform were shaken thoroughly for 15min in a separating funnel. The organic phase was concentrated to dryness. To this 1ml of methanol was added and 0.2ml was placed on activated TLC and run in a solvent (Chloroform: Methanol; 97:3) mixture. The spot thus developed was eluted in 5ml of methanol. The intensity of fluorescence thus developed was read at 360nm with the help of spectrophotometer and the amount of aflatoxin produced was calculated by the following formula.

$$\text{Amount of Aflatoxin } (\mu g/ml) = \frac{D \times M \times 10}{E \times I \times 1000}$$

D = Optical density
M=Molecular weight of aflatoxin
E=Molar extraction coefficient
I=Path length

Table 1

Effect of aqueous leaf extracts of different plants on growth and aflatoxin production by A. flavus.

Name of the plant	Concentration (mg/ml)	pH	Dry weight (mg/ml)	Aflatoxin (µg/ml)
Acalypha indica	100	5.0	7.09	3.43
	300	4.9	6.02	2.01
Allium cepa	100	6.5	9.24	3.96
	300	6.5	7.12	3.12
Annona squamosa	100	5.5	7.21	2.61
	300	5.0	5.91	1.52
Azadirachta indica	100	5.5	9.14	3.16
	300	5.0	8.01	1.81
Caesalpinia bonduc	100	7.0	7.25	4.51
	300	7.5	8.76	4.23
Coriandrum sativum	100	6.5	6.98	2.96
	300	6.0	6.56	2.20
Cymbopogon martinii	100	7.0	8.95	39.2
	300	7.0	8.87	34.2
Elettaria cardamomum	100	7.5	8.21	39.7
	300	7.5	8.01	35.2
Emblica officinalis	100	7.0	8.79	33.5
	300	6.7	8.65	22.1
Gossypium herbaceum	100	6.5	9.51	32.4
	300	5.5	8.59	21.6
Hemidesmus indicus	100	7.0	7.21	40.1
	300	7.0	7.10	35.1
Hibiscus rosa-sinensis	100	6.0	9.14	42.2
	300	5.5	7.72	29.0
Ocimum sanctum	100	6.5	6.02	29.1
	300	5.0	5.02	21.0
Pterocarpus santalinus	100	6.5	9.01	42.9
	300	7.0	9.13	36.7
Phaseolus atropurpureus	100	6.5	8.91	9.7
	300	7.0	8.61	1.6
Rauvolfia serpentina	100	7.0	7.89	39.0
	300	7.5	8.01	29.1
Trigonella foenum-graecum	100	6.5	6.55	25.8
	300	5.0	6.60	----
Tylophora asthmatica	100	6.5	8.83	36.7
	300	7.0	8.10	22.1
Vitex negundo	100	6.5	9.94	40.8
	300	5.0	7.00	29.1
Zingiber officinalis	100	5.5	4.91	5.02
	300	5.0	3.91	----
Control	-----	7.5	9.42	54.5

Table 2
Effect of different leaf solvent extracts of some plants on growth and aflatoxin production by A. flavus.

Name of the plant	Solvent fraction (500µg/ml)	Dry weight (mg/ml)	Aflatoxin (µg/ml)
Coriandrum sativum	Petroleum ether	8.23	12.30
	Benzene	6.50	6.30
	Chloroform	5.32	1.40
	Acetone	8.10	9.30
	Methanol	2.40	0.80
Euphorbia microphylla	Petroleum ether	9.90	16.20
	Benzene	7.24	5.20
	Chloroform	4.41	1.80
	Acetone	8.53	6.20
	Methanol	2.94	1.20
Oxalis corniculata	Petroleum ether	8.91	7.90
	Benzene	8.35	7.40
	Chloroform	5.40	3.10
	Acetone	9.02	8.20
	Methanol	--	--
Phaseolus atropurpureus	Petroleum ether	9.76	15.20
	Benzene	7.23	9.20
	Chloroform	5.42	1.90
	Acetone	8.31	7.90
	Methanol	2.01	0.90
Trigonella foenum-graecum	Petroleum ether	9.90	14.40
	Benzene	9.50	10.60
	Chloroform	4.78	5.30
	Acetone	7.92	9.60
	Methanol	1.90	0.35
Zingiber officinalis	Petroleum ether	8.41	11.20
	Benzene	5.81	5.30
	Chloroform	6.94	2.10
	Acetone	7.41	6.70
	Methanol	--	--
Control		10.37	53.0

RESULTS AND DISCUSSIONS

Aqueous extracts of some local plants were screened against *A. flavus* for their efficacy in checking the growth and aflatoxin production and the results are presented in Table 1.

Table 1 reveals that out of 20 aqueous plant extracts tried, rhizome of *Zingiber officinalis* and *Trigonella foenum-graecum* were effective inhibitors of aflatoxin production by *A. flavus* as they caused total inhibition at 300 mg/ml concentration. Similarly *Coriandrum sativum* was responsible for significant inhibition of aflatoxin production at 300mg/ml concentration while the growth of *A. flavus* was affected marginally. Leaf extracts of *Acalypha indica*, *Allium cepa, Annona squamosa* and *Azadirachta indica* have inhibited the aflatoxin production by *A. flavus* to a significant level. Though leaf extracts of

Acalypha indica caused significant inhibition of aflatoxin production, the growth was inhibited only marginally. *Gossypium herbaceum, Rauvolfia serpentina* and *Tylophora asthmatica* were moderate in their fungitoxicity and affected aflatoxin and growth of *A. flavus* only marginally. Rest of the plants screened in the present investigations were lacking fungicidal activity against *A. flavus*. In most of the media the final pH was alkaline or near neutral. However, the pH remained at 5.0 or below 5.0 when there was not much mycelial growth. From the present investigations it can be concluded, that the plant extracts tried proved to be promising and can be exploited in the management of mould infestation and mycotoxin contamination of foods, feeds and fodders with minimum residual effects.

Table 2 reveals that the methanol and chloroform fractions of *Coriandrum sativum* were highly toxic. Petroleum ether and acetone fractions were mild in their toxicity, while benzene fraction proved to be intermediate in its inhibitory activity. Similarly the methanol and chloroform fractions of *Euphorbia microphylla* inhibited aflatoxin production to a significant level, while petroleum ether was least fungitoxic. Benzene and acetone fractions were almost same in their low fungicidal activity. Methanol fraction of *Oxalis corniculata* was also responsible for total inhibition of growth and aflatoxin production by *A. flavus*. Chloroform extracts come next in their fungi toxicity. Fractions of petroleum ether and benzene were almost same in their low fungitoxicity. Acetone fraction was least toxic to *A. flavus*. Methanol and chloroform fractions of *Phaseolus atropurpureus* exhibited strong fungicidal activity, while petroleum ether fractions were least toxic to aflatoxin production. The methanol fraction of *Trigonella foenum-graecum* was highly toxic and caused almost total inhibition of aflatoxin production and permitted only marginal growth of *A. flavus.* Chloroform fraction also exhibited significant fungicidal action against *A. flavus* and aflatoxin production, while acetone fraction was low in its fungicidal activity. The fractions of petroleum ether and benzene exhibited least inhibitory effect on *A. flavus*. The methanol fraction of *Zingiber officinalis* caused total inhibition of growth and aflatoxin production by *A. flavus*, while petroleum ether fraction was lacking fungicidal principle. Chloroform fraction was effective inhibitor of aflatoxin production but it inhibited the growth of *A. flavus* only marginally. Benzene fraction also exhibited significant fungitoxicity against *A. flavus* and aflatoxin production.

It is clear from the above observations that all the plants under investigation proved to be useful in the management of aflatoxin problem. The fungi toxicity of *Coriandrum sativum*[17,19], *Euphorbia microphylla*[20], *Oxalis corniculata*[16], *Phaseolus atropurpureus*[21], *Trigonella foenum-graecum*[20] and *Zingiber officinalis*[17] was also established, and can be exploited in the protection of foods from mycotoxins contamination.

ACKNOWLEDGEMENTS

Thanks are due to Head, Department of Botany and Microbiology for providing necessary facilities and the financial assistance of UGC is gratefully acknowledged.

REFERENCE

1. Zaki.N, 1992. Effect of some natural antifungal extracts on growth and aflatoxin production by *Aspergillus Parasiticus*. Mansoura J.Agri Sci.17:1069-1079.
2. Satish, S., Mohana, D.C., Ranhavendra, M.P. and Raveesha, K.A. (2007). Antifungal activity of some plant extracts against important seed borne pathogens of *Aspergillus* sp. Journal of Agricultural Technology 3(1): 109-119.
3. Bhatnagar, D., & Cormick, S. P. (1988). The inhibitory effect of neem (Azadirachta indica) leaf extracts on aflatoxin synthesis in Aspergillus

parasiticus. Journal of American Oil Chemical Society, 65, 1166–1168.
4. Bhatnagar, D., Zeringue, H. J., & Cormick, S. P. (1990). Neem leaf extracts inhibit aflatoxin biosynthesis in Aspergillus flavus and A. parasiticus. In Proceedings of the USDA neem workshop (pp. 118–127). Beltsville, Maryland: US Department of Agriculture.
5. Ramesh, V.M., A.Hilda and V.K Manjula. 1995. Fumgi - Toxic effect of leaf extract of *Croton sparsiflorumorong* on phyto pathogenic fungi Acta Botanica Indica 63-66.
6. Bambawale, O.M., Punit Mohan and Mukta Chakraborty 1995. Efficacy of some medicinal plant extracts against cotton pathogens. Ad. Plant.Sci.224-229.
7. Murali, N. D., & Mathela, C. S. (1978). Antifungal study of some indigenous essential oils. Indian Perfume, 4, 291–292.
8. Reddy, C. S., Reddy, K. R. N., Prameela, M., Mangala, U. N., & Muralidharan, K. (2007). Identification of antifungal component in clove that inhibits Aspergillus spp. colonizing rice grains. Journal of Mycology and Plant Pathology, 37(1), 87–94.
9. Paster, N., Menasherov, M., Ravid, U., & Juven, B. (1995). Antifungal activity of oregano and thyme essential oils applied as fumigants against fungi attacking stored grain. Journal of Food Protection, 58, 81–85.
10. Bouamama, H., Noel, T., Villard, J., Benharref, A. and Jana, M. (2006) Antimicrobial activities of the leaf extracts of two Moroccan *Cistus* L. species. Journal of Ethnopharmacology 104: 104-107.
11. SINGHP, .1983 Control of aflatoxin through natural plant extracts. In The Proceeding of Symposium on Mycotoxin in Food & Feed ed. Bilgrami, K.S., Prasad. T. & Sinha, K.K. pp. 307-315. Bhagalpur: Allied Press.
12. KUMAR, S. & PRASAD, G. 1992. Efficacy of medicinal plant *(Andrographis peniculata)* extract on aflatoxin production and growth of *Aspergillusflauus.* Letters in Applied Microbiology 15, 131-132.
13. Okigbo, R.N. and Ogbonnaya, U.O. (2006) Antifungal effects of two tropical plant leaf extracts (*Ocimum gratissimum* and *Aframomum melegueta*) on post harvest yam (*Dioscorea* spp.) rot. African Journal of Biotechnology 5: 727-731.
14. Sánchez, E., Heredia, N., & Garcia, S. (2005). Inhibition of growth and mycotoxin production of Aspergillus flavus and Aspergillus parasiticus by extracts of Agave species. International Journal of Food Microbiology, 98, 271–279.
15. Bilgrami,K.S., R.S Misra, K.K Sinha and Premlata Singh 1980. Effect of some wild and medicianal plant extracts on aflatoxin production and growth of *Aspergillus flavus* in liquid culture. Indian J. Bot. Sci., 59; 123-126.
16. Surekha .M and S.M Reddy 1996. Efficacy of some plant extracts on *Penicillium crustosum.* Nat. Acad. Sci., Letter 125; 69-71.
17. Giridhar,P and S.M Reddy 1996. Effect of some plant extracts on citrinin production by *P. citrinum* in vitro. J.Indian Bot.Sci.75. 153-154.
18. Nabney, J. and B.F Nesbitt. 1965. A spectrophotometric method for determining the aflatoxins.
19. B. Pradeep Kumar, M.A.S Charya and S.M Reddy 1979 Screening of some plant extracts for anti fungal properties. New Botanist. 6: 41-45.
20. M.P Cary, E.J.S Reddy and S.M Reddy 1984. Screening of indigenous plants for their antifungal principle. Pesticides. 10: 17-18.
21. M.M Reddy, S.M. Reddy and V.M Reddy 1982. Anti microbial activity of various solvent extracts of leafs of *Phaseolus atropurpureus* . Indan J. Microbiol. 22: 229-23