J. Phytol. Res. 24(1) : 67-69, 2011

EFFICACY OF SOME PLANT EXTRACTS AGAINST GROWTH AND AFLATOXIN (AFB1) PRODUCING POTENTIAL OF *ASPERGILLUS FLAVUS*

RASHMI PANT
Botany Department, Govt. P.G. College, Kota, India.

Aflatoxin B1 produced by *Aspergillus flavus* is a carcinogenic, mutagenic and teratogenic substance. Extracts of seven plants rich in phenolic compounds were evaluated for their potential to inhibit aflatoxin production by fungi, three different concentrations 5, 10 and 15 percent of each extract were tested. All tested plant extracts were found capable of reducing aflatoxin production with a range of (23.48% to 78.13%). Lower concentration (5%) of the plant extracts were not very effective in reducing aflatoxin production but (10%) concentration of *B. diffusa, T .cordifolia, M. koenzie* and *E. hirta* inhibited aflatoxin production more than 50 percent. At higher concentration (15%) of *Murraya koenigii* and *Tinospera cordifolia,* aflatoxin production showed an inhibition of more than 75 percent. Plant extract of *Crotolaria juncea* did not show any significant effect on mycelial growth and aflatoxin production .All extracts inhibited mycelial growth of *Aspergillus flavus,* but no direct co-relation between mycelial growth of *A. flvaus* and aflatoxin production was observed.

Keywords : Aflatoxin B1; Bio-control; Phenolic plant extracts; Toxigenic *A. flavus.*

Introduction

Aflatoxin B1 is produced as secondary metabolite by toxigenic *Aspergillus flavus*[1]. Aflatoxin can be produced in pre-harvest as well as post harvest conditions in the infected plant part. Aflatoxin B1 not only affect seed germination, seedling growth and other physiological process of crops[2] but is also responsible for causing liver cancer, gastro-intestinal bleeding, enlargement of liver and spleen[3] causing several death every year. Prevention of aflatoxin production is the best mode to control this problem. Inhibition of aflatoxin through natural plant extract is a cheap and eco -friendly method. Plant extracts having phenols are very effective in reducing aflatoxin production[4]. Many researchers have evaluated a number of plant extracts for their inhibitory properties[5-9].

Present study deals with the evaluation of some more plant extracts for their inhibitory action against aflatoxin production. Aqueous plant extracts of *Crotolaria juncea, Catharanthus roseus, Boerhavia diffusa, Tinospora cordifolia, Murraya koenigii, Euphorbia hirta* and *Lawsonia inermis* were tested for their inhibitory action against aflatoxin production. All these plants are well known for their medicinal properties. Three different concentrations of 5, 10 and 15 percent of each plant extract were tested for mycelial growth and aflatoxin production by *Aspergillus flavus.*

Material and Methods

1. Culture of Aspergillus flavus-Aspergillus flavus was isolated from diseased plant parts and cultured in SMKY media (Sucrose - 200 gm, $MgSO_4 7H_2O$ - 0.5 gm, KNO_3 - 3 gm and yeast extract -7 gm per lit.) for 10 days at 28 ± 2°C. After 10 days culture were filtered and extracted with chloroform[1].

2.Qualitative and quantitative estimation of aflatoxin- Qualitative estimation was done by thin layer chromatography[10]. 10 day old cultures extracted with chloroform were used for estimation. Toluene, Iso-amyl Alcohol and Methanol (90:32:2) were used as solvent. Silica gel-c-coated plates were used for TLC. Spots were observed under UV light at 365 nm. These spots were eluted in cold methanol diluted to 5 ml and OD was determined at 365 nm. For qualitative estimation aflatoxin concentration was calculated from standard curve[11].

3. Preparation of plant extract- Fresh leaves of plants were used for plant extract. 50 gm leaves of each plant were taken, washed, sterilised with 5% solution of sodium hypochloride and again washed with distilled water. These leaves were then crushed in 50 ml of distilled water and filtered with double layered muslin cloth. These filtered extracts were autoclaved at 1.2 Kg pressure for 20 minutes. These plant extracts were used as stock solution.

4. Test for efficacy of plant extract- Plant extract was mixed

Table 1. Effect of plant extracts on growth of *A. flavus* and aflatoxin production.

Plant Extract	Inhibition of Aflatoxin production at concentration (of plant extract)			Percentage reduction in growth (over control) at 15% concentration of plant extracts	Percentag. inhibition of aflatoxin production (over control)
	5%	10%	15%		
Crotolaria juncea	+	+	+	31.64	23.48
Catharanthus roseus	+	++	++	39.24	56.16
Boerhavia diffusa	++	++	++	44.83	61.27
Tinospora cordifolia	++	+++	+++	61.73	78.13
Murraya koenigii	++	++	+++	63.89	76.27
Euphorbia hirta	++	++	++	48.06	62.79
Lawsonia inermis	+	+	+	40.39	36.62

+ = 25% Reduction; ++ = 50% Reduction; +++ = 75% Reduction

with SMKY in three different parts to make three concentrations.

(i) One part plant extract + 19 part medium for 05 percent.
(ii) Two part plant extract + 18 part medium for 10 percent.
(iii) Three part plant extract + 17 part medium for 15 percent.

25 ml of each concentration was taken in conical flask. 25 ml of media without plant extract was set as control. 10 days old culture of Alfatoxigenic A. flavus was inoculated in each flask at 28±2°C. After ten days culture was extracted with chloroform and qualitative and quantitative estimation of aflatoxin B-1 was done and dry mycelial weight were recorded. Inhibition percentage was calculated against control.

Results and Discussion

In general all plant extracts in all concentrations reduced the growth of *A.flavus* and aflatoxin production. Maximum inhibition of fungal mycelium was found in *Murraya Koenigii* (63.89 percent) and minimum in *Crotolaria Juncea* (31.64 percent). Maximum inhibition in aflatoxin production was observed in higher concentration (15 percent) of *Tinaspora Cordifolia* (78.13 percent) and the minimum inhibition was observed in *Crotolaria Juncea* (23.48%). Although no direct co-relation between mycelial growth of *A. flavus* and aflatoxin production was observed but *Crotolaria Juncea* gave maximum results in both cases. Lower concentration (5 percent) of plant extracts were not very effective in all test plant extract. 10 percent concentration of *B. diffusa, T. Cordifolia, M. koenigii* and *E. Hirta* showed up-to 50% inhibition in aflatoxin production. 15 percent concentration of all plant extracts except *C. Juncea* and *L. inermis* gave more than 50% inhibition of aflatoxin production.

Sudharameshwari and Radhika[12] found *Lawsonia inermis* very effective against many fungal pathogens. In present study also *L. inermis* plant extract reduced mycelia growth of *A. flavus* significantly (40.39 %) but was not much effective in inhibiting aflatoxin production (< 25%).

Murugan[13] working on *Euphorbia milli* and *E. pulcherrima* reported successful inhibition of *A. flavus* and aflatoxin production. In this study also *E. hirta* reduced the mycelia growth (48.06%) and inhibited aflatoxin production (>50 %). This inhibitory action may be due to water soluble glycoside and flavonoids[14] present in the leaf extract of *Euphorbia* plant.

Leaf extract of *Murraya koenigii* were very effective in both reducing the mycelial growth (63.89%) and inhibiting aflatoxin production (up to75 %). Huda Faujan[15] had reported antioxidant activity of leaf extract of *Murraya* due to its phenolic contents (38.60 mg/TAC/ 100g). These factors may be responsible for their inhibitory effect on aflatoxin bio-synthesis.

Leaf extract of *Tinaspora cordifolia* showed equally good results. It gave maximum inhibition of

aflatoxin production (>75%) and also reduced mycelial growth (61.73%). Subramanian *et al.*[16] reported high antioxidant properties of *T. cordifolia* leaf extract and several workers had confirmed its antimicrobial properties.

Leaf extract of *Boerhavia diffussa* contain quercetin and many other flavonoids and phenolic compounds[17] that attribute to its inhibitory effect on aflatoxin production (>50%) mycelial growth reduction was not much (44.83%) in *B. diffusa* extract. Although Jain and Khanna[18] reported the presence of many flavonoides in plant extract of *Crotolaria juncea,* its activity against mycelial growth of *A. Flavus* (31.64%) and aflatoxin production (<25%) was not very significant. Leaf extract of *Catharanthus roseus* showed good results (>50%) for inhibition of aflatoxin production and 39.24% reduction in mycelial growth of *A. flavus.* It contain 2,3 dihydroxy benzoic acid and cinnamic acid[19] that may inhibit production of secondary metabolite in fungal mycelium.

Present study showed that plant extract with high content of phenols, alkaloids and flavonoids have possibility to develop as good inhibitor of aflatoxin production.

Refrences

1. Diener U L and Davis M D 1966, Aflatoxin production by isolates of *Aspergillus flavus. Phytopath.* **55** 1390-1393.
2. Sinha Kushal K and Sinha Ashok K 1993, Effect of aflatoxin B1 on germination index and seedling growth in wheat varieties. *Mycopathologia* **123**(3) 165-169.
3. Krishnamacharie K A *et al.* 1975, Hepatitis due to Aflatoxicosis . "An outbreak in Western India". *Lancet.* **1**(7915) 1061-1063.
4. Bilgrami K S 1984, Mycotoxins in food. *J. Indian Bot. Soc.* **63** 976-977.
5. Bilgrami K S, Singh K K and Singh Premlata 1982, Prevention of aflatoxin production on some cereals and oil seeds by *O. vanillin.Curr. Sci.***51** 138
6. Thanaporipat D, Waranong N and Purapakon N 1989, Efficacy of some herbs on growth of *A. flavus* and aflatoxin production. *Srinakarinwirot J. Sci.* **18** 203-205.
7. Kumar S and Prasad G 1992, Efficacy of medicinal plants *(Andrographis peniculata)* extract on aflatoxin production and growth of *A. flavus. Letters in Applied Microbiology* **15**(4) 131-132.
8. Yin and Cheng *et al.* 1998, Inhibition of *A. niger* and *A. flavus* by some herbs and spices. *J. Food Protection* **61** 123-125.
9. Dorner J W 2010, Efficacy of a bio pesticide for control of aflatoxin in corn. *J. Food Protection* **73**(3) 495-499.
10. Reddy T V, Vishwanathan L and Venkitasubramanian T A 1970, Thin Layer Chromatography of Aflatoxin. *Anal. Bio. Chem.* **38** 568-571.
11. Nabney J and Nesbit B F 1965, A spectrophotometric method for determining the aflatoxins. *Analyst Lond.* **90** 155-160.
12. Sudharameshwari K and Radhika J 2007, Antibacterial Screening of *Agle marmelos, Lawsonia inermis* and *Albizzia libbeck. African J. Tradit. Complement Altrm. MEI.* **4**(2) 199-204.
13. Murugan S *et al.* 2007, Efficacy of *Euphorbia milli* and *E. pulcherrima* on aflatoxin producing fungi *Aspergillus flavus* and *A. parasitica. J. Biotech.* **6**(6) : 718-719.
14. Gvazava L N and Alaniya N P 1997, Flavonoids of *Euphorbid armena. Chem. Nat. Comp.* **33** 210.
15. Huda Faujan *et. al.* 2007, Antioxidant activity of plants, methanolic extracts containing phenolic compounds. *African J. Bio-technology.* **8**(3) 484-489.
16. Subramanian M, Chintalwar G J and Chattupadhyay S 2002, Antioxident properties of *Tinospora cordifolia* polysaccharides against iron mediated lipid damage and gamma ray induced protein damage. *Redox Reports* 7 137-143.
17. Maurya Rakesh *et al.* 2007, Flavonoids and phenol glycosides from *Boerhavia diffusa. Nat. Prod. Res.* **21**(2) 126-134.
18. Jain S C and Khanna P 1974, Quercetin from *Crotolaria juncea* L. tissue culture. *Indian J. Exp. Biol.* **35** 163-164.
19. Natali Rianika Mustafa and Robert Verpoorte 2007, Phenolic compounds in *Catharanthus roseus. Phytochem. Rev.* **6** 246-248.