JOURNAL OF PURE AND APPLIED MICROBIOLOGY, April 2009. Vol. **3**(1), p. 223-226

Antifungal Potential of some Botanical Extracts to Reduce Aflatoxin Producing "Storage Fungi"

Ankita Sharma*, Madhu Rathore, Naveen Sharma, Jashoda Kumari and Kanika Sharma

Microbial Research Laboratory, Department of Botany, MLS University, Udaipur - 313 001, India.

(Received: 23 January 2009; accepted: 19 February 2009)

This study characterizes the antifungal potential of crude extract of different plant leaves against aflatoxin producing strains of *Aspergillus*. Fifteen local plants were used for preparation of crude aqueous, 100% alcoholic and 50% alcoholic extract of leaves. A poisoned food technique was adopted to assess the inhibitory effect of extracts against growth of test fungi. Among fifteen plants tested *Lawsonia inermis*, *Origanum majorana, Adathoda vasica*, *Emblica officinalis*, *Eucalyptus globules*, *Euphorbia pulcherima*, *Murraya koenigii*, *Azadirachta indica* and *Phycus elasticus* gave significant inhibition of *Aspergillus flavus* and *Aspergillus parasiticus*. Observations of present study suggest the possible use of selected extracts as potential fungitoxicant in ecofriendly control of biodeterioration of food commodities from "storage fungi".

Key words: Antifungal activity, Botanical extracts, Aflatoxin, Storage fungi.

Aspergillus flavus and *Aspergillus parasiticus* are considered as dominant storage fungi due to major role in biodeterioration of cereal grains, legume seeds, dry fruits, fresh fruits and raw herbal drugs during post harvest processing, transport and storage (Shukla *et al*., 2008). These fungi are ubiquitous causing significant economic loss of food products mainly through hydrolytic enzymes (Mishra and Dubey, 1994). Lipid peroxidation is also a major cause of deterioration of food products during processing and storage (Donnelly and Robinson, 1995). It alters the chemical and nutritional characteristic of agricultural products due to production of aflatoxins. Aflatoxins are one of the most common and dangerous mycotoxin produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxins cause liver cirrhosis, depressed food efficiency, depressed immune response, carcinogenesis and teratogenesis in animal system (Thanaboripat *et al*., 2004). In plants seed germination, seedling growth, root elongation, chlorophyll and carotenoid synthesis are inhibited in presence of aflatoxins (Shukla *et al*., 2008).

Use of synthetic chemicals against microorganisms contributed to management of such losses but these chemical fungicides attributed

* To whom all correspondence should be addressed.

the emergence of resistance microorganisms (Shah *et al*., 1992, Akimpelu, 2001) and led to many ecological and medical problems due to residual toxicity, carcinogenicity and hormonal imbalance (Omura *et al*. 1995, Pandey, 2003). To reduce this problem, there is a need of alternative biological method including use of botanicals. Plants contain antimicrobial secondary metabolites such as alkaloids, flavonoids, sterols and tannins. (Thanaboripat *et al*. 2006, Murugan *et al*. 2007, Kumar *et al*. 2006). These biocontrol agents are biodegradable, renewable, safe to human and plants and simple in application.

Present study was an attempt to explore the antifungal potential of some local plants i.e. *Lawsonia inermis, Psidium pyrifera, Murraya koenigii, Euphorbia pulcherima, Polyalthia longifolia, Calotropis procera, Adathoda vasica, Azadirachta indica, Origanum majarana, Lantana camara*. Results of *in vitro* inhibitory effect of these plant extracts against *Aspergillus flavus* and *Aspergillus parasiticus* are presented herein.

MATERIAL AND METHODS

Preparation of leaf extracts

Fresh leaves of different plants were collected from local regions of Udaipur. These leaves were thoroughly washed with 2% sodium hypochlorite solution and sterile distilled water. Leaves were shade dried at room temperature and pulverized into fine powder using an electric blender. Powdered leaves (40 gm) were soaked in 200 ml of water, 100% alcohol and 50% alcohol for 24 h. The mixtures were filtrate through whatman no. 1 filter paper; the filtrates were vacuum dried in rotary evaporator. The dried extracts were stored at 4ºC.

Test pathogen

Two toxigenic fungal pathogens *Aspergillus flavus* Navjot 4NSt (procured from Department of Botany, BHU, Varanasi) and *Aspergillus parasiticus* MTCC 411 (procured from IMTECH, Chandigarh) were used in experiment.

Antifungal assay of crude extracts

The inhibitory activity of different plant extracts against *Aspergillus flavus* and *Aspergillus parasiticus* was tested using poison food technique (Grover and Moore, 1962). The requisite amount of different extracts in methanol was mixed with 20 ml of sterilized Sabourd's Dextrose Agar (SDA) medium to achieve final concentration of 10 mg/ml of growth medium. Two types of control were used. The 1st control set had only methanol with medium in place of plant extract whereas in 2nd set inoculation was done on media. The extract amended medium was inoculated at the centre with 5 mm disc of 7 day old culture of test fungus and incubated at 28 ± 1ºC(7 days). The comparison of antifungal activity of different extracts was done with standard antibiotics thiram and bavistin at the same concentration (10 mg/ml).

RESULTS AND DISCUSSION

Antifungal activity of different leaf extracts against *Aspergillus flavus* and *Aspergillus parasiticus* are listed in Table 1. Among 15 crude extracts tested, alcoholic and aqueous extract of *Origanum majorana, Lawsonia inermis* and *Eucalyptus globule* are found to be show highest inhibitory effects. Appreciable (50%) inhibition was observed against *Aspergillus flavus* and *Aspergillus parasiticus* in case of *Euphorbia pulcherima, Phycus elasticus, Murraya koenigii, Azadirachta indica, Adathoda vasica* and *Calotropis procera*.

Antifungal potential of all extracts may be due to presence of secondary metabolites as reported by some authors (Kumar *et al*. 2006, Mishra *et al*. 1994, Thanaboripat *et al.* 2004). Present investigation is a first step in developing plant based formulation against seed borne pathogens. In comparison of standard synthetic fungicide tested, all extract shows low efficacy but it can be argued that standard antibiotics are purified form where all crude extract contain very small amount of active compounds. The results of fungal growth after treatment with acetone and standard antibiotics are presented in table no. 2.Lysis of mycelia and spores of the toxigenic fungi is one of the characteristics of aflatoxin deactivation process which may proliferate under favorable conditions (Namazi *et al.* 2002). Absence of fungal growth in the presence of these extracts would be useful since success in this aspect could also provide the means for the control of aflatoxin contamination in food stuffs.

All plants used in study are widely grows in India, so may be recommended as an easily

Table 1.

S. No.	Plant name	Growth diameter after 7 days					
		Aspergillus flavus			*Aspergillus parasiticus*		
		100% alcoholic	50% alcoholic	100% aqueous	100% alcoholic	50% alcoholic	100% aqueous
1.	*Lawsonia inermis*	27±0.33	24±0.33	20±0.33	20±0.33	24±1	19±0.33
2.	*Psidium pyrifera*	26±0.33	29±0.33	29±0.33	24±0.66	23±0.66	29±0.33
3.	*Murraya koenigii*	24±0.66	24±0.66	27±0.66	27±1.45	27±1.20	20±0.33
4.	*Euphorbia pulcherima*	20±0.33	24±0.66	20±0.33	18±0.66	22±0.33	24±1.20
5.	*Polyalthia longifolia*	22±1.20	22±0.66	25±0.57	24±0.33	27±0.33	25±1.85
6.	*Calotropis procera*	20±0.33	30±0.57	31±0.33	20±0.33	27±1.45	20±0.88
7.	*Azadirachta indica*	25±0.57	24±0.33	20±0.33	24±1.20	19±0.66	18±0.33
8.	*Origanum majarana*	18±0.33	25±1	21±0.33	19±0.33	25±1	20±0.33
9.	*Adathoda vasica*	22±0.33	28±0.33	26±1.20	24±0.33	29±0.33	26±0.57
10.	*Lantana camara*	24±0.33	29±0.33	27±0.57	22±0.33	28±0.33	27±1.20
11.	*Thevetia peruviana*	28±0.88	22±0.66	26±0.57	24±0.66	23±1.20	25±1.66
12.	*Phycus elasticus*	25±0.66	23±1.20	26±1.66	27±0.88	22±0.66	25±0.57
13.	*Eucalyptus globule*	19±0.33	23±0.33	27±0.33	20±0.66	24±0.33	26±0.33
14.	*Cassia fistula*	22±1.15	28±0.88	29±1.66	23±1.20	28±0.88	25±0.33
15.	*Emblica officinalis*	26±0.88	29±0.33	30±0.88	27±0.57	30±0.57	32±1.20

Values are Mean (n=3) ± standard error

Table 2.

S. No.	Test Fungi	Growth diameter (mm) after 7 days			
		C1*	C2*	S1*	S2*
1.	*A. flavus*	45	45	13	15
2.	*A. parasiticus*	46	45	11	13

* C1= only media, C2= Media+ Acetone, S1= Media+Thiram (10 mg/ml)
S2= Media+ Bavistin (10mg/ml)

available and renewable source of fungitoxicant in place of synthetic chemicals used for this purpose. However, some experiments are in process to isolate and characterize the active compound from selected plants in the study.

REFERENCES

1. Akimpelu, D.A. Antimicrobial activity of *Anacardium occidentale* bark. *Fitoterapia.* 2001; **72**: 286-287.
2. Donnelly, J.K., Robinson, D.S. Free radicals in foods. *Free radical research.* 1995; **22**: 147-176.
3. Grover, R.K., Moore, J.D. Toxicometric studies of fungicides against the brown rot organisms *Sclerotinia fructivola* and *S. laxa*. *Phytopatho*. 1962; **52**: 876-880.
4. Kumar R., Mishra A. k., Dubey N. K., Tripathi Y.B. Evaluation of *Chenopodium ambrosioides* oil as a potential source of antifungal, antiaflatoxigenic and antioxidant activity. *International journal of Food Microbiology*, 2007; **115**: 159-164.
5. Mishra, A. K., Dubey, N.K. Evaluation of some essential oils for their toxicity against fungi causing deterioration of stored food commodities. *Applied and Environmental*

microbiology. 1994; **60**: 1101-1105.

6. Murugan, S., Anand, R., Uma devi, P., Vidhya, N. and Rajesh, K.A., Efficacy of *Euphorbia milli* and *Euphorbia pulcherima* on aflatoxin producing fungi (*Aspergillus flavus* and *Aspergillus parasiticus*). *AJB*, 2007; **6**(6): 718-719.
7. Namazi, M., Allameh, A., Aminshahidi, M., Nohee, A., Malekzadeh, F., Inhibitory effects of ammonia solution on growth and aflatoxin production by *Aspergillus parasiticus* NRRL-2999. *Acta Poloniae Toxicologia* 2002; **10**: 65-72.
8. Omura, M., Hirata, M., Zhao, M., Tanaka, A. and Inoue, N. Comparative testicular toxicities of 2 isomers of dichloropropanol, 2, 3-dichloro-1-propanol and 1, 3-dicholoro-2-propanol and their metabolites alpha chlorohydrin and epichlorohydrin and the potent testicular toxicant 1,2- dibromo-3-chloropropane. *Bulletin of environmental contamination and Toxicology.* 1997; **55**: 1-7.
9. Pandey, R. Pesticides and sterility. *Everyman's Science.* **XXXVVIII:** 2003; 84-86.
10. Shah, N.H., Khan, M.I., Azam, M.F. Seed mycoflora of cow pea and its control with extract of *Argemone maxicana*. *Bioved.* 1992; **3**: 176-178.
11. Shukla, R., Kumar, A., Prasad, C.S., Srivastava, B. and Dubey, N.K. Antimycotic and antiaflatoxigenic potency of *Adenocalymma alliaceum Miers*. on fungi causing biodeterioration of food commodities and raw herbal drugs. *International biodeterioration and biodegradation.* 2008; **62**: 348-351.
12. Thanaboripat, D., Mongkontanawut, N., Suvathi, Y. and Ruangrattanamatee, V. Inhibition of aflatoxin production and growth of *Aspergillus flavus* by *Citronella* oil. *KMITL Sci. Tech. J.* 2004; **49**(1): 1-8.
13. Thanaboripat, D., Chareonsettasilp, S., Pandee, K. and Udomwongsup, K. Inhibitory effect of Kaffir lime, Bitter cucumber and Tobacco extracts on the growth of *Aspergillus flavus. KMILT Sci. Tech. J*. 2006; **6**(1):18-24.