Acta Scientific Microbiology (ISSN: 2581-3226)
Volume 1 Issue 6 June 2018
Research Article

Studies on the Inhibitory Effect of some Plant Extracts on Mycoflora and Aflatoxin Production

Basant Kumar Sinha*

Ex Professor and Head (Microbiology), Bihar Veterinary College, Patna, Bihar, India

***Corresponding Author:** Basant Kumar Sinha, Ex Professor and Head (Microbiology), Bihar Veterinary College, Patna, Bihar, India.

Received: April 20, 2018; **Published:** May 17, 2018

Abstract

Aqueous extract of different plant extracts was exploited in SKMY liquid medium to observe their effect on aflatoxin production either partly or completely. The substances included were various parts of Neem *(Azadirachta indica),* such as dried and fresh levees, seeds and its oil. Other substances were Chiraita *(Swertia chirata),* leaves of tulsi *(Osmium sanctum),* dried Bhant leaves *(Clerodendrum inerme)* and Giloi *(Tinosporo cordifolia).* Dried neem leaves and chiraita were found to be more effective both against AFB1¬ and AFB2 as dried neem leaves showed 87.82 and 80.81 percent inhibition of AFB1 and AFB2 respectively. Chiraita showed 67.24% inhibition and 100% inhibition of AFB2. In both the cases there was complete inhibition of sporulation and only growth of white mycelial matt was observed. Tulsi leaves had no effect on aflatoxin. *Clerodendrum inerme* and *Tinospora cordifolia* showed less than 50% inhibition of aflatoxin.

Keywords: Aspergillus flavus; Aflatoxin production; Plant Extracts; Chromatography

Abbreviation

SMKY: Sucrose, Magnesium Sulphate, Potassium Nitrate and Yeast Extract; AFB: Aflatoxin; TLC: Thin Layer Chromatography

Introduction

Aflatoxins are secondary metabolites produce by certain toxigenic strains of *Aspergillus flavus* (Link ex-Fries) and *Aspergillus parasiticus* (Speare), while colonizing on different agricultural products and animal and human eatables of animal origin, like meat, egg, fish, milk produced by livestock and poultry, when fed with aflatoxin contaminated food [1,2]. Aflatoxins have been recognized as one of the most potent teratogens [3], carcinogens [4], mutagens [5] and immunosuppressant [6] and also cause some metabolic disorders resulting into damage of heart, lungs and kidney finally causing death both in human and animals [2].

As aflatoxins are ubiquitous and has been isolated from different food and feed used for human and animal consumption [7] so it constitutes an international health hazards problem as well as fascinating scientific challenge for scientists. The food contaminated with the toxigenic fungi and presence of aflatoxin is a major concern, which has received worldwide attention due to their deleterious effect on the health of human and animals [8]. In the present era there is craze for herbal medicine's as 80% Asian and African communities rely on traditional herbal medicines for primary health care. There are increasing evidences of the efficiency of many crude plant drugs used by the tribal or traditional societies in preventing and curing different diseases. As control of aflatatoxin has become a global problem in spite of a variety of physical and chemical approaches to counteract the mycotoxin [9], so the present study is to exploit the potential use of aqueous extract of some commonly used plant extract in controlling aflatoxin production.

Materials and Methods

Plants and leaves used

Leaves of *Azadirachta indica* (Neem), *Osmium sanctum, Clerodendrum inerme* and *Tinospora cordifolia* were obtained from local area, whereas chirata was purchased from local shop dealing with indigenous drugs. All these materials were treated with 2% aqueous solution of calcium hypochlorite [10].

Preparation of aqueous extract

Aqueous extracts of different leaves were prepared in glass homogenizer. The homogenized liquid was centrifuged at 3000 rpm and sterilized by passing through Millipore filter 0.22 µm pore size (Sigma, USA). Standard strain of *Aspergillus flavus* (BUML-2) was obtained from Postgraduate department of Botany, Bhagalpur and was maintained on potato dextrose agar [11].

Procedure of test

One ml. of test extract was mixed with 49 ml of the liquid SMKY (sucrose, magnesium sulphate, potassium nitrate and yeast extract) in 250 ml. Erlenmeyer flask. For control 1 ml. distilled water was mixed with 49 ml liquid SMKY medium. Each set was inoculated with 0.1 ml of spore suspension (107 spore/ml) of *Aspergillus flavus* and were incubated at room temperature. Each set was run in triplicate. These flasks were examined daily for the presence of mycelia and spores. After two weeks of incubation the content

of each flask was filtered with Whatman filter paper No.1. From the culture filtrate aflatoxin was extracted in chloroform. 0.05 ml chloroform extract was spotted and separated by thin layer chromatography (TLC) on silica gel (G) using Toluene: Isoamyl alcohol: Methanol, 90:30:3 (v/v/w) solvent system [12]. Quantitative estimation of aflatoxin was done spectrophometrically [13].

Percent inhibition of aflatoxin was calculated as follows:

$$\text{Percent inhibition} = \frac{\text{Aflatoxin level in control- treated x 100}}{\text{Aflatoxin level in control}}$$

Student "t" test was applied for testing the significance. Level of significance at which data were significant or not have been denoted by p-level of significance for the test.

Result and Discussion

India is bestowed with unique diversity in culture and natural vegetation exhibiting rich plant diversity. Herbs have always been the principal form of medicine in India and use of certain plant extracts has been used for controlling the growth of aflatoxigenic fungi and aflatoxin production [14-17].

Effect of aqueous extract of all the above plants have been presented in table 1.

S.N.	Name of plant parts/extracts	Concentration of Aflatoxin (μgm/ml)				% Inhibition	
		Aflatoxin B_1		Aflatoxin B_2			
		Control ± SE	Test ± SE	Control ± SE	Test ± SE	AFB_1	AFB_2
1.	Neem (Azadirachta indica) leaves (fresh)	12.56 ± 0.65	11.12 ± 1.09^{NS}	8.95 ± 1.90	4.97 ± 1.29^{NS}	11.46	50.05
2.	Neem (Azadirachta indica) leaves (dried)	13.12 ± 0.28	1.65 ± 0.31**	9.13 ± 1.47	1.75 ± 0.06^{NS}	87.42	80.81
3.	Neem (Azadirachta indica) oil (1ml/dl)	11.38 ± 0.75	8.91 ± 0.76^{NS}	8.23 ± 0.11	1.90 ± 0.11***	21.47	76.91
4.	Neem (Azadiracht indica) oil (0.5ml/dl)	11.38 ± 0.75	9.27 ± 0.24^{NS}	8.24 ± 0.11	1.65 ± 0.78*	18.54	80.07
5.	Neem (Azadirachia indica) seeds	12.43 ± 1.38	9.8 ± 0.46^{NS}	7.39 ± 0.67	1.73 ± 0.06*	20.69	76.76
6.	Osmium sanctum (Tulsi) (fresh leaves)	12.75 ± o.54	12.75 ± 1.42^{NS}	10.66 ± 1.47	10.54 ± 0.76^{NS}	0.00	0.00
7.	Swertia chirata (Chirata)	9.87 ± 0.32	3.23 ± 0.45**	9.18 ± 0.78	0.00 ± 0.00**	67.29	100.0
8.	Clerodendrum inerme	11.89 ± 1.33	8.96 ± 0.23^{NS}	7.36 ± 0.36	1.89 ± 0.10**	27.234	74.33
9.	Tinospora cordifolia	11.75 ± 0.54	10.67 ±0.52^{NS}	9.77 ± 0.23	5.52 ± 0.23*	9.19	43.50

Table 1: Evaluation of some plant extracts against aflatoxin production.

N.B: NS = Non-significant, * - $p < 0.05$, ** = $p < 0.1$, *** $p < 0.001$.

Neem (*Azadirachta indica*) has reputed value for its antifungal properties. Among the different components of *Azadirachta indica* (neem) studied, the maximum inhibition of AFB1 (87.42%) was exhibited by dried neem leaves showing highly statistical significant level ($p < 0.01$) whereas minimum inhibition of AFB1 (18.54%) was caused by neem oil in 0.5% dilution. The percent inhibition by fresh neem leaves, neem oil (1% concentration) and neem seeds was found to be 11.46, 21.47 and 20.69 respectively (neem leaves, neem oil (Table 1). The extract of dried neem leaves was also responsible for 80.81% inhibition of AFB2 whereas fresh neem leaves, neem oil (both 1% and 0.5% concentration) inhibited AFB2 production above 50%. Inhibition of aflatoxin production in *Aspergillus parasiticus* grown in presence of various concentration of neem extract has been observed [18]. Aflatoxin was at its lowest level (> 90%) inhibition when the concentration of neem extract was adjusted 50% (v/v). Other workers also showed inhibitory effect of different preparation of neem on aflatoxin production [19].

It is interesting to note that almost all parts of the neem studied in the present investigation inhibited sporulation and only mycelial matts were observed. Though sporulation has no direct effect on aflatoxin production, but reduced sporulation certainly reduces quantum of fungi produced and thus can minimize elaboration of aflatoxin. Ranjan*., et al.* [20] have also observed no correlation between inhibition of fungal spores and toxin production. It was observed that the growth of *Aspergillus flavus* decreased progressively with increasing concentration of essential oil from leaves of neem and seeds when incorporated in SKMY medium [21]. Other studies also revealed that volatile neem leaf constituents are known to potentially inhibit aflatoxin biosynthesis in *Aspergillus parasiticus* without affecting fungal growth [22,23].

Other plants used to evaluate anti-toxic activity was *Osmium sanctum, Swertia chirata, Clerodendrum inerme* and *Tinospora cordifolia*. It is evident from table 1 that out of the above substances Swertia chirata was found to be most useful for inhibiting significantly both AFB_1 ($P < 0.01$) and AFB_2 ($p < 0.01$). The percent inhibition for AFB_1 and AFB_2 was found to be 67.29 and 100.00 respectively. It also inhibited sporulation leaving white matt of mycelia only. *Swertia chirata* is a most common herb used in many parts of India in the treatment of various ailments of human beings including liver disorder [24] and hepatoprotective activity [25] and one of the important ingredients in animal herbal medicine for protection against aflatoxicosis. *Osmium sanctum* was found to be ineffective on the inhibition of AFB_1 and AFB_2, however, some worker observed that in the presence of ocimum extract, the culture of *Aspergillus flavus* in yeast extract sucrose medium showed an increase in mycelial mass and reduction of aflatoxin B1 production up to 64% [26]. *Clerodendrum inerme* inhibited AFB2 significantly ($p < 0.01$) but has no effect on AFB1. The percent inhibition of AFB_1 and AFB_2 was 27.24 and 74.33 respectively. The reason for inhibiting AFB_2 and not inhibiting AFB cannot be explained and requires further elucidation. The antifungal activity of *Clerodendrum inerme* and *Clerodendrum phlomidis* against *Aspergillus flavus* and *Aspergillus niger* has been reported but their effect on aflatoxin production/inhibition was not studied [27]. Lot of works have been done on *Tinospora cordifolia* as immunostimulant and protective properties it's extract has been reported to scavenge free radicles gen-

erated during aflatoxicosis and thus has protective effect against aflatoxicosis [28]. In the present investigation it's percent inhibition for AFB_1 and AFB_2 was found to be 9.19 and 43.50 respectively.

As various indigenous plant extracts offer many promising prospects both for traditional and modern medicine, so it is suggested that more and more indigenous plants should be exploited for prevention of aflatoxin, as aflatoxin is of great concern in many countries throughout the world.

Conclusion

It is concluded that the extreme toxicity and carcinogenicity of the aflatoxin and the ubiquitous occurrences of toxigenic strains of *Aspergillus flavus* indicate that it is not the mere agent of deterioration but equally responsible for human and animal health hazards. As it is difficult to get rid of aflatoxin problem due to ubiquity of *Aspergillus flavus*, so it is essential to take some steps in order to control aflatoxicosis. On the basis of the present study it was observed that aqueous extract of *Azadirachta indica* and Swertia chiretta was found to be effective inhibitory substance for aflatoxin production under laboratory condition. These findings can be exploited for prevention of aflatoxin contamination. It is further suggested if various food and feed products if treated with these extracts may inhibit and prevent the production of aflatoxin and thus hence human and animal population can be saved from the toxic effect of aflatoxin.

Bibliography

1. Bennett JW and Klinch M. "Mycotoxins". *Client Microbiology* 16.3 (2003): 497-451.

2. Herzallah SM. "Aflatoxin B_1 residue in eggs and flesh of laying hens fed aflatoxin B_1 contaminated diet". *American Journal of Agricultural and Biological Sciences* 8.2 (2013): 156-161.

3. Wagnikar PB., *et al*. "Teratogenic effects in rabbits of simultaneous exposure tp ochratoxin A and aflatoxin B_1 with special reference to microscopic effects". *Toxicology* 215.1-2 (2005): 37-40.

4. Harris CC and Sun T. "Multifactorial etiology of human liver cance". *Carcinogenesis* 5.6 (1984): 697-712.

5. Ong T. "Aflatoxin Mutagenesis". *Mutation Research* 32.1 (1975): 35-58.

6. Norbury KC. "Immunotoxicological evaluation: An overview". *Journal of the American College of Toxicology* 4 (1985): 279-290.

7. Sinha BK and Singh RN. "Studies on aflatoxigenic fungi from human animal feed". *Toxin Reviews* 8 (1989): 293-300.

8. Mishra HN and Das C. "A Review on biological control and metabolism of aflatoxin". *Critical Reviews in Food Science and Nutrition* 43.3 (2003): 245-264.

9. Samarajeeva V., *et al*. "Detoxification of aflatoxin by physical and chemical method". *Journal of Food Protection* 53.6 (1990): 483-501.

10. Seenappa M., *et al*. "Aspergillus infection and aflatoxin production in beans (*Phaseolus vulgaris*): in Tanzania". *International Biodiversity Bulletin* 17.3 (1981): 79-82.

11. Diener UL and Davis ND. "Aflatoxin production by isolates of Aspergillus flavus". *Phytopathology* 56 (1966): 1390-1394.

12. Reddy TV., *et al*. "Thin layer chromatography of aflatoxin". *Analytical Biochemistry* 38.2 (1970): 568-571.

13. Nabney J and Nesbitt BF. "A spectrophometrically method for determining the aflatoxin". *Analyst* 90 (1965): 155-160.

14. Sapkota D., *et al*. "Efficacy of *Andrographis* paniculata in amleorating aflatoxicosis in broilers". *Indian Veterinary Journal* 82.5 (2005): 529-532.

15. Krisnamurthy YL and Shashikala J. "Inhibition of aflatoxin B_1 production of *Aspergillus flavus*, isolated from soybean seeds by certain natural plant products". *The Journal of Applied Microbiology* 43.5 (2006): 469-474.

16. Sandosskumar R., *et al*. "Inhibition of Aspergillus flavus growth and detoxification of aflatoxin B_1 by the medicinal plant Zimmu (Allium sativum L x Allium cepa L):". *World Journal of Microbiology and Biotechnology* 23.2 (2007): 1007-1014.

17. Vijayenand S., *et al*. "Detoxification of aflatoxin $B_1$by an aqueous extract from leaves of Adhatodavasico Nees". *Microbiological Research* 169.4 (2014): 249-300.

18. Allameh A., *et al*. "Effect of neem leaf extract on production of aflatoxins and activities of fatty acid synthetase, isocitrate dehydrogenase and glutathione S-transferase in Aspergillus parasiticus". *Mycopathologia* 154.2 (2002): 79-84.

19. Ghorbanian M., *et al*. "Study on the effect of neem (Azadirachta indica A.juss): leaf extract on the growth of Aspergillus parasiticus and production of aflatoxin by it at different incubation time". *Mycosis* 51.1 (2008): 35-39.

20. Ranjan KS., *et al*. "Evaluation of some bitter plant extracts against aflatoxin production and growth of *Aspergillus flavus*". *National Academy Science Letters* 14 (1991): 241-243.

21. Bankola SA. "Effect of essential oils from two Nigerian medicinal plants (*Azadirachia indica* and *Morida lucida*): on growth and aflatoxin B_1 production in maize grain by a toxigenic *Aspergillus flavus*". *Letters in Applied Microbiology* 24 (1997): 190-192.

22. Bilgrami KS., *et al*. "Effect of some wild and medicinal plant extract on aflatoxin production and growth of *Aspergillus flavus* in liquid". *International Journal of Botany* 59 (1980): 123-126.

23. Bhatnagar D and Mc Cormic SP. "The inhibitory infect of neem (*Azadirachia indica*): leaf extract on aflatoxin systhesis in *Aspergillus parasiticus*". *Journal of the American Oil Chemists' Society* 65.7 (1988): 1166-1168.

24. Kumar V and Staden JV. "A Review of *Swertia chirata* (Gentianceae): as a Traditional Medicine Plant". *Frontiers in Pharmacology* 6 (2015): 308-318.

25. Nagalekshmi R., *et al*. "Hepatoprotective activity of *Andrographis paniculata* and *Swertia chirayita*". *Food and Chemical Toxicology* 49.2 (2011): 3367-3373.

26. Panda P and Mehta A. "Aflatoxin detoxification potential of ocimum Tenuiflorum". *Journal of Food Safety* 33.3 (2013): 229-377.

27. Anitha R and Kannan P. "Antifungal activity of *Clerodendrum inerme*(L): and *Clerodendrum phlomidis* (L):". *Turkish Journal of Biology* 30.3 (2006): 139-142.

28. Patni K. "The amazing pharmacological properties of *Tinospora cordifolia*-A short Review". *International Journal of Ayurveda and Pharmaceutical Chemistry* 4.2 (2015): 105-129.

Volume 1 Issue 6 June 2018