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Effect of aqueous extracts of some Nepalese plants on the growth of and aflatoxin production by *Aspergillus flavus*

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The effect of aqueous extracts of 40 Nepalese plants representing 25 families was studied on the growth of and aflatoxin production by a toxigenic strain of *Aspergillus flavus* in SMKY liquid medium. Twenty two plants inhibited aflatoxin production to the extent of 50 per cent or more. Maximum inhibition in aflatoxin production (82.71%) was recorded by the leaf extract of *Zanthoxylum armatum* followed by *Aloe vera* (78.25%) and *Drymaria cordata* (76.06%). Extracts of 2 plants viz. *Cucumis sativus* and *Capsicum annuum* were, however, found to stimulate toxin production. Aflatoxin production had no correlation with the growth of *A. flavus* because maximum inhibition in mycelial growth was recorded by the extract of *Coriandrum sativum* (47.36%) followed by *Gingiber officinale* (42.10%).

Key words : Aflatoxin, *Aspergillus flavus*, aqueous extracts, Nepalese plants

INTRODUCTION

Aflatoxins are a group of highly toxic secondary metabolites produced by the toxigenic strains of *Aspergillus flavus* Link ex Fries and *A. parasiticus* Speare. Their natural contamination in a wide variety of food commodities has posed serious health hazards to the consumers due to their carcinogenic nature (IARC, 1976, 1987; Bhat *et al.*, 1978). Considerable concern has, therefore, been shown towards their control. Although, several physical, chemical and biological methods have been suggested to prevent aflatoxin production, they have not been satisfactory from economical and practical view points.

In the recent years, use of plant extracts has also been suggested as an effective method of controlling aflatoxin contamination in agricultural commodities due to their inhibitory effects on aflatoxin production (Bilgrami *et al.*, 1980, 1992; Singh, 1981; Sinha, 1985, 1990; Singh and Sinha, 1985, 1986). Such effects of plant extracts on aflatoxin production have not been reported yet form Nepal, although the growth suppression of *Aspergillus flavus* has been studied using some plant extracts, essential oils and some chemicals (Joshi, 2000; Shrestha, 2000). In the present investigation an attempt has, therefore, been made to screen extracts of some Nepalese plants against growth of and aflatoxin production by a toxigenic strain of *A. flavus* in liquid SMKY medium.

MATERIALS AND METHODS

Different parts of plant species which are generally considered non-toxic and commonly found as wild or cultivated in Nepal, were collected from Kathmandu valley and Biratnagar, during the month of July, 2002. The collected plant materials were sealed in sterile polythene bags and were immediatily brought to the University Department of Botany, T. M. Bhagalpur University, Bhagalpur, for the preparation of aqueous extracts.

Aqueous extracts (2:10, w/v) of the plant materials were prepared with sterilized distilled water in glass homogenizers. The homogenate was filtered and subsequently centrifuged and the supernatant was used as test extract. One ml of the test extract was mixed with 24 ml SMKY liquid medium comprising Sucrose - 200 g ; Magnesium sulphate -

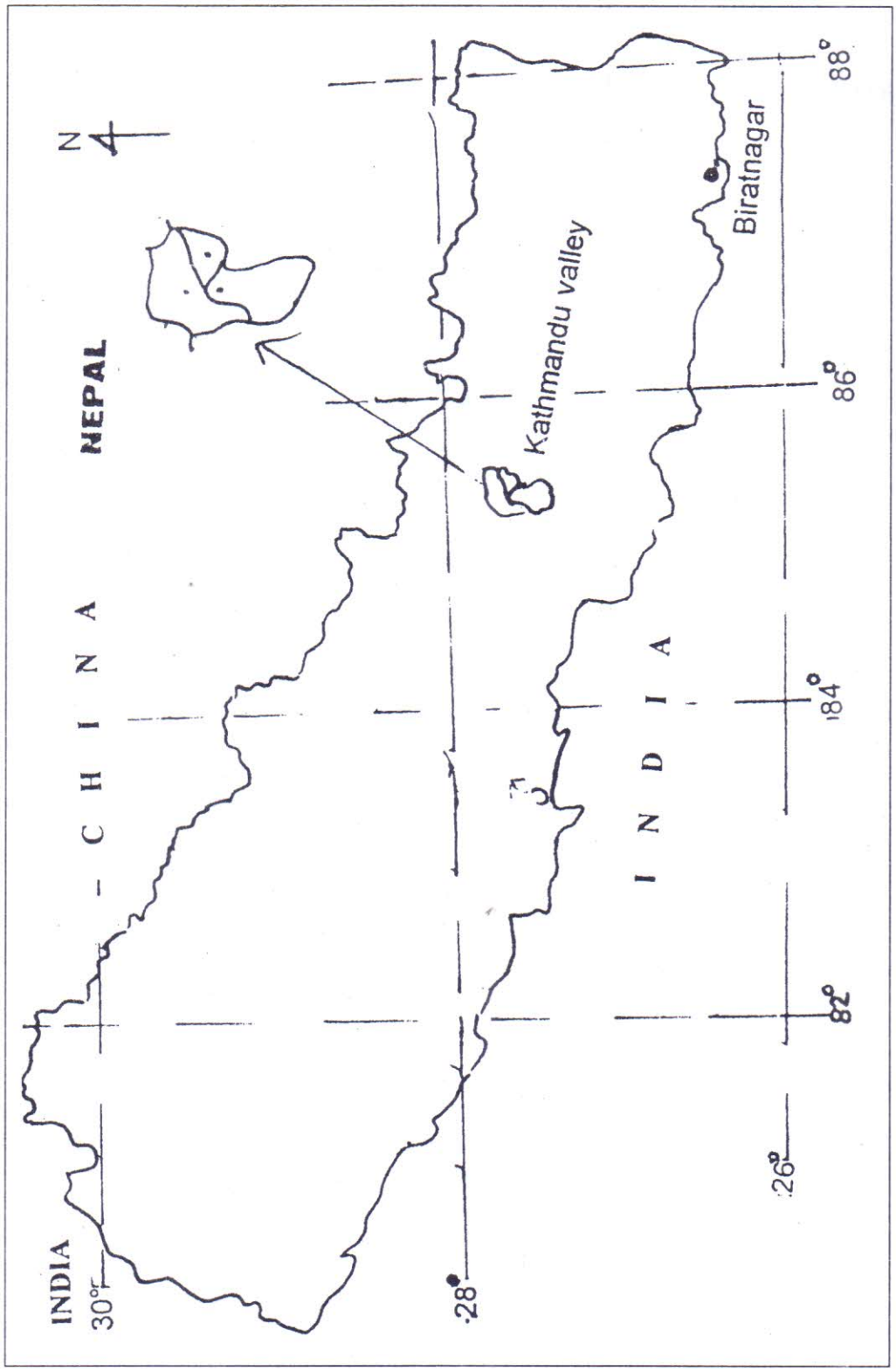

Fig. 1 : Map of Nepal showing areas of collection of plant materials for their aqueous extracts.

0.5 g; Potassium nitrate - 3 g; Yeast extract - 7 g/l distilled water (Diener and Davis, 1966) contained in a 250 ml Erlenmeyer conical flask. Control lots were maintained by adding 1 ml sterile distilled water in 24 ml of the medium. Each set had three replicates. The flasks were inoculated with 0.5 ml spore suspension of culture of a known toxigenic strain of *Aspergillus flavus* (AF - 63), which was previously isolated from wheat kernels. The flasks were then incubated at 28 ± 2°C for 10 days after which the cultures were filtered through Whatman no 1 filter paper. Mycelial mat was dried at 60°C for 48 hours and weighed. Average dry weight of the mycelial mat was used ad the criterian of the fungal growth. The filtrate was extracted with chloroform and chloroform extract was evaporated to dryness over a water bath. The residue was dissolved in 1 ml chloroform. Qualitative estimation of aflatoxins was done by TLC method using toluene: iso-amyl alcohol: methanol (90 : 32 : 2) as mobile phase (Reddy *et al*., 1970). The quantitative estimation of aflatoxin was done spectrophotometrically (AOAC, 1984).

RESULTS AND DISCUSSION

Table 1 and Fig. 1 shows the inhibition of the growth of *Aspergillus flavus* and subsequent aflatoxin B_1 production by different plant extracts. Out of 40 plant species representing 25 families studied in the present investigation, 22 plant species inhibited aflatoxin production to the extent of 50 per cent or more. The extract of *Zanthoxyhum armatum* showed maximum inhihition of aflatoxin B_1 production by 82.71% followed by those of *Aloe vera* (78.25%) and *Drymaria cordata* (76.06%). Extracts of some plants like *Adiantum capillus-veneris, Acalypha indica, Cymbopogon* sp., *Allium cepa, Melia azedarach, Raphanus sativus, Swertia chirayita, Nephrolepis cordifolia, Asparagus recemosus* and *Coriandrum sativum* also showed marked inhibition (> 60%) of aflatoxin production. The present findings of inhibitory effect of aflatoxin production by extracts of *Acalypha indica, Allium cepa, Melia azedarach, Raphanus sativus* showed resemblances with earlier investigations (Bilgrami *et al*., 1980; Sinha, 1985; Ranjan *et al*., 1991; Kumar and Prasad, 1993). However the extracts of some plants like *Ocimum sanctum, Oxalis corniculata, Ficus religiosa, Eclipta alba* which were reported to exhibit negligible inhibitory effect (Sinha, 1985) had shown marked inhibitory effect on aflatoxin production in this investigation. Extracts of some plants like *Ephedra gerardiana, Acorus calamus* and *Phyllanthus emblica* showed reduced inhihitory effect (< 25% inhibition) on aflatoxin production. However extract of *P. emblica* was earlier reported to show marked inhibitory effect (> 60%) on aflatoxin production (Sinha, 1985; Ranjan *et al*., 1991) Interestingly, the extracts of two plants viz. *Cucumis sativus* and *Capsicum annuum* were found to stimulate aflatoxin production. Extract of *C. annuum* has, however, been reported earlier to inhibit aflatoxin production (Singh and Sinha, 1986).

The growth of *A. flavus* was not correlated with toxin production. Extracts of some plants like *Cymbopogon* sp. and *Ficus religiosa* showed inhibitory effect on aflatoxin production but stimulatory effect on the growth of *A. flavus*. On the other hand, extracts of some plants like *Cucumis sativus* and *Capsicum annuum* showed inhibitory effect on the growth of *A. flavus* with stimulatory effect on aflatoxin production. Similarly extracts of *Momordica charantia, Bauhinia purpurea* showed reduced inhibitory effect of growth of *A. flavus* but marked inhibitory effect on aflatoxin production. However, extracts of some plants like *Coriandrum sativum, Asparagus racemosus, Azadirachata indica, Nephrolepis cordifolia, Gingiber officinale* etc. showed significant correlation between aflatoxin B_1 production and growth of *A. flavus.*

It is clear from the above results that some plants possess antifungal and/or antitoxic properties. This may be due to the effect of certain active compounds present in the aqueous extracts of these plants on the metabolism of the fungi.

On the basis of earlier reports (Singh and Sinha, 1985, 1986; Ghewande and Nagaraj. 1987; Sinha, 1990; Bilgrami *et al*., 1992), it is expected that the plants screened as effective inhibitors of aflatoxin production in the present investigation may show similar results with agricultural commodities both in the laboratory as well as in field conditions.

Table 1 : Effect of plant extracts on growth of and aflatoxin production by *Aspergillus flavus* in Liquid SMKY medium.

Family	Plants Species			Plant parts used	Growth of *A. flavus*		Aflatoxin B_1 Production	
	Botanical Name	Common Name	Local Name		Average Mycelial weight (g/25ml)	% inhibition	Amount (µg/l)	% inhibition
	CONTROL				1.90	—	1625.00	
1. Amaranthaceae	*Achyranthes aspera* L.	Prickly chaff flower	Apamarg	Lf.	1.42	25.26	1006.20	38.08
2. Anacardiaceae	*Choerospondias axillaris* (Roxb.) Brutt & Hill	Hog plum	Lapsi	Lf	1.43	24.73	989.30	39.12
	Mangifera indica L.	Mango	Anp	Lf.	1.79	5.78	845.81	47.95
3. Araeea	*Acorus calamus* L.	Sweet Flag	Bojho	Lf.	1.86	2.10	1236.14	23.93
	Colocasia esculenta (L.) Sehott	Co-Co Yam	Karkalo	Lf.	1.51	20.53	938.73	42.25
4. Berberidaceae	*Mahonia napaulensis* DC.		Jamani mandro	Lf.	1.41	25.79	989.30	39.12
5. Caryophyllaceae	*Drymaria cordata* (L.) Willd ex Roem & Schult.		Abhijalo		1.33	30.00	389.03	76.06
6. Compositae	*Eclipta alba* (L.) Hassk.		Bhringraj	Lf.	1.39	26.84	914.06	43.75
7. Cruciferae	*Raphanus sativus* L.	Radish	Moola	Lf.	1.35	28.94	559.98	65.54
8. Cucurbitaceae	*Cucumis sativus* L.	cucumber	Kankro	Fr.	1.30	31.57	2152.22	**32.44
	Momordica charantia L.	Bitter gourd	Tite Karela	Fr.	1.85	2.63	688.35	57.64
9. Euphorbiaceae	*Acalypha indica* L.		Khakali	Lf.	1.51	20.52	435.99	73.17
	Phyllanthus emblica L.		Amla	Lf.	1.92	*1.05	1519.38	6.5
10. Gentianaceae	*Swertia chirayita* (Roxb. ex Flem.) Karst.	Chiretta	Chirato	Lf. & St.	2.00	*5.26	561.60	65.44
11. Gingiberaceae	*Gingiber officinale* Rose.	Ginger	Aduwa	Rh.	1.10	42.10	811.85	50.04
12. Gnetaceae (Ephedraceae)	*Ephedra gerardiana* Wall ex Stapf	Ephedrine	Somlata	St.	1.30	31.58	1272.38	21.70
13. Gramineae (Poaceae)	*Cymbopogon* sp.	Lemon grass	Pirhe ghans	Lf.	1.95	*2.63	423.84	69.61
14. Labiatae	*Mentha arvensis* L.	Field Mint	Pudina	Lf.	1.42	25.26	215.84	43.66
	Ocimum sanctum L.	Sacred Basil	Tulsi	Lf.	1.60	15.78	723.78	55.46
15. Leguminosae	*Bauhinia purpurea* L.	Camle's foot tree	Tanki	Lf.	1.76	7.36	654.39	59.73
	(Trigonella foenum-graecum L. (Papilionaceae)	Fenugreek	Methi	Sd.	1.62	14.74	1111.18	31.62
16. Liliaceae	*Allium ascalonicum* L.	Shallot	Chhyapi	B.	1.91	*0.52	706.88	56.50
	A. cepa L.	Onion	Pyaj	B.	1.60	15.78	529.43	67.42
	A. sativum L.	Garlic	Lasun	B.	1.60	15.78	724.43	55.42
	Aloe vera (L.) Bumf. f	Indian Aloe	Ghyu Kumari	Lf.	1.51	20.53	353.44	78.25
	Asparagus racemosus Willd.	Asparagus	Satawari	Lf. & St.	1.35	28.94	566.48	65.14
17. Meliaceae	*Azadirachta indica* A. Juss.	Margosa tree	Neem	Lf.	1.36	28.42	671.45	58.68
	Melila azedarach L.	Peepul	Pipal	Lf.	1.48	22.11	530.08	67.38
18. Moraceae	*Ficus religiosa* L.	Peepul	Pipal	Lf.	1.95	*2.63	645.39	59.73
19. Oleaceae	*Nyctanthes arbortristis* L.	Night Jasmine	Parijat	Lf.	1.53	19.47	832.37	48.78
20. Oxalidaceae	*Oxalis corniculata* L.	Indian Sorrel	Chari Amilo	Lf.	1.62	14.73	935.19	42.45
21. Polypodiaceae	*Adiantum capillusveneris* L.	Maidean hair fern	Hansraj	Lf. & St.	1.40	26.32	424.25	73.88
	Nephrolepis cordifolia Presl.	Sword fern	Pani Amala	Lf.	1.36	28.42	564.69	65.25
22. Rutaceae	*Murraya koenigii* (L.) Spreng	Curry Leaf tree	Mitha neem	Lf.	1.22	35.78	892.61	45.07
	Zanthoxylum armatum DC	Nepal Pepper	Timur	Lf.	1.43	24.73	280.96	82.71
23. Solanaceae	*Capsicum annuum* L.	Red Pepper	Khursani	Fr.	1.62	14.73	1751.55	**7.78
	Withania somnifera (L.) Dunal		Ashwa gandha	Lf.	1.33	29.62	789.90	51.40
24. Umbelliferae	*Centella asiatica* (L.) Urb.	Asiatic penny-wort	Ghodtapre	Lf.	1.65	13.15	1129.70	30.48
	Coriandrum sativum L.	Coriander	Dhaniya	Lf.	1.00	47.36	600.44	63.05
25. Valerianaceae	*Valeriana wallichii* DC.	Valerian	Suggandhawal	Lf.	1.41	25.79	847.93	47.82

Note : Lf. = Leaf ; B = Bulb ; St. - Stem ; Fr. = Fruit ; Sd. = Seed ; Rh. = Rhizome

* = % stimulation of growth of *A. flavus*

** = % stimulation of aflatoxin B1 production

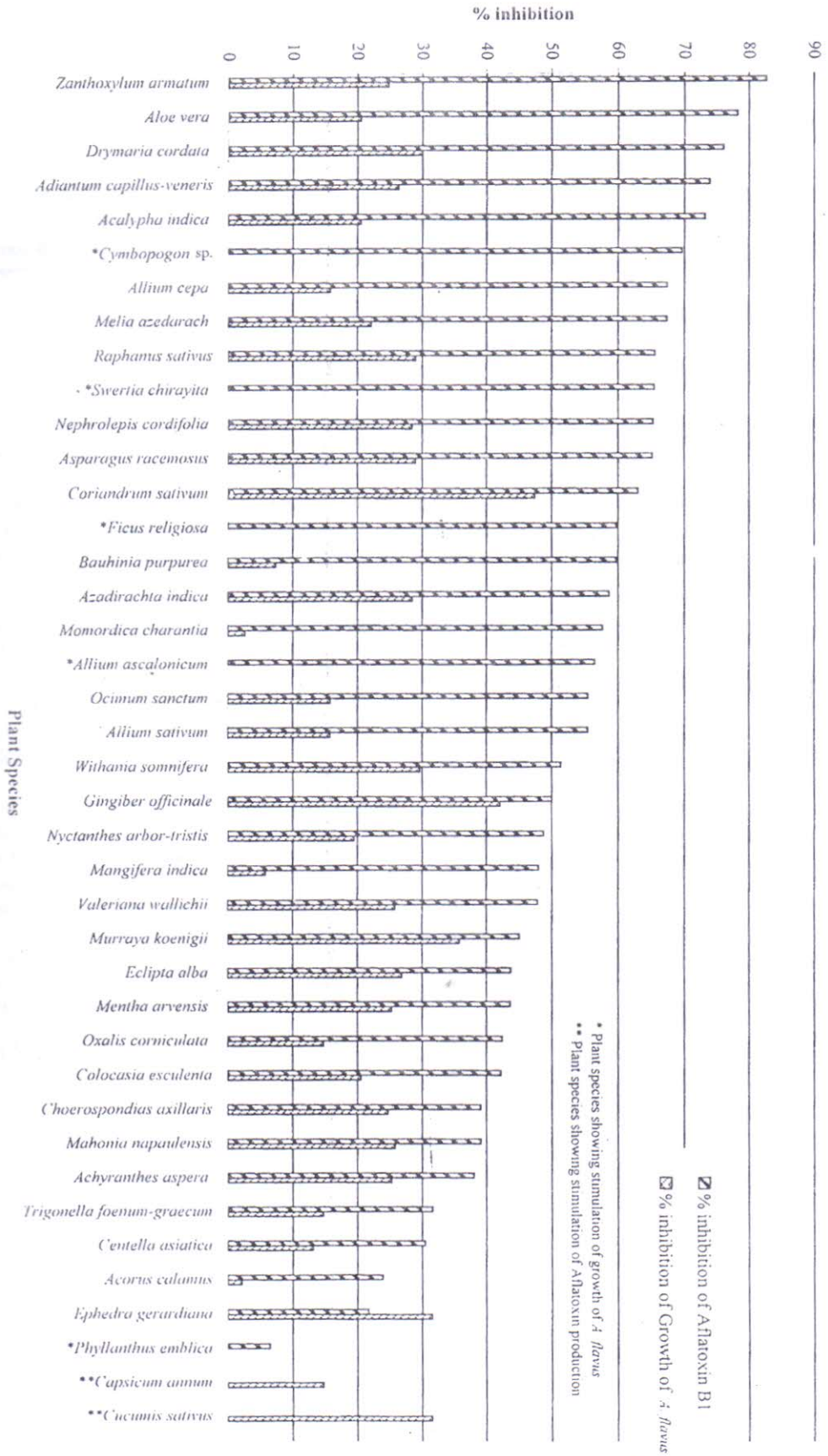

Fig. 1 : Effect of Plant Extracts on Aflatoxin Production and Growth of *Aspergillus flavus* in Liquid SMKY medium

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