Pak.J.Bot., 31(1): 227-230, 1999.

DETECTION OF AFLATOXIN IN ALMOND SEED

ZAKIA BILGRAMI AND A. GHAFFAR

Department of Botany
University of Karachi, Karachi-75270, Pakistan.

Abstract

TLC technique and plug method were used for the detection of aflatoxin in almond seed. Of the 25 seed samples examined, 5 were found to be contaminated with aflatoxin B_1 (5.8-140 μg/kg) which included 2 samples that showed the presence of B_2 (7.0-140 μg/kg) also. Out of 29 isolates of *A. flavus* tested, 9 showed the presence of aflatoxin B_1 with 1:2 ratio of toxigenic and non-toxigenic strains.

Introduction

Almond (*Prunus amygdalus* L.), a native of Central Asia is now cultivated throughout Southern Europe, California (USA), Australia and South Africa (Krishnamurthi, 1969). In Pakistan, almond trees are grown over 7500 ha in the northern areas of Pakistan with an average yield of 30,900 tons per annum (Anon., 1990). The kernels being a rich source of fat (58.9%), protein (20.8%), carbohydrates (10.5%) and mineral matter (2.9%) with a calorific value of 655 cal/100g, are eaten fresh or as a dessert and extensively used in confectionary. Almond oil extracted from sweet as well as bitter almonds is used in pharmaceutical and cosmetic preparations (Krishnamurthi, 1969). During the studies on seedborne mycoflora of almond seed, out of 39 species of fungi isolated, *Aspergillus flavus* Link., was found to be predominant (Bilgrami & Ghaffar, 1996). The fungus is known to produce aflatoxin B_1, B_2, G_1 and G_2 which cause degeneration of kidney and liver cancer (Newberne & Butler, 1969). Toxigenic strains of *A. flavus* not only produce aflatoxins inside the dry fruits but also cause considerable loss in the nutritive value by changing the concentration of vital components (Bilgrami *et al.*, 1983). The present report describes the frequency of aflatoxin contamination in almond seed.

Material and Methods

Twenty five samples of almond seeds collected from different localities of Pakistan were used for the detection of aflatoxins. Aflatoxin was extracted from 50g seed samples using the method as laid down by Association of Officials Analytical Chemist (Anon., 1975). The toxin obtained was redissolved in known quantity of Benzene: Acetonitrile (98:2) solution and spotted on the TLC plate. The TLC plates were developed in chloroform: Xylene: Acetone (60:30:10, v/v) solution and qualitative and quantitative analysis was carried out using standard of aflatoxins provided by Dr. C.J. Mirocha of the Department of Plant Pathology, University of Minnesota, U.S.A.

Isolates of *Aspergillus flavus* were also tested for the presence of aflatoxin using plug method (Frisvad & Filtenborg, 1983). *A. flavus* isolates were inoculated on Yeast

Table 1. Extraction of aflatoxins from seed samples of almond collected from different localities of Pakistan.

Sample No.	Aflatoxin B_1 (μg/kg)	Aflatoxin B_2 (μg/kg)	Sample No.	Aflatoxin B_1 (μg/kg)	Aflatoxin B_2 (μg/kg)
1	---	---	14	---	---
2	---	---	15	---	---
3	---	---	16	---	---
4	---	---	17	---	---
5	11.6	---	18	---	---
6	---	---	19	---	---
7	23.3	---	20	---	---
8	---	---	21	---	---
9	---	---	22	---	---
10	5.8	---	23	---	---
11	---	---	24	7.0	7.0
12	---	---	25	140.0	140.0
13	---	---	--	---	---

Extract Sucrose (YES) Agar containing sucrose 150g, yeast extract 20g, agar 15g in 1000 ml distilled water and incubated for 7 days. Four mm diameter discs from the centre of culture were removed and a drop of extraction liquid (benzene: acetonitrile, 98: 2 v/v) was placed directly on the disc while still wet. The mycelium side of the disc was gently pressed against the application line on a precoated TLC plate (Silicagel 60 G, Merck Art, thickness of silicagel 0.25 mm on 20x20 cm glass plate). The disc was removed from the TLC plate after few seconds when a liquid front appeared. The spots were allowed to dry and the TLC plate developed in chloroform: xylene: acetone (60:30:10) solvent system. After drying the TLC plate in an oven, they were examined before and after spray treatment with 50% H_2SO_4 in visible light as well as under shortwave UV light (254 nm) and long wave UV light (366 nm) (Hashmi, 1988).

Results and Discussion

Of the 25 almond seed sample analysed, 5 were found to be contaminated with aflatoxin B_1 (5.8-140 μg/kg) which included 2 samples that showed the presence of B_2 (7.0-140 μg/kg) also (Table 1). Occurrence of *A. flavus* group on almond has been reported from USA where aflatoxin were detected in harvested Kernels (Philips *et al.*, 1976). According to Shade *et al.*, (1981) a sample of diced almond was reported to contain about 500 ppb aflatoxin with over 80% of the total as B_1 and B_2 whereas more than 20 ppb of aflatoxin present in the food stuff is injurious for health (Purchase, 1974). Out of 29 different isolates of *A. flavus*, 9 were found to be toxigenic and

Table 2. Detection of Aflatoxin from *Aspergillus flavus* Isolates obtained from Almond seed using plug method.

Isolate	Aflatoxin	Isolate	Aflatoxin
1	---	16	B_1^{++}
2	---	17	B_1^{++}
3	B_1^{++}	18	---
4	---	19	---
5	---	20	---
6	---	21	---
7	---	22	B_1^{+}
8	B_1^{++}	23	B_1^{+}
9	---	24	---
10	---	25	B_1^{+}
11	---	26	---
12	B_1^{++}	27	---
13	---	28	---
14	B_1^{+++}	29	---
15	---	---	---

+, ++, +++ shows the relative intensity of the spot.

showed the presence of aflatoxin B_1 when compared with B_1 standard. Of these, one fluosesced extensively (represented by B_1+++), 5 fluoresced moderately (B_1++) and 3 showed fade colour (B_1+) (Table 2). Toxigenic and nontoxigenic isolates of *A. flavus* were found to be present in the ratio of approximately 1:2. Since almond is used in human diet, there is need to ensure that the seeds sold in the market are free from aflatoxin contamination. There is also need to develop an awareness amongst the masses on the adverse effects of mold fungi with special reference to mycotoxin produced.

References

Anonymous. 1975. AOAC Methods, 12th ed. Chapter 26.

Bilgrami, K.S., K.K. Sinha and A. Singh. 1983. Chemical changes in dry fruits during aflatoxin elaboration by *Aspergillus flavus* Link ex Fries. *Curr. Sci.*, 52: 960-964.

Bilgrami, Z. and A. Ghaffar. 1996. Detection and control of seed borne mycoflora in almond. *Proc. 2nd Int. Conf. on Impact of Food, Research on New Product Development*. B.C.C. & T. Press, University of Karachi, pp305.

Frisvad, J.C. and O. Filtenborg. 1983. Classification of terverticillate penicillia based on profiles of mycotoxins and other secondary metabolites. *Appl. Environ. Microbiol.*, 46: 1301-1310.

Hashmi, M.H. 1988. *Seedborne mycoflora of some* Fusarium *species, detection techniques and pathogenicity*. Ph.D. Thesis, Department of Botany, University of Karachi, Pakistan, pp. 164.

Krishnamurthi, A. 1969. *The wealth of India. A dictionary of Indian Raw Materials*, Vol. VIII. Publication Information Directorate CSIR, New Delhi, pp. 394.

Newberne, P.M. and W.H. Butler. 1969. Acute and chronic effects of aflatoxin on the liver of domestic and laboratory animals: a review. *Cancer Research*, 29: 236.

Philips, D.J., M. Uota, D. Monticelli and C. Curtis. 1976. Colonization of almond by *Aspergillus flavus*. *Journal of the American Society for Horticulture Science*, 101: 19-23.

Shade, J.E., A.D. King Jr., B.E. Machey, W.U. Halbrook and G. Fuller. 1981. Sampling diced almonds for aflatoxin. *J. Am. Oil Chem. Soc.*, 58: 852.

Purchase, I.R.H. 1974. *Mycotoxin*. Elsevier Scientific Publ. Com. Amsterdam. pp. 443.

(Received for publication 20 December 1998)