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LOCATION OF FUNGI IN ALMOND (*PRUNUS AMYGDALUS*) SEED

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Abstract

Using component plating technique, 7 genera and 16 species of fungi were isolated from almond seeds collected from different localities of Pakistan. Most of the fungi were located on seed coat followed by cotyledons, shell and axis. The cotyledons were more infected by *Fusarium moniliforme* and *Aspergillus flavus*. Surface disinfection reduced the infection of *Aspergillus* spp., with increase in *Alternaria* sp. Component plating technique could be used to determine the depth of infection and the suitability of dry fruits for human consumption.

Introduction

Almond trees are grown on 7,500 ha in the northern areas of Pakistan with an average yield of 30,900 tons per annum (Anon., 1990). The kernels are eaten fresh or as a dessert in confectionery and for the preparation of almond milk. The kernels are a rich source of fat (58.9%) and protein (20.8%) with a calorific value of 655 Cal./100g (Krishnamurthi, 1969). In a previous study, 37 species of fungi belonging to 13 genera were detected from almond seed (Bilgrami, 1994). Of these *Trichothecium roseum*, the cause of storage rot of almond seed has been reported from India (Sumbali, 1989) and *Aspergillus flavus* from U.S.A. (Philips *et al.*, 1976), the rest of the fungi appear to be new record on almond. There are reports where substrates such as pea nuts, Brazil nuts, walnuts, almonds, pistachio nuts, cotton seeds, corn, sorghum, millet and figs support aflatoxin production by *A. flavus* (Stoloff, 1977) It would therefore suggest that almond seeds contaminated with toxin producing mold fungi would have adverse effect on human health. Experiments were therefore, carried out to study the presence of mold fungi in different parts of almond seed the results of which are presented in this paper.

Materials and Methods

Four samples of almond (*Prunus amygdalus*) seed collected from different parts of Pakistan viz., Karachi (1), Islamabad (1), Muslimbagh (1) and Peshawar (1) were used to study the location of seedborne fungi. One hundred seeds from each sample after removing the shell were washed in distilled water in a test tube and soaked for 10-12 hours in distilled water. The seeds were then dissected into component parts like seed coat, cotyledons and axis (rodicle & plumule). Seed parts treated with 1% solution of $Ca(OCl)_2$ and untreated parts were plated on blotters. Different parts of one seed were plated in a dish and incubated for 5-7 days at 24°C. Fungi growing on the seed parts

Table 1. Location of fungi in different parts of seeds of *Prunus amygdalus* collected from different parts of Pakistan

	Fungi	City	Sterilized				Non sterilized			
			Shell	Seed coat	Cotyledon	Axis	Shell	Seed coat	Cotyledon	Axis
1.	*Alternaria* sp.	Islamabad	—	—	—	—	—	—	—	—
		Karachi	—	—	—	—	—	—	—	—
		Peshawar	6	—	12	—	—	—	4	—
		Muslimbagh	—	—	—	—	—	—	—	—
2.	*Aspergillus candidus*	Islamabad	—	2	2	—	—	8	4	—
		Karachi	—	—	2	—	2	2	2	—
		Peshawar	6	6	—	—	—	12	6	—
		Muslimbagh	—	—	—	2	2	—	2	—
3.	*A. flavipes*	Islamabad	—	—	—	—	—	2	—	—
		Karachi	4	4	—	—	2	10	—	—
		Peshawar	—	—	—	—	—	6	2	—
		Muslimbagh	—	—	—	—	—	2	—	—
4.	*A. flavus*	Islamabad	—	—	—	—	—	16	18	—
		Karachi	2	—	2	—	4	4	—	—
		Peshawar	—	—	—	—	—	4	6	—
		Muslimbagh	—	4	—	—	—	4	4	—
5.	*A. fumigatus*	Islamabad	—	—	—	—	—	2	2	—
		Karachi	2	—	—	—	—	4	2	
		Peshawar	—	—	—	—	—	—	—	—
		Muslimbagh	—	—	—	—	—	4	—	—
6.	*A. niger*	Islamabad	—	—	—	—	—		—	—
		Karachi	—	—	—	—	2	—	—	—
		Peshawar	—	—	—	—	—	—	—	—
		Muslimbagh	—	—	—	—	—	—	—	—
7.	*A. ochraceus*	Islamabad	—	—	—	—	—	—	—	—
		Karachi				—	—		—	
		Peshawar	—	—	—	—				—
		Muslimbagh	—	—	—	2	—	—	2	—
8.	*A. wyndowi*	Islamabad	—	—	—	—		2	—	—
		Karachi	—	2	—	—	—	—	2	—
		Peshawar	—	—	—	—	—	—	—	—
		Muslimbagh	—	—	—	—	—	—	—	—
9.	*A. wentii*	Islamabad	—	—	—	—	—	8	—	—
		Karachi	—	—	—	—	—	—	—	—
		Peshawar	—	—	4	2	—	—	—	—
		Muslimbagh	—	—	—	—	—	—	—	—
10.	*Chaetomium* sp	Islamabad	—	—	—	—	—	—	—	—
		Karachi	—	—	—	—	4	—	—	—
		Peshawar	—	—	—	—	—	—	—	—
		Muslimbagh	—	—	—	—	—	—	—	—
11.	*Fusarium moniliforme*	Islamabad	—	—	6	—	—	—	2	—
		Karachi	—	—	8	2	—	—	14	2
		Peshawar	4	—	—	—	—	—	2	—

Table 1 (Cont'd.)

	Fungi	City	Sterilized				Non-sterilized			
			Shell	Seed coat	Cotyledon	Axis	Shell	Seed coat	Cotyledon	Axis
		Muslimbagh	-	---	---	---	--	4	---	--
12	*Fusarium* spp	Islamabad	---	4	18	---	2	---	16	2
		Karachi		---	6	2	4	4	8	2
		Peshawar	--	6	10	4	6	14	10	10
		Muslimbagh	---	8	2	---	4	6	14	2
13	*Penicillium camemberti*	Islamabad	---	---	---	---	---	---	---	---
		Karachi		--	---	---	---	---	---	---
		Peshawar	---	---	---	---	8	20	---	2
		Muslimbagh	---	---	---	---	---	---	---	---
14	*Penicillium* sp	Islamabad	---	14	---	---	4	8	---	---
		Karachi	---	---	---	---	2	---	---	---
		Peshawar	-	---	2	---	---	2	---	---
		Muslimbagh	---	4	---	---	2	14	---	---
15	*Paecilomyces lilacinus*	Islamabad	-	---	---	---	---	---	---	---
		Karachi	- -	---	---	---	---	---	---	---
		Peshawar	--	--	---	---	---	2	---	---
		Muslimbagh	---	---	---	---	---	---	---	---
16	*Rhizopus* sp	Islamabad	--	---	---	---	10	16	16	6
		Karachi	4	4	---	2	6	14	4	2
		Peshawar	---	---	---	---	---	---	---	---
		Muslimbagh	---	4	---	---	6	8	2	2
			28	62	74	16	70	202	144	30

were examined and identified after reference to Barnett (1960), Booth (1971), Ellis (1971), Nelson *et al.*, (1983). Raper & Thom (1949) and Raper & Fennel (1965).

Results and Discussion

Of a total number of 7 genera and 16 species of fungi isolated from different parts of almond seed, most of the fungi were found to be located on seed coat followed by cotyledons, shell and axis (Table 1). All the samples were infected with *Fusarium moniliforme* where cotyledons were more infected than other parts. Mathur *et al.*, (1975) and Sultana *et al.*, (1988) also found that infection of *F. moniliforme* was greater in the endosperm than seed coat and embryo of sorghum seed. *Aspergillus flavus* was detected from seed coat and cotyledons in all the samples but no infection of *A. flavus* was seen where sterilized seed samples from Islamabad were used. Sterilization of the seed parts reduced the incidence of *Aspergillus* spp. *Alternaria* spp., was detected from Peshawar sample only, where infection was greater in sterilized cotyledons (12%) than non-sterilized (4%). *Penicillium* spp., *Fusarium* spp., and *Rhizopus* sp., were detected from all parts of the seed.

Study of mycoflora by component plating method was helpful in detecting the depth of infection which may be used to determine the suitability of dry fruits for human consumption. Removal of seed coat could eliminate superficial infection and dry fruits with deep seated infection by fungi especially those which produce mycotoxins can be avoided. There are reports where mycotoxins produced by different fungi have physiological and cytotoxic effects on plant system like inhibition of seed germination and seedling growth, fall in protein and chlorophyll synthesis (Bilgrami, 1995).

Apart from *Aspergillus flavus* which produces aflatoxin B1, B2, G1 and G2, known to cause of liver cancer (Purchase, 1974), the association of mycotoxin producing strains of *Fusarium* sp., with a number of food items also pose a serious threat to human and animal health (Bilgrami *et al.*, 1990). There is therefore need to use healthy seed free from contamination with mold fungi.

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