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FUNGI ASSOCIATED WITH *PISTACIA VERA*

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Abstract

Using ISTA techniques, 9 genera and 22 species of fungi viz., *Alternaria alternata, A. tenuissima, Aspergillus candidus, A. clavatus, A. flavus, A. fumigatus, A. niger, A. sulphureus, A. terreus, A. wentii, Aspergillus spp., Chaetomium spp., Cladosporium sp, D. state of Cochliobolus spicifer, Fusarium equiseti, F. oxysporum, Fusarium spp., Penicillium camemberti, P. decumbens, Penicillium spp., Rhizopus sp.*, and *Trichoderma harzianum* were isolated from different parts of pistachio nuts collected from different parts of Pakistan. Of these, *A. flavus* and *A. niger* were found predominant. The cotyledons were infected by greater number of fungi with less infection in shell, seed coat and axis (radicle and plumule). Less number of fungi were isolated by deep freezing method as compared to agar plate and blotter methods. Sterilization of seeds with 1% $Ca(OCl)_2$ reduced the infection of *Aspergillus* species only.

Introduction

Pistachio kernels are rich in proteins, fats and carbohydrates having a calorific value of 626 cal/100 g (Krishnamurthi, 1969). Pistachio nuts are attacked by *Nematospora coryli* which produces stigmatomycosis (massu disease) in Greece (Kouyeas, 1979), Iran (Ershad & Barkhordary, 1976) and U.S.S.R (Kutlunina, 1975) producing brown necrotic areas and malformation of cotyledons. Pistachio nuts are also prone to infection by *Aspergillus flavus* and *A. parasiticus* where the fungi produce aflatoxin mostly detected when pistachios were stored at 100% RH (Sommer *et al.*, 1986; Mujtahedi *et al.*, 1978). There are reports that increased infection of *A. flavus* was correlated with exposure of the nut since endocarp (shell) and often mesocarp (hull) were split during maturation (Thomson & Mehdy, 1978). Experiments were therefore, carried out to study the association of mold fungi with different component parts of pistachio nut, the results of which are presented in this paper.

Materials and Methods

Ten samples of *Pistacia vera* seeds collected from different localities of Pakistan viz., Islamabad (1) Quetta (1) Muslimbagh (1) Risalpur (1) Peshawar (1) Swat (1) Hyderabad (1) and Karachi (3) were used. From 150 seeds of each sample, the kernels taken out easily by separating hard shell, were soaked in sterilized water for 1 h (Mathur *et al.*, 1975) and seed parts i.e., seed coat, cotyledon and axis were separated (Lawrence, 1951). Agar plate, blotter and deep freezing method as recommended by ISTA (Anon., 1976) were used for isolation of fungi. In agar plate method, the seed parts untreated and after treatment with 1% $Ca(OCl)_2$ were plated on potato dextrose

Table 1. Location o Fungi in different parts of *Pistacia vera* seed collected from different parts of Pakistan. (infection %)

Fungi	Province	Shell						Seed Coat						Cotyledons						Axis					
		Agar		Blotter		Deep F.		Agar		Blotter		Deep F.		Agar		Blotter		Deep F.		Agar		Blotter		Deep F.	
		S	N	S	N	S	N	S	N	S	N	S	N	S	N	S	N	S	N	S	N	S	N	S	N
Alternaria alternata	Baloch-istan	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-
	N.W.F.P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	20	-	-	-	-	-	-
A. tenuis-sima	Sindh	-	-	-	-	-	-	-	-	-	-	-	10	-	-	-	-	-	-	-	-	-	-	-	-
Aspergillus candidus	Baloch-istan	-	-	-	-	-	-	10	-	-	-	-	-	-	-	16	8	4	-	10	-	10	-	-	-
	Federal	-	-	-	-	-	-	-	-	-	20	-	-	-	-	-	-	4	-	-	-	-	-	-	-
	N.W.F.P.	-	-	-	-	2	-	-	-	-	-	-	-	-	-	25	-	2.5	2	-	10	-	-	-	-
	Sindh	-	-	-	-	-	-	-	-	-	10	-	-	10	8	-	-	12	30	-	-	-	10	10	-
A. clavatus	Bloch-istan	16	2	-	-	-	-	-	-	-	-	-	-	20	10	2	-	-	-	-	-	-	-	-	-
	N.W.F.P	-	-	-	-	-	-	-	-	-	-	-	-	26	10	-	-	-	-	-	-	-	-	-	-
A. flavus	Baloch-istan	8	-	-	-	-	-	30	10	-	-	-	-	-	14	10	16	10	12	10	60	-	-	-	-
	Federal	-	-	4	36	8	12	-	8	10	20	-	-	8	28	4	24	4	16	40	-	-	-	-	-
	N.W.F.P	2	4	-	2	-	2.5	-	30	-	-	-	-	24	48	20	22.5	-	-	-	-	-	-	-	-
	Sindh	-	-	5	4	-	-	30	40	20	50	20	50	26	34	26	33	7	17	-	-	28	40	10	-
A. fumiga-tus	Baloch-istan	-	6	-	-	-	-	-	-	-	-	-	-	-	-	6	-	-	-	-	-	-	-	-	-

Table 1 (Cont'd)

Fungi	Province	Shell						Seed Coat						Cotyledons						Axis					
		Agar		Blotter		Deep F.		Agar		Blotter		Deep F.		Agar		Blotter		Deep F.		Agar		Blotter		Deep F.	
		S	N	S	N	S	N	S	N	S	N	S	N	S	N	S	N	S	N	S	N	S	N	S	N
	Federal	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-
A. niger	Baloch-istan	12	42	32	68	48	48	20	100	-	-	-	-	44	14	20	30	10	14	-	50	-	-	-	-
	Federal	64	88	60	100	20	64	32	30	-	-	-	-	40	76	36	88	12	20	60	-	-	-	-	-
	N.W.F.P	26	82	12	32	12	50	40	-	-	-	-	-	30	52	32	23	1	3	-	-	-	-	-	-
	Sindh	12	29	-	13	-	8	60	100	-	-	20	-	27	40	2	9	2.5	10	-	-	-	-	-	-
A. sulphur-eus	Baloch-istan	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-
A. terreus	Baloch-istan	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	2	-	-	-	-	-	-
	Federal	-	-	-	-	-	4	-	-	10	-	8	-	-	-	4	-	-	4	-	-	-	20	-	-
	N.W.F.P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	15	-	-	2	-	-	-	-	-	-
	Sindh	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9	-	1	7	-	-	-	-	-	-
A. wentii	Baloch-istan	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16	6	2	-	50	-	-	-	-	-
	Federal	-	-	-	-	-	-	-	-	-	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	N.W.F.P	-	-	-	-	-	2	-	80	40	60	-	-	-	-	-	-	-	2	-	60	-	-	-	-
	Sindh	-	-	-	-	-	-	-	100	20	50	-	-	-	-	-	-	-	-	-	-	-	50	-	-
Aspergillus spp.	N.W.F.P.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	20	2	-	-	-	-	-	-	-	-
	Sindh	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	8	-	-	-	-	-	-
Chaetomium spp.	Baloch-istan	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 1 (Cont'd)

Fungi	Province	Shell						Seed Coat						Cotyledons						Axis					
		Agar		Blotter		Deep F.		Agar		Blotter		Deep F.		Agar		Blotter		Deep F.		Agar		Blotter		Deep F.	
		S	N	S	N	S	N	S	N	S	N	S	N	S	N	S	N	S	N	S	N	S	N	S	N
Cladospo-rium	N.W.F.P	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D. state of Cochliobolus spicifer	N.W.F.P	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-
Fusarium equiseti	N.W.F.P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.2	-	-	-	-	-	-	-	-	-
F.oxysporum	Federal	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	80	-
	N.W.F.P.	-	-	-	-	-	-	-	-	-	-	10	-	-	-	-	-	-	-	-	-	-	-	-	-
Fusarium sp.	Federal	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-
	N.W.F.P	-	-	-	-	-	-	-	-	30	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-
	Sindh	2	-	-	-	-	-	-	-	-	-	-	-	-	16	-	-	-	1	-	-	-	100	-	-
Penicillium spp.	Baloch-istan	-	6	4	10	6	32	-	10	-	-	-	-	2	-	2	8	2	6	10	10	-	-	20	-
	Federal	12	24	-	8	16	52	-	-	-	-	16	-	-	-	-	-	4	20	20	-	-	20	-	-
	N.W.F.P	-	-	2	22	4	22	-	10	20	40	-	-	7	26	-	2	4	5	24	20	-	-	-	-
	Sindh	-	-	-	11	-	-	30	50	30	30	100	20	-	-	-	1	-	-	40	100	60	50	60	10
P. camem-berti	Baloch-istan	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-
	N.W.F.P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8	-	-	-	-	-	-	-
	Sindh	-	-	-	-	-	-	-	-	-	-	20	-	-	-	-	-	-	-	-	-	-	-	-	-
P. decum-bens	Federal	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	30	-	-	-	-

Table 1 (Cont'd)

Fungi	Province	Shell						Seed Coat						Cotyledons						Axis					
		Agar		Blotter		Deep F.		Agar		Blotter		Deep F.		Agar		Blotter		Deep F.		Agar		Blotter		Deep F.	
		S	N	S	N	S	N	S	N	S	N	S	N	S	N	S	N	S	N	S	N	S	N	S	N
Rhizopus sp.	Baloch-istan	18	12	-	-	-	-	100	50	30	30	-	-	10	24	18	56	-	-	-	100	-	-	-	-
	Federal	-	76	-	-	-	-	100	100	40	100	-	-	20	82	32	100	16	52	100	-	-	100	-	-
	N.W.F.P	30	14	-	-	2	-	100	100	-	-	-	-	84	100	74	64	42	42	100	100	-	-	-	-
	Sindh	20	70	-	-	-	2	50	-	100	-	100	-	30	50	2	27	-	3	50	100	100	-	-	-
Trichoderma harzianum	N.W.F.P	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-

F = Freezing, S = Sterilized, N = Non-sterilized

agar (PDA). In blotter method the seed parts were placed on three layers of sterilized moistened blotter and the dishes were incubated at 25°C for 5 days. In deep freezing method, the seed parts placed on blotter, were incubated for one day each at 25°C and -4°C followed by 5 days incubation at 25°C. Fungi growing from different seed part were identified after reference to Barnett (1960), Booth (1971), Ellis (1971), Nelson *et al.*, (1983), Raper & Thom (1949) and Thom & Raper (1945).

Results and Discussion

Using agar plate, blotter and deep freezing methods as recommended by ISTA (Anon, 1976), 9 genera and 22 species of fungi viz., *Alternaria alternata* (Fr.) Keissler, *A. tenuissima* (Kunze ex Pers) Wiltshire, *Aspergillus candidus* Link, *A. clavatus* Desm, *A. flavus* Link, *A. fumigatus* Fresenius, *A. niger* van Tieghem, *A. sulphureus* (Fres) Thom and Church, *A. terreus* Thom, *A. wentii* Wehmer, *Aspergillus* spp., *Chaetomium* sp., *Cladosporium* sp., *D. state* of *Cochliobolus spicifer* Nelson, *Fusarium equiseti* (Corda) Sacc, *F. oxysporum* Schlecht, *Fusarium* spp., *Penicillium camemberti* Thom, *P. decumbens* Raper, *Penicillium* spp., *Rhizopus* sp., and *Trichoderma harzianum* Rifai were isolated from different parts of *Pistacia vera* seed (Table 1).

Except *A. flavus and Alternaria alternata*, all the other species of fungi do not appear to have been reported from *Pistacia* vera seeds. *Aspergillus flavus* and *A. niger* were found to be predominant. Most of the fungi were located on the cotyledons (18) followed by shell (12) seed coat (11) and axis (10). At least 15 species of fungi were isolated where blotter method was used followed by agar plate (14) and deep freezing

Table 2. Occurence of *Aspergillus flavus* in Pistachio collected from different parts of Pakistan.

Province	City	Agar plate		Blotter method		Deep freezing	
		S	N	S	N	S	N
				(Infection %)			
Balochistan	Muslimbagh	-	12	-	-	-	4
	Quetta	-	16	20	32	20	20
Federal	Islamabad	8	28	4	24	4	16
N.W.F.P.	Peshawar	-	-	-	20	-	5
	Risalpur	32	8	-	12	-	-
	Sawat	16	88	20	50	-	-
Sindh	Hyderabad	100	100	68	100	60	100
	Karachi	4	20	36	32	-	12

S = Sterilized

N = Non-sterilized

method (10). Surface sterilization of seed parts with 1% $Ca(OCl)_2$ showed an increase in isolation of *A. candidus* with a reduction in the incidence of other *Aspergillus* species. Limonard (1961) also reported that microbial contamination was eliminated by Chlorine disinfection. *Aspergillus niger* and *Penicillium* spp., were dominant in pistachio shell, *A. flavus*, *A. niger* and *A. wentii* in seed coat, *A. flavus* and *A. niger* in cotyledons while the axis was heavily infected by *Fusarium* and *Penicillium* spp., showing an infection range of upto 100%.

The samples collected from Swat and Hyderabad were heavily infected with *A. flavus* showing an infection range of upto 88% and 100%, respectively (Table 2). Of the fungi isolated, *A. flavus* which was found to be predominant is known to produce aflatoxin B1, B2, G1, G2 responsible for causing liver cancer (Purchase, 1974). Other mycotoxin producing fungi such as *A. candidus* produces citrinin, patulin and ochratoxin A which are carcinogenic and damage kidney. *Fusarium oxysporum* produces Zearalenone A and B, Zearelenol, Trichothecene which cause alimentary aleukia, haemorrhage and necrosis of bone marrow (Domsch, 1980). There is therefore, need for better storage of dry fruits to protect them from mold growth and production of toxic metabolites harmful for human health.

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