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LOCATION OF FUNGI IN DIFFERENT PARTS OF WALNUT (*JUGLANS REGIA*) SEED COLLECTED FROM DIFFERENT PARTS OF PAKISTAN

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Abstract

Using agar plate, blotter and deep freezing method, 11 genera and 23 species of fungi were isolated from walnut (*Juglans regia*) seeds. Agar plate method yielded greater number of fungi. Of the fungi isolated, *Aspergillus niger* and *Penicillium* spp., were found preponderant. Greater number of fungi were isolated from cotyledons followed by shell and axis. Component plating was helpful in detecting the depth of infection which may be used to determine the disease free seeds and their suitability for human consumption.

Introduction

Walnut (*Juglans regia*) is an esteemed dry fruit used as dessert during the winter. The edible kernel which constitutes about half the weight of the whole nut contains 5.6% moisture, 15.6% protein, 64.5% ether extract, 11.0% carbohydrate, 2.6% fibre and 1.8% mineral matter. Vitamins of the B group are reported to be present in the kernel. The kernels yield 60-70% of walnut oil used for edible purposes and by the artists in paints, printing ink, varnishes and for making soap (Krishnamurthi, 1969). Mold fungi have been found to grow on walnut seeds sold in the market. Experiments were therefore carried out to study the location of fungi in different part of walnut seed collected from different parts of Pakistan.

Materials and Methods

Ten samples of *Juglans regia* seed collected from different localities of Pakistan viz., Balochistan (2), Islamabad (1), N.W.F.P (2), Punjab (1) and Sindh (4) were used. The hard endocarp was broken to remove large cotyledons and axis (Lawrence, 1951). The endocarp cotyledons and axis of each seed were divided into 10 equal pieces and 150 pieces of each seed part from each sample were used to study the seed-borne mycoflora.

ISTA techniques (Anon., 1976) were used for isolation of fungi where a set of seed parts with or without surface disinfection with $Ca(OCl)_2$ for 2 minutes were used. For the standard blotter technique seed parts were placed on three layers of moistured blotter. For the agar plate method, the seed parts were placed on potato dextrose agar (PDA) and the Petridishes incubated at 25°C for 5 days. In the deep freezing method, the seed parts were incubated for one day each at 25°C and at -40°C followed by 5 days incubation at 25°C. Fungi growing from seed parts were identified after reference to Barnett (1960), Booth (1971), Ellis (1971), Nelson *et al.*, (1983), Raper & Thom (1949) and Thom & Raper (1945).

Table 1. Locaton of fungi in different parts of seeds of *Juglans regia* collected from different parts of Pakistan.

	Fungi	Province	Shell						Cotyledon						Axis					
			Agar		Blotter		D.f.		Agar		Blotter		D.f.		Agar		Blotter		D.f	
			S	N	S	N	S	N	S	N	S	N	S	N	S	N	S	N	S	N
1.	*Alternaria*	Federal	4	8	—	—	—	—	—	20	—	—	—	—	—	—	—	—	-	-
	alternata	Sindh	14	—	—	—	—	—	—	—	—	—	12	40	—	—	—	-	-	16
2.	*A. tenuissima*	N.W.F.P.	—	—	—	—	—	12	—	—	—	—	12	40	—	—	—	—	—	16
		Sindh	—	—	—	—	—	—	—	—	—	—	—	24	—	—	—	—	—	-
3.	*Amblyospo-*	Federal	8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	rium sp.																			
4.	*Aspergillus*	Balochistan	—	—	—	—	—	—	4	—	—	—	—	—	—	—	—	—	—	—
	candidus	N.W.F.P.	—	—	—	—	4	4	4	—	—	—	—	—	—	—	—	—	—	16
		Sindh	—	—	—	—	—	—	4	—	—	—	—	—	—	—	—	—	—	—
5.	*A. clavatus*	Balochistan	40	—	—	—	—	—	16	10	—	—	—	—	50	60	—	—	—	—
		N.W.F.P.	16	—	—	—	—	4	—	—	—	—	—	16	—	—	—	—	—	—
		Punjab	4	—	20	60	—	—	—	—	28	44	—	8	30	—	20	—	20	—
6.	*A. flavus*	Balochistan	—	—	—	—	—	—	8	20	16	20	—	—	—	—	—	—	—	—
		Federal	—	—	—	—	—	—	—	—	—	12	—	—	6	—	—	—	—	—
		N.W.F.P.	—	—	—	—	—	—	6	4	—	—	—	4	—	—	—	—	—	—
		Punjab	8	4	4	8	—	—	4	8	20	12	—	—	—	—	—	—	—	—
		Sindh	4	—	—	76	—	—	16	14	36	44	4	—	—	—	44	—	—	—
7.	*A. fumigatus*	Balochistan	—	8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
		Punjab	—	—	—	—	—	—	4	—	—	—	—	—	—	—	—	—	—	—
8.	*A. niger*	Balochistan	—	—	—	—	4	—	12	72	80	100	—	—	30	50	—	—	—	—
		Federal	8	92	12	20	—	12	20	32	76	76	8	4	100	100	—	—	—	—
		N.W.F.P.	8	24	—	4	10	6	22	58	18	24	14	6	—	30	—	—	64	32

Table 1 (Cont'd.)

	Fungi	Province	Shell						Cotyledon						Axis					
			Agar		Blotter		D.f.		Agar		Blotter		D.f.		Agar		Blotter		D.f	
			S	N	S	N	S	N	S	N	S	N	S	N	S	N	S	N	S	N
		Punjab	8	32	44	60	—	—	—	40	16	60	8	—	10	—	24	—	24	30
		Sindh	38	42	—	28	—	4	66	74	8	20	12	—	20	30	5	—	—	52
9.	*A. sulphureus*	Balochistan	—	—	—	—	—	—	4	—	—	4	—	—	—	—	—	—	—	—
		N.W.F.P.	—	—	—	—	—	—	—	—	—	4	—	—	—	—	—	—	—	—
		Punjab	—	—	—	—	—	—	—	—	—	24	—	—	—	—	—	—	—	—
10.	*A. terreus*	N.W.F.P.	—	—	—	—	—	—	—	—	—	—	20	8	—	—	—	—	—	—
		Punjab	—	—	—	—	—	—	4	—	—	—	8	12	—	—	—	—	—	—
		Sindh	—	—	—	—	—	—	—	—	—	—	4	—	—	—	—	—	—	—
11.	*A. wentii*	N.W.F.P.	—	—	—	—	—	—	—	4	—	—	16	16	—	—	—	—	—	—
		Punjab	—	—	—	—	—	—	—	—	—	—	8	—	—	—	—	—	—	—
12.	*Botryodiploi-dia theobromae*	Sindh	20	—	—	—	—	—	—	—	—	—	—	—	20	20	—	—	—	—
13.	*Cephalos-porium* sp.	Federal	—	20	—	—	—	—	12	32	—	—	—	—	—	—	—	—	—	—
		Punjab	4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
14.	*Chaetomium* sp.	Sindh	4	12	—	—	—	—	8	12	—	—	—	—	—	—	—	—	—	—
15.	*Paecilomyces*	Balochistan	12	—	—	—	16	—	—	—	4	28	4	—	—	—	20	16	—	25
	lilacinus	N.W.F.P.	4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
16.	*Penicillium*	Balochistan	—	4	—	—	—	—	—	—	—	—	—	—	10	15	—	—	—	—
	spp.	Federal	—	20	16	60	64	60	—	—	64	76	20	76	6	10	12	50	—	60
		N.W.F.P.	14	6	64	96	76	80	46	64	58	50	50	52	—	10	40	—	—	48
		Punjab	4	16	—	—	—	—	—	12	—	—	—	—	—	—	4	—	—	—
		Sindh	—	—	92	—	—	—	56	92	12	44	50	48	10	10	5	100	—	36
17.	*P. camem-*	Federal	—	—	—	—	—	—	—	—	—	—	—	24	—	—	—	—	—	—

Table 1 (Cont'd.)

	Fungi	Province	Shell						Cotyledon						Axis					
			Agar		Blotter		D.f.		Agar		Blotter		D.f.		Agar		Blotter		D.f	
			S	N	S	N	S	N	S	N	S	N	S	N	S	N	S	N	S	N
	berti	N.W.F.P.	—	—	—	—	—	—	—	—	—	—	25	40	—	—	—	—	—	—
		Sindh	—	—	—	—	—	—	—	—	—	—	25	23	—	—	—	—	—	—
18.	*P. decumbens*	Sindh	16	8	—	—	—	—	—	24	—	—	—	—	—	—	—	—	—	—
19.	*P. frequentans*	Sindh	8	4	—	—	—	—	40	48	—	—	—	—	—	—	—	—	—	—
20.	*Rhizopus* sp.	Balochistan	—	8	12	—	—	—	—	40	100	100	—	30	100	100	—	—	—	—
		Federal	8	100	—	—	—	20	8	28	100	100	20	40	75	20	—	—	—	—
		N.W.F.P.	28	84	—	—	—	—	70	68	38	76	12	—	100	—	—	20	—	—
		Punjab	20	52	—	—	—	—	4	44	—	—	—	—	—	—	—	—	—	—
		Sindh	—	—	12	—	—	—	48	32	28	—	12	—	—	—	—	—	—	—
21.	*Trichoderma*	Balochistan	—	—	—	—	—	—	4	—	—	—	—	—	—	—	—	—	—	—
	harzianum	Federal	—	—	—	—	—	—	20	—	—	—	—	—	—	—	—	—	—	—
		Punjab	—	4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
22.	*T. viride*	Sindh	6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
23.	*Verticillium*	Federal	52	8	—	—	—	—	12	40	—	—	—	—	—	—	—	—	—	—
	sp.	Punjab	—	—	—	—	—	—	—	—	—	—	—	—	10	—	—	—	4	—

D.f. = Deep freezing, S = Sterilized, N = Non-sterilized

Table 2. Infection of *Penicillium* spp. in walnut (*Juglans regia*) collected from different parts of Pakistan.

Province	City	Agar plate method		Blotter		Deep freezing	
		S	N	S	N	S	N
				(Infection %)			
Balochistan	Muslimbagh	—	—	—	—	—	—
	Quetta	—	—	—	—	—	—
Federal	Islamabad	—	20	64	76	20	76
N.W.F.P.	Peshawar	80	100	28	80	32	84
	Risalpur	40	28	16	—	4	4
	Swat	52	100	100	100	95	100
Punjab	Lahore	—	12	4	—	—	—
Sindh	Hyderabad	88	92	88	100	100	96
	Karachi	64	78	12	50	100	66

S = Sterilized, N = Non-sterilized

Results and Discussion

Using agar plate, blotter and deep freezing method, 11 genera and 23 species of fungi isolated from *Juglans regia* seed were *Alternaria alternata* (Fr.) Keissler, *A. tenuissima* (Kunze ex Pers.) Witshire, *Amblyosporium* sp., *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, *Botryodiplodia theobromae* Pat., *Cephalosporium* sp., *Chaetomium* sp., *Paecilomyces lilacinus* Bainier, *Penicillium camemberti* Thom, *P. decumbens* Thom, *P. frequentans* Thom, *Penicillium* sp., *Rhizopus* sp., *Trichoderma harzianum* Rifai, *T. viride* Pers. ex Grey and *Verticillium* sp. (Table 1). Of these only 2 fungi. viz., *Alternaria* sp., from India (Verma *et al.*, 1975) and *Trichothecium roseum* Link from Kashmir (Mukerji, 1986) have been reported on *Juglans regia*. The remaining 21 species of fungi appear to be new report on *Juglans regia*.

Of the fungi isolated, *Aspergillus niger* and *Penicillium* spp., were found to be predominant showing an infection range from 4-100% (Table 2). Greater number of fungi were isolated from cotyledons (20 species) followed by shell (17 species) and axis (11 species). Of the three methods used, agar plate method yielded highest number of fungi (17 species) followed by deep freezing method (10 species) and blotter method (7 species). Where non-sterilized seed parts were used, greater number of fungi were isolated from seed samples collected from Sindh yielding 14 species of fungi with 9 species of fungi isolated from samples collected from Balochistan. *Trichoderma viride* (6%) and *Amblyosporium* sp., (8%) were isolated from shell only. *Verticillium* sp., was isolated only from cotyledons of Islamabad sample (52%) and axis of Punjab sample (10%). *Alternaria alternata* was found to be present in shell and cotyledons (4.20%) but not in the axis while *A. tenuissima* was isolated from all parts of the seed (12.40%).

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. If the infection is superficial, removal of outermost covering or use of proper cleaning methods could be recommended. Use of dry fruit with deep seated infection by fungi especially those producing mycotoxins can be avoided. It will also help in selecting healthy seed lots for raising new plants.

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