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ISOLATION OF DEEP-SEATED FUNGI FROM *LENS CULINARIS.* L (LENTIL) SEEDS COLLECTED FROM PAKISTAN

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ABSTRACT

Forty six fungal species belonging to 21 genera were isolated from seed samples of lentil collected from different localities of Pakistan viz; Karachi (2), Swabi (1) and Ghazi (1) by component plating technique on potato dextrose agar. Highest number of storage and pathogenic fungi were isolated from cotyledons followed by seed coat and embryo. The most dominant fungi were the species of *Aspergillus* on all the three parts. Fourteen species belonging to 7 genera was isolated from seedling symptoms test conducted on samples collected from Karachi (1), Faisalabad (1), Ghazi (1) and Sukkur (1). Highest post emergence rot on seedlings was caused by the species of *Aspergillus*. Surface sterilization with 1% Na $(OCl)_2$ reduced the incidence of pathogenic fungi. *Macrophomina phaseolina*, *Fusarium* and *Trichoderma* species were isolated from the embryo which showed that they were deep seated and caused the death of seedlings by appearing in later stages of growth.

Key words: lentil seeds, deep-seated mycoflora, seed components, seedlings symptoms.

INTRODUCTION

Lens culinaris. L (lentils) seed is a pulse crop. In Pakistan during 2007- 2008 it was cultivated in an area of 30.4 thousand hectares and the production of 14.6 thousands tonnes with an average yield of 480 Kg/hectare (Anon, 2007). Lentil, a Rabi crop (Hussain *et al.,* 2007) it is rich in minerals like calcium, phosphorous, sodium, potassium as well as in vitamins (Sastri, 1962). It is good nitrogen fixers from air specially when cultivated in rotation with cereals (Robyne, 2011). *Ascochyta* blight due to *Ascochyta fabae* f. sp. *Lentis* , seedling blight due to *Rhizoctonia solani* , stem and root rot due to *Fusarium* species, stem rot due to *Sclerotinia sclerotiorum,* grey mold and pod rot (*Botrytis cinerea), Anthracnose* (*Collectotrichum truncatum)* (Robyne, 2011) were reported from fields in Alberta before harvest. 42 fungal species belonging to 18 genera were reported as storage fungi from lentil seed samples collected from Pakistan (Rahim *et al*., 2010). Storage fungi are present as dormant mycelium or as contaminant within the tissues of pericarp or seed coat and attack mostly after harvest (Neergard, 1977). Most of the fungi are present as dormant mycelium in the tissues of seed coat followed by cotyledons and embryo. Tariq *et al*., (2006) reported 9 fungal species isolated from various components of soybean seeds. Similar results were also reported by Dawar and Ghaffar (1990) on sunflower seeds and Rasheed *et al*., (2004) on groundnut. Tariq *et al.,* (2006) also reported pre-emergence and post emergence rot on soybean seeds due to pathogenic fungi like *Fusarium* and *Macrophomina phaseolina.* A number of pathogenic as well as storage fungi were found to be associated with lentils, experiments were therefore carried out to isolate the deep-seated fungi from different parts of seed using component plating technique and to check the fungal attack on seedlings by using seedling symptoms test.

MATERIALS AND METHODS

Component plating technique: For the isolation of deep-seated fungi component plating technique as given by Mathur *et al*., (1975) with slight modifications (Dawar and Ghaffar, 1992) was used. Seed soaked for three hours in sterilized distilled water in test tubes were dissected aseptically into seed coat, cotyledons and axis. Agar plate method as given by ISTA (Anon, 1993) was used to detect the fungal infection on seed parts where 50 untreated seeds and 50 seeds treated with 1% Na $(OCl)_2$ were used. The untreated and treated seeds components were plated on PDA. The dishes were incubated at room temperature (25-30°C) for seven days. Fungi growing on different parts of seeds were identified.

Seedling symptoms test: For the pre and post emergence seedling rot using seedling symptoms test, one seed was placed in a test tube containing 10ml of 1% plain water agar. The tube was closed by loose cotton plug and incubated for 14days at 20°C under 12hr alternating cycles of ADL and darkness. The cotton plug was removed when the seedlings reached the mouth of the tube. After 12 to 14 days of incubation, seedlings showing fungal infection were studied (Khare *et al.,* 1977).

Fungi growing on seeds were identified after reference to Barnett (1960), Booth (1971), Domsch *et al.,* (1980), Ellis (1971), Nelson *et al*., (1983), Raper *et al.* (1965).

Table 1. Isolation of deep-seated fungi on seed components of lentil.

FUNGI	SEED COAT		COTYLEDONS		EMBRYO	
	St.	N.St	St.	N.St	St.	N.St
	I%±SD	I%±SD	I%±SD	I%±SD	I%±SD	I%±SD
Alternaria alternata	0.5±0.0	0.25±0.0	-	0.25±0.0	-	-
A.dianthicola	-	0.5±0.0	-	-	0.5±0.0	-
A.longipes	0.5±0.0	-	0.5±0.0	-	-	-
A.passiflorae	0.75±0.0	-	0.5±0.0	-	-	-
A.sonchi	0.25±0.0	-	-	0.5±0.0	-	-
A.tenuissima	-	-	-	0.25±0.0	-	-
Arthrobotrys oligospora	-	-	-	0.25±0.0	-	-
Aspergillus candidus	-	-	-	1.0±0.0	-	-
A.flavus	5.5±6.14	23±24.66	4±6.66	29.25±29.60	8.5±2.08	18.5±4.04
A.fumigatus	5.5±11.3	14.75±8.62	6.5±12.73	18.5±14.29	6±0.0	23.5±7.57
A.niger	9.25±9.25	12±7.16	10.5±11.70	11.25±8.22	8.5±0.57	14.5±7.41
A.ochraceus	-		-	0.5±0.0	-	-
A.sulphures	-	0.25±0.0	-	-	-	-
A.terreus	-	1±0.0	0.25±0.0	1.5±2.82	1±0.0	0.5±0.0
A.versicolor	-	-	-	0.75±0.0	-	1±0.0
A.wentii	0.25±0.0	3.5±6.35	-	2.5±2.51	-	0.5±0.0
Brachysporium obovatum	-	-	-	0.25±0.0	-	-
B.bloxami	-	-	-	0.25±0.0	-	-
Chaetomium crispatum	-	-	-	0.25±0.0	-	-
C.elatum	0.25±0.0	-	-	-	-	-
Cladosporium cladosporoides	2.75±3.75	1±0.0	2±0.0	0.5±0.0	2±0.0	-
C.macrocarpum	0.25±0.0	2±0.0	-	1.25±0.58	-	0.5±0.0
C.sphaerospermum	-	2±1.53	-	-	-	-
Cochliobolus sativus	0.25±0.0	-	-	-	-	-
C.spicifer	-	-	0.25±0.0	-	-	-
Drechslera australiensis	0.25±0.0	-	0.25±0.0	-	-	-
D.biseptata	-	-	-	0.75±0.71	-	0.5±0.0
D.hawaiiensis	-	-	0.25±0.0	0.25±0.0	-	-
D.papendorfii	-	-	0.25±0.0	0.25±0.0	-	-
Macrophomina phaseolina	-	-	-	1.25±0.0	-	-
Monilia sp.	-	3.25±0.71	-	2.25±4.95		2±1.41
Monodictys glauca	-	-	-	-	0.5±0.0	-
Mucor sp	-	-	-	1±0.0	-	-
Myrothecium roridum	-	-	-	-	0.5±0.0	-
Nigrospora khuskia	2±0.0	0.5±0.0	1.75±0.0	-	1.5±0.0	-
N.sphaerica	-	0.25±0.0	-	-	-	-
Penicillium citrinum	1±0.0	0.25±0.0	-	-	-	-
Penicillium spp.	1.25±2.12	1±0.0	0.5±0.0	1.5±0.0	2±0.0	1.41±0.0
Rhizopus arrhizus	0.25±0.0	-	1±0.0	-	-	-
R.oryzae	4±0.0	1.75±0.71	3±3.05	1±0.0	2.5±0.0	-
R.stolonifer	14.75±0.0	3.5±0.0	15.25±32.62	1.25±0.0	0.5±0.0	2±0.0
Scopulariopsis brumpii	-	0.25±0.0	-	-	-	-
Scytilidium sp	-	0.25±0.0	-	-	-	-
Stemphylium sp	-	0.25±0.0	-	-	-	-
Stachybotrys atra	-	-	-	0.25±0.0	-	-
Trichoderma polysporum	-	-	-	-	0.5±0.0	-

St =sterilized seeds; N.St = non-sterilized seeds; I% = Infection percentage; S.D= Standard deviation.

RESULTS AND DISCUSSION

Location and identification of fungi: Total number of 47 fungal species belonging to 21 genera was isolated from different components of seeds. Most of the fungi were located on pericarp (cotyledons) followed by seed coat (outer covering) and axis (embryo). Species of *Aspergillus* were the most dominant on all three parts; however surface

sterilization has greatly reduced the incidence of storage fungi. *A. flavus* was most dominant on cotyledons, seed coat while *A. fumigatus* was dominant on embryo. The incidence of saprophytic fungi like *Alternaria, Cladosporium, Chaetomium, Drechslera, Monilia, Mucor, Nigrospora, Penicillium*, and *Rhizopus* species were higher on cotyledons, seed coat and axis of untreated seeds. The incidence of pathogenic fungi was mostly observed on the seed coat and cotyledons of seeds which includes *Arthrobotrys oligospora*, *Macrophomina phaseolina, Trichoderma, Cochliobolus* sp., *Stachybotrys* sp., *Stemphylium* sp., *Monodictys glauca, Scopulariopsis* sp., *Trichocladium* sp. The highest infection percentage was observed on cotyledons (29.25%) and embryo (23%) due to *A. fumigatus* followed by *A. flavus* which infected the seed coat (23%) (Table 1).

Seedling symptom test: An experiment was carried out to determine the pre and post-emergence infection of lentil seeds. One seed per test tube were allowed to grow on 1% plain water agar for 12-14 days after which seedlings showing fungal infection were counted. *Aspergillus fumigatus* (39%) followed by *A. flavus* (33%) and *A. niger* (29%) were the most dominant fungi causing pre-emergence and post-emergence death of seedlings (Table 2). Seeds having the infection caused by *A. fumigatus* either did not germinate at all or were rotten. Among the pathogenic fungi were present the species of *Fusarium, M. phaseolina*, *Myrothecium,* and *Diplococcium. M. phaseolina* has produced charcoal-rot effect on stem and seed while roots have shown discoloration. *F. oxysporum* has caused stem rot and wilting of seedlings. The incidence of *Myrothecium cinctum* has prevented the germination of seeds, *Diplococcium* and *Alternaria* spp. too has caused rotting of seedlings. Some healthy seedlings were observed to have shown wilting effect near stem tip, also a slight discoloration of water agar was observed on the incidence of pathogenic fungi. Seed sample from Karachi was infected only by storage fungi, while seed sample from Sukkur showed the highest incidence of pathogenic fungi followed by Faisalabad and Ghazi seed samples. The pathogenic fungi caused infection after several days of incubation which showed that the pathogenic fungi were deep-seated. Over all 38% death of seedlings were caused by the species of *Aspergillus* while *F. oxysporum* caused 3%, *M. phaseolina* caused 1%death of seedlings and 21% seedlings remain healthy.

Table 2. Seedling symptom test of lentil.

NAME OF FUNGI	PRE-EMERGENCE SEED ROT %		POST-EMERGENCE SEED ROT %			
			Dead seedlings %		Healthy seedlings %	
	St.	N.St	St.	N.St	St.	N.St
Alternaria alternata	-	-	1	-	-	-
A .longipes	-	1	-	-	-	-
A .tenuissima	1	-	1	-	-	-
Aspergillus candidus	-	-	1	-	-	-
A .flavus	8	1	10	33	17	-
A. fumigatus	7	8	37	39	-	11
A .niger	4	6	3	29	3	1
A .terreus	-	-	-	1	4	-
A .wentii	-	-	4	-	-	-
Diplococcium spp.	-	-	1	-	-	-
Drechslera australiensis	-	-	1	-	-	-
Fusarium oxysporum	-	-	3	-	-	-
Macrophomina phaseolina	-	-	-	1	-	-
Myrothecium cinctum	-	-	-	-	-	1

St = sterilized seeds' N.St = non-sterilized seeds

Species of *Aspergillus* were the most dominant fungi on all parts of seeds and as well as in seedlings symptoms test causing pre and post emergence death of seedlings. *Aspergillus* species are known to produce mycotoxins known as aflatoxins. Lentils contain Aflatoxins B1, B2, G2 and G2 @ 14.3 µ/KG (El-nagerabi and El-shafie, 2000). Other fungi are also important because they also produce mycotoxins which are harmful for both animals and plants (Hiscocks, 1965). Infection by storage fungi is the primary cause of loss of germination (Mills and Frydman, 1980; and Barton, 1961; Harrington, 1963). Storage fungi preferentially attack the embryo of seed causing discoloration and finally out right decay (Golumbic and Laudani, 1966). *Aspergillus* and mould metabolites are carcinogenic.

Species of *Aspergillus* are known to cause aspergillosis in man, animal and birds. Ear infection in man is caused by *A. niger* while ear pulmonary infection is caused in pigeons by *A. fumigatus* (Raper *et al.*, 1965).

A number of pathogenic as well as storage fungi were isolated from seed samples of lentils which caused seedlings rot. Seeds should be stored at cool places to avoid the growth of fungi. There is need to control both storage and pathogenic fungi for obtaining good quality seeds.

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