

## Mycoflora Detected from Seeds of *Sesamum indicum* L. in Sialkot, Pakistan

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**Abstract:** Sesame seeds (*Sesamum indicum* L.) from various localities of Sialkot were analysed for their mycoflora by using Agar plate, Blotter paper and Deep freezing method. Agar plate and Deep freezing method yielded 22 and 21 fungal species respectively followed by Blotter paper method yielded 19 species. A total number of 36 species belonging to 10 genera of fungi were isolated. The prevalent genera were *Penicillium* (10 species), *Alternaria* (7 species), *Fusarium* (5 species), *Cercospora* and *Cladosporium* (4 species each). *Penicillium* was predominant followed by *Alternaria* and *Fusarium*. All detected fungi are the first record of mycoflora from sesame seeds in Sialkot, Pakistan.

**Key words:** *Sesamum indicum*, seed-borne, Agar plate, Blotter paper, Deep freezing method

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### I. Introduction

Sesame (*Sesamum indicum* L.) or “til” is one of the world’s oldest spice and oilseed crop. It was originated in Africa and is considered to be one of the primeval oil seed plant brought into cultivation in various parts of the world. Presently, China, India, and Myanmar are the leading producers of sesame, followed by Sudan, Nigeria, Pakistan, Bangladesh, Ethiopia, Thailand, Turkey, and Mexico (15). The world production is anticipated at 3.66 million tones with Asia and Africa producing 2.55 and 0.95 million tons, respectively (5). In Pakistan, sesame seeds were refined on over 100,000 hectares of territory during 2001-2002. Entire production that year was 50.7 thousand tones (18). Over 90 per cent of Pakistan's production is exported. Substantial progress in sesame production for the period 2001-2002 is worth mentioning because of the phenomenal growth of 37.2 per cent and Pakistan earned about US\$ 20 million from exports. Of the total sesame production of Pakistan, Punjab contributes 88.5 per cent followed by Balochistan 6.9 per cent, Sindh 4.3 per cent and Khyber Pakhtun Khwa only 0.3 per cent. Gujrat, Sialkot, Bahawalpur, Bhakkar, Chichawatni and Layyah are the main sesame seed growing areas in Punjab (6).

Sesame seeds are prosperous source of protein (20%) and edible oil (50%), and contain about 47% oleic acid and 39% linolenic acid (35). Seeds having shells are loaded with calcium (1.3%) and adequate mineral supply. Sesame seeds are added to lysine rich soybean meal to make appropriate animal feed (19). They are used as ingredients in manufacturing of bread, candies, chips and health foods whereas Sesame oil is used as cuisine oil, it has brilliant permanence due to the presence of innate antioxidants sesamol, sesamoline and sesamin hence known as the “king of oils” (27). It is also used in cooking, salad preparation, margarine, and raw materials for the manufacturing of paints, varnishes, soaps, perfumes, pharmaceuticals, and insecticides.

Although sesame is extensively used for numerous purposes, the crop has very low yielding capacity as compared to other plants due to various factors specially disease susceptibility (7). Numerous deteriorative microbes particularly fungi have created so many problems in production and storage conditions. Many authors reported the incidence of *A. flavus* in sesame seeds along with other fungi (21, 22). These fungi secrete toxic secondary metabolites, out of them Aflatoxin is the most important (2). Other fungi viz., *Aspergillus niger*, *A. viridis*, *A. alba*, *Fusarium* sp., *Alternaria* *redicina*, *A. brassicola*, *Drechslera* sp., *Curvularia* sp., *Cephalosporium* sp., *Penicillium* sp. have also been isolated from sesame (4) which have hazardous effects on this crop. Due to which, sesame agriculture is facing numerous problems like root rot, wilting disease, and damping off for seedlings, which is caused by transfer of fungi from soil to plant and thus effect the crop (16).

Revealing seed borne pathogen is a main factor in production and quality to ensure health and vitality. Thus, proper identification of these pathogens is a primary step in planning management. The present work was planned to study the mycoflora of Sesame seeds of Sialkot which is the major growing area of sesame in Punjab. No previous report on seed borne mycoflora of sesame from this region is found.

## II. Materials And Methods

### Collection of seed samples

Fifteen seed samples of Sesame were collected from various localities of the Sialkot, Punjab and were preserved in plastic bags and stored in refrigerator until used. For sterilization, seeds were treated with 2% sodium hypochlorite (NaOCl) for 2 minutes and were rinsed three times with distilled water to remove sanitizing particles. Four hundred seeds were used for each experiment.

### Detection of Seed Mycoflora

Standard blotter, agar plate and deep freezing methods were used for the detection of seed borne fungi as recommended by International Seed Testing Association (17). For agar plate method, the untreated seeds and seeds treated with 2% NaOCl were placed on potato dextrose agar (PDA) at the rate of 25 seeds per plate. For blotter and deep freezing methods, the treated and untreated seeds were placed on three layers of moistened blotters placed in Petri plates at the rate of 25 seeds per plate. Then the plates were incubated at  $25 \pm 1$  °C for seven days except in deep freezing method in which the plates were incubated for 24 hours at  $25 \pm 1$  °C and then transferred to  $-20$ °C in a freezer for 24 hours followed by incubation at  $25 \pm 1$  °C for 7 days. After incubation period, the number of infested seeds and fungal colonies developed were recorded in term of percentage frequency. Based on macroscopic and microscopic observations, the fungal flora was identified after the reference of Thom and Raper (38), Ellis (13, 14), Domsch *et al.*, (12).

## III. Results

The occurrence of fungi most frequently encountered is recorded in terms of mean value with standard error (Table 1).

### a) Agar Plate Method

A total of 22 species belonging to nine genera viz. *Alternaria alternata*, *Al. chlamydospora*, *A. flavus*, *A. niger*, *Cercospora* sp., *C. chenopodii*, *C. koepkei*, *Cladosporium variable*, *C. herbarium*, *C. tenuissimum*, *Curvularia richardiae*, *Dreschlera hawaiienses*, *Fusarium oxysporum*, *F. redolens*, *F. reticulatum*, *F. tabacinum*, *Penicillium egyptiacum*, *P. herqui*, *P. janthinellum*, *P. lanso-coerellum*, *P. paxilli*, *R. oryzae* were detected from both surface sterilized and unsterilized seeds with almost same number of colonies and species diversity. Of the fungi isolated *P. lanso-coerellum*, *R. oryzae*, *C. koepkei* and *F. oxysporum* were few predominant species.

### b) Blotter Paper Method

A total of 19 species belonging to eight genera viz. *Alternaria alternata*, *Al. cinerariae*, *Al. citri*, *A. flavus*, *A. niger*, *Cercospora* sp., *C. bolleana*, *C. chenopodii*, *C. koepkei*, *C. herbarium*, *C. sphaerosporum*, *Fusarium oxysporum*, *F. proliferatum*, *Penicillium egyptiacum*, *P. janthinellum*, *P. lilacinum*, *P. waksmani*, *R. oryzae*, *Verticillium albo-atrum* were detected from both surface sterilized and unsterilized seeds. The most prevalent fungi were *Cercospora* sp. and *P. egyptiacum*. Unsterilized seeds yielded more number of colonies and species.

### c) Deep Freezing Method

A total of 21 species belonging to six genera *Alternaria alternata*, *Al. chlamydospora*, *Al. cinerariae*, *Al. citri*, *Al. pluriseptata*, *Al. radeina*, *Al. triticina*, *A. flavus*, *A. niger*, *Cercospora* sp., *C. herbarium*, *C. sphaerosporum*, *Fusarium oxysporum*, *F. proliferatum*, *F. reticulatum*, *F. tabacinum*, *Penicillium egyptiacum*, *P. expansum*, *P. italicum*, *P. vermiculatum*, *P. waksmani*, were detected from both surface sterilized and unsterilized seeds. *Al. chlamydospora*, *Al. cinerariae*, *Al. radeina* and *Cercospora* sp. were few predominant species. Here also unsterilized seeds yielded more number of species as compared to sterilized seeds.

**Table 1: Percent frequency of fungi in Agar plate, Blotter paper and Deep freezing method**

S. No.	NAME OF FUNGI	AGAR PLATE METHOD		BLOTTER PAPER METHOD		DEEP FREEZING METHOD	
		S.D*	S.N.D**	S.D*	S.N.D**	S.D*	S.N.D**
		Mean± S.E	Mean± S.E	Mean± S.E	Mean± S.E	Mean± S.E	Mean± S.E
1	<i>Alternaria alternata</i> (Fr.) Keissl.	5.5±1.78	7.7±0.86	33.9±2.25	28.5±0	19.7±2.67	22.4±3.14
2	<i>Alternaria chlamydospora</i> Mouchacca	9.3±0.20	20.1±0			30±0	
3	<i>Alternaria cinerariae</i> Hon & Enjoji			6±0		34±0	23±0
4	<i>Alternaria citri</i> Ellis & Pierce			38.5±0		17.5±0	
5	<i>Alternaria pluriseptata</i> Karst. & Har.					20±0	16±0
6	<i>Alternaria radicina</i> Meier.					34±0	26.4±0
7	<i>Alternaria triticina</i> Prada & Prabhu						20.3±0
8	<i>Aspergillus flavus</i> Link ex Gray	14.3±0	26.5±0.80		34.1±0		3.4±0
9	<i>Aspergillus niger</i> Van Tieghem	10±0	14.8±4.37		20.1±0		40±0
10	<i>Cercospora</i> spp.	21.3±1.36	17±1.64	21.9±2.76	23.1±2.47	33±0.36	18.4±3.64
11	<i>Cercospora bolleana</i> Thüm.				3.9±0		
12	<i>Cercospora chenopodii</i> Fresen.	1.8±0	8.8±0		27.8±0		
13	<i>Cercospora koepkei</i> Krüger	27.5±0			33.4±0		
14	<i>Cladosporium variable</i> Cooke	22.2±0					
15	<i>Cladosporium herbarum</i> (Pers.) Link, Fr.		11±0		4.9±0		2.5±0

\*S.D= Surface disinfected  
\*\* S.N.D= Surface non disinfected

**Table 1 (Cont'd.)**

S. No.	NAME OF FUNGI	AGAR PLATE METHOD		BLOTTER PAPER METHOD		DEEP FREEZING METHOD	
		S.D*	S.N.D**	S.D*	S.N.D**	S.D*	S.N.D**
		Mean± S.E	Mean± S.E	Mean± S.E	Mean± S.E	Mean± S.E	Mean± S.E
16	<i>Cladosporium sphaerosporum</i> Penz.				9.8±0		11.5±0
17	<i>Cladosporium tenuissimum</i> Cooke	10±0	14.4±0				
18	<i>Curvularia richardiae</i> Alcom		6.9±0				
19	<i>Drechlera hawaiiensis</i> (Bugn.) Sub. Jain	11±0					
20	<i>Fusarium oxysporum</i> Schlecht.	22.5±2.93	15.9±3.2	24.3±1.19	24.5±1.0	13.8±1.36	32.1±0.43
21	<i>Fusarium proliferatum</i> (Mats.) Nirenberg			20.1±3.2		16±1.46	29.3±0.37
22	<i>Fusarium redolens</i> Wollenv.		15.7±3.3				
23	<i>Fusarium reticulatum</i> Mont.		17.6±0			10.5±0	
24	<i>Fusarium tabacinum</i> App. & Wr.		22.3±0			30±0	25±0
25	<i>Penicillium egyptiacum</i> (Beyma) Palm <i>et al.</i>	9.8±1.78	17.3±2.42	33±2.92	50±1.04	33±2.92	9.2±0.60
26	<i>Penicillium expansum</i> Link.					25±0	7.5±0
27	<i>Penicillium herqui</i> Baimier & Sartory	11.8±0					
28	<i>Penicillium italicum</i> Wehmer						5±0
29	<i>Penicillium janthinellum</i> Bioturge	17.5±0	13±0	24±0			
30	<i>Penicillium lanoso-coerellum</i> Thom	36±0	6.5±0				
31	<i>Penicillium lilacinum</i> Thom			8±0			
32	<i>Penicillium paxilli</i> Baimier	5.8±0	3.2±0				
33	<i>Penicillium vermiculatum</i> Dangeard						27.5±0
34	<i>Penicillium waksmani</i> Zaleski			4±0.36	2±0		9.3±0.60
35	<i>Rhizopus oryzae</i> Went & Geel.	32.5±0	30.8±1.1		27.8±0		
36	<i>Verticillium albo-atrum</i> Reinke & Berth.			22.4±0			

**IV. Discussion**

Overall in this study Agar plate and Deep freezing method yielded more number of fungi than blotter method but in deep freezing method infection rate was the lowest (Fig.1). Blotter paper method showed moderate rate of infection and found to be suitable for isolation of *Cercospora* sp. Panchal and Dhale (26) reported that isolation of seed borne fungi by Agar plate method was more favourable than blotter paper method for the maximum counts of saprophytic fungi and for detection of some specific fungi. Such similar results were also observed by Kassim (20) on sorghum, Bilgrami and Ghaffar (9) on pinus, Perveen and Ghaffar (28) on tomato and Rathod *et al.*, (31) on legume seeds.

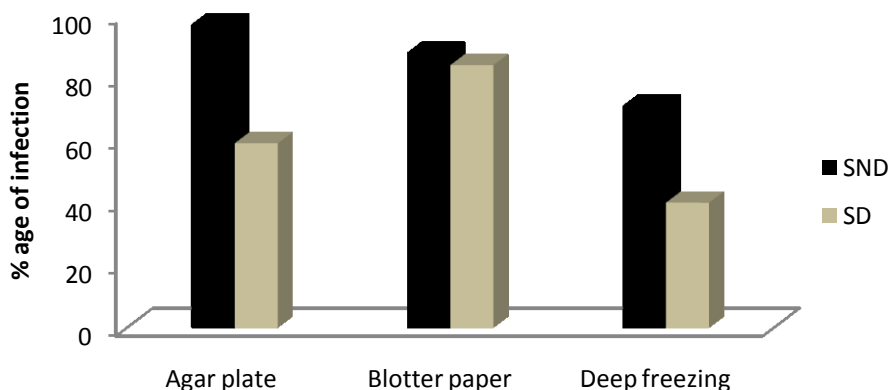


Fig. 1. Percentage of fungal infection in sterilized and unsterilized seeds by all three methods

Deep freezing method was found most suitable for the detection of *Alternaria* species. The average percent incidence and the range of occurrence of fungi in seed samples tested revealed that *A. alternata*, *A. flavus*, *A. niger*, *Cercospora* sp., *F. oxysporum*, *P. egyptiacum* and *R. oryzae* were most frequent and detected from all three methods. Few workers in Pakistan isolated fungi from sesame seeds by blotter paper method only (4, 32) and they recorded fungi like *A. flavus*, *A. niger* and *F. oxysporum* which is in confirmatory with present study. Surface disinfection of seeds with 2% NaOCl reduced the incidence of *Aspergillus* spp. in blotter and deep freezing methods hence give similarity with the results of Bilgrami and Ghaffar (9). Species of *Aspergillus*, *Penicillium* and *Rhizopus* are reported to reduce the germination of seeds and damage the seeds in storage (10). *A. flavus* and *A. niger* were the predominant storage fungi of groundnut seeds (25), bottle gourd seeds (36), sunflower seeds (34) and soybean seeds (37). *Rhizopus* has been reported on ground nut seeds (30) and conifers (24). These species have been reported to reduce the germination of seed and damage the seeds in storage (10). *F. oxysporum* has been isolated in high frequencies from seeds of water melon (23), sponge gourd (33), and sunflower (1) causing wilting and seedling rot. *A. alternata* detected from sunflower cause characteristic leaf spot (8) and reduction in germination in wheat seeds (29).

The result of present study shows that seed samples are highly infected with pathogens which cause various diseases in sesame. On the other hand, these fungi are known to produce mycotoxins which are harmful for human health. *A. flavus* produces aflatoxin B1, B2, G1, G2 which are carcinogenic and mutagenic toxins (3). *F. oxysporum* produces Zearalenone  $\alpha$  and  $\beta$  causing haemorrhage and necrosis of bone marrow (11). *A. niger* attacks human skin and is a parasite of human ear. Thus there is a need for the control of these pathogens by employing various management techniques to ensure improvement of seed health which ultimately increase crop quality and human health.

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