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Seed Mycoflora of Sesame (*Sesamum indicum* L.) and their Phytopathogenic Effect

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A B S T R A C T

Sesame (*Sesamum indicum* L.) which belongs to the pedaliaceae family, is one of the oldest oilseed crops cultivated in tropical and subtropical regions of Asia, Africa and South America. India ranks first in both acreage and production (about 8 lakh MT) of sesame in the world. The seed mycoflora which causes deteriorative effects like reduction in seed quality and their germination in sesame. Seed samples of sesame were collected from five tehasils of Jaipur district, revealed variation in germination, seedling symptoms and incidence of seed mycoflora which finally effect seed quality. Discolouration (dirty black), deformity (Shrivelled), along with damaged seeds and impurities were commonly found in all the seed samples. A total number of eight seed mycoflora viz., *Alternaria sesami*, *Aspergillus flavus*, *Aspergillus niger*, *Colletotrichum* spp., *Fusarium* spp., *Macrophomina phaseolina*, *Mucor* spp. and *Penicillium* spp. were obtained in both Blotter and Agar Plate Method. The disease incidence of *Alternaria sesami* were found predominant in among the inoculation method. Maximum disease incidence of mycoflora was observed in seed sample 'A' whereas minimum observed in seed sample 'C'. Among these, *Alternaria sesami* was found to be highly pathogenic as it showed maximum reduction in seed germination and vigour index with enhanced pre and post-emergence mortality. The pathogenicity of *Alternaria sesami* highest was observed in seed cum foliar inoculation technique.

Introduction

Sesame (*Sesamum indicum* L.*)* which belongs to the pedaliaceae family, is one of the oldest oilseed crops cultivated in tropical and subtropical regions of Asia, Africa and South America. Sesame seeds are very small in size, pearl shaped, ovate, small and thinner at the hilum. The seed contain testa (hull), endosperm, cotyledons and embryo. The seed is exalbuminous in nature. The seed colour ranges from white, light brown and dark brown to black. Sesame seed has long shelf life due to the presence of lignans, which play the role of antioxidant function. The seed, remarkably rich in quality proteins and essential amino acids. The seed is also a rich source of linoleic acid, vitamin E, A, B1 and B2 and minerals including calcium and phosphorus (Pathak *et al.*, 2014). India ranks first in both acreage and production (about 8 lakh MT) of sesame in the world. Considerable changes were observed in

the extent of crop coverage at state as well as national levels. In Rajasthan, among the 13 districts, the highest yield (335 kg/ha) was estimated for Kota and the lowest (165 kg/ha) for Tonk. The highest production was estimated for Pali, the district with the largest acreage in the state and was followed by Jodhpur, the district with the second largest acreage in the state.

Pali and Jodhpur jointly accounted for one-third (33.4%) of the state acreage. The total estimated production of sesame crop in Rajasthan was 73,548 MT with an average yield of 270 kg/ha (Anonymous, 2017).

The mycoflora associated with seeds has a pronounced effect on germination as many pathogenic fungi are reported to produce toxins as their metabolic by products. Production of such toxic metabolites by seed borne fungi gains much importance as they cause considerable influence on seed germination and seedling health.

The seed borne fungi also deteriorate both the physical and chemical constituents of the seed. The fungi occurring on sesame seeds also affect its seed colour and quality parameter. Due to seed borne fungi there is some reduction in carbohydrates and protein content. Healthy and pathogen free seed is therefore, basic requirement along with suitable fungicides for seed treatment (Saxena *et al.*, 1991).

In view of the seed borne pathogens, causing substantial damage and to study the different aspects of the fungal pathogens and their effects on seed the following objectives were formulated.

Collection of sesame seed samples from five tehasils of Jaipur district

isolation, purification, identification, and pathogenicity of major seed mycoflora associated with sesame from Jaipur district

Effect of isolated major fungi on seed germination and seedling vigour.

Seed borne mycoflora are carried over by infected seeds and they cause deterioration of seed and affecting seed germination, causing seedling mortality. Fungi including *Alternaria*, *Curvularia*, *Fusarium*, *Helminthosporium*, *Penicillium*, *Mommoniella* and *Rhizophus* spp. have been found associated with sesame. Among these, *Alternaria* is the most destructive pathogen of sesame it causes Leaf spot of sesame (*Alternaria sesami*).

Collection, isolation, purification, identification and pathogenicity of major seed mycoflora associated with sesame from Jaipur district

Seed is considered as a symbol and foundation of life as it is a container of embryo(s) of a new generation and carrier for the spread of new life. Therefore healthy seeds gives a good picture of their glorious golden era (Saskatchewan, 2013).

Rathod (2012) worked with seed borne *Alternaria* spp. and concluded that species of *Alternaria* cause range of diseases with great economic importance on large variety of commercially cultivated tropical crop plants which include cereals, legumes, oil seeds and large number of post-harvest crops. *Alternaria* species are dominant seed borne fungi and these species are responsible for changes in physical properties of seeds. Ghosh *et al.,* (2018) studied the seed borne mycoflora associated with oilseed crops (such as groundnut, sesame, mustard) and isolated *Alternaria* sp., *Curvularia* sp., *Fusarium* sp., *Helminthosporium* sp., *Penicilium* spp., *Mommoniella* spp., *Aspergillus* spp., *Mucor* spp. and *Rhizophus* spp. Among these, *Alternaria* spp. as well as *Aspergillus* spp. were the most destructive pathogen of oilseed crop.

Effect of isolated major fungi on seed germination and seedling vigour

Althaf *et al.,* (2004) reported seed borne mycoflora of sesame are carried over by infected seeds. They cause deterioration in seed in soil before germination, causing seedling mortality and cause infection of foliage at adult stage fungi including

Alternaria, Curvularia, Fusarium, Helminthosporium, Memnoniella, Penicillum and *Rhizophus spp.* have been found associated with sesame. *Alternaria sesami* is the most destructive pathogen of sesame. The seed-borne diseases are most disastrous as they reduce the seed vigour and weaken the plant at the initial of its growth. Seed-borne diseases caused by fungi are relatively difficult to control as the fungal hyphae gets established and become dormant.

Srikantappa *et al.*, (2009) investigated the detection of seed-borne fungi of sesame seeds. The following fungal genera were isolated as *Alternaria alternata, A. sesamicola, Fusarium moniliforme, F. oxysporum, A. tenuis, Verticillium dahliae, Sclerotinia sclerotiorum, S. rolfsi, Cercospora sesami, Curvularia lunata, Macrophomina phaseolina, Cladosporium cladosporioides, C. herbarum, C. fulvum, C. chlorocephalum, Acremonium* spp., *Helminthosporium* spp., *Gliocladium roseum, Neurospora glabra, Cunigamella elegans, Chaetomium globosum, Stachybotrys chartarum, S. atra, Pestalotia macrotricha, Aspergillus niger, A. flavus, A. ochraceus, A. versicolor, A. terreus, A. candidus, Haplosporangium* spp., *Penicillium citratum, Rhizopus nigricans* and *R. stolonifer*. The isolated pathogenic fungi were highly decreased the seedling germination in *in vitro*.

Kakde and Chavan (2011) stated that *Alternaria dianthicola, Curvularia lunata, Fusarium oxysporum, Fusarium equiseti, Macrophomina phaseolina* and *Rhizopus stolonifer* decrease in reducing sugar of oilseeds. *Alternaria dianthicola, Curvularia pellescens, Macrophomina phaseolina, Penicillium digitatum* and *Penicillium chrysogenum* reduces the fat content of oilseeds.

Curvularia lunata, Curvularia pellescens, Fusarium oxysporum, Macrophomina phaseolina, Rhizopus stolonifer and *Penicillium digitatum* enhances the fiber content in oilseeds.

Materials and Methods

The present investigation was carried out in the Plant Pathology laboratory of Department of Plant Pathology, SKNAU, Jobner during 2018-19. The details of materials used and the methodology followed in conducting the experiments are presented in this chapter.

General laboratory procedure

Glass wares are sterilized in hot air oven at 180°C for 2 hours. The inoculation needle and other metallic instruments such as cork borer, forceps, were sterilized by dipping in alcohol and heating over the flame. The culture media were sterilized in autoclave at 1.02 kg pressure/cm2 (15 lbs PSI) for 15 minutes.

Collection of Seed Samples

Total 5 seed samples of sesame were collected from five tehsils of Jaipur districts *viz*, Chomu, Sambhar lake, Sanganer, Renwal and Phagi. From each tehsils, seed samples were collected randomly from farmers and mixed them to represent composite sample. All the collected seed samples were kept in cloth bags, brought to the laboratory and stored at 10°C for further studies. Samplings were done as method suggested in ISTA (1976).

Examination of Dry seed

The method suggested by Agarwal and Sinclair (1987) and ISTA (1985) were followed. Twenty gram seeds per sample were drawn at random and divided into four fractions. Each fraction was spread on the bottom of the Petri dish and examined with the help of a hand lens or if needed under stereo binocular microscope. The inspected seed materials were categorized as follows:

a. Deformed seeds
i) Shrivelled
ii) Over sized seed
b. Discoloured seeds
i) Light brown
ii) Dirty brown

c. Damaged seeds(insect)

d. Impurities
i) Plant debris
ii) Seed of other crop/ Weed
iii) Inert material

e. Apparently healthy seeds

Incubation Method

Mycoflora associated with seeds samples of sesame were isolated by using two incubation methods i.e. Blotter Method and Agar Plate Method (ISTA, 1985 and Agarwal and Sinclair, 1987)

Blotter Method

From each sample, hundred seeds selected at random were analysed. White blotter papers were cut into circles of 9 cm diameter and sterilized at 1.045 kg/cm2 for 15 minutes. Three circles of blotter papers were placed at the bottom of sterilized Petri-dishes aseptically moistened by sterilized distilled water. Twenty five seeds were placed at an equal distance in each petri-dish with fifteen seeds made outer ring, nine seeds made middle ring and one at center. These Petri-dishes were incubated at 25±1°C with 12 hours of light alternating with 12 hours of dark period. The seeds were examined on 7th day of incubation for emanating fungal colonies.

Agar Plate Method

Hundred seeds from each sample were taken for isolation of seed mycoflora. Seeds were surface sterilized with 0.1 per cent mercuric chloride solution for 1-2 minutes followed by 3 washing with sterilized distilled water. Sterilized Petri dishes each containing 20 ml Potato dextrose agar (PDA) medium were used for incubation of seeds. Ten seeds per Petri dish were equispaced/aseptically and incubated at 25±1°C with 12 hours of light alternating with 12 hours of dark period. The fungal colonies emanating from seeds were examined from 7th day of incubation. Isolation of mycoflora from sesame seeds was carried out and maintained on 2 per cent Potato Dextrose Agar (PDA) medium. Observations on mycoflora were recorded in both Blotter and Agar Plate Methods.

Seedling Symptom Test

Symptoms due to seed mycoflora on seedling were observed by using Seedling Symptom Tests (Agar Test in Test Tubes). (Agarwal and Sinclair, 1987) was employed for this purpose with slight modification.

Agar Test in Test Tubes

One hundred seeds of each sample selected at random were tested. The seedling were raised in 160×16 mm culture tubes, each containing 10 ml, 1 per cent Water Agar (10g Agar in 1000 ml distilled water) and plugged with a loose cotton plug and sterilized at 1.045 kg/cm2 for 15 minutes. One seed was placed in each test tube and incubated at 25±1°C under alternating cycle of 12 hours light and 12 hours darkness. After 15 days, plugs were removed and on 21st day of incubation, seedlings were examined for symptoms.

Per cent mortality and seedling vigour index were calculated by using following formula (Abdul-Baksi and Anderson, 1973):

Seedling Vigour Index = Germination(%) × (Root length + Shoot length)

$$\text{Mortality}(\%) = \frac{\text{Mortality with Test Fungi - Mortality in Check}}{\text{Mortality with Test Fungi}}$$

Among mycoflora tested, *Alternaria sesami* showed maximum reduction in germination per cent and vigour index (seedling vigour), less than 50 per cent of the vigour index observed in control considered as highly pathogenic, so further studies were carried out with *Alternaria sesami* only.

Isolation, purification and identification

Isolation

Isolation was done from seeds showing different types of symptoms to find out the association of particular mycoflora with particular type of symptoms. Mycoflora isolated from infected tissues of seedling symptoms tests and from seeds in incubation test, respectively were purified and identified as described below.

Purification

Pure culture of each mycoflora isolated from seeds/infected tissues was obtained by hyphal tip technique. For this, hyphal tips were obtain from culture slants after 96 hours of incubation and were suspended in sterilized distilled water.

The dilution of suspension was adjusted such that in one loopful, 5-10 spores could be counted under the low power objective of the microscope.

One ml of above suspension was spread in Petri plates containing 20 ml sterilized PDA medium. After 12-24 hours of inoculation, the germinating spores were located under the microscope and marked with the help of dummy objective and then transferred to PDA slant and kept in BOD for further growth. The culture was maintained by periodical transfer on PDA slants for further studies.

Identification of fungi

The identification of fungi was done based on the spore morphology and colony character (Ram Nath *et al.*, 1970). Further, the identification of the isolated fungi was confirmed from Indian Type Culture Collection (ITCC-5218), Division of Plant Pathology, ICAR- IARI, New Delhi.

Pathogenicity test

Three tests were performed as follows:

Seed Inoculation Technique

Hundred apparently healthy surface sterilized seeds were taken. The seeds were then rolled, separately on 7 days old sporulating culture of *Alternaria sesami* thriving on PDA in Petri dishes. The inoculated seeds were air dried and sown separately in 30 cm earthen pots filled with sterilized soil (Soil: FYM 3:1, sterilized at a pressure of 1.045kg/cm2 for 1 hour on 3 consecutive days). Five seeds were sown at equal distance in each pot with 4 replications. The un-inoculated apparently healthy surface sterilized seeds served as check. After sowing pots were kept in a cage house. These pots were watered regularly. Observations on per cent seed germination, Pre- and Post- emergence mortality. Seedling vigour and per cent seedling showing disease symptoms were recorded 60 days of sowing.

Foliar spray Inoculation technique

The method suggested by Mathur *et al.*, (1973) was followed to observed the symptoms on seedlings and grown up plants. 15 days old seedling, raised from disinfected seeds in plastic pots having autoclaved soil (soil : FYM= 3 : 1) were inoculated by spraying with spore suspension of 10 days old culture of *Alternaria sesami*, having concentration @ 106 spores/ ml.

The seedlings were sprayed to run off. Plastic pots having inoculated seedlings were kept in a humid chamber for 48 hours. Check plants were sprayed with distilled water only. Each treatment comprised of 100 seedlings (5 seedlings / pots × 20).

The sprayed seedlings were accommodated in a polythene house where temperature ranged from 25-

30°C. Plants were inspected on 10th and 15th day of inoculation. Isolation were carried out from inoculated plant parts showing disease symptoms.

Seed + Foliar spray Inoculation Techniques

In this method combination of above two methods (seed inoculation and foliar spray inoculation) are used.

Formula to calculate per cent disease intensity was as follows:

$$PDI = \frac{\text{Summation of all numerical ratings}}{\text{Total no. of plant} \times \text{Maximum rating scale observed.}} \times 100$$

Where, PDI= Per cent disease intensity

Results and Discussion

Collection of seed samples

Seed samples were collected from five tehasils of Jaipur district (Figure 1) and it marked as, A= Chomu, B= Sambhar Lake, C= Sanganer, D= Renwal, E= Phagi. From each tehasils, collected five seed samples from farmers and mixed them to represent the composite sample of correspond tehasil level.

The collected seed samples were kept in cloth bags, brought to the laboratory and stored at 10°C temperature for further studies. Sampling was done by the method suggested in ISTA (1976).

Examination of dry seeds

Each sample was categorised in to five groups (Table 4.1 and Plate 1). Deformities (Shrivelled and over sized seed) and discolouration (Dirty brown and light brown) were noticed in all the samples. Maximum deformity, in the form of shrivelling (14.75%) was observed in sample 'A' followed by samples 'B' (14.00%) and it was minimum in sample 'C' (8.20), While seeds deformity in the form of over sized on seeds was also noted in the samples examined and it was observed to be maximum (11.00%) in samples 'A' and minimum (3.45%) in sample 'C'. Discolouration in the form of dirty brown and light brown was maximum in sample 'A' (8.50/8.25%) while it was minimum in the sample 'C' (4.50/3.75%), respectively. Maximum damaged seeds (3.25%) occurred in sample 'B' and it was minimum in sample 'C' (0.90). Impurities in the form of plant debris and inert materials were observed in all the samples. Maximum apparently healthy seeds were observed in sample 'C' (81.90%) and it was minimum (48.75) in sample 'A'.

Incubation tests

Blotter Method

Eight mycoflora were isolated from sesame seeds (Table 4.2 and Plate 2). Fungi and their respective per cent incidence were *Alternaria sesami* (1.85-5.00%), *Aspergillus flavus* (2.10-3.75%), *Aspergillus niger* (1.80-2.50%), *Colletotrichum* spp. (1.20-2.00%), *Fusarium* spp. (1.00-2.00%), *Macrophomina phaseolina* (0.80-1.75%), *Mucor* spp. (1.10-2.50%), and *Penicillium* spp (0.80-1.60%).

Total per cent mycoflora was maximum in sample 'A' (20.40%) followed by 'B' (18.42%), 'D' (16.63%), 'E' (15.22%) and 'C' (6.60%). Average per cent incidence observed to be maximum of *Alternaria sesami (*3.40), *Aspergillus flavus* (2.80), *Aspergillus niger* (2.00), *Colletotrichum* spp. (1.50), *Fusarium* spp. (1.45) *Macrophomina phaseolina* (1.30), *Mucor* spp. (1.76), *Penicillium* spp.(1.24).

Agar Plate Method

Eight mycoflora were detected by Agar Plate Method from all the five seed samples (Table 4.3 and Plate 3). Mycoflora and their per cent occurrence recorded were *Alternaria sesami* (2.25-5.25%), *Aspergillus flavus* (2.70-3.50%), *Aspergillus niger* (1.97-3.00%), *Colletotrichum* spp. (1.76-2.75%), *Fusarium* spp. (1.00-2.75%),

Macrophomina phaseolina (1.25-2.50%), *Mucor* spp (1.52-2.50%) and *Penicillium* spp. (1.00-1.60%). Total per cent mycoflora was maximum in sample 'A' (22.35%) followed by 'B' (20.40%), 'D' (18.25%), 'E' (17.45%) while it was minimum in sample 'C' (8.65%). Average per cent incidence observed to be maximum of *Alternaria sesami* (3.90), followed by *Aspergillus flavus* (2.95), *Aspergillus niger* (2.15), *Colletotrichum* spp. (2.00), *Fusarium* spp. (1.90), *Macrophomina phaseolina* (1.55), *Mucor* spp (1.80), while it was minimum of *Macrophomina phaseolina* (1.29), respectively.

Seedling Symptom Test

Agar Test in Test Tubes

Symptoms like seed rot, yellowing, seedling blight, leaf blight and root rot were observed on seedlings. Range of such seedlings were 8.00 to 18.00 per cent (Table 4.4a and Plate 4) and it was maximum in sample 'A' (18.00%) followed by sample 'B' (16.00%) and minimum in 'C' (8.00%).

It is evident from Table 4.4b that seeds treated with individual fungus caused lowest germination, higher pre and post-emergence mortality, lowest reduced seedling length and vigour index as compared to control. Common fungi that showed higher per cent incidence in incubation tests and already reported as pathogenic were used to see their phytopathological effects.

Among these *Alternaria sesami*, *Aspergillus flavus*, *Aspergillus niger*, *Mucor spp*., *Colletotrichum spp*., *Fusarium spp*., *Macrophomina phaseolina* cause lowest seed germination and high per cent of pre and post-emergence mortality as compared to control. Lowest seed germination (50.00%) and maximum pre-emergence (28.00%) and post-emergence mortality (13.50%) with lowest seedling length (0.90 cm / 0.45 cm) and vigour index (67.50) was observed in seeds inoculated with *Alternaria sesami* followed by *Aspergillus niger* and *Aspergillus flavus*. Highest seed germination (74.00%) and lowest pre and post-emergence mortality (9.60% and 8.60%, respectively) with highest seedling length (2.10 cm / 1.03 cm) and vigour index (231.62) were observed in seeds inoculated with *Penicillium* spp. The degree of infection was directly correlated with the amount of fungal growth on seed. Heavy growth of the fungi resulted in complete failure of seed germination while sparse and moderate growth produced symptomatic seedlings. Moderate fungal growth often arrested seed germination at an early stage, cotyledons failed to emerge out of the seed coat.

Pathogenicity Test

Among various fungi isolated from seeds and seedlings showing symptoms, *Alternaria sesami* was most predominantly associated with them. Hence for further studies on pathogenicity was tested for *Alternaria sesami* only.

The *Alternaria sesami* isolated from seeds of sesame found pathogenic when seed, foliar and seed + foliar was inoculated artificially to sesame plant under pot conditions. The typical symptoms of Alternaria leaf spot disease appeared as small, dark brown, water soaked and round to irregular lesions, 1-8 mm in diameter with target board appearance on the leaves and spread fast to cover the entire leaf, giving a blighted appearance.

The lesions may also appear on the midrib and veins of the leaves without showing the typical leaf spotting from artificially inoculated plant shows *Alternaria sesami* which was identical to original once, (Table 4.5 and Plate 5).

Pathogenicity of *Alternaria sesami* was tested by seed, foliar inoculation and seed + foliar inoculation techniques. Maximum 72.53 per cent disease incidence was recorded in seed + foliar inoculation technique followed by foliar inoculation techniques (62.92%). Minimum 70.00% seed germination was observed in seed + foliar inoculation technique over control (86.00 per cent) at 40 days.

Sesame (*Sesamum indicum L.)* which belongs to the

pedaliaceae family, is one of the oldest oilseed crops cultivated in tropical and sub tropical regions of Asia, Africa and South America. Sesame seed has long shelf life due to the presence of lignans, which play the role of antioxidant function.

The seed, remarkably rich in quality proteins and essential amino acids. The seed is also a rich source of linoleic acid, vitamin E, A, B1 and B2 and minerals including calcium and phosphorus.

Seed play a vital role in the production of healthy crops. Seed is considered as a symbol and foundation of life as it is a container of embryo(s) of a new generation and carrier for the spread of new life. Therefore healthy seeds gives a good picture of their glorious golden era (Saskatchewan, 2013).

Any pathogen present in a seed that causes either failure of germination of seed or rotting of emerged seedlings or produce other kind of disease symptoms on adult plants may, in a broad sense be called as a seed borne pathogen.

A pathogen carried through the seed has a very good chance of being transmitted to next generation. Sesame seed being very tender and small have greater chances of attack of seed borne fungi causing seed or seedling rot and transmission to growing plant.

Examination of dry seed samples revealed the presence of deformed (shrivelled and over sized), discoloured (dirty brown and light brown), damaged seeds (insect damage), impurities (plant debries, seed of other crops, inert material) and apparently healthy seed. Presencce of such seeds & other impurities as seed contaminent in chilli, cumin, taramira, sesame & pearl millet have also been reported by singh and singh (1983); Singh (1993); and kumhar (1997), respectively.

Eight fungi including *Alternaria sesami, Aspergillus flavus, A.niger, Colletotrichum* spp., *Fusarium* spp., *macrophomina phaseolina, Mucor* spp. and *Penicillium* spp. were found on the different seed samples in which sample 'C' shows minimum fungal load from other samples. These are also reported by Singh and Singh (1990) who examined sesame seed samples from 18 districts of Rajasthan and found 108 samples to be infected.

They reported that sixty five seed samples contained with microsclerotia of *Macrophomina phaseolina.* Incubation test yielded 24 fungi. Majority of these were saprophytes but *Alternaria sesami, Cephalosporium acremonium, Fusarium oxysporum f.sp. sesami, F. solani and Macrophomina phaseolina* were most important pathogenic fungi.

During present investigation, Blotter Method, Agar Plate Method were employed for detection of fungi associated with seed samples collected from them at storage stage of their collection. The studies reveal that all eight fungi were detected in both Blotter Method, Agar Plate Method from all five samples and these fungi were *Alternaria sesami, Aspergillus flavus, A.niger, Colletotrichum* spp., *Fusarium spp.*, *Macrophomina phaseolina, Mucor* spp.

Penicillium spp.. among these, some of the species already been reported by Mathur and Kabeere (1975) on sesame seeds on which these five fungi viz. *A.sesami, Cercospora sesami, C. cassicola, Fusorium moniliforme and M. phaseolina* associated. Kushi and Khare (1979) also reported several seed samples of 21 sesame varieties and found 17 fungi associated of which *M. phaseolina was the most important along with Corynespora cassicola and Alternaria sesami*.

Isolation from infected tissues of various symptoms developed on seedling symptoms test yielded *Alternaria sesami, Aspergillus flavus, Aspergillus niger, Colletotrichum spp., Fusarium spp., Macrophomina phaseolina, Mucor spp., Penicillium spp.* It shows seed rot, seedling blight, yellowing, types of symptoms.

Table.1 Sesame seed samples were collected from different tehsils of Jaipur district with their code number

Sr. No.	Tehsils	Code No.
1.	Chomu	**A**
2.	Sambhar Lake	**B**
3.	Sanganer	**C**
4.	Renwal	**D**
5.	**Phagi**	**E**

Table.2 Seed abnormalities and impurities in different sesame seed samples

S. No.	Categories of seed/ impurities	Per cent content (by weight basis / sample)*					
		A	B	C	D	E	Mean
1.	**Deformed**						
	(i) Shrivelled	14.75	14.00	8.20	13.25	10.25	**14.75**
		(22.59)	(21.97)	(16.64)	(21.35)	(18.67)	**(22.59)**
	(ii) Over sized seed	11.00	10.25	3.45	5.00	7.50	**11.00**
		(19.37)	(18.67)	(10.70)	(12.92)	(15.89)	**(19.37)**
2.	**Discoloured**						
	(i) Dirty brown	8.50	7.75	4.50	7.50	5.25	**8.50**
		(16.95)	(16.16)	(12.25)	(15.89)	(13.25)	**(16.95)**
	(ii) Light brown	8.25	6.50	3.75	7.00	4.75	**8.25**
		(16.69)	(14.77)	(11.17)	(15.34)	(12.59)	**(16.69)**
3.	**Damaged seeds**						
	(i) Insect	1.75	3.25	0.90	2.25	2.75	**1.75**
		(7.60)	(10.39)	(5.44)	(8.63)	(9.55)	**(7.60)**
4.	**Impurities**						
	(i) Plant debries	2.75	1.90	1.35	2.50	1.50	**2.75**
		(9.55)	(7.92)	(6.67)	(9.10)	(7.03)	**(9.55)**
	(ii) Seeds other crops	2.75	1.50	0.70	1.75	2.50	**2.75**
		(9.55)	(7.03)	(4.80)	(7.60)	(9.10)	**(9.55)**
(iii) Inert material							
		1.50	0.90	0.45	1.00	1.25	**1.50**
		(7.03)	(5.44)	(3.85)	(5.74)	(6.42)	**(7.03)**
5.	**Apparently healthy seed**	48.75	53.95	81.90	59.75	64.25	**48.75**
		(44.28)	(47.27)	(64.82)	(50.62)	(53.28)	**(44.28)**
SEm±		**2.22**					
CD at 5%		**6.83**					

* Average of 5 replications (5g seeds/ replication)

A = Chomu, B = Sambhar Lake, C = Sanganer, D = Renwal E= Phagi

Table.3 Per cent incidence of mycoflora of sesame seeds isolated by Blotter Method

Mycoflora	Per cent incidence /sample*					Average
	A	B	C	D	E	
Alternaria sesami	5.00	4.00	1.85	3.75	3.25	**3.40**
Aspergillus flavus	3.75	3.00	2.10	3.25	3.00	**2.80**
Aspergillus niger	2.50	2.25	1.80	2.00	2.00	**2.00**
Colletotrichum spp.	2.00	1.75	1.20	1.50	1.75	**1.50**
Fusarium spp.	1.50	1.50	1.00	2.00	1.25	**1.45**
Macrophomina phaseolina	1.75	2.00	0.80	1.00	1.25	**1.30**
Mucor spp.	2.30	2.50	1.10	1.75	1.50	**1.76**
Penicillium spp.	1.60	1.42	0.80	1.38	1.20	**1.24**
Per cent mycoflora	**20.40**	**18.42**	**6.60**	**16.63**	**15.22**	

*Average of 400 seeds; A = Chomu, B = Sambhar Lake, C = Sanganer, D = Renwal E= Phagi

Table.4 Per cent incidence of mycoflora of sesame seeds isolated by Agar Plate Method

Mycoflora	Per cent incidence /sample*					Average
	A	B	C	D	E	
Alternaria sesami	5.25	5.00	2.25	4.25	3.75	**3.90**
Aspergillus flavus	3.25	3.50	2.70	3.75	3.25	**2.95**
Aspergillus niger	3.00	2.00	1.97	2.25	2.25	**2.15**
Colletotrichum spp.	2.75	2.25	1.76	2.00	2.25	**2.00**
Fusarium spp.	2.75	2.00	1.00	1.75	1.50	**1.90**
Macrophomina phaseolina	1.25	2.50	1.37	1.50	1.50	**1.55**
Mucor spp.	2.50	1.75	1.52	2.00	1.75	**1.80**
Penicillium spp.	1.60	1.40	1.00	1.35	1.20	**1.29**
Per cent mycoflora	**22.35**	**20.40**	**8.65**	**18.25**	**17.45**	

* Average of 200 seeds; A = Chomu, B = Sambhar Lake, C = Sanganer, D = Renwal E= Phagi

Table.5 Symptoms on seedlings raised from different seed samples (Agar test in test tubes)

Sample No.	Per cent seedlings showing symptoms	Types of symptoms
A	18.00	**Seed rot, seedling blight/decay, leaf blight and yellowing**
B	16.00	**Seed rot, seedling blight, leaf blight and yellowing**
C	8.00	**Seed rot, leaf blight and yellowing**
D	14.00	**Seed rot, seedling leaf blight and yellowing**
E	**10.00**	**Seed rot, seedling blight and yellowing**

No. of seeds tested = 100; * Based on the emerged seedlings
A = Chomu, B = Sambhar Lake, C = Sanganer, D = Renwal E= Phagi

Table.6 Effect of seed mycoflora on seed germination, pre and post-emergence mortality and seedling vigour tested by test tube method

S. No	Mycoflora	Per cent germination	Per cent mortality		Seedling length*		Vigour index
			Pre-emergence	Post-emergence	Shoot length (cm)	Root length (cm)	
1	*Alternaria sesami*	50	28.00	13.50	0.90	0.45	**67.50**
2	*Aspergillus flavus*	57	20.0	9.46	1.40	0.75	**122.55**
3	*Aspergillus niger*	60	18.60	8.20	1.60	0.70	**138.00**
4	*Colletotrichum* spp.	64	12.00	6.75	1.80	1.10	**185.60**
5	*Fusarium* spp.	66	10.00	13.00	1.90	1.20	**204.60**
6	*Macrophomina phaseolina*	69	11.00	7.33	2.04	1.27	**228.39**
7	*Mucor* spp.	62	17.50	8.53	1.70	1.00	**167.40**
8	*Penicillium* spp.	74	9.60	8.60	2.10	1.03	**231.62**
9	**Control**	**82**	**0.00**	**0.00**	**2.50**	**1.60**	**336.60**

*Average of 100 seedlings

Table.7 Pathogenicity of *Alternaria sesami* with sesame

Inoculation method	Per cent germination	Per cent disease intensity
Seed inoculation	72.00	**60.01**
	(58.05)	**(50.77)**
Foliar inoculation	83.00	**62.92**
	(65.65)	**(52.49)**
Seed + foliar inoculation	70.00	**72.53**
	(56.79)	**(58.39)**
Control	86.00	**0.00**
	(68.03)	**(0.00)**
SEm±	0.86	**0.77**
CD (P=0.05)	**2.65**	**2.39**

*Average of four replications; Figures given in parentheses are angular transformed values

Fig.1 Map showing sesame seed collection from different tehsils of jaipur district

sesame seed collection from different tehsils of jaipur district

Fig.2

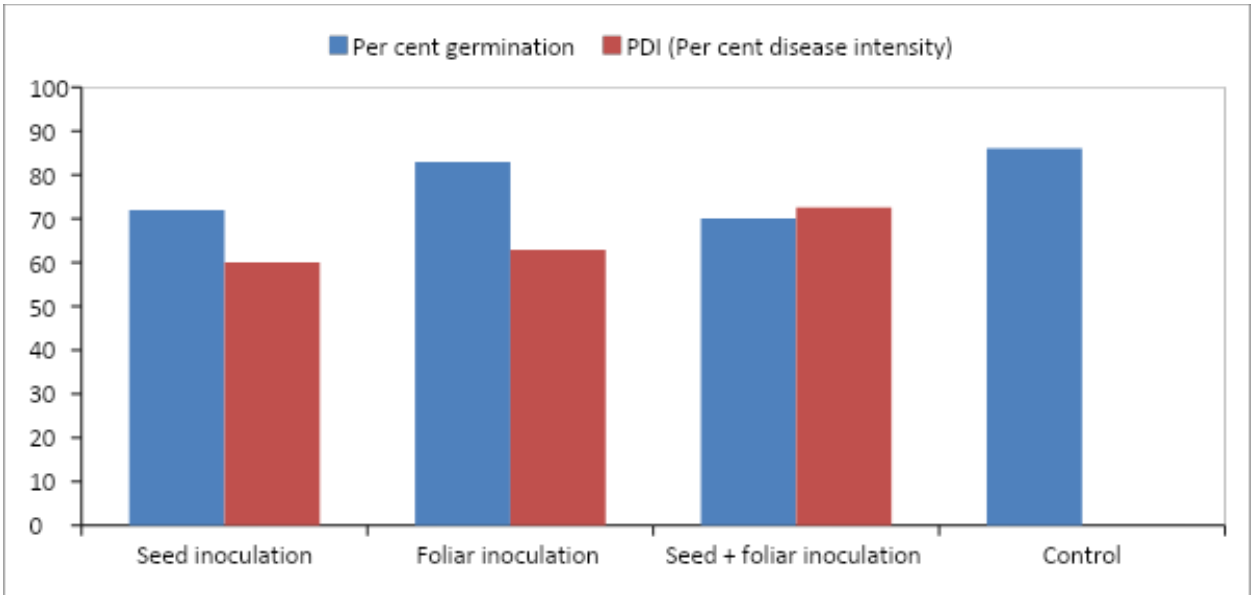


Plate.1 Seed abnormalities and impurities in different sesame seed samples

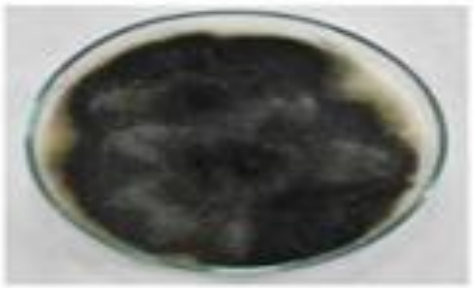
Plate.2 Incubation Methods

(a) Blotter Method

(b) Agar Plate Method

Plate.3 Sesame Seed Showing Growth of Seed Mycoflora

CONIDIA OF *Alternaria sesami*



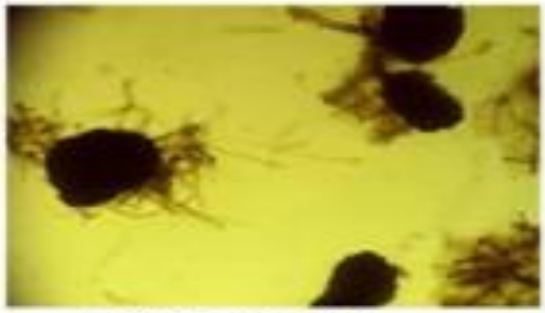
PURE CULTURE OF
Alternaria sesami

ACERVULUS OF
Colletotrichum spp.

PURE CULTURE OF
Colletotrichum spp.

CONIDIA OF *Fusarium* spp.

PURE CULTURE OF
Fusarium spp.



SCLEROTIA OF
Macrophomina phaseolina

PURE CULTURE OF
Macrophomina phaseolina

Plate.4 Symptoms of seedlings raised from different seed samples (Agar test in test tubes)

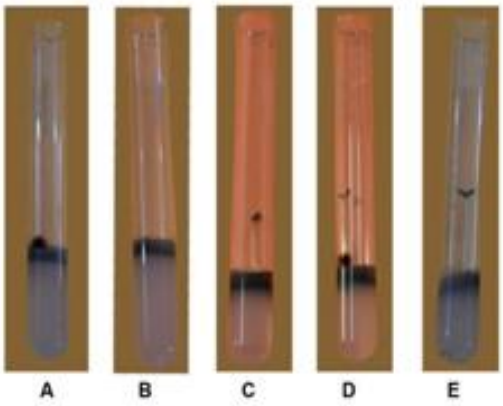
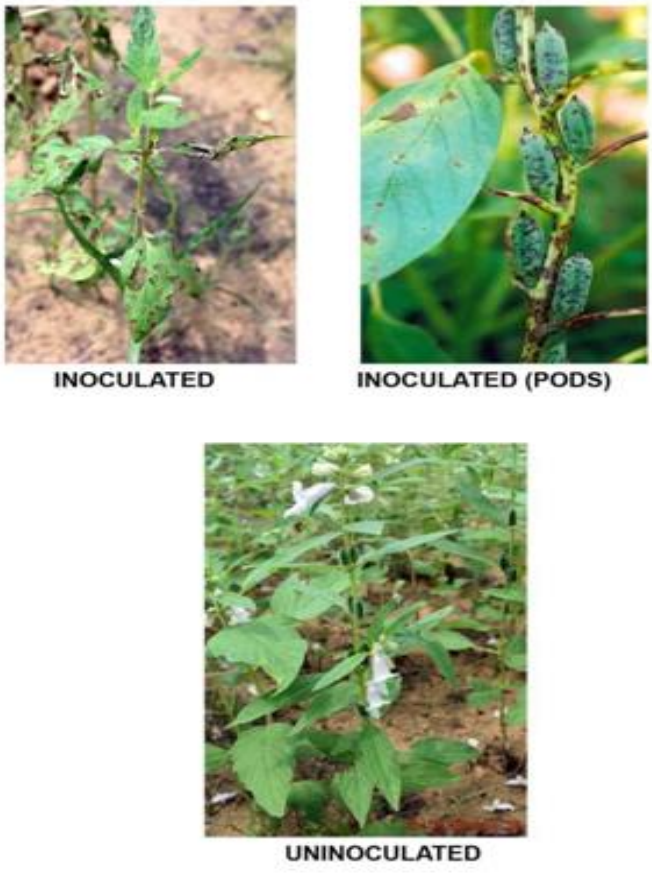


Plate.5 Proving pathogenicity of *Alternaria sesami* causing leaf blight



Result of Test Tube Seedling Symptom Test revealed that among all the fungi tested, *Alternaria sesami* showed minimum seed germination with maximum pre and post-emergence mortality and reduced vigour index, therefore *Alternaria sesami* was recognized as highly pathogenic seed borne fungi. Srikantappa *et al.,* (2009) also reported the detection of seed-borne fungi of sesame seeds. The following fungal genera were isolated as *Alternaria alternata, A. sesamicola, Fusarium moniliforme, F. oxysporum, A. tenuis, Verticillium dahliae, Sclerotinia sclerotiorum, S. rolfsi, Cercospora sesami, Curvularia lunata, Macrophomina phaseolina, Cladosporium cladosporioides, C. herbarum, C. fulvum, C. chlorocephalum, Acremonium* sp., *Helminthosporium* spp., *Gliocladium roseum, Neurospora glabra, Cunigamella elegans, Chaetomium globosum, Stachybotrys chartarum, S. atra, Pestalotia macrotricha, Aspergillus niger, A. flavus, A. ochraceus, A. versicolor, A. terreus, A. candidus, Haplosporangium* spp., Penicillium *citratum, Rhizopus nigricans* and *R. stolonifer.* The isolated pathogenic fungi were highly decreased the seedling germination in *in vitro*.

In the present study, the isolation of the pathogen was made from sesame seeds which are plated on Agar plates. The culture was purified by hyhal tip techniques.

On the basis of cultural and morphological characteristics, the fungus was identified as *Alternaria sesami. Alternaria sesami causing Alternaria* blight is *a* seed-borne disease reported by Agrawal (1961); Choudhery (1945); Jain and Kulkarni (1965) on sesame in India. Pathogenicity was proved by following three methods of inoculation i.e. seed, foliar and seed + foliar was inoculated artificially to sesame plant under pot conditions. Among these, seed + foliar inoculation method was proved highly effective. Conformity of this study is earlier findings of Pradhan (2017) who reported seed mycoflora of mung bean that reduced germination and seedling vigor index. Seedling vigour was markedly decreased by some of the seed borne mycoflora when evaluated by seed inoculation techniques.

The present investigation entitled “Seed mycoflora of sesame (*Sesamum indicum L.*), their Phytopathogenic effect” was carried out at Department of Plant Pathology, S.K.N. College of Agriculture, Jobner with following objectives:

Collection of sesame seed samples from five tehasils of Jaipur district

Isolation, purification, identification and pathogenicity of major seed mycoflora associated with sesame from Jaipur district.

Effect of isolated major fungi on seed germination and seedling vigour.

Sesame (*Sesamum indicum* L.) which belongs to the pedaliaceae family, is one of the oldest oilseed crops cultivated in tropical and subtropical regions of Asia, Africa and South America. Sesame seeds are very small in size, pearl shaped, ovate, small and thinner at the hilum.

The seed colour ranges from white, light brown and dark brown to black. Sesame seed has long shelf life due to the presence of lignans, which play the role of antioxidant function. The seed, remarkably rich in quality proteins and essential amino acids. The seed is also a rich source of linoleic acid, vitamin E, A, B1 and B2 and minerals including calcium and phosphorus.

Alternaria species are dominant seed borne fungi and these species are responsible for changes in physical properties and quality of seeds.

Total 5 composite samples of sesame seeds were collected from five tahasils of Jaipur districts *viz*, Chomu, Sambhar lake, Sanganer, Renwal and Phagi. These samples showing deformed (shriveled and over sized seeds), discoloured (dirty brown and light brown), damaged (by insects) seeds. Impurities of one kind or other were also found in all the five samples.

Total of eight seed mycoflora were obtained in Blotter and Agar Plate tests. These mycoflora were *Alternaria sesami, Aspergillus flavus, Aspergillus niger, Colletotrichum* spp., *Fusarium* spp., *Macrophomina phaseolina, Mucor* spp., *Penicillium* spp.

Average per cent incidence of seed mycoflora was more in Agar Plate Method in comparison to Blotter Test and amongest these fungi obtained, incidence of *Alternaria sesami* was maximum.

In general, seeds of sample 'A' found to be highly contaminated as the incidence of fungi in seeds were maximum in amongst the sample analysed. Minimum incidence of fungi was in sample 'C'.

The seed samples used for seedling symptoms test exhibited various types of disease symptoms with the variation in per cent seedling showing symptoms with in samples and stages of their analysis. Maximum number of seedlings of sample' A 'showed symptoms of disease, while they were minimum in 'C'.

The isolation of *Alternaria sesami* was made from infected seeds of sesame and purified by hyphal tip technique. The pathogenicity of the pathogen was proved by seed inoculation, foliar inoculation and seed + foliar inoculation techniques. Highest disease incidence was observed in seed+ foliar inoculation technique.

In Test Tube Seedling Symptom Test, among the fungi tested, *Alternaria sesami* found highly pathogenic ones as it showed maximum reduction of per cent germination and vigour index with enhanced pre and post-emergence mortality.

Seed samples of sesame collected from five tehasils of Jaipur district (Chomu, Sambhar lake, Sangner, Renwal, and Phagi).

Among the seed samples collected highest per cent apparently healthy seed was found in Sanganer and least in Chomu. Highest per cent mycoflora isolated from both Blotter and Agar Plate Method was *Alternaria sesami*.

Alternaria sesami was found to be highly pathogenic as it showed maximum reduction in seed germination and vigour index with enhanced pre and post-emergence mortality.

Seed+ foliar inoculation technique was found most effective in producing higher disease intensity.

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