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Detection and identification of seed associated fungi of sesame (*Sesamum indicum*) during storage and their management

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Abstract

Sesame (*Sesamum indicum L.*) is one of the most ancient oilseed crop and rich source of oil, protein, calcium as well as phosphorus, which is cultivated throughout Pakistan. Different pathogens affect the sesame crop and causing diseases including some post-harvest and storage fungi causing serious losses in production. These seeds borne fungi causes heavy damage by decreasing seed germination. Infected germinated seeds do not grow properly which increased the chance of seedling concision and pre or post emergence diseases consequences into inhibited growth and yield losses. By considering the above evidences the current research is intended to determine the seed borne fungi related with sesame seeds and their management. For this purpose seed samples were collected from seed market of Faisalabad and Ayub Agriculture Research Institute (AARI) (250 g/sample). These seed samples were platted on blotter paper and agar plates. These plates were incubated at 25±2ºC and number of fungi were observed and identified with fungal colony color, sporulation type and relevant literature after 6-8 days of incubation. During management trials the evaluation of treated and non-treated seeds were done by using different seed dressing fungicides and plant extracts. These research trials were carried out under Complete Randomized Design (CRD) and the statistical significance between mean values were recorded using LSD test. During the detection experiments, *Alternaria alternata, Fusarium moniliforme, F. oxysporum, A. tenuis, S. rolfsi, Cercospora sesami, Curvularia lunata, Macrophomina phaseolina, Aspergillus niger, A. flavus, A. ochraceus, A. versicolor, A. terreus, A. candidus, Haplosporangium sp., Penicillium citratum, Rhizopus nigricans, and R, stolonifer* were isolated from local variety of sesame seeds. Among all the seed health test methods, standard blotter method is most superior for detection of seedborne fungi over the other methods.

Key words: Sesame, seed associated fungi, seed health test methods

Introduction

Sesame *(Sesamum indicum L.)* is the oldest oil plant. Sesame is the 6th most important oilseed crop in the world. It is grown in all major crop-growing seasons namely *kharif, pre-rabi, rabi* and summer. Sesame oil is used as edible oil, in paints and soap industry and also has great medicinal value. This crop faces many pest and diseases like other crops. Fusarium wilt (*Fusarium oxysporum*), charcoal rot (*M. phaseolina*], leaf spot (A. sesami, powdery mildew and phytophathora blight are the most common diseases. Most of the diseases are known to be seed borne in nature. *A. sesami*, *F. oxysporum*, *M. phaseolina* and eight other fungi are known to be seed borne and are cause of different diseases (Richardson, 1990). No systematic work has been carried out on the seed borne fungi of sesame and their effects on yield in Pakistan. The present studies were initiated to study the seed borne fungi on sesame prevalent in the Punjab, Pakistan.Sesame crop is infected by a number of fungal, bacterial, viral, mycoplasma and non-parasitic diseases around the world. Among the most important fungal pathogens, *Fusarium oxysporum f.* sp. sesami causes seedling blight and Fusarium wilt of sesame by blocking the root xylem vessels. *Macrophomina phaseolina* is a virulent fungus causing charcoal rot or stem rot disease in sesame, which leads to heavy economic losses to the grower by significantly reducing the oil content if the crop is attacked at maturity stage. Alternaria sesami causes damage by

reducing photosynthesis through leaf damage and premature defoliation and by producing elongated lesion on capsules and reducing yield. Additionally fungi such as *Aspergillus sp. Penicillium citrinum and Fusarium sp*. might Salso infect sesame seeds and cause immediate damage as the seeds germinate (Jonsyn, 1988). Seed borne mycoflora are carried over by infected seeds and they cause deterioration in seed, in soil affecting germination, causing seedling mortality and further infection of foliage is observed at adult stage. Fungi including *Alternaria*, *Curvularia, Fusarium, Helminthosporium, Penicillium, Mommoniella* and *Rhizophus* sp. have been found associated with sesame seeds (ISTA, 1999). Among these, *Alternaria* is the most destructive pathogen of sesame; as it produces small brown spots on leaf ranging from 1-8 mm in diameter. It reduces the viability of seeds and the seed borne pathogens are the most disastrous as they reduce the seed vigour and weaken the plant at the initial growth. Numerous microorganisms, especially fungi, pose a challenge to both sesame production and seed storage. Sesame agriculture faces numerous disease problems, including vascular wilt, root rot, leaf blight, leaf spot and damping off in young seedlings (Sahab et al. 2008). Soil is the major source of pathogenic and non-pathogenic fungi which later on associated with seeds at germination and seedling stages. These pathogens including *Trichophyton tonsurans, Trichophyton mentagrophytes, Trichophyton rubrum, Trichophyton equinum, Microsporum gypseum, Microsporum nanum, Microsporum audouinii* and *Aspergillus niger* which damage the crop profile of sesame. Different type of seed borne fungi including *Aspergillus flavus, Aspergillus niger, Aspergillus ochraceous, Drechslera australiensis, Fusarium sporotrichioides, Rhizopus oryzae* and *Trichoderma viride* which damage the crop profile at post-harvest stages (Khokhar *et al*., 2013).*Alternaria alternata, Alternaria longissima, Alternaria sesami, Alternaria sesamicola, Aspergillus flavus, Aspergillus niger, Aspergilllus ochraceus, Corynespora cassicola, Cercospora sesami, Chaetomium globosum, Colletotrichum dematium, Curvularia lunata, Drechslera sesami, Fusarium oxysporum, Fusarium moniliforme, Macrophomina, phaseolina, Penicillium chrysogenum, Phoma spp, Rhizopus nigricans, Rhizopus stolonifer, Phytophthora nicotianae var. Parasitica*, these seed borne pathogens have been found to cause diseases of various plant parts like root, stem, leaf and pod of sesame seed. However, some seed borne pathogens of sesame dwell in soil, where their inoculam potential is increased which helps in the attack of other plant parts. (Elewa *et al* 2011). Keeping in view the above facts the fundamental purpose of the study is to observe and record seed mycoflora of sesame in field and storage condition and also to check the effectiveness of several seed treatment fungicides.

The objectives were attained via following line of work.

1. Collection of seed sample from Grain market Faisalabad and AARI Faisalabad
2. Determination of seed borne mycoflora by Blotter paper and agar Media plate method
3. Effect of isolated fungi on seed germination and health
4. Management of seed borne fungi through chemicals and plant extracts by means of seed treatment

Materials and methods

The study of fungi associated with seeds and field crop sesame were conducted in seed pathology laboratory, Department of Plant Pathology, University of Agriculture, Faisalabad. The details of materials used and the methods followed in conducting the experiments are described in this chapter.

Collection of Seeds:

Different sesame growing areas were visited to collect the seeds of sesame.

Fungal Growth:

Sterilized petri plates were taken and blotter paper was paced on each and every petri plate. Then with the help of sterilized needle all the seeds were placed on blotter paper in several petri plates. Wrapping of petri plates were done properly and the plates were placed in incubator for fungal growth for 5 to seven days.

DETECTION OF SEED-BORNE FUNGI BY SEED TESTING METHODS

Fungal Growth on PDA:

First of all 200 g of unpeeled potatoes were taken in 1000 ml of distilled water and subjected to heat. After boiling filtration was done by cheese cloth by saving the potato infusion in media bottle. After that 20 g of Agar and 15 g of dextrose were added in it and shaking was done for proper mixing of all the ingredients. For sterilization, media was autoclaved at 121°C and 15 Psi pressure for 15 minutes. After cooling, media was poured in already sterilized plastic petri plates in Laminar flow cabinet and infected small pieces of stem and root are placed properly on PDA media and plates were wrapped properly by cling film to avoid the contamination. Tagging on plates was done by permanent marker with the name of location from where the samples were collected and petri plates were kept in incubator at 25°C.

Seed health testing of different samples of sesame

Untreated sesame seed samples were collected from different sesame growing districts of northern Karnataka *viz*., Dharwad, Bijapur, Gulbarga, Bidar, Belgaum, Raichur and Koppal. Following are the details of the collected seed samples:

Standard blotter method

The standard blotter method was developed by Doyer in 1938 which was later included in the International Seed Testing Association Rules of 1966. Four hundred seed of each variety were tested by employing standard blotter method in three replications. Three pieces of blotting paper of 90 mm size were moistened with distilled water and placed in 90 mm sterilized Petri plates after draining excess water. Untreated seeds were placed at the rate of 20 seeds per Petri plate at equal distance. The plates were incubated at room temperature (25± 2º C) under alternate cycles of 12 hrs NUV lightand darkness.

Identification of fungi:

The identification of fungi was done based on the spore morphology and colony character (Ram Nath*et al.*, 1970). The culture was further purified by following single spore isolation technique (Tuite, 1969), thus obtained pure culture was maintained on potato dextrose agar slants. Such culture tubes were preserved in a refrigerator at 5º C and renewed once in a month for further studies.

Evaluation of seed health testing methods:

To know the efficacy of different seed health testing methods in detecting seed borne fungi of sesame, following methods were employed as described below.

Standard Blotter Method:

Four hundred seeds of sesame cv. E -8 were placed at the rate of 20 seeds per Petri plate on moistened blotters as described under standard blotter method. Such seeds were examined under stereoscopic-binocular microscope for the infection of *A. sesami*.

Agar plate method with potato dextrose agar:

Four hundred seeds of sesame variety E-8 were surface sterilized with 1 per cent sodium hypochlorite solution for 1-2 min and then placed at the rate of 20 seeds per Petri plate containing 20 ml of potato dextrose agar. The Petri plates were incubated for seven days as described under standard blotter method. After seven days of incubation the fungal growth on seeds was examined under stereoscopic binocular microscope.

Management strategies to overcome seed borne fungal infections of sesame:

Evaluation of seed dressing fungicides:

This study was carried out to know the efficacy of different seed dressing fungicides in eliminating the seed-borne fungal infections in the infected seed sample of sesame (variety E -8). No. of conidia in the diluted suspension/milliliter Average no of conidia above one large square × 1ml / 0.004 mm3 =

The fungicides were tested initially under *in vitro* condition by following using poisoned food technique (Nene and Thapliyal, 1973) and rolled towel method.

***In vitro* evaluation of fungicides against *A. sesami*by poisoned food technique:**

All the systemic fungicides were tested at 0.025, 0.05 and 0.1 per cent and combine products were tested 0.05, 0.1, 0.2 per cent concentration by adopting poisoned food technique. In this technique the fungus *A. sesami*was grown on potato dextrose agar medium in Petriplates for seven days prior to setting up of experiment. The fungicidal suspension was added to the melted potato dextrose agar medium to obtain the desired concentration on the basis of active ingredients present in the chemical.

Twenty ml of poisoned medium was poured in each sterilized Petri plates. Suitable checks were maintained without addition of fungicides. Five mm mycelia disc taken from the periphery of seven days old colony was placed in the centre and incubated at 25 ± 2ºC for full growth of the fungus. Four replications were maintained for each treatment, the diameter of the colony growth was measured in two directions after seven days of inoculation at which maximum growth was observed in control and average was recorded. Per cent inhibition was calculated by using the following formula given by Vincent (1947).

C-T I = ---------- X 100 C

Where,

I = Per cent inhibition, C = Growth in control, T = Growth in treatment

Statistical analysis

The experiment was carried out under complete randomized design (CRD) in in vitro and randomized complete block design (RCBD) in vivo, statistical analysis was performed using standard analysis of variance (ANOVA). The means and standard error of means (SEM) were calculated and statistical significance between the mean values was assessed by using Least Significant Difference (Steel and Torrie, 1997).

Results and discussion

The study was conducted in the Seed Health Testing lab. Department of Plant Pathology, U.A.F. during the year 2016-18 to assess the association of different seed borne fungi of sesame seeds. A total of 10 seed samples were assessed through Agar Plate Method and Blotter Paper Method for detection and isolation of seed associated fungi. By this trials genera of *Aspergillus niger, Aspergillus flavus, Alternaria alternate, Botrytis* spp., *Pencillium* spp., and *Fusarium oxysporum* were isolated in different percentage. Data about various experiments were collected and analyzed about which results are presented below (Table 1).

Table 1 (a): Analysis of Variance of UF-32

SOV	DF	SS	MS	F-values	Probability
Replications	2	5.21	2.60		
Method	1	431.65	431.65	167.30	0.0000**
Fungus	6	1691.21	281.86	109.24	0.0000**
F × M	6	771.47	128.57	49.83	0.0000**
Error	26	67.24	2.58		
Total	41	2966.78			

SOV = Source of variation; DF = Degree of freedom; SS = Sum of square; MSS = Mean sum of square; ** = Highly Significant at p ≤ 0.01

Table 1 (b): Comparison of mean values for isolation of fungi from UF-32

Fungus	Methods		Means
	Blotter paper method	**Agar plate method**	
Aspergillus flavus	13.23 c	35.65 a	**24.44 A**
Aspergillus nigar	11.45 cd	23.63 b	**17.55 B**
Pencillium spp	14.32 c	18.62 b	**16.47 B**
Alternaria alternate	12.76 cd	12.35 d	**12.55 C**
Rhizopus spp	10.78 d	14.66 cd	**12.72 C**
Botrytis spp	12.65 cd	12.27 cd	**12.46 C**
Fusarium oxysporum	1.64 f	4.45 e	**3.04 D**
Total	**10.98 B**	**17.38 A**	

LSD: Method = 2.07; Fungus = 0.53; Interaction = 2.50

Table 2 (a): Analysis of Variance of PF-35

SOV	DF	SS	MS	F-values	Probability
Replications	2	4.22	2.11		
Method	1	231.61	231.62	88.40	0.0000**
Fungus	6	594.21	99.03	45.01	0.0000**
F × M	6	171.41	28.57	10.90	0.0000**
Error	26	68.28	2.62		
Total	41	859.73			

SOV = Source of variation; DF = Degree of freedom; SS = Sum of square; MSS = Mean sum of square; ** = Highly Significant at p ≤ 0.01

Table 2 (b): Comparison of mean values for PF-35

Fungus	Methods		Means
	Blotter paper method	**Agar plate method**	
Aspergillus flavus	11.23 c	31.61 a	**21.42 A**

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Aspergillus nigar	12.41 c	21.64 b	**17.03 B**
Pencillium spp	13.31 c	17.66 b	**15.49 C**
Alternaria alternata	10.56 c	13.34 c	**11.96 D**
Rhizopus spp	11.19 c	13.67 c	**12.43 D**
Botrytis spp	13.61 c	10.26c	**11.94 D**
Fusarium oxysporum	5.64 d	3.45 c	**4.55 E**
Total	**11.14 B**	**15.95 A**	

LSD: Method = 2.01; Fungus = 1.57; Interaction = 2.47

Table 3 (a): Analysis of Variance of Gujrat Sesame -1

SOV	DF	SS	MS	F-values	Probability
Replications	2	16.23	8.11		
Method	1	430.12	430.12	126.87	0.0000**
Fungus	6	1494.15	249.02	73.45	0.0000**
F × M	6	571.44	95.24	28.09	0.0000**
Error	26	88.29	3.39		
Total	41	2600.23			

SOV = Source of variation; DF = Degree of freedom; SS = Sum of square; MSS = Mean sum of square; ** = Highly Significant at $p \leq 0.01$

Table 3 (b): Comparison of mean values for Gujrat Sesame

Fungus	Methods		Means
	Blotter paper method	Agar plate method	
Aspergillus flavus	12.43 cd	24.66 a	**19.5482 A**
Aspergillus nigar	11.43 cd	23.15 b	**17.2835 B**
Pencillium spp	12.33 cd	15.61 b	**14.9701 B**
Alternaria alternata	13.17 c	14.15 d	**14.6544 C**
Rhizopus spp	10.29 d	10.98 cd	**11.6287 C**
Botrytis spp	11.61 cd	17.17cd	**15.3842 C**
Fusarium oxysporum	6.13 e	8.46 e	**8.2973 D**
Total	**11.06 B**	**15.94 A**	

LSD: Method = 2.23; Fungus = 1.64; Interaction = 2.91

Table 4 (a): Analysis of Variance for UF-32 fresh harvested seed samples

SOV	DF	SS	MSS	F-values	Probability
Replications	2	115.23	57.61		
Method	1	190.67	190.67	7.43^{NS}	0.0620
Fungi	6	6095.26	1015.87	39.58**	0.0051
Method × Fungi	6	280.45	46.74	1.82^{NS}	0.6210
Error	26	667.29	25.66		
Total	41	7348.90			

SOV = Source of variation; DF = Degree of freedom; SS = Sum of square; MSS = Mean sum of square; ** = Highly Significant at $p \leq 0.01$; NS = Non Significant

Table 4 (b): LSD All-Pairwise Comparisons Test of UF-32 for Blotter Method

Fungus	Means
Aspergillus flavus	14.23 b
Aspergillus nigar	12.45 d
Pencillium spp	15.32 a
Alternaria alternate	13.76 c
Rhizopus spp	11.78 e

Botrytis spp	13.65 c
Fusarium oxysporum	2.64 f

LSD (value) = 0.45

There are 6 groups (a-f) in which the means are not significantly different from one another but groups are significantly different from each other.

Table 5 (a): Analysis of Variance for PF-35 fresh harvested seed samples

SOV	DF	SS	MSS	F-values	Probability
Replications	2	0.26	0.13		
Method	1	4.03	4.03	15.50*	0.0390
Fungi	6	13.80	2.30	5.11^{NS}	0.0820
Method × Fungi	6	3.13	0.52	1.15*	0.0210
Error	26	11.73	0.45		
Total	41	32.96			

SOV = Source of variation; DF = Degree of freedom; SS = Sum of square; MSS = Mean sum of square; ** = Highly Significant at $p \leq 0.01$; NS = Non Significant

Table 5 (b): LSD All-Pairwise Comparisons Test of PF-35 for Blotter Method

Fungus	Means
Aspergillus flavus	12.23 c
Aspergillus nigar	13.41 b
Pencillium spp	14.31 a
Alternaria alternate	11.56 d
Rhizopus spp	12.58 c
Botrytis spp	14.61 a
Fusarium oxysporum	6.64 e

LSD (value) = 0.33

There are 4 group (a-d) in which the means are not significantly different from one another but group significantly different from each other.

Table 6 (a): Analysis of Variance for Gujrat Ajwain fresh harvested seed samples

SOV	DF	SS	MSS	F-values	Probability
Replications	2	0.52	0.26		
Method	1	4.25	4.25	12.87*	0.0100
Fungi	6	12.61	2.10	6.36*	0.0320
Method × Fungi	6	2.25	0.37	1.12*	0.0210
Error	26	8.64	0.33		
Total	41	32.96			

SOV = Source of variation; DF = Degree of freedom; SS = Sum of square; MSS = Mean sum of square; ** = Highly Significant at $p \leq 0.01$; NS = Non Significant

Table 6 (b): LSD All-Pairwise Comparisons Test of Gujrat Ajwain for Blotter Method

Fungus	Mean
Aspergillus flavus	13.42 b
Aspergillus nigar	12.42 c
Pencillium spp	13.33 b
Alternaria alternate	14.16 a
Rhizopus spp	11.28 d
Botrytis spp	12.60 c
Fusarium oxysporum	7.14 e

LSD (value) = 0.21

There are 5 groups (a-e) in which the means are not significantly different from one another but groups are significantly different from each other.

Table 7 (a): Analysis of Variance for UF-32 fresh harvested seed samples

SOV	DF	SS	MSS	F-values	Probability
Replications	2	1.86	0.93		
Method	1	2.70	2.70	4.57*	0.0310
Fungi	6	10.46	1.74	2.94*	0.0360
Method × Fungi	6	0.46	0.07	0.11^{NS}	0.5210
Error	26	15.46	0.59		
Total	41	30.96			

SOV = Source of variation; DF = Degree of freedom; SS = Sum of square; MSS = Mean sum of square; ** = Highly Significant at $p \leq 0.01$; NS = Non Significant

Table 7 (b): LSD All-Pairwise Comparisons Test of UF-32 for Agar Plate Method

Fungus	Mean
Aspergillus flavus	36.65 a
Aspergillus nigar	24.65 b
Pencillium spp	19.61 c
Alternaria alternata	13.35 e
Rhizopus spp	15.66 d
Botrytis spp	13.26 e
Fusarium oxysporum	5.45 f

LSD (value) = 1.22

There are 3 groups (C, D and E) in which the means are not significantly different from one another but groups are significantly different from each other.

Table 8 (a): Analysis of Variance for PF-35 fresh harvested seed samples

SOV	DF	SS	MSS	F-values	Probability
Replications	2	0.26	0.13		
Method	1	4.03	4.03	8.95*	0.0210
Fungi	6	13.80	2.30	5.11*	0.0310
Method × Fungi	6	3.13	0.52	1.15^{NS}	0.4810
Error	26	11.73	0.45		
Total	41	32.96			

SOV = Source of variation; DF = Degree of freedom; SS = Sum of square; MSS = Mean sum of square; ** = Highly Significant at $p \leq 0.01$; NS = Non Significant

Table 8 (b): LSD All-Pairwise Comparisons Test of PF-35 for Agar Plate Method

Fungus	Means
Aspergillus flavus	21.61 c
Aspergillus nigar	27.64 a
Pencillium spp	25.65 b
Alternaria alternata	13.34 e
Rhizopus spp	18.67 d
Botrytis spp	10.26 f
Fusarium oxysporum	18.45 d

LSD (value) = 1.10

There are 6 groups (a-f) in which the means are not significantly different from one another but groups are significantly different from each other.

Table 9 (a): Analysis of Variance for Gujrat Ajwain fresh harvested seed samples

SOV	DF	SS	MSS	F-values	Probability
Replications	2	0.52	0.26		
Method	1	4.25	4.25	12.87*	0.0261
Fungi	6	12.61	2.10	6.36*	0.0290
Method × Fungi	6	2.25	0.37	1.12^{NS}	0.4610

Error	26	8.64	0.33		
Total	41	28.29			

SOV = Source of variation; DF = Degree of freedom; SS = Sum of square; MSS = Mean sum of square; ** = Highly Significant at $p \leq 0.01$; NS = Non Significant

Table 9 (b): LSD All-Pairwise Comparisons Test of Gujrat ajwain-1for Agar Plate Method

Fungus	Means
Aspergillus flavus	17.67 c
Aspergillus nigar	21.14 b
Pencillium spp	14.60 e
Alternaria alternata	13.14 e
Rhizopus spp	9.97 f
Botrytis spp	26.16 a
Fusarium oxysporum	16.45 d

LSD (value) = 2.110; Fungus = 5.321; Interaction = 0.8794

There are 6 groups (a-f) in which the means are not significantly different from one another but groups are significantly different from each other.

Comparison of mean value of different fungi isolated from fresh harvested and stored seed sample of UF-32

Table 10 (a): Fungal growth on Blotter Paper

Fungi	Harvested seed sample	Stored seed sample
Aspergilus niger	3.29	5.10
Aspergilus flavus	1.61	2.31
Alternaria alternata	0.61	1.10
Fusarium oxysporum	0.39	0.65
Botrytis spp	0.07	0.34

Table 10 (b): Fungal growth on PDA

Fungi	Harvested seed sample	Stored seed sample
Aspergilus niger	2.10	3.33
Aspergilus flavus	1.62	2.31
Alternaria alternata	1.37	2.33
Fusarium oxysporum	1.10	1.67
Botrytis spp	0.67	1.33

Comparison of mean value of different fungi isolated from harvested and stored seed sample of PF-35

Table 11 (a): Fungal growth on Blotter Paper

Fungi	Harvested seed sample	Stored seed sample
Aspergilus niger	2.33	2.67
Aspergilus flavus	1.00	2.33
Alternaria alternata	1.66	2.33
Fusarium oxysporum	1.00	1.33
Botrytis spp	0.67	0.67

Table 11 (b): Fungal growth on PDA

Fungi	Harvested seed sample	Stored seed sample
Aspergilus niger	1.33	4.00
Aspergilus flavus	2.66	2.33
Alternaria alternata	0.67	0.33
Fusarium oxysporum	1.00	0.67
Botrytis spp	0.67	1.00

Comparison of mean value of different fungi isolated from harvested and stored seed sample of Gujrat Sesame

Table 12 (a): Fungal growth on Blotter Paper

Fungi	Harvested seed sample	Stored seed sample
Aspergilus niger	1.33	3.00
Aspergilus flavus	0.67	2.67
Alternaria alternata	1.33	2.33
Fusarium oxysporum	1.45	1.67
Botrytis spp	1.67	1.00

Table 12 (b): Fungal growth on PDA

Fungi	Harvested seed sample	Stored seed sample
Aspergilus niger	4.33	4.67
Aspergilus flavus	3.67	4.31
Alternaria alternate	1.00	0.33
Fusarium oxysporum	0..67	0.67
Botrytis spp	1.33	0.33

Effect of different fungicides on seed germination

Table 13 (a): Analysis of variance of variance for seed germination

SOV	DF	SS	MSS	F-values	Probability
Replications	2	115.23	57.60		
Fungi	3	190.67	63.55	3.61^{NS}	0.0790
Fungicides	4	6095.26	1523.81	86.77**	0.0000
Fungi × Fungicides	12	280.45	23.37	1.33^{NS}	0.0810
Error	38	667.29	17.56		
Total	59	7348.90			

SOV = Source of variation; DF = Degree of freedom; SS = Sum of square; MSS = Mean sum of square; ** = Highly Significant at $p \leq 0.01$; NS = Non Significant

Table 13 (b): Comparison of mean values of fungicides on seed germination

Fungicides	Fungi				Means
	Aspergillus niger	***Aspergillus flavus***	***Fusarium oxysporum***	***Alternaria alternata***	
Carbendazim	66.61 a	51.65d	21.23 f	27.72 e	**41.80 C**
Mancozeb	55.66 d	50.30 c	51.45 d	50.73 c	**52.04 B**
Raydar	65.32 a	59.60 d	21.98 f	61.51 b	**52.10 B**
Copper oxychloride	64.61 a	62.71 c	23.52 f	56.70 b	**52.88 B**
Control	61.60 b	59.01 b	60.15 b	61.45 b	**60.55 A**
Total	**62.75 A**	**57.657 B**	**35.67 D**	**51.61 C**	

LSD: Fungicides = 1.1.0; Fungi = 2.10; Interaction = 0.90

According to the table, Carbedazim showed the best effect on germination (66.61 %) of fennel seeds. Raydar also show approximately same result as of Mancozeb while the seeds sown without treatment of any fungicides suspension and infested only with fungal isolates showed the lowest germination.

Effect of different fungicides on the recovery of test fungi

For recovery percentage of fungi, dead seedling pieces were platted on PDA for confirmation of fungal infestation and plates were examined under stereoscopic binocular microscope; observations recorded were statistically analyzed (CRD design).

Table 14 (a): Analysis of variance of recovery of test fungi

SOV	DF	SS	MSS	F-values	Probability
Fungi	3	2514.67	838.22	2444.41**	0.0000
Fungicides	4	2095.26	523.87	1092.31**	0.0000
Fungi×Fungicides	12	312.45	26.03	67.50^{NS}	0.9000

Error	40	19.29	0.48		
Total	59	4941.67			

SOV = Source of variation; DF = Degree of freedom; SS = Sum of square; MSS = Mean sum of square; ** = Highly Significant at p ≤ 0.01; NS = Non Significant

Table 14 (b): Comparison of mean values for recovery of test fungi

Fungicides	**Fungi**				**Means**
	Aspergillus niger	***Aspergillus flavus***	***Fusarium oxysporum***	***Alternaria alternate***	
Carbendazim	86.62	81.63	87.23	83.78	**84.83 A**
Mancozeb	74.64	70.34	75.45	71.76	**73.04 B**
Raydar	77.324	79.61	75.98	71.56	**76.63 C**
Copper oxychloride	73.62	68.79	70.52	65.76	**71.68 D**
Control	54.61	54.6	56.65	51.65	**54.90 E**
Total	**73.76 A**	**71.01 B**	**73.13 A**	**69.30 C**	

LSD: Fungicides = 1.22; Fungi = 0.91Figures sharing the same case letters don not differ significantly at p < 0.05

The above mention table showed that all the four used fungicides suspensions have the same result against the tested fungi. Above mention table and graph showed that fungicides treated seeds provided different recovery of fungi and control also recovery of fungi.

Evaluation of plant extracts

For the recovery percentage of fungi, damaged or dead pieces of seedling were plated for the validation of fungal infestation, plates were examined directly under stereoscopic microscope; observation recorded by statistically analyzed (CRD design). Healthy seeds of (UF-32 and PF-35) were surface sterilized with 1 % sodium hypochlorite and then infested with isolates of *Aspergillus niger, A. flavus, Alternaria alternate, Fusarium oxysporum* and *Botrytis* spp.The inoculated seeds were dipped in allopathic aqueous plant extract for 20-30 minutes and controlled seeds in distil water for 20 minutes. Seeds were sown on blotter paper 10 seeds per plate and germination was recorded after 10 days. **Table 15 (a): Analysis of variance of seed germination**

SOV	**DF**	**SS**	**MSS**	**F-values**	**Probability**
Replications	3	145.23	48.41		
Fungi	3	89.67	29.89	1.90*	0.0290
Fungicides	4	5091.26	1272.81	81.17**	0.0000
Fungi × Fungicides	12	240.45	20.03	1.27^{NS}	0.0810
Error	37	627.29	15.68		
Total	59	6193.9			

Table 15 (b): Comparison of mean values of Plant Extracts on seed germination

Plant extracts	**Fungi**				**Means**
	Aspergillus nigar	***Aspergillus flavus***	***Fusarium oxysporum***	***Alternaria alternata***	
Neem	64.65	52.25	65.13	71.18	**63.30 A**
Onion	43.14	72.22	74.15	63.76	**63.32 A**
Ginger	58.12	40.62	51.18	68.16	**54.02 B**
Garlic	38.25	42.18	71.12	47.56	**49.78 C**
Control	54.75	52.05	53.15	53.65	**53.65 B**
Total	**51.78 C**	**51.85 C**	**62.72 A**	**60.81 B**	

LSD values = Plant extracts = 0.90; Fungi = 0.67

Figures sharing the same case letters do not differ significantly at p<0.05

According to the table, Neem showed the best effect on germination (71.18 %) of ajwain seeds. Ginger also show approximately same result as of Garlic while the seeds sown without treatment of any plant extract suspension and infested only with fungal isolates showed the lowest germination (52.05 %).

Effects of different plant extract on the recovery of test fungi

Table 16 (a): Analysis of variance of recovery of test fungi

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SOV	DF	SS	MSS	F-values	Probability
Replications	3	70.28	23.42		
Fungi	3	79.67	26.55	2.30^{NS}	0.0990
Fungicides	4	6291.76	1572.94	136.30**	0.0000
Fungi × Fungicides	12	245.15	20.42	1.76^{NS}	0.0610
Error	37	427.19	11.54		
Total	59	7113.05			

SOV = Source of variation; DF = Degree of freedom; SS = Sum of square; MSS = Mean sum of square; ** = Highly Significant at $p \leq 0.01$; NS = Non Significant

Table 16 (b): Comparison of mean values of plant extracts for recovery of test fungi

Plant extracts	Fungi				Means
	Aspergillus nigar	***Aspergillus flavus***	***Fusarium oxysporum***	***Alternaria alternate***	
Neem	44.62	41.95	45.13	63.18	**48.72 C**
Onion	58.12	42.82	48.10	46.71	**48.94 C**
Ginger	56.42	34.12	43.18	55.06	**47.19 D**
Garlic	66.21	62.10	56.12	67.06	**62.87 B**
Control	64.05	69.00	52.00	73.00	**64.51 A**
Total	**58.88**	**51.00**	**49.90**	**62.00**	

LSD = Plant extracts = 0.54, Figures sharing the same case letters do not differ significantly at $p<0.05$

Plants extracts have significant role in inhibition of seed-borne pathogens such as *Alternaria alternate* and development of seed quality and emergence of seed embryo (Nwachukwe and Umechuruba, 2001). This study was carried out to check the allelopathic effect of various weed extract on seed germination of 3 crop species revealed that most of the weed (*Allium sativum* L; *Azadirachta indica* A.Juss, *Eucalyptus globes* labil and *Allium cepa* L.) tested had inhibitory effect on seeds germination of common fennel at different application rates (25%, 20%, 22% and 30% respectively) as compared with 10 % acetone control. At the end of 21 days of incubation seed germination rate were 88%, 89%, 92% and 89% respectively as compared to control.

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