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Seed-borne fungi of sesame (*Sesamum indicum* L.) seeds in Davanagere district and their effect on germination

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ABSTRACT

An investigate to detect the seed-borne fungi of sesame and their effect on germination. Thirty four fungal pathogens against sesame were conducted in the department of Applied Botany, Plant pathology laboratory, Kuvempu University Shivamogga during 2008. A total of twenty eight seed samples were collected from five taluks of Davanagere district. The seed samples were also obtained from fields, farmers, retail shops and APMC markets. Blotter method, potato dextrose agar method, water agar and 2,4-D methods were used for detection of seed-borne fungi of sesame seeds. Altogether thirty four fungi belonging to three genera namely *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 sp., 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 sp., Penicillium citratum, Rhizopus nigricans, R, stolonifer* and Mycella sterilliae were isolated from local variety of sesame seeds. Among all the seed health test methods standard blotter method is most superior for detection of seed-borne fungi over the other methods. All the collected seed samples fields and farmers samples shows higher incidence of pathogenic fungi than others. The effect of seed-borne fungi on germination capacity was evaluated by sand method and rolled paper towel method. The associated fungi decreased the germination potential. All the pathogenic and storage fungi were isolated from abnormal seedlings, un germinated seedlings and rotted seeds.

KEYWORDS

Sesame seed;
Seed health test;
Germination;
Survey;
A. alternate.

INTRODUCTION

Sesame (*Sesamum indicum* L.) is one of the major kharif season oilseed crop in India. Sesame is regarded as "*queen of oilseed*" by users become of the quality (Fatty acid composition) of its oil. It belongs to

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the family Pedaliaceae[7]. It is an important source of oil and protein. The quality and quantity of oil and protein is adversely affected by biological agents, which consequently influence the product manufactured by sesame seed and its derivatives. The total area under the oilseed crops is about 17 million ha, which accounts for approximately 11 percent of the total cultivated area of the Indian subcontinent. The total annual production of oilseeds is about 11 million tones, accounting for about 10 percent of Indian agricultural economy[19]. In Davanagere, the crop is cultivated over an area 1574 with a production of 1805 tones with 1207 productivity[3]. In the health of sesame plant affected by number of fungal diseases. The diseases are leaf spot, blight-*A. alternata, A. sesamicola*, wilt- *F. moniliforme* & *V. dahliae,* cercospora leaf spot-*Cercospora sesami*, root rot-*M. phaseolina*, Phyllody-*Mycoplasma like organism* (MLO), damping off-*Rhizoctonia solani*. These diseases are reduced seed yield, vigour, germination and oil quality[25]. Seed-borne fungi are carried over by infected seeds. They cause deterioration in soil in before germination causing seedling mortality and infection of foliage at adult stage fungi including *Alternaria, Fusarium, Curvularia, Cladosporium* sp., *Cercospora, Verticillium, Sclerotinia, Macrophomina, Aspergillus* and *Rhizopus* sp., have been found associated with sesame. *A. alternata* and *A. sesamicola* is the most destructive pathogen of sesame, as it cause leaf spot and blight. Infection of seeds reduces viability of seeds. *F. moniliforme, V. dahliae* and *M. phaseolina* reduced the seed germination by causing seed rot, wilts, blights and root rots. Seed-borne diseases caused by fungi are relatively difficult to control as the fungal hyphae gets established and become dormant. Information on seed-borne fungi of sesame in Karnataka is lacking and need to be addressed. Therefore, present study was to investigate the incidence of seed associated fungi, their frequency of association and their effect on germination.

MATERIALS AND METHODS

Collection of sesame seed sample

Seeds of sesame were collected from five taluks of Davanagere district during kharif, 2008. Total twenty eighty seed samples were collected from fields, farmers, retail shops and APMC markets of Davanagere, Harihara, Harapanahally, Jagalur and channagiri. (TABLE 1). The collected seeds were stored in cloth bags. The seeds were brought to the plant pathology laboratory of Applied Botany, Kuvempu University and stored at room temperature for subsequent studies.

TABLE 1: Seed samples collected in Davanagere district during kharif-2008

Place of collection	Source of collection				
	Fields	Farmers	Retail shops	APMC markets	Total samples
Davangere	1	-	-	4	5
Harihara	2-	-	-	-	2
Harapanahally	4	-	5	-	9
Jagalur	3	1	3	2	9
Channagiri	-	-	2	1	9
Total	10	1	10	7	28

Detection of seed-borne fungi by seed health tests methods

SBM method

Seed samples were analyzed for the detection of seed-borne fungi by blotter method following ISTA, 1993 with some modifications[6]. In this method three layers of blotter paper were soaked in sterilized and placed at the bottom of the Petri plates. Hundred seeds were sterilized with 0.2% sodium hypo chloride solution for 2 to 3 minuets and seeds taken randomly from each sample and were placed in five Petri plates (20 seeds per plate). The Petri plates with seeds were than incubated at room temperature for seven days in the laboratory. The plates were alternating cycles of 12 hrs light and 12 hrs darkness for seven days. After incubation every fourth day the distilled water is added for the blotter is sufficiently moist. Germination and fungi associated with the seeds were recorded during the incubation period. Each of the incubated seeds was examined under stereo binocular microscope to ascertain the presence of fungi. Some times were not apparent even after seven days of the incubation. In such condition, the Petri plates were allowed for further incubation. A temporary slide was prepared from each colony, which could not be identified stereo binocular microscope. Fungi were identified by preparing temporary slides and examined under labomed vision 2000 compound microscope. In fewer cases the fungi from the incubated seeds were transferred to

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TABLE 2 : Seed mycoflora of sesame in different seed health test methods during kharif -2008

Place of collection	Methods	Germ %	Seed mycoflora (%)														
			A.al	A.se	F.mo	C.se	C.gl	V.da	S.sc	M.ph	A.fl	A.ni	A.oc	R.st	Mean	S D	S E
Shivapura	SBM	28.0	41.0	23.0	14.0	18.0	6.0	2.0	10.0	13.0	9.0	4.0	3.0	13.0	13	4.47	1.29
	PDA	23.0	35.0	17.0	10.0	11.0	0	0	0	5.0	3.0	0	0	0	6.75	0.50	0
	WA	27.0	36.0	13.0	0	12.0	2..0	0	0	0	0	0	0	0	2.5	10.79	3.11
	2,4-D	0	16.0	4.0	0	0	0	0	0	2.0	3.0	4.0	1.0	5.0	2.91	4.52	1.30
Agasana katti	SBM	19.0	53.0	18.0	6.0	21.0	17.0	10.0	11.0	19.0	13.0	19.0	9.0	17.0	17.75	12.06	3.48
	PDA	13.0	45.0	13.0	2.0	3.0	11.0	0	0	3.	8.0	11.0	0	0	8	12.62	3.48
	WA	21.0	39.0	10.0	0	2.0	0	0	0	0	0	0	3.0	10.0	5.33	11.25	2.24
	2,4-D	0	23.0	5.0	2.0	14.0	12.0	0	0	0	3.0	2.0	8.0	13.0	6.83	2.22	0.54
Hulikatti	SBM	22.0	48.0	29.0	18.0	10.0	13.0	8.0	5.0	3.0	10.0	4.0	11.0	16.0	14.58	12.70	3.66
	PDA	15.0	36.0	19.0	9.0	13.0	16.0	0	0	5.0	6.0	2.0	0	0	8.83	4.87	1.40
	WA	21.0	37.0	18.0	4.0	0	0	0	0	0	2.0	0	0	0	5.08	11.29	3.25
	2,4-D	0	13.0	10.0	0	0	11.0	0	3.0	0	3.0	4.0	1.0	0	3.75	4.82	1.39
Thumbigere	SBM	8.0	58.0	32.0	31.0	21.0	4.0	3.0	6.0	7.0	4.0	3.0	9.0	10.0	15.66	16.34	4.71
	PDA	12.0	49.0	23.0	16.0	17.0	3.0	0	3.0	0	0	0	0	0	9.25	14.97	4.32
	WA	13.0	50.0	19.0	2.0	0	0	0	0	1.0	6.0	8.0	4.0	5.0	7.91	14.78	4.26
	2,4-D	0	51.0	20.0	8.0	0	12.0	0	0	0	6.0	3.0	2.0	7.0	9.08	14.51	4.18
Duthidurga	SBM	12.0	37.0	13.0	11.0	14.0	5.0	14.0	3.0	0	0	0	0	12.0	9.08	10.61	3.06
	PDA	11.0	28.0	5.0	14.0	0	0	0	3.0	0	0	12.0	5.0	12.0	6.58	8.56	2.46
	WA	19.0	19.0	5.0	9.0	10.0	0	0	0	0	6.0	3.0	5.0	3.0	5	5.60	1.61
	2,4-D	0	12.0	0	0	0	0	0	0	10.0	8.0	5.0	3.0	10.0	4	4.76	1.37
Siddanuru	SBM	18.0	10.0	11.0	0	0	6.0	3.0	4.0	0	0	1.0	3.0	5.0	3.58	3.84	1.11
	PDA	13.0	9.0	3.0	3.0	2.0	0	0	0	2.0	5.0	2.0	3.0	10.0	3.25	5.35	1.54
	WA	21.0	6.0	5.0	0	0	1.0	3.0	5.0	5.0	3.0	5.0	10.0	10.0	4.41	3.31	0.95
	2,4-D	0	9.0	5.0	8.0	7.0	0	0	0	0	0	0	0	12.0	3.41	4.35	1.25
Bilichodu	SBM	45.0	13.0	9.0	3.0	0	0	0	0	9.0	4.0	0	0	8.0	3.83	4.70	1.35
	PDA	41.0	9.0	5.0	3.0	0	0	0	0	0	2.0	3.0	4.0	14.0	3.33	4.29	1.23
	WA	51.0	6.0	3.0	6.0	2.0	0	2.0	5.0	9.0	3.0	2.0	4.0	10.0	4.33	2.71	0.78
	2,4-D	0	15.0	2.0	3.0	0	0	0	0	6.0	2.0	5.0	6.0	11.0	4.16	1.05	0.74
Syagalahatti	SBM	62.0	11.0	8.0	2.0	0	3.0	0	0	0	3.0	3.0	0	5.0	2.91	3.54	1.02
	PDA	60.0	8.0	6.0	0	0	2.0	5.0	6.0	0	0	0	2.0	6.0	2.91	3.04	0.87
	WA	65.0	6.0	0	0	0	0	0	2.0	0	5.0	2.0	5.0	3.0	1.91	2.31	0.66
	2,4-D	0	10.0	9.0	0	0	3.0	1.0	2.0	4.0	6.0	2.0	3.0	11.0	4.25	3.87	1.11
Yellapura	SBM	63.0	12.0	4.0	6.0	0	0	10.0	12.0	6.0	5.0	0	0	6.0	5.08	4.54	1.31
	PDA	51.0	13.0	0	3.0	12.0	10.0	0	3.0	2.0	6.0	8.0	9.0	14.0	6.66	3.02	0.87
	WA	63.0	5.0	3.0	2.0	0	0	0	2.0	3.0	2.0	5.0	4.0	14.0	3.33	3.79	1.09
	2,4-D	0	3.0	2.0	4.0	8.0	9.0	0	0	0	0	5.0	3.0	4.0	3.16	2.83	0.82
Jammapura	SBM	71.0	9.0	12.0	1.0	6.0	5.0	0	0	3.0	4.0	16.0	16.0	14.0	7.16	4.78	1.38
	PDA	74.0	10.0	6.0	0	0	0	0	0	6.0	3.0	5.0	5.0	4.0	3.25	3.30	0.95
	WA	69.0	8.0	3.0	2.0	4.0	5.0	3.0	4.0	3.0	2.0	5.0	2.0	3.0	3.66	1.72	0.49
	2,4-D	0	3.0	0	0	0	0	2.0	3.0	2.0	2.0	5.0	2.0	12.0	2.58	4.29	1.23

Data based on 100 seeds for each sample, (each sample for four replications). A.al-*Alternaria alternata,* F.mo-*Fusarium moniliforme,* A.se-*Alternaria sesamicola,* V.da-*Verticillium dahliae,* C.se-*Cercospora sesami,* M.ph- *Macrophomina phaseolina,* S.sc- *Scerotinia sclerotiorum,* C.gl- *Chaetomium globosum,* A.ni-*Aspergillus niger,* A.fl-*A. flavus,* A.oc-*A. ochraceus,* R.st-*Rhizopus stolonifer*. SBM-standared blotter method, PDA-potato dextrose agar method,WA- water agar method, 2,4-D- 2,4-dichlorophenoxy acetic acid method.

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TABLE 3 : Effect of seed borne fungi on germination by Sand method

Place of collection	Germ (%)	Normal seedlings	Abnormal seedlings	Un germinated seedlings	Rotted seedlings	Fungal pathogens
Shivapura	92.0	62.0	30.0	5.0	3.0	*A. alternata, A. sesamicola, C. sesami, C. globosum, V. dahliae, M. phaseolina, C. cladosporioides, A. niger,*
Agasanakatti	88.0	50.0	38.0	10.0	2.0	*A. alternata, F. moniliforme, C. sesami, A. flavus, R. Stolonifer, A. sesamicola, M. phaseolina, A. ochraceus.*
Thumbigere	89.0	40.0	41.0	19.0	0	*A. alternata, F. moniliforme, C. sesami, A. flavus, R. Stolonifer, A. sesamicola, M. phaseolina, A. ochraceus.*
Duthidurga	91.0	53.0	37.0	8.0	2.0	*A. alternata, A. sesamicola, C. sesami, C. globosum, V. dahliae, M. phaseolina, C. cladosporioides, A. niger,*
Hulikatti	90.0	49.0	33.0	3.0	3.0	*A. alternata, F. moniliforme, C. sesami, A. flavus, R. Stolonifer, A. sesamicola, M. phaseolina, A. ochraceus.*
Mean	90	50.8	35.8	9	2	
SD	1.584	7.918	4.324	6.204	1.224	
SE	0.547	2.179	1.248	1.790	0.353	

PDA medium in Petri plates aseptically and incubated under controlled temperature (28±1°C) for 3 to 10 days and than examined under labomed vision 2000 compound microscope.

PDA method

For potato dextrose agar method, 100 seeds were sterilized with 0.2% sodium hypo chloride solution for 2 to 3 minuets. Seeds were plated on sterile glass Petri plates containing PDA medium[13]. 25 seeds per Petri plates and than the plates were incubated at 40°C in alternating cycles of 12 hrs light and 12 hrs darkness for seven days. After incubation eighth days the seeds were examined by stereo binocular microscope.

Water agar method

For agar plate method, 100 seeds were sterilized with 0.2% sodium hypo chloride solution for 2 to 3 minuets. Seeds were plated on sterile glass Petri plates containing (2.5%., i.e 12.5 gms in 1000ml of distilled water) water agar medium. These Petri plates were incubated 25±2°C for seven days. After seven days these seeds were examined under stereo binocular microscope[16].

2, 4-D method

In this method, 100 seeds were sterilized with 0.2% sodium hypo chloride solution for 2 to 3 minuets. The three layers of blotter paper discs were dipped in 0.2% of 2, 4-Dichloro Phenoxy acetic acid solution[11]. Twenty five seeds were placed equidistantly on moist blotter discs using sterilized forceps in laminar air flow wood under aseptic condition. The plates were incubated at 25±2°C at room temperature for seven days. Observation was done in seventh day and then seeds were examined under stereo binocular microscope.

Effect of seed borne fungi on germination

Sand method

The required quantity of sand sterilized for pressure cocker for 20 min. The sterilized soils are transferred in to plastic trays up to 5 cm square. Take 100 seeds randomly for each sample are pressed into surface of the sand. The amount of water is added to sand, just moistened to 50 percent water holding capacity, pH 6.0-7.5 for seven days. At the end of incubation the number of germinated, un germinated, normal, abnormal and rotted seedlings were recorded (TABLE 3).

TABLE 4 : Effect of seed borne fungi on germination by rolled paper towel method

Place of collection	Germ (%)	Normal seedlings	Abnormal seedlings	Un germinated seedlings	Rotted seedlings	Fungal pathogens
Shivapura	65.0	33.0	47.0	13.0	7.0	*A. alternata, F. moniliforme, C. sesami, A. flavus, R. Stolonifer, A. sesamicola, M. phaseolina, A. ochraceus.*
Agasanakatti	62.0	28.0	52.0	10.0	10.0	*A. alternata, A. sesamicola, C. sesami, C. globosum, V. dahliae, M. phaseolina, C. cladosporioides, A. niger,*
Thumbigere	69.0	37.0	43.0	8.0	12.0	*F. moniliforme, C. sesami, A. flavus, R. Stolonifer, A. sesamicola, M. phaseolina, A. ochraceus.*
Duthidurga	71.0	26.0	54.0	9.0	11.0	*F. moniliforme, C. sesami, A. flavus, R. Stolonifer, A. sesamicola, M. phaseolina, A. ochraceus.*
Hulikatti	74.0	29.0	51.0	3.0	17.0	*A. alternata, F. moniliforme, C. sesami, A. flavus, R. Stolonifer, A. sesamicola, M. phaseolina, A. ochraceus.*
Mean	68.2	30.6	49.4	8.6	11.4	
SD	4.764	4.393	4.393	3.646	3.646	
SE	1.375	1.268	1.268	1.052	1.053	

Rolled paper towel method

100 un treated seeds of each sample were randomly selected and allow to germinate between two layers of blotter paper[15]. Which may placed in a flat or upright position at 25±2°C for room temperature for seven days. At the end of incubation the number of germinated, un germinated, normal, abnormal and rotted seedlings were recorded (TABLE 4).

Screening of seeds for associated mycoflora

The incubated seeds were screened on eighth day using stereo binocular microscope and labomed vision 2000 compound microscope. The germination, associated fungi were recorded and identified with the help of standard guides and manuals like[8-10,13,20-23].

RESULT AND DISCUSION

The analysis of seed-borne fungi of sesame showed thirty four fungal species belonging to five genera namely *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 sp., Gliocladium roseum, Neurospora glabra, Cunigamella elegans, Chaetomium globosum, Stachybotrys chartarum, S. atra, Pestaltia macrotricha, Aspergillus niger, A. flavus, A. ochraceus, A. versicolor, A. terreus, A. candidus, Haplosporangium sp., Penicillium citratum, Rhizopus nigricans, R, stolonifer* and Mycella sterilliae. Twelve fungi are predominant in sesame seeds (TABLE 2). Among the samples screened and Jagalur taluk shows a higher incidence of pathogenic and storage fungi. Among the isolated fungi *A. alternata, F. moniliforme, A. sesamicola, V. dahliae, C. sesami, M. phaseolina, S. sclerotiorum, C.globosum, A. niger, A. flavus, A. ochraceus* and *R.stolonifer* ranged from 09-58%, 01-31%, 04-32%, 03-14%, 06-21%, 03-13%, 03-11%, 03-19%, 04-13% and 05-17% respectively. These fungi were isolated from local variety of sesame seeds. Among the seed health test methods standard blotter method found to be suitable for detection of seed-borne fungi. These samples screened farmers and

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field samples showed higher incidence of pathogenic and storage fungi. Harapanahally and Jagalur taluk seed samples showed higher incidence of fungi then lower in Channagiri taluk of Davanagere district. Seed-borne mycoflora plays an important role in determining the quality and longevity of seeds. Microbial invasion can lead to the rotting, loss of seed viability, vigour, germination and oil quality.

These fungi were reported to seed-borne in sesame by a number of other workers. Epidomology of sesamum phyllody disease caused by Mycoplasma like organisms (MLO)[21]. Rhizoctonia dumping off in sesame caused by *Rhizoctonia solani* [1]. Seed mycoflora of sesame and their role in plant health[2,18]. Seed born infection of *A. alternata* and its role in disease development in sesame[5]. Seed born fungi of sesame in Uganda. *A. sesami, C. sesami* are most destructive pathogen in sesame it causes to seed rot, seedling mortality and poor emergence of seedlings[14]. Seed borne fungi of sesame and their significance[12]. The seeds isolates of *M. phaseoseolina, Corynespora cassicola* and *A.sesami* were pathogenic and resulted in seed rot,pre and post emergence losses, stem rot and leaf spots, Alternaria leaf spot was monitored in plots planted with *A. sesami* infected seed at six different infection levels[17]. *Alternaria* sp., *Helminthosporium* sp., *A. alternata* and *Corynespora cassicola* which cause blights and leaf spots are of economic importance[24].

All the associated fungi decreased the germination potential[15]. The effect of associated fungi on germination capacity was evaluated by the sand method and rolled paper towel method (TABLE 3 & 4). In sand method, Shivapura sample showed a 62.0% normal, 30.0% abnormal, 5.0% un germinated and 3.0% of rotted seedlings. Agasanakatti sample 50.0% normal, 38.0% abnormal, 10.0% un germinated and 2.0% of rotted seedlings. Thumbigere sample 40.0% normal, 41.0% abnormal, 19.0% un germinated seedlings. Duthidurga sample 53.0% normal, 37.0% abnormal, 8.0% un germinated and 2.0% of rotted seedlings. While Hulikatti sample 49.0% normal, 33.0% abnormal, 3.0% un germinated and 3.0% rotted seedlings including the fungus *A. alternata, F. moniliforme, A. sesamicola, V. dahliae, C. sesami, M. phaseolina, S. sclerotiorum, C. globosum, A. niger, A. flavus, A. ochraceus* and *R. stolonifer* respectively. In rolled paper towel method, Shivapura sample showed a 33.0% normal, 47.0% abnormal, 13.0% un germinated and 7.0% of rotted seedlings. Agasanakatti sample 28.0% normal, 52.0% abnormal, 10.0% un germinated and 10.0% of rotted seedlings. Thumbigere sample 37.0% normal, 43.0% abnormal, 8.0% un germinated and 12.0% rotted seedlings. Duthidurga sample 26.0% normal, 54.0% abnormal, 9.0% ungerminated and 11.0% of rotted seedlings. While Hulikatti sample 29.0% normal, 51.0% abnormal, 3.0% un germinated and 17.0% rotted seedlings including the fungus *A. alternata, F. moniliforme, A. sesamicola, V. dahliae, C. sesami, M. phaseolina, S. sclerotiorum, C. globosum, A. niger, A. flavus, A. ochraceus* and *R. stolonifer* respectively. Thirty four fungi were isolated from abnormal seedling, un germinated and rotted seedlings. Heavily infected seeds did not germinate. Among the germination tests, standard rolled paper towel method is superior for germination. Seeds should be treated with suitable chemicals before sowing to reduce the fungal infection. *A. alternata, F. moniliforme, A. sesamicola, V. dahliae, C. sesami, M. phaseolina, S. sclerotiorum, C. cladosporioides, C. globosum, A. niger, A. flavus, A. ochraceus* and *R. stolonifer* are significantly reduced the seed germination. Seeds of many oilseed crops are known to harbour to large number of mycoflora. The seed-borne fungi associated with sesame seeds are known to adversely affect the seed germination, vigour, quality and oil quality. Fungus isolated from sesame seeds were similar to fungus isolated from abnormal seedlings. It suggests that seeds are major agent of fungal transmission, Seeds should be treated with suitable chemical before sowing to reduce the fungal infection.

CONCLUSION

The results reveled that sesame (*Sesamum indicum* L.) is one of the major kharif season oilseed crop in India. Sesame is regarded as "*queen of oilseed*" by users become of the quality (Fatty acid composition) of its oil. The quality and quantity of oil and protein is adversely affected by biological agents. The associated fungi decreased the seed germination, viability, vigour and oil quality. It suggests that seeds are major agent of fungal transmission, Seeds should be treated with suitable chemical before sowing to reduce the fungal infection.

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