

SEED BORNE FUNGI OF SUNFLOWER

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ABSTRACT:

The deterioration in sunflower seed due to fungi is of the great importance in the present study nine seeds were isolated from abnormal sunflower seed. Which is collected from a different location the different variety like, SS- 56, surya, KBSH-1, LSH-1, LSH-3, MSFH-1, AP5H-1, BSH-1, MSFH -17 were studied. These seeds were associated with 45 species of fungi belonging to 19 general the Broadest species spectrum on most cultivars consist of genera *Aspergillus*, *Alternaria*, and *Fusarium* followed by *penicillium*, *chaetomium*, *Trichoderma*, and *ulocladium*, *Aspergillus niger*, *A flavus*, *Chaetomium globosum*. *Alternaria alternate*, *A Funmigatus*, *Ch. Atrobrunneum*, *A terruss*, *penicillium expansum*, *P. brevicompactum*, *Fusarium oxysporum*. *F. solani*, *Rhizopus Stolonifer*, *Mucor hiemalis* and *A. Ochraceus* were the most frequent species. The species composition, percentage of seed infection and seed germination percentage differed among cultivars 55-56 show the lowest number of detected species where as the highest number was isolated from surya species and the highest fungal infestation was recorded in unidentified local cultivar 3 maximum seed germination occur in MSFH-1. The seed borne pathogenic species *Macrophomina phaseolina* was detected.

KEYWORDS: Deterioration, Cultivar, Species Spectrum.

INTRODUCTION

Sunflower (*Helianthus annuus L.*) Which is considered as commercial oil crop. This crop is cultivated widely overall the world. Sunflower is particularly used for production of edible oil as well as for seed consumption. Sunflower is an important member of family Asteraceae. The sunflower seed used for cultivation of the crop are mostly hybrid also these seeds used for cultivated on the crop mostly imported cultivars or inbreds. While during the last some decades attempts were made at several scientific research foundation to produce sunflower genotype. Sunflower seed contains over more than 40% of good edible oil and 23% proteins it also constituent an excellent source of unsaturated fats crude protein, fiber, and important nutrient like, vitamin E, selenium, copper, Zinc and B – complex vitamins. sunflower is one of most important producers of oil among different oil crop all over the world. Sunflower is affected by a large number of diseases caused by many fungi and other phytopathogenic microorganisms. Several pathogenic and saprotrophic fungal species have been reported on sunflower seed. The most important seed borne pathogens represented the genera *Alternaria* (*A. Alternata*, *Abelianthi*) *Fusarium* (*F. Chlomydosporum*, *F. Solani*, *F. sporotrichioides*, *F. Subglutin* and *F. Verticillioides*) *Macrophomina* (*M. Phaseolina*), and *verticillium* (*V alboatum* and *V dahlia*) most of the sunflower fungal species are reported to be seed borne thirteen isolated phytopathogenic fungal species were isolated from different stored

sunflower varieties externally seed borne mycoflora and saprophytes but a few were parasites. The internal seed mycoflora is composed of parasites as well as saprophytic organism during the storage condition sunflower seeds are exposed to various infections by microorganisms like fungi which may lead to various damage including reducing yield of seed in both quantitatively and qualitatively beside these decreases in germination percentage mycotoxin production and total decay has been observed. The economic value of sunflower seed is greatly influenced by associated saprotrophic fungi. Which may reduce oil quality due to increase of free fatty acid amount in the seeds during storage. The studies on the mycobiota associated with sunflower seeds and their significance have been made by researchers in different part of the world. The present study was carried out to survey fungi associated with seeds of sunflower with the help of different method.

MATERIALS AND METHOD:

Sample collection: Nine sunflower (*Helianthes annuus L.*) seed samples were collected from local market of different locations obtained samples brought to the laboratory and kept at room temperature.

Isolation of Fungi: The seeds which are kept at room temperature were surface disinfected with 1% of sodium hypochloride in a beaker for 10 min and then rinse three times in sterile distilled water after that the surface disinfected seeds were placed on water soaked blotters in sterilized aluminium trays for this one hundred seeds were placed in each tray the tray were autoclave cellophane sheets and the seeds in the tray were incubated for 7 – 10 days at 25⁰ C.

That seeds were examined individually under a dissecting microscope the frequency of occurrence for each fungus species was calculated by applying the formula.

$$\text{p.f. \%} = \frac{\text{number of seeds on which fungus appeared}}{\text{Total Number of seeds}} \times 100$$

1) STANDARD BLOTTER METHOD:

Non – sterilized and surface sterilized seeds were plated in 9 cm diameter sterile petri dishes containing three layers of sterile blotter moistend with sterilized tap water ten seeds were placed in each petri dish and incubated at 20 ± 2⁰ C. for seven days. Under cool white fluorescent light with alternating cycles of 12 hrs. light and 12 hrs. dark.

2) DEEP FREEZING BLOTTER METHOD:

This method is used to detect a wide range of fungi which are able to arise easily from seeds in presence of humidity after plating in stand are blotter method the dishes were incubated at 20 ± 2⁰ C. for 24 hrs. and transfeced to -20⁰C. freezer for 24 hrs. this was done for 5 days incubation at 20 ± 2⁰C under cool white fluroscent light with alternating cycles of 12 hrs. light and 12 hrs. darkness the single spore isolation technique were followed to obtain pure culture. The fungi were identified by recording their cultural properties for the seeds of Nine cultivars total 45 fungal species were isolated by using standard blotter and deep freezing blotter method. The common genera was *Aspergillus*, *Alternaria* and *Fusarium* and *Denicillium*, *chae omium*, *Trihchoderma* and *ulocaladium* were represented by three

species each and the other genera were represented by single species.

3) PATHOGENICITY TEST:

The fungi isolated are most common in our survey as well as worldwide known pathogenic fungi on sunflower were selected for pathogenicity test flask containing 50 ml of potato dextrose broth were inoculated which disc taken from the growing edge of 5 day old colony of each fungus. The flask were incubated in dark for 10 days at $25 \pm 2^{\circ}$ C. fifty grams of each mycelia mat were harvested and blended in 500 ml. of sterile distilled water to produce fungal suspensions.

Pathogenicity of selected fungi isolated from sunflower seed.

Fungus	Pathogenicity (%)		
	Seed rot	Infected seeding	Wealthy seed
Control	2.40 ^{d2}	0.00 ^d	96.56 ^a
M. Phaseding	45.82 ^a	23.82 ^a	28.33 ^e
F. Solani	39.00 ^b	18.92 ^b	41.21 ^d
F. oxysporum	37.12 ^{bc}	15.28 ^c	46.32 ^c
F. incarnatum	31.05 ^c	12.10 ^c	58.00 ^b

RESULT AND DISCUSSION

The most common species were *Alternaria alternate*, *Aspergillus flavus*, *A. fumigates*, *A. niger*, *A. Ochraceus*, *A. terrus*. *Chaetomium atrobrunneum*, *ch. Globosum*, *Fusarium oxysporum*, *F. solani* *Mucor hiemalis*, *Penicillium brevicompactum*, *P. expansum* *Rhizopus stoloniyer* and *Ulocladium chartarum* which were isolated from almost every cultivar *Aspergillus flavus*, *A. fumigatus* and *A. niger* show the highest incidence on nine

cultivars with the frequency of occurrence 1.4-11.4, 0.6 – 7.8 and 2.1 – 11.9% respectively high frequency of species such as *Alternaria alternate* (1.3 – 7.1%) *Chaetomium atrobrunneum* (0.5 – 6.9%) *ch. Globosum* (0.8 – 8.9%) and *Fusarium oxysporum* (0.3 – 4.1%) was also recorded.

Among the nine species of *Aspergillus* reported, *A. flavus*, *A. niger* and *A. Fumigatus* show the highest incidence. A high incidence of *A. flavus* was reported on sunflower seed from India. It is also investigated that *A. Niger* is the dominant fungi associated with sunflower seed grown in Rajasthan region of India. *Aspergillus* spp. were followed by *Alternaria* and *Fusarium* represented by six species each *Alternaria alternate* was the most frequent among those of the former and covered from almost all samples. While *A. chlamydospora* and *A. longipes* were detected seven cultivars however *A. helianthi* and *A. helianthi* and *A. Tenuissima* were detected in six and five cultivars respectively. Six *Fusarium* species were identified among those *F. solani* were found common in seeds of all cultivars whereas *F. Culmorum*, *F. semitectum* and *F. Verticillioides* were common in seven cultivars. All the reported *Fusarium* species are known to be pathogenic to sunflower causing various symptoms.

Four species of *Penicillium* were detected viz. *P. brevicompactum*, *P. Chrysogenum*, *P. Expansum* and *P. oxalicum*. *Penicillium brevicompactum* and *P. expansum* were common in all cultivars, whereas *P. chrysogenum* and *P. oxalicum* were detected in four detected in four and six cultivars respectively. *Penicillium* species are commonly detected on sunflower seeds. Three species of *Chaetomium* were identified viz, *Ch.*

Atrobrunneum, Ch. Globosum and Ch. Elatum their frequencies were much higher in the three unidentified local cultivars as compared to identified cultivars. *Curvularia lunata* was detected in six cultivars, *Bipolaris hawaiiensis* and *B. spicifera* were detected only in unidentified local cultivars. The degree of seed infestation ranged from 10 to 43% and share of germinated seed range from 36 to 100%. The three unidentified

sunflower cultivars which have been collected from local market displayed the highest fungal infestation and the smallest share of germinated seed ranges from 35 to 62% fungal species composition and percentage of fungal infestation varied among sunflower cultivars.

Percentage Frequency of occurrence of fungi on sunflower seed

Fungal Species	Percentage of infected seeds of the particular cultivars								
	Surya	55-56	KBSH-1	MSFH-17	APSH-1	LSH	Unidentified Cultivars		
<i>Alternaria alternate</i>	1.1	2.8	2.2	2.4	2.4	3.6	5.0	6.3	7.4
<i>Alternaria chlamydospora</i>	0.0	0.4	0.4	0.3	0.2	0.5	0.8	1.4	1.8
<i>Alternaria helianthi</i>	0.1	0.3	0.1	0.0	0.3	0.4	0.8	1.6	1.4
<i>Alternaria longipes</i>	0.0	0.6	0.4	0.2	0.4	0.7	1.6	1.3	2.6
<i>Alternaria raphani</i>	0.0	0.1	0.0	0.1	0.0	0.1	0.7	1.0	0.3
<i>Alternaria tenuissima</i>	0.2	0.2	0.2	0.0	0.0	0.4	0.7	0.3	0.5
<i>Aspergillus conchidus</i>	0.0	0.1	0.0	0.2	0.1	0.0	0.0	0.5	0.6
<i>Aspergillus flavus</i>	1.2	3.8	3.2	2.2	3.6	4.3	4.5	6.3	8.4
<i>Aspergillus fumigatus</i>	0.8	2.1	2.1	1.8	2.4	2.8	8.2	10.5	9.6
<i>Aspergillus niger</i>	2.0	4.2	3.6	3.4	4.3	5.1	0.6	0.7	0.4
<i>Aspergillus niveus</i>	0.0	0.0	0.0	0.0	0.0	0.3	1.7	3.1	1.4
<i>Aspergillus ochraceus</i>	0.1	0.7	0.6	0.4	0.6	1.0	0.4	0.5	0.3
<i>Aspergillus parasiticus</i>	0.0	0.0	0.0	0.2	0.1	0.0	2.6	3.4	2.3
<i>Bipolaris spicifera</i>	0.0	1.6	1.5	1.1	1.3	2.1	0.2	0.4	0.5
<i>Bipolaris hawaiiensis</i>	0.0	0.0	0.0	0.2	0.0	0.3	0.4	0.6	1.1
<i>Chaetomium atrobrunneum</i>	0.4	0.0	0.0	0.0	0.1	0.0	0.3	0.4	0.2
<i>Chaetomium globosum</i>	0.8	0.1	0.1	1.3	0.0	0.1	5.3	6.1	9.2
<i>Cladosporium herbarum</i>	0.0	0.3	1.2	0.6	1.5	2.3	0.4	0.6	0.3
<i>Curvularia lunata</i>	0.1	0.2	0.2	1.8	0.6	0.7	0.7	1.3	1.5
<i>Doratomyces microspores</i>	0.1	0.0	2.4	0.4	2.2	3.4	1.1	1.0	0.8
<i>Emericella quacrilineata</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.5	0.4
<i>Fusarium</i>	0.1	0.2	0.1	0.4	0.4	0.6	1.3	1.2	1.1

graminearum									
Fusarium oxysporum	0.0	0.7	0.4	0.1	0.3	0.5	0.6	0.7	0.4
Fusarium culmorum	0.4	0.2	0.4	0.5	0.6	0.7	1.8	2.5	4.1
Fusarium Semitectum	0.2	0.4	0.3	0.1	0.4	0.6	1.2	1.0	0.7
Fusarium solani	0.4	1.1	0.7	1.1	1.1	1.3	1.6	1.7	2.5
Fusarium verticillioides	0.0	0.4	0.6	0.2	0.4	0.6	1.4	1.6	2.1
Macrophomina phaseolina	0.1	0.0	0.0	0.0	0.3	0.4	0.8	1.6	2.4
Mucor hie malis	0.3	0.7	0.2	0.6	0.8	0.7	1.3	2.1	2.6
Myrothecium roridum	0.0	0.0	0.1	0.8	0.0	0.0	0.4	0.6	0.4
Oedocephalum glomerulosum	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.4
Penicillium brevicompactum	0.3	0.8	0.6	0.1	0.7	1.3	2.4	2.6	3.1
Penicillium chrysogenum	0.1	0.3	0.1	0.7	0.0	0.4	0.3	0.7	0.2
Pericillium expansum	0.3	1.2	0.7	0.3	1.1	1.6	2.6	3.2	3.6
Penicillium oxalicum	0.1	0.6	0.4	1.2	0.2	0.4	1.0	0.5	1.3
Rhizopus stolonifer	0.2	1.3	1.2	0.3	1.1	1.4	1.4	1.5	2.1
Stachybotrys atra	0.0	0.4	0.0	0.1	0.1	0.5	1.3	1.2	0.6
Trichoderma homatum	0.1	0.0	0.4	0.4	0.4	0.4	1.2	0.6	0.4
Trichoderma horizianum	0.0	0.3	0.2	0.1	0.4	0.6	1.3	0.9	1.4
Trichoderma viride	0.1	0.5	0.0	0.0	0.0	0.5	0.6	0.7	0.4
Vlocladium atrum	0.2	0.0	0.1	0.2	0.4	0.1	0.1	0.2	0.5
Ulocladium botrytis	0.1	0.3	0.4	0.3	0.3	0.6	2.1	1.6	1.5
Ulocladium chartarum	0.3	0.6	0.2	0.4	0.4	0.7	1.2	0.8	0.3

Seed flower seed germination (%)

No.	Cultivar	Germination seed	Seed infected by fungi
1	Surya	1-5	12
2	55-56	95	24
3	MSFH-17	93	15
4	KI3SH – 1	100	13

5	BSH – 1	95	19
6	APSC – 1	96	22
7	Unidentified cultivar	69	36
8	Unidentified cultivar	42	39
9	Unidentified cultivar	37	46

CONCLUSION:

The present study investigated that there is a large number of fungal species associated with seeds for sunflower the greatest number of fungi were recorded from seeds purchased from local market. Some of them are well known as seed borne potentially pathogenic fungi.

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