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TP Uchitkar
Student (M. Sc. Agriculture), Department of Plant Pathology and Microbiology, Post Graduate Institute, Mahatma Phule krishi Vidyapeeth, Rahuri, Dist., Ahilyanagar, Maharashtra, India

HS Shirsath
Student (M. Sc. Agriculture), Department of Plant Pathology and Microbiology, Post Graduate Institute, Mahatma Phule krishi Vidyapeeth, Rahuri, Dist., Ahilyanagar, Maharashtra, India

SR Zanjare
Senior Scientist (Seed Pathology), Department of Plant Pathology and Microbiology, Post Graduate Institute, Mahatma Phule krishi Vidyapeeth, Rahuri, Dist., Ahilyanagar, Maharashtra, India

AV Suryawanshi
Assistant Seed Research Officer, Department of Plant Pathology and Microbiology, Post Graduate Institute, Mahatma Phule krishi Vidyapeeth, Rahuri, Dist., Ahilyanagar, Maharashtra, India

Corresponding Author:
TP Uchitkar
Student (M. Sc. Agriculture), Department of Plant Pathology and Microbiology, Post Graduate Institute, Mahatma Phule krishi Vidyapeeth, Rahuri, Dist., Ahilyanagar, Maharashtra, India

Isolation and pathogenicity of different seed borne mycoflora associated with seeds of sesamum

TP Uchitkar, HS Shirsath, SR Zanjare and AV Suryawanshi

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Abstract
The present investigation entitled "Studies on seed borne mycoflora of sesamum (*Sesamum indicum* L.)" was undertaken with a view to detect and isolation of seed borne mycoflora and their pathogenicity. Six externally seed borne fungi detected *viz*., *Fusarium oxysporum, Macrophomina phaseolina, Alternaria alternata, Penicillium* spp., *Aspergillus niger* and *Aspergillus flavus* on sesamum seeds. Internally seed borne fungi were detected *viz.*, *Fusarium oxysporum, Macrophomina phaseolina* and *Alternaria alternata.* All the isolated pathogens were found to be pathogenic to sesamum seeds resulting into reduction in seed germination and seedling vigour index. The seed borne mycoflora *viz*., *Aspergillus niger, Aspergillus flavus, Fusarium oxysporum, Macrophomina phaseolina* and *Alternaria alternata* were found more damaging.

Keywords: *Fusarium oxysporum, Macrophomina phaseolina, Alternaria alternata, Penicillium* spp., *Aspergillus niger, Aspergillus flavus*

Introduction
Sesamum (*Sesamum indicum* L.) is an old and important oilseed crop. It belongs to the Pedaliaceae family and comes under the order Tubiflorae. Although sesamum originally came from Africa, it is now grown in many parts of the world including Myanmar (Burma), China and Japan. It can be grown during the *kharif*, *rabi* and *summer* seasons. For good growth and yield, it needs a temperature range of 26–30 °C, well-drained soil and a soil pH between 6.5 and 7.5. Sesamum commonly known as the "Queen of Oilseeds," is valued for its high economic importance. The seeds are mainly used for oil extraction, while a smaller portion is used for direct consumption as food. (El Khier *et al*., 2008) [3]. Sesamum seeds are also nutritious, containing oil 50–62 per cent, protein 18–25 per cent, carbohydrates 13.4–25.0 per cent, fiber 9.8 per cent, calcium 1 per cent, phosphorus 0.7 per cent. They are also a good source of vitamin E and important minerals like iron, copper, magnesium, zinc and potassium.

Sesamum production is heavily affected by various diseases around the world. These diseases can attack the crop at any stage from planting and growing to harvest and storage leading to the loss of millions of tons each year. Along with diseases, insect pests also pose major challenges for sesamum farmers. Sesamum crops are affected by various diseases caused by fungi, bacteria and phytoplasma which lead to a reduction in yield. These diseases can cause a loss of about 15–20 per cent in production. In India, the major diseases affecting sesamum include *Alternaria* leaf spot, root and stem rot, bacterial blight, phyllody, powdery mildew, *Cercospora* leaf spot and damping-off. Among these leaf spots, phyllody and root and stem rot are the most serious causing significant yield losses (Maiti *et al*., 1985) [6].

Oilseeds infected by various mycoflora undergo several harmful changes that make them unsuitable for both human consumption and sowing. The presence of these fungi also reduces seed quality and health. In seed samples, various fungal species were found including *Alternaria*, *Curvularia*, *Fusarium*, *Helminthosporium*, *Penicillium*, *Mommoniella*, *Aspergillus*, *Mucor* and *Rhizopus*. Among these, *Alternaria* and *Aspergillus* were identified as the most harmful fungi affecting oilseeds like sesamum, groundnut and mustard (Ghosh *et al*., 2018) [5]. Infected seeds can spread mycoflora from one seed to another. These fungi can damage seeds in the soil before they germinate leading to seedling death. Therefore,

identifying seed-borne fungi in sesamum seeds is necessary to prevent these problems and reduce losses.

Material and Methods

The seeds of sesamum was collected from Seed Cell, MPKV., Rahuri. Externally seed borne fungi of the sesamum variety JLT-408 were detected using the ISTA's standard blotter test, as described by Neergard (1979). ISTA's standard agar plate method was used to detect internally seed borne mycoflora associated with seeds of the sesamum. The Koch's postulate of the mycoflora isolated from seeds of sesamum were proved in Seed Pathology Laboratory at MPKV, Rahuri. The method followed to study the pathoginicity of seed borne mycoflora is as below.

1. The seeds were surface sterilised for 5 minutes with 1.0 per cent NaOCl solution, then washed three times with sterilised water to eliminate any corrosive sublimate.
2. To inoculate the seeds with the individual pathogen, seeds were dipped in concentrated suspension of spores (10^6 cfu/ml) for 12 hours. Then these seeds were dried in shade for 12 hours (Agarwal and Sinclair, 1993) [1].
3. For each isolate, seeds were placed to sterilised plastic petri plates containing three discs of blotter paper.
4. Seeds which were not smeared with fungal isolates served as control.
5. In the incubation room, these petri plates were incubated for seven days at 20 ± 20C.
6. On the seventh day after incubation observations on symptoms produced and the per cent occurrence of seed borne infections were recorded.
7. Care was taken to keep the blotter moist by adding sterilized water as per requirement.

Reisolation of pathogens

By transferring fungal growth on sterilized petri plates containing potato dextrose agar, different seed borne fungi were reisolated from pathogenic growth on rotten seeds. The reisolation was done to ensure that the original isolates were true. Pathogenic fungi were selected and used in further research work.

Results and Discussion

The results showed that total six fungi i.e. *Fusarium oxysporum, Macrophomina phaseolina, Alternaria alternata, Penicillium* spp., *Aspergillus niger* and *Aspergillus flavus* were detected on seeds of sesamum. The result of internally seed borne fungi in seeds of sesamum found three fungal species *Fusarium oxysporum*, *Macrophomina phaseolina* and *Alternaria alternata*, which were internally present in the seeds.

Gangrade (2003) [4] noticed *Alternaria* spp., *Aspergillus* spp., *Fusarium* spp., *Curvularia* spp., *Macrophomina* spp., *Penicillium* spp. and *Rhizopus* spp. were detected and isolated from sesamum seeds. Bharathi *et al*. (2013) [2] detected that sesamum seed collected from farmers of Nizamabad and Karimanagar districts of Andhra Pradesh were infested with ten pathogenic and five saprophytic fungi. Among the pathogenic fungi *Fusarium*, *Alternaria* and *Macrophomina* were the most frequent followed by *Curvularia* spp.

All the pathogens identified were pathogenic to sesamum seeds. Table 1 presents the list of these pathogenic organisms, along with the symptoms they caused and their percentage incidence on artificially inoculated sesamum seeds. All the seed borne fungi (mycoflora) of sesamum were pathogenic and produced various symptoms on the seeds. Among all seed borne mycoflora, *Aspergillus niger* showed highest incidence (66%) followed by *Aspergillus flavus* (62%), *Fusarium oxysporum* (52%), *Macrophomina phaseolina* (49%), *Alternaria alternata* (46%), *Penicillium* spp. (41%).

Nayyar *et al*. (2014) [7] reported during the mycological analysis of sesamum seeds in our previous report, a total number of 36 species belonging to 10 genera of fungi were isolated. The prevalent genera were *Alternaria*, *Aspergillus*, *Cercospora*, *Fusarium*, *Penicillium* and *Rhizopus*. The pathogenicity of isolates was evaluated at seedling stage which confirmed the incidence of prevalent fungi.

Conclusions

1. Six seed borne fungi *Fusarium oxysporum*, *Macrophomina phaseolina*, *Alternaria alternata*, *Penicillium* spp., *Aspergillus niger* and *Aspergillus flavus* were detected and isolated externally from the seeds of the sesamum.
2. All the isolated pathogens were found to be pathogenic to sesamum seeds, leading to reduced seed germination and lower seedling vigour index. Among them, *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporum* and *Macrophomina phaseolina* caused the most damage.

Table 1: Pathogenicity test of seed borne mycoflora associated with seeds of sesamum

Sr. No.	Mycoflora associated with seeds	Incidence of seed borne mycoflora (%)	Symptoms produced by mycoflora (7 days of inoculation)
1.	*Fusarium oxysporum*	52.00	White fungal growth on seeds. Seed becomes rotted and turn pinkish brown.
2.	*Macrophomina phaseolina*	49.00	Black to greyish mycelium growth on seeds which convert into black to brown circular spots leads to rotting of seed.
3.	*Alternaria alternata*	46.00	Infected seed show water soaked appearance, discoloured and shrivelled appearance.
4.	*Penicillum* spp.	41.00	Seeds covered with blue to green mycelium and spores. Rotting of seeds.
5.	*Aspergillus niger*	66.00	Seeds covered with black fungal growth completely. Seedling shoot showed browning symptoms
6.	*Aspergillus flavus*	62.00	Seeds completely covered with green mycelium growth and rotting of seed occur.

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