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SV Gawarkar
Student, Department of Plant Pathology, College of Agriculture, Nagpur, Maharashtra, India

RG Deshmukh
Student, Department of Plant Pathology, College of Agriculture, Nagpur, Maharashtra, India

AD Matte
Student, Department of Plant Pathology, College of Agriculture, Nagpur, Maharashtra, India

MJ Patil
Assistant Professor, Department of Plant Pathology, College of Agriculture, Nagpur, Maharashtra, India

Corresponding Author:
SV Gawarkar
Student, Department of Plant Pathology, College of Agriculture, Nagpur, Maharashtra, India

In vitro management of *Aspergillus niger* of Sesamum (*Sesamum indicum*)

SV Gawarkar, RG Deshmukh, AD Matte and MJ Patil

Abstract
Sesamum (*Sesamum indicum*) known variously as sesame, til, gingelly, simsin, gergelin etc, belong to family pedaliaceae. In view Sesamum is rich source of oil content (40-50%), seed protein (20%). Infected seeds carry seed-borne mico-organisms that may cause seed degradation, reduced seed germination, seed vigour and weakening of the plant, resulting in a reduction in plant population. Therefore it is necessary to do investigation on seed mycoflora associate with sesamum.
Five varities were tested to detect the seed borne mycoflora by standard blotter paper method, pre treatment blotter method and agar plate method. In that *Aspergillus niger* is dominant fungi associate with seeds of sesamum. Three fungicides and two botanicals were tested against *Aspergillus niger* under *in vitro* condition using poison food technique. In three fungicides carbendazim+mancozeb and tebuconazole completely inhibited the mycelial growth (100%) followed by hexaconazole (79.25%). In botanicals maximum mycelial growth inhibited by *Allium sativum* (73.46%) than neem leaf extract (62.58%). Four bioagents *viz., Trichoderma viride, Trichoderma harzianum, Pseudomonas fluorescens* and *Bacillus subtilis* were tested against *Aspergillus niger* by dual culture method. Maximum mycelium growth inhibition was observed in *Trichoderma viride* (78.20%) followed by *Trichoderma harzianum* (73.07%) *Pseudomas fluorescens* a whereas, *Bacillus subtilis* were less effective exhibited (28.20, 41.66%) against *Aspergillus niger*.

Keywords: Sesamum seeds, seed mycoflora, *Aspergillus niger*, chemicals control, botanical control, bioagents control

Introduction
Sesamum (*Sesamum indicum*) known variously as sesame, til, gingelly, simsin, gergelin etc, belong to family pedaliaceae. It is an important oil yielding crop cultivated in India, Myanmar, Indo-China, China and Japan. Sesamum is regarded as oldest oil yielding plant known to man. Sesamum is called as "Queen of edible oils" in view of the rich oil content (40-50%), seed protein (20%), carbohydrates and minerals such as calcium (1%), and phosphorous (0.7%). It is rich source of vitamin E. In India crop is grown in all seasons *viz.*, kharif, semi-rabi and summer. Uttar Pradesh leads in area and production followed by Rajasthan, Gujrat, Orissa and Karnataka. It has become a popular summer crop in West Bengal and recently Bihar., biological control using bio-agents and phytoextract has received much attention in both conventional and organic farming to suppress plant disease and to overcome some extent the public concern regarding chemical fungicides (Samnels, 2006) [6]. Fungicides and botanicals inhibits the growth of seed borne mycoflora of sesame *in vitro* (Hosen and Shamsi 2017) [3]. Fungicides have been reported for many years to control plant pathogens and the use of fungicide seed dressing chemicals and bio-agents has become an inevitable method of disease and pest control in Sesame (Anandu *et al.*, 2010) [1].
Mycoflora associated with seeds reduce the seed germination, final stand of crop in the field and overall growth of plant. Infected seeds play an important role in dissimination of pathogens and establishment of disease take place which will lead to increase a cost of cultivation as well as reduce a quality of product, to avoid all these problems and losses it felt necessary to detect and manage a seed borne mycoflora associate with seed.

Materials and Methods
The present investigations were carried out in the plant pathology section of College of Agriculture, Nagpur. The details of materials used and the methodology followed in conducting the experiments are presented here under.

Isolation of *Aspergillus niger*

This study was carried out on seeds of 5 cultivars of sesamum including PKV-NT-11, AKT-101, N-8, JLT-408, GT-10 were collected from oilseed research unit Dr. P.D.K.V. Akola. Varieties were analyzed for their association of seed-borne mycoflora by standard blotter paper, Pre treatment and agar plate method. Isolate a *Aspergillus niger* associate with seeds of sesamum.

***In vitro* evaluation of fungicides and botanicals against *Aspergillus niger* by poisoned food technique**

Three different fungicides and two botanicals were evaluated under *in vitro* against test pathogen by adopting poison food technique. Requisite quantity of each of the fungicides (as per concentration) was added in sterilized melted PDA separately so as to obtain desired concentration.. PDA was poured in sterilized petri plate and allow to solidify. Five mm disc of fungus culture was transferred aseptically in the center of petri plate containing the poisoned media with test fungicide. The control plates were kept the culture disc grown in same condition on PDA without fungicides. Treated plates were incubated at room temperature (26±2 °C) for a period of seven days. Colony diameter was recorded in mm and percent mycelial growth inhibition was calculated as per Vincent's formula based on the average colony diameter. The data was subjected to statistical analysis wherever necessary.

$$I = \frac{C - T}{C} X\ 100$$

Where,
I = Percent growth inhibition
C = Fungal growth in control plate (mm)
T = Fungal growth in treatments (mm)

***In vitro* evaluation of bio agents against *Aspergillus niger* by dual culture technique**

PDA was autoclaved and poured into a 90 mm petri plate, where it solidified. Then, on one end of the plate, a 5 mm disc of test species was set, and on the other end, an antagonistic disc and striking bacterial culture. Along with the control, the plates were incubated at room temperature (26$\pm$2^{0}C) for seven days. The data was subjected to statistical analysis wherever necessary.

$$I = \frac{C - T}{C} X\ 100$$

Where,
I = percent growth inhibition
C = Fungal growth in control plate (mm)
T = Fungal growth in treatments (mm)

Result and Discussion

Management of *Aspergillus niger in vitro*

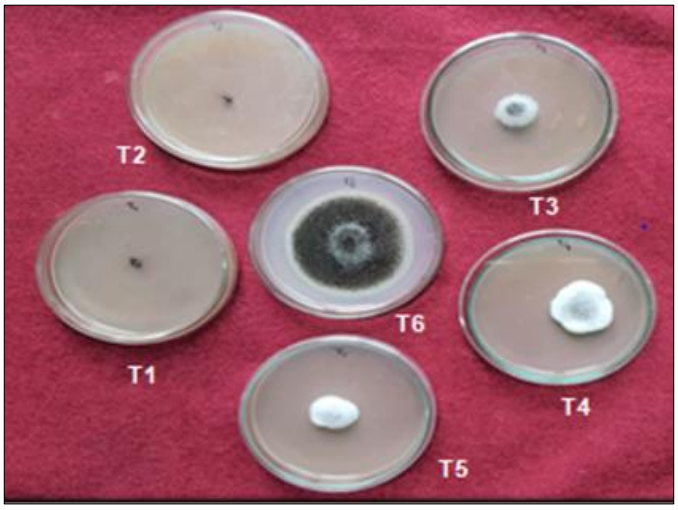
1. Through poison food technique

Result obtained were presented in Table 1. it was clearly indicated that all the treatments were significantly superior over control in inhibiting the growth of *Aspergillus niger.* In *Aspergillus niger* carbendazim+mancozeb and tebuconazole completely inhibited the mycelial growth (100%) followed by hexaconazole (79.25%).In botanicals maximum mycelial growth inhibited by *Allium sativum* (73.46%) than neem leaf extract (62.58%).

It was observed that among all the treatments, carbendazim+mancozeb (T1) and tebuconazol (T2) were best treatment followed by hexaconazole (T3) and neem leaf extract (T4). *Allium sativum* (T5) was less effective among the treatments over a control. Similar result were reported by Saranya *et al.,* (2017) [7].

Table 1: Efficacy of fungicides and botanicals against *Aspergillus niger in vitro*

Tr. No.	Treatment detail	Conc. Percent	Mean colony diameter (mm)	Percent growth inhibition
1	Carbendazim 12% + Mancozeb 63% WP	0.25	0.00	100
2	Tebuconazole 25% EC	0.1	0.00	100
3	Hexaconazole 5% EC	0.1	15.25	79.25
4	Neem leaf extract	10	27.50	62.58
5	*Allium sativum* (Clove)	10	19.50	73.46
6	Control	--	73.50	--
	SE(m)±	--	0.33	--
	CD (P=0.01)	--	1.38	--



T1: Carbendazim + Mancozeb, T2: Tebuconazole, T3: Hexaconazole T4: Neem leaf extract, T5: *Allium sativum*, T6: Control

Plate 1: Efficacy of fungicides and botanicals against *Aspergillus niger in vitro*

2. Dual Culture Technique

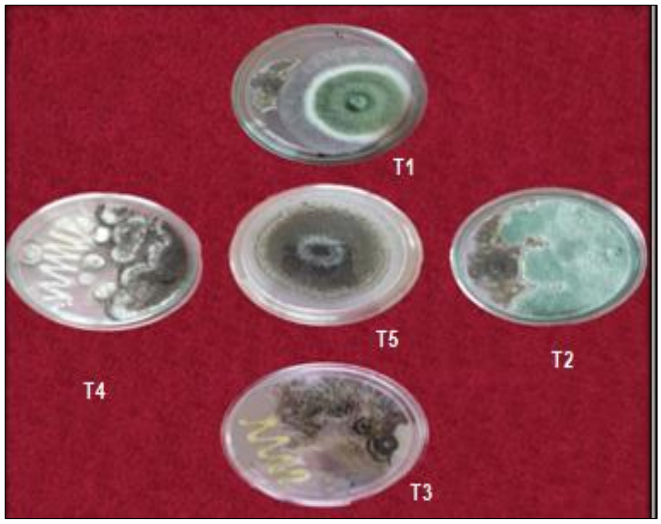
Efficacy of *Trichoderma viride, Trichoderma harzianum, Pseudomonas fluorescens* and *Bacillus subtilis* were tested against *Aspergillus niger* by dual culture technique. Result depicted in Table 2, Maximum mycelia growth inhibition of *Aspergillus niger* was observed in *Trichoderma viride* (78.20%) followed by *Trichoderma harzianum* (73.07%). The next best effective treatment were found *Pseudomonas fluroscens* and *Bacillus subtilis* exhibiting (28.20, 41.66%) inhibition of *Aspergillus niger*.

Similar result were also earlier given by Gajera *et al.,* (2011) [2] observed antagonistic effect of 12 isolates of 3 *Trichoderma strain (T. virens, T. harzianum, T.viride*) against collar rot of pathogen in vitro and observed that *T. viride* inhibited maximum (86.2%) growth of test fungus followed by *T. harzianum* (80.4%). Lone *et al.,* (2012) [4] reported that *T. harzianum* exhibited maximum growth inhibition of *A. niger* (75%) followed by *C. spherospermum* (72.2%) and *F. oxysporum* (25%), which justifies that *T. harzianum* is

promising biological agent for restricting a wilt and other fungal disease. Rohtas *et al.*, (2016) [5] reported that three bioagents *viz.*, *T. viride* inhibited a mycelium growth up to 78.32% followed by *T. harzianum* 72.50%, while bacteria *P. fluorescence* only managed to inhibit 23.80 percent mycelium growth of *Aspergillus niger.*

Table 2: Efficacy of bioagents against *Aspergillus niger in vitro*

Tr. No.	Treatment detail	Mean colony diameter (mm)	Percent growth inhibition
1	*Trichoderma viride*	17.00	78.20
2	*Trichoderma harzianum*	21.00	73.07
3	*Pseudomonas fluorescens*	56.00	28.20
4	*Bacillus subtilis*	45.50	41.66
5	Control	78.00	--
	SE(m)±	0.43	--
	CD(P=0.01)	1.85	--



T1: *Trichoderma viride*, T2*: Trichoderma harzianum*
T3*: Pseudomonas fluorescens*, T4*: Bacillus subtilis* T5*: Control*

Plate 2: Efficacy of bioagents against *Aspergillus niger in vitro*

Conclusion

Sesamum varities were highly associated with *Aspergillus niger.* Maximum inhibition of *Aspergillus niger* was recorded in carbendazim + mancozeb and tebuconazol followed by hexaconazole in posion food technique. *Trichoderma viride* and *Trichoderma harzianum* had shown maximum inhibition of *Aspergillus niger* by dual culture method.

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