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Effect of fungicides and plant extracts on the sporulation and spore germination per cent of *A. sesami*

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Abstract
Laboratory studies were conducted to study the effect of different fungicides and plant extracts on the sporulation and spore germination per cent of *Alternaria sesami.* The minimum mycelial growth was observed on Babool leaf extract amended medium whereas minimum sporulation on Babool, Neem and Gajar Grass. Spore germination was the minimum on Gajar grass leaf extract amended medium. In inhibition of mycelial growth Propiconazole was most effective whereas Propiconazole, Hexaconazole, Tebuconazole, Difenoconazole and Mancozeb were most effective in inhibition of sporulation at tested concentrations. Spore germination was most inhibited by Propiconazole.

Keywords: Mycelial growth, sporulation and spore germination, *Alternaria sesami*

Introduction
Sesame is an important oil seed crop of this country and thought to have originated in Africa (Brar and Ahuja 1979; Ram *et al*. 1990) [19, 21]. It is widely grown in tropical and subtropical regions. Its production is often concentrated in marginal and sub marginal lands (Ashri 1998) [20]. Most of the sesame seeds are used for oil extraction and the rest are used for edible purposes (El Khier *et al*. 2008) [1]. Sesame is grown primarily for its oil-rich seeds. Before seeds were appreciated for their ability to add nutty flavour or garnish foods, they were primarily used for oil and wine (Ghandi 2009) [2]. Sesame suffers from many diseases during the growth period. Alternaria blight is one of the serious disease which causes the considerable qualitative and quantitative yield losses in the crop. In case of severe infection of Alternaria blight, severe yield losses occur.

Material and Methods
The present investigation on ―Studies on Alternaria blight of sesame‖ was carried out during 2014 - 15 at Department of Plant pathology, College of Agriculture, JNKVV, Jabalpur. The diseased samples collected during the season from various locations were used for isolation in the laboratory. They were cut into small pieces and surface sterilized with 0.1 per cent HgCl2 for 30 seconds. The pieces were then given three washings in sterile water and transferred into petri plates containing PDA. In each plate three pieces were kept in three replications. The plates were incubated at 28+2 °C and examined for the fungal growth after 4 days. The associated fungi was re-isolated, purified and identified.

Efficacy of plant extracts on the mycelial growth, sporulation and spore germination of *A. sesami*
The fresh plant samples of 100 g were washed in tap water then washed with alcohol and finally washed thrice using sterile distilled water. They were crushed in a sterile mortar and pestle by adding 100 ml of sterile distilled water. The extracts were collected by filtering through Whatman No. 1 filter paper. Finally, filtrate thus obtained from the sample was used as 100 per cent stock solution. To study antifungal mechanism of plant extracts. The stock solution 5 ml, 10 ml and 15ml was taken separately and was mixed with 50ml sterile molten PDA medium respectively, so as to get 10, 20 and 30 per cent concentrations. The plant extract amended PDA medium was poured aseptically into sterilized petri plates while the plates containing PDA alone served as control. All the plates were inoculated with 5 mm mycelial disc of 7 days old actively growing culture of *A. sesami.*

Each treatment was replicated thrice, and incubated at 28+2 °C till the pathogen completely occupied the control plate. To test the spore germination, two drops of each suspension at 10, 20 and 30 per cent concentration along with spores were placed in a cavity slide and incubated at 28+2 °C for 24 hours and thereafter germination percentages were recorded. The per cent inhibition over control was calculated according to formula given by Vincent (1947) [6].

Effect of fungicides on the mycelial growth, sporulation and spore germination of *Alternaria sesami*

The fungicides were weighed as per the desired concentration and mixed thoroughly in the 250 ml conical flasks containing warm PDA under aseptic conditions. The uniform mixture was then poured in the sterilized plates @ 20 ml per petri plate and allowed to solidify. Mycelial disc of five mm diameter was cut from 7 days old culture of pathogen and was inoculated in the centre of each plate. The inoculated plates were incubated at 28+2 °C till the pathogen completely occupied the control plate. Three replications were maintained for each treatment, while the plates without fungicides served as control. An observation on radial growth (colony diameter) and sporulation was recorded in the end of the experiment. To test the spore germination, two drops of fungicide suspensions along with spores were placed in a cavity slide and incubated at 28+2 °C for 24 hours and thereafter germination percentage were recorded. Percent growth inhibition of pathogens was calculated as described by Vincent (1947) [6].

I = C-T/C x100 27

Where,

I= Percent inhibition

C= Radial growth of control

T= Radial growth in treatment

Results and Discussion

Preparations derived from higher plants are known to possess disease controlling properties. Frequently, it is reported that many plants possess antifungal properties against *Venturia inequalis* (Gulliver 1947) [7]. Hence there is urgent need to develop alternate method of managing the plant diseases using plant based derivatives which are eco-friendly, cost-effective, bio-degradable and easily accessible along with many additional advantages to suit the organic based agriculture. The antifungal potential of plant extracts results from the presence of secondary metabolites in leaf extracts. In the present investigation, efficacy of seven plant extracts was evaluated against *A. sesami* at three concentrations 10, 20 and 30 per cent. Among the seven plant extracts maximum per cent growth inhibition was recorded on the leaf extracts amended medium of the Babool followed by Neem and Gajar grass. The maximum inhibition of radial growth was observed at 30 per cent concentration (Table 1). In the leaf extract of Gajar grass, alkaloid, flavonoid, tannin, glycoside, saponin, steroid, terpenoid, phenol and antharaquinone are major secondary metabolites which probably resulted in inhibition of mycelial growth as well as inhibition of spore germination (Table 2). Among all the secondary metabolites major bioactive ingredients present in the leaves of the Gajar grass are as camphor, parthenin, parthenolide and alpha-pinene. Similar ingredients were extracted by Singh and Shrivastava (2013) [8]; Roy and Shaik (2013) [9]. In neem leaf extract, presence of some natural products called triterpenoids (Limonoids) are the major bioactive ingredients in the form of nimbidin, gedunin, cyclic trisulphide, cyclic tetrasulphide which shows antifungal propertieshave already been reported by Kumar *et al*. (2010) [10]. The inhibitory effect of Neem and Prosopis on mycelial growth of *A. tenuissima* was reported by Raja (2010) [11]. Poor sporulation was observed in Neem, Khejri, Babool, Thuja and Amarbel leaf extracts amended media at 30 per cent concentration. Whereas maximum inhibition of spore germination was observed in Gajar grass leaf extracts followed by Thuja and Babool leaf extracts. Similarly Patni *et al*. (2006) [12] recorded effect of leaf extract of *Parthenium hysterophorus* on sporulation and spore germination of *Alternaria brassicae*. Antifungal activities of plant extracts on *Alternaria* sp. were also reported by Seetharaman *et al*. 2001, [13] Raghvendra *et al*. 2009 [14]; Sharma and Pandey 2011 [15]; Meena 2012 [16].

Use of fungicides is an alternative method of controlling the diseases of crop in the absence of resistant cultivars. Hence, fungicides would continue to be one of the major tool of integrated disease management (IDM). Evaluation of different fungicides *in vitro* is a handy tool to screen a large number and thus can serve as guide for field testing. The data presented (in Table 9A) shows that all the fungicides significantly inhibited the growth of fungus at all the concentrations. Radial growth ranged from 0.0 to 51.7 mm in treatments as compared to control (90.00mm). Among all the fungicides evaluated Propiconazole and Difenoconzazole were found to be most effective followed by Hexaconazole and Tebuconazole in inhibition of mean radial growth of *A. sesami* at 1000 ppm (Table 3). However mycelial growth decreased as the concentration increased. Similarly same results were also reported by Sharma and Pandey (2011) [15] on *Alternaria burnsii*.

All the fungicides significantly inhibited the sporulation as well as spore germination. Among all the fungicides evaluated Hexaconazole, Propiconazole, Difenoconazole and Mancozeb completely inhibited the sporulation. Among all the fungicides Propiconazole (0.0%) and Difenoconazole (0.0%) were found most effective followed by Hexaconazole (4.6%) and Mancozeb (5.0%) spore germination at 1000 ppm concentration (Table 4). Similar results were also reported by Kumar *et al*.2005 on *A. alternata* and *Alternaria carthami*.

Table 1: Efficacy of plant extracts on mycelial growth and per cent growth inhibition of *A. sesame*

S. No.	Plants	Mean radial growth (mm)* Concentration (%)			Percent growth inhibition at 30% concentration
		10	20	30	
1	Khejri	60.0	40.0	30.3	66.3
2	Neem	50.5	38.0	28.3	68.5
3	Thuja	69.5	50.7	40.3	55.2
4	Babool	46.7	31.7	24.5	72.8
5	Amarbel	73.3	46.0	32.5	63.9
6	Gajar grass	54.2	45.7	29.3	67.4
7	Ashoka	79.7	60.8	50.3	44.1
8	Control	90.0	90.0	90.0	-
S. Em±		0.80	0.71	0.61	
C. D. (P = 0.05%)		2.43	2.176	1.869	

Table 2: Efficacy of plant extracts on sporulation and spore germination per cent of *A. sesame*

S. No.	Plants	Sporulation			Spore germination per cent		
		10%	20%	30%	10%	20%	30%
1	Neem	++	++	+	24.0	18	11.5
2	Khejri	+++	++	++	26.5	29.5	20.0
3	Thuja	+++	++	+	20.0	9.5	7.0
4	Babool	++	++	+	28.0	13.5	8.5
5	Gajar grass	++	+	+	19.0	7.5	4.0
6	Amarbel	+++	++	+	27.0	17.5	12.0
7	Ashoka	+++	+++	++	22.0	20.0	11.0
8	Control	++++	-	-	80.0	80.0	80.0

*Mean of three replications

- Nil, +poor, ++Moderate, +++ Good, ++++ Abundant

Table 3: Efficacy of fungicides on the mycelial growth and per cent inhibition of *A. sesame*

S. No.	Fungicides	Mean radial growth(mm)* Concentration (ppm)			Per cent growth inhibition at 1000 ppm
		250	500	1000	
1	Mancozeb	50.3	25.0	16.1	82.0
2	Hexaconazole	40.0	21.7	11.5	87.2
3	Chlorothalonil	51.7	32.1	22.5	75.0
4	Azoxystrobin	47.8	30.1	20.5	77.2
5	Propiconazole	34.1	17.0	0.0	100
6	Tebuconazole	44.8	21.5	13.3	85.2
7	Difenoconazole	36.1	17.8	0.0	100
8	Control	90.09	90.0	90.0	
S. Em±		0.64	0.57	0.42	
C. D. (P = 0.05%)		1.95	1.72	1.29	

Table 4: Efficacy of fungicides on the sporulation and spore germination per cent of *A. sesame*

S. No.	Fungicides	Sporulation			Spore germination per cent		
		250	500	1000	250	500	1000
1	Mancozeb	++	+	–	20.0	11.5	5.0
2	Azoxystrobin	+++	++	+	23.0	16.0	10.0
3	Chlorothalonil	+++	++	+	28.5	19.0	12.0
4	Hexaconazole	++	+	–	18.5	13.0	4.6
5	Propiconazole	++	+	–	9.5	5.5	0.0
6	Tebuconazole	+++	++	–	21.5	16.0	2.0
7	Difenoconazole	++	+	–	12.5	80.0	0.0
8	Control	++++	–	–	82.0		-

*Mean of three replications

- Nil, + Poor, ++Moderate, +++ Good, ++++Abundant

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