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Chitali S Vighe

Department of Plant Pathology,
Jawaharlal Neharu krishi
Vishwa Vidyalaya, Jabalpur,
Madhya Pradesh, India

Sushma Nema

Department of Plant Pathology,
Jawaharlal Neharu krishi
Vishwa Vidyalaya, Jabalpur,
Madhya Pradesh, India

Devashish R Chobe

Department of Plant Pathology,
Jawaharlal Neharu krishi
Vishwa Vidyalaya, Jabalpur,
Madhya Pradesh, India

Gaurav A Thakare

Department of Plant Pathology,
Jawaharlal Neharu krishi
Vishwa Vidyalaya, Jabalpur,
Madhya Pradesh, India

Evaluation of botanicals against the mycelial growth, sporulation and spore germination of *Alternaria cassiae* causing alternaria leaf blight of cowpea

Chitali S Vighe, Sushma Nema, Devashish R Chobe and Gaurav A Thakare

Abstract

Cowpea *Vigna unguiculata* (L) walp is an important leguminous vegetable crop for the livelihood of poor people in underdeveloped countries and can be used for various purposes such as food crop, cash crop and animal feed. In recent year *Alternaria* blight of cowpea is the most important disease in India. It occurs in endemic form in some region. The disease has gained importance because of changing climate. To achieve resistance against alternaria or other foliar diseases, botanical fungicides are most widely used as they are ecofriendly and harmless to humans. So keeping in mind this aspects present study was aimed to identify suitable botanicals use to find out there antagonistic efficacy against mycelial growth, sporulation and spore germination of *Alternaria cassiae*. Present experiment was conducted at plant pathology laboratory JNKVV Jabalpur with the help of Completely Randomise Block Design with three replication. Botanicals like *Azadirachta indica*, *Pongamia pinnata*, *Bougainvillea sp.*, *Acacia nilotica*, *Eucalyptus globules*, *Polyanthia longifolia*, *Lawsoniainermeris*, *Thuja compacta*, *Camera lantanawere* used to identify the efficacy of botanicals against *Alternaria cassiae* using poisoned food technique. Findings revealed that Inhibition of Mycelial growth, sporulation and spore germination percentage of *A. cassiae* was minimum in Babool leaf extract followed by thuja leaf extract.

Keywords: *Vigna unguiculata*, *Alternaria cassiae*, Botanicals, PDA

Introduction

Cowpea *Vigna unguiculata* (L) walp is an important leguminous vegetable crop for the livelihood of poor people in underdeveloped countries and can be used for various purposes such as food crop, cash crop and animal feed (Singh *et al* 1971) [1]. The crop suffers due to a number of diseases caused by bacteria, virus and fungi, Some 40 spp of fungi are reported on Cowpea. *Septoria* leaf spot (*Septoria vignae*), Brown blotch (*Sphaceloma spp.*), *Cercospora* laef spot (*Cercospora spp.*), Ashy stem blight (*Macrophominaphaseolina*), are the important fungal diseases of cowpea as reported by (Emechebe and Florini 1997) [2]. *Alternaria cassiae* as foliar pathogen causing *Alternaria* blight on cowpeas in South Africa. Foliar symptoms begin as semicircular water soaked lesions at leaf margins. Lesions enlarged towards centre of leaf, margin becoming necrotic. The mycelia of *Alternaria cassiae* are composed of branched, septate, pale brown to almost hyaline (David, 1991) [2]. Conidiophores may be septed branched or unbranched and 36-112x4-5.5um in size. The cells walls are generally smooth but occasionally slightly viscous (David, 1991) [2]. In recent year *Alternaria* blight is the most important disease in India. It occurs in endemic form in some region. The disease has gained importance because of changing climate. Very little work has been done on this pathogen in India. Now a days botanical fungicides are most widely used to control the foliar diseases like alternaria. So keeping in mind this aspects present study was aimed to identify suitable botanicals use to find out there antagonistic efficacy against mycelial growth, sporulation and spore germination of *Alternaria cassiae*.

Material and Methods

Materials: - During the study Corning and Borosil make glasswares, and mortar and pestle were used. They were cleaned with detergent powder. After cleaning the glasswares with the detergent, they were thoroughly washed with tap water, rinsed with water and dried before use whenever required.

Correspondence**Chitali S Vighe**

Department of Plant Pathology,
Jawaharlal Neharu krishi
Vishwa Vidyalaya, Jabalpur,
Madhya Pradesh, India

The petriplates were sterilized in oven at 180 °C for two hours. The inoculation needle and other metallic instruments were sterilized by dipping them in alcohol and heating over a flame surface sterilization of plant parts and diseased materials were done by dipping them in 0.1 per cent mercuric chloride for 30 seconds followed by three washings in sterilized water.

Collection and purification of *Alternaria cassia*:- 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 to petriplates containing PDA. In each plate five pieces were kept in three replications. The plates were incubated at 28±2 °C and examined for the association of fungi after 10 days. The associated fungi were re-isolated, purified and identified. The diseased plant bits were examined and associated fungi were recorded and percent associated was calculated. The pathogen *Alternaria cassiae* was purified by Single spore isolation. The fungus was sub cultured on potato dextrose agar slants and allowed to grow at 28±2 °C degree celcius for 10 days and such slant were preserved in the refrigerator at 4 degree celcius and sub cultured once in 30 days. Such cultured was used throughout the study period. The identification of the isolated fungus was done on the basis of morphological characters.

Methodology

Present investigation was carried out using poisson food technique to evaluate the efficacy of botanicals. The fresh plant samples of 100 gm 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 percent 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, 30 percent concentration. 20 ml of this PDA medium was poured aseptically into each sterilized petri plates. All the plates were inoculated with 5 mm mycelial disc of 7 days old actively growing culture of *A. cassiae*. Each treatment was replicated thrice using stastical model CRD, and incubated at 28±2°C for 8 dyas. To test the spore germination, two drops of each suspension at 10, 20, 30 percent concentration along with spores were placed in a cavity slide and incubated at for 24 hours, germination percentages were recorded. Percent growth inhibition of pathogens was calculated as described by Vincent (1947) [8]

$$I = C - T / C \times 100$$

Where,

I= Percent inhibition

C= Radial growth of control

T= Radial growth in treatment

Result and Discussion

Findings revealed that babool leaf extract amended medium showed (43.33mm) radial growth of the test fungus at 10% concentration that was minimum against maximum (90mm) in control. Thuja and Bougainvillea were significantly at par and stood second in order of efficiency where (48mm) and (54mm) radial growth was recorded. Karanj and camera lantana leaf extract recorded (56.66mm) and (59.33mm) radial growth of the test fungus respectively. Rest of the treatments were comparatively less effective for the inhibition of mycelial growth of the pathogen. Babool leaf extract at 20% concentration inhibited the mycelial growth of *A. cassiae* significantly higher over control followed by Thuja and Bougainvillea of the test fungus was (42.66mm), (46mm), and (46mm) respectively against maximum (90.00mm) in control and was followed by Karanj (52.66mm) and camera lantana (46.66mm). The Minimum (39.33mm) radial growth of *A. cassiae* was recorded in Babool leaf extract followed by Thuja (46mm), Bougainvillea (42.66mm) and Nilgiri (48mm) against maximum (90.00mm) in control after 10 days at 30% concentration. All the botanicals inhibit the sporulation and spore germination. Out of this Babool, Thuja, and Camera lantana was the most effective. The sporulation of Babool, Thuja, and Camera lantana at all the concentration was moderate to poor. Also at 30% concentration all the botanicals showed poor sporulation.

In spore germination Babool found to be most effective followed by Thuja and Camera lantana. Spore germination percentage of Babool at 10% was (12), 20% (9.3), 30% (5.3) was observed. Thuja significantly inhibited the spore germination percentage at 10% (9.3), 20% (5.3) and 30% was (4). Spore germination percentage of *camera lantana* at 10 % was (20), 20% was (18.6), 30% was (4) respectively. The antifungal activity of Neem, Datura, and Polyathia against *Alternaria brassicicola* has been also reported by Shivpuri *et al.* (1997) [6]. Similarly Polyathia and Azadirachta effectively inhibited the mycelial growth of *Alternaria solani* (Seetharaman *et al.*, 2001) [5]. The efficacy of leaf extracts of *Eucalyptus globulus*, *Datura stramonium*, and *Azadirachta indica* was reported against *Alternaria alternata* by Chaudhary *et al.*, (2003) [1]. Inhibitory effect of *Datura stramonium*, *Eucalyptus globules* and *Azadirachta indica* against *A. burnsii* was also observed by Patni and Kolte (2006) [4].

Table 1: The list of botanicals used *in-vitro*

S. No	Botanicals	Common name	Plant parts used
1	<i>Azadirachta indica</i>	Neem	Leaf
2	<i>Pongamia pinnata</i>	Karanj	Leaf
3	<i>Bougainvillea sp.</i>	Bougainvillea	Leaf
4	<i>Acacia nilotica</i>	Babool	Leaf
5	<i>Eucalyptus globules</i>	Nilgiri	Leaf
6	<i>Polyanthia longifolia</i>	Ashoka	Leaf
7	<i>Lawsoniainermeris</i>	Heena	Leaf
8	<i>Thuja compacta</i>	Tessu	Leaf
9	<i>Camera lantana</i>	Camera lantana	Leaf

Table 2: Efficacy of botanicals on mycelial growth, and percent growth inhibition of *A. cassiae*

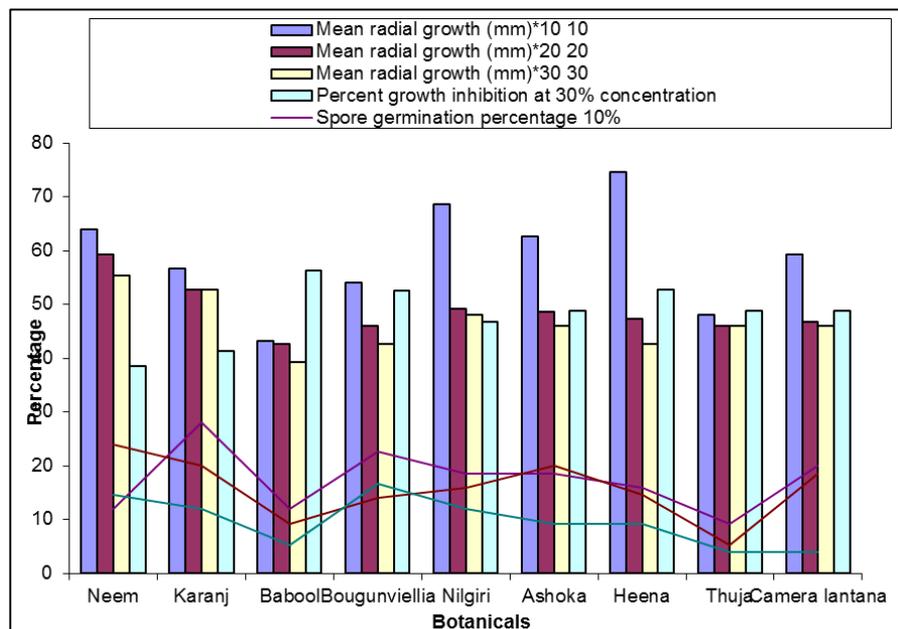
S. No	Plants	Mean radial growth (mm)*			Percent growth inhibition at 30% concentration
		Concentration (%)			
		10	20	30	
1	Neem	64.0	59.3	55.3	38.5
2	Karanj	56.7	52.7	52.7	41.4
3	Babool	43.3	42.7	39.3	56.3
4	Bougunviellia	54.0	46.0	42.7	52.6
5	Nilgiri	68.7	49.3	48.0	46.7
6	Ashoka	62.7	48.7	46.0	48.9
7	Heena	74.7	47.3	42.7	52.7
8	Thuja	48.0	46.0	46.0	48.9
9	Camera lantana	59.3	46.7	46.0	48.9
	Control	90.0	90.0	90.0	
	S. Em±	0.515	0.557	0.471	
	C. D. (P = 0.05%)	1.523	1.644	1.396	

Table 3: Efficacy of botanicals on sporulation and spore germination percentage of *A. cassiae*

S. No	Plants	Sporulation			Spore germination percentage		
		10%	20%	30%	10%	20%	30%
1	Neem	+++	++	+	12.0	24.0	14.6
2	Karanj	+++	++	+	28.0	20.0	12.0
3	Babool	++	+	+	12.0	9.3	5.3
4	Bougunviellia	+++	++	+	22.6	14.0	16.6
5	Nilgiri	+++	++	+	18.6	16.0	12.0
6	Ashoka	+++	++	+	18.6	20.0	9.3
7	Heena	+++	++	+	16.0	14.6	9.3
8	Thuja	++	+	+	9.3	5.3	4.00
9	Camera lantana	+++	++	+	20.00	18.6	4.00

*Mean of three replication

-nil, +poor, ++Moderate,+++ good, ++++Abundant

**Fig 8:** Efficacy of botanicals on sporulation and spore germination percentage of *A. cassiae***Conclusion**

Inhibition of Mycelial growth, sporulation and spore germination percentage of *A. cassiae* was minimum in Babool leaf extract followed by thuja leaf extract.

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