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Bioefficiency of Botanicals against *Colletotrichum lindemuthianum* causing Anthracnose in Blackgram

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A B S T R A C T

Black gram anthracnose caused by *Colletotrichum lindemuthianum* is the most devasting disease. Due to its severe infection during humid climatic conditions spraying of chemical fungicides is essential to control the disease. Frequent application of synthetic chemicals, leads to the detoriation of environment and development of resistance. To overcome these situations, a study was carried out to examine the effect of twelve plant extracts *viz*., *Acalypha indica, Ocimum sanctum, Coleus amboinicus, Phyllanthus niruri, Tribulus terrestris, Allium sativum, Parthenium hysterophorus, Lawsonia inermis, Senna alexandrina, Azadirachta indica, Anisomeles malabarica, Zingiber officinale* were tested against the blackgram anthracnose under *in vitro* condition through poison food technique at 5% and 10% concentration. Among the plant extracts tested, *Anisomeles malabarica* recorded lowest mycelial growth of 3.2 cm and 1.0 cm and highest mycelial inhibition of 64.4% and 88.8% followed by *Allium sativum* recorded the mycelial growth of 3.5 cm and 3.0 cm and mycelial inhibition of 61.1% and 66.6 % against control @ 5% and 10% concentration respectively.

Introduction

Black gram (*Vigna mungo* (L.) Hepper) popularly known as urd bean, mash, black maple is an annual semi erect to spread herb of the *Fabaceae* family grown in tropical and sub-tropical countries as a kharif crop (Gopalan *et al.*, 1971). Dehulled and defatted flour of urdbean (*V. mungo),* contains 25% protein and majorly rich in globulins (63%) (Mahajan *et al.*, 1988). In India, black gram is cultivated around 4.47 million hectares with a production of 2.83 million tones and a productivity of 632 kg ha^{-1}. In Tamil Nadu, black gram is cultivated in 4.30 lakh hectares with a production of 2.74 lakh tones and an

average productivity of 637 kg ha^{-1} (Indiastat, 2019). Black gram anthracnose caused by *C. lindemuthianum* is one of the most devasting fungal diseases which causes more yield losses. In India, the pathogen particularly under cool and humid environmental conditions, results in 80 to 100% yield losses (Sharma *et al*., 2007). The teleomorph stage of fungus is *Glomerella* and both teleomorph and anamorph stages were widely prevalent in hot and humid climate. Nowadays anthracnose disease in black gram which shows severe yield losses and it can affect the plant at all stages. Symptoms appear mostly on leaves with water-soaked lesions with brick red colour and the lesions also appears on twigs and stem. On severe cases it leads to defoliation.

Anthracnose disease can be easily and effectively controlled by using the synthetic fungicides like Benomyl, Mancozeb etc. Synthetic pesticides lead to environmental side effects like, the production of resistant strains of pathogens and toxicity to non-target species. To overcome this, botanical extracts were tested to control phytopathogens. Many scientists were reported that extracts of many higher plants showed antifungal and antibacterial activity against plant pathogens. Since, plant-based products are highly degradable and non-hazardous to environment and humans, plant extracts can be used as alternative to synthetic fungicides (Varma and Dubey, 1999), the present study was carried out to manage black gram anthracnose pathogen using plant extracts.

Materials and Methods

Isolation of pathogen

Black gram leaves with typical anthracnose symptoms were collected from the field of Agricultural College and Research Institute, Killikulam. A small portion of 4-5 mm size of infected leaf was cut and sterilized with 0.1% mercuric chloride solution and then rinsed with sterile water thrice. The sterilized leaf bits were dried by placed on sterilized blotter paper and then transferred to the Petri plates containing sterilized Potato Dextrose Agar (PDA) medium under aseptic conditions. The plates were incubated at 28±2℃ for a period of ten days for growth. Afterwards, the culture was confirmed morphologically by observing the conidia under microscope and compared with reference cultures.

In vitro efficacy of Plant extracts against *C. lindemuthianum*

Preparation of plant extracts

Twelve medicinal plants *viz.,* Kuppaimeni, Thulasi, Coleus, Keezhanelli, Nerunji, Garlic, Parthenium, Henna, Senna, Neem, Perunthumbai and Ginger were selected and its efficacy against *C. lindemuthianum* was tested through Poisoned Food Technique. Details of botanicals were appended in Table 1.

Five and ten grams of each selected plant were taken and ground separately into a fine paste with equal volume of the sterile water with the help of the sterilized pestle and mortar. Then the extract was first filtered through the double layered cotton muslin cloth and centrifuged for 10 minutes at 5000 rpm. Finally, the supernatant was filtered by using the bacterial proof filter in order to avoid the bacterial contamination.

Effect of plant extracts on the mycelial growth of *C. lindemuthianum*

To get five and ten percent concentration of plant extracts five and ten ml of each plant extracts were mixed with 95 and 90 ml PDA medium respectively. Then they were poured onto the sterilized Petri plate and allowed to

solidify. A nine mm size of mycelial disc of *C. lindemuthianum* was taken from the periphery of the 10 days old culture by using sterilized cork borer and then placed at the centre of the Petri Plates. Plates containing PDA medium without plant extracts and inoculated with the mycelial disc of *C. lindemuthianum* (test fungus) served as control. Three replications were maintained in each treatment. Then the plates were incubated at the room temperature of 28±2℃ until the control plate were fully covered with the test fungus. The radial growth of the mycelium was measured in each treatment on 10 days after inoculation when the fungus was fully grown (9cm) in the control plate. The mean diameter of the mycelial growth of the pathogen was recorded and the percent inhibition was calculated by using the formula,

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

where,

I = Per cent inhibition

C = Fungal growth in control plate (mm)

T = Fungal growth in treatment plate (mm)

Results and Discussion

Twelve plant extracts viz., *Acalypha indica, Ocimum sanctum, Coleus amboinicus, Phyllanthus niruri, Tribulus terrestris, Allium sativum, Parthenium hysterophorus, Lawsonia inermis, Senna alexandrina, Azardirachta indica, Anisomeles malabarica, Zingiber officinale* at 5 and 10% concentration were tested against black gram anthracnose pathogen *C. lindemuthianum* under *in vitro* condition and the results are appended in Table.1.

Among the plant extracts tested, *Anisomeles malabarica* recorded lowest mycelial growth of 3.2 cm and 1.0 cm and highest mycelial inhibition of 64.4% and 88.8% followed by *Allium sativum* recorded the mycelial growth of 3.5 cm and 3.0 cm and mycelial inhibition of 61.1% and 66.6 % against control @ 5% and 10% concentration respectively (Fig.1 &Fig.2).

Antifungal activity of various plant extracts has been used to control various plant pathogens reported by many scientists. *Invitro* studies were carried out against *C. lindemuthianum* by using the plant extracts of Garlic bulb extract, Neem leaf extract, Ginger rhizome extract, Dhatura leaf extract, Mehandi leaf extract (Choudhary *et al.*, 2017). Botanicals like garlic, onion, ginger, neem, mehandi, parthenium, bougainvillea were also reported as fungistatic against several *Colletotrichum* species causing anthracnose disease (Chavan *et al.*, 2016). Ethanolic extract of *Datura stramonium* showed significant antifungal potential against plant pathogenic fungi and thus could be used as alternate to chemical fungicides for management of fungal infection in plants (Sharma and Sharma, 2013). In the present study also extracts of *Anisomeles malabarica* at 5 and 10 percent concentration showed highest inhibition (64.4% and 88.8%) of *Colletotrichum lindemuthianum* against control.

From this study, it was concluded that 5 and 10 percent concentration of *Anisomeles malabarica* leaf extract was found to be most effective against blackgram anthracnose under *invitro* condition when compared to untreated control. Use of plant extract for the control of plant pathogen is less economical and less harmful to the environment.

Table.1 List of botanicals tested against *C. lindemuthianum*

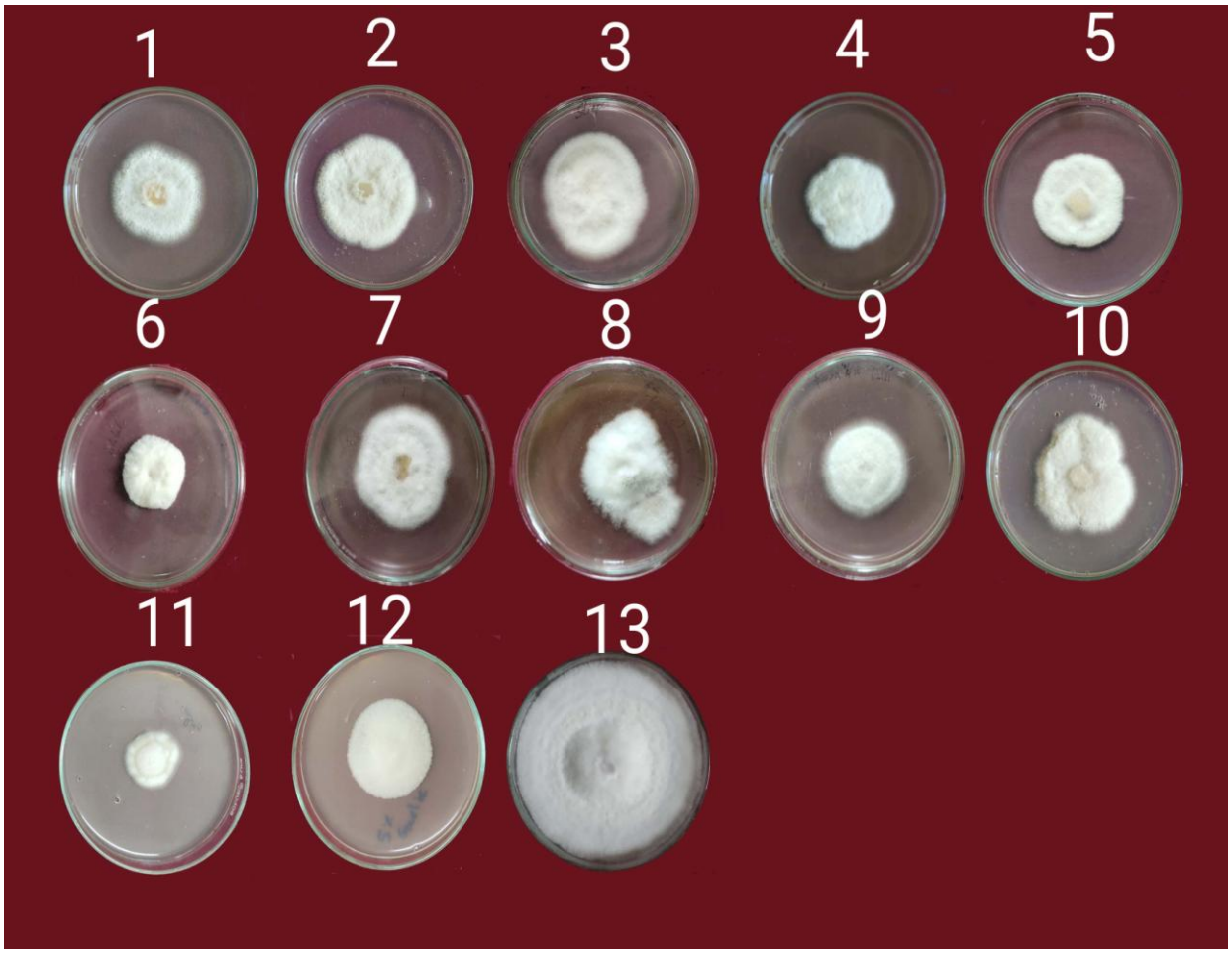
S. No	Scientific Name	Common Name	Parts used
1.	*Acalypha indica*	Kuppaimaeni	Leaf
2.	*Ocimum sanctum*	Thulasi	Leaf
3.	*Coleus amboinicus*	Coleus	Leaf
4.	*Phyllanthus niruri*	Keezhanelli	Leaf
5.	*Tribulus terrestris*	Nerunji	Leaf
6.	*Allium sativum*	Garlic	Bulb
7.	*Parthenium hysterophorus*	Parthenium	Leaf
8.	*Lawsonia inermis*	Henna	Leaf
9.	*Senna alexandrina*	Senna	Leaf
10.	*Azadirachta indica*	Neem	Leaf
11.	*Anisomeles malabarica*	Perunthumbai	Leaf
12.	*Zingiber officinale*	Ginger	Rhizome

Table.1 *In vitro* assay of different plant extracts against black gram anthracnose caused by *Colletotrichum lindemuthianum*

S.No	Scientific name	Common name	Mycelial growth (cm)*		Mycelium growth inhibition over control (%)*	
			5%	10%	5%	10%
1	*Acalypha indica*	Kuppaimaeni	3.7^{c}	5.9^{g}	58.8 $(50.1)^{bc}$	34.4 $(35.9)^{g}$
2	*Ocimum sanctum*	Thulasi	4.5^{d}	4.4^{e}	50.0 $(44.9)^{d}$	51.1 $(46.9)^{e}$
3	*Coleus amboinicus*	Coleus	5.3^{e}	4.5^{e}	41.1 $(39.44)^{e}$	50.0 $(44.9)^{e}$
4	*Phyllanthus niruri*	Keezhanelli	4.5^{d}	6.0^{d}	50.0 $(44.9)^{d}$	33.3 $(48.6)^{d}$
5	*Tribulus terrestris*	Nerunji	4.2^{d}	3.6^{c}	53.3 $(48.2)^{d}$	60.0 $(50.4)^{c}$
6	*Allium sativum*	Garlic	3.5^{b}	3.0^{b}	61.1 $(51.4)^{ab}$	66.6 $(54.7)^{b}$
7	*Parthenium hysterophorus*	Parthenium	5.0^{e}	5.2^{d}	44.4 $(41.5)^{e}$	42.2 $(48.8)^{d}$
8	*Lawsonia inermis*	Henna	4.1^{d}	3.5^{c}	54.4 $(46.8)^{d}$	61.1 $(51.4)^{c}$
9	*Senna alexandrina*	Senna	4.4^{d}	4.9^{f}	51.1 $(46.9)^{d}$	45.5 $(42.4)^{f}$
10	*Azadirachta indica*	Neem	5.6^{e}	3.1^{b}	37.7 $(38.5)^{e}$	65.5 $(54.0)^{b}$
11	*Anisomeles malabarica*	Perunthumbai	3.2^{a}	1.0^{a}	64.4 $(53.3)^{a}$	88.8 $(69.5)^{a}$
12	*Zingiber officinale*	Ginger	4.6^{d}	3.8^{d}	48.8 $(47.5)^{cd}$	57.7 $(45.63)^{d}$
13	Control(untreated)		9.0	9.0	0.00	0.00
	CD (P=0.05)		0.479	0.480	2.809	1.208

*Mean of three observations
The treatment means are compared using Duncan multiple range test (DMRT)
Values in parentheses are arc sine transformed
In a column, mean followed by a common letter (s) are not significantly different (p=0.05)

Figure.1 *In vitro* assay of different plant extracts against black gram anthracnose caused by *C. lindemuthianum* at 5% concentration



1. *Acalypha indica*
2. *Ocimum sanctum*
3. *Coleus amboinicus*
4. *Phyllanthus niruri*
5. *Tribulus terrestris*
6. *Allium sativum*
7. *Parthenium hysterophorus*
8. *Lawsonia inermis*
9. *Senna alexandrina*
10. *Azadirachta indica*
11. *Anisomeles malabarica*
12. *Zingiber officinale*
13. *Control.*

Figure.2 *In vitro* assay of different plant extracts against black gram anthracnose caused by *C. lindemuthianum* at 10% concentration

1.Acalypha indica
2.Ocimum sanctum
3.Coleus amboinicus
4.Phyllanthus niruri
5.Tribulus terrestris
6.Allium sativum
7. Parthenium hysterophorus
8. Lawsonia inermis
9. Senna alexandrina
10. Azadirachta indica
11.Anisomeles malabarica
12. Zingiber officinale
13. Control.

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