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Efficacy of fungicides and botanicals against *Colletotrichum lagenarium* (pass.) Ellis & Halsted causing anthracnose of bottle gourd

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Abstract
Bottle gourd (*Lagenaria siceraria*) is one of the most important cucurbitaceous vegetable crop grown extensively throughout the world. The anthracnose of bottle gourd caused by *Colletotrichum lagenarium* (Pass.) Ellis & Halsted causes extensive damage rendering its cultivation difficult, required effective management. Therefore, the present investigation was carried out to evaluate fungicides and botanicals for eco-friendly management of the disease. The experiment was carried out through standard procedure of poison food technique under laboratory conditions and through foliar spray under screen house conditions. Seven fungicides and six botanicals were evaluated under laboratory conditions against pathogen. The results reported that, carbendazim and propiconazole completely inhibited mycelial growth up to 100 per cent at all the three concentrations. Copper oxychloride was found least effective among all seven fungicides. Among the botanicals evaluated neem leaf extract was found most effective in inhibition of fungal growth up to 75.56 and 69.72 per cent at 20 and 10 per cent concentrations (w/v), respectively. Garlic leaves extract at 20 per cent concentration (w/v) inhibited mycelial growth up to 67.50 percent. The best performing two fungicides and two botanicals when tested on two bottle gourd varieties *viz.*, Pusa summer prolific long and Pusa naveen under screen house conditions, significantly controlled disease when sprayed on 45 and 60 days old vines of Pusa summer prolific long (PSPL) and Pusa naveen varieties of bottle gourd.

Keywords: Bottle gourd, *Colletotrichum lagenarium*, disease severity, mycelium

Introduction
Bottle gourd (*Lagenaria siceraria*) is one of the most important cucurbitaceous vegetable crop being grown both during warm and rainy season in northern parts of India. It has wide genetic diversity and is grown throughout the tropics and subtropics of the world. In India, bottle gourd is cultivated in an area of 103.23 thousand ha with productivity of 17.61 ton/ha (Anonymous, 2016) [1]. In Haryana, bottle gourd is cultivated during summer and rainy season. Bottle gourd is prone to various fungal, bacterial and viral diseases. Among them anthracnose caused by *Colletotrichum lagenarium* (Pass.) Ellis & Halsted is most serious fungal disease that causes maximum damage in bottle gourd. This disease regularly occurs in different bottle gourd growing area during both the seasons. The pathogen is seed borne in nature but initiation as well as spread of disease largely depends upon the environmental factors. This disease is widespread under both greenhouse and field cultivation resulting in poor fruit quality and yield. Direct infection on the fruit also resulted in loss of market value. Synthetic pesticides are being used in general to manage anthracnose of bottle gourd and only few reports are available in the literature for prevention of *C. lagenarium* using plant extracts. Gopinath *et al*. (2006) [4] reported that propiconazole was found most effective in inhibition of mycelial growth event at low concentration. Bavistin completely inhibited mycelial growth at 250 ppm concentrations (Kumar 2008; [6] Gawade *et al*. 2009) [3]. Meera *et al*. (2004) [8] reported that extract of *Allium sativum* completely inhibited the mycelial growth and conidial germination of *Colletotrichum capsici* and its 20 per cent spray on chilli crop gave maximum control of disease and higher yields. Gawade *et al*. (2009) [3] recorded highest inhibition (72.56%) of mycelial growth of *Colletotrichum truncatum* by neem followed by parthenium (61.31%), mehandi (46.03%) and bougainn veilia (28.98%). The extent of damage inflicted by the disease has necessitated conducting studies on some important aspects of the disease. The evaluation of new fungicide and few plant extracts is also emphasized to identify the most efficacious ones.

The aim of the present investigation was to evaluate the host resistance and antifungal activities of plant extracts and fungicides to manage bottle gourd anthracnose. Keeping in view the importance of this disease in this region, the present study has been taken up with the following objectives to evaluation of fungicides and botanicals against *C. lagenarium* causing anthracnose of bottlegourd under laboratory and screen house conditions.

Materials and Methods

In vitro evaluation of fungicides against *Colletotrichum lagenarium*

The efficacy of seven fungicides *viz*, manozeb, carbendazim, copper oxychloride, hexaconazole, propiconazole, chlorothalonil and carboxin + thiram was evaluated against *C. lagenarium* by poison food technique (Mayer, 1962) [7]. Sock solution of each fungicide was prepared in double strength *i. e.* 100, 200 and 500 ppm by dissolving weighed or measured quantity of fungicide in a measured volume of sterilized water. The double strength potato dextrose agar medium was also prepared and sterilized at 15 lbs pressure for 20 minutes. An equal volume of each test chemical solution and PDA was mixed in a sterilized conical flask and poured aseptically in the Petri plates. After solidification of medium, each Petri plate was centrally inoculated with 5 mm disc of fungus taken from 10 days old culture of *C. lagenarium* with the help of sterilized cork borer and incubated at 25±1 °C. Suitable controls were maintained for each chemical. Four replications of each fungicide were maintained and completely randomized design (CRD) was followed. Colony diameter of the fungus of each treatment along with control was recorded with metric scale (mm) till the fungus of controlled treatment occupied the full area of Petri plate within which it was growing. The per cent inhibition of mycelial growth over control was calculated by following formula given by Vincent (1947) [12].

$$\text{Growth inhibition (\%)} = \frac{(C\text{-}T)}{C} \times 100$$

Where,
C= Radial growth of *C. lagenarium* mycelium in control.
T= Radial growth of *C. lagenarium* mycelium in treatment.

In vitro evaluation of plant extracts

The efficacy of six aqueous plant extracts of neem leaves, onion leaves, garlic leaves, jamun leaves, lantana leaves and safeda were evaluated against *C. lagenarium* under *in vitro* conditions using the standard procedure of poison food technique as given by Mayer (1962) [7]. Fresh leaves of respective plants were collected and washed thoroughly with distilled water. The leaves were macerated in appropriate volume of water separately in a pestle mortar (1:1 w/v to obtain 100 per cent concentration of plant extract). The macerated material obtained was passed through double fold muslin cloth. The filtrate obtained after passing through muslin cloth was filtered through Whatman filter paper number 1. The filtrate was centrifuged at 10,000 rpm for 20 minutes. The supernatant obtained were filtered through Millipore filter of 0.22 µm porosity attached to a glass syringe of 20 ml. The plant extract obtained were collected in sterile flask for further use. Stock solution of each plant extract was further diluted to desired concentrations of 10, 20 and 40%. The per cent growth inhibition of *C. lagenarium* by different plant extracts was calculated with the formula given by Vincent (1947) [12] as mentioned earlier.

Evaluation of plant extracts and fungicides on disease control under screen house conditions

The experiment was conducted on bottle gourd cultivars Pusa Naveen and PSPL during *kharif*, 2016, in screen house of Department of Plant Pathology, CCS HAU, Hisar was sown on 15th June 2016 and two vines per pot were maintained in completely randomized design with four replications. Plant extracts (neem and garlic) at 20% (w/v) and fungicides (carbendazim 50% WP and propiconazole 25% EC) at 100 ppm concentration were sprayed twice (30th July and 15th August, 2016) on the vines. The first spray (pre inoculation) of plant extracts and fungicides was done at 4-5 leaf stage of bottle gourd. Vines were inoculated with standard spore suspension of pathogen after one week of first spray of plant extracts and fungicides. Second spray (post inoculation) of plant extracts (neem and garlic) and fungicides (carbendazim 50% WP and propiconazole 25% EC) was done one week after pathogen inoculation.

Disease intensity (%) was recorded on 30th August and 15th September, 2016 using 0-4 scale as suggested by Chauhan, (2002) [2].

Grade	Description
0	No spots/leaf or fruit
1	1-10 pots/leaf or fruit
2	11-20 spots/leaf or fruit
3	21-50 spots/leaf or fruit
4	More than 50 spots/leaf or fruit

Disease intensity (%) was calculated by using the formula:

$$\text{Disease intensity (\%)} = \frac{\text{Sum of all numerical ratings}}{\text{Total no.of leaves or fruits observed} \times \text{Maximum rating}} \times 100$$

Results and Discussion

In vitro evaluation of fungicides

The evaluation of seven fungicides was tested *in vitro* conditions; carbendazim and propiconazole completely inhibited mycelia growth up to 100 per cent at 100 ppm concentration (Table 1), while chlorothalonil at 500 ppm concentration completely inhibited up to 100 per cent. Hexaconazole provided inhibition up to 57.22 per cent even at 500 ppm concentration. Copper oxychloride was found least effective among all seven fungicides, as this fungicide inhibited 26.39 per cent of mycelia growth even at 500 ppm concentration.

Table 1: Evaluation of fungicides against *C. lagenarium in vitro*

Fungicides	Growth inhibition (%)			
	Concentration (ppm)**			Mean
	100	200	500	
Carbendazim (50% WP)	100(89.39)*	100(89.39)	100(89.39)	100(89.39)
Mancozeb (75% WP)	21.94(27.91)	28.33(32.14)	40.83(39.67)	30.37(33.25)
Hexaconazole (5% EC)	23.06(28.65)	39.45(38.88)	57.22(49.14)	39.91(38.89)
Carboxin + thiram(75% WP)	43.33(41.15)	59.45(50.43)	81.11(64.25)	61.27(51.94)
Chlorothalonil (75% WP)	42.22(40.51)	79.72(63.24)	100(89.39)	73.98(64.38)
Propiconazole (25% EC)	100(89.39)	100(89.39)	100(89.39)	100(89.39)
Copper oxychloride (50% WP)	0.01(0.57)	0.01(0.57)	26.39(30.88)	8.78(10.68)
Control	0.01(0.57)	0.01(0.57)	0.01(0.57)	0.01(0.57)
Mean	41.32(39.77)	50.87(45.58)	63.19(56.59)	
	Treatment(F)	Concentration(C)	Interaction (F X C)	
C. D (p=0.05)	1.37(0.86)	0.84(0.53)	2.38(1.50)	
SE(m)	0.48(0.30)	0.29(0.18)	0.84(0.53)	

Figures in parenthesis are angular transformed values ****** mean of three replications

***In vitro* evaluation of botanicals**

Efficacy of six plant extract was tested *in vitro;* neem leaf extract was found most effective in inhibition of fungal growth up to 75.56 per cent at 20% (w/v) concentration (Table 2).Garlic leaf extract at 20 per cent (w/v) that recorded 67.50% growth inhibition at 20% concentration. Among all the six plant extracts safeda leaves extract was found least effective in inhibition of mycelial growth, up to 30.56 per cent even at 20% (w/v) concentration.

Table 2: Evaluation of plant extracts against *C. lagenarium in vitro*

Plant extracts	Mycelial growth inhibition (%)			
	Concentration%(w/v)**			Mean
	5	10	20	
Neem leaves	59.72 (50.59)*	69.72 (56.60)	75.56 (60.36)	68.33 (55.85)
Onion leaves	33.89 (35.58)	51.39 (45.78)	60.83 (51.24)	48.70 (44.20)
Garlic leaves	45.84 (42.59)	56.67 (48.81)	67.50 (55.23)	56.67 (48.88)
Jamun leaves	26.39 (30.87)	37.50 (37.74)	51.95 (46.10)	38.61 (38.24)
Lantana leaves	23.61 (29.04)	37.78 (37.91)	55.00 (47.85)	38.80 (38.27)
Safeda leaves	10.56 (18.87)	17.22 (24.47)	30.56 (33.53)	19.44 (25.63)
Control	0.01 (0.57)	0.01 (0.57)	0.01 (0.57)	0.01 (0.57)
Mean	28.57 (29.73)	38.61 (35.98)	48.77 (42.13)	
	Treatment (T)	Concentration (C)	Interaction (TXC)	
C.D (p=0.05)	1.64 (1.06)	1.07 (0.69)	2.85 (1.83)	
SE (m)	0.58 (0.37)	0.38 (0.24)	1.00 (0.64)	

Figures in parenthesis are angular transformed values, ** mean of three replications

Evaluation of fungicides and plant extracts against *Colletotrichum lagenarium* under screen house conditions

The best result obtained under *in vitro* conditions was the fungicides carbendazim (100 ppm), propiconazole (100 ppm) and botanicals evaluated under screen house conditions, significantly controlled disease when sprayed on 45 and 60 days old vines of Pusa summer prolific long (PSPL) and Pusa naveen varieties of bottle gourd. The observations on anthracnose severity (%) are presented in the Table 3.

Table 3: Efficacy of plant extracts and fungicides against anthracnose disease under screen house conditions

Treatment	Per cent disease intensity			
	PSPL		Pusa naveen	
	30-Aug	15-Sept	30-Aug	15-Sept
Carbendazim (100 ppm)	7.19 (15.47)*	21.56 (27.64)	5.63 (13.61)	16.56 (23.97)
Propiconazole (100 ppm)	9.38 (17.78)	23.44 (28.90)	8.76 (16.87)	20.94 (27.20)
Garlic leaves (20%)	20.63 (26.99)	50.49 (45.52)	18.13 (25.17)	52.19 (46.25)
Neem leaves (20%)	25.33 (30.16)	55.94 (48.41)	22.19 (28.06)	56.25 (48.58)
Control	49.38 (44.62)	84.00 (66.44)	43.25 (41.10)	82.88 (65.57)
	Variety	Date of spray	Treatment	
C. D (p=0.05)	1.25 (0.85)	1.25 (0.85)	1.98 (1.34)	
SE (m)	0.44 (0.30)	0.44 (0.30)	0.70 (0.47)	

Figures in parenthesis are angular transformed values

The disease intensity was minimum on 30th August (7.19 and 5.63% on PSPL and Pusa Naveen variety, respectively) when sprayed with carbendazim 50% WP at 100 ppm concentration followed by propiconazole 25% EC (100 ppm) which recorded 9.38 and 8.76% disease intensity on PSPL and Pusa Naveen variety, respectively. It was observed that anthracnose intensity increased with passage of time even after spraying. The observation recorded on 15th September revealed that carbendazim (100 ppm) recorded minimum disease intensity on both the varieties followed by propiconazole (100 ppm)

that resulted in significantly lower disease severity as compared to control. It is also evident from results that garlic and neem leaves extracts (20% w/v) also significantly reduce anthracnose disease in comparison to control, however disease intensity on vines sprayed with plant extracts was higher than sprayed with fungicides.

Several workers have studied the management of disease through chemicals. In the present study, different fungicides were evaluated with a view to find out the suitable fungicides for the inhibition of the growth of *C. lagenarium in vitro*. Result of present study revealed that, among seven fungicides tested *in vitro*; carbendazim 50% WP and propiconazole 25% EC were found most effective and recorded complete inhibition of growth of *C lagenarium* even at 100 concentration followed by chlorothalonil that recorded 100 per cent inhibition of growth at 500 ppm concentration. The results of present investigation are in positive agreement with the findings of Singh *et al*. (2008) that carbendazim was most effective in reducing the *C. gloeosporiodes* incidence on guava. Results also found in positive relation to Prakash (2011) [10] who observed that carbendazim was significantly effective *in vitro* as well as *in vivo* conditions against *C. capsici*. Kamble *et al*. (2015) [5] reported that propiconazole (0.1%) recorded least mean disease incidence and intensity of *C. capsici* on chilli. Mukund (2006) [9] reported that neem leaf extract (50%) reduced the disease severity (11.70%) when sprayed at the appearance of symptoms of bottle gourd anthracnose.

Conclusion

In conclusion, carbendazim was found to be the most effective *in vitro* and under screen house conditions for the control of *C lagenarium* in bottle gourd. So, this fungicide can be used for the control of bottle gourd anthracnose disease.

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