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Efficacy of botanicals against *in vitro* growth of *Corynespora cassiicola*, cause of Target leaf spot in cotton

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ABSTRACT

Corynespora leaf spot (target leaf spot) of cotton caused by *Corynespora cassiicola* (Berk. and Curt.) Wei. emerged as a major disease in cotton growing areas of Andhra Pradesh. Management of the disease by locally available botanicals is an ecofriendly approach in view of resistance developed against effective fungicides. Hence an effort was made to observe the antifungal activity of botanicals *in vitro*. Five aqueous leaf extracts *viz*., neem, datura, lantana, tulasi and pongamia along with commercially available herbal mixture i.e., Ezee cotton (Perfekt) were tested at 5% and 10% concentrations under *in vitro* conditions. Herbal mixture, Perfekt induced complete inhibition (100%) growth of *C. cassiicola* at both the concentrations. At 10% concentration the highest inhibition percent was shown by pongamia leaf extract (51.47%) followed by neem leaf extract (49.98%). At 5% concentration highest per cent of inhibition was shown by neem leaf extract (39.90%) followed by pongamia leaf extract (26.09%).

Key words: *Botanicals,Cotton, Corynespora cassiicola and Mycelial inhibition*

Cotton is referred as 'King of fibres' and also known as 'White Gold'. Cotton is an important commercial crop and has applications ranging from textiles to industrial usage and belongs to the family Malvaceae. Cotton fibre is used as raw material in textile, pulp and paper industries and oil extracted from cotton seed is used in food, chemicals, cosmetics, pharmaceuticals *etc*. Cotton seed cake is used as cattle feed (Proto *et al*., 2000). The cotton hulls serve as roughage for livestock and the fuzz (short seed hair) is used in the manufacture of papers, plastics, carpets, rayon, explosives and cotton wools etc. (Prasad, 2015).

In world, cotton area is 318.78 lakh ha, production is 1429.84 lakh bales and yield is 763 Kg ha^{-1}. The cotton production in India during 2023-24 was estimated to produce 323.11 lakh bales of 170 kg from 124.69 lakh ha with a productivity of 441 kg lint/ha. In Andhra Pradesh, cotton area was 4.27 Lakh ha, with production of 11.58 Lakh bales and productivity of 461 Kg ha^{-1} (ICAR-AICRP (Cotton) Annual Report (2023-24)).

The various diseases that affect cotton in India are Fusarium wilt, Verticillium wilt, root rot, Anthracnose, bacterial blight, Alternaria leaf blight, Corynespora leaf spot, Cercospora leaf spot, Helminthosporium leaf spot, grey mildew, rust and tobacco streak virus disease.

In India, cotton is cultivated under rainfed conditions and the cultivation is highly fluctuating due to biotic as well as abiotic stresses and competition from other crops in recent years. Foliar diseases in cotton (fungal, bacterial, viral and boll rot) have been estimated to cause yield losses upto 20 to 30% in India (Mayee and Mukewar, 2007).

The lint yield loss due to target spot on an apparently susceptible cotton cultivar had been estimated as high as 448 kg lint/ha (Hagan *et al*., 2015). Target spot damage and yield losses can be highly variable (Mehl *et al*., 2017).

MATERIAL AND METHODS

Cotton leaves with typical target leaf spot symptoms were collected from the field. The pathogen confirmation was done by scraping the leaves and then observing under the microscope. Leaf bits of five mm^2 with infected and healthy leaf portion were cut, surface sterilized using 1% sodium hypo chlorite

for a minute and rinsed with three changes of sterile distilled water to remove the disinfectant. Leaf bits were dried on blotting paper, transferred aseptically on to PDA plates and incubated at 27±1°C in an incubator. Fresh tender leaves of healthy plants (100 g) were washed under running tap water followed by sterile distilled water and blot dried. Leaf tissues were macerated with 100 ml of sterile distilled water (1:1) and filtered through double layered sterile muslin cloth to collect leaf extracts. It was centrifuged at 5000 rpm for 10 min to get clear supernatant which was further sterilized through G-3 filter. The resultant extracts were considered as standard leaf extract solution with 100% concentration (Kurucheve *et al.,* 1997). The 5 ml and 10 ml of commercially available herbal mixture was added to 100 ml PDA to obtain 5 and 10% concentrations respectively. Potato dextrose agar (PDA) was prepared and autoclaved at 15 psi for 15 min. 5 and 10 ml of aqueous leaf extracts were added to lukewarm PDA of 100 ml to obtain 5 and 10% concentrations respectively. PDA was transferred to the previously sterilized Petri plates and allowed to solidify. The efficacy of leaf extracts at 5 and 10% concentrations was evaluated by employing poisoned food technique (Grover and Moore, 1962). Mycelial discs of 5 mm diameter from five day old culture was inoculated into the centre of poisoned medium and incubated at 27±1°C. Radial growth of the test fungus was measured at 24 h interval and inhibition per cent was calculated by comparing with control plates using the formula given by Vincent (1927). Three replications of each treatment were maintained. I=((C - T) /C) x100 Where, I = Inhibition per cent, C = radial growth of pathogen in control, T = Radial growth of pathogen in treatment

RESULTS AND DISCUSSION

Observations on the radial growth of *C. cassiicola* were recorded when control culture Petri plate with *C. cassiicola* showed full growth of 9 cm diameter after 13 days of inoculation. There was reduction in growth of *C. cassiicola* in leaf extract amended medium compared to un-amended medium. Variation in the inhibition of mycelial growth by different leaf extracts was observed.

Among the various treatments, the commercially available Herbal mixture, Perfekt showed zero growth of *C. cassiicola i.e.,* complete inhibition (100%) was observed in the two test concentrations. (Table 1) It was found highly effective in inhibiting the mycelial growth. (Plate 1) Among the various leaf extracts, pongamia leaf extract at 10% concentration showed the highest inhibition per cent of 51.47% followed by neem leaf extract (49.98%) and remaining were found less effective in inhibiting growth of *C. cassiicola.* (Fig) The inhibition per cent of mycelial growth were recorded as 30.94 % by lantana leaf extract, 25.72% by datura leaf extract and 19.37% by ocimum leaf extract at 10% concentration.

The per cent inhibition of mycelium at 5% concentration of different leaf extracts ranged between 9.29% and 39.90% (Fig. 1). Among the various leaf extracts at 5%, neem leaf extract showed significant and highest inhibition per cent of 39.90 % followed by pongamia leaf extract (26.09%), lantana leaf extract (15.64 %), datura leaf extract (14.15 %) and ocimum leaf extract (9.29%).

The above results were in accordance with Manju *et al*. (2019) where datura leaf extract showed 31.20% @ 10% concentration. Ishwari *et al.* (2020) reported that Perfekt showed complete inhibition of mycelium of *C. cassiicola in vitro* and datura leaf extract by 12.74%. Paras *et al*. (2023) reported that 10% concentration of neem and datura leaf extracts showed inhibition per cent of 38.53% and 18.95%, against *C. cassiicola* mycelium *in vitro*, respectively.

The contents of Perfekt herbal mixture include Pongamia leaf extract also, which might be one of the reasons for its efficacy as experiment results revealed pongamia leaf extract recorded the higher inhibition of *C. cassiicola* mycelium (51.47%).

In vitro evaluation of different botanicals revealed that the herbal mixture, Perfekt induced complete inhibition (100%) of *C. cassiicola* growth in the two concentrations (5 and 10%). At 10% concentration the highest inhibition per cent was showed by pongamia leaf extract (51.47%) followed by neem leaf extract (49.98%). At 5% concentration the highest inhibition percent was recorded in neem leaf extract (39.90%) followed by pongamia leaf extract (26.09%).

Table 1. Effect of different botanicals against the mycelial growth of *Corynespora cassiicola* in *in vitro*

S. No.	Treatment	Botanical	Mycelium growth (cm)*		Per cent Inhibition	
			5% Conc.	10% Conc.	5% Conc.	10% Conc.
1	T_1	Neem leaf extract	5.37 (2.52)^{b}	4.47 (2.34)^{bc}	39.9	49.98
2	T_2	Datura leaf extract	7.67 (2.94)^{de}	6.63 (2.76)^{e}	14.15	25.72
3	T_3	Lantana leaf extract	7.53 (2.92)^{d}	6.17 (2.68)^{d}	15.64	30.94
4	T_4	Ocimum leaf extract	8.10 (3.02)^{f}	7.20 (2.86)^{f}	9.29	19.37
5	T_5	Pongamia leaf extract	6.60 (2.76)^{c}	4.33 (2.31)^{b}	26.09	51.47
6	T_6	Perfekt	0.00 (1.00)^{a}	0.00 (1.00)^{a}	100	100
7	T_7	Control	8.93 (3.15)^{g}	8.93 (3.15)^{g}		
		SEm±	0.012	0.009		
		CD *p* ±5%	0.04	0.03		
		CV (%)	1.75	1.38		

*Mean of the three replications and figures in parentheses are square root transformed values. Figures with the same alphabets are statistically not significant

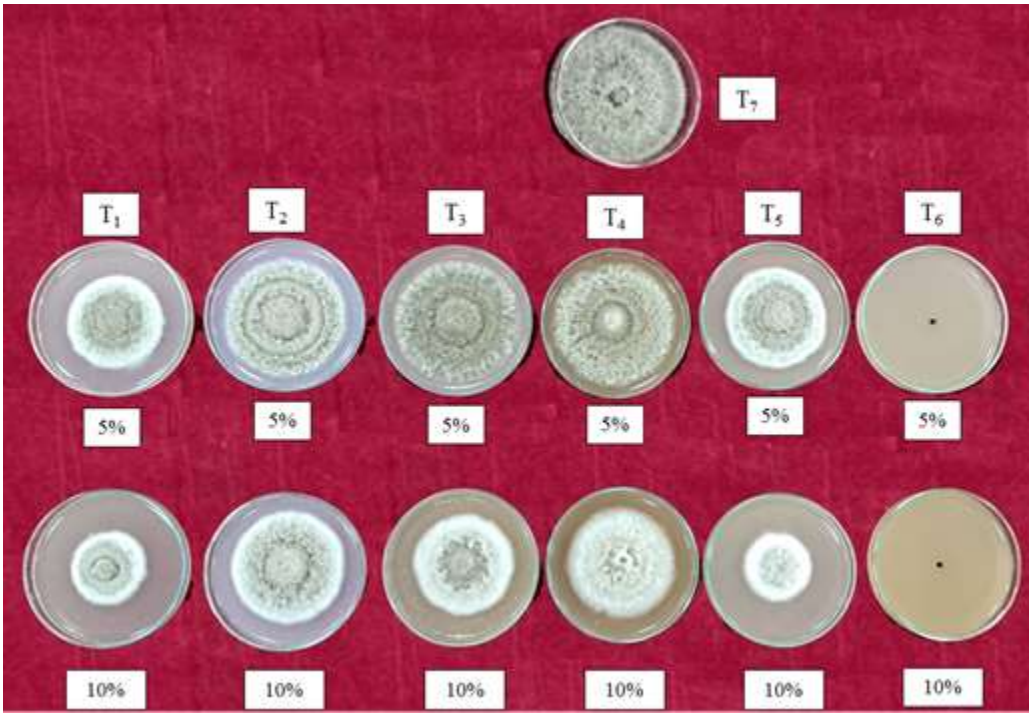

Plate 1. Efficacy of botanicals against the mycelial growth of *Corynespora cassiicola* under *in vitro*

T_1 – Neem Leaf Extract, T_2 – Datura Leaf Extract, T_3 – Lantana Leaf Extract, T_4 –Tulasi Leaf Extract, T_5 – Pongamia Leaf Extract, T_6 –Perfekt, T_7 – Control

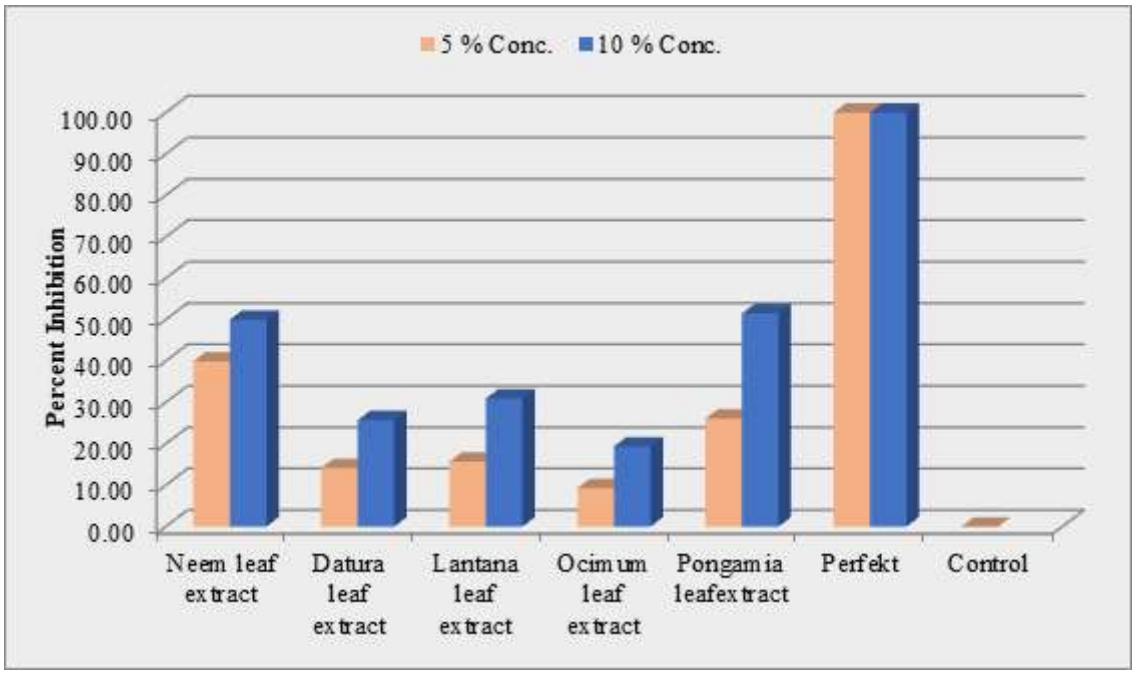

Fig. 1. Per cent Inhibition of mycelia growth of *Corynespora cassiicola* by different botanicals

CONCLUSION

In vitro evaluation of botanicals against *Corynespora cassiicola* causing cotton leaf spot (target leaf spot) revealed that botanicals individually or herbal mixture are effective in inducing mycelial growth inhibition and thus provide alternative diseases management in an eco-friendly way.

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