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In vitro Evaluation of Plant Extracts and Fungicides against *Colletotrichum capsici* causing Anthracnose Disease of Chilli

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ABSTRACT: Anthracnose disease of chilli is one of the major disease of chilli and responsible for causing pre-harvest and post-harvest losses to the produce. Limited availability of the resistant cultivars make the farmers to rely on fungicidal use to control the disease. The present investigation was carried out with an aim to develop some eco-friendly control measures and judicious use of fertilizers. Eight plant extracts *viz.* garlic (*Allium sativum*), marigold (*Tagetes* sp.), neem (*Azadirachta indica*), ginger (*Zingiber officinale*), milk weed (*Calotropis* sp.), ashwagandha (*Withania somnifera*), lantana (*Lantana camara*) and tulsi (*Ocimum tenuiflorum*) were evaluated by poisoned food technique at three different concentrations (5, 10, 20 per cent). Also, eight fungicides *viz*. Difenoconazole, Azoxystrobin, Propiconazole, Copper Oxychloride, Hexaconazole, Mancozeb, Carbendazime + Mancozeb and Pyraclostrobin + Epoxiconazole were evaluated under in vitro conditions at four concentrations (10, 25, 50, 100 ppm). Among all plant extracts neem at 20% was found to be the most effective with maximum growth inhibition (43.37%). Among fungicides Propiconazole and Pyraclostrobin + Epoxiconazole at 100ppm showed the maximum per cent growth inhibition of 100 per cent followed by Difenoconazole with growth inhibition of 87.08 per cent.

Keywords: *C. capsici*, dual culture, botanicals, fungicides.

INTRODUCTION

Anthracnose of chilli caused by *Colletotrichum capsici* is one of the major disease of chilli. Pathogen is responsible for causing the disease in tropical and sub tropical regions of the world including India. The disease cause pre- and post-harvest damage to chilli causing lesions on fruits and reduces the market value. Anthracnose of chilli is one of the major devastating disease of chilli causes severe losses up to 10-60 per cent in yield and quality parameters (Bansal and Grover 1969). Pakdeevaraporn *et al*. (2005) reported that yield losses may occur up to 50 per cent. Characteristically, small-circular lesions are formed on mature fruits. Spots on the leaves leads to premature leaf fall in case of severe infection. Die back symptoms are also reported in which the infection on the growing branches progresses backwards and kill the whole plant (Abeygunawardhana, 1969; Kumar and Bhaskaran 2007). In later stage black dots (acervuli) are formed on necrotic surface which bear concentric rings containing conidial masses.

Due to the limited availability of the chilli cultivars resistant to the disease, farmers mostly rely on fungicides to control disease. But the indiscriminate use of the chemicals for crop protection is leading to their accumulation of residues in soil, water and plants, and also causing the ecological imbalance by killing the beneficial microorganisms (Rajathilagam and Kannabiran 2001). Hence, efforts should also be made to use some eco-friendly disease control measures and judicious use of effective fungicides to maintain the ecological balance. Plant extracts with toxic properties against plant pathogens are now being explored. Several plant species have been identified for showing antifungal activity against the phytopathogens. The plant extracts are responsible for retarding the germination of fungal spores and inhibit the fungal growth (Islam *et al*., 2003). Hence, use of plant extracts is getting a special attention as an ecologically safe method for plant disease management.

No effective management strategies with botanicals or chemicals are available. Therefore, the present investigation was undertaken to find out the effective

botanical and fungicide agent against anthracnose pathogen of chilli under *in vitro* condition. The investigation includes the *in vitro* evaluation of eight plant extracts *viz.,* garlic (*Allium sativum*), marigold (*Tagetes* sp.), neem (*Azadirachta indica*), ginger (*Zingiber officinale*), milk weed (*Calotropis* sp.), ashwagandha (*Withania somnifera*), lantana (*Lantana camara*) and tulsi (*Ocimum tenuiflorum*) and eight different fungicides *viz*., Difenoconazole 25% EC, Azoxystrobin 23% SC, Propiconazole 25% EC, Copper Oxychloride 88% W/W, Hexaconazole 5% EC, Mancozeb 75% WP, Carbendazime 12% + Mancozeb 63% and Pyraclostrobin 12.5% + Epoxiconazole 4.7% SC.

MATERIAL AND METHODS

The present investigation was carried out in department of Plant Pathology, college of Agriculture, JNKVV, Jabalpur (M.P.) during 2021. In vitro evaluation of eight plant extracts was done at three different concentrations (5%, 10% and 20%) while, for fungicides four different concentrations used are 10 ppm, 25 ppm, 50 ppm and 100 ppm. The efficacy of plant extracts and the fungicides were tested by using Poisoned Food Technique described by Nene and Thapliyal (1993) on potato dextrose agar (PDA) medium.

Isolation and identification of *Colletotrichum capsici.* Chilli fruits having anthracnose symptoms were collected from the fields. Isolation was done by cutting small pieces from the margin of lesions which were then surface sterilized by immersing in 1 per cent Sodium Hypochlorite solution for 30 seconds and washed with sterilized distilled water. To remove excess moisture from the samples the pieces were transferred on to sterilized blotter paper. These pieces were then transferred to Petri plates containing PDA medium under aseptic conditions followed by incubation at 26±2ºC.

Purification of *Colletotrichum capsici.* The fungus was further purified by single hyphal tip method. They are grown by inoculating in the centre of a plain agar plate. The fungus spreads out with its hyphal strands in search of nutrients. These hyphal strands could be located under low power of the microscope, and the isolated hyphal tips marked. These tips were carefully transferred to potato dextrose agar slants to obtain the pure cultures of *Colletotrichum capsici.* The culture was maintained by sub-culturing on potato dextrose agar medium at room temperature.

Preparation of Botanicals and Fungicidal solutions. To prepare the plant extract healthy and fresh leaves, cloves or rhizomes of selected plants were taken and washed with tap water followed by sterile distilled water and were chopped into small bits with sterilized sharp knife. Mechanical grinder was used to separately grind each sample and homogenize it with equal quantity of sterile distilled water 1:1 (w/v). The obtained homogenate was strained through double layered sterilized muslin cloth followed by filtration through Whatman's filter paper No. l. The obtained clear leaf extracts serve as the stock solution of 100 per cent and subsequently 5, 10 and 20 per cent concentrations were made. The desired quantity of each extract was separately mixed in molten PDA medium in conical flask to get the desired concentrations (5, 10 and 20 %).

Similarly, stock solution of 1000 ppm for each fungicide was made. The desired quantity of fungicide from stock solution was amended in molted PDA medium to get the concentrations *i.e.* 10 ppm, 25 ppm, 50 ppm and 100 ppm for each fungicide.

The PDA medium amended with plant extract and fungicides were poured separately @ 20ml per Petri plate. After solidification of poisoned medium, the plates were inoculated with 0.5mm mycelium disc of *C. capsici* obtained from seven days old culture of pathogen. Plates containing um-amended medium served as control. The inoculated plates were incubated in B.O.D incubator at 26±2˚C. The colony diameter of culture was recorded when plates under control were fully covered. The efficacy of plant extracts and fungicides was expressed as per cent inhibition of mycelial growth over control, which was calculated by using the following formula (Vincent, 1947).

$$\text{Per cent Inhibition}(PI) = \frac{C - T}{C} \times 100$$

Where,

PI = per cent inhibition over control

C = diameter of fungal growth in control plate

T = diameter of fungal growth in treatment plate.

RESULT AND DISCUSSIONS

***In vitro* evaluation of botanicals.** Eight botanicals viz. Neem (*Azadirachta indica*), Tulsi (*Ocimum tenuiflorum*), Marigold (*Tagetes* sp.), Garlic (*Allium sativum*), Ginger (*Zingiber officinale*), Ashwagandha(*Withania somnifera*), Milk weed (*Calotropis* sp.) and Lantana (*Lantana camara*) were evaluated at three different concentrations *viz*., 5.0, 10.0 and 20.0 per cent concentration by Poisoned food technique. Observations were taken on seventh day (168 hrs) of inoculation.

Data presented in Table 1 shows that at 20 per cent concentration, maximum per cent growth inhibition of mycelium was recorded in Neem with 43.37 per cent and the minimum radial growth of mycelium 42.46 mm followed by Tulsi with 42.22 per cent growth inhibition and 43.33 mm of radial growth. Minimum per cent growth inhibition was recorded in Milk weed, where per cent growth inhibition 16.88 per cent and radial growth of mycelium 62.33 mm was observed.

Similarly, at 10 per cent concentration neem leaf extract showed maximum per cent growth inhibition of 41.77 per cent and minimum radial growth of 43.66 mm and 38.22 per cent growth inhibition with 46.33 mm radial

growth at 5 per cent concentration respectively followed by Tulsi leaf extract. In Tulsi leaf extract, the per cent growth inhibition was 34.22 and 25.77 per cent at 10 per cent and 5 per cent concentration of plant extract with radial growth of 49.33 mm and 55.66 mm, respectively. Milk weed showed minimum inhibition per cent of 15.11 and maximum radial growth of mycelium *i.e.* 63.66 mm at 10 per cent concentration and 11.11 per cent growth inhibition and 66.66 mm radial growth at 5 per cent concentration.

Neem as compared to other botanicals was found to be most effective at all concentrations. But, at 20 per cent concentration it was highly effective as compared to 5 and 10 per cent followed by Tulsi at 20 per cent concentration was found to be effective against the pathogen among other concentrations (Plate 1).

Table 1: Efficacy of botanicals on growth of *C. capsici.*

Trt.	Botanicals	Concentration (%) / Radial growth (mm)			Concentration (%)/ Per cent Growth inhibition (%)		
		5%	10%	20%	5%	10%	20%
T1	Garlic (*Allium sativum*)	54.33	53.83	50.66	27.55	28.22	32.44
T2	Marigold (*Tagetes*sp.)	61.33	58.50	52.33	18.22	22	30.22
T3	Neem (*Azadirachta indica)*	46.33	43.66	42.46	38.22	41.77	43.37
T4	Ginger (*Zingiber officinale*)	54.33	53.33	50	27.55	28.88	33.33
T5	Ashwagandha (*Withania somnifera*)	66	61.66	59.66	12	17.77	20.44
T6	Milk weed (*Calotropis*)	66.66	63.66	62.33	11.11	15.11	16.88
T7	*Lantana camara*	59.66	57	55	20.44	24	26.66
T8	Tulsi (*Ocimum tenuiflorum*)	55.66	49.33	43.33	25.77	34.22	42.22
T9	Control	75	75	75	0	0	0
S.E(m)±		0.67	0.76	0.76	0.83	1.17	1.23
C.D. at 5%		2.00	2.28	2.29	2.66	3.77	3.95

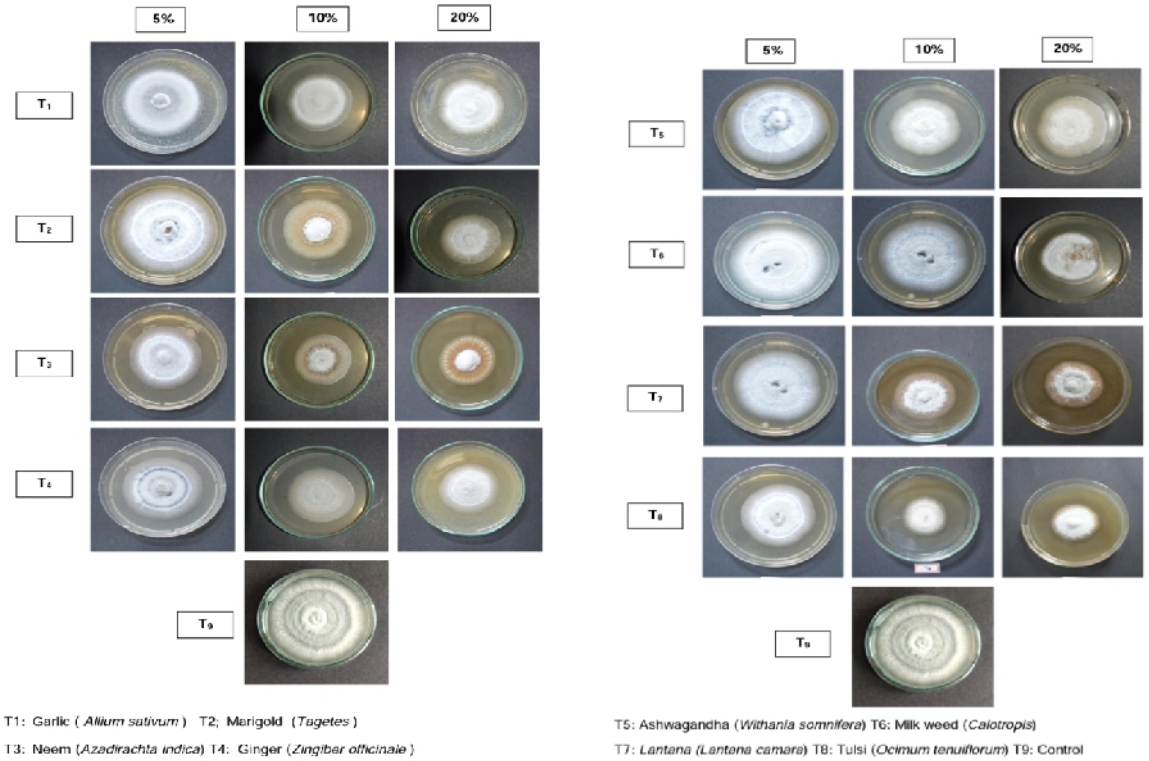

Plate 1: *In vitro* evaluation of botanicals (T1-T9).

***In vitro* evaluation of fungicides.** Eight fungicides and their combinations *viz.*, Difenoconazole 25% EC, Azoxystrobin 23% EC, Propiconazole 25% EC, Copper oxychliride 88% W/W, Hexaconazole 5% EC, Mancozeb 75% WP, Carbendazim 12% WP + Mancozeb 63% WP, Pyraclostrobin 12.5% + Epoxiconazole 4.7% SC were evaluated at four different concentrations *viz.*, 10 ppm, 25ppm, 50ppm and 100ppm for each fungicide by Poisoned food technique. Observations were recorded daily and the final observations were taken after seven days of inoculation.

Data presented in Table 2 shows that with increase in the concentration of the fungicide there is increase in the inhibition of mycelial growth of *C. capsici*. Significantly minimum growth was noticed at 100 ppm

concentration followed by 50 ppm, 25 ppm and 10 ppm. In control *C. capsici* showed the radial growth of 80 mm after seven days of inoculation. At 100 ppm concentration of fungicides maximum per cent growth inhibition of 100 per cent was obtained in two treatments *i.e.* Propiconazole25% EC and Pyraclostrobin + Epoxiconazole without any growth of mycelium followed by Difenoconazole (10.33mm) with growth inhibition of 87.08 per cent, Carbendazim + Mancozeb (11.33mm) with 85.83 per cent growth inhibition, Hexaconazole (18.33mm) with 76.82 per cent growth inhibition, Azoxystrobin (29 mm) with growth inhibition of 63.75 per cent and Mancozeb (29.33) with growth inhibition of 63.33 per cent. The minimum per cent growth inhibition at 100 ppm concentration of fungicides was observed in treatment Copper oxychloride with 41.66 per cent and radial growth of 46.66 mm was recorded.

At 50 ppm concentration of fungicides maximum growth inhibition of 100 per cent was obtained in Propiconazole followed by Pyrachlostrobin + Epoxiconazole with growth inhibition of 96.66 per cent and radial growth 2.66mm. The minimum per cent growth inhibition at 50 ppm concentration of fungicides was observed in treatment with Copper oxychloride 88% W/W where growth inhibition of 36.66 per cent and radial growth of 50.66 mm was recorded.

At 25 ppm concentration of fungicides maximum per cent growth inhibition of 100 per cent was obtained in Propiconazole where no mycelia growth was observed, it was followed by Pyrachlostrobin + Epoxyconazole with growth inhibition of 88.95 per cent and radial growth 8.83mm. The minimum per cent growth inhibition at 25 ppm concentration of fungicides was observed in treatment with Copper oxychloride 34.58 per cent and radial growth of 52.33 mm was recorded.

Similar trend was also recorded with 10 ppm concentration where maximum growth inhibition of 100 per cent was obtained in Propiconazole followed by Pyrachlostrobin + Epoxyconazole with growth inhibition of 83.33 per cent and radial growth 13.33mm. The minimum per cent growth inhibition was observed with Copper oxychloride 88% W/W with growth inhibition of 33.75 per cent and radial growth of 53 mm was recorded.

The complete inhibition in the growth of mycelium was recorded in case of Propiconazole at all the four concentrations (10ppm, 25ppm, 50ppm and 100ppm) while in Pyrachlostrobin + Epoxyconazole complete inhibition was observed at 100 ppm concentration (Plate 2).

Table 2: Mycelial growth and Per cent Inhibition in mycelial growth over control at different concentrations of fungicides.

Treatment	Fungicides	Concentration (ppm) / Radial growth (mm)				Concentration (ppm) / Per cent inhibition			
		10ppm	25ppm	50ppm	100ppm	10ppm	25ppm	50ppm	100ppm
T1	Difenoconazole 25% EC	21	20	15.33	10.33	73.75	75	80.83	87.08
T2	Azoxystrobin 23% SC	43.33	36.33	31.5	29	45.83	54.58	60.62	63.75
T3	Propiconazole 25% EC	0	0	0	0	100	100	100	100
T4	Copper oxychloride 88% W/W	53	52.33	50.66	46.66	33.75	34.58	36.66	41.66
T5	Hexaconazole 5% EC	44	40	25.2	18.53	45	50	68.50	76.82
T6	Mancozeb 75%WP	42.16	37	31.66	29.33	47.29	53.75	60.41	63.33
T7	Carbendazim 12% + Mancozeb 63%	18.16	17.66	16.5	11.33	77.29	77.91	79.37	85.83
T8	Pyraclostrobin 12.5% + Epoxiconazole 4.7% SC	13.33	8.83	2.66	0	83.33	88.95	96.66	100
T9	Control	80	80	80	80	0	0	0	0
S.E(m)±		0.69	0.61	0.60	0.91	0.80	0.76	0.78	0.67
C.D. at 5%		2.05	1.95	1.79	2.70	2.56	2.44	2.49	2.15

Eight botanicals *viz.,* Neem (*Azadirachta indica*), Tulsi (*Ocimum tenuiflorum*), Marigold (*Tagetes* sp.), Garlic (*Allium sativum*), Ginger (*Zingiber officinale*), Ashwagandha (*Withania somnifera*), Milk weed (*Calotropis* sp.) and Lantana (*Lantana camara*) were evaluated at three different concentrations viz. 5.0, 10.0 and 20.0 per cent concentration by Poisoned Food technique. Also, Eight different fungicides and their combinations *viz.,* Difenoconazole 25% EC, Azoxystrobin 23% EC, Propiconazole 25% EC, Copper Oxychloride 88% W/W, Hexaconazole 5% EC, Mancozeb 75% WP, Carbendazim 12% WP + Mancozeb 63% WP, Pyraclostrobin 12.5% + Epoxiconazole 4.7% SC were evaluated at four different concentrations *viz.,* 10 ppm, 25ppm, 50ppm and 100ppm for each fungicide by Poisoned Food technique. Results showed that out of eight botanicals used Neem was found to be the most effective one followed by Tulsi. At 20 per cent concentration, maximum per cent growth inhibition of mycelium of the pathogen was recorded in Neem with inhibition of 43.37 per cent and the minimum radial growth of mycelium was 42.46 mm.

Similar results were also recorded in the findings of Kumar and Yadav (2007); Reman *et al.* (2011), they reported that the plant extract of neem contain some antifungal compounds. Rajput *et al.* (2014) reported that in addition to neem tulsi at 10 per cent

concentration was found to be effective in inhibiting the mycelial growth of *C. capsici*.

The results of *in vitro* application of fungicides showed that with increase in the concentration of the fungicide there is increase in the inhibition of mycelial growth of *C. capsici*. The efficacy of propiconazole was demonstrated by other workers (Shovan *et al.,* 2008; Jagtap *et al.,* 2013). Anand *et al.* (2020) reported that the protective field spray of Hexaconazole was found most effective in reducing fruit anthracnose (PDC 83.3%) and increasing fruit yield (225.50 q ha^{-1}). Whereas, Santoshreddy and Nargund (2015) observed that Pyraclostrobin 20 WG was at par with Difenoconazole 25 EC (8.18 PDI) followed by Propiconazole (11.77 PDI) at 0.1% concentration.

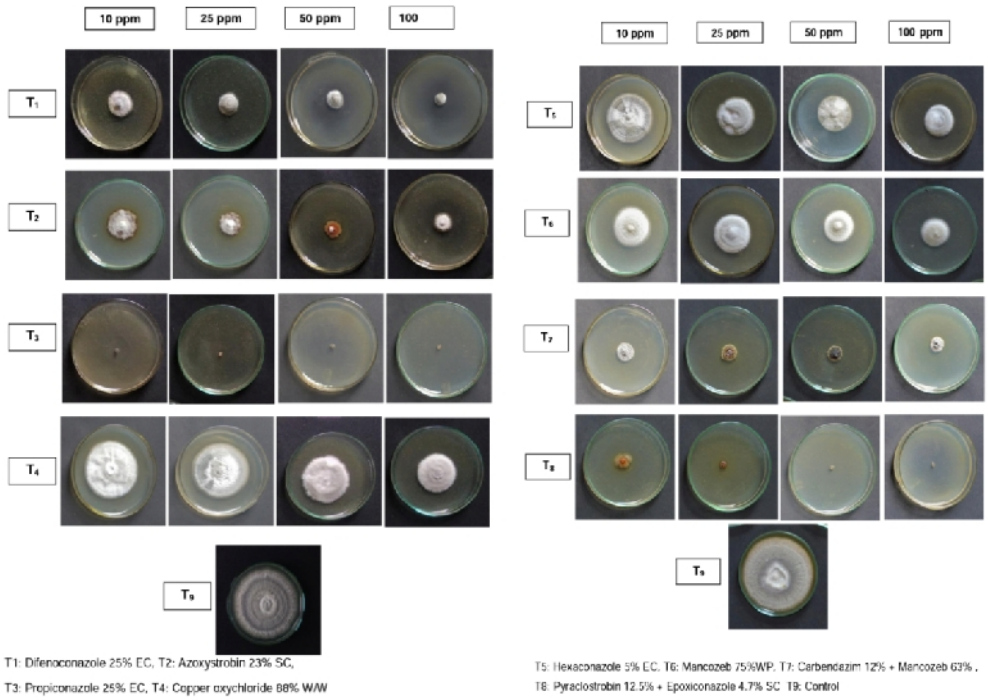

Plate 2: *In vitro* evaluation of fungicides (T1-19).

CONCLUSION

Among eight selected fungicides Propiconazole 25% EC was found to be the most effective showing complete inhibition in the mycelia growth of *C. capsici.* Neem leaf extract at 20 % concentration under *in vitro* condition was found very promising in inhibiting the growth of pathogen as it caused 43.37 per cent growth inhibition of *C. capsici.*

FUTURE SCOPE

— Assessment of losses caused by anthracnose disease in Madhya Pradesh.
— To ascertain the epidemiological factors responsible for development of disease
— To study an integrated disease management strategy for effective management of the disease.

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Conflict of Interest. None.

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