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Management of Cercospora leaf spot disease in mungbean using novel fungicides, botanicals and bio-agent

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Abstract
This study aimed to investigate the disease management of Cercospora leaf spot of Mungbean [*Vigna radiata* (L.) Wilezek] by fungicides, plant extract and bio-agent. The mungbean crop is infected by a large number of pathogens such as fungi, bacteria, virus and nematodes in which mungbean Cercospora Leaf Spot causes significantly losses. At present Cercospora Leaf Spot is being managed by using fungicide through seed and soil treatment. However, fungicides are more costly and pollutant to environment. Many plant extracts are known to have antifungal activity. During the present investigation of eight treatments: Chlorothalonil @ 0.1% /lit (T_1), Copper hydroxide @ 0.1% /lit (T_2), Azoxystrobin @ 0.1% /lit (T_3), Neem (*Azadirachta indica*) @ 10 ml /lit (T_4), Madar (*Calotropsis gigantea*) @ 10 ml /lit (T_5), Tulsi (*Ocimum sanctum*) @ 10 ml /lit (T_6), and *Trichoderma viride* @ 4 gm/lit (T_7) were evaluated for fungi toxicity against *C. canescens* by using poison food technique. The percent inhibition in radial growth of *Cercospora canescens* was maximum in Chlorothalonil (3.83 to 6.45 mm) to *T. viride* (15.66 to 18.86 mm) at 48 to 120 hrs of incubation period. The radial growth in check was (38.00 to 85.00 mm). Maximum percent inhibition of mycelial growth was varies from Chlorothalonil (89.92 to 92.41%) to *T. viride* (58.78% to 77.81%) at 48 to 120 hrs of incubation period.

Keywords: Botanicals, Cercospora, leaf, bio-agent, chemicals

Introduction
Mungbean (*Vigna radiata* L.) is short duration legume crop belongs to family Leguminosae, Mungbean is important pulse crop of India, it is widely cultivated throughout the primarily a rainy season crop but with the development of early maturing varieties, though they get highly affected from the disease. The leaf spot symptoms of *C. canescens* are circular, while those of *P. cruenta* are angular. Although *C. canescens* is comparatively a weaker parasite than *P. cruenta*, the former has a wider host range under tropical climates than the latter (Fery *et al.*, 1977) [2]. Average yield of the crop is very low mainly due to low inherent yield potential and susceptibility of the crop to diseases (Thakur *et al.*, 1977) [13]. Leaf spot disease caused by *Cercospora canescens* Ellis & Martin is a serious disease in the mungbean growing areas of the country where high humidity prevails during the growing season (Bashir & Zubair 1985) [1]. Cercospora leaf spot is one of the important diseases that cause serious losses to mungbean crop and 23% losses in yield have been reported (Quebral & Cagampang 1970). [9] Maximum loss of 61% was observed in case of grain yield (Iqbal *et al.*, 1995) [5]. The disease starts appearing about 30-40 days after planting. Depending upon the temperature and humidity, it spreads rapidly in susceptible varieties causing premature defoliation and reduction in size of pods and grains (Grewal *et al.*, 1980) [3]. Several workers had reported the effective control of the disease with the application of fungicides (Singh & Naik, 1977; Singh & Singh 1978) [11, 12].
Mungbean is an indigenous vegetable legume and one of the most important pulse crops in Southeast Asia. Being rich in digestible protein (24%), mungbean is utilized in the cereal-based diets (Khattak *et al.*, 2003) [8]. It contains vitamin A (94 mg), iron (7.3 mg), zinc (3 mg), calcium (124 mg) and folate (549 mg) per 100 g dry seed. Usually it is used in split form (Dhal) and in other different food products (Rasul *et al.*, 2012) [10]

Materials and Methods

For the management of Cercospora disease, three experiments *viz*., evaluation of botanicals, chemicals and bio-agent were carried out at Student's Research Farm, Pili Kothi and in the laboratory Department of Plant Pathology T. D. P. G., College, Jaunpur (U.P.) Kharif-2019. Three fungicides, namely Chlorothalonil, Copperhydroxide, Azoxystrobin and three botanicals, namely Neem, Calotropsis, Tulsi and one bio-agent *Trichoderma viride*, were evaluated against *C. caneascens in-vitro* by the poison food technique.

The radial growth of the regular colonies was measured at a right angle in two directions using a linear scale. Measurements were obtained at the widest and narrowest diameters in the event of irregular colonies, and the average of two distinct directions was used to determine growth. After seven days of incubation, radial development was seen in every case. It was discovered after five and seven days of incubation in the instance of the poison food technique. A 100 millilitre container of sterilized melted potato dextrose agar (PDA) was mixed with the necessary amount of different fungicides. After being exposed to each fungicide, this PDA was divided into four sterile Petri-plates at a volume of 20 milliliters each, and left to solidify. Petri-plates with PDA were filled with no fungicide added to create control plates. After solidifying, 5 mm discs from cultures of *Cercospora canescens* that were seven days old were carefully positioned in the centre of each Petri plate. The plates were then incubated at 28 ± 1 °C.

After five and seven days, observations on the test fungus's radial growth were recorded using the previously developed methods. After incubation, measurements of the radial colony expansion were taken every 48, 72, 96, and 120 hours. The following formula was then used to translate the radial growth data into percentage growth inhibition.

Percent growth inhibition $I = \left(\frac{C - T}{C} \right) \times 100$

Where,
C = Colony diameter (mm) in check plate
T = Colony diameter (mm) in the treated plate

The percent inhibition data was transformed into Arcsin √Percentage transformation and then analyzed statistically using completely randomized design (CRD).

Results and Discussion

(A) Effect of fungicides, plant extract and bio-agent at different concentrations against *C. canescens* on mycelial growth in *in vitro*

(i) At 48 hrs of incubation

The experiment's results (Table 1) demonstrated the considerable effects of chemicals, botanicals, and bioagents on *C. canescens* mycelia growth. Chlorothalonil (3.83 mm) and Azoxystrobin (5.83 mm) had the lowest radial growth, followed by Tulsi (12.17 mm), Madar (14.16 mm), Copper hydroxide (8.33 mm), Neem (10.66 mm), and *T. viride* (15.66 mm). 38.00 mm was the radial growth in check.

(ii) At 72 hrs of incubation

The use of chemicals, botanicals, and bioagents dramatically reduced the pathogen's radial growth at 72 hours of incubation, according to data shown in Table 1. In compared to the check (45.00 mm), the lowest radial growth was seen in Chlorothalonil (4.33 mm) and Azoxystrobin (6.33 mm), which were followed by Copper hydroxide (9.33 mm), Neem (11.33 mm), Tulsi (12.67 mm), Madar (15.16 mm), and *T. viride* (16.66 mm).

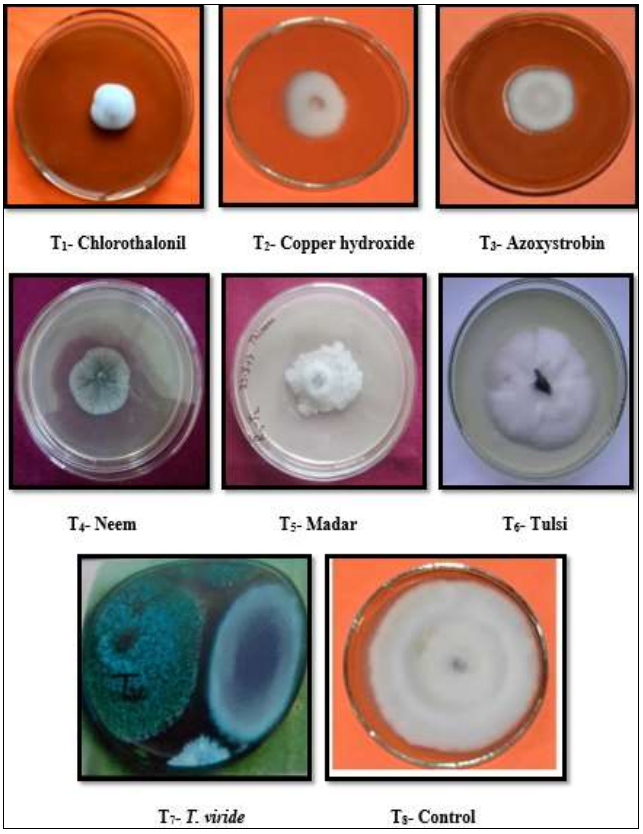

Fig 1: Effect of fungicides, botanicals and bio-agent on mycelial growth and percent inhibition of C. canescens at different period

(iii) At 96 hrs of incubation

Based on a comparison of check (60.00 mm) and radial growth, the lowest values were found for Chlorothalonil (5.46 mm) and Azoxystrobin (7.42 mm), followed by Copper hydroxide (10.41 mm), Neem (12.28 mm), Tulsi (13.65 mm), Madar (16.66 mm), and *T. viride* (17.66 mm). There were notable differences in the radial growth between the treatments (Fig. 1 & 2).

(iv) At 120 hrs of incubation

The experiment's findings indicated that the test pathogen's radial growth was significantly impacted by chemicals, botanicals, and bioagent. The effect was least noticeable in the cases of chlorothalonil (6.45 mm) and azoxystrobin (8.43 mm), and was subsequently followed by copper hydroxide (12.21 mm), neem (13.30 mm), tulsi (14.65 mm), madar (18.72 mm), and *T. viride* (18.86 mm). The check's radial growth was 85.00 mm. (Table 1).

Khandar *et al*. (1986) [7] recorded maximum inhibition of mycelial growth of *C. canescens* with benzimedazoles and captafol. They also recorded maximum inhibition of spore germination with benomyl followed by zineb and copper-oxychloride. The maximum inhibition of mycelial growth of *C. canescens* was also recorded by Jamadar and Padaganur (1992) [6].

(B) Efficacy of chemicals, botanicals and bio-agents against *C. canescens* on percent inhibition *In-Vitro*:

(i) At 48 hrs of incubation

The results displayed in Table 1 demonstrated that Chlorothalonil (89.92) had the highest percent suppression of

mycelial development, followed by Azoxystrobin (84.65), Copper hydroxide (78.07), Neem (71.94), Tulsi (68.00), Madar (62.73), and *T. viride* (58.78). Between the treatments, the percent inhibition was statistically significant (Fig. 1 & 2).

(ii) At 72 hrs of incubation

Chlorothalonil (90.37) exhibited the highest percent inhibition when tested against C. canescens, with Azoxystrobin (85.93), Copper hydroxide (79.26), Neem (74.82), Tulsi (71.84), Madar (66.31), and *T. viride* (62.97) following closely behind. Between treatments, the percentage inhibitions were significantly different from one another (Table 1).

(iii) At 96 hrs of incubation

Based on Table 1 data, it was determined that Chlorothalonil (91.11) had the highest percentage of tested pathogen inhibition, followed by Azoxystrobin (87.63), Copper hydroxide (82.65), Neem (79.53), Tulsi (77.25), Madar (72.23), and *T. viride* (70.56). Between treatments, the percentage inhibitions were significantly different from one another.

(iv) At 120 hrs of incubation

As can be seen from the data in Table-1, the test pathogen with the highest percent inhibition was Chlorothalonil (92.41), followed by Azoxystrobin (90.08), Copper hydroxide (85.63), Neem (84.35), Tulsi (82.76), Madar (77.97), and *T. viride* (77.81). Between the treatments, the % inhibition was significantly different (Fig. 1 & 2).

Hedge *et al.* (2002) determined the efficacy of extracts of Neem (leaf), Ocimum (leaf), Onion (bulb), Bougainvillea (leaf) and Lantana (leaf) against the inhibition of spore germination of *Cercaspora zinniae* causing leaf spot disease of Zinnia (*Zinnia elegans*).

Table 1: Influence of different fungicides, botanicals and bio-agent treatment on radial growth and percent inhibition of *C. canescens* at different period

S.N.	Treatment	48 hrs.		72 hrs		96 hrs.		120 hrs.	
		Radial growth (mm)	% inhibition	Radial growth (mm)	% inhibition	Radial growth (mm)	% inhibition	Radial growth (mm)	% inhibition
T_1	Chlorothalonil	3.83	89.92	4.33	90.37	5.46	91.11	6.45	92.41
T_2	Copper hydroxide	8.33	78.07	9.33	79.26	10.41	82.65	12.21	85.63
T_3	Azoxystrobin	5.83	84.65	6.33	85.93	7.42	87.63	8.43	90.08
T_4	Neem	10.66	71.94	11.33	74.82	12.28	79.53	13.30	84.35
T_5	Madar	14.16	62.73	15.16	66.31	16.66	72.23	18.72	77.97
T_6	Tulsi	12.17	68.00	12.67	71.84	13.65	77.25	14.65	82.76
T_7	*T. viride*	15.66	58.78	16.66	62.97	17.66	70.56	18.86	77.81
T_8	Control	38.00	0.00	45.00	0.00	60.00	0.00	85.00	0.00
S.Em±		0.54	2.86	0.80	2.31	0.77	2.87	0.77	3.06
CD at 5%		1.60	8.57	2.41	6.94	2.30	8.61	2.32	9.18

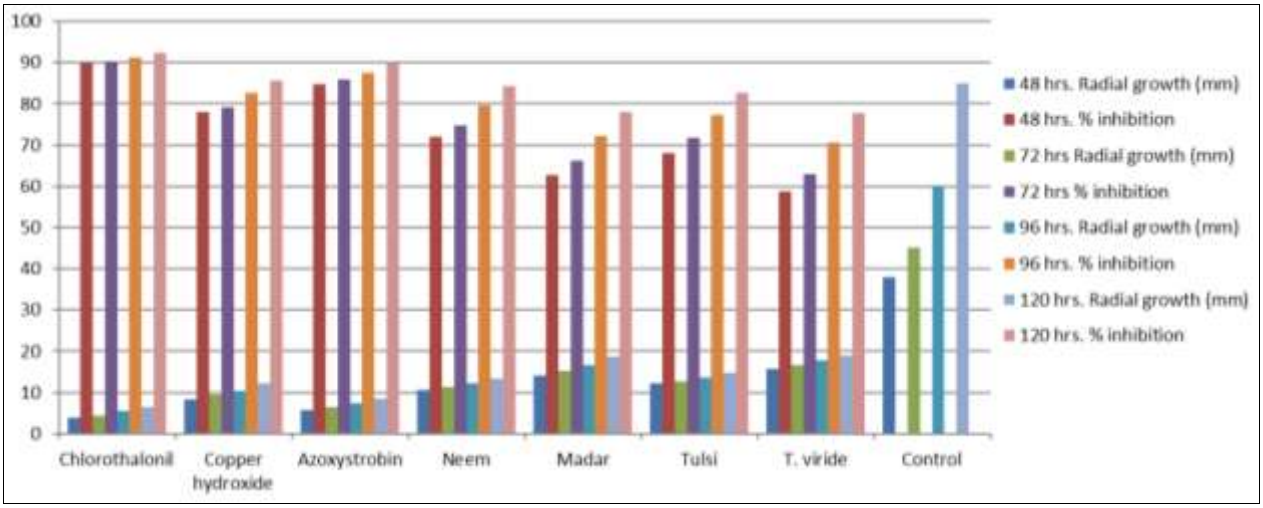

Fig 2: Influence of different fungicides, botanicals and bio-agent treatment on radial growth and percent inhibition of *C. canescens* at different period

Conclusion

In conclusion, the study examined the impact of fungicides, plant extracts, and bio-agents on the mycelial growth of *C. canescens in vitro* over 120 hours of incubation. Results revealed significant variations in radial growth and percent inhibition across different treatments and incubation periods. Chlorothalonil consistently demonstrated the most pronounced suppression of mycelial growth and highest percent inhibition compared to other treatments. Azoxystrobin also showed considerable efficacy in inhibiting *C. canescens* growth. These findings highlight the potential of Chlorothalonil and Azoxystrobin as effective agents for controlling *C. canescens* and contribute to the ongoing research on eco-friendly solutions for fungal disease management in agriculture. Further investigations into the mechanisms underlying the observed effects and field trials are warranted to validate these findings for practical application in disease management strategies.

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