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Rapid evaluation of different fungicides against *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyder and Hansen. Causing Fusarium wilt of Tomato

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Abstract

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important vegetable crops grown all over the world. It is world's 3rd largest vegetable crop after potato and sweet potato which is highly threatened by the Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* causing considerable economic losses in India as well as in the world. Six fungicides belonging to different groups and at different concentrations viz. 0.05%, 0.1%, 0.15% & 0.2% were screened, against the pathogen under *in vitro* condition to find out their relative efficacy in inhibiting the growth of the pathogen among which Benfil (Carbendazim 50% WP) was found effective at 0.1% concentration which inhibited the mycelial growth upto 92.5% as compared to control, followed by Matco (Metalaxyl (8%) + Mancozeb (74%). 72% WP) at 0.2% concentration with an inhibition of 84.64% and Blitox-50 (Copper oxy chloride 50% WP) at 0.2% with an inhibition of 83.92% over control. Fungicides like Manfil (Mancozeb 75% WG) and Daconil (Chlorothalonil) were found moderately effective. Among the treatments Srilaxyl (Metalaxyl 35% WS) was found least effective in inhibiting the mycelial growth of pathogen.

Keywords: *Fusarium oxysporum* f. sp. *lycopersici*, fusarium wilt, Tomato

1. Introduction

Tomato (*Lycopersicon esculentum* Mill.) is considered as one of the most important and remunerative vegetable crops cultivated throughout the world and a major contributor to the fruits and vegetables diet of humans (Kapasiya *et al.*, 2015) [4]. Which is susceptible to several diseases like damping off, early blight, late blight, Fusarium wilt, Verticillium wilt, bacterial wilt, tomato mosaic virus etc. Among them, Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyder and Hansen is an economically important disease of tomato crop worldwide (Hanaa *et al.*, 2011) [2]. Various measures which are generally used to manage the disease includes cultural, chemical, biological and use of resistant varieties. Cultural practice like field sanitation, summer ploughing, soil solarisation, soil amendments and crop rotation etc. can minimize the possibility of disease but cannot completely control the disease in standing crops. Another alternative method of disease management strategy is biological control. In this context, *Trichoderma harzianum*, *Trichoderma viride*, *Bacillus spp.*, *Penicillium spp.* etc. have been exploited for management of diseases but biological control alone cannot manage the disease completely because a little fluctuation in temperature, pH, moisture etc. largely affects the efficacy of bio agent. The use of resistant variety is another important method which is reliable and cheap for management of plant disease but due to development of new races of pathogen, the resistant variety becomes susceptible one. Hence the use of fungicides was the most dominant form of plant disease management.

2. Materials and Methods

The present investigation was done at the Department of Plant Pathology, Chandra Shekar Azad University of Agriculture and Technology Kanpur during September 2017. The procedure and techniques applied during the course of investigation was elucidated as below.

Isolation, purification, identification and maintenance of *Fusarium oxysporum* f. sp. *Lycopersici*.

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2.1 Collection of infected plant samples

Fusarium wilt infected plant samples were collected from the tomato field at Vegetable Research Farm, C. S. Azad University of Agriculture and Technology, Kanpur. Infected plants which are partially and complete wilted were taken from the field and washed in sterilized water. The wilt infected plants parts were then placed in a humidity chamber. They were incubated at $25\pm 2^{\circ}\text{C}$ in BOD, for further studies.

2.2 Isolations of pathogen

The diseased plant's roots were taken and washed thoroughly with tap water and finely with distilled water to remove all dust particles. The diseased part of the root is cut into small pieces by sterilized knife in such a way that each piece had small bits of diseased and healthy parts. The chopped pieces were dipped in mercuric chloride solution (0.1%) for 30 seconds rinsed in sterilized distilled water thrice and dried off with sterilized filter paper. The small pieces were then placed on PDA based media which was previously poured and sterilized in Petri plates. The plates were finally sealed with par film tape and were incubated at $25\pm 1^{\circ}\text{C}$. The Petri plates were observed daily to find out the presence of mycelium around the bits. As soon as mycelia growth is noticed around the bits, the pathogen was purified by hyphal tip culture method.

2.3. Purification of pathogen

The pathogen was purified by transfer of hyphal tip in Petri plates which were previously poured with sterilized PDA under aseptic condition.

2.4. Identifications of pathogen

After appearance of colony in petri plate, the pathogen was identified under compound microscope. The pathogen was identified on the basis of its morphological and cultural characteristics as described by (Sacc.) Synder and Hansen (1940).

2.5 Collection of fungicides

Fungicides viz., Carbendazim 50% WP, Mancozeb 75% WG (Manfil) Copper oxy chloride 50% WP (Blitox-50), Metalaxyl (8%)+ Mancozeb (74%). 72% WP (Matco), Metalaxyl 35% WS (Srilaxyl), Chlorothalonil (Daconil) were collected from Departmental store, Department of Plant Pathology, Chandra Shekhar Azad University of Agriculture & Technology, Kanpur to conduct the present study.

Effect of different fungicides on mycelial growth of *Fusarium oxysporum f. sp. lycopersici*

Six fungicides belonging to different groups and at 4 different concentrations viz. 0.05%, 0.1%, 0.15% & 0.2% were screened, against the pathogen under *in vitro* condition to find out their relative efficacy in inhibiting the growth of the pathogen in culture by the "Food Poison Technique". Required quantity of each fungicide was incorporated in already prepared two per cent PDA medium prior to solidification and thoroughly mixed them by shaking prior to pouring in sterilized Petri plates. The medium was allowed to solidify and then 5 mm. bits of fungal culture from seven days old culture were placed at the centre of Petri plates. The fungal disc was reversed so that the pathogen could come in direct contact with the medium. Three replications were kept for each treatment. The Petri plates were incubated at $25\pm 1^{\circ}\text{C}$. One set of control was maintained in which the medium was not mixed with any fungicide but simply inoculated with the

pathogen. The data on radial growth of fungal colony was measured in mm. after every 24 hours till the control petri plates were not filled up. The per cent inhibition over control was calculated by the following formula as given by Schimtz (1930)^[5].

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

Where,

I= Per cent inhibition in mycelia growth

C = Growth of pathogen in control plates.

T = Growth of pathogen in dual culture plates.

3. Results and Discussion

The average diameters of the fungal colonies were noted in the poured plates containing different fungicides and inhibition percentage was recorded in Table-1 & Table-2.

3.1 Effect of Carbendazim 50% wet table powder on radial growth of *F.o.f. sp. lycopersici* at 24, 72 and 120 hrs. (*In vitro*)

Carbendazim is a systemic fungicide with protective action. It was evaluated *in vitro* against *F.o. f. sp. lycopersici* by Poison Food Technique at 0.05%, 0.1%, 0.15%. 0.2% concentrations after 24,72 & 120 hrs. of incubation. Data presented in Table - 1&2, showed that minimum radial growth of 0.42 cm in diameter with 92.50% inhibition over control was recorded at 0.1% concentration after 120hrs followed by 0.01% (0.60 cm, 80.35% inhibition) From the Table-1&2 it is clear that concentration of fungicide is inversely proportion with radial growth of mycelium. Jha *et al.*, 2018^[3] also found Carbendazim as an effective fungicide in inhibiting the growth of the pathogen. Statistical analysis of the data revealed that each treatment varied invariably and significantly at 0.05 level of significance.

3.2 Effect of Mancozeb 75% WG on Radial Growth of *F.o. f.sp. lycopersici* at 24, 72 and 120 hrs. (*In vitro*)

Mancozeb is a non-systemic fungicide with protective and curative action. It was evaluated *in vitro* against *F.o. f. sp. lycopersici* by Poison Food Technique at 0.05%, 0.1%, 0.15%. 0.2% concentrations after 24, 72 & 120 hrs. of inoculation. Data presented in Table-1&2, indicated that minimum radial growth of 3.10 cm in diameter was recorded at 0.2% concentration which was followed by 0.15%, 0.1% and 0.05% concentration with 41.78%, 37.85% & 33.92% inhibition respectively. Chandel and Tomar (2007)^[1] also found that a combination of Mancozeb + carbendazim was effective in inhibiting the growth of pathogen. From the Table-1&2 it is also clear from that there were significance differences at 0.05 level of significance among all the treatments.

3.3 Effect of Copper oxy chloride 50% Wettable Powder on Radial Growth of *F.o. f. sp. lycopersici* at 24, 72 and 120 hrs. (*In vitro*)

Copper oxy chloride is a systemic fungicide with protective and curative action. It was evaluated *in vitro* against *F.o. f.sp. lycopersici* by using Poison Food Technique at 0.05%, 0.1%, 0.15%. 0.2% concentrations after 24, 72 & 120 hrs. of inoculation. Data presented in Table-1&2 indicated that minimum radial growth of 0.90cm in diameter of mycelium growth was recorded at 0.2% concentration after 120hrs after inoculation which is inhibited by 83.92% over control. The

rest of the treatments like 0.15%, 0.1% and 0.05% concentration with 81.07%, 75% & 71.42% inhibition respectively at 120 hrs after inoculation. It is evident that each treatment varied in variably and significantly at 0.05 level of significance.

3.4 Effect of Metalaxyl (8%) + Mancozeb (74%).72% Wettable Powder on Radial growth of *F.o. f. sp. lycopersici* at 24, 72 and 120 hrs. (*In vitro*)

Metalaxyl (8%) + Mancozeb (74%).72% was evaluated *in vitro* against *F.o. f.sp. lycopersici* by using Poison Food Technique at 0.05%, 0.1%, 0.15%. 0.2% concentrations after 24,72 &120 hrs. of inoculation. Data presented in Table-1&2, indicated that minimum radial growth (0.86cm) of 84.64 % inhibition was recorded at 0.2% concentration at 120 hrs after inoculation followed by 0.15% (1.0 cm, 82.14%), 0.1 (1.20cm, 78.57%), 0.05 % (1.20cm, 78.57%) respectively as compare to control. It is also clear from the Table-1&2 that each treatment varied in variably and significantly at 0.05 level of significance.

3.5 Effect of Metalaxyl 35%WS on Radial Growth of *F.o. f. sp. lycopersici* at 24, 72 and 120 hrs. (*In vitro*)

Metalaxyl was evaluated *in vitro* against *F.o. f. sp. lycopersici* by using Poison Food Technique at 0.05%, 0.1%, 0.15%. 0.2% concentrations after 24, 72 &120 hrs. of inoculation.

Data presented in Table-1&2, indicated that minimum radial growth of 4.24cm in diameter of mycelium growth with was recorded at 0.2% concentration which is inhibited upto 24.28% over control at 120 hrs after inoculation The rest of the treatments like 0.15%, 0.1%, 0.05% concentrations also influenced the mycelium growth of fungi showing 4.40cm, 4.75cm, 4.70cm respectively. It is also cleared from the Table-1, 2 that each treatment varied in variably and significantly at 0.05 level of significance.

3.6 Effect of Chlorothalonil on Radial growth of *F.o.f. sp. lycopersici* at 24, 72 and 120 hrs. (*In vitro*)

Chlorothalonil was evaluated *in vitro* against *F.o. f. sp. lycopersici* by using Poison Food Technique at 0.05%, 0.1%, 0.15%. 0.2% concentrations after 24, 72 & 120 hrs. of inoculation. Data presented in Table-1 & 2, indicated that minimum radial growth (3.90cm) of 30.35 % inhibition was recorded at 0.2% concentration at 120 hrs after inoculation followed by 0.15% (4.10 cm, 26.78%), 0.1 (4.30cm, 23.21%), 0.05%(4.60cm, 17.85%) respectively as compare to control. Urban and Fipowicz (2004) [6] also found that chlorothalonil was effective in inhibiting the mycelial growth in *in-vitro* @10ppm concentration. It is also clear from the Table-1&2 that each treatment varied in variably and significantly at 0.05 level of significance.

Table 1: Effect of different fungicides on mycelial growth of *Fusarium oxysporum* f. sp. *lycopersici* at various concentrations (0.05% & 0.1%) and at different time intervals (24 hrs, 72 hrs and 120 hrs).

S. No.	Treatments	Radial mycelial growth(cm) at 0.05% concentration at different time intervals			% inhibition	Radial mycelial growth(cm) at 0.1% concentration at different time intervals			% inhibition
		24 Hrs.	72 Hrs.	120 Hrs.		24hrs	72 hrs	120 hrs	
1.	Carbendazim 50% WP (Benfil)	0.20	0.60	1.10	80.35	0.18	0.26	0.42	92.50
2.	Mancozeb 75% WG (Manfil)	0.90	2.30	3.70	33.92	0.86	1.92	3.48	37.85
3.	Copper oxy chloride 50%WP (Blitox-50)	0.90	1.20	1.60	71.42	1.00	1.10	1.40	75.00
4.	Metalaxyl(8%)+ Mancozeb (74%).72% WP (Matco)	0.50	0.80	1.20	78.57	0.42	0.74	1.20	78.57
5.	Metalaxyl 35% WS (Srilaxyl)	1.50	2.90	4.70	16.07	1.40	2.60	4.75	15.17
6.	Chlorothalonil (Daconil)	1.30	2.70	4.60	17.85	1.26	2.42	4.30	23.21
7.	Control	1.60	4.20	5.60		1.60	4.20	5.60	
8.	C.D.	0.267	0.291	0.313		0.234	0.285	0.213	
9.	SE(m)	0.087	0.095	0.102		0.076	0.093	0.069	
10.	SE(d)	0.123	0.135	0.145		0.108	0.131	0.098	
11.	C.V.	15.338	7.845	5.515		13.797	8.509	3.980	

Table 2: Effect of different fungicides on mycelial growth of *Fusarium oxysporum* f. sp. *lycopersici* at various concentrations (0.15% & 0.2%) and at different time intervals (24 hrs, 72 hrs and 120 hrs).

S. No.	Treatments	Radial mycelial growth(cm) at 0.15% concentration at different time intervals			% inhibition	Radial mycelial growth(cm) at 0.2% concentration at different time intervals			% inhibition
		24 Hrs.	72 Hrs.	120 Hrs.		24hrs	72 hrs	120 hrs	
1.	Carbendazim 50% WP (Benfil)	0	0	0	-	0	0	0	-
2.	Mancozeb 75% WG (Manfil)	0.74	1.78	3.26	41.78	0.52	1.62	3.10	44.64
3.	Copper oxy chloride 50%WP (Blitox-50)	0.90	1.00	1.06	81.07	0.80	0.85	0.90	83.92
4.	Metalaxyl(8%)+ Mancozeb (74%).72% WP (Matco)	0.30	0.60	1.00	82.14	0.20	0.54	0.86	84.64
5.	Metalaxyl 35% WS (Srilaxyl)	1.30	2.50	4.40	21.42	1.10	2.10	4.24	24.28
6.	Chlorothalonil (Daconil)	1.10	2.40	4.10	26.78	0.90	1.82	3.90	30.35
7.	Control	1.60	4.20	5.60		1.60	4.20	5.60	
8.	C.D.	0.314	0.250	0.158		0.125	0.098	0.143	
9.	SE(m)	0.103	0.082	0.052		0.041	0.032	0.047	
10.	SE(d)	0.145	0.116	0.073		0.058	0.045	0.066	
11.	C.V.	20.959	7.944	3.218		9.681	3.469	3.044	

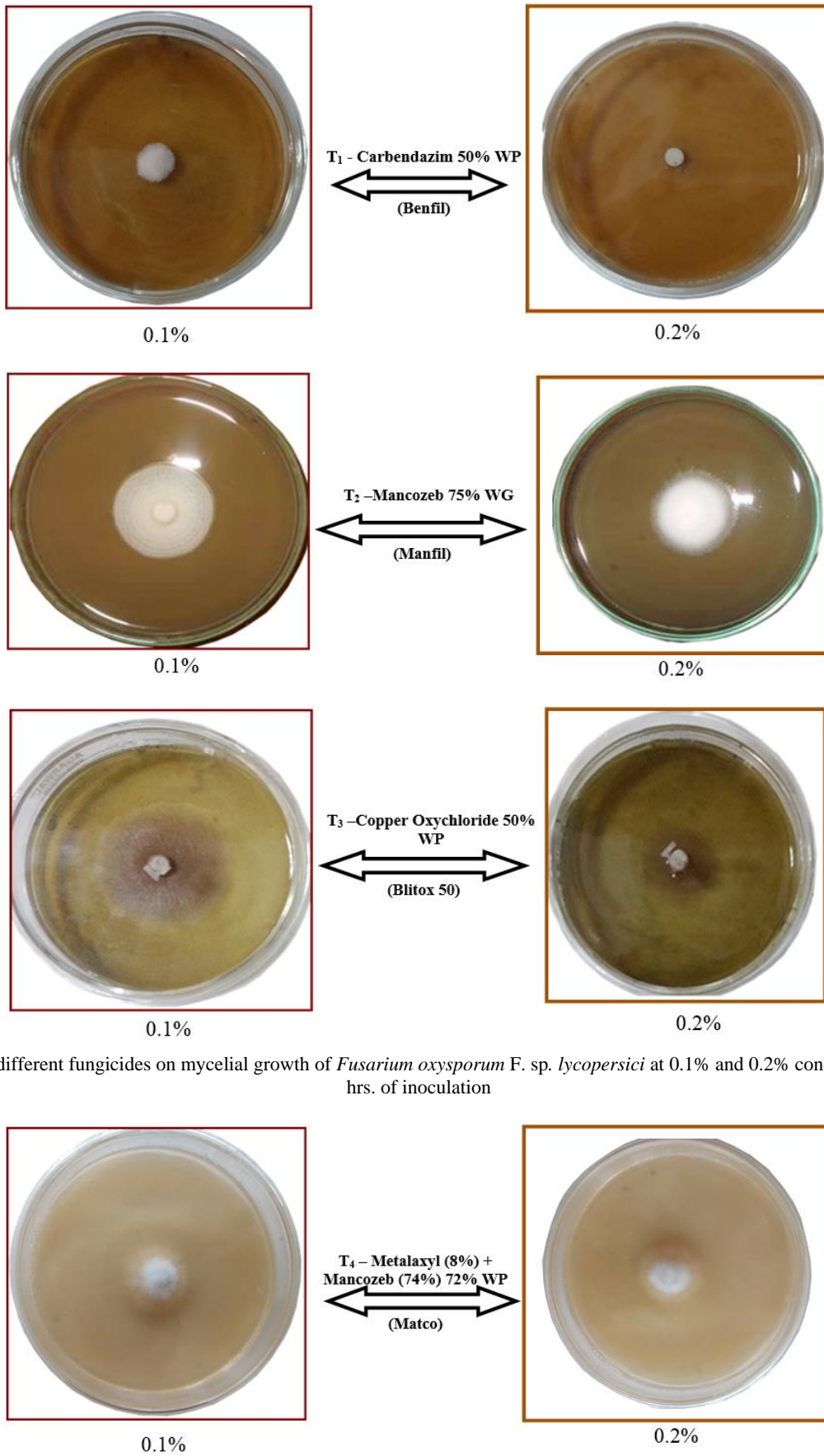


Plate 1: Effect of different fungicides on mycelial growth of *Fusarium oxysporum* F. sp. *lycopersici* at 0.1% and 0.2% concentrations after 120 hrs. of inoculation

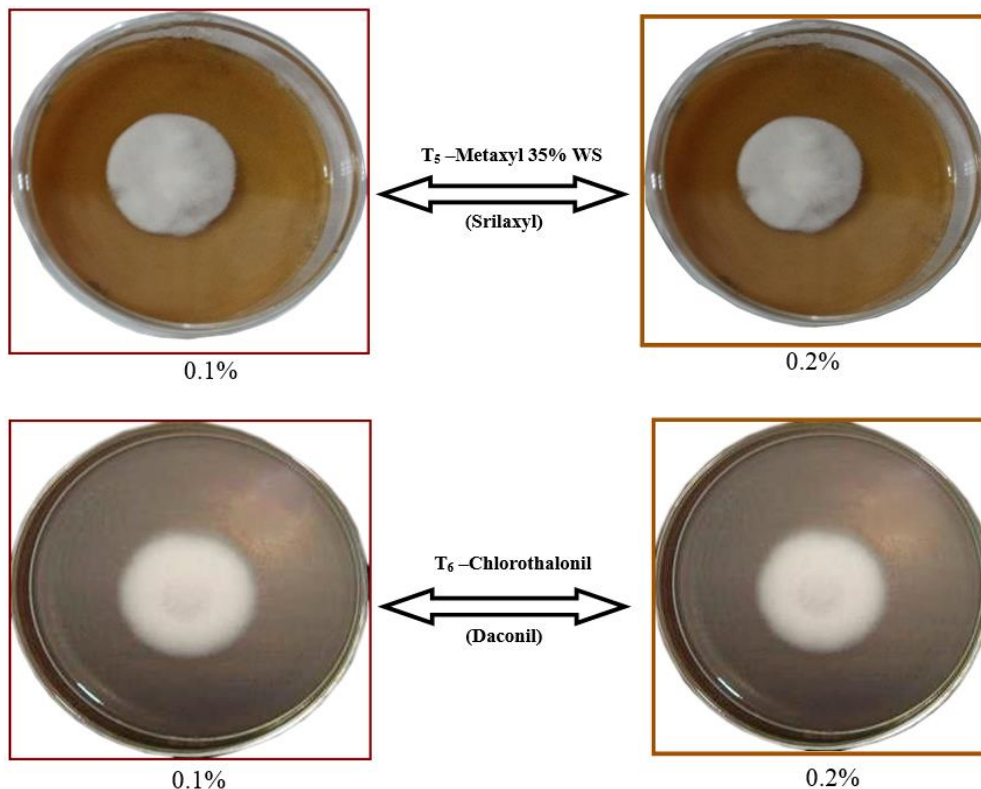


Plate 2: Effect of different fungicides on mycelial growth of *Fusarium oxysporum* F. sp. *lycopersici* at 0.1% and 0.2% concentrations after 120 hrs. of inoculation



Plate 3: Effect of different fungicides on mycelial growth of *Fusarium oxysporum* F. sp. *lycopersici* at 0.1% and 0.2% concentrations after 120 hrs. of inoculation

4. Conclusion

It is evident that out of six fungicides tested, Benfil (Carbendazim 50% WP) was found effective at 0.1% concentration which inhibited the mycelial growth upto 92.5% as compared to control, followed by Matco (Metalaxyl (8%) + Mancozeb (74%). 72%WP) at 0.2% concentration with an inhibition of 84.64% and Blitox-50 (Copper oxy chloride 50%WP) at 0.2% with an inhibition of 83.92% over control. Fungicides like Manfil (Mancozeb 75% WG) and Daconil (Chlorothalonil) were found moderately effective. Among the treatments Srilaxyl (Metalaxyl 35% WS) was found least effective in inhibiting the mycelial growth of pathogen (Plate-1).

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