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Comparative analysis of various strategies for management of early blight of tomato incited by *Alternaria solani* (Ellis and Martin) Jones and Grout

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Abstract
The present experiment was conducted to test the efficacy of bio-control agents, plant extracts and fungicides *in vitro* against *Alternaria solani* causing early blight of tomato. The efficacy of two fungal and one bacterial antagonist (*T. harzianum*, *T. viride* and *P. fluroresenes*) were evaluated through dual culture technique. Six plant extracts *viz.*, *Azadirachta indica* (Neem), *Zingiber officinale* (Ginger), *Allium sativum* (Garlic), *Eucalyptus spp* (Eucalyptus), *Dhatura stramonium* (Datura) and *Ociumum sanctum* (Tulsi) at five concentrations *i.e.* 1%, 2%, 3%, 4%, and 5% and six combination of systemic and non-systemic fungicides *viz.*, Mancozeb, Roko, Carbendazim, Companion, Blitox 50 and Sanchar also at five concentrations *i.e.* 50ppm, 100ppm, 250ppm, 500ppm and 1000ppm were evaluated through poison food technique. All bio-control agents, highest inhibition of radial growth of test fungus was recorded in *P. fluorescens* showed maximum inhibition (56.03%) followed by *Trichoderma harzianum* (51.58 %), while minimum mycelium growth inhibition was observed in *Trichoderma viride* (47.78%). Among different plant extracts used, *Allium sativum* (Garlic) was significantly inhibit the mycelial growth of pathogen in all concentrations followed by *Azadirachta indica* (Neem). Among combination of systemic and non-systemic fungicides at different concentrations, the significant maximum growth inhibition was recorded in Metalaxyl + Mancozeb (Sanchar) at 100, 250, 500 and 1000 ppm concentration which inhibited the fungal growth as 100 per cent respectively, followed by Mancozeb and Carbendazim + Mancozeb was most effective in this study.

Keywords: Fungal pathogen, Mycelial growth, Bio control agents, Plant extracts, Fungicides

Introduction
Naturally, the tomato (*Lycopersicon esculentum* Mill.) (2n = 24) is a perennial plant, but it is cultivated annually because of having a great economical and commercial advantages. The crop is cultivated across all continents in the fields as well as in protected conditions. It is widely grown in China, India, United States, Turkey, Egypt, USA, Italy, Philippines, Indonesia, Central East and West Africa, Tropical America and throughout tropics. Tomato is grown for its edible fruits, which can be consumed either fresh salads or in the form of various processed products such as paste, powder, ketchup, sauce, chutney, soup, puree and canned whole fruits. Tomato also has high medicinal value; the pulp and juice are digestible, promoter of gastric secretion and blood purifier. Additionally, its nutrients and metabolites (Folate, potassium, and vitamins A and C) that are important for human health. Tomato seed contains 2.4 percent oil which has great medicinal value. Tomato fruits content about 95 percent water and 5 percent other component mainly carbohydrates and fibers. Tomato is highly sensitive to abiotic stresses especially extreme temperature, salinity, drought, excessive moisture and environmental pollution and biotic stresses. Tomato plants are suffered with large number of biotic stresses including insect pests and diseases from the time of emergence to harvest. Tomato is commonly affected by numerous diseases *viz*., Early blight [*Alternaria solani* (Ellis and Mart.)], Late blight [*Phytophthora infestans* (Mont.) de Bary], Septoria leaf blight [*Septoria lycopersici* Speg.], Anthracnose [*Colletotrichum phomoides* (Sacc.) Chester], Fusarium wilt [*Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyder and Hansen], Verticillium wilt [*Verticillium dahilae* Klebalum], Phoma rot [*Phoma lycopersic* Cooke] etc. Among the diseases early blight caused by *A. solani* was most destructive causing heavy losses in yield of tomato sometimes as high as 78 per cent of fruit loss (Datar and Mayee, 1981) [7]. The pathogen *Alternaria solani* belongs to phylum: Ascomycota, class: Deuteromycetes, order: Moniliales, family: Dematiaceae (Jones and Grout, 1986) [10]. Symptoms of early blight

are small, dark, necrotic lesions that usually appear on the older leaves and spread upward of the plants. As lesions enlarge, they commonly have concentric rings with a target-like appearance, and they are often surrounded by a yellowing zone (Sherf and MacNab, 1986) [16]. So, management of this disease is very important. Use of resistant varieties is the ultimate control of this disease. However, farmers in pursuance of high yield are inclined to cultivate some varieties which may be less resistant to disease. Also unplanned and wide use of fungicides often leads to serious environmental problems besides affecting the health of users and consumers. So, it is necessary to minimize the use of chemicals for controlling disease. Hence, the attempt has been made to evaluate some new agro chemicals, plant extracts and bio agents against *Alternaria solani*, as it is use full in short listing the effective fungicides for field experiments and also integrating the bio agents and botanicals to come up with a eco-friendly management strategy to manage the early blight of tomato. Keeping the importance of this disease in view the Comparative analysis of various strategies for management of early blight of tomato incited by *Alternaria solani* (Ellis and Martin) Jones and Grout.

Materials and Methods

Isolation and Purification of pathogen

The infected leaves showing typical early blight symptoms was used for isolation of pathogen. The diseased plant leaves cut into small pieces and sterilized with mercuric chloride solution for 30 seconds. The pieces were washed repeatedly thrice in sterilized distilled water. Taken sterilized Petri plates and placed in laminar air flow chamber and poured 20 ml PDA in each Petri plates. Leaves pieces were placed in each plate and incubated at 25±1°C in a BOD incubator for 12 days and observed periodically for fungal growth and sporulation. Colonies were identified by microscopic observation on the basis of mycelial and spore characters. After identification they were transferred to PDA slants and incubated at 25±1ºC for further use. The culture was purified by both hyphal tip method (Pathak, 1972) [14] and single spore technique.

In-vitro efficacy of bio-control agents on mycelial growth of *Alternaria solani*

The growth of the causal agent of tomato early blight caused by (*Alternaria solani*) was investigated by dual culture in laboratory condition. In this step, the maximum and minimum inhibitory effect was caused by *T. harzianum*, *T. viride* and *P. fluroresenes*. The efficacy of two fungal and one bacterial antagonist were evaluated against *Alternaria solani* for radial growth inhibition on the potato dextrose agar medium using dual culture technique under *in vitro* condition. The test organism and pathogen was grown separately on PDA medium. 20 ml of PDA was poured into 90 mm diameter Petri dishes and allowed to solidify. Five mm disc of *A. solani* taken from 12 days old culture was placed at one end of Petri dish and respective antagonistic organisms were inoculated at the opposite side. Petri plates without antagonist served as control. The per cent growth inhibition of the fungus in each treatment in comparison with control was calculated by using the following equation (Vincent, 1927) [18].

$$P.I. = \frac{C - T}{C} X\ 100$$

Where,
P.I. = Percent mycelial growth inhibition
C = Colony diameter in control (mm)
T = Colony diameter in respective treatment (mm)

In case of bacterial antagonist *A. solani* was placed at both ends of Petri plates and bacterial culture was streaked at the centre of Petri plates. Each treatment was replicated three times and incubated for 12 days at 25 ± 1ºC. The growth of antagonistic organisms was recorded by measuring the colony diameter of *A. solani* in each treatment and compared with control.

In-vitro efficacy of plant extracts on mycelial growth of *Alternaria solani*

The present investigation was carried out to evaluate the plant extracts collected from different plant species to know the toxicant properties against early blight pathogen (Table-1). The plant extracts were evaluated *in vitro* through poisoned food technique (Carpenter, 1942) [3]. Fresh and healthy plant parts of 100 g (leaves, rhizome and bulb) were collected from field, and washed thoroughly with tap water and then with sterile distilled water, air dried and respective plant parts were crushed in grinder mixer by adding 100 ml distilled water to obtained 1:1 extract separately. Each Phyto-extracts thus obtained was centrifuged and filtered through double layered sterile muslin cloth in conical flasks and plugged. 100 ml PDA was taken in 150 ml conical flasks, plugged and sterilized by autoclaving at 15 psi for 20 minutes. 100 ml PDA culture filtrate (100%) was further diluted to required concentrations of 1%, 2%, 3%, 4%, and 5%. Potato dextrose agar was used as nutrient medium and required quantity of each botanical extract was added separately to get a required concentration of the plant extract. The botanical extracts were thoroughly mixed in the flask containing PDA individually and sterilized. About 20 ml of poisoned medium was poured to each of the 90 mm Petri dishes and three plates per treatment was kept and allowed for solidification. The actively growing periphery of the 12 old culture of *Alternaria solani* was carefully cut 5.0 mm disc with the help of a cork borer and transferred aseptically to the centre of each Petri dish containing the poisoned solid medium. Suitable control was maintained by growing the cultures on PDA without the plant extract. The plates were incubated at 25±1 °C for twelve days and the colony diameter was recorded. The per cent growth inhibition of the fungus in each treatment in comparison with control was calculated by using the following equation (Vincent, 1927) [18].

$$P.I. = \frac{C - T}{C} X\ 100$$

Where,
P.I. = Percent mycelial growth inhibition
C = Colony diameter in control (mm)
T = Colony diameter in respective treatment (mm)

Table 1: List of plant extracts used in the experiment

No.	Botanical name	Common name	Plant part used
1.	*Azadirachta indica* Juss.	Neem	Leaf
2.	*Zingiber officinale* Roscoe	Ginger	Rhizome
3.	*Allium sativum* L.	Garlic	Bulb
4.	*Eucalyptus spp.*	Eucalyptus	Leaf
5.	*Dhatura stramonium* L.	Datura	Leaf
6.	*Ociumum sanctum* L.	Tulsi	Leaf

In-vitro efficacy of fungicides on mycelial growth of *Alternaria solani*

The efficacy of six combination of systemic and non-systemic fungicides at different recommended concentrations (50ppm,

100ppm, 250ppm, 500ppm, 1000ppm) were evaluated on potato dextrose agar medium using poisoned food technique against *Alternaria solani* in laboratory (Table-2). The radial growth of the fungus on the poisoned medium was recorded at 12 days after inoculation. The poisoned food technique (Shravelle, 1961) [17] was followed to evaluate the efficacy of chemical fungicides in inhibiting the mycelial growth of *A. solani*. The measured quantities of fungicides were incorporated in melted sterilized PDA medium aseptically to obtain desired concentration of the respective fungicide at the time of pouring of medium. The fungus was grown on PDA medium for eight days prior to setting up the experiment. The PDA medium was prepared and melted. The fungicidal suspension was added to the melted medium to obtain the required concentrations on commercial formulation basis of the fungicide. 20ml of poisoned medium was poured in each sterilized Petri plates. Mycelial disc of 5 mm was taken from the periphery of eight days old colony was placed in the centre of Petri plates and three replications were maintained for each treatment. Then incubated at 25±1^{0}C for 12 days and the diameter of the colony was measured in two directions and average was recorded.

The per cent growth inhibition of the fungus in each treatment in comparison with control was calculated by using the equation is described in bio control agents and plant extracts.

Table 2: List of fungicides used in the experiment

Sl. No	Chemical Name	Trade name
1	Thiophanate methyl	Roko
2	Carbendazim	Bavistin
3	Metalaxyl+ Mancozeb	Sanchar
4	Carbendazim + Mancozeb	Companion
5	Copper oxy chloride	Blitox 50
6	Mancozeb	Indofil M-45

Result and Discussion

In-vitro efficacy of bio-control agents on mycelial growth of *Alternaria solani*

Three bio-agents, two fungal bio-agents and one bacterial antagonist were obtained from different sources were used for these experiments. The experiments were conducted to know their antagonistic potential against *A. solani* through dual culture technique.

Table 3: *In vitro* efficacy of bio-control agents against test pathogen

S. No.	Bio-control agents	Average radial growth of pathogen (mm)	Per cent inhibition
1.	*A. solani* + *T. viride*	45.50	47.78
2.	*A. solani* + *P. fluorescens*	38.21	56.03
3.	*A. solani* + *T. harzianum*	42.18	51.58
4.	Control	87.13	-
	CV (%)	4.75	
	SE. m.±	1.46	
	CD @ 0.01%	4.78	

The results presented in above Table-3 and its corresponding bar diagram Fig.-1 and also represented with photograph Plate-1 indicated that the significant different was observed among the all bio-control agents. The data pertaining to the effect of bio agents on the fungus growth taken at 7 days after inoculation. The per cent inhibition of the bio agents was higher at 7 days after inoculation. It ranged per cent inhibition from 47.78 to 51.58 at 7 days after inoculation. Among these bio-control agents, *P. fluorescens* showed maximum inhibition in mycelium growth (56.03%) followed by *Trichoderma harzianum* (51.58 %), while minimum mycelium growth inhibition was observed in *Trichoderma viride* (47.78%). On the other hand, maximum colony growth of *A. solani* was observed in *Trichoderma viride* (45.50mm) followed by *T. harzianum* (42.18mm) and *P. fluorescens* (38.21mm).

The result of present findings is supported with the finding of Babu *et al.*, (2000) [2] they reported that all the six isolates of *P. fluorescens* used, were significantly inhibited the growth of *A. solani* compared to control**.** Casida and Lukezie (1992) [4] reported that *Pseudomonas* strain 679-2 was able to reduce the severity of the leaf spot disease caused by *A. solani*. Similar type of results was reported also by Leifort *et al., (*1992), Koley *et al.,* (2015) [12, 11].

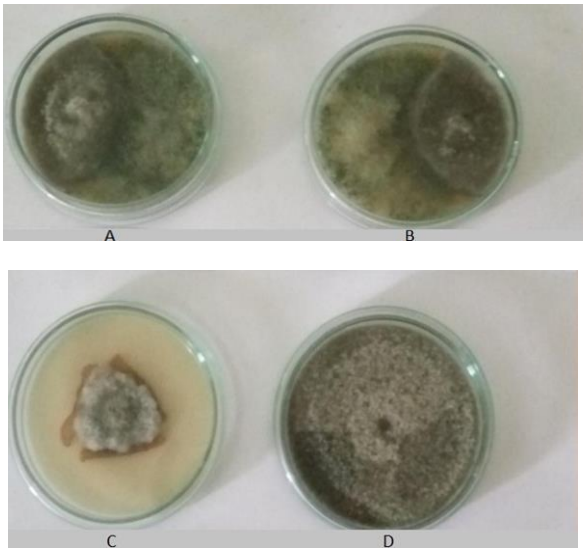

Plate 1: Antagonism of *bio-agents (A- A. solani +T. viride, B- A. solani + T. harzianum, C - A. solani + P. fluorescens,* D -Control

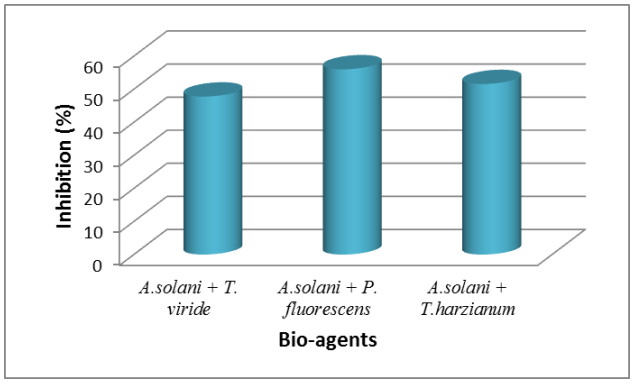

Fig 1: *In vitro* efficacy of bio-control agents against test pathogen

In vitro efficacy of different natural plant extracts on mycelial growth of *Alternaria solani*

Experimental data pertaining to *in vitro* efficacy of plant extracts against *Alternaria solani* have been presented in Table-4 and its corresponding bar diagram Fig.-2 and also represented with photograph Plate-2 indicated that significant difference on mycelium growth was recorded from different plant extracts in at all the concentrations. Among the six-plant extracts tested, most effective plant extracts were found with *Allium sativum* which exhibited minimum mycelium growth (53.95mm). It was significantly lower of over rest of the plant extracts. However, maximum mycelial growth (78.19mm) was

observed in *Datura stramonium*. In case of concentrations, minimum growth of the mycelium was observed in higher concentration (5%) in all the plant extracts. It's indicated that the mycelium growth was reduced with gradually increased in concentration of plant extract. Interaction was found significant. In case of interaction between plant extract and concentration, minimum mycelium growth (48.36mm) was found in *Allium sativum* @ 5% which was at par with *Azadirachta indica* @ 5% (50.65mm), *Eucalyptus spp.* @ 4% (53.26mm), *Azadirachta indica* @ 4% (54.00mm), *Eucalyptus spp.* @ 3% (54.12 mm), and *Zingiber officinale* @ 5% (67.03mm and significantly lower over rest of concentrations of plant extracts. On the other hand, maximum inhibition in mycelium growth was noticed in *Allium sativum* @ 5% (44.67%) followed by @ 5% *Azadirachta indica* (42.05%), *Eucalyptus spp.* @ 4% (39.06%), *Azadirachta indica* @ 4% (38.23%), *Eucalyptus spp.* @ 3% (38.08%), *Ociumum sanctum* @ 3% (31.60mm), while minimum inhibition in mycelium growth of 5.27% was recorded in *Datura stramonium*. @ 1%. Ganeshan (2009) [8], reported that the garlic extracts significantly reduce the leaf blight. The botanicals inhibited the mycelial growth production. The present investigation of various botanicals inhibiting the growth of *A. solani* is in line with the earlier findings similar type of results was reported by Nashwa *et al.*, (2012), Patni *et al.*, 2005, Dalpati *et al.*, 2010, Anamika and Simon, 2011 [13, 15, 6, 1].

Table 4: *In vitro* efficacy of plant extracts at different concentrations against *Alternaria solani*

S. No.	Botanicals/Concentration	Colony diameter (mm)*					Mean	Per cent inhibition					Mean
		1%	2%	3%	4%	5%		1%	2%	3%	4%	5%	
1.	*Zingiber officinale*	74.75	73.10	71.58	70.36	67.03	71.36	14.48	16.37	18.11	19.50	23.31	18.35
2.	*Azadirachta indica*	69.80	66.49	62.01	54.00	50.65	60.59	20.15	23.93	29.05	38.23	42.05	30.66
3.	*Allium sativum*	60.89	56.68	53.44	50.39	48.36	53.95	30.34	35.15	38.86	42.35	44.67	38.27
4.	*Ociumum sanctum*	72.21	64.70	59.78	58.20	52.00	61.37	17.34	25.98	31.60	33.41	40.51	29.76
5.	*Eucalyptus spp.*	62.42	59.57	54.12	53.26	51.25	56.12	28.58	31.84	38.08	39.06	41.36	35.78
6.	*Datura stramonium*	82.80	79.62	78.01	76.38	74.15	78.19	5.27	8.91	10.75	12.61	15.16	10.54
7.	Control	87.41	87.41	87.41	87.41	87.41	87.41	-	-	-	-	-	
	Mean	71.61	69.65	62.33	64.28	61.55		19.36	23.67	27.74	31.36	34.51	
	SE(d)	3.28											
	CV%	7.76											
	SE.m.±	2.32											
	CD @ 0.01%	6.73											

* mean of three replicates

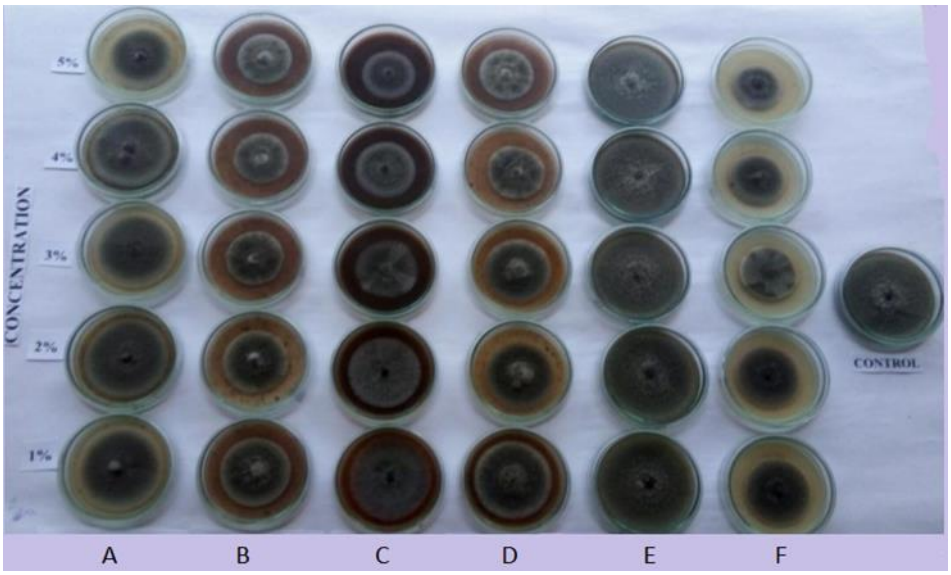

Plate 2: *In vitro* efficacy of plant extracts against *Alternaria solani* (A- *Zingiber officinale*, B- *Eucalyptus spp.*, C- *Ociumum sanctum*, D- *Azadirachta indica*, E- *Datura stramonium*, F- *Allium sativum)*

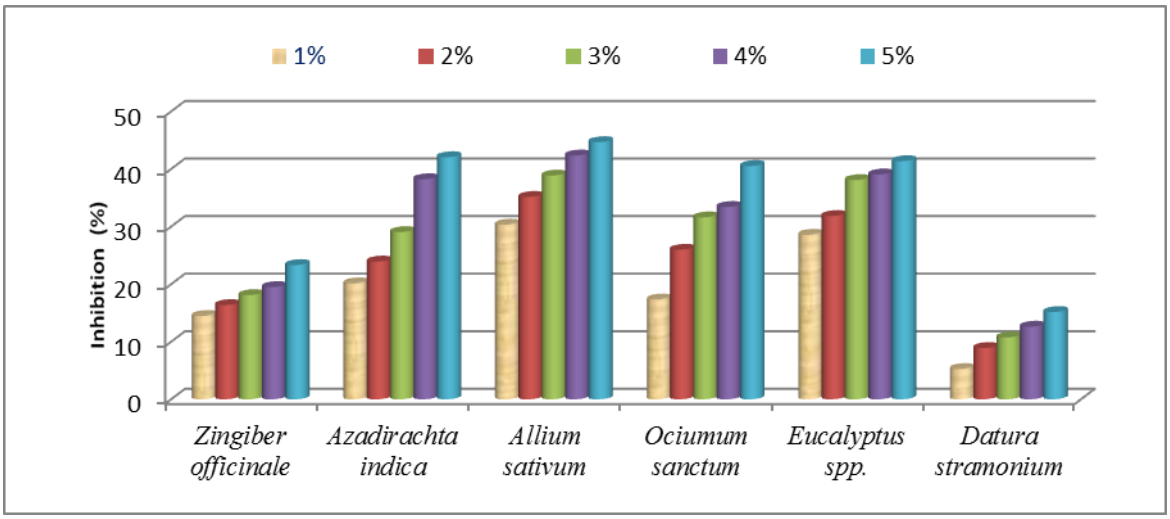

Fig. 2: *In vitro* efficacy of different natural plant extracts against pathogen

In vitro* efficacy of different fungicides on mycelial growth of *Alternaria solani

Six combination of systemic and non-systemic fungicides of different groups were assayed *in vitro* to find out the most effective fungicide against *A. solani* at different concentrations *viz.*, 50, 100, 250, 500, and 1000 ppm using poison food technique. The analyzed data pertaining to *in vitro* efficacy different chemical fungicides against *Alternaria solani* have been presented in Table-5 and its corresponding bar diagram Fig.-3 and also represented with photograph Plate-3 revealed that all treatments are given better response in minimizing the redial growth of pathogen over control. The significant difference among fungicides against *A. solani* was observed. Minimum growth was checked with Metalaxyl + mancozeb at 100 ppm, while in mancozeb at 500 ppm and Carbendazim + mancozeb at 500 ppm concentration. Among the six fungicides, most effective fungicides were found in Metalaxyl + mancozeb which exhibited 100.00 percent inhibition in mycelial growth at 100 ppm followed by in mancozeb at 500 ppm and Carbendazim + mancozeb at 500 ppm concentration. However, other fungicides were unable to check 100.00 per cent mycelium growth up to 1000 ppm concentration.

Effectiveness of different fungicides for inhibition of growth of *A. solani*, have been reported by many research workers. choulwar *et al.*, (1989) [5] reported Mancozeb (1000 ppm) as effective fungicide in reducing the mycelial growth (77.00 %) of *Alternaria solani* causing early blight of tomato, followed by copper oxychloride, carbendazim and Thiophanate methyl. Hassan *et al.* (2014) [9] determined the efficacy of commonly available fungicides at six different concentrations against *Alternaria solani*. Results showed that Chlorothalonil has better effectiveness as compared to others followed by Clipper and Antracol.

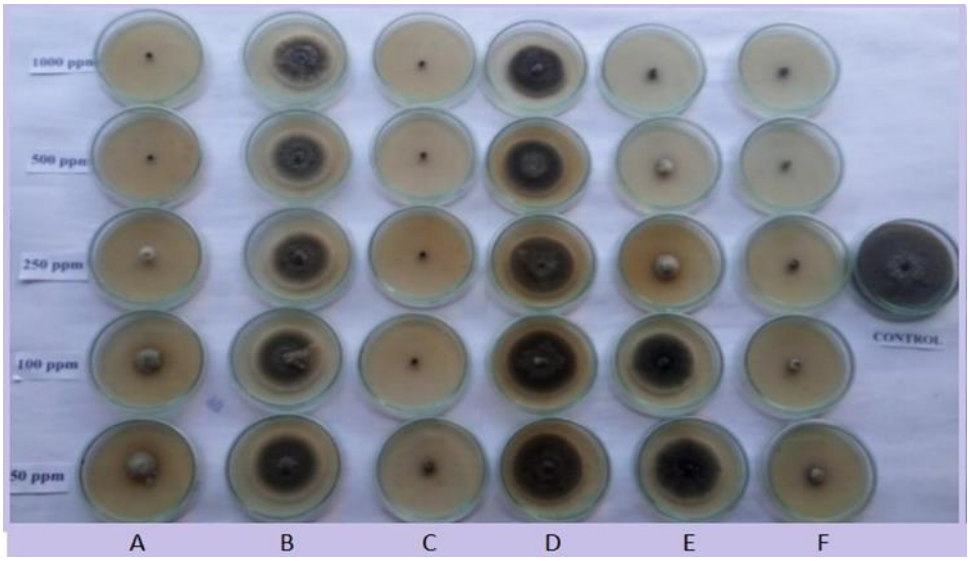

Plate 3: *In vitro* efficacy of fungicides against *Alternaria solani (*A-Carbendazim + mancozeb, B-COC, C- Metalaxyl + mancozeb, D-Carbendazim, E- Thiophanate methyl, F- Mancozeb)

Table 5: *In vitro* efficacy of different fungicides at different concentrations against *Alternaria solani*

S. No.	Fungicides/Concentration	Colony diameter (mm)*					Mean	Per cent inhibition					Mean
		50 ppm	100 ppm	250 ppm	500 ppm	1000 ppm		50 ppm	100 ppm	250 ppm	500 ppm	1000 ppm	
1.	Carbendazim + mancozeb	28.80	22.11	15.31	0.00	0.00	13.24	66.70	74.43	82.30	100	100	84.69
2.	Copper oxy chloride	65.91	65.36	63.16	60.48	55.56	62.09	23.80	24.43	26.98	30.08	35.76	28.21
3.	Metalaxyl + mancozeb	15.34	0.00	0.00	0.00	0.00	3.07	82.26	100	100	100	100	96.45

4.	Carbendazim	65.05	63.65	60.41	58.21	51.48	59.76	24.79	26.41	30.16	32.70	40.48	30.91
5.	Thiophanate methyl	62.45	54.74	30.43	16.38	8.45	34.49	27.80	36.72	64.82	81.06	90.23	60.13
6.	Mancozeb	18.52	13.74	9.30	0.00	0.00	8.31	78.58	84.11	89.24	100	100	90.38
7.	Control	86.50	86.50	86.50	86.50	86.50	86.50	-	-	-	-	-	
	Mean	48.93	43.72	37.87	31.65	28.85		50.65	57.68	65.58	73.97	77.74	
	SE(d)	7.10											
	SE.m.±	5.02											
	CD @ 0.01%	14.56											

* mean of three replicates

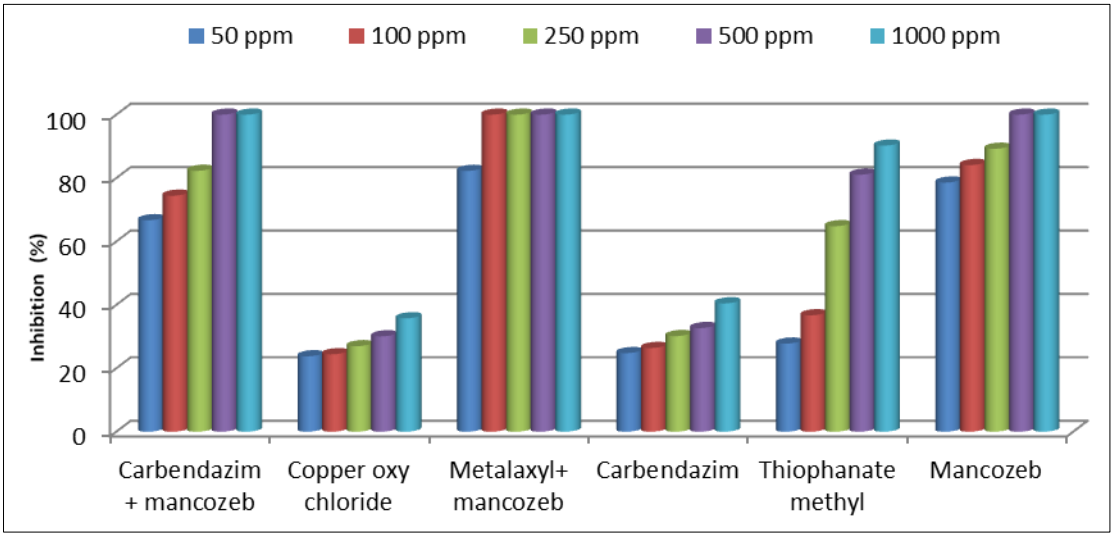

Fig 3: *In vitro* efficacy of different fungicides against pathogen

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