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MANAGEMENT OF EARLY BLIGHT OF TOMATO UNDER *IN VITRO* AND FIELD CONDITIONS

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ABSTRACT

Early blight of tomato is a very serious disease in tomato growing areas causing up to 80 percent crop loss. To evaluate the efficacy of botanicals and fungicides against early blight experiments were conducted during rabi season 2024 (February to April) at P.G. Department of Agriculture, Khalsa college Amritsar under *in vitro* and field conditions. Among all the botanicals, *Melia azedarach* was found to be most effective with average mycelial inhibition of 71.20 per cent followed by Garlic (59.26 %). Lantana was found least effective and among fungicides, maximum average mycelial growth inhibition of 88.44 per cent was recorded in Opera followed by Nativo (85.71%). Under field conditions, among botanicals and fungicides evaluated, minimum disease incidence (11.57 %) and disease severity (6.54%) were recorded in plots with treatment of Opera. Nativo were found next in efficacy with disease incidence of 16.87 per cent and disease severity of 11.57 per cent. Among botanicals, Darek was found most effective with disease incidence of 19.23 per cent and disease severity of 15.32 per cent followed by Garlic (25.67%, 19.85%, respectively) along with enhanced fruit quality and yield.

Keywords : Botanicals, fungicides, *in vitro*, *in vivo*, early blight, tomato.

Introduction

Tomato (*Lycopersicon esculentum* L.) is the second most important remunerable solanaceous vegetable crop after potato. Tomato contains 95.30 percent water, 0.07 percent calcium, and niacin all of which are crucial for human metabolism. It has a high nutritional content and offers a balanced supply of vitamins A, C, and E, all of which are essential for human health. Tomato is affected by number of diseases caused by fungi, bacteria, viruses and other pathogens. Diseases could be soil borne and above ground infections while in some cases it could be transmitted by insects (TNAU, 2023). Amongst fungal diseases, damping off (*Pythium aphanidermatum*), septoria leaf spot (*Septoria lycopersici*), early blight (*Alternaria solani*), late blight (*Phytophthora infestans*), anthracnose (*Colletotrichum gloeosporoides*), powdery mildew (*Oidium lycopersicum*), fusarium wilt (*Fusarium oxysporum f.sp. lycopersici*), verticillium wilt (*Verticillium dahliae*) and buckeye rot (*Phytophthora nicotianae*

parasitica) are important diseases that cause huge crop losses (Kumar *et al.*, 2018). Among these diseases, early blight caused by *Alternaria solani* is the most destructive disease of tomato which severely affects the growth and yield of tomato. The disease occurs at all stages of plant growth and affect all above ground parts of tomato including leaf, stem and fruits (Akhtar *et al.*, 2019; Mphahlele *et al.*, 2020). Tomato early blight has been reported in many parts of the world.

The initial symptoms typically manifest as small, brown water soaked lesions on the older leaf of the plant. Brown water soaked lesion may enlarge and concentric rings can form in the middle of the spots. As the disease progress, the color of the spot changes from brown to dark brown while, adjacent spots also merge together forming large irregular spots. The infection starts on the lower leaves and gradually progress upwards. In the later stages, symptoms can progress and change in appearance. The disease is managed by the use of several conventional fungicides but due to

development of resistance in pathogenic fungi against fungicides and human health risk has given a push for obtaining alternatives to control plant pathogenic fungi. There is need for alternative methods of disease management in tomato as use of chemical fungicides results in retention of residues in the fruit due to its succulent nature. Use of botanical pesticides is one of the best alternatives for the management of foliar pathogen. Botanicals are widely available, less costly, accessible and rapidly biodegradable and have little toxic effect to beneficial micro-organism (Ngegba *et al.*, 2022). Botanical pesticides have a low toxicity to mammals, a low chance of causing resistance in target pests and good effects on environmental preservation (Ahmad *et al.*, 2013; Neeraj *et al.*, 2017; Liu *et al.*, 2017). The benefits of botanical pesticides are their rapid degradation and lack of persistence and bioaccumulation in the environment, which have been major problems in case of synthetic fungicide (Arson *et al.*, 2012). Botanicals namely *Azadirachta indica*, *Eucalyptus chamadulonsis* and *Allium sativum* were found to be quite effective in reducing the disease incidence of tomato early blight and subsequently

increasing the fruit yield of tomato (Chourasiya *et al.*, 2013).

Material and Methods

Present investigation was carried out at P.G. Department of Agriculture, Khalsa College, Amritsar under *in vitro* and *in vivo* conditions to evaluate the efficacy of botanicals and fungicides against *Alternaria solani* rabi season 2024. *In-vitro* study was laid-out with Complete Randomized Design (CRD) and *in-vivo* study was laid-out with Randomized Block Design (RBD) with three replications. The details are as follow:

In vitro evaluation of Botanicals

The treatments comprised of application of selected botanicals extracts selected from local flora, evaluated *in vitro* at different concentrations (10, 20, 50) for their efficacy on inhibition in mycelial growth of *Alternaria solani* by Poisoned food technique (Falck, 1907) and inhibitory effect of these extracts on the mycelial growth of *Alternaria solani* was observed.

Table 1 : List of botanicals used and their concentrations.

Sr.No	Plants	Scientific name	Plant Part used	Concentrations (%)
1	Neem	<i>Azadirachta indica</i>	Leaves	10,20,50
2	Tulsi	<i>Ocimum sanctum</i>	Leaves	10,20,50
3	Darek	<i>Melia azaderach</i>	Leaves/seeds	10,20,50
4	Lantana	<i>Lantana camera</i>	Leaves	10,20,50
5	Eucalyptus/ Safeda	<i>Eucalyptus spp</i>	Leaves	10,20,50
6	Garlic	<i>Allium sativum</i>	Bulb/ cloves	10,20,50

Preparation of plant extracts

Fresh leaves and seeds (200g) of each plant were taken and properly washed. The leaves were crushed using mixer and blender by adding small quantity of sterilized distilled water to help in effective grinding. After grinding 200ml of distilled water was added to the mixture and homogenized in orbital shaker at 2000rpm for half an hour and plant material was then filtered through double layered muslin cloth. After extraction the botanicals of different plants were sterilized in autoclave at 5 psi pressure for 30 minutes for 3 consecutive days and the extract were kept in refrigerator for further use.

The autoclaved extract was mixed individually in Potato Dextrose Agar medium at the time of pouring and allowed to solidify. The mixtures of different concentrations (10%, 20%, 30%) were prepared one by one and poured aseptically into the Petri pates. The test pathogen (*Alternaria solani*) which was isolated from early blight infected leaves was taken and a small

culture bit of 5mm size was cut using cork borer and placed on solidified poisoned media. Poured double strength PDA on a Petri plate and were served as control after inoculating it with test fungus. Each treatment was replicated thrice using CRD (Completely Randomised Design). Finally, Petri plates were incubated at 26±1°C for 10-12 days.

Per cent mycelial growth inhibition will be calculated by the following formula given by

Vincent (1947) as below:

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

I = Per cent mycelial inhibition

C = Linear growth in control

T = Linear growth in treatment

***In-vitro* evaluation of fungicides**

Three fungicides at different concentrations (50 ppm, 100 ppm, 150 ppm) were evaluated under *in vitro*

condition for their efficacy against mycelial growth of *Alternaria solani* through Poisoned food technique (Falck, 1907).

Table 2 : List of fungicides used and their concentrations.

Chemical name	Trade name	Concentration (ppm)
Pyraclostrobin 13.3% + Epoxyconazole 5% SE	Opera	50,100,150
Propineb 70% WP	Antracol	50,100,150
Tebuconazole 50% + Trifloxystrobin 25% WG	Nativo	50,100,150

For fungicide evaluation, buffer stock of 1000 ppm was prepared using the test fungicide. For 50 ppm, using micropipette 50ml of fungicide from buffer stock was added to 50ml of sterilized distilled water. After mixing, the poisoned water was mixed with the 50ml autoclaved double strength PDA medium. The poisoned PDA media was poured into the Petri plates and left for solidification. In the same way 100 and 150ppm concentrations were prepared. A small culture bit of 5mm size of *Alternaria solani* was cut with sterilized cork borer, picked and finally placed in center of Petri plate under aseptic conditions in laminar air flow. For control, use Petri plates with media without fungicide. Incubate the Petri plates at 25°C for 10-12 days in BOD incubator. Radial growth of the fungal mycelium (mm) was recorded from the incubated Petri plates and per cent mycelial growth inhibition was calculated by the following formula given by Vincent (1947)

Management of early blight of tomato under field conditions

Disease management studies were carried out at the experimental farm of PG Department of Agriculture, Khalsa College Amritsar, Punjab. The experiment was conducted in RBD (Randomized Block Design) in 3×3m² plot size. Distance from plant to plant and row to row was (60×30cm).

Field evaluation of Botanicals and Fungicides

Botanicals extracts of Darek, Garlic and Eucalyptus at 10 per cent concentration, along with Propineb, Pyraclostrobin + Epoxyconazole and Tebuconazole + Trifloxystrobin were evaluated under field conditions for comparison. There were total 7 treatments including control. Sprays of different treatments were given starting with the appearance of the disease and the control plants were sprayed with water only at 10 days' interval during 2024.

After the sprays, disease incidence and severity was recorded in each treatment. Per cent disease incidence and severity were calculated by the following formula.

$$\text{Disease incidence (\%)} = \frac{\text{Total number of infected plant}}{\text{Total number of plant observed}} \times 100$$

$$\text{Disease severity (\%)} = \frac{\text{Sum of all individual ratings}}{\text{Total number of rating}} \times 100 \\ \times \text{Maximum disease grade}$$

Yield (quintals/ha), height of the plants from bottom to the top apex of the plant (cm) and diameter of tomatoes per plant were also recorded in each treatment.

Results and Discussion

***In vitro* evaluation of botanicals**

Results of the present investigation as presented in Table 4 indicate that all the tested botanicals inhibited the mycelial growth of the *Alternaria solani*. Among all Darek was found to be most effective showing an average mycelial inhibition of 71.20 per cent followed by Garlic (59.26%) and Eucalyptus (52.52%). Lantana was found to be least effective with average mycelial inhibition of 24.63 per cent. Different workers have reported the efficacy of different botanicals against *Alternaria solani*. Our results are in accordance with Kumar *et al.* (2017) who evaluated different botanicals against *Alternaria solani* under *in vitro* conditions. Results showed that among all the tested botanicals, Drake was found to be the most effective with maximum per cent mycelial inhibition of 63.52. Bashir *et al.* (2020) research showed that *Melai azedarach* was found to be most efficient with 73.18 per cent inhibition at 20 per cent concentration followed by *Cannabis sativa*, *Datura stramonium* and *Achillia millefolium* (55.79%, 37.12%, 31.90%, respectively).

Table 4: *In vitro* efficacy of botanicals (Neem, Lantana, Darek, Euclyptus, Tulsi, Garlic) against early blight of tomato

Botanicals	Mycelial growth (mm) at different concentrations (%)				Mycelial inhibition (%)
	10	20	50	Mean	
Neem	69.43 (56.42)	56.27 (52.12)	34.77 (35.29)	53.49 (47.95)	40.57
Tulsi	62.33 (67.86)	44.33 (50.04)	33.17 (43.84)	46.61 (53.91)	48.21
Darek	33.43 (48.58)	24.37 (41.73)	19.97 (29.56)	25.92 (39.96)	71.20
Lantana	85.77 (57.57)	71.27 (39.98)	46.47 (37.80)	67.83 (45.12)	24.63
Eucalyptus	58.77 (36.12)	41.33 (35.14)	28.10 (26.39)	42.73 (32.55)	52.52
Garlic	48.00 (42.96)	37.58 (31.96)	24.42 (29.60)	36.67 (34.84)	59.26
Mean	59.62 (51.58)	45.86 (41.83)	31.15 (33.75)		

Figure in parentheses arc sine transformed values

CD_(0.01)

Treatment = 1.582

Concentration = 1.118

Treatment × Concentration = 2.740

In vitro evaluation of fungicides

Among fungicides evaluated under *in vitro* conditions, maximum average mycelial growth inhibition of 88.44 per cent was recorded in Opera followed by Nativo (85.71%). Whereas, minimum mycelial inhibition of 75.99 per cent was recorded in Antracol as shown in Table 5. Our results are in accordance with Choudhary *et al.* (2021) who

evaluated efficacy of various fungicides at four different concentrations under *in vitro* conditions against *Alternaria solani*. The results showed that Opera (Pyraclostrobin 133 G/L + Epoxiconazole 50 G/L SE) was found to be highly effective, inhibiting 89.62 per cent mycelial growth at 250 ppm concentration and 100 per cent mycelial growth inhibition at 500, 750 and 1000 ppm concentrations.

Table 5: *In vitro* evaluation of different fungicides (Opera, Antracol, Nativo) against mycelial growth of *Alternaria solani*

Fungicides	Mycelial growth (mm) at different concentrations (ppm)				Mycelial inhibition (%)
	50	100	150	Mean	
Opera	13.75 (21.75)	10.67 (34.33)	6.81 (26.60)	10.41 (27.56)	88.44
Antracol	31.83 (19.04)	20.67 (27.02)	12.33 (19.20)	21.61 (21.75)	75.99
Nativo	20.08 (15.09)	10.83 (20.55)	7.67 (16.05)	12.86 (17.23)	85.71
Mean	21.89 (18.63)	14.06 (27.30)	8.94 (20.62)		

Figure in parentheses arc sine transformed values

CD_(0.01)

Treatment = 0.951

Concentration = 0.951

Treatment × Concentration = 1.647

Efficacy of botanicals and fungicides under field conditions

After *In vitro* evaluation of botanicals and fungicides, three most effective botanicals (Darek, Garlic, Eucalyptus) and all three fungicides were evaluated under field conditions. Botanicals were

Table 6 : Field evaluation of various botanicals (Darek, Garlic, Eucalyptus) and fungicides (Opera, native, Antracol) against early blight of tomato.

Treatments	Disease incidence (%)	Disease severity (%)	Fruit diameter (cm)		Plant height (cm)	Yield (q/hac.)
			Healthy fruit	Diseased fruit		
Darek	19.23 ^d	15.32 ^c	5.30	4.87	81.67	211.15
Garlic	25.67 ^b	19.85 ^b	5.25	4.53	76.67	199.35
Euclyptus	29.5 ^a	21.33 ^a	4.60	4.21	65.00	190.94
Opera	11.57 ^f	6.54 ^f	4.77	4.10	71.33	215.30
Antracol	20.87 ^c	13.67 ^d	4.47	3.93	58.33	195.86
Nativo	16.87 ^e	11.57 ^e	4.33	3.21	53.33	204.98
Control	40.67	35.76	3.89	3.02	50.45	85.89
C.D _(0.05)	1.165	0.554	0.392	0.195	10.02	6.480

Figure in superscripts are DMRT test ($p=0.05$) values

The investigation on effect of fungicides and botanicals on various parameters under field conditions is indicated in Table 6. Among all the treatments, minimum disease incidence (11.57 %) and disease severity (6.54%) were recorded in plots with treatment of Opera. Nativo were found next in efficacy with disease incidence of 16.87 per cent and disease severity of 11.57 per cent. While, Antracol was found to be least effective fungicides under field evaluation. Among botanicals, Darek was found most effective with disease incidence of 19.23 per cent and disease severity of 15.32 per cent followed by Garlic with disease incidence of 25.67 per cent and disease severity of 19.85 per cent. Eucalyptus was least effective botanical under field evaluation with disease incidence and severity of 29.50 and 21.33 respectively. Although disease incidence and disease severity were comparatively lower in the treatments where chemical fungicides were sprayed but the plant growth and fruit yield was quite significantly observed in treatments where botanical were sprayed. Maximum fruit diameter of 5.30cm was found in the plot where Darek was sprayed followed by Garlic (5.25cm) and Eucalyptus (4.60cm). Similarly, highest average plant height of 81.67cm was recorded in treatment with sprays of Darek followed by Garlic and Opera with plant height of 76.67 and 71.33cm respectively. Highest yield of 215.30q/ha was recorded in fungicide Opera followed by Darek i.e. 211.15q/ha. Minimum yield was found in plots sprayed with Eucalyptus (190.94 q/ha) when compared with control plots.

evaluated at 10 per cent concentration while the fungicides were evaluated at different rates. The data on per cent disease incidence, disease severity, fruit diameter (cm), plant height (cm) and yield (q/ha) were recorded.

The results are in corroboration with Bashir *et al.* (2020) who conducted *in vivo* evaluation of four plant extracts (*Melia azedarach*, *Cannabis sativa*, *Achillia millefolium*, *Datura stramonium*) against early blight, results showed that Darek showed highest reduction of disease severity (67.93%) followed by *Cannabis sativa*, *Datura stramonium* and *Achillia millefolium* (53.63%, 43.28%, 31.78%, respectively). Ganeshan and Chetana (2009) who evaluated efficacy of Pyraclostrobin (25%EC) @ 50, 75 and 100ga.i/ha against early blight of tomato. The results showed that Pyraclostrobin at all concentrations was found significantly effective in reducing the disease level. Mesara *et al.* (2021) reported that tomato plants showed a significant increase in the height of the plant, leaf number and flower number when cumulative Pyraclostrobin application were given.

Conclusion

The results of the research conducted shows that, botanicals and fungicides both are effective in controlling early blight of tomato. Botanicals when used effectively and at regular intervals are highly effective in controlling the disease *in vitro* and in field conditions. The botanicals are eco-friendly, biodegradable and no remains in the succulent tomatoes, making them fit for consumption and there is no residual effect.

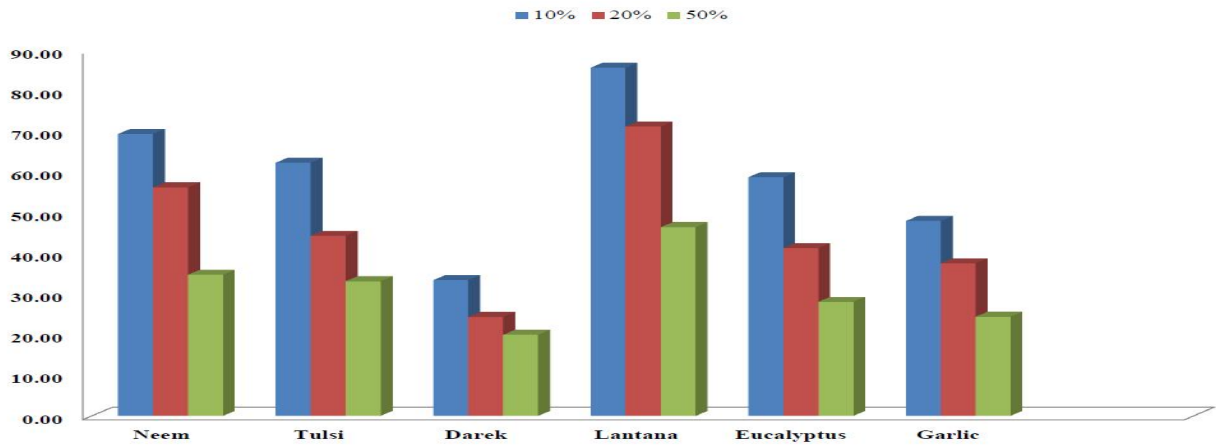


Fig. 1 : Efficacy of different botanicals against mycelial growth of *Alternaria solani*

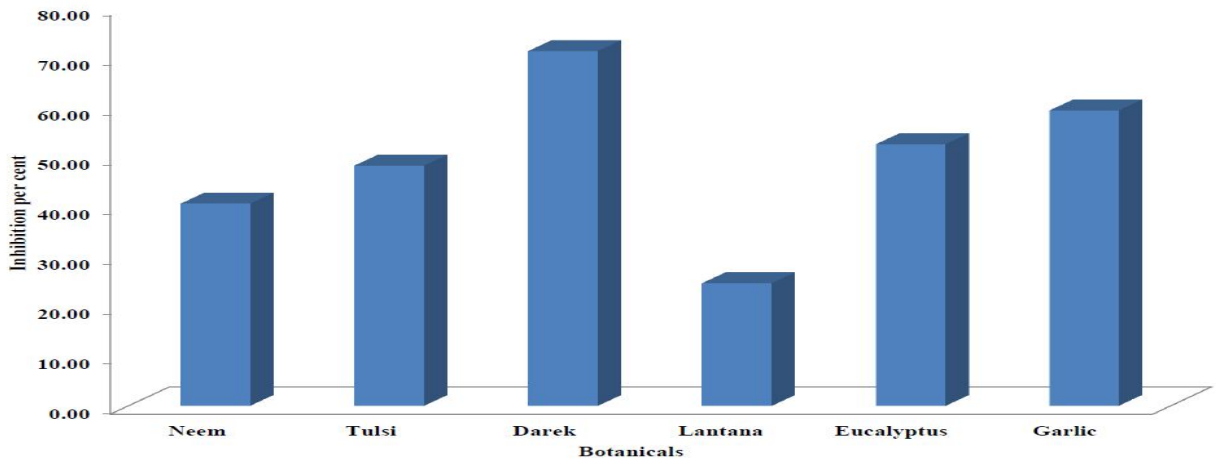


Fig. 2 : Inhibition per cent of *Alternaria solani* by different botanicals

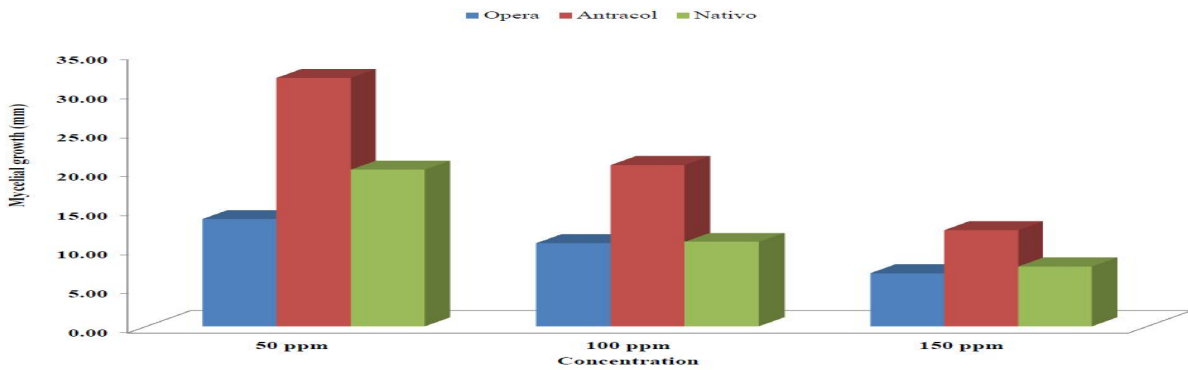


Fig. 3 : Efficacy of fungicides against mycelial growth of *Alternaria solani*

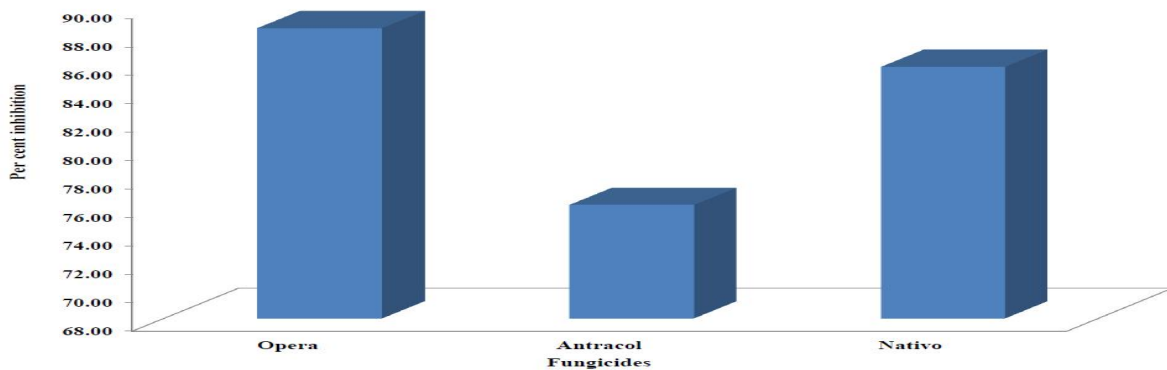


Fig. 4 : Inhibition per cent of *Alternaria solani* by different fungicides



Plate 1: Botanicals evaluated

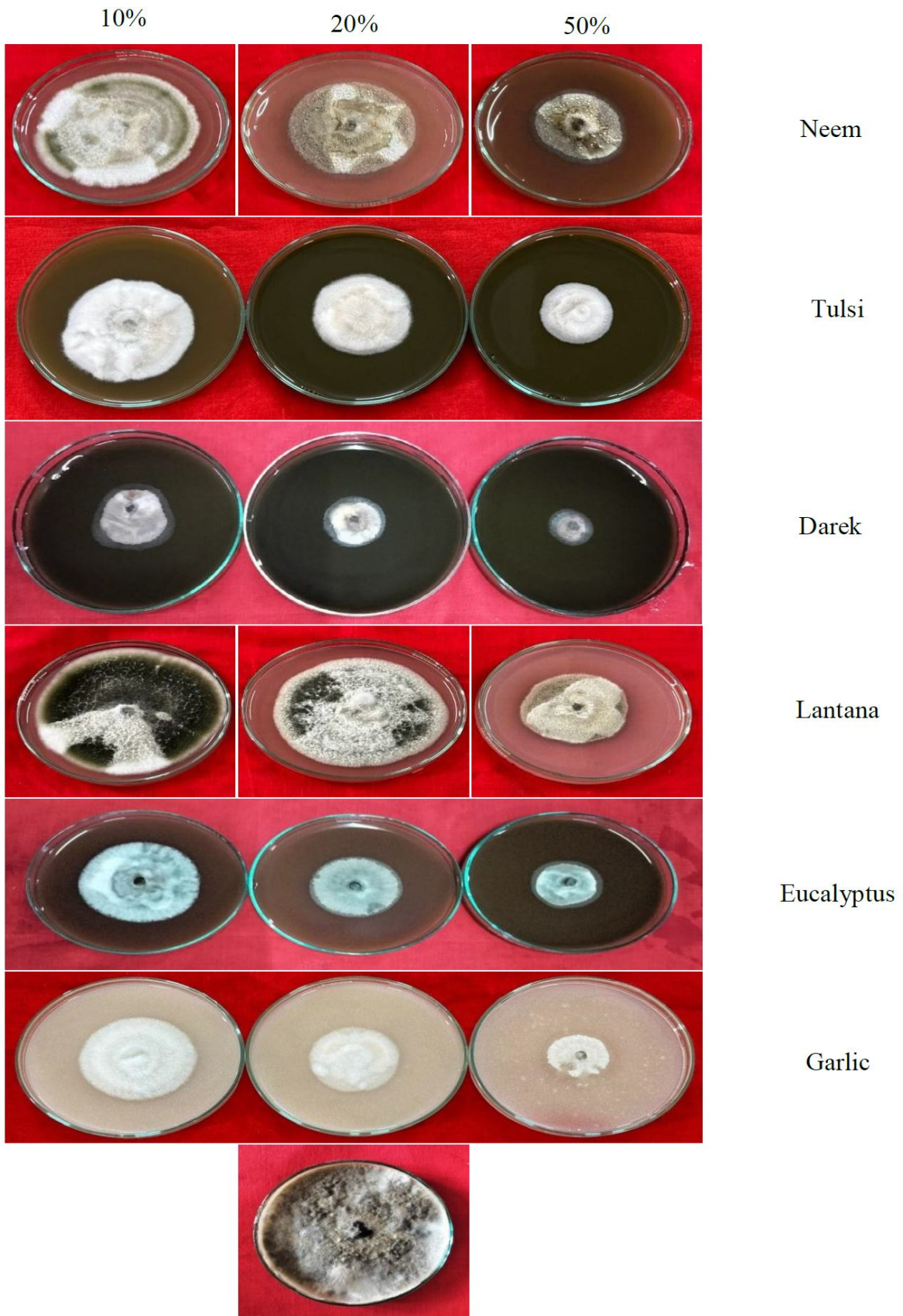


Plate 2: *In vitro* efficacy of botanicals (Neem, Tulsi, Darek, Lantana, Eucalyptus, Garlic) against *Alternaria solani*

50 ppm

100 ppm

150 ppm



Opera



Antracol



Nativo



Control

Plate 3 : *In vitro* efficacy of fungicides (Opera, Antracol, Nativo) against *Alternaria solani*

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