

Original Research Article

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***In Vitro* Evaluation of Fungicides and Plant Extract against *Alternaria solani* (Ellis) Causing Early Blight in Tomato (*Lycopersicon esculentum* Mill.)**

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ABSTRACT

Early blight disease of tomato caused by *Alternaria solani* (Ellis) is an economically important disease causing huge losses throughout Country. In the present investigation six fungicides viz. Trifloxystrobin 25% w/w + Tebuconazole 50% WG, Difenoconazole 25% EC, Hexaconazole 5 % SC, Propineb 70 WP, Azoxystrobin 23%SC, Thiafluzamide 24%SC at 50, 100, 250, 500 and 1000ppm concentration and Six plants extracts viz. *Datura stramonium* (Dhatura) green fruit extract, *Azadirachta indica* (Neem) seed kernel extract, *Allium sativum* (Garlic) bulb extract, *Eucalyptus spp.* (Eucalyptus) dry leaf extract, *Crotalaria juncea* (Sunhemp) seed extract, *Euphorbia hirta* (Bara Dudhi) whole plant extract at 1, 2, 3, 4 and 5 percent concentration were tested against *A. solani* under laboratory condition. Among the six fungicides, most effective fungicides was found Hexaconazole 5% EC which exhibited 100.00 percent inhibition in mycelium growth at 100ppm followed by followed by Thiafluzamide 24% SC and Trifloxystrobin 25% w/w + Tebuconazole 50% WG at 500ppm. Out of six plant extracts tested against *A. solani in vitro*, most effective plant extract was found *Allium sativum* @ 5% which exhibited maximum inhibition in mycelium growth (45.15%) followed by *Crotalaria juncea* @ 5% (44.40%), while minimum inhibition in mycelium growth was recorded in *Euphorbia hirta*.

Keywords

Alternaria solani,
Concentrations,
Fungicides, *In vitro*,
Plant extracts.

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Introduction

Tomato (*Lycopersicon esculentum* Mill.) is one of the most remunerative and widely grown vegetables in the world. It is a small annual or short lived perennial herb belonging to the family Solanaceae, probably native of 'Peru-Ecuador'. It is a regular kitchen component of Indian diet which is used as raw fruit and also as cooked processed products like soup, ketchup, sauce, pickle, pastes and powder. The pulp and juice of tomato is very digestible, promoter of gastric secretion and blood purifier, additionally it

nutrients and metabolites (Folate, potassium, and vitamins A and C) that are important for human health. Tomato is being extensively grown as an annual plant all over the world. Tomato has ranks second next to potato in world acreage but it has rank first among processing crops. It is cultivated in an area of 4.73 million hectares all over the world with production of 163.96 million tones and an average yield of 34.66 tones ha⁻¹. In India, it is grown in a wide range of climate across states of Andhra Pradesh, Odisha, Karnataka,

Maharashtra, West Bengal, Bihar, Gujarat, Uttar Pradesh, Madhya Pradesh and Chhattisgarh, accounting total production of 18732 thousand tones from an area of 774 thousand hectares with an average productivity of 24.20 tones ha⁻¹ during 2015-16 (Anonymous, 2016).

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. More than 200 diseases have been reported to infect tomato in the world (Atherton and Rudich, 1986). Large number of fungal diseases such as early blight (*Alternaria solani*), Late blight (*Phytophthora infestans*), Septoria leaf blight (*Septoria lycopersici*), Powdery mildew (*Oidiopsis taurica*), Fusarium wilt (*Fusarium oxysporum* f. sp. *lycopersici*), Collar rot (*Sclerotium rolfsii*), and Damping off (*Pythium* sp.) are causes severe losses in tomato. Among the fungal diseases, early blight caused by *Alternaria solani* is one of the most important and frequent occurring disease of the crop nation and worldwide (Jones *et al.*, 1991).

The early blight was the most catastrophic diseases incurring loss under field and post-harvest stages causing 50 to 86 percent reduction in fruit yield (Mathur and Shekhawat, 1986). Saha and Das (2012) reported losses in yield 0.75 to 0.77 tons ha⁻¹ with 1per cent increase in disease severity. Once early blight is established in the crop, it is very difficult to be controlled (Smith and Kotcon, 2002). Fungicide treatments are generally the most effective control measures, but are not economically feasible in all areas of the world and may not be effective under weather conditions favorable for disease epidemics. It is very essential to determine the efficacy of different fungicides and plant

extracts against early blight of tomato. Therefore, keeping in view of above facts present experiments were conduct on “*in vitro* efficacy of fungicides against *Alternaria solani* causing early blight disease in Tomato (*Lycopersicon esculentum* Mill.)”.

Materials and Methods

Isolation, identification and purification of *Alternaria solani*

Tomato leaves showing typical early blight symptoms were collected from growing tomato plants from different tomato growing fields during crop season. Standard tissue isolation technique was followed to obtain *A. solani* culture described by Naik *et al.*, (2010). The leaves were microscopically examined to confirm the presence of the fungus. After confirming for the presence of fungal spores, isolation was done by following standard tissue isolation. The culture was purified by single spore technique described by Johnson and Booth (1983). In case of single spore technique 2 to 3 drops from spore suspension prepared from 10 days old culture with sterilized distilled water. Later 1 ml of suspension was spread on the surface of plain agar medium in Petri plates and incubated at 25±2°C for 24 h. The plates were observed for germinating spores under stereoscopic microscopic and finally germinating spores were lifted by inoculation needle and transferred aseptically to potato dextrose agar slants for further growth.

Evaluation of fungicides

The poisoned food technique (Nene and Thapliyal, 1993) was followed to evaluate the efficacy of six systemic fungicides *viz.* Trifloxystrobin 25% w/w + Tebuconazole 50%WG, Difenconazole 25% EC, Hexaconazole 5 % SC, Propineb 70 WP, Azoxystrobin 23%SC, Thiafluzamide 24%SC against *A. solani* at five concentrations (50,

100, 250, 500 and 1000ppm). Fungicides were added to the potato dextrose agar medium before sterilization as per treatment detail. Five mm disc of *A. solani* was taken from seven days old culture and placed at center of petri dish. The activity of fungicides were recorded by measuring the colony diameter of *A. solani* in each treatment and compared with control.

Evaluation of plant extracts

Tested efficacy of plant extracts against *Alternaria solani* using poisoned food technique under *in vitro* conditions. Six plants extracts viz. *Datura stramonium* (Dhatura) green fruit extract, *Azadirachta indica* (Neem) seed kernel extract, *Allium sativum* (Garlic) bulb extract, *Eucalyptus spp.* (Eucalyptus) dry leaf extract, *Crotalaria juncea* (Sunhemp) seed extract, *Euphorbia hirta* (Bara Dudhi) whole plant extract were tested at 1, 2, 3, 4 and 5 percent concentration. Fresh healthy plant parts of 100g (leaves/fruit/bulb) were collected from field, then they were washed with tap water, air dried and crushed in 100 ml of sterile water. Potato dextrose agar medium was used as nutrient medium and required quantity of each plant extract was added separately to get a required concentration of the plant extract. The plant extract were thoroughly mixed with PDA medium and sterilized at 121⁰C for 20 minutes. Twenty milliliter of poisoned medium was poured to each of the 90 mm petri dishes and allowed for solidification.

Simultaneously without plant extract PDA was poured in petri dishes as control. Actively growing periphery of the 7 day old culture of *A. solani* was carefully cut using a cork borer and transferred aseptically to the centre of each petri dish containing the poisoned/non-poison solid medium. The plates were incubated at 25±2°C. Each treatment was replicated three times.

Observation recorded

The radial growth of the fungus on the poisoned medium was recorded at time of mycelium growth reached 90 mm in control. Per cent inhibition of mycelium growth of the fungus was calculated by using the formula described by Vincent (1927).

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

Where,

I = Per cent inhibition

C = Radial growth in control

T = Radial growth in treated (fungicide/ botanicals/ bioagents).

Results and Discussion

Evaluation of fungicides

Six fungicides were assessed *in vitro* to find out the most effective fungicide against *A. solani* at different concentrations viz. 50, 100, 250, 500, and 1000ppm using poison food technique.

The results are presented in table 1 reveal that the significant difference among fungicides against *A. solani* was observed. Fungus growth was checked in Hexaconazole 5% EC at 250ppm, in Trifloxystrobin 25% w/w + Tebuconazole 50%WG at 500ppm concentration and in Thiafluzamide 24% SC at 500ppm concentration (Plate 1). Among the six fungicides, most effective fungicides was found Hexaconazole 5% EC which exhibited 100.00 percent inhibition in mycelium growth at 100 ppm followed by followed by Thiafluzamide 24% SC and Trifloxystrobin 25% w/w + Tebuconazole 50%WG at 500 ppm. However, other fungicides were unable

to check 100.00 percent mycelium growth up to 1000 ppm concentration (Fig. 1). The present findings are similar with the result of Singh and Singh (2006) reported that the Hexaconazole 5% EC was very effective as it caused 100% growth inhibition of *A. alternata*. Similar type of result were also

obtained by Mesta *et al.*, (2009) who reported that the maximum inhibition of mean fungal growth of *Alternaria helianthi* in hexaconazole (72.87%) followed by difenoconazole (72.61%), mancozeb (58.29%), chlorothalonil (51.54%) and captan (50.43%).

Table.1 *In vitro* efficacy of fungicides against *Alternaria solani*

Fungicides	Colony diameter (mm) at different concentration					
	50ppm	100ppm	250ppm	500ppm	1000ppm	Mean
Trifloxystrobin 25% w/w + Tebuconazole 50% WG	26.33	20.67	13.00	0.00	0.00	12.00
Difenconazole 25 EC	67.00	64.33	62.00	61.00	59.00	62.67
Hexaconazole 5 EC	12.33	0.00	0.00	0.00	0.00	2.47
Propineb 70 WP	65.67	65.00	62.67	61.33	55.33	62.00
Azoxystrobin 23%SC	62.67	60.67	26.33	13.33	6.33	33.87
Thiafluzamide 24%SC	13.67	11.33	7.33	0.00	0.00	6.47
Control	89.27	89.27	89.27	89.27	89.27	89.27
Mean	48.10	44.47	37.23	32.13	29.99	
	CV (%)		SEm±		CD at 5%	
Fungicides	9.51		0.94		2.65	
Concentration			0.79		2.24	
Fungicides X Concentration			2.10		5.93	

Table.2 *In vitro* efficacy of botanicals against *Alternaria solani*

Botanicals	Mycelium diameter (mm) at different concentrations					
	1%	2%	3%	4%	5%	Mean
P ₁ – <i>Datura stramonium</i>	76.00	74.00	71.00	71.67	67.67	72.07
P ₂ – <i>Azadirachta indica</i>	70.33	66.33	63.67	55.33	52.00	61.53
P ₃ – <i>Allium sativum</i>	60.33	56.00	52.33	50.67	49.33	53.87
P ₄ – <i>Eucalyptus spp.</i>	73.67	66.00	59.67	57.00	52.33	61.73
P ₅ – <i>Crotalaria juncea</i>	61.33	59.67	54.33	53.00	50.00	55.53
P ₆ – <i>Euphorbia hirta</i>	87.33	86.00	84.67	83.33	82.00	84.67
Control	89.93	89.93	89.93	89.93	89.93	89.93
Mean	74.13	71.13	67.94	65.85	63.32	
		CV (%)	SEm±		CD at 5%	
Botanicals		5.37	0.95		2.68	
Concentration			0.80		2.27	
Botanicals X Concentration			2.12		5.99	

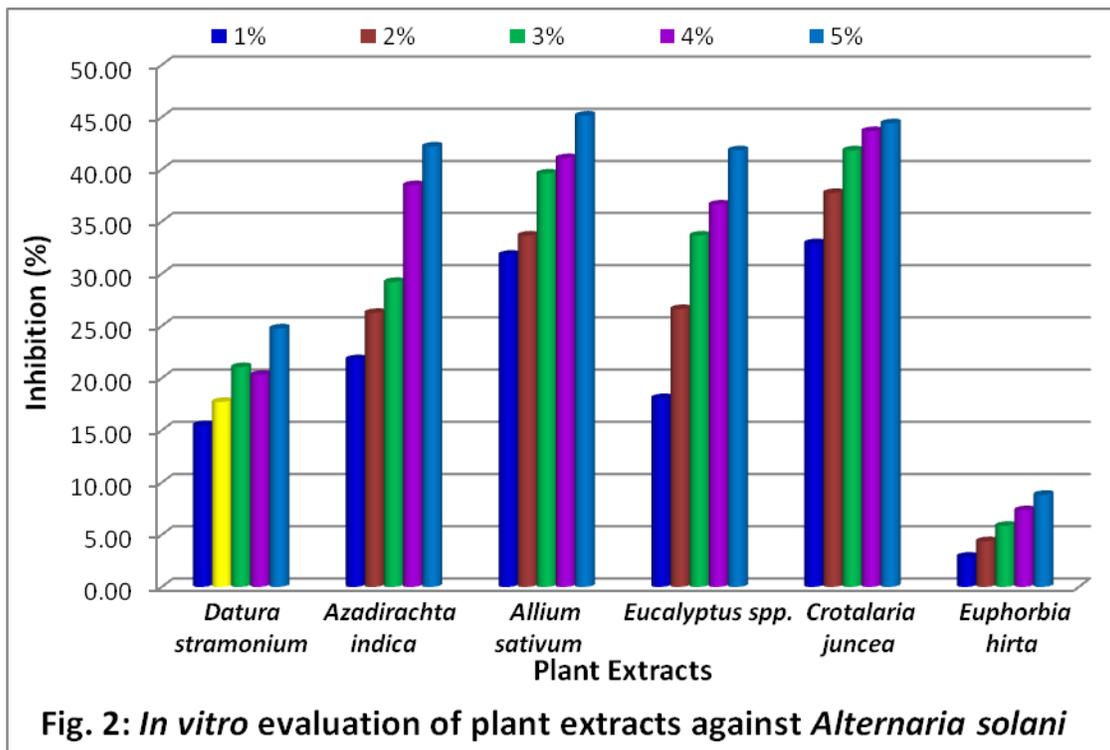
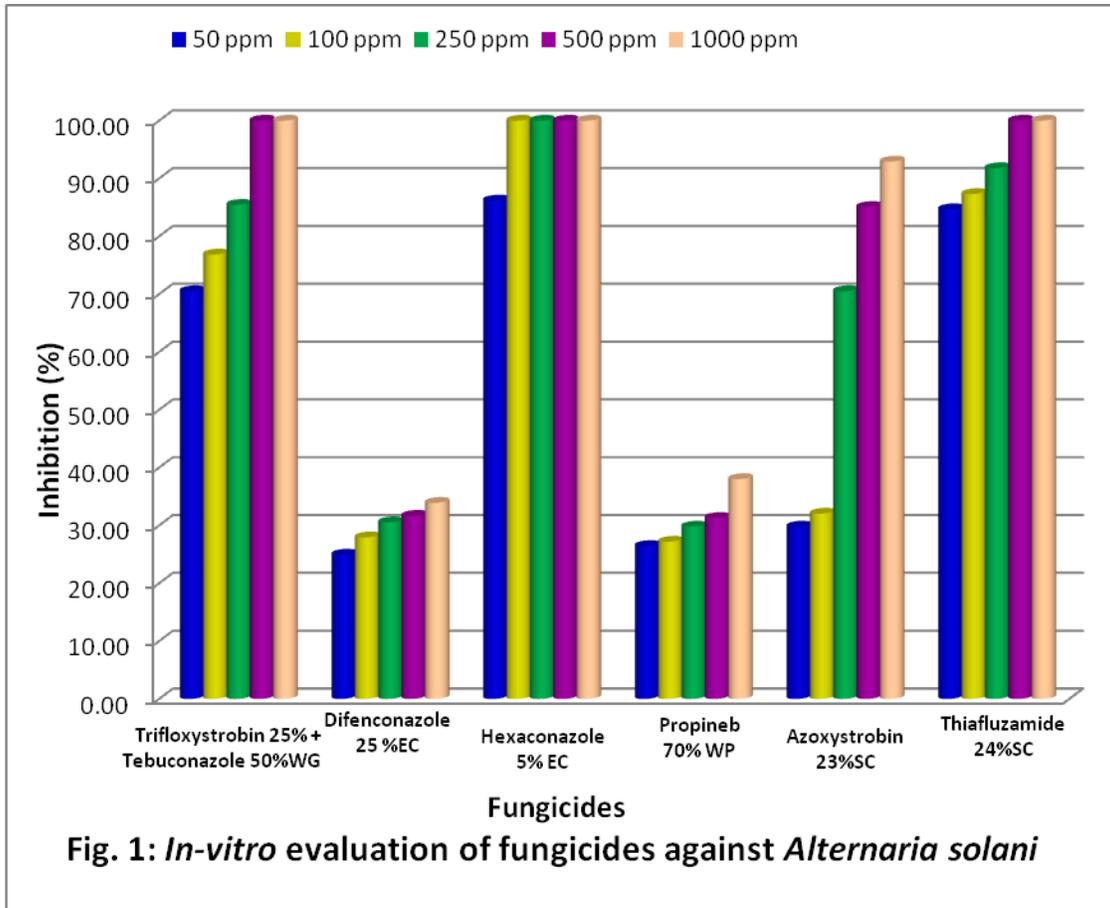


Plate.1 *In vitro* efficacy of fungicides against *Alternaria solani*

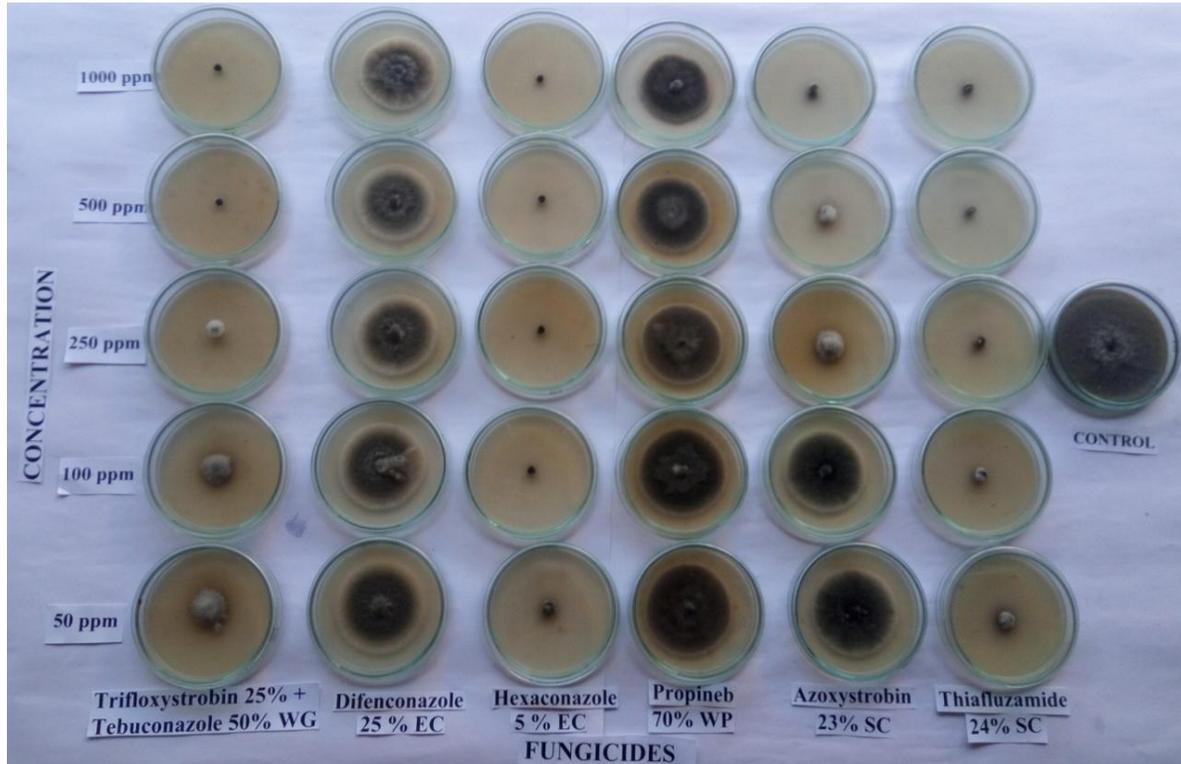
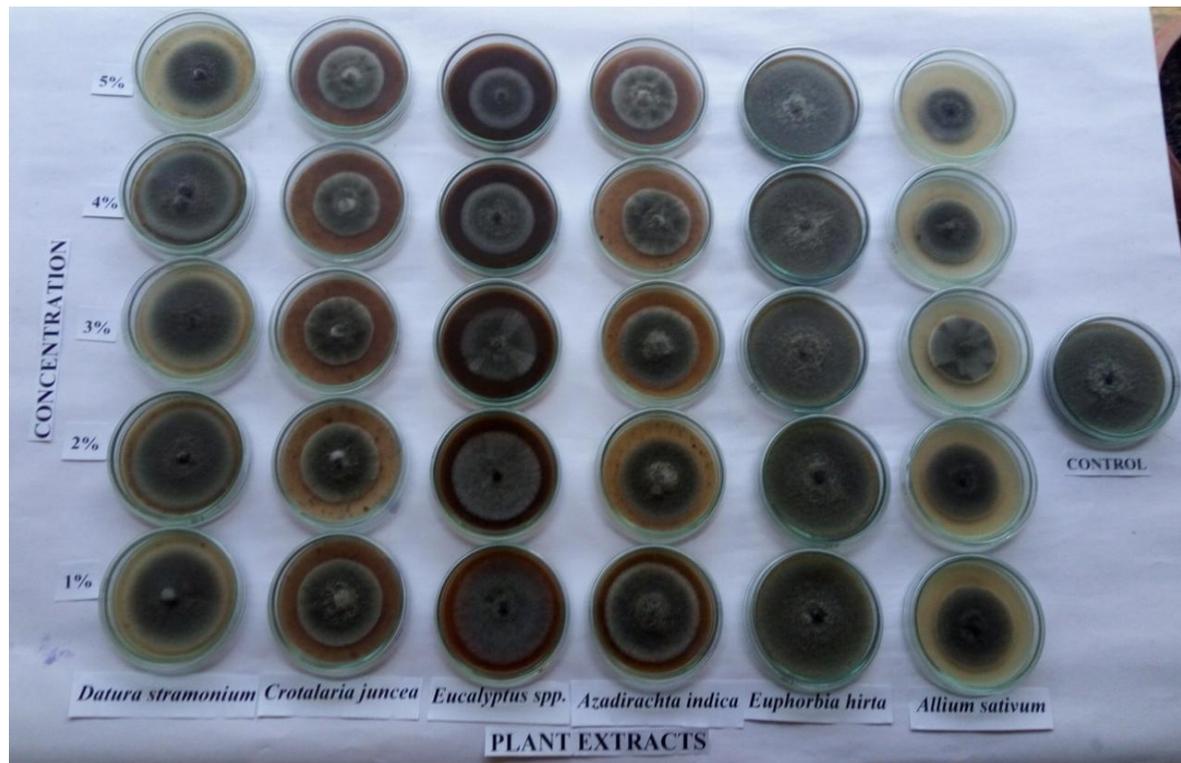


Plate.2 *In vitro* efficacy of plant extracts against *Alternaria solani*



Evaluation of plant extracts

Data regarding on *in vitro* efficacy of plant extracts against *Alternaria solani* have been presented in table 2 indicated that significant difference on mycelium growth was recorded in different plant extract in at all the concentrations. Among the six plant extract tested, most effective plant extract was found *Allium sativum* which exhibited minimum mycelium growth (53.87mm). It was significantly lower of over rest of the plant extracts. However, maximum mycelium growth (84.67mm) was observed in *Euphorbia hirta*. 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 (Plate 2). Interaction was found significant. In case of interaction between plant extract and concentration, minimum mycelium growth (49.33mm) was found in *Allium sativum* @ 5% which was at par with *Crotalaria juncea* @ 5% (50.00mm), *Allium sativum* @ 4% (50.67mm), *Azadirachta indica* @ 5% (52.00mm), *Allium sativum* @ 3% (52.33mm), *Eucalyptus spp.* @ 5% (52.33mm), *Crotalaria juncea* @4% (53.00 mm), *Crotalaria juncea* @3% (54.33 mm) and *Azadirachta indica* @ 5% (55.33mm) 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% (45.15%) followed by *Crotalaria juncea* @ 5% (44.40%), *Allium sativum* @ 4% (43.66%), *Azadirachta indica* @ 5% (42.18%), *Allium sativum* @ 3% (41.81%), *Eucalyptus spp.* @ 5% (41.81), while minimum inhibition in mycelium growth of 2.89% was recorded in *Euphorbia hirta* @ 1% (Fig. 2).

The present findings are confirmed with the results of Nashwa *et al.*, (2012) they reported

leaf extracts of *D. stramonium*, *A. indica*, and *A. sativum* @ 5% inhibited highest mycelial growth of *A. solani* (44.4, 43.3 and 42.2%, respectively). Dalpati *et al.*, (2010) tested ten botanicals *viz.* Neem, Custard apple, Lantana, Eucalyptus, Marigold, Tamarind, Kanher, Garlic, Datura and Congress grass against the *Alternaria macrospora* causing leaf spot of cotton. The percent inhibition of botanicals ranged from 44.59 to 8.25 percent. Similar results were also reported by Sadana, and Didwania (2015) he reveal that the fresh aqueous extract of *Eucalyptus obliqua* @ 15% was most effective which exhibited 88 percent inhibition of mycelial growth of *A. solani* strain A1 followed by *Datura stamonium*, *Azadirachta indica*, *Calotropis procera* and *Polyalthia longifolia*.

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