

Original Research Article

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***In Vitro* Evaluation of Bacterial Biocontrol Agents and Botanicals against *Alternaria* Leaf Spot Caused by *Alternaria macrospora* in Cotton**

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Four bacterial biocontrol agents (*Pseudomonas fluorescens* strain 1, strain 2, strain 3 and *Bacillus subtilis* strain 1) and six botanicals {Neem (*Azadirachata indica*), Lantana (*Lantana camera*), Calotropis (*Calotropis procera*), Tulasi (*Ocimum sanctum*), Onion bulb (*Allium cepa*), Garlic glove (*Allium sativum*)} were evaluated *in vitro* against *Alternaria macrospora* causing leaf spot of cotton. All the treatments significantly inhibited mycelial growth of the test fungus over untreated control. Among different bacterial biocontrol agents, *B. subtilis* strain caused 51.68% inhibition with 1.73cm radial growth of *A. macrospora* followed by *P.f* strain 1, *P.f* strain 3 and *P.f* strain 2 with radial growth and inhibition of *A. macrospora* 2.08 cm, 41.89%; 2.15 cm, 39.94% and 2.45 cm, 31.56% respectively. Among botanicals, neem leaf extract was superior to other treatments with 2.83 cm radial growth and 68.52% inhibition of *A. macrospora* followed by garlic clove with mean radial growth 2.84 cm and 68.40% inhibition of *A. macrospora* compared to control (9.0 cm).

Introduction

Cotton (*Gossypium* spp.) is the most important commercial crops of the world, which belongs to the botanical family *Malvaceae*. Cotton is referred to as “King of Fibres” and also known as “White Gold”. Cotton crop is affected by fungal, bacterial and viral diseases. Among fungal diseases, leaf spot/blight caused by *Alternaria macrospora* Zimm, is the most commonly occurring disease in Andhra Pradesh. Under congenial conditions the disease causes severe defoliation, cracking and breaking of stems and reduction in boll formation. The disease caused losses to the tune of 38.23% in

LRA 5166 (Bhattiprolu and Prasada Rao, 2009) and 33.43% in Jayadhar varieties of cotton (Chattannavar *et al.*, 2010). In recent years, there is a change in trend towards the organic farming to get high quality produce and to protect the environment and public health. Therefore, there is a need and scope for exploiting sources of alternative chemotherapeutants which are reserved in many plant species Based on the importance of *Alternaria* leaf spot, the present investigation was carried out with a view to find out the efficacy of bacterial biocontrol agents and botanicals against *A. macrospora*.

Materials and Methods

Effect of bacterial bio-control agents on radial growth of *A. macrospora*

Three isolates of *Pseudomonas fluorescens*, which were native to RARS, Lam, Guntur and one isolate of *Bacillus subtilis*, native to Agricultural Research Station, Amaravathi were evaluated *in vitro* for their antagonistic activity against mycelial growth of *A. macrospora* by employing dual culture technique (Dhigra and Sinclair, 1985). Sterilized potato dextrose agar medium, melted and cooled at 45°C, was poured aseptically into sterilized Petri dishes. Mycelial discs of 5 mm diameter from the edge of actively growing culture of *A.*

macrospora was separately cut with the help of a sterilized cork borer and the disc was placed in the centre of the Petri dishes (9 cm diameter) and streaking the bacteria using sterilized inoculation loop on the periphery about one cm from the edge of Petri dishes on both sides.

The Petri dishes containing potato dextrose agar medium inoculated with the pathogen alone served as control. All the Petri dishes were incubated at room temperature of 25 ± 1°C. Ten days after incubation, the colony diameter of the pathogen was measured and the per cent inhibition of *A. macrospora* was calculated by adopting the following formula given by Vincent (1927).

Per cent inhibition =

$$\frac{\text{Radial growth in control (C)} - \text{Radial growth in treatment (T)}}{\text{Radial growth in control (C)}} \times 100$$

The experiment was repeated twice and the mean data on percent inhibition was calculated.

Effect of botanicals on fungal growth

Six botanicals *viz.*, neem, lantana, calotropis, ocimum, onion, garlic were tested for their efficacy against *A. macrospora*.

Preparation of extracts

Fresh leaf material of neem, lantana, calotropis and ocimum, onion bulb and garlic cloves were thoroughly cleaned, surface sterilized with ethanol and washed well with sterile water. The plant tissue was ground with sterile water at the rate of 1 ml/g of plant tissue using sterilized pestle and mortar, and the macerate was filtered through a muslin cloth to get the crude extract.

The extracts of each plant species were tested at 5, 10 and 15 per cent concentration against the mycelial growth of *A. macrospora* by poisoned food technique (Nene and Thapliyal, 1993). The radial growth of fungal colony was measured and per cent inhibition of growth over control was calculated (Vincent, 1927).

To the sterilized potato dextrose agar medium, melted and cooled at 45°C, required quantity of the vacuum filtered crude leaf extract was added so as to get the required concentrations (5, 10 and 15 per cent) of the extract in the medium. The molten potato dextrose agar medium containing leaf extracts was stirred well and poured into Petri dishes and allowed to solidify. Mycelium disc of 5 mm size cut from 10 days old colony of *A. macrospora* was grown on Petri plate containing potato dextrose agar with different leaf extracts at different concentrations.

Potato dextrose agar medium without any plant extract was inoculated with *A. macrospora* to serve as control. Four replicates were maintained for each leaf extract at each concentration. All the Petri dishes were incubated at room temperature of $25 \pm 1^{\circ}\text{C}$.

Results and Discussion

Effect of bacterial bio-control agents on the growth of *A. macrospora*

All the strains of bacterial biocontrol agents (*Pseudomonas fluorescens* and *Bacillus subtilis*) reduced the radial growth of *A. macrospora* compared to control (Table 1).

Among these bacterial biocontrol agents, *B.s* strain, *P.f* strain 1, *P.f* strain 3 and *P.f* strain 2 recorded 1.73 cm, 2.08 cm, 2.15 cm and 2.45

cm radial growth with 51.68%, 41.89%, 39.94% and 31.56% inhibition respectively (Fig. 1 and Plate 1).

Effect of botanicals on the growth of *A. macrospora*

All the botanicals, at all the concentrations tested, significantly reduced the radial growth of *A. macrospora* compared to check (9.00 cm) (Table 2). Among the botanicals, garlic clove and neem leaf extract with mean radial growth of 2.84 cm and 2.83 cm respectively, were significantly more effective followed by onion bulb and tulasi leaf with 3.27 cm and 3.44 cm mean radial growth respectively. The significantly least effective botanicals, calotropis and lantana recorded mean radial growth of 3.52 cm and 3.58 cm, respectively (Plate 2).

Fig.1 Effect of bacterial biocontrol agents (*Pseudomonas fluorescens* and *Bacillus subtilis*) on the growth of *Alternaria macrospora* *in vitro*

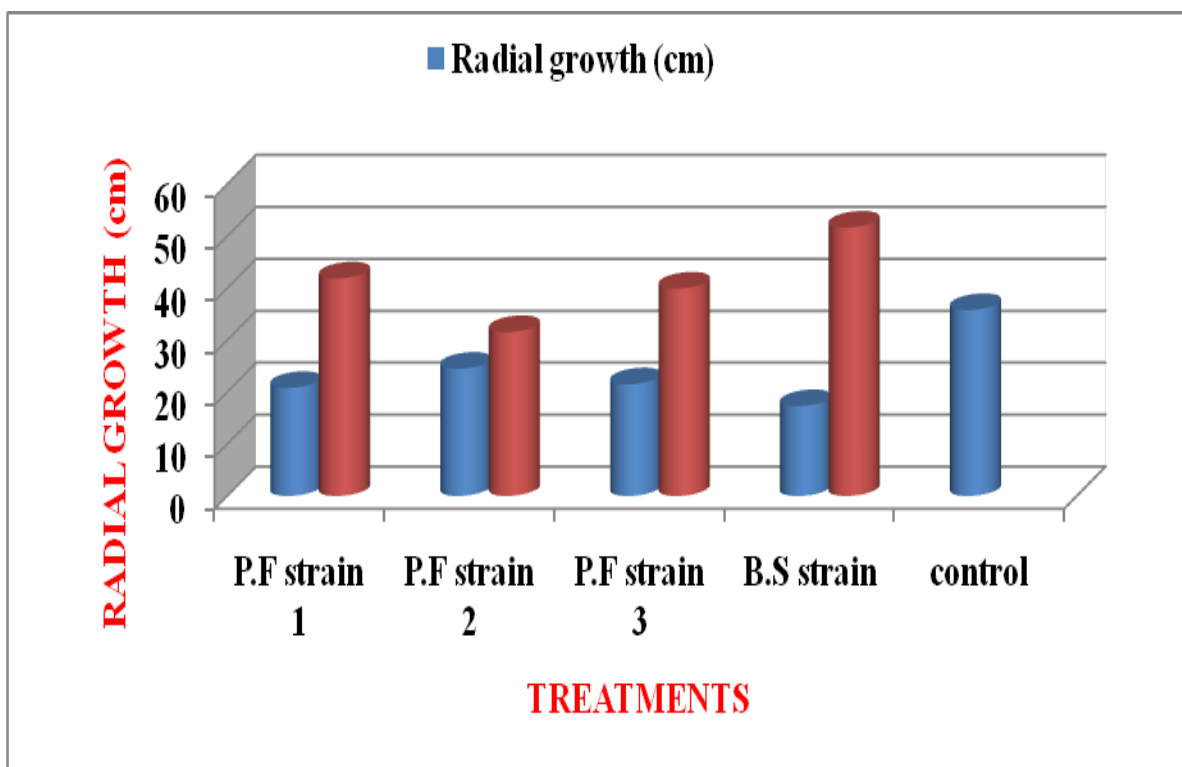


Fig.2 Effect of plant extracts on growth of *Alternaria macrospora* in vitro

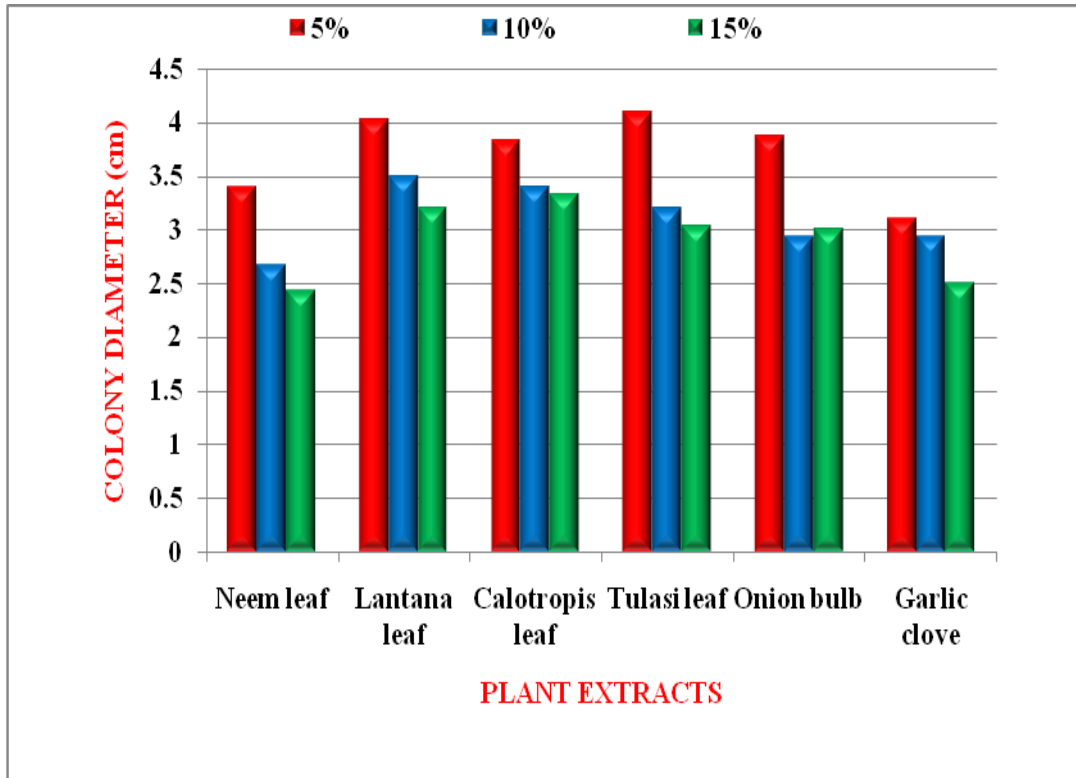


Fig.3 Effect of plant extracts on per cent growth inhibition of *Alternaria macrospora* in vitro

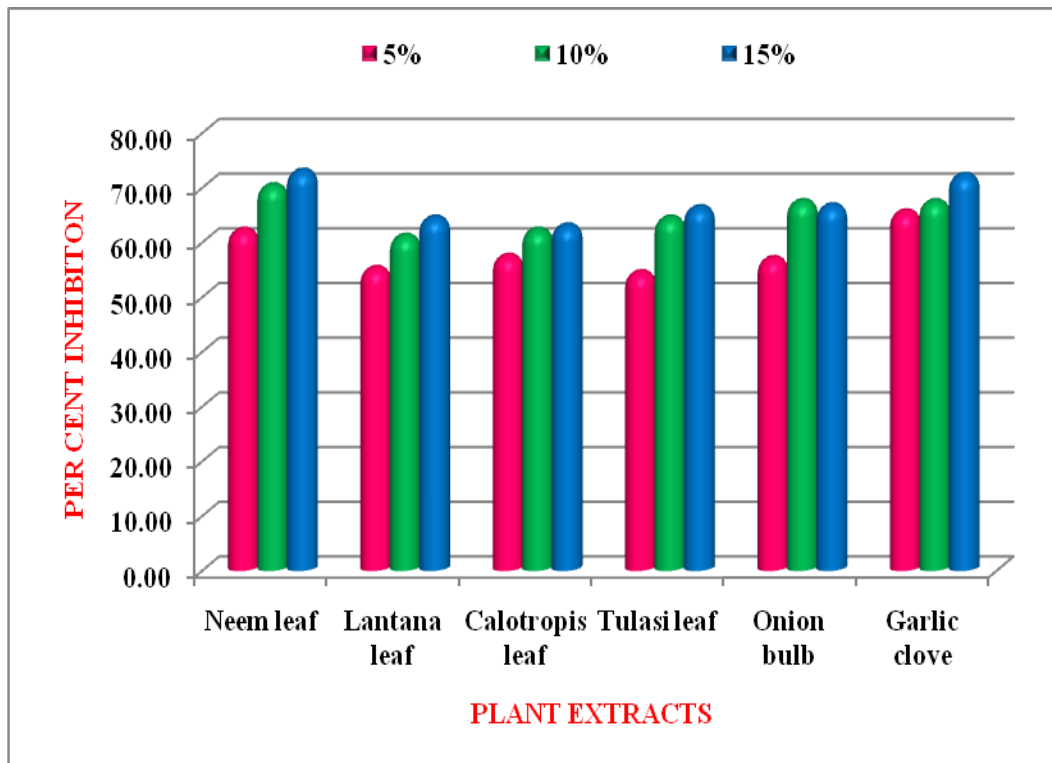


Plate.1 Effect of bacterial biocontrol agents (*Pseudomonas fluorescens* strains and *Bacillus subtilis*) on radial growth of *Alternaria macrospora*

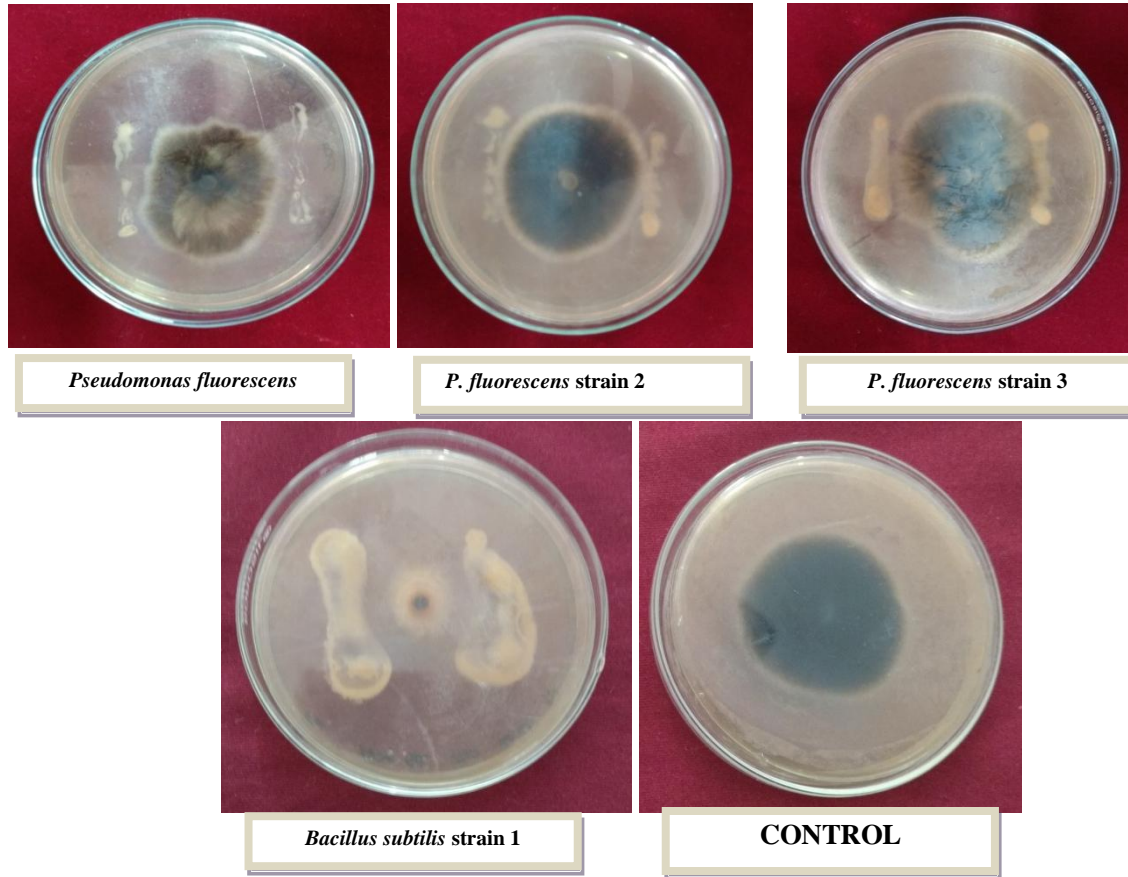


Table.1 Radial growth of *Alternaria macrospora* dual cultured with bacterial biocontrol agents (*Pseudomonas fluorescens* and *Bacillus subtilis*) *in vitro*

S. No.	Treatment	Radial growth (cm)*	Inhibition (%)
1	<i>P.f</i> strain 1	2.08 (1.44) ^b	41.89
2	<i>P.f</i> strain 2	2.45 (1.57) ^d	31.56
3	<i>P.f</i> strain 3	2.15 (1.47) ^{cb}	39.94
4	<i>B.s</i> strain	1.73(1.31) ^a	51.68
5	Control (<i>A. macrospora</i>)	3.58 (1.89) ^e	
	SEm ±	0.02	
	CD (P ≤ 0.05)	0.05	
	CV (%)	2.10	

P.f - *Pseudomonas fluorescens*, *B.s* - *Bacillus subtilis*

*Mean of four replications

Figures in parentheses are square root transformed values

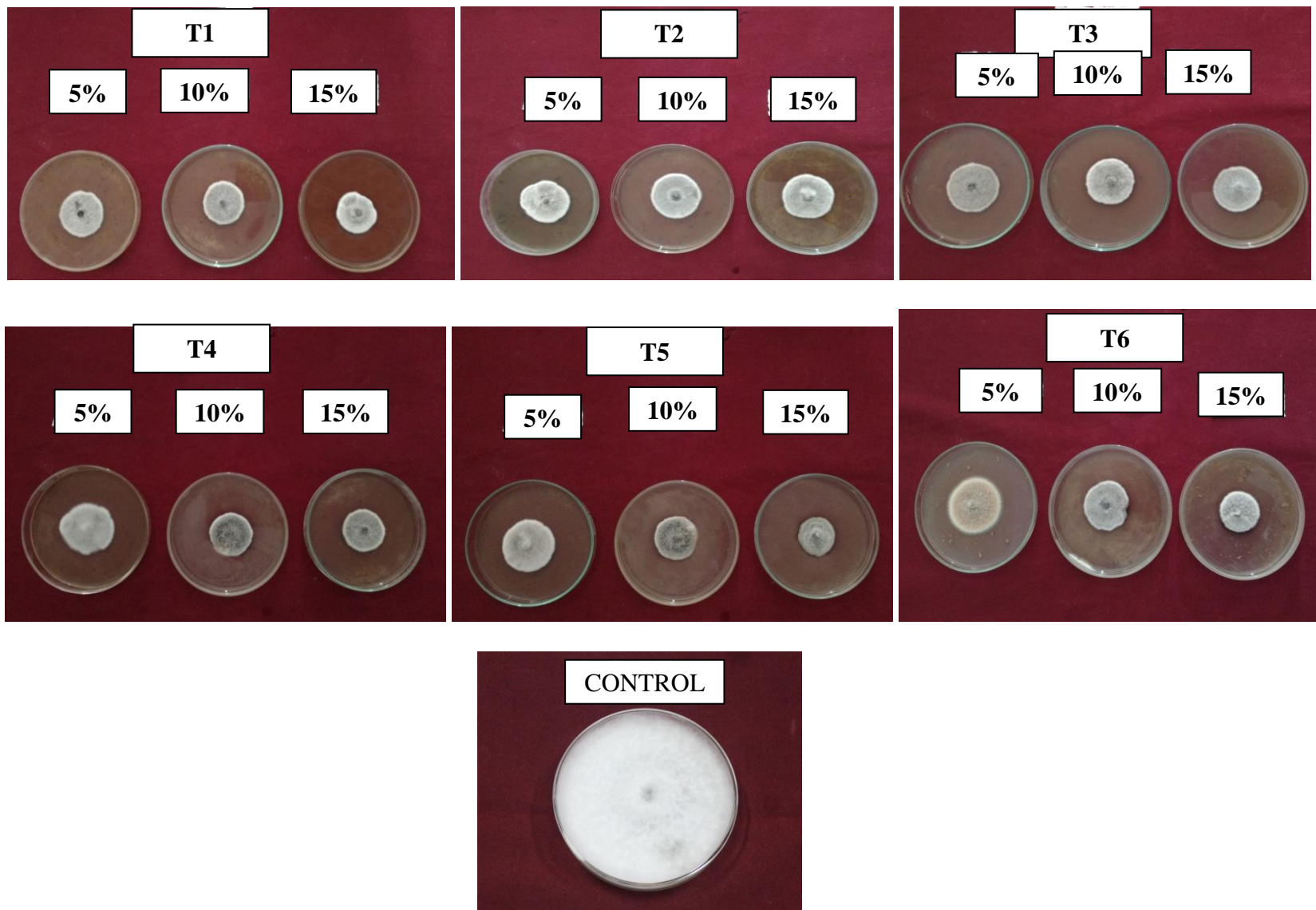
Treatment means with same alphabet do not differ significantly

Table.2 *In vitro* evaluation of plant extracts at different concentrations against *Alternaria macrospora*

S. No.	Botanicals/ Concentration	Colony diameter (cm)*			Mean	Per cent Inhibition			Mean
		5%	10%	15%		5%	10%	15%	
1	Neem leaf	3.40 (1.84)	2.67 (1.63)	2.43 (1.56)	2.83 (1.68)	62.22	70.33	73.00	68.52
2	Lantana leaf	4.03 (2.01)	3.50 (1.87)	3.20 (1.79)	3.58 (1.89)	55.22	61.11	64.44	60.26
3	Calotropis leaf	3.83 (1.96)	3.40 (1.84)	3.33 (1.83)	3.52 (1.88)	57.44	62.22	63.00	60.89
4	Tulasi leaf	4.10 (2.02)	3.20 (1.79)	3.03 (1.74)	3.44 (1.85)	54.44	64.44	66.33	61.74
5	Onion bulb	3.87 (1.97)	2.93 (1.71)	3.00 (1.73)	3.27 (1.80)	57.00	67.44	66.66	63.70
6	Garlic clove	3.10 (1.76)	2.93 (1.71)	2.50 (1.58)	2.84 (1.68)	65.55	67.44	72.22	68.40
	Mean	3.72 (1.93)	3.11 (1.76)	2.92 (1.71)		58.65	65.50	67.61	
	Control	9.00 (3.00)							
		Botanical	Concentration	B X C	Check vs Others				
	SEm ±	0.01	0.01	0.02	0.02				
	CD (P ≤ 0.05)	0.03	0.02	0.05	0.05				
	CV (%)	1.54%							

*Figures in parentheses are square root transformed values

Plate.2 Effect of plant extracts on radial growth of *Alternaria macrospora*



Significant decrease in radial growth with increase in concentration was recorded with all botanicals. Significantly the least radial growth of *A. macrospora* was obtained with 15% (2.92 cm) and the highest with 5% concentration (3.72 cm). The mean radial growth for each concentration was significantly lesser than that of the next higher concentration (Fig. 2).

Interaction between botanicals and concentrations revealed that inhibition of growth at 5% concentration of botanicals ranged between 54.44% in tulasi and 65.55% in garlic. The next superior botanical, neem recorded 62.22% inhibition followed by calotropis (57.44%), onion bulb (57.00%) and lantana (55.22%) respectively.

At 10% concentration of plant extracts inhibition of growth ranged between 61.11% in lantana and 70.33% in neem. The next superior botanical, onion bulb and garlic clove recorded 67.44% inhibition. At 15% concentration of plant extracts inhibition of growth ranged between 63.0% in calotropis and 73.00% in neem. The next superior botanical, garlic clove recorded 72.22% inhibition (Fig. 3).

Prasad and Naik (2003) reported that most of the plant extracts showed fungicidal activity at higher concentration (7.5%) with garlic bulb extract being the most effective causing 90.7 per cent of mycelial growth inhibition followed by *Prosopis juliflora* leaf extract (79.9%) against *A. solani*. Ramegowda (2007) reported that among botanicals, garlic bulb extract was effective against *A. macrospora* under *in vitro* conditions. Arun Kumar (2008) reported that six botanicals evaluated against *A. alternata* were found to be effective. NSKE (43.67%) was significantly superior over all other plant extracts evaluated. The next best treatment was neem leaf extract (15.18%). Sanjeev *et al.* (2017) reported that

among different botanicals tested onion and garlic extracts showed significant reduction in mycelia growth of *A. alternata in vitro*

The present results suggest utilization of the effective isolates of *B. subtilis* and *P. fluorescens*; and extracts of neem and garlic in the integrated management of *Alternaria* leaf spot in cotton.

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