In Vitro Evaluation on Efficacy of Bioagents and Plant Extracts Against Early Blight of Tomato (*Alternaria alternata*)

ABSTRACT:

AIMS: *In vitro* evaluation on efficacy of Bioagents and plant extracts against Early blight of tomato (*Alternaria alternata*)

Study design:CRD (Completely Randomized Design)

Place and Duration of Study:A trial was conducted in plant pathology laboratory at HRS Venkataramannagudem, Dr. Y.S.R. Horticultural University, Venkataramannagudem, during 2022.

Methodology: The efficacy of bioagents were tested against isolatesfor radial growth inhibition on suitable media using dual culture technique under *in vitro* conditions. The poisoned food technique was followed to evaluate theefficacy of botanicals in inhibiting the mycelial growth of test pathogen.

Results: Early blight disease of tomato caused by *Alternaria solani*(Ellis) is an economically important disease causing huge losses throughout Country. In the present investigation total nine bioagents ands four plants extracts were tested against *Alternaria alternata* under *in vitro*.Results revealed that among the nine antagonists tested against *A. alternata*maximum reduction in colony growth of *A. alternata*was observed in A10 (*T. asperellum*) (69.50%)and significantly superior over all other bioagents tested. Total four plant extracts tested against *A. alternata,*onion bulb extract (44.07%) which was found superior to all other tested botanicals.

Key words: Tomato, Bio control agents, *Alternariaalternata,Trichoderma asperellum*, onion bulb extract

INTRODUCTION:

Tomato (*Solanum lycopersicum* L.) is one of the most popular vegetable crop in the world, shares a coveted position in India. It is one of the important food and cash crop for many low-income farmers in the tropical countries so also regarded as poor man's apple. Among the vegetable crops tomato ranks the second position in world and rank first among the processing crops. It is a native of tropical America and is cultivated in about 130 different countries. Brazil, China, Cuba, Egypt, Indonesia, Russia, Spain *etc.,* are the leading producers of tomato.

Tomato is one of the versatile vegetable with wide usage in Indian culinary tradition. It is used as a fresh vegetable and also variety of processed products such as juice, ketchup, sauce, canned fruits, puree, paste, *etc*.Tomato is rich source of vitamins and minerals mainly rich in vitamin C and minerals especially phosphorus, potassium and calcium. Nutritive value per 100 g of edible part is carbohydrate 3.9 g, protein 0.9 g and fat 0.2 g. Besides, it is a good source of "lycopene" pigment which is largely responsible for the red colour of fruit.

The major limiting factors towards production of optimum yield are considerable biotic stresses caused by fungi, bacteria, viruses, viroids, nematodes and insect-pests in existing varieties and hybrids. Open field and protected cultivation of tomato is seriously impaired due to increasing infections of early blight (*Alternaria solani*), late blight (*Phytophthora infestans*), Septoria leaf spot *(Septoria lycopesici)*, Fusarium wilt *(Fusarium oxysporum*sp.*lycopersici),* Anthracnose (*Colletotrichum coccodes)*Collar rot (*Sclerotium rolfsii*), and Damping off (*Pythium sp.).* Among the fungal diseases, early blight caused by *Alternaria alternata*is one of the most important and frequent occurring disease of the crop. Therefore, keeping in view present experiments were conduct on *in vitro* evaluation on efficacy of bioagents and plant extracts against early blight of tomato (*Alternaria alternata*).

2. Materials and methods

2.1 General laboratory procedures

2.1.1 Glassware

Different types of glassware used in the present study *viz.*, petri plates (90 mm diameter), conical flasks (250, 500, 1000 ml), measuring cylinder (25, 250 and 500 ml), test tubes *etc*.

2.1.2 Glassware cleaning

For all the laboratory experimental studies, borosilglasswares were used. The glasswares were kept overnight in the cleaning solution prepared by dissolving 60 g of potassium dichromate ($K_2Cr_2O_7$). Then, they were washed with detergent powder followed by rinsed 3-4 times with running tap water, air dried and sterilized before use.

2.1.3 Equipments

Different types of laboratory equipments were used for the present investigation. Compound microscope (10x, 40x, 100x magnifications Olympus) was used to identification of the pathogen. Hot air oven was used for the sterilization of glassware at 180^0C for 20 min. Autoclave was used for the sterilization of the media at 121^0 C (15lb psi) for 15 min. Incubator was used for the incubating test materials at different temperatures. Refrigerators were used for storage of cultures and samples. Electronic balance was used for the measuring the chemicals. Other types of tools used in the present investigation for various purposes include surgical knife, inoculation needle, corkborer, scalpel, forceps *etc*.

2.1.4 Sterilization of glassware and media

Petri plates were sterilized in hot air oven at 180^o C for 20 minutes. Work benches were sterilized with 70 per cent ethyl alcohol. Cork borer, scalpel and inoculation loop were sterilized over flame. Media and water used in the study were sterilized at 15 lb psi (121^0C) for 15 minutes in an autoclave.

2.1.5 Culture media used

The following culture media was used for isolation, culturing and maintenance of pathogen in the laboratory. Potato dextrose agar (PDA) media was used for the isolation and maintenance of the fungi. Nutrient agar media was used for the maintenance of the bacterial bioagents.

2.2 Preparation of media for pathogen

2.2.1 Potato dextrose Agar medium (Ricker and Ricker, 1936)

Materials required

Peeled potato pieces: 200 g

Dextrose: 20 g

Agar agar: 20 g

Distilled water: 1000 ml

pH– 6.5

Preparation:

Potatoes (200 g) were peeled, made into small pieces and boiled in 500 ml of distilled water for 20 minutes. The extract obtained was filtered through muslin cloth which was squeezed in to beaker. Equal amount *viz*., 20 g of dextrose and agar-agar were melted in 500 ml of distilled water. Potato infusion was prepared by adding dextrose and agar solution into beaker and made up to 1000ml using distilled water. After melting of agar, 200 ml of solution was dispensed into 500 ml capacity conical flasks and plugged with non-absorbent cotton and sterilized in autoclave at 15 lbs pressure at 121°C for 15 minutes. Potato dextrose broth was prepared utilizing the similar makeup of PDA but without agar.

2.2.2 Nutrient Agar medium

Peptone - 5 g.

Beef extract - 3 g.

Agar Agar: 15 g

Distilled water: 1000 ml

pH – 7.0

The required amount of peptone and beef extract were weighed and dissolved in 500 ml of distilled water. Then agar was added and dissolved by constant heating. pH of the medium was adjusted to 7.0 and the volume should be making up to one liter by adding distilled water. This one liter of medium is distributed in to conical flasks, plugged with non absorbent cotton and sterilized in autoclave at 15 lbs pressure at 121°C for 15 minutes.

2.3 Bioagents:

The efficacy of bioagents were tested against isolatesfor radial growth inhibition on suitable media using dual culture technique under *in vitro* conditions.

Table 1. List of bioagents used against test isolates are mentioned below.

S.no	Bioagent	Source
1	*Trichoderma harzianum*	COH, Ananthrajupeta
2	*Trichoderma viride*	

3	*Trichoderma virens*	
4	*Trichoderma longibrachiatum (TCT4)*	CRS, Tirupati
5	*Trichoderma reesi (TCT10)*	
6	*Trichoderma asperellum (A10)*	IARI, New Delhi
7	*Trichoderma harzianum (A28)*	
8	*Pseudomonas fluorescens*	HRS, Ambajipeta
9	*Bacillus subtillis*	

2.3.1 Dual culture test

Bioagents were evaluated for their efficacy through dual culture technique. Twenty ml of sterilized suitable medium melted and cooled to 45^0 C was poured aseptically into sterilized petri dishes of nine cm diameter. Mycelial discs of five mm diameter cut from the edge of actively growing seven days old culture of pathogen and mycelial discs (5 mm) of bioagent cut from actively growing colony with the help of a sterilized cork borer, these were placed on the periphery about one cm from the edge of the petri dish at opposite sides. All the treatments were replicated and incubated at room temperature (27 ± 1^0C). After incubation when the growth of the pathogen was completed in the control, the colony diameter of antagonists was measured in each treatment and the per cent inhibition of the pathogen over control was calculated by adopting the formula given by Vincent (1947). Later data were analyzed statistically after suitable transformation.

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

Where, I= Per cent inhibition

C= Radial growth in control

T= Radial growth in treatment

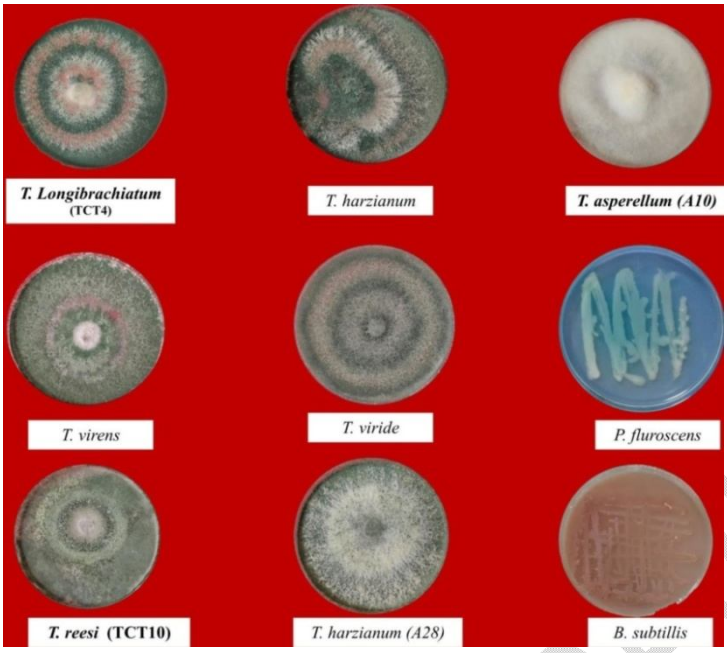

Plate 1. Bio agents used for *in vitro* assay against test pathogens.

2.4 *In vitro* evaluation of plant extracts:

2.4.1 Extraction of plant extracts:

Fresh healthy 100g plant parts mentioned in Table .2 were collected and thoroughly washed with distilled water and air dried and crushed in 100 ml of sterile water with the help of mortar and pestle. The crushed product was tied in muslin cloth and the filtrate was collected in small beaker. The prepared solution was considered 100 per cent, which was further diluted to required concentrations of 5, 7.5 and 10 per cent. The following extracts were tested against test pathogenon PDA using poisoned food technique under *in vitro* condition as described below. The percent inhibition of growth of the test fungus was calculated by using the below formula given by Vincent (1947). The data were analyzed statistically after transformation.

2.4.2 Poisoned food technique:

The poisoned food technique (Nene and Thapliyal, 1982) was followed to evaluate theefficacy of botanicals in inhibiting the mycelial growth of test pathogen. The technique involves culturing of test pathogen on a medium containing the test chemical. The fungus was grown on PDAmedium for seven days prior to setting up the experiment. The required quantity of each extract was addded aseptically in 100 ml of PDA in 250 ml flasks at the time of pouring, then supplemented with streptomycin to avoid bacterial contamination. Twenty ml of poisoned medium was poured in each sterilizedpetriplate. Suitable check was maintained without addition of any plant extract. Mycelial disc of 5mm wastaken from the periphery of fugal colony was placed in the center of petriplates. Plate incubatedat 27±1^{0}C and three

replications were maintained for each treatment. The diameter of thecolony was measured in two directions and average was recorded. Percent inhibition of myceliagrowth of the fungus was calculated by using the formula by Vincent (1947).

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

Where, I= Per cent inhibition

C= Radial growth in control

T= Radial growth in treatment

Table 2 List of plant extracts used against test isolates are mentioned below.

S.No	Description	Part used	Scientific name	Concentration (%)		
				1	2	3
1	Neem	Leaves	*Azadirachta indica*	5%	7.5%	10%
2	Garlic	Bulb	*Allium sativum*	5%	7.5%	10%
3	Turmeric	rhizome	*Curcuma longa*	5%	7.5%	10%
4	Onion	Bulb	*Allium cepa*	5%	7.5%	10%

3. Result and discussion:

***In vitro* evaluation of bioagents:**

In the present investigation, the antagonistic effects of nine bio-agents (Table 1) were assessed against *A. alternata*by dual culture technique. Maximum reduction in colony growth of *A. alternata*was observed in *T. asperellum* (*A10*) which was significantly superior over all the other bioagents tested followed by *T. harzianum*(*A28*)and next best was *T. virens*and least inhibition was noticed in *B. subtillis.*(Table 3, Plate2 and Fig 1)The results of present findings supported with findings of Ganie *et al.* (2013), Singh *et al.* (2018) and Devi *et al.* (2017).

This could be obviously attributed to several possibilities of existence of mechanisms to exhibit antibiosis and secrete many antifungal compounds, cell wall degrading enzymes and compete for space and nutrients according to the findings of Mukhopadhyay and Kumar (2020), Mendoza *et al.* (2015), Shi *et al*. (2012), Sunpapao*et al*. (2018), and Baiyee*et al*. (2019). The antagonism of *Trichoderma* spp against many fungi is mainly due to production of acetaldehyde, a carbonyl compound (Robinson and Park, 1966; Dennies and Webster, 1971). This may also be the reason for its antagonistic effect on test pathogens.*Bacillus* spp have also been reported to produce antibiotic substances and lytic enzymes, which were directly, inhibit pathogens according to Raaijmakers *et al*. (2002), Siddiqui (2006).

Table 3.*In vitro* evaluation of bio agents against *Alternaria alternata*causing early blight of tomato.

Trt	Bioagent	Linear mycelial growth of the pathogen (mm)	% Inhibition over control
T1	***T. longibrachiatum (TCT4)***	37.88	52.64 (46.51)*
T2	***T. reesi (TCT10)***	35.33	55.83 (48.35)
T3	***T. harzianum***	34.11	57.36 (49.23)
T4	***T.viride***	38.88	51.39 (45.80)
T5	***T. virens***	31.77	60.28 (50.93)
T6	***T. harzianum (A28)***	33.33	62.96 (52.51)
T7	***T. asperellum (A10)***	27.44	69.5 (56.48)
T8	***B. subtilis***	42.89	46.69 (42.93)
T9	***P. fluorescens***	39.78	50.28 (45.16)
T10	**Control**	80.00	-
	SE m±		1.656
	C.D (*P* 0.05)		4.957

*Figures in the parenthesis are angular transformed values.

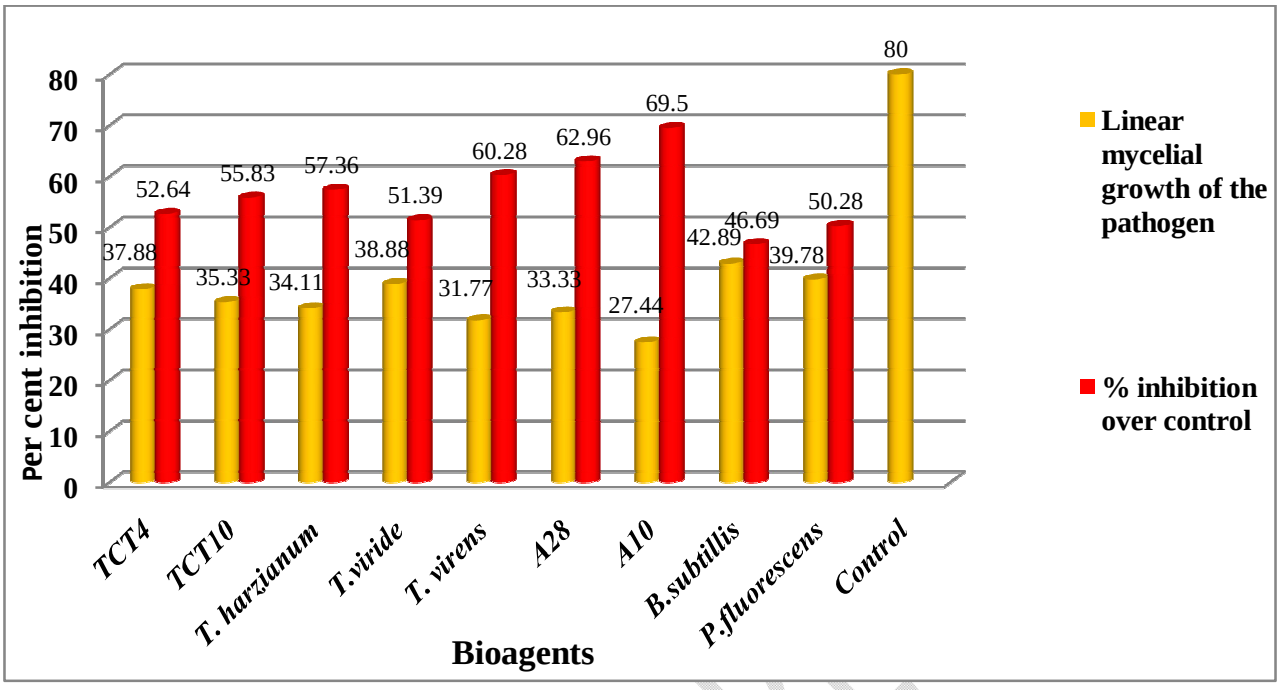

Fig 1. *In vitro* evaluation of bio agents against *A. alternata*causing early blight of tomato.

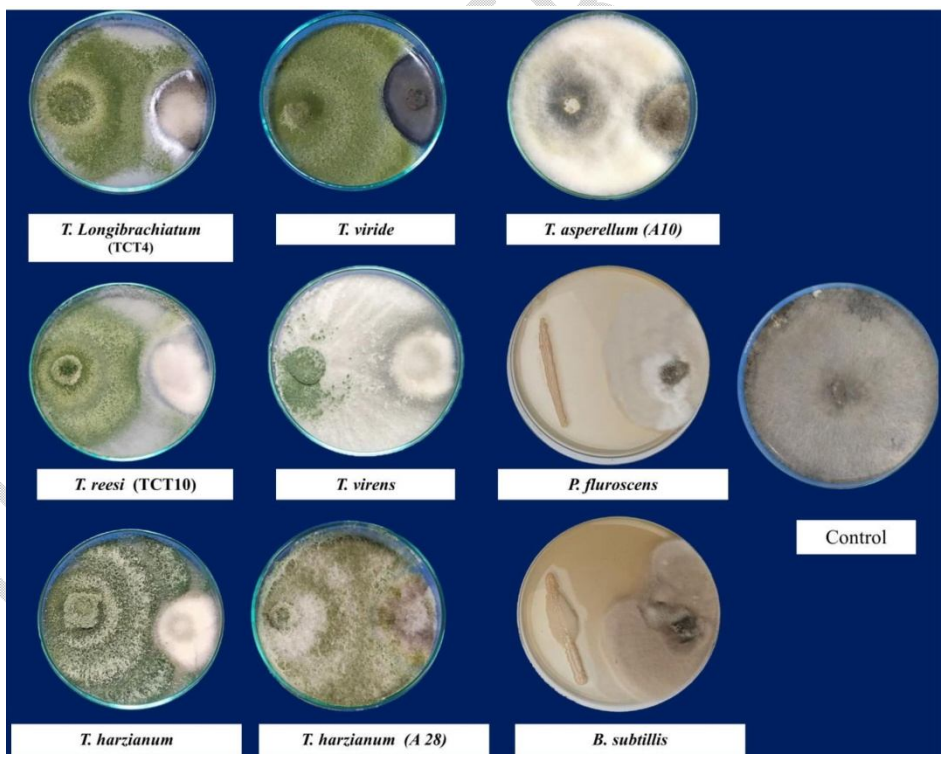

Plate 2*In vitro* of evaluation bioagents against *Alternaria alternata* causing early blight of tomato.

***In vitro* study evaluation of Plant extracts:**

Plant extracts derived from neem leaves (*Azadirachta indica*), onion bulb (*Allium cepa*), garlic bulb (*Allium sativum*), and turmeric rhizome (*Curcuma longa*) were known to suppress the growth and multiplication of various fungi.In the present study all the plant extracts tested at three concentrations (5, 7.5, 10 %) with suitable control by poisoned food technique to evaluate their efficacy on the growth of the test pathogen *Alternaria alternata*(Table 2).

Results showed that the increase concentrations of each plant extract resulted a proportionate reduction in radial growth of test pathogen. The results revealed that, the plant extracts were effective at 10 per cent than 5 per cent and 7.5 per cent concentrations. Among four plant extracts evaluated against *A.alternata* highest mean per cent inhibition (44.07 %) with onion which was found superior to all other tested botanicals. This was followed by garlic bulb extract (40.97 %). Least mean per cent inhibition was recorded with turmeric (21.34 %).

The results (Table 4 Figure 2 and Plate 3) revealed that the all plant extracts at 5, 7.5 and 10 per cent concentrations inhibited the radial growth of the test pathogen (*A. alternata)* significantly when compared with control. Maximum reduction in colony growth of *A. alternata*was observed in onion (85.41 %) at 10 per cent which was followed by garlic (57.64 %) at 10 per cent concentration and onion (40.00 %) at 7.5 per cent concentration.

The present investigation of various botanicals inhibiting the growth of *A. alternata*is in line with the earlier findings of Cornago*et al.* (2011), Devi *et al.* (2017), Nashwa and Sallam (2011), Kumar and Singh (2017) and Yadav *et al*. (2020).

Plants have inherent ability to synthesize aromatic secondary metabolites, like poly phenols, phenolic acids, quinones, flavonoids, flavonols, tannins, saponins and coumarins (Cowan, 1999). The components with phenolic structures, like carvacrol, eugenol, and thymol, were highly active against the pathogen. These groups of microbial compounds shows antimicrobial effect and serves as plant defence mechanisms against pathogenic microorganisms (Das *et al.* 2010). Extracts from neem plant parts contains a number of chemical compounds *viz.*, nimbin (0.04%), nimbicidin (0.4%), nimibicidin (0.001%), nimbosterol (0.03%), essential oil (0.02%) tannin (6.0%) and margosine. Neem oil yield, various acids and sulphur*etc.* Meliantiol and azadiractin are obtained from seeds and decatylimbin also contains quercetin and sitosterol (Hossain *et al.* 2013). The fungicidal spectrum of *Azadirachta indica* has been attributed to azadiractin which belongs to C25 terpenoides (Subramaniam and Shrinivas, 1953). Inhibition of test pathogeninthe present investigation by neem may also because of the same reason.

Garlic bulb extracts antifungal activity is due to the presence of allicin (diallyl-thiosulfinate) as prime antimicrobial constituent as noted by Bayan *et al*. (2014). Fungicidal and fungistatic effects of allicin ruin the structure of fungal cell wall as stated by Khan and Zhihui (2010). Borlinghaus*et al*. (2014) revealed that allicin can arrest the growth or kill the fungi. Feldberg *et al*. (1988), Ankri and Mirelman (1999), Coppi *et al*. (2006) and Wallock-Richards *et al*. (2014) reported that garlic exhibits antifungal properties. Extracts

of garlic bulb are documented to control several plant diseases as reported by Khair *et al.* (2007) and Ting *et al*. (2011).

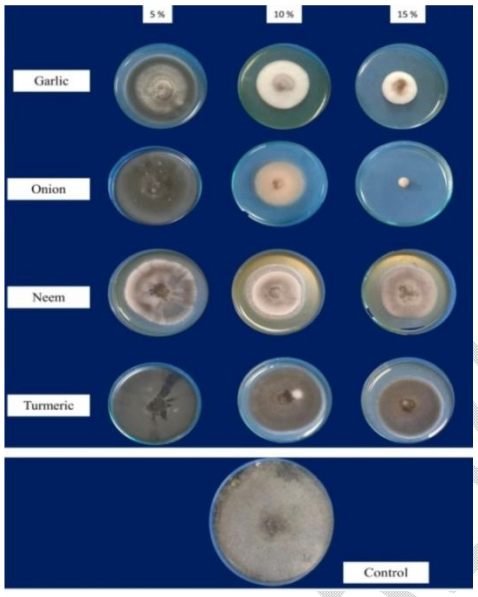

Plate 3. Bio efficacy of plant extracts on inhibiting *A. alternata*in poisoned food method.

Table 4. Bio efficacy of plant extracts on inhibiting *Alternaria alternata*in poisoned food method.

Trt	Plant extract	Plant part used	Concentration (%)	Linear mycelial growth of the pathogen(mm)	Per cent inhibition over control
T1	Onion	Bulb	5	74.55	6.81 (15.13)*
			7.5	48	40 (39.23)
			10	11.88	85.41 (67.54)
	Mean			44.81	44.07 (41.59)
T2	Garlic	Bulb	5	58.22	27.22 (31.45)
			7.5	49.55	38.06 (38.09)
			10	33.88	57.64 (49.39)
	Mean			47.22	40.97 (39.80)
T3	Neem	Leaves	5	62.22	22.22 (28.12)
			7.5	54.44	31.94 (34.41)
			10	50.44	36.94 (37.43)

	Mean			55.7	30.37 (33.44)
T4	Turmeric	Rhizome	5	73.11	8.61 (17.06)
			7.5	60.44	24.44 (29.63)
			10	55.22	30.97 (33.81)
	Mean			62.92	21.34 (27.51)
T5	Control	-	-	80.00	-
SE (m)±					0.907
C.D (*P* 0.05)					2.664

*Figures in the parenthesis are angular transformed values.

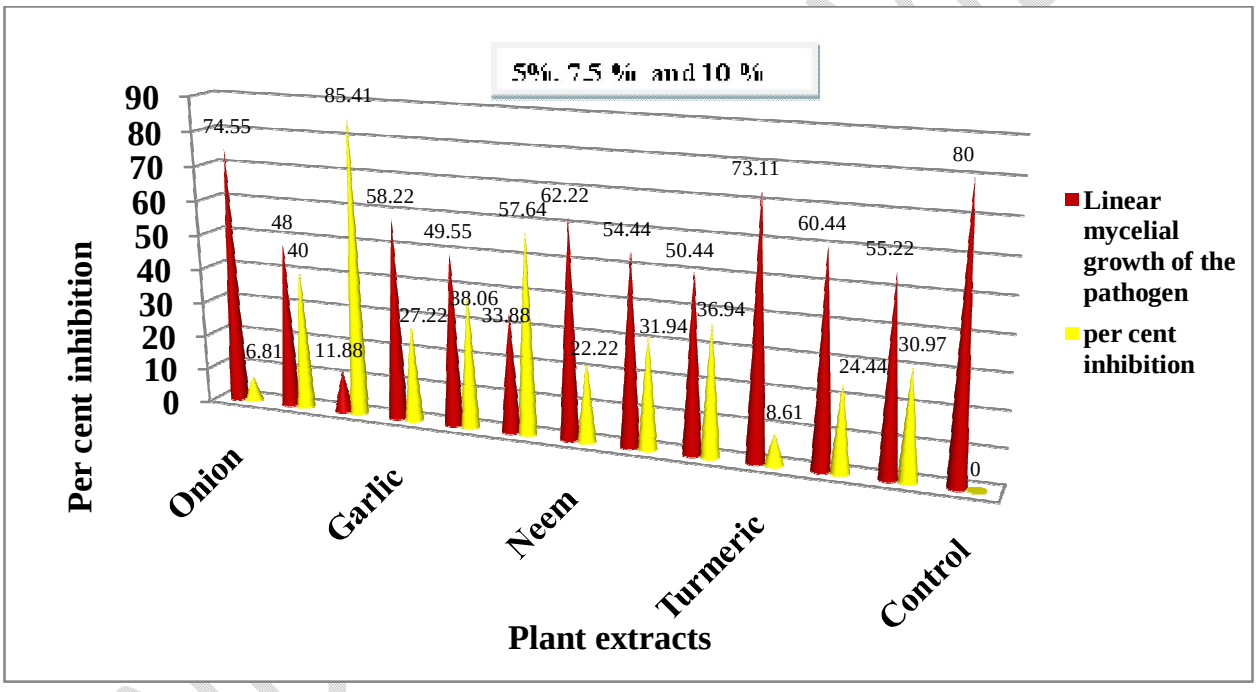

Figure 2*In vitro* evaluation of plant extracts against *A. alternata*causing early blight of tomato.

Conclusion:

Total nine antagonists tested against *A. alternata*under laboratory condition in dual culture technique maximum reduction in colony growth of *A. alternata*was observed in A10 (*T. asperellum)* and significantly superior over all other bioagents tested, which was followed by A28 (*T. harzianum).* Least inhibition was noticed in *Bacillus subtillis.* Total four plant extracts tested against *A. alternata*onion which was found superior to all other tested botanicals. This was followed by garlic bulb extract. Least mean per cent inhibition was recorded with turmeric

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