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

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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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 isolates for radial growth inhibition on suitable media (Potato dextrose media and Nutrient agar 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: In the present investigation total nine bioagents and four plants extracts were tested against *Alternariaalternata* under *in vitro*. Results revealed that among the nine antagonists., *Trichoderma harzianum, Tichoderma viride, Tichoderma longibraciatum* (TCT4), *Trichoderma reesi* (TCT10), A10 (*Tichoderma asperellum*), A28 (*Trichoderma harzianum*), *Trichoderma virens* and two bacterial bioagents *Pesudomonas fluorescens and Baciilus subtillis* 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 which was followed by A28 (*T. harzianum)* (62.96%) and the next best was *T. virens* (60.28%). Least inhibition was noticed in *Bacillus subtillis* (46.69%). Total four plant extracts neem leaves (*Azadirachta indica*), onion bulb (*Allium cepa*), garlic bulb (*Allium sativum*), and turmeric rhizome (*Curcuma longa*) tested at three concentrations (5, 7.5, 10 %) with suitable control by poisoned food technique against *A. alternata,* onion bulb extract (44.07%) which was found superior to all other tested botanicals. Least mean per cent inhibition was recorded with turmeric (21.34%).

Keywords: Tomato; bio control agents; Alternaria alternate; Trichoderma asperellum; onion bulb extract.

1. 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" [1,2].

"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" [3,4].

"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 [1]. 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 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.

2.2 Culture Media Used

Potato dextrose agar (PDA) media was used for the isolation and maintenance of the fungal bio control agents and *A.alternta*. Nutrient agar media was used for the maintenance of the bacterial bioagents.

2.3 Preparation of Media for Pathogen

2.3.1 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.

2.3.2 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.

2.4 Bioagents

The efficacy of bioagents were tested against *A. alternata* for radial growth inhibition on potato dextrose agar (PDA) media and nutrient agar media using dual culture technique under *in vitro* conditions.

2.4.1 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 [5]. 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

2.5 *In vitro* Evaluation of Plant Extracts

2.5.1 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 (v/v). The following extracts were tested against

test pathogen on 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 [6]. The data were analyzed statistically after transformation.

2.5.2 Poisoned food technique

"The poisoned food technique [5] 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 PDA medium 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 sterilized petriplate. 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 [6].

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

Where
I= Per cent inhibition
C= Radial growth in control
T= Radial growth in treatment

Table 1. List of bioagents used against test isolates

S.no	Bioagent	Source
1	*Trichodermaharzianum*	College of Horticulture, Ananthrajupeta
2	*Trichodermaviride*	
3	*Trichoderma virens*	
4	*Trichodermalongibrachiatum (TCT4)*	Citrus Research Station, Tirupati
5	*Trichodermareesi (TCT10)*	
6	*Trichodermaasperellum (A10)*	Indian Agricultural Research Institute, New Delhi
7	*Trichodermaharzianum (A28)*	
8	*Pseudomonas fluorescens*	Horticultural Research Station, Ambajipeta
9	*Bacillus subtillis*	

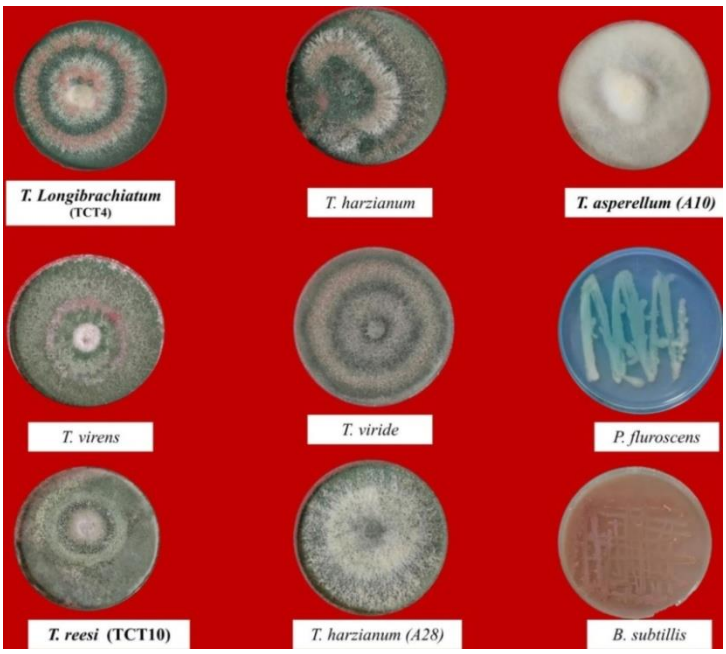

Plate 1. Bio agents used for *in vitro* assay against test pathogens

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	*Azadirachtaindica*	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

3.1 *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, Plate 2 and Fig. 1). The results of present findings supported with findings of Ganie et al. [7], Singh et al. [8] and Devi et al. [9].

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 [10], Mendoza et al. [11], Shi et al. [12], Sunpapao et al. [13], and Baiyee et al. [14]. The antagonism of *Trichoderma* spp against many fungi is mainly due to production of acetaldehyde, a carbonyl compound [15,16]. 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. [17], Siddiqui [18].

3.2 *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 [19]. 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 alternate* (Table 2).

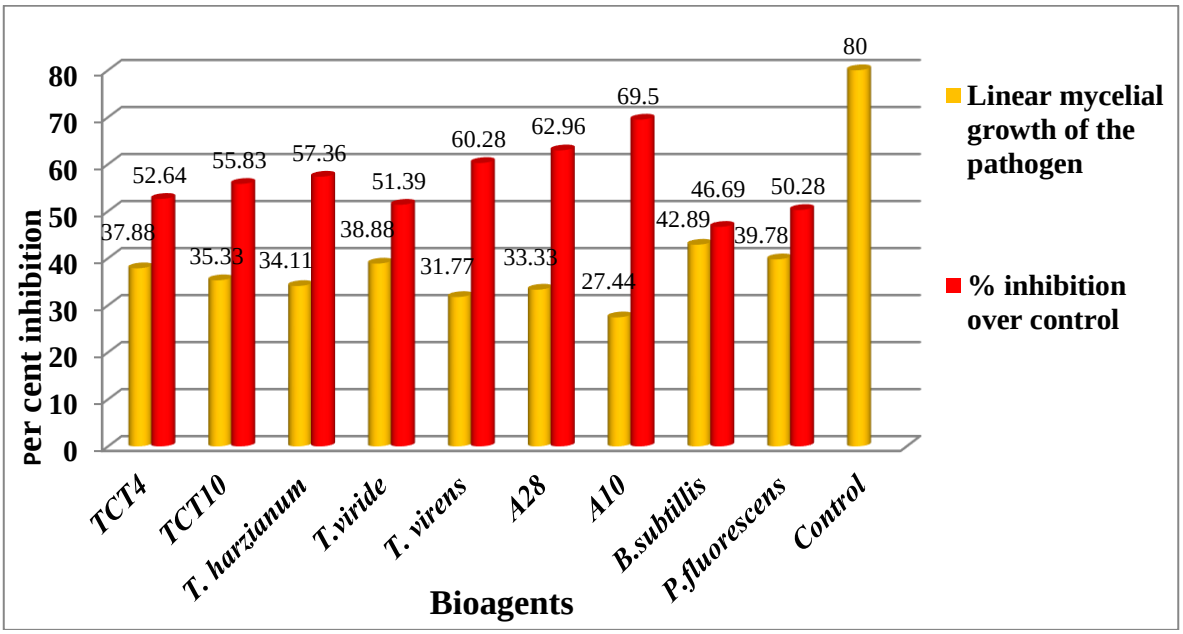

Fig. 1. *In vitro* evaluation of bio agents against *A. alternata*causing early blight of tomato

Table 3. *In vitro* evaluation of bio agents against *Alternariaalternata*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*

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

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 Fig. 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*.* [20], Devi et al*.* [9], Nashwa and Sallam [21], Kumar and Singh [2] and Yadav et al. [22].

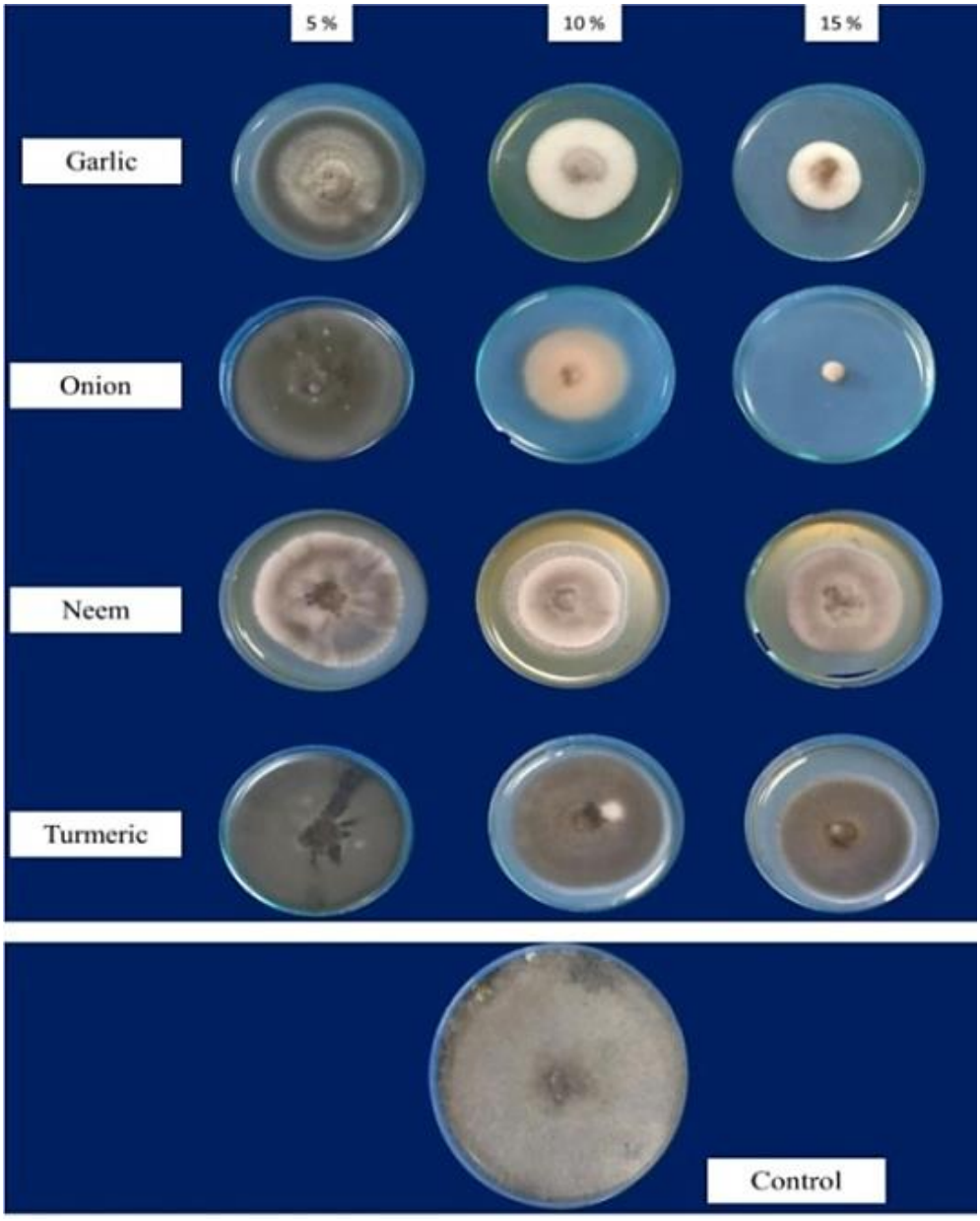

Plate 3. Bio efficacy of plant extracts on inhibiting *A. alternata* in poisoned food method

"Plants have inherent ability to synthesize aromatic secondary metabolites, like poly phenols, phenolic acids, quinones, flavonoids, flavonols, tannins, saponins and coumarins" [23]. 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 [24]. 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 [25]. The fungicidal spectrum of *Azadirachtaindica*has been attributed to azadiractin which belongs to C25 terpenoides [26]. Inhibition of test pathogeninthe present investigation by neem may also because of the same reason.

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*

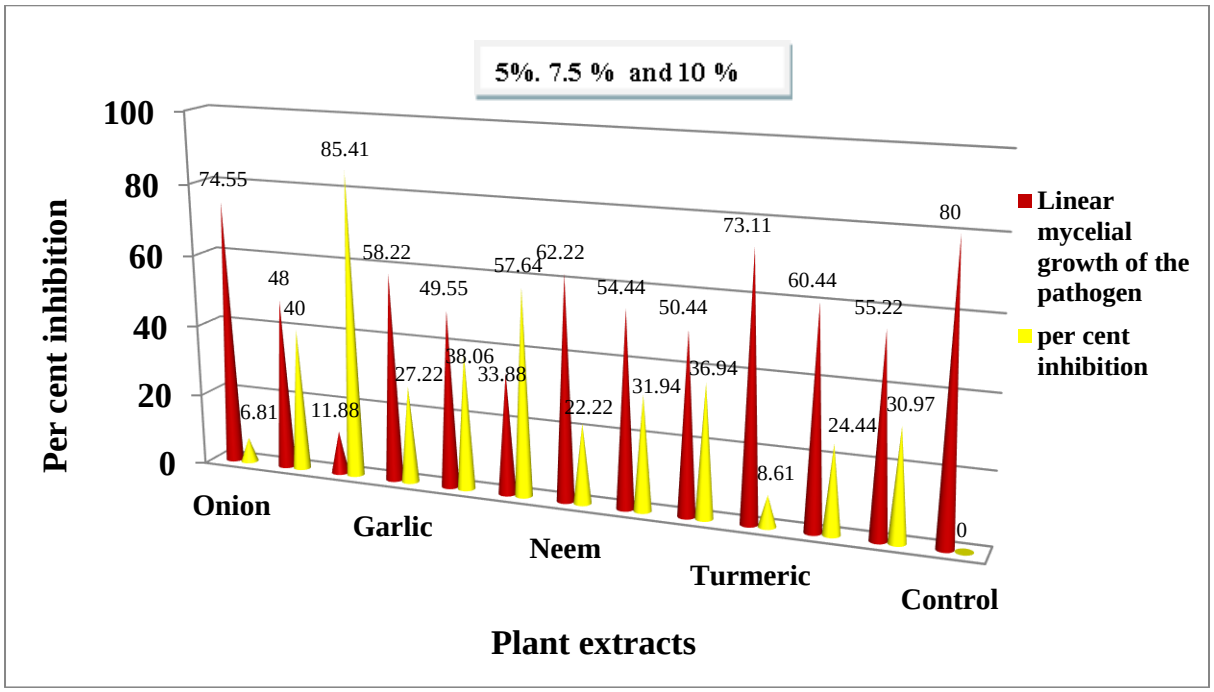

Fig. 2. *In vitro* evaluation of plant extracts against *A. alternata*causing early blight of tomato

"Garlic bulb extracts antifungal activity is due to the presence of allicin (diallyl-thiosulfinate) as prime antimicrobial constituent" as noted by Bayan et al. [1]. Fungicidal and fungistatic effects of allicin ruin the structure of fungal cell wall as stated by Khan and Zhihui [27]. Borlinghaus et al. [3] revealed that allicin can arrest the growth or kill the fungi. Feldberg et al. [28], Ankri and Mirelman [29], Coppi et al. [30] and Wallock-Richards et al. [31] reported that garlic exhibits antifungal properties. Extracts of garlic bulb are documented to control several plant diseases as reported by Khair et al. [32] and Ting et al. [33].

4. 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. The present investigation revealed that extracts of many plants had inhibitory effect against *Alternaria alternta*. Phytochemicals liberated from the plant extracts act better against the pathogen. Hence these could be exploited as an alternate management strategy for chemical pesticides in the management of early blight of tomato*.* The future studies focusing on identification and elucidation of the active ingredients present in these medicinal plants having potential antimicrobial properties will be the need of hour.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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