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Eco-friendly Management of Alternaria Leaf Spot of Gerbera (*Gerbera jamesonii* Bolus ex. Hooker F) Caused by *Alternaria alternata* (Pers.; Fr.) in North East India

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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

Gerbera (*Gerbera jamesonii* Bolus ex. Hooker F) includes the family Asteraceae, is an enormous and extremely profitable cut flower, which is grown in a wide range of environmental conditions globally. Based on *in vitro* studies the highest percentage of mycelial growth inhibition was seen in *Allium sativum* (10% conc.) and *T. harzianum* (86.66% and 81.38%), respectively. Concurrently, Hexaconazole (0.1%) showed a maximum mycelial growth inhibition of 100.00% against *A. alternata.* The *in vivo* field experiments were conducted for two seasons at Horticulture experimental farm, AAU, Jorhat-13 in the year 2022 and

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Hexaconazole (0.1%) were recorded lowest disease incidence (16.52%) and per cent disease severity (11.76%) with highest per cent disease reduction (79.43% and 85.59%) of leaf spot caused by *A. alternata.* When gerbera was treated with *T. harzianum* [Org-Trichojal (@ 5ml/l)], the yield-attributing traits and growth parameters gradually enhanced.

Keywords: Gerbera; Alternaria alternata; hexaconazole; org-trichojal.

1. Introduction

Floriculture represents the most rapidly expanding sector in the North Eastern Region, including Assam, due to its diverse topography, altitude, favorable agro-climatic conditions, and fertile soil, which facilitate year-round production of tropical, sub-tropical, and temperate flowers. Assam ranks top with 7.13 thousand tons of production, accounting for 3.99% of overall production in India (Anonymous, 2021–2022). Gerbera a popular commercial cut flower, is a member of the Asteraceae family and belongs to order Asterales. It is grown all over the world in a variety of climates. Its graceful beauty, hardiness, ability to withstand transit, and long shelf life make it a popular choice for cut flowers and attractive potted plants on the international market. It also has good export potential (Saikia et al., 2022).

Gerbera is susceptible to numerous fungal diseases, with Alternaria leaf spot, stemming from *Alternaria alternata*, being the most destructive disease. It affects all gerbera varieties of both open and protected environments and affects both the quantity and quality of flowers. In general, using chemicals to control disease is more commercially viable; but, using chemicals continuously and carelessly poses major risks to both human and the environment. Bio-agents are becoming increasingly important in plant disease control techniques because of growing awareness of the dangers associated with chemical pesticides and the adoption of alternative bio-approaches, such as the usage of plant extract and beneficial biocontrol agents (Sobia et al., 2015). The current study aims to isolate and characterize the most serious fungal pathogen *Alternaria alternata* associated with gerbera, as well as assess the effectiveness of various botanicals, bioagents, and chemicals against the pathogen both *in vitro* and *in vivo*. This is because Alternaria leaf spot is so common and there is not enough information about it.

2. Materials and Methods

2.1 Isolation and Purification of the Pathogen

The tissue isolation protocol from (Riker and Riker, 1936) was followed for isolating the pathogen. The fungal culture underwent purification via single-spore isolation methodology (Johnston and Booth, 1983).

2.2 Morpho-cultural Characteristics

Pathogen identification and characterization was performed using standard reference methods (Mathur and Kongsdal, 2003, Agrios, 2005). The Methuen Handbook of Color was used to assess the fungus's colony color.

Observations on the morphological characteristics of mycelium, conidiophores, and conidia were made using the reference paper (Dipak et al., 2012). The size of the conidia was determined and microphotographed taken using a computer-generated micrometre. For taxonomic confirmation and species-specific classification, the purified fungal culture was delivered to the National Centre of Fungal Taxonomy (NCFT).

2.3 Molecular Characteristics

Species identification through molecular analysis was performed by Bioserve Biotechnologies India Pvt. Ltd., utilizing 18s ITS gene sequencing. Evolutionary relationships were established by comparing sequences against the NCBI (National Centre for Biotechnology Information) nucleotide database through BLAST (Basic Local Alignment Search Tool) analysis. The obtained sequences were deposited in NCBI to receive accession numbers. Sequence alignment was conducted using MEGA 6.0 software (Tamura et al., 2013) with multiple

sequence alignment tools. A phylogenetic tree was generated using the Maximum-Likelihood approach in MEGA 6.0, employing 500 bootstraps.

2.4 Pathogenicity Test

Healthy gerbera saplings of highly susceptible variety (Red Gem) were chosen to perform the pathogenicity test. The healthy gerbera leaves were surface sterilized with NaOCl (1%) before being washed using sterilized distilled water. Small puncture wounds were created on the leaf surfaces using a sterilized needle (pin-pricking approach). The organism's spore suspension ($1X10^9$ spore/mL of water) was sprayed on the upper surface of the leaves using a hand sprayer and bit inoculation method was done using a sterile cork borer and bit was positioned on the leaves upper surface. Each inoculated plant was individually enclosed in clear, ventilated polypropylene covers to maintain humidity above 90% for 48 hours. Control involving sterile distilled water spraying were included with three replications. Symptom development was observed and reported at routine intervals. Upon typical symptoms appearance on inoculated specimens, the causal agents were reisolated to confirm Koch's postulates.

2.5 *In vitro* Efficacy of different botanicals against *A. alternata*

2.5.1 Preparation of Plant Extracts with Cold Water

Fresh rhizomes, bulbs, and leaves of *A. indica*, *C. longa*, and *A. sativum* were thoroughly rinsed using sterile distilled water. A 100 g of cleaned plant tissues were ground using a pre-chilled pestle and mortar with an equivalent volume of (100 mL) sterilized water (1:1 W/V). The resulting extract was filtered with muslin cloth and centrifuged for 20 minutes at 10, 000 rpm at room temperature. The supernatant was used as a 100 per cent basic stock solution. The plant extract was purified using a bacterial membrane filter (RanDisc, PVDF 0.22µm) under sterile conditions. The efficacy of the 10% concentration was tested against *A. alternata* using the poisoned food technique (Nene and Thapliyal, 1979). Each of the treatments was replicated in five times. The percentage inhibition of mycelial growth was calculated using the formula proposed by (Vincent, 1947). Based on the *in vitro* effectiveness of the botanicals against *A. alternata*, the most effective one was chosen for field evaluation.

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

Where, I= Inhibition of mycelial growth, C= Growth in control, T= Growth in treatment

2.6 *In vitro* Efficacy of Biocontrol Agents against *A. alternata*

Four different biocontrol agents *viz.*, *Trichoderma harzianum*, *Trichoderma viride*, *Trichoderma pseudokoningii*, and *Pseudomonas fluorescens* were assessed against the pathogen by dual culture technique (Dennis and Webster, 1971). All the biocontrol agents were collected from the Nano Laboratory, Department of Plant Pathology, Assam Agricultural University, Jorhat-13.

2.7 *In vitro* Efficacy of Chemicals against *A. alternata*

Three systemic chemical fungicides such as Hexaconazole (0.1%), Difenoconazole (0.1%), and Propiconazole (0.1%), were taken and an *in vitro* efficacy test against pathogens was performed by the poisoned food technique. To attain the required fungicide concentration, an adequate amount of tested fungicides was systematically mixed with molten cooled PDA media in conical flasks based on the presence of the active ingredient. A 5 mm diameter culture disc from a week-old pure culture of *A. alternata* was placed on petri plates and incubated in a BOD incubator at 28±1ºC until complete growth was achieved in the control; each treatment was performed in five replications.

2.8 *In vivo* Field Experiments

The best botanical, biocontrol agent and chemical found in the above *in vitro* were further selected for efficacy test as foliar spray for the management of *A. alternata* in gerbera (var. red gem) under open conditions.

2.9 Artificial Inoculation of Pathogen

Three-month-old gerbera plants (var. red gem) were grown in field conditions and the pathogen was inoculated into the plants employing the Micro Droplet Inoculation Technique (MDIT) described by (Munaut et al., 1997). Treatments were given as a foliar spray within 7 days of the onset of symptoms and subsequent applications were done at 14-day intervals. Disease incidence was recorded 30 days after the second foliar spray.

2.10 Observations Recorded for Field Experiment

i) Disease Incidence (%)

The disease incidence was calculated using the following formula (Agrios, 2005):

$$\text{Disease incidence (DI)} = \frac{\text{Number of infected leaves}}{\text{Total number of leaves observed}} \times 100$$

ii) Per cent Disease severity (PDS)

Disease intensity was evaluated using a 0 – 5 rating scale (Dar et al., 2024) with six categories established based on the percentage of leaf area affected.

Chart 1. Per cent Disease severity

Category	Leaf area infected (%)
0	Disease free
1	0.1-10.0
2	10.1-25.0
3	25.1-50.0
4	50.1-75.0
5	>75

The Per cent disease severity was computed by using the following formula (Wheeler, 1969).

$$\text{PDI} = \frac{\text{Sum of individual disease rating}}{\text{Total number of leaves observed} \times \text{Maximum disease grade}} \times 100$$

iii) Per cent disease reduction (PDR)

The disease control was calculated with the following formula:

$$\text{PDR} = \left(\frac{\text{Disease (\%) in control} - \text{Disease (\%) in treatment}}{\text{Disease (\%) in control}} \right) \text{X}100$$

2.11 Yield Parameters

The yield parameters from each plot were recorded 30 days after the application of the second treatment and data are means of two seasons. Yield and growth parameters like Suckers per plant, Flower per plant, Diameter of flower (cm), Length of the flower stalk (cm), Self-life of flower (days), Vase life of flower (days), Shoot length (cm) and root length (cm) were recorded.

2.12 Statistical Analysis

CRD was employed for *in vitro* and RBD was followed for field experiments. The data were collected and analyzed using Fisher's method of analysis of variance. Variance in the data was examined following the

procedure outlined by (Snedecor and Cochran, 1989). The percentage values were converted after (Gomez and Gomez, 1984) and transformed by the angular transformation.

3. Results and Discussion

3.1 Symptomatology

Gerbera leaves infected with *A. alternata* developed brown, tiny, dispersed spots that enlarged and merged over time to produce massive, oval, round, or uneven brown to black spots showing concentric ring patterns. In severe infections, lesions coalesce rapidly until the leaves appeared blighted (Supplementary Fig. 1). Similar types of symptoms in gerbera have been reported by several researchers (Borah et al., 2019, Hoque et al., 2023).

3.2 Morpho-cultural Characteristics

The colony colour on PDA medium showed whitish aerial mycelium that progressively turned light greyish and brown to dark greyish. The reverse side of the culture in the petri dish was observed to be dark black in colour. Microscopic examination showed simple, septate, light to dark brown conidiophores with long, pale brown conidia measuring 22.35 - 30.52 x 5.27-8.95 µm, with 3-5 transverse and 0-3 longitudinal septa. Conidia are typically ovoid to ellipsoid in shape, with a cylindrical beak and dark brown coloration. They are known to be borne only or in groups of 5 to 8 (Supplementary Fig. 2). Based on these morpho-cultural characteristics, the fungal pathogen was identified as an *Alternaria alternata* (Fr.) Keissler. This identification of fungus was confirmed by the National Centre of Fungal Taxonomy (NCFT), New Delhi as *Alternaria alternata* (Fr.) Keissler and assigned the NCFT No. 9629.19. All these parameters corresponded with those reported by Nagrale who investigated comparable morphological characters of the pathogen [Borah et al., 2019, Hoque et al., 2023, Nagrale et al., 2012, Bhat et al., 2013) and also recorded the similar observations.

3.3 Molecular Characterization

Based on the molecular characterization and nBLAST results depicts that isolate Alt01 having 91.69% similarity with *Alternaria alternata* and in the basis of ITS region sequencing the fungi were confirmed as *Alternaria alternata (Farhood and Hadian, 2012)*. Phylogenetic analysis was carried out by comparing the query sequence with database of NCBI (Fig. 1).

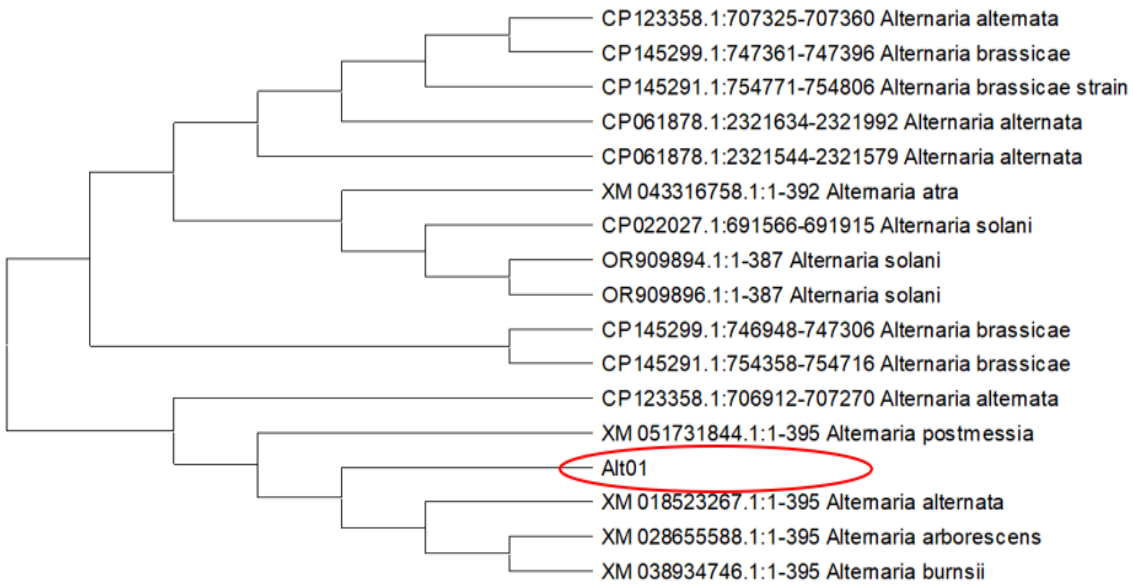
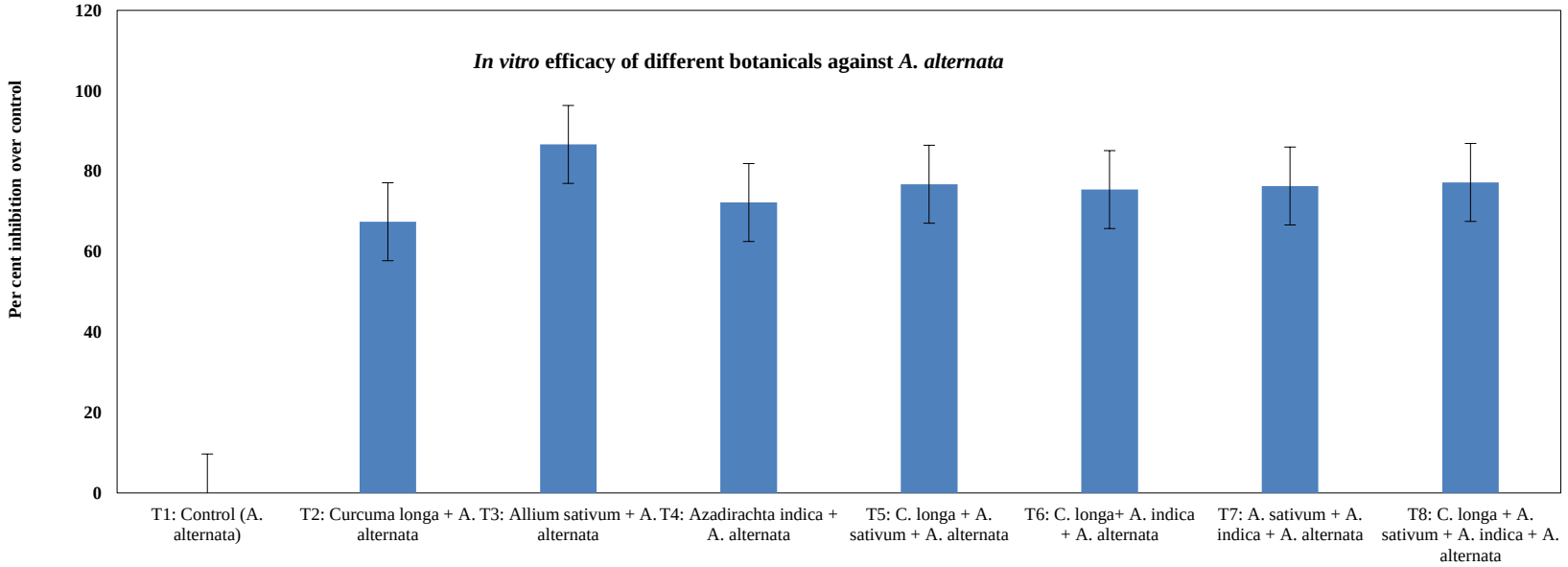
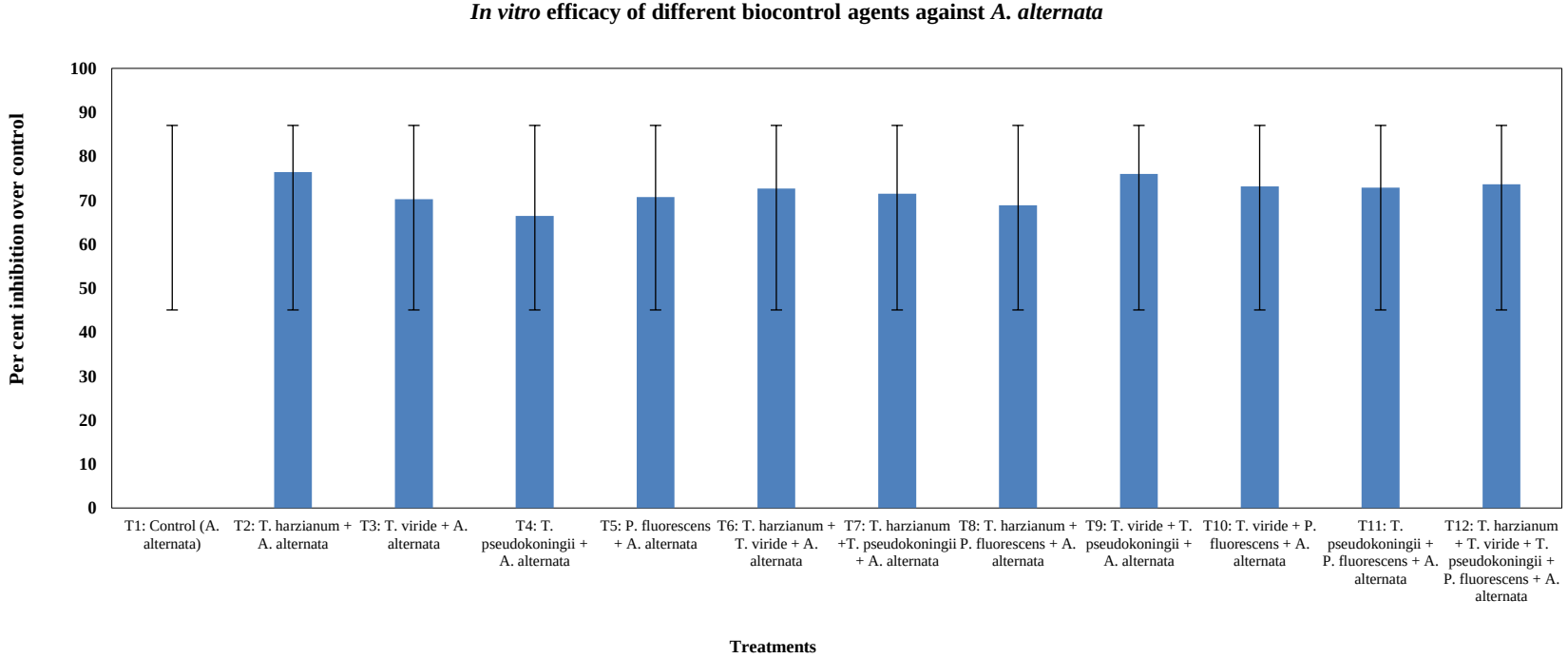


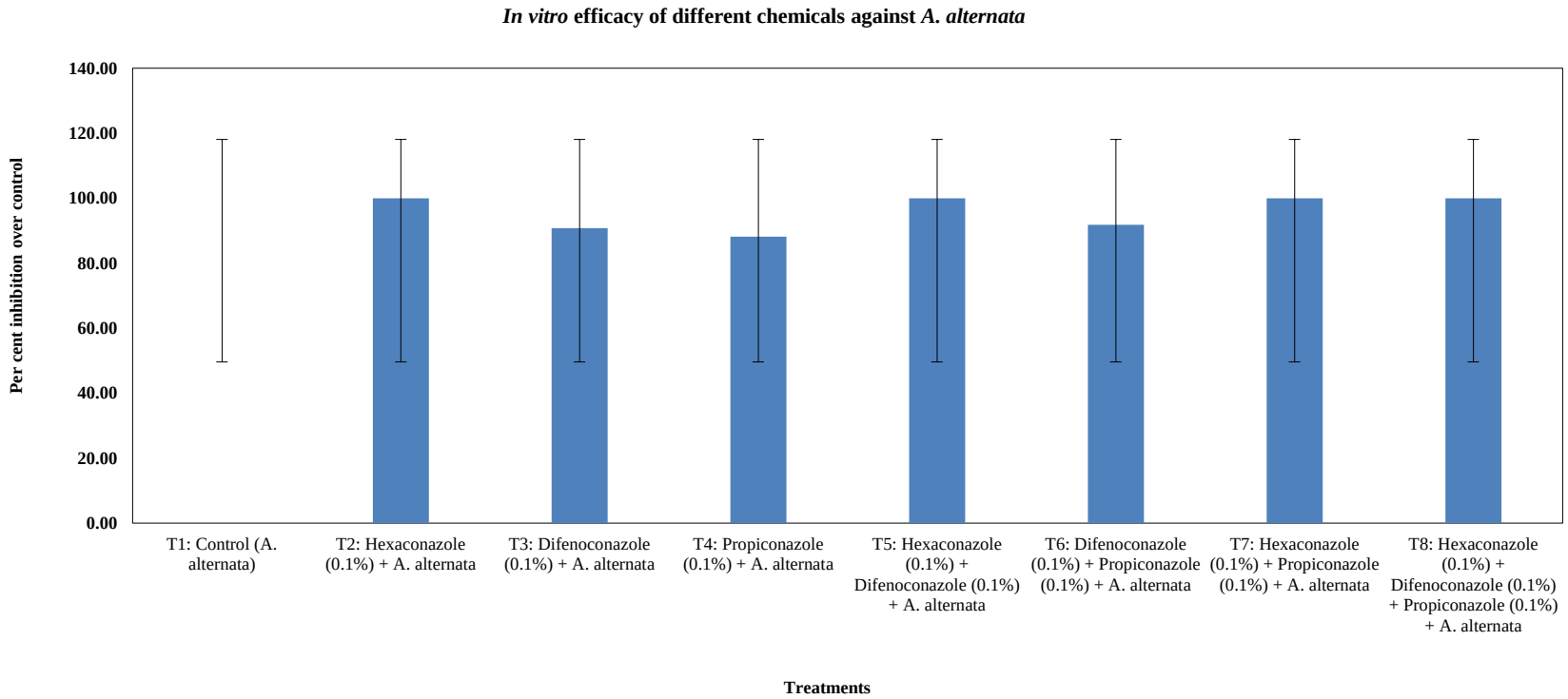
Fig. 1. A phylogenetic tree constructed with 500 bootstrap replicates and the maximum likelihood approach is used to showing the genetic relationships between the fungal strain "Alt01" and other isolates


In vitro efficacy of different botanicals against A. alternata
Per cent inhibition over control
120
100
80
60
40
20
0
T1: Control (A. alternata)
T2: Curcuma longa + A. alternata
T3: Allium sativum + A. alternata
T4: Azadirachta indica + A. alternata
T5: C. longa + A. sativum + A. alternata
T6: C. longa+ A. indica + A. alternata
T7: A. sativum + A. indica + A. alternata
T8: C. longa + A. sativum + A. indica + A. alternata
Treatments

(A)


In vitro efficacy of different biocontrol agents against *A. alternata*
Per cent inhibition over control
100
90
80
70
60
50
40
30
20
10
0
T1: Control (A. alternata)
T2: T. harzianum + A. alternata
T3: T. viride + A. alternata
T4: T. pseudokoningii + A. alternata
T5: P. fluorescens + A. alternata
T6: T. harzianum + T. viride + A. alternata
T7: T. harzianum +T. pseudokoningii + A. alternata
T8: T. harzianum + P. fluorescens + A. alternata
T9: T. viride + T. pseudokoningii + A. alternata
T10: T. viride + P. fluorescens + A. alternata
T11: T. pseudokoningii + P. fluorescens + A. alternata
T12: T. harzianum + T. viride + T. pseudokoningii + P. fluorescens + A. alternata
Treatments

(B)



(C)

Fig. 2 (A-C). *In vitro* efficacy of botanicals, biocontrol agents and chemicals against *Alternaria alternata*

Table 1. Effect of treatments on disease occurrence of Alternaria leaf spot under field condition

Treatments	30 Days after spraying			
	PDI in field condition	**PDR over control in field condition**	**Per cent disease severity in field condition**	**Per cent disease severity reduction over control in field condition**
T_1: Control (*A. alternata*)	80.32 (63.70) *	---	81.65 (64.67) *	---
T_2: *T. harzianum* (Org-Trichojal @ 5ml/l)	19.40 (26.15)	75.84	17.85 (25.01)	78.13
T_3: *A. sativum* (10 conc.)	22.11 (28.06)	72.47	20.11 (26.65)	75.37
T_4: Hexaconazole (0.1%)	16.52 (24.00)	79.43	11.76 (20.06)	85.59
T_5: *T. harzianum* (Org-Trichojal) + *A. sativum*	22.62 (28.41)	71.83	21.26 (27.47)	73.96
T_6: *A. sativum* + Hexaconazole	21.76 (27.82)	72.90	21.06 (27.33)	74.20
T_7: *T. harzianum* (Org-Trichojal) + Hexaconazole	23.43 (28.96)	70.82	22.51 (28.34)	72.43
T_8: *T. harzianum* (Org-Trichojal) + *A. sativum* + Hexaconazole	21.17 (27.41)	73.64	19.41 (26.15)	76.22
S. Ed (±)	**0.937**		**1.216**	
CD $_{(P=0.05)}$	**2.334**		**3.029**	

(Data are mean of four replications, PDR-Per cent disease reduction over control, ***** *Data in the parentheses are angular transformed value)*

Table 2. Effect of treatment combinations on plant growth parameters under field condition

Treatments	No. of suckers per plant	No. of Flower per plant	Diameter of flower (cm)	Length of the flower stalk (cm)	Self-life of flower (days)	Vase-life of flower (days)	Shoot length (cm)	Root length (cm)
T_1	14.00*	31.50	5.02	31.97	14.75	11.50	26.65	13.47
T_2	21.00	42.25	6.25	39.32	18.75	14.75	34.72	18.30
T_3	17.50	40.00	5.75	36.80	16.25	14.25	29.35	17.30
T_4	20.25	42.00	6.05	38.62	18.00	14.50	33.50	18.22
T_5	19.75	39.75	5.25	36.55	17.75	13.00	31.40	16.60
T_6	18.75	39.75	5.80	37.40	17.50	13.50	29.65	17.65
T_7	19.50	41.00	5.90	36.17	17.00	12.75	30.07	15.97
T_8	20.00	41.75	6.10	38.00	18.25	14.00	32.15	17.50
S. Ed (±)	**0.601**	**2.043**	**0.161**	**0.639**	**0.657**	**0.925**	**0.747**	**0.736**
$CD_{(P=0.05)}$	**1.258**	**4.277**	**0.388**	**1.338**	**1.337**	**1.936**	**1.563**	**1.542**

(**Data are mean of four replications)***

3.4 Pathogenicity Test

The pathogenicity of the fungus was ascertained using Robert Koch's theory on potted gerbera plants. Initial symptoms were developed at 7 days post-inoculation, and characteristic symptoms were occurred after 10 days. The control plants exhibited no symptoms (Supplementary Fig. 3). The first indication of *A. alternata* was the minute circular spots appearance at the tips and borders of the leaflets. Such dark brown patches grew to cover a wide region of the leaflets. Re-isolation of the pathogen were confirmed a similarity to the original culture of the pathogen. Accordingly, *Alternaria alternata* was confirmed as the causal agents of the diseases (Nagrale et al., 2012, Farhood and Hadian, 2012).

3.5 *In vitro* efficacy of Botanicals against *A. alternata*

According to the findings of present study, all the botanical considerably reduced the pathogen mycelial growth rate in comparison to the control Fig. 2(A). Among the botanicals evaluated against *A. alternata* the treatment T3 inhibited mycelial growth of (86.66%), followed by T8 and T5, which inhibited mycelial growth by 77.22% and 76.77%, respectively. The lowest inhibition of mycelial growth of the pathogen was detected in T_2 (67.44%) (Supplementary Fig. 4). The findings of (Raja, 2010) who found that garlic was the best in inhibiting the mycelial growth of *A. tenuissima* causing leaf spot and fruit rot of brinjal which showed 100.00%, 88.70% and 76.10% inhibiting over control at 15%, 10%, 5% (V/V) respectively. Mycelial growth inhibition of *A. alternata* might be due to presence alkaloid, phenol, tannin, glycoside, saponin, flavonoid, terpenoidetc in the plant extracts which possess antimicrobial activity against a number of microorganisms (Gandhiraja et al., 2009).

3.6 *In vitro* Efficacies of Biocontrol agents against *A. alternata*

The results shown in Fig. 2 (B) revealed that all biocontrol agents significantly suppressed the mycelial growth of *A. alternata* relative to control. Treatment T2 inhibited the highest mycelial growth rate (76.41%) among the biocontrol agents. The lowest inhibition (66.41%) on mycelial growth of the pathogen was noted in T_4 (Supplementary Fig. 5). (Yamuna et al., 2020) studied *in vitro* bioefficacy of different bioagents against *A. alternata* causing fungal fruit rot in pomegranate and reported *T. harzianum* proved to be most effective exhibiting highest mycelial inhibition (82.11%). This inhibition of mycelial growth of the pathogen might be due to the principle of mycoparasitism with the antagonist for nutrition by secreting cell wall degrading enzymes. *Trichoderma* spp. synthesizes cellulase and chitinase which degrade the pathogen's cellulose and chitin and utilized the derived carbon source and thus inhibits the growth of the pathogen (Singh, 2025).

3.7 *In vitro* Efficacy of Chemicals against *A. alternata*

The results shown in Fig. 2(C) depicted that all the chemicals substantially diminished the mycelial growth as compared to the control (Supplementary Fig. 6). Treatment T2 was found to exhibit the highest per cent inhibition (100%) on the mycelial growth of *A. alternata* and followed by T3 and T4 with inhibitions of 90.74% and 88.14%, respectively. The findings of the present investigation are correspondence with (Gholve et al., 2014) who observed 94.5 per cent inhibition of *A. alternata* of gerbera with Hexaconazole 5EC (0.1%). Similarly, (Bhat et al., 2017) observed inhibitory effect of Tebuconazole 250EC and Hexaconazole 5EC against *A. alternata* of gerbera which are agreeable with the present study.

3.8 Effect of Treatments on Disease Occurrence of Alternaria Leaf Spot under Field Condition

Based on the findings of *in vitro* experiments, the most effective treatment was selected for efficacy test against *A. alternata* under field conditions. The effect of treatments on field condition were displayed in Table 1 and results showed that all the treatment could significantly reduce the disease incidence and disease severity of the *A. alternata* except control in field condition. Per cent disease incidence (16.52%) and disease severity (11.76%) were significantly low in treatment T_4 with maximum (79.43% and 85.59%) percent disease reduction (PDR) respectively compared with untreated control. Highest per cent disease incidence (80.32%) and disease severity (81.65%) were recorded in the treatment T_1 (Control) where no treatment was applied (Supplementary Fig. 7). The findings of the present investigations were corresponded with (Nagrale et al., 2012) who conducted field experiment using fungicides against Alternaria leaf blight of gerbera and reported effectiveness of Difenoconazole (0.1%), Propiconazole (0.25%) and Hexaconazole (0.1%) with 94.21, 93.09 and 92.41 percent

disease control. Likewise, several other workers also noticed the effectiveness of Hexaconazole (0.1%), Carbendazim (12%) + Mancozeb (63%) (0.2%), Propineb 70WP (0.2%) in reducing the incidence of chrysanthemum leaf blight caused by *A. alternata* in field conditions (Ravikumar et al., 2016). Several workers have detailed the efficiency of *T. harzianum* for the control of diseases resulted by *A. alternata (Saikia et al., 2022)*. These reports are consistent with the current investigation. *Trichoderma* spp. suppressing the growth of *A. alternata* and *Fusarium* spp. and they will compete for nutrient and space, mycoparasitism and antibiosis are the most common mode action. *Trichoderma* spp. secretes many antifungal compounds like pyrones, isopyrones, peptides, and trichothenes that kill pathogens. The relatively high concentration of cell wall degrading enzymes is produced by *T. harzianum* which inhibit and kill the pathogen (Philip et al., 2024).

3.9 Effect of the Treatment on Plant Growth Parameters in Field Condition

The data showed in the Table 2 suggested that all the treatments consisting of botanical, biocontrol agent and chemical were found to be significantly superior as compared to control. Maximum number of suckers (21.00 nos./plant), flower (42.25 nos./plant), Diameter of flower (6.25 cm), Length of the flower (39.32 cm), Shelf life of the flower (18.75 days), Vase life of the flower (14.50 days), shoot and root length (34.72 and 18.30), was observed in T_2: *T. harzianum* (Org-Trichojal @ 5ml/l). The outcomes of this field study are consistent with the research conducted by (Deka and Talukdar, 2014), who examined the performance of twelve gerbera cultivars (*Gerbera jamesonii* Bolus) under open field conditions in Assam. The cultivars were found varied significantly in terms of vegetative, flowering, and floral characteristics. The cultivar Red Gem produced the highest number of leaves per plant (46.55 nos), suckers per plant (24.04 nos), and the highest plant spread (54.10 cm), according to the mean performance of the cultivars. The results indicated that bio-agents might be having some growth promoting effects on plants. Growth promoting effects by *Trichoderma* spp. has been reported in other crops by several workers. On the other hand, *Trichoderma* secretes growth promoting hormones *viz*., zeatin, gibberelic acid, gluconic acid, citric acid and fumaric acid and has good capacity to uptake soil nutrients with higher productivity (Saikia et al., 2022). Several previous researchers agree on these aspects of enhanced growth promotion on application of bioagents due to a potential synergistic effect. Bio agents like *Trichoderma* spp. and *P. fluorescens* has a great potentiality in the enhancement of reproductive as well as vegetative status of treated plants (Mathivanan et al., 2000). Strains of *Trichoderma* spp. have been reported to solubilize insoluble forms of Iron, Manganese, Zinc and rock phosphate and thus making them available for plants (Altomare et al., 1999). (Shetty et al., 2025) found that the application of *Trichoderma* spp. for disease control in vegetable crops resulted in enhanced germination, leaf area, dry weight of roots and shoots, and early flowering.

4. Conclusion

From the present study, it can be concluded that among the fungal diseases of gerbera, the incidence of leaf spot caused by *Alternaria alternata* was found to be most common in Assam condition. The severity of *Alternaria* leaf spot of gerbera can be significantly reduced by using Hexaconazole (0.1%). *Trichoderma harzianum* based bio formulation Org-Trichojal (@5mL/L) was also found to be effective against the pathogens. Application of Org-Trichojal (*T. harzianum*) significantly increased the flower yield with enhanced plant growth parameters under both pot and field conditions. Although, foliar application of fungicides was found effective in reducing the incidence of *Alternaria* leaf spot but its application alone should be preferably avoided because chemical control measures are not only uneconomical but also potentially hazardous for the ecological and environmental health.

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 the writing or editing of this manuscript.

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Competing Interests

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary

1. **Symptomatology of Alternaria leaf spot**

Fig. 1. Typical symptoms observed in leaves

2. Morpho-cultural characteristics

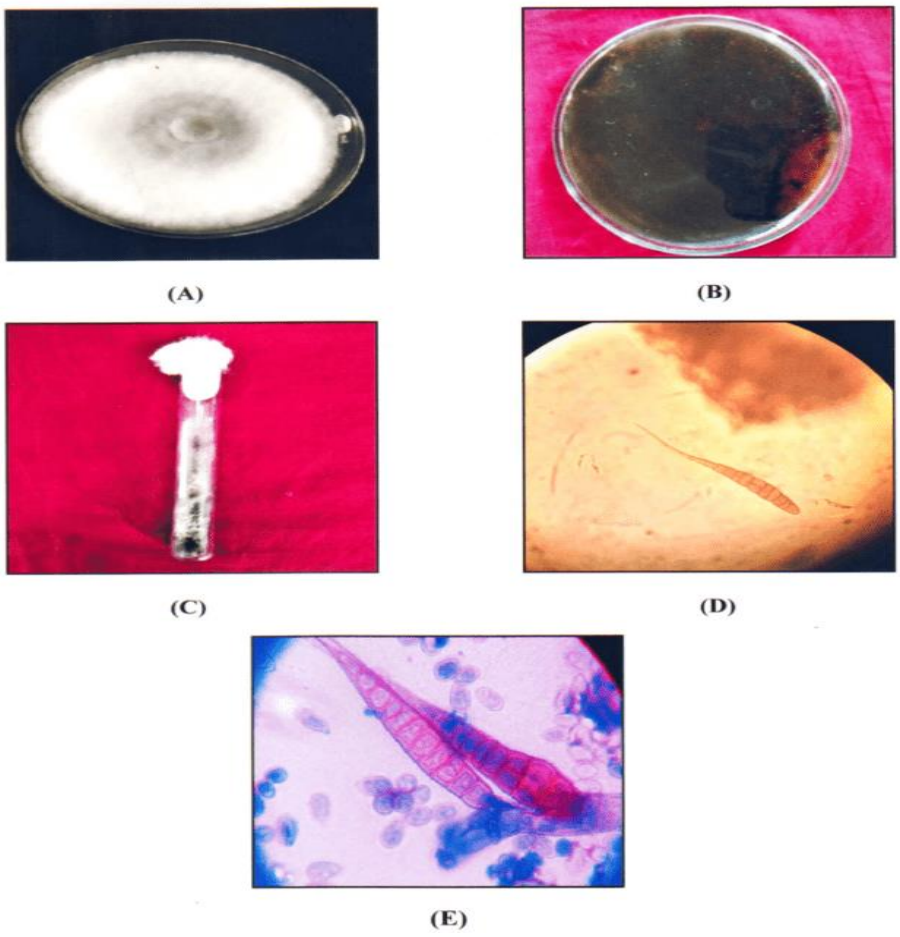

Fig. 2. (A-E). Cultural and morphological characteristics of *A. alternata* on PDA media

A. Pure culture of A. alternata in PDA (7 days old culture), B. Reverse view of A. alternata in Petri plate, C. A. alternata on culture tube, D. Microscopic view of conidia (10X), E. Microscopic view of conidia (40X)

3. Pathogenicity test

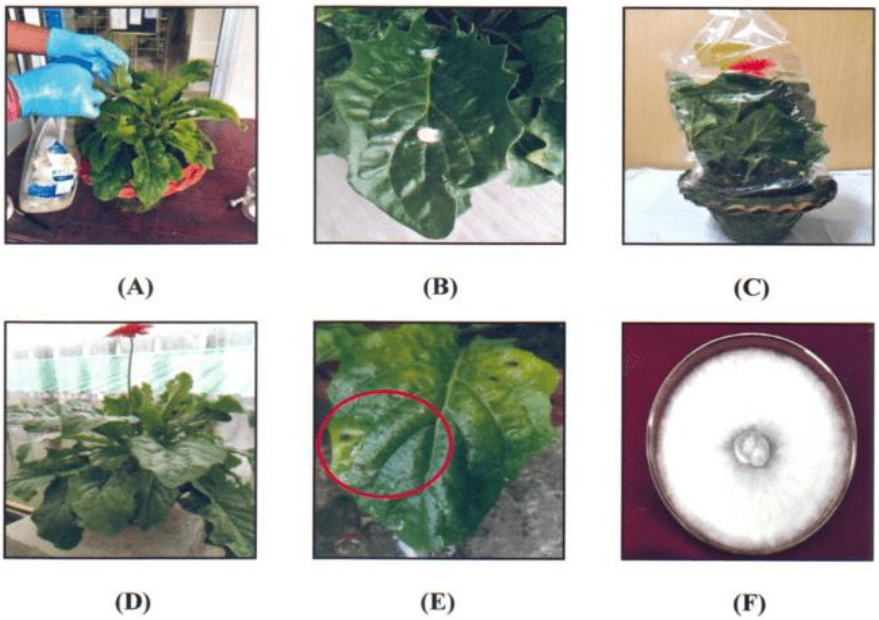

Fig. 3 (A-F). Pathogenicity test of *A. alternata* on healthy gerbera plant

A. Pathogenicity test by Pin-pricked method, B. Pathogenicity test by Mycelial Bit Inoculation Technique (MBIT), C. Plants covered with transparent perforated polypropylene bag, D. Control plant E.7 days after inoculation of A. alternata developed initial symptoms, F. Re-isolation of A. alternata from infected leaf

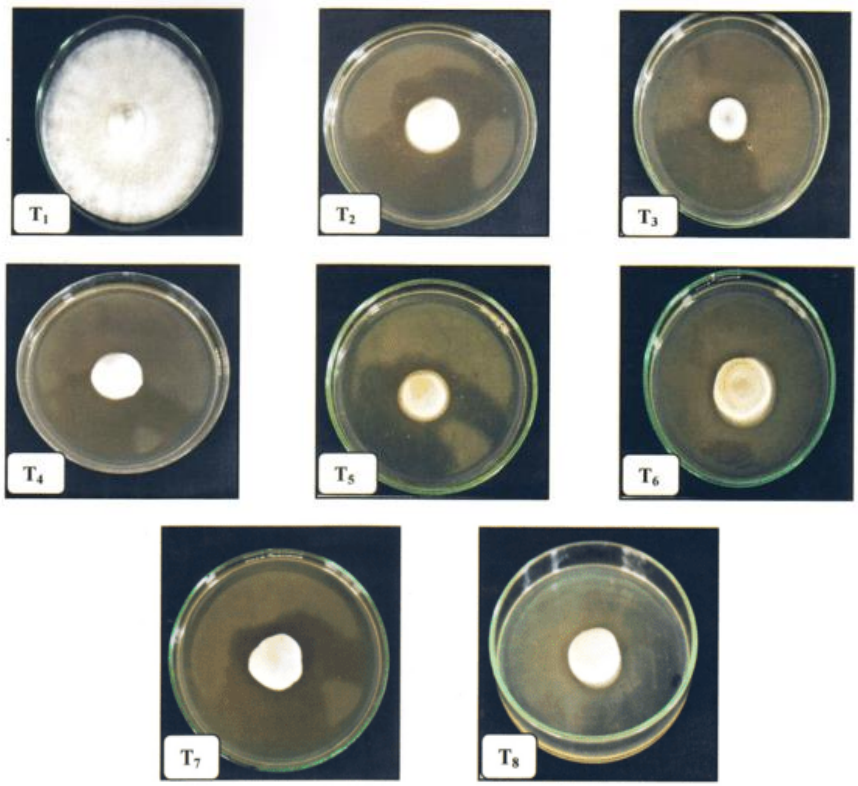

Fig. 4. *In vitro* efficacy of different botanicals against mycelial growth of *A. alternata*

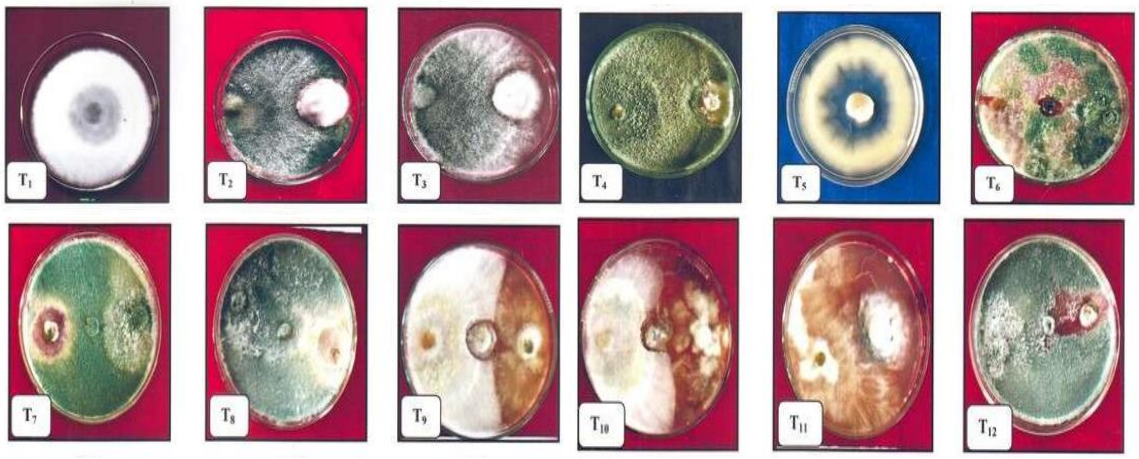

Fig. 5. *In vitro* efficacy of different biocontrol agents against mycelial growth of *Alternaria alternata*

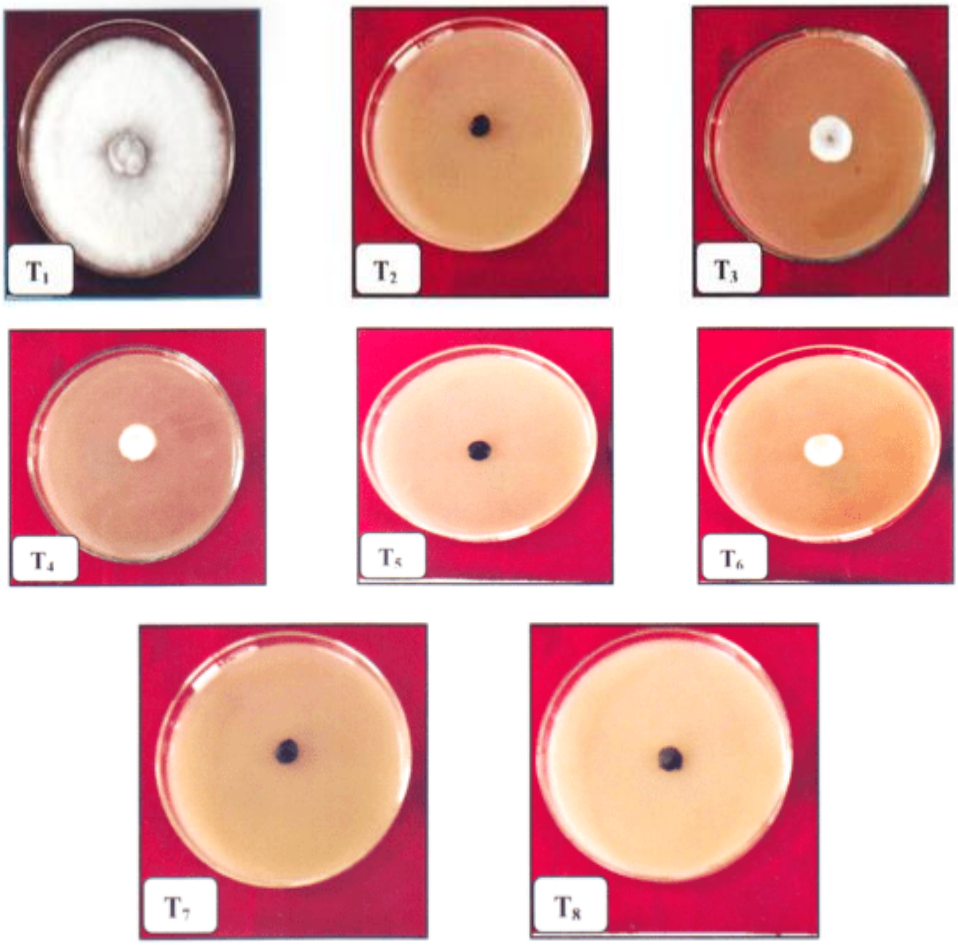

Fig. 6. *In vitro* efficacy of different chemicals against on mycelial growth of *A. alternata*

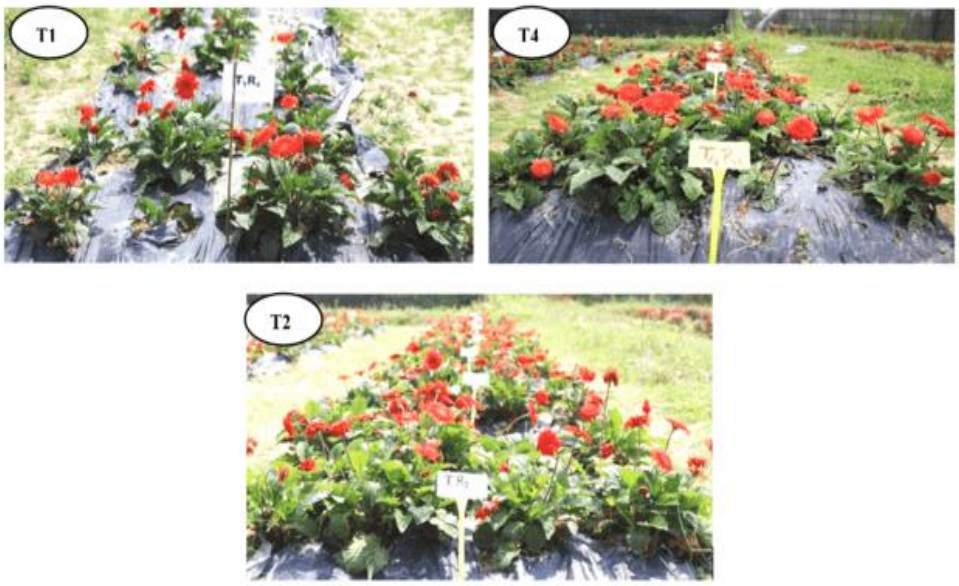

Fig. 7 (A-C). *In vivo* effect of different treatment combinations against *Alternaria* leaf spot under field condition

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