Original Research Article

Potential of Phyto Extracts and Essential Oils in Controlling of Leaf Blight Pathogen *Alterneria alternata* of Gerbera (*Gerbera jamesonii* L.): An *In-vitro* Approach

Abstract

Gerbera jamesonii L.is a widely cultivated ornamental plant valued for its vibrant flowers and long vase life, playing a vital role in the global floriculture market. However, its commercial production is significantly constrained by foliar diseases, particularly leaf blight caused by *Alternaria alternata* (Fr.) Keissler. This pathogen is known to cause necrotic lesions, defoliation, and substantial yield and aesthetic losses, ranging from 40–60% under favorable conditions. Increasing concerns over fungicide resistance, environmental impact, and consumer preference for residue-free products have emphasized the need for sustainable, plant-based disease management strategies. The present investigation was undertaken to evaluate the *in vitro* antifungal efficacy of selected Phyto-extracts and Essential oils as eco-friendly alternatives to synthetic fungicides. The pathogen was isolated from infected gerbera leaves and identified based on cultural and microscopic characteristics. The poisoned food technique was used to assess the efficacy of six Phyto-extracts (nettle grass, vach, bitter melon, jatropha, turmeric, and garlic) at 5%, 10%, and 15% concentrations, and six essential oils (thyme, lemongrass, eucalyptus, tea tree, moringa, and ginger) at 200, 400, and 600 ppm. Among Phyto-extracts, garlic extract at 15% showed the highest inhibition of mycelial growth (74.22%), followed by turmeric (59.21%) and vach (51.70%). Among essential oils, thyme oil at 600 ppm exhibited the highest antifungal activity (93.42%), followed by tea tree (78.43%) and ginger oil (76.69%). The findings demonstrate the significant antifungal potential of garlic extract and thyme oil against *A. alternata*, suggesting their utility in developing sustainable disease management practices for gerbera cultivation.

Key words: *Gerbera jamesonii*, *Alternaria alternata*, Phyto-extracts, Essential oils, antifungal activity,

INTRODUCTION

Gerbera (*Gerbera jamesonii* L.) is one of the most commercially valued ornamental crops, cultivated worldwide for its vibrant colors, prolonged shelf life, and market demand as a cut flower (Mahanta and Gantait, 2024). However, its production is adversely affected by a range of biotic stresses, particularly fungal pathogens that compromise both yield and flower quality (Gautam *et al*., 2020). Among these, leaf blight caused by *Alternaria alternata* (Fr.) Keissler is one of the most

destructive foliar diseases, capable of causing 40–60% loss under favorable conditions (Surbhi *et al*., 2021). *Alternaria alternata* is a necrotrophic fungus belonging to the phylum Ascomycota, known for its wide host range, morphological adaptability, and production of dark, septate, multicellular conidia (Mamgain *et al.*, 2013). It thrives in warm, humid environments, with optimal growth observed between 20–30°C and high relative humidity (>85%), often spreading rapidly through airborne spores and rain-splash (Jain and Sandhu, 2019). The pathogen infects leaf tissues, leading to necrotic lesions, defoliation, and eventually plant death, significantly reducing the ornamental and commercial value of the crop (Mirkova and Konstantinova, 2003).In addition to visible symptoms, *A. alternata* produces several mycotoxins, including alternariol (AOH), alternariol monomethyl ether (AME), tenuazonic acid (TeA), tentoxin (TEN), and altertoxin-II (ATX-II), which contribute to its pathogenicity by impairing photosynthesis, disrupting cellular metabolism, and accelerating tissue necrosis (Meena *et al*., 2017). These toxins not only intensify disease severity but may also reduce the post-harvest quality and safety of ornamental products.

Excessive dependence on chemical fungicides for disease management poses serious environmental risks and can lead to fungicide resistance. Therefore, the use of natural plant-based antifungal agents—such as Phyto-extracts and Essential oils—is gaining momentum as a sustainable alternative (Navale and Sawant, 2021). These botanical products are eco-friendly, biodegradable, and effective in suppressing a wide range of pathogens, making them suitable for integrated disease management in floriculture systems. (Deka *et al*., 2024).In this context, the present study was undertaken to evaluate the *in vitro* antifungal efficacy of selected Phyto-extracts and Essential oils against *A. alternata* infecting *Gerbera jamesonii,* with the aim of identifying potent botanical alternatives for sustainable leaf blight management.

MATERIALS AND METHODS

The present investigation, entitled “Potential of Phyto Extracts and Essential Oils in Controlling Leaf Blight Pathogen *Alterneria alternata* of Gerbera (*Gerbera jamesonii* L.): An *In-Vitro* Approach” was undertaken with the objective of identifying effective, sustainable, and environmentally friendly alternatives for the management of this economically significant foliar disease. The study was conducted under controlled laboratory conditions at the Department of Plant Pathology, College of Horticulture, VCSG UUHF, Bharsar, located in Pauri Garhwal, Uttarakhand.

Infected leaves of *Gerbera jamesonii* exhibiting typical symptoms of *Alternaria* blight were collected from the Floriculture Block of the College of Horticulture, VCSG UUHF, Bharsar, Pauri Garhwal, Uttarakhand. The samples were placed in clean, sterile polyethylene bags, labeled with collection date and location, and transported to the Plant Pathology Laboratory under cool conditions. To minimize further deterioration, the samples were stored at 4 °C until further processing for fungal isolation. For purification, the samples were initially washed under running tap water to remove surface debris and air-dried. Approximately 5 mm segments containing both diseased and adjacent healthy tissue were excised using a sterile scalpel. These segments were surface sterilized in 0.1% mercuric chloride ($HgCl_2$) for 2–3 minutes, followed by triple rinsing with sterile distilled water to remove residual sterilant. Under aseptic conditions, the sterilized tissue segments were transferred onto sterile Petri plates containing solidified Potato Dextrose Agar (PDA) medium.

The plates were incubated at 25 ± 2 °C and monitored daily for fungal growth. Emerging colonies were sub-cultured by transferring hyphal tips from the actively growing margins onto fresh PDA plates. This process was repeated until pure cultures were obtained. The purified isolates were maintained on PDA slants and stored at 4 °C for further studies.

The identification of the fungal pathogen was carried out based on its cultural and morphological characteristics. Purified isolates were cultured on Potato Dextrose Agar (PDA) medium and incubated at 25 ± 2 °C for 7 days. Observations were made on colony color, texture, margin, and growth pattern. For microscopic examination, a small portion of the fungal mycelium was mounted in lactophenol cotton blue on a clean glass slide, covered with a coverslip, and examined under an Olympus compound microscope. Key diagnostic features such as conidiophores, conidial shape, septation, and arrangement were recorded. Identification was conducted using standard taxonomic keys and descriptions available in the relevant mycological literature.

In-vitro Evaluation of Phyto-Extracts against *Alternaria alternata*

The antifungal efficacy of six phyto-extracts—*Urtica dioica* (nettle grass), *Acorus calamus* (vach),*Momordica charantia* (bitter melon), *Jatropha curcas* (jatropha), *Curcuma longa* (turmeric), and *Allium sativum* (garlic)—was assessed at 5%, 10%, and 15% concentrations using the poisoned food technique. Healthy, disease-free plant parts were collected, washed thoroughly with distilled water, and shade-dried. The dried material was ground into a fine paste using a sterile mortar and pestle. Extraction was carried out by mixing the paste with distilled water, methanol, or ethanol in a 1:1 (w/v) ratio, depending on solubility. The mixture was left at room temperature for 24 hours to allow phytochemical extraction, then filtered sequentially through muslin cloth and Whattman No. 1 filter paper to obtain a clear extract.

In-vitro Evaluation of Phyto-Extracts against *Alternaria alternata*

Six essential oils—Thyme, Lemongrass, Eucalyptus, Teatree, Moringa, and Ginger—along with an untreated control were tested at concentrations of 200, 400 and 600 ppm using the poisoned food technique. Essential oils used in the experiment—Thyme, Lemongrass, Eucalyptus, Teatree, Moringa, and Ginger—were procured from certified online suppliers to ensure purity and quality. Upon receipt, the oils were stored in amber-colored glass bottles at room temperature in a cool, dry place to prevent degradation from light and heat.

Poisoned food technique

The *in-vitro* antifungal activity of selected phyto-extracts and essential oils against *Alternaria alternata* was evaluated using the poisoned food technique. For phyto-extracts, stock solutions were prepared and incorporated into sterilized molten Potato Dextrose Agar (PDA) to achieve final concentrations of 5%, 10%, and 15% by mixing 5, 10, and 15 mL of extract into 95, 90, and 85 mL of PDA, respectively. Essential oils were tested at 200, 400, and 600 ppm concentrations. Due to their hydrophobic nature, a few drops of Tween 20 were added as an emulsifying agent before mixing the oils thoroughly into the PDA.

Approximately 20 mL of the amended medium was poured into sterile Petri plates and allowed to solidify. A 5 mm mycelial disc from a 3-week-old culture of *A. alternata* was aseptically placed at the center of each plate. The plates were incubated at 25 ± 2°C for 7 days. An untreated control (PDA without any botanical treatment) was maintained under identical conditions. The antifungal efficacy of the phyto-extracts and essential oils was expressed as percent inhibition of mycelial growth over control, calculated using the formula suggested by Vincent (1947):

$$\mathbf{PGI = C-T/C \times 100}$$

Where:

PGI = Percent growth inhibition

C = Radial growth of the fungus in control (mm)

T = Radial growth of the fungus in treatment (mm)

The data were analyzed using ANOVA under a Completely Randomized Design (CRD) as per Gomez and Gomez (1984), using OPSTAT and Excel. Significant differences at $P = 0.05$ were determined using the Critical Difference (CD) test, and results were presented through tables.

RESULTS AND DISCUSSION

1.Cultural and Morphological Characteristics of *Alternaria alternata*

1. 1. Cultural Characteristics

The fungal isolate exhibited rapid growth on Potato Dextrose Agar (PDA), attaining an average colony diameter of 8.2 cm within 7 days at room temperature (25 ± 2 °C). Initially, the colony appeared dull white with profuse mycelial growth, which gradually turned light grey to dark black with a cottony texture. The colony margins were irregular and wavy, and concentric zonation was distinctly observed. Ahmad *et al*. (2024) observed that *A. alternata* isolates from infected sweet cherry fruits grew at an average rate of 8 mm/day, producing circular, dark olive green colonies with grey to white aerial mycelium on PDA, matching the growth pattern seen in the present study. Fagodiya *et al*. (2024) reported radial growth ranging from 72.4 mm to 87.2 mm after 7 days on PDA, with colony colours varying from grey to dark green, and highlighted isolate-specific morphological differences. Shingne *et al*. (2020) found that different *A. alternata* isolates exhibited colony colours from light grey to olive green**,** dense aerial mycelium, and both regular and irregular colony margins on PDA.

1.2. Morphological Characteristics

Microscopic examination of the fungal isolate revealed septate, multicellular, and irregularly branched mycelium, initially hyaline and later turning grey to brown. Conidiophores arose singly or in clusters (2–6), varying in length, straight to slightly curved, geniculate, and pale olivaceous to olivaceous brown in color, with a slightly swollen apex. The average size of

conidiophores was 40.33 µm in length and 5.22 µm in width. Conidia were produced in long chains (up to 10 or more), obclavate in shape, and light olivaceous to dark brown. They were multicellular with muriform septation and bore a short apical beak, characteristic of *Alternaria alternata*. The morphological traits observed in the present study were similar to those reported by Nagrale *et al.* (2013) and Ahmad *et al.* (2024), particularly in colony appearance and growth pattern. Conidial and hyphal characteristics also correspond well with the findings of Kamalakannan *et al*. (2008) and Toppo and Kothasthane (2023), indicating consistency with previous observations of *A. alternata*.

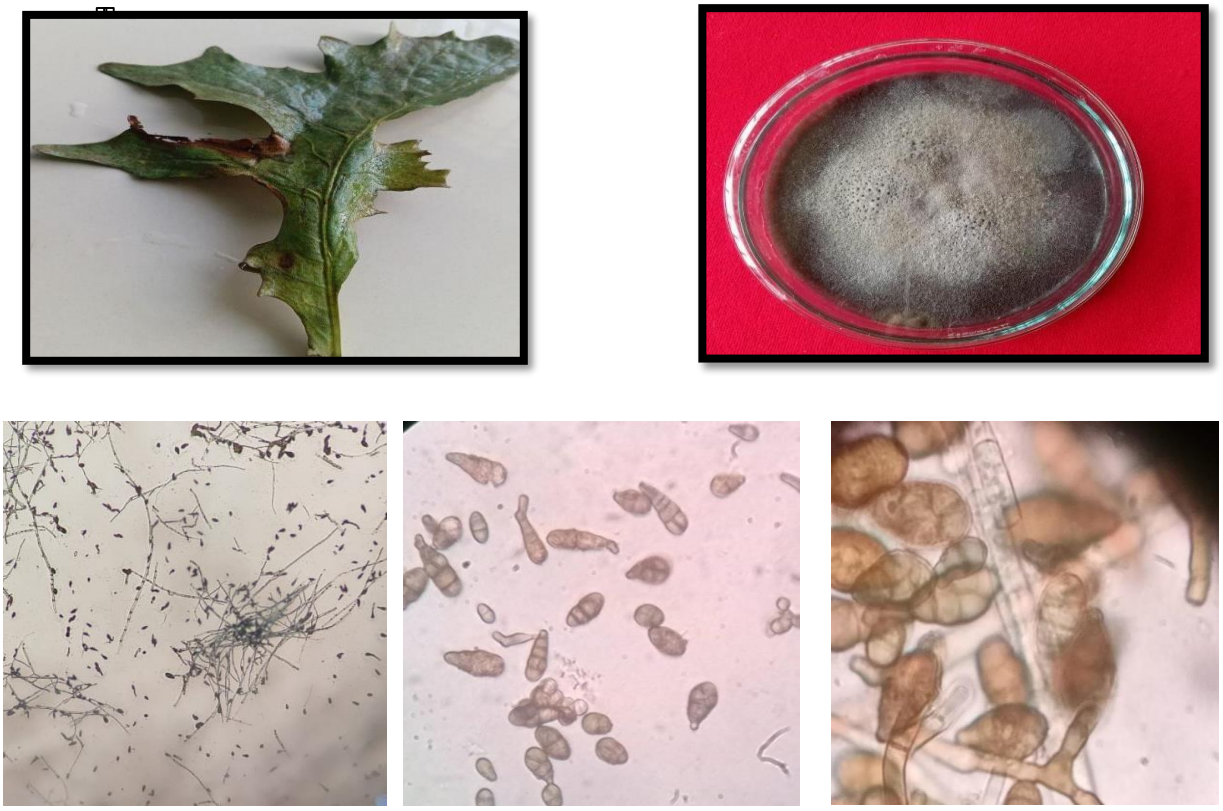

fig.1 Microscopic examination of the fungal Alternaria alternata

1.3 Effect of Phyto extracts on per cent mycelial inhibition of *Alterneria alternata.*

The antifungal activity of various phyto-extracts was evaluated at 5%, 10%, and 15% concentrations against *A. alternata*. At all concentrations, the untreated control (T1) exhibited no inhibition (0.00%), while the efficacy of the treatments increased with concentration. Garlic extract (T_7) consistently demonstrated the highest inhibitory effect, recording 69.90%, 71.33%, and 74.22% inhibition at 5%, 10%, and 15% concentrations, respectively. Turmeric (T_6) followed with 50.80%, 54.51%, and 59.21% inhibition, while vach (T_3) showed 40.25%, 45.01%, and 51.70%,

respectively. Nettle grass (T_2) displayed moderate efficacy with 30.48%, 35.53%, and 38.44% inhibition across the concentrations. In contrast, jatropha (T_5) and bitter melon (T_4) consistently showed lower antifungal activity, recording 9.39%, 14.24% and 20.97% for jatropha, and 7.66%, 10.39%, and 15.84% for bitter melon, at increasing concentrations. These results indicate a dose-dependent response, with garlic, turmeric, and vach extracts showing promising potential as botanical antifungal agents. These findings are in line with earlier studies by Kutawa *et al*. (2018) and Chaudhary and Singh (2021), who also reported potent antifungal action of garlic due to compounds like allicin and ajoene. The efficacy of turmeric and vach extracts is supported by the reports of Qi *et al*. (2024), Kipkogei *et al*. (2019) and Bhagat and Yadav (2023), which attributed their activity to curcumin and asarones, respectively. Moderate inhibition by nettle grass, jatropha, and bitter melon corroborates findings by Behiry *et al.* (2022), Ingle *et al*. (2017) and Filho *et al*. (2020), suggesting that these extracts also contain bioactive compounds with potential antifungal properties. (Table 1).

1.4 Effect of Essential oils on per cent mycelial inhibition of *Alterneria alternata*.

The antifungal activity of essential oils against *A. alternata* was assessed at 200, 400, and 600 ppm concentrations. At all levels, the untreated control (T_1) showed no inhibition (0.00%), confirming the absence of any natural suppression. At 200 ppm, thyme oil (T_2) exhibited the highest inhibition (87.42%), followed by tea tree oil (T_5) and ginger oil (T_7) with 72.08% and 70.31% inhibition, respectively. Moringa oil (T_6), eucalyptus oil (T_4), and lemongrass oil (T_3) showed moderate efficacy, recording 65.49%, 55.72%, and 50.35% inhibition. At 400 ppm, antifungal activity improved across all treatments, with thyme oil increasing to 91.16%, tea tree oil to 75.49%, and ginger oil to 74.38%. Moringa, eucalyptus, and lemongrass oils also showed enhanced inhibition at 71.31%, 57.47%, and 53.51%, respectively. The highest inhibition levels were recorded at 600 ppm, where thyme oil (T_2) reached 93.42%, maintaining its superior efficacy. Tea tree and ginger oils followed with 78.43% and 76.69% inhibition. Moringa oil remained effective at 74.16%, while eucalyptus and lemongrass oils reached 62.91% and 57.26%, respectively. These findings indicate a concentration-dependent response, with thyme, tea tree, and ginger oils being the most potent in suppressing the mycelial growth of *A. alternata.* These results are consistent with earlier reports by Pedrotti *et al.* (2022), Hendges *et al.* (2021) and Ghuffar *et al.* (2022), who also observed strong antifungal effects of these oils against phytopathogenic fungi. The superior efficacy of thyme oil may be attributed to its high thymol and carvacrol content (Halat *et al*., 2022), while tea tree and ginger oils exert their effects through membrane disruption and inhibition of fungal development (Hussein and Joo, 2018; Hendges *et al*., 2021). Moringa and eucalyptus oils also showed moderate inhibition, corroborating findings by Kachelo *et al*. (2022) and Guleria *et al.* (2011), respectively. These observations support the potential of essential oils as sustainable alternatives to synthetic fungicides in plant disease management. (Table 2).

Table 1 Effect of Phyto extracts on per cent mycelial growth inhibition of *A. alternata* at different concentrations

Treatments	Average per cent growth inhibition		
	Concentration(%) ± S.E.(m)		
	5%	10%	15%
Control	0.00±0.00 (0.00)	0.00±0.00 (0.00)	0.00±0.00 (0.00)
Nettle grass	30.48*±0.45 (33.5)	35.53*±0.38 (35.37)	38.44*±0.45 (39.37)
Vach	40.25*±0.28 (39.36)	45.01*±0.53 (42.11)	51.70*±0.56 (45.96)
Bitter melon	7.66*±0.10 (16.06)	10.39*±0.17 (18.79)	15.84*±0.11 (23.44)
Jatropha	9.39*±0.28 (17.84)	14.24*±0.10 (22.16)	20.97*±0.24 (27.24)
Turmeric	50.80*±0.49 (45.44)	54.51*±0.54 (47.57)	59.21*±0.69 (50.29)
Garlic	69.90*±0.63 (56.70)	71.33*±0.55 (57.60	74.22*±0.52 (59.46)
SE(d)	**0.54 (0.35)**	**0.55 (0.33)**	**0.62 (0.37)**
C.D.$_{(0.05)}$	**1.17 (0.76)**	**1.20 (0.73)**	**1.34 (0.81)**

() = values in parentheses are angular transformed

* Significant at 5% level of significance as compared with control

Table 2 Effect of Essential oils on per cent mycelial inhibition of *A. alternata* at different concentrations

Treatments	Average per cent growth inhibition		
	Concentration(ppm) ± S.E.(m)		
	200	**400**	**600**
Control	0.00±0.00 (0.00)	0.00±0.00 (0.00)	0.00±0.00 (0.00)
Thyme oil	87.42*±0.39 (69.20)	91.16*±0.28 (72.67)	93.42*±0.56 (75.14)
Lemongrass oil	50.35*±0.54 (45.18)	53.51*±0.25 (46.99)	57.26*±0.28 (49.15)
Eucalyptus oil	55.72*±0.56 (48.26)	57.47*±0.46 (49.27)	62.91*±0.61 (52.46)
Teatree oil	72.08*±0.49 (58.08)	75.49*±0.78 (60.30)	78.43*±0.70 (62.31)
Moringa oil	65.49*±0.31 (54.00)	71.31*±0.75 (57.56)	74.16*±0.63 (59.43)
Ginger oil	70.31*±0.53 (56.96)	74.38*±0.35 (59.57)	76.69*±0.71 (61.11)
SE(d)	**0.63 (0.40)**	**0.76 (0.49)**	**0.78 (0.59)**
C.D.$_{(0.05)}$	**1.37 (0.87)**	**1.66 (1.07)**	**1.70 (1.28)**

() = values in parentheses are angular transformed

* Significant at 5% level of significance as compared with control

Conclusion

The present study demonstrated that *Alternaria alternata* is the causal agent of leaf blight in *Gerbera jamesonii*, confirmed through cultural and morphological characterization. Among the tested treatments, garlic extract (15%) and thyme essential oil (600 ppm) exhibited the highest antifungal activity under *in vitro* conditions, significantly inhibiting mycelial growth of the pathogen. The strong efficacy of these botanicals can be attributed to their bioactive compounds—allicin in garlic and thymol and carvacrol in thyme. These findings highlight the potential of natural plant-based products as effective, eco-friendly alternatives for the management of gerbera leaf blight. Further field-level evaluations are recommended to standardize formulations for practical use in sustainable floriculture practices.

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