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 commercially important ornamental crop, but its production is severely constrained by leaf blight caused by *Alterneria alternata*, leading to 40-60 % yield and quality losses. Increasing concerns over fungicide resistance and chemical residues necessitate sustainable, eco-friendly alternatives. The present investigation was undertaken to evaluate the *in vitro* antifungal efficacy of selected Phyto-extracts and Essential oils against *Alterneria alternata*. 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*, supporting their use as plant-based biofungicides. This study provides a baseline for incorporating phyto-extracts and essential oils into integrated, eco-friendly disease management strategies for sustainable 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 globally for its striking color diversity, extended vase life, and consistent market demand as a premium cut flower (Mahanta and Gantait, 2024). Its wide adaptability to diverse agro-climatic conditions and aesthetic appeal have made it a preferred choice in both domestic and international floriculture industries. India has emerged as one of the major producers of gerbera, with expanding cultivation under protected conditions to meet export standards and consumer preferences. However, the productivity and post-harvest quality of gerbera are often constrained by several biotic and abiotic stresses, among which fungal pathogens are of paramount concern due to their potential to cause substantial yield and quality losses (Gautam *et al*., 2020).

Among the major fungal pathogens, *Alternaria alternata* (Fr.) Keissler -a necrotrophic fungus belonging to the phylum Ascomycota — is recognized as the principal causal agent of leaf blight in gerbera. The pathogen produces dark, multicellular, beak-shaped conidia and thrives under warm (20–30 °C) and humid conditions (>85% relative humidity), spreading primarily through air-borne conidia and rain splashes (Jain and Sandhu, 2019). The disease typically appears as small necrotic lesions that enlarge concentrically, leading to severe blighting, premature senescence, and defoliation. Consequently, photosynthetic activity declines, and both the aesthetic value and marketability of the flowers are compromised (Mirkova and Konstantinova, 2003). Additionally, *A. alternata* produces a range of mycotoxins—such as alternariol (AOH), alternariol monomethyl ether (AME), tenuazonic acid (TeA), tentoxin (TEN), and altertoxin-II (ATX-II)—that disrupt metabolic processes and accelerate tissue necrosis (Meena *et al*., 2017).

Post-harvest deterioration is another significant challenge in the floriculture industry, where nearly 10–30% of the total global production of cut flowers is lost due to microbial spoilage and improper handling (Kumar *et al*., 2022). The vase life and aesthetic quality of cut flowers are strongly influenced by preharvest cultural practices, harvesting methods, packaging, storage, and transportation conditions. High relative humidity, poor ventilation, and temperature fluctuations during storage often promote the proliferation of fungal pathogens such as *A. alternata*, *Botrytis cinerea*, and *Penicillium* spp., leading to rapid tissue degradation and reduced shelf life (Navale and Sawant, 2021). Hence, maintaining optimal storage conditions—including temperature regulation (1–4 °C), humidity control, and proper ventilation—is crucial to minimize fungal growth and post-harvest losses (Zhang *et al*., 2024).

Conventionally, pre- and post-harvest diseases in cut flowers are managed through chemical fungicides and bactericides. Although these agents provide effective short-term disease suppression, their indiscriminate and repeated use has resulted in several ecological and economic drawbacks. These include the development of fungicide-resistant pathogen strains, phytotoxic effects, contamination of soil and water, and the accumulation of chemical residues on ornamental produce (Rahman *et al*., 2025). Furthermore, the growing consumer awareness regarding environmental sustainability and human safety has led to increasing demand for residue-free and eco-friendly management practices in floriculture (Abdullah *et al*., 2025).

Similarly, over the past five decades, chemical pesticides have been extensively employed across agricultural systems to reduce pest- and pathogen-induced losses in crops and stored produce. However, their continuous use has led to several environmental and health-related issues, including contamination of food, soil, and water. These concerns have driven researchers to explore safer alternatives with minimal adverse effects. Among such approaches, the application of plant-based or herbal pesticides in both field and storage systems has shown promising potential. Nevertheless, before employing these eco-friendly technologies, essential storage parameters—such as temperature, moisture, and oxygen content—must be carefully regulated, as these factors play a critical role in determining the susceptibility of stored products to microbial spoilage (Nag, 2024).

In this context, plant-derived antifungal agents such as phyto-extracts and essential oils have gained considerable attention as sustainable and biodegradable alternatives to synthetic fungicides. These natural products contain diverse bioactive compounds—including alkaloids, terpenoids, flavonoids, and phenolics—that exhibit broad-spectrum antimicrobial activity. They can be

effectively incorporated into pre- and post-harvest disease management systems to enhance the shelf life and quality of cut flowers while minimizing chemical dependency (Deka *et al*., 2024). The integration of such botanical formulations also presents opportunities for developing safe storage technologies and natural preservative treatments that align with eco-conscious production systems.

Therefore, the present investigation was undertaken to evaluate the *in vitro* antifungal efficacy of selected phyto-extracts and essential oils against *A. alternata* infecting gerbera (*G. jamesonii*). The ultimate goal of the study is to identify potent botanical alternatives for sustainable management of leaf blight and post-harvest fungal deterioration, thereby contributing to environmentally responsible and economically viable floriculture practices.

This manuscript holds significant importance as it addresses one of the key challenges in gerbera cultivation—disease management with minimal chemical input. By identifying effective plant-based antifungal agents such as garlic extract and thyme oil, the study promotes eco-friendly disease control strategies that can enhance flower quality, reduce chemical residues, and support sustainable post-harvest management. The findings provide a scientific foundation for developing botanical biofungicides and advancing green floriculture practices globally.

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* showing typical *Alternaria* blight symptoms were collected from the Floriculture Block, College of Horticulture, VCSG UUHF, Bharsar, Pauri Garhwal, Uttarakhand. Samples were placed in sterile polyethylene bags, labeled, and transported to the Plant Pathology Laboratory under cool conditions. After washing and air-drying, 5 mm diseased leaf segments were surface sterilized with 0.1% $HgCl_2$ for 2–3 min, rinsed thrice with sterile distilled water, and aseptically transferred onto Potato Dextrose Agar (PDA) plates for fungal isolation.

The inoculated plates were incubated at 25 ± 2 °C and observed daily for fungal growth. Actively growing colonies were sub-cultured on fresh PDA to obtain pure cultures, which were maintained on PDA slants at 4 °C for further study. The pathogen was identified based on cultural and morphological characteristics, including colony color, texture, and growth pattern on PDA after 7 days of incubation. Microscopic observations of lactophenol cotton blue–stained mounts were made to examine conidiophores and conidial morphology, and identification was confirmed using standard mycological keys.

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 Essential Oils 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 $25 \pm 2°C$ 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.

Experimental Workflow

Collection of infected gerbera leaves

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Isolation of pathogen on PDA medium

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Purification and maintenance of pure culture

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Identification of pathogen (cultural and morphological characteristics)

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Preparation of phyto-extracts (nettle, vach, bitter melon, jatropha, turmeric, garlic)

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Preparation of essential oils (thyme, lemongrass, eucalyptus, tea tree, moringa, ginger)

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In-vitro evaluation using poisoned food technique

↓

Incubation at 25 ± 2 °C for 7 days

↓

Measurement of radial growth and calculation of % inhibition (Vincent, 1947)

↓

Statistical analysis (ANOVA – CRD using OPSTAT and Excel)

RESULTS AND DISCUSSION

1.Cultural and Morphological Characteristics of *Alternaria alternata*

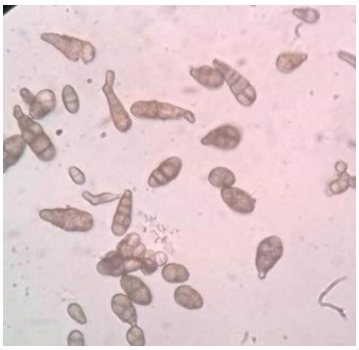
1. 1. Cultural Characteristics

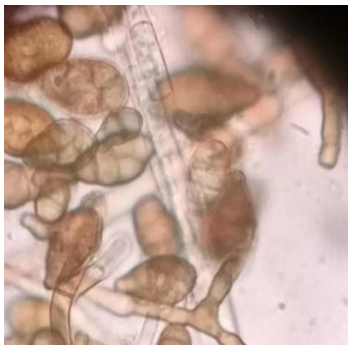
The fungal isolate exhibited rapid growth on Potato Dextrose Agar (PDA), attaining an average colony diameter of 8.2 cm within 7 days at room temperature (25 ± 2 °C). Initially, the colony appeared dull white with profuse mycelial growth, which gradually turned light grey to dark black with a cottony 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*.





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 dosedependent 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).

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

Research Gaps and Contributions of the Present Study in Managing *Alternaria alternata*

Aspect	Existing Research	Research Gap / Limitation	How Present Study Addresses It
Disease characterization	Morphology, cultural traits of *A. alternata* in other crops	Limited studies specifically on gerbera	Conducted cultural & morphological characterization of isolates from gerbera
Chemical control	Synthetic fungicides widely used	Fungicide resistance, chemical residues, environmental contamination	Tested eco-friendly botanical alternatives to reduce chemical dependency
Plant-based antifungals	Some studies on phyto-extracts & essential oils	Limited information on effectiveness against *A. alternata* in gerbera	Evaluated multiple phyto-extracts and essential oils for *in-vitro* efficacy
Concentration & comparative efficacy	Few studies compare multiple extracts/oils	Lack of systematic evaluation of optimal concentrations	Tested three concentrations for each extract/oil to identify most effective dose
Integration with post-harvest management	General suggestions for floriculture	Scarce research on combining botanical agents with storage and shelf-life improvement	Study provides baseline for integrating botanical agents in pre- and post-harvest management

Conclusion

This study confirmed *Alternaria alternata* as the causal agent of leaf blight in *Gerbera jamesonii*. Among the treatments tested, garlic extract (15%) and thyme essential oil (600 ppm) emerged as the most potent inhibitors of fungal growth, likely due to the bioactive compounds allicin, thymol, and carvacrol. These findings showcase the promise of plant-based botanicals as eco-friendly, sustainable alternatives to chemical fungicides. Future research should focus on field validation, formulation refinement, and integration into comprehensive disease management strategies to enhance flower quality, extend vase life, and minimize chemical use.

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