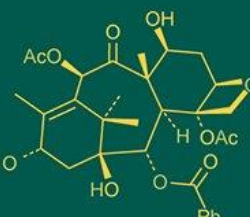
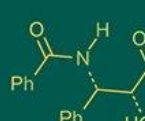
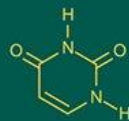


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Assessment of anti-fungal properties of extracts derived from different parts of *Tagetes erecta* L.

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Abstract

The purpose of this work is to assess the anti-fungal efficacy of marigold (*Tagetes erecta* L.) plant part extracts against phytopathogenic fungi such as *Alternaria brassicae* and *Pestalotiopsis psidii*. Extracts of various parts of the marigold plant, including petals, leaves and roots were prepared using suitable solvent extraction method. The anti-fungal potential of these extracts was assessed *in-vitro* using poisoned food method to determine their ability to suppress fungal growth. Marigold plants are containing several biologically active compounds such as flavonoids, terpenoids, lutein and essential oils, which may contribute to their anti-fungal activity. The study was carried out using a CRD (Completely Randomized Design) with 7 treatments each replicated 3 times. The mycelial radial growth was monitored at specified intervals and the percentage of growth inhibition was calculated by comparing treated samples with the control. Leaf extract at 5% was observed to be more effective for inhibition followed by flower (petals) extract at 5%. On the 9th day minimal radial mycelial growth 24.57 mm was noted in T₄, closely accompanied by 24.80 mm growth in T₂ and 26.53 mm in T₆ respectively against *Alternaria brassicae* with utmost growth of 76.77 mm in T₇. However, against *Pestalotiopsis psidii*, least radial growth of 24.00 mm was recorded in T₄ in succession to 26.83 mm in T₂ and 28.00 mm in T₆ respectively, with the maximum radial growth of mycelium of 74.50 mm was recorded in T₇ on 9th day, thus making it to be an effective management strategy.

Keywords: Phytopathogenic fungi, anti-fungal, *Alternaria brassicae*, *Pestalotiopsis psidii*

Introduction

Marigold (*Tagetes* spp.) is commercially significant flower crop which is extensively grown for its vivid blooms, religious rituals, ornamental gardens, and extraction of carotenoids for use in industry and medicine (Selvan *et al.*, 2025) [16]. By serving in form of a supporting plant that deters insect pests, draws beneficial organisms, and enhances soil quality, marigold also supports sustainable agriculture (Rime *et al.*, 2025) [12]. *Tagetes* genus is one of the most important plants which have some anti-microbial properties. There are over fifty species of seasonal or recurring foliar plants in the genus *Tagetes* (Family- Compositae/ Asteraceae), the *Tagetes erecta* L. plant locally known as Genda (Marigold) being one of them. Linnaeus classified *Tagetes* as a distinct genus in 1753. The term *Tagetes* is thought to be derived from Tages, a mythological character associated with Etruscan tradition (Shetty *et al.*, 2015) [18]. Because of their different uses, well-known species including *Tagetes minuta*, *Tagetes erecta*, *Tagetes patula*, are especially significant (Salehi *et al.*, 2018) [15]. There are yellow, orange and golden flowers and the plant can reach a height of 1 to 3 feet and a width of half a foot. Their blooming tops *i.e.*, flowers are rich in carotenoids, such as lutein esters, that are highly valued in medication, food as well as animal nutrition sector (Saini *et al.*, 2023) [14].

In agricultural and forestry industries, plant illnesses caused by various pathogens such as bacteria, fungi, viruses result in significant production and financial losses. Fungal pathogens are responsible for about two-thirds to 85% of plant disease outbreaks. One of the devastating phytopathogen is *Alternaria brassicae* triggering a major disease in mustard *i.e.*, Alternaria blight resulting in a reduction in yield of up to 47% (Meena *et al.*, 2010) [8]. Anthracnose is also one of the major plant diseases that can attack guava fruits. Branches die as a result of anthracnose infestation on all plant sections above ground. Guava anthracnose is believed to be caused by *Pestalotiopsis psidii* and *Colletotrichum gloeosporioides* (Rahman *et al.*, 2003) [11].

Management of these diseases is very important to reduce agricultural yield losses. However, the intensive use of various industrial pesticides has led to significant ecological challenges, including soil and water pollution, biodiversity losses and the development of anti-fungal resistance (Deeksha *et al.*, 2025) [4]. Extracts, aromatic oils, resins and other plant-based substances have shown fungicidal activity against plant fungal infections under laboratory conditions and in real time, according to a number of publications published over past ten years (Sharma *et al.*, 2022) [17]. Some of such products with antifungal action have been demonstrated to come from the Asteraeae/ Compositae family. The anti-microbial qualities of marigold leaves are attributed to a wide range of bioactive substances. The leaves and flowers of marigold are rich in essential oils like, Ocimene, Linalool and caryophyllene which have anti-fungal as well as anti-bacterial properties (Chatterjee *et al.* 2025) [3]. Therefore, this study focuses on the anti-fungal properties of marigold plant part extract on two major fungal pathogens i.e., *Alternaria brassicae* causing Alternaria blight in mustard and *Pestalotiopsis psidii*, causing Anthracnose in guava.

Materials and Methods

Materials

Plant Material

Marigold plants (*Tagetes erecta* L.) were obtained from home garden in Bhaniyawala, Dehradun. Various parts of the plant such as, flowers (petals), leaves and roots were collected. They were rinsed thoroughly under running tapwater to eliminate adhering soil and dirt, followed by rinsing with distilled water. The plant parts were subsequently oven-dried at 30-35°C for 1 day until a constant weight was achieved.

Equipments and glasswares

Various equipments such as, Autoclave, Laminar air flow, Hot air oven, Microwave oven, Bio-oxygen Demand (BOD) incubator, Microscope, Digital balance, Refrigerator and Water bath were used. Also, glasswares like Petri plates, Beakers, Graduated cylinder, Conical flasks, Volumetric flasks were utilized during the experiment.

Miscellaneous Materials

0.1% Sodium Hypochlorite (NaOCl), Ethanol, Micropipette, Potato Dextrose Agar (PDA) media, solvent such as Methanol, forceps, Mechanical blender, Falcon tubes etc., were used.

Methods

Isolation, identification and purification of phytopathogens

Fungal pathogens were isolated from infected plant parts such as leaves of mustard (*Brassica juncea*) and guava (*Psidium guajava*). Plant parts were collected from home garden, and washed to remove dirt and debris. The parts were then cut into small pieces containing infected as well as healthy part. The small pieces were then washed using 0.1% Sodium hypochlorite and distilled water and kept on tissue paper for complete drying of pieces. The pieces were then transferred to the PDA media in petri plates. The entire process was performed inside Laminar Air Flow with sterilized instruments. The plates were then sealed with parafilm and kept in BOD incubator at 25±2°C for 3 days. The plates were observed daily to check the growth of fungal pathogens.

To confirm the fungal pathogen, it was observed under microscope by preparing a slide. Various morphological and colony characteristics were studied to confirm the pathogen *Alternaria brassicae* and *Pestalotiopsis psidii*. After the growth of the fungal pathogen, purification was done to obtain a pure culture of both fungal pathogens i.e., *Alternaria brassicae* and *Pestalotiopsis psidii*. Purification of the fungal culture was done using hyphal tip method.

Plant Part Extract Extraction

Extraction method mentioned by Hamza *et al.*, (2006) [6] was utilized. Cold maceration was done by weighing dried plant part powder i.e., petals, leaves and roots. The powder was then placed in clean glass beaker. Methanol was added as solvent with the standard ratio of 1:10 (w/v). The beaker was sealed tightly by wrapping aluminum foil to prevent evaporation. The beaker was then stored in a cool, dark place to protect from light-induced degradation. The beaker was shaken vigorously 3-4 times a day to ensure effective solvent penetration. After 3- 4 days, the mixture was filtered through Whatman filter paper to obtain a clear liquid and collected in a small beaker. Later, the extract was subjected to evaporation for 2-2.5 hours at 40°C in water bath to obtain a desired volume of extract. The final extract was weighed and stored in a sealed, falcon tubes at 4°C for subsequent antifungal bioassays.



Fig 1: Marigold Plant parts extract

Anti-fungal bioassay

The antifungal activity was evaluated using the Poisoned Food Technique, which is standard for measuring mycelial inhibition in plant pathology (Vincent, 1947) [21]. A 10% stock solution of Tween20 (1 ml) and sterile distilled water (10 ml) was prepared to use as a surfactant for proper dispersion of plant extract.

Further to prepare stock solution of plant part extract of desired concentration i.e., 2.5%, 1.5 ml plant extract was added in 1.5 ml of tween20 stock solution and shaken vigorously to get a homogenous solution. Similarly, to prepare stock solution extract of 5%, 3 ml of extract was added in 3 ml of tween 20 stock solution. Then, the mixed content was added in PDA media along with small amount of Streptomycin, mixed and poured in petri plates. The plates were then left to solidify. After solidification, fungal discs were placed on surface of poisoned media plates and kept in BOD incubator at 25±2°C. The plates were then observed on 3rd, 6th and 9th day for their mycelia growth and the readings were recorded in millimeters (mm). Inhibition percentage of the mycelia growth was calculated using the formula proposed by Vincent, 1947 [21].

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

In which

- I = Percentage Inhibition of mycelial growth
- C = Colony Diameter in untreated control (mm)
- T = Colony Diameter in Treatment (mm)

Statistical Analysis

There were total 7 treatments with 3 replications for each of the fungal culture used. Completely Randomized Design (CRD) was used for the experiment (Gomez and Gomez, 1984). The experimental data was statistically analyzed using graph. The difference in the mean values among different treatment groups was determined by applying one-way ANOVA.

Results and Discussion

The entire set of experiments took place at the Uttaranchal University's Plant Pathology Laboratory within the School of Agriculture. The results and observations of the experiment were recorded here as under.

Isolation, Identification and Purification of fungal pathogens

After the isolation of fungal pathogens from the infected plant parts i.e., leaves of mustard and guava, and fungal cultures were obtained. Later, the fungal pathogens were characterized based on their microscopic traits. The isolated fungal pathogens were identified as *Alternaria brassicae* and *Pestalotiopsis psidii* which was done by making slide and observing the spores under the microscope. Various characters were observed and noted.



Fig 2: Fungal culture of *Alternaria brassicae*



Fig 3: Fungal culture of *Pestalotiopsis psidii*



Fig 4: Conidia of *Alternaria brassicae*

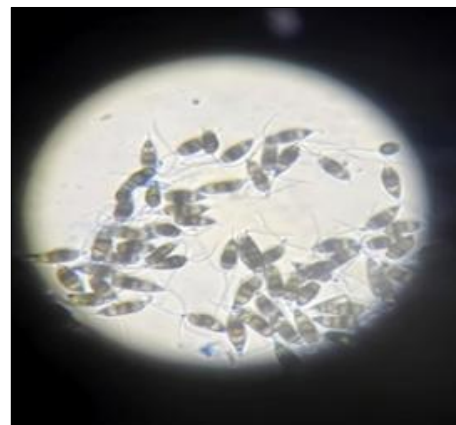


Fig 5: Conidia of *Pestalotiopsis psidii*

During identification of *Alternaria brassicae*, Rahimloo (2015) [10] recorded identical olivaceous-to-dark-brown colony margins for *Alternaria* species grown on standard agar media. The colony of the fungus was observed to be dark-olive to grayish which was quite similar to the findings of Singh *et al.*, (2022) [19]. In similar way, the causal organism of anthracnose of guava was identified as *Pestalotiopsis psidii* as described by Rahman *et al.* (2003) [11]. It was observed that the colony was white to off-white with cottony-velvety mycelium. The characteristics observed matches the classic diagnostic characters documented by Keith *et al.* (2006) [7] for *Pestalotiopsis* strains isolated from tropical fruit hosts.

Anti-fungal activity of Marigold plant part extract against *Alternaria brassicae* and *Pestalotiopsis psidii*

Various plant part extract of marigold (Flower i.e., petals, Leaf, and Root) at two different concentrations (2.5% and 5%) were used for evaluating the effect of anti-fungal activity. The mycelial growth was recorded at 3, 6, and 9 days after inoculation (DAI). The data revealed that all tested plant part extracts restricted the growth of the fungus significantly compared to untreated control (T_7) across all observation intervals.

On 3rd day the minimum radial growth of 6.33 mm was found in T_4 followed by 6.50 mm in T_2 and 6.67 mm in T_6 respectively. The maximum radial growth of mycelium 17.17 mm was recorded in T_7 against *Alternaria brassicae* as mentioned in Table 1 below references. In case of *Pestalotiopsis psidii*, the minimum mycelial growth 5.37 mm on 3rd day was witnessed in T_4 which was followed by growth of 5.83 mm in T_2 and of 6.50 mm in T_6 respectively. Also, the utmost radial growth of mycelia of 21.23 mm was observed in T_7 as shown in Table 2 below references.

In *Alternaria brassicae*, on 6th day the least radial growth of mycelium of 12.77 mm was observed in T₄ further followed by 13.40 mm in T₂ and 13.67 mm in T₆ respectively, and the highest radial mycelial growth of 43.57 mm was recorded in T₇ (control) as shown in Table 1 below references. While in *Pestalotiopsis psidii*, on the 6th day, the lowest radial growth of mycelium of 8.20 mm was observed in T₄ which was followed by 8.67 mm in T₂ and 9.67 mm in T₆ respectively and peak radial growth of mycelium of 41.63 mm was recorded in T₇ as mentioned in Table 2 below references.

Later, on the 9th day minimal radial mycelial growth 24.57 mm was noticed in T₄ followed by 24.80 mm in T₂ and 26.53

mm in T₆ respectively against *Alternaria brassicae* with utmost radial mycelial growth of 76.77 mm in T₇ (as mentioned in Table 1 below references). However, against *Pestalotiopsis psidii*, least radial growth 24.00 mm was recorded in T₄ in succession to 26.83 mm in T₂ and 28.00 mm in T₆ respectively, with the maximum radial growth of 74.5 mm recorded in T₇ on 9th day (as shown in Table 2 below references). Graphical representation of mycelial growth of *Alternaria brassicae* and *Pestalotiopsis psidii* is shown in Graph 1 and Graph 2 respectively below references.

a. *Alternaria brassicae* b. *Pestalotiopsis psidii*

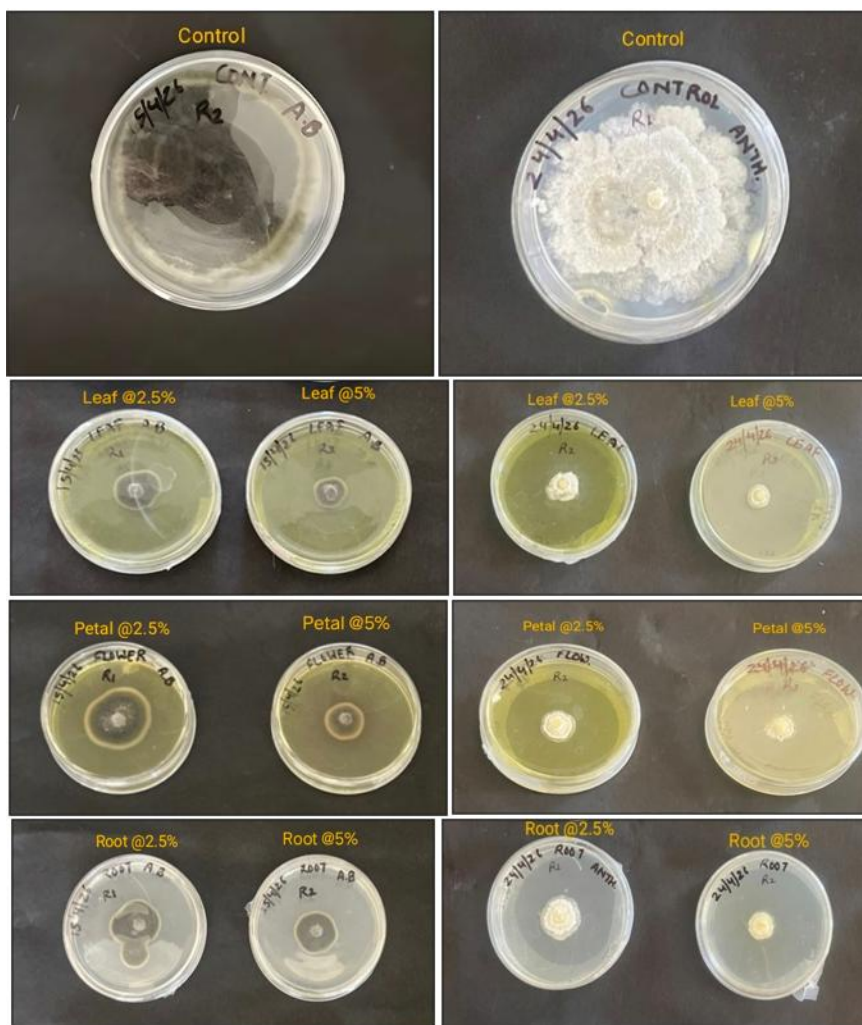


Fig 6: Inhibition zones observed in different treatments of marigold plant part extracts at two different concentrations (2.5% and 5%) along with control

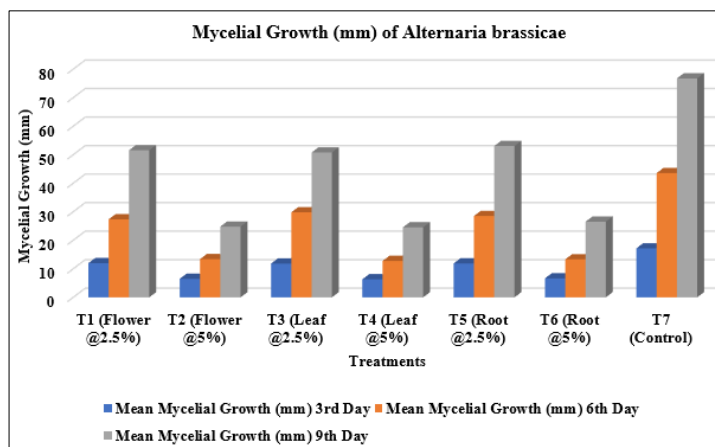
In this study leaf extract was found to be more effective against both fungal pathogens which were in alignment to the findings of Swer *et al.* (2023) [20] who assessed the antimicrobial properties of various *Tagetes patula* biological components. According to their research, leaf extracts have higher potency than Petals extracts against a number of fungal pathogens. They also explained this by pointing out that the vegetative covering of marigold plant had greater initial concentrations of some oxygenated monoterpenes and bioactive thiopenes.

Research on various botanical pesticides has recorded similar dose-dependent anti-fungal activity from various extracts from the Asteraceae family (Abood, 2021; Podgórska-

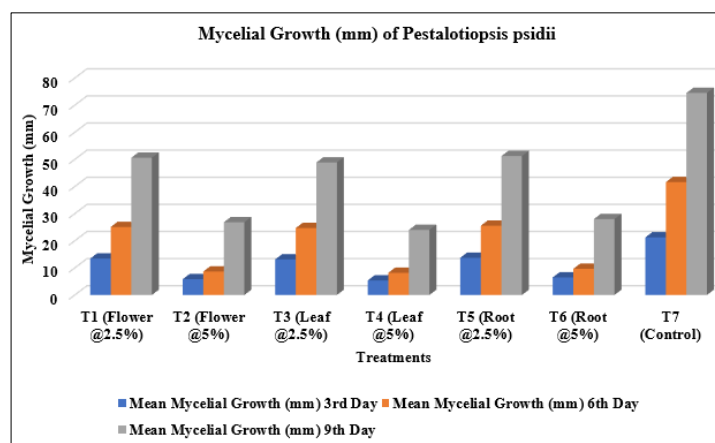
Kryszczuk and Pankiewicz, 2023). Romagnoli *et al.* (2025) [1, 9] examined *Tagetes* leaf essential oils' anti-fungal properties against plant pathogenic fungi. According to their characteristics, chemical originating from leaves significantly alters morphology of fungal hyphae, causing cell wall disintegration. This is one of the reasons for why leaf extract treatments performed better than other plant part extracts in this experiment. Also, according to our study the foliar extracts of Asteraceae species have been proven to offer protection against necrotrophic fungus such as *Alternaria* spp. which was in compliance with the findings of Ben Jabeur *et al.* (2021) [2].

Table 1: Anti-fungal activity of Marigold (*Tagetes erecta* L.) plant parts extract against *Alternaria brassicae* at different concentrations.

<i>Alternaria brassicae</i>				
Treatments	Mycelial Growth (mm)			Inhibition Percentage (%) at 9 th Days
	3 rd Day	6 th Day	9 th Day	
T1 (Petals @2.5%)	12.00	27.37	51.57	32.83
T2 (Petals @5%)	6.50	13.40	24.80	67.69
T3 (Leaf @2.5%)	11.83	29.83	50.80	33.83
T4 (Leaf @5%)	6.33	12.77	24.57	68.00
T5 (Root @2.5%)	11.90	28.53	53.07	30.87
T6 (Root @5%)	6.67	13.67	26.53	65.44
T7 (Control)	17.17	43.57	76.77	-
C.D.	1.35	0.54	0.64	-
S.E (M)	0.44	0.17	0.21	-
S.E (d)	0.62	0.25	0.29	-
C.V	7.41	1.28	0.83	-

**Graph 1:** Graphical Representation of Mycelial growth of *Alternaria brassicae***Table 2:** Anti-fungal activity of Marigold (*Tagetes erecta* L.) plant parts extract against *Pestalotiopsis psidii* at different concentrations.

Treatments	Mean Mycelial Growth (mm)			Inhibition Percentage (%)
	3 rd Day	6 th Day	9 th Day	
T1 (Petals @2.5%)	13.43	25.10	50.63	32.04
T2 (Petals @5%)	5.83	8.67	26.83	63.98
T3 (Leaf @2.5%)	13.13	24.70	48.87	34.41
T4 (Leaf @5%)	5.37	8.20	24.00	67.79
T5 (Root @2.5%)	13.73	25.57	51.23	31.23
T6 (Root @5%)	6.50	9.67	28.00	62.42
T7 (Control)	21.23	41.63	74.50	-
C.D.	2.01	3.71	5.50	-
S.E (M)	0.65	1.21	1.79	-
S.E (d)	0.93	1.71	2.54	-
C.V	10.07	10.24	7.16	-

**Graph 2:** Graphical Representation of Mycelial growth of *Pestalotiopsis psidii*

Conclusion

The present *in-vitro* study Assessment of Anti-Fungal Properties of Extracts Derived from Different Parts of *Tagetes erecta* L., specifically flower petals, leaves, and roots at two concentrations (2.5% and 5.0%) against two destructive phytopathogenic fungi: *Alternaria brassicae* (isolated from mustard) and *Pestalotiopsis psidii* (isolated from guava anthracnose) was entirely carried out in Plant Pathology Laboratory, School of Agriculture, Uttaranchal University. *Tagetes* genus is one of the most important plants which have some anti-microbial properties. For evaluating these properties for the study purpose, the isolation and morphological identification of the pathogens was done. Antifungal bioassays were performed using the Poisoned Food Method, and radial growth of mycelia was tracked at 3, 6, and 9 Days After Inoculation (DAI). The experimental data revealed a distinct concentration-dependent inhibitory trend across all treatments. Against *Alternaria brassicae*, at 9 DAI, the highest mycelial inhibition was achieved by T₄ (Leaf extract @5%) at 68.00% with radial growth of 24.56 mm which showed remarkable mycelial growth inhibition followed by T₂ (Petal extract @5%) at 67.69%. Similarly, against *Pestalotiopsis psidii* Leaf extract @5% outperformed other extracts by showing mycelial inhibition of about 67.79% further followed by Petal extract @5%.

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