***Original Research Article***

**Effect of botanical and bio-resources on management of Alternaria leaf spot disease in *Stevia rebaudiana* (Bertoni)**

**Abstract**

The experiments were conducted research laboratory of Department of Biological Sciences, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj during 2023-2024. Observations were recorded at screening of plant leaf extracts and their antimicrobial activity against *Alternaria* *alternata*, assayed of secondary metabolites and active compound from the selected 10 botanicals. Results obtained that *Azadirachta indica* was significantly inhibited the mycelial growth of *Alternaria alternata* over untreated control. The treatment T9 - *Azadirachta* *indica* was significantly recorded least mean colony diameter at 15% concentration (9.53, 12.93, 21.51 and 23.71 mm), at 20% concentration (8.56, 11.31, 16.16 and 20.11 mm), at 25% concentration (8.06, 9.78, 10.78 and 13.28 mm) at 48hr, 72, hr, 96 hr and 120 hr after incubation respectively. While, *Azadirachta indica* @ 15%, 20% and 25% concentration was found least radial growth and more % inhibition of *Alternaria* *alternata*. Results summarized that *Azadirachta indica* was recorded minimum mycelial radial growth and maximum % inhibition as compared with all the botanicals leaf extract at 48, 72, 96 and 120 hrs after incubation.

Key words: *Alternaria alternata*, bio-resources, botanical extracts, microalgae and *Stevia* *rebaudiana* (Bertoni)

1. **INTRODUCTION**

**Stevia rebaudiana,** commonly called Stevia, is among India's most promising medicinal plants, earning the nation the title “medicinal garden of the world” due to its rich therapeutic biodiversity. Of the more than 35 species of Stevia present, S. rebaudiana is especially valued for its natural sweetness and health-promoting qualities. It has demonstrated the ability to lower postprandial blood glucose in individuals with Type II diabetes (an 18% reduction in glucose response) and exert antihypertensive effects in adults with moderate hypertension . Clinical trials and meta-analyses support these benefits, showing improvements in fasting and post-meal glucose levels, though effects on insulin and HbA1c remain moderate or inconsistent **Zare *et al.,* (2024)*.*** Crucially, S. rebaudiana and its purified steviol glycosides (SGs) are considered safe for human consumption, with ADI guidelines set at 4 mg/kg body weight/day by JECFA, FDA, and EFSA

The surge in demand for natural, non-caloric sweeteners has led to regulatory approvals of S. rebaudiana-derived SGs by bodies like the FDA and EFSA. Consequently, the global stevia market was projected to reach around USD 781.6 million by 2023. This opportunity is driving genetic improvement research targeting SG biosynthesis to optimize yield and sweetness quality.

In India, cultivation of Stevia has expanded in states such as Maharashtra, Rajasthan, and West Bengal. However, cultivation is significantly hampered by plant pathogens—mainly fungi, along with bacteria, viruses, and nematodes. Chemical fungicides are the principal control strategy, but lingering chemical residues pose environmental risks and may compromise herbal product purity.

Among these diseases, **Alternaria leaf spot,** caused by Alternaria alternata, is the most devastating, severely compromising the leaves—the crop’s economically valuable part. Maiti *et al.,* (2007) reported first in 2007 in West Bengal, severe infections occur during February (20–25 °C). Initial symptoms are small circular, light-brown spots (2–18 mm), which later enlarge, becoming irregular or forming concentric rings; advanced infection leads to necrotic coalescing lesions **Anil *et al.,* (2023)**. *Microscopic* examination reveals characteristic branched, septate brownish mycelia, olive-brown conidiophores, and solitary or chained conidia.

In light of these challenges, biological control agents are gaining attention as safer, eco-friendly alternatives for disease management in medicinal plants like Stevia. Their use can help reduce reliance on chemical fungicides and support the production of cleaner, residue-free herbal products.

**2. Materials and Methods**

**2.1 Experimental site**

The laboratory experiment was conducted at the laboratory of the Department of Plant biological Sciences and lab experiment was carried out at the central research lab, Sam Higginbottom University of Agriculture and Sciences, Prayagraj.

**2.2 Isolation of *Alternaria alternata* from collected samples**

 The infected leaf showing typical symptoms of disease were used for the isolation of pathogen. The standard tissue isolation procedure was followed to isolate the pathogen. The infected parts were surface sterilized with 1:1000 mercuric chloride (HgCl2) solution for 60 seconds and washed separately in sterilized distilled water to remove the traces of mercury if any and then transferred to sterilized petri-plates containing potato dextrose agar (PDA). The Petri plates were incubated at room temperature (27 ± 1°C) and observed periodically for the growth of pure colonies **Gupta *et al*., (2010).** The pure colonies which developed from the bits were transferred to PDA slants and incubated at 27 ± 1°C for 5 – 7 days. Then such slants were used to study characters.

**2.3 Identification of the pathogen:**

 Morphological studies of the pathogen was conducted from pure culture. Spore suspension in sterilized distilled water is to be made from pure culture of the pathogen grown on PDA. One drop of the spore suspension is to be placed on a slide and morphological characters is to be noted with the help of microscope.

**2.4 Morphological characteristics of *Alternaria alternata***

 Conidiophores of A. alternata were simple, light brown, variable in length ranging from 17.10 to 61.56 μm and mostly 2- 3 septate rarely 4-5 septate. Conidia were found light to dark brown in colour, uniform with 0-2 longitudinal septa and 1-6 transverse septa, and variable in shape and size, mostly oval shape with rudimentary beak and in size measuring about 10.26-77.52 x 4.56-14.82 μm. Based on the morphological characters, the organism was identified as *Alternaria alternata* (**Poonam *et al*., 2020**).

**2.5 Poisoned food technique**

In poison food technique the following treatments were used:

T0-Control, T1*-Zingiber officinale,* T2*-Phyllanthus emblica,* T3*-Eucalyptus globulus,* T4*-Moringa oleifera,* T5*-Allium sativa,* T6*-Mentha piperita,* T7*-Catharanthus roseus,* T8*-Citrus limon,* T9*-Azadirachta indica* andT10*-Calotropis procera.*

Five mm diameter of culture disc of *Alternaria alternata* was kept at the center of each Petri plate containing the essential oil of required concentrations dissolved in PDA media. Three replications were maintained. The plates will be incubated at 27°C for two days and colony diameter was recorded. Percent mycelia growth inhibition of test fungus over untreated control was calculated by using the formula.

 (C-T)

Percent Inhibition(I)= ×100

 C

Where,

C = Growth (mm) of the test fungus in untreated control plate.

T = Growth (mm) of the test fungus in treated control.

**3. Results and Discussion**

**3.1 *In vitro* efficacy of botanical extracts at 15 % concentration on mycelial growth of *Alternaria alternata*:**

Data presented in Table1, shows that all the selected plant extracts at 15% concentration was significantly reduced the growth of *Alternaria alternata* at 48, 72, 96 and 120 hrs after incubation.

At 48 hrs after incubation, results shows that treatment T9 - *Azadirachta indica* was significantly recorded least mean colony diameter (9.53 mm) as compared with T5 – *Allium sativa* (10.93 mm), T6 – *Mentha piperita* (12.96 mm), T3 – *Eucalyptus globulus* (14.66 mm), T1 – *Zingiber officinale* (16.51 mm), T7 – *Catharanthus roseus* (17.88 mm), T4 – Moringa oleifera (19.08 mm), T2 – *Phyllanthus emblica* (19.51 mm), T8 – Citrus limon (19.31 mm), including with T0 - control (21.86 mm). Among plant extracts, the treatments *Azadirachta indica* and *Eucalyptus globules* were recorded significantly maximum % inhibition of mycelial growth followed by other botanicals treatments. Whereas, treatments (T4, and T8), (T4, and T2) and (T8 and T2) were found non-significant among themselves but they are significant from each other. While, control petri plates were maximum mycelial growth of *A. alternata*.

At 72 hrs after incubation, results shows that treatment T9 - *Azadirachta indica* was significantly recorded least mean colony diameter (12.93 mm) as compared with T5 – *Allium sativa* (14.68 mm), T6 – *Mentha piperita* (17.96 mm), T3 – *Eucalyptus globulus* (21.53 mm), T1 – *Zingiber officinale* (27.68 mm), T7 – *Catharanthus roseus* (33.06 mm), T4 – *Moringa oleifera* (34.41 mm), T2 – *Phyllanthus emblica* (38.01 mm), T8 – Citrus limon (37.43 mm), including with T0 -control (47.86 mm). Among all the botanicals, *Azadirachta indica* was observed significant highest inhibition of growth (12.93) as compared with T9, T5, T6, T3, T1, T7, T4, T8 and T2. These botanicals are significant in results from T9, T5 and T2. Whereas, treatments (T8, T2) were found non-significant with each other.

At 96 hrs after incubation, results shows that treatment T9 - *Azadirachta indica* was significantly recorded least mean colony diameter (21.51 mm) as compared with T5 – *Allium sativa* (23.73 mm), T6 – *Mentha piperita* (26.13 mm), T3 – *Eucalyptus globulus* (23.78 mm), T1 – *Zingiber officinale* (35.48 mm), T7 – *Catharanthus roseus* (35.53 mm), T4 – *Moringa oleifera* (43.26 mm), T8 – Citrus limon (50.61 mm), T2 – *Phyllanthus emblica* (51.26 mm), including with T0 -control (74.06 mm). Among the treatment *Azadirachta indica* (21.51), *Allium sativa* (23.73) and *Eucalyptus globulus* (23.78) significantly reduced the growth of *Alternaria alternata* from other leaf extracts. Whereas, treatment (T5, T3), (T1, T7) and (T8, T2) were shows non-significant among themselves but they are significant to each other. While, treatments T8, T6, T4 and T5 were found significant from each other.

At 120 hrs after incubation, results shows that treatment T9 - *Azadirachta indica* was significantly recorded least mean colony diameter (23.71 mm) as compared with T5 – *Allium sativa* (24.58 mm), T3 – *Eucalyptus globulus* (25.93 mm), T6 – *Mentha piperita* (27.91 mm), T1 – *Zingiber officinale* (38.66 mm), T7 – *Catharanthus roseus* (38.93 mm), T4 – *Moringa oleifera* (57.58 mm), T8 – Citrus limon (63.16 mm), T2 – *Phyllanthus emblica* (65.71 mm), including with T0 -control (90.89 mm). Among the treatment *Azadirachta indica* (23.71 mm) and *Allium sativa* (24.58) were showed least mycelium growth of *Alternaria alternata* followed by all the botanicals including with over control (90.00). However, treatments (T9, T5), (T5, T3), and (T1, T7) were found non- significant among themselves but they are significant to each other.

**3.2 *In vitro* efficacy of botanical extracts at 20% concentration on mycelial growth of *Alternaria alternata*:**

Data are presented in the Table 2, results shows that all the aqueous plant extracts at 20% concentration was significantly reduced the growth of *Alternaria alternata* at 48, 72, 96 and 120 hrs after incubation.

At 48 hrs after incubation, results shows that treatment T9 - *Azadirachta indica* was significantly recorded least mean colony diameter (8.56 mm) as compared with T5 – *Allium sativa* (8.91 mm), T6 – *Mentha piperita* (9.8 mm), T3 – *Eucalyptus globulus* (10.42 mm), T1 – *Zingiber officinale* (10.39 mm), T7 – *Catharanthus roseus* (12.01 mm), T4 – Moringa oleifera (12.61 mm), T2 – *Phyllanthus emblica* (13.43 mm), T8 – Citrus limon (13.91 mm), including with T0 - control (18.63 mm). Among plant extracts, the treatments *Azadirachta indica* and *Allium sativa* were recorded significantly maximum % inhibition of mycelial growth followed by other botanicals treatments. Whereas, treatments (T9 and T5), (T6 and T1) and (T6, T3 and T1), (T7. and T4), (T4.T2 and T8) were found non-significant among themselves but they are significant from each other.

At 72 hrs after incubation, results shows that treatment T9 - *Azadirachta indica* was significantly recorded least mean colony diameter (11.31 mm) as compared with T5 – *Allium sativa* (12.01 mm), T6 – *Mentha piperita* (13.08 mm), T3 – *Eucalyptus globulus* (11.86 mm), T1 – *Zingiber officinale* (13.26 mm), T7 – *Catharanthus roseus* (15.76 mm), T4 – Moringa oleifera (17.48 mm), T2 – *Phyllanthus emblica* (15.68 mm), T8 – Citrus limon (21.88 mm), including with T0 - control (47.76 mm). Among all the botanicals, *Azadirachta indica* was observed significant highest inhibition of growth as compared with T8, T3, T10, T1, T6, T9 and T4. These botanicals are significant in results from T5, T8 and T4. Whereas, treatments (T9, T3 and T5) and (T3, T5), (T5, T6) and (T2, T7) were found non-significant among themselves.

At 96 hrs after incubation, results shows that treatment T5 - *Allium sativa* was significantly recorded least mean colony diameter (15.16 mm) as compared with T9 – *Azadirachta indica* (16.16 mm), T1 – *Zingiber officinale* (17.20 mm), T6 – *Mentha piperita* (17.25 mm), T3 – *Eucalyptus globulus* (17.78 mm), T2 – *Phyllanthus emblica* (19.61 mm), T7 – *Catharanthus roseus* (23.71 mm), T4 – Moringa oleifera (25.13 mm), T8 – Citrus limon (32.13 mm), including with T0 - control (75.63 mm). Among the treatments *Allium sativa* (15.16), *Azadirachta indica* (16.16) and *Mentha piperita* (17.23) significantly reduced the growth of *Alternaria alternata* from other leaf extracts. Whereas, treatment (T5, and T9,), (T9, T1, and T6) and (T1, T6 and T3) (T6, and T3,), were shows non-significant among themselves but they are significant to each other. While, treatments T3, T2, T7, T4 and T8 were found significant from each other.

At 120 hrs after incubation, results shows that treatment T5 - *Allium sativa* was significantly recorded least mean colony diameter (19.68 mm) as compared with T9 – *Azadirachta indica* (20.11 mm), T3 – *Eucalyptus globulus* (21.46 mm), T6 – *Mentha piperita* (21.76 mm), T1 – *Zingiber officinale* (21.86 mm), T2 – *Phyllanthus emblica* (24.11 mm), T7 – *Catharanthus roseus* (26.86 mm), T4 – Moringa oleifera (30.18 mm), T8 – Citrus limon (39.21 mm), including with T0 - control (90.89 mm). Among the treatments *Azadirachta indica* (19.68mm) and *Allium sativa* (20.11) were showed least mycelium growth of *Alternaria alternata* followed by all the botanicals including with over control (90.89). However, treatments (T5, T9), (T9, T3), (T3, T6, T1) and (T6, T1) were found non- significant among themselves but they are significant to each other.

**3.3 *In vitro* efficacy of botanical extracts at 25% concentration on mycelial growth of *Alternaria alternata*:**

Data are presented in the Table 3, results shows that all the aqueous plant extracts at 25% concentration was significantly reduced the growth of *Alternaria alternata* at 48, 72, 96 and 120 hrs after incubation.

At 48 hrs after incubation, results shows that treatment T9 - *Azadirachta indica* was significantly recorded least mean colony diameter (8.06 mm) as compared with T5 – *Allium sativa* (9.16 mm), T6 – *Mentha piperita* (11.21 mm), T3 – *Eucalyptus globulus* (12.56 mm), T1 – *Zingiber officinale* (15.13 mm), T7 – *Catharanthus roseus* (15.63 mm), T4 – Moringa oleifera (16.18 mm), T2 – *Phyllanthus emblica* (16.51 mm), T8 – Citrus limon (16.63 mm), including with T0 - control (23.18 mm). Among plant extracts, the treatments *Azadirachta indica* and Eucalyptus globules were recorded significantly maximum % inhibition of mycelial growth followed by other botanicals treatments. Whereas, treatments (T4, and T8), (T4, and T2) and (T8 and T2) were found non-significant among themselves but they are significant from each other.

At 72 hrs after incubation, results shows that treatment T9 - *Azadirachta indica* was significantly recorded least mean colony diameter (9.78 mm) as compared with T5 – *Allium sativa* (11.43 mm), T6 – *Mentha piperita* (13.21 mm), T3 – *Eucalyptus globulus* (14.66 mm), T1 – *Zingiber officinale* (17.06 mm), T7 – *Catharanthus roseus* (17.88 mm), T4 – Moringa oleifera (19.08 mm), T8 – Citrus limon (19.31 mm), T2 – *Phyllanthus emblica* (19.51 mm), including with T0 - control (47.71 mm). Among the treatments *Azadirachta indica* was observed significant highest inhibition of growth (76.32) as compared with T9, T5, T6, T3, T1, T7, T4, T8 andT2. These botanicals are significant in results from T9, T3, T7 and T2. Whereas, treatments (T4, T8 andT2) and (T8, T2) were found non-significant among themselves.

At 96 hrs after incubation, results shows that treatment T9 - *Azadirachta indica* was significantly recorded least mean colony diameter (10.78 mm) as compared with T5 – *Allium sativa* (12.23 mm), T6 – *Mentha piperita* (14.18 mm), T3 – *Eucalyptus globulus* (16.16 mm), T1 – *Zingiber officinale* (17.56 mm), T7 – *Catharanthus roseus* (18.88 mm), T4 – Moringa oleifera (19.98 mm), T2 – *Phyllanthus emblica* (20.26 mm), T8 – Citrus limon (20.56 mm), including with T0 - control (72.01 mm). Among the treatments *Azadirachta indica* treatment (10.78mm) followed by all other botanical leaf extracts. Aqueous leaf extract of neem was showed minimum mycelium growth and maximum inhibition as compared with rest botanical treatments. *Allium sativa* (12.23), *Mentha piperita* (14.18) and (16.16) significantly reduced the growth of *Alternaria alternata* from other leaf extracts. Whereas, treatment (T7, T7), (T4, T2) and (T4, T2, T8) and (T2, T8), were shows non-significant among themselves but they are significant to each other.

At 120 hrs after incubation, results shows that treatment T9 - *Azadirachta indica* was significantly recorded least mean colony diameter (13.28 mm) as compared with T5 – *Allium sativa* (14.18 mm), T6 – *Mentha piperita* (15.98 mm), T3 – *Eucalyptus globulus* (17.31 mm), T1 – *Zingiber officinale* (18.33 mm), T7 – *Catharanthus roseus* (18.88 mm), T4 – Moringa oleifera (19.96 mm), T2 – *Phyllanthus emblica* (21.06 mm), T8 – Citrus limon (22.56 mm), including with T0 - control (90.86 mm). Among the treatments *Azadirachta indica* (13.28mm) and *Allium sativa* (14.18) were showed least mycelium growth of *Alternaria alternata* followed by all the botanicals including with over control (90.86). However, treatments (T9, T5), (T5, T6), (T6, T3), (T3, T1, T7) and (T2, T8) were found non- significant among themselves but they are significant to each other.

One of the most effective measures to control the disease caused by *Alternaria* is the effective application of fungicides. The arbitrary usage of the common fungicides has made the man and wild life susceptible to a wide array of diseases. The use of various herbal extracts and natural products is being encouraged because these cause no health hazard or pollution. The natural plant products are bio-degradable and thus eco-friendly, are catching the concentration of the scientists worldwide. Our results showed that leaf extracts of *Azadirachta indica* and *Eucalyptus globules* are found effective in the management of leaf spot disease. Similar finding results have reported by **Sharma *et al.* (2007).** That is why, because plants contain various kinds of phytochemicals like, Saponins, Alkaloids, Flavanoids *etc.,* (commonly called secondary metabolites) are bringing the antimicrobial effects. In the current study it is well proved that all most plants used in the study have at least little effect in controlling the fungus. Yet, it is clearly shown that except neem and eucalyptus no other extracts have the similar effect of chemical fungicide (positive controls).

This finding is also supported by **Hassanein *et al.* (2008)** reported that the neem leaf extract showed maximum inhibition percentages were 17.88%, 23.66, 52.77 % and 70.55% for *A. solani* at 5%, 10%, 15% and 20 % concentrations, while those for *F. oxysporum* were 14.77 %, 23.88%,

31.22 % and 100%, respectively.

Sheikh and Agnihotri (1972) were among the first to show that a wide range of plant extracts—such as those from Canna indica, Convolvulus arvensis, Ipomoea palmata, Cenchrus catharticus, Mentha piperita, Prosopis spicigera, Allium cepa, A. sativum, Lawsonia inermis, Argemone mexicana, Datura stramonium, and Clerodendron inerme—were highly effective in completely inhibiting the spore germination of Alternaria brassicae, a major pathogen of cauliflower. They also noted that the antifungal effect became stronger as the concentration of these extracts increased.

**Deshmukh *et al.* (2020)** reported similar findings showed that aqueous extracts of garlic (Allium sativum) and Datura stramonium significantly reduced both the radial growth and spore germination of A. brassicae, with more pronounced effects observed at higher extract concentrations.

Other recent research supports the antifungal potential of common plants like garlic and onion. For example, Allium sativum and Allium cepa have been shown to possess strong, broad-spectrum antifungal properties, including against species like Candida, Malassezia, and various dermatophytes. Studies using agar dilution methods have demonstrated complete inhibition of fungal growth at certain concentrations **Sharma *et al.* (2007).** Furthermore, garlic extract in particular has been found to be more effective than both onion and mint when tested against Alternaria species **Kumar *et al.* (2018).**

Datura stramonium, traditionally known for its antibacterial and acaricidal activity, has also shown promising antifungal effects. Its leaf extracts have been tested successfully against pathogens such as Alternaria, Fusarium, Aspergillus, and Candida, with results consistently indicating that higher concentrations lead to stronger antifungal activity **Gul *et al.* (2012).**

Peppermint (Mentha piperita) also continues to stand out for its antifungal properties. **Yasmeen and Saxena (1990)** observed significant fungitoxic effects of peppermint leaf extracts against A. brassicae, and more recent studies have confirmed its ability to inhibit fungal spore germination across various Alternaria species.

**Table 1 *In vitro* efficacy of botanical extracts at 15 % concentration on mycelial growth of *Alternaria alternata* at different hrs of interval**

|  |  |
| --- | --- |
| Treatments | **Mycelial radial growth (mm)** |
| **48 hrs** | **%****Inhibition over control** | **72 hrs** | **%****Inhibition over control** | **96 hrs** | **%****Inhibition over control** | **120 hrs** | **%****Inhibition over control** |
| **T1** | ***Zingiber officinale***  | 16.510**c** | 24.47 | 27.68 e | 42.16 | 35.48 d | 52.09 | 38.66 e | 57.47 |
| **T2** | ***Phyllanthus emblica*** | 19.510**a** | 10.75 | 38.01 b | 20.58 | 51.26 b | 30.79 | 65.71 b | 27.70 |
| **T3** | ***Eucalyptus globulus*** | 14.660**d** | 32.94 | 21.53 f | 55.01 | 23.78 f | 67.89 | 25.93 g | 71.47 |
| **T4** | ***Moringa oleifera*** | 19.080**a** | 12.72 | 34.41 c | 28.10 | 43.26 c | 41.59 | 57.58 d | 36.65 |
| **T5** | ***Allium sativa***  | 10.930**f** | 50.00 | 14.68 h | 69.33 | 23.73 f | 67.96 | 24.58 gh | 72.96 |
| **T6** | ***Mentha piperita***  | 12.960**e** | 40.71 | 17.96 g | 62.47 | 26.13 e | 64.72 | 27.91 f | 69.29 |
| **T7** | ***Catharanthus roseus*** | 17.880**b** | 18.21 | 33.06 d | 30.92 | 35.53 d | 52.03 | 38.93 e | 57.17 |
| **T8** | ***Citrus limon***  | 19.310**a** | 11.67 | 37.43 b | 21.79 | 50.61 b | 31.66 | 63.16 c | 30.51 |
| **T9** | ***Azadirachta indica*** | 9.530**g** | 56.40 | 12.93 i | 72.98 | 21.51 g | 70.96 | 23.71 h | 73.91 |
| **T0** | **Control** | 21.86 | - | 47.86 a | - | 74.06 a | - | 90.89 a | - |
|  | F-test | S |  | S |  | S |  | S |  |
|  | S. Ed (+) | 0.398 |  | 0.578 |  | 0.653 |  | 0.725 |  |
|  | CD (P = 0.05) | 0.831 |  | 1.206 |  | 1.363 |  | 1.512 |  |

**Table 2 *In vitro* efficacy of botanical extracts at 20% concentration on mycelial growth of *Alternaria alternata* at different hrs of interval.**

|  |  |
| --- | --- |
| **Treatments** | **Mycelial radial growth (mm)** |
| **48 hrs** | **%****Inhibition over control** | **72 hrs** | **%****Inhibition over control** | **96 hrs** | **%****Inhibition over control** | **120 hrs** | **%****Inhibition over control** |
| **T1** | ***Zingiber officinale***  | 10.39 e | 44.18 | 13.26 e | 72.24 | 17.20 e | 77.25 | 21.86 f | 75.95 |
| **T2** | ***Phyllanthus emblica*** | 13.43 bc | 27.91 | 15.68 d | 67.17 | 19.61 d | 74.07 | 24.11 e | 73.47 |
| **T3** | ***Eucalyptus globulus*** | 10.42 e | 44.11 | 11.86 ef | 75.17 | 17.78 e | 76.49 | 21.46 fg | 76.39 |
| **T4** | ***Moringa oleifera*** | 12.61 cd | 32.31 | 17.48 c | 63.40 | 25.13 c | 66.77 | 30.18 c | 66.80 |
| **T5** | ***Allium sativa***  | 8.91 fg | 52.17 | 12.01 ef | 74.85 | 15.16 f | 79.96 | 19.68 g | 78.35 |
| **T6** | ***Mentha piperita***  | 9.8 ef | 47.34 | 13.08 e | 72.61 | 17.25 e | 77.20 | 21.73 f | 76.09 |
| **T7** | ***Catharanthus roseus*** | 12.01 d | 35.53 | 15.76 d | 67.00 | 23.71 c | 68.65 | 26.86 d | 70.45 |
| **T8** | ***Citrus limon***  | 13.91 b | 25.34 | 21.88 b | 54.19 | 32.13 b | 57.52 | 39.21 b | 56.86 |
| **T9** | ***Azadirachta indica*** | 8.56 g | 54.05 | 11.31 f | 76.32 | 16.16 ef | 78.63 | 20.11 fg | 77.87 |
| **T0** | **Control** | 18.63 a | - | 47.76 a | - | 75.63 a | - | 90.89 a | - |
|  | F-test | S |  | S |  | S |  | S |  |
|  | S. Ed (+) | 0.506 |  | 0.650 |  | 0.768 |  | 0.800 |  |
|  | CD (P = 0.05) | 1.056 |  | 1.356 |  | 1.601 |  | 1.669 |  |

**Table 3 *In vitro* efficacy of botanical extracts at 25 % concentration on mycelial growth of *Alternaria alternata* at different hrs of interval**

|  |  |
| --- | --- |
| **Treatments** | **Mycelial radial growth (mm)** |
| **48 hrs** | **%****Inhibition over****control** | **72 hrs** | **%****Inhibition over****control** | **96 hrs** | **%****Inhibition over****control** | **120 hrs** | **%****Inhibition over****control** |
| **T1** | ***Zingiber officinale***  | 15.13 b | 34.73 | 17.06 c | 64.24 | 17.56 d | 75.61 | 18.33 de | 79.83 |
| **T2** | ***Phyllanthus emblica*** | 16.51 b | 28.77 | 19.51 b | 59.11 | 20.26 b | 71.87 | 21.06 bc | 76.82 |
| **T3** | ***Eucalyptus globulus*** | 12.56 c | 45.82 | 14.66 d | 69.27 | 16.16 e | 77.56 | 17.31 ef | 80.95 |
| **T4** | ***Moringa oleifera*** | 16.18 b | 30.20 | 19.08 b | 60.01 | 19.98 bc | 72.25 | 19.96 cd | 78.03 |
| **T5** | ***Allium sativa***  | 9.16 d | 60.48 | 11.43 f | 76.04 | 12.23 g | 83.02 | 14.18 gh | 84.39 |
| **T6** | ***Mentha piperita***  | 11.21 c | 51.64 | 13.21 e | 72.31 | 14.18 f | 80.31 | 15.98 fg | 82.41 |
| **T7** | ***Catharanthus roseus)*** | 15.63 b | 32.57 | 17.88 c | 62.52 | 18.88 c | 73.78 | 18.88 de | 79.22 |
| **T8** | ***Citrus limon***  | 16.63 b | 28.26 | 19.31 b | 59.53 | 20.56 b | 71.45 | 22.56 b | 75.17 |
| **T9** | ***Azadirachta indica*** | 8.06 d | 65.23 | 9.78 g | 79.50 | 10.78 h | 85.03 | 13.28 h | 85.38 |
| **T0** | **Control** | 23.18 a | - | 47.71 a | - | 72.01 a | - | 90.86 a | - |
|  | F-test | S |  | S |  | S |  | S |  |
|  | S. Ed (+) | 0.647 |  | 0.382 |  | 0.539 |  | 0.865 |  |
|  | CD (P = 0.05) | 1.349 |  | 0.797 |  | 1.124 |  | 1.805 |  |

**Conclusion**

Based on the results obtained, Azadirachta indica (neem) demonstrated the most effective antifungal activity against Alternaria alternata in the food poisoning technique at concentrations of 15%, 20%, and 25%. Among all the botanical extracts tested, neem leaf extract and neem oil exhibited the smallest mean colony diameter and the highest percentage of fungal growth inhibition, indicating their superior efficacy.

**DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

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

**References**

1. Anil, I. A., & Reddy, Y. N. Kamatham Neeharika and Savitha, M. (2023). Fungal bioagents and botanicals efficacy against *alternaria alternata* responsable for leaf blight disease of *stevia rebaudiana.* [*International Journal of Plant & Soil Science*](https://journalijpss.com/index.php/IJPSS/index)*. 35*(22), 254-260.

# Deshmukh, H. V., Deokar, C. D., Khaire, P. B., & Brahmane, P. R. (2020). Efficacy of different botanicals against the *alternaria solani* under *in-vitro* conditions. *Journal of Pharmacognosy and Phytochemistry*, *9*(6), 1986-1989.

# Gul, H., Qaisrani, R. N., Khan, M. A., Hassan, S., & Younis, N. (2012). Antibacterial and antifungal activity of different extracts of *datura stramonium* (branches and leaves sample). *Journal of Biotechnology and Pharmaceutical Research*, *3*(9), 141-148.

# Gupta Vikas,  Kumar Deepak, . Razdan V. K (2010). Occurrence of root rot of stevia caused by *Sclerotium rolfsii* in Jammu and Kashmir, India*.  Plant Disease Research (Ludhiana)*, 2012, Vol. 27, No. 1, 97-98 ref. 7.

1. Hassanein, N. M., Abou Zeid, M. A., Youssef, K. A. and Mahmoud, D. A. (2008). Efficacy of leaf extracts of neem (*azadirachta indica*) and chinaberry (*melia azedrach*) against early blight and wilt diseases of tomato. *Australian Journal of Basic and Applied Sciences*, 2(3): 763-772.Lelliott and Stead, 1987

# Kumar, S. P., Mishra, M. K., & Mishra, P. R. (2018). *In vitro* efficacy of botanicals and biocontrol agents against fusarium leaf blight of tomato. *JEZS*, *6*(5), 2415-2418.

1. Maiti, C. K., Sen, S., Acharya, R., & Acharya, K. (2007). First report of *alternaria alternata* causing leaf spot on *stevia rebaudiana*. *Plant Pathology*, *56*(4), 723.
2. Poonam, K., Amit, T., Sakshi, M., Akansha, D. and Shivam, M. (2020). Identification of *alternaria alternata. International Journal of Current Microbiology and Applied Sciences,* 9(2): 1011-1015.
3. Pryor, B. M. and Michailides, T. J. 2002. Morphological, pathogenic, and molecular characterization of alternaria isolates associated with alternaria late blight of pistachio. *Phytopathology*.;92:406–416.

# Ramezani, H., & Abdollahi, M. (2015). Management of Alternaria brassicae through some plants extract. *International Journal of Pure and Applied Biosciencs*, *3*(2), 108-112.

1. Sharma, A., Dass, A. and Pau, M. S. (2007). Antifungal effect of neem extract on some common phytopathogenic fungi. *Advances of Plant Sciences*, *20*(2): 357-358.
2. Sheikh, R. A. and Agnihotri, J. P. (1972). Antifungal properties of some plant extracts. Indian *Journal of Mycology and Plant Pathology*, 2: 143-146.
3. Simmons EG. 1999; Alternaria themes and variations (236-243). *Mycotaxon*. 70: 325–369.
4. Yasmeen and Saxena, S. K. (1990). Effect of fern extracts on growth and germination of fungi. *Current Science*, 15: 798-799.
5. Zare, M., Zeinalabedini, M., Koujan, S. E., Bellissimo, N., & Azadbakht, L. (2024). Effect of stevia on blood glucose and HbA1C: A meta-analysis. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*, 103092.