**Assessment of botanical cakes in managing *Alternaria* *brassicae* in Broccoli leaf spot disease *in-vivo***

**ABSTRACT**

In India, Broccoli (*Brassica oleracea* var. *italica* L.) is a prominent vegetable crop cultivated during the *Rabi* season. A prevalent pathogen affecting broccoli is *Alternaria brassicae*, which causes Alternaria blight and has a global distribution. To mitigate the adverse effects of this disease on crop health, an effort has been made to assess the efficacy of specific botanical cakes and fungicide. The present study aimed to evaluate the efficacy of botanical oilcakes in managing *Alternaria brassicae*, the causal agent of leaf spot disease in broccoli (*Brassica oleracea* var. *italica* L.), under field conditions during the 2022–23 *Rabi* season at Sam Higginbottom University, Prayagraj. Eight treatments, including three botanical oilcakes (Neem, Mustard, and Groundnut cakes), their combinations, and a chemical control (Bavistin @ 0.2%), were tested against a control (untreated). Results showed that all treatments significantly reduced disease intensity and improved growth and yield parameters compared to the control. The best botanical treatment, Mustard cake @ 400 kg/ha (T2), reduced disease intensity by 52.38% (from 40.6% to 19.33% at 75 DAT), and increased plant height by 18.65% (from 27.13 cm to 32.2 cm), head weight by 82.76% (from 138.8 g to 253.7 g), and head diameter by 49.07% (from 9.73 cm to 14.5 cm), compared to the untreated control. Although Bavistin (T7) was most effective overall, the use of mustard cake demonstrated considerable potential as a sustainable and eco-friendly alternative to chemical fungicides. This organic approach offers a viable option for integrated disease management in broccoli cultivation. Further multi-season and multi-location trials are recommended to validate these findings for broader applicability

**Keywords*:*** *Alternaria brassicae*, broccoli, botanical cakes, leaf spot disease.

**INTRODUCTION**

Broccoli (*Brassica oleracea* var. *italica* L.) originates from the Mediterranean region, specifically evolving from wild cabbage. This wild cabbage is believed to have originated thousands of years ago along the northern and western Mediterranean coastlines, where it was first domesticated **(Cai *et al.*, 2022)**.

Broccoli is primarily composed of 89% water, along with 6.27% carbohydrates, 2.57% protein, 0.34% lipids, and 0.83% ash, while the remaining 1% includes essential vitamins, minerals, and bioactive compounds. Its high fibre content and nutrient density contribute to its health-promoting properties **(Nagraj *et al*., 2020).**

There are many diseases which affect the yield of broccoli both in terms of quality and quantity such as damping-off caused by *Pythium* spp. **(Stanghellini *et al.,* 2014),** downy mildew caused by *Peronospora parasitica* **(Singh *et al.,* 2025)**, black rot caused by *Xanthomonas campestris* **(Nagai *et al.,* 2017)**, black leg caused by *Phoma lingam* **(Sultana and Hossain*,* 2021)**, wire stem by fungus *Rhizoctonia solani* **(Jagadeesh *et al.,* 2022)**, club root *Plasmodiophora brassicae* **(Khalid *et al.,* 2022)**, sclerotinia stem rot *Sclerotinia sclerotiorum* **(Jiang *et al.,* 2019)**, powdery mildew *Erysiphe cruciferarum* **(Borges *et al.,* 2023)** and Alternaria leaf spot caused by *Alternaria brassicae* (Berk) Sacc. **(LAfi *et al.*, 2022)**.

**Berkely (1836)** noticed the fungal infection on plant belonging to the family Brassicaceae and identified as *Macrosporium brassicae* Berk. later renamed as *A. brassicae* (Berk.) Sacc. by **Saccardo (1886)**.

Alternaria is a genus of Deuteromycetes fungi known for causing significant agricultural spoilage and losses, estimated at 20% to 80% of yield. It has approximately 300 known species, divided into 26 sections and cause diseases in economically important crops, including broccoli **(Woudenberg *et al.,* 2015; Wang *et al.,* 2022)**.

*Alternaria brassicae* and *Alternaria brassicola* are two species that can infect commercial broccoli crops, posing significant challenges **(Saharan *et al.*, 2016; Ahammad *et al.,* 2024)**. In recent years, these diseases have become increasingly problematic in vegetable brassica crops. Historical data indicates that *A. brassicola* can cause yield losses of up to 70% in vegetable brassicas, while *A. brassicae* can lead to losses exceeding 50% in oilseed rape crops. Effective management strategies are crucial to mitigate these losses**(Doklega and El-hady, 2017)**.

Recently, leaf spot disease in broccoli has emerged as a significant issue, resulting in substantial yield losses ranging from 23% to 30% in India **(Ingale, 2013)**. Similarly, other cruciferous crops such as cauliflower and rapeseed-mustard have also been affected, with reported yield losses of up to 30% **(Sharma *et al.*, 2024)** and 35-60% **(Harde and Atar, 2014)** respectively. This highlights the need for effective disease management strategies to mitigate these losses **(Chand and Singh, 2005)**.

The application of organic amendments such as farm yard manure (FYM), poultry manure, and neem cake has been shown to reduce the incidence of Alternaria leaf spot and boost broccoli yields **(Kumar *et al.*, 2024)**. Notably, poultry manure and FYM significantly enhanced plant growth and yield. These organic manures contribute to improved soil productivity, structure, and nutrient content, ultimately supporting sustainable crop production practices **(Nuka and Veenapani, 2011)**.

Among the oils and oil cakes Eucalyptus oil (2%) and Mahua cake extract (10%) were found superior in reducing the fungal growth of *Alternaria alternata* in Aloe vera **(Kannan, 2016)**.

Efficacy of nine phytoextracts and four oil cakes against the pathogen *Alternaria solani*, causing early blight of tomatoes. Among the phytoextracts, Azadirachta indica at 30% concentration was most effective and inhibited mycelial growth by 74.67% after 120 h of inoculation. In the case of oil cakes, maximum inhibition (51.51%) was recorded after neem cake-based preparations, followed by mustard (44.56%) and cotton (42.75%). Minimum inhibition (34.48%) after 120 h was recorded when linseed cake-based product was used **(Dhaka *et al.*, 2022)**.

Raw oil-cakes and their composts increased plant growth and yield and considerably decreased disease incidence and severity of *A. tenuissima* leaf spot in chilli grown in pot and field. The composted oil-cakes of sima­rouba were most effective in improving plant growth and yield and decreasing leaf spot disease in chilli, followed by madhuca and neem oil-cake compost (**VasudhaUdupa *et al.,* 2022)**.

To overcome the undesirable effects of chemical usage, use of organic amendments and agents to control the infection came at rescue. Investigations proved that application of organic amendments significantly reduced the leaf spot incidence and enhanced the head yield. The different organic amendments *viz*., fish compost (12.5 t/ha), farm yard manure (FYM) (12.5 t/ha), neem cake (250 kg/ha), mahua cake (250 kg/ha), sheep manure (12.5 t/ha), paddy husk (3 t/ha), press mud (12.5 t/ha), poultry manure (12.5 t/ha), coir pith compost (12.5 t/ha) and groundnut cake (250 kg/ha), efficacy against Alternaria leaf spot of broccoli. The experiment was conducted in vitro and micro-plot to test the efficacy of six organic manures *viz.,* FYM, poultry manure, water hyacinth compost, spent mushroom compost. goat dung and pigeon excreta among them effect of organic manures on plant growth parameter on, poultry manure treated field was significantly higher in plant height, followed by FYM, pigeon excreta, goat dung, water hyacinth compost and spent mushroom compost. The use of manure application enhances soil productivity, increases the soil organic carbon content, soil micro-organism, improves soil crumb structure, the nutrient status of the soil and enhance crop yield. Organic manure is also very cheap and effective as good as source of nitrogen for sustainable crop production **(Nuka and Veenapani, 2011).**

Nowadays increasing use of chemicals tremendously for the management of broccoli Alternaria blight has resulted in growing concern of both in public health and environmental hazards. Thus, emphasis is now on the use of indigenous sources for the management of plant disease which is less costly and doesn’t affect public health and the environment. Considering the above-mentioned facts, a study entitled **“Assessment of botanical cakes in managing *Alternaria* *brassicae* in Broccoli leaf spot disease *in-vivo*”** is proposed.

**MATERIAL AND METHODS**

**Isolation of Pathogen:**

Infected leaves were collected and washed thoroughly with running tap water. The diseased portions were cut into small pieces under aseptic conditions, surface-sterilized with 0.1% HgCl2 for 1 minute, and rinsed three times with sterile distilled water. Excess moisture was removed using sterilized blotting paper, and the pieces were placed on Potato Dextrose Agar (PDA) in Petri dishes. Streptomycin (100 ppm) was added before innoculation of the pathogen to the medium to prevent bacterial contamination. The plates were incubated at 25±2°C for 7 days, with mycelial growth observed after 3 days. The hyphal tip method was used for sub-culturing to obtain a pure culture, which was periodically transferred to fresh media to maintain the pure culture and kept at deep freezer at 4° C. Afterwards slides were made from the obtained culture and observed under microscope, based on morphological features it was confirmed that the causal agent is *Alternaria brassicae.*

**Field Trials**

The study was conducted under field conditions at the Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology and Sciences, during the 2022-23 *Rabi* season. The location's elevation was 98 meters above sea level, with geographical coordinates of 25.87°N latitude and 81.25°E longitude. The field experiment was designed using a randomized block design (RBD) with three replications.

The region is located in the South-Eastern part of Uttar Pradesh and has subtropical climate with extreme of summer and winter. During the winter season especial in month of December and January the temperature drops down to as low as 1°C while during the summer temperature reaches up to 48-50°C. The average rainfall in area is around 1013.4 mm annually and maximum concentration during July to September with a few occasions of shower and drizzles in winter also. The soil type of experimental site was sandy loam, low in organic carbon, nitrogen and phosphorus.

A local hybrid variety named Green Magic was chosen for this experiment and was purchased from seed shop in Prayagraj. All the package of practices were followed as per the general agronomic practices.

Three trays, each measuring 76 cm × 46 cm, were prepared for seedling growth, which would later be transplanted and used for gap filling in the field experiment. Sowing occurred on November 1, 2022, with seeds planted in lines on raised beds that offered superior water retention, excellent aeration, and high nutrient content. A seed rate of 400-450 g/ha was used, and the beds were irrigated as needed. After 25-30 days, uniform and healthy seedlings were selected for transplantation, spaced 45 cm × 50 cm apart, on December 3, 2022 **(Basak *et al.*, 2022)**. The field was naturally infested with *Alternaria brassicae*, which causes leaf spot in broccoli (*Brassica oleracea* var. *italica* L.).

The experiment was designed as eight treatments with 3 replicates. T0 represented as control treatment, T1: Neem cake @400 kg/ha, T2: Mustard cake @400 kg/ha, T3: Groundnut cake @400 kg/ha,T4: Neem cake and Mustard cake @400 kg/ha, T5: Neem cake and Groundnut cake @400 kg/ha, T6: Neem cake, Groundnut cake and Mustard cake @400 kg/ha and T7: Bavistin @0.2% per hectare (2 ml/litre of water). The oilcakes and fungicide were applied at 45, 60 and 75 DAT. Botanical cakes and the fungicide were purchased from fertilizer shop in Prayagraj.

Data collection involved observing key characteristics to compare the impact of oilcakes and fungicides. Five randomly selected plants from each plot were tagged for consistent observation. The mean values of recorded data were considered the actual values for each trait. Measurements included disease intensity (%) and plant height (cm) at 45, 60, and 75 days after transplanting (DAT) (**Cassity-Duffey *et al.,* 2024)**. Additionally, head diameter (cm) was recorded at harvest time, and head weight (g) was calculated post-harvest. The crop was harvested in three pickings, spaced 8-10 days apart.

**Statistical analysis**

The data obtained from the laboratory and field experiments were statistically analysed by following the standard procedures **(Panse and Sukhatme, 1967)**. The percentage values were converted to arcsine values wherever required.

**Analysis of variance**

The analysis of variance was worked out to test the significance of F and t-tests. It was carried out according to procedure of RBD analysis for each character as per methodology suggested by **Panse and Sukhatme (1967)**.

**RESULTS AND DISCUSSION**

**Symptomatology and Identification of the Pathogen**

Typical symptoms of Alternaria leaf spot disease was formation of spots on leaves. Alternaria leaf spot occurs during warm, moist conditions. On older plants, the bottom leaves are infected first with brown circular spots on the leaves. Spots have characteristic concentric rings; infected leaves turn yellow and drop soon. On seedling the symptoms are small dark spots on the stem that can cause damping off or stunting of plant. In later infection, brown to black spots are appears on the head of the broccoli (*Brassica oleracea* var. *italica* L.).

The disease initially manifests as small, pinhead-sized spots on lower leaves, which gradually expand and develop concentric rings, resembling a target board. This distinctive pattern is a key diagnostic feature that distinguishes it from other diseases. Similar symptoms have been observed in other studies, where small, dark to light brown spots appear on leaves, stems, and siliquae, often progressing from lower to upper leaves **(Giri *et al*., 2013)**. *Alternaria brassicae*, the causative agent, produces spots that can vary in colour, with some reports indicating grey spots compared to the black sooty velvety spots typically associated with the disease **(Meena *et al*., 2016)**. Research has consistently shown that Alternaria blight affects the aerial parts of host plants, causing small, circular, dark spots on leaves that can grow up to 1 cm or more in diameter, often appearing grey or black **(Nayyar *et al*., 2014)**.

Studies conducted in different geographical regions have also reported Alternaria blight on cotyledonary leaves, appearing as black spots **(Shrestha *et al*., 2005)**. These findings highlight the importance of recognizing the disease's symptoms and characteristics to develop effective management strategies.

Sporulating culture of the test pathogen was mounted on glass slide and observed using microscope at 10X and 40X. Conidia are obclavate, muriform with long beak and longer with more septation. They were constricted at one or more (commonly three) of the major transverse septa, Conidia were medium brown to brownish grey, in Petri plates the culture was black in colour. Based on typical symptoms on foliage and foliage sheath, cultural characteristics of the fungus and microscopic observations, the fungus has been identified as *Alternaria brassicae* (Berk.) Sacc., the cause of Alternaria leaf spot of broccoli (*Brassica oleracea* L.)



1. **(B) (C)**

**Figure 1:** (A) Pure culture of *Alternaria* brassicae in Petri plates, (B) conidia images in 40X and (C) leaf spot symptom in broccoli (*Brassica oleracea* var. *italica* L.) crop.

**Effect of treatments on disease intensity (%) of Alternaria leaf spot of broccoli at different time intervals of DAT**

Disease intensity is a crucial parameter for assessing the extent of disease infestation and pathogen impact on crops. In this study, disease intensity (%) was recorded at various time intervals to evaluate the pathogen's infestation across different stages of the crop's growth period.

1. **Disease intensity (%) at 45, 60 and 75 DAT:**

 The data in Table 1 reveals that all treated plots with oilcakes and fungicide at 45, 60 and 75 DAT significantly reduced *Alternaria brassicae* incidence compared to the control (T0 - 26.6%). Among the treatments, T7 (Bavistin @ 0.2%) showed the most significant reduction in disease intensity (10.2%, 15.66%, and 18.33%) at 45, 60, and 75 DAT. In contrast, T3 (Groundnut cake @ 400 kg/ha) had the highest disease intensity at 45, 60 and 75 DAT (21.95%, 27.5%, and 32%).

This study found that **mustard cake** (T2) showed the lowest Alternaria leaf spot intensity in broccoli (**19.33%**), followed by neem cake (22.5%), performing comparably to Bavistin (fungicide control). These results align with **VasudhaUdupa *et al*. (2022)**, who studied 3 oilcakes neem, simarouba and madhuca which effectively reduced leaf spot severity in chilli.

**Effect of treatments on the plant height (cm) of broccoli at 45, 60 and 75 DAT**

1. **Plant height (cm) of broccoli at 45, 60, 75 DAT**

 The data presented in table 1 shows that all the treated plots with oilcakes and fungicide at 45, 60 and 75 DAT significantly reduced the plant height of broccoli from control T0 – (26.6%). Among the treatments T7 - Bavistin @ 0.2% (12.4%, 22.53% and 34.13 %) at 45, 60 and 75 DAT significantly increased the plant height of broccoli from other treatments and plant height (cm) was lowest at 45 and 60 DAT was of T3– Groundnut cake @ 400kg/ha (11.47%, 17.6% and 28.27%).

According to **Naorem *et al*. (2024)**, mustard cake @ 1 t ha⁻¹ (T2) recorded the highest plant height (30.01 cm), surpassing other organic inputs. Similarly, Singh et al. (2018) found that mustard cake (T2) maximized plant height (32.2 cm) in broccoli, performing comparably to neem cake (30 cm) and Mancozeb. **Mostarin *et al*. (2014)** also reported that mustard cake enhanced growth, likely due to its disease-suppressing properties and nutrient-rich composition. These findings collectively highlight mustard cake's efficacy in promoting crop growth.

|  |  |  |
| --- | --- | --- |
| **Treatments** | **Disease intensity (%)** | **Plant height (cm)** |
| **45 DAT** | **60 DAT** | **75 DAT** | **45 DAT** | **60 DAT** | **75 DAT** |
| Control | 26.66 | 33.35 | 40.6 | 11.23 | 17.13 | 27.13 |
| Neem Cake | 14.33 | 21 | 22.5 | 12.07 | 20.27 | 30 |
| Mustard Cake | 12.8 | 17.63 | 19.33 | 12.23 | 21.37 | 32.2 |
| Groundnut Cake | 21.95 | 27.5 | 32 | 11.47 | 17.6 | 28.27 |
| Neem Cake + Mustard Cake | 15.66 | 21.33 | 23.66 | 11.93 | 19.03 | 29.33 |
| Neem Cake + Groundnut Cake | 18.26 | 24.66 | 26.66 | 11.70 | 17.87 | 28.73 |
| Neem Cake + Groundnut Cake + Mustard Cake | 16.88 | 22 | 24.3 | 11.83 | 18.5 | 28.90 |
| Bavistin | 10.2 | 15.66 | 18.33 | 12.40 | 22.53 | 34.13 |
| S.Ed.(±) | 0.71 | 0.80 | 1.38 | 0.11 | 0.88 | 1.19 |
| CD @5% | 1.51 | 1.71 | 2.9 | 0.23 | 1.89 | 2.54 |
| CV (%) | 5.07 | 4.27 | 0.385 | 1.11 | 5.60 | 4.87 |

**Table 1:** Disease Intensity (%) andPlant height (cm) at 45, 60 and 75 DAT as affected by treatments

1. **Effect of treatments on the head weight (gm) of broccoli**

 The data presented in table 2 shows that all the treated plots with oilcakes and fungicide significantly increased the head weight (gm) of Broccoli from control T0 – (138.8 gm). Among the treatments T7 – Bavistin @ 0.2% (272 gm) significantly increased the head weight (gm) of broccoli from other treatments. Among the oilcakes, T2 – Mustard cake @ 400kg/ha (253.7 gm) shows significantly increased head weight (gm) from other treatments followed by T1 – Neem cake @ 400kg/ha (218.5 gm), T4 – Neem cake @ 200kg/ha + Mustard Cake @ 200kg/ha (204.6 gm) and T3– Groundnut cake @ 400kg/ha (160.8 gm).

According to **Naorem *et al*.** (**2024)** reported bythe maximum net head weight (253.38 g) was recorded with mustard cake 1 t ha-1(T2) which was found superior to all other concentrated organic inputs. Similar findings are also reported by **Dipika *et al*. (2014)** in broccoli.

The results were in agreement with findings of **Rattan *et al.* (2012)**who reported that mustard and neem cake were highly effective in increasing the head weight of cabbage. Similar findings were also in agreement with **Chander *et al.*, (2020)** who reported that organic manure amendments of neem cake in the soil results in superior head weight of cauliflower.

1. **Effect of treatments on the head diameter (cm) of broccoli**

The data presented in table 2 shows that all the treated plots with oilcakes and fungicide significantly increased the head diameter (cm) of Broccoli from control T0 – (9.73 cm). Among the treatments T7 – Bavistin @ 0.2% (15.73 cm) significantly increased the head diameter (cm) of broccoli from other treatments. Among the oilcakes, T2 – Mustard cake @ 400kg/ha (14.5 cm) shows significantly increased head diameter (cm) from other treatments.

In line with the current study, **Kayesh (2019)** found that Mustard cake was most effective in increasing broccoli head diameter (16.17 cm). This is likely due to the nutrient-rich composition of Mustard cake, particularly its high levels of nitrogen, phosphorus, and potassium, which are vital for promoting plant growth.

|  |  |  |
| --- | --- | --- |
| **Treatments** | **Head weight (gm)** | **Head diameter(cm)** |
| Control | 138.8 | 9.73 |
| Neem Cake | 218.5 | 13.77 |
| Mustard Cake | 253.7 | 14.50 |
| Groundnut Cake | 160.8 | 10.47 |
| Neem Cake + Mustard Cake | 204.6 | 13.43 |
| Neem Cake + Groundnut Cake | 163.2 | 11.37 |
| Neem Cake + Groundnut Cake+ Mustard Cake | 178.8 | 12.53 |
| Bavistin | 272.0 | 15.73 |
| S.Ed.(±) | 2.42 | 0.25 |
| CD @5% | 5.18 | 0.54 |
| CV (%) | 1.49 | 2.43 |

**Table 2:** Head weight (gm) andHead diameter (cm) of broccoli as affected by treatments

**CONCLUSION**

The study concluded that while chemical inputs may enhance crop growth, they carry environmental and health risks, whereas organic amendments like mustard cake, neem cake, and groundnut cake provide a sustainable and eco-friendly alternative. The results demonstrated that botanical oilcakes, particularly mustard cake @ 400 kg/ha (T2), significantly reduced Alternaria brassicae infection (19.33% at 75 DAT) while improving plant height (32.2 cm), head weight (253.7 g), and head diameter (14.5 cm). These findings suggest that mustard cake is a viable organic substitute for chemical fungicides in managing Alternaria leaf spot in broccoli. However, since the study was conducted only during the *Rabi* season in Prayagraj, further multi-season and multi-location trials are necessary to confirm these results for wider applicability.

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