**Original Research Article**

**Assessment of the botanical cakes in managing broccoli leaf spot disease *in-vivo***

**ABSTRACT**

The *Alternaria* leaf spot of broccoli has become a serious problem as it reduced productivity in recent years by causing serious yield loss in India. The research was conducted to investigate the combined impact of different oil cakes and one fungicide (Neem, Mustard and Groundnut) alone and in combination, respectively in combating *Alternaria* spp. on broccoli *in-vivo* during *Rabi* season (2022-2023) under the agro-climatic conditions of Prayagraj, Uttar Pradesh. The results revealed that, among the 7 different treatments the maximum plant height (12.23 cm, 21.37 cm, 32.2 cm), head weight (253.7 gm) and head diameter (14.50 cm) at 45, 60 and 75 DAT were recorded in Mustard oil cake @ 400kg/ha followed by disease intensity (14.33%, 21%, 22.5%), plant height (12.07 cm, 20.27 cm, 30 cm), head weight (218.5 gm) and head diameter (13.77 cm) in Neem cake @ 400kg/ha as compared to treated check Bavistin @ 0.2% which recorded disease intensity (10.2%, 15.66%, 18.33%), plant height (12.40 cm, 22.53 cm, 34.13 cm), head weight (272 gm) and head diameter (15.73 cm) and minimum plant height (11.23 cm, 17.13 cm, 27.13 cm), head weight (138.8 gm) and head diameter (9.73 cm).

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

**INTRODUCTION**

Broccoli 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 an exceptionally nutritious vegetable, rich in vitamins A and C, and essential minerals like potassium, calcium, and iron. It's also a notable source of thiamine, riboflavin, and niacin. Among Cole crops, broccoli stands out for its high protein content. Furthermore, it's a valuable source of chromium, which plays a crucial role in regulating insulin and blood sugar levels, while also exhibiting anti-viral and anti-ulcer properties, and supporting heart health **(Allen and Allen, 2007)**.

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

*Alternaria brassicae* and *Alternaria brassicola* are two species that can infect commercial broccoli crops, posing significant challenges. 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. Similarly, other cruciferous crops such as cauliflower and rapeseed-mustard have also been affected, with reported yield losses of up to 30% and 35-60%, 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. 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 **(Lata and Veenapani, 2011)**.

**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 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 viability.



1. (B)

**Figure 1:** (A) Pure culture of *Alternaria* and (B) leaf spot symptom in broccoli crop.

**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.

Three trays, each measuring 2.5 ft × 1.5 ft, 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. The field was naturally infested with Alternaria spp., which causes leaf spot in broccoli.

The experiment was designed as eight treatments with 3 replicates. T0 represented as control treatment, T1: Neem Cake @400 kg/hectare, T2: Mustard Cake @400 kg/hectare, T3: Groundnut Cake @400 kg/hectare,T4: Neem Cake and Mustard cake @400 kg/hectare, T5: Neem Cake and Groundnut cake @400 kg/hectare, T6: Neem Cake, Groundnut cake and Mustard cake @400 kg/hectare and T7: Bavistin @0.2% per hectare. The oilcakes and fungicide were applied at 45, 60 and 75 DAT.

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). 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)**. The total variance and degree of freedom were partitioned into three components viz. replications, treatments and error.

**RESULTS AND DISCUSSION**

**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 2 and Figure 2 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%).

Among the oilcakes (T4 and T1) and (T6 and T4) are found non-significant from one another at 60 DAT.

Among the oilcakes (T5 and T6) and (T6, T4 and T1) and (T2 and T7) are found non-significant from one another at 75 DAT.

**TABLE 1. The C.D. values of eight treatment numbers and the corresponding treatment averages**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Treatment No** | T0 | T3 | T5 | T6 | T4 | T1 | T2 | T7 |
| **Treatment Average** | 26.66 | 21.95 | 18.26 | 16.88 | 15.66 | 14.33 | 12.8 | 10.2 |

**C.D. (0.05) = 1.51**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Treatment No** | T0 | T3 | T5 | T6 | T4 | T1 | T2 | T7 |
| **Treatment Average** | 33.35 | 27.5 | 24.66 | 22 | 21.33 | 21 | 17.63 | 15.66 |

**C.D. (0.05) = 1.71**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Treatment No** | T0 | T3 | T5 | T6 | T4 | T1 | T2 | T7 |
| **Treatment Average** | 40.6 | 32 | 26.66 | 24.33 | 23.66 | 22.5 | 19.33 | 18.33 |

**C.D. (0.05) = 2.9**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Treatment number** | **Treatments** | **Dosages** | **Disease intensity (%)** | | |
|  | **45 DAT** | **60 DAT** | **75 DAT** |
| T0 | Control | - | 26.66 | 33.35 | 40.6 |
| T1 | Neem Cake | 400kg/ha | 14.33 | 21 | 22.5 |
| T2 | Mustard Cake | 400kg/ha | 12.8 | 17.63 | 19.33 |
| T3 | Groundnut Cake | 400kg/ha | 21.95 | 27.5 | 32 |
| T4 | Neem Cake + Mustard Cake | 400kg/ha | 15.66 | 21.33 | 23.66 |
| T5 | Neem Cake + Groundnut Cake | 400kg/ha | 18.26 | 24.66 | 26.66 |
| T6 | Neem Cake + Groundnut Cake + Mustard Cake | 400kg/ha | 16.88 | 22 | 24.3 |
| T7 | Bavistin | 0.2% | 10.2 | 15.66 | 18.33 |
| S.Ed.(±) | |  | 0.71 | 0.80 | 1.38 |
| CD @5% | |  | 1.51 | 1.71 | 2.9 |
| CV (%) | |  | 5.07 | 4.27 | 0.385 |

**Table 2:** Disease intensity (%) at 45, 60 and 75 DAT as affected by treatments

**Figure 2:** Disease intensity (%) at 45, 60 and 75 DAT as affected by treatments

**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 and 75 DAT**

The data presented in table 4 and depicted in figure 3 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%).

Among the oilcakes (T7 and T2), (T2 and T1), (T1 and T4), (T4 and T6) and (T6 and T5) are found non-significant from one another.

Among the oilcakes (T7 and T2), (T2 and T1), (T1, T4 and T6), (T4, T6, T5 and T3) and (T6, T5 and T3) are found non-significant from one another.

Among the oilcakes (T7 and T2), (T2 and T1), (T1, T4 , T6 , T5 andT3) and (T4, T6, T5 and T3) are

found non-significant from one another.

**TABLE 3. The C.D. values of eight treatment numbers and the corresponding treatment averages**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Treatment No** | T7 | T2 | T1 | T4 | T6 | T5 | T3 | T0 |
| **Treatment Average** | 12.40 | 12.23 | 12.07 | 11.93 | 11.83 | 11.70 | 11.47 | 11.23 |

**C.D. (0.05) = 0.23**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Treatment No** | T7 | T2 | T1 | T4 | T6 | T5 | T3 | T0 |
| **Treatment Average** | 22.53 | 21.37 | 20.27 | 19.03 | 18.5 | 17.87 | 17.6 | 17.13 |

**C.D. (0.05) = 1.89**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Treatment No** | T7 | T2 | T1 | T4 | T6 | T5 | T3 | T0 |
| **Treatment Average** | 34.13 | 32.2 | 30 | 29.33 | 28.90 | 28.73 | 28.27 | 27.13 |

**C.D. (0.05) = 2.54**

**Table 4:** Plant height (cm) at 45, 60 and 75 DAT as affected by treatments

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Treatment number** | **Treatments** |  | **Plant height (cm)** | | |
| **Dosages** | **45 DAT** | **60 DAT** | **75 DAT** |
| T0 | Control | - | 11.23 | 17.13 | 27.13 |
| T1 | Neem Cake | 400kg/ha | 12.07 | 20.27 | 30 |
| T2 | Mustard Cake | 400kg/ha | 12.23 | 21.37 | 32.2 |
| T3 | Groundnut Cake | 400kg/ha | 11.47 | 17.6 | 28.27 |
| T4 | Neem Cake + Mustard Cake | 400kg/ha | 11.93 | 19.03 | 29.33 |
| T5 | Neem Cake + Groundnut Cake | 400kg/ha | 11.70 | 17.87 | 28.73 |
| T6 | Neem Cake+ Groundnut Cake + Mustard Cake | 400kg/ha | 11.83 | 18.5 | 28.90 |
| T7 | Bavistin | 0.2% | 12.40 | 22.53 | 34.13 |
| S.Ed.(±) | |  | 0.11 | 0.88 | 1.19 |
| CD @5% | |  | 0.23 | 1.89 | 2.54 |
| CV (%) | |  | 1.11 | 5.60 | 4.87 |

**Figure 3:** Plant height (cm) at 45, 60 and 75 DAT as affected by treatments

According to the study's findings, T2 (Mustard oil cake) resulted in the maximum plant height (32.2 cm). Among the three oilcakes tested, Mustard cake (32.2 cm) and Neem cake (30 cm) performed best, comparable to Mancozeb. These results align with **Mostarin *et al*. (2014)**, who found that Mustard cake yielded maximum growth parameters. The likely reason for this outcome may be attributed to the chemical properties of the oilcakes, which suppressed disease and promoted crop growth.

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

The data presented in table 6 and depicted in figure 4 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).

Whereas among the oilcakes (T5 and T3) are found non-significant from one another.

**TABLE 5. The C.D. value (5.18) derived from the eight treatment numbers and the corresponding treatment averages**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Treatment No** | T7 | T2 | T1 | T4 | T6 | T5 | T3 | T0 |
| **Treatment Average** | 272.0 | 253.7 | 218.5 | 204.6 | 178.8 | 163.2 | 160.8 | 138.8 |

**C.D. (0.05) = 5.18**

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.

|  |  |  |  |
| --- | --- | --- | --- |
| **Treatment code** | **Treatments** | **Dosages** | **Head weight (gm)** |
| T0 | Control | - | 138.8 |
| T1 | Neem Cake | 400kg/ha | 218.5 |
| T2 | Mustard Cake | 400kg/ha | 253.7 |
| T3 | Groundnut Cake | 400kg/ha | 160.8 |
| T4 | Neem Cake + Mustard Cake | 400kg/ha | 204.6 |
| T5 | Neem Cake + Groundnut Cake | 400kg/ha | 163.2 |
| T6 | Neem Cake + Groundnut Cake+ Mustard Cake | 400kg/ha | 178.8 |
| T7 | Bavistin | 0.2% | 272.0 |
| S.Ed.(±) | |  | 2.42 |
| CD @5% | |  | 5.18 |
| CV (%) | |  | 1.49 |

**Figure 4:** Head weight (gm) of broccoli as affected by treatment

Table 6: Head weight (gm) of broccoli as affected by treatments

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

The data presented in table 8 and depicted in figure 5 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.

Whereas among the oilcakes (T5 and T3) are found non-significant from one another.

**Table 7. The C.D. value (0.54) derived from the eight treatment numbers and the corresponding treatment averages**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Treatment No** | T7 | T2 | T1 | T4 | T6 | T5 | T3 | T0 |
| **Treatment Average** | 15.73 | 14.5 | 13.77 | 13.43 | 12.53 | 11.37 | 10.47 | 9.73 |

**C.D. (0.05) = 0.54**

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.

**Table 8:** Head diameter (cm) of broccoli as affected by treatments

|  |  |  |  |
| --- | --- | --- | --- |
| **Treatment code** | **Treatments** | **Dosages** | **Head diameter(cm)** |
| T0 | Control | - | 9.73 |
| T1 | Neem Cake | 400kg/ha | 13.77 |
| T2 | Mustard Cake | 400kg/ha | 14.50 |
| T3 | Groundnut Cake | 400kg/ha | 10.47 |
| T4 | Neem Cake + Mustard Cake | 400kg/ha | 13.43 |
| T5 | Neem Cake + Groundnut Cake | 400kg/ha | 11.37 |
| T6 | Neem Cake + Groundnut Cake + Mustard Cake | 400kg/ha | 12.53 |
| T7 | Bavistin | 0.2% | 15.73 |
| S.Ed.(±) | |  | 0.25 |
| CD @5% | |  | 0.54 |
| CV (%) | |  | 2.43 |

**Figure 5:** Head diameter (cm) of broccoli as affected by treatment

**CONCLUSION**

The study concluded that while chemicals can benefit crops, they pose environmental and health risks, and harm soil properties due to residual effects. In contrast, using organic amendments like Mustard cake, Neem cake, and Groundnut cake offers a sustainable, economical, and residue-free alternative. Mustard cake, in particular, showed promise in controlling Alternaria leaf spot disease and boosting broccoli yields. However, since this investigation was limited to a single crop season (Rabi) in Prayagraj's climate, further trials are needed to validate these findings and inform broader recommendations.

**REFERENCES**

**Allen, R., and Allen, Z. (2007).** Broccoli: The crown jewel of nutrition. *Vegetarians in Paradise*.

**Cai, C., Bucher, J., Bakker, F. T., and Bonnema, G. (2022).** Evidence for two domestication lineages supporting a middle-eastern origin for Brassica oleracea crops from diversified kale populations. *Horticulture research*, *9*, uhac033.

**Chand, G. and Singh, V. K. (2005).** Eco-friendly management of Alternaria blight of carrot (Daucus carota L.). *Journal of Hill Agriculture*. *2*(2): 201-203.

**Chander, A. S., Anitha, T., Madhuri, S. B., Alexander, G. and Venkateswarlu, M. (2020).** Detection of urea adulteration in oilseed cakes using 4-Dimethylaminobenzaldehyde reagent and foldscope. *Asian Journal of Dairy and Food Research*. 42(1): 65-69.

**Doklega, M. A. and El-hady, M.A. (2017).** Impact of organic, mineral and bio-fertilization on broccoli. *Journal of Plant Production*. 8(9): 945-951.

**Kayesh, E., Sharker, M. S., Roni, M. S. and Sarker, U. (2019).** Integrated nutrient management for growth, yield and profitability of broccoli. *Bangladesh Journal of Agricultural Research*. 44(1): 13-26.

**LAfi, O. I., El-Hamarnah, H. A., Al-Saloul, N. J., Radwan, H. I., and Abu-Naser, S. S. (2022).** A Proposed Expert System for Broccoli Diseases Diagnosis.

**Lata, N., and Veenapani, D. (2011).** *Response of water hyacinth manure on growth attributes and yield in Brassica juncea*. **Asian Journal of Agricultural Sciences, 3**(2), 96–99.

**Mostarin, T., Khatun, K., Hossain, F., Saha, S. R. and Begum, F. (2014).** Yield of broccoli as influenced by cowdung and mustard oilcake. J. Sher-e-Bangla Agric. Univ. 8(2): 47-54.

**Panse, V. G. and Sukhatme, P. V. (1967).** Statistical methods of agricultural workers. 2nd Endorsement. *ICAR Publication, New Delhi, India*, *381*.

**Rattan, P., Sharma, J. P. and Kumar, S. (2012).** Response of vegetable crops to use of integrated nutrient management practices. *SABB Journal of food and Agriculture Science*. 2(1): 15-19.

**Singh, H. P., and Malhotra, S. K. (2013).** Trend of horticultural research particularly vegetables in India and its regional prospects. In *Proceedings of the regional symposium on high value vegetables in Southeast Asia: production, supply and demand (SEAVEG 2012). Bangkok* (pp. 321-343).

**Ulger, T. G., Songur, A. N., Çırak, O. and Çakıroğlu, F. P. (2018).** Role of vegetables in human nutrition and disease prevention. *Veg. Importance Qual. Veg. Hum. Health*. 7-32.