Original Research Article

Sustainable Approaches for Managing Alternaria Blight in Mustard (*Brassica juncea L.*) Caused by *Alternaria brassicae* (Berk.) Sacc.

ABSTRACT

Brassica juncea, also known as Indian mustard, is a globally used oilseed, vegetable, and condiment. *Alternaria brassicae*, a filamentous fungus, causes Alternaria black spot, affecting crop productivity. This research endeavours to investigate environmentally sustainable methods for managing the Alternaria blight on mustard. The experiment was analyzed in randomized block design (RBD) with three replications in a plot size 2x1 m^2. The field experiment was conducted at the research plot in the Central Research Field, SHUATS, Prayagraj, U.P. during *Rabi* season 2022-2023 to test the effect of *Trichoderma viride, Pseudomonas fluorescens,* eucalyptus oil, neem leaf extract, salicylic acid, and *Ascophyllum nodosum* on Alternaria blight of mustard (*Brassica juncea* L.) caused by *Alternaria brassicae* (Berk.) Sacc**.** Treatments included seed treatment and foliar applications optimized from prior laboratory trials. Parameters like disease intensity and plant growth were assessed, and data were statistically analyzed. Among the treatments, *Trichoderma viride* @ 10 g/L was the most effective followed by salicylic acid. The seed treatment and foliar spray of *T. viride* @ 10g/L thrice at 15-day intervals significantly reduced disease intensity on leaves (37.20%) and pods (18.73%), AUDPC (1245) and significantly increased the yield (1.631 t/ha), and cost-benefit ratio (2.37). However, the maximum height of the plant was recorded in Ascophyllum nodosum (189cm). The current experiment proved that, without using any chemicals, the management of Alternaria blight disease in mustard can be profoundly possible through the use of different bio-agents, essential oils, botanicals, and elicitors.

Keywords: *Alternaria brassicae,* bio-agents, elicitors, Indian mustard, oilseed crop, salicylic acid*, Trichoderma viride*

1. INTRODUCTION

The contribution of oilseeds to the agriculture economy of India ranks second to food grains (Rathore et al., 2018). Mustard is a Latin term ‘must’/ ‘mustum’ denotes the expressed juice of

grapes and ‘ardens' means hot and burning. Mustard occupies a prominent place as the next most important to groundnut, both in area and production. Mustard crop is grown in both tropical and subtropical countries. Mustard oil has several fatty acids, among which erucic and linoleic acids are particularly significant. In *Brassica juncea* (L.) Czern and Coss, oil content is usually about 30–38 % (Thomas et al., 2012). The crop can be ravaged by several diseases, among them, Alternaria blight disease, caused by *Alternaria brassicae* (Berk.) Sacc. and *A. brassicicola* (Schw.). Wiltshire, which has been reported from all the continents of the world, causing 10-70% yield losses depending on the crop species and affects most of the cruciferous crops (Kolte et al., 1987; Chattopadhyay, 2008; Meena et al., 2016; Kumar et al., 2019). In India, it is one of the most important and widespread disease of all mustard growing areas of the Uttar Pradesh (Wadhwani and Dudheja, 1982). The symptoms of *A. brassicae* appear on leaves and stem and mature plants also in siliquae during ripening stage. Dark spots appear on leaves and siliquae, which adversely affect seed production and quality of mustard (Kumar et al., 2014). Pod infection is major factor that reduces seed yield and its management is necessary to increase seed yield (Hossian and Mian, 2004). Fungicide sprays are although effective in controlling the various fungal diseases but their extensive use is environmentally unsafe and also uneconomical. However, with increasing environmental pollution and the present-day public perception on pesticide contaminants of foods, especially edible oils, development of alternate economical and eco-friendly approaches for disease management is needed. Bio-agents offer an alternative to use of costly agrochemicals by producing low-cost environmental friendly control measures using antagonistic microorganisms that reduces the number and activity of plant pathogens (Sindhu et al., 2016; Bach et al., 2016). In this research, the limitations of chemical management have highlighted the need for exploring alternative eco-friendly approaches within the framework of Integrated Disease Management (IDM), aiming to effectively control Alternaria blight of Indian mustard while safeguarding the ecosystem.

2. METHODOLOGY

The field experiment was conducted at the research plot of the Central Research Field, Sam Higginbottom University of Agriculture, Technology And Sciences, Prayagraj (Uttar Pradesh) during *Rabi* season 2022-2023. The experiment was analyzed in randomized block design (RBD) with three replications in a plot size 2x1 m^2. The chosen field was excavated, weeded, cleaned and the dirt pulverized. NPK fertilizers were sprayed at rates of 80 N, 60 P, and 60 K kg/ha, respectively. At the time of sowing, half of the nitrogen, full doses of phosphate and potash were applied as basal, and the remaining half dose of nitrogen was top dressed at 30

DAS. The seeds were drilled at a depth of 3–4 cm according to the treatment guidelines, with a plant-to-plant spacing of 10 cm and a row-to-row spacing of 30 cm. The SRM - 777 (Mustard variety seed) was used for the research.

2.1 Isolation, purification and maintenance of culture of the pathogen

The leaf spot and lesions, showing the initial and conspicuous characteristic symptoms of Alternaria blight were selected for isolation of the pathogen. These selected infected spots were washed 3-4 times in sterilized distilled water and then surface sterilized by dipping in 1% NaOCl solution for 1 minute, followed by washing with sterilized water 3-4 times. These pieces were placed between two folds of sterile blotter paper in the inoculation chamber under aseptic conditions in order to eliminate excess moisture. Surface sterilized leaf spot pieces were then, aseptically transferred into 9 cm petri dishes containing Potato Dextrose Agar (PDA) and incubated at 25±2°C for 7 days. Thereafter, growing mycelia from margin of apparently distinct colonies of the leaf spot pieces on the medium were aseptically transferred into another petri plate containing PDA medium, where it was grown for 7 days at 25±2°C in the BOD incubator. The culture of *Alternaria brassicae* was purified by single spore technique and maintained by periodic sub-culturing on PDA petri plates and slants for the morphological studies.

2.2 Morphological characterization

Microscopic examination was conducted following the procedure described by Grahovac et al. (2012). The identification of Alternaria was based on morphological characteristics, including the size and shape of conidia, as well as cultural characteristics such as colony outline, shape, color, and texture, as described by Meena et al. (2010).

2.3 Preparation and artificial inoculation of the pathogen

One disc of actively growing culture of *A. brassicae* was seeded in 90 mm petri plates containing sterile and solidified PDA medium and incubated at 25±1°C for 7 days. The pathogen culture was harvested in sterilized distilled water upon full growth at the rate of one plate per litre (inoculum load of approximately 10^6 spores per ml). The conidial concentrations were adjusted to 1 x 10^6 ml by adding sterile distilled water and observed with haemocytometer. Using a power sprayer, the suspension was applied to the crop's foliar regions at 30 days after sowing (DAS) and twice more at intervals of 7 days, continuing until the suspension began to drip off the sides of the leaves at dusk to avoid rapid evaporation and ensure better pathogen establishment, when the temperature was relatively low.

2.4 Preparation of treatments:

Bio-agent powdered formulation was brought to the laboratory and the viability was checked by serial dilution method. Polysorbate 20 (Tween 20) was added to water along with 0.2 % eucalyptus oil to bind oil in water (o/w) emulsion prior field spray. Neem leaf extract was prepared by using the method of standard procedure given by Mahapatra and Das (2013). Matured leaves were collected and sterilized with distilled water, the leaves were homogenized in a pre-chilled pestle and mortar using chilled and sterilized distilled water. Aqueous extract of this botanical (1% w/v) was prepared by mixing 100g fresh leaves of plant with 100ml of sterile distilled water and crushing in warring blender. The extract was filtered through Whatman grade 42 filter paper. The filtrate thus obtained was considered as 100% concentration.

2.5 Evaluation of treatments *in vivo*

The efficacy of bio-agents, essential oil, botanical, and elicitors on *Alternaria brassicae* was carried out in field condition. Seeds were treated and spread over a clean paper and dried in cool and shade place were sown immediately after drying. The laboratory trials conducted previously (Barath et al., 2023; Ann Rose et al., 2023) provided the basis for selecting the optimum concentration for the field study by testing three different concentrations. The lab tested eucalyptus oil @ 2% shows the phytotoxicity effect on plants. So, the concentration has been changed to 0.2%. Treatments were applied uniformly across all plots using a hand-operated knapsack sprayer fitted with a flat-fan nozzle at a pressure of 2 kg/cm². Spraying was carried out in the early morning to minimize evaporation losses. Observations were taken at 15 days interval after initiation of disease. Observations of the characters were recorded at 45, 60, 75 and 90 DAS.

Table 1. Details of treatments

Sr. No.	Tr. no.	Treatments	Concentrations
1	T_0	Control (untreated check)	-
2	T_1	*Trichoderma viride*	10 g/L
3	T_2	*Pseudomonas fluorescens*	10 g/L
4	T_3	Eucalyptus oil	0.2 %
5	T_4	Neem leaf extract	15 %
6	T_5	Salicylic acid	100 ppm
7	T_6	*Ascophyllum nodosum*	2 ml/L
8	T_7	Mancozeb (treated check)	0.2 %

2.6 Per cent disease intensity

Per cent disease intensity was calculated by following formula (Wheeler, 1969).

$$\text{Per cent disease intensity} = \frac{\text{Sum of total numerical ratings}}{\text{Total no. of leaves observed} \times \text{Maximum disease garde}} \times 100$$

Observations were recorded on leaf blight severity (0-9 disease rating scale based on blighted area), on five randomly selected plants from each plot and per cent disease intensity (PDI) was calculated. The disease severity was recorded using following scale as per recommendation of All India Coordinated Research Project on Rapeseed- Mustard, 2018 which is as under:

Table 2. Grade chart for calculating PDI on leaves

Grade	Leaf area covered	Reaction
0	No lesion on leaves	Immune (I)
1	Non sporulating pinpoint size or small brown necrotic spots, less than 5% leaf area covered by the lesions	Highly resistant (HR)

3	small roundish slightly sporulating larger brown necrotic spot, about 1-2mm in diameter with a distinct margin or yellow halo, 5-10% leaf area covered by lesions	Resistant (R)
5	moderate sporulation, non-coalescing larger brown spots, about 2-4 mm in diameter with a distinct margin or yellow halo, 11-25% leaf area covered by the lesions	Moderately resistant (MR)
7	moderately sporulating, coalescing, larger brown spots about 4-5 mm in diameter, 26-50% leaf area covered by the lesions	Susceptible (S)
9	profusely sporulating, rapidly coalescing, brown to black spots measuring more than 6 mm in diameter without margins covering more than 50% leaf area	Highly susceptible (HS)

2.7 Area under the disease progress curve (AUDPC)

The area under the disease-progress curve (AUDPC) value was calculated according to formula (Jeger and Viljanen- Rollinson, 2001; Tratwal and Bocianowski, 2014):

$$AUDPC = \sum_{i=1}^{n} \left[\left(\frac{y_i + y_{i-1}}{2} \right) (x_i - x_{i-1}) \right]$$

Where, AUDPC is the area under disease progress curve, y_i is the percentage of visible infected area (y_i/ 100) at the i-th observation, x_i day of the i-th observation, and n the total number of observations (modified from Shaner and Finney, 1977).

2.8 Per cent disease reduction over control

Per cent disease reduction over control was worked out by applying the formula:

$$\text{Per cent disease reduction over control} = \frac{\text{PDI in control plot} - \text{PDI in treatment plot}}{\text{PDI in control plot}}$$

2.9 Pod disease intensity

The pod disease intensity (%) was recorded as per the scale suggested by Conn *et al*. (1990).

Table 3. Grade chart for calculating pod disease intensity

Grade	Pod area covered	Reaction
0	No symptoms on pods	Immune (I)
1	Small light brown spots covering 1% or less leaf area	Highly resistant (HR)
2	Small spots (up to 5mm in size) covering 1-10% of the leaf area	Resistant (R)

3	Large spots, brown, irregular with concentric rings covering 10-25% of leaf area	Moderately resistant (MR)
4	Large brown irregular lesions with typical blight symptoms covering 25-50% of leaf area	Moderately susceptible (MS)
5	Spots enlarging, covering more than 50% of leaf area	Highly susceptible (HS)

2.10 Cost benefit ratio:

Gross returns will be calculated by multiplying total yield with the market price of the produce. Cost of cultivation and cost of treatment imposition will be deducted from the gross returns, to find out net returns and cost benefit ratio by following formula (Reddy et al., 2004).

$$\text{Cost benefit ratio} = \frac{\text{Gross return}}{\text{Total cost of cultivation}}$$

2.11 Statistical analysis

The data was analyzed using ANOVA in WASP 2.0 (Web Agri Stat Pack), ICAR, Goa. Assumptions of normality were checked using the Shapiro-Wilk test, while homogeneity of variances was assessed using Levene's test. Tukey's HSD test was performed for pairwise comparison of means to identify significant differences between treatments. All analyses were conducted at a 5% level of significance.

3. RESULTS AND DISCUSSION

Effect of bio-agents, essential oil, botanical and elicitors were evaluated on disease parameters like per cent disease intensity (PDI) (%) at 45, 60, 75, 90 DAS, area under disease progress curve (AUDPC), per cent disease reduction over control and pod disease intensity (PDI) at 110 DAS and growth parameters like height of the plant (cm) at 45, 60, 75, 90 DAS, number of branches per plant at 110 DAS, number of siliquae per plant at 110 DAS, number of seeds per siliqua at 115 DAS, yield (t/ha), avoidable yield loss and test weight of seeds (1000 number) (gm). The following results were observed under field conditions.

3.1 Symptomalogy

Symptoms were first visible on lower leaves with appearance of black points, which later enlarged to develop into prominent, round, concentric spots of various sizes. They were characterized by formation of spots on leaves, stem and siliquae. *Alternaria brassicae* can affect host species at all stages of growth including seed. On seedlings, symptoms appeared as dark lesions on stem immediately after germination that can result in damping-off, or stunted seedlings. The symptoms produced by *A. brassicae* were usually grey in colour compared with black sooty velvety spots by *A. brassicicola*. Later, round black conspicuous spots appeared on

siliquae and stem. These spots coalesced, leading to complete blackening of siliquae or weakening of the stem with formation of elongated lesions. Spots on mustard siliquae are brownish black with a distinct grey centre. When older plants became infected, symptoms often occured on the older leaves, since they were closer to the soil and are more readily infected as a consequence of rain splash or wind-blown main. The infection of Alternaria blight on leaves and siliquae reduced the photosynthetic area drastically. The phase of infection on siliquae adversely affected the normal seed development, seed weight, colour of seed and percent oil content in seed and the quality of seed. The symptoms observed in the research work were also reported earlier by Meena et al. (2010), Kumar and Shete (2021), Pandey et al. (2024) and Meena et al. (2024).

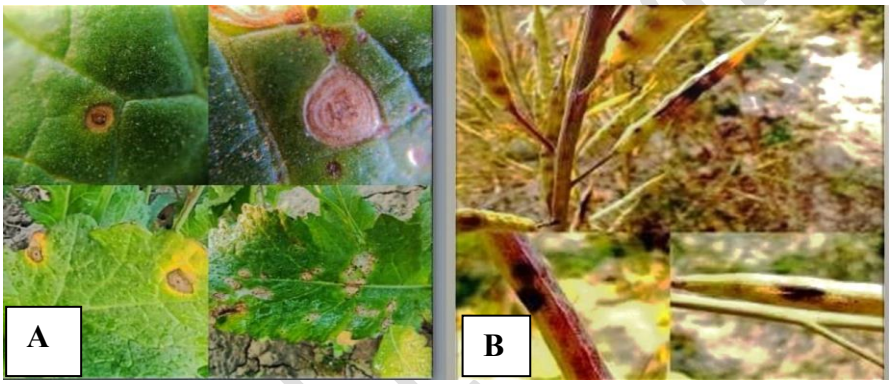

Fig 1. Typical disease symptom on leaves (A) and pods (B)

3.2 Morphological Studies of the Pathogen Mycelium

The *Alternaria brassicae* mycelium is unique with its dendroid (tree-like), septate growth and transitional color from hyaline to dark brown, 2–8 µm in diameter, offering a measurable character for distinguishing from other *Alternaria* species. The conidiophores are normally unbranched, sometimes branched, cylindrical with minimal basal swelling, pale to mid-olivaceous brown, 4–6 µm x 6–8 µm, and straight or slightly sigmoid, helping in identification. *A. brassicae* conidia are dark, obclavate, and muriform with both longitudinal and transverse septa, less frequent in other *Alternaria* species, and hence this is a key diagnostic character. In short chains of up to four spores, the conidia also have a characteristic beak, occupying nearly one-third of their length, with a greenish-brown to colorless basal part and narrow shape, further aiding in distinguishing *A. brassicae*.

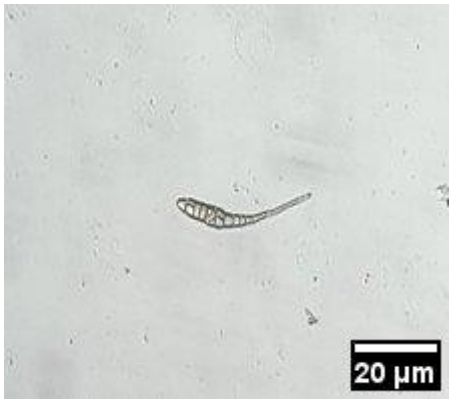

Fig 2. Microscopic view *Alternaria brassicae* conidium under 40X

3.3 Effect of selected treatments on Alternaria leaf blight disease intensity

The data presented in table 4 reveals that all the treatments were superior to T_0 - control in reducing disease intensity @ 90 DAS, per cent disease intensity was recorded minimum in T_1 - *Trichoderma viride* @ 10g/L (32.20%) followed by T_3 - Eucalyptus oil @ 0.2 % (40.66%), T_5 - Salicylic acid @ 100ppm (40.83%), T_2 - *Pseudomonas fluorescens* @ 10g/L (43.63%), T_4 - Neem leaf extract @ 15% (45.03%) and T_6 - *Ascophyllum nodosum* @ 2ml/L (47.93%) as compared to treated check T_7 - Mancozeb @ 0.2 % (34.93%) and T_0 - control (74.10%). Comparing the treatments with CD value (1.85), all the treatments are statistically significant over control. Among the treatments, T_1, T_6 and T_7 are statistically significant over other treatments and the treatments, (T_3 and T_5) and (T_2 and T_4) are statistically non-significant with each other.

3.4 Effect of selected treatments on area under disease progress curve (AUDPC)

The data presented in table 4 and depicted in figure 3 reveals that all the treatments were superior to T_0 - control in reducing area under disease progress curve, minimum AUDPC was found in T_1 - *Trichoderma viride* @ 10g/L (1245) followed by T_3 - Eucalyptus oil @ 0.2 % (1431), T_2 - *Pseudomonas fluorescens* @ 10g/L (1451), T_5 - Salicylic acid @ 100ppm (1475), T_4 - Neem leaf extract @ 15% (1566), and T_6 - *Ascophyllum nodosum* @ 2ml/L (1645) as compared to treated check T_7 - Mancozeb @ 0.2 % (920) and T_0 - control (2351).

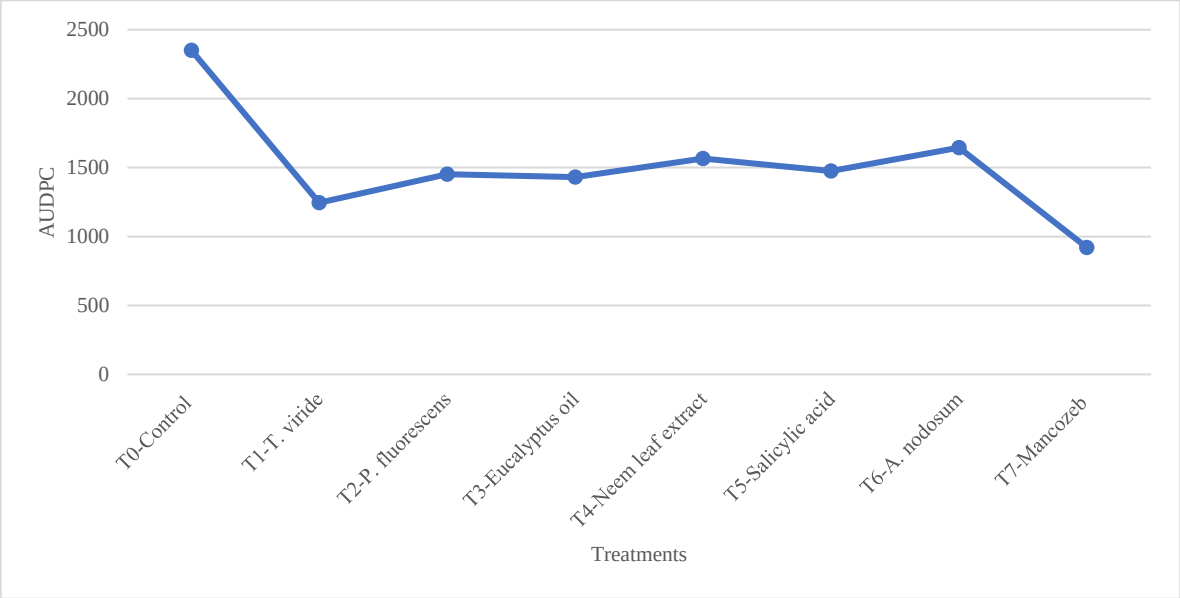

Figure 3. Effect of bio-agents, essential oil, botanical and elicitors on area under disease progress curve (AUDPC)

3.5 Effect of selected treatments on per cent disease reduction over control

The data presented in table 4 reveals that all the treatments were superior to control - T_0 in reducing the disease. Per cent disease reduction over control was found maximum in T_1 - *Trichoderma viride* @ 10g/L (48.87%) followed by T_3 - Eucalyptus oil @ 0.2 % (44.11%), T_5 - Salicylic acid @ 100ppm (43.88%) T_2 - *Pseudomonas fluorescens* @ 10g/L (40.03%), T_4 - Neem leaf extract @ 15 % (38.11%) and T_6 - *Ascophyllum nodosum* @ 2ml/L (34.10%) as compared to treated check T_7 - Mancozeb @ 0.2 % (51.99%) and T_0 - control.

3.6 Effect of selected treatments on avoidable yield loss

The data presented in table 4 reveals that all the treatments were superior to T_0 - control in reducing yield loss, maximum avoidable yield loss was found in T_1 - *Trichoderma viride* @ 10g/L (35.70%) followed by T_3 - Eucalyptus oil @ 0.2 % (31.43%), T_5 - Salicylic acid @ 100ppm (30.36%), T_2 - *Pseudomonas fluorescens* @ 10g/L (27.79%), T_4 - Neem leaf extract @ 15 % (27.33%) and T_6 - *Ascophyllum nodosum* @ 2ml/L (20.05%) as compared to treated check T_7 - Mancozeb @ 0.2 % (38.14%) and T_0 - control.

3.7 Effect of selected treatments on per cent disease intensity on pods

The data presented in table 4 and figure 4 reveals that all the treatments were superior to T_0 - control in reducing per cent disease intensity on pods @ 110 DAS, minimum per disease intensity was recorded in T_1 - *Trichoderma viride* @ 10g/L (18.73%) followed by, T_3 -

Eucalyptus oil @ 0.2 % (19.80%), T_5 - Salicylic acid @ 100ppm (20.46%), T_2 - *Pseudomonas fluorescens* @ 10g/L (25.33%), T_4 - neem leaf extract @ 15 % (25.46%) and T_6 - *Ascophyllum nodosum* @ 2ml/L (27.33%) as compared to treated check T_7 - Mancozeb @ 0.2 % (13.0%) and T_0 - control (35.46%). Comparing the treatments with CD value (2.10), all the treatments are statistically significant over control. Among the treatments, T_7 is statistically significant over other treatments and the treatments, (T_4 and T_6), (T_2 and T_4), (T_3 and T_5) and (T_1 and T_3) are statistically non- significant with each other.

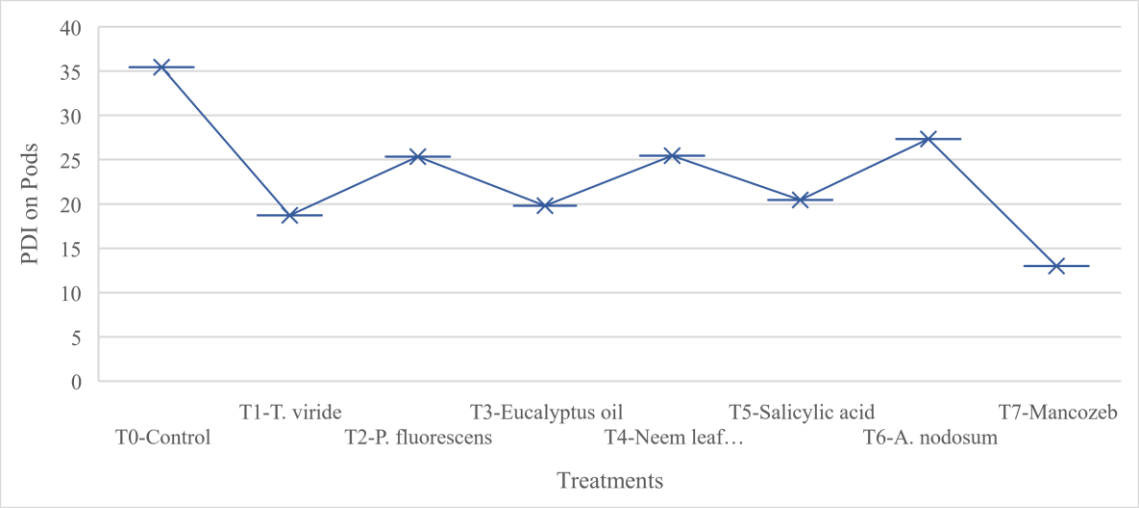

Figure 4. Effect of bio-agents, essential oil, botanical and elicitors on per cent disease intensity on pods

3.8 Effect of selected treatments on plant height @ 90 DAS

The data presented in table 5 reveals that among all treatments, maximum plant height (cm) @ 90 DAS was found in @ 2ml/L (189 cm) which was superior over all treatments, followed by T_1 - *Trichoderma viride* @ 10 g/kg (178.5cm), T_2 - *Pseudomonas fluorescens* @ 10 g/kg (176cm), T_5 - Salicylic acid @ 100mg (175.6cm), T_3 - Eucalyptus oil 0.2 % (171.3cm) and T_4 – Neem leaf extract @ 15% (170cm) as compared to treated check T_7 - Mancozeb @ 0.2 % (173cm) and T_0 - control (159.7cm). Comparing the treatments with CD value (1.94), all the treatments are statistically significant over control. Among the treatments, T_1 and T_6 are statistically significant over other treatments and the treatments (T_4 and T_3), (T_3 and T_7) and (T_5 and T_2) are statistically non-significant with each other.

3.9 Effect of treatments on number of branches per plant of mustard

The data presented in table 5 reveals that among all treatments, highest number of

branches/plant @ 110 DAS was found in T_1 - *Trichoderma viride* @ 10g/L (8.20) followed by, T_3 - Eucalyptus oil @ 0.2 % (8.13), T_5 - Salicylic acid @ 100ppm (6.53), T_2 - *Pseudomonas fluorescens* @ 10g/L (5.80), T_4 - Neem leaf extract @ 15% (5.40) and T_6 - *Ascophyllum nodosum* @ 2ml/L (4.60) as compared to treated check T_7 - Mancozeb @ 0.2 % (8.86) and T_0 - control (3.66). Comparing the treatments with CD value (0.58), all the treatments are statistically significant over control. Among the treatments, T_5, T_6 and T_7 are statistically significant over other treatments and the treatments (T_4 and T_2) and (T_3 and T_1) are statistically non-significant with each other.

3.10 Effect of treatments on number of siliquae per plant of mustard

The data presented in table 5 reveals that among all treatments, highest number of siliquae/plant @ 110 DAS was found in T_1 - *Trichoderma viride* @ 10g/L (246.2) followed by T_3 - Eucalyptus oil @ 0.2 % (227.8), T_5 - Salicylic acid @ 100ppm (214), T_2 - *Pseudomonas fluorescens* @ 10g/L (196.5), T_4 - Neem leaf extract @ 15% (185.3) and T_6 - *Ascophyllum nodosum* @ 2ml/L (172.4) as compared to treated check T_7 - Mancozeb @ 0.2 % (265.6) and T_0 - control (131.1). Comparing the treatments with CD value (4.15), all the treatments are statistically significant over control and are statistically significant with each other.

3.11 Effect of treatments on number of seeds per siliqua of mustard

The data presented in table 5 reveals that among all treatments, highest number of seeds/siliqua @ 115 DAS was found in T_1 - *Trichoderma viride* @ 10g/L (13.13) followed by T_3- Eucalyptus oil @ 0.2 % (12.93), T_5- Salicylic acid @ 100ppm (12.53), T_2- *Pseudomonas fluorescens* @ 10g/L (12.33), T_4 - Neem leaf extract @ 15 % (11.40) and T_6 - *Ascophyllum nodosum* @ 2ml/L (10.40) as compared to treated check T_7- Mancozeb @ 0.2 % (15.06) and T_0 - control (7.53). Comparing the treatments with CD value (0.90), all the treatments are statistically significant over control. Among the treatments, T_6 and T_7 are statistically significant over other treatments and the treatments (T_4 and T_2), (T_2 and T_5), (T_5 and T_3) and (T_3 and T_1) are statistically non-significant with each other.

3.12 Effect of treatments on the yield of mustard

The data presented in table 5 and figure 5 reveals that among all treatments, yield (t/ha) was found maximum in T_1 - *Trichoderma viride* @ 10g/L (1.631 t/ha) followed by T_3 - Eucalyptus oil @ 0.2 % (1.521 t/ha), T_5 - Salicylic acid @ 100ppm (1.498 t/ha), T_2 - *Pseudomonas fluorescens* @ 10g/L (1.445 t/ha), T_4 - Neem leaf extract @ 15 % (1.436 t/ha) and T_6 - *Ascophyllum nodosum* @ 2ml/L (1.305 t/ha) as compared to treated check T_7 -

Mancozeb @ 0.2 % (1.686 t/ha) and T_0 - control (1.043 t/ha). Comparing the treatments with CD value (0.16), all the treatments are statistically significant over control. Among the treatments (T_6 and T_4), (T_4 and T_2), (T_2 and T_5), (T_5 and T_3), (T_3 and T_1) and (T_1 and T_7) are statistically non-significant with each other.

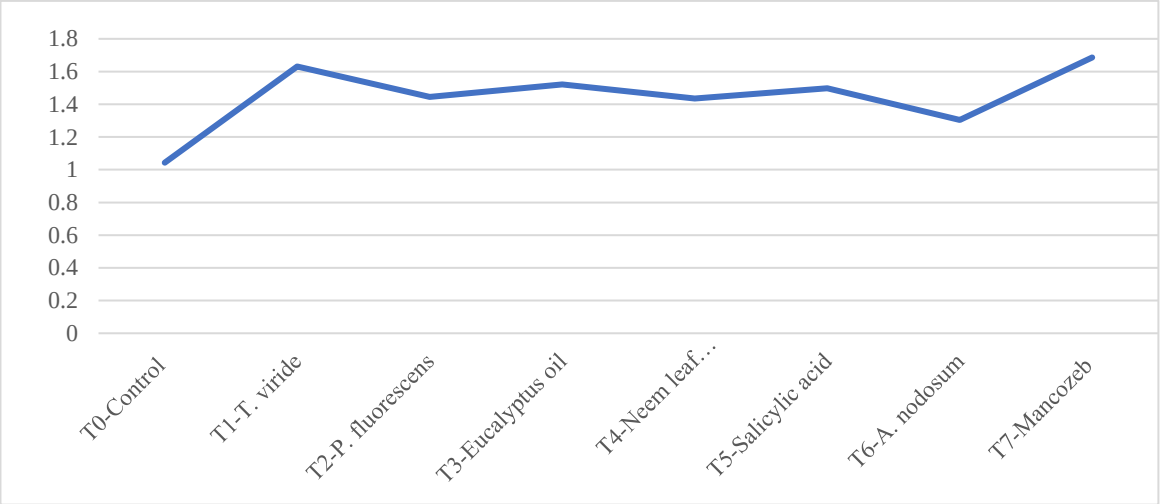

Figure 5. Effect of treatments on the yield of mustard

3.13 Effect of treatments on test weight (1000 seeds) (gm) of mustard

The data presented in table 5 reveals that among all treatments, maximum test weight (1000 seed) (gm) was found in T_1 - *Trichoderma viride* @ 10g/L (4.46 gm) followed by T_3 - Eucalyptus oil @ 0.2 % (4.23 gm), T_5 - Salicylic acid @ 100ppm (4.23 gm), T_2 - *Pseudomonas fluorescens* @ 10g/L (4.06 gm), T_4 - Neem leaf extract @ 15% (3.90 gm) and T_6 - *Ascophyllum nodosum* @ 2ml/L (3.63 gm) as compared to treated check T_7 - Mancozeb @ 0.2 % (4.66 gm) and T_0 - control (3.10 gm).Comparing the treatments with CD value (0.50), all the treatments are statistically significant over control. Among the treatments, (T_6 and T_4), (T_4 and T_2), (T_2 and T_5), (T_5 and T_3), (T_3 and T_1) and (T_1 and T_7) are statistically non-significant with each other.

3.14 Cost-benefit ratio of mustard as influenced by selected treatments

The data presented in table 6 reveals that among all treatments, highest cost benefit ratio was found in T_1 - *Trichoderma viride* @ 10g/L (1:2.37) followed by T_5 - Salicylic acid @ 100ppm (1:2.23), T_4 - Neem leaf extract @ 15% (1:2.11), T_3 - Eucalyptus oil @ 0.2 %

(1:2.04), T_2 - *Pseudomonas fluorescens* @ 10g/L (1:2.00) and T_6 - *Ascophyllum nodosum* @ 2ml/L (1:1.90) as compared to treated check T_7 - Mancozeb @ 0.2 % (1:2.46) and T_0 - control (1:1.58).

The seed treatment and foliar spray of *T. viride* @ 10g/L thrice at 15 days interval significantly reduced disease intensity on leaves and pods, AUDPC and significantly increased the per cent disease reduction over control, avoidable yield loss, number of branches, number of siliquae per plant, number of seeds per siliqua, yield, test weight and cost benefit ratio as compared to all other treatments. The most likely reason is that *Trichoderma viride* species have many qualities and have a high potential for use in agriculture, such as amending abiotic stresses, improving physiological response to stresses, and assisting in the improvement of photosynthetic efficiency, mycoparasitism and antibiosis, extracellular enzyme secretion, and hyphae penetration and lysis. Antagonism could be caused by nutrient and niche competitors, antibiosis caused by the generation of volatile components, and non-volatile antibiotics Lahlali et al. (2022). Inhibitory activity of *Trichoderma* spp. may be due to secretion of extracellular cell degrading enzymes such as chitinase, β-1,3-glucanase, cellulose, lectin and other secondary metabolites such as glioviridin, viridian and gliotoxin which may help mycoparasites in colonization of host Kakraliya et al. (2018). Similar results were discovered by Raghuvanshi et al. (2021); Yarasani and Zacharia (2021) and Devi et al. (2024). *Ascophyllum nodosum* applied at 2 ml/L was found to be effective in increasing plant height. It showed greater effectiveness during the vegetative phase; however, its efficacy declined during the reproductive phase against the pathogen compared to bioagents. Under pathogen stress, bioagents such as beneficial fungi or bacteria may provide a more targeted defense response by directly antagonizing the pathogen through mechanisms like antibiosis, competition, and induction of systemic resistance. In contrast, the indirect, growth-promoting effects of *A. nodosum* may not effectively counter the pathogen during this critical phase, resulting in comparatively lower efficacy.

Table 4. Effect of selected treatments on disease parameters

Treatment no.	Per cent disease intensity on leaves				AUDPC	Per cent disease reduction over control	AYL	PDI on Pods
	45 DAS	60 DAS	75 DAS	90 DAS				110 DAS
T_0	19.96	44.86	56.96	74.10	2351	-	-	35.46
T_1	10.40[a]	24	30	37.20	1245	48.87	35.72	18.73[a]
T_2	11.53[a]	27.50[bc]	35.90[b]	43.63[b]	1451	40.03	27.79	25.33[c]
T_3	16.50[b]	25.80[a]	32.80[a]	40.66[a]	1431	44.11	31.43	19.80[ab]
T_4	17.80[de]	27.70[a]	36.83[b]	45.03[b]	1566	38.11	27.33	25.46[cd]
T_5	18.36[e]	26.63[ab]	33.23[a]	40.83[a]	1475	43.88	30.36	20.46[b]
T_6	16.53[bc]	30.26	38.93	47.93	1645	34.12	20.05	27.33[d]
T_7	16.53[cd]	22	27.80	34.93	920	51.99	38.14	13.00
S.Em(±)	0.51	0.52	0.60	0.60	-	-	-	0.69
C.D.(p=0.05)	1.58	1.63	1.83	1.85	-	-	-	2.10

***** Average of three replications.

Values in the same column followed with similar alphabet are non-significant to each other at (p=0.05).

Table 5. Effect of selected treatments on growth and yield parameters

Treatment No.	Number of leaves				No. of branches	No. of siliquae/ plant	No. of seeds/ siliqua	Yield t/ha	Test weight 1000 seeds (gm)
	45 DAS	60 DAS	75 DAS	90 DAS	110 DAS	110 DAS	115 DAS	120 DAS	
T_0	19.40	92.1	148.8	159.7	3.66	133.1	7.53	1.043	3.10
T_1	34.00	121.6^{c}	169.2^{cd}	178.5	8.20^{b}	246.2	13.13^{d}	1.631^{ef}	4.46^{ef}
T_2	32.10	116.6	168.3^{bc}	176^{c}	5.80^{a}	196.5	12.33^{ab}	1.445^{bc}	4.06^{bc}
T_3	21.10^{ab}	107.7^{ab}	161.8^{a}	171.3^{ab}	8.13^{b}	227.8	12.93^{cd}	1.521^{de}	4.23^{de}
T_4	22.00^{bc}	106.7^{a}	159.8	170^{a}	5.40^{a}	185.3	11.40^{a}	1.436^{ab}	3.90^{ab}
T_5	20.80^{a}	111.5	167.8^{b}	175.6^{c}	6.53	214	12.53^{bc}	1.498^{cd}	4.23^{cd}
T_6	23.30	122.9^{c}	171^{d}	189	4.60	172.4	10.40	1.305^{a}	3.63^{a}
T_7	22.00^{c}	109^{b}	163.2^{a}	173^{b}	8.86	265.6	15.06	1.686^{f}	4.66^{f}
S.Em(±)	0.37	0.60	0.61	0.64	0.19	1.37	0.29	0.05	0.16
C.D.(p=0.05)	1.19	1.85	1.89	1.94	0.58	4.15	0.90	0.16	0.50

***** Average of three replications.

Values in the same column followed with similar alphabet are non-significant to each other at (p=0.05).

Table 6. Cost-benefit ratio of mustard as influenced by selected treatments

Tr. No.	Total cost of cultivation	Yield t/ha	Gross return	Net return	C:B ratio
T_0	34956	1.043	55336	20380	1:1.58
T_1	36410	1.631	86540	50130	1:2.37
T_2	37682	1.445	76642	38960	1:2
T_3	39456	1.521	80705	41249	1:2.04
T_4	36056	1.436	76165	40109	1:2.11
T_5	35562.2	1.498	79453	43890.8	1:2.23
T_6	36242	1.305	69217	32975	1:1.9
T_7	36323	1.686	89425	53102	1:2.46

***** Average of three replications.

Values in the same column followed with similar alphabet are non-significant to each other at (p=0.05).

CONCLUSION

The present research focuses on demonstrating the alternative eco-friendly approaches to be potential to counter *Alternaria brassicae*, one of the critical pathogens that cause significant yield loss in mustard. *T. viride* demonstrated its inherent ability to enhance plant defences through the induction of necessary defense enzymes while in parallel supporting plant growth development. Mostly all the treatments act as a sustainable and eco-friendly alternative to chemical fungicides in line with the international call for IDM approaches aimed at minimizing plant diseases while maintaining ecological integrity. However, this research is limited to just one crop season under agro-climatic conditions of Prayagraj. For broad applicability and validation, multi-seasonal trials in different agro-climatic regions, molecular and hormonal analysis are required. Such extended studies will confirm the consistency and reliability of the results and pave way for comprehensive recommendations for large-scale adoption in mustard disease management practices.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declares 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 manuscript.

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Supportive Figures

Plate 1. Pure culture of *Alternaria brassicae*

Plate **2. Disease grade chart on mustard leaves**

Plate 3. Experimental site