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# Efficacy of selected bio-agents and botanicals against Alternaria blight (*Alternaria brassicae*) of mustard (*B. juncea* L.)

## Geethika Yarasani and Sunil Zacharia

### Abstract
Mustard (*Brassica juncea* L.) is one among the major oil seed crops in India grown during *Rabi* season. Alternaria blight caused by *Alternaria brassicae* is one of the commonly found pathogen in mustard. The pathogen can affect host species at all stages of growth, including seed. A field experiment was conducted at Central Research Field, Department of Plant Pathology, SHUATS, Prayagraj, Uttar Pradesh during *Rabi* 2020- 2021 to evaluate the effect of selected bio-agents and botanicals *in-vivo* by foliar spray on Alternaria blight incidence. Maximum plant height (166.73 cm), maximum number of branches per plant (13/plant), maximum number of number of siliques (217.46), maximum number of seeds per siliques (7.73), minimum disease intensity (%) (48.8), maximum test weight (4.48 gm) and maximum yield (1.356 t ha-1) were recorded in the treatment $T_1$ – *Trichoderma viride* @10g/l followed by $T_2$ – *Pseudomonas fluorescens*@10g/l, $T_4$ – Mycel @ 250 gm/acre and $T_3$ – leaf extract of *Lantana camara* @10% as compared to $T_5$ – mancozeb @0.2% and untreated control $T_0$. Higher gross return value (Rs. 54240 ha-1), net return value (Rs. 24640 ha-1), and B: C ratio (1.83) was found in the treatment $T_1$ – *Trichoderma viride* @10g/l as compared to $T_5$–Mancozeb and $T_0$–control.

**Keywords:** Alternaria blight, *Alternaria brassicae*, bio-agents, botanicals, *Pseudomonas fluorescens*, *Trichoderma viride*

### Introduction
Mustard (*Brassica juncea* L.) is the principle oilseed crop in India grown during *Rabi* season. Diseases like Alternaria blight, white rust, downy mildew and powdery mildew attacks the crop and disturbs the normal physiological function of the crop during growth which leads to the lower productivity. Among these diseases, Alternaria blight is the major disease and is distributed worldwide and is reported to cause losses up to 70% depending on the crop species (Kolte *et al.*, 1987; Meena and Sharma, 2012) [7, 11]. The most commonly found pathogen *Alternaria* is responsible for the disease which is a polyphagous fungus and occurs most frequently as a saprobe on dead and decaying organic material on/in seed and is liable for causing leaf spot diseases (Mehrotra and Aneja, 2003) [12].

Four species of *Alternaria, viz.*, *A. brassicae* (Berk.) Sacc., *A. brassicicola* (Schw.), *A. raphani* Grooves and Skolka, *A. alternate* (Fr.) Keissler are reported for the cause of Alternaria blight. Among the species, *Alternaria brassicae* is found wide spread and infect all parts of the plant such as leaves, pods, branches and stem but the special target point of fungus are leaves and pods. Symptoms of the disease are characterized by the formation of spots on leaves, stem and siliqua (Prasad and Lallu, 2006 and Nayyar *et al.*, 2014) [15, 13]. Often lesions are produced on green leaves and due to sever attack in pods, seeds become shrivel and early ripening or shattering. Infection of seeds by the pathogen may result in poor germination and can also deteriorate both quality and quantity of oil content (Kolte, 1985 and Gagandeep *et al.*, 2020) [8, 3]. Conventionally plant diseases are controlled by applying fungicides, but this practice increase input cost on the crop on one hand and on the other hand cause environmental pollution. So an attempt was made to evaluate the efficacy of bio-agents and botanicals against Alternaria blight of mustard *in-vivo*.

### Materials and Methods
**Experimental site:** The experiment was conducted at the Central Research Field, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, Uttar Pradesh. The experiment was conducted during *Rabi* season 2020 – 2021. The field was laid out in random block design with three replications.

**Methodology Collection of the disease samples**

Plants showing typical symptoms, in the field of standing crop i.e., the infected plant parts of mustard were selected. These infected plant materials were brought to the lab for further investigation.

**Identification of the fungus**

Examination of the fungal colony characteristics was done through microscopic examination. Symptoms are characterized by the formation of spots on leaves, stem and siliqua. Often lesions are produced on green leaves and during severe attack in pods, seeds become shrivel and early ripening or shattering. Foliar infection by the pathogen can be identified by greyish center brown to black spots with concentric rings with difference in the symptoms according to the host and environmental conditions. The tissues, with typical symptoms were mounted on compound microscope, were used for the examination of morphological characteristics of fungal structures (Grahovac *et al*., 2012) [4].

**Morphological characters of *Alternaria brassicae***

Mycelium is septate, brownish to grey and the conidiophores arise in fascicles and the conidia are dark, obclavate, muriform, borne singly or in short chains and conidia contains long beak which was usually pale brown, short, cylindrical (Manika *et al*., 2013) [10].

**Observations recorded**

Pre-harvest and post-harvest observations were recorded during the period of experiment. Preharvest observations were height of the plant (cm), number of branches per plant, number of siliquae per plant, number of seeds per siliqua, disease intensity (%) and post-harvest observations were yield (t/ha), test weight of seeds (1000 number) (gm) and economics of the treatments.

**Disease intensity scale**

Assessment of disease intensity was done by score card method following a five-point scale (0-5) for scoring leaf spot diseases. From each plot, 5 plants were randomly selected and tagged.

Disease intensity (%) was recorded at 45, 60, 75 days after incidence of Alternarial blight.
Disease intensity (%) is calculated using the formula as mentioned by Conn *et al*. (1990) [2].

**Table 1:** Grade chart

| Grade | Leaf and Pod area covered | Reaction |
|---|---|---|
| 0 | No symptoms on leaves | Immune |
| 1 | Small light brown spots covering 1% or less leaf area | Highly resistant |
| 2 | Small spots (up to 5mm in size) covering 1-10% of the leaf area | Resistant |
| 3 | Large spots, brown, irregular with concentric rings covering 10-25% of leaf area | Moderately resistant |
| 4 | Large brown irregular lesions with typical blight symptoms covering 25-50% of leaf area | Moderately susceptible |
| 5 | Spots enlarging, covering more than 50% of leaf area | Highly susceptible |

**Results and Discussion**

**Effect of treatments on plant height (cm) of Mustard**

**Plant height (cm)**

The statistical analysis of data presented in the table 2 showed that all the treatments were significantly effective and increased plant height (cm) as compared to untreated control. The plant height of mustard was significantly increased in $T_1$-foliar spray of *Trichoderma viride*@10g/l (166.73 cm), followed by $T_2$ -foliar spray of *Pseudomonas fluorescens*@ 10g/l (158.66 cm), $T_4$ foliar spray of Mycel @ 250g/acre (156.00 cm) and $T_3$ -foliar spray of leaf extract of *Lantana camara* @ 10% (151.66 cm) as compared to treated $T_5$ -foliar spray of Mancozeb @ 0.2% (180.46 cm) and $T_0$- untreated check (108.20 cm). All the treatments were significant over untreated control ($T_0$).

**Number of branches per plant**

The statistical analysis of data presented in the table 2 showed that all the treatments were statistically significant as compared to untreated control. The highest number of branches was recorded in $T_1$-foliar spray of *Trichoderma viride* @10g/l (13/plant), followed by $T_2$-foliar spray of *Pseudomonas fluorescens* @ 10g/l (12.8/plant), $T_4$-foliar spray of Mycel @ 250g/acre (12.6/plant) and $T_3$-foliar spray of leaf extract of *Lantana camara* @ 10% (12.2/plant) as compared to treated $T_5$ -foliar spray of Mancozeb @ 0.2% (15.26/plant) and $T_0$ - untreated check (9.53/plant). All the treatments were significant over the untreated control ($T_0$), among the treatments ($T_1$, $T_2$ and $T_4$; $T_2$, $T_4$ and $T_3$) were non-significant to each other.

**Number of siliquae per plant**

The statistical analysis of data presented in the table 2 showed that all the treatments were statistically significant as compared to untreated control. The maximum number of siliques was observed in $T_1$ -foliar spray of *Trichoderma viride* @10g/l (217.46), followed by $T_2$ -foliar spray of *Pseudomonas fluorescens* @ 10g/l (210.13), $T_4$ -foliar spray of Mycel @ 250g/acre (206.66) and $T_3$ -foliar spray of leaf extract of *Lantana camara* @ 10% (202.86) as compared to treated $T_5$ -foliar spray of Mancozeb @ 0.2% (233.33) and $T_0$ - untreated check (194.20). All the treatments were significant over the untreated control ($T_0$), among the treatments ($T_2$ and $T_4$; $T_4$ and $T_3$) were non-significant to each other.

**Number of seeds per siliqua**

The statistical analysis of data presented in the table 2 showed that all the treatments were statistically significant as compared to untreated control. The maximum number of seeds per siliqua per plant was observed in $T_1$-foliar spray of *Trichoderma viride*@10g/l (7.73), followed by $T_2$ -foliar spray of *Pseudomonas fluorescens* @ 10g/l (7.66), $T_4$ -foliar spray of Mycel @ 250g/acre (7.06) and $T_3$ -foliar spray of leaf extract of *Lantana camara* @ 10% (6.46) and as compared to treated $T_5$ -foliar spray of Mancozeb @ 0.2% (8.40) and $T_0$ - untreated check (6.44). All the treatments were significant over the untreated control ($T_0$), among the treatments ($T_1$ and $T_2$; $T_3$ and $T_0$) were non-significant to each other.

**Table 2:** Effect of bio-agents and botanicals on plant height (cm), No. of branches per plant, No. of siliquae per plant and No. of seeds per siliqua

| Tr. No. | Treatments | Plant height (cm) | | | No. of branches per plant | No. of siliquae per plant | No. of seeds per siliqua |
|---|---|---|---|---|---|---|---|
| | | 45DAS | 60DAS | 75DAS | | | |
| T0 | Control (untreated check) | 29.06 | 55.46 | 108.20 | 9.53 | 194.20 | 6.44 |
| T1 | *Trichoderma viride* | 40.54 | 82.00 | 166.73 | 13.00 | 217.46 | 7.73 |
| T2 | *Pseudomonas fluorescens* | 34.33 | 76.46 | 158.66 | 12.80 | 210.13 | 7.66 |
| T3 | Leaf extract of *Lantana camara* | 30.43 | 66.70 | 151.66 | 12.20 | 202.86 | 6.46 |
| T4 | Mycel | 30.88 | 71.13 | 156.00 | 12.60 | 206.66 | 7.06 |
| T5 | Mancozeb | 41.40 | 89.89 | 180.46 | 15.26 | 233.33 | 8.40 |
| | S.Em+ | 0.22 | 0.27 | 0.41 | 0.33 | 0.597 | 0.23 |
| | CD (5%) | 0.68 | 0.85 | 1.28 | 0.76 | 3.93 | 0.54 |

**Disease intensity (%)**

The statistical analysis of data presented in the table 3 showed that all the treatments were significantly effective and minimized the disease intensity (%) as compared to untreated control. Result showed that the minimum disease intensity was observed in $T_1$ -foliar spray of *Trichoderma viride* @10g/l (48.8%), followed by $T_2$ -foliar spray of *Pseudomonas fluorescens* @10g/l (51.8%), $T_4$ -foliar spray of Mycel @ 250g/acre (53.2%) and $T_3$ -foliar spray of leaf extract of *Lantana camara* @ 10% (57.7%) as compared to treated $T_5$ -foliar spray of Mancozeb @ 0.2% (37.20%) and $T_0$ - untreated check (64.40%). All the treatments were significant over the untreated control ($T_0$), among the treatments ($T_2$ and $T_4$) found non-significant with each other.

**Seed yield (t/ha)**

The results presented in the table 3 showed that all the treatments significant over control and increased the yield (t/ha). The maximum yield was observed in $T_1$ -foliar spray of *Trichoderma viride* @10g/l (1.356 t/ha), followed by $T_2$ -foliar spray of *Pseudomonas fluorescens* @ 10g/l (1.28 t/ha), $T_4$ -foliar spray of Mycel @ 250g/acre (1.106 t/ha) and $T_3$ -foliar spray of leaf extract of *Lantana camara* @ 10% (1.076 t/ha) as compared to treated $T_5$ -foliar spray of Mancozeb @ 0.2% (1.406 t/ha) and $T_0$ - untreated check (0.924 t/ha). All the treatments were significant over untreated control ($T_0$).

**Test weight of seeds (1000 number) (gm)**

The results presented in the table 3 showed that all the treatments significant over control and increased the test weight (1000 number) (gm). The highest test weight was found in $T_1$ -foliar spray of *Trichoderma viride*@10g/l (4.48 gm), followed by $T_2$ -foliar spray of *Pseudomonas fluorescens*@ 10g/l (4.48), $T_4$ -foliar spray of Mycel @ 250g/acre (4.40 gm), and $T_3$ -foliar spray of leaf extract of *Lantana camara* @ 10% (4.36 gm) as compared to treated $T_5$ -foliar spray of Mancozeb @ 0.2% (5.20 gm) and $T_0$ - untreated check (3.97 gm). All the treatments were significant over the untreated control ($T_0$), among the treatments ($T_1$, $T_2$ and $T_4$; $T_4$ and $T_3$) non- significant to each other but significant overall other treatments.

**Cost-benefit ratio**

The results presented in the table 2 showed that the cost of cultivation (Rs. 29600/-) with highest gross returns (Rs. 54240/-) and B: C ratio (1.83) were recorded in treatment $T_1$ - Foliar spray with *Trichoderma viride*@10g/l followed by treatment $T_2$ - Foliar spray with *Pseudomonas fluorescens* @10g/l with cost of cultivation (Rs. 29600/-) with gross returns (Rs. 51240/-) and B: C ratio (1.73). The lowest was recorded in $T_0$ untreated control with cost of cultivation (Rs, 26100/-) with gross return (Rs. 36960/-) and B: C ratio (1.41).

**Table 3:** Effect of bio-agents and botanicals on disease intensity (%), test weight (gm), yield (t/ha) and B:C ratio

| Tr. No. | Treatments | Disease intensity (%) | | | Test Weight of seeds (1000 number) (gm) | Yield (t/ha) | B:C ratio |
|---|---|---|---|---|---|---|---|
| | | 45DAS | 60DAS | 75DAS | | | |
| T0 | Control (untreated check) | 37.30 | 47.96 | 64.40 | 3.97 | 0.924 | 1.41 |
| T1 | *Trichoderma viride* | 24.66 | 29.73 | 48.80 | 4.48 | 1.356 | 1.83 |
| T2 | *Pseudomonas fluorescens* | 28.16 | 31.20 | 51.86 | 4.48 | 1.281 | 1.73 |
| T3 | Leaf extract of *Lantana camara* | 32.86 | 37.73 | 57.86 | 4.36 | 1.076 | 1.55 |
| T4 | Mycel | 28.80 | 35.33 | 53.20 | 4.40 | 1.106 | 1.57 |
| T5 | Mancozeb | 13.33 | 21.33 | 37.20 | 5.20 | 1.406 | 2.03 |
| | S.Em+ | 0.22 | 0.649 | 1.15 | 1.18 | 0.063 | |
| | CD (5%) | 0.68 | 1.441 | 2.562 | 2.652 | 0.108 | |

**Discussion**

Use of bio-agents for controlling plant diseases is an age-old practice all over the world and also fast gaining importance due to its efficiency (Jackson and Kumar, 2019) [5]. Biocontrol agents contain anti-fungal properties that suppress the growth of the fungus. It is suggested that constituents of bio-control agents can be used as eco-friendly natural compounds for controlling Alternaria leaf spot of Mustard. Among the bio-agents, *Trichoderma viride* and *Pseudomonas fluorescens* are most widely used against *Alternaria brassicae* (Yogita *et al.,* 2017) [18]. The probable reason may be species of the genus *Trichoderma viride* possess many qualities and they have great potential use in agriculture such as amend abiotic stresses, improving physiological response to stresses, alleviating uptake of nutrients in plants, enhancing nitrogen use efficiency in different crops, and assisting to improve photosynthetic efficiency and mycoparasitism which includes, chemotropic growth of *Trichoderma viride*, recognition of the host by the mycoparasites, secretion of extra cellular enzymes, penetrations of the hyphae and lysis of the host (Rai *et al.,* 2014 [16] and Mahapatra and Das, 2013 [9]). *Pseudomonas fluorescens* embodies an attractive biocontrol agent because of their catabolic adaptability, their outstanding root-colonizing abilities, and their capacity to produce a wide range of antifungal metabolites. *Pseudomonas fluorescens* exerts its bio-control activity through direct antagonism of phytopathogens and induction of disease resistance in the host (Neetam *et al.*, 2018 and Shakywar *et al.*, 2012) [14, 17]. The use of bio-agents in the control of Alternaria species have many advantages over the agrochemical traditional use, such as the fast degradation, low toxicity in the environment low cost of production, and lack of health risks to the producer and final consumer. It has the ability to inhibit the growth of *Alternaria brassicae*. It lowers the health risk along with being economical and eco-friendly makes the bio-agents a suitable ingredient for the development of a natural fungicide, which can reduce the use of chemical fungicides. The results obtained proved that application of botanicals could be bio-pesticidal and eco-friendly substitute for chemical fungicides in management of Alternaria blight of mustard along with bio-agents as observed by Kalidas *et al*. (2017) [6] and Biswas and Gosh (2018) [1].

## Conclusions

The *in-vivo* results revealed that plant height (cm) 45, 60, 75 DAS, number of branches per plant, number of siliquae per plant, number of seeds per siliqua, test weight of seeds (1000 number) (gm) and yield (t/ha) significantly increased and disease intensity (%) in mustard at 45, 60,75 DAS significantly decreased in the treatment $T_1$ -foliar spray of *Trichoderma viride*@10g/l. The higher gross return value, net return value and B:C ratio were also recorded. To reduce the use of fungicides and to control the environmental pollution and soil health many researches are undergoing, one such attempt is the use of bio-agents and botanicals. Thus, the present study indicated that suitable integration of more efficient eco-friendly treatments like bio-agents and botanicals may provide a better and effective management of Alternaria blight disease of mustard but there is a scope for more experimental trials to further validate the results on larger basis. The findings of the present experiment are limited to one crop season under Prayagraj agro-climatic conditions, as such to validate the present findings more such trials should be carried out in future.

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## References

1. Biswas MK, Ghosh T. Evaluation of Phyto-extracts, Botanical Agents and Chemicals against the Development of Alternaria brassicae *in-vitro* and *in-vivo*. European Journal of Medicinal Plants. 2018;22(3):1-9.
2. Conn KL, Tewari JP, Awasthi RP. A disease assessment key for Alternaria blackspot in rapeseed and mustard. Canadian Plant Disease Survey. 1990;70:1.
3. Gagandeep SS, Amanpreet S, Bahaderjeet S. Symptoms and Management of Alternaria blight of Indian Mustard (*Brassica juncea*). Journal of Plant Development Sciences. 2020;12(3):165-170.
4. Grahovac M, Indic D, Vukovic S, Hrustic J, Gvozdenac S, Mihajlovic M, *et al*. Morphological and ecological features as differentiation criteria for different species. Zemdirbyst Agriculture. 2012;99:189-196.
5. Jackson KS, Kumar A. Management of Alternaria leaf spot on Indian mustard through chemical and biological agents. Plant Cell Biotechnology and Molecular Biology. 2019;20(3-4):162-178.
6. Kalidas DK, Lal AA, Simon S. Efficacy of Bio-Agents and Plant Extracts against the Alternaria leaf spot of cabbage (*Alternaria brassicae*). Journal of Pharmacognosy and Phytochemistry. 2017;6(4):1980-1982.
7. Kolte SJ, Awasthi RP, Vishwanath. Assessment of yield losses due to Alternaria blight in rapeseed and mustard. Indian Phytopath. 1987;40:209-211.
8. Kolte SJ. Diseases of Annual Edible Oilseed Crops, Rapeseed-Mustard and Sesame Diseases, CRC Press Inc. Boca Raton, Florida. 1985;2:135.
9. Mahapatra S, Das S. Bio efficacy of botanicals against Alternaria Leaf Blight of Mustard under Field Condition. The Bioscan. 2013;8(2):675-679.
10. Manika S, Swati D, Dinesh SB, Chowdappa P, Selvamani R, Sharma P. Morphological, cultural, pathogenic and molecular studies of *Alternaria brassicae* infecting cauliflower and mustard in India. African Journal of Microbiology Research. 2013;7(26):3351-3363.
11. Meena PD, Sharma P. Antifungal activity of plant extracts against *Alternaria brassicae* causing blight of *Brassica* spp. Annals of Plant Protection Science. 2012;20(1):256-257.
12. Mehrotra RS, Aneja KR. An Introduction to Mycology. New Age International (P) Ltd. New Delhi, 2003, 594-610.
13. Nayyar BG, Akhund S, Akram A. Management of Alternaria and its mycotoxins in crops. Scholarly Journal of Agricultural Science. 2014;4(7):432-437.
14. Neetam RS, Mukherjee SC, Salam JL, Neetam PS. Management of Alternaria leaf and pod blight diseases of Mustard through combination of bio-agents, Fungicides, Micronutrients and Cultural operations in Bastar Plateau of Chhattisgarh. International Journal of Current Microbiology and Applied Sciences. 2018;7(11):1239-1243.
15. Prasad R, Lallu. Management of Alternaria blight of mustard with combination of chemicals and botanicals. Annals of Plant Protection Science. 2006;14(2):313320. Publisher. 527.
16. Rai D, Mishra AK, Pandey A. Integrated Management of Alternaria blight in Indian Mustard. The Bioscan. 2014;9(3):1247-1249.
17. Shakywar RC, Dinesh S, Pathak M, Singh AK. Integrated approaches for management of foliar diseases in Indian Mustard. Journal of Plant Disease Sciences. 2012;7(2):154157.
18. Yogita T, Zacharia S, Chauhan BS. Efficacy of bio-agents and plant extracts against Alternaria leaf blight of mustard (*Brassica juncea* L.). European Journal of Biotechnology and Bioscience. 2017;5(4):29-35