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Effects of Bio-inoculation on the Growth of Cabbage as Well as the Disease Progression of Alternaria Leaf Spot

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Cabbage is one of the common Cole crop which is widely grown throughout India. It is affected various disease including, Alternaria leaf spot, black rot, club root, downy mildew and powdery mildew. Alternaria leaf spot, caused by Alternaria species such as *A. brassicae* and *A. brassicicola*,

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has been documented on every continent and is a prevalent disease affecting cabbage. Among the various diseases impacting cabbage, it stands out as a major contributor to yield loss, averaging between 32-57%. An experiment Effects of bio-inoculation on the growth of cabbage seedlings, as well as the disease progression of Alternaria leaf spot under challenge inoculation was conducted at Department of Plant Pathology, Rama University, Kanpur during 2023. Result of the study revealed that. application of Trichoderma as a seed treatment led to higher root and shoot dry biomass (0.080 and 0.021 mg/ seedling respectively), higher % increase in root and shoot dry weight (61.25and 82.78%) was found in T_1 (Seed treatment with *trichoderma harzianum*) whereas highest root: shoot ratio (0.222) was reported in treatment T_2.

Keywords: Alternaria leaf spot; Trichoderma; P. fluorescence; seed treatment.

1. INTRODUCTION

Cabbage is one of the most popular crucifer vegetable grown in world. India is one of the important cabbage growing country in Asia. India is next to China in cabbage production besides, good technology and certified seeds, the desirable production is not achieved because of damages caused by insect pests and diseases [1]. Alternaria black leaf spot disease is one the most destructive disease of cabbage and brassicas worldwide [2]. A complex of Alternaria species (*A. brassicicola* (Schw.) Wiltsh., *A. brassicae* (Berk.) Sacc., *A. alternata* (Fr.) Kreissler and *A. raphani* Groves and S kolko) are responsible for considerable yield loss [2]. The pathogens are greatly influenced by weather with the highest disease incidence reported in mild, wet seasons and in areas with relatively high rainfall the pathogen appears on leaves and stems of cabbage seedlings and adult plants). *A. brassicae* and *A. brassicicola* can affect host species at all stages of growth, including seeds. On seedlings symptoms include dark stem lesions immediately after germination that result in damping-off, or stunted seedlings [3]. In addition to destruction of a seed crop, the pathogens can live within the seed, spread the disease to other fields, and cause loss seedlings [4].

2. MATERIALS AND METHODS

The impact of bio-inoculation with *Trichoderma harzianum* and Pseudomonas fluorescence on the physical health of cabbage seedlings under potted conditions was investigated during the 2022-23 *rabi* crop season at the Department of Plant Pathology, Rama University, Kanpur. Nine different treatments were applied: seed treatment, soil application, seed treatment + soil application, seed treatment + foliar application, and foliar application, using Trichoderma harzianum, Pseudomonas fluorescence, and a combination of Trichoderma harzianum + Pseudomonas fluorescence.

2.1 Bio Agents

Pure cultures of *Trichoderma harzianum* (UBT18) and Pseudomonas fluorescence (VPF-1) were obtained from the Department of Plant Pathology, Rama University, Kanpur. Pseudomonas fluorescence strain VPF-1 was mass multiplied in King´s B broth in 250 mL Erlenmeyer flasks, incubated at 28ºC for 48 hrs in a shaker incubator. The bacterial strain's cell suspension was adjusted to a concentration of 106 CFU/ml and mixed with talc and Carboxy methyl cellulose. *Trichoderma harzianum* (UBT18) was mass multiplied in potato dextrose broth, and after full mycelial growth, the media were mixed with talc and Carboxy methyl cellulose.

2.2 Preparation of Pathogen Inoculums

Alternaria sp. was isolated from cabbage leaves affected by leaf spot. The fungal inoculums were obtained from petri dishes and transferred to potato dextrose broth (PDB), left for 7 days of incubation. After complete pathogen growth, the mycelial mat was harvested and homogenized. Conidial concentration was determined using a haemocytometer and adjusted to 5 x 105 conidia per mL. Plants were labeled and spray-inoculated with a conidial suspension of the pathogen using a 1000 mL handheld atomizer directed at the central part of the upper leaf side. Approximately 0.3 mL of conidial suspension per leaf was applied to the plants.

2.3 Mode of Application

Seeds were treated with *Trichoderma harzianum* and Pseudomonas fluorescence at a rate of 5 grams per kg of seed, applied 30 minutes before sowing. *Trichoderma harzianum* and Pseudomonas fluorescence were applied to the

soil at a rate of 12.5 kg per hectare. Foliar spray of *Trichoderma harzianu*m and Pseudomonas fluorescence, at a 1% concentration of talc-based formulation, was done 5 days before pathogen inoculation.

2.4 Plant Growth Promotion

The effectiveness of *Trichoderma harzianum* and Pseudomonas fluorescence in promoting plant growth was evaluated through an assessment of seedling health. The vigour index was determined using the formula outlined by Abdul Baki and Anderson (Abdul et al., 1973), which involves calculating the sum of the mean root length and the product of the mean shoot length and germination percentage.

2.5 Shoot and Root Dry Weight

Seedlings at the transplanting stage kept at hot air over for 7 days and measured the shoot and root dry biomass.

2.6 Percent Disease Index

Percent disease index (PDI) was calculated for each plot by summing the scores of twenty leaves and analyzing using rating scale. The value was expressed as percentage using the following formula:

$$\text{PDI (\%)} = \frac{\text{Sum of all rating X 100}}{\text{No. of leaves examined x maximum rating score}}$$

Scoring of disease was done according to (Kulibaba, 1972)

Rating	Symptoms
0	Up to 5% leaf area covered
1	Up to 5% leaf area covered
3	5-10% leaf area covered
5	11-25% leaf area covered
7	26-50% leaf area covered
9	More than 50% leaf area covered

3. RESULTS AND DISCUSSION

3.1 Effect on Cabbage Seedling

Shoot dry wt. (mg/seedling): As the data presented in Table 1 and displayed Fig. 1 showed that among various treatments highest shoot dry wt. (0.080 mg/seedling) was reported in treatment T_1 = Seed treatment with *trichoderma harzianum* followed by T_4 = Seed treatment with trichoderma *harzianum* + Seed treatment with Trichoderma *harzianum* (0.074mg/seedling) whereas minimum shoot dry wt. was found in T_2 = Soil application of trichoderma. These findings are accordance with Dharmendra et al. [5].

Root dry wt. (mg/ seedling): Data regarding to root dry wt. is presented in Table 1 and Fig. 2 showed that maximum root dry wt. (mg/ seedling) was reported in T_1 = seed treatment with *trichoderma harzianum* (0.021 mg/ seedling) followed by T_3 = Seed treatment with trichoderma *harzianum* + Seed treatment with trichoderma *harzianum* (0.020 mg/ seedling) whereas minimum root dry wt. (mg/ seedling) in T_5 =Soil application of *Pseudomonas fluorescence* (0.016 mg/ seedling). Similar findings were reported by Abhijit et al. [1].

Root: shoot: The data of root: shoot ratio varies 0.185- 0.222. Maximum ratio of root: shoot was reported in T_2 = Soil application of trichoderma (0.222) followed by T_7 = Seed treatment with *Trichoderma harzianum*+ *Pseudomonas fluorescence* (0.217) while minimum root: shoot was reported in 0.185. (Table 1 and Fig. 3.). These results are closely related with the findings of Chaddha et al. [6].

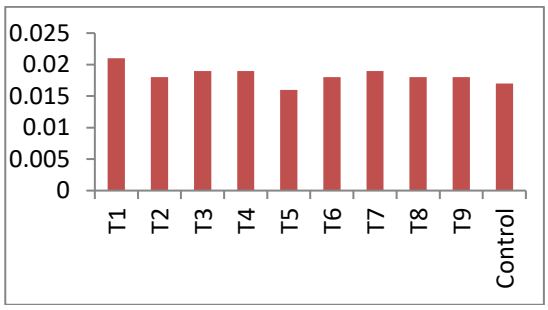

Fig. 1. Shoot dry wt.(mg/seedling)

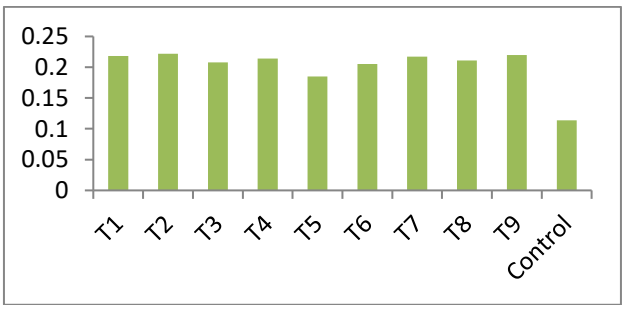

Fig. 2. Root dry wt.(mg/ seedling)

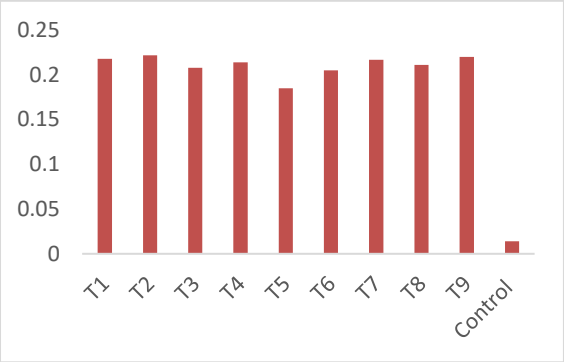

Fig. 3. Root: Shoot

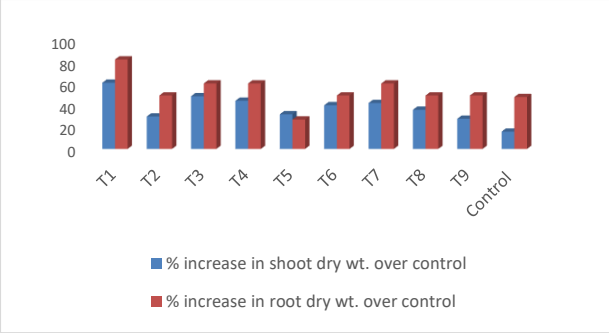

Fig. 4. % increase in shoot and root dry wt. over control

Table 1. Effect of bio- inoculation on dry bio mass of cabbage seedlings

Treatment	Shoot dry wt. (mg/seedling)	Root dry wt.(mg/ seedling)	Root: shoot	% increase in shoot dry wt. over control	% increase in root dry wt. over control
T_1 = Seed treatment with *trichoderma harzianum*	0.080	0.021	0.218	61.25	82.78
T_2 = Soil application of trichoderma	0.060	0.018	0.222	30.00	49.44
T_3 = Seed treatment with trichoderma *harzianum* + Seed treatment with *trichoderma harzianum*	0.074	0.020	0.208	48.75	60.56
T_4 = Seed treatment with *Pseudomonas fluorescence*	0.072	0.019	0.214	44.58	60.55
T_5= Soil application of *Pseudomonas fluorescence*	0.066	0.016	0.185	32.08	27.22
T_6 = Seed treatment with *Pseudomonas fluorescence* + Soil application with *Pseudomonas fluorescence.*	0.070	0.018	0.205	40.42	49.44
T_7 = Seed treatment with trichoderma *harzianum+ Pseudomonas fluorescence*	0.071	0.019	0.217	42.5	60.53
T_8=, Soil application of trichoderma *harzianum+ Pseudomonas fluorescence*	0.068	0.018	0.211	36.25	49.44
T_9 = Seed treatment and Soil application of trichoderma *harzianum+ Pseudomonas fluorescence*	0.064	0.018	0.220	27.92	49.44
T_{10} = Control	0.058	0.0027	0.014	16.00	48.00
SEm±	0.00367	0.00428	0.00576	0.00616	0.00428
CD (P=0.05)	0.00124	0.01219	.00358	0.00219	0.00567

% increase in shoot dry wt. over control: Values of % increase in shoot dry wt. are showed in Table 1 and Fig. 4. T_1 = Seed treatment with *trichoderma harzianum* (61.25%) followed by T_3 = Seed treatment with trichoderma *harzianum* + Seed treatment with trichoderma *harzianum.* (48.75%) whereas minimum in control (16.00%). These findings are partially related with Kucuk et al*.,* [7] and Tra et al*.* [8] % increase in root dry wt. over control: The data about % increase in root dry wt. over control in presented in Table 1 and Fig. 4. Maximum % increase in root dry wt. was found in T_1 = Seed treatment with *trichoderma harzianum* (82.78%) followed by T_3 = Seed treatment with trichoderma *harzianum* + Seed treatment with trichoderma *harzianum* (60.56%) and minimum in control (48.00%). These result are accordance with Sharma et al*.,* [9], Harman et al. [10], Singh et al. [11].

4. CONCLUSION

Seed treatment with *Trichoderma harzianum* proved to be the most effective treatment in promoting both shoot and root growth, as evidenced by the highest shoot and root dry weights and the highest percentage increases over the control group. Additionally, soil application of *Trichoderma harzianum* showed a significant impact on root development, as indicated by the highest root: shoot ratio. These findings suggest that *Trichoderma harzianum*, especially when applied to seeds, can be a

valuable tool in enhancing plant growth and productivity. Further research could explore the mechanisms underlying the observed effects and optimize application methods for agricultural practices.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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