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Effect of biochar and seed priming with *Trichoderma* spp. on the occurrence of Alternaria blight in rapeseed mustard (*Brassica juncea* L.)

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Abstract
Brassica juncea is an important oilseed crop of the family Brassicaceae. In India, its productivity is limited by various plant diseases including Alternaria blight. To evaluate the effect of biochar application and seed priming with *Trichoderma* spp. (*T. harzianum* and *T. viride*) on the growth, yield, and disease parameters of rapeseed mustard (*B. juncea* var. PBR 91), the present study was conducted during the *rabi* season of 2024-25. The field experiment was laid out in a randomized block design (RBD) with ten different treatments of biochar, *Trichoderma* spp., and the combinations thereof. Out of different combinations tested, the treatment T_4 *i.e.*, RDF (recommended dose of fertilizers) + seed priming (SP) with *T. viride* 50% + *T. harzianum* 50%, significantly reduced the number of days to 50% germination (4.50 DAS), and increased plant height (208.93 cm at 90 DAS), number of leaves (58.51), number of branches (28.33), promoted early flowering (61.50 DAS), and improved plant and shoot fresh weights (91.20 g at 90 DAS and 44.43 g, respectively). It was also useful in increasing the number of siliquae (235.50), seeds per siliqua (15.80), and seed yield per plot (668.16 g). The treatment T_4 also delayed the initial appearance of Alternaria blight (83 DAS) and showed the lowest disease incidence (48.29%), severity (21.09%), and AUDPC value (447.51). In contrast, the control treatment, T_1 (RDF only) recorded the poorest performance in all parameters including maximum disease incidence (54.54%), severity (29.12%), and AUDPC value (716.40). Interestingly, biochar treatments (alone or in combination with *Trichoderma* spp.) showed some improvement in all the parameters than the control, but were less effective than seed priming with the fungal biocontrol agent, *Trichoderma* spp. (especially, the treatment T_4). Therefore, it may be concluded that seed priming with a balanced mixture of *T. harzianum* and *T. viride* (both at 50% dose) is quite effective in enhancing mustard growth, yield, and in reducing Alternaria blight incidence under field conditions.

Keywords: Biocontrol agent, sustainable agriculture, seed priming, plant disease, area under disease progress curve, disease management

Introduction
Brassica juncea (L.) Czern., commonly known as brown mustard, is a major oilseed crop of South Asia and China, and an important nutraceutical [1]. Mustard is rich in glucosinolates, flavonoids, anthocyanins, chlorophylls, β-carotene, and ascorbic acid [2], and is also used in herbal medicine [3]. Mustard has been cultivated since 3000 BC and was introduced from China to Northern India [1,4]. It plays a vital role in the Indian oilseed economy, contributing 28.6% to total oilseed production [5], with a record 12 million tonnes produced in 2023-24 [6]. However, its productivity is limited by several diseases *viz.*, Alternaria blight, White rust, Downy mildew, Sclerotinia rot and Powdery mildew. Alternaria blight caused by *A. brassicae* is one of the most devastating disease of mustard [7]. Yield losses range between 10-70% depending on severity [8]. The disease produces concentric brown spots on leaves, stems, and siliquae [9,10]. It is generally spread via seeds, plant debris, soil, and weed hosts. The spread of the disease is affected by various climatic conditions, and its incidence peaks during wet seasons with relatively high rainfall. [7]. Infection of the siliqua impairs seed development, leading to reductions in seed weight, alterations in seed coloration, lower oil content, and a general decline in seed quality [11].

Chemical fungicides like Mancozeb and Hexaconazole are generally used to manage the disease [12,13,14], but excessive use leads to environmental hazards [15,16] and can also lead to the development of fungicide-resistant pathogen strains [17,18]. Biochar, a solid carbon rich product obtained from the process of thermo-chemical conversion of the organic-biomass in oxygen limited condition, generally referred to as pyrolysis, improves soil fertility, microbial dynamics, and induces systemic resistance [19,20,21]. It induces systemic resistance in plants (ISR/SAR) by activating different defense pathways like salicylic acid, ethylene signaling, enhancing antioxidant enzyme activity and priming defense gene expression [22]. Further, the use of biochar results in an increase in population of beneficial fungi such as *Pseudomonas* spp., or Arbuscular mycorrhizal fungi (AMF) [23].

Integrating biological control agents (such as beneficial fungi and bacteria), in plant disease management strategies, is becoming increasingly important as a sustainable alternative for conventional plant disease management tactics. They employ various mechanisms, including competition, parasitism, and induction of plant resistance for their action [24]. Seed bio-priming, an eco-friendly and sustainable technique, has emerged as a promising strategy for enhancing crop resilience against diseases. Bio-priming involves treating seeds with beneficial microbes including bio-control agents, such as *Trichoderma* spp., to improve germination, vigor, and pathogen resistance. Seed treatments with biocontrol agents (BCAs) is an effective method for controlling soil-borne diseases [25].

Use of microbial products in agriculture is an age-old practice, but has recently received increased attention owing to its sustainable and eco-friendly nature [26]. The potential use of the *Trichoderma* spp., as a biocontrol agent was suggested about 85 years ago by Weindling [27]. Various species of the fungal genera *Trichoderma* are considered very important ecologically, as they serve as sources of variety of antibiotics, enzymes, act as plant growth promoters, xenobiotic degraders, and most importantly, have been now well-established as the commercial bio-fungicides [28]. *Trichoderma* spp. also act as plant growth stimulator due to their inherent properties of mineral solubilization, root colonization, symbiosis, nutrient uptake, production of phytohormones and secretions of enzymes [29]. Its interaction with crop plants can result in root colonization, which triggers morphological changes in the plant roots [30] and promote growth of plant in the form of increased root density, improved nutrient uptake, mineral solubilization, and induced defense response against various biotic and abiotic stresses [31]. *Trichoderma* spp. particularly *T. harzianum* and *T. viride*, exhibit strong antagonistic activity against *A. brassicae*, with dual culture assays reporting over 75% inhibition of pathogen mycelial growth [32]. By combining direct parasitic interactions with induced systemic resistance and growth promotion, *Trichoderma* spp. not only suppresses disease, but also enhances host resilience, validating its role as a potent bio-fungicide in integrated disease management strategies [33]. Considering the above benefits of *Trichoderma* spp. and Biochar, the present experiment was conducted to evaluate the effect of biochar (as soil amendment) and seed priming with *Trichoderma* spp. on the growth, yield, and the occurrence of Alternaria blight in mustard.

Materials and Methods

Details of Experiment

The field experiment was conducted during *rabi* season of 2024-25 at the experimental farm, Faculty of Agricultural Sciences, DAV University, Jalandhar, Punjab. The experiment was laid out in a randomized block design (RBD) with ten treatments and six replications. The seed material comprises of brown mustard, *B. juncea*, variety PBR 91 obtained from Punjab Agricultural University (PAU), Ludhiana, Punjab.

Table 1: Detail of treatments

Treatment	Treatment details
T_1	RDF
T_2	RDF + SP # with *Trichoderma viride* 100%
T_3	RDF + SP# with *Trichoderma harzianum* 100%
T_4	RDF + SP# with *Trichoderma viride* 50% and *Trichoderma harzianum* 50%
T_5	RDF + SP# with *Trichoderma viride* 100% and *Trichoderma harzianum* 100%
T_6	RDF + Biochar*
T_7	RDF + Biochar* + SP# with *Trichoderma viride* 100%
T_8	RDF + Biochar* + SP# with *Trichoderma harzianum* 100%
T_9	RDF + Biochar*+ SP# with *Trichoderma viride* 50% and *Trichoderma harzianum* 50%
T_{10}	RDF + Biochar* + SP# with *Trichoderma viride* 100% and *Trichoderma harzianum* 100%

Seed Priming @ 1 g/ml
*Biochar @ 0.9 kg/ m^2
RDF: Recommended dose of fertilizers *i.e.*, MOP @2.5g/m^2, Urea @ 21.6g/m^2 and SSP @ 18g/ m^2

Seed priming and biochar application

Seeds of mustard were bio-primed by treating them with a solution of *T. harzianum* and *T. viride* @ 1g/ml. Treated seeds were incubated at 28±2°C for 24 hours and sown in the field. Sugarcane biochar @0.9 kg/m^2 was also applied to the field, during the field preparation stage, before sowing by mixing the desired amount of biochar in the top layer of soil of the selected plots

Observations

Growth parameters recorded included days to 50% germination, days to 50% flowering, shoot fresh weight, plant height, plant fresh weight, number of leaves per plant and number of branches per plant. Yield attributes included number of siliquae per plant, seeds per siliqua, and seed yield per plot.

The percent disease incidence (PDI), percent disease severity (DS) and Area under disease progress curve (AUDPC) of Alternaria blight were calculated by using following formulae:

Percent disease incidence

$$\text{Disease incidence (\%)} = \frac{Number\ of\ Plants\ infected}{Number\ of\ Plants\ inspected} \times 100$$

Percent disease severity

$$\text{Disease severity (\%)} = \frac{Sum\ of\ all\ disease\ Rating}{Total\ no.\ of\ Ratings \times Maximum\ Disease\ rating} \times 100$$

Area under disease progress curve (AUDPC)

$$AUDPC = \sum_{i=1}^{n} \left[\frac{Y(i+1)+Yi}{2}\right] [X(i+1) - Xi]$$

where,
Y_i = disease index (per unit) at i^{th} observation

$Y_{(i+1)}$ = disease index (per unit) at $(i+1)^{th}$ observation
X_i = time of i^{th} observation (in days)
X_{i+1} = time of $(i+1)^{th}$ observation (in days)
n = total number of observations

Statistical analysis
The means of various treatments were compared using analysis of variance (ANOVA) using OPSTAT tool (https://14.139.232.166/opstat) at $p \leq 0.05$. The means of individual treatments were compared using critical difference (CD) value. The data of DS and PDI was subjected to the Arcsine transformation, prior to ANOVA in Microsoft Excel for Windows®.

Results

Days to 50% germination
It was observed that the treatment T_4 (RDF + *Tv* 50% and *Th* 50%), took least number of days (4.50 DAS) to achieve 50% germination, which was at par with treatments T_3 (RDF + *Th* 100%), T_2 (RDF + *Tv* 100%) and T_5 (RDF + *Tv* 100% and *Th* 100%) (*i.e.*, 4.66, 4.83, and 4.50 DAS, respectively) and was significantly lower than all other treatments (Table 2). Whereas the highest number of days (6.16 DAS) was observed in treatment T_1 (RDF), which was at par with treatments T_{10} (RDF + Biochar + *Tv* 100% and *Th* 100%), T_9 (RDF + Biochar + SP with *Tv* 50% and *Th* 50%), T_8 (RDF + Biochar + SP with *Th* 100%), T_7 (RDF + Biochar + SP with *Tv* 100%), and T_6 (RDF + Biochar) with 6.00, 5.66, 5.83, 5.66 and 5.83 DAS, respectively.

Days to 50% flowering
The least number of days (61.50) to achieve 50% flowering was observed in the treatment T_4 (RDF + SP with *Tv* 50% and *Th* 50%), which was at par with the treatments T_3 (RDF + SP with *Th* 100%), T_2 (RDF + SP with *Tv* 100%) and T_5 (RDF + SP *Tv* 100% and *Th* 100%) with 61.50, 61.66, and 62.16 DAS, respectively (Table 2). Maximum number of days (63.83) to achieve 50% flowering were observed in the control *i.e.*, treatment T_1 (RDF), which was at par with treatments T_{10} (RDF + Biochar + SP with *Tv* 100% and *Th* 100%), T_7 (RDF + Biochar + SP with *Tv* 100%), T_8 (RDF + Biochar + SP with *Th* 100%) and T_9 (RDF + Biochar+ SP with *Tv* 50% and *Th* 50%) with 63.50, 63.66, 63.66, and 63.33 DAS, respectively.

Shoot fresh weight
The highest shoot fresh weight at harvest *i.e.*,140 DAS was recorded in the treatment T_4 (RDF + SP with *Tv* 50% and *Th* 50%) with 44.43 g, significantly higher than all other treatments (Table 2). The lowest shoot fresh weight (24.81 g) was recorded in the control *i.e.*, treatment T_1 (RDF), which was significantly lower than all other treatments.

Table 2: The effect of different treatments on days to 50% germination (DAS), days to 50% flowering (DAS), and shoot fresh weight.

Treatments	Mean number of days to 50% germination (DAS)	Mean number of days to 50% flowering (DAS)	Shoot fresh weight (g)
T_1	6.16^b	63.83^c	24.81^j
T_2	4.83^a	61.66^a	40.78^c
T_3	4.66^a	61.50^a	41.86^b
T_4	4.50^a	61.50^a	44.43^a
T_5	5.00^a	62.16^a	37.00^d
T_6	5.83^b	62.66^b	26.30^h
T_7	5.66^b	63.66^c	27.68^g
T_8	5.83^b	63.66^c	30.11^f
T_9	5.66^b	63.33^c	35.86^e
T_{10}	6.00^b	63.50^c	26.40^i
SE(m)±	0.22	0.45	0.26
CD ($p \leq 0.05$)	0.64	1.29	0.74

Different letters (a, b, c ….) as superscripts on the values indicate that the mean values of the treatments are significantly different from each other at $p \leq 0.05$.

Plant height (cm)
The mean data for plant height recorded at 30 days interval *i.e.*, 30 DAS, 60 DAS and 90 DAS was presented in the table 3. The maximum plant height was recorded in the treatment T_4 (RDF + SP with *Tv* 50% and *Th* 50%) on all sampling dates (*i.e.*, 98.60, 148.93, and 208.93 cm, respectively), which was significantly higher than all the treatments on respective sampling dates. However, minimum plant height (78.63 cm) on 30 DAS was observed in the control *i.e.*, treatment T_1 (RDF), which was at par with treatment T_{10} (RDF + Biochar + SP with *Tv* 100% and *Th* 100%) with 79.31 cm height. Moreover, the least plant height (130.61 cm) at 60 DAS was observed in control *i.e.*, treatment T_1 (RDF), which was significantly lower than all other treatments. Further, at 90 DAS also, the minimum height of plant (188.23 cm) was recorded in the control *i.e.*, treatment T_1 (RDF), which was at par with the treatment T_{10} (RDF + Biochar + SP with *Tv* 100% and *Th* 100%) with 189.03 cm height.

Plant fresh weight (g)
The highest mean plant fresh weight in all three sampling dates *i.e.*, 30 DAS, 60 DAS and 90 DAS were recorded in treatment T_4 (RDF + SP with *Tv* 50% and *Th* 50%) as 19.04, 35.67, and 91.20 g, respectively; which was significantly higher than all other treatments on respective sampling dates (Table 3). The lowest plant fresh weight at 30 DAS was recorded in the control *i.e.*, treatment T_1 (RDF), with 9.90 g mean plant fresh weight, which was significantly lower than all the other treatments. Similarly, at 60 DAS, the lowest plant fresh weight (25.38 g) was recorded in the control *i.e.*, treatment T_1 (RDF), which was at par with the treatment T_{10} (RDF + Biochar + SP with *Tv* 100% and *Th* 100%), with 25.79 g mean fresh weight. Further, the lowest plant fresh weight at 90 DAS was recorded in the control *i.e.*, treatment T_1 (RDF) with 77.46 g mean fresh weight, which was significantly lower than all other treatments.

Number of branches per plant
The mean number of branches per plant recorded at 30 days interval *i.e.*, 30 DAS, 60 DAS, 90 DAS are presented in table 4. The highest number of branches (3.73, 20.43 and 28.33, respectively) on all three sampling dates were recorded in the treatment T_4 (RDF + SP with *Tv* 50% and *Th* 50%), which is significantly higher than all other treatments on respective

sampling dates. The lowest number of branches in all three sampling dates were observed in the control *i.e.*, treatment T_1 (RDF), with the mean number of branches 1.81, 13.48, and 22.08, respectively, which was significantly lower than the other treatments on all respective sampling dates.

Table 3: The effect of different treatments on plant height (cm) and plant fresh weight (g).

Treatments	Plant height (cm)			Plant fresh weight (g)		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
T_1	78.63^{d}	130.61^{h}	188.23^{g}	9.90^{j}	25.38^{g}	77.46^{j}
T_2	93.51^{b}	144.67^{b}	205.11^{b}	16.23^{c}	33.02^{b}	87.48^{c}
T_3	94.10^{b}	145.65^{b}	206.08^{b}	17.08^{b}	33.41^{b}	88.76^{b}
T_4	98.36^{a}	148.93^{a}	208.93^{a}	19.04^{a}	35.67^{a}	91.20^{a}
T_5	87.15^{c}	142.33^{c}	203.10^{f}	15.34^{d}	30.86^{c}	85.76^{d}
T_6	80.21^{c}	134.51^{f}	192.93^{e}	11.31^{h}	27.25^{f}	80.26^{h}
T_7	81.53^{c}	136.56^{e}	194.31^{d}	12.61^{g}	28.85^{e}	82.03^{g}
T_8	84.56^{c}	137.88^{e}	195.10^{d}	13.61^{f}	30.19^{d}	83.05^{f}
T_9	85.30^{c}	139.78^{d}	198.38^{c}	14.20^{e}	30.40^{cd}	83.70^{e}
T_{10}	79.31^{d}	132.67^{g}	189.03^{g}	10.18^{i}	25.79^{g}	78.08^{i}
SE(m)±	0.22	0.60	0.37	1.11	0.11	0.20
CD ($p \leq 0.05$)	0.64	1.73	1.07	3.17	0.33	0.59

Different letters (a, b, c ….) as superscripts on the values indicate that the mean values of the treatments are significantly different from each other at $p \leq 0.05$.

Number of leaves per plant

The number of leaves recorded thrice, at 30 days interval *i.e.*,30 DAS, 60 DAS and 90 DAS and the highest number of leaves was observed in the treatment T_4 (RDF + SP with *Tv* 50% and *Th* 50%) with 11.56, 37.23 and 58.51, respectively; which was significantly higher than the mean values of all other treatments on respective sampling dates (Table 4). The lowest number of leaves (6.10) at 30 DAS was recorded in the control *i.e.*, treatment T_1 (RDF), which was at par with the treatment T_{10} (RDF + Biochar + SP with *Tv* 100% and *Th* 100%) with the mean number of leaves as 6.46. Moreover, the lowest number of leaves (24.53) at 60 DAS was recorded in the control *i.e.*, treatment T_1 (RDF), which was also at par with the treatment T_{10} (RDF + Biochar + SP with *Tv* 100% and *Th* 100%) with 24.90 average number of leaves. Further, at 90 DAS also, the lowest number of leaves (44.16) was recorded in the control *i.e.*, treatment T_1 (RDF) which was significantly lower than all the other treatments.

Table 4: The effect of different treatments on number of branches per plant and number of leaves per plant.

Treatments	Number of branches			Number of leaves		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
T_1	1.81^{j}	13.48^{i}	22.08^{j}	6.10^{g}	24.53^{i}	44.16^{j}
T_2	3.25^{c}	18.30^{c}	26.20^{c}	9.73^{b}	32.76^{c}	55.16^{c}
T_3	3.38^{b}	18.96^{b}	26.60^{b}	10.20^{b}	34.46^{b}	56.50^{b}
T_4	3.73^{a}	20.43^{a}	28.33^{a}	11.56^{a}	37.23^{a}	58.51^{a}
T_5	2.90^{d}	17.03^{d}	25.60^{d}	8.76^{c}	30.40^{d}	53.83^{d}
T_6	2.20^{h}	14.71^{g}	23.33^{h}	6.73^{f}	25.43^{h}	48.66_{h}
T_7	2.33^{g}	15.86^{f}	24.06^{g}	7.10^{f}	26.33^{g}	49.83^{g}
T_8	2.46^{f}	16.13^{ef}	24.45^{f}	7.90^{e}	27.40^{f}	51.16^{f}
T_9	2.76^{e}	16.46^{e}	24.85^{e}	8.20^{d}	29.10^{e}	52.50^{e}
T_{10}	1.95^{i}	13.93^{h}	22.46^{i}	6.46^{g}	24.90^{i}	45.83_{i}
SE(m)±	0.16	0.04	0.12	0.16	0.23	0.39
CD ($p \leq 0.05$)	0.47	0.12	0.34	0.33	0.66	1.11

Different letters (a, b, c ….) as superscripts on the values indicate that the mean values of the treatments are significantly different from each other at $p \leq 0.05$.

Number of siliquae per plant

It was observed that the highest number of siliquae per plant was found in treatment T_4 (RDF + SP with *Tv* 50% and *Th* 50%) with 235.50 mean number of siliquae, which is significantly higher than all other treatments (Table 5). The lowest number of siliquae per plant (154.76) was observed in the control *i.e.*, treatment T_1 (RDF), which was at par with treatment T_{10} (RDF + Biochar + SP with *Tv* 100% and *Th* 100%) with 157.30 mean number of siliquae.

Number of seeds per siliquae

The highest number of seeds per siliquae (15.80) was recorded in treatment T_4 (RDF + SP with *Tv* 50% and *Th* 50%), which was significantly higher than all other treatments (Table 5). The lowest number of seeds per siliquae was observed in the control *i.e.*, treatment T_1 (RDF), with 9.51 seeds per siliquae, which was significantly lower than all other treatments.

Seed yield per plot (g)

In terms of yield, treatment T_4 (RDF + SP with *Tv* 50% and *Th* 50%) produced the highest yield per plot at 668.16 g, which was significantly higher than all other treatments (Table 5). The lowest yield per plot (456.50 g) was recorded in the control *i.e.*, treatment T_1 (RDF), which was at par with treatment T_{10} (RDF + Biochar + SP with *Tv* 100% and *Th* 100%) with 474.16 g seeds per plot.

Table 5: The effect of different treatments on mean number of siliquae per plant, number of seeds per siliquae and seed yield (g).

Treatments	No. of Siliquae	No of Seeds/Siliquae	Seed yield (g)
T_1	154.76^{h}	9.51^{j}	456.50^{g}
T_2	206.63^{c}	14.56^{c}	619.00^{bc}
T_3	214.43^{b}	14.93^{b}	628.16^{b}
T_4	235.50^{a}	15.80^{a}	668.16^{a}
T_5	198.73^{d}	13.50^{d}	600.33^{cd}
T_6	164.56^{g}	11.26^{h}	511.83^{f}
T_7	168.13^{g}	11.78^{g}	536.16^{f}
T_8	178.83^{f}	12.40^{f}	566.16^{e}
T_9	184.16^{e}	12.90^{e}	589.83^{de}
T_{10}	157.30^{h}	9.96^{i}	474.16^{g}
SE(m)±	1.64	0.10	9.07
CD ($p \leq 0.05$)	4.68	0.30	25.92

Different letters (a, b, c ….) as superscripts on the values indicate that the mean values of the treatments are significantly different from each other at $p \leq 0.05$.

Appearance of Alternaria blight and identification of the pathogen

During the course of experiment, the earliest disease incidence was recorded 79 days (Table 6) after sowing in treatment T_1 (RDF) on February 1st (5th week of the Standard Metrological week). The latest was found in treatment T_4 (RDF + SP with *Tv* 50% and *Th* 50%) at 83 DAS (6th week of the Standard Metrological week).

Table 6: Appearance of Alternaria blight on different treatments in rapeseed mustard in the *rabi* season 2024-25.

Treatment (Mean DAS for initial appearance of disease)	Replicate	Initial appearances of disease (DAS)		
		Days after sowing (DAS)	Date	Standard Metrological week, 2025
T_1 (79.5)	R_1	79	01/02/25	5
	R_2	79	01/02/25	5
	R_3	79	01/02/25	5
	R_4	80	02/02/25	5
	R_5	80	02/02/25	5
	R_6	80	02/02/25	5
T_2 (81.16)	R_1	80	02/02/25	5
	R_2	79	01/02/25	5
	R_3	80	02/02/25	5
	R_4	81	03/02/25	5
	R_5	80	02/02/25	5
	R_6	81	03/02/25	5
T_3 (81.5)	R_1	80	02/02/25	5
	R_2	79	01/02/25	5
	R_3	79	01/02/25	5
	R_4	82	04/02/25	5
	R_5	82	04/02/25	5
	R_6	81	03/02/25	5
T_4 (82)	R_1	80	02/02/25	5
	R_2	80	02/02/25	5
	R_3	81	03/02/25	5
	R_4	82	03/02/25	5
	R_5	83	05/02/25	6
	R_6	83	05/02/25	6
T_5 (81.66)	R_1	80	02/02/25	5
	R_2	80	02/02/25	5
	R_3	80	02/02/25	5
	R_4	81	03/02/25	5
	R_5	82	04/02/25	5
	R_6	81	03/02/25	5
T_6 (80.66)	R_1	79	01/02/25	5
	R_2	79	01/02/25	5
	R_3	79	01/02/25	5
	R_4	80	02/02/25	5
	R_5	80	02/02/25	5
	R_6	80	02/02/25	5
T_7 (81.16)	R_1	80	02/02/25	5
	R_2	79	01/02/25	5
	R_3	79	01/02/25	5
	R_4	80	02/02/25	5
	R_5	80	02/02/25	5
	R_6	82	04/02/25	5
T_8 (81.16)	R_1	80	02/02/25	5
	R_2	79	01/02/25	5
	R_3	80	02/02/25	5
	R_4	80	02/02/25	5
	R_5	81	03/02/25	5
	R_6	81	03/02/25	5
T_9 (81)	R_1	80	02/02/25	5
	R_2	79	01/02/25	5
	R_3	79	01/02/25	5
	R_4	80	02/02/25	5
	R_5	81	03/02/25	5
	R_6	81	03/02/25	5
T_{10} (80.66)	R_1	79	01/02/25	5
	R_2	79	01/02/25	5
	R_3	79	01/02/25	5
	R_4	80	02/02/25	5
	R_5	80	02/02/25	5
	R_6	81	03/02/25	5

Effect of different treatments on percent disease incidence (PDI)

During the course of present investigation, the disease incidence was measured thrice *i.e.*, 80, 95 and 110 DAS after 15 days interval. The lowest disease incidence was recorded in treatment T_4 (RDF + SP with *Tv* 50% and *Th* 50%) both at 80 and 95 DAS (15 days interval) with values 3.34 (Arcsine transformed value: 10.31) and 48.84 (Arcsine transformed value: 44.32), which was significantly lower than all other treatments. However, the highest disease incidence at 80 DAS *i.e.*, 7.86 (Arcsine transformed value: 16.18) was recorded in the control *i.e.*, treatment T_1 (RDF), which was at par with treatments T_{10} (RDF + Biochar + SP with *Tv* 100% and *Th* 100%), T_7 (RDF + Biochar + SP with *Tv* 100%) and T_6 (RDF + Biochar) with values 7.43 (Arcsine transformed value: 15.71), 7.13 (Arcsine transformed value: 15.44), and 7.25 (Arcsine transformed value: 15.49), respectively. Further, at 95 DAS, the highest disease incidence *i.e.*, 70.88 (Arcsine transformed value: 57.44) was recorded in the control *i.e.*, treatment T_1 (RDF) which was at par with treatment T_{10} (RDF + Biochar + SP with *Tv* 100% and *Th* 100%) with value 66.12 (Arcsine transformed value: 54.43). But on the third sampling date *i.e.*, 110 DAS, percent disease incidence (PDI) was 100 (Arcsine transformed value: 90) in all of the treatments and F-test was not significant (Table 7). The lowest overall mean of percent disease incidence (PDI) *i.e.*, 48.29 was recorded in the treatment T_4 (RDF + SP with *Tv* 50% and *Th* 50%), whereas the highest was recorded in the control *i.e.*, treatment T_1 (RDF), with value 54.54.

Effect of different treatments on percent disease severity (DS)

The lowest disease severity *i.e.*, 1.19 (Arcsine transformed value: 6.18) at 80 DAS was observed in the treatment T_4 (RDF + SP with *Tv* 50% and *Th* 50%), which was at par with treatments T_3 (RDF + SP with *Th* 100%) and T_2 (RDF + SP with *Tv* 100%) with values 1.41 (Arcsine transformed value: 6.80) and 1.55(Arcsine transformed value: 7.12). At 95 DAS, the lowest disease severity *i.e.*, 5.49 (Arcsine transformed value: 13.51) was observed in the treatment T_4 (RDF + SP with *Tv* 50% and *Th* 50%), which was significantly lower than all other treatments (Table 7). Further, at 110 DAS, the lowest severity *i.e.*, 47.51 (Arcsine transformed value: 43.57) was recorded in the treatment T_4 (RDF + SP with *Tv* 50% and *Th* 50%), which was at par with the treatment T_3 (RDF + SP with *Th* 100%) with value 49.57 (Arcsine transformed value: 44.75). However, the highest disease severity *i.e.*, 4.92 (Arcsine transformed value: 12.80) at 80 DAS was recorded in the control *i.e.*, treatment T_1 (RDF), which was at par with treatment T_{10} (RDF + Biochar + SP with *Tv* 100% and *Th* 100%) with value 12.05 (Arcsine transformed value: 20.30). Similarly, the highest disease severity *i.e.*, 13.15 (Arcsine transformed value: 21.25) at 95 DAS was recorded in the control *i.e.*, treatment T_1 (RDF), which was significantly higher than all other treatments. Moreover, at 110 DAS, the highest disease severity *i.e.*, 64.29 (Arcsine transformed value: 53.31) was recorded in the control *i.e.*, treatment T_1 (RDF), which was at par with treatment T_{10} (RDF + Biochar + SP with *Tv* 100% and *Th* 100%) with value 63.57 (Arcsine transformed value: 52.87). Further, the lowest overall mean of percent (%) disease severity (DS) *i.e.*, 21.09 was observed in the treatment T_4 (RDF + SP with *Tv* 50% and *Th* 50%), whereas the highest overall mean was observed in the control *i.e.*, treatment T_1 (RDF), with value 29.12.

Table 7: Percent disease incidence (PDI) and disease severity (DS) on three different dates (15 days interval) after appearance of disease.

Treatments	PDI (%)				Disease Severity (DS)			
	80 DAS	95 DAS	110 DAS	Overall mean	80 DAS	95 DAS	110 DAS	Overall mean
T_1	7.86 (16.18)^{ghi}	70.88 (57.44)^{d}	100 (90)	54.54	4.92 (12.80)^{e}	13.15 (21.25)^{i}	64.29 (53.31)^{i}	29.12
T_2	4.38 (11.96)^{bc}	50.04 (45.03)^{a}	100 (90)	49.03	1.55 (7.12)^{a}	7.15 (15.47)^{c}	52.37 (46.36)^{bc}	22.98
T_3	4.22 (11.70)^{b}	49.30 (44.64)^{a}	100 (90)	48.81	1.41 (6.80)^{a}	6.20 (14.40)^{b}	49.57 (44.75)^{ab}	21.98
T_4	3.34 (10.31)^{a}	48.84 (44.32)^{a}	100 (90)	48.29	1.19 (6.18)^{a}	5.49 (13.51)^{a}	47.51 (43.57)^{a}	21.09
T_5	5.01 (12.86)^{cd}	52.51 (46.46)^{a}	100 (90)	49.79	2.40 (8.90)^{b}	7.55 (15.91)^{d}	55.35 (48.07)^{cd}	24.29
T_6	7.25 (15.49)^{fgh}	61.69 (51.77)^{c}	100 (90)	52.46	3.85 (11.27)^{d}	11.03 (19.39)^{g}	60.98 (51.36)^{gh}	27.34
T_7	7.13 (15.44)^{fg}	58.81 (50.10)^{bc}	100 (90)	51.85	3.49 (10.71)^{cd}	10.06 (18.48)^{e}	59.76 (50.62)^{fg}	26.60
T_8	6.45 (14.65)^{ef}	54.40 (47.53)^{ab}	100 (90)	50.74	3.27 (10.41)^{c}	9.14 (17.58)^{e}	58.86 (50.10)^{ef}	26.03
T_9	5.84 (13.89)^{de}	53.29 (46.90)^{a}	100 (90)	50.29	3.00 (9.95)^{c}	8.49 (16.92)^{e}	57.12 (49.09)^{de}	25.32
T_{10}	7.43 (15.71)^{fghi}	66.12 (54.43)^{cd}	100 (90)	53.40	4.47 (12.15)^{de}	12.05 (20.30)^{h}	63.57 (52.87)^{hi}	28.44
CD ($p \leq 0.05$)	0.96	0.50	1.44	NA*	-	0.86	2.01	-
SE(m)±	0.33	1.43	4.13	NA*	-	0.30	0.70	-

Different letters (a, b, c ….) as superscripts on the values indicate that the mean values of the treatments are significantly different from each other at $p \leq 0.05$.

* CD and SE(m) were calculated from the Arcsine transformed values.

NA: CD and SE(m) were not calculated as the F-test was not significant ($p \leq 0.05$)

Value in parenthesis '()' represent the arcsine transformed values for the respective PDI and DS values.

Area under disease progress curve (AUDPC)

The A-value representing area under disease progress curve (AUDPC) calculated for disease incidence of Alternaria blight of mustard in ten different treatments revealed that treatment T_4 (RDF + SP with *Tv* 50% and *Th* 50%) has the minimum A-value *i.e.*, 447.51 ± 12.21. Whereas, the maximum A-value (716.40 ± 6.84) was recorded in treatment T_1 (RDF) (Figure 1).

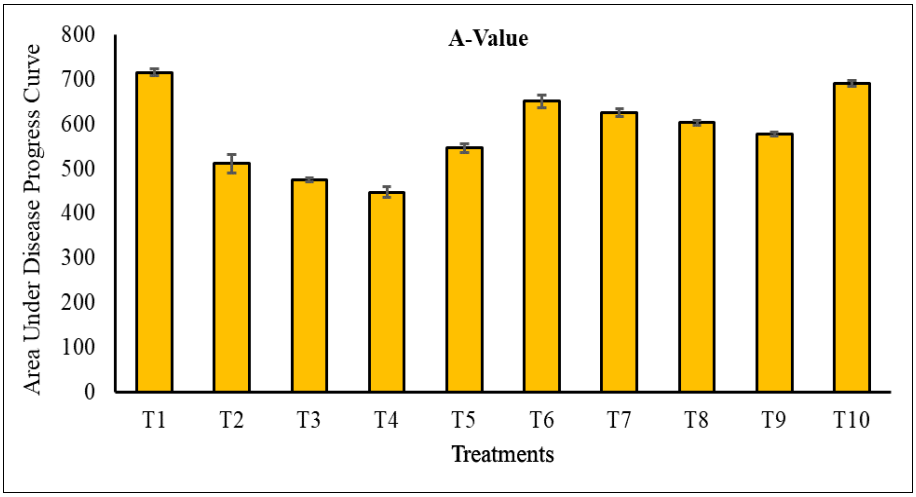

Fig 1: A-value (representing area under disease progress curve) of *A. brassicae* infecting brown mustard (*Brassica juncea* L. var. PBR 91)

Discussion

Rapeseed-mustard is one of the most important oilseed crops; However, its productivity is largely limited by Alternaria blight. Recently, a number of eco-friendly and sustainable methods of plant disease management have been popularized. In this regard, the present study was performed to evaluate the effectiveness of biochar application and seed priming with *Trichoderma* spp. (*T. harzianum* and *T. viride*) on the growth, yield, and disease parameters of rapeseed mustard (*B. juncea* var. PBR 91) in a randomized block design with ten different treatments of the combinations thereof and the results described above are discussed below under suitable headings.

Growth parameters

The data recorded for the growth attributes mentioned above revealed that plants in the treatment T_4 (RDF + SP with *Tv* 50% and *Th* 50%), had the fastest germination (4.5 days). This is likely because *Trichoderma* spp. produces plant growth regulators (PGRs) like auxins and gibberellins, which help seeds to start their metabolism more quickly [34]. In experiment in chickpeas, it was recorded that the combined treatment *T. harzianum* and *T. viride* to the seeds resulted in the improved germination rate of approximately 92 %, which was higher than all other treatments [11]. In another experiment in Chilli (*Capsicum annuum* L.), the seeds treated with spore suspensions of various *Trichoderma* strains showed significantly higher germination rates compared to control. Faster germination gives plants the initial growth advantage and allows a better utilization of nutrients from soil. In the present experiment, the seeds of the control group (T_1) and in the biochar treatments (T_6-T_{10}), germination was found to be slower, than those without biochar treatment. This could be attributed to the ability of biochar to temporarily adsorb the nutrients [35], making them unavailable. Moreover, biochar can inhibit germination, due to the presence of soluble phenolic compounds, polycyclic aromatic hydrocarbons (PAHs), and salts, which it may release into the soil, creating an osmotic stress and phytotoxicity [36].

Further, the plants under treatment T_4 (RDF + SP with *Tv* 50% and *Th* 50%). were taller, had more leaves and branches, and had more shoot and plant weight at all stages as compared with the biochar induced treatments. Seed priming by the combined use of *T. harzianum and T. viride* improved these growth parameters in *B. juncea* and suggested that they help plants to grow more biomass. *Trichoderma* spp. is known to colonizes roots, helps absorb nutrients like nitrogen and phosphorus, and supports plant hormone balance [37]. Further, Abdelmoaty *et al*., (2022)[38] had also reported that the soil inoculation with both *T. harzianum* and *T. viride* under reduced NPK regime in lemon (*Citrus aurantifolia*) increased plant height by approximately 50%, while the number of branches increased by 107 % as compared to untreated controls.

On the other hand, the application of biochar (alone or in combination with the seed priming using *T. harzianum* and *T. viride*) improved the above growth parameters than the control (RDF only), but were failed to perform to a level that of the combined use of both *T. harzianum* and *T. viride* (without biochar). The performance of *B. juncea* in terms of growth parameters in the present study was better when the seed priming using *T. harzianum* and *T. viride* was performed without biochar treatment than with their combination of biochar. This could be because it has been reported previously that the biochar's benefit depends heavily on its quality (feedstock, pyrolysis temperature), and biochar prepared from a feedstock may fail to support *Trichoderma* spp. effectively or could even inhibit microbial activity, that some other feedstocks [39]. Therefore, separate evaluation of different types of biochar depending on their feedstock is required to select the most appropriate type to be used alongside *Trichoderma* spp.

Yield Performance

The data recorded for the yield attributes revealed that treatment T_4 (RDF + SP with *Tv* 50% and *Th* 50%), produced the highest number of siliquae, more seeds per siliquae, and highest total yield per plot. Various other workers have also reported the use of *Trichoderma* spp. in improving the yield and yield related attributes in different crop plants. Srivastava *et al*., (2006) [40] reported that combining *T. harzianum* and *T. viride* significantly increased yield of sugarcane by 6-38%. Further, treatment with *Trichoderma* spp. was also reported to improve the yield of rapeseed mustard than the untreated [7].

Trichoderma spp., not only protect the plants from plant diseases but also improve plant health to mitigate the harmful effects of abiotic stresses like salt *etc*. Saha *et al*., (2025) [41] reported that the application of *Tric hoderma* substantially reduced the salt stress and enhanced growth of *B. juncea*, under saline conditions and increased yield by 25.13%. Moreover, an increase in the yield by the application of *Trichoderma* spp. has been found in a number of plants including, wheat, corn, tuberose, sugarcane, tomato, mustard, and okra *etc*., by several workers [42,43,44,45,46,47].

The control (T_1) and T_{10} treatments showed statistically low yield, likely due to weak growth and more disease. Similar outcomes were reported by Lehmann and Joseph, (2015) [48] who

stated that biochar may often have inconsistent or negligible effects on crop yield. Further, in a meta- analysis conducted by Biederman and Harpole, (2013) [49] biochar application resulted in increased crop yield only in 50% of crops, while the rest 50% has no effect on yield. Moreover, biochar's nutrient release kinetics is slow, and its benefits are typically seen in the long term [50].

Disease management

Trichoderma spp. have been widely used as biological control agent against many plant pathogens, particularly against the soil borne diseases [51]. Biochar, on the other hand, may also suppress plant disease in some cases by enhancing the growth of antagonistic microorganisms in the soil, such as plant beneficial bacteria and fungi that outcompete pathogens or induce systemic resistance (*e.g.*, *Pseudomonas* spp., *Trichoderma* spp., arbuscular mycorrhizal fungi (AMF) *etc*.) [23].

The treatment T_4 (RDF + SP with *Tv* 50% and *Th* 50%) was found to be most effective in suppressing Alternaria blight disease, which T_4 significantly delayed onset of disease, reduced percent disease incidence (PDI), percent disease severity (DS) and area under disease progress curve (AUDPC) compared to the other treatments. In an experiment in mustard crops, seed treatment plus soil application of *T. viride* along with garlic spray significantly slowed Alternaria leaf spot than the chemical controls [52]. In the present experiment, it was found that the combination of two *Trichoderma* spp., in optimal dose (50% each) was most effective in managing Alternaria blight. Similar results have been shown by some other workers as well.

It has been reported that the combination of *T. viride* and *T. harzianum* reduced the disease incidence (DI) by 54.9% in Fusarium wilt of chickpea, which is higher than the control as well as all other treatments [53]. Although, other researchers such as Narayanasamy, (2013) [54] and Shoresh *et al*., (2010) [55] reported that *Trichoderma* spp. strengthens plant defenses through various other mechanisms too. It helps in suppression of disease through various mechanisms such as antibiosis, mycoparasitism, competition, induced systemic resistance (ISR) [56,57]. *Trichoderma* spp. has been known to suppress foliar pathogens including *A. brassicae* in mustard (*B. juncea*) [11].

On the other hand, in the present experiment, biochar slightly improved growth, yield and disease suppression (T_6- T_9), but when used in high amounts or mixed with too many microbes such as T_{10} (RDF + SP with *Tv* 100% and *Th* 100%), sometimes its benefits were reduced. This may be because fresh biochar can absorb nutrients or even the helpful chemicals made by microbes [58]. Studies by Biederman and Harpole, (2013) [49] show that biochar effects vary with soil type, biochar quality, and how it is applied.

Overall, the treatment T_4 (RDF + SP with Tv 50% and Th 50%) was the most effective because the two *Trichoderma* strains worked well together in improving growth, yield and imparted disease tolerance. Research have already demonstrated that mixed strains sometimes work better than single ones by covering more roles in the soil [59]. Microbial products combining more than one species tend to perform better in fields with variable conditions [60,61]. Studies have showed that *Trichoderma* consortia (*e.g.*, combinations of *T. harzianum*, *T. viride*, *T. hamatum*) offer higher disease inhibition and growth enhancement than any single species alone, even under variable environmental or pathogen pressures [62]. Although *Trichoderma* spp., can be used by different modes (like seed primming, root dipping, soil application and foliar spray), using *Trichoderma* spp. by seed priming can reduce the quantity required as well as the time and effort to use other modes of treatment [63,64,65], and also reduce the dependence on chemical plant protectant [66]. Further, it is safer for the environment and helps farmers get better yields [67].

Conclusion

From the present experiment, it was concluded that the seed priming with a mixture of *T. harzianum* and *T. viride* is an effective method to improve growth, yield, and reduce the occurrence of Alternaria blight in Indian mustard (*B. juncea*). However, the addition of biochar (with or without seed priming with *Trichoderma* spp.) was capable of improving, growth, yield and disease related parameters than the control, but were less effective than the use of *Trichoderma* spp., alone; especially when both the species of BCAs were used together at their 50% dose. In the nutshell, it was concluded that seed priming with *Trichoderma* spp. (*i.e.*, *T. harzianum* and *T. viride*, both at 50% dose) is a simple, affordable, and environment-friendly method, which can be recommended as a sustainable alternative to chemical fungicides.

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Conflict of Interest

The authors declare that they do not have any conflict of competing interests.

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