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CONTENTS

Comparative studies of the effect of microbial inoculants and inorganic chemicals on growth, yield, yield contributing traits and disease suppression in two varieties of mustard green (*Brassica juncea* L.) under open field conditions in mid hills of Uttarakhand

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ABSTRACT: Mustard green (*Brassica juncea* L.) is a popular green vegetable grown in the plains and hills of Northern India. It is a rich source of vitamins, minerals, and protein. The present investigation was conducted with an aim to study the effect of microbial inoculants and inorganic chemicals on growth, yield, yield contributing traits and disease suppression in two varieties of mustard green *viz*., Local (Hathikaan) and UHF VR 12-1 under open field conditions in mid hills of Uttarakhand. The experiment was laid out in Randomized Block Design comprising nine treatments and one control with three replications. For the experiment, bio-agents (*Trichoderma asperellum Th-14, Pseudomonas fluorescens Psf-4,* and *Bacillus subtilis Bs-2)*, fertilizers (Sulphur 800 WG and Boron), and the fungicide (Ridomil Gold) were used. With respect to plant growth and yield parameters, treatment T_4 (Seed bio-priming with Th-14 + FYM pre-colonized by Th-14 + one drenching of Th-14 at 45 days of the transplanting) recorded the maximum chlorophyll content, leaf length, leaf width, leaf yield, plant height, number of branches per plant, number of siliquae per plant and seed yield followed by T_7 *(*Seed dressing with Sulphur 800 WG + FYM + one foliar spray of Sulphur 800 WG at 45 days of the transplanting*)*. Whereas, control recorded lowest with respect to all the studied growth and yield parameters. T_4 showed minimum disease incidence (Damping off and Alternaria blight) and disease severity (Alternaria blight) followed by T_9 {Seed treatment with Ridomil Gold (Metalaxyl 4% w/w + Mancozeb 64% w/w) + FYM + one foliar spray of Ridomil Gold at 45 days of the transplanting} whereas control (untreated check) revealed maximum disease incidence and severity. From the present investigation, it may be concluded that the application of seed bio-priming with *Th-14* + FYM pre-colonized by *Th-14* + one drenching of *Th-14* at 45 days of the transplanting was found to be the most effective and promising treatment for enhancing growth, yield and resistance to damping off and Alternaria blight disease in *Brassica juncea* in both the studied varieties under open field conditions. However, the variety UHF VR 12-1 responded better towards the given treatments when compared with the local variety under present materials and environmental conditions.

Key words: *Bacillus subtilis, Brassica juncea*, FYM*,* microbial inoculants, *Pseudomonas fluorescens, Trichoderma asperellum*

Green leafy vegetables are a boon for a safe and healthy life and have been used by mankind for centuries. Mustard green [*Brassica juncea* (L.) Czern & Coss] is a cruciferous vegetable used for young leaves and folk medicine worldwide (Tian and Deng, 2020). Mustard green (*Brassica juncea*) commonly known as leaf mustard, broad leaf mustard, Chinese mustard, Indian mustard and vegetable rai. It is one of the most consumed vegetables in several Indian states like Jammu & Kashmir, Uttarakhand, Himachal Pradesh, Punjab and North-eastern hill states (Tian and Deng, 2020). Mustard green is one of the most nutritious green leafy vegetables available around in the winter months. It contains phytochemicals such as vitamin A, vitamin K, vitamin C, dietary fibre, essential minerals, carotenes, antioxidants, glucosinolates, beta carotene and ascorbic acid (Punetha and Adhikari, 2020). In India, the mustard green crop is affected by various diseases *viz*., Alternaria blight, white rust, downy mildew, sclerotinia rot and powdery mildew, which can cause severe losses under vulnerable conditions and can destroy the entire crop (Meena *et al.,* 2013). Alternaria blight is one of the important diseases of Indian mustard which is responsible for an average yield loss of 10 to 70% in different parts of Northern India depending upon the severity (Giri *et al.*, 2013). The major challenge, which lies ahead, is to develop technologies that can enhance quality and productivity of vegetables under reducing land, declining natural resources, increasing biotic and abiotic stresses and ever-increasing population. To fulfil this purpose, reduction in use of chemical fertilizers and use of bio fertilizers along with the use of eco friendly method of nutrient application would be beneficial both economically and ecologically (Banerjee *et al.,* 2011; Beenish *et al.*, 2019). The beneficial microbes colonizing the plant root, designated as plant growth promoting rhizobacteria (PGPR), increase the crop productivity and present an attractive and promising way to substitute chemical fertilizers, pesticides and other harmful supplements (Vishwakarma *et al.,* 2018). A large number of PGPR and PGPF (plant growth promoting fungi) have been reported to enhance plant growth and other traits. Use of bio-inoculants having broad action spectrum through various modes of application viz., seed treatment, seed biopriming, drenching, foliar spray and colonized FYM/compost not only provide higher productivity, resistance against biotic and abiotic stresses but also sustain the soil fertility (Rawat *et al.,* 2021). It is understood that commercial fertilizers have assisted in improving yield of crops and addressed soil fertility problems (Dubey *et al.,* 2021) but on the other side are also posing dangerous threats to humans as well as environment health.

Therefore, the present study was undertaken to investigate the comparative effect of microbial inoculants, fertilizers and fungicide on growth, physiological traits, yield (leaf and seed) and yield contributing traits and on disease suppression in two varieties of mustard green under open field conditions to estimate and find out the most effective variety and strategy for enhancing growth, yield and suppression of endemic diseases in *Brassica juncea* under open field conditions in hills of Uttarakhand.

MATERIALS AND METHODS

Experimental site

The study was carried out at the Vegetable Research Block (1700 m above msl), College of Forestry, Ranichauri, Tehri Garhwal, VCSG Uttarakhand University of Horticulture and Forestry, Uttarakhand during the crop season 2021.

Experimental materials

The seed materials, comprised of two varieties of mustard green namely UHF VR 12-1(IC0598459) and *local* (Hathikaan), were taken from Vegetable Research Block, College of Forestry, Ranichauri. The bioagents (*Trichoderma asperellum Th-14*, *Pseudomonas fluorescens Psf-4* and *Bacillus subtilis Bs-2), fertilizers* (Sulphur 800 WG and Boron), and the fungicide (Ridomil Gold) used under the present investigation were obtained from the Plant Pathology Laboratory, College of Forestry, Ranichauri, Tehri Garhwal, V.C.S.G. Uttarakhand University of Horticulture and Forestry, Uttarakhand.

Preparation of value added FYM (colonization of FYM by bio-control agent)

FYM was supplemented separately with bioagents *viz.*, *Trichoderma asperellum (Th-14)*, *Pseudomonas fluorescens (Psf-4)* and *Bacillus subtilis (Bs-2)* @ 250gm/ 100 kg FYM. The mixture was then spread up to 6-10-inch layer under the shade and covered with leaves or rice straw. The supplemented FYM was left for 2 to 3 weeks, with water poured at regular intervals just to keep the FYM heap moist as described by Singh *et al*., 2003. The FYM got colonized by bio-agents after 2 to 3 weeks and was ready to use as it had a large population of inoculated bio-agent.

Bio-priming of seed with talc-based formulation of *Trichoderma, Pseudomonas* and *Bacillus*

Seeds of vegetable rai were bio-primed separately by treating them with the talc-based formulations of *Trichoderma asperellum (Th-14), Pseudomonas fluorescens (Psf-4)* and *Bacillus subtilis* (*Bs-2*) @ 10g/kg seed. Talc based formulations with optimum CFU were obtained from the Biocontrol Laboratory, Plant Pathology Department, College of Forestry, Ranichauri. Treated seeds were then incubated at 27±1°C for 24 hours until prior to radicle emergence to allow *Trichoderma asperellum (Th-14)*, *Pseudomonas fluorescens (Psf-4)* and *Bacillus subtilis* (*Bs-2*) to sporulate and colonize the seed.

Seed treatment with fertilizers and chemical

Seeds of vegetable rai were treated with sulphur, boron and ridomil gold before use as mentioned in Table 1. Untreated seed was used in control.

The experimental plot and plant geometry

The seed materials of both the varieties of mustard green were raised in the nursery and thereafter, 28-30 days seedlings were transplanted in open field. At the nursery before seed sowing, all the treatments were done as seed bio-priming with bio inoculants and seed treatment with fertilisers and fungicide as mentioned in Table 1.

The field experiment was laid out in randomized block design consisting of nine treatments and one control along with three replications and each treatment was assigned randomly in plots of the experimental field during the experimentation. The plot size of 1.5 m × 1.5 m with a spacing of 45 cm from row to row and 20 cm from plant to plant were maintained. In open field conditions, drenching from T_1 to T_6 and foliar spray from T_7 to T_9 were performed 45 days after transplanting.

Treatment details

The details of different treatments given in nursery as well as in open field conditions along with dosage are presented in Table 1

Observations and analysis

The observations were recorded by randomly selecting five plants in each replication of every treatment and the average of the readings was evaluated for the computation of the data. The observations were recorded for the following parameters: chlorophyll content (SPAD value), leaf area (cm^2), leaf length (cm), leaf width (cm), leaf yield (kg/plot), plant height (cm), number of branches per plant, number of siliquae per plant, seed yield (kg/plot), disease incidence (%) and disease severity (%).

Chlorophyll content and leaf area were measured 60 days after transplanting using chlorophyll meter (Fig. 1.A) and leaf area meter (Fig. 1.B), respectively. Leaf length, leaf width and leaf yield were taken at 30, 60, 90 and 120 days after transplanting. Plant height, number of branches per plant and the number of siliquae per plant were taken 180 days after transplanting whereas seed yield for each plot was measured separately after harvesting.

In nursery, per cent disease incidence of damping-off was observed in each plot by measuring the number of infected plants per square meter area. In open field conditions, Alternaria blight disease appeared 40 days after transplanting. Twenty-five leaves taken randomly per plot to record disease severity by using a 0-5 scale on leaf as depicted in Table 2 (Bal and Kumar, 2014), whereas disease incidence was calculated by observing the number of infected plants out of total plants per plot. The following formulas were used to calculate disease incidence (%) and disease severity index (%).

$$\text{Per cent disease incidence} = \frac{\text{Number of plants infected}}{\text{Total number of plants observed}} \times 100$$

$$\text{Per cent disease severity index} = \frac{\text{Sum of numerical ratings}}{\text{Total number of leaves observed x Maximum grade}} \times 100$$

The data were analysed statistically through OPSTAT programme, described by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Physiological and growth attributes in two varieties of *Brassica juncea* in response to different treatments

Chlorophyll content significantly differed with different treatments in both local and UHF VR 12-1 which ranged from 7.17 SPAD to 12.06 SPAD and 11.07 SPAD to 15.87 SPAD respectively. In local variety**,** highest chlorophyll content (12.06 SPAD) was found in T_4 (Seed bio-priming with *Trichoderma asperellum Th-14* + FYM pre-colonized by *Th-14* + one drenching of *Th-14* at 45 days of the transplanting) followed by T_7 (Seed dressing with Sulphur 800 WG + FYM + one foliar spray of Sulphur 800 WG at 45 days after transplanting) with 11.43 SPAD, while minimum chlorophyll content (7.17 SPAD) was observed in T_{10} (Control) followed by T_8 with 8.60 SPAD (Table 4). In variety UHF VR 12-1, treatment T_4 resulted in the maximum (15.87 SPAD) chlorophyll content followed by T_7 with value of 15.43 SPAD while minimum chlorophyll content (11.07 SPAD) was observed in T_{10} (Control) followed T_8 with 12.53 SPAD (Table 6).

In both the varieties of rai i.e., local and UHF VR 12-1, the effect of different treatments observed a significant effect on leaf area that ranged from 411.88 cm^2 to 576.67 cm^2 (Table 4) and 540.37 cm^2 to 875.91 cm^2 (Table 6) respectively. Among the treatments, T_4 (Seed bio-priming with *Trichoderma asperellum Th-14* + FYM pre-colonized by *Th-14* + one drenching of *Th-14* at 45 days of the transplanting) resulted in the maximum (576.67 cm^2) leaf area followed by T_7 with 544.00 cm^2, while minimum leaf area was observed in T_8 (421.22 cm^2) followed by T_9 with 434.55 cm^2 observed in local variety. In variety UHF VR 12-1**,** among the treatments, T_4 resulted in maximum (875.91 cm^2) leaf area followed by T_7 (843.11 cm^2), while minimum leaf area was observed in T_8 (595.07 cm^2) followed by T_9 with 624.02 cm^2. All the treatments were found to be significantly superior over Control (Untreated check) in both the varieties.

Leaf length was recorded in four different pickings in both the varieties of mustard. In local variety as shown in Table 4 and Table 5, maximum leaf length in Ist, IInd, IIIrd and IVth picking was recorded in treatment T_4 with 16.03 cm, 17.97 cm, 24.70 cm and 24.93 cm respectively followed by T_7 with 15.43 cm, 17.47 cm, 24.43 cm and 24.77 cm leaf length respectively. Minimum leaf length in Ist, IInd, IIIrd and IVth picking was recorded in T_{10} (Control) with 11.70 cm, 13.93 cm, 21.03 cm and 21.03 cm respectively. In variety UHF VR 12-1 also the highest leaf length in all the four pickings was recorded in the treatment T_4 followed by T_7 as mentioned in Table 6 and Table 7. Lowest leaf length in Ist, IInd, IIIrd and IVth picking was recorded in T_{10} (Control) with 15.17 cm, 20.67 cm, 24.10 cm and 23.97 cm respectively.

Leaf width significantly differed with different treatments in all four pickings in both the local (Table 4 and Table 5)

Table 1: Details of treatments along with dosage used in the present study

Symbol	Treatment Details	Dosage
T_1	Seed biopriming with *Trichoderma asperellum Th-14* + FYM + one drenching of *Th-14* at 45 days of the transplanting.	10g/Kg seed +5Kg FYM/Plot +10 g/Litre
T_2	Seed biopriming with *Pseudomonas fluorescens Psf-4* + FYM + one drenching of *fluorescens Psf-4* at 45 days of the transplanting.	10g/Kg seed +5Kg FYM/ Plot+ 10 g/Litre
T_3	Seed biopriming with *Bacillus subtilis Bs-2* + FYM+ one drenching of *Bs-2* at 45 days of the transplanting.	10g/Kg seed +5Kg FYM/ Plot+ 10 g/Litre
T_4	Seed bio-priming with *Trichoderma asperellum Th-14* + FYM pre-colonized by *Th-14* + one drenching of *Th-14* at 45 days of the transplanting.	10g/Kg seed +5Kg FYM/Plot + 10 g/Litre
T_5	Seed bio-priming with *Pseudomonas fluorescens Psf-4* + FYM pre-colonized by *Psf-4* + one drenching of *fluorescens Psf-4* at 45 days of the transplanting.	10g/Kg seed +5Kg FYM/plot + 10 g/Litre
T_6	Seed bio-priming with *Bacillus subtilis* Bs-2+ FYM pre-colonized by *Bacillus subtilis Bs-2* + one drenching of *Bs-2* at 45 days of the transplanting.	10g/Kg seed +5Kg FYM/plot + 10 g/Litre
T_7	Seed dressing with Sulphur 800 WG + FYM + one foliar spray of Sulphur 800 WG at 45 days after transplanting.	3g/ Kg seed + 5Kg FYM/Plot + 2g/Litre
T_8	Seed treatment with Boron + FYM + one foliar spray of Boron at 45 days after transplanting.	3g/Kg seed + 5Kg FYM/Plot + 2g/Litre
T_9	Seed treatment with Ridomil Gold (Metalaxyl 4% w/w + Mancozeb 64% w/w) + FYM + one foliar spray of Ridomil Gold at 45 days after transplanting.	1Kg/Quintal seed+ 5Kg FYM/ Plot + 1g/Litre
T_{10}	Control (Untreated check)	

and UHF VR 12-1 variety (Table 6 and Table 7). In local variety, the maximum leaf width Ist, IInd, IIIrd and IVth picking was recorded in T_4 (Seed bio-priming with *Trichoderma asperellum Th-14* + FYM pre-colonized by *Th-14* + one drenching of *Th-14* at 45 days of the transplanting) with 13.07 cm, 15.37 cm, 20.27 cm and 19.33 cm respectively, followed by T_7 with 12.73 cm, 15.03 cm, 19.77 cm and 19.17 cm leaf width respectively, while minimum leaf width was recorded in T_{10} (Control) with 10.07 cm, 12.03 cm, 16.27 cm and 16.67 cm respectively. Similarly, in variety UHF VR 12-1, the maximum leaf width Ist, IInd, IIIrd and IVth picking was recorded in treatment T_4 with 15.20 cm, 21.13 cm, 24.07 cm and 22.03 cm respectively, followed by T_7 with 14.63 cm, 20.73 cm, 23.77 cm and 21.57 cm leaf width respectively, while minimum was observed in T_{10} with 11.13 cm, 16.93 cm, 19.93 cm and 18.43 cm leaf width respectively.

Table 2: Rating scale used for scoring Alternaria blight disease (Bal and Kumar, 2014)

Disease rating (G)	Disease severity description
0	No symptoms on leaf
1	Small light brown spots scattered covering ≤5% leaf area
2	Spots small, brown, with concentric rings, covering 5.1 to 10% leaf area
3	Spots large, brown, irregular, with concentric rings, covering 10.1 to 25% leaf area
4	Large, brown, irregular lesions with typical blight symptoms, covering 25.1 to 50% leaf area
5	Large, brown, irregular lesions with typical blight symptoms, covering more than 50% leaf area

A thorough inspection of data revealed that plant height was significantly influenced by different treatments in both the varieties i.e., local and UHF VR 12-1 which ranged from 123.33 cm to 254.00 cm (Table 5) and 162.33 cm to 269.33 cm (Table 7) respectively. In local variety, the maximum (254.00 cm) height was recorded in T_4 followed by T_7 with 211.33 cm, while minimum was recorded in T_{10} (Control) with 23.33 cm which was statistically at par to T_8 with 126.33 cm. In variety UHF VR 12-1, the maximum (269.33 cm) plant height was recorded in T_4

Fig. 1: Vegetable rai cultivation in open field cultivation: (A) Local variety (B) UHF VR 12-1 (C) Control plot (D) Treatment T_4 plot of UHF VR 12-1 variety

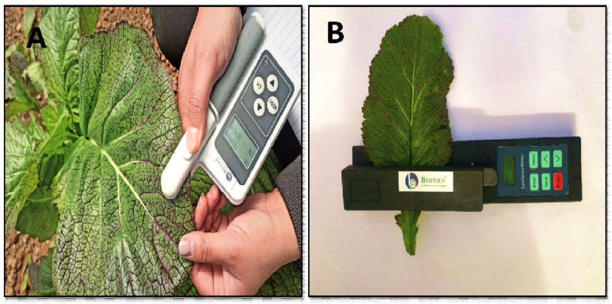

Fig. 2: (A) Measuring chlorophyll content (SPAD value) using chlorophyll meter (B) Measuring leaf area with leaf area meter.

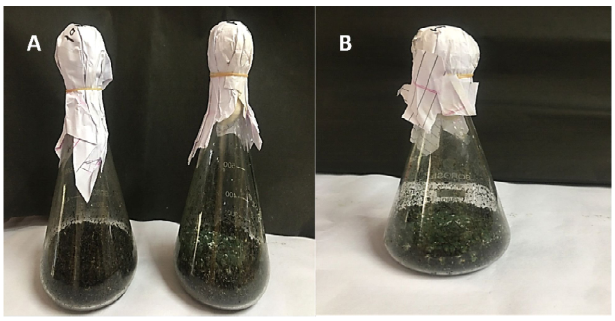

Fig. 3: Colonization of FYM by bioagents. Bioagents multiply and proliferate on FYM, which serves as organic source for the former, and enhancing nutritional composition.

followed by T_7 with 251.33 cm plant height, while the minimum was recorded in T_{10} (control) with 162.33 cm followed by T_8 with 197.00 cm plant height.

It is evident from the results that all the treatments were effective in increasing the number of primary and secondary branches in both the varieties of mustard. In local variety (Table 5), the number of primary and secondary branches per plant ranged from 5.67 to 10.67 and 7.33 to 12.83 respectively. The maximum primary and secondary branches were recorded in T_4 with 10.67 and 12.83 respectively, followed by T_7 with 9.67 and 11.67 numbers respectively. The minimum primary and secondary branches were in T_{10} (Control) with 5.67 and 7.33 respectively. Similarly, in UHF VR 12-1, the maximum primary and secondary branches recorded in T_4 with 12.17 and 14.00 respectively, followed by T_7 with 11.00 and 12.67 respectively, while the minimum was recorded in T_{10} (control) with 6.67 and 8.67, respectively, which was statistically at par with T_8 with 7.33 and 9.33 number of primary and secondary branches in variety UHF VR 12-1 (Table 7).

Fig. 4: (A) Damping-off disease of *Brassica juncea* observed in Nursery (B) Alternaria blight disease of *Brassica juncea* observed in field

Harman (2000) demonstrated the role of *T. harzianum* strain in enhancement of leaf greenness in maize. The enhancement in leaf area, leaf length, leaf width in both the varieties of mustard green might be due to the production of plant growth regulators such as GA and IAA, solubilisation of insoluble minor nutrients and, increase in nutrient transfer from soil to root due to actions of incorporated *Trichoderma* spp. (Yedidia *et al*., 2001; Gravel *et al.,* 2007) and enhanced root development and increase root hair formation during seedling. The present results are also in close agreement with the findings of Singh *et al.* (2016) and Rawat *et al.* (2013). Growth and growth-related factors are an essential component in determining the overall performance of plant. However, among the two varieties of mustard, variety UHF VR 12-1 showed better growth (Fig. 2.B & 2.D) when compared to local variety (Fig. 2.A) under field conditions. The other possible reason for an increase in various growth-related parameters might be due to production of Harzianic acid, a secondary metabolite produced by *Trichoderma* as earlier reported to have increased the growth of canola (Vinale *et al.,* 2009). Similar findings were also observed by Rawat *et al*. (2012), Haque *et al*. (2012) in mustard and Singh *et al*. (2011) in sweet pepper.

Yield and yield contributing attributes in two varieties of *Brassica juncea* in response to different treatments

Yield is an important factor in determining the potential growth of plant which can be considered as an outcome of overall growth of plant. It was observed that, in local variety, T_4 (Seed bio-priming with *Trichoderma asperellum Th-14* + FYM pre-colonized by *Th-14* + one drenching of *Th-14* at 45 days of the transplanting) recorded significantly higher leaf yield in I^{st}, II^{nd}, III^{rd} and IV^{th} pickings with 0.54, 0.70, 2.49 and 1.88 Kg/ plot respectively, followed by T_7 with 0.53, 0.68, 2.28 and 1.82 Kg/ plot leaf yield respectively (Table 4). In case of variety UHF VR 12-1, T_4 recorded maximum leaf yield in I^{st}, II^{nd}, III^{rd} and IV^{th} pickings with 0.69, 1.06, 3.36 and 2.24 Kg/ plot respectively, followed by T_7 with 0.68, 0.99, 3.02 and 1.99 Kg/plot leaf yield respectively, while minimum was observed in T_{10} (Control) which gave leaf yield of 0.46,

Table 3: Effect of microbial inoculants on nutritional composition of FYM

S. No.	Treatment	Organic content %	Nitrogen (mg/Kg)	Phosphorous (mg/Kg)	Potassium (mg/Kg)	pH
1.	FYM pre-colonized by *Trichoderma asperellum Th-14*	1.81	253.42	166.51	108.36	5.51
2.	FYM pre-colonized by *Pseudomonas fluorescens Psf -4*	1.52	222.02	158.12	102.53	6.83
3.	FYM pre-colonized by *Bacillus subtilis Bs*-2	1.43	219.28	150.14	97.60	6.78
4.	Non-colonized FYM	1.40	211.45	141.04	90.62	6.46
	CD at 5%	**0.015**	**2.589**	**1.655**	**3.231**	**0.056**
	SEM (±)	**0.004**	**0.782**	**0.500**	**0.976**	**0.017**
	CV (%)	**0.496**	**0.266**	**0.251**	**0.756**	**0.457**

Table 4: Effect of different treatments on chlorophyll content, leaf area, leaf length, leaf width and leaf yield of *Brassica juncea* (var. Local) at various stages of leaf picking

Treatments	Chlorophyll content (SPAD)	Leaf area (cm^2)	I[nd] Leaf picking			II[nd] Leaf picking		
			Leaf length (cm)	Leaf width (cm)	Leaf yield (Kg/plot)	Leaf length (cm)	Leaf width (cm)	Leaf yield (Kg/plot)
T_1	10.43	481.00	14.23	11.67	0.46	16.33	14.07	0.61
T_2	10.03	465.88	13.10	11.47	0.44	15.93	13.63	0.59
T_3	9.50	451.81	12.77	11.03	0.42	15.63	13.27	0.57
T_4	12.06	576.67	16.03	13.07	0.54	17.97	15.37	0.70
T5	11.10	517.51	15.10	12.47	0.50	16.93	14.67	0.67
T_6	10.73	497.18	14.67	12.07	0.48	16.77	14.37	0.64
T_7	11.43	544.00	15.43	12.73	0.53	17.47	15.03	0.68
T_8	8.60	421.22	12.03	10.37	0.27	14.43	12.47	0.53
T_9	9.17	434.55	12.43	10.63	0.30	15.17	12.83	0.55
T_{10}	7.17	411.88	11.70	10.07	0.25	13.93	12.03	0.51
CD at 5%	**0.278**	**3.044**	**0.203**	**0.145**	**0.005**	**0.050**	**0.135**	**0.008**
SEM (±)	**0.093**	**1.017**	**0.068**	**0.048**	**0.002**	**0.017**	**0.045**	**0.003**

Table 5: Effect of different treatments on leaf length, leaf width, leaf yield, plant height, number of branches, number of siliquae per plant and seed yield of *Brassica juncea* (var. Local)

Treatments	III[rd] Leaf picking			IV[th] Leaf picking			Plant height (cm)	Number of branches		No. of siliquae/ plant	Seed yield (Kg/plot)
	Leaf length (cm)	Leaf width (cm)	Leaf yield (Kg/plot)	Leaf length (cm)	Leaf width (cm)	Leaf yield (Kg/plot)		Primary	Secondary		
T_1	23.37	18.30	1.96	22.73	18.07	1.74	207.33	8.33	10.33	1166.67	0.36
T_2	22.77	17.97	1.91	22.37	17.73	1.71	197.67	7.67	9.67	1156.00	0.34
T_3	22.37	17.77	1.89	22.03	17.33	1.67	185.00	7.33	9.00	1140.67	0.33
T_4	24.70	20.27	2.49	24.93	19.33	1.88	254.00	10.67	12.83	1271.67	0.43
T5	24.07	19.27	2.07	23.27	18.73	1.78	232.33	9.33	11.00	1206.67	0.39
T_6	23.57	18.73	1.98	22.93	18.43	1.75	218.67	9.00	10.67	1185.33	0.38
T_7	24.43	19.77	2.28	24.77	19.17	1.82	211.33	9.67	11.67	1238.33	0.41
T_8	22.03	16.97	1.65	21.47	16.63	1.62	126.33	6.33	7.67	1087.67	0.31
T_9	22.57	17.47	1.76	21.77	17.00	1.64	172.33	7.00	8.67	1114.33	0.32
T_{10}	21.03	16.27	1.54	21.03	16.67	1.53	123.33	5.67	7.33	1085.00	0.30
CD at 5%	**0.278**	**0.071**	**0.065**	**0.108**	**0.61**	**0.019**	**36.229**	**0.886**	**0.817**	**31.013**	**0.005**
SEM (±)	**0.093**	**0.024**	**0.022**	**0.036**	**0.204**	**0.006**	**12.100**	**0.296**	**0.273**	**10.358**	**0.002**

0.76, 1.81 and 1.62 Kg/plot respectively (Table 6).

Different treatments were effective in improving the number of siliquae per plant in both the varieties i.e., local and UHF VR 12-1 as given in Table 5 and Table 7. In local variety, T_4 recorded significantly higher (1271.67) number of siliquae per plant followed by T_7 with 1238.33. The minimum with respect to this parameter was in T_{10} (Control) with 1085.00 followed by T_8 with 1087.67 number of siliquae per plant which were statistically at par with each other (Table 5). However, in variety UHF VR 12-1, the maximum number of siliquae per plant was

Table 6: Effect of different treatments on leaf area, chlorophyll content, leaf length, leaf width and leaf yield of *Brassica juncea* (var. UHF VR 12-1) at various stages of leaf picking

Treatments	Leaf area (cm^2)	Chlorophyll content (SPAD)	Ist Leaf picking			IInd Leaf picking		
			Leaf length (cm)	Leaf width (cm)	Leaf yield (Kg/plot)	Leaf length (cm)	Leaf width (cm)	Leaf yield (Kg/plot)
T_1	697.10	14.33	17.73	13.27	0.59	23.33	19.37	0.91
T_2	665.46	13.93	17.53	12.47	0.57	22.73	18.97	0.86
T_3	635.37	13.53	17.17	12.07	0.55	22.23	18.57	0.86
T_4	875.91	15.87	19.07	15.20	0.69	24.97	21.13	1.06
T5	820.54	14.93	18.47	14.23	0.65	24.07	20.37	0.96
T_6	734.53	14.63	18.07	13.63	0.62	23.77	19.87	0.93
T_7	843.11	15.43	18.67	14.63	0.68	24.43	20.73	0.99
T_8	595.07	12.53	16.07	11.43	0.50	21.27	17.57	0.81
T_9	624.02	13.23	16.47	11.83	0.53	21.77	18.03	0.83
T_{10}	540.37	11.07	15.17	11.13	0.46	20.67	16.93	0.76
CD at 5%	**14.840**	**0.148**	**0.218**	**0.170**	**0.005**	**0.117**	**0.083**	**0.027**
SEM (±)	**4.956**	**0.049**	**0.073**	**0.057**	**0.002**	**0.039**	**0.028**	**0.009**

Table 7: Effect of different treatments on leaf length, leaf width, leaf yield, plant height, number of branches, number of siliquae per plant and seed yield of *Brassica juncea* (var. UHF VR 12-1)

Treatments	IIIrd Leaf picking			IVth Leaf picking			Plant height (cm)	No. of branches		No. of siliquae/ plant	Seed yield (Kg/plot)
	Leaf length (cm)	Leaf width (cm)	Leaf yield (Kg/plot)	Leaf length (cm)	Leaf width (cm)	Leaf yield (Kg/plot)		Primary	Secondary		
T_1	26.63	22.77	2.68	25.63	20.40	1.94	232.00	9.33	11.333	1363.33	0.39
T_2	26.27	22.57	2.64	25.43	20.13	1.89	224.67	8.67	10.667	1313.33	0.38
T_3	25.53	22.07	2.59	25.17	19.60	1.84	233.00	8.33	10.333	1309.33	0.37
T_4	28.07	24.07	3.36	26.97	22.03	2.24	269.33	12.17	14.00	1492.33	0.46
T5	27.37	23.43	2.98	26.47	21.13	1.99	244.00	10.33	12.00	1426.67	0.42
T_6	27.03	22.97	2.76	26.07	20.77	1.95	242.33	9.67	11.67	1395.00	0.41
T_7	27.63	23.77	3.02	26.73	21.57	1.99	251.33	11.00	12.67	1463.67	0.43
T_8	24.63	20.63	2.22	24.83	19.07	1.75	197.00	7.33	9.33	1254.00	0.36
T_9	25.07	21.57	2.46	24.77	19.27	1.78	205.33	7.67	9.67	1275.67	0.36
T_{10}	24.10	19.93	1.81	23.97	18.43	1.62	162.33	6.67	8.67	1232.33	0.34
CD at 5%	**0.200**	**0.337**	**0.025**	**0.360**	**0.185**	**0.030**	**4.444**	**0.954**	**0.974**	**28.189**	**0.008**
SEM (±)	**0.067**	**0.112**	**0.008**	**0.120**	**0.062**	**0.010**	**1.484**	**0.319**	**0.325**	**9.415**	**0.003**

Table 8: Effect of different treatments on percent disease incidence of Damping-off, per cent disease incidence and percent disease severity index of Alternaria blight in *Brassica juncea*

Treatments	Damping-off disease		Alternaria blight disease			
	Local	UHF VR 12-1	Local		UHF VR 12-1	
	% Disease incidence	% Disease incidence	% Disease incidence	% Disease severity index	% Disease incidence	%Disease severity index
T_1	14.00	12.33	19.33	17.67	17.33	13.67
T_2	14.67	14.33	20.67	18.33	18.00	14.00
T_3	17.33	16.33	21.67	19.67	19.67	15.33
T_4	0.00	0.00	16.33	12.33	14.33	10.33
T5	0.00	0.00	17.33	14.00	15.67	11.67
T_6	11.33	10.67	18.67	16.00	16.67	12.67
T_7	38.67	37.67	22.67	20.00	20.67	17.00
T_8	41.33	38.67	24.67	21.67	21.33	19.33
T_9	0.00	0.00	16.67	13.33	14.67	11.00
T_{10}	47.33	46.67	49.67	39.33	47.33	36.67
CD at 5%	**1.435**	**1.129**	**1.657**	**2.124**	**1.534**	**2.676**
SEM (±)	**0.483**	**0.38**	**0.558**	**0.715**	**0.516**	**0.901**

recorded in T_4 with 1492.33 followed by T_7 with 1463.67, while minimum (1232.33) was in T_{10} which was statistically at par with the T_8 with 1254.00 number of siliquae per plant (Table 7).

Seed yield in both the varieties of rai i.e., local and UHF VR 12-1 ranged from 0.30 to 0.43 Kg/ plot and 0.34 to 0.46 Kg/ plot, respectively. In local variety, the maximum seed yield (0.43 Kg/ plot) was recorded in T_4 followed by T_7 with 0.41 Kg/ plot, while minimum was recorded in T10 (Control) with 0.30 Kg/ plot followed T_8 with 0.31 Kg/ plot (Table 5). In variety UHF VR 12-1, T_4 recorded highest seed yield (0.46 Kg/ plot) followed by T_7 with 0.43 Kg/ plot respectively, whereas minimum seed yield was observed in T_{10} (Control) with 0.34 Kg/ plot followed by T_8 with 0.36 Kg/ plot (Table 7).

With respect to yield parameters, all the treatments were observed to be effective in enhancing the yield and yield related traits. It is well documented that the interaction of *Trichoderma* strains with the plant promote growth and improves crop yield (Harman *et al*., 2004; Benítez *et al.,* 2004). Increase in yield and yield contributing traits might possibly be due to various actions of inoculated bio agents which helped in the overall improvement of plant growth, development and yield along with stem length, thickness and chlorophyll content (Rawat *et al*., 2011). Also, the inoculation of bio agents along with pre colonized FYM in plants have helped in increasing the colonies of *Trichoderma* by providing nutrients to fungal bio-control agent, thereby increasing the plant growth and yield along with increased deep roots, which helped in more water acquisition and uptake of nutrients and thereby increased the plant's ability to resist abiotic stresses and triggered defence pathway would have helped to resist to biotic stresses (Azarmi *et al*., 2011; Rawat *et al*., 2021). In the present study the FYM got colonized by bio-agents after 2 to 3 weeks (Fig. 3). This technique boosted the FYM nutritional content at the same time allowed the bio-agent to proliferate faster on the FYM (Table. 3). The present findings are in line with the previous findings of other workers *viz*., Rawat *et al.,* (2013), Sharma *et al*. (2012) in wheat and Hossain and Aktar (2020) in brinjal.

Disease assessment under field conditions

Damping-off incidence was observed under nursery condition (Fig. 4.A) and Alternaria blight disease (Fig. 4.B) was observed under open field condition. All treatments were reported to be responsive in reducing the per cent disease incidence and severity in both the varieties as depicted in Table 8.

In local variety, treatments T_4, T_9 and T_5 were found immune with 0.00 % disease incidence of damping off, while maximum disease incidence (47.33 %) was recorded in T_{10} (Control) followed by T_8 with 41.33 % disease incidence. In variety UHF VR 12-1, treatments T_4, T_9 and T_5 were again found immune to damping off disease with 0.00 % incidence followed by T_6 (10.67 %), while maximum disease incidence was again recorded in T_{10} (Control) with 46.67 % disease incidence.

Different treatments were observed to be effective in reducing the per cent disease incidence and per cent disease severity index of Alternaria blight in both the varieties of vegetable rai as presented in Table 8. It was observed that in local variety, T_4 recorded minimum disease incidence (16.33 %) and disease severity index (9.33 % with 2G) followed by T_9 with disease incidence of 16.67 % and disease severity index of 13.33 % with 3G, while maximum disease incidence (49.67 %) and disease severity index (39.33 % with 4G) was recorded in T_{10} (Control). Similarly, in variety UHF VR 12-1, the data revealed that the minimum disease incidence (14.33 %) and disease severity index (8.66 % with 2 G) was depicted in the treatment T_4 while the treatment T_{10} (Control) exhibited 47.33 % disease incidence and 36.67 % disease severity index with 4G.

Kumhar *et al.* (2022) reported that *T. asperellum* formulation improved seed germination and controlled the damping-off diseases in tomatoes when compared with control. It might be due to the fact that *Trichoderma* species having a special ability to parasitize other fungi and involves a direct attack by the process called mycoparasitism. This process involves different complex sequential events from recognition of the fungal strain by *Trichoderma*, effective penetration into the host fungi, attack on cellular machinery to finally killing off the host (Benítez *et al*., 2004; Waghunde *et al*., 2016). Other probable reasons for reduced disease incidence and disease severity might be due to elicitors released by *Trichoderma spp*., involved in triggering expressions of defence related proteins within the plant (Thakur and Sohal, 2013). In this way, plant immunity against pathogens is induced and in turn, improves plant growth, yield and suppresses diseases. Surekha *et al.,* 2014 concluded that plant defence enzymes play a vital role in mitigating pathogen-induced stress in legume, *Vigna mungo* by *T. viride.*

Several *Trichoderma* spp. have been reported to induce systemic resistance (Baiyee *et al*., 2019; Singh *et al.,* 2016), triggering defences such as high phenylpropanoid activities and lignification against phytopathogens (Amira *et al.,* 2017). Sood *et al.* (2020) concluded that

Trichoderma inhibited the growth of other pathogenic microbes through the biosynthesis of targeted metabolites like growth regulators, enzymes, antibiotics and siderophores. The isolates of biocontrol agents differ in their biocontrol and plant growth promotion ability in different crops (Joshi *et al*., 2010). The results of present study are also in close agreement with the findings of Jambhulkar *et al.* (2015) in chickpea, Kumhar *et al*. (2022) in tomato and Yarasani and Zacharia (2022) in mustard.

CONCLUSION

The results obtained from the present investigation revealed that the treatments exerted significant effect on all the studied traits. However, the application of seed bio-priming with *Trichoderma asperellum Th-14* + FYM pre-colonized by *Th-14* + one drenching of *Th-14* at 45 days of the transplanting was found to be the most effective and promising treatment for enhancing growth, yield and yield contributing traits and inducing resistance against damping- off and Alternaria blight disease in both the studied varieties of *Brassica juncea*. The variety UHF VR 12-1 was found best with respect to all the studied traits when compared with the local variety under present materials and environmental conditions. In the present era, humans are becoming more conscious for own health that's why more ecological approaches are now being researched. In recent years, there has been a worldwide swing to the use of eco-friendly methods for enhancing growth, yield and protecting the crops from various pests and diseases. The challenge today is how to achieve not only food security but also food safety by employing effective, economic and eco-friendly measures for control of plant pathogens in agriculture. From the present study, it may be concluded that the use of bio agents through various modes of application in combination (seed bio-priming, in form of pre colonized FYM and drenching) proved to be beneficial in enhancing the growth and yield of mustard green along with the suppression of endemic diseases in mid hills of Uttarakhand.

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