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Effect of Organic Manure and Biofertilizer on Growth and Yield of Brown Mustard (*B. juncea* subsp. *integrifolia* var. crispifolia)

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ABSTRACT

The study was conducted at Department of Horticulture, Sikkim University, Gangtok, Sikkim during 2020–2021 with an objective to observe impact of organic manure and biofertilizers on growth and yield of brown mustard. The experiment had 7 treatments and 3 replications and statistical design used was Randomized Block Design. The best results were observed in treatment T_6 (FYM 100%) + *Azospirillium* @5kg/ha) in terms of leaf length, leaf width, plant girth, number of leaf/plant, leaf weight, fresh weight of plant, dry weight of plant, leaf area index, leaf NPK content, germination percentage, root length. The second best treatment in this study was T_3 (FYM 100%+ *Azotobacter* @500 ml/ha). The study is helpful in improvement of production of brown mustard.

Keywords Biofertilizer, Brown mustard, Leaf, Organic manure, Yield.

INTRODUCTION

Brassica juncea L. belongs to family Brassicaceae or Cruciferae and is also known as Chinese mustard, oriental mustard and raayo saag. The own circle of relatives of mustard presently consist of 338 genera and 3790 species (Warwick *et al.* 2006). The *B. juncea* subsp. *integrifolia* var. crispifolia is also known as "curled mustard", "American mustard", "Southern mustard", "Texas mustard" and "Southern curled mustard". Brown mustard is normally grown in Sikkim for its sparkling foliage and the climatic and soil conditions of this state are favorable for its cultivation. The leaves of brown mustard/raayo saag are ovate or obovate and petiolated. The inflorescence is raceme and has bisexual flowers with 4 unfastened sepals and 4 yellow petals, together with longer and shorter stamens. This annual herb originates from the hybridization of black mustard (*Brassica nigra* L. Koch) (2n=18) and turnip mustard (*Brassica rapa* L.) (2n=20) and it is amphidiploid (2n=36). It originated in middle east however, it is largely found in Europe, Africa, North America and Asia. Several

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authors have reported eastern India, the Caucasus as its origin (Dixon 2007). Organic agriculture supports health of agro-ecosystem which includes biodiversity, biological cycles and biological activity of soil (Aggani 2013, Malusà *et al*. 2016, Malve *et al.* 2017). At present, the production of sufficient number of "ecologically clean" food products is one of the most important global challenges facing humanity (Dubey and Shukla 2014, Malve *et al.* 2017). Biofertilizers include microbes and are one of the crucial additives of integrated nutrient management, as they are very powerful and renewable source of plant nutrients and are ecologically secure too (Raja 2013, Malve *et al.* 2017). Balanced application of natural farming and biofertilizers facilitate sustainable production. The natural substances typically used to enhance soil fertility include farmyard manure (FYM), animal wastes, crop residues, city natural wastes (composted), manures, biofuel, slurry, microbial preparations, vermicompost and biodynamic preparations. Organic manure allows in preserving C:N ratio by decomposing organic matter and mineralization within soil and additionally increases the soil fertility and productivity. They solubilize the insoluble phosphates like tricalcium, iron and aluminium phosphate into soluble forms. They fix atmospheric nitrogen within the soil and root nodules of legume crop and make it available to the plants. *Azotobacter* infuse the soil with antibiotic pesticide and inhibit the soil profile from getting soil-borne diseases caused by *Pythium* sp. and *Phytophthora* sp. Aquatic cyanobacteria increase hormone, protein and nutrients. Verma and Pandey (2022) also reported that biofertilizers have ability to make environment clean by bioremediation. Gupta *et al.* (2024) reported that application of biofertilizers with chemical fertilizers improves mustard crop productivity along with environmental sustainability. By keeping above mentioned points, the present study was undertaken with an objective to observe impact of natural manure and biofertilizers on growth and yield of brown mustard.

MATERIALS AND METHODS

The present experiment was carried out at the farmer's field, Singtam, East Sikkim at an altitude of 665 m amsl (longitude 27°15'44"N and latitude 88°35'45"E). Details of material used and techniques employed during the course of study are being mentioned in this section. The soil pH, soil Nitrogen, Potassium, Phosphorus and organic content was analyzed in Soil Testing Laboratory at ICAR, Tadong, Gangtok, Sikkim. The number of treatments were seven (07) and number of replications were three (03) and the spacing between plants was 30cm x15cm. The experimental design used was Randomized Block Design. The local variety of brown mustard i.e. crispifolia was used. The seeds were sown in November, 2020 in the farmer's field and the harvesting was done in last week of January, 2021. The treatment details were as follows:-T_0- Control (FYM 100%) (25 tonnes/ha) , T_1- Vermicompost (100%), T_2- Farmyard manure (50%) + Vermicompost (50%), T_3 –Farmyard manure (100%) + *Azotobacter* (@500 ml/ha) T_4: Farmyard manure(100%)+ *Trichoderma viridae* (@ 2g/kg of seeds), T_5- Farmyard manure (100%) + BioSAR NPK, T_6- Farmyard manure (100%)+ *Azospirillium* (@ 5kg/ha). The following observations were recorded during the period of study.

Growth characters

***Plant height, leaf length, leaf width and plant girth*:-** The plant height was recorded at 15 days, 30 days, 60 days and at the time of harvesting. The plant height, leaf length and leaf width were measured in cm by measuring tape. The girth of plant was taken by vernier callipers.

***Number of leaves/plant, leaf weight, fresh weight of plant, dry weight of plant*:-** Individual leaves were counted after the harvesting of the mustard greens. Leaf weight was taken after harvesting of the plant with the help of digital weighing machine. The fresh weight of the plant was taken by the digital weighing machine after its harvesting along with its roots. Dry weight of the plant along with its roots was taken after drying it in 40-50^{0}C for a week until it was completely dry by digital weighing machine.

Fresh weight of leaf, gross weight of plant, leaf area index: Individual leaves were collected from the plant and then the fresh weight was taken by the weighing balance. The gross weight of the plant was recorded similarly. Leaf area index was recorded by leaf area meter.

Leaf NPK and micro nutrient content : Individual leaves were placed in the sun for 2-3 days and then they were placed in the hot air oven at 40-50^{0}C and then made into a powder form and labelled. The powdered samples were sent to ICAR NEH Regional Center, Sikkim located at Tadong, Gangtok for finding out the leaf NPK and micro nutrient content.

Leaf NPK uptake : The leaf NPK content was calculated by the amount of NPK content in the soil- NPK content in plant.

Leaf micronutrient uptake: The leaf micronutrient content was taken by calculating the amount of NPK content in the soil- NPK content in plant.

Germination percentage, root length and root volume: After emergence of the seedling (i.e.9-10 days) the germination percentage was taken by counting the number of seedlings per treatment. After uprooting the whole mustard greens the roots were measured from the base below. The root volume was taken after harvesting the brown mustard from the field. Individual roots per plant were weighed on the weighing scale.

Economics of crop production

Cost of cultivation(Rs): Total fixed cost + Total variable cost.

Gross income (Rs): Gross income represents the income or profit remaining after the production costs have been subtracted from revenue.

Net income (Rs): Net income is the profit that remains after all expenses and costs have been subtracted from revenue.

B:C (Benefit:Cost) ratio: A benefit-cost ratio (B: C) is a ratio used in a cost-benefit analysis to summarize the overall relationship between the relative costs and benefits of a proposed project.

Before transplantation

Seed treatment

The jaggery was melted with some water in a pan. It was cooled at room temperature, then it was mixed with the required treatment. The mustard seeds were poured in solution and it was mixed well and kept at room temperature for 24 hours. Seed of mustard greens were treated according to details of treatments mentioned above i.e. in T_0, T_1 and T_2- no seed treatment was required ; in T_3, T_4, T_5 and T_6, *Azotobacter* (@500ml/ha), *Trichoderma viridae* (@ 2g/kg of seeds), Biosar, *Azospirillium* @ 5kg were given respectively.

The pro trays were filled with vermicompost 50% (20g) and soil 50% (20g) weighing 40g in each pro cells. The seeds were sown by placing 2-3 seeds in a pro cell. The soil was prepared with 50%soil and 50% vermicompost and the pro trays were filled. The trays were used until the seedlings were ready to be transplanted. Germination was observed after 2-3 days of planting. The germination percentage was calculated with formula:- Germination % = (Number of seeds sown /Number of seeds germinated)×100. The irrigation was done alternatively as and when required with a small sprinkler. Weeding was done once in the pro tray.

After transplantation

The soil was ploughed twice or thrice before transplanting. At the time of transplanting as mentioned in details of treatments above in T_0 Control FYM 100% was given. Similarly in T_1- Vermicompost (100%), T_2- Farmyard manure (50%) + Vermicompost (50%), T_3, T_4, T_5, T_6 -Farmyard manure (100%) was given.

The transplanting was done after 20 days of germination when it had 3-4 true leaves and 10 cm height. The planting distance was kept as 15 cm (plant-plant) × 30 cm (row-row). Irrigation was done every alternate day till harvesting. Weeding was done after every 10 days initially and later after every 5 days. Neem oil (10%) in water was used to reduce the blight, the solution was sprayed on individual leaves twice. One spraying was done after 80 days and another after 90 days of transplanting. Harvesting was done after 110 days of transplanting.

RESULTS AND DISCUSSION

The results of present study are being presented as follows under various sub-headings:

Table 1. Effect of organic manure and biofertilizer on plant height, leaf length, leaf width, plant girth and germination% of brown mustard.

Treatments	Plant height 30 DAT	Plant height 60 DAT	At harvest stage	Leaf length (cm)	Leaf width (cm)	Plant girth (cm)	Germination (%)
T_0	6.00	17.6	22.6	16.27	9.5	7.33	82
T_1	6.00	21.1	26.0	18.05	10.0	7.77	82
T_2	7.00	20.7	25.6	19.79	10.4	8.42	83
T_3	8.00	22.1	27.1	24.41	11.8	9.56	84
T_4	7.00	21.5	26.4	22.00	10.9	9.37	83
T_5	8.00	21.6	26.5	21.07	10.9	8.97	84
T_6	8.01	22.4	27.6	26.73	13.3	10.1	85
SEm±	0.97	0.72	0.81	0.88	0.33	0.28	1.38
LSD (p=0.05)	NS	2.11	2.38	2.59	0.97	0.81	NS

(Note: Treatment details same as in materials and methods and Tables 2–6).
[T_0: Control (FYM 100% @25 t/ha) , T_1: Vermicompost (100%); T_2: FYM (50%) +Vermicompost (50%); T_3: FYM (100%)+ *Azotobacter* @500 ml/ha, T_4 : FYM (100%)+ *Trichoderma viridae* @ 2g/kg of seeds, T_5: FYM 100%+ Bio SAR, T_6: FYM (100%)+ *Azospirillium* @ 5 kg/ha].

Plant height, leaf length, leaf width, plant girth and germination%: It is clear from Table 1 that the treatment T_6(FYM 100% + *Azospirillium* @5kg/ha) performed best regarding plant height (8.01 cm (30 DAT), 22.4 cm (60 DAT), 27.6 cm (harvesting stage), leaf length (26.73 cm), leaf width (13.3 cm), plant girth (10.1 cm), germination (85%) followed by treatment T_3 (FYM 100% + *Azotobacter* @ 500 ml/ha). The treatment T_0 i.e. control (FYM 100%) showed least performance.

Mangmang *et al.* (2014) in an experiment showed that the treatment by using *Azospirillium* showed that the percentage of increase of plant height was seen to be 19 over control. These results confirm the results given by Quadros (2014) who tested hybrids of corn and verified interaction between the hybrids and inoculation of *Azospirillum brasilense* for plant height. Bacteria of the genus *Azospirillum,* besides the ability to fix N, help in the availability of hormones e.g. auxins, which are related to root development. When the nutritional needs of plant are fulfilled, it mobilizes resources for shoot growth too. This may explain the increase in N concentration in the leaves, a sum of hormonal factors and N fixation by the bacteria (Taiz and Zeiger 2010). Bugalia *et al.* (2017) also reported maximum plant height recorded by foliar spray of *Trichoderma viridae* @2%. The present findings were similar to Malve *et al.* (2017) who reported that silicate solubilizing bacteria enriched biofertilizer improves photosynthetic function of *B. juncea.*

Azospirillium enhances plant growth, however its complete mechanism is yet to be understood properly. The hormonal effect may be one of the phytostimulators (Puente *et al.* 2009, Prongjunthuek *et al.* 2019). *Azospirillum* sp. inoculation has shown results in improvement in yield and different growth parameters like plant height, leaf size, root length, nutrient uptake, tissue N content of cereals (Bashan *et al.* 2004, Noumavo *et al.* 2013, Prongjunthuek *et al.* 2019). Also, *Azospirillum* has shown good results to be used as natural fertilizer (Cakmakci *et al.* 2006, Prongjunthuek *et al.* 2019).

The organic manure and biofertilizer affected plant girth significantly. The percentage of increase of leaf number due to inoculation over control was similar to the findings of Mangmang *et al.* (2014). The effect on maximum germination % was found to be insignificant. Shaukat *et al.* (2006) reported that *Azospirillum, Pseudomonas,* and *Azotobacter* strains could improve seed germination and seedling growth (Prongjunthuek *et al.* 2019). The findings of Yadava *et al.* (2023) revealed that the treatment 100% RDN through Poultry Manure +Biofertilizer; Vermicompost +Biofertilizer and FYM +Biofertilizer demonstrated significantly higher yield of mustard. Kalita *et al.* (2019) reported that application of *Azotobacter*

Table 2. Effect of biofertilizers and organic manure on no. of leaves per plant, green leaf yield, dry matter accumulation, leaf area index, root length and root volume.

Treatment	No. of leaves per plant	Green leaf yield (g/plant)	Green leaf yield (t /ha)	Dry matter accumulation (g/plant) 60 DAT	Dry matter accumulation At harvest stage	Leaf area index 60 DAT	Leaf area index At harvest stage	Root (at harvesting stage) Length	Root (at harvesting stage) Volume (cm^3)
T_0	5.10	171	6.19	22.6	31.4	1.97	3.04	20.0	11.5
T_1	5.17	188	6.98	26.0	38.3	2.12	3.10	25.4	12.9
T_2	5.40	186	6.71	25.6	37.9	2.19	3.17	27.9	13.3
T_3	8.60	197	8.00	27.1	39.4	2.42	3.40	31.8	14.4
T_4	6.13	192	7.41	26.4	38.8	2.37	3.35	30.9	13.8
T_5	6.03	193	7.50	26.5	38.8	2.40	3.38	29.5	13.9
T_6	9.37	200	8.34	27.6	39.9	2.48	3.46	33.3	15.0
SEm ±	0.33	5.53	0.17	0.81	1.19	0.10	0.13	0.93	0.48
LSD (p=0.05)	0.98	16.2	0.50	2.38	3.49	0.30	0.38	2.74	1.41

+PSB+50-75%NPK+FYM @2 t ha^{-1} showed good results for toria cultivation in hilly parts of Assam.

Number of leaves per plant, green leaf yield, dry matter accumulation, leaf area index and root length

It can be observed from Table 2 also that the maximum result was observed in T_6 (FYM 100% + *Azospirillium* @5 kg/ha) with maximum no. of leaves /plant (9.37), green leaf yield (200g/plant, 8.34 t/ha), dry matter accumulation [27.6 (DAT), 39.9 (at harvesting stage)], LAI [2.48 (60 DAT), 3.46 (at harvesting stage)], root length (33.3 cm) and root volume (15.0 cm^3) at harvesting stage. The second best treatment in all parameters studied was T_3 (FYM 100% + *Azotobacter* @ 500 ml/ha). The results were significantly superior over control T_0 (FYM 100%). Inoculated seedlings produced more developed and bigger leaves than those without inoculation as reported by Mangmang *et al.* (2014).

Azospirillum changes morphology of root of its host plant. It might be due to phytohormones i.e. auxin-IAA produced by the bacteria. Dobbelaere *et al.* (1999) also reported that production of auxins, gibberellins, cytokinins, and other growth substances by the bacteria could be responsible for the growth effects associated with *Azospirillum.* The improvement of root morphology could enhance the absorption of water and essential minerals by plant roots from the surrounding environment which in turn increases plant growth (Mangmang *et al.* 2014). Laslo *et al.* (2012) and Shaharoona *et al.* (2006) reported that bacteria isolated from maize rhizosphere had various plant growth promoting (IAA) and biocontrol activities and they have shown increase in shoot and root length (Prongjunthuek *et al.* 2019). Widnyana *et al.* (2018) also reported significant effect of soil seed immersion with *Bacillus* sp. on all parameters studied except root length of plant.

Leaf NPK content and NPK uptake

It is evident from Table 3 that the NPK content and NPK uptake of the plant was observed as non-significant. But the highest NPK value was observed in T_6(0.45:0.183:0.92) and the lowest was seen in T_0(0.41:0.142:0.80). The highest NPK uptake in the

Table 3. Effect of organic manure and biofertilizer on NPK content of the leaves and NPK uptake of plant.

Treatments	NPK content of leaves (g)			NPK uptake of the plant ($kg\ ha^{-1}$)		
	N	P	K	N	P	K
T_0	0.41	0.142	0.80	25.6	8.79	49.6
T_1	0.42	0.151	0.80	29.5	10.5	56.1
T_2	0.43	0.153	0.84	29.0	10.3	56.5
T_3	0.45	0.180	0.90	36.0	14.4	72.1
T_4	0.43	0.160	0.80	32.2	11.9	59.5
T_5	0.44	0.171	0.89	33.0	12.8	67.1
T_6	0.45	0.183	0.92	37.4	15.3	76.9
LSD (p=0.05)	NS	NS	NS	NS	NS	NS

Table 4. Effect of organic manure and biofertilizer on micro nutrient content of the leaves (g).

Treatment	Ca	Mg	S	Fe	Mn	Zn	Cu	Bo
T_0	0.31	0.131	0.126	73.2	51.2	19.2	4.10	20.2
T_1	0.33	0.143	0.127	75.1	51.5	19.6	4.32	22.1
T_2	0.38	0.133	0.129	78.2	52.7	20.0	5.17	22.3
T_3	0.42	0.164	0.132	80.3	55.2	21.3	7.12	25.0
T_4	0.40	0.150	0.130	82.4	53.0	20.2	5.29	23.3
T_5	0.41	0.162	0.131	82.2	56.2	20.7	6.12	24.2
T_6	0.46	0.171	0.134	83.5	58.1	22.5	7.15	25.4
LSD (p= 0.05)	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S

Table 5. Effect of organic manure and biofertilizer on micro nutrient uptake of the plant (kg ha^{-1}).

Treatment	Ca	Mg	S	Fe	Mn	Zn	Cu	Bo
T_0	19.2	8.11	7.80	0.453	0.317	0.025	0.025	0.125
T_1	23.1	10.0	8.87	0.524	0.360	0.030	0.030	0.154
T_2	25.7	8.93	8.66	0.525	0.354	0.035	0.035	0.150
T_3	33.8	13.1	10.6	0.643	0.442	0.057	0.057	0.200
T_4	29.7	11.1	9.64	0.611	0.393	0.039	0.039	0.173
T_5	31.0	12.2	9.83	0.617	0.422	0.046	0.046	0.182
T_6	38.5	14.3	11.2	0.696	0.484	0.060	0.060	0.212
LSD (p= 0.05)	N/S	N/S	N/S	N/S	N/S	N/S		N/S

column was observed in T_6 (FYM100% + *Azospirillium* @5 kg/ha) followed by T_3 (FYM 100% + *Azotobacter* @500 ml/ha) and the lowest was seen in T_0(Control-FYM 100%). The findings of this study were similar to study conducted by Kumar and Singh (2019) who reported that the available N content in post harvest soil was improved with inoculation of biofertilizers in Indian mustard (*Brassica juncea*). It has already been mentioned in the first part of results and discussion earlier under sub heading plant height that *Azospirillum* sp. inoculation results in significant improvement in growth parameters, leads to improved yield and can be used as natural fertilizer.

Leaf micro nutrient content

It is clear from Table 4 that the micro nutrient content of the plant was shown as insignificant. The maximum output was seen in T_6 (FYM 100% + *Azospirillium* @ 5 kg/ha) where the nutrient were shown as follows Ca-0.46, Mg-0.171, S-0.134, Fe-83.5, Mg-58.1, Zn-22.5, Cu-7.15, Bo-25.4 followed by T_3 (FYM 100% + *Azotobacter* @ 500 ml/ha). The minimum output was seen in T_0 control (FYM 100%).

Leaf micronutrient uptake of plant

Similarly, it is clear from Table 5 that the effect of organic nutrients and biofertilizers on micronutrient uptake of plant was also observed as non-significant. However, the highest value was seen in T_6 (FYM 100% + *Azospirillium* @ 5 kg/ha) followed by T_3 (FYM 100%+ *Azotobacter* @500 ml/ha). The lowest was observed in T_0 [(Control (FYM 100%)].

The findings of present study are similar to the findings of Sahoo *et al.* (2010), Yadav *et al.* (2010), Alami *et al.* (2018), Kalita *et al.* (2019) who supported the significant effect of biofertilizers on growth and yield of Indian mustard (*Brassica juncea*). Haque *et al*. (2012) reported significant effect of biofertilizers on mustard (*Brassica rapa*).

Economics of production of brown mustard

It is clear from Table 6 that the yield (tonnes/ha) and cost of cultivation was recorded as highest i.e. 8.34 tonnes/ha and Rs 32,700 respectively in T_6 (FYM 100%+*Azospirillium* @5 kg/ha). The minimum value was observed in control T_0 (FYM 100%) as the cost of FYM was only Rs 50/kg.

It is also evident from Table 6 that the gross income (Rs 69,769/-) net income (Rs 37,069/-) and B:C ratio (2.1) was also observed to be highest in T_6 (FYM 100%+*Azospirillium* @5 kg/ha). The treatment

Table 6. The economics of production of brown mustard (*Brassica juncea* subsp. *integrifolia* var. *crispifolia*).

Treatments	Yield (t/ha)	Cost of cultivation (Rs)	Gross income (Rs)	Net income (Rs)	B:C ratio
T_0	6.19	10,986	19,000	8,014	1.6
T_1	6.98	20,675	35,000	14,325	1.6
T_2	6.71	21,879	38,559	16,680	1.7
T_3	8.00	29,546	53,675	24,129	1.8
T_4	7.41	23,865	39,890	16,025	1.6
T_5	7.50	24,000	38,087	14,087	1.7
T_6	8.34	32,700	69,769	37,069	2.1

T_3 (FYM 100% + *Azotobacter* @500 ml/ha) was observed as second best treatment with gross income (Rs 53,675/-), net income (Rs 24,129/-) and B: C ratio (1.8). The lowest gross income (Rs 19000/-) net income (Rs 8,014/-) and B:C ratio (1.6) was observed in T_0 (control).

The present study was found similar to the findings of Hadiyal *et al.* (2017) who reported that seed inoculation with *Azotobacter* sp.+ PSB sp. (each @10 ml/kg seed) promoted growth parameters with ultimately higher seed and stover yield with higher net returns of Rs 86,629 Rs/ha and B:C ratio 3.40 over control (no inoculation).

CONCLUSION

The effect of organic manure and biofertilizers was observed to be the best with reference to all parameters studies i.e. number of leaves per plant, green leaf yield, dry matter accumulation, leaf area index, root length, root volume, leaf NPK and micronutrient content, NPK and micronutrient uptake by plant, gross income, net income and B: C ratio in treatment T_6 (FYM 100% + *Azospirillum* @ 5 kg/ha) followed by treatment T_3 (FYM 100%+ *Azotobacter* @ 500 ml/ha) and they were significantly superior over control. Brown mustard production can be increased by use of organic manures and biofertilizers in Sikkim and it can be introduced to non-traditional areas also.

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