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Influence of Bio-fertiliser (*Rhizobium radiobactor*) in Association with Organic Manures on Qualitative Traits of Broccoli (*Brassica oleracea* L. var. *italica* Plenck) cv. Palam Samridhi under Lucknow Conditions

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Authors' contributions

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

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ABSTRACT

The field experiment was conducted at Horticultural Research Farm of the Department of Applied Plant Science (Horticulture), Babasaheb Bhimrao Ambedkar University (A Central University), Vidya-Vihar, Rai-Bareli Road, Lucknow (U.P.) – 226025, India during Rabi season of 2016-17. The research material comprised of 8 treatments replicated three times in a Randomized Block Design. The chlorophyll content in leaves (108.27 mg/l), Proline content in leaves (33.99), Nitrate content in leaves (156.75 mg/kg), Carbohydrate content in leaves (108.27 mg/l), Protein content in leaves (0.38 mg/l), Carbohydrate in curd (107.49 mg/l), Chlorophyll in curd (108.62 mg/l), Antioxidant in curd (61.90 %), Flavonoid in curd (34.49 %), Nitrate in curd (172.96 mg/kg), Protein in curd (0.40 mg/l), T.S.S. in curd (10.9 ºBrix) was maximum recorded under T_7 (Biofertilizer + Vermicompost) treatment. The fresh weight of plant (1132.66 g), Dry weight of plant (0.203 g), Fresh weight of root (82 g) Dry weight of root (0.021 g), Root length (17.80 cm), Root spread (24.7 cm), was maximum recorded under T_7 (Biofertilizer + Vermicompost) treatment. It is recommended for higher production of sprouting broccoli was under Lucknow conditions.

Keywords: Broccoli; chlorophyll; Proline; nitrate; protein; carbohydrate; antioxidant; flavonoid and T.S.S.

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1. INTRODUCTION

Broccoli is an Italian vegetable, cultivated in Italy in ancient Roman times and in England in about 1720. On the other hand, in the USA it first appeared in 1806 but was commercially cultivated around 1923 [1]. Broccoli is an Italian word derived from Latin word *brachium* which means "an arm or branch" [2]. Cruciferous vegetables are an excellent dietary source of antioxidant vitamins and glucosinolates, precursors to a group of isothiocyanates shown to be anticarcinogenic [3]. Dietary antioxidants, vitamins and non-nutrient components such as flavonoids, are present in crucifers and may decrease the risk for certain cancers [4]. Glucosinolates are the main bioactive compounds found in the Brassicaceae family. Broccoli flower head extract also possesses an antimutagenic property [5]. Broccoli (Brassica oleracea var Italica) is a nutritionally important crop grown all over the world, and it is a floral vegetable with an important nutritional value due to its content of vitamins, antioxidants, glucosinolates and anticarcinogenic compounds (Parente et al*.,* 2013). Broccoli (Brassica oleracea. var italica plenck; 2n=x=18), which is originated from the Mediterranean region commonly known as Hari ghobi in Hindi and a member of Cole group belongs to the family Brassicaceae or Cruciferae. The term Cole has originated from the word "Colewart'' meaning wild cabbage while the broccoli derived its name from the Latin word Brachium meaning an arm or branch. United States of America (USA) is the largest producer of Sprouting broccoli in the world. It is a new crop in India and its cultivation is negligible but now it is becoming increasingly popular in hotels in Mumbai, Calcutta, Delhi and Chennai. The nutritive value of sprouting broccoli per 100g of edible portion is given below: water (89.3%), protein (3.6%), fat (0.2%), carbohydrates (5.5%), fiber (1.2%), vitamin A (900 I.U.), vitamin B (33 I.U.), vitamin C(137 I.U.), vitamin E (2.3 I.U.), vitamin K (3.5 I.U.), calcium (1.29 mg), manganese (20 mg), Iron (1.3 mg), phosphorus (0.79 mg),and sulphur (1.26 mg). Vegetables are a great source of antioxidants because almost all contain one or more of the following: vitamin C, vitamin E, selenium and beta-carotene, all hailed for having high antioxidant properties. Organic fertilisation is becoming increasingly important particularly regarding health benefits. Organic manures can serve as an alternative to mineral fertilisers as reported by Naeem et al. [6] for improving soil structure [7] and microbial biomass [8]. Biofertilizers have a great potential to bridge the gap between demand and supply of nutrient. Bio-fertilisers contain microorganism which is capable of mobilising nutritive elements from non-usable form to usable form through biological processes. Bio-fertilisers play a multifaceted role by not only enriching the soil micro-organism but also as nutrients, stabilisers, hormones and insulators [9]. The organic manures are the beneficial response of yield might also be attributed to the availability of sufficient amounts of plant nutrients throughout the growth period and especially at critical growth period of the crop, resulting in better uptake, plant vigour and superior yield attributes. Organic manures are a considerable amount of major nutrients in the soil beside improving the soil properties. Further, decomposition of organics in the soil leads to different types of biological reactions which are helpful in preventing various disease-causing pathogens [10].

2. MATERIALS AND METHODS

The present field experiment was carried out at the Horticulture Research Farm-I of Department of Applied Plant Science (Horticulture), Babasaheb Bhimrao Ambedkar University, Vidya-Vihar, Rae-Bareli Road, Lucknow (U.P.), India. The location of the farm is situated on the main campus of the University. The experiment was conducted at Rabi season of 2016-17. Broccoli seeds Palam Samridhi were sown in the nursery beds of Horticulture Research Farm-I, BBAU, Lucknow, by sowing in row method on 7 October 2016. Raised bed about 5-6 meter long, one-meter width and 15 cm above ground level, was prepared. A thin layer of powdered leaf mould was applied to cover the seeds. Regular watering, hoeing, weeding, plant protection measures etc. were done from time to time. The seedlings were ready for transplanting within five-six weeks. The NPK done by RDF method (100:60:60) and organic manures viz. Farm Yard Manure (FYM) @ 20t/ha, Vermicompost @ 8t/ha were applied before transplanting as per the treatment and mixed thoroughly in the soil. Biofertilizer *Rhizobium radiobacter* was obtained from the Department of Environmental Microbiology, Babasaheb Bhimrao Ambedkar University, Lucknow. Before transplanting the seedlings were treated with bio-fertiliser *Rhizobium radiobactor* for 30 minutes by dipping the roots of seedlings in the solution culture. The full dose of FYM & vermicompost should be applied in the soil before the transplanting. The distance between row to row and plant to plant

was kept as 45 × 30 cm, respectively. Thus, nine plants were accommodated in each plot. The experiment was laid out in Randomized Block Design (RBD) with three replication and experiment comprised of 8 treatment combinations. The qualitative character - Chlorophyll content in leaves, Proline content in leaves, Nitrate content in leaves, Carbohydrate content in leaves, Protein content in leaves, Carbohydrate in curd, Chlorophyll in curd, Antioxidant in curd, Flavonoid in curd, Nitrate in curd, Protein in curd, T.S.S. in curd, Fresh weight of plant, Dry weight of plant, Fresh weight of root, Dry weight of root, Root length, Root spread. The statistical analysis of the data was carried out as per the method described by Cochran and Cox, 1963. The treatment effect was tested at 5% level of significance. The data obtained from selected plants were subjected to analysis of variance.

Biochemical Analysis of Broccoli (Leaves and Curd)

1. Nitrite estimation (Stevens and Oaks, 1973)

Reagents required

- ✓ $NaNO_2$ stock solution: - Dissolved 0.001 g of $NaNO_2$ in 100 ml distilled water.
- ✓ 1 % Sulphailamide (LR) Solution (4-amono benzene sulphonamide, ($C_6H_8N_2O_2S$): Dissolved 1 g sulphanilamide in 100 ml 1N HCl.
- ✓ 0.01% NED (N -1-napthyl ethyldiamine dihydrochloride (GR) $C_{12}H_{16}C_{l2}N_2$ Dissolved 0.01 g of NED in 100 ml distilled water.

2. Protein Estimation (Bradford Assay)

The Bradford protein assay is used to measure the concentration of total protein in a sample.

Reagents required

- ✓ Bovine Serum Albumin (BSA) (Sigma-Aldrich)
- ✓ Coomassie Brilliant Blue G-250
- ✓ Methanol
- ✓ Phosphoric acid (H_3PO_4)
- ✓ Bradford reagent (see Recipes)

3. Proline Assay Protocol (Bates et al., 1973; Hamid et al., 2003)

Preparation of crude plant extract

Ten grams of each leaf were extracted with cold water and boiled water (100°C in 100ml) for 10, 30 and 60 minutes. Both extractions were then filtered (Whatman No. 1). The filtrates were analysed for proline and antioxidant activity by DPPH free radical scavenging assay.

Reagents

- ✓ Acid-ninhydrin Prepared by warming 1.25 g ninhydrin in 30 ml glacial acetic acid and 20 ml 6 M phosphoric acid, with agitation, until dissolved. Kept cool (stored at 4°C) the reagent remains stable 24 hours.
- ✓ Extraction buffer: 3% (w/v) aqueous sulfosalicylic acid.
- ✓ glacial acetic acid
- ✓ Toluene
- ✓ Proline

4. Extraction of Antioxidants

The extraction of antioxidants was carried out with 1 g of sample in 25 ml of 30% methanol in water bath with stirring for 1 h at 60°C (Dubnoff bath ET-053, Tecnal) after centrifuged (HIMAC CR22CII, Hitachi) for 30 min at 27.000 *g* and filtered directly into a 25 ml volumetric flask. The extracts were stored at -18°C (Gomes et al*.,* 2013).

DPPH radical scavenging activity (Antioxidant activity)

The free radical – scavenging activity of each extract was determined as described by Braca et al., (2001). Plant leaves extracts were added to 3ml of a0.004% methanol solution of DPPH. Absorbance at 517 nm was measured under constant mixing at room temperature after 30 min and percent inhibitory activity was calculated.

5. Chlorophyll estimation (Arnon 1949)

- ✓ 0.5gm fresh plant materials were homogenised in 20 ml of 80% chilled acetone with the help of mortar and pestle in dark.
- ✓ A pinch of MgCO3 powder added. The extract filtered through Whatman no.1 filter paper.
- ✓ The final volume of the filtrate was made to 100 ml with 80% acetone in conical flask wrapped with black carbon paper.
- ✓ Absorbance readings are done at the 645 nm and 663 nm wavelengths respectively with 80% acid used as a blank.

Phenol sulphuric acid method for total carbohydrate: The phenol-sulphuric acid method to estimate total carbohydrates is described below:

Principle: In hot acidic medium glucose is dehydrated to hydroxymethyl furfural. This forms a green coloured product with phenol and has an absorption maximum at 490nm.

Materials

- ✓ Phenol 5%: Redistilled (reagent grade) phenol (50g) dissolved in water and diluted to one sulphuric acid 96% reagent grade.
- ✓ Standard Glucose: Stock – 100 mg in 100 ml of water.
- ✓ Working standard – 10 ml of stock diluted to 100 ml with distilled water.

3. RESULTS AND DISCUSSION

Qualitative traits of curd

1. Carbohydrate in curd

Regarding the carbohydrate in curd, it was observed (Table 1) that the effect of different treatment of organic manure and biofertilizer. Table 1 indicated that the carbohydrate in curd was maximum (107.49 mg/l) under treatment T_7 (Biofertilizer + Vermicompost) followed by T6 (105.35 mg/l) and the minimum observation (96.09 mg/l) was recorded under treatment T_1 (control). Carbohydrate (sugars and starch) is known to be an end product of photosynthesis synthesise from carbon dioxide and water, reducing CO_2 at the expense of the energy and reducing power stored in the ATP and NADPH generated from photosynthesis [11].

2. Chlorophyll in curd

Regarding the chlorophyll in curd, it was observed (Table 1) that the effect of different treatment of organic manure and biofertilizer. Table 1 indicated that the chlorophyll in curd was maximum (108.62 mg/l) under treatment T_7 (Biofertilizer + Vermicompost) followed by T6 (106.72 mg/l) and the minimum observation (82.57 mg/l) was recorded under treatment T_1 (control). The vermicompost and farmyard manure along with biofertilizer helps in effective uptake and assimilation of magnesium in chlorophyll. Changes of chlorophyll level in photosynthetic cells are good indicators of senescence, occurring in green vegetables after harvest. Chlorophyll content degradation increased with temperature in broccoli florets [11] Chlorophyll is the principal pigment of photosynthesis in which magnesium is present in its heterocyclic protoporphyrin ring [12].

3. Antioxidant in curd

Regarding the antioxidant in curd, it was observed (Table 1) that the effect of different treatment of organic manure and biofertilizer. Table 1 indicated that the antioxidant in curd was maximum (61.90 %) under treatment T_7 (Biofertilizer + Vermicompost) followed by T6 (57.21 %) and the minimum observation (32.64 %) was recorded under treatment T_1 (control).

4. Flavonoid in curd

Regarding the flavonoid in curd, it was observed (Table 1) that the effect of different treatment of organic manure and biofertilizer. Table 1 indicated that the flavonoid in curd was maximum (34.49 %) under treatment T_7 (Biofertilizer + Vermicompost) followed by T6 (32.59 %) and the minimum observation (25.76 %) was recorded under treatment T_1 (control).

5. Nitrate in curd

Regarding the nitrate in curd, it was observed (Table 1) that the effect of different treatment of organic manure and biofertilizer. Table 1 indicated that the nitrate in curd was maximum (172.96 mg/kg) under treatment T_7 (Biofertilizer + Vermicompost) followed by (168.52 mg/kg) under treatment T_6 and the minimum observation (113.71 mg/kg) was recorded under treatment T_1 (control).

6. Protein in curd

Regarding the nitrate in curd, it was observed (Table 1) that the effect of different treatment of organic manure and biofertilizer. Table 1 indicated that the protein in curd was maximum (0.40 mg/L) under treatment T_7 (Biofertilizer + Vermicompost) followed by (0.38 mg/L) under treatment T_6 and the minimum observation (0.28 mg/L) was recorded under treatment T_1.

7. T.S.S. in curd

Regarding the T.S.S. in curd, it was observed (Table 1) that the effect of different treatment of organic manure and biofertilizer. Table 1 indicated that the maximum T.S.S. (10.9 ^{0}Brix) is found in treatment T_7 (Biofertilizer + Vermicompost) and which is followed by treatments T_6 (10.2 ^{0}Brix) and the minimum T.S.S. (8.5 ^{0}Brix) is found in treatment T_1 (control).

Table 1. Influence of bio-fertiliser (*Rhizobium radiobactor*) in association with organic manures on qualitative traits of broccoli

S. No.	Treatment combinations	Biochemical analysis of curd						
		Carbohydrate (mg/l)	Chlorophyll (mg/l)	Antioxidant (%)	Flavonoid (%)	Nitrate (mg/kg)	Protein (mg/l)	T.S.S. (^{0}Brix)
T_1	Control	96.09	82.57	32.64	25.76	113.71	0.28	8.56
T_2	BF-100%	100.96	101.78	45.54	29.21	155.85	0.32	9.51
T_3	NPK (RDF)	103.40	105.31	52.28	32.12	164.88	0.35	9.86
T_4	FYM 100%	99.83	100.70	41.98	29.00	129.21	0.30	9.35
T_5	VC – 100%	97.99	92.57	37.36	27.39	123.10	0.29	8.80
T_6	BF (50%) + FYM (50%)	105.35	106.72	57.21	32.59	168.52	0.38	10.24
T_7	BF (50%) + VF (50%)	107.49	108.62	61.90	34.49	172.96	0.40	10.90
T_8	BF (50%) + NPK (50%)	102.62	103.54	49.84	30.83	162.72	0.34	9.69
SE(d)		2.05	1.34	1.03	0.68	1.01	0.02	0.17
CD (5%)		4.45	2.90	2.24	1.24	2.19	0.04	0.37

Table 2. Influence of bio-fertiliser (*Rhizobium radiobactor*) in association with organic manures on qualitative traits of broccoli

S. No.	Treatment combinations	Biochemical analysis of leaves				
		Chlorophyll (mg/l)	Proline (%)	Nitrate (mg/kg)	Carbohydrate (mg/l)	Protein (mg/l)
T_1	Control	93.92	25.07	105.93	93.92	0.26
T_2	BF-100%	99.71	27.80	132.47	99.71	0.31
T_3	NPK (RDF)	105.11	30.21	144.48	105.17	0.34
T_4	FYM 100%	98.09	27.27	130.56	98.09	0.29
T_5	VC – 100%	95.44	26.25	112.87	95.44	0.28
T_6	BF (50%) + FYM (50%)	105.86	31.93	150.55	105.86	0.36
T_7	BF (50%) + VF (50%)	108.27	33.99	156.75	108.27	0.38
T_8	BF (50%) + NPK (50%)	103.52	28.55	136.14	103.52	0.33
SE(d)		1.34	1.22	1.84	1.34	0.01
CD (5%)		2.91	2.64	3.98	2.91	0.02

8. Chlorophyll content in leaves

Regarding the chlorophyll content in leaves, it was observed (Table 2) that the effect of different treatment of organic manure and biofertilizer. Table 1 indicated that the chlorophyll content in leaves was maximum recorded (108.27 mg/l) under treatment T_7 (Biofertilizer + Vermicompost) followed by T6 (105.86 mg/l) and the minimum observation (93.92 mg/l) was recorded under treatment T_1 (control). The similar finding reported in rice [13]. Al-Tarawneh, [14] was found that the application the highest dosages of organic manure (80 ton ha-1) with the highest dose of inorganic fertiliser (60 kg ha-1) induced the highest leaf chlorophyll content, while the lowest chlorophyll content obtained by control treatment.

9. Proline content in leaves

Regarding the proline content in leaves, it was observed (Table 2) that the effect of different treatment of organic manure and biofertilizer. Table 1 indicated that the proline content in leaves was maximum recorded (33.99%) under treatment T_7 (Biofertilizer + Vermicompost) followed by T6 (31.93 %) and the minimum observation (25.07 %) was recorded under treatment T_1 (control).

10. Nitrate content in leaves

Regarding the nitrate content in leaves, it was observed (Table 2) that the effect of different treatment of organic manure and biofertilizer. Table 1 indicated that the nitrate content in leaves was maximum recorded (156.75 mg/kg) under treatment T_7 (Biofertilizer + Vermicompost) followed by T6 (150.55 mg/kg) and the minimum observation (105.93 mg/kg) was recorded under treatment T_1 (control).

11. Carbohydrate content in leaves

Regarding the carbohydrate content in leaves, it was observed (Table 2) that the effect of different treatment of organic manure and biofertilizer. Table 1 indicated that the maximum carbohydrate content in leaves was recorded (108.27 mg/l) under treatment T_7 (Biofertilizer + Vermicompost) followed by T6 (105.86 mg/l) and the minimum observation (93.92 mg/l) was recorded under treatment T_1 (control). Hammoda [15] they observed that the application of compost and biofertilizer increases carbohydrate percentage in *Glossostemon bruguieri*.

12. Protein content in leaves

Regarding the protein content in leaves, it was observed (Table 2) that the effect of different treatment of organic manure and biofertilizer. Table 1 indicated that the maximum protein content in leaves was observed (0.38 mg/l) under treatment T_7 (Biofertilizer + Vermicompost) followed by T6 (0.36 mg/l) and the minimum observation (0.26 mg/l) was recorded under treatment T_1 (control).

4. CONCLUSION

From the present investigation, it can be concluded that the application of T7 (Biofertilizer 50% + Vermicompost 50%) followed by T6 in (Biofertilizer 50% + Farm yard manuers 50%) proved best for higher curd yield. It is recommended for higher production of broccoli in Lucknow condition.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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