

RESEARCH ARTICLE

Comparative Study of Biofertilizers on Growth and Yield of Broccoli in Acid Soil

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Abstract

This study assessed the effectiveness of two different biofertilizer consortia in terms of growth and yield of Broccoli crop (var. NSC Apollo IUS) and their impact on soil properties in a strongly acid Inceptisol. A field experiment on broccoli crop during the Rabi season, 2023-24, was conducted at the farmer's field, Madan Mawkhar village, Ri-Bhoi, Meghalaya. The experimental layout was a randomized block design incorporating six treatments with four replications. The treatments were comprised of native biofertilizer consortium (NBC), non-native biofertilizers consortium (NNBC), either alone or in combinations with 50% RDF (Recommended dose of fertilizers) and 100% RDF as control. Biofertilizer was applied by the seedling root-dip method for one hour just before transplanting. Application of NBC and NNBC consortia improved growth and yield attributes of broccoli crop, but the effect of NBC on yield attributes of broccoli curd was significantly higher compared to that under NNBC. Leaf length, leaf breadth, leaf area and number of leaves per plant at 30, 45, 60 days after transplanting (DAT) and harvest were higher in 50% RDF+NBC as compared to other treated plots. The fresh curd weight, dry curd weight, and plant biomass were significantly higher in 50% RDF+NBC treated plots, with values of 184.9 g, 19.97 g, and 1036 g plant⁻¹, respectively. Values of soil available N, available P and available K were the highest in 50% RDF+NBC treated plots as compared to other treated plots at harvest. In conclusion, NBC performed better than NNBC in strongly acid Inceptisol and the dual inoculation of NBC and NNBC exhibited an antagonistic effect on the growth and yield of broccoli. Further, integrated use of NBC and 50% RDF reduced physiological stress in broccoli plants, and supported higher yield and better quality of curds.

Keywords: Consortia, Fertilizer dose, Native biofertilizer, Non-native biofertilizer.

Introduction

Broccoli (*Brassica oleracea* var. *italica* Plenck; $2n=x=18$) originates from the Mediterranean region, and is commonly referred to as "Hari Ghobi" in Hindi. A member of the Cole group, it belongs to the Brassicaceae or Cruciferae family. "Broccoli" is derived from the Latin word "Brachium," meaning arm or branch. Sprouting broccoli features a terminal head with green buds and thick, fleshy flower stalks, resembling cauliflower in structure but with secondary heads that form in leaf axils, contributing up to 50% of the total yield. Brassica vegetables like broccoli are valued for their antioxidant and anti-carcinogenic properties (Cartea *et al.*, 2008). Known as the "Crown Jewel of Nutrition," broccoli is packed with essential vitamins and minerals, including high levels of vitamins A and C, carotenoids, fiber, calcium, and folic acid. It has roughly 130 times more vitamin A than cabbage. Additionally, broccoli contains key phytonutrients such as beta-carotene and indole-3-carbinol, which help combat breast and lung cancer. Phytochemicals in broccoli work by preventing the formation of carcinogens. Broccoli also contains sulforaphane, which can inhibit tumour growth and reduce cancer risk. Broccoli sprouts are especially rich in glucosinolates, which are precursors to chemo-protective isothiocyanates compounds associated with a lower risk of cancer (Aires *et al.*, 2006).

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How to cite this article: Dutta, P., Thakuria, D., Hareesh, D., Singh, L.M., Majumdar, S., Dutta, P., Das, A. 2025. Comparative Study of Biofertilizers on Growth and Yield of Broccoli in Acid Soil. *Indian J. Hill Farm.*, 38(2):21-26.

Source of support: Nil

Conflict of interest: None.

Received: 25/03/2025 **Revised:** 12/04/2025 **Accepted:** 24/04/2025

The management of integrated nutrient systems and sustained agricultural productivity is greatly influenced by

microbial inoculants (Sammauria *et al.*, 2020). According to Malusa and Vassilev (2014), the term “biofertilizer” refers to a product that contains living microorganisms in the form of carrier based solid or liquid that are beneficial to agriculture in terms of nitrogen-fixation, phosphorus solubilization, or nutrient mobilisation. Microbial inoculants or biofertilizers contain living microorganisms that colonize the rhizosphere and help in the promotion of plant growth. The microbes convert the insoluble elements in the soil to a soluble form by a biological process similar to rock phosphate solubilization and nitrogen fixation (Macik *et al.*, 2020). Beneficial microorganisms utilized in biofertilizers improve microflora, soil health, plant growth, plant disease control, and protect the plant from pests. There are beneficial microbial inoculants, such as nitrogen fixers, phosphate, sulfur, zinc solubilizers (VAM), and plant growth-promoting rhizobacteria, in biofertilizers. Plant growth-promoting rhizobacteria are bacteria that live freely on rhizosphere soil and promote plant growth. They also act as biopesticides, based upon the ability or behaviour of the crops and biocontrol agents (Nitrogen fixer, PSB, and SSB) (Labuschagne *et al.*, 2010).

It's possible for the many processes promoting plants to be strain and host-specific. Moreover, after being incorporated into the soil, plant growth-promoting microorganisms (PGPM) encounter competitive environments that could significantly diminish their advantageous impacts (Bashan, 1998). Therefore, the benefits of using a specific biofertilizer can differ substantially depending on the agro-environmental conditions, leading to debates and discrepancies regarding the efficacy of microbial-based solutions (Cummings, 2009; Owen *et al.*, 2015). In this regard, combination products based on microbial consortia are becoming more and more popular as a means of taking advantage of complementary or even synergistic interactions. Microbial consortia products (MCPs) offer a wide range of applications by combining suitable microbial strains with distinct mechanisms of action. Additionally, the MCPs are essential in overcoming phosphorous and zinc fixation in acid soil (Thakuria *et al.*, 2009). The North East Indian acid soils are a good fit for the N-P-K-Zn biofertilizer consortium because of a number of characteristics, including low agricultural production in the area due to the presence

of toxic heavy minerals, poor soil fertility, and deficiencies in phosphorus, boron, manganese, calcium, and magnesium. By keeping this in view, the present study was undertaken to assess the effect of native and non-native biofertilizer on soil fertility status and growth and yield of broccoli.

Materials and Methods

To assess the effect of biofertilizers on the growth and yield of broccoli, a field experiment was conducted at a farmer's field of Madan Mawkhal village (25°41'01.6"N, 91°57'44.8"E), which is situated in the Ri-Bhoi district belonging to the Meghalaya plateau. Soils of Ri-Bhoi district, including the experimental site belongs to the Red Soil group and the dominant soil order is *Inceptisol* (USDA Soil Taxonomic Classification) with depth ranging from deep to very deep. The majority of the soils had light to medium textures (clay loam and sandy loam), and their depths ranged from very deep to deep. Over 80% of the region's soils have an acidic pH (less than 5.5). A composite soil sample (0–15 cm depth) was taken prior to the experiment, taking into account the required soil sampling protocols, in order to ascertain the chemical characteristics of the experimental soil. The composite soil samples were analyzed for chemical properties (Table 1). A field experiment was carried out in micro-plot conditions (individual plot area was 1.8 x 1.8 m; four plots per treatment as replication). The treatment combinations were T₁ – Recommended dose of fertilizers (100% RDF), T₂ – Native biofertilizer consortia (NBC), T₃ – Non-native biofertilizer consortia (NNBC), T₄ – 50% RDF + NBC, T₅ – 50% RDF + NNBC, T₆ – 50% RDF + NBC+NNBC. A full dose of fertilizer for T₁ (120-80-60: N-P₂O₅-K₂O kg ha⁻¹) was applied (Devi *et al.*, 2021). Likewise, we used an hour root dip Seedling for T₂ and T₄ from native biofertilizer (CAU Bioenhancer) and for T₃ and T₅ non-native biofertilizer (NBAIM biofertilizer) applied pot respectively. For growth parameters analysis, ten (10) plants were selected from each replicate plot at the curd initiation and at the harvest stage for measuring various plant growth parameters, *viz.*, plant height, leaf length, leaf breadth and number of leaves per plant at 30 and 45 days after transplanting (DAT) and harvest and yield attributes were also measured at harvest. Dynamics of soil chemical properties were observed in the rhizosphere soils of broccoli under biofertilizer treatment.

Table 1: Basic soil properties of experimental field plot

Soil properties and units	Value	Fertility rating
pH (1:2.5 soil water suspension)	4.85 ± 0.1	Acidic
Soil organic carbon (%ww-1)	2.89 ± 0.06	High
Soil available N (kg ha-1)	489.21 ± 10.66	Medium
Soil available P ₂ O ₅ (kg ha-1)	21 ± 0.4	Low
Soil available K ₂ O (kg ha -1)	176.8 ± 5.4	Medium
DTPA extractable Zn (mg kg-1)	2.13 ± 0.04	Sufficient

Table 2: Effect of native and non-native biofertilizer on growth parameters of broccoli

Treat- ments	Plant height(cm)		Leaf length (cm)		Leaf breadth (cm)		No of leaves per Plant					
	30 DAT	45 DAT	At harvest	30 DAT	45 DAT	At harvest	30 DAT	45 DAT				
T1	32.8 ± 0.2b	40.4 ± 0.4a	50.8 ± 0.4ab	17.2 ± 0.2b	23.5 ± 0.3b	35.9 ± 06ab	11.3 ± 0.2a	12.8 ± 0.2a	17.7 ± 0.6ab	12.5 ± 0.1b	40.4 ± 0.4a	21.2 ± 0.2ab
T2	37.0 ± 0.3c	47.4 ± 0.4b	51.6 ± 0.8ab	16.8 ± 0.2c	25.9 ± 0.8cd	36.2 ± 07ab	13.4 ± 0.08c	15.6 ± 0.3b	17.4 ± 0.7ab	13.4 ± 0.4b	47.4 ± 0.4b	21.1 ± 0.6ab
T3	36.6 ± 0.2c	45.3 ± 0.4b	51.4 ± 0.5ab	18.1 ± 0.1b	24.6 ± 0.5bc	36.1 ± 0.6ab	12.5 ± 0.1b	15.4 ± 0.2b	18.5 ± 0.6ab	13.3 ± 0.1b	45.3 ± 0.4b	20.7 ± 0.2a
T4	37.4 ± 0.2c	47.5 ± 0.5b	52.2 ± 0.6b	18.5 ± 0.08c	26.8 ± 0.7d	38.0 ± 0.2ab	14.1 ± 0.2c	16.4 ± 0.1c	19.2 ± 0.6b	15.2 ± 0.2c	47.5 ± 0.5b	22.4 ± 0.4b
T5	34.0 ± 1.1b	45.9 ± 0.5b	51.4 ± 0.3ab	17.2 ± 0.1b	24.3 ± 0.3bc	35.2 ± 0.5a	11.7 ± 0.3ab	15.3 ± 0.4b	17.4 ± 0.5ab	12.7 ± 0.2b	45.9 ± 0.5b	20.8 ± 0.3a
T6	31.0 ± 0.3a	39.8 ± 0.7a	49.7 ± 0.3a	15.5 ± 0.1a	21.7 ± 0.4a	33.9 ± 0.7a	11.8 ± 0.3ab	13.4 ± 0.08a	16.4 ± 0.5a	11.2 ± 0.5a	39.8 ± 0.7a	19.8 ± 0.2a

Values are means ± standard error (n = 4). Each mean represents values from 4 replicate plots and from each plot data derived from 6 random plants. Within a column, values followed by different letters are statistically significant at P<0.05 as determined by one-way ANOVA incorporating Student-Newman-Keuls (SNK) test for multiple pair-wise comparison among means. *Note: T₁-100% RDF, T₂- Native biofertilizer consortia, T₃- Non-native biofertilizer consortia, T₄- 50% RDF + Native biofertilizer consortia, T₅- 50% RDF + Native biofertilizer consortia, T₆- 50% RDF + Non-native biofertilizer consortia

Available nitrogen (N) was determined by the alkaline permanganate oxidation method as described by Subbiah and Asija (1956). Bray's P in soil was determined by following the stannous chloride blue colour method (Bray and Kurtz, 1945). The soil available potassium (K) was extracted with 1N neutral ammonium acetate (NH₄OAc) solution, as prescribed by Hanway and Heidel (1952). DTPA-extractable zinc from soil samples was extracted with DTPA-extractant (0.005 M Diethylene triamine penta acetic acid + 0.01 M CaCl₂ + 0.1 M triethanolamine), pH 7.3, at 1:2 soil to extractant ratio as described by Lindsay and Norvell (1978).

Statistical analysis

Univariate statistical analysis was performed using SPSS v.21 (Statistical Packages for Social Science Inc., Chicago, IL, USA). For each parameter, the effects of different treatments at each time point were determined by performing one-way analysis of variance (ANOVA) incorporating Student-Newman-Keuls (SNK) test for the pair-wise comparison among treatment means. The repeated measures-ANOVA was also performed to test the significant difference between treatments, considering the different time intervals as replicate measurements for each parameter analysed.

Results

Effect of biofertilizers on growth parameters

Plant height (cm)

Plant height, leaf length, leaf breadth and number of leaves per plant at 30 and 45 days after transplanting (DAT) and at harvest as influenced by native and non-native biofertilizer on broccoli are presented in Table 2. It was observed that at 30 DAT (37.4 cm) and 45 DAT (47.5 cm), 50% RDF + native biofertilizer recorded significantly higher plant height as compared to 100% RDF and 50% RDF + native biofertilizer+ non-native biofertilizer. At harvest, 50% RDF + native biofertilizer (52.2 cm) recorded significantly higher plant height as compared to 50% RDF + native biofertilizer + non-native biofertilizer (49.7 cm) and it was comparable with native biofertilizer consortia (51.6 cm), non-native biofertilizer consortia (51.4 cm) and 50% RDF + non-native biofertilizer consortia (51.4 cm).

Leaf length (cm)

At 30 and 45 DAT, leaf length in 50% RDF + native biofertilizer was significantly higher as compared to 100% RDF and 50% RDF + native biofertilizer + non-native biofertilizer. At 30 and 45 DAT, it was comparable to native biofertilizer treatment. At harvest, the highest value of leaf length (38.0 cm) was recorded in 50% RDF + native biofertilizer.

Leaf breadth (cm)

At 30 DAT and 45 DAT, leaf breadth was recorded significantly higher in 50% RDF + native biofertilizer as compared to other

Table 3: Effect of native and non-native on yield parameters after harvesting of broccoli

Treatments	Fresh Curd Weight (g)	Curd Diameter (cm)	Biomass wt. (g)	Dry Curd wt. (g)	Fresh Curd yield (tha ⁻¹)
T1 – 100% RDF	133.4 ± 3.8c	8.96 ± 0.3a	998 ± 5.3d	16.42 ± 0.5c	11.13 ± 0.4cd
T2 – Native biofertilizer consortia (NBC)	164.8 ± 3.2d	10.84 ± 0.3b	946 ± 3.5c	17.98 ± 0.7c	12.21 ± 0.4d
T3 – Non-native biofertilizer consortia (NNBC)	119.8 ± 4.2b	9.30 ± 0.1a	918 ± 9.5b	14.00 ± 0.6b	9.37 ± 0.3ab
T4 – 50% RDF + NBC	184.9 ± 3.7e	11.24 ± 0.4b	1036 ± 13e	19.97 ± 0.7d	13.69 ± 0.5e
T5 – 50% RDF + NNBC	140.2 ± 4.1c	8.92 ± 0.3a	977 ± 2.0d	16.42 ± 0.4c	10.38 ± 0.3b
T6 – 50% RDF + NBC+NNBC	94.9 ± 4.1a	8.23 ± 0.1a	826 ± 3.0a	11.39 ± 0.5a	8.53 ± 0.3a

Values are means ± standard error (n = 4). Each mean represents values from 4 replicate plots and from each plot data derived from 6 random plants. Within a column, values followed by different letters are statistically significant at $P < 0.05$ as determined by one-way ANOVA incorporating Student-Newman-Keuls (SNK) test for multiple pair-wise comparison among means

Table 4: Effect of native and non-native biofertilizer consortia on soil properties after harvesting of broccoli

Treatments	Available N(kg ha ⁻¹)	Available P (kg ha ⁻¹)	Available K(kg ha ⁻¹)	DTPA Zn (mg kg ⁻¹)
T1 – 100% RDF	500 ± 5.2bc	21.6 ± 0.8a	196 ± 3.6d	1.36 ± 0.05a
T2 – Native biofertilizer consortia (NBC)	486 ± 3.6b	24.3 ± 0.7ab	170 ± 4.2ab	1.52 ± 0.09a
T3 – Non-native biofertilizer consortia (NNBC)	462 ± 3.7a	23.6 ± 0.5ab	160 ± 5.6a	1.60 ± 0.05a
T4 – 50% RDF + NBC	511 ± 4.7c	25.5 ± 0.8b	192 ± 3.6cd	1.43 ± 0.07a
T5 – 50% RDF + NNBC	508 ± 5.8c	22.8 ± 0.5ab	178 ± 5.5bc	1.49 ± 0.02a
T6 – 50% RDF + NBC + NNBC	501 ± 2.8bc	21.9 ± 0.6a	186 ± 3.3cd	1.49 ± 0.04a

Values are means ± standard error (n = 4). Each mean represents values from 4 replicate plots. Soil sample belong to each plot represents the composite of 6 random plant rhizosphere soil. Within a column, values followed by different letters are statistically significant at $P < 0.05$ as determined by one-way ANOVA incorporating Student-Newman-Keuls (SNK) test for multiple pair-wise comparison among means.

treatments ($P < 0.05$, one-way ANOVA). However, it was comparable with native biofertilizer at 30 DAT. At harvest, the highest (19.2 cm) and lowest (16.4 cm) values of leaf breadth were recorded in 50% RDF + native biofertilizer and 50% RDF + native biofertilizer + non-native biofertilizer, respectively.

Number of leaves per plant

The number of leaves per plant at 30 DAT was significantly higher in 50% RDF + Native biofertilizer consortia among all other treatments. At 45 DAT and at harvest, the highest and lowest value of the number of leaves per plant was recorded in 50% RDF + native biofertilizer and 50% + native biofertilizer + non-native biofertilizer, respectively.

Effect of biofertilizers on yield parameters

Fresh curd weight and Dry curd weight (g)

The fresh curd and dry curd weights of broccoli were presented in Table 3. The 50% RDF + native biofertilizer consortia recorded significantly higher fresh and dry curd weight (185 g and 20.0 g, respectively) among other treatments. Dry and fresh curd weight followed the order as 50% RDF + native biofertilizer > native biofertilizer > 50% RDF + non-native biofertilizer > 100% RDF > non-native biofertilizer > 50% RDF + native biofertilizer + non-native biofertilizer.

Curd diameter (cm)

The 50% RDF + native biofertilizer consortia showed significantly better curd diameter as compared to other treatment which were comparable with native biofertilizer consortia.

Biomass weight (g)

Biomass weight was reported significantly higher in 50% RDF + native biofertilizer consortia as compared to other treatments and the trend was 50% RDF + native biofertilizer > 100% RDF > 50% + non-native biofertilizer > native biofertilizer > non-native biofertilizer > 50% + native biofertilizer + non-native biofertilizer.

Dry curd yield (kg ha⁻¹)

Fresh curd yield was reported significantly higher in 50% RDF + native biofertilizer consortia as compared to other inorganic and biofertilizer treatments. The trend of dry curd yield followed the order as 50% RDF + native biofertilizer > native biofertilizer > 100% RDF > 50% RDF + non-native biofertilizer > non-native biofertilizer > 50% + native biofertilizer + non-native biofertilizer.

Available N, P, K and micronutrient content in soil

Soil available N, P, and K after harvest as influenced by native and non-native biofertilizers were analysed and data were presented in Table 4. The results indicated that 50%

RDF + native biofertilizer consortia (511 kg ha^{-1}) showed a significant difference in available N as compared to solely applied native and non-native biofertilizer consortia ($p < 0.05$, one-way ANOVA). Likewise, available P content in 50% RDF + native biofertilizer consortia (25.5 kg ha^{-1}) showed significantly different than 100% RDF and Mix biofertilizer consortia. On the other hand, soil available K was recorded higher in T_1 (100% RDF) compared to that in other treatments ($P < 0.05$, one-way ANOVA), which was comparable with T_4 (50% RDF+NBC) and T_6 (50% RDF + native biofertilizer consortia + non-native biofertilizer). There was no significant relation on DTPA Zn content among all application method and it was registered higher in 50% RDF + native biofertilizer consortia (1.60 mg kg^{-1}).

Discussion

Results getting from micro plot field experiment clearly showed that plant height, leaf length, leaf breadth, leaf number of broccoli and yield parameters in 50% recommended dose of fertilizer along with native biofertilizer over 100% recommended dose and mixed biofertilizer consortia which might be due to biofertilizer with half dose fertilizer help to robust root system that help to nitrogen availability, enhanced by microbial secretions and atmospheric nitrogen fixation via *Azotobacter* and *Phosphate-Solubilizing Bacteria* (PSB) likely contributed to the proliferation of leaf buds and promoted plant growth. Additionally, the secretion of growth-promoting substances by microbes may have played a role in enhancing leaf production. These findings align with previous research by Kumar and Biradar (2017) in broccoli and Mishra *et al.* (2018) in brinjal, which reported similar increases in leaf number and plant growth in response to microbial inoculation and nitrogen augmentation. Plant height unveiled a remarkable response to biofertilizers in conjunction with inorganic fertilizers. The experiment showed that the highest available nitrogen after harvest resulted from biofertilizer application and mineralization, attributed to microbial activity converting organic nitrogen to inorganic forms. This study aligns with Yoldas *et al.* (2008). Biofertilizers and organic sources also enhanced nutrient release by narrowing the C: N ratio (Sarangthem *et al.*, 2011; Bhardwaj *et al.*, 2012). The 50% RDF + native biofertilizer treatment resulted in the highest phosphorus availability, due to biofertilizer-fertilizer synergy that reduced phosphorus fixation and enhanced its mobilization via microbial action (Sarangthem *et al.*, 2011; Sharma *et al.*, 2008). Organic matter and increased microbial activity also contributed to higher phosphorus availability. The highest available phosphorus was observed in treatment T_4 , which received 50% RDF + native biofertilizer. This increase in available phosphorus can be attributed to the combined application of inorganic fertilizer and biofertilizers, leading to reduced fixation of water-soluble phosphorus, enhanced mineralization of organic phosphorus through

microbial action, and improved mobility of phosphorus. Additionally, the overall increase in available phosphorus may be due to the addition of organic matter, NPK and increased microbial population, which can convert bound phosphorus to available forms. Decomposition of organic matter and phosphorus solubilization from the native soil pool, as well as the formation of an organic cover on sesquioxide, reducing the soil's phosphate-fixing capacity, may also contribute to this increase (Sharma *et al.*, 2008). The highest potassium levels were observed in 100% RDF, similar to 50% RDF with biofertilizers. The integrated use of biofertilizers and inorganic fertilizers reduced potassium fixation and increased its availability through organic matter-clay interactions, as noted by Urkurkar *et al.* (2010) and Gupta *et al.* (2019).

Conclusion

This study clearly demonstrated that the native biofertilizer consortium performed better than the non-native biofertilizer consortium at least in the strongly acid soil and the combined application of both native and non-native biofertilizer consortia was detrimental on growth and yield of broccoli. Further, the application of native biofertilizer consortium could reduce 50% of inorganic fertilizer inputs with 2 times cost reduction and maintain a comparable yield and better quality of broccoli heads under NBC+50%RDF treatment than that in 100%RDF alone. Overall, this study demonstrated the vast potential of native biofertilizer consortium in increasing growth, yield, and better quality of produce, improving soil fertility and maintenance of soil quality in strongly acid *Inceptisol*.

Acknowledgment

The authors acknowledge the College of PG Studies in Agricultural Sciences, Central Agricultural University (Imphal), Umiam, Meghalaya, for students' fellowship and laboratory infrastructures and the Department of Biotechnology, Ministry of Science and Technology, Govt. of India, for funding (No.BT/PR45218/NER/95/1903/2022 dtd.12/03/2022).

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