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Bio-Priming with Compatible Rhizospheric Microbes Enhances Growth and Micronutrient Uptake of Red Cabbage

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Abstract: Red cabbage is known as the millennium's functional food, which has a lot of importance in our diet because of the health-promoting ingredients present in it. The current study investigated the synergistic relationship of rhizospheric-competent microbial agents (*Trichoderma harzianum*, *Pseudomonas fluorescens*, and *Bacillus subtilis*) in modulating the performance of red cabbage under the field conditions of Middle Gangetic Plains, India. Growth parameters were studied at three developmental stages, viz., pre-cupping, early head formation, and maturity. Our results suggested that the dual application of *T. harzianum* + *P. fluorescens* along with the 75% recommended dose of fertilizers (RDF) increased the number of leaves (24.6), leaf area (537.2 cm²), root length (19.8 cm), and micronutrient uptake (Fe, Mn, and Cu) by head of the crop, whereas the co-inoculation of *P. fluorescens* and *B. subtilis* along with 75% RDF enhanced plant spread (39.0 cm), earliness (95.2 days), and Zn uptake. Maximum plant height (28.7 cm) and chlorophyll (SPAD, 77.3) were recorded in 100% RDF (120:60:60 kg ha⁻¹) and the combination of *T. harzianum* + *B. subtilis* along with 75% RDF, respectively. Interestingly, consortium (*T. harzianum* + *P. fluorescens*) bio-primed plants recorded about 14% higher root length in comparison to plants receiving sole fertilizers. The regression analysis revealed a significant relationship of Fe and Mn uptake with chlorophyll (SPAD) and between Zn uptake and the earliness of the crop. The present study indicated that seedling bio-priming with the dual consortium of efficient bio-agents is a viable strategy to lessen our dependence on chemical fertilizers for improving red cabbage production.

Keywords: red cabbage; rhizosphere engineering; plant-microbe symbiosis; growth parameters; micronutrient uptake



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1. Introduction

Red cabbage [*Brassica oleracea* L. var. *capitata* L. f. *rubra* (L.) Thell], commonly known as the modern multitasker's dream food, has recently gained increased attention around the world. This remarkable nutritious vegetable is an excellent source of health-promoting ingredients that can boost our immune system [1]. The choice of crops is also an important armament in our arsenal against degenerative diseases that have devastated human livelihoods and crippled the global economy. The abundance of anthocyanins, carotenoids,

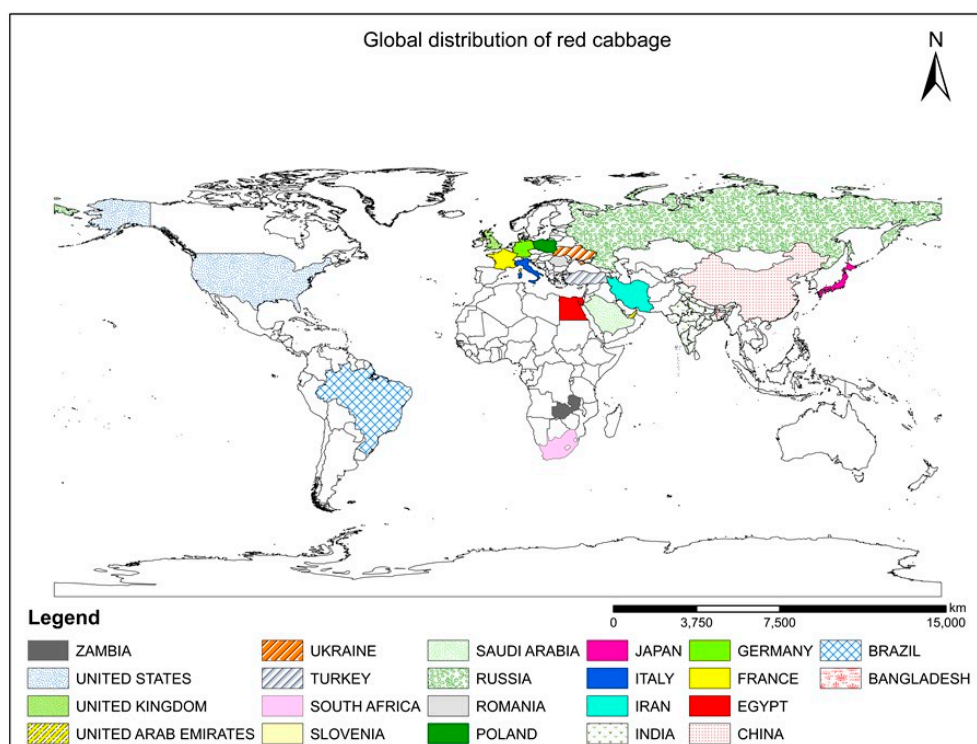
glucosinolates, antioxidants, vitamins (C, E, A, and K), minerals (K, Ca, Mg, Fe, and Mn), and phenols in red cabbage make it a suitable supplement for preventions against oxidative stress, cardiovascular disease, cancer, obesity, and diabetes [2,3]. Therefore, it is directly consumed as microgreens (Figure 1) and salads and also cooked as a vegetable. The microgreens of this plant contain higher β -carotene (~260-fold), vitamin C (2.4–6-fold), and vitamin E (>40 times) than the matured ones [4]. Natural dyes are extracted from red cabbage due to the presence of high levels of anthocyanins (40–188 mg Cy 3-glcE 100 g⁻¹ FW), which are suitable for coloring food products [5]. The nutritional value of red cabbage is greater than white cabbage in terms of antioxidants [6], vitamins, and minerals [7]. This will fetch farmers higher market prices and sell their products in supermarkets [8]. Although cole crops are native to the Mediterranean region, currently, red cabbage is cultivated globally [3,5] (Figure 2a). The geographical distribution maps can highlight the crop's popularity and direct the need for suitable production strategies to maintain sustainable agriculture. Red cabbage is grown in all of Europe [2], North America, Asia, China [7], Brazil [9], South Africa [1], Japan, and many other countries of Asia and Africa [3,10,11]. Growing belts of this cruciferous leafy vegetable can be found in almost all states of India [12,13] (Figure 2b).



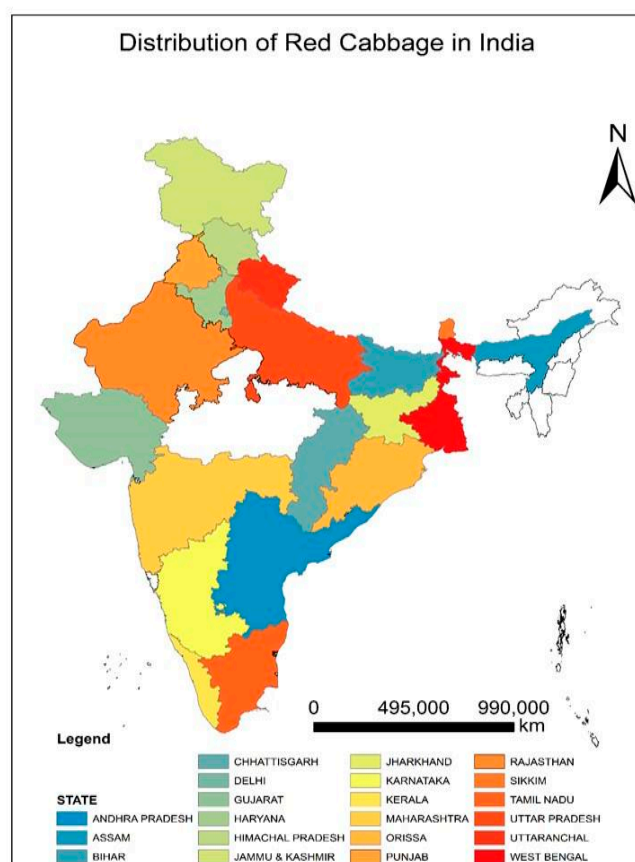
Figure 1. Transition of tender immature seedlings (microgreens) of red cabbage to mature seedlings.

Fertilization is an important strategy to meet the demands of the burgeoning population, which is expected to rise from the current state of 7.8 billion to 9.7 billion by 2050 [14]. Our entire dependence on these costly chemicals has severely affected the physical, chemical, and biological properties of soil all over the world [15]. In the case of nitrogen, poor management results in its loss in the form of volatilization (NH_3), denitrification (NO and N_2O), and leaching (NO_3^-). This indicates that we should search for alternative strategies or planet-friendly food production practices that could minimize environmental pollution and ensure sustainable and profitable food production hand in hand [16].

Rhizosphere modification has great relevance to soil fertility and plant nutrition for sustainable agriculture. The establishment of rhizo-microbiome will provide better nutrient supply, water absorption, and nutrient assimilation in plants with greater anchorage in soil [17,18]. Bio-priming is the best microbial delivery system because it requires a small volume of inoculums [19]. Bio-priming is a pre-sowing treatment of plants with beneficial microorganisms [20]. This technology triggers microbial colonization along with their proliferation in the rhizosphere which increases seedling vigour, nutrient and water uptake, systemic resistance to diverse stresses, and vegetative growth resulting in uniform stand [21,22]. Basically, it is a combined package covering several physiological facets and biological aspects of plant-microbe interactions. Several reports accredit plant growth promotion through modified metabolic pathways in bio-primed plants and enhanced nutrient use efficiency with changes in root architecture and other mechanisms [8,23,24]. Eligible candidates of bio-priming comprising species of *Trichoderma*, *Pseudomonas*, *Bacillus*, *Azotobacter*, *Azospirillum*, *Glomus*, etc., have been found to enhance plant growth [25,26]. Furthermore, co-inoculation studies claimed the enhancement of synergistic effects with the application of microbial consortiums, as compared to a single inoculation of bio-agents [27,28].



(a)



(b)

Figure 2. (a) World map showing the major countries adapting red cabbage cultivation. (b) Different states of India promoting red cabbage cultivation.

According to the latest estimate by the World Health Organization (WHO), micronutrient deficiency has affected more than 2 billion people in the world [29]. Unfortunately, we have less provisions to track the micronutrient removal from the soil by crops. The proper evaluation of micronutrient status is very imperative because their deficiencies and toxicity range/limit in soil are narrow. Red cabbage is a nutrient exhaustive crop [1,8].

There are few published works on bio-primed red cabbage plants. In this backdrop, an attempt was made to examine the effect of bio-priming intervention on integrated nutrient management on growth and micronutrient uptake of red cabbage. We were also interested in monitoring the priming effect of individual bio-agent as well as the significance of a microbial consortium on the growth promotion of this exotic crop.

2. Materials and Methods

2.1. Seedling Bio-Priming

Seeds (truthful label) of red cabbage (*B. oleracea*, variety F1 Hybrid Red Ruby-2) were sown in a nursery (raised bed) about 1 month prior to transplanting. *Pseudomonas fluorescens* (OKC; GenBank accession No. JN128891) and *Bacillus subtilis* (BHHU100; GenBank accession No. JN099686) were grown in nutrient broth at 150 rpm, while *Trichoderma harzianum* (BHU P4; GenBank accession No. MH730446) was grown on a potato dextrose agar at 28 ± 2 °C. These three rhizosphere bio-agents were collected from the Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University. The process of selection was based on their plant growth-promoting traits and compatibility with each other [30]. Seedlings were handpicked when there were 5–6 leaves and then inoculated with bio-agents (liquid culture) using an adhesive agent (2% carboxymethyl cellulose). Bacterial and fungal populations were adjusted to 4×10^8 CFU mL⁻¹ and 2×10^7 CFU mL⁻¹, respectively. Cell suspensions and/or spore suspension were mixed in 1:1 ratio for dual consortium and 1:1:1 ratio for triple consortium.

2.2. Study Area Description

The present investigation was carried out at the Vegetable Research Farm of the Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India, during two successive *rabi* (winter) seasons of 2016–2017 and 2017–2018. The experimental site (25°26' N, 82°99' E, and 80.7 m above mean sea level) represents the Middle Gangetic Plains (agro-ecological region) of Eastern India. The soil of the experimental area is classified as Typic Ustochrept (order Inceptisol). Pre-cropping soil analysis revealed that the soil is slightly alkaline in reaction (7.58); low in organic carbon (4.04 g kg⁻¹) and available N (203.21 kg ha⁻¹) content; medium in available P (20.86 kg ha⁻¹) and available K (217.73 kg ha⁻¹) content; and sandy loam in texture [31]. The mean maximum and minimum temperatures recorded during the study period were 30.0 °C and 7.06 °C, respectively (Table 1). Meteorological data were obtained from the Department of Agronomy of our Institute.

Table 1. Monthly meteorological data of Varanasi for the study period.

Parameter	2016				2017			
	December	January	February	March	December	January	February	March
Maximum temperature (°C)	23.3	24.4	29.7	38.5	26.2	23.1	30.0	35.4
Minimum temperature (°C)	9.8	8.2	10.8	12.3	8.2	5.9	9.5	13.8
Relative humidity (%)	80.1	74.4	65.7	55.0	70.0	73.8	65.0	50.5
Sunshine hours (h)	1.2	2.0	6.3	6.6	6.1	6.6	7.9	8.5

2.3. Experimental Details

Altogether, 9 treatments, viz., T₁: Absolute control N:P₂O₅:K₂O @ 0:0:0 kg ha⁻¹; T₂: Recommended dose of fertilizers (RDF) of N:P₂O₅:K₂O @ 120:60:60 kg ha⁻¹; T₃: 75% RDF + *Trichoderma harzianum*; T₄: 75% RDF + *Pseudomonas fluorescens*; T₅: 75%

RDF + *Bacillus subtilis*; T₆: 75% RDF + *T. harzianum* + *P. fluorescens*; T₇: 75% RDF + *P. fluorescens* + *B. subtilis*; T₈: 75% RDF + *T. harzianum* + *B. subtilis*; and T₉: 75% RDF + *T. harzianum* + *P. fluorescens* + *B. subtilis*, were laid out in a randomized block design with three replications. Seedlings (five-weeks-old) were inoculated with bio-agents and transplanted in plots ($4 \times 2 \text{ m}^2$) at a spacing of 50 cm \times 50 cm intervals. The full doses of P and K in the form of diammonium phosphate (DAP; 18% N, 46% P₂O₅) and muriate of potash (MOP; 60% K₂O), respectively, were applied as basal. Nitrogen in the form of urea was applied in 3 splits, i.e., basal and top dressing at 30 and 45 days after transplanting (DAT) of red cabbage seedlings.

2.4. Biometric Observations

All data related to the growth response of cabbage were collected at different stages (Figure 3) of crop growth, viz., 30 (pre-cupping), 60 (early head formation), and 90–110 (maturity or harvesting) days after transplanting (DAT), except root length and earliness.

2.4.1. Plant Height

A measuring scale was used to measure the height (cm) of the plant from ground level to top surface of the highest leaf.

2.4.2. Number of Leaves

At monthly intervals, the number of leaves per plant was counted.

2.4.3. Plant Spread

The average distance (cm) between the two outermost leaves of the crop was recorded as its spread.

2.4.4. Chlorophyll (SPAD)

A portable Leaf Chlorophyll Meter or Soil Pant Analysis Development (SPAD) 502 Plus Chlorophyll Meter (Konica Minolta, Japan) was used for measuring the chlorophyll content (greenness) of leaves.

2.4.5. Leaf Area

Leaf Area Meter (Model 211, Systronics) was used to measure the area of leaves in cm² unit.

2.4.6. Root Length

The root length was estimated by line intersect method as outlined by Tennant [32]. Roots were spread out in a box containing square grids and a thin layer of water. The number of intersections between grid lines (vertical and horizontal) and roots was counted and converted to measurements (length) using the following formula.

$$\text{Root length (cm)} = 11/14 \times \text{Number of intercepts} \times \text{Grid unit} \quad (1)$$

2.4.7. Earliness

The number of days to head maturity was calculated from the date of transplanting to the date of the formation of maximum marketable heads.

2.5. Micronutrient Uptake

The uptake of micronutrients by the head of red cabbage was determined by multiplying their respective concentration with head biomass.

$$\text{Micronutrient uptake (kg ha}^{-1}\text{)} = \frac{\text{Micronutrient content (\%)} \times \text{Yield (kg ha}^{-1}\text{)}}{100} \quad (2)$$

The contents of micronutrients (Fe, Mn, Zn, and Cu) were estimated in an atomic absorption spectrophotometer (Model 240 FS, Agilent Technologies, Mulgrave, Australia) after the diacid digestion ($\text{HNO}_3\text{:HClO}_4$: 9:4) of plant samples.



30 DAT (Pre-cupping)



60 DAT (Cupping)



90 DAT (Maturity)

1st Year (2016-17)



30 DAT (Pre-cupping)



60 DAT (Cupping)



90 DAT (Harvesting)

2nd Year (2017-18)

Figure 3. Photographs showing the three prominent growth stages at which growth parameters were studied.

2.6. Statistical Analysis

The experimental setup consists of nine treatments and three replications. Data presented as mean were analyzed through Statistical Package for Social Science (SPSS,

version 20) software. Data were subjected to analysis of variance (ANOVA) F test with the total degrees of freedom being 26. The significance of the difference between treatment means was analyzed using Duncan's multiple range test (DMRT) at $p \leq 0.05$ significance level. Pooled data represented the average value of two years. Regression analysis was executed to find out the relationship between the chlorophyll, root length, earliness, and micronutrient uptake (Fe, Mn, Zn, and Cu).

3. Results

3.1. Effect on Plant Height

Data on plant height were periodically collected at 30 DAT, 60 DAT, and maturity (Table 2). At the early stage (30 DAT), no significant variation ($p \leq 0.05$) was noticed in the height of the crop. The highest plant height of 24.1 and 24.4 cm was observed under 100% RDF (T_2), and the lowest plant height of 21.6 and 21.7 cm was observed under absolute control (T_1) during first and second season, respectively. During the early head formation stage (60 DAT), the tallest plants (28.7 cm) were recorded under T_2 , followed by the plants (28.7 cm) under 75% RDF + *P. fluorescens* + *B. subtilis* (T_7). Pooled analysis at harvest revealed maximum height (30.7 cm) in T_7 , which was 3% higher over T_2 . Results were recorded in following order: $T_7 > T_8 > T_2 > T_3 > T_6 > T_5 > T_9 > T_4 > T_1$.

3.2. Effect on Number of Leaves

The treatments did not show any significant variation ($p \leq 0.05$) in the number of leaves during the pre-cupping stage (Table 3). The maximum (16.3) and minimum (14.2) numbers of leaves at this stage were registered in 75% RDF + *P. fluorescens* + *B. subtilis* (T_7) and absolute control (T_1), respectively. The number of leaves was the highest during the early head formation stage (60 DAT), and it varied from 21.2 to 24.6 (pooled data). At 60 DAT, the application of 75% RDF + *T. harzianum* + *P. fluorescens* (T_6) and 75% RDF + *P. fluorescens* + *B. subtilis* (T_7) showed an equivalent effect on this growth parameter. Compared to 100% RDF (T_2), these two bio-priming treatments recorded a 4% increment in leaf number. Again, pooled analysis revealed a similar trend between 75% RDF + *T. harzianum* (T_3) and 75% RDF + *T. harzianum* + *P. fluorescens* + *B. subtilis* (T_9). Results revealed the superiority of the dual consortium over the triple consortium, single-species bio-priming treatments, and non-primed treatments. Among the three priming agents, the number of leaves was highest in *P. fluorescens*-treated plants. The number of leaves reduced at the maturity stage. Furthermore, more leaves at this stage will be hampered in head formation. Therefore, the number of leaves was the highest (12.2) in the control plants. The lowest number of leaves (9.9) was observed in 75% RDF + *T. harzianum* (T_3).

3.3. Effect on Plant Spread

The application of 75% RDF + *P. fluorescens* + *B. subtilis* (T_7) recorded the maximum (28.4 cm) spread of plants at 30 DAT (Table 4). Results also demonstrated that the spread of single-species and triple-species bio-primed plants were at par with each other. Among the individual bio-priming agents, *P. fluorescens* attained the highest value (27.4 cm). At 60 DAT, plant spread varied from 34.3 to 39.1 cm. The dual consortium attained more plant spread (Figure 4) than other treatments, and their performance was in the order of $T_7 > T_6 > T_8$ (as revealed from pooled analysis). Plants spread increased by 13% in T_7 as compared to the control treatment. A full dose of chemical fertilizer yielded at par results with the combined use of a reduced fertilizer and *P. fluorescens*. The average plant spread at maturity was 45.5 cm. The maximum (48.5 cm) spread at this stage was observed under T_7 during the first season, while T_6 attained maximum (48.5 cm) spread during the second season. The performance of single-species bio-priming treatments was in the following order: $T_4 > T_3 > T_5$.

Table 2. Effect of bio-priming and fertilization on the height of red cabbage at different growth stages.

Treatments	Plant Height (cm)								
	Pre-Cupping (30 DAT)			Early Head Formation (60 DAT)			Maturity		
	2016–2017	2017–2018	Pooled	2016–2017	2017–2018	Pooled	2016–2017	2017–2018	Pooled
T ₁ : Absolute control N:P ₂ O ₅ :K ₂ O @ 0:0:0 kg ha ^{−1}	21.6	21.7	21.7	24.8 ^b	24.6 ^b	24.7 ^d	26.2 ^b	26.1 ^b	26.1 ^d
T ₂ : RDF of N:P ₂ O ₅ :K ₂ O @ 120:60:60 kg ha ^{−1}	24.1	24.4	24.3	28.5 ^a	28.8 ^a	28.7 ^a	29.8 ^a	29.7 ^a	29.8 ^{abc}
T ₃ : 75% RDF + <i>Trichoderma harzianum</i>	23.5	23.4	23.5	28.2 ^a	28.3 ^a	28.3 ^{abc}	29.7 ^a	29.7 ^a	29.7 ^{abc}
T ₄ : 75% RDF + <i>Pseudomonas fluorescens</i>	22.2	22.6	22.4	27.4 ^a	27.5 ^a	27.4 ^c	28.9 ^a	29.2 ^a	29.1 ^c
T ₅ : 75% RDF + <i>Bacillus subtilis</i>	23.3	22.9	23.1	27.7 ^a	27.6 ^a	27.6 ^{bc}	29.5 ^a	29.5 ^a	29.5 ^{abc}
T ₆ : 75% RDF + <i>T. harzianum</i> + <i>P. fluorescens</i>	22.7	23.5	23.1	27.8 ^a	28.2 ^a	28.0 ^{abc}	29.4 ^a	29.9 ^a	29.7 ^{abc}
T ₇ : 75% RDF + <i>P. fluorescens</i> + <i>B. subtilis</i>	23.8	23.5	23.6	28.4 ^a	28.7 ^a	28.5 ^{ab}	30.6 ^a	30.7 ^a	30.7 ^a
T ₈ : 75% RDF + <i>T. harzianum</i> + <i>B. subtilis</i>	23.0	23.5	23.3	28.3 ^a	28.6 ^a	28.5 ^{ab}	30.5 ^a	30.4 ^a	30.5 ^{ab}
T ₉ : 75% RDF + <i>T. harzianum</i> + <i>P. fluorescens</i> + <i>B. subtilis</i>	22.4	22.7	22.6	27.5 ^a	27.9 ^a	27.7 ^{bc}	29.3 ^a	29.3 ^a	29.3 ^{bc}

Means followed by different alphabets within a column differed significantly ($p \leq 0.05$) among the treatments as per DMRT.

Table 3. Effect of bio-priming and fertilization on the number of leaves of red cabbage at different growth stages.

Treatments	Number of Leaves								
	Pre-Cupping (30 DAT)			Early Head Formation (60 DAT)			Maturity		
	2016–2017	2017–2018	Pooled	2016–2017	2017–2018	Pooled	2016–2017	2017–2018	Pooled
T ₁ : Absolute control N:P ₂ O ₅ :K ₂ O @ 0:0:0 kg ha ^{−1}	14.2	14.1	14.2	21.1 ^d	21.2 ^c	21.2 ^d	12.2 ^a	12.1 ^a	12.2 ^a
T ₂ : RDF of N:P ₂ O ₅ :K ₂ O @ 120:60:60 kg ha ^{−1}	15.9	16.3	16.1	23.5 ^{abc}	23.7 ^{ab}	23.6 ^{bc}	10.3 ^b	10.3 ^b	10.3 ^b
T ₃ : 75% RDF + <i>Trichoderma harzianum</i>	15.3	15.6	15.4	22.9 ^{bc}	22.7 ^{bc}	22.8 ^c	9.8 ^b	9.9 ^b	9.9 ^b
T ₄ : 75% RDF + <i>Pseudomonas fluorescens</i>	15.7	15.7	15.7	23.4 ^{abc}	23.3 ^{ab}	23.4 ^c	10.1 ^b	10.0 ^b	10.0 ^b
T ₅ : 75% RDF + <i>Bacillus subtilis</i>	15.5	15.7	15.6	22.7 ^c	22.8 ^{bc}	22.7 ^c	10.1 ^b	10.1 ^b	10.1 ^b
T ₆ : 75% RDF + <i>T. harzianum</i> + <i>P. fluorescens</i>	15.3	15.9	15.6	24.4 ^{ab}	24.7 ^a	24.6 ^a	9.9 ^b	10.3 ^b	10.1 ^b
T ₇ : 75% RDF + <i>P. fluorescens</i> + <i>B. subtilis</i>	16.2	16.3	16.3	24.5 ^a	24.6 ^a	24.6 ^a	10.1 ^b	10.2 ^b	10.2 ^b
T ₈ : 75% RDF + <i>T. harzianum</i> + <i>B. subtilis</i>	16.1	15.9	16.0	24.5 ^a	24.1 ^{ab}	24.3 ^{ab}	10.2 ^b	10.3 ^b	10.2 ^b
T ₉ : 75% RDF + <i>T. harzianum</i> + <i>P. fluorescens</i> + <i>B. subtilis</i>	15.27	15.4	15.3	22.8 ^c	22.7 ^{bc}	22.8 ^c	10.1 ^b	10.0 ^b	10.0 ^b

Means followed by different alphabets within a column differed significantly ($p \leq 0.05$) among the treatments as per DMRT.

Table 4. Effect of bio-priming and fertilization on spread of red cabbage at different growth stages.

Treatments	Plant Spread (cm)								
	Pre-Cupping (30 DAT)			Early Head Formation (60 DAT)			Maturity		
	2016–2017	2017–2018	Pooled	2016–2017	2017–2018	Pooled	2016–2017	2017–2018	Pooled
T ₁ : Absolute control N:P ₂ O ₅ :K ₂ O @ 0:0:0 kg ha ^{−1}	25.3 ^d	25.2 ^c	25.2 ^d	34.5 ^b	34.3 ^b	34.4 ^c	37.1 ^d	36.8 ^e	36.9 ^d
T ₂ : RDF of N:P ₂ O ₅ :K ₂ O @ 120:60:60 kg ha ^{−1}	27.5 ^{abc}	27.4 ^{ab}	27.5 ^{bc}	38.6 ^a	38.5 ^a	38.5 ^{ab}	46.4 ^{bcd}	46.5 ^{bcd}	46.4 ^{bc}
T ₃ : 75% RDF + <i>Trichoderma harzianum</i>	26.7 ^c	26.8 ^b	26.8 ^c	37.6 ^a	37.6 ^a	37.6 ^b	45.8 ^c	45.9 ^{cd}	45.9 ^c
T ₄ : 75% RDF + <i>Pseudomonas fluorescens</i>	27.5 ^{abc}	27.3 ^{ab}	27.4 ^{bc}	38.2 ^a	38.2 ^a	38.2 ^{ab}	46.0 ^{cd}	46.1 ^{bcd}	46.0 ^{bc}
T ₅ : 75% RDF + <i>Bacillus subtilis</i>	26.9 ^{bc}	26.9 ^b	26.9 ^c	37.3 ^a	37.46 ^a	37.4 ^b	45.4 ^c	45.4 ^d	45.4 ^c
T ₆ : 75% RDF + <i>T. harzianum</i> + <i>P. fluorescens</i>	27.7 ^{abc}	28.0 ^{ab}	27.8 ^{ab}	39.1 ^a	38.8 ^a	39.0 ^a	48.5 ^a	47.6 ^{ab}	48.1 ^a
T ₇ : 75% RDF + <i>P. fluorescens</i> + <i>B. subtilis</i>	28.3 ^a	28.4 ^a	28.4 ^a	38.9 ^a	39.2 ^a	39.0 ^a	47.6 ^{ab}	48.5 ^a	48.0 ^a
T ₈ : 75% RDF + <i>T. harzianum</i> + <i>B. subtilis</i>	28.14 ^{ab}	27.9 ^{ab}	28.0 ^{ab}	39.1 ^a	38.7 ^a	38.9 ^a	47.4 ^{abc}	47.3 ^{abc}	47.3 ^{ab}
T ₉ : 75% RDF + <i>T. harzianum</i> + <i>P. fluorescens</i> + <i>B. subtilis</i>	26.9 ^{bc}	26.8 ^b	26.9 ^c	37.5 ^a	37.5 ^a	37.5 ^b	45.8 ^c	45.7 ^d	45.73 ^c

Means followed by different alphabets within a column differed significantly ($p \leq 0.05$) among the treatments as per DMRT.

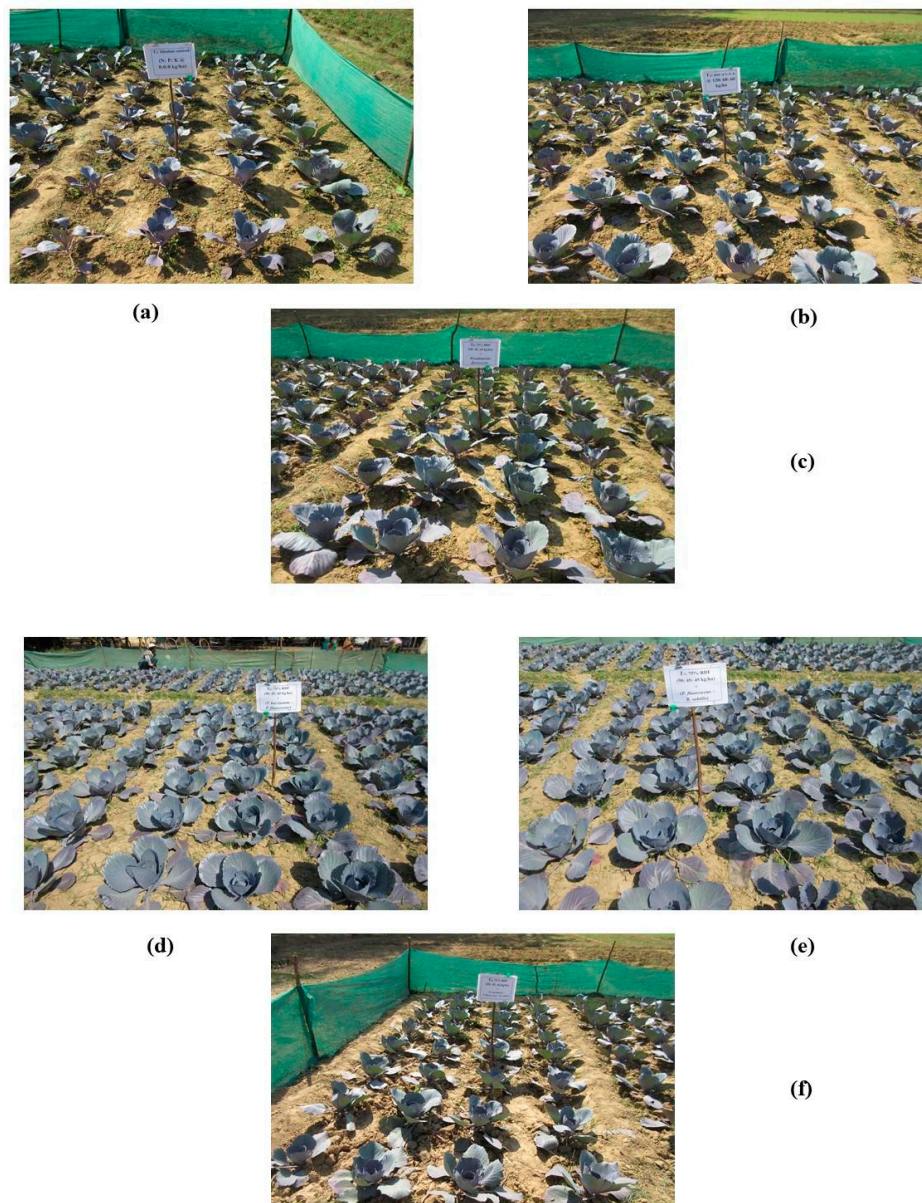


Figure 4. Effective photographs of field experiment involving treatments; T₁: Absolute control N:P₂O₅:K₂O @ 0:0:0 kg ha⁻¹ (a); T₂: RDF of N:P₂O₅:K₂O @ 120:60:60 kg ha⁻¹ (b); T₄: 75% RDF + *Pseudomonas fluorescens* (c); T₆: 75% RDF + *T. harzianum* + *P. fluorescens* (d); T₇: 75% RDF + *P. fluorescens* + *B. subtilis* (e); T₉: 75% RDF + *T. harzianum* + *P. fluorescens* + *B. subtilis* (f).

3.4. Effect on Chlorophyll (SPAD)

The chlorophyll (SPAD) data of the red cabbage plant were recorded at 30 DAT, 60 DAT, and harvest (Table 5). Throughout the growth period, treatments remained at par with each other except the absolute control. At 30 DAT, the highest SPAD value (60.0) was noted in the T₇ treatment (75% RDF + *P. fluorescens* + *B. subtilis*), while the lowest value (47.7) was reported in the T₁ treatment (absolute control). As revealed from pooled data, the maximum value (77.3) was observed at 60 DAT under 75% RDF + *T. harzianum* + *B. subtilis* (T₈), which was significantly ($p \leq 0.05$) higher (23%) than the absolute control (T₁). This treatment also showed the highest value (69.9) at harvest stages. It was significantly greater (21%) than compared to the absolute control. On average, our study showed the following trend: T₈ > T₆ > T₇ > T₄ > T₂ > T₅ > T₉ > T₃ > T₁.

Table 5. Effect of bio-priming and fertilization on chlorophyll (SPAD) of red cabbage at different growth stages.

Treatments	Chlorophyll (SPAD)								
	Pre-Cupping (30 DAT)			Early Head Formation (60 DAT)			Maturity		
	2016–2017	2017–2018	Pooled	2016–2017	2017–2018	Pooled	2016–2017	2017–2018	Pooled
T ₁ : Absolute control N:P ₂ O ₅ :K ₂ O @ 0:0:0 kg ha ^{−1}	47.5 ^b	47.9 ^b	47.7 ^d	62.4 ^b	62.7 ^b	62.5 ^c	57.4 ^b	57.7 ^b	57.6 ^b
T ₂ : RDF of N:P ₂ O ₅ :K ₂ O @ 120:60:60 kg ha ^{−1}	58.4 ^a	58.7 ^a	58.5 ^{abc}	75.4 ^a	75.1 ^a	75.3 ^b	69.2 ^a	68.9 ^a	69.0 ^a
T ₃ : 75% RDF + <i>Trichoderma harzianum</i>	56.3 ^a	56.1 ^a	56.2 ^c	72.8 ^a	73.2 ^a	73.0 ^b	66.7 ^a	67.1 ^a	66.9 ^a
T ₄ : 75% RDF + <i>Pseudomonas fluorescens</i>	59.0 ^a	58.5 ^a	58.8 ^{abc}	75.3 ^a	75.6 ^a	75.4 ^{ab}	68.9 ^a	68.6 ^a	68.8 ^a
T ₅ : 75% RDF + <i>Bacillus subtilis</i>	57.5 ^a	57.9 ^a	57.7 ± 1.1 ^{abc}	73.2 ^a	74.6 ^a	73.9 ^{ab}	67.5 ^a	67.8 ^a	67.7 ^a
T ₆ : 75% RDF + <i>T. harzianum</i> + <i>P. fluorescens</i>	57.1 ^a	57.8 ^a	57.4 ^{abc}	76.7 ^a	77.7 ^a	77.2 ^a	69.6 ^a	70.2 ^a	69.9 ^a
T ₇ : 75% RDF + <i>P. fluorescens</i> + <i>B. subtilis</i>	60.3 ^a	59.8 ^a	60.0 ^a	76.9 ^a	77.0 ^a	77.0 ^a	69.1 ^a	68.7 ^a	68.9 ^a
T ₈ : 75% RDF + <i>T. harzianum</i> + <i>B. subtilis</i>	59.3 ^a	60.1 ^a	59.7 ^{ab}	78.1 ^a	76.4 ^a	77.3 ^a	70.4 ^a	69.5 ^a	69.9 ^a
T ₉ : 75% RDF + <i>T. harzianum</i> + <i>P. fluorescens</i> + <i>B. subtilis</i>	56.7 ^a	57.0 ^a	56.9 ^{bc}	75.4 ^a	75.0 ^a	75.2 ^{ab}	67.3 ^a	66.9 ^a	67.1 ^a

Means followed by different alphabets within a column differed significantly ($p \leq 0.05$) among the treatments as per DMRT.

3.5. Effect on Leaf Area

Data pertaining to leaf area revealed no significant ($p \leq 0.05$) difference among different treatment combinations (Table 6). At 30 DAT, the leaf area ranged between 185.9 and 190.9 cm² (pooled data). With the advancement in crop age, the leaf area increased progressively, but the rate of increment slowed after 60 DAT. Similarly to chlorophyll (SPAD) data, treatments remained at par with each other except the absolute control during the crop growth period. However, the maximum value (537.2 cm²) at 60 DAT showed a significant ($p \leq 0.05$) effect of 75% RDF + *T. harzianum* + *P. fluorescens* (T₆) over the absolute control (T₁) and 75% RDF + *T. harzianum* (T₃) as per the pooled analysis. At harvest, the leaf area ranged from 639.2 to 661.8 cm² with an average value of 657.9 cm². The application of 75% RDF + *T. harzianum* + *B. subtilis* (T₈) resulted in the highest leaf area of 661.9 cm² in the first year, while highest value of 661.8 cm² was observed in the second year under 75% RDF + *P. fluorescens* + *B. subtilis* (T₇) during the maturity of the crop. Plants bio-primed with *P. fluorescens* exhibited higher leaf area than other single inoculated plants at all growth stages.

3.6. Effect on Root Length

A perusal of root length data indicated a positive influence of dual microbial priming when applied along with the reduced dose of fertilizers over the application of a full dose of fertilizers and absolute control (Figure 5). The longest roots of 19.8 cm and shortest roots of 15.8 cm were found under 75% RDF + *T. harzianum* + *P. fluorescens* (T₆) and absolute control (T₁), respectively. In comparison to 100% RDF (T₂) and 75% RDF + *T. harzianum* + *P. fluorescens* + *B. subtilis* (T₉), about 14% and 5%, respectively, a higher length of the root was recorded in T₆. However, all dual consortium treatments were at par with each other. Plants bio-primed with *P. fluorescens* exhibited higher root length than other single inoculated plants. The effect of triple consortium was non-significant ($p \leq 0.05$) when compared with single-species and dual-species bio-priming treatments.

3.7. Effect on Earliness

The minimum number of days (95.2) to head maturity was taken by T₇ (Figure 5b). The plants of absolute control plots (T₁) significantly delayed in producing marketable heads (104.8 days). Bio-primed plants (T₃–T₉) demonstrated the early maturation of heads than non-primed plants (T₁–T₂). On the other hand, the higher dose of N increases the undue vegetative stage of growth period. A statistically equivalent effect on earliness was recorded under triple-species and single-species and bio-priming treatments.

3.8. Effect on Micronutrient Uptake

Results revealed that the application of 75% RDF + *T. harzianum* + *P. fluorescens* (T₆) significantly ($p \leq 0.05$) enhanced Fe uptake (Table 7) by the head of the crop over 100% RDF (T₂), 75% RDF + *T. harzianum* + *P. fluorescens* + *B. subtilis* (T₉), and absolute control (T₁). The plant uptake of Mn was the highest under 75% RDF + *T. harzianum* + *P. fluorescens* (T₆) for both years of the experiment. The pooled analysis confirmed the equivalent effect of dual inoculation in Mn uptake, but they were significantly ($p \leq 0.05$) higher than the other treatments. The uptake of Zn varied from 2.82 to 65.49 g ha⁻¹. The significantly lowest Zn uptake of 10.34 g ha⁻¹ was observed in absolute control (T₁) followed by 100% RDF (T₂). The co-inoculation of *P. fluorescens* + *B. subtilis* along with 75% RDF (T₇) showed the highest Zn uptake, but it was at par with 75% RDF + *T. harzianum* + *P. fluorescens* (T₆). Similarly, Zn uptake recorded in 75% RDF + *T. harzianum* (T₃) was at par with *T. harzianum* + *P. fluorescens* + *B. subtilis* (T₉). Significantly highest (17.70 g ha⁻¹) and lowest (2.11 g ha⁻¹) Cu uptakes were obtained in 75% RDF + *T. harzianum* + *P. fluorescens* (T₆) and absolute control (T₁), respectively. The triple combination of bio-agents (*T. harzianum* + *P. fluorescens* + *B. subtilis*) showed lower micronutrient uptake than the other bio-primed plants. Among single inoculations, *P. fluorescens* resulted in higher uptake by the head. The micronutrient uptake followed the pattern of Fe > Zn > Mn > Cu.

Table 6. Effect of bio-priming and fertilization on leaf area of red cabbage at different growth stages.

Treatments	Leaf Area (cm ²)								
	Pre-Cupping (30 DAT)			Early Head Formation (60 DAT)			Maturity		
	2016–2017	2017–2018	Pooled	2016–2017	2017–2018	Pooled	2016–2017	2017–2018	Pooled
T ₁ : Absolute control N:P ₂ O ₅ :K ₂ O @ 0:0:0 kg ha ^{−1}	186.3	185.6	185.9	517.7 ^b	518.0 ^b	517.9 ^c	639.2 ^b	638.9 ^b	639.1 ^b
T ₂ : RDF of N:P ₂ O ₅ :K ₂ O @ 120:60:60 kg ha ^{−1}	190.6	190.4	190.5	535.3 ^a	535.6 ^a	535.4 ^{ab}	661.1 ^a	660.6 ^a	660.8 ^a
T ₃ : 75% RDF + <i>Trichoderma harzianum</i>	189.4	189.8	189.6	533.8 ^a	534.0 ^a	533.9 ^b	658.3 ^a	658.7 ^a	658.5 ^a
T ₄ : 75% RDF + <i>Pseudomonas fluorescens</i>	190.4	190.5	190.5	535.5 ^a	534.9 ^a	535.2 ^{ab}	660.4 ^a	659.8 ^a	660.1 ^a
T ₅ : 75% RDF + <i>Bacillus subtilis</i>	189.8	190.0	189.9	533.7 ^a	534.3 ^a	534.0 ^b	658.5 ^a	659.6 ^a	659.0 ^a
T ₆ : 75% RDF + <i>T. harzianum</i> + <i>P. fluorescens</i>	189.5	189.9	189.7	537.4 ^a	537.0 ^a	537.2 ^a	661.6 ^a	661.5 ^a	661.6 ^a
T ₇ : 75% RDF + <i>P. fluorescens</i> + <i>B. subtilis</i>	190.7	190.6	190.7	536.6 ^a	537.7 ^a	537.1 ^a	660.7 ^a	661.8 ^a	661.3 ^a
T ₈ : 75% RDF + <i>T. harzianum</i> + <i>B. subtilis</i>	190.6	191.1	190.9	537.5 ^a	536.7 ^a	537.1 ^a	661.9 ^a	660.8 ^a	661.3 ^a
T ₉ : 75% RDF + <i>T. harzianum</i> + <i>P. fluorescens</i> + <i>B. subtilis</i>	189.8	189.9	189.9	534.8 ^a	534.8 ^a	534.8 ^{ab}	659.4 ^a	659.6 ^a	659.5 ^a

Means followed by different alphabets within a column differed significantly ($p \leq 0.05$) among the treatments as per DMRT.

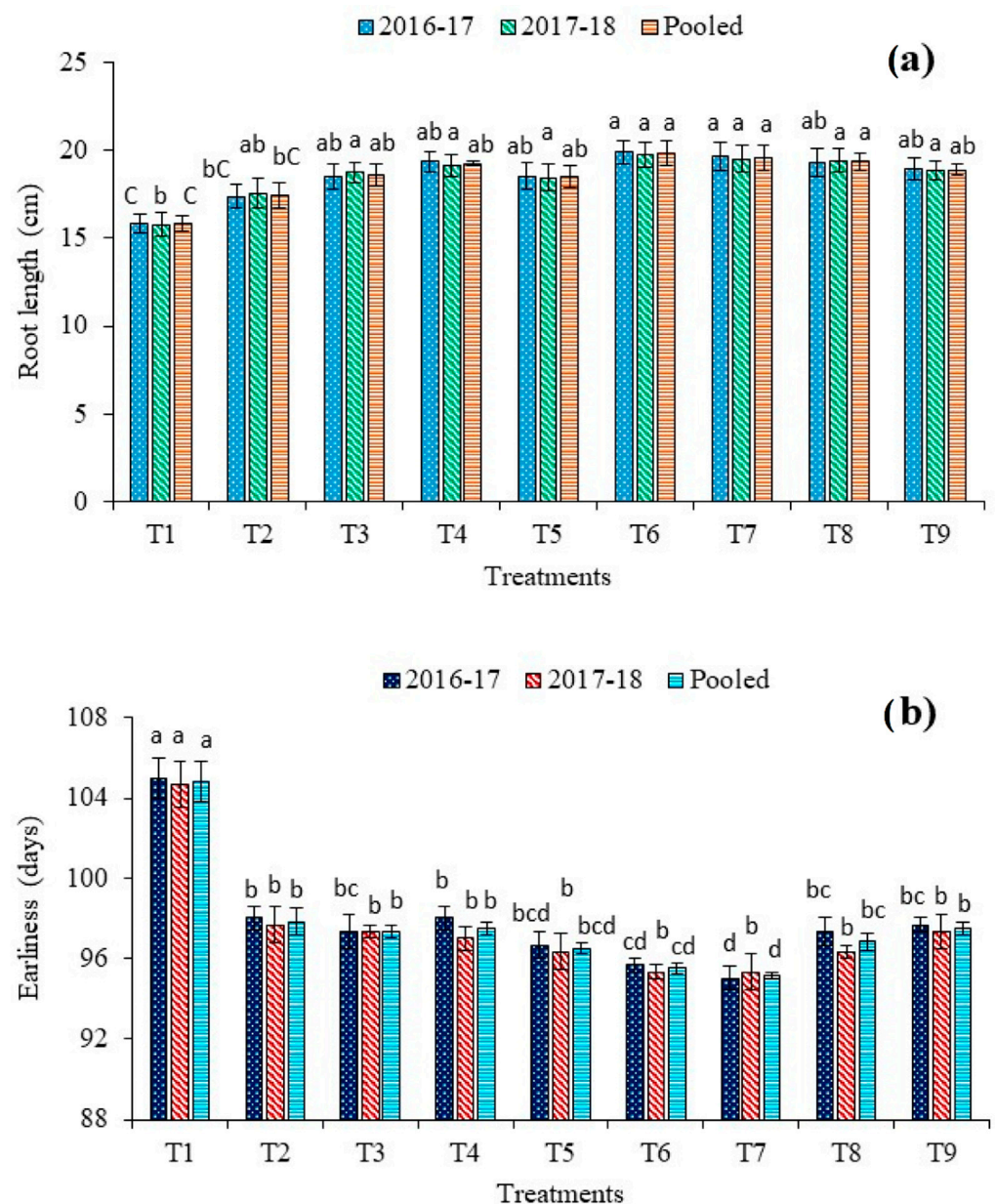


Figure 5. Effect of bio-priming and fertilization on (a) root length and (b) earliness of red cabbage. Bars (mean \pm SE; $n = 3$) followed by different alphabets significantly differ ($p \leq 0.05$) among the treatments as per DMRT. Treatments: T₁: Absolute control N:P₂O₅:K₂O @ 0:0:0 kg ha⁻¹; T₂: RDF of N:P₂O₅:K₂O @ 120:60:60 kg ha⁻¹; T₃: 75% RDF + *Trichoderma harzianum*; T₄: 75% RDF + *Pseudomonas fluorescens*; T₅: 75% RDF + *Bacillus subtilis*; T₆: 75% RDF + *T. harzianum* + *P. fluorescens*; T₇: 75% RDF + *P. fluorescens* + *B. subtilis*; T₈: 75% RDF + *T. harzianum* + *B. subtilis*; T₉: 75% RDF + *T. harzianum* + *P. fluorescens* + *B. subtilis*.

3.9. Regression Analysis

The statistical relationship between key growth parameters and micronutrient uptake is presented in Table 8. The Fe and Mn uptake of the crop showed strong relationships with chlorophyll (SPAD) ($Y = 0.057X + 59.81$, $R^2 = 0.94$; $Y = 0.450X + 59.70$, $R^2 = 0.94$). Among micronutrients, the uptake of Cu had a significant relationship with root length ($Y = 0.277X + 15.27$, $R^2 = 0.93$), while the uptake of Zn had significant relationship with the earliness ($Y = -0.170X + 106.06$, $R^2 = 0.96$) of the crop.

Table 7. Micronutrient uptake by head of red cabbage as influenced by bio-priming and mineral fertilization.

Treatments	Micronutrient Uptake (g ha ⁻¹)											
	Fe			Mn			Zn			Cu		
	2016–2017	2017–2018	Pooled	2016–2017	2017–2018	Pooled	2016–2017	2017–2018	Pooled	2016–2017	2017–2018	Pooled
T ₁ : Absolute control N:P ₂ O ₅ :K ₂ O @ 0:0:0 kg ha ⁻¹	69.1 ^d	46.8 ^d	57.9 ^d	9.9 ^d	5.5 ^d	7.7 ^c	13.1 ^c	7.6 ^d	10.3 ^d	2.8 ^e	1.4 ^e	2.1 ^e
T ₂ : RDF of N:P ₂ O ₅ :K ₂ O @ 120:60:60 kg ha ⁻¹	245.8 ^{bc}	243.5 ^c	244.6 ^c	32.9 ^{bc}	29.6 ^c	31.2 ^b	48.4 ^b	42.7 ^c	45.6 ^c	11.0 ^d	8.8 ^d	9.9 ^d
T ₃ : 75% RDF + <i>Trichoderma harzianum</i>	245.7 ^{bc}	257.5 ^{bc}	251.6 ^c	33.2 ^{bc}	32.1 ^{bc}	32.7 ^b	50.1 ^b	48.5 ^{bc}	49.3 ^c	12.4 ^{cd}	11.1 ^{bcd}	11.7 ^c
T ₄ : 75% RDF + <i>Pseudomonas fluorescens</i>	268.2 ^{abc}	271.8 ^{abc}	270.0 ^{bc}	34.9 ^{abc}	32.1 ^{bc}	33.5 ^b	50.2 ^b	53.3 ^{abc}	51.7 ^{bc}	13.7 ^{bcd}	11.6 ^{bcd}	12.6 ^c
T ₅ : 75% RDF + <i>Bacillus subtilis</i>	234.5 ^c	250.7 ^{bc}	242.6 ^c	33.1 ^{bc}	32.31 ^{bc}	32.7 ^b	50.8 ^b	51.2 ^{abc}	51.0 ^{bc}	11.7 ^d	11.4 ^{bcd}	11.5 ^{cd}
T ₆ : 75% RDF + <i>T. harzianum</i> + <i>P. fluorescens</i>	324.0 ^a	318.6 ^a	321.3 ^a	42.7 ^a	40.1 ^a	41.4 ^a	68.6 ^a	62.3 ^a	65.5 ^a	20.1 ^a	15.3 ^a	17.7 ^a
T ₇ : 75% RDF + <i>P. fluorescens</i> + <i>B. subtilis</i>	311.7 ^{ab}	307.1 ^{ab}	309.4 ^{ab}	41.3 ^{ab}	36.1 ^{abc}	38.7 ^a	69.0 ^a	62.9 ^a	65.9 ^a	17.1 ^{ab}	12.8 ^{abc}	15.0 ^b
T ₈ : 75% RDF + <i>T. harzianum</i> + <i>B. subtilis</i>	282.7 ^{abc}	292.1 ^{abc}	287.4 ^{abc}	40.1 ^{ab}	37.8 ^{ab}	39.0 ^a	58.7 ^{ab}	55.7 ^{ab}	57.2 ^b	16.0 ^{bc}	13.9 ^{ab}	14.9 ^b
T ₉ : 75% RDF + <i>T. harzianum</i> + <i>P. fluorescens</i> + <i>B. subtilis</i>	233.3 ^c	243.3 ^c	238.3 ^c	31.3 ^c	29.5 ^c	30.4 ^b	49.3 ^b	45.6 ^{bc}	47.4 ^c	12.8 ^{cd}	10.3 ^{cd}	11.5 ^{cd}

Means followed by different alphabets within a column differed significantly ($p \leq 0.05$) among the treatments as per DMRT.

Table 8. Relationship between key growth parameters and micronutrient uptake of red cabbage.

Parameters		Regression Equation	R ²	p Value	n
Chlorophyll	Fe	$Y = 0.057X + 59.81$	0.94	0.00001	9
	Mn	$Y = 0.450X + 59.70$	0.94	0.00002	9
	Zn	$Y = 0.266X + 60.95$	0.90	0.00008	9
	Cu	$Y = 0.969X + 62.55$	0.85	0.00034	9
Root length	Fe	$Y = 0.015X + 14.80$	0.87	0.00019	9
	Mn	$Y = 0.110X + 14.78$	0.86	0.00025	9
	Zn	$Y = 0.072X + 14.90$	0.89	0.00011	9
	Cu	$Y = 0.277X + 15.27$	0.93	0.00002	9
Earliness	Fe	$Y = -0.035X + 106.54$	0.94	0.00001	9
	Mn	$Y = -0.280X + 106.62$	0.94	0.00001	9
	Zn	$Y = -0.170X + 106.06$	0.96	0.00000	9
	Cu	$Y = -0.600X + 104.88$	0.87	0.00022	9

4. Discussion

Screening efficient microbial strains to address numerous agro-environmental challenges will help in achieving the targets of sustainable development goals. Their capacity to promote plant growth by increasing nutrient availability and acquisition has huge potential to reduce the use of chemical fertilizers in agroecosystems [8,26]. Moreover, they also serve as a good option to produce healthy foods and increase marketable head yield [30]. However, the efficacy of microbial products is often found to be low under field conditions [33]. In the current study, the effects of *T. harzianum*, *P. fluorescens*, and *B. subtilis* as single-species bio-primers and combination treatments (consortia) along with 75% RDF on crop growth and micronutrient uptake by red cabbage were studied.

At 60 DAT, the plant height (Table 2) was the highest in 100% RDF (T₂); however, at maturity, the effect was significantly ($p \leq 0.05$) higher with 75% RDF + *P. fluorescens* + *B. subtilis* (T₇). Similar results in plant height was observed by Verma et al. [34] due to a combined application of chemical fertilizer and organics (*Pseudomonas fluorescens* + humic acid). According to Adesemoye et al. [35], 75% RDF was the optimum rate to which the addition of bacterial inoculants (*Bacillus* spp.) augmented the plant height of tomato. Recently, Houida et al. [36] reported the increased seedling growth of maize due to seed bio-priming. The yield of red cabbage is highly dependent on the number of leaves because they act as the main site for the assimilation of carbohydrates. At 60 DAT, dual inoculated treatments (T₆–T₈) were higher in the number of leaves than other treatments (Table 3). The increased absorption of nutrients in bio-primed plants triggered cell division, cell elongation, and overall metabolism in their system [21]. Our findings are in conformity with the reports of Verma et al. [34] and Thakur et al. [37]. At maturity stage, the number of leaves was reduced in bio-primed plants, which indicated a better head formation of these plants.

The plant spread was maximum with 75% RDF + *P. fluorescens* + *B. subtilis* (T₇) at all three stages, i.e., pre-cupping, early head formation, and maturity (Table 4). Our observations are in partial agreement with the findings of Das et al. [12]. Chlorophyll (SPAD) was found to be highest under 75% RDF + *T. harzianum* + *B. subtilis* (T₈) at all stages (30 DAT, 60 DAT, and harvest) (Table 5). Root system modulation in bio-primed plants led to a greater absorption of nutrients and the production of carbohydrates. However, the effect was not significant in comparison to plants receiving 100% RDF. Contrary to our results, Chatterjee et al. [38] observed significantly higher SPAD values in cabbage due to a combined application of inorganic fertilizers and organics (organic manure and biofertilizer) in comparison to the sole application of inorganic fertilizers. However, our results are in partial agreement with the reports of Vafadar et al. [39] who claimed positive effects of triple inoculations on chlorophyll content over single inoculations, but the effects

were inferior to dual inoculations. Seed inoculation with *Bacillus megaterium* enhanced chlorophyll (SPAD) by 5.8% in cabbage [40].

During early head formation, leaf area (Table 6) was at its maximum with 75% RDF + *T. harzianum* + *P. fluorescens* (T₆). Among single species bio-priming, *P. fluorescens* inoculated plants exhibited higher leaf area over other individual inoculated plants at all growth stages. A similar effect of *Pantoea agglomerans* over *B. subtilis* was reported in cabbage by Turan et al. [40]. However, significant ($p \leq 0.05$) variations between bio-primed plants and 100% fertilized plants were not documented. In contrast to our findings, the significant effect of bio-priming in combination with mineral fertilizer on leaf area was reported in baby corn [19]. Furthermore, the positive effect of conjoint use of inorganic and organic biofertilization in cauliflower was also reported by Thakur et al. [37]. Thus, the improvement in morphological and physiological parameters in the host plants are a resultant of bio-priming induced seed germination, seedling vigor, activation of enzymes, root growth, nutrient uptake, etc. [41].

Root length is also a vital parameter that is directly linked to plant growth. In our investigation, higher root length (Figure 5a) was recorded in 75% RDF + *T. harzianum* + *P. fluorescens* (T₆). Results also clearly suggested that bio-priming was effective in improving the root growth of red cabbage crop. The positive effect of organic and inorganic fertilizers on root length was also reported by Islam et al. [42]. Microbial agents such as *Trichoderma* have the ability to colonize the epidermis and outer cortical layers of the root and enhance root development for increasing the uptake of nutrients [43]. However, chemotactic signals from root exudates are also responsible for root colonization [44,45]. The significant enhanced root length of baby corn was detected with the application of 75% RDF in combination with *Trichoderma viride* and *Glomus intraradices* [19]. Increased root colonization by fungal strains (*Glomus intraradices* and *Acaulospora laevis*) in the presence of mycorrhiza helper bacteria (*P. fluorescens*) was recorded in broccoli plants [46]. The minimum number of days to head maturity (earliness) was recorded with 75% RDF + *P. fluorescens* + *B. subtilis* (T₇) (Figure 5b). Our results are in agreement with the observation of Kumari et al. [47] and Thakur et al. [37], who noticed a significant reduction in the number of days to maturity of cabbage head and cauliflower curd, respectively, with the use of bio-inoculants.

Results showed that bio-priming not only promoted the growth (Figure 4) of red cabbage but also influenced micronutrient uptake (Fe, Mn, Zn, and Cu) by the head (Table 7). The plant uptake of Fe, Mn, and Cu was the highest with 75% RDF + *T. harzianum* + *P. fluorescens* (T₆), while the highest Zn uptake was observed with 75% RDF + *P. fluorescens* + *B. subtilis* (T₇). The micronutrient bioavailability to plants is a consequence of microbial activities including siderophore production, exudation of organic ligands and organic acids, rhizospheric acidification, etc. [48,49]. The bacterial inoculation of *Pseudomonas* spp. and *Bacillus* spp. showed increased uptake of Fe, Mn, Zn, and Cu by maize plants [50]. Modifications in root architecture, viz., higher as well as longer secondary root hairs induced by qid74 gene in *T. harzianum*-treated cucumber plants, facilitated the enhanced uptake of macronutrients and micronutrients as a resultant of the increment in total absorptive surface and, consequently, led to better plant biomass [51]. The solubility of Zn increased due to the secretion of organic acids (citrate and malate) by rhizospheric microbes [52]. Diffusible metabolites produced by *T. harzianum* 1295-22 (T-22) have the ability to solubilize Fe, Mn, Zn, and Cu [53]. The increased uptake of micronutrients in bio-primed plants indicated the existence of positive interactions among the growth promotion, root colonization, and micronutrient uptake. The Fe and Mn uptake of the crop showed strong relationships (Table 8) with chlorophyll (SPAD). The micronutrients Fe and Mn are important for photosynthesis, carbohydrate metabolism, phytohormone synthesis, and chlorophyll formation [49,54,55]. Cu and Zn uptakes showed a significant relationship with root length and earliness, respectively. Zinc is essential for the synthesis of carbohydrates, protein, and phytohormones [50,52,56].

5. Conclusions

The present investigation confirms that bio-priming in combination with mineral fertilizer triggered growth promotion in red cabbage. Dual inoculations of *T. harzianum* and *P. fluorescens* followed by *P. fluorescens* and *B. subtilis* had synergistic activities and were found to be the most effective bio-priming agents in the enhancement of growth and the micronutrient uptake of red cabbage. The formulations of novel biofertilizers will have a significant impact for the production of exotic crops under sustainable agriculture. Furthermore, improving nutrient use efficiency in crops by using pragmatic solutions such as bio-priming reduces fossil fuel consumption (fertilizers, pesticides, etc.) and promotes the fundamental goals of sustainable agriculture. Future studies should also target to determine the root colonization pattern of bio-agents under the rhizosphere engineering of exotic crops.

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