

Article

Application of *Trichoderma viride* and *Pseudomonas fluorescens* to Cabbage (*Brassica oleracea* L.) Improves Both Its Seedling Quality and Field Performance

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Abstract: Inoculating cabbage (*Brassica oleracea* L.) plants with bio-control agents and plant growth-promoting rhizobacteria (PGPR) can considerably improve seedling quality, growth, yield, and yield-related parameters over time. An experiment was conducted to evaluate the bio-fertilizer efficiency of a bio-control agent (*Trichoderma viride*) alone or in combination with PGPR (*Pseudomonas fluorescens*). Accordingly, various seedling quality and yield parameters were studied, and the results suggested that all the co-inoculation treatments displayed beneficial effects. Still, the combination of *Trichoderma viride* and *Pseudomonas fluorescens* showed the maximum increment in all the parameters considered, i.e., seedling emergence, seedling height, stem diameter, leaf area, root length, seedling vigour index, seedling fresh weight, seedling dry weight, total chlorophyll content, plant height at 30 DAT, plant height at 60 DAT, leaf numbers, leaf area index, root length, root dry weight, number of non-wrapping leaves, number of wrapping leaves, head weight, head diameter, and head yield. The findings appear to offer a viable bio-control technique for crop protection as bio-fertilizers bundled in a single formulation.

Keywords: cabbage; drought; bio-control; *Trichoderma viride*; *Pseudomonas fluorescens*



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1. Introduction

Cabbage (*Brassica oleracea* var. *capitata* L.) is one of the foremost crops belonging to the *Cruciferae* family [1]. It is used for culinary purposes and has various medicinal and nutritional properties. It contains significant amounts of dietary fibre, phenols, carotenoids, carbohydrates, flavonoids, glucosinolates, anthocyanin, minerals, protein, and vitamins with excellent caloric value [2,3]. This crop is frequently consumed for salad purposes, juiced, in stir-fry, as boiled leaves, and as a fermented food. However, the production through excessive use of synthetic fertilizers cannot be overpowered [4]. This is because the plants take up only a partial amount of nutrients due to low fertilizer-use efficiency in the soil, and the fertilizers that remain lead to environmental pollution [5]. Additionally, these synthetic fertilizers contain heavy metals that cannot degrade easily, acting as a persistent pollutant for the environment, further causing eutrophication of water sources [6].

To limit the use of synthetic fertilizers, organic inputs are needed, which can upsurge the nutrient availability in soil and show significant effects on the health and horticultural traits of plants [7]. Amongst all the organic inputs, bio-fertilizers can serve as a substitute

for synthetic fertilizers. Bio-fertilizers are comprised of living microorganisms that facilitate faster colonization and augment nutrient availability to encourage plant growth [8]. Various vegetable crops, such as cabbage, broccoli, etc., were benefited from the application of bio-fertilizers, including strains of *Azospirillum*, *Azotobacter*, *Bacillus*, and *Rhizobium*, to the soil and as a seed treatment [9]. They enhanced the yields of organic systems through rapid breakdown of organic matter, increased nutrients' availability, and improved soil characteristics through the release of metabolites stimulating the growth. Bio-fertilizers can help improve soil structure by increasing the microbial biomass that ultimately augments crop production and productivity. These bio-fertilizers can convert essential nutrient elements through biological processes to make them available for the plants' consumption [10]. Apart from this, numerous studies validated that a plant treated with bio-agents performed better in early growth stages and showed other improved traits, such as the breakdown of dormancy, improvised synchronization of seedling emergence, vigour index, growth indices, vegetative growth, early flowering, etc. [11].

As a heavy feeder crop, cabbage uptakes nitrogen, phosphorus, and potassium to a greater extent [12]. Most suitable bio-agent species belong to either bacterial or fungal groups [13,14]. Amongst various bacterial bio-agents, *Pseudomonas* spp. is the most frequently distributed gram-negative bacteria that can colonize in different environmental conditions [15]. Due to its versatile metabolic capacity and widespread occurrence, this genus is mainly used as a crop inoculant that enhances the plant's growth-promoting activities in numerous ways [16]. *Pseudomonas fluorescens* may help stimulate the growth-related aspects of cabbage, particularly encouraging the rapid growth of seedlings and minimizing the transplanting shock [17]. Additionally, in a sequence of fungal bio-agents, *Trichoderma* is frequently used because of its fast growth, well-built spore production, ease in isolation, and secretion of enzymes related to cell wall degradation [18]. These traits may improve nutrient solubilization, nutrient uptake capacity, root growth, plant health, enhanced microbial activity, and degradation of xeno-biotic compounds and polysaccharides, rendering them efficient bio-control agents [19]. Essentially, the efficacy of these bio-fertilizers depends on their counts, soil type, and environmental conditions [20]. To level up the sub-optimal activity of microorganisms, bio-fertilizers are multiplied artificially to accelerate the biological activity that ultimately increases the plant's nutrient availability [21]. Hence, our study was conducted to demonstrate the beneficial effects of *T. viride* and *P. fluorescens* on cabbage seedlings' morphological quality and field performance so as to present them as potential bio-fertilizers for yield enhancement.

2. Material and Methods

From mid-November 2018 to mid-November 2019, field experiments were conducted at Ranchi University, at 23°22'18" N longitude, 85°19'27" E latitude, Ranchi, Jharkhand, India. The experiment was conducted in a randomized complete block design (RCBD) with three replications. The seeds were pre-treated with *P. fluorescens* in a 100 mL solution containing 1 mL *P. fluorescens* inoculum (IMTECH, Mohali, India) at 37 °C for 5 h on a rotary shaker. The seeds were then air-dried and used for subsequent analysis. The four-week-old seedlings were maintained under relative humidity of 80% at 30 °C. Nutrient-broth medium made in sterilized water and incubated for 48 h at 32 °C was used to create *P. fluorescens* inoculum [22]. The healthy seedlings were transplanted in pots with the dimensions of 3.0 m × 2.7 m × 45 cm, with 45 cm spacing between rows and within each plot.

Sterilized inoculum sand–soil mixture was added to each pot, 1 kg to the control and 100 g less to other treatments, such as the *Trichoderma* soil inoculum (SriRam fertilizer, Kota, India). The soil dilution plate method was used to create the inoculum on potato dextrose agar medium, and *T. viride* was identified manually. The plates were incubated for four days at a temperature of 30 °C. Wheat bran, sawdust, and water were added to the inoculum in a 3:1:1 ratio. In the experiment, seeds treated with 5% *T. viride* were referred to as T1, seeds treated with 5% *P. fluorescens* were referred to as T2, and seeds treated with 5%

T. viride and 5% *P. fluorescens* were referred to as T3. All the experiments were conducted with at least three biological replicates.

To ensure the best possible results, seedling characteristics, such as seedling emergence percentage, days to 2-true-leaf stage, seedling height, stem diameter, leaf area, days to transplant, root length, seedling vigour index, seedling fresh weight, seedling dry weight, and total chlorophyll content, were all measured just before transplanting. A portable leaf chlorophyll meter SPAD-502 (Konica Minolta, Osaka, Japan) was used to determine the quantity of chlorophyll across the entire leaf's surface [23]. Seedling height (cm), stem diameter (mm), and root length (cm) were determined from 20 randomly chosen seedlings at the time of final germination count. A leaf area meter (LI-3100, LI-COR, Lincoln, NE, USA) was used to measure the leaf area (cm²) [24]. Seedling dry weight (g) was recorded by keeping the seedlings in a hot-air oven for 48 h at 60 °C. Based on the seed germination and seedling dry weight data, the seedling vigour index was calculated as the seedling germination (%) × seedling dry weight (g) [25].

The crop received a homogenous dose of fertilizers at rates of 130 kg N, 80 kg P₂O₅, and 80 kg K₂O per hectare. The appropriate cultural and plant protection measures were executed uniformly in all plots as needed. Ten randomly selected plants from each plot were observed in the main field. Data were collected for the following variables: days to head initiation, days to head maturity, head weight, and head yield.

Statistical Analysis

All analyses were conducted using the statistical tool INDOSAT version 8.0. The mean of each individual year and the pooled mean were determined. We compared the treatment averages using the least significant difference (LSD) test at the 0.05 level of significance [26].

3. Results

3.1. Effect on Seedlings' Morphological Quality Characteristics

The percentage of seedling emergence was found to be the greatest (93.13%) for those with the T3 treatment, in which seed treatment was done with 5% *Trichoderma viride* and with 5% *Pseudomonas fluorescens* before transplanting. Seedling emergence increased by 8% in T1, 10% in T2, and 12% in T3 compared with the control treatment; days to 2-true-leaf stage increased by 22% in the control, 2% in T1, and 32% in T2 compared with the T3 treatment (Figure 1). The combined efficiency of the 5% *Trichoderma viride* and the 5% *Pseudomonas fluorescens* treatments before transplanting was further confirmed with the increased seedling height, the greatest being 39% in T1, then 29% in T2, and 56% in T3 compared with the control treatment (Figure 1). The stem diameter of the seedling was also increased by 50% in T1, 28% in T2, and 57% in T3 compared with the control treatment (Figure 1). Further, the leaf area of the seedlings increased by 89% in T1, 72% in T2, and 98% in T3 (Figure 1). The number of days it took to transplant the seedlings by the seed treatment with 5% *Trichoderma viride* was decreased by 31% in T2, 9% in control, and 1% in T3 compared with T1 (Figure 1).

The root length of the seedlings also showed a probable increase by 25% in the control, 94% in T1, and 168% in T3 compared with T2 (Figure 2). However, the most significant variations were recorded in the seedling vigour index, seedling fresh weight, and the seedling dry weight, where T3 remarkably increased by 89%, 143%, and 171%, respectively; T1 increased by 72%, 128%, and 157%, respectively; and T2 increased by 10%, 64%, and 64%, respectively, compared with the control (Figure 2). In addition, T3 also significantly enhanced chlorophyll content with an increase of 26%, followed by 17% in T1, and 1% in the control, which was very close to T2 (Figure 1). The days taken to establish the seedlings were increased by 27% in the control, 61% in T2, and <1% in T3 compared with T1, which fewer days to establish the seedlings of cabbage (Figure 2). All these observations pointed toward the fact that although all the treatments produced significant results, T3 was found to be the most efficient in enhancing the seedlings' morphological characteristics (Figures 1 and 2).

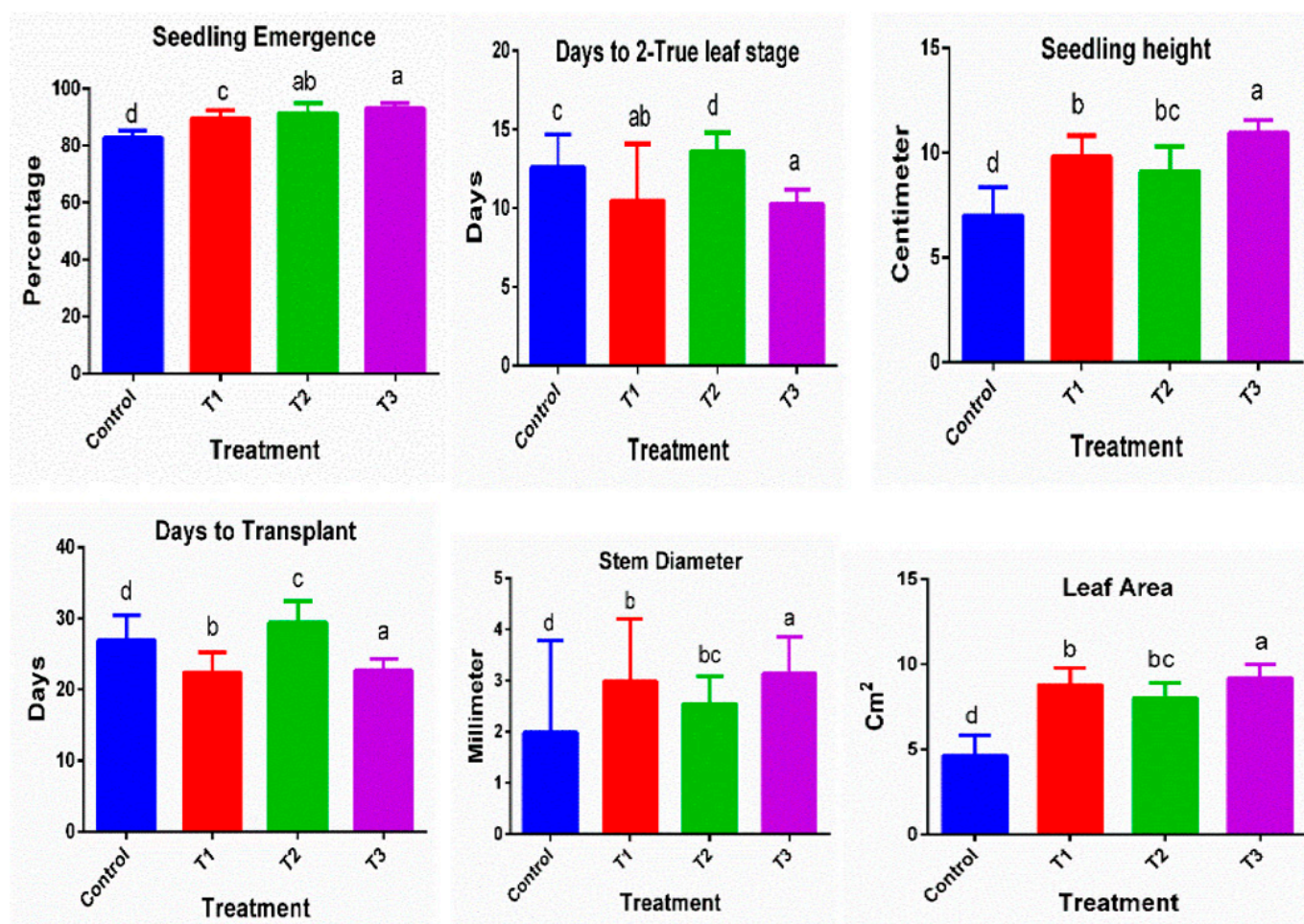


Figure 1. Effect of different treatments involving *Trichoderma viride* (T1), *Pseudomonas fluorescens* (T2), and their combination (T3) on seedling emergence (%), days to 2-true-leaf stage, seedling height (cm), days to transplant, stem diameter (mm), and leaf area (cm²) traits of cabbage compared with the control (C). Means with the different letter (a, b, etc.) are significantly different at $p < 0.05$ based on the Student–Newman–Keuls test.

3.2. Effect on the Field Performance of Cabbage

The plant height at 30 DAT and 60 DAT increased by 30% and 16%, respectively, in T3; by 25% and 13%, respectively, in T1; and by 4% and 2%, respectively, in T2 compared with the control (Figure 3). The number of leaves increased by 31% in T3, 26% in T1, and 4% in T2 compared with the control; the leaf area index increased by 38% in T3, 16% in T1, and 5% in T2 compared with the control (Figure 2). Root length increased by 31% in T3, 18% in T1, and 1% in the control, and root dry weight increased by 33% in T3, 24% in T1, and 4% in the control (Figure 3). The number of non-wrapping leaves and wrapping leaves exhibited a maximum increase in T3 of 18% and 35%, respectively, followed by T1 with 14% and 30%, respectively, and then T2 with 1% and 5%, respectively (Figure 3).

In the case of days to head initiation and days to head harvesting, there were nominal increments of 5% and 10%, respectively, in the control; 4% and 8%, respectively, in T2; and 1% and 3%, respectively, in T1 (Figure 4). Further, the increase of 40%, 24%, and 8% was observed in the head weights of T3, T1, and T2, respectively, when subjected to treatments (Figure 4). In addition to the different upgraded head features of the cabbage plant, the inoculation with 5% *Trichoderma viride* and seed treatment with 5% *Pseudomonas fluorescens* also caused an improvement in head diameter, which increased by 23% after treatment in T3, 13% in T1, and a minimum increase of <0.07% in the control (Figure 4). However, it is worth mentioning that the head compactness also showed significant variations and

exhibited only a nominal decrease of 65% in T2, 47% in the control, and 23% in T1 compared with the effects of 5% *Trichoderma viride* and seed treatment with 5% *Pseudomonas fluorescens* inoculations (Figure 4). Furthermore, the head yield also increased by 37%, 24%, and 9% after the treatment of the plants in T3, T1, and T2, respectively (Figure 4). Therefore, as far as the yield and yield-related characteristics of cabbage are concerned, the dual combination of 5% *Trichoderma viride* and seed treatment with 5% *Pseudomonas fluorescens* was found to be more effective than both their separate applications and no application.

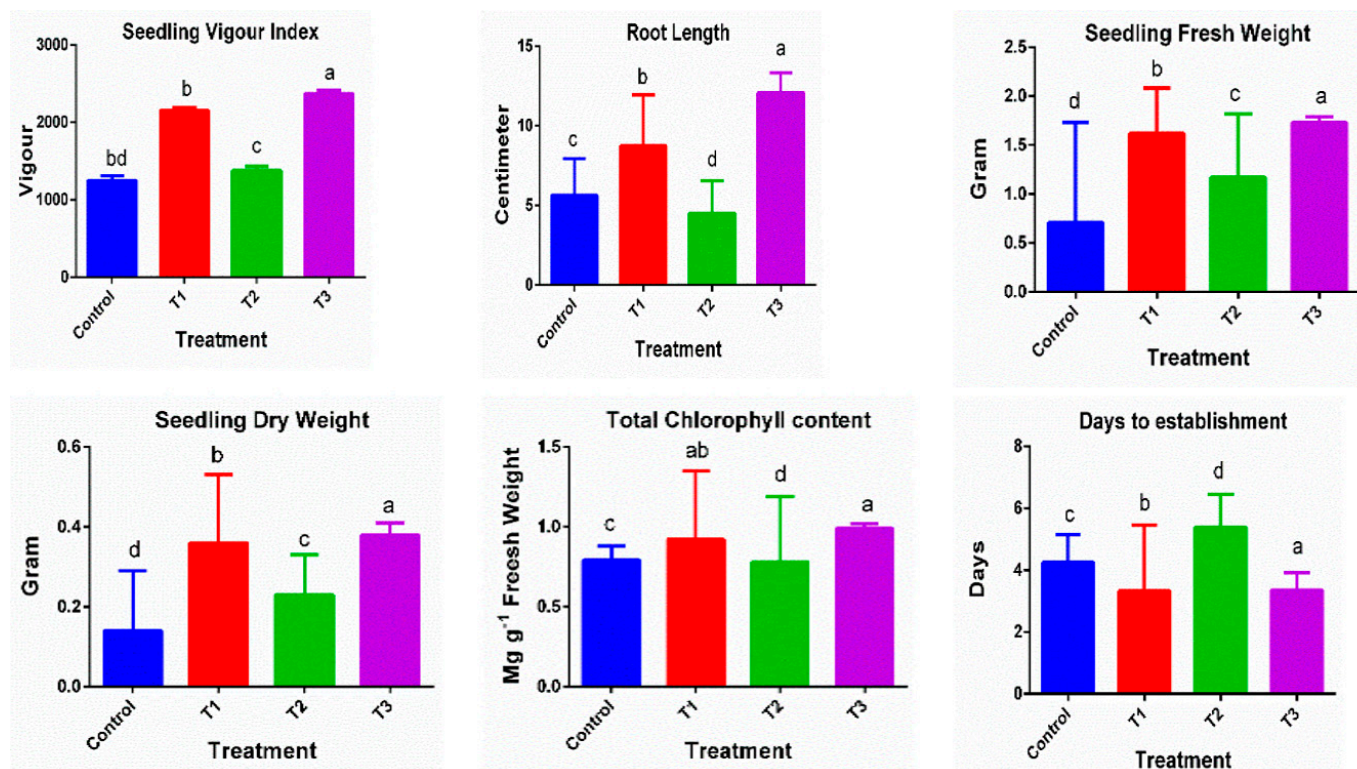


Figure 2. Effect of different treatments involving *Trichoderma viride* (T1), *Pseudomonas fluorescens* (T2), and their combination (T3) on seedling vigour index, root length (cm), seedling fresh weight (gm), seedling dry weight (gm), total chlorophyll content (mg/g of fresh weight), and days to establishment traits of cabbage compared with the control (C). Means with the different letter (a, b, etc.) are significantly different at $p < 0.05$ based on the Student–Newman–Keuls test.

3.3. Correlations among the Traits

In total, there were 325 correlations, of which only 140 were significant with $p < 0.1$. The days to 2-true-leaf stage was positively correlated with days to transplant (0.995), days to establishment (0.968), and head compactness (0.959), and it was negatively correlated with total chlorophyll content (−0.953) and root dry weight (−0.972) (Figure 5). Seedling height showed a positive correlation with stem diameter (0.981), leaf area (0.967), fresh seedling weight (0.971), seedling dry weight (0.95), and head yield (0.952) (Figure 5). Stem diameter was found to be positively correlated with leaf area (0.965), seedling fresh weight (0.999), seedling dry weight (0.99), and head yield (0.959), and it was negatively correlated with days to head initiation (−0.957) (Figure 5). However, leaf area and days to transplant showed positive correlation with seedling fresh weight (0.952) and days to establishment (0.981) respectively (Figure 4). Root length was positively correlated with total chlorophyll content (0.987), leaf area index (0.958), root length (0.992), root dry weight (0.986), number of non-wrapping leaves (0.952), and head diameter (0.989), while it was negatively correlated with head compactness (−0.99) (Figure 5).

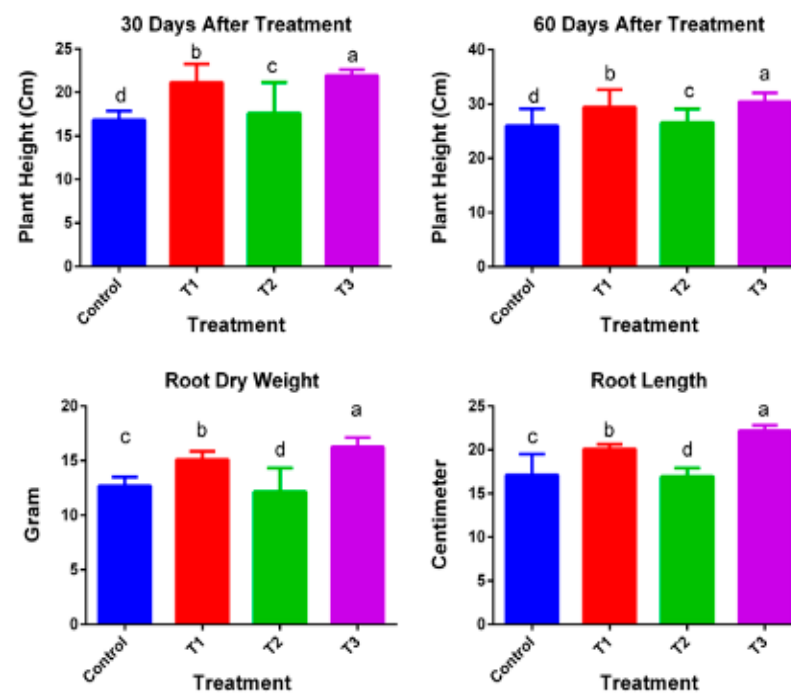


Figure 3. Plant height, root length, and root dry weight after 30 and 60 days of treatment with *Pseudomonas fluorescens* and *Trichoderma viride* and their combination (T3). The treated samples had significant differences compared with the control (C). Means with the different letter (a, b, etc.) are significantly different at $p < 0.05$ based on the Student–Newman–Keuls test.

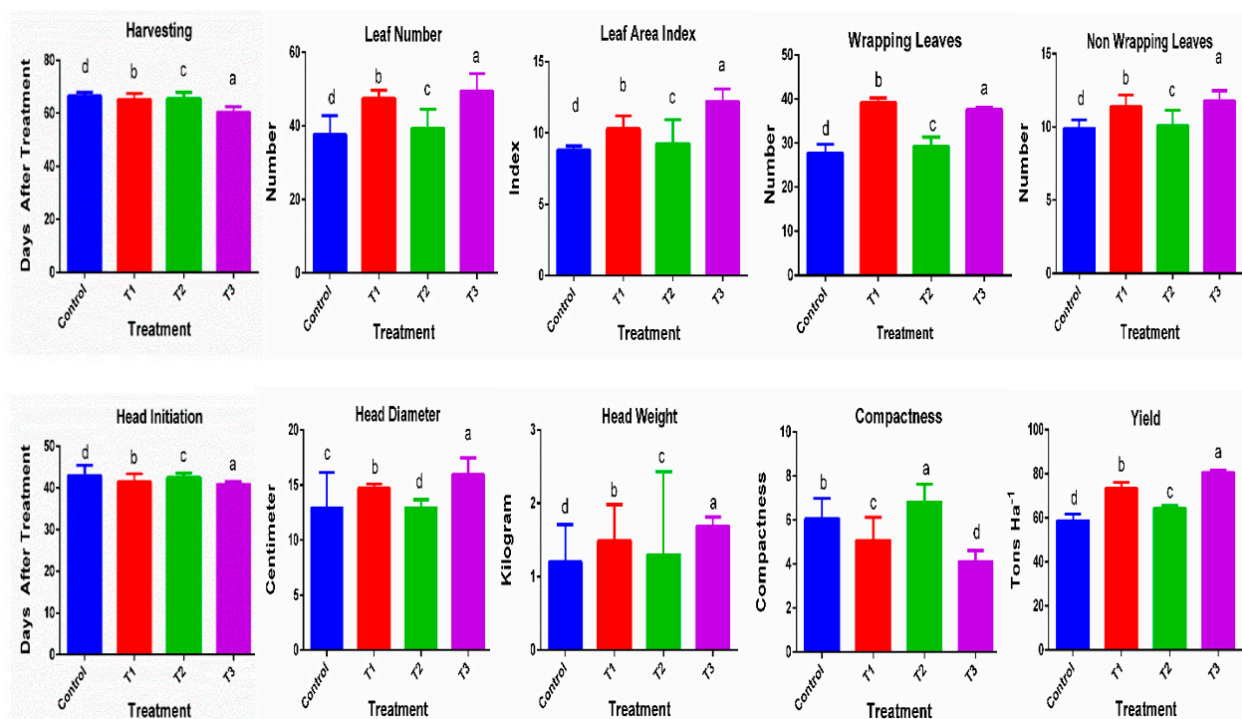


Figure 4. Bio-mass details of cabbage plants at the harvesting stage. Treatment involving *Trichoderma viride* (T1), *Pseudomonas fluorescens* (T2), and their combination (T3) performed better than control (C). Means with the different letter (a, b, etc.) are significantly different at $p < 0.05$ based on the Student–Newman–Keuls test.

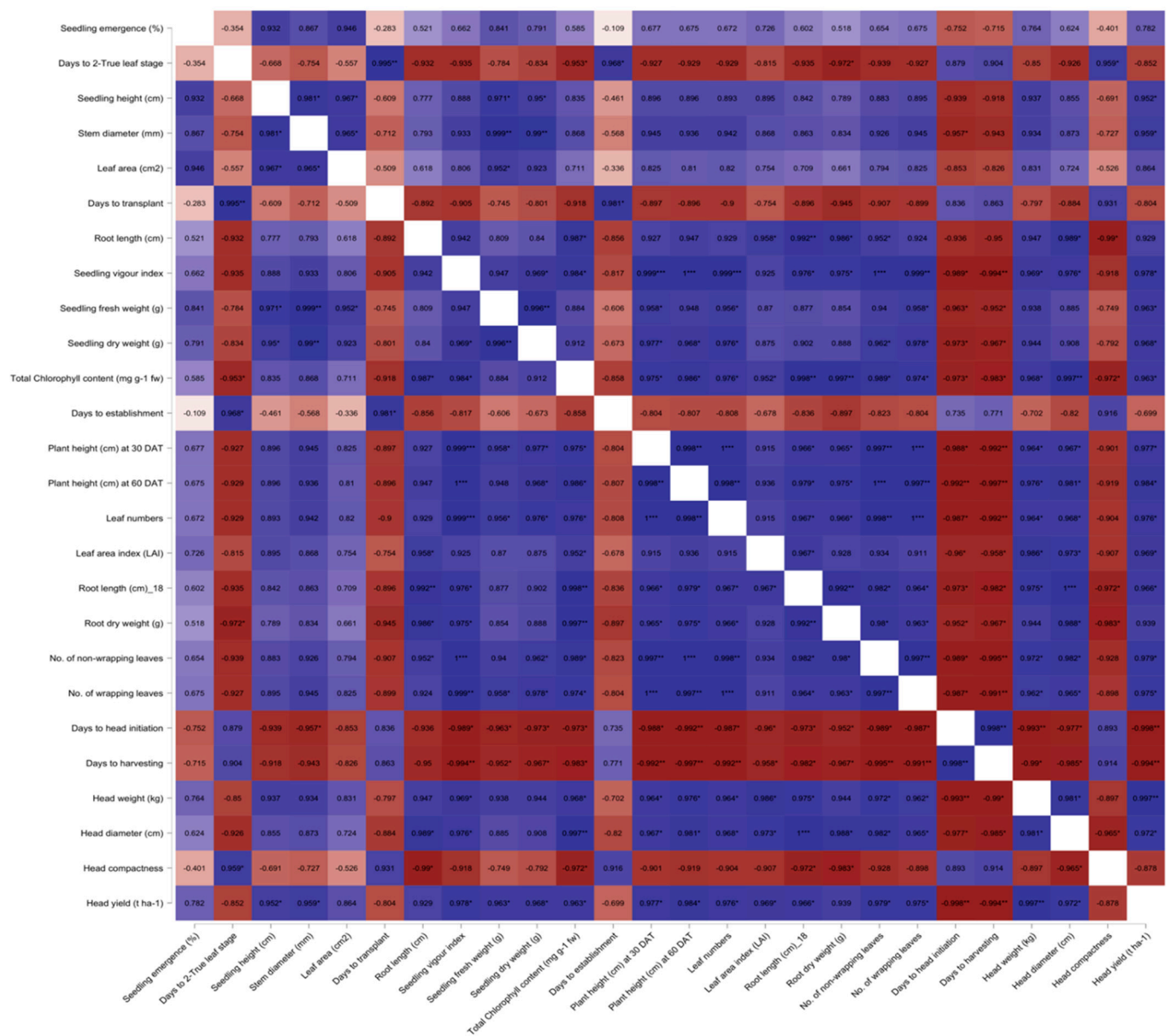


Figure 5. Pearson's correlations among the seedling quality, yield, and yield-contributing factors of cabbage studied with *T. viride* and *P. fluorescens*. *, **, *** denote significance at the 10, 5 and 1 percent level, respectively.

In the case of seedling vigour index, positive correlation was observed for seedling dry weight (0.969), total chlorophyll content (0.984), plant height at 30 DAT (0.999), plant height at 60 DAT (1), leaf numbers (0.999), root length (0.976), root dry weight (0.975), number of non-wrapping leaves (1), number of wrapping leaves (0.999), head weight (0.969), head diameter (0.976), and head yield (0.978) (Figure 5). In contrast, negative correlation was observed for days to head initiation (−0.989) and days to harvesting (−0.994) (Figure 5). Seedling fresh weight was positively correlated with seedling dry weight (0.996), plant height at 30 DAT (0.958), leaf numbers (0.956), number of wrapping leaves (0.958), and head yield (0.963) but was negatively correlated with days to head initiation (−0.963) and days to harvesting (−0.952) (Figure 5). Likewise, seedling dry weight was positively correlated with plant height at 30 DAT (0.977), plant height at 60 DAT (0.968), leaf numbers (0.976), number of non-wrapping leaves (0.962), number of wrapping leaves (0.978), and head yield (0.968) but was negatively correlated with days to head initiation (−0.973) and days to harvesting (−0.967) (Figure 5).

Furthermore, total chlorophyll content showed a direct correlation with plant height at 30 DAT (0.975), plant height at 60 DAT (0.986), leaf numbers (0.976), leaf area index (0.952), root length (0.998), root dry weight (0.997), number of non-wrapping leaves (0.989), number of wrapping leaves (0.974), head weight (0.968), head diameter (0.997), and head yield (0.963), whereas it showed an inverse correlation with days to head initiation (−0.973), days to harvesting (−0.983), and head compactness (−0.972) (Figure 5). Plant height at 30 DAT was positively correlated with plant height at 60 DAT (0.998), leaf numbers (1), root length (0.966), root dry weight (0.965), number of non-wrapping leaves (0.997), number of wrapping leaves (1), head weight (0.964), head diameter (0.967), and head yield (0.977) but was negatively correlated with days to head initiation (−0.988) and days to harvesting (−0.992) (Figure 5).

In the same way, days to harvesting exhibited a negative correlation with head weight (−0.99), head diameter (−0.985), and head yield (−0.994) (Figure 5). Finally, head weight showed a positive correlation with head diameter (0.981) and head yield (0.997), whereas head diameter showed a negative correlation with head compactness (−0.965) and a positive correlation with head yield (0.972) (Figure 5).

4. Discussion

As can be seen in Figure 1, the study's findings confirm that inoculating cabbage plants with *Trichoderma viride* and *Pseudomonas fluorescens* resulted in significant improvements in seedling quality, plant growth, yield, and yield-related indicators. Our results corroborate the findings of earlier researchers, who found that different crops inoculated with bio-inoculants, such as *Pseudomonas*, *Azospirillum*, and *Azotobacter* strains, showed similar increases in root and shoot weight, stem diameter, and leaf area [27]. This could be ascribed to the fact that these bio-control agents increased the surface area of roots, thereby improving seedling emergence, reducing the number of days required to reach the 2-true-leaf stage, and significantly impacting seedling height, stem diameter, leaf area, root length, seedling vigour index, seedling fresh and dry weight, and total chlorophyll content of cabbage seedlings [28]. Despite all these seedling quality metrics, the number of days required to transplant the seedlings and the days required to establish cabbage seedlings were also lowered by the combined action of the bio-control agent and PGPRs (Plant Growth-Promoting Rhizobacteria) [29]. All of this is linked to the improvements in water and nutrient uptake and the mineral absorption efficiency, most notably for sluggish mineral ions including phosphate, resulting in enhanced plant development in response to all bio-stimulants and rhizosphere microorganisms compared with the control [30].

Trichoderma is a kind of fungi present in all types of soils. This may improve the seed germination rate, growth, and vigour traits of plants. The result was confirmed with the finding of Sarkar et al. [31], where the dual inoculation of *T. harzianum* and *P. fluorescens* had a positive impact on root length, as it increased the microbial activity in the soil. PGPRs boost plant growth and increase root biomass or root morphology in general [32]. Plant root exudates are an intricate type of natural acid ionic species, phytosiderophores, sugar content, vitamin supplements, essential nutrients, purines, nucleosides, inorganic salts (e.g., HCO_3^- , OH^- , H^+), gaseous molecules (CO_2 , H_2), enzymes, and root border cells that have considerable consequences on the mineral nutrient acquisition required for plant growth [33]. Faruk [34] elucidated that the application of biofungicides *T. harzianum* showed the seedling emergence, shoot and root length, and weight were improved. Further, the total chlorophyll content was increased in cabbage because the application of bio-fertilizers increases the content of nitrogen and phosphorus, which enhance the nutrient availability to the crops [35]. This increased amount of nitrogen in plants increased chlorophyll, coupled with increased net photosynthetic rates and carbohydrates content [36].

As per the findings of Marulanda et al. [37], these microbial treatments may also help to enhance the levels of IAA growth regulators, which also contribute to improvised seedling growth parameters, including root and shoot fresh weight and leaf area [38]. Further, the infusion of vermicompost with *T. harzianum* in cabbage greatly enhanced the

seedling's establishment rate [39]. In addition, the seedling height of the cabbage plant was significantly improved by incorporating the soil amendments and microbes [40]. Etesami and Maheshwari [41] previously revealed the positive influence of seed inoculation with rhizobacteria and biological control agents on the plant shoot's dry weight and yield of various crops. They also proposed that the probable reason for this enhancement might be related to bacteria's N₂-fixing and phosphate-solubilizing abilities, as well as its capacity to create growth-promoting chemicals such as IAA [42]. Therefore, capacity of PGPRs to fix nitrogen may have been the key factor impacting seedling development and improving yield performance of cabbage in this investigation [43].

In general, the seeds' and plant seedlings' root dip before inoculating with the bio-control agent and PGPRs enabled them to possess better seedling quality in terms of emergence, days to 2-true-leaf stage, seedling growth (height and diameter), crop leaf area, root length, seed vigour index, fresh and dry weight, chlorophyll content, and number of days taken to establish the seedlings [44]. For all this, the possible explanation could be better photosynthetic activity as well as better efficiency and better availability of photosynthates, which is directly associated with the chlorophyll content of the plant [45]. The improvement in seedling quality as well as plant growth and yield-performing characteristics after inoculation with *Trichoderma viride* and *Pseudomonas fluorescens* are supported by earlier researchers, who also worked on cauliflower [46]. The plant growth-promoting effects and yield enhancement of cabbage crops due to dual effect of PGPRs and bio-control agents on plants can be described in several different ways, including biological control and microbial activity implantation, physiological nitrogen metabolism, phosphorus emulsification, and/or endogenous hormone synthesis [47].

Further, the study also indicated a significant increase in the yield and yield-performing traits. Previous observations in cauliflower detailing an increase in numerous plant growth metrics with improved seedling quality corroborate our findings [48]. This may be explained by the fact that mycorrhizae symbiosis develops a better photosynthetic source for plants through increased leaf area, allowing the cabbage crop to produce a greater yield [46]. The increase in plant height and the head diameter was ascribed to the improved uptake of some nutrients such as boron through root colonization with bio-control agents and PGPRs [4]. The increase in total yield, as per our observations, might also have resulted from the improved root structure [49].

5. Conclusions

Our experiment revealed significant increases in seedling quality and production performance of cabbage after *T. viride* and *P. fluorescens* inoculation. Our findings confirm that seed treatment with 5% *T. viride* and seed treatment with 5% *P. fluorescens* before transplanting is efficient in improving seedling quality, including bio-mass and yield performance. The improvement in several metrics may be associated with greater nutrient availability due to the addition of a bio-control agent and PGPR with plant roots and improved root structures for greater water absorption. PGPR influences plant growth directly either by stimulating plant metabolism or by producing plant hormones such as auxins and gibberellins, solubilizing minerals and fixing atmospheric nitrogen, or indirectly by mitigating the negative effects of phytopathogenic microbes and improving stress tolerance. They also influence plant development by boosting nutrient availability and decreasing pathogenic infestation. Therefore, the application of *T. viride* and *P. fluorescens* as bio-control agents may be beneficial for obtaining higher crop yields in a sustainable manner to upgrade their seedling quality and yield-performing characteristics with low chemical input.

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