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RESEARCH ARTICLE

Endophytic seed cube technology for enhanced seed germination, crop establishment and soil microbial dynamics in ridge gourd (*Luffa acutangula*)

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Abstract

Ridge gourd (*Luffa acutangula*), which is often called a nutritional powerhouse, is a widely cultivated and valued crop across the globe. The use of synthetic chemicals in agriculture poses serious risks to both environment and human health, urging the need for eco-friendly techniques. Thus, providing an optimum growth medium enriched with beneficial organisms can enhance seedling establishment, microbial interactions and plant health sustainably. Therefore, this study aims to investigate the potential of endophyte enriched seed cubes to improve seedling vigour, microbial activity and crop establishment in ridge gourd. The seed cubes were enriched with different endophytes *viz., Bacillus subtilis, Bacillus licheniformis, Pseudomonas fluorescens, Pseudomonas putida, Trichoderma harzianum, Rhizobium japonicum* and *Bacillus megaterium* each at 2 %, 4 % and 6 % concentrations. Among the endophytes, it was found that *Rhizobium japonicum* 6 % significantly enhanced seed germination (95 %), root length (15.2 cm), shoot length (18.7 cm), dry matter (14.8 g/100 seedlings), seedling vigour (3221 & 14.1) and microbial load (35, 47 & 23 cfu/g) over dry seeds under shadenet condition. Similarly, field evaluation also confirmed that *R. japonicum* 6 % improved seedling growth, biomass and chlorophyll content over control plants. In addition, the enhanced soil dehydrogenase (66 %) and phosphatase (46 %) activities favour soil microbiome improvement, organic matter enrichment and nutrient cycling which is ultimately essential for crop establishment. Thus, the findings of this study highlight the potential of *Rhizobium japonicum* 6 % enriched seed cubes as an eco-friendly alternative to enhance ridge gourd cultivation with reduced environmental hazards. Furthermore, the feasibility of this technology across various crops offers a promising solution for enhancing crop productivity, while maintaining environmental sustainability.

Keywords: endophyte; ridge gourd; seed cube; seedling vigour; soil enzymatic activity; soil microbiome

Introduction

Antioxidants and minerals play a crucial role in human health by supporting various biological functions. An appropriate food practice can provide the necessary nutrients to meet daily dietary requirements. Ridge gourd (*Luffa acutangula*), often called as nutritional powerhouse, is a cucurbitaceous vegetable cultivated globally for its value as food, fibre and indigenous medicines. The chemical constituents of ridge gourd fruits include carbohydrates, carotene, vitamins, minerals, fat, protein, phytin, flavonoids, saponin and amino acids (1). It also exhibits several pharmacological properties like hepatoprotective, antidiabetic, antioxidant and antifungal activities, which has been involved in regulating digestion, supporting immune system and in treatment of ulcers and sores (2). Thus, from the overall nutritive view, ridge gourd is widely favoured as a valuable agricultural produce worldwide. On the other hand, the global population has already surpassed 8.1 billion and is expected to reach approximately 9.7 billion by 2050 (3). Billions of people in developing countries suffer from hidden hunger due to micronutrient deficiencies. Therefore, the only solution to bridge the gap between food production and consumption is maximising the crop yield and productivity amid diminishing agricultural land. The future of crop growth and productivity is mainly decided by good quality seeds, initial seedling establishment, subsequent crop growth and timely nutrient availability. To meet these demands, farmers rely mainly on synthetic agrochemicals, which were meant for their immediate benefits.

Although synthetic fertilizers have immediate nutrient benefits, their high solubility leads to rapid nutrient release and long-term accumulation of residues in the ecosystem (4).

Macro nutrients mainly nitrogen (N), phosphorus (P) and potassium (K) applied to the soil are lost at rates of 40-70 %, 80-90 % and 50-90 %, respectively due to various reasons, resulting in the reduced nutrient availability for crops (5). Excess nitrogen contributes to global warming (6), while phosphorus often gets fixed in soil by forming complexes with minerals like calcium, magnesium, aluminium, iron and zinc, makes the nutrients inaccessible to plants (7). Similarly, potassium is lost mainly through surface runoff and leaching (8). These synthetic agrochemicals also disrupt the normal soil ecosystem and its functions, which creates toxic effects on soil microbiome, degrade soil health and adversely affect crop productivity (9). Furthermore, these residues can enter into the food chain, posing serious health risks such as acute poisoning, cancer, neurological disorders and tumour associated diseases (10). Therefore, a sustainable and eco-friendly approach balancing the food production and demand with minimal risks to environment and human health has become the need of an hour.

Many eco-friendly techniques have started to revolutionise in the field of modern agriculture and one among them is the seed cube technology. Seed cube is a biodegradable, cubical shaped growth media enriched with soil, coirpith, sawdust, bonemeal, VAM fungi and vermicompost each in different proportions (11). Seed cubes provide a self-sustained microenvironment, ensuring the optimal delivery of nutrients and moisture during the early period of crop establishment which is very much essential for its further development. This nutrient enriched seed cubes can attract the beneficial microbes in the rhizosphere region, thereby supporting the initial crop growth through nutrient cycling. Recent studies have also demonstrated that, seedlings of *Albizia lebbeck* and *Thespesia populne* grown from seed cubes showed an improved emergence, seedling growth, dry weight and vigour compare to control seedlings (11, 12) .

Endophytes are plant associated microbes that establish a symbiotic relationship with the host plant by residing inside the plant. They offer wide range of benefits to plants and environment like enhanced plant growth, biotic and abiotic stress tolerance, improved soil fertility and soil microbiome and reduced chemical toxicity (13). Plant Growth Promoting Rhizobacteria (PGPR) with endophytic nature helps in enhancing plant growth and development by following various mechanisms such as nitrogen fixation, solubilisation of phosphorus, potassium and zinc, production of siderophores, ammonia and phytohormones. Additionally, they also produce various hydrolytic enzymes and bioactive compounds that support plant growth and protection (14). PGPR significantly enhanced the rapeseed seedling growth, biochemical activities and overall crop yield due to the enhanced photosynthetic activity and nutrient absorption (15). Similar improvement in germination and seedling vigour due to application of PGPR was also evidenced in carrot capsicum and wheat (16-18). *Trichoderma harzianum* was reported to activate jasmonic acid and ethylene pathways, thereby inducing systemic resistance and enhancing plant immunity through pathogenesis-related (PR) protein accumulation and lignification (19).

Having all these as a base, a hypothesis was framed that include beneficial endophytes as one of the components in biodegradable seed cube media to enhance the initial seedling establishment, soil microbiome and overall crop establishment with minimal environmental hazards. With this hypothesis, the present study was outlined to study the effect of endophyte fortified seed cubes on ridge gourd with the following objectives (i) screening of seed cubes fortified with endophytes on seedling growth and microbial activities under shade net condition; (ii) Field evaluation of endophyte fortified seed cubes on seedling growth, microbial and enzyme activities. Overall, this study will contribute for developing a sustainable and eco-friendly input in the form of endophyte fortified seed cubes for enhancing seedling establishment, soil health, crop growth and productivity in ridge gourd.

Materials and Methods

Experimental site

All experiments were conducted in the Department of Seed Science and Technology at Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India, situated at 11°N latitude and 77° E longitude. The experiment was conducted during January and February 2025 and the weather prevailed during the study period was given in Table 1.

Preparation of beneficial endophyte fortified seed cubes

The seed cubes were prepared using a seed cube making machine (Design patent no: 400717-001) by incorporating all the media components in their respective proportions as standardised by Jawahar and Umarani (2019) along with different beneficial endophytic microbes (11). The microbes include *Bacillus subtilis*, *Bacillus licheniformis, Pseudomonas fluorescens, Pseudomonas putida, Trichoderma harzianum, Rhizobium japonicum* and *Bacillus megaterium,* each added at 2 %, 4 % and 6 % concentrations. A single ridge gourd (COH 1) seed was placed in each cube. Then, the seed cubes were subjected to shade drying, followed by sun drying until they reached a moisture content of 5.8 %, while the embedded seeds retained 9.0 % moisture content.

Table 1. Weather parameters prevailed during January and February 25

Date	Maximum temperature (°C)	Minimum temperature (°C)	Rainfall (mm)
1st week-Jan-2025	29.2	18.8	0
2nd week-Jan-2025	28.9	21.1	0
3rd week-Jan-2025	28.7	20.1	0
4th week-Jan-2025	31.4	19.8	0
1st week-Feb-2025	32.2	18.8	0
2nd week-Feb-2025	32.2	18.1	0
3rd week-Feb-2025	33.6	18.4	0
4th week-Feb-2025	33.6	20.5	0

Evaluation of endophyte fortified seed cubes on seedling vigour and microbial population in ridge gourd (COH 1) under shade net condition

Ridge gourd seed cubes fortified with different endophytes were evaluated under shade net conditions along with control (seed cube without endophytes) and an absolute control (dry seeds). The experiment was employed with randomized block design with four replications, each consisting of 25 seed cubes.

The nursery beds were maintained with adequate moistening for two weeks and final observations were recorded on the 14^{th} day after sowing (DAS). Speed of emergence was calculated based on the number of seedlings emerge daily (20). The number of normal seedlings emerged at 14^{th} day were counted and represented as germination percentage (21). Seedling length was measured using measuring scale and expressed in centimetre (cm). For root length it was measured from the point of attachment of seed to the tip of primary root and for shoot length it was measured from point of attachment of seed to the tip of the primary leaf. Similarly, seedling dry matter production (g/100 seedlings) and vigour index were also recorded (22). One day after sowing, the microbial population in the spermosphere region was assessed in the media adhering to the seeds. While at 14 DAS, the microbial population in rhizosphere and phyllosphere regions were evaluated in the soil adhering to the roots and leaf samples respectively. Then the microbial populations in the spermosphere, rhizosphere and phyllosphere regions were quantified using serial dilution and plating techniques and expressed as colony forming units (CFUs) per gram of sample (23).

Evaluation of seed cubes fortified with endophytes on physiological, microbial and enzyme activities in ridge gourd (COH 1) under field condition

The most effective endophytic seed cube combinations from initial screening namely *Pseudomonas putida* 6 %, *Rhizobium japonicum* 6 % and *Bacillus megaterium* 4 % were forwarded to field evaluation. These treatments along with control (seed cube without endophytes) and absolute control (dry seeds) were sown in the field following randomized block design with four replications.

Observations on speed of emergence, field emergence percentage, plant height (cm) and seedling fresh and dry weight (mg/seedling) were recorded for assessing the growth status of the plants. Likewise, the physiological status of the plants was assessed using the following parameters. The chlorophyll content of the plants was measured using SPAD (Soil Plant Analysis Development) photometer (Model Minotta SPAD 502) and plant vigour was assessed using a GreenSeeker instrument that uses Normalized Difference Vegetation Index (NDVI) principle. The microbial load in the spermosphere, rhizosphere and phyllosphere regions were also quantified. The quantity of root dehydrogenase activity was assessed using the method described by (24). Additionally, two key soil enzymes *viz.,* soil dehydrogenase (25) and soil alkaline phosphatase (26) were also ascertained. All the above observations were recorded on 14^{th} DAS.

Statistical analysis

All statistical analyses were performed using R software version 4.3.2 and analysis of variance (ANOVA) was conducted at a significance level of $P < 0.05$. Least Significant Difference (LSD) test and Duncan's Multiple Range Test (DMRT) were conducted to confirm the significant differences between treatments (27, 28) . The data represented in the table are mean values. Within each column, different letters at each treatment indicate significant differences by DMRT at $P<0.05$ and the non-significant treatments were not grouped with letters. Origin Ver.8.5 software and Microsoft Excel were used to prepare the graphical representations and Principal Component Analysis (PCA).

Results and Discussion

The study revealed a significant increase in germination, seedling vigour, soil microbiome and soil enzyme activity due to *Rhizobium japonicum* 6 % fortified seed cube in ridge gourd. Each component in seed cube media plays a specific role in crop establishment and growth *viz.,* soil as a medium for plant growth and root anchorage; coirpith for more water retention and providing aeration to root surface; sawdust for improving soil porosity and root penetration by reducing soil bulk density; bonemeal for nutrient supply (29-32). Vesicular arbuscular mycorrhiza (VAM) fungi for enhancing plant nutrient uptake, improving soil properties and plant defence mechanisms and vermicompost as nutrient supplementation and boosting soil health (33, 34). Thus, seed cubes provide a self-sustained microenvironment, which ensure optimal and timely delivery of nutrients and moisture during the early crop development period.

PGPR was found to have more influence on seed physiology, plant growth and yield. Embedding the seeds in microbial media helps in softening the seed coat by providing consistent moisture and facilitates early emergence and good seedling growth. The results of initial shade net screening revealed that seed cubes fortified with endophytes significantly improved the speed of emergence, germination percent, seedling growth, seedling vigour and microbial population of ridge gourd compared to dry seeds and seed cubes without endophytes. Among the treatments, *Rhizobium japonicum* 6 % (T_{20}) showed the highest emergence speed (3.6) and germination percent (95 %). In contrast, the lowest speed of emergence and germination percent (60 %) was recorded in dry seeds (Fig. 1) (2). PGPR enhances the activity of hydrolytic enzymes such as amylase, protease and cellulase which play a crucial role in seed germination and early seedling vigour. Among these, IAA, the active form of auxin produced by most of the PGPR plays a vital role in shoot elongation, stimulation of cell division and initiation of lateral roots (35). Furthermore, *Rhizobium japonicum* 6 % fortified cubes also produced seedlings with highest root length (15.2 cm), shoot length (18.7 cm), dry matter production (14.8 g/100 seedlings) and vigour index (3221 & 14.1), followed by *Pseudomonas putida* 6 % and *Bacillus megaterium* 4 % (Fig. 2, 3). This is mainly due to the synthesis of phytohormones like auxin, siderophore and exopolysaccharides which intervenes with different mechanisms that regulate nutrient uptake efficiency and food translocation within the plant (36). Similar improvement in germination and plant growth was also observed in bottle gourd, sweet corn and lettuce and carrot due to the application of *Trichoderma harzianum*, *Bacillus subtilis* and *Rhizobium laguerreae* respectively (37-39).

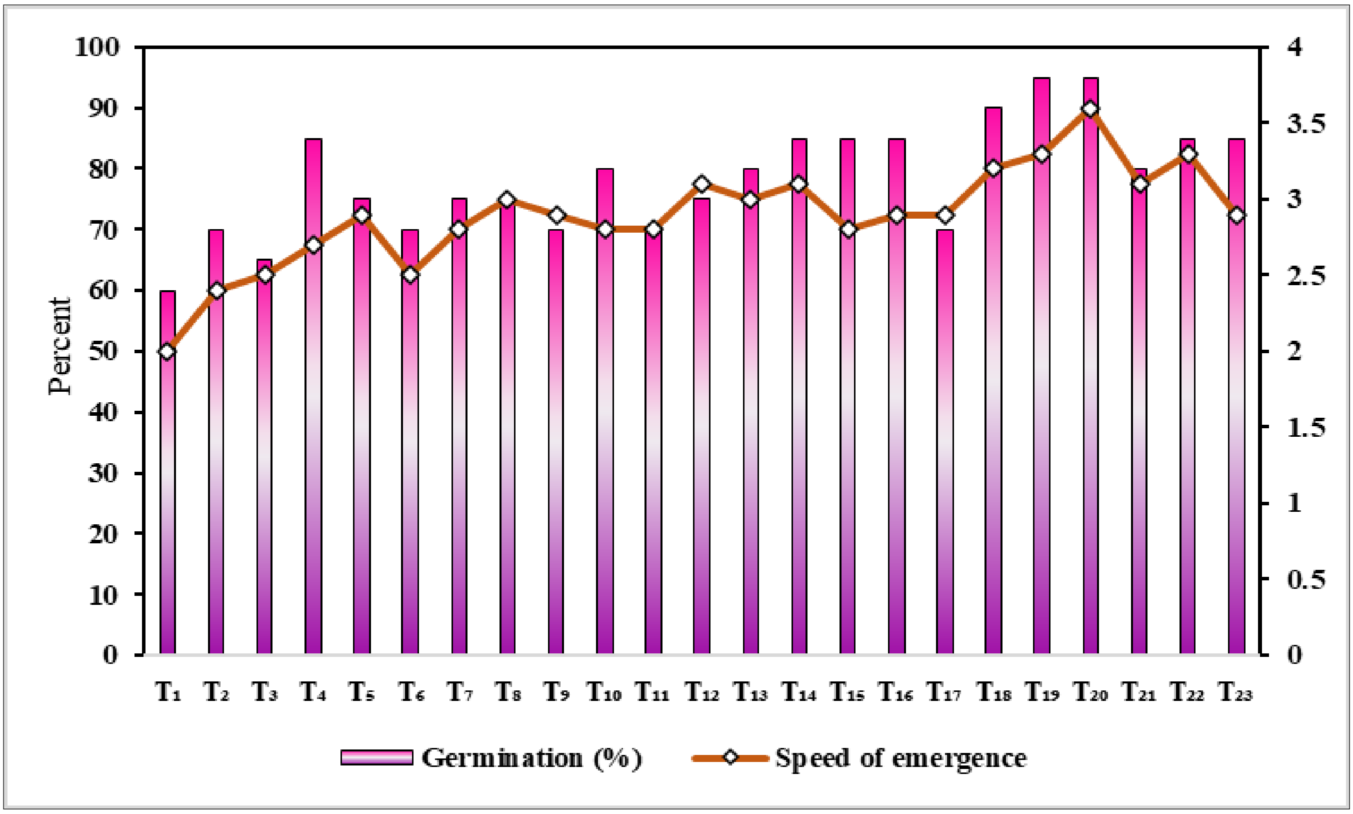

Fig. 1. Effect of endophyte fortified seed cubes on speed of emergence and germination in ridge gourd (COH 1) under shadenet condition.

T_1 - Dry seed (Absolute control), T_2 - Seed cube (Control), T_3 - *Bacillus subtilis* 2 %, T_4 - *B. subtilis* 4 %, T_5 - *B. subtilis* 6 %, T_6 - *Bacillus licheniformis* 2 %, T_7 - *B. licheniformis* 4 %, T_8 - *B. licheniformis* 6 %, T_9 - *Pseudomonas fluorescens* 2 %, T_{10} - *P. fluorescens* 4 %, T_{11} - *P. fluorescens* 6 %, T_{12} - *Pseudomonas putida* 2 %, T_{13} - *P. putida* 4 %, T_{14} - *P. putida* 6 %, T_{15} - *Trichoderma harzianum* 2 %, T_{16} - *T. harzianum* 4 %, T_{17} - *T. harzianum* 6 %, T_{18} - *Rhizobium japonicum* 2 %, T_{19} - *R. japonicum* 4 %, T_{20} - *R. japonicum* 6 %, T_{21} - *Bacillus megaterium* 2 %, T_{22} - *B. megaterium* 4 %, T_{23} - *B. megaterium* 6 %

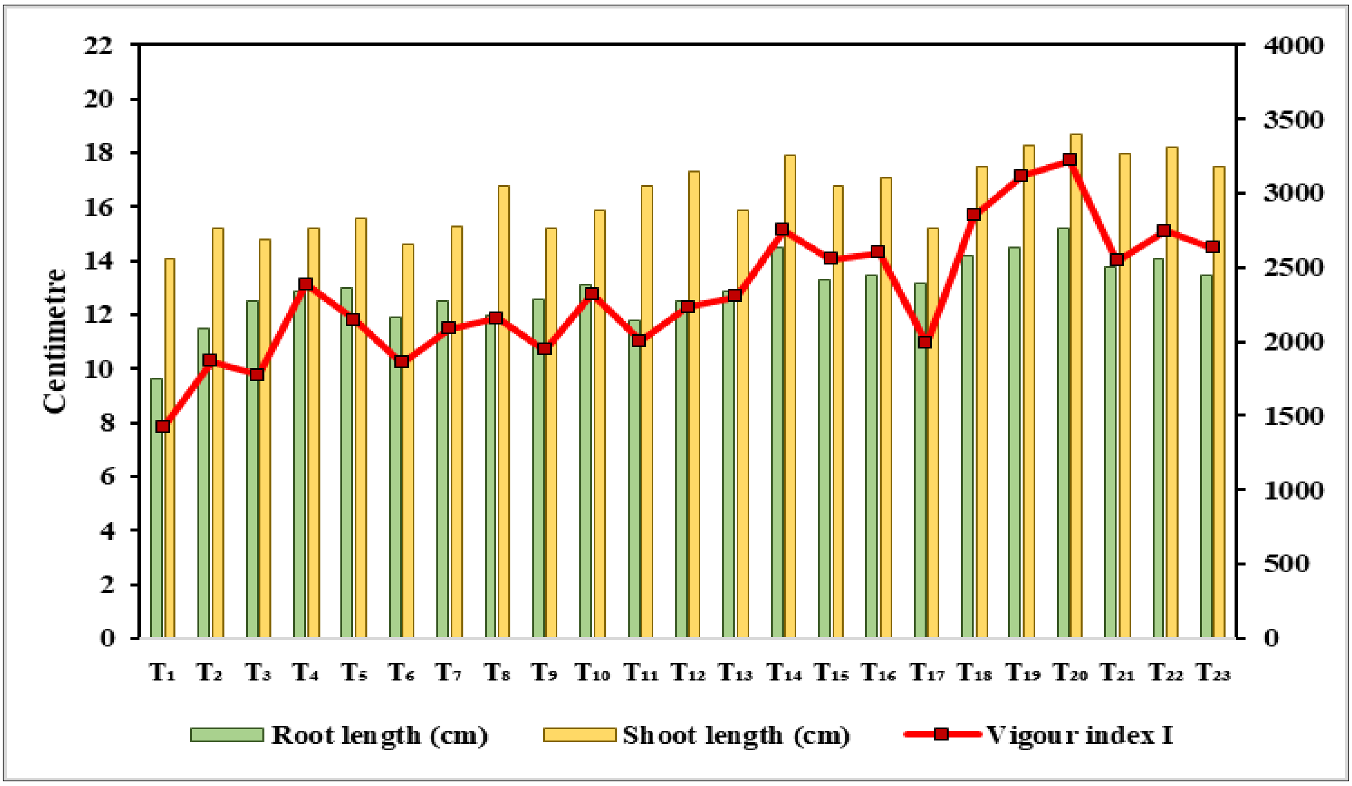

Fig. 2. Effect of endophyte fortified seed cubes on root length (cm), shoot length (cm) and vigour index I in ridge gourd (COH 1) under shade net condition.

T_1 - Dry seed (Absolute control), T_2 - Seed cube (Control), T_3 - *Bacillus subtilis* 2 %, T_4 - *B. subtilis* 4 %, T_5 - *B. subtilis* 6 %, T_6 - *Bacillus licheniformis* 2 %, T_7 - *B. licheniformis* 4 %, T_8 - *B. licheniformis* 6 %, T_9 - *Pseudomonas fluorescens* 2 %, T_{10} - *P. fluorescens* 4 %, T_{11} - *P. fluorescens* 6 %, T_{12} - *Pseudomonas putida* 2 %, T_{13} - *P. putida* 4 %, T_{14} - *P. putida* 6 %, T_{15} - *Trichoderma harzianum* 2 %, T_{16} - *T. harzianum* 4 %, T_{17} - *T. harzianum* 6 %, T_{18} - *Rhizobium japonicum* 2 %, T_{19} - *R. japonicum* 4 %, T_{20} - *R. japonicum* 6 %, T_{21} - *Bacillus megaterium* 2 %, T_{22} - *B. megaterium* 4 %, T_{23} - *B. megaterium* 6 %

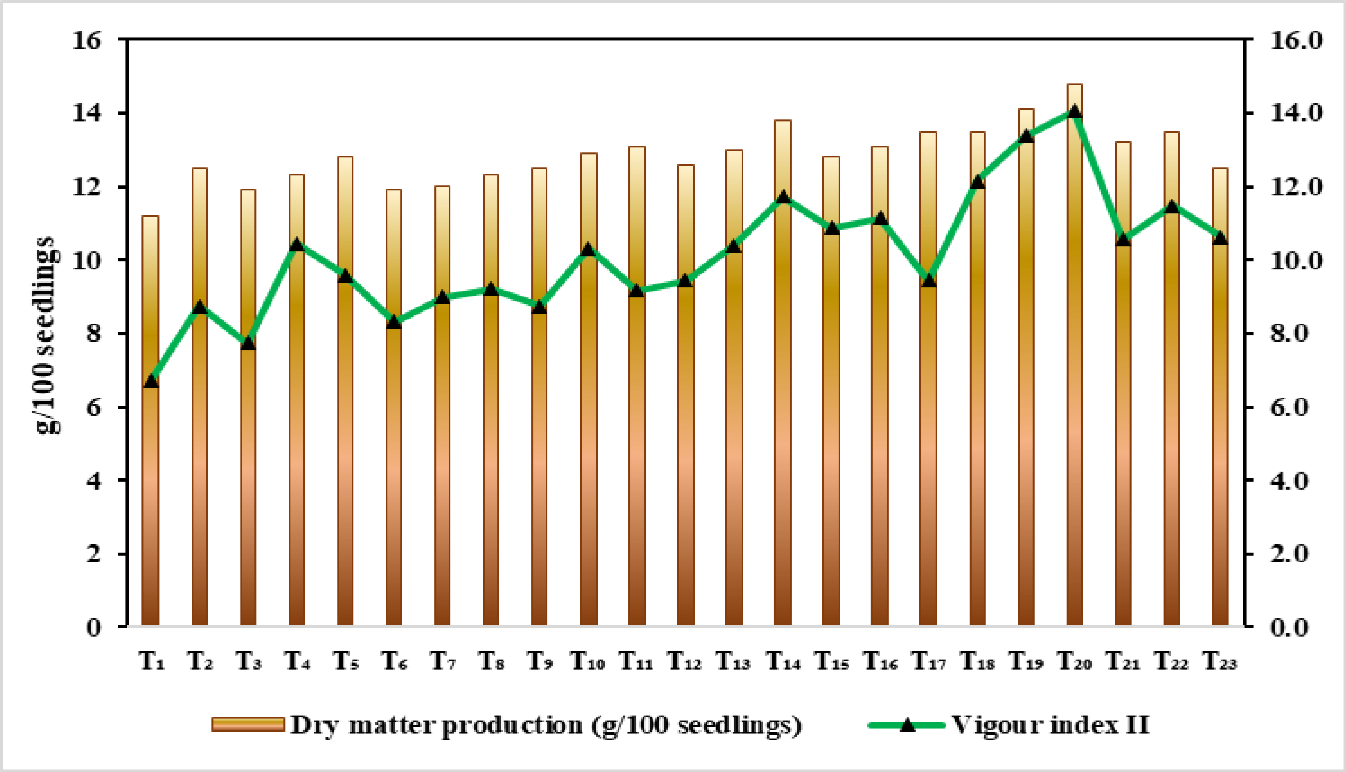

Fig. 3. Effect of endophyte fortified seed cubes on dry matter production (g/100 seedlings) and vigour index II in ridge gourd (COH 1) under shadenet condition.

T_1 - Dry seed (Absolute control), T_2 - Seed cube (Control), T_3 - *Bacillus subtilis* 2 %, T_4 - *B. subtilis* 4 %, T_5 - *B. subtilis* 6 %, T_6 - *Bacillus licheniformis* 2 %, T_7 - *B. licheniformis* 4 %, T_8 - *B. licheniformis* 6 %, T_9 - *Pseudomonas fluorescens* 2 %, T_{10} - *P. fluorescens* 4 %, T_{11} - *P. fluorescens* 6 %, T_{12} - *Pseudomonas putida* 2 %, T_{13} - *P. putida* 4 %, T_{14} - *P. putida* 6 %, T_{15} - *Trichoderma harzianum* 2 %, T_{16} - *T. harzianum* 4 %, T_{17} - *T. harzianum* 6 %, T_{18} - *Rhizobium japonicum* 2 %, T_{19} - *R. japonicum* 4 %, T_{20} - *R. japonicum* 6 %, T_{21} - *Bacillus megaterium* 2 %, T_{22} - *B. megaterium* 4 %, T_{23} - *B. megaterium* 6 %

The application of PGPR enhances the soil organic matter decomposition, microbial synergy and results in better plant establishment. This aligns well with our results, in which the spermosphere microbial population at 1 DAS and rhizosphere microbial population at 14 DAS were also found to be highest in *Rhizobium japonicum* 6 %, followed by *Pseudomonas putida* 6 % and *Bacillus megaterium* 4 %, compared to control. The highest spermosphere (35 cfu/g) and rhizosphere microbial population (47 cfu/g) might have contributed to a significantly higher phyllosphere microbial population of 23 cfu/g in *Rhizobium japonicum* 6 %, compared to dry seeds (12 cfu/g) and control seed cubes (15 cfu/g) (Fig. 4). When microbe enriched seeds come into contact with the rhizosphere soil, they form a mutual association with beneficial microbes that already exist near the rhizosphere region (40). Moreover, the microbial colonization on seed surfaces and root tissues by PGPR promotes the development of systemic resistance in plants, which helps in protecting the germinating seeds and young seedlings from adverse environmental conditions and pathogenic attacks (41).

The data obtained during field evaluation also showed that seed cubes fortified with *Rhizobium japonicum* 6 % exhibited higher field emergence and speed of emergence compared to dry seeds and control seed cubes. It was found that the modulation of hormone signaling pathways is a key mechanism through which IAA producing rhizobacteria enhance seed germination by interacting with other plant hormones such as abscisic acid (ABA) and gibberellins (GAs) (42). At 14 DAS, plants from *Rhizobium japonicum* 6 % fortified seed cubes recorded the highest plant height (17.3 cm), which was 28 % higher than dry seeds (13.5 cm). The main mechanisms that are directly involved in promoting the plant growth include nitrogen fixation, production of phytohormones, solubilization of potassium and phosphate and synthesis of enzymes that regulate ethylene levels (43). Chlorophyll content serves as a key indicator of plant vigour, as it is directly linked to the plant's photosynthetic activity. It also helps in improving the electron transporters and uptake of magnesium which is much needed for chlorophyll synthesis and thus increasing the photosynthetic activity of plants (44). In our study, the *Rhizobium japonicum* 6 % fortified seed cubes resulted in a significantly higher chlorophyll index (28.3) and plant vigour (0.42), compared to dry seeds (21.6 & 0.30) and control seed cubes (23.1 & 0.33), respectively (Table 2). Additionally, the seedling weight parameters namely shoot fresh weight, shoot dry weight, root fresh weight and root dry weight were also found to be highest in *Rhizobium japonicum* 6 % followed by *Pseudomonas putida* 6 % (Fig. 5). Consistently on the 14th day, *Rhizobium japonicum* 6 % maintained the highest values of 33, 43 & 19 nos. for microbial population in spermosphere, rhizosphere and phyllosphere region compared to other treatments (Fig. 6). It was also found that when PGPR comes in contact with soil, it promotes organic acid production, lowers the soil pH and enhances the growth of beneficial microorganisms (45). Similar reports of enhanced microbial activity were also reported in rice and *Pisum sativum* (46, 47). The exopolysaccharide produced by PGPR facilitates the effective utilization of root exudate carbon during colonization, which enriches the soil carbon and nutrients reservoir, enhances nutrient availability and positively influences the subsequent crop growth through plant-soil feedback (48).

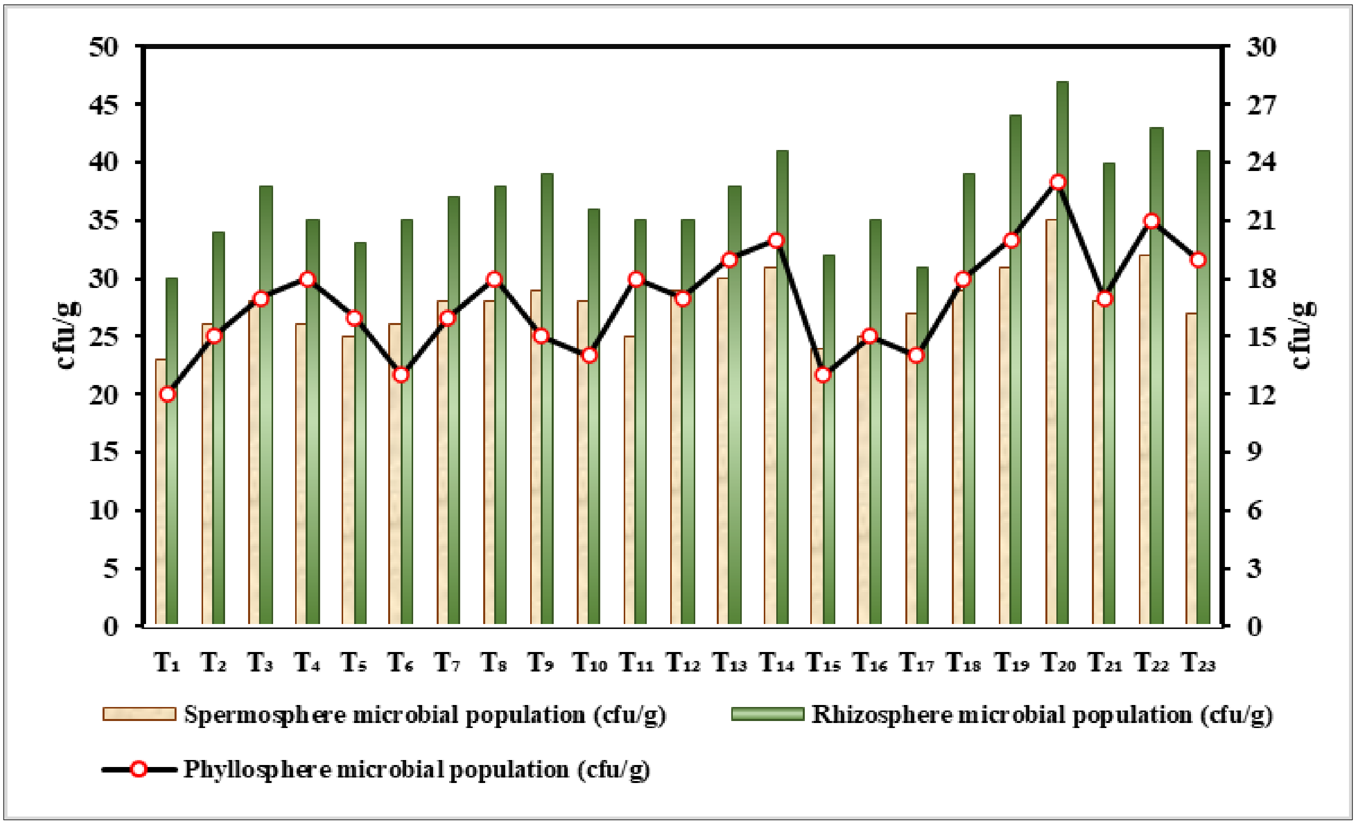

Fig. 4. Effect of endophyte fortified seed cubes on spermosphere, rhizosphere and phyllosphere microbial activity (cfu/g) in ridge gourd (COH 1) under shadenet condition.

T_1 - Dry seed (Absolute control), T_2 - Seed cube (Control), T_3 - *Bacillus subtilis* 2 %, T_4 - *B. subtilis* 4 %, T_5 - *B. subtilis* 6 %, T_6 - *Bacillus licheniformis* 2 %, T_7 - *B. licheniformis* 4 %, T_8 - *B. licheniformis* 6 %, T_9 - *Pseudomonas fluorescens* 2 %, T_{10} - *P. fluorescens* 4 %, T_{11} - *P. fluorescens* 6 %, T_{12} - *Pseudomonas putida* 2 %, T_{13} - *P. putida* 4 %, T_{14} - *P. putida* 6 %, T_{15} - *Trichoderma harzianum* 2 %, T_{16} - *T. harzianum* 4 %, T_{17} - *T. harzianum* 6 %, T_{18} - *Rhizobium japonicum* 2 %, T_{19} - *R. japonicum* 4 %, T_{20} - *R. japonicum* 6 %, T_{21} - *Bacillus megaterium* 2 %, T_{22} - *B. megaterium* 4 %, T_{23} - *B. megaterium* 6 %

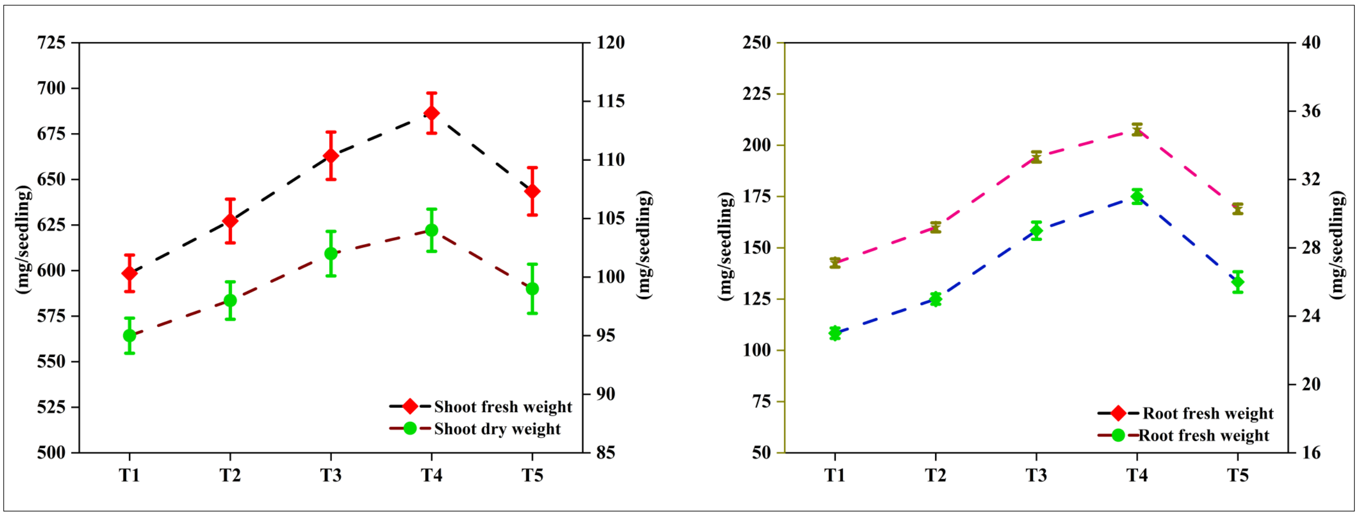

Fig. 5. Effect of endophyte fortified seed cubes on (a) shoot fresh and dry weight (mg/seedling) and (b) root fresh and dry weight (mg/seedling) in ridge gourd (COH 1) under field condition.

T_1 - Dry seed (Absolute control), T_2 - Seed cube (Control), T_3 - *Pseudomonas putida* 6 %, T_4 - *Rhizobium japonicum* 6 %, T_5 - *Bacillus megaterium* 4 %

Table 2. Effect of endophyte fortified seed cubes on field emergence (%), plant height (cm) and vigour in ridge gourd (COH 1) under field condition

Treatments	Speed of emergence	Field emergence (%)	Plant height (cm)	Chlorophyll index	Plant vigour
T_1	1.30 ± 0.02^{d}	$70\ (56.79 \pm 0.82)^{d}$	13.5 ± 0.19^{d}	21.6 ± 0.31^{e}	0.30 ± 0.004^{d}
T_2	1.59 ± 0.02^{c}	$78\ (62.03 \pm 0.69)^{c}$	14.2 ± 0.16^{d}	23.1 ± 0.26^{d}	0.33 ± 0.004^{c}
T_3	1.71 ± 0.04^{b}	$85\ (67.22 \pm 1.43)^{b}$	16.5 ± 0.25^{b}	26.7 ± 0.40^{b}	0.36 ± 0.005^{b}
T_4	1.79 ± 0.03^{a}	$93\ (74.66 \pm 1.33)^{a}$	17.3 ± 0.31^{a}	28.3 ± 0.50^{a}	0.42 ± 0.007^{a}
T_5	1.63 ± 0.02^{bc}	$80\ (63.44 \pm 0.94)^{c}$	15.4 ± 0.33^{c}	25.4 ± 0.54^{c}	0.38 ± 0.008^{b}

Mean values presented in the table with similar superscripts in the column were not significantly different at 5 % probability level

T_1 - Dry seed (Absolute control), T_2 - Seed cube (Control), T_3 - *Pseudomonas putida* 6 %, T_4 - *Rhizobium japonicum* 6 %, T_5 - *Bacillus megaterium* 4 %

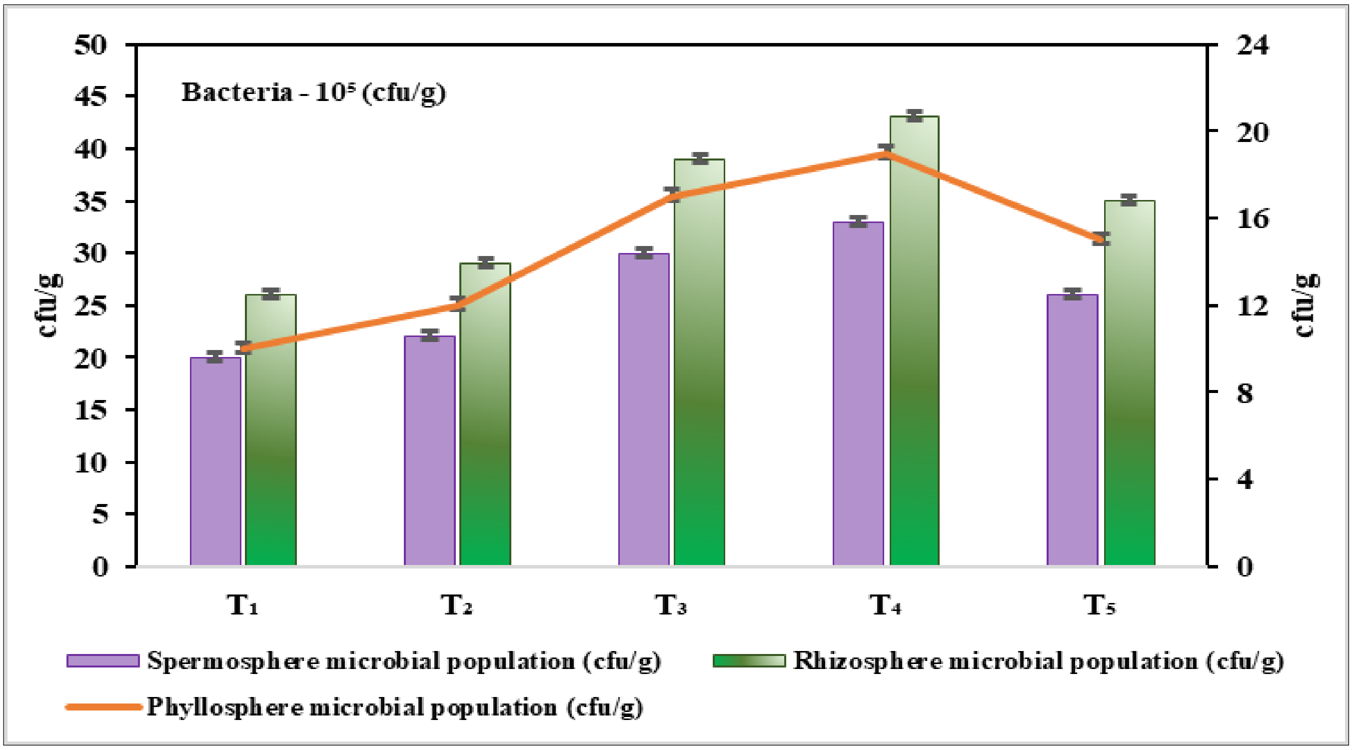

Fig. 6. Effect of endophyte fortified seed cubes on spermosphere, rhizosphere and phyllosphere microbial activity (cfu/g) in ridge gourd (COH 1) under field condition.

T_1 - Dry seed (Absolute control), T_2 - Seed cube (Control), T_3 - *Pseudomonas putida* 6 %, T_4 - *Rhizobium japonicum* 6 %, T_5 - *Bacillus megaterium* 4 %

Soil enzyme activity is essential for the decomposition of organic matter and nutrient cycling, with dehydrogenase and phosphatase activity serving as a general indicator of microbial activity and organic matter decomposition (49). Our study has also proved that the endophyte fortified seed cubes has enhanced the soil enzymatic activity. Among the different treatments, *Rhizobium japonicum* 6 % fortified seed cubes showed a significant difference for root dehydrogenase, soil dehydrogenase and soil alkaline phosphatase activities. The highest root dehydrogenase activity (25.8 µg TTC reduction root^{-1} g h) observed in *Rhizobium japonicum* 6 % seed cubes was found to be 56 and 31 % higher than that of dry seeds and control seed cubes, respectively (Fig. 7). Similarly, at 14 DAS, *Rhizobium japonicum* 6 % recorded the highest soil dehydrogenase activity (25.4 µg TTC reduction g^{1} soil hr^{-1}), which was followed by *Pseudomonas putida* 6 % (22.9 µg), while the lowest value of 15.3 µg TTC reduction g^{1} soil hr^{-1} was noted in dry seeds. Regarding soil alkaline phosphatase activity, an increase of 46 and 26 % was found in *Rhizobium japonicum* 6 % fortified seed cubes compared to dry seeds and control seed cubes, respectively (Fig. 8). This is mainly due to the mechanism that endophytic microbes are known to exhibit 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity, which helps host plants tolerate stressful environmental conditions (50). Thus, altogether endophytic seed cubes make the nutrients more available to the plants by nutrient cycling and enhances the plant growth which was evident from the present study (Fig. 9).

The PCA biplot depicted in Fig. 10. illustrates the relationship between different treatments (T_1-T_{23}) and seedling growth parameters. The treatments such as T_{20}, T_{18}, T_{19}, T_{21} and T_{22} demonstrated strong associations with key growth parameters namely germination, seedling length, seedling vigour and dry matter production and can be considered the most effective. In contrast, T_1, T_2, T_3, T_4 and T_6 were the least effective in promoting germination and seedling vigour, as indicated by their distance and direction from the growth-related vectors. This indicates the significance of PGPR in promoting the overall seedling establishment. In addition, the correlation matrix also further established the strong positive correlations between crop growth parameters, photosynthetic activity and enzymatic traits, thereby confirming the holistic role of PGPR in enhancing crop performance (Fig. 11).

Overall, it could be confirmed that ridge gourd seeds embedded in seed cubes fortified with *Rhizobium japonicum* 6 % enhanced the seed germination, soil microbiome and early seedling vigour with positive plant-soil feedback in a sustainable way.

Conclusion

In conclusion, incorporating *Rhizobium japonicum* at a 6 % concentration into biodegradable seed cubes will offer an eco-friendly and sustainable approach for enhancing ridge gourd cultivation. *Rhizobium japonicum* 6 % enriched seed cubes (T_4) can facilitate early crop establishment through enhanced seed germination, photosynthetic activity and root and soil enzyme levels, with minimal external inputs. Additionally, the increased microbial population in the spermosphere, rhizosphere and phyllosphere regions will positively help in supporting soil health and long-term agricultural productivity. Thus, these findings highlight the potential of organic and biodegradable inputs to reduce the reliance on synthetic inputs and ensure sustainable food production. Future research in this area could be focused on assessing the feasibility of this technology across various crops and agro climatic conditions, as well as its long-term influence on soil health and microbial community.

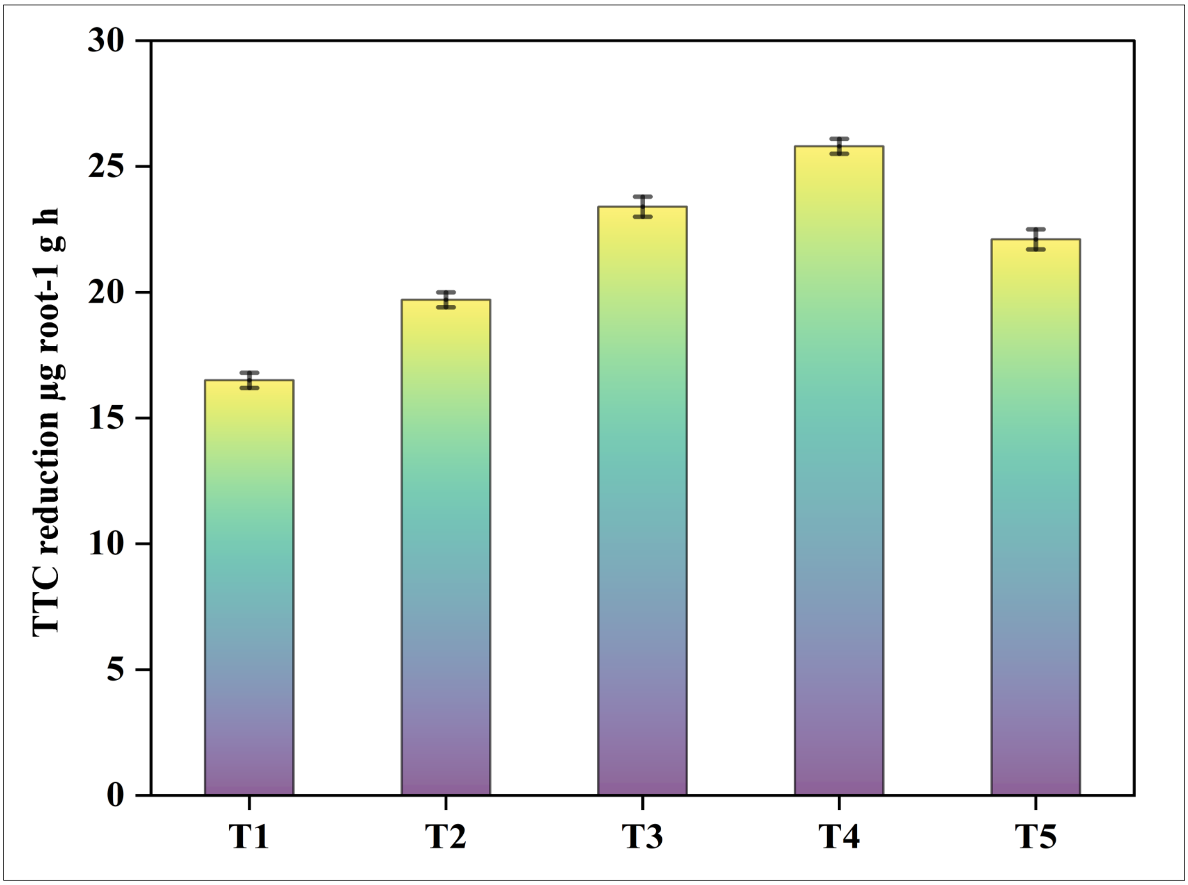

Fig. 7. Effect of endophyte fortified seed cubes on root dehydrogenase activity (TTC reduction µg root^{-1} g h) in ridge gourd (COH 1) under field condition.

T_1 - Dry seed (Absolute control), T_2 - Seed cube (Control), T_3 - *Pseudomonas putida* 6 %, T_4 - *Rhizobium japonicum* 6 %, T_5 - *Bacillus megaterium* 4 %

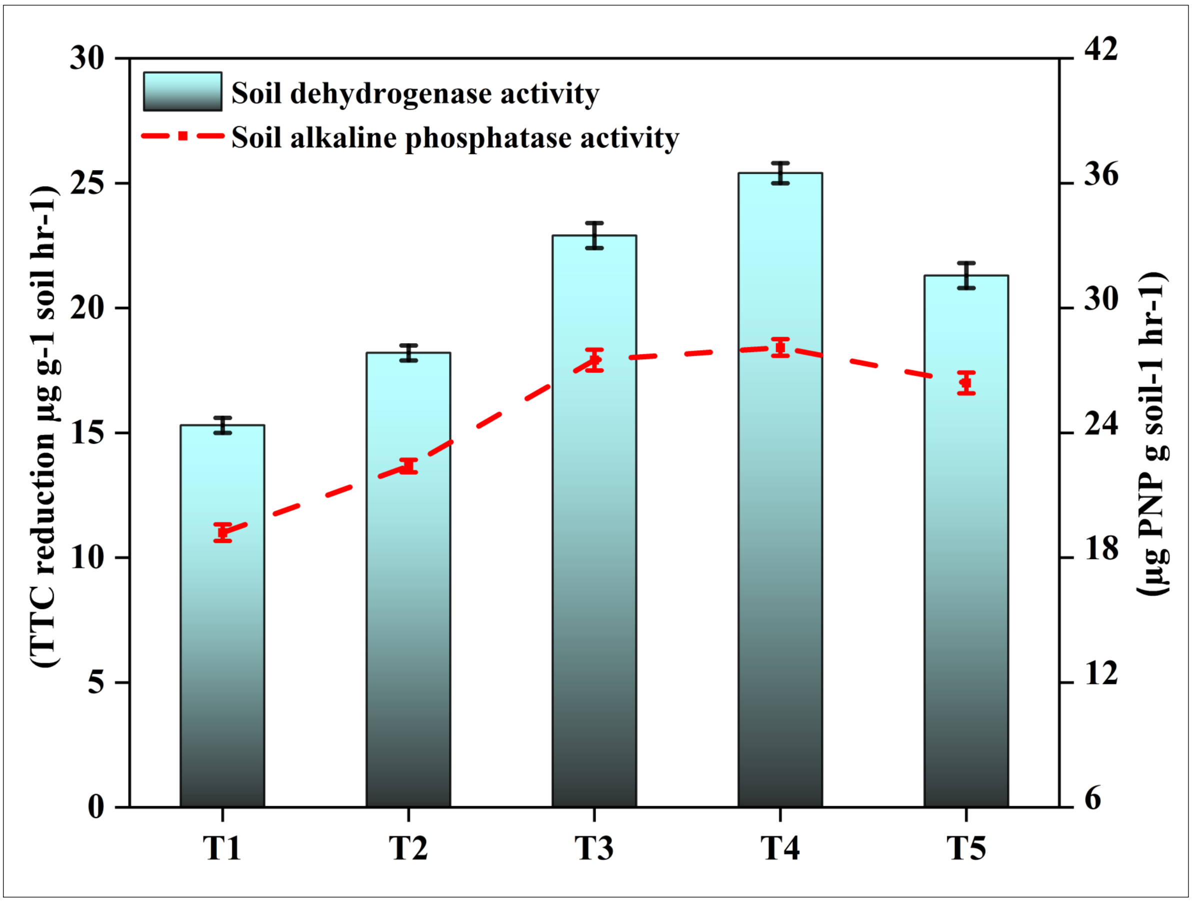

Fig. 8. Effect of endophyte fortified seed cubes on soil dehydrogenase activity (TTC reduction µg g^{-1} soil hr^{-1}) and soil alkaline phosphatase activity (µg PNP g soil^{-1} hr^{-1}) in ridge gourd (COH 1) under field condition.

T_1 - Dry seed (Absolute control), T_2 - Seed cube (Control), T_3 - *Pseudomonas putida* 6 %, T_4 - *Rhizobium japonicum* 6 %, T_5 - *Bacillus megaterium* 4 %

(a) Dry seed

(b) *Rhizobium japonicum* 6 %

Fig. 9. Effect of endophyte fortified seed cubes on seedling growth and root architecture in ridge gourd (COH 1) seedlings under shadenet condition.

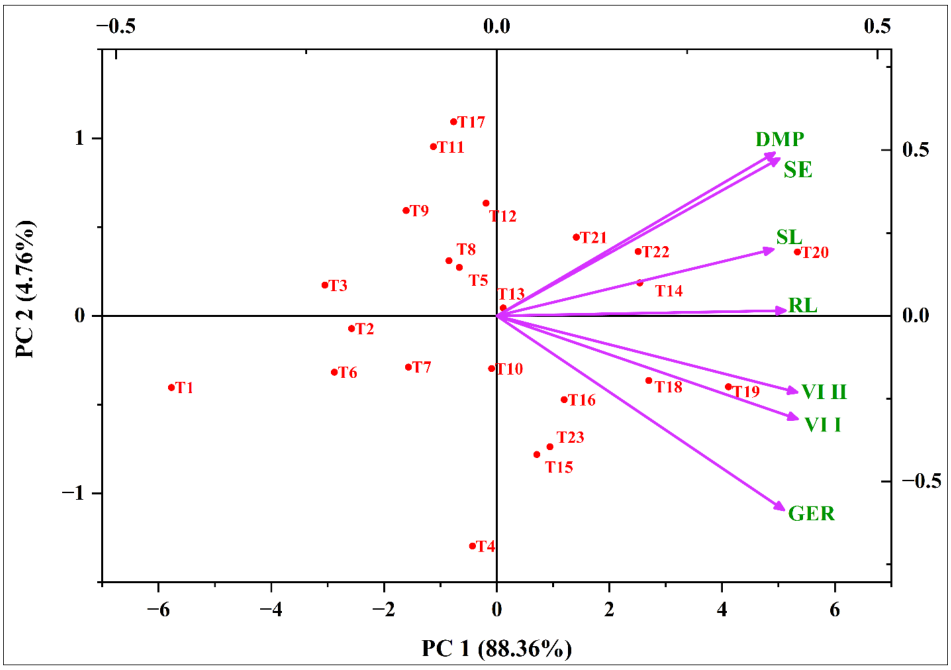

Fig. 10. PCA - biplot of endophyte fortified seed cubes on seedling vigour and other physiological parameters in ridge gourd (COH 1) under shadenet condition.

T_1 - Dry seed (Absolute control), T_2 - Seed cube (Control), T_3 - *Bacillus subtilis* 2 %, T_4 - *B. subtilis* 4 %, T_5 - *B. subtilis* 6 %, T_6 - *Bacillus licheniformis* 2 %, T_7 - *B. licheniformis* 4 %, T_8 - *B. licheniformis* 6 %, T_9 - *Pseudomonas fluorescens* 2 %, T_{10} - *P. fluorescens* 4 %, T_{11} - *P. fluorescens* 6 %, T_{12} - *Pseudomonas putida* 2 %, T_{13} - *P. putida* 4 %, T_{14} - *P. putida* 6 %, T_{15} - *Trichoderma harzianum* 2 %, T_{16} - *T. harzianum* 4 %, T_{17} - *T. harzianum* 6 %, T_{18} - *Rhizobium japonicum* 2 %, T_{19} - *R. japonicum* 4 %, T_{20} - *R. japonicum* 6 %, T_{21} - *Bacillus megaterium* 2 %, T_{22} - *B. megaterium* 4 %, T_{23} - *B. megaterium* 6 %

SE - Speed of emergence, GER - Germination (%), RL - Root length, SL - Shoot length, DMP - Dry matter production, VI I - Vigour index I, VI II - Vigour index II

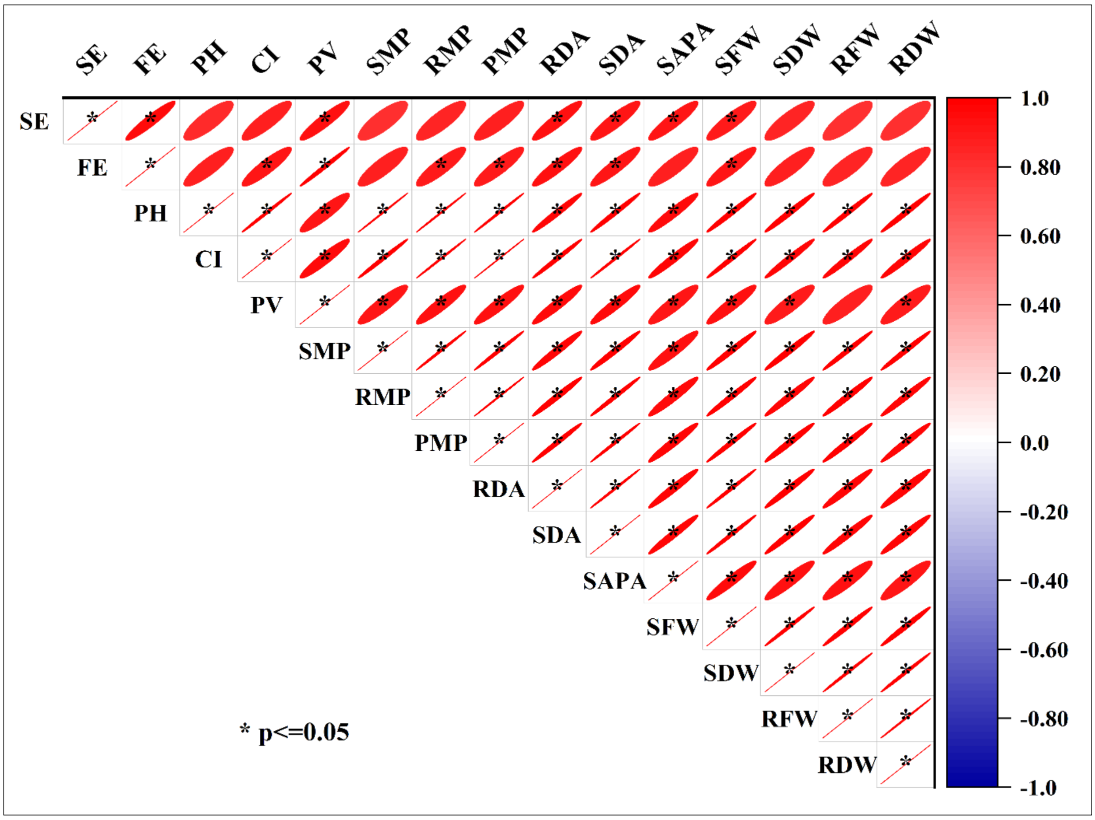

Fig. 11. Triangular correlation matrix depicting the relationship between different physiological, microbial and enzymatic parameters under field condition.

SE - Speed of emergence, FE - Field emergence, PH - Plant height, CI - Chlorophyll index, PV - Plant vigour, SMP - Spermosphere microbial population, RMP - Rhizosphere microbial population, PMP - Phyllosphere microbial population, RDA - Root dehydrogenase activity, SDA - Soil dehydrogenase activity, SAPA - Soil alkaline phosphatase activity, SFW - Shoot fresh weight, SDW - Shoot dry weight, RFW - Root fresh weight, RDW - Root dry weight

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Authors' contributions

The research article conceptualized by RU and GJ was written by GJ under the supervision of RU, reviewed by KNN, TA, CI and MD and edited by RU and GJ.

Compliance with ethical standards

Conflict of interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical issues: None

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this manuscript, the author G Jagadeeshkumar used QuillBot paraphrasing tool for language editing. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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