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Integrative approaches to improve litchi (*Litchi chinensis* Sonn.) plant health using bio-transformations and entomopathogenic fungi

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Abstract

Bio-transformations refer to the chemical modifications made by an organism on a chemical compound that often involves the interaction of plants with microbes to alter the chemical composition of soil or plant. Integrating bio-transformations and entomopathogenic fungi into litchi cultivation can enhance symbiotic relationships, microbial enzymatic activity in rhizosphere, disease suppression and promote overall plant health. The integration of biological formulations and entomopathogenic fungi can significantly influence growth, nutrient dynamics, physiology, and rhizosphere microbiome of air-layered litchi (*Litchi chinensis* Sonn.) saplings. Biological modifications included, K-mobilizers, AM fungi, *Pseudomonas florescence* and *Azotobacter chroococcum* along with *Metarhizium*, entomopathogenic fungi have been used. The treatments included, T_1-Litchi orchard soil + sand (1:1); T_2-Sand + AM fungi + *Azotobacter chroococcum* (1:2:1); T_3-Sand + *Pseudomonas florecence* + K-mobilizer (1:1:1); T_4- AM fungi + K-mobilizers (1:1); T_5, *P. Florecence* + *A. chroococcum* + K-mobilizer (1:1:1); T_6-Sand + *P. florecence* (1:2) and T_7-Uninoculated control for field performance. Treatments T_4-T_6 were further uniformly amended with drenching of *Metarrhizium* in rhizosphere. T_2 application significantly increased resident microbe survival, total chlorophyll content and root soil ratio in seedlings. *A. chroococcum*, *Pseudomonas*, K-mobilizers and AM fungi increased in microbial biomass of 2.59, 3.39, 2.42 and 2.77 times, respectively. Acidic phosphatases, dehydrogenases and alkaline phosphatases were increased in rhizosphere. Leaf nutrients reflected through DOP were considerably altered by T_2 treatment. Based on *Eigen* value, PCA-induced changes at biological modifications showed maximum total variance. The study inferred that the bio-transformations through microbial inoculants and entomopathogenic fungi could be an encouraging strategy to enhance the growth of plants, health and productivity. Such practices align well with the goals of sustainable agriculture through biological means by reducing dependency on chemical inputs. By delving into these aspects, the research gaps including microbial processes, competitive and symbiotic relationships, resistance in microbes and how complex interactions among bio-transformations, entomopathogenic fungi and microbes can significantly impact the health and productivity of litchi. Understanding and harnessing these interactions can lead to more effective and sustainable farming practices.

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Keywords Air-layering, Microbial inoculants, *Litchi chinensis* Sonn., Solubilizers

Introduction

Litchi (*Litchi chinensis* Sonn.), is a worldwide most familiar sub-tropical fruit, belongs to the Sapindaceae family believed to be originated in the provinces of Kwantung and Fukien in South China. India accounts for 99,000 ha of the total cultivable area and productivity of 7.57 MT/ha, ranking second China. The crop contributed significantly to the farmer economies of Indian states, with 30,500 ha in Bihar accounting for 78.4 per cent of the nation's total production, followed by West Bengal, Assam, and Jharkhand. In Himachal Pradesh, it occupies an area of 5,407 ha, yielding 6,071 MT annually. Although the crop's productivity is higher in India than in other countries however, there is a wider gap exists. In comparison to the 14–15 tons/ha of realizable potential production, the productivity in the foothills is only 7–8 tons/ha. Litchi saplings experience a variety of challenges during nursery establishment. Mortality exceeded a rate of 40 per cent. Additionally, nursery plants exhibit weak root formation, limited growth, and susceptibility to soil diseases. In order to induce roots without removing the parent plant's stem, air layers, *gootees*, or marcottages are the most economically feasible propagation techniques [1, 2]. Using this technique, it is possible to develop plants that mature faster than seeds or cuttings and are bigger as well. Main bottlenecks include the establishment of the saplings' due to high mortality when they separated from mother plant following air-layering operation and the saplings' development of their own root system during hardening process in field conditions [3]. There was a significant variability in the rooting capacity of various seed sources (genotypes) through air-layering [4]. Earlier researches have documented the symbiotic association of biological supplements in rhizosphere.

Litchi plantlets have high dependence on AM fungi and plant growth promoting rhizobacteria (PGPR) association because farmers inoculate new plantations with soil from existing litchi orchards. When layering on mother shoots is done, air-layered saplings are certainly lacking from microbial consortium [5]. Furthermore, in spite of native inoculants, these air-layered plants typically need three to four years to establish themselves, even in fields with fertile soils and irrigation. Because of their own root systems, the layers exhibited significant rates of mortality when they separated from the mother plant [6]. Bio-transformations through microbial actions can alter soil chemistry, nutrient availability or convert biological materials into more useful forms. Plant growth and development processes can be promoted by biological supplements including PGPR that inhabit the rhizosphere and colonize the roots of plants [7]. The green muscardine genus entomo-pathogenic fungus (EPF), *Metarhizium*, has long been recognized as a biological pesticide that penetrates the roots of a number of plants owing to the establishment of genetic profiling [8]. Soil saprophytes are the most common form of fungus species. EPF are primarily known as insect populations regulators with considerable potential use as mycopesticides. Some EPF strains can colonize plant roots and compete with or inhibit the growth of plant-pathogenic fungi and bacteria, thus promoting a healthier plant environment [9]. Recent studies have revealed their function in promoting plant growth after artificial inoculation [10]. EPF in the rhizosphere zone associate with plant roots to increase long-term survival, which minimizes the effects of drought. Moreover, the introduction of beneficial microbes through bio-transformations and the action of EPF can alter the microbial diversity and functionality in the rhizosphere, leading to improved plant health and soil quality. Concerning the absorption of nutrients and the recycling of organic matter, the microbial communities dwelling in the rhizosphere soil have direct interactions with the host plants. Using EPFs as endophytic fungi has aroused increasing interest from researchers and has unfolded many unique benefits as compared to conventional analogues. Many attempts have already been made to identify air-layers in a natural ecosystem. Endophytic bacteria that support plant growth have recently been shown to assist their host plants to overcome biotic and abiotic barriers [11]. The purpose of this study was to evaluate bio-transformations induced inoculants along with entomo-pathogenic fungus, *Metarhizium* in air-layered litchi saplings for growth, survival, physiological profile and rhizosphere stoichiometry during the nursery stage.

Materials and methods

Study area

Air-layered litchi seedlings were transplanted between late September to early October until June. The study site (RHRTS, Dhaulakuan, Himachal Pradesh, India) was typically with sub-tropical climate with cool winters and extremely scorching summers, with maximum and minimum temperatures of 39.5 and 17.3^{0}C, respectively. Average annual rainfall is 1100 mm. July and August are humid and rainy. At end of June month experienced the ninety per cent the south west monsoon, which ends in the first week of September. In December and January, there is considerable foggy weather conditions. Maximum temperature of the soil was 28.4 °C. Cooler nights and fairly warmer days have provided novelty to the crop's growth and development.

The edaphic conditions

Five rows, each spaced 0.5 m apart (4×2 m plot size) were included. Soil samples were collected from 0 to 15 cm depth for the analysis. The experimental soil was deep, humus-rich and sandy clay loam in texture with neutral soil reaction (pH 6.9, 1:2 soil water suspension), 21 dSm^{-1} of electrical conductivity, organic carbon (5.8 g kg^{-1}), alkaline $KMnO_4$ extractable-N (135.7 mg kg^{-1}), available NaHCO3-extractable P (9.2 mg kg^{-1}), and available NH_4OAC-K (11.4 mg kg^{-1}). DTPA-extractable micronutrient cations viz., Zn, Mn, Fe and Cu were 1.07, 35.8, 50.3 and 1.07 mg kg^{-1}, respectively. The trial site contained an initial microbial count of *Azotobacter chroococcum* (12.2×10^6 cfu g^{-1}), *Pseudomonas* sp. (8.6×10^5 cfu g^{-1}) and K-mobilizers (9.1×10^4 cfu g^{-1}).

The progeny orchard

For the air layering operation, the elite trees of 25 years old trees were selected as the mother orchard. The progeny orchard was provided with proper plant protection measures during cropping season based upon yield potential, free from any incidence of diseases, insect-pests and desirable quality parameters. Mother plants were pruned regularly to keep them for longer vegetative (juvenile) phase for the production of new shoots for air-layering around the year. Air layering operation was carried out on shoots of pencil thickness (2 cm diameter) in the months of August and September vis-à-vis minimum (19ºC) and maximum temperature (35ºC) along with 90 per cent of relative atmospheric humidity.

Soil solarization

Between May and mid-June, the soil was sterilized prior to transplanting of air-layer saplings in the field. A layer of 60 cm thickness of potting material after being well mixed was applied. In May and June, the mixture was thoroughly saturated with water before being covered with UV-stabilized polythene sheets of 100 mm thickness. Trenches (45–60 cm deep) around the perimeter and between the plots were dug and covered with polythene sheet. The edges of polythene sheet were completely covered with soil to maintain moisture content within the plots.

Substrate for layering

Air-layers of uniform size were propagated. During the months of August and September, the air-layering technique was used to remove the bark along with cambium layer (2 cm) from shoots of pencil-thickness. *Sphagnum* moss, a naturally sterile media was used. The purpose of using biological supplements was to produce high-quality planting material with improved root systems and the survival of air-layers inside the nursery. 10 g of the rooting media was used in the substrate. The substrate contained the inocula of AM fungi (200 spores per 50 g), 5 ml culture carrier slurry (10% gur) for *A. chroococcum* ($x10^6$ cfu g^{-1}), *P. florecence* ($x10^5$ cfu g^{-1}) and K-mobilizer ($x10^6$ cfu g^{-1}). The substrate (treatment-wise) was wrapped with polythene sheet (200-gauge thickness) during air-layer operation. Subsequently, the observations were recorded on the efficacy of substrates used along with bio-inoculants and rooting media on root emergence after air-layering and total root length of air-layered shoots. The rooted air-layered shoots detached from the mother trees during second fortnight of October and kept for hardening at nursery stage.

Field trial

Biological supplements namely, *Pseudomonas florescence*, *Azotobacter chroococcum*, AM fungal consortia and potassium (K) mobilizers along with local orchard's soil (LOS) and *Metarrhizium* were included. The treatments comprises of [T_1-LOS+sand (1:1)]; [T_2-sand+AM fungi+*A. chroococcum* (1:2:1)]; [T_3-sand+*P. florecence*+K-mobilizer (1:1:1)]; [T_4-AM fungi+K-mobilizers (1:1)]; [T_5-*P. florecence*+*A. chroococcum*+K-mobilizer (1:1:1)]; [T_6-sand+*P. florecence* (1:1)] and [T_7-uninoculated control with N: P:K (60:30:30 kg ha^{-1})]. Following the double application of PGPR in each treatment combination, the air-layered saplings were root dipped with *Metarrhizium* for 10 min. The rooted air layers separated from the mother plants were then transplanted. The uniform air-layers were transplanted at a size of 30×60 cm, using the double-row planting technique in October. Supplementary bio-inoculants were applied to 75 plants per replication, for a total of 225 plants. The rooted seedlings were submerged in the suitable microbial treatments for 20 min before being transplanted. To improve the viability of the propagation time and humidity, the nursery air-layered plantlets were also protected for 7–8 months beneath a shade net and success in regulating the micro-climate with temperature.

Sampling protocol and chemical studies

Composite soil samples (1 kg weight) were drawn at 0–15 cm depth with four soil cores in each bed (treatment-wise) using an auger (5 cm diameter) and were collected carefully to avoid the bands of microbial inoculants applied. Samples were air-dried and sieved in 2 mm size. Samples were stored in refrigerator (at 4ºC) to record observations on arable resident microbial indicators. The chemical characteristics of soils were determined according to standard methods. Soil pH and EC (1:2 soil-water) were measured. Soil organic carbon (OC) was determined using wet oxidation method [12], available N by alkaline potassium permanganate method [13], Olsen P (0.5 M $NaHCO_3$ extractable) [14] and 1 N neutral ammonium acetate extractable K estimated using

flame photometer [15]. Exchangeable Ca and Mg were determined using ammonium acetate method. DTPA-extractable micronutrients cations viz., Fe, Cu, Zn, Mn, buffered at pH 7.3±0.05 were analyzed on atomic absorption spectrophotometer [16]. For leaf analysis, each foliage sample comprising of 25 leaves was taken from middle of the shoot in the month of June. Newly matured leaves samples from the shoots were collected [17]. Leaf N was estimated using Kjeldahl method and P by phospho-vanado-molybdate method. Leaf K content was determined by flame photometer, whereas, the micronutrient cations were quantified on atomic absorption spectrophotometer.

Quantification of resident microbial community

The cultivable microorganisms were counted to calculate the rhizosphere resident microbial population. To monitor variations in PGPR population, the viable plate count method was used. Triplicate analyses of each soil sample were conducted. Using the serial dilution method, a pure and viable microbiological count was isolated on modified Aleksandrov medium for K-mobilizers, Jensen's N-free agar for *A. chroococcum* and nutrient agar for *Pseudomonas* sp. 10 g of soil from each sample was diluted at 1:10 and the suspensions were homogenized for 1 h. Dilutions were prepared and 0.1 ml of diluted sample of 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} dilution were spreaded on specified medium. The colony forming units (cfu) were counted after 72 h of the incubation period at 25±2°C. AM fungal spores were recovered using wet sieving and decanting process [18], and were expressed as the number of spores 50 g^{-1} of moist soil. In addition, the PGPR dependency was computed using the formula: (biomass of the inoculated seedling-biomass of un-inoculated seedling) divided by the inoculated seedling biomass x 100 and the values were expressed as per cent [19].

Microbial biomass and root: Soil

Microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were monitored in different bio-amendments treated plots. Chloroform-fumigation incubation method was employed for the determination of MBC in the rhizosphere zone [20]. Soil sample (50 g) was fumigated by spraying chloroform, after defumigation, it was incubated with 1.0 g non-fumigated soil for 10 days in the presence of NaOH (in vial) to trap the evolved CO_2. In another beaker, 50 g of soil was taken without fumigation and incubated as control. The following equations were used to calculate MBC and MBN. MBC= (F_c - UF_c)/ Kc, where, F_c=CO_2 evolved from fumigated soil, UF_c= CO_2 evolved from non-fumigated soil, K_c=0.45, and MBN= (F_N - UF_N)/ K_N, where, F_N= NH_4-N mineralized during 10 days from fumigated soil, UF_N= NH_4–N mineralized during 10 days from non fumigated soil, K_N= 0.54) [21]. Root: Soil of microbes was calculated by dividing the indigenous microbial population in the rhizospheric zone by the microbial population in non-rhizospheric zone.

Determination of soil enzymes

One gram of moist soil was taken into polypropylene vials (five-replicate of sub-samples). Enzymatic activity of acid phosphatase (AcP) and alkaline phosphatase (AlP) was determined by taking 4 ml of buffer (pH 6.5) for AcP and pH 11.0 for AlP and 1 ml of 0.1 M disodium p-nitrophenol phosphate (substrate). Both enzymes hydrolyzed p-nitrophenol phosphate to p-nitrophenyl and enzyme assays were performed [22]. Mixture of the polypropylene vials was incubated at 37 °C for 1 h. After that, 1 ml of 0.5 M $CaCl_2$ and 4 ml of 0.5 M NaOH were added to the mixture and filtered through Whatman filter paper. The yellow color intensity of p-NP (supernatant) measured at 420 nm wavelength for both AcP and AlP and compared with p-nitrophenol standards for calculation. Accordingly, dehydrogenase (DHA) enzyme activity in rhizosphere was assessed.

Growth indices and leaf chlorophyll content

Saplings plant height was determined at 90 and 120 days of transplanting and the average was taken. Number of leaves per plant, root surface area and root fresh weight (g/plant) were also determined. At the end of crop cycle, a representative sample size of 25 fully matured leaves were collected to measure leaf area using leaf area meter (LI-COR-3100) and the values were expressed as cm^2. Total leaf chlorophylls were determined and were calculated using formula: {(20.2A_{645}+8.02A_{663})/ a x 1000 x W} x V, where, A_{645}=absorbance at 645 nm, A_{663}=absorbance at 663 nm, a=length of light path in cell, W=weight of the sample, V=volume of the extract prepared and the values were expressed as gram per g of fresh weight [23].

Deviation from optimum percentage (DOP)

DOP nutrient index and the respective blade nutrient concentration were calculated as optimum (DOP=0), deficiency (DOP<0) or excess (DOP>0). A negative DOP index designates a deficiency, whereas a positive DOP index designates an excess. DOP index based on leaf analysis is calculated as: DOP= [C_n /C_o-1] x 100, where, C_n= foliar concentration of the tested nutrient and C_o= critical (reference) optimum nutrient concentration. The C_0 was taken from optimum values. Besides, general status of nutrients through the ΣDOP index can be obtained by adding the values of DOP index irrespective of sign. The larger is the ΣDOP value, the greater is the intensity of imbalances among nutrients and the lower the ΣDOP value, and the greater is the intensity of balance among nutrients.

Table 1 Conjoint effect of microbial inoculants on root emergence and total root length of layered litchi saplings

Treatment	Root emergence (days)	Total root length air-layered shoots (m)
T_1	39 ± 0.68bc	2.03 ± 0.07b
T_2	28 ± 0.58a	2.57 ± 0.11c
T_3	42.2 ± 1.03 cd	1.88 ± 0.11b
T_4	37.9 ± 0.47b	1.98 ± 0.11b
T_5	42.5 ± 0.74d	2.00 ± 0.10b
T_6	40.8 ± 0.46bcd	1.80 ± 0.18b
T_7	46.8 ± 0.68e	0.98 ± 0.03a

The values represent standard error of mean (± SE) of ten saplings per replicate (n = 75). The values followed by the same letters within each column are not significantly different from each other according to Duncan's Multiple Range Test (DMRT, $p \leq 0.05$). T_1, LOS + sand (1:1); T_2, sand + AM fungi + *Azotobacter chroococcum* (1:2:1); T_3, sand + *Pseudomonas florecence* + K-mobilizer (1:1:1); T_4, AM fungi + K-mobilizers (1:1); T_5, *P. florecence* + *A. chroococcum* + K-mobilizer (1:1:1); T_6, sand + *P. florecence* (1:2); T_7, Uninoculated control (*Sphagnum* moss)

Statistics

Statistical analysis of the data was done by linear model of the standard errors of the mean. Mean values were tested by ANOVA and the difference was compared by least significant difference (LSD) test at 5 per cent level of confidence (probability), wherever the results were significant using DSAASTAT version 1.101. Correlation analysis was worked out between all possible combinations including, agro-morphometric characters and soil properties [24]. The data reduction using the principle component analysis (PCA) of morphometric traits were also worked out according to Statistic XL software.

Results

The extrapolated data on the stimulatory effect of biological supplements' inoculation have demonstrated a large potential to enhance the formation of air layers, plant establishment, rhizosphere stochiometry, microbial biomass, and physiological profiling of layered transplants.

Rooting behavior during air-layering operation

In the current experiment, the observations have been undertaken to determine the effects of root emergence and total root length on the inoculation of biological supplements, whether it be dual or triple inoculation for successful air layering. The treatment T_2 considerably exhibited better root emergence with less time after layering among microbial consortium used, as compared to the uninoculated control (Table 1; Figs. 1 and 2). Maximum days taken for root appearance and for minimum root length were observed in the uninoculated control, whereas T_2 treatment indicated minimum days to root appearance and maximum root length. Total root length of seedlings grown in air layers was greatly increased by 162.2 per cent by dual inoculation with AM fungi and A. chroococcum in the ratio of 1:2. Additionally, T_2 treatment had the greatest number of roots, compared to the uninoculated control, which had the least.

Growth, leaf chlorophylls and root characteristics

Survival, vegetative growth and total leaf chlorophyll of transplanted layered saplings have been determined to assess the potential benefits of biological supplements. The data showed that T_2 treatment, which administered the saplings, produced the highest survival (89.2%) of

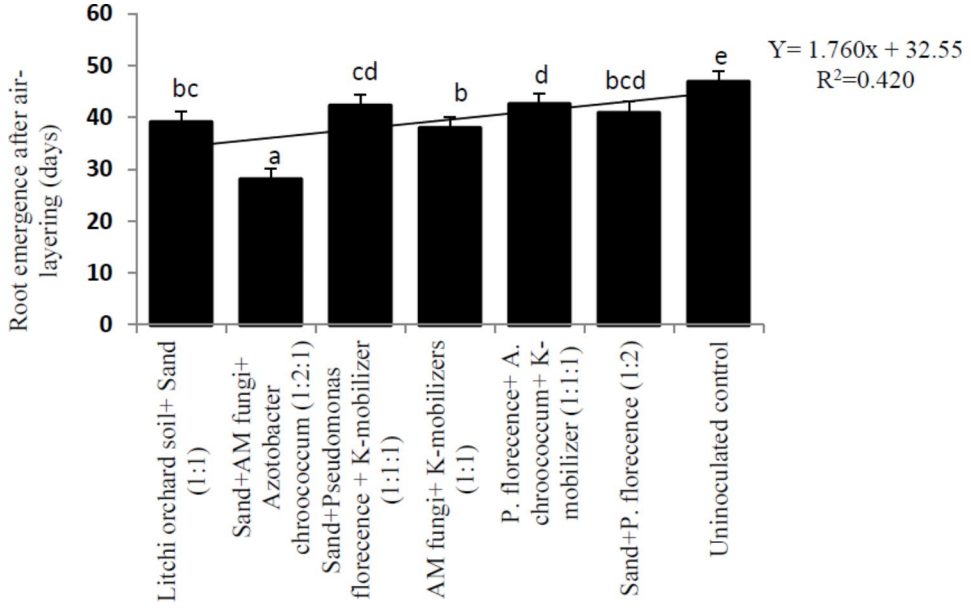

Fig. 1 The effect of biological amendments on root emergence after layering process (days) in litchi

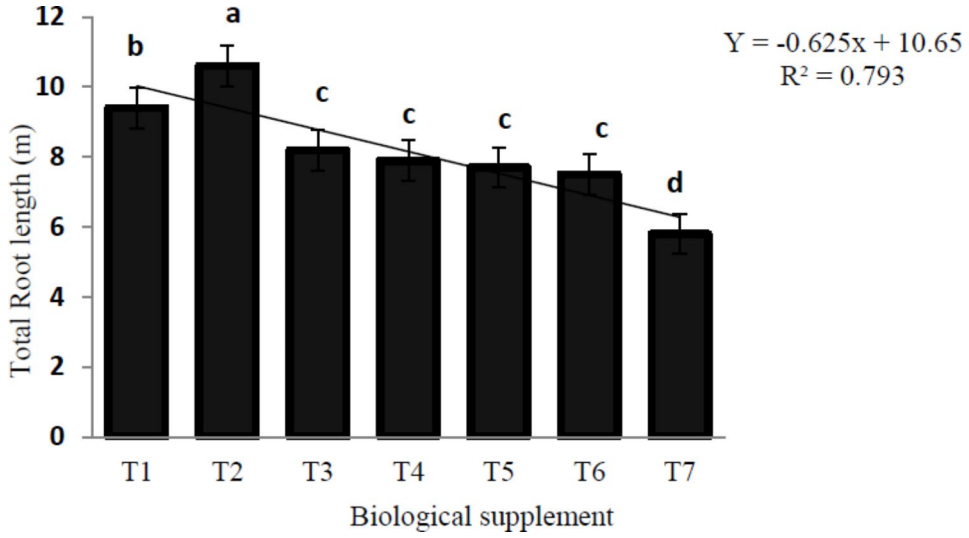

Fig. 2 The effect of biological supplements on total root length of transplanted litchi layers in field. T 1, LOS + sand (1:1); T 2, sand + AM fungi + Azotobacter chroococcum (1:2:1); T 3, sand + Pseudomonas florecence + K-mobilizer (1:1:1); T 4, AM fungi + K-mobilizers(1:1); T 5, P.florecence + A. chroococcum + K-mobilizer (1:1:1); T 6, sand + P. florecence (1:2); T 7, Control (FYM supplemented along with inorganic fertilizers NPK @ 60:30:30)

Table 2 The effect of biological inoculants on total chlorophylls and root characteristics of transplanted litchi layers

Treatment	Total chlorophylls (mg g^{-1})	Number of lateral roots	Thickness of lateral roots (mm)	FWR (g)	DWR (g)	FWS (g)	DWS (g)	*R*: S (FW basis)	*R*: S (DW basis)
T_1	1.03b	27.5b	18.8a	9.2b	4.4b	67.2bc	38.3b	0.137b	0.115 cd
T_2	1.26a	30.1a	19.3a	11.2a	6.2a	74.7a	45.8a	0.150a	0.135d
T_3	0.97bc	24.6c	17.5b	8.2bc	3.4bc	68.4b	39.5b	0.120c	0.086ab
T_4	0.90 cd	24.8c	16.9c	7.2 cd	2.6 cd	63.8bc	36.9b	0.113 cd	0.070a
T_5	0.83de	22.6c	17.5b	8.2bc	3.5bc	66.8bc	34.9bc	0.123c	0.100bc
T_6	0.94bcd	23.8c	16.6 cd	7.8c	3.1c	63.2c	32.3c	0.123c	0.096b
T_7	0.70e	16.8d	16.3d	5.9d	1.7d	55.6d	23.7d	0.106d	0.072a

The values represent mean of ten replicates ($n=75$). The values followed by the same letters within each column are not significantly different from each other according to DMRT ($p \leq 0.05$). FWR, Fresh weight of roots; DWR, Dry weight of roots; FWS, Fresh weight of shoots; DWS, Dry weight of shoots; FW, fresh weight; DW, dry weight, RS; Root: shoot ratio. T_1, LOS + sand (1:1); T_2, sand + AM fungi + *Azotobacter chroococcum* (1:2:1); T_3, sand + *Pseudomonas florecence* + K-mobilizer (1:1:1); T_4, AM fungi + K-mobilizers (1:1); T_5, *P. florecence* + *A. chroococcum* + K-mobilizer (1:1:1); T_6, sand + *P. florecence* (1:2); T_7, Control (FYM supplemented along with inorganic fertilizers NPK @ 60:30:30)

transplanted rooted layers and the lowest number of days to begin growth after 95.4 days after transplantation. In addition, T_2 showed a maximum increase of (15.6 cm) in plant height, stem diameter (20.5 mm), leaf number (15.1), leaf area (14.4 cm2), and total leaf chlorophylls (0.56 mg g-1) compared to the uninoculated control. According to the data depicted in Table 2, the saplings in treatment T_2 that had been dual inoculation with AM fungi and *A. chroococcum* in rooting media (sand) showed the greatest number of lateral roots. Air layers also showed the greatest thickness of lateral roots and total root length.

Microbial biomass, R: S and soil enzymes

In general, the double or even triple inoculation of different PGPRs in air layered transplants improved the resident microbial population count in rhizosphere and non-rhizosphere soils compared to uninoculated control. The overall culture microbial population in the rhizospheric and non-rhizospheric zone had been significantly affected by PGPRs inoculation. In case of AM fungi, *A. chroococcum*, *Pseudomonas* and K-mobilizers microbial population was observed at less than 5% of propagules of the total culturable microbial population in the soil which was significantly higher in rhizospheric zone than non-rhizospheric zone ($p<0.05$). Among different combinations of treatments, the respective plate count of *A. chroococcum*, *Pseudomonas sp*., K-mobilizers and AM fungi (per 50 g) also varied in rhizospheric and non-rhizospheric moist soil. The combination of treatment T_2 resulted in tremendous increase in resident microbial population of *A. chroococcum Pseudomonas*, K-mobilizers and AM fungi by 2.59, 3.39, 2.42 and 2.77 times, respectively over non-rhizosphere zone (Fig. 3).

Number of biochemical activities are also promoted by MBC, MBN and soil enzymes in the rhizosphere

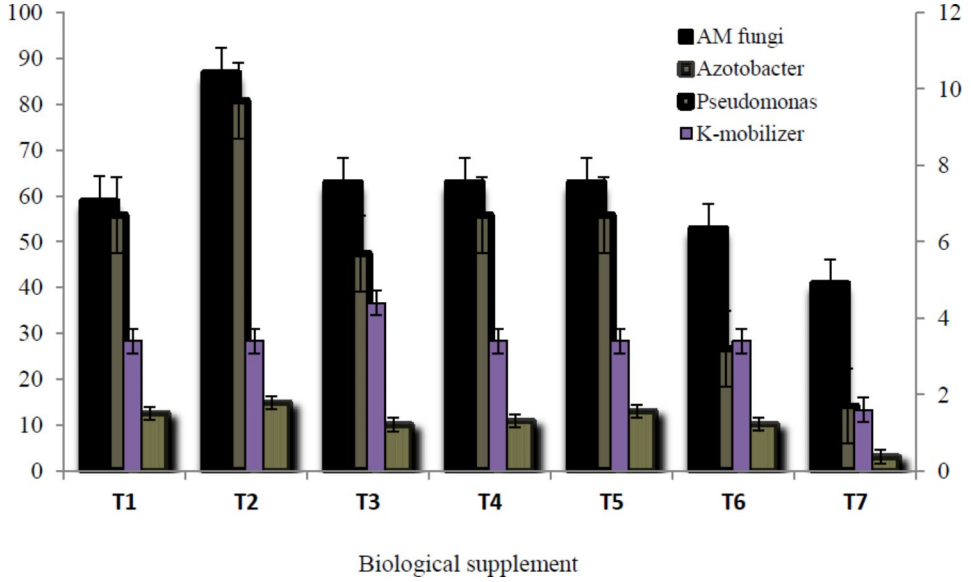

Fig. 3 Trends in difference of litchi rhizosphere and non-rhizosphere microbial communities affected by biological supplements. T 1, LOS + sand (1:1); T 2, sand + AM fungi + Azotobacter chroococcum (1:2:1); T 3, sand + Pseudomonas florecence + K-mobilizer (1:1:1); T 4, AM fungi + K-mobilizers(1:1); T 5, P. florecence + A. chroococcum + K-mobilizer (1:1:1); T 6, sand + P. florecence (1:2); T 7, Control (FYM supplemented along with inorganic fertilizers NPK @ 60:30:30)

Table 3 Microbial biomass C, microbial biomass N, and Microbial quotient at 0–15 cm litchi soil profile* affected by PGPR transplant amendments

Treatment	MBC (mg kg^{-1})	MBN (mg kg^{-1})	MBC: MBN	MQ
T_1	375.7 ± 1.13b	26.2 ± 1.08 cd	14.3 cd	0.057bc
T_2	424.8 ± 1.13c	26.8 ± 0.96d	15.9a	0.062c
T_3	378.0 ± 2.26b	27.1 ± 1.42d	13.9d	0.056bc
T_4	360.4 ± 1.60b	23.9 ± 1.27b	15.1abc	0.053ab
T_5	372.9 ± 2.26b	25.3 ± 0.92c	14.7bcd	0.056bc
T_6	355.6 ± 3.35b	23.1 ± 0.91b	15.4ab	0.055bc
T_7	289.2 ± 2.66a	20.8 ± 1.02a	13.9d	0.047a

Treatment-wise composite sampling (n = 21); MBC, microbial biomass carbon; MBN, microbial biomass nitrogen, MQ, microbial quotient. The values represent mean (± SE) of three replicates. The values followed by the same letters within each column are not significantly different from each other according to DMRT ($p \leq 0.05$). T_1, LOS + sand (1:1); T_2, sand + AM fungi + *Azotobacter chroococcum* (1:2:1); T_3, sand + *Pseudomonas florecence* + K-mobilizer (1:1:1); T_4, AM fungi + K-mobilizers (1:1); T_5, *P. florecence* + *A. chroococcum* + K-mobilizer (1:1:1); T_6, sand + *P. florecence* (1:2); T_7, Control (FYM supplemented along with inorganic fertilizers NPK @ 60:30:30)

soil, which are possible markers for soil productivity to maintain soil fertility[25]. Under various biological supplements, the transplanted air-layered litchi shoots showed significant shifts in MBC and MBN (Table 3). Compared to the treatments T_3, T_1 and T_5, which had corresponding MBC values of 378.0, 375.7, and 372.9 mg kg^{-1}, respectively, with no apparent differences among themselves, the treatment T_2 had highest MBC (424.8 mg kg^{-1}) value. Comparatively, T_2 had MBC of 12.4, 13.1 and 13.9 per cent higher than uninoculated control (46.9%) than T_3, T_1 and T_5. MBN far exceeded uninoculated control in T_3 (1.30), which was statistically comparable to T_2 (1.28), T_1 (1.25), and T_5 (1.21) times, respectively. The ratio for MBC: MBN for the various components ranged between 15.9 and 13.9 in the order followed was $T_2 > T_6 > T_4 > T_5 > T_1$. The ratio of MBC to soil organic carbon determined as the microbial quotient was observed no significant differences among different PGPR transplant amendments inoculated. R: S was highest in PGPR transplant layered saplings compared to uninoculated control (Table 4). The highest and the lowest R: S ratio of the resident microflora was expected based on respective PGPR probiotic combination inoculated in the litchi transplanted layers. Among different PGPR combinations, R:S ratio was recorded highest in T_1 (1.46) for AM fungi, T_2 (2.38) for *A. chroococcum*, T_1 (1.63) for *Pseudomonas* and T_3 (1.51) for K-mobilizers compared to uninoculated control. Similarly, the activities of AcP in rhizospheric soil was significantly higher in treatment T_2 i.e. 163.2 followed by treatment T_1 (149.3), treatment T_3 (139.3) and treatment T_4 (138.5) µg PNP g^{-1} h^{-1} in comparison to control (uninoculated) (Table 5). Treatment T_2 when compared to control, AcP activity was observed 1.65 and 2.53 times more between rhizospheric and non-rhizospheric zone. The order of AlP activity varied significantly among the PGPRs treatments in rhizospheric zone as $T_2 > T_3 > T_1 > T_6 > T_5$ and $T_2 > T_3 > T_1 > T_5 > T_6 > T_4$ in non-rhizospheric zone. In addition, DHA activity showed the same trends w.r.t AcP and AlP both in rhizospheric and non-rhizospheric zone. In rhizospheric zone, DHA activity measured in terms of µg TPF g^{-1} h^{-1} was significantly higher in treatment T_2 (11.5) followed by treatment T_1 (10.6), treatment T_3 (10.0) and treatment T_4 (9.6) and with no significant differences between

Table 4 PGPR dependency and root to soil ratio in terms of indigenous microflora at 0–15 cm soil profile affected by biological amendments

Treatment	Root: Soil				PGPR dependency (%)
	AM fungi	A. chroococcum	Pseudomonas sp.	K-mobilizers	
T_1	1.46a	2.22ab	1.65a	1.38b	19.5b
T_2	1.44a	2.38a	1.60ab	1.21d	28.4a
T_3	1.42b	1.95 cd	1.58bc	1.51a	19.7b
T_4	1.44a	2.07bc	1.63ab	1.31bc	13.4c
T_5	1.40c	2.27a	1.52c	1.25 cd	18.0bc
T_6	1.45c	2.02c	1.39d	1.26 cd	13.1c
T_7	1.38c	1.82d	1.18e	1.18d	0.00d

The values followed by the same letters within each column are not significantly different from each other according to DMRT ($p \leq 0.05$) ($n=21$). T_1, LOS + sand (1:1); T_2, sand + AM fungi + *Azotobacter chroococcum* (1:2:1); T_3, sand + *Pseudomonas florecence* + K-mobilizer (1:1:1); T_4, AM fungi + K-mobilizers (1:1); T_5, *P. florecence* + *A. chroococcum* + K-mobilizer (1:1:1); T_6, sand + *P. florecence* (1:2); T_7, Control (FYM supplemented along with inorganic fertilizers NPK @ 60:30:30)

Table 5 Soil enzyme activities in rhizosphere and non-rhizosphere zone at 0–15 cm depth affected by PGPR transplant amendments in transplanted litchi air layers

Treatment	Acid phosphatase (μg PNP g^{-1} h^{-1})		Alkaline phosphatase (μg PNP g^{-1} h^{-1})		Dehydrogenase (μg TPF g^{-1} h^{-1})	
	R	NR	*R*	NR	*R*	NR
T_1	149.3 ± 1.13b	107.8 ± 1.32b	99.7ab	74.2ab	10.6b	6.7b
T_2	163.2 ± 1.16a	119.7 ± 1.13a	104.6a	81.1a	11.5a	7.6a
T_3	139.3 ± 1.36bc	94.8 ± 1.30c	100.8ab	75.3ab	10.0bc	6.2bc
T_4	138.5 ± 0.91bc	96.0 ± 0.14c	92.4b	69.9b	9.6c	6.0c
T_5	130.6 ± 1.15c	83.1 ± 1.13d	95.0ab	72.5b	9.3 cd	5.7c
T_6	128.8 ± 1.05c	80.3 ± 0.86d	97.8ab	70.3b	8.6d	5.0d
T_7	98.8 ± 1.33d	64.3 ± 0.84e	68.9c	50.4c	6.5e	3.8e

R, rhizosphere; NR, non-rhizosphere. The values followed by the same letters within each column are not significantly different from each other according to DMRT ($p \leq 0.05$) ($n=21$); T_1, LOS + sand (1:1); T_2, sand + AM fungi + *Azotobacter chroococcum* (1:2:1); T_3, sand + *Pseudomonas florecence* + K-mobilizer (1:1:1); T_4, AM fungi + K-mobilizers (1:1); T_5, *P. florecence* + *A. chroococcum* + K-mobilizer (1:1:1); T_6, sand + *P. florecence* (1:2); T_7, Control (FYM supplemented along with inorganic fertilizers NPK @ 60:30:30)

treatments T_1, T_3, T_4 and T_5, whereas, it was least in treatment T_7 (9.6). Similar trends of DHA activity were determined in non-rhizospheric zone with the orders of $T_2 > T_1 > T_3 > T_4 > T_5$, however, the differences among these were found non significant.

Post harvest soil chemical indicators

Application of bio-organic amendments has significantly changed chemical characteristics of rhizosphere soils in transplanted litchi layers. Chemical characteristics of soil were found highest in treatment T_2 which further reduced through T_1, T_2 while, uninoculated control has the least. The data also depicted the significant effect of PGPR amedements on soil reaction and EC ($P<0.05$), but differences were negligible. PGPR probiotics inoculated whether dual or triple inoculation in transplanted litchi layers changed pH of the soil towards neutral. Soil pH varied from 6.94 (T_7) to 7.09 (T_2). Besides, the probiotics tested were found effective in decreasing soil EC level in all the treatments. Similarly, OC content of the soil showed a significant increase due varied double or triple inoculation concentration of tested PGPR amendments. Maximum increased SOC build up was noticed in T_2 treatment. The extent of SOC in general, was greater when the consortia of PGPR were inoculated litchi transplanted layers during hardening in field conditions. Maximum soil OC increased by in T_2, T_3, T_4 with corresponding values of 18.6, 16.9 and 16.2 per cent over initial (5.8 g kg^{-1}), respectively. In all tested PGPR, treatment T_2 showed highly available macronutrients viz., nitrogen, phosphorus and potassium, exchangeable calcium, magnesium and DTPA-extractable micronutrient cations (Zn, Fe, Cu, Mn) followed by treatments T_1, T_3, T_5 compared to control (uninoculated) which recorded the least. The initial available nitrogen content was determined as 135.7 mg kg^{-1}. After PGPR applications however, available total N content was 16.8, 14.8, 14.1 and 13.8 per cent increase when layered transplant saplings inoculated with T_2, T_1, T_3, T_4 treatments over initial, respectively. Available P content in soil significantly affected by PGPR applications and calculated as 13.4, 12.3 and 12.2 mg kg^{-1} P_2O_5 in treatments T_2, T_1, T_3 treatment application, while, it was 9.8 mg kg^{-1} P_2O_5 in the control (uninoculated). While, the initial available phosphorus content present in soil was 9.2 mg kg^{-1} at the beginning of treatment. Mean values of available potassium in the soil significantly increased under different combinations of probiotic inoculations being the

highest in T_2 (22.4%) followed by T_1 (18.5%), T_3 (18.3%) over initial (11.4 mg kg^{-1}). The superior T_2 treatment also showed maximum exchangeable Ca and Mg content increased with corresponding values of 58.4 and 34.1 per cent over control (uninoculated). In concern to the availability of DTPA extracted Fe, Cu, Zn and Mn however, the double or triple inoculation of treatment T_3 increased 29.6, 41.7, 32.1, 69.9 per cent, respectively, over uninoculated control. Similarly, Deviation from optimum percentage (DOP) is the one of the most important methods for interpretation of nutritional need in terms of quantity and quality of each nutrient in crops through leaf chemical analysis. In the present study, the DOP indexing showed the excess and deficiency in the order of N>Ca>Mg>Fe>Cu>Zn>Mn and P>K, respectively irrespective of treatment. DOP indices for N, Ca, Zn, Mg, Fe, Mn and Cu was positive, whereas, it was negative for leaf P and leaf K regardless of biological amendments supplemented (Table 6).

Discussion

The effect of biological amendments has shown a great potential to improve survival and growth traits of layered litchi saplings in nursery. The results revealed that maximum number of roots was observed in layers made on third week of June month, which actually appeared in early September. Change in the intensity of rooting emergence in layers could be due to variations in temperature especially high temperature coupled with high relative humidity in June, and thus increased the rate of respiration of the plant with low net photosynthates for rooting. However, air temperatures had dropped which provided least utilization of carbohydrates for respiration and the extra energy has been diverted and utilized in development. The treatment T_2 contributed more nutrients especially P and N which favored an ideal condition for the growth of roots in air layers [25]. The potential of AM fungi and its ability to association with roots appears to depend upon relationship of fungus and host. Effectiveness of this colonization might be due to better root colonization, which had direct relationship with growth [26]. Application of bio-organics especially PGPR enhanced the absorption of nutrients by plants, especially availability of N, which led to higher levels of proteins, thereby, increased in photosynthetic pigments which could accordingly strengthened photosynthetic activities and ultimately posed balanced nutrition compared to traditional fertilizers for the conversion process and sink-source relations. The restorative characteristics of biological amendments enhanced the acquisition and uptake of nutrients, release of growth promoters in rhizosphere of the plant and the suppression of harmful soil borne pathogenic communities due to application of *Metarrhizium* by following double application of biological amendments in each combination of treatments. Sapling's growth rate after transplant stimulated the litchi rooted layers had taken 95.4 days for the establishment of plant to accomplish better survival and vegetative growth traits. Our results were also in accordance with earlier studies which documented that PGPR produced the plant growth regulators like indole-acetic acid, gibberellins and cytokinins, Increased microbial activity upon PGPR interaction [27], the type of microbial consortium in the plant rhizosphere [28] caused effective nutrient acquisition through mobilization [29] and root morphology and physiology [30], beside insoluble phosphates into soluble form through acidification and chelation and exchange reactions [31], release of organic acids by PGPR transplant amendments contributed towards conversion into soluble $H_2PO_4^-$ and HPO_4^{2-} ions [32] and thus stimulated the availability of immobile and partial mobile nutrients [33].

According to the experimental results, all the biological amendments inoculated have positive influence on the rooting characteristics of the saplings. Our findings also emphasized that application of PGPR produced growth promoting substances in rhizosphere zone especially, indole-3-acetic acid which influencing root growth and

Table 6 DOP indices and ΣDOP determined from litchi saplings leaf nutrients at various biological inoculations

Treatment	*N*	*P*	K	Ca	Mg	Fe	Cu	Zn	Mn	ΣDOP
T_1	+72.09	-0.52	-9.74	+151.39	+22.43	+200.00	+341.74	+687.83	+100.87	+1566.09ab
T_2	+63.39	+2.87	+3.30	+194.61	+36.43	+1262.61	+453.91	+852.17	+265.22	+3134.51b
T_3	+48.96	+1.13	-5.04	+189.57	+30.61	+515.65	+174.78	+696.52	+186.09	+1838.27ab
T_4	+44.96	-1.13	-3.22	+182.70	+28.43	+584.35	+93.91	+540.87	+134.78	+1605.65ab
T_5	+45.91	-1.83	-7.65	+161.74	+32.52	+365.22	+13.91	+75.65	+37.39	+722.86ab
T_6	+39.83	-3.39	-2.87	+212.87	+21.65	+106.96	-63.48	+137.39	+165.22	+614.18ab
T_7	+34.61	-4.87	-14.17	+94.78	+-0.09	-834.78	-83.48	+-52.17	-93.04	-953.21a
Mean	+49.96	-1.11	-5.63	+169.67	+24.57	+314.29	+133.04	+419.75	+113.79	

Leaf standards for air-layered litchi plants based on whole leaf along with petioles sampled; sign (–) indicates deficiency level, sign (+) indicates excessive (Menzel et al., 1992); mean followed by same small and capital letters within columns indicate significant differences among ΣDOP indexes within each PGPR treatment are not significant according to DMRT ($p \leq 0.05$). T_1, LOS + sand (1:1); T_2, sand + AM fungi + *Azotobacter chroococcum* (1:2:1); T_3, sand + *Pseudomonas florecence* + K-mobilizer (1:1:1); T_4, AM fungi + K-mobilizers (1:1); T_5, *P. florecence* + *A. chroococcum* + K-mobilizer (1:1:1); T_6, sand + *P. florecence* (1:2); T_7, Control (FYM supplemented along with inorganic fertilizers NPK @ 60:30:30)

morphology and initiation of lateral root development [34]. Moreover, the release of metabolites stimulated by plant growth promoting rhizobacteria that directly stimulated root growth, increased photosynthesis, carbohydrates accumulation and nutrients acquisition by roots [35]. Maximum fresh weight and dry weight of roots i.e., 11.2 and 6.2 g were attained with PGPR transplant amendments media treatment (T_2) followed by treatment T_1, T_5, whereas minimum was obtained with control (uninoculated). Moreover, the promotional effects on root characteristics ascribed to the variation in the intensity of colonization due to capacity by forming extensive and effective network of external hyphae around the root zone for nutrient acquisition [36]. The production of plant growth promoting hormones viz., auxins, gibberellins and cytokinins by PGPR interfere on resident soil's microbial diversity especially AM fungi by associating the roots leads increased root growth rate and exudation rate. AM fungi promoted plant growth and improved plant establishment by increasing nutrient and water relations especially ascribed to increased uptake of immobile P and plant tolerance to biotic and abiotic stresses [37].

In the present investigation, the quantitative analysis of resident microbial diversity from rhizosphere and non-rhizosphere zone recorded is in close conformation with the respective microbial propagules in general, has been well documented. Earlier literature also depicted a pronounced positive rhizospheric effect for substantial increased native soil microbial community through PGPR amendments in litchi [34]. Treatment T_2 showed the highest amount of microbial quotient when air-layered transplants were inoculated in the order of $T_1 > T_3 > T_5 > T_6 > T_4$, whereas, it was least in T_4, and however, there were no differences between T_3, T_5 and T_6. In the present investigations, the addition of PGPR transplant amendments and subsequent degradation of organic residues had increased MBC in soil which might be due to the stimulatory effects coupled with favorable environment for microbial growth and development to perform biochemical processes within rhizosphere. Besides, the decomposition of soil organic material by exogenous PGPR inoculation has also caused changes in rhizosphere for physico-chemical and biological properties resulted in increased MBC and MBN and their ratio in litchi transplanted layers [38]. The variation in microbial biomass coupled with more microbial density and their involvement in decomposition of the organic matter emphasized considerably more microbial turnover with increased MBC and MBN in rhizosphere compared to non-rhizosphere [39]. Soil microorganisms were involved in many biological processes in rhizosphere [40] and helped in biological conversion, assimilation and nutrient cycling process in soils. Microbial biomass was acted both as the labile nutrient pool and the medium for the transformation, cycling of organic matters and plant nutrients in soils [41]. Earlier studies also revealed that the application of organic amendments changed physical, chemical, and biological properties of soils, increased soil microbial population [42] and biological microbial diversity in rhizosphere [43]. Besides, microbial biomass also maintained soil enzymes that regulate transformation processes of elements in rhizosphere [44]. The addition of organic matter vis-à-vis the process however, altered through PGPR amendments towards increased microbial biomass carbon in soil [45]. Our findings have also shown that the resident microbial diversity was significantly benefited by their proximity towards roots of the plants and therefore, the R: S ratio increased to 1:5. These results are also confirmed when strawberry plantlets were rhizo-inoculated with PGPR transplant amendments in solarized soils [34]. Moreover, maximum R: S ratio for respective resident microbes in varied treatment combinations were recorded might be due to rooted nature of the layered saplings, which has effectively influenced the nutrient dynamics in the rhizosphere, and thus enhanced indigenous density of beneficial microflora and thereby positively contributed for their growth [46]. Moreover, microbial density and growth have shown to occupy low volume of soil in rhizosphere, where microflora has a continuous access to a flow of organic substrates derived from roots [47]. Considering physico-chemical and biological properties in rhizosphere, the flow of organic substrates markedly influenced higher microbial population densities and also the microbial community structure [48]. The treatment T_2 in case of rhizosphere for activities of AcP, AlP and DHA in layered transplants were observed 2.53, 2.08 and 30.2 times higher than non-rhizosphere control (uninoculated), respectively. Soil enzymatic bioassay was critically important for soil productivity which has provided indications of changes in metabolic capacity and nutrient cycling [49]. Earlier literature is well documented on the distribution of enzyme activities in rhizosphere and non-rhizosphere [50]. Increase of acid phosphatases, alkaline phosphatases and dehydrogenases enzymes due to increased population of resident beneficial soil microorganisms when rhizoinoculation with PGPR in strawberry plantlets in solarized soils was carried out. Generally, enzyme concentration in rhizosphere soils is higher compared to bulky non-rhizosphere soils which might be due to rhizo-deposition and the stimulation of root-associated microbial diversity and the production of enzymes during root cells lysis. The enzymes so produced in the zone catalyzed the formation of secondary products to be utilized by roots and or rhizosphere microorganisms [51]. Besides, several evidences are also available on the accumulation of enzymes between soil and plant roots, the participation

of enzymes originating from proteins produced by roots of plants, its further discharge in rhizosphere and the contribution of mycorrhization on enzyme activities were also directly related to the inhabitant microbial community for improving nutrient acquisition [52]. Higher enzyme activity in rhizosphere, on account of enhanced microbial activity sustained by root exudates or due to the release of enzymes from roots [53]. Moreover, the resultant post-harvest soil chemical indicators in the treated microbial inoculants plots revealed variation in soil pH due to inoculation of bacterial and fungal consortia which caused more production of organic acids such as oxaloacetic acids, alpha-ketoglutaric acid, aspartic acid etc. in rhizosphere [54]. Nitrogen, phosphorus and potassium are the most required essential nutrients for the growth of plants and their development in crop production. Appreciable number of bacterial and other PGPR, mainly those associated with rhizosphere zone as compared to non-rhizosphere zone, are able to exert potential beneficial effects on the growth of plants. Further, phosphate solubilizing microorganisms especially AM fungi and *Pseudomonas* species rendered insoluble phosphates into soluble form through acidification, chelation and exchange reactions. The tested PGPR secreted carboxylic acid, thus lowered pH in the rhizosphere and consequently released the bound forms of phosphates especially $Ca_3(PO_4)_2$ in calcareous soils. Further, available P content of soil easily forms insoluble complexes with cations and incorporated into soil organic matter by microbial population which in turn increased the availability of accumulated phosphates through increased mineralization, solubilization, biological nitrogen fixation and through production of plant growth promoting substances, therefore, rendered availability of Fe, Zn, Cu and Mn content of soil [55]. Positive values for DOP indices in leaf N, P, Mg, Zn and Mn, whereas, it was negative (deficient) for leaf P and K were in strawberries growing in sub-tropical agro-climatic conditions were also recorded. Besides, the saplings fertilized with treatment T_3 tend to have a positive DOP value except DOP_K. However, leaf nitrogen, phosphorus, potassium, Magnesium contents, in general, was in excessive range, when leaf Ca was in lowest amount has been previously reported, probably a consequence of lower K competition, which was universally trait of leaf Mg. Further, P and K had the most negative indices among nutrients attributed to low mobility and low availability in soil and thereby decreased availability in the soil through fixation by clay particles. Earlier studies carried out in pistachio nut [56], apple [57], strawberry [58] and guava [59] where did not observe any significant differences for nutritional balances.

Pearson correlation coefficient (r) depicted significant correlations among some variables obtained. The results shown that stem diameter exhibited a positive relationship with number of leaves (r=0.896), area of leaf (r=0.912), fresh weight of roots (r=0.875), fresh weight of shoots (r=0.959) and dry weight of shoots (r=0.905) of the saplings. Number of leaves of the saplings also attained a positive as well as significant relationship with total chlorophylls content of the leaf, number of lateral roots, thickness of lateral roots, total root length, fresh and dry weight of roots, fresh and dry weight of shoots, whereas, all other variables had shown positive relationship among themselves. The effect of biological amendments on soil enzymes was also significant with various chemical and microbiological properties studied. Acid phosphatase and dehydrogenases has shown a positive and highly significant correlation with SOC, available N, P, K and Ca content, whereas, it was positive but significant between DHA and DTPA Cu, alkaline phosphatase and available P and Mg content (Table 7). The positive and significant relationship between *A. chroococcum* and MBC, alkaline phosphatase, dehydrogenases and microbial quotient, *Pseudomonas* and MBC and MBN in rhizosphere was recorded [60, 61]. The data reduction technique with PCA of the original factors drawn as

Table 7 Pearson's correlation coefficient (r) between soil enzymes, microbial biomass and survival of layered litchi saplings

Variable	Survival rate	AM fungi	Achr	Pseudo	K-mobilizer	MBN	MBC	MQ	AcP	AlP	DHA
Survival rate	-										
AM fungi	0.869	-									
Achr	0.698	0.812	-								
Pseudo	0.701	0.950*	0.877*	-							
K-mobilizer	0.791	0.790	0.801	0.756	-						
MBC: MBN	0.538	0.554	0.598	0.575	0.874						
MBN	0.691	0.753	0.837	0.746	0.472	-					
MBC	0.809	0.881*	0.959**	0.887*	0.789	0.891*	-				
MQ	0.753	0.827	0.944*	0.844	0.791	0.864	0.982**	-			
AcP	0.658	0.779	0.922*	0.844	0.654	0.865	0.960**	0.936*	-		
AlP	0.734	0.673	0.910*	0.671	0.691	0.870	0.931*	0.926*	0.906*	-	
DHA	0.666	0.796	0.928*	0.856	0.617	0.916*	0.961**	0.931*	0.992*	0.904*	-

The values with * are significantly different by the CORR Test (P<0.05), and values with ** are significantly different by the CORR Test (P<0.01), MBC, microbial biomass C; MBN, microbial biomass N; Ach, *A. chroococcum;* Pseudo, *Pseudomonas* AcP, acid phosphatase; AlP, alkaline phosphatase; DHA, dehydrogenase

'*Eigen vectors*' summarized the correlation between the variables including vegetative growth traits, root characteristics and total leaf chlorophylls during hardening of saplings. PCA studies showed the first components which accounted for higher total variance based on the *Eigen* value (>1) for 15.63 per cent (PC1) and 1.13 per cent (PC2) and explained 86.82% (PC1) and 93.11% (PC2) of the cumulative variance. PC4 accounted for maximum total cumulative variances score of 98.22 per cent among morphometric (vegetative growth), root characteristics and total leaf chlorophyll contents in the saplings. PCA studies considered variables with equal or greater values which also explained the variability in PCs, showing that PGPR are considered significant in the fourth PC, this means that the PGPR group was influenced by the plots and environmental conditions. In the present investigation, a very clear separation of PGPR treatments was shown by PC1, which accounted for 86.82 per cent of the total variation, which was strongly associated with all morphometric and leaf chlorophylls of the saplings whether positive or negative. Besides, it also inferred that PGPR inoculation caused higher variation in the measured parameters, than uninoculated control, which accounted only for 93.11 per cent of the total variation along PC2. The factor loadings greater than 0.40 among different PCs being the highly weighted variables were appreciated. The sign of the factor loading shows about the relationship between a component and a variable. Minimum data set suggested vegetative growth traits namely, survival rate, root emergence, total root length of air-layered, height of plant, diameter of stem, number of leaves transplanted layer, area of leaf, characteristics of roots including number of lateral roots, thickness of lateral roots, total root length, roots fresh weight, roots dry weight, fresh weight of shoots, shoots dry weight, fresh weight, dry weight, root/ shoot ratio and leaf chlorophyll content by PCA studies.

PCA was also performed to clarify the relationship between variables viz., chemical, microbiological and enzymatic activities of soil. The *Eigen* values, variability and cumulative variance rate were also gathered. According to the 96.19 per cent accumulative contribution (cumulative variance), the information on most of the soil properties affected by PGPR inoculation can be summarized in the first four PCs. PC1 and PC2 explained 73.86 and 14.84 per cent of the total variance, respectively. The different soil properties were observed to have the same approximate weight in the definition of the factor scores irrespective of positive and negative values. All of the PGPR supplemented had lower weights (component score coefficients), thus, they contributed less to explain the data variance (Table 8). Various soil properties viz., organic carbon, available N, P, K, DTPA Mn, Zn, MBC, MBN, acidic AcP, AlP and DHA enzymatic activity in rhizosphere of saplings were highly loaded in PC1. This indicated that PC1 reflected the main factor for hardening of air-layered litchi saplings with an *Eigen* value of 25.08, and the contribution rate (variability) was 73.76 per cent of the total variation.

Conclusion

The application of a sand mixture with AM fungi and *Azotobacter chroococcum* in a 1:2:1 ratio, along with *Metarhizium*, significantly improved the survival rates, vegetative growth, and total leaf chlorophyll content of litchi saplings compared to the uninoculated control. This biological amendment not only enhanced the post-harvest soil conditions by improving both chemical and microbiological properties in the rhizosphere but also markedly increased the biomass of resident microbial communities and soil enzyme activities, particularly with adequate phosphorus nutrition. The study indicates that integrating bio-inoculations with the entomopathogenic fungus *Metarhizium* can be an effective strategy for maintaining a healthy rhizosphere during the nursery stage of litchi cultivation. This approach supports sustainable pest management and boosts overall productivity and sustainability in litchi farming, particularly in the Shiwalik foothills of the north-west Himalayas. The findings highlight the transformative impact of combining bio-transformations and entomopathogenic fungi in litchi cultivation, demonstrating significant improvements in plant health, growth, and productivity. Biological formulations, such as K-mobilizers, AM fungi, *Pseudomonas fluorescens*, and *Azotobacter chroococcum*, used in conjunction with *Metarhizium*, effectively enhanced the rhizosphere microbiome and plant vigor. Among the treatments, T2, which included sand, AM fungi, and *Azotobacter chroococcum*, was particularly effective in boosting microbial survival, total chlorophyll content, and root-to-soil ratios. The integration of these components significantly increased microbial biomass and the activity of critical soil enzymes like acidic phosphatases, dehydrogenases, and alkaline phosphatases, positively affecting leaf nutrient levels and plant health as measured by Differential Optical Properties (DOP). Principal Component Analysis (PCA) revealed that these biological modifications accounted for the highest variance in plant and soil responses, underscoring their substantial impact. The research supports the notion that bio-transformations and the use of entomopathogenic fungi are not only advantageous for plant growth and resilience but also align with sustainable agricultural practices by reducing reliance on chemical inputs.

Future research should delve deeper into the complex interactions among microbial processes, competitive and symbiotic relationships, and their effects on plant health and productivity. Such insights will be crucial for

Table 8 PCA depicting microbiological properties and soil enzyme activities in rhizosphere and non-rhizosphere zone of air-layered litchi saplings

Parameter	Principal Component							
	PC1	PC2	PC3	PC4				
Eigen value	25.08	5.04	1.61	0.98				
Variability (%)	73.76	14.84	4.72	2.89				
Cumulative variance (%)	73.76	88.59	93.30	96.19				
Soil properties	Factor loadings (Pattern Matrix)				Eigen vectors (Component Score Coefficients)			
	F1	F2	F3	F4	F1	F2	F3	F4
AM fungi (R)	0.889	0.300	0.301	0.091	0.178	0.134	0.238	0.091
AM fungi (NR)	0.862	0.306	0.361	0.125	0.172	0.136	0.285	0.126
A. chroococcum (R)	0.950	0.145	-0.080	-0.006	0.190	0.064	-0.064	-0.006
A. chroococcum (NR)	0.891	-0.051	0.335	0.172	0.178	-0.023	0.264	0.173
Pseudomonas sp. (R)	0.891	0.321	0.288	-0.139	0.178	0.143	0.228	-0.140
Pseudomonas sp. (NR)	0.813	0.465	0.341	-0.068	0.162	0.207	0.269	-0.068
K-mobilizers (R)	0.706	0.612	-0.126	0.252	0.141	0.273	-0.100	0.254
K-mobilizers (NR)	0.563	0.787	-0.101	0.178	0.112	0.350	-0.080	0.180
MBC	0.989	0.097	-0.037	0.105	0.197	0.043	-0.030	0.106
MBN	0.918	-0.299	0.148	0.152	0.183	-0.133	0.117	0.154
MBC: MBN	0.517	0.709	-0.388	-0.031	0.103	0.316	-0.307	-0.031
AM fungi (R: S)	0.614	-0.161	-0.729	-0.250	0.123	-0.072	-0.576	-0.252
A. chroococcum (R: S)	0.819	0.410	0.022	-0.176	0.163	0.183	0.017	-0.178
Pseudomonas sp. (R: S)	0.883	-0.294	-0.020	-0.256	0.176	-0.131	-0.016	-0.258
K-mobilizers (R: S)	0.337	-0.916	-0.008	0.181	0.067	-0.408	-0.006	0.183
AcP (R)	0.980	-0.018	-0.135	-0.120	0.196	-0.008	-0.107	-0.121
AcP (NR)	0.937	-0.045	-0.054	-0.261	0.187	-0.020	-0.043	-0.263
AlP (R)	0.918	-0.113	-0.289	0.211	0.183	-0.050	-0.228	0.213
AlP (NR)	0.969	-0.045	-0.143	0.151	0.194	-0.020	-0.113	0.153
DHA (R)	0.989	-0.084	-0.042	-0.101	0.198	-0.037	-0.034	-0.102
DHA (NR)	0.982	-0.041	0.030	-0.152	0.196	-0.018	0.024	-0.153
PGPR dependency	0.987	0.036	-0.002	0.124	0.197	0.016	-0.002	0.125

R, rhizosphere; NR, non-rhizosphere; R:S, root to soil ratio; MBC, microbial biomass C; MBN, microbial biomass N; AcP, acid phosphatase; AlP, alkaline phosphatase; DHA, dehydrogenase PC1, Principal component-1; PC2, Principal component-2; PC3, Principal component-3; PC4, Principal component-4, F1, factor-1; F2, factor-2; F3, factor-3; F4, factor-4

optimizing agricultural practices, enhancing sustainability, and improving efficiency. This study establishes a foundation for integrating biological approaches into litchi cultivation, paving the way for more effective and eco-friendly farming practices.

Supplementary Information

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Supplementary Material 1

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Conceptualization-PK, AKJ, SL; Visualization, supervision & project administration-PK, AKJ; Software, Writing-original draft preparation-PK, NS, SL, YKA, AM; Writing-review and editing-NS, YKA, AK, PS, SM, Funding acquisition: YKA, IMM All authors have read and agreed to the published version of the manuscript.

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Data availability
The data that support the results of this study are available from the corresponding author, upon reasonable request.

Declarations

Ethics approval and consent to participate
All methods were in compliance with relevant institutional, national, and international guidelines and legislation.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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