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Proceeding Paper

Biological Amendments Improved Survival, Growth Traits, and Microbial Properties of Air-Layered *Litchi chinensis* Sonn. cv. Early Large Red Saplings †

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Abstract: Biological amendments namely, *Pseudomonas florescence, Azotobacter chroococcum*, K-mobilizers and AM fungi were expedited during air-layering operation on litchi (*Litchi chinensis* Sonn.). Twenty-five years healthy progeny of mother plants were maintained for air layering operation. The treatments comprised of the combinations namely, 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. florescence* + *A. chroococcum* + K-mobilizer (1:1:1); T_6, sand + *P. Florecence* (1:2); T_7, uninoculated control. Treatment T_2 significantly improved survival rate, plant height, stem diameter, leaf number, leaf area and total leaf chlorophylls of the saplings. Microbial biomass of *A. chroococcum Pseudomonas*, K-mobilizers and AM fungi were tremendously increased. Soil enzymes activity in rhizosphere was increased which indicated better P nutrition. The study indicated that biological amendments inoculation can be a promising technology to improve survival rate to produce elite litchi planting material.

Keywords: bio-inoculants; rhizosphere; soil enzymes; air-layering

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1. Introduction

Biological amendments have the well-known property of symbiotic effects in the rhizosphere. Litchi trees have been reported to have a very high dependence for AM fungi and associated beneficial PGPRs because of farmers' inoculation for new plantations with soil of old litchi orchards. Air-layered saplings are devoid of microbial consortium at the time of layering on shoots [1]. Further, these air-layered litchi plants in the absence of natural inoculants usually take 3–4 years for establishment after transplanting in fields even when provided with well fertile soils and irrigated conditions. The bottlenecks were high mortality of layers once detached from mother plant of their own root system [2]. Biological amendments including PGPRs are rhizosphere-inhabiting bacteria, colonizing the root system of plants and could stimulate plant growth and development processes [3]. The genus *Metarhizium* (also known as green muscardine) is an entomo-pathogenic fungus with a worldwide distribution. The fungi have long been recognized as biological pesticide (myco-insecticide) with advent of genetic profiling known to colonize roots of different species [4]. The fungal species are most frequently found as soil saprophytes in agricultural fields [5]. Earlier literature has suggested that the fungi form associations with

plant roots in the rhizosphere zone [6] for better survival over extended periods of time [7] which alleviated in drought stress conditions [8]. In rhizosphere soil, the microbial communities inhabiting have both direct interactions with the host plants in relation to nutrient acquisition and organic matter recycling. Several experimental attempts have been made for the establishment of air-layers in field conditions [1].

It has been shown that plant growth promoting endophytic bacteria can help their host plants to coup with various biotic and abiotic stresses [9]. The present investigations therefore, were planned and focused with the objective to study the effectiveness of PGPRs coupled with interactive association of entomo-pathogenic fungus, *Metarrhizium* inoculation at nursery stage on growth, survival, physiological profiling and rhizosphere stochiometry of transplanted air-layered litchi saplings. PGPR are rhizosphere-inhabiting bacteria, colonizing the root system of plants and could stimulate plant growth and development processes. The saplings planted during the initial phase of air layering results in very-very low survival due to poorly developed root system. Besides, the genus *Metarhizium* is an entomo-pathogenic fungus with a worldwide distribution. The fungi have long been recognized as biological pesticide with advent of genetic profiling known to colonize roots of a variety of plants. The fungal species are most frequently found as soil saprophytes in agricultural fields as compared to forest ecosystems. Earlier literature has suggested that the fungi form association with plant roots in the rhizosphere and survive better in that environment over extended periods of time. Several experimental attempts on rooting media have been already made towards the success for the establishment of air-layers in field conditions. The present investigations therefore, were planned and focused with the objective to study the effectiveness of PGPR probiotics on air-layers root development on mother litchi plants and further evaluation of their interactive effects along with *Metarrhizium* inoculation on survival and physiological profiling of transplanted air-layered saplings at nursery stage which could be an innovative scientific approach with consistent outcome for the production of elite planting material.

2. Materials and Methods

The experiment was conducted in RHRTS of Dr YS Parmar University of Horticulture and Forestry at Dhaulakuan, Sirmour (HP). The trial site is located at an elevation of 468 m above mean sea level with geographical coordinates of 28°25′ North (latitude) and 75°48′ East (longitude). The experiment was carried out on *Litchi chinensis* Sonn. cv. 'Early Large Red' between late September and October until June commencing monsoon for two consecutive years of 2015 and 2016. The climate of the experimental area was typically sub-tropical. Winters are cold and the summers are very hot. Maximum mean temperature was 39.5 °C, while, the minimum mean temperature was 17.3 °C during the growth periods. Normal annual rainfall is 1100 mm, which is almost unevenly distributed. The south west monsoon contributes 90 per cent which sets in the last week of June and withdraws in middle of September. July and August are rainy months. Maximum soil temperature was 28.4 °C. Soil solarization increased the maximum daily temperature to 35.3 °C and the average minimum daily soil temperature to 22.7 °C.

Biological amendments in growing media were used with the purpose to produce quality planting material with better root system in air-layers and enhanced final survival of litchi air layers within the nursery. PGPR bioinoculants namely, *Pseudomonas florescence*, *Azotobacter chroococcum*, AM fungi consortia (*Glomus fasciculatum*, *G. clarum* and *G. mosseae*) and Potash (K) mobilizers were included. The treatments comprised of the combinations namely, T_1—litchi orchard soil + sand + *Metarrhizium* (1:1:1); T_2—sand + AM fungi + *A. chroococcum* + *Metarrhizium* (1:2:1:1); T_3—sand + *P. florecence* + K-mobilizer (1:1:1); T_4—AM fungi + K-mobilizers + *Metarrhizium* (1:1:2); T_5—*P. florescence* + *A. chroococcum* + K-mobilizer + *Metarrhizium* (1:1:1:1); T_6—Sand + *P. florescence* + *Metarrhizium* (1:2); T_7—Uninoculated control supplemented with farmyard manure along with recommanded dose of fertilizers of N:P:K in the ratio of 60:30:30) for field performance. Besides, the air layers saplings were root dip with *Metarrhizium* for 10 min following dual application of

PGPR probiotics in each treatment combination in bulk as well as rhizosphere soil. The detached rooted air layers from the mother plants were further transplanted in different rooting media. The uniform air-layers were transplanted at 30 × 60 cm using double-row planting method replicated thrice during the month of October. The probiotics application was performed using a dipping method in which plant roots were inoculated with the respective microbial suspensions for about 20 min prior to transplantation. Control plants were dipped into sterile water. The nursery air-layered plantlets were also covered for 7–8 months under shade net for improved viability of propagation period and success percentage to regulate micro-climate with temperature and humidity for improved survival of detached layers.

3. Results and Discussion

3.1. Survival and Growth Traits of Saplings

It is further depicted that maximum number of roots was obtained in layers made on the third week of June, which actually appeared early September (Figure 1). Variation in the intensity of rooting emergence in layers could be due to fluctuations in temperature especially high temperature coupled with high relative humidity in the month of June, and thus increased the respiration of the plant with low net photosynthates for rooting. Besides, air temperatures had dropped which provided lesser the utilization of carbohydrates for respiration; thus, an extra energy has been diverted to root 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. Moreover, the potential of AM fungi and its ability to colonize 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 [10]. Application of bio-organics especially PGPR enhanced the absorption of nutrients by plants, especially availability of N, which led to higher levels of proteins [11], 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 stimulative effects of biological amendments improved the acquisition and uptake of nutrient, release of growth promoting substances in rhizosphere and the suppression of deleterious soil borne microbial communities due to inoculation of *Metarrhizium* following dual application of biological amendments in each treatment combination. Moreover, sapling's growth after transplant encouraged the litchi rooted layers had taken 95.4 days for plant establishment to achieve better survival and vegetative growth traits.

Table 1. Effect of microbial inoculants on growth traits of *Litchi chinensis* Sonn. cv. 'Early Large Red' saplings.

Treatment	Survival (%)	Plant Height (cm)	Stem Diameter (mm)	Leaf Number Transplanted Layer^{-1}	Leaf Area (cm^2)
T_1	69.9	52.1	34.2	25.5	48.8
T_2	89.2	62.5	42.8	31.2	53.6
T_3	85.7	52.9	35.3	26.1	46.9
T_4	78.2	55.6	32.8	22.4	42.8
T_5	81.2	50.6	38.8	22.8	47.3
T_6	78.3	53.2	31.4	20.6	42.8
Control (T_7)	66.2	46.9	22.3	16.2	39.2
LSD ($p \leq 0.05$)	9.03	4.08	5.82	2.97	3.22

Figure 1. The effect of PGPR transplant treatments on root behaviour during layering process in *Litchi chinensis* Sonn. cv. Early Large Red saplings, (**A**) Sand + AM fungi + *Azotobacter chroococcum* (1:2:1), (**B**) Litchi orchard soil + Sand (1:1), (**C**) Uninoculated control (*Sphagnum* moss), (**D**) hardening.

3.2. Rooting Characteristics

Highest fresh and dry weight of roots (11.2 g and 6.2 g) was achieved with PGPR transplant amendments media (T_2) followed by T_1, T_5, whereas lowest was recorded with 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 [12,13]. Besides, the production of plant growth regulating substances like auxins, cytokinins and gibberellins by PGPR probiotics interfere on resident soil microbial communities especially AM fungi by colonizing the roots causes increased root growth 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 [8].

3.3. Microbial Population and Soil Enzymes

In general, the resident microbial population count in rhizosphere and non-rhizosphere soils with the dual and or triple inoculation of different PGPR in air layered transplants was increased over uninoculated control. PGPR probiotics had significant effect on the total cultural microbial population both of the rhizosphere and non-rhizosphere zone. The microbial biomass estimated less than 5 per cent of propagules of total culturable

bacterial population in soil in terms of AM fungi, *A. chroococcum*, *Pseudomonas* and K-mobilizers were significantly higher in rhizosphere than non-rhizospheric zone. Among different treatment combinations, the respective plate count of *A. chroococcum*, *Pseudomonas sp*. and K-mobilizers also varied between the corresponding values of 13.0 × 10^6–25.7 × 10^6 cfu g^{-1}, 9.3 × 10^5–25.8 × 10^5 cfu g^{-1} and 9.7 × 10^4–19.6 × 10^4 cfu g^{-1} in rhizosphere and 9.9 × 10^6–10.8 × 10^6 cfu g^{-1}, 7.6 × 10^5–16.1 × 10^5 cfu g^{-1} and 8.1 × 10^4–16.2 × 10^4 cfu g^{-1} non-rhizospheric zone. Similarly, the propagules of AM fungi (per 50 g) ranged between 143.0, 282.8 and 102.0, 195.8 per 50 g of rhizosphere and non-rhizosphere moist soil (Figure 2).

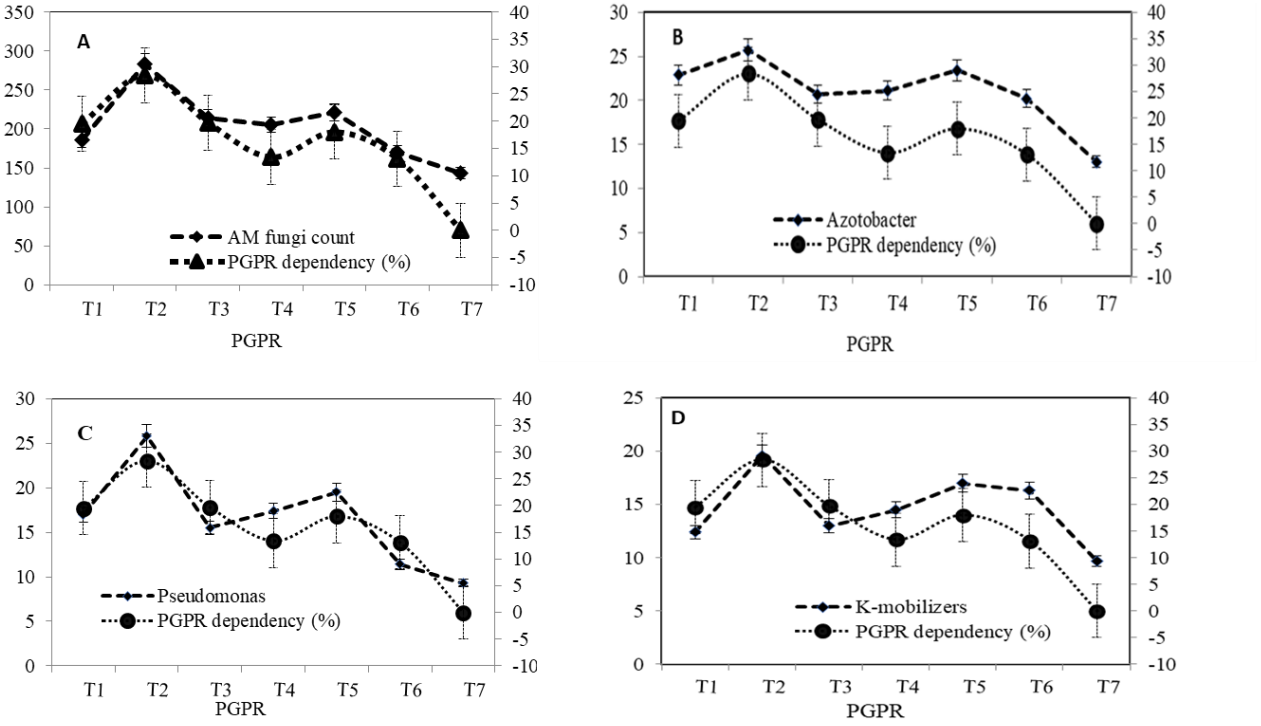

Figure 2. PGPR dependency and indigenous microflora affected by biological amendments in *Litchi chinensis* Sonn. cv. Early Large Red saplings.

In rhizosphere zone, acid phosphatase (AcP) activity was significantly higher in T_2 (163.2) followed by T_1 (149.3), T_3 (139.3) and T_4 (138.5) μg PNP g^{-1} h^{-1} than uninoculated control. Treatment T_2 when compared to uninoculated control, AcP activity was 1.65 folds within and 2.53 times more between rhizosphere and non-rhizosphere zone. The order of alkaline phosphatase (AlP) activity varied significantly among the PGPR treatments in rhizosphere 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-rhizosphere zone. In addition, dehydrogenases (DHA) activity showed the same trends with respect to AcP and AlP both in rhizosphere and non-rhizosphere zone. In rhizosphere, DHA activity expressed in terms of μg TPF g^{-1} h^{-1} was observed significantly higher in T_2 (11.5) followed by T_1 (10.6), T_3 (10.0) and T_4 (9.6) and with no significant differences between T_1, T_3, T_4 and T_5 treatments, whereas, it was least in T_7 (9.6). Similar trends of DHA activity were observed in non-rhizosphere zone with the orders $T_2 > T_1 > T_3 > T_4 > T_5$, however, the differences among these were not significant. The superior treatment of T_2 in rhizosphere for activities of AcP, AlP and DHA in layered transplants recorded 2.53, 2.08 and 30.2 times higher than non-rhizosphere uninoculated control, respectively (Figure 3). 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 [12]. The treatment of T_2 in rhizosphere for activities of AcP, AlP and DHA in layered transplants recorded 2.53, 2.08 and 30.2 times higher than non-rhizo-

sphere uninoculated control, respectively. Soil enzymatic bioassay was critically important for soil productivity which has provided indications of changes in metabolic capacity and nutrient cycling [14].

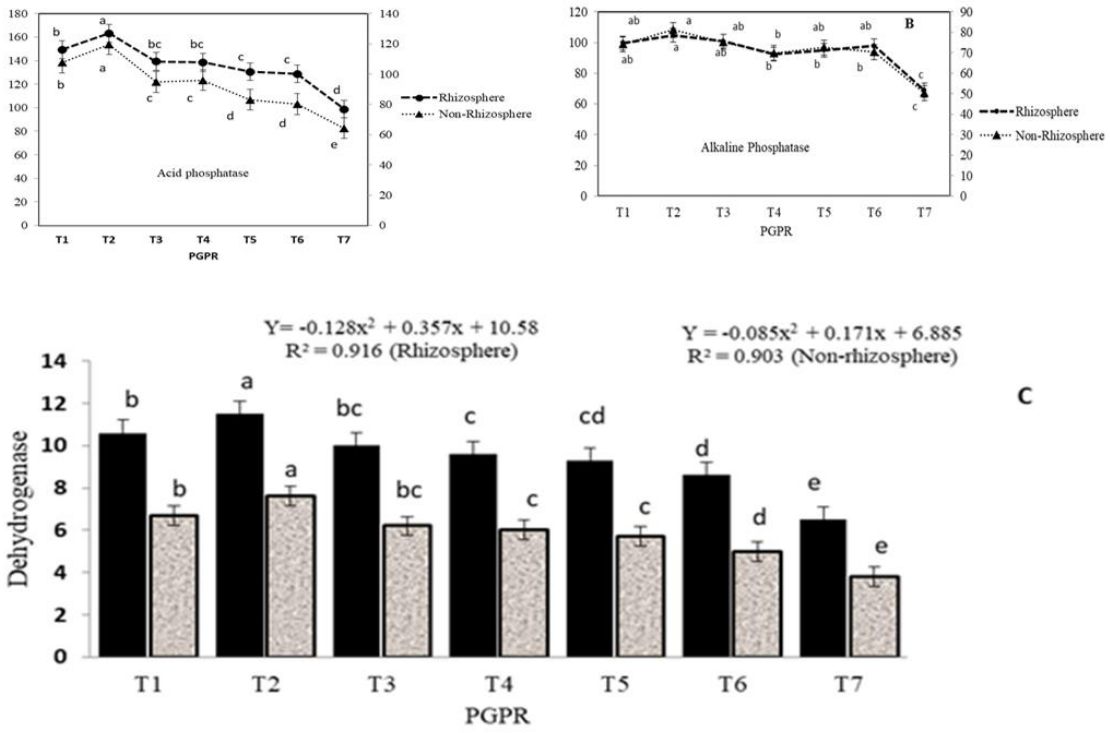

Figure 3. Soil enzymes affected by biological amendments in *Litchi chinensis* Sonn. cv. Early Large Red saplings.

4. Conclusions

Application of AM fungi + *A. chroococcum* + *Metarrhizium* (2:1:1) at hardening stage in nursery recorded maximum per cent survival (34.7%) of air layered litchi plantlets over control and better rooting, survival and the establishment of the layered *guttee* in soil. The study indicated PGPR transplant amendments coupled with soil solarization as a promising technology to maintain healthy rhizosphere in litchi at nursery stage which could be an innovative scientific approach with consistent outcome for the production of elite planting material in Shiwalik foothills of north-west Himalayas.

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References

1. Sharma, S.D.; Kumar, P.; Gautam, H.R.; Bhardwaj, S.K. Isolation of arbuscular mycorrhizal fungi and *Azotobacter chroococcum* from local litchi orchards and evaluate their activity in air-layers system. *Sci. Hortic.* **2009**, *123*, 117–123.
2. Sarita, B.P.; Kour, K.; Mehla, U.; Bhawana, S.S.; Jasrotia, A.; Bushan, B. Effect of different potting media on survival and growth of air layered litchi cv. Dehradun. *Int. J. Curr. Microbio. App. Sci.* **2019**, 8, 1196–1204.

3. Dinesh, R.; Anandaraj, M.; Kumar, A.; Srinivasan, V.; Bini, Y.K.; Subila, K.P.; Aravind, R.; Hamza, S. Effects of plant growth-promoting rhizobacteria and NPK fertilizers on biochemical and microbial properties of soils under ginger (*Zingiber officinale*) cultivation. *Agric. Res.* **2013**, *2*, 346–353.
4. Sasan, R.K.; Bidochka, M.J. The insect-pathogenic fungus *Metarhizium robertsii* (Clavicipitaceae) is also an endophyte that stimulates plant root development. *Am. J. Bot.* **2012**, *99*, 101–107.
5. Meyling, N.; Eilenberg, J. Ecology of the entomopathogenic fungi Beauveria bassiana and *Metarhizium anisopliae* in temperate agro-ecosystems: Potential for conservation biological control. *Biol. Cont.* **2007**, *43*, 145–155.
6. Hu, G.; St. Leger, R.A. Field studies using a recombinant mycoinsecticide (*Metarhizium anisopliae*) reveal that it is rhizosphere competent. *Appl. Environ. Microbiol.* **2002**, *68*, 6383–6387.
7. Bruck, D.J. Ecology of *Metarhizium anisopliae* in soilless potting media and the rhizosphere: Implications for pest management. *Biol. Cont.* **2005**, *32*, 155–163.
8. Behrooz, A.; Vahdati, K.; Rejali, F.; Lotfi, M.; Sarikhani, S.; Leslie, C.A. Arbuscular mycorrhiza and plant growth-promoting bacteria alleviate drought stress in walnut. *Hort Sci.* **2019**, 54, 1087–1092.
9. Forouzi, A.; Ghasemnezhad, A.; Ghorbani Nasrabad, R. Effects of growth stimulator microbes on growth and ions concentration of Stevia under salinity stress conditions. *Int. J. Hort. Sci. Tech.* **2019**, 6, 217–236.
10. Abbott, L.K.; Robson, A.D. Factors influencing the occurrence of VA-mycorrhizae. *Agric. Ecosyst. Environ.* **1985**, *35*, 121–150.
11. Zaferanchi, S.; Salmasi, S.; Salehi Lisar, S.; Sarikhani, M. Influence of organics and biofertilizers on biochemical properties of *Calendula officinalis* L. *Int. J. Hortic. Sci. Tech.* **2019**, 6, 125–136.
12. Sharma, S.D.; Devi, M.; Kumar, P.; Bhardwaj, S.K.; Raj, H. Potential use of bio-organic and inorganic nutrient source dynamics for improving cropping behavior, soil biological properties, nutrient content and quality attributes of apricot. *Commun. Soil Sci. Plant Anal.* **2011**, *42*, 1659–1674.
13. Sharma, S.D.; Kumar, P.; Bhardwaj, S.K.; Chandel, A. Symbiotic effectiveness of arbuscular mycorrhizal technology and Azotobacterization for citrus nursery management under soil disinfestations and moisture conservation mulch practices. *Sci. Hortic.* **2011**, *132*, 27–36.
14. Zhou, X.G.; Yu, G.B.; Wu, F.Z. Effects of intercropping cucumber with onion or garlic on soil enzyme activities, microbial communities and cucumber yield. *Eur. J. Soil Bio.* **2011**, 47, 279–287.