Biological Forum
an International Journal 17(8): 80-85(2025)
ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

# Influence of Biochar Amended Media on Root Development and Microbial Dynamics in Guava Propagation

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*(Received: 06 May 2025; Revised: 20 June 2025; Accepted: 16 July 2025; Published online: 07 August 2025)*
*(Published by Research Trend)*

**ABSTRACT: Propagation through softwood cuttings often suffers from low rooting efficiency, largely due to suboptimal nursery media conditions. Traditional substrates, such as red soil, sand and FYM, lack of adequate water retention, aeration and microbial support. Incorporating organic amendments such as cocopeat, tank silt, red soil, sand and biochar, particularly biochar derived from guava waste, has shown promise in improving media quality. Biochar enhances root zone aeration, nutrient retention and microbial activity. Inspite of its potential, few studies have assessed biochar-enriched substrates for guava propagation.**

**This study aims to evaluate the effectiveness of different biochar-based media on rooting and early growth of guava softwood cuttings, to establish a more sustainable and efficient propagation method.**

**The experiment was conducted at the nursery of SRM College of Agricultural Sciences, located in Baburayanpettai, Chengalpattu, Tamil Nadu, India, which experiences a tropical climate conducive to nursery operations. A Completely Randomized Design (CRD) was adopted with 5 treatment combinations and four replicates for each treatment. The treatment combinations (2:1:1) included different substrate mixtures: $T_1$ (Sand + Biochar + FYM), $T_2$ (Cocopeat + Biochar + FYM), $T_3$ (Red Soil + Biochar + FYM), $T_4$ (Tank Silt + Biochar + FYM) and $T_5$ Control (Red Soil + Sand + FYM). Each substrate was thoroughly mixed before planting guava softwood cuttings to ensure homogeneity. The results of the experiment were statistically analyzed using the General R-Based Analysis Platform Empowered by Statistics (GRAPES), developed by the Department of Agricultural Statistics, Kerala Agricultural University, Kerala (www.kaugrapes.com). Analysis of Variance (ANOVA) was performed to compare the means, using a significance level of $P \leq 0.05$.**

**Among the treatments, $T_2$ resulted in the highest plant height (36.34 cm), sturdy stem girth (4.74 mm), more number of leaves (4.66), maximum root volume (10.23 cm$^3$), and longest root length (24.07 cm). Both dehydrogenase activity DHA (1.08 µg TPF $g^{-1}$ $h^{-1}$) and microbial biomass carbon MBC (0.37 µg C $g^{-1}$) were also better in the media $T_2$ (Cocopeat + Biochar + FYM).**

**The study demonstrated that biochar-enriched growing media, particularly the combination of cocopeat, biochar, and FYM, significantly improved root development, vegetative growth, and microbial activity in guava (*Psidium guajava* L.) cv. Lucknow-49 softwood cuttings. This $T_2$ (Cocopeat + Biochar + FYM) media showed the highest rooting percentage with superior physiological performance, highlighting its potential for sustainable and efficient propagation of guava cuttings. These findings support the use of guava-derived biochar as a viable substrate amendment to enhance nursery production under tropical conditions.**

**Keywords:** Guava, Biochar, Media, Morphological traits, Dehydrogenase activity and Microbiome.

## INTRODUCTION

Guava (*Psidium guajava* L.), commonly known as the "Apple of the Tropics," is a highly valued tropical fruit cultivated widely in India. Guava, a crop belonging to the Myrtaceae family and native to tropical America, is rich in vitamin C and pectin, making it a nutritious and economically important fruit (Khairiyah *et al.*, 2022; Guntarti *et al.*, 2021). Among the several varieties grown in India, 'Lucknow-49' (L-49) is distinguished by its large fruit size, white pulp, few seeds and excellent flavour profile. This variety is appreciated for its extended post-harvest shelf life, regular market availability, and high yield potential, making it highly desirable for both fresh consumption and processing (Kumar and Sharma 2020; Kumar Sharma, 2019). As of 2024, guava cultivation in India spans approximately 3.59 lakh hectares, with an annual production of 5.59 million tonnes and a productivity range of 15–20 MT $ha^{-1}$ (APEDA, 2023). Additionally, India exported over 111.76,000 metric tonnes of guava in 2023–24 to countries such as Mexico, Saudi Arabia and Thailand (APEDA, 2024), underscoring its global commercial relevance.

Vegetative propagation is a crucial aspect of guava cultivation that maintains genetic uniformity and reduces the juvenile phase (Siddiqui *et al*., 2014). Air layering and stem cuttings, especially softwood to semi-hardwood cuttings, are widely practiced methods. Although air layering ensures high rooting success and genetic fidelity, it is limited by low propagation output (Pereira *et al*., 2017; Vilchez *et al*., 2011). Guava propagation through softwood cuttings under mist conditions supports large-scale multiplication and shorter establishment periods, although it requires precise environmental controls. The success of cutting propagation largely depends on the choice of rooting medium, which must balance aeration, moisture retention and nutrient availability. Substrates such as red soil, tank silt, sand, coco peat, biochar and farmyard manure (FYM) have shown significant potential for influencing root development and plant growth promotion (Patil *et al*., 2018; Bunt, 2019; Singh and Singh 2020; Yadav *et al*., 2017).

Furthermore, guava pruned residues, often considered agricultural waste, present an opportunity for sustainable waste management through biochar production. Biochar production through the pyrolysis of guava-pruned shoots in the absence of oxygen generates biochar, a stable, carbon-rich material known as 'black gold' that improves soil health by nutrient retention, adsorbing heavy metal toxins through its porous nature, enhancing water retention and stimulating microbial activity (Lehmann and Joseph 2015). Even though, growing interest in biochar applications, limited studies have explored its specific effects on root development during guava propagation. Existing research often focuses on mature plant growth, overlooking early-stage root architecture and establishment. The interaction between biochar and rhizosphere microbial communities in fruit crops like guava remains underexplored. Most findings are crop-specific, with few transferable insights into tropical fruit tree propagation. There is a need to understand how biochar amendments modulate soil biology and structure to enhance guava seedling vigour and rooting success. In this context, integrating guava propagation practices with biochar application offers a promising approach to sustainable horticulture and environmental stewardship (Steiner *et al*., 2007; Kammann *et al*., 2015).

## METHODOLOGY

*A. Experimental Site*

The experiment was conducted at the nursery complex of SRM College of Agricultural Sciences, Baburayanpettai, Chengalpattu, Tamil Nadu, India, which experiences a tropical and conducive climate for nursery level propagation. This study was conducted between January 2025 to July 2025. The location is positioned at a latitude of 12.70°N and a longitude of 79.97°E. The relative humidity in the area is 65 – 85 % and the average annual rainfall is 1200 mm. The temperatures in the region typically range from 27°C to 29°C.

*B. Experimental Design*

The experimental design was a Completely Randomized Design (CRD) comprising five treatment combinations (2:1:1 ratio) - dry weight (w/w) replicated four times: $T_1$ (Sand + Biochar + FYM), $T_2$ (Cocopeat + Biochar + FYM), $T_3$ (Red Soil + Biochar + FYM), $T_4$ (Tank Silt + Biochar + FYM) and $T_5$ Control (Red Soil + Sand + FYM). Softwood cuttings of *guava (Psidium guajava* L. cv. Lucknow-49 was selected for uniformity and planted in pre-mixed potting mixture homogenized substrates. The observation recorded plant growth parameters were analyzed using these protocols.

Plant height (cm) was measured from the soil surface to the apical tip of the main shoot using a graduated ruler (Alam *et al*., 2019), and stem girth (mm) was recorded 2 cm above ground level using a digital vernier calliper (Khan *et al*., 2021). The number of fully expanded green leaves per cutting was counted weekly (Raza *et al*., 2018). For root assessment, secondary roots were manually counted after gentle washing, and root length (cm) was recorded using the thread-and-scale method (Ali *et al*., 2022). Root volume (ml) was estimated using water displacement in a graduated cylinder (Singh and Meena 2017). The soil biological properties were analyzed post-harvest.

Dehydrogenase activity (µg TPF $g^{-1}$ $h^{-1}$), an indicator of microbial respiration, was quantified using the TTC (2,3,5-triphenyltetrazolium chloride) reduction method (Casida *et al*., 1964). After 24-hour incubation at 37°C, the triphenyl formazan (TPF) formed was extracted with methanol and the absorbance was measured at 485 nm using a UV-Vis spectrophotometer. Microbial biomass carbon (MBC, µg C $g^{-1}$ soil) was estimated using the chloroform fumigation–extraction method (Anderson and Domsch 1978; Vance *et al*., 1987). Fumigated and non-fumigated soil samples (10–15 g) were extracted with 0.5 M $K_2SO_4$ and digested with 0.1 N $K_2Cr_2O_7$ and concentrated $H_2SO_4$. Absorbance was measured at 600 nm, and MBC was calculated using the following equation: MBC = (C fumigated – C non-fumigated) / $K_ec$, where $K_ec$ = 0.38.

Data were statistically analyzed using the General R-Based Analysis Platform Empowered by Statistics (GRAPES), developed by the Department of Agricultural Statistics, Kerala Agricultural University, Kerala (www.kaugrapes.com). Analysis of Variance (ANOVA) was performed to compare the means, using a significance level of $P \leq 0.05$ (Gopinath *et al*., 2021).

## RESULTS AND DISCUSSION

*A. Plant Height (cm)*

Significant differences in plant height were observed among the different treatments. The highest plants were recorded in $T_2$ (Cocopeat + Biochar + FYM), with an average height of 36.34 cm, was recorded indicating superior vegetative growth. This was statistically on par with $T_4$ (Tank Silt + Biochar + FYM), which recorded an average height of 34.03 cm. In contrast, the shortest plants were observed in the treatment $T_5$ Control (Red Soil + Sand + FYM), with an average height of only 25.41 cm. These results suggest that the inclusion of cocopeat or tank silt along with biochar and FYM provided a better physical and nutrient environment for vertical-shoot growth development. This improvement may be due to enhanced moisture retention, aeration and nutrient availability in the media, which are known to promote shoot elongation in cuttings (Chandra *et al*., 2021).

*B. Stem Girth (mm)*

The thickest stems were recorded in $T_2$ (Cocopeat + Biochar + FYM) was (4.74 mm), which was statistically at par with $T_4$ (Tank Silt + Biochar + FYM) (4.56 mm) and $T_1$ (Sand + Biochar + FYM) at 4.48 mm. The thinnest stem girth (3.84 mm) was noted in the $T_5$ Control (Red soil + Sand+ FYM). The increased stem girth in $T_2$ (Cocopeat + Biochar + FYM) may attribute to enhanced nutrient availability and better root-shoot coordination fostered by cocopeat and biochar-enhanced media (Fig. 1). Stem thickening is a physiological response linked to auxin transport and assimilate partitioning, which improves under improved rooting and nutrient conditions (Bhargava *et al*., 2020). The porous structure of biochar enhances microbial colonization and mineralization, facilitating better nutrient uptake and supporting stem thickness.

*C. Number of Leaves (per cutting)*

Number of new leaf production, an indicator of vegetative vigour, was highest in $T_2$ (Cocopeat + Biochar + FYM), with an average of 4.66 new leaves per cutting. This was followed by $T_4$ (Tank Silt + Biochar + FYM) (3.98) and $T_1$ (Sand + Biochar + FYM) (3.23), which performed significantly better than the control. The lowest number of new leaves was observed in $T_5$ Control (Red soil + Sand + FYM) (2.15), indicating poor vegetative initiation in traditional media. Enhanced aeration and moisture retention in $T_2$ (Cocopeat + Biochar + FYM) likely contributed to improved metabolic and photosynthetic activities. According to Bera *et al*. (2019), leaf emergence is highly influenced by substrate porosity and microbial interactions, both of which are enhanced by the inclusion of biochar and organic matter.

*D. Number of Secondary Roots (per cutting)*

The number of secondary roots, a crucial parameter for anchorage and nutrient absorption, was also significantly influenced by the growth media. $T_2$ (Cocopeat + Biochar + FYM) recorded more number of roots (23.05), followed by $T_4$ (Tank silt + Biochar + FYM) (21.76) and $T_1$ (Sand + Biochar + FYM) (20.97), indicating effective root proliferation in media containing biochar and organic amendments. $T_5$ Control (Red soil + Sand + FYM) had a lesser root count (17.25), which may reflect suboptimal aeration and microbial support for root branching. Rhizospheric stimulation by Plant Growth – Promoting Rhizobacteria (PGPR), enhanced in biochar-rich substrates, promotes lateral root formation, which is consistent with prior reports on improved root traits under biochar application (El-Naggar *et al*., 2020).

*E. Root Volume (cm³)*

Root volume was greatest in $T_2$ (Cocopeat + Biochar + FYM) (10.23 cm³), which was statistically on par with $T_4$ (Tank Silt + Biochar + FYM) (9.85 cm³), and closely followed by $T_1$ (Sand + Biochar + FYM) (9.23 cm³). The lowest root volume was recorded in $T_5$ Control (Red soil + Sand + FYM) (8.15 cm³). A higher root volume implies better water and nutrient absorption capacity, and the improvement observed in $T_2$ (Cocopeat + Biochar + FYM) reflects the combined effect of cocopeat's water retention and biochar's porous structure, which enhances root development. Biochar promotes root architecture complexity by facilitating microbial symbiosis and buffering against environmental stresses, as noted by Bolan *et al.* (2024).

*F. Root Length (cm***)**

The lengthiest roots were observed in $T_2$ (Red soil + Biochar + FYM) (24.07 cm), followed by $T_4$ (Tank Silt + Biochar + FYM) (22.95 cm) and $T_1$ (Sand + Biochar + FYM) (21.57 cm). $T_5$ Control (Red soil + Sand + FYM) treatment had the shortest roots at 19.26 cm (Table 1). These findings indicate that treatments containing biochar facilitated deeper root penetration, likely due to improved soil structure and aeration. The influence of biochar on root elongation is supported by its role in reducing soil compaction and promoting beneficial microbial activity, both of which are essential for healthy root growth (Schmidt *et al*., 2015).

*G. Dehydrogenase Activity (µg* TPF *$g^{-1}$ $h^{-1}$)*

Dehydrogenase activity, a key indicator of microbial respiration and overall soil biological health, was highest in $T_1$ (Sand + Biochar + FYM) (1.08 µg TPF $g^{-1}$ $h^{-1}$) $T_4$ (Tank Silt + Biochar + FYM) (0.96 µg TPF $g^{-1}$ $h^{-1}$) and $T_1$ (Sand + Biochar + FYM) (0.74 µg TPF $g^{-1}$ $h^{-1}$) also showed considerable microbial activity compared to the $T_5$ Control (Red soil +Sand + FYM), which had the lowest activity (0.48 µg TPF $g^{-1}$ $h^{-1}$). The enhanced dehydrogenase activity in biochar-amended treatments suggests improved microbial colonization and organic matter utilization by the microbes. This agrees with the findings of Singha *et al*. (2022), who emphasized the sensitivity of dehydrogenase activity as a biological indicator of microbial metabolism under enhanced organic matter inputs.

*H. Microbial Biomass Carbon (MBC) (µg C $g^{-1}$)*

Microbial biomass carbon levels were significantly higher in $T_2$ (Cocopeat + Biochar + FYM) (0.37 µg C $g^{-1}$), followed by $T_4$ (Tank Silt + Biochar + FYM) (0.26 µg C $g^{-1}$), indicating greater microbial biomass and activity in these treatments than in the others. The lowest MBC was recorded in $T_5$ Control (Red Soil + Sand +FYM) at 0.05 µg C $g^{-1}$. The elevated MBC values in biochar-enriched media underscore the role of biochar as a microbial habitat that supports higher microbial loads and better nutrient cycling. The high surface area and porosity of biochar facilitate microbial retention and activity, thereby enhancing microbe-mediated nutrient dynamics and root–microbe interactions (Debnath *et al*., 2021).

**Table 1: Influence of Different Media for Plant Growth.**

| Treatments | Plant height (cm) | Stem girth (mm) | No. of new leaves | No. of secondary roots | Root volume ($cm^3$) | Root length (cm) | Dehydrogenase activity (µg TPF $g^{-1}$ $h^{-1}$) | Microbial biomass carbon (µg C $g^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| **$T_1$ - Sand + biochar + FYM** | 31.19 | 4.48 | 3.23 | 20.97 | 9.23 | 21.57 | 0.74 | 0.19 |
| **$T_2$ - Coco peat + biochar + FYM** | 36.34 | 4.74 | 4.66 | 23.05 | 10.23 | 24.07 | 1.08 | 0.37 |
| **$T_3$ - Red soil + biochar + FYM** | 28.3 | 4.23 | 2.87 | 18.73 | 8.75 | 20.33 | 0.61 | 0.11 |
| **$T_4$ - Tank silt + biochar + FYM** | 34.03 | 4.56 | 3.98 | 21.76 | 9.85 | 22.95 | 0.96 | 0.26 |
| **$T_5$ - Control (Red soil + sand + FYM)** | 25.41 | 3.84 | 2.15 | 17.25 | 8.15 | 19.26 | 0.48 | 0.05 |
| **SE(d)** | 1.56 | 0.24 | 0.20 | 1.14 | 0.41 | 1.22 | 0.04 | 0.009 |
| **CD** | 3.49 | 0.53 | 0.45 | 2.55 | 0.93 | 3.86 | 0.10 | 0.019 |

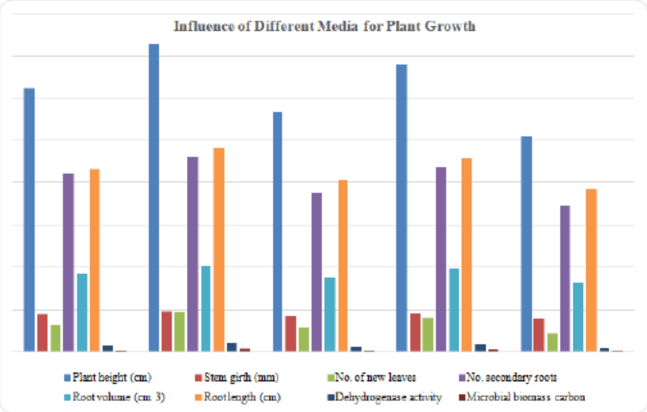


**Fig. 1.** Influence of Different Media for Plant Growth.

## CONCLUSIONS

The present study demonstrated that the composition of the growing media plays a critical role in the successful propagation of guava cv. Lucknow-49 through softwood cuttings. Among all treatments, the media comprising cocopeat, biochar and farmyard manure ($T_2$) significantly enhanced shoot growth, root development and soil biological activities, including dehydrogenase activity and microbial biomass carbon. The superior performance observed in this treatment can be attributed to improved substrate aeration, moisture retention, nutrient availability and a favourable microbial environment created by the synergistic interaction of its components. These results underscore the potential of guava-based biochar as a sustainable, value-added input in nursery media, contributing not only to plant growth but also to soil health. These findings open new avenues for environmentally sound propagation practices and advocate the recycling of orchard biomass for biochar production. However, further studies should be conducted to evaluate the long-term effects of biochar-based substrates on field performance, nutrient dynamics, and microbial ecology under diverse agro-climatic conditions in the future. Additionally, assessing the scalability of such media for commercial nursery use and organic cultivation systems remains an applicable area of future research.

## FUTURE SCOPE

The promising results achieved through biochar-amended growing media for softwood cuttings of guava (*Psidium guajava* L.) cv. Lucknow-49, several critical research gaps remain unaddressed. Notably, semi-hardwood and hardwood cuttings of guava are known to be more recalcitrant and physiologically less responsive to rooting compared to softwood cuttings. The limited rooting potential in these harder tissues is primarily attributed to their lower endogenous auxin levels, reduced meristematic activity and lignified vascular structure, which collectively hinder root initiation and emergence. Further investigation is needed to develop effective propagation techniques for these harder cutting types, possibly through advanced media formulations, optimized hormonal treatments, or microbial consortia that enhance rooting competency.
Future research should also focus on the interaction between biochar properties (e.g., feedstock type, pyrolysis temperature) and plant physiological responses to identify ideal combinations for different cutting types. Additionally, long-term studies under field conditions are essential to validate the transplant success, growth stability and soil health impacts of

biochar-based propagation systems. Exploring the molecular and anatomical mechanisms governing adventitious root formation in difficult-to-root cuttings could open new avenues for genetic and agronomic interventions. These efforts will be crucial in broadening the applicability of sustainable nursery technologies for commercial guava propagation and other woody fruit crops.

**Acknowledgement.** I would like to extend my heartfelt thanks to the Department of Fruit Science and Dept. of Natural Resources Management (Microbiology) Laboratory at SRM College of Agricultural Sciences in Chengalpattu, Tamil Nadu, for offering the essential facilities and support that enabled me to successfully conduct this research.
**Conflict of Interest.** None.

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**How to cite this article:** Nithiyasri S., Akino A., Gopu B. and Anbukkarasi Kaliyaperumal (2025). Influence of Biochar Amended Media on Root Development and Microbial Dynamics in Guava Propagation. *Biological Forum, 17*(8): 80-85.