



# Effect of Enriched Soilless Media on Corm Propagation of Red Banana

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## ABSTRACT

**Background:** Macropropagation is a farmer friendly technology complementing field sucker production. An investigation was conducted to examine the impact of biocontrol-enriched growing media on the corm propagation of red bananas in order to utilize the biopesticide or bionematicide potential in producing disease-free plantlets as well as the plant multiplication potential of soilless substrates.

**Methods:** Polybags containing sawdust, cocopeat, or a 50:50 mixture of sawdust and cocopeat media, enhanced with varying concentrations of Vesicular Arbuscular Mycorrhizal, *Bacillus subtilis* and *Pochonia*, were used to plant the decapitated and decorticated suckers.

**Result:** The growing medium consisting of cocopeat + sawdust (1:1) + VAM (30 g/corm) + *Pochonia chlamydosporia* (60 g/corm) produced the best results in terms of the number of days taken for first bud emergence (24.11 days), secondary bud emergence (46.34 days), tertiary bud emergence (67.35 days), total number of buds/ corm (9.54), plant height (73.56 cm) and girth of the pseudostem (12.34 cm). The plantlets regenerated from the same growing media showed superior performance for survival percentage, number of leaves/plant, plant height, pseudostem girth, number of roots, plant fresh and dry weight, chlorophyll content (SPAD) and total phenol content at 45 days of hardening. The population of *Bacillus subtilis* was found maximum ( $2.4 \times 10^7$  cfu/g) in the Cocopeat and Sawdust media enriched with *Bacillus subtilis* (30 g/corm) and *Pochonia chlamydosporia* (60 g/corm), while the population of *Pochonia chlamydosporia* ( $9 \times 10^7$  cfu/g) and higher root colonization (100.00%) was found maximum in the growing media Cocopeat + Sawdust (1:1) + VAM (30 g/corm) + *Pochonia chlamydosporia* (60 g/corm) at 120 days after planting.

**Key words:** Biocontrol agents, Colonization, Corm propagation, Growing media, Red banana.

## INTRODUCTION

Macro-propagation, or corm propagation, is promoted as an efficient alternative method that necessitates little capital and expertise to generate substantial quantities of superior grade banana seedlings. According to Njeri *et al.* (2010), scarification or the removal of the apical meristem of growing lateral buds, can increase the average number of seedlings produced by a single corm by three to four times, depending on the cultivar. This approach possesses the capability to connect the disparity between the requirement and availability of cost-effective, healthy banana seedlings. According to Kwa (2003), it serves as a viable substitute for both traditional and tissue culture methods. While, corm propagation techniques have been utilized for many cultivars of bananas in India, the potential of the red banana has not been fully realized. Macropropagation is cost effective, rapid propagation technique, where repression of apical meristem will stimulate the regeneration of lateral meristem (Uma *et al.*, 2008). So, for rapid production of quality planting material, macropropagation techniques can be adopted (Faturoti *et al.*, 2002). The physical composition of the soilless growth media has a significant impact on supply of water and air for successful plant growth (Beardsell and Nichols, 1982) as well as it improves anchorage, nutrient and water holding capacity of the

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medium (Dayarani *et al.*, 2013). Nematodes constitute a significant pest for bananas, with crop losses attributed to

them estimated at 30-60% (Sikora *et al.*, 2018). Sahoo (2016) observed that *Pochonia chlamydosporia*, a facultative parasite of root-knot nematodes can provide varying levels of control on populations of root-knot nematodes (*Meloidogyne* spp.). Combining the inoculation with other measures such as crop rotation and the use of resistant cultivars is likely to be needed if sufficient levels of control are to be obtained. Due to the growing demand for red banana planting materials, it is essential to rapidly produce cost-effective, disease-free and robust planting materials. This investigation aims to explore the plant multiplication capabilities of soilless substrates and the potential of biopesticides or bio-nematicides from biocontrol agents in generating disease-free plantlets of red banana through corm propagation techniques.

## MATERIALS AND METHODS

This experiment was carried out at SRM College of Agricultural Sciences, Chengalpattu during 2024-25. The corms of Red banana (1.0 to 1.5 kg) were carefully cleaned in tap water for 15 to 20 minutes. After removing the pseudostem and roots, the corms were scrapped off with sharp knife to eliminate the nematodes and other diseases, which spreads through soil. The prepared corms were planted polybags filled with cocopeat, sawdust or mixture of both as per the treatment mentioned. These were supplemented with Vesicular Arbuscular Mycorrhizal, *Bacillus subtilis* and *Pochonia chlamydosporia* in the necessary quantities as specified in the treatment details (Table 1). The corms were buried to a depth of 10 cm and were maintained in a shadenet providing 50 per cent shade and were regularly watered. Thirteen treatments and three replications were included in this completely randomized design (CRD) trial. The standard protocol of ICAR - National Research Centre for Banana, Tiruchirapalli, was followed for corm propagation.

### Observations recorded

The number of primary, secondary and tertiary buds per corm, the total number of plantlets per corm, plant height (cm), pseudostem girth (cm) and the number of days it takes for primary, secondary and tertiary bud emergence were recorded during the initiation period. The following plant morphological, physiological and biochemical parameters were measured at 45 days of hardening using secondary decapitated plantlets: survival percentage of hardened plants (per cent), plant height (cm), number of leaves/plant, pseudostem girth (cm), leaf area/plant (cm<sup>2</sup>), number of roots/plant, root length (cm), fresh and dry weight of plant (g/plant), total phenol content (mg/100 g) and chlorophyll content.

### Isolation and enumeration of biocontrol agents

The number of colony forming units of biocontrol agents in the treated growing media alone was studied. Microscopic and morphological analysis of the bioagents was

performed by culturing on specific culture media after 120 days of inoculation in the growing media. *Bacillus* endophytes were isolated from healthy roots by the method described by Zinniel *et al.* (2002) with slight modifications and the antagonists were isolated by serial dilution technique using Nutrient Agar (NA) medium. The colony forming units (CFU) of *Pochonia chlamydosporia* were determined using serial dilution technique with Potato Dextrose Agar (PDA) as culture media following the procedure of Sindhu *et al.* (2019). The root samples were collected from the VAM treated polybags were processed as per Phillips and Hayman (1970). Root colonization was assessed using Giovannetti and Mosse's Grid Line Intersect Method (1980).

### Statistical procedures and analysis of data

The data was subjected to analysis of variance (ANOVA) following the approach suggested by Panse and Sukhatme (1967). At a 5% level of significance, critical difference values were computed, especially in cases where the 'F' test yielded a significant result.

## RESULTS AND DISCUSSION

Table 1 shows the effects of growing media and biocontrol agents on the number of primary, secondary and tertiary buds per corm. The primary bud (22.77 days), secondary bud (46.34 days) and tertiary bud (67.35 days) appeared much faster in corms planted in T12 [Cocopeat + Sawdust (1:1) + VAM (30 g/corm) + *Pochonia chlamydosporia* (60 g/corm)]. However, primary bud emergence (28.33 days), secondary bud emergence (66.48 days) and tertiary bud emerging (92.46 days) took longer in T13 (Control). The highest number of primary buds/corm (1.53) was found in treatments T12 and T11, followed by 1.43 in treatments T8, T2 and T1. The minimum primary buds/corm (1.00) were seen in T13 (Control). Treatment T12 produced more number of secondary buds/corm (3.67), followed by T8 and T4 (3.33). In both T7 and T13, a minimum of 1.67 secondary buds/corm was noted. The treatment with the largest total number of buds/corm (9.54) was T12; followed by T8 and T11 with 8.43 and 7.75, respectively. T13 (Control), on the other hand, had the fewest buds overall (4.23) per corm. There was a significant variation between the different treatments in the data pertaining to plant height (cm) and pseudostem girth (cm). The height (73.56 cm) and girth (12.34 cm) were highest in treatment T12, followed by T11 and T8. On the other hand, treatment T13 [Control] had the smallest plant height (37.89 cm) and pseudostem girth (5.59 cm).

### Performance of regenerated plantlets at hardening stage

The data corresponding to the morphological parameters at hardening stage indicated significant difference among the treatments (Table 2). The highest survival (100.00%) was recorded in T12 followed by T11 and T8, which recorded 99.08% and 98.06% respectively. The treatment T13 (Control) exhibited a low survival (75.61%). The plants

**Table 1:** Impact of biocontrol agents and growth media on the morphological traits of corm-propagated Banana cv. Red Banana (Days).

| Treatments | Days taken for emergence |               |              | No. of buds/corm |           |          | Total buds/corm | Plant height (cm) (90 DAP) | Pseudostem girth (cm) (90 DAP) |
|------------|--------------------------|---------------|--------------|------------------|-----------|----------|-----------------|----------------------------|--------------------------------|
|            | Primary bud              | Secondary bud | Tertiary bud | Primary          | Secondary | Tertiary |                 |                            |                                |
| T1         | 25.22                    | 61.63         | 88.56        | 1.43             | 3.00      | 1.77     | 6.21            | 57.00                      | 7.16                           |
| T2         | 25.55                    | 53.66         | 79.86        | 1.43             | 2.00      | 2.66     | 6.10            | 59.65                      | 6.42                           |
| T3         | 25.00                    | 59.78         | 86.32        | 1.10             | 2.33      | 2.44     | 5.87            | 43.39                      | 7.31                           |
| T4         | 25.11                    | 55.46         | 76.54        | 1.20             | 3.33      | 2.66     | 7.20            | 63.17                      | 8.34                           |
| T5         | 24.77                    | 60.64         | 81.32        | 1.20             | 2.00      | 2.66     | 5.87            | 45.48                      | 6.89                           |
| T6         | 24.77                    | 62.31         | 83.47        | 1.23             | 2.33      | 2.11     | 5.67            | 58.07                      | 8.11                           |
| T7         | 24.66                    | 57.34         | 80.21        | 1.23             | 1.67      | 2.55     | 5.46            | 56.33                      | 6.15                           |
| T8         | 26.22                    | 49.54         | 70.53        | 1.43             | 3.33      | 3.66     | 8.43            | 69.18                      | 10.36                          |
| T9         | 25.88                    | 58.67         | 82.78        | 1.20             | 3.00      | 2.77     | 6.98            | 49.65                      | 8.46                           |
| T10        | 24.66                    | 55.78         | 73.12        | 1.10             | 2.00      | 3.00     | 6.10            | 67.13                      | 7.45                           |
| T11        | 24.11                    | 50.47         | 75.56        | 1.53             | 2.33      | 3.88     | 7.75            | 70.46                      | 11.45                          |
| T12        | 22.77                    | 46.34         | 67.35        | 1.53             | 3.67      | 4.33     | 9.54            | 73.56                      | 12.34                          |
| T13        | 28.33                    | 66.48         | 92.46        | 1.00             | 1.67      | 1.55     | 4.23            | 37.89                      | 5.59                           |
| S.E(d)     | 0.77                     | 4.54          | 2.36         | 0.10             | 0.19      | 0.40     | 0.50            | 1.35                       | 0.18                           |
| CD at 5%   | 1.58                     | 9.35          | 4.86         | 0.20             | 0.40      | 0.81     | 1.02            | 2.79                       | 0.37                           |

T1: Cocopeat + *Bacillus subtilis* (30 g/corm)T2: Cocopeat + *Bacillus subtilis* (30 g/corm) + *Pochonia chlamydosporia* (60 g/corm)

T3: Cocopeat + VAM (30 g/corm)

T4: Cocopeat + VAM (30 g/corm) + *Pochonia chlamydosporia* (60 g/corm)T5: Saw dust + *Bacillus subtilis* (30 g/corm)T6: Saw dust + *Bacillus subtilis* (30 g/corm) + *Pochonia chlamydosporia* (60 g/corm)

T7: Saw dust + VAM (30 g/corm)

T8: Saw dust + VAM (30 g/corm) + *Pochonia chlamydosporia* (60 g/corm)T9: Cocopeat + Sawdust (1:1) + *Bacillus subtilis* (30 g/corm)T10: Cocopeat + Sawdust (1:1) + *Bacillus subtilis* (30 g/corm) + *Pochonia chlamydosporia* (60 g/corm)

T11: Cocopeat + Sawdust (1:1) + VAM (30 g/corm)

T12: Cocopeat + Sawdust (1:1) + VAM (30 g/corm) + *Pochonia chlamydosporia* (60 g/corm)

T13: Control [Cocopeat + Sawdust (1:1)]

**Table 2:** Performance of the secondary decapitated plantlets of Banana cv. Red Banana at 45 days of hardening.

| Treatments | Survival percentage | Plant height (cm) | Pseudostem girth (cm) | Number of leaves /plant | Leaf area/ plant (cm <sup>2</sup> ) | Number of roots/ plant | Root length (cm) | Fresh weight of plant (g/plant) | Dry weight of plant (g/plant) | Chlorophyll content (SPAD value) | Total phenol content (mg 100g) |                          |
|------------|---------------------|-------------------|-----------------------|-------------------------|-------------------------------------|------------------------|------------------|---------------------------------|-------------------------------|----------------------------------|--------------------------------|--------------------------|
|            |                     |                   |                       |                         |                                     |                        |                  |                                 |                               |                                  | Growing media (90 DAP)         | Hardening media (45 DAP) |
| T1         | 90.80               | 63.00             | 10.60                 | 4.26                    | 33.27                               | 52.06                  | 30.40            | 167.36                          | 24.20                         | 38.36                            | 83.33                          | 97.23                    |
| T2         | 96.45               | 69.30             | 12.10                 | 5.12                    | 53.95                               | 50.35                  | 23.54            | 150.07                          | 13.70                         | 40.84                            | 69.46                          | 90.30                    |
| T3         | 98.04               | 75.60             | 12.70                 | 4.26                    | 43.39                               | 44.38                  | 15.69            | 129.47                          | 12.50                         | 39.54                            | 76.40                          | 104.16                   |
| T4         | 95.61               | 69.60             | 13.20                 | 6.68                    | 59.36                               | 53.49                  | 33.67            | 176.54                          | 21.40                         | 43.17                            | 90.26                          | 111.10                   |
| T5         | 88.55               | 68.00             | 13.40                 | 4.96                    | 45.48                               | 61.47                  | 21.11            | 181.99                          | 26.50                         | 40.18                            | 104.16                         | 118.06                   |
| T6         | 91.14               | 79.60             | 11.54                 | 5.58                    | 58.07                               | 58.63                  | 60.58            | 197.60                          | 15.70                         | 34.11                            | 97.23                          | 131.93                   |
| T7         | 95.12               | 73.30             | 11.60                 | 4.77                    | 33.09                               | 51.58                  | 39.79            | 185.57                          | 24.00                         | 39.52                            | 111.10                         | 125.00                   |
| T8         | 98.06               | 72.40             | 14.49                 | 6.68                    | 69.18                               | 67.82                  | 29.59            | 237.70                          | 37.50                         | 44.56                            | 131.93                         | 173.60                   |
| T9         | 93.72               | 69.80             | 13.70                 | 5.72                    | 46.27                               | 64.00                  | 44.90            | 178.59                          | 30.50                         | 38.55                            | 118.06                         | 152.76                   |
| T10        | 97.60               | 70.50             | 12.30                 | 7.64                    | 69.47                               | 55.40                  | 64.29            | 187.06                          | 19.50                         | 38.77                            | 125.00                         | 159.73                   |
| T11        | 99.08               | 79.10             | 14.70                 | 6.68                    | 74.56                               | 69.73                  | 13.26            | 260.63                          | 39.20                         | 47.08                            | 138.90                         | 187.50                   |
| T12        | 100.00              | 87.30             | 15.60                 | 8.59                    | 76.32                               | 72.59                  | 39.79            | 362.15                          | 44.60                         | 49.97                            | 145.83                         | 194.43                   |
| T13        | 75.61               | 57.30             | 10.39                 | 4.77                    | 33.27                               | 45.85                  | 26.53            | 147.39                          | 16.50                         | 33.30                            | 62.50                          | 83.33                    |
| S.E(d)     | 1.57                | 5.58              | 1.00                  | 0.42                    | 1.21                                | 4.32                   | 2.09             | 10.82                           | 1.82                          | 1.51                             | 7.53                           | 9.57                     |
| CD at 5%   | 3.23                | 11.47             | 2.07                  | 0.86                    | 2.48                                | 8.89                   | 4.30             | 22.25                           | 3.74                          | 3.11                             | 15.48                          | 19.69                    |

regenerated from treatment T12 recorded maximum plant height (87.30 cm) and pseudostem girth (15.60 cm), number of leaves per plant (8.59) and leaf area per plant (76.32 cm<sup>2</sup>), number of roots (72.59), while the treatment T13 [Control] recorded minimum plant height (57.30 cm) and pseudostem girth (10.39 cm), number of leaves per plant (4.77), number of roots per plant (45.85), while T7 recorded minimum leaf area per plant (33.09 cm<sup>2</sup>).

The length of root was the highest (64.29 cm) in the plantlets regenerated from T10 followed by (T6) which recorded (60.58 cm). Smallest root length (13.26 cm) was recorded in the T11. The fresh weight (362.15 g/plant) and dry weight (44.60 g/plant) of plant was maximum in plantlets regenerated from T12 followed by T11 which registered 260.63 g/plant and 39.20 g/plant respectively. T3 registered minimum fresh weight (129.47 g/plant) and dry weight (12.50 g/plant) of the plant.

#### Physiological and biochemical parameters

The chlorophyll content recorded with SPAD meter was maximum (49.97) in the treatment T12 followed by T11 and T8 with 47.08 and 44.56 respectively. The minimum value (33.30) was recorded in T6. The mean values for total phenol content ranged from 145.83 to 62.50 mg/100 g among the treatments during the corm propagation in initiation media. The mean values for total phenol content ranged from 194.43 to 83.33 mg/100 g among the treatments during hardening stage. The maximum value was recorded in T12, while minimum value was recorded in the control at both the stages.

#### Microbial population of *Bacillus subtilis* (cfu/g of soil)

*Bacillus subtilis* (population in the rhizosphere of Red Banana (120 DAP) was enumerated in the treatment T1, T5, T10 and T13. The maximum colony forming units of *Bacillus subtilis* ( $2.4 \times 10^7$  cfu/g) was recorded in T10 followed by T1 and T5 with the population of  $6 \times 10^6$  cfu/g and  $3.2 \times 10^6$  cfu/g respectively. The uninoculated control [T13] also recorded ( $1.7 \times 10^6$  cfu/g) the colony forming units of *Bacillus subtilis* (Table 3).

#### Microbial population of *Pochonia chlamydosporia*

The microbial population of *Pochonia chlamydosporia* (cfu/g of soil) in the rhizosphere of Red Banana (120 DAP) was enumerated in the treatment T8, T10, T12 and T13. The maximum ( $9 \times 10^7$  cfu/g) and minimum ( $1.1 \times 10^6$  cfu/g) colony forming units was recorded in T12 and T8 respectively.

#### Root colonization of VAM (%)

The root colonization (%) of VAM in the inoculated growing media (T3, T4, T7, T8, T11 and T12) was examined and compared with the control (T13). Root colonization was increased upon inoculation with VAM fungus in all the inoculated growing media (Table 4). Higher root colonization (100.00%) was seen in the growing media T12 and followed by T8. The minimum number of root colonization

was recorded (77.66%) in T7. Root colonization was not observed in control (T13).

#### Influence of growing media on corm multiplication of Banana cv. Red Banana

The physical composition of the growing medium has a significant influence on the availability of air and water, which are essential for effective plant growth (Beardsell and Nichols, 1982), in addition to enhancing anchoring, nutrient retention and water holding capacity (Dayarani *et al.*, 2013). The results indicated that there were notable differences among the treatments regarding the time taken for bud initiation in the corm. The earliest emergence of buds has been seen in the media comprising cocopeat and sawdust enriched with VAM (30 g/corm) and *Pochonia chlamydosporia* (60 g/corm). Similar result was given by Oselebe *et al.* (2008), who reported that the fastest method of producing plantlets for *Musa species* at the farm level relies on soilless media. In the current study, the bud emergence was observed within one month of planting. Sannigrahi *et al.* (2017) also reported that, banana varieties Grand Naine and Bagda took 19.75 days and 28.25 days to produce primary shoots. Sawdust is best initiation media for macro-propagation of Banana (Sudeshna *et al.*, 2015), while, Pujar *et al.* (2017) reported, Cocopeat serves as the ideal growing medium for the macro-propagation of 'Malbhog' Banana.

Growing medium are regarded as essential factors in regulating both the physiological patterns and morphological characteristics of several plants. The media comprising

**Table 3:** Microbial population of *Bacillus subtilis* and *Pochonia chlamydosporia* in the rhizosphere of Banana cv. Red Banana in growing media (120 DAP).

| Treatment                             | cfu/g of soil     |
|---------------------------------------|-------------------|
| <b><i>Bacillus subtilis</i></b>       |                   |
| T1                                    | $6 \times 10^6$   |
| T5                                    | $3.2 \times 10^6$ |
| T10                                   | $2.4 \times 10^7$ |
| T13                                   | $1.7 \times 10^6$ |
| <b><i>Pochonia chlamydosporia</i></b> |                   |
| T8                                    | $1.1 \times 10^6$ |
| T10                                   | $2.4 \times 10^7$ |
| T12                                   | $9 \times 10^7$   |
| T13                                   | 0                 |

**Table 4:** Root colonization (per cent) of VAM in growing media during corm propagation of Banana cv. Red Banana (120 DAP).

| Treatment | Root colonization % |
|-----------|---------------------|
| T3        | 78.45               |
| T4        | 78.10               |
| T7        | 77.66               |
| T8        | 99.33               |
| T11       | 98.33               |
| T12       | 100.00              |
| T13       | 0.00                |

cocopeat and sawdust enriched with VAM (30 g/corm) and *Pochonia chlamydosporia* (60 g/corm) has produced more primary (1.53), secondary (3.67), tertiary (4.33) and total buds per corm (9.54) than other treatment. This study shows that suppressing apical dominance promotes the rapid growth of tiny buds as sprouts, which later mature into high-quality suckers.

In this study, it was observed that the plants/corm was significantly higher in all treatments that were enriched with VAM alone or in conjunction with *Pochonia chlamydosporia*. This results from their mutualistic relationship with vascular plants, facilitating the absorption and assimilation of less soluble and unavailable elements, such as phosphorus, zinc and copper, from the rhizosphere, thereby enhancing plant growth and productivity (Neelima *et al.*, 2002). According to Kiran (2018), 9.80 plantlets per corm were produced when Red Banana was macropropagated using a combination of sawdust, cocopeat and potting media.

#### Morphological characteristics in growth/initiation media

The growing media enriched with VAM and *Pochonia chlamydosporia* exhibited the highest morphological traits, including pseudostem girth (12.34 cm) and plant height (73.56 cm). The pseudostem of the banana plant is made up of leaf sheaths, which are most prominent at the collar. This trait affects the quantity of leaves, the pseudostem's circumference and the plant's total vigor (Blomme *et al.*, 2003). The treatments including VAM and *Pochonia chlamydosporia* exhibited a substantial shift in morphological characteristics due to the infection and proliferation of endophytic AMF fungi and *Pochonia chlamydosporia* within the root system. While the vesicles, which resemble sacs, store phosphorus as phospholipids, the root system receives nutrients from the soil through the arbuscules. Bagyaraj (1984) observed the enhanced development of plants attributable to the connection with arbuscular mycorrhizae (AM). Additionally, microelements such as zinc are likely involved, whereas arbuscular mycorrhizal fungi obtain photosynthetic carbon from their host (Smith and Smith, 2011). In the current research, the growth medium supplemented with *Bacillus subtilis* demonstrated enhanced plant height and increased pseudostem girth. This may result from enhanced nutrient absorption, root development and plant proliferation facilitated by *Bacillus subtilis* (Arunkumar and Kiruthika, 2021). *Bacillus subtilis* has been recognized as a biofertilizer, phytoestimulator and biopesticide (Perez-Montano *et al.*, 2014). Mingot-Ureta *et al.* (2020) demonstrated that *Pochonia chlamydosporia* can endophytically colonize banana roots, significantly promoting root, corm and leaf growth in banana plantlets. The study highlighted that *Pochonia chlamydosporia* enhances plant growth parameters, such as root and leaf length and weight, particularly when inoculated with conidia and chlamydospores.

#### Effect of growing media and biocontrol agents on plant characteristics during hardening

In the current study, plant growth characteristics such as survival percentage, plant height, number of leaves, roots, pseudostem girth, leaf area/plant, root length, fresh and dry weight of the plant at 45 days of hardening were improved by the inoculation of *Bacillus subtilis*, *Pochonia chlamydosporia* and VAM in the growing or initiation media. The endophytic characteristics of biocontrol agents enable their existence within the plant host, encompassing both aerial and subterranean plant components, thereby enhancing plant growth (Chebotar *et al.*, 2015). Endophytic bacteria that can promote the growth of a variety of plants, such as wheat, rice, canola, potatoes and tomatoes, have been reported in many research investigations (Mei and Flinn, 2010).

In the present study, the plantlets regenerated from VAM-inoculated sawdust and sawdust combined with cocopeat, either alone or in combination with *Pochonia chlamydosporia*, exhibited enhanced plant attributes recorded in this study. This aligns with the findings of Swamy *et al.* (2005) and Singhal *et al.* (2012), which enhances the absorption of phosphorus through the VAM's extensive hyphal network and also facilitates the uptake of zinc, copper, iron, manganese and other elements.

In present study, the increased fresh weight and dry weight of the plant was observed in the plantlets regenerated from the VAM inoculated media. This is due to the fact that essential nutrients are better absorbed when VAM colonizes plant roots (Allen *et al.*, 2003). Increased biomass is the outcome of improved plant growth and vigor. These results closely match with the report of Tanvar *et al.* (2013) and Ghori *et al.* (2014). The significant growth parameters during the hardening stage of plantlets regenerated in VAM + *Pochonia chlamydosporia* inoculated media are associated with microbes's endophytic behavior, encompassing the synthesis of hydrolytic enzymes, transporters, proteases, chitinases and a plethora of secondary metabolites (Larriba *et al.*, 2014). The current study's improved growth characteristics attributed to the endophytic behavior of *Pochonia chlamydosporia* corroborates with Dallemole-Giaretta *et al.* (2015).

Plantlets regenerated from the *Bacillus subtilis* supplemented growing media either alone or in combination with *Pochonia chlamydosporia* also showed enhanced shoot and root characteristics at hardening stage. *B. subtilis* acts as a biostimulant by generating phytohormones, auxin and cytokinin, which support plant growth and development. It also forms a thin biofilm on roots enabling extended colonization of the rhizosphere (Zubair *et al.*, 2019).

In accordance to the current study, better photosynthetic efficiency of the leaves is a result of increased chlorophyll levels, more leaves per plant and larger leaf area per plant in all media treated with bio-agents. The elevated chlorophyll concentration serves as compelling evidence for the development of disease resistance due to the application

of endophytic biocontrol agents, constituting one of the observable indicators (Abdelaziz *et al.*, 2022). Similar reports were given by Thakur and Jasrai (2002) in micropropagated banana. Spore inoculation of *Pochonia chlamydosporia* strains from diverse origin (Pc21, Pc123, Pc399 and Pccat), significantly increase root, corm and leaf length and weight in banana plantlets (Mingot-Ureta *et al.*, 2020).

An increase in phenolic content was noted across all treatments in conjunction with biocontrol agents, specifically *Bacillus subtilis*, *Pochonia chlamydosporia* and VAM, whether administered alone or in combination. The biosynthesis of phenolic compounds from phenylalanine is a metabolic alteration that consistently occurs in infected plants. The swift elevation in phenolic concentrations has also been shown in plants following the inoculation of beneficial microorganisms in response to disease threats (Singh *et al.*, 2011).

### Microbial population

Corm regeneration of Red Banana, growth parameters, chlorophyll content, total phenol content and disease free plantlets are directly correlated with the composition of the rhizobial microbes present in the growing or initiation media. *Bacillus subtilis* and *Pochonia chlamydosporia* population was found higher in T12 after 120 days of planting. Organic growth media can enhance antagonistic colonization in the Banana rhizosphere by serving as a nutrient source for bacteria and fungi involved in biocontrol, hence raising soil microbial biomass and their activity lead to the generation of disease-free plantlets (Janvier *et al.*, 2007). This result was in line with other studies involving Banana (Xue *et al.*, 2015). The microbial population observed in the control might be the naturally available lignin and cellulose degrading bacteria (Dewi *et al.*, 2020).

Root colonization by VAM was recorded in all the inoculated growing media. Fungal colonization of organic growth media is frequent (Koohakan *et al.*, 2004); however, in this investigation, the complete root colonization seen in treatment T12 may be attributed to the enhanced porosity of the sawdust and cocopeat (Corkidi *et al.*, 2004). Comparable findings of enhanced root colonization by VAM were documented by Kiran (2018) in Red Banana and Chandalinga *et al.* (2013) in turmeric.

### CONCLUSION

Macropropagation presents an economical alternative with significant potential for producing high-quality planting material in bananas. It has been found that adding materials biocontrol agents to the soilless substrate has enhanced the regeneration, growth and development of plantlets and raising the field survival rate.

### Compliance with ethical standards

#### Ethical issues

None.

#### Conflict of interest

The authors have disclosed no conflicts of interest.

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