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Synergistic Effect of Different Soilless Substrates on the Hardening of *In-vitro* Raised Banana (*Musa* sp.) Saplings of Grand Naine

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The present study was carried out to investigate the impact of different substrates on the primary and secondary hardening of tissue culture raised banana plantlets. Among eight different treatment combinations of potting mixtures, treatment PHT7 which contained cocopeat with the combination of perlite and vermiculite (2:1:1) emerged as the best potting media with the survival percentage of

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98.90% with comparative better growth during primary hardening. Simultaneously maximum growth and development including plant height (15.36 cm), number of leaves (5.71), pseudostem girth (3.88 cm), maximum number of roots (5.90), maximum root length (3.69 cm) has been observed during primary hardening. For secondary hardening, eight combinations of substrates were used in conjunction with soil. Among all the treatments in the secondary hardening, SHT4 (soil+ bonemeal; 3:1) along with the application of Jeevamrit have shown highest field survivability (100%). Moreover a maximum shoot length (33.40 cm) has been observed during secondary hardening with maximum number of leaves were recorded (9.25) with pseudo stem girth 6.98 cm. The outcome of the present study suggested that the treatment PHT7 performed better growth during primary hardening. However the treatment SHT4 has shown comparatively higher growth during secondary hardening.

Keywords: Banana; in-vitro; potting media; jeevamrit; primary hardening; secondary hardening.

1. INTRODUCTION

Banana (*Musa sp.*) belongs to the family *Musaceae* and ranks as an important and leading fruit crop of various sub-tropical and tropical climatic conditions with abundant amount of vitamins and minerals [1]. The current global banana production was estimated to be around 31504 million metric tons having total area of 878 ha [2]. For large scale production of banana with healthy and disease free planting material, *in-vitro* multiplication has proved to be a boon with high yield over the conventional method of banana production [3]. The *in-vitro* raised banana plantlets are exposed to multiple stress conditions after field transplantation.

1.1 Tisuue Culture Banana Plantlets

Due to the consistent yield and disease-free nature, *in-vitro* plantlets are increasingly being used as planting material [4]. Numerous commercial tissue culture facilities have been built in India in order to meet the demand for banana plantlets. In 2016-17, nearly 28.607 million tissue culture banana plants produced by Indian tissue culture units. Farmers in India are adopting tissue culture banana plants despite knowing that the initial cost is high because of their benefits in terms of high yield, better quality, homogeneity, disease free plantlets and good market price [5].

Micropropagation is a technique that allows rapid multiplication of disease-free planting material with high quality and uniformity in planting material irrespective of weather and season. But high mortality rate has been recorded by micropropagated plantlets during lab-to-land transfer which is the major limitation in adopting the technique in large scale [6]. Acclimatization of *in-vitro* grown plantlets to natural environment before transplantation of plants to the field is known as hardening. The *in-vitro* plantlets need to be hardened before transplanting by providing them water, shade, nutrients, light and potting media in semi- controlled conditions so that they can withstand natural climatic conditions [7].

1.2 Acclimatization and Hardening

Acclimatization is the most important step in the development of tissue culture plants. The *in-vitro* raised plantlets are exposed to different stress factors when they are transferred to the field [8]. The use of the best growth media and bio stimulants during acclimation could help in some extent to overcome these stress conditions [9].

Along with other growth medium and substrates, Jeevamrit act as a natural bio-fertilizer that can improve the fertility and nutrient availability of soil and can stimulate the root-shoot growth. It activates the microorganisms present in the rhizosphere such as various NPK fixers and solublizers [9,10].

2. MATERIALS AND METHODS

2.1 Site of Experiment

The present study was investigated under the Centre For Tissue Culture Technology, Jacob Institute of Biotechnology and Bio-Engineering, Sam Higginbottom University of Agriculture Technology and Sciences, Prayagraj, Uttar Pradesh for a period of 6 months in greenhouse conditions. *In-vitro* plantlets were taken from the tissue culture laboratory and were subjected to hardening and acclimatization by providing different treatments combinations to get maximum survival and its growth. The experiment was laid out under completely randomized design with the following objectives:

- To develop the efficient potting mixture (combination+ concentration) for the hardening of *in- vitro* raised banana saplings.
- To assess the effect of Jeevamrit on hardening of tissue culture raised banana saplings.

2.2 Experimental Details

During primary hardening the pro trays were filled with different treatment combinations as shown in Table 1.

After primary hardening of banana plantlets, they were transferred to secondary hardening growth media as given in Table 2.

2.3 Hardening of *In-vitro* Plantlets

The plantlets were acclimated in various substrates, including cocopeat mixed with different proportions of sand, soil, vermicompost, vermiculite, and perlite in the pro trays. The plantlets were transferred into a greenhouse under semi-controlled conditions for 3-weeks. Further the morphological characteristics were observed such as; root length, shoot length, number of leaves, number of roots, pseudostem girth with the help of measuring scale. The plantlets were further transferred into polythene bags for secondary hardening, which included soil and potting mixtures (bonemeal, neem cake, and vermicompost). The morphological parameters has been observed to elucidate the synergistic effect of Jeevamrit with the potting media on *in-vitro* raised banana plantlets during secondary hardening.

2.4 Data Analysis

Five percent level of significance was used to test the "null hypothesis" for the significance of results. The data was statistically analyzed using the analysis of variance (ANOVA) [11]. Values presented are having SD (∓) mean of seven treatments having 10 replications followed by secondary hardening having eight treatments with three replications.

3. RESULTS AND DISCUSSION

3.1 Effect of Primary Potting Mixtures on Hardening of *in-vitro* Banana Plantlets

Under greenhouse conditions, plantlets were hardened and a significant effect has been observed in morphological parameters such as plant height (cm), pseudo stem girth (cm), number of leaves, number of roots, length of roots (cm) among the different potting mixtures and the mean values was represented in Table 3. The treatment PHT7 have shown significant growth during primary hardening when compared to control and other treatments followed by PHT4.

Table 1. Treatment details for primary hardening

Treatment No.	Potting mixtures	Ratio
PHT0	Control (cocopeat)	100%
PHT1	Cocopeat+ Vermicompost	(3:1)
PHT2	Cocopeat+ Vermicompost	(1:1)
PHT3	Cocopeat+ Vermicompost+ Sand	(2:1:1)
PHT4	Cocopeat+ Vermicompost+ Soil	(2:1:1)
PHT5	Cocopeat+ Vermicompost+ Perlite	(2:1:1)
PHT6	Cocopeat+ Vermicompost+ Vermiculite	(2:1:1)
PHT7	Cocopeat+ Perlite+ Vermiculite	(2:1:1)

Table 2. Treatment details for secondary hardening

Treatment No.	Potting media	Ratio
SHT1	Soil	100%
SHT2	Soil+Bonemeal	(3:1)
SHT3	Soil+ Jeevamrit	(3:1)
SHT4	Soil+Bonemeal +Jeevamrit	(3:1)
SHT5	Soil+Neemcake	(3:1)
SHT6	Soil+Neemcake+ Jeevamrit	(3:1)
SHT7	Soil+Vermicompost	(3:1)
SHT8	Soil+Vermicompost+ Jeevamrit	(3:1)

Similar results were found in an experiment conducted by Yao et al. [12]. In an experiment conducted by Hassan *et al.*, it was evident that the combinations of equal amount of perlite and vermiculite as a potting media led to better results in plant development than either used the substrates alone [13].

3.2 Effect of Different Potting Mixtures on Shoot Length of Banana Saplings during Primary Hardening

During primary hardening, the growth of shoots in banana plants was recorded in response to various potting mixtures. The impact of the different treatments on growth and development of banana plants is presented in Table 3. Among all the treatment combination PHT7 have shown the maximum shoot length (15.36cm) that was recorded 51% higher than the control (10.16cm). However PHT4 treatment have also shown a significant impact on the shoot length. The shoot length in PHT4 treatment was recorded 14.03 cm which was 38.09% higher than the control. In contrast, the PHT6 led to significantly lower shoot length growth 9.84 cm observed among all the treatments.

3.3 Effect of Different Potting Mixtures on Number of Leaves of Banana Saplings during Primary Hardening

The variation in the number of leaves per plant was recorded during the primary hardening of banana plantlets and the maximum number of leaves per plant was observed in PHT7 (5.71) that was 16.76% more when compared with control (4.89). However, PHT4 and PHT2 have also shown significant impact on number of leaves that was 5.52 leaves/shoot, and 5.51 leaves/shoot which was 12.88% and 12.67% more than the control. It was observed that more number of leaves results in good physiological and biochemical processes in the plants such as respiration, transpiration, photosynthesis, etc [14].

3.4 Effect of Different Potting Mixtures on Root Length (cm) of Banana Saplings during Primary Hardening

During the primary hardening the maximum root length was measured in PHT7 (3.69 cm), which was found to be 7.89% more as compared to the control (3.42cm) in the 30-days study period as shown in Table 3. Where as the root length in treatment PHT4 was recorded to 3.62 cm which was 5.847% more than the control (Table 3).

3.5 Effect of Different Potting Mixtures on Number of Roots during Primary Hardening

The maximum number of roots (5.9) was observed in PHT7 treatment which was 7.27% higher as compared to control (5.5), followed by PHT4 (5.6) which was 1.81% higher than the control as shown in Table 3. More number of roots brings adequate amount of nutrients which are needed for better growth and development to the plants due to the nutrients uptake from soil [15,16].

3.6 Effect of Different Potting Mixtures on Pseudostem Girth (cm) during Primary Hardening

The pseudostem girth in primary hardened banana saplings have shown varied results under different treatments (Table 3). The maximum pseudostem girth was recorded after 30 days of transplantation in PHT7 treatment and it was recorded as 3.88 cm. The pseudostem girth was 64.60% more in PHT7 as compared to the control plants having pseudostem girth of 2.36 cm. Treatment PHT4 also showed varied results having pseudostem girth of 3.66 cm which was 55.08% more than the control plants. Similar results were also provided by Sindhupriya et al. [17].

3.7 Effect of different potting mixtures and Jeevamrit on secondary hardening of Banana saplings

The primary hardened saplings of banana were transferred to secondary hardening. The secondary hardening mixture comprised of different substrates. The SHT4 showed a significant effect on the morphological attributes such as; shoot length, number of leaves, pseudo stem girth as compared to other treatments. The Jeevamrit treatment enhances soil biology by altering the structure of microbial communities and the soil's chemical and microbiological properties. The results of present study were in accordance with the findings of Saharan et al. [18].

3.8 Effect of Different Potting Mixtures and Jeevamrit on the Shoot Length of Banana Plants during Secondary Hardening

The maximum shoot length was recorded in the SHT4 treatment and it was measured as 29.96

cm which was 56.69% higher as compared to control SHT1 (19.12 cm) whereas the shoot length in the treatment SHT8 was measured as 26.86 cm which was 40.48% higher as compared to control. The treatments with Jeevamrit application have shown better shoot length when compared to the plants with no Jeevamrit treatment as shown in Table 4. Similar results were found in Maher et al. [19]. The results of the present study indicated that there was a variation in the growth of plant height with time, that have recorded highest growth rate in the initial days and during later stages of development the growth rate was comparatively slow [21]. Similar results were also provided by Sarrwy et al., Reginio et al., and Agarwal et al. [20,21,22].

3.9 Effect of Different Potting Mixture and Jeevamrit on the Number of Leaf of Banana Plants during Secondary Hardening

The variation in the number of leaves per plant was recorded during the application of Jeevamrit in secondary hardening stage. The highest number of leaves per shoot was recorded in SHT4 and it was recorded as 7.12 leaves/shoot which was 33.83% more than control (5.32 leaves/shoots), followed by SHT8 that have shown 6.89 leaves/shoot which was 29.51% more than the control. The results of the present study suggested that the maximum number of leaves per shoot was observed in the plants having potting mixture irrigated with Jeevamrit.

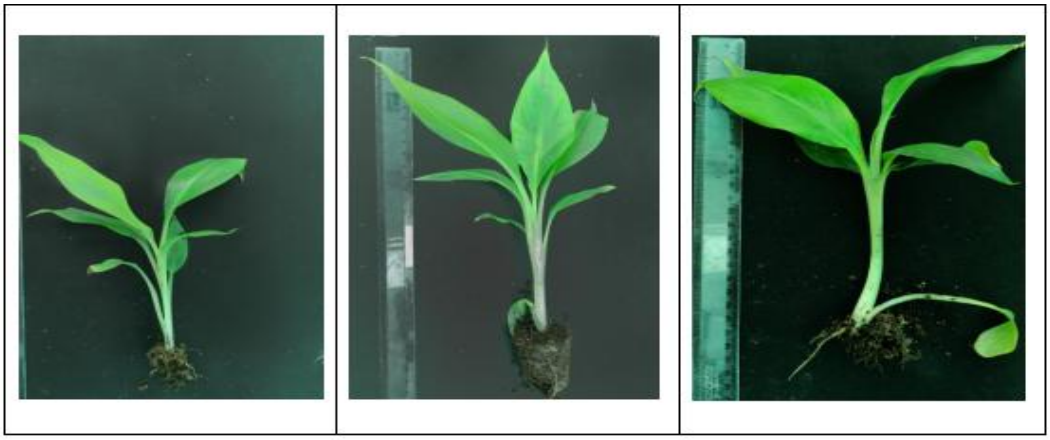

Fig. 1. Effect of primary potting media on the growth of primary hardened banana plantlets of *Grand naine*

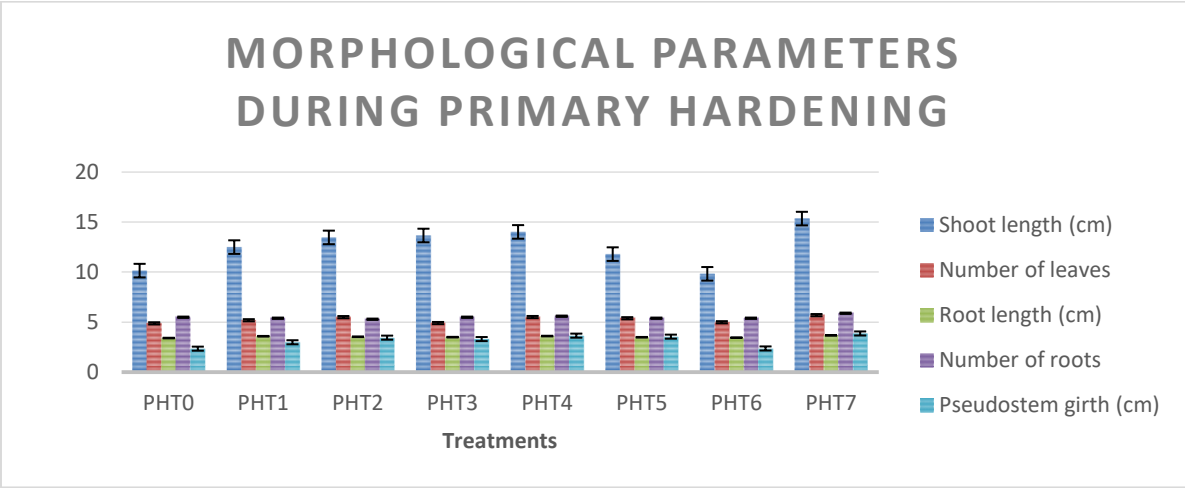

Fig. 2. Effect of different substrates on morphological parameters of banana plantlets of Grand naine during primary hardening 3.7 Effect of different potting mixtures and Jeevamrit on secondary hardening of Banana saplings

Table 3. Effect of different potting mixtures on morphological characteristics of primary hardened banana saplings of *Grand naine*

Treatment	Potting mixture	Shoot length (cm)	Number of leaves	Root length (cm)	Number of roots	Pseudostem girth (cm)
PHT0	Coco peat (Control)	10.16	4.89	3.42	5.5	2.36
PHT1	Coco peat + Vermicompost (3:1)	12.51	5.2	3.60	5.4	3.0
PHT2	Coco peat+ Vermicompost (1:1)	13.48	5.51	3.55	5.3	3.46
PHT3	Coco peat + Vermicompost +Sand (2:1:1)	13.67	4.92	3.51	5.5	3.32
PHT4	Coco peat + Vermicompost +Soil (2:1:1)	14.03	5.52	3.62	5.6	3.66
PHT5	Coco peat+ Vermicompost + Perlite (2:1:1)	11.81	5.4	3.50	5.4	3.56
PHT6	Coco peat + Vermicompost + Vermiculite (2:1:1)	9.84	5.0	3.46	5.4	2.38
PHT7	Cocopeat + Perlite + Vermiculite (2:1:1)	15.36	5.71	3.69	5.9	3.88
F-Test		S	S	NS	S	S
S. Ed. (±)		0.160	0.082	0.180	0.062	0.076
C.D. at 5%		0.338	0.175	0.382	0.131	0.162
CV		4.460	2.302	5.038	1.733	2.134

**PH=Primary Hardening, T=Treatments*

SHT2 SHT4

Fig. 3. Effect of Jeevamrit on the growth of secondary hardened banana plantlets

3.10 Effect of Different Potting Mixture on the Pseudo Stem Girth of Banana Plants during Secondary Hardening

The pseudostem girth of secondary hardened banana plantlets varied depending on the planting mixture. The maximum growth was observed on the 30th day in the treatment SHT4 with a pseudostem girth of 5.945 cm that was 55.26% more than the control (3.829 cm). A similar growth pattern was observed in the treatment SHT6 and SHT8, which exhibited a girth of 5.501 cm and 5.937 cm that was 43.66% and 55.05% more than the control. A similar study was reported by Sathiamoorthy et al. They took different organic amendments including bonemeal, neemcake, vermicompost in which bonemeal have shown greater effects on pseudostem girth and shoot length [23].

Fig. 4. Effect of different substrates on the plants of *Grand naine* during secondary hardening

Table 4. Effect of substrates on morphological parameters of banana plantlets of *Grand naine during secondary hardening*

Treatment	Potting mixture	Shoot length (cm)	Number of leaves	Pseudostem girth (cm)
SHT1	Soil (control)	19.12	5.321	3.829
SHT2	Soil+Bonemeal	20.04	5.977	3.897
SHT3	Soil+ Jeevamrit	26.07	6.375	4.723
SHT4	Soil+Bonemeal +Jeevamrit	29.96	7.125	5.945
SHT5	Soil+Neemcake	24.98	6.652	4.821
SHT6	Soil+Neemcake+Jeevamrit	25.31	6.534	5.501
SHT7	Soil+Vermicompost	26.23	6.526	4.992
SHT8	Soil+Vermicompost+Jeevamrit	26.86	6.891	5.937
Due to treatments	F-Test	S	S	S
	Em. (±)	0.040	0.044	0.050
	S. Ed. (±)	0.056	0.062	0.071
	C.D. at 5%	0.120	0.133	0.151
	CV	1.064	0.306	1.800

**SH=Secondary hardening, T=Treatments*

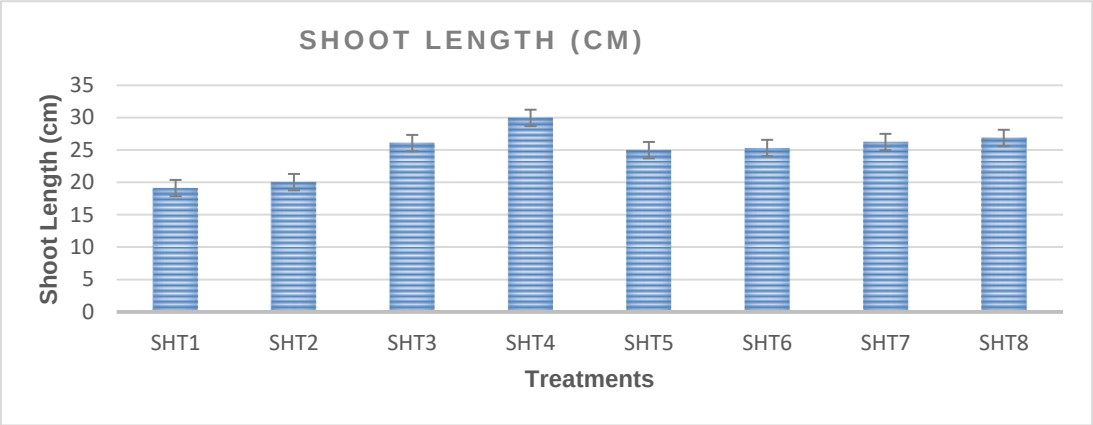

Fig. 5. Effect of substrates on shoot length of banana saplings of Grand naine during secondary hardening

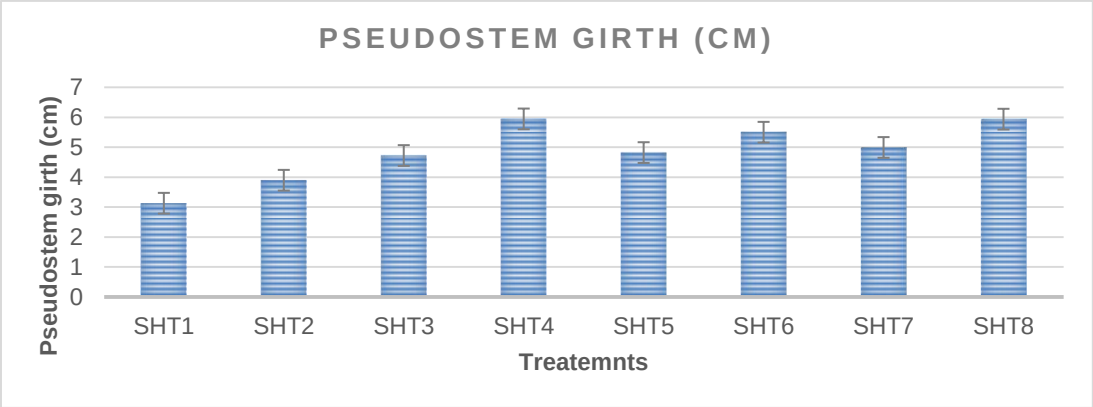

Fig. 6. Effect of substrates on pseudostem girth of banana saplings of Grand naine during secondary hardening

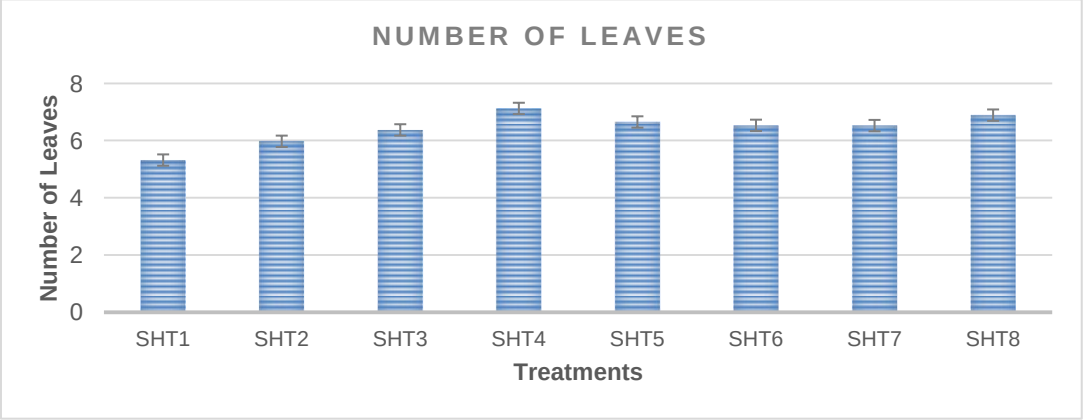

Fig. 7. Effect of substrates on number of leaves of banana saplings of Grand naine during secondary hardening

4. CONCLUSION

The present study was aimed to develop a best suited composition of primary and secondary hardening of banana saplings which could lead to maximize the survival of banana plants. This work could contribute to commercial Tissue Culture Labs for the production of best planting material with high survival rate.

In conclusion, the use of different growth substrates such as cocopeat, vermiculite, perlite, sand, vermicompost, soil, neemcake and bonemeal have showed significant impact on the growth of banana saplings during primary and secondary hardening.These substrates provide different levels of water holding capacity, nutrient availability, and aeration to the plants, which affect their growth and development. Mixture of cocopeat-vermiculite-perlite have been found to promote better growth of banana plantlets of *Grand naine* during hardening of plant. In secondary hardening of banana plantlets grown under the influence of soil-neem cake mixture shows good results in later stage but slower growth than soil-bonemeal. While soil-vermicompost grown plants have shown average response during secondary hardening.The effect of Jeevamrit have also been identified in the plantlets. Plants those were treated with Jeevamrit have shown better growth than the plants that were not treated with Jeevamrit. It was observed that the good shoot length with a better pseudostem girth can promote strength to the plant so at the time of fruiting plant can hold the bunch weight more efficiently [24].

From the above findings it has been concluded that for primary hardening, the potting mixture comprises of cocopeat+vermiculite+perlite found to be best option. Simultaneously for secondary hardening, soil+bonemeal+Jeevamrit was found best for growth and development of banana saplings.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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