

RESEARCH ARTICLE

EFFECT OF EPIDERMAL MUCOUS SECRETION OF EARTHWORMS AND OTHER SELECTED ORGANIC SUBSTANCES ON ROOTING OF SEMI-HARDWOOD CUTTINGS OF *Citrus aurantifolia* AND TOP CUTTINGS OF *Dracaena sanderiana*

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ABSTRACT

A recent trend in plant growth and propagation is to use natural substances rather than synthetic plant growth regulators. Two experiments were conducted to find out the effect of epidermal mucous secretion (EMS) of earthworms and other organic substances including *Aloe vera* gel, coconut water and ripe banana on rooting of semi-hardwood cuttings of *Citrus aurantifolia* and top cuttings of *Dracaena sanderiana* compared to commercially available plant growth regulators (PGRs). Eleven treatments were prepared using sole application of EMS solution, *Aloe vera* gel, coconut water and ripe banana, and mixtures of EMS and other organic substances in two different ratios by allocating equal and more inputs from EMS. The present experiments were set up adopting a completely randomized design. The number of roots per cutting, total root length per cutting (cm), length of the longest root per cutting (cm) and root vigor score were recorded for both plant species where destructive sampling was done at two months after planting for *C. aurantifolia* and one and two months after planting for *D. sanderiana*. The number of roots per cutting, length of the longest root per cutting and the root vigor score were significantly greater in the control treatment that used commercially available PGR and the cuttings treated with EMS: coconut water (1:1) for *C. aurantifolia* suggesting the possibility to use EMS and coconut water mixture to substitute the PGR for root induction in *C. aurantifolia*. The number of roots, total root length and the length of the longest root per cutting were significantly high in the treatments administered with *Aloe vera* gel, ripe banana, and a mixture of EMS: ripe bananas (1:1) together with the PGR at two months after planting for *D. sanderiana*. Hence, the ingredients listed above could be utilized as alternative organic substances to produce adventitious roots in *D. sanderiana* top cuttings as same as the PGR. The results of the present study could be extremely valuable for developing a unique organic rooting replacement for the synthetic root inducing materials for *C. aurantifolia* and *D. sanderiana*. Nonetheless, additional research is required to validate the results of this study.

Keywords: *Aloe vera* gel, Coconut water, *Dracaena sanderiana*, epidermal mucous secretions, ripe banana

INTRODUCTION

Plant Growth Regulators (PGRs) are defined as small and simple substances that produced artificially to regulate the growth and development of plants. Plant growth regulators can be grouped into five classes; compounds related to auxins, gibberellins, cytokinin, abscisic acid, and ethylene. A large number of PGRs are utilized in commercial agriculture globally. The usage of plant growth regulators

has increased drastically and now accounts for a vital part of the production of agricultural commodities (Lopez-Lauri, 2016). The PGRs are used in horticulture, agriculture and viticulture to achieve a number of benefits, including inducing of rooting, reduced susceptibility to biotic and abiotic stresses, improved morphological structure, ease of harvest, increases in yield that are both quantitative and qualitative, and modification of plant constituents (Rademacher, 2015).

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Despite the use of synthetic PGRs, shifting to natural PGR alternatives has recently become a favor for several reasons. Synthetic plant growth regulators and phytohormones can be replaced with natural plant extracts since they are less expensive and more environmentally friendly (Sherif, 2017). Specially, organic components have become very popular to accelerate the initiation of the roots from cuttings. Natural rooting agents can be applied for multiplication of hardwood and softwood cuttings of horticulture crops (Sany *et al.* 2020). Coconut water, bee honey, humic acid, seaweed extract and cinnamon powder are some commonly used natural substances for inducing rooting of cuttings. To encourage the roots of semi-hardwood cuttings of *Citrus aurantifolia* and air layering plants of *Syzygium jambos*, *Aloe vera* leaf gel, as a substitute root inducing agent was suggested by Mirihagalla and Fernando (2020). Agampodi & Jayawardena (2009) reported that *Dracaena purplecompacta* L. canes can be made to root by using coconut water, which contains natural IAA. As reported by Pacholczak & Nowakowska (2020), Algamin plant and Goteo, two biostimulators used in the study, had positive effects on both the amount and percentage of rooting as well as the overall growth of cuttings of two cultivars of ground cover Roses.

Several studies have revealed that epidermal mucous of *E. fetida* contains various chemicals (Zhang *et al.* 2016). A variety of natural PGRs and phytochemicals, including cytokinins, auxin, gibberellins, brassinosteroids, and phenolic acid, have been examined and found to be present in vermicompost leachate (Sany *et al.* 2020). Epidermal mucous secretion produces by earthworms as they move through the soil, which soil microorganisms break down and use as a source of nutrients for plants (Guhra *et al.* 2020). The clitellar epithelium of *Eisenia foetida* has eight distinct types of secretory cells, including large granular, fine granular, metachromatic, orthochromatic, and small granular proteinaceous cells (Morris, 1985). The epidermal mucous secretion of earthworms significantly accelerated the decomposition and humification of vermicomposting materials, and could even

promote microbial activity, growth, and increase community diversity in vermicomposting systems (Huang & Xia, 2018). Flores (2014) states that vermi-extract was observed to improve root length in grape vine propagation compared to vermicompost or ordinary compost. The epidermal mucous secretion of earthworms holds significant potential as a natural and sustainable root-inducing substance. Its effectiveness is attributed to the presence of growth-promoting hormones, enzymes, and other bioactive compounds. However, limited evidence is available of using of epidermal mucous secretion of earthworm for inducing rooting of cuttings. Therefore, the present study was planned with the objective to compare the efficacy of epidermal mucous secretion of earthworms and other types of organic substances including *Aloe vera* gel, coconut water and ripe banana together with commercially available synthetic PGRs for rooting of semi-hardwood cuttings of *Citrus aurantifolia* (lime) and top cuttings of *Dracaena sanderiana*.

MATERIALS AND METHODS

Preparation of epidermal mucous secretion solution of earthworms and other organic substances

Red earthworms (*Eisenia fetida*) were collected near the cattle shed of the farm of Faculty of Agriculture, University of Ruhuna, Mapalana. Then the collected earthworms were multiplied in a container with a substrate consisting of cattle manure, chopped ripe banana peels and cabbage for four weeks. During multiplication of earthworms, the substrate temperature was maintained in the range of 25-28 °C, and the substrate humidity was 70-80%. After four weeks, mature earthworms were separated from the substrate and cleaned by rinsing gently with sterile distilled water to remove debris and dirt (Striganova and Ponomareva, 2002). Earthworms are kept on a moist and sterile surface while keeping the environment humid. According to Balamurugan *et al.* (2004), cotton swabs were used to gently brush the earthworms' soft bodies to encourage the creation of epidermal mucous. After an hour, as mucus is secreted, 100 g of earthworms

were put into a clean beaker filled with 100 ml sterile water and thoroughly rinsed for an hour. The solution of epidermal mucous secretion was filtered through a filter paper (Doornbos *et al.* 2011) and stored in a refrigerator for future use (Rastogi *et al.* 2013). After extraction of EMS, earthworms were returned to their natural habitat for recovery. Ripe banana solution and *Aloe vera* gel liquid was prepared by mixing 50g of materials (wet weight) with 100 ml of distilled water using an electric blender. Tender coconuts were used to collect fresh coconut water. Different treatments were prepared by using sole application of EMS solution, coconut water, *Aloe vera* gel and ripe banana and mixed application of EMS and other organic substances in two different ratios by allocating equal and more inputs from EMS solution (Table 1). A treatment having commercially available root inducing hormone (*Rapidroot*®) was considered as a control, since the objective of the present study was to identify organic substitute for synthetic PGRs on root induction and production. A simple completely randomized design was used to arrange the 11 defined treatments in both sub-experiments.

Sub- experiment 1: Effect of different treatments on rooting of Semi-hardwood cuttings of *Citrus aurantifolia* (lime)

Semi-hardwood cuttings of *Citrus aurantifolia* with the length of 12-15 cm with 3-5 leaves were treated by dipped them into different treatments for 10 minutes before been established in 12 x 30 cm transparent

polythene single propagators filled with a general potting mixture (coir dust 1: topsoil 1: sand 1: compost 1/4) sterilized by 1% of fungicide solution. Five replicates were used for each treatment and each replicate consisted of four experimental units. All propagators were kept in a net house to provide a cool and shade environment for inducing rooting. The survival rate, number of roots, total root length (cm) and length of the longest root (cm) were recorded using destructive sampling at two months after planting. The survival rates of cuttings were calculated using the below equation. Root vigor was recorded as a five-scale visual score (no callus formation = 0, Formation of callus = 1, Callus and root initiation = 2, Callus and few adventitious roots = 3, more than four adventitious roots = 4).

$$\text{Survival rate} = \frac{\text{Number of cuttings having roots/callus}}{\text{Total number of cuttings established}} \times 100$$

Sub- experiment 2: Effect of different treatments on rooting of top cuttings of *Dracaena sanderiana*

The same treatments as in sub-experiment one were used in the sub-experiment two for top cutting of *Dracaena sanderiana*. Each treatment had ten replicates while each replicate contained five experimental units. *D. sanderiana* top cuttings with 20–25 cm in length having 6-7 leaves, were placed in 12 x 20 cm black polythene bags filled with a general potting mixture consisting of coir dust, topsoil, sand, and compost (1:1:1:1/4) after treated in respective treatment by

Table 1: Details of different treatments used in the sub-experiments

Treatment	Detail description
T ₁	Commercial hormone only (<i>Rapidroot</i> ®, 0.3% Indole 3-butyric acid) (Control)
T ₂	Epidermal Mucous secretion (EMS) solution only
T ₃	<i>Aloe vera</i> gel only
T ₄	Coconut water only
T ₅	Ripe banana solution only
T ₆	EMS: <i>Aloe vera</i> gel (1:1)
T ₇	EMS: <i>Aloe vera</i> gel (3:1)
T ₈	EMS: Coconut water (1:1)
T ₉	EMS: Coconut water (3:1)
T ₁₀	EMS: Ripe banana solution (1:1)
T ₁₁	EMS: Ripe banana solution (3:1)

T1: Only commercial hormone, T2: Only Epidermal Mucous secretion (EMS), T3: Only *Aloe vera* gel, T4: Only coconut water, T5: Only ripe banana solution, T6: EMS : *Aloe vera* gel (1:1), T7: EMS : *Aloe vera* gel (3:1), T8: EMS : Coconut water (1:1), T9: EMS : Coconut water (3:1), T10: EMS : Ripe banana solution (1:1), T11: EMS : Ripe banana solution (3:1)

dipping for ten minutes. The number of roots, total root length and length of the longest root per cutting were recorded at one and two months after establishment through destructive sampling. However, a different visual scoring scale having five-scale was used to evaluate the root vigor of for top cutting of *D. sanderiana* (no callus formation = 0, callus formation = 1, callus and root initiation = 2, callus and few roots adventitious roots = 3, less than ten adventitious roots = 4, more than ten adventitious roots = 5). Five replicates were used to collect data at one month after establishment and rest was used at two months after establishment.

Data analysis

All of the data of the above 2 experiments that distributed normally were analysed using *SAS* software by one-way analysis of variance and the Duncan's multiple range test was used to compare treatment means at probability of 5%. The number of roots per cutting was identified as non-normally distributed data and transformed by log transformation technique followed by analysed using one-way analysis of variance. Root vigor data was analysed using *Statistix* 10 software by the Kruskal–Wallis test followed by comparison mean separation.

RESULTS AND DISCUSSION

Sub- experiment 1: Effect of different treatments on rooting of semi-hardwood cuttings of *Citrus aurantifolia*

The survival percentage of the cuttings was significantly different among treatments ($P < 0.05$). Figure 1 shows that all cuttings of *Citrus aurantifolia* established in single propagators treated by T₁, T₃, T₅, T₇, T₈, T₉, and T₁₁ survived (100%) whereas T₆ treatment had the lowest survival rate of 60%.

The number of roots per cutting ($P < 0.01$), total root length ($P < 0.01$), length of the longest root of the cutting ($P < 0.05$) and the root vigor score of the root system ($P < 0.05$) were significantly different among treatments of the experiment. The cutting treated with equal proportion of EMS and coconut water (T₈) reported significantly same number of

roots per cutting as the control treatment (T₁) where synthetic PGRs were used suggesting potential of T₈ to be used equally as a commercially available root-inducing growth regulator. However, the number of roots per cutting observed in all other treatments were significantly low compared to the control (T₁) and T₈.

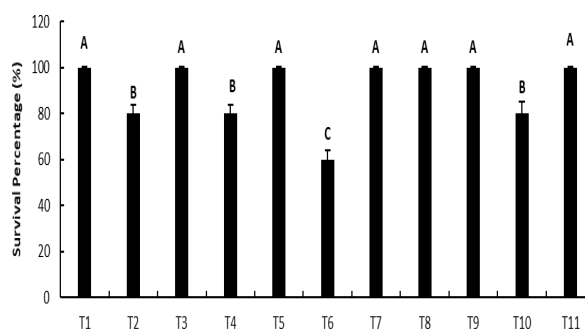


Figure 1: Effect of different treatments on the survival percentage of the semi hardwood cuttings of *C. aurantifolia* at two months after establishment in single propagators. The graph includes error bars representing the standard error of the mean. Mean values with different letters (A, B, C) are significantly different ($\alpha = 0.05$) according to DMRT.

T1: Only commercial hormone, T2: Only Epidermal Mucous secretion (EMS), T3: Only *Aloe vera* gel, T4: Only coconut water, T5: Only ripe banana solution, T6: EMS : *Aloe vera* gel (1:1), T7: EMS : *Aloe vera* gel (3:1), T8: EMS : Coconut water (1:1), T9: EMS : Coconut water (3:1), T10: EMS : Ripe banana solution (1:1), T11: EMS : Ripe banana solution (3:1)

The greatest total root length was observed in the cuttings treated by the commercial root-inducing agent (T₁- 12.7 cm) while all other treatments recorded lower values (Fig 2B). The length of the longest root per cutting was greater in T₁, T₈, T₉ and T₁₀ when compared to other treatments (Fig 2C). According to the visual scoring scale for root vigor, the greater values were recorded in the control treatment (T₁) and the treatment comprised 1:1 ratio of EMS and coconut water (T₈) while the lowest value was observed in the treatment having 3:1 ratio of EMS and *Aloe vera* gel (T₇) (Fig 2D). According to the observations of the present study, it is interesting to note that 1:1 ratio of EMS and coconut water (T₈) mixture

performed well by producing greater number of roots, length of the longest root per cutting and higher value for the root vigour score as same as the control treatment where cuttings were treated with commercially available PGR. Therefore, it is conceivable to use EMS: coconut water (1:1) to induce rooting of *C. aurantifolia* a manner similar to that of commercially available PGR, making a simple replacement possible. Muscolo *et al.* (2007), found that earthworm mucous and vermicasts contain substances similar to humic acids that significantly promoted root elongation and overall plant growth.

The successful rooting of cuttings depends upon many factors that are associated with plants *i.e.*, the age of the mother plant, parts used of tree, time of planting, rainfall, humidity, temperature, rooting media and

after care operations (Maurya *et al.* 2022). According to Singh *et al.* (2008), rooting of citrus cuttings could be affected by propagation media, type of cuttings, relative humidity and temperature of the environment and root inducing material (hormone/plant growth regulator). In the present experiment, all the above-mentioned factors are constant for all experimental units and only variable factor was root inducing compound. Therefore, in the present study, it can be assumed that the rooting of cuttings of lime in single propagator was affected only by root inducing materials. Similar experiments have been conducted by different researchers related to citrus species on various parameters affiliated with root formation. According to Mirihagalla & Fernando (2020), semi-hardwood cuttings of and *C. aurantifolia*, synthetic PGRs can be substituted with *Aloe*

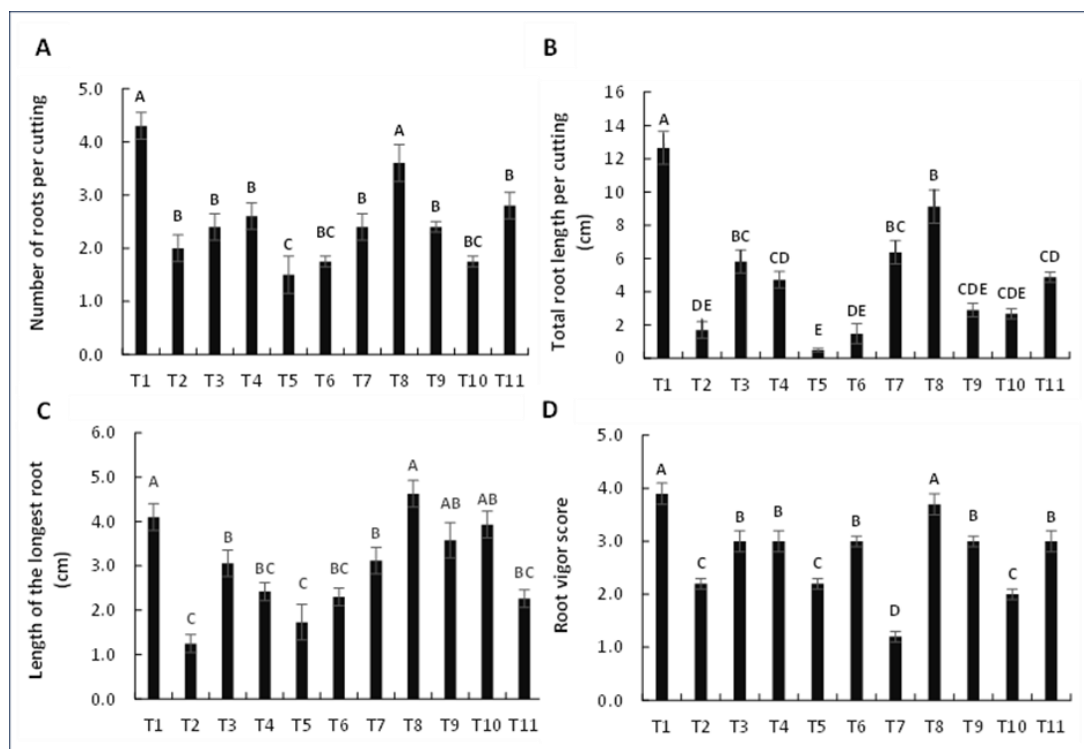


Figure 2: Effects of different treatments on root traits (A) number of roots (B) total root length (cm) (C) length of longest root (cm) (D) root vigor score of semi-hardwood cuttings of *C. aurantifolia* at two months after establishment in single propagators. The graph includes error bars representing the standard error of the mean. Mean values with different letters within the same column are significantly different ($\alpha = 0.05$) according to DMRT.

T1: Only commercial hormone, T2: Only Epidermal Mucous secretion (EMS), T3: Only *Aloe vera* gel, T4: Only coconut water, T5: Only ripe banana solution, T6: EMS : *Aloe vera* gel (1:1), T7: EMS : *Aloe vera* gel (3:1), T8: EMS : Coconut water (1:1), T9: EMS : Coconut water (3:1), T10: EMS : Ripe banana solution (1:1), T11: EMS : Ripe banana solution (3:1)

vera gel, a natural alternative root promoting material. Krikorian (1988), discuss the applications of coconut water in plant tissue culture, emphasizing its role in promoting root and shoot development. According to the findings of the present study, it can be

suggested that commercial PGR might be replaced by 1:1 ratio of EMS and coconut water on rooting of stem cuttings of *C. aurantifolia*. Plate 2 shows the variation of root formation in the cuttings of *C. aurantifolia* in different treatments.



Plate 1: Root formation as affected by different treatments in the stem cuttings of *C. aurantifolia* at two months after establishment in single propagators.

T1: Only commercial hormone, T2: Only Epidermal Mucous secretion (EMS), T3: Only *Aloe vera* gel, T4: Only coconut water, T5: Only ripe banana solution, T6: EMS : *Aloe vera* gel (1:1), T7: EMS : *Aloe vera* gel (3:1), T8: EMS : Coconut water (1:1), T9: EMS : Coconut water (3:1), T10: EMS : Ripe banana solution (1:1), T11: EMS : Ripe banana solution (3:1)

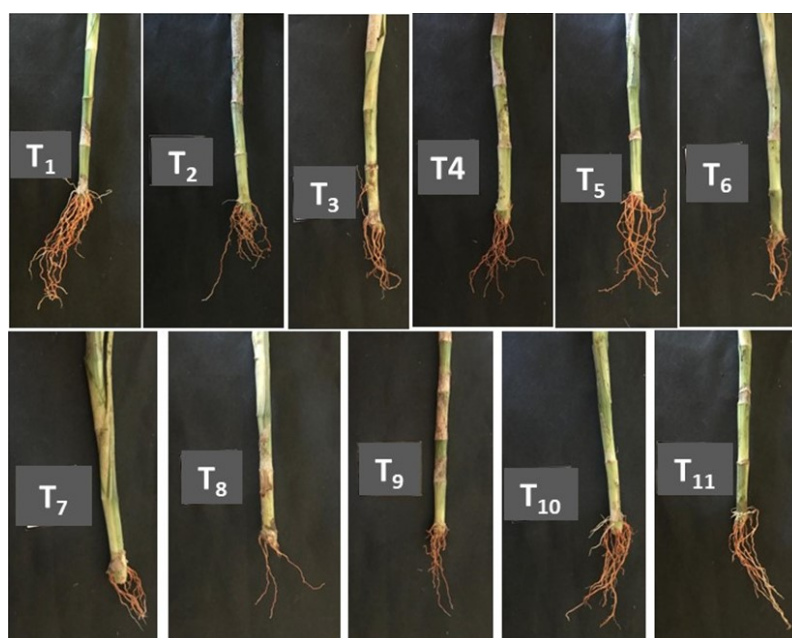


Plate 2: Root formation as affected by different treatments in *D. sanderiana* at one month after planting

T1: Only commercial hormone, T2: Only Epidermal Mucous secretion (EMS), T3: Only *Aloe vera* gel, T4: Only coconut water, T5: Only ripe banana solution, T6: EMS : *Aloe vera* gel (1:1), T7: EMS : *Aloe vera* gel (3:1), T8: EMS : Coconut water (1:1), T9: EMS : Coconut water (3:1), T10: EMS : Ripe banana solution (1:1), T11: EMS : Ripe banana solution (3:1)

Sub-experiment 2: Effect of different treatments on rooting of top cuttings of *Dracaena sandriana*

Figure 3 displays the variations in root characteristics of top cuttings of *D. sanderiana* subjected to various treatments at a month after establishment. Accordingly, the number of roots per cutting (Fig 3A) and root vigor score (Fig 3D) were not significantly different among treatments. However, the total root length (Fig 3B) and length of the longest root (Fig 3C) showed a significant difference among treatments. Highest values for total root length per cutting (cm) were recorded in T₁ (32.3), T₃ (24.0), T₄ (24.6), T₅ (269) and T₁₀ (27.5) treatments ($P<0.05$). According to the results, it can be suggested that production of total root length per cutting of *D. sanderiana* was influenced equally by sole application of *Aloe vera* gel, coconut water, ripe banana solution and EMS: ripe banana mixture (1:1) as same as commercially available PGR for top cuttings

at early stage of their establishment. Behaviour of the treatments on the length of the longest root per cutting was similar to the total root length per cutting. Plate 2 shows the appearance of the rooted cuttings of *D. sanderiana* in different treatments at one month after establishment.

After two months of establishment, the number of roots per top cuttings of *D. sanderiana* was significantly influenced by the treatments ($P<0.05$). The highest number of roots per cutting was reported in T₁ (17.2), T₂ (14.1), T₃ (16.9), T₄ (15.3), T₅ (14.6), T₇ (15.7) and T₁₀ (15.9) (Fig 4A). Accordingly, the number of roots per cutting was influenced EMS (T₂), *Aloe vera* gel (T₃), coconut water (T₄), ripe banana (T₅), the combination of EMS: *Aloe vera* gel (3:1) (T₇) and EMS: ripe banana (1:1) (T₁₀) as same as the control (T₁) treatment having commercially available PGR. The greater total root length (cm) was observed in the cutting treated by T₁ (155.5),

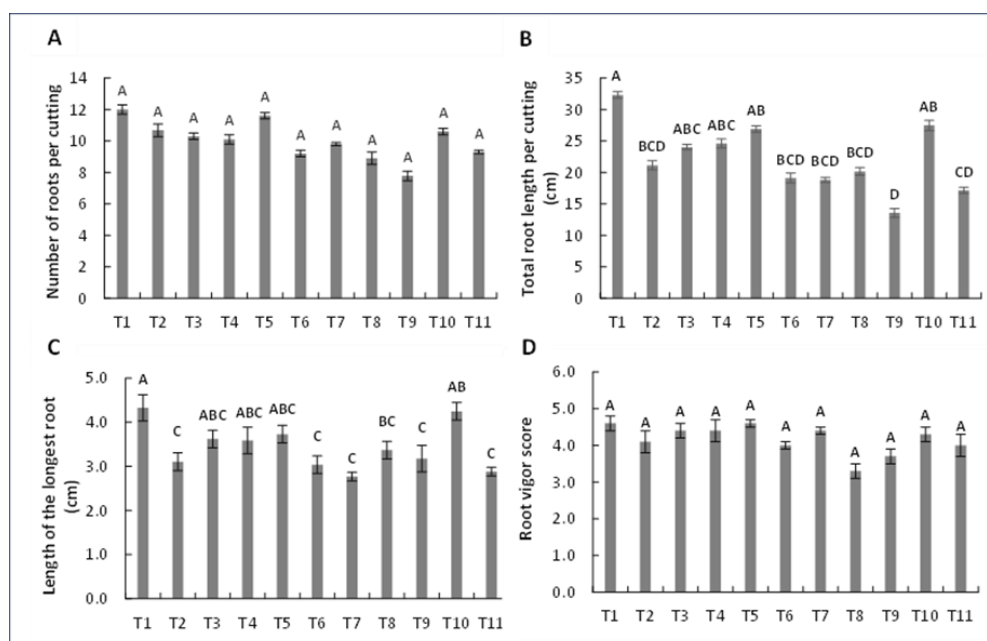


Figure 3: Effect of different treatments on (A) number of roots (B) total root length (cm) (C) length of longest root (cm) (D) root vigor score of *D. sanderiana* at one month after establishment. The graph includes error bars representing the standard error of the mean. Mean values with different letters within the same column are significantly different ($\alpha = 0.05$) according to DMRT.

T1: Only commercial hormone, T2: Only Epidermal Mucous secretion (EMS), T3: Only *Aloe vera* gel, T4: Only coconut water, T5: Only ripe banana solution, T6: EMS : *Aloe vera* gel (1:1), T7: EMS : *Aloe vera* gel (3:1), T8: EMS : Coconut water (1:1), T9: EMS : Coconut water (3:1), T10: EMS : Ripe banana solution (1:1), T11: EMS : Ripe banana solution (3:1)

T₃ (147.5), T₅ (127.5) and T₁₀ (125.5) (Fig 4B) ($P<0.05$). The greater values for the length of the longest root per cutting (cm) was recorded in T₁ (11.0), T₃ (10.7), T₄ (10.2), T₅ (10.0), T₈ (9.5), T₁₀ (10.8) and T₁₁ 9.2) (Fig 4C) ($P<0.01$). However, the root vigor scale was not significantly influenced by the treatments (Fig 4D). Treatments with significantly greater values for number of roots, total root length and length of the longest root per cutting is summarized in the Table 3. Based on the summary, it can be suggested that sole use of *Aloe vera* gel, ripe banana solution and 1:1 mixture of EMS and ripe banana solution can be successfully used as commercially available synthetic root inducing substance used in the present study for root production of top cuttings of *D. sanderiana*. However, written evidence are limited to prove the findings of the present experiment. Mukhtar *et al.* (2020) investigates the use of ripe banana extract for *in-vitro* rooting of olive cultivars, demonstrating notable improvements in root production as

an alternative to synthetic auxin. Furthermore, Negi & Saxena (2011) reported that banana pulp significantly enhances the *in-vitro* propagation of *Chlorophytum borivillianum*, including improved root development. At two months after establishment, variation in root system development of top cuttings of *D. sanderiana* was shown in plate 3.

Table 3: Treatments with significantly greater values for number of roots, total root length and length of the longest root per cutting of *D. sanderiana* is summarized

Root trait	Treatments with significantly greater values
Number of roots per cutting	T ₁ , T ₂ , T ₃ , T ₄ , T ₅ , T ₇ , T ₁₀
Total root length per cutting (cm)	T ₁ , T ₃ , T ₅ , T ₁₀
Length of the longest root per cutting (cm)	T ₁ , T ₃ , T ₄ , T ₅ , T ₈ , T ₁₀ , T ₁₁



Plate 3: Root formation as affected by different treatments in *D. sanderiana* at two months after planting

T1: Only commercial hormone, T2: Only Epidermal Mucous secretion (EMS), T3: Only *Aloe vera* gel, T4: Only coconut water, T5: Only ripe banana solution, T6: EMS : *Aloe vera* gel (1:1), T7: EMS : *Aloe vera* gel (3:1), T8: EMS : Coconut water (1:1), T9: EMS : Coconut water (3:1), T10: EMS : Ripe banana solution (1:1), T11: EMS : Ripe banana solution (3:1)

CONCLUSION

The results of this study show that the purpose of root production in semi-hard wood cuttings of *Citrus aurantifolia*, a combined application of epidermal mucous secretion (EMS) and coconut water (1:1) functioned same as the control treatment using commercially available PGR. Therefore, it is suggested that in order to induce and establish roots in semi-hardwood cuttings of *C. aurantifolia*, EMS and 1:1 mixture of coconut water can be used in place of commercially available root inducing synthetic PGR. When concerning the root formation of *Dracaena sanderiana* top cuttings at one month after establishment, sole application of *Aloe vera* gel, coconut water and ripe banana together with EMS: ripe banana (1:1) performed equally as commercially available PGR. After two months, however, the administration of just *Aloe vera* gel, ripe bananas, and a mixture of EMS: ripe bananas (1:1) demonstrated encouraging outcomes for *D. sanderiana* root production, comparable to that of commercially available PGR. As a result, the ingredients listed above could be utilized as a substitute to produce adventurous roots in *D. sanderiana* top cuttings. However, more and continuous investigations are needed to verify the findings of this study.

AUTHOR CONTRIBUTION

KMCF conceptualized and designed the study. PDGIHK performed the experiment and analysed data. KMCF supervised the study. KMCF and WRSJ drafted the manuscript and KMCF critically revised the manuscript.

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