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Research Paper

Natural growth stimulants for *Sanchezia spp.* stem cuttings

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Abstract: *Sanchezia spp* is an important ornamental plant in Sri Lanka. The species is also grown as a medicinal plant in other countries. This investigation was aimed at evaluating the growth and development of *Sanchezia spp* stem cuttings with the treatment of Coconut water, moringa (*Moringa oleifera* Lam) leaf extract and Bee honey as natural growth stimulants. Cuttings were planted without dipping in any solution (control: T1), water (T2), coconut water (T3), Bee honey (T4) and moringa leaf extract (T5). The outcome of the study revealed that stem cuttings dipped in Bee honey had higher leaf greenness (SPAD reading), plant height and number of roots. The maximum root length and higher root dry weight were observed when the stem cuttings were dipped in moringa leaf extract. Most of the growth parameters including number of leaves, total plant fresh weight, and root and shoot dry weights were superior when stem cuttings were dipped in coconut water compared to the rest of the treatments. Therefore, coconut water was considered the most suitable natural growth stimulants for treating the stem cuttings of *Sanchezia spp.*

Keywords: Moringa leaf extract, Coconut water, Bee honey, *Sanchezia spp.*

Introduction

Successful propagation of plants is a vital in plant establishment. Propagation by cuttings produces quick and large number of plants similar to the mother plant conserving the plant genetic sources. However, high rate of rooting is an important characteristic to establish successful propagation method through stem cuttings. Several factors such as propagation material, genotype, type of cutting and the rooting environment influence rooting ability in cuttings (Hartmann *et al*., 2011). Application of exogenous hormones is commonly practiced in stem cuttings. It accelerates the cell division, promotes synthesis of endogenous hormones and salicylic acid, stimulate carbohydrate accumulation and consequently rooting (Zhang *et al*., 2017).

Hormones are chemicals produced by plants that regulate growth. They stimulate root development, control plant height and increase size of flower and fruit. Many cuttings will root naturally due to the presence of auxins, the hormones that help plants to produce adventitious roots in the shoot tips. Synthetic forms of auxins are available commercially in the form of Indolebutyric acid (IBA) and Napthalene Acetic acid (NAA). However, the cost of rooting hormones tends to be a concern in rural poor farmers. Further, the world currently seeks for plants grown using less or no synthetic

formulations owing due to their adverse effect. Use of synthetic growth regulators frequently in higher concentrations can lead to harm to the environment and human beings. These materials can mix with the soil through agricultural applications, contact with groundwater due to infiltration, and could cause toxic effects resulting in accumulation in living bodies through food chain. Hence, the environmental effects of growth regulators are similar to those of pesticides (Sezgina and Kahya 2018).

Alternative hormones are natural materials that possess the ability to stimulate rooting of cuttings. They are a suitable substitute to the synthetic hormones such as auxins, cytokinins, gibberellins, which are popular rooting hormones. Honey, coconut water, willow tea, aspirin, moringa extract and saliva are examples for such materials that contain growth regulators (Shield, 2012; Carusetta, 2014). Further, compared to synthetic hormones, those natural substances are available at low cost.

Honey contains sugars, amino acids, minerals, enzymes, phenol antioxidants and organic acids (Solayman *et al.*, 2016). Due to the presence of these chemicals, honey possess both antibacterial and antifungal properties helping to prevent root rot and retain a cleaner environment needed for root growth (Olaitan *et al*., 2007; Mandal, 2011). Honey might prove to be a safe, cost effective, and easily used method to help cuttings grow adventitious roots. Moringa leaf extract is gaining a significant position in agriculture where it is used to enhance seed germination, growth and yield of crops (Foidl *et al*., 2001; Phiri and Mbewe, 2010). Martin (2000) and Foidl *et al*. (2001) reported that juice from fresh Moringa leaves effectively control the growth of various plants. Coconut water has growth- promoting activities as it contains phytohormones (Prades *et al*., 2012). The wide applications of coconut water can be justified by its unique chemical composition of sugars, vitamins, minerals, amino acids and phytohormones (Yong *et al.*, 2009), which directly or indirectly promote plant growth due to gibberellins, auxins and cytokinins (Kurepin *et al.*, 2014).

Sanchezia spp. is used mainly as an ornamental plant that can be grown outdoors in households and in many other places. It can serve as a component of ecological safety. Further to enhanced rooting, use of natural rooting stimulants could also provide a preventive mechanism for most of the plant diseases since field establishment, owing to their antifungal and antibacterial properties. Hence, this study was conducted to examine the effect of natural rooting substances on the propagation of *Sanchezia spp*. from cuttings in the plant nursery.

Materials and Methods

A pot experiment was carried out at the Crop Farm of the Eastern University, Sri Lanka during 2017/2018. The pots were filled with top soil: red soil: cow dung at the ratio of 1:1:1 and leaving 2.5 cm at the top for watering. Uniform cuttings were taken from a vigorous and healthy single mother plant of *Sanchezia* spp. At the Crop Farm, early in the morning. The cuttings were separated from mother plant by a sterile secateurs to prevent the xylem from being crushed, given it a very smooth surface and preventing microbial infection. The cuttings of uniform diameter and length were selected for the experiment and all leaves were removed to reduce transpiration from the cuttings. Treatments were T1: without dipping in any solution (control), T2: water dipping, T3: coconut water dipping, T4: Bee honey dipping and T5: moringa (*Moringa oleifera* Lam) leaf extract dipping.

Sufficient amount of fresh pure coconut water obtained from a mature coconut fruit was used for the experiment. Pure bee honey was obtained from a local shop and was mixed with two cups of boiling water, and allowed to cool for few hrs. The cooled honey water was used as a growth stimulant (Melia, 2016). Moringa leaf extract was prepared by using fresh moringa leaves that were shade dried for a period of 4 days. The dried leaves were powdered using lab blender (11, blender, USA), and the powdered material was mixed with distilled water at a ratio of 1:20 (w/v). After mixing, the

mixture was filtered through muslin cloth, and the filtrate was centrifuged at 5000 x g for 15 min (Legend Micro 17, Germany). Then the supernatant was collected and considered as 100% moringa leaf extract (Rao, 1990). Thereafter, 1 ml of extract was mixed with 30 ml distilled water and used for dipping the stem cuttings.

The stem cuttings were dipped in the treatments for two min (except control T1) and planted into the prepared poly bags and labelled. The design of the experiment was a Complete Randomized Design with each treatment having twenty five replicates. Poly bags were randomly selected for destructive sampling that was done at ten-day intervals starting from 10 days after planting (DAP) up to 30 DAP. The parameters *viz*., plant height, length of roots, number of roots and leaves, leaf greenness (SPAD meter readings, SPAD502), fresh weights of plant and root and dry weights of shoot, root and leaf (oven dried at 70 °C until constant weight) were measured. The data collected were analysed using parametric (ANOVA) and non-parametric statistical procedures. Mean separation was done using DMRT at p=0.05.

Results and Discussion

Plant height

There was a significant difference (p<0.01) in plant height of *Sanchezia spp.* among treatment (Table 1). Plant height was high in T5 (32 cm/plant) followed by T4 (31 cm/plant) at 10 days after planting, while tallest plants were noted in T4 (62 cm/plant) and shortest in T1 (50.3 cm/plant) at 1 month after planting. The rate of growth in plant height would be determined by various specialized factors such as hormones, except fertilization. Number of roots per plant showed a higher value in T4 indicating that *Sanchezia spp.* cuttings dipped in honey produced roots that can absorb more nutrients from soil, which may have contributed for the higher plant height in this treatment.

Table 1: Plant height of *Sanchezia spp*.

Dipping Treatment	Plant height (cm/plant)		
	10th day	20th day	30th day
Control (T1)	24.00 ± 1.02^{e}	43.25 ± 1.26^{c}	50.30 ± 1.79^{e}
Water (T2)	25.00 ± 0.99^{d}	54.00 ± 1.65^{b}	61.00 ± 2.01^{c}
Coconut water (T3)	28.00 ± 1.21^{c}	57.46 ± 1.78^{ab}	61.50 ± 1.98^{b}
Bee honey (T4)	31.00 ± 1.09^{b}	59.33 ± 1.65^{a}	62.00 ± 2.65^{a}
Moringa leaf extract (T5)	32.00 ± 1.28^{a}	44.00 ± 1.45^{c}	52.20 ± 1.79^{d}
F test	**	**	**

Values presented are mean ± standard error of five replicates. F test: ** p<0.01; Within a column, means followed by the same letter are not significantly different by the DMRT test at p=0.05.

Leaf greenness

A significant difference (p<0.01) was observed in SPAD meter readings (which is proportionate to leaf chlorophyll content) of *Sanchezia spp*. among treatments (Table 2). The SPAD meter reading was high in T4 (78.8) followed by T3 (75) at 30 DAP. The chlorophyll synthesis requires many elements such as nitrogen (N), phosphorous (P), magnesium (Mg) and iron (Fe) (Li *et al*., 2018; Chatzistathis and Therios, 2013). Bee honey contains a higher proportion of P (84.1 mg/kg), Mg (74.31 mg/kg) and Fe (30.34 mg/kg) (Solayman *et al*., 2016), which are useful in the synthesis of chlorophyll. This could have resulted in higher SPAD meter reading in *Sanchezia spp*. in T4. Wolff and Price (1960) reported that certain sugars, prominently glucose and sucrose, can stimulate the biosynthesis of chlorophyll in leaves. Bee honey contains sugars such as glucose and sucrose in superior quantities and thus, may have increased the chlorophyll content in T4 at each 10 days interval providing higher SPAD meter readings.

Number of roots and leaves

Among the several factors that affect the rooting of cuttings, growth regulators or rooting hormones play an effective role (Attia *et al*., 2015). Natural rooting substances significantly affected the number of roots and leaves at 10, 20 and 30 DAP (Table 3). The highest number of roots/plant was obtained in the treatment T4 at each 10-day intervals, while the minimum was observed in T1 at 21, 40 and 45/plant, at 10, 20 and 30 DAP, respectively.

Table 2: Leaf greenness of *Sanchezia spp*.

Dipping treatment	SPAD meter reading		
	10th day	20th day	30th day
Control (T1)	22.36 ± 1.02^{e}	59.00 ± 1.25^{e}	65.10 ± 2.07^{d}
Water (T2)	26.40 ± 2.45^{d}	61.40 ± 1.35^{c}	67.70 ± 1.91^{c}
Coconut water (T3)	38.40 ± 1.68^{b}	62.90 ± 1.47^{b}	75.00 ± 2.47^{b}
Bee honey (T4)	56.60 ± 1.73^{a}	64.40 ± 1.68^{a}	78.80 ± 2.39^{a}
Moringa leaf extract (T5)	32.00 ± 2.76^{c}	59.50 ± 1.29^{d}	64.23 ± 2.38^{e}
F Test	**	**	**

Values presented are mean ± standard error of five replicates. F test: ** p<0.01; Within a column, means followed by the same letter are not significantly different by the DMRT at p=0.05

Table 3: Number of roots and leaves in *Sanchezia spp*.

Dipping Treatment	Number of roots/plant			Number of leaves/plant		
	10th day	20th day	30th day	10th day	20th day	30th day
Control (T1)	21	40	45	1	4	8
Water (T2)	21	56	67	1	5	8
Coconut water (T3)	25	61	82	2	6	12
Bee honey (T4)	52	72	88	2	6	11
Moringa leaf extract (T5)	39	69	74	2	4	10
Chi square value	15	15	15	15	14	15
P value	0.005	0.005	0.005	0.005	0.007	0.005

There is a possibility to use be honey as a natural rooting hormone as bee honey consists of enzymes that can promote root growth in plants. Bee honey can help in rooting and maintain the strength and health of plants as it exhibits antifungal and antiseptic properties. Further, use of bee honey to dip plant cuttings will help as a guard against bacterial or fungal problems (Ibironke and Victor, 2016).

Mahros *et al*. (1994) reported that rooting behavior in stem cuttings is associated with carbohydrate:nitrogen (C/N) ratio in cutting base tissue. With the increase of C/N ratio, rooting on cuttings would increase. Bee honey contains higher amount of carbohydrates (glucose and sucrose) and lesser amount of nitrogen (Solayman *et al*., 2016) resulting in higher C/N ratio. Both these reasons may have resulted in a higher number of roots in the treatment T4 at each 10-day intervals. Contradictory to the present experiment, Dunsin *et al*. (2016) reported that the number of roots in *Parkia biglobosa* was the highest with the application of Moringa leaf extract.

The number of leaves was highest and similar in T3, T4 and T5 at 10 DAP. At 20 DAP, the maximum number of leaves was reported in treatments T3 and T4, while T3 recorded the highest value at 30 DAP. Both auxin and cytokinin are important as development regulators in plants. Phyllotaxis of leaf initiation from the shoot apical meristem is coordinated by auxin and it determines the initiation of leaflets and lobes from the margin of leaf primordia. Cytokinin is also important in maintaining shoot apical meristem. A complex relationship between auxins and cytokinins involves in the differentiation of leaf initiation

(Shwartz *et al*., 2016). Coconut water also contains higher quantities of auxins and cytokinins (Dunsin *et al*., 2016) and may be the reason for higher number of leaves observed in treatment T3.

Root Length

Root length per plant showed a significant difference ($p<0.01$) among treatments at each 10-day intervals (Table 4). Root length was high in the cuttings treated with Moringa leaf extract (17.2 cm/plant) at 30 DAP. Moringa leaf extract is rich in cytokinins in the form of zeatin, which can promote the rate of cell division (Dunsin *et al*., 2016). This could have attributed to the higher root length in treatment T5. Dunsin *et al*. (2016) also reported that the maximum root length in *P. biglobosa* was obtained with the application of Moringa leaves.

Table 4: Root length (cm) of *Sanchezia spp*.

Dipping Treatment	Root length (cm)		
	10th day	20th day	30th day
Control (T1)	0.8 ± 0.01^{d}	11.30 ± 1.25^{d}	11.40 ± 1.03^{d}
Water (T2)	2.2 ± 0.05^{c}	14.00 ± 1.23^{c}	16.00 ± 1.48^{c}
Coconut water (T3)	4.0 ± 0.81^{b}	15.10 ± 1.68^{ab}	16.00 ± 1.26^{c}
Bee honey (T4)	4.0 ± 0.85^{b}	14.73 ± 1.46^{b}	16.20 ± 1.46^{b}
Moringa leaf extract (T5)	5.0 ± 0.65^{a}	15.50 ± 1.75^{a}	17.20 ± 1.75^{a}
F test	**	**	**

Values presented are mean ± standard error of five replicates. F test: ** $p<0.01$; Within a column, means followed by the same letter are not significantly different by the DMRT at $p=0.05$.

Fresh weight of total plant and root

Total plant fresh weight of *Sanchezia spp*. showed a significant difference ($p<0.01$) among the tested treatments (Table 5). The maximum values for total plant weight was observed in the cuttings dipped in coconut water (55.21 g/plant) at 30 DAP. The plant growth rate is accelerated with the presence of crude proteins and growth promoting hormones such as auxins and cytokinins in higher proportions as crude proteins are useful for the formation of protoplasm, while growth hormones enhances the rapid cell division, cell multiplication and enlargement (Abdalla, 2013). Coconut water also contains higher amounts of crude protein, auxins and cytokinins (Yong *et al.*, 2009). Further, nitrogen is an essential nutrient in the growth of plants. Sandy soils are usually acidic in nature. Plants that are adapted to lower pH and grown in reducing soils absorb ammonium or amino acid (Masclaux-Daubresse *et al.*, 2010).

Table 5: Fresh weights of total plant of *Sanchezia spp*.

Dipping Treatment	Total plant fresh weight (g/plant)		
	10th day	20th day	30th day
Control (T1)	11.75± 1.02^{d}	23.50 ± 1.23^{e}	32.25 ± 1.37^{e}
Water (T2)	15.74 ± 0.99^{b}	32.56 ± 1.37^{b}	53.25 ± 1.46^{b}
Coconut water (T3)	9.63 ± 0.85^{e}	32.68 ± 1.69^{a}	55.21 ± 1.76^{a}
Bee honey (T4)	15.82 ± 1.25^{a}	30.25 ± 1.76^{c}	50.36 ± 1.93^{c}
Moringa leaf extract (T5)	12.00 ± 1.87^{c}	25.36 ± 1.83^{d}	40.48 ± 1.52^{d}
F value	**	**	**

Values are mean ± standard error of five replicates. F test: ** $p<0.01$; Within a column, means followed by the same letter are not significantly different by the DMRT at $p=0.05$.

Coconut water is an acidic component with a pH value of 4.6-5.9 (Yong *et al*. 2009). Within this range, *Sanchezia spp*. cuttings can maintain a comparatively a higher lower pH value in sandy regosol compared to the control, while giving more absorbance for ammonium or amino acid help in protein synthesis. Therefore, with both the reasons, coconut water supported the higher plant fresh

weight in *Sanchezia spp.* Although Moringa leaf extract contained auxins and cytokinins, it is an alkaline solution because of higher mineral content (Gopalakrishnan *et al*., 2016). Hence, it cannot produce a higher plant fresh weight relative to coconut water, due to the lower absorption rate of nitrogenous substances from reducing soil.

Propagation using the stem cuttings is a method of vegetative multiplication of plants, while inducing roots as the first step in growth. When the K concentration is increased, the relative water content and water storage tissues in plants are increased in plants. With increased level of K, stomatal opening could be reduced (Lin and Yeh, 2008). Coconut water, bee honey and Moringa leaf extract contain K at levels of 312 mg/100g (Yong *et al*., 2009), 0.118 mg/100g (Altun *et al*, 2017), and 259 mg/100g (Gopalakrishnan *et al*., 2016), respectively. Higher quantity of K in coconut water would lead higher absorption and storage of water thus, increasing the total fresh weight of plant.

Roots are one of the important organs in plants that help in absorbing nutrients and water, while providing a support for the standing in soil. Root fresh weight of *Sanchezia spp*. was superior in values in T3 (8.03 g/plant) at 30 DAP (Table 6). Cytokinins produced in roots are participated in nitrogen accumulation in roots itself (Fageria and Moreira, 2011). Further, hormonal signals are responsible for the inducing and controlling of vascular system development.

Auxin in the form of Indole Acetic Acid (IAA) performs as the inducing and controlling agent of xylem vessels. Tracheid and vessel elements are important transporting cells in xylem. Tracheid differentiation from cambium is induced by IAA. As coconut water contain auxin in the form of IAA, coconut water can assist in the formation of xylem vessels, especially tracheid vessel and subsequent absorption of water from root system. Hence, roots of *Sanchezia spp* plants produced by the stem cuttings treated with coconut water can produce higher root fresh weight (Aloni, 2013).

Table 6: Fresh weights of root (g) of *Sanchezia spp*.

Dipping Treatment	Root fresh weight (g/plant)		
	10th day	20th day	30th day
Control (T1)	0.37 ± 0.01^{e}	2.36 ± 1.24^{e}	4.51 ± 1.23^{e}
Water (T2)	0.98 ± 0.25^{d}	4.12 ± 1.98^{c}	7.86 ± 1.75^{b}
Coconut water (T3)	1.08 ± 0.75^{c}	5.75 ± 1.79^{a}	8.03 ± 1.99^{a}
Bee honey (T4)	2.35 ± 1.25^{b}	4.74 ± 1.89^{b}	6.60 ± 1.73^{c}
Moringa leaf extract (T5)	2.49 ± 1.38^{a}	3.56 ± 1.42^{d}	5.30 ± 1.72^{d}
F test	**	**	**

Values presented are mean ± standard error of five replicates. F test: ** p<0.01; Within a column, Mmeans followed by the same letter are not significantly different by the DMRT at p=0.05.

Dry weights of shoot, root and leaf

Tables 7 and 8 show significant differences (p<0.05) of the shoot and root dry weights of *Sanchezia spp*. among tested dipping treatments. Shoot cuttings dipped in coconut water showed the highest shoot dry weight at 20 and 30 DAP. Werner *et al*. (2001) reported that cytokinins accelerate plant growth. Further, cytokinins are associated in many aspects of plant growth and development including nitrogen signalling. When the cytokinins are provided exogenously, growth limiting factors caused by nitrogen deficiency can be exhausted (Kiba *et al*., 2011). Coconut water can provide cytokinins and perform as a supplement of nitrogen to give a higher shoot biomass.

The root length or root density is closely related to root dry weight with the proper growth and development of a plant (Fageria and Moreira, 2011). Stem cuttings dipped in Moringa leaf extract have produced higher root length at 30 DAP. Thus, the lengthier roots at higher volumes could penetrate into higher depths in soil reaching the rhizosphere, acquiring more nutrients from soil.

Table 7: Shoot dry weight of *Sanchezia spp*.

Dipping Treatment	Shoot dry weight(g/plant)		
	10th day	20th day	30th day
Control (T1)	0.14 ± 0.01^{d}	0.41 ± 0.01^{d}	1.41 ± 0.11^{e}
Water (T2)	0.14 ± 0.03^{d}	0.22 ± 0 .01^{e}	1.52 ± 0.18^{c}
Coconut water (T3)	0.15 ± 0.02^{c}	0.68 ± 0.02^{a}	1.69 ± 0.18^{a}
Bee honey (T4)	0.29 ± 0.01^{a}	0.51 ± 0.03^{c}	1.51 ± 0.13^{d}
Moringa leaf extract (T5)	0.27 ± 0.01^{b}	0.55 ± 0.01^{b}	1.55 ± 0.17^{b}
F test	**	**	**

Values presented are mean ± standard error of five replicates. F test: ** p<0.01; Within a column, means followed by the same letter are not significantly different by the DMRT at p=0.05.

Table 8: Root dry weight of *Sanchezia spp*.

Dipping Treatment	Root dry weight(g/plant)		
	10th day	20th day	30th day
Control (T1)	1.09 ± 0.01^{e}	1.56 ± 0.02^{d}	1.66 ± 0.01^{d}
Water (T2)	1.48 ± 0.01^{c}	0.64 ± 0.03^{e}	1.64 ± 0.02^{e}
Coconut water (T3)	3.23 ± 0.05^{a}	1.64 ± 0.01^{c}	1.74 ± 0.02^{c}
Bee honey (T4)	1.46 ± 0.02^{d}	1.93 ± 0.02^{b}	1.93 ± 0.01^{b}
Moringa leaf extract (T5)	2.37 ±0.09^{b}	2.12 ± 0.04^{a}	2.12 ± 0.01^{a}
F test	**	**	**

Values presented are mean ± standard error of five replicates. F test: ** p<0.01; Within a column, means followed by the same letter are not significantly different by the DMRT at p=0.05.

The changes in leaf dry weight of *Sanchezia spp* with each dipping treatment is shown in Table 9. Leaf dry weight showed a significant difference (p<0.01) among different dipping treatments at 10-day intervals. The treatment T2 produced higher leaf dry weights at 20 and 30 DAP. Leaf dry matter content is an important trait in plant ecology due to its association with plant growth and survival (Li *et al*., 2005). Leaf dry matter content is predicting the leaf nitrogen content and is closely related in the expression of specific leaf area in plants (Hodgson *et al*., 2011). With a higher leaf area, plants are capable of having a higher photosynthesis and producing higher leaf biomass.

Table 9: Leaf dry weight of *Sanchezia spp*.

Dipping Treatment	Leaf dry weight(g/plant)		
	10th day	20th day	30th day
Control (T1)	0.10 ± 0.010 b	1.07 ± 0.10 c	1.57 ± 0.11 e
Water (T2)	0.01 ± 0.001 d	2.71 ± 0.01 a	2.85 ± 0.12 a
Coconut water (T3)	0.16 ± 0.001 a	1.85 ± 0.11 b	2.65 ± 0.11 b
Bee honey (T4)	0.07 ± 0.010 c	0.85 ± 0.02 d	1.94 ± 0.10 c
Moringa leaf extract (T5)	0.01 ± 0.001 d	0.73 ± 0.03 e	1.73 ± 0.10 d
F test	**	**	**

Values presented are mean ± standard error of five replicates. F test: ** P<0.01; Within a column, means followed by the same letter are not significantly different by the DMRT at p=0.05.

Conclusion

The present study revealed that coconut water can be used as an growth stimulant to induce number of leaves, fresh weights of total plant and root and dry weight of shoot. Moringa leaf extract produced higher root length together with higher dry weights among the tested dipping treatments. Leaf

greenness (representing the leaf chlorophyll content), plant height and number of roots were higher in stem cuttings dipped in Bee honey. With the overall performance of *Sanchezia spp* stem cuttings, coconut water could be identified as the most promising natural growth stimulant for *Sanchezia spp*.

Coconut water usually wasted daily from most of the households in Sri Lanka. Thus, this is an easily available natural growth stimulant, which can be a good alternative to chemical stimulants available in the market. Coconut water can also be introduced as an alternative natural growth stimulant for other crops through further research.

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