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Evaluation of Coconut Water as a Natural Biostimulant for Enhancing Root and Shoot Development in Acerola Cherry (*Malpighia emarginata* L.) Stem Cuttings

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***Abstract* - An experiment was conducted in a home garden in the North Central Province of Sri Lanka to evaluate the response of coconut water (CW) on the growth of stem cuttings of acerola cherry and to determine the optimal CW concentration for improved root and shoot formation. Acerola cuttings with three nodes were treated with various CW concentrations, while water was used as a control. These cuttings were planted in polybags containing a mixture of topsoil, red soil, and cow dung in a ratio of 1:1:1 (v/v/v). The experiment was laid out in a completely randomized design with eight replications. The treatments included five CW concentrations (2%, 4%, 6%, 8%, and 10%) along with a control. Cuttings soaked in CW showed superior growth parameters compared to the control. At 60 days after planting, the cuttings treated with 10% (v/v) CW concentration exhibited significantly higher values in the measured growth parameters, including newly formed shoot length (7.29 cm), root number (5.25), longest root length (5.64 cm), rooting percentage (93.75%), fresh shoot weight (15.19 g), fresh root weight (2.5 g), dry shoot weight (8.85 g), and dry root weight (1.31 g). Lower values were recorded in the control for these parameters. The results demonstrated that CW had a positive impact on the growth of acerola cherry stem cuttings, with a 10% (v/v) CW concentration being the most effective for root and shoot formation among the tested treatments.**
Keywords: **Coconut Water (CW), Acerola Cherry, Stem Cuttings, Root and Shoot Formation, Growth Parameters**

I. INTRODUCTION

The acerola tree (Malpighia emarginata L.) is a well-known bonsai tree that produces small, red fruits. Acerola plants are commonly grown in home gardens throughout Sri Lanka and other tropical regions of Asia and Africa. The tree's fruit, often referred to as Barbados cherry, has significant economic and nutritional value due to its high vitamin C content [1]. Acerola fruits are also rich in phenolics, carotenoids, and anthocyanins [1], [2], and contain high levels of phytochemicals with notable antioxidant activity [3].

Immature fruits serve as a source of pectin in confections and as an enriched source of dietary fiber [4], [5]. Acerola contains natural vitamin C levels that are 50-100 times higher than those found in lemons or oranges [6]-[8]. Propagation of the acerola tree can be achieved through cutting, seed, grafting, or other methods. However, seed propagation often results in poor fruit quality, low germination rates, and reduced productivity [9].

A. Stem Cutting Propagation

Stem cutting is an essential method for large-scale propagation of improved materials while preserving the qualities of the parent plant [10]. This technique is simpler and more cost-effective than budding, grafting, or micropropagation. Organic materials are commonly added to potting media to enhance crop growth while minimizing environmental impact [11]. Microbial activities in the potting medium can further reduce environmental contamination [12].

The success of stem cutting propagation depends largely on the age and physiological status of the mother plant, the composition of the potting medium, and environmental conditions. According to the literature, shoot and root growth of stem cuttings can be improved by using optimal concentrations of appropriate plant growth regulators. Naturally occurring auxin (indole-3-acetic acid) has shown positive effects on the growth of citrus stem cuttings [13].

B. Natural Biostimulators

Natural substances, such as biostimulators, can effectively induce rooting and promote root development in stem cuttings [14], [15]. Coconut water, a natural biostimulator, contains phytohormones such as auxins, cytokinins, and gibberellins, which promote plant growth activities [14], [16]. Phytohormones can directly or indirectly enhance plant growth [17]. Coconut water stimulates root and shoot growth [18] and is a cost-effective and easily accessible alternative to synthetic plant growth regulators [19].

C. Research Gap and Objectives

Research on propagating acerola cherry through stem cuttings using natural biostimulators is limited in Sri Lanka. Coconut water, as a natural biostimulator, is a low-cost source of nutrients that could enhance the growth of acerola cherry stem cuttings. This study aims to evaluate the effect of natural biostimulators on the growth of acerola cherry stem cuttings and to identify the optimal concentration of coconut water for superior root and shoot formation.

II. MATERIALS AND METHOD

This experiment was conducted in a home garden in the North Central Province of Sri Lanka during March to September 2020 to evaluate the effect of coconut water (CW) application on the establishment of stem cuttings in acerola cherry. The home garden is located at a latitude of 8.3114˚N and a longitude of 80.4037˚E. The mean annual rainfall ranges from 1200 mm to 1900 mm, and the temperature varies between 26.5˚C and 28.5˚C. The soil type at the experimental site is classified as reddish-brown earth according to soil taxonomy.

A preliminary experiment was conducted to select a suitable biostimulator for rooting and survival of acerola cherry stem cuttings. Different stock solutions were prepared using natural biostimulators, including coconut water, honey, aloe vera gel, cinnamon powder, and vinegar, as well as pure water. The cuttings were planted in polybags and treated with 5% solutions of the various biostimulators. The preliminary study revealed that the rooting medium containing coconut water (CW) resulted in a higher rooting percentage of cuttings compared to other liquid media. Based on these findings, different concentrations of CW (0%, 2%, 4%, 6%, 8%, and 10%) were assessed to determine the optimal CW concentration for the growth of acerola cherry stem cuttings.

The experiment was laid out in a completely randomized design with six treatments and eight replicates. Semihardwood cuttings (10 cm long) with three nodes were taken using a sharp knife from the newly grown branches of a healthy 5-year-old mother plant of acerola cherry. A slant cut was made at the base of the stem cutting just below the third node. A mixture of topsoil, red soil, and cow dung in a ratio of 1:1:1 (v/v/v) was added to each polybag up to three-fourths of its height. Coconut water was diluted with distilled water to prepare 2%, 4%, 6%, 8%, and 10% concentrations.

The basal parts of the stem cuttings were soaked in the respective CW concentrations for one week, while the control cuttings were dipped in distilled water for the same duration. After soaking, the cuttings were planted in the soil mixture, with two cuttings per polybag. The polybags were kept in a shade house, and 100 ml of different CW concentrations were applied as foliar and soil treatments at two-week intervals. Water was sprayed on the cuttings daily.

At 60 days after planting, various growth parameters, including newly formed shoot length (cm), number of leaves per shoot, leaf area (cm²), rooting percentage (%), root number, longest root length (cm), and fresh and dry weights of shoots, leaves, and roots (g), were measured. The data were analyzed using a statistical analysis system, and treatment means were compared using Tukey's Honest Significance Test at P = 0.05.

III. RESULTS AND DISCUSSION

In a preliminary study, the coconut water (CW) medium showed superior results for rooting of cuttings compared to other liquid media (Table I), with cuttings treated with 5% (v/v) CW exhibiting the best rooting performance. Consequently, different concentrations of CW were evaluated in this study to determine the optimal concentration for the improved growth of acerola cherry cuttings. Coconut water contains various rooting hormones and valuable substances with the ability to initiate rooting in cuttings [14]. As a result, CW enhanced the rooting of acerola cherry cuttings effectively.

TABLE I ROOTING PERCENTAGE OF STEM CUTTINGS IN A PRELIMINARY STUDY

Biostimulant	Concentration (v/v)	Rooting Percentage (%)
Pure Water	-	20.0%
Coconut Water	5%	73.3%
Honey	5%	60.0%
Aloe Vera Gel	5%	46.7%
Cinnamon Powder	5%	33.3%
Vinegar	5%	33.3%

Based on the results of the preliminary study, this work was conducted to evaluate the effect of coconut water as a biostimulant on the growth of acerola cherry cuttings and to determine the optimal concentration of coconut water for achieving high rooting and shoot growth in acerola cherry stem cuttings.

A. Number of Leaves

Coconut water (CW) plays a crucial role in root and shoot growth, as illustrated in Fig. 1, which shows the effect of CW on the leaf number of acerola cherry at 60 days after planting. No significant difference in the number of leaves per newly developed shoot was observed among the treatments ($P > 0.05$).

The mean values ranged from 4.1 to 5.4. The maximum number of leaves (5.4) per newly formed shoot was recorded in cuttings treated with 10% CW, while the minimum number of leaves (4.1) were recorded in cuttings treated with 0% CW.

Several hormones are known to accelerate leaf growth. CW contains plant growth regulators, including auxins, gibberellins, and cytokinins, as well as natural inhibitors [20]. Cytokinins play a vital role in cell expansion during the expansion stages of leaf cell development [21].

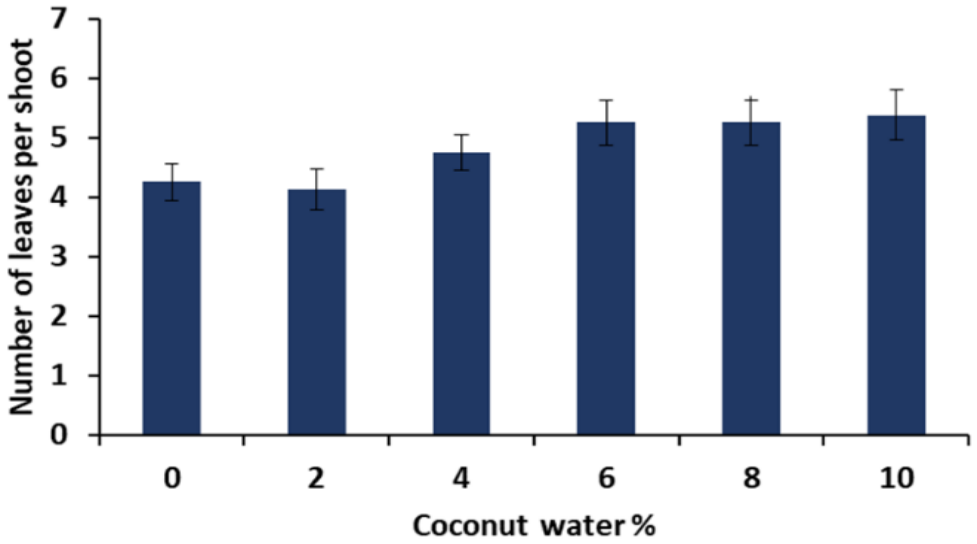

Fig 1: Effect of Coconut Water Application on Number of Leaves per Newly Formed Shoot in Acerola Cuttings at 60 Days After Planting

B. Leaf area

Fig. 2 shows the effect of coconut water (CW) application on the leaf area of acerola cuttings. The foliar application of CW increased the leaf area to 4.58 cm² in cuttings treated with 10% (v/v) CW. However, statistical analysis indicated that the treatments did not significantly influence the leaf area ($P > 0.05$). The mean values ranged from 4.28 cm² in the control treatment (0% CW) to 4.58 cm² in cuttings treated with 10% CW.

The application of CW at two-week intervals, particularly at a 10% concentration, enhanced the leaf area of acerola cuttings. This effect is likely due to the presence of naturally occurring cytokinins in CW, which promote cell expansion and leaf growth.

Leaves are crucial for the growth of the entire plant, as they capture sunlight necessary for the initial stages of photosynthesis. Additionally, there is a strong relationship between rooting success and the net photosynthetic rate, highlighting the importance of leaf development for overall plant health and productivity [22].

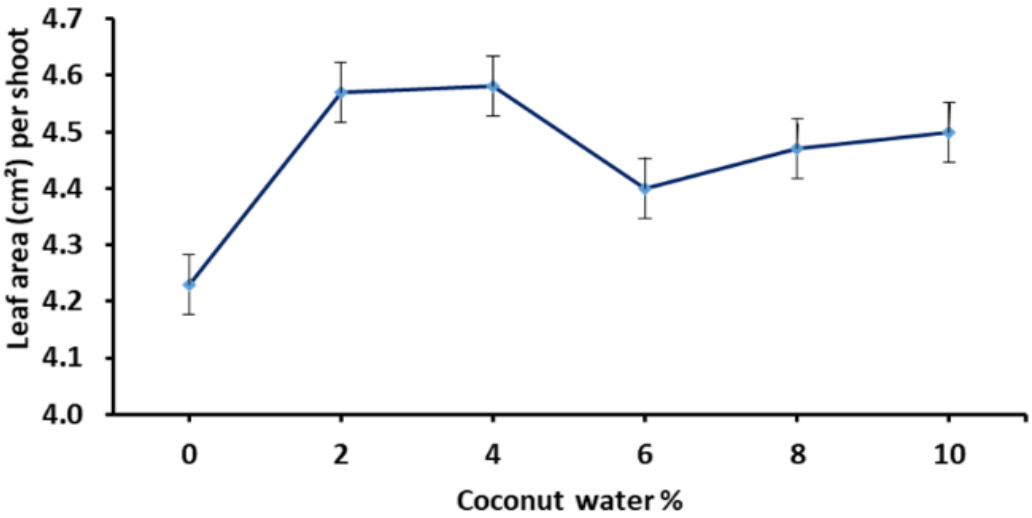

Fig 2: Leaf Area per Newly Formed Shoot in Acerola Cuttings as Affected by Coconut Water Application at 60 Days After Planting

C. Newly Formed Shoot Length

The results indicated significant variation ($P < 0.01$) among treatments with respect to the newly formed shoot length of acerola cuttings (Table II). Cuttings treated with 10% CW significantly differed from those treated with 0% and 2% CW. The highest shoot length of 7.29 cm was recorded in cuttings treated with 10% CW, followed by 6.79 cm in the 8% CW treatment. The lowest shoot length (4.14 cm) was observed in the control treatment (0% CW).

The presence of cytokinin in coconut water, a natural growth regulator, may have stimulated cell division, leading to an increase in shoot length. The formation of shoots appears to

depend on the concentration of CW, with higher concentrations promoting greater shoot elongation. In general, cytokinins are known to stimulate shoot formation, while auxins primarily promote root development.

As the CW concentration increased, both shoot and root formation were observed. The auxins present in CW contributed to shoot initiation and elongation, consistent with findings in rose cuttings where CW enhanced shoot formation [23].

D. Longest Root

The results showed that the length of roots increased significantly ($P < 0.01$) with the application of different concentrations of coconut water (CW) (Table II). The longest root length was observed in cuttings treated with 10% (v/v) CW solution, followed by the 8% CW treatment, while the minimum root length was recorded in the control treatment (0% CW).

Coconut water had a significant effect on root length [18]. Roots are a critical plant organ responsible for nutrient and water absorption. Hence, an increase in root length enhances the surface area available for nutrient absorption. The data regarding the length of the longest root indicated a progressive increase in root length with higher CW concentrations. The presence of plant growth regulators and essential amino acids in CW [14] likely contributed to this effect. The variation in the longest root length between treatments can be attributed to the auxins in CW, which promote root elongation. Seran and Thiresh [24] reported that auxin application to stem cuttings of dragon fruit significantly increased root length compared to untreated controls.

TABLE II NEWLY FORMED SHOOT LENGTH AND LONGEST ROOT LENGTH IN ACEROLA CUTTING AS INFLUENCED BY COCONUT WATER APPLICATION

Count water (%)	Newly formed Shoot length (cm)	Length of longest root (cm)
0	4.14 ± 0.44^{c}	2.96 ± 0.18^{c}
2	5.65 ± 0.32^{bc}	3.91 ± 0.28^{bc}
4	6.16 ± 0.23^{ab}	4.19 ± 0.35^{bc}
6	6.53 ± 0.33^{ab}	4.46 ± 0.35^{ab}
8	6.79 ± 0.35^{ab}	5.05 ± 0.35^{ab}
10	7.29 ± 0.46^{a}	5.64 ± 0.39^{a}
F test	**	**

F test: ** - P<0.01. Means having same letter are not remarkably variation from each other at P=0.05 level based on Tukey's honest significance Test.

E. Rooting Percentage

The results for rooting percentage revealed a significant ($P < 0.01$) difference between treatments (Table III). The application of 10% (v/v) CW resulted in a notable increase in the average rooting percentage compared to other treatments. This effect is likely due to the presence of organic acids and auxins in CW, which are essential for root development. The average rooting percentage ranged from 93.75% in the 10% CW treatment to 50% in the control (0% CW) treatment. Auxins, whether naturally occurring or applied exogenously via CW, are critical for initiating root formation in stem cuttings [25]. These results underscore the effectiveness of CW as a biostimulant for promoting root development in acerola cherry cuttings.

TABLE III ROOTING PERCENTAGE AND NUMBER ROOTS PER ACEROLA CUTTING AS INFLUENCED BY COCONUT WATER APPLICATION

Coconut Water %	Rooting %	Number of Roots Per Cutting
0	50.0^{f}	2.6 ± 0.3^{c}
2	56.3^{e}	$3.8\pm0.3b^{c}$
4	68.8^{d}	$3.6\pm0.4b^{c}$
6	75.0^{c}	$3.5\pm0.3b^{c}$
8	87.5^{b}	4.5 ± 0.2^{ab}
10	93.8^{a}	5.2 ± 0.3^{a}
F test	**	**

F test: ** - P<0.01. Means having the similar letter are not remarkably varied from each other at P=0.05 level according to the Tukey's honest significance Test

F. Number of Roots

Root hairs on the taproot are essential for absorbing the required amounts of water and nutrients. The data on the number of lateral roots at 60 days after planting are presented in Table III. The analysis of variance for the number of roots per cutting revealed that the treatments had a significant impact ($P < 0.01$) on root number.

Cuttings treated with 10% CW exhibited a significantly higher number of roots compared to other treatments. The number of roots per cutting ranged from 5.25 in the 10% CW treatment to 2.625 in the control treatment (0% CW) (Fig. 3). The data indicated a gradual increase in the number of roots per cutting with increasing CW concentration.

Fig 3: Root growth of cuttings in 0% (A) and 10% (B) concentrations of coconut water.

An increase in indole-3-acetic acid (IAA) concentration up to a certain level has been shown to enhance the root number in stem cuttings treated with IAA [13]. Similarly, prolonged soaking of seeds in coconut water (CW) has been reported to increase root production [18]. Indole-3-butyric acid (IBA), present in CW, has a greater ability to promote the formation of adventitious roots compared to IAA [26], [27]. This is likely due to the higher stability of IBA, which allows it to induce a greater number of adventitious roots [27]. IAA was the first plant hormone used for stimulating rooting in cuttings [28]. Its role, along with other hormones in CW, highlights the importance of natural biostimulants in enhancing root development. Additionally, the use of labour remains a predominant factor in agricultural activities, including propagation practices [29].

G. Fresh Weights of Newly Formed Plant Parts

The effect of coconut water (CW) on the fresh weight of newly formed shoots, leaves, and roots at 60 days after planting is presented in Table IV. The fresh weights of shoots and roots exhibited significant variations ($P < 0.05$) among the treatments, while the fresh weight of leaves showed no considerable variation ($P > 0.05$).

The fresh weight of shoots ranged from 12.93 g in the control treatment (0% CW) to 15.19 g in the 10% CW treatment. The fresh weight of leaves ranged from 3.64 g (0% CW) to 4.55 g (10% CW), while the fresh weight of roots ranged from 2.01 g (0% CW) to 2.52 g (10% CW). These results indicate that CW application, particularly at a 10% concentration, positively influences the fresh weight of shoots, leaves, and roots.

TABLE IV IMPACT OF COCONUT WATER ON FRESH WEIGHTS OF NEWLY FORMED LEAVES, SHOOT AND ROOT PER ACEROLA CUTTING

Coconut Water %	Fresh weights (g) of newly formed plant parts per cutting		
	Shoot	Leaves	Roots
0	12.93±0.38^{b}	3.64±0.28	2.01±0.09^{b}
2	13.79±0.85^{ab}	3.43±0.26	2.51±0.05^{a}
4	14.09±0.14^{ab}	4.00±0.25	2.19±0.09^{ab}
6	14.24±0.15^{ab}	4.35±0.25	2.27±0.09^{ab}
8	14.36±0.31^{ab}	4.52±0.30	2.35±0.07^{ab}
10	15.19±0.37^{a}	4.55±0.24	2.52±0.03^{a}
F test	*	ns	*

F test: * - P<0.05; ns - not significant. Means having the same letter are not remarkably varied from each other at P=0.05 level based on Tukey's honest significance Test.

All treatments resulted in improved fresh weights of shoots, leaves, and roots compared to the control. The presence of auxins in coconut water (CW) contributed to increased fresh root weight by promoting the production of a greater number of roots and longer root lengths. These findings confirm that the use of CW positively impacts plant growth [30].

H. Dry Weight of Newly Formed Plant Parts

The results recorded on dry weight of newly formed shoot and roots clearly showed considerable differences ($P<0.05$) among treatments and leaves showed no significant variation ($P>0.05$) among treatments (Table V). 10% of CW recorded highest dry weight of shoots, leaves and roots. Dry weight of roots increased by increasing concentration of coconut water. Application of CW had substantial effects on plant growth. Water stress at various stages of plant growth affects the biological yield of crop [31]. More than half of the water that enters the soil returns to the atmosphere through evapotranspiration [32].

TABLE V INFLUENCE OF COCONUT WATER ON DRY WEIGHTS OF NEWLY FORMED LEAVES, SHOOT AND ROOT PER ACEROLA CUTTING

Coconut Water %	Dry Weights (g) of Newly Formed Plant Parts Per Cutting		
	Shoot	Leaves	Roots
0	7.01±0.35[b]	0.95±0.12	0.73±0.18[b]
2	7.61±0.65[ab]	0.91±0.11	1.02±0.15[ab]
4	8.07±0.14[ab]	1.17±0.14	1.07±0.10[ab]
6	8.14±0.16[ab]	1.39±0.19	1.05±0.11[ab]
8	8.26±0.22[ab]	1.44±0.24	1.15±0.05[ab]
10	8.85±0.27[a]	1.45±0.24	1.31±0.11[a]
F test	*	ns	*

F test: * - $P<0.05$; ns - not significant. Means having same letter are not considerably varied from each other at $P=0.05$ level based on Tukey's honest significance Test.

IV. CONCLUSION

In this study, root formation in acerola stem cuttings was significantly affected by the application of different concentrations of coconut water (CW). The use of CW resulted in a well-developed root system compared to the control treatment, as observed in the measured agronomic parameters. The results demonstrated that increasing CW concentrations had a positive effect on newly formed shoot length, longest root length, rooting percentage, root number, and the fresh and dry weights of shoots and roots. Among the tested treatments, a 10% (v/v) concentration of CW showed the best response for rooting of acerola cuttings. Coconut water, being a locally available and low-cost biostimulator, can serve as an effective plant growth regulator for promoting rooting in cuttings. This makes it a sustainable and economical alternative for agricultural practices.

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