Application of Coconut (Cocos Nucifera) Water as an alternative source of Plant growth Hormones for Ginger (Zingiber Officinale) tissue culture

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Research Article

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Posted Date: May 18th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9507088/v1

Additional Declarations: No competing interests reported.

Application of Coconut (*Cocos Nucifera*) Water as an alternative source of Plant growth Hormones for Ginger (*Zingiber Officinale*) tissue culture

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Abstract

Background Tissue culture provides a solution for the quick propagation of ginger to overcome its constantly increasing demand due to its medicinal, spicy, and confectionery uses. Phytohormones are crucial growth regulators in plant tissue culture, and coconut water is a promising, economical, and available alternative source. This research investigated the effects of different concentrations (5 %, 10%, and 20 %) of coconut water obtained from two varieties (Samoan tall and Nias green) at different maturity stages (Mature and Immature) from three locations (Aboh, Ahiazu, and Ezinihitte LGA) on the height, number of leaves, vigor, survival rate, and root/shoot development of ginger plants.

Results The variety, location, and maturity levels of coconut water had no significant ($p>0.05$) effect on the development of the ginger plants. However, the concentration of coconut water had a significant ($p>0.05$) effect on ginger development. Ginger plants cultured in MS basal medium supplemented with 10 % coconut water performed as well as those in the control medium (conventional phytohormones) and showed no significant ($p<0.05$) difference when compared with the 20% supplementation. Conversely, ginger cultured in 5 % coconut water failed in vigor compared to the auxin-containing control.

Conclusions Coconut water proved to be an effective and reliable, local, and cheap alternative source of phytohormones for the tissue culture of ginger.

Keywords: Coconut Water, Ginger, Tissue Culture, Phytohormone, Alternative Source**.**

1. INTRODUCTION

Ginger is an essential cash crop in Nigeria whose production and propagation is on the decline despite our abundant land and human resources. With global demand for high-quality ginger soaring, a critical 26% production deficit averaging just 2.5 tons/hectare against a 3.4

tons/hectare requirement coupled with the devastating 2023/2024 blight, has plunged supply into a persistent decline, severely limiting its availability for vital medicinal and confectionary uses [1]. The commercial multiplication of ginger rhizome is done predominantly by asexual propagation to avoid genetic segregation and long juvenile phase of plants originated through rhizome [2]. This contributes to the decline in production but tissue culture provides solution to quick propagation to overcome the decline. Therefore *in vitro* propagation in aseptic culture medium shall be an alternative method to speed up multiplication or even selection of new ginger genotypes. In addition, in vitro culture of plant embryos are used as an important research tool for several purposes, such as rescue of rare hybrids; genetic manipulation; propagation of elites and disease free germplasm etc. One of the most important factors ruling the growth and morphogenesis of plant tissues in vitro is the composition of the culture medium. The basic nutrient requirements of cultured plant cells are very similar to those of whole plants.

Tissue culture broadly refers to the *in vitro* culture of explants under aseptic conditions (sterility) in appropriate nutrient medium under optimum environmental conditions [3, 4]. Among the most usual compounds used to supplement the culture media are the yeast and malt extracts, potato and banana pulps, tomato juice and coconut water [5, 6].

Phytohormones are important component of tissue culture which are chemical substances that control growth and developments of plants [7,8]. However, conventional phytohormones in tissue culture can induce abnormal growth (malformation, apical dominance), cause toxicity at high concentrations (like 2,4-D acting as a herbicide), promote excessive vegetative growth over fruiting, induce ethylene buildup (inhibiting growth), leads to genetic instability (somaclonal variation), cause undesirable morphology (like stunted roots or malformed leaves), redirect plant energy [9], lead to genetic or epigenetic changes, potentially causing issues like hyperhydricity

(water logging) or reduced plant resilience and adding significant costs and labor to the culture media which coconut water is promising to be an alternative and cheap source for economic and availability reasons.

Coconut (*Cocos nucifera)* water is a clear liquid inside of the fruit of a coconut palm. In early developmental stage, coconut water serves as a suspension for the endosperm of the coconut during their nuclear stage of development. As growth progresses, the endosperm matures into their cellular phase and deposit into the rind of coconut meat which is one of the basic parameters to distinguish between the mature and immature coconut water [10, 11] Coconut water contains several organic compounds and mineral nutrients important for plant developments and plays significant roles as physiological buffer. It is rich in magnesium, phosphate and contains high amount of sugar, about 2.5% [12, 13]. Besides that, coconut water has high level of nitrogen in the form of amino acids and phyto-hormones in an adequate balance for plant requirements [14]. In consideration of high content of potassium and other trace metals (e.g. magnesium and phosphorus), low level of fat, mineral content of coconut water, anti-oxidant, L-arginine, ascorbic acid, vitamins, carbohydrates, cytokinins which promote plant cell division and growth and high percentage sterility of coconut water [15]. This study therefore verified the effectiveness and reliability of coconut water as a suitable alternative source of plant growth hormones in tissue culture of ginger in a perfect sterile condition.

2. MATERIALS AND METHODs

2.1 Sources of coconut water

Two varieties of coconut water (Samoan tall and Nias green from three different local government areas of Mbaise (Aboh, Ahiazu and Ezinihitte), in Imo State, Nigeria, where sourced

from private farms and used for the study. The Plant materials were identified and verified by a botanist, Dr. C. M. Duru, of the department of Biology, Federal University of Technology, Owerri (FUTO), Imo State, Nigeria, with voucher specimens deposited as noted below. The two varieties of coconut water (the Samoan tall and Nias green) were harvested at two different physiological stages of maturity (the mature and immature). The physiological stages were determined by the number of months of the coconut from flowering. Four coconuts were used per sample and a total of 48 coconuts were used for the study. The coconut water was carefully drained from the mature and immature coconuts by drilling holes through the germination pore with a sterile stainless-steel knife punch under laminar flow conditions [16]. The coconut water was carefully harvested and checked to ensure that there was no fermentation before adding to the bulk. The extracts collected were heated at 50-100 ^{o}C for 10 minutes with continuous stirring to precipitate the fat, protein and other materials [17,18]. The precipitates were separated by filtration and were used immediately for medium preparation following the method of [17,19].

Plate 1

plate 2

Plates 1 & 2: Immature and Mature Samoan tall coconuts respectively

Plate 3 plate 4

Plates 3& 4: Immature and Mature Nias green coconuts respectively.

2.2 Source of ginger explants

The ginger explants used for this study were sourced from plantlets already established *in vitro*, using meristem tip culture, at the plant tissue culture laboratory of National Root Crop Research Institute (NRCRI) Umudike, Ikwuano Local Government Area, Abia State.

2.3 Experimental design

Coconut water from the two different sampled varieties of coconut designated as V1 and V2 were collected. Each of the two samples were sourced from three different locations designated as

V1L1	V1L2	V1L3
V2L1	V2L2	V2L3

Each of the two varieties of coconut (V1 and V2) from three specific locations (L1, L2 and L3) where collected at two different physiological stages; mature and immature (V1L1M, V1L1I, V1L2M, V1L2I, V1L3M, V1L3I, V2L1M, V2L1I, V2L2M, V2L2I, V2L3M, V2L3I.) were

V1L1M represents Coconut water from mature Samoan tall coconut sourced randomly from private farms in Mbutu community, Aboh Mbaise Local Government Area, Imo State, Nigeria.
V1L1I represents Coconut water from immature Samoan tall coconut sourced randomly from private farms in Mbutu community, Aboh Mbaise Local Government Area, Imo State, Nigeria.
V1L2M represents Coconut water from mature Samoan tall coconut, sourced randomly from private farms in Ogbe community, Ahiazu Mbaise Local Government Area, Imo State, Nigeria
V1L2I represents Coconut water from immature Samoan tall coconut sourced randomly from private farms in Ogbe community, Ahiazu Mbaise Local Government Area, Imo State, Nigeria.
V1L3M represents Coconut water from mature Samoan tall coconut, sourced randomly from private farms in Eziudo community, Ezinihitte Mbaise Local Government Area, Imo State, Nigeria
V1L3I represents Coconut water from immature Samoan tall coconut, sourced randomly from private farms in Eziudo community, Ezinihitte Mbaise Local Government Area, Imo State, Nigeria

V2L1M represents Coconut water from Mature Nias green coconut sourced from private farms in Mbutu community, Aboh Mbaise Local Government Area, Imo State, Nigeria.

V2L1I represents Coconut water from immature Nias green coconut sourced from private farms in Mbutu community, Aboh Mbaise Local Government Area, Imo State, Nigeria.

V2l2M represents Coconut water from Mature Nias green coconut, sourced randomly from private farms in Ogbe community, Ahiazu Mbaise Local Government Area, Imo State, Nigeria

V2L2I represents Coconut water from immature Nias green coconut sourced randomly from private farms in Ogbe community, Ahiazu Mbaise Local Government Area, Imo State, Nigeria

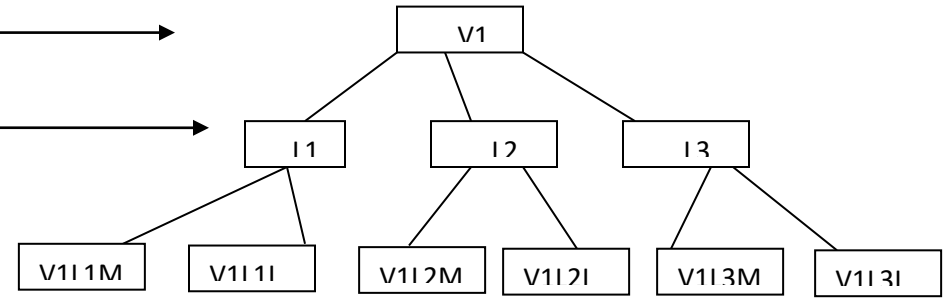
V2L3M represents Coconut water from Mature Nias green coconut, sourced randomly from private farms in Eziudo community, Ezinihitte Mbaise Local Government Area, Imo State, Nigeria and

V2L3I represents Coconut water from immature Nias green coconut sourced randomly from private farms in Eziudo community, Ezinihitte Mbaise Local Government Area, Imo State, Nigeria.

Sample 1

Locations

Maturity



Sample 2

Locations

Maturity

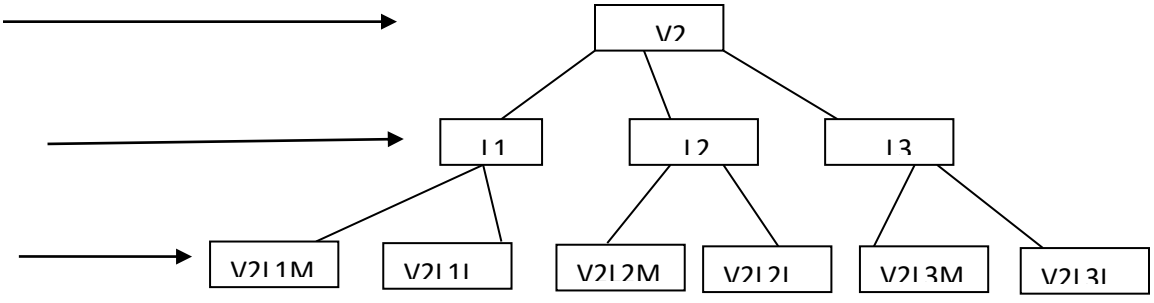


Fig. 2.1 Diagrammatic representation of the entire experimental design

2.4 Medium composition and preparation

i). MS medium for tissue culture of ginger as the control C

Table 1: Medium composition

MS Stock solution	Quantity used	
NH_4NO_3	33000 mg/L	
KNO_3	38000 mg/L	
$CaCl_2.2H_20$	8800 mg/L	50 mL/L
$MgSO_4.7H_2O$	7400 mg/L	
KH_2PO_4	3400 mg/L	
K1	160 mg/L	
H_3BO_3	1240 mg/L	
$MnSO_4.4H_2O$	4460 mg/L	
$ZnSO_4.7H_2O$	1720 mg/L	5 mL/L
$NaMoO_4.2H_2O$	50 mg/L	
$CoCl_2.6H_2O$	5 mg/L	
$CuSO_4.5H_2O$	5 mg/L	
$FeSO_4.7H_2O$	5.560 mg/L	
$Na_2.EDTH$	7460 mg/L	5 mL/L
MS Vitamin mixture	5 mL/L	
Sucrose	30 g/L	

Myoinositol	100 mg/L
L-cystine	10 mg/L
Agar	7 g/L

Auxin(1-napthaleneacetic acid),and Cytokinin (BAP) 2 mg/L.

ii). MS Basal medium supplemented [19] with 5 %, 10%and 20% of coconut water of two varieties of coconut (Samoan tall and Nias green), from three locations and two maturity stages designated as V1L1M, V1L1I, V1L2M, V1L2I, V1L3M, V1L3I; V2L1M, V2L1I, V2L2M, V2L2I, V2L3M, V2L3I.

2.5 Medium preparation procedure:

In the course of medium preparation, 500 mL of distilled water (DW) was placed in one liter beaker containing a magnetic bar. Each beaker was placed on the magnetic stirrer hot plate; MS stock solutions and MS vitamin stock solution were added as stipulated in the medium composition. The stirrer was kept on, all the time, sucrose, myoinositol were also added (MS basal medium does not contain growth regulator). Each basal medium prepared was supplemented with 5 %, 10 % and 20 % of coconut water from different samples of coconut used for the experiment. Distilled water was added to approximately 1000 mL mark. Each beaker containing approximately 1000 mL medium solution was adjusted to pH 5.8 with drop wise of 0.5 mL NaOH and 0.5 mL HCl. Each solution was poured to a one liter measuring cylinder and DW was added to 1000 mL mark. Each medium was transferred to a one liter flat bottom flask. The flask containing media sample were properly labeled and placed in a micro wave oven for 10 minutes for the media to melt. Each medium was dispensed in a 25 x 150 mm test tube bearing the name of the medium sample as stated above. The test tubes with the media were autoclaved for 15 minutes at a temperature of 121 ^{o}C [18,19]. The sterilized media in test tubes

were removed when the pressure reached zero and temperature below 100 ºC. The medium samples were stored in the aseptic manipulation room.

2.6 Plantlet multiplication

Explants from established ginger plantlets were transferred to the experimental media comprised of full strength MS basal medium [19] supplemented with 5 %, 10 % and 20 % (varying concentration) coconut water and MS medium for ginger multiplication that serves as the control.

Excess folia leaves were removed from each plantlet using sterile forcep and scapel, the base of the explants placed in a sterile petri-dish was cut off before inoculation into the medium. This was done in the aseptic room under the laminar airflow cabinate. The explants were inoculated individually in 25 x 150 mm text tube. The tubes were thoroughly sealed and labeled before taking the cultures to the growth room. Cultures were maintained at 28 $\pm$2 ºC, and 16 hour photoperiod [19, 20] for 8 weeks. The plant height in cm, number of leaves, number of roots, survival rate, number of shoots and vigor were assessed for each concentration for eight consecutive weeks.

2.7 Statistical Analysis

The data were analyzed using a one-way ANOVA, while mean separation was done using Duncan's Multiple Range Test. Probability level at $P>0.05$ was considered significant.

225

226 **3.0 Result**

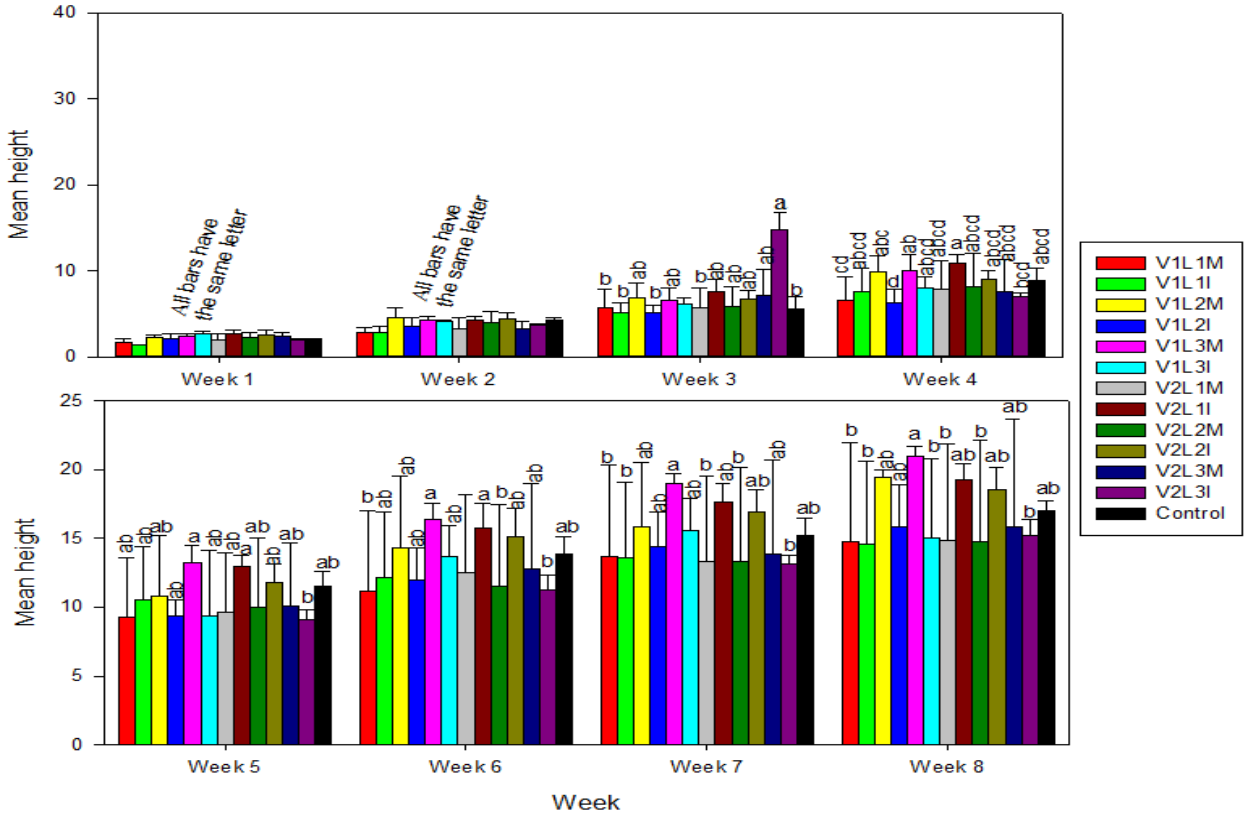


Figure 1: Effect of 50 mL of coconut water supplementation basal medium on the height of giger culture. n = 5.

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228 Bars with the same letters within each week are not significantly different at 0.05 probability.

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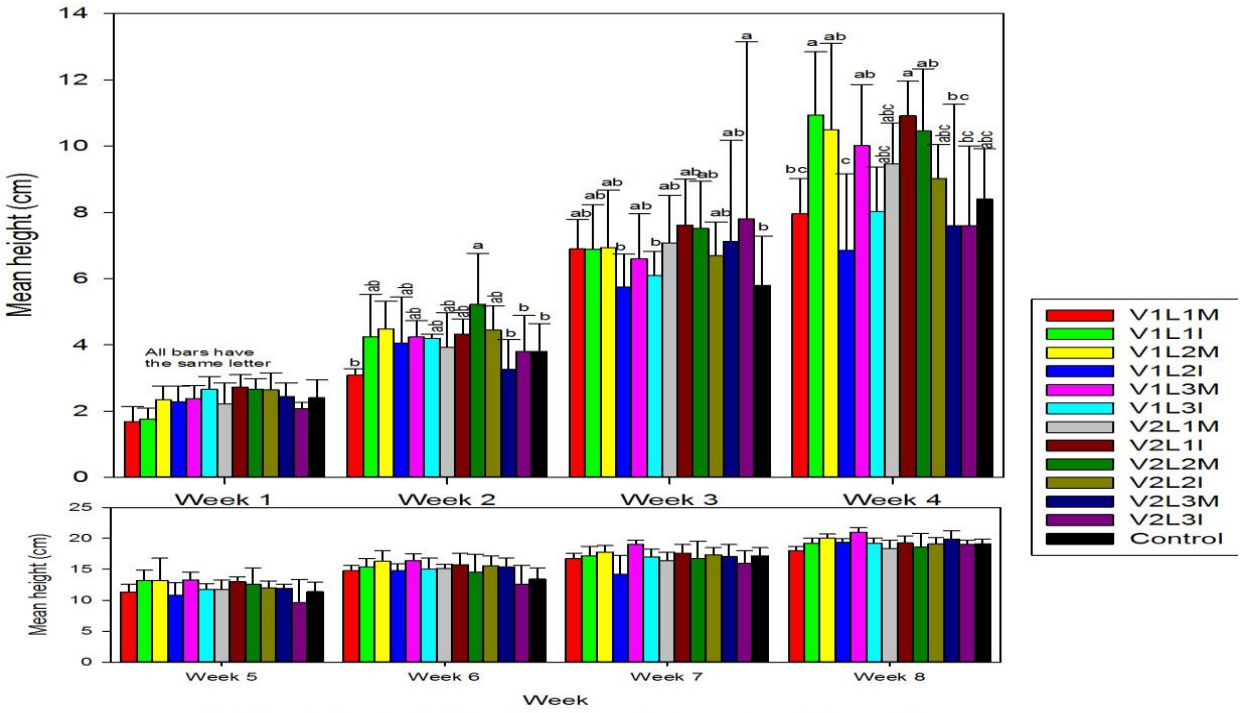

Figure 2: Effect of 100 mL of cocont water supplemental medium on the height of tissue culture of ginger. n= 5

Bars with the same letter within each week are not significantly different at $P<0.05$

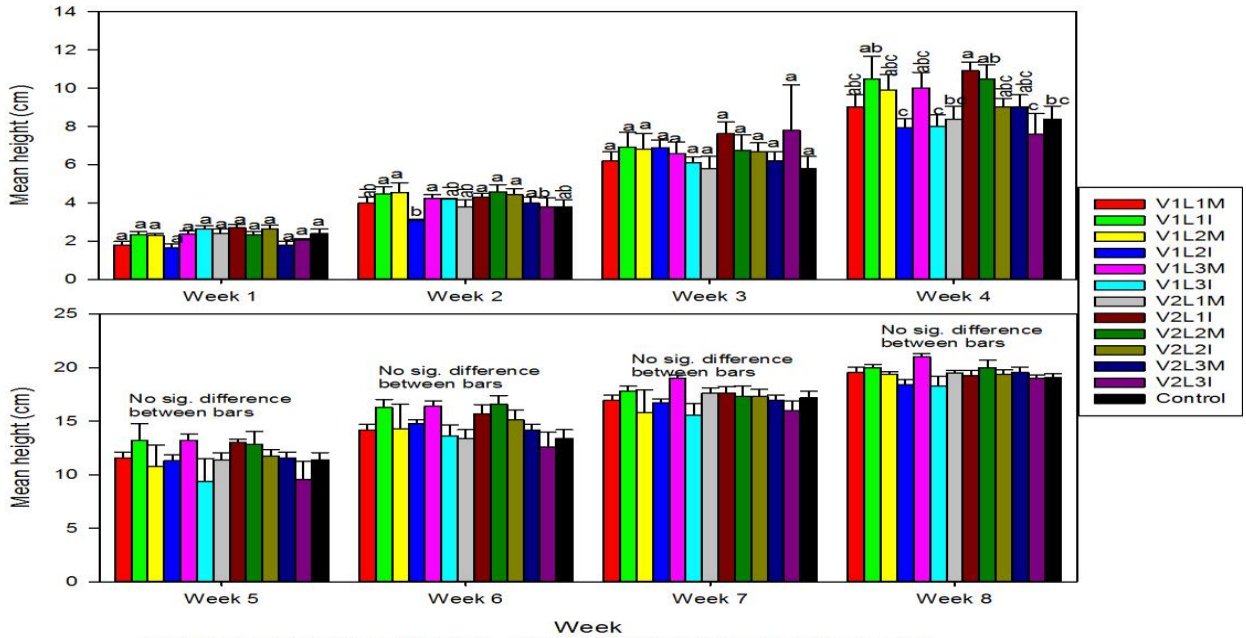

Figure 3: Effect of 200 mL of coconut water supplemental basal medium on the height of ginger culture. n = 5.

Bars with the same letter within each week are not significantly different at P<0.05

The effect of 5 % coconut water supplementation to basal medium on the height (cm) of ginger plants, using two varieties of coconut from three different locations and two maturity stages, as an alternative source of phytohormone in tissue culture of ginger was shown in Fig. 1.

From week one up to week eight, all the samples grew competitively with the control (C) despite the varieties, location and maturity stages of the coconut water used as alternative source of phytohormones. Sample V2L3I (ginger cultured in coconut water from immature Nias green coconut, Ezinihitte) showed greatest height above all the samples even above the control (C) but not significantly (P<0.05) different from samples VIL2M, VIL3M, V2L1I, V2L2I, V2L3M, V2L2M at week three. Rapid growth was experienced in all the samples from week four up to week eight with samples V1L3M, V2L1I, V2L2I and V1L2M showing excellent growth above

246 others till the end of the research period. Interestingly, when compared with the control, there
247 was no significant ($P<0.05$) difference in all the samples.
248 Effect of 10 % coconut water supplementation on the height of ginger plants is shown in Fig 2.
249 All the samples grew well in the basal medium supplemented with 100 mL coconut water and
250 produced appreciable height at week one and there was no significant ($P<0.05$) difference when
251 compared with the control (C) that contained phytohormones at the end of first week. Sample
252 V2L2M (ginger cultured in coconut water from mature Nias green from Ahiazu) showed the
253 highest growth rate at week two even above the control (C). High competitive growth was
254 observed from week three up to week eight in all the samples, despite the variety and location of
255 the coconut waters used. At week three, sample V2L3I showed the highest height production
256 while samples V1L2I and V1L3I showed the least height (cm) production but there were no
257 significant ($P>0.05$) difference when compared with the control (C). Samples V1L1I and V2L1I
258 produced the highest height while sample V1L2I showed the least growth but when compared to
259 the control (C) containing phytohormones, there was no significant ($P>0.05$) difference at week
260 four. All the samples followed the same trend of growth from week five up to week eight,
261 producing and maintaining maximum height (cm) till the end of the research period, and in all
262 samples there were no significant ($P>0.05$) difference in heights .
263

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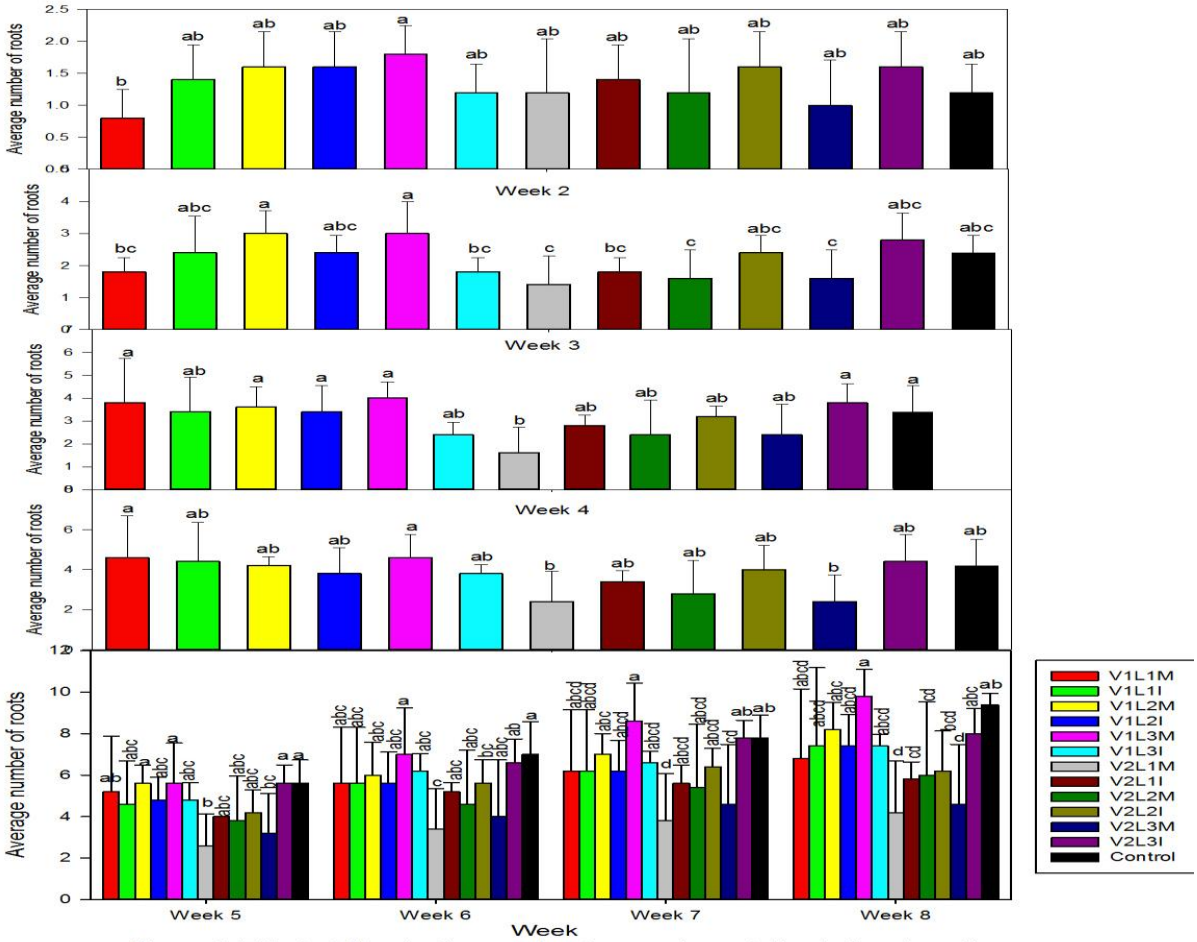

Figure 4: Effect of 50 mL of coconut water supplementation to basal medium on the number of roots in ginger culture (n = 5).

Bars with the same letter within each week are not significantly different at P<0.05 probabilities

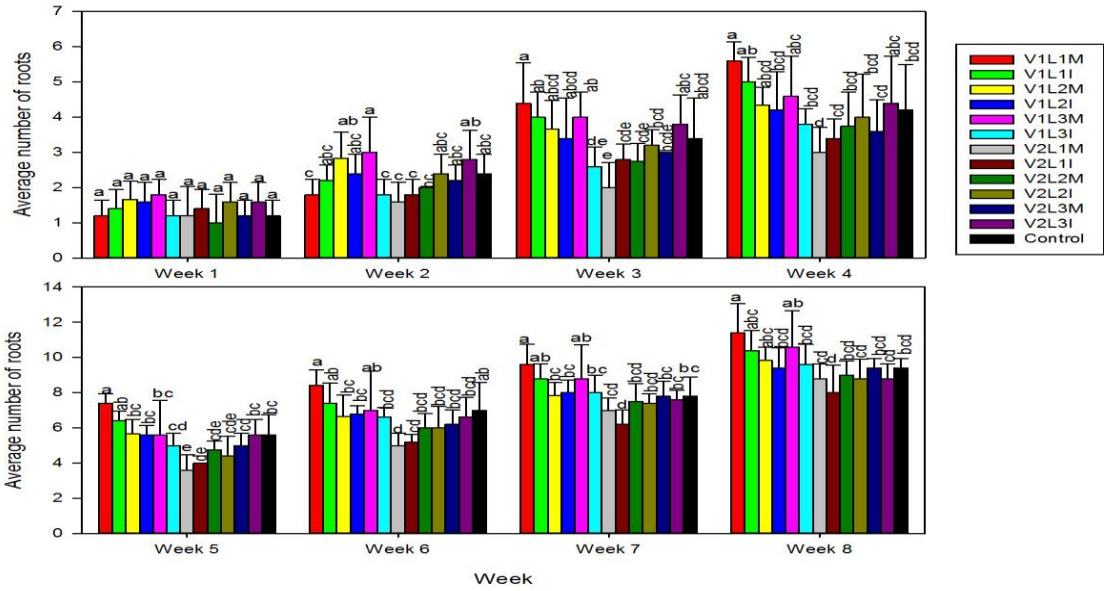

Figure 5: Effect of 100 mL of coconut water on the number of roots in ginger culture. n = 5.

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2D Graph 1

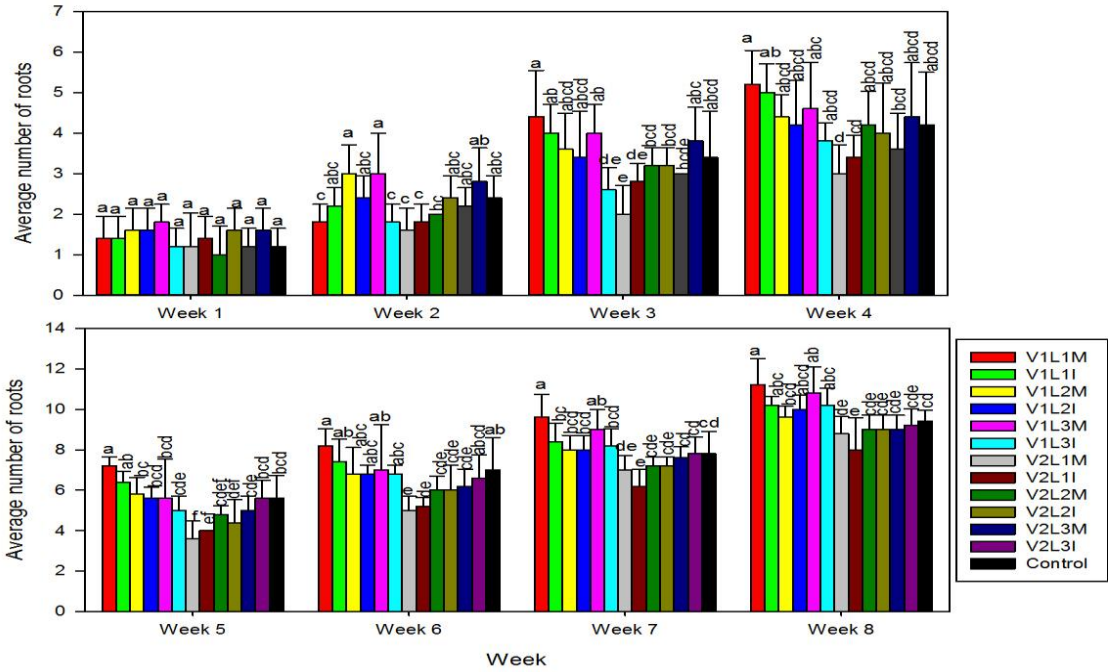

Figure 6: Effect of 200 mL of coconut water supplementation basal medium on the number of roots in ginger culture. n = 5.

270 Bars with the same letters within each week are not significantly different at P<.05 probabilities

271

The effect of 5 % coconut water was represented in figure 4. Maximum numbers of roots were observed in all the samples with sample V1L3M proliferating the highest number of roots even above the control (C) throughout the experimental period. At week two, samples V2L1M, V2L2M and V2L3M cultured in Nias green coconut water basal medium supplementation produce the least number of roots but when compared to the control, showed no significant ($p>0.05$) difference. Rapid increase in root production was observed from week three up to week eight of the experiment in all the samples. At the third and fourth week, V2L1M produced the least number of roots. At week six, seven and the last week of the experiment, sample V2L1M produced the least number of roots and there was significant ($p<0.05$) difference among the samples and when compared to the control.

Effect on the number of roots of ginger cultured *in vitro* in MS basal medium supplemented with 10 % coconut water was presented in figure 5**.** *In vitro* ginger cultures supplemented with 10 % of coconut water showed consistent root proliferation from weeks one through eight. Samples from the Samoan Tall variety (V1L1M, V1L1I, and V1L3M) consistently produced the highest number of roots, often exceeding the control group. Samples from the Nias Green variety (V2L1M and V2L1I) generally produced the fewest roots. While no significant difference from the control was noted in the first two weeks, a significant difference ($p<0.05$) emerged by weeks five and six among the various samples and the control groups.

Figure 6 illustrates the effect of MS basal medium supplemented with 20 % of coconut water on the root count of *in vitro* ginger cultures. During the first week, all samples produced root numbers comparable to the control, with no significant statistical difference ($p>0.05$). By week two, samples V1L2M and V1L3M outperformed the control, while V1L1M, V1L3I, V2L1M**,**

and V2L1I showed the least proliferation. Significant root proliferation continued from week three through week eight. During weeks three and four, V1L1M, V1L1I**,** and V1L3M (Samoan Tall variety) yielded the highest root counts, whereas V2L1M and V2L1I yielded the lowest. By week five, all ginger plants cultured in Samoan Tall coconut water showed superior root production regardless of maturity stage or location, while those in Nias Green coconut water (specifically V2L1M) performed poorly. In weeks six and seven, V1L1M maintained the highest production levels. Notably, all samples showed appreciable root growth, though V1L1M remained the most productive and V2L1Ithe least. Statistical analysis confirmed a significant difference ($p<0.05$) among the treatments and when compared to the control group.

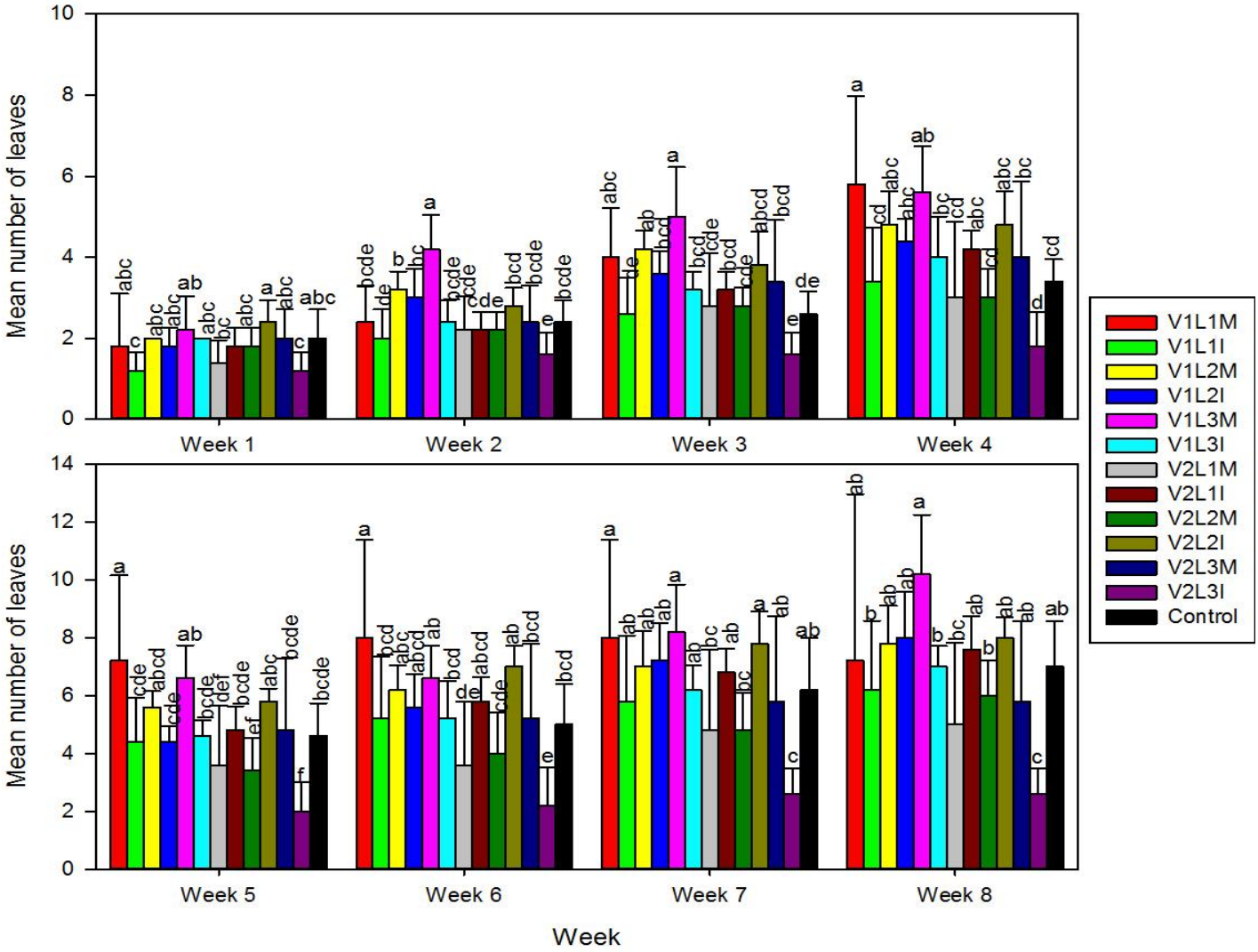

Figure 7: Effect of 50 mL of coconut water supplemental basal medium on the number of leaves of ginger culture. n = 5

304 Bars with the same letters within each week are not significantly different at 0.05 probabilities.

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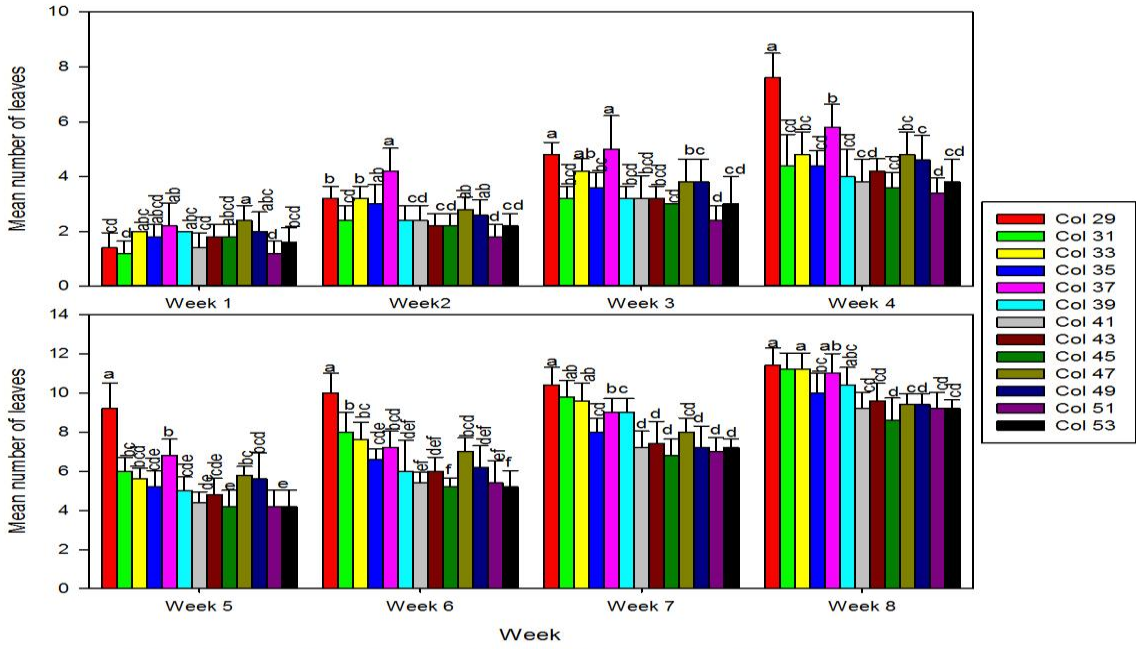


Figure 8: Effect of 100 mL of coconut water supplemental basal medium on the number of leaves of ginger plant (n = 5)

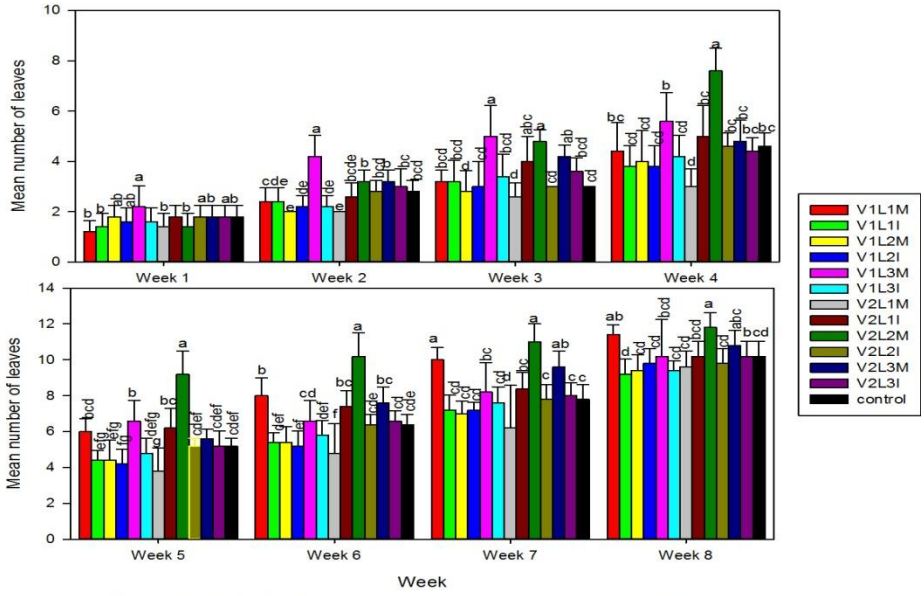


Figure 9: Effect of 200 mL of coconut water supplementation basal medium on the number of leaves of ginger plant (n = 5)

Bars with the same letters within each week are not significantly different at P<.05 probabilities

Figure 7 illustrates how supplementing basal medium with 5 % of coconut water affects leaf production in ginger plants within a tissue culture setting. When used as an alternative phytohormone source, immature Nias green coconut water (V2L2I) initially led to the most leaves at week one, though this difference was not statistically significant ($p>0.05$) compared to the control (C). By the second week, Samoan tall coconut water (V1L3M) showed a higher number of leaves than the control, a trend that persisted until the fourth week, with no significant differences observed relative to the control group. From week five to six, sample V1L1M consistently produced the highest number of leaves. Notably, sample V2L3I stopped developing new leaves after the second week and did not produce any more growth through week eight. All other samples produced considerable leaf growth, with no statistically significant differences from the control, regardless of the coconut water's variety, maturity, or location.

Ginger tissue culture experiments (Figure 8) demonstrate that 10 % of coconut water supplementation can effectively serve as an alternative phytohormone source, often outperforming the control medium containing synthetic hormones. Notably, ginger cultured in both immature Nias green (sample V2L2I) and mature Samoan tall coconut water (samples V1L1M, V1L3M, V1L2M) exhibited superior leaf proliferation over several weeks. Sample V1L1M consistently showed the highest number of leaves across the study. Conversely, several samples, including V1L1, V2L3I, and V2L2M, produced the fewest leaves, with some results being statistically comparable to the control ($p>0.05$).

Figure 9 illustrates the time course of the effect of supplementing basal medium with 20 % coconut water on ginger leaf production in a tissue culture setting. All samples exhibited good leaf production at week one, with V1L3M showing the highest count. At weeks two and three,

V1L3M and V2L2M produced the most leaves, significantly outperforming the control (C), while V2L1M and V1L2M produced the fewest. Though all samples produced a considerable number of leaves compared to the control, V2L1M and V1L3M showed exceptional performance, yielding very high leaf counts above the control at weeks four and five, whereas V2L1M produced the least number of leaves during the same period. At weeks six and seven, V2L1M and V1L1M produced a great number of leaves, again above the control, while V2L1M produced the least, falling below the control's count. By the study's conclusion, V2L2M and V1L1M displayed the highest number of leaf proliferation, and V1L1M showed the lowest, though this difference was not statistically significant (P>0.05) when compared with the control.

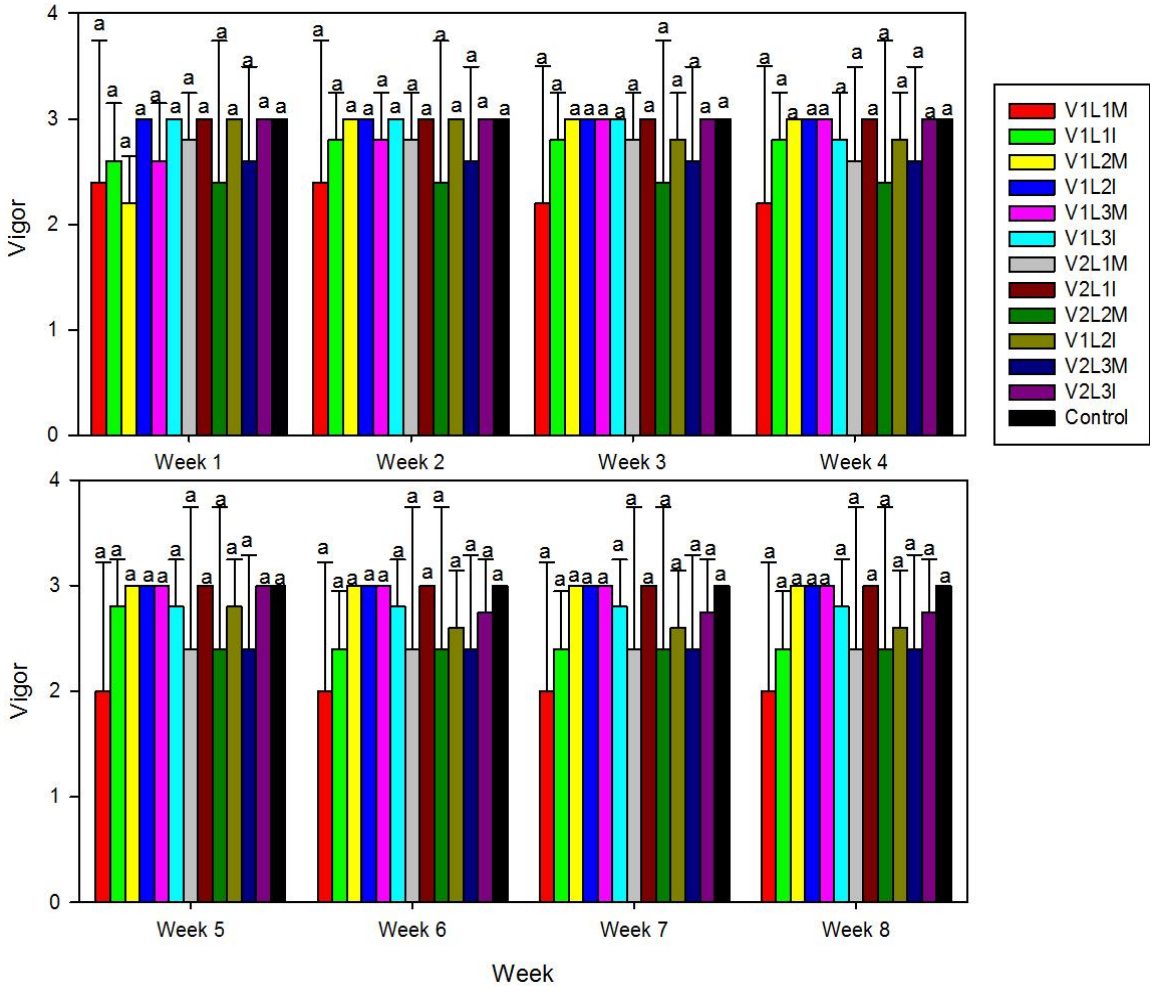


Figure 10: Effect of 5 % of coconut water supplemental basal medium on the vigor of ginger growth. (n = 5)

Bars with the same letters within each week are not significantly different at 0.05 probabilities**.**

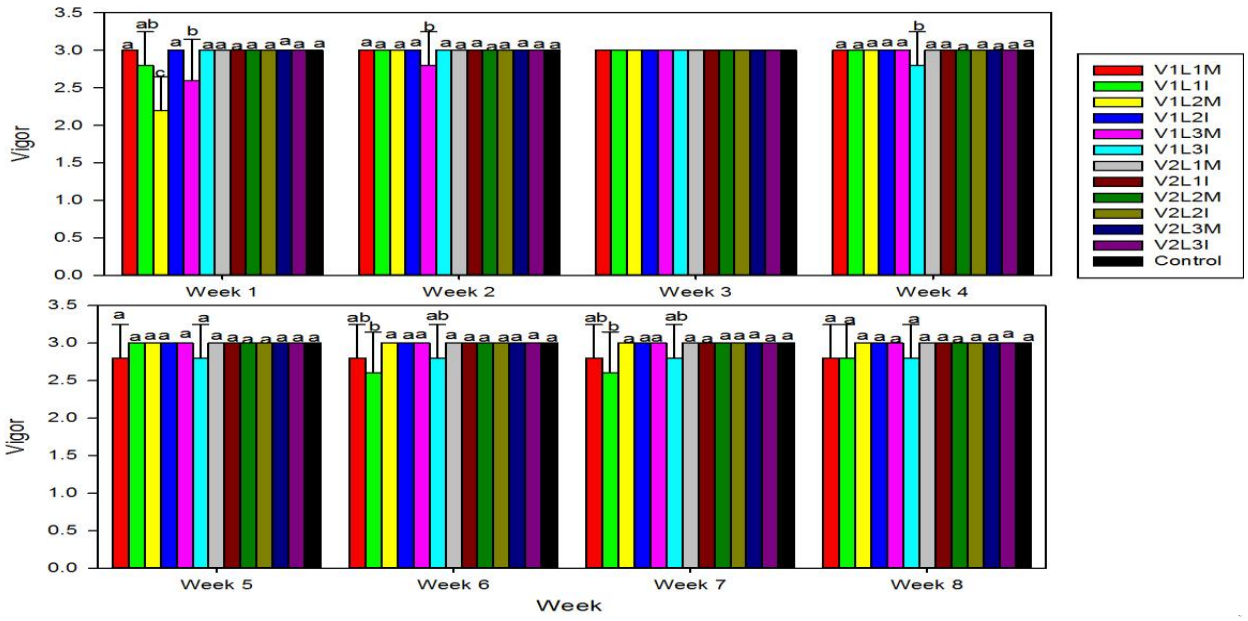


Figure 11: Effect of 10 % of coconut water supplemental basal medium on the vigor of ginger culture. (n = 5)

Bars with the same letters within each week are not significantly different at 0.05 probabilities

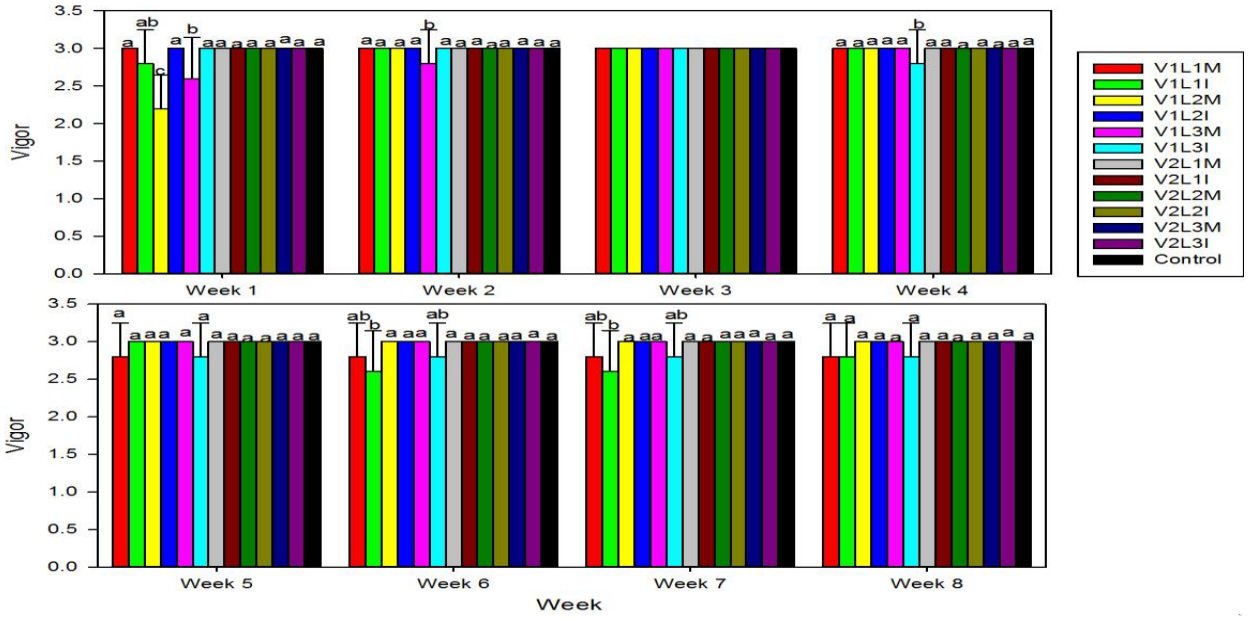


Figure 12: Effect of 20 % of coconut water supplemental basal medium on the vigor of ginger culture. (n = 5)

Bars with the same letters within each week are not significantly different at 0.05 probabilities

Figure 10 illustrates the effect of 5 % coconut water supplementation as an alternative source of phytohormones and undefined nutrients on the vigor of ginger plants in tissue culture. While all twelve samples remained vigorous and viable throughout the first week, specific samples (V1L2I, V1L3I, V2L1I, V2L2I, and V2L3I) exhibited the highest vigor by the end of week one. Overall, all samples maintained excellent vigor; however, samples V1L1I, V1L2M, V1L3M, V1L2I, and V2L1I appeared most vigorous from week two through week eight. Statistical analysis showed no significant difference ($p > 0.05$) among the samples or when compared to the control (C), which contained synthetic phytohormones for in vitro ginger culture.

Figure 11 illustrates the effect of 10 % coconut water supplementation as an alternative source of phytohormones and undefined nutrients on ginger vigor in tissue culture. Although samples V1L2M and V1L3M exhibited the lowest vigor during the first week, they maintained excellent growth from week three through week eight. By weeks two and three, all twelve samples showed appreciable vigor, with the exception of V1L3M, which was significantly less vigorous than the control at week two. By week three, all samples outperformed the control in vigor. While V1L3I appeared less vigorous than other samples at week four, all samples remained vigorous from week five until the end of the study. Statistical analysis revealed no significant difference ($p > 0.05$) between the samples and the standard at the final eight-week mark.

The growth response of ginger plantlets to 20 % coconut water is shown in Figure 12. Initial observations at week one identified V1L2M and V1L3M as the least vigorous; however, these samples demonstrated sustained high vigor from the third week onwards. Most treatments displayed significant growth by the second week, though V1L3M initially lagged behind the control. By week three, all supplemented samples showed superior vigor compared to the control group. Despite a temporary reduction in vigor for sample V1L3I at week four, all cultures stabilized by week five. At the conclusion of the eight-week research period, there were no statistically significant differences ($p > 0.05$) in vigor between the treatment groups and the established standard.

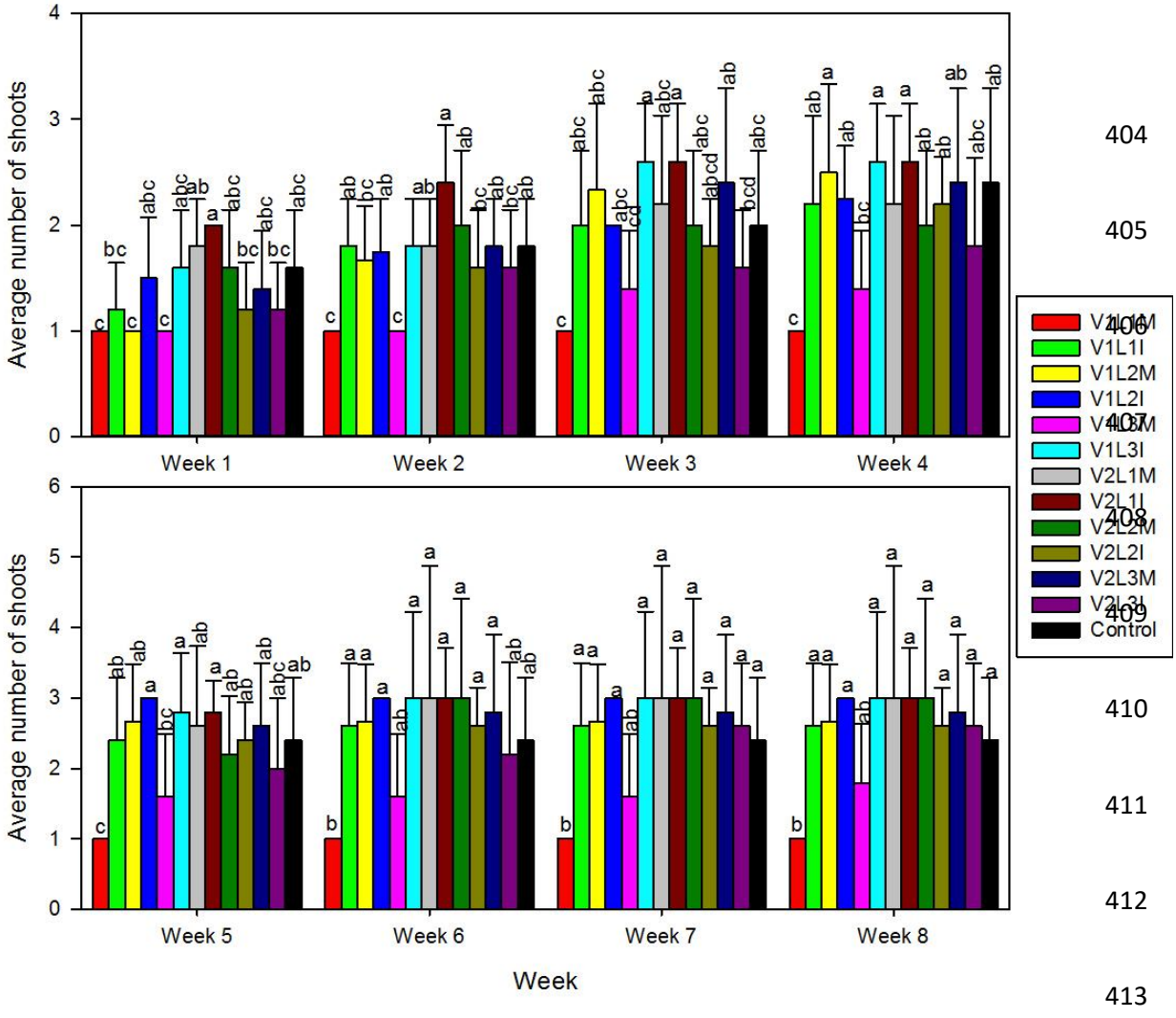
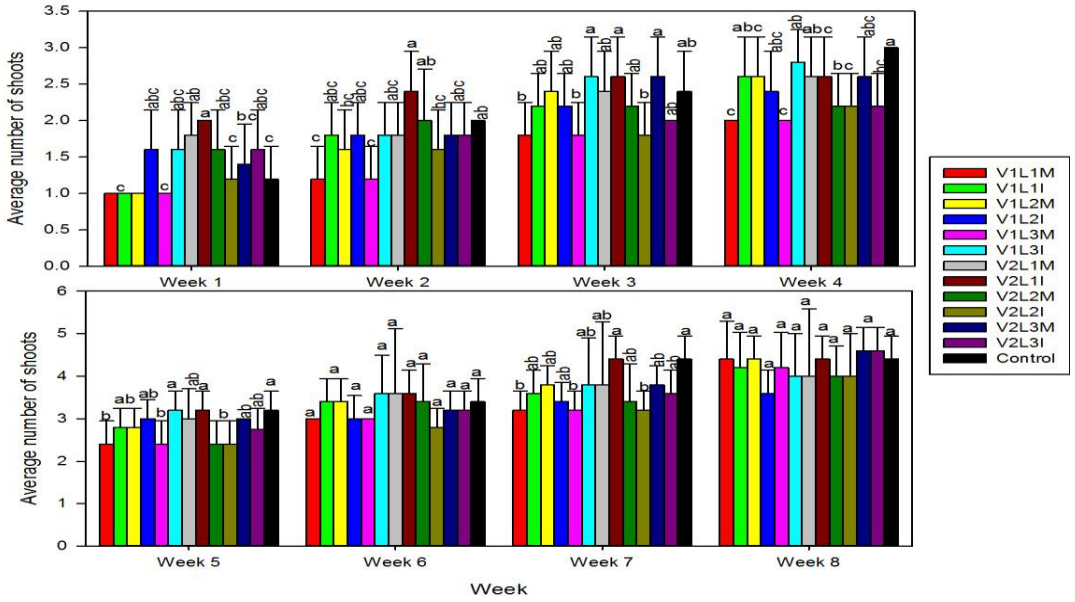


Figure 13: Effect of 5 % of coconut water supplemental basal medium on shoot development of ginger. (n = 5)

Bars with the same letters within each week are not significantly different at 0.05 probabilities



418

419 **Figure 14: Effect of 10 % of coconut water supplemental basal medium on shoot**

420 **development of ginger. (n = 5)**

421 Bars with the same letters within each week are not significantly different at 0.05 probabilities

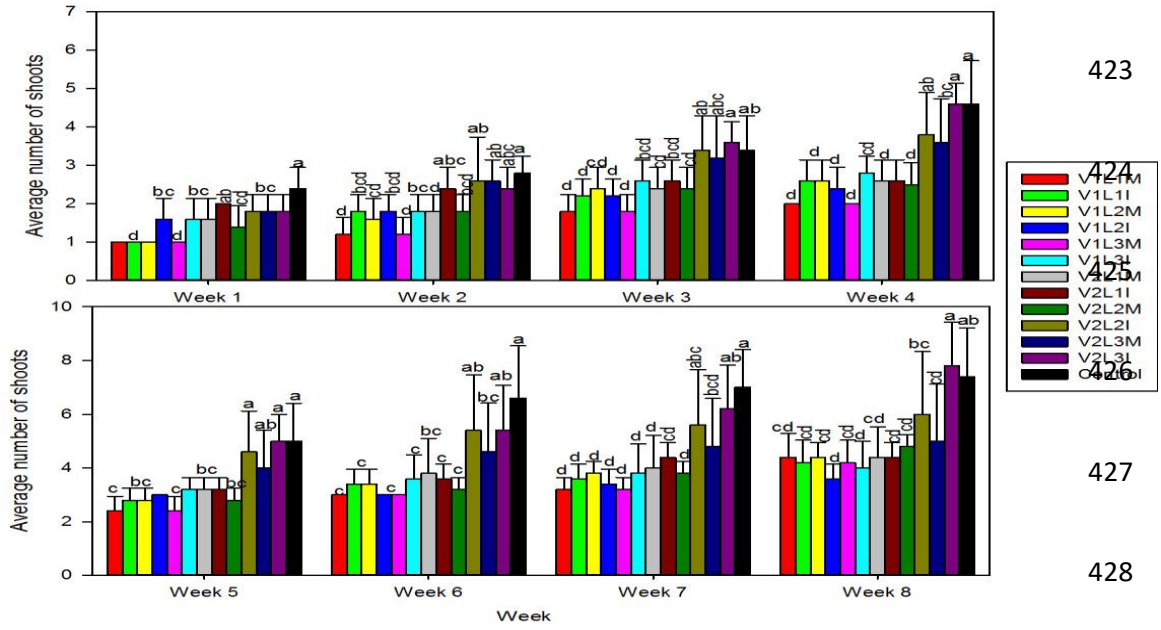


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Figure 15: Effect of 20 % of coconut water supplemental basal medium on shoot development of ginger. (n = 5)

Bars with the same letters within each week are not significantly different at 0.05 probabilities

Figure 13 presents the effects of 5 % of coconut water derived from two varieties, three different locations, and two maturity stages on ginger shoot development in ginger tissue culture. The ginger cultured in V2L1I produced the highest number of shoots during week one, while samples V1L1M, V1L2M, and V1L3M produced the fewest shoots. Compared to the control, there was no significant difference ($P>0.05$) at week one. By week two, sample V2L1I maintained the highest rate of shoot proliferation, while V1L1M and V1L3M had the lowest number of shoots. Rapid shoot production was observed from week three up to week eight, except in V1L1M, which ceased producing shoots at week one. From week three down to week eight, there was appreciable shoot development across all treatments. Treatments V1L3I, V2L1I, and V1L2M exhibited the highest number of shoot productions, even exceeding the control (C); however, there was no significant difference ($P>0.05$) when compared with the control, except in V1L1M.

Figure 14 illustrates the effects of supplementing MS basal medium with 10 % of coconut water as a phytohormone alternative for ginger shoot development. By week one, sample V2L1I produced the most shoots, exceeding the control (C), though differences were not statistically significant ($p>0.05$). This trend continued through week two, while V1L1M and V1L3M recorded the fewest shoots. By week three, shoot development peaked across all samples with no significant differences observed. During weeks four and five, most samples showed robust growth, except for V1L1M, V1L3M, and V2L2I; which lagged but remained statistically comparable to the control. By week six, all samples achieved shoot numbers equivalent to the

control. At week seven, V2L1I matched the control's peak performance, while V1L1M, V1L3M, and V2L2I produced fewer shoots. Ultimately, by week eight, all samples demonstrated strong development with no significant difference ($p>0.05$) compared to the hormone-enriched control.

Figure 15 illustrates the time course of shoot development in ginger plants using 20 %coconut water as an alternative phytohormone source. By week one, only V2L1I showed shoot development comparable to the control, while other samples remained minimal. From weeks two to five, samples V2L2I, V2L3I, and V1L2I produced appreciable shoots similar to the control; however, most other samples performed poorly, showing a significant difference ($P<0.05$**).** Between weeks six and eight, only V2L2I and V2L3I maintained development comparable to the phytohormone-enriched control. Most samples, including V1L2I and V1L3I**,** exhibited significantly lower shoot counts through the final week.

4. DISCUSSION

This research investigated the effect of different concentrations (5 %, 10 % and 20 %) of coconut water obtained from two varieties of coconut (Samoan tall and Nias green) at different maturity stages (Mature and Immature) obtained from three locations (Aboh, Ahiazu and Ezinihitte Local Government Area) on the height, number of leaves, vigor, survival rate, root and shoot development of ginger plants.

The results indicate that coconut water effectively serves as an alternative, cheap source of phytohormones in ginger tissue culture. The exceptional growth observed, particularly at the 50 mL concentration in specific samples, suggests the presence of sufficient plant hormones within the coconut water [21].

Enhanced shoot proliferation, vigorous performance, with significant leaf bearing and consistent growth rate suggests the presence of sufficient plant hormones in the coconut water [21] to support vegetative development. However, a specific trend noted was the subdued root proliferation, which was particularly evident at the 10% and 20% concentrations in certain samples. Leaves serve as the primary photosynthetic organs in vascular plants and exhibit substantial inter-specific geometric diversity [22]. In many dicotyledons, the foliar architecture typically consists of a lamina and a petiole that anchors the organ to the stem [23]. Morphogenesis initiates at the shoot apical meristem, where the iterative emergence of leaf primordia is mediated by polar auxin transport [24]. Subsequent expansion and the attainment of final leaf dimensions are governed by localized meristematic activity, characterized by coordinated cell division and differentiation [25].

In *in vitro* tissue culture, the exogenous application of thiamine is essential; it functions as a metabolic cofactor that optimizes callus induction, rhizogenesis, and caulogenesis. The requirement for supplemental thiamine is particularly pronounced under conditions of low cytokinin availability, as cytokinin deficiency significantly impairs mitosis and inhibits axillary shoot proliferation [26].

Investigation into natural growth regulators, such as those conducted by [27] indicates that cytokinins remain bioactive and effective even at reduced concentrations within the culture medium. The endogenous cytokinins identified in coconut water are hypothesized to facilitate foliar development. Specifically, research involving *Curcuma xanthorrhiza* by [28] demonstrated that a 15% concentration of coconut water yielded the most significant increase in shoot multiplication.

The subdued root proliferation observed across all concentrations, despite adequate shoot growth, can be explained by the auxin: cytokinin (IAA/kinetin) ratio [5]. A high IAA: kinetin ratio favors root formation while a low ratio favors shoot formation. The results therefore suggest that the cytokinin level is higher than the auxin level in the coconut water used, leading to extensive shoot, leaf, and vigor development [19, 21].

Most of the ginger plants planted in MS basal medium coconut water started well and maintained a consistent growth rate in all the growth parameters down to week eight of the research, while others performed slightly lower than the control but there was no significant ($p>0.05$) difference from the first week to the eight week of the research proving that coconut water basal medium supplementation contains plant growth regulators capable of facilitating adequate growth in ginger when used as an alternative source of phytohormones in tissue culture which, is in accordance with [21] who elucidated that coconut water contains high percentage of cytokinins.

Although both 10 % and 20 % concentrations supported competitive ginger growth and leaf development with no significant difference from the control, confirming these ranges are practical for supporting ginger growth. The lack of significant difference in most parameters across locations (e.g., Mbaise areas) suggests location has little or no effect on the efficacy of the coconut water. However, variety seems to have a slight effect, with the Samoan tall variety appearing more preferable as it consistently showed superior performance in root production and overall growth rate compared to the Nias green variety, particularly from week five to week eight. This is in line with the work of [29] who compared coconut water from four different Thai coconut varieties (two commercially available, two traditional) and found non-statistically significant differences in the total phenolic content and antioxidant activity between them.

519 Nevertheless, experimental results indicate that 5% and 10% coconut water concentrations
520 effectively support ginger shoot development [30], with several samples matching or exceeding
521 hormonal controls by week eight. While lower concentrations (5% and 10%) showed no
522 significant difference compared to the control, 20% coconut water led to poor performance in
523 most samples ($P<0.05$). These findings suggest that the efficacy of coconut water as a
524 phytohormone alternative is highly dependent on both concentrations, with V2L1I consistently
525 demonstrating strong performance. [31] reported that addition of coconut water on the growth of
526 micro potato cutting (*Solanumtuberosum L.*) showed a significant effect on the observed
527 parameters i.e the formation of roots, number of leaves, plantlets height and weight. Treatment
528 with the addition of coconut water volume of 150 ml/l on MS medium gave the best effect on the
529 growth of *in vitro* potato micro cutting.

530

531 **4.0 CONCLUSION**

532 The concentration of coconut water has a very significant effect on ginger plants in ginger tissue
533 culture. In all the phenotypic parameters accessed during ginger tissue culture, a concentration of
534 10%–20% coconut water proved most effective and reliable accepts on shoot development. As
535 such, 10%–20% coconut water can be effectively and reliably used as an alternative source of
536 phytohormone in ginger tissue culture. Ginger cultured in MS basal medium supplemented with
537 coconut water developed as effectively as ginger grown in standard MS medium. Due to its cost-
538 effectiveness and accessibility, coconut water serves as a viable alternative plant hormone for
539 tissue culture. Ginger plants grew well in both mature and immature coconut water, with no
540 significant difference ($p > 0.05$) observed. The plants were most vigorous when treated with a
541 20% concentration of coconut water. However, a 10% concentration proved most effective for

shoot development. This indicates that careful consideration should be given to the desired outcome when using coconut water as a phytohormone source.

5.0 List of abbreviations

V1L1M Coconut water from mature Samoan tall coconut sourced randomly from private farms in Mbutu community, Aboh Mbaise Local Government Area, Imo State, Nigeria.
V1L1I Coconut water from immature Samoan tall coconut sourced randomly from private farms in Mbutu community, Aboh Mbaise Local Government Area, Imo State, Nigeria.
V1L2M Coconut water from mature Samoan tall coconut, sourced randomly from private farms in Ogbe community, Ahiazu Mbaise Local Government Area, Imo State, Nigeria
V1L2I Coconut water from immature Samoan tall coconut sourced randomly from private farms in Ogbe community, Ahiazu Mbaise Local Government Area, Imo State, Nigeria.
V1L3M Coconut water from mature Samoan tall coconut, sourced randomly from private farms in Eziudo community, Ezinihitte Mbaise Local Government Area, Imo State, Nigeria
V1L3I Coconut water from immature Samoan tall coconut, sourced randomly from private farms in Eziudo community, Ezinihitte Mbaise Local Government Area, Imo State, Nigeria

V2L1M Coconut water from Mature Nias green coconut sourced from private farms in Mbutu community, Aboh Mbaise Local Government Area, Imo State, Nigeria.
V2L1I Coconut water from immature Nias green coconut sourced from private farms in Mbutu community, Aboh Mbaise Local Government Area, Imo State, Nigeria.
V2l2M Coconut water from Mature Nias green coconut, sourced randomly from private farms in Ogbe community, Ahiazu Mbaise Local Government Area, Imo State, Nigeria
V2L2I Coconut water from immature Nias green coconut sourced randomly from private farms in Ogbe community, Ahiazu Mbaise Local Government Area, Imo State, Nigeria
V2L3M Coconut water from Mature Nias green coconut, sourced randomly from private farms in Eziudo community, Ezinihitte Mbaise Local Government Area, Imo State, Nigeria
V2L3I Coconut water from immature Nias green coconut sourced randomly from private farms in Eziudo community, Ezinihitte Mbaise Local Government Area, Imo State, Nigeria

6.0 Declearations

6.1 Ethics approval and consent to participate

Not applicable.

6.2 Consent for publication

Not applicable.

6.3 Availability of data and materials

All data generated or analysed during this study are available from the corresponding author on reasonable request.

6.4. Competing interests

The author declares no conflicts of interest of any kind and affirms there are no compromises involving any other parties.

6.5. Funding

No funding was obtained to carry out this study.

6.6. Authors contributions

Conceptualization: JN; Data analysis: EE and NO; Data interpretation: EE and JN; Drafting the initial manuscript: NO; Review and editing: RO. All authors contributed to the article and approved the submission.

6.7 Acknowledgements

Not applicable

6.8 Necessary Remark

Coconut palms are not listed in CITES Appendices I, II, or III. CITES focuses on protecting endangered species from unsustainable international trade; cultivated, and as such, widespread plants like coconuts do not meet this criteria. Because it is not a protected species, using planted coconut for scientific research or experiments does not require CITES permits or special, standardized recommendations from the CITES Secretariat. Research using coconut palms, such as genetic studies, pest studies, tissue culture or breeding trials, is strictly governed by national agricultural research authorities and local environmental laws, rather than international treaties. Field studies were conducted in accordance with local legislation, of Imo State Government and the plant tissue culture laboratory of National Root Crop Research Institute (NRCRI) Umudike, Ikwuano Local Government Area, Abia State, Nigeria.

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