International Journal of
Agriculture
(IJA)

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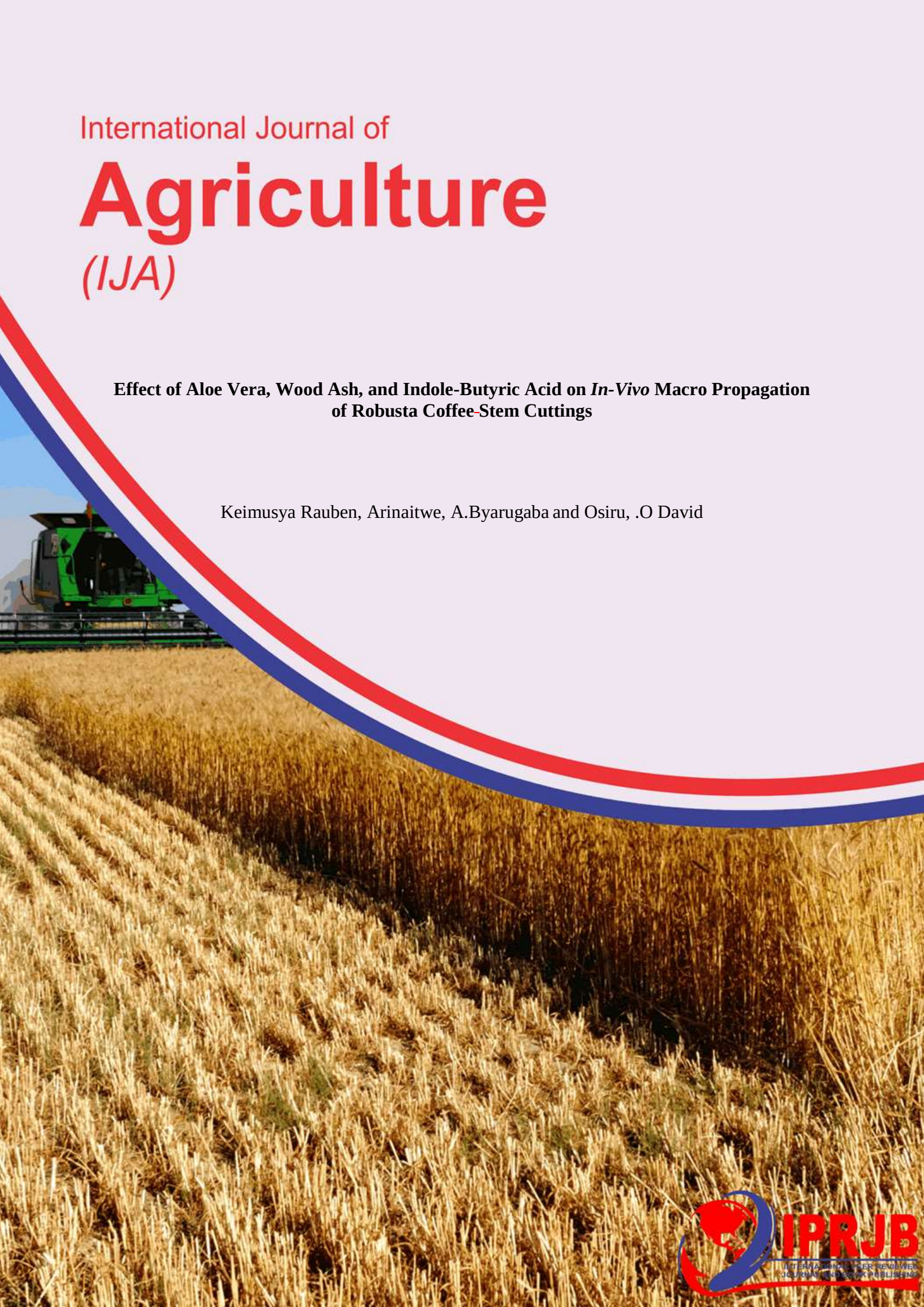

IPRJB
INTERNATIONAL PEER REVIEWED JOURNAL AND BOOK PUBLISHING

International Journal of Agriculture
ISSN 2520-4629X (Online)
Vol.9, Issue 2, No. 5. PP. 55 - 67, 2024

www.iprjb.org

Effect of Aloe Vera, Wood Ash, and Indole-Butyric Acid on *In-Vivo* Macro Propagation of Robusta Coffee-Stem Cuttings

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Article History

Received 9th June 2024

Received in Revised Form 12th July 2024

Accepted 7th August 2024

How to cite in APA format:

Keimusya, R., Arinaitwe, B., & Osiru, D. (2024). Effect of Aloe Vera, Wood Ash, and Indole-Butyric Acid on In-Vivo Macro Propagation of Robusta Coffee Stem Cuttings. *International Journal of Agriculture*, *9*(2), 55–67. https://doi.org/10.47604/ija.2840

Abstract

Purpose: This study investigated the influence of Aloe Vera, Wood ash and a synthetic growth hormone Indole-butyric acid (IBA) on root and shoot in the proliferation of Coffee Wilt disease-resistant (CWD-R) Robusta coffee stem cuttings.

Methodology: Stem cuttings of CWD-R Robusta coffee were treated with five levels of rooting natural substances and IBA independently and in combination. The treated cuttings were propagated in a uniform potting medium under humidity chamber conditions. Data was collected on the number of surviving cuttings, shoot and root number, and root and shoot lengths. At 90 days after planting, data on root proliferation, the number, and lengths of roots were collected and analyzed for variance (ANOVA) using GenStat 11th Edition.

Findings: The survival, root, and shoot development were significantly ($p<0.05$) influenced by the rooting substances applied. A combination of Aloe Vera and IBA, 0.8% IBA, and Aloe Vera gel applied alone gave the highest percentages (90, 70 and 70%, respectively) of stem cuttings that survived to form roots and shoots. A mixture of aloe Vera and IBA, 0.8% IBA, and Aloe Vera gave the highest mean number of shoots and roots per cutting; that is (8.75, 8.5, and 8.5 cm) and (13.87, 12.85 and 12.39 cm) for shoots and roots respectively. Cuttings treated with wood ash and control gave the lowest mean number of shoots and roots that formed per cutting; that is (5.25 and 4.50 cm) and (11.75 and 11.38 cm) for shoots and roots respectively.

Unique Contribution to Theory, Practice and Policy: Propagation of *Coffea canephora*, *by* stem cuttings can be adequately achieved by using Aloe Vera, IBA, or their mixture, but not with wood ash.

Keywords: *Aloe Vera, Coffee Canephora, Indole-Butyric Acid, Cuttings, Wood Ash*

JEL Codes of Classification: *Q10, Q16, Q13, Q16, `Q19*

International Journal of Agriculture
ISSN 2520-4629X (Online)
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INTRODUCTION

Coffee is the oldest, most significant commercial agricultural commodity and major foreign exchange earner, contributing an annual average of 15% to Uganda's total export revenue (FAOSTAT, 2019). Uganda is the third largest Robusta coffee producer in the world, after Vietnam and Brazil (IMF, 2022). Among the traded commodities, coffee is the largest contributor to exports, which for Financial Year 2021/22 was valued at US$ 512 million, representing 17% of total exports. Currently, global demand for coffee stands at about 160 million bags against 148 million bags in exports, giving a deficit of 12 million bags. Demand is estimated to rise to 185.8 million bags by 2025 (ICO, 2020). Given the untapped production potential in Uganda, the country has the opportunity to benefit from increasing the volumes to tap this demand.

There are two types of coffee grown commercially in Uganda; Arabica coffee, grown in highland areas and Robusta coffee in lowland areas. Robusta coffee accounts for 80% of the coffee grown in Uganda. However, Robusta coffee is under threat due to coffee wilt disease (CWD). There has been intensified research by UCDA and NARO to produce coffee wilt disease-resistant (CWD-R) varieties.so far, 10 resistant lines have been developed, but the challenge has been on propagation to produce enough planting materials of the same. In order to maintain varietal purity and quality, these CWD-R lines can only be propagated using vegetative means; that is by using tissue culture or stem cuttings. Since tissue culture is cumbersome in terms of investment, cost and technical expertise, the viable option remains the use of stem cuttings. However, coffee does not naturally regenerate from vegetative parts, and therefore, requires artificial inducement using rooting enhancers.

Currently, coffee farmers face difficulties in producing quality coffee seedlings that can give good yields. Coffee seedlings produced through the vegetative propagation method offer greater advantages to both coffee farmers and the coffee industry (UCDA, 2020. Such seedlings have high resistance to diseases and grow at a uniform rate making management more predictable as well as producing more uniform and higher quality coffee beans. Such beans have good harvesting and processing efficiency, and also have high market demand.

Robusta coffee plants grown from seed will have a high degree of heterogeneity, which alters the desirable characteristics of the resultant coffee plants. This variation may be manifested in the physical appearance of leaves, size of the fruits and beans, tree vigour, productivity, tolerance to drought, fruit formation, internode lengths, ripening time and uniformity. The method of vegetative propagation eliminates this progressive loss of the desired traits in the descendant coffee trees.

Since vegetative propagation of coffee requires artificial inducement, there was a need to study the various options to be used by farmers. Aloe Vera leaves have been reported to have natural substances (plant hormones and organic acids) that are believed to play a fundamental role in enhancing root and shoot initiation and development (Ofori-Gyamfi, 2018). However, limited scientific information is available on the applicability and efficacy of Aloe Vera extract on propagation performance, particularly of nodal cuttings of CWD-R robusta coffee. Similarly, farmers in Bushenyi and Sheema districts report achieving about 65% propagation success when they dip the cuttings in wood ash before inserting them in the potting mixture. However, this information is verbally reported and has not been tested or documented.

This study evaluated the efficacy of Aloe Vera gel, wood ash, and Indole-acetic acid (IBA) on the propagation performance of CWD-R robusta coffee under controlled humidity chamber conditions.

METHODOLOGY

Experimental site

This study was conducted at the coffee nursery structure located at Nyaruteme Trading Centre, Nyamukana Town Council, Ntungamo district located at 0° 768" N and 30° 299" E. The experiment run for two seasons from May to August 2022, and from September 2022 to February 2023. CWD-R Robusta coffee shoot cuttings of KR Lines 1, 3, 4, 5, 6, and 7 were used in this study.

Preparation of Nursery Beds and Cuttings

Nursery plots were made using wooden frames that formed sides of the raised beds in the nursery with 75% shade. The raised beds were then filled with well-mixed proportions of sand, forest soil, and decomposed pine sawdust in the ratio of 1:2:1. Each of the raised beds formed an experimental block.

CWD-R robusta coffee cuttings were prepared to consist of an internode and a node with one pair of leaves. The leaf blades were cut by two-thirds to minimize water loss by reducing the area available for transpiration. The CWD-R robusta coffee cuttings were preferred because the study was interested in coming up with suitable rooting media for the propagation of CWD-R seedlings. Healthy cuttings were selected from a mother garden with a pool of 6 CWD-R lines (KR1, KR3, KR4, KR5, KR6 and KR7).

The cuttings were then trimmed at an angle of 45 degrees above the leaf axil to facilitate moisture drainage and prevent terminal fungal and bacterial infection that would result from water collecting on the cut ends (Ofori, 1996). The cuttings were then dipped in the respective treatment substances and left for three minutes to ensure sufficient contact with the treatment substance. They were then inserted into the well-watered potting medium to ensure that the leaf petioles on the stem just rested on top of the potting medium.

A clear plastic polyethylene sheet of 800 gauge was laid on top of the treated cuttings to cover all the beds, and was held onto the ground with red bricks to minimize air entry into the humidity-tight chamber. The humidity chamber conditions created were essential for mimicking the physiological environment for plant cell division and callusing (Newton, 2002).

Nordox and Harvestor XL at 2000 ppm were sprayed on all the cuttings to control fungal infection (Yeboah and Amoah, 2009). The experimental setup was inspected every week to remove dead leaves and debris. The cuttings in the beds were watered weekly to keep the potting media with sufficient moisture throughout the experiment.

Experimental Design and Treatments

The Completely randomized block design (CRD) was used with five treatments and replicated four times. The treatments comprised a control, where no rooting enhancer was applied (C), Aloe Vera gel (A), 8% Indole-butyric acid (B), Aloe Vera gel + IBA (50 %v/v) (A/B), and wood ash (D)

International Journal of Agriculture
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The aloe Vera gel was extracted physically from the mesophyll portion of fresh aloe Vera leaves using a sterile scooping spoon and then collected into a clean container. The extracted gel was divided into two equal portions of 100mls each. One portion (100mls) was applied onto the cuttings without dilution, by dipping the cuttings into the gel for three minutes (Treatment A). The second portion was mixed with 50% of the pre-mixed Rootex (8% IBA) to make it 50% Rootex and 50% aloe Vera gel by volume (Treatment A/B). The 8% IBA (Rootex powder) is manufactured by Osho Agrochemicals Ltd and is the mostly used commercial rooting hormone available on the East African market. The lower end tips of the coffee cuttings were dipped into the IBA Rootex Powder to cover ¼ inch. The excess rootex was removed by tapping the cutting on the container to avoid excess application (Treatment B).

Wood ash was obtained from home kitchens where different wood types are used for cooking, and was applied on a set of cuttings as another treatment (Treatment D). The lower ends of the cuttings were dipped into wood ash powder before inserting them into the potting medium. A control treatment (Treatment C) where no rooting enhancer was applied on the cuttings was also included. All the treated cuttings were then planted in the potting medium, and each treatment contained 40 stem cuttings.

Data Collection and Analysis Procedures

The experiment was opened on the 21st, 75^{th,} and 90th day after the treatment/planting, and data were collected on the number of cuttings that had survived (green, callusing, healthy), number and length of shoots emanating from auxiliary buds, and on the number of roots developed and root lengths. The percentage of the number of cuttings that had survived was obtained by counting, and then converting into percent. The lengths of shoots were obtained by measuring shoot height from the point of initiation of the axillary bud from the stem to the apex of the youngest leaf using ruler.

The data for each cutting was separately recorded, tallied, compiled in the field notebook, and then entered in Excel sheet version 2010. The Excel data were further grouped into the average number of cuttings that survived at 21, 75 and 90 days after treatment, the number and length of shoots on the cuttings at 21, 75 and 90 days after treatment), and the average number and lengths of roots that developed per rooted cutting at the 90th day for all the treatments (Yeboah and Amoah, 2009).

Data Analysis

The Excel data was imported into GenStat version 11.1 for the one-way analysis of variance (ANOVA). Treatment means for the different parameters were separated using Fisher's least significant difference (LSD) procedure at 5 % level of significance.

RESULTS AND DISCUSSION

Survival of Robusta Coffee Stem Cuttings

Cuttings treated with IBA, Aloe vera and Aloe+IBA mixture gave the highest ($p<0.05$) percentage of stem cuttings that survived at 90 days after treatment when compared with the control (Fig. 1). Treating the cuttings with wood ash did not ($p>0.05$) affect the survival of the cuttings when compared with the control, but the number of cuttings that survived was lower ($p<0.05$) than those of other treatments. However, there were no significant differences in survival among the cuttings treated with Aloe Vera, IBA and Aloe-IBA mixture.

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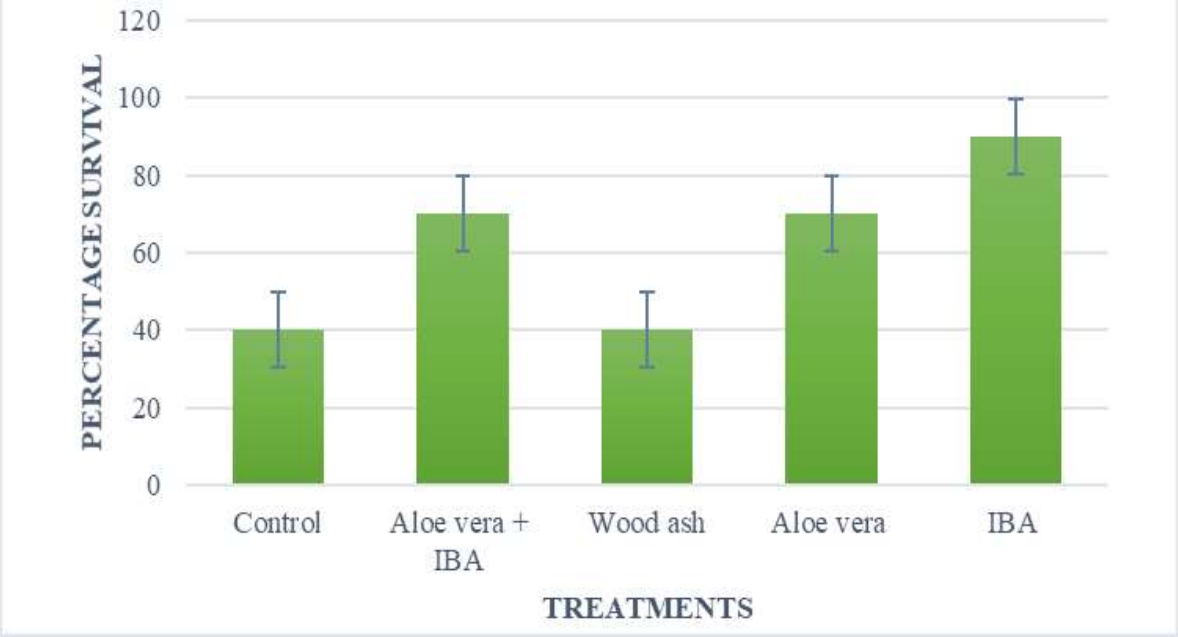

Figure 1: Percentage of Cuttings Surviving at 90 Days after Treatment with Growth Regulators

The ANOVA for the number of cuttings surviving at 90 days after treatment with different growth regulators showed significant differences ($p<0.05$) among the different treatments. Thus the different growth regulators applied to the robusta coffee stem cuttings significantly affected their survival under the *in-vivo* conditions in the nursery.

The percentages of surviving cuttings at 90 days after treatment were related to the number of stem cuttings that formed the callus at 60 days after treatment. All the treated cuttings that formed the root callus by the 60th day after treatment were able to survive up to 90 days.

The significant survival of robusta coffee stem cuttings treated with Aloe Vera, IBA and Aloe+IBA treatment combination could be attributed to the following;

Aloe Vera (*Aloe barbadensis*) used in this experiment has been shown to contain essential nutrients and over 75 beneficial compounds including amino acids, antioxidants, complex carbohydrates, calcium, magnesium, zinc, vitamins A, C, E, and B-vitamins, among others (Zhang *et al*., 2018). These substance, probably became available to the Coffee stem cuttings when the aloe Vera gel was extracted and the cuttings inserted in the gel for three minutes before planting in the soil media (Holzapfel, 2022). In addition, the ingredient called salicylic acid contained in aloe Vera gel is believed to enhance the immune system (systemic immune response or SAR) within the plant tissues of stem cuttings and enabled them to fend off disease causing pathogens that could have infected the cuttings and caused tissue mortality. (Van-Doorn, 2011).

Noting that survival of tissues and cells depends on their ability to escape programmed cell death (PCD), which is an integral part of plant development, and of its response to associated abiotic stresses or pathogens, the anti-oxidant components and salicylic acid in aloe Vera could have played a vital role in maintaining live cells and tissues of the cuttings (Bajaj, 1995).

The anti-oxidative properties of aloe Vera prevented vacuolar cell death and necrosis which would otherwise cause tissue mortality by a combination of autophagy process. Such necrosis

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is characterized by early rupture of the plasma membrane and shrinkage of the protoplast (Hae, 2011).

The recorded high rate of survival of the cuttings treated with IBA can be attributed to the plant hormone influence by indole-butyric acid (IBA). This is in tandem with a study by Akwatulira *et al*. (2011) in which different IBA hormone concentrations were used on root and shoot development in stem cuttings of *Warburgia ugandensis*. The results revealed that root and shot development were positively influenced by increasing IBA concentrations up to the optimum concentration of 0.8% IBA (Akwatulira *et al.*, 2011)

The low survival rate of coffee stem cuttings treated with wood ash might have been due to necrosis which results from cuttings suffering abiotic stresses particularly water stress due high salinity of wood ash, which could have caused osmotic pressure differences between the cutting tissues and wood ash. (Bozhkov, 2015). The other factors that would otherwise cause a low survival rate were controlled under the *in-vivo* humidity chamber conditions in the nursery. Such factors primarily involve the physical environmental conditions of moisture, light, temperature, and humidity, among others (Ziv, 2005). Mahipal and Machokali (2013) obtained similar results extracted plant-extracted auxins and leaf extracts on vegetative propagation through stem cuttings of *Couroupita guianensis*. In their experiments, aloe Vera extract was proved to contain a derivative of auxins that prolonged the life of *Couroupita guianensis* cuttings in moist environmentsnvironment (Mahipal and Machokali, 2013)

Number of Sprouted

Treatment of robusta coffee cuttings with IBA, aloe vera and a combination of aloe vera and IBA yielded the highest ($p<0.05$) average number of sprouted shoots when compared with the control (Fig. 2). However, these three treatments were not significantly ($p>0.05$) different from each other. A non-significant difference was observed between the cuttings treated with wood ash and the control.

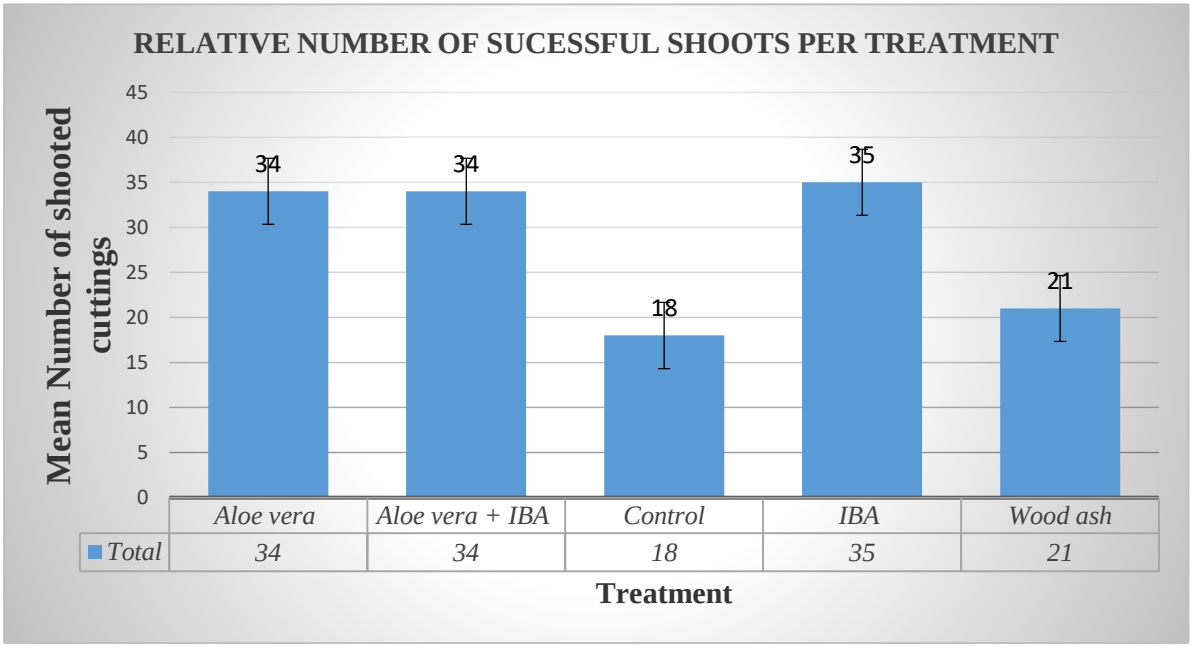

Figure 2: Number of Sprouted Shoots as a Result of Treatment with Growth Enhancers

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The ANOVA for the effect of rooting enhancers on the number of sprouted shoots of robusta coffee stem cuttings at 90 days after treatment revealed significant ($p<0.05$) differences among the different treatments (Fig. 2).

The highest number of sprouted shoots on coffee stem cuttings treated with IBA, aloe Vera and Aloe+IBA treatment combination could be due to the same factors relating to the survival as explained above. Aloe vera extract contains sugars and phytochemicals that improve cell metabolism and stimulate plant tissue growth in addition to being a natural pesticide and biocide. Also, the high concentration of *Aloin* in aloe vera leaves is believed to provide nutrients that strengthen the immune system of plants and promote growth (Zhang, 2018)

The positive effect of IBA on the number of sprouted shoots at 90 days could have resulted from IBA speeding up root initiation on the lower cut ends of stem cuttings. There is also mechanism by which IBA influences plant growth through the reduction of its carbon chain to convert to Indole acetic acid (IAA) (Strader and Bartel, 2011). This occurs during the conversion of IBA to IAA via a fatty acid β-oxidation process that is contained within the peroxisome, and this results in increased rate of cell elongation in the meristems (Zolman *et al*., 2000; Strader *et al*., 2010).

This is in agreement with the study by Ziv (2005) in which he confirmed that shoot initiation and growth were significantly related to the different bioreactors that were used in the experiment for mass propagation of plants in liquid culture systems for *in vitro* plant propagation (Ziv, 2005). Therefore, in this study, the observed results in shoot induction after pre-treatment of the coffee stem cuttings with IBA and aloe Vera extract could be attributed to the constituent plant hormones in IBA and Aloe Vera.

Number of Roots per Cutting

The ANOVA confirmed the existence of significant differences in the number of roots on the stem cuttings as a result of treatment with the growth enhancers (Table 7).

Further confirmation of the differences in root lengths was done using Tukey's test at the significance level of $\alpha = 0.05$. the optimal Average number of roots formed on *Coffee Canephora* stem cuttings was observed with Aloe+ IBA (4.8), followed by 8% IBA (4.75), Aloe Vera (4.50), Wood ash (3.25) and control (2.10) as portrayed in Table 7 below.

Table 7: Tables of Means for the Average Number of Roots of Coffea Canephora Stem Cuttings as a Result of Treatments with Growth Enhancers

Treatment	Aloe vera	Aloe vera + IBA	Control	IBA	Wood ash
	4.50	4.80	2.10	4.75	2.25
$LSD_{(0.05)}$.	1.027				

Mean Root Length

Analysis of variance (ANOVA) revealed a great significant difference in the mean root length of *Coffea Canephora* stem cuttings as a result of different treatments with natural and synthetic growth enhancers (Table 9).

The difference was highly significant between the cuttings under the control experiment and those to which different rooting enhancers were applied apart from those treated with Wood ash. However, there was no significant difference in the mean root length of Coffea Canephora stem cuttings among the cuttings treated with Aloe Vera, IBA and Aloe+IBA.

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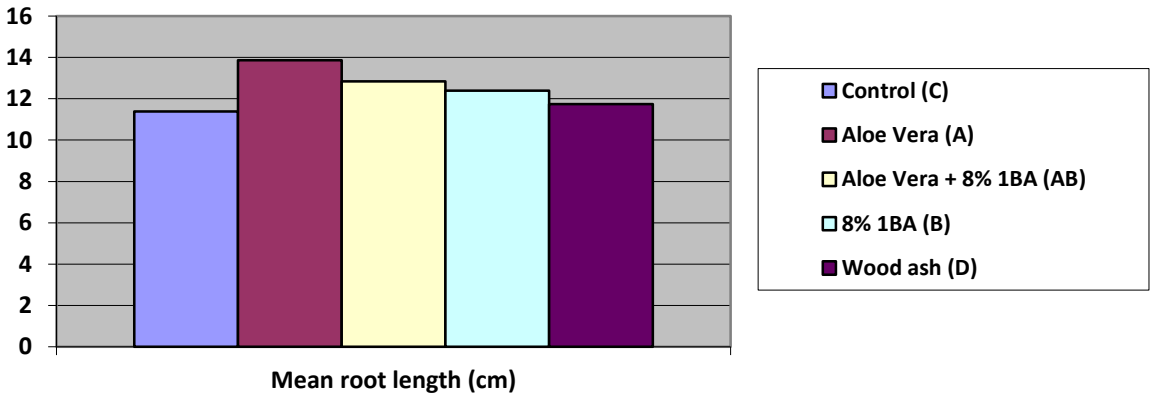
The least average root lengths were recorded on cuttings under the control experiment (11.38), followed by those treated with Wood ash (11.75), followed by Aloe+IBA (12.85), 8% IBA (13.39), and the highest Average root length from treated with Aloe Vera (13.87) as portrayed in Figure 3 below.



$LSD_{(0.05)}$ 1.38cm

Figure 3: Mean Root Lengths of Robusta Coffee Stem Cuttings as a Result of Treatments with Growth Enhancers

The average number of lateral roots were highly related to the treatments. The highest number of lateral roots were achieved on the *Coffea canephora* stem cuttings treated with a combination of Aloe vera and IBA. This was followed by those treated with IBA, Aloe Vera alone and lastly Wood ash.

IBA is an endogenous compound in plants and when applied exogenously has a number of various effects on growth and development of different plant tissues. Indole-butyric acid is the mostly used commercial substance for the induction of adventitious roots especially in cuttings (Alvarez, 1989).

The positive effect of IBA on lateral root initiation originates from the fact that *IBA* is a synthetic form of Auxin hormone that naturally exist in many plants. IBA, therefore, regulates the development of tissues, especially the root. Within the root, IBA stimulate xylem tissue differentiation (Wareing, 1958; Aloni *et al*., 2006). Treatment of *Coffea canephora* stem cuttings with 8% IBA could have induced cell division in the cambium, thereby promoting callusing and root primordium development. Auxins, such as IBA are also known to promote xylem tissue differentiation, thus enhancing multiple lateral root formation on the lower cut ends of *Coffea Canephora* stem cuttings where 8% IBA was applied (Wareing, 1958; Digby & Wareing, 1966). This is consistent with a study by Digby and Wareing in 1996 where it was found that Auxins were responsible for stimulating the elongation of vessels in *Populus robusta*, and also in increasing the radius of the xylem tissues (Digby & Wareing, 1966).

In fact, Indole butyric acid (IBA) was used in this study as a basis for comparing its efficacy with aloe Vera and wood ash because it is the mostly used synthetic auxin in the vegetative multiplication of plants using plant sections/cuttings (Hartmann *et al*., 1997).

However, IBA treated cuttings exhibited a relatively shorter average root length per cutting compared the cuttings treated with Aloe Vera, wood ash and control experiment. This is

explained by the fact that IBA inhibits primary root elongation and stimulates lateral root formation, as confirmed in the study by Chee in 1995 (Chee, 1995b)

The observed positive effect of aloe Vera on both the average number of lateral roots and average root lengths is attributed to the nutrients in Aloe Vera which encourages root initiation, rapid root development, improved cell strength, and contribution to overall superior plant health, growth, and vigor. This is in agreement with the study by Mohammed between 2014 and 2015 in Erbil, Iraq, in a greenhouse experiment using hardwood stem cuttings of olive (*Olea europea*) (Mohammed, 2015).

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

The following are the major conclusions that emerge;

IBA, Aloe Vera and a combination of IBA and Aloe Vera have a positive influence on survival, shoot development and root proliferation of *Coffea Canephora* (CWD-R.) stem cuttings.

Combining aloe Vera with IBA improves the efficacy and effectiveness of each (Aloe Vera and IBA) particularly when the equivalent volume of crude Aloe Vera extract and 8% IBA is used.

Recommendations

Aloe Vera, having been proven in this study as a great substance when used a rooting agent and at promoting growth, it can replace the synthetic hormones and growth regulators to become the commonly used natural rooting hormone to help plant cuttings establish new roots.

In areas, especially the upcountry nursery stations where synthetic rooting powders such as IBA are not available, propagation of *Coffea canephora* stem cuttings can be satisfactorily achieved by treating the cutting with Aloe vera extract.

In view of the highly raising global environmental concerns of using synthetic in puts in Agriculture, such as hormones and growth regulators, Aloe vera products are particularly recommended.

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International Journal of Agriculture
ISSN 2520-4629X (Online)
Vol.9, Issue 2, No. 5. PP. 55 - 67, 2024

www.iprjb.org

APPENDICES

Appendix Table 1*:* The ANOVA for the Number of Robusta Coffee Stem Cuttings Surviving at 90 Days after Treatment

Source of variation	d.f.	s.s.	m.s.	v.r.	F Pr.
BLOCK stratum	3	8.200	2.733	2.56	
TREATMENT	4	20.800	5.200	4.87	**0.014**
Residual	12	12.800	1.067		
Total	19	41.800			

Appendix Table 2: The ANOVA for the Number of Shooted Robusta Coffee Stem Cuttings Treated With Growth Enhancers

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
BLOCK stratum	3	8.200	2.733	3.18	
TREATMENT	4	67.300	16.825	19.60	**0.011**
Residual	12	10.300	0.858		
Total	19	85.800			

Appendix Table: 3: The ANOVA for the Root Lengths of Coffee Stem Cuttings Treated With Rooting Enhancers

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
BLOCK stratum	3	1.6767	0.5589	0.70	
TREATMENT	4	17.9786	4.4947	5.63	**0.009**
Residual	12	9.5852	0.7988		
Total	**19**	**29.2405**			

Appendix Table 4: Tables of Means for Root Lengths at 90 Days of *Coffea Canephora* Stem Cuttings under Different Treatments with Rooting Enhancers

Treatment	Mean root length (cm)
Control (C)	11.38
Aloe Vera (A)	13.87
Aloe Vera + 8% 1BA (AB)	12.85
8% 1BA (B)	12.39
Wood ash (D)	11.75
Grand mean	**12.65**
$LSD_{(0.05)}$	1.38