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Rooting and Shooting of *Coffea canephora* Stem Cuttings in Response to Clonal Chamber Conditions and Rooting Hormone in Ghana

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Abstract

Mass propagation of *Coffea canephora* through stem cuttings using various rooting hormones and clonal chambers is an innovative practice to hasten and mass produce planting materials of coffee for commercial production. The study was conducted to determine the rooting and shooting performance of *C. canephora* cuttings in response to rooting hormone and the clonal chamber using semi-hardwood stem cuttings. The experiment was laid out in a randomized complete block design (RCBD) with four treatments and replicated four times. The different treatments were: cuttings treated with hormone + propagated off clonal chamber condition), control (cuttings without hormonal treatment + propagated off clonal chamber condition), cuttings treated with hormone + propagated under clonal chamber condition), and cuttings without hormonal treatment + propagated under clonal chamber condition. Data collection started on the 8th week after propagation and data were collected on leaf number, root number, root length and mortality of cuttings for a period of ten weeks. The study indicated that, the effect of rooting hormone on the number of roots, root length and cutting mortality was significant ($p \leq 0.05$) with the exception of the number of newly developed leaves. More so, the clonal chamber had a significant effect on the number of leaves, root number and root length as well as mortality ($p \leq 0.05$). The cuttings treated with rooting hormone propagated under clonal chamber conditions significantly improved the number of roots, root length, number of leaves with the least mortality. However, cuttings propagated off the clonal chamber conditions had the highest mortality, least root number, least root length and least leaf number. Therefore, it is recommended that the ideal treatment for *C. canephora* cuttings for mass propagation is to treat cuttings with rooting hormone and propagated under clonal chamber condition.

Keywords: Coffee (Robusta), clonal chamber, rooting hormone, stem cuttings

INTRODUCTION

Coffee (*Coffea canephora*), commonly known as Robusta coffee, holds paramount economic importance in Ghana's agricultural sector (Akpertey *et al*., 2022). Coffee ranks as a highly sought-after commodity in the global trade market, second only to oil, according to the International Coffee Council (2015). With over 100 coffee genera, this agricultural product is classified as a species of flowering plant within the Rubiaceae family (Bareke *et al*., 2021; López *et al*., 2021). While coffee has origins in Africa, particularly

Ethiopia, where it is indigenous, it has become a widespread crop globally (Krishnan, 2022; Melese & Kolech, 2021). In Ghana, the cultivation of coffee dates back to the 18th century, coinciding with the introduction of cocoa, and despite its historical presence, coffee has not achieved the same economic prominence as cocoa in the country (Mohammed *et al*., 2023). In Ghana, coffee cultivation primarily occurs in small holdings scattered throughout cocoa-growing regions, with only a limited number of large plantations (Zaney, 2011; Wongnaa *et al*., 2021).

Coffee production in Ghana, like many tropical countries, has witnessed a reduction, which has been linked to insufficient extension services officers available for training coffee farmers in effective management practices (Abebe, 2021; Kabita *et al*., 2021). Additionally, factors contributing to this decline include the absence of improved planting materials, the disappearance of elite coffee plants, and the susceptibility of aging coffee trees to diseases and pests (Córdoba *et al*., 2023). Cecon *et al.* (2005) emphasize the importance of conducting divergence analysis to enhance the selection of progeny in coffee tree genetics, aiming to improve and conserve coffee species with desired traits. Additionally, Denham *et al*. (2020) and Shahnawaz *et al*. (2021) assert that plants propagated vegetatively exhibit higher productivity and genetic consistency compared to those propagated from seeds. Malabadi *et al*. (2023) and Gupta *et al*. (2020) further highlight the significance of factors like plant rooting hormones and alterations in environmental conditions, such as temperature, humidity, and light, in influencing successful vegetative propagation methods like cuttings. According to Adams *et al*. (2021), the utilization of clonal chambers, designed to create optimal microenvironments for rooting, and rooting hormones, such as Ferti Lome, known for their potential to stimulate root development, presents a promising avenue for addressing these challenges. In Ghana, the propagation of clonal cuttings has been employed for decades to boost forest tree populations, showing significant promise. However, this method has faced challenges, including poor rooting and plant mortality. Additionally, the propagation of cuttings through vegetative means is hampered with the inability of cuttings to root effectively to attain maximum yields (Sarpong *et al*., 2020).

Furthermore, understanding the combined impact of clonal chambers and Ferti Lome on the rooting and shooting processes of *C. canephora* stem cuttings is essential for refining propagation techniques and ultimately enhancing the efficiency of coffee cultivation. Given this, it becomes crucial to investigate how the use of clonal chambers and rooting hormones influences the rooting and shooting processes of *C. canephora* stem cuttings. The study aimed to achieve two main objectives: first, to assess the effectiveness of clonal chamber conditions and rooting hormones in enhancing the rooting and shooting capabilities of *C. canephora* stem cuttings; and second, to evaluate the impact of clonal chamber conditions and rooting hormones on the mortality rate of *C. canephora* stem cuttings.

MATERIALS AND METHODS

Location of the Study Area

The experimental study took place at the demonstration farm of the Agroforestry Department, situated within the premises of the Kwame Nkrumah University of Science and Technology (KNUST) in Kumasi, Ghana. The location of the farm falls within the humid Semi-Deciduous Forest region of Ghana, and its geographical coordinates are latitude 6.40°N and longitude 1.37°W.

Rainfall

The region has a unique pattern of rainfall that occurs in two main periods every year. The average amount of rainfall is usually between 1250 to 1500 mm annually. The first period, known as the big wet season, happens from May to July, while the second period, known as the minor rainy season, occurs from September to November. Additionally, there are two dry seasons, with the longer one lasting from December to March and the shorter one happening in August.

Temperature and Humidity

According to Adu and Asiamah's report from 1992, the average daily temperature of the site is 25.6 °C. During the coldest months of December to February, the average temperature drops to 20 °C, while the hottest month of March has an average high temperature of 33 °C. The site's average yearly temperature is 26.61 °C with a relative humidity of 67.6%.

Soil Type

Neina and Agyarko-Mintah (2022) reported that the soil type present at the location of the experiment is Ferric Acrisol, which is strongly acidic and well-drained. The soil texture is described as sandy-loam.

Experimental Procedures

Land clearing

The experiment was conducted in an open environment, and the site was manually cleared of all weeds and foreign materials using tools such as a hoe, cutlass, and rake.

Source of cuttings and hormones

Coffea canephora clonal cuttings were obtained from the Cocoa Seedlings Production Division in Fumso, while the rooting hormone was sourced from the Horticulture Department in the Faculty of Agriculture in the Ashanti Region of Ghana.

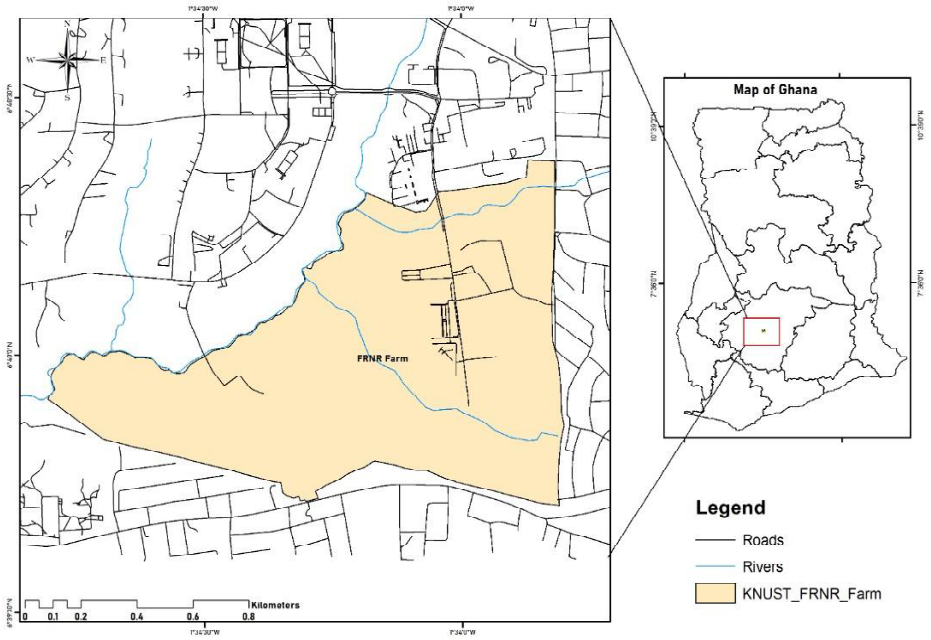

Figure 1. Map of the Agroforestry Department Demonstration Farm at the Kwame Nkrumah University of Science and Technology

Clonal chambers construction

Clonal chambers were constructed with dimensions of 50 cm in length, 40 cm in ovidth, and 50 cm in height with an open base. The side covers were made of transparent polythene and entirely covered with transparent polythene of equal size as the length of the clonal chamber.

Filling of polybags and cutting propagation

Polypots were filled with sterilized river sand as the rooting medium to the brim and the pots were perforated to aid drainage of excess water. Clonal cuttings (semi hardwood) of height 10 cm and diameter of 3 mm were used, the leaves were stripped off leaving only two functional ones but with reduced surface area.

The cuttings were transported to the experimental site that same day. Upon arrival, all spoil cuttings were discarded, after which new cuttings were made. The new fresh cuttings made were subjected to treatments; those to be treated with rooting hormones were treated as such. This treatment was done by dipping the respective cuttings into a poured hormone solution on a dish to a distance of 2 cm from the base, and any excess hormones on the cuttings were gently tapped off. Clonal cuttings were propagated immediately after removal from rooting hormones in a hole of depth 6 cm in each poly pot filled with the rooting medium. Firm contact with the cuttings was ensured. The propagation of both cuttings—those with hormones and no-hormones—was carried out spontaneously, and shade was projected 2 m high above the propagated cuttings. The experiment lasted for 18 weeks.

Experimental design and treatments

The experimental design was laid out in a randomized complete block design (RCBD) with four treatments and four replicates. This land area was used to propagate 400 clonal cuttings of *C. canephora* of sample size. Two hundred clonal cuttings of *C. canephora* were under clonal chamber conditions, and 200 clonal cuttings were under off-clonal chamber conditions (thus, the general environment). One cutting was propa-gated per polypot; each treatment unit contained 25 polypots.

The treatments were as follows:

1. HnC are cuttings treated with hormone and propagated under no clonal chamber
2. nHnC are cuttings treated with no hormone and propagated under no clonal chamber
3. HC are cuttings treated with hormone and propagated under clonal chamber
4. nHC are cuttings treated without hormone and propagated under clonal chamber

Data collection

Data on rooting and shooting parameters of *C. canephora* clonal cuttings were collected once every two weeks after eight weeks of cutting propagation and were done five times. Data were collected on root length, number of roots, number of newly developed leaves, and mortality. The length of the roots was measured with respect to the longest one with a 30 cm ruler from the tip of the root to the basal area of the cuttings where rooting started. Roots and the number of shoots per cutting were determined by visual counting, respectively.

Data analysis

Data collected on growth parameters were analyzed for differences using analysis of variance (ANOVA) technique based on STATISTIX 10 software at 5% level of significance. The least significance difference (LSD) was employed to compare the means

which were significantly different. Results were presented in graphs using excel.

RESULTS AND DISCUSSION

Rooting of Cuttings

Statistical analysis revealed that there were significant differences ($p \leq 0.05$) in the number of roots produced with regards to the application of rooting hormone and clonal chamber conditions. Cuttings treated with hormone and propagated under clonal chamber conditions recorded the highest number of roots (12.14), followed by clonal chamber condition + no-hormones (2.85), off-clonal chamber condition + hormones and off-clonal chamber condition + no-hormones had no roots (Figure 2).

The highest number of roots per cutting (reaching 12.14), as seen in cuttings treated with hormone and propagated under clonal chamber conditions is likely due to the combined effects of the clonal chamber and the rooting hormone, as depicted in Figure 2. This aligns with findings from Husin *et al*. (2022), suggesting that the heat buildup within clonal chamber conditions encourages the formation of adventitious roots at the basal ends, there by promoting root development in cuttings. Moreover, the significant difference observed between treatment with hormone under chamber condition and treatment without hormone under chamber condition may be attributed to the impact of the rooting hormone in stimulating root initiation. This finding is consistent with reports by Abdel-Rahman *et al*. (2020), El-Gedawey (2021), and Quan *et al.* (2022), which suggest that hormones act as chemical stimulants in cuttings, enhancing certain physiological processes and inhibiting others, thereby resulting in a greater number of roots compared to untreated cuttings. Additionally, these studies highlight that the application of rooting hormone leads to an exponential increase in the number of roots per cutting.

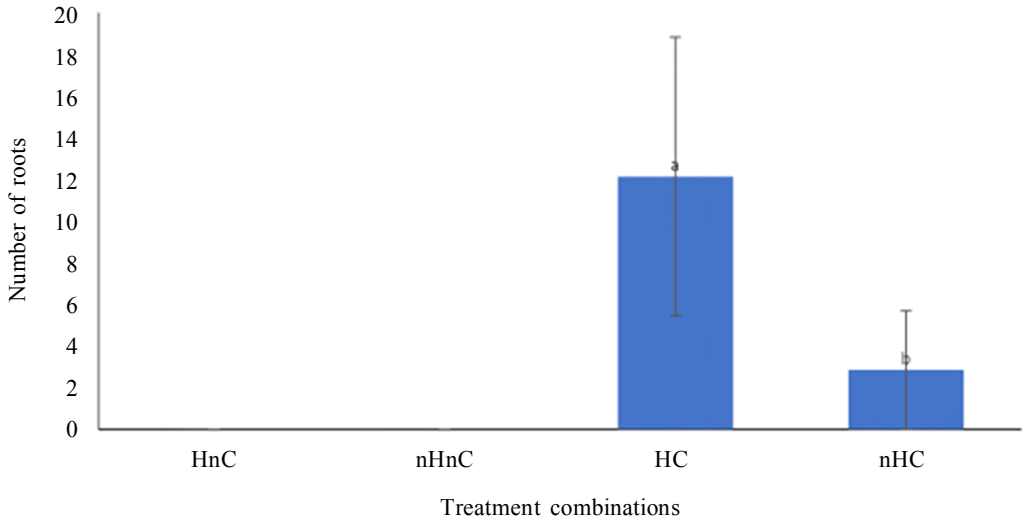

Figure 2. Effect of clonal chamber and rooting hormone on the number of roots of *Coffea canephora* cuttings

Notes: HnC with hormone and no chamber; nHnC no hormone no chamber; HC with hormone under chamber; nHC no hormone and under chamber.

The absence of roots in treatments without chamber although with and without hormone may be attributed to unfavorable environmental conditions to which the cuttings were exposed, leading to the poor performance and mortality of most cuttings. This observation aligns with the findings documented by Bahru & Derero (2023), Druege (2020), and Gazzana *et al*. (2020), indicating that the success of cuttings in vegetative propagation depends on providing suitable environmental conditions conducive to both shoot and root formation. Observations revealed that cuttings propagated under clonal chamber conditions dried up more rapidly compared to those under non-chamber conditions. This discrepancy can be attributed to the clonal chambers' ability to retain evaporated and transpired water from both the polypot soils and cuttings, maintaining a moist environment within the chambers. This finding is consistent with the findings of Tao *et al*. (2021), who emphasized the crucial role of water throughout the rooting process and the plant's lifespan. Furthermore, since physiological processes within living cells occur in an aqueous medium, successful rooting would not occur if cuttings were unable to absorb sufficient water from their surroundings.

The absence of roots observed in treatments without chamber although with and without hormone may be attributed to underlying factors elucidated in prior research. This study's findings are consistent with the conclusions drawn by Al-Jabbari *et al.* (2020) and Campbell *et al*. (2021), indicating that the rooting of cuttings, regardless of hormone application, is hindered in unsuitable environmental conditions. In contrast to the observations made by Politu & Aviko (2016), who proposed that coffee cuttings typically initiate rooting after twelve weeks of propagation (approximately three months), our study demonstrates that rooting initiation occurred as early as the 10th week after propagation, with an average of 1.63 roots per cutting. Additionally, Politu & Aviko (2016) emphasized that for a plant to be considered rooted, the number of roots per plant should exceed one.

Root Length of Cuttings

Statistical analysis revealed that there were significant differences in the root length of coffee stem cuttings with the applications of rooting hormone and clonal chamber conditions ($p<0.05$) (Figure 3). Cuttings treated with hormone and propagated under clonal chamber conditions recorded the longest elongated root length of 5.73 cm and was significantly different from the other treatments.

The notably greater root length observed in treatment with hormone under chamber condition (5.73 cm) may be attributed to the favorable conditions within the clonal chamber and the application of hormonal treatments aimed at promoting early root initiation. This finding aligns with the conclusions drawn by Cai *et al.* (2023), El-Banna *et al*. (2024), and Wang *et al*. (2022), which suggest that rooting hormones serve as accelerators for cuttings, facilitating the initiation of new roots more rapidly than untreated cuttings. Consequently, the application of rooting hormone is shown to significantly enhance rooting compared to untreated counterparts. Furthermore, this study corroborates the findings of Kaviani *et al*. (2023) and Politu & Aruko (2016), indicating that the combined effects of naphthalene acetic acid (NAA) and clonal conditions exert an influence on both the quantity and length of roots in cuttings. This combined treatment effectively promotes root formation and subsequent growth, resulting in increased root length in cuttings.

Treatment of cuttings propagated under clonal chamber conditions without hormonal treatment, exhibited a root length of 1.86 cm.

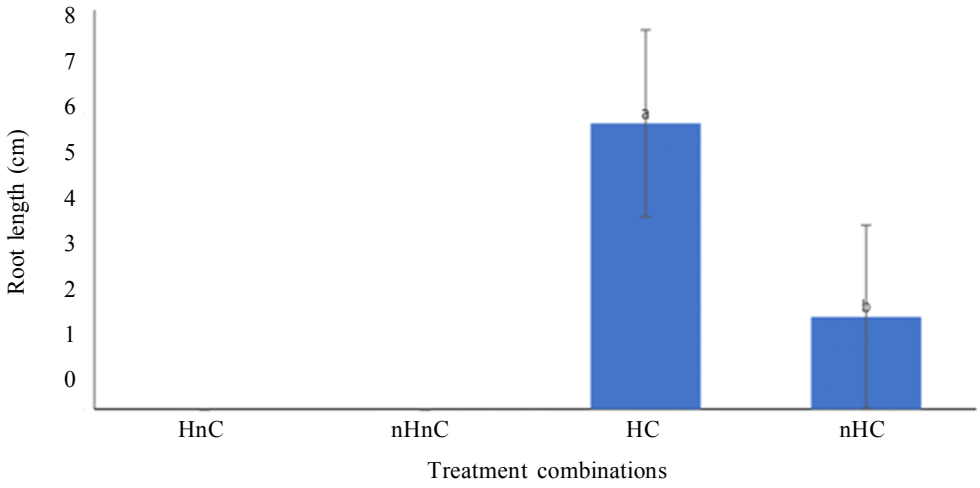

Figure 3. Effect of clonal chamber conditions and rooting hormone on root length of *Coffea canephora* cuttings

Notes: See Figure 2.

This length was attributed to the favorable environmental conditions within the clonal chamber, which promoted early root initiation. According to Hartmann *et al*. (2005) and Husin *et al*. (2022), the heat accumulation inside the chamber triggers the development of adventitious roots at the basal ends, thereby facilitating root formation even without hormone treatment. However, contrary to findings by Okonkwo *et al*. (2020) and Rauter (1994), who reported limited success with hormone application for rooting improvement, our study's analysis of variance indicated a significant effect of hormonal treatment on root length. This discrepancy may come from variations in experimental conditions and species-specific factors. Our study demonstrates the positive influence of hormones on root development and length, presenting promising results compared to previous findings.

Leaves number

The number of leaves produced by coffee cuttings in response to rooting hormone and clonal chamber conditions is presented in Figure 4. Cuttings treated with clonal chamber conditions + hormones and cuttings treated with clonal chamber conditions + no hormones registered the highest leaf numbers of 9.3 and 8.09, respectively, were statistically similar but significantly different from cuttings treated with off-clonal chamber condition + hormones (0.54) and the control (0.56).

The increased leaf count observed in treatment with hormone under chamber condition and treatment without hormone under chamber condition may be attributed to the controlled warm and humid conditions provided by the clonal chambers. These conditions help minimize water loss through transpiration and maintain optimal air and soil temperatures and light levels, which are conducive to the initiation of new leaves (Patial *et al*., 2021; Tanner *et al*., 2021). Additionally, the clonal chambers play a role in regulating air and water balance around the cuttings, facilitating respiration and preventing dehydration (Wang *et al*., 2023). This aligns with previous findings by Morales *et al*. (2020) and Moore *et al*. (2021), which demonstrated that elevated temperatures promote faster plant processes, thereby accelerating leaf development for efficient photosynthesis. Moreover, the higher number of leaves observed in treatment with hormone under chamber condition and treatment without hormone under chamber

condition may be attributed to the favorable atmospheric conditions created by the clonal chambers and the level of stored carbohydrates in each cutting. These conditions provide the necessary energy for cuttings to undergo metabolic and physiological processes, facilitating the initiation of new leaves. Studies by Kumar *et al*. (2022) and Shamsuddin *et al*. (2021) have highlighted the significance of sufficient stored carbohydrates in promoting successful shoot production from cuttings during propagation. Additionally, Muniandi *et al*. (2022) have emphasized the complex interaction between environmental and internal factors in determining the shooting response of cuttings. They noted that optimal conditions, including temperature, light, humidity, oxygen supply to the base of the cuttings, and suitable substrate, are essential for the successful formation of shoots and roots. Therefore, the clonal chambers utilized in this study provide the necessary environmental conditions for cuttings to promote leaf production.

However, Treatments 1 and 2 resulted in a low number of leaves, potentially due to unfavorable environmental conditions that led to the death of most cuttings. The study findings suggest that the rooting hormone used did not statistically influence leaf initiation in the cuttings. The results also indicate that the formation of new leaves is challenging when environmental conditions are not optimal. For instance, Treatment 4, which involved cuttings without hormonal treatment propagated under clonal chamber conditions, exhibited a higher number of leaves compared to Treatment 1, where cuttings were treated with hormone but propagated off clonal chamber conditions. This suggests that maximum leaf production is attributed to the stabilized control environment provided by the clonal chambers and the availability of sufficient stored carbohydrates in the cuttings. This availability of energy facilitates various metabolic and physiological processes necessary for leaf initiation. Moreover, the study underscores the significance of environmental conditions highlighted by Awotedu *et al*. (2021), which include adequate water, suitable temperature, light, appropriate rooting medium, and gas composition, with oxygen being particularly crucial. Failure to meet these environmental parameters can lead to poor performance of cuttings across different species.

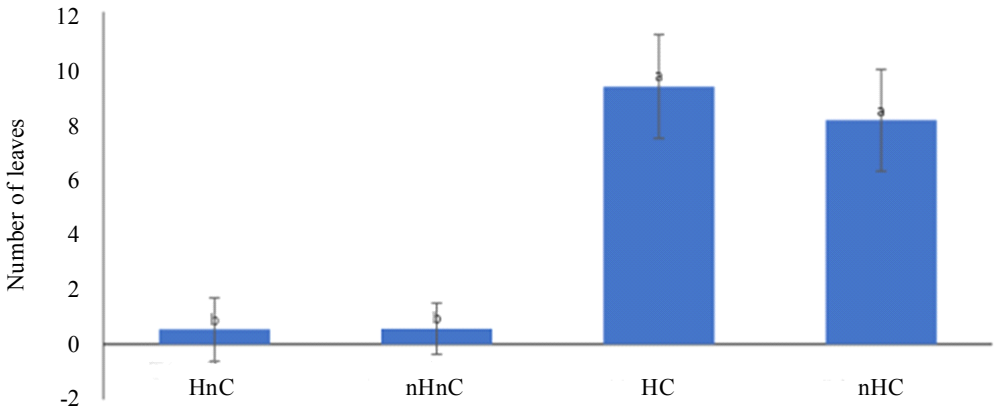

Figure 4. Effect of clonal chamber conditions and rooting hormone on the number of leaves of *Coffea canephora* cuttings

Notes: See Figure 2.

Mortality

The mortality of coffee stem cuttings was significantly ($p<0.05$) affected by both environmental conditions and hormonal treatment. Treatment under chamber without hormone recorded the least mortality (3.25) and was significantly different from treatment with hormone under chamber, without both hormone and chamber and with hormone without chamber, with mortality of 3.75, 19.2 and 20.22%, respectively. Also, T3 was statistically different from treatments without chamber although with and without hormone, and likewise, without both hormone and chamber was also different from with hormone without chamber statistically (Figure 5).

However, treatments without chamber although with and without hormone resulted in a low number of leaves, potentially due to unfavorable environmental conditions that the cuttings were exposed to, leading to a high mortality rate. Statistical analysis revealed that the rooting hormone used had no significant influence on the initiation of leaf growth in the cuttings. Moreover, the findings indicated that the formation of new leaves was hindered when the cuttings were not subjected to appropriate environmental conditions, as evidenced by the comparison between T4 and T1 treatments. Cuttings without hormonal treatment propagated under clonal chamber conditions, exhibited the highest number of leaves compared to T1. The mortality observed in treatment with hormone under chamber condition may have been caused by the application of rooting hormone on the cuttings, leading to leaf discoloration and subsequent leaf drop upon contact with hormonal chemicals. Additionally, mortality in both treatment with hormone under chamber condition and treatment without hormone under chamber condition could have been exacerbated by the excessive heat generated within the clonal chambers, as excessive heat buildup is known to negatively impact the longevity of cuttings or seedlings, as reported by Hartmann *et al*. (2005) and Milner *et al*. (2023). This finding aligns with previous reports by Aflakpui *et al*. (1998) and Balliu *et al*. (2021), indicating that the rooting and shooting of cuttings increase with temperature up to a certain threshold level.

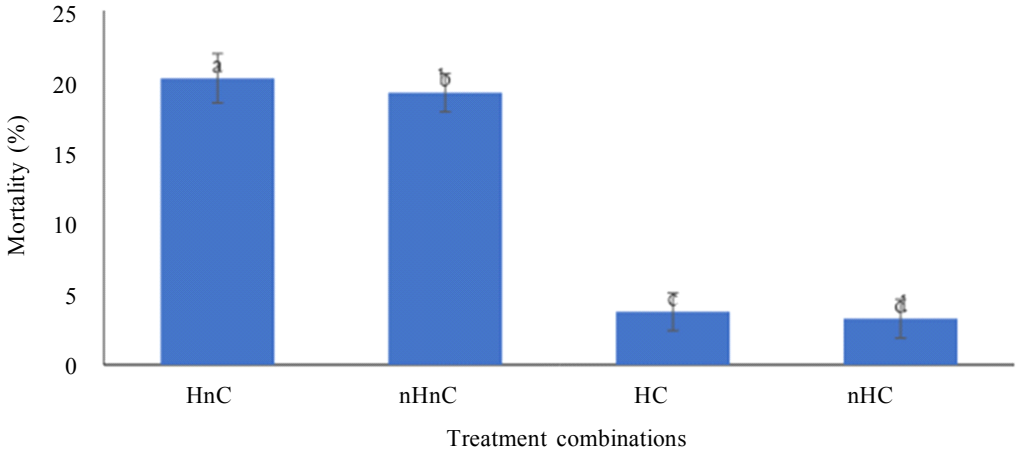

Figure 5. Effect of clonal chamber conditions and rooting hormone on mortality of *Coffea canephora* cuttings

Notes: See Figure 2.

Treatment without chamber although with and without hormone experienced elevated mortality rates due to adverse environmental conditions to which the cuttings were exposed. Insufficient measures were implemented to mitigate the effects of temperature fluctuations, soil moisture levels, evaporation, and humidity, leading to rapid drying of the applied water and potential water stress. This observation aligns with findings by Sahmat *et al*. (2022), who noted the challenge of rooting performance as soil moisture content nears the permanent wilting point. They emphasized the critical role of water throughout the rooting process and plant life cycle. The inability of cuttings to absorb adequate water from the surrounding medium may have contributed to the heightened mortality in treatments without chamber although with and without hormone. These factors collec-tively underline the reasons behind the increased mortality observed in these treatments.

CONCLUSIONS

It could be concluded that *C. canephora* can be propagated vegetatively. Also, the cuttings treated with rooting hormone and propagated under clonal chamber conditions significantly improved the number of roots, root length, number of leaves per cuttings as well as recorded the least mortality. However, cuttings propagated off the clonal chamber conditions had the highest mortality, least root number and least root length as well as leaf number. It is recommended that, during cuttings propagation involving rooting hormone, hormones should be prevented from touching the leaves of the coffee cuttings to avoid increase in cuttings mortality. Also, the ideal treatment for *C. canephora* cuttings for mass coffee propagation is to treat cuttings with rooting hormone and propagated under clonal chamber condition. However, in regards to the recorded mortality with treatment with hormone under chamber condition and treatment without hormone under chamber condition, studies can be conducted to determine the best poly ethylene to be used in construction of the clonal chambers for creating optimum ideal environment for cuttings propagation. This may reduce the mortality of cuttings during propagation under clonal chambers.

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Conflicts of Interest

All authors declared no conflict of interest.

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