



PATTERN OF ROOTING AND GROWTH OF CUTTINGS OF SOME SPECIES OF INSECTICIDAL AND MEDICINAL IMPORTANCE AS AFFECTED BY GROWTH PROMOTING SUBSTANCES

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Abstract: This study was designed to examine the effects of promoting substances on bud retention, rooting and plantlets survival of rooted cuttings on selected tree species using growth promoting substances. Stem cuttings were treated with rooting-promoting substances, planted in plastic pots filled with top soil and arranged using a completely randomized design (CRD) in a screen house. Stem cuttings of pepper fruit, guava, bush mango and cashew were obtained from the Teaching and Research Farm, Federal University of Technology, Akure, Nigeria. The experiments were conducted in 2010. The effects of growth promoting substances namely indole-3-butyric acid (IBA), indole-3-acetic acid (IAA) and 1-naphthalene acetic acid (NAA) applied 50 μ M concentration and coconut water (50% dilution) were evaluated on adventitious root formation, shoot growth (bud retention) and survival of plantlets of hard- to semi-hardwood cuttings of tested tree species. The effects of growth promoting substances were significant on emergence of plantlets (rooting), growth and survival of plantlets. Number of sprouts (plantlets) produced were highest in guava and pepper fruit followed by bush mango and cashew. The cuttings from guava and pepper fruit treated with coconut water and NAA promoted the survival of plantlets. Coconut water and NAA were found better than IBA and IAA in terms of bud retention and rooting, leaf development and survival of plantlets. In most of the tested species, wilting of leaves commenced 6 weeks after planting (WAP) and attained 100% mortality thereafter except for pepper fruit cuttings dipped in coconut water. The treatments modified the rooting response among species by promoting percentages of rooting, reduction in bud abscission and plantlet survival. The results affirmed the possibility of propagating plantlets from stem cuttings of the tested species using growth promoting substances.

Keywords: Bud retention, conservation, growth substances, propagation, rear plants, rooting.

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INTRODUCTION

Majority of plant species are of medicinal and insecticidal importance. The vegetative propagation of traditional tropical plant species of medicinal and insecticidal importance is a tool for the valorization and conservation of local genetic resources. In particular, tropical plant species had been employed for ages in plant, livestock, food and in human health (traditional medicine) and as protestants of agricultural products against pests and diseases (alternatives to chemical insecticides), in

pharmaceutical and natural product industries (Ogunjobi and Ofuya, 2008). In the recent past and presently, huge research efforts are directed at the screening and use of these plants as protectants of agricultural products and in human health (traditional medicine). To date, many identified and known plant species with medicinal/insecticidal importance and properties are collected from the wild, little efforts have been directed at their conservation/domestication/propagation in or ex situ (Negash, 2003). Ecosystem disturbance and habitat destruction (natural or human induced) from activities of such as slash and burn agriculture, logging, foraging habits of man and animals (livestock) for medicinal/bioactive plant species erode species richness due to massive clearing of natural vegetation (Agele, 2007; Ogunjobi and Ofuya, 2009). Thus these plant species with medicinal/insecticidal importance are pushed further into isolated pockets in the landscape, thus most of these species have become endangered. It is necessary to save the unique flora of the tropics especially species of medicinal/insecticidal importance. Concerted efforts should be focused on the conservation and regeneration of medicinal plants and promotion of sustainable harvesting.

In circumstances of declining availability of suitable habitats and changing environmental conditions, botanical investigations are necessary on successional processes and status of each species (Negash, 2003; Agele, 2007). Efforts geared towards obtaining a complete picture of natural plant species distribution (biogeographical ecology) may include botanical survey aimed at concerted monitoring of the status of these plant species either as rare, threatened, vulnerable etc. It is important to establish a list/compendium of these priority species, design their transplantation experiments and take active measures to ensure their survival. Majority of plant species are of medicinal and insecticidal importance, despite the significance of these plants, they are mostly collected from the wild and little attention had been given thus far to their domestication and large scale cultivation (Ogunjobi & Ofuya, 2009). The continued harvesting of these species from the wild coupled with large scale destruction of their habitat due to deforestation for example, would increase their rarity and promote their extinction (Negash, 2003; Agele, 2007).

The ease with which the stem cuttings can root and establish varies with species and cultivars, and also with physiological factors related to plant growth (Hartmann *et al.*, 1990; Zhao and Hasenstein, 2009; Coutessa and Valentini, 2011). Based on the pioneer work of Njoku (1963), interest has been generated in using stem cuttings as propagative material in view of their potentialities for clonal propagation, replication and uniformity of clonal materials (plantlets) produced. Literature has reported various research works on the vegetative propagation using stem cuttings treated with auxin or rooting hormones such as indolebutyric acid (IBA) and naphthaleneacetic acid (NAA) (Al – Salem and Koram, 2001; Ercisli and Read, 2001; Acha *et al.*, 2004; Zhao and Hasenstein, 2009; Coutessa and Valentini, 2011). It has been widely reported that treating stem cuttings with different hormone concentrations before planting in a suitable rooting medium is required for effective rooting (Kelen and Ozkan, 2003; Coutessa and Valentini, 2011). There are great differences in the rooting potential among plant species, in particular, tree species are often categorised into groups, easy, moderate and hard-to-root species (Wiesman and Lavee, 1995a; Denaxa *et al.*, 2012). In some plant species, root formation initiates without use of rooting or growth promoting substances, while in others it requires the application of growth regulators, usually auxins (Coutessa and Valentine, 2011). Stem cuttings from most tree species hardly roots simply by planting cuttings in substrates as reported by several researchers, this has prompted the use of auxins which has been shown to have effects on rooting of stem cuttings of most species (Ercisli and Read, 2001; Hartmann *et al.*, 2001; Kelen and Ozkan, 2003; Cristofori *et al.*, 2010). However, auxins have slight effects on root initiation of hard-to-root species (Wiesman and Lavee, 1995a). The rooting ability of cuttings is strongly influenced by collection time, age of the cutting and genotype (Cristofori *et al.*, 2010). Bud abscission is a limiting factor to propagation of stem cuttings, even though the rooting percentage may be acceptable (Bassil *et al.*, 1991; Coutessa and Valentini, 2011). It has been reported that a balance between endogenous stimulatory and inhibitory factors, as

well as, nutritional factors is required to promote rooting of cuttings (Wiesman and Lavee, 1995a; Denaxa et al., 2012). Since rooting process in most species is often lengthy, it is important for the cuttings to be continuously supplied with high amount of energy (Wiesman and Lavee, 1995b; Denaxa et al., 2012). Carbohydrates have been considered as optimal markers since they are the direct products of photosynthetic activity and constitute an important source of energy (Sivaci, 2006; Aslmoshtaghi and Shahsavari, 2010) as well as cell structural materials for the initiation of root primordia (Yoo and Kim, 1996). In addition, it has been suggested that carbohydrates promote rooting in a way unconnected with their role as energy-supplying substrates (Haissig, 1989). Thus, carbohydrates have been shown to affect auxin metabolism indicating that they can improve the stimulatory effect of IBA in the rooting process (Wiesman and Lavee, 1995b; Denaxa et al., 2012). Many studies have shown a positive correlation of rooting capacity with carbohydrate content of cuttings (Yoo and Kim, 1996; Aslmoshtaghi and Shahsavari, 2010), while some others failed to establish such a correlation (Tsipouridis et al., 2006). It has been also demonstrated that the carbohydrate content was high in easy-to-root than in difficult-to-root cultivars of olive cuttings (Yoo and Kim, 1996; Denaxa et al., 2012).

However, the cost implication of using the synthetic hormones (IBA, IAA, NAA) is high and the ease with which they are obtained is limited. This therefore means suitable and cheaper natural compounds (growth – promoting substances) for vegetative propagation of cuttings of plant species are required. It is therefore necessary to assess locally sourced root - promoting substances as a substitute to synthetic products (auxins). The main objective of this study was to propagate plantlets from stem cuttings of some selected tree species of insecticidal and medicinal importance using growth promoting substances in terms of rooting, bud retention, leaf production, growth and survival/mortality of plantlets of selected tree species. The species (*Denitia tripetala*), guava (*Psidium guajava*), bush Mango (*Irvingia gabonensis*) and cashew (*Anacardium occidentale*) were treated with growth promoting substances: indole-3-butyric acid (IBA), indole-3-acetic acid (IAA), 1-naphthalene acetic (NAA) and coconut water.

MATERIALS AND METHODS

Collection and handling of plant materials

Semi hardwood shoots of four selected tree species were obtained from the Teaching and Research Farm, Federal University of Technology, Akure, Nigeria. The tree species are: Pepper fruit (*Denitia tripetala*), Africa bush mango (*Irvingia gabonensis*), Guava (*Psidium guajava*) and Cashew (*Anacardium occidentale*). From the selected tree species, semi hardwood shoots were chosen as propagation materials following literature (Ercisli and Read, 2001). Shoots were collected and maintained wet in black plastic bags. Semi-hardwood cuttings were collected from mature and fruiting plants (hard wood) and each stem cuttings had at least four nodes and 2- 4 leaves and 15–20 cm in length. These cuttings were obtained from shoots of the species same day as their arrival to the nursery (one day after collection). Leaves were removed from the cuttings except for two leaves at the top, which were trimmed to two leaflets each. Twenty-five semi-hardwood stem cuttings obtained from each of the tested tree species were treated with the tested rooting-promoting substances from which data were obtained at each sampling date. The experiment was carried out between February and May, 2010.

Plant material: preparation of cuttings and rooting-promoting substances

The semi hardwood cuttings were collected, sprayed with water and maintained wet overnight in white plastic bags, the following day the materials were treated with rooting-promoting substances. The rooting-promoting substances tested were: IBA (Indole – 3 – buytric acid), IAA (3 – indole acetic acid) and NAA (1 – naphthalene acetic acid) and additionally, coconut water (CW) was also tested. IBA, IAA and NAA solutions were freshly prepared by dissolving the powders 1 g each of the rooting-promoting substances in 2 ml of 0.1M NaOH and diluted to 1000 ml of distilled water. Using the stock

solution, 50 μM was prepared by diluting 5ml of the stock into 100 ml distilled water while coconut water was mixed with distilled water to obtain 50 % dilution.

Treatments and propagation (rooting-promoting substances)

The basal portion (3 cm) of each cutting was dipped in the solutions of the rooting-promoting substances for 1 min. The untreated cuttings were used as control. All cuttings were planted in 4 kg polyethylene pots filled with top soil collected from fallow forest under a screenhouse covered with a shading net (60%) where temperature ranged between 30 and 32 °C and relative humidity was 70 – 80 %. The cuttings were dipped into respective growth promoting substances and were planted immediately to avoid prolonged dehydration. The rooted cuttings were watered, and thereafter, watering continued twice weekly. Cuttings were grown in pots filled with filtered soil and sprinkled with the water and the cuttings were placed in the screenhouse of the Department of Crop, Soil & Pest management, Federal University of Technology, Akure, Nigeria. The rooted cuttings were observed for 20 weeks and parameters were taken on patterns of sprouting (onset, number and mortality of plantlets), leaves (number of leaves per sprout), root development and mortality of plantlets.

Sampling scheme and statistical analysis

Sampling of semi-hardwood cuttings was performed 2 and 3 weeks after the beginning of rooting treatments and each cutting was scored for the presence of living cuttings (rooted, callused and un-rooted). The number of sprouts (retained buds) and leaves per cutting were counted. At 15 weeks after planting (WAP), cuttings were removed, observed and classified as: rooted, callused, living un-rooted, and dead. The number of cuttings with retained buds was counted. The percentage survival of sprouts per cutting (number of cuttings with retained buds per species) was calculated across all living cuttings (rooted, callused and un-rooted). The mean number of retained bud was calculated across the number of cuttings with at least one retained buds per species (number of retained buds per cutting). The quality of rooting was rated from the observation of the presence of callus and roots and further evaluated by measuring root length (root length per rooted cutting), using a ruler and the fresh weight of roots using a weighing balance (Metler Toledo). The number of cuttings with callus and the number of rotten cuttings were observed.

The experimental design was completely randomized. There were five single-plant replications (experimental units) within each species by rooting promoting substances (species by rooting promoting substances treatment combinations). Measurements made on single-plant replicates (1,2,3,4 and 5 replicates) were combined and the averages used for statistical analysis of data collected from the measurements of plant parameters. The experiment was repeated between August and November, 2010. The cuttings were taken and propagated following similar procedure of the first experiment (February and May, 2010), and the rooting percentage was measured. At each sampling date, data were subjected to Chi square (χ^2) test to statistically examine the effects of rooting treatment within each species. Data collected were further subjected to analysis of variance (ANOVA) while significant treatment means were separated using the Least Significance Difference (LSD) test at 5% level of probability.

RESULTS AND DISCUSSION

The trends in shoot emergence and leaf growth of cuttings obtained from the tested species are summarized in the Tables (1, 2 and 3). The responses of the species to the growth promoting substances (IBA, IAA, NAA and CW) are however different for the various growth promoting substances. Shorter number of days for plantlet emergence were observed for cuttings obtained from guava, cashew and bush mango than for pepper fruit. In these species, emergence was generally noticed 8 days after planting. However, emergence of plantlets was first noticed for cuttings of pepper fruit (Table 1).

Table 1. Trends in rooting percentage of the tested species as affected by growth promoting substances

Time (WAP)	Plant species	Growth hormones				Control
		IBA	IAA	NAA	CW	
2	Pepper fruit	0	0	0	0	10
	Guava	50	75	100	100	15
	Bush mango	50	0	25	50	10
	Cashew	25	50	25	0	13
3	Pepper fruit	25	0	0	0	14
	Guava	50	75	100	100	21
	Bush mango	50	0	25	50	15
	Cashew	25	50	25	10	23
5	Pepper fruit	50	50	50	75	20
	Guava	50	75	100	100	30
	Bush mango	25	0	25	0	27
	Cashew	25	50	25	0	33
6	Pepper fruit	50	50	75	100	20
	Guava	50	75	100	100	40
	Bush mango	25	0	25	50	20
	Cashew	25	50	25	0	42
7	Pepper fruit	75	75	100	100	30
	Guava	50	75	100	100	37
	Bush mango	25	0	25	50	31
	Cashew	25	50	25	0	35

IBA (Indole – 3 – buytric acid), IAA (3 – indole acetic acid), NAA (1 – naphthalene acetic acid), CW (Coconut water).

Guava cuttings treated with NAA and dilute coconut water produced 100% healthy shoots while cuttings dipped in IAA and IBA had 75% and 50% shoots respectively. The pattern of shoot emergence was the same for pepper fruit cuttings except for cuttings dipped in IBA which had 75% healthy shoots. In the case of bush mango, cuttings dipped in IBA and NAA gave 25% sprouts while those in coconut water had 50%. In the control experiment, little emergence was obtained from most cuttings from tested species. The results also showed that the growth promoting substances affected the number of leaves produced by the cuttings (Table 2). It was observed that the number of leaves on cuttings increased with time (3 – 5 WAP). Mean number of leaves on the cuttings of guava were higher from the 3 WAP than on cuttings from other species. After 35 days (5 WAP), the number of leaves initiated increased in all the cuttings while sprouts on guava cuttings increasing considerably than other cuttings. The highest number of leaves were obtained from guava cuttings dipped in NAA and coconut water. It was however observed that leaves on all cuttings from most tested species began to wither from the 6 WAP except for pepper fruit cuttings where the number of leaves increased. However at 10 WAP almost all the leaves in all the cuttings had withered. Only pepper fruit cuttings dipped in coconut water retained their leaves.

Table 2. Effect of growth promoting substances on number of leaves/plant among the tested species

Time (WAP)	Plant species	Growth hormones				Control
		IBA	IAA	NAA	CW	
1	Guava	4	6	8	8	2
	Bush mango	4	0	2	4	0
	Cashew	2	4	2	0	2
	Pepper fruit	1	3	4	4	0
2	Guava	8	12	16	16	4
	Bush mango	4	0	2	4	1
	Cashew	2	4	2	0	3
	Pepper fruit	3	3	4	4	0
3	Guava	10	15	20	19	4
	Bush mango	4	0	4	8	2
	Cashew	4	8	4	0	3
	Pepper fruit	3	3	4	4	1
4	Guava	0	0	8	8	3
	Bush mango	4	0	4	8	1
	Cashew	4	8	4	0	4
	Pepper fruit	3	3	4	4	1
5	Pepper fruit	3	3	5	6	0
	Guava	0	0	0	0	3
	Bush mango	0	0	0	0	1
	Cashew	0	0	0	0	3
6	Pepper fruit	3	3	5	9	0
	Guava	0	0	0	0	1
	Bush mango	0	0	0	0	0
	Cashew	0	0	0	0	0
7	Pepper fruit	3	3	5	11	0
	Guava	0	0	0	0	0
	Bush mango	0	0	0	0	0
	Cashew	0	0	0	0	0

IBA (Indole - 3 - butyric acid), IAA (3 - indole acetic acid), CW (Coconut Water), NAA (1 - naphthalene acetic acid).

The rooting ability of cuttings is influenced by age of the cutting and species or genotype (Cristofori *et al.*, 2010). Bud abscission which limits success of propagation of stem cuttings is linked to carbohydrate status in the rooted cuttings as well as cell structural materials for the initiation of root primordia (Yoo and Kim, 1996). Bud abscission is a limiting factor to propagation of stem cuttings, even though the rooting percentage may be acceptable (Bassil *et al.*, 1991; Coutessa and Valentini, 2011). For all species evaluated, trends in plantlet survival counts and leaves per plantlet and root development were similar among the rooting promoting substances that were tested at the close of experiment (15 WAP). Highest number of sprouts, better root and leaf development and survival of sprouted cuttings were obtained from cuttings treated with IBA and coconut water (Table 3). These substances promoted sprouting (and rooting) and the development of cuttings more than others. Among the species, the number of sprouts and survival of sprouted plantlets were highest in guava and

pepper fruit and least in Bush mango (*Irvingia*) across rooting promoting substances. This response can be attributed to differences in the species ability to root via stem cutting propagation (Melanta and Sulladmath, 1990; Cristofori et al., 2010; Gautam et al., 2010; Coutessa and Valentini, 2011).

Table 3. Effects of growth promoting substances on the growth and survival of plantlets (sprouts) on stem cuttings of tested species

Growing media	Onset of sprouting (DAP)	No. of sprouts/ Cutting	Total No. of leaves/ sprouts	Survival count and percent at 15 WAP	Root fresh weight (g) at 15 WAP	Total root length (cm) at 15 WAP
<u>Pepper fruit</u>						
IBA	35	5	21	3(30%)	7	18
IAA	35	4	18	2(20%)	6	17
NAA	21	4	18	2(20%)	8	18
Coconut water	35	4	20	2(20%)	7	17
Control	38	2	11	1(10%)	4	12
LSD (0.05)	2.3	1.2	3.7	1.2	2.2	3.1
<u>Guava</u>						
IBA	8	9	22	3(30%)	10	22
IAA	8	11	26	3(30%)	8	18
NAA	8	13	24	5(50%)	8	17
Coconut water	8	12	22	6(60%)	9	19
Control	9	5	15	3(30%)	6	11
LSD (0.05)	1	1.3	2.2	1.5	2.0	3.3
<u>Irvingia spp.</u>						
IBA	14	5	14	4(40%)	8	20
IAA	14	3	12	2(20%)	7	18
NAA	8	5	13	4(40%)	7	16
Coconut water	8	8	17	6(60%)	8	18
Control	5	3	9	2(20%)	6	12
LSD (0.05)	2.1	1.2	2.7	1.4	1.3	4.6
<u>Cashew</u>						
IBA	8	6	12	4(40%)	9	22
IAA	8	6	17	8(80%)	8	17
NAA	8	5	13	4(40%)	8	20
Coconut water	10	3	9	2(20%)	10	23
Control	11	2	6	1(10%)	6	13
LSD (0.05)	2.1	2.4	4.2	2.2	3.1	5.3

IBA (Indole – 3 – buytric acid), IAA (3 – indole acetic acid), NAA (1 – naphthalene acetic acid)

The results of this study showed that growth promoting substances (IBA, IAA) markedly stimulated shoot initiation (sprouting) and survival of plantlets from species. Under the experimental condition, all the growth substances induced or stimulated responses in cuttings obtained from guava and cashew. The regrowth of shoots from buds was also influenced by NAA, IAA and coconut water treatments, in particular, for Cashew, Guava and Bush mango, emergence commenced within 8 to 14 days after planting. There were significant differences in the number of days for shoot emergence among pepper fruit and the other plant species. In pepper fruit, shoots emerged on cuttings 3 WAP for all growth promoting substances while in Guava and Bush mango, shoots emerge in the cuttings within 1 to 2 WAP respectively. Carbohydrates as a source of energy have been shown to affect auxin

metabolism indicating that they can improve the stimulatory effect of IBA in the rooting process (Wiesman and Lavee, 1995b; Denaxa *et al.*, 2012). It has been also demonstrated that the carbohydrate content was high in easy-to-root than in difficult-to-root cultivars of olive cuttings (Yoo and Kim, 1996; Denaxa *et al.*, 2012),

Although, differences were found between NAA and coconut water treatments, the emergence of shoots from NAA and coconut water were 100% over IBA and IAA which gave 50% and 75% respectively. The use of hormone IAA was not effective in initiating shoot on the stem cuttings of bush mango. It is reported that coconut water contains sugar, amino acid, myo-inositol, and micro constituents of phenyl urea (Acha *et al.*, 2004; Agele *et al.*, 2010). Stem cuttings of various species treated with coconut water had been reported to promote root formation and shoot initiation (Komamine *et al.*, 1990; Agele *et al.*, 2010). Optimum sprout emergence and growth was obtained with NAA and coconut water.

Different auxins had been found to promote the rooting percentage of stem cuttings (Ercisli and Read, 2001; Kelen and Ozkan, 2003; Zhao and Hasenstein, 2009; Coutessa and Valentini, 2011). For example, cuttings of *Alnus meritina* (Schrader and Graves, 2000) and of *Arbutus andrachne* (Al-salem and Koram, 2001) and yam vine cuttings (Acha *et al.*, 2004; Agele *et al.*, 2010). IITA in her annual report (2000) reported the use of coconut water (10%) as an important components of basal medium supplements in tissue culture for obtaining rooting and plantlet formation from immature yam seeds. The tested species were not significantly different in terms of the number of shoots produced from them.

Significant differences were found in the rooting responses of the tested plant species to auxin treatment, stem cutting dipped in water (where stem cuttings were neither treated with auxins nor coconut water) and planted in top soil produced low rooting percentage (< 15%). This could be attributed to the hard nature of most of the tested species (hard-to – root plants) conventionally, they are propagated by seeds. However, almost all the leaves on all the various cuttings started to wither from 6 WAP, however, leaves on sprouts from pepper fruit cuttings were retained for longer time, leaf drop commenced about 7 WAP. Since rooting process in most species is often lengthy (2–3 months), it is important for the cuttings to be continuously supplied with high amount of energy (Wiesman and Lavee, 1995b; Denaxa *et al.*, 2012). Carbohydrates have been implicated as an important source of energy (Sivaci, 2006; Aslmoshtaghi and Shamsavar, 2010) as well as cell structural materials for the initiation of root primordia (Yoo and Kim, 1996; Bassil *et al.*, 1991; Kelen and Ozkan, 2003; Denaxa *et al.*, 2012).

CONCLUSION

This study aimed at identifying characteristics of genotypes that determine bud retention, rooting and plantlets survival of rooted cuttings obtained from selected tree species of insecticidal and medicinal importance using growth promoting substances. The results showed that application of growth promoting substances enhanced bud retention, rooting and plantlets survival in the tested semi-hardwood cuttings of tropical tree species. Although, the rooting percentage of the species treated with rooting-promoting substances varied, however, coconut water at 50% dilution in water was also found useful in the vegetative propagation of stem cuttings of the tested tree species. Chemical growth substances (IBA, IAA and NAA) and coconut water dilution can be used for rapid multiplication of cuttings of tested species. In this study, treating stem cuttings with growth promoting substances (NAA, IAA, IBA and coconut water) before planting in top soil as rooting medium enhanced rooting and plantlets survival of the selected tree species. Seeds are the conventional planting material for three of the tested plant species (pepper fruit, guava, cashew and bush mango), however, obtaining these propagules which are limited in supply, are not only difficult but also costly. Vegetative propagation of

traditional tropical plant species of medicinal and insecticidal importance is a tool for the valorization and conservation of local genetic resources. The use of stem cuttings would enable farmers produce more from the lateral branches of mother plants. This would augment the hitherto limited quantity of planting materials (stem cuttings) needed by the farmers for rapid multiplication. The propagation of these plant species of insecticidal/medicinal importance using stem cuttings creates an avenue for method diversification other than the usual conventional means of propagating them. The results of this study affirmed the possibility of propagating plantlets from stem cuttings obtained from selected tree species of insecticidal and medicinal importance using growth promoting substances.

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