Effect of seed extracts and Indole-3-butyric acid on the rooting potential of *Dialium guineense* Willd stem cuttings

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Abstract
The effects of some post severance treatments on the rooting potentials of juvenile stem cuttings of *Dialium guineense* Willd was assessed in an experiment conducted at the Physiology and Tree breeding Section's Nursery of the Forestry Research Institute of Nigeria, Jericho, Ibadan, Oyo State, Nigeria. 160 single node softwood cuttings of *D. guineense* were randomly assigned to 4 treatments namely orange seed extract, pawpaw seed extract, 250 ppm indole-3-butyric acid (IBA) dissolved in industrial alcohol and a control (water) applied by dipping the base of the cuttings for 10 seconds. Although the results displayed no pronounced effect of seed extracts and IBA on the leaf abscission and cutting mortality at 0.05 level of probability but there was a significant difference in shoot formation with the control showing a mean of 2.50 followed by pawpaw (1.75), IBA (1.50) and orange (1.0) respectively. Also there were significant differences in root formation, number of roots and root length with pawpaw seed extract recording higher values than the rest of the treatments. The results therefore, suggest that pawpaw seed extract can replace synthetic IBA in inducing rooting in cuttings of *D. guineense* for mass clonal propagation.

Keywords: Seed extracts, *D. guineense,* vegetative propagation, Indole-3-Butyric Acid (IBA)

Introduction
Forest trees act as buffer solution against poverty and provide protection against adverse environmental conditions. Forest trees help to provide the stable environmental condition for sustainable food production. They restore soil productivity through nutrient recycling and provide a store house for genetic diversity. At the farm level, forest trees improve micro-climate, reduce wind damage to crops, reduce erosion and restore soil fertility. Forest trees reduce sedimentation in streams and water bodies and improve water quality (Adekunle *et al*., 2008).

On a broader scale, trees influence regional climate patterns. Afforestation is one means of slowing global warming by creating carbon sinks, reducing global warming and lead to conservation of healthy and diverse forest ecosystems, thus improving the ability to deal with climate change and to prevent environmental degradation. (Adekunle *et al*., 2008).

Indigenous plants have enormous nutritional, medicinal and social economic potential. One of the indigenous fruit trees with enormous potential for plantation establishment and food insecurity is *Dialium guineense* Willd.

Dialium guineense (Velvet tamarind) belongs to the Family Fabaceae. *D. guineense* is an important leguminous plant that is widely distributed in the tropics. It is common in humid dense forests, dry dense forest and forest galleries in West and Central Africa (Arogba, *et al*. 1994). On farmland, *D. guineense* is predominantly a fallow species. It restores lost soil fertility as it usually emerges as one of the pioneer species in abandoned lands. It is used in outlying farms in the forest area as an agroforestry tree crop.

The fruits are widely sold on local markets and are consumed fresh by people of all ages as a snack. Some old people consume non-alcoholic drink made from the fruit. According to the producers and sellers of medicinal plants of Benin, the leaves, bark and roots can be used to treat a variety of health problems. They are used in the management of fever, diarrhoea and palpitations, and as an antibacterial treatment. Extracts from velvet tamarind plants growing in Nigeria have been shown to possess both antimutagenic and molluscicidal activities. Fruits are rich in minerals, sugars and tartaric, citric, malic and ascorbic acids (Ubani and Tewe, 2001).

Velvet tamarind wood is hard, heavy and fine textured, and is used for flooring and other local construction. It is also a high-quality fuel and is used for making charcoal. Velvet tamarind is a source of substantial income to the population in rural and suburban zones in Benin, Nigeria and Togo. The fruits are traded locally and across borders. Velvet tamarind grows best on well-drained iron-rich acidic soils within a temperature range of 25 to 32°C and an annual rainfall of 900-3000 mm (Ubani and Tewe, 2001).

The wood is a good source of charcoal. In Nigeria, *D. guineense* flowers from September to October and fruits from October to January while in Ghana, the tree is covered with small white flowers in panicles from September to November. Fruits ripen in March to May but could extend beyond July. Animals, which like to eat pulp in which the seeds are embedded, help disperse the fruit. However, the fruit can also be transported by water since it floats; transporting by sea currents may lead to long-distance dispersal (Orwa *et al.*2009).

D. guineense is one of the under-utilized fruit trees in Nigeria which is of great importance and has very high potential for many uses. However, if the plant is propagated through seeds, it takes many years before it starts producing fruits. Therefore, vegetative propagation remains a good alternative to multiplying a mature tree that has superior qualities.
There is a dearth of information on the effects of different seed extracts and synthetic IBA on the rooting of the stem cuttings of *D. guineense.*

This study therefore, aims at investigating the effects of different seed extracts (pawpaw and orange) and synthetic IBA on the rooting potentials of the stem cuttings of *Dialium guineense*

Materials and Methods

The experiment was carried out at the Physiology and Tree Breeding Section's Nursery of the Department of Sustainable Forest Management in the Forestry Research Institute of Nigeria

(FRIN) in Ibadan. It lies between latitude $7^{0}24'$N and longitude $3^{0}55'$E. The climate is typically dominated by rainfall pattern ranging between 1400mm – 1500mm and the average temperature of 32^{0}C with two distinct seasons: Dry season (usually between November and March) and Rainy season (from April to October) (FRIN Annual Metrological Report 2011)**.**

A propagation unit was established at the Nursery, for the experiments. The propagator consisted of a wooden frame enclosed in a clear polyethylene with a water-tight block work base. The base of the propagator was covered by a thin layer of sand (10 cm depth) and then successive layers of small and medium size granite (0.5–5 cm, to a depth of 25 cm) and then filled with water. The non-mist propagation unit was placed in a screen-house.

Seedlings Collection

The fruits of *D. guineense* were collected from the Botanical Garden, University of Ibadan, Oyo State. The fruits collected were taken to Forestry Research Institute of Nigeria, Ibadan where seeds were extracted by manual depulping and were later planted on germination tray. Of the germinated seedlings, one hundred seedlings were selected and used for the experiment. The stock plants were sprayed with systemic fungicides and insecticides prior to severance.

Seed Extracts Collection And Preparation

Mature fruits of *Carica papaya* (pawpaw) and *Citrus spp* (orange) were collected from fruit trees in Forestry Research Institute of Nigeria. The seeds were extracted from the fruits and washed thoroughly in clean water and sun dried for 2 weeks after which they were ground separately in a sterile mortar to obtain 100g each of the dry seed powder. Ethanol extracts of the seeds was obtained by adding 100g each of the seed powder to 100 ml of ethanol in a beaker to obtain 100g (wt/v) of the extracts, and was left to settle for 5 hours at room temperature before the extracts was filtered using cheesecloth.

Sterilization of the extracts was done by applying 125 mg of streptopenicillin, a mixture of 62.5 mg of streptomycin & 62.5 mg of penicillin as described by Gupta and Banerjee (1990). 160 single nodal cuttings were harvested from the stock seedlings. 10 cuttings each were randomly assigned to the four treatments namely orange seed extract, pawpaw seed extract, 250 ppm indole-3-butyric acid (IBA) dissolved in industrial alcohol and water as control. The base of the cuttings were dipped quickly in the extracts, evaporated in a gentle air before been set in washed and sterilized river sand in the propagator in a Completely Randomized Design with four replicates (Plate 1) making 160 cuttings in all.

Cuttings were assessed weekly for leaf abscission, cutting mortality and shoot formation, while the presence and number of roots ($\geq$ 2 mm in length), rooting percentage, and root length were assessed at the end of the experiment.

Numbers of leaves abscised were literally determined by physically counting the number of dead leaves. Cuttings mortality was determined by physically counting the number of dead cuttings. Shoot formation was done by physically counting the number of new shoots from the cuttings.

Presence and number of roots were determined at the end of the experiment by physically checking the presence of roots and counting the number of roots from each of the cuttings, while root length was determined using meter rule.

Data collected were subjected to analysis of variance (ANOVA) and significant means were separated using Duncan Multiple Range Test (DMRT) at 5% level of probability.

Plate 1: Cuttings newly set in the Propagator

Results

The result showed that leaf abscission was not significantly affected by the treatment (Table 1). IBA recorded the least value (8.5) of leaf abscised (Fig. 1). The proportion of leaf death ranged between 8.5 to 10.75 in IBA and the control respectively (Fig. 1). In cutting mortality, there was no treatment effect overall (Table 1) with proportion of cutting death ranging from 5.75 to 7.5 in pawpaw and orange seed extracts respectively (Fig. 2). Treatment effect on shoot formation was highly significant (Table 1) with the control showing a mean of 2.50 followed by pawpaw (1.75), IBA (1.50) and orange (1.0) respectively (Table 2 & Fig. 3)

Treatment effect on root formation was highly significant (Table 1) at the final assessment with pawpaw recording a higher root formation (2.75) than the rest of the treatments (Table 2 & Fig. 4). The effect of treatment on number of roots was also highly significant (Table 1) with pawpaw (10.25) higher than control (5.75), which in turn was higher than the rest treatments, which were not different from each other (Table 2 & fig. 5). Treatment effect on root length was highly significant (Table 1) with pawpaw recording highest value of 7.30 followed by IBA (4.02) (Plates 2a, 2b & 2c). Fig. 6 also revealed that treatment effects were highest in pawpaw.

Table 1. Analysis of Variance for Leaf Abscission, Cutting Mortality, Shoot Formation, Root Formation, Number of Roots and Root Length.

Source of variation	Df	Leaf Abscission	Cutting mortality	Shoot formation	Root formation	Number of roots	Root length
Media	3	4.16	2.50	1.52*	3.84*	46.27*	23.69*
Error	12	15.54	3.70	4.20	1.13	28.65	11.43
Total	15						

*significant at 5% probability level

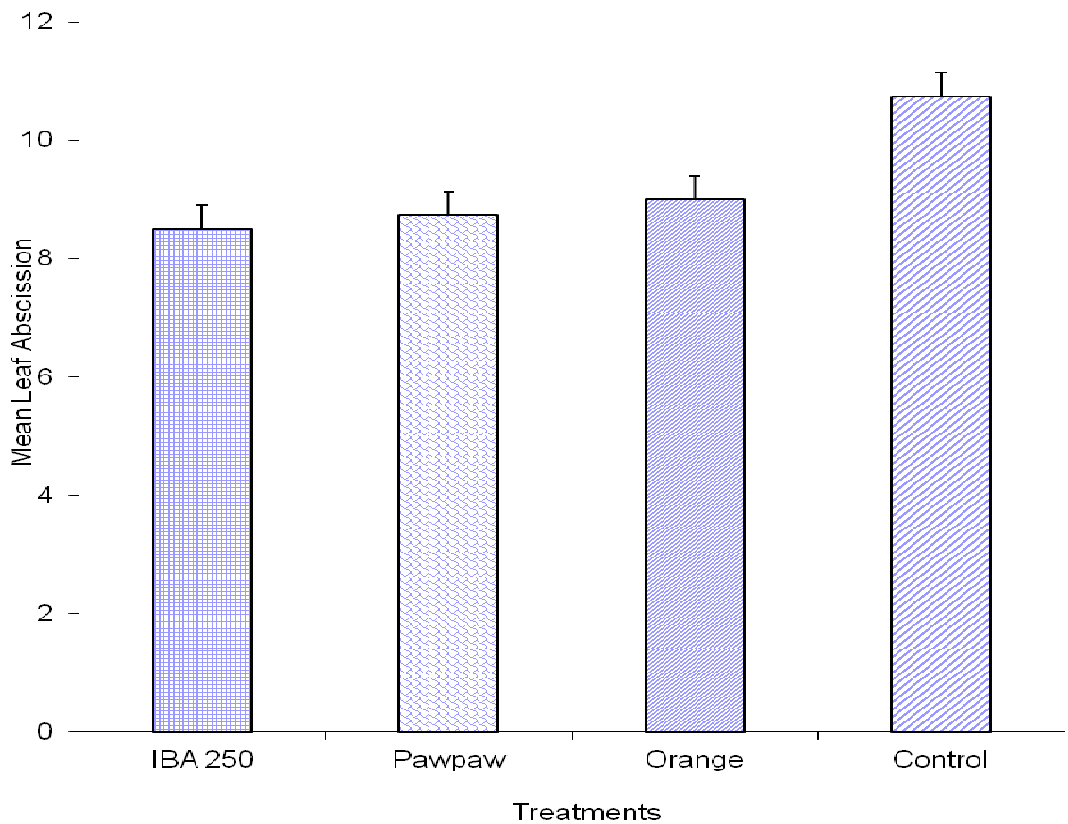

Fig. 1: Mean Effect of Treatments on Leaf Abscission of *Dialium guineense* cuttings.

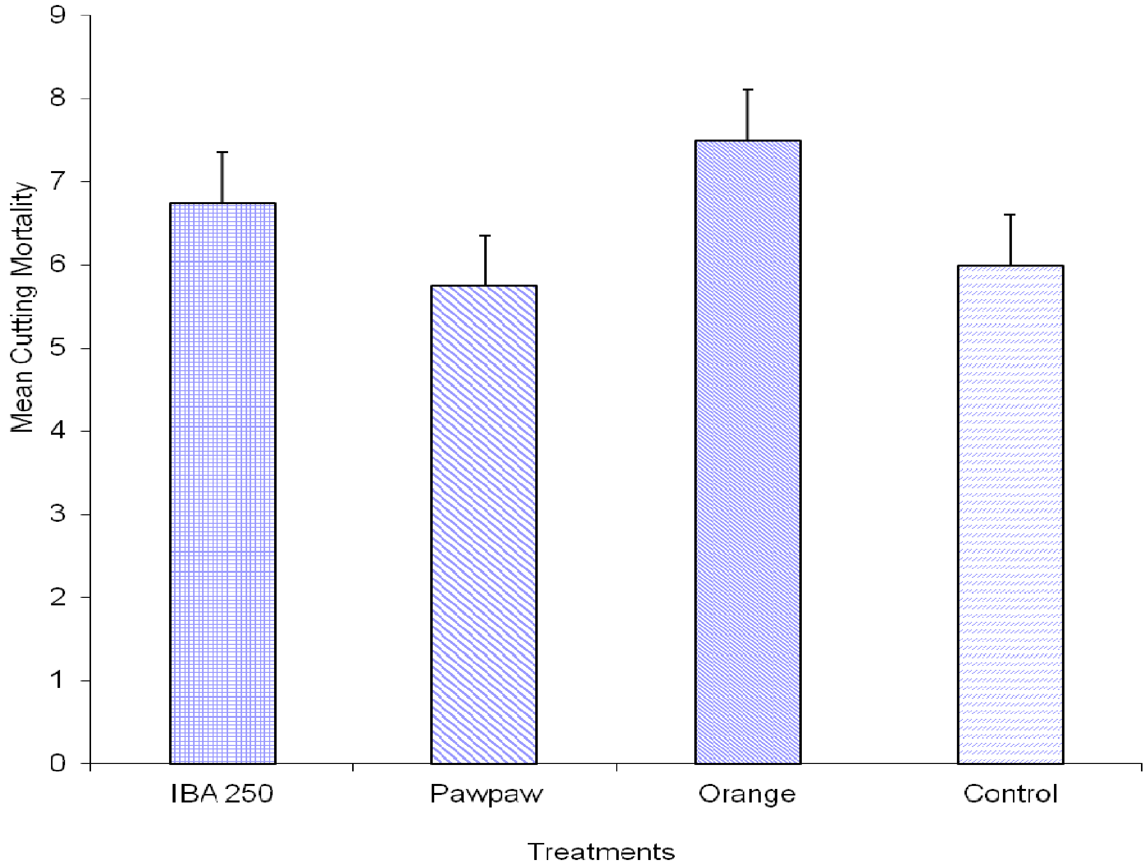

Fig. 2: Mean Effect of Treatments on Cutting Mortality of *Dialium guineense* cuttings.

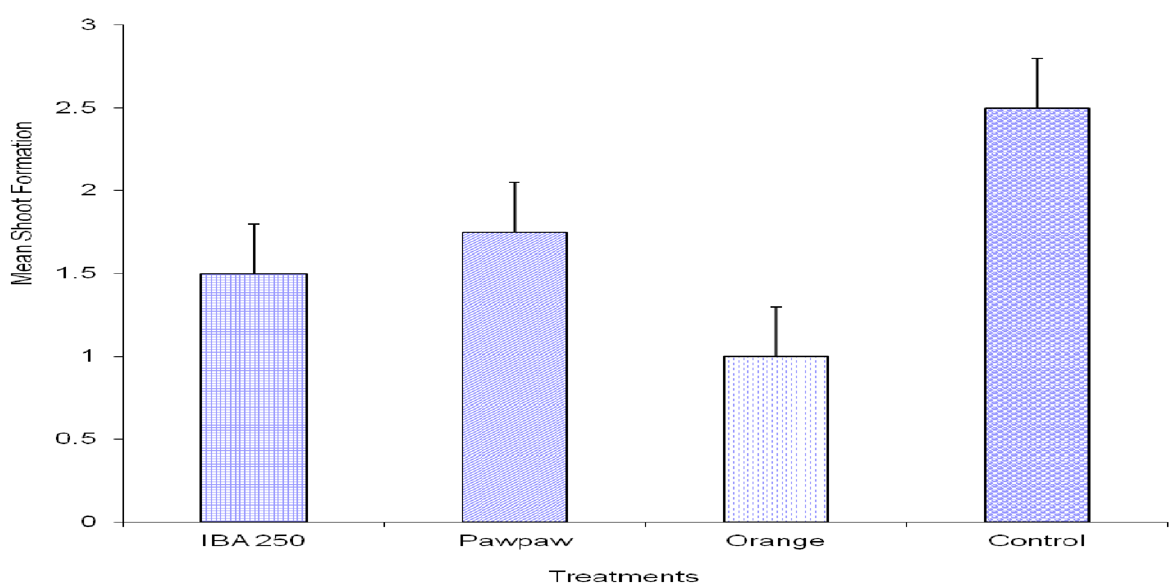

Fig. 3: Mean Effect of Treatments on Shoot Formation of *Dialium guineense* cuttings.

Table 2. Duncan Multiple Range Test of Shoot Formation, Number of Root, Root Formation and Root Length of *Dialium guineense* Subjected to Four Treatments.

Variable	Planting Media	Mean	± SE	DMRT Rating
	Orange	1.00	0.70	D
Shoot Formation	IBA 250	1.50	0.50	C
	Pawpaw	1.75	0.83	B
	Control	2.50	1.32	A
	Orange	3.00	2.38	C
	IBA 250	3.75	1.93	C
Number of Root	Pawpaw	10.25	5.50	A
	Control	5.75	3.14	B
	Orange	1.25	0.75	B
	IBA 250	0.75	0.25	C
Root Formation	Pawpaw	2.75	0.62	A
	Control	1.50	0.64	B
	Orange	2.95	1.92	C
Root Length (cm)	IBA 250	4.02	1.04	B
	Pawpaw	7.30	2.69	A
	Control	2.15	1.04	C

Means with the same alphabet are not significantly different from each other at 0.05 level of probability for the four media treatments separately

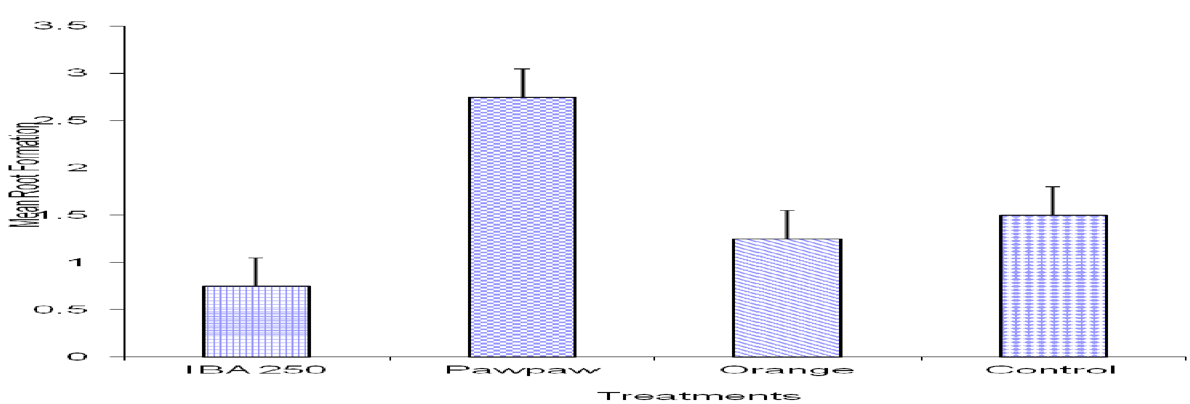

Fig. 4: Mean Effect of Treatments on Root Formation of *Dialium guineense* cuttings.

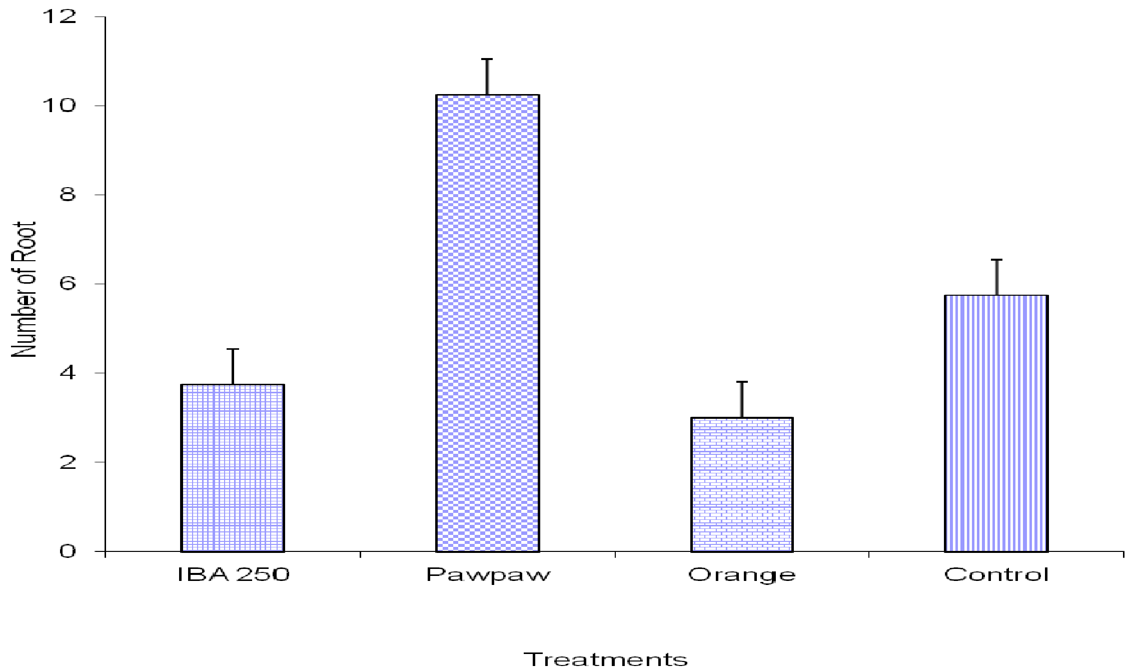

Fig. 5: Mean Effect of Treatments on Number of Root of *Dialium guineense* cuttings.

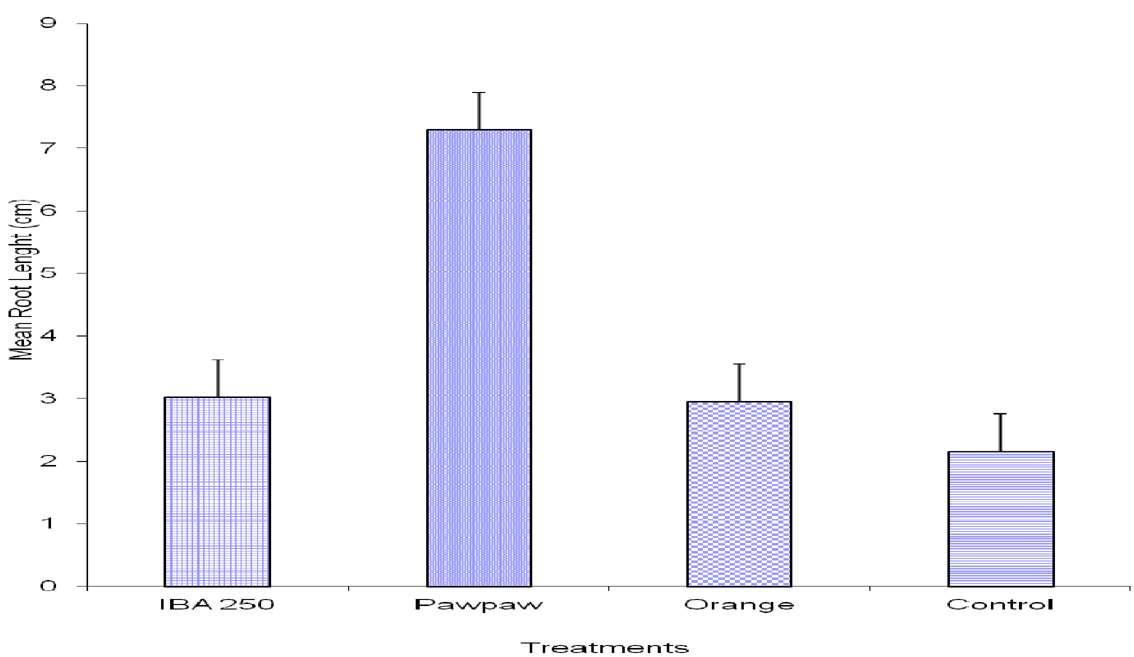

Fig. 6: Mean Effect of Treatments on Root Length of *Dialium guineense* cuttings.

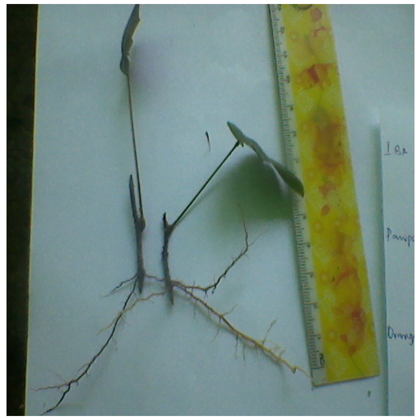

Plate 2a: Root Length of *D. guineense* cuttings formed at the end of the experiment using IBA.

Plate 2b: Root Length of *D. guineense* cuttings formed at the end of the experiment using Orange Seed Extract

Plate 2c: Root Length of *D. guineense* cuttings formed at the end of the experiment using Pawpaw Seed Extract.

Discussion

Results of this study have revealed that *D. guineense* can be successfully propagated by leafy stem cuttings in a mist propagation system, which suggests that vegetative multiplication of this species is feasible. The results clearly established the potentials of pawpaw seed extracts in enhancing the rooting of leafy stem cuttings of *D. guineense*. The results also indicated that pawpaw seed extracts could replace synthetic indole-3-butyric acid (IBA) in enhancing the rooting of the stem cutting of the species for multiplication. The significantly higher rooting percentage in pawpaw seed extracts compared to the other treatments may be due to the ability of plants to store Indole-3-Acetic Acid (IAA) in the form of conjugates and IBA in their seeds which can provide free IAA upon hydrolysis or β-oxidation respectively (Zolman *et al*., 2000).

The fact that orange extracts did not do well may not be strange. According to Sztein *et al*., (1999), among divergent plants, endogenous auxin levels are quite variable. Furthermore, different plant species have distinct IAA conjugate profiles (Cohen and Bandurski, 1982; Slovin *et al*., 1999), cited by Woodward and Bartel (2005). According to Normanly *et al*. (1993) and Tam *et al*. (2000), cited by Woodward and Bartel (2005), in experiments using alkaline hydrolysis to release free IAA from conjugates revealed that Arabidopsis maintains approximately 90% of IAA in amide linkages, 9% as ester-linked conjugates and 1% as free IAA. Ljung *et al*. (2002) stated that most of the amide-linked conjugates in Arabidopsis seeds are solvent insoluble, pointing out that a certain IAA-peptide are present; the large size of this conjugate may contribute to the solvent insolubility of amide conjugates. This may lead one to suggest that even when auxins are present in high levels, it may not available to the cuttings.

Another reason for the poor performance of orange seed extracts may be due to the oily nature of the extracts which may have created a film around the base of the cuttings thereby cutting off access to much needed oxygen for respiration. The profound influence of root formation, number of roots and root length by pawpaw seed extracts agrees with Middleton *et al*. (1980), who attributed it to the effect of auxins mobilization of carbohydrates and subsequent transfer to the root zone. This suggests that the application of pawpaw seed extracts to the base of the cuttings of the species could enhance the number of roots produced. The inability of orange seed extracts to influence the root formation, number of roots produced and length of roots produced may not be unconnected with the reasons adduced earlier.

The result also showed that the pronounced effect of treatment on the shoot formation was influenced by the control. The reason for this effect is not clear. Future studies focusing on increasing the seed extract concentrations could reveal the potentials of pawpaw seed extract in enhancing the formation of shoots. Although no pronounced effect of seed extracts and IBA on leaf abscission and cutting mortality was obtained, there is an indication from the trend in the results that pawpaw seed extracts may have the potentials to lower leaf abscission and cutting mortality, while enhancing the number of cuttings forming new shoots, inspite of the lack significant effect of treatments recorded.

Conclusion and Recommendation

The results from the experiment revealed that *D. guineense* can be successfully propagated by leafy stem cuttings in a mist propagation system, which suggests that vegetative multiplication of this species is feasible. The present study has also demonstrated that pawpaw seed extract can enhance the rooting of the leafy stem cuttings of *D. guineense* for mass clonal propagation and could therefore replace the expensive synthetic IBA in enhancing the rooting of the stem cuttings of the species and it is therefore recommended.

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