

EFFECT OF SOME COMBINATION OF PHYTOHORMONES ON SOME GROWTH PARAMETERS AND VITAMIN C, CARBOHYDRATE, PROTEIN AND CHLOROPHYLL CONTENTS OF *SPONDIAS MOMBIN* (LINN) SEEDLINGS .

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(Received: ; Accepted)

ABSTRACT

The study was carried out to investigate the responses of *Spondias mombin*(Linn.) seedlings to combined phytohormonal treatments. The plant vegetable parameters, mineral constituents, water status, vitamin C, chlorophyll and biochemical contents of *S.mombin* were determined using standard methods. The combined hormonal treatments used for this experiment comprised of 100mg/L GA₃ + 15% coconut water, 100mg/L GA₃ + 50 mg/L NAA, 50mg/L NAA + 15% coconut water and 50mg/L ABA + 100mg/L Quinine. A total of five foliar applications were made at four weeks intervals commencing from four weeks after planting. The control seedlings were sprayed with distilled water. 100 mg/L GA₃ + 15% coconut water was significantly different from the control at 22 weeks after planting with the highest values of plant height (75.60 cm), number of leaves (73.00), shoot-root ratio (5.56), leaf relative water (70.60), vitamin c (37.64 mg/100 g), carbohydrate (13.87%) and Chlorophyll content (2.00 mg/g) while both 100mg/L GA₃ + 15% coconut water and 50mg/L NAA + 15% coconut water recorded protein content of 6.56%. Also 100 mg/L GA₃ + 50 mg/L NAA elicited highest value of stem circumference (2.05 cm). Sodium, calcium, potassium, and phosphorus levels were highest in the leaves of *S. mombin* seedlings treated with 100 mg/L GA₃ + 15% coconut water whereas magnesium level was greater in 100 mg/L GA₃ + 50 mg/L NAA. The study revealed that 100mg/L GA₃ + 15% coconut water has the potential to increase growth and nutritional value of *S. mombin*(Linn.) so that *S. mombin* leaves can be potential source of highly nutritious feed stuff and phytomedicine.

Key words: *Spondias mombin*, seedling, phytohormone.

INTRODUCTION

Spondias mombin(Linn.) is a tree with habitat in Nigeria, Brazil and several other tropical forests of the world. This plant is common in South West of Nigeria. *S. mombin* commonly called Hog plum is of the plant family Anacardiaceae. In Nigeria, it is 'iyeye' for the fruit and 'akika' for the tree in Yoruba; oheeghe (the fruit) in Edo; nsukakara in Efik; tsadar masar in Hausa; ijikara, ogo, ngwu or ungwu in Igbo; aginiran in Ijaw; kakka in Tiv. *Spondias mombin* measures up to 20 metres tall. It grows in the rainforest and in the coastal area. The fruit is like the temperate plum, 3.7 centimetres long, ovoid, one-seeded, yellow-skinned when ripe. The fruit is commonly sold in local markets. Young leaves are cooked as greens and excessive indulgence in the fruit is said to cause dysentery (Ayoka, *et al*, 2008). This tree crop has not been purposely cultivated as other food or cash crops, but is usually protected when found growing in a farmland. Indeed, *S. mombin* is widely relied on for various herbal remedies for numerous conditions and virtually every part of the tree is used from its

thick corky bark to its leaves, fruits, roots, to even its flowers (Moronkola, *et al*, 2003). This plant has been traditionally noted for its medicinal and food values (Ayoka, *et al*, 2006). The current awareness of the potentials of the species has increased the demand for its seedlings (Moronkola, *et al*, 2003; Ademola, *et al*, 2005). In an attempt to develop improved techniques for mass production of uniform and vigorously growing seedlings, seeds are usually pretreated with hormones. Phytohormones play an essential role in regulating plant growth and development. Cytokinnins have been implicated in the control of many developmental processes and environmental responses of plants, including leaf senescence, apical dominance, chloroplast development and regulation of cell division (Hutchison and Kieber, 2002). Abscisic acid (ABA) regulates various aspects of plant growth and development, including seed maturation and dormancy, as well as adaptation to abiotic environmental stresses (Beaudoin *et al*, 2000). Auxins are primary regulators of plant form while

gibberellins and brassinosteroids stimulate elongation (Dugardeyn *et al.*, 2008). Gibberellic acid has been used to stimulate stem and petiole extension in rhubarb, celery and water cress (Thomas, 1976). Treatment of radish and onion seeds with auxin or a mixture of gibberellic acid (GA₃) and kinetin have been found to increase the germination of the seeds (Thomas 1976). Application of gibberellic acid, 4-chloroindole and 6-benzyl amino purine (BAP) on to the standard petal and calyx of *Vicia faba* var. major was found to significantly enhance pod set (Rylott and Smith, 1990). Likewise, spraying of *Vicia faba* cv. Troy reproductive structure with indole-3-acetic acid, gibberellic acid or 6-benzyl aminopurine (BAP) resulted in increased pod number (Clifford *et al.*, 1992). Others are Ethylene and compounds with hormone-like properties such as thiourea, phenols, alkaloids e.g. caffeine and Quinine (Jones, 1973; Khan, 1980; Agboola and Adedire, 1998; Fasidi *et al.*, 2000; Ebofin *et al.*, 2003). This study was carried out to determine the combined effects of some phytohormones on the growth of the seedlings of *Spondias mombin* (Linn.).

MATERIALS AND METHODS

Fruit Collection and Pretreatment

Ripe (yellow) fruits of *Spondias mombin* (Linn.) (Plate 1) were collected from a farmland, besides the proposed secretariat site of Senior Staff Association of Nigerian Universities (SSANU) at the University of Agriculture, Abeokuta, Ogun State, Nigeria (7°N 3°E). Fruits were pretreated for dormancy release according to the method of Agboola (2002). This involved open fermentation of the seeds for 7 days due to its tough and corky endocarp which together served as the planting material (Agboola, 2002). Fermented fruits were later washed thoroughly in running water, the seeds extracted and sundried for 5 days and later used for studies on hormonal effects on seedlings of *S. mombin* raised from them.

Soil

The soil sample used for raising the seedlings was collected from a *Teak* and *Gmelina* plantation along the main gate of the University of Agriculture, Abeokuta, Ogun State. The soil from the *Teak* and *Gmelina* plantation represented "fallow soil".

Experimental Site and Seedling Raising

The seedlings of *S. mombin* were raised from treated seeds using perforated black polythene bag of 37cm by 31cm, under shade afforded by crowns of tall trees at the forest nursery site along the main gate of the University of Agriculture, Abeokuta, Ogun State. Seedlings were thinned to three per polythene bag after emergence (Plate 2). Ten polythene bags were used for each of the combined phytohormonal treatment and replicated five times for both the control and the treated seedlings.

Phytohormonal Treatments and Seedling Growth

The combined hormonal treatments used for this experiment were 100mg/L GA₃ + 15% coconut water, 100mg/L GA₃ + 50mg/L NAA, 50mg/L NAA + 15% coconut water and 50mg/L ABA + 100mg/L Quinine. The seedlings of *S. mombin* were sprayed with 500ml of each of the combined hormonal treatments until the leaf surfaces of the seedlings were properly wet and dripping using a hand "Harry Brand" sprayer at 4 weeks intervals for 22 weeks, commencing from 4 weeks after planting (WAP) while the control seedlings were also sprayed with 500ml of distilled water. The treated seedlings were harvested at 22 WAP for dry weight measurement of root shoot, leaf relative water content, plant height, stem circumference and number of leaves. Plant height was measured with a ruler from the soil level to the tip of the apical bud. The stem circumference was measured according to Kadiri (1999) using a thread and a metre rule after which the mean per treatment was recorded. Vitamin C was carried out using the indophenol method described by Association Official Analytical Chemists (1994). The leaves of the treated seedlings and controls were harvested at 22 weeks after sowing, dried in an oven at 80°C for 48 hrs, ground into powder and the powder was used for carbohydrate, protein and mineral elements except for chlorophyll contents and vitamin C in which fresh leaves were used. Total carbohydrate was analysed using the anthrone method of Southgate (1969) following extraction for 4 hrs with 250ml of 1% sulphuric acid, protein was determined using the micro kjeldahl method, Chlorophyll content was determined using the method of Witham *et al.* (1971) and mineral elements were determined using atomic phosphovanado-molybdate reaction according to I.I.T.A manual 1979. The experiment was a

Completely Randomized Design. The data were subjected to ANOVA and separation of means using Duncan Multiple Range Test (DRMT) at $\alpha=0.05$.

RESULTS

The mean plant height, stem circumference and number of leaves of all the treated seedlings were significantly different from the control except 50mg/L ABA + 100mg/L quinine (Table 1). 100mg/L GA₃ + 15% coconut water gave the highest value of 75.60cm and 73.00cm for plant height and number of leaves respectively at 22WAP while the highest value of 2.14cm for stem circumference was observed in seedlings given treatment of 50mg/L NAA + 15% Coconut water (2.14cm; Table 1). The highest value of shoot root ratio (5.56) and leaf relative water content (70.60) at 22WAP were observed in seedlings treated with combined hormonal treatments of 100mg/L GA₃ + 15% coconut water (Table 2). It was observed that 100mg/L GA₃ + 15% coconut water was significantly different from the controls for both shoot root ratio and leaf relative water content at 22WAP. The highest values of vitamins C (37.64

mg/100g), carbohydrate (13.87%) and chlorophyll content (2.00 mg/g) in combined hormonal treatments at 22WAP were observed in seedlings treated with 100mg/L GA₃ + 15% coconut water whereas the highest protein content (6.56%) was observed in seedlings treated with both 100mg/L GA₃ + 15% coconut water and 50mg/L NAA + 15% coconut water. All the combined treatments were significantly different from the controls at 22WAP (Table 3). Similarly, the highest values of sodium (0.113mg/g), calcium (0.184mg/g), potassium (1.361mg/g) and phosphorus (0.218mg/g) were induced by 100mg/L GA₃ + 15% coconut water in the combined hormonal treatments at 22WAP while magnesium (0.273mg/g) was induced by 100mg/L GA₃ + 50mg/L NAA.(Table 4). Furthermore, mineral contents in 100 mg/L GA₃ + 15% coconut water; 100 mg/L GA₃ + 50 mg/L NAA and 50 mg/L NAA + 15% coconut water were significantly different from their controls while those in 50 mg/L ABA+100 mg/L quinine were not significantly different from their controls (Table 4).

Table 1: Effect of Combined Hormonal Treatments on the Vegetative Growth of *S mombin* Seedlings at 22weeks after Sowing.

HORMONAL TREATMENT	Plant height (cm) Mean \pm S.E.	Stem circumference (cm) Mean \pm S.E.	Number of leaves Mean \pm S.E.
100 mg/L GA ₃ + 15% Coconut water	75.60 \pm 0.71 ^a	1.95 \pm 0.41 ^a	73.00 \pm 0.03 ^a
100 mg/L GA ₃ + 50mg/ L NAA	69.06 \pm 1.35 ^a	2.05 \pm 0.04 ^a	64.80 \pm 1.02 ^b
50mg/ L NAA + 15% Coconut water	72.31 \pm 1.24 ^a	2.14 \pm 0.52 ^a	71.00 \pm 1.05 ^a
50 mg/L ABA + 100 mg/L Quinine	61.27 \pm 0.38 ^b	1.62 \pm 0.16 ^b	56.40 \pm 0.07 ^c
Distilled water (control)	58.15 \pm 0.14 ^b	1.61 \pm 0.03 ^b	55.10 \pm 1.03 ^c

Mean followed by the same letters on the same columns are not significantly different according to Duncan's Multiple Range Test at 5% probability. Means are of 5 replicates. GA₃ = GIBBERELLIC ACID; NAA = NAPHTHHALENE ACETIC ACID AND ABA = ABSCISIC ACID.

Table 2: Effect of Combined Hormonal Treatments on the Shoot/Root Ratio and Leaf Relative Water

HORMONAL TREATMENT	Mean shoot/root ratio (mean \pm S.E.)	Mean leaf relative water content (Mean \pm S.E.)
100 mg/L GA ₃ + 15% Coconut water	5.56 \pm 0.34 ^a	70.60 \pm 0.12 ^a
100 mg/L GA ₃ + 50mg/ L NAA	2.63 \pm 0.61 ^c	64.30 \pm 0.02 ^a
50mg/ L NAA + 15% Coconut water	3.32 \pm 0.08 ^b	64.60 \pm 0.37 ^a
50 mg/L ABA + 100 mg/L Quinine	1.13 \pm 1.43 ^d	54.60 \pm 0.85 ^b
Distilled water (control)	0.68 \pm 0.32 ^d	47.60 \pm 1.07 ^b

Mean followed by the same letters on the same columns are not significantly different according to Duncan's Multiple Range Test at 5% probability. Means are of 5 replicates. Ga₃ = GIBBERELLIC ACID; NAA = NAPHTHHALENE ACETIC ACID AND ABA = ABSCISIC ACID

Table 3: Effect of Combined Hormonal Treatments on the Vitamin C, Carbohydrate, Protein and Chlorophyll Contents of *S mombin* Seedling Leaves at 22weeks after Sowing.

HORMONAL TREATMENTS	Vitamin C (mg/100g) (Mean \pm S.E.)	Carbohydrate (%) (Mean \pm S.E.)	Protein (%) (Mean \pm S.E.)	Chlorophyll (mg/g) (Mean \pm S.E.)
100 mg/L GA ₃ + 15% Coconut water	37.64 \pm 0.20 ^a	13.87 \pm 0.11 ^a	6.56 \pm 0.16 ^a	2.00 \pm 0.06 ^a
100 mg/L GA ₃ + 50mg/ L NAA Coconut water	30.93 \pm 0.70 ^b	10.03 \pm 0.45 ^c	6.55 \pm 0.04 ^a	1.69 \pm 0.11 ^b
50mg/ L NAA + 15% Coconut water	33.14 \pm 0.10 ^b	12.45 \pm 0.29 ^b	6.56 \pm 0.35 ^a	1.78 \pm 0.27 ^b
50 mg/L ABA + 100 mg/L Quinine	29.72 \pm 0.30 ^b	6.37 \pm 0.13 ^d	5.74 \pm 0.13 ^b	1.31 \pm 0.05 ^c
Distilled water (control)	25.38 \pm 0.50 ^c	2.53 \pm 0.05 ^c	4.13 \pm 0.05 ^c	1.10 \pm 0.02 ^d

Mean followed by the same letters on the same columns are not significantly different according to Duncan's Multiple Range Test at 5% probability. Means are of 5 replicates Ga₃ = GIBBERELIC ACID; NAA = NAPHTHALENE ACETIC ACID AND ABA = ABSICISIC ACID.

Table 4: Effect of Combined Hormonal Treatments on the Mineral Elements Contents of *S mombin* Seedling Leaves at 22weeks after Sowing.

HORMONAL TREATMENTS	SODIUM	CALCIUM	POTASSIUM	PHOSPHORUS	MAGNESIUM
100 mg/L GA ₃ + 15% Coconut water	0.113 \pm 0.03 ^a	0.184 \pm 0.61 ^a	1.361 \pm 0.01 ^a	0.218 \pm 0.16 ^a	0.266 \pm 0.23 ^a
100 mg/L GA ₃ + 50mg/ L NAA Coconut water	0.096 \pm 0.11 ^b	0.142 \pm 0.17 ^b	0.845 \pm 0.38 ^c	0.126 \pm 0.32 ^b	0.273 \pm 0.10 ^a
50mg/ L NAA + 15% Coconut water	0.092 \pm 0.54 ^{bc}	0.177 \pm 0.02 ^a	1.090 \pm 0.22 ^b	0.148 \pm 0.40 ^b	0.219 \pm 0.31 ^b
50 mg/L ABA + 100 mg/L Quinine	0.084 \pm 0.32 ^{cd}	0.101 \pm 0.14 ^c	0.643 \pm 0.25 ^d	0.069 \pm 0.01 ^c	0.187 \pm 0.50 ^c
Distilled water (control)	0.075 \pm 0.14 ^d	0.093 \pm 0.05 ^c	0.621 \pm 0.07 ^d	0.076 \pm 0.05 ^c	0.188 \pm 0.30 ^c

**Plate 1:** Ripe fruits of *Spondias mombin* plant before extraction of seed.**Plate 2:** One month old seedlings of *Spondias mombin*

DISCUSSION

Present findings indicate that there were significant differences in plant height, stem circumference and number of leaves for all the seedlings of *S. mombin* grown under control and those treated with hormonal combination, at 22WAP except for 50 mg/L ABA + 100 mg/L quinine. These results conform with those of Geekiyanage *et al.* (2006). Alamu and McDavid (1979), observed in their studies with tannia (*Xanthosoma sagittifolium*) that application of GA₃, auxin and cytokinins increased the number of leaves of the plant by promoting the development of axillary leaf systems. An increase in stem circumference was likewise observed by Kadiri (1991) in his studies with *Abelmoschus esculentus* and *Lycopersicum esculentus* treated with various concentrations of GA₃ and 2, 4-D. The role of GA₃ has been highlighted in both somatic embryogenesis and organogenesis resulting in cell division and elongation (Al-Khayri *et al.*, 1992; Komai *et al.*, 1996; Molvig and Rose, 1994 and Geekiyanage *et al.*, 2006). Ebofin *et al.* (2004) similarly recorded enhancement in leaf number and plant height in *Prosopis africana* and *Albizia lebbbeck*. This might explain why GA₃ singly and in combination with other hormones increased the stem circumference and plant height. The highest shoot-root ratio and leaf relative water content recorded at 22WAP in *S. mombin* seedlings treated with 100 mg/L GA₃ + 15% coconut water treatments was similar to the result obtained by Mukhtar, 1993.

Akhtar *et al.*, (2008) explained that increases in shoot-root ratio by hormone treatments are due to the fact that they enhance the stem elongation of plants. In addition, cytokinins such as those contained in coconut water, 6-benzylaminopurine (BAP) also facilitate cell division and sprouting (Pan, 2001). Combined hormone treatments in this study stimulated significant differences in the vitamin C contents, carbohydrate contents, chlorophyll contents and protein contents of the seedlings of *S. mombin* at 22 WAP compared with the controls except for 50 mg/L ABA + 100 mg/L quinine. Similar result was reported for *Hibiscus sabdariffa* by Mukhtar (2008). The sodium, calcium, potassium and phosphorus contents of the seedlings treated with combined hormone treatments of 100 mg/L GA₃ + 15% coconut water were significantly different from the controls. This agreed with Njoku and Akumefula (2007); Okwu and Nnamdi (2008). 100 mg/L GA₃

+ 50 mg/L NAA produced the highest magnesium contents at 22WAP. This value of magnesium was lower in *Raphia bookeri* (Okwu and Nnamdi, 2008).

It is worthy of note that magnesium, sodium, phosphorus, calcium and potassium are present in leaves of combined hormonal treated seedling of *S. mombin* at 22 weeks after planting. The combination of these elements together with fluoride may have therapeutic, protective and preventive roles in teeth (Olabanji *et al.*, 1996; Okwu and Ekeke, 2003). It could be inferred from this study that combined hormone treatment of 100mg/L GA₃ + 15% coconut water elicited the highest growth in seedlings of *S. mombin*.

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