

Effect of Some Growth Hormones on Seed Germination and Seedling Growth of *Tetrapleura tetraptera* (Thaub)

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Abstract Timber and non-timber tree species with medicinal properties often suffer from extensive exploitation. In order not to allow these species to go into extinction there is a need for rapid replacement. The study assessed the effect of plant growth hormones viz: indole acetic acid (IAA), indole butyric acid (IBA), naphthalene acetic acid (NAA) and gibberellic acid (GA₃), on seed germination and seedling growth of *Tetrapleura tetraptera* (Thaub). Seeds were collected from Akungba Akoko, Western Nigeria (Lat. 7° 27'N and 5° 44'E). Nine hundred and forty-five seeds (945) of *T. tetraptera* were sown in germination trays containing washed-sterilized river sand in the screen house. Before sowing, seeds were treated with different plant growth hormones (IAA, IBA, NAA and GA₃) at different rates (0.005g/ml, 0.01g/ml, 0.015g/ml, 0.02g/ml and 0.03g/ml). Germination count was taken daily and seedlings were later transferred to open nursery at two leaf stage and watered daily. Growth parameters such as height of the plant, collar diameter and number of leaves were taken fortnightly. The effect of different hormone concentrations were highly significant ($P \leq 0.05$) on mean height growth of *T. tetraptera* seedlings, seedlings collar diameter and number of leaves. Different growth hormone concentration had no significant ($P \geq 0.05$) effect on leaf dry weight, stem dry weight, root dry weight and total dry weight. However, IAA, IBA and NAA at low concentrations enhanced the growth of *T. tetraptera* seedlings. In conclusion, IAA, IBA and NAA could be used for rapid regeneration of *T. tetraptera* in natural forest and GA₃ should be excluded in the pre-treatment procedure of *T. tetraptera* prior sowing.

Keywords Growth hormones, *Tetrapleura tetraptera*, Germination percentage, Growth parameters

1. Introduction

The urgent need to conserve the tropical rainforest cannot be overemphasized. The tropical rainforest has been identified as the most biologically diverse terrestrial ecosystem on earth ([1];[2]). According to [3] a typical tropical rainforest is a complex community whose framework is provided by tree of many sizes, and this has subjected the tropical rainforest to severe exploitation for their timber and economically valuable non-timber resources which has consequently put several species of plants in a great danger of extinction.

The tropical rainforest ecosystem is a major source of livelihoods of millions of people from time immemorial. This is because many of the plant species in the tropical rainforests produce a variety of highly valuable non-timber products like edible and medicinal fruits, seeds, nuts and oils. A considerable number of trees like *Tetrapleura tetraptera* have the traditional usage in the management and control of adult-onset type 2 Diabetes mellitus; and based on the

studies using the dry fruit of this plant, it has been suggested that the plant should be used in formulating drugs[4].

The timber trees producing economically valuable non-timber products have been christened 'timber plus tree'[5]. Evaluating from a long-term standpoint, non-timber products from those trees are more valuable than their timber as the former can be harvested for many years without cutting down the trees, in contrast to timber harvesting and their harvesting activity or extraction has imperceptible perturbation on the ecosystem. *Tetrapleura tetraptera* is a deciduous tree generally found in the lowland rainforest of tropical Africa. Its distribution extends over large parts of tropical Africa, especially West and East Africa[6]. It is commonly known as Aridan in Nigeria. It belongs to the family *fabaceae* – *mimosoideae* which is widely distributed across Nigeria and Africa. It is widely used in Africa traditional medicine for the management and control of an array of human ailments[7].

Tetrapleura tetraptera is one of the most valued forest species in Nigeria that is facing ecological threat of extinction caused by deforestation as a result of timber and non-timber values. Ecological consequences of deforestation have been reported by many authors, ([8];[9];[10],[11]). It has led to the extinction of much wildlife and destruction of some plant species which would have helped in restoring the

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ecological balance of the ecosystem. However, in order to mitigate the human influence on the biological structure of the forest, there is a need for regeneration of the plant species. Seeds are best means of regeneration and this could be achieved through several pretreatments such as acids, distilled water and hormonal preparations at different low concentrations to induce the seed germination and seedling growth/ development. Plant hormones are chemicals that regulate plant growth, which, in the United Kingdom, are referred to as 'plant growth substances'[12]. Plant hormones are not nutrients, rather they are chemicals that in small amounts promote and influence the growth, and development of plant cells and tissues[12]. Examples of hormones used include Indole acetic acid (IAA), indole butyric acid (IBA), naphthalene acetic acid (NAA) and gibberellic acid (GA₃).

The fruits and seeds of *T. tetraptera* add good aroma and flavor to food, thereby increasing the pleasure of food consumption. In addition, the species is highly medicinal. It is used to cure fever and as an enema for constipation in many parts of Nigeria. Due to the medicinal values, problem of regeneration and over-exploitation of the species alongside the use of forest for agricultural purpose; industrialization or urbanization, existing stands of the species are mainly in the wild and traditional agro-forestry system. There are no known large scale plantations of the species anywhere in Nigeria. Anthropogenic activities have had great effects on the ecosystem over the years as a result of the use of forest plant species for several purposes. Many of these species are difficult to regenerate from seeds as a result of dormancy problem. Before a meaningful regeneration of such plant species could be accomplished, such seeds must be pretreated to hasten germination and seedling establishment. It is good to state at this point that the germination of seed and large scale production of *T.tetraptera* seedlings have been retarded without any pretreatment in previous study.

Therefore this study was carried out to determine the effect of different growth hormones on the seed germination and seedling growth of *Tetrapleura tetraptera* for subsequent recommendation in agroforestry and to enhance large scale propagation of the species for plantation establishment.

2. Materials and Methods

Matured seeds of *T. tetraptera* used for this study were collected from Akungba Akoko located on latitude 7° 27' N and longitude 5° 44' E. The seedlings used for this study were raised from matured seeds collected for this study were raised from matured seeds collected from the source above. The study was conducted in the screen house of the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba Akoko, located in the northern part of Ondo State, Nigeria on latitude 7° 27' 3" N and longitude 5° 44' E with elevation of 336 to 390m (Relief 54m). The climate is of the West Africa monsoon type with dry and

wet seasons. The dry season starts from November through March and is characterized by dry cold wind of harmattan. The rainy season normally starts from the month of April to October. The mean annual rainfall is approximately 12mm, while the mean average temperature is about 26°C.

The site topography is not plain as a result of presence of hills and mountains of rocks. The site has a sparse type of vegetation which varies from one area to another. A total of nine hundred and forty five (945) seeds were pretreated for dormancy release according to the method of [13]. The seeds were soaked in concentrated sulphuric acid for 2 minutes with continuous stirring due to problem of hard seed coats. Pretreated seeds were air-dried for 2 days and later used for studies on hormonal effects on seed germination and seedling raised. Pretreated seeds were divided into Twenty-one (21) lots, each of Forty-five (45) seeds and soaked in five concentrations at 0.005, 0.01, 0.015, 0.02 and 0.03g/ml of IAA, IBA, NAA and GA, for 24 hrs. Five replicate samples of Forty-five (45) seeds each were then prepared for germination in washed river sand. Water-treated seeds served as control. The experiment was a Complete Randomized Block Design (CRBD) of 5 concentrations x 4 hormones x 1 tree species.

A total of Nine hundred and Forty-five seed and Twenty-one germination trays were used in all for the seedling production. Sowing of the seeds took place at the Department of Plant Science and Biology screen house under controlled environmental and growth conditions. During the period of the seedlings production, the planted seeds were watered daily and cumulative germination count was equally taken daily.

Five hundred and thirteen (513) healthy and uniformly growing seedlings were transplanted into small sized polythene bags filled with top soil. The transplanted seedlings were allowed to acclimatize under shade for two weeks before taking them to the open nursery where they were also subjected to daily watering. Immediately after the seedlings were transferred to the open nursery, the following metric characteristics were taken: Height of the plant, collar diameter and number of leaves. These growth parameters were fortnightly throughout the period of the study.

The seedlings heights were measured from the collar to the tip of the apical bud using a ruler calibrated in centimeters and the data obtained were recorded. The seedlings stem diameters at the collar were measured and recorded using a Vernier Caliper. The number of leaves on each seedling were counted and recorded. Biomass assessments of the seedlings were carried out at the end of the experimental period of 3 months. Five seedlings were randomly selected from different treatments and control fortnightly for the assessment of biomass production. These seedlings were uprooted by placing them in a wide/flat spacious bowl containing water and the polythene bags were carefully cut to wash off the soil around the roots painstakingly. The uprooted seedlings were divided into root, shoot and leaf components. Each component of the seedlings was put into separate envelopes and labeled for ease of

identification. The envelopes containing the parts of the plant were weighed in the laboratory to determine the fresh weights of the samples. The envelopes containing the leaf, root and shoot components of the seedlings were over-dried for 24 hours at 80°C. The samples were allowed to cool to a constant temperature (room temperature) before the dry weight of the samples were measured using an electronic weighing balance for the determination of net carbon gain and productivity of *T. tetraptera*.

3. Results

Seeds under different treatments germinated six days after sowing (DAS) except those treated with GA₃ at 0.005g/ml, 0.015g/ml, 0.02g/ml and 0.03g/ml which germinated seven

DAS data not shown. Among IAA treated seeds of *T. tetraptera*, the highest percentage germination of 73% was recorded in 0.005g/ml treated seeds while the lowest percentage germination of 51% was obtained in seeds treated with 0.03g/ml (Table 1). Among seeds treated with IBA, those treated with 0.005g/ml and 0.015g/ml gave the highest percentage germination of 71% and the lowest percentage germination of 57% was observed in 0.03g/ml (Table 1) and (figure 1). Among NAA treated seeds of *T. tetraptera*, 0.005g/ml gave the highest percentage germination of 66% and the lowest percentage germination of 42% was observed in 0.03g/ml (Table 1). Among GA₃ treated seeds of the species, the highest percentage germination was observed in 0.03g/ml with 48% and 0.015g/ml gave the lowest with 33% compared to the control which gave 60% (Table 1).

Table 1. Effect of different hormones on seed germination percentage of *Tetrapleura tetraptera*

Hormone	Concentration (g/ml)	Percentage germination (%)
IAA	0.005	77
	0.01	55
	0.015	66
	0.02	60
	0.03	51
IBA	0.005	71
	0.01	68
	0.015	71
	0.03	68
	0.03	57
NAA	0.005	66
	0.01	64
	0.015	53
	0.02	41
	0.03	42
GA ₃	0.005	44
	0.01	46
	0.015	33
	0.02	46
	0.03	48
CONTROL	0	60

*Generally, seeds of *T. tetraptera* germinated better at lower hormonal concentration

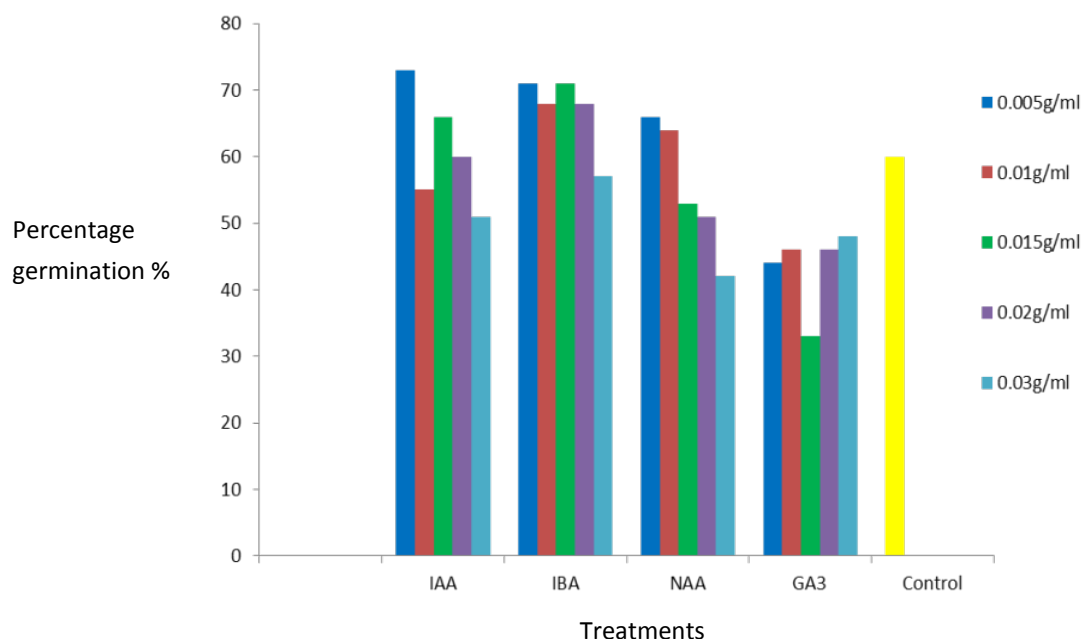


Fig 1: Seeds of *T. tetraptera* treated with IAA at 0.005g/ml had the highest germination percentage of 73%, followed by IBA at 0.005g/ml and 0.015g/ml with 71%. The lowest germination percentage was observed in seeds treated with GA₃ at 0.015g/ml which gave 33%

Figure 1. Cumulative germination percentage of *T. tetraptera* seeds under four different treatments and dosage at day 30 after sowing (30DAS)

Table 2. Mean values of seedlings height, collar diameter and number of leaves in *Tetrapera tetraptera* under different hormone treatments

TREATMENTS Hormone conc. (g/ml)	HEIGHT (cm)	COLLAR DIAMETER (cm)	NUMBER OF LEAVES.
IAA 0.005	7.46±0.14 ^e	2.07±0.04 ^e	21.60±1.07 ^c
0.01	7.23±0.15 ^{de}	1.88±0.04 ^{bc}	19.56±1.08 ^{abc}
0.15	7.19±0.14 ^{de}	1.88±0.04 ^{bc}	19.13±0.97 ^{abc}
0.02	7.63±0.17 ^e	2.03±0.05 ^{de}	20.66±1.13 ^{bc}
IBA 0.005	6.58±0.16 ^{abc}	1.92±0.05 ^{cd}	19.15±1.01 ^{abc}
0.01	7.27±0.15 ^{de}	1.91±0.04 ^{cd}	21.40±1.15 ^e
0.15	6.13±0.15 ^a	1.86±0.03 ^{bc}	21.06±1.17 ^c
0.02	6.29±0.19 ^{ab}	1.67±0.03 ^a	19.60±1.17 ^{abc}
NAA 0.005	6.78±0.14 ^{bcd}	1.92±0.03 ^{cd}	19.31±0.86 ^{abc}
0.01	6.59±0.15 ^{abc}	1.87±0.04 ^{bc}	19.16±0.96 ^{abc}
GA ₃ 0.005	6.12±0.16 ^a	1.80±0.04 ^{bc}	17.31±0.90 ^{ab}
0.01	6.86±0.16 ^{cd}	1.86±0.04 ^{bc}	18.33±1.01 ^{abc}
CONTROL	6.38±0.17 ^{abc}	1.77±0.45 ^{ab}	16.85±0.89 ^a

*abcde: The superscript letters that are the same within each column show that the values are not significantly different from each other by the Duncan Multiple Range test at (P>0.05)

The effects of different hormone concentrations were highly significant (P<0.05) on the mean height growth of *T. tetraptera* seedlings (Table 2). *T. tetraptera* seedlings treated with 0.02g/ml of IAA produced the highest mean height value of 7.63±0.17cm, followed by seedlings supplied with IAA at the rate of 0.005g/ml (7.46±0.14cm). The lowest mean height values of 6.12±0.16cm and 6.13±0.15cm were

recorded among seedlings treated with GA₃ at 0.005g/ml and 0.015g/ml of IBA respectively (Table 2). There were no significant (P<0.05) differences in mean height of seedlings treated with 0.005g/ml of IAA, 0.01g/ml, 0.15g/ml of IAA, 0.02g/ml of IAA and 0.01g/ml of IBA (Table 2). Different hormone concentrations differ significantly (P<0.05) in their effects (Table 2). The highest mean collar diameter value of

2.07±0.04cm was obtained among seedlings of *T. tetraptera* treated with 0.005g/ml of IAA, followed by seedlings that were fed with IAA at 0.02g/ml having the mean diameter value of 2.03±0.05 and 1.67±0.03 at 0.02g/ml of IBA gave the lowest (Table 2). Seedlings supplied with 0.005g/ml of IAA and 0.02g/ml were significantly different ($P<0.05$) from other treatments (Table 2). Different hormone concentrations had no high significant ($P>0.05$) effect on number of leaves production among seedlings of *T. tetraptera* (Table 2). The highest mean values of number of leaves were recorded among *T. tetraptera* seedlings treated with 0.005g/ml of IAA (21.60±1.07), followed by seedlings that had 0.01g/ml of IBA with (21.40±1.15), while the lowest mean number of leaves value of 16.85±0.89 was obtained from seedlings treated with the control (Table 2).

The effect of different concentrations of hormones had no significance ($P>0.05$) on Leaf Dry Weight (LDW) seedlings of *T. tetraptera* (Table 3). Seedlings treated with 0.015g/ml of IBA gave the highest (LDW) of 0.89±0.26g, followed by seedlings supplied with IBA at 0.01g/ml (0.89±0.19g). The lowest (LDW) mean value of 0.36±0.11g was obtained from seedling under 0.005g/ml of GA₃ (Table 3). The different hormone concentrations had no high significant ($P>0.05$) effect on SDW of seedlings of *T. tetraptera* (Table 3). Seedlings supplied with 0.01g/ml of IBA had the highest SDW of 0.32±0.10g, followed by seedlings treated with IAA at 0.005g/ml which gave (0.29±0.05g). The lowest SDW

mean value of 0.11±0.03g and 0.11±0.02g were obtained among seedlings under 0.005g/ml NAA and 0.01g/ml GA₃, respectively (Table 3). There was a significant difference between the SDW mean value of seedlings treated with 0.01g/ml of IBA and seedlings under other treatments (Table 3). The effect of different concentrations of hormone had a significant difference ($P<0.05$) on RDW of *T. tetraptera* seedlings (Table 3). The highest RDW mean values of 0.87±0.11g and 0.72±0.11g were obtained from seedlings treated with IAA at 0.005g/ml and IBA at 0.02g/ml respectively. The lowest RDW mean value of 0.31±0.08g was obtained among seedlings under 0.005g/ml of NAA (Table 3). The mean RDW values among seedlings supplied with 0.005g/ml of NAA, 0.005g/ml of GA₃ and 0.01g/ml of GA₃, were significantly different from other treatments (Table 3). The effect of different concentrations of growth hormone had no significant difference ($P>0.05$) on Total Dry Weight (TDW) of *T. tetraptera* seedlings (Table 3). Seedlings treated with IAA at 0.005g/ml had the highest mean TDW value of 1.97±0.25g, followed by seedlings supplied with IBA at 0.01g/ml (1.88±0.43g). The lowest TDW mean value of 0.82±0.23g was obtained among seedlings under GA₃ at 0.005g/ml (Table 3). The TDW mean values of seedlings treated with 0.005g/ml of NAA, 0.005g/ml of GA₃ and 0.01g/ml of GA₃ were significantly different from other treatments (Table 3).

Table 3. Mean values of seedlings biomass accumulation in *Tetrapleura tetraptera* under different hormone concentrations

TREATMENTS	LEAF DRY WEIGHT (g)	STEM DRY WEIGHT (g)	ROOT DRY WEIGHT (g)	TOTAL DRY WEIGHT (g)	
Hormone conc. (g/ml)					
IAA	0.005	0.81±0.09 ^a	0.29±0.05 ^{ab}	0.87±0.11 ^c	1.97±0.25 ^b
	0.01	0.78±0.16 ^a	0.24±0.07 ^{ab}	0.65±0.12 ^{abc}	1.67±0.35 ^{ab}
	0.015	0.77±0.20 ^a	0.20±0.05 ^{ab}	0.63±0.15 ^{abc}	1.60±0.40 ^{ab}
	0.02	0.74±0.06 ^a	0.19±0.07 ^{ab}	0.69±0.14 ^{bc}	1.62±0.27 ^{ab}
IBA	0.005	0.80±0.25 ^a	0.22±0.08 ^{ab}	0.68±0.16 ^{abc}	1.70±0.49 ^{ab}
	0.01	0.86±0.19 ^a	0.32±0.10 ^b	0.70±0.14 ^{bc}	1.88±0.43 ^b
	0.015	0.89±0.26 ^a	0.13±0.04 ^a	0.55±0.07 ^{abc}	1.57±0.31 ^{ab}
	0.02	0.65±0.16 ^a	0.15±0.03 ^{ab}	0.72±0.11 ^{bc}	1.52±0.30 ^{ab}
NAA	0.005	0.70±0.17 ^a	0.11±0.03 ^a	0.31±0.08 ^a	1.12±0.28 ^{ab}
	0.01	0.66±0.19 ^a	0.17±0.03 ^{ab}	0.62±0.12 ^{abc}	1.45±0.34 ^{ab}
GA ₃	0.005	0.36±0.11 ^b	0.12±0.03 ^a	0.34±0.09 ^{ab}	0.82±0.23 ^a
	0.001	0.48±0.13 ^b	0.11±0.02 ^a	0.32±0.06 ^a	0.91±0.21 ^a
CONTROL		0.55±0.09 ^c	0.14±0.06 ^a	0.41±0.09 ^{ab}	1.10±0.24 ^{ab}

*abc: The superscript letters that are the same within each column show that the values are not significantly different from each other by the Duncan Multiple Range test at ($P>0.05$)

4. Discussion

Seed germination is a complex process, which starts with the absorption of water, followed by a short pause, and ending with the synthesis and activation of enzymes. Germination is regulated through a series of interactions of hormonal and environmental factors, and this is possible only when appropriate conditions are met [14]. The differences observed in the germination percentage of seed subjected to different hormonal treatments at different concentrations shows significant impacts of the various pre-treatments on seed germination. Indole Acetic Acid (IAA) treated seeds had the highest rate of germination which agrees with the report that states that some concentrations of IAA enhanced seed germination in *Alibzia lebbeck* and *Parkia biglobosa* and to some extent in *Prosopis africana* and *Senne siamea*.

Germination must have occurred as a result of the partial exposure of the cotyledons of the seeds which permits the process of hydrolysis whereby hormones such as auxins and ethylene, which could increase nucleic acid metabolism and protein synthesis, are released [15]. The seeds treated with indole butyric acid (IBA) and naphthalene acetic acid (NAA) also induced germination progressively which must have occurred as a result of its effects on the seed coat of *T. tetraptera*. Seeds under control treatment had better germination results than the GA₃ treated seeds. This seems to negate the report of [16], which stated that gibberellic acid at different concentrations has positive effect on germination and can take on the task of a 5°C chilling treatment in breaking seed dormancy.

The variations observed among the seedling growth characteristics such as seedling height, collar diameter and number of leaves can be attributed to the effect of the treatments the seeds were subjected to. Observations showed that the same auxin treatments that gave the highest germination also resulted in seedlings with the best growth parameters. [17] observed the significant relationships between seed morphological characteristics and seedling vigor in *Terminalia invorensis* (A. Chiv). These parameters may be very useful in the promotion of rapid production of vigorous seedlings for nursery establishment or species for plantation establishment. Based on the results of this study, treatment of seeds with IAA and IBA enhanced seedling height; collar diameter and number of leaves of *T. tetraptera* seedlings. Best performance in terms of leaf dry weight (LDW) was observed among the seedlings treated with IBA. *T. tetraptera* seedlings treated with IBA and IAA at low concentrations also produced a better stem dry weight (SDW) biomass accumulation in the species. The least performance both in germination percentage and seedling growth characteristics was observed in seeds treated with (GA₃) compared to the seedlings under control treatment.

5. Conclusions

From the results of this study, it could be deduced that

IAA, IBA and NAA at low concentrations were the best treatments suitable for the enhancement of seed germination and seedling growth of *T. tetraptera* for rapid establishment of its plantation. On the other hand, GA₃ should be excluded in the pre-treatment procedure of *T. tetraptera* prior sowing.

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