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Effects of Indole-3-Acetic Acid (IAA) on the Vegetative Propagation and Phytochemical Properties of Bushbuck (*Gongronema Latifolium* Benth.)

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Abstract

The effects of indole-3-acetic acid (IAA) on the vegetative propagation and phytochemical properties of *Gongronema latifolium* were investigated at Imo State University Botanical Garden, Owerri. Different stem cuttings of *Gongronema latifolium* namely: tip, middle and butt were used for the investigation. Randomized Complete Block Design was used as the experimental design. Plants were treated with 0 ppm, 10 ppm, 25 ppm, 40 ppm and 55 ppm, with 0 ppm as the control. The results showed that IAA increased the growth parameters which are number of leaves, dry weight of leaves (g), leaf area (cm^2), number of roots, root dry weight (g), number of buds and affected the concentration of the phytochemicals such as alkaloids, flavonoids, tannins and saponins positively. It was observed that there were no root initiations from the tip cuttings indicating no survival of the cuttings. There was a decrease in alkaloids at 10 ppm with 5%. Also, a decrease in saponins as 10 ppm had 4.3% and control 6.05%. there was an increase in the percentage of all concentrations of flavonoids and tannins. 40 ppm and 55 ppm have the same percentage of flavonoids. Also, there were significant differences between the treated plants and control at $P \leq 0.05\%$. In all cases of growth parameters, 10 ppm of IAA of butt cuttings had the highest mean values followed by 25 ppm, 40 ppm and 55 ppm. From the results obtained, butt cutting is recommended for the vegetative propagation of *Gongronema latifolium* at 10 ppm of IAA.

Keywords: Indole-3-acetic acid; Vegetative propagation; Phytochemical properties; Bushbuck

Introduction

Non-wood Forest Products (NWFPs) provide safety in health for the rural poor in many developing countries especially Nigeria, where these products serve as a dependable source of food security, income and medicare. Infact, poverty has led over 90% of rural dwellers in Nigeria to depend entirely on harvests of forest products for their livelihood and economic survival [1]. *Gongronema latifolium* is a non-wood forest product of West African origin [2] and is known as "Utazi" in southeastern Nigeria and "Arokeke" in Yoruba. It is used as a leafy vegetable as well as spice in soup preparations and often eaten as a dessert with other preparations in southeastern Nigeria.

Although the plant serves many nutritional and medicinal purposes, its availability is on the decline and in some places threatened to extinction. Osemeobo and Ujor [3] reported that "Utazi is one of the major NWFPs found in Nigeria which is primarily harvested from forest and has become scarce and threatened. Therefore, to ensure sustainable conservation of *Gongronema latifolium,* there is need to develop a vegetative propagation method to ensure its continuous availability. Vegetative propagation has the advantage of rapid dissemination of selected clones or new varieties resulting from breeding programs. Stem cutting is one type of vegetative propagation and is classified into three based on the physiological age of the wood from which they are taken. They are hardwood, semi-hard wood and softwood cuttings [4,5]. Many internal factors influence root initiation [6] and shoot development in stem cuttings. These include auxins, carbohydrates and nitrogen levels in the rooting stock [7]. They explained further that the rooted cuttings have high correlation with carbohydrate level in the stems. Indole-3-acetic acid is a type of auxin which helps in root initiation.

The plant is nutritionally high in minerals, vitamins, proteins, and amino acids [8]. The proximate analysis of leaves of *G. latifolium* reveals that the plant is a valuable source of proteins, fats and oil and minerals such as Na, K, Mg and Fe [8]. The plant contains 62.66%, 10.94%, 18.77%, 58 ppm, 336 ppm, 20.75 ppm, 50 ppm, 8.17 ppm 0.90 ppm and 0.12 ppm of proteins, ash, oil, Na, K, Ca, Mg, Fe, Zn and Cu respectively. Okeke and Elekwa [9] reported that *G. latifolium* has phytochemical properties–alkaloids, saponins, flavonoids and tannins. Also, Whiteside and Milner [10] showed that *G. latifolium* has tannin 0.3% and nitrogen free extracts 44.3%. *G. latifolium* is an important medicinal plant and vegetable. A range of pharmacological teste have shown its promising hypoglycemic activities [11]. Also, antibacterial, antioxidants, anti-inflammatory [12], antiplasmodial, anti-ulcer analgesic and antipyretic activities [13,14]. It is widely used in the West African sub-region for several nutritional and medicinal purposes. Its uses are:

1. In Sierra Leone, a decoction or cold infusion of the pounded stem is used for colic and intestinal symptoms [15].

2. The leaf extracts have been shown to be high in protein (62.66%) and vitamins [8].

3. The plant is used in the treatment of cough, stomach problems, dysentery, high blood pressure, malaria, typhoid fever and helps in boosting appetite [8,16].

4. The leaves are used in the management of diabetes mellitus and high blood pressure [16,17].

5. In Ghana and Senegal, the leaves are rubbed on the joints of small children to help them walk.

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6. It also serves as a source of income for the teeming population in the society.

There is a rising need for conservation of plants that are going into extinction. *G. latifolium* is one of such plants as it seems difficult to be propagated by seeds. Due to its nutritional and medicinal values, there is an increase in the demand for the plant. From existing literature, the demand is greater than the supply. All these problems are caused by poor management system, overexploitation, destruction of its habitat where it thrives easily and other human activities. It can also be propagated by *in vitro* propagation. To sustain its availability for its economic uses, there is need to employ an alternative method of propagation which is vegetative propagation.

Materials and Methods

G. latifolium plants were obtained from Forestry Research Institute of Nigeria, Umudike, Umuahia, Abia State, Nigeria. The voucher specimen was prepared and deposited at Imo State University Herbarium with herbarium number IMSUH 032. The growth hormone, indole-3-acetic acid was also obtained from the tissue culture laboratory at National Root Crop Research Institute (NRCRI), Umudike, Umuahia, Abia State, Nigeria. The research work was carried out as a pot-experiment in the Botanical Garden of Imo State University, Owerri with an annual rainfall varying from 1500 mm to 2200 mm. An average temperature of about 27°C to 28°C, an annual humidity of 75%.

Soil sample that was used for the planting was obtained from Imo State University Botanical Garden, Owerri. It was left to dry and was sieved to remove all unwanted materials. The physical and chemical properties were determined. The soil was put into empty black nursery bags. The bags were made to have drainage holes to avoid water logging. The experiment incorporated 5 treatments. Each treatment was replicated 15 times. 1 g of IAA salt was dissolved in 100mls of distilled water to give 1000 ppm in a volumetric flask. The indole-3-acetic acid was prepared at four different concentrations 0, 10, 25, 40 and 55 all in part per million (PPM). *G. latifolium* plant was cut with a sharp knife. The plant was divided into three parts: tip, middle and butt. The stem cuttings were re-cut at an interval of 2 nodes before treatment. The solution was applied by soaking the base of the cuttings in the different IAA concentrations for 12 hours before pinning into the potting medium. Growth parameters were measured at 2 week intervals, namely: number of leaves formed, the dry weight of leaves (g), leaf area (cm^2), number of roots formed, root dry weight (g), number of buds. The phytochemical properties alkaloids, tannins, saponins and flavonoids of the leaves of *G. latifolium* were determined at the end of the experiment. The leaves of the control and the treated ones were used. This was to assess the impact of IAA on the phytochemicals of the leaves. The experimental design was based on Randomized Complete Block Design (RCBD). The different stem parts: tip, middle and butt constitute the blocks and the concentration of IAA the treatment. The data obtained was subjected to analysis of variance and least Significant Difference (LSD) was used to partition the means at $P \leq 0.05\%$.

Results and Discussion

The three parts of the cuttings tip, middle and butt from this study were used with five different concentrations of indole-3-acetic acid (IAA) on the vegetative propagation. The results of the study revealed that the mode of application of the treatment which is exogenous, got into the plant parts well. The results obtained showed that the control had the longest root initiation and 10 ppm of the treated ones had the earliest root initiation followed by 25 ppm, 40 ppm and 55 ppm of both the middle and the butt cuttings. There were no root initiations in the tip cuttings, an indication of no survival of the cuttings. Indole-3-acetic acid was observed to quicken the root initiation on vegetative propagation and growth of *G. latifolium*. This agrees with Okorie [18] on the effect of IAA on the rapid growth of *Pinus sylversis*. Chinnappan [19] reported that all the treatment of IBA and IAA increased the percentage of rooting in *Andrographis elongata* in comparison with the control. Results obtained from the vegetative propagation of *G. latifolium* showed that the treatments highest rooting mean value was recorded at 10 ppm (11.266) which was followed by 25 ppm (10.266), 40 ppm (9.266) and 55 ppm (7.932). Kijar [20] studied rooting ability of coppice shoots of *Azadirachta indica*. The study showed that 1000 ppm of IBA had the highest rooting percentage compared to control. Kamaluddin and Ali [21] concluded that treatment with 0.2% and 0.4% IBA had effect on root and growth of *Azadirachta indica* cutting. Bassiri et al. [22] reported that 40 ppm of IAA is required to induce rooting in chickpea varieties. Results obtained also showed that the highest mean value of number of buds was produced in plants treated with 10 ppm of the IAA at 2.534 in butt cuttings and 2.334 in middle cuttings. This was followed by 25 ppm, 40 ppm and 55 ppm. The lowest mean value of the treated ones was obtained at 55 ppm with 1.400 butt cuttings and 1.266 middle cuttings. The differences between 0 ppm, 10 ppm, 25 ppm and 40 ppm were significant while no significant difference was obtained between 0 and 55 ppm at ($P \leq 0.05\%$).

The highest number of leaves was obtained at 10 ppm with mean of 5.268 in butt cuttings and 4.470 in middle cuttings. This was followed by 25, 40 and 55 ppm. The differences between 10, 25 and 40 ppm were not significant but significant difference 0 and 10, 25, 40 ppm. In all cases, the highest number of buds, number of leaves, dry weight of leaves (g), leaf area (cm^2), number of roots and dry weight of root (g) were obtained with butt cuttings at 10 ppm and followed by 25, 40 and 55 ppm. Similar trends were observed in middle cuttings while the tip cuttings did not survive at the period of this study. Several internal and external factors influence the process of adventitious root formation. Among the internal factors, the most important role is ascribed to phytohormones especially the auxins. It is accepted that auxins have a certain role in the rooting initiation [23]. Furthermore, Pulak et al. [24] reported that the application of GA3 and IAA on plants of soybean increased its yield. The result showed that both GA3 and IAA enhanced the plant height, number of leaves, flowers, pods, branches, number of seed per plant as compared to control plants. Hina et al. [25] studied the effect of IAA and NAA on the vegetative propagation of chickpea stem cutting and the result showed that NAA was the better root-inducing hormone for chickpea than IAA and is required in very low concentration. These results are in conformity to this study.

The results also showed the presence of phytochemicals which are of medicinal value both in the leaves of the control and the treated plants. The report of Okwu [26] indicates some indigenous plants are used for treatment of pregnant and nursing mothers, of which *G. latifolium* is one of such plants. In this study, there were changes in the concentrations of alkaloids, tannins, saponins in the leaves due to the application of indole-acetic acid (IAA). From this study, there was decrease in the alkaloids and saponins content. An increase in the flavonoids and tannins content of the leaves. The observed changes in the metabolism of the IAA on the treated plants. Leopold [27] concluded that auxin played a role on physiological process and development requiring other substances as rooting co-factor that will stimulate growth. Treated levels of concentrations had effects on phytochemical contents of *G. latifolium* leaves. 10 ppm and 25 ppm

Treatment (ppm)	Alkaloid (%)	Flavonoid (%)	Tannin (%)	Saponin (%)
0	5.100	2.000	1.400	6.050
10	5.000	6.600	3.000	4.300
25	4.800	4.600	2.914	4.100
40	4.400	3.800	1.780	3.800
55	3.400	3.800	1.480	3.600

Table 1: Effect of IAA on the phytochemical properties of *G. latifolium*.

produced the highest concentrations of alkaloids, flavonoids and saponins. Also, tannin content increased at 10 ppm and 25 ppm but decreased at 40 ppm and 55 ppm (Table 1). This indicates that the plant reacts to different concentrations of IAA for their metabolic differential activities especially on their metabolites. However, the results of the study contrast with the report of Ahuzi [28] on the vegetative propagation of *Pinus caribaea*. The report revealed that 25 ppm should be used in the propagation of *Pinus caribaea*. This difference may be because *Pinus caribaea* is a woody plant and *Gongronema latifolium* is a liana plant (woody climber).

Conclusion

Conclusively, evidence from this study showed that 10 ppm of IAA is essential for the cultivation of *G. latifolium* and is needed for the adequate production of these phytochemicals that are of medicinal value. IAA should not be indiscriminately applied exogenously, rather 10 ppm of it should be applied at butt cutting for enhanced propagation of *G. latifolium*. From the research investigated, we recommend the use of 10 ppm of IAA and the butt cutting for effective propagation of *G. latifolium* to farmers who are interested for mass propagation of this plant.

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