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Effects of Nano Silver and Indole Butyric Acid Application on Growth and Some Physiological Characteristics on Hardwood Cutting of *Dalbergia sissoo* Roxb

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ABSTRACT

Nanosilver (NS) and indole-3-butyric acid (IBA) can improve cutting performance and subsequent growth. This study was performed in Taqtaq city in a randomized complete block design to study the effects of NS (30, 60, and 90 mg/L) and IBA (50, 100, and 200 mg/L) in addition to distilled water as a control on growth and some characteristics of hardwood cutting of *Dalbergia sissoo* Roxb. Application of IBA enhanced significantly buds sprouting, where the cutting treated with 50 mg/L IBA sprouted after 35.53 days. IBA at 200 mg/L increased plant leaf area significantly to 204.11 dm^2 in comparison to the control cuttings (122.00 dm^2). Furthermore, IBA at 50 and 200 mg/L increased the number of leaves to 194.66 and 193.00 leaves/plant, compared to control cuttings (158 leaves/plant). The lowest peroxidase activity (902.00 and 903.30 absorbing units/ g fresh leaves) was observed in the cuttings soaked in 30 and 60 mg/L NS, respectively. Both NS and IBA had a significant effect on macro and microelements in the shoot except Mg and Fe. The shoot content of elements was different in response to NS and IBA applications, whereas the high level of IBA decreased significantly K content (25.80 %) it increased significantly the shoot content of Zn (0.22%). However the lowest concentration of NS (30 mg/L) decreased significantly the Cu content (0.02 %) and increased significantly the shoot content of Mn (0.58%). Root response to NS and IBA also was different, where 90 mg/L NS increased significantly each of K (26.40%) and Zn content (0.68 %), whereas it decreased significantly the root content of Fe (8.78%). The enhancement effects of IBA were more than that of NS on most studied characteristics.

Index Terms: Auxins, *Dalbergia sissoo*, Hardwood Cutting, Indole-3-Butyric Acid, Silver Nanoparticles, Macro Elements, Micro Elements

1. INTRODUCTION

Blackwood, sissoo or shisham *Dalbergia sissoo Roxb.* is an important *Fabaceae* legume tree, it is strongly recommended for afforestation and reforestation programs in dry and water conservation areas [1]. It is used widely as a windbreak, for nitrogen fixation, their wood for furniture and construction purposes, and important source of animal fodder [2], [3]. Their roots and seed oil contain tectoridin which is used medicinally [4]. Recently, its leaves, stem barks, and root extracts are used for the green synthesis of silver nanoparticles (AgNPs) for different biological and catalyst activities [5]–[7].

The developmental processes in the agricultural sector for plant growth and development are continuous including

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nanotechnology and plant hormones. Nanotechnology is a modern field of science which plays an important role in everyday life. Nanoparticles have a small size of 1–100 nm, and a large surface area, different types and shapes of agricultural nano-materials are used depending upon the needs and nature of the work, such as pesticides, fertilizers, food industries, control of insects, fungi and weeds, and remediation of heavy metals, AgNPs are in great interest, which is the most popular type of nanomaterials [8], [9]. Many studies were conducted using nanosilver (NS) to improve cutting performance and subsequent growth [10], [11].

The plant hormone indole-3-butyric acid (IBA) belongs to the auxin group and it is known as the rooting hormone. It is used traditionally to stimulate root initiation on stem cuttings [12] Numerous researchers have demonstrated the potential of propagation of *Dalbergia* sp. by gaining more vigorous trees within a short period, they also indicated that rooting and sprouting percent, number of roots, and length of shoots and roots increased with increasing concentration of IBA in different clones of *D. sissoo* Roxb. [13]–[16].

Due to a lack of research about the effects of NS on *D. sissoo Roxb.*, and as an attempt to increase the cultivated area of this important tree species in Iraq and Iraqi Kurdistan, this study was conducted as a complement to our previous study [10] which was on the effect of NS and gibberellic acid on *D. sissoo* seeds, whereas the aim of this part of the study is to investigate the effects of NS and IBA in different concentrations on the growth and some physiological characteristics of *D. sissoo* hardwood cutting.

2. MATERIALS AND METHODS

2.1. Plant Materials and Experiment Treatments

Cuttings of *D. sissoo* Roxb. from 15-year-old trees were taken on February 14, 2019, from Mnara Park/Erbil, Iraq with 36°11'21.7"N, 44°00'05.2"E, and 400 m AMSL. Approximately, 30 cm of hardwood cuttings about 1–1.5 cm in diameter were taken from the base and middle of 1-year-old branches. The study was conducted in a private field in Taqtaq city, Koysinjaq, Erbil, where the mean of maximum and minimum temperature throughout the study period was 38.5, and 25.03°C, and the average rainfall was 74.82 mm.

The NS particles were purchased from US Research Nanomaterials, Inc (CAS: 7440-22-4, USA), prepared at 30, 60, and 90 mg/L in distilled water, and dispersed using ultrasonic vibrations (100 W, 30 kHz) [17] for 30 min. The IBA was purchased from Fine Chemicals Expert (CAS: 133-32-4, Germany), and prepared at 50, 100, and 200 mg/L in distilled water. The stalk solutions for each of the NS and the IBA were prepared by dissolving the powder in a little ethanol then completed by distilled water, then kept in dark bottles. The experiment consists of seven treatments (distilled water as a control, NS30, NS60, NS90, IBA50, IBA100, and IBA200). Each treatment consists of three replications and each experimental unit consists of 10 poly bags sized (10 × 30) cm which filled with sandy soil (74% sand, 16.9% clay, and 9.1% silt, with 0.1 Ec, 7.83 pH, and 0.3% organic matter) mixed at (1:1) with peat moss. On February 19, 2019, 10 cm of the cuttings were submerged in the NS and IBA solutions at room temperature for 24 h (Fig. 1). On February 20, 2019, the cuttings were cultivated by putting 2–3 nodes in the soil. The experiment was finished on July 4, 2019.

2.2. Studied Characteristics

The bud sprout percentage and velocity are determined as mentioned by Ranal and Santana [18]. Plant height, number of leaves and branches per plant, length of longest root, and fresh and dry matter of shoot and root were measured as they demonstrated by Al-Barzinji *et al.* [19]. Leaf area was calculated using the method described by Watson and Watson [20]. Pigments of chlorophyll a and b and total carotenoids are calculated as they mentioned by Lichtenthaler and Wellburn [21]. Total carbohydrate was determined using the method outlined by Joslyn [22]. Peroxidase enzyme activity in leaves was quantified according to Nezih [23]. Shoot and root chemical contents were determined using X-ray fluorescence (XRF) spectroscopy [24].

Fig. 1. Preparation and planting of (1) preparing bags for cultivation, (2) prepared cutting, (3) soaking in treatment solutions, (4) cutting planting, (5) bud outgrowth, and (6) plant stalks.

2.3. Statistical Analysis

The experiment was laid out using a one-way Randomized Complete Block Design with three replications. The measured data were analyzed using the SAS statistical program, and Duncan's multiple range test ($P \leq 0.05$) was used [25].

3. RESULTS AND DISCUSSION

The results in Table 1 show that the effect of each of NS and IBA on the bud sprout, plant height, leaf area, leaf number, number of branches, and root length was significant. NS and IBA had non-significant effects on the percent of bud sprouting, whereas the velocity of bud sprouts was enhanced significantly by the application of IBA and NS. The cutting treated with 50 mg/L IBA had the fastest bud sprout (after 35.53 days) compared to the control cutting (40.00 days), but treatment with NS at 60 mg/L retarded bud sprout (after 44.03 days). IBA at 200 mg/L resulted in the highest leaf area (204.11 dm^2) in comparison to control cuttings with (122 dm^2); however, NS at 90 mg/L showed the lowest leaf area (95.13 dm^2). Furthermore, IBA at 50 and 200 mg/L showed the maximum number of leaves (194.66 and 193 leaves/plant, respectively) compared to control cuttings with (158 leaves/plant). The effect of different NS and IBA concentrations was not significant on the number of branches compared to the control cuttings. Although, NS at 30 mg/L gained the maximum number of branches (eight branches/plant) related to the 100 mg/L IBA with (4.66 branches/plant). Using 50 and 100 mg/L IBA increased significantly longest root significantly to (30.53 and 25.50 cm, respectively) over the control cuttings with (21.3 cm). Meanwhile using NS at 30 and 60 mg/L decreased the longest root (11.5 and 11.56 cm, respectively) significantly compared to all other treatments.

Different concentrations of IBA had a significant effect on each velocity of bud sprout, plant height, leaf area, number of leaves, and root length, and this was confirmed by Yeshiwas *et al.* [26]. The positive effects of IBA may be due to they have indirect influence by enhancing the speed of translocation and movement of sugar to the base of cuttings and consequently stimulating rooting [27]. Increasing plant growth parameters by adding IBA may be due to increasing photosynthesis process as a result of increasing root growth and chlorophyll a and b (Table 2). The efficiency of IBA in increasing root performance and some growth characteristics may be due to the slow and continuous release of IAA from IBA [28], thus accelerating carbohydrate translocation to the base of cuttings [29]. It may be expected that the reduction in plant growth and length of root caused by NS may lead to a reduction in above-ground biomass, due to decreased nutrient uptake [30].

Different shoot fresh and dry weights, root fresh and dry weights, carbohydrate ratios, and peroxidase activity were achieved when various concentrations of NS and IBA were applied (Table 3). In this context, shoot fresh and dry weights were the highest (71.3 and 33.45 g) at 200 mg/L IBA, but the control cuttings recorded the minimum shoot fresh and dry weights (33.6 and 13.15). Moreover, IBA at 50 mg/L was the best dose to obtain the maximum weights of fresh and dry root (4.8 and 2.23 g), respectively, and it was significantly in contrast to the control cuttings with root fresh weight (3.05 g) and dry weight (1.65 g), whereas, the lowest root fresh weight (0.75 g) and dry weight (0.51) were found in the cuttings treated with 30 mg/L NS. The results of carbohydrate analysis explained that 50 mg/L IBA and 60 mg/L NS were the best treatments to raise carbohydrates in shoots (31.1%) and roots (24.9%) in comparison with the control cuttings. Minimal carbohydrates in shoots (15.2%) and in roots (3.56) were quantified in the cuttings soaked in 30 mg/L NS and 100 mg/L IBA, respectively. Peroxidase activity was maximal (2276.7 absorbing unit/ g fresh leaves) in the cuttings soaked in 50 mg/L IBA which was significantly higher than control

Table 1: Effects of NS and IBA on *Dalbergia sissoo* Roxb. cutting performance and some vegetative characteristics.

Treatments	Cutting sprout (%)	Duration of sprouting (days)	Plant height (cm)	Leaf area (dm^2)	Number of leaves/plant	Number of branches/plant	Longest root (cm)
Control	93.33 a	40.00 b	45.00 e	122.96 cd	158.00 c	6.33 abd	21.30 c
NS30	96.67 a	37.92 bc	47.00 cd	111.85 d	177.00 bc	8.00 a	11.50 f
NS60	93.33 a	44.03 a	50.23 b	118.15 cd	171.66 bc	6.00 bcd	11.56 f
NS90	96.67 a	39.32 bc	44.00 e	95.13 e	174.00 bc	7.00 abc	16.00 e
IBA50	100.00 a	35.53 c	55.00 a	151.91 b	194.66 a	5.33 cd	30.53 a
IBA100	96.67 a	36.41 bc	48.23 bc	127.48 c	173.66 bc	4.66 d	25.50 b
IBA200	96.67 a	38.31 bc	50.06 b	204.11 a	193.00 a	7.33 ab	18.13 d

*Means followed by the same letters within columns are not significantly different at $P \leq 0.05$ according to the Duncan test. NS: Nano silver, IBA: Indole-3-beutyric acid

cuttings with (1593). Contrarily, the lowest peroxidase activity (902 and 903.3 absorbing units/ g fresh leaves) was observed in the cuttings soaked in 30 and 60 mg/L NS, respectively.

Low IBA concentration significantly increased carbohydrate contents as compared to the control, which is in accordance with the results of Massoud *et al.* [31]. The decreasing carbohydrate in shoots and roots with increasing IBA concentration may be ascribed to the inhibition of carbohydrate transport from roots to leaves and their metabolism in leaves to other compounds, or their consumption during aerobic respiration [32]. According to Ahkami *et al.* [33], a relationship exists among carbohydrate concentration, photosynthesis, and root number, carbohydrates participate in root formation since the accumulation of soluble and insoluble carbohydrates was observed during the rhizogenesis process. However, IBA concentrations and genotypes used affect leaf chlorophyll and carbohydrate concentration in both leaves and roots in different ways. According to Sarropoulou *et al.* [34], the rooting ability of cuttings is correlated with their carbohydrate content because as seen in Tables 2 and 3 where decreasing carbohydrate content leads to reduced root length because the free-reducing sugars and storage carbohydrates are important in root formation as energy sources and structural materials of cells.

NS reduced peroxidase activity, in our study as same as the results of Kumar *et al.* [35] and Hatami and Ghorbanpour [36]. Besides acidic effects, NS could act as an anti-ethylene agent, Ag^+ is an effective ethylene action inhibitor, whereas Kim *et al.* [37] suggested that NS acted as an anti-ethylene agent on cut Asiatic hybrid *Lilium*. While the peroxidase activity in all treatments of IBA started to increase, Pan and Tian [38] got the same result.

Photosynthetic pigments were affected significantly by each of the NS and IBA applications compared to control cuttings. Accordingly, 100 mg/L IBA increased significantly each of chlorophylls *a* (1.3 mg/g fresh weight) and *b* (0.71 mg/g fresh weight) compared to all other treatments. The lowest chlorophyll *a* (0.31 mg/g fresh weight) and chlorophyll *b* (0.08 mg/g fresh weight) were found at 60 and 30 mg/L NS, respectively. Whereas the highest total carotenoids were recorded at 90 mg/L NS, the minimum (0.17 mg/g fresh weight) carotenoids were observed at 60 mg/L NS. Increasing chlorophyll content by IBA treatment may be due to increasing Fe content in roots (Table 4), this result is considered by El-Shraiy and Hegazi [39].

Table 5 shows that different concentrations of NS and IBM had significant effects on macro and microelements in shoots except Mg and Fe. The treatments of 90 mg/L NS and 100 mg/L IBA had the highest values for K (29.7 and 29.9% respectively). Inversely, IBA at 200 mg/L caused the lowest K (25.8%). IBA at 50 and 100 mg/L were the best doses for Cl (1.35 and 1.26%) respectively, but increasing NS concentration to 90 mg/L reduced Cl content to 0.35%. In addition, IBA at 100 mg/L was an outstanding dose for Cu (0.05%). The lowest concentration of NS (30 mg/L) and IBA (50 mg/L)

Table 2: Effects of NS and IBA on some photosynthetic pigments of *Dalbergia sissoo* leaves

Treatments	Mg/g fresh weight		
	Chlorophyll *a*	Chlorophyll *b*	Total Carotenoids
Control	0.46 d	0.13 cd	0.20 d
NS30	0.64 c	0.19 c	0.26 c
NS60	0.31 e	0.08 d	0.17 e
NS90	0.97 b	0.31 b	0.32 a
IBA50	0.99 b	0.28 b	0.29 b
IBA100	1.30 a	0.71 a	0.26 c
IBA200	0.91 b	0.32 b	0.27 c

*Means followed by the same letters within columns are not significantly different at $P \leq 0.05$ according to the Duncan test. NS: Nano silver, IBA: Indole-3-beutyric acid

Table 3: Effects of NS and IBA on fresh and dry weights of shoot and root, total carbohydrate in shoot and root, and peroxidase enzyme activity of *Dalbergia sissoo* Roxb.

Treatments	Shoot (g)		Root (g)		Total carbohydrate (%)		Peroxidase activity (absorbing units/ g fresh leaves)
	Fresh weight	Dry weight	Fresh weight	Dry weight	Shoot	Root	
Control	33.60 e	13.15 d	3.05 c	1.65 b	20.83 c	5.73 d	1593.00 c
NS30	47.40 b	22.45 b	0.75 e	0.51 d	15.20 d	12.50 bc	902.00 d
NS60	38.95 cd	15.15 d	3.12 c	1.23 c	33.30 a	24.90 a	903.30 d
NS90	35.50 ed	15.75 cd	1.40 d	1.24 c	26.73 b	13.66 b	1450.00 c
IBA50	39.60 cd	16.30 cd	4.80 a	2.23 a	31.10 a	10.60 c	2276.70 a
IBA100	41.75 c	18.85 c	4.35 ab	2.40 a	22.20 c	3.56 e	1852.00 b
IBA200	71.30 a	33.45 a	4.30 b	1.65 b	15.06 d	7.06 d	1852.30 b

*Means followed by the same letters within columns are not significantly different at $P \leq 0.05$ according to the Duncan test. NS: Nano silver, IBA: Indole-3-beutyric acid

Table 4: The effect of NS and IBA on some macro and microelements in roots of *Dalbergia sissoo*

Treatments	Macroelements (%)		Microelements (%)				
	K	Mg	Cl	Cu	Fe	Mn	Zn
Control	14.17 c	4.91 a	2.50 ab	0.10 a	11.02 a-c	0.328 b	0.33 d
NS30	21.40 b	4.49 a	2.87 ab	0.06 a	10.30 bc	0.346 b	0.48 bc
NS60	23.90 ab	4.53 a	2.75 ab	0.07 a	9.54 bc	0.297 bc	0.57 ab
NS90	26.40 a	4.58 a	2.63 ab	0.08 a	8.78 c	0.248 cd	0.68 a
IBA50	11.60 c	3.06 a	0.90 b	0.07 a	13.87 a	0.465 a	0.40 cd
IBA100	21.90 b	3.96 a	3.35 a	0.07 a	10.20 bc	0.243 bc	0.55 b
IBA200	14.10 c	4.89 a	3.15 a	0.07 a	11.80 ab	0.241 d	0.42 cd

*Means followed by the same letters within columns are not significantly different at $P \leq 0.05$ according to the Duncan test. NS: Nanosilver, IBA: Indole-3-butyric acid

Table 5: The effect of NS and IBA on some macro and microelements in shoots of *Dalbergia sissoo*

Treatments	Macroelements (%)		Microelements (%)				
	K	Mg	Cl	Cu	Fe	Mn	Zn
Control	28.07 abc	4.73 a	0.65 bc	0.03 cd	0.77 a	0.39 bc	0.08 d
NS30	28.60 ab	5.09 a	0.63 bc	0.02 d	0.51 a	0.58 a	0.12 bcd
NS60	26.40 bcd	2.85 a	0.88 b	0.04 b	1.07 a	0.47 ab	0.17 abc
NS90	29.70 a	3.00 a	0.53 c	0.04 bc	1.10 a	0.33 c	0.13 bcd
IBA50	26.20 cd	4.42 a	1.35 a	0.02 d	1.25 a	0.14 d	0.10 cd
IBA100	29.90 a	4.92 a	1.26 a	0.05 a	0.74 a	0.39 bc	0.18 ab
IBA200	25.80 d	4.42 a	0.62 bc	0.03 bcd	0.90 a	0.39 bc	0.22 a

*Means followed by the same letters within columns are not significantly different at $P \leq 0.05$ according to the Duncan test. NS: Nanosilver, IBA: Indole-3-butyric acid

resulted in the least Cu (0.02%). NS at 30 mg/L and IBA at 50 mg/L were the best and worst doses for Mn ratio with (0.58%) and (0.14%), respectively. In comparison to control cuttings, the cuttings supplied with 200 mg/L IBA had the highest Zn (0.22%), but control cuttings contained 0.08% Zn.

Root macro and microelements demonstrated in Table 4 confirmed that the NS and IBA concentrations applied in this study were not effective regarding Mg and Cu content. Furthermore, the effect of the NS and IBA treatments was not significant on Cl and Fe related to control cuttings. Although, 50 mg/L IBA elevated Fe to the peak value (13.87%), but declined Cl to the lowest value (0.9%). Moreover, Cl was the highest at 100 mg/L IBA (3.35%) followed by 200 mg/L IBA (3.15%). Treatment with 90 mg/L NS reduced Fe content to 8.78%. In addition, IBA at 50 mg/L raised Mn to 0.465% in comparison to control cuttings, contrarily 200 mg/L IBA declined Mn to 0.241%. NS at 90 mg/L improved the Zn amount (0.68%), at the same time the lowest Zn (0.33%) was measured in control cuttings.

A high concentration of NS (Table 5) reduces Mg and Zn in the shoot, the same result was obtained by Zuverza-Mena *et al.* [40]. The uptake of Mn and Zn by roots is mediated by putative transporters, natural resistance-associated macrophage protein (Nramp), and the Zinc/Iron Permease (ZIP) family [41]. In addition, Magesky and Pelletier [42] mentioned that silver is a membrane disruptor that breaks down cellular homeostasis, very likely, this disruption affected the uptake of essential elements. It is also possible that NS down-regulates the genes encoding for metal transporters. Results showed that NS, especially at high concentrations, reduced the accumulation of macroelements and microelements in the purslane plants, it has been shown that Ag ion damages the cell membranes and disrupts ionic homeostasis in cells, which negatively affects the absorption of nutrients. Since the mechanism of the effect of the NS on the accumulation of nutrient elements is unclear [43]. These results corroborate with the findings of Zuverza-Mena *et al.* [40] on *Raphanus sativus*.

Increasing macroelements in shoot was agrees with El-Sayed *et al.* [44] and Ashour [45]. IBA has a significant effect on micro and macroelements, this result agrees with [31]. Decreasing chemical elements by IBA may be due to highly varied macronutrient content in plants, which may have been caused by different climatic and growing conditions during the period of the experiment [46].

4. CONCLUSIONS

From the results of this study, we could conclude that the application of NS on *D. sissoo* cutting affected significantly

on most studies' characteristics. Treated cuttings with IBA show faster bud sprout, higher vegetative growth, chlorophyll *a* and *b*, and total carotenoids, shoot and root carbohydrate content, peroxidase enzyme activity, and most elements content of root and shoot. Furthermore, the IBA effect was higher than NS on *D. sissoo* cutting performance. Further studies are required to estimate some plant hormones and some other enzyme activities. In addition, the effect of some soil adapters like chemical fertilizers and peat moss and their interactions with NS on the cutting and plant performance will also needed.

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