

## Effect of Cutting Types, Cutting Dates, and IBA Concentrations on The Propagation of Buddleia Shrubs (*Buddleia davidii*)

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### ABSTRACT:

This factorial experiment was conducted in a glasshouse of Horticulture Department, College of Agricultural Engineering Sciences, University of Sulaimani, Sulaymaniyah, Kurdistan region of Iraq, during the growing seasons of 2024-2025 from 15<sup>th</sup> of September to 15<sup>th</sup> of February, to investigate the effects of cutting dates, cutting types, and IBA concentrations on the propagation of buddleia shrubs. Study design was in RCBD design, cuttings were collected from Sitak, Sulaymaniyah, Kurdistan region of Iraq. The factorial experiment included three factors, cutting dates which consisted of 15<sup>th</sup> of September (D1), 1<sup>st</sup> of October (D2), 15<sup>th</sup> of October (D3), and 1<sup>st</sup> of November (D4). The second factor was cutting types which consisted of softwood cuttings (A1), semi-hardwood (A2), and hardwood cuttings (A3). The third factor was IBA concentrations, included treatment without IBA (C0), 500 mg kg<sup>-1</sup> (C1), 1000 mg kg<sup>-1</sup> (C2), and 1500 mg kg<sup>-1</sup> (C3). Comparisons among the means were performed using LSD ( $\alpha=0.05$ ). The results indicated that D1 had the best results in rooting percentage, root fresh and dry weights, shoot length, shoot fresh and dry weights by recording (88.88%, 1.22g, 0.25g, 11.53cm, 2.56g, and 0.65g respectively), D2 obtained highest results in longest root, number of leaves per plant (22.07cm and 48.69 leaf plant<sup>-1</sup>). A2 gave the best result in rooting percentage (85%), A3 achieved highest results in rooting percentage, root fresh weight, number of leaves per plant, longest shoot fresh and dry weight by recording (81.66%, 0.74g, 37.14 leaf plant<sup>-1</sup>, 6.75 cm, 1.97g, and 0.60 g respectively). C2 (1000 mg kg<sup>-1</sup>) gave maximum results in longest root (19.25 cm) and number of leaves per plant (37.76 leaf plant<sup>-1</sup>), C3 (1500 mg kg<sup>-1</sup>) gave the best result in root fresh weight (0.73 g).

**Keywords:** Butterfly shrubs, stem cuttings, perlite, peatmoss, auxin.

### INTRODUCTION

*Buddleia davidii* also known as butterfly bush is an ornamental plant that belongs to the Buddlejaceae family that originated in Asia (specifically southern China), and was introduced to different countries as ornamental plant, and they are distributed in the temperate, tropical and subtropical climates [1-3]. Generally, butterfly bushes are free from pests however when they are under stress, they will be

susceptible to spider mites [4]. Beyond butterfly bush's role in commercial horticulture, improving propagation technique for this plant is important due its ecological impact on environment as well as pollinators as it can act as a good source of food for pollinators [2]. Butterfly bush can be propagated via seeds and cuttings [1].

Propagation by stem cuttings is a method used for rapid multiplication and as an economical technique that is utilized to create genetically homogenous plants. However, the

success of this process depends on external and internal factors. Cuttings are divided into three types according to their maturity such as softwood, semi-hardwood and hardwood cuttings, which directly affect auxin sensitivity and cellular differentiation [5]. Improving root characteristics can enhance nutrient and water uptake resulting in better plant growth and the need for seed propagation [6].

There are many methods exploited to enhance rooting success, one of these methods is the use of rooting hormones such as synthetic auxin, these hormones like indole-3-butyric acid (IBA) are commonly known for their role in promoting and improving rooting in various plants species [7, 8]. IBA can regulate the concentration of auxin at the base of cuttings, as it can reduce oxidative stress while promoting cell division and elongation [9]. Optimal dosage of auxin vary from one species to another, for that the assessment for each woody or herbaceous plant is necessary in order to use the correct concentration [10]. When a high concentration of auxin is used it may inhibit adventitious roots initiation and might cause tissue browning and necrosis, while a low concentration of auxin (below the signaling threshold) might fail to initiate adventitious root formation [11, 12].

## MATERIALS AND METHODES

The experiment was conducted in the glasshouse of Horticulture Department, College of Agricultural Engineering Sciences, University of Sulaimani, Sulaymaniyah, Kurdistan Region of Iraq, during 2024-2025 growing season from 15<sup>th</sup> of September to 18<sup>th</sup> of February. The site of the experiment is situated between 35°32'15.8" N latitude and 45°21'53.4" E longitude, at altitude of 737 m

above sea level. This study used healthy *B. davidii* mother plants as the source of cuttings, obtained from Sitak, Sulaymaniyah, Kurdistan region of Iraq. The site in which cuttings were obtained is situated between 35°38'34.4" N latitude and 45°28'59.8" E longitude. softwood, semi-hardwood, and hardwood cuttings were selected for the experiment. The cuttings were collected and prepared to a uniform length of 12 cm, each cutting contained 3 nodes, and was carefully chosen for uniform thickness and maturity. After preparing the cuttings, they were treated with Tapsen antifungal by submerging them for 10 minutes, then left in a shady place so the excessive water evaporates before treating them with IBA. The rooting substrate used for the experiment consisted of a mixture of 70% peat moss and 30% perlite, the content of the peatmoss showed in Table 1. The prepared substrate was placed in a plastic pot with a diameter of 12 cm, then treated with Tapsen antifungal to prevent fungal contamination.

**Table 1: Nutrient content and pH level of the peatmoss used as rooting media for the experiment.**

Name	Ratio
pH	5.5
N	110 (mg L <sup>-1</sup> )
P <sub>2</sub> O <sub>5</sub>	130 (mg L <sup>-1</sup> )
K <sub>2</sub> O	140 (mg L <sup>-1</sup> )
Mg	80 (mg L <sup>-1</sup> )
B	0.2 (mg L <sup>-1</sup> )
Cu	0.3 (mg L <sup>-1</sup> )

Indole-3-butyric acid with 99.99% purity (IBA) was used as a rooting hormone to promote root development in cuttings of butterfly bush. Four different concentrations of IBA were prepared for the study: 0 mg kg<sup>-1</sup>, 500 mg kg<sup>-1</sup>, 1000 mg kg<sup>-1</sup>, 1500 mg kg<sup>-1</sup>. The IBA powder was weighed with a sensitive scale and dissolved in a 500ml of

70% ethanol to ensure it is fully dissolved. The solution was then diluted with distilled water to one litre.

### Experiment Design and Parameters

The design of the factorial experiment was Randomized Complete Block Design (RCBD) with 36 treatments divided into three replicates (twelve treatment per replication) and each treatment contained five cuttings planted in five separate pots. Factors of the experiment were cuttings dates (D) which it included 15<sup>th</sup> of September (D1), 1<sup>st</sup> of October (D2), 15<sup>th</sup> of October (D3), and 1<sup>st</sup> of November (D4), second factor were cutting types included softwood cuttings (A1), semi-hardwood cuttings (A2), and hardwood cuttings (A3), and the third factor was IBA concentration which consisted of treatment without IBA (C0), 500 mg kg<sup>-1</sup> (C1), 1000 mg kg<sup>-1</sup> (C2), and 1500 mg kg<sup>-1</sup> (C3). Data were analysed statically using a computer application XLSTAT v21.2.59614 [13]. Least Significant Difference (LSD) at 5% level was used to compare the means of the results. Data were collected after 14 weeks from planting the cuttings, date one 1/1/2025, date two 15/1/2025, date three 1/2/2025, and date four 15/2/2025.

### RESULTS AND DISCUSSIONS

According to Table (2) The highest rooting percentage was observed from cuttings collected during the first date (D1) which was 88.88% while the least rooting percentage was observed from the third date (D3) 62.77%. Semi-hardwood cuttings gave the highest rooting percentage while hardwood cuttings gave the least rooting percentage. Applying IBA showed no

significant impact on rooting percentage. Effect of interaction between two study factors on the rooting percentage of the cuttings. As showed in Table (2) the highest rooting percentage in cutting dates and cutting types combination was observed from treatment D4A2 which recorded 96.66% while the least result was from D3A1 33.33%. Interaction between cutting dates and IBA concentration gave the highest result in treatment D1C3 91.11% while the least result observed from D3C1 51.11%. Combination of cutting types and IBA concentration gave the highest result in treatment A2C3 by recording 88.33% and the least result observed was from treatment A1C3 53.33%. Table (2) illustrate the significant impact of interaction between all study factors on rooting percentage of the cuttings. According to the result the highest rooting percentage was observed from treatment D4A2C2, D1A3C1, and D4A2C1 which they recorded 100%, while the least rooting percentage was observed from treatment D4A1C3 as it recorded only 20%.

Table (3) shows the effect of study factors on longest root, according to the results (D2) gave the longest roots by recording 22.1 cm which was significantly higher than cuttings collected during the third date (D3) by 55.30% as D3 recorded the shortest roots (14.23 cm). While cutting types did not show any significant difference between them. On the other hand, IBA concentration affected longest root as cuttings treated with 1000 mg kg<sup>-1</sup> (C2) gave the longest root (19.3 cm) compared to cuttings treated with 500 mg kg<sup>-1</sup> (C1) which gave the least record of 16.72cm. Interaction between D2 and A1 gave the longest root (23.70 cm) compared to D3 and A1 (12.40 cm), while combination of cutting dates and IBA concentration recorded 23.04

cm in D2C3 which was significantly higher than D3C1 by 100.35% as it recorded 11.50 cm. On the other hand, interaction between A2 and C3 gave the highest record (19.80 cm) in comparison with A2C1 as it recorded the shortest root (15.53 cm). Three-ways interaction significantly affected longest root as the interaction between cutting dates, cutting types and IBA concentrations gave the highest result (28.95 cm) in treatment D2A1C3 which was significantly higher than D3A1C1 by 236.63% as D3A1C1 recorded only 8.60 cm.

According to (Table 4) cuttings collected during the first date (D1) showed a significant difference in root fresh weight as it surpassed other dates by recording 1.23 g of root fresh weight which was higher than D3 by 382% as D1 treatment recorded only 0.25 g of root fresh weight. Same table also shows the impact of cutting types on root fresh weight as A3 and A2 recorded 0.75 g and 0.71 g respectively which was higher than A1 cuttings (0.60 g). On the other hand, C3, C2 and C0 IBA concentration showed similar result in root fresh weight as they recorded 0.74, 0.72 and 0.70 g respectively, while C1 showed the least result by recording 0.60 g. Interaction between cutting dates and cutting types, as in D1A3 treatment was significantly higher than D3A1 combination by achieving 1.40 g of root fresh weight compared to 0.10 g which was lower than D3A1 by 92.86%. Combination of cutting dates and IBA concentration gave a significant result in D1C3 as it obtained 1.34g compared to 0.14 g in D3C1 combination. While combination of cutting types and IBA concentration gave 0.85 g in A3C0 combination as it was higher

than A1C0 (0.40 g) by 45%. As indicated in Table (4), the interaction between all study factors showed a significant result as treatment D1A3C0 was significantly higher than D3A1C0 by 4000% as it obtained 2.05 g of root fresh weight compared to 0.05 g in D3A1C0.

Results showed in Table (5) indicates the impact of study factors on root dry weight of buddleia shrubs. According to the results, D1 and D2 was significantly higher than D3 and D4 by achieving 0.25 and 0.24 g of root dry weight respectively, while D3 and D4 achieved the least root dry matter as they recorded 0.12 and 0.10 g respectively. On the other hand, cutting types and IBA concentrations showed no significant impact on root dry weight of buddleia shrubs cuttings. According to the results obtained from interaction between two study factors as in D1A1 treatment was significantly higher than D3A1 treatment by 800% as it recorded 0.27 g of root dry weight compared to 0.03 g of D3A1. Interaction between cutting dates and IBA concentration in D2C3 treatment obtained the highest root dry weight by recording 0.28 g compared to 0.05 g in D3C1 which was less than D2C3 by 82.14%. Table (5) also shows the interaction between cutting types and IBA concentration, as A2C2 gave the highest result (0.21 g) compared to A1C0 (0.14 g). Three-way interaction showed a significant impact on root dry weight of buddleia shrubs, as D2A2C3 treatment gave highest root dry weight by recording 0.34 g compared to D3A1C0 which gave the least result (0.02 g).

**Table 2: Effect of cutting types, cutting dates and IBA concentration on rooting percentage of buddleia shrubs.**

Rooting Percentage (%)						
D x C		C				Mean D
		C0	C1	C2	C3	
D1		88.88 a*	88.88 a	86.66 a	91.11 a	88.88 a
D2		86.66 a	86.66 a-c	80.00 a-c	75.55 a-c	82.22 ab
D3		64.44 c-e	51.11 e	68.88 b-d	66.66 b-e	66.77 c
D4		82.22 ab	77.77 a-c	77.77 a-c	57.77 de	73.88 b
A x C		C0	C1	C2	C3	Mean A
A1		73.33 b-d	68.33 cd	61.66 de	53.33 e	64.16 b
A2		85.00 ab	80.00 a-c	86.66 ab	88.33 a	85 a
A3		88.33 ab	80.00 a-c	86.66 ab	76.66 a-c	81.66 a
Mean C		80.55 a	76.11 a	78.33 a	72.77 a	
D	A	C				D x A
		C0	C1	C2	C3	
D1	A1	80 a-c	86.66 a-c	93.33 ab	80 a-c	88.33 a-c
	A2	86.66 a-c	80 a-c	86.66 a-c	86.66 a-c	86.66 a-c
	A3	100 a	100 a	80 a-c	100 a	91.66 ab
D2	A1	93.33 ab	93.33 ab	73.33 a-c	93.33 ab	81.66 b-d
	A2	86.66 a-c	80 a-c	86.66 a-c	86.66 a-c	81.66 b-d
	A3	80 a-c	86.66 a-c	80 a-c	80 a-c	83.33 a-d
D3	A1	33.33 ef	26.66 f	40 d-f	33.33 ef	33.33 f
	A2	73.33 a-c	60 c-e	73.33 a-c	73.33 a-c	75.00 cd
	A3	86.66 a-c	66.66 b-d	93.33 ab	86.66 a-c	80.00 b-d
D4	A1	86.66 a-c	66.66 b-d	40 def	86.66 a-c	53.33 e
	A2	93.33 ab	100 a	100 a	93.33 ab	96.66 a
	A3	66.66 b-	66.66 b-	93.33 ab	66.66 b-	71.66 d

		d	d		d	
LSD: D= 8.43	LSD: A= 7.30			LSD: C= 8.43		
LSD: D x A= 14.60	LSD: D x C= 16.86		LSD: A x C= 14.60		D x A x C= 29.21	
*Similar mean letters in each column indicates there is no significant difference between them, while different mean letters indicate there is a significant difference between the means according to LSD multiple range test at 95% level ( $\alpha=0.05$ ).						

**Table 3: Effect of cutting types, cutting dates and IBA concentration on longest root of buddleia shrubs.**

Longest root (cm)						
D x C		C				Mean D
		C0	C1	C2	C3	
D1		18.74 a-e	19.83 a-d	19.99 a-c	19.10 a-e	19.41 b
D2		22.84 a	19.90 a-d	22.60 b	23.04 a	22.1 a
D3		12.50 fg	11.50 g	17.03 c-e	15.92 d-f	14.23 d
D4		17.45 c-e	15.71 ef	17.43 c-e	15.72 ef	16.60 c
A x C		C0	C1	C2	C3	Mean A
A1		17.02 a-c	16.70 a-c	19.80 a	19.70a	18.30 a
A2		19.65 a	15.53 c	19.30 ab	19.80 a	18.60 a
A3		16.96 a-c	17.95 a-c	18.73 a-c	15.84 bc	17.40 a
Mean C		17.90 ab	16.72 b	19.30 a	18.43 ab	
D	A	C				D x A
		C0	C1	C2	C3	
D1	A1	16.42 f-k	20.98 b-h	24.62 a-c	21.33 b-h	20.84 ab
	A2	20.83 b-h	16.99 d-k	18.80 b-j	18.55 b-j	18.80 bc
	A3	18.97 b-j	21.52 b-g	16.60 e-k	17.30 d-k	18.60 bc
D2	A1	25.42 ab	21.32 b-h	19.10 b-j	28.95 a	23.70 a
	A2	23.60 a-e	17.03 d-k	23.85 a-d	21.90 b-f	21.60 ab
	A3	19.53 b-i	21.22 b-h	24.80 a-c	18.30 c-k	20.95 ab
D3	A1	8.73 l	8.60 l	18.60 b-j	13.55 i-l	12.40 e
	A2	15.20 f-l	11.32 kl	16.12 f-k	18.70 b-j	15.32 c-e
	A3	13.55 i-l	14.53 h-l	16.40 f-k	15.55 f-l	15.01 de
D4	A1	17.50 d-k	15.80 f-k	16.80 e-k	14.82 g-l	16.22 cd
	A2	19.05 b-	16.80 e-	18.33 c-	20.10 b-	18.60 bc

		j	k	j	i	
	A3	15.80 f- k	14.53 h- l	17.20 d- k	12.30 j-l	14.94 de
LSD: D= 2.01		LSD: A= 1.74				LSD: C= 2.01
LSD: D x A= 3.49		LSD: D x C= 4.03		LSD: A x C= 3.49		D x A x C= 6.98
*Similar mean letters in each column indicates there is no significant difference between them, while different mean letters indicate there is a significant difference between the means according to LSD multiple range test at 95% level ( $\alpha=0.05$ ).						

**Table 4: Effect of cutting types, cutting dates and IBA concentration on root fresh weight of buddleia shrubs.**

Root fresh weight (g)						
D x C		C				Mean D
		C0	C1	C2	C3	
D1		1.30 a	0.99 b	1.32 a	1.34a	1.23 a*
D2		0.73 c	0.82 bc	0.71 c	0.91 bc	0.80 b
D3		0.21 de	0.14 e	0.40 d	0.30 de	0.25 d
D4		0.45 d	0.40 de	0.45 d	0.44 d	0.43 c
A x C		C0	C1	C2	C3	Mean A
A1		0.40 e	0.60 b-d	0.70 a-d	0.62 b-d	0.60 b
A2		0.80 a-d	0.60 cd	0.70 a-d	0.80 a-c	0.71 a
A3		0.85 a	0.60 de	0.80 a-d	0.80 ab	0.75 a
Mean C		0.70 ab	0.60 b	0.72 a	0.74 a	
D	A	C				D x A
		C0	C1	C2	C3	
D1	A1	0.32 l-o	1.15 c-f	1.60 b	1.50 bc	1.4 b
	A2	1.41 b-d	0.98 e-i	1.15 c-f	1.10 c-g	1.20 b
	A3	2.05 a	0.84 e-k	1.21 b-f	1.45 bc	1.40 a
D2	A1	0.51 j-m	0.93 e-j	0.81 f-k	0.50 k-o	0.70 de
	A2	0.99 e-i	0.70 g-l	0.65 h-m	1.30 b-e	0.90 c
	A3	0.70 g-l	0.83 f-k	0.70 g-l	1.02 d-h	0.80 cd
D3	A1	0.05 o	0.10 no	0.10 no	0.05 o	0.10 g
	A2	0.35 l-o	0.10 no	0.43 k-o	0.30 l-o	0.30 cf
	A3	0.30 l-o	0.30 l-o	0.70 h-m	0.45 k-o	0.41 f
D4	A1	0.60 i-m	0.25 m-o	0.32 l-o	0.50 k-n	0.40 f
	A2	0.33 l-o	0.60 i-m	0.52 j-m	0.55 j-m	0.50 ef
	A3	0.42 k-o	0.33 l-o	0.53 j-m	0.30 l-o	0.40f
LSD: D= 0.12		LSD: A= 0.10				LSD: C= 0.12
LSD: D x A= 0.21		LSD: D x C= 0.24		LSD: A x C= 0.21		D x A x C= 0.42
*Similar mean letters in each column indicates there is no significant difference between them, while different mean letters indicate there is a significant difference between the means according to LSD multiple range test at 95% level ( $\alpha=0.05$ ).						

**Table 5: Effect of cutting types, cutting dates and IBA concentration on root fresh weight of buddleia shrubs.**

Root dry weight (g)						
D x C		C				Mean D
		C0	C1	C2	C3	
D1		0.25 a	0.26 a	0.27 a	0.23 a	0.25 a*
D2		0.22 ab	0.24 a	0.21 ab	0.28 a	0.24 a
D3		0.08 cd	0.05 d	0.15 bc	0.10 cd	0.12 b
D4		0.13 c	0.11 cd	0.12 cd	0.10 cd	0.10 b
A x C		C0	C1	C2	C3	Mean A
A1		0.14 b	0.18 ab	0.17 ab	0.15 ab	0.16 a
A2		0.19 ab	0.15 ab	0.21 a	0.20 ab	0.19 a
A3		0.19 ab	0.17 ab	0.19 ab	0.18 ab	0.18 a
Mean C		0.17 a	0.16 a	0.19 a	0.18 a	0.16 a
D	A	C				D x A
		C0	C1	C2	C3	
D1	A1	0.23 a-k	0.32 a	0.30 a-c	0.23 a-k	0.27 a
	A2	0.23 a-l	0.24 a-i	0.27 a-e	0.22 a-m	0.24 a
	A3	0.31 ab	0.21 a-m	0.25 a-h	0.25 a-h	0.25 a
D2	A1	0.17 c-n	0.29 a-d	0.24 a-i	0.22 a-m	0.23 a
	A2	0.24 a-i	0.19 b-m	0.26 a-g	0.34 a	0.26 a
	A3	0.24 a-j	0.26 a-f	0.15 e-o	0.28 a-e	0.23 a
D3	A1	0.02 p	0.02 p	0.05 n-p	0.02 p	0.03 c
	A2	0.13 g-p	0.04 op	0.16 d-o	0.10 k-p	0.11 b
	A3	0.11 j-p	0.09 m-p	0.25 a-h	0.17 c-n	0.15 b
D4	A1	0.13 g-p	0.10 l-p	0.10 l-p	0.13 g-p	0.11 b
	A2	0.17 d-o	0.13 h-p	0.14 f-p	0.13 g-p	0.14 b
	A3	0.11 k-p	0.10 m-p	0.12 i-p	0.05 n-p	0.09 b
LSD: D= 0.03		LSD: A= 0.03				LSD: C= 0.03
LSD: D x A= 0.06		LSD: D x C= 0.07		LSD: A x C= 0.06		D x A x C= 0.12
*Similar mean letters in each column indicates there is no significant difference between them, while different mean letters indicate there is a significant difference between the means according to LSD multiple range test at 95% level ( $\alpha=0.05$ ).						

Results in Table (2) shows that cuttings collected during the first day had the best rooting percentage compared to other dates due to the suitable collecting time as well as

donor plant status in term of nutrients, water and hormones, these in turn improved rooting percentage. While A2 and A3 cuttings gave the highest value, this can be due to the

degree of tissues maturity and content of nutrients, carbohydrates, water and non-structural carbohydrates. Two way interaction shows that D4A2 gave the highest rooting percentage, this can be due to reduced transpiration demands during late season, increased tissue lignification and conservative metabolism, in which they reduced water loss and allowed the cuttings to utilize the remaining reserves efficiently for root development [14, 15]. While applying various doses of IBA to cuttings collected during D1 further improved rooting percentage, this might be due to the favorable timing and large pools of non-structural carbohydrates that amplified root primordia formation, therefore raised rooting percentage because the tissues are already had enough energy and hormonal context to convert IBA into organized adventitious roots [16]. While cuttings collected at D2 might require only 500 mg kg<sup>-1</sup> IBA as in this date a low IBA does can be sufficient to promote root primordial formation without excessive callus formation or phytotoxic effect [7, 17]. In the three-ways interaction the highest rooting percentage was observed in D4A2C2 and D4A2C1 treatments, this is might be due to collecting date of semi-hardwood at D4 which had reduced shoot water demand, an anatomical and maturational state that favored organized primordium formation, and both IBA concentration (C2) and (C1) can efficiently trigger adventitious root initiation without provoking phytotoxicity or excessive callus, differences in uptake may also make nominal doses produce similar effective internal auxin levels in this tissue. On the other hand, D1A3C1 likely reflects synergic effect between high carbohydrate reserves and non-structural carbohydrates at 15<sup>th</sup> of September collecting date and hardwood stems have more developed xylem and phloem, and

thicker stem, therefore once adventitious roots form they rapidly reconnect hydraulically and nutritionally to the shoot, improving establishment and apparent rooting success, combined with 500 mg kg<sup>-1</sup> IBA that provided the necessary exogenous signal to trigger that efficiently induced organized root primordia without promoting excessive callus formation and phytotoxicity [7, 11, 15].

Results in Table (3) shows how study factors significantly affected longest root of the cuttings, as cuttings collected during the second date showed its superiority in longest root this might be due to phenological timing and condition of the donor plant, as it contained sufficient amount of carbohydrates, soluble sugars and optimal cambial and meristematic activity, in turn these factors were in favor of root initiation and elongation of adventitious roots, while applying 1000 mg kg<sup>-1</sup> of IBA improved root initiation, cell expansion and root elongation without causing excessive callus formation or phytotoxicity, as auxin accumulation at the base of the cuttings helped in enhancing callus formation and primordial roots, thereby increased longest root [15]. Similarly, interaction between two study factors showed that higher concentration of IBA can further improve root elongation, however the effect is based on cutting type and tissue maturity as application of IBA can improve elongation only when the collected cuttings has sufficient metabolic activities and proper tissue maturity to support better response and root growth [18]. On the other hand, three way interaction improved longest root, this might be due to the proper collecting date of the soft wood and high IBA dosage, however, longer roots might not necessarily mean good root system as it might lead to longer, thinner and weaker

root system compared to shorter thicker and denser roots [19].

Results in Table (4) shows that root fresh weight was the highest in hardwood cuttings (A3) that collected during the first date (D1), this might be due to the high content of water and mobile carbohydrates, both in turn promoted rapid root growth and development of adventitious roots which are rich in water [20]. On the other hand, C3 increased root fresh weight in the single and two combination factors, this can be due to the role of IBA in promoting the initiation of adventitious roots, root primordium and cell expansion [7]. Furthermore, high content of carbohydrates is essential for a successful adventitious rooting and early root growth as carbohydrates services as a skeleton and energy reservoir for cell division and elongation during the process of root initiation and early growth [21].

Results in Table (5) indicates that D1 treatment gave the highest dry weight, this can be due to the higher carbohydrates and nutrients accumulated in the cuttings during the first date, and cutting collected during the first date might had experienced better environmental conditions which in turn resulted in an increase in photosynthesis process and better carbohydrates accumulation [15]. On the other hand, semi-hardwood collected in the second date and IBA application significantly affected root dry weight of the cuttings, this can be due to the significant effect of auxins such as IBA on increasing root lignification, thickness and carbon accumulation in semi-hardwood cuttings that collected during the second date, it is also known for increasing meristematic activity and structural tissues that in turn increase dry weight of roots, such increase in

dry weight usually indicates good root structure and quality [22].

According to Table (6) the highest leaf number observed from cuttings collected during the second date (48.69), while the least leaf number was observed from the cuttings collected during the third and fourth dates (27.88 and 27.46 leaf plant<sup>-1</sup> respectively). Hardwood cuttings (A3) gave the highest leaf number (37.14 leaf plant<sup>-1</sup>) while semi-hardwood cuttings (A2) gave the least leaf number by recording 33.14 leaf plant<sup>-1</sup>. Similar table shows that different concentrations of IBA affected number of leaves, as the highest result recorded from cuttings treated with 1000 mg kg<sup>-1</sup> (C2) as which recorded 37.76 leaf plant<sup>-1</sup>, while cuttings without IBA treatment (C0) gave the least number of leaf (32.52 leaf plant<sup>-1</sup>). Table (6) also illustrate the interaction effect between two study factors of the experiment on number of leaves per plant. The highest number of leaves was observed from treatment (D2A1) by recording 54.68 leaf plant<sup>-1</sup>, while the least number observed from D3A1 (17.61 leaf plant<sup>-1</sup>) which was less than D2A1 by 67.79%. Interaction effect between cutting dates and IBA concentration showed the highest leaf number in treatment D2C3 (52.34 leaf plant<sup>-1</sup>) and the least leaf number observed from D3C1 (24.38 leaf plant<sup>-1</sup>). Interaction between cutting types and IBA concentration affected leaf number by recording 40.02 leaf plant<sup>-1</sup> in A3C2 which was significantly higher than A2C0 (29.32 leaf plant<sup>-1</sup>) as showed in Table (6). Interaction between all study factors significantly affected number of leaves as indicated in Table (6) as the highest leaf number was observed from treatment combination of D2A1C3 (62.01 leaf plant<sup>-1</sup>) which was significantly higher than that

observed from treatment combination of D3A1C0 by 316.17% as D3A1C0 recorded 14.90 leaf plant<sup>-1</sup>.

Results from Table (7) shows the effect of study factors on longest shoot. According to the results, D1 was significantly higher than D3 by 370.601% as D1 recorded 11.53cm and D3 recorded 2.45cm. Hardwood cuttings (A3) obtained the longest shoot (6.75cm) while softwood cuttings (A1) obtained the shortest shoot (5.24cm). While IBA application showed no significant impact on longest shoot. Interaction between cutting dates and cutting types gave the longest shoot in treatment D1A3 (12.49cm) while the shortest shoot obtained from D3A1(1.25cm). Interaction between cutting dates and IBA concentration gave the longest shoot in treatment D1C0 (12.57 cm) and the shortest shoot recorded from treatment D3C1 (1.83 cm). The longest shoot recorded from the interaction between cutting types and IBA was from treatment A2C2 (7.37 cm) and minimum shoot length recorded from treatment A1C0 (4.55 cm). Table (7) also indicates the significant effect of interaction between all study factors on longest shoot. As indicated in the table below, the longest shoot was recorded from treatment D1A3C0 (16.63 cm) which was significantly higher than D3A1C0 as it recorded the shortest shoot among all treatment (0.70 cm), this result was lower than D1A3C0 by 95.79%.

Table (8) shows the effect of study factors on shoot fresh weight of buddleia shrubs. According to the results, cuttings collected in the first date (D1) gave the highest shoot fresh weight (2.56 g) compared to D2 (0.76 g) which was lower than D1 by 70.31%. Hardwood cuttings (A3) recorded the highest shoot fresh weight (1.97 g) and it was

significantly higher than A1 cuttings (1.34 g). On the other hand, IBA concentration had no significant impact on shoot fresh weight. Interaction effect between two study factors on shoot fresh weight. Highest shoot fresh weight recorded from interaction between cutting dates and cutting types as in D1A3 (2.88 g) which was significantly higher than D3A1 combination as it recorded roughly 0.27 g. The maximum result from interaction between cutting dates and IBA concentration was obtained from D1C0 by recording 2.87g while the minimum shoot fresh weight achieved from D3C1 (0.43 g). Interaction between cutting types and IBA concentration recorded the highest shoot fresh weight in treatment A3C2 (2.13 g) which was higher than A1C3 (1.17 g) by 82.05%. Three-ways interaction between study factors gave the highest result in D1A3C0 treatment as it was significantly higher than treatment D3A1C3 by 2342% as treatment D3A1C3 recorded only 0.17g of shoot fresh weight.

Table (9) shows the results from impact of cutting dates, cutting types and IBA concentration on shoot dry weight. According to the results, cuttings collected during the first dates (D1) showed a significant impact on shoot dry weight as it recorded 0.65g compared to the third date (D3) which recorded the least shoot dry weight (0.27 g). Hardwood cuttings (A3) obtained the highest shoot dry weight (0.60 g) and the minimum shoot dry weight obtained from softwood cuttings (A1) by recording 0.36 g. IBA concentration showed no significant impact on shoot dry weight. Effect of interaction between two study factors on shoot dry weight gave the highest shoot dry weight in treatment D1A3 by obtaining 0.75 g, and the minimum result obtained from D3A1 (0.06 g). The highest shoot dry weight recorded

from interaction between cutting dates (D) and IBA concentration (B) was in treatment D1C0 (0.70 g) and the least dry weight recorded from D3C1 (0.15 g). Interaction between cutting types (A) and IBA concentration (B) gave the best results in treatment A3C2 by recording 0.64g and least result recorded from treatment A1C3 (0.30 g). Interaction between all study factors gave the

highest shoot dry from treatment D1A3C0 (0.99 g) as it was significantly higher than D3A1C0 which recorded the least shoot dry weight (0.02 g), this result was lower than D1A3C0 by 97.97%.

**Table 6: Effect of cutting types, cutting dates and IBA concentration on number of leaves per plant of buddleia shrubs.**

Number of leaves per plant (leaf plant <sup>-1</sup> )						
D x C		C				Mean D
		C0	C1	C2	C3	
D1		37.09 bc	35.79 bc	36.74 bc	31.48 cd	35.28 b*
D2		44.00 ab	50.01 a	48.42 a	52.34 a	48.69 a
D3		24.38 de	21.55 e	35.79 bc	29.79 c-e	27.88 c
D4		24.60 de	31.22 cd	30.07 cde	23.96 de	27.46 c
A x C		C0	C1	C2	C3	Mean A
A1		32.91 ab	36.56 ab	34.67 ab	32.66 ab	34.20 ab
A2		29.53 b	30.45 b	38.40 a	34.17 ab	33.14 b
A3		35.11 ab	36.92 ab	40.20 a	36.34 ab	37.14 a
Mean C		32.52 b	34.64 ab	37.76 a	34.39 ab	
D	A	C				D x A
		C0	C1	C2	C3	
D1	A1	43.17 c-i	40.35 c-j	36.44 d-m	28.33 i-o	37.07 c
	A2	29.44 h-o	27.56 j-o	48.35 a-e	32.78 f-n	34.53 cd
	A3	38.67 c-k	39.46 c-k	25.45 j-o	33.34 e-n	34.23 cd
D2	A1	44.45 c-h	61.55 ab	50.70 a-d	62.01 a	54.68 a
	A2	47.11 a-f	43.56 c-i	46.13 b-g	47.23 a-f	46.01 b
	A3	40.44 c-j	44.91 c-g	48.44 a-e	47.77 a-f	45.39 b
D3	A1	14.90 o	15.33 o	22.23 l-o	18.00 no	17.61 f
	A2	21.34m-	24.66 k-	31.57 g-	25.57 j-	25.79 e

		o	o	n	o	
	A3	36.90 d-l	24.66 k-o	53.57 a-c	45.80 c-g	40.23 bc
D4	A1	29.13 h-o	29.01 i-o	29.33 h-o	22.33 l-o	27.45 de
	A2	20.23 no	26.01 j-o	27.55 j-o	31.11 g-n	26.22 e
	A3	24.44 k-o	38.66 c-k	33.33 e-n	18.44 no	28.72 de
LSD: D= 4.45		LSD: A= 3.86			LSD: C= 4.45	
LSD: D x A= 7.72		LSD: D x C= 8.91	LSD: A x C= 7.72		D x A x C= 15.44	
*Similar mean letters in each column indicates there is no significant difference between them, while different mean letters indicate there is a significant difference between the means according to LSD multiple range test at 95% level ( $\alpha=0.05$ ).						

**Table 7: Effect of cutting types, cutting dates and IBA concentration on longest shoot of buddleia shrubs.**

Longest shoot (cm)						
D x C		C				Mean D
		C0	C1	C2	C3	
D1		12.57 a	11.33 ab	12.37 a	9.84 b	11.53 a
D2		4.53 c-e	5.74 c	4.77 c-e	6.05 c	5.27 b
D3		2.51 f	1.83 f	2.35 f	3.12 ef	2.45 c
D4		3.60 def	5.27 cd	5.63 c	4.96 c-e	4.87 b
A x C		C0	C1	C2	C3	Mean A
A1		4.55 c	6.16 a-c	4.63 c	5.64 bc	5.24 b
A2		5.73 a-c	5.74 a-c	7.37 a	5.56 bc	6.10 ab
A3		7.12 ab	6.24 a-c	6.85 ab	6.78 ab	6.75 a
Mean C		5.80 a	6.04 a	6.28 a	5.99 a	
D	A	C				D x A
		C0	C1	C2	C3	
D1	A1	8.91 b-f	11.70 b	9.64 b-e	11.00 bc	10.31 b
	A2	12.17 b	12.10 b	16.17 a	6.67 e-h	11.78 ab
	A3	16.63 a	10.20 b-d	11.31 b	11.84 b	12.49 a
D2	A1	4.59 g-n	6.71 e-h	3.67 h-o	4.28 g-n	4.81 cd
	A2	4.49 g-n	4.36 g-n	5.46 g-l	7.04 d-h	5.34 cd
	A3	4.51 g-n	6.16 f-i	5.18 g-l	6.83 d-h	5.67 c
D3	A1	0.70 o	1.26 no	1.46 no	1.60 m-o	1.25 f
	A2	2.63 j-o	1.45 no	2.37 l-o	2.45 k-o	2.22 ef
	A3	4.21 h-n	2.77 i-o	3.21 i-o	5.32 g-l	3.87 de
D4	A1	4.01 h-o	4.96 g-m	3.73 h-o	5.70 f-l	4.60 cd
	A2	3.63 h-o	5.04 g-l	5.46 g-l	6.06 f-j	5.05 cd
	A3	3.16 i-o	5.82 f-k	7.71 c-g	3.13 i-o	4.95 cd
LSD: D= 0.92		LSD: A= 0.85				LSD: C= 0.92
LSD: D x A=1.71		LSD: D x C= 1.98		LSD: A x C= 1.71		D x A x C= 3.43
*Similar mean letters in each column indicates there is no significant difference between them, while different mean letters indicate there is a significant difference between the means according to LSD multiple range test at 95% level ( $\alpha=0.05$ ).						

**Table 8: Effect of cutting types, cutting dates and IBA concentration on shoot fresh weight of buddleia shrubs.**

Shoot fresh weight (g)						
D x C		C				Mean D
		C0	C1	C2	C3	
D1		2.83 a	2.12 bc	2.73 a	2.50 ab	2.56 a*
D2		1.59 de	2.01 b-d	1.83 c-e	2.00 cd	1.86 b
D3		0.88 gh	0.43 h	0.88 gh	0.82 gh	0.76 d
D4		1.00 fg	1.54 de	1.46 ef	1.46 ef	1.36 c
A x C		C0	C1	C2	C3	Mean A
A1		1.52 bc	1.46 bc	1.21 c	1.17 c	1.34 c
A2		1.46 bc	1.27 c	1.84 ab	1.81 ab	1.59 b
A3		1.78 ab	1.85 ab	2.13 a	2.11 a	1.97 a
Mean C		1.59 a	1.53 a	1.73 a	1.69 a	
D	A	C				D x A
		C0	C1	C2	C3	
D1	A1	2.82 a-c	2.42 b-f	2.15 c-h	2.25 b-h	2.24 b
	A2	2.35 b-g	1.61 f-m	3.37 a	2.22 b-h	2.38 b
	A3	3.45 a	2.35 b-f	2.68 a-d	3.04 ab	2.88 a
D2	A1	1.40 h-p	2.37 b-f	1.68 f-l	1.03 j-r	1.62 de
	A2	1.76 f-k	1.49 g-n	1.85 d-j	2.33 b-g	1.86 cd
	A3	1.62 f-m	2.19 b-h	1.97 c-i	2.65 a-e	2.10 bc
D3	A1	0.44 q-s	0.23 rs	0.24 rs	0.17 s	0.27 g
	A2	0.67 n-s	0.30 rs	0.55 p-s	0.51 q-s	0.53 g
	A3	1.45 h-o	0.77 m-s	1.87 d-j	1.79 e-k	1.47 def
D4	A1	1.43 h-o	0.82 l-s	0.79 m-s	1.23 i-q	1.07 f
	A2	0.98 k-s	1.70 f-k	1.58 f-n	0.18 c-h	1.61 de
	A3	0.60 o-s	2.10 c-h	2.01 c-i	0.96 k-s	1.42 ef
LSD: D= 0.24		LSD: A= 0.21				LSD: C= 0.24
LSD: D x A= 0.42		LSD: D x C= 0.49		LSD: A x C= 0.42		D x A x C= 0.85
*Similar mean letters in each column indicates there is no significant difference between them, while different mean letters indicate there is a significant difference between the means according to LSD multiple range test at 95% level ( $\alpha=0.05$ ).						

**Table 9: Effect of cutting types, cutting dates and IBA concentration on shoot dry weight of buddleia shrubs.**

Shoot dry weight (g)						
D x C		C				Mean D
		C0	C1	C2	C3	
D1		0.70 a	0.67 a	0.66 ab	0.58 a-d	0.65 a
D2		0.48 b-f	0.68 a	0.54 a-e	0.62 a-c	0.58 a
D3		0.26 gh	0.15 h	0.39 e-g	0.30 f-h	0.27 c
D4		0.36 e-g	0.46 c-f	0.45 c-f	0.43 d-g	0.42 b
A x C		C0	C1	C2	C3	Mean A
A1		0.36 ef	0.46 b-e	0.34 ef	0.30 f	0.36 c
A2		0.44 c-f	0.41 def	0.55 a-d	0.53 a-d	0.49 b
A3		0.55 a-d	0.59 a-c	0.64 a	0.62 ab	0.60 a
Mean C		0.45 a	0.49 a	0.51 a	0.48 a	
D	A	C				D x A
		C0	C1	C2	C3	
D1	A1	0.48 d-k	0.71 a-e	0.55 b-j	0.42 e-k	0.54 b-d
	A2	0.64 b-e	0.58 b-h	0.85 ab	0.60 b-g	0.67 ab
	A3	0.99 a	0.72 a-e	0.58 b-h	0.71 a-e	0.75 a
D2	A1	0.45 e-k	0.79 a-d	0.50 d-k	0.30 g-l	0.51 c-e
	A2	0.56 b-i	0.48 d-k	0.60 b-g	0.71 a-e	0.59 b-d
	A3	0.45 e-k	0.77 a-d	0.52 c-j	0.85 ab	0.65 a-c
D3	A1	0.02 l	0.08 l	0.07 l	0.05 l	0.06 g
	A2	0.28 h-l	0.10 l	0.27 h-l	0.19 kl	0.21 fg
	A3	0.47 d-k	0.26 h-l	0.84 a-c	0.67 b-e	0.56 b-d
D4	A1	0.48 d-k	0.27 h-l	0.24 i-l	0.42 e-k	0.35 ef
	A2	0.30 f-l	0.50 d-k	0.50 d-k	0.64 b-e	0.48 de
	A3	0.30 f-l	0.62 b-f	0.62 b-f	0.24 j-l	0.44 de
LSD: D= 0.09		LSD: A= 0.08				LSD: C= 0.09
LSD: D x A= 0.16		LSD: D x C= 0.18		LSD: A x C= 0.16		D x A x C= 0.31
*Similar mean letters in each column indicates there is no significant difference between them, while different mean letters indicate there is a significant difference between the means according to LSD multiple range test at 95% level ( $\alpha=0.05$ ).						

Results show that D1 advantage is clearly based on quantity and timing of non-structural carbohydrates (NSC) reserves in donor plant, these non-structural carbohydrates include sucrose, glucose, fructose and starch which are stored in donor plant's stem and nodes that

provided carbon and ATP required for cell wall biosynthesis, cell division, and osmotic activity during the early stage after collecting the cuttings when xylem continuity and root uptake of nutrients and water are still limited. Therefore, high NSC during D1 collecting

date supported two essential processes: rapid cell enlargement due to internal water pressure (turgor-driven cells), the second process is the biosynthetic phase of metabolism (anabolic phase), involving the deposition of structural carbohydrates and lignin precursors that might have had increased shoot dry weights (Table 9). In counterpart, D1 hormonal profile (auxin: cytokinin optimum ratio) and active cambial and meristematic tissues allowed organized roots primordia formation and efficient vascular differentiation as when the roots developed, they rapidly became functional for water and nutrients uptake rather than just being bypassed by callus. Thus, this might have had denser leaves and shoots (Tables 6 and 8 respectively) in cuttings collected during D1 [15, 21]. Single factor of cuttings type and specifically A3 (hardwood cuttings) clarifies how structural and storage traits of lignified stems that contain starch and minerals reserves and possess further developed xylem and phloem network as well as thicker stem cross section, which in turn increased hydraulic conductivity and assimilate transport once root system is re-established. These traits allowed rapid allocation of assimilates to growing sinks such as leaves and shoots, and support maintenance of leaf pigments and density per unit area, this might be the cause of yielding more leaves per plant, longer shoot, and higher shoot fresh weight as showed in Tables (6-8 respectively). Regarding IBA concentration influence as a single factor, in most occasions IBA regardless the doses that used, had no significant effect, however in some cases such as number of leaves per plant (Table 6), implementing 1000 mg kg<sup>-1</sup> (C2) might have affected root initiation, division, and vascular differentiation stimulation without inducing excessive undifferentiated callus or

phytotoxicity. Furthermore, improved early root initiation at 1000 mg kg<sup>-1</sup> dosage might have led to improved water and nutrients uptake specifically nitrogen and magnesium in which they are an essential element for chlorophyll biosynthesis, this in turn enabled higher leaves formation and higher shoot biomass [7, 17].

Interaction combination of two study factors showed contrasting outcomes. For instance, interaction between D and A had various outcomes (Tables 6-9). Such outcomes suggests that it might be due to the phenological stage of donor plant during D1 and D2 collecting dates as they might have provided ideal internal physiological condition such as balanced carbohydrates, nutrients (specifically N and Mg), and endogenous hormones. Cuttings collected during D1 and D2 might exhibit high metabolic activities leading to better overall vegetative growth. Furthermore, suitable environment condition within the place of propagation might also contributed in having better growth result [21]. While the syringic effect of cutting dates and IBA concentrations produced contrasting outcomes, different concentration had different impact on each parameter, this might be due to the physiological state of the donor plant during D1 and D2, hormonal balance and metabolic activities, as IBA acted as a trigger to initiate root formation and efficiently channeling these abundant resources toward roots primordial. While applying the same concentration to other dates did not yield the same results, thus phenological timing, hormonal balance and metabolic activities were at best during D1 and D2. Similarly, the synergic effect of cutting types and IBA concentrations showed different outcomes, this is probably due to it

dependency on cutting maturity, as each type had different concentration of stored carbohydrates, endogenous auxin levels, degree of lignification and vascular development. For instance, interaction effect in number of leaves per plant (Table 6), and shoot fresh and dry weights (Tables 8 and 9 respectively), this might be due to high NSC reserves and better xylem and phloem network, thus when applying moderate exogenous auxin (1000 mg kg<sup>-1</sup> IBA) it helped in quickly developing adventitious roots and quickly became functional that resumed water and nutrients uptake, which in turn increased vegetative performance [7, 15, 23].

Three-ways interaction of D2A1C3 showed the highest leaf number (Table 6), this might

### Conclusions

Based on the findings from this experiment, it can be concluded that cuttings collected during 15<sup>th</sup> of September (D1) and 1<sup>st</sup> of October (D2) significantly affected root and vegetative growths, hardwood cuttings (A3) had overall the best performance in terms of root and vegetative growths, an IBA concentration of 1000 mg kg<sup>-1</sup> (C2) improved some root and vegetative growths

### Recommendations

It is recommended collecting cuttings for buddleia shrubs during 15<sup>th</sup> of September and 1<sup>st</sup> of November in order to gain the best root and vegetative growths. Use semi-hardwood

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be due to active meristems and leaf primordia that retained in soft wood cuttings collected during D2, when applying 1500 mg kg<sup>-1</sup> of IBA it promoted strong root initiation elongation which in turn rapidly supplied the plant with water and nutrients to the new shoots which it affected number of leaves and significantly increased it [7, 14]. D1A2C2 showed highest shoot fresh weight (Table 8), this is likely due to the positive effect of moderate IBA concentration and suitable collecting time of A2 cuttings, as it was able to promote functional adventitious roots without excessive callus formation, and with the sufficient supply of carbohydrates to support rapid water retention and cell expansion, they led to water rich shoots, longer shoot, and high leaf dry weight [7].

characteristics. Interaction between two study factors (D x A), (D x C), and (A x C), and three-ways interaction between study factors (D x A x C) achieved various outcomes, and the best outcomes noted was from interaction between cutting dates (D1 and D2), cutting types (A2 and A3), and IBA concentration (C2).

and hardwood cuttings when propagating buddleia shrubs. Utilizing 1000 mg kg<sup>-1</sup> IBA for ten seconds to get the best outcomes.

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