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Efficacy of Different IBA Concentrations and Medium Types on Rooting of *Euphorbia milii* Cutting

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Abstract

Cuttings of *Euphorbia milii* were treated with different concentrations (0, 1000, 2000 or 4000 mg/l) of indole butyric acid (IBA) before culturing in different media types [sand: peat moss (1:1, v/v), sand or peat moss] to investigate the impact of these treatments on rooting and shooting growth characters. Results indicated that treating cuttings with 4000 mg/l IBA before culturing in sand: peat moss (1:1, v/v) medium or sand medium gave the ultimate values of all investigated traits (rooting%, No. of roots/cutting, root length, root fresh and dry weights, No. of shoots/cutting, shoot length, No. of leaves/shoot as well as shoot fresh and dry weights).

Conclusion: from the economical point of view sand medium is preferable because it is cheaper than sand + peat moss (1:1, v/v) medium.

Key words: *Euphorbia milii*, indole butyric acid (IBA), peat moss, sand, cutting, propagation

Introduction

Euphorbia milii is belonging to family *Euphorbiaceae*. It has many common names such as; Christ thorn, Christ plant and Crown of thorns. This specie is a flowering succulent plant. It is highly estimated for its elegance inflorescence, extended flowering season and the tolerance to inapplicable conditions (Jankalski, 2000). Also, it can grow around the year in dry, temperate and intense sun light radiation locations as pot plant, bedding or garden plants (Jankalski, 2000). *Euphorbia milii* is widely used as pot plant for its attractive inflorescences. Moreover, many important medicinal ingredients have been identified in this plant such as; anthraquinones, saponins, cardiac glycosides, and flavonoids (Ashfaq *et al*., 2022). The leaves of *E. milii* are used in folk medicine as antiseptic, antibacterial, anticancer, anti-inflammatory and for mild hypersensitive (Okwu, 2005 and Pradyutha *et al*., 2015).

Discovering of auxin effect on stimulation the adventitious root formation in cuttings was a major development for commercial plant propagation (Arteca, 1996). The manufactured auxins such as indole-3-butyric acid and 1-naphthaleneacetic acid were proved to be more efficient than indole acetic acid in root formation (Zimmerman and Wilcoxon, 1935). So that, IBA and NAA are the most commonly used auxins to encourage root formation on stem cutting. Treating with auxins is widely used for commercial plant propagation to improve rooting percentages, accelerate root initiation and enhances the number of initiated roots and its quality as well as to promote homogeneity of rooting (Hartmann *et al*., 2002 and Macdonald, 1987).

Rooting medium is one of the most important factors affecting cutting rooting. A rooting medium has four functions. These are water supply, cutting support, oxygen supply for the metabolic processes of root formation and growth, and provision of a dark environment for root formation (Loach, 1985; Hartmann *et al*., 2002). Rooting percentage and root development on cutting are impacted by rooting medium, which is an essential part for propagation by cutting. Insufficient rooting can be caused by the rooting medium used, and in

spite of its obvious disadvantages, pure sand has recently become more popular for economic reasons (Loach, 1985).

E. millii is mainly propagated by tip cuttings however progeny numbers are limited (Dewir *et al*., 2005). However, *in vitro* propagation of this plant represents an alternative method for propagation; micropropagation is highly expensive and often requires a lot of work (Saranga and Cameron, 2007).

So that the main objective of this paper is to evaluate the impact of indole butyric acid concentration and propagation medium type on *Euphorbia milii* cutting rooting ability and root growth.

MATERIALS AND METHODS

This work was carried out during the two consecutive seasons of 2019 and 2020 under the green-house conditions of Faculty of Agriculture, Zagazig University, Egypt, to study the effect of different indole butyric acid (IBA) concentrations (0, 1000, 2000 or 4000 mg/l), media types [sand: peat moss (1:1, v/v, M1), sand (M2) or peat moss (M3)] and their combinations on *Euphorbia milii* cutting rooting and shooting growth characters.

The experiment included 12 treatments, which were the combinations between four IBA concentrations and three media types. This experiment was designed as factorial experiment between the above mentioned IBA concentrations and media types in a complete randomized block design with three replicates, each replicate contained twelve pots.

Terminal cuttings were obtained on 30th of March, for both tested seasons, from three years old *Euphorbia milii* stock plants grown under open field conditions. Cuttings had uniform length (10 cm) and diameter (about 1-1.5 cm) were collected and maintained overnight to prevent latex accumulation at basal end. After that leaves were removed from cutting.

Cutting bases (5.0 cm) were dipped in different IBA treatments for 10 seconds. Then, cuttings were planted in 20 cm diameter plastic pots, one cutting/pot, filled with one of the above mentioned media. Pots were maintained in the green-house conditions with air temperature ranged from 25 to 30 ^{o}C, relative humidity between 70 to 85%. Throughout the experimental period during both seasons, pots were irrigated whenever needed.

After six weeks the following data were recorded; rooting (%), No. of roots/cutting, root length (cm), root diameter (mm), root fresh and dry weights/ cutting (g), number of shoots/cutting, shoot length (cm), number of leaves/shoot as well as shoot fresh and weights (g).

The obtained data were statistically analyzed with analysis of variance (ANOVA) procedure using the MSTAT-C Statistical Software Package (Michigan State University, 1983). Differences between means were compared by using Duncan multiple range test (Gomez and Gomez. 1984).

RESULTS

As shown in Fig. 1 and 2 the maximum rooting percentage (100%) was recorded during first season as cuttings were treated with 4000 mg/l IBA before culturing in M1 medium. The same treatment gave the ultimate value (100%) of this parameter during second season; however there was no difference between this treatment and the treatment of 4000 mg/l IBA + M2.

The data presented in Table 1 show that increasing of IBA concentration was concomitant with gradual significant increase in number of roots/cutting. The maximum main value (38.90 and 40.38 root/cutting) of this parameter was recorded with the highest IBA concentration (4000 mg/l) during both seasons, respectively. The main effect of rooting medium indicated that M2 medium was the most suitable medium for elevating the number of initiated roots on cutting. The analysis of interaction effect demonstrated that the interaction between the highest IBA concentration (4000 mg/l) and M2 medium gave the ultimate value (51.66 and 53.50 root/cutting) of this character during both seasons, respectively.

With the reference to Table 2 it is clear that there was a positive linear relationship between IBA concentration and root length. The main of longest root (10.55 and 12.25 cm) was detected with the highest IBA

concentration during both seasons, respectively. The main effect of medium type proved that M1 medium was the best medium for root elongation. Analysis of variance of the interaction effect stated that the interaction between the ultimate IBA concentration and M1 medium surpassed all other tested combinations and gained the tallest root length (12.70 and 14.83 cm) during both seasons, respectively.

Root diameter was enhanced by increasing of IBA concentration since the thickest root (9.02 and 9.02 mm) was observed with the maximum IBA concentration during both seasons, respectively (Table 3). The main effect of medium type cleared that M3 medium encouraged root thickness compared with other investigated media. Concerning the impact of interaction between IBA concentration and medium type it is clear that the combination between 4000 mg/l IBA and M3 medium resulted in the thickest root (11.58 and 10.90 cm) during both seasons, respectively.

Root fresh weight was improved as IBA concentration increased. The highest IBA concentration produced the heaviest fresh root (6.66 and 7.01 g) during both seasons, respectively (Table 4). The main effect of medium type show that the maximum fresh root weight obtained with M2 medium. The interaction between different IBA concentrations and medium types clearly demonstrate that using of 4000 mg/l IBA combined with M2 medium attained the heaviest root fresh (8.91 and 9.08 g) during both seasons, respectively.

Root dry weight followed the similar trend of root fresh weight since the heaviest root dry weight (1.666 and 1.583 g) was discovered when cuttings were treated with 4000 mg/l IBA and cultured in M2 medium during both seasons, respectively (Table 5).

As shown in Table 6 there was a gradual increase in number of shoots/cutting as IBA concentration enhanced. The highest IBA concentration surpassed the other concentrations in this regard and recorded 2.30 and 2.27 shoot/cutting as main values during both seasons, respectively. M1 medium seems to be the best medium for raising the number of produced shoots on cutting. Although, there was no significant difference between this medium and M2 medium during first season only. The interaction between different media and different IBA concentrations referred to the superiority of the combination between 4000 mg/l IBA and M1 medium compared with all investigated combinations. This combination gained the maximum values (3.16 and 3.00 shoot/cutting) of this trait during both seasons, respectively.

Elevating of IBA concentration resulted in stimulating of shoot elongation, but without significant difference between 2000 and 4000 mg/l concentrations (Table 7). M1 medium substantiated to be the best medium to improve this parameter. Consulting the interaction combination treatments shows that the combinations between 2000 or 4000 mg/l and M1 medium were exceeded all other examined combinations without significant difference between both treatments in this respect.

Linear relationship between IBA concentration and shoot fresh weight was detected (Tale 8). The heaviest fresh shoot main value (29.36 and 31.61 g) was recorded with 4000 mg/l IBA treatment during both seasons, respectively. However, during first season the highest value of this parameter was discovered with M1 medium but without significant differences between this medium and other examined media. During second season M1 medium proved to be the best medium in this regard. Analysis of variance for interaction between both investigated factors demonstrated that cuttings treated with 4000 mg/l IBA and cultured in M1 medium recorded the heaviest fresh weight of shoot (31.3 and 36.5 g) during both seasons, respectively.

As IBA level increased shoot dry weight improved (Table 9). The maximum shoot dry weight (3.28 and 5.00 g) was observed with the highest IBA concentration during both seasons, respectively. While there were no significant differences among tested media during first season, M1 medium seems to be the most suitable medium for enhancing shoot dry weight. The interaction combinations results clear that the combination of 4000 mg/l IBA + M1 medium produced the maximum values (4.75 and 6.00 g) of this character during both seasons, respectively.

DISCUSSION

A perusal of the above mentioned results show that rooting percentage and all recorded root initiation and growth parameters as well as shoot growth traits were improved by increasing IBA applied concentration.

These results were in accordance with those noticed by; Rehana *et al*. (2020), Kumar *et al*. (2020), Otiende and Maimba (2020), Rajamanickam *et al*. (2021) and Solgi *et al*. (2022).

This promotive impact of IBA on root initiation and growth may be attributed to the stimulatory effect of auxin on cambial activity which resulted in the translocation of stored assimilates to root initiation location as mentioned by Gurumurthy *et al*. (1984). Also, treating of cutting with auxin resulted in degradation of starch in to monosaccharides, which is required to a further extent for new cells formation and for enhancing respiratory activity in the new produced tissue during the emergence of new root primordia (Nanda, 1975). The improvement in root growth as a result to auxin application could be attributed to the buildup of metabolites at treating zone which encourage cell division, cell enlargement and stimulate carbohydrates hydrolysis which supply the meristematic tissues of root primordia with energy (Ezekiel, 2010).

The stimulatory effect of auxin on shoot growth may be as a reflection to the improve in rooting percentage and root growth after treating cutting with auxin which resulted in enhancing the uptake of water and nutrients from the culture medium. Also, auxin has an importance for cell division, enlargement and differentiation. Moreover, auxin is used as signals between cells, tissues and organs (Davies, 2010).

Consulting the abovementioned results concerning the effect of culture medium type on cutting, it is clear that the maximum values of rooting percentage, root length, shoot length, shoot fresh and shoot dry weight were recoded when cutting was cultured in M1 medium (Sand: Peat moss, 1: 1, v/v); while the ultimate values of No. of roots/cutting, root fresh weight and root dry weight were estimated by using M2 medium (sand). On the other side, M3 medium (peat moss) gave the lowest values of all measured characters, except root diameter. Both media (M1 and M2) were better than M3 medium (peat moss).

Similar results were earlier mentioned by Dolor *et al*. (2009) and Hammo *et al*. (2013). The superiority of sand or the mixture of sand and peat moss over peat moss alone may be attributed to the fact that M1 and M2 media had better aeration and drainage compared to peat moss medium (M3).

Hartmann *et al*. (2002) reported that the ideal propagation media must provide porosity to supply good aeration. They also revealed that rooting is declined when cutting is cultured in propagation medium has high holding water capacity with small air pore space. When more than 80% to 90% of the soil's pore space is filled with water, aeration is poor and plant growth is inhibited (Brady and Weil, 1999).

From our data it is clear that the combinations of 4000 mg/l IBA + M1 or M2 medium gave the ultimate values of all investigated traits. However, from the economical point of view M2 medium (sand) is preferable because it is cheaper than M1 medium (sand + peat moss, 1:1, v/v).

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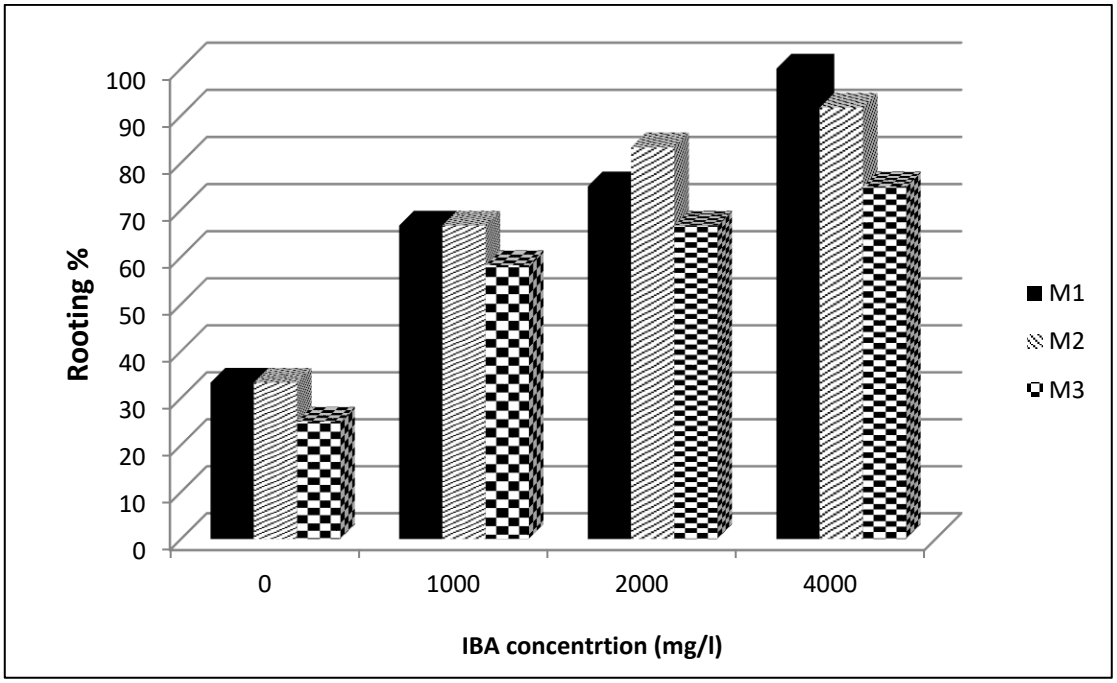

Fig. 1. Effect of medium type and IBA concentration on rooting % of *Euphorbia milii* cutting after six weeks during first season. (M1) sand: peat moss (1:1, v/v), (M2) sand and (M3) peat moss.

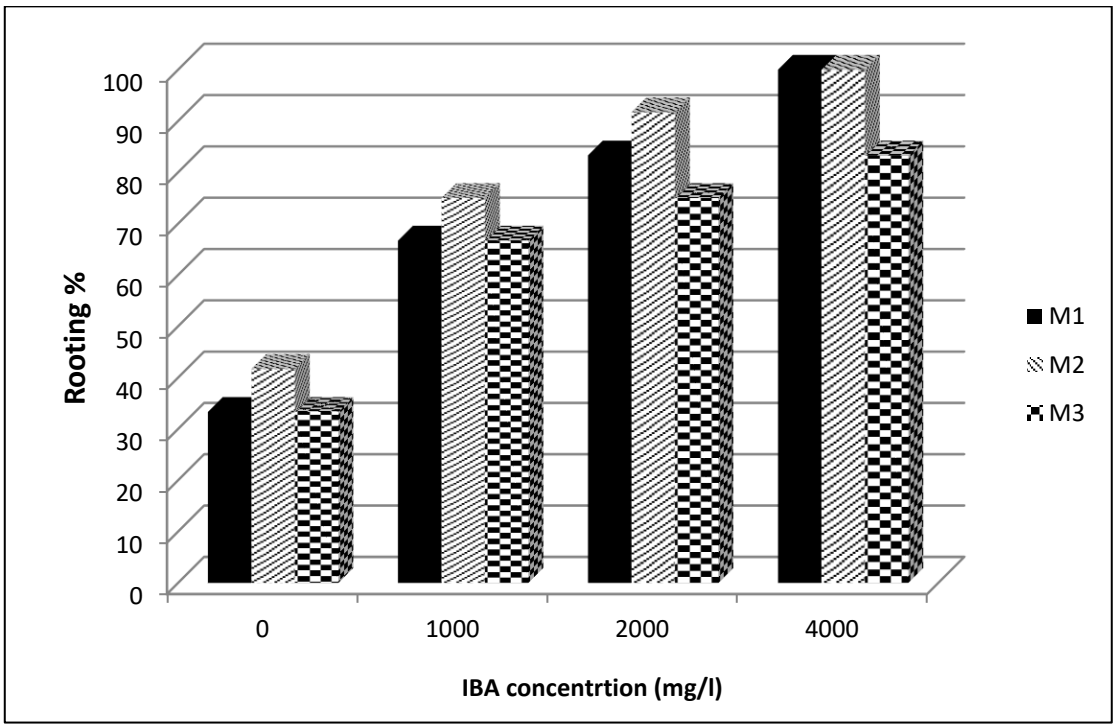

Fig. 2. Effect of medium type and IBA concentration on rooting % of *Euphorbia milii* cutting after six weeks during second season. (M1) sand: peat moss (1:1, v/v), (M2) sand and (M3) peat moss.

Table 1. Effect of medium type and IBA concentration on number of roots/cutting of *Euphorbia milii* after six weeks during both seasons

IBA Conc. (mg/l)	First season				Second season			
	Medium type			Mean	Medium type			Mean
	M1	M2	M3		M1	M2	M3	
0.0	8.16 e	16.91 d	9.66 e	11.58 D	8.58 ef	14.91 d	7.66 f	10.38 D
1000	12.58 de	24.00 c	13.04 de	16.54 C	13.75 d	26.33 c	12.66 de	17.58 C
2000	12.50 de	39.08 b	12.33 de	21.30 B	14.00 d	41.25 b	12.16 de	22.47 B
4000	41.33 b	51.66 a	23.70 c	38.90 A	42.58 b	53.50 a	25.08 c	40.38 A
Mean	18.64 B	32.91 A	14.68 C		19.72 B	34.00 A	14.39 C	

M1 (sand: peat moss, 1:1, v/v), (M2) sand and (M3) peat moss

Table 2. Effect of medium type and IBA concentration on root length (cm) of *Euphorbia milii* cutting after six weeks during both seasons

IBA Conc. (mg/l)	First season				Second season			
	Medium type			Mean	Medium type			Mean
	M1	M2	M3		M1	M2	M3	
0.0	3.50 g	3.70 g	5.16 fg	4.12 D	3.91 g	4.25 g	4.25 g	4.34 D
1000	7.75 cde	4.91fg	5.95 ef	6.20 C	8.04 cd	6.00 ef	6.00 ef	6.75 C
2000	8.16 cd	7.95 cd	6.37 def	7.50 B	8.87 c	8.45 c	8.45 c	8.02 B
4000	12.70 a	10.37 b	8.58 bc	10.55 A	14.83 a	11.29 b	11.29 b	12.25 A
Mean	8.03 A	6.73 B	6.52 B		8.91 A	7.50 B	7.11 B	

M1 (sand: peat moss, 1:1, v/v), (M2) sand and (M3) peat moss

Table 3. Effect of medium type and IBA concentration on root diameter (mm) of *Euphorbia milii* cutting after six weeks during both seasons

IBA Conc. (mg/l)	First season				Second season			
	Medium type			Mean	Medium type			Mean
	M1	M2	M3		M1	M2	M3	
0.0	4.33 f	4.35 f	5.06 ef	4.58 C	5.61 ef	4.65 f	5.80 ef	5.35 C
1000	6.48 cde	5.24 def	7.30 c	6.34 B	5.98 ef	5.33 f	6.10 def	5.80 C
2000	7.15 cd	4.96 ef	9.78 ab	7.30 B	7.13 cde	5.53 ef	9.11 b	7.26 B
4000	8.01 bc	7.46 c	11.58 a	9.02 A	8.50 bc	7.68 bcd	10.90 a	9.02 A
Mean	6.49 B	5.50 B	8.43 A		6.80 B	5.80 C	7.97 A	

M1 (sand: peat moss, 1:1, v/v), (M2) sand and (M3) peat moss

Table 4. Effect of medium type and IBA concentration on root fresh weight (g) of *Euphorbia milii* cutting after six weeks during both seasons

IBA Conc. (mg/l)	First season				Second season			
	Medium type			Mean	Medium type			Mean
	M1	M2	M3		M1	M2	M3	
0.0	1.95 d	2.08 d	1.75 d	1.93 D	1.91 de	2.00 de	1.50 e	1.80 D
1000	2.25 d	4.28 c	2.29 d	2.94 C	2.20 de	4.58 c	2.20 de	3.00 C
2000	2.41 d	6.87 b	2.29 d	3.86 B	2.37 d	6.70 b	2.41 d	3.83 B
4000	7.08 b	8.91 a	4.00 c	6.66 A	7.20 b	9.08 a	4.75 c	7.01 A
Mean	3.42 B	5.53 A	2.58 C		3.42 B	5.59 A	2.71 C	

M1 (sand: peat moss, 1:1, v/v), (M2) sand and (M3) peat moss

Table 5. Effect of medium type and IBA concentration on root dry weight (g) of *Euphorbia milii* cutting after six weeks during both seasons

IBA Conc. (mg/l)	First season				Second season			
	Medium type			Mean	Medium type			Mean
	M1	M2	M3		M1	M2	M3	
0.0	0.500 fg	0.508 fg	0.458 g	0.488 D	0.500 e	0.541 e	0.500 e	0.513 D
1000	0.625 d-g	0.700 def	0.583 efg	0.636 C	0.583 e	0.958 d	0.583 e	0.708 C
2000	0.750 de	0.958 c	0.625 d-g	0.777 B	0.625 e	1.208 c	0.583 e	0.805 B
4000	1.375 b	1.666 a	0.825 cd	1.288 A	1.375 b	1.583 a	1.000 d	1.319 A
Mean	0.812 B	0.958 A	0.622 C		0.770 B	1.072 A	0.666 C	

M1 (sand: peat moss, 1:1, v/v), (M2) sand and (M3) peat moss

Table 6. Effect of medium type and IBA concentration on number of shoots/cutting of *Euphorbia milii* cutting after six weeks during both seasons

IBA Conc. (mg/l)	First season				Second season			
	Medium type			Mean	Medium type			Mean
	M1	M2	M3		M1	M2	M3	
0.0	1.00 d	1.00 d	1.00 d	1.00 C	1.00 e	1.00 e	1.00 e	1.00 C
1000	2.08 b	1.91bc	1.83bc	1.94 B	2.16 bc	2.00 bcd	1.50 de	1.88 B
2000	2.25 b	2.16 b	1.25 cd	1.88 B	2.25 b	1.66 cd	1.83 bcd	1.91 B
4000	3.16 a	2.16 b	1.58 bcd	2.30 A	3.00 a	1.75 bcd	2.08 bc	2.27 A
Mean	2.12 A	1.81 A	1.41 B		2.10 A	1.60 B	1.60 B	

M1 (sand: peat moss, 1:1, v/v), (M2) sand and (M3) peat moss

Table 7. Effect of medium type and IBA concentration on shoot length (cm) of *Euphorbia milii* cutting after six weeks during both seasons

IBA Conc. (mg/l)	First season				Second season			
	Medium type			Mean	Medium type			Mean
	M1	M2	M3		M1	M2	M3	
0.0	6.41 ef	5.91 ef	5.08 f	5.80 C	6.87 fgh	6.25 gh	5.50 h	6.20 C
1000	9.70 ab	7.00 de	7.29 cde	8.00 B	10.33 bc	7.20 fg	7.95 ef	8.50 B
2000	10.58 a	8.66 bc	8.66 bc	9.30 A	11.33 ab	9.12 cde	8.83 de	9.76 A
4000	11.04 a	9.54 ab	8.25 bcd	9.61 A	12.25 a	9.91 cd	8.83 de	10.33 A
Mean	9.43 A	7.78 B	7.32 B		10.19 A	8.12 B	7.78 B	

M1 (sand: peat moss, 1:1, v/v), (M2) sand and (M3) peat moss

Table 8. Effect of medium type and IBA concentration on shoot fresh weight (g) of *Euphorbia milii* cutting after six weeks during both seasons

IBA Conc. (mg/l)	First season				Second season			
	Medium type			Mean	Medium type			Mean
	M1	M2	M3		M1	M2	M3	
0.0	10.2 f	14.9 def	13.0 ef	12.7 D	13.9 f	12.2 f	11.4 f	12.5 D
1000	21.3 bcd	19.3 cde	20.1 bcd	20.2 C	24.8 d	18.5 e	20.1 e	21.1 C
2000	23.6 bc	24.0 bc	23.8 bc	23.8 B	32.5 b	25.1 d	23.8 d	27.1 B
4000	31.3 a	26.6 ab	23.7 bc	27.2 A	36.5 a	29.5 bc	28.7 c	31.6 A
Mean	21.6 A	21.2 A	20.1 A		26.9 A	21.3 B	21.0 B	

M1 (sand: peat moss, 1:1, v/v), (M2) sand and (M3) peat moss

Table 9. Effect of medium type and IBA concentration on shoot dry weight (g) of *Euphorbia milii* cutting after six weeks during both seasons

IBA Conc. (mg/l)	First season				Second season			
	Medium type			Mean	Medium type			Mean
	M1	M2	M3		M1	M2	M3	
0.0	1.16 f	1.58 ef	1.75 def	1.50 C	2.04 fg	1.87 g	1.83 g	1.91 D
1000	2.25 c-f	1.70 def	1.91 def	1.95 C	4.00 cd	2.66 ef	2.66 ef	3.11 C
2000	3.08 bcd	3.55 abc	2.83 b-e	3.15 B	5.16 b	3.25 de	3.16 e	3.86 B
4000	4.75 a	4.12 ab	3.37 abc	4.08 A	6.00 a	4.54 bc	4.45 bc	5.00 A
Mean	2.81 A	2.74 A	2.46 A		4.30 A	3.08 B	3.03 B	

M1 (sand: peat moss, 1:1, v/v), (M2) sand and (M3) peat moss