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Effect of plant growth regulators on the rooting of lemon verbena cutting

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Abstract
Lemon verbena oil is used with great advantage in certain types of flavors, as well as in the preparation of some pharmaceuticals. Because of propagation of the difficult to root limon verbena plant this study included revealing the effect of growth regulators (IBA and NAA) dissolved in dist. water or mixed talc powder as well as the type of cutting (terminal and middle) on the rooting of limon verbena. The highest values of root cutting and root length were recorded with the treatments of 250 ppm level of IBA dissolved in dist. water x middle cutting type, 1500 ppm level of IBA mixed with talc powder x middle cutting type, 200 ppm level of NAA dissolved in dist. water x middle cutting type and 500 ppm level of NAA mixed with talc powder x middle cutting type.

Keywords: Lemon verbena, IBA, NAA, terminal, middle, root cutting, root length.

1. Introduction
Lippia citriodora Kunth (family Verbenaceae), it also called "Limon verbena" a small shrub that grows to 1.5 m high although native to South America today it widespread also in Europe as an ornamental plant in the gardens. Limon verbena shrub has woody stems, opposite lanceolate leaves, externally white and internally reddish-blue flowers, and dry drupes [1-2]. Lemon verbena oil is used with great advantage in certain types of flavors [1], as well as in the preparation of some pharmaceuticals [3].
Propagation of the difficult to root plant species i.e limon verbena becomes commercially feasible with the establishment of the optimal propagation method. A key step in vegetative propagation is adventitious root formation losses occur because of the poor quality of the root system or of the shoot and because of poor or slow rooting, as well as vegetative propagation practiced in horticulture for mass production of improved material within a short time, and to perpetuate the characteristics of the parent plants. The rooting potential of cuttings of different plant species, however, varies considerably [4-5]. Some of them root easily others with difficulty and still others do not root even with the application of synthetic growth substances. Even among the easy-rooting species, the ability of stem cuttings to root varies considerably with season. In many cases, profuse rooting occurs when cuttings are taken from trees in an active season [6].

Auxins are commonly used to stimulate root initiation in plants [7-8]. It has been reported that application of exogenous auxins to roots increased root regeneration of oak seedlings up to six fold [9]. Application of Indole Buteric Acid (IBA) and Naphthalene Acetic Acid (NAA) to root system of Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco] by soil drench method increased lateral root production of the seedlings [10]. Also, application of IBA to the root system of *Pistacia chinensis* seedlings prior to transplanting greatly increased root regeneration potential [11]. Audus [12] stated that IBA and NAA were much superior to the inducement of rooting because of their greater chemical stability and their low mobility in the plant. Kelly [13], stated that dipping the base of the *Magnolia stellata* cuttings into 0.8% IBA powder before planting resulted in 40% rooting, whereas untreated cuttings achieved only 10% rooting. Kelly [13] reported that the use of IBA stimulated rhododendron root production. Salikhov [14] stated that IBA favorably affected rooting in softwood cuttings of *Hinpahae rhamnoids* taken from the base of shoot but had no marked effect on apical cuttings. Morini [15]

found that cuttings of *Actinidia chinensis* gave the best rooting with IBA. Also, Singh [16] reported that sweet lime cuttings taken in July and treated with IBA (1500 ppm) gave the highest rooting stimulated and rooting to greater extent than NAA or IAA (each at 1000 or 2000 ppm). Farmer [17] mentioned that IBA treatments increased the number of roots cuttings of tamarack. Uno [18] mentioned that IBA at 3000 ppm increased the number and length of roots produced from cuttings of *Berberis atropurpurea*. Pimpini [19] and Memoni [20] stated that NAA increased rooting percentage and root number of *Ficus elastica* and Bougainvillea. Bhattacharijee [21] reported that NAA (4000 ppm) increased rooting of bougainvillea by 80% compared with untreated cuttings. IAA and IBA influenced the root formation of *Prunus sp*. cuttings [22].

Various workers investigated the effect of the position from which the cuttings were taken and found differences in rooting which varied from plant to another. Byers [23] carried experiment on *Laqerstroemia indica* and stated that soft - wood cuttings taken in July and treated with IBA gave more rooting percentage, while Chi [24] mentioned that, semi hard wood cuttings of *Simmondisia* sp treated with 2000 PPM IBA rooted successfully.

Because of propagation of the difficult to root limon verbena plant this study included revealing the effect of growth regulators (IAA and NAA) dissolved in dist. water or mixed with talc powder as well as the type of cutting on the rooting of limon verbena cuttings.

2. Materials and methods

2.1. Experimental

Experiments were carried out at the National Research Centre (NRC), Giza, Egypt, during two seasons, 2010 and 2011. Two types of limon verbena cutting (terminal and middle) selected during February of each season. Then, the uniform cutting (20 cm length) of both types subjected to different levels of Indole-3n-butric acid (IBA), or Napthalin acetic acid (NAA). To prepare IBA and NAA, dist. water or talc powder were used. The concentrations of IBA were 0, 150, 250 and 350 ppm for water solution or 0, 1500, 2500 and 3500 ppm for talc powder. On the other hand the concentrations of NAA were 0, 50, 100 and 200 ppm for water solution or 0, 500, 1000 and 2000 ppm for talc powder. Rooted cuttings (%) and Root length (cm) were recorded.

2.2. Statistical analysis

In these experiments, two factors were considered: growth regulators i.e. IBA or NAA (either dissolved in dist. water or mixed with talc powder) and 2 cutting types (middle and terminal). For each treatment there were 4 replicates, each of which had 8 pots; in each pot 3 individual cuttings were planted. The experimental design followed a complete random block design. According to Snedecor [25] the averages of data were statistically analyzed using 2-way analysis of variance (ANOVA).

3. Results and discussion

3.1 Effect of IBA (dissolved in dist. water), cutting type and their interactions on root cutting and root length

IBA and/or cutting type affected root cutting and root length in both first and second seasons (Table 1). Root cutting and root length were affected by changes in IBA with or without cutting type. Thus the root cutting and root length in general increased under the various IBA levels, especially at 250 ppm. Greatest values of root cutting and root length at each season were obtained from the 250 ppm X middle cutting treatment (Table 1). ANOVA indicated that the increases in root cutting and root length were significant in the first and second seasons for IBA, cutting type and IBA x cutting type interaction treatments (Table 1).

3.2. Effect of IBA (mixed with talc powder), cutting type and their interactions on root cutting and root length

As shown in Table 2, root cutting and root length increased at all IBA, cutting type and IBA x middle cutting during both seasons. The highest values of root cutting and root length were recorded at 1500 ppm level of IBA x middle type of cutting interaction compared with control treatments during the first and second seasons. ANOVA indicated that the increases in root cutting and root length were significant in the first and second seasons for IBA, cutting type and IBA x cutting type interaction treatments (Table 2).

Table 1: Effect of IBA (dissolved in dist. water), cutting type and their interactions on root cutting and root length.

Treatments		IBA dissolved in dist. Water			
		Rooted cuttings		Root length (cm)	
Cutting type	IBA (ppm)	1ST Season	2nd Season	1ST Season	2nd Season
Terminal	0	10.0	12.5	7.7	6.3
	150	20.0	23.8	11.8	8.7
	250	25.0	27.5	13.5	12.2
	350	17.5	20.0	10.6	7.5
Over all Terminal		18.1	21.0	10.9	8.7
Middle	0	30.0	35.0	11.6	8.9
	150	72.5	77.5	19.4	18.1
	250	77.5	78.8	21.7	20.7
	350	50.0	55.0	17.8	16.3
Overall Middle		57.5	61.6	17.6	16.0
Overall IBA	0	20.0	23.8	9.6	7.6
	150	46.3	50.7	15.6	13.4
	250	51.3	53.2	17.6	16.5
	350	33.8	37.5	14.2	11.9
LSD: 0.05					
IBA		4.1	4.0	0.8	0.4
Cutting type		2.9	2.8	0.3	0.3
IBA x Cutting type		5.8	5.6	1.2	0.6

Table 2: Effect of IBA (mixed with talc powder), cutting type and their interactions on root cutting and root length.

Treatments		IBA mixed with talc powder			
		Rooted cuttings		Root length (cm)	
Cutting type	IBA (ppm)	1ST Season	2nd Season	1ST Season	2nd Season
Terminal	0	10.0	11.3	7.1	7.0
	1500	25.0	21.3	9.2	8.7
	2500	15.0	18.8	8.7	8.2
	3500	15.0	17.5	8.0	7.9
Over all Terminal		16.3	17.2	8.3	8.0
Middle	0	28.8	35.0	10.8	8.8
	1500	37.5	42.5	14.0	13.3
	2500	32.5	33.0	11.4	9.6
	3500	25.0	35.3	11.1	9.5
Overall Middle		31.0	36.5	11.8	10.3
Overall IBA	0	19.4	23.2	9.0	7.9
	1500	31.3	31.9	11.6	11.0
	2500	23.8	25.9	10.1	8.9
	3500	20.0	26.4	9.6	8.7
LSD: 0.05					
IBA		2.8	3.4	0.6	0.4
Cutting type		2.0	2.4	0.4	0.3
IBA x Cutting type		4.0	4.8	0.9	0.5

3.3. Effect of NAA (dissolved in dist. water), cutting type and their interactions on root cutting and root length
The characters of root cutting and root length of Limon verbena roots during the first and second seasons were promoted by applying various levels of NAA. It also increased by middle cutting type with NAA (Table 3). The highest values of root cutting and root length were resulted from the treatment of 200 ppm of NAA x middle cutting type interaction. ANOVA indicated that the increases in root cutting and root length were significant in the first and second seasons for NAA, cutting type and NAA x cutting type interaction treatments (Table 3).

Table 3: Effect of NAA (dissolved in dist. water), cutting type and their interactions on root cutting and root length.

Treatments		NAA dissolved in dist. Water			
		Rooted cuttings		Root length (cm)	
Cutting type	NAA (ppm)	1ST Season	2nd Season	1ST Season	2nd Season
Terminal	0	10.0	12.5	7.7	6.3
	50	35.0	32.5	9.3	9.1
	100	17.5	20.0	8.7	8.9
	200	17.5	21.3	8.8	9.0
Over all Terminal		20.0	21.6	8.6	8.3
Middle	0	30.0	35.0	11.7	9.8
	50	62.5	57.5	15.0	11.2
	100	55.5	58.8	13.1	13.7
	200	72.5	66.3	17.2	12.2
Overall Middle		55.1	54.4	14.3	11.7
Overall NAA	0	20.0	23.8	9.7	8.1
	50	48.8	45.0	12.2	10.2
	100	36.5	39.4	10.9	11.3
	200	45.0	43.8	13.0	10.6
LSD: 0.05					
NAA		4.4	3.2	0.5	0.6
Cutting type		3.1	2.5	0.3	0.7
NAA x Cutting type		6.2	4.6	0.7	1.0

3.4. Effect of NAA (mixed with talc powder), cutting type and their interactions on root cutting and root length
NAA and / or cutting type affected the root cutting and root length in both seasons (Table 4). Thus, various characteristics of the rooting increased under the various NAA levels, especially at 500 ppm. Greatest values in each season for both variables were obtained at 500 ppm (NAA) x middle cutting (Table 4). ANOVA indicated that the increases in root cutting and root length were significant in the first and second seasons for NAA, cutting type and NAA x cutting type interaction treatments (Table 4).
The obtained results are in accordance with those obtained by previous literature. Byers [23] stated that soft wood cuttings (middle cutting) treated with IBA gave more rooting percentage of *Laqerstroemia indica,* while Chi [24] mentioned that, semi hard wood cuttings of *Simmondisia* sp treated with 2000 ppm IBA rooted successfully. Singh [16] found that IBA (4000 ppm) gave the best rooting and the length of roots of *Jasmminum sambac*. Also, application of IBA to the root system of *Pistacia chinensis* seedlings prior to transplanting greatly increased root regeneration potential [11]. Kelly [13], stated that dipping the base of the *Magnolia stellata* cuttings into 0.8% IBA powder before planting resulted in 40% rooting, whereas untreated cuttings achieved only 10% rooting. Kelly [13] reported that the use of IBA stimulated rhododendron root production. Salikhov [14] stated that IBA favorably affected rooting in softwood cuttings of *Hinpahae rhamnoids* taken from the base of shoot but had no marked effect on apical cuttings. Morini [15] found that cuttings of *Actinidia chinensis* gave the best rooting with IBA. Some investigators such as Singh [16] reported the promoting effect of NAA on root formation; they found that NAA was much superior to the inducement of

rooting of *Cailistemen lanceolatus* and some ornamental shrubs. The same effect was noticed when *Callistemon lanceolatus* cuttings were treated with 400 ppm of NAA [16].

Table 4: Effect of NAA (mixed with talc powder), cutting type and their interactions on root cutting and root length.

Treatments		NAA mixed with talc powder			
		Rooted cuttings		Root length (cm)	
Cutting type	NAA (ppm)	1ST Season	2nd Season	1ST Season	2nd Season
Terminal	0	10.0	11.3	7.1	7.0
	500	7.5	13.8	5.4	5.3
	1000	8.8	8.8	5.2	5.3
	2000	5.0	5.0	5.0	4.8
Over all Terminal		7.8	9.7	5.7	5.6
Middle	0	28.3	35.0	10.8	8.8
	500	35.0	40.0	11.1	10.3
	1000	22.5	25.0	8.3	6.1
	2000	10.1	13.8	6.1	5.0
Overall Middle		24.0	28.5	9.1	7.6
Overall NAA	0	19.2	23.2	9.0	7.9
	500	21.3	26.9	8.3	7.8
	1000	15.7	16.9	6.8	5.7
	2000	7.6	9.4	5.5	4.9
LSD: 0.05					
NAA		2.4	3.2	0.8	0.4
Cutting type		1.7	2.3	0.6	0.3
NAA x Cutting type		3.4	4.6	1.1	0.6

Pimpini [19, 20] stated that NAA increased rooting percentage and root number of *Ficus elastica* and Bougainvillea. Bhattacharijee [21] reported that NAA (4000 ppm) increased rooting of bougainvillea by 80% compared with untreated cuttings [9]. Application of IBA and naphthalene acetic acid (NAA) to root system of Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco] by soil drench method increased lateral root production of the seedlings [10]. Bhattacharijee [21] reported that NAA (4000 ppm) increased rooting of bougainvillea by 80% compared with untreated cuttings. IAA and IBA influenced the root formation of *Prunus* sp. cuttings [22].

Conclusion

It may be concluded that IBA, NAA and cutting type affected root cutting and root length of limon verbena. Root cutting and root length were affected by changes in IBA or NAA with or without cutting type. Thus the root cutting and root length in general increased under the various IBA or NAA levels. Middle cutting type resulted higher values of root cutting and root length compared with terminal cutting type.

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