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Evaluation the activity of *Petroselinum crispum* aqueous extract as promoter rooting for stem cuttings of some plants

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ABSTRACT

This study was conducted in the botanical garden, Department of biology, College of Science/ Mustansiriyah University in from (15 February to 15 March, 2019) under the natural environmental conditions in the greenhouse in order to evaluate the effectiveness of parsley aqueous extract as a promoter for rooting. The study included the use of aqueous extract of a plant Parsley (*Petroselinum crispum*) extract was used in concentrations (1.25, 2.5 g / l), compare with IBA in concentration (100 mg / L) with dipping time 24 hour for all treatments. The cutting stems were included *Rosmarinus officinalis*, *Nerium oleander*, *Olea europaea*, *Plumeria alba*, *Hibiscus rosa*, *Pelargonium graveolens*, and *Myrtus communis*. The following measurements were taken after 30 days from the beginning of the experiment: rooting percentage, (number and length of roots), number of new (leaves and branches). Plant hormone concentrations, oxidative enzyme activity (SOD, POS) and amino acids were also analyzed for the extract that gave the highest values for all traits. The results showed that there were significant differences between treatments, and indicated that parsley extract in the concentration (2.5 g / L) was more exceeded compared with other treatments. The chemical analysis of parsley extract showed the presence of the hormones IAA, GA3, cytokinin and ABA as well as the highest activity of the antioxidant enzymes (POS and SOD), also the analysis of parsley indicated the presence of several types of amino acids, including Tryptophan, Glutamic acid, alanine, valine, Tyrosine, Phenylalanine Histadine, Ornithine, Arginine and other amino acids. In conclusion, the Parsley extract can be used as promoter rooting for stem cuttings as a new method instead of using plant growth regulators , Replace the IBA which used in the rooting of stem cuttings by some natural extracts which can stimulate the rooting process.

KEYWORDS: parsley, stem cuttings, rooting response.

الخلاصة

أجريت هذه الدراسة في الحديقة النباتية ، في قسم علوم الحياة ، كلية العلوم / الجامعة المستنصرية من (١٥ شباط الى ١٥ اذار من عام ٢٠١٩) في ظل الظروف البيئية الطبيعية في البيت الزجاجي بهدف تقييم فعالية المستخلص المائي للمعدنوس كمحفز لتجذير العقل الساقية.شملت الدراسة استخدام المستخلص المائي لنبات (المعدنوس *Petroselinum crispum*) بتراكيز (١,٢٥ , ٢,٥ غم / لتر) و منظم النمو IBA بتركيز (١٠٠ ملغم / لتر) وبفترة تنقيع بلغت (٢٤) ساعة .تم استخدام العقل الساقية التالية : (اكليل الجبل, الدفلة, الزيتون, الياسمين الهندي, الجمال , العطرة و نبات الياس). في هذه الدراسة أخذت قياسات التالية بعد فترة ٣٠ يوما من الزراعة وهي : (النسبة المئوية للتجذير,عدد الجذور, طول المجموع الجذري وعدد الافرع و الاوراق الجديدة).ودلت النتائج على تفوق المستخلص المائي للمعدنوس بتركيز ٢,٥\لتر على معاملة IBA في كل الصفات المدروسة.تم تقدير تراكيز الهرمونات النباتية و فعالية انزيمات مضادات الاكسدة (SOD,POD)، مضافا اليها تحليل الأحماض الامينية لمستخلص المعدنوس . وقد اظهرت النتائج وجود هورمونات IAA, GA3، وانواع السايتوكاينين في مستخلص المعدنوس *Petroselinum crispum* , فضلا عن الفعالية العالية لإنزيمات مضادات الاكسدة (POS, SOD)، واظهر تحليل الاحماض الامينية هذا المستخلص عن وجود انواع عديدة من الاحماض الامينية منها,Tyrosine, Phenylalanine, Histadine, Ornithine, **Tryptophan**, Glutamic acid, Alanine, Valine, Arginine وغيرها من الاحماض الامينية. يمكن استنتاج من هذه الدراسة ان مستخلص المعدونس ذات تاثير محفز لتجذير العقل النباتية اذ يعد احدى الطرق الحديثة بديلا عن منظمات النمو , حيث يمكن استبدال IBA الذي يستخدم لتجذير العقل الساقية بواسطة بعض المستخلصات الطبيعية التي تحفز على عملية التجذير.

INTRODUCTION

Stem cuttings are considered the most important part used in vegetative propagation. This technique is favorable, easy and an inexpensive, suitable for producing a lot of plants in a short time [1].

The growth hormones responsible for the rooting of cuttings are called auxins. They are produced

primarily in the plant's growing points the meristems zone. These hormones stimulate root initiation and development. Tip cuttings, which include the apical meristem area, are the primary site of hormone production. Auxins are transported down the stems to other plant parts. The amount of hormone varies from plant to plant, tissue maturity, the time of year, and the environment. The primary auxin produced in the plant is indole acetic acid (IAA), but synthetic auxins are commonly used to propagate plants. The one most common synthetic auxins is indole-3-butyric acid (IBA) [2].

Adventitious root formation in stem cuttings is affected by many factors, including endogenous factors such as phytohormones and external supplied of plant growth regulator factors including the indole butyric acid which improve rooting and promoted the development of the root system, in the cuttings [3].

Hence, the necessity of exogenous auxin application to induce root formation in cuttings has been reported in many species [4,5]. Application of auxin, particularly indole-3-butyric acid (IBA) is a synthetic root-promoting and one of the most popular and efficient plants rooting to stimulate the rooting

of cuttings in a large number of plant species over a wide concentration range. Indole butyric acid (IBA) is an important auxin used to increase cuttings rooting ability. However, IBA sometimes don't stimulate rooting of cuttings[6,7,8].

However, IBA is not recommended in organic agriculture, as a synthetic product. The main objective of organic agriculture system is to obtain high-quality products while protecting the environment and land fertility. There are natural auxin sources which contains various compounds, which may affect root formation [9].

The objective of this work was to study the possibility of using available plant extracts such as parsley in rooting stem cutting for seven plants instead of the growth regulators.

MATERIAL AND METHODS

Preparation of stem cuttings

Healthy and uniform apical cuttings of different stem cuttings difficult and non-difficult stem cuttings such as *Nerium*, *Olea*, *Plumeria*, *Hibiscus*, *Pelargonium*, *Myrtus* and Rosemary were prepared, these plants are farmed and have been classified by Dr. Hadeel Radawy. These cuttings were collected from one year old plant, during February 2019. The length and diameter of these cuttings were 12 (cm) and 3-4 (mm) respectively; these cuttings were slanted cut at the base to increase the surface area of absorbing to the treatments, and to distinguish from the top of the stem cuttings. The soil was prepared by mixing the sandy soil with the peat moss in a ratio of (1:1), the soil placed in (9) cm diameter pots, with (6) replication for each treatment in greenhouse.

Plants Extracts

The selected plant was Parsley (*Petroselinum crispum)* leaves, were collected from Baghdad /Awirij. The plants extract was prepared as described by Harborne [10]. The plants were washed under the running water 2.5g for each plant (fresh weight) mixed with (500) ml distil water using an electronic blender for (10) minutes, the extract was lifted for one hour then filtered by cheesecloth. Finally, centrifuged at 3000 round/minutes for 15 minutes. The filtrate solution was completed to 1000 ml, to get stock solution in concentration 2.5g/L. The 1.25 concentration prepared by the equation: (Volume1x Concentration1= Volume 2 x Concentration 2).

The stock solution was stored in black container in the refrigerator for further use.

Preparation of IBA solution

One gram of IBA was dissolved in small quantity (10-15 ml) of absolute alcohol in a (50) ml beaker, thoroughly mixed them with one liter of distilled water with continuous stirring to form clear solution from1000 mg / L IBA concentration (stock solution), the desired concentration was prepared from the stock solution by the following equation:

$$Volume1 \times Concentration1 = Volume\ 2 \times Concentration\ 2$$

The stock solution was stored in a black container in cool condition for further use.

Stem cutting treatments

The leaves were removed from the bottom of the cuttings were dipping in one period time 24 hour for all treatments. Then inserted in the pots containing sand medium mixed with peat moss. Six replicates for each treatment and each replicate have four cuttings. On 15 February the temperatures ranged from (15 to 20) °C, the pots

covered with plastic bags to keep the moisture of the cuttings.

The rooting and vegetative parameters studies

Rooting percentage
Determined by counting the number of the rooted cuttings per replicate and was then divided by the total number cuttings per replicate.

Average Number of Roots per Rooted Cutting
All produced roots from the rooted cuttings were counted and then the total numbers of roots were divided by the total number of rooted cuttings.

Average Root Length per Rooted Cutting in (cm)
All produced roots were measured and the summation of the roots length was divided by the total number of rooted cuttings.

Numbers of new leaves and branches.

Estimation of some Chemical Composition

The analysis was conducted at the Ministry of Science and Technology. Quantitative analysis was done for the extract which gave the highest values in all studied traits.

Endogenous hormones
According to the methods of Ünyayar and others [10]. Extraction, purification and quantitative determination the endogenous hormones to the highest results for all studied traits. According to our fast FLC fast liquid chromatographic separation which was modified. The sample (1 gm) the sample was taken and were crushed into fine paste using clean pestle, mortar and combined with 60 ml of combined extract. The extract contains methanol: ammonia: chloroform solution in ratio (12:5:3 v/v/v). After that the combined extract filtered. The filtrate centrifuged at (6000 rpm) for (15 min). The combined extract filter ate was treated with (25 ml) deionized water. The chloroform phase was discarded. The water methanol phase was evaporated to dryness in rotary evaporator (Buchi Switzerland) at (30 C) and re-dissolved in known volume of the mobile phase the water phase was adjusted the extract to (pH 2.5) and (20 ul) were injected to HPLC system.

Estimation of antioxidant activity enzymes POD and SOD
According to [11], the growth of total plant were freezed in liquid nitrogen, reduced to small pieces and blended. Then extracted with (0.1 M) Na2HPO4 buffer solution have performed. The filtrate after centrifugation half-saturated with solid Ammonim-Sulfate(A.S), then 35% saturated with solid ammonium phosphate pH between (7-8). The mixture were passed through (2.5 um) disposable filter, and stored at (4 C) for further analysis, then (20 ul) of the sample injected into HPLC system according the optimum condition. The extract were separated on FLC (Fast Liquid Chromatographic) column , 7um particle size , NUCLOSIL 4000-7 PEI , anion exchange for protein and peptides (125x 4.0 mm I.D) column, Mobile phase ; solvent A:2 mM/L tris-acetate Ph 8.0, solvent B:20 mM/L tris-acetate Ph 8.0,0.01 M phosphate buffer + 1.5 mM KCL, linear gradients from 0%B -100% B in 10 min. Detection UV set at 280 nm, flow rate 1.5 ml/min.

Amino acids analysis in parsley extract
Depending on Fierabracci and others [12], the amino acid was estimated in the parsley leaf extract. Aliquots of standard or unknown sample (10 ul) were mixed with (10 ul) of PTIC reagent after (1 min), (50 ul) of (0.1 M) sodium acetate PH (7) were added. The sample shaking and agitated in Ultrasonic bath for (10 min), the extract were filtered on disposable filters (0.2 um) (supelco company cat No. 16534K) then (20 ul) were injected on HPLC column. The concentration for each compound were quantitatively determined by comparison the peak area of the standard with that of the samples. The column used was Shimpack XR-ODS (50 x 4.6 mm l.d), 3um particle size. Gradient were formed between tow degassed solvents. Solvent A 5% methanol in (0.1) N sodium acetate buffer PH (7.0) , B methanol, linear gradients in from (0-20)minutes. Detection (UV set at 254nm), flow rate (1 ml/min), injection (20 µl).

Statistical analysis and experimental design

The Statistical Analysis System- SAS (2012) [13]. program was used to detect the effect of different factors in study parameters. Least significant difference–LSD test was used to significant compare between means in this study. The experiment is designed according to the complete

randomize design (CRD). The rooting and vegetative parameters, after one month of planting were counted and the data was recorded for all treatments.

RESULTS AND DISCUSSION

The results in (Table 1) showed that parsley extract had the highest values in rooting % for all stem cuttings with significance different. The parsley extract showed its superiority over IBA treatment in all stem cuttings in 2.5 gm/l concentrations, this extract gave 80.9%, 66.9, 66.9, 100, 96.6 and 96.0 for *Nerium*, *Olea*, *Plumeria*, *Hibiscus*, *Pelargonium*, *Myrtus* and rosemary. Compare with 69.6, 66.3, 38.6, 39.3, 76.9, 66.9 and 91.6% respectively in IBA treatment. Such results were probably due to internal plant hormones such as auxins and other cofactors in parsley extract stimulating rooting process [14].

The results in (Table 2) showed the effect of different treatments on roots number and (Table 3) for roots length these tables indicated that parsley extract has a positive effect in increasing the number of roots and their length compare to IBA treatment for all stem cuttings.

The results in (Table 2) showed the mean of IBA was 8.19 compare to13.71 roots number in 2.5 gm/l of parsley treatment, also the results in table 3 obtained 5.66 in IBA treatment compare to7.75 cm root length in parsley treatment.

The results in (tables 4,5) the vegetative growth (new leaves and new branch numbers), indicated that the treatments have the same trained as the previous tables in its influence on the vegetative growth of the different stem cuttings. The results in table 4 showed that the mean value of 2.5 gm/l of parsley treatment was 5.34 while the mean value of IBA treatment was 3.01. As for the number of new branches table 5. Showed the mean value of 2.5 gm/l of parsley treatment was 2.82 compare to 1.83 in IBA treatment.

The plant growth regulators IBA is essential treatment to enhance rooting of cuttings in nurseries. Plant extracts are natural products which contains various compounds such as vitamins have long been found to promote root formation in numerous plant species, carbohydrates, nucleic acid , lipids , different minerals, in addition to amino acids, pyridoxine, hormones and other growth regulating substances such as cytokinins and auxin which helps to stimulate the growth of roots and shoots in addition to gibberellins.

These compounds enhance uptake of macro and microelements and their translocation within plants also increase the respiration rate and root growth, photosynthesis and other metabolic processes [15,16,17].

These components of plant extracts vary between plants so the effect on the rooting process is different from the extract to another depending on the type of extract components.

Table 1. Effect of different treatments on rooting percentage in different stem cuttings

Rooting Percentage								
Treat.	**Cons.**	***Nerium***	***Olea***	***Plumeria***	***Hibiscus***	***Pelargonium***	***Myrtus***	***Rosmarinus***
Water	0	36.3	33.3	0.0	0.0	30.3	33.3	60.6
IBA	100 mg/l	69.6	66.3	38.6	39.3	76.9	66.9	916
Parsley	1.25 gm./l	66.3	69.6	59.3	56.6	90.0	83.3	93.6
	2.5 gm./l	80.9	86.0	66.9	66.9	100	96.6	96.0
LSD		7.54*	7.63*	6.44*	7.52*	7.35*	7.81*	6.24*

*(P<0.05).

Table 2. Effect of different treatments on root numbers in different stem cuttings.

Roots number									
Treat.	**Cons.**	***Nerium***	***Olea***	***Plumeria***	***Hibiscus***	***Pelargonium***	***Myrtus***	***Rosmarinus***	**Mean of treat.**
Water	0	1.6	3.0	0.0	0.0	1.5	3.5	9.8	2.77
IBA	100	11.1	7.6	1.6	1.1	10.9	11.3	13.8	8.19
Parsley	1.25 gm./l	15.0	8.8	2.8	2.2	15.0	16.0	15.4	10.74
	2.5 gm./l	20.2	10.6	3.5	3.3	17.7	18.7	18.2	13.71
LSD		2.93*	2.07*	0.933*	0.872*	3.26*	3.63*	2.97*	2.34*
*(P<0.05).									

Table 3. Effect of different treatments on roots length in different stem cuttings.

Roots Length									
Treat.	**Cons.**	***Nerium***	***Olea***	***Plumeria***	***Hibiscus***	***Pelargonium***	***Myrtus***	***Rosmarinus***	**Mean of treat.**
Water	0	1.1	3.6	0.0	0.0	1.0	3.0	6.3	2.14
IBA	100	6.6	6.0	1.9	1.3	6.6	9.0	8.1	5.66
Parsley	1.25 gm./l	8.9	6.9	3.0	2.9	7.8	10.3	9.9	7.10
	2.5 gm./l	9.3	7.3	3.9	3.6	8.6	10.6	11.0	7.75
LSD		2.41*	1.88*	0.864*	0.873*	2.08*	2.33*	2.57*	1.82*
*(P<0.05).									

Table 4. Effect of different treatments on new leaves numbers in different stem cuttings.

New leaves numbers									
Treat.	**Cons.**	***Nerium***	***Olea***	***Plumeria***	***Hibiscus***	***Pelargonium***	***Myrtus***	***Rosmarinus***	**Mean of treat.**
Water	0	0.0	0.0	0.0	0.0	1.3	3.0	0.9	0.74
IBA	100	2.3	3.0	2.3	2.0	3.3	5.3	2.9	3.01
Parsley	1.25 gm./l	3.3	4.6	3.6	2.9	6.3	6.9	6	4.80
	2.5 gm./l	3.6	5.3	3.6	3.3	6.6	9.0	6	5.34
LSD		0.923 *	1.09 *	0.844 *	0.781 *	1.92 *	2.48 *	2.39 *	1.13*
*(P<0.05).									

Table 5. Effect of different treatments on new branch numbers in different stem cuttings.

New branch numbers									
Treat.	**Cons.**	***Nerium***	***Olea***	***Plumeria***	***Hibiscus***	***Pelargonium***	***Myrtus***	***Rosmarinus***	**Mean of treat.**
Water	0	0.0	0.0	0.0	0.0	1.0	1.3	0.6	0.41
IBA	100	1.6	0.6	1.0	1.0	2.0	3.0	3.6	1.83
Parsley	1.25 gm./l	2.6	1.0	2.0	1.6	3.0	3.9	4.0	2.16
	2.5 gm./l	3.1	1.3	2.3	2.3	3.6	4.6	4.6	2.82
LSD		0.703*	0.588*	0.591*	0.504*	0.796*	1.18*	1.52*	0.63*
*(P<0.05).									

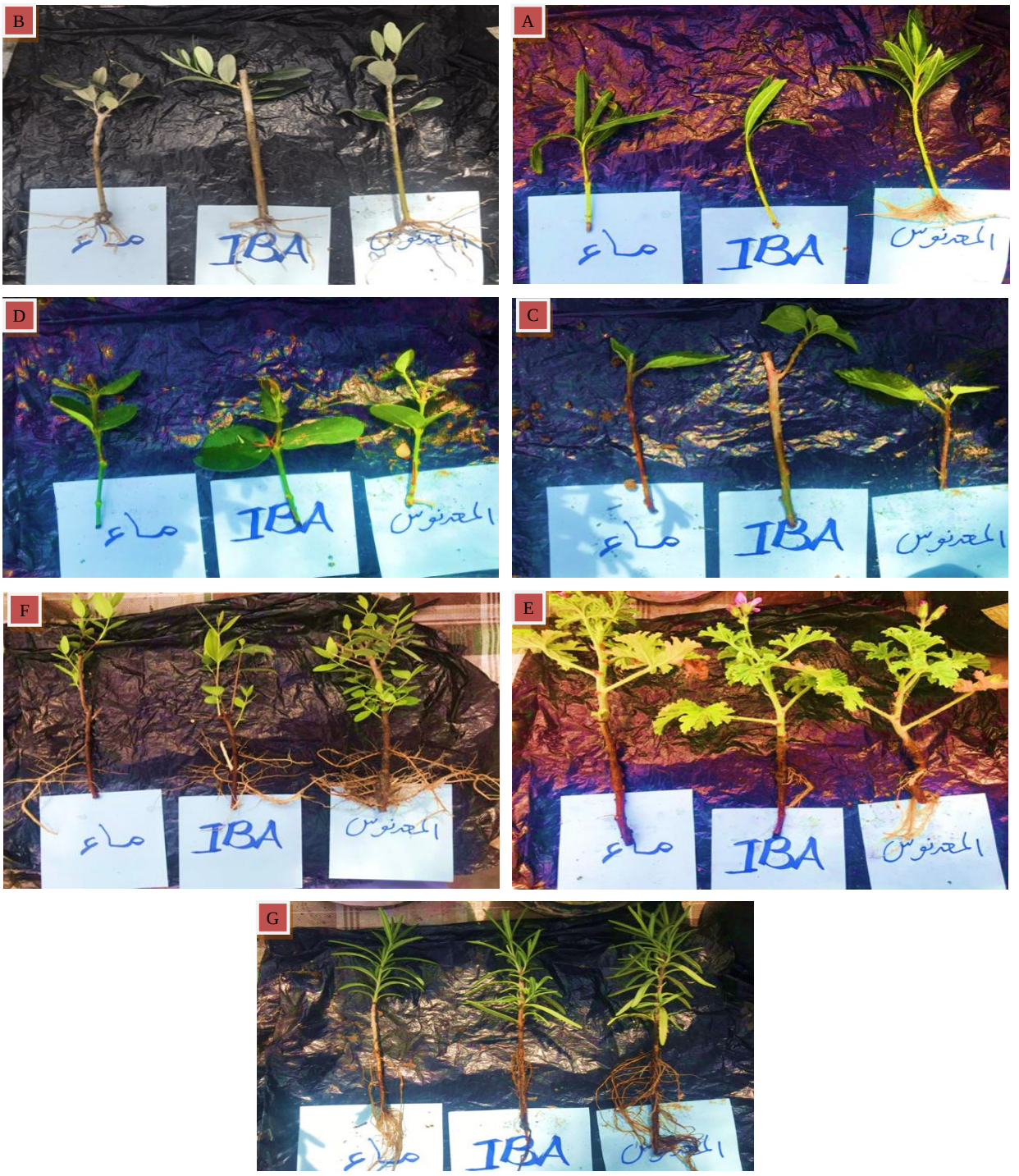

Figure 1. Effect of different treatments (parsley extract at concentration 2.5 g/l, IBA at concentration 100 mg/l and control treatment) on different stem cuttings taken after one month.
A- *Nerium oleander*, B-*Olea europaea, C- Hibiscus rosa, D- Plumeria alba, E- Pelargonium graveolens, F- Myrtus communis , G- Rosmarinus officinalis.*

Hormones types and quantity of endogenous hormones in Parsley extract

The results in (Table 6) showed that the presence of IAA and two types of cytokinin (zeatin and kinetin) in addition to GA3 and ABA. Auxin is the primary endogenous hormone which regulator the adventitious root induction [18,19]. IAA content has a direct correlation with rooting, appearing as the adventitious roots were breaking through the epidermis in cuttings. The endogenous hormones cytokinin and auxin regulate each other signaling and metabolism, IAA controls cytokinin content, by regulation of their biosynthesis, and positively effect on rooting number, length in addition to play roles in promoting cell division and expansion [20,21]. Gibberellins could play a role in the initiation of shoot growth by dormant plants in the spring, and has an indirect promotion for causing bud activity and increased the

endogenous auxin supply from the axillary buds as mentioned by (Erikson, 1971). ABA levels were positively correlated with rooting, and particularly in seasonal variations, also had a promotive effect on adventitious root formation. Other study the suggested that ABA concentration has a stimulate effect for rooting of cuttings, in addition to has antagonizes the effects of gibberellins and cytokinins [22].
These results can explain the increasing of the values in rooting and vegetative characters in the previous tables.

Table 6. Hormones type and quantity of endogenous hormones in parsley extract.

Hormones quantity in parsley extract in µg\gm	
IAA	9.157
Zeatin	4.987
Kinetin	5.584
GA3	6.335
ABA	2.443

The antioxidant enzymes peroxidase (POD) and superoxidase dismutase (SOD) in (Table 7) the cell wall is important defence barrier against the invasion of pathogen and expansion [23]. Lignin synthesis and accumulation facilitate cell wall formation and improve its strength [24]. Peroxidase activity promotes lignin biosynthesis in addition to promotes the production of iso-2-tyrosin [25,26]. The reduction in POD enzyme activity could decrease cell wall strength, while the increase in POD enzyme activity increases the resistance of cells to stress [27]. The increase in POD activity at the early stage facilitates in scavenging for H_2O_2 molecules, increases cell wall strength, and subsequently increases resistance to stress. At a later stage, POD activity decreases, which in turn facilitates cell expansion and growth [28]. Superoxidase dismutase (SOD) an important antioxidant in the defines line which catalyzes the excess of H_2O_2 to form H_2O and O_2, and therefore to prevent the cells from damages of their structural, componential and functional which caused by free oxygen radicals [29]. Increased SOD activity can increase the defines of cuttings against stress by scavenging reactive oxygen [30]. SOD activity continuously increases before root formation to enhance the resistance of cuttings against stress. After adventitious root emergence the activity of SOD start to decline. After adventitious root emergence, the absorption function of the roots is recovered, thereby relieving the plant from stress, whereas SOD activity starts to decrease [28].

Table 7. The activity some important antioxidant in parsley extract.

Antioxidant enzymes activity in µmole/g	
peroxidase	3.382
superoxidase dismutase	3.319

The results in (Table 8) showed the amino acids in parsley extract. The amino acids such as (aspartic acid, glutamic acid and ornithine) found that they had significantly enhanced the number of roots and rooted shoots [31]. Other study found that glutamine and arginine, are also beneficial to plant tissue growth and used for shoot and root induction, in addition to that they found there is increase in the IAA production with the presence of L-tryptophan in the media culture [32]. Another amino acid can stimulate rooting is arginine this amino acid has ability to stimulate rooting process in vitro, and Influencing on leaf chlorophyll content, in addition to get involved in carbohydrate, biosynthesis and play role in proline accumulation in both leaves and roots [33,34]. From the biochemical analysis for hormones, antioxidant enzymes and amino acids these components taken together and their effects on rooting process, we can explain the better performance for rooting and vegetative growth of the different stem cuttings and also the possibilities for using this extract as an alternative method and can replacing from use the chemically manufactured plant growth regulators.

Table 8. Aminoacides type and quantity in parsley extract.

Amino Acid	Concentration in µg/g
Taurine	612.12
Aspartic acid	1344.18
Threonine	326.95
Serine	268.61
Asparagine	182.44
Glutamine	91.81
Glutamic acid	263.75
glycine	715.07
alanine	879.43
valine	350.62
isoleucine	277.46
leucine	195.62
tyrosine	195.34
phenylalanine	70.11
ornithine	1046.07
tryptophan	34.31
histadine	256.11
arginine	383.18

CONCLUSIONS

1- The parsley aqueous extract in 2.5 m/L gave the highest values in all trait studies (rooting percentage, number and length of roots, number of new leaves and branches) than IBA treatment.

2- This study used parsley aqueous extract as a natural, very cheap and available in any time with no toxicity compared to use the chemical synthetic and expensive IBA.

3- Comparing hormonal concentrations that propagate plants in the research with the concentration of the extract to determine the best substance in the plant root.

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