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EVALUATION THE ACTIVITY OF SOME MEDICINAL PLANTS EXTRACTS AS PROMOTER ROOTING FOR STEM CUTTINGS OF ROSEMARY (*ROSMARINUS OFFICINALIS* L.)

Shahad Basheer Bahedh* and Amal Abdul Salam AL Habib

Department of Biology, College of Science, Mustansiriyah University, Baghdad, Iraq.

Abstract

This study was conducted in the botanical garden, Department of biology, College of Science / Mustansiriyah University in spring season, where the starts from (15 February to 15 March, 2019). Under the natural environmental conditions in the greenhouse in order to evaluate the effectiveness of some plant extracts as a promoter for rooting the apical stem cutting of rosemary plants at different concentrations compared with the IBA growth regulator. Plant extracts are Parsley (*Petroselinum crispum*), Dill (*Anethum graveolens*) and date palm fruits (*Phoenix dactylifera*) were used with concentrations (0, 1.25, 2.5 g / l). The IBA concentration was (100 mg / L) with dipping time 24 hour for all treatments. The following measurements were taken after 30 days from the beginning of the experiment: rooting percentage, (number and length of roots), (soft and dry) weight of roots, number of new (leaves and branches) and the longer leaves rate, for all treatments. Plant hormone concentrations, 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) exceeded on the other treatments. The results also showed that the apical cuttings surpassed the basal cuttings in all studied trait. The chemical analysis of parsley extract showed the presence of the hormones IAA, GA3 and cytokinin. It can be concluded from this study the possibility of using available plant extracts and inexpensive such as parsley in rooting instead of the growth regulators which are not available and expensive.

Key words: parsley, dill, date palm fruit extracts, IBA, roots responses.

Introduction

Rosemary (*Rosmarinus officinalis* L.) is a medicinal herb which known as a spice and belongs to the Lamiaceae (labiatae) family. Rosemary is a appreciated and has receiving attention for its aromatic, antioxidant, antimicrobial or antitumoural properties,antioxidant activity that is linked to the presence of substances arising from the secondary metabolism mainly to phenolic compounds (Randhir *et al.,* 2004). Recent studies found that the essential oil extracts of rosemary can delay lipid oxidation in biological systems and food also is used as food preservation and in pharmaceutical products (Hsu, 2005). As well as the rosemary diterpenes found to inhibit neuronal cell death, which induced by a variety of agents both *in vitro* and *in vivo*, the therapeutic potential of these compounds for Alzheimer's disease. These antioxidant protections to other mechanisms including brain inflammation and amyloid beta formation (Nieto *et al.,* 2018). Rosemary herb is characterized by short seeds as well low germination (Habtemariam, 2016). To solve this problem is to use the production of plants through the vegetative propagation. The vegetative propagation of plants is the process, when an exact copy of the genome of a mother plant is made and continued in new individuals (Nicola *et al.,* 2005). Adventitious root formation is affected by several factors including endogenous factors such as phytohormones which auxin play an central role of this processes. Growth regulators commonly are synthetic which are expensive and sometimes rare. Several previous studies have shown that plant extracts have an effect on the vegetative growth of many plants. An alternative of natural compounds can work as phytohormones (which their extracts contain bioactive compounds can be used as plant growth regulators with no risk to human health, organisms and the environment (Wiesman and Jaenicke, 2002). The seasonal variations

***Author for correspondence :** E-mail: shahaddashir@gmail.com

can also play an important role in rooting, which response to the changes in the levels of endogenous rooting promoter and inhibitors.

Therefore the aims of this study were to investigate the followings:

1. To evaluate the efficiency of some plant extracts including *Anethum graveolens*, *Petroselinum crispum* and *Phoenix dactylifera* (Zahdi variety), in rooting of rosemary stem cuttings compare with IBA treatment.

2. Studying the differences between cuttings position to evaluate the best position in rooting of rosemary stem cuttings.

3. Quantity estimate for endogenous hormones, to the plant aqueous extract which had the highest rooting values.

Material and Methods

Preparation of stem cuttings

Healthy and uniform apical and basal cuttings of rosemary were prepared,these cuttings were collected from one year old rosemary 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.

Preparation of IBA solution

One gram of IBA was dissolved in small quantity (10-15ml) of absolute alcohol in a (50) ml beaker, thoroughly mixed them with one litter of distilled water with continuous stirring to form clear solution from 1000 mg/L IBA concentration (stock solution), the desired concentration were prepared from the stock solution by the following equation: Volume 1 × Concentration 1 = Volume 2 × Concentration 2. The stock solution was stored in a black container in cool condition for further use.

Plants Extracts

The selected plants were Dill *Anethum graveolens* and Parsley (*Petroselinum crispum*) leaves, Date fruits *Phoenix dactylifera* (Zahdi variety) fruit, were collected from Baghdad /Awirij. The plants extract were prepared as described by (Fachirah *et al.*, 2013).

Stem cutting treatments

The leaves were removed from the bottom of the cuttings. Each cuttings of rosemary were dipping in one period time 24 hour for each treatment (IBA 100 mg/ L) and (2.5, 1.25 g/L) for each plant extracts. The cuttings of rosemary were insertion 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 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.

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 highest results extract.

• Endogenous hormones: According to the methods of (Unyayar *et al.*, 1996), 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 contain 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 (Pourmorad *et al.*, 2006), the growth of total plant were freezed in liquid nitrogen,

reduced to small pieces and blended. Then extracted with (0.1 M) Na_2HPO_4 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.

• Amino acids analysis in parsley extract: Depending on (Fierabracci *et al.*, 1991), the amino acid was estimated in the *Petroselinum crispum* 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 shaked 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.

• Statistical analysis and experimental design: The Statistical Analysis System-SAS (SAS, 2012) program was used to detect the effect of difference factors in study parameters. Least significant difference-LSD test was used to significant compare between means in this study.

Results and Discussion

The results in table 1, showed that the apical cuttings was the best in rooting than the basal cuttings in all treatments and have significant differences at the level of 5% with the basal cuttings. The parsley extract was the highest in stimulating the roots formation in apical rosemary cuttings which gave 93.6% rooting while date palm fruits gave 91.65 compare with 91.4% in IBA treatment. In basal rosemary cuttings parsley extract in 2.5gm.\L with date palm fruits in the same concentration they gave the highest value 86.2% and 82.2% respectively compare with 80.2% in IBA treatment. Rooting percentage of apical cuttings was highest than basal cuttings, the position of stock plant stem was effective on rooting of rosemary cuttings. May be probably due to internal plant hormones such as auxins and other cofactors which are concentrated on plants' apex, where meristematic cells abound (Hartmann *et al.*, 2002) and were more actively dividing cells which could be easily influenced by the hormonal treatment (Raji and Mohamad, 2012).

Table 2, showed roots number in spring season the results showed that the average numbers of roots in parsley extracts in 2.5 and 1.25\L concentration had the highest results (18.2 and 15.4), the second highest result given by date palm fruits (16.86) in concentration 2.5gm\L compare with 13.8 in IBA treatment. The same trend in the results indicated with basal cuttings with significant difference between the parsley and date palm fruits extracts with IBA treatment. The result showed there is no significant difference between control treatment and dill extract, the significant difference present only between control and the rest treatments.

Table 3, demonstrated the effect of different treatments on root length of rosemary stem cuttings, the same effect of different extracts on roots length. The parsley and date palm fruit extract were the best and have significance different in their effect on roots length, they gave mean 7.96 and 7.10 cm mean for date palm fruit extract compare with 6.69 cm given by IBA treatment.

Table 1: Effect of different treatments on rooting percentage in spring season (*P<0.05).

Rooting percentage					
Treatments	**Conc.**	**Apical cuttings**	**Basal cuttings**	**Means of Conc.**	**Means of treatments**
Control	0.00 mg/L	60.0	49.0	54.50	70.15
IBA	100	91.4	80.2	85.8	
Parsley	0.00 g/L	60.2	47.4	54.35	76.91
	1.25	91	82.2	86.5	
	2.5	93.6	86.2	89.9	
Dill	0.00 g/L	60.0	49.0	54.50	70.75
	1.25	83.0	72.8	77.9	
	2.5	85.2	74.0	79.6	
Date palm fruits	0.00 g/L	60.0	49.0	54.50	75.28
	1.25	88.0	81.4	84.45	
	2.5	91.6	82.2	86.9	
L.S.D for Conc.	6.255 *				
L.S.D for Treat.	4.0562 *				
Means of cutting types		85.45	73.49		
L.S.D	3.163 *				

Table 2: Effect of different treatments on root numbers.

Root numbers					
Treatments	**Conc.**	**Apical cuttings**	**Basal cuttings**	**Means of Conc.**	**Means of treatments**
Control	0.00 mg/L	9.8	2.58	6.19	8.40
IBA	100	13.8	7.4	10.6	
Parsley	0.00 g/L	9.6	2.6	6.1	11.35
	1.25	15.4	10.4	12.9	
	2.5	18.2	11.9	15.05	
Dill	0.00 g/L	9.16	3.44	6.3	8.23
	1.25	11.8	6	8.9	
	2.5	12	7	9.5	
Date palm fruits	0.00 g/L	9.6	3	6.3	10.74
	1.25	15.2	9.2	12.2	
	2.5	16.86	10.6	13.73	
L.S.D for Conc.	2.635 *				
L.S.D for Treat.	2.077*				
Means of cutting types		12.85	6.74		
L.S.D	1.674 *				

The results in table 4, indicated shoot length that parsley and date palm fruit extract still with the apical cuttings position gave the highest values in all traits study. The results showed a significant variation in the propagation ability of rosemary cuttings, the parsley extract with apical was the best from IBA treatment because this treatment in 25gm/L concentration, this extract gave mean of 7.35 cm while the IBA treatment gave 6.24cm. Using auxin mainly 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 enhances uptake of macro and microelements and their translocation within plants also increase the respiration rate and root growth, photosynthesis and other metabolic processes (Nagodawithana, 1991; Dunsin *et al*., 2016). 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. The results in tables (4,5) gave the same trend of plant extracts effect in terms of influence of parsley and date palm fruit extracts on means the number of leaves and shoot length with the interaction of apical cuttings.

Table 3: Effect of different treatments on root length.

Root length					
Treatments	**Conc.**	**Apical cuttings**	**Basal cuttings**	**Means of Conc.**	**Means of treatments**
Control	0.00 mg/L	6.62	5.6	6.09	6.69
IBA	100	8.0	6.6	7.30	
Parsley	0.00 g/L	6.1	5.5	5.80	7.96
	1.25	9.8	8.1	8.95	
	2.5	10.7	9.6	9.15	
Dill	0.00 g/L	6.3	5.2	5.78	7.04
	1.25	8.4	6.5	7.45	
	2.5	9	6.8	7.90	
Date palm fruits	0.00 g/L	6.1	5.1	5.60	7.10
	1.25	8.2	6.1	7.13	
	2.5	9.2	7.9	8.58	
L.S.D for Conc.	1.258 *				
L.S.D for Treat.	0.894*				
Means of cutting types		8.04	6.65		
L.S.D	0.633 *				

Analyses of parsley extract

Hormones types and quantity of endogenous hormones in parsley extract.

Table 4: Effect of different treatments on shoot length.

Shoot length					
Treatments	**Conc.**	**Apical cuttings**	**Basal cuttings**	**Means of Conc.**	**Means of treatments**
Control	0.00 mg/L	5.64	5.12	5.38	6.24
IBA	100	7.7	6.5	7.1	
Parsley	0.00 g/L	5.74	5.16	5.45	7.35
	1.25	8.4	7.3	7.85	
	2.5	9.6	7.9	8.75	
Dill	0.00 g/L	5.2	4.8	5	6.65
	1.25	7.54	7.04	7.29	
	2.5	7.9	7.44	7.67	
Date palm fruits	0.00 g/L	5.56	4.78	5.17	7.08
	1.25	8.32	7.14	7.73	
	2.5	9.18	7.54	8.63	
L.S.D for Conc.	0.861 *				
L.S.D for Treat.	0.653*				
Means of cutting types		7.19	6.34		
L.S.D	0.490 *				

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, the formation of roots from non-root tissues (Steffens and Rasmussen, 2016). Adventitious root formation consist from many steps: the root induction, root initiation and root emergence (Nag *et al.*, 2001). The induction phase is the period which the biochemical changes in the first cell division happen, initiation due to the cell divisions that lead to the formation of root primordial, emergence stage refers the growth of adventitious roots through the cortex and epidermis. Endogenous IAA levels increase temporarily during the induction phase,in the other hand simultaneously of the plant tissue increased sensitivity to auxin signals (Štefanˇciˇc *et al.*, 2007). IAA content has a direct correlation with rooting, appearing as the adventitious roots were breaking through the epidermis in cuttings. Zeatin and GA3 promoted adventitious rooting. The peak values of the IAA: ABA and IAA: ZT ratios appeared at the same time to the occurrence of adventitious roots (Shao *et al.*, 2017).

The Antioxidant enzymes peroxidase and superoxidase dismutase (SOD) as in table 7, the cell wall is important defense barrier against the invasion of pathogen and expansion (Lewis and Yamamoto, 1990). Lignin synthesis and accumulation facilitate cell wall formation and improve its strength (Jackson *et al.*, 2001). Peroxidase activity promotes lignin biosynthesis in addition to promotes the production of iso-2-tyrosin (Rout, 2006). The reduction in POD enzyme activity could decrease

Table 5: Effect of different treatments on leaves number.

Number of leaves					
Treatments	**Conc.**	**Apical cuttings**	**Basal cuttings**	**Means of Conc.**	**Means of treatments**
Control	0.00 mg/L	0.4	0	0.2	0.75
IBA	100	2.6	0	1.3	
Parsley	0.00 g/L	0.6	0	0.3	1.93
	1.25	4	0.8	2.4	
	2.5	5	1.2	3.1	
Dill	0.00 g/L	0.2	0	0.1	1.47
	1.25	3.6	0.4	2	
	2.5	4	0.6	2.3	
Date palm fruits	0.00 g/L	0.6	0	0.3	1.60
	1.25	3.4	0.4	1.9	
	2.5	4.2	1	2.6	
L.S.D for Conc.	0.923 *				
L.S.D for Treat.	0.697*				
Means of cutting types		2.6	0.4		
L.S.D	0.527 *				

Table 6: Endogenous hormones quantity Parsley extract.

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

cell wall strength, while the increase in POD enzyme activity increases the resistance of cells to stress (Syros *et al.*, 2004). 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 (Zhang *et al.*, 2017). Superoxidase dismutase (SOD) an important antioxidant in the defense 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 (Ueda *et al.*, 2013). Increased SOD activity can increase the defense of cuttings against stress by scavenging reactive oxygen (Zhao *et al.*, 2013).

Table 8, showed the amino acids in parsley extract. The amino acids (aspartic acid, glutamic acid and ornithine) found significantly enhanced the number of roots and rooted shoots (Orlikowska, 1992). 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 an increase in the IAA production with the presence of L-tryptophan in the media culture. The maximum production of IAA in concentration 0.5% of L-tryptophan in the medium (Harikrishnan *et al.*, 2014). 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 metabolism, biosynthesis and play role in proline accumulation in both leaves and roots (Virginia *et al.*, 2014). Previous tables show the existence of hormones, antioxidant enzymes and a mino acids. These components taken together can explain the better performance for rooting, roots and vegetative growth of rosemary stem cuttings and also the possibilities for using this extract as alternative plant growth regulators.

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

Antioxidant enzymes activity in µ mole\g	
Peroxidase	3.382
Superoxidase dismutase	3.319

Table 8: Amino acids type and quantity in parsley extract.

Amino Acids	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. Apical rosemary cuttings are the highest rooting than the basal cuttings.

2. The parsley aqueous extract in 2.5 m./L. give the highest values in all trait studies than IBA treatment.

3. The parsley aqueous extract increase the endogenous hormones (IAA, Zeatin, kinetine, GAs and ABA) in the base of the rosemary cuttings.

4. Use the parsley aqueous extract for promoting rooting cuttings in the first time in this study.

5. Date palm fruits aqueous extract also were better from IBA in rosemary rooting cuttings and vegetative trait studies in addition to be the first time used in promoting rooting in this studies.

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