



Adverse Effects of Salt Stress on Rootability of *Rosmarinus officinalis* Cuttings and their Alleviation by Indole-3-Butyric Acid (IBA) and *Bacillus subtilis*



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THIS STUDY was conducted at the Horticulture Farm, Faculty of Veterinary and Agricultural Science, El-Zawia University, Libya during 2014 and 2015 seasons, to investigate the effect of indole-3-butyric acid (IBA) concentrations (0, 50 and 100 ppm) alone or in combination with *Bacillus subtilis* on the rootability, root and shoot growth of *Rosmarinus officinalis* L. cuttings under salt stress conditions (control, 1000, 2000 and 4000 ppm NaCl). The obtained data revealed that irrigation of cuttings with NaCl at 2000 and 4000 ppm resulted in a significant reduction in rooting percentage, root and vegetative growth characteristics, contents of N, P and K as well as C/N ratio in cutting tissues compared to control and the lowest salinity level used, whereas Na % and Na/K ratio were high, especially at high salinity levels. Treatment of cuttings with IBA alone or combined with *B. subtilis* modified and alleviated most of the harmful effects of salt stress, especially at the lower salinity level. Generally, the combination of IBA at 50 ppm with *B. subtilis* had a profound effect in increasing rootability, root and vegetative growth characteristics of plants compared to the control and all other treatments. The same treatment increased contents of N, P and K as well as C/N ratio, whereas reduced Na % and Na/K ratio in the rooted cutting tissues. So, it could be recommended to use this treatment for alleviation of adverse effects of irrigation water salinity on rooting and growth of *R. officinalis* cuttings.

Keywords: *Rosmarinus officinalis*, Cutting, Rooting, IBA, *Bacillus subtilis*, Salt Stress.

Introduction

Medicinal and aromatic plants such as *Rosmarinus officinalis* L. are characterized by low seed viability and low germination percentage (Nicola et al., 2005). In addition, propagation through seeds is undesirable because of enormous heterozygosity in the plants resulting from cross pollination (Anis et al., 2003). Therefore, the vegetative propagation is widely preferred rather than propagation by seeds (Hartmann et al., 2014). Propagation of medicinal and aromatic plants by stem cuttings is the most vital method to reproduce plants (Anderson & Woods, 1999 and Waman et al., 2019). It offers production of true-to-type plants in a short time and availability of superior individuals for large scale commercial plantation with quick productive gains (Kiuru et al., 2015).

Regeneration of roots in the cutting is largely controlled by endogenous and exogenous factors. Endogenous factors include phytohormones and carbohydrates. On the other hand, exogenous factors such as amount of soluble salts in the irrigation water, beneficial microorganisms, rooting substrate, collection time, temperature, humidity and light (Hartmann et al., 2014 and Martorello et al., 2019).

Use of saline water in nurseries can seriously affect the success rates of sexual and asexual propagation (Barthwal et al., 2005 and Li et al., 2010 and Mohamed and Gomaa, 2012). Small amounts of salt in commercial nurseries can lead to enormous economic losses over longer period of time. The amount of loss depends on the species ability to tolerate salt stress (Mostafa,

2002 and Martorello et al., 2019). The salt tolerance of unrooted cuttings, germinating seeds and tissue culture explants is much lower than that of established plants, which can be grown under minor irrigation salinity by modifying cultural conditions (Hartmann et al., 2014). Rooting of *Chrysanthemum morifolium* cuttings from the salt stressed mother plants was depressed with a 45% decrease in root number, almost 70% in root length and 52% for root weight (Prabucki et al., 1999). In this respect, the use of plant growth regulators such as IBA and plant growth promoting rhizobacteria (PGPR) like *Bacillus subtilis* to alleviate salt stress are better options (Mostafa, 2002, Mohamed & Gomaa, 2012 and Martorello et al., 2019).

Little available information in the literature about mitigation salt stress-induced adverse effects on rooting and growth of cuttings by plant growth regulators. Martorello et al. (2019) reported that salinity was the main limiting factor for rooting of cuttings from different *Salix spp.* clones. At high salinity level (6.4 g/l NaCl), rooting was totally inhibited, while it was possible at moderate salinity level (3.2 g/l NaCl). These adverse effects of salinity on rooting could be alleviated by IBA addition in some particular genotypes. In addition, Mostafa (2002) showed that irrigation of *Acalypha welkesiana*, *Euphorbia pulcherrima* and *Lantana camara* cuttings with saline water reduced rooting percentage, root and vegetative characteristics progressively with increasing the level of salinity. Dipping of the used cuttings in IBA solutions alleviated most of the harmful effects of salt stress, especially under the lower levels of salinity. Similarly, Wang (1989) reported that misting the cuttings of *Buxus microphylla* cv. Japonica with the low salinity water (EC = 0.01 to 0.03 dS/m) increased rooting percentages and roots fresh weight compared with the high salinity water (EC = 0.96 to 1.5 dS/m). Treating the used cuttings with IBA at 2500 ppm increased rooting percentage, roots number, root length and roots fresh weight under salt stress conditions.

On the other hand, many studies have showed that inoculation of plants with PGPR such as *B. subtilis* improves plant growth, yield and nutrient uptake under salt stress conditions (Abdel-Rahman et al., 2011, Mohamed & Gomaa, 2012 and Abd Allah et al., 2017). In addition, PGPR in the genera of *Bacillus*, *Agrobacterium*, *Azospirillum* and *Pseudomonas* have recently been used to induce adventitious root formation

in stem cuttings (Abdel-Rahman & El-Naggar, 2014 and González et al., 2018). These bacteria are able to exert a beneficial effect upon plant growth such as increases root growth and root weight (Karakurt et al., 2009 and Abdel-Rahman et al., 2019). Growth promotion and increase in adventitious root formation in response to PGPR inoculation could be result from production of phytohormones such as auxins, cytokinins and gibberellins by bacteria (Goto, 1990 and Hussein et al., 2016). Moreover, several studies have also showed that combined IBA-bacteria treatments are more effective in increasing rootability of cuttings compared to IBA or bacteria alone (Karakurt et al., 2009, Kınık & Çelikel, 2017 and Zenginbal & Demir, 2018).

Although several studies have been conducted to investigate the effects of PGPR on growth and productivity of plants under salt stress conditions as well as inducing adventitious root formation in stem cuttings under normal conditions, no available reports have been published on effects of PGPR alone or in combination with IBA on rootability of *R. officinalis* stem cuttings under salt stress conditions. Therefore, the present study was aimed alleviating salt stress-induced adverse effects on rooting and growth of *R. officinalis* cuttings by indole-3-butyric acid (IBA) alone or combined with *B. subtilis*.

Materials and Methods

This study was conducted during the two successive seasons (2014 and 2015) at the Horticulture Farm, Faculty of Veterinary and Agricultural Science, El-Zawia University, Libya.

On March 1st of both seasons, softwood terminal cuttings of *R. officinalis* were harvested and trimmed to a 9-10 cm length. About 4-5 leaves were left on cuttings to increase the carbohydrates content in cutting tissues during rooting period .

Broth inoculant of *B. subtilis* contains 10⁸ CFU/ml was obtained from the Lab of Fac. Sci., El-Zawia Univ., Libya. Bacterial suspension prepared of *B. subtilis* (10⁸ CFU/ml) was added as a drench into the rooting substrate at a rate of 10 ml/pot before sticking the cuttings. For IBA treatments, the basal portion of the cuttings was soaked in aqueous solutions of 0, 50 and 100 ppm IBA for 4 hr. The combined treatments were applied by sticking IBA-treated cuttings into the rooting substrate which contains inoculums of *B. subtilis*. Cuttings in the control group were

treated with remediation water since it is the available irrigation water in Libya. The cuttings were irrigated regularly with remediation water (EC= 0.675 dS/m) for a week, and then they were subjected to four different salinity levels (control "remediation water", 1000, 2000 and 4000 ppm NaCl). Some chemical analysis of the remediation water used in this study were done according to the methods described by Jackson (1973) and Black et al. (1982) as shown in Table 1.

The experiment was arranged in a split-plot design, with three replicates. The salinity levels (control "remediation water", 1000, 2000 and 4000 ppm NaCl) represented in the main plots, meanwhile treatments of IBA and *B. subtilis* (control, 50 ppm IBA, 100 ppm IBA, 50 ppm IBA + *B. subtilis* and 100 ppm IBA + *B. subtilis*) represented in the sub-plots. Each experimental subunit consisted of 10 cuttings were planted in plastic pot of 12 cm. diameter filled with peat moss and perlite (1:1 v/v) and placed in a plastic house.

The cuttings in present investigation were held in rooting substrate almost two and half months. Data were collected on the rooting percentage, number of roots, root length, stem length, number of branches and number of leaves per plant as well as shoot fresh and dry weights. Then, leaf samples were collected and dried at 70 °C for 48 h to determine the chemical constituents of leaves which taken at the end of experiment. Also, one centimeter sample of the basal end of cutting were taken and dried for determination of carbohydrates and nitrogen. Total carbohydrate content was colorimetrically determined with the anthrone sulphuric acid method; Fales (1951). Total nitrogen was determined by using semi-micro Kjeldahl method described by Black et al. (1982). Total phosphorus was determined using Spectrophotometer according to Jackson (1973). Leaf content of potassium was determined photometrically using a flame photometer according to the method of Jackson (1973). Sodium content was determined according to the method described by A.O.A.C (1990).

The obtained data during the two seasons of the study were statistically analyzed using MSTAT computer software and the means were compared using a least significant difference (LSD) test at 5% level according to Gomez and Gomez (1984).

Results and Discussion

Percentage of rooted cuttings

Data presented in Table 2 show clearly that the rooting percentage of *R. officinalis* cuttings was significantly affected by salinity levels used during both seasons. The maximum rooting percentage (77.4 %) was obtained from cuttings irrigated with the lower salinity level (1000 ppm NaCl), followed by control treatment (70.7%) as average of both seasons. Meanwhile, the lowest values of rooting percentages (65.7 and 55.5%) were observed mainly at the higher salinity levels (2000 and 4000 ppm NaCl, respectively) with significant differences between them in the two seasons. These results are in agreement with the findings of Li et al. (2010) on *Tamarix chinensis* and Martorello et al. (2019) on *Salix* spp., who found that irrigation with water containing high levels of salinity resulted in low rooting success and may inhibit adventitious root formation on cuttings. Reduction in rooting percentage with increased salinity may be due to increasing the osmotic water potential of the rooting substrate which resulted in reducing water influx and translocation into cutting (Gulnaz et al., 1999), as well as accumulation of Na⁺ and Cl⁻ to toxic levels in plant tissues (Munns et al., 1995). Another possible reason for increasing the ethylene production (Moe & Andersen, 1988 and Javid et al., 2011), as well as reduction of auxin synthesis (Sakhabutdinova et al., 2003).

Statistical analysis showed that application of IBA alone or in combination with *B. subtilis* significantly increased rooting percentage of *R. officinalis* cuttings compared to untreated cuttings (Table 2). Obviously, the combined treatments of IBA either at 50 or 100 ppm with *B. subtilis* were superior to the individual IBA treatments and produced the highest rooting percentages (82.5% and 75.1%, respectively). There are several authors (Abdel-Rahman & El-Naggar, 2014, Kınık & Çelikel, 2017 and Zenginbal & Demir, 2018) showed that application of IBA alone or in combination with PGPR improves adventitious root formation on stem cuttings. Positive effects of IBA application and *B. subtilis* inoculum on rootability of *R. officinalis* cuttings may be attributed to IAA produced by *B. subtilis* and exogenous IBA treatments. It is known that auxins stimulate adventitious root formation on stem cuttings through their ability to promote the initiation of lateral roots primordia and starch hydrolysis and to enhance transport of

carbohydrates and nutrients from upper part of the cuttings to their basal ends by increasing the activity of enzymes (Davies, 2004). Besides, IBA may also enhance rooting via increased internal-free IBA, or may synergistically modify the action of IAA or the endogenous synthesis of IAA; IBA can enhance tissue sensitivity for IAA and increase rooting (Babaie et al., 2014). On the other hand, root stimulation by *B. subtilis* is thought to be due to the production of IAA, inhibition of ethylene synthesis, a decrease IAA oxidase activity and mineralization of nutrients by the bacteria (Grichko & Glick, 2001, Han & Lee, 2005 and Hussein et al., 2016). IAA produced by the bacteria works in conjunction with the internal auxin plant to stimulate root proliferation and division of cells and nutrient uptake from the rooting substrate (Leveau and Lindow, 2005).

However, the interaction between different salinity levels and IBA with or without *B. subtilis* exerted significant differences in rooting percentage during both seasons. Obviously, the lowest rooting percentage (34.4%) was obtained from untreated cuttings with IBA or combined with *B. subtilis* at the highest salinity level (4000 ppm NaCl). Treating cuttings with IBA alone or in combination with *B. subtilis* could alleviate the adverse effects of salinity and considerably improved rootability

of *R. officinalis* cuttings compared to untreated cuttings. Overall, the best rooting percentages (86.1 and 96.7%) were obtained from the combined treatment of 50 ppm IBA plus *B. subtilis* at low salinity levels (control and 1000 ppm NaCl, respectively), followed by 100 ppm IBA plus *B. subtilis* (82.2 and 83.9%) at the same salinity levels. The increments in rooting percentage reached 58.4, 59.3, 105.2 and 84.0% for 50 ppm IBA, 100 ppm IBA, 50 ppm IBA + *B. subtilis*, 100 ppm IBA + *B. subtilis*, respectively over the untreated cuttings at the highest salinity level (4000 ppm NaCl). Similar results were obtained by Mostafa (2002), Karimi et al. (2012) and Martorello1 et al. (2019), who also reported that exogenous IBA application resulted in an improvement in the rooting percentage of cuttings compared to untreated cuttings under salt stress conditions. The improvement in the rooting percentage under salt stress conditions when *R. officinalis* cuttings were treated with IBA alone or combined with *B. subtilis* may be related to the role of IBA at suitable concentration and IAA produced by *B. subtilis* in promoting the initiation of roots premordia, induction the meristematic activity in roots and alleviation of salinity stress by compensation of the shortage in endogenous hormones levels as a result of salinity (Carpenter & Cornell, 1992 and Mohamed & Gomaa, 2012).

TABLE 1. Some chemical analysis of the remediation water used in the cuttings irrigation.

Properties	Value	Properties	Value
Soluble cations (meq/L):		Soluble anions (meq/L):	
Ca ⁺⁺	0.099	SO ₄ ⁼	0.728
Mg ⁺⁺	0.089	Cl ⁻	4.313
Na ⁺	5.185	HCO ₃ ⁻	0.349
K ⁺	0.098	CO ₃ ⁼	0.081
TDS (ppm)	371.4	E.C. (dS/m)	0.675

TABLE 2. Rooting percentage of *Rosmarinus officinalis* cuttings as affected by indole-3-butyric acid (IBA) and *Bacillus subtilis* under salt stress conditions during the 2014 and 2015 seasons.

Salinity levels (ppm) "A"	First season (2014)					Second season (2015)						
	IBA (ppm) and <i>B. subtilis</i> "B"											
	Cont.	50 IBA	100 IBA	50 IBA + <i>Bacillus</i>	100 IBA + <i>Bacillus</i>	Mean	Cont.	50 IBA	100 IBA	50 IBA + <i>Bacillus</i>	100 IBA + <i>Bacillus</i>	Mean
Cont.	46.7	66.7	68.9	88.9	84.4	71.1	48.9	68.9	70.0	83.3	80.0	70.2
1000	56.7	72.2	73.3	97.8	87.8	77.5	64.4	73.3	73.3	95.5	80.0	77.3
2000	50.0	65.5	66.7	80.0	72.2	66.9	48.9	63.3	66.7	73.3	70.0	64.4
4000	33.3	53.3	53.3	71.1	63.3	54.9	35.5	55.6	56.2	70.0	63.3	56.1
Mean	46.7	64.4	65.6	84.4	76.9		49.4	65.3	66.6	80.6	73.3	
LSD _{0.05}	A = 2.9		B = 3.0		A*B = 6.0		A = 2.5		B = 2.7		A*B = 5.4	

Root and shoot characteristics

Data presented in Tables 3, 4 and 5 indicate that irrigation of *R. officinalis* cuttings with the high salinity levels (2000 and 4000 ppm NaCl) badly affected the all root and vegetative growth characteristics studied during the two seasons. It was clear also that low salinity level (1000 ppm NaCl) recorded the highest significant increase in number of roots, root length, stem length, number of branches and number of leaves per plant as well as shoot fresh and dry weights compared with non-salinized cuttings and the other salinity levels. Meanwhile, the lowest significant means in root and vegetative characteristics studied were obtained at the highest salinity level (4000 ppm NaCl). These results are in accordance with those obtained by Li et al. (2010) and Martorello et al. (2019). The observed reduction in root number and root length per rooted cutting of *R. officinalis* as a result of high salinity levels may be related to the toxic effects of Na⁺ and Cl⁻ ions accumulated in the cytoplasm of root cells, besides the reduction of the total water used by the rooted cuttings leading to a reduction in the root cells division and elongation (Khan et al., 2000). Also, salinity reduced cell size, cell turgor and the number of cells per unit area (Greenway and Munns, 1980) as well as transpiration and closure of stomata which is associated with vegetative growth reduction (Sánchez-Blanco et al., 2002). On the other hand, The stimulatory effect of low salinity levels on root and shoot growth of *R. officinalis* rooted cuttings in this study was recorded by several authors as Wang (1989), Mostafa (2002) and Li et al. (2010), who recorded stimulatory effect of moderate salinity on growth of some plants, these may be due to improve shoot osmotic status as a result of increasing nutrient elements uptake.

It was noticed also from the obtained data in the same Tables 3, 4 and 5 that treatment of *R. officinalis* cuttings with IBA with or without *B. subtilis* caused a significant increase in root and vegetative growth characteristics compared to untreated cuttings in both seasons. The highest number of roots, root length, stem length, number of branches and leaves per plant as well as shoot fresh and dry weights were obtained when cuttings treated with 50 ppm IBA + *B. subtilis*, followed by 100 ppm IBA + *B. subtilis* treatment compared with IBA alone treatments and untreated cuttings. The results are in conformity with (Kınık & Çelikel, 2017, Zenginbal & Demir, 2018 and Abdel-Rahman et al., 2019), who

stated that combined IBA-bacteria treatments are more effective on increasing root and vegetative growth characteristics compared to IBA alone. The improvement in root characteristics of *R. officinalis* cuttings may be attributed to role of auxins (externally added IBA and IAA produced by bacteria) in increasing the cambial activity, root initial formation, primordial differentiation and elongation as well as acceleration root formation and increase root number and quality per cutting (Zengibal and Özcan, 2006).

Better vegetative characteristics were obtained when *R. officinalis* cuttings were treated with IBA alone or in combination with *B. subtilis*. This might associated with the increased number of roots and root length in treated cuttings which enhances uptake of water and nutrients from the rooting substrate as well as perhaps consequent to higher carbohydrate production and assimilation reflected as vigorous vegetative growth (Sharma et al., 2015). Also, Scott (1972) reported that the increment in vegetative growth may be due to the influence of auxin in the synthesis of nucleic acid and metabolites and enzymes synthesis and activation, leading to accumulation of the biosynthesates.

The interaction effect between different salinity levels and IBA treatments with or without *B. subtilis* on root and vegetative growth characteristics was significant during both seasons. The most promising effect of IBA and *B. subtilis* on alleviation of salt stress on *R. officinalis* cuttings was found when they were applied in combination. However, combined treatment of 50 ppm IBA + *B. subtilis* provided higher tolerance to salinity compared with the other treatments, where cuttings treated with 50 ppm IBA + *B. subtilis* showed greater number of roots, root length, stem length, number of branches and number of leaves as well as shoot fresh and dry weights compared to the other treatments under saline conditions, especially at low salinity level (1000 ppm NaCl) and control. Meanwhile, the lowest values in root and vegetative characteristics were recorded with untreated cuttings at the highest salinity level (4000 ppm NaCl) in both seasons. Similar results have been reported by Mostafa (2002), Abdel-Rahman et al. (2011) and Martorello et al. (2019). They found that treatment of cuttings with IBA and inoculation of plants with *B. subtilis* resulted in an improvement in root and vegetative

growth characteristics compared to the control under salinity conditions. The improvement in the root and vegetative characteristics under salinity conditions when *R. officinalis* cuttings were treated with IBA may be related to IBA role in increasing lateral root production, inducing adventitious root formation and improving root characteristics and hence increasing uptake of water and nutrients from rooting substrate under the lower saline conditions, leading to enhancing

the vegetative growth characteristics (Mostafa, 2002). On the other hand, many studies have showed that inoculation of plants with *B. subtilis* improves plant growth under salt stress conditions (Abdel-Rahman et al., 2011 and Abd Allah et al., 2017) by influencing IAA production, enhancing the stability of the cell membrane, raising the root vigor of plant and improving photosynthesis under salt stress (Mayak et al., 2004, Mohamed and Gomaa, 2012).

TABLE 3. Mean of number of roots and length of root (cm) per rooted cutting of *Rosmarinus officinalis* as affected by indole-3-butyric acid (IBA) and *Bacillus subtilis* under salt stress conditions during the 2014 and 2015 seasons.

Salinity levels (ppm) "A"	First season (2014)					Second season (2015)						
	IBA (ppm) and <i>B. subtilis</i> "B"											
	Cont.	50 IBA	100 IBA	50 IBA + <i>Bacillus</i>	100 IBA + <i>Bacillus</i>	Mean	Cont.	50 IBA	100 IBA	50 IBA + <i>Bacillus</i>	100 IBA + <i>Bacillus</i>	Mean
Number of roots/cutting												
Cont.	29.3	37.6	39.1	45.4	42.7	38.8	32.3	40.3	42.3	47.8	45.5	41.7
1000	33.7	44.6	46.3	53.5	48.1	45.2	35.3	43.0	45.2	50.8	46.2	44.1
2000	24.0	32.8	38.2	46.4	43.7	37.0	30.5	38.2	40.0	43.2	40.8	38.5
4000	22.7	33.7	35.3	41.0	38.3	34.2	23.3	33.7	35.5	39.1	35.5	33.4
Mean	27.5	37.2	39.7	46.6	43.2		30.4	38.8	40.8	45.2	42.0	
LSD _{0.05}	A = 1.0	B = 1.1	A*B = 2.3				A = 0.6	B = 0.8	A*B = 1.6			
Root length/cutting (cm)												
Cont.	11.8	13.7	13.9	14.9	13.9	13.6	12.0	13.1	13.6	15.8	14.2	13.7
1000	13.4	14.3	15.4	15.8	15.4	14.9	12.5	14.6	14.8	16.7	14.7	14.7
2000	11.2	13.1	12.9	13.8	13.3	12.9	10.3	13.0	12.7	13.7	13.4	12.6
4000	10.5	12.6	12.0	12.8	12.6	12.1	8.4	12.3	12.2	13.1	12.8	11.8
Mean	11.7	13.4	13.6	14.3	13.8		10.8	13.2	13.3	14.8	13.8	
LSD _{0.05}	A = 0.05	B = 0.06	A*B = 0.13				A = 0.31	B = 0.53	A*B = 1.06			

TABLE 4. Mean of stem length (cm), number of branches and leaves per plant for *Rosmarinus officinalis* as affected by indole-3-butyric acid (IBA) and *Bacillus subtilis* under salt stress conditions during the 2014 and 2015 seasons.

Salinity levels (ppm) "A"	First season (2014)					Second season (2015)					Mean	
	IBA (ppm) and <i>B. subtilis</i> "B"											
	Cont.	50 IBA	100 IBA	50 IBA + <i>Bacillus</i>	100 IBA + <i>Bacillus</i>	Cont.	50 IBA	100 IBA	50 IBA + <i>Bacillus</i>	100 IBA + <i>Bacillus</i>		
Stem length (cm)												
Cont.	12.2	12.8	13.2	14.1	13.4	13.1	12.6	13.8	14.0	15.3	14.3	14.0
1000	13.0	13.2	14.0	15.9	14.7	14.2	13.5	14.0	14.9	16.2	15.3	14.8
2000	11.1	12.5	12.7	13.9	12.8	12.6	11.1	13.3	14.0	14.9	14.7	13.6
4000	9.7	11.8	12.4	13.0	12.6	11.9	9.6	12.3	12.5	13.0	12.8	12.0
Mean	11.5	12.6	13.1	14.2	13.4		11.7	13.3	13.9	14.8	14.2	
LD _{0.05}	A = 0.2	B = 0.2	A*B = 0.4			A = 0.3	B = 0.4	A*B = 0.8				
Number of branches/plant												
Cont.	7.8	8.1	9.5	11.6	10.4	9.5	8.2	10.0	10.2	12.8	10.4	10.3
1000	8.8	9.2	9.4	12.6	9.9	10.0	8.9	10.6	11.1	13.5	11.0	11.0
2000	6.9	8.2	9.0	9.4	8.5	8.4	7.6	8.8	9.0	11.6	9.5	9.3
4000	6.6	7.3	8.1	9.1	8.3	7.9	6.8	8.0	8.0	9.4	8.4	8.1
Mean	7.5	8.2	9.0	10.7	9.3		7.9	9.3	9.6	11.8	9.8	
LSD _{0.05}	A = 0.3	B = 0.3	A*B = 0.7			A = 0.2	B = 0.3	A*B = 0.4				
Number of leaves/plant												
Cont.	81.3	100.7	103.3	112.6	108.0	101.2	85.7	101.7	106.3	116.9	109.0	103.9
1000	100.3	120.0	130.3	137.9	133.1	124.3	90.9	130.3	135.0	148.0	140.1	128.9
2000	82.8	98.3	99.0	114.9	103.0	99.6	84.3	96.3	98.3	115.8	103.0	99.6
4000	74.7	87.0	91.3	104.0	95.5	90.5	70.7	84.3	88.3	95.9	89.0	85.7
Mean	84.8	101.5	106.0	117.4	109.9		82.9	103.2	107.0	119.1	110.3	
LSD _{0.05}	A = 1.3	B = 1.6	A*B = 2.6			A = 0.9	B = 1.5	A*B = 3.0				

TABLE 5. Mean of shoot fresh and dry weights (gm) for *Rosmarinus officinalis* as affected by indole-3-butyric acid (IBA) and *Bacillus subtilis* under salt stress conditions during the 2014 and 2015 seasons.

Salinity levels (ppm) "A"	First season (2014)					Second season (2015)						
	IBA (ppm) and <i>B. subtilis</i> "B"											
	Cont.	50 IBA	100 IBA	50 IBA + <i>Bacillus</i>	100 IBA + <i>Bacillus</i>	Mean	Cont.	50 IBA	100 IBA	50 IBA + <i>Bacillus</i>	100 IBA + <i>Bacillus</i>	Mean
Shoot fresh weight (gm)												
Cont.	15.4	18.2	18.7	21.5	19.4	18.7	13.3	17.8	18.8	23.6	21.2	18.9
1000	18.2	20.8	21.7	25.4	23.3	21.9	16.7	18.8	19.4	25.0	21.6	20.3
2000	15.1	18.3	18.5	20.2	19.2	18.3	15.3	17.4	18.2	21.0	20.2	18.4
4000	11.4	15.5	15.9	18.5	16.9	15.7	10.7	14.3	14.8	16.8	15.4	14.4
Mean	15.0	18.2	18.7	21.4	19.7		14.0	17.1	17.8	21.6	19.6	
LSD _{0.05}	A = 0.3	B = 0.3	A*B = 0.6				A = 0.6	B = 0.8	A*B = 1.2			
Shoot dry weight (gm)												
Cont.	3.5	4.8	5.0	5.6	5.2	4.8	2.7	4.2	5.0	6.4	5.3	4.7
1000	4.3	5.6	5.7	6.7	6.1	5.7	3.3	4.3	5.3	6.9	5.6	5.1
2000	3.4	3.8	4.0	5.2	4.2	4.1	3.2	3.5	4.0	5.5	4.8	4.2
4000	2.0	3.2	3.6	4.6	3.7	3.4	2.0	2.5	2.7	3.6	3.4	2.8
Mean	3.3	4.4	4.6	5.5	4.8		2.8	3.7	4.3	5.6	4.8	
LSD _{0.05}	A = 0.1	B = 0.2	A*B = 0.3				A = 0.2	B = 0.2	A*B = 0.4			

Minerals content

The obtained results in Tables 6 and 7 showed that contents of N, P and K in leaves of *R. officinalis* rooted cuttings were increased as a result of increasing salinity concentration up to 1000 ppm NaCl, followed by significant decrease with further increase in salinity levels (2000 and 4000 ppm NaCl). Content of Na⁺ and Na⁺/K⁺ ratio were pronouncedly increased with increasing salinity levels compared to the lowest salinity level (1000 ppm NaCl) and control in both seasons. Similar results were reported by Li et al. (2010) and Abdel-Rahman et al. (2011), who

reported that high NaCl concentration decreased N, P and K contents, and increased content of Na⁺ ion. The reduction in N, P and K contents at high salinity levels may be attributed to the excess presence of Na⁺ ion which has the ability to enter the root cells through several channels via plasma membrane Na⁺/H⁺ antiports that are energized by the proton gradient generated by the plasma membrane ATPase, hence translocated and accumulated in plant tissues exposed to high NaCl concentrations (Blumwald et al. 2000). Entry of both Na⁺ and Cl⁻ into the cells causes severe ion imbalance, decrease of water potential

and specific ion toxicity (Arzani, 2008). In addition, NaCl decreases N concentration in the shoot tissues (Cordovilla et al., 1995) and it has a negative influence on the nitrogen acquisition and utilization (Lewis, 1986). The negative effect of NaCl on the nitrogen content in plant tissues could be explained by the antagonism between Cl^- and NO_3^- as reported by Wehrmann and Hahndel (1984). On the other hand, the increment in Na^+/K^+ ratio at high salinity levels may be due to the presence of excess Na^+ ions in the rooting substrate, which absorbed and accumulated in cutting tissues. Besides, Na^+ competes with the uptake of K^+ and reduces its absorption, leading to a higher Na^+/K^+ ratio (Benito et al., 2014).

In addition, the present results also showed that treatment of *R. officinalis* cuttings with IBA alone or combined with *B. subtilis* led to increase the contents of N, P and K and significantly decreased the Na% and Na/K ratio compared to untreated cuttings in both seasons. Generally, adding IBA either at 50 ppm or 100 ppm combined with *B. subtilis* gave the highest percentages of N, P and K, as well as the lowest percentage of Na^+ and Na^+/K^+ ratio compared to the control and single treatments of IBA. Similar results were obtained by Mostafa (2002) and Mohamed & Gomaa (2012), who reported that adding IBA at suitable level or inoculation with *B. subtilis* caused a decrease in the uptake of Na^+ and an increase in the uptake of nutrient elements such as N, P and K.

The interactions between the different salinity levels and IBA with or without *B. subtilis* treatments indicated that all treatments of IBA and/or *B. subtilis* significantly increased contents of N, P, K, but decreased Na^+ content and Na^+/K^+ ratio in the leaves under salt stress conditions compared to untreated cuttings in both seasons. The combined treatment of 50 ppm IBA + *B. subtilis* provided higher tolerance to salinity, where cuttings treated with 50 ppm IBA + *B. subtilis* showed greater N, P and K% as well as lower Na% and Na/K ratio under the lowest salinity level (1000 ppm NaCl) and control compared to the other treatments. Meanwhile, the lowest values of N, P and K%, as well as the highest values of Na% and Na/K ratio were recorded with untreated cuttings at high salinity level of 4000 ppm NaCl. These results are in accordance with Mostafa (2002), who found that treatment of cuttings with IBA increased N, P and K contents in cuttings tissues, but decreased Na and Cl contents under salt stress conditions. The increment in percentages of N, P and K in leaves

of *R. officinalis* rooted cuttings as a response to IBA treatment may be due to the role of IBA in alleviation of adverse effects of salt stress via improving the root system of cuttings, leading to increasing the absorbed amount of N, P and K from the rooting substrate, especially under the lower salinity levels. On the other hand, many studies have showed that inoculation of plants with *B. subtilis* improves nutrient uptake under salt stress conditions (Abdel-Rahman et al., 2011 and Abd Allah et al., 2017). In addition, PGPR strains such as *B. subtilis* can produce bacterial exopolysaccharides (EPSs) that bind cations, including Na^+ (Geddie and Sutherland, 1993), it may be envisaged that increasing the population density of EPS-producing bacteria in the root zone would decrease the content of Na^+ available for plant uptake and thus help alleviating salt stress in plants growing in saline environments (Ashraf et al., 2004).

C/N Ratio

Statistical analysis of the data regarding C/N ratio (Table 8) revealed that different salinity levels and the interaction between salinity and IBA with or without *B. subtilis* significantly affected C/N ratio in basal part of stem cuttings of *R. officinalis*. The highest C/N ratio was obtained when cuttings were irrigated with low salinity level (1000 ppm NaCl), followed by non-salinized cuttings. By increasing salinity levels from 1000 to 2000 and 4000 ppm NaCl, C/N ratio was decreased. Strong reduction was observed when cuttings were irrigated with the highest salinity level (4000 ppm NaCl). These results are in agreement with those obtained by Rahneshan et al. (2018), who stated that a total carbohydrate was adversely affected due to salinity effect. This reduction in C/N ratio within tissues of *R. officinalis* cuttings at high salinity levels could be attributed to a decrease in photosynthesis rate, chlorophyll content and leaf area expansion (Heidari, 2012). On the other hand, adventitious root formation depends on a sufficient supply of carbohydrates to adventitious roots formation zone where the roots processed energy and carbon necessary to initiation and development adventitious roots formation (Hartmann et al., 2014). Abdel-Rahman & El-Naggar (2014) revealed that a positive relationship was found among carbohydrate content, total nitrogen, growth promoters and the rooting response. Therefore, the reduction in rooting percentage of *R. officinalis* cuttings at high water salinity is considered to be a result of a decrease in C/N ratio.

TABLE 6. Effect of indole-3-butyric acid (IBA) and *Bacillus subtilis* on nitrogen, phosphorus and potassium percentages in dry leaves of *Rosmarinus officinalis* under salt stress conditions during the 2014 and 2015 seasons.

Salinity levels (ppm) "A"	First season (2014)					Second season (2015)						
	IBA (ppm) and <i>B. subtilis</i> "B"											
	Cont.	50 IBA	100 IBA	50 IBA + <i>Bacillus</i>	100 IBA + <i>Bacillus</i>	Mean	Cont.	50 IBA	100 IBA	50 IBA + <i>Bacillus</i>	100 IBA + <i>Bacillus</i>	Mean
Nitrogen %												
Cont.	1.51	1.85	1.88	1.95	1.94	1.83	1.48	1.75	1.80	1.90	1.81	1.75
1000	1.66	1.87	1.89	1.96	1.92	1.86	1.61	1.87	1.92	1.98	1.93	1.86
2000	1.61	1.74	1.77	1.85	1.79	1.75	1.52	1.64	1.67	1.81	1.67	1.66
4000	1.45	1.62	1.65	1.75	1.67	1.63	1.38	1.58	1.61	1.75	1.77	1.62
Mean	1.56	1.77	1.80	1.88	1.83		1.50	1.71	1.75	1.86	1.79	
LSD _{0.05}	A = 0.02	B = 0.03	A*B = 0.06				A = 0.03	B = 0.03	A*B = 0.06			
Phosphorus %												
Cont.	0.197	0.221	0.225	0.236	0.232	0.222	0.185	0.210	0.216	0.227	0.212	0.210
1000	0.203	0.230	0.236	0.242	0.240	0.230	0.199	0.218	0.212	0.235	0.220	0.217
2000	0.188	0.211	0.223	0.232	0.229	0.217	0.180	0.202	0.210	0.220	0.213	0.205
4000	0.171	0.182	0.185	0.198	0.190	0.185	0.167	0.186	0.180	0.189	0.182	0.181
Mean	0.190	0.211	0.217	0.227	0.223		0.183	0.204	0.204	0.218	0.207	
LSD _{0.05}	A = 0.001	B = 0.003	A*B = 0.005				A = 0.002	B = 0.003	A*B = 0.005			
Potassium %												
Cont.	1.13	1.24	1.25	1.28	1.25	1.23	1.10	1.21	1.21	1.27	1.23	1.20
1000	1.15	1.26	1.27	1.30	1.28	1.25	1.15	1.23	1.25	1.30	1.25	1.24
2000	1.12	1.23	1.24	1.26	1.24	1.22	1.10	1.21	1.22	1.24	1.23	1.20
4000	1.09	1.15	1.15	1.22	1.18	1.16	1.01	1.13	1.13	1.22	1.14	1.13
Mean	1.12	1.22	1.23	1.27	1.24		1.09	1.19	1.20	1.26	1.21	
LSD _{0.05}	A = 0.01	B = 0.01	A*B = 0.02				A = 0.01	B = 0.01	A*B = 0.02			

TABLE 7. Effect of indole-3-butyric acid (IBA) and *Bacillus subtilis* on sodium percentage and Na/K ratio in dry leaves of *Rosmarinus officinalis* under salt stress conditions during the 2014 and 2015 seasons.

Salinity levels (ppm) "A"	First season (2014)					Second season (2015)					Sodium %	
	IBA (ppm) and <i>B. subtilis</i> "B"											
	Cont.	50 IBA	100 IBA	50 IBA + <i>Bacillus</i>	100 IBA + <i>Bacillus</i>	Mean	Cont.	50 IBA	100 IBA	50 IBA + <i>Bacillus</i>		100 IBA + <i>Bacillus</i>
Cont.	1.09	0.97	0.95	0.92	0.96	0.98	1.10	0.94	0.93	0.90	0.92	0.96
1000	1.10	1.05	1.01	0.97	1.04	1.03	1.10	0.98	0.98	0.80	0.90	0.95
2000	1.12	1.08	1.09	1.01	1.06	1.07	1.13	1.05	1.05	1.02	1.05	1.06
4000	1.12	1.11	1.10	1.06	1.09	1.10	1.11	1.08	1.08	1.05	1.09	1.08
Mean	1.11	1.05	1.04	0.99	1.04		1.11	1.01	1.01	0.94	0.99	
LSD _{0.05}	A = 0.01		B = 0.01		A*B = 0.02		A = 0.02		B = 0.02		A*B = 0.04	
Na/K ratio												
Cont.	0.96	0.78	0.76	0.72	0.77	0.80	1.00	0.78	0.77	0.71	0.75	0.80
1000	0.96	0.83	0.79	0.75	0.81	0.83	0.96	0.80	0.79	0.62	0.72	0.77
2000	0.99	0.88	0.88	0.80	0.86	0.88	1.02	0.87	0.86	0.82	0.85	0.89
4000	1.03	0.97	0.96	0.87	0.92	0.95	1.10	0.96	0.96	0.86	0.96	0.97
Mean	0.99	0.87	0.85	0.78	0.84		1.02	0.85	0.84	0.75	0.82	
LSD _{0.05}	A = 0.01		B = 0.01		A*B = 0.02		A = 0.02		B = 0.02		A*B = 0.04	

TABLE 8. Effect of indole-3-butyric acid (IBA) and *Bacillus subtilis* on C/N ratio in basal end of *Rosmarinus officinalis* cutting under salt stress conditions during the 2014 and 2015 seasons.

Salinity levels (ppm) "A"	First season (2014)					Second season (2015)						
	IBA (ppm) and <i>B. subtilis</i> "B"											
	Cont.	50 IBA	100 IBA	50 IBA + <i>Bacillus</i>	100 IBA + <i>Bacillus</i>	Mean	Cont.	50 IBA	100 IBA	50 IBA + <i>Bacillus</i>	100 IBA + <i>Bacillus</i>	Mean
Cont.	11.6	13.7	14.1	14.8	14.6	13.8	13.6	14.3	14.3	15.0	14.5	14.3
1000	13.0	14.1	14.4	15.6	14.8	14.4	14.0	14.7	15.0	15.5	15.1	14.8
2000	11.2	11.8	12.8	13.6	12.8	12.4	12.7	13.6	13.8	14.5	14.1	13.7
4000	9.6	10.4	11.3	11.6	11.8	10.9	10.9	11.8	12.3	13.1	13.1	12.2
Mean	11.4	12.5	13.1	13.9	13.5		12.8	13.6	13.8	14.5	14.2	
LSD _{0.05}	A = 0.30		B = 0.32		A*B = 0.63		A = 0.19		B = 0.23		A*B = 0.46	

Apparently, it is clearly appeared that treatment of *R. officinalis* cuttings with IBA alone or in combination with *B. subtilis* significantly increased C/N ratio in basal part of the cuttings compared to untreated cuttings in both seasons. The combined treatments of IBA with *B. subtilis* were more effective on increasing C/N ratio compared to the control and single IBA treatments. The maximum value of C/N ratio was obtained from cuttings treated with 50 ppm IBA + *B. subtilis*, followed by 100 ppm IBA + *B. subtilis*. These results are in accordance with those obtained by Abdel-Rahman & El-Naggar (2014) and Hussein et al. (2016), who found that the C/N ratio in cuttings tissues increased as a result of IBA application and/or inoculation with *B. subtilis* which lead to increase rootability and to improve root and shoot characteristics.

Regarding the interaction effect between different salinity levels and IBA treatments with or without *B. subtilis* on C/N ratio in the basal part of *R. officinalis* cuttings, it is noticeable that the application of IBA alone or in combination with *B. subtilis* significantly increased C/N ratio compared to untreated cuttings under salt stress conditions. The highest C/N ratio was obtained in the cutting bases treated with 50 ppm IBA + *B. subtilis* at low salinity level (1000 ppm NaCl) and control. Otherwise, the lowest value of C/N ratio was recorded with untreated cuttings with IBA or combined with *B. subtilis* at the highest salinity level (4000 ppm NaCl). The increment in C/N ratio in cutting bases of *R. officinalis* caused by IBA treatment and inoculation with *B. subtilis* may be due to increase in starch hydrolysis and/or to increase carbohydrates transport towards the rooting zone (Davies, 2004).

Conclusions

From the obtained results in the current study, it could be concluded that increasing irrigation water salinity from 1000 ppm to 2000 and 4000 ppm NaCl caused a significant decrease in rooting percentage, root and vegetative growth characteristics, contents of N, P and K as well as C/N ratio in cutting tissues, whereas Na % and Na/K ratio were increased. Application of IBA alone or in combination with *B. subtilis* could alleviate adverse effects of salinity, especially at the low salinity level (1000 ppm NaCl). IBA-bacteria treatments were more effective in increasing rootability of cuttings and improving the root and shoot characteristics compared to the individual IBA treatments. The combination of

IBA at 50 ppm with *B. subtilis* is recommended to achieve maximum rooting percentage, root and vegetative characteristics of *R. officinalis* cuttings under salt stress conditions.

Acknowledgements: Special thanks and deep gratitude to Dr. Omer Hosni Mohamed, Associate Prof. of Floriculture, Department of Ornamental Plants and Landscape Gardening, Fac. Agric., Assiut Univ., Egypt for his revision of this the manuscript.

Funding statements: The author declares that there is no received external funding for this study.

Conflicts of interest: The author declares that there are no conflicts of interest related to the publication of this study.

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التأثيرات الضارة للإجهاد الملحي على قدرة عقل نبات حصالبان على التجذير وتخفيفها باستخدام إندول حمض البيوتيريك وبكتيريا باسيليس ساتلس

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أجريت هذه الدراسة بمزرعة البساتين- كلية البيطرة والعلوم الزراعية – جامعة الزاوية - ليبيا خلال موسمي ٢٠١٤، ٢٠١٥ وذلك بهدف دراسة تأثير التركيزات المختلفة لإندول حمض البيوتيريك (صفر، ٥٠، ١٠٠ جزء في المليون) بمفرده أو بالاشتراك مع بكتيريا باسيليس ساتلس على قدرة عقل نبات حصالبان على التجذير وكذلك الصفات الخضرية والجزرية تحت ظروف الإجهاد الملحي (كنترول، ١٠٠٠، ٢٠٠٠، ٤٠٠٠ جزء في المليون كلوريد صوديوم). وأظهرت النتائج أن رى العقل بالماء المالح (٢٠٠٠ و ٤٠٠٠ جزء في المليون كلوريد صوديوم) أدى إلى حدوث نقص معنوي في نسبة التجذير، والصفات الخضرية والجزرية للنباتات الجديدة الناتجة، كما أدى إلى نقص محتوى أنسجة العقلة من النيتروجين، الفوسفور والبوتاسيوم، ونسبة الكربوهيدرات إلى النيتروجين مقارنة بالكنترول والمستوى المنخفض من الملوحة، بينما نسبة الصوديوم ونسبة الصوديوم إلى البوتاسيوم كانت مرتفعة عند المستويات المرتفعة من الملوحة. من ناحية أخرى، فإن معاملة العقل بإندول حمض البيوتيريك بمفرده أو بالاشتراك مع بكتيريا باسيليس ساتلس أدى إلى تخفيف معظم التأثيرات الضارة للإجهاد الملحي، خاصة مع المستوى المنخفض من الملوحة. بصفة عامة، المعاملة المشتركة لإندول حمض البيوتيريك بتركيز ٥٠ جزء في المليون + بكتيريا باسيليس ساتلس أظهرت تفوقا ملحوظا في زيادة قدرة العقل على التجذير وكذلك الصفات الخضرية والجزرية للنباتات الناتجة، كما أدت إلى زيادة محتوى أنسجة العقلة من النيتروجين، الفوسفور والبوتاسيوم وكذلك نسبة الكربوهيدرات إلى النيتروجين، بينما أدت إلى نقص في نسبة الصوديوم ونسبة الصوديوم إلى البوتاسيوم مقارنة بالكنترول وجميع المعاملات الأخرى. لذا يمكن أن يوصى باستخدام هذه المعاملة لتخفيف التأثيرات الضارة لملوحة ماء الري على تجذير ونمو عقل نبات حصالبان.

الكلمات الدالة: حصالبان، العقلة، التجذير، إندول حمض البيوتيريك، باسيليس ساتلس، الإجهاد الملحي.