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EFFECTS OF NACL ON GROWTH, ESSENTIAL OIL AND CHEMICAL COMPOSITION OF *PLECTRANTHUS AMBOINICUS*

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Abstract

Plectranthus amboinicus (Lour.) Spreng. is a plant used both as spice or as traditional herbal medicine. Its cultivation is expected to grow in the future, driven by the growing interest toward nutraceuticals and alternative medicine. Mediterranean Region is amply characterized by salt irrigation water, so posing limitations to the introduction of new crops. However, if properly managed, this feature can be used to improve the secondary metabolites profile of many crops. For these reasons a greenhouse experiment was conducted to determine the effect of four NaCl concentrations in the irrigation water (corresponding to an EC of 0.4, 5, 10 and 15 dS m^{-1}) on some physiological variables, as well as on essential oil content, yield and chemical composition in *P. amboinicus*. Saline irrigation progressively increased Na^+, Cl^- and proline concentration into the plant (up to +235, +231 and +959%, respectively), highlighting a growing condition of metabolic disturbance in salt-stressed plants. Accordingly, salt stress gradually reduced *P. amboinicus* fresh herb and essential oil yield as compared to control plants (up to -68 and -62%, respectively). However salt-stressed plants increased their essential oil content up to 0.129% at 10dS m^{-1}. Thymol, γ-terpinene, p-cymene and trans-caryophyllene were the major components of *P. amboinicus* and the concentration of these compounds was significantly affected by salt stress. Our results show positive modifications in the essential oil composition of *P. amboinicus* in response to salt stress, a feature that could be properly supported by means of agronomical and breeding strategies.

Key words: salinity stress, herb, essential oil, proline, ionic content.

Introduction

Plectranthus amboinicus (Lour.) Spreng, also known as Cuban oregano or Mexican mint, is a pubescent, succulent perennial herbnative to Southern and Eastern Africa (El-Leithy *et al*., 2019), belonging to the *Lamiaceae* family. The plant is used both as spice or as traditional herbal medicine to treat inflammation related diseases, particularly for skin, infective, digestive and respiratory problems (Lukhoba *et al*., 2006; Chen *et al*., 2014). Indeed, *P. amboinicus* essential oil shows insecticidal, antileptospiral, antioxidant, anti-inflammatory, analgesic, diuretic, cytotoxic, antparasites and antimicrobial activities (Valera *et al*., 2003; Nirmala *et al*., 2008; El-hawary *et al*., 2013; Hikal and Said-Al Ahl, 2019), so the growing interest of consumers toward nutraceutical products and alternative medicine is expected to prompt in the future its cultivation in several regions worldwide.

Over recent years, the cultivation of medicinal and aromatic plants in the Mediterranean Region is experiencing a phase of increasing interest, due to the possibilities of transforming them into high value-added products for nutraceutical and pharmaceutical purposes (Mittal and Singh, 2007). However, due to its climatic peculiarities often leading to over-exploitation of groundwater resources, this area accounts for million of ha of salt-affected soils, both in South Europe and North Africa (Abou-Hadid, 2003; Libutti *et al*., 2018), so posing potential limitations to the introduction of new species in the cultivation systems.

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Indeed, salinity stress is one of the most serious challenges facing the agricultural production, causing considerable yield loss in agricultural farmlands (Passioura, 2007; Munns and Tester, 2018). These are primarily due to the osmotic stress, which generates the disruption of homeostasis and distribution of ions in plant cells (Munns and Tester, 2018). In addition to the osmotic component, salinity causes ion toxicity because of the increased intracellular concentration of toxic ions such as Na^+ and Cl^- (Grattan and Grieves, 1998), which leads to nutrient disorders including imbalances and/or competition with nutrient uptake (Maathuis and Amtmann, 1999). Competition between these and other anions and cations results in reduced growth and stomatal aperture of different crops (De Pascale *et al*., 2005; Paz *et al*., 2014).

On the other hand, it has been demonstrated that irrigation with saline water is able to improve some quality characteristics of several crops (Rouphael *et al*., 2012; Di Gioia *et al*., 2018), including the biosynthesis of secondary metabolites in response to the osmotic and nutritional stress (Giuffrida *et al*., 2018). Therefore, if properly managed, irrigation with salt water could represent adoption to improve the quality of the raw materials of these emerging crops, provided thatin the future adequate and reliable knowledge will become available to growers. For these reasons, the objective of this study was to determine the effect of NaCl saline irrigation on *P. amboinicus* fresh herb yield, physiological response, essential oil content and chemical composition.

Materials and Methods

Site and treatment descriptions

The experiment was carried out during two growing seasons (2018 and 2019), under the natural conditions of a greenhouse of the Farm Station of Faculty of Agriculture, Cairo University, Egypt. The local climate is subtropical desert (*Bwh* climate according to Köppen classification), with mild, wet winters and warm, dry summers. Homogeneous *P. amboinicus* stem cuttings (~15 cm long and having 8 expanded leaves) have been taken from mother plants on early February 2018 and 2019 andtransplanted into pots (30 cm diameter, 50 cm depth, 3.5 L volume). The pots contained two stem cuttings each and were placed in the greenhouse on 15 February of each year. Each pot was previously filled with 10 kg of air-dried soil. The soil texture was sandy-loam, having a physical composition as follows: 45.5% sand, 27.6% silt, 26.9% clay and 0.87% organic matter. Before the start of the experiment, the results of soil chemical analysis were as follows: pH = 8.05; EC (dSm^{-1}) = 0.80; total nitrogen = 0.10%; available phosphorus and potassium were 2.11 $mg100g^{-1}$ and 0.021 $mg100g^{-1}$, respectively; Sodium = 3.55 meq $100g^{-1}$ soil and Chloride = 0.52 $meq100g^{-1}$ soil. All soil properties were determined by using the standard methods.

Within each growing season, the experimental layout was a randomized blocks design with three replicates. Each experimental unit contained fifteen pots and each pot contained two plants. The study regarded four NaCl saline irrigation treatments, namely control (0.40 dSm^{-1}, tap water), 5 dSm^{-1}, 10 dSm^{-1} and 15 dSm^{-1}, hereafter referred as S_0, S_5, S_{10} and S_{15}, respectively. Before transplanting, each pot was fertilized with 200 g cattle manure. The use of salt solutions started 7days after transplanting and each final EC was gradually achieved 10days after by adding the 10% day^{-1} of the final NaCl concentration (which was 2300, 4800 and 7300ppm in S_5, S_{10} and S_{15}, respectively).

Physiological and chemical variables

In each growing season, Na^+ and Cl^- were measured in dry herb according to Cottenie*et al*., (1982), while proline determination was carried out in fresh leaves according to Bates (1973). Plant fresh weight ($gplant^{-1}$), essential oil content (%) and essential oil yield ($mlplant^{-1}$) were determined in the fresh herb at the end of the experiment (on June 15 of each year, *e.g*. 4 months after transplantation). Essential oil was extracted by hydro-distillation for 3 h. (Guenther, 1961). The essential oils were collected and dehydrated over anhydrous sodium sulphate and kept in a refrigerator until GC-MS analysis.

GC-MS analyses and identification of components

GC-MS analysis of the essential oil was performed using HP 5890 series II gas chromatograph and HP 5973 mass detector. A TRFAME (Thermo 260 M142 P) capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) with helium as the carrier gas, at a flow rate of 1.5 $mlmin^{-1}$ was used. The initial GC oven temperature was set at 40°C for 5 minutes and then heated up to 140°C at a rate of $5°Cmin.^{-1}$ and held at 140°C for 5 minutes. Then the temperature was increased to 280°C at a rate of $10°Cmin.^{-1}$ and held for 5 additional minutes. Injector and detector temperatures were 250°C. Diluted samples (1/100, v/v in heptane) of 1.0 μl were injected automatically. Mass spectrometry was run in the electron impact mode (EI) at 70 eV. Identification of components was based on the comparison of their GC retention times. Mass spectra interpretation was confirmed by mass spectral library search using the National Institute of Standards and Technology (NIST) database (Masada, 1976; Adams, 2007).

Statistical analysis

A 1-way analysis of variance (ANOVA) related to a randomized blocks design with 6replicates was used to determine the effect of NaCl concentration in the irrigation water on the studied variables. The 6 replicates were combinations of the three blocks within each growing season and the two growing seasons. The GLM Procedure of SAS, (2014) was used to complete the statistical analysis. For each response variable, the validity of normal distribution and constant variance assumptions on the error terms was verified by examining the residuals as described in Montgomery, (2017). Independence assumption was met through randomization within each block. Percentage data were subjected to Bliss' transformation (untransformed data are reported and discussed). Since the effect of NaCl saline irrigation was significant ($P< 0.05$) on all response variables, multiple means comparison was completed and letter groupings generated using Tukey's honestly significant difference (HSD) test at 5% level of significance. A polynomial regression analysis was performed, up to third degree, to define the trend of the variables in response to the increase in salinity level. A multiple correlation analysis was also performed, in order to check any significant relationship among the physiological variables and chemical composition of *P. amboinicus* tissues.

Results and Discussion

Na^+ and CI^- content in plant tissues

The increase in NaCl concentration in the irrigation water generated a linear increase in the content of both ions into the plant tissues (Table 1). Comparing the extreme treatments, such increase was similar for both ions, *i.e*. equal to +235% for Na^+ (from 0.870 to 2.915%) and +231% for Cl^- (from 311 to 1028 ppm) (Table 1). However, when considered at S_5 treatment, it was recorded a higher increase for Cl^- concentration (+124%) than for Na^+ (+50%), meaning that up to this salinity level, *P. amboinicus* plants were able to partially buffer the cation entrance inside the plant, a mechanism which is noticeable for glycophytes (Liang *et al*., 2018). Beyond this threshold, both ions showed a similar accumulation trend, so suggesting a raising alteration in the mechanisms of ion homeostasis, transport and partitioning inside the plant. In the case of Na^+ and Cl^-, the ion accumulation is able to generate nutrient deficiencies or imbalances, due to their competitive action toward other nutrients (Pardo and Quintero, 2002). Moreover, their specific toxicity once they get into the cytoplasm, is able to inhibit the activities of many cellular enzymes, so negatively acting on biophysical and/or metabolic components of plant growth (Grattan and Grieves, 1998; Bayuelo-Jimenez *et al*., 2003).

Table 1: Proline (μmol g^{-1} fresh weight), Na^+ (%), CI^- (ppm), mean herb fresh weight ($gplant^{-1}$), essential oil content (%) and essential oil yield ($mlplant^{-1}$) obtained from *Plectranthus amboinicus* subjected to four NaCl saline irrigation treatments. L: linear, C: cubic. *, ** and ***: significant a $P\leq 0.05$, 0.01 and 0.001, respectively.

Treatment	Na^+	Cl^-	Proline	Plant fresh weight	Essential oil content	Essential oil yield
S_0	0.870 d	311 d	0.302 d	559 a	0.098 c	0.550 a
S_5	1.305 c	698 c	0.997 c	397 b	0.107 bc	0.426 b
S_{10}	2.242 b	822 b	2.202 b	297 c	0.129 a	0.384 b
S_{15}	2.915 a	1028 a	3.197 a	179 d	0.110 b	0.208 c
Trend	L^*	L^*	L^{**}	L^{**}	C^{***}	L^*

Within each column, means sharing the same letter are not significantly different at Tukey's HSD test ($P\leq 0.05$).

Proline content

Table 1, shows that each increment in NaCl concentration in the irrigation water significantly increased the proline content in plant tissues, with a high significance of the linear component of the linear regression. These increases were equal to +230%, +629% and +959% at S_5, S_{10} and S_{15}, respectively, meaning that at each of these salinity levels proline concentration (and the energy expense for its biosynthesis) increased proportionally more than the accumulation of Na^+ and Cl^- inside the plant. From an ecophysiological viewpoint, such steep accumulation of proline seems to suggest the need of *P. amboinicus* plants to sustain an adequate water transpiration flux in order to maintain their leaves succulence, since the known protective action of proline against the hyperosmotic stress (Nounjan *et al*., 2012). On the other hand, the increase in proline content due, for example, to the decrease in proline oxidase activity in saline conditions, is one of the main adaptation ways of plants to salinity, since the multifunctional protective role of this amino acid, acting as free radical scavenger and antioxidant compound (Said-Al Ahl and Omer, 2011), so playing a key role in stabilizing proteins, DNA and helping to maintain the structure and function of cellular macromolecules (Matysik *et al*., 2002).

Herb and essential oil yield and content

The results shown in table 1 indicate that NaCl affected the studied variables in different ways. Increasing the NaCl concentration in the irrigation water led to a significant, linear decrease in fresh weight and oil yield of *P. amboinicus* plants. The highest fresh weight and oil yield values were obtained from S_0 treated plants, whereasboth variables showed the least values at S_{15}, showing a decrease of 68% and 62%, respectively, when compared to unstressed plants (Table 1). On the other hand, the oil content increased passing from S_0 to S_{10} (from 0.098 to 0.129%) and then decreased (Table 1).

Saline conditions reduces the ability of the plants to absorb water and minerals from the soil and induces many metabolic changes, which leads to reduced leaf transpiration and closure of stomata (Ben-Asher *et al*., 2006). According to Meiri and Shahavet, (1973), salinity affects plant growth with different mechanisms: (a) the distribution of salts within the plant cells may result in turgor reduction and growth retardations, (b) the balance between root and shoot hormones changes considerably under saline conditions, (c) salinity changes the structure of the chloroplasts and mitochondria and such changes may interfere with normal metabolism and growth and (d) salinity increases respiration and reduces photosynthesis products available for growth. However, as we found in our study, oil yield may be decreased by salinity due to the decrease in plant growth (Table 1). Previous studies have confirmed that NaCl salt condition has reduced growth and oil yield in *P. amboinicus* (Abdelrazik *et al*., 2016) and *Anethum graveolens* L. (Ünver and Tilki, 2012).

As shown in table 1, NaCl salt condition increased plant oil content, likely as a result of a higher oil gland density and an increase in the absolute number of glands produced prior to leaf emergence and the effect of salinity on either net assimilation or the partitioning of assimilates among growth and differentiation processes[36]. Another reason for the increase in oil content with increasing salinity, up to S_{10}, is probably due to a decline in the primary metabolites, causing intermediary products to become available for secondary metabolites synthesis (Charles *et al*., 1990) and may be due to its effects on enzyme activity and metabolism (Burbott and Loomis, 1969). Stimulating oil content due to salinity in aromatic species has been reported in several previous studies (Omer *et al*., 2014; Abdelrazik *et al*., 2016).

GC-MS analysis

The results shown in table 2 indicate that thymol (36.32- 48.55%), γ-terpinene (10.02-25.35%), p-cymene (6.76-13.25%) and trans caryophyllene (7.74-10.07%) were the major components in *P. amboinicus* (more than 10%), α-thujene (0.17-1.52%), α-pinene (0.57-1.01%), β-myrcene (1.28-2.31%), 1-octen-3-ol (2.11-5.12%), α-terpinene (3.66-7.23%), α-phellandrene (0.27-1.53%), α-terpinolene (2.15-3.69%), α-copaene (1.00-1.89%), α-bergamotene (0.47-1.61%), α-humulene (1.13-3.32%) were categorized as minor components (less than 10% and more than 1%) and the remaining components (α-phellandrene, terpinene-4-ol, β-selinene, β-patchoulene and β-cadinene) were trace components (less than 1%).

Table 2: GC-MS percentage quantification of *P. amboinicus* components. L: linear; Q: quadratic; C: cubic. *, ** and ***: significant a $P \leq 0.05$, 0.01 and 0.001, respectively.

Component	S_0	S_5	S_{10}	S_{15}	Trend
α-thujene	0.58 a	0.15 c	0.17 c	0.28 b	Q *
α-pinene	0.57 b	0.63 b	1.00 a	1.01 a	C ***
β-myrcene	2.31 a	1.63 b	1.28 c	2.12 a	Q *
1-octen-3-ol	3.72 b	0.25d	5.12 a	2.11 c	C ***
α-phellandrene	0.31c	0.45b	0.80 a	0.83 a	Q *
α-terpinene	3.99 c	3.66 d	5.11 b	7.23 a	Q **
p-cymene	10.34 b	13.25a	6.98 c	6.76 c	C ***
β-phellandrene	0.70 b	0.03d	1.53 a	0.51 c	C ***
γ-terpinene	25.35 a	19.75b	13.12c	10.02d	L*
α-terpinolene	3.06 b	2.15 c	3.69 a	3.10 b	C ***
terpinen-4-ol	0.76 a	0.03c	0.63 b	0.66 b	C ***
thymol	36.32 c	39.65bc	43.21 ab	48.55 a	L **
α-copaene	1.11 b	1.89 a	1.86 a	1.00 b	Q ***
trans-caryophyllene	8.99 b	10.07 a	7.74 c	8.00 c	C ***
α-bergamotene	0.47 c	0.90b	0.47 c	1.61 a	C ***
α-humulene	1.13 c	0.33d	1.39 b	2.21 a	C ***
β-selinene	ND	0.58b	0.98 a	0.30 c	Q ***
β-patchoulene	0.53 a	0.49a	0.29 c	0.41 b	C ***
β-cadinene	0.60 b	0.71a	ND	0.33 c	Q ***

ND = not detected. Within each row, means sharing the same letter are not significantly different at Tukey's HSD test ($P \leq 0.05$).

Previous studies on *P. amboinicus* revealed the identification of several components including carvacrol, thymol, p-cymene, γ-terpinene and (Z)-caryophyllene as major (Pinheiro *et al*., 2015; Santos *et al*., 2015). Sabra *et al*., (2018) also reported that thymol, γ-terpinene, p-cymene and trans caryophyllene were the four major components of *P. amboinicus* essential oil.

The results in table 2, show that the concentrations of p-cymene and trans-caryophyllene increased with NaCl saline irrigation from control to 5 dSm^{-1}, then decreased at 10 and 15 dSm^{-1}. On the other hand, thymol and γ-terpinene showed opposite response to salinity (as NaCl concentration increases, the concentration of thymol increased while that of γ-terpinene decreased) (Table 2). In other words, the control plants gave the highest concentration of γ-terpinene (25.35%), but the

Table 3: Pearson's product-moment correlation coefficients (r) among physiological variables and the aromatic components identified in P. amboinicus. *and ** indicate significance at $P \leq 0.05$ and 0.01, respectively. NS: not significant.

Component	Na+ concentration	Cl^- concentration	Proline concentration	Plant fresh weight	Essential oil content	Essential oil yield
α-thujene	-0.484^{NS}	-0.728^{NS}	-0.500^{NS}	0.647^{NS}	-0.691^{NS}	0.521^{NS}
α-pinene	0.955*	0.860^{NS}	0.946^{NS}	-0.904^{NS}	0.768^{NS}	-0.825^{NS}
β-myrcene	-0.207^{NS}	-0.375^{NS}	-0.206^{NS}	0.311^{NS}	-0.854^{NS}	0.101^{NS}
1-octen-3-ol	0.114^{NS}	-0.156^{NS}	0.079^{NS}	0.067^{NS}	0.496^{NS}	0.188^{NS}
α-phellandrene	0.967*	0.913^{NS}	0.962*	-0.944^{NS}	0.767^{NS}	-0.867^{NS}
α-terpinene	0.929^{NS}	0.782^{NS}	0.926^{NS}	-0.849^{NS}	0.289^{NS}	-0.901^{NS}
p-cymene	-0.797^{NS}	-0.549^{NS}	-0.775^{NS}	0.645^{NS}	-0.568^{NS}	0.600^{NS}
β-phellandrene	0.282^{NS}	0.072^{NS}	0.250^{NS}	-0.142^{NS}	0.728^{NS}	0.021^{NS}
γ-terpinene	-0.984*	-0.970*	-0.985*	0.988*	-0.680^{NS}	0.937^{NS}
α-terpinolene	0.445^{NS}	0.166^{NS}	0.413^{NS}	-0.264^{NS}	0.601^{NS}	-0.161^{NS}
terpinen-4-ol	0.245^{NS}	-0.110^{NS}	0.215^{NS}	-0.017^{NS}	0.052^{NS}	-0.038^{NS}
thymol	0.989*	0.953*	0.993**	-0.982*	0.481^{NS}	-0.987*
α-copaene	-0.171^{NS}	0.076^{NS}	-0.164^{NS}	0.019^{NS}	0.564^{NS}	0.202^{NS}
trans-caryophyllene	-0.735^{NS}	-0.482^{NS}	-0.710^{NS}	0.577^{NS}	-0.623^{NS}	0.509^{NS}
α-bergamotene	0.677^{NS}	0.719^{NS}	0.697^{NS}	-0.721^{NS}	-0.136^{NS}	-0.855^{NS}
α-humulene	0.793^{NS}	0.541^{NS}	0.779^{NS}	-0.643^{NS}	0.224^{NS}	-0.693^{NS}
β-selinene	0.364^{NS}	0.502^{NS}	0.362^{NS}	-0.451^{NS}	0.921^{NS}	-0.245^{NS}
β-patchoulene	-0.708^{NS}	-0.654^{NS}	-0.693^{NS}	0.676^{NS}	-0.976*	0.504^{NS}
β-cadinene	-0.681^{NS}	-0.526^{NS}	-0.658^{NS}	0.584^{NS}	-0.886^{NS}	0.433^{NS}

lowest concentration of thymol (36.32%) (Table 2). Conversely, plants treated with the highest level of NaCl gave the highest concentration of thymol (48.55%) and the lowest one of γ-terpinene (10.02%).

For p-cymene and trans-caryophyllene components, S_5 treated plants gave the highest percentages of both p-cymene (13.25%) and trans-caryophyllene (10.07%) and the lowest percentages of p-cymene (6.76%) and trans-caryophyllene (7.74%) were obtained from S_{15} and S_{10} treated plants, respectively (Table 2). It was reported that thymol, p-cymene, γ-terpinene and caryophyllene concentrations from *Origanum vulgare* L. increased with NaCl saline irrigation (Said-Al Ahl *et al*., 2010). However, thymol, p-cymene and ã-terpinene increased with increasing salinity level, but the increase in thymol continued up until the level below the highest salinity level and then it decreased in ajwian oil seeds (Burbott and Loomis, 1969). On the contrary, γ-terpinene decreased with increasing NaCl stress in summer savory (*Satureja hortensis* L.) plants (Najafi *et al*., 2010). These results indicate that the accumulation of essential oil components in *P. amboinicus* is largely influenced by salt irrigation in different ways. In this regard, the differential thymol : γ-terpinene ratio as a function of NaCl salinity irrigation may be due to chemical conversion from one component to another, or due to the differences in the regulation of the activities of some key enzymes involved in the biochemical biosynthesis of these components.

Correlation among physiological variables and chemical constituents of P. amboinicus plants

Overall 114 were analyzed, of which 13 (11.4% of total) showed significance, revealing 6 negative and 7 positive relationships (Table 3).Thymol and γ-terpinene were those constituents showing the highest number of significant correlations (5 and 4, respectively), followed by α-phellandrene (2 significant correlations) and by α-pinene and β-patchoulene (1 significant correlation each). Among the negative correlations, the lowest r values were recorded among thymol and essential oil yield (-0.987 *) and amongg-terpinene, proline and Na^+ content (-0.985* and -0.984*, respectively). The strongest relationship in the data frame of positive correlations was found among thymol, proline (0.993**) and Na^+ content (0.989*) in plant tissues and among g-terpinene and plant fresh weight (0.988*) (Table 3). On the whole, the correlation analysis showed that the content of thymol and γ-terpinene in *P. amboinicus* green tissues were the most reliable indicators of plants' stress under salt conditions, with thymol likely playing a key role in modulating the plants' protective response against the salt-derived stresses. Interestingly, thymol was significantly correlated to the essential oil yield and its related variable of plant fresh weight, while γ-terpinene and β-patchoulene were significantly correlated to the essential oil yield component of plant fresh weight and essential oil content in plant tissues. This suggests the possibility to use the concentration of these three molecules as predictive markers of plant's adaptation and essential oil yield in large scale cultivation of the species under salt-stressed conditions.

Conclusions

P. amboinicus is a promising crop from both a nutraceutical and pharmacological view point, whose diffusion could help to diversify the Mediterranean agricultural systems and to respond to the growing consumer demands toward natural, health-promoting products. Our results demonstrate that the use of salt irrigation water, which characterizes ample areas of this Region, generates a condition of stress to the crop, resulting in an increase of the essential oil content and in a modification of its composition, but in a reduction of the overall plant growth, with a subsequent reduction of the essential oil yield. However, when taken together, all these features suggest the possibility to buffer the detrimental effects of salt stress by means of agronomical factors, such as the increase in plant density (at least up to an EC of 10 dS $m^{-1}$of the irrigation water). Such indication appears consistent with the good growth performance of the genus *Plectranthus* with respect to seasonal light interception and radiation use efficiency (Zhang *et al.*, 2017). Our study highlighted, in *P. amboinicus*, the existence of a mechanism of tolerance to salt stress, acting up to 5 dS m^{-1} to limit di Na^+ entrance inside the plant and more importantly, a positive response of the main constituent thymol to the increasing salt stress. Since the wide range of functional possibilities in pharmacy, food and cosmetic industry of this phenol monoterpene (Salehi *et al*., 2018), our results suggest the need to intensify the studies to identify genes and enzymes that modulate the quantitative and qualitative production of essential oil in this herb, along with the plant's tolerance to salinity.

Conflict of interest

The authors declare that there is no conflict of interest.

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