International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 8, Issue 8, 2016

Original Article

RESPONSE OF GROWTH, ESSENTIAL OIL CONTENT AND ITS CONSTITUENT'S OF *PLECTRANTHUS AMBOINICUS* TO IRON AND/OR UREA FOLIAR APPLICATION UNDER SALINE IRRIGATION

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Received: 18 Apr 2016 Revised and Accepted: 20 June 2016

ABSTRACT

Objective: To study the response of *Plectranthus amboinicus* plants to iron and/or urea foliar application using tap water and Nacl saline water for irrigation, a pot experiment was conducted during two successive seasons (2014 and 2015) under the natural conditions of the greenhouse of the National Research Center, Dokki, Giza, Egypt.

Methods: *Plectranthus amboinicus* cuttings were transplanted in the pots in February of each season**.** After one month, seedlings were irrigated with (2 levels: Tap water (0.40 dsm-1), and Nacl solution (4 dsm-1)) and FeUrea (4 levels: None, EDTA (200 ppm), urea (1000 ppm), and mixture of urea (1000 ppm) and Fe EDTA (200 ppm)) were applied one month from transplanting. The foliar application treatments were sprayed at 60, 90, 120 and 150 d from transplanting**.** Plant fresh mass (g/plant) and proline content were determined in the first and second cuts after 120 and 180 d from transplanting, respectively. The essential oil was obtained by hydrodistillation and essential oil % was expressed as ml 100 g/fresh herb. The chemical composition of the essential oil was studied using GC-MS and compounds were identified based on their mass spectra and literature.

Results: Repeated measures analysis of the data showed significant effects of irrigation and iron/urea on fresh weight of herb, essential oil (%), oil yield, and proline in both first and second cuts. Plectranthus amboinicus plants sprayed with urea/or iron under tap and Nacl saline conditions were better than the control (unsprayed) plants. While saline irrigation decreased fresh weight, essential oil and oil yield, it increased proline; and 200Fe+1000Urea gave significantly higher proline for both tap and Nacl irrigations. Within each irrigation, the highest fresh mass and oil yield were obtained from 200Fe+1000Urea. Fresh mass for tap irrigation was significantly higher during the second cut than during the first cut, but it was the opposite for oil yield. This research demonstrated that application of iron and/or urea decreases salinity stress, and increases proline content. The GC/MS analysis revealed the major components of Plectranthus amboinicus to be p-cymene, carvacrol, (+)-epibicyclosesquiphellandrene, β-cadinene and α-cadinol.

Conclusion: It may be concluded that Plectranthus amboinicus is tolerant to Nacl irrigations, thus we may recommend its cultivation in slain soil of Egypt. Foliar spraying with iron and/or urea under these conditions could be much more efficient than the not application of nutrients. So, we recommended that foliar application of iron and/or urea to raise the salt stress tolerance of *Plectranthus amboinicus.*

Keywords: Plectranthus amboinicus, Foliar application, Urea, Iron, Salinity, Essential oil

INTRODUCTION

Genus *Plectranthus* (Lamiaceae family), distributed in Tropical Africa, Asia and Australia and comprises about 350 species worldwide was cultivated as ornamentals or as sources of essential oils, whereas others are used as edible tubers, or as food flavorings [1]. *Plectranthus amboinicus* (Lour.) is a perennial herb native to Indonesia and used as a traditional herbal medicine [2]. The volatile oil of Plectranthus amboinicus was reported to exhibit insecticidal [3]; anti-leptospiral [4]; and antioxidant, anti-inflammatory, analgesic, diuretic, cytotoxic and antimicrobial [5]. *Plectranthus amboinicus* has been used for decades to treat inflammation related diseases, particularly for skin, infective, digestive, and respiratory problems [1]. It has been used to treat convulsive, coughs, sore throats, nasal congestion, epileptic, bronchodilator syndromes, cutaneous leishmaniasis and a range of other problems such as rheumatism and flatulence [6-8]. *Plectranthus amboinicus* extract has been shown to exhibit analgesic, anti-inflammatory activities, diabetic foot ulcers and rheumatoid arthritis [9-11].

Salinity is one of the major factors that affect plant growth and is a serious problem in many areas of the world causing considerable loss in agricultural production [12]. The deleterious effects of soil salinity resulting from natural processes or from crop irrigation with saline water on plant growth are associated with (1) low osmotic potential of soil solution (water stress), (2) nutritional imbalance, (3) specific ion effect (salt stress), or (4) a combination of these factors [13]. In a saline environment, ion homeostasis can be disturbed by excessive uptake of Na^+ and Cl^-. Competition between these and other anions and cations resulted in reduced growth [14]. Salinity can reduce N accumulation in plants and an increase in Cl^- uptake, which is attributed to Cl^-antagonism of $NO3^-$uptake [15, 16]. Inhibition of nitrogen uptake may occur by $NO3^-$/Cl^-interaction at the sites of ion transport because sodium results in severe membrane depolarization in plants [17], which in turn has been linked to non-competitive inhibition of nitrate uptake [18].

The addition of N improves plant growth and yield regardless of whether the crop is salt-stressed or not. Some studies showed that N-fertilizer additions alleviate the deleterious effect of salinity on plants and improved growth and/or yield [19]. Urea is the most suitable nitrogen source for foliar applications, characterized by high leaf penetration rate due to its low salt index and high solubility as well as most plants can absorb rapidly and hydrolyze urea in the cytosol [20]. Urea has been shown to stimulate absorption of other nutrients by increasing the permeability of leaf tissue and also increase the level of storage of N compounds such as amino acids and proteins [21]; thus, foliar urea could directly affect N metabolism under saline conditions and therefore amino acids synthesis. Moreover, the application of urea directly to leaves can be a potential alternative to conventional soil fertilization and does not contribute to soil salinity or potential groundwater contamination

[22]. Del Amor and Cuadra-Crespo [23] showed that foliar spray of urea to the salinized plants appeared to counteract the deleterious effects of salinity partially.

Iron (Fe) is a cofactor for approximately 140 enzymes that catalyze unique biochemical reactions [24]. Hence, iron fills many essential roles in plant growth and development, including chlorophyll synthesis, thylakoid synthesis and chloroplast development [25]. In saline soils, the solubility of micronutrients is particularly low, and plants grown on such soil often suffer from deficiencies in these elements. Soil salinity may reduce micronutrients uptake due to stronger competition by salt cations at the root surface [26]. Abbas *et al.* [27] concluded that in the salt affected areas, the iron application could alleviate possible Na and Cl injury in plants.

Moreover, little is known about salinity interaction with iron and nitrogen fertilizer (urea). The present study aimed to test the hypothesis that exogenous, foliar application of N-fertilizer (urea) or iron could counteract the NaCl concentration, and to elucidate their responses when foliar N-fertilizer or iron are applied to *Plectranthus amboinicus* plants. Also, the present study describes the composition of the essential oils of *Plectranthus amboinicus* cultivated in Egypt.

MATERIALS AND METHODS

The experiment was carried out under the natural conditions of the greenhouse of the National Research Center, Dokki, Giza, Egypt, during the two growing seasons. The physical and chemical analyses of the soil were determined according to Jackson [28]. The soil texture was sandy loam, having the following physical composition: 46.80% sand, 28.20% silt, 25.0% clay and 0.85% organic matter. The results of soil chemical analysis were: pH = 8.12; E. C (mmohs/cm) = 0.73; and total nitrogen = 0.09 %; available phosphorus = 2.0 mg/100gram; potassium = 20.8 mg/100gram and iron = 21.0 ppm. In February of each season, stem cuttings were immediately placed in the pots deep enough so that the cuttings will not fall over. The cuttings were irrigated, and the soil was kept damp. The experimental unit was a bench that has five pots, and each pot contained three plants. Eight treatment combinations of Irrigation (2 levels: Tap water (0.40 dsm-1), and Nacl solution (4 dsm-1)) and FeUrea (4 levels: None, EDTA (200 ppm), urea (1000 ppm), and mixture of urea (1000 ppm) and Fe-EDTA (200 ppm)) were applied one month from transplanting. The foliar application treatments were sprayed at 60, 90, 120 and 150 d from transplanting. The experimental layout was 2x4 Factorial design with three replications in two Year blocks.

Herb and chemical composition

Plant fresh mass (g/plant) of each experimental unit was determined in the first and second cuts after 120 and 180 d from transplanting, respectively. Determination of proline was carried out in the fresh herb according to the method given by Bates [29]. The essential oil percentage was determined in the fresh herb of each experimental unit. To extract and quantify the essential oils, a mass of 100 g of fresh herb, during each of the two cuts, and in each year was separately subjected to hydrodistillation for 3 h using a modified Clevenger apparatus. Essential oil percentage of each experimental unit was determined and expressed as (%), while essential oil yield per plant was expressed as ml/plant. 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 analyses were carried out on a Varian 450-GC connected with a Varian 220-MS. Separation was achieved using a Factor Four TM capillary column VF 5ms (30m× 0.25 mm i.d., 0.25 µm film thickness). Injector type 1177 was heated to a temperature of 220 °C. Injection mode was splitless (1 µl of a 1:1000 n-hexane solution). Helium was used as a carrier gas at a constant column flow rate of 1.2 ml min−1. The column temperature was programmed: initial temperature was 50 °C for 10 min, then increased to 100 °C at 3 °C min−1, maintained isothermal for 5 min and then increased to 150 °C at 10 °C min−1. The total time for analysis was 46.7 min. The mass spectrometer trap was heated to 200 °C, manifold 50 °C and transfer line 270 °C. Mass spectra were scanned every 1 s in the range 40–650m/z. Components were identified by comparison of their mass spectra with those stored in NIST 02 (software library) or with mass spectra from the literature [30, 31], and a home-made library, as well as by comparison of their retention indices with standards.

Statistical analysis

The statistical analyses were conducted on fresh mass (g/plant), proline, essential oil (%), and oil yield (ml plant-1) response variables that were collected during the two cuts and in the two years (2014 and 2015). Since the two cuts were made from the same experimental unit on Day 120 and Day 180 after transplanting, the response measurements were analyzed as repeated measures in a two-factor (Irrigation and FeUrea) factorial design with two-year blocks. Since the responses measured during the second cut depend on the values measured during the first cut, the data were analyzed as repeated measures by identifying the most appropriate type of covariance structure (dependence) and incorporating it in the model [32] using the Mixed procedure of SAS [33]. This was followed by multiple means comparison for significant (P-value<0.05) and marginally significant (0.05<P-value<0.1) effects by comparing the least squares means of the corresponding treatment combinations using the LSmeans statement of Proc Mixed with the PDIFF option to produce P-values for all pairwise differences. Letter groupings were generated using a 1% level of significance for two-factor and three-factor interaction effects to protect the over inflation of Type I experiment-wise error rate, and a 5% level of significance was used for main effects. For each response, the validity of normal distribution and constant variance model assumptions on the error terms were verified by examining the residuals as described in Montgomery [34]. A power transformation was needed for two of the four response variables, but the means reported are back-transformed to the original scale.

RESULTS AND DISCUSSION

The P-values shown in table 1 suggest that the factors under study have significant effects on fresh mass, essential oil (%), oil yield and proline in both first and second cuts. However, the extent and interaction of these factors depended on the response variable. For fresh mass and oil yield, the three-way interaction effect of irrigation, FeUrea treatment and cut was significant, whereas the interaction of irrigation and FeUrea was significant on proline. There was a significant difference in proline from the two cuts regardless of the irrigation and FeUrea treatment used (table 1). On the other hand, the interaction effects of irrigation and cut, and FeUrea and cut were highly significant on essential oil (table 1).

Table 1: ANOVA P-values are showing the main and interaction effect of Irrigation (Irrig), Fe and Urea (FeUrea), and Cut on fresh mass, proline, essential oil (%) and oil yield. P-values of significant effects that require multiple means comparison are shown in bold

Source of variation	Fresh mass	Proline	Essential oil (%)	Oil yield
Year	0.135	0.504	0.804	0.531
Irrig	0.001	0.001	0.044	0.001
FeUrea	0.001	0.001	0.001	0.001
Irrig*FeUrea	0.001	**0.001**	0.368	0.005
Cut	0.001	**0.006**	0.001	0.001
Irrig*Cut	0.001	0.794	**0.001**	0.001
FeUrea*Cut	0.088	0.688	**0.009**	0.002
Irrig*FeUrea*Cut	**0.040**	0.870	0.297	**0.082**

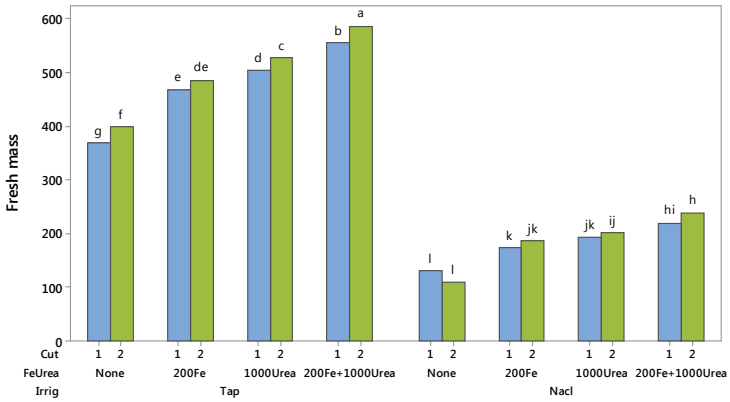

Fig. 1: Mean Fresh mass (g/plant) obtained from the 16 combinations of irrigation, FeUrea and Cut. Means sharing the same letter are not significantly different

As shown in fig. 1, fresh mass during the second cut was significantly higher for tap irrigation, but not for Nacl irrigation. Within each irrigation, the highest fresh mass was obtained from 200Fe+ 1000Urea (587 and 557 g plant^{-1} from Cut 2 and Cut 1 respectively from tap irrigation; and 239 and 220 g plant^{-1} from Cut 2 and Cut 1 respectively from Nacl irrigation). Although tap irrigation and 200Fe+1000Urea gave higher oil yield (fig. 2), the differences between the two cuts were opposite to what was seen in fresh mass. Oil yield from Cut 2 was either equal or less than that obtained from Cut 1 (fig. 2). For the tap irrigated plants, the highest oil yield was obtained from 200Fe+1000Urea (0.812 and 0.806 ml plant^{-1} from Cut 1 and Cut 2 respectively, which were not significantly different). But, for the Nacl irrigated plants, the highest oil yield was obtained from Cut 1 treated with 1000Urea and 200Fe+ 1000Urea (0.307 and 0.262 ml plant^{-1} respectively, which were not significantly different).

Table 2: Mean proline obtained from the 8 combinations of irrigation and FeUrea, and the two cuts. Means sharing the same letter are not significantly different

Irrig	FeUrea	Proline	Essential oil (%)	Cut	Prolne	Irrig	Cut	Essential oil (%)
Tap	None	0.117 g	0.122 bc	1	0.525 b	Tap	1	0.128 a
Tap	200Fe	0.123 f	0.087 e	2	0.532 a	Tap	2	0.117 b
Tap	1000Urea	0.119 g	0.129 bc			Nacl	1	0.135 a
Tap	200Fe+1000Urea	0.217 e	0.101 d			Nacl	2	0.100 c
Nacl	None	1.486 c	0.133 ab					
Nacl	200Fe	1.529 b	0.117 c					
Nacl	1000Urea	1.445 d	0.143 a					
Nacl	200Fe+1000Urea	1.584 a	0.129 bc					

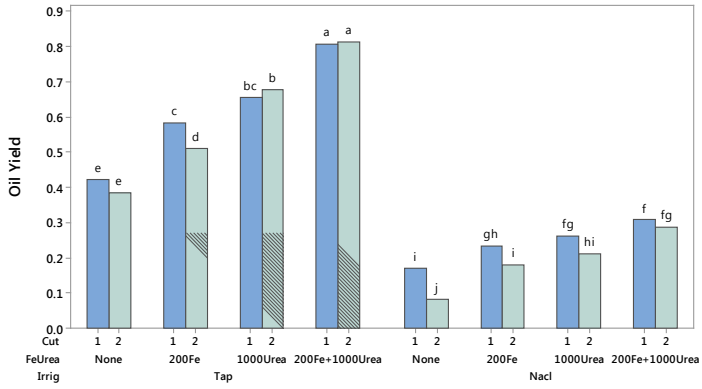

Fig. 2: Mean Oil Yield (ml plant^{-1}) obtained from the 16 combinations of irrigation, FeUrea and Cut. Means sharing the same letter are not significantly different

Unlike fresh mass and oil yield, Nacl irrigation gave significantly higher proline than tap irrigation (fig. 3). Although the differences among proline from the different FeUrea treatments were not as much magnified, 200Fe+1000Urea gave significantly higher proline for both tap and Nacl irrigations. However, regardless of irrigation and FeUrea treatment, Cut 2 gave significantly higher (0.532) proline than Cut 1 (0.525; table 2).

The results are shown in fig. 1 and fig. 2 demonstrate that fresh weight of herb, essential oil and oil yield decrease with saline irrigation conditions. The inhibitory effect of salinity demonstrated in this study was also reported by several researchers [35-38]. Saline conditions reduce the ability of plants to absorb water causing rapid reductions in growth rate, and induce many metabolic changes therefore; the reduction in growth was explained by lower osmotic potential in the soil, which leads to decreased water uptake, reduced transpiration, and closure of stomata, which is associated with reduced growth [39].

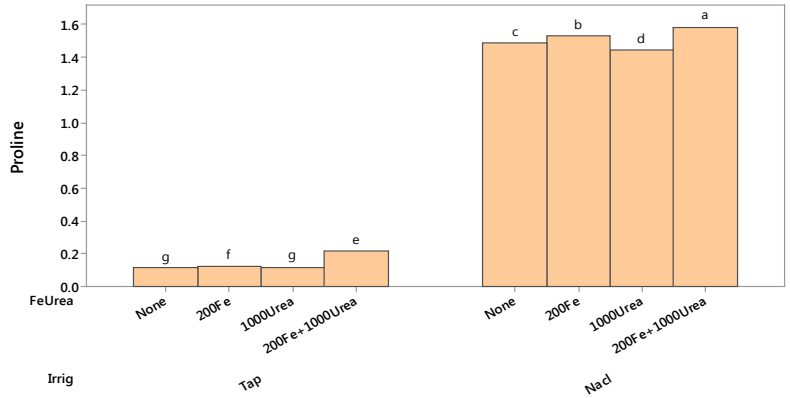

Fig. 3: Mean proline obtained from the 8 combinations of irrigation and Fe Urea. Means sharing the same letter are not significantly different

NaCl salt condition significantly decreased total biomass and essential oil percent and yield [40-42]. Salt stress may also affect the essential oil accumulation indirectly through its effects on either net assimilation or the partitioning of assimilate among growth and differentiation processes [43]. Penka [44] showed that the formation and accumulation of essential oil in plants was due to the action of environmental factors. It can be postulated that the formation and accumulation of essential oil were directly dependent on ideal growth and development of the plants producing oils. The decrease in oil production might be due to the decrease in plant anabolism.

Plectranthus amboinicus plants sprayed with urea/or iron under tap and Nacl saline conditions were superior compared to unsprayed plants (fig. 1 and fig. 2). The results also indicated that treated plants with urea+iron were much superior in fresh mass, essential oil and oil yield compared to the untreated plants. However, plants treated by urea produced fresh mass, essential oil and oil yield greater than those treated by iron. The stimulatory effect of urea and/or iron was reported by [45]. The stimulation effects of applying urea and iron on vegetative growth may be attributed to the well-known functions of urea and iron in plant life, as described in the Introduction section. Younis *et al.* [46] reported that, with urea fertilizer, all growth components increased significantly by up to 4%compared to control plants treated with NaCl alone. Puttanna *et al.* [47] demonstrated that foliar application of urea fertilizer significantly enhanced the growth and yield of citronella plants. The stimulation effects of spraying iron on plant growth, yield and quality products were recorded by [48, 49].

Accumulation of solutes like proline is one of the adaptation ways of plants to salinity [50]. Proline is known as a beneficial solute, which is accumulated in many plant species during water stress conditions [51]. The increase in proline content could be attributed to a decrease in proline oxidase activity in saline conditions [52]. Yildiz and Terzi [53]; Said-Al Ahl and Omer [38] and Sivritepe *et al.* [54] reported that the proline contents increased significantly under salinity. Aymen *et al.* [55] showed that proline content of safflower shoot was higher from plants treated with NaCl (5 g L-1) than that in non-salinized plants. Proline acts as a free radical scavenger and antioxidant activity [56]. Therefore, proline is able to stabilize proteins, DNA as well as membranes [57]. It is suggested that the higher proline concentration under salt stress conditions may help maintain structure and function of cellular macromolecules.

It is evident from fig. 3 that application of iron and/or urea decreased salinity stress and significantly affected proline content. However, proline contents were much higher in plant sprayed with Nacl and urea+iron. Foliar-applied urea could induce an increased proline [58].

Essential oil composition

The results of the GC/MS analysis of the essential oils of the *Plectranthus amboinicus*in the second season is shown in table 3 and table 4. Identified compounds in the first and second cuts were grouped into three categories, namely, major compounds (more than 10%), minor compounds (less than 10% and more than 1%) and trace compounds (less than 1%). In this respect, it is evident that, carvacrol (6.7-15.6%) and β-cadinene (8.3-11.0%) in the first cut and p-cymene (3.2-10.9%), (+)-epi-bicyclosesquiphellandrene (8.6-14.5%), β-cadinene (1.4-12.9%) were majors in the second cut. The results in table 3 showed that irrigated plants with Nacl had lower carvacrol but higher β-cadinene than plants irrigated with tap water in the first cut. Similarly, irrigated plants with Nacl decreased (+)-epibicyclosesquiphellandrene but higher P-cymene and β-cadinene than plants irrigated with tap water in the second cut. The highest content of carvacrol and β-cadinene in the first cut were obtained from plants irrigated with tap water and plants sprayed with iron, respectively. In the second cut, plants sprayed with urea gave the highest % of p-cymene, whereas irrigated with tap water gave the highest % of (+)-epi-bicyclosesquiphellandrene. But, the highest % of β-cadinene was obtained from plants irrigated with Nacl and sprayed with iron. The highest % of carvacrol p-cymene and β-cadinene were obtained in the first cut, but the highest % of p-cymene, (+)-epibicyclo-sesquiphellandrene were obtained in the second cut.

The chemical composition of Plectranthus amboinicus essential oil from various origins has been the subject of many studies. The literature reveals the occurrence of several chemotypes. In Egypt, El-Hawary *et al.* [5] reported that, in the leaves, δ-cadinene was the major component in the oils of spring (18.66%) and autumn (12.52%), while, β-caryophyllene (12.65%) and thymol (8.75%) were the major components in the oils of winter and summer, respectively. In the stems, α-humulene was the major component in the oil samples of winter (11.14%) and summer (12.70), while, β-copaene-4-α-ol (9.37%) and thymol (13.02%) were the major constituents in the oil samples of spring and autumn respectively. Also in another study in Egypt, El-Ahmady [59] reported that Plectranthus amboinicus oil produced a high content of thymol (11.90%), α-copaene (9.11 %), β-caryophyllene (7.62%), germacrene D (19.86%), δ-cadinene (8.79%) and α-caryophyllene (8.36%). In India, carvacrol (28.65%), thymol (21.66%), α-humulene (9.67%), undecanal (8.29%), γ-terpinene (7.76%), ρ-cymene (6.46%), caryophyllene oxide (5.85%), α-terpineol (3.28%) and β-selinene (2.01%) were the main components [60]. In another report in India, thymol (18.88%), carvocrol (14.21%), cis-caryophyllene (18.06%), P-cymene (10.83), γ-terpinene (6.58%),α-terpinene(1.11%)were the major constituents [61]. In Brazil, the main constituents of Plectranthus amboinicus essential oil were carvacrol and thymol [62]. In another study in Brazil, p-cymene (12.01%), γ-terpinene (14.74%), carvacrol (37.70%), and (Z)-caryophyllene (14.07%) were the major constituents [63]. Also, in another study in Brazil, (Z)-caryophyllene (25.53%) and caryophyllene oxide (9.76%) was the predominant compounds in the essential oil [64]. In Malaysia, the main components of the oil of Plectranthus amboinicus were 3-carene (20.78), α-terpinene (6.04), o-cymene (5.06) γ-terpinene (8.94), camphor (17.96) and carvacrol (19.29) [65]. In Morocco, carvacrol (23.0 %), camphor (22.2 %), Δ-3-carene (15.0 %), λ-terpinene (8.4 %), o-cymene (7.7 %) and α-terpinene (4.8 %) were the major constituents of the oil [66].

1-octen-3-ol; terpinolene; α-terpinene; p-cymene; ocimene; γ-terpinene; linalyl anthranilate; α-copaene; β-elemene; isocaryo-phillene; α-guaiene; (+)-epibicyclosesquiphellandrene; α-amorphene; bicyclo(4.4.0)dec-1-ene, 2-isopropyl-5-methyl-9-methylene; isoledene; α-gurjunene; α-muurolene; γ-cadinene; neidentif; (-)-spathulenol; tau. muurolol; tau. cadinol; 6-isopropenyl-4,8a-dimethyl; Cubenol; 2,2,7,7-tetramethyltricyclo (6.2.1.0(1,6))undec-4-en3-onel and neoisolongifolene in the first cut; and 1-octen-3-ol; α terpinene; γ-terpinene; linalyl anthranilate; isoledene; β-elemene; β-caryophyllene; γ-muurolene; α-guaiene; α-amorphene; β-gurjunene; α-gurjunene; α-muurolene; δ-cadinene; γ-cadinene; ledol; spathulenol; β-guaiene; tau. cadinol; tau. muurolol; α-cadinol; 7-tetracyclo(6.2.1(3.8)0(3.9)undecanol, 4,4,11,11, tetramethyl; Cubenol; 2,2,7,7'tetramethyltricyclo; neoisolongifolene, 8,9-dehydro; cadina-1(10),6,8-triene; cycloiso-longifolene,8,9-dehydro in the second cut were represented as major or minor compounds; and the remaining compounds (less than 1%) were considered as traces.

A different effect was observed on minor compounds in the first and second cuts and by irrigated with tap water/or Nacl; the highest % of α terpinene in the two cuts was obtained from plants sprayed with Urea. The highest % of cubenol in the two cuts was obtained from plants irrigated with Tap water. Irrigated plants with Tap water gave the highest % of ocimene in the first cut α-muurolene, γ-cadinene in the second cut. β-cadinene increased when plants irrigated with Nacl than irrigated with Tap water in the first cut and second cut, whereas, sprayed plants irrigated Nacl with iron+urea gave the highest % in the first cut and plants irrigated by Nacl gave the highest % in the second cut.

Table 3: The essential oil compounds of *plectranthus amboinicus* herb at the first cut in 2014 season

Compound	Rt	RI[a)]	T1	T2	T3	T4	T5	T6	T7	T8	Identification[b)]
α-thujene	9.16	933	0.1	0.1	0.1	0.2	0.1	0.1	0.2	0.2	RI, MS, Co-I
1R-α-pinene	9.61	934	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.2	RI, MS, Co-I
1-octen-3-ol	13.47	979	1.2	1.1	1.0	1.3	1.1	0.9	1.4	0.6	RI, MS
α-phellandrene	14.86	1009	tr	tr	tr	tr	0.3	tr	tr	0.4	RI, MS, Co-I
myrcene	14.93	1011	0.2	0.4	0.2	0.3		0.2	0.2		RI, MS, Co-I
terpinolene	15.48	1015	6.1	0.7	tr	0.9	6.0	5.3	6.5	5.2	RI, MS, Co-I
α-terpinene	15.52	1016	-	-	6.7	-	-	-	-	-	RI, MS
p-cymene	16.08	1023	5.0	5.2	3.4	3.8	4.6	3.6	0.5	5.6	RI, MS
trans-β-ocimene	16.97	1031	0.2	0.2	0.1	0.2	0.1	0.1	0.1	0.1	RI, MS
ocimene	17.60	1044	2.5	2.1	2.1	2.7	2.0	1.7	2.1	1.2	RI, MS, Co-I
γ-terpinene	18.21	1050	5.3	5.3	4.8	5.9	5.2	4.6	5.3	6.8	RI, MS
fenchone	20.06	1110	0.3	0.2	0.1	tr	0.4	0.2	0.3	0.5	RI, MS, Co-I
linalyl anthranilate	20.94	1116	1.7	1.3	1.2	1.4	1.6	1.2	1.9	1.2	RI, MS
1-terpinen-4-ol	25.16	1176	0.3	0.3	0.3	0.3	0.3	0.3	0.4	0.4	RI, MS
carvacrol	32.90	1287	15.6	9.0	8.2	13.9	8.3	10.0	9.7	6.7	RI, MS, Co-I
β-patchoulene	33.28	1292	tr	tr	tr	tr	tr	tr	tr	tr	RI, MS
(+)-valencene	33.47	1303	tr	0.1	0.1	tr	tr	0.1	tr	tr	RI, MS
γ-elemene	33.68	1309	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.1	RI, MS
α-cubebene	34.21	1316	0.3	0.3	0.3	0.3	0.3	0.2	0.1	0.2	RI, MS
clovene	34.67	1334	tr	tr	tr	rt	-	tr	0.2	tr	RI, MS
ylangene	34.91	1355	0.3	0.3	0.3	0.4	0.3	0.2	0.4	0.2	RI, MS
α-copaene	35.12	1366	3.7	4.7	4.8	4.6	4.3	4.6	3.2	4.0	RI, MS
2-isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,7-octahydronaphtalene	35.20	1367	tr	0.1	tr	0.1	tr	tr	0.1	tr	RI, MS
β-bourbonene	35.33	1369	0.3	0.2	0.2	0.2	0.3	0.3	0.5	0.4	RI, MS
β-elemene	35.53	1397	1.2	1.4	1.5	1.4	1.3	1.2	1.0	1.6	RI, MS
isoledene	35.64	1404	0.2	0.2	0.2	0.2	0.2	0.2	0.3	-	RI, MS
isocaryophillene	36.24	1412	2.5	3.1	2.8	2.8	3.1	2.9	2.5	3.7	RI, MS
β-chamigrene	36.34	1415	0.3	0.5	0.4	0.5	0.4	0.4	0.4	0.3	RI, MS
β-cubebene	36.48	1417	0.7	0.6	0.7	0.6	0.7	0.5	0.5	0.6	RI, MS
γ-gurjunene	36.58	1418	0.4	0.5	0.5	0.5	0.5	0.4	2.9	0.3	RI, MS
valencene	36.68	1420	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.2	RI, MS
α-guaiene	37.09	1422	3.9	5.0	4.6	4.7	5.9	4.8	7.0	5.3	RI, MS
γ-muurolene	36.56	1419	0.3	0.3	-	0.3	0.7	0.3	0.3	1.5	RI,MS
valencene	36.67	1420	-	0.1	0.5	0.1	-	-	-	-	RI,MS
α-guaiene	37.09	1422	4.9	4.9	5.1	4.8	tr	6.0	5.4	5.6	RI,MS
(+)-epibicyclosesquiphellandrene	37.23	1427	2.5	2.9	2.7	2.9	2.7	2.6	1.9	2.1	RI, MS
β-gurjunene	37.39	1438	0.2	-	0.1	0.1	0.2	0.1	0.5	0.2	RI, MS
α-amorphene	37.60	1480	1.1	1.2	1.3	1.3	1.3	1.2	-	1.1	RI, MS
bicyclo(4.4.0)dec-1-ene, 2-isopropyl-5-methyl-9-methylene	37.76	1483	7.1	8.1	9.8	9.1	7.4	8.5	5.2	5.6	RI, MS
isoledene	38.01	1497	1.4	1.6	1.7	1.8	1.7	1.8	-	1.3	RI, MS
α-gurjunene	38.12	1502	2.1	2.6	2.4	2.7	2.0	2.5	1.8	2.3	RI, MS
α-muurolene	38.18	1503	1.1	1.3	1.4	1.0	-	1.3	1.2	1.3	RI, MS
δ-cadinene	38.30	1504	0.3	0.5	0.4	0.5	0.4	0.4	0.4	0.3	RI, MS
γ-cadinene	38.58	1507	2.2	2.7	2.3	2.7	2.5	2.4	1.9	2.1	RI, MS
β-cadinene	38.75	1518	8.3	11.0	9.9	9.6	9.8	11.0	8.5	9.5	RI, MS
epizonarene	38.86	1520	-	0.4	0.5	-	0.3	0.5	0.4	0.6	RI, MS
(+)-ledol	38.94	1524	0.9	0.5	0.6	0.6	0.6	0.4	0.4	0.3	RI, MS
neidentif	39.24	1526	1.4	1.9	1.5	1.9	1.7	1.8	1.6	1.5	RI, MS
cadala-1(10),3,8-triene	39.41	1527	0.1	0.1	0.1	tr	0.2	0.1	0.2	0.2	RI, MS
4,6,6-trimethy-2-(3-methylbuta-1,3dienyl)	39.61	1531	0.2	0.2	0.1	0.1	0.2	0.1	0.2		RI, MS
(-)-spathulenol	40.60	1570	1.1	0.8	0.7	0.7	1.0	0.8	1.1	1.3	RI, MS
caryophyllene oxide	40.74	1580	0.3	0.1	tr	0.1	0.2	0.1	0.1	0.2	RI, MS

β-guaiene	40.91	1588	0.2	0.2	0.2	0.1	0.3	0.2	0.2	0.4	RI, MS
isoaromadendrene epoxide	41.57	1595	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.3	RI, MS
cubenol	42.48	1608	0.6	0.7	0.6	0.5	0.6	0.6	2.9	0.9	RI, MS
tau. muurolol	43.23	1629	1.3	1.8	1.4	1.2	1.7	1.8	1.9	2.6	RI, MS
δ-cadinol	43.28	1629	0.3	0.5	0.6	0.4	0.4	0.6	0.6	0.6	RI, MS
tau. cadinol	43.78	1642	4.9	7.2	6.6	5.3	6.9	7.3	7.6	9.7	RI, MS
aromadendrene oxide	44.30	1645	tr	0.1	-	tr	0.1	0.1	0.8	0.1	RI, MS
neidentif	45.06	1662	0.8	0.9	0.8	0.8	0.8	0.7	0.7	0.6	RI, MS
6-isopropenyl-4,8a-dimethyl	45.52	1669	0.4	0.6	0.3	0.4	0.5	0.5	0.7	1.0	RI, MS
cubenol	45.68	1674	2.4	1.5	2.1	1.9	1.6	1.0	1.8	0.9	RI, MS
2,2,7,7-tetramethyltricyclo (6.2.1.0(1,6))undec-4-en3-one	46.65	1981	1.4	1.8	1.8	1.7	1.6	1.7	1.7	1.1	RI, MS
aromadendrene,dehydro	48.36	1692	0.3	0.4	0.4	0.3	0.4	0.5	0.4	0.4	RI, MS
neoisolongifolene	49.93	1719	2.1	2.9	2.7	2.4	2.7	2.7	2.8	2.7	RI, MS
cycloisolongifolene,8,9-dehydro	50.04	1721	0.4	0.4	0.3	0.3	0.5	0.3	0.6	0.6	RI, MS
isolongifolene	51.24	1732	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	RI, MS
7-tetracyclo(6.2.10(3.80(3.9)	53.96	1754	0.5	0.5	0.5	0.4	0.6	0.6	0.7	0.6	RI, MS
cembrene	55.57	1782	0.1	0.2	-	0.2	0.2	0.2	0.1	0.2	RI, MS
total identified			98.7	99.2	99.1	98.7	99.0	99.3	97.0	98.9	

Where, T1, T2, T3, T4 = Control plants (Irrigated with tap water); T5, T6, T7, T8=Irrigated with NaCl: T1, T5(spraying with tap water);T2, T6(sprayin gwith Fe);T3, T7(spraying with Urea);T4, T8(sprayin gwith Fe Urea), a) RI: Kovats retention index determinate relative to the series of n-alcanes (C10-C35)onVF-5MS Capillary Column, b) Identification method: RI, comparison on Kovats retention indices with published data; MS, comparison of mass spectra with those listed in the NIST[02]library and with published data; Co-I, co-injection with authentic compound. c)blank: Not detected. d209)tr: Traces(< °.1%), With authentic compound. c): Not detected d)tr: Traces(< °.1%).

Table 4: The essential oil compounds of *plectranthus amboinicus* herb at the second cut in 2014 season

Compound	Rt	RI[a)]	T1	T2	T3	T4	T5	T6	T7	T8	Identification[b)]
α-thujene	9.16	932	tr	0.1	0.3	0.1	tr	tr	0.2	tr	RI,MS, Co-I
α-pinene	9.61	935	tr	0.3	0.3	0.1	tr	tr	0.2	tr	RI,MS, Co-I
1-octen-3-ol	13.43	977	0.5	0.9	0.4	0.4	0.2	0.7	1.3	0.6	RI,MS
1-pentanol,5-cyclopropylidene	13.82	989	-	-	0.2	0.2	-	-	-	-	RI,MS
m-cymene	14.34	1002	-	0.5	-	-	-	-	-	-	RI,MS,
myrcene	14.90	1009	0.3	0.3	0.6	0.5	0.3	0.4	0.4	0.2	RI,MS, Co-I
α terpinene	15.47	1014	3.7	4.9	5.8	4.0	2.8	3.6	4.1	2.4	RI,MS, Co-I
p-cymene	16.05	1020	4.0	5.2	10.9	4.8	5.7	4.5	9.2	3.2	RI,MS
trans-β-ocimene	16.95	1033	tr	0.9	0.1	0.7	tr	tr	0.1	tr	RI,MS
β-ocimene	17.58	1042	0.9	0.7	0.9	0.5	0.6	0.6	0.9	0.5	RI,MS
α-phellandrene	17.88	1047	-	-	-	tr	-	-	-	-	RI,MS, Co-I
γ-terpinene	18.18	1052	5.0	6.4	6.0	6.4	3.4	5.8	6.4	4.3	RI,MS, Co-I
L-fenchone	20.00	1108	0.2	0.5	0.7	0.7	0.6	0.4	0.8	0.7	RI,MS, Co-I
fenchyl acetate	20.17	1110	-	0.3	-	-	-	-	-	-	RI,MS
linalyl anthranilate	20.91	1114	1.0	1.1	1.3	1.1	1.1	1.3	1.9	1.2	RI,MS
1-terpinene-4-ol	25.15	1178	0.2	0.4	0.3	0.4	0.3	0.4	0.4	0.4	RI,MS
6-isopropyl-3-methyl-7-oxabicyclo(4.1.0)heptan-2-one	29.52	1250	tr	tr	0.1	0.1	0.1	0.1	0.1	0.1	RI,MS
thymol	32.43	1290	0.6	0.9	0.6	0.5	0.4	0.7	0.6	0.6	RI,MS, Co-I
carvacrol	32.89	1297	7.8	5.4	7.4	7.0	7.7	9.3	5.2	8.9	RI,MS, Co-I
eremophillene	33.44	1300	0.1	tr	0.1	tr	tr	tr	tr	tr	RI,MS
γ-elemene	33.66	1308	0.2	0.2	0.1	0.1	0.2	0.1	0.1	0.1	RI,MS
gama gurjunene	33.77	1312	0.1	tr	0.1	tr	0.2	0.1	0.1	0.1	RI,MS
α-cubebene	34.20	1318	0.4	0.7	0.4	0.3	0.3	0.3	0.3	0.3	RI,MS
ylangene	34.91	1352	0.6	0.2	0.2	0.4	0.3	0.4	0.3	0.4	0.3
isoledene	35.11	1367	4.2	2.4	4.1	6.5	9.8	4.9	5.1	4.9	6.8
β-bourbonene	35.32	1369	0.2	0.2	0.3	0.5	0.3	0.5	0.5	0.5	0.4
β-elemene	35.51	1397	1.7	0.6	1.2	1.4	1.2	1.5	1.3	1.3	1.3
β-patchoulene	35.64	1404	0.2	0.1	0.1	0.2	0.1	0.2	0.2	0.2	0.2
β-cubebene	36.04	1409	0.1	tr	0.1	tr	-	-	tr	tr	RI,MS
β-caryophyllene	36.24	1412	3.3	4.1	4.1	4.0	4.5	4.7	4.4	4.7	RI,MS, Co-I
β-chamigrene	36.35	1415	0.5	0.3	0.5	0.4	0.3	0.4	0.4	0.4	RI,MS
germacrene D	36.48	1417	0.7	0.4	0.6	0.5	0.7	0.6	0.6	0.5	RI,MS, Co-I
γ-muurolene	36.56	1419	0.3	0.3	-	0.3	0.7	0.3	0.3	1.5	RI,MS
valencene	36.67	1420	-	0.1	0.5	0.1	-	-	-	-	RI,MS
α-guaiene	37.09	1422	4.9	4.9	5.1	4.8	tr	6.0	5.4	5.6	RI,MS
β-gurjunene	37.38	1438	-	0.1	0.3	0.3	-	-	-	-	RI,MS
α-amorphene	37.57	1478	1.4	0.9	1.2	1.0	1.2	1.4	1.2	1.3	RI,MS
(+)-epi-bicyclosesquiphellandrene	37.73	1483	14.5	8.6	10.3	8.6	10.0	8.9	8.8	8.7	RI,MS
β-gurjunene	37.98	1495		1.4	0.6	0.6		1.7	1.3		RI,MS
α-gurjunene	38.11	1501	0.8	2.4	2.9	2.8	2.4	2.9	2.5	2.9	RI,MS
α-muurolene	38.19	1502	5.1	2.0	2.3	1.6	2.8	3.3	2.7	3.2	RI,MS
δ-cadinene	38.30	1504	0.6	0.3	0.5	0.4	0.5	0.5	0.6	1.3	RI,MS
γ-cadinene	38.57	1508	3.1	2.0	2.5	2.2	2.3	2.6	2.5	2.6	RI,MS

β-cadinene	38.761	1519	11.1	9.6	1.4	8.7	11.9	12.9	9.9	11.9	RI,MS
epizonarene	38.85	1522	-	0.5	0.6	0.4	-	-	-	-	RI,MS
ledol	38.93	1523	1.4	0.3	0.8	0.7	1.0	1.1	0.4	0.3	RI,MS
α-cubebene	39.11	1525	0.9	0.5	0.7	0.6	0.7	0.8	0.7	0.7	RI,MS
cadala-1(10),3,8-triene	39.41	1527	-	0.2	0.2	0.2	0.3	0.3	0.2	0.3	RI,MS
4,6,6-trimethyl.	39.62	1530	-	0.2	-	0.2	-	-	-	-	RI,MS
longipinocarveol, trans	39.91	1537	-	tr	tr	0.1	-	-	-	-	RI,MS
spathulenol	40.60	1570	1.4	1.0	1.6	1.0	1.8	1.4	1.0	4.8	RI,MS
caryophyllene oxide	40.73	1579	0.1	0.3	0.5	0.2	0.3	0.2	0.4	0.2	RI,MS
β-guaiene	40.88	1584	0.2	1.1	0.3	0.2	0.3	0.2	0.2	0.3	RI,MS
aristolene epoxide	41.14	1589	-	tr	0.2	0.1	-	-	-	-	RI,MS
isoaromadendrene epoxide	41.55	1593	0.2	0.3	0.2	0.2	0.3	0.2	0.2	0.5	RI,MS
ledene oxide	42.75	1617	0.5	0.4	0.6	0.5	0.5	0.4	0.4	0.5	RI,MS
tetracyclo(6.3.2.0(2.5)0(1.8)tridecan-9-ol,4,4-dimethyl	42.96	1625	-	tr	0.2	tr	-	-	-	-	RI,MS
tau. cadinol	43.09	1626	2.7	4.2	1.3	1.3	1.5	2.1	1.4	4.6	RI,MS
tau. muurolol	43.19	1627	-	-	1.4	1.6	2.4	-	1.5	-	RI,MS
δ-cadinol	43.32	1629	-	0.6	0.5	0.5	0.9	-	0.4	-	RI,MS
α-cadinol	43.73	1641	-	6.8	0.4	4.5	7.8	-	4.1	-	RI,MS
alloaromadendrene oxide(2)	44.28	1644	-	0.1	0.1	tr	0.1	-	tr	-	RI,MS
humulane-1,6-dien-3-ol	44.45	1652	0.3	0.2	0.3	0.3	0.4	0.3	0.3		RI,MS
unidentified	45.03	1661	1.0	0.6	0.7	0.5	0.7	0.6	0.6	0.6	RI,MS
unidentified	45.18	1662	-	-	0.3	0.1	-	-	-	-	RI,MS
7-tetracyclo(6.2.1(3.8)0(3.9)undecanol, 4,4,11,11,tetramethyl	45.50	1667	tr	0.5	1.1	0.9	3.4	0.6	0.7	0.6	RI,MS
cubenol	45.64	1675	5.2	2.2	3.4	2.7	0.6	1.6	1.9	1.8	RI,MS
2,2,7,7'tetramethyltricyclo.	46.63	1681	1.6	1.2	1.1	1.1	0.5	1.5	0.8	1.4	RI,MS
aromadendrene, dehydro	49.02	1702	0.3	tr	tr	0.4	0.1	0.1	tr	0.1	RI,MS
neoisolongifolene,8,9-dehydro	49.19	1708	3.1	2.7	0.1	0.2	0.3	0.3	0.1	0.3	RI,MS
cadina-1(10),6,8-triene	49.62	1712			2.2	2.3	3.3	3.2	2.1	2.9	RI,MS
cycloisolongifolene,8,9-dehydro	50.03	1721	1.0	0.8	1.1	0.9	1.0	0.9	0.8	0.7	RI,MS
isolongifolene,9,10-dehydro	51.24	1732	0.2	0.2	0.3	0.2	0.2	0.2	tr	0.2	RI,MS
6-isopropenyl,4,8a-dimetyl	54.47	1775	0.5	0.2	0.3	0.4	0.7	0.7	0.3	0.6	RI,MS
cembrene	55.56	1781	0.3	0.3	0.2	0.3	0.3	0.3	0.2	0.3	RI,MS
total identified			99.1	98.2	98.7	98.3	97.4	98.6	98.8	98.8	

Where, T1, T2, T3, T4=Control plants (Irrigated with tap water); and T5, T6, T7, T8=Irrigated with NaCl: T1, T5 (spraying with tap water); T2, T6 (spraying with Fe); T3, T7 (spraying with Urea); T4, T8 (spraying with Fe Urea). a) RI: Kovats retention index determinate relative to the series of n-alcanes (C10-C35) on VF-5MS Capillary Column. b) Identification method: RI, comparison on Kovats retention indices with published data; MS, Comparison of mass spectra with those listed in then ist [02] library and with published data; Co-I, coinjection with the authentic compound. c) blank: Not detected. d209)tr: Traces(< °.1%), With authentic compound. c}:Not detected. d) tr: Traces(< °.1%).

We found that the foliar spraying treatments under tap irrigation water led to an increase in (+)-epibicyclosesquiphellandrene, β-cadinene, p-cymene and decrease of both carvacrol and p-cymene. While irrigating plants with salt water led to an increase in p-cymene, (+)-epibicyclosesquiphellandrene and β-cadinene, this treatment led to a decrease in carvacrol during the first cut. In the second cut, the foliar spraying treatments under tap irrigation water or irrigated with Nacl led to an increase in p-cymene and carvacrol, respectively. Foliar spraying treatments under irrigation tap water led to a decrease in (+)-epibicyclosesquiphellandrene. Control plants gave the highest % of carvacrol (15.6%) and (+)-epibicyclo-sesqui-phellandrene (14.5%) in the first and second cuts, respectively. FeUrea treatment under irrigated plants with tap water gave the highest % of β-cadinene (11.0%) in the two cuts and carvacrol in the second cut. Fe spraying under irrigated with Nacl gave the highest % of p-cymene (5.6%) and α-cadinol (9.7%) in the first cut. Urea spraying under irrigated with Nacl gave the highest % of α-cadinol (11.0%) in the second cut. FeUrea treatment under irrigated plants with Nacl gave the highest % of (+)-epibicyclosesquiphellandrene (12.5%) in the first cut and p-cymene (10.9%) in the second cut.

However, essential oil quantity and chemical composition varies depending on many factors such as climate, cultivar, nutrition, seeding date, management practices, plant parts, developing stage of the plant and harvest time [67, 68]. Said-Al Ahl and Hussein [69] reported a decrease in carvacrol and an increase in p-cymene, γ-terpinene, α-terpinene and caryophyllene when Nacl saline water irrigation was applied on *Origanum vulgare*. Also, values of carvacrol, p-cymene and γ-terpinene were affected by cuttings. For carvacrol, the third cut recorded the highest mean value followed by the second cut and then first cut. γ-terpinene has adverse behavior; first cut resulted in the highest value followed by a second cut and then third cut.

However, the highest value of p-cymene resulted from the second cut, and the third cut recorded the lowest value. According to urea and Fe foliar application, Nurzyńska-Wierdak [70] found that foliar nitrogen application (0.5% urea solution) decreased linalool in volatile basil oil. Said-Al Ahl and Mahmoud [71] reported that 1,4-terpineol, caryophyllene, germacrene D, cadinene and cadinol increased with Fe spraying on basil plants under non-saline conditions. Also, 1, 4-terpineol, caryophyllene, germacrene D and cadinene increased, but cadinol decreased under salt stress. Jabbari *et al.* [72] reported after treating plants with Fe increased thymol and p-cymene of thyme. Also, Yadegari [73] on thyme indicated that Fe foliar application increased carvacrol and decreased thymol.

CONFLICT OF INTERESTS

Declared none

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How to cite this article

- Abdelrazik TM, Sabra AS, Astatkie T, Hegazy MH, Grulova D, Said-Al Ahl Hah. Response of growth, essential oil content and its constituent's of *Plectranthus amboinicus* to iron and/or urea foliar application under saline irrigation. Int J Pharm Pharm Sci 2016;8(8):223-231.