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# Changes in the essential oil content and terpene composition of rosemary (*Rosmarinus officinalis* L.) by using plant biostimulants

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## ABSTRACT

Plant biostimulants can stimulate the increase of growth, metabolism and the biosynthesis of metabolites in plants. This study investigated the changes of rosemary essential oil and its components composition under use of biostimulants for the possible reduction in use of chemical fertilizers. Treatments included biostimulants based on amino acids in four formulations, Aminolforte, Kadostim, Humiforte, and Fosnutren (each of them at 0.75 and 1.5 L $ha^{-1}$), and application of N.P.K fertilizer as a control treatment (by applied complete fertilizer at 100 kg per hectar with proportion of 15:8:15 percentage of N:P:K in the fertilizer). Results showed that the essential oil content and its components were significantly affected by biostimulants application. The maximum content of essential oil was obtained at 1.5 L $ha^{-1}$ Humiforte and both concentrations of Aminolforte. While, the highest content of *α*-pinene, 1,8-cineole, and camphor as major components of rosemary essential oil were obtained at 1.5 L $ha^{-1}$ Fosnutren. In addition, the maximum content of linalool, *z*-pinocamphone, bornyl acetate, and caryophyllene oxide were observed at 1.5 L $ha^{-1}$ Fosnutren. Although, the highest content of myrcene and verbenone was obtained in the treatment with N.P.K fertilizer, but the maximum contents of *β*-pinene, camphene, borneol, and *α*-terpineol were related to the both concentrations of Aminolforte. We can conclude that biostimulants based on amino acids can be an effective alternative in reducing the use of chemical fertilizer and increasing the quantity and quality of rosemary essential oil.

**Key words:** amino acids, biostimulants, essential oil, monoterpenes hydrocarbon, oxygenated monoterpenes, *Rosmarinus officinalis* L.

## IZVLEČEK

**SPREMEMBE V VSEBNOSTI ETERIČNIH OLJ IN SESTAVI TERPENOV V ROŽMARINU (*Rosmarinus officinalis* L.) PO UPORABI RASTLINSKIH BIOSTIMULATORJEV**

Rastlinski biostimulatorji lahko pospešijo rast, presnovo in biosintezo metabolitov v rastlinah. V raziskavi smo preučevali spremembe v sestavi eteričnih olj rožmarina po uporabi biostimulatorjev, ki so bili uporabljeni kot možnost zmanjšanja rabe mineralnih gnojil. Obravnavanja so obsegala biostimulatorje na osnovi aminokislin v štirih komercialnih pripravkih: Aminolforte, Kadostim, Humiforte in Fosnutren (vsakega od njih 0.75 in 1.5 L $ha^{-1}$). Kontrola je bila uporaba N.P.K gnojila (100 kg $ha^{-1}$, 15:8:15 N:P:K). Rezultati so pokazali, da je uporaba biostimulatorjev značilno vplivala na vsebnost eteričnih olj in njihovo sestavo. Največja vsebnost eteričnih olj je bila dosežena pri uporabi biostimulatorjev Humiforte in Aminolforte v koncentraciji 1.5 L $ha^{-1}$. Največja vsebnost *α*-pinena, 1,8-cineola in kamfora, kot glavnih sestavin eteričnega olja rožmarina, je bila dosežena pri uporabi biostimulatorja Fosnutren, v koncentraciji 1.5 L $ha^{-1}$. Tudi največja vsebnost linaloola, *z*-pinokamfona, bornil acetata in kariofilen oksida je bila dobljena pri isti koncentraciji biostimulatorja Fosnutren. Največja vsebnost mircena in verbenona je bila dobljena pri obravnavanju z N.P.K gnojili, toda največja vsebnost *β*-pinena, kamfena, borneola in *α*-terpineola je bila dobljena pri uporabi obeh koncentracij biostimulatorja Aminolforte. Zaključimo lahko, da so lahko biostimulatorji na osnovi amino kislin učinkovita alternativa pri zmanjšanju rabe mineralnih gnojil za povečanje količine in kakovosti rožmarinovega eteričnega olja.

**Ključne besede:** amino kisline, biostimulatorji, eterična olja, monoterpenski ogljikovodiki, oksigenirani monoterpeni, *Rosmarinus officinalis* L.

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## 1 INTRODUCTION

Rosemary (*Rosmarinus officinalis* L.) is an evergreen branched, aromatic and medicinal plant that belongs to the Labiatae family. Rosemary has white-blue flowers and dark green small leaves. It grows along the north and south coasts of the Mediterranean sea and in the sub-Himalayan areas as wild type (AI-Sereiti et al., 1999). Rosemary essential oil is an almost colorless to pale yellow liquid with a characteristic and pleasant odor. Major components characterized for the essential oil are $\alpha$-pinene, 1,8-cineole and camphor (Boutekedjiret et al., 2003). A systematic study identified about 38 compounds from the rosemary essential oil using gas chromatographymass spectrometry (GC/MS), and antimicrobial properties of the essential oil (mixture) were evaluated against 19 microbial strains (Bozin et al., 2007). Another comprehensive study identified 22 antioxidant compounds from 24 commercial rosemary extracts using high-pressure liquid chromatography with UV detector (HPLC-UV/MS), including polyphenolic acids such as vanillic acid, caffeic acid, and rosmarinic acid; phenolic diterpenes such as carnosic acid, carnosol, and rosmadial; and flavonoids such as genkwanin and cirsimaritin (Cuvelier et al., 1996).

Plant biostimulants include diverse substances and microorganisms that enhance plant growth. On the basis of the European Biostimulants Industry Council (EBIC), the following definition is proposed and each of its elements is justified: “Plant biostimulants are substances and materials, with the exception of nutrients and pesticides, which, when applied to plant, seeds or growing substrates in specific formulations, have the capacity to modify physiological processes of plants in a way that provides potential benefits to growth, development and/or stress response” (Du Jardin, 2012). In general, biostimulants are substances that stimulate plant metabolism and metabolic processes in order to improve plant growth (Starck, 2005). The base structure of biostimulants consists the mixture of various amino acids or their mixed with nutrition, hydrolyzed proteins, humic acid, seaweed extract and other ingredients (Gawronska et al., 2008; Thomas et al., 2009).

Some studies showed that primary metabolites such as sugars, amino acids, and carboxylic acids have important biological effects on plant physiology and their application on plants may change the secondary metabolite composition indirectly (Drew and Damon, 1977). Amino acids are involved in the synthesis of other organic compounds, such as proteins, amines, alkaloids, vitamins, enzymes, terpenoids (Ibrahim et al., 2010). Amino acids are crucial for stimulating cell growth, act as buffers, provide a source of carbon and energy and protect the cells from ammonia toxicity, with amid formation (Abdel Aziz et al., 2010).

Amino acids use can stimulate plant performance (Abdel-Mawgoud et al., 2011). Many studies have reported that foliar application of amino acids caused an increase in the growth and development of plants (Haj Seyed Hadi, 2010; Fawzy et al., 2012a; Fawzy et al., 2012b). Neeraja et al., (2005) found that amino acid treatment increased the number of flowers, fruit setting and fruit yield in tomato. Besides facts mentioned above, amino acids can be mixed with nutrients, protein hydrolyzate, triacontanol, humic acid, seaweed extract and brassinolides and they are formulated as a plant biostimulants. A study found that indicators of physiological, biochemical and yield parameters in tea bushes were improved by spraying with Humiforte, Aminolforte, Kadostim, and Fosnutren as biostimulants (base formulation of this biostimulants is consisted from different amino acids which were combined with organic matters) (Thomas et al., 2009). Kadostim caused the increase of germination, root formation, accelerated the formation of leaves and reduced the effects of stress (Anonymous, 2014).

Although plant biostimulants based on amino acids can influence the phytochemistry of plants, there is no research work done on the effect of plant biostimulants on the quantity and quality of rosemary essential oil in field. Therefore, this study aimed to evaluate the changes of terpenes content in rosemary essential oil by using plant biostimulants as a possibility to reduce the use of chemical fertilizers.

# 2 MATERIALS AND METHODS

## 2.1 Location and treatments

This study was performed at the experimental farm of Medicinal Plants Institute (MPI) of Academic Center for Education, Culture & Research (ACECR) located in Karaj region during 2013-2014 based on a randomized complete block design (RCBD) with 9 treatments and 3 replications. The geographical location of the station was 35° 54′ 17″ N and 50° 53′ 7″ E with about 1461 m (elevation) altitude above the mean sea level. The soil was loam-silt with 0.071 % N, 48.9 mg $kg^{-1}$ phosphorous, 33.6 mg $kg^{-1}$ potassium, EC 2.71 dS $m^{-1}$, and pH 8.3. Each experimental plot was 4 m long and 2.5 m wide, prepared after tillage operations. There was 1 m space between the plots and 3 meters between replications. Rosemary cuttings were planted in plots with a density of 20,000 plants per hectare. Other agronomic practices such as irrigation, pest and weed management were regularly designed according to the rosemary plant needs.

Treatments included plant biostimulants with four formulations of Aminolforte, Kadostim, Humiforte, and Fosnutren (each of them at 0.75 and 1.5 L $ha^{-1}$, respectivily), and application of N.P.K fertilizer as a control treatment (by application of complete fertilizer at 100 kg per hectar with proportion of 15:8:15 percentage of N:P:K in the fertilizer). The commercial formulations of biostimulants based on amino acids were supplied by Inagrosa Industries Agro Biological, Madrid of Spain (Anonymous, 2014). The details of the formulations are shown in Table 1. All treatments involved spraying in 4 times with 15 days intervals. The first spraying was 60 days after planting. To increase the absorption of solutions by plants, the foliar application of bio-stimulants was done in conditions without wind and rain and before sunrise when plant stomata are open.

**Table 1:** The formulation of biostimulants used in the experimental treatments

| Biostimulants* | Formulation of compounds** |
|---|---|
| Aminolforte | 3750 mg $L^{-1}$ free amino acids, 2 % organic components, and 1.1 % total N (0.8 % urea N, and 0.3 % organic N) |
| Kadostim | 3750 mg $L^{-1}$ free amino acids, 2 % organic components, and 4.2 % total N (0.8 % ammonia N, 3.1 % nitric N, and 0.3 % organic N), and 6 % potassium ($K_2O$) |
| Humiforte | 3750 mg $L^{-1}$ free amino acids, 2 % organic components, and 6 % total N (1.4 % ammonia N, 3.7 % urea N, 0.5 % nitric N, 0.3 % and organic N), 5 % potassium ($K_2O$), and 3 % phosphorous ($P_2O_5$) |
| Fosnutren | 3750 mg $L^{-1}$ free amino acids, 2 % organic components, and 3.8 % total N (2.1 % ammonia N, 1.4 % nitric N, and 0.3 % organic N), and 6 % phosphorous ($P_2O_5$) |

* Biostimulants supplied by Inagrosa Industries Agro Biological are compatible with the climate of Iran.
** Quantity and kind of free amino acids applied in the formulation of biostimulants in this experiment based on the percent of total amino acids are as follows: 11.2 % glycine, 5.1 % valine, 8.3 % proline, 13.2 % alanine, 4.4 % aspartic acid, 8.3 % arginine, 0.9 % glutamic acid, 5.1 % lysine, 16.4 % leucine, 4.4 % isoleucine, 5.1 % phenylalanine, 4.2 % methionine, 3.9 % serine, 0.3 % treonine, 0.3 % histidine, 1.5 % tyrosine, 0.9 % glutamine, 0.3 % cysteine, 0.4 % asparagine, and 0.4 % tryptophan.

## 2.2 Essential oils analysis

The aerial parts of the plants were harvested at the beginning of flowering stage. The harvested materials were air-dried in a shaded place at a convenient temperature and in an air-flow during 5 days. Samples were transferred to phytochemical analysis laboratory, for determine the percentage of essential oils according to the British pharmacopoeia method. Essential oils of the aerial parts were extracted by hydro-distillation method for 3 h, using Clevenger-type apparatus. The oils were dried over anhydrous sodium sulphate and kept on 4 °C until it was analyzed (Great Britain medicines commission, 1988).

The extracted essential oils were identified by gas chromatography (GC) and gas chromatographymass spectrometry (GC/MS) analysis. GC/MS analysis was carried out on an Agilent Instrument coupled with a 5973 Mass System equipped with flame ionization detector (FID) and a HP-5 capillary column (30 m × 0.25 mm; 0.25 μm film thicknesses). Temperature program included: oven temperature held for 3 minutes at 60 ºC and was enhanced to 220 ºC with 5 ºC per min rate. Then, enhancement of temperature was programmed up to 300 ºC with 15 ºC per min rate and this temperature held for 3 minutes. Other operating conditions included: carrier gas was *He* with a flow rate of 1 mL $min^{-1}$; injector and detector temperatures were 250 ºC, and split ratio, 1:50. Mass spectra were taken at 70 eV (Socaci et al., 2008). The components of the essential oils were identified by comparison of their mass spectra and retention indices with those published in the literature and presented in the MS computer library (Adams, 2007; Swigar and Silverstein, 1981). Each extraction was replicated three times and the compound percentages are the means of the three replicates.

### 2.3 Statistical analysis

All data were subjected to the statistical analysis (one-way ANOVA) based on a randomized complete block design (RCBD) with nine treatments and three replications by using the program of SAS software. The probabilities of significance were used to test for significance among treatments. The difference among treatments means were compared by Duncan's multiple range test at 5 % confidence interval. The obtained values were expressed as mean ± SD (standard deviation) from three replications (n = 3) of each treatment.

## 3 RESULTS AND DISCUSSION

Analysis of variance showed that the use of biostimulants had a significant ($p \leq 0.01$) effect on the essential oil content, *α*-pinene, camphene, *β*-pinene, myrcene, *p*-cymene, limonene, 1,8-cineole, linalool, camphor, borneol, pinocamphone, *α*-terpineol, bornyl acetate, caryophyllene and caryophyllene oxide contents. They had also a significant ($p \leq 0.05$) effect on the verbenone content. Application of various biostimulants had a significant ($p \leq 0.01$) effect on the aggregation of monoterpene hydrocarbon (MH) and oxygenated monoterpene (OM) (Table 2). Overall, all measured traits of this study have been significantly affected by the concentrations of biostimulants based on amino acids.

Mean comparison of data revealed that the content of rosemary essential oil was increased by the application of 1.5 L $ha^{-1}$ Humiforte (1.4 %), and both concentrations of Aminolforte (1.3 %) compared to N.P.K treatment (1 %). Of course, there were no significant differences between the other treatments of biostimulants with N.P.K treatment. The highest content of *α*-pinene was obtained in the treatment of 1.5 L $ha^{-1}$ Kadostim (27.37 %). While, the lowest content of it was showed in the treatment of 1.5 L $ha^{-1}$ Fosnutren (21.74 %). Increasing the concentrations of Kadostim and Aminolforte caused to the improvement of *α*-pinene content. But, increasing the concentration of Fosnutren led to the decreasing of *α*-pinene content (Table 3).

Among the different biostimulants, the maximum content of camphene and *β*-pinene was caused by application of the Aminolforte treatment at 1.5 L $ha^{-1}$ (5.93 and 1.78 %, respectively), but the minimum content of camphene and *β*-pinene was achieved when the Aminolforte treatment was applied at 0.75 L $ha^{-1}$ (4.99 and 1.15 %, respectively). In contrast with N.P.K treatment, reduction of the myrcene content was observed with the use of various biostimulants. It is interesting that the increasing of Aminolforte and Kadostim concentrations had not significant effect on myrcene content, but its content by the increasing of Fosnutren and Humiforte concentrations was enhanced. The greatest and lowest content of *p*-cymene was detected at 1.5 L $ha^{-1}$ Kadostim (1.33 %) and 0.75 L $ha^{-1}$ Aminolforte (1.01 %), respectively (Table 3). In comparison with N.P.K treatment, using of the Aminolforte at 0.75 L $ha^{-1}$ decreased the *p*-cymene content up to 15 percent, whiles the applied treatment of Aminolforte at 1.5 L $ha^{-1}$ had not significant difference with N.P.K treatment. Also, there was no significant difference between N.P.K treatment and Kadostim, Fosnutren, and Humiforte at 0.75 L $ha^{-1}$ for the content of *p*-cymene. Moreover, increasing the concentration of Humiforte and Fosnutren caused to decreasing the content of *p*-cymene up to 6.7 and 13 percent, respectively. The content of *p*-cymene was also increased up to 11 percent at high concentration of Kadostim. The maximum content of limonene was obtained with the application of Kadostim at 1.5 L $ha^{-1}$ which had not significant difference with N.P.K treatment. The lowest content of limonene was acquired at 1.5 L $ha^{-1}$ Fosnutren. The content of limonene was increased with increasing the concentrations of various biostimulants, except Fosnutren. The application of Fosnutren at 1.5 L $ha^{-1}$ improved the content of 1,8-cineol to 23 %. In contrast, it was reduced to 11 percent when used at 0.75 L $ha^{-1}$ Aminolforte. The effect of other biostimulants application on 1,8-cineol content was similar to the treatment of N.P.K. The content of linalool increased with increasing the Fosnutren concentration, but it was decreased with increasing the Humiforte and Aminolforte concentration to 1.5 L $ha^{-1}$. Therefore, the highest content of linalool was related to application of Aminolforte and Fosnutren at 1.5 L $ha^{-1}$ and the lowest content of it was recorded in the treatment of N.P.K and various concentrations of Kadostim biostimulants (Table 3). Among different biostimulants applied for rosemary, Fosnutren at 1.5 L $ha^{-1}$ led to the high content of camphor that it was 33 percentages more than N.P.K treatments. Although, the camphor content in comparison with biostimulants had not a significant difference with the N.P.K. treatment. Increasing the concentration of each of the biostimulants had not a significant effect on the borneol content except the Aminolforte application. Thus, the highest and the lowest content of borneol was found in the concentration of Aminolforte at 0.75 L $ha^{-1}$ (7.97 %) and 1.5 L $ha^{-1}$ (5.5 %), respectively. The content of pinocamphone in each of the Aminolforte and Humiforte concentrations was alike to N.P.K treatment, whiles, the highest content of pinocamphone was obtained at 1.5 L $ha^{-1}$ Fosnutren (1.46 %), and the lowest content of it was studied at 0.75 L $ha^{-1}$ Fosnutren (1.21 %) and at 1.5 L $ha^{-1}$ Kadostim (1.2 %). The content of α-terpineol was highest at 0.75 L $ha^{-1}$ Aminolforte (2.01 %) and it was the lowest at 1.5 L $ha^{-1}$ Kadostim (0.3 %). Though, the other biostimulants had not significant difference with N.P.K treatment. The maximum verbenone content was obtained with the application of N.P.K treatment (9.2 %), and the means of other biostimulants were less than N.P.K treatment. Therefore, the lowest content of verbenone was related at 1.5 L $ha^{-1}$ of Kadostim (7.41 %) (Table 3). By the application of 1.5 L $ha^{-1}$ Fosnutren, the content of bornyl acetate was reached to the maximum own value. The lowest content (4.09 %) of bornyl acetate was observed at 1.5 L $ha^{-1}$ Kadostim that had not significant difference with N.P.K treatment (4.36 %). The highest content (1.94 %) of caryophyllene was observed at low concentration (0.75 L $ha^{-1}$) of Aminolforte, despite the fact that the lowest content (0.74 %) of caryophyllene was obtained at high concentration (1.5 L $ha^{-1}$) of Fosnutren. There was not significant effect on caryophyllene content when the concentrations of Kadostim and Fosnutren were increased, whereas the caryophyllene content was reduced with the enhancement of Aminolforte and Fosnutren concentration. The use of Fosnutren and Kadostim at 0.75 L $ha^{-1}$ directed to the highest content of caryophyllene oxide, although, the lowest content was observed in N.P.K treatment (Table 3).

**Table 2:** Analysis of variance (ANOVA) for the biostimulants effect on essential oil components of rosemary

| Source of variance (S.O.V) | D.f. | Mean squares | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Essential oil | MH | MO | *α*-pinene | camphene | *β*-pinene | myrcene | *p*-cymene | limonene | 1,8-cineole | linalool |
| Replication | 2 | 0.054 ns | 0.47 ns | 5.39 ** | 0.001 ns | 0.011 ns | 0.016 ns | 0.12 ** | 0.002 ns | 0.086 ns | 0.018 ns | 0.073 ** |
| Biostimulants | 8 | 0.083 ** | 13.4 ** | 7.38 ** | 9.720 ** | 0.224 ** | 0.096 ** | 0.289 ** | 0.031 ** | 0.568 ** | 1.510 ** | 0.179 ** |
| Erorr | 16 | 0.019 | 0.98 | 0.41 | 0.846 | 0.018 | 0.006 | 0.009 | 0.002 | 0.037 | 0.055 | 0.006 |
| CV | - | 12.03 | 2.24 | 2.02 | 3.69 | 2.37 | 5.25 | 2.59 | 4.09 | 5.93 | 3.16 | 2.51 |

MH; Monoterpene Hydrocarbons, MO; Oxygenated Monoterpenes , and ns, * and **; showed non-significant and significant at the 5% and 1% probability, respectively.

**Table 2:** Continued.

| Source of variance (S.O.V) | D.f. | Mean squares | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | camphor | borneol | pinocamphone | *α*-terpineol | verbenone | bornyl acetate | caryophyllene | caryophyllene oxide |
| Replication | 2 | 0.458 * | 0.423 * | 0.001 ns | 0.04 ** | 0.423 ** | 0.29 * | 0.004 ns | 0.011 * |
| Biostimulants | 8 | 1.84 ** | 1.47 ** | 0.02 ** | 0.73 ** | 0.93 * | 1.05 ** | 0.341 ** | 0.043 ** |
| Erorr | 16 | 0.096 | 0.070 | 0.002 | 0.006 | 0.024 | 0.051 | 0.018 | 0.002 |
| CV | - | 3.94 | 4.10 | 3.04 | 4.76 | 1.86 | 4.65 | 9.87 | 4.59 |

ns, * and **; showed non-significant and significant at the 5% and 1% probability, respectively.

**Table 3:** Means comparison* based on percentages for the biostimulants effect on essential oil components of rosemary

| Biostimulants | Essential oil | *α*-pinene | camphene | *β*-pinene | myrcene | *p*-cymene | limonene | 1,8-cineole | linalool |
|---|---|---|---|---|---|---|---|---|---|
| 0.75 L $ha^{-1}$ Aminolforte | $1.3^{ab} \pm 0.1$ | $22.76^{de} \pm 1.1$ | $4.99^{d} \pm 0.32$ | $1.15^{e} \pm 0.16$ | $3.54^{de} \pm 0.09$ | $1.01^{e} \pm 0.08$ | $3.1^{bc} \pm 0.065$ | $6.66^{c} \pm 0.4$ | $3.37^{a} \pm 0.16$ |
| 1.5 L $ha^{-1}$ Aminolforte | $1.3^{ab} \pm 0.2$ | $26.56^{ab} \pm 0.8$ | $5.93^{a} \pm 0.15$ | $1.78^{a} \pm 0.15$ | $3.43^{e} \pm 0.14$ | $1.22^{b} \pm 0.02$ | $3.47^{ab} \pm 0.12$ | $7.16^{b} \pm 0.15$ | $2.63^{d} \pm 0.21$ |
| 0.75 L $ha^{-1}$ Fosnutren | $1^{c} \pm 0.1$ | $25.64^{abc} \pm 0.34$ | $5.7^{abc} \pm 0.03$ | $1.34^{d} \pm 0.06$ | $3.6^{de} \pm 0.06$ | $1.21^{b} \pm 0.02$ | $3^{c} \pm 0.11$ | $7.48^{b} \pm 0.01$ | $3.05^{bc} \pm 0$ |
| 1.5 L $ha^{-1}$ Fosnutren | $1.1^{bc} \pm 0.2$ | $21.74^{e} \pm 1.61$ | $5.72^{abc} \pm 0.08$ | $1.45^{bcd} \pm 0.01$ | $3.22^{f} \pm 0.25$ | $1.04^{de} \pm 0.07$ | $2.19^{d} \pm 0.52$ | $9.22^{a} \pm 0.48$ | $3.43^{a} \pm 0.19$ |
| 0.75 L $ha^{-1}$ Humiforte | $1.1^{bc} \pm 0.2$ | $24.11^{cd} \pm 0.42$ | $5.64^{bc} \pm 0$ | $1.38^{d} \pm 0.045$ | $4.02^{b} \pm 0.15$ | $1.24^{b} \pm 0.03$ | $3.43^{ab} \pm 0.1$ | $7.41^{b} \pm 0.02$ | $3.17^{b} \pm 0.06$ |
| 1.5 L $ha^{-1}$ Humiforte | $1.4^{a} \pm 0.1$ | $25.68^{abc} \pm 0.36$ | $5.87^{ab} \pm 0.12$ | $1.57^{bc} \pm 0.05$ | $3.62^{d} \pm 0.05$ | $1.12^{cd} \pm 0.03$ | $3.39^{ab} \pm 0.08$ | $7.28^{b} \pm 0.09$ | $3.01^{c} \pm 0.02$ |
| 0.75 L $ha^{-1}$ Kadostim | $1.1^{bc} \pm 0.2$ | $24.97^{bc} \pm 0.01$ | $5.66^{bc} \pm 0.01$ | $1.59^{b} \pm 0.06$ | $3.83^{c} \pm 0.06$ | $1.21^{b} \pm 0.02$ | $3.39^{ab} \pm 0.08$ | $7.08^{b} \pm 0.19$ | $2.97^{c} \pm 0.04$ |
| 1.5 L $ha^{-1}$ Kadostim | $0.9^{c} \pm 0.1$ | $27.37^{a} \pm 1.32$ | $5.73^{abc} \pm 0.05$ | $1.43^{cd} \pm 0.02$ | $3.96^{bc} \pm 0.12$ | $1.33^{a} \pm 0.08$ | $3.63^{a} \pm 0.2$ | $7.33^{b} \pm 0.06$ | $2.93^{c} \pm 0.06$ |
| N.P.K treatment | $1^{c} \pm 0.1$ | $25.76^{abc} \pm 0.4$ | $5.5^{c} \pm 0.07$ | $1.54^{bc} \pm 0.035$ | $4.19^{a} \pm 0.24$ | $1.2^{bc} \pm 0.01$ | $3.47^{ab} \pm 0.12$ | $7.5^{b} \pm 0.02$ | $2.92^{c} \pm 0.07$ |

*Means with the same letters in each column indicate no significant difference between treatments according to Duncan's multiple range test at the 5 % level of probability. The obtained values were expressed as mean ± SD (standard deviation) from three replications (n = 3).

**Table 3:** Continued.

| Biostimulants | camphor | borneol | pinocamphone | *α*-terpineol | verbenone | bornyl acetate | caryophyllene | caryophyllene oxide |
|---|---|---|---|---|---|---|---|---|
| 0.75 L $ha^{-1}$ Aminolforte | $7.79^{b} \pm 0.04$ | $7.97^{a} \pm 0.76$ | $1.37^{b} \pm 0.04$ | $2.01^{a} \pm 0.22$ | $7.86^{d} \pm 0.23$ | $4.84^{bc} \pm 0.003$ | $1.94^{a} \pm 0.3$ | $1.06^{bc} \pm 0.017$ |
| 1.5 L $ha^{-1}$ Aminolforte | $7.35^{b} \pm 0.26$ | $5.5^{e} \pm 0.47$ | $1.32^{bc} \pm 0.01$ | $1.55^{d} \pm 0.01$ | $8.46^{bc} \pm 0.075$ | $4.84^{bc} \pm 0.003$ | $1.63^{b} \pm 0.14$ | $0.98^{cd} \pm 0.023$ |
| 0.75 L $ha^{-1}$ Fosnutren | $7.86^{b} \pm 0.01$ | $6.47^{bcd} \pm 0.01$ | $1.21^{d} \pm 0.04$ | $1.69^{bc} \pm 0.06$ | $8.74^{b} \pm 0.22$ | $4.5^{cd} \pm 0.167$ | $1.39^{bc} \pm 0.02$ | $1.19^{a} \pm 0.082$ |
| 1.5 L $ha^{-1}$ Fosnutren | $9.87^{a} \pm 1$ | $6.86^{b} \pm 0.21$ | $1.46^{a} \pm 0.08$ | $1.64^{cd} \pm 0.04$ | $8.53^{bc} \pm 0.11$ | $6.09^{a} \pm 0.628$ | $0.74^{e} \pm 0.1$ | $1.14^{ab} \pm 0.057$ |
| 0.75 L $ha^{-1}$ Humiforte | $7.91^{b} \pm 0.02$ | $6.71^{bc} \pm 0.13$ | $1.27^{cd} \pm 0.01$ | $1.81^{b} \pm 0.12$ | $8.48^{bc} \pm 0.085$ | $4.47^{cd} \pm 0.182$ | $1.08^{d} \pm 0.13$ | $0.92^{de} \pm 0.053$ |
| 1.5 L $ha^{-1}$ Humiforte | $7.86^{b} \pm 0.01$ | $6.3^{cd} \pm 0.07$ | $1.25^{cd} \pm 0.02$ | $1.71^{bc} \pm 0.07$ | $7.71^{d} \pm 0.3$ | $5.2^{b} \pm 0.183$ | $1.22^{cd} \pm 0.06$ | $0.96^{d} \pm 0.033$ |
| 0.75 L $ha^{-1}$ Kadostim | $7.42^{b} \pm 0.23$ | $6.25^{cd} \pm 0.1$ | $1.26^{cd} \pm 0.02$ | $1.73^{bc} \pm 0.08$ | $8.4^{c} \pm 0.045$ | $5.11^{b} \pm 0.138$ | $1.28^{cd} \pm 0.03$ | $1.17^{a} \pm 0.072$ |
| 1.5 L $ha^{-1}$ Kadostim | $7.43^{b} \pm 0.22$ | $6.01^{d} \pm 0.22$ | $1.2^{d} \pm 0.05$ | $0.3^{e} \pm 0.04$ | $7.41^{e} \pm 0.45$ | $4.09^{d} \pm 0.372$ | $1.47^{bc} \pm 0.06$ | $0.97^{d} \pm 0.028$ |
| N.P.K treatment | $7.4^{b} \pm 0.24$ | $5.97^{d} \pm 0.24$ | $1.32^{bc} \pm 0.01$ | $1.68^{bcd} \pm 0.06$ | $9.2^{a} \pm 0.44$ | $4.36^{d} \pm 0.237$ | $1.34^{c} \pm 0$ | $0.85^{e} \pm 0.088$ |

*Means with the same letters in each column indicate no significant difference between treatments according to Duncan's multiple range test at the 5% level of probability. The obtained values were expressed as mean ± SD (standard deviation) from three replications (n = 3).

With regard to the compound groups of essential oil, the maximum content of monoterpene hydrocarbons (MH) was obtained at 0.75 L ha$^{-1}$ Kadostim (47.15 %) that it was identical with the monoterpene hydrocarbon content in N.P.K treatment (45.69 %) and Aminolforte at 1.5 L ha$^{-1}$ (46.08 %). The lowest content of monoterpene hydrocarbons was observed at 1.5 L ha$^{-1}$ Fosnutren (42.39 %) which it had no significant difference with Humiforte at 0.75 L ha$^{-1}$ (43.8 %). Accumulation of monoterpene hydrocarbons was observed by increasing the Aminolforte and Kadostim concentration. However, increasing the Fosnutren concentration caused to reduce the accumulation of monoterpene hydrocarbon (Figure 1). In contrast, the content of oxygenated monoterpenes (OM) was reduced by increasing the concentration of Aminolforte, Humiforte and Kadostim. But the accumulation of oxygenated monoterpenes enhanced up by increasing the concentration of Fosnutren. Therefor, the greatest content of oxygenated monoterpenes was found at 1.5, and 0.75 L ha$^{-1}$ for Fosnutren (33.98 %), and Aminolforte (33.47 %), respectivily. Also, the lowest content of oxygenated monoterpenes was attained at 1.5 L ha$^{-1}$ Kadostim (28.91 %) (Figure 2).

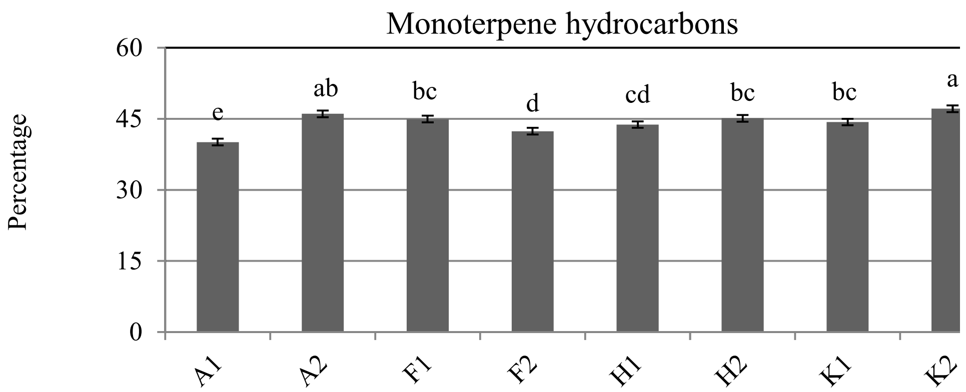


**Figure 1:** The effect of various biostimulants on monoterpenes hydrocarbon (MH %). Means with the same letter are not significantly different according Duncan's multiple range test ($p \leq 0.05$). Error bars represent standard errors (n = 3). A1: 0.75 L ha$^{-1}$ Aminolforte, A2: 1.5 L ha$^{-1}$ Aminolforte, F1: 0.75 L ha$^{-1}$ Fosnutren, F2: 1.5 L ha$^{-1}$ Fosnutren; H1: 0.75 L ha$^{-1}$ Humiforte, H2: 1.5 L ha$^{-1}$ Humiforte, K1: 0.75 L ha$^{-1}$ Kadostim, K2: 1.5 L ha$^{-1}$ Kadostim, and C: N.P.K treatment.

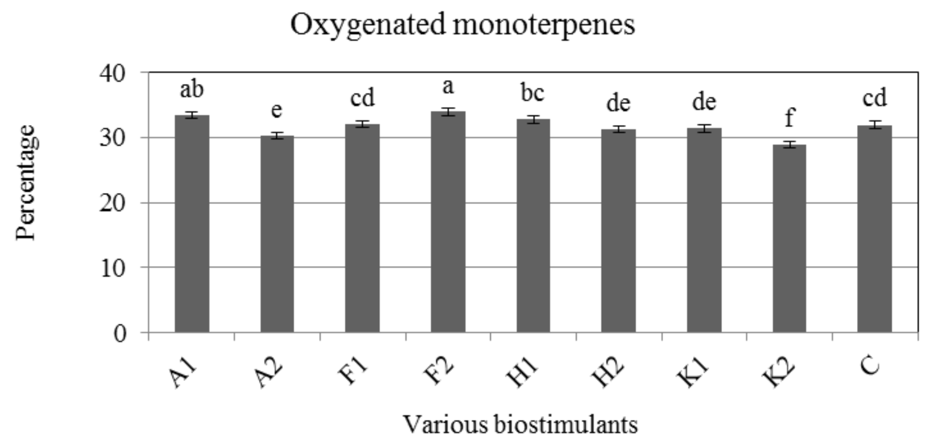


**Figure 2:** The effect of various biostimulants on oxygenated monoterpenes (OM %). Means with the same letter are not significantly different according Duncan's multiple range test ($p \leq 0.05$). Error bars represent standard errors (n = 3). A1: 0.75 L ha$^{-1}$ Aminolforte, A2: 1.5 L ha$^{-1}$ Aminolforte, F1: 0.75 L ha$^{-1}$ Fosnutren, F2: 1.5 L ha$^{-1}$ Fosnutren; H1: 0.75 L ha$^{-1}$ Humiforte, H2: 1.5 L ha$^{-1}$ Humiforte, K1: 0.75 L ha$^{-1}$ Kadostim, K2: 1.5 L ha$^{-1}$ Kadostim, and C: N.P.K treatment.

The results demonstrated that the application of various biostimulants increased the most components of rosemary essential oil in relationship to N.P.K treatment. The comparison of chemical composition in the essential oil of rosemary showed the significant differences in important components. The highest content of *α*-pinene, 1,8-cineole and camphor as major components of rosemary essential oil were obtained at 1.5 L $ha^{-1}$ Fosnutren. In addition, the maximum content of linalool, *z*-pinocamphone, bornyl acetate and caryophyllene oxide were observed at 1.5 L $ha^{-1}$ Fosnutren. Although the maximum content of myrcene and verbenone were acquired in N.P.K treatment. However, the maximum content of *β*-pinene, camphene, borneol, and *α*-terpineol was related to the both concentrations of Aminolforte.

This research reported that the quantity and quality of rosemary essential oil was changed by application of the plant biostimulants based on amino acids. These biostimulants which had various amino acids and other organic matters affected essential oil components of rosemary plant. Another study confirmed this finding (Neeraja et al., 2005; Toosi et al., 2014). Some authors reported that increased the soybean oil content was obtained by spraying with Kadostim (Toosi et al., 2014). Also, polyphenols and amino acid content in the tea plant by using of Aminolforte as bio-stimulant was increased (Thomas et al., 2009). Mehrabi et al., (2013) reported that use of Aminolforte, Kadostim, and Fosnutren in savory cultivation (*Satureja hortensis* L.) could increase the essential oil content. Maini (2006) summarized the early literature showing enhanced activity of NAD-dependent glutamate dehydrogenase, nitrate reductase and malate dehydrogenase in maize following application of Siapton (protein hydrolyzed from animal epithelial tissues). Also, biostimulants may through improvement of plant nutrients uptake, induction of phytohormones biosynthesis, biotic and abiotic stress reduction and enhancing of enzymes activity related to tricarboxylic acid cycle (TCA cycle) and nitrogen metabolism caused increassing essential oil components of the rosemary plant (Calvo et al., 2014).

Amino acids have a major role in protein structure formation and their presence is necessary for the proper function of metabolic and biological processes. For example, the asparagine and glutamine connect the two important metabolic cycles of the plants, the carbon and nitrogen cycles, and they have an influence both on sugars and proteins. The glycine is an amino acid that inhibits the photorespiration done by $C_3$ plants (Taiz and Zeiger, 2010). Also, in plants the amino acids decomposed and intermediate metabolites formed and afterward, these metabolites will transform into glucose or are oxidized in the citric acid cycle (Miflin, 1993). Unlike sugars and fatty acids, the extra amino acids are not stored or disposed, but they are used as energy compounds (Thomas et al., 2009). In plants, amine group of the additional amino acids can be converted to urea and will be used as a nitrogen source. The backbone of amino acids can be transformed to seven molecules including pyruvate, acetyl-CoA, ketoglutarate, succinyl-CoA, fumarate, oxaloacetate or an intermediate metabolite of urea cycle (Miflin, 1993). According to several studies, the foliar application of amino acids causes an improvement in plant growth and fruit yield in cucumber (El-Shabasi et al., 2005) and garlic (Fawzy et al., 2012). On the other hand, amino acids have important role in the biosynthesis of secondary metabolite and phytohormones. The methionine is the ethylene precursor and the tryptophan is responsible for regulation of auxin biosynthesis. Also, the glutamic acid is important for the synthesis of the auxin (Taiz and Zeiger, 2010).

## 4 CONCLUSION

The use of plant biostimulants to improve plant phytochemistry is one of the goals of ecological and sustainable agriculture. The maximum content of essential oil was obtained at 1.5 L $ha^{-1}$ Humiforte, and both concentrations of Aminolforte. While, the highest content of α-pinene, 1,8-cineole, and camphor as major components of rosemary essential oil were found at 1.5 L ha$^{-1}$ Fosnutren. In addition, the maximum content of linalool, *z*-pinocamphone, bornyl acetate, and caryophyllene oxide were observed at 1.5 L ha$^{-1}$ of Fosnutren. Therefore, application of the various biostimulants could increase the major components of rosemary essential oil in comparison with N.P.K treatment. According to the results, the use of amino acids in combination with other nutrients based on the formulation of plant biostimulants can help to improve the phytochemical traits of essential oil in rosemary at different stages of plant development. In general, the plant biostimulants as environmentally friendly products can be the effective alternative to reducing the use of chemical fertilizer and increasing the quantity and quality of rosemary essential oil.

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