The Influences of Bio-stimulators Compounds on Growth Traits and Essential Oil Content of Rosemary (*Rosmarinus officinalis* L.)

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Abstract

Background: **Rosemary (*Rosmarinus officinalis* L.) as a member of the Lamiaceae family is a valuable aromatic and medicinal plant which is cultivated in large scale in Iran. Therefore, it is necessary to determine influence of factors affecting plant growth and drug metabolite production.**

Objectives: **This experiment was carried out to evaluate the effects of bio-stimulators compounds on growth and phytochemical traits of rosemary (*Rosmarinus officinalis* L.).**

Methods: **This experiment was conducted on the basis of randomized complete blocks design with three replications at Medicinal Plants Institute (MPI) of ACECR in 2013. The treatments included commercial formulations of aminolforte, kadostim, fosnutren, humiforte (each of them at 0.75 and 1.5 L.ha^{-1}), 120 kg.ha^{-1} chemical fertilizers (N.P.K, 15:8:15%), and control treatment (without any bio-stimulators and chemical fertilizer applications).**

Results: **The effects of treatments were significant ($p \leq 0.01$) on all of the traits in a way that the maximum value of plant height (42.94 cm), leaf length (34.92 mm), leaf width (4.2 mm), number of leaves per plant (465 leaves.plant^{-1}), leaf SPAD value (56.1 SPAD), total dry weight (246.78 kg.ha^{-1}) essential oil (1.4%), α-pinene (27.35%), was obtained by foliar application of 1.5 L.ha^{-1} fosnutren. Also, the highest of collar diameter of stem (10.77 mm) and number of branches per plant (24.63 branches.plant^{-1}) and camphor (9.87%) was observed in chemical fertilizers treatment, 1.5 L.ha^{-1} aminolforte and humiforte, respectively. In contrast, the least amounts of traits were observed in control treatment.**

Conclusion: **The growth and phytochemical traits of rosemary increased due to foliar application of bio-stimulators compounds.**

Keywords: ***Rosmarinus officinalis* L., Bio-stimulators, Camphor, Essential oil content, α-pinene**

Introduction

Rosemary (*Rosmarinus officinalis* L.) is belonging to Lamiaceae family. It is endemic to the Mediterranean regions and commonly cultivated in many parts of Iran [1]. Rosemary is an aromatic evergreen shrub that its abundant branches are soft and fluffy when young leaves are thin and mutual. It is reported that the essential oil of plants including rosemary have anti-microbial properties [2, 3]. Rosemary is anti-emphysema and it has disinfectant properties. Indeed it increases the secretion of digestive juices and bile and also blood circulation. It is effective on rheumatism and migraine. Rosemary is used in the production of cologne and as deodorant in shampoos, creams, soaps and cosmetics. It is used as an antispasmodic in real colic and dysmenorrhea, in relieving respiratory disorders and to stimulate growth of hair. Rosemary extract relaxes smooth muscles and has choleric hepatic protective and anti-tumorigenic activity [4]. It is also antiviral and inhibits even HIV protease at very low concentrations. The potent antioxidant properties of rosemary extracts have been attributed to its phenolic compounds, mainly rosmarinic acid and diterpenes carnosic acid and carnosol [5, 6].

Bio-stimulators as biological substances stimulate metabolism and metabolic processes to increase plants yield and these compounds like commercial formulations of aminolforte, kadostim, fosnutren and humiforte have the basis of amino acid and they improve quantitative and qualitative growth [7]. The amino acids are essential constituents in all cells. In addition to their role in protein synthesis, they participate in both primary and secondary metabolic processes associated with plant development and in responses to stress. For example, glutamine, glutamate, aspartate, and asparagines serve as pools and transport forms of nitrogen, as well as in balancing the carbon/nitrogen ratio. Other amino acids such as tryptophan, methionine, proline and arginine contribute to the tolerance of plants against biotic and abiotic stresses either directly or indirectly by serving as precursors to secondary compounds and hormones [8]. Manival *et al*. (1994) reported that application of bio-stimulators on tea plant increased photosynthetic CO_2 uptake. Also, stomata control and photosynthetic CO_2 uptake are directly related to each other [9, 10]. Mandal *et al*. (2007) stated that the most common growth sources include amino acid, a mixture of nutrients, hydrolysis proteins, triacotanol, humic acids, extracts of seaweed, and brassinolids [11]. The positive effect of bio-stimulators on production, quality and growth of vegetables, *Camellia* species and forage crops was reported [12, 13]. Therefore, the aim of this study is to investigate the effects of bio-stimulators on growth and phytochemical parameters of rosemary (*Rosmarinus officinalis* L.).

Materials and Methods

This experiment was carried out in 2013 at Medicinal Plants Institute (MPI) of Academic Center for Education, Culture & Research (ACECR) (56° 35′ N and 50° 58′ E; 1500 m elevation). The soil was loam-silt with 0.06% nitrogen, 8.4 p.p.m phosphorous, 163.4 p.p.m potassium, 0.71 $ds.m^{-1}$ EC, and pH 7.74.

This study has been conducted on the base of randomized complete blocks design with three replications. The treatments were foliar application of bio-stimulators with commercial formulations at 0.75 and 1.5 $L.ha^{-1}$ of aminolforte (A_1, and A_2), kadostim (K_1, and K_2), fosnutren (F_1, and F_2), and humiforte (H_1, and H_2), and 120 $kg.ha^{-1}$ chemical fertilizer

(15:8:15%; N.P.K), and also, control treatment (without any application of bio-stimulators and chemical fertilizer). Four commercial formulations of bio-stimulators (including humiforte, aminolforte, kadostim and fosnutren) were supplied by Inagrosa Industries Agro Biologicals, Madrid, Spain. The details of the formulations are mentioned in Table 1.

The plants were supplied from Gene Bank of Medicinal Plants Institute (MPI-ACECR). The same of rosemary plants was sown in rows 25 cm apart with inter-row spacing of 15 cm apart. Each experimental plot contained of 5 rows. The replicates with distance of 1.5 m from each other and plots with distance of 1 m from every side were considered. Irrigation and other field practices had been done as needed.

Foliar application was applied 4 times during growth stages and cultivation season of rosemary with 12 days intervals on shoot of rosemary. The first spray applied 40 days after sowing and other applied 52, 64 and 76 days after sowing. Sprays were carried out in a way that all above ground parts of rosemary plants were covered. To increase the absorption of solutions by plants, foliar application of bio-stimulators was done in conditions without wind and rain and before sunrise when plant stomata are open [14].

The samples in nylon bags were sent to laboratory for measuring parameters. The studied parameters were plant height (cm), collar diameter of stem (mm), number of leaves per plant (leaves.plant^{-1}), leaf length (mm), leaf width (mm), number of branches per plant (branches.plant^{-1}), leaf dry/fresh weight (kg.ha^{-1}), stem dry/fresh weight (kg.ha^{-1}), total dry/fresh weight (kg.ha^{-1}), SPAD value (SPAD), essential oil content (%) and major components of α-pinene (%) and camphor (%). For measurement of leaf SPAD value, 10 leaves of each plant were selected and mean of leaf SPAD value was measured by device of SPAD (Minolta, 50 II). Determination of essential oils was achieved by using a combination of the well tested Gas Chromatography analysis (GC) method described by Jankovsky *et al.* (1989) and the Continuous Distillation Extraction (CDE) method after Jankovsky *et al.* (1993) [15, 16].

Analysis of variance of the results was done using the SPSS software (ver.17), and means in the results were compared using the Fisher's protected Least Significant Differences (FLSD) Test.

Table 1 - Formulation of bio-stimulators used in the experimental treatments

Biostimulators*	Formulation of compounds**
Aminolforte	3750 mg.L^{-1} free amino acids, 2% organic components and 1.1% total N (urea 0.8% N and organic 0.3% N)
Kadostim	3750 mg.L^{-1} free amino acids, 2% organic components and 5% total N (amonia 1.6% N, nitric 3.1% N and organic 0.3% N) and potassium 6% K_2O
Humiforte	3750 mg.L^{-1} free amino acids, 2% organic components and 6% total N (amonia 1.5% N, urea 3.7% N, nitric 0.5% N and organic 0.3% N), potassium 5% K_2O and phosphorous 3% P_2O_5
Fosnutren	3750 mg.L^{-1} free amino acids, 2% organic components and 3.8% total N (amonia 2.1% N, nitric 1.4% N and organic 0.3% N) and phosphorous 6% P_2O_5

*Bio-stimulators supplied by Inagrosa Industries Agro Biologicals are compatible to the climate of Iran.

**Quantity and kind of free amino acids applied in the formulation of bio-stimulators in this experiment based on the percent of total amino acids are as follows: Glysin 11.2%, Valine 5.1%, Proline 8.3%, Alanin 13.2%, Aspartic acid 4.4%, Arginine 8.3%, Glutamic acid 0.9%, Lysine 5.1%, Lucine 16.4%, Isolucine 4.4%, Phenylalanin 5.1%, Methionine 4.2%, Serin 3.9%,Treonine 0.3%, Histidine 0.3%, Tyrosine 1.5%, Glutamine 0.9%, Systein 0.3%, Aspargine 0.4%, and Tryptophan 0.4%.

Results

According to results of analysis variance, the effect of bio-stimulators and chemical fertilizers on plant height, collar diameter of stem, number of leaves per plant, leaf length, leaf width, number of branches per plant, leaves and stems dry/fresh weight, total dry/fresh weight was statistically significant ($p \leq 0.01$) (Table 2).

In relation to the mean comparisons, the maximum plant height, number of leaves per plant, leaf length, leaf width, yield of stem dry weight, yield of leaf fresh weight and yield of total fresh weight was obtained in treatment of 1.5 $L.ha^{-1}$ fosnutren. The most collar diameter of stem, and yield of stem fresh weight was observed in treated plants with chemical fertilizer. The highest number of branches per plant was recorded in treated plants with 1.5 $L.ha^{-1}$ aminolforte. Also, the least amount of all mentioned parameters was achieved in treatment of control (Table 3). The maximum value of leaf dry weight, total dry weight was related to treated plants by 1.5 $L.ha^{-1}$ fosutren, while the lowest amount of these parameters was attained by treatment of control (Fig. 1, 2).

The results of analysis variance showed the significant effect ($p \leq 0.01$) of bio-stimulators and chemical fertilizers on SPAD value, essential oil content and components of α-pinene and camphor (Table 2). The highest amount of SPAD value, essential oil content, and camphor was found in treatment of 1.5 $L.ha^{-1}$ humiforte. The maximum value of α-pinene was obtained in treatment of 1.5 $L.ha^{-1}$ fosnutrn. On the other hand, the lowest value of those traits was realized in treatment of control (Fig. 3, 4, 5 & 6).

Discussion

The foliar application of bio-stimulators was appeared to have significant and positive effect on growth parameters and essential oil content of rosemary. The plants height increased in treatment of 1.5 $L.ha^{-1}$ fosnutren (34.14 cm) in compared to control (42.94 cm). These results are according to Karima & Abdel Wahed (2005) [17]. They reported that use of amino acids significantly increased height of German chamomile (*Matricaria chamomilla* L.). Abou Dahab & Abd El-Aziz (2006) suggested that use of amino acids (especially tryptophan) were significantly effective on height of philodendron so that in 1st and 2nd year, the height of samples increased from 25.60 to 46.30 cm and from 27.30 to 47.30 cm, respectively [18].

The collar diameter of stem, number of leaves and number of branches per plant in rosemary were increased with application of 1.5 $L.ha^{-1}$ fosnutren. The results of this study are in line with experiment results of Mazher *et al*., (2011) on *Codiaeum variegatum* L., Farooqi *et al*., (1996) on *Artemisia annua* L. [19, 20]. Nahed *et al*., (2010) concluded that all growth parameters improved with increase in concentration of amino acids [21]. Positive effect of amino acids on plant yield might be due to stimulating effect of amino acids on plant cells growth. However, amino acids were introduced by Goss, 1973 as a source of energy during lack of carbohydrates [22]. These results are in according to Christopher *et al*., 2007 on *Basella rubra* L. They reported that application of bio-fertilizers increased collar diameter of stem, number of branches per plant and leaves number [23]. However,

Table 2 - Analysis of variance for effects of bio-stimulators on measured parameters of rosemary *(Rosmarinus officinalis* L.)

Source of variance	d.f.	Plant height	Collar diameter of stem	Number of leaves per plant	Leaf length	Leaf width	Number of branches per plant	Yield of stem fresh weight	Yield of stem dry weight
Rep. (block)	2	2.98	0.71	30.64	0.04	0.77	0.25	451.93	9.438
Treatment	9	1.94**	5.09**	27.07**	1.03**	0.25**	0.72**	619.833**	177.389**
Error	18	0.75	0.38	17.51	0.37	0.38	0.25	21.893	5.971
CV (%)		2.25	7.87	1.15	1.85	16.84	2.5	3.81	4.09

ns, *and **: not significant, significant at the 5% and 1% probability levels, respectively

Table 2 - continued

Source of variance	d.f.	Yield of leaf dry weight	Yield of leaf fresh weight	Total dry weight	Total fresh weight	SPAD value	Essential oil	α-pinene	Camphor
Rep.(block)	2	0.893	1.335	10.944	215.082	0.17	1.68	0.00007	0.0003
Treatment	9	59.394**	5.233**	87.717**	878.987**	0.81**	3.42**	15.511**	1.658**
Error	18	1.608	0.812	4.341	99.380	0.13	1.01	1.301	0.139
CV (%)		0.9	0.3	1.10	2.41	0.7	8.97	4.66	4.71

ns, *and **: not significant, significant at the 5% and 1% probability levels, respectively

Table 3 - Mean comparison for effects of different treatments on measured parameters*

Treatment	Plant height (cm)	Collar diameter of stem (mm)	Num. of leaves per plant (leaves.plant^{-1})	Leaf length (mm)	Leaf width (mm)	Num. of branches per plant (branches.plant^{-1})	Stem fresh weight (kg.ha^{-1})	Stem dry weight (kg.ha^{-1})	Leaf fresh weight (kg.ha^{-1})	Total fresh weight (kg.ha^{-1})
C	34.13^{e}	6.33^{d}	265^{d}	30.86^{d}	3.26^{d}	14.3^{d}	92^{c}	45.93^{c}	247^{c}	339^{d}
CF	39.6^{ab}	10.77^{a}	376^{abc}	34.4^{a}	3.84^{abc}	23^{ab}	156.8^{a}	69.88^{ab}	330^{2}	486^{ab}
A_1	36.48^{bcde}	7.22^{cd}	266^{d}	31.96^{cd}	3.38^{d}	23^{ab}	94^{c}	47^{c}	253^{c}	347^{d}
A_2	39.28^{bc}	7.48^{bcd}	406^{ab}	31.31^{d}	3.31^{d}	24.63^{a}	139.2^{ab}	64.33^{bc}	291.2^{bc}	430.4^{bc}
H_1	34.8^{de}	7.15^{cd}	365^{bc}	33.43^{abc}	3.78^{bc}	20^{b}	97.2^{c}	52.92^{cd}	250.3^{c}	347^{d}
H_2	39.86^{ab}	8.02^{bc}	456^{ab}	34.06^{ab}	3.86^{ab}	21.3^{ab}	148^{ab}	67.32^{abc}	315.2^{b}	463.2^{b}
K_1	36^{cde}	6.74^{cd}	285^{cd}	32.02^{cd}	3.46^{cd}	18.6^{bc}	93.86^{c}	46.86^{c}	200.4^{c}	335^{d}
K_2	38.8^{bcd}	7.41^{bcd}	452^{ab}	32.31^{bcd}	3.77^{bc}	19.3^{b}	120^{bc}	59.2^{b}	260^{c}	380^{cd}
F_1	39.58^{ab}	8.25^{bc}	285^{cd}	33.36^{abc}	3.81^{bc}	18^{cd}	130.4^{ab}	63.2^{bc}	312.6^{b}	452^{b}
F_2	42.94^{a}	8.99^{b}	465^{a}	34.92^{a}	4.2^{a}	17.3^{cd}	154.8^{a}	80.16^{a}	388^{a}	453.2^{a}

* Means in each column followed by the same letter are not significantly different ($p < 0.01$).

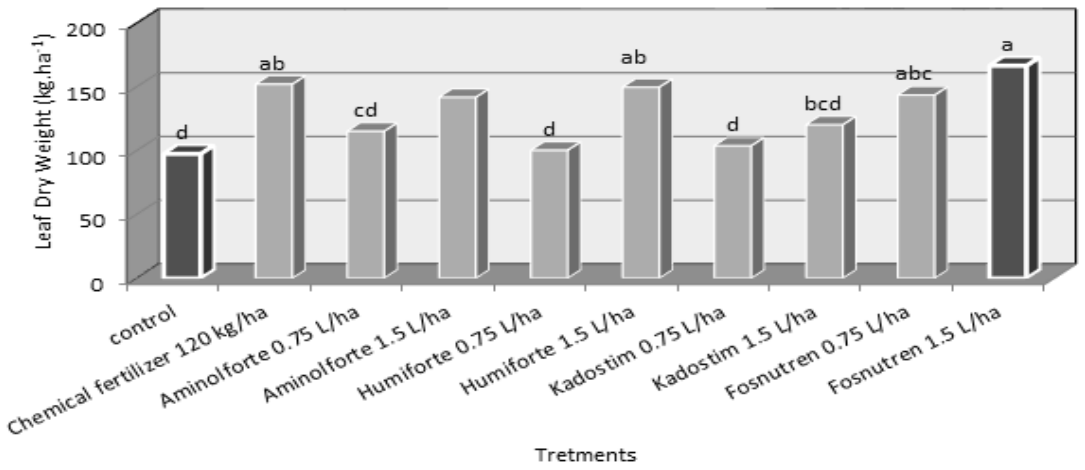

Figure 1 - Mean comparison of bio-stimulators and fertilizers effect on leaf dry weight (kg.ha^{-1})

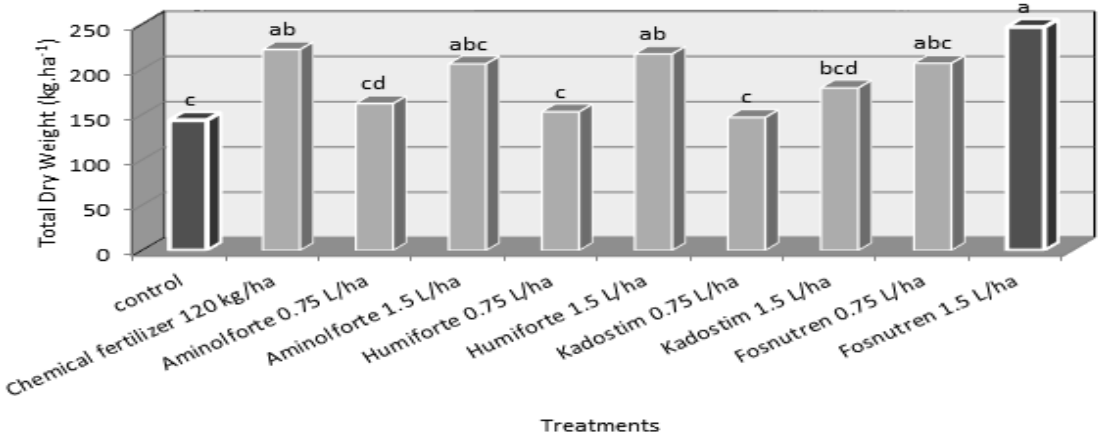

Figure 2 - Mean comparison of bio-stimulators and fertilizers effect on total dry weight (kg.ha^{-1})

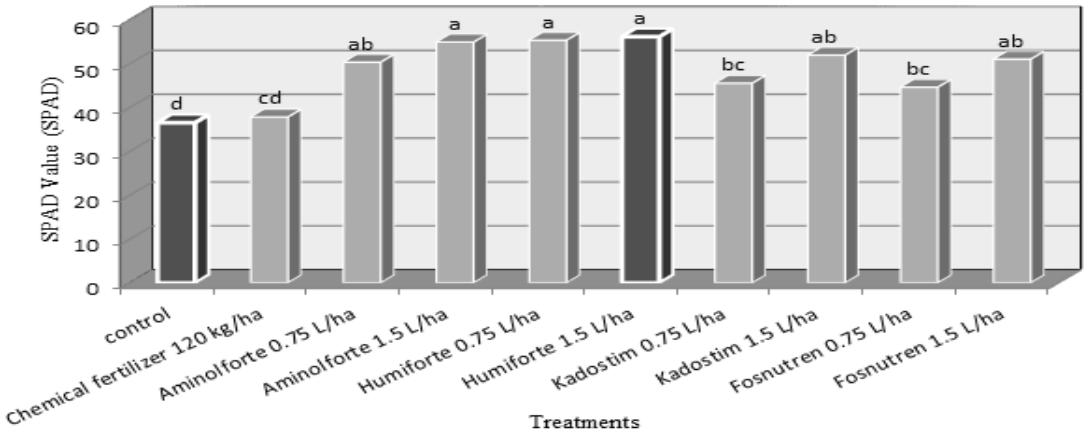

Figure 3- Mean comparison of bio-stimulators and fertilizers effect on SPAD value.

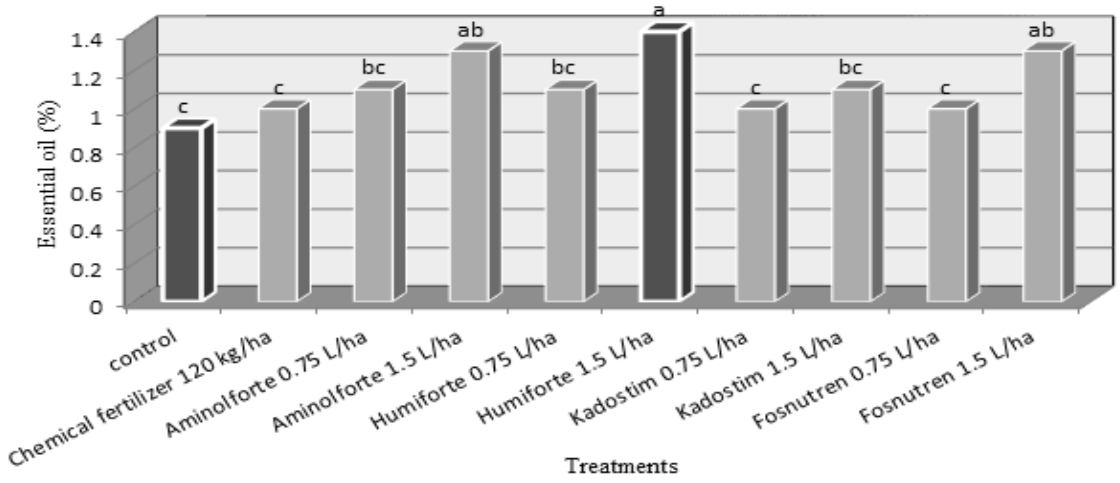

Figure 4 - Mean comparison of bio-stimulators and fertilizers effect on essential oil (%)

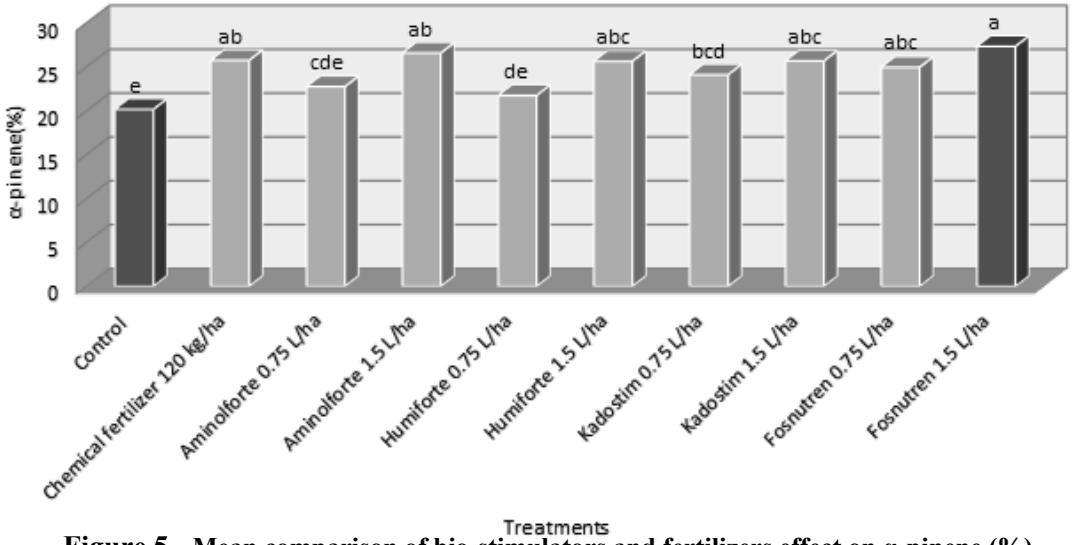

Figure 5 - Mean comparison of bio-stimulators and fertilizers effect on α-pinene (%)

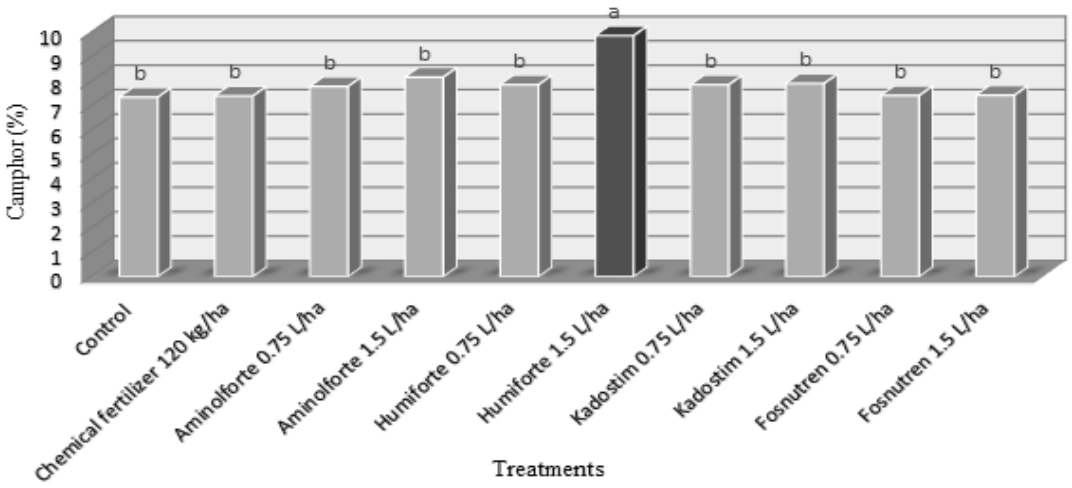

Figure 6 - Mean comparison of bio-stimulators and fertilizers effect on camphor (%)

the results are in agreement with that of Ayman *et al*. (2009) experiment on *vicia faba* L. concerning interaction effect of humic acid and amino acid in isolation and in presence of chelated micro nutrients and the results by Shekari *et al*. (2012) on *plantago psyllium* L. with bio-stimulator spray [24, 25]. Increase in yield and growth parameters is proved to be feasible using amino acids. Therefore, supply of nutritious sources to form protein tissue is essential [26].

The yield of leaf dry and fresh weight reached the highest amount with 1.5 $L.ha^{-1}$ fosnutren. According to the results of Celik and katkat (2007) experiment, application of macro element of phosphorous (existed in fosnutren formulation) had positive effect on leaves and roots dry weight of Zea mays [27]. Amino acids are the precursor of polyamines that are essential in the regulation of plant growth and development [28, 29]. The yield of total fresh and dry weight increased about 37.59 and 41.68% with application of 1.5 $L.ha^{-1}$ fosnutren. The results showed that all the treatments, especially treatment of fosnutren were capable of increasing biomass (i.e., stem, branches with leaves) in comparison to the control. The above finding agrees with the study done by Hlava and Matejka (1988) [30]. In an experiment, Sanchez *et al*., 2005 reported that application of bio-stimulators could increase yield of *Matricaria recutita* [31]. These results are according to study of Dinoo *et al*., 2009 on application of perfectoz bio-stimulator on pepper [32]. Also, Rafiee *et al*., 2011 reported that application of bio-stimulators increased total dry weight of pot marigold seedlings [33]. Amino acid compounds can improve fertilizer assimilation, increase uptake of nutrients and water, enhance the photosynthetic rate and dry matter partitioning, and hence increase crop yield [34].

SPAD value of rosemary was increased by application of humiforte. These results are in line with Nahed *et al*., 2010. They concluded that the use of amino acids tyrosine, thiamin and tryptophan had positive effect on SPAD value of *Thuja orientalis* L. Exogenous application of Putrescine to several plant species have been shown to inhibit chlorophyll loss and senescence [35] and protect plants against environmental stress [36]. Ma *et al.*, (1996) suggested that the effect of Pas in inhibiting chlorophyll degradation may be related to the inhibition of peroxidase activity [37]. Treated plants with 1.5 $L.ha^{-1}$ humiforte showed higher content of essential oil compared to other treatments and control. Our results are similar to experiment results of Sani (2011) and he resulted that application of amino acid compounds increased content of essential oil in flixweld. Because nitrogen, which is a primary constituent of proteins, is extremely susceptible to loss when considering that average recovery rates fall in the range of 20 to 50% for dry matter production systems in plants. Amino acids generally cause deficiency of potassium, increased carbohydrate storage and reduced proteins, alteration in amino acid balance and consequently change the quality of proteins [38]. Major components of essential oil in rosemary plants, α-pinene and Camphor with range of 20.17 to 27.35% and 7.35 to 9.87%, reached the highest amount by application of 1.5 $L.ha^{-1}$ fosnutren and humiforte, respectively. In an experiment foliar application of some amino acids on chamomile plants was investigated. Gas liquid chromatographic analysis revealed that the main components of essential oil increased with application of amino acids [39]. Talaat

and Yousef (2002) obtained similar results on basil plants [40].

Conclusion

In this experiment, commercial formulation of bio-stimulators with basis of bio-active amino acid compound accompanied by macronutrients of N, P and K had positive effect on growth and phytochemical parameters of rosemary plants (*Rosmarinus officinalis* L.).

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