Phytochemical, Agronomical and Morphological Responses of Pot Marigold (*Calendula officinalis* L.) to Foliar Application of Bio-stimulators (Bioactive Amino Acid Compounds)

Rafiee H (M.Sc.)[1], Mehrafarin A (Ph.D.)[2], Qaderi A (Ph.D.)[3], Kalate Jari S (Ph.D.)[1], Naghdi Badi H (Ph.D.)[2]*

1- Department of Horticulture, Science and Research branch, Islamic Azad University, Tehran, Iran
2- Cultivation & Development Department of Medicinal Plants Research Center, Institute of Medicinal Plants, ACECR, Karaj, Iran
3- Biotechnology Department of Medicinal Plants Research Center, Institute of Medicinal Plants, ACECR, Karaj, Iran
* Corresponding author: Medicinal Plants Research Center, Institute of Medicinal Plants, Iranian Academic Center for Education, Culture and Research (ACECR), P.O.Box: 33651/66571, Karaj, Iran
Tel: +98-26-34764010-9, Fax: +98-26-34764021
Email: Naghdibadi@yahoo.com

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Abstract

Background: Bio-stimulators as biological substances stimulate metabolism and metabolic processes, which can be increase plants yield and secondary metabolites content.

Objective: The aim is to introduce bio-stimulators as bioactive amino acid compounds to increase yield and help to sustainable agriculture.

Methods: This experiment was conducted at ACECR on the base of randomized complete blocks design with three replications in 2011. The treatments were commercial formulations of Aminolforte, Kadostim, Fosnutren, Humiforte (each of them 0.75 and 1.5 L.ha^{-1}), chemical fertilizer NPK (70 kg.ha^{-1}), and control treatment (no foliar application).

Results: The treatments had significant effect on the studied parameters except SPAD value. Humiforte 1.5 L.ha^{-1} caused increase in plant dry weight to 37.46%, capitula dry weight to 36.92%, leaf area to 34.44%, relative water content to 32.03%, harvest index to 20.40%, capitula number/plant to 40.64%, total carbohydrates of capitula to 18.43%, total flavonoids of leaves to 19.35%, and total flavonoids yield of leaves to 38.63% compared to control. The highest amount of total flavonoids in capitula (0.25%, with increase to 32% compared to control) was related to Aminolforte 1.5 L.ha^{-1}. Kadostim 1.5 L.ha^{-1} increased plant height to 36.83%, leaves number to 26.65% and total flavonoids yield of capitula to 38.82%, total carbohydrates of leaves to 13.52%. Content of N, P and K increased with foliar application of bioactive amino acid compounds.

Conclusion: Humiforte and Kadostim 1.5 L.ha^{-1} were the best treatments in regard to existence of amino acid compounds and macro-nutrients of N, P and K in their formulations.

Keywords: ***Calendula officinalis*** **L., Bioactive amino acid compounds, Morphological and Phytochemical traits**

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Introduction

Pot marigold (*Calendula officinalis* L.) is from Asteraceae family and complete capitulum or separated florets from floral receptacle compose the medicinal part of this plant. This plant has medicinal properties and it is used for treatment of skin diseases [1]. Pot marigold can be used as a colorant because it primarily contains two classes' pigments, the flavonoids and carotenoids, which can be used as yellow and orange natural colors, respectively. Of course, flavonoids have antioxidant activities which play an important role in food preservation and human health by combating damage caused by oxidizing agents [2, 3, 4].

To increase crop production, different agricultural operations like application of chemical fertilizers is conducted. The result of these activities in recent years was the crisis of environmental pollutions especially pollution of soil and water sources that is penetrated to the nutrition sources of human beings and it threatened human health [5]. Therefore, extensive attempts are being conducted to find suitable solutions for improving the soil quality, agricultural products and polluters' elimination. Decrease of these environmental threats in line with increase of crops yield needs application of new agricultural techniques. One of these techniques is application of bio-stimulators that is purification of proteins/ amino acid compounds from natural sources and alteration to specific oligopeptides [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].

Bio-stimulators made of plants natural extracts are mainly composed of Amino acids and Poly peptides with low molecular weight, vitamins, enzymes and hormons (auxin, cytokinin and giberlin), carbohydrates, betains and antioxidants and other substances and also animal extracts like main amino acids and peptides, and stimulator compounds of enzyme activity in plant tissues [8]. Mandal et al., (2002) reported that the most currently used bio-stimulators are amino acid substances, compounds of elements, hydrolyzed proteins, triacotanol, humic acids, alga extracts and brassinolides. Mandal et al. (2007) emphasized on increase of biochemicals in tea plant in response to growth regulators. Asad et al., (2002), Fraser and Percival (2003), Sabirov et al., (2003) and Yildrim (2007) reported that the bio-stimulators application had positive effects on production, quality and growth of vegetables, tea species and fodder crops.

The aim of this study is to investigate the effects of foliar application of bio-stimulators on morphological and phytochemical parameters of medicinal plant *Calendula officinalis* L.

Material and Methods

Field experiment

To investigate the effects of bio-stimulators on growth and phytochemical characteristics of *Calendula officinalis* L., a field experiment was conducted at Academic Center for Education, Culture & Research (ACECR)-Institute of Medicinal Plants in 2011. This study was done on the base of randomized complete blocks design and three replicates.

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The treatments were 0.75 $L.ha^{-1}$ of Aminolforte (A_1), Kadostim (K_1), Fosnutren (F_1), Humiforte (H_1) and also 1.5 $L.ha^{-1}$ of Aminolforte (A_2), Kadostim (K_2), Fosnutren (F_2), Humiforte (H_2), 70 $kg.ha^{-1}$ of NPK (before sowing) and control treatment (without foliar application). The geographical characterization of experimental field is as follows: 56° 35´ N and 50° 58´ E; 1500 m elevation. 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. The properties of experimental field are shown in Table 1.

The seeds were sown in rows 50 cm apart with inter-row spacing of 20 cm apart in 28 April 2011. 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. The seeds with proper quality of germination were supplied from seed bank of Institute of Medicinal Plants. Irrigation and other field practices had been done as needed.

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 [42]. Foliar application was done in 3 intervals every 15 days. First sample was collected 60 days after emergence. Samples in nylon bags were sent to laboratory for measuring parameters.

Four commercial formulations of Bio-stimulators including Aminolforte, Kadostim, Fosnutren and Humiforte) were supplied by Inagrosa Industries Agro Biologicals, Madrid, Spain. The details of the formulations are mentioned in Table 2.

Table 1 - Characterizations of experimental field

Mean of Yearly temp. (°c)	Mean of yearly rain (mm)	pH	EC ($ds.m^{-1}$)	Soil texture	Height above sea level (m)	Geographical latitude	Geographical longitude
13.21	263	7.8	0.95	Silty loam	1426	35° 36′	50° 56′

Table 2- Formulation of bio-stimulators used in the experimental treatments

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

*Biostimulators 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%, Tryptophan 0.4%

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Studied parameters

The measured parameters are as follows: plant height (cm), capitula dry weight ($g.m^{-2}$), plant dry weight ($g.m^{-2}$), relative water content (RWC) (%) of leaves, SPAD value, leaf area index (LAI), harvest index (%), capitula number per m^2, leaves number per plant, total flavonoids of leaves and capitula (%), flavonoids yield of leaves and capitula ($mg.m^{-2}$), total carbohydrates of leaves and capitula ($mg.g^{-1}$ DW), and content of N (%), P ($mg.g^{-1}$ DW) and K ($mg.g^{-1}$ DW).

The relative water content (RWC %) was calculated using the following equation:

RWC = (FW-DW) / (TW-DW) ×100

Where, FW, DW and TW are the fresh, dry and turgid weight of leaves, respectively. To measure turgid weight, the leaves were kept in water saturated condition and then dried with a cloth and weighed [15].

To measure leaf area index (LAI), device of field leaf area meter was used with manual scan. For measurement of SPAD value, 5 leaves of each plant were selected and mean of SPAD value was measured by device of SPAD (Minolta, 502).

The content of flavonoids was determined using spectrophotometric method [16]. The sample contained 1 ml of methanol solution of the extract in the concentration of 1 $mg.ml^{-1}$ and 1 ml of 2% $AlCl_3$ solution dissolved in methanol. The samples were incubated for an hour at room temperature. The absorbance was determined using spectrophotometer at λmax = 415 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of rutin and the calibration line was construed. Based on the measured absorbance, the concentration of flavonoids was read ($mg.ml^{-1}$) on the calibration line; then, the content of flavonoids in extracts was expressed in terms of rutin equivalent (mg of $RU.g^{-1}$ of extract) [17].

Total carbohydrates were determined in the methanolic extract by using the phenol – sulphoric method according to Dubois *et al*., (1966). Nitrogen, phosphorus and potassium were determined in dried leaves according to Wahing et al., 1989 and Chapman and Pratt, 1961.

Statistical Analysis

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 (LSD) Test.

Results

The results indicated that application of amino acid compounds had significant effect on plant height, capitula dry weight and plant dry weight at significant level of 0.05 and also relative water content (RWC), leaf area index (LAI), harvest index, capitula number, leaves number, total flavonoids of leaves and capitula and their yield, total carbohydrates of Leaves and capitula and content of N, P and K at significant level of 0.01. Of course, its effect wasn't significant on SPAD value (Table 3).

With consideration of mean comparisons, the maximum plant height (13.41 cm, with 36.83% increase compared to control) was observed in Kadostim 1.5 $L.ha^{1-}$ and its minimum (8.47 cm) was obtained control treatment. Highest capitula dry weight (46 $g.m^{-2}$, with 35.21% increase in comparison with control) was observed in Humiforte

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Table 3 - Analysis of variance for effects of different treatments on the measured parameters

S.O.V	df	Mean square								
		Plant height	Capitulum dry weight	plant dry weight	SPAD Value	Leaf area	Relative water content (RWC)	Harvest index	Num. of capitula.m^{-2}	Num. of leaves.plan t^{-1}
Rep. (block)	1	290.080	0.132	0.829	24.75	1524.92	53.47	0.003	8.133	0.137
treatment	9	651.985*	0.317*	2.864*	16.26^{ns}	6748.13**	156.003**	0.023**	23.64**	16.554**
error	29	219.334	0.121	0.825	16.94	1311.91	18.58	0.004	3.39	2.042
CV (%)		13.89	18.21	13.93	13.25	8.43	5.79	16.64	13.98	8.89

*, **, ^{ns} shows significant in 5%, 1%, and insignificant, respectively

Table 3 - continued

S.O.V	df	Mean square								
		Total flavonoids		Yield of flavonoids		Total carbohydrates		Elements content in leaves		
		Leaves	Capitula	Leaves	Capitula	Leaves	Capitula	N	P	K
Rep.(block)	1	2.622	13.24	0.0000097	0.00007	6.304	13.175	0.634	443.889	44389.772
treatment	9	12.124**	51.82**	0.000061**	0.0013**	12.814**	13.776**	0.126**	278.575**	4518.579**
error	29	1.152	7.80	0.0000085	0.00002	2.283	1.695	0.034	45.139	1116.992
CV (%)		13.79	13.47	19.43	9.75	7.27	5.32	10.84	6.92	22.88

*, **, ns shows significant in 5%, 1%, and insignificant, respectively

1.5 L.ha^{-1}and the lowest ones (28.6 g.m^{-2}) resulted by Fosnutren 1.5 L.ha^{-1}. Highest and lowest plant dry weight (161.2 and 100.8 g.m^{-2}, respectively) was obtained by treatment of Humiforte 1.5 L.ha^{-1} and control, respectively (Table 4). In this treatment, the capitula dry weight increased about 35% in comparison with control treatment. The maximum leaf area (492.3 mm^2.plant^{-1}) was observed in Humiforte 1.5 L.ha^{-1} and its least (322.73 mm^2.plant^{-1}) with control treatment (Table 4).

The most relative water content (88.58%, with 32.03% increase compared to control) was occurred with Humiforte 1.5 L.ha^{-1} in comparison with control and chemical fertilizer treatments. Highest harvest index (9.8%) was observed with application of Humiforte 1.5 L.ha^{-1}, while the lowest index was obtained in Fosntren 0.75 L.ha^{-1} (Table 4). This treatment caused increase in this parameter to 20.40% in comparison with control. Maximum of capitula number (380 capitula.m^{-2}) were related to in Humiforte 1.5 L.ha^{-1} and the lowest number (220 capitula.m^{-2}) to Aminolforte 0.75 L.ha^{-1}. Maximum of leaves number was observed in Kadostim 1.5 L.ha^{-1} and the least number were achieved in control. With application of Humiforte 1.5 L.ha^{-1}, the highest total flavonoids content and yield of leaves (with increase to 19.35% and 38.63% compared to control, respectively) were obtained respectively (Fig. 3 and 4). Of course, the highest total flavonoids content and yield of capitula were observed in the highest concentration of Aminolforte (1.5 L.ha^{-1}) and Kadostim (1.5 L.ha^{-1}), respectively (Fig. 1 and 2). These parameters were increased to 32% and 38.82% in comparison with control, respectively Maximum carbohydrates content of leaves (0.244 mg.g^{-1} DW, with increase to 13.52% in comparison with control) was obtained in Kadostim 1.5 L.ha^{-1} and the least content (0.174 mg.g^{-1} DW) was observed in Aminolforte 0.75 L.ha^{-1}. Highest and lowest carbohydrates content of capitula (0.282 and 0.209 mg.g^{-1} DW, respectively) resulted from Humiforte 1.5 L.ha^{-1} and Aminolforte 1.5 L.ha^{-1}, respectively (Table 4). Humiforet 1.5 L.ha^{-1} caused increase to 18.43% in this parameter compared to control.

The most content of nitrogen (2.07%, with increase to 22.70% in comparison to Control treatment) was observed in Fosnutren 1.5 L.ha^{-1} and the lowest content (1.44%) was related to Aminolforte 1.5 L.ha^{-1}. Highest content of phosphorous (1.11 mg.g^{-1} DW) was related to Kadostim 1.5 L.ha^{-1} that caused increase in content to 25.67% and the lowest content was observed in control and chemical fertilizer treatments. Highest potassium content was observed in Kadostim 1.5 L.ha^{-1} (2.13 mg.g^{-1} DW, with increase to 38.02% compared to control treatment) and the minimum amount was related to chemical fertilizer treatment (Table 4).

Discussion

This study indicated that Kadostim 1.5 L.ha-1 increased some of vegetative parameters such as plant height and leaves number per plant to 36.83 and 26.65% in comparison with control, respectively. These results were in line with those obtained by Thomas et al. (2009) on tea plants and Rahimi et al. (2013) on basil plants. The increasing plant height and leaves number per plant by Kadostim 1.5 L.ha^{-1} is due to existence of nitrogen and potassium and their synergistic effect with amino acids in this bio-stimulator

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Table 4 - Mean comparison for effects of different treatments on the measured parameters*

Treatment	Plant height (cm)	Capitula dry weight ($g.m^{-2}$)	Plant dry weight ($g.m^{-2}$)	SPAD Value	Leaf area (mm^2)	Relative water content (RWC)	Harvest index (%)	Num. of capitula.m^{-2}	Num. of leaves.$plant^{-1}$
C	8.47^{d}	29.8^{cd}	100.8^{d}	25.73^{d}	322.73^{c}	60.2^{d}	7.8^{abc}	233.4^{cd}	12.88^{d}
CF	9.3^{cd}	44.2^{ab}	109.4^{cd}	31.56^{ab}	451^{ab}	74.12^{bc}	8.6^{ab}	300^{bc}	16.44^{bc}
A_1	11.63^{abc}	31.4^{bcd}	131^{abcd}	31.06^{ab}	450^{ab}	75.19^{bc}	6.2^{cd}	220^{d}	15^{bcd}
A_2	12.22^{ab}	40^{abcd}	150.4^{ab}	34.3^{a}	474.3^{a}	77.43^{b}	8.6^{ab}	253.4^{bcd}	16.33^{bc}
K_1	11.17^{abcd}	40.2^{abcd}	124.8^{bcd}	29.73^{ab}	400.6^{b}	67.29^{cd}	5.4^{cd}	240^{bcd}	13.22^{d}
K_2	13.41^{a}	44.4^{ab}	140.6^{abc}	31.4^{ab}	422.6^{ab}	76.02^{b}	9.4^{a}	306.6^{b}	21^{a}
F_1	9.68^{bcd}	28.6^{d}	118.6^{bcd}	31.2^{ab}	431.3^{ab}	74.65^{bc}	4.8^{d}	200^{d}	14.57^{cd}
F_2	10.34^{bcd}	35.2^{abcd}	117.4^{bcd}	33.83^{a}	401.3^{b}	74.79^{bc}	6.4^{bcd}	206.6^{d}	17.04^{bc}
H_1	9.85^{bcd}	43^{abc}	148.8^{ab}	30.8^{ab}	447.6^{ab}	75.85^{b}	8.8^{ab}	293.4^{bc}	16.66^{bc}
H_2	10.49^{bcd}	46^{a}	161.2^{a}	31.03^{ab}	492.3^{a}	88.58^{a}	9.8^{a}	380^{a}	17.56^{b}

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

Table 4- continued

Treatments	Total carbohydrates (mg.g^{-1} DW)		Elements content in leaves		
	Leaves	Capitula	N %	P (mg.g^{-1} DW)	K (mg.g^{-1} DW)
C	0.211^{bc}	0.23^{bc}	1.6^{cd}	0.825^{d}	1.32^{bcd}
CF	0.226^{ab}	0.22^{cd}	1.75^{abcd}	1^{abc}	0.815^{d}
A_1	0.174^{d}	0.239^{bc}	1.68^{bcd}	0.975^{bc}	1.52^{abc}
A_2	0.188^{cd}	0.209^{d}	1.44^{d}	0.905^{cd}	1.93^{ab}
K_1	0.215^{bc}	0.225^{cd}	1.71^{bcd}	0.875^{cd}	1.37^{bcd}
K_2	0.244^{a}	0.249^{bc}	1.82^{abc}	1.11^{a}	2.13^{a}
F_1	0.203^{bc}	0.257^{b}	1.49^{cd}	1^{abc}	1.32^{bcd}
F_2	0.193^{cd}	0.258^{b}	2.07^{a}	1.05^{ab}	1.72^{abc}
H_1	0.197^{bcd}	0.262^{ab}	1.53^{cd}	1.07^{ab}	1.32^{bcd}
H_2	0.222^{ab}	0.282^{a}	1.96^{ab}	0.875^{cd}	1.11^{cd}

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

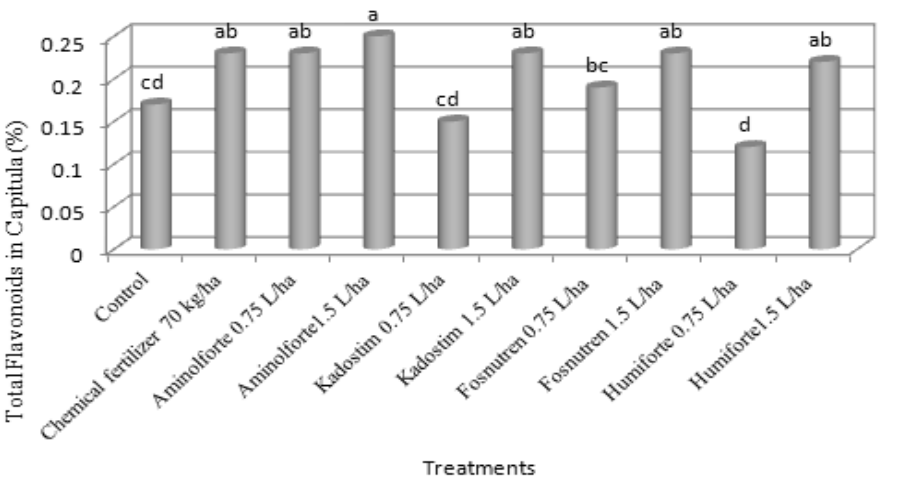


Figure 1- Mean comparison of bio-stimulators and fertilizers effect on the total flavonoids in capitula (%)

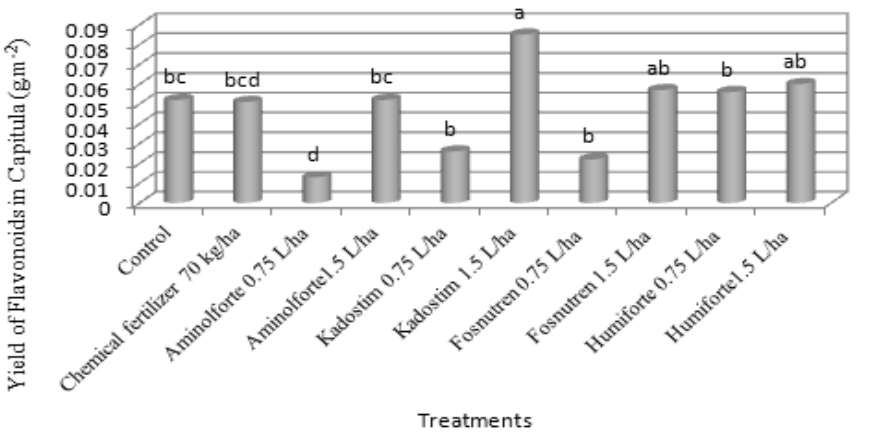


Figure 2- Mean comparison of bio-stimulators and fertilizers effect on the yield of flavonoids in capitula (g.m^{-2})

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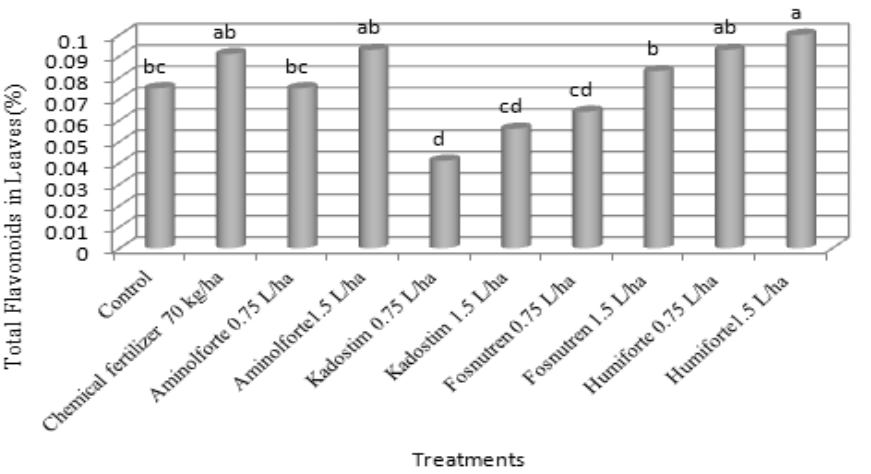


Figure 3 - Mean comparison of bio-stimulators and fertilizers effect on the total flavonoids in leaves (%)

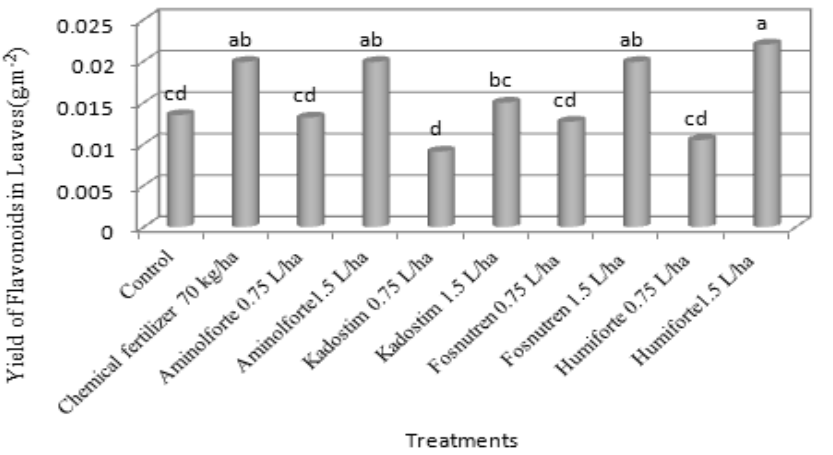


Figure 4 - Mean comparison of bio-stimulators and fertilizers effect on the yield of flavonoids in capitula ($g.m^{-2}$)

that promotes the growth of aerial parts of the plant. 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 cm to 46.30 cm and from 27.30 cm to 47.30 cm, respectively.

On parameters regarding to weight, the treatment of Humiforte 1.5 $L.ha^{-1}$ was the best treatment and significantly increased the yield of capitula dry weight and plant dry weight to 35.21 and 37.46% in comparison with control, respectively. According to Abdul Qados (2010) research on mung bean plants, application of amino acids had the most effect on dry weight of plants. Arginine, an amino acid (11.7%) in Humiforte, is one of the essential amino acids considered the main precursor of polyamines which produced by decarboxylation of arginine via arginine decarboxylase to form putrescine [24, 25]. Polyamines and their precursor arginine have been implicated as vital modulators in a variety of growth, physiological and

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developmental processes in higher plants [26]. Humiforte contains coenzymes which can join macro- and micro-nutrients with the amino acid chains and transform polysaccharides into stable humic and fulvic acid molecules. Such humic substances have been reported to have direct and indirect effects on plant growth and productivity: direct effects are on plant metabolism through root uptake, and indirect effects through alterations to the chemical and physical properties of the soil [27]. This regulatory effect of amino acids on growth could be explained by the notion that some amino acids (e.g. phenylalanine, ornithine) can affect plant growth and development through their influence on gibberellin biosynthesis [43]. In addition, Bidwell (1972) and Fowden (1973) reported that amino acids acting as the building blocks of proteins can serve in number of additional functions in regulation of metabolism, transport and storage nitrogen. Highest leaf area, relative water content, harvest index and capitula numbers were obtained in treatment of Humiforte 1.5 $L.ha^{-1}$. Slavik (2005) reported that Humiforte stimulated shoot growth of *Picea abies* L. Also these findings are in agreement with Shahriari and khayatnezhd (2011) results on wheat and Fisher and Wilson (1975) findings on *Sorghum bicolor*. Humiforte is a high-tech soluble liquid nutrient, with rapidly absorption via leaves or roots, and a high concentration of free amino acids and biologically active oligopeptides, especially recommended for shock treatments. In addition, application of bio-stimulators in environmental stress conditions has decreased stress effects and increased water retention efficiency, radicle growth and product yield that it can be due to existence of proline amino acid in these bio-stimulators. Humiforte causes more water absorption and retention in plant cells while exposed to environmental stress conditions [30, 31]. Amino acids with activation of effective hormones in capitol initiation and fruit set, improvement of pollen germination and increase in rate of flowering cause improve in pollination and capitula number [46].

In regard to phytochemical parameters, yield content of total flavonoids and also total carbohydrates of leaves and capitula increased with application of bio-stimulators compounds. These results are similar to Golzadeh et al., (2011) research findings on *Matricarai recutita*. Previously reported that the application of arginine significantly promoted the growth and increased the fresh and dry weights, certain endogenous plant growth regulators, chlorophylls a and b and carotenoids in bean and wheat [33, 34, 35]. Asparagines and glutamine connect to the two important metabolic cycles of the plant, the carbon and nitrogen cycles, and they have an influence on both sugars and proteins [40]. The positive effect of amino acids on growth showed that amino acids can serve as a source of carbon and energy. When carbohydrates become deficient in the plants, amino acids are determinate, releasing the ammonia and organic acid from which the amino acid was originally formed. The organic acids then enter the Krebs cycle, to be broken down to release energy through respiration [41]. Content of N, P and K increased with application of amino acid compounds. Highest amount of nitrogen was observed in treatment of Fosnutren 1.5 $L.ha^{-1}$ which can be due existence of phosphorous and nitrogen in this compound and their synergistic effect with each other

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[36]. Highest content of P and K resulted from Kadostim 1.5 $L.ha^{-1}$ which can be due to the content of N and P in this formulation and antagonistic or synergistic effect with other elements in leaves [22, 37]. According to these results it is found that these compounds have not only the effect of fertility and reproduction for plant cells but also they can enter the process of protein synthesis and cause arrangement in metabolism and catabolism of plant cells. Also these compounds act as an increasing agent in DNA transcription. On the other hand these compounds are recovered naturally in regard to their composition and application and in contrary to chemical fertilizers, they are compatible to environment. Overuse of chemical fertilizers and herbicides in recent years caused accumulation of heavy metals in soil and extinction of useful micro organisms. Bio-stimulators in order to their high compatibility and with decrease in need to chemical fertilizers, not only decrease bad effects of chemical fertilizers but also cause fertile soil with good structure and texture, absorption, reduction and refining of these compounds in soil [22].

Conclusion

According to these results, the bio-stimulators can promote morphological, agronomical and biochemical properties of *Calendula officinalis* L. These abilities were due to existence of macro-nutrients including NPK and their synergistic effect with amino acid compounds in bio-stimulators. Thus with consideration of these compounds role in plants biological activities, it is recommended to have more studies on absorption and transport of amino acid compounds separately in different parts of the plants.

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