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EFFECT OF PLANT GROWTH REGULATORS AND ORGANIC MANURE ON SOME MORPHOLOGICAL CHARACTERS, BIOMASS AND ESSENTIAL OIL YIELD OF DRAGONHEAD (*DRACOCEPHALUM MOLDAVICA*)

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Abstract

Nasiri Y., 2016: Effect of plant growth regulators and organic manure on some morphological characters, biomass and essential oil yield of dragonhead (*Dracocephalum moldavica*) [Augalų augimo reguliatorių ir organinių trąšų poveikis darželinės žiomenės (*Dracocephalum moldavica*) morfologiniams požymiams, biomasei ir eterinio aliejaus išeigai]. – Bot. Lith., 22(2): 123–132.

To study the effect of farmyard manure and plant growth regulators on yield and essential oils of dragonhead, an outdoor experiment was conducted in Maragheh, Iran. The experiment was carried out based on randomized complete block design with three replications. Three amounts of farmyard manure (FYM) including 0, 10 and 20 t/ha together with foliar growth regulators including 0, salicylic acid (SA), ascorbic acid (ASc) and SA + ASc were used. The results revealed that application of 10 and 20 t/ha farmyard manure significantly increased plant height and the number of lateral stems. However, application of 20 t/ha led to the increase of the number of the main and flowering stems, biomass and essential oil percentage of dragonhead compared to control. The effect of growth regulators on the number of lateral and flowering stems, and biomass was significant: foliar application of SA and ASc separately or in combination increased the number of lateral stems; however, the number of flowering stems and biomass of dragonhead increased only when both growth regulators were used in combination (SA + ASc). The results also indicated that the highest length of inflorescence (13.57 cm) and essential oil yield (41.83 kg/ha), by 31.4 and 126.3% increment, respectively, were observed under foliar application of 20 t/ha farmyard manure together with SA + ASc application compared to the control.

Keywords: ascorbic acid, *Dracocephalum moldavica*, essential oils, farmyard manure, salicylic acid, yield.

INTRODUCTION

Dragonhead (*Dracocephalum moldavica* L.; syn. Moldavian balm) is an annual herbaceous aromatic plant belonging to the Lamiaceae family. This crop is native to southern Siberia and central Asia and has naturalized in eastern and central Europe (Dastmalchi et al., 2007) and it has been cultivated for a long time as a medicinal plant, honey-plant, and for ornamental purposes (Kakasy et al., 2006). In Iran, dragonhead is known by the names of Badrshby, Badrshbo and Savory and mainly found in the north of the country, especially in the Albourz Mountains and West Azerbaijan (Miraldi et al., 2001; Mozafarian, 2003). As a medicinal plant is used to treat stomach and liver disorders, headaches, toothache (Rechinger, 1986), coronary heart disease, hypertension, etc. (Zeng et al., 2010). Due to the presence of geraniol and citral in essential oils of this plant, it also has tranquilizer properties (Matinez-Vazquez et al., 2012).

The soils of arid and semi-arid regions such as Iran are predominantly shallow and low in organic matter content, water holding capacity and plant nutrients. The organic matter and nutrients of these soils are declining due to intensive farming, non-application of manure and prevalence of erosion, so the fertility of such soils is consequently decreasing (GEORGIS & ALEMU, 1994; SHIRANI et al., 2002). It is necessary to enrich the soil organic matter by application of organic manure such as green manure and farmyard manure (FYM). Application of organic manure promotes the soil physical properties by improving the soil stability, decreasing the microspores, increasing the macrospores and hydraulic conductivity and improving the soil water-holding capacity (CHAUDHARY & NARWAL, 2005; BAYU et al., 2006; SINGH BRAR et al., 2015). Also, many reports have illustrated that organic manure application increases the yield and quality of products.

Application of FYM (15 t/ha) increased the biomass and essential oil yield (EOY) of *Cymbopogon martinii* by 10.7% and 0.3%, respectively, over control (RAJESWARA RAO, 2001). AL-FRAIHAT et al. (2011) reported that plant height, the number of branches, fresh biomass, essential oil (EO) percentage and EOY of marjoram (*Majorana hortensis* L.) increased due to organic manure application. Based on KHALID et al. (2006) findings, growth characters, seed yield, EO and EOY of marigold (*Calendula officinalis* L.) were increased by cattle manure application. IZHAR et al. (2015) also reported that FYM fertilizers had positive effect on plant height, the number of leaves, leaf area, herbal yield, EO and menthol contents of mint (*Mentha arvensis*). Application of 25 t/ha FYM + bio-fertilizers resulted in the increase of plant height, the number of branches and fresh weight of sweet basil (JAYSARY & ANUJA, 2010).

Many factors such as age, nutrition and temperature influence the accumulation and metabolism of secondary metabolites in plants. Many reports have proved that plant growth regulators (PRGs) such as gibberellic acid (GA3), kinetin, indole acetic acid (IAA), salicylic acid (SA), ascorbic acid (ASc) influence the growth, EO production and chemical composition of aromatic plants (PRINS et al., 2010; EL-LETHY et al., 2011; HESAMI et al., 2012; KHAN et al., 2015). Although since 1940 PGRs have been used to improve the agriculture production, however, there is little knowledge about the effects of such substances on secondary metabolite of medicinal plants (BASRA, 2000; POVH & ONO, 2006).

Salicylic acid (SA) or ortho hydroxyl benzoic acid, as an endogenous plant growth regulator, has been found to generate a wide range of metabolic and physiological responses in plants thereby affecting their growth and development. SA plays a key role in the regulation of plant growth, development and in the responses to environmental stresses (HAYAT et al., 2010; KALANTAR AHMADI et al., 2014). Moreover, SA is involved in seed germination, fruit yield, glycolysis, flowering in thermogenic plants (KLESSIG & MALAMY, 1994), photosynthetic, stomata conductance and transpiration (KHAN et al., 2015). SAHARKHIZ & GOUDARZI (2014) have reported that 150 mg·L^{-1} SA application significantly increased the EO content and some EO components (menthone and menthol) of peppermint (*Mentha piperita* L.) compared to control plants. Foliar application of sweet basil (*Ocimum basilicum* L.) by SA led to the increase of EO content in leaves and stems, improving of flowering parameters, thousand seeds weight and seed mucilage when compared to control treatment (MIRZAJANI et al., 2015).

Ascorbic acid (ASc) as an antioxidant protects plant cells. It has been reported that ASc is an abundant component of plants. It is involved in chloroplasts and all cell compartments including cell wall and cell division as well as differentiation. It has been proposed that ASc acts in photosynthesis as an enzyme cofactor (SMIRNOFF & WHEELER, 2000; BLOKHINA et al., 2003; ISHIKAWA et al., 2006; MAZHER et al., 2011). Stem and root length, fresh and dry weight, relative water content, extract percentage and extract yield of purslane (*Portulaca oleracea* L.) were increased by 20 mM ascorbate foliar application (NIKI ESFAHLAN et al., 2013). EL-LETHY et al. (2011) have reported that plant height, the number of branches, fresh and dry weight, chlorophyll content (a, b and total), EO, EOY, total phenols and flavonoids as well as antioxidant activity of geranium (*Pelargonium graveolens* L.) were increased by foliar spray of 50 to 150 mg·L^{-1} ASc. EO and EOY of German chamomile (*Matricaria chamomilla* L.) were increased significantly by 100 ppm ASc foliar application (RANJBAR et al., 2014). ASc foliar application resulted in a significant increment of total chlorophyll and water stress resistance of sunflower (EBRAHIMIAN & BYBORDI, 2012). BARAKAT et al. (2015) have reported that exogenous application of

ASc significantly increased canopy dry weight, leaf area, weight of 100 seeds, dry seed yield and total chlorophylls and carotenoids of common bean.

There are little findings about the effect of PGRs and organic material application on aromatic plant production. Therefore, the present study was set out to determine the effects of SA and ASc under FYM application conditions on yield, EO and some growth characteristics of *Dracocephalum moldavica*.

MATERIALS AND METHODS

Location of the experiment

The field experiment was conducted during the growing season of 2014 at the Research Farm of the University of Maragheh, Maragheh (46°16' E, 37°23' N), East Azarbaijan, Iran. The experimental location experiences cold and semi-arid climate (Peel et al., 2007). In the study period, the average rainfall was 11.4 mm, relative humidity – 39.4%, daily sunshine – 389.3 hours, temperature – 20.4°C and the number of days of rain – 5.4. The soil of the experimental field was of relatively shallow and sandy loam texture, pH 7.75, electrical conductivity (EC) – 0.51 dS/m, organic matter – 0.2%, total nitrogen – 0.02%, available phosphorus (P) – 4.78 ppm, available potassium (K) –360.0 ppm, iron – 3.24 ppm and zinc – 0.79 ppm. Chemical properties of farmyard manure included 0.68% of total nitrogen, 0.44% of available P and 1.08% of available K, pH 6.83.

Plant material

Dracocephalum moldavica seeds were obtained from the Pakan Bazr Esfahan Co., Esfahan, Iran. Salicylic acid (SA) and ascorbic acid (ASc) were manufactured by Merck, Germany. The factorial experiment was laid out in randomized complete block design (RCBD) with three replications. The treatments included three rates of FYM application (zero, 10 and 20 t/ha) as first factor and four levels of growth regulator application (0, SA, ASc and SA +ASc). SA and ASc were sprayed on plants at 1 millimolar (mM) concentration and the control plants were sprayed with distilled water.

Planting and treatment operation

Each experimental plot included five rows, the row spacing was 50 cm and plant-to-plant spacing within a row was 10 cm. FYM treatments were applied to the plots one month before sowing. Seeds were directly sown on the rows by hand on 21 April 2014. SA and ASc were applied on plants two times during vegetative (stem elongation and beginning of flowering) stages. Plants were thoroughly sprayed with considered solutions until the excess solution was dripping.

All field practices were carried out on time whenever they were needed. No disturbing pests or disease were observed on dragonhead plants during the experiment. Weed control was done manually throughout the all vegetative stages. At the flowering stage, seven plants were randomly harvested from each plot to assess the morphological characters such as plant height, main stem diameter, inflorescence length, the number of lateral and flowering stems. Biomass was obtained by cutting plants from ground level (1 m^2) at 50% flowering stage from each plot. Then plants were dried by natural air flow in a shade place and finally weighted using digital weighing scales. In each plot, at flowering stage, plants were harvested in 1 m^2 and were dried for essential oil analysis.

Extraction of essential oils

The essential oils (EO) of *D. moldavica* shoot (50 g) were extracted by hydro distillation using Clevenger-type apparatus for 4 h (Clevenger, 1928). The dehydration of the collected EO was performed by anhydrous sodium sulphate (Fayed, 2015). The extracted EO were weighted and converted to a percentage. Essential oil yield (EOY) was calculated multiplying EO by plant dry weight (Gharib et al., 2008).

Statistical analysis

The data were subjected to the analysis of variance by MSTAT-C software, the means were compared using the Least Significant Difference (LSD) test and graphs were plotted using Excel software.

RESULTS AND DISCUSSION

The results of the analysis of variance (Table 1) revealed that application of FYM had significant effect on the height of *D. moldavica* plants, thus, application of 10 and 20 t/ha FYM increased the plant height by 7.94% on average compared to control plants (Table 2). These results are in accordance with

Table 1. Analysis of variance for morphological characters, biomass and essential oils of dragonhead as affected by farmyard manure and plant growth regulators

Experiment	Degree of free-dom	Plant height	Main stem diameter	Length of inflorescence	Number of lateral stems	Number of flowering stems	Biomass	Essential oils	Essential oil yield
Replication	2	**1.30** 0.29	**0.92** 0.40	**0.71** 0.504	**0.20** 0.821	**0.26** 0.78	**5.84** 0.009	**11.09** 0.0005	**2.73** 0.087
Farmyard manure (FYM)	2	**4.58** 0.02	**4.28** 0.02	**11.27** 0.002	**3.93** 0.034	**8.09** 0.002	**8.44** 0.001	**7.65** 0.003	**16.71** 0.001
Plant Growth Regulators (GRs)	3	**0.61** 0.62	**0.84** 0.46	**2.21** 0.11	**5.14** 0.007	**3.05** 0.04	**3.22** 0.042	**1.98** 0.14	**3.52** 0.032
FYM × GRs	6	**0.44** 0.84	**0.31** 0.94	**2.67** 0.042	**1.10** 0.391	**0.92** 0.533	**0.241** 0.958	**2.39** 0.06	**2.57** 0.048
Mean Square of Error	22	13.1	1.07	1.64	1.73	1.55	479070	0.003	17.8

Data presented inside the table are F (bold) and *p*-value (not bold), respectively.

Table 2. Effect of farmyard manure on morphological characters, biomass and essential oils of dragonhead

FYM application	Plant height (cm)	Main stem diam-eter (mm)	Number of lateral stems	Number of flow-ering stems	Biomass (kg/ha)	Essential oils (%)
0 t/ha	47.44b	8.70b	11.57b	8.79b	5197.7b	0.43b
10 t/ha	51.74a	9.49ab	12.92a	10.02ab	5947.2ab	0.47ab
20 t/ha	50.68a	9.93a	12.82a	10.82a	6340.9a	0.52a

Means in the same column with different letters differ significantly at 0.05 probability levels according to Least significant difference (LSD).

those obtained by Zariri et al. (2014), who found that application of FYM increased the height of peppermint plants. According to the mentioned authors, the positive effect of organic manure on plant height has resulted from providing balanced plant nutrition and imposed a direct effect on the number of nods and the length of internodes that finally lead to the increase of plant height. Our results also revealed that there was no significant effect of GRs on plant height of dragonhead.

The obtained results showed that there were significant differences between FYM application treatments for the main stem diameter at 5% level (Table 1). The highest stem diameter (9.93 mm) was measured after application of 20 t/ha FYM, and the lowest stem diameter (8.7 mm) was registered for control plants (Table 3). This increase may be due to the increase of the number and diameter of cells because of the availability of water and enough nutrients for plants (Agamy et al., 2012). There was no significant difference between 10 t/ha FYM application and control for recent parameter. Enhancing effect of organic fertilizers on stem diameter of sunflower and corn has also been reported in other studies (Ahmad & Jabeen, 2009; Solomon Wisdom et al., 2012). No difference was observed between the stem diameters of the plants treated with salicylic acid or ascorbic acid, and control plants.

The length of inflorescences of *D. moldavica* was significantly influenced by FYM and FYM × GRs application (Table 1). In Fig. 1, it is shown that the

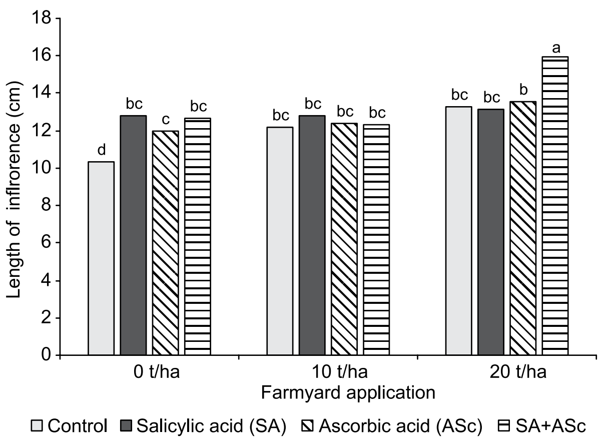

Fig. 1. Effect of farmyard manure and growth regulators on the length of inflorescences of dragonhead (Letters on the charts: means followed by the same letter are not significantly different according to LSD)

maximum length of inflorescence was recorded for the plants grown at 20 t/ha FYM application conditions and treated with foliar application of SA + ASc. Thus, this treatment increased the length of inflorescences by about 33.1% compared to control (no FYM and no GRs application). The results also showed that other treatments led to a significant increase in the mentioned parameter compared to the control. Therefore, as it is shown in Fig. 1, such results indicate that SA and ASc have positive effect on the increase of length of inflorescences.

The results also showed that FYM and GRs considerably affected the number of both lateral and flowering stems (Table 1). The plants grown at 10 and 20 t/ha FYM application produced more lateral stems than control plants (Table 2). The largest number of lateral stems was recorded for SA + ASc application treatment (Table 3). Also, the largest number of flowering stems was recorded for the plants grown at 20 t/ha farmyard manure application (Table 2). In addition, the plants sprayed with GRs (SA, ASc or SA + ASc) had more lateral stems, by 17.1% on average, compared to control (Table 3). Foliar application of SA and ASc increased the number of flowering stems when applied together. In that case, SA + ASc increased this trait by 20% as compared to control plants (Table 3).

Table 3. Effect of plant growth regulators on morphological characters and biomass of dragonhead

GRs application	Number of lateral stems	Number of flowering stems	Biomass (kg/ha)
Control	11.02b	9.00b	5262.1b
SA	12.87a	9.67ab	5879.7ab
ASc	12.53a	10.03ab	5916.7ab
SA + ASc	13.31a	10.80a	6255.8a

SA: Salicylic acid, ASc: Ascorbic acid. Means in the same column with different letters differ significantly at 0.05 probability levels according to Least Significant Difference (LSD).

Organic fertilizers, especially FYM, could improve plant rhizosphere through increasing the soil organic matter, enhancing the nutrients availability as well as improving the soil physical properties (Singh Brar et al., 2015). Therefore, in an appropriate environment, nutrients and water were well absorbed by roots and finally led to better growth of shoot organs. In this respect, it has been reported that the number of branches of marjoram increased due to organic manure application (Al-Fraihat et al., 2011). Balemi (2012) has reported that application of 20 and 30 t/ ha FYM significantly increased the stem number of potato compared to the absence of FYM application.

On the other hand, there are many reports suggesting that SA and ASc in plants act as endogenous growth regulators and stimulate both physiological processes and growth of plants (Barth et al., 2006; Jabbarzadeh et al., 2009; Bayat et al., 2012). In this regard, Gharib (2006) has reported that application of SA increased photosynthetic activity in basil and marjoram, which enhanced the plant height, the number of branches and some other growth parameters.

Nahed et al. (2009) found that spraying gladiolus plants with ASc at different concentrations (50, 100 and 200 ppm) stimulated growth and flowering parameters of plants. The results are also in agreement with those obtained by Salehi Sardoei et al. (2014), who have reported that the total number of flowers per *Gazania rigens* plant was strongly increased by application of ASc.

The results showed that both farmyard manure and growth regulators (SA and ASc) considerably affected the biomass (dry weight) of *D. moldavica* plants (Table 1). The plants grown under high amount of farmyard manure (20 t/ha) had maximum biomass, 6340.9 kg/ha or the increase by 22%, compared to the control (Table 2). This effect of the farmyard manure on biomass production could be a result of the nutrient availability and increased their uptake by the plants. In line with these findings, it has been reported that manure fertilizer application significantly increased the biomass of sage (Kaplan et al., 2009) and rosemary (Valiki & Ghanbari, 2015) compared to control plants. This increment linked to the improvement of the physical and biological conditions of the soil and creating of more favourable environment for root growth and nutrient availability. The results also revealed that biomass of dragonhead significantly increased when subjected to SA + ASc foliar application compared to control plants (Table 3). This treatment increased biomass by 18.9% compared to non-sprayed plants. Similarly, Elwan & El-Hamahmy (2009) and Hesami et al. (2012) have reported that SA application improved the growth and biological yield of pepper (*Capsicum annuum*) and coriander

(*Coriandrum sativum*), respectively.

Also, it has been reported that dry weight of basil (KHALIL et al., 2010) and common bean (BARAKAT et al., 2015) were increased by ASc foliar application compared to control plants. It seems that the increase in the fresh and dry weight of plants whenever treated with SA and ASc might be attributed to an increase in the number of branches and leaves as well as leaf area, leading to increased photosynthetic activity (GHARIB, 2006).

The data presented in Table 1 show that the content of the essential oils in dragonhead plants was significantly influenced by FYM treatments. In this respect, the highest essential oil content (0.52%) was recorded in plants grown in the plots, where 20 t/ha FYM application was performed (Table 2). Meanwhile, the lowest essential oil content was obtained in control plants (0.43%). This is in agreement with the findings of AL-FRAIHAT et al. (2011) on marjoram, KHALID et al. (2006) on marigold and IZHAR et al. (2015) on mint, who opined that the use of FYM had a promoting influence on essential oil content of the mentioned plants. Such results may be due to the influence of nutrients present in the farmyard manure in accelerating the metabolism reactions as well as stimulating enzymes (MALIK et al., 2013).

The effect of different amounts of FYM and GRs (SA and ASc) on essential oil yield of dragonhead was significant at 5% level (Table 1). The results presented in Fig. 2 show that the highest essential oil yield was obtained from plants treated with 20 t/ ha FYM and SA + ASc (41.8 kg/ha) and the lowest essential oil yield was obtained from control plants (18.5 kg/ha). It should be noted that other treatments also increased the EOY compared to control, but with a lower increase. The increase in essential oil yield might be due to either the increase in biomass or the increase in essential oil percentage under application of farmyard manure and GRs. Similar increases in EOY by farmyard manure application have been reported on marjoram (AL-FRAIHAT et al., 2011) and mint (UPADHYAY et al., 2012). In this regard, SAHARKHIZ & GOUDARZI (2014) have reported that EOY of *Mentha piperita* significantly increased due to SA application compared to control plants.

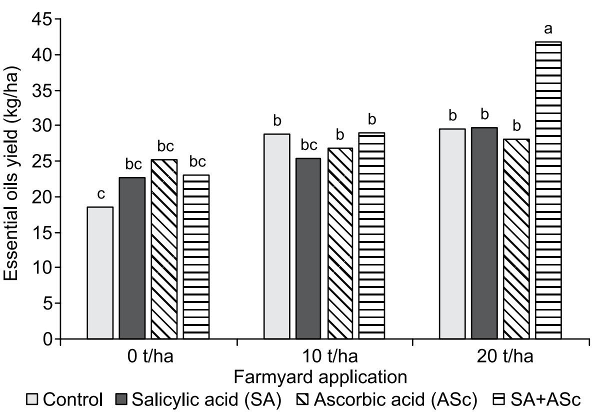

Fig. 2. Effect of farmyard manure and growth regulators on essential oil yield of dragonhead (Letters on the charts: means followed by the same letter are not significantly different according to LSD)

Salicylic acid is known as a factor that influences several physiological and biochemical processes in plants and may play vital role in the regulation of their growth and productivity (HAYAT et al., 2010). The increment of essential oil yield in essential oil bearing plants by SA application might be due to the increase in vegetative growth, nutrient uptake or density changes in oil glands in leaves, carbohydrate content, beneficial effect of SA on metabolism and enzyme activities and monoterpene biosynthesis (GHARIB, 2006; DONG et al., 2010; NADEEM et al., 2012).

Furthermore, in regarding to ascorbic acid, it has been reported that the essential oil yield of rosemary (YOUSSEF & TALAAT, 2003) and geranium (EL-LETHY et al., 2011) increased when subjected to ASc. This effect might be attributed to enhanced capacity of meristematic cells to synthesis of active substrate needed for biosynthesis of essential oils (REDA et al., 2005). FARAHAT et al. (2007) also reported that foliar application of ASc promoted vegetative growth and essential oil yield of *Cupressus sempervirens*. Ascorbic acid is known as a growth regulator that influences various bioprocesses and acts as a co-enzyme factor in reactions in which proteins and carbohydrates are metabolized and involved in photosynthesis and respiration processes (ROBINSON, 1973).

CONCLUSIONS

Based on our results, it can be concluded that application of farmyard manure and growth regulators (salicylic acid and ascorbic acid) are not only suitable ways for improving the growth and biomass of dragonhead plants, but also are the ways for increasing the essential oil productivity per unit area in this plant. The results also indicated that application of salicylic acid + ascorbic acid had better effect on essential oil yield of dragonhead in combination with high amount of farmyard manure.

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AUGALŲ AUGIMO REGULIATORIŲ IR ORGANINIŲ TRĄŠŲ POVEIKIS DARŽELINĖS ŽIOMENĖS (*DRACOCEPHALUM MOLDAVICA)* MORFOLOGINIAMS POŽYMIAMS, BIOMASEI IR ETERINIO ALIEJAUS IŠEIGAI

Yousef NASIRI

Santrauka

Tirta mėšlo ir augimo reguliatorių įtaka darželinės žiomenės žaliavos derliaus rodikliams ir eterinių aliejų išeigai. Lauko eksperimentas atliktas trimis pakartojimais Maragheh, Iranas. Eksperimento schemą sudarė trys tręšimo variantai: 0 (kontrolė), 10 ir 20 t / ha mėšlo, kartu naudojant keturis augimo reguliatorių variantus – 0 (kontrolė), salicilo rūgštis, askorbo rūgštis ir salicilo + askorbo rūgštis. Gauti rezultatai parodė, kad augalų tręšimas mėšlu gerokai padidino augalų aukštį ir šoninių ūglių skaičių, tačiau tik naudojant didesnę tręšimo normą (20 t / ha) reikšmingai padidėjo pagrindinių ir žydinčių stiebų skaičius, augalų biomasė ir eterinių aliejų išeiga, lyginant su kontrole. Augimo reguliatoriai reikšmingai teigiamai įtakojo šoninių ir žydinčių stiebų skaičių bei augalų biomasę, tačiau jų poveikis buvo skirtingas. Askorbo ir salicilo rūgštis visais atvejais padidino šoninių ūglių skaičių, tačiau žydinčių žiomenių stiebų skaičius ir biomasė reikšmingai padidėjo tik naudojant abu augimo reguliatorius. Rezultatai parodė, kad ilgiausiais žiedynais (13,57 cm) ir didžiausia eterinio aliejaus išeiga (41,83 kg / ha) pasižymėjo augalai, kurie buvo tręšti didžiausia mėšlo norma, kartu naudojant abu augimo reguliatorius: 20 t / ha mėšlo + salicilo + askorbo rūgštis. Žiedynų ilgis ir eterinio aliejaus kiekis atitinkamai padidėjo 31,4 ir 126,3 %, lyginant su kontrole.