

How Different Concentrations of Humic acid, Zinc, Nitrogen and Boron Influence Quantitative and Qualitative Yield of German Chamomile (*Matricaria chamomilla* L.)?

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Received: June 20, 2017

Accepted: August 27, 2017

ABSTRACT

The aim of this study was to evaluate the effects of different concentrations of humic acid, zinc, nitrogen, and boron on quantitative and qualitative yield of German chamomile (*Matricaria recutita* L.) on research site of Darou Darman Salefchegan Company in Shahriar in Randomized Complete Block Design (RCBD) with four replicates in 2011. For this aim, treatments involved humic acid in tree levels of 3, 5, 10; zinc in tree levels of 2, 3, 5; nitrogen in tree levels of 2, 5, 10 and boron in 3, 5 10 gr/Lit, zinc in 2,3,5 gr/Lit, nitrogen in 2, 5, 10 gr/Lit, boron in 2, 3,5 gr/Lit as well as mixture of humic acid, zinc, nitrogen and boron in two concentrations of 2 and 5 gr/Lit were applied. some of vegetative, reproductive and functional traits of German chamomile including main stem height, number of leaf in plant, number of flower in m², flower fresh and dry weight in hectare, weight of 1000 seeds, diameter of receptacle, essential oils per kg F.W., weight of flowering stem per hectare, and chamazulene in milliliter essential oil were measured. The results indicated that effect of all the treatments on the foregoing traits was significant in comparison with the control (in probability level of one percent). Also, the highest fresh weight of flower was related to 10 gr/lit humic acid, the highest weight of flowering stem was related to 5 gr/lit humic acid and the highest dry weight, flower receptacle diameter, essence, essential oils, and chamazulene was related to treatment 5 gr/lit mixture.

KEYWORDS: Chamazulene, Essential oils, Medicinal plant, plant nutrition, Secondary metabolites

1.INTRODUCTION

Scientific-based cultivation and utilization of standard medicinal plants is of high paramount. This is attributed to increasing trend of medicinal plants consumption to treat and cure various diseases thanks to their low side effects in rather to chemical drugs, unique climatic, and geographical conditions of Iran for growing different species and finally degradation caused by excessive utilization of medicinal plants in rangeland species. German chamomile (*Matricaria recutita* L.) belongs to Asteraceae family that has a branched, erect and smooth stem. The long and narrow leaves are bipinnate or tripinnate. Chamomile is one of the most widely used medicinal plants known in the world [9]. It is also one of the few plants that has industrial applications [19]. Chamomile is a plant with great medicinal properties. It has long history in traditional medicine and used as an ergogenic substance, insecticide, and disinfectant. It has many health benefits such as painkilling and indigestion reliever, pyretic, treatment of arthritis and rheumatism, treatment of asthma, anti-spasmodic effects especially blood vessel walls in the brain and smooth muscle relaxant effects, preventing the aggregation of platelets in the blood, and preventing headaches especially chronic migraine headaches [12]. Chamomile flowers as a raw material containing nearly 120 chemical compounds such as terpenoids, flavonoids and mucilage [15]. The main ingredients of the essential oil of chamomile are Chamazulene, α -bisabolol, Farnesene [15]. Other metabolites in flowers of chamomile are Flavonoids belonged to methoxy flavonoids and methoxy flavanols and the most important of which are Apigenin and Luteolin. Other substances of chamomile are coumarin derivatives (Umbelliferone, Herniarin) and mucilage [6]. medicinal plants are rich in secondary metabolites as the main active ingredients of drugs. these metabolites are synthesized primarily by genetic processes, but also they are significantly affected by environmental factors, so that such factors lead to some changes in the growth of medicinal plants and also in concentration and quality of their ingredients such as alkaloids, glycosides, steroids, and essential oils [21]. A medicinal plant product is cost-effective when its secondary metabolites reach an optimal level. proper application of fertilizers serves as a major factor in the successful cultivation of medicinal

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plants[3]. The maximum yield can be reached from appropriate use of chemical fertilizers and soil fertility. Nutrients play a major role in yield, quality and quantity of secondary metabolites in medicinal plants[10, 15]. Today, foliar application of nutrient as supplements of rhizosphere application is an effective method in the application of soil trace micronutrients and macronutrients, amino acids, humic acids and fulvic acid, plant growth regulators, extracts of seaweed, and carbohydrates. Organic and biological compounds are applied for different purposes such as increasing yields, alleviation of effects of harmful chemicals, and act as hormones for plant growth[8]. Nitrogen is the main constituent of plant protein and is essential for aboveground parts of growth [11]. Khan, Samiullah [7] pointed out that application of nitrogen fertilizer as a foliar spray on green parts of the plant compared to the direct application in the soil, increased essential oil yields in fennel and also types of essential oil was affected by fertilization method. Furthermore, humic substances (humic acid and fulvic acid) accounts for 65 to 70 percent of soil organic matter[18]. Therefore, this study aimed to study the effect of different fertilizers on growth, vegetative organs yield and essential oil of chamomile.

2. MATERIALS AND METHODS

This experiment was conducted to evaluate effects of nutrients humic acids, zinc, nitrogen, and boron on phenological traits, yield, and essential oil of German chamomile. The study area was on the research site from of Darou Darman Salefchegan Company in Shahriar in Randomized Complete Block Design (RCBD) with four replicates in 2011. After plowing and land preparation, commercial German chamomile seeds were planted in plots in 2*4 m dimension and in 30 cm row spacing and 10 cm seeds in May. The distance between each block was 1.5 meters and plots space was 0.5 meters. After planting operations, each plot was fully covered approximately by 0.5 cm sand. To stick the seeds into the soil, the roller was used in plots and then plots were irrigated. Irrigation was performed once every 5 days until emergency and after plant establishment, the next irrigation was performed within once per 10 days. During the planting, no pest and disease were not found on the farm. The applied treatments were humic acid in tree levels of 3, 5, 10; zinc in tree levels of 2, 3, 5; nitrogen in tree levels of 2, 5, 10 and boron in 3, 5, 10 gr/Lit, zinc in 2, 3, 5 gr/Lit, nitrogen in 2, 5, 10 gr/Lit, boron in 2, 3, 5 gr/Lit as well as mixture of humic acid, zinc, nitrogen and boron in two concentrations of 2 and 5 gr/Lit. All fertilizers were applied by foliar spraying method on the branches and leaves of chamomile. Spraying since the five-leaf stage to flowering stage was performed twice, at intervals of one month. After the floral stage, flowers were harvested. Two side rows and 0.5 m from the beginning and end of each row were considered as marginal. Harvesting was done on 6 stages so that 3 harvests was considered a major harvesting. Then harvested flowers were placed in a paper packet and were dried for 2 days in the dark and in the open air. After drying they were sieved to obtain uniform particle size. To extract German chamomile essence, Clevenger apparatus using distillation with steam distillation in laboratory medicine treatment was used so that every 50 grams of crushed dried flowers per treatment was poured in flask Distillers Clevenger and 1,000 ml of distilled water was added. The flask containing herbal powder and distilled water, and was connected to Clevenger apparatus and placed on a heater. Heating operation at a temperature of 70-60 ° C was continued for 3 hours and then the essential oil extracted from plant flowers were placed in dark glass containers and were kept in the refrigerator and spectrophotometer was used for determining chamazulene content [4].

Dichloromethane solution was used to calibrate the device for measuring chamazulene. After calibration, oil was moved to a 10 ml volumetric flask and was brought to volume 10 ml by dichloromethane and was transferred to the apparatus. Then wavelength was set at 603 nm (chamazulene absorption wavelength) and was measured by a 1 cm cuvette. Chamazulene molar absorption constant and a molecular weight were considered about 420 and 184.3, respectively.

Chamazulene percentage was determined using the following equation:

$$C = \frac{50 \times 10 \times E \times 184.3}{\varepsilon \times 1000}$$

C = percentage of chamazulene in essential oil

50 = flower dry weight in grams of essential oil

E = number read in spectrophotometer

184.3 = chamazulene molecular weight

ε = molar absorption constant chamazulene which is equal to 420 nm.

Other traits such as plant height and diameter of the main stem were calculated by caliper once the flowers were fully open and one thousand seed weight, fresh weight and dry weight (after drying petals at 60 ° C in an oven) were measured by the digital scale in precision 0.01 g. The number of leaves and flowers were determined by counting. The volume of oil extraction after extraction was determined by measuring cylinder. Data were entered in Excel and then were analyzed by SAS program and means were compared using LSD test.

3.RESULTS

Results of analysis of variance for experimental data are presented in table 1. Based on this table, the consumption of nutrients influenced all traits at a probability significant level of one percent.

Mean comparison showed that the highest plant height, leaf number, flower number, flowers fresh weight, flower dry weight, seed weight, diameter of flower receptacle, essential oils, and chamazulene were found in mixture treatment (humic acid, zinc, nitrogen, boron) at the level of 5 grams per liter and the maximum weight of the stem was found in the humic acid fertilizer treatment at 5 grams per liter. the lowest number of leaves in boron treatment was observed at the level of 2 grams per liter and in other traits the lowest was reached in control (without fertilizer) (Table 2).

3.1 PLANT HEIGHT

Our results showed that the mixture fertilizer treatments (humic acid, zinc, nitrogen, boron) at 5 grams per liter accounted for the highest increase in height followed by 2 grams per liter of fertilizer treatment. In fertilizer treatment, 10 grams per liter of humic acid had an increasing trend in comparison with the other levels. Also, at different levels of zinc and boron fertilizer treatments, the lowest level (2 grams per liter) had the highest increasing trend compared to other levels which indicated that this concentration is more cost-effective than other levels. In nitrogen fertilizer treatment, the concentration of 2 grams per liter was much more cost-effective than others and control treatment had the lowest height than other treatments (Table 2).

3.2 LEAF NUMBER PER PLANT

The greatest number of leaves per plant in mixed fertilizers treatments (humic acid, zinc, nitrogen, boron) was achieved at 5 grams per liter, followed by 2 grams per liter of mixed fertilizer treatments. In humic acid fertilizer treatment, the concentration of 10 grams per liter showed more leaf number per plant than the other two concentrations. At the same time in different levels of zinc and boron fertilizer treatment, 5 grams per liter showed the largest leaf number per plant than other levels. At 10 and 5 grams per liter of nitrogen fertilizer treatment, slight difference in the number of leaves per plants was observed which suggesting that 5 grams per liter are cost-effective than other levels (Table 2).

3.3 FLOWERS NUMBER M²

Mixed treatment had a massive impact on the number of flowers per m² which indicated that the concentration of 5 grams per liter was the best with 172.5 flowers per m² and control with about 147.5 m² had the least number of flowers. In zinc fertilizer treatment, 5 grams per liter was much better than others. As for nitrogen fertilizer treatment, the level of 2 grams per liter nitrogen was better than the other levels. Also, There was no significant difference between the different levels of boron in the fertilizer treatment (Table 2).

3.4 FLOWERS FRESH AND DRY WEIGHT Kg/ ha

The statistical analysis showed that humic acid fertilizer treatment at the level of 10 grams per liter with 2163.25 kg per hectare and control treatment with 1985.25 kg fresh weight accounted for the highest and lowest flowers fresh weight dry Kg/ ha, respectively. In zinc fertilizer treatment, 5 grams per liter was better than other levels and in nitrogen fertilizer treatment, 10 grams per liter was better than other levels. Among different levels of boron in 5 grams per liter accounted for the highest flower fresh weight. As for dry weight, the statistical analysis showed that mixed fertilizer treatments at 5 grams per liter with 425.25 kg per hectare and control with 398.5 kg had the highest and lowest dry weight, respectively (Table 2).

3.5 THOUSAND KERNEL WEIGHT

The effect of the mixed fertilizer treatments on thousand kernel weight showed that the 5 grams per liter of this treatment with 0.325 g and 2 grams per liter with 0.307 were the best and cost effective treatment. Boron treatment in 5 gram per liter with 0.282 g was followed by mixed and control treatment had the lowest thousand kernel weight with 0.207 (Table 2).

3.6 FLOWER RECEPTACLE DIAMETER

The effect of mixed treatment on flower receptacle diameter indicates that the level of 5 grams per liter with 2.535 cm was the best treatment and also 2 grams per liter with 2.435 cm and control with 2.090 cm diameter receptacle showed the lowest diameter (Table 2).

3.7 ESSENTIAL OILS

Our results showed that the mixed treatment at the level of 5 grams per liter with 3.44 ml per kg of essential oil accounted for the highest yield followed by 2 grams per liter with 3.73 ml. Also, the control treatment with the production of 2.32 ml per kg had the least amount of essential oils (Figure 1), (Table 2).

3.8 ESSENCE CONCENTRATION

Mixed treatment at the level of five grams per liter with 4.187 ml of the extract per kg accounted for the highest yield followed by two grams per liter with 10 grams per liter of humic acid fertilizer treatment 3.927 and 3.910 respectively. Control treatment with 2.930 ml per kg fresh weight control treatment had the least amount of extracts (Figure 1), (Table 2).

3.9 CHAMAZULENE PER MILLILITER ESSENTIAL OIL

The control and mixed treatment accounted for the lowest and highest Chamazulene per milliliter essential oil, respectively followed 10 grams per liter of humic acid levels and mixed treatment at the level of 2 grams per liter (Figure 1), (Table 2).

3.10 FLOWERING STEM WEIGHT (kg/ha)

As for flowering stem weight, mean comparison showed that humic acid treatments at 5 grams per liter with 622.25 kg per hectare followed by 10 grams per liter and humic acid and mixed fertilizer treatments at 5 grams per liter and control with 497.5 kg per hectare accounted for the highest and lowest flowering stem weight (kg/ha), respectively among other treatments (Table 2).

Figure 1. the Effect of different

treatments to the Extracts (A),

Essential Oil (B), and Chamazulene

(C)

1 = control

2 = humic acid (3 g/liter)

3 = humic acid (5 g/liter)

4 = humic acid (10 g/liter)

5 = Zinc (2 g/liter)

6 = Zinc (3 g/liter)

7 = Zinc (5 g/liter)

8 = N (2 g/liter)

9 = N (5 g/liter)

10 = N (10 g/liter)

11 = boron (2 g/liter)

12 = boron (3 g/liter)

13 = boron (5 g/liter)

14 = Mixed treatment (2 g/liter)

15 = Mixed treatment (5 g/liter)

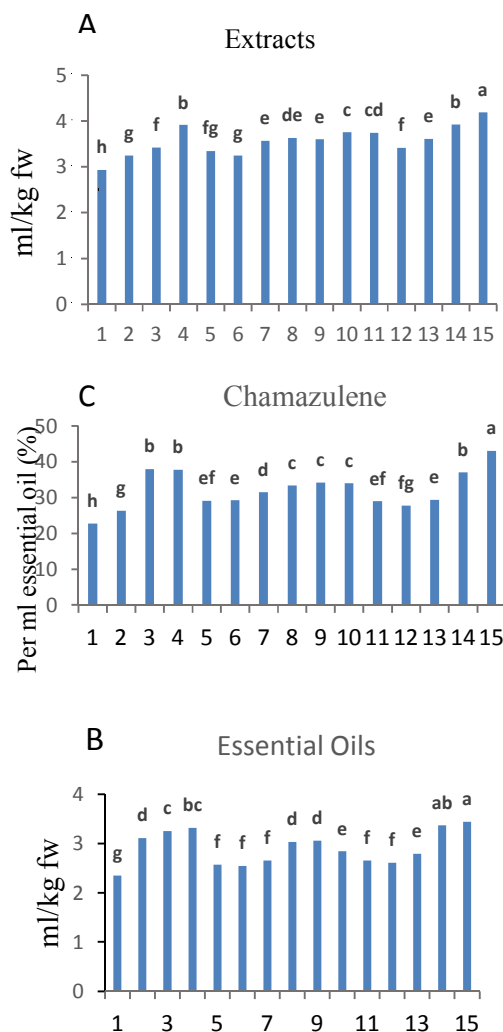


Table 1. Analysis of variance (MS) on studied traits in *Matricaria chamomilla* L.

| SV | df | Plant height (cm) | Leaf number (per plant) | Flower number (m ²) | Flower fresh weight (kg/ha) | Flower dry weight (kg/ha) | Thousand Kernel Weight (g) | Flower receptacle diameter (cm) | Essential oils (ml/kg F.W.) | Extract (ml/kg F.W.) | Chamazulene (per ml essential oil) % | Flowering stem weight (kg/ha) |
|-------------|----|--------------------|-------------------------|---------------------------------|-----------------------------|---------------------------|----------------------------|---------------------------------|-----------------------------|----------------------|--------------------------------------|-------------------------------|
| replication | 3 | 2/50 ^{ns} | 3/26 ^{ns} | 5/84 ^{ns} | 17/65 ^{ns} | 48/28 ^{ns} | 0/0002 ^{ns} | 0/006 ^{ns} | 0/012 ^{ns} | 0/004 ^{ns} | 0.78 ^{ns} | 145/75 ^{ns} |
| treatment | 14 | 99/28** | 38/71** | 205/7** | 12583/84** | 991/42** | 0/004** | 0/05** | 0/47** | 0/41** | 112.79** | 5122/94** |
| error | 42 | 3/97 | 2/19 | 7/90 | 81/69 | 28/04 | 0/0002 | 0/004 | 0/007 | 0/007 | 1.07 | 38/11 |

Table 2. the response of *Matricaria chamomilla* L. to the different treatment.

| | Plant height (cm) | Flower number (per plant) | Leaf number (m ²) | Flower fresh weight (kg/ha) | Flower dry weight (kg/ha) | Thousand Kernel Weight (g) | Flower receptacle diameter (cm) | Essential oils (ml/kg f.w.) | extract | Chamazulene (per ml essential oil%) | Flowering stem weight (kg/ha) |
|--------|----------------------|---------------------------|-------------------------------|-----------------------------|---------------------------|----------------------------|---------------------------------|-----------------------------|--------------------|-------------------------------------|-------------------------------|
| 1 | 51/5 ^j | 25 ^{ij} | 147/5 ⁱ | 1985/25 ^j | 398/5 ^k | 0/207 ^f | 2/09 ^f | 2/35 ^g | 2/93 ^h | 22.8 ^h | 497/50 ⁱ |
| 2 | 54 ^{hij} | 29/5 ^{def} | 160/25 ^{cd} | 2097/5 ^d | 430/25 ^{de} | 0/225 ^{ef} | 2/19 ^{de} | 3/11 ^d | 3/24 ^g | 26.3 ^g | 581/75 ^c |
| 3 | 59/25 ^{de} | 31/5 ^{bcd} | 162/75 ^c | 2148/75 ^b | 437 ^{cd} | 0/252 ^{cd} | 2/13 ^{ef} | 3/25 ^c | 3/42 ^f | 38.01 ^b | 622/25 ^a |
| 4 | 62/75 ^{bc} | 33 ^{ab} | 163/25 ^{bc} | 2163/25 ^a | 440/5 ^{bc} | 0/260 ^c | 2/28 ^{cd} | 3/32 ^{bc} | 3/91 ^b | 37.8 ^b | 616/5 ^{ab} |
| 5 | 60 ^{cd} | 26 ^{hij} | 149/5 ^{ghi} | 2043/25 ^f | 413 ^{hi} | 0/232 ^{de} | 2/15 ^{ef} | 2/57 ^f | 3/34 ^{fg} | 29.01 ^{ef} | 556/75 ^{de} |
| 6 | 57 ^{efg} | 26 ^{hij} | 153/5 ^{efg} | 2040/5 ^{fg} | 408/5 ^{ij} | 0/217 ^{ef} | 2/30 ^c | 2/55 ^f | 3/24 ^g | 29.3 ^c | 554/75 ^{de} |
| 7 | 58/5 ^{def} | 29 ^{efg} | 157/5 ^{de} | 2061 ^e | 423 ^{ef} | 0/247 ^{cd} | 2/25 ^{cd} | 2/66 ^f | 3/56 ^e | 31.5 ^d | 542/75 ^{fg} |
| 8 | 53/5 ^{hij} | 27/75 ^{fgh} | 152/3 ^{fgh} | 2019/3 ^{hi} | 407/5 ^{ij} | 0/237 ^{ed} | 2/13 ^{ef} | 3/03 ^d | 3/63 ^{de} | 33.4 ^c | 531/50 ^h |
| 9 | 52/75 ^{ij} | 30/75 ^{cde} | 150/3 ^{ghi} | 2011/5 ⁱ | 414 ^{ghi} | 0/247 ^{cd} | 2/24 ^{cd} | 3/06 ^d | 3/60 ^e | 34.2 ^c | 548/00 ^{ef} |
| 10 | 54 ^{hij} | 31 ^{bcd} | 149 ^{hi} | 2030/5 ^{fgh} | 405 ^{jk} | 0/222 ^{ef} | 2/28 ^{cd} | 2/84 ^e | 3/76 ^c | 34 ^c | 532/50 ^h |
| 11 | 55/75 ^{fgh} | 24/25 ^j | 155/25 ^{ef} | 2028/5 ^{gh} | 423/75 ^{ef} | 0/237 ^{de} | 2/2 ^{de} | 2/66 ^f | 3/74 ^{cd} | 29.01 ^{ef} | 532/75 ^{gh} |
| 12 | 55 ^{ghi} | 26 ^{hij} | 155/25 ^{ef} | 2042 ^f | 417/5 ^{fgh} | 0/235 ^{de} | 2/2 ^{de} | 2/61 ^f | 3/42 ^f | 27.7 ^{fg} | 542/75 ^{fg} |
| 13 | 55/75 ^{fgh} | 27 ^{ghi} | 156/5 ^{de} | 2061/25 ^e | 420/75 ^{fg} | 0/282 ^b | 2/22 ^{de} | 2/79 ^e | 3/61 ^e | 29.4 ^c | 558/5 ^d |
| 14 | 65/25 ^b | 31/75 ^{bc} | 167 ^b | 2120 ^c | 444/75 ^{ab} | 0/307 ^a | 2/44 ^b | 3/37 ^{ab} | 3/93 ^b | 37.07 ^b | 588/25 ^c |
| 15 | 69/25 ^a | 34/25 ^a | 172/5 ^a | 2153/75 ^{ab} | 452/25 ^a | 0/325 ^a | 2/54 ^a | 3/44 ^a | 4/19 ^a | 43.05 ^a | 612/75 ^b |
| LSD 5% | 2/9 | 2/1 | 2 | 12/9 | 7/6 | 0/021 | 0/09 | 0/11 | 0/12 | 1.478 | 8/81 |

4.DISCUSSION

As for the traits, control treatment was characterized by low nutrients application and leads to low yield. Because of the availability of nutrients for the treatments that received good fertilizer levels, the plant takes up more nutrients and spent more energy to increase yield. For this reason, the flowering and vegetative growth per plot in mixed fertilizers treatment were increased significantly. Different levels of acid humic on all traits had best performance, which is important in economic terms. There is conclusive evidence that small amounts of specific organic materials such as humic acid, a definite positive effect on plant growth and development. Research to understand the mechanisms of humic acid still needs to be developed. In the supply of rare and trace elements to plants, humic acid plays an important role in combining with these elements and can absorb them for plants by chelating these elements. It also increases the permeability of plant membranes, thus increasing the uptake of nutrients [16]. Researchers have suggested that the application of humic acid under different levels and in application alone or in combination with other fertilisers has increased the yield in some of the measured indices in different plants [16]. Atieh *et al.*, [2] reported Mixing the container media with increasing concentrations of vermicompost-derived humic acids increased plant growth. It is clear from the above that humic acids may positively influence higher plant metabolism. This function seems to be carried out more readily by humic acid fractions, because they are able to reach the plasma membrane of root cells and then to be translocated [13]. According to the results, due to provide necessary food for chamomile tested, the plant is better absorbed and appropriate nutrients to intercropped all elements and humic acid alone showed and better growth and increased the number of flowering branches and flowers and also had an increasing trend in plant growth. Application of humic acids with other elements used to increase the yield and crop quality because it provides nutrients to the plant and simultaneously increase the rate of photosynthesis and other plant physiological activities [5]. Given that mix treatment at 5 grams per liter has the highest amount of oil and chamazulene per unit area it can be introduced as a cost-effective and applicable treatment among other treatments. Also the mix treatment at 5 grams per liter can increase the efficiency of photosynthesis and respiration, which is very important for producing plant secondary metabolites [14, 20]. Physical and chemical properties of humic acid and nutrient holding capacity and increasing growth regulators elevated the concentration of nitrogen in plant and by increasing nitrogen, plant growth traits such as height, a number of nodes, internodes length and leaf area will be increased significantly [1, 17].

5.CONCLUSION

As a whole, the effect of nitrogen fertilizers, humic acid, zinc and boron on studied traits were found to be positive and it was observed that mixed treatment is characterized by a higher yield. Because mixed treatment at the level of 5 grams per liter in many traits have had a positive influence, one can recommend this treatment in order to increase Essence concentration, Essential oils, and Flower receptacle diameter.

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