

## THE EFFECT OF CHEMICAL FERTILIZERS ON THE QUANTITATIVE AND QUALITATIVE TRAITS OF OREGANO (*Origanum vulgare* L.) PLANT

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### ABSTRACT

This experiment was conducted to investigate the effects of NPK fertilizer on oregano (*Origanum vulgare* L.) plant for two years (2017 and 2018). Various levels of nitrogen (0, 50 and 100 kg ha<sup>-1</sup>), potash (0, 40 and 80 kg ha<sup>-1</sup>), and phosphorus (0 and 30 kg ha<sup>-1</sup>) fertilizers were applied. The results revealed that the use of NPK fertilizers significantly increased the dry weight of the oregano plant's aerial parts (leaves, inflorescences, and whole aerial parts). In the first year, the dry weight of the whole shoot increased by 82 to 124 g m<sup>-2</sup>, and in the second year, it increased by 82 to 129 g m<sup>-2</sup>. Moreover, regarding essential oil concentration, this parameter in inflorescence ranged from 2.38% to 3.66% in the first year, while in the second year, it ranged from 3.02% to 4.14%. Notably, the inflorescence had a higher essential oil concentration compared to the leaves. The study also found that the use of NPK fertilizer at a ratio of 100:20:40 kg ha<sup>-1</sup> resulted in the highest percentage of essential oil in the aerial. Conversely, the control treatment led to a decrease in essential oil yield. Among the essential oil compounds, Carvacrol and p-Cymene were the predominant components, with concentrations ranging from 49.36% to 60.32% and 2.03% to 4.56%, respectively, in various oregano plant organs.

**Keywords:** Dry weight, Essential oil, Inflorescence, Oregano, Nitrogen.

### INTRODUCTION

Oregano (*Origanum vulgare* L.) is one of the most solded culinary and medicinal herb throughout the world. Flowering aerial parts and leaves of oregano have been used as a popular flavoring of food stuffs and as an antioxidant agent in cosmetics (Morshedloo et al, 2018). One of the most important issues in the field of agriculture and medical sciences and even global trade is attention to the production, processing and use of medicinal plants (Pirzad et al. 2006). The importance of medicinal plants is due to the production of effective substances that play an important role in pharmaceutical, cosmetic, perfumery, dyeing and flavoring industries (Kim et al., 2005). Oregano medicinal plant belongs to the lamiaceae family and has been registered as a plant used in traditional medicine as well as an effective plant in the world's authoritative pharmacopoeias (Skoula and Harborne, 2002; Azizi et al., 2009).

The study of different aspects of crop plants, including the quantitative and qualitative changes of these plants in response to different nutritional sources, is of particular importance. Proper nutrition of medicinal plants along with compliance with the principles of organic production, while preserving the environment, increases the quantitative and

qualitative yield of effective substances in these plants (Sahoo, 2001).

In addition to the fact that nutrients should be easily used by the plant, the balance between their amounts is also very important. Nitrogen is the most important food element that is needed in large quantities by higher plants. Nitrogen plays a role in the formation of proteins, nucleic acids, chlorophyll, coenzymes, photosynthesis and plant secondary metabolism (Lynch et al., 2012). The production of hydrocarbon materials increases with the increase of nitrogen, and its consumption for protein fuel and the production of secondary metabolites increases (Qranjik and Galeshi, 2001). Sotiropoulou and Karamanos (2010) showed by examining four nitrogen levels (zero, 40, 80 and 120 kg ha<sup>-1</sup>) that with increasing nitrogen fertilization, vegetative yield and essential oil yield of oregano also increased. Yazdani Bioki et al. (2014) investigated the effect of urea fertilizer and azocompost on marjoram plant, and their results showed that the use of 120 kg ha<sup>-1</sup> of nitrogen will result in the highest economic yield in marjoram plant.

Phosphorus is one of the essential elements involved in many vital processes of plants. Phosphorus, one of the components of ATP, plays an important role in energy transfer. Phosphorus helps to absorb nutrients by increasing

the growth of roots, so the accumulation of dry matter in the plant increases (Nahar et al., 2014). In addition, phosphorus plays an important role in energy transfer and regulation of enzyme activity and signal transmission (Sarker et al., 2015). Optimum nutrition of plants with potassium can have a positive role in improving water relations, chlorophyll level, increasing leaf area, increasing root growth and increasing plant photosynthetic activity and thus increasing plant yield. Also, the potassium in the fertilizer improves the yield and quality of the product (Hasanuzzaman et al., 2018). The studies conducted so far on medicinal plants show that the use of chemical fertilizers improves the growth and absorption of elements in these plants (Kumawat et al., 2006). Also, studies showed that a number of physical and chemical characteristics of soil affect the concentration and composition of oil in Greek oregano and other related species (Panagopoulos, 2012).

To produce effective medicinal substances, medicinal plants need a suitable nutritional system including all kinds of nutrients, and by increasing soil fertility, the effectiveness of inputs can be increased. Considering the increasing need for medicinal plants and the effect of fertilizers on their quality and quantity, it is necessary to identify the most suitable amount of fertilizers to determine the plant's needs in the conditions of the region. On the other hand, in the west of Mazandaran, every year, many fields are cultivated with oregano, and due to the lack of information on the amount of chemical fertilizers used in

this plant, there was a difference between the farmers in the region in terms of the amount of fertilization. Also, the effect of using chemical fertilizers on the amount of essential oil and components of essential oil has been paid less attention. Therefore, according to the environmental conditions of the region, providing optimal plant needs and environmental risks in excessive use of chemical fertilizers, this study has been conducted to determine the most suitable amount of NPK fertilizers in the yield and essential oil of oregano plant.

## MATERIALS AND METHODS

### *Location and geographic location of the experiment*

The experiment was conducted at a research farm at Islamic Azad University, Chalous Branch, located at 82° 40' 58' N and 53° 69' E with an altitude of 3 m above sea level, during the 2017-18 crop year. Also, oregano seedlings were produced by seed propagation in a greenhouse and cultivated in a research field

### *Characteristics of the soil and weather of the experiment site*

To determine the soil characteristics, samples were taken from a depth of 0-30 cm before cultivation (Table 1). The mean temperature, precipitation and relative humidity of the experimental years are shown in Table 2 (Prepared from the meteorological office of Mazandaran Province).

**Table 1.** Physical and chemical properties of soil

Texture	Organic matter (%)	active cation elements (%)	N (%)	K (ppm)	P (ppm)	pH	Ec (ds m <sup>-1</sup> )
Sandy Clay Loam	0.92	14.24	0.13	76	14.36	7.4	0.52

**Table 2.** Meteorological parameters for the field sites during experiment

Months	Mean temperature (°C)		Relative humidity (%)		Precipitation (mm)	
	2017	2018	2017	2018	2017	2018
March	9.85	8.25	83.00	86.50	99.70	119.20
April	14.20	11.65	82.50	80.00	62.60	114.70
May	18.00	15.35	78.50	83.30	21.20	51.20
June	24.90	21.50	73.00	78.00	10.30	29.30
July	28.30	26.00	71.00	75.00	7.10	15.30

### *Treatments and experimental design*

This experiment was conducted as a factorial design in the form of a randomized complete block design in 3 replications. Nitrogen fertilizer was added to the plots at three levels (0, 50 and 100 kg ha<sup>-1</sup>) in the form of pure urea (45%) and in two stages before planting and 2 weeks after planting and rooting. Potash at three levels (0, 40 and 80 kg ha<sup>-1</sup>) as K<sub>2</sub>O 20% and phosphorus at two levels (0 and 30 kg ha<sup>-1</sup>) before planting were added to the plots.

### *Preparation of seedlings*

Oregano seeds were obtained from Karaj Agricultural Research, Education and Development Institute. The seeds

were planted in wooden boxes in the greenhouse in early March. First, the seed bed was wet, then the seeds were planted on the bed and covered with 0.5 cm of light soil. The seeds were irrigated with a geyser in the form of misting. After growing and at the five-leaf stage, the seedlings were transferred to the field.

### *Land preparation and cultivation*

The desired land was plowed at the end of November using a reversible iron bull to a depth of 25-30 cm and then it was plowed twice. Then the plotting of the land was done in early April. The total test plots were 54 plots and each test block consisted of 18 plots measuring 2.50 x 3.50 m.

The distance between the blocks was 3 m and the distance between the plots was 1 m. Transplanting was done manually in early the late April. The distance between rows of crops was 50 cm and the distance between plants was 30 cm. The plots were watered regularly and every week. Weed control was done manually. Harvesting was done at the stage of full bloom (the highest amount of essential oil in the aerial parts of the oregano plant is at the time of full inflorescences).

#### Measurements

After harvesting, 1 m<sup>2</sup> of each plot was selected after removing the margins and the wet parts of the plant were determined, and after drying in natural conditions (shade and dry air), the dry weight of leaves, inflorescences and the whole plant was measured.

The extraction of essential oil was done in the flowering stage using a clevenger apparatus for 4 hours and by water distillation method (Said-Al Ahl et al., 2009). First, 10 grams of the dried samples were poured into a 1000 ml flask and about 100 ml of distilled water was added to it, and extraction and essential oil extraction was done. The essential oil yield of the plant organs was calculated from the product of the essential oil content and its total dry weight.

The analysis of all essential oils was performed using a Hewlett Packard 5890 II GC (single injector (split/split less or Packed) single FID detector), equipped with a HP-5 capillary column (30 m, 0.25 mm i.d., 0.25 μm film thickness) and a mass spectrometer HP 5972 as detector (Agilent Brand). The carrier gas was helium, at a flow rate of 1 ml min<sup>-1</sup>. The column temperature was initially 55 °C for 3 min, then gradually increased to 200 °C at 3 °C/min and finally increased to 220 °C at 5 °C min<sup>-1</sup>. For GC-MS detection, an electron ionization system was used with an ionization energy of 70 eV. The extracts were diluted 1:100 (v/v) with acetone and 1 l of the diluted samples was injected automatically in splitless mode. Injector and detector temperatures were set at 220 and 290 °C, respectively.

#### Statistical analysis

Analysis of data variance was done with SAS v.3 software and Duncan's multiple range tests were used at a probability level of 5% to compare the mean of the desired traits (Steel and Torrie, 1980).

### RESULTS AND DISCUSSION

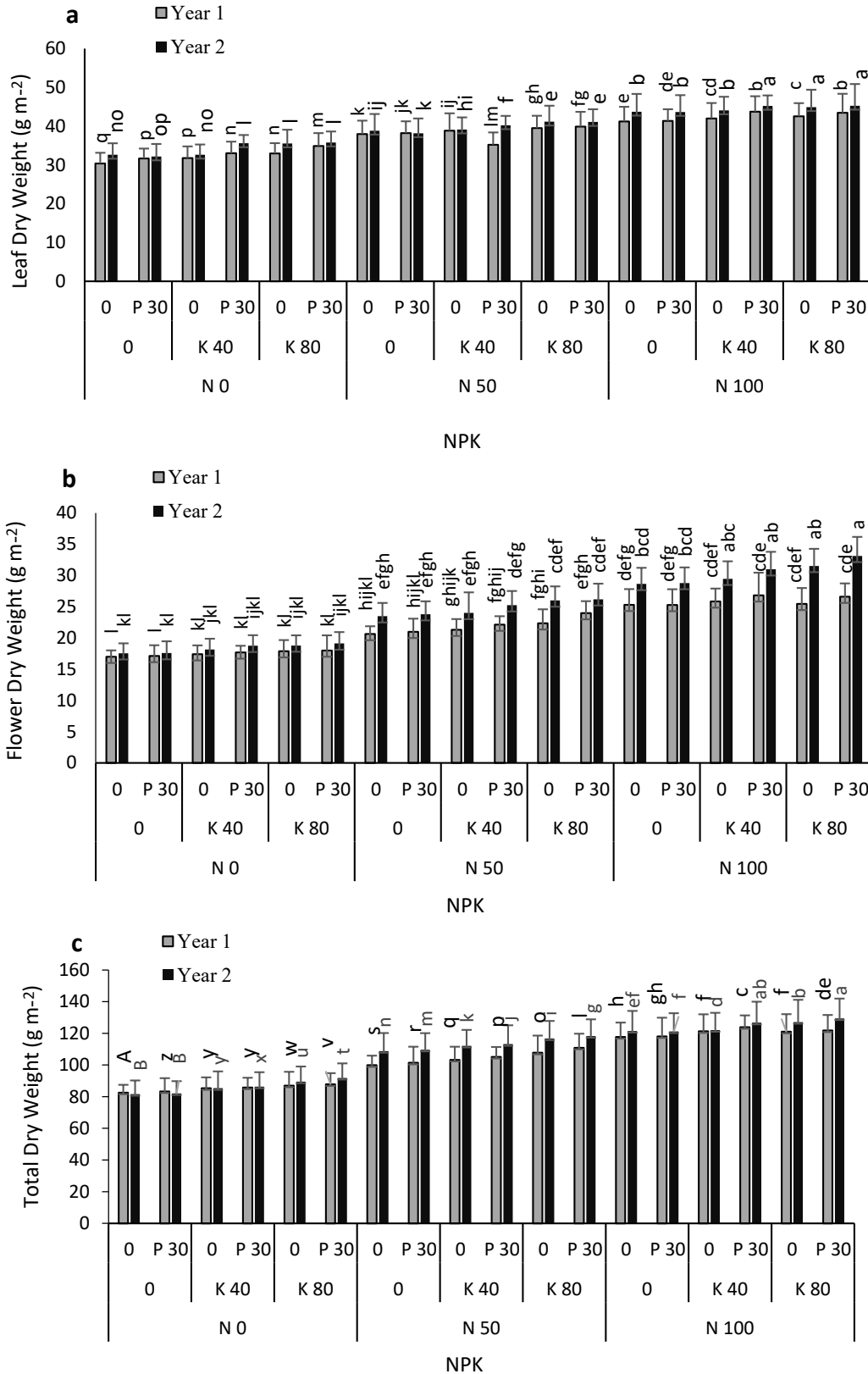
#### Dry weight

Results of variance analysis of the effect of chemical fertilizers on the dry weight of oregano is shown in Appendix Table 1. The results showed that the interaction effect of year, nitrogen, phosphorus and potassium fertilizer on dry weight of leaf, inflorescences and whole plant was significant. With the increase in nitrogen, phosphorus and potash fertilizer consumption, the dry weight of leaves has increased. The average increase in leaf dry weight was significantly between 30.38 and 43.75 g m<sup>-2</sup> in the first year and between 32.18 and 45.25 g m<sup>-2</sup> in the second year. The increase in leaf dry weight in the second year was higher than in the first year. The maximum dry weight of leaves with the application of N<sub>100</sub>P<sub>30</sub>K<sub>80</sub>, N<sub>100</sub>P<sub>30</sub>K<sub>40</sub> and N<sub>100</sub>P<sub>0</sub>K<sub>80</sub> in the second year of the experiment was 45.25, 45.21 and 44.90 g m<sup>-2</sup>, respectively. The lowest dry weight of leaves was observed in the condition of no application of chemical fertilizers (Figure 1). It has been reported that nitrogen deficiency causes leaf fall from the lower part of the plant (Hopper, 1996). Nitrogen plays an effective role in the development of new cells and increases vegetative growth and the number of secondary shoots in the plant. By increasing root growth, phosphorus helps the plant absorb nutrients, so there is a greater accumulation of dry matter (Nahar et al., 2014). The use of phosphorus fertilizers can increase plants' uptake of phosphorus and lead to improved plant yield (Sumer et al., 2023). Plants that are fertilized with low levels of nitrogen usually have short height, few foliage and yellow leaves. They have thin and weak plant cover, which ultimately leads to a decrease in plant yield (Diepen Brock, 2000). In this study, it was observed that the lack of application of chemical fertilizers, especially nitrogen, has led to the lack of development of leaves and the fall of leaves in oregano.

**Appendix Table 1.** The results of analysis of variance of the effect of NPK on the dry weight of different organs of oregano plant

S.O.V	df	Leave dry weight	inflorescences dry weight	Whole dry weight
Year	1	80.46*	203.97**	524.79*
Block(Year)	4	6.07	1.52	55.28
N	2	936.84**	944.96*	12549.23**
K	2	45.95*	32.60	415.74*
P	1	6.43*	10.82*	57.25*
Year*N	2	0.85	31.40	130.96
Year*K	2	1.02	3.25	19.25
Year*P	1	0.01	0.02	0.20
N*K	4	4.11	1.62	12.24
Year*N*K	4	1.63	2.06	7.85
N*P	2	6.41	10.04*	1.63
Year*N*P	2	2.01	0.32	2.47
K*P	2	1.12	1.54	4.71
Year*K*P	2	6.08	0.26	0.90
N*K*P	4	2.07	0.28	3.41
Year*N*K*P	4	43.08*	51.25*	460.70**
Error	68	15.56	15.12	120.43

\*, \*\* and ns: significant at the level 0.05, 0.01



**Figure 1.** Application of NPK fertilizers in different amounts on leaf dry weight (a), inflorescence dry weight (b) and total dry weight (c) during the years 2017 and 2018. Values represent means  $\pm$  S.E. Significant differences among treatments were measured by the least significant difference (LSD) at  $P < 0.05$  and indicated by different letters.

The dry weight of inflorescences increased significantly with the increase in the use of chemical fertilizers. The average increase in dry weight of inflorescences was

significantly about 16.98 to 26.60 g m<sup>-2</sup> in the first year and between 17.55 to 33.13 g m<sup>-2</sup> in the second year. The dry weight increase of inflorescences in the second year was

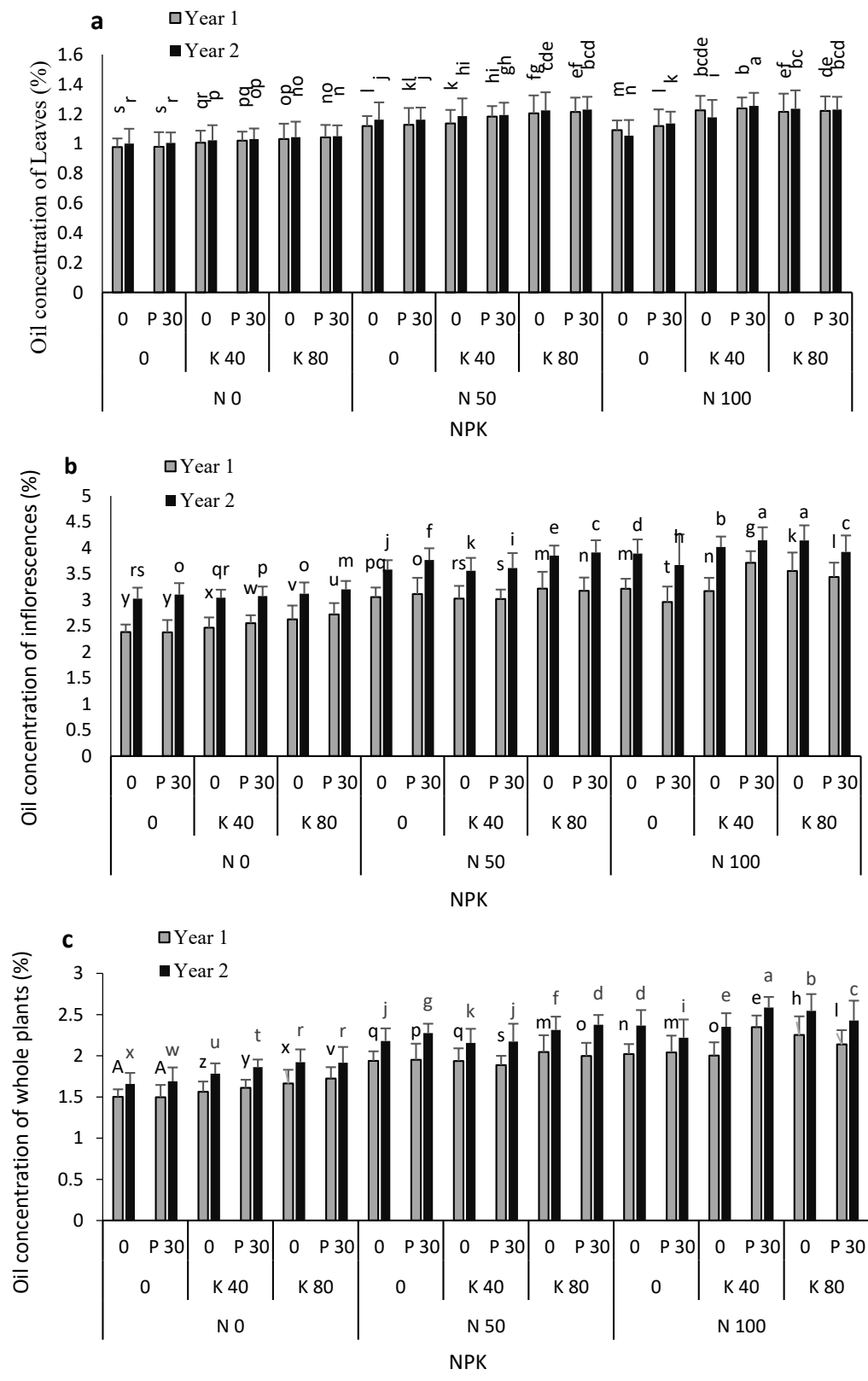
higher than the first year (Figure 1b). The results showed that the highest dry weight of inflorescences was observed in the  $N_{100}P_{30}K_{80}$  treatment in the second year of the experiment at the rate of  $33.13 \text{ g m}^{-2}$ . In Figure 1b, the role of nitrogen fertilizer in increasing the dry weight of oregano inflorescences is well shown, so that in the absence of nitrogen fertilizer, the dry weight of inflorescences did not increase significantly with the use of potash and phosphorus fertilizers, but the application of 50 and  $100 \text{ kg ha}^{-1}$  of nitrogen fertilizer especially in the second year, it has increased the number of inflorescences. The use of nitrogen fertilizer has made the absorption of nutrients potassium and phosphorus better. Some researchers attributed the reduction of nutrients due to nitrogen deficiency as premature aging of leaves, reduction of chlorophyll and photosynthesis and therefore reduction of transfer of microbes to seeds, lack of root growth and development in the soil and therefore reduction of the ability to absorb elements from the soil (Uauy et al., 2006; Yuan et al., 2005).

Increasing the consumption of nitrogen, phosphorus and potash fertilizers has increased the dry weight of the whole plant. The average increase in dry weight of the whole shoot was significantly around 82 to  $124 \text{ g m}^{-2}$  in the first year and between 82 and  $129 \text{ g m}^{-2}$  in the second year. The role of nitrogen in increasing the total dry weight can be seen in Figure 1c. The absence of nitrogen application despite the use of phosphorus and potash fertilizers did not significantly increase the dry weight of the oregano plant, but the use of nitrogen fertilizer significantly increased the dry weight of the whole plant. The highest total dry weight was obtained in  $N_{100}P_{30}K_{80}$  at the rate of  $129.4 \text{ g m}^{-2}$ , then  $N_{100}P_{30}K_{40}$  treatment in the second year of the experiment. The increase in total dry weight and inflorescence in the second year compared to the first year was probably due to the better temperature conditions for the growth and development of the oregano plant. The lowest dry weight of the whole plant was observed in the condition of no application of chemical fertilizer (Figure 1c). The lack of nutrients by affecting the photosynthetic activity of the plant and increasing respiration leads to the decrease in hydrocarbon substances as a result of the decrease in growth and yield. The increase in dry weight of the whole plant in the second year was higher than in the first year. Pal et al. (2016) showed yield generally increase in accordance with the increases in N, P and K fertilizer rates. Sotiropoulou and Karamanos (2010) showed that the application of nitrogen fertilizer increases the vegetative yield of oregano plant, which is consistent with the results of this study. The yield and quality of the oregano were influenced by nitrogen, potassium and phosphorus (Matłok et al., 2020). In the early stages of plant growth, phosphorus increases nitrogen absorption (Naomi et al., 2021). Studies have shown that there is a close relationship between the supply of nutrients and the increase of plant dry matter (Malakooti and Nafisi, 1995; Kumawat et al., 2006). In case of nitrate deficiency, the level of cytokinin hormone in the plant is reduced, and as a result, growth is reduced by

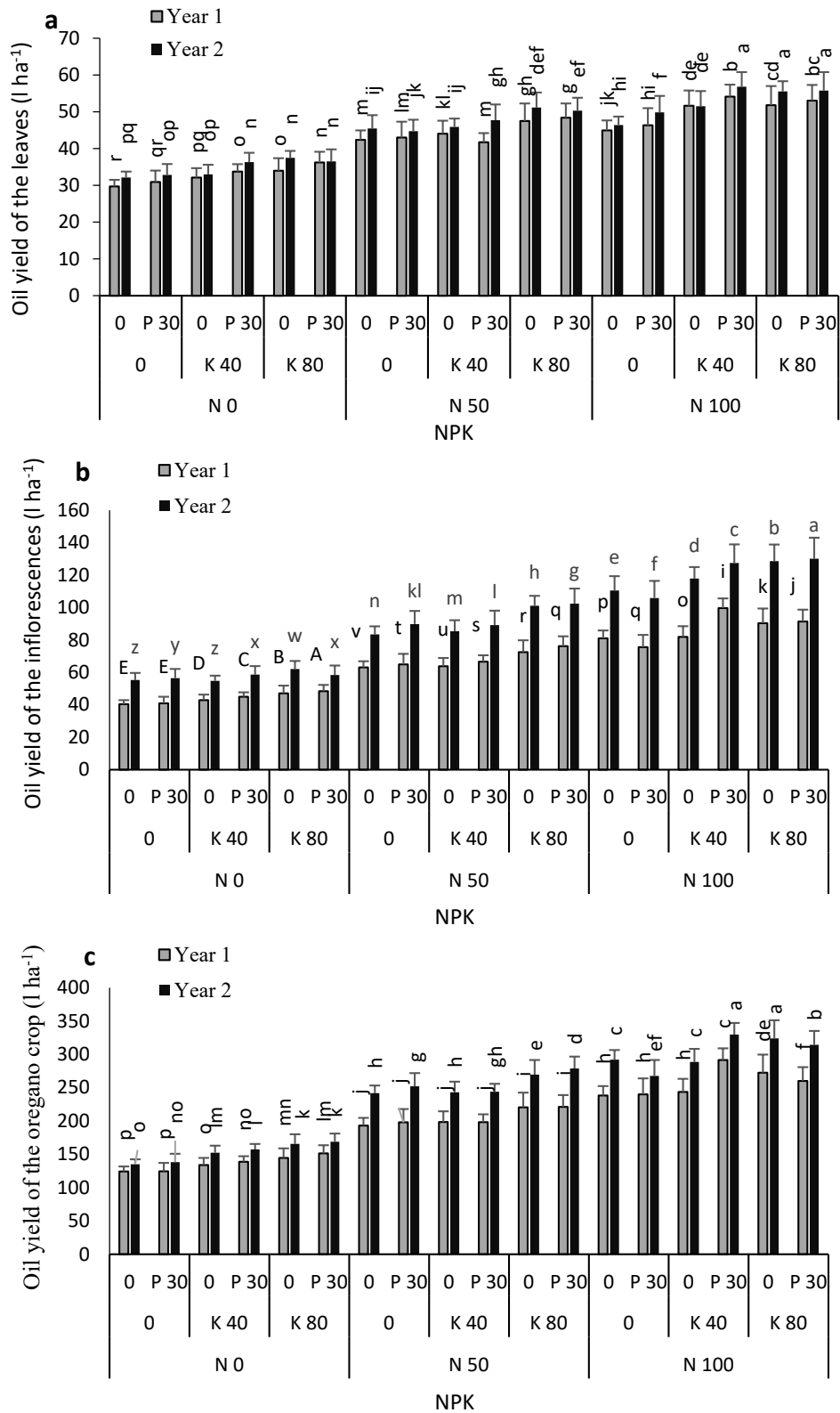
affecting cell proliferation and expansion (Rahayu et al., 2005). On the other hand, the use of nutrients in optimal concentrations increases the rate of plant photosynthesis and the absorption of nutrients, which leads to the development of the leaf area and the number of secondary branches. As a result, the dry weight of plants treated with chemical fertilizers has increased.

#### *Concentration of essential oil*

The results showed that the interaction effect of year, nitrogen, phosphorus and potassium fertilizer on concentration of essential oil of leaf, inflorescences and whole plant was significant (Appendix Table 2). The effect of using chemical fertilizers on the concentration of essential oil in different organs of oregano plant is shown in Figure 2. The results showed that with the increase in the use of chemical fertilizers, the percentage of essential oil in oregano organs has increased. The average increase in leaf essential oil percentage was about 0.98% to 1.22% in the first year and between 1.00% and 1.23% in the second year (Figure 2a). The concentration of essential oil in inflorescences varied between 2.38 and 3.66% in the first year and between 3.02 and 4.14% in the second year (Figure 2b). Also, the concentration of essential oil in all parts of the plant was measured between 1.50 and 2.35% in the first year and between 1.66 and 2.58% in the second year. When fertilizer was applied at 50 and  $100 \text{ kg ha}^{-1}$ , oregano aerial parts had higher levels of essential oil than when nitrogen was not applied. (Figure 3c). The use of NPK in the order of 100:20:40  $\text{kg ha}^{-1}$  had the highest percentage of essential oil in aerial parts. In the presence of low or non-use of chemical fertilizers, oregano leaves had a decreased percentage of essential oil. Essential oils' content and composition are affected by a number of factors, including genetic makeup and cultivation conditions, including climate, habitat, harvesting time, water stress, and fertilizer use. An increase in the essential oil content of plants with an increase in the concentration of nutrients has also been reported in previous studies (Sifola and Babieri, 2006). Nitrogen is one of the essential nutrients in plants, which is used for the synthesis of many organic compounds in plants, such as amino acids, proteins, enzymes, and nucleic acids. Since enzymes and amino acids play an important role in the biosynthesis of plant essential oils (Koeduka et al., 2006). The presence of nitrogen as a key factor can affect the production of essential oils in aromatic plants (Briat et al., 2015; Gedik, and Akgul., 2023). It is well known that P and K is an essential element in reproductive and vegetative growth can increase by the increased P and K applications. also, Phosphorus has many other cellular functions in plants and affects the primary and secondary metabolites (Mengel and Kirkby, 2001). In this study, it seems that the use of chemical fertilizers, especially nitrogen, has increased the essential oil by continuing the activity of the leaf area, participating in the structure of chlorophyll and enzymes involved in photosynthetic carbon metabolism.



**Figure 2.** The application of NPK fertilizers in different amounts on the content of leaf essential oil (a), the inflorescences (b) and the whole plants (c) during the years 2017 and 2018. Values represent means  $\pm$  S.E. Significant differences among treatments were measured by the least significant difference (LSD) at  $P < 0.05$  and indicated by different letters.



**Figure 3.** Application of NPK fertilizers in different amounts on essential oil yield of leaf (a), inflorescence (b) and whole plants (c) during the years 2017 and 2018. Values represent means  $\pm$  S.E. Significant differences among treatments were measured by the least significant difference (LSD) at  $P < 0.05$  and indicated by different letters.

**Appendix Table 2.** The results of analysis of variance of the effect of NPK on the essential oil and oil yield of different organs of oregano plant

S.O.V	df	concentration of essential oil leave	concentration of essential oil inflorescences	concentration of essential oil whole plant	Essential oil yield of the leaves	Essential oil of the inflorescences	Essential oil of the whole plant
Year	1	0.0052	9.81**	1.824	158.28*	15084.30**	37364.63
Block(Year)	4	0.0019	0.13	1.230	11.27	102.94	17059.34
N	2	0.3183**	6.81**	3.140**	2968.01**	24898.87**	169138.56**
K	2	0.0681**	0.47*	0.248**	299.75**	1250.88*	7502.17*
P	1	0.0086	0.02	0.022	39.10	169.98	725.44
Year*N	2	0.0024	0.01	0.016	2.79	895.46	2901.57
Year*K	2	0.0002	0.02	0.004	0.31	41.57	143.44
Year*P	1	0.0002	0.002	0.0043	0.69	3.39	20.99
N*K	4	0.0093*	0.13*	0.040*	28.95*	206.88*	711.42**
Year*N*K	4	0.0006	0.02	0.002	2.33	25.82	46.02
N*P	2	0.0017	0.02	0.001	13.72	17.31	31.61
Year*N*P	2	0.0017	0.04	0.018	2.16	6.39	134.53
K*P	2	0.0011	0.08	0.049	5.57	118.20	871.25
Year*K*P	2	0.0003	0.03	0.0005	13.39	12.56	42.25
N*K*P	4	0.0009	0.11	0.051	2.41	83.89	1139.33
Year*N*K*P	4	0.30**	0.81*	0.24*	196.15*	792.69*	6569.52**
Error	68	0.04	0.29	0.09	71.04	287.60	1316.03

\*, \*\* and ns: significant at the level 0.05, 0.01

#### Essential Oil yield

The results showed that the interaction effect of year, nitrogen, phosphorus and potassium fertilizer on the essential oil yield of leaf, inflorescences and whole plant was significant (Appendix Table 2). The effect of using chemical fertilizers on the yield of essential oil in different organs of oregano plant is shown in Figure 3. The results showed that with the increase in the use of chemical fertilizers, the yield of essential oil in oregano organs has increased. The average yield of leaf essential oil increased from 29.72 to 54.14 l ha<sup>-1</sup> in the first year and between 32.15 to 56.79 l ha<sup>-1</sup> in the second year (Figure 3a). The yield of essential oil in inflorescences varied between 40.41 and 99.66 l ha<sup>-1</sup> in the first year and between 55.26 and 130.07 l ha<sup>-1</sup> in the second year (Figure 3b). Also, the yield of essential oil in all plant organs was measured between 124.21 and 291.51 l ha<sup>-1</sup> in the first year and between 134.99 and 329.51 l ha<sup>-1</sup> in the second year (Figure 3c). A significant difference was observed in the yield of essential oil between fertilizer treatments, so that the application of 50 and 100 kg ha<sup>-1</sup> of nitrogen fertilizer has increased the yield of essential oil of the aerial parts of oregano compared to the absence of nitrogen application. The use of NPK in the order of 100:20:40 kg ha<sup>-1</sup> had the highest yield of essential oil in aerial parts. Gharib et al. (2008) reported that the weight of essential oil in oregano plant increased with the increase in total nitrogen concentration. Arango et al. (2012) showed using phosphorus chemical fertilizer and mycorrhiza, the most effective fertilizer combination was phosphorus chemical fertilizer and mycorrhiza. Essential oils are terpenoid compounds whose building blocks (isoprenoids) such as isopentenyl pyrophosphate and dimethylallyl pyrophosphate have an urgent need for ATP and NADPH, and considering the fact that the presence of

elements such as nitrogen and phosphorus is necessary for the formation of the latter compounds, finally the improvement of essential oil yield was observed following the increase of nutrients in plants (Rezvani Mogadam et al., 2013; Aslani et al., 2014). Nutrients play a key role in the primary and secondary processes of plants, and nutrients increase the fresh and dry weight of plants by increasing the rate of photosynthesis; And considering that the essential oil content of plants is affected by the dry weight of plants, as a result, nutrients increase the essential oil content of plants by increasing the dry weight of plants treated with chemical fertilizers.

#### Oil composition

The components of the essential oil of leaves and inflorescences of oregano plants harvested from the best treatment during the years 2017 and 2018 are shown in Table 3. In the table, the essential oil components that were higher than 1% are given. Among the essential oil compounds, Carvacrol with 58.36 to 82.32% had the highest concentration in oregano plant organs. After that, p-Cymene with 2.03% to 7.56%,  $\gamma$ -Terpinene with 1.65% to 4.52% and Carvacrylacetate with 0.68% to 1.46%. The amount of Carvacrol,  $\beta$ -Pinene, 1-Octen-3-ol, Myrcene,  $\alpha$ -Terpinen and Carvacrylacetate was higher in inflorescences than in leaves. Also,  $\alpha$ -Thujene,  $\beta$ -Pinene, Camphene, Sabinene, p-Cymene,  $\gamma$ -Terpinen, cis-Sabinene hydrate, Borneol,  $\alpha$ -Terpineol, Geraniol, Thymol,  $\beta$ -Caryophyllen,  $\beta$ -Bisabolene, Spathulenol and  $\alpha$ -Cadinol in the leaves were higher than the oregano inflorescences. The main components of essential oil mainly depend on genetic factors, plant root morphology, nutritional status and different parts of the plant (stem, leaf and inflorescences) (Rattanachaikunsopon and Phumkhachorn, 2010).



**Table 3.** The constituents of the oregano (*Origanum vulgare* L.) essential oil (% v/v) extracted from leaves and inflorescences on harvest during 2017 and 2018. The plants harvested from the application of NPK fertilizers in order of 100:30:40 kg ha<sup>-1</sup>

Compounds	Inflorescences		Leaves	
	2017	2018	2017	2018
$\alpha$ -Thujene	0.10	0.32	0.08	0.45*
$\beta$ -Pinene	0.20	0.54	0.41	0.38
Camphene	0.10	0.09	0.13	0.15
Sabinene	0.41	0.52*	1.02	1.95*
$\beta$ -Pinene	0.41	0.39	0.02	0.03
1-Octen-3-ol	0.19	0.23	0.31	0.49*
3-Octanone	0.12	0.13	-	-
Myrcene	0.73	1.23*	0.42	0.38
$\alpha$ -Phellandrene	0.08	0.12	-	-
$\delta$ -3-Carene	0.02	0.03	-	-
$\alpha$ -Terpinen	0.98	1.08*	0.52*	0.43
<i>p</i> -Cymene	2.03	3.12*	6.12	7.56*
Limonene	0.29	0.34	0.41*	0.30
$\beta$ -Phellandrene	0.16	0.15	0.21	0.19
(Z)- $\beta$ -Ocimene	0.06	0.04	-	-
(E)- $\beta$ -Ocimene	0.01	0.03	-	-
$\gamma$ -Terpinen	1.65	2.36	4.52*	2.98
cis-Sabinene hydrate	0.52	0.65	2.03	1.98
Terpinolen	0.09	0.12	-	-
trans-Sabinene hydrate	-	0.06	0.09	0.07
Borneol	0.19	0.21	0.36	0.56*
$\alpha$ -Terpineol	0.08	0.09	0.12	0.23*
Geraniol	0.06	-	1.03*	0.05
trans-para-mentha-2-one	-	0.09	0.12	0.34*
trans-Dihydrocarvone	0.03	0.1	0.23*	0.05
Carvacrol methylether	0.09	0.1	0.12	0.23*
Thymoquinon	0.10	0.13	0.21	0.16
Thymol	0.23	0.36*	0.65	0.97*
Carvacrol	82.32*	73.06	66.03*	58.36
Carvacrylacetate	0.68	1.26*	0.93	1.46*
$\beta$ -Caryophyllen	0.03	0.32*	0.25	1.46*
$\alpha$ -Humulene	-	0.19	1.10	0.13
Allo-Aromadendrene	0.54*	0.36	1.03*	0.09
$\alpha$ -Muurolol	0.58	-	1.02*	0.03
$\beta$ -Bisabolene	0.32*	0.09	2.06*	0.65
$\gamma$ -Cadinene	1.05*	0.09	0.24	2.21*
$\delta$ -Cadinene	0.09	0.68*	0.06	0.98*
Spathulenol	1.32*	0.65	3.31*	1.81
Globulol	0.04	0.01	0.34*	0.06
Veridiflorol	1.03*	0.32	0.95*	0.09
Humulene epoxide II	0.03	0.1	0.02	0.05
Caryophyllene oxide	0.03	0.40*	0.5	0.65*
epi- $\alpha$ -Cadinol	0.03	0.5*	0.4	0.9
epi- $\alpha$ -Muurolol	-	-	0.6	1.32*
$\alpha$ -Cadinol	0.17	1.03*	1.14	2.01*
Total	97.19	91.69	99.11	92.49

\* The year exhibiting significantly higher percentage ( $p < 0.05$ ) of each constituent.

The effect of year had a significant effect on some components of oregano essential oil. The content of compounds Sabinene,  $\alpha$ -Terpinen, *p*-Cymene, Thymol, Carvacrylacetate,  $\beta$ -Caryophyllen, Caryophyllene oxide and  $\alpha$ -Cadinol in 2018 was higher than in 2017. An increase in rainfall and a decrease in temperature lead to an increase in the percentage of oregano essential oil (Karamanos and

Sotiropoulou, 2013). In this study, the percentage of oregano essential oil was higher in the second year than in the first year. One of the reasons can be attributed to the higher rainfall and lower temperature in the second year of the experiment compared to the first year. Carvacrol, which is the major constituent of oregano essential oil, increases its amount in the plant in conditions of low humidity and

rainfall and high temperature (Panagopoulos, 2012), and in this study, the amount of Carvacrol in the first year of the experiment was higher because The temperature during the growth period was higher than the second year of the experiment. Similar results were reported by Karamanos and Sotiropoulou (2013).

## CONCLUSION

The application of chemical fertilizer has increased the dry weight of leaves, inflorescences and the entire shoot of oregano plant in both years of testing. The increase in the dry weight of the aerial parts was higher in the second year than in the first year of the experiment. With the application of maximum NPK fertilizer, the highest dry weight of aerial parts was obtained.

The concentration of essential oil in the leaves, inflorescences and the entire shoot of the plant was also affected by the use of chemical fertilizers. With the application of chemical fertilizer, the concentration of essential oil in the plant increased. The highest concentration of essential oil in aerial parts under the conditions of NPK application was 100:20:40 kg ha<sup>-1</sup> respectively. Also, the results showed that the lack of application of chemical fertilizer has reduced the yield of essential oil in the plant. Thus, the lowest yield of essential oil was observed in the condition of not using NPK. The components of oregano essential oil varied in different years. Among the compounds, Carvacrol, p-Cymene,  $\gamma$ -Terpinen and Carvacrylacetate had the highest percentage of essential oil.

**Conflict of Interest:** The authors declare that they have no conflict of interest.

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