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Effect of nitrogen and phosphorus application on herb and essential oil composition of *Satureja montana* L. 'carvacrol' chemotype

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ABSTRACT

A field experiment was carried out during two successive seasons to study the effects of nitrogen fertilizer (0, 50 and 100 kg N/fed.) and phosphorus fertilizer (0, 15.5 and 30 kg P/fed.) as well as 1st and 2nd cuts on dry matter, essential oil percentage and composition of Satureja montana under Egyptian conditions. In the two seasons, herb dry weight/plant, essential oil % and oil yield in two cuts were significantly increased with the rise in nitrogen and/or phosphorus fertilizers. 2nd cut gave higher values for herb dry weight/plant, essential oil % and oil yield than 1st cut. The maximum herb dry weight/plant and oil yield was obtained from plants fertilized (50 kgN+30 kg p/fed.) and harvested at 2nd in both seasons, while maximum essential oil percentage was obtained from plants harvested in the 1st cut with fertilized at (100 kgN /fed.) and from plants harvested in the 2nd cut with fertilized at (30 kg p/fed.) in the first and second seasons, respectively. Carvacrol was the major marker compound in Satureja montana. Other important compounds were p-cymene (7.78 to 9.95% and 4.10 to 6.44%), γ-terpinene (3.45 to 6.43% and 2.03 to 4.02%), linalool (0.56 to 1.70% and 0.81 to 2.60%), thymol (1.78 to 2.58% and 1.28 to 3.93%) and β-caryophyllene (1.88 to 2.34% and 2.32 to 3.20%) considered as minors in the first and second cuts, respectively. The highest carvacrol percentage was recorded from Satureja montana harvested at 2nd cut and fertilized by (50 kgN+30 kg p/fed.). This study showed that the accumulation of essential oil constituents is influenced by the nitrogen and phosphorus fertilizers as well as 1st and 2nd cuts.

Keywords: *Satureja Montana*, Nitrogen fertilizer, Phosphorus fertilizer, Essential oil, Carvacrol

INTRODUCTION

Winter or mountain savory (*Satureja montana* L. family Lamiaceae) is a perennial herb native to the Mediterranean regions of Europe [1, 2] cultivated worldwide as medicinal and spice plants [3-5] and used as traditional medicinal herb for bronchitis, antiseptic, carminative, digestive, expectorant stimulant, gastrointestinal, anti-diarrheic, aphrodisiac and stomachic [6-10]. *Satureja Montana* is rich source of biologically active phytochemicals and has positive effects on human health attributed to its active constituents such as essential oil, triterpenes, flavonoids and rosmarinic acid [11-14]. The essential oil is used in the food industry as a flavoring agent and perfumery and as active ingredients in medical preparations [15-17]. Essential oil, obtained from the whole herb, possess antibacterial, antifungal, antiviral, antioxidant, antispasmodic, antidiarrhoeal activities antiproliferative activity on human erythroleukemic K562 cells [17, 18-29].

Yield and active compounds of medicinal and aromatic plants are affected by genetic variation, environmental influences, agronomic conditions, fertilization and harvesting time. Nitrogen (N) is the most important plant nutrient for crop production. It stimulates root growth, crop development as well as uptake of the other nutrients. It is an essential component of chlorophyll, enzymes, proteins, etc. Additionally, nitrogen is found in such important molecules as purines, pyrimidines, porphyrines and coenzymes. Purines and pyrimidines are found in nucleic acids, RNA and DNA, which are essential for protein synthesis. The porphyrin structure is found in such metabolically important compounds as chlorophyll pigments and the cytochromes, both essential in photosynthesis and respiration [30] Application of nitrogen fertilizer was influenced the herb yield, oil content and the essential composition in aromatic and medicinal plants [31]. Phosphorus (P) is required in relatively large amounts for the biosynthesis of primary and secondary metabolites, [32]. Since P has essential functions as a constituent of nucleic acids and phospholipids (biomembranes) and plays a key role in the energy metabolism of cells. From this viewpoint, phosphorus element is an essential nutrient for crop growth and high yield with good quality. In this respect, increasing phosphorus application increased growth characters, oil percentage and oil yield [33].

Harvest time is an influential factor on yield, quality and quantity of active substances in medicinal plants and that environmental factors impact, in addition, for maximum oil production, long days and high light intensities are required during the maturation period for maximum oil production, and a lot of research has demonstrated the importance of harvest time and its impact factor on the quantity and quality of essential oil [34-40].

However, agricultural studies on *Satureja montana* plants in Egypt have not been investigated thoroughly until now, and the lack of previous reports on the impact of agronomic factors such as nitrogen and phosphorus fertilization on yield and essential oils of *Satureja montana*. This study aimed to evaluate the effect of nitrogen and phosphorus fertilization on the dry herb yield and essential oil content and their main constituents of *Satureja montana* L.

EXPERIMENTAL SECTION

Plant material and optimization of growing conditions

Seeds of *Satureja montana* were obtained from the HEM ZADEN B.V - P.O. Box 4 - 1606 ZG Venhuizen - The Netherlands. The seeds were sown in the nursery on 15 March, 2011 and 2012. Two months after seed sowing on 15 March, the seedlings were subsequently transplanted into the field on 15^{th} May of both seasons into 3×3.5m plots, on rows with 60 cm apart and 20 cm between seedlings, the seedlings were thinned 30 days after transplanted to leave one plant per hill to give 85 plants/plot. The design of this experiment was factorial and planned in a complete randomized block with three replications. The study contained 9 treatments, which represented all combinations between nitrogen fertilizer treatments [(N0) = 0, (N1) = 50 and (N2) = 100 kg N/fed., as urea (46% N)] was applied as a dressing application and divided into four equal portions at 30, 45, 75 and 90 days from transplanting and phosphor application treatments [(P0) = 0, (P1) = 100 and (P2) = 200 kg as calcium super phosphate (P_2O_5, 15.5%)] were applied before transplanting. This study was carried out during 2013 and 2014 growing seasons at the experimental farm of the Faculty of Pharmacy, Cairo University, Giza governorate, Egypt. Before sowing, physical and chemical properties of the soil of the experiment were determined according to Jackson [41]. The soil texture was sandy loam, having a physical and chemical composition as follows: 51.1% sand, 25% silt, 23.9 % clay and 0.47% organic matter. Soil chemical analysis was as follows: E.C (ds/m) = 4.9; pH= 8.05 and available N, P and K =0.07, 0.53 and 2.8 mg/kg, respectively.

Sample preparation

During each growing season, the plants were harvested 2 times on 15 July and 15 September, 2 and 4 months respectively, after transplantation. The plants were harvested before flowering stage at 5-10 cm above the soil. The fresh plant materials from each replicate were dried at room temperature, in a shady and well ventilated place for 3 weeks until reaching a constant weight. Dry material of whole aerial parts was weighted (g $plant^{-1}$) and essential oil content of the dry samples of each cut was determined.

Isolation of essential oils

Representative plant samples were submitted hydro distillation using a Clevenger-type apparatus according to the method described in the British Pharmacopoeia [42]. Essential oil yield was expressed as ml 100 g^{-1} dry materials), while essential oil yield per plant was expressed as ml $plant^{-1}$. The essential oils were collected and dehydrated over anhydrous sodium sulphate and kept in refrigerator until GC-MS analyses.

GC-MS analyses and identification of components

Chromatography-mass spectrometry (GC-MS) instrument stands with the following specifications. Instrument: a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a THERMO mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC/MS system was equipped with a TGWAX MS column (30 m x 0.25 mm i.d., 0.25 μm film thickness). The carrier gas was helium at a flow rate of 1.0 ml/min and a split ratio of 1:10 using the following temperature program: 40 oC for 1 min; rising at 4.0 oC/min to 160 oC and held for 6 min; rising at 6 oC/min to 210 oC and held for 1 min. The injector and detector temperatures were held at 210 oC. Diluted samples (1:10 hexane, v/v) of 0.2 µL of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40-450. Most of the compounds were identified using mass spectra (authentic chemicals, Wiley spectral library collection and NIST library).

Statistical analysis

Except for the constituents of the essential oil, the data in this study were analyzed with the analysis of variance (ANOVA) using JMP 10 program (SAS Institute, NC, USA). The mean values of treatments were compared using Tukey's HSD test. Values accompanied by different letters are significantly different at $p \leq 0.05$.

RESULTS AND DISCUSSION

Biomass Productivity

It is evident from Table (1) that N and P fertilizers significantly increased dry weight in both seasons. For individual fertilizer, fertilized plants at 100kgN/fed resulted the highest value of dry weight followed by that of 50 kgN/fed, 200 kg calcium super phosphate, and 100 kg calcium super phosphate then plants without fertilizer. Nitrogen and phosphorus is essential elements for plant growth and needed in large amounts for the biosynthesis of primary and secondary metabolites [32]. N fertilizer increased growth characters that may be due to the positive effects of N on activation of photosynthesis and metabolic processes of organic compounds in plants, which, in turn, encourage plant vegetative growth [43]. These results are in agreement with previous studies [44-48]. Phosphorus has essential functions as a constituent of nucleic acids and phospholipids (biomembranes) and plays a key role in the energy metabolism of cells. Applied P enhanced the photosynthesis and plant growth [49]. Phosphorus fertilizer increased growth and herb yield [50, 51]. While added nitrogen and phosphorus fertilizers collectively gave the best results than adding any of them singly, except (100 kg N+100 kg calcium super phosphate /fed) in the first cut of both seasons which gave lower value comparing with fertilizer treatments. Where plants fertilized with 30 Kg P (200 kg calcium super phosphate + 50 kg Nitrogen/fed) gave the heaviest weights of dry herbs followed by plats treated with (100 kg calcium super phosphate + 50 kg Nitrogen/fed), while control plants gave the lowest values in the first and second cuts of both seasons. The interaction treatments also led to significant differences in herb dry weight, however plants fertilized with (200 kg calcium super phosphate + 50 kg Nitrogen/fed) in the 2nd cut gave the best dry weight in both seasons compared to other treatments. Alsafar and Al-Hassan [52] pointed out that the impact of a mixture of nitrogen and phosphate fertilizers was best for herb and essential oil from adding both individually. Mohammadreza et al. [53] showed that both nitrogen and phosphorus fertilizers had significant affects on flowers yield, essential oil and chamazulene percent. The highest yield of dried flowers was obtained using 100 kg/ha nitrogen plus 50 kg/ha phosphorus fertilizer.

Table 1. Effect of nitrogen and/or phosphorus fertilizer on dry weight (g/plant) of *Satureja montana* in the 1st and 2nd cuts two during two seasons

Treatments	1St season		2nd season	
	1st cut	2nd cut	1st cut	2nd cut
Control (without fertilizer)	20.6±0.31h	40.9±0.5de	18.2±0.60f	38.4±0.4d
50 kg N/fed.	23.6±0.70gh	57.3±1.9c	24.2±0.79ef	60.1±0.47c
100 kg N/fed.	27.3±0.85g	63.2±1.82b	27.3±0.67e	65.2±1.89bc
15.5 kg P/fed.	22.2±0.83gh	45.3±1.20d	24.5±1.01ef	43.0±1.53d
30 kg P/fed.	25.4±1.21gh	57.8±1.30c	25.7±1.20e	59.8±2.51c
50 kg N+15.5 kg P/fed.	33.6±0.30f	74.1±0.4a	37.7±1.45d	72.5±2.3ab
50 kg N+30 kg P/fed.	36.5±1.01ef	76.6±0.9a	38.7±1.86d	72.8±1.4a
100 kg N+15.5 kg P/fed.	21.5±0.50h	58.9±1.0bc	22.5±1.04ef	63.5±0.8c
100 kg N+30 kg P/fed.	23.5±0.87gh	58.3±0.9bc	26.7±1.2e	63.6±1.9c

Numbers with different letters within the season are significantly different at $p \leq 0.05$ by Tukey's HSD test.

From the same table (1), the results also showed that plants harvested in the second cut gave higher values of dry weight compared to plants harvested in the first cut in both seasons. The increment of herb weight was significant between the two cuts. Previous studies have shown that harvest time is an important factor for the attributes growth, yield and active substances. Hegazy et al. [38] found that harvested plants at second cut gave the best values from herb in dragonhead, hyssop and sages plants compared to the first one. Said-Al AHl et al. [37] showed that harvest time affects significantly on the herb weight of parsley cultivars. The same results was obtained on *Melissa officinalis* plants, since different cutting interval dates was significantly effect on the herb dry weight.

Harvesting frequently affecting on biomass yield and essential oil yield of aromatic plants, thus it can be useful or harmful to oil production, depending on environmental factors. Kothari et al. [54] found that essential oil content was lower in the first harvest and increased gradually in subsequent harvests. Harvesting of secondary branches of *Ocimum tenuiflorum* led to maximum plant spread and number of secondary branches during second and subsequent harvests. Cutting plants above ground to allow reestablishment of new leaves for the process of photosythesis [55, 56].

Temperature plays an important role in plant growth and yield and changes in most of their metabolic activities such as photosynthesis, respiration and transpiration [57]. Weiss [55] concluded that geranium maximum leaf growth with high oil content was obtained under warm sunny conditions. The same result have acquired from Kumar et al. [58] who reported that warm conditions favored for vegetative growth of geranium. However, the growth in geranium increased under long-day photoperiods and plant dry mass as a consequence of increased chlorophyll content [59]. Letchamo and Xu [60] found that variability in shoot yield and essential oil of thyme was associated with photosynthetic activities.

Essential Oil Percentage

It seems clear from the table (2) that, fertilization treatments led to significant differences in the essential oil % and plants treated with phosphorus fertilizer (200 kg calcium super phosphate /fed.) gave the highest % of volatile oil, except of (100 kg N/fed) at the second cut in the first season. No significant differences between plants that have been fertilized with nitrogen and phosphorus individually and plants treated with interaction treatment (100 kg N/fed+100 kg calcium super phosphate /fed).While, control plants (without fertilizer) gave the lowest essential oil %. In general we can say that phosphate fertilization was more effective on oil content, followed by nitrogen fertilization and then mixed fertilization between phosphate and nitrogen. However, plants treated with phosphorus fertilizer (200 kg calcium super phosphate /fed.) in the second cut gave the highest % of volatile oil in both seasons. While control plants gave the lowest essential oil %. Previous studies have shown that application of phosphorus fertilizer increased essential oil content and oil yield [50, 51]. Phosphorus may have an impact on enzymes involved in the mevalonate pathway, which are important in the biosynthesis of terpenes [61] and thus the essential oil, seem to be affected by P fertilization [49]. Nitrogen fertilization enhances the essential oil biosynthesis processes through its role in plant metabolism and plays an important role in synthesis of the plant constituents through the action of different enzymes. Baranauskienne et al. [62] and Omer et al. [63] found that nitrogen fertilizer increased essential oil of thyme and basil. These results are in agreement with previous studies which demonstrated that the addition of phosphate and nitrogen fertilization, or the interaction between them affect positively on essential oil content [31, 33, 44, 49- 51].

From previous research the data indicated that the essential oil % in *Satureja montana* dry herb was varied depending on the origin. In Croatia, the content of essential oil (1.2 to 2.2%) [28]. In Montenegro, the content of essential oil in the herb of *Satureja montana* (1.1 to 1.9%) [7]. In Albania, essential oil % varied from 0.22 to 1.61% [64]. In Italy, (0.59%) [65]. In Poland, the content of essential oil was (1.67 to 2.37%) [9].

Table 2. Effect of nitrogen and/or phosphorus fertilizer on essential oil % of *Satureja montana* dry herb in the 1st and 2nd cuts two during two seasons

Treatments	1st season		2nd season	
	1st cut	2nd cut	1st cut	2nd cut
Control (without fertilizer)	1.02±0.02h	1.43±0.02d-f	1.07±0.02h	1.33±0.04e-g
50 kg N/fed.	1.45±0.03c-e	1.93±0.02a	1.40±0.06de	1.95±0.03a
100 kg N/fed.	1.23±0.02e-h	2.05±0.05a	1.13±0.03gh	1.98±0.06a
15.5 kg P/fed.	1.43±0.03d-f	2.03±0.07a	1.33±0.03e-g	1.93±0.04a
30 kg P/fed.	1.67±0.07bc	1.90±0.03a	1.53±0.03c-e	2.02±0.04a
50 kg N+15.5 kg P/fed.	1.12±0.04gh	1.67±0.06bc	1.15±0.03f-h	1.60±0.03cd
50 kg N+30 kg P/fed.	1.18±0.02gh	1.85±0.05ab	1.15±0.03f-h	1.85±0.03ab
100 kg N+15.5 kg P/fed.	1.28±0.08e-g	1.95±0.03a	1.37±0.09ef	1.92±0.02a
100 kg N+30 kg P/fed.	1.2±0.02f-h	1.55±0.03cd	1.2±0.03f-h	1.65±0.05bc

Numbers with different letters within the season are significantly different at $p \leq 0.05$ by Tukey's HSD test.

Essential oil content of *Melissa officinalis* was significantly affected by harvesting times [37]. Saeb and Gholamrezaee [66] reported that the essential oil yields vary considerably from month-to-month and is also influenced by the micro-environment (sun or shade) in which the plant was growing. Also, Simionatto et al. [67] reported that the oil % of *Melissa officinalis* varies greatly with a range of 0.12 % to 0.25 % during the first and second cutting. However, the essential oil of peppermint was influenced by changes in temperature. Plant dry matter, frequency of oil glands on leaves, morphological development and oil yield responded positively to higher temperatures and the leaf mass ratio showed an increase with increasing day temperature. The combination of high day and low night temperatures produced the greatest leaf mass ratio [68]. As well as, photoperiod has a strong effect on plant growth and yield production [69]. Runkle [70] concluded that photoperiod influence plant height, branching and other growth characteristics. It also had a direct impact by adjusting the metabolic pathways relevant, of photosynthetic carbon production and its partitioning to the Rohmer route (non-mevalonate pyruvateglyceraldehyde-3-phosphate driven isopentenyl pyrophosphate synthesis), which leads to generation of essential oil terpenoids [69]. These metabolic processes have a direct relevance to essential oils obtained from mints. This was evident in the three different Mentha species (*Mentha arvensis*, *Mentha citrate*, *Mentha cardiaca*) were long-day plants, exhibiting substantially higher vegetative proliferation under long day conditions. Shorter-day conditions resulted in slower growth and reduced herbage yield.

As mentioned in the herb weight, the differences in essential oil % between the first and the second cut were significant. 2nd cut gave the highest % of volatile oil extracted from dry herb in both seasons. Hegazy et al., [38] found that harvested plants at second cut gave the best values from essential oil % in dragonhead, hyssop and sages plants compared to the first one. Said-Al Ahl et al. [39] showed that harvest time affects significantly on the essential oil % of parsley cultivars. The same result was recorded on *Melissa officinalis* plants [40].

Essential Oil Yield

As is evident from the table (3), essential oil yield affected significantly by fertilizer treatments in 1st and 2nd cuts of both seasons. However, plants fertilized with (200 kg calcium super phosphate + 50 kg N/fed) was the best followed by plants treated with (200 kg calcium super phosphate/fed) in the first season or (100 kg calcium super phosphate + 50 kg N/fed) in the second season from plants harvested in 1st cut. While, in 2nd cut, plants fertilized with (200 kg calcium super phosphate + 50 kg N/fed) was the best followed by plants treated with (50 kg N/fed) in both seasons. Herein it is clear that generally the mixed fertilization of nitrogen at higher dose and phosphorus treatments were was not featured for oil yield. However, *Satureja montana* plants that have not fertilizer (control) gave the lowest oil yield values in both seasons. The same result was recorded previously by [31, 33, 44, 49- 51].

Fertilized plants with (200 kg calcium super phosphate + 50 kg N /fed) in the second cut was the best and gave the highest oil yield output of dry herb in both seasons. Harvested *Satureja montana* plants in the 2nd cut gave the more essential oil yield than plants harvested in the 1st cut in both seasons as expected result to increase of the herb dry weight and the percentage of essential oil in the 2nd cut comparing with 1st cut. The differences between 1st and 2nd cuts were significant. Hegazy et al., [38] found that harvested plants at second cut gave the best values from essential oil yield in Dragonhead, hyssop and sages plants compared to the first one. Said-Al Ahl et al. [39] showed that harvest time affects significantly on the essential oil yield of parsley cultivars. The same result was recorded on *Melissa officinalis* plants [40].

Table 3. Effect of nitrogen and/or phosphorus fertilizer on essential oil yield of *Satureja montana* dry herb in the 1st and 2nd cuts two during two seasons

Treatments	1st season		2nd season	
	1st cut	2nd cut	1st cut	2nd cut
Control (without fertilizer)	0.21±0.01g	0.59±0.01e	0.19±0.01g	0.51±0.01e
50 kg N/fed.	0.34±0.0fg	1.11±0.04c	0.34±0.03fg	1.17±0.02bc
100 kg N/fed.	0.34±0.01fg	1.30±0.07ab	0.31±0.01fg	1.29±0.07ab
15.5 kg P/fed.	0.32±0.02fg	0.92±0.02d	0.33±0.01fg	0.83±0.03d
30 kg P/fed.	0.42±0.03f	1.10±0.04c	0.39±0.03ef	1.20±0.05a-c
50 kg N+15.5 kg P/fed.	0.38±0.02f	1.24±0.045bc	0.43±0.02ef	1.16±0.02bc
50 kg N+30 kg P/fed.	0.43±0.01ef	1.42±0.05a	0.45±0.03ef	1.35±0.04a
100 kg N+15.5 kg P/fed.	0.28±0.011fg	1.15±0.01bc	0.31±0.01fg	1.22±0.01ab
100 kg N+30 kg P/fed.	0.30±0.01fg	0.90±0.03d	0.30±0.01fg	1.05 ±0.04c

Numbers with different letters within the season are significantly different at p≤0.05 by Tukey's HSD test.

The decrease in essential oil content at higher temperature was attributed to the loss of oil during water movement from the leaves epidermis as well as the evaporation of essential oil from glands when get ruptured from high temperature. It is also expected that the degree of essential oil loss by high temperature will differ based on the species, localaization of secretory structures, moisture content and other drying factors [37]. The month of harvesting had an effect on the oil yield. Weiss [55] reported that the highest oil content of geranium was observed in July and lowest in February in southern India. Furthermore, the effect of harvest month, Doimo et al. [71] reported that location area that plant grow also affected oil yield. Changes of the photoperiod may benefit essential oil yield and composition at the expense of the plant biomass. The photosynthetic input of CO_2 increased the number or the density of essential oil glands per given leaf area, or it increased both the number and the size at the same time [72]. High or low temperatures may favor the development of oil glands on leaf surfaces. In the study of Japanese mint appeared that the number of oil glands per unit leaf area on the adaxial leaf surface responded differently to high day temperature treatment than those on the abaxial leaf surface. The oil glands on the adaxial surfaces were greater in number at 35^{o}C day temperature and it was observed that they increased in number with increases in night temperature while those on the abaxial surface remained costant at all temperatures [68].

GC/MS analysis of essential oil

Sixteen compounds were detected in the dry herb at vegetative stage essential oil. The main compound of the essential oil of dry herb in the vegetative stage was carvacrol ranged from 69.15 to 80.39%. Percentage of the main compound (carvacrol) in the essential oil was affected by either nitrogen fertilizer and/or phosphorus treatments and also harvests time at the first and second cuts. Generally, carvacrol was found in the essential oil only as major component. In the same time, the maximum content of carvacrol (80.39%) was observed in the essential oil of the herb that received 50 kg N+30 kg P/fed. in the second cut. While, control plants gave the lowest content of carvacrol (69.15%) in the first cut.

From previous studies, it has proven to convert the biosynthesis from γ-terpinene to *p*-cymene and further and further to thymol [73]. Carvacrol, which similar to thymol, can be produced by a similar biosynthesis in *Satureja montan* from γ-terpinene via *p*-cymene. Chizzola [74] concluded that various chemtypes such as carvacrol or linalool or p-cymene or thymol, carvacrol and linalool chemotypes has been found in Spain [75, 76], borneol chemotype which found in France [75], thymol, geraniol and carvacrol also, found [77].Besides carvacrol, p-cymene and γ-terpinene chemotype [78]. Therefore these three compounds may be considered as the main chemotype of *S. Montana*. The amount of these three compounds is influenced by climatic conditions in different sites. Milos et al 2001 found the carvacrol content decreases whereas *p*-cymene content tends to increase, in the same time interval the γ-terpinene may increase or decrease [76]. Moreover, higher temperature and less precipitation may increase the carvacrol content [80].

The typical phenolic compound of *S. montana* is carvacrol and the prevailing carvacrol chemotype occurs [75, 81, 82] and also have carvacrol (up to 84.19%) as the main constituent [83]. Other important compounds were *p*-cymene, γ-terpinene, 1-octen-3-ol, β-myrcene, thymol, β-phellandrene, β-caryophyllene, carvacrol methyl ether, linalool, geraniol, and borneol were evidenced [29, 64, 78, 81, 84, 85]. Composition of the essential oil of *S. montana* depends on many factors, such as environmental conditions, harvest time, geographic origin, and fertilizers which seem to have a significant influence on the essential oil relative compounds concentration in *S. montana* [77, 86].

Table (4) explained that carvacrol which is the main compound (more than 10%) in the volatile oil influenced under the study treatments. Meaning that that carvacrol % increased as a result of fertilizers treatments compared to control plants (without fertilization). However, plants fertilized with (50 kg N+30 kg P/fed.) gave the highest % from carvacrol followed by plants fertilized with (30 kg P/fed.), plants fertilized with (50 kg N/fed.) and then control plants in the two cuts. Similar results from the analysis of thyme oil proved the effect of nitrogen fertilization on carvacrol content through the study carried out by Sharafzadeh [87] they found that Urea treatment when used as 100 mg N/kg (200 kg N/ha), increased p-cymene, γ-terpinene and carvacrol but decreased thymol when compared to control. In another study on thyme, Jabbari et al. [88] indicated that N application increased thymol, carvacrol and p-cymene. Kılıç et al. [89] reported that P fertilizer increased the content of thymol, p-cymene, and carvacrol in thyme oil. Hendawy et al.[90] concluded that using rock phosphate led to increase thymol, p-cymene, and carvacrol contents and decrease the contents of γ-terpinene and myrecen in thyme oil.

Table (4) also explained clearly that 1^{st} and 2^{nd} cuts have an impact on % of carvacrol compound. The second cut was the best to get the highest % of carvacrol compared to first cut and treatment (50 kgN+30 kg p/fed) in the second cut gave the highest % of carvacrol compound compared to other treatments. The rest of compounds did not have a clear trend. Thymol was decresed when carvacrol increased and vice versa. Said-Al Ahl [31] found that the volatile oil content of oregano plant from carvacrol increased in the second cut than the first cut and vice versa in p-cymene, γ-terpinene.

For the minor compounds (less than 10% and more than 1%), p-cymene (7.78 to 9.95% and 4.10 to 6.44%), γ-terpinene (3.45 to 6.43% and 2.03 to 4.02%), linalool (0.56 to 1.70% and 0.81 to 2.60%), thymol (1.78 to 2.58% and 1.28 to 3.93%) and β-caryophyllene (1.88 to 2.34% and 2.32 to 3.20%) in the first and second cuts, respectively considered as minors. The percentages of these compounds differ under the study treatments. P-cymene increased in the first cut then the second cut and vice versa in the γ-terpinene compound. The highest % of p-cymene (9.95%) and γ-terpinene (6.43%) was obtained from control plants in the first cut. While, the highest % of linalool (2.60%) and thymol (3.93%) was obtained from plants fertilized with (100 kg N/fed.) in the second cut. Moreover, harvested control plants in the second cut gave the highest of β-caryophyllene (3.20%). The rest of the compounds in the table (4) are considered as trace ones (less than 1%).

Carvacrol compound increase in hot seasons at the expense of their preceding precursors. In other words, the relative percentage of carvacrol was higher in the second cut than in the first cut. This may be attributed to the effect of the environmental factors especially non-edaphic factors, since these plants grew in summer months under high temperatures and received more solar energy than those grown in the spring months (Table 1). These conditions accelerate the transformation of terpinene and p-cymene to phenolic compounds. These findings agree with those of Omer [91] on *Origanum syriacum* and Omer et al. [92] on marjoram. In this respect, Piccaglia and Marotti [93] reported that during their two-year study differences in relative amounts of thymol, carvacrol, γ-terpinene, and p-cymene, essential oils of Satureja montana which is grown in Italy, could be attributed to the effects of environmental conditions.

The most interesting observation that the treatments showed was that the maximum carvacrol content resulted from the minimum content of γ-terpinine (2.03%). However, the maximum content of γ-terpinine (6.43%) was observed with the treatment that resulted in the minimum content of carvacrol. This observation supports the idea that terpinene and p-cymene are the biogenetic precursors of thymol and carvacrol and that there is a negative relationship between γ-terpinene and carvacrol.

The data of previous studies showed that, essential oil composition of Satureja montana differed in the relative concentration of major components depending on the geographic origin and shows of different chemotypes [86]. In Egypt, Hassanein et al. [81] found that carvacrol (79.75%), o-cymene (4.26%), 1-octen-3-ol (2.33%) and thymol (2.26%) were the major component in essential oil of S. montana. In Poland, carvacrol (76.16%), p-cymene (12.51%) and γ-terpinene (6.03%) [78]. In Spain, Similarly, carvacrol (52.2%), p-cymene (12.8%) and γ-terpinene (8.9%) [78] also, in Albania: carvacrol (2.21-55.95%), p-cymene (1.13-16.22%), γ-terpinene (0.31-8.86%) [64]. In Italy, the major components were carvacrol (18.00%), p-cymene (14.30%), thymol (9.92%), β-phellandrene (5.60%), β-caryophyllene (4.97%), carvacrol methyl ether (4.86%) and linalool (4.81%) [65]. From two different localities in Bosnia and Herzegovina, essential oil contained thymol (3.8-31.7%), carvacrol (10.6-23.3%), geraniol (0.1-22.3%) and caryophyllene oxide (5.2-7.7%) as major components [29]. In Croatia, carvacrol (63.4%), thymol (19.4%) and borneol (4.2%) as the main components [85]. In another study in Croatia, Bezic et al. [94] reported that, carvacrol (13.7%), p-cymene (11.8%) and γ-terpinene (10.6%) as the main components. In Serbia, the essential oil of S. montana contained carvacrol only in 0.4-1.1% [95]. In contrast Wesolowska et al. [9] reported that the content of carvacrol (65.43-69.99%) found S. Montana. In Tunisia, carvacrol (53.35%), γ-terpinene (13.54%), and p-cymene (13.03%) [96]. Another study in Montenegro by Damjanovic-Vratnica et al. [7] found that thymol (37.36% and 27.68%), carvacrol (15.47% and 4.40%), γ-terpinene (11.75% and 8.66%) and p-cymene (7.86% and 31.37%) before and after flowering, respectively.

Table 4. Effect of nitrogen and / or phosphorus fertilizer on the constituents of *Satureja montana* during the 1st and 2nd cuts

Component	1st cut				2nd cut			
	T1	T3	T5	T7	T1	T3	T5	T7
α-thujene	0.23	0.29	0.32	0.40	0.07	0.20	0.10	0.22
α-pinene	0.24	0.56	0.69	0.89	0.12	0.26	0.18	0.44
β-pinene	0.90	0.87	0.47	0.89	0.49	0.32	0.12	0.20
1-octen-3-ol	0.28	0.45	0.68	0.46	0.35	0.71	0.51	0.30
β-myrcene	0.66	0.56	0.39	0.94	0.61	0.30	0.95	0.54
α-terpinene	0.65	0.29	1.03	0.68	0.52	0.60	0.45	0.36
p-cymene	9.95	9.58	7.78	8.14	5.70	6.44	4.80	4.10
limonene	0.56	0.57	0.78	0.25	0.38	0.11	-	0.06
eucalyptol	0.55	0.43	0.31	0.43	0.52	0.22	0.14	0.65
γ-terpinene	6.43	5.82	6.30	3.45	4.02	2.12	3.80	2.03
linalool	1.70	1.45	1.23	0.56	1.34	2.60	1.80	0.81
1-terpinen-4-ol	0.56	0.78	0.83	0.59	0.90	0.98	0.79	0.86
thymol	1.78	2.58	2.56	2.48	3.23	3.93	1.75	1.28
carvacrol	69.15	70.22	70.90	72.83	75.10	76.13	78.05	80.39
β-caryophyllene	2.04	1.89	1.88	2.34	3.20	2.40	2.32	2.94
caryophyllene oxide	0.37	0.23	0.35	0.31	0.13	-	0.56	0.22

Since; T1=control, T3= plants fertilized with 100kg N/fed., T5= plants fertilized with 30kg P/fed., T7= plants fertilized with 50 kgN+30 kg p/fed.

CONCLUSION

Results from the essential oil analysis from Satureja montana plant harvested in the 1st and 2nd cuts had similar compositions. The major compound was carvacrol. Other compounds were found with percentage less than 10% and more than 1%, which were p-cymene, γ-terpinene, linalool, thymol and β-caryophyllene. The second cut and fertilized plants with (50 kgN+30 kg p/fed) was the best for harvesting the plants with respect to high dry matter, oil yield and carvacrol. Overall, Chemical composition of Satureja montana essential oil may be linked to the geographic origin of the population and environmental conditions in which they grow such as temperature, light, and nutrient supply from the rest of the environment that affect the composition of the essential oils. Satureja montana essential oils characterized by high amounts of carvacrol up to 80% , carvacrol is very important, since this aromatic monoterpene holds a significant commercial interest, as it is a known antimicrobial, antiseptic and antioxidant agent with low acute toxicity and weak genotoxic potential. It is approved by both the Food and Drug Administration (FDA) and the European Commission (EC) as an additive [97]. So we recommended cultivation Satureja montana in Egypt as an interesting source of carvacrol component used as potent agents in food preservation and for therapeutic or nutraceutical industries.

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