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AGRONOMIC YIELD AND ESSENTIAL OIL PROPERTIES OF PURPLE CONEFLOWER (*Echinacea purpurea* L. MOENCH) WITH DIFFERENT NUTRIENT APPLICATIONS

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ABSTRACT

***Echinacea purpurea* is cultivated around the world due to its unique pharmacological effects. The aerial parts of the plant, especially its flowers, contain a wide variety of beneficial bioactive substances. The objective of this study was to evaluate the effect of different nutrient applications on the growth parameters, essential oil yield, and compounds of *Echinacea purpurea* L. Moench in Afyonkarahisar/Turkey. The experiment was conducted over a 3-year period (2016-18), including four experimental treatments (F_0: control, F_1: 75 kg ha^{-1} N; F_2: 150 kg ha^{-1} N; and F_3: 75 kg ha^{-1} N + foliar fertilizer). As a general result of five cuttings, F_2 and F_3 had a positive effect on agronomic yield. F_2 and F_3 recorded the highest plant height (91 and 90 cm, respectively) and yields for fresh bud (578 and 543 kg ha^{-1}), dry bud (118 and 112 kg ha^{-1}), fresh flower (8,595 and 7,449 kg ha^{-1}), dry flower (2,021 and 1,745 kg ha^{-1}), fresh herb (32,645 and 29,291 kg ha^{-1}), dry herb (8,746 and 7,745 kg ha^{-1}) and essential oil (4.55 and 3.57 L ha^{-1}). Sesquiterpene hydrocarbons were the most abundant chemical group compound of *E. purpurea* essential oil. Germacrene D (20.4-50.6%) was the predominant constituent, recording its maximum level in F_1. Other major compounds were β-pinene, β-myrcene, α-humulene, δ-cadinene, spathulenol, and α-cadinol. The application of 150 kg ha^{-1} N as well as the combined use of 75 kg ha^{-1} N and foliar application of macro and micro elements resulted in the highest agronomic yield and essential oil production.**

Keywords: *Echinacea purpurea*, fertilizer, foliar, GC-MS, nitrogen.

INTRODUCTION

Purple coneflower or echinacea (*Echinacea purpurea* L. Moench) is a perennial herbaceous plant belonging to the Asteraceae family from North America (Abdallah, 2016; Ghatas and Abdallah, 2016). At present, this plant is cultivated commercially worldwide, being widely used in the pharmaceutical industry, especially in Europe and the United States (Parsons et al., 2018). *Echinacea* species have been traditionally used by Native Americans for their analgesic properties to treat headaches, stomachaches, and coughs (Yeşil and Kan, 2013). Various studies have described the immunostimulatory, anti-inflammatory, antianxiety, antidepression, cytotoxicity, antimutagenicity, antioxidant, antibacterial, antiviral, and larvicidal activities of *E. purpurea* (Manayi et al., 2015). Its roots, leaves, and flowers synthesize various secondary

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metabolites (Senica et al., 2019). Terpenes, flavonoids, phenolic compounds, and fatty acids have been identified as the predominant chemical constituents of *E. purpurea*, with varying contents in different parts of the plant (Coelho et al., 2020). p-Cymene (0.8-2.4%), β-caryophllyene (2.1-5.5%), α-humulene (1.5-4.2%), germacrene D (12.7-42.3%), α-muurolene (0.9-2.2%), δ-adinene (0.5-2.5%), nerolidol (0.4-3.6%), (E)-β-farnesene (1.0-2.8%), germacra-4 (15),5,10 (14)-trien-1-α-ol (1.1-4.7%), and shyobunol (1.7-4.9%) are among the major components of essential oil obtained from flowers of *E. purpurea* (Kaya et al,. 2019).

The genetic characteristics of the plant, climatic conditions, edaphic factors, agricultural practices, harvest time, and post-harvest management affect both the growth and chemical properties of medicinal and aromatic plants (Soltanbeigi and Sakartepe, 2020). Fertilizing is one of the most critical agricultural practices that improve the yield and secondary metabolite concentration of medicinal and aromatic plants (Omidbeigi, 2013). Tillage in accordance with the geographical conditions and balanced use of different macro and micronutrients in the cultivation of echinacea is one of the most important requirements for achieving optimal yield (Yarnia et al., 2012). Nitrogen, phosphorus, and potassium are the structural constituents of proteins and vital for the natural growth of medicinal plants, especially in the reproductive organs (Arvin, 2019). The adequate supply of nitrogen and phosphorus fertilizers in plants, whose flowers are part of their economic performance, is critical. In fact, it directly affects flowering continuity, fresh and dry weight of flowers, and essential oil yield (Fariborzi, 1999). Micronutrients (such as B, Zn, Mn, Mo, Cl, Cu, and Fe), as the main constituents of enzymes and act as functional, structural, or regulatory cofactors, are related to saccharide metabolism, protein synthesis, and photosynthesis ratio (Marschner, 1995). Although conventional fertilization is done through the roots, foliar application of nutrients has been considered as an alternative practice, with an immediate impact on nutrition management, especially for micro elements (Bernal et al., 2007). Various studies have positively evaluated the effect of different fertilizer sources on yield components and some chemical properties of *E. purpurea* (Abdallah, 2016; Ghatas and Abdallah, 2016; Ahmadi-Samadi and Rahimi, 2020).

The objective of this study was to evaluate the effect of different nutrient applications on the growth parameters, essential oil yield, and compounds of *Echinacea purpurea* L. Moench in Afyonkarahisar/Turkey. The experiment was conducted over a 3-year period (2016-18). Two doses of nitrogen (75 kg ha^{-1} and 150 kg ha^{-1}) and a foliar application of various macro and micro elements were used.

MATERIALS AND METHODS

Plant material and experimental location

Young seedlings obtained from *E. purpurea* seeds with 85% germination capability and 100% purity were used. The experiment was conducted in the research field of Afyonkarahisar Medicinal and Aromatic Plants Center/Turkey (38° 46′ N, 30° 30′ E). The climate of the region is harsh and moderately rainy. Most precipitation occurs in winter and spring. Summers are hot and dry, while winters are cold and snowy. Meteorological data of the region are given in Table 1.

Treatments and design

After field preparation, such as plowing (at a depth of 45 cm), soil samples were taken at depths of 30 and 60 cm and physico-chemical properties were determined (Table 2). The experiment was set up in a randomized complete block design (RCBD) with three replications. The study included a total of twelve experimental plots. The seedlings were transferred on 16 May 2016 (8-10 leaves stage) with a plant density of 50 × 60 cm. Each plot was formed from six planting lines and ten plants on the lines. Four experimental treatments were used: F_0: 0 fertilizer/control; F_1: 75 kg ha^{-1} N; F_2: 150 kg ha^{-1} N; and F_3: 75 kg ha^{-1} N+ foliar fertilizer. The chemical characteristics of the foliar fertilizer are provided in Table 3. Two-thirds of the N was applied at a depth of 5 cm to the root area simultaneously with transplanting. The remaining fertilizer (N) was applied when the first buds appeared. In the following years, half of N was applied at the beginning of the growing season in two steps (1st step: at the end of winter; 2nd step: at the budding stage). The remaining half was added after the 1st cutting (immediately after cutting and at the beginning of the budding stage). A drip irrigation system was installed to ensure that all plots receive equal amounts of water. The first foliar fertilizer was applied 77 days after transplanting and repeated two times at 14 days intervals. Thus, spraying was done three times in each cutting (Table 4). Weed control was conducted mechanically during the growing seasons.

Harvesting and records

During the 3-year trial, a total of 5 cuttings were made (Table 4) at the flowering stage. To determine plant height, eight plants were randomly selected after removing the border effect. The selected plants were cut from 8-10 cm soil surface and

Table 1. Local meteorological data in the study site during the study period (2016-18).

Month/Year	Temperature (°C)								
	Minimum			Maximum			Mean		
	2016	2017	2018	2016	2017	2018	2016	2017	2018
January	-2.8	-5.6	-1.4	5.7	1.2	7.3	1.1	-2.4	2.2
February	2.4	-1.9	2.1	14.1	7.8	11.7	7.7	2.6	6.4
March	2.1	2.3	5	13.5	13.5	15.8	7.5	7.6	9.8
April	6.8	3.9	6.7	21.3	16.8	21.5	14	10.3	14.3
May	8.8	9.0	11.3	21.2	20.8	23.3	14.8	14.6	16.9
June	14.2	12.7	13.5	28.2	26	26.1	21.4	19.4	19.5
July	15.7	16.9	15.8	31.6	31.8	29.7	23.6	24.5	22.7
August	16.3	15.9	16.2	31	29.9	30.2	23.4	22.6	23.1
September	11.3	13.0	12.4	25.5	29.3	26	18.2	21	19.1
October	8.1	6.4	7.7	20.6	18.3	20.5	13.9	11.9	13.4
November	1.2	2.0	3.2	14.2	12.8	13.1	7.2	6.6	7.7
December	-4	0.6	-0.5	3.1	10	5.8	-0.8	4.5	2.5

Month/Year	Rain (mm)			Average Relative Humidity (%)			Insolation (h)		
	2016	2017	2018	2016	2017	2018	2016	2017	2018
January	63	39.7	37.1	71.6	78.2	75.4	86.2	64.1	92.8
February	12.5	1.3	19.7	63.3	68	69.4	140	102.6	73.2
March	48.2	29.2	45.3	59.3	58.9	58.1	142.1	135.7	129.6
April	31.5	43.9	15.5	49.7	55.8	47.8	241.4	178.3	228.4
May	60.1	0.6	80.1	60.5	64.8	60.1	185.5	123.7	161
June	13.8	32.2	131.4	48.8	61	60.1	277.7	200.4	196.9
July	28.2	4.4	11.9	46.0	44.2	51.9	312.2	275.3	281.1
August	29.7	59.1	7.8	53.1	52.6	50.2	271.4	255.7	262.3
September	30.4	7.1	1.5	54.6	38.9	51	228.4	228.2	234.4
October	6.7	38.3	37.2	57.9	60.3	65	179.9	153.1	182.4
November	28.1	28.5	44.7	58.2	68.8	70.1	152.6	127.3	107.1
December	42.5	24.7	84.1	74.8	73.6	82	74.8	95.2	57.1
Total	394.7	309	516.3						

Source: Regional Directorate of State Meteorology

Table 2. Physico-chemical properties of experimental field soil at depths of 30 and 60 cm.

Properties	30 cm	60 cm	Elements	30 cm	60 cm
Organic matter (%)	0.34	0.27	Ca (ppm)	3952	4999
Total N (%)	0.10	0.08	Mg (ppm)	624	747
Sand (%)	52.99	48.61	K (ppm)	344	307
Clay (%)	32.70	32.89	Na (ppm)	50	838
Dust (%)	14.31	18.50	Fe (ppm)	1.07	1.08
Lime (%)	1.88	2.01	P (ppm)	73	49
EC (mS cm^{-1})	0.15	0.17	Cu (ppm)	0.75	0.62
pH	8.44	8.74	Zn (ppm)	1.12	0.86
			Mn (ppm)	6.03	3.50

Soil class: sandy clay-loam

Table 3. Components of the foliar fertilizer used.

Element	(% v w^{-1})
Total N	9.2
Nitrate nitrogen (N)	4.4
Ammonium nitrate (N)	1.4
Urea nitrogen (N)	3.4
Water-soluble phosphorus pentoxide (P_2O_5)	6.8
Water-soluble potassium oxide (K_2O)	18.2
B	0.10
Cu* (EDTA chelated)	0.021
Fe** (EDTA chelated)	0.05
Mn* (EDTA chelated)	0.02
Mo	0.005
Zn* (EDTA chelated)	0.051
* pH range that chelate is stable:	pH 2-11
** pH range that chelate is stable:	pH 2-6.5

Table 4. Cutting times and foliar fertilizer application dates.

Replication	1st year (2016)	2nd year (2017)		3rd year (2018)		Dosage
	1st Cutting	2nd Cutting	3rd Cutting	4th Cutting	5th Cutting	
1	1 Aug	22 May	28 Aug	7 May	20 Aug	300 ml 100 lt^{-1}
2	15 Aug	5 Jun	11 Sep	21 May	3 Sep	300 ml 100 lt^{-1}
3	29 Aug	19 Jun	25 Sep	4 Jun	17 Sep	300 ml 100 lt^{-1}
Cuttings Date	24 Oct	31 Jul	30 Oct	17 Jul	10 Nov	

weighed for yield estimation. Then leaves, stems, flowers, and buds were separated and weighed again. Primary and secondary branches, flowers, and buds were dried in a drying cabinet at 37 °C for 96 hours and weighed.

Isolation of essential oils

For isolation of the essential oil, 200 g of dried and powdered flowers were extracted with 2000 ml of distilled water using a neo-Clevenger type apparatus. Hydro-distillation was performed for 3 h. The obtained essential oils were dried over anhydrous sodium sulfate and stored in amber vials at +4 ºC.

Identification and quantification of compounds of essential oil

A gas chromatography (GC) system (Agilent Technologies, 7890B), which was equipped with a flame ionization detector (FID) and coupled to a mass spectrometry detector (MSD) (Agilent Technologies, 5977A), was used to identify the chemical components of the essential oils. Compounds were separated using an HP-Innowax column (Agilent 19091N-116: 60 m × 0.320 mm internal diameter and 0.25 **µm** film thickness). The carrier gas was helium (99.999%) with 1.3 mL min^{-1} flow rate. Injection volume was set at 1 **µl** (20 **µL** essential oil was dissolved in 1 mL n-Hexane). The solvent delay time was 8.20 min. The injection was performed in split mode (40:1). The samples were analyzed with the column held initially at 70 °C after injecting with 5 min hold time. Then, the temperature raised to 160 °C with 3 °C min^{-1} heating ramp and 5 min hold time. Eventually, the temperature reached 250 °C with 6 °C min^{-1} heating ramp and 5 min hold time. The detector, injector, and ion source temperatures were 270 °C, 250 °C, and 230 °C, respectively. MS scan range was (m z^{-1}): 50-550 atomic mass units (AMU) under electron impact (EI) ionization (70 eV).

The retention indices (RI) were determined by injecting C7-C30 n-alkanes (Sigma-Aldrich) to (GC/FID) system (Agilent Technologies, 7890B) under the same conditions of the analyses of the essential oils. The components of the essential oil were identified by comparison of retention indices, mass spectra by the computer library database of the US National Institute of Standards

and Technology (NIST), Wiley libraries, and other published mass spectra data (Adams, 2017), and our database. Relative abundance (% area) was calculated based on the ratio between the peak area of each compound and the sum of the areas of all the compounds.

Data analysis

The MSTAT-C computer software program was used for the analysis of variance (ANOVA). To compare means, the Least Significant Differences (LSD) test was used at $p \leq 0.05$ probability level. The analysis of variance was conducted on the samples to determine variations in the parameters between the effects of the fertilizers and the number of cuttings.

RESULTS AND DISCUSSION

Plant height

Mean comparisons indicated that fertilizers and cuttings had significant effects on plant height (Table 5). F_2 (150 kg N) and F_3 (combined application of 75 kg N and foliar fertilizer) treatments with the same statistical group had the highest plant height. F_0 (control group) recorded the lowest values. As the amount of fertilizer applied increased, plant height also increased. The tallest and shortest plants were observed in the 2nd and 3rd cuttings, respectively (Table 5).

Macro elements such as N, P, and K are essential for plant growth and development. As a principal constituent of chlorophyll, N also has a vital role in cell division and enlargement (Purbajanti et al., 2019). In the present study, plants with access to different nutrient sources, especially nitrogen, grew more morphologically. According to Youssef (2014), consumption of micronutrients resulted in increased plant height of *E. purpurea* (75-82 cm) compared to the control treatment (71-72 cm). A study conducted by Ghatas and Abdallah (2016) showed that increasing nitrogen rates produced taller plants (65 to 86 cm). In our study, the highest values in terms of plant height were observed in the 1st cuttings of each year, which can probably be related to a more extended period of vegetation, high spring precipitation, and relative humidity (Soltanbeigi and Özgüven, 2021). As a morphological feature, plant height can be affected by intrinsic characteristics, ecological factors, and agricultural management.

Fresh and dry herb yield

Fresh and dry herb yields (aerial parts of the plant) were affected by fertilizer levels and cutting times (Table 5). The maximum fresh and dry herb yields of *E. purpurea* were recorded in the F_2 and F_3 treatments followed by F_1. The difference between maximum and minimum (control group) fresh and dry herb yields was about 45%. Similar trends were observed in dry herb yield and fresh yield. The 2nd and 4th cuttings recorded the highest production of biomass. This finding showed that the 1st cuttings of each growing season resulted in a higher production potential than the following cuttings. However, this was not observed in the first year, in which only one cutting was performed. Since dry biomass is directly related to fresh biomass, the results of these parameters have the same variation pattern. In addition, dry biomass is also affected by ecological conditions. While it seems that herb production gradually reduced with the aging of plants, the 1st cuttings of each year had the highest fresh and dry herb yields. The low herb yield recorded in the 1st year is probably due to poor plant growth and development after planting, particularly of the root system. The decreasing yield in the 2nd cuttings of each year is probably related to short day length, the difference between night and day temperature, low temperatures during the growing season, and lack of rainfall. As the vital plant nutrient source, N controls plant growth and has the largest share in the structure of plant protein molecules (Barzegar et al., 2020). A study conducted by Akanbi et al. (2007) showed that N also causes an increase in production and accumulation of dry matter in plants. Similarly, another study revealed that increasing amounts of N (238, 476, and 714 kg ha^{-1}) resulted in increased fresh and dry yield of *Echinacea paradoxa* (El-Sayed et al. 2012). Isazadeh-Hajagha et al. (2017) reported that by adding 120:60:60 kg ha^{-1} of NPK, fresh herb weight of *E. purpurea* increased from 7,242 to 11,500 kg ha^{-1} in 1st year and from 18,120 to 28,770 kg ha^{-1} in the 2nd year. The authors reported that dry herb weight increased from 2,852 to 4,735 kg ha^{-1} and from 5,257 to 9,441 kg ha^{-1} for the 1st and 2nd year, respectively. Furthermore, El-Sayed et al. (2012) and Isazadeh-Hajagha et al. (2017) reported that yield tended to be higher in the 2nd year compared to the 1st year of the experiments.

Fresh and dry flower yield

The comparative assay of fertilizers and cuttings showed a significant difference in fresh and dry flower yield (Table 5). F_2 produced the highest fresh and dry flower yield, followed by F_3, F_1, and F_0 with the different statistical groups. Data related to different fertilization regimes on flower yield in *E. purpurea* were divided into various statistical groups. Comparison of these results shows that generative plant growth, rather than herb yield, is extremely affected by fertilization. The difference between the minimum and maximum flower

Table 5. Agronomic parameters and essential oil yields of *E. purpurea* affected by various fertilizer levels and cutting times.

Treatments	Plant Height (cm)	Fresh Herb Yield (kg ha^{-1})	Dry Herb Yield (kg ha^{-1})	Fresh Flower Yield (kg ha^{-1})	Dry Flower Yield (kg ha^{-1})	Fresh Bud Yield (kg ha^{-1})	Dry Bud Yield (kg ha^{-1})	Essential Oil Yield (L ha^{-1})
Fertilizers (F)								
F_0 (Control / 0 N)	64±29.2c	14109±10768c	3817±3277c	3832±3468c	913±880c	253±77.4b	52±20.9b	1.54±1.5d
F_1 (75 kg ha^{-1} N)	78±34.7b	19972±14983b	5366±4624b	5664±5134b	1372±1353b	333±103.9b	68±21.3b	2.44±2.5c
F_2 (150 kg ha^{-1} N)	91±34.9a	32645±22888a	8746±6947a	8595±7185a	2021±1800a	578±156a	118±45.1a	4.55±4.2a
F_3 (75 kg ha^{-1} N + Foliar)	90±36.7a	29291±20183a	7745±6069a	7449±6349a	1745±1583a	543±162a	112±38.8a	3.57±3.4b
Probability level	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01
LSD $_{(\% 5)}$	4.269	4598	1395	1266	314.3	148.5	32.80	0.86
Cutting Time								
1st Cut. (1st year)	78±16.6b	15318±5606b	3693±1373c	4684±1709b	978±360c	537±169a	108±33.2a	1.74±0.9c
2nd Cut. (2nd year)	117±18.6a	43775±12696a	12930±3600a	12383±3431a	3256±870a	417±177ab	98±40.6ab	6.68±2.5a
3rd Cut. (2nd year)	44±9.7d	9612±3986c	2100±831d	1156±339c	256±81d	372±173ab	66±29.3bc	0.49±0.23d
4th Cut. (3rd year)	112±11.8a	41417±16264a	11136±4545b	12161±4203a	2750±951b	513±248a	114±56.4a	5.59±2.63b
5th Cut. (3rd year)	52±7.8c	9898±4043c	2233±917d	1540±938c	323±158d	293±101b	51±18.8c	0.63±0.37d
Probability level	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.05	P≤0.01	P≤0.01
LSD* $_{(\% 5)}$	4.77	5141	1560	1415	351.4	166.0	36.67	0.96

*LSD: least significant difference

yields was about 45%. In addition, the highest amount of fresh and dry flower yields in the present experiment was recorded in the 2nd and 4th cuttings. Most of the bioactive ingredients of *E. purpurea*, which have an important role in the pharmacy industry, accumulate in the flowers of the plant. Thus, flowers of *E. purpurea* have primary economic importance. As can be deduced from the results, the increase in the amount of fertilizer resulted in increased flower production. The size and number of flowers are the determining factors of flower yield. Goldani et al. (2016) reported an increase in the number and weight of *E. purpurea* flowers due to increased nitrogen doses and explained that the availability of nutrients, particularly nitrogen, reduces inter-plant competition and improves flower yield. According to Ghasemi et al. (2019), the application of nitrogenous organic compounds improved growth parameters such as number of flowers, and in turn flower yield. Nitrogen is involved in the biosynthesis of protein compounds, enzymes, metabolic intermediates, compounds presented in the production of assimilate and energy transfer, and even in DNA structure, which is responsible for transmitting hereditary properties (Khorsand, 2011). Therefore, nitrogen deficiency disrupts the biosynthesis of basic compounds and affects the vegetative and reproductive growth of the plant. On the other hand, plants use micro nutrients in negligible amounts. These elements have a strong bond with other soil components. Therefore, if the edaphic conditions are not suitable, the plant will not absorb these elements optimally. Therefore, foliar application of micronutrients is readily available to the plant and is spent on physiological activities (Khathutshelo et al., 2016). In the present results, the increasing effect of foliar application of micro and macro elements was evident in the production of *E. purpurea* flowers.

Fresh and dry bud yield

The fresh and dry bud yield means revealed significant differences in fertilizers and cuttings (Table 5). The highest fresh and dry bud yields were observed in the F_2 and F_3 treatments in the same statistical group and in the 1st and 4th cuttings. The cuttings of the 2nd year of the experiment had moderate bud yield. The last cutting also produced the lowest number of buds. The application of different amounts of nitrogen and the simultaneous foliar application of essential plant growth elements showed an additive effect in improving bud yield. Although the bud has no economic value compared to the mature flower, it is considered delete one of the yield components that affects the final yield. The reason for observing buds at harvest time is the heterogeneous flowering process of *E. purpurea*. Since *E. purpurea* is a cross-pollinated plant, disruption of the pollination process can alter flower formation trends (Chen et al. 2008). Isazadeh-Hajagha et al. (2017) indicated that the number of buds in *E. purpurea* increased when different fertilizer sources were applied. The maximum increase was observed in the NPK treatment. The authors reported that *E. purpurea* fresh and dry bud yields were 525-1,334 and 260-591 kg ha^{-1}, respectively. Since the number of flowers or buds is related to vegetative growth, changes in growth parameters also affect reproductive production. Therefore, changes in bud yield can be explained in different cutting times. The highest bud yield was observed in the 1st and 4th cuttings. It seems that the lack of full development of the plants in the 1st year and possibly the aging of the plants in the last year of the experiment led to the heterogeneous flowering of the plant and the incomplete maturation of the flowers. When the plants reached their maximum yield (2nd year), bud yield was lower.

Essential oil yield

The effect of different fertilizer levels and the number of cuttings on the yield of *E. purpurea* essential oil was significant (Table 5). The highest essential oil yield was related to the F_2 treatment, followed by the F_3, F_1, and F_0 treatments. Most of the essential oil production was observed in the 2nd cutting. The 4th cutting ranked statistically in second place, followed by the 1st, 5th, and 3rd cuttings. The 1st cutting of each year recorded values that were considerably higher than those of the 2nd cutting. The essential oil yield of oil-bearing species, in addition to its intrinsic and environmental characteristics (essential oil percentage), is a function of dry flower yield. The action of essential oil yield data was similar to the flower yield of *E. purpurea*. Although secondary metabolites are primarily made by directing genetic processes, their production is considerably affected by environmental factors, agricultural practices, and post-harvesting management (Sedlakova et al., 2003; Soltanbeigi, 2020). Furthermore, the essential oil is a terpenoid compound. The constituent units of terpenoids such as isopentenyl pyrophosphate and Dimethylallyl pyrophosphate are in dire need of NADPH and ATP. In addition, elements such as nitrogen are necessary for the formation of bioactive compounds (Janmohammadi et al. 2014). Essential oil biosynthesis increases in long days and with high light intensity (Fernandes et al. 2013). Since the 1st cutting of each year has a long vegetative period, plants can use

food sources effectively. Hence, the amount of essential oil production also increases. Sati (2012) determined the essential oil yield of *E. purpurea* was 0.3-0.9 and 1.1-1.8 L ha^{-1} for the 1st and 2nd years, respectively.

Essential oil components

A total of 44 compounds were identified for all the samples of *E. purpurea* essential oil during the chromatographic analysis (Table 6). The highest number (38-40) of chemical compounds was observed in the control treatments. Conversely, F_2 had the lowest number of identified compounds. It seems the increasing amounts of nitrogen and foliar fertilizer reduce the biosynthesis of some compounds of *E. purpurea* essential oil. Sesquiterpene hydrocarbons (32.6-63.8%) were the most abundant compounds in *E. purpurea* essential oil (Table 7). The highest amount of sesquiterpene hydrocarbons was observed in the F_1 treatments. In general, as the number of cuttings increased, the amounts of this chemical group also increased. Oxygenated sesquiterpenes formed the next high category of important compounds (11.3-41.6%). The high amounts of these compounds were observed in the F_3 treatments except for the 2nd cutting. Unlike sesquiterpene hydrocarbons, oxygenated sesquiterpenes were significantly reduced by a higher number of cuttings. Monoterpene hydrocarbons (8.2-30.6%) as the 3rd most abundant compounds were found in *E. purpurea* essential oil. Except for the 1st cutting, most of these compounds were present in the F_2 treatments. Oxygenated monoterpenes (0.9-6.5%) were other constituents of *E. purpurea* essential oil. These compounds decreased sharply as the number of cuttings increased. Phenolics (0.7-2.5%) and esters (0.4-3.2%) were also other constituents of *E. purpurea* essential oil. Except for the 1st cutting, esters were not present in the F_1 and F_2 treatments (Table 7). As a sesquiterpene hydrocarbon, Germacrene D (20.4-50.6%) was the predominant constituent in all essential oil samples. The minimum and maximum germacrene D levels were observed in the F_3 treatment of the 1st cutting and the F_1 treatment of the 2nd cutting, respectively. In general, the highest amounts of germacrene D were observed in the F_1 treatment of the 5th cutting (Table 6). l-Phellandrene (1.9-14.3%), caryophyllene (2.9-8.5%), o-cymene (1.5-9.3%), α-pinene (1.9-7.3%), caryophyllene oxide (1.8-6.6%), salvial-4(14)-en-1-one (1.3-6.7%), spathulenol (1.6-5.7%), β-pinene (1.8-4.1%), α-cadinol (0.7-4.0%), δ-cadinene (1.4-3.5%), α-humulene (1.4-2.9%) and germacrene D-4-ol (0.6-3.0%) were also identified as main components (Table 6 and 7). The chemical components of essential oils are affected by many endogenous and exogenous factors. Production of secondary metabolites and their qualities are directly related to genetic characteristics, climatic conditions (light, temperature, rainfall, irrigation, soil, height, location, etc.), environment organisms, applied agro-techniques, and post-production processing (Soltanbeigi and Sakartepe, 2020). Based on German Homeopathic Pharmacopoeia, *E. purpurea* contains 0.08-0.32% essential oil. Borneol, bornyl acetate, pentadeca-8-(Z)-en-2-one, germacrene D, caryophyllene, and caryophyllene epoxide are the main compounds of this plant's plant essential oil (EMA/HMPC, 2017). A study by Diraz et al. (2012) reported that germacrene D (11.3%), caryophyllene oxide (8.7%), β-caryophyllene (7.2%) and α-cadinol (6.2%), δ-cadinene (3.3%), 1,5-epoxysalvial-4(14)-ene (3.3%), α-phellandrene (2.9%), *p*-cymene (2.65), 3,4,-Difloro-4-methoxybiphenyl (2.7%), *trans*-(Z)-α-bisaboleneepoxide (2.3%), α-bisabolene (2.3%), β-elemene (2.1%) and α-cadinene (2.0%) were the major components of essential oil obtained from flowers of *E. purpurea*. The authors also determined the amounts of monoterpene hydrocarbon, sesquiterpene hydrocarbons, and oxygenated sesquiterpene, reporting values of 5.4, 22.8, and 10.4%, respectively. A study conducted by Mirjalili et al. (2006) revealed that germacrene D (57%) was the major compound of *E. purpurea* essential oil, while the following compounds were also identified: sesquiterpene hydrocarbons (70.9%), oxygenated sesquiterpenes (15.4%), and monoterpene hydrocarbons (6.4%). In another study on *E. purpurea* germacrene D (7.2-33.5%), myrcene (10.5-26.1%), β-pinene (tr-13.0%), β-caryophyllene (0.5-9.3%), α-pinene (1.7-10.3%) and limonene (1.0-6.1%) were identified as the main components of flower essential oil (Thappa et al. 2004). As previously discussed, the production and variations of secondary metabolites depend on many endogenous and exogenous factors. Hence, changes in essential oil constituents are hard to interpret.

CONCLUSIONS

This study evaluated the agronomic yield and biochemical properties of *Echinacea purpurea* L. Moench by applying different fertilizers over a three-year period under the ecological conditions of Afyonkarahisar/Turkey. Fertilizer applications (75 kg ha^{-1} N, 150 kg ha^{-1} N, and 75 kg ha^{-1} N + foliar fertilizer) resulted in improved growth and chemical properties of the plant compared to the control treatment. The application of 150 kg ha^{-1} N and 75 kg ha^{-1} N + foliar fertilizer produced the greatest aerial part yields and essential oil production. However, the use of 150 kg ha^{-1} N was relatively effective. The final yield of the

Table 6. Compounds of *E. purpurea* essential oil affected by various fertilizer levels and cutting times.

RI*	Compounds (%)	1st Cutting				2nd Cutting				3rdCutting				4th Cutting				5th Cutting			
		F_0	F_1	F_2	F_3	F_0	F_1	F_2	F_3	F_0	F_1	F_2	F_3	F_0	F_1	F_2	F_3	F_0	F_1	F_2	F_3
1031	α-Pinene	4.72	4.27	4.32	4.14	5.52	2.95	5.04	7.31	5.34	2.9	5.18	4.18	4.32	2.83	3.17	1.96	3.58	2.5	4.19	5.6
1120	β-Pinene	2.5	3.22	3.65	1.99	3.02	2.5	4.15	3.76	2.53	2.43	2.36	2.33	2.62	2.4	3.82	2.39	2.06	2.14	1.82	3.19
1130	Sabinene	0.48	-	-	-	0.55	-	-	0.8	0.49	-	-	-	0.42	-	-	-	0.32	-	-	-
1167	β-Myrcene	2.02	-	-	-	0.44	-	1.36		1.9	-	2.79	-	0.65	-	4.95	-	3.53	0.58	4.94	1.45
1174	l-Phellandrene	5.88	5.71	9.27	3.99	4.21	5.36	10.7	3.71	6.2	5.2	11.4	-	2.08	5.16	14.4	1.96	8.73	6	14.4	7.4
1209	Limonene	0.63	0.8	-	-	0.71	1.01	-	0.94	0.6	0.98	-	-	0.82	0.97	-	-	0.63	0.85	-	1.24
1281	o-Cymene	4.97	3.02	2.12	1.54	6.68	3.88	6.48	6.54	9.3	3.8	8.94	1.73	4.76	3.77	2.18	4.86	3.37	3.4	3.2	3.8
1501	Copaene	0.6	0.66	1.09	-	0.72	-	-	-	0.72	1.07	-	1.14	0.94	1.06	-	-	0.77	0.95	-	0.92
1547	β-Cubebene	0.61	-	-	-	1.01	-	-	-	0.93	-	-	0.92	0.71	-	-	-	0.67	0.71	-	-
1591	Zingiberene	0.48	-	-	-	0.62	-	-	-	0.67	-	-	-	0.55	-	-	-	0.41	-	-	-
1599	β-Elemene	0.99	1.04	-	1.07	1.46	1.37		1.55	1.19	1.35	1.27	1.11	1.16	1.35	0.73	1.26	0.71	1.19	0.72	0.9
1608	Caryophyllene	4.96	3.82	2.96	3.7	5.7	4.46	3.66	4.35	8.57	7.2	4.35	6.72	7.65	5.97	4.36	4.36	7.69	5.3	4.89	4.5
1681	α-Humulene	1.7	1.65	1.96	1.44	2.06	2.08	2.77	2.04	2.06	2.04	2.35	2.59	2.02	2.04	1.89	2.51	1.79	2.3	2.91	1.77
1685	*trans*-Verbenol	1.09	2.73	2.61	2.07	1.38	-	1.98	1.92	1.25	-	1.17	1.26	0.74	-	-	-	0.49	-	-	-
1698	γ-Muurolene	1.54	1.9	-	1.07	1.65	1.32	-	1.95	1.77	1.3	3.46	-	1.38	1.29	2.79	-	1.79	1.15	2.79	1.27
1720	Germacrene D	34.2	36.8	23.9	20.4	30.2	50.6	30.8	32	22.1	47	29.8	28.1	32.4	48.8	41.7	37.4	40.1	46	43.7	44
1743	Bicyclogermacrene	0.76	0.87	-	1.08	0.74	1.38	-	-	-	1.34	-	-	0.94	1.34	1.33	-	1.07	1.21	1.33	1.37
1764	δ-Cadinene	2.01	2.29	2.79	3.52	2.16	2.07	2.08	2.61	2.1	2.03	2.33	2.1	1.5	2.01	2.03	1.9	1.74	1.81	2.05	1.49
1781	α-Curcumene	0.93	1.39	-	1.97	1.65	-	-	1.27	2.36	-	2.28	1.54	2.61	-	-	-	0.43	0.59	-	-
1894	o-Guaiacol	0.73	-	2.04	2.52	1.09	-	-	-	1.12	-	1.27	1.86	1.26	-	-	1.88	0.72	1.1	-	1.22
1934	1,5-Epoxysalvial-4(14)-ene	1.02	2.62	2.38	2.79	1.7	-	2.68	1.51	1.65	-	1.79	2.54	1.55	-	-	1.03	0.74	0.95	-	-
1949	β-Calacorene	0.58	1.48	-	1.51	0.83	-	-	-	0.76	-	-	-	0.83	-	-	-	0.51	-	-	-
1995	Caryophyllene oxide	2.14	2.44	5.8	6.67	2.96	2.63	4.19	2.96	3.51	2.6	3.88	5.9	3.4	2.63	4.64	5.16	1.88	2.4	2.64	2.8
2022	Salvial-4(14)-en-1-one	2.47	2.88	6.08	4.82	3.68	1.83	4.32	3.91	2.13	1.8	1.65	5.02	3.13	1.82	1.36	6.7	1.62	1.6	1.36	2.7
2038	Ethyltetradecanoate	1.77	-	-	-	1.24	-	-	3.21	1.43	-	-	-	0.86	-	-	2.92	0.44	-	-	1.16
2045	Nerolidol	-	1.61	0.79	1.2	-	1.51	2.98	-	0.38	1.49	-	1.28	0.96	1.52	-	1.19	0.68	1.35	-	1.2
2054	α-Humulene oxide	0.72	-	2.01	2.34	1.04	-	1.99	1	1.12		1.28	1.83	1.09	-	-	1.87	-	0.89	-	0.96
2060	Germacrene D-4-ol	1.36	2.73	1.33	1.94	1.83	1.8	-	2.3	1.47	1.77	1.66	1.52	1.67	1.78	1.95	3.02	0.62	1.58	0.95	1.94
2087	Elemol	2.57	0.41	1.17	1.47	0.82	-	1.37	1.05	0.75	-	-	1.14	0.39	-	-	-	-	-	-	-
2101	Viridiflorol	1.24	0.51	1.41	1.48	0.67	-	-	-	0.59	-	0.72	1.13	0.65	-	-	-	0.43	0.43	-	-
2134	Spathulenol	4.01	1.63	5.05	4.84	3.1	3.26	3.87	2.8	3.58	3.23	3.03	5.75	3.68	3.22	1.74	5.51	2.11	2.97	1.74	2.85
2181	Eugenol	0.93	2.62	2.56	3.47	1.23	-	-	1.28	1.13	1.29	-	1.36	1.23	1.31	-	1.75	1.18	1.16	-	-
2189	β-Bisabolol	0.74	1.51	1.62	2.09	0.98	0.99	-	0.98	0.86	1.09	1.11	1.74	1.13	1.09	1.7	1.69	0.88	1.19	0.66	0.95
2195	t-Muurolol	1.45	-	-	1.16	1.59	1.03	-	1.03	0.73	1.03	-	0.96	0.82	1.03	0.6	1.09	0.75	1.11	0.58	-
2200	δ-Decalactone	0.81	0.69	1.11	1.04	0.93	1.44	1.84	1.74	2.17	1.41	2.04	1.06	1.19	1.44	-	-	1.13	1.35	-	0.97
2219	α-Eudesmol	0.53	-	-	-	0.39	1.39	-	-	0.36	1.34	-	1.32	0.81	1.39	0.97	-	0.94	1.23	0.97	1.15
2241	Caryophylla-3,8(13)-dien-5β-ol	-	0.87	2.07	2.41	-	-	1.67	-	-	-	-	1.76	-	-	-	-	-	-	-	-
2243	α-Cadinol	2.75	0.78	1.43	2.38	2.05	4.04	2.22	3.07	2.82	2.98	2.66	2.53	2.98	2.96	2.45	3.1	2.37	3.7	2.43	2.62
2260	β-Eudesmol	0.42	1.38	1.35	1.58	0.5	-	-	-	0.48	-	-	1.08	0.52	-	-	-	0.47	-	-	-
2270	Eugenol acetate	-	1.46	0.45	0.59	-	-	-	-	-	-	-	0.61	-	-	-	-	-	-	-	-
2299	γ-Gurjunenepoxide	0.53	0.12	0.93	1.1	0.31	-	-	-	0.7	-	-	0.96	0.86	-	-	-	0.88	-	-	-
2306	(Z)-α-Santalol	-	0.49	1.58	1.76	-	-	1.96	0.6	-	-	-	1.48	-	-	-	1.32	-	-	-	-
2372	Vulgarol B	0.48	1.86	1.65	1.25	0.87	-	0.65	-	0.53	-	-	1.26	0.65	-	-	1.55	0.38	-	-	-
2376	Ledene oxide (II)	0.31	0.25	0.92	0.38	0.39	-	-	-	0.39	-	-	1	0.81	-	-	-	0.29	-	-	-
	Total Identified (%)	98.5	98.5	98.3	98.7	98.72	98.9	98.7	98.2	98.7	98.7	98.7	98.8	98.7	99.1	98.7	98.3	98.9	99.3	98.2	98.9
	Number of compounds	39	34	30	35	39	21	21	27	39	23	23	34	40	23	19	23	38	30	19	25

*RI: Retention indices calculated against n-alkanes (C7-C30) on HP-Innowax column

Table 7. Compounds of *E. purpurea* essential oil by classes affected by various fertilizer levels and cutting times.

Grouped components (%)	1st Cutting				2nd Cutting				3rd Cutting				4th Cutting				5th Cutting			
	F_0	F_1	F_2	F_3	F_0	F_1	F_2	F_3	F_0	F_1	F_2	F_3	F_0	F_1	F_2	F_3	F_0	F_1	F_2	F_3
Monoterpene hydrocarbons	21.2	17.0	19.3	11.6	21.13	15.7	27.7	23.0	26.3	15.2	30.6	8.24	15.6	15.1	28.4	11.1	22.2	15.43	28.5	22.6
Oxygenated monoterpenes	2.83	6.04	6.28	6.58	3.54	1.44	3.82	4.94	4.55	2.7	3.21	3.68	3.16	2.75	-	1.75	2.8	2.51	-	0.97
Sesquiterpene hydrocarbons	49.3	51.9	32.6	35.7	48.84	63.3	39.2	45.8	43.2	63.5	45.8	44.2	52.7	63.8	54.8	47.4	57.7	60.87	58.3	55.8
Oxygenated sesquiterpenes	22.7	22.0	37.5	41.6	22.88	18.4	27.9	21.2	22.0	17.3	17.7	40.2	25.1	17.4	15.4	33.2	15.0	19.39	11.3	17.1
Phenolics	0.73	-	2.04	2.52	1.09	-	-	-	1.12	-	1.27	1.86	1.26	-	-	1.88	0.72	1.1	-	1.22
Esters	1.77	1.46	0.45	0.59	1.24	-	-	3.21	1.43	-	-	0.61	0.86	-	-	2.92	0.44	-	-	1.16

plant decreased with the aging of the plants. The essential oil profile indicated that the quality of secondary metabolites is in the range of reliable international standards. The results suggest that the cultivation of *E. purpurea* in the Afyonkarahisar climate is cost-effective under regular irrigation.

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LITERATURE CITED

Abdallah, W.H. 2016. Effect of some fertilization and micro-nutrients treatments on growth and chemical constituents of *Echinacea purpurea* plant. Journal of Plant Production 7(7): 709-719. https://doi.org/10.21608/JPP.2016.46143

Adams, R.P. 2017. Identification of essential oil components by gas chromatography/mass spectrometry. 4.1st ed. Allured Publishing Co., Illinois, USA.

Ahmadi-Samadi, F., and A. Rahimi. 2020. Improving growth properties and phytochemical compounds of *Echinacea purpurea* (L.) medicinal plant using novel nitrogen slow release fertilizer under greenhouse conditions. Sci. Rep. 10: 13842. https://doi.org/10.1038/s41598-020-70949-4

Akanbi, W.B., C.O. Adebooye, A.O. Togun, J.O. Ogunrinde, and S.A. Adeyeye. 2007. Growth, herbage and seed yield and quality of *Telfairia occidentalis* as influenced by cassava peel compost and mineral fertilizer. Agricultural Journal 2(5): 588-595.

Arvin, P. 2019. Study of different levels of nitrogen, phosphorus and potassium on physiological and morphological parameters and essential oils in savory plant (*Satureja hortensis* L.). J. Plant Res. 32(2): 464-473.

Barzegar, T., S. Mohammadi, and Z. Ghahremani. 2020. Effect of nitrogen and potassium fertilizer on growth, yield and chemical composition of sweet fenne. J. Plant Nutr. 43(8): 1189-1204. https://doi.org/10.1080/01904167.2020.1724306

Bernal, M., R. Cases, R. Picorel, and I. Yruela. 2007. Foliar and root Cu supply affect differently Fe and Zn-uptake and photosynthetic activity in soybean plants. Environ. Exp. Bot. 60(2): 145-150. https://doi.org/10.1016/j.envexpbot.2006.09.005

Chen, C.L., S.C. Chang, and J.M. Sung. 2008 Biomass and caffeoyl phenols production of *Echinacea purpurea* grown in Taiwan. Exp. Agric. (4): 497-507. https://doi.org/10.1017/S0014479708006753

Coelho, J., L. Barros, M.I. Dias, T.C. Finimundy, J.S. Amaral, M.J. Alves, R.C. Calhelha, et al. 2020. *Echinacea purpurea* (L.) Moench: chemical characterization and bioactivity of its extracts and fractions. Pharmaceuticals 13(6): 125. https://doi.org/10.3390/ph13060125

Diraz, E., S. Karaman, and N. Koca. 2012. Fatty acid and essential oil composition of *Echinacea purpurea* (L.) Moench growing in Kahramanmaras-Turkey. pp. 35-37. International Conference on Environmental and Biological Sciences (ICEBS'2012), 21-22 December, Bangkok, Thailand. https://www.researchgate.net/profile/Emel-Diraz/publication/289527127_Fatty_Acid_and_Essential_Oil_Composition_of_Echinacea_Purpurea_L_Moench_Growing_in_Kahramanmaras-Turkey/links/568e96bb08ae78cc0515f804/Fatty-Acid-and-Essential-Oil-Composition-of-Echinacea-Purpurea-L-Moench-Growing-in-Kahramanmaras-Turkey.pdf

El-Sayed, A.A., A.S. Shalaby, H. El-Hanafy, T.A. El-Razik. 2012. Effects of chemical fertilizers on growth and active constituents of *Echinacea paradoxa* L. plants. Journal of Horticultural Science & Ornamental Plants. 4(2): 125-133.

EMA/HMPC (European Medicines Agency-Committee on Herbal Medicinal Products). Assessment report on *Echinacea purpurea* (L.) Moench, herba recens, 2017. https://www.ema.europa.eu/en/documents/herbal-report/final-assessment-report-echinacea-purpurea-l-moench-herba-recens_en.pdf

Fariborzi A. 1999. Effects of nitrogen fertilizer and harvest dates on yield and essential oil content of chamomile flower (*Matricaria chamomile*). Master of Science Thesis, Faculty of Agriculture, University of Mash'had, Iran.

Fernandes, V.F., L.B. Almeida, E.V.R.S. Feijó, C.S. Delmira, A.O. Rosilene, S.M. Marcelo, C.B.C. Larissa. 2013. Light intensity on growth, leaf micromorphology and essential oil production of *Ocimum gratissimum*. Rev. Bras. Farmacogn. 23(3): 419-424. https://doi.org/10.1590/S0102-695X2013005000041

Ghasemi, A., N. Esfandiari, S.A.M. Modarres-Sanavy, and M. Safari. 2019. Foliar application of nitrogenous organic compounds (Humiforte and Fosnutren) on growth parameters and caffeicacid derivatives of *Echinacea purpurea*. J. Plant. Nutr. 42(7): 783-794. https://doi.org/10.1080/01904167.2018.1544259

Ghatas, Y.A.A., and W.H. Abdallah. 2016. Effect of some fertilization and micro-nutrients treatments on growth and chemical constituents of *Echinacea purpurea* plant. J. Plant. Prod. 7(7): 709-719. https://doi.org/10.21608/JPP.2016.46143

Goldani, M., H. Zare, and M. Kamali. 2016. Evaluation of different levels of nitrogen and phosphorus fertilizers on shoot and root characteristics of *Echinacea purpurea*. J. Hortic. Sci. 30(3): 366-375. https://doi.org/10.22067/jhorts4.v30i3.26615

Isazadeh-Hajagha, R., S. Kırıcı, L. Tabrizi, A. Asgharzadeh, and A. Hamidi. 2017. Evaluation of growth and yield of Purple Coneflower (*Echinacea purpurea* L.) in response to biological and chemical fertilizers. J. Agric. Sci. 9(3): 160-171. https://doi.org/10.5539/jas.v9n3p160

Janmohammadi, M., Z. Sufi-Mahmoudi, A. Ahadnezhad, S. Yousefzadeh, and N. Sabaghnia. 2014. Influence of chemical and organic fertilizer on growth, yield and essential oil of dragonhead (*Dracocephalum moldavica* L.) plant. Acta. Agric. Slov. 103(1): 73-81. https://doi.org/10.14720/aas.2014.103.1.08

Kaya, M., M. Merdivan, P. Tashakkori, P. Erdem, and J.L. Anderson. 2019. Analysis of Echinacea flower volatile constituents by HS-SPME-GC/MS using laboratory-prepared and commercial SPME fibers. J. Essent. Oil Res. 31(2): 91-98. https://doi.org/10.1080/10412905.2018.1545707

Khathutshelo, M.V., N. Mpumelelo, N. Wonder, and M.N. Fhatuwani. 2016. Effects of foliar spray application of selected micronutrients on the quality of Bush Tea. Hortic. Sci. 51(7): 873-879. https://doi.org/10.21273/HORTSCI.51.7.873

Khorsand, A. 2011. Medicinal plants. Pajvak Andishe Pub. Tehran.

Manayi, A., M. Vazirian, and S. Saeidnia. 2015. *Echinacea purpurea*: Pharmacology, phytochemistry and analysis methods. Pharmacogn. Rev. 9(17): 63-72. https://doi.org/10.4103/0973-7847.156353

Marschner, H. 1995. Mineral nutrition of higher plants. 2nd ed. Academic Press. New York, USA.

Mirjalili, M.H., P. Salehi, H. Naghdi-Badi, and A. Sonboli. 2006. Volatile constituents of the flower heads of three Echinacea species cultivated in Iran. Flavour Fragr. J. 21(2): 355-358. https://doi.org/10.1002/ffj.1657

Omidbeigi, R. 2013. Production and processing of medicinal plants I. 8th ed. Behnashr Pub. Msh'had.

Parsons, J.L., S.I. Cameron, C.S. Harrisa, and M.L. Smith. 2018. Echinacea biotechnology: advances, commercialization and future considerations. Pharm. Biol. 56(1): 485-494. https://doi.org/10.1080/13880209.2018.1501583

Purbajanti, E.D., W. Slamet, E. Fuskhah, and R. Rosyida. 2019. Effects of organic and inorganic fertilizers on growth, activity of nitrate reductase and chlorophyll contents of peanuts (*Arachis hypogaea* L.). IOP Conf. Ser. Earth Environ. Sci. 250(1): 1-7. https://doi.org/10.1088/1755-1315/250/1/012048

Sati, M. 2012. Effects of different plant densities on yield and quality characteristics of *Echinacea purpurea* L. Master of Science Thesis, University of Ege, Institute of Natural and Applied Sciences, Field Crops Department, İzmir, Turkey. https://tez.yok.gov.tr/UlusalTezMerkezi/tezSorguSonucYeni.jsp

Sedlakova, J., B. Kocourkova, L. Lojkova, and V. Kuban. 2003. The essential oil content in caraway species (*Carum carvi* L.). Hortic. Sci. 30(2): 73-79. https://doi.org/10.17221/3818-HORTSCI

Senica, M., G. Mlinsek, R. Veberic, and M. Mikulic-Petkovsek. 2019. Which plant part of purple coneflower (*Echinacea purpurea* (L.) Moench) should be used for tea and which for tincture? J. Med. Food 22(1): 102-08. https://doi.org/10.1089/jmf.2018.0026

Soltanbeigi, A. 2020. Qualitative variations of lavandin essential oil under various storage conditions. J. Essent. Oil-Bear. Plants 23(6): 1237-1252. https://doi.org/10.1080/0972060X.2020.1871076

Soltanbeigi, A., and M. Özgüven. 2021. Influence of Planting Date on Growth, Yield, and Volatile Oil of Menthol Mint (*M. arvensis*) Under Marginal Land Conditions, J. Essent. Oil-Bear. Plants 24(2): 360-373. https://doi.org/10.1080/0972060X.2021.1923575

Soltanbeigi, A., and E. Sakartepe. 2020. Chemical specification of wild *Salvia tomentosa* Mill. collected from Inner Aegean Region of Turkey. Z Arznei Gewurzpfla 24(1): 31-35.

Thappa, R.K. S.K. Bakshi, P.L. Dhar, S.G. Agarwal, S. Kitchlu, M.K. Kaul, K.A. Suri. 2004. Significance of changed climatic factors on essential oil composition of *Echinacea purpurea* under subtropical conditions. Flavour Fragr. J. 19(5): 452-4. https://doi.org/10.1002/ffj.1352

Yarnia, M., M. Farzanian, V. Rashidi, A. Aziz-Javanshir, and N. Aliasgharzad. 2012. Effects of microelement fertilizers and phosphate biological fertilizer on some morphological traits of Purple coneflower in water stress condition. Afr. J. Microbiol Res. 6(22): 4825-4832. https://doi.org/10.5897/AJMR12.1262

Yeşil, R., and Y. Kan. 2013. The effects of applications of organic and inorganic fertilizers in different doses on yield and components of essential oil of Echinacea species (*E. pallida* and *E. purpurea*) cultivated in Konya ecological conditions. Selcuk J. Agric. Food Sci. 27(1): 14-23.

Youssef, A.S.M. 2014. Influence of some amino acids and micro-nutrients treatments on growth and chemical constituents of *Echinacea purpurea* plant. J. Plant. Prod. 5(4): 527-543. https://doi.org/10.21608/JPP.2014.53703