ISSN print: 1224-5984
ISSN online: 2248-3942

Rom Biotechnol Lett. 2021; 26(3): 2722-2735
doi: 10.25083/rbl/26.3/2722.2735

Received for publication, January, 13, 2020
Accepted, December, 12, 2020

Original paper

Investigation on the effects of different concentrations of some fertilizers on yield, quality and essential and fixed oil composition of Nigella damascena

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Abstract

In this study, the effect of application of fertilizers with different ingredients in different doses in cultivation of *Nigella damascena* plant on morphological, yield and quality was investigated. According to the obtained results; plant height was 25.07-37.54 cm, and the number of branches per plant was on average 2.33-5.60, besides, it has been observed that root lengths vary between 4.33-8.62 cm. On the other hand, while the average amount of capsules per plant was between 3.53-7.10, the capsule width and length varied by 12.92-15.06 (mm) and 13.85-16.29 (mm), respectively. When evaluated in terms of seed yield, approximately 45.68-113.57 kg da^{-1} yield has been obtained also, seed weights in the capsule are 0.104-0.158 g, thousand grain weights 2.31-2.84 g and seed weights per plant ranged from 32 to 0.87 g/plant. While the fixed oil rate were seen between 34-36.48% in the application groups, the fixed oil yield was calculated as 15.53- 41.43 kg da^{-1}. As a result of GC-MS analysis, it was observed that the main compound of essential oils was between 19.47-61.13% β-elemene (sesquiterpen), followed by 6.91-34.53% damascenine (alkaloid). In addition, 17 fatty acids were observed in GC-FID analysis and it showed that fertilizer applications cause a variable effect on saturated and unsaturated fatty acid compositions.

Keywords *Nigella damascena*, Fertilizer, Essential Oil, Yield and Quality.

To cite this article: ULUSU F, ŞAHIN A. Investigation on the effects of different concentrations of some fertilizers on yield, quality and essential and fixed oil composition of *Nigella damascena*. *Rom Biotechnol Lett*. 2021; 26(3): 2722-2735. DOI: 10.25083/rbl/26.3/2722-2735

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Introduction

Medicinal and aromatic plants have been used in traditional and complementary medicine in developing countries for centuries to treat diseases and continue to be an important resource for drugs produced today. Theset ype of plants generally contain bioactive secondary metabolites such as alkaloids, flavonoids, steroids, terpenes, sesquiterpenes, diterpenes, phenolics and saponins. These secondary metabolites have many protective properties such as antimalarial, anthelmintic, anti-inflammatory, analgesic (BAYDAR, 2009; CROTEAU et al, 2000; HANSON, 2003; SHAKYA, 2016; KABERA et al, 2014).

There are more than 300 flowering plant families spread around the world (HEYWOOD, 1979). *Ranunculaceae* are mostly herbaceous and annual or perennial, and they have many medicinal and aromatic properties. *Nigella* species belonging to this family have pharmacological properties (anti-inflammatory, antidiabetic, hepatoprotective, antimicrobial, antioxidant, etc.) and they are also used worldwide as spices (AYHAN, 2012; IKHSAN et al, 2018; EL RABEY et al, 2017; AMIN and HOSSEINZADEH, 2016, ERDOGRUL et al, 2009). The most commonly used species of the genus *Nigella* are *N. sativa*, *N. damascena* and *N. arvensis* (BAYTOP, 1999). *Nigella damascena* L. is anannual, flowering, herbaceous and valuable medicinal-aromatic plant belonging to the *Ranunculaceae* family and it can develop well in sandy-loamy soils ranging from 20 to 50 cm. Its homeland is North Africa and Southwest Asia and its special name comes from the province of Syria-Damascus. *N. damascena* is frequently used in the treatment of many diseases (ALAMGIR, 2018; FICO et al, 2004), especially in traditional Eastern medicine, and in the field of gastronomy in Asian cultures (FICO et al, 2003). Among the *Nigella* species, the most studied species is *N. sativa*, and studies on the seed content of other species are more limited. *Nigella damascena* L. seeds contain valuable secondary metabolites such as fixed oil, essential oil, alkaloids, phenolic compounds and saponins (FICO et al, 2000; SAJFRTOVA et al, 2014; FARAG, 2014; FICO, 2001).

Many studies in recent years have shown that *N. damascena* seeds contain a large number of medicinal essential oils (β-elemene, germacrene A, Damascenine, α-pinene, β-pinene, myrcene, p-cymene, limonene, etc.) (FICO et al, 2003, WAJS et al, 2009, SIENIAWSKA et al, 2018). It has been observed that β-elemene and Damascenine, which are the main components of these seeds, have antitumor, analgesic, antiproliferative effect in phytotherapy (BEKEMEIER et al, 1968), as well as anti-inflammatory effect on human neutrophils (SIENIAWSKA et al, 2019). In addition, it has been stated that β-element has a synergistic effect with anticancer drugs such as paclitaxel or docetaxel (ZHAO et al, 2007).

Natural or chemical fertilizers are applied to the soil or directly to the plant in order to increase the efficiency and quality in herbal production activities and at the same time to regain the nutrients lost or decreased in the soil (MERRIAM-WEBSTER, 2016). As a result of factors such as improper soil cultivation and frequent cultivation during a period of herbal production, conditions such as deterioration of soil aggregate structure, decrease in soil fertility and decrease in the amount of organic matter can occur. With the increase in the use of synthetic fertilizers used to obtain more products from the unit area, the sustainability of soil fertility has become a problem as a result of the decrease in the treatment of organic fertilizers. Toxic substances such as nitrates and nitrites, which are formed as a result of intensive use of chemical fertilizers, harm not only the soil, but also the entire ecosystem life. Organic fertilizers such as farm manure, green manure, vermicomposting increase the biological nitrogen fixation, minimizing the formation of toxic compounds, thus contributing to sufficient soil fertility for sustainable crop production (TANVEER et al, 2019).

It has been stated in many studies that environmental factors on morphological, yield-quality characteristics and application of different cultivation techniques such as irrigation amount, fertilizer type-amount, sowing-time-frequency, and seed amount have been significantly affected in the black cumin plant (DAS et al, 1991; GEREN et al, 1997; ÖZEL and DEMIRBILEK, 2000; TELCI, 1995; TUNCTURK et al, 2012; TONCER and KIZIL, 2004; FOROUZANDEH, 2014). Studies on the effect of fertilization on secondary metabolite production in medicinal and aromatic plants are quite limited. In this study, the effect of different fertilizers (in different rates) applied to *N. damascena* plant in the greenhouse conditions on morphological features, yield-quality and essential oil components were investigated.

Material and Method

This study investigating the effect of fertilizers of different types (Table 1) and doses on the morphological and yield-quality of *N. damascena* plant was carried out according to four repetitive randomized block design Karamanoglu Mehmetbey University research greenhouse in Turkey at the appropriate temperature (24-25°C) and under humidity (55-60%) conditions in 2018-2019 period (Figure 1).

Figure 1. *N. damascena* grown in the greenhouse

Table 1. Fertilizer types and application dosages

Groups	Chemical Content	Application Dosages		
Control		*Half*	*Recommended*	*Double*
Liquid chemical	N: P: K (%7: 7: 7)	1-1,5 L/da	2-3 L/ da	4-6 L/ da
Liquid organic	Total organic matter: %45 Organic C: %19,5 N: %3 K_2O: %7	150-300 mL/da	300-600 mL/da	600-1200 mL/da
Liquid vermicompost	Total organic matter: %5 N: %1 Humic + fulvic acid: %10	0,75-1 L/da	1,5-2 L/da	3-4 L/da

Cultivation of *Nigella damascena*

After surface sterilization, *N. damascena* seeds (Vilmorin-Anadolu Seed, Turkey) were kept in 10% fertilizer solutions for 6 hours, then seeds were planted in pots containing peat and garden soil in a ratio of 50:50 according tofour repetitive randomized block design and were grown by watering once in every 3-4 days. In the experiment, three different fertilizers (liquid synthetic, liquid organic, and vermicompost) were applied in three different ratios, and 10 different treatment groups were formed together with the control group (w/o fertilizer). The types and quantities of fertilizers applied have shown in Table 1. The germination in pots occurred on average 15 days later, after approximately 80% of the plants reached 10-15 cm length of the plant height, the first fertilizer treatments were applied to the soil at the dosage recommended by the manufacturers, at half and double the concentration of this dose. The second fertilizer treatment to the soil was carried out when more than 50% of the plants began to bloom. The plants were harvested by hand after the capsules matured. The harvested capsules were kept at +4℃ for morphological, yield – quality analysis and extraction studies.

Morphological Analysis

Morphological evaluations were carried out to evaluate the effects of fertilizers applied on black seed plants development. These morphological observations were plant height (cm), branch number, root length (cm), number of capsules in the plant (pieces plant^{-1}), capsule width (mm), and capsule length (mm).

The plant height was recorded by measuring the distance from the ground level to the top point of the plant. In order to determine the average number of branches in the grown plants, the branches of the plants in all pots that are connected to the main stem are counted and their averages are calculated (ARSLAN, 1993). The width and length of the harvested capsules were measured with a caliper and their averages were calculated. After the plants were harvested and removed from the soil, the part from the tip of the root to the soil surface was measured in cm to determine the root lengthfor each treatment groups.

Yield and Quality Analysis

As yield and quality analysis; seed weight per plant (g plant^{-1}), thousand grain weight (g), seed weight per capsule (g capsule^{-1}), seed yield (kg da^{-1}), plant elemental analysis, fixed oil rate (%), fixed oil yield (kg da^{-1}), essential oil characterization and rate have been evaluated.

Seed weight per plant

The seeds obtained from the plants in the pots during the harvest period were weighed on a sensitive scale and the seed weight per plant was determined as average using the following formula.

$$SWPP = \frac{TSWPP}{PPP}$$

SWPP: Seed weight per plant

TSWPP: Total seed weight per pot

PPP: Plant per pot

1000 seed weight (g)

From the seeds obtained from each pot (4 repeats), 100 seeds were counted and weighed in the analytical balance, and the result was multiplied by 10 and 1000 grain weight was calculated (AHMED and HAGUE, 1986).

Seed weight per capsule (g capsule^{-1})

The seeds obtained from the maturing capsules were weighed and the average of the data per capsule was calculated and the seed weight in the capsule was calculated from the formula below.

$$SWPC = \frac{TSW}{TCN}$$

SWPC: Seed weight per capsule

TSWPC: Total seed weight

TCN: Total capsule number

Plant Elemental Analysis

For the quantitative elemental analysis determination by dismantling the plants at the end of the harvest, analyses of 9 different elements were performed by service procurement. N analysis was performed by Kjeldahl method, P_2O_5 analysis by spectrophotometric and K_2O, Mg, Fe, Zn, Cu, Mn, Ca by atomic absorption spectroscopy.

Essential Oil Extraction

Crushed seeds of *N. damascena* (5 g) were subjected to hydro-distillation for 4 h using a clevenger-type apparatus (WAJS et al, 2009). The sample obtained as a result of distillation was 3 times subjected to liquid-liquid extraction with n-Hexane. The obtained n-Hexane extract (upper phase) was taken to the flask and subjected to evaporation (40℃) and dried over anhydrous sodium sulfate at the end of evaporation and kept in deep freezer at −20°C under N atmosphere until GC-MS analysis (FICO et al, 2003; WAJS et al, 2009; NICKAVAR et al, 2003).

GC-MS Analysis

GC-MS analyses were carried out on an Agilent Technologies 7890A Network GC System equipped with a HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm) and interfaced with an Agilent G4513A series auto sampler and a 5975C VL Mass Selective Detector. The injector was set at 250°C and the carrier gas was helium at a flow rate of 0.8 mL/min. The oven temperature was initially at 60°C, increased at a rate of 4°C min^{-1} up to 260°C the split ratio was 1:10. The ion source, quadrupole and transfer line temperature were set at 280℃. The mass spectrometer was operated at 70 eV in electron impact (EI) mode. Transfer line temperature 280℃; ion source temperature 210℃; carrier gas helium at a linear velocity of 1.5 mL/min; ionization energy 70eV; scan range 15-550 amu. The constituents were identified by matching their mass spectra in the Wiley275. Llibrary and by comparison of their retention indices with literature values (FICO et al, 2003; SİENİAWSKA et al, 2018). Relative percentage amounts of essential oils were calculated from the total area under the peaks by the software of the apparatus.

Fixed Oil Extraction and Preparation of Fatty Acid Methyl Esters (FAMEs)

For fixed oil extraction from seeds obtained after harvest, 5 g dried and powdered seed samples from each treatment group were extracted with n-hexan for 8 hours using soxhlet apparatus and the collected solvent was removed under vacuum using a rotary evaporator at 40℃ for determining the fixed oil content (%) (NICKAVAR et al, 2003; BAYTÖRE, 2011). Fixed oil yield (kg da^{-1}) was calculated by multiplying the seed yields calculated by unit area (da) in each group by the fixed oil rate of that group. For methylation, 20 mg of extracted oil was dissolved in a tube containing n-hexane. Then 2 M KOH (2 mL) was added and vortexed vigorously for 2 minutes. After phase formation, the upper layer containing fixed oil methyl esters was taken into another tube and stored at -20°C until the GC-FID analysis (PALABIYIK and AYTAÇ, 2018).

GC-FID Analysis

Analysis of fatty acids was performed on Perkin Elmer Clarus 500 gas chromatography with FID (flame ionization detector) and Restek (Rtx-2330) capillary column (30 m x 0.25 mm x 0.2 µm). Identification of fatty acids was carried out as standard using a mixture of 37 fatty acids with methyl esters. The samples were given to the device by adjusting the detector temperature of GC device as 250℃, injector temperature 250℃, injection split 50/1, carrier gas flow rate helium 1 ml / min. The oven temperature program was kept at 100℃ for 2 minutes, and then it increased to 2℃ per minute up to 150℃. Then it was kept at 150℃ for 10 minutes and the temperature was increased up to 170℃ at 2℃ per minute. After standing at 170℃ for

10 minutes, the temperature was heated up to 200℃ and 1.5℃ and the oven program was terminated. Thus, the total analysis time is completed in 77 minutes.

Statistical Analysis

The differences among the control and treaments were analyzed with one-way ANOVA analysis according to Duncan multiple interval test and calculated at the significance value of $p < 0.05$. In the study, 4 replications were done for each group (n = 4) (DUNCAN, 1955).

Results and Discussion

Morphological data, yield and quality analysis results, elemental analysis results, essential oil composition obtained in the treatments were given in Tables 2, 3, 4 and 5, respectively. In terms of both morphological features and yield-quality analyzes, statistically significant differences were observed among treatments. In addition, essential oil composition and amount (%) varied depending on the fertilization treatments.

Morphological Analysis

According to the results of the analysis, plant height varied from 25.03 to 37.2 cm in negative control and in fertilizer treatments.The lowest plant height was observed in the control and chemical double fertilizer treatment, and the highest plant height was observed in the chemical half, followed by the organic double group. The other treatments, were not statistically different for plant height. While there was an increase in all fertilizer treatments compared to negative control, a decrease in plant height was observed only in the chemical double group. At this point, it is thought that the fertilizer treatment of chemical fertilizer double as much as the recommended amount regresses vegetative development by creating toxicity in the plant. As a result, biomass production decreased in this treatment. The findings are lower than those reported by Telci (1995) and Tektaş (2015). However, it is compatible with Baytöre (2011), Şahin (2013), Arslan (1993), Kalçın (2003) and Selicioğlu (2018). These differences may have resulted from dissimilarity in conditions such as cultivation, climate, ecological factors, soil characteristics and genotype (SADEGHI, 2009).

The number of branches in the plants grown in the study varies on average between 2.30-5.62 per plant. While the number of branches formed by the plant in the chemical double treatment group was in parallel with the negative control, the number of branches was statistically significantly increased according to negative control in all other groups where fertilizer was applied. Average number of branches found in previous studies were between 3.00-5.42 in *N. damascena* (KALÇIN, 2003) and 3.10-4.57 in *N. sativa* (PALABIYIK and AYTAÇ, 2018).

In the study, average root lengths in the plant groups vary between 5.33-8.62 cm. The root length of the plants in all fertilized groups were higher than in the negative control ($p < 0.05$). It has been observed that as the amount of vermicompost increased the length of the root increased, whereas the longest root lenth was observed in organic and chemical fertilizer treatments in the recommended dosages, being not statistically different. In this respect, it has been observed that all fertilizer treatments positively contributed to the development of subsoil parts in the plant (Table 2).

Table 2. Morphological data of fertilizer treatment groups

MORPHOLOGICAL DATA						
GROUPS (Types and Doses of fertilizers)	**Plant height (cm)**	**Number of branches (branches plant^{-1})**	**Root length (cm)**	**Number of capsule (capsule plant^{-1})**	**Capsule length (mm)**	**Capsule width (mm)**
Control (w/o fertilizer)	28.00 ± 1.4c	2.3 ± 0.49b	5.33 ± 0.80b	4.16 ± 0.56bc	15.90 ± 0.57ab	15.06 ± 0.53a
Organic Half	33.04 ± 1.49ab	5.00 ± 0.41a	6.88 ± 0.53a	4.55 ± 0.62bc	14.09 ± 0.33cd	13.25 ± 0.28de
Organic Recommended	33.58± 1.86ab	5.41 ± 0.42a	7.91 ± 0.47a	4.75 ± 0.41bc	14.55 ± 0.22cd	13.54 ± 0.18cde
Organic Double	32.69 ± 1.3b	4.91 ± 0.42a	7.45 ± 0.51a	5.13 ± 0.21bc	15,09 ± 0.26bc	14.37 ± 0.23abc
Vermicompost Half	33.46 ± 1.6ab	4.25 ± 0.67a	7.56 ± 1.11a	4.75 ± 0.52bc	14.65 ± 0.42cd	14.06 ± 0.42bcd
Vermicompost Recommended	36.20 ± 1.35ab	4.77 ± 0.48a	8.30 ± 0.47a	4.61 ± 0.42bc	16.29 ± 0.34a	14.70 ± 0.29ab
Vermicompost Double	33.35 ± 1.64ab	5.4 ± 0.54a	8.60 ± 0.40a	5.70 ±0.40ab	15.94 ± 0.28ab	14.54 ± 0.21ab
Chemical Half	37.20 ± 1.54a	5.62 ±0.38a	7.85 ± 0.39a	7.08 ± 0.41a	14.60 ± 0.22cd	13.50 ± 0.19cde
Chemical Recommended	33.00 ± 1.45ab	5.45 ±0.62a	8.62 ± 0.68a	7.10 ± 0.60a	14.05 ± 0.21cd	12.92 ± 0.19e
Chemical Double	25.03 ±1,96c	2.61 ±0.33b	8.61 ± 0.67a	3.53 ± 0.20c	13.85 ± 0.36d	12.93 ± 0.33e

The average number of capsules obtained per plant varies between 3.53-7.10 and is parallel to the previous study results of Palabıyık and Aytaç (2018) with 5.60-9.17 capsules plant^{-1}, Baytöre (2011) with 5.40-7.22 capsules plant^{-1}, Taqi (2013) with 4.5-4.9 capsules plant^{-1}, Safaei et al. (2014) with 5.6-6.1 capsules plant^{-1}. While the chemical fertilizer caused a significant increase in the number of capsules among the fertilizer treatments, a dramatic decrease in the number of capsules was observed when applying double the recommended dose. Like in the results of other morphological analysis, the dosage adjustment is very important in the chemical fertilizer, as well as the growth retardation both vegetatively and generatively as a result of the physiological effects occurring at high doses.

For morphological analysis, the width and length measurements of seed capsules developed on grown plants were also evaluated. Within the scope of the study, the average capsule width and length in the fertilizer treatments were determined in the range of 12.92-15.06 (mm) and 13.85-16.29 (mm), respectively. In terms of average capsule size, the recommended dosing group of vermicompost and negative control separated positively from the others. Similar to other morphological data, the smallest average capsule size was found in the chemical double dosing fertilizer treatment group. These values were found to be higher than Iqbal et al. (2010) and Hosseini et al. (2018), Ali et al. (2015).

Yield and Quality Analysis

Seed Yield (kg da^{-1})

Within the scope of this study, the amount of seeds obtained per decare was calculated and the seed yield varied between 45.68 and 113.57 kg da^{-1} among fertilizer treatments (Table 3).

Among the fertilizer treatments, the highest seed yield is in the chemical recommended group and it is observed that other treatments increase the yield of seed compared to negative control except for chemical double and vermicompost half treatment. In organic and vermicompost fertilizer treatment, as the dose increases, the seed yield increases, in chemical fertilizer treatment, the seed yield increases as much as the concentration, and after a certain limit, a dramatic decrease has occurred due to the toxicity occurring in the plant. At this point, it was determined that the most advantageous treatment in terms of seed yield is the recommended dose of chemical fertilizer, since the highest seed yield was found in the chemical recommended treatment. In previous studies, Özgüven ve Tansı (1989) at 140.0 kg da^{-1} in *N. damascena* and 135.5 kg da^{-1} in *N. sativa*. İlisulu (1992), ranges between 80-200 kg da^{-1}, even in fertile and irrigable soils this yield can reach 250 kg da^{-1}. Kalçın (2003), 68.39-77.48 kg da^{-1}, Özel et al. (2009) 143.63-248.23 kg da^{-1}, Telci (1995), 104.38-151.95 kg da^{-1}, Taqi (2013), 82.86-126.96 kg da^{-1}and Akgören (2011), 90.53-188.13 kg da^{-1}, Selicioğlu (2018) 58.4-122 kg da^{-1}reported. The results obtained in the current study were lower than the values of Özel et al. (2009) and Akgören (2011), and were similar to the results of other studies (KALÇIN, 2003; TELCI, 1995; SELICIOĞLU, 2018).

Seed Weight in Capsule (g capsule^{-1})

Capsules obtained from plants in each pot were counted and seeds removed from the capsule were weighed on an analytical balance. As a result of the calculation, although the average seed weights in the capsule vary between 0.104-0.158 g capsule^{-1} according to statistical analysis ($P < 0.05$), there was no difference between the groups. In studies conducted with the *N. sativa*; the seed weight in per capsule obtained by Baytöre (2011) (1.27-1.64 g capsule^{-1}) and Telci (1995) (0.822-0.967 g capsule^{-1}) was higer than the seed weight of *N. damascena* obtained in the current study. In terms of seed weight in capsule, growing *Nigella damascena* plants in our study was grown green house conditions and potting medium, and the fact that *N. damascena* seeds are smaller and lighter than *N. sativa* seeds, those can explain the difference from the previous studies.

1000 seed weight (g)

Average 1000 seed weights between treatment groups varied between 2.31 and 2.84 g. While the lowest thousand grain weight was in the chemical half, the highest was organic recommended treatment group. Although other fertilizer treatment, except the chemical half fertilizer treatment, have an effect on increasing 1000 seed weight compared to the control group, the difference between treatments were not statistically significant. In the *N. sativa*, thousand grain weights were found in the range of 1.23-2.62 g (PALABIYIK and AYTAÇ, 2018), 2.40-2.90 g (TEKTAŞ, 2015), 1.86-2.19 g (ÖZEL and DEMIRBILEK, 2000), 2.40-2.65 g (TUNÇTÜRK et al, 2005), 2.05 g (ÖZEL et al, 2000), 2.30-2.40 g (ÖZEL et al, 2009), 2.49-3.27 g (ABDOLRAHIMI et al, 2012), 1,95-2,96 g (SELICIOĞLU, 2018), 1.97-2.30 g (BAYTÖRE, 2011), 2.8-3.1 g (D'ANTUONO et al, 2002). Findings obtained in our study higher than Kalcin (2003) values (1.59-2.06 g). The effect of nitrogen and phosphorus fertilization on yield and quality is parallel to the thousand seed weights obtained in the study of Horvat (2017). As a result, it was observed in the study that fertilizer treatments caused a rise in weight by making a positive effect compared to the thousand grain weight of black seed in the literature.

Seed Weight per Plant

An average of 0.32-0.87 g plant^{-1} seeds were obtained from plants grown with different treatments (Table 3). In this study, in which the effects of different concentrations of different fertilizers were grown in a controlled environment under greenhouse conditions, it was observed that the highest seed amount was obtained from plants grown by applying chemical fertilizer at the recommended dose, while the lowest seed amount was obtained from plants grown by applying chemical fertilizer double and control. It has been observed that in organic and vermicompost fertilizer treatments, as the amount of fertilizer increases, seed weights per plant increase, but a sudden decrease in chemical double dose treatment. At this point, as in other yield and quality analyzes, when the chemical fertilizer treatment rises above a certain limit it affects to the plant rapidly and causes toxicity. Obtained values are higher than Selicioğlu (2018) and similar to the Kulan et al. (2012).

In other studies, the seed weight per black seed was found between 0.23-0.61 g $plant^{-1}$ by Selicioğlu (2018) and 0.26-1.59 g $plant^{-1}$ by Kulan et al. (2012) while it was found as 0.23 g $plant^{-1}$ in *N. damascena* species in the current study. The values obtained in the study carried out were similar to the literature data. In plant production studies, it is supported by many studies that the treatment of different cultivation techniques such as environmental factors, irrigation amount, type and amount of fertilization, planting time-frequency and seed amount significantly affect phenological, morphological and yield-quality characteristics (GEREN et al, 1997; ÖZEL and DEMİRBİLEK, 2000; TELCİ, 1995; TELCİ et al, 2014; ÖZGÜVEN, 1989; TUNCTURK et al, 2012; TONCER and KİZİL, 2004; FOROUZANDEH, 2014).

Table 3. Yield and Quality data of fertilizer treatment groups

YIELD AND QUALITY DATA						
Groups	**Seed weight per plant (g $plant^{-1}$)**	**1000 seed weight (g)**	**Seed yields (kg da^{-1})**	**Fixed oil rate (%)**	**Fixed oil yield (kg da^{-1})**	**Seed weight per capsule (g $capsule^{-1}$)**
Control	0.33 ± 0.02d	2.40 ± 0.25a	45.68 ± 8.9c	34 ± 0.25d	15.53 ± 1.03e	0,144 ± 0,024a
Organic Half	0.36 ± 0.05cd	2.78 ± 0.16a	59.72 ± 9.2bc	35.01 ± 0.32bc	20.9 ± 1.2d	0,111 ± 0,006a
Organic Recommended	0.44 ± 0.04c	2,84 ± 0.25a	69.38 ± 10.31bc	36.04 ± 0.31a	25 ± 2.31c	0,154 ± 0,019a
Organic Double	0.55 ± 0.03bc	2.42 ± 0.25a	86.41 ± 7.4b	36.23 ± 0.21a	31.3 ± 2.4b	0,127 ± 0,029a
Vermicompost Half	0.36 ± 0.03cd	2.51 ± 0.25a	53.17 ± 11.34c	34.76 ± 0.26c	18.48 ± 1.34d	0,158 ± 0,024a
Vermicompost Recommended	0.41 ± 0.04cd	2.34 ± 0.23a	65.53 ± 7.8bc	35.68 ± 0.29b	23.38 ± 2.8c	0,128 ± 0,016a
Vermicompost Double	0.59 ± 0.06bc	2.33 ± 0.18a	77.47 ± 11.2bc	36.2 ± 0.2a	28.04 ± 1.4bc	0,104 ± 0,011a
Chemical Half	0.66 ± 0.06b	2.31 ± 0.15a	104.55 ± 11.25ab	35.44 ± 0.2b	37.05 ± 2.05ab	0,117 ± 0,019a
Chemical Recommended	0.87 ± 0.04a	2.66 ± 0.29a	113.57 ± 5.7a	36.48 ± 0.37a	41.43 ± 2.7a	0,154 ± 0,019a
Chemical Double	0.32 ± 0.03d	2.33 ± 0.10a	50.21 ± 11.1c	34.6 ± 0.23c	17.3 ± 1.6e	0,133 ± 0,026a

Plant Elemental Analysis

After the capsule harvesting period of the trial, 9 different element analysis of the dry plants were performed in BSK Analysis Laboratory (Konya, Turkey) through service procurement. While the highest nitrogen content among the groups was found in the treatment at the concentration of double the recommended dose of the chemical fertilizer, the negative control was found to contain less amount of nitrogen than all the treatment groups.

On the other hand, phosphorus was found in the highest recommended organic dosing treatment. While potassium increased significantly with organic fertilizer treatment, the amount of magnesium was determined at similar rates in all fertilizer treatments.

In addition, there was a significant increase in the amount of calcium, in the chemical fertilizer double treatment group, while the lowest amount was found in negative control for all of these elements.

According to elemental analysis results, the highest copper and manganese content was found in plants grown by applying double the dosage of chemical fertilizer. In iron and zinc analysis, it was found in plants obtained by applying double the dose of vermicompost.

As a result, elemental analysis data showed that plants can uptake more elements from the soil in general in all fertilizer treatments.

Table 4. Plant Elemental Analysis

GROUPS	N (%)	P (%)	K (%)	Mg (%)	Ca (%)	Cu (ppm)	Fe (ppm)	Mn (ppm)	Zn (ppm)
Control	1.42 ± 0.015g	0.55 ± 0.03f	0.35 ± 0.01h	0.25 ± 0.005b	2.06 ± 0.31e	10.4 ± 1.87e	25 ± 1.9e	90.4 ± 0.47f	75.1 ± 1.02g
Organic Half	1.58±0.025ef	0.25 ± 0.02d	1.80 ± 0.01b	0.26 ± 0.01a	2.4 ± 0.02cd	12.2 ± 0.26d	35 ± 0.17bc	97.1 ± 0.78e	81.9 ± 0.37ef
Organic Recommended	1.79 ± 0.02c	0.63 ± 0.01a	1.83 ± 0.01b	0.26 ± 0.005a	2.38 ± 0.01cde	7.9 ± 0.25f	36.5 ± 0.56b	77.5 ± 0.58h	123.5 ± 0.57c
Organic Double	1.62 ± 0.005e	0.45 ± 0.01c	1.97 ± 0.01a	0.27 ± 0.01a	2.32 ± 0.02de	17.8 ± 0.29c	25.6 ± 0.31e	94.8 ± 0.80e	124.3 ± 1.03c
Vermicompost Half	1.88 ± 0.015b	0.34 ± 0.005e	1.78 ± 0.005b	0.26 ± 0.01a	2.15 ± 0.01de	16.8 ± 0.24c	29.3 ± 0.23d	74.1 ± 0.71i	99.8 ± 0.64d
Vermicompost Recommended	1.59 ± 0.005ef	0.45 ± 0.02c	0.54 ± 0.01f	0.26 ± 0.005a	2.35 ± 0.01cde	6.3 ± 0.08g	34 ± 0.26c	120.54 ± 1.88b	135.8 ± 0.26b
Vermicompost Double	1.73 ± 0.01d	0.56 ± 0.01b	0.44 ± 0.005g	0.27 ± 0.01a	2.65 ± 0.005bc	19.9 ± 0.22b	55 ± 0.19a	115.9 ± 0.82c	159.2 ± 0.27a
Chemical Half	1.57 ± 0.01f	0.51 ± 0.02bc	1.41 ± 0.01d	0.27 ± 0.02a	2.39 ± 0.005cde	5.7 ± 0.07h	26 ± 0.43e	81.4 ± 0.49g	85.2 ± 0.32e
Chemical Recommended	1.74 ± 0.01d	0.53 ± 0.01b	0.88 ± 0.01e	0.27 ± 0.01a	2.88 ± 0.01b	12.4 ± 0.3d	25.4 ± 0.39e	101 ± 0.68d	96.9 ± 0.45d
Chemical Double	1.93 ± 0.01a	0.33 ± 0.01e	1.62 ± 0.02c	0.26 ± 0.01a	3.62 ± 0.02a	23.3 ± 0.56a	26.8 ± 0.13e	132.3 ± 0.69a	79.9 ± 0.43f

Essential Oil Analysis

Before analyzing the extracts obtained by hydrodistillation, GC-MS analysis was performed using the standard solution for β-elemene (Figure S1), which is usually a characteristic dominant compound in *N. damascena*, and the chromatogram of this compound is presented in Figure 2. As a result of the analysis performed, the peak obtained from the relevant standard was compared with the Wiley275.L library used in the evaluation of the data.

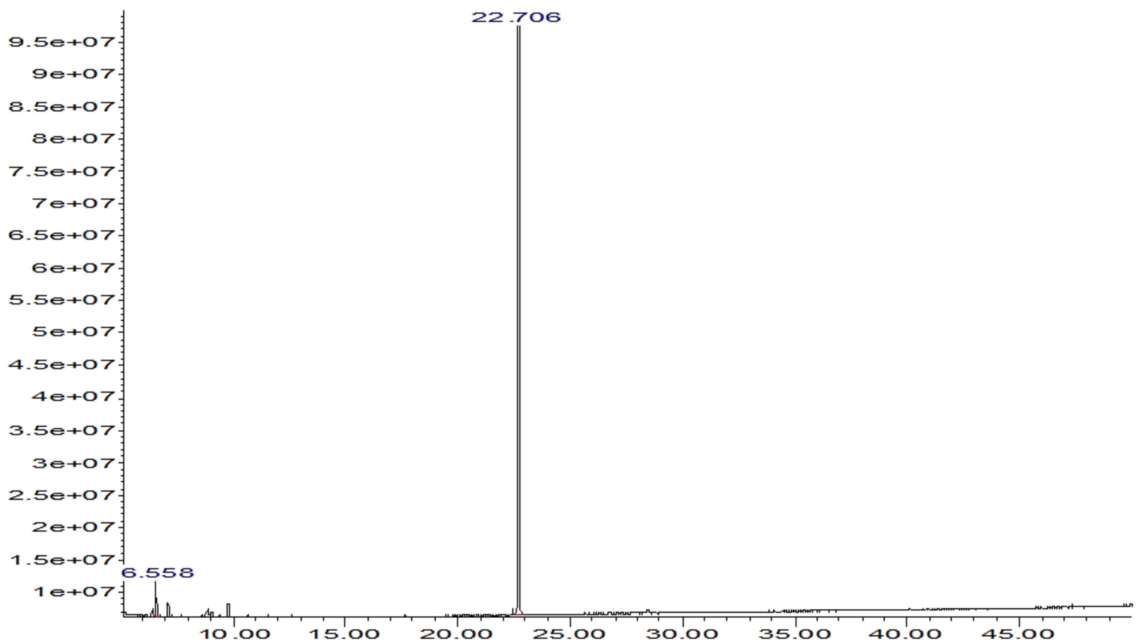

Figure 2. GC-MS chromatogram of β-elemene

As a result of the essential oil analysis of the *N. damascena* plant, it was observed that there were 18 different compounds in the chromatogram and their % amounts were determined. In the study, the main compound was defined as β-elemene (sesquiterpen) in the range of 19.47-62.32%, which was observed to be followed by the Damascenine (alkaloid) compound in the range of 6.91-34.53%. These data obtained as a result of GC-MS analysis also showed compatibility with the studies previously reported in the literature (SIENIAWSKA, 2018; WAJS et al, 2009). In addition to sesquiterpenes (bourboene, elemene, cryophyllene, selinene, 7-epi-selinene) and alkaloids (damascenine, methyl 2-amino-3-methoxyl benzoate), which are found in the essential oil content, also there are trace amount of monoterpene (p-Cymene) (Figure 3).

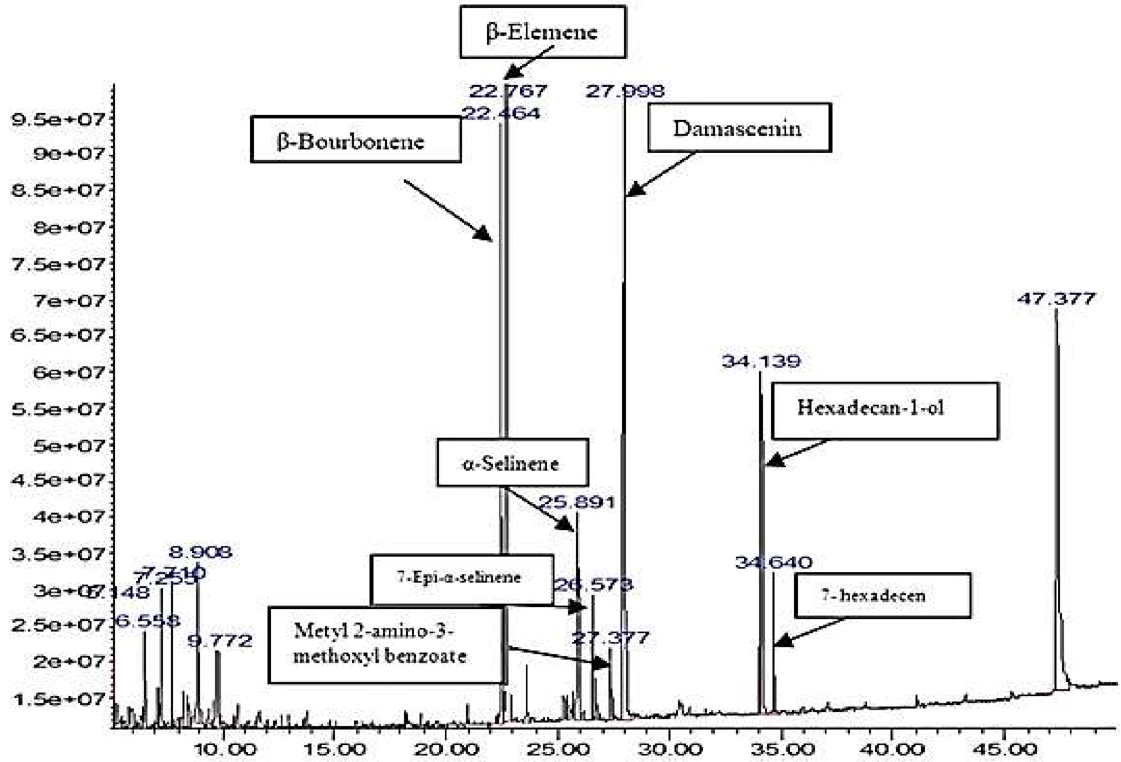

Figure 3. Composition of *N. damascena* seed essential oils

It has been reported in many studies on medicinal aromatic plants that certain doses of fertilization increase essential oil and fixed oil components and decreases in high doses (RIOBA et al, 2015, SEYYEDI et al, 2015) as in our study, even higer content of essential oils were obtained in recommended dosage of chemical fertilizer treatments (Table 5).

Table 5. Essential Oil Composition

Compounds	Retention Time	Control (%)	Organic Half (%)	Organic Recommended (%)	Organic Double (%)	Vermicompost Half (%)	Vermicompost Recommended (%)	Vermicompost Double (%)	Chemical Half (%)	Chemical Recommended (%)	Chemical Double (%)
p-Cymene	12,623	0,51	-	-	-	-	-	-	-	-	0,58
β-Bourbonene	22,464	2,1	6,41	7,52	4,29	5,76	8,87	8,44	4,79	16,7	0,73
β-Elemene	22,767	37,34	47,45	48,24	62,32	60,77	61,13	42,41	53,09	32,59	19,47
β-Caryophyllene	23,647	-	-	-	-	-	-	-	-	1,92	-
α-Selinene	25,891	1,06	3,04	3,97	1,88	2,83	2,4	3,77	2,18	9,03	0,41
7-Epi-α-selinene	26,573	0,52	1,3	1,78	0,82	1,27	1,62	1,6	0,95	4,03	0,42
Metyl 2-amino-3-methoxyl benzoate	27.377	1,3	0,9	1,38	0,71	0,89	1,43	0,72	1,36	1,48	2,04
Damascenine	27.998	20,29	17,61	23,09	16,04	12,71	6,91	17,17	23	18,13	34,53
Unknown	30.864	1,14	-	-	-	-	-	-	-	-	1,69
Unknown	31.850	0,65	-	-	-	-	-	-	-	-	0,95
Unknown	32.062	0,43	-	-	-	-	-	-	-	-	0,53
Unknown	32.244	0,93	-	-	-	-	-	-	-	-	0,54
Unknown	32.866	0,38	-	-	-	-	-	-	-	-	0,48
1,13 tetradecadiene	34.048	0,55	-	-	-	1,01	-	0,96	-	2,15	-
Hexadecan-1-ol	34.139	2,22	3,5	4,02	2,58	4,98	4,13	4,71	2,71	9,02	0,69
Unknown	34.549	0,67	-	-	-	-	-	-	-	-	1,05
7- hexadecen	34.640	1,31	1,38	1,5	1,14	1,8	1,55	1,81	1,08	3,67	0,41
Adipic acide	47.377	4,15	11,14	-	-	0,84	2,37	3,48	-	-	-
Total compounds		75,55	92,73	91,5	89,78	92,86	90,41	85,07	89,16	98,72	64,52

Fixed Oil Rate (%)

In the harvested seeds, constant oil extraction was carried out by the soxhlet method. As a result of the calculation, constant oil yield varied between 34-36.48% among treatment groups (Table 3). Among the fertilizer treatment groups, the highest percentage of fixed oil was observed at the chemical recommendation (36.48%) and the lowest was at the chemical double (34.46%). In all fertilizer treatments, the amount of fixed oil obtained from seeds increased compared to negative control.

In the study of Selicioğlu (2018) on different populations of black seed plant, while the fixed oil rate was in the range of 33.8-35.5%, the highest fixed oil rate among the populations was found in *N. damascena* type as 35.5%. In addition, Kökdil (2005) presented 32.9% fixed oil in this type. In other studies, fixed oil rates were reported in the range of 27.1-34.6% (KÜÇÜKEMRE, 2009), 38.91-40.58% (KULAN et al, 2012), 27.87-31.16% (TAQI, 2013), 16.71-30.08% (BAYTÖRE, 2011), 28.08-34.29% (KALÇIN, 2003), 38.91-40.58% (KULAN et al, 2012), 25.29-29.20% (BANNAYAN et al, 2008), 20.6-33.6% (KARA et al, 2015).

Fixed Oil Yield (kg da^{-1})

Seed yield and fixed oil ratio are important criteria in order to evaluate the seeds obtained after harvest in terms of constant oil yield. In this respect, it is an estimated result that the factors (fertilization, irrigation, genetics, etc.) that can increase seed yield and constant oil rate also affect fixed oil yield.

Since the constant oil rate in the study has very close values in all treatment groups, the most important factor affecting oil yield was seed yield. In this sense, the constant oil yield varies between 15.53-41.43 kg da^{-1} among fertilizer treatments (Table 3).

In the literature, fixed oil yield varied according to treatment differences (sowing frequency, fertilization, sowing time, growing area, etc.) (ERTAŞ, 2016; SELICIOĞLU, 2018; TELCI, 1995; AKGÖREN, 2011). In previous studies, fixed oil yield was generally in the range of 36.78-52.73 kg/da (TELCI, 1995), 13.4-21.0 kg/da (ERTAŞ, 2016), 19.7-43.3 kg/da (SELICIOĞLU, 2018), 6.12-48.94 kg/da (KAMÇI, 2019), 18.78-41.08 kg/da (AKGÖREN, 2011) and our results were found to be compatible with these studies.

Fatty Acids Profile

Fatty acid profile of fixed oil samples of *N. damascena* seeds are shown in Table 6. Linoleic acid (18:2) had the highest amount in the range of 47.34-57.21%. Oleic acid (18: 1) with 23.2-32.5% and palmitic acid (16:0) with 9.58-10.77% accounted for the highest percentage of seed oil after linoleic acid compared to other fatty acids. Also, among the fertilizer treatments, Caprylic (8:0), Myristic (14:0), Pentadecanoic (15:0), Heptadecanoic (17:0), Stearic (18:0), Arachidic (20:0), Henicosanoic (21:0), Tricosanoic (23:0), Liqnoseric (24:0) acids were determined, while Palmitoleic (16:1), Linolenic (18:3), Eicosenoic (20:1), Eicosadienoic (20:2), Nervonic (24:1) acids were investigated as unsaturated fatty acids. Total unsaturated and saturated fatty acids range from 80.08-82.44% and 17.55-19.93%, respectively. In other studies, unsaturated and saturated fatty acids were reported as 79-21% (TELCİ et al, 2014), 80.47-16.83 (KÖKDİL and YILMAZ, 2005), 73.5-13.2% (MATTHAUS and ÖZCAN, 2011), respectively. While linoleic acid was determined as the highest (57.21%) in organic double fertilizer treatment, it was seen in the chemical half treatment in the lowest (47.34%). However, when evaluated in terms of oleic acid, it was determined that it was the highest (32.5%) in chemical half treatment and the lowest in vermicompost double (23.2%). Palmitic acid was found in the highest chemical half treatment group (10.77%) and the lowest in the vermicompost double treatment group (9.58%). It was observed that the organic fertilizer group had the highest total unsaturated fatty acids (82.44%) in the experimental groups, and as the amount increased, the percentage of Linoleic (18:2) and oleic (18:1) acids increased. In addition to being close to total saturated and unsaturated fatty acids obtained in vermicompost and chemical fertilizer treatments, the highest total saturated fatty acid is in vermicompost double (19.93%) treatment group. Although the findings obtained were similar to the data previously published, 4 saturated fatty acids and 1 unsaturated fatty acid (15:0, 21:0, 23:0, 24:0, 24:1) were determined different from the findings of Kökdil and Yılmaz (2005). In the presented study, the presence of 6 saturated fatty acids and 3 unsaturated fatty acids (8:0, 14:0, 15:0, 18:3n3, 20:1, 21:0, 23:0, 24:0, 24:1) different from Daukšas et al. (2002) findings, while 6 saturated fatty acids and 1 unsaturated fatty acids (8:0, 14:0, 15:0, 17:0, 23:0, 24:0, 24:1) were determined from Matthaus and Özcan (2011) findings. On the other hand, 6 saturated fatty acids and 3 unsaturated fatty acids different from the findings of Telci et al. (2014), were detected.

Table 6. Fixed Oil Composition

GROUPS	Rt	Control	Organic Half	Organic Recommended	Organic Double	Vermicompost Half	Vermicompost Recommended	Vermicompost Double	Chemical Half	Chemical Recommended	Chemical Double
C8:0 Caprylic	4.38	0,14 ± 0,005a	0,07 ± 0,005b	0,08 ± 0,005b	0,07 ± 0,005b	0,07 ± 0,005b	0,13 ± 0,005a	-	0,05±0c	0,05±0c	0,07 ± 0,005b
C14:0 Myristic	19.26	0,11 ± 0,005a	0,1 ± 0,0033a	0,1 ± 0,005a	0,1 ± 0,005a	0,1 ± 0,010a	0,1 ± 0,005a	-	0,09±0,003a	0,1 ± 0,002a	0,1 ± 0,01a
C15:0 Pentadecanoic	22.99	0,12± 0,005f	0,33 ± 0,006b	0,29 ± 0,011c	0,06 ± 0,005g	0,18 ± 0,011e	0,14 ± 0,005f	0,55 ± 0,008a	0,31±0,008bc	0,14 ± 0,005f	0,23 ± 0,008d
C16:0 Palmitic	26.41	10,06 ± 0,011c	9,99 ± 0,005d	10,07 ± 0,008c	10,02 ± 0,005d	10,15 ± 0,008b	10,16 ± 0,011b	9,58 ± 0,005f	10,77±0,018a	10,16±0,011b	9,92 ± 0,011e
C16:1 Palmitoleic	27.81	0,14 ± 0,006ab	0,14 ± 0,005ab	0,16 ± 0,012a	0,16 ± 0,011a	0,15 ± 0,014ab	0,15 ± 0,005a	-	0,13 ± 0,007b	0,16± 0,008a	0,14 ± 0,006ab
C17:0 Heptadecanoic	30.05	-	0,71 ± 0,011b	-	-	-	-	1,94 ± 0,005a	-	0,41± 0,012c	-
C18:0 Stearic	35.79	3,85±0,011abc	3,8 ± 0,115bc	3,84 ± 0,008abc	3,82 ± 0,012bc	3,9 ± 0,06a	3,79 ± 0,057bc	3,69 ± 0,057c	3,21 ± 0,011d	3,82±0,017bc	3,9 ± 0,005ab
C18:1 Oleic	37.79	24,25 ± 1,23b	23,71 ± 1,03bc	23,94 ± 1,07bc	24,23 ± 1,21b	24,14 ± 1,32bc	23,99 ± 1,08bc	23,2 ± 1,023c	32,5 ± 2,21a	24,13 ± 1,82bc	24,2 ± 1,24bc
C18:2 Linoleic	41.67	56,67 ± 1,24ab	55,33 ± 1,05b	56,02 ± 1,03ab	57,21 ± 1,28a	56,52 ± 1,33ab	56,66 ± 1,12ab	53,42 ± 1,07c	47,34 ± 2,32d	56,3 ± 1,78ab	56,55 ± 1,35ab
C20:0Arachidic	45.25	0,29 ± 0,005ab	0,28 ± 0,012ab	0,28 ± 0,0115ab	0,28 ± 0,005ab	0,27 ± 0,0115b	0,28 ± 0,0120ab	-	0,21±0,0088c	0,29 ± 0,0145ab	0,31 ± 0,005a
C18:3n3alpha Linolenic	46,31	0,28 ± 0,012b	0,26 ± 0,003c	0,3 ± 0,008b	0,28 ± 0,012bc	0,29 ± 0,013bc	0,28 ± 0,015bc	-	0,42 ± 0,005a	0,28±0,012bc	0,28 ± 0,003bc
C20:1 Eicosenoic	47.52	0,54 ± 0,033g	1,22 ± 0,11b	0,94 ± 0,02c	0,44 ± 0,06g	0,57 ± 0,012f	0,75 ± 0,08d	3,46 ± 0,12a	0,69 ± 0,01e	0,82 ± 0,05d	0,46 ± 0,07g
C21:0 Henicosanoic	50.81	3,24 ± 0,035ab	3,09 ± 0,045c	3,27 ± 0,030a	3,2 ± 0,058abc	3,19 ± 0,0371abc	3,17 ± 0,038abc	3,12± 0,0152bc	2,61 ± 0,14d	3,16 ± 0,023abc	3,25 ± 0,020a
C20:2 cis 11,14 eicosadienoic	51.39	0,14 ± 0,005d	0,35 ± 0,02b	-	-	-	-	-	0,78 ± 0,012a	-	0,24 ± 0,011c
C23:0 Tricosanoic	60.76	-	-	-	-	-	0,02 ± 0,003a	-	-	-	-
C24:0 Liqnoseric	70.24	0,14 ± 0,006g	0,62 ± 0,012d	0,72 ± 0,017c		0,47 ± 0,012e	0,39 ± 0,005f	1,05 ± 0,020a	0,88 ± 0,0145b	0,18 ± 0,005g	0,35 ± 0,0185f
C24:1 Nervonic	70.31	-	-	-	0,12 ± 0,007a	-	-	-	-	-	-
Total unsaturated fatty acids (%)		82,02	81,01	81,36	82,44	81,67	81,83	80,08	81,86	81,69	81,87
Total saturated fatty acids (%)		17,95	18,99	18,65	17,55	18,33	18,18	19,93	18,13	18,31	18,13
Total known (%)		99,97	100	100	99,99	100	99,98	100	99,99	100	100

Conclusion

In the presented study, the effect of fertilizer treatments on yield and quaality properties of *N. damascena* was evaluated and it was seen that the different fertilizers applied (variable concentrations) had different effects. The results obtained in morphological, yield and quality analyzes showed the positive effect of fertilizer compared to the control group in general. It was observed that it developed in morphological characteristics in parallel with the increase of organic and vermicompost fertilizer amounts. However, treatments of chemical fertilizers at double dosage have partially prevented plant growth by showing toxic effects and were statistically in the same group as the control group ($p < 0.05$). In terms of yield and quality characteristics, fertilizer treatments showed an increase in parallel with the dose increase, especially by showing the effect on seed yield, seed weight per plant, constant oil rate (%) and constant oil yield (kg da^{-1}), but again, a negative effect of the chemical double treatment was observed. On the other hand, the best results have been obtained in the chemical recommended treatments for these properties. β-elemene production, which is the main component of *N. damascena* essential oil, was observed in the highest organic double treatment. Although the percentages of the total essential oil components were low in the control and chemical double treatment groups, the components not seen in other treatments were observed in these groups. While there is a limited number of studies in the literature on fatty acid composition in *N. damascena*, the effect of fertilizer type and amount to fatty acid composition was investigated for the first time with this study. It is extremely important that the unsaturated fatty acids, which are valuable for human health and should be consumed in nutrition, are sufficient in the structure of foods (LIN, 2006; HOSSEINI et al, 2018). In this sense, knowing to what extent the applications to be done during the cultivation of herbal products will change these saturated and unsaturated fatty acids will both increase the quality of the grown products and increase the possibility of using these products in the field of pharmacology. Consequently, fertilizers needs to be applied in the right amount and variety in the cultivation of this species, which has valuable compounds such as β-elemene and damascenine, will help increase both its morphological properties and yield-quality and will allow it to be used more frequently in the field of pharmacology.

Acknowledgments

This study partially produced from Funda Ulusu's PhD thesis. This work was supported by Karamanoğlu Mehmetbey University Scientific Research Projects Commission. Grant Number: BAP 03-D18

Conflicts of Interest disclosure

There is no conflict of interest.

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