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Effects of Nitrogen Fertilization and Plant Density on Leaf Mineral Element Contents, Capsule Yield, Seed Yield and Morphine Ratio of Poppy (*Papaver somniferum* L.) Genotypes

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Abstract

Plant density for poppy (*Papaver Somniferum* L.) and impacts of genotype and nitrogen fertilizer dose applications on total ash, N, P, K, Ca, Mg, Na, Fe, Mn, Zn, Cu contents on poppy leaves, capsule yield, seed yield and morphine ratio were investigated. Two plant density (SD_1: 25 plants per m^2, SD_2: 16 plants per m^2), four poppy genotypes (Zaferyolu, Tınaztepe, Ofis 3 and Local line) and four nitrogen fertilizer dose (0, 6, 12 and 18 kg da^{-1}) were used. Experiments were conducted in randomized complete block, split-split plots design with 3 replications for 2 years (2009/10 and 2010/11). While the effect of plant density x nitrogen dose x genotype interaction was statistically significant in terms of the total ash ($p<0.01$), mineral elements of poppy leaves, capsule yield and seed yield, it was not significant for morphine ratio. The highest capsule yield and seed yield (134.30 kg da^{-1} and 161.11 kg da^{-1}) were obtained in 12 kg da^{-1} N treatment of local line genotype at SD_1 plant density and the lowest capsule yield and seed yield (67.22 kg da^{-1} and 76.53 kg da^{-1}) were observed in the without nitrogen of Tınaztepe genotype at SD_2 plant density, respectively. Morphine ratios were determined as 0.445%, 0.508%, 0.570% and 0.597% according to N doses (0, 6, 12 ve 18 kg da^{-1}), respectively. With regard to plant density, SD_1 (25 plants per m^2) was prominent for majority of investigated parameters. As compared to other genotypes, Ofis 3 genotype can be recommended 12 kg da^{-1} N treatment in terms of mineral nutrient contents of poppy leaves and morphine ratio at SD_1 plant density.

Keywords: Genotype, mineral elements, morphine ratio, nitrogen, poppy, plant density, yield

Haşhaş (*Papaver Somniferum* L.) Genotiplerinde Ekim Sıklığı ile Azotlu Gübrelemenin Haşhaş Yapraklarının Mineral Madde İçerikleri, Kapsül Verimi, Tohum Verimi ve Morfin Oranı Üzerine Etkisi

Öz

Haşhaşta (*Papaver Somniferum* L.) ekim sıklığı, genotip ve azotlu gübre dozu uygulamalarının haşhaş yapraklarında toplam kül, N, P, K, Ca, Mg, Na, Fe, Mn, Zn, Cu içerikleri, kapsül verimi, tohum verimi ve morfin oranı üzerine olan etkileri araştırılmıştır. Çalışmada iki ekim sıklığı (SD_1: serpme ekim, m^2'de yaklaşık 25 bitki, SD_2: sıraya ekim, m^2'de 16 bitki), dört haşhaş genotipi (Zaferyolu, Tınaztepe, Ofis 3 ve Yerel hat) ve dört azotlu gübre dozu (0, 6, 12 ve 18 kg da^{-1}) kullanılmıştır. Deneme tesadüf bloklarında bölünen bölünmüş parseller deneme desenine göre 3 tekerrürlü olarak kurulmuştur. Ekim sıklığı x azot dozu x genotip interaksiyonu haşhaş yapraklarının toplam kül, mineral elementler, kapsül ve tohum verimi

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bakımından istatistiksel olarak önemli iken ($p<0.01$), morfin oranı bakımından önemsiz bulunmuştur. En yüksek kapsül verimi ve tohum verimi (134.30 kg da^{-1} and 161.11 kg da^{-1}) SD_1 ekim sıklığında 12 kg N da^{-1} uygulanan yerel hat genotipinde ve en düşük kapsül verimi ve tohum verimi (67.22 kg da^{-1} and 76.53 kg da^{-1}) SD_2 ekim sıklığında N uygulanmayan Tınaztepe genotipinden elde edilmiştir. Bu uygulamadan elde edilen morfin oranları N dozlarına (0, 6, 12 ve 18 kg da^{-1}) göre sırası ile %0.445, %0.508, %0.570 ve %0.597 olarak belirlenmiştir. Diğer genotip çeşitleriyle (Zaferyolu, Tınaztepe ve Yerel hat) karşılaştırıldığında, Toprak Mahsülleri Ofisinin tavsiye ettiği Ofis 3 çeşidine ait haşhaş bitki yaprağının makro ve mikro besin içeriklerinin ve morfin oranının daha yüksek olduğu belirlenmiştir.

Anahtar Kelimeler: Azot dozları, ekim metotları, haşhaş, genotip, mineral elementler, morfin oranı, verim

INTRODUCTION

Poppy (*Papaver somniferum* L.) is a plant which has been traditionally cultured since ancient times in Anatolia, and today half of the legal production areas of the world under the control of the United Nations is located in Turkey. The other common poppy producer countries include India, Japan, China, France and Spain. In Turkey, Afyon, Kütahya, Isparta, Burdur, Konya, Balıkesir, Çorum, Amasya, Manisa, Tokat, Denizli, Uşak and Eskişehir provinces are main opium poppy production areas. Turkey and India are the leading producer countries. Poppy is cultivated over 101 874 hectares worldwide (FAOSTAT, 2015). In Turkey, poppy is cultivated over 61 591 hectares with an annual production of 30 730 tons and an average poppy yield of 500 kg ha^{-1} (TURKSTAT, 2015). More than 50% of the total sowing areas in the world is located in Turkey according to the data of the year 2015 year (FAOSTAT, 2015).

Sowing is performed either manually or with sowing machines. Manual seeding has already been vanished in developed countries of the world, but still is practiced for some economic crops and poppy in Turkey. Such seeding methods are usually practiced because of small land sizes and insufficient machinery opportunities. The optimum plant density should be reached in order to get optimal yields. Plant density is highly effective on yield and yield components (Yılmaz, 1999).

Nitrogen (N) nutrition is crucial for poppy; thus, it is necessary to select a suitable dose, form and date of application (Ehsanipour et al., 2012). Among the fertilizers, nitrogen is the most significant one. Nitrogen positively influences plant growth and development, improves capsule and seed yield, promotes synthesis of nitrogenous substances and regulates some quality and pharmacological parameters (Katar and Yılmaz, 1997). Therefore, sufficient nitrogen should be supplied to plants. N uptake by plants from the soil solution in the form of NO_3^- anion and NH_4^+ cation is associated with the mutual effects of other nutrients on inorganic N bonds. The nitrogen use efficiency is affected by the availability of other plant nutrients and the maximum benefits from N application can only be achieved when adequate supply of other macronutrients as well as micronutrients is assured keeping in view their synergistic or antagonistic effects in different crops (Manchanda and Aulakh, 2007). One of the essential conditions for good utilisation of soil N is good phosphorous (P) and potassium (K) availability in the soil. The N x P interaction can, thus, be termed the single most important nutrient interaction of practical significance. This interaction is often synergistic, occasionally additive, and, in rare cases, may be antagonistic (Biswas and Prasad, 1991). The synergistic interactions between N and P help explain the effect, when applied as a banding beneath seed, on root growth and proliferation (Biswas and Prasad, 1991). Increased N application generally increases production and the content of nitrogen and proteins in plants (Ehsanipour et al., 2012). Disturbances of N metabolism, resulting from K deficiency, are manifested in changes in the proportions of N fractions and in the accumulation of harmful amino substances in plants (Nurzyńska and Wierdak, 2006). The significance of N x K interaction and its optimum management is increasing due to increasing cropping intensity, higher crop yield and greater depletion of soil K (Singh, 1992).

Ecological factors play a great role in seed yield and capsule yield of the poppy genotypes. Besides, genotype, cultural practices and growing conditions also play an important role in capsule yield and seed yield. Poppy genotypes are sometimes broadly classified as culinary, industrial or dual purpose. There are differences among the operational procedures (i.e. fertilization, plant density) adopted by poppy growing areas across the world (Bernáth and Németh, 2010). Since varietal development is an essential prerequisite for achieving success in the commercial cultivation of any medicinal plant species, over the years, many genotypes of poppy have also been developed for different beneficial purposes. Among the cultural practices, fertilization and irrigation may improve the capsule and seed yields significantly. However, fertilization plays more significant role in improving capsule yield, seed yield and morphine ratio. Poppy nutrition largely depends on soil macro and micro element contents (Yadav et al. 1984).

The main component of the poppy's alkaloids was morphine, and its ratio varied between 0.45% and 1.00% depending on the genotypes (Skalicky et al., 2014). Skalicky et al. (2014) reported that the morphine ratio of the industrial poppies was approximately 1%. Morphine ratio of the capsule is low in Turkey when compared to other poppy producer countries. The morphine contents of the poppy populations in Turkey varied with 0.25-0.89% (Arslan et al., 2000). The morphine ratio of the poppy capsules obtained from the different provinces in Turkey varied with 0.093- 0.263% (Erdemoglu et al., 2002). Recently, the ten poppy genotypes with high morphine ratio were registered by the Soil Products Office. The poppy quality is mostly evaluated with its morphine ratio. Prajapati et al. (2002) reported that poppy varieties with high morphine ratio are used for medicinal purpose and varieties with low morphine ratio are used for food production. The capsule yield, seed yield and morphine ratio of the poppy genotypes varied between 0.30-6.48 g plant^{-1}, 0.26-11.66 g plant^{-1} and 0.22-1.225%, respectively (Karadavut and Arslan, 2006).

In this study, effects of genotypes, plant density and nitrogen doses on total ash, N, P, K, Ca, Mg, Na, Fe, Mn, Zn, Cu contents of poppy leaves, capsule yield, seed yield and morphine ratio were investigated.

MATERIALS AND METHODS

Experimental site

Experiments were conducted over the farmer's field within Yalnız village of Merzifon town of Amasya province as winter sowing in 2009/10 and 2010/11 growing seasons. Amasya is located in the middle of Black Sea region between 35°00'-36° 30' east longitudes and 40°15'-41°03' north latitudes. Soil characteristics of the research site are given in Table 1. Experimental soils were clay in texture with slight alkaline soil reaction. Soils were unsaline, poor in organic matter and rich in available phosphorus and potassium (Soil Survey Staff, 2014).

Table 1. Some chemical and physical properties of the experimental site
Çizelge 1. *Deneme alanine ait bazı kimyasal ve fiziksel özellikler*

Properties	Amount	Properties	Amount
Sand (%)	15.58	P (ppm)	19.059
Silt (%)	20.12	K (meq 100 g^{-1})	1.533
Clay (%)	64.30	Ca (meq 100 g^{-1})	23.171
pH (1:1)	7.90	Mg (meq 100 g^{-1})	13.851
EC (dS m^{-1})	0.689	Na (meq 100 g^{-1})	0.954
OM (%)	1.708		

Climate characteristics of the research site

Some climate data for two growing seasons (2009/10 and 2010/11) are provided in Table 2. Amasya province generally had dominant Black Sea climate, but exhibits terrestrial climate characteristics since there is no sea cost of the province. Annual average precipitation is below 500 mm with the greatest precipitations in winter. Precipitations decrease in spring, there is drastic decline in July, the least precipitation was observed in August and precipitations increased again in September and so on. Long-term (40 years) vegetation period average temperature is 9.82 °C with the greatest temperatures in June and July and the least in January. Long-term vegetation period relative humidity is 69.26%. Total precipitation of the experimental years was 360.4 and 404.5 mm.

Table 2. Some climate data of the experiment area in the poppy growing seasons (Amasya Meteorology Office Records)
Çizelge *2.* *Haşhaş gelişme sezonunda deneme alanının bazı iklim verileri*

Climatic factors	Years	Months										Total or Average
		October	November	December	January	February	March	April	May	June	July	
Average Temperature (°C)	2009-10	12.9	7.0	2.6	0.8	2.3	6.1	11.3	15.3	18.7	21.2	9.82
	2010-11	15.9	7.2	6.2	3.5	6.6	6.9	10.6	16.3	20.1	23.1	11.64
	Long terms	11.8	10.3	5.4	1.7	2.5	5.4	8.7	13.9	17.9	22.7	10.03
Precipitation (mm)	2009-10	37.4	34.8	45.8	37.1	28.0	36.1	55.7	58.4	48.5	20.6	402.4
	2010-11	19.2	57.6	47	16.8	36.2	9.4	45.2	28.8	99.8	0.4	360.4
	Long terms	40.0	26.3	118.4	35.6	9.8	21.8	26.6	59.8	48.2	18.0	404.5
Relative humidity (%)	2009-10	68.5	72.9	77.4	76.5	72.7	68.0	64.8	65.4	64.3	62.1	69.26
	2010-11	57.5	76.5	75.9	76.2	72.3	66.7	64.5	57.3	66.5	60.3	61.94
	Long terms	73.1	65.6	79.7	78.0	69.2	67.6	69.2	68.8	64.6	58.2	69.40

Plant material

In present experiments, seeds of 4 opium (Papaver somniferum L.) genotypes (Ofis 3 genotype supplied from Soil Products Office; Zaferyolu and Tınaztepe genotypes supplied from Eskişehir Agricultural Research Institute and a local opium line used in Merzifon town of Amasya province) were used as the plant materials.

Field experiments

Experimental factors included 4 poppy materials (3 genotypes and 1 line), 2 plant densities (SD_1: 25 plants per m^2; SD_2: 16 plants per m^2 with 60 cm row spacing and 10 cm on-row plant spacing) and 4 different N doses (0, 6, 12 and 18 kg da^{-1}). Experiments were conducted in randomized blocks split-split plots design with 3 replications for 2 years (2009/10 and 2010/11). Plant density was sown on the 25 October in the both growing years. Harvest was carried out on 1 July 2010 in the first year and 3 July 2011 in the second year. The capsules in the center of each plot in the full ripeness period according to the maturity stage of the cultivars were manually harvested.

Seeds were sown in 3x3 m plots as to have 300 g seed per decare in SD_1 and thinning was performed at the first hoeing as to have 25 plants per m^2. In SD_2, manual seeding was performed at 60 cm row spacing, then thinning was performed as to have 16 plants per m^2 with 10 cm on-row plant spacing. Total experimental area was 1519 m^2, main plot size 465 m^2, sub-plot size was 225 m^2 and sub-sub plot size was 9 m^2. Harvest was performed from inner rows. Side rows and 50 cm sections from the top and bottom of each plot were omitted as to consider side effects.

Cultural practices

Following the harvest of preceding plants, fields were tilled with moldboard plows, then made ready for sowing through sweeps and harrows. Ammonium sulphate (21% N) was used as the N source and half of 0, 6, 12 and 18 kg da^{-1} N doses were applied after sowing and remaining half was applied after hoeing and thinning processes at around bolting period. Irrigation was not practiced in both years. Representative leaf samples were taken from each plot following the flowering period.

Leaf analyses

Collected opium leaves were brought to laboratory in paper bags, washed through distilled water, a portion was separated for analyses to be conducted on fresh leaves and the rest was dried at 65 °C in an air-circulating drying cabin until a constant weight. Dried leaf samples were ground and stored in labeled polyethylene bags. Dried samples were dry-ashed in accordance with Kacar and Inal (2008) to get ash content. Leaf total nitrogen (N) content was determined in a Leco TruSpec-CHN device in accordance with Dumas method (Kacar and Inal, 2008). Samples (0.25 g) were subjected to wet digestion with nitric acid (HNO_3) in a microwave device for P, K, Ca, Fe, Cu and Zn analyses. These samples were transferred to 50 ml cups and final volume was completed with deionized water and filtered through blue-band filter paper. Total K was determined in wet-digestion solution with a Jenway PFP 7 Flamephotometer (Kacar and Inal, 2008). Total phosphorus of wet-digestion solution was determined in accordance with yellow-color method in a Shimadzu UV-160 Spectrophotometer (Kacar and Inal, 2008). Wet-digestion filtrates were subjected to Ca, Fe, Cu and Zn analyses with a Varian 720-ES ICP-OES device (Kacar and Inal, 2008).

Capsule and seed yields

The capsule yield (kg da^{-1}) and seed yield (kg da^{-1}) were determined as described by Karabuk (2012).

Morphine ratio

The capsule of the poppy was dried for 24 hours at 70 ^{O}C, and the capsules were powered by grinding. The morphine ratio was analyzed according to the spectrophotometric method of the Afyonkarahisar- Bolvadin Poppy and Alkaloid Office (Karabuk, 2012).

Data assessment

Experimental results were subjected to statistical analyses with SPSS Version 16.0 statistics software. Data were subjected to ANOVA. Treatment means were compared with Duncan's multiple range test at 0.01 significance level and correlation analyses were performed to express the relationships between experimental parameters (Yurtsever, 2011).

RESULTS AND DISCUSSION

Plant density x nitrogen doses x genotype interactions had significant effects on leaf total ash, N, P, K, Ca, Mg, Na, Fe, Mn, Zn and Cu contents ($p<0.01$; Table 3 and 4). The difference between the mineral element contents of poppy leaf, seed yield and morphine ratio was not significant between years.

Effects of plant density, nitrogen doses and genotypes on macronutrients contents of poppy leaves

Total nitrogen (N)

The greatest total N value (3.73%) was obtained in 18 kg da^{-1} N treatment of Ofis 3 genotype at SD_1 plant density and the lowest total N value (2.25%) was obtained in the control (without N) of Zaferyolu genotype at SD_2 plant density. In general, total N values of poppy leaves increased as nitrogen dose increased in all poppy genotypes at both plant density. The majority of soils around the globe are deficient in available N and are either low or medium in available P (IFA, 2003). These two nutrients account for a major share of the current annual fertilizer consumption (IFA, 2003). Nitrogen nutrition is crucial for poppy; it is necessary to select a suitable dose, form and date of application (Ehsanipour et al., 2012). Total N is responsible for the development of leaf area (Escalante, 1999) and is a major mineral element (Sedano-Castro et al., 2011) used in agricultural fertilization. Total N is the element most absorbed from soil by plants growing under normal conditions. Within the plant, N serves in the same ways as it does in other organisms as a component of amino acids and nucleic acids. N also plays a critical role in the structure of chlorophyll, the primary light harvesting compound of photosynthesis. This, along with its structural role in amino acids, explains why plants require large amounts of N, and thus why it is often the limiting nutrient for plant growth. In this study, increasing the N levels significantly increased the N content in the plant leaves.

Phosphorus (P)

P values of poppy leaves varied between 0.15-0.35% with a mean value of 0.23%. The greatest value (0.35%) was obtained in 18 kg da^{-1} N treatment of Ofis 3 genotype at SD1 plant density, while the lowest value (0.15%) was obtained in the control treatment (without N) of Zaferyolu genotype at SD_1 plant density. In general, P values of poppy leaves increased as nitrogen dose increased in all poppy genotypes at both plant density. N can increase P uptake in plants by increasing root growth, by increasing the ability of roots to absorb and translocate P, and by decreasing soil pH as a result of absorption of NH4- and thus increasing solubility of fertilizer P (Wilkinson et al., 1999). The interaction between P and N has been found to be synergistic (Wilkinson et al., 1999). P is a necessary component of the photosynthetic processes which are systematically implicated in creation of oils, sugars and starches. It also improves the rapid growth of plants as well as root systems (Brown and Weselby, 2010).

Table 3. Plant density x nitrogen dose x genotype interaction on total ash, N, P, K, Ca, Mg and Na contents of poppy leaves

Çizelge 3. *Haşhaş yapraklarının toplam kül, N, P, K, Ca, Mg ve Na içerikleri üzerine ekim sıklığı x azot dozu x genotip interaksiyonu*

Plant density	N doses	Genotypes	Total ash, %	N, %	P, %	K, %	Ca, %	Mg, %	Na, %
SD_1	N_0	Zaferyolu	20.2 b-e	2.32 m	0.15 f	3.66 g-j	1.03 d-g	0.52 b-f	0.028 gh
		Ofis 3	19.8 b-g	2.93 g-j	0.25 b-e	4.01 b-f	1.04 c-g	0.51 b-g	0.023 h
		Tınaztepe	20.0 b-f	2.86 h-j	0.21 c-f	3.56 ıj	1.05 c-g	0.53 b-f	0.023 h
		Local line	19.3 c-j	2.72 ı-l	0.20 def	4.02 bf	1.07 c-f	0.52 b-f	0.021 h
	N_6	Zaferyolu	19.8 b-g	2.36 lm	0.17 ef	3.91 d-h	1.07 c-f	0.50 c-h	0.027 gh
		Ofis 3	18.1 ıjk	3.43 a-e	0.24 b-e	4.03 b-f	1.18 c	0.53 b-f	0.022 h
		Tınaztepe	19.0 d-j	3.23 b-h	0.26 bcd	3.95 c-h	1.09 c-f	0.52 b-f	0.025 h
		Local line	19.7 b-h	3.20 b-h	0.22 b-f	4.21 a-d	1.09 c-f	0.51 b-g	0.044 f
	N_{12}	Zaferyolu	18.4 g-k	2.98 f-j	0.27 bcd	3.94 c-h	1.00 efg	0.48 e-h	0.078 d
		Ofis 3	14.3 l	3.39 a-f	0.29 abc	4.31 ab	1.18 c	0.58 abc	0.075 d
		Tınaztepe	17.2 k	3.38 a-f	0.23 b-f	3.82 e-ı	0.99 fg	0.48 e-h	0.060 e
		Local line	18.3 h-k	3.11 d-ı	0.23 b-f	4.22 a-d	1.14 cde	0.51 b-g	0.040 fg
	N_{18}	Zaferyolu	19.6 b-ı	3.25 b-h	0.34 a	3.91 d-h	1.14 cde	0.49 d-h	0.026 h
		Ofis 3	17.8 jk	3.73 a	0.35 a	4.38 a	1.32 b	0.65 a	0.022 h
		Tınaztepe	17.9 jk	3.19 c-h	0.26 bcd	4.28 abc	1.01 d-g	0.42 h	0.017 h
		Local line	19.7 b-h	3.59 abc	0.26 bcd	4.00 b-g	1.15 cd	0.43 gh	0.022 h
SD_2	N_0	Zaferyolu	21.9 a	2.25 m	0.19 def	3.40 j	1.09 c-f	0.55 b-e	0.026 h
		Ofis 3	20.2 b-e	3.05 e-ı	0.23 b-f	3.87 d-ı	1.09 c-f	0.57 bcd	0.023 h
		Tınaztepe	20.4 bcd	2.30 m	0.17 ef	3.61 hıj	1.10 c-f	0.53 b-f	0.024 h
		Local line	20.8 abc	2.27 m	0.17 ef	4.13 a-e	0.97 fg	0.47 e-h	0.021 h
	N_6	Zaferyolu	21.1 ab	2.46 klm	0.19 def	3.75 f-ı	0.92 g	0.53 b-f	0.042 f
		Ofis 3	19.1 d-j	3.03 e-ı	0.23 b-f	4.28 abc	1.06 c-g	0.48 e-h	0.048 f
		Tınaztepe	20.0 b-f	2.91 g-j	0.17 ef	3.76 f-ı	1.06 c-g	0.47 e-h	0.040 fg
		Local line	20.1 b-f	2.85 h-k	0.17 ef	4.08 a-f	1.03 d-g	0.55 b-e	0.029 gh
	N_{12}	Zaferyolu	18.6 f-k	2.60 j-m	0.17 ef	4.04 a-f	1.02 d-g	0.59 ab	0.159 a
		Ofis 3	18.6 f-k	3.49 a-d	0.25 b-e	4.09 a-f	1.01 d-g	0.57 bcd	0.124 b
		Tınaztepe	19.6 b-h	3.05 e-ı	0.22 b-f	4.17 a-e	1.01 d-g	0.55 b-e	0.112 c
		Local line	20.6 abc	3.15 d-h	0.23 b-f	4.03 b-f	1.11 c-f	0.50 c-h	0.063 e
	N_{18}	Zaferyolu	20.5 a-d	2.84 h-k	0.30 ab	3.95 c-h	1.09 c-f	0.49 d-h	0.020 h
		Ofis 3	18.8 e-j	3.61 ab	0.29 abc	4.02 b-f	1.45 a	0.49 d-h	0.020 h
		Tınaztepe	20.2 b-e	3.33 a-g	0.30 ab	4.08 a-f	1.00 efg	0.45 fgh	0.023 h
		Local line	20.1 b-f	3.12 d-ı	0.22 b-f	4.10 a-f	1.18 c	0.47 e-h	0.028 gh

Different letters between plant density x nitrogen dose x genotype interaction donete significant differences (Duncan test, $p< 0.01$)

Potassium (K)

K values of poppy leaves varied between 3.40-4.38% with a mean value of 3.99%. The greatest value (4.38%) was obtained in 18 kg da^{-1} N treatment of Ofis 3 genotype at SD_1 plant density and the lowest value (3.40%) was obtained in the control treatment (without N) of Zaferyolu

Table 4. Plant density x nitrogen dose x genotype interaction on Fe, Mn, Zn and Cu contents of poppy leaves
Çizelge 4. *Haşhaş yapraklarının Fe, Mn, Zn ve Cu içerikleri üzerine ekim sıklığı x azot dozu x genotip interaksiyonu*

Plant density	N doses	Genotypes	Fe. ppm		Mn. ppm		Zn. ppm		Cu. ppm	
SD_1	N_0	Zaferyolu	36.37	lm	36.19	ghı	20.56	cde	5.19	e
		Ofis 3	43.63	cd	36.43	ghı	18.44	cde	5.82	cde
		Tınaztepe	40.00	e-j	39.83	cde	18.95	cde	5.78	cde
		Local line	36.86	klm	37.55	fgh	18.62	cde	6.09	cde
	N_6	Zaferyolu	35.14	m	33.32	jk	20.91	cde	6.22	cde
		Ofis 3	50.95	b	36.32	ghı	23.56	cde	7.17	a-e
		Tınaztepe	38.35	ı-l	36.88	ghı	17.53	de	8.17	abc
		Local line	45.78	c	35.06	ıj	19.78	cde	9.22	ab
	N_{12}	Zaferyolu	39.74	f-k	36.78	ghı	16.08	e	7.61	a-e
		Ofis 3	55.80	a	39.20	def	20.18	cde	6.83	b-e
		Tınaztepe	45.85	c	35.91	hı	20.00	cde	8.18	abc
		Local line	50.35	b	45.38	a	20.36	cde	8.18	abc
	N_{18}	Zaferyolu	41.77	d-h	35.92	hı	32.80	c	9.75	a
		Ofis 3	51.22	b	45.38	a	56.24	ab	8.96	ab
		Tınaztepe	42.84	de	31.56	kl	46.31	b	8.96	ab
		Local line	43.11	cd	39.80	cde	31.53	cd	7.93	a-d
SD_2	N_0	Zaferyolu	34.52	m	30.11	l	16.21	e	5.78	cde
		Ofis 3	39.01	g-l	35.19	ıj	17.99	cde	5.49	De
		Tınaztepe	36.43	lm	35.65	hı	15.05	e	5.18	e
		Local line	39.02	g-l	35.02	ıj	20.15	cde	6.09	cde
	N_6	Zaferyolu	40.73	d-ı	33.32	jk	21.71	cde	7.61	a-e
		Ofis 3	41.97	d-g	39.79	cde	24.69	cde	8.33	abc
		Tınaztepe	42.82	de	32.10	k	15.34	e	7.64	a-e
		Local line	40.69	d-ı	35.03	ıj	26.34	cde	7.37	a-e
	N_{12}	Zaferyolu	37.17	j-m	36.28	ghı	18.29	cde	9.75	a
		Ofis 3	50.37	b	43.00	b	28.71	cde	8.96	a-d
		Tınaztepe	36.14	lm	36.53	ghı	26.43	cde	8.16	abc
		Local line	45.80	c	41.41	bc	25.66	cde	7.93	a-d
	N_{18}	Zaferyolu	38.83	h-l	35.60	hı	62.41	a	6.96	b-e
		Ofis 3	42.06	def	40.84	cd	56.98	ab	7.11	b-e
		Tınaztepe	41.95	d-g	37.62	fgh	65.08	a	8.11	a-d
		Local line	41.73	d-h	37.98	efg	65.47	a	9.22	ab

Different letters between sowing density x nitrogen dose x genotype interaction donete significant differences (Duncan test, p<0.01).

genotype at SD_2 plant density. Domino effects of K are very common in the plant system due to the complex relationship between K and other nutrients. Synergistic interactions are well known for N x K and N x P interactions (Aulakh and Malhi 2005; Manchanda and Aulakh, 2007). In addition to N, K is the major plant nutrient absorbed and removed by crops in the largest amounts among all essential nutrients (Zlatev and Lidon, 2012). After N x P interactions, N x K interactions are the second most significant interaction in crop production (Manchanda and Aulakh, 2007). The important of N x K interaction and its optimum management is increasing due to increasing cropping intensity, higher crop yield and greater depletion of soil K (Singh, 1992; Manchanda and Aulakh, 2007). K is extremely important in many ways to the productivity of plant. It not only performs the important physiological functions, but it improves nitrogen use efficiency.

In conclusion, nitrogen is directly related to yield. In this study, increasing the nitrogen levels significantly increased the K content, and thus capsule and seed yield.

Effects of plant density, nitrogen doses and genotypes on micronutrients contents of poppy leaves

Iron (Fe)

Fe contents of poppy leaves varied between 34.52-55.80 ppm with a mean value of 42.09 ppm. The greatest value (55.80 ppm) was obtained in 12 kg da^{-1} N treatment of Ofis 3 genotype at SD_1 plant density and the lowest value (34.52 ppm) was obtained in the control treatment (without N) of Zaferyolu genotype at SD_2 plant density. Iron has direct synergistic relationships with N, P and K. Optimum supply of N ensures optimum uptake of iron as well as K, P, Mg, Mn and Zn from the soils. Excessive amounts of N decrease the uptake of P, K, Fe and almost all secondary and micronutrients like Ca and Mg, Fe, Zn, Mn and Cu (Malvi, 2011). Fe is an importance element in crops, because it is essential for many significant enzymes, including cytochrome that is involved in synthesize chlorophyll, electron transport chain, enzyme activity and maintain the structure of chloroplasts. In general, solubility of trivalent Fe reduces by increasing pH (Rubio et al., 2005). It is a component of ferrodoxin which is responsible for oxidation/reduction reactions in the plant system like-nitrate and sulphate reduction and N fixation (Malvi, 2011). Fe deficiency has a powerful effect on chloroplast protein, so that chloroplast protein is decreased importantly by Fe deficiency. In conditions of severe Fe deficiency, cell division stops and therefore leaf growth reduces. Fe solution concentrations in flooding soils can be increased several-folds due to low redox potential. In these conditions, large amounts of Fe may be available for plant, and can be toxic to plants. Brown plant tissues, black and soft roots are the Fe toxicity symptoms.

Manganese (Mn)

Mn contents of poppy leaves varied between 30.11-45.38 ppm with a mean value of 37.09 ppm. The greatest value (43.58 ppm) was obtained in 12 kg da^{-1} treatment of the local line and 18 kg da^{-1} treatment of Ofis 3 genotype at SD1 plant density, the lowest value (30.11%) was obtained in the control treatment (without N) of Zaferyolu genotype at SD_2 plant density. Mn concentration tended to increase with N fertilization. The highest concentration of Mn was observed in leaves indicating that N fertilizer might promote the absorption of Mn by roots and its translocation from roots to shoot (Hu-Lin et al., 2007). Mn has direct synergistic relationships with K (Manchanda and Aulakh, 2007). Mn is a very significant component of photosynthesis, N metabolism and N assimilation; it activates decarboxylase, dehydrogenase and oxidase enzymes. Fe plays a very significant part in chlorophyll formation. It is a component of ferrodoxin which is responsible for oxidation/ reduction reactions in the plant system like-nitrate and sulphate reduction and N fixation. Each essential element may perform its role in plant nutrition properly only when the other necessary elements are available in balanced ratios for plant. Divalent Mn^{2+} are converted into Mn^{3+} or Mn^{4+} easily, thus Mn plays a significant role in oxidation and reduction processes and in electron transport in photosynthesis. Moreover, Mn acts as an activator of many enzymes (Sharifianpour et al., 2013). Mn has an effective role in lipids metabolism. Moreover, amount of lignin in the plant will decrease due to Mn deficiency, such a reduction is more severe in roots. Mn deficiency symptoms first appear on younger leaves and then seen in older leaves (Mousavi et al., 2013).

Zinc (Zn)

Zn contents of poppy leaves varied between 15.05-65.47 ppm with a mean value of 28.39 ppm. The greatest value (65.47 ppm) was obtained in 18 kg da^{-1} N treatment of Local Line at SD_2 plant density and the lowest value (15.05 ppm) was obtained in the control treatment (without N) of Tınaztepe genotype at SD_2 plant density. N treatment has been determined to effect Zn absorption by plants and vice versa. N x Zn interaction is an important factor in nutrient management for all field crops that require moderate to high amounts of N. The synergistic N x Zn interaction has also been determined to increase the N concentration in different crops as Zn helps to accelerate protein synthesis and the biological N_2 fixation (Verma and Bhagat, 1990). Zn uptake of soil solution is generally in divalent cation form (Zn^{2+}), but in calcareous soils with high pH zinc uptake may be in valence ion form. The main function of Zn is tendency to make up tetragonal complexes with N, sulfur and oxygen, thus Zn have a catalytic, activating and building role in the enzymes. Zn deficiency symptoms appear on young leaves of the plants first; because Zn cannot be transferred to younger tissues from older tissue (Mousavi et al., 2013).

Copper (Cu)

Cu contents of poppy leaves varied between 5.18-9.75 ppm with a mean value of 7.49 ppm. The greatest value (9.75 ppm) was obtained in 12 kg da^{-1} N treatment of Zaferyolu genotype at SD_2 plant density and the lowest value (5.18 ppm) was obtained in the control treatment of Tınaztepe genotype at SD_2 plant density. Nutrient interaction in crops is probably one of the most significant factors affecting yields of annual crops. Optimal levels of copper and Zn increase uptake of N and P (Fageria 2014). An increase in Cu uptake by wheat due to N application has also been reported (Singh and Swarup, 1982). However, antagonistic N x Cu interaction was observed only when both were in excess supply. At lower levels of Cu, the effects of N on Cu uptake were synergistic (Antil et al., 1988). Cu is contained in cell wall formation, several enzyme systems, oxidation reactions and electron transport. Cu is not readily transferred from older to younger leaves. Cu deficiencies will most likely show up first in barley, wheat, canary or oats seed, as these crops are highly sensitive to Cu deficiency (Ask Saskatchewan Agriculture, 2012). Crop genotypes may differ widely in sensitivity to Cu deficiency (Ask Saskatchewan Agriculture, 2012).

Effects of plant density, genotypes and nitrogen doses on capsule yield, seed yield and morphine ratio of poppy

Capsule yield

Plant density x nitrogen doses x genotypes interactions had significant effects on capsule yield ($p< 0.05$, Figure 1). The greatest capsule yield and seed yield (134.30 kg da^{-1}) was obtained in 12 kg da^{-1} N treatment of local line genotype at SD_1 plant density and the lowest capsule yield and seed yield (67.22 kg da^{-1}) was obtained in the control (without N) of Tınaztepe genotype at SD_2 plant density. In general, capsule yield increased as nitrogen dose increased in all poppy genotypes (Zaferyolu, Ofis 3, Tınaztepe and Local line) at both plant density (SD_1 and SD_2). Jain et al. (1990) determined after a change of N dose from 30 to 90 kg N ha^{-1}, an increase in the volume of capsules by as much as 58%. Turkhede et al (1981) and Camcı (1983) determined that increased capsule and seed yield of poppy by nitrogen fertilization. Camcı (1983) determined that the capsule yield varied between 68.6 kg da^{-1} in the control plots and 130 kg da^{-1} in the 15 kg N da^{-1} treatment. Kara (2017) reported that the capsule yield and seed yield of the poppy genotypes in the autumn sowing varied between 416.7-1043.3 kg ha^{-1} and 523.5-1276.3 kg ha^{-1} in the first year, between 465.3-1375.6 kg ha^{-1} and 596.7-1520.4 kg ha^{-1} in the second year, respectively. The highest capsule yield, seed yield and morphine ratio of Afyon Kalesi-95 genotype were determined as 141.0%, 180.7% and 0.74% in Afyonkarahisar condition, respectively (Aytekin and Önder, 2006). The

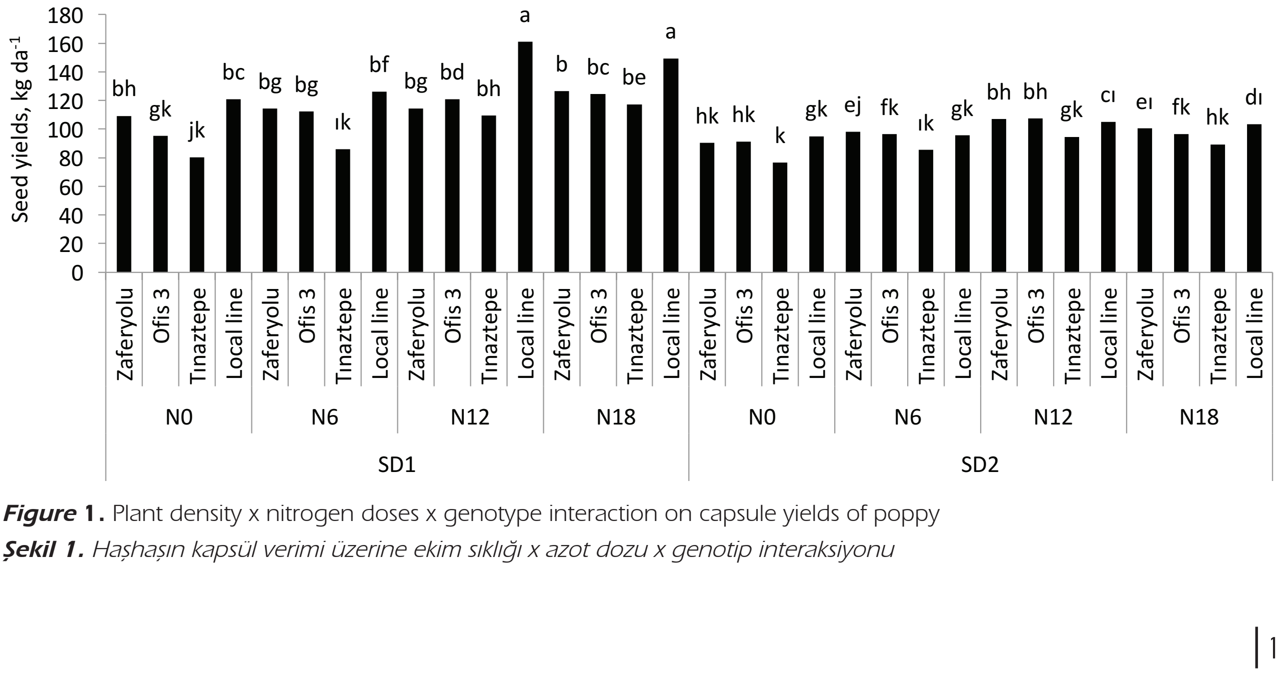

Figure 1. Plant density x nitrogen doses x genotype interaction on capsule yields of poppy

Şekil 1. *Haşhaşın kapsül verimi üzerine ekim sıklığı x azot dozu x genotip interaksiyonu* previous researchers determined that the capsule yield of poppy genotypes varied between 114.5 kg da^{-1} (Sülümenli) and 150.7 kg da^{-1} (Şuhut) in the 20 kg N da^{-1} treatment (Camcı and Arslan, 1984), 73.54-173.56 kg da^{-1} (Erdurmuş, 1989),113.3 kg da^{-1} (Eyüpoğlu, 1995), 145.5-255.8 kg da^{-1} (Katar and Yılmaz, 1997), 141.0 kg da^{-1} (Aytekin and Önder, 2006). In this study, increasing the nitrogen levels significantly increased the capsule yield. The marked effect of nitrogen on capsule and seed yields might be due to the cumulative stimulating effect of nitrogen. In addition, Nasto et al. (2009) and Khasmakhi-Sabet et al. (2009) reported that the greatest plant yield were obtained from plants grown at high density. In this study, the capsule yield increased with higher planting densities. This was probably due to increase in the number of plants per unit area, which might contribute to the production of yield per unit area leading to high yield (Law and Egharevba, 2009).

Seed yield

Plant density x nitrogen doses x genotypes interactions had significant effects on seed yield ($p<0.05$, Figure 2). The greatest seed yield (161.11 kg da^{-1}) was obtained in 12 kg da^{-1} N treatment of local line genotype at SD1 plant density and the lowest seed yield (76.53 kg da^{-1}) was observed in the control (without N) of Tınaztepe genotype at SD_2 plant density. In general, seed yield increased as nitrogen dose increased in all poppy genotypes at SD_1 plant density. These results correspond with findings of Yadav et al. (1984) where doses of nitrogen increasing 50, 100, 150 and 200 kg N ha^{-1} increased seed yields compared to the control. In addition, Jain et al. (1990) detected yields increased by 37.5% when nitrogen doses increased from 30 to 90 kg N ha^{-1}. Kharwara et al. (1988) noted a significant stimulation in seed yields when the nitrogen dose was increased from 75 kg N ha^{-1} to 150 kg N ha^{-1}. Bahandari et al. (1989) reported that seed yield increased from 64.7 kg da^{-1} to 98.0 kg da^{-1} when the nitrogen dose was increased from 3 kg N da^{-1} to 9 kg N da^{-1} in field experiments in which they examined the effects of plant density and nitrogen levels on yields of poppy genotypes, respectively. Eyüpoğlu (1995) reported that seed yield with increasing nitrogen doses increased from 164.8 kg da^{-1} to 215.4 kg da^{-1} under irrigated conditions. Rahimi et al. (2011) reported that the seed yields of the lines varied between 93.96-131.46 kg da^{-1}. Kadar and Földesi (2001) determined that the seed yield varied between 200 kg ha^{-1} in the control plots and 800 kg ha^{-1} in the NPK treatment. The previous researchers determined that the capsule yield and seed yield of the poppy genotypes varied between 92.9-140.5 kg ha^{-1} and 577.4-1046.4 kg ha^{-1} (Boydak and Kavurmacı, 2015), 450.3-1331.0 and 512.0-1511.0 kg ha^{-1} (Gumuscu and Arslan, 2008), 1200.0 kg ha^{-1} and 1500.0 kg ha^{-1} (Kosar et al., 2012), 61.4-697.2 kg ha^{-1} and 82.2-767.5 kg ha^{-1} (Gumuscu and Arslan, 1999), 2029.7-2781.5 kg ha^{-1} and 1088.0-1550.0 kg ha^{-1} (Ipek, 2011). Koc et al. (2014) reported that the capsule yield and seed yield of the autumn poppy genotypes varied with 420- 980 kg ha^{-1} and 720-1170 kg ha^{-1}, respectively.

Morphine ratio

The effect of N doses on morphine ratio was found statistically important at $p<0.05$ level and the effect of genotypes at $p<0.01$ level. On the other hand, plant density and plant density x

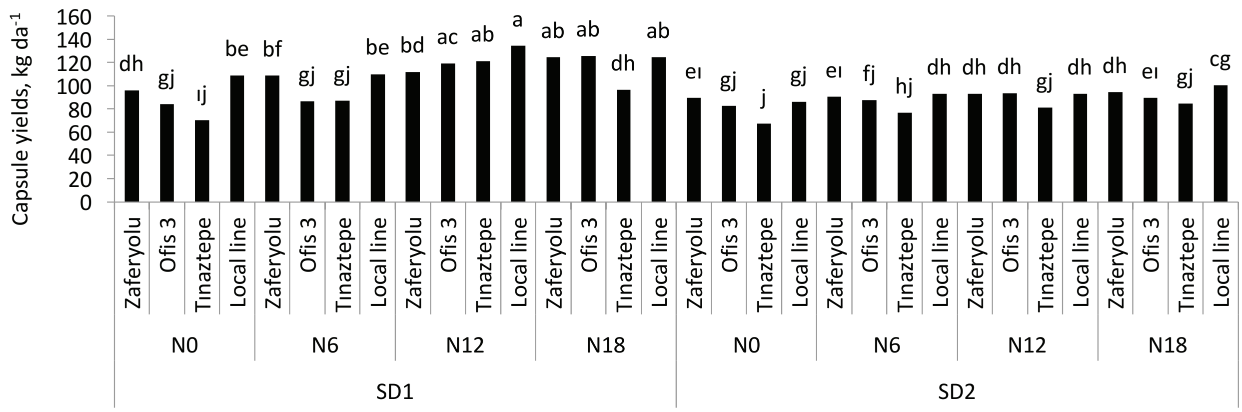

Figure 2. Plant density x nitrogen doses x genotype interaction on capsule yields of poppy
Şekil 2. *Haşhaşın kapsül verimi üzerine ekim sıklığı x azot dozu x genotip interaksiyonu*

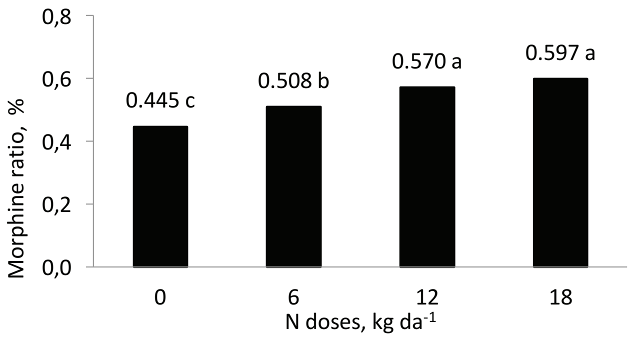

***Figure* 3.** Effects of nitrogen doses on the morphine ratio of poppy
Şekil 3. *Haşhaşın morfin oranı üzerine azot dozlarının etkileri*

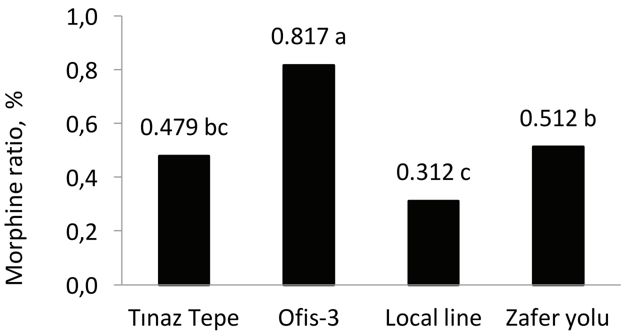

***Figure* 4.** Effects of poppy genotypes on the morphine ratio
Şekil 4. *Morfin oranı üzerine haşhaş genotiplerinin etkileri*

nitrogen dose and genotype interaction on the morphine ratio were not statistically significant ($p<0.01$). Morphine ratio of the poppy capsules increased with nitrogen fertilization (Figure 3). Losak and Richter (2004) reported that the increasing dose of nitrogen increased morphine content. The highest morphine ratio (0.597%) was observed in 18 kg da^{-1} N treatment and the lowest value (0.445%) was obtained in the control (without N). There was no difference between 12 kg da^{-1} N and 18 kg da^{-1} N treatments in terms of morphine content. This is in accordance with a number of literary sources, which indicated that the increasing content of morphine is dependent on the increasing level of N fertilization (Engin, 1995). The morphine ratio in the poppy can be affected by the genetic capabilities of the nitrogen nutrition, the genotype, diseases, the water regime and pests (Harvest et al., 2009; Jaszberenyi and Nemeth, 2012; Skalicky et al., 2014).

In this study, there were large variations in the morphine ratio of the poppy genotypes ($p<0.01$). The greatest morphine ratio (0.817%) was obtained in the Ofis 3 genotype and the lowest morphine ratio (0.312%) was obtained in the local line genotype (Figure 4). The differences in morphine ratio could be the result of soil and climatic conditions, agronomic practices, genotypes and sowing times. The previous researchers reported that these differences among the poppy genotype are due to the root structures, the plant characteristics, genetic potential and the nutrient uptake capacities of the varieties (Karadavut and Arslan, 2006; Gumuscu et al., 2008; Ipek, 2011). Skalicky et al. (2014) reported that the morphine content of the industrial poppies was approximately 1%. Kara (2017) reported that the morphine ratio of Ofis-3, TMO-3, TMO-T, Bolvadin-95 and Ofis-8 genotypes in the spring sowing were determined as 0.95%, 0.97%, 0.95%, 0.90%, and 0.95%, respectively. The morphine content of the poppy genotypes varied between 0.22-1.225% (Karadavut and Arslan, 2006), 0.110-1.140% (Gumuscu et al., 2008), 0.45–1.30% (Koc et al., 2014), 0.23- 0.83% (Boydak and Kavurmaci, 2015), 0.45%-1.00% depending on the genotypes (Skalicky et al., 2014). The results of this study are in accordance with the results of the researchers mentioned above.

CONCLUSION

While the effect of plant density x nitrogen dose x genotype interaction was statistically significant in terms of the total ash, mineral elements of poppy leaves, capsule yield and seed yield ($p<0.01$), it was found insignificant for morphine ratio. As compared to the control treatments without N, higher N doses increased macro-micro nutrients of poppy leaves. Genotypes had also significant effects on nutrient contents. The greatest capsule and seed yield were obtained in 12 kg da^{-1} N treatment of local line genotype at SD_1 plant density and the lowest capsule and seed yield was observed in the control (without N) of Tınaztepe genotype at SD_2 plant density. In generally, capsule yield, seed yield and morphine ratio of the poppy in the all genotypes at both plant density increased due to increased nitrogen fertilization. There were not any studies in literature about the effects of plant density, genotypes and nitrogen doses on macro and micro nutrients of poppy leaves. Therefore, present study will fill up a significant gap in literature and will have significant contributions to further studies on poppy. With regard to plant density, SD_1 (25 plants per m^2) was prominent for majority of investigated parameters.

As compared to other genotypes, Ofis 3 genotype can be recommended 12 kg da^{-1} N treatment in terms of mineral nutrient contents of poppy leaves and morphine ratio at SD_1 plant density.

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