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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Comparison of Bulb Yield, Some Bioactive Compound and Elemental Profile of Taşköprü Garlic (*Allium sativum* L.) Grown in Greenhouse and Open Field Conditions

Sera ve Açık Tarla Koşullarında Yetiştirilen Taşköprü Sarımsağının (***Allium sativum*** **L.**) Verim, Bazı Biyoaktif Bileşikler ve Element İçeriklerinin Kıyaslanması

Nezahat TURFAN

Abstract

Taşköprü garlic, which is among the Geographical Indications of Turkey, is one of the important sources of income for the people in the Kastamonu region. It (*Allium sativum* L.) has become an indispensable food in the kitchen with taste, a high peculiar pungent smell, a specific colour, flavon enhancing molecules. Also, it has been used for medicinal purposes due to having antioxidant capacity, sulphur compounds, and minerals. Moreover, it can preserve its superior properties for a long time due to its high dry matter compared to other garlic genotypes. This study was conducted to reveal the yield, storage duration, and nutritional elements of Taşköprü garlic bulbs grown in an open field (OF-TD; OF-KUC) and greenhouse conditions (GH-TD; GH-KUC) as well as to determine how some bioactive components including pigment, secondary metabolites, nitrogenous compound, simple reduced sugar, pyruvic acid, lipid peroxidation (MDA-malondialdehyde) and H_2O_2 (hydrogen peroxide), vary according to the growing environment in both cloves and fresh leaves. The results revealed that there were significant differences in the effects of habitat on all the parameters investigated ($p<0.001$). For instance, while nitrogenous compounds are high in OF-KUC and GH-KUC samples, OF-TD and GH-TD showed enrichment in pigment, pyruvate, glucose and secondary metabolites in the fresh leaves. In the cloves, while pigments and secondary metabolites content were found to be higher in OF-KUC and GH-KUC samples, the content of nitrogenous compounds, pyruvate and carbon were higher in OF-TD and GH-TD samples. In terms of nutrients, the GH-KUC cloves were rich in K and P, while OF-TD samples were rich in N, Mg, S, and Si, and OF-KUC samples were the richest group in Ca, Na, Cl, Mn, Fe, Zn, Cu and Se. In terms of bulb yield and storage properties, the highest values were noted with OF-KUC and GH-KUC samples, and the lowest values of them were recorded with GH-TD samples. As a result, considering the first two highest values of the parameters analyzed in garlic samples, the OF-KUC and the OF-TD samples had the maximum values, but the GH-TD samples showed the lowest value. It can be concluded that by cultivating garlic in greenhouses, fresh green leaves with high nutritional value can be provided to the consumer at all seasons and products with high nutritional value, as well as products with a storage process / long shelf life can be obtained as in plants obtained from open areas.

Keywords: Biochemical, Garlic, Greenhouse, Field, Mineral

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Öz

Türkiye'nin Coğrafi İşaretleri arasında yer alan Taşköprü sarımsağı, Kastamonu yöresi halkının önemli gelir kaynaklarından biridir. Kendine has tadı, kendine has keskin keskin kokusu, kendine özgü rengi, flavon arttırıcı molekülleri ile (*Allium sativum* L.) mutfakların vazgeçilmezi haline gelmiştir. Ayrıca antioksidan kapasitesi, kükürt bileşikleri ve mineraller içermesi nedeniyle tıbbi amaçlar için de kullanılmıştır. Üstelik sayılan üstün vasıflarını, yüksek kuru madde içeriği diğer sarımsak genotiplerine göre uzun süre koruyabilir. Bu çalışma, tarlada (OF-TD; OF-KUC) ve sera koşullarında (GH-TD; GH-KUC) yetiştirilen Taşköprü sarımsağında baş verimi, depolanma süresi, besin elementlerinin değişimini belirlemek ve ayrıca taze yapraklarda ve dişlerde pigmentler, sekonder metabolitler, azotlu bileşikler, indirgen şeker, piruvik asit, lipit peroksidasyonu (malondialdehit) ve H_2O_2 (hidrojen peroksit) gibi bazı biyoaktif kimyasal bileşiklerin yetişme ortamına göre nasıl değiştiğini ortaya koymak için gerçekleştirilmiştir. Sonuçlar yetişme ortamlarının incelenen tüm parametreler üzerinde önemli etki yaptığını göstermiştir ($p<0.001$). Örneğin, azotlu bileşikler OF-KUC ve GH-KUC örneklerinin taze yapraklarında, pigment, pürivik asit, glikoz ve sekonder metabolitler ise OF-TD ve GH-TD örneklerinin taze yapraklarında zengindir. Sarımsak dişlerinde ise OF-KUC ve GH-KUC örneklerinde pigment ve sekonder metabolit içerikleri daha fazla iken OF-TD ve GH-TD örneklerinde azotlu bileşikler, pürivik asit ve toplam karbon miktarı yüksektir. Mineraller açısından GH-KUC örnekleri K ve P, OF-TD örnekleri N, Mg, S ve Si ve OF-KUC örnekleri ise Ca, Na, Cl, Mn, Fe, Zn, Cu ve Se miktarınca zengindir. Sarımsak örneklerinde baş verimi ve depolama özellikleri açısından OF-KUC ve GH-KUC örnekleri en yüksek değere sahip iken, GH-TD örnekleri ise en düşük değere sahip bulunmuştur. Tüm değerler göz önünde bulundurulduğunda OF-KUC ve GH-KUC örneklerinin incelenen parametreler açısından daha zengin olduğu, GH-TD örneklerinin ise fakir olduğu söylenebilir. Ayrıca Taşköprü sarımsağının seralarda sarımsak yetiştirilerek tarlada olduğu kadar besin değeri yüksek yeşil sarımsağın tüketiciye her mevsim sağlanabileceği ve depola süresi/raf ömrü uzun ürünlerin de elde edilebileceği sonucuna varılmıştır.

Anahtar Kelimeler: Biyokimyasal, Mineral, Sarımsak, Sera, Tarla

1. Introduction

Recently, greenhouse cultivation is among the most widely used methods in plant production, which is used to obtain higher yield and quality products per unit area and to increase vegetable production in periods when climatic conditions affect plant growth in open land (Kontopoulou et al., 2015; Basal and Szabo, 2020). Evaluation of the smallest suitable areas is important for providing the nutritional demands of population density, which is constantly increasing. In this context, greenhouse cultivation facilitates access to all kinds of vegetables in markets in all seasons of the year and provides a source of income, given the need for regular labour. In this aspect, greenhouse cultivation enables the population to stay in the countryside and indirectly contributes to today's most up-to-date environmental problems (Gianquinto et al., 2013; Katsoulas et al., 2015). Garlic is a plant that is grown by people from all geographical regions and cultures. It is consumed fresh or dried and is used in the treatment of diseases, as well as in reducing pathogenic damage in plants (Obiadalla-Ali et al., 2016; Atif et al., 2020).

In Turkey, the region identified with garlic is the district of Taşköprü in Kastamonu province named Taşköprü Garlic, which is characterized by a pungent odour and rich in soluble dry matter, amino acids, vitamins, macro-microelements, and sulfur-containing compounds and has a long shelf life or storage period. Garlic cultivation is mostly done within February-March in the district, but it is also planted in October (Turan et al., 2013). While the cloves of garlic planted in October are harvested in the first weeks of June, garlic cultivated within the February-March period is generally harvested after the second week of July. According to the data, garlic is grown in 61 provinces in Turkey. The total garlic cultivation area in 2017 was 131, 451 thousand decares and in 2018 it was 121, 805 thousand decares (TUIK, 2019, Akan and Univar, 2020). In 2019, the dry garlic planting area in Turkey was 120 thousand decares, and Kastamonu was the province with the largest planting area with 26,6 thousand decares. Although Kastamonu was the province with the highest garlic production until 2018, Gaziantep took the lead in garlic production in the following years. Kastamonu's total garlic production was 25,97 thousand tons in 2017 and 20, 54 thousand tons in 2018. In Taşköprü district, the cultivation area of garlic, which was 24 thousand decares in 2017, decreased to 23 thousand decares in 2018, while the total production amount decreased from 24 thousand tons (2017) to 18 thousand tons (2018) (Akan et al., 2019; Akan and Univar 2020). In 2019, dry garlic production in Turkey is 103 thousand tons, fresh garlic production is 23,3 thousand tons and the total garlic production is 126 thousand tons (TUIK, 2019). While China ranks first in world garlic production, India ranks second and Bangladesh ranks third. Turkey, on the other hand, ranks 13th in garlic production (TUIK, 2019, Akan and Univar 2020). In recent years, both climatic conditions, improper agricultural practices, and problems regarding production policy have caused various problems in garlic production and the demand for garlic cannot be supplied. The most important factor that causes a decrease in yield and quality (by causing mechanical damage and stimulation of various diseases in green parts of garlic) is the rain and hail rains observed in May in Kastamonu. This situation causes the garlic producer or farmers to prefer different garlic varieties, thus leading to the import of garlic. Therefore, the use of greenhouses can serve as an important alternative for improving the yield and quality of garlic and finding markets for garlic in all seasons with its edible green leaves (Tüzel et al., 2004). Garlic production is generally based on dry garlic production and, unfortunately, greenhouses are not produced. In addition, it reduces irrigation and controls heat and humidity requirements (Keyhaninejad et al., 2012; Katsoulas et al., 2015). There are many studies on bulb yield in garlic, the factors affecting development and yield, and the main problems in marketing (Tüzel et al., 2004; Turan et al., 2013). However, there is no study to compare the yield, nutritional and bioactive constituents of Taşköprü garlic grown in the greenhouse and open fields. Therefore, the purpose of this study was to determine (1) the yield of bulbs, (2) the nutritional levels of fresh leaves and cloves, and (3) to compare the storage duration of garlic samples grown in the open field and greenhouse conditions. This investigation is the first study conducted in this scope in Kastamonu.

2. Materials and Methods

2.1. Sowing of garlic, harvesting, and preparation of the garlic for chemical analysis

A total of four experiments; two in PE covered greenhouse (GH) and two in an open field (OF) were conducted in two different areas, Central Villiage of Taşköprü (TD) and Kastamonu University Campus (KUC) between the third week of February and the second week of July of 2017. Soil samples for the greenhouse study were taken from Taşköprü District and Kastamonu University Campus and spread over soil in a greenhouse in KUC to establish experimental plots. Since the soil sample of the experimental greenhouses were the soils taken from their related district, the properties of experimental soils given in *Table 1* are representative of these areas. In all four experiments, garlic cloves were planted on plots of 2.86 m^2 (1.6mx1.8m) according to a randomized block design with three replications. Similar caliber garlic cloves were planted at 3-4 cm distances between the rows and between the cloves in the rows with the end parts visible above the ground (Vural et al., 2000). The N (nitrogen), C (carbon) and pH of KUC and TD soils used for cultivation were 0.44-0.52%, 27.44-33.558% and 6.54-6.76, respectively *(Table 1).* Water was applied twice a week in the greenhouse and open field and irrigation was continued until the garlic physiological maturity. When the garlic samples reached the 5-6 leaf stage, fully developed healthy leaves were selected from each application in each repetition. These samples were cleaned with distilled water, dehumidified, and used fresh for chemical analysis. The garlic bulbs were harvested in the second week of July when the leaves completely turned yellow and began to dry. The harvested garlic samples were kept on coarse blotting papers for 10-12 days to lose moisture at room temperature, after which they were freed from soil particles and kept until they became air dry. Stems and roots of dried samples were separated and cleaned. Air-dried bulbs were used to determine the fresh weight of bulb (g), as well as the polar and equatorial diameter of bulbs (cm). For the fresh weight of bulbs, ten samples were weighed using an electronic scale.

Table 1. Chemical properties of the experimental soils

Locations	%		mg kg^{-1}											
	N	C	K	Ca	P	S	Mg	Na	Cl	Mn	Fe	Zn	Cu	pH
KUC	0.44	27.44	27543 ± 30	12754 ± 100	5394 ± 2.3	12066 ± 4.3	12950 ± 60	120.50 ± 3	6250 ± 40	536 ± 2.5	42444 ± 30	69 ± 0.7	22.6 ± 0.5	6.54
TD	0.52	33.55	11540 ± 30	111700 ± 100	5400 ± 2.3	13374 ± 1.3	16927 ± 60	123.76 ± 20	6975 ± 60	461 ± 2	34960 ± 30	71 ± 0.7	37.8 ± 0.7	6.76

The polar and equatorial diameter of bubs (cm) was recorded using a millimeter ruler. After the measurement of bulb yield, the garlic samples were cleaned in running tap water, washed with distilled water, and moisture was removed with blotter paper. Some of the garlic bulbs were used in chemical analysis, while some were used in determining the storage duration. The samples reserved for the determination of the storage period were garlic selected based on the uniformity in size from each repetition in each experimental site as ten bulbs. Selected bulbs were kept in paper packages at room temperature (22°C ± 2°C), with aeration and shadow conditions for 6 months. The samples were checked at intervals until the end of February 2018. After 6 months of the storage period, bulbs were compared in terms of colour loss, the number of sprouting, and cloves decay.

2.2. *Chemical Analyses*

To examine the chlorophyll concentration of garlic leaves, 0.5 g of a fresh leaf was crushed in liquid nitrogen and homogenized by adding 10 ml of 80% acetone in an ice bath (Lichtenthaler, 1987). For β-carotene and lycopene content, the fresh samples and cloves were extracted with acetone-hexane (4:6) at once, then the optical density of the supernatant at 663 nm, 645 nm, 505 nm, and 453 nm was measured via a spectrophotometer at the same time. The concentrations of β-carotene (βc) and lycopene (Ly) in the garlic homogenate (in mg per 100 ml) were estimated spectrophotometrically using the following equations (Eq.1,2) (Nagata and Yamashita, 1992):

$$\beta c = 0.216 \times A1 - 1.22 \times A2 - 0.304 \times A3 + 0.452 \times A4 \quad \text{(Eq.1)}$$

Where; βc =β-Carotene in 100 ml A1 =A663; A2 =A645; A3 =A505; A4 =A453

$$Ly = -0.0458 \times A1 + 0.204 \times A2 + 0.372 \times A3 - 0.0806 \times A4 \quad \text{(Eq.2)}$$

Where; Ly =Lycopene in 100 ml A1 =A663; A2 =A645; A3 =A505; A4 =A453

The Bates method (Bates et al., 1973) was used to estimate the proline content of the leaves and cloves, and the Bradford method (Bradford 1976) was used to measure the soluble protein content of leaves and cloves. The total free amino acid content of both garlic parts was measured following the method of Moore and Stein (1948).

The total phenolic amount was performed following the Folin-Ciocalteu method via spectrophotometric (Singleton et al., 1999). Total flavonoid measurement was done spectrophotometrically (Kumaran and Karunakaran, 2006). The level of lipid peroxidation of leaf and cloves were determined and expressed as MDA (malondialdehyde) content following the method of Çakmak and Horst (1991). The H_2O_2 (hydrogen peroxide) concentration was determined according to the method of (Velikova et al., 2000). The pyruvic acid concentrations were estimated according to the colourimetric method developed by Anthon and Barrett (2003) with some modifications. The total soluble carbohydrate was estimated by spectrophotometry at 620 nm following the Antron Method (McCready et al., 1950). Glucose and sucrose contents of leaves and cloves were measured following the Antron Method by spectrophotometry at 630 nm for glucose and 620 nm for sucrose (Handel, 1968). To determine the enzyme activities of cloves, 0.5 g of a fresh leaf was crushed in liquid nitrogen and then homogenized with 5 ml of 50 mM (pH= 7.6) KH_2PO_4 (pH=7) buffered solution containing 0.1 mM Na-EDTA (Etilendiamin tetraacetic acid). The mixtures were centrifuged for 10 minutes at 10.000 g and 4°C. Enzyme activities in this supernatant were estimated. APX (ascorbate peroxidase) was determined following the method of Nakano and Asada (1981) by measuring the oxidation rate of ascorbate at 290 nm (E = 2.8 mM cm^{-1}) and SOD (superoxide dismutase) enzyme activity was measured following the method of Çakmak and Horst (1991).

2.3. Mineral Analyses of Cloves and Soil samples

For mineral analyses, samples were chosen from cloves from 15 bulbs from each group and all the samples were separated in order to obtain separate cloves and the outer skins were removed, which were further peeled and cut in slices. Then, all samples were placed in an oven at 70 °C for 24 h to dry, and the dried samples were powdered in a laboratory mill. After that, they were put into polyethene bags. Soil samples to levels of 20 cm taken from Taşköprü and Campus were air-dried, sieved. Later, both garlic and soil samples were used in mineral analysis measurements using the SPECTRO brand XEPOS model XRF instrument at the Central Research Laboratory of Kastamonu University.

2.4. ***Statistical Analyses***

All the experimental data obtained from four experimental sites were subjected to one-way analysis of variance (ANOVA) using SPSS statistical software (SPSS for Windows, Version 16). Following the results of ANOVAs, Tukey's honestly significance difference (HSD) test (α = 0.05) was also applied.

3. Results and Discussion

3.1. Changing of pigment and secondary metabolites in garlic fresh leaves and cloves

Chlorophyll pigments, carotenoids, phenolic compounds, flavonoids, soluble sugars, and nitrogenous compounds play important roles in the growth and development stages, as well as in the storage duration or shelf life of garlic bulbs (Mashayekhi et al., 2016; Yüzbaşıoğlu et al., 2017; Atif et al. 2020). In this study, significant differences in the amount of photosynthetic pigment, non-photosynthetic pigments and secondary metabolites were observed in the garlic samples with the growing sites ($p<0.001$). The amount of chlorophyll in the leaves ranged from 0.263-0.443 mg g^{-1}; carotenoid ranged from 10.76-14.53 mg g^{-1}; β-carotene ranged from 0.091-0.807 µg g^{-1}; total phenolic ranged from 17.39-42.46 mg g^{-1} (*Table 2*).

OF-TD samples had the highest β-carotene, lycopene, total flavonoid content, but poorest in total chlorophyll and carotenoid. In cloves, the recorded level varied between 0.935-10.53 µg $g^{-1}$for β-carotene, 3.46-8.25 µg g^{-1} for lycopene, 164.33-309.70 mg 100 g^{-1} for total phenolic, and 145.67-386.02 mg 100 g^{-1} for flavonoid (*Table 2*). The amount of pigment and secondary metabolites differed by growing sites and growing stages of garlic, such as seedling and bulb (Beato et al., 2011; Gmaa, 2016) (*Table 2*). These results coincide with those of Gianquinto et al. (2013); Gadel-Hak et al. (2015); Çelebi (2019), who observed that plant bioactive compounds may vary depending on environmental conditions, developmental stage of organs, and growing season. There were higher levels of pigment, total phenolic, and flavonoids in OF-TD leaf samples (*Table 2*). The higher levels of these above-mentioned parameters in garlic cloves from OF-KUC and GH-KUC associated with the fact that light conditions stimulated more bioactive compounds in the green leaves of the garlic seedling (Yuan et al., 2015; Atif et al., 2020). For field-grown garlic seedling, factors, such as sudden drops in air temperatures, heavy precipitation, and high light stress, is associated with an increase in carotenoid, lycopene, flavonoid, and phenolic to protect against photo-oxidative stress in the chloroplast (Schirrmacher et al., 2004; Hörtensteiner, 2013). Keyhaninejad et al. (2012) showed that the amount of leaf chlorophyll and fruit total carotenoids levels of pepper plants grown in the open field is lower than those grown in greenhouse and shaded greenhouse conditions. Alternatively leaf carotenoids higher in open field conditions. It has been found that the amount of flavonoids in tomato fruit (Stewart et al., 2000) and *Gynura bicolour* plant (Schirrmacher et al., 2004) were much higher than those in samples grown in the greenhouse. The high level of total chlorophyll in GH-TD and GH-KUC samples was associated with the higher temperature inside the greenhouse in April when the leaves of garlic were harvested (Gururani et al., 2015).

Table 2. Variation of pigment (total chlorophyll, total carotenoid, β-carotene, lycopene), total flavonoid, and total phenolic content in garlic fresh leaves and cloves grown in different sites

Growing sites	Leaves						Cloves			
	*Total Chlorophyll mg g^{-1}	Total carotenoid mg g^{-1}	β-carotene µg g^{-1}	Lycopene µg g^{-1}	Total flavonoids mg g^{-1}	Total phenolic mg g^{-1}	β-carotene µg g^{-1}	Lycopene µg g^{-1}	Total phenolic mg 100 g^{-1}	Total flavonoid mg 100 g^{-1}
OF-KUC	0.264a ± 0.001	14.53c ± 0.013	0.145 b ± 0.001	0.268b ± 0.001	33.83b ± 0.001	17.39a ± 0.31	3.011 b ± 0.001	8.25d ± 0.001	309.70d ± 0.16	230.38b ± 0.23
GH-KUC	0.371b ± 0.002	10.76a ± 0.008	0.091 a ± 0.002	1.519c ± 0.001	14.57a ± 0.002	42.46c ± 0.89	10.53 c ± 0.002	6.38c ± 0.001	269.15b ± 0.17	386.02d ± 1.21
OF-TD	0.263a ± 0.001	10.82a ± 0.015	0.807 d ± 0.001	1.716d ± 0.001	37.57c ± 0.001	33.45b ± 0.52	2.967 b ± 0.001	3.46a ± 0.001	288.64c ± 0.30	145.67a ± 0.54
GH-TD	0.443c ± 0.001	12.60b ± 0.065	0.723 c ± 0.001	0.221a ± 0.001	37.40c ± 0.002	18.20a ± 0.36	0.935 a ± 0.001	4.90b ± 0.001	164.33a ± 0.23	343.91 c± 0.56
F	2742.63	32703.79	1057.90	911.68	366502.11	166.80	1057.90	911.68	847.54	224.50
Sig.	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*

*Any two means within a row not followed by the same letter are significantly different at *$P < 0.05$*

3.2. Changing of proline, free amino acid, soluble protein content in garlic fresh leaves and cloves

MDA and H_2O_2 are normally produced in physiological events such as differentiation, maturation, and decreased cellular water, in both leaves and garlic cloves. However, in the green leaves of garlic and developing cloves, osmolytes with antioxidant properties eliminated the MDA and H_2O_2 damage and provided homeostasis to the cell (Hossain et al., 2015; Munoz and Munn´e-Bosch, 2018). In this investigation, MDA and H_2O_2 concentrations varied significantly with all growing conditions (p<0.001). The amount of MDA in cloves was lowest in GH-TD samples (33.15 µmol g^{-1}), while H_2O_2 was lowest in OF-TD (33.73 µmol g^{-1}) samples (*Table 3*). In terms of antioxidant osmolytes, such as total soluble protein, proline, and free amino acids in garlic seedling, OF-KUC and GH-KUC leaf samples had the higher values (*Table 3*). In garlic cloves, OF-TD and GH-TD samples were found to be richer in osmolytes (*Table 3*). Results of total soluble protein of cloves in the current investigation were similar to those of Chen et al. (2019), who reported that protein concentration was in the range of 21.09-50.01 mg g^{-1} and that light, temperature, and soil characteristics have a very strong effect on the content of bioactive compound. It was thought that the amount of MDA and H_2O_2 is implicated in the higher nitrogen compounds in OF-KUC and GH-KUC groups when compared to leaf samples of OF-TD and GH-TD (Dietz et al., 2016; Davies, 2016). The low amount of proline and protein in GH-KUC and GH-TD samples confirms this situation (*Table 3*) (Hossain et al., 2014; Gujjarı et al., 2019). It has been reported that soluble amino acids, proteins, and other nitrogenous compounds, which are among the bioactive components found in organs, such as leaves, roots, rhizomes, stems, and fruits, are effective in the adaptation of the plant to the environment in which it lives due to the prevention of injuries caused by intracellular toxic compounds (Davies, 2016; Gujjarı et al., 2019). In this study, proline, amino acids, and soluble protein of cloves may have played a role in maintaining the osmotic balance in garlic cloves, as well as in reducing the effect of toxic compounds, such as MDA and H_2O_2 (Hossain et al., 2014; Davies, 2016).

Table 3. Variation of proline, free amino acid, soluble protein, MDA, and H_2O_2 content in garlic fresh leaves and cloves grown in different sites

Growing sites	Leaf					Cloves				
	*Proline µmol g^{-1}	Free amino acid µmol g^{-1}	Total soluble protein mg g^{-1}	MDA µmol g^{-1}	H_2O_2 µmol g^{-1}	Proline µmol g^{-1}	Free amino acid µmol g^{-1}	Total soluble protein mg g^{-1}	MDA µmol g^{-1}	H_2O_2 µmol g^{-1}
OF-KUC	87.70c ± 0.04	60.78c ± 0.030	151.19c ± 0.27	73.66c ± 0.15	14.20a ± 0.17	56.58b ± .04	94.88b ± 0.33	23.54b ± 0.28	50.38d ± 0.002	71.05d ± 0.42
GH-KUC	100.76d ± 0.90	50.69b ± 0.002	152.35d ± 0.09	55.95a ± 0.07	49.65d ± 0.27	40.46a ± 0.02	82.49a ± 0.26	23.60b ± 0.15	41.34b ± 0.002	57.41b ± 0.17
OF-TD	41.38a ± 0.06	86.29d ± 0.167	89.45a ± 0.33	83.50d ± 0.25	21.46b ± 0.21	79.06d ± 0.02	146.79c ± 0.34	19.34a ± 0.08	44.25c ± 0.002	33.73a ± 0.90
GH-TD	66.20b ± 0.04	39.88a ± 0.024	106.53b ± 0.18	69.03b ± 0.07	40.20c ± 0.04	70.56c ± 0.02	145.27c ± 0.31	25.35c ± 0.15	33.15a ± 0.002	65.12c ± 0.37
F	70494.20	536.63	10699.85	401372.41	115757.47	14629.68	994.14	199.864	222044.75	941.38
Sig	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*

*Any two means within a row not followed by the same letter are significantly different at *$P < 0.05$*

According to these results, the interaction of location-growing environment factors was found to be important. Because while total soluble protein in cloves did not change in Kastamonu, it increased in Taşköprü greenhouse. Similarly, H_2O_2 was found to be low in Kastamonu greenhouse and high in Taşköprü greenhouse. During cell-tissue differentiation, the amount of soluble compounds as protein and amino acids increases in plant organs and balances their osmotic potential and energy requirements. H_2O_2 is synthesized predominantly in plant cells during metabolic reactions and therefore, play crucial role as signaling molecule in the differentiation of cells, growth, development processes. In this study, since the temperature factor in greenhouse conditions stimulates the cell-tissue differentiation rate, it may cause an increase in the amount of proline, protein and H_2O_2 (Wang et al., 2014; Hossain et al., 2015; Gujjarı et al., 2019).

The photoperiod is defined as the ratio of light and dark hours in a 24-hour period, which has a great effect on the photosynthetic activity, accumulation of bioactive compounds and formation and development of bulbs. As it is known, photosynthesis provides primary metabolites including carbohydrates as glucose, sucrose, total carbohydrates, proteins, fatty acids, which leads to the synthesis of many organic molecules related to growth (Caretto et al., 2015; Pöhnl et al., 2019). Soluble sugars as glucose, fructose, and sucrose are stored in the cloves during bulb formation, which is synthesized in leaves by photosynthesis metabolism, and are used as energy sources through respiration reactions during the germination or sprouting of the cloves (Pöhnl et al., 2019; Oku et al., 2019).

The amount of glucose of fresh leaves ranged from 79.15 to 118.15 mg g^{-1}and from 36.60 to 44.53 mg g^{-1} in the cloves (*$p<0.001$*) while no significant change was observed in the amount of sucrose in the leaves and cloves in all the groups (*Table 4*). The total amount of carbohydrates varied between 86.92-91.88 mg g^{-1} in leaf samples and between 81.955-101.90 mg g^{-1} in the cloves. Furthermore, the total carbon content (C%) of cloves varied between 38.64-43.79% in GH-KUC and OF-TD, respectively. According to data, the amount of C% of OF-TD and GH-TD are higher than those from OF-KUC and GH-KUC (Table 4). But the total amount of soluble carbohydrate was high in the fresh leaves of OF-KUC, OF-TD and GH-KUC seedlings, it was found to be high in the garlic cloves from GH-TD.

Table 4. Variation of pyruvic acid, glucose, sucrose, total soluble carbohydrate (total carb.), and total carbon (%) content in garlic fresh leaves and cloves grown in different sites

Growing sites	Leaves				Cloves				
	*Pyruvic acid µmol g^{-1}	Glucose mg g^{-1}	Sucrose mg g^{-1}	Total carb mg g^{-1}	Pyruvic acid µmol g^{-1}	Glucose mg g^{-1}	Sucrose mg g^{-1}	Total carb. mg g^{-1}	C %
OF-KUC	66.45b±0.002	105.80c±0.04	11.23b±0.11	91.88b± 0.43	3.85a±0.001	44.53c±0.04	17.37b±0.03	81.95a±0.48	40.28b±0.23
GH-KUC	56.67a±0.066	79.75a±0.04	10.84a± 0.05	89.66a± 0.56	5.33b±0.001	36.60a±0.11	16.71a±0.02	91.41b±0.12	38.64a±0.19
OF -TD	74.63c±0.034	118.15d±0.11	11.22b ±0.07	91.40b± 0.74	7.89d±0.002	42.64b±0.04	17.36b±0.05	89.60b±0.26	43.79c±0.24
GH-TD	65.26b±0.037	95.91b±0.02	10.65a ± 0.08	86.92a±0.65	6.78c±0.002	38.28a±0.08	16.93a±0.03	101.90c±0.51	42.76c±0.15
F	33363.08	103818.40	4895.97	31648.19	1538055.82	2259.58	4895.972	31648.184	131.97
Sig	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*

*Any two means within a row not followed by the same letter are significantly different at $P < 0.05$

This phenomenon has been associated with the light and temperature conditions in the growing environment of the garlic (Ikeda et al. 2019). Atif et al. (2019), Wu et al. (2016), Atif et al. (2019) reported that in warm and short-day conditions, garlic bulb yield reduces, but in temperate zones, the bulb productivity enhances due to increasing size and weight. Similarly, Chen et al. (2019) showed that the amount of total carbohydrate of cloves varied between 32.01-186.91 mg. Atif et al. (2020) showed that the amount of soluble sugar varied between 14.25-26.29% and that sucrose varied between 15.91-18.59 mg. They opined that light duration and intensity and temperature caused an increase in soluble protein, sugars, phenolic compounds, as well as pyruvic acid, which contributed to the tolerance against the environmental alterations, as well as the nutritive properties of garlic (Van den Ende and El-Esawe, 2014; Bian et al., 2015; Pöhnl et al., 2019). Pyruvic acid is one of the key organic acids produced during the initial stage of cellular respiration in the cytoplasm, called glycolysis (Avgeri et al., 2020). It undergoes oxidation through many catalytic enzymes in the mitochondria to form the universal energy (ATP) as well as precursors of many organic molecules as terpenoids, amino acids, hormones, and pigments (Oku et al., 2019; Wall and Corgan, 2019).

Studies have been revealed that the quality of garlic clove is significantly affected by the amount of pyruvic acid both during the growth and development phase and during the storage period, which can cause a negative effect through stimulating the sprouting rate, spoiling of tissue and deterioration of colour by the oxidations of chemical constituents and water loss (Angeletti et al., 2010; Wall and Corgan, 2019).

In this study, the amount of pyruvic acid in the leaves ranged from 56.67 and 74.63 µmol g^{-1} from 3.85 to 7.89 µmol g^{-1} in the cloves, respectively (*Table 4*). The concentration of pyruvic acid is the highest in the samples of OF-TD in both leaves and cloves. The high amount of sucrose and total carbohydrate in leaf samples of OF-KUC and OF-TD group was evaluated as a strategy for tolerance developed by the leaves against climatic changes that may occur in the open field (Yuan et al., 2015; Atif et al., 2020). As a matter of fact, in OF-TD fresh leaf samples, β-carotene, flavonoid, and phenolic compounds were also high, while glucose, sucrose and total carbohydrate are the highest. The quantities of total carotenoids, flavonoids, proline, sucrose, and total carbohydrate were high in OF-KUC leaf samples, which also confirmed this finding (Keyhaninejad et al., 2012; Yan et al., 2017; Gujjarı et al., 2019). These compounds were also reported to have a high effect on the shelf life of garlic, with their properties of balancing water content in tissues and cells (Wang et al., 2014; Gmaa, 2016). In garlic cloves, the amount of sucrose was high in OF-KUC and OF-TD samples and total carbohydrate content was high in GH-KUC and GH-

TD samples (*Table 3*). This result was associated with a higher respiration rate of tissues in garlic grown in greenhouse conditions (Mashayekhi et al., 2016; Avgeri et al., 2020).

3.3. Mineral status of garlic samples

Kinds of Garlic are rich in minerals, such as N, K, P, Mg, S, Ca, Fe, Mn, Zn, and Cu, which are of huge benefits to human health as well as the development of garlic seedling and bulbs and its adaptation to the change of environmental conditions (Bloem et al. 2011; Drissi et al. 2015). In this study, N concentration in garlic ranged from 1.87 to 2.36% (GH-KUC and OF-KUC, respectively), from 1.89 to 2.52% (GH-TD and OF-TD, respectively) (Table 5). Soil N level varied between 0.44 (KUC) and 0.52% (TD). The nitrogen level was found to be higher in the garlic samples of OF-TD and OF-KUC. Variations of N contents were similar to the amino acid, pyruvic acid, glucose and sucrose *(Table 4, Table 5).* One possible explanation for this situation is that light conditions can stimulate the synthesis of precursors of macromolecules via respiration (Bian et al., 2015; Caretto et al., 2015; Basal and Szabo, 2020). K and Ca contents of soil samples were found to be high in the KUC soil mixture, while P, S, and Mg were found in the TD soil mixture, which was used in garlic cultivation (*Table 1*). The highest K (22333 mg kg^{-1}) and P (5312 mg kg^{-1}) in garlic samples were found in GH-KUC samples; the highest Ca (11255 mg kg^{-1}) was found in OF-KUC samples, and the highest S (10122 mg kg^{-1}) and Mg (157.52 mg kg^{-1}) were found in OF-TD samples (*Table 5*). K and Ca content of cloves from OF-KUC and GH-KUC, is parallel to the amount of soil elements, while the Mg and S of gloves from OF-TD and GH-TD, contents are parallel with the TD soil Mg and S content (*Table 5*). When micronutrient element changes in garlic samples are examined, as seen in *Table 6*, OF-KUC samples were observed to be richer in terms of all ions, except the Si element. The highest Si content was determined as 186.3 mg kg^{-1} in OF-TD samples, while the lowest Si content was determined as 127.2 mg kg^{-1} in OF-KUC samples. Microelement content of soil samples revealed that, Mn (536 mg kg^{-1}) Fe (42444 mg kg^{-1}), Si (17844 mg kg^{-1}), and Se (0.61 mg kg^{-1}) content of KUC soil were higher than TD soil; whereas, TD soil were found to be richer in Na (123.76 mg kg^{-1}), Cl (6975 mg kg^{-1}), Zn (71 mg kg^{-1}), and Cu (37.8 mg kg^{-1}) (*Table 6*).

Table 5. Macronutrient contents of harvested garlic bulbs (mg kg^{-1})

Growing sites	%	mg kg^{-1}				
	N	K	Ca	P	S	Mg
OF-KUC	2.36±0.02	18478 ± 20	11255 ± 20	4635 ± 6	7377 ± 5	37.35 ±0.001
GH-KUC	1.87±0.001	22333 ± 30	10234 ± 18	5312 ± 6	8489 ± 6	27.87 ±0.001
OF-TD	2.52±0.003	19422 ± 20	2992 ± 9	4958 ± 6	10122 ± 10	157.52 ± 4.8
GH-TD	1.89±0.015	19886 ± 19	2916 ± 9	4879 ± 6	10088 ± 9	153.27 ± 4.6

The amount of macronutrients and trace elements detected in this study coincide with the results recorded in other investigations, which revealed that the most abundant macronutrient elements in plant tissues are generally K, Ca, Na, Mg, P, S, Fe, Zn, Cl, Cu, and Si, such that to the development stage of organs, as well as soil nutrients concentration (Anschütz et al. 2014; Demidchik and Shabala, 2018). In previous studies, it has been shown that K and Ca elements in fruits, seeds, leaves, and rhizomes play a role in the promotion of shelf life (Bloem et al., 2011; Vadalà et al., 2016), wall resistance, and osmotic potential (Demidchik et al. 2014; Hossain et al., 2014), and thus contribute to the extension of bioactive components in food for longer periods (Martins et al., 2016). Also, S is involved in the synthesis of organosulfur compounds in garlic and P is involved in the synthesis of amino acids and proteins (Chen et al., 2013; Drissi et al., 2015). And also, S and pyruvic acid are the key factors in the synthesis of organosulfur molecules, amino acids, and flavour substances in Allium spp. It has been reported that pyruvate is produced as a stable primary organic acid from the enzymatic breaking of each of the flavour precursors (Sardar and Kempken, 2018; Yoo et al., 2019).

Table 6. Micronutrient contents of harvested garlic bulbs (mg kg^{-1})

Growing sites	Na	Cl	Mn	Fe	Zn	Cu	Si	Se
OF-KUC	116.54 ± 0.0030	817.87 ± 1.11	34.24 ± 0.23	167.45 ± 1.34	64.49 ± 0.23	10.57 ± 0.3	127.2 ± 2.6	0.507 ± 0.001
GH-KUC	101.23 ± 0.910	767.72 ± 0.93	29.57 ± 0.22	164.79 ± 0.23	36.89 ± 0.31	8.98 ± 0.3	129.3 ± 2.4	0.503 ± 0.001
OF-TD	99.88 ± 0.0020	703.57 ± 0.97	30.48 ± 0.33	35.06 ± 0.44	32.97 ± 0.31	9.23 ± 0.3	186.3 ± 2.7	0.467 ± 0.001
GH-TD	97.56 ± 0.0340	700.46 ± 0.97	30.26 ± 0.31	34.11 ± 0.44	30.86 ± 0.31	9.17 ± 0.3	184.9 ± 2.2	0.465 ± 0.001

The high amount of proline and amino acids and pyruvic acid in the samples with high S content in the study strengthens this result (Hossain et al., 2014; Yan et al., 2017). Trace elements are ions necessary for the maintenance of many physiological processes in the plant, but these ions contribute to the preservation of health by participating in many reactions in human metabolism (Hossein et al., 2014; Drissi et al., 2015). The values of micronutrients from the garlic samples are in accordance with the results of Drissi et al. (2015), and Özer and Aksoy (2019), indicating those trace elements are found in fewer amounts in plants compared to the essential nutrients.

3.4. Yield changes in garlic bulbs and differences in storage duration of the samples

The bulb yield of garlic varies depending on soil characteristics in the growing environment, climatic parameters, such as temperature, light, humidity, and preharvest treatments (Atif et al., 2020). The average weight (39.83 g), length (3.39 cm), width (3.78 cm), and the number of cloves per head were highest in OF-KUC samples (*Table 7*).

Table 7. Some yield and storage parameters of garlic grown in different sites

Growing sites	After harvesting Bulb			After storage		
	*Length (cm)	Width (cm)	Weight (gr)	Number of total cloves per head	Number of Healthy cloves	Number of sprouting
OF-KUC	3.39c ± 0.04	3.78d ± 0.06	39.83d ± 1.72	23c ± 0.57	17.33 c± 0.67	4a±0.33
GH-KUC	2.86b ± 0.11	3.06c ± 0.11	21.53 c± 1.46	11.33a ± 0.66	8.33a ± 0.33	5a±0.33
OF -TD	1.96a± 0.15	2.11b ± 0.12	12.66b ± 0.44	15.00 b± 0.57	9.33b ± 0.33	8b±0.35
GH-TD	1.91a ± 0.12	1.70a ± 0.08	8.92a ± 0.43	11.00 a± 0.57	8.33 a± 0.33	8b±0.35
F	27.424	42.994	54.224	86.23	9.72	48.25
Sig	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*

*Any two means within a row not followed by the same letter are significantly different at $P \leq 0.05$

These values were lowest in GH-TD samples. Considering the growing areas, bulb properties and clove numbers were higher in OF-KUC samples. Considering the length, diameter and weight measurements, the data were found to be higher in OF-KUC samples, but OF-KUC and OF-TD samples showed a better value based on the number of cloves (*Table 7*). It has been proven that vegetables grown in the greenhouse can show a rapid growth period in the vegetative part, with the advantage of controlled conditions; whereas, the underground parts, including root, rhizome, or storage stems, such as onion and garlic, develop better in the open field (Beato et al., 2011; Gururani et al., 2015). The shelf life or storage duration of garlic and onion bulbs is closely related to the applications during preharvest and postharvest periods, conditions of the storage environment, water ratio in the cloves, the concentration of bioactive compounds, and amount of elements, such as K, Ca, P, and S (El-Morsy et al., 2004; Chen et al., 2019; Drissi et al., 2015). The number of healthy cloves without any colour loss and sprouting in garlic samples were found to be high in OF-KUC and OF-TD samples (*Table 7*). However, sprouting rate and colour loss were the highest in OF-TD and GH-TD samples. The values related to the storage life of garlic are similar to those of the studies on the storage life of bulbous species. In a study by Gmaa (2016), in which the effects on garlic storage duration, colour loss, deterioration rate, and amount of some bioactive compounds in garlic samples treated with foliar K, Ca, and chitosan was investigated, it was observed that all three applications extended the storage life of garlic, reduced the rate of decay, and increased the rate of chemical compounds. Similarly, it has been reported that Ca and K applications significantly extend the shelf life of strawberries (Angeletti et al., 2010), onions (Obiadalla-Ali et al., 2016) and garlic (El-Morsy et al., 2004), as well as prevent oxidation of chemical content and stimulate wall resistance.

However, it was thought that the high number of healthy cloves without any decay in OF-KUC and OF-TD samples and the low number in GH-TD and GH-KUC samples may also be related to the total amount of soluble carbohydrates in these samples (Yuan et al., 2015; Oku et al., 2019; Pöhnl et al., 2019). Moreover, the number of healthy cloves is higher in samples with high K and Ca and low S content. This result is similar to that of Bloem et al. (2011), Vadala et al. (2016) which shows that while K and Ca content is high and S content is low, the amount of allicin is higher in garlic and the weight loss in garlic is also reduced.

4. Conclusion

In this study, it was found that the behaviour of green parts and bulbs growth and development patterns of garlic is quite differed by growing conditions and the mineral level of soils. In terms of fresh leaves, nitrogenous compounds are high in OF-KUC and GH-KUC samples, but the quantities of pigments, pyruvate, glucose and secondary metabolites are high in OF-TD and GH-TD samples. In the cloves, while pigments and secondary metabolites were found to be richer in OF-KUC and GH-KUC samples, the status of nitrogenous compounds, pyruvate and carbon were found to be higher in OF-TD and GH-TD samples. The richest group in terms of bioactive components was recorded as OF-TD and GH-KUC, respectively. The amount of major minerals in garlic cloves varied widely. The highest level of K and P were obtained from GH-KUC samples, the highest N, S and Mg levels were recorded with the OF-TD samples, but OF-KUC samples were the richest in Ca, Na, Cl, Mn, Fe, Zn, Cu and Se elements. In terms of bulb yield and storage properties, the highest values were noted with OF-KUC and GH-KUC samples, and the lowest values of them were recorded with GH-TD samples. Considering the first two highest values of the parameters measured in garlic samples, OF-KUC and OF-TD samples had the highest values and GH-TD samples showed the lowest values. As a result of this study, it can be concluded that, by cultivating garlic in greenhouses, fresh green leaves with high nutritional value can be provided to the consumer at all seasons and products with high nutritional value and high storage resistance can be obtained as well as in open fields. Besides, it can be said that by growing garlic in a greenhouse, the economic gain of the producers can be increased by obtaining high-quality and high-yield products due to the shortening of vegetation period, earliness, and also by protecting it from adverse climatic conditions. In addition, reliable products in terms of human health can be supplied with organic production under greenhouse conditions without causing any environmental damaging. However, more studies of the effective parameters on garlic cultivation in a greenhouse environment may be beneficial in revealing the results in a healthier and more scientifically way.

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