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Research Article

Effect of Different Organic Manures Application on the Bioactive Compound and Yield of Taşköprü Garlic (*Allium sativum* L.) under 50% Drought

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Abstract. This study was carried out to assess the contribution of different organic fertilizer mixtures [(CONTROL (open field), CATTLE, CHICKEN, GOAT, İMPORTED PEAT (IPT), NATİVE PEAT (NPT), FİELD SOİL (FS: in greenhouse)] to drought tolerance of Taşköprü garlic, which was exposed to 50% water deficiency under greenhouse conditions. For this purpose, the measurement of yield was performed with bulbs, but the amount of some bioactive chemicals as chlorophyll, carotenoid, flavonoid, phenolic, proline, protein, free amino acid, sugars, pyruvic acid, malondialdehyde (MDA), hydrogen peroxide (H_2O_2), ascorbate peroxidase (APX) and superoxide dismutase (SOD) activities obtained from the fresh leaf and cloves grown in the different soil mix. According to the result, while the amount of pigment was found to be higher in the leaf of FS and FS-IPT, the flavonoid in FS-CHICKEN and total phenolic in FS-CATTLE-GOAT samples, nitrogenous compounds, and sugars (glucose, sucrose) were found to be rich in the control group (open field). The measurement of bulb weight, length, diameter, and the amount of lycopene, flavonoid, total phenolic, and free amino acid of garlic cloves obtained from FS-CHICKEN-IPT, FS-CATTLE-GOAT, FS-CHICKEN-GOAT, and FS-GOAT-NPT was higher when compared to the control. Furthermore, APX and SOD increased, whereas malondialdehyde lowered in these samples. As a result, the amounts of bulb yield, enzymatic and non-enzymatic compound, and sugars estimated using organic fertilizer were higher than the control, which was grown with inorganic fertilizer in an open field. In addition, the most drought-susceptible examples are the samples of FS, FS-ITP, FS-NTP, and FS-CATTLE soils considering all data.

Farklı Organik Gübre Uygulamalarının %50 Kurak Stresi Altındaki Taşköprü Sarımsağının (*Allium sativum*) Biyoaktif bileşenleri ve Verim Üzerine Etkileri

Anahtar kelimeler:
Kimyasal, kurak, gübre, sarımsak, taşköprü

Özet. Bu çalışma, farklı organik gübre karışımlarının [(kontrol (tarla), sığır, tavuk, keçi, ithal torf (PT), yerli torf (NPT), tarla toprağı (FS: serada tarla toprağı)] sera koşulları altında %50 su eksikliğine maruz bırakılan Taşköprü sarımsağında kuraklığa dayanımdaki etkilerini ölçmek için gerçekleştirilmiştir. Bu amaçla sarımsak başlarında verim ölçümleri yapılırken yaprak ve dişlerde pigment, flavonoit, fenolik bileşik, prolin, protein, serbest amino asit, şekerler (glikoz, sukroz), pürivik asit, malondialdehit (MDA), hidrojen peroksit (H_2O_2), askorbat peroksidaz (APX) ve süperoksit dismutaz (SOD) gibi bazı biyoaktif bileşiklerin miktar tayinleri ölçülmüştür. Sonuçlara göre fotosentetik pigment içeriği FS ve FS-IPT yapraklarında yüksek bulunurken flavonoit miktarı FS-CHICKEN, toplam fenolik FS-CATTLE örneklerinde, azotlu bileşikler ve şeker miktarı ise kontrol grubu (açık alan) bitkilerde yüksek bulunmuştur. Baş ağırlığı, uzunluk, çap ölçümleri ve ayrıca likopen, flavonoit, toplam fenolik ve serbest amino asit miktarı FS-CHICKEN-IPT, FS-CATTLE-GOAT, FS-CHICKEN-GOAT ve FS-GOAT-NPT örneklerinde daha yüksek belirlenmiştir. Hatta bu örneklerde askorbat peroksidaz ve süperoksit dismutaz aktivitesi artış göstermiş ancak MDA azalmıştır. Sonuç olarak, baş verimi, şeker içerikleri, enzimatik ve enzimatik olmayan biyoaktif bileşenler organik gübre ile yetiştirilen bitkilerde açık alanda inorganik gübre ile yetiştirilen örneklerden daha yüksek bulunmuştur. Ayrıca tüm veriler göz nünde bulundurulduğunda kuraklığa en duyarlı örnekler FS, FS-ITP, FS-NTP ve FS-CATTLE olmuştur.

INTRODUCTION

Garlic is a widely cultivated spice crop that is used for food and medicinal purposes. The green parts of this crop are freshly consumed or cooked, especially in tropical areas and the raw use of immature bulbs in the salad is also popular (Adewale *et al.*, 2011). The economic importance of the garlic crop has greatly increased in the entire world in recent years. Despite its importance, several problems, such as drought, organic matter and mineral deficiency/toxicity, salinity, or some diseases, are encountered during the growth period (Shafeek *et al.*, 2015). In Kastamonu, the selenium level identified with garlic is sufficient in the soils of the garlic cultivation areas; however, the organic matter and some trace elements are insufficient (Kaçar *et al.*, 1998; Akça *et al.*, 2017). Taban *et al.* (2004) reported that 67.5% of the soil is insufficient for N (40%), P (85.5%), K (95%), Zn and Mn (7.5%), and Fe and B (67.5%). In addition, the use of natural manures (sheep/goat, chicken, and cattle) and peat, enrich the organic status of the soil. Studies have shown that the use of manure (cattle, chicken, goat, or sheep), herbal and urban wastes (Can *et al.,* 2019; Doğan *et al.* 2020), and composts in the cultivation of many crops, increases the tolerance of plants to drought by improving soil properties and water-holding capacity, which results in the availability of the nutrients available to plants by root cells, thus increasing the yield and quality per unit area (Mahmoody *et al.*, 2014; Zhang *et al.*, 2016). Garlic can only be planted in areas where its root can uptake maximum water and mineral, with the soil moisture level close to the field capacity (60 cm from the soil surface), since garlic has a shallow root structure (Taban *et al.*, 2004; Acharya and Kumar, 2018). In addition, the stage at which garlic mostly requires water is during bulb formation and water deficiency, since this stage can result in reduced bulb quality, yield, and storage time (Rekowska *et al.*, 2008; Abou El-Magd *et al.*, 2012). Drought stress is among the most important suppressing factors that adversely affect growth and development, which results in reduced yield, quality and storage time of some fruits, bulbous species, roots, and stems that store nutrients. It is reported that the severity of the damage caused by drought on plants is closely related to the phases of the life cycle of the species. For instance, the drought that occurs at the initial growth phases (where energy consumption at the highest level can be lethal for the plant (Yazgan *et al.*, 2008; Hussein *et al.*, 2018). A most common effect of water deficiency on crops is a reduction in fresh weight which results in declination of both elongation and expansion of leaf (Farooq *et al.*, 2009; Temesgen *et a*l., 2018; Kurt *et al.*, 2020). Producers during the drought periods may reduce the number of hectares cultivated, thereby cultivating only drought-resistant cultivars. However, the use of organic fertilizers and the selection of resistant genotypes is now a common practice it effectively increases the yield and quality of agricultural products by stimulating drought tolerance (Shafeek *et al.*, 2013; Rahman *et al.*, 2018). There are studies investigating the factors that affect the growth, development, bulb yield, and quality of garlic; however, there are limited studies on the effects of organic manure application on drought-resistant Taşköprü garlic. The main purpose of this study was to investigate the effects of natural organic manures (cattle, chicken, and goat) and peat on drought stress tolerance level of Taşköprü garlic grown in an open field and greenhouse conditions under the drought. In this context, the most important quality criteria for garlic were examined and they include: the amount of bioactive compounds in the leaves and cloves; bulb weight, length, and diameter measurement, and cloves number. This investigating is the first study conducted to determine the effects of organic fertilizers on the drought-stress resistance of Taşköprü garlic in Kastamonu.

MATERIAL AND METHOD

Cultivation Experiment

This study was performed in an open field and under greenhouse conditions, in Kastamonu for approximately 5 months (24 February-13 July 2016). The field experiment was performed in the Taşköprü district, while the greenhouse experiment was set up in the Kastamonu University plastic greenhouse. For the open field experiment, big sized cloves were cultivated with a spacing of 10 x 30 cm in 2 x 2 sized plots (Mengesha and Tesfaye, 2015). The experiment was performed using a randomized block design (RBD) with three replicates. The samples were treated with the recommended dose (100%) of 100, 60, and 50 kg ha^{-1} NPK (Mirzaei *et al.*, 2007).

For the greenhouse experiments, the cultivation order was arranged in RBD in three replicates, with six types of organic components: manure (cattle, chicken, and goat) and peat (native and imported) from February 2016 to July 2016. First, soil samples in the field were collected from the topsoil layer (20 cm) in Taşköprü and mixed with other organic manures. Second, the soil mixtures (the soil sample and organic manures) were placed into 40 x 50 diameter pots, with a total of 5000 g, as shown in Table 1. Homogeneous big sized garlic cloves were planted in triplicates, with 6-8 cloves and nine tubes used in each replica (Table 1). The cloves were planted in the soil mixture, such that the ends of the cloves and bulbs were visible on the soil surface (Vural *et al.*, 2000). Irrigation of the garlic samples was performed twice a week with tap water according to the water-holding capacity of each pot until the seedling had 5-6 leaves. Application of 50% water restriction to the garlic samples started when the seedlings reached the 5-6 leaf stage. They were kept in the greenhouse with an average temperature of 26 ± 2°C and 65-70% humidity. Water given to the samples was estimated based on the water-holding capacity of the pots and values determined for each group were given in Table 1.

Table 1. Doses of soil mixtures where Taşköprü garlic samples were cultivated and amount of water to be applied for each group (50% water shortage).
Çizelge 1. Taşköprü sarımsak örneklerinin ekildiği toprak karışımlarının dozları ve su miktarları (%50 eksikliği).

Groups	Control	FS Field soil	IP Imported peat	NP Native peat	Cattle	Goat	Chicken	Water, ml
Control		-	-	-	-	-	-	270
FS	-	5000 g	-	-	-	-	-	135
FS-IPT	3000 g		2000 g					140
FS-NPT	3000 g			2000 g				140
FS-CATTLE	3000 g			1000 g	1000 g			140
FS-CATTLE-NPT	3000 g		1000 g		1000 g			145
FS-GOAT-NPT	3000 g			1000 g		1000 g		140
FS-CHİCKEN-GOAT	2000 g			1000 g		1000 g	1000 g	140
FS-CHİCKEN-IPT	3000 g			1000 g			1000 g	140
FS-CATTLE-GOAT	2000g				1000g	1000g	1000g	145

Control: only soil in the field in Taşköprü district of Kastamonu.

To perform chemical analysis on fresh leaves of garlic samples, fully developed healthy leaves were collected from seedlings exposed to drought for 4 weeks. All analysis was done with fresh leaf tissue in triplicate. Application of 50% water stress to the garlic seedling was stopped before harvest when the leaves turn yellow and begin to twist. The mature bulbs were harvested when the top leaves were dried completely with a notable yellow colouration. The garlic samples were collected in the second week of July, 2016. All samples were dried in a place that is not exposed to sunlight (carton paper) for 12-13 days (Francois, 1994). Ten mature bulbs were randomly chosen from each soil mixture to determine the fresh weight of bulb (g), as well as the polar and equatorial diameter of bulbs (cm) per plant. For the fresh weight of bulbs, ten samples were weighed using an electronic weighing machine. The polar and equatorial diameter of bulbs (cm) was estimated with a millimeter ruler.

Table 2. Properties of organic manures and field soil used in garlic cultivation.
Çizelge 2. Sarımsak ekiminde kullanılan toprak karışımlarının bazı özellikleri.

Nutrients of Soils	Cattle	Chicken	GOAT	IPT	NPT	Field soil (control)
%N	0.36	1.74	0.78	15	14	0.44
%C	28.31	34.32	35.22	33.42	35.28	24.44
%P	0.24	1.58	0.34	10	10	0.86
pH	7.13	6.76	7.08	6.44	6.32	6.76
mg Fe kg^{-1}	88.74	144.56	124.46	2088	2046	34960
mg Zn kg^{-1}	43.34	46.34	88.46	108.22	116.44	459.42
mg Mn kg^{-1}	78.36	276.46	124.32	108.22	116.44	459.42

Soil samples to levels of 20 cm taken from Taşköprü and organic manures were air-dried, sieved, and used in elemental analysis at the Central Research Laboratory of Kastamonu University, which is shown in Table 2. The N, C, P, K of fertilizer used were 0.36-15%, 28.31-35.28%, 0.24-10% and 0.84-20%, respectively. Other microelements were 88.74-34960 mg Fe kg^{-1}, 43.34-88.68 mg Zn kg^{-1}, and 78.36-459.42 mg Mn kg^{-1}, respectively. The pH level of soils ranged from 6.32 to 7.17 (Table 2).

Chemical Analyses

To determine the chlorophyll content of garlic leaves, 0.5 g of the fresh leaf was crushed in liquid nitrogen and homogenized by adding 10 ml of 80% acetone in an ice bath (Lıchtenthaler, 198). The mixture was centrifuged for 10 minutes at 3,000 rpm, and triplicate spectrophotometric (Shimadzu UV-260) readings of the supernatants noted were recorded at values of 652 and 450. 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 (Nagata and Yamashita, 1992):

$$\beta c = 0.216 \times A1 - 1.22 \times A2 - 0.304 \times A3 + 0.452 \times A4$$

Where; βc =β-Carotene in 100 ml A1 =A663; A2 =A645; A3 =A505; A4 =A453 (1)

$$Ly = -0.0458 \times A1 + 0.204 \times A2 + 0.372 \times A3 - 0.0806 \times A4$$

Where; Ly =Lycopene in 100 ml A1 =A663; A2 =A645; A3 =A505; A4 =A453 (2)

The Bates method (1973) was used to estimate the proline content of the leaves and cloves, and the Bradford method (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). 500 mg sample was boiled in 10 ml of 80% ethanol. Homogenate was centrifuged at 3500 g for 15 min and the clear supernatant was taken. Then it was completed to10 ml with ethanol. 1 ml of extract was put into 25 ml tube and 0.1 N NaOH with methyl red and 1 ml of ninhydrin reagent was added. Then, the mixture was incubated for 20 min. Afterwards, 5 ml of ninhydrin reagent was put again and it was cooled in an ice bath. After completing with distilled water to 25 ml, the absorbance was read at 570 nm. The standard was prepared by glycine and total free amino acid content was expressed as mg/g. 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 the 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 content was determined via the colourimetric method following Schwimmer and Weston (1961). 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 Anthron 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 the fresh leaf was crushed in liquid nitrogen and then homogenized with 5 ml of 50 mM (pH = 7.6) KH2PO4 (pH=7) buffered (potassium phosphate) solution containing 0.1 mM Na-EDTA (Sodium-Ethylene Diamine Tetra Acetic 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). The data were analyzed through one-way analysis of variance (ANOVA) to POİNT OUT the effect of different organic soil samples on Taşköprü garlic yield and chemical composition of them, using SPSS statistical software (SPSS for Windows, Release 16).

RESULTS AND DISCUSSION

The amount of photosynthetic pigments of leaves has been estimated as an index for evaluation of the source. Therefore, a reduction in this amount can be considered as nonstomatal impressions in drought stress conditions (Hussein *et al.,* 2018; Kibar, 2020). In this study, chlorophyll (Chl) and carotenoid content obtained from garlic seedling, which was cultivated in different organic manures mixtures, were given in Table 3. There was a significant difference between the garlic samples that were cultivated on different soil mixtures ($p < 0.05$). Chl a, Chl b, and total chlorophyll showed an increase in leaves under drought when compared to the control group. Chl a level in garlic leaves ranged from 0.091-0.160 mg, Chl b from 0.067-0.267 mg, and total chlorophyll from 0.158-0.415 mg.

Table 3. The amount of photosynthetic pigment, flavonoid and total phenolic in Taşköprü garlic leaves under 50% drought.
Çizelge 3. %50 kuraklık altındaki Taşköprü sarımsağı yapraklarında fotosentetik pigmentler, toplam flavonoit ve toplam fenolik bileşim miktarları.

Groups	Chl a mg g^{-1}	Chl b mg g^{-1}	Total Chlorophyll mg g^{-1}	Chl a: Chl b	Total Carotenoid mg g^{-1}	Flavonoid µg g^{-1}	Total phenolic mg g^{-1}
Control	0.091±0.001a*	0.067±0.001a	0.158±0.00a1	1.36±0.003e	10.76±0.02a	116.33±0.06a	23.17±0.06c
FS	0.148±0.001b	0.267±0.001g	0.415±0.00h	0.55±0.001a	10.66a±0.01a	152.57±0.18b	10.77a±0.02a
FS-IPT	0.159±0.001d	0.174±0.001g	0.333±0.00g1	0.92±0.003b	15.74±0.02c	268.65±0.05f	25.32±0.02d
FS-NPT	0.157±0.001c	0.078±0.00b1	0.234±0.001b	2.03±0.005h	15.89±0.01c	202.99±0.11d	23.41±0.04c
FS-CATTLE	0.160±0.001d	0.091±0.001c	0.251±0.001c	1.76±0.004g	13.20±0.01b	272.55±0.13f	14.80±0.03b
FS-CATTLE-NPT	0.160±0.001d	0.105±0.001	0.264±0.001d	1.53±0.002f	12.42±0.02b	182.22±0.09c	27.58±0.03e
FS-GOAT-NPT	0.158±0.001c	0.123±0.001e	0.281±0.001e	1.29±0.003d	12.78±0.01b	238.94±0.34e	21.93±0.05c
FS-CHICKEN-GOAT	0.157±0.001c	0.142±0.001f	0.298±0.001f	1.10±0.001c	13.06±0.02b	243.38±0.05e	20.13±0.02c
FS-CHICKEN-IPT	0.160±0.001d	0.102±0.001d	0.262±0.00d	1.58±0.003f	13.19±0.01b	314.60±0.05g	26.21±0.02d
FS-CATTLE-GOAT	0.155±0.001c	0.145f±0.001	0.300±0.001f	1.07c±0.002	13.84±0.01b	208.97±0.04d	31.79±0.03f
F.	32654.20	44876.88	32703.80		2742.64	366502.12	168.84
Sig.	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

*Means within a group that has a different small letter are significantly different from each other. P <0.01. Chl a:chlorophyll a; Chl b:chlorophyll b; Chl a:Chl b:ratio of Chla to Chl b.

When compared to the control samples (0.091 mg), the highest Chl a was estimated in FS-CATTLE and FS-CATTLE-NPT samples (0.160 mg), while the highest Chl b and total chlorophyll were estimated in samples of FS and FS-IPT (0.415-0.333 mg). Although the ratio of Chl a to Chl b reduced in FS and FS-IPT samples when compared to the control, the ratio was increased in the other samples. As observed in Table 3, drought induced carotenoid accumulation when compared to the control, except in FS samples. In general, the estimated values of pigments in this study agree with those cited in the literature, in which the concentration of chlorophyll and carotenoid increases in the tolerant species exposed to water deficiency (Hancı and Cebecı, 2014; Badran, 2015; Husseind and El-Faham, 2018). Similarly, Kıran (2019) found that chlorophyll and carotenoid level in lettuce leaves grown in vermiculite under drought stress were higher when compared to the control plants. Kibar (2018) found that chlorophyll content in lettuce was higher in vermicomposting applications than that of a control application, which was compatible with our findings. In this study, a high level of photosynthetic pigments in stressed garlic seedlings was associated with improvement of nitrogen, mineral, the water content of the soil, as well as organic manures (Wang *et al*., 2017; Amiri *et al*., 2017). Nitrogen is generally considered to be effective on chlorophyll synthesis in cultivated leafy species, especially in winter (Kopsell *et al*., 2007; Rambo *et al*., 2010).

In addition to the pigments, green leaves, non-enzymatic antioxidant (secondary metabolites) (Zoratti et al., 2014; Türk and Şen, 2020), soluble nitrogenous compounds (proline and protein), and sugar (glucose and sucrose) (Gulen *et al*., 2018) are molecules responsible for tolerance of plants to drought and other stresses in grown conditions (Garcia *et al*., 2014; Wang *et al*., 2017). As shown in Table 3, the amount of flavonoid in garlic seedlings was between 10.66 and 15.89 mg, whereas the total phenolic concentration was between 10.77 and 31.90 mg. The highest level of flavonoid (314.60 µg) and total phenolic (31.79 mg) was recorded with FS-CHICKEN-IPT and FS-CATTLE-GOAT samples. While the amount of flavonoid increased in all groups compared to control, the total phenolic molecules in FS and FS-CATTLE samples decreased to very low levels. Water shortage caused a decrease in the amount of proline, protein, glucose, and total soluble carbohydrate level of garlic seedling, which are the most important osmolytes that play a role in turgor and osmotic regulation in cells in response to drought (Garcia *et al*., 2014; Hancı and Cebei, 2015; Amiri *et al*., 2017).

However, proline concentration was high with FS-GOAT-NPT, sucrose was high with FS-CATTLE and FS-CATTLE-GOAT samples, but MDA and H_2O_2 were elevated only in FS samples (Table 4). These findings support the previous results that the content of proline, protein, glucose, sucrose, and carbohydrate are lower under water restriction, but the amount of MDA and H_2O_2 is higher (Rahbarian *et al*., 2011; Gulen *et al*., 2017). The low osmolytes in garlic seedlings have been associated with the use of these compounds to prevent reactions that induce the accumulation of MDA and H_2O_2 molecules, which can be stimulated to accumulate in the cell by the combined effect of drought and nitrogen-rich soil mixtures (Table 4). As a matter of fact, the high amount of flavonoid and carotenoids in garlic samples strengthens this result (Riahi and Hdider, 2013; Edreva *et al*., 2015).

Table 4. The amount of proline, protein, MDA, H_2O_2, glucose, sucrose and total carbohydrate concentrations in Taşköprü garlic leaves under drought.

Çizelge 4. Kurak stresi altındaki Taşköprü sarımsağı örneklerinin yapraklarında prolin, protein, MDA, H_2O_2, glikoz, sukroz ve toplam karbohidat miktarları.

Groups	Proline µmol g^{-1}	Protein mg g^{-1}	MDA µmol g^{-1}	H_2O_2 µmol g^{-1}	Glucose mg g^{-1}	Sucrose mg g^{-1}	Soluble carbohydrate %
Control	5.39±0.06d*	98.79±0.09d	192.49±0.25f	126.20±0.06g	131.65±0.01c	113.78±0.38c	65.82±0.05c
FS	4.05±0.04c	60.30±0.11a	199.26±0.07f	268.35±0.06h	79.96±0.02a	67.58±0.01b	39.98±0.08a
FS-IPT	2.36±0.20a	81.30±0.02c	42.43±0.08b	113.89±0.06f	79.33±0.01a	67.37±0.09b	39.66±0.04a
FS-NPT	2.46±0.14a	85.59±0.05d	52.88±0.05d	58.87±0.06a	82.68±0.06b	67.19±0.09b	41.34±0.29b
FS-CATTLE	2.85±0.21b	89.74±0.04	28.69±0.09a	65.38±0.08b	80.56±0.01a	132.73±0.03d	40.28±0.04a
FS-CATTLE-NPT	2.19±0.27a	87.26±0.40c	45.26±0.05c	110.47±0.03f	79.78±0.01a	67.69±0.04b	39.89±0.04a
FS-GOAT-NPT	5.42±0.26d	79.15±0.03c	40.65±0.18b	78.95±0.02c	79.47±0.05a	67.05±0.16b	39.73±0.23a
FS-CHICKEN-GOAT	3.32±0.15b	90.06±0.02d	114.65±0.10e	125.48±0.03g	81.42±0.01a	111.42±0.06c	40.71±0.04a
FS-CHICKEN-IPT	4.34±0.06c	90.76±0.02d	35.30±0.06b	98.40±0.06e	79.80±0.10a	63.67±0.01a	39.90±0.49a
FS-CATTLE-GOAT	4.76±0.04c	71.80±0.08b	30.79±0.05a	84.65±0.03d	81.58±0.01a	146.27±0.02e	40.79±0.04a
F	5242.28	10699.86	401372.42	115757.47	14629.69	4895.96	31648.20
Sig.	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

*Means within a group that has a different small letter are significantly different from each other. P <0.01.

Bulbs Yield, Cloves and Chemicals

As shown in Table 5, bulb yield parameters, such as number, average bulb weight, individual bulb weight, diameter, length and width of the bulb, and clove number were influenced with the soil mixtures under drought condition. The maximum bulb number of 34, 28, 27, and 26 were recorded with FS-NPT-FS-CATTLE, FS-CHİCKEN-GOAT, and FS-IPT, but the poorest value of it was noticed with FS-CATTLE-NPT and FS-CHİCKEN-IPT as 17 and 19, respectively (Table 5).

Also, individual bulb weight was found to be higher than control, while length and diameter of bulbs were recorded as low in the first three samples coded as FS, FS-IPT, and FS-NPT (Table 5). The maximum number of cloves were noted in FS-CHICKEN-IPT and FS-CATTLE-GOAT samples (9.27, 9.12), whereas the lowest number was recorded in FS-NPT, FS-IPT, and FS samples as 3.77, 3.84, and 3.86, respectively (Table 5).

Table 5. The effects of 50% drought on bulb yield of Taşköprü garlic grown different soil mixture.

Çizelge 5. Farklı toprak karışımlarında yetişen Taşköprü sarımsağında %50 kuraklık uygulamasının baş verimine etkisi.

Groups	Bulb				Clove
	Number	Fresh weight (g)	Length (cm)	Width (cm)	Number
Control	20±0.30b*	10.71±0.29b	1.89±0.13ab	2.38±0.15b	4.44±0.70b
FS	27±0.26e	9.34±0.78b	1.72±0.08a	1.68±0.99a	3.86±0.99a
FS-IPT	26±0.27e	9.87±0.31b	1.72±0.10a	1.84±0.13a	3.84±0.63a
FS-NPT	34±0.45e	10.38±0.16b	1.79±0.09a	2.14±0.15b	3.77±0.64a
FS-CATTLE	28±0.27d	10.55±0.29b	1.93±0.12ab	1.84±0.10a	5.35±0.82bc
FS-CATTLE-NPT	17±0.22a	14.23±1.49b	2.15±0.07b	2.57±0.07c	7.13±0.88c
FS-GOAT-NPT	23±0.25c	15.15±0.85c	2.13±0.07b	2.61±0.09c	7.21±0.88c
FS-CHICKEN-GOAT	28±0.27d	16.32±0.43c	2.16±0.13b	2.85±0.13d	8.65±1.08d
FS-CHICKEN-IPT	19±0.22b	21.88±0.96e	2.14±0.04a	2.92±0.16d	9.27±1.06e
FS-CATTLE-GOAT	20±0.22b	19.25±1.22d	2.82±0.16c	2.56±0.11c	9.15±0.63e
F	28.88	28.86	8.715	11.272	83.823
Sig.	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

*Means within a group that has a different small letter are significantly different from each other. P <0.01.

Based on the yield data, the drought resistance of FS-CATTLE-GOAT, FS-CHICKEN-GOAT, FS-CATTLE, and FS-GOAT-NPT samples were found to be higher than control, but FS, FS-IPT, and FS-NPT samples were observed to be susceptible (Alphonse *et al*., 2015; Badran, 2015). The presence of goat and chicken manures in the groups with the highest measured values in garlic bulbs indicated that these soil mixtures make a significant contribution toward overcoming drought stress (Daba *et al*., 2018; Doğan *et al*., 2020). Results of this study have confirmed the results of Adewale *et al*. (2011) and Acharya and Kumar (2018) who revealed that organic fertilizers, such as poultry manure, sheep/goat manure, and cattle manures significantly enhanced the yield of garlic in terms of bulb weight, diameter, and clove number. Similarly, Kibar (2018) found that vermicompost applications have positive effects on plant growth parameters such as plant height, plant wet weight, plant dry weight and number of marketable leaves in lettuce. Other studies have revealed that organic manure can provide good soil fertility, such as rich nutrient, water-holding capacity, and aerobic conditions for plant growth and productivity, depends on manure types and doses, as well as season and genotype (Clautildea *et al*., 2017; Abou-El magd *et al*., 2012; Can *et a*l., 2019).

Photosynthesis products accumulate in the subsoil parts of garlic and participate in the substances by respiration and synthesis reactions, which will play crucial roles during the storage time and germination stages. All of them are essential for plant and human life, since they are involved in many functions, such as eliminating or alleviating biotic and abiotic stresses injuries (Jaleel *et al*., 2008; Alphonse *et al*., 2015).

In this study, the amount of β-carotene, lycopene, flavonoid, and total phenolic content of garlic bulbs were significantly ($p \leq 0.05$) affected by the soil mixtures and drought (Table 6). The drought reduced the amount of β-carotene in the garlic cloves in all groups when compared to the control, but lycopene level increased in FS-CATTLE-NPT, FS-GOAT-NPT, FS-CHICKEN-GOAT, and FS-CHICKEN-IPT samples and the flavonoid level in FS-CHICKEN-GOAT, FS-CHICKEN-IPT, and FS-CATTLE-GOAT (Table 6). The highest lycopene was detected in FS-CATTLE-IPT and FS-CHICKEN-GOAT samples (2.085-1.969 µg) and maximum flavonoid was observed in FS-CATTLE-GOAT and FS-CHICKEN-IPT samples (46.37-46.22 mg). Total phenolic content of samples varied between 33.82-56.62 mg and decreased in FS, FS-GOAT-NPT, and FS-CATTLE-NPT samples (33.2, 37.60, and 41.64 mg) when compared to control (42.56 mg) (Table 6). As seen in Table 6, the highest level of lycopene was produced by FS-CHICKEN-IPT and FS-CHICKEN-GOAT; the highest flavonoid was produced by FS-CATTLE-GOAT and FS-CHICKEN-IPT, while total phenolic was produced by FS-IPT and FS-CATTLE-GOAT compared to the control. These results are in agreement with the results of other studies, which expressed enhanced non-enzymatic compounds with the application of organic manure types under drought. Zhang et al. (2016) showed that the application of mixed organic manure with N increased the level of total phenolic and flavonoid in tomato.

Table 6. The effects of %50 droughts on β-carotene, lycopene, total flavonoid, total phenolic, proline, protein and free amino acid concentrations in the cloves of Taşköprü garlic grown different soil mixture.
Çizelge 6. %50 kuraklık uygulamasının farklı toprak karışımlarında yetişen Taşköprü sarımsak dişlerinde β-karoten, likopen, toplam flavonoit, toplam fenolik bileşik, prolin, protein ve serbest amino asit konsantrasyonları üzerine etkileri.

Groups	β-carotene µg g^{-1}	Lycopene µg g^{-1}	Flavonoid mg g^{-1}	Total phenolic mg g^{-1}	Proline µmol g^{-1}	Protein mg g^{-1}	Free amino acid µmol g^{-1}
Control	0.81±0.004h*	1.644±0.003g	40.36c±0.06c	42.56±0.14c	9.38±0.02e	83.61c±0.03c	13.12a±0.04
FS	0.73±0.002f	1.184±0.001d	31.80b±0.06b	33.82±0.09a	9.64±0.03e	96.72d±0.16d	14.22b±0.18
FS-IPT	0.06a±0.003a	0.905±0.001a	23.22a±0.14a	62.47±0.12f	2.38±0.03a	102.24e±0.23e	16.44c±0.24
FS-NPT	0.06±0.001a	1.052±0.002c	21.66a±0.07a	45.14±0.06d	5.91±0.02c	85.16c±0.10c	12.32a±0.13
FS-CATTLE	0.53±0.001c	0.981±0.001b	31.08b±0.05b	48.82±0.21	5.97±0.03c	75.61b±0.25b	14.46b±0.22
FS-CATTLE-NPT	0.33±0.001b	1.708±0.001h	23.93a±0.07a	41.64±0.17b	4.32±0.06b	80.13c±0.17c	14.28b±0.18
FS-GOAT-NPT	0.57±0.002d	1.552±0.002f	27.78b±0.11b	37.60±0.16b	7.22±0.02d	61.46a±0.16a	18.44c± 0.18
FS-CHICKEN-GOAT	0.65±0.010e	1.969±0.001ı	44.33d±0.10d	43.69±0.14c	8.34±0.02e	92.37d±0.22d	20.28d±0.21
FS-CHICKEN-IPT	0.68±0.00e1	2.085±0.001i	46.22e±0.22e	47.77±0.17d	8.20±0.01e	64.24a±0.17	20.24d±0.18
FS-CATTLE-GOAT	0.80±0.002g	1.350±0.002d	46.37e±0.15e	56.62±0.26e	6.68±0.01d	92.65d±0.28	22.56e±0.24
F	12443.32	75967.51	7154.82	11982.53	10513.68	837.14	4466.45
Sig.	0.000	0.000	0.000	0.000	0.000	0.000	0.000

*Means within a group that has a different small letter are significantly different from each other. $P < 0.01$.

Alphonse *et al*. (2015) tested the effect of organic manures as vegetable waste, cattle dung, and inorganic fertilizer (NPK) in *Solanum nigrum* and they found that higher level of vitamin c, flavonoids, total phenolics, tannins, and saponins were produced by organic manure compared to NPK fertilizer.

Garlic bulbs contain a different compatible compounds, such as nitrogen-containing compounds, including proline, free amino acid, soluble proteins, reduced soluble sugars, organic acid (pyruvate), and antioxidant enzymes, which are important for growth and development as building substances, energy sources, osmotic regulation, and even protection against the negative effects of drought stress (Jaleel *et al.*, 2008; Salehi *et al.*, 2016). It has been shown that these compounds are also very effective in preventing water loss, oxidation of bioactive chemicals, and tissue decay under the storage time of garlic (Azzini *et al.*, 2014; Can *et al.*, 2019).

In this study, the amount of proline decreased overall with drought, but showed a partial increase in FS samples and amino acid increased in all samples compared to control, except for the FS-NPT sample (Table 6). The highest amino acid was recorded in FS-CATTLE-GOAT, FS-CHICKEN-GOAT, and FS-CHICKEN-IPT samples as 22.56, 20.28, and 20.24 mg, respectively. Total soluble protein amount was lowest in FS-GOA-NPT and FS-CHICKEN-IPT (61.46-64.24 mg) samples when compared to the control (83.61 mg), whereas it reached the highest value in FS-IPT and FS samples (102.24-96.72 mg) (Table 6).

The fact that water stress caused a decrease in the proline content while increasing the free amino acid level was associated with the use of proline to prevent water loss in garlic cloves (Mafakheri *et al.*, 2010; Vidya vani *et al.*, 2019). As a matter of fact, the general low MDA and pyruvate content of garlic cloves compared to the control confirms this idea (Table 7). Pyruvate, a key molecule of the Krebs cycle in respiratory reactions, is an important indicator of respiratory rate, which occurs easily under drought conditions (Gupta and Igamberdiev, 2016). MDA accumulates in cells as a result of enzymatic and non-enzymatic destruction of cellular membrane lipids-induced drought and H_2O_2 as a result of oxidative stress and may cause irreversible damage to the structures of organic molecules indispensable for cell viability (Smith and Dukes, 2013; Gulen *et al.*, 2018).

Table 7. The effects of %50 drought on pyruvic acid, MDA, H2O2, sucrose and soluble carbohydrate concentrations, and APX and SOD activities in the cloves of Taşköprü garlic.

Çizelge 7. %50 kurak uygulamasının Taşköprü sarımsağı dişlerinde pürivik asit, MDA, H2O2, sukroz ve toplam karbohidrat konsantrasyonları ve APX ve SOD aktiviteleri üzerine etkileri.

Groups	Pyruvic Acid µmol g^{-1}	MDA µmol g^{-1}	H_2O_2 µmol g^{-1}	APX EU mg Protein $^{-1}$	SOD EU mg Protein $^{-1}$	Glucose mg g^{-1}	Sucrose mg g^{-1}	Total Soluble Carbohydrate %
Control	16.64±0.6c*	8.35d±0.01d	21.46±0.22b	2.33±0.002a	58.65±0.23c	190.33±0.26c	178.85c±0.07c	91.88±0.01d
FS	16.88±0.14d	6.91c±0.01c	40.21±0.12e	2.64±0.006b	49.56±0.22b	157.66±0.17b	173.65±0.14a	87.78±0.01c
FS-IPT	15.22±0.22c	3.35b±0.01b	12.46±0.19a	2.35±0.020a	49.64±0.39b	158.52±0.20b	175.81±0.12b	83.58±0.02b
FS-NPT	15.68±0.14c	2.42a±0.01a	11.43±0.07a	2.95±0.010d	47.85±0.11a	161.89±0.28b	169.32±0.14a	78.17±0.01a
FS-CATTLE	18.26±0.18d	4.22b±0.01b	25.00±0.04b	2.24±0.002a	49.78±0.06b	155.80±0.28b	169.77±0.08a	84.48±0.02b
FS-CATTLE-NPT	18.34±0.17d	5.42b±0.01b	26.62±0.15c	2.88±0.002c	59.84±0.08c	184.82±0.08c	172.29±0.08a	84.90±0.02b
FS-GOAT-NPT	12.46±0.18a	4.47b±0.01b	31.76±0.06d	2.75±0.005b	45.36±0.03b	147.58±0.16a	178.52±0.14b	87.29±0.01c
FS-CHICKEN-GOAT	12.64±0.22ab	4.43b±0.01b	39.52±0.12e	2.86±0.006c	66.78±0.06d	159.83±0.11b	176.55±0.22b	86.40±0.02b
FS-CHICKEN-IPT	12.42±0.16a	5.34b±0.01	23.93±0.04b	3.62±0.006e	72.26±0.05e	158.90±0.14b	175.32±0.13a	86.88±0.02b
FS-CATTLE-GOAT	16.22±0.22d	5.11b±0.01	46.59±0.32f	2.74±0.011b	68.348±0.14e	187.26±0.24c	174.11±0.01a	88.00±0.20c
F	11213.68	4012888.34	116646.47	6314.24	6374.56	418.81	289.91	418.81
Sig.	<0.001	<0.001	<0.001	0.000	0.000	0.000	0.000	0.000

*Means within a group that has a different small letter are significantly different from each other. P <0.01.

The generally low MDA content in garlic samples may be related to the soil samples used, as well as the drought resistance. In previous studies, it has been shown in detail that organic manure mixed into the soil increases yield and quality in plant production by enriching the soil's organic matter composition, water-holding capacity, and mineral status (Adewale *et al*., 2011; Garcia *et al*., 2014; Can *et al*., 2019). Salehi *et al*. (2016) and Baddour *et al*. (2017) stated that proline, amino acid, and soluble protein increased with the application of organic manure types. According to variation of antioxidant enzymes, such as ascorbate peroxidase (APX) and superoxide dismutase (SOD) in this present investigation, APX activity reduced only in FS-CATTLE samples with respect to control, while SOD activity was found to be significantly higher in FS-CHICKEN-IPT, FS-CATTLE-GOAT, and FS-CHICKEN-GOAT (Table 7). Considering the enzyme values, animal fertilizers increased the enzyme activities in garlic samples under drought by improving the nitrogen, mineral content, and water status of the soil. These values were similar to the results from Ahmed *et al*. (2010) and Abd El-Ghany (2007) who noted a significant increase in activities of enzymes, such as CAT, POD, and SOD in response to organic crop management in sorghum and wheat genotypes. Also, Siavoshi and Laware (2013) studied the role of soil mixtures with cow manure, poultry manure, rice straw, and husk on antioxidant enzymes activities of rice grain. Soluble carbohydrates, such as glucose, sucrose, fructose, and fructans, are the main reserved substances of garlic and onion bulbs (Bizuayehu *et al*., 2018; Burritt, 2019). Sugar profiles of the garlic samples are shown in Table 6. The results showed that the amount of glucose, sucrose, and total soluble sugars were significantly lower in all groups with respect to the control cloves (Table 6). In our opinion, the low sugar content in the garlic samples compared to the control is a strategy for the regulation of turgor and osmosis in garlic cloves, with the combined effect of soil mixtures and drought (Amiri *et al*., 2017). A certain amount of water loss observed in the garlic tissues after harvest prevents decay, loss of colour and weight and early sprouting during storage of the cloves (Rohkin Shalom *et al*., 2015; Lisciani *et al*., 2017).

CONCLUSION

This present study has demonstrated that the mixture of different organic manures, peat, and soil significantly increased the amount of photosynthetic pigment, carotenoid, and flavonoid in the garlic leaf samples under greenhouse conditions exposed to drought treatments seedling. Also, according to the results, the measurement of bulb weight, length, and diameter and also the amount of lycopene, flavonoid, total phenolic, the free amino acid of garlic cloves obtained from FS-CHICKEN-IPT, FS-CATTLE-GOAT, FS-CHICKEN-GOAT, and FS-GOAT-NPT was found to be higher compared to non-stressed samples. Moreover, the activities of APX (ascorbate peroxidase) and SOD (superoxide dismutase) were high, but MDA and pyruvate concentrations were low in these samples. These soil mixtures increased the resistance of Taşköprü garlic against drought application by 50%, thus resulting in an increase in the head yield and the amount of bioactive components in the garlic. In contrast, soil mixtures of FS, FS-IPT, FS-NPT, and FS-CATTLE caused water to flow through the mixture, thus reducing drought stress tolerance. According to the results, it can be concluded that the mixture of the field soil with the chicken, goat, and cattle manures can be used to obtain higher yield and better chemical compound in the garlic production, which is underwater limitation areas and nutrient-deficient.

CONFLICT OF INTEREST

As an author, I declare that there is no conflict of interest in the planning, execution and writing of the article.

DECLARATION OF AUTHOR CONTRIBUTION

As the author, the planning, execution and writing of the articles was carried out by me.

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