Effect of Fusarium Infection on Physiological, Phytochemical, and Nutrient Responses in Garlic (#110327)

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Effect of Fusarium Infection on Physiological, Phytochemical, and Nutrient Responses in Garlic

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Fusarium species are significant pathogens in many crops, including garlic (*Allium sativum*), threatening yield and food safety through mycotoxin production. This study investigates the physiological, phytochemical, and nutrient responses of garlic genotypes (Local-Konya, Babaeski-Kırklareli, and Iranian-Balıkesir) to *Fusarium proliferatum* infection. Phenolic compounds, antioxidant activity, protein content, and macro- and microelement levels were assessed in healthy and infected garlic genotypes. Infestation of *Fusarium* significantly increased phenolic compounds, especially resveratrol and catechin, and the highest response was obtained by Iranian-Balıkesir genotype with 110.9% increase in total phenolic content. Regarding antioxidant activity, DPPH inhibition also rose in all genotypes with the rate of 41,57 - 55.5% in diseased groups in comparison with healthy groups. However, the protein content of garlic was declined by infection of *F. proliferatum* in all genotypes. Elemental analysis revealed that there were notable drops in potassium and calcium levels, particularly in Local-Konya genotype, but the other elements in plants were either increased or decreased accordingly. It was observed that garlic genotypes responded differently to *F. proliferatum* infection in organic acid components. These findings highlighted that *F. proliferatum* infestation in garlic enhanced phenolic production and antioxidant activities as a defense mechanism, but the amount of nutrient content of plants according to fertilization will also affect developing resilient to disease physiologically.

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Abstract

Fusarium species are significant pathogens in many crops, including garlic (*Allium sativum*), threatening yield and food safety through mycotoxin production.
This study investigates the physiological, phytochemical, and nutrient responses of garlic genotypes (Local-Konya, Babaeski-Kırklareli, and Iranian-Balıkesir) to *Fusarium proliferatum* infection. Phenolic compounds, antioxidant activity, protein content, and macro- and microelement levels were assessed in healthy and infected garlic genotypes.
Infestation of *Fusarium* significantly increased phenolic compounds, especially resveratrol and catechin, and the highest response was obtained by Iranian-Balıkesir genotype with 110.9% increase in total phenolic content. Regarding antioxidant activity, DPPH inhibition also rose in all genotypes with the rate of 41,57 - 55.5% in diseased groups in comparison with healthy groups. However, the protein content of garlic was declined by infection of *F. proliferatum* in all genotypes. Elemental analysis revealed that there were notable drops in potassium and calcium levels, particularly in Local-Konya genotype, but the other elements in plants were either increased or decreased accordingly. It was observed that garlic genotypes responded differently to *F. proliferatum* infection in organic acid components. These findings highlighted that *F. proliferatum* infestation in garlic enhanced phenolic production and antioxidant activities as a defense mechanism, but the amount of nutrient content of plants according to fertilization will also affect developing resilient to disease physiologically.

Introduction

Fusarium represents one of the fungi with hosts in the widest range, causing diseases in plants, humans, and animals. Besides, it also produces mycotoxins in its hosts, which bring huge threats to food safety. Recently, it has been noticed that the species of *Fusarium* cause serious problems even in garlic (Dean et al., 2012, Summerell, 2019). *Fusarium proliferatum* has been described as the most common species causing *Fusarium* basal root rot among *Allium* species and is generally associated with *F. oxysporum, F. solani, F. acuminatum, F. redolens, F. verticillioides, F. equiseti, F. culmorum, F. falciforme,* and other species of *Fusarium* (Le et al., 2021). Liamas et al. (Liamas et al. 2013) presented *F. proliferatum* as the etiological agent of the postharvest disease and dry rot of garlic during long-term storage. In fact, *F. proliferatum* infection was found to accompany *F. oxysporum* and jointly cause basal rot of the garlic bulbs. More recently, this organism has been reported to cause postharvest dry rot, resulting in losses as high as 30% in yields throughout the world. The rotten condition that results in the principal causes of loss in quality of garlic bulbs during storage is related to practices in postharvest handling such as drying, storage, transportation, and marketing (Mondani et al., 2021).
Various phytohormones, phytochemicals, and peptide hormones participate in plant defense through alterations in their respective expression levels to impede pathogen colonization during infection (Adie et al., 2007; Bari and Jones, 2009). Salicylic acid (SA), ethylene (ET), and jasmonic acid (JA) are the major important plant defense-controlling hormones against *F. oxysporum* disease. The interactions between *Arabidopsis–F. oxysporum* f.sp. conglutinans and *F. oxysporum* f.sp. lycopersici were estimated by Berrocal-Lobo and Molina (2004). By using several mutants impaired in the ET, JA and SA signaling they noticed that a positive cooperation among SA, JA, and ET is needed to assure an effective plant resistance against the evaluated pathogen. SA content in tobacco plants is increased in post infection of *F. solanii* (Luu et al., 2015). Similarly, representatives of the genus *Fusarium* can provoke the biosynthesis of phenolic compounds such as chlorogenic acid, caffeic acid, and resveratrol, well known for their abilities

to strengthen cell walls and reduce oxidative stress in plants. These phytochemicals have a very vital role in limiting the damage caused by *Fusarium* and further prevent the spread of the pathogen.

In addition, significant responses of plant nutrient content to *Fusarium* infection have been reported. Lobna et al. (2017) observed a significant decrease in the contents of Cu, Zn, Fe, Mn, Mg, and K in the plants of tomato due to co-inoculation with *F. oxysporum* f. sp. *radicis lycopersici* and *Meloidogyne javanica*. On the other hand, Ca content in tomato root significantly increased. Resistance of plants to pathological organisms can be partial or systemic; it may provide fast and gradual physiological changes in plants and nutrient metabolism, for example, changes in root nutrient uptake and photosynthesis in leaf after infection. Nutrient redistribution is quite common for infected plants, which often redirect nutrients for defense at the expense of growth, thus causing nutrient imbalances of critical macro and microelements such as nitrogen, phosphorus, potassium, and calcium (Nadeem et al., 2018).

Moreover, some common physiological symptoms related to *Fusarium* infection include physiological changes such as protein degradation and increased markers of oxidative stress. Among the major physiological markers of resource reallocation by the plant toward defense are reduced protein synthesis and increases in antioxidant activities and scavenging of ROS. These physiological and biochemical changes therefore reflect a complex interrelationship among *Fusarium* infection, phytochemicals production, and nutrient content change. Despite considerable studies on *Fusarium*, very little information is available on the physiological, phytochemical, and nutrient-based responses of garlic after *Fusarium* infection. This study deals with the investigation of *F. proliferatum* infection effects on the production of phenolic compounds, antioxidant activity, protein content, macro-, and microelements of three different garlic genotypes under healthy and infected conditions. This work aims to explain the changes in these parameters due to *Fusarium* infection and outlines the defensive mechanisms that are switched on within garlic plants.

Materials & Methods

Plant and fungal materials. Three garlic genotypes from different regions of Türkiye, where garlic has been grown for a long time, were studied. Details about location and genotype characteristics were given in Table 1. Plant samples were collected from garlic common storages, which were owned by garlic growers, in August – September 2022. Sampling method was done according to Mondani et al. (2021). Garlic genotypes were harvested at the end of July 2022, when plants reached their physiological maturity. After harvest, garlic plants were sun dried on field ground for two weeks, then garlic was taken to common storages of growers in each region. After two months of storage at each sampling, 20 bulbs were collected in three replicates (total 60 bulbs) according to regions. After sampling, the bulbs were separated as clean and diseased. Fungal isolations were made after sampling to assess disease progress on sampled bulbs.

The basal part of garlic bulbs was used for the isolation of *Fusarium* spp. (Altınok and Can,2010; Datta et al., 2011). The plant samples were first washed under tap water and the small parts of bulbs (explant) containing symptomatic tissue were cut into 0.5-1 cm pieces with the help of sterile scalpel. Explants were soaked in 1.5% NaOCl solution (0.5ml L^{-1}, Tween 20 added) for 5 minutes in a lamin-air flow cabinet in order to surface sterilization. For removing sterilant, they were rinsed with sterile distilled water 3 times. Then, they were put in sterile filter papers for 4-5 hours to dry (Altınok and Can,2010). Explants were cultured to PDA (Potato Dextrose Agar) and

FMM (*Fusarium* Minimal Media) media containing antibiotics (Stretomycin Sulphate, 100 g ml^{-1}) and incubated 24±2°C for 5-7 days. At the end of incubation, the fungal colonies developing around the plant tissues were purified and single spore isolation was done.

For the purpose of genus and species characterization of *Fusarium* spp. isolates developed from single spores, it was carried out according to the species identification key of (Datta et al., 2011). The structure of the colonies of the cultures which were reseeded on PSA medium to determine the colony color and on SNA medium to encourage sporulation were examined. For this purpose, colony color, chain structures, macroconidia, microconidia, conidiophore and phialid structures were examined and *Fusarium* spp. isolates were identified (Altınok and Can,2010).

In the molecular diagnosis of the fungal isolates, translation elongation factor 1-α (Tef-1α), and RNA polymerase second large subunit (RPB2) regions were used. The fungal isolates were assigned as *Fusarium proliferatum* after comparison with representative sequences available in NCBI (National Center for Biotechnology Information) (Dedecan et al. 2022). The obtained sequences were deposited in GenBank and accession numbers were assigned (Table 1).

Total phenolic content extraction and measurement. The extraction of the phenolic compounds was done by homogenizing 10 g garlic samples in 80 mL of 80% methanol solution. The homogenate was left at room temperature in the dark for 24 hours and later centrifuged for 10 minutes at 4000 rpm. The resulting supernatant was filtered to discard impurities, and total phenolic content was quantified using the Folin-Ciocalteu method. Absorbance of the resulting solution was measured at 765 nm. The total phenolic content was expressed as mg GAE g^{-1} fresh weight, using a calibration curve prepared with standard gallic acid solutions (Erol, 2024-a).

Extraction and DPPH free radical scavenging method. For antioxidant activity, garlic samples were extracted with 85% ethanol in an amount equal to 2 grams. Homogenization was done for samples collected and kept away from light at room temperature for a period of 24 hours. Then, centrifugation was done for 10 minutes with 5000 rpm, after which solution was obtained for extraction. The DPPH radical scavenging activity was carried out using the procedure previously described with some modifications. To this end, 0.1 mM DPPH radical was reconstituted in methanol and 100µL of the extract from each sample was added to 3 mL of the DPPH solution. The resulting mixture was thereafter kept in the dark for 30 minutes. The absorbance was consequently measured at an absorbance wavelength of 517 nm using a spectrophotometer while ascorbic acid was used as the positive control. The DPPH radical scavenging capacity was thereafter computed by formula (Ozdenefe et al., 2024). The % inhibition may be calculated using the formula:

% Inhibition = (Absorbance of control – Absorbance of sample) / (Absorbance of control) × 100. (1)

Assays were done in triplicate, and data are expressed as mean ± SD. Data were analyzed by ANOVA, and a probability of less than 0.05 was considered significant.

Protein analysis. The protein content in garlic samples was determined by the Kjeldahl method. A 0.25 g sample was digested with sulfuric acid (H_2SO_4) and a catalyst, with combustion for 3.5 h. The digested solution was then distilled in an alkaline environment created by 40% sodium hydroxide, NaOH, and the ammonia collected with 4% boric acid. The distillate obtained was then titrated against 0.1 N hydrochloric acid (HCl) for protein content. The calculations were performed according to the guidelines laid out by Casado et al. (2004).

Mineral content analysis. The samples of garlic were dried at 72°C and ground into powder form for the analysis of their mineral content. A 0.25 g portion of each sample was then subjected to digestion using a mixture of 9 mL nitric acid (HNO_3) and 3 mL hydrogen peroxide (H_2O_2) in a microwave digestion system operating with 200 W for 30 minutes. The digested samples were filtered and diluted to a final volume of 25 mL. The concentration of Ca, Mg, Fe, Mn, Zn, and Cu was done by atomic absorption spectroscopy, while for Na and K, flame photometry was done (Besirli et al., 2022). The level of N and P was determined in a UV spectrophotometer. All the measurements were carried out in triplicate for reliability.

Extraction method for HPLC analysis of phenolic compounds. The phenolic compounds were extracted from garlic samples using a method optimized for maximum recovery. About 10 g of each garlic sample was homogenized in 80 mL of 80% methanol and incubated at room temperature in the dark for 24 hours. On incubation, the mixture was centrifuged at 4000 rpm for 10 minutes to separate the supernatant. Filtration was subsequently carried out on the supernatant to remove impurities and improve the solubility of the phenolic compounds (Erol, 2024-b).

HPLC conditions of phenolic compounds. The separation and quantification of phenolic compounds were performed on an RP-HPLC system fitted with a C18 column (4.6 x 250 mm, 5 µm particle size). The column temperature was kept at 30°C, and a diode array detector (DAD) was set to monitor the phenolic peaks. A gradient elution system was performed when using two mobile phases: A, 0.1% phosphoric acid in water, and B, 100% acetonitrile. The volume of injection was 10 µL, and the flow rate was 1 mL/min. Identification and quantification of phenolic compounds were performed in accordance with comparison of retention times with those of external standards. Firstly, standard solutions with known concentrations of phenolic compounds were plotted in calibration curves. Results were given as milligrams of phenolic content per kilogram of fresh garlic weight. All analyses were run in triplicate in order to assure precision and repeatability (Erol, 2024-b).

Extraction method and HPLC analysis conditions of organic acids and sugars. Organic acids and sugars were extracted from freshly harvested garlic bulbs. Approximately 2.5 g of finely crushed garlic samples were placed into 50 mL Falcon tubes, to which 25 mL of a deionized water/methanol mixture (7:3, v/v) was added. The mixture was thoroughly homogenized using a high-speed homogenizer. The homogenate was then incubated in a water bath at 40°C for 30 minutes to aid extraction. The solution was then centrifuged for 10 minutes at a speed of 10,000 rpm at 4°C and the supernatant filtered via a 0.45-micron syringe filter. The filtered extracts were kept under -20°C until further analysis (Erol, 2024-b).

Organic acids and sugars in the garlic extracts were determined by a high-performance liquid chromatography system. Separation of sugars was done on a Rezex RCM-Monosaccharide Ca^{2+} (8%) LC Column (300 x 7.8 mm). Column temperature was maintained at 80°C, with analysis done in an isocratic mode by using ultrapure water as a mobile phase at a flow rate of 0.5 mL/min. The analysis was completed within 15 minutes, and the glucose, fructose, and sucrose concentrations were calculated from calibration curves prepared using standard sugar solutions. Results were presented as milligrams of sugar per gram of fresh weight, and three replicates of each sample were analyzed (Erol, 2024-b).

Organically bound acid was determined by the same HPLC system, only with a different column: Rezex ROA-Organic Acid H^+ (8%) LC Column (300 x 7.8 mm). The column temperature was 50°C, and the UV detector was set at 210 nm. A gradient elution system was used for separating a number of organic acids. The analytical results for organic acids were

determined using calibration curves built from standard solutions of the target organic acids. Milligrams of organic acid were expressed per gram fresh weight (Erol, 2024-b).

Statistical analysis. The correlation between all the parameters was calculated using a Pearson correlation coefficient. The parameters that were considered in this correlation analysis include: phenolic compounds, organic acids, sugars, antioxidant activity, elements, total phenolic content, and protein levels. The data set on the analysis of elements was used to construct a correlation matrix by summarizing and visualizing it through a heatmap showcasing relationships: positive/negative. Then, PCA was applied to gauge the variation among phenolic compounds, reducing data dimensions into two main components. The resulting biplot showed the distribution of genotypes and their effect regarding infection caused by *Fusarium* on the selected phenolic compounds. Correspondingly, changes in the organic acid concentration within different genotypes were compared by applying line charts for healthy and diseased genotypes. Triplicated measurements for all experimental data were carried out for better accuracy and reliability. Mean comparisons were done by analysis of variance (ANOVA), followed by Tukey's HSD test to establish the significant difference among the samples. Any results with a p-value below 0.05 were considered significant. Furthermore, PCA and other data visualization techniques were carried out using appropriate software, including JMP 14 and OriginPro.

Results

Total phenolic contents. Total phenolic content was significantly higher in the infected than in the healthy plants (Fig. 1a, Table 2). Total phenolic content in the Local-Konya genotype of healthy plants was 5400.96 mg/kg and increased by 36.0% to 7347.44 mg/kg in infected plants ($P \leq 0.01$). While the phenolic content was 5264.72 mg/kg in healthy plants for the Babaeski-Kırklareli genotype, the infection increased it by 66.9% and reached the value 8790.07 mg/kg ($P \leq 0.001$). For the genotype Iranian-Balıkesir, the content of total phenolic was found to be 3634.33 mg/kg in healthy plants and increased by 110.9% to 7664.61 mg/kg in infected plants ($P \leq 0.001$). According to the statistical analysis, it was found that the total phenolic compounds increased significantly in all the studied genotypes after *Fusarium* infection. It is obvious that the 110.9% increase in the Iranian-Balıkesir genotype might show a meaningful increase in phenolic compound production due to resistance against this disease. Overall, an increase in the total phenolic content was statistically significant (at least at $P \leq 0.05$) in all studied genotypes.

Antioxidant activity. *Fusarium* infection enhanced the antioxidant activity of all genotypes (Fig 1b, Table 2). While the DPPH radical inhibition was 39.07% in healthy plants of the Local-Konya genotype, it increased to 51.25% in the infected plants, reflecting a 31.1% increase ($P \leq 0.05$). In the genotype Babaeski-Kırklareli, antioxidant activity increased from 33.63% in healthy plants to 41.57% after the infection, marking a 23.6% rise ($P \leq 0.05$). In the Iranian-Balıkesir genotype, antioxidant activity increased from 29.94% in healthy plants to 46.57% in infected plants, which is a 55.5% increase ($P \leq 0.01$). Statistical analysis carried out with respect to *Fusarium* infection showed that antioxidant activities increased significantly. It can be said that with the increase of 55.5% observed in the Iranian-Balıkesir genotype, this genotype developed a good antioxidant defense against oxidative stress caused by *Fusarium* infection. Increases in antioxidant activities in all genotypes were significant statistically at least at $P \leq 0.05$.

Total protein content. The *Fusarium* infection effects on the protein content of garlic plants showed lower protein contents of all genotypes in comparison to that of the healthy ones (Fig. 1c, Table 2). The protein content of healthy plants was 4.23% for the Local-Konya genotype and declined by 2.6% to 4.12% in the infected ones, significantly lower at $P \leq 0.05$. In the healthy

plants of the Babaeski-Kırklareli genotype, protein contents were determined as 4.93%, but it was observed to decrease by 7.5% to 4.56% due to *Fusarium* infection ($p<0.01$). In the Iranian-Balıkesir genotype, it was 4.18% in the healthy plants, while it also decreased by 15.5% in the infected ones as in the first group to 3.53% ($p<0.001$).

Whereas the reduction in protein content was observed for all genotypes, the Iranian-Balıkesir genotype showed a greater reduction. One-way ANOVA showed that these reductions were at a statistically significant level in all genotypes ($P \leq 0.05$). Among the genotypes, the highest reduction was obtained in the Iranian-Balıkesir genotype. This definitely shows that *Fusarium* infection may interfere with the synthesis and metabolism of proteins by the plants, hence causing such a sharp loss of the protein content.

Element analysis. *Fusarium* infection caused significant variations in both macro and microelement levels among all the garlic genotypes tested (Table 3). The nitrogen (N) level was stable for both the healthy and infected Local-Konya plants at 0.81% ($p > 0.05$); regarding phosphorus, it increased by 43.6% to 430.62 mg/100 g ($P \leq 0.01$). K decreased slightly by 1.4% for 5607.23 mg/kg, whereas for Ca, there was a 13.3% decrease, which was 1374.31 mg/kg ($P \leq 0.05$). In the Babaeski-Kırklareli genotype, N decreased from 0.96% to 0.91% ($P \leq 0.05$), while P increased by 10.4% to 477.32 mg/100 g ($P \leq 0.01$). K dropped by 7.5% to 6472.62 mg/kg ($P \leq 0.05$), and Ca decreased by 16.4% to 1712.5 mg/kg ($P \leq 0.01$). In the Iranian-Balıkesir genotype, N dropped to 0.67% ($P \leq 0.05$), and K levels fell by 13.9% to 4798.5 mg/kg ($P \leq 0.01$). P levels showed a slight increase to 498.35 mg/100 g ($p > 0.05$), while Ca levels decreased by 24.3% to 1337.88 mg/kg ($P \leq 0.01$). These results highlight that *Fusarium* infection caused significant differences in macro element levels between genotypes, particularly in potassium and calcium levels, which showed notable reductions.

Regarding microelements, copper (Cu) levels varied significantly between genotypes (Table 3). In the Local-Konya genotype, Cu levels increased by 145.2% from 1.79 mg/kg in healthy plants to 4.39 mg/kg in infected plants ($P \leq 0.01$). In the Babaeski-Kırklareli genotype, Cu levels showed a slight decrease to 2.28 mg/kg, which was not statistically significant ($p > 0.05$). In the Iran-Balıkesir genotype, Cu levels dropped sharply from 2.24 mg/kg to 0.20 mg/kg ($P \leq 0.001$). Iron (Fe) levels showed a slight increase to 16.7 mg/kg in the Local-Konya genotype ($p > 0.05$), while they decreased by 52.3% to 13.41 mg/kg in the Iranian-Balıkesir genotype ($P \leq 0.01$). Magnesium (Mg) levels decreased across all genotypes, with the largest reduction observed in the Babaeski-Kırklareli genotype, where Mg levels fell by 34.2% to 279.71 mg/kg ($P \leq 0.01$). In the Iranian-Balıkesir genotype, Mg levels decreased by 25.6% to 204.97 mg/kg ($P \leq 0.05$). These findings indicate that *Fusarium* infection significantly affected microelement levels, particularly in copper and iron, with notable differences observed between genotypes.

HPLC analysis of phenolic compounds. The HPLC analysis of phenolic compounds revealed significant effects of *Fusarium* infection on phenolic content across the Local-Konya, Babaeski-Kırklareli, and Iranian-Balıkesir genotypes (Table 4). In the Local-Konya genotype, chlorogenic acid levels in healthy plants were 210.99 mg/kg, but after *Fusarium* infection, they decreased by 29.0% to 149.78 mg/kg ($P \leq 0.05$). In the Babaeski-Kırklareli genotype, chlorogenic acid levels dropped by 65.7% from 220.46 mg/kg in healthy plants to 75.48 mg/kg in infected plants ($P \leq 0.01$). Conversely, in the Iranian -Balıkesir genotype, chlorogenic acid increased by 55.7% from 88.35 mg/kg in healthy plants to 137.60 mg/kg in infected plants ($P \leq 0.05$). Catechin, which was undetectable in healthy plants, was found to be 276.43 mg/kg in infected Babaeski-Kırklareli plants ($P \leq 0.001$). Similarly, catechin levels rose to 252.55 mg/kg in infected Iranian-Balıkesir plants, where it was also absent in healthy plants ($P \leq 0.001$).

Caffeic acid was undetectable in healthy Local-Konya plants but measured at 3.01 mg/kg in infected plants (Table 4). In the Babaeski-Kırklareli genotype, caffeic acid levels increased by 47.3% from 0.91 mg/kg in healthy plants to 1.34 mg/kg in infected plants ($P \leq 0.05$). In the Iran-Balıkesir genotype, caffeic acid rose slightly from 0.36 mg/kg in healthy plants to 1.32 mg/kg in infected plants ($P \leq 0.05$). 4-hydroxybenzoic acid was only detected in infected Iranian-Balıkesir plants at 0.36 mg/kg, while it was absent in the other genotypes. Vanillin levels increased to 0.23 mg/kg in infected Local-Konya plants ($P \leq 0.05$), with no significant differences observed in the other genotypes (Fig. 4). Rutin levels remained low in the Iranian -Balıkesir genotype and showed no significant changes after *Fusarium* infection. T-ferulic acid, which was undetectable in healthy Babaeski-Kırklareli plants, increased to 0.67 mg/kg following infection ($P \leq 0.01$), while remaining at low levels in the other genotypes. Naringin, found at 2.04 mg/kg in healthy Babaeski-Kırklareli plants, disappeared in infected plants, while only low levels were detected in the Iranian-Balıkesir genotype. O-coumaric acid levels in the Babaeski-Kırklareli genotype decreased by 23.1%, from 0.13 mg/kg in healthy plants to 0.10 mg/kg in infected plants ($P \leq 0.05$), while in the Iranian -Balıkesir genotype, this compound showed a slight increase to 0.03 mg/kg following infection ($P \leq 0.05$).

Resveratrol levels increased dramatically in the Iranian-Balıkesir genotype, rising by 998.5% from 0.042 mg/kg in healthy plants to 0.461 mg/kg in infected plants (Table 4). A significant increase in resveratrol levels was also observed in the Local-Konya genotype, from 0.051 mg/kg in healthy plants to 0.144 mg/kg in diseased plants, representing a 182.4% increase ($P \leq 0.01$). Quercetin levels in the Babaeski-Kırklareli genotype decreased by 14.8%, from 1.89 mg/kg in healthy plants to 1.61 mg/kg in infected plants ($P \leq 0.05$), with no significant changes in quercetin levels observed in the other genotypes. These results indicate differential responses in the biosynthesis of phenolic compounds by garlic plants according to their genotype after *Fusarium* infection. While in some phenolic compounds, such as resveratrol and catechin, significant enhancements were observed, in others, such as quercetin and O-coumaric acid, there was a reduction. Most of these changes were statistically significant ($P \leq 0.05$) by statistical analysis. A strong defense response can be seen in compounds such as resveratrol against *Fusarium* infection.

HPLC analysis of organic acids and sugars. Organic acid profiles determined with HPLC analysis showed that *Fusarium* infection significantly affects garlic genotypes (Table5, Fig 2). The oxalic acid level increased from non-detected in healthy Local-Konya plants to 15.17 mg/kg in infected plants. For the Iranian-Balıkesir genotype, too, the oxalic acid level increased from 7.01 mg/kg in healthy plants to 10.56 mg/kg in infected ones ($P \leq 0.05$). Citric acid levels in the Babaeski-Kırklareli genotype increased from 4175.95 mg/kg in healthy plants to 6291.02 mg/kg following infection ($P \leq 0.01$). In the Local-Konya genotype, citric acid levels rose from 4322.72 mg/kg in healthy plants to 4781.87 mg/kg in infected plants ($P \leq 0.05$). Succinic acid showed significant increases in all genotypes, especially in Local-Konya plants, where it rose from 9281.79 mg/kg in healthy plants to 11579.81 mg/kg in infected plants ($P \leq 0.01$).

Among other organic acids, malic acid was not detected in healthy Babaeski-Kırklareli plants but measured at 305.87 mg/kg in infected plants ($P \leq 0.05$). Lactic acid levels also increased significantly; in the Babaeski-Kırklareli genotype, levels rose from 13639.16 mg/kg in healthy plants to 20840.81 mg/kg in infected plants ($P \leq 0.01$), while in the Iranian-Balıkesir genotype, lactic acid increased to 2031.90 mg/kg in infected plants.

The sugar analysis revealed that *Fusarium* infection had significant effects on sucrose levels (Table 2, Fig. 1d). In the Local-Konya genotype, sucrose levels increased by 57.2% from 2.40

mg/kg in healthy plants to 3.77 mg/kg in infected plants ($P \leq 0.01$). However, in the Babaeski-Kırklareli genotype, sucrose levels dropped by 59.5%, from 2.84 mg/kg in healthy plants to 1.15 mg/kg in infected plants ($P \leq 0.05$). Similarly, in the Iranian-Balıkesir genotype, sucrose levels decreased by 38.5%, from 2.58 mg/kg in healthy plants to 1.59 mg/kg in infected plants ($P \leq 0.05$). These results suggest that *Fusarium* infection influences the synthesis of organic acids and sugars differently depending on the genotype. Valuably enough, the increased levels detected at succinic acid, lactic acid, and sucrose upon infection might suggest providing a more direct indication of the metabolic response of the plant. Most of these changes have already been statistically proven ($P \leq 0.05$) to be significant.

Multivariate analyses: Multivariate analyses were performed in order to obtain an overview of the interactions among the studied parameters, garlic genotypes, and the disease pathogen. The heatmap very well demonstrated great changes between the healthy and diseased states of plants in a level of both macro and microelements (Fig. 3), where red color indicates a higher and blue lower level, thus showing great differences among genotypes. In the case of the Local-Konya genotype, one can observe a marked decline in Ca and K levels between healthy and diseased plants. With respect to the Babaeski-Kırklareli genotype, high levels of elements in the healthy state dropped to low values when under a diseased state. In diseased plants, a serious decrease of copper, along with other microelements, was observes in the Iran-Balıkesir genotype. The Ni levels were significantly reduced at $P \leq 0.001$, hence showing a great deal of difference between healthy and diseased plants. Overall, this heatmap clearly presented the consequence of *Fusarium* infection on plant nutrient levels and the importance of such changes.

The result of PCA biplot analysis (Fig. 4) showing differentiation among genotypes because of *Fusarium* infection by two major principal components is as follows: It is obvious that, under healthy and diseased conditions, respectively, the Local-Konya genotype clustered closely, which means minimal changes in phenolic compounds. On the other hand, the Babaeski-Kırklareli genotype showed clear differentiation between healthy and diseased states. Among them, the variables chlorogenic acid, caffeic acid and resveratrol contributed a lot to genotype separation, whereas catechin and quercetin showed highest variance especially under diseased conditions. The first principal component explained 58.9% of total variance, while the second principal component accounted for 24.5%, which indicates an increased production of these compounds upon *Fusarium* infection. Overall, this biplot effectively showed the variation in the effect of *Fusarium* infection on phenolic compounds regarding the variability among the genotypes.

Analysis provides some relationships of all the measured parameters, across three genotypes (Local-Konya, Babaeski-Kırklareli, and Iran-Balıkesir) and health/disease conditions (Fig. 5). In most cases, it was a positive correlation for the phenolic compounds and antioxidant activity. For instance, for such compounds as resveratrol and DPPH % compound inhibition, strong correlations are found. A weaker correlation was observable between the total phenolic content and protein level. Within organic acids of the same genotype, citric acid and succinic acid showed correlations. In terms of elements, potassium (K) had a good positive relationship with other macroelements: phosphorus (P) and calcium (Ca). In the diseased genotypes, the increase in antioxidant activity was well related to the increase in phenolic compounds—an indication that the plant's defense mechanism effectiveness in response to *Fusarium* infection was up-regulated.

Discussion

The phenolic compounds are very significant to plants through their intervening roles in the defense mechanisms against the pathogen and stress conditions. In this study, *Fusarium* infection was found to increase the total phenolic content in the studied garlic plants. For example, in the Local-Konya genotype (Table 4), the total phenolic contents rose by 36% from 5400.96 mg/kg in healthy plants to 7347.44 mg/kg in infected plants ($P \leq 0.01$). Present findings give a signification of the paramount importance of phenolic compounds in plant defense systems against pathogens. The literature shows that phenolic compounds strengthen cell walls and reduce the oxidative stress by scavenging the reactive oxygen species. The role of phenolic compounds in plants for defense against biotic and abiotic stresses was widely reviewed by Tuladhar et al. (2021). Moreover, according to Miedes et al. (2014), phenolics participate in lignin synthesis that reinforces cell walls against the penetration of the pathogen. Increased total phenolic content suggests that plants enhance the biosynthesis of secondary metabolites as a result of their reaction against the infectious agent. In connection with this, Tuladhar et al. (2021) have also found that there has been enhanced biosynthesis of phenolics in plants treated under *Fusarium* pathogens. Some phenolic compounds have a very significant role in plant defense; these prevent pathogen attack through promoting lignin synthesis and cell wall thickening. In this context, Tak and Kumar (2021) reported that biosynthesis of phenolic compounds is induced through pathogen attack and in some cases is effective in counteracting pathogen attack. Increased antioxidant activity was observed in all genotypes following *Fusarium* infection (Table 2). Antioxidants help protect plants from oxidative stress in defense mechanisms. The DPPH radical inhibition in plants of the Iranian-Balıkesir genotype increased by 55.5%, from 29.94% in healthy plants to 46.57% in infected plants; the change was statistically significant ($P \leq 0.01$). This, probably, means that under the influence of *Fusarium* infection, defense against oxidative stress in plants has increased due to better antioxidant defense. It was further said that oxidative stress causes the initiation of plant defense responses against pathogens and the role in this process is being taken by antioxidant enzymes (Biswas et al, 2020). Suamn et al. (2021) elaborated that antioxidants act to protect the plant cell under oxidative stress by neutralizing ROS. The increase in antioxidant activity as found in this study means that the plant produces the antioxidant compound is to neutralize ROS in the defense response of pathogens. Chrpová et al. (2021) therefore said that infection from *Fusarium* species would provoke increased ROS generation in the plants, and they in turn activated their antioxidant defense systems. *Fusarium* infection had a negative effect on the protein synthesis of garlic plants, as their protein levels were reduced in all the tested genotypes (Table 2). In the Iranian-Balıkesir genotype, protein content was reduced by 15.5%, from 4.18% in healthy plants to 3.53% in infected plants ($P \leq 0.001$). This shows that *Fusarium* infection suppresses plant metabolism and protein synthesis. Literatures show that pathogens have negative effects on the protein synthesis in plants, which thereby shifts energy and other resources towards defense responses. Ferreira et al. (2007) reported that the pathogen attacks suppress protein synthesis in plants with the activation of a series of defense responses. Similarly, Dutta et al. (2023) found that the soil-borne pathogens, *Fusarium*, negatively affected protein synthesis within the plants by disrupting metabolic processes. This reduction in protein synthesis may indicate that plants utilize their energy resources in growth and development to incorporate defense mechanisms. Matyssek et al. (2012) stated that plants, during pathogen attacks, utilize their energy in defense mechanisms instead of growth-related metabolic activities. Rojas et al.(2014) further explained the

relationship between reduced protein synthesis and the plant defense response by indicating energy utilization is altered during pathogen defense.

Infection with *Fusarium* significantly altered the contents of both macro and microelements in garlic plants (Table 3). Considering the macroelements, the most serious changes took place according to the concentration of phosphorus and potassium. In the Local-Konya genotype, P increased by 43.6% from 299.82 mg/100 g in healthy plants to 430.62 mg/100 g in infected plants ($P \leq 0.01$). That might indicate that infected plants require more phosphorus to increase energy production as a result of pathogen inoculation. Ahanger et al. (2016) pointed out that P is one of the main mineral elements used in energy metabolism and for proper plant development. Lower values of potassium, on the other hand, are indicative of an impaired water balance and ion exchange post-infection, since potassium is considered a very important cation for the health of the plant. Its reduction during the infection impairs the plant mechanism for the maintenance of water balance. Sardans and Peñuelas (2021), reported that potassium maintains for water balance and stomatal action in plant cells. Moreover, potassium deficiency reduces vigor in plants and their resistance to pathogenic attacks (Tripathi et al., 2022). It has been documented that potassium-starved plants are highly susceptible to different pathogenic agents due to disturbances of ion balance and defense mechanisms against *Fusarium* spp.

Regarding microelements (Table 3), it manifested considerable changes in the level of copper (Cu) and iron (Fe). Copper levels increased by 145.2% in the infected plants as compared to those in the healthy plants of the Local-Konya genotype, representing 1.79 mg/kg and 4.39 mg/kg, correspondingly ($p<0.01$). This is an indication that copper is very vital in the plant's mechanisms of defense against oxidative stress and pathogens. Copper being a cofactor of the antioxidant enzyme also participates in this very important battle against oxidative stress. According to Liu et al. (2018), copper considerably contributes to a decline in oxidative stress and scavenging of ROS in plants. Further, copper contributes to the synthesis of lignin, which strengthens plant cell walls against the penetration of the pathogen (Liu et al. 2018). Also noteworthy are changes in iron (Fe) levels. Within the Iranian-Balıkesir genotype, iron decreased by 52.3%, from 28.15 mg/kg in healthy plants to 13.41 mg/kg in infected plants ($P \leq 0.01$). Iron deficiency has a negative effect on chlorophyll synthesis and photosynthetic activity, slowing the growth and development of the plants. As stated by Abbas et al.(2021), it plays an important role in the synthesis of chlorophyll, hence in energy production, and its deficiency weakens the plant's defense capacity. Iron deficiency reduces photosynthetic capacity and thus weakens plant mechanisms set up for defense against *Fusarium*.

The infection of garlic plants with *Fusarium* significantly altered the level of phenolic compounds (Table 4). In general, increased levels of phenolic compounds, such as resveratrol and catechin, are indicative of increased synthesis in response to the infecting pathogen. In the genotype Iranian -Balıkesir (Table, the resveratrol increased from 0.042 mg/kg in healthy plants to 0.461 mg/kg in infected ones, with an increase rate of 998.5% ($P \leq 0.001$). Mattio et al. (2020) noted that resveratrol is one of the most important stilbenoids involved in plant defense, while stilbenoids have various other activities against pathogens. The increase of catechin levels is of interest. In the Babaeski-Kırklareli genotype, while it was not detected in the healthy plants, the level reached 276.43 mg/kg in infected plants ($P \leq 0.001$). Among flavonoids, catechin is one type involved in the plant defense mechanisms against oxidative stress. According to Tuladhar et al. (2021), catechins enhance the plant defense mechanism against oxidative stress and pathogen attack through their antioxidant properties. In this respect, it has also been observed that during

oxidative stress, catechins play a role in neutralizing reactive oxygen species (ROS), thereby enhancing the antioxidant defense systems in plants.

Fusarium infection significantly affected the organic acids and sugars profile of garlic plant (Table 5). Some organic acids, like succinic acid and lactic acid, showed great increases with infection. Succinic acid increased from 9281.79 mg/kg in a healthy plant to 11579.81 mg/kg in an infected one with the Local-Konya genotype ($P \leq 0.01$). Succinic acid is one of the major organic acids participating in plant energy metabolism, thus showing increased energy demand and hence accelerated metabolic processes under infection (Wang et al., 2019). As Müller et al. (2012) pointed out, succinic acid is among those metabolites that afford great importance toward generating energy under stress conditions, considered to have a critical role in mitochondrial energy metabolism. Increased lactic acid points out that metabolic pathways are turned on for enhancing stress tolerance (Moons, 2008). In the case of the Babaeski-Kırklareli genotype, lactic acid values showed an increase from 13639.16 mg/kg for healthy plants to 20840.81 mg/kg in infected plants ($P \leq 0.01$). Lactic acid would, therefore, be one of the major intermediates in the process of energy production under the conditions of stress in plants and play a major role in their metabolic response.

The line graph has compared the concentrations of organic acids in both healthy and diseased genotypes (Table 5, Fig. 2). There has been a significant difference during the determination of acids like oxalic acid, citric acid, succinic acid, propionic acid, and lactic acid in healthy and diseased plants. For instance, lactic acid showed its highest concentration in genotype Babaeski-Kırklareli (Diseased), which means a higher response against *Fusarium* infection. In the organic acid group, citric acid was highly present in the case of diseased conditions of all genotypes, most especially in the Babaeski-Kırklareli genotype. Some organic acids, on the other hand, such as malic acid and fumaric acid, were only present in certain genotypes with a given infection status; this result underlines differences among genotypes and points out the action of *Fusarium* infection.

Changes in sucrose levels indicate that *Fusarium* infection affected carbohydrate metabolism in garlic plants (Table 2, Fig. 1d). In the Local-Konya genotype, sucrose increased by 57.2%, from 2.40 mg/kg in the healthy plants to 3.77 mg/kg in the infected plants ($P \leq 0.01$). This increase might suggest that sucrose is an important energy source for the plant during its strengthened defense mechanisms under these stress conditions. Sucrose is one of the critical carbon sources produced within energy production in plants. As explained by Anisimova et al. (2024), its production increases due to responses of defense. According to Mathan et al. (2021), sucrose acts as an energy sink during stress, where increased levels are recorded during stress. These results indicated that *Fusarium* infection strongly affected the organic acid and sugar metabolisms of the garlic plants, which varied greatly among genotypes. This elevation in organic acids would suggest accelerated energy production and a defense response for infected plants.

Conclusions

The present work studied in detail the biochemical and physiological effects of the infection caused by *Fusarium* in garlic plants. Infection caused significant changes in the levels of total phenolic compounds, antioxidant activity, synthesis of proteins, macro and microelements, phenolic profiles, organic acids, and sugar metabolism. Increase in phenolic compounds and antioxidant activity refers to the enhanced mechanisms of plant defense against pathogens. Besides, the lowered protein synthesis and deficiencies in key elements such as potassium and iron indicate that the infection had a deterring impact on growth and development of these plants.

Further, profiling organic acids and sugars demonstrated that *Fusarium* infection significantly modified plant energy metabolism.

Soil-borne diseases, especially *Fusarium* wilt, seriously threaten the yield and quality of economically important crops such as garlic. Knowledge gained from this work is of paramount importance for identifying the genotypes that are resistant to *Fusarium* infection and the mechanisms involved in improving plant defense responses. It has to be pointed out that the induction of plant immunity against any kind of pathogen infection, such as *Fusarium*, through phenolic compounds and antioxidant defense mechanisms was clearly demonstrated. These results provide evidence that there is great potential for developing biochemical defense strategies in garlic cultivation and other such crop production in order to fight against such pathogens like *Fusarium*. Overall, this work forms a good backbone for sustainable agriculture in the direction of developing strains of crops with higher resistance, along with improvement in disease management practices.

Acknowledgements

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Table 1(on next page)

Location, GPS, (global positioning system) data and genotypes characteristics of plant and fungal materials.

1 **Table 1.** Location, GPS, (global positioning system) data and genotypes characteristics of plant
2 and fungal materials.

Provinces	Coordinates	Altitude (m)	Genotype Name	Genotype Characteristics	*Fusarium proliferatum* NCBI Numbers
Balıkesir	39° 30’ 40” N 27° 50’ 53” E	231	Iranian-Balikesir	Hardneck, White colored, Larger bulbs	JQ762611.1
Konya	38° 18’ 46” N 31° 29’ 30” E	1047	Local-Konya	Hardneck, White colored, Medium sized bulbs, Long-term storage	MT095058.1
Kırklareli	41° 34’ 33” N 27° 50’ 43” E	314	Babaeski-Kirklareli	Hardneck, White colored, Smaller bulbs	MH383509.1

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Table 2 (on next page)

Comparison of total phenolic, antioxidant activity, total protein, and total sugar content of diseased/healthy garlic genotypes

Table 2. Comparison of total phenolic, antioxidant activity, total protein and total sugar content of diseased/healthy garlic genotypes.

	Total phenolics (mg/kg GAE)	Antioxidant Activity (Inhibition, DPPH %)	Total protein (%)	Total sugar (Sucrose, mg/kg)
Disease status (DS)	***	***	**	*
Diseased	7934,04 A	46,46 A	4,45 A	2,61 A
Healthy	4766,67 B	34,21 B	4,07 B	2,17 B
Genotypes (G)	**	*	*	*
Local-Konya	6374,20 b	45,16 a	4,17 a	3,09 a
Babaeski-Kirklareli	7027,39 a	37,60 b	4,74 a	1,99 c
Iranian-Balikesir	5649,47 c	38,25 b	3,85 b	2,08 b
Mean	**6350,35**	**40,34**	**4,26**	**2,39**
DS x G	**	*	*	*

Letters show the mean values of different groups in each column. * $p \leq 0.5$, ** $p \leq 0.01$, ***$p \leq 0.001$, as indicated by the Tukey's HSD test.

Table 3(on next page)

Comparison of macro- and microelements contents of diseased/healthy garlic genotypes

Table 3. Comparison of macro- and microelements contents of diseased/healthy garlic genotypes.

	N (%)	P (mg/100g)	K (mg/kg)	Ca (mg/kg)	Cu (mg/kg)	Mn (mg/kg)	Fe (mg/kg)	Zn (mg/kg)	Mg (mg/kg)	Na (mg/kg)	Ni (mg/kg)
Disease status (DS)	*	*	**	**	**	**	**	***ns***	**	**	**
Diseased	0,80 B	468,76 A	5626,11 B	1474,90 B	2,29 A	6,33 B	20,06 B	17,62 A	235,44 B	133,37 B	0,012 B
Healthy	0,86 A	408,13 B	6087,02 A	1800,10 A	2,18 B	9,28 A	25,01 A	17,91 A	314,58 A	160,89 A	0,080 A
Genotypes (G)	**	**	**	**	**	**	**	**	**	**	*
Local-Konya	0,81 b	365,22 c	5646,88 b	1479,89 c	3,09 a	6,15 b	16,29 c	16,75 b	232,27 b	123,46 c	0,045 b
Babaeski-Kirklareli	0,93 a	454,83 b	6734,55 a	1880,52 a	2,39 b	11,45 a	30,52 a	21,22 a	352,55 a	175,58 a	0,055 a
Iranian-Balikesir	0,74 c	495,28 a	5188,27 c	1552,08 b	1,22 c	5,80 c	20,78 b	15,31 c	240,20 b	142,33 b	0,040 b
Mean	0,83	438,44	5856,57	1637,49	2,23	7,80	22,53	17,76	275,01	147,13	0,046
DS x G	**	**	**	**	**	**	**	*	**	**	*

Letters show the mean values of different groups in each column. ^{ns}: non-significant* $p \leq 0.5$, ** $p \leq 0.01$, ***$p \leq 0.001$, as indicated by the Tukey's HSD test.

Table 4(on next page)

Comparison of phenolic compounds of diseased/healthy garlic genotypes

Table 4. Comparison of phenolic compounds of diseased/healthy garlic genotypes.

Organic Acids	Diseased Genotypes			Healthy Genotypes			
	Local-Konya	Babaeski-Kirklareli	Iranian-Balikesir	Local-Konya	Babaeski-Kirklareli	Iranian-Balikesir	Mean
Chlorogenic acid	210,99 a	220,46 a	88,35 b	149,78 a	75,48 c	137,60 b	**147,110**
Catechin hydrate	nd	nd	nd	nd	276,43 a	252,55 b	**88,163**
Caffeic acid	nd	0,91 a	0,36 b	3,01 a	1,34 b	1,32 b	**1,157**
4-hydroxy benzoic acid	nd	nd	nd	nd	nd	0,36 a	**0,060**
Vanillin	nd	nd	nd	0,23 a	nd	nd	**0,038**
Routine	nd	nd	nd	nd	nd	nd	**0,000**
trans-ferulic acid	nd	nd	nd	nd	nd	nd	**0,000**
Hydroxy cinnamic acid	nd	nd	nd	0,69 b	0,67 b	5,19 a	**1,092**
Naringin	nd	2,04 a	0,00	nd	nd	nd	**0,340**
orto-coumaric acid	nd	0,13 a	0,01 b	nd	0,10 a	0,03 b	**0,045**
Rosmarinic acid	2,23 a	nd	nd	nd	nd	nd	**0,372**
Salicylic acid	nd	nd	nd	nd	nd	nd	**0,000**
Resveratrol	0,05 a	0,04 a	0,04 a	0,14 b	0,17 b	0,46 a	**0,153**
Quercetin	1,23 a	1,89 a	0,00	nd	1,61 b	2,74 a	**1,245**
trans-cinnamic acid	nd	nd	nd	nd	nd	3,92	**0,654**
Naringenin	nd	nd	nd	nd	nd	nd	**0,000**
Chrysin	3,62 b	28,07 a	0,00	3,62 a	1,47 b	nd	**6,632**
Flavones	nd	nd	nd	4,04 b	9,76 a	nd	**2,300**
Mean	**12,118**	**14,086**	**4,931**	**9,288**	**20,391**	**22,454**	

Letters indicate the mean values of different groups in each row. nd: not detected.

Table 5(on next page)

Comparison of organic acid compounds of diseased/healthy garlic genotypes

Table 5. Comparison of organic acid compounds of diseased/healthy garlic genotypes.

Organic Acids	Diseased Genotypes			Healthy Genotypes			Mean
	Local-Konya	Babaeski-Kirklareli	Iranian-Balikesir	Local-Konya	Babaeski-Kirklareli	Iranian-Balikesir	
Oxalic acid	nd	4,01 b	7,01 a	15,17 a	nd	10,56 b	**6,12**
Citric acid	4322,72 a	4175,95 b	3654,26 c	4781,87 c	6291,02 a	5498,69 b	**4787,41**
Tartaric acid	1100,43 a	nd	nd	219,92 b	377,59 a	nd	**282,99**
Malic acid	nd	nd	nd	nd	305,87 a	274,54 b	**96,73**
Succinic acid	9281,79 b	1386,63 a	580,82 c	11579,81 a	1159,36 b	951,96 c	**4156,73**
Lactic acid	nd	13639,16 a	11168,82 b	0,00	20840,81 a	2031,90 b	**7946,78**
Formic acid	nd	nd	nd	147,37 a	0,00	176,03 a	**53,90**
Acetic acid	nd	nd	nd	1455,32 b	3675,93 a	nd	**855,21**
Fumaric acid	5,18 a	nd	nd	8,02	nd	nd	**2,20**
Propionic acid	292,11 b	353,52 a	262,93 c	272,63 c	795,44 a	483,47 b	**410,02**
Isobutyric acid	nd	nd	nd	nd	nd	nd	**0,00**
Butiric acid	nd	892,23 a	nd	nd	nd	nd	**148,70**
Mean	**1250,19**	**1704,29**	**1306,15**	**1540,01**	**2787,17**	**785,60**	

Letters indicate the mean values of different groups in each row. nd: not detected.

Figure 1

Changes in (a) total phenolic content, (b) antioxidant activity, (c) protein content (mg/kg) and (d) total sugar content of garlic genotypes. Values are mean ± standard deviation. Different letters indicate statistically significant differences ($p \leq 0.05$)

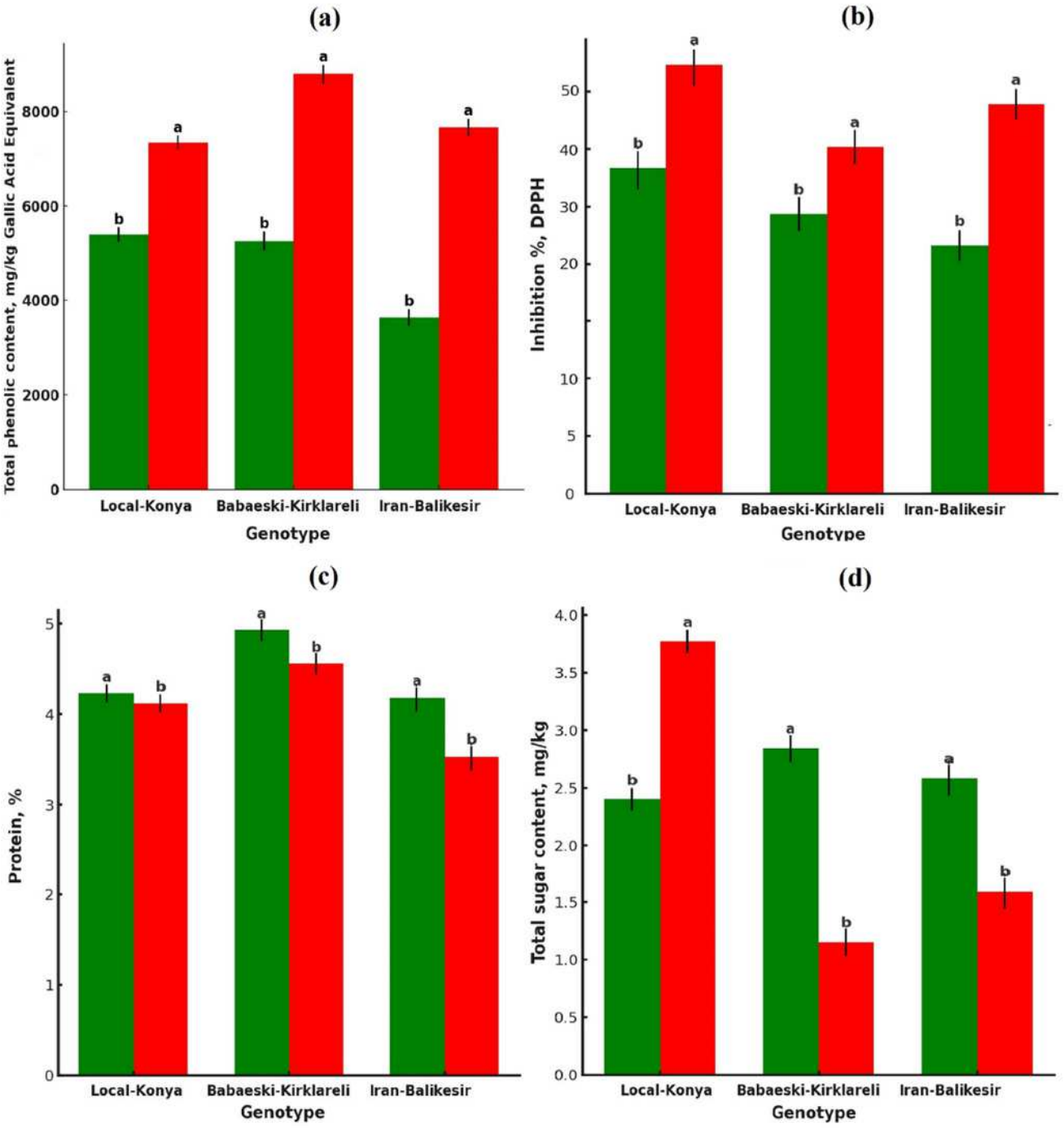
(a)
Total phenolic content, mg/kg Gallic Acid Equivalent
0
2000
4000
6000
8000
a
b
a
b
a
b
Local-Konya
Babaeski-Kirklareli
Iran-Balikesir
Genotype
(b)
Inhibition %, DPPH
0
5
10
20
30
40
50
a
b
a
b
a
b
Local-Konya
Babaeski-Kirklareli
Iran-Balikesir
Genotype
(c)
Protein, %
0
1
2
3
4
5
a
b
a
b
a
b
Local-Konya
Babaeski-Kirklareli
Iran-Balikesir
Genotype
(d)
Total sugar content, mg/kg
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
a
b
a
b
a
b
Local-Konya
Babaeski-Kirklareli
Iran-Balikesir
Genotype

Figure 2

Line chart variation graph of organic acids of healthy and diseased garlic genotypes. Values are mean ± standard deviation.

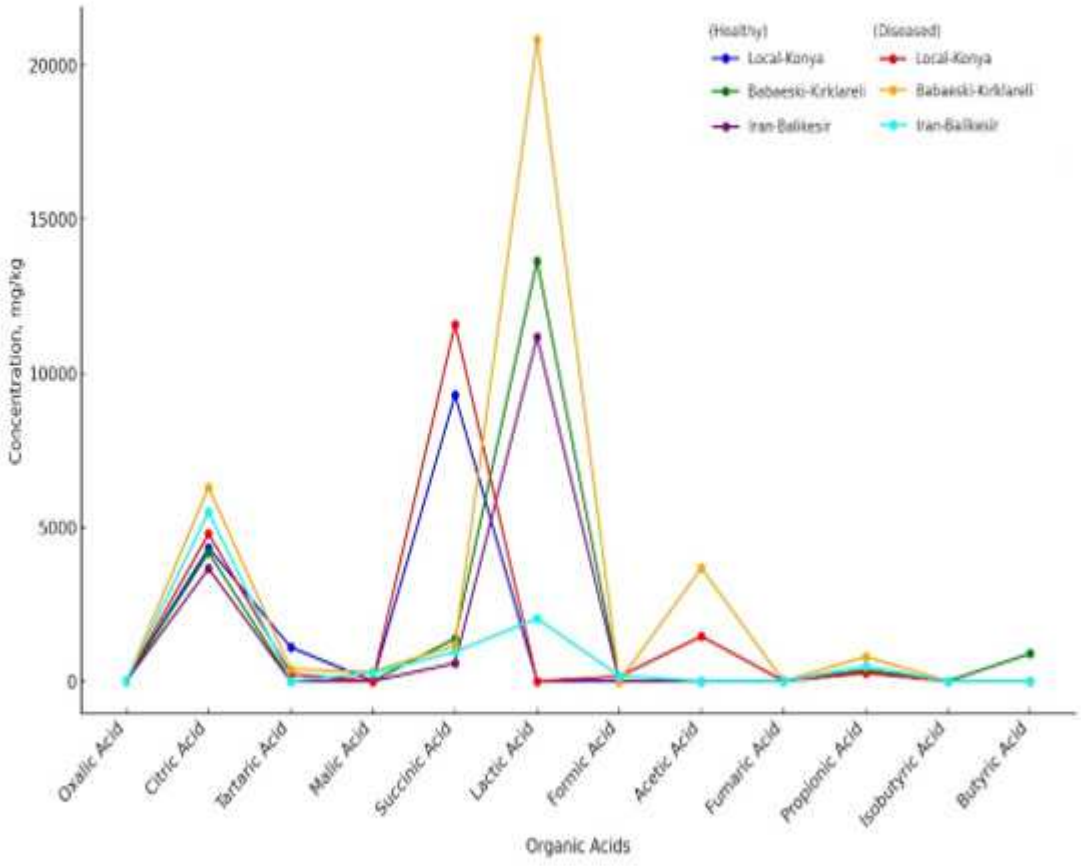

Figure 2. Line chart variation graph of organic acids of healthy and diseased garlic genotypes. Values are mean ± standard deviation.

Figure 3

Heatmap plot of macro- and microelements of garlic genotypes. Values are mean ± standard deviation.

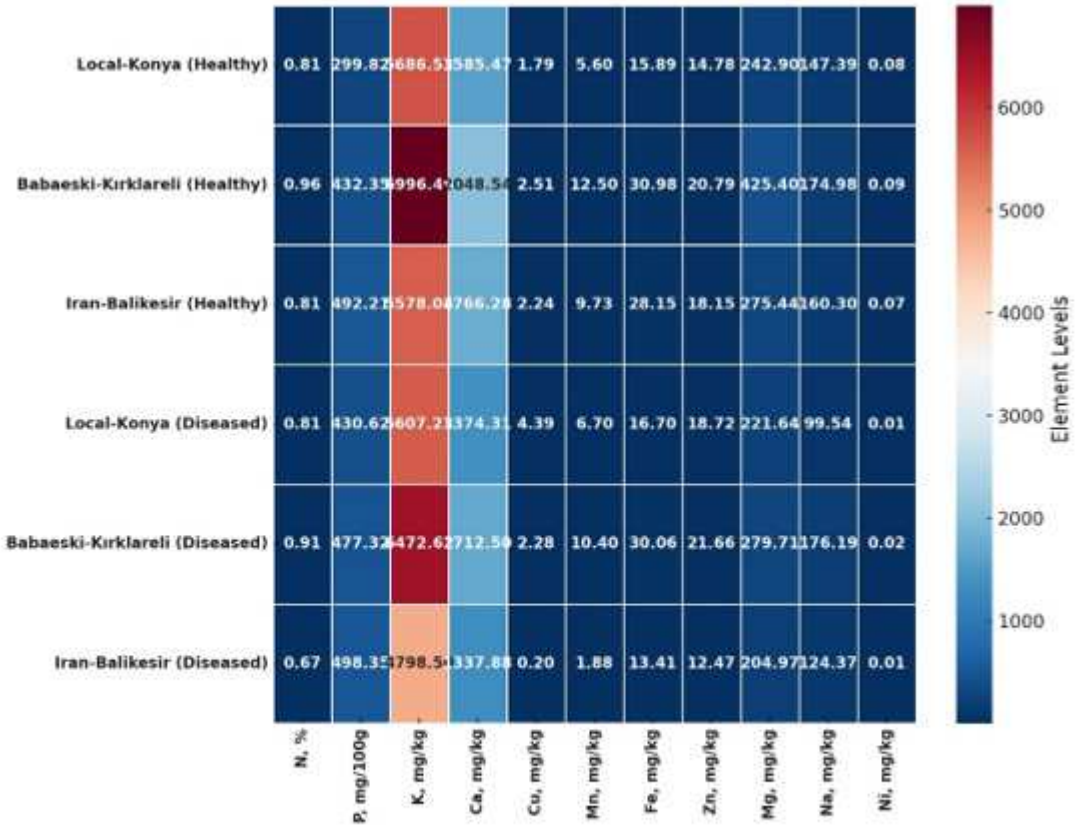

Figure 3. Heatmap plot of macro- and microelements of garlic genotypes. Values are mean ± standard deviation.

Figure 4

Principal component analysis (PCA) results of the phenolic contents of the analyzed genotypes according to disease/health status.

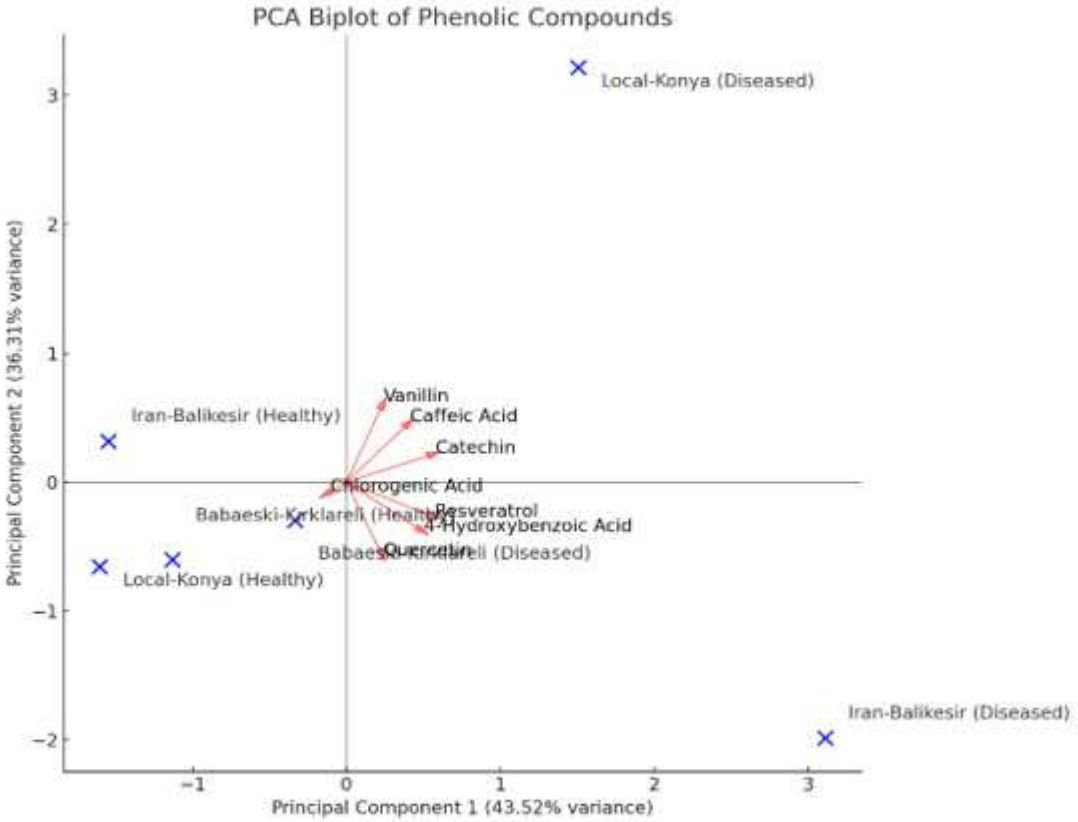

Figure 4. Principal component analysis (PCA) results of the phenolic contents of the analyzed genotypes according to disease/health status.

Figure 5

The Pearson correlation analysis results of analyzed parameters according to the status of different garlic genotypes. Each cell depicts the value of the correlation coefficient (r) between two components and is positive or negative.

(Red) and (Shade of Red) represent positive correlation

(Blue) and (Shade of Blue) show negative correlation

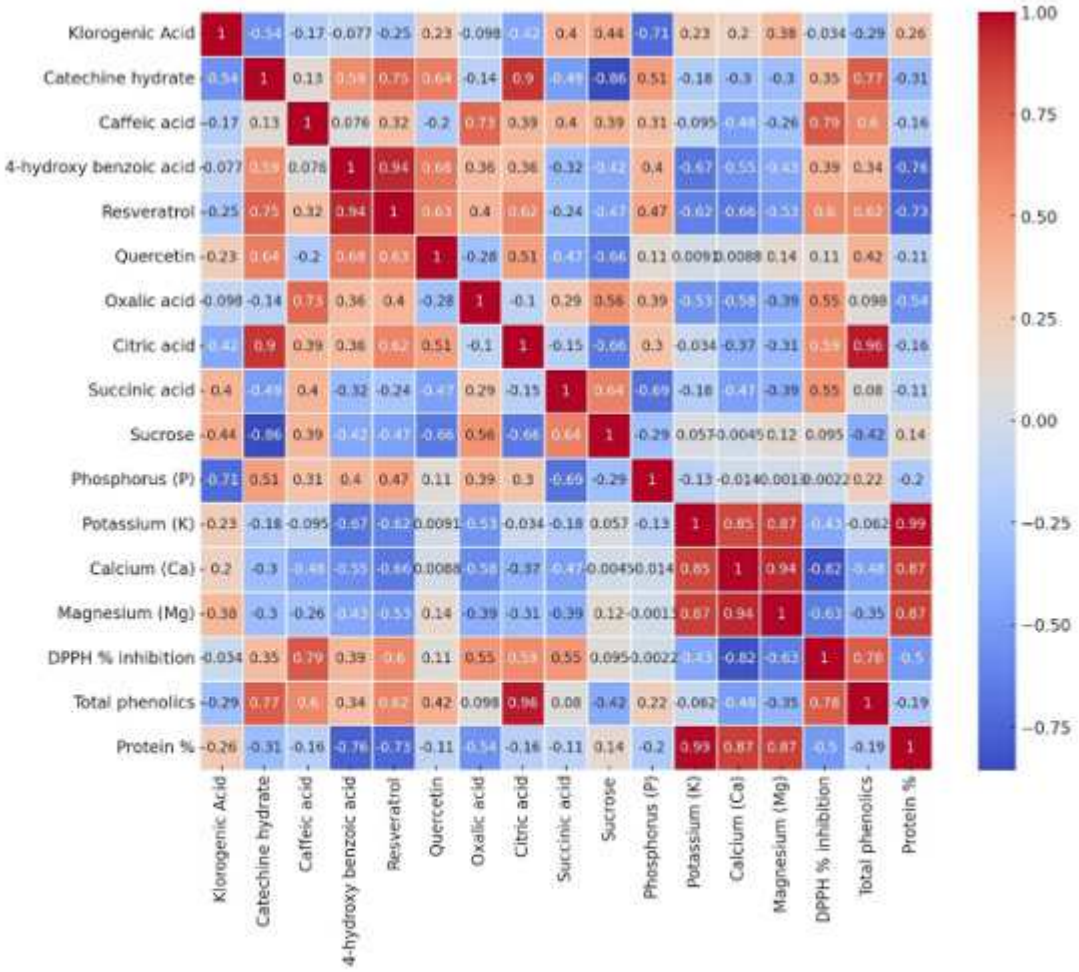

Figure 5. The Pearson correlation analysis results of analyzed parameters according to the disease/health status of different garlic genotypes. Each cell depicts the value of the correlation coefficient (r) between two components and is positive or negative. The colors red and the shade of red represent positive correlations with parameters, while blue and the shades of blue show negative ones.