

The Effect of *Cuscuta babylonica* Aucher on Chemical Compounds of Lice Tomato

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ABSTRACT

Tomato (*Lycopersicon esculentum* L.) is one of the most widely used products in nutrition. But this plant suffers significant loss of yield due to parasitic plant infection. *Dodder* (*Cuscuta* spp.), a flowering plant belonging to the *Convolvulaceae* family, is one of the most harmful parasites for tomatoes. Although some tomato varieties have developed resistance to this, *Cuscuta* is the most important disease that has a negative impact on tomato yield. In this study, it was investigated that how Lice tomato is affected by *Cuscuta babylonica* Aucher infection and whether it develops resistance to it. For this purpose, following *Cuscuta* infection, the amount of fatty acids, calcium (Ca²⁺) and some phenolic compounds in the leaves of the tomatoes were investigated. The results of the study showed that the *Cuscuta* infection increased the amount of 16:1, 18:0 and 18:1 fatty acids, Ca²⁺ and some phenolics such as chlorogenic acid, rutin, quercetin and salicylic acid which are effective in defense against pathogens.

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Cuscuta babylonica Aucher'in Lice Domates Genotipinin Kimyasal Bileşiklerine Etkisi

ÖZET

Domates (*Lycopersicon esculentum* L.) beslenmede en çok kullanılan ürünlerden biridir. Fakat, parazit bitki saldırısına bağlı olarak önemli verim kaybına uğramaktadır. *Convolvulaceae* familyasına ait çiçekli bir bitki olan küsküt (*Cuscuta*), domates için en zararlı parazitlerden biridir. Her ne kadar bazı domates çeşitleri buna karşı direnç geliştirmiş olsalar da küsküt, domates verimi üzerinde olumsuz etkisi olan en önemli zararlıdır. Bu çalışmada, Lice domates genotipinin *Cuscuta babylonica* Aucher enfeksiyonundan nasıl etkilendiği ve buna karşı bir direnç geliştirip geliştirmediği araştırılmıştır. Bunun için enfeksiyondan sonra bitkinin yapraklarında bulunan yağ asitleri, kalsiyum (Ca²⁺) ve bazı fenolik bileşiklerin miktarları araştırılmıştır. Çalışmanın sonuçları, küsküt enfeksiyonunun patojenlere karşı savunmada etkili olan: 16:1, 18:0 ve 18:1 yağ asitleri, kalsiyum (Ca²⁺) ve klorojenik asit, rutin, kuersetin ve salisilik asit gibi fenolik bileşiklerin miktarının arttığını göstermiştir.

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INTRODUCTION

Tomato (*Lycopersicon esculentum* L.) is the second most consumed vegetable in the world after potatoes. Tomatoes are rich in vitamins like A, C, K, B6 and folate and thiamine and minerals like potassium, manganese, magnesium, phosphorus and copper. It also contains dietary fiber, protein and organic components such as lycopene, polyphenolic compounds and phenolic acid which are very important for human health (Bergougnoux 2014; Dūma et al., 2018). Due to

the above mentioned features, tomato consumption continues to increase every year. In fact, global tomato production has increased by about 300% in the last forty years.

Despite some diseases, pests and parasitic plants occurring at different stages of vegetation cause significant losses in crops. Parasitic plants have immense negative effects on the yield of tomatoes. One of the most well-known and agriculturally damaging parasitic plants is the genus *Cuscuta* belonging to the

Convolvulaceae family. For example, this parasitic plant can cause yield loss of up to 72% in tomatoes (Marambe et al., 2002). *Cuscuta* species, which are common in almost all over the world, do not have roots and leaves. The physical connection between the parasite and the host plant is established through an organ called the haustorium, which connects the vascular system of both plants. *Cuscuta* is connected to the xylem and phloem of host plants and absorbed organic nutrients, water and minerals via its haustorium (Marvier, 1996).

Research has shown that plants have two important signaling pathways in their defense systems. Systemic resistance due to salicylic acid (SA) which is the path of resistance to pathogen attacks and resistance pathway due to jasmonic acid (JA) which is effective against herbivores (Jones and Dangl, 2006; Palmer et al., 2017).

Tomato is one of the few plants with active resistance to dodder. A hypersensitivity like response occurs in response to dodder, resulting in an increase in calcium (Ca^{2+}), salicylic acid (SA) and jasmonic acid (JA) contents (Jones and Dangl, 2006; Kaiser et al., 2015). Calcium acts as an active second messenger for plants to respond to biotic and abiotic stress.

The amount of SA synthesized in response to pathogen attack is regulated by a mechanism directed by the calcium-calmodulin complex (Seybold et al., 2014; Aldon et al., 2018). It has long been known that salicylic acid plays a central role in plant pathogen interactions. Genetic studies have shown that some resistance genes involved in defense require SA to activate. In addition, it has been reported that SA, which plays a central role in the resistance mechanism of tomato against pathogens, suppresses the synthesis of jasmonic acid and suspends the associated reactions (Pena-Cortés et al., 1993; Runyon et al., 2010). Foliar application of SA has been reported to stimulate the synthesis of many proteins. The accumulation of SA is essential for both local defense responses to the pathogen in plant tissues and for the establishment of systemic acquired resistance (SAR) (Koç and As, 2008).

Lice tomato is one of important genotypes which is consumed fondly in Southeast Anatolia Region. As a result of ecological conditions and production method, one of the most important plant protection problems seen in Lice tomato plantation areas is weeds, including dodder (Özaslan and Kendal, 2014).

Previous studies have shown that some tomato varieties develop resistance to dodder and thus prevent some of the yield loss.

In this study, it investigated that how Lice tomato cultivar acts against *C. babylonica* infection and whether it develops a certain resistance and how fatty acids, calcium and phenolic compounds which are involved in resistance formation mechanism are

affected by *Cuscuta* infection.

MATERIAL and METHOD

Materials

In this study, the seed of tomato (*Lycopersicon esculentum* L.) that collected from Lice district and *Cuscuta* plants (*Cuscuta babylonica* Aucher) that collected from Dicle University vicinity were used. The study was conducted at August 2018, in a growth room of Biotechnology Laboratory, Dicle University.

Method

Germination of seeds

The tomato seeds were soaked in water for one day and they were planted in pots filled with soil, fertilizer and sand mixture. The seeds were germinated on 5-6 days. When seedlings were grown at about 4-5 cm height, they were transferred to separate pots and kept in growth room with light intensity of 3000 lux for 16 hours day (25-27 ° C) and 8 hours dark (16-18 ° C).

The *Cuscuta* seeds were left in concentrated sulfuric acid for 30 minutes for hard seed coat weakens. It was then washed with tap water and placed in a container of moist filter paper and stored in refrigerator at 4 ° C for 15 days. At the end of this period, they were removed from the refrigerator and germinated in damp filter papers on 5-6 days at room temperature.

Infection of Tomato Plants with *Cuscuta*

The germinated dodder was brought into contact with the leaves of the tomato plant and wrapped. The attacked tomato leaves were harvested 10 days after the start of the infection, dried at room temperature and stored in the refrigerator.

Fatty Acid Analysis

Chloroform/methanol (2:1) was added to the milled plant samples and kept in the dark for three days and the lipids were extracted. The solvent in the filtered samples was evaporated in the evaporator until 1 ml remained. Overall, 4 ml of methanol and 4-5 drops of sulfuric acid were added to the homogenate and methylated in the reflux system for two hours. After methylation, the homogenate was extracted three times with 5 ml of hexane. The hexane was evaporated to 1 ml thick and transferred to brown vials to prevent light exposure and stored in the refrigerator.

Gas Chromatography Conditions

Fatty acid methyl esters of Lice tomato leaves were analyzed by gas chromatography (AtiUnicam 610) with a flame ionization detector (FID) and capillary column (Quqdrex, 007-23 (78% cyanopropyl) methyl polysiloxan capillary column [column length, 30 m; inner diameter, 0.25m). Fatty acid methyl esters

analyzed using a temperature program. Fatty acid methyl esters were chromatographed using a temperature program. It was set Column starting temperature 100 °C, final temperature 260 °C, ramp 5

°C min⁻¹. The exit rate of gases; nitrogen + makeup, 30 ml min⁻¹; dry air, 330 ml min⁻¹. Injection was performed with split (40: 1), 1 µg. nitrogen was used as the carrier gas.



Şekil 1. Domates bitkisinin Kusküt ile enfeksiyonu
Figure 1. Infection of Tomato plants with Cuscuta

Calcium (Ca²⁺) Analysis

Dried leaf samples (0.5 g) were placed in Berghof MWS-3 microwave tubes. Then 6 ml HNO₃ (65%) and 4 ml H₂O₂ were left on. The gas was allowed to stand until exhaustion was concluded. The lids of the containers were closed and left to microwave resolution device and worked according to the program given below. Samples were completed to 50 ml and measured in atomic absorption device.

Unicam 929 atomic absorption device was used for calcium analysis. The standard solutions (2 ppm, 4 ppm, 8 ppm and 16 ppm) were prepared from 1000 ppm (mg ml⁻¹) Ca stock solution and measurements were taken at 422.7 nm. The calibration curve against absorption was plotted and the samples were read.

Phenolic Compound Analysis

Extraction of phenolic compounds

The dried leaf samples (200 mg) were taken to 10 mL of methanol (80% v:v) and then sonicated for 20 min (Sanyo MSE-Soniprep 150, UK). Sonicated samples were centrifuged for 5 min. (Thermo Scientific Labofuge, 200). The supernatant fraction (100 µL) was taken from and completed to 1000 µL with methanol and filtered through a 0.22 µm nylon filter and delivered to LC-MS/MS.

LC-MS / MS device

On the phenolic compound analysis, the LC-MS/MS system consists of Shimadzu Nexera model UHPLC and Shimadzu LCMS 8040 triple quadrupole mass spectrometer was used. It consists of LC-30 AD model gradient pump, DGU-20A3R model degaser, CTO-10ASvp model column furnace and SIL-30AC model auto sampler. Chromatographic separation was

performed on the Agilent Poroshell 120 (EC-C18 2.7 µm, 4.6 mm × 150 mm) column. The triple quadrupole mass spectrometer is equipped with an electrospray ionization (ESI) source operating in both negative and positive mode. LC-ESI-MS / MS data were collected and processed with LabSolutions (Shimadzu, Kyoto, Japan) software registered on the instrument (Akdeniz, 2018).

Statistical Analyses

The percentages of fatty acid were compared by one-way ANOVA. Differences between means were assessed by Tukey HSD test (p < 0.05). Each treatment contained three independent replicates. For the phenolic compound analysis, to evaluate and quantify the uncertainty sources of the applied LC-MS/MS method were performed according to earlier reports (Akdeniz, 2018; Ertas et al., 2014).

RESULTS and DISCUSSION

Effects of *Cuscuta* Infection on The Fatty Acid Components of Tomato

The leaves of the Lice tomato were harvested to determine the effect of infection on the fatty acid components, after 10 days. Fatty acid content of infected and control group tomato plants are given in Table 1.

The abundant fatty acids in both control and infected plants were linolenic acid (48.86%), palmitic acid (23.12%) and linoleic acid (13.14%). The results of the present study demonstrate that, statistically increases were observed in palmitoleic, heptadecanoic, stearic and oleic acids compared to control (Table 1.).

Increases in the amounts of some free fatty acids in plants are observed in response to various stress

factors. It was found that the cuticle layer in the area of infection was strengthened after the *Cuscuta* infection. Since the cuticle layer originates from the

fatty acids of sixteen and eighteen carbons, the increase in the amount of these fatty acids seems to be a natural result (Kachroo et al., 2005).

Tablo 1. Küsküt Enfeksiyonu sonrası Lice domatesi yapraklarındaki yağ asidi bileşenleri
Table 1. Fatty acid components of Lice tomato leaves after Cuscuta infection.

Fatty Acid%	Miktar(%) Quantity(%)	
	Control	Infected
14:0 (Myristic acid)	0.74±0.02 ^a	0.73±0.02 ^a
15:0(Pentadecanoic acid)	0.88±0.03 ^a	0.58±0.02 ^b
16:0 (Palmitic acid)	23.12±0.67 ^a	23.67±0.68 ^a
16:1(Palmitoleic acid)	1.01±0.03 ^a	1.38±0.04 ^b
17:0 (Heptadecanoic acid)	4.42±0.13 ^a	4.88±0.14 ^b
18:0 (Stearic acid)	5.08±0.15 ^a	5.56±0.16 ^b
18:1 (Oleic acid)	1.29±0.04 ^a	2.91±0.08 ^b
18:2 (Linoleic acid)	13.14±0.38 ^a	12.14±0.35 ^b
18:3 (Linolenic acid)	48.86±1.41 ^a	46.69±1.34 ^b
20:1 (Eicosenoic acid)	1.44±0.04 ^a	1.43±0.04 ^a

Each data is the average of three replicates. Differences between the means indicated by different letters in the same line are significant (P <0.05).

Results of this study indicated that the amounts of oleic acid increased on the infected tomato plants after dodder infection. It is known that the oleic (18:1), linoleic (18:2) and linolenic (18:3) acids are also fatty acids involved in defense against pathogens (Walley et al., 2013; Furuhashi et al., 2014). In particular, oleic acid (18: 1) levels regulate plant defense response to pathogens, including programmed cell death and systemic defense mechanisms (Upchurch, 2008). Similarly, in a study conducted with tomato plants, after the bacteria attack, an increase in oleic (18:1) and linoleic (18:2) acid amounts was observed in these plants (Kolomiets et al., 2016). According to this result, it can be said that Lice tomato cultivar developed a resistance mechanism against *C. babylonica*.

Our study showed that the amount of linolenic acid (the precursor of JA) was not increased, this result may be explained by the absence of JA.

The Effect of *Cuscuta* Infection on the Calcium (Ca²⁺) contents of Tomato

Calcium is an important secondary precursor that serves as signal transduction. For this reason, the amount of calcium in tomato during *Cuscuta* infection was also measured (Table 2).

Based on results, the amount of Ca²⁺ also increased on the 10th day (44.47ppm) of the *Cuscuta* infection compared to the control (31.91ppm). Similarly, increases in the amount of free calcium after the attack have been observed in studies conducted with tomato and *Cuscuta* (Albert et al., 2010; Lecourieux et al., 2006). It is known that the Ca²⁺, acts as a secondary messenger in the synthesis of defense compounds and increased Ca²⁺ content has been suggested to activate the SA synthesis pathway (Albert et al., 2010; Li and Zou, 2017). Therefore, increasing the amount of Ca²⁺

during infection in plants containing SA resistance pathway is a naturally expected outcome and seems to be in agreement with our findings. It has also been reported that calcium in tomato leaves may be increased since it is used to strengthen the cell wall after infection (Albert et al., 2004; Goldwasser et al., 2001).

Tablo 2. Küsküt enfeksiyonu sonrası Lice domatesi yapraklarındaki Ca²⁺ içeriği
Table 2. Ca²⁺ content of Lice tomato leaves after Cuscuta infection

Ca ²⁺ (ppm)	Miktar (ppm) Quantity (ppm)	
	Control	Infected
Ca ²⁺ (ppm)	31.91±0.92 ^a	44.47±1.28 ^c

Each data is the average of 3 replicates. Differences between the means indicated by different letters in the same line are significant (P <0.05).

The Effects of *Cuscuta* Infection on Phenolic Compounds of Tomato

The effect of *Cuscuta* infection on phenolic contents of Lice tomato leaves was also determined on 10th day (Table 3).

The chlorogenic acid and rutin were the quantitatively dominant compounds in the both plant groups. Besides, significant increases in chlorogenic acid, hyperoside, rutin, quercetin, and salicylic acid amounts in tomato plant were observed on the 10th day of the attack compared to control. The amount of hesperidin compound was lower than control.

It is observed that the 9-11 days after dodder infection, browning was observed in the area where the haustorium infected to the tomato stem. Additionally, the amount of phenolic compounds especially chlorogenic acid was also increased in this period.

Tablo 3. Küsküt Enfeksiyonu sonrası Lice domatesi yapraklarındaki fenolik bileşik içerikleri
 Table 3. Phenolic compound contents of Lice tomato leaves after *Cuscuta* infection

Phenolic compounds	Miktar ($\mu\text{g kg}^{-1}$)	
	Quantity ($\mu\text{g kg}^{-1}$)	
	Control	Infected
Hesperidine	3.41±0.10 ^a	3.27±0.09 ^c
Chlorogenic acid	7.32±0.21 ^a	10.36±0.30 ^b
Hyperoside	1.57±0.05 ^a	2.69±0.08 ^c
Rutin	14.04±0.40 ^a	14.67±0.42 ^a
Quercetin	0.04±0.00 ^a	0.13±0.01 ^c
JA	ND	ND
SA	0.13±0.01 ^a	0.31±0.01 ^{cc}

ND: Not detected. Each data is the average of three replicates. Differences between the means indicated by different letters in the same line are significant ($P < 0.05$).

Studies have shown that compounds that increase the synthesis of tomato plants against various pathogens help to defend (Beimen et al., 1992). Quercetin, rutin and campferol are some phenolic compounds commonly found in tomatoes (Stewart et al., 2000; Barros et al., 2012). It has been reported that the amount of chlorogenic acid is widely increased in tomato plants against dodder and other biotic pest infections (Sahm et al., 1995; Wojciechowska et al., 2014).

SA is an important component of the resistance mechanism developed by plants and tomato in particular. The results of the LC-MS/MS analysis showed that the amount of SA increased (2.3-fold), but JA was not detected in infected plants. SA and JA serve as defense compounds against parasitic plant infection (Lattanzio et al., 2006; Runyon et al., 2010). SA and JA are antagonistic to each other and one suppresses the synthesis of the other. Therefore, it is considered normal that JA could not be detected (Thaler et al., 2002). In some studies, examining the effects of dodder attack on tomato, resistance pathways related to SA have been developed (Jones and Dangl, 2006; Kaiser et al., 2015).

CONCLUSION

The studies have shown that the increase of some compounds on the infection time was helpful to defend systems. Data of this study revealed that the amount of fatty acids, Ca^{2+} , and phenolic compounds chlorogenic acid, hyperoside, rutin, quercetin and SA increased in tomato leaves that infected with dodder on the 10th day.

According to the results of this study, it can be said that Lice tomato genotype has developed resistance against *C. babylonica* by means of these compounds and SA-linked resistance pathway.

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Statement of Conflict of Interest

Authors have declared no conflict of interest.

Author's Contributions

The contribution of the authors is equal.

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