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# The Effect of Salicyclic Acid and Endomycorrhizal Fungus *Glomus etunicatum* on Plant Development of Tomatoes and Fusarium Wilt Caused by *Fusarium oxysporum* f.sp *lycopersici*

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**Abstract** : The effect of salicylic acid (SA) and *Glomus etunicatum* (GE) on plant development of tomatoes and infection potential of wilt disease *Fusarium oxysporum* f.sp *lycopersici* (Fol) were studied. The effects of different SA concentrations on mycelial development of Fol were tested in vitro and two concentrations of SA and GE were included in pot experiment. SA completely inhibited the mycelial development of Fol in vitro at concentrations from 0.6 mM to 1.0 mM and ED50 value was found as 0.51 mM. GE could increase dry weight of plant, length of shoot and root growth irrespective whether Fol infected the tomato plants. The root colonization by GE was determined as 62.3% when the Fol was absent and as 53.2% when the plants were infected. However, in different combinations of GE and SA, the root colonization was determined between 19.1 and 34.2%. In pot experiments, the combination of GE and 1 mM SA had the highest effect on infection of Fusarium wilt and disease severity was reduced by 70%. Results indicate that GE increases the growth of tomato plants, and could be used against Fusarium wilt of tomato. While SA is effective against the pathogen, the root colonization of GE is, however, affected negatively by SA.

Key Words : Tomato, *Fusarium oxysporum* f.sp. *lycopersici, Glomus etunicatum* induced resistance, salicyclic acid

## Salisilik Asit ve Endomikorizal Fungus *Glomus etunicatum*' un Domates Bitkilerinin Gelişimine ve *Fusarium oxyporum* f.sp *lycopersici* Tarafından Neden Olunan Fusarium Solgunluğuna Etkisi

**Özet :** Salisilik asit (SA) ve *Glomus etunicatum* (GE)'un domateslerde bitki gelişmesi ve solgunluk hastalığı etmeni *Fusarium oxysporum* f.sp. *lycopercisi* (Fol)'nin infeksiyon potansiyeli üzerine etkileri araştırılmıştır. Fol'nin miseliyal gelişimi üzerine farklı SA konsantrasyonlarının etkisi in vitro'da test edilmiş ve saksı çalışmalarına SA'in iki konsantrasyonu ve GE dahil edilmiştir. SA, in vitro'da Fol'nin miseliyal gelişmesini 0.6 mM'dan 1.0 mM'a kadar olan konsantrasyonlarda tamamen engellemiş ve ED50 değeri 0.51 mM olarak bulunmuştur. Fol domates bitkilerini infekte etse de etmese de, GE sürgün kuru ağırlığını, sürgün uzunluğunu ve kök gelişimini arttırabilmiştir. GE tarafından kök kolonizasyonu, Fol olmadığında %62.3 ve infekteli bitkilerde 53.2% olarak belirlenmiştir. Ancak, GE ve SA'in farklı kombinasyonlarında, kök kolonizasyonu %19.1-34.2 arasında belirlenmiştir. Saksı denemelerinde, GE ve 1 mM SA kombinasyonu Fusarium solgunluğu üzerine en yüksek etkiyi göstermiş ve hastalık şiddeti %70 oranında azaltılmıştır. Sonuçlar, GE'un domates bitkilerinin büyümesini artırdığını ve domateste Fusarium solgunluğuna karşı kullanılabileceğini göstermektedir. SA patojone karşı etkili olurken, GE'un kök kolonizasyonunu da olumsuz şekilde etkilediği gözönünde tutulmalıdır.

Anahtar Sözcükler : Domates, *Fusarium oxysporum* f.sp. *lycopersici Glomus etunicatum,* dayanıklılığın teşviki, salisilik asit

## Introduction

One of the important diseases of tomatoes is Fusarium wilt caused by *Fusarium oxysporum* f.sp *lycopersici* (Sacc) W.C. Snyder & H.N. Hans and due to the disease, substantial economic losses may occur especially in greenhouses in many tomato growing countries.

Control of the disease is provided by chemical, biological and cultural methods. Although applications of fungicides have helped control plant disease, chemical control is very expensive and environmentally undesirable. In addition, the pathogen has shown resistance against some fungicides. Therefore, using tomato varieties resistant to Fusarium wilt is the only practical measure to control the disease (1). However, the characteristics such as high yield, quality and adaptation to regions have taken into consideration firstly in cropping without regard to resistance to disease. For this reason, the most viable method is to protect the plants against pathogen through inducing resistance

without changing any characteristics. Recently, there are some studies about this subject (2, 3).

It was first reported that, some substances such as potassium- and sodium-2-benzothiazolylthioglycolate, 4-chloro-3,5-dimethyl-phenoxyethanol induced the resistance mechanism of tomato plants and reduced Fusarium wilt symptoms (4, 5). Then, it was determined that, many different plant-growth regulating substances induced resistance of tomato plants against Fusarium wilt (6). SA, a phenolic compound, also regulates plant growth and confers resistance to plants to some viral, bacterial and fungal diseases (7). Therefore, it provides multiple disease protection with only one application.

The roots of most plants are generally infected by vesicular-arbuscular mycorrhizal (VAM) fungi which are beneficial to their host plant (1). Several studies indicated that, VAM fungi influenced plant disease caused by root pathogens. Most of them concluded that, root infections by VAM fungi reduced the disease severity (8, 9, 10, 11).

The objectives of the study were to determine the effect of SA and GE on development of tomato plants and Fusarium wilt and to provide new strategies to control the wilt disease of tomatoes.

## Materials and Methods

### Materials

Susceptible cultivar Ontario 7710 grown commonly in the region was used in this study. Fol was obtained from tomato seedlings showed wilt symptoms. GE, produced on *Medicago sativa* L. was used as mycorrhizal inoculum and the inoculum was consisted of infested soil mixed with root fragment.

### Methods

#### In vitro Test of SA on Mycelial Development of Fol

Different SA concentrations from 0.1 to 1.0 mM were incorporated 100 ml PDA media and poured in 9 cm Petri dishes. A 5 mm diameter agar disc containing fungal mycelium of Fol was transferred to the test medium (six plates for each concentration and control). Then plates were incubated at 25°C in a growth chamber. Colony diameters of Fol were measured daily until control Petri dishes were covered by Fol during incubation period.

#### The Effects of SA and GE on Plant Development and Disease Severity of Fol

For determining the effects of SA and GE on plant development and disease severity of Fol, 50 g soil inoculum mixed with root fragments was incorporated 2-3 cm below the seed before sowing, then tomato seeds were sown (12). The following characters were studied with four replicates per character in the pot experiment conditions.

1) 0.5 mM SA, 2) 1 mM SA, 3) GE, 4) Fol, 5) GE plus 0.5 mM SA, 6) GE plus 1 mM SA, 7) 0.5 mM SA plus Fol, 8) 1 mM SA plus Fol, 9) GE plus Fol, 10) GE, 0.5 mM SA plus Fol, 11) GE, 1 mM SA plus Fol, 12) C: Control

Plants were watered with distilled water during the experiment. At the 6-7 leaf stage, SA stock solutions were prepared in distilled water at 0.5 and 1.0 mM concentrations and tomato seedlings were drenched with different SA concentrations for four days (100 ml/pot). Control plants were treated only with 100 ml distilled water. After 4 days, Fol inoculum, grown in sand-cornmeal culture (13) during 2 weeks was incorporated in each pot (30 g/pot). Experiment was conducted under controlled conditions at 25°C with a photoperiod of 16 h and symptom development was observed.

During the experiment, the length of shoot was measured periodically. Dry weight of shoot, length of root and weight of root were measured after harvesting. Dry weight of shoot was determined after drying plant material at 80°C for 48 hours. Evaluations of effects of SA and GE and the effects of SA on root colonization of GE were done both in the case of absence of Fol and infection of Fol.

Symptoms of the disease were evaluated according to the leaf, vascular discoloration and wilting using 0-4 scale (14). The scale used was:

0: No wilting symptoms

1: Plant slightly wilted, vascular discoloration only in main root region

2: Plant moderately wilted, yellowing of old leaves, spreading of vascular browning

3: Plant severely wilted, dying of all leaves except end leaves

4: Dead plant, seedling entirely wilted

The disease severity was calculated using disease scale values.

For determining the effect of SA on root colonization by GE on the plant inoculated and non-inoculated with Fol, after harvesting, plants roots were washed with tap water and then distilled water carefully. To determine the mycorrhizal colonization of root, clearing and staining were done as described by Koske and Gemma (15). After staining with Trypan blue, endomycorrhizal colonization was estimated using the gridline intersection method (16). Roots placed on a grid of 10 mm divisions and examined under dissecting microscope (40x). Total number of roots and colonized roots by GE were counted and percentage of colonization was determined.

## Results and Discussion

### In vitro Test of SA on Mycelial Development of Fol

The inhibitory effect of SA on the development of Fol increased linearly with increasing concentration. On the 9th day, while the colony diameter of control was 89 mm, it was reduced to 74.5 mm by 0.5 mM SA concentration. However, the mycelial development of Fol was completely inhibited at 0.6 mM SA concentration (Table 1).

Depending on the SA concentration, the data of colony development of Fol were subjected to linear regression analyse and ED50 value was found as 0.51 mM. Candela et al (17) indicated that, SA and related compounds inhibited the development of *Phytopthora capsici* in vitro. On the other hand, except inhibitory effect in vitro, when SA was applied by irrigation or spray, SA had inhibitory effect on development of bacterial speck of tomato (18).

Table 1. Effects of Salicyclic Acid on mycelial development of *Fusarium oxysporum* f.sp *lycopersici* in vitro.

| Concentration of SA (mM) | Means of Colony diameter (mm)** |
|---|---|
| 0.0 (control) | 84.8 f* |
| 0.1 | 81.2 e |
| 0.3 | 78.0 d |
| 0.4 | 76.3 c |
| 0.5 | 69.5 b |
| 0.6 | 0.0 a |
| 0.7 | 0.0 a |
| 0.8 | 0.0 a |
| 1.0 | 0.0 a |

* Mean values within a column are significantly different based on LSD (0.05) test.

** 5 mm colony disc diameter transferred to petri dishes was subtracted from measured values

### The Effects of SA and GE on Plant Development and Disease Severity of Fol

First of all, the effects of two doses of SA, 0.5 and 1.0 mM, GE alone and GE combining with the two SA concentrations on the development of plant were evaluated. Results are shown in Table 2.

The experiment was evaluated in the case of without and with the infection of Fol. In non-infected plants with Fol, plants inoculated with GE only produced the highest shoot dry weight and it was found as 15.4 g. However, GE plus 0.5 mM and 1 mM SA showed lower effect as 11.6 and 8.3 g, respectively. This may be related with the effect of SA on GE colonization of tomato plants. As indicated that, SA and such related compounds have negative effect on plant pathogens and also on vesicular-arbuscular mycorrhizal fungi which have a mutualistic association with plants (19). In addition to this, 0.5 and 1.0 mM SA treatments had no phytotoxic effect on plants. The highest shoot and root length were obtained from GE inoculation and they were found as 125 cm and 34.25 cm, respectively. While other treatments had lower effect than those of GE treatment, shoot length obtained were higher than control plants in all treatments. Similar to that, Tosi et al (20) indicated that, *Glomus mosseae* increased shoot dry weight, shoot length and root length in sunflower seedlings.

When dry weight of shoot evaluated in infected plants, it increased with mycorrhizal inoculation (12.4g) and decreased with pathogen inoculation. This indicate that GE was effective for increasing dry weight of shoot when SA treatments were not applied. However, length of shoot was effected by GE plus 1 mM SA, 1 mM SA and GE only with a high degree as 117.5, 116 and 113.8cm, respectively. Shoot length of Fol infected plants decreased with respect to control plants. After Fol infection, there was no significant effect of GE on the length of root, but the highest weight of root was obtained from both GE alone and its combination with SA. In addition, the lowest weight of root was obtained from Fol treatment only because of the damage of the roots by the pathogen. Other authors have obtained similar results that vesicular-arbuscular mycorrhizal fungi had positive effects on vegetative development of plant (21, 22). However, some studies indicated that, SA induced the plant resistance against pathogen or inhibited pathogen development directly (3, 23, 24, 25).

Table 2. Effects of Salicyclic Acid and *Glomus etunicatum* on development of tomato plants.

| Treatments | Dry weight of plants (g) | Length of shoots (cm) | Length of roots (cm) | Weight of roots (g) |
|---|---|---|---|---|
| in the absence of Fol infection | | | | |
| 0.5 mM SA | 9.7 bc* | 104.3 cd | 26.0 bc | 28.4 a |
| 1 mM SA | 8.7 c | 118.5 ab | 31.0 ab | 17.7 b |
| GE | 15.4 a | 125.0 a | 34.3 a | 21.1 ab |
| GE+0.5 mM SA | 11.6 b | 122.5 ab | 33.0 a | 20.7 ab |
| GE+1 mM SA | 8.3 c | 112.5 bc | 30.0 ab | 18.6 b |
| CONTROL | 9.4 bc | 98.8 d | 23.5 c | 6.5 c |
| in the presence of Fol infection | | | | |
| 0.5 mM SA+Fol | 8.6 bc | 110.3 ab | 32.5 a | 14.0 c |
| 1 mM SA+Fol | 6.7 c | 116.0 a | 24.5 b | 16.6 bc |
| GE+Fol | 12.4 a | 113.8 a | 21.5 c | 21.6 a |
| GE+0.5 mM SA+Fol | 9.8 b | 108.8 ab | 26.8 bc | 20.7 ab |
| GE+1 mM SA+Fol | 10.2 ab | 117.5 a | 26.0 bc | 21.4 ab |
| Fol | 9.4 b | 87.8 c | 17.5 d | 3.6 d |
| CONTROL | 9.4 b | 98.8 bc | 23.5 b | 6.5 d |

*Mean values within a column are significantly different based on LSD (0.05) test.

The effects of SA, GE, SA plus GE on Fol are shown in Table 3.

As it shown in Table 3, the combination of GE and 1 mM SA concentration was the most effective, resulting significant reduction in disease severity and this treatment reduced the disease severity by 70%. It was followed by GE plus 0.5 mM SA and 1 mM SA treatments as 60%. GE only and 0.5 mM SA had lower effect as 40 and 30, respectively. In a previous study, *Glomus mosseae* and GE had a marked effect on Fusarium wilt of tomato by reducing wilt symptoms, vascular invasion and sporulation of the pathogen (10). In another study, root necrosis caused by *Fusarium oxysporum* f.sp *radicis-lycopersici* was prevented in tomato roots previously inoculated with the mycorrhizal fungus *Glomus intraradices* and the population of the pathogen were suppressed in these roots (9)

Vesicular-arbuscular mycorrhizal fungus GE is a good colonizer of roots of many plants. Depending on this characteristic, besides the improving the growth of plant, it makes the roots more resistant to infection of soilborne plant pathogens (10). The roots of tomato plants inoculated with Fol were colonized by GE with 62.3%, on the other hand, in roots of plants with Fol treatment, the rate of colonization was found as 53.2% with the lowest decrease (Table 4). However, in infected or non-infected plants with Fol, exogenously applied SA caused significant reduction on root colonization of GE.

Table 3. Effects of Salicyclic Acid and *Glomus etunicatum* on disease severity of *Fusarium oxysporum* f.sp *lycopersici*.

| Treatments | Disease Severity (%) | % Effects of treatments |
|---|---|---|
| GE+Fol | 37.50 | 40 |
| GE+0.5mM SA+Fol | 25.00 | 60 |
| GE+1mM SA+Fol | 18.75 | 70 |
| 0.5mM SA+Fol | 43.75 | 30 |
| 1mM SA+Fol | 25.00 | 60 |
| Fol | 62.50 | |

Table 4. Effects of Salicyclic Acid on root colonization of *Glomus etunicatum*.

| Treatments | Root Numbers | Colonized Root Numbers by GE | Colonization (%) |
|---|---|---|---|
| in the absence of Fol infection | | | |
| GE | 168.3 | 104.8 | 62.3 a* |
| GE+0.5 SA | 94.3 | 32.3 | 34.2 b |
| GE+1 SA | 123.3 | 23.5 | 19.1 c |
| in the presence of Fol infection | | | |
| GE+Fol | 175.8 | 93.5 | 53.2 a |
| GE+0.5 SA+Fol | 128.5 | 25.0 | 19.5 b |
| GE+1 SA+Fol | 136.0 | 34.8 | 25.6 b |

*Mean values within a column are significantly different based on LSD (0.05) test.

The results of this study showed that SA and GE had a potential for using in the control of Fol of tomatoes. Future studies should be focused on the effect of SA and GE on the control of this disease under greenhouse and field conditions including the yield parameters.

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