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# Effects of *Alternaria alternata* Toxins (AOH, AME and TA) on Apple (*Malus sylvestris* Miller Cv. Golden ) Pollen

*Nazmi Gür, Suat Karamanoglu and Seher Gür*

Department of Biology, Faculty of Arts and Sciences,
University of Fırat, Elazığ, Turkey

**Abstract:** Effects of *Alternaria alternata* metabolites alternariol (AOH), Alternariol Monomethyl Ether (AME) and tenuazonic acid (TA) of Alternaria on *in vitro* pollen germination and tube growth in apple were studied. The effect of different concentrations of toxins on pollen germination and pollen tube growth in apple was investigated. All mycotoxins at 100 ppm concentrations inhibited pollen germination and tube growth completely or almost completely. At 10 ppm the toxins had significant, but much reduced effects. TA was the most effective toxin. These results suggest that *Alternaria alternata* toxins, even at low concentrations, may adversely affect plant reproduction by inhibiting pollen germination and tube growth in apple.

**Key words:** AAL toxin · pollen germination · AME · AOH · TA

## INTRODUCTION

The genus *Alternaria* encompasses both saprophytic and pathogenic species. *Alternaria alternata* is common plant pathogen known to contaminate a wide variety of foods, including fresh or stored fruits, ripe vegetables, nuts and cereal grains grown or stored under wet conditions. *Alternaria alternata* species produces several mycotoxins such as alternariol (AOH), Alternariol Monomethyl Ether (AME) and Tenuazonic Acid (TA). Natural occurrences of AOH, AME and TA have been reported in various fruits, including tomatoes, olives, mandarins, melons, peppers, apples and raspberries [1]. *Alternaria* mycotoxins are important in the disease process, e.g. black spot, seedling chlorisis [2]. Witsenboer et.al. described effects of *A. alternata* toxins on leaves, shoots, leaf disks, calli, suspension cells and protoplasts [3].

*Alternaria* toxins affecting sporophytic plant tissue may also affect pollen germination and growth. Thus, the toxin from *Alternaria brassicicola* has been shown to inhibit pollen germination and pollen tube growth of Brassica [2]. Bino *et al*. showed AAL toxins inhibited both germination and tube growth of pollen from several *Lycopersicon esculantum* genotypes [4]. *Alternaria helianthi* culture filtrate reduced pollen germination and tube growth in seven sunflower genotypes [5]. In the present paper we report effects of *A. alternata* toxins on *in vitro* germination and tube growth of pollen in apple plant, which has economic importance.

## MATERIALS AND METHODS

In this study, pollens of apple flowers (*Malus sylvestris* Miller cv. Golden) were used as material. Flowers were placed in polyethylene containers and the experiments were done immediately in the laboratory. Flowers from the same tree were used in every experiment. Also, flowers were from the same maturity level. Alternariol (Sigma), alternariol monomethyl ether (Sigma), tenuazonic acid (Sigma) and deionised water were used for preparing Alternaria toxin solutions. Thus, toxin values of solutions were as 10-100 ppm (10 different concentrations with 10 ppm increments) deionised water has been used as control (CG).

Pollen was germinated in Brewbaker and Kwack [6] culture solution (culture medium). Alternaria toxin and culture medium at the same volumes were used. Three sterile microscope slides were prepared for each toxin (2 for experimental compounds, 1 for the control). A 50 µl culture solution was dripped to 2 various areas on each slide. 50 µl toxin for experiment groups and 50 µl deionised water for Control Group ( CG ) were added onto slides. Pollen was harvested from anthers homogeneously with a sterile syringe into culture medium under a stereomicroscope. Petri dishes (15 cm diameter) with a

**Corresponding Author:** Dr. Nazmi Gür, Department of Biology, Faculty of Arts and Sciences, University of Fırat, Elazığ, Turkey

moist filter paper lining the lower plate served as an improvised humidity chamber. Two glass rods were placed parallel at about 4 cm apart on the moist filter paper to facilitate the handling of the pollen cultures. Then, the petri dishes were settled in incubator at 22±°C. Each germination medium was fixed with 10% ethyl alcohol after 3 hours [6] and then, lamella were closed. Germination percentages and tube lengths of pollens were determined under light microscope by method of Shivanna and Rangaswamy [6].

All experiments were repeated three times and results were statistically analyzed by calculating variance and the standard error (Sx) of the mean. Statistical analysis was performed based on SPSS (version 10.0) program. In order to detect the significance of differences ($p<0.01$ or $p<0.05$) of variables, a multiple comparison (LSD) test was performed.

## RESULTS

In the germination medium series which contained 50 µl culture solution and 50 µl toxin pollen germination and tube length are given Table 1-3. AOH, AME and TA toxins inhibited both germination and tube length of apple pollen.

TA caused the highest toxin effect, whereas pollen germination and tube growth rate was less affected by AME (Table 1-3). Significant differences ($P<0.05$) are observed between all *A. alternata* toxin concentrations on apple pollen germination and tube growth Table 1-3).

Pollen germination at this range was determined 68.3±2.0% (10 ppm AOH) maximum and 0.6±0.1% (100 ppm AOH) in AOH toxin (Table 1). Although pollen germination was decreased 30% at 10 ppm AOH to CG, this was statistically significant ($P<0.05$). A decrease (98.4%) has been determined for tube elongation at 100 ppm AOH to 10 ppm AOH. Table 2 shows that both pollen germination and tube growth were affected by the application of AME in increasing with the toxin concentrations. Significant differences ($P<0.05$) are observed between treatments at all AME concentrations on pollen germination and tube growth. AME was more effective on pollen of apple than AOH. TA was determined to most reduce the pollen germination rate and tube growth on apple (Table 3). The pollen germination and tube growth completely stopped at 100 ppm TA concentration. Growth of pollen was decreased 96.8% at 90 ppm TA compared to 10 ppm TA. The tube growth of

Table 1: The effects of Alternariol (AOH) on pollen germination and tube growth of apple (*Malus sylvestris* Miller cv. Golden)

| Treatment (ppm) | Germination (%) | Tube length (m) |
|---|---|---|
| CG | 97.7±1.4a | 555.0±21.7a |
| 10 | 68.3±2.0b | 436.6±16.0b |
| 20 | 44.3±3.2c | 343.3±20.2c |
| 30 | 34.6±3.5d | 245.0±15.1d |
| 40 | 25.6±3.0e | 201.6±7.6e |
| 50 | 18.0±2.6f | 148.3±10.4f |
| 60 | 13.3±1.1g | 108.3±7.6g |
| 70 | 5.6±2.0h | 61.3±6.1h |
| 80 | 2.0±0.3i | 35.0±5.0i |
| 90 | 1.3±0.2i | 15.0±5.0i |
| 100 | 0.6±0.1j | 6.6±2.8j |

P<0.001 Significant differences (P<0.05) are indicated by different letters according to multiple range test (Duncan)

Table 2: The effects of Alternariol Monomethyl Ether (AME) on pollen germination and tube growth of apple (*Malus sylvestris* Miller cv. Golden)

| Treatment (ppm) | Germination (%) | Tube length (m) |
|---|---|---|
| CG | 97.7±1.0a | 555.0±20.0a |
| 10 | 87.6±2.5b | 491.6±20.8b |
| 20 | 58.8±1.2c | 343.3±24.4c |
| 30 | 43.5±1.4d | 291.6±12.2d |
| 40 | 28.4±2.4e | 216.6±10.8e |
| 50 | 17.8±1.3f | 143.3±10.0f |
| 60 | 15.2±1.1g | 115.0±11.2g |
| 70 | 9.2±0.9h | 90.0±9.1h |
| 80 | 7.9±0.8i | 60.0±5.5i |
| 90 | 2.8±0.3i | 33.0±2.7i |
| 100 | - | - |

P<0.001 Significant differences (P<0.05) are indicated by different letters according to multiple range test (Duncan)

Table 3: The effects of tenuazonic acid (TA) on pollen germination and tube growth of apple (*Malus sylvestris* Miller cv. Golden)

| Treatment (ppm) | Germination (%) | Tube length (m) |
|---|---|---|
| CG | 97.7±1.0a | 555.0±20.0a |
| 10 | 92.1±1.4b | 480.0±15.0b |
| 20 | 74.3±1.5c | 338.3±15.2c |
| 30 | 56.0±3.0d | 226.6±12.5d |
| 40 | 37.3±1.5e | 178.3±20.2e |
| 50 | 22.0±1.0f | 130.0±15.0f |
| 60 | 18.1±0.7g | 110.0±11.4g |
| 70 | 15.6±0.6h | 90.0±5.0h |
| 80 | 10.3±0.6i | 70.0±8.0i |
| 90 | 6.0±0.2i | 46.6±4.6i |
| 100 | 1.4±0.3j | 25.0±5.0j |

P<0.001 Significant differences (P<0.05) are indicated by different letters according to multiple range test (Duncan)

pollen at this toxin showed smilar results to the growth of pollen. Tube growth at 90 ppm was decreased 93.2% compared to 10 ppm.

## DISCUSSION

The results show that *Alternaria alternata* toxin (AOH, AME and TA) inhibit pollen germination and tube growth in apple (*Malus sylvestris* Miller cv. Golden). TA demonstrates highest toxin effect following AOH and AME.

*Alternaria* are commonly parasitic on plants and other organic materials. *Alternaria alternata* is a frequently occurring species of particular interest to mycotoxicologist because it produces a number of mycotoxins, including alternariol (AOH), Alternariol Monomethyl Ether (AME) and Tenuazonic Acid (TA). AOH, AME, TA and in some cases other Alternaria toxins has been found naturally occurring grains [7], seeds [8, 9], flowers [10] and various fruits, including apples, tomatoes, mandarins, raspberries, peppers, melons [11-13]. *A. alternata* toxins play an important role in pathogenesis of plants. AAL toxins inhibited plant cell development at various levels of differention: protoplasts, calli, suspension cells, shoot induction on leaf disks, rooting of shoots and leaves [3]. Park *et al.* reported that the first effect of *Alternaria alternata* toxin were on endoplasmic reticulum and mitochondrial membranes in the leaves of susceptible tomato plants [14]. AME is cytotoxic and AOH and AME show synergetic effects [15]. *Alternaria alternata* toxins influenced carrot seed germination negatively [9] and TA had the greatest negative effects on carrot seed germination. This was also observed in present study.

Compounds toxic to sporophytic plant tissue may also affect pollen germination and growth *in vitro*. Thus, the toxin from *Helminthosporium maydis* has been shown to inhibit pollen germination of maize [16]. In another work by Bino *et al.* studied the effects of *Alternaria alternata* toxins on pollen germination and tube growth of several *Lycopersicon esculantum* genotypes and showed AAL toxin inhibited pollen germination and tube growth [4]. The results of the present study are similar.

Sexual reproduction in flowering plants requires germination of pollens on the stigma. In this study, these toxins have been shown to have negative effects on pollen germination and tube growth of apple.

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