The effect of activator application on the anatomy, morphology, and viability of *Lycopersicon esculentum* Mill. pollen

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**Abstract:** The present study examined the effect of ACT-2, a K-vitamin group activator intensively applied to tomato (*Lycopersicon esculentum* Mill.), on the anatomy, morphology, and viability of tomato pollen from plants grown under greenhouse conditions. The activator was applied to tomatoes at the recommended dosage (150 cc/100 L of tap water) and double the recommended dosage (300 cc/100 L of tap water). Pollen width and length, exine and intine layer thickness, pore width and length, and crevice width and length were measured using an ocular micrometer. Reductions were observed in all the measured parameters in all application groups, except for pollen width and length values at the 150 cc/100 L dosage, as compared to the control group. The viability of the pollen, as determined by triphenyl tetrazolium chloride, decreased in all the application groups as the dosage increased, as compared to the control group, and the toxic effect became more evident at the 300 cc/100 L dosage. In particular, non-viable pollen types, such as wrinkled pollen and abnormally shaped pollen, were observed in the fungicide groups. Additionally, the activator caused changes in the morphological structure of the tomato pollen. The morphological structures of some pollen, such as subprolate and suboblate, which were not observed in the control group, were seen in pollen in the polar view at the 150 cc/100 L ACT-2 dosage.

**Key words:** Tomato, activator, anatomy, morphology, pollen viability

Aktivatör uygulamasının *Lycopersicon esculentum* Mill. poleninin anatomisi, morfolojisi ve canlılığı üzerine etkileri

Özet: Bu çalışmada, sera koşullarında yetiştirilen domates (*Lycopersicon esculentum* Mill.) bitkisine uygulanan bir aktivatör olan ACT-2 (K-vitamin grubu)'nin domates poleninin anatomisi, morfolojisi ve canlılığı üzerine etkileri incelenmiştir. Aktivatör domates bitkilerine üretici firma tarafından önerilen dozda (150 cc/100 L musluk suyu) ve önerilenin iki katı dozda (300 cc/100 L musluk suyu) uygulanmıştır. Polen en-boy, eksin-intin tabaka kalınlığı, por en-boy ve yarık en-boy ölçümleri micrometrık oküler kullanılarak yapılmıştır. 150 cc/100 L dozdaki polen en-boy değerleri düşündaki tüm uygulama gruplarının değerlerinde kontrol göre bir azalma gözlenmiştir. Trifenil tetrázolium klorit yoluyla belirlenen polen canlılığı kontrolde göre tüm uygulama grubunda doz miktari arttıkça azalmış ve toksik etki 300 cc/100 L dozda daha da belirginleşmiştir. Özellikle fungisit gruplarında buruşuk polen, anormal şekilli polen gibi canlı olmayan polen tipleri gözlenmiştir. Diğer taraftan, activator domates poleninin morfolojik yapısında da değişikliklere neden olmuştur. Subbrolat ve suboblat gibi kontrol grubunda gözlenmemeyen bazı polen morfolojik yapları ACT-2'nin 150 cc/100 L dozunun kutupalı görünümülü polenlerinde görülmüştür.

Anahtar sözcükler: Domates, aktivatör, anatomi, morfoloji, polen canlılığı
Introduction

The rapid increase in the world population in recent years has caused many problems, including hunger. Therefore, obtaining the maximum yield from available land has become a major goal. For this purpose the use of chemicals that increase plant defensive capacity against pathogenic fungi, viruses, and bacteria has become widespread.

The chemicals used for agricultural purposes have some harmful effects on pollen structure. It was reported that pyrimethanil resulted in some changes in the morphological structure of tomato plant pollen and caused a reduction in the percentage of fertile pollen, as compared to the control (1). It was also reported that pesticides could have negative effects on pollen germination and tubes (2). Additionally, it was determined that pollen germination decreased by 20%, as compared to the control group, in apples treated with captan (3). Moreover, excessive use of triazole on some fruit trees during the flowering period negatively affected pollen germination and fruit formation (4,5). In another study, pollen germination and pollen tube growth decreased considerably in tomato flowers treated with chlorothalonil 75% WP (6).

The present study investigated the effects of different concentrations of ACT-2 activator, a new chemical widely used in greenhouses in Turkey, on the anatomy, morphology, and pollen viability in tomato in consideration of the fact that improper use of agricultural chemicals is by no means at minimal levels. Research on the numerous problems caused by excessive use of agricultural chemicals is increasing in importance.

Material and Methods

The study was conducted in a 970-m² greenhouse in the village of Karaçulha, Fethiye, Turkey. Healthy tomato seedlings were obtained from M-38 F₁-type domestic seeds. The activator used was ACT-2 (K-vitamin group), a new and patent protected active ingredient and specific organic metabolic activator. In total, 4 applications at 10-day intervals were made: 150 cc/100 L of tap water, as recommended by the manufacturer, and 300 cc/100 L of tap water (twice the recommended dosage). The first application of activator was performed at the stage after 2 weeks. The seedlings were planted in the greenhouse when they did not bear fruit. Flower bud specimens were randomly collected from different plants and fixed in Carnoy's solution. The flowers were removed from Carnoy's solution and then the anthers were mounted in a glycerin-gelatin-liquid safranin mixture (7).

In all, 100 pollen grains from each group were used for measurements using an ocular micrometer on a 100-Prior microscope. The pollen grains were divided into classes on the basis of shape, and the ratio of the pollen's polar axis in the equatorial and polar view to the equatorial diameter (8). The level of pollen viability of 100 pollen grains was determined in each group. This level was determined using the TTC test (9), using 2,3,5-triphenyl tetrazolium chloride solution. The solution prepared with this chemical, which is normally colorless, turned into triphenylformazan, which is insoluble and appears red in living tissue to which it is applied. This reaction is formed by some reductase enzymes in living tissue. The activity of redox-enzyme occurs via the same mechanism in other tetrazolium salts, such 2,3,5 triphenyl tetrazolium chloride; therefore, according to the level of liveliness in tissue, tissues stain red in accordance to the density of enzyme activity. When the enzyme activity increases a dark color is observed. If the same activity decreases, a light color is observed (10). One drop of this solution was placed on a slide, pollen grains were spread with a brush on the slide, and a cover slip was placed on top. Counting was performed after TTC application. It was divided into 3 groups based on staining density. Pollen grains that stained dark red were referred to as viable, light red as semi-viable, and unstained as non-viable (11). Viable pollen grains in the control group and non-viable pollen grains in the application groups were photographed using a JEOL JSM-6060 scanning electron microscope (12,13).

Statistical analysis of the values related to all measurements in the study was performed using SPSS v.11.0 for Windows and Tukey's multiple range test was used for variance analysis (14). Differences between a,b,c with the control group, a,b with the 150 cc/100 L ACT-2 group, and a,c with the 300 cc/100 L ACT-2 group were statistically significant (P < 0.05), as indicated in the tables.
Results and discussion

An examination of the effects of the activator used in the present study on the width and length measurements of pollen grains observed in the equatorial and polar view indicates that the values obtained in the 300 cc/100 L ACT-2 group were lower than those in the control group. Values obtained in the application groups decreased as the ACT-2 dosage increased (Table 1). This decrease in the 300 cc/100 L ACT-2 group was statistically significant, as compared to the 150 cc/100 L group. Various chemicals interfered with pollen development, and resulted in a delay in flowering and leaf formation (15). It was reported that width and length measurements of pollen grains observed in the equatorial and polar view at 250 mL/100 L, a higher dosage of Mythos SC 300 (300 g/L pyrimethanil), were lower than in the control group (16). It was determined that in the present study 300 cc/100 L, a higher dosage of ACT-2, also resulted in a decrease in width and length measurement values in pollen grains observed in the equatorial and polar view.

In the present study width and length measurements related to pores and cracks in pollen grains observed in the polar view were lower in all the application groups than in the control group (Table 2); these decreases were statistically significant, as compared to the control group. Pore width and length, and crevice length values in the pollen observed in the polar view in the application groups decreased as the dosage increased. The decrease observed in the 300 cc/100 L dosage group was statistically significant, as compared to that in the 150 cc/100 L dosage group. The literature contains only a few studies on the effects of pesticides on width and length measurements related to pollen pores and crevices. It was reported that width and length measurement values related to the pores and crevices of tomato pollen observed in the polar view decreased in the Switch 62.5 WG (37.5% cyprodinil + 25% fludioxonil) group at dosages of 60 g/100 L and 120 g/100 L, as compared to the control group (16).

A number of studies have reported detrimental effects of chemical sprays on pollen germination (17,18) and pollen tube growth (19,20). When the results of pollen exine and intine layer thicknesses observed in the equatorial view are evaluated it can be seen that all the values obtained in the application groups were again lower than those in the control group (Table 3). The observed decreases in exine and

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### Table 1. Width/length measurements of pollens in equatorial and polar view (μ).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Equatorial View</th>
<th>Polar View</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Width (μ) ↓</td>
<td>Length (μ)</td>
<td>Width (μ) ↓</td>
</tr>
<tr>
<td>Control</td>
<td>21.916 ± 0.230</td>
<td>22.583 ± 0.145</td>
<td>21.000 ± 0.227</td>
</tr>
<tr>
<td>ACT-2 150 cc/100 L</td>
<td>21.683 ± 0.187</td>
<td>22.000 ± 0.165</td>
<td>21.333 ± 0.223</td>
</tr>
<tr>
<td>ACT-2 300 cc/100 L</td>
<td>20.266 ± 0.130</td>
<td>20.708 ± 0.204</td>
<td>20.150 ± 0.111</td>
</tr>
</tbody>
</table>

### Table 2. Measurements of pores and colpi (μ) in polar view.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Polar View</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pore Width (μ)</td>
<td>Pore Length (μ)</td>
</tr>
<tr>
<td>Control</td>
<td>7.541 ± 0.111</td>
<td>7.833 ± 0.157</td>
</tr>
<tr>
<td>ACT-2 150 cc/100 L</td>
<td>6.666 ± 0.330</td>
<td>7.066 ± 0.241</td>
</tr>
<tr>
<td>ACT-2 300 cc/100 L</td>
<td>4.525 ± 0.230</td>
<td>4.458 ± 0.235</td>
</tr>
</tbody>
</table>
intine layer values were statistically significant. As the dosage increased exine and intine layer values observed in the equatorial view decreased in the application groups. The decrease in the values in the 300 cc/100 L dosage group was statistically significant, as compared to that in the 150 cc/100 L group.

An examination of the effects of the activator used in the present study on pollen viability showed that the percentage of dark red (viable) pollen decreased, as compared to the control group, whereas the percentage of light red (semi-viable) and unstained (non-viable) pollen increased (Table 4). Furthermore, various non-viable pollen types, such as wrinkled pollen and abnormally shaped pollen, were observed in the application groups (Figures 1 and 2). It was thought that decreases in the percentage of dark red pollen in the application groups that were observed as the dosage increased might have been due to the toxic effect of ACT-2. In particular, the decrease observed in the percentage of dark red pollen in the application groups, as compared to the control, could have a negative effect on fruit productivity and quality of tomato in the future. It was reported that captan and some chemicals reduced pollen viability in many apple cultures (21). The combined effect of 2 organophosphate insecticides and a dinitro herbicide resulted in a reduction of viability of about 60% in pollen of the eggplant *Solanum melongena* (22).

Only a few studies on the effects of chemicals on pollen morphology have been published. It was reported that some chemicals, such as myclobutanil, iprodione, and cyprodinil, resulted in substantial damage to stigma morphology (23). When the effects of ACT-2 on the morphological structure of the pollen were examined, it was noted that the percentage of oblate spheroidal pollen grains observed in the equatorial view was lower, whereas the percentage of prolate spheroidal pollen grains was higher in the application groups, as compared to the control group (Table 5). In both application groups the percentage of oblate spheroidal pollen grains observed in the equatorial view decreased as the ACT-
Table 5. Pollen shape classification in the control and application groups.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Equatorial view</th>
<th>Polar view</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Oblate Spheroidal (%)</td>
<td>Prolate Spheroidal (%)</td>
</tr>
<tr>
<td>Control</td>
<td>93.33</td>
<td>6.66</td>
</tr>
<tr>
<td>ACT-2 150 cc/100 L</td>
<td>63.33</td>
<td>36.66</td>
</tr>
<tr>
<td>ACT-2 300 cc/100 L</td>
<td>20</td>
<td>80</td>
</tr>
</tbody>
</table>

2 dosage increased, while the percentage of prolate spheroidal pollen grains increased. When the pollen shape class of pollen grains observed in the polar view was evaluated the percentage of oblate spheroidal pollen grains was higher and that of prolate spheroidal pollen grains was lower in both applications groups, as compared to the control group. In both application groups the percentage of oblate spheroidal pollen...
grains observed in the polar view increased as the dosage increased, while the percentage of prolate spheroidal pollen grains decreased. However, unlike in the control group and the 300 cc/100 L ACT-2 dosage group, subprolate and suboblate pollen grains were noted in all pollen groups observed in the polar view at the 150 cc/100 L dosage. It was reported that, unlike other application groups, subprolate and suboblate pollen grains in tomato treated with a 60 g/100 L dose of Switch 62.5 WG were observed (16).

The ACT-2 used in the present study resulted in some changes in the morphological features of tomato pollen grains. The activator, which is used to increase yield, negatively affected tomato pollen viability. In particular, the percentage of pollen viability decreased in response to the higher dosage application. This observed negative effect on tomato pollen viability could cause a decrease in the productivity of fruits in the future.

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**References**


