The effects of fosetyl-Al application on morphology and viability of *Lycopersicon esculentum* Mill. pollen

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ABSTRACT

In the present study, the effects of fungicide Aliette WG 800 [80% fosetyl-Al (aluminium tris-o-ethyl phosphonate)], widely used against *Phytophtora infestans* on tomatoes grown in greenhouse in Turkey, were studied on the morphology and viability of tomato (*Lycopersicon esculentum* Mill.) pollens. The fungicide was applied to tomatoes grown in greenhouse at recommended dosage (200 g/100 l water) and at double the recommended dosage (400 g/100 l water). The fungicide caused changes in the morphological structures of tomato pollens. Some pollen morphological structures that are not observed in the control group were encountered in the pollens in equatorial view and in polar view at 200 g/100 l treated groups. On the other hand, pollen viability level decreased as the dosage increased. Especially, non-viable pollen types such as wrinkled pollen or pollen with abnormal shape were encountered in the fungicide groups. It was expected that the pollen fertility as well as yield would decrease in future.

Keywords: fosetyl-Al; tomato, morphology, pollen viability

Rapidly increasing world population in recent years brings about an increasing demand for nutrition, which is one of the most important problems for mankind. Various methods have been discussed in order to solve this problem. One of these methods is using chemicals against harmful organisms in plants. The use of pesticides in agricultural areas increases the plant yield; however, some chemical substances may result in pollution in nature and health problems.

The effects of pesticides used in high concentrations in the world have been dealt with and investigated by various researchers from different aspects. For this reason, a number of studies were carried out to determine harmful effects of pesticides (Soriano 1984, El-Khodary et al. 1987). Detrimental effects of fungicide sprays on fruit set and yield in crops such as cranberry (*Vaccinium macrocarpon* Ait.) were reported (Özgen and Palta 2001). Furthermore, it was stated that pesticides had often prevented cell division (Yoshida et al. 1983). A lot of work was done to study the effects of fungicides on pollen germination and pollen tube growth (He et al. 1995). Harmful effects of fungicides on pollen germination (Watters and Sturgeon 1990) and pollen tube growth (Marcucci et al. 1983) were demonstrated for commercially important plants. Although many studies have been conducted on the effects of fungicides on pollen germination and pollen tube growth, very few studies have been carried out handling the impact of fungicides on the pollen morphology and viability.

This study was thus conducted to determine whether fungicide fosetyl-Al affects morphology and viability of *Lycopersicon esculentum* Mill pollen.

Fosetyl-Al is rapidly absorbed, predominantly through the leaves but also through the roots, with translocation both acropetally and basipetally. Fosetyl-Al (aluminium salt of ethyl hydrogen phosphonate) is $C_6H_{18}AlO_9P_3$, or $(H(CH_3CH_2)P(O)O)_3Al$, respectively. The metabolism of fosetyl in plants proceeds the hydrolytic cleavage of the ethyl ester bond. Phosphoric acid is detected as the major metabolite in plants; for fosetyl-Al the major metabolites are $Al(H_2PO_4)$ (Tomlin 1994).

MATERIAL AND METHODS

The flower buds of tomato were obtained at a 970-m² greenhouse in the village of Karaçulha

in Fethiye. Healty tomato seedlings were grown from M-38 F_1 type domestic seeds. Aliette WG 800 [80% fosetyl-Al (aluminium tris-o-ethyl phosphonate)], the fungicide used in the trial, was applied on tomato seedlings grown in the greenhouse conditions. In total, 4 applications were made at 10-day intervals, i.e. 200 g/100 l water as the recommended dosage and 400 g/100 l water as double the recommended dosage. The fungicide was first applied at the stage of fifth flower of bunch; after the fungicide application, the plant was left to develop until the first fruits were egg sized and before ripening. Specimens randomly collected from different plants were fixed in the Carnoy's solution. The flowers were removed from Carnoy and then the anthers taken from ripe floral buds with the help of a dissection needle were mounted on glycerine-gelatin-liquid safranine mixture (Wodehouse 1965). A total of 1000 pollens from each group were used for the determination of pollen shape classification. The pollens were divided into classes on the basis of shape and rate of the polar axis of the pollens in equatorial and polar view to the equatorial diameter (Erdtman 1966). These were made with the help of a micrometric ocular on a 100-Prior microscope. To determine pollen viability level, 1000 pollen grains of each group were counted under a light microscope. This level was determined with the TTC test (Norton 1966); 2,3,5-triphenyl tetrazolium chloride solution was used in the test. The solution prepared with this chemical and normally colourless turns into triphenylformazan that is insoluble and has red colour in applied living tissue; the reaction is caused by some reductase enzymes in living tissue. The activity of redox-enzyme is effective with the same mechanism in other tetrazolium salts, such as 2,3,5-triphenyl tetrazolium chloride, too. Hence, according to the level of liveliness in tissue, tissues are painted in red colour parallel to the density in enzyme activity. When the enzyme activity increases, dark colour occurs. If the same activity decreases, light colour is formed (Smith 1951). One drop of this solution was placed on slide and pollens were spread by brush on the slide and a cover slip was placed on it. Counting was made after the TTC application and it was divided into three groups based on staining density. Dark red stained pollens were referred to as viable, light red as semi-viable, and unstained as non-viable (Eti 1991). Viable pollens in the control and non-viable pollens in the application groups were photographed using a JEOL JSM-6060 Scanning Electron Microscope (Nepi et al. 1995, Giuseppe 1999).

RESULTS AND DISCUSSION

Results of pollen shape classification in the control and in the fungicide application groups are given in Table 1. According to these results, the percentage of oblate spheroidal pollens was lower but that of prolate spheroidal pollens was higher in treated groups as compared to the control group in equatorial view. The percentage of oblate spheroidal pollens seen in equatorial view decreased in parallel with the increase in dosage, while the percentage of prolate spheroidal ones increased. The percentage of oblate spheroidal pollens was higher but that of prolate spheroidal pollens was lower in fungicide groups according to the control in polar view. However, unlike other fungicide groups, subprolate as well as suboblate pollens were encountered in pollen groups seen in polar view at 200 g/100 l fosetyl-Al group; moreover, pollens belonging to subprolate shape class were seen at 200 g/100 l fungicide group in equatorial view.

This study has verified that the application of the fungicide fosetyl-Al to tomato can directly affect

Table 1. Pollen shape classification in the control and the fungicide application groups

Treatment	Pollen shape classification (%)						
	equatorial view			polar view			
	oblate spheroidal	prolate spheroidal	subprolate	oblate spheroidal	prolate spheroidal	subprolate	suboblate
Control	93.33	6.66	0	50	50	0	0
Fosetyl-Al (200 g/100 l)	76.66	16.66	6.66	76.66	3.33	10	10
Fosetyl-Al (400 g/100 l)	73.33	26.66	0	63.33	36.66	0	0

Table 2. Pollen viability level in the control and the fungicide groups

Treatment	Pollen viability percentage (%)		
	dark red	light red	unstained
Control	91	7	2
Fosetyl-Al (200 g/100 l)	61	19	20
Fosetyl-Al (400 g/100 l)	35	31	34

pollen morphology. In the trial, various pollen structures not seen in the control, such as "subprolate" and "suboblate", were determined in the fungicide groups. Besides, both in equatorial and polar views, the percentages of oblate and prolate spheroidal pollens in treated groups were different from the percentages of oblate and prolate spheroidal pollens in the control group. Pollen morphology is an important variable affected by fungicides; however, there were very few studies carried out on the effects of pesticides on pollen morphology (Yı et al. 2003). According to Yı et al. (2003), fungicides can be detrimental to flower development, pollen function and fruit set in a number of crops. It was demonstrated that Deltan application resulted in some abnormal features at pollen cells (Prakash et al. 1988); similarly, propiconazole treatments induced abnormal tube morphology in *Tradescantia virginiana* pollens (He et al. 1995). It was also found that the effects of Chorus 50 WG (50% cyprodinil) fungicide on the morphological structures of the tomato pollens revealed that the percentage of oblate spheroidal type pollens was higher and that of prolate spheroidal pollens was lower at the dosages of 40 g/100 l and 80 g/100 l in equatorial and polar view as compared to the control. In the same study, pollen morphological structures that were not observed in the control group were encountered in the pollens in both equatorial and polar view at 40 g/100 l Chorus 50 WG group (Öztürk Çalı 2005).

The results of the TTC test for pollen viability are shown in Table 2. The percentage of viable pollens was lower whereas the percentages of semi-viable and non-viable pollens were higher in all application groups as compared to the control group. This decrease in the values of viable pollens and the increase in the values of semi-viable and non-viable pollens occurred in parallel to dosage increase. On the other hand, various non-viable pollen types such as wrinkled pollen or pollen with abnormal shape were encountered in the fungicide groups (Figures 1 and 2).

A number of studies reported detrimental effects of fungicide on pollen germination (Watters and Sturgeon 1990, Wetzstein 1990) and pollen tube growth (Abbott et al. 1991, He et al. 1996). It was stated that the combined effect of two organophosphate insecticides and a dinitro herbicide indicated a loss of viability of about 60% in the pollen of the egg plant *Solanum melongena* (Dubey et al. 1984). Captan and various other fungicides, which belong to the family *Pthalamide*, reduced pollen viability in many apple cultures (Church and Williams 1977). In the trial, fosetyl-Al resulted in a decrease in the values of pollen viability level compared to the control group while the same fungicide increased the percentages of semi-viable and non-viable pollens.

Plants have to bind Al to compounds for example to phytochelatines and metalloproteines.

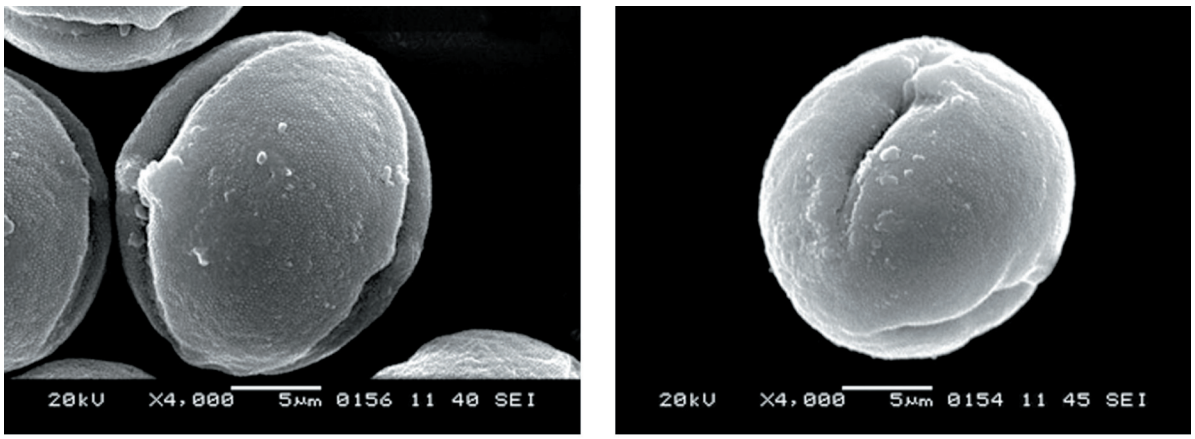

Figure 1. SEM photographs of viable pollens in the control group

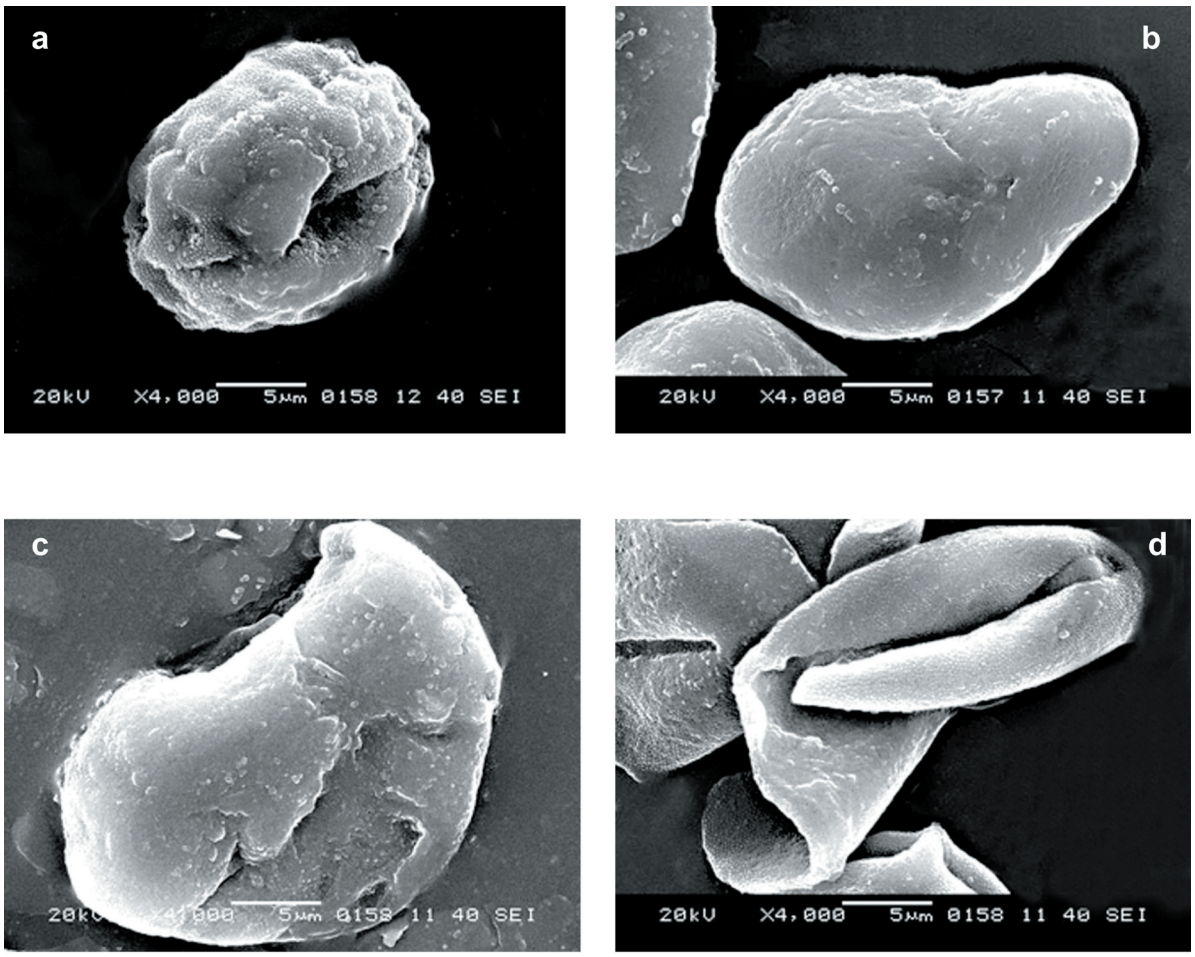

Figure 2. SEM photographs of non-viable pollens in the fosetyl-Al groups: (a) wrinkled pollen in 200 g/100 l dosage; (b) abnormal shape pollen in 400 g/100 l dosage; (c) abnormal shape pollen in 400 g/100 l dosage; (d) abnormal shape pollen in 400 g/100 l dosage

Phytochelatin compounds contain glutamic acid and cysteine and production of glutamic acid can be a reason for a lack of proline. Pavlíková et al. (2007, 2008) reported that this amino acid is significant for production of glycoproteines and the lack of glycoproteines consequently affected non-fertile pollen. The effect of Al and its toxicity corresponds to the toxicity of microelements tested using pollen as shown by Pavlík and Jandurová (2001). This method is more sensitive than the methods used by Dr. He (Pavlík and Jandurová 2000).

In the present study, the fungicide used in the trial negatively affected the pollen shape and the pollen viability level of *Lycopersicon esculentum* Mill. "Subprolate" and "suboblate" classes were not observed in the pollen shape classes of the control group, but they were encountered in the 200 g/100 l fungicide group. In particular, the pollen viability level in treated groups was lower than in the control. It is obvious that this situation will reduce pollen fertility and yield in general.

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