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EFFECTS OF A FUNGICIDE ON THE MORPHOLOGY AND VIABILITY OF POLLENS OF TOMATO (*LYCOPERSICON ESCULENTUM* MILL.)

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Abstract

Effects of Agri Fos 400 (Mono and di-potassium phosphanate), a fungicide widely used on tomatoes grown in greenhouses in Turkey against *Phytophthora infestans* were studied on the morphology and viability of tomato (*Lycopersicon esculentum* Mill.) pollens. The fungicide was applied on tomato plants at recommended dosage (4 ml/l water) and double the recommended dosage (8 ml/l water). The fungicide caused changes in the morphological structures of pollens. Some pollen morphological structures that are not observed in the control group were encountered in the pollens due to application of Agri Fos 400 in equatorial view at 8 ml/l and in polar view at 4 ml/l. Level of pollen viability decreased as the dosage increased.

Introduction

In recent years, many chemicals are used as fungicides and as well as insecticides. Pavlik and Jandurova (2000) reported that fungicides could have negative effects on pollen germination. Öztürk (2006) stated that a fungicide, Equation Pro (22.5% Famoxadone + 30% Cymoxanil), increased number of abnormal stomata in tomato plants. On the other hand, chlorothalonil fungicide impeded pollen germination of muskmelon and this situation could cause negative effect on fruit development (Abbott *et al*. 1991).

In the present study, effects of Agri Fos 400, a fungicide against *Phytophthora infestans,* were studied on the morphology and viability of pollen in tomato plants.

Materials and Methods

The fungicide Agri Fos 400 (Mono and di-potassium phosphanate, active ingredient) was applied against *Phytopthora infestans*. A total of three groups, one untreated (control) and other two were treated with the fungicide at dosages recommended by the manufacturer (4 ml/l water) and double of the recommended dosage (8 ml/l water). A total of 228 healthy tomato seedlings obtained from M-38 F_1 type domestic seeds were planted on April 3, 2006 in a greenhouse in Muğla, Fethiye, Turkey. Each treatment had 76 seedlings as replicates. The two fungicide solutions were sprayed four times at an interval of ten days starting from DAS until the end of the flowering period using a sprayer between 0700 and 0900 hr.

Flower samples for the pollen analyses were randomly collected between 1030 and 1130 hr starting from the day after the first treatment until the day of the final treatment. These were put in Karnoy fixative (3 parts 96% ethyl alcohol : 1 part glacial acetic acid) and kept in a refrigerator. The flowers were removed from Karnoy and then the anthers taken from ripe floral buds with the help of a dissecting needle, and mounted on gylcerine-gelatin-liquid safranine mixture (Wodehouse 1965). A total of 100 pollens from each group were used for the determination of changes in pollen shape. The pollens were divided into classes on the basis of shape and rate of the polar axis of the pollens in equatorial and polar views to the equatorial diameter (Erdtman 1966).

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To determine viability level of pollens, 100 pollen grains of each group were counted under a light microscope. The level was determined with 2, 3, 5-triphenyl tetrazolium chloride (TTC) solution (Norton 1966). One drop of this solution was placed on a slide and pollens were spread and a cover slip was placed on it. Counting was made after TTC application and it was divided into three groups based on staining density. Dark red stained pollens were referred as viable, light red as semi-viable and unstained as non-viable (Eti 1991, Stosser 1984). Viable pollens in the control and non-viable pollens in the treatments were photographed using a JEOL JSM-6060 Scanning Electron Microscope (Nepi *et al.* 1995, Giuseppe 1999).

Results and Discussion

Effects of Agri Fos 400 fungicide on pollen shape in tomato is given in Table 1. Results revealed that 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 increased with the increase in dosage, while the percentage of prolate spheroidal pollens decreased with the increase in dosage.

Table 1. Effect of Agri Fos 400 on the percentage of pollen shape in tomatos.

Treatment	Pollen shape						
	Equatorial view			Polar view			
	Oblate spheroidal (%)	Prolate spheroidal (%)	Subprolate (%)	Oblate spheroidal (%)	Prolate spheroidal (%)	Subprolate (%)	Suboblate (%)
Control	93.33	6.66	-	50.00	50.00	-	-
4 ml/l	66.66	33.33	-	86.66	6.66	3.33	3.33
8 ml/l	73.33	23.33	3.33	96.66	3.33	-	-

The percentage of oblate spheroidal pollens was higher than prolate spheroidal pollens in polar view. The percentage of oblate spheroidal pollens in polar view increased with the increase in dosage, while that of prolate spheroidal pollens decreased. However, unlike other fungicide groups, a small percentage of both subprolate and suboblate pollens were found in pollen groups seen in polar view at 4 ml/l Agri Fos 400 group; moreover only a small percentage of subprolate pollens were seen at 8 ml/l Agri Fos in equatorial view.

Pollen morphology has been reported to be affected by fungicides (Erdtman 1966, Yı *et al.* 2003). He and Wetzstein (1994) demonstrated that fungicide applications caused problems in pollen development and delayed flower and leaf formation. Öztürk Çalı (2005) found that due to application of Chorus 50 WG (50 % Cyprodinil) fungicide percentage of oblate spheroidal type pollens was higher but that of prolate spheroidal pollens was lower at the dosages of 4 ml and 8 ml in equatorial and polar view as compared to the control. Similarly, Tort *et al.* (2005) reported that Switch 62.5 WG (37.5% Cyprodinil + 25% Fludioxonil) and Mythos SC 300 (300 g/l Pyrimethanil) fungicides caused abnormal morphological structures as compared to the control in tomato plants.

Table 2. Percentage of pollen viability in the control and treatments with Agri Fos 400.

Treatments	Viable	Semi-viable	Non-viable
Control	91	7	2
4 ml/l	70	14	16
8 ml/l	45	23	32

The results of TTC test for percentage of pollen viability are shown in Table 2. The percentage of viable pollens was lower whereas the percentage of semi-viable and non-viable pollens were higher in all application groups as compared to the control group. Viable pollens were more or less round in shape without any wrinkles or abnormality (Figs 1a-b). Following fungicide applications pollen morphology of non-viable pollen types showed wrinkled pollen and also pollens with abnormal shape (Figs 1c-f).

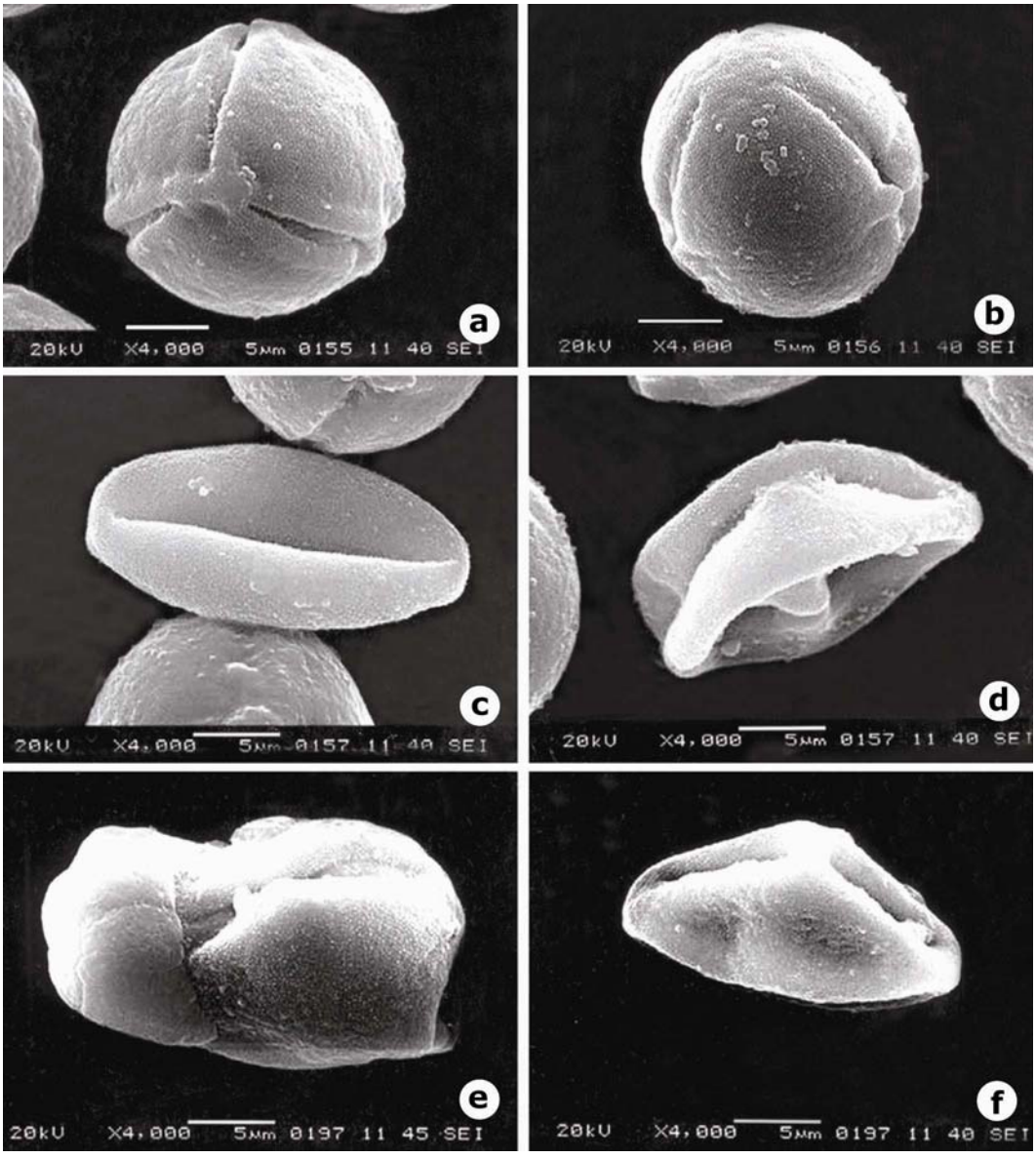

Fig. 1a-f. SEM photographs of tomato pollens. a-b. Viable pollens in the control. c-d. Wrinkled pollens in 4 ml Agri Fos 400/l dosage. e-f. Abnormal shaped pollens in 8 ml Agri Fos 400/l dosage.

Several authors have reported detrimental effects of fungicide spray on pollen viability, pollen germination and pollen tube growth in many crops such as 'Hass' avocado (*Persea americana*), Nartvaranant *et al.* (2004); almond (*Prunis dulcis*), Yı *et al.* (2003) and tomato (Lacerda *et al.* 1994). Furthermore, Church and Williams (1977) reported that Captan and various other fungicides which belong to the family Phthalamide, reduce pollen viability in many apple cultures.

Results obtained in the present study, established that Agri Fos 400 fungicide even at recommended dose affected the pollen shape and viability level of tomato.

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