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EFFECTS OF A FUNGICIDE ON POLLEN MORPHOLOGY AND FERTILITY OF TOMATO (*LYCOPERSICON ESCULENTUM* MILL.)

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Abstract

Effects of Equation Pro (22.5% Famoxadone + 30% Cymoxanil), a fungicide widely used on tomatoes (*Lycopersicon esculentum* Mill.) grown in greenhouses in Turkey against *Phytopthora infestans* and *Alternaria solani,* were studied on the morphology and fertility of tomato pollens. The fungicide was applied to tomatoes grown in pots in a greenhouse at recommended dosage (0.4 g/l water) and double the recommended dosage (0.8 g/l water). At the recommended dosage of Equation Pro, pollens were affected but at double the recommended dose, fertility is decreased to 41% with morphological deformity and thinner wall layers.

Introduction

A number of chemical compounds are being used to protect agricultural crops from diseases and weeds. Residues of these chemicals lead to environmental pollution and pose some threat to non-terget organisms, human and animals.

Pesticides produce a toxic effect on plant leaves resulting morphological, anatomical and physiological changes; inhibit pollen germination and pollen tube formation; and thus affect fruit production (He *et al*. 1995). Detrimental effects of fungicides on pollen germination (Watters and Sturgeon 1990) and pollen tube growth (Marcucci *et al*. 1983) have been demonstrated for commercially important plants. Çali (2008) showed that Equation Pro fungicide caused anomalies in pollen mieosis in parallel with the increase in application dose. It was reported that Chlorothalonil fungicide impeded pollen germination of muskmelon and this situation could cause negative effect on fruit development (Abbott *et al*. 1991). Although studies have been conducted on the effects of fungicides on pollen germination and pollen tube growth, very few have been carried out on the effects of fungicides on the pollen morphology and fertility.

In this study, the effects of Equation Pro fungicide, widely used on tomatoes grown in greenhouses in Turkey against *Phytopthora infestans* and *Alternaria solani,* were investigated on the morphology and fertility of tomato pollens.

Materials and Methods

Tomato (*Lycopersicon esculentum* Mill.) seedlings were raised by sowing M-19 F_1 type domestic seeds. Eighty one healthy seedlings were randomly planted in a total of 27 pots in the greenhouse of Plant Protection Department, Agricultural Faculty, Ege University. Each pot contained three seedlings. Each treatment had nine replicates. Fungicides were applied at 12-day intervals until the end of the flowering period using a sprayer between 7 and 9 a.m. in the morning. The fungicide Equation Pro (22.5% Famoxadone [5-methyl-5-(4-phenoxyphenyl)-3-(phenylamine)-2,4-oxazolidimedione] + 30% Cymoxanil [2-cyano-N-[(ethylamino) carbonyl]-2-(methoxyamino) acetamide] active ingredient) was applied against *Phytopthora infestans* and *Alternaria solani*. The fungicide was applied at dosages recommended by the manufacturer (0.4 g/1 water) and double the recommended dosage (0.8 g/1 water).

Flower samples for the pollen analyses were randomly collected between 10.30 and 11.30 a.m. starting from the day after the treatment until the day of the next treatment. These were put in Karnoy fixative and stored in a refrigerator. The flowers were removed from the fixative and then the anthers were taken from ripe floral buds with the help of a dissection needle and were mounted on gylcerine-gelatin-liquid safranine mixture (Wodehouse 1965).

A total of 100 pollens from each treatment were studied to determine fertility, pollen morphology and shape. The pollens were divided into classes on the basis of shape and dimensions of the pollens in equatorial and polar views (Erdtman 1966).

To determine pollen viability level, 100 pollen grains of each group were counted under a light microscope. This level was determined with 2, 3, 5-triphenyl tetrazolium chloride (TTC) solution (Norton 1966). One drop of this solution was placed on slide and pollens were spread by brush 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).

Statistical analyses were made on a SPSS 11.0 for Windows statistical program and Multiple Range Tukey Test was used for variance analyses (Tukey 1954).

Results and Discussion

Examination of the effects of the fungicide used in the present study on the width and length of pollens in equatorial and polar views showed that the values obtained in both the treatments were lower than those in the control (Table 1). Such a decrease in dimension of pollens seen in equatorial and polar views were statistically significant as compared to the control in the 0.8 g/l Equation Pro.

Table 1. Effects of Equation Pro at two concentrations on width and length of pollens of *L. esculentum*.

Treatment (g/l)	Equatorial view		Polar view	
	Width (µm)	Length (µm)	Width (µm)	Length (µm)
Control	18.733 ± 0.157	19.065 ± 0.176 [c]	18.924 ± 0.147	19.065 ± 0.162
0.4	18.533 ± 0.146	18.650 ± 0.157 [b]	18.401 ± 0.160	18.509 ± 0.221
0.8	18.069 ± 0.202	18.260 ± 0.144 [a]	17.571 ± 0.141	18.177 ± 0.162

Means followed by common letters are not significantly different at 5% level.

A number of studies have reported detrimental effects of fungicide on fruit set or yield in many crops such as apple (Hutcheon *et al*. 1986), cranberry (Ozgen and Palta 2001), raspberry (Redalen 1980) and strawberry (Kovach *et al*. 2000).

An examination of the results of width and length measurements related to pores of pollens seen in polar view reveals that the values in question are again lower in all treatments (Table 2). This decreased in the width and length of pores of pollens in polar view in 0.8 g/l Equation Pro was statistically significant as compared to the control. The pore width and length values of the pollen in polar view decreased in treated groups as dosage increased. This decrease in 0.8 g/l was statistically significant as compared to 0.4 g/l Equation Pro. Tort *et al*. (2005) observed that the values of width and length measurements related to pores of tomato pollens in polar view decreased in the Mythos SC 300 (300 g/l Pyrimethanil) fungicide at the dosages of 1.25 ml/l and 2.50 ml/l as compared to the control.

Evaluation of present results regarding the exine and intine layer thickness of the pollen seen in equatorial view depicts that all the values obtained in the treatments are significantly lower than the control (Table 2). As the dosage increased, the values of exine-intine layer thicknesses decreased in all treatments. However, all values at higher dosages are not significantly different compared to recommended dosage.

Table 2. Effects of Equation Pro at two concentrations on dimension of pores and thickness of exine and intine of the tomato pollens.

Treatments (g/l)	Polar view		Equatorial view	
	Pore width (μm)	Pore length (μm)	Exine (μm)	Intine (μm)
Control	4.838 ± 0.108^{c}	4.755 ± 0.082^{c}	0.929 ± 0.057^{bc}	0.550 ± 0.035^{bc}
0.4	4.565 ± 0.104^{c}	4.623 ± 0.104^{c}	0.796 ± 0.014^{a}	0.409 ± 0.016^{a}
0.8	4.042 ± 0.093^{ab}	4.091 ± 0.095^{ab}	0.730 ± 0.023^{a}	0.403 ± 0.013^{a}

Means followed by common letters are not significantly different at 5% level.

It was reported that the exine and intine layer thicknesses of the tomato pollens seen in equatorial view were lower in the Switch 62.5 WG (37.5% cyprodinil + 25% fludioxonil) fungicide groups at the dosages of 0.6 and 1.2 g/l than the control (Tort *et al*. 2005).

An evaluation of the percentage fertility results showed that values in general decreased compared to the control (Table 3). Various sterile pollen types such as wrinkled pollen, unstained pollen, pollen with abnormal shape and pollen with invisible pore were encountered in fungicide treatments. Çalli (2008) showed that Equation Pro causes anomalies in pollen meiosis and this lead to pollen sterility. This decrease in the percentage of fertile pollens in both the treatments as the dosage increases can be attributed to their toxic effects on pollens. In fact, this toxic effect becomes more evident at higher dosages. The decrease observed in the percentage of fertile pollens in treatment compared to the control could produce a negative effect on fruit productivity and quality of tomato in the long run.

Table 3. Effect of Equation Pro on pollen fertility and morphology of tomato.

Treatments (g/l)	Fertile pollen (%)	Wrinkled pollen (%)	Unstained pollen (%)	Pollen without visible pore (%)	Pollen with abnormal shape (%)	Total sterile pollen (%)
Control	89	4	0	6	1	11
0.4	50	23	0	23	4	50
0.8	41	22	0	33	4	59

According to Dubey *et al*. (1977), the combined effect of two organophosphate insecticides and a dinitro herbicide indicated a loss of viability of about 60% in the pollen of eggplants. Propiconazole fungicide caused detrimental effects on pollen germination and pollen tube growth of *Tradescantia virginiana* (He *et al*. 1995).

Adequate pollination and fertilization are critical in numerous economically important crops (Gonzales *et al*. 1998). It was reported that phosphate fungicide treatment reduced the pollen fertility of eight plant species during either after spring and fall applications or after summer applications of this fungicide (Fairbanks *et al*. 2002).

The effects of fungicide on the morphological features of the pollens revealed that the percentage of oblate spheroidal pollens was higher but that of prolate spheroidal pollens was lower in all treated groups as compared to the control (Table 4). The percentage of oblate spheroidal pollens seen in equatorial view decreased in parallel with the increase in dosage. The percentage of prolate spheroidal pollens seen in equatorial view is the same both 0.4 and 0.8 g/l Equation Pro groups. The percentage of oblate spheroidal pollens seen in polar view increased as dosage increase while that of prolate spheroidal pollens decreased in parallel with the applied dosage. However, unlike other treated groups, subprolate pollens were encountered in pollen groups seen in equatorial view at 0.8 g/l Equation group; besides, belonging to oblate shape class seen at 0.4 g/l Equation Pro in polar view.

Table 4. Effects of Equation Pro on the shape of pollens of *L. esculentum.*

Treatments (g/l)	Pollen shape classification (%)					
	Equatorial view			Polar view		
	Oblate spheroidal (%)	Prolate spheroidal (%)	Subprolate (%)	Oblate spheroidal (%)	Prolate spheroidal (%)	Oblate (%)
Control	63.33	36.66	0	60	40	0
0.4	73.33	26.66	0	63.33	33.33	3.33
0.8	70	26.66	3.33	70	30	0

Studies on the effects of fungicides on the stigma morphology of the almond tree, stigma surface treated with four types of fungicides (azoxystrobin, myclobutanil, ipradione and cyprodinil) has been examined under an electron microscope after 4 and 24 hours of application. Regional deformations on stigma surfaces and breakdown in stigmatic cells have been observed 24 hr after Azoxystrobin treatment. Similarly, Myclobutanil, Ipradione and Cyprodinil have also resulted in the substantial damage of stigma (Yı *et al.* 2002). According to Erdtman (1966) and Yı *et al.* (2003), pollen morphology is another important variable affected by fungicides. Application of Mythos SC 300 fungicide on the morphological features of the tomato pollens revealed that the percentage of oblate spheroidal pollens was higher but that of prolate spheroidal pollens was lower at the dosages of 1.25 and 2.50 ml/l in equatorial and polar views compared to the control and pollen morphological structures that were not observed in the control group were encountered in the pollens in polar view at 125 ml/l Mythos application (Tort *et al.* 2005).

During the present investigation fungicide application led to some changes in the morphological features of tomato pollens. The Equation Pro fungicide caused a decrease in width-length of tomato pollens, exine-intine layer thickness and pore width-length. The study shows that the tomato pollen fertility is seriously affected by the fungicide used even at the recommended dose. The toxic effect was particularly severe at higher dosages of the fungicide applied.

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