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EFFECTS OF SOME FUNGICIDES ON POLLEN MORPHOLOGY AND ANATOMY OF TOMATO (*LYCOPERSİCON ESCULENTUM* MILL.)

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Abstract

In this investigation, the effects of fungicides Switch 62.5 WG (%37.5 Cyprodinil + % 25 Fludioxonil) and Mythos SC 300 (300 g/ L Pyrimethanil); widely used on greenhouse grown tomatoes against grey mould (*Botrytis cinerea*); were studied on the morphology and anatomy of tomato (*Lycopersicon esculentum* Mill.) pollens. Application of excessive dosages of fungicides affected fruit production and quality, as such possible effects due to overdose applications were put forth. Fungicides were applied to tomatoes grown in pots in the greenhouse at recommended dosage (60 g/100 L water for Switch; 125 mL/100 L water for Mythos) and double the recommended dosage (120 g/100 L water for Switch; 250 mL/100 L water for Mythos). The pollen width-length, exine-intine layer thickness, pore width-length, crevice width-length were measured using an ocular micrometre. The data obtained revealed that there was a reduction in the values of the groups getting fungicide application as compared to the control. Increased dosage resulted in a decrease in the values of fertile pollen percentages in all fungicide applied groups and the toxic effect became more evident at higher doses. Generally there was an overall increase in the percentage of oblate spheroidal type pollens at higher dosages as compared to the control, but the percentage of prolate spheroidal types decreased. Furthermore, some pollen morphological structures that are not observed in the control group were encountered in the pollens in polar view at 60 g/ 100 L Switch and 125 mL/ 100 L Mythos.

Introduction

Rapidly increasing world population is regarded as one of the most important problems for mankind in the decades to come. A decline in the agricultural areas plays an important role in this direction and lead to certain restrictions in feeding the growing population. Therefore, an evaluation of the available land for obtaining the maximum yield has become a major goal. One of the applications in this connection has been the use of pesticides in plants against harmful organism for improving agricultural productivity. However, in addition to the benefits they provide, an excessive use of these chemicals, wrong applications as well as their side-effects have started creating serious environmental problems as well as posing toxicity threat to living organisms.

Pesticides used for eliminating various pests cause harmful effects on agricultural plants as well. They produce a toxic effect on the leaves where crucial functions such as photosynthesis and transpiration are carried out; cause morphological, anatomical and physiological changes; inhibit pollen germination and pollen tube formation; and thus affect the fruit production. A lot of work has been done on the effects of fungicides on pollen germination (He *et al.*, 1995). In pollens treated with fungicides under *in vitro* conditions, a decrease in pollen germination, deformation and cracks in pollen tubes have been reported (Lacerda *et al*., 1994; Pavlik & Jandurova, 2000). The pollen germination in apples treated with Captan decreases by 20 % as compared to the control (Yı *et al.*, 2003). A decrease in the pollen germination and pollen tube growth has been recorded in tomato flowers as well when treated with Chlorothalonile 75% WP (3.200 ppm of a.i.), Mancozeb 80% WP (2.400 ppm of a.i.), Mancozeb 48% WP (1,680 ppm of a.i.)+Metalaxyl 10% WP (350 ppm of a.i.) and Dibrom 86% EC (1.030 ppm of a.i.) (Lacerda *et al.*, 1994). According to He & Wetzstein (1994) fungicide applications interfere in the pollen development and result in a delay in the flowering and leaf formation. In the studies on the effects of fungicides on the stigma morphology of the almond tree (*Prunus dulcis*), stigma surface treated with 4 types of fungicides (azoxystrobin, myclobutanil, ipradione and cyprodinil) has been examined under an electron microscope 4 and 24 hours after the application. Regional deformations on stigma surfaces and breakdown in stigmatic cells have been observed 24 hour after Azoxystrobin treatment. Similarly, Myclobutanil, Ipradione and Cyprodinil have also resulted in the substantial damage of stigma (Yı *et al.*, 2002).

Effects of pesticides used in large concentrations in the world as well as in Turkey have been investigated by many workers from different perspectives. A number of studies conducted recently have reported that fungicides create a toxic effect on pollens by upsetting pollen germination and thus fruit formation (Pavlik & Jandurava, 2000). The excessive use of triazole fungicide on some fruit trees during the flowering period has been observed to cause a negative effect on pollen germination and fruit formation (Marcucci & Filiti, 1984; Redalen, 1980). Similarly Captan and some other fungicides have been reported to reduce pollen vitality in many apple cultures (Church & Williams, 1977). Although many studies have been conducted on the effects of pesticides on pollen germination and pollen tube growth, very few studies have been carried out on the effects of pesticides on the pollen morphology and anatomy. The present study covers the effects of Switch 62.5 WG and Mythos SC 300 fungicides, widely used on greenhouse grown tomatoes in Turkey against grey mould caused by the fungus *Botrytis cinerea*.

Materials and Methods

Ninety healthy tomato (*Lycopersicon esculentum* Mill.) seedlings obtained from M-19 F_1 type domestic seeds were sown @ 3 seedlings per pot. These were randomly planted in a total of 30 pots on August 27, 2003 in the greenhouse of Plant Protection Department, Agricultural Faculty, Ege University. Six pots were allocated for each group. The fungicides Switch 62.5 WG (37.5% Cyprodinil and 25 % Fludioxonil active ingrediant) and Mythos SC 300 (300 g/ L Pyrimethanil active ingrediant) were applied against grey mould caused by *Botrytis cinerea* (Table 1,2). A total of 5 groups, one control (untreated) and 2 treated groups for each fungicide were used during this study. The fungicide applications were prepared in 1 litre of water and applied at dosages recommended by the manufacturer and double the recommended dosage (Table 1). Applications were made 5 times at 10-day intervals until the end of the flowering period using a sprayer between 7:00-9:00 hours in the morning (Table 3).

Flower samples for the pollen analyses were randomly collected between 10:30-11:30 in the morning starting from the day after the treatment until the day of the next treatment. These were put in Karnoy fixative (3 parts 96% ethyl alcohol; 1 part glacier acetic asid) 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 dissection needle were mounted on gylcerine-gelatin-liquid safranine mixture (Wodehouse, 1965).

Table 1. Recommended dozes of fungicides and those applied in this study.

Fungicide	Applied dosage		Recommended dosage
Switch 62.5 WG	R.D.*	0.6 g /L water	60 g /100 L water
	R.D.X2**	1.2 g /L water	
Mythos SC 300	R.D.*	1.25 cc /L water	125 mL/100 L water
	R.D.X2**	2.50 cc /L water	

*R.D.: Dosage recommended; **R.D.X2: Double the recommended dosage.

Table 2. Description and characteristics of fungicides used.

Trade name	Active ingrediant	Chemical formula	Rates %	Class
Switch 62.5 WG	Cyprodinil	4-cyclopropyl-6-methyl-N-phenyl-2-Pyrimidinamine	37.5	Systemic
	Fludioxonil	4-(2,2-difluoro-1,3-benzodioxol-4-yl)-1H-pyrrole=3-carbonitrile	25	Contact
Mythos SC 300	Pyrimethanil	(4,6-dimethylpyrimidin-2-yl)aniline	300 g/L	Systemic-Contact

Table 3. Application dates of fungicides.

Application number	Switch 62.5 WG	Mythos SC 300
First	9.09.2003	9.09.2003
Second	19.09.2003	19.09.2003
Third	29.09.2003	29.09.2003
Fourth	8.10.2003	8.10.2003
Fifth	18.10.2003	18.10.2003

A total of 100 pollens from each group were used for the measurements of equatorial-polar length/width, exine - intine thickness in equatorial view, pore length/ width, colpus length/width in polar view. These were made with the help of a micrometric ocular on a 100-Prior microscope. The numbers and percentages of fertile and sterile pollens among 100 pollens were determined in each group. Sterile pollen types were photographed using a Jena and Olympus microscope. 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)

Statistical analyses of the values related to all measurements in our study were made on a SPSS 11.0 for Windows statistical program and Multiple Range Tukey Test was used for variance analyses (Tukey, 1954). The difference between "[a]" and the control group, "[b]" and the 60 g/100 L Switch group, "[c]" the 120 g/100 L Switch group "[d]" and the 125 mL/100 L Mythos group, and "[e]" the 250 mL/100 L Mythos group is statistically significant ($p<0.05$).

Results and Discussion

Fungicide applications interfere in the pollen development and result in a delay in the flowering and leaf formation (He & Wetzstein, 1994). For example Myclobutanil, Ipradione and Cyprodinil resulted in the substantial damage of stigma (Yı *et al.*, 2002).

Table 4. Length / width measurements of pollens in equatorial and polar view (µm).

Treatment	Equatorial view		Polar view	
	Width (µ)	Length (µ)	Width (µ)	Length (µ)
Control	18.733±0.157 [be]	19.065±0.176 [bce]	18.924±0.147 [e]	19.065±0.162 [e]
Switch (60 g/ 100 L)	17.903±0.233 [a]	17.952±0.232 [a]	18.094±0.246	18.284±0.232
Switch 120 g/ 100 L	18.172±0.211[a]	18.094±0.206 [a]	17.762±0.696	18.616±0.196 [e]
Mythos 125 mL/100 L	18.284±0.148[a]	18.367±0.115[a]	18.069±0.298	18.260±0.144
Mythos 250 mL/100 L	17.703±0.146 [a]	17.737±0.112 [a]	17.430±0.166 [a]	17.454±0.122 [a]

Table 5. Measurements of pores and colpi (µ)in polar view.

Treatment	Polar view			
	Pore width (µ)	Pore length (µ)	Colpus width (µ)	Crevice length (µ)
Control	4.838±0.108 [de]	4.755±0.082 [de]	3.012±0.122 [bcde]	16.990±0.377
Switch (60 g/ 100 L)	4.457±0.122	4.399±0.098	2.514±0.102 [a]	16.541±0.415
Switch 120 g/ 100 L	4.482±0.113	4.457±0.131	2.407±0.111 [a]	16.434±0.402
Mythos 125 mL/100 L	4.374±0.095 [a]	4.257±0.133 [a]	2.265±0.095 [a]	16.600±0.368
Mythos 250 mL/100 L	4.208±0.106 [a]	4.256±0.115 [a]	2.182±0.089 [a]	16.492±0.295

Table 6. Exine and intine measurements (µ) of the pollens.

Treatment	Equatorial view	
	Exine (µ)	Intine (µ)
Control	0.929 ± 0.057 [bcde]	0.550 ± 0.035 [bcde]
Switch 60 g/ 100 L	0.805 ± 0.018 [a]	0.381 ± 0.015 [a]
Switch 120 g/ 100 L	0.780 ± 0.025 [a]	0.392 ±0.021 [a]
Mythos 125 mL/ 100 L	0.747 ± 0.026 [a]	0.387 ± 0.014 [a]
Mythos 250 mL/ 100 L	0.771 ± 0.017 [a]	0.404 ± 0.015 [a]

An examination of the effects of the fungicides used in the present study on the length and width of pollens seen in equatorial and polar view showed that the values obtained in all treated groups were lower than those in the control group. Values obtained in the Mythos application showed a significant ($p<0.05$) decrease in the width and length of pollen as the dosage increased (Table 4). This decrease was found to be statistically significant at the higher dosage of Mythos 250 mL/100 L. However, the situation was a little different in the Switch group, in which the decrease in these parameters as seen in equatorial view at 60 and 120 g/100 L was statistically significant ($p<0.05$) as compared to the control group. In the Switch group, as the dosage increased, width and length values of the pollen seen in equatorial view as well as the length of the pollen seen in polar view also increased while a decrease was observed in the width value of the pollen seen in polar view with parallel to increasing in application dosage. Nevertheless, the increase and decrease in these had no significant ($p<0.05$) impact when the higher dosages in the treated groups were compared with recommended doses.

An examination of the results of width-length measurements related to pores and cracks of pollens seen in polar view reveals that the values in question are again lower in all treated groups (Table 5). Such a decrease in the pore width and pore length of the pollens was statistically significant as compared to the control ($p<0.05$) in the Mythos group. Meanwhile, the decrease that occurred in the colpus width of the pollen as compared to the control was found to have a statistical significance ($p<0.05$) in treated groups. The pore width value of the pollen seen in polar view increased in the Switch group as the dosage increased, while it decreased in the Mythos group. However, these values had no significance ($p<0.05$) as regards the recommended dosages of these fungicides. Pore length values increased in parallel with the dosage in the Switch group, but decreased in the Mythos group. These values at higher dosages of fungicides too had no significance ($p<0.05$) as compared to recommended dosages. The colpus width and colpus length values of pollens seen in polar view showed that values decreased in both fungicide groups as the dosage increased which was statistically insignificant ($p>0.05$) as compared to the recommended dosages.

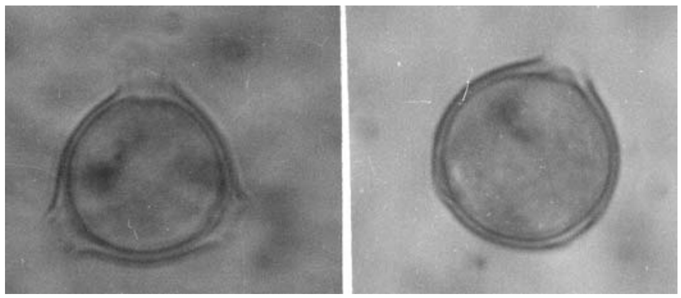

Fig. 1. Fertile pollen in the control group a) Equatorial View (6.3x100) b) Polar View (6.3x100).

The pollen germination in apples treated with Captan decreased by 20 % as compared to the control (Yı *et al.*, 2003). An evaluation of our results regarding the exine- intine layer thicknesses of the pollen seen in equatorial view depicts that all the values obtained in the treated groups are lower than the control (Table 6; Fig. 1). The decrease in the values for both exine and intine layers was found to be significant ($p<0.05$). As the dosage increased, the exine layer value of the pollen seen in equatorial view decreased in the Switch treated group, but increased in the Mythos group. The intine layer value, on the other hand showed a parallel increase with dosage. However, all values at higher dosages had no significance ($p<0.05$) as compared to recommended dosages. Our studies showed that fungicides result in the changes in pollen anatomical features of tomato plants as regards the values related to width-length of pollens seen in equatorial and polar view; exine-intine layer thickness of pollens seen in equatorial view; and pore width-length and colpus width length of pollens seen in polar view. A decrease was observed in all values in treated groups.

In pollens treated with fungicides under *in vitro* conditions, a decrease in pollen germination, deformation and cracks in pollen tubes have been reported (He *et al.*, 1995; Pavlik & Jandurova, 2000). An evaluation of the percentage fertility results showed that values in general decreased as compared to the control group and that this decrease occured in inverse proportion to the increase in dosage (Table 7). Various sterile pollen types such as wrinkled pollen, unstained pollen, pollen with abnormal shape and pollen with invisible pore were encountered in fungicide treated groups (Fig. 2). This decrease in the percentage of fertile pollens in the treated groups as the dosage increases can be attributed to the 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 treated groups as compared to the control could produce a negative effect on fruit productivity and quality of tomato in the long run. Our findings agree with those of Fairbanks *et al*., (2002) who report that Phosphite treatment reduces the pollen fertility of *Dryandra sessilis, Adenanthos barbiger, Boronia cymosa, Hovea elliptica, Phyllanthus calycinus, Comesperma calymega, Eramaea astrocarpa* and *Hibbertia hypericoides* during either after spring and fall applications of this fungicide or after summer applications.

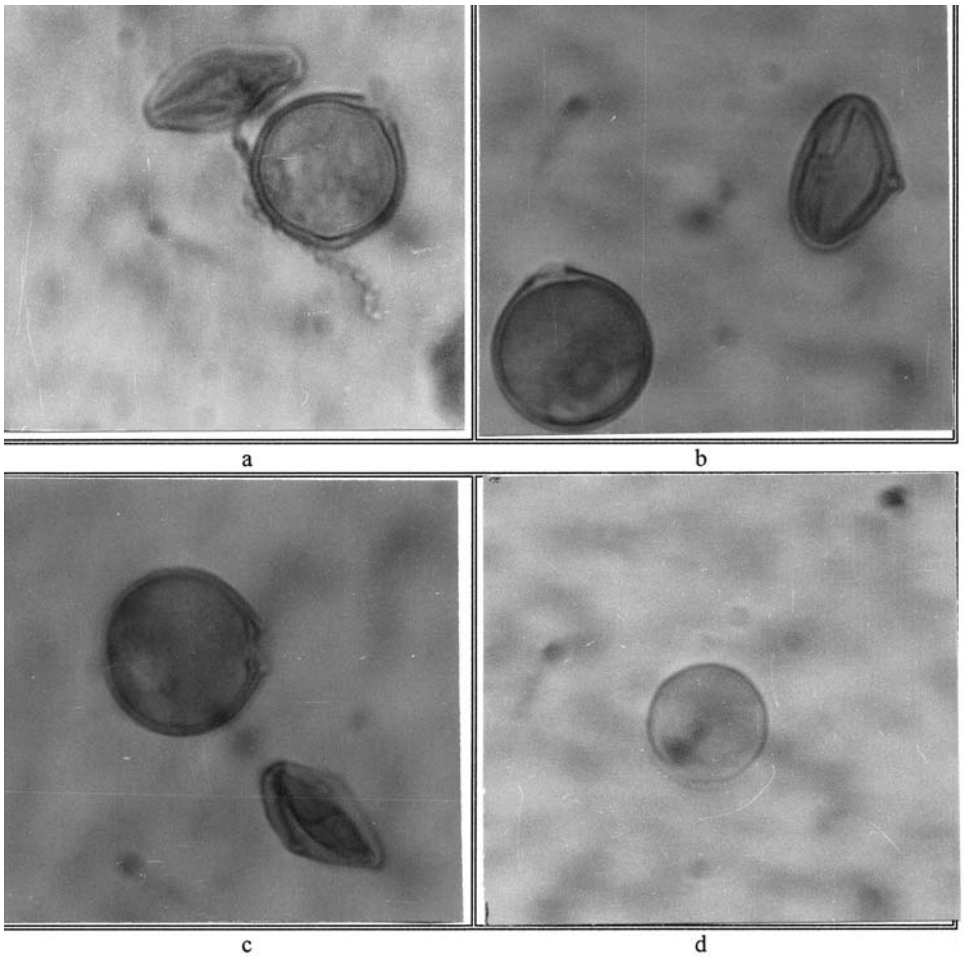

Fig. 2. Sterile pollen types in fungicide application groups a) Switch 60 g/100 L group, wrinkled sterile pollen (6.3x100) b) Switch 120 g/100 L group, sterile pollen with abnormal shape (6.3x100) c) Mythos 125 mL/ 100 L group, wrinkled sterile pollen (6.3x100) d) Mythos 250 mL/ 100 L group, unstained sterile pollen (6.3x100).

Pollen morphology is another important variable affected by fungicides (Erdtman, 1966; Yi *et al*., 2003). The effects of fungicides 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 treated groups as compared to the control except for the 120 g/ 100 L dosage of Switch in polar view (Table 8). The percentage of oblate spheroidal pollens seen in equatorial view increased in parallel with the increase in dosage, while the percentage of prolate spheroidal pollens decreased in an inverse proportion to the increase in dosage. Similarly, the percentage of oblate spheroidal pollens in polar view was higher and that of prolate spheroidal ones lower in all treated groups except for the 120 g/100 L dosage of Switch. The percentage of oblate spheroidal pollens seen in polar view decreased as the dosage increased, while the percentage of prolate spheroidal pollens increased in parallel with the applied dosage. However, unlike

Table 7. Pollen fertility of the control and fungicide application groups.

Treatment	Fertile pollen (%)	Wrinkled pollen (%)	Unstained pollen (%)	Pollen with unvisible pore (%)	Pollen with abnormal shape (%)	Total sterile pollen (%)
Control	89	4	0	6	1	11
Switch 60 g/ 100 L	69	12	2	14	3	31
Switch 120 g/ 100 L	67	14	2	12	5	33
Mythos 125 mL/100L	68	8	1	21	2	32
Mythos 250 mL/100L	61	6	1	26	6	39

Table 8. Pollen shape classification in the control and fungicide application groups.

Treatment	Pollen shape classification (%)						
	Equatorial view		Polar view				
	Oblat Spheroidal (%)	Prolate Spheroidal (%)	Oblat Spheroidal (%)	Prolate Spheroidal (%)	Subprolate (%)	Suboblate (%)	Prolate (%)
Control	63.33	36.66	60.00	40.00	0.00	0.00	0.00
Switch 60 g/ 100 L	73.33	26.66	63.33	30.00	3.33	3.33	0.00
Switch 120 g/ 100 L	80.00	20.00	56.66	43.00	0.00	0.00	0.00
Mythos 125 mL/ 100 L	66.66	33.33	76.66	20.00	0.00	0.00	3.33
Mythos 250 mL/ 100 L	76.66	23.33	73.33	26.66	0.00	0.00	0.00

other treated groups, subprolate as well as suboblate pollens were encountered in pollen groups seen in polar view at 60 g/ 100 L Switch; moreover, pollens belonging to prolate shape class were seen at 125 mL/100 L Mythos in polar view. The fungicides used in our treatments led to some changes in the morphological features of tomato pollens. Our studies show that the tomato pollen fertility is seriously affected by fungicides used to control various fungal diseases affecting the tomato plant. This could lead to a decrease in the productivity of fruits.

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