

# The Effect of Fungicide Application on Pollen Structure in Tomato (*Nıeqr gt ukəqp 'ğüewıgp wwo 'O knı*) Plant

İlkay Öztürk ÇALI\*<sup>1</sup>

Feyza CANDAN <sup>2</sup>

<sup>1</sup> Amasya University, Faculty of Art and Science, Department of Biology, Amasya-Turkey

<sup>2</sup> Celal Bayar University, Faculty of Art and Science, Department of Biology, Manisa-Turkey

Corresponding Author.

E-mail: ilkay.cali@amasya.edu.tr

Received: July 10, 2008

Accepted: September 22, 2008

## Abstract

The aim of the study was to investigate effect of Agri Fos 400 (400 g/ L Mono and di-potassium phosphanate) which is a fungicide widely used on tomatoes grown in greenhouse on pollen structure of tomato (*Lycopersicon esculentum* Mill.). The fungicide was applied to tomatoes at recommended dosage (400 mL/ 100 L tap water) by the manufacturing company. Measurements of pollen width-length, pore-crevice width-length and exine-intine layer thicknesses were made using a micrometric ocular. A reduction was observed in the values of fungicide group except for intine layer thickness when compared to the control. On the other hand, the fungicide caused changes on the surface layer of pollen. Papillae seen on the surface layer of pollen was damaged in the fungicide group.

**Key Words:** Fungicide, *Lycopersicon esculentum*

## INTRODUCTION

Pesticides applied at excessive dosages ignorantly caused many problems. Application of excessive dosages of pesticides bring on utmost residue problems which damage human and animal health. Besides pesticides used ignorantly pollute nature and result in that there is a decrease in sensitivity of organism against these chemicals [1].

Pesticide applications that are made against pests seen in agricultural areas have harmful effects on pollens of agricultural plants too.

It was reported that fungicides have detrimental effects on pollen germination [2; 3] and pollen tube growth [4, 5].

It was stated that morphological structures not seen in the control group were observed in tomato plants applied with 40 g/ 100 L dosage of Chorus 50 WG (50 % Cyprodinil) fungicide [6].

Besides Captan and various other fungicides which belong to the family Phthalamide have reduced pollen viability in many apple cultures [7].

Moreover a lot of insecticides have caused chromosomal anomalies in mitotic and meiotic systems [8].

It was reported that pollen fertility was reduced in *Pterocheata paniculata*, *Podotheca gnaphalioides* and *Hyalosperma cotula* applied with Phosphite fungicide [9].

The scope of present study covers pesticide applications that are frequently made against diseases and pests seen in agricultural areas. In the study the effects of the Agri Fos 400 fungicide at recommended dosage were investigated on the pollens of tomato plants.

## MATERIALS AND METHODS

The study material selected was comprised of the tomato (*Lycopersicon esculentum* Mill.) plant obtained from M-38 F1 type domestic seeds. Agri Fos 400 (400 g/ L Mono and Di-Potassium Phosphanate) which is a fungicide used against *Phytophthora infestans* in tomato was applied.

A total of two groups one control and one application group for fungicide were formed for the study. The control group was not treated with any chemicals. As for the application group, application was made at dosage recommended (400 mL/ 100 L water) by the manufacturer.

Work for obtaining the tomato flowers to be used for determining the effects of fungicide on tomato pollens was conducted at 970 m<sup>2</sup> – greenhouse in the village of Karaçulha in Fethiye. 152 healthy seedlings were grown from M-38 F1 type domestic tomato seeds. 76 healthy seedlings were used for each group. A total of 4 applications were made on 10-day intervals.

The treatment was made using a sprayer between 7:00-9:00 hours in the morning. 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 and then fixed in Karnoy fluid (3 parts 96 % ethyl alcohol; 1 part glacier acetic acid). After that the anthers were taken from ripe floral buds with the help of a dissection needle and they were placed on a slide containing glycerine-gelatin with liquid safranin [10].

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. Pollens in the control and the application group were photographed using a JEOL JSM-6060 Scanning Electron Microscope [11].

Statistical analyses of the values related to all the measurements in the study were made on a SPSS 11.0 for Windows statistical program and Multiple Range Tukey Test was used for variance analyses [12]. The difference between "a" and the control group, "b" and the 400 mL/ 100 L Agri Fos 400 group is statistically significant ( $p < 0.05$ ).

## RESULTS AND DISCUSSIONS

It was determined that 400 mL/ 100 L dosage of the Agri Fos 400 fungicide caused some changes on the surface structure of tomato pollen.

tion group (Table 2).

When the results are to be evaluated as regards the exine-intine layer thicknesses of the pollen seen in equatorial view, it can be seen that exine layer thickness decreased as compared to the control, but intine one increased (Table 3).

On the other hand in the present study, it was found that the fungicide resulted in changes on the surface layer of the tomato pollen.

It was determined that papillae seen on the surface layer of the pollen in the control group was damaged in the fungicide group (Fig. 1, 2).

Although many studies have been carried out on the effects of fungicides on pollen germination and pollen tube growth, there are very few studies on the effects of fungicides on pollen structure.

It was demonstrated that fungicide applications caused problems in the development of pollen [13].

In pollens treated with fungicides under in vitro conditions, pollen germination often decreased and deformation and cracks occur in pollen tubes [14].

**Table 1.** Length-Width measurements of pollens in equatorial and polar view ( $\mu$ ).

Treatment	Equatorial view		Polar view	
	Width ( $\mu$ )	Length ( $\mu$ )	Width ( $\mu$ )	Length ( $\mu$ )
Control	21.916 $\pm$ 0.230 <sup>b</sup>	22.583 $\pm$ 0.145 <sup>b</sup>	21.000 $\pm$ 0.227 <sup>b</sup>	22.333 $\pm$ 0.166 <sup>b</sup>
Agri Fos 400 400 mL/ 100 L	20.375 $\pm$ 0.148 <sup>a</sup>	21.125 $\pm$ 0.218 <sup>a</sup>	19.916 $\pm$ 0.231 <sup>a</sup>	19.958 $\pm$ 0.203 <sup>a</sup>

**Table 2.** Measurements of pores and colpi in polar view ( $\mu$ ).

Treatment	Polar view			
	Pore width ( $\mu$ )	Pore length ( $\mu$ )	Colpus width ( $\mu$ )	Crevice length ( $\mu$ )
Control	7.541 $\pm$ 0.111 <sup>b</sup>	7.833 $\pm$ 0.157 <sup>b</sup>	5.500 $\pm$ 0.372 <sup>b</sup>	20.333 $\pm$ 0.198 <sup>b</sup>
Agri Fos 400 400 mL/ 100 L	6.683 $\pm$ 0.241 <sup>a</sup>	7.125 $\pm$ 0.181 <sup>a</sup>	2.641 $\pm$ 0.097 <sup>a</sup>	17.250 $\pm$ 0.277 <sup>a</sup>

**Table 3.** Exine-intine measurements of the pollens ( $\mu$ ).

Treatment	Equatorial view	
	Exine ( $\mu$ )	Intine ( $\mu$ )
Control	2.316 $\pm$ 0.044 <sup>b</sup>	1.266 $\pm$ 0.053 <sup>b</sup>
Agri Fos 400 400 mL/ 100 L	1.716 $\pm$ 0.073 <sup>a</sup>	1.386 $\pm$ 0.010 <sup>a</sup>

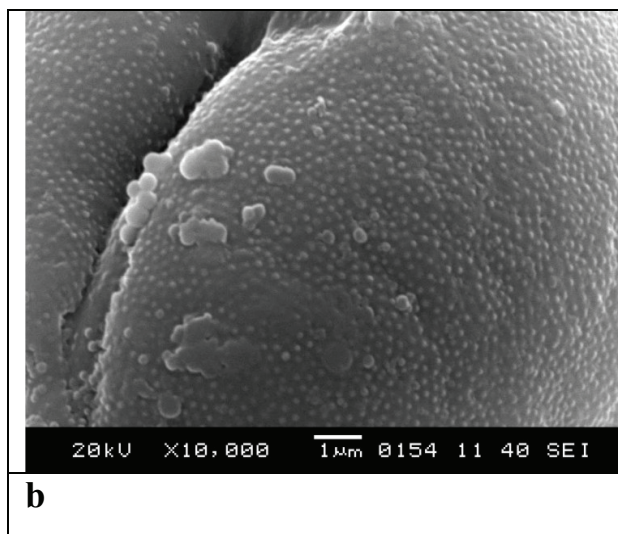
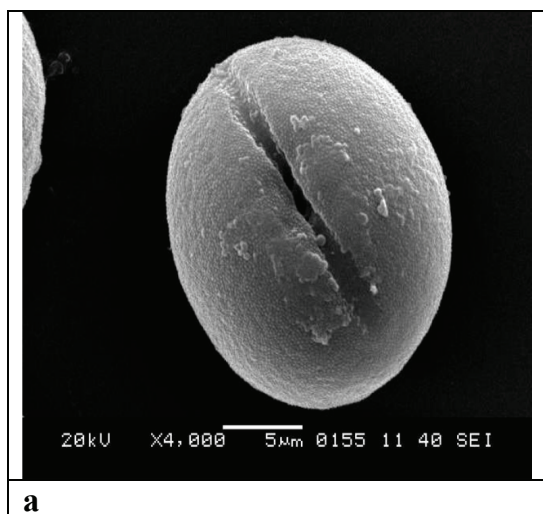
An examination of the effects of the fungicide used in the present study on the width-length measurements of pollens seen equatorial and polar view showed that the values obtained in the application group were lower than those in the control group (Table 1). When results of width-length measurements related to pores and cracks of pollens seen in polar view are examined the values mentioned are again lower in the applica-

In another study investigating the effects of fungicides on the stigma morphology of the almond tree (*Prunus dulcis*), stigma surface treated with Ipradione and Cyprodinil fungicides was examined under an electron microscope 4 and 24 hours after the application. It was emphasized that the fungicides led to harmful effects on the morphology of the stigma and stigmatic papillae were also harmed in treatments with the fungicides

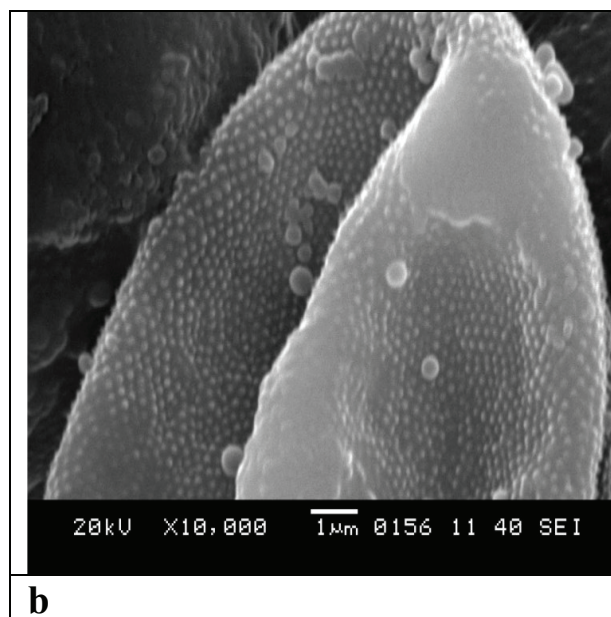
[15].

It was reported that changes in pollen shapes were occurred in tomato plant treated with Mythos SC 300 (300 g/ L Pyrimethanil) and in the same study increasing number of abnormal pollen shape was observed [16].

Width-length measurements of pollens, pore-crevice width-length and exine-intine layer thicknesses decreased in tomato plants applied with 40 g, 80 g and 120 g/ 100 L water dosages of Chorus 50 WG (50 % Cyprodinil) according to the control [18].



**Figure 1.** SEM photographs of pollens in the control group. a) General appearance of pollen. b) Papillae seen on the surface layer of pollen.



**Figure 2.** SEM photographs of pollens in the fungicide group. a) General appearance of pollen treated with 400 mL/ 100 L dosage of Agri Fos 400 group. b) Papillae seen on the surface layer of pollen was damaged in the fungicide group.

It was reported that equatorial and polar width-length of pollens, as well as pore-crevice width-length seen in polar view were decreased in the Switch 62.5 WG (37.5 % Cyprodinil + 25 % Fludioxonil) fungicide group at the dosages of 60 g and 120 g/ 100 L as compared to the control [17]. On the other hand, in the same study it was determined that the values of exine-intine layer thicknesses of pollen seen in equatorial view decreased in the application groups treated with 125 mL and 250 mL/ 100 L dosages of Mythos SC 300 fungicide according to the control.

The results of the studies above are in agreement with the results of the present study.

In the present study, it was determined that 400 mL/ 100 L water dosage of Agri Fos 400 fungicide caused important changes in pollen structure of tomato plant. The values except for intine layer thickness decreased in the application group according to the control. Besides 400 mL/ 100 L dosage of the fungicide gave rise to changes on the surface layer of the pollen. Papillae observed on the surface layer of the pollen were

damaged in the fungicide group.

## REFERENCES

- [1] Durmuşoğlu E. 2002. Studies on the residue of some organophosphorus insecticides in tomato and cucumber in market of Izmir province. *Turkish Journal of Entomology*. 26(2): 93-104.
- [2] Bristow PR, Windom GE. 1987. Effects of selected fungicides, insecticides and adjuvants on in vitro germination of high bush blueberry pollen. *Plant Disease*. 71: 326-328.
- [3] Watters BS, Sturgeon SR. 1990. The toxicity of some foliar nutrients and fungicides to apple pollen cv. golden delicious. *Tests of Agrochemicals and Cultivars 11. Annals of Applied Biology*. 116: 70-71.
- [4] He Y, Palevitz BA, Wetzstein HY. 1996. Pollen germination, tube growth and morphology, and microtubule organization after exposure to benomyl. *Physiologia Plantarum*. 96: 152-157.
- [5] Abbott JD, Bruton BD, Patterson CL. 1991. Fungicidal inhibition of pollen germination and germ-tube elongation in muskmelon. *HortScience*. 26: 529-530.
- [6] Öztürk Çalı İ. 2005. The effects of Cyprodinil application on morphology and fertility of tomato (*Lycopersicon esculentum* Mill.) Pollen. Cumhuriyet University, The Faculty of Art and Science, The Journal of Science. 26(1): 26-34.
- [7] Church BM, Williams RR. 1977. The toxicity to apple pollen of several fungicides as demonstrated by in vivo and in vitro techniques. *Journal of Horticultural Science*. 52: 429-436.
- [8] Nicloff N, Kappas A. 1987. Benomyl induced mitotic disturbances in *Hordeum vulgare*. *Mutation Research*. 189: 271-275.
- [9] Fairbanks MM, Hardy GESTJ, McComb JA. 2002. Mitosis and meiosis in plants are affected by fungicide phosphite. *Sexual Plant Reproduction*. 13(6): 315-321.
- [10] Wodehouse RP. 1965. *Pollen Grains*, Hamer Press., New-york, 249 pp.
- [11] Nepi M, Ciampolini F, Pacini E. 1995. Development of Cucurbita pepo pollen: Ultrastructure and histochemistry of the sporoderm. *Canadian Journal of Botany*. 73: 1046-1057.
- [12] Tukey JW. 1954. Some Selected Quick and Easy Methods of Statistical Analysis, *Trans of New York Acad. Sci.*, pp. 88-97.
- [13] He Y, Wetzstein HY. 1994. Pollen degeneration and retarded leaf development from fungicidal sprays applied during microspore development and shoot expansion. *Journal of Horticultural Science*. 69: 975-983.
- [14] Pavlik M, Jandurova OM. 2000. Fungicides cytotoxicity expressed in male gametophyte development in *Brassica campestris* after in vitro application of converted field doses. *Environmental Experimental Botany*. 44: 49-58.
- [15] Yı W, Law SE, Wetzstein HY. 2002. Fungicide sprays can injure the sitigmatic surface during receptivity in almond flowers. *Annals of Botany*. 91:1-7.
- [16] Kesercioğlu T, Öztürk Çalı İ. 2007. Effects of Pyrimethanil on pollen meiosis of tomato plant (*Lycopersicon lycopersicum* Mill.). *Bangladesh Journal of Botany*. 36(1): 85-88.
- [17] Tort N, Öztürk İ, Güvensen A. 2005. Effects of some fungicides on pollen morphology and anatomy of tomato (*Lycopersicon esculentum* Mill.). *Pakistan Journal of Botany*. 37(1): 23-30.
- [18] Öztürk İ. 2006. The effect of fungicide on the structure of tomato (*Lycopersicon esculentum* Mill.) pollen. The Book of 18. National Biology Congress, 26-30 June 2006 Kuşadası/Aydın-Turkey, Nobel Publication, pp. 121.
- [33] Rossignol, M., Lamant, D., Salsac, L., and Heller, R. 1977. Calcium fixation by the roots of calcicole and calcifuge plants: the importance of membrane systems and their lipid composition. In: Thellier, M., Monnier, A., Demarty, M., Dainty, J. (eds) *Transmembrane ionic exchange in plants*. CNRS Paris., P 483.
- [34] Montoro, P.H.E., Michaux-Ferriere, N., and Carron, M.P. 1993. Callus friability and somatic embryogenesis in *Hevea brasiliensis*. *Tiss. Org. Cult*. 33: 331-338.
- [35] Montoro, P., Teinseree, N., Rattana, W., Kongsawadworakul, P., and Michaux-Ferriere, N. (2002). Effect of exogenous calcium on *Agrobacterium tumefaciens*-mediated gene transfer in *Hevea brasiliensis* (rubber tree) friable calli. *Plant Cell Rep*. 19: 851-855.
- [36] Chai, M.L., Xu, C.J., Senthil, K.K., Mo, S.Y., Chung, Y.S., Cho, S.H., Shin, J.S., Park, M.H., and Kim, D.W. 2002. Stable transformation of protocorm like-bodies in *Phalaenopsis orchid* mediated by *Agrobacterium tumefaciens*. *Sci. Hort*. 96: 213-224.
- [37] Mohanty, A., Sarma, N.P., Tyagi, A.K. 1999. *Agrobacterium* mediated high frequency transformation of an elite Indica rice variety Pusa Basmati 1 and transmission of the transgenes to R2 progeny. *Plant Sci*. 147: 127-137.
- [38] Wilson, T.L.Y., Janna, O. A., and Maziah, M. 2006. Optimization of *Agrobacterium*-mediated transformation parameters for *Melastomataceae* spp. Using green fluorescent protein (GFP) as a reporter. *Sci. Hort*. 38: 101-105