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EFFECTS OF FUNGICIDE ON MEIOSIS OF TOMATO (*LYCOPERSICON ESCULENTUM* MILL.)

İLKAY ÖZTÜRK ÇALI

Department of Biology, Faculty of Art and Science,
Amasya University, Amasya, Turkey
E-mail: <ilkayozt@yahoo.com>

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Abstract

The effects of Equation Pro (22.5 % Famoxadone + 30 % Cymoxanil) fungicide applied to tomato grown in greenhouse at recommended dosage (40 g/100 l tap water) by manufacturing company and double the recommended dosage (80 g/100 l tap water) were investigated on pollen meiosis. It was observed that the fungicide used in trial applications caused anomalies in pollen meiosis in parallel with the increase in dosage. It is obvious that this condition will have a negative effect on pollen fertility.

Introduction

A number of chemical compounds are used to protect agricultural products from diseases and weeds. Residues of these chemicals lead to environmental pollution and pose threat to people and animals. Although chemical control creates several problems, use of pesticides is still maintaing its popularity for obtaining effective results.

Pesticide applications in agricultural plants have harmful effects on their cytological mechanisms. Pollen germination was reduced by *in vitro* fungicide applications and degeneration in pollen tubes occurred (Pavlik and Jandurova 2000). Tort *et al*. (2005) observed that pyrimethanil fungicide caused changes in the structure of tomato pollens. Moreover, it was reported that fungicides resulted various chromosomal abnormalities, such as Deltan applications (0.3%) gave rise to chromatid and subchromatid breaking in red pepper (Prakash *et al.* 1988). Thiodan, Folithion, Lebaycid and Kitazin caused a fragmentation, lagging chromosomes, bridges, tripolar and tetrapolar spindle in barley (Grover and Tyagi 1980). Therefore, it is necessary to study the effect of overdose application of common pesticides on plants. In this study, the effects of different concentrations recommended and double to recommend dosages of Equation Pro fungicide on meiosis of tomato were investigated.

Materials and Methods

The study was carried out in a 970 m² greenhouse in the village of Karaçulha in Fethiye, Turkey. Healthy tomato seedlings were obtained from M-19 F_1 type domestic seeds. The fungicide used in the study was Equation Pro (22.5% Famoxadone + 30% Cymoxanil). A total of five applications, 40 g/100 l tap water as recommended by the manufacturing company on the label and 80 g/100 l tap water as double the recommended dosage, were made at ten-day intervals. Flower bud randomly collected from different plants were fixed in Carnoy's fluid. For meiotic study smear preparation method was applied. Anthers were stained with 2 % aceto-orcein before being smeared (Östergren and Heneen 1962).

Cell shapes, cell division patterns, cell sizes and bivalent arrangements of a total of 100 pollens from each group were examined and photographed using a Jena microscope. Statistical analysis of the values obtained from all the measurements in the study were made on a SPSS 11.0 for Windows Statistical Program, and the variance analyses were made using the Chi-Square Test, a nonparametric test widely utilized in such procedures. The tables show that the differences among control, 40 g/100 l Equation Pro group and 80 g/100 l Equation Pro group are statistically significant ($p < 0.05$).

Results and Discussion

It was observed that 40 g/100 l and 80 g/100 l dosages of Equation Pro fungicide brought about some changes with increased dosage in shape and size of pollen, chromosome structure and cell division patterns of tomato plant.

Effect of Equation Pro on the pollen shape revealed that the number of round pollens in both application groups was lower than that observed in the control group, whereas the number of oval pollens was higher (Table 1). These fluctuations in the values were also found to be significant as compared to the control group. When the application groups were assessed among one another, it was seen that the number of round pollens decreased in parallel with the increase in dosage whereas that of oval pollens increased as the dosage increased. On the other hand, presence of "triangular" and "rectangular shaped" not seen in the control was observed in the application groups. Similarly, the number of abnormally shaped pollens was higher in the application groups as compared to the control (Figs. 1, 2). It was stated that abnormal main pollen cells were observed in various pesticide applications (Prakash *et al.* 1988). Liang *et al.* (1967) reported that these abnormal main pollen cells occurred sterile pollen grains.

Table 1. Cell shapes of pollens in control and fungicide application groups.

Application groups	Cell shape				
	Round	Oval	Triangular	Rectangular	Abnormal shaped
Control	87 ^{bc}	11 ^{b}	0	0	2 ^{bc}
Equation Pro 40 g/100 l	50 ^{ac}	23 ^{ac}	1	0	26 ^{a}
Equation Pro 80 g/100 l	20 ^{ab}	43 ^{ab}	1	1	35 ^{a}

In a vertical column numericals having same alphabet do not differ significantly at 5% level.

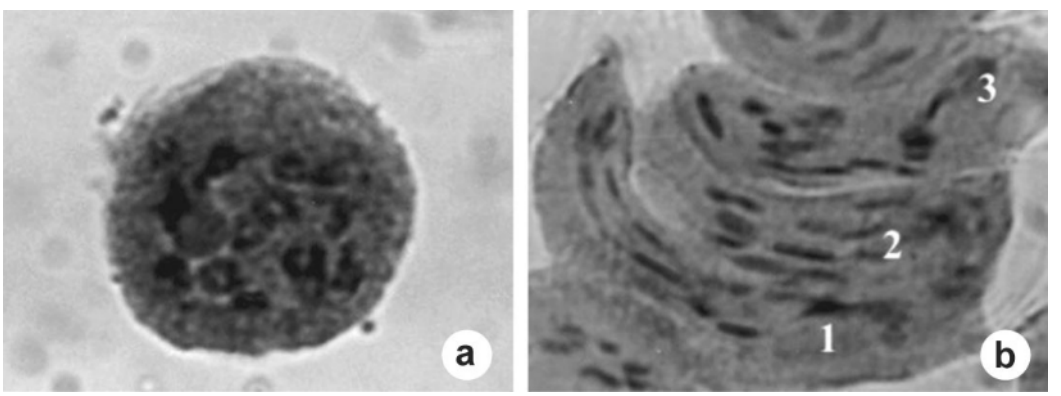

Fig. 1. Meiotic division abnormalities in the Equation Pro 40 g/100 l group. Ring-shaped (a); Large cell (b 1); Linear chromosome (b 1, 2, 3); Abnormal shaped (b 1, 2, 3).

The number of distinct bivalents in the 80 g/100 l fungicide group was lower as compared to the control group, whereas the number of indistinct ones higher in all applied groups (Table 2). These increase and decrease observed in the values were statistically significant in the 80 g/100 l dosages as compared to dosage recommended on the label. The number of chromosome anomalies

except for polar distortion was found to increase in all application groups as compared to the control with increasing dosage (Figs. 1, 2).

It was stated that there was an increase in the frequency of chromosome anomalies with parallel to increase in fungicide dosage (Ma 1982). In the present study, chromosome anomalies increased with parallel to increase in dosage as well. It is thought that this situation can effect pollen germination, pollen tube growth and pollen fertility negatively. Reddy and Rao (1981) reported that there was a positive relationship between chromosome anomalies and pollen sterility. Chromosome anomalies observed in the trial applications was seen in some pesticide application as well. It was also reported that application of certain insecticide had caused ring-shaped chromosome anomalies in *Hordeum vulgare* (Pusztai 1983). Bavistin and Deltan fungicides had led to chromosome anomalies in the form of binding in red pepper (Prakash *et al.* 1988). On the other hand, it was stated that Nitralin had brought about abnormal polarization in *Allium cepa* (Badr 1979) and CIPC herbicide had resulted in "ski-shaped" chromosomes in *Vicia faba* (Yoshida *et al.* 1983).

Table 2. Arrangement of bivalents in pollens of control and fungicide application groups.

Application groups	Arrangement of bivalents						
	Distinct	Indistinct	Thread-like	Ring-shaped	Linear	Binding	Polar distortion
Control	86 [c]	14 [bc]	1 [bc]	4 [bc]	3 [bc]	2 [bc]	0
Equation Pro 40 g/100 l	64 [c]	36 [ac]	13 [ac]	15 [ac]	15 [ac]	20 [ac]	4
Equation Pro 80 g/100 l	43 [ab]	57 [ab]	29 [ab]	31 [ab]	40 [ab]	38 [ab]	7

In a vertical column numericals having same alphabet do not differ significantly at 5% level.

Values of cell division patterns and cell sizes related to control and fungicide application groups were given in Table 3. The number of properly divided cells and the number of normal size cells decreased in the 80 g/100 l group, whereas the numbers of improperly divided and large cells in all application groups increased (Figs. 1, 2). The decrease in the numbers of properly divided cells and normal size cells was statistically significant in the 80 g/100 l dosage of Equation Pro as compared to the control. The increase in the numbers of improperly divided and large cells was found to have statistically significant in both fungicide groups as compared to the control. On the

Table 3. Cell division patterns and cell sizes of pollens in control and fungicide application groups.

Application groups	Cell division patterns		Cell size		
	Proper	Improper	Normal	Large	Small
Control	98 [c]	2 [bc]	96 [c]	4 [bc]	0
Equation Pro 40 g/100 l	74	26 [a]	83	17 [ac]	0
Equation Pro 80 g/100 l	65 [a]	35 [a]	65 [a]	35 [ab]	0

In a vertical column numericals having same alphabet do not differ significantly at 5% level.

the other hand, the number of large cells increased in parallel with the increase in dosages in application groups (Fig. 1). It was stated that improperly divided cells were observed in various pesticide applications as well. For example, Clorpyriphos insecticide caused abnormal anaphase where improperly dispersial of chromosome was seen (Rao *et al.* 1988). Furthermore, it was

reported that Topsin and Vitavax fungicides led to spindle-fibrile anomalies in root cells of *Allium cepa* (Somashaker and Gowda 1984).

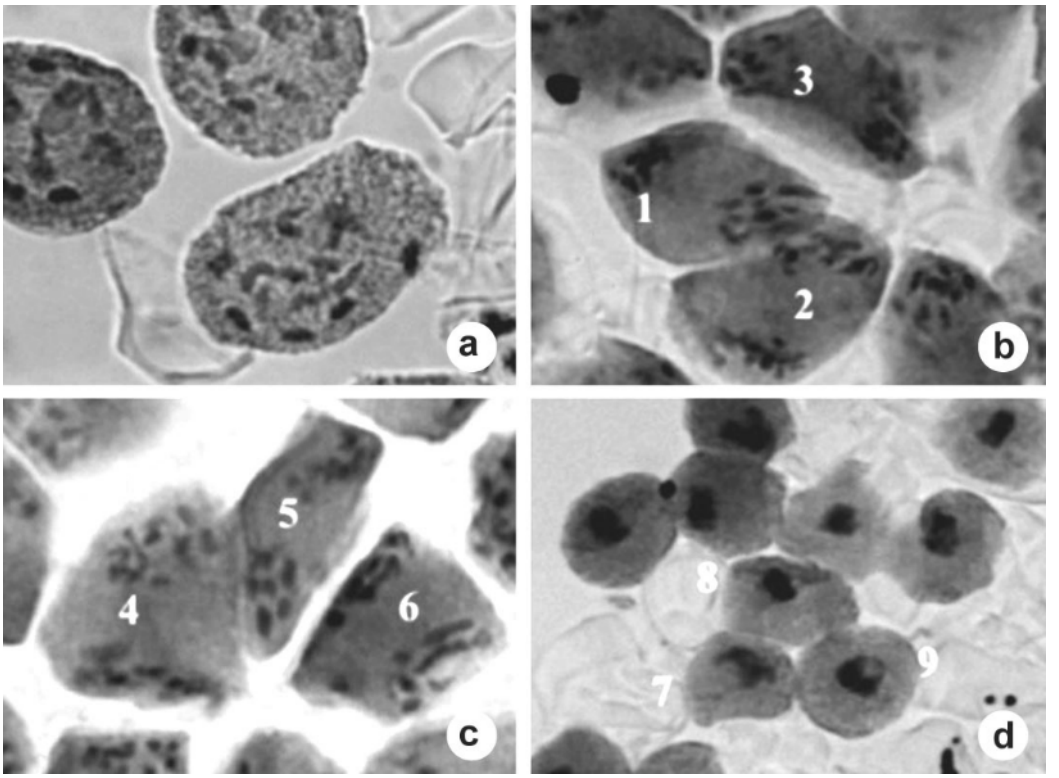

Fig. 2. Meiotic abnormalities in the Equation Pro 80 g/100 l group. Thread-like chromosome (a); Linear chromosome (a), (b 1,3), c (4, 5, 6); Abnormal shaped (b 1, 2, 3), (c 4, 5, 6), (d 7); Binding (d 7, 8, 9); Improper cell division (b 1, 2 ,3), (c 4, 5 ,6); Polar sliding (b 2), (d 7, 8).

Mohandas and Grant (1972) stated that pesticides could give rise to mutation by changing genetic structure of plants. Chromosomal changes resulted due to pesticides application had stimulated mutation similar to mutation occurred by mutagenous chemical substance (Kerkenaar 1981). In this study, it was found that Equation Pro fungicide applied to tomato even at the recommended dose resulted various anomalies in chromosomes structures. Therefore, farmers must take maximum care about the concentration of Equation Pro during application. Since chromosomal abnormalities were found in the recommended dose, the manufacturer is suggested to revise the recommended dose for this fungicide.

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