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Cytogenetic effects of fungicide applications on meiosis of tomato (*Lycopersicon esculentum* Mill.)

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Abstract: In the present study, the cytogenetic effects of the fungicide Agri Fos 400 (400 g/l mono- and di-potassium phosphonate) on meiosis of *Lycopersicon esculentum* Mill. (Family: Solanaceae) were studied. The fungicide was applied to tomatoes grown in greenhouse conditions at the dosage (400 ml/100 l water) recommended by the manufacturing company and double the recommended dosage (800 ml/100 l water). All dosages of the fungicide Agri Fos 400 caused various abnormalities in meiosis when compared with the control. This situation could lead to a decrease in the productivity of fruits.

Key words: Agri Fos 400, Cytotoxicity, Tomato, Meiocytes

Domatesin (*Lycopersicon esculentum* Mill.) polen mayozu üzerine fungisit uygulamalarının sitogenetik etkileri

Özet: Bu çalışmada *Lycopersicon esculentum* Mill.'in (Familya: Solanaceae) mayotik kromozomları üzerine Agri Fos 400 (400 g/l mono ve di-potassium phosphonate) fungisitinin sitogenetik etkileri incelenmiştir. Bu fungisit, sera koşullarında yetiştirilen domates bitkilerine üretici firmaca önerilen dozda (400 ml/100 l suya) ve önerilenin iki katı (800 ml/100 l suya) dozunda uygulanmıştır. Agri Fos 400 fungisitinin uygulanan tüm dozları kontrole göre polen mayozunda çeşitli anomalilere neden olmuştur. Bu durum meyve veriminde bir azalmaya yol açabilir.

Anahtar sözcükler: Fungisit, Sitogenetik etki, Domates, Mayoz, Polen

Introduction

Modern agriculture and industry depend on a wide variety of synthetically produced chemicals, including insecticides, fungicides, herbicides, and other pesticides. Continual widespread use and release of such synthetics has become an everyday occurrence, resulting in environmental pollution (1). It was indicated that many cytogenetic studies have been carried out to detect the harmful effect of different pesticides on different plants (2,3,4,5). These chemicals used at excessive dosages can give rise to abnormal chromosomes and degeneration in mitotic cycle, such as micronucleus formation, chromosome bridges, and polyploidy (6). Benomyl, a systemic fungicide, affects germination, mitotic and meiotic activity, and pollen fertility in barley (7). Besides, the fungicide phosphite reduced pollen fertility in *Petunia hybrida* Vilm., *Tradescantia*

virginiana L. and *Vicia faba* L., while phosphite application increased the number of abnormal meiotic cells at all stages in *Tradescantia virginiana* L. microspores (8). Furthermore, some common pesticides (Thiodan, Folithion, Lebaycid, and Kitazin) caused a spectrum of cytogenetic abnormalities such as chromosome fragmentation, lagging of chromosomes, anaphase bridge formation as well as tripolar and tetrapolar spindle formation in barley (9).

The abnormalities occurring in meiosis are very important because they cause sterility in pollens (10,11) and genetic damage can be transmitted to the offspring via male gametes, leading to congenital abnormalities (12).

The present study was performed to determine the cytogenetic effects of the fungicide Agri Fos 400 on the meiotic behavior of *Lycopersicon esculentum* Mill.

Materials and methods

Tomato flower buds were obtained from a 970 m²-greenhouse in the village of Karaçulha in Fethiye. Healthy tomato seedlings (2-week-old) were obtained from M-38 F_1 type domestic seeds. Agri Fos 400 (400 g/l mono and di-potassium phosphonate active ingredient), the fungicide used in the trial, was applied on tomato seedlings grown under greenhouse conditions. A total of 4 applications of 400 ml/100 l water as the recommended dosage and 800 ml/100 l water as double the recommended dosage were administered at 10-day intervals. Specimens randomly collected from different plants were fixed in Carnoy's solution. Anthers were stained with 2% aceto-orcein before being squashed (13).

For meiotic division examinations, cell shapes, cell division patterns, cell sizes, and bivalent arrangements of a total of 100 meiocytes from control and 400 ml/100 l and 800 ml/100 l fungicide groups were reviewed and photographed using a Jena microscope. Statistical analyses were made using SPSS 11.0 for Windows and the variance analyses were made using the Chi-Square test, a nonparametric test. The tables show that the differences among "a" and control group, "b"and 400 ml/100 l Agri Fos 400 group, "c" and 800 ml/100 l Agri Fos 400 group are statistically significant ($P < 0.05$).

Results and discussion

In the study, 400 ml and 800 ml/100 l dosages of Agri Fos 400 fungicide gave rise to some changes in cell shapes, chromosome structures, cell division patterns, and cell sizes of tomato meiocytes.

Cell shapes of pollens in the control and treatment groups are presented in Table 1. The number of round pollens in the treatment group was lower than that observed in the control group, whereas the number of oval and abnormally shaped meiocytes was larger. These fluctuations in the values were also found to be significant compared to the control group. When the treatment groups were compared with each other, the number of round meiocytes decreased whereas the numbers of oval and abnormal cells increased as the dosage increased. On the other hand, presence of "triangular and rectangular" meiocytes not seen in the

Table 1. Meiocyte shapes in the control and fungicide treatment groups (100%).

Groups	Cell Shape				
	Round	Oval	Triangular	Rectangular	Abnormal Cells
Control	85 [bc]	11 [bc]	0	0	4 [bc]
Agri Fos 400 400 ml/100 l	46 [ac]	25 [ac]	1	0	28 [ac]
Agri Fos 400 800 ml/100 l	12 [ab]	41 [ab]	1	1	45 [ab]

The differences among "a" and control group, "b" and 400 ml/100 l group, "c" and 800 ml/100 l group are statistically significant ($P < 0.05$).

control was observed in the treatment group. Meiocyte shapes not observed in the control group were seen in the tomato plant which is treated with 125 ml/100 l dosage of Mythos SC 300 (300 g/l Pyrimethanil) fungicide (14). The applications of Deltan and Bavistin caused abnormal pollen development (15). These abnormal main pollen cells lead to the formation of sterile pollen grains (16). In the trial, Agri Fos 400 fungicide brought about meiocytes shapes not seen in the control which resulted in an increase in the number of meiocytes with abnormal shapes.

The effects of the fungicide Agri Fos 400 on bivalents in tomato pollen are seen in Table 2. According to these results, the number of distinct bivalents in the treatment group was smaller compared to the control, whereas the number of indistinct ones was larger in the applied group. In both treatment groups, the number of distinct bivalents decreased whereas that of indistinct ones increased parallel with the increase in the dosages used in the applications. On the other hand, the number of chromosome abnormalities, such as thread-like, ring-shaped, linear, binding, and polar distortion, increased in the applied groups compared to the control [Table 2, Figures a(1,2), (b), c(3,4), and d(5)]. The number of abnormalities in the treatment groups increased with an increase in dosages applied and was found to have a statistical significance in the 800 ml/100 l fungicide application compared to the 400 ml/100 l fungicide dosage. Abnormalities occurring in meiosis are very important because they cause sterility in pollens (17). Similarly there was a positive relationship between chromosomal damages and pollen sterility (18). It is also thought that meiocytes having chromosomal damages will lead to the formation of sterile pollens.

Similar cytogenetic abnormalities have been observed in other studies, too. For example, the application of insecticide resulted in ring-shaped chromosome abnormalities in *Hordeum vulgare* L. (19), whereas Phosphomidon application led to linear abnormalities in red pepper (20) and Metosystox was responsible for chromosome abnormalities in the form of binding in red pepper (21). Moreover, Basudin in *Hordeum vulgare* L. (22) and Nitralin in *Allium cepa* L. (Badr 1979) caused abnormal polarization.

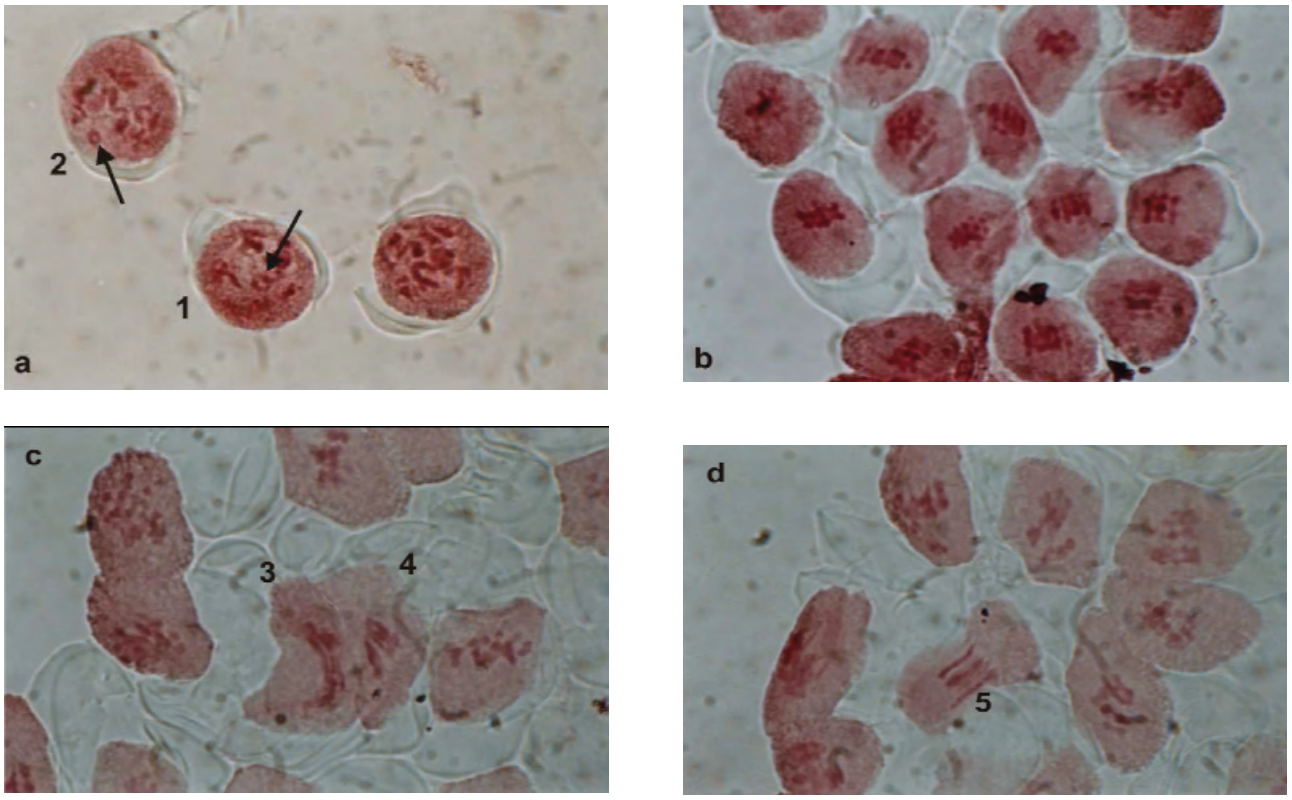
Cell division patterns and cell sizes of the control plants and the fungicide treatment groups are presented in Table 3. The numbers of properly divided cells and normal-size cells decreased in the 800 ml/100 l fungicide group compared to the control, whereas the numbers of improperly divided as well as large and small cells increased in all treatment groups. Unlike normal-size cells, the numbers of improperly divided large and small cells in the treatment groups increased as the dosage increased.

Topsin and Vitavax fungicides led to spindle abnormalities in root cells of *Allium cepa* L. (23) and abnormal anaphase with improper division of chromosome occurred due to Endosulfan application (24). In the present study, improperly divided cells with improper division of chromosome as well as large and small cells were observed. Abnormal anaphase results

Table 2. Arrangement of bivalents in meiocytes of control and fungicide treatment groups (100%).

Groups	Arrangement of bivalents						
	Distinct	Indistinct	Thread-like	Ring shaped	Linear	Binding	Polar Distortion
Control	85 [bc]	15 [bc]	1 [bc]	4 [bc]	3 [bc]	2 [bc]	0
Agri Fos 400 400 ml/100 l	60 [ac]	40 [ac]	13 [ac]	15 [ac]	15 [ac]	20 [ac]	1 [c]
Agri Fos 400 800 ml/100 l	39 [ab]	61 [ab]	29 [ab]	31 [ab]	40 [ab]	38 [ab]	7 [b]

Differences among "a" and control group, "b"and 400 ml/100 l group, "c" and 800 ml/100 l group are statistically significant ($P < 0.05$).



Figures. Meiotic division abnormalities in 400 ml/100 l and 800 ml/100 l groups. Chromosomes are generally stained with a lighter shade of orcein in 800 ml/100 l group. Ring-shaped chromosomes in 400 ml/100 l group a (1, 2); Binding chromosomes in 800 ml/100 l group (b), c (3, 4); Abnormal shape in 800 ml/100 l group c (3, 4) d (5); Linear chromosomes in 800 ml/100 l group d (5). (×600).

Table 3. Cell division patterns and meiocyte sizes of pollens in the control and fungicide treatment groups (100%).

Groups	Cell Division Patterns		Cell Size		
	Proper	Improper	Normal	Large	Small
Control	99 ^{c}	1 ^{bc}	99 ^{c}	1 ^{bc}	0
Agri Fos 400 400 ml/100 l	94	6 ^{ac}	84 ^{c}	11 ^{ac}	5 ^{c}
Agri Fos 400 800 ml/100 l	73 ^{a}	27 ^{ab}	51 ^{ab}	24 ^{ab}	25 ^{b}

Differences among "a" and control group, "b"and 400 ml/100 l group, "c" and 800 ml/100 l group are statistically significant ($P < 0.05$).

from degeneration in spindle (25) and large and small cells have been observed in some pesticide applications (26).

In conclusion, it seems that all dosages of Agri Fos 400 fungicide led to some abnormalities in chromosomes which in turn bring about production

of sterile pollens and will negatively affect yield in the future.

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