

Ultrastructural changes in the root tip and leaf cells of *Lens culinaris* treated with fluazifop-p-butyl

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Abstract: In this study, ultrastructural changes in root tip cells and leaf cells of lentil plants treated with 4 different concentrations (0.25%, 0.5%, 1%, and 1.5%) of the herbicide fluazifop-p-butyl (FPB) were determined. The experiments were performed with light and transmission electron microscopy (TEM) and the chlorophyll content of the leaves was determined using spectrophotometric methods. The effect of FPB included alterations in cell walls, nuclei, plastids, cuticle, and epidermis. The obtained results indicate that the herbicide FPB has the ability to cause different kinds of abnormalities in various cell organelles, especially the cell walls in root tips. The effect of FPB was not limited only to the anatomy and plastid ultrastructure of the leaf, but the chlorophyll content was also affected.

Key words: *Lens culinaris*, Leguminosae, root tip, leaf, organelles, chlorophyll

Fluazifop-p-butyl ile muamele edilmiş mercimeğin kök ucu ve yaprak hücrelerindeki ince yapı değişimleri

Özet: Bu çalışmada bir herbisit olan fluazifop-p-butyl'in (FPB) 4 farklı (% 0,25, % 0,5, % 1 ve % 1,5) konsantrasyonu ile muamele edilmiş mercimek kök ucu ve yaprak hücrelerindeki ince yapı değişimleri belirlendi. Deneyler ışık ve geçirgen elektron mikroskobu (TEM) ile gerçekleştirildi ve yapraklardaki klorofil içeriği spektrofotometrik yöntemle saptandı. FPB'in etkileri hücre duvarı, hücre çekirdeği, plastidler, kütikula ve epidermisdeki farklılıkları içermektedir. Elde edilen sonuçlar FPB'in özellikle kök uçlarında hücre duvarı olmak üzere çeşitli hücre organellerinde farklı anormalliklere neden olabileceğini göstermiştir. FPB'in etkileri yaprak anatomisi ve plastid ince yapısı ile sınırlı olmayıp, aynı zamanda klorofil içeriğini de kapsamaktadır.

Anahtar sözcükler: *Lens culinaris*, Leguminosae, kök ucu, yaprak, organeller, klorofil

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Introduction

Plants make up a large portion of our biosphere and constitute a vital link in the food chain (Kumar & Kumar, 2010). Lentil (*Lens culinaris* Medik.) is a major grain legume crop and an important protein source for people in many developing countries in western Asia, northern Africa, and many other parts of the world. It also improves soil fertility by fixing atmospheric nitrogen, thereby providing an excellent break crop, profitable in its own right to the intensive cereal farmer. However, lentil production is threatened by many weeds (Khawar & Özcan, 2002). Both annual and perennial grasses can be controlled by fluzifop-p-butyl (FPB) in lentil cultivation (Rahman, 2009). FPB is a grass-specific postemergence herbicide that inhibits the synthesis of enzymes required for lipid synthesis. It inhibits acetyl CoA carboxylase, the enzyme responsible for catalysing an early step in fatty acid synthesis (Ma et al., 2004). FPB may induce pathological changes in testes and the daily no-observed-adverse-effect level (NOAEL) for the compound was estimated to be 55.72 ± 4.13 mg/kg for female rats and 22.05 ± 1.56 mg/kg for male rats (Yang et al., 2007).

When herbicides are applied to plants, they must cross the cuticle in order to reach the site of action. Generally, this cuticle layer is composed of cutin and embedded waxes with epicuticular waxes on the outer surface (Chamel, 1986; Chamel & Vitton, 1996). Because of their physical structure, cuticular waxes represent the main barrier that controls the diffusion rate of lipophilic compounds (Santier & Chamel, 1998). Cutin, on the other hand, is a polyester of hydroxylated fatty acids and does not constitute a limiting factor for diffusing substances because its components leave spaces between chains big enough to be crossed by all kinds of molecules (Kerstiens, 1996; Malpassi, 2006). The herbicide FPB, applied at a double dose rate, has been shown to exert a phytotoxic effect on *Phaseolus vulgaris* L. and is expressed in the growth suppression and inhibition of leaf gas exchange and chlorophyll fluorescence parameters (Berova & Zlatev, 2002).

When treated with a lethal dose of the herbicide quizalofop, the *Eleusine indica* (L.) Gaertn. cuticle

shows areas where the epicuticular waxes disappear, especially in the youngest individuals (Malpassi, 2006). In a pot culture experiment conducted with a rice (*Oryza sativa* L.) cultivar, acetochlor significantly inhibited the growth of the roots and shoots, and the total chlorophyll content and chlorophyll a-to-b ratio were both decreased (He et al., 2006). Another 4 herbicides, chlorsulfuron, tribenuron-methyl, dimethylamine, and isooctyl ester, were tested on *Scenedesmus kissi* Hortob. and *Chlorella vulgaris* Beij. and were found to affect microalgal growth depending on the concentration (Çetinkaya Dönmez & Öztürk, 1997).

The symptoms induced by fluzifop-butyl in *Acanthospermum hispidum* DC. were wilting and necrosis. In *Avena sativa* L., lipid biosynthesis and acetyl-CoA carboxylase (ACCCase) activity were inhibited and electrolyte leakage from the shoots was increased as a result of exposure to fluzifop-butyl (Xiao & Hiroshi, 2002). At half to quadruple the strength of recommended field application concentrations, FPB adversely affected the development of both native and introduced species, grasses and nongrasses (Rokich et al., 2009). Fayez and Kristen (1996) used electron microscopy to study the effects of 3 herbicides (chlorsulfuron, norflurazon, and triallate) on the root caps of young seedlings of *Pisum sativum* L., *Phaseolus vulgaris* L., and *Vicia faba* L. Subcellular cell components, cell walls, and membranes constitute the cell interface, contributing to structural and metabolic compartmentalisation of the cell, as well as its perception of external and internal stimuli, generation of signals, and building up of defence barriers against stress factors. However, there are not any ultrastructural studies in the literature that have used this herbicide to point out the side effects in nontarget plants. Herbicide absorption occurs through the roots by passive diffusion through the epidermis and cortex. Therefore, the present study was undertaken in order to yield a better understanding of the mode of action of the herbicide FPB; light and transmission electron microscopy were used to detect the various abnormalities and changes in organelles of lentil root tip cells and leaf cells.

Material and methods

Treatment of lentil seeds and leaves with FPB

The herbicide FPB was tested in its commercial formulation, Fusilade Forte (2004). Fusilade Forte is an emulsifiable concentrate containing 45% of the active ingredient fluazifop-p-butyl, butyl(R)-2-[4-(5-trifluoromethyl-2-pyridinyloxy)phenoxy]propionate (IUPAC), produced by Syngenta (Olszewska, 2004). The FPB Forte used in this study was provided by the Agricultural Research Institute of Thrace. In accordance with the label instructions, FPB was prepared in concentrations between 0.25% and 1.5% (in modified Hoagland nutrient solution) for range-finding studies (Olszewska, 2004). After 3 replications, we found that the half maximal effective concentration (EC₅₀) for seed germination (Auletta et al., 1993) was between 1% and 1.5%, and then we began our study using 4 different FPB concentrations: 0.25%, 0.5%, 1%, and 1.5%.

Seeds of lentil (*Lens culinaris* 'Sultan') were pretreated with 5% NaOCl for 10 min for seed surface sterilisation, then placed under sterile bench conditions in petri dishes. Control groups were filled with modified Hoagland nutrient solution and experimental groups were filled with different concentrations of FPB at room temperature for soaking and germination (Ouzounidou et al., 1997). The EC₅₀ (the concentration at which germination was 50% of the control) values were calculated according to seed germination percentages using the trimmed Spearman-Kärber method after treatment for 72 h (Bartakova et al., 2001). Root tips that were 0.5 cm or higher were determined as germinated. All experiments were done in 3 repetitions.

A pot experiment was conducted with lentil (*Lens culinaris* 'Sultan') for the treatment of lentil leaves with FPB. Germinated seeds were taken to pots and the experiments were performed in 2 main groups: the plants treated with FPB at the beginning (seed treatment) and the plants treated with FPB after the 6-8 leaf stage (leaf treatment). Using a modified Hoagland nutrient solution, 4 different FPB butyl(R)-2-[4-(5-trifluoromethyl-2-pyridinyloxy)phenoxy]propionate (IUPAC) concentrations (0.25%, 0.5%, 1%, and 1.5%) were prepared (Ouzounidou et al., 1997). The control groups were treated only with the modified Hoagland nutrient solution.

Cytological analysis using light and transmission electron microscope (TEM)

After the experiment, the roots and leaves were sliced into 1 mm³ pieces, fixed in glutaraldehyde (3%, w/v) in a cacodylate buffer, and postfixed with osmium tetroxide (1%, w/v) in Millonig's phosphate buffer (0.1 M). The samples were then rinsed in buffer solution and distilled water 3-4 times, postfixed in 5% uranyl acetate, and rinsed again in distilled water 2-3 times. The samples were dehydrated in graded acetone solutions of 30%, 50%, 70%, and 90% for 10 min each, 100% acetone for 30 min, 1:1 acetone and propylene oxide for 20 min, and 100% propylene oxide for 20 min. Following dehydration, the samples were infiltrated with 1:1 propylene oxide and epon 812 for 1 h and 1:3 propylene oxide and epon 812 for 1 h, and then transferred into pure epon 812 in a desiccator overnight. The samples were then embedded in capsules and polymerized for 3 days at 40 °C, 1 day at 45 °C, and 3 days at 60 °C.

Semithin sections, 1 µm thick and prepared using an ultramicrotome, were placed on slides, stained with toluidine blue (0.1%, w/v) and azure II (0.2%, w/v) for 1-2 min, and dried on a hot plate for 5 min. After mounting with Entellan, the semithin sections were examined with an Olympus photomicroscope.

Using an ultramicrotome, thin sections of 150-300 Å (15-30 nm) of the identified regions were obtained and placed on film-coated grids before being stained with uranyl acetate (2%, w/v). The samples on the grids were examined using a JEOL JEM-1010 electron microscope and electron micrographs were taken. The ultrastructure of meristematic cells in both the control and FPB-treated plants were observed and the number of cells with altered nuclei, cell walls, amyloplasts, and vacuoles were determined by examination of 1000 cells per slide on semithin sections from each concentration. The ultrastructure of the leaf cells was observed by examination of the plant's cuticle, epidermis, and chloroplasts.

Chlorophyll a, chlorophyll b, and total chlorophyll determination

Chlorophyll was extracted from leaf tissue samples with 80% acetone and the absorbance of the extracts was measured at 645 and 663 nm using a Jenway 6105 UV/VIS scanning and recording spectrophotometer.

Chlorophyll concentrations were calculated from the spectrophotometric data using the formulas of Arnon (1949).

$$\text{Chlorophyll a (mg/g)} = [12.7 (A_{663}) - 2.69 (A_{645}) \times \text{mL acetone}] / (\text{mg tissue sample})$$

$$\text{Chlorophyll b (mg/g)} = [22.9 (A_{645}) - 4.68 (A_{663}) \times \text{mL acetone}] / (\text{mg tissue sample})$$

$$\text{Chlorophyll a (mg/g)} + \text{Chlorophyll b (mg/g)} = \text{Total chlorophyll (mg/g)}$$

Results

Observations of the root tip cells and leaf cells with light microscopy

Prior to the electron microscopic observations, we investigated the semithin sections obtained with an ultramicrotome. The most noticeable difference between the control group and groups treated with various concentrations of FPB was a decreased amount of amyloplasts following the exposure to FPB (Figure 1). Starting from the FPB concentration of 0.5% mL, deformations were observed, especially in nuclei and cell membranes. We found that nuclei

and cell membranes were undulated in almost all of the investigated cells. At higher concentrations, micronuclei were seen in some of the cells and large vacuoles were observed completely filling up the cells (Figure 1).

The percentages of altered nuclei, cell walls, amyloplasts, and vacuoles within cells observed from semithin sections were calculated. In the control group, no vacuoles were observed, whereas in 1.5% FPB-treated cells, none of the organelles except the nuclei and cell walls could be observed because of large vacuoles within the cytoplasm. The number of cells with altered amyloplasts was significantly increased starting from the lowest FPB concentration, and the number of altered cells increased parallel to the increase in FPB concentrations (Table 1).

Control plants had a normal leaf anatomy, with chloroplasts appressed to the plasmalemma of mesophyll cells. The leaf anatomy of plants treated with FPB showed several abnormalities; epidermis cells were swollen and the expansion of the cuticle could be seen in the cross section of the lentil leaves. While the cuticle and epidermis were uniform in the

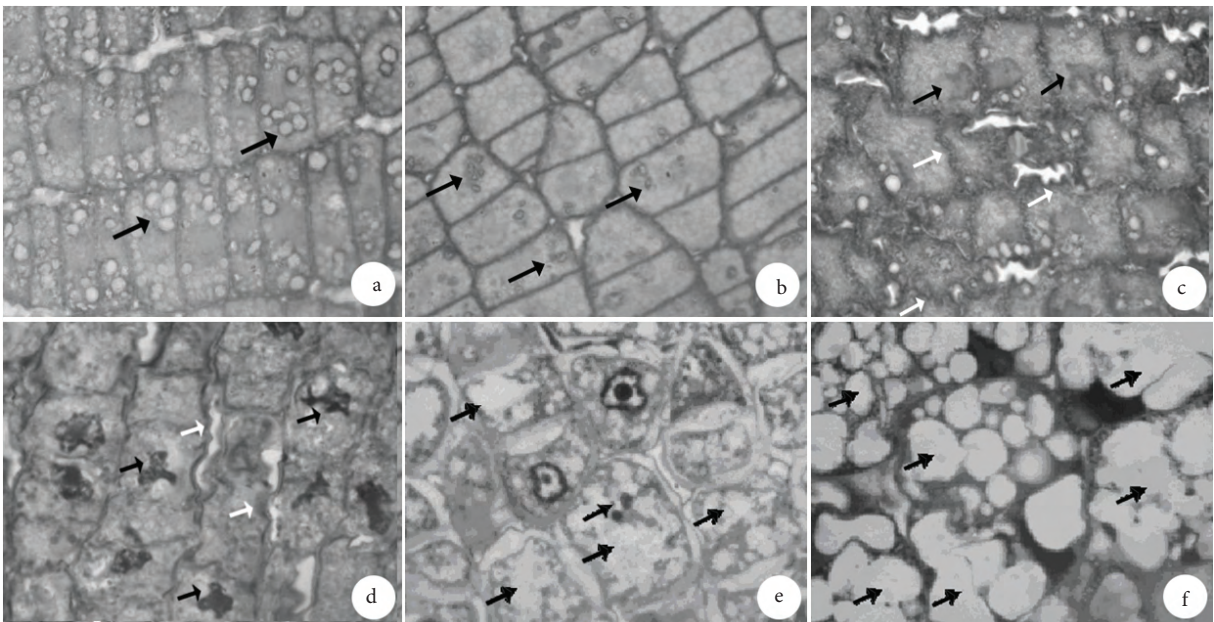


Figure 1. *Lens culinaris* root tip longitudinal section: a- control, b- 0.25% mL FPB, c- 0.5% mL FPB, d- 1% mL FPB, e- 1.5% mL FPB, and f- 1.5% mL FPB (magnification 1000×). a and b: black arrows show amyloplasts; c and d: intermittent black arrows show nuclei, intermittent white arrows show cell walls; e and f: intermittent large black arrows show vacuoles, intermittent black arrow shows micronuclei.

Table 1. The percentages of altered nuclei, cell walls, amyloplasts, and vacuoles within cells observed from semithin sections.

Fusilade conc. (mL %)	Number of cells (%)			
	With altered nuclei	With altered cell wall	With altered amyloplasts	With vacuoles
Control	4	6	8	**
0.25	10	8	72	4
0.5	70	86	80	6
1	82	94	90	90
1.5	86	98	*	96

*Could not be seen because of large vacuoles. **No vacuoles were observed.

control group, there was a slight expansion in the cuticle and epidermis cells of FPB-treated groups, and the vascular bundle was also segmented into

parts (Figure 2). In the final FPB concentration, segmentation in the vascular bundle was distinctive and degenerations were observed (Figure 2).

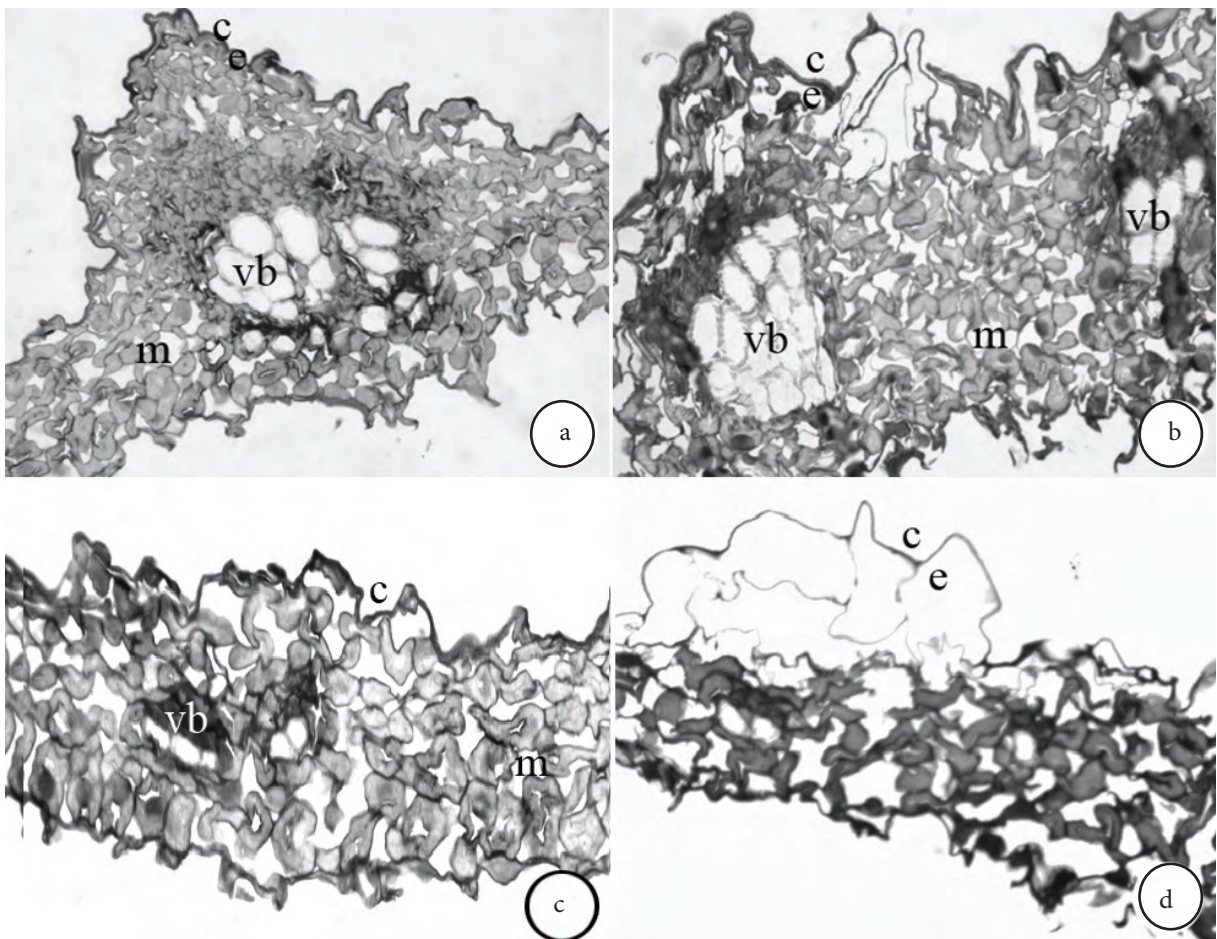


Figure 2. Cross section of *Lens culinaris* leaf: **a**- control, **b**- control, **c**- 1% FPB treatment after 6-8 leaf stage, and **d**- 1% FPB treatment after 6-8 leaf stage (magnification 400×). **c**: cuticle, **e**: epidermis, **m**: mesophyll, **vb**: vascular bundle.

Observations of the root tip cells and leaf cells with electron microscopy

In ultrastructural studies of root tip cells, we observed that the cells in the control group had regular shapes, normal nuclei, and regular cell and nuclear membranes, and contained many well-developed mitochondria and amyloplasts. Large starch grains were noticeable inside amyloplasts, as was an abundance of Golgi vesicles (Figure 3). Different kinds of abnormalities were observed in FPB-treated root tip cells. The number of mitochondria and the vesicles around the amyloplasts were decreased and lipid vesicles near the cell wall were increased with increasing FPB concentrations. Furthermore, the shapes of mitochondria were irregular and they were filled with osmiophilic granules (Figure 3). The number of lipid bodies was increased in

the cytoplasm, especially near the undulated cell walls (Figure 3). The cell wall was damaged and the nucleolar membrane was disintegrated in the 1.5% FPB-treated cells and none of the cell organelles could be clearly seen because of the large vacuoles (Figure 3). In some of the treated cells, osmiophilic vesicles were observed.

Columella cells of treated lentil roots showed a large number of vacuoles. In this area, the cells were apparently still dividing, but there was no longitudinal elongation. Nucleolar chromatin and peripheral chromatin were rough, the number of pores was increased, and the layers of the lipid bilayer around the nuclei were separated from one another in some parts of the nucleolar membrane (Figure 4). The number of cell organelles was decreased, most notably the mitochondria. Vacuole-like small

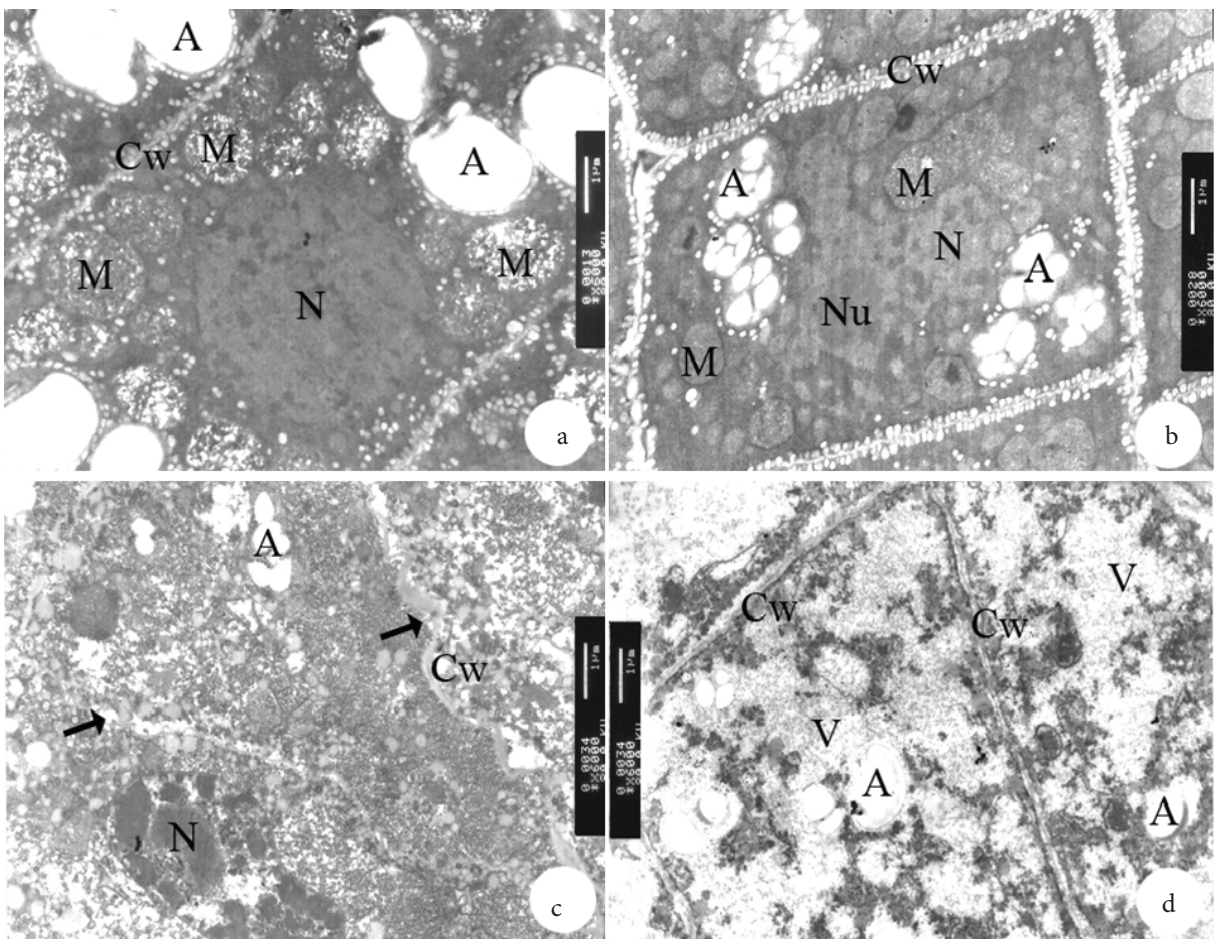


Figure 3. Transmission electron micrographs of *Lens culinaris* root tip cells: **a**- control, **b**- 0.25% mL FPB, **c**- 0.5% mL FPB (black arrows show crinkles in cell wall), and **d**- 1% mL FPB (magnification 6000×). A: amyloplasts, Cw: cell wall, N: nucleus, Nu: nucleolus, M: mitochondria, V: vacuole.

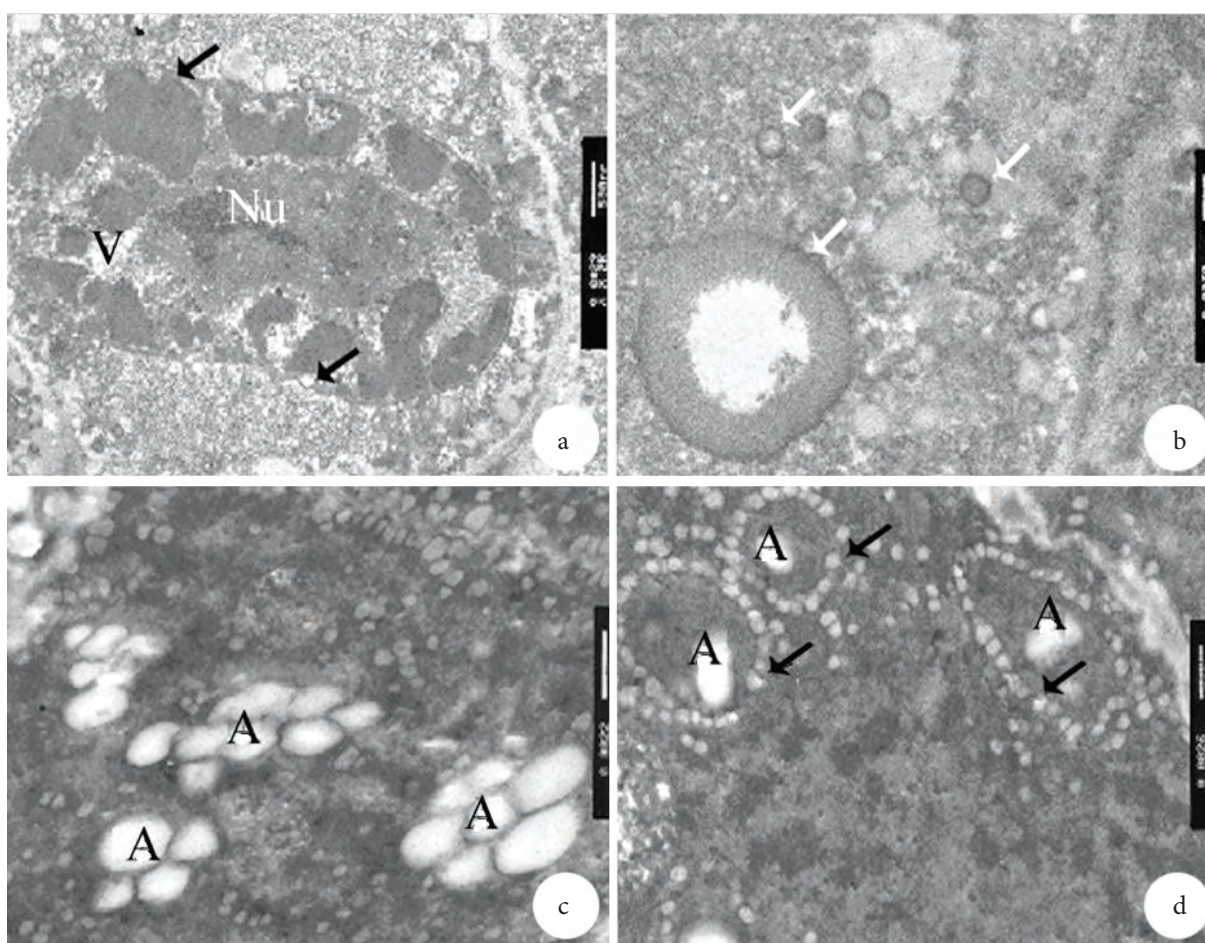


Figure 4. Transmission electron micrographs of *Lens culinaris* root tip cells: **a**- nucleus, 1% mL FPB (black arrows show separated parts of lipid bilayer); **b**- cytoplasm, 1% mL FPB (white arrows show thick layered vesicles); **c**- amyloplasts, 0.25% mL; **d**- amyloplasts, 0.5% mL FPB (black arrows show the vesicles around the reduced amyloplasts) (magnification of **a** and **d** = 12,000 \times ; **b** = 25,000 \times ; **c** = 15,000 \times). A: amyloplasts, Nu: nucleolus, V: vacuole.

vesicles surrounded by thick layers were observed in the cytoplasm (Figure 4). In 0.25% FPB-treated cells, the starch grains in amyloplasts were smaller (Figure 4) and the number of mitochondria was decreased. In 0.5% FPB-treated cells, degenerative changes were observed in cell shape and in cytoplasm. Cell organelles were reduced in number and also became smaller in shape. The starch grains in the amyloplasts were smaller and almost disappeared (Figure 4).

While the cell wall and membrane were uniform in the control group (Figure 5), they were undulated in the treated concentrations and lipid bodies containing osmiophilic granules were formed densely within them (Figure 5). Crinkles in the cell wall were

noticeable and an increased number of osmiophilic (lipid) globules were observed (Figure 5). In 1% FPB-treated cells, large vacuoles containing vacuolar inclusions like fibrillar structures were noticeable (Figure 5).

The effect of FPB on the ultrastructure of the cuticle and chloroplasts of lentil leaf cells was determined. The epicuticular wax, cuticle, and epidermal cells were normal in control groups (Figure 6), the borders of cutin and wax were prominent and uniform, and the cell wall was connected with the plasma membrane. Many deformations were observed after leaf treatment, however; the cuticles got thinner and began to intertwine, and borders could not be seen

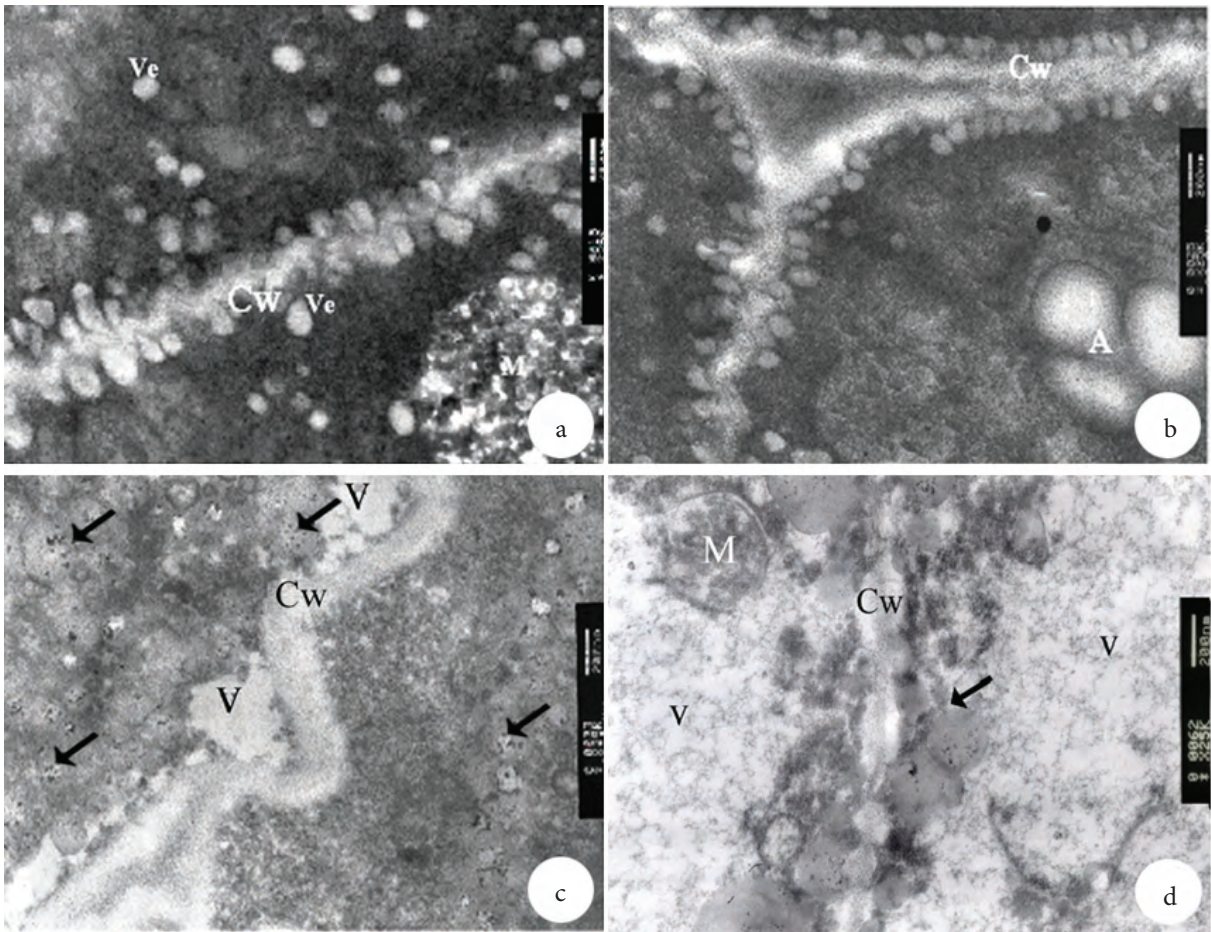


Figure 5. Transmission electron micrographs of *Lens culinaris* root tip cells: **a**- control, **b**- 0.25% mL FPB, **c**- 0.5% mL FPB, **d**- 1.5% mL FPB (magnification = 25,000 \times). A: amyloplasts, Cw: cell wall, V: vacuole, Ve: vesicles.

between the epicuticular wax and cuticle (Figure 6). These deformations showed morphological differences based on the concentration of FPB. While chloroplasts and grana were uniform in the control group (Figure 7), thylakoid membranes were degenerated and the amount and size of the osmiophilic vesicles were increased in FPB-treated leaves (Figure 7).

The effects of different FPB concentrations on the chlorophyll contents of lentil leaves

The effect of different concentrations of FPB on the chlorophyll content of *L. culinaris* leaves after seed and leaf treatment can be seen in Tables 2 and 3. By looking at the evidence presented in the Tables, we can see that leaf treatment with FPB caused a greater effect on plants than seed treatment. There was also a

distinguishable difference between the colours of the chlorophyll extracts.

Discussion

FPB inhibits acetyl CoA carboxylase, an enzyme that catalyses an early step in fatty acid synthesis. Lipids are important components of cellular membranes and when they cannot be produced in sufficient quantities, cell membrane integrity fails, especially in regions of active growth such as meristems (Tu et al., 2001). It is generally recognized that herbicidal responses vary based on differences in their site of action. A detailed analysis and comparison of the symptoms would thus enable us to clearly understand their mode of action. AOPP herbicides are rapidly absorbed by plant foliage,

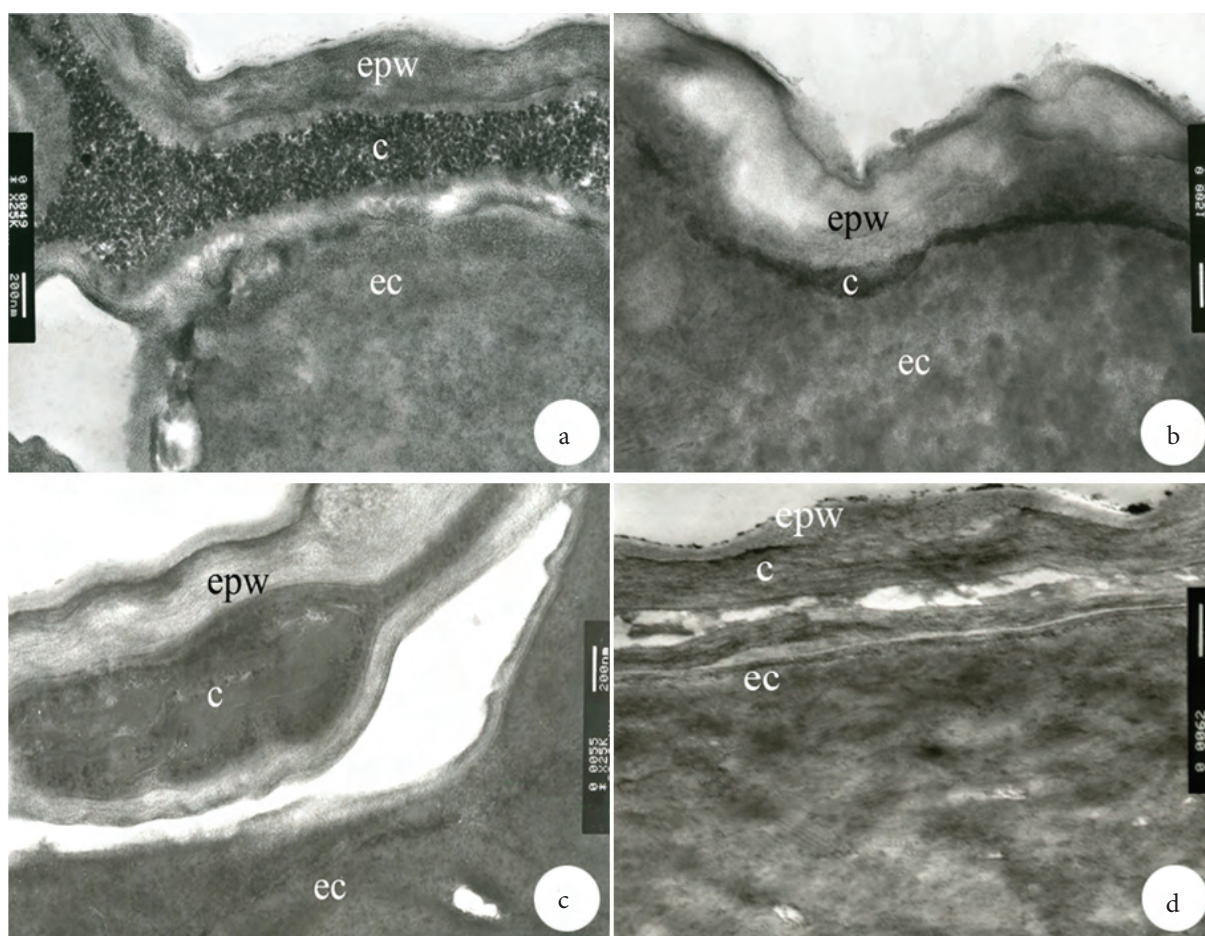


Figure 6. Transmission electron micrographs of *Lens culinaris* cuticle and epidermis cell after 6-8 leaf stage FPB treatment: a- control, b- 0.25% FPB, c- 0.5% FPB, d- 1% FPB (magnification = 25,000 \times). c: cuticle, ec: epidermis cell, epw: epicuticular waxes.

translocated throughout the plant, and accumulated in the meristems (Kim et al., 2005).

Herbicides such as Fusilade[®] and Fusilade Forte[™], used throughout the world, were shown to adversely affect seed germination, seedling emergence, growth, and the health of species native to southwest Australia (Rokich et al., 2009). We found similar results after the examination of FPB-treated lentil root tip cells with light microscopy, with abnormalities observed in the cell shape, cell wall, nuclei, and amyloplasts. Additionally, at higher FPB concentrations vacuolisation was noticeable in the cytoplasm. Chabot and Leopold (1985) investigated the ultrastructural aspects of chilling injury in the soybean seed radicle and found similar results in the cell wall and nucleus. Swisher and Corbin (1982)

observed necrotic zones and noticed deformations in the cell wall structure after their research on the physiological effects of some herbicides in the roots and shoots of soybean.

The subchronic toxicity of FPB on rats was determined and increases in the organ coefficients of the brain, liver, and kidney were observed. At the same time, a decrease was seen in the organ coefficients of the spleen of male rats in the middle dose group when compared with those in the control group (Yang et al., 2007). Fayez and Kristen (1996) studied the effects of 3 herbicides (chlorsulfuron, norflurazon, and triallate) on the root caps of young seedlings of *Pisum sativum* L., *P. Vulgaris* L., and *Vicia faba* L. All 3 herbicides caused changes in the root cap ultrastructure, and the outermost cell layer of the secretory tissue was

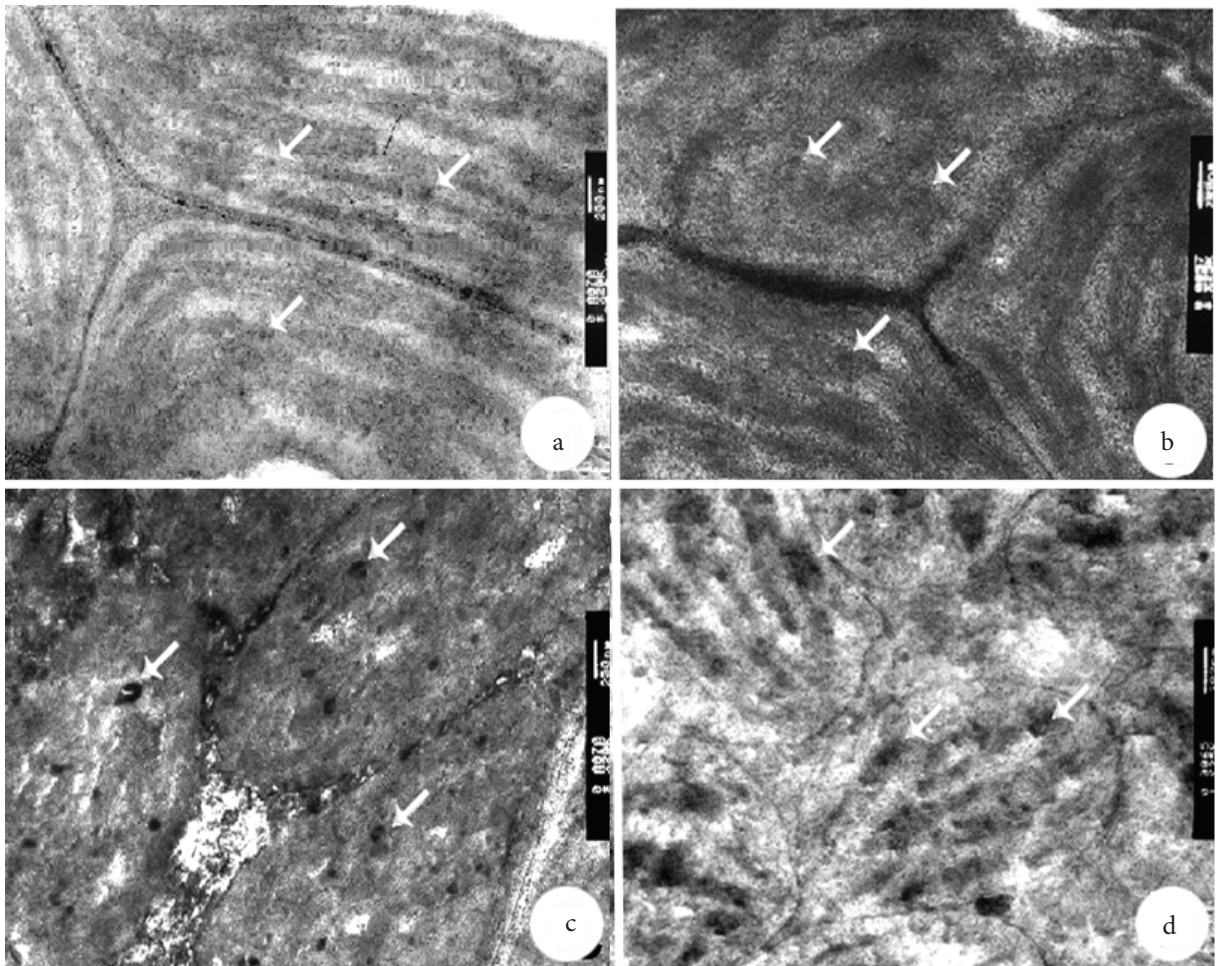


Figure 7. Transmission electron micrographs of chloroplasts in *Lens culinaris* leaves after 6-8 leaf stage FPB treatment: **a-** control, **b-** 0.25% FPB, **c-** 0.5% FPB, **d-** 1% FPB (magnification = 20,000 \times). White arrows show osmiophilic vesicles.

Table 2. The effect of different concentrations of Fusilade on the chlorophyll content of *L. culinaris* leaves (after leaf treatment).

Fusilade conc. (mL %)	Chl a (mg/g) \pm SD	Chl b (mg/g) \pm SD	Total chl (mg/g) \pm SD
0.25	0.78 \pm 0.44	0.35 \pm 0.60	1.13 \pm 0.40
0.5	0.75 \pm 0.61	0.36 \pm 0.31	1.11 \pm 0.25
1	0.58 \pm 0.22	0.27 \pm 0.34	0.85 \pm 0.36
1.5	0.43 \pm 0.45	0.23 \pm 0.67	0.66 \pm 0.58
Control	1.15 \pm 0.56	0.50 \pm 0.21	1.65 \pm 0.28

destroyed. Chlorsulfuron damaged amyloplasts in gravity-sensing cells (statocytes). Radicle growth was reduced by all 3 herbicides. This inhibition may be

partially due to root cap damage, as the radicle is in direct contact with herbicides in the soil. Flaburiari and Kristen (1996) exposed seeds of *Zea mays* L. to

Table 3. The effect of different concentrations of Fusilade on the chlorophyll content of *L. culinaris* leaves (after seed treatment).

Fusilade conc. (mL %)	Chl a (mg/g) \pm SD	Chl b (mg/g) \pm SD	Total chl (mg/g) \pm SD
0.25	0.98 \pm 0.21	0.42 \pm 0.44	1.40 \pm 0.56
0.5	0.91 \pm 0.59	0.47 \pm 0.38	1.38 \pm 0.43
1	0.79 \pm 0.20	0.46 \pm 0.24	1.25 \pm 0.29
1.5	0.66 \pm 0.49	0.44 \pm 0.28	1.10 \pm 0.60
control	1.15 \pm 0.50	0.50 \pm 0.32	1.65 \pm 0.23

low doses of the sulfonylurea herbicides chlorsulfuron and metsulfuron-methyl. Transmission electron microscopy revealed obvious changes in the nuclei and deformation of radial cell walls in the primary root cortex at 0.012 and 1.5 mg/L for both herbicides.

According to our observations, the occurrence of altered amyloplasts was high in comparison to the control group, even starting from the lowest FPB concentration. The percentages of altered cell walls were also found to be high in all FPB concentrations except the lowest one. Similar results were obtained from studies of dichlobenil, a herbicide that has been shown to inhibit glucose incorporation into cellulose (Demler & Stone, 1987). Another study postulated that protein synthesis and cell wall biosynthesis might be the primary sites of action for the herbicide isoxaben (Heim et al., 1990). Comparing these studies with our present results, we are able to say that FPB affects cell wall biosynthesis in a way that is similar to the herbicides dichlobenil and isoxaben. The percentages of altered nuclei were almost the same in the 2 highest FPB concentrations, and from our previous studies we also identified mitotic changes in lentil root tip cells treated with FPB. Cell membranes are referred to as sensors of the environment. The overall membrane functions are coordinately expressed in stress situations. Pesticides may cause different kinds of stress situations in plants. According to the mode of action, FPB can be placed in both the "grass meristem destroyers" and "lipid biosynthesis inhibitors" groups (Ross & Childs, 2009). In our previous publication, we showed that different FPB concentrations had a negative effect on mitotic division in *Lens culinaris* Medik. root tip cells. The mitotic index was 13.93 in the control

group and decreased to 8.9 and 6.69, respectively, at FPB concentrations of 0.25% and 0.5% (Aksoy et al., 2007).

We observed an increase in the number of vacuoles in FPB-treated lentil root tip cells, especially at higher concentrations. Lovett (1982) observed increased vacuolation and other apparent disruptions in the root tip cells of linseed (*Linum usitatissimum* L.). Similar alterations were observed in our study with lentil roots. These results suggest that general responses, such as the highly vacuolated cells, occur when root tip cells are exposed to chemical toxins. Another herbicide, aminoprophosmethyl (1 μ M, 1 day), induced marked vacuolation of tobacco root cells and disturbed polarity of their growth, as evident from the reduced cell areas on longitudinal sections (Baskin et al., 1994). Vigil et al. (1989) researched cotton embryos and found that as a result of drought stress, cells of the apical and basal regions of radicles were highly vacuolated, particularly in the basal region. The vacuolar compartment occupied a considerable area and often consisted of small rather than (typically) large vacuoles. TEM studies of the shoot apical meristem of caffeine-treated plants revealed that the cell wall formation of some meristematic cells was incomplete and only some parts of the wall were fully formed. The vacuolar system of the treated meristematic cells was also found to be more extended when compared to the controls (Lahouti et al., 2007).

The herbicide-induced subcellular changes that we observed in FPB-treated lentil roots may be an indication of alterations at the gene expression level or a consequence of an effect on protein synthesis.

This assumption is based on our previous study about the effects of FPB on the phases of mitosis, mitotic index, and α -amylase activity of lentil root tip cells, in which several mitotic aberrations and changes were observed (Aksoy et al., 2007).

The reactions of ER and polyribosomes may be related to the induction of stress during protein synthesis in root cells; this condition may result from all kinds of stresses. The reason for such complex relationships may be that the primary effect of a stress is on the cell membranes and possibly also on the cytoskeleton or nucleus, which then induces secondary or tertiary effects with a variety of structural manifestations.

Deposits of a less dense and less compact material found behind the plasmalemma were associated with the cell wall, as was an accumulation of dense material attached to the cell walls; cells with such deposits on their walls revealed extensive cytoplasmic damage. Wheeler and Hanchey (1971) reported that in roots exposed to 1 mM uranyl acetate for 1 h, some cells in the interior of the root cap had walls with very irregular surface contours and numerous cytoplasmic vesicles associated with crinkles in the plasmalemma. They also reported that the plasmalemma and the membranes around the cytoplasmic vesicles were much thicker than normal. In another study, changes occurring in response to oryzalin (a selective preemergence surface-applied herbicide) treatment included vacuolation, the induction of cytoplasm fragmentation, and the formation of vesicles directed to the vacuoles (Abdrakhimova et al., 2006).

In 1% FPB-treated root tip cells, the nuclear membrane was degenerated and vacuole-like structures were observed in some parts. The absence of intact organelles was striking and those that were in evidence showed severe damage. Treated cells displayed nuclei clearly and most contained nucleoli. In the nuclear membranes of 1% and 1.5% FPB-treated cells, it appeared that the double membrane had separated, leaving small vacuole-like spaces between the 2 membranes. These spaces ranged in size from being just visible to more than 1 μm in diameter.

Prominent in every micrograph of FPB-treated tissue were large aggregates of what appeared to be lipid globules. They were found throughout the

cytoplasm, especially near the cell membrane, but not in the nucleus. Muller and Hauge (1967), in commenting upon the appearance of lipids in their preparations, suggested that these may arise as a result of the poor utilisation of food or the blocking of a metabolic pathway by the toxins themselves. On the other hand, FPB inhibits acetyl CoA carboxylase, the enzyme responsible for catalysing an early step in fatty acid synthesis (Ma et al., 2004). Lorber and Muller (1976) suggested yet another possible origin for these globules, theorising that they may be the by-products of organelle and membrane decomposition. The absence of a variety of intact organelles indicates that structural breakdown and decomposition are occurring within FPB-treated root tip cells. As lipids are a known structural component of membranes, it is reasonable to suppose that membrane degradation could result in the freeing of lipids within the cytoplasm of affected cells.

An important factor in determining the tolerance of plants to herbicide stress is probably the regulation of photosynthesis (Terzi et al., 2010). We observed that the thickness of the chloroplast peripheral reticulum (PR) was increased in the FPB-treated concentrations. The chloroplast PR was first recognised as a discrete structure by Shumway and Weier (1967), and in 1968 Laetsch proposed that it might be involved in the rapid transport of metabolites into or out of the chloroplast. The chloroplast PR frequently appears as a consequence of chilling injury; Musser et al. (1984) concluded that the PR develops to increase inner chloroplast membrane surface area during a chilling-induced decrease in transport capacity. We observed that the degree of destruction of the internal structure of chloroplasts varies widely, depending on the FPB concentration used, although total chlorophyll content was reduced at all FPB concentrations. The development of prolamellar bodies and thylakoid membranes indicated the appearance of ultrastructural disorders. We could hypothesise that FPB leads to the destruction of the polyunsaturated fatty acids of the galactolipids and accelerates the unfolding of thylakoid membranes. When we evaluated the chlorophyll contents, we found that a foliar application of FPB showed more deleterious effects than root application. It was obvious that the foliar application of FPB herbicide alone at dosages of 40 g/fedan slightly decreased the fresh and dry weights of

3 soybean cultivars as compared to the corresponding controls (Kawther, 2006).

Our studies demonstrate that the ultrastructure of lentil root tip cells and leaf cells changes with the application of herbicide FPB at different concentrations. As has been found in research on animal cells, characteristics of plant apoptosis are a series of successive structural and morphological cell changes: chromatin condenses with subsequent nucleus degradation, the plasma membrane becomes blebby, and the cytoplasm bubbles and produces giant vacuoles. We observed such distinct changes in our samples. Unlike in findings resulting from the study of animal cells, the envelopes of plant apoptotic cells did not disappear due to the hard cell walls.

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