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Fusilade herbicide causes alterations in chloroplast ultrastructure, pigment content and physiological activities of peanut leaves

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Abstract

Fusilade (fluazifop-*p*-butyl) is one of the herbicides that inhibit acetyl-CoA carboxylase. The exogenous effect of 30, 60, and 90 ppm fusilade on peanut (*Arachis hypogaea* L. cv. Giza 5) leaves was studied. With increasing fusilade concentration, the peanut leaf chlorosis appeared after 7–10 d. Declined leaf pigment contents confirmed the leaf chlorosis. Electron microscopic observation of the fusilade-treated (FT) leaves revealed disorganization in the ultrastructure of mesophyll cell chloroplasts. An increase of plastoglobuli occurrence within chloroplasts and degenerated grana thylakoids were observed in FT leaves. Fusilade treatments induced mainly the enhancement of malondialdehyde content and the activities of peroxidases (guaiacol and ascorbate). On contrary, a decrease in H_2O_2 content, catalase and superoxide dismutase activities was recorded. Enhancements of the guaiacol and ascorbate peroxidase activities were associated with the decreasing H_2O_2 content in the FT leaves. Hydrogen peroxide seems not to be involved in the oxidative stress of FT leaves. In the FT leaves, the oxidative stress confirmed by chlorophyll degradation and lipid peroxidation might be caused by the other reactive oxygen species probably due to the decrease of superoxide dismutase activity.

Additional key words: antioxidants; *Arachis hypogeae*; fusilade; lipid peroxidation; ultrastructure.

Introduction

Peanut (*Arachis hypogaea*, Fabaceae) is an economically important food crop in many countries worldwide. It is native to South Africa, Mexico, South America, and Mediterranean region. The post-emergence herbicides used in peanuts for weed control are paraquat, 2,4-dichlorophenoxybutanoic acid, acifluorfen, and graminicides such as clethodim, fusilade (fluazifop-*p*-butyl), and sethoxydim (Wilcut *et al*. 1994, Gnanamurthy and Balasubramaniyan 1998, Horbowicz *et al*. 2013). However, injuries of plant cell organelles and alteration in the physiological activities have resulted from the application of herbicides (Fayez and Kristen 1996, Fayez 2000, Fayez and Hassanein 2000, Radwan 2012).

Fusilade (R)-2-[4-[[5-(trifluoromethyl)-2-pyridinyl] oxy] phenoxy] propanate is sold under the various trade names, such as Fusilade 5, Fusilade 2000, Fusilade Super, Horizon 2000, Ornamec, Fusion, and Tornado. Fusilade is one of the systemic herbicides belonging to the aryloxy-phenoxypropionates "FOPS" family of herbicides. The main target site of fusilade is the inhibition of acetyl-CoA carboxylase (ACCase; E.C. 6.4.1.2) activity. This enzyme catalyses the carboxylation of acetyl-CoA to malonyl-CoA. It is required for the biosynthesis of fatty acids and secondary metabolites (Harwood 1996, Nikolskaya *et al*. 1999, Yu *et al*. 2004), thereby inhibiting the biosynthesis of fatty acids necessary for membrane building and function (Harwood 1988). Poaceae are killed by ACCase-inhibiting herbicides since they have only the herbicide-sensitive, eukaryotic ACCase. In nearly all grass species, the plastidic ACCase is strongly inhibited by commercial ACCase-inhibiting herbicides (Zhang and Powles 2006). Most dicotyledonous plants are resistant to herbicides, which inhibit ACCase since they have both eukaryotic ACCase and herbicide-insensitive, prokaryotic ACCase. (Konishi *et al*. 1996, Christopher and Holtum 1998). However, there are exceptions among dicotyledonous plants, such as rape and soybean, which are characterized by the presence of a plastidial homomeric ACCase (Christopher and Holtum 2000, Belkebir and Benhassaine-Kesri 2013).

Herbicide inhibitor of ACCase induces the accumulation of reactive oxygen species (ROS), such as

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Abbreviations: APX – ascorbate peroxidase; Car – carotenoids; CAT – catalase; Chl – chlorophyll; FM – fresh mass; FT – fusilade-treated; MDA – malondialdehyde; POX – guaiacol peroxidase; ROS – reactive oxygen species; SOD – superoxide dismutase.
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superoxide radicals ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($^{\bullet}OH$), and singlet oxygen ($^1O_2$). ROS are highly reactive and without effective protective mechanisms, they can seriously disrupt normal metabolism by causing oxidative stress to the membrane lipids, proteins, nucleic acids, chlorophylls, leading to leaf chlorosis, necrosis, and cell death (Banaś *et al*. 1993, Rout and Shaw 2001, Cui and Yang 2011, Chen *et al*. 2012).

Oxidative stress induced by abiotic and biotic factors through ROS may disrupt the membrane integrity as a consequence of lipid peroxidation (Feierabend and Winkelhüsener 1982, Lambert and Bondy 1989, Banaś *et al*. 2000, Fayez and Mahmoud 2011, Lukatkin *et al*. 2013, Fayez and Bazaid 2014). Malondialdehyde (MDA) content is widely used to measure the extent of lipid peroxidation and as an indicator of oxidative stress and membrane damage (Ohkawa *et al*. 1979).

In order to confer protection against oxidative stress, plant cells and its organelles, such as chloroplast, mitochondria, and peroxisomes employ enzymatic and nonenzymatic antioxidant defense systems. The most important antioxidant enzymes include superoxide dismutase (SOD, E.C. 1.15.1.1), ascorbate peroxidase (APX, E.C. 1.11.1.11), guaiacol peroxidase (POX, E.C. 1.11.1.7), and catalase (CAT, E.C. 1.11.1.6). SOD catalyzes the dismutation of $O_2^{\bullet -}$ to O_2 and H_2O_2 that is scavenged by APX and CAT; they work together to control $O_2^{\bullet -}$ and H_2O_2 to limit the generation of $^{\bullet}OH$ (Mittler 2002). APX reduces H_2O_2 to water by utilising ascorbate as specific electron donor. CAT is involved in the conversion of H_2O_2 into water and oxygen (Foyer and Noctor, 2000, Di Cagno *et al*. 2001, Mhamdi *et al*. 2012). Wang *et al*. (2008) reported that activities of POX and APX of *Vicia faba* seedlings exposed to exogenous lead were upregulated to remove excess H_2O_2 under the condition of reduced CAT activity.

In the present study, we investigated the cytotoxic and physiological effects of fusilade herbicide on chloroplast ultrastructure, photosynthetic pigment content, oxidative stress, and activity of antioxidative enzymes in peanut leaves relative to untreated ones. Assessments of oxidative stress were monitoring through estimation of H_2O_2 and MDA contents, and the activity of antioxidant enzymes in FT and untreated peanut leaves.

Materials and methods

Plants and treatments: Seeds of peanut (*Arachis hypogaea* L. cv. Giza 5) were kindly provided by the Agriculture Research Center, Ministry of Agriculture, Shandawil, Sohag, Egypt. Seeds were sown in a mixture of soil and sand (2:1, v/v) in clean plastic pots. The fusilade used was commercially available (12.5% a.i.) from *Syngenta* (Egypt) and frequently used for weed control. After four weeks from sowing, plants of the same growth were chosen and divided into the following four treatment groups as follows:

Group	Treatment
1	water (control)
2	30 ppm fusilade (78.24 μM)
3	60 ppm fusilade (156.49 μM)
4	90 ppm fusilade (234.74 μM)

The plants were treated for two weeks. Each treatment was replicated four times (each replicate was one pot containing four plants). The leaves were sprayed once with fusilade concentrations until saturation.

Leaf symptoms and chloroplast ultrastructure: For symptom observation, the leaves of control and treated plants with 90 ppm herbicide concentration were photographed. For transmission electron microscope observation, the control and 90 ppm FT leaves were submerged in 0.05 M phosphate buffer, pH 7.0. Samples were cut (about 1–2 mm in diameter) with a new scalpel blade and fixed immediately in 3% solution of glutaraldehyde in the buffer solutions for 3 h at room temperature. The samples were rinsed several times with the same phosphate buffer and then postfixed in 1% OsO_4 in the same buffer for another 2 h at room temperature. The samples were rinsed several times in 0.05 M phosphate buffer, pH 7.0, and dehydrated using 10-min steps in a graded series of cold acetone. The dehydrated samples were then transferred to a mixture of Spurr's embedding medium (Spurr 1969), and 100% acetone (1:1, v/v) for 12 h at room temperature, with the sample vials clipped onto a rotating mixing wheel. Acetone/Spurr's medium mixture was replaced with pure Spurr's medium and the samples were incubated for 48 h at room temperature. Samples were then transferred into the fresh Spurr resin and polymerized at 70°C for 8–10 h to complete the embedding. Ultrathin sections (60 nm thick) were cut by ultramicrotome (*Reichert Supernova*, Germany), stained with uranyl acetate and lead citrate, and examined by transmission electron microscope *Jeol-1010* (*Jeol*, Tokyo, Japan) at 80 kV.

Photosynthetic pigments: The pigments were extracted from peanut leaves with 80% acetone. Chlorophyll (Chl) *a*, Chl *b*, and carotenoids (Car) were quantitatively determined spectrophotometrically (*Jenway 6300 UV/VIS, Jenway,* UK) at wavelengths 663, 647, and 470 nm, respectively, according to Lichtenthaler (1987):

$$\text{Chl } a = 12.25\ A_{663} - 2.79\ A_{647}$$
$$\text{Chl } b = 21.50\ A_{647} - 5.10\ A_{663}$$
$$\text{Car} = (1{,}000 \times A_{470} - 1.82 \times \text{Chl } a - 95.15 \times \text{Chl } b)/225$$

The concentrations for Chl *a*, Chl *b*, and the sum of leaf Car (xanthophylls and carotenes) were expressed as mg per g of fresh matter (FM).

Hydrogen peroxide content in leaves was determined colorimetrically (Jana and Choudhuri 1981). Hydrogen peroxide was extracted by homogenizing 0.5 g of FM with 3 cm^3 of phosphate buffer (50 mM, pH 6.5). The homogenate was centrifuged at 6,000 × *g* for 25 min. To determine H_2O_2 content, 3 cm^3 of extracted solution was mixed with 1 cm^3 of 0.1% $Ti_2(SO_4)_3$ in 20% H_2SO_4. The mixture was then centrifuged at 6,000 × *g* for 15 min. The pellet was then dissolved in 5 cm^3 of 2 M H_2SO_4 and the intensity of the yellow color of the supernatant was measured at 410 nm (*Spekol 11, Carl Zeiss*, Jena, Germany). H_2O_2 concentration was calculated using the extinction coefficient ($\varepsilon = 0.28\ \mu mol^{-1}\ cm^{-1}$).

Lipid peroxidation: Malondialdehyde (MDA) content was determined as an indication of leaf lipid peroxidation (Hernández and Almansa 2002). Fresh leaf samples (500 mg) were homogenized in 5 cm^3 of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 15,000 × *g* for 20 min at 4°C. Aliquots of the supernatant (1 cm^3) were mixed with 3 cm^3 of 0.5% thiobarbituric acid (TBA) prepared in 20% TCA, and incubated at 90ºC for 20 min. After stopping the reaction in an ice bath, samples were centrifuged at 10,000 × *g* for 5 min. The supernatant absorbance was then measured at 532 nm (*Jenway 6300*, UK). After subtracting the nonspecific absorbance at 600 nm, MDA concentration was determined using the extinction coefficient ($\varepsilon = 155\ mM^{-1}\ cm^{-1}$).

Antioxidant enzyme assays: Guaiacol peroxidase (POX, EC 1.11.1.7) activity was measured by the following change in the absorption due to guaiacol oxidation ($\varepsilon = 26.1\ mM^{-1}cm^{-1}$) at 470 nm (Polle *et al*. 1994). The activity was assayed for 1 min in a reaction solution (3 cm^3 final volume) composed of 100 mM potassium phosphate buffer (pH 7.0), 20 mM guaiacol, 10 mM H_2O_2, and 50 mm^3 of crude extract. Enzyme activity was calculated as µM of guaiacol oxidized $min^{-1}\ g^{-1}$(FM).

Catalase (CAT, E.C. 1.11.1.6) activity was measured according to Chandlee and Scandalios (1984) with some modifications. The assay mixture contained 2.6 cm^3 of 50 mM potassium phosphate buffer (pH 7.0), 0.4 cm^3 of 15 mM H_2O_2, and 40 mm^3 of crude enzyme extract. The decomposition of H_2O_2 was followed by the decline in absorbance at 240 nm ($\varepsilon = 0.036\ mM^{-1}\ cm^{-1}$). The enzyme activity was expressed in units, where 1 unit of catalase converts 1 µM of H_2O_2 $min^{-1}\ g^{-1}$(FM) under the above-mentioned assay conditions.

Ascorbate peroxidase (APX, E.C. 1.11.1.11) activity was determined according to Nakano and Asada (1981) with the modified procedure of Hernández *et al*. (2004). The decrease in ascorbate concentration was followed by decline in absorbance at 290 nm and activity was calculated using the extinction coefficient ($\varepsilon = 2.8\ mM^{-1}\ cm^{-1}$ at 290 nm) for ascorbate. The assay medium consists of 3 cm^3 containing 50 mM phosphate buffer (pH 7.0), 0.1 mM EDTA, 0.3 mM ascorbate, 0.06 mM H_2O_2, and 0.1 cm^3 of the enzyme extract. Activity was expressed as µM of ascorbate oxidized $min^{-1}\ g^{-1}$(FM).

Superoxide dismutase (SOD, E.C. 1.15.1.1) activity was assayed according to Beauchamp and Fridovich (1971) with some modifications. The leaf samples (500 mg) were homogenized in 5 cm^3 extraction buffer consisting of 50 mM phosphate, pH 7.8, 0.1% (w/v), ascorbate, 0.05% (w/v) β-mercaptoethanol. The assay mixture of 3 cm^3 contained 50 mM phosphate buffer (pH 7.8), 9.9 mM L-methionine, 57 mM nitroblue tetrazolium (NBT), 0.025% (w/v) Triton X-100, and 0.0044% (w/v) riboflavin. The photoreduction of NBT (formation of purple formazan) was measured at 560 nm. One unit of SOD activity was defined as the extract volume that caused 50% inhibition of NBT reduction. Activity was expressed as µM of NBT reduced $min^{-1}\ g^{-1}$(FM). All spectrophotometric analyses of enzyme activities were performed on a spectrophotometer *Jenway 6300 UV/VIS* (*Jenway*, UK).

Statistical analysis: The obtained data were tested for significance by using one-way of variance (*ANOVA*) test. Means were compared by least significant differences (LSD) test at levels $P<0.05$ and $P<0.01$. All statistical tests were carried out using *SPSS 9.0* statistical software for *Windows*.

Results

Leaf symptom and chloroplast ultrastructure: Leaves of the untreated peanut (Fig. 1*A*) were dark green, while leaf chlorosis appeared in 90 ppm FT peanut (Fig. 1*B*). Chlorosis followed by necrosis appeared after 7 d from the beginning of the fusilade treatment. In plants treated with 30 and 60 ppm fusilade, leaf chlorosis was observed after 10 d of the fusilade treatment (data not shown). The symptoms started appearing from the leaf petiole and the midvein, and then they were spread around the midvein. The severity of the bronze color or burn was more obvious in the lower part of leaves. Cup-shaped leaves appeared in response to the highest concentration of fusilade (90 ppm).

Ultrastructural alterations occurred in 90 ppm FT (Fig. 2*B*) peanut leaves compared with those of the control (Fig. 2*A*). Chloroplast of the untreated leaves showed a condensed stroma matrix. In contrast, the chloroplast of the 90 ppm FT leaves had low electron opaque of stroma matrix (Fig. 2*B*). Formation of vesicles and degenerated grana thylakoids were noticed in chloroplasts of the FT leaves. Moreover, the chloroplasts of FT plants changed to spherical shape and contained a higher number of plastoglobuli compared with the control. In the FT plants (Fig. 2*B*), the mesophyll cell contained many peroxisomes.

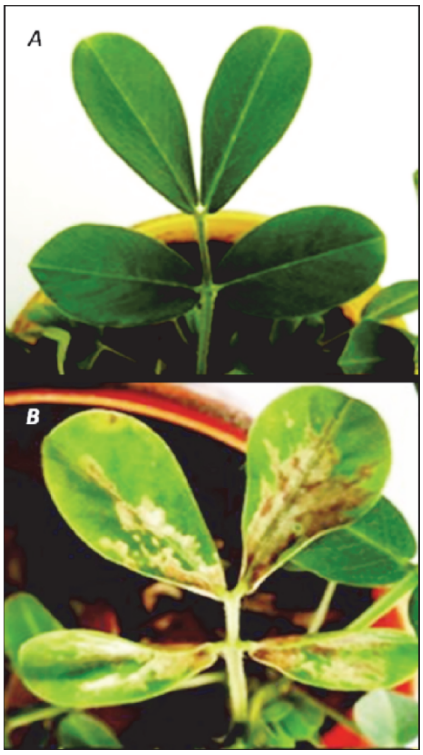

Fig. 1. Light micrographs of the control (*A*) and 90 ppm fusilade-treated leaves (*B*) of peanut leaves (*Arachis hypogaea* L. cv. Giza 5).

Photosynthetic pigments: Chl *a*, Chl *b*, and Car contents in peanut leaves were significantly reduced in response to fusilade application relative to the control (Table 1). Moreover, the decrease in pigment contents was much more pronounced with the increasing fusilade concentration. For example, Chl *a* content decreased by 38.3, 56.5, and 63.6% in response to 30, 60, and 90 ppm of fusilade, respectively, compared with the control. Chl *b*, Car, Chl *a*/*b* ratio, and the total pigment contents were also lowered in a dose dependent manner, mostly with 90 ppm of fusilade (Table 1). Chl *a* content decreased more than Chl *b* content in response to fusilade treatments, and it caused a decline in the Chl *a*/*b* ratio. Total pigment contents decreased by 62.6% in the 90 ppm FT leaves compared with the control.

H_2O_2 and MDA: H_2O_2 content of peanut leaves increased by 8% with 60 ppm of fusilade (Table 2), but decreased by 7.4 and 22. 4% with 30 and 90 ppm, respectively, compared with the control (Table 2). The MDA content increased with the increasing concentration of fusilade. MDA contents increased by 24.5, 99, and 109.4% in the 30, 60, and 90 ppm FT leaves, respectively, compared with the control.

Antioxidant enzymes: Activities of POX and CAT varied greatly in the FT leaves compared with the control (Table 3). The POX activity increased significantly with the applied fusilade concentrations. In response to 30, 60, and 90 ppm of fusilade, the POX activities increased by 186.3, 405.4, and 445.4%, respectively, relative to the control. In contrast to POX activity, the CAT activity of FT leaves was significantly lower only with 30 ppm, but no significant changes were observed with 60 and 90 ppm compared with the control. The activity of APX and SOD significantly increased and decreased, respectively, only with 90 ppm of fusilade (Table 3). Compared with the control, leaf APX activity declined by 32.2% in response to 30 ppm, but it was stimulated by 86.9% in response to the 90 ppm fusilade treatment (Table 3). With 60 ppm of fusilade, the APX was approximately the same as the activity of the control. The SOD activity decreased with the increasing concentration of fusilade (Table 3). The highest concentration of fusilade (90 ppm) reduced SOD activity of peanut leaves by 57.5% compared with the control.

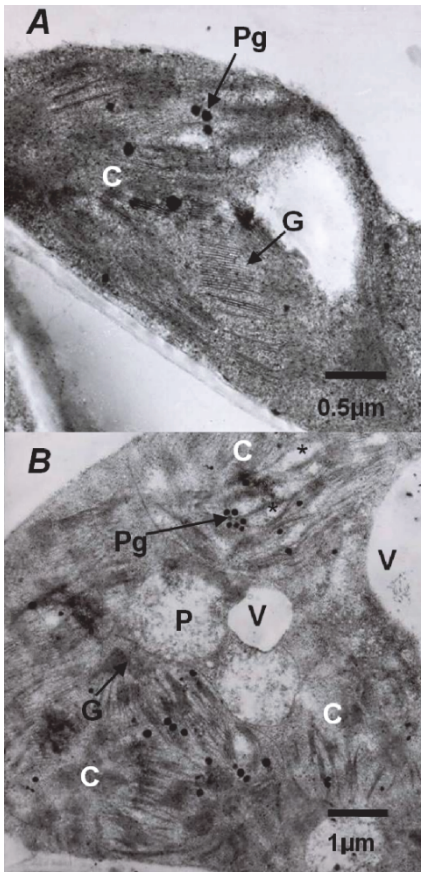

Fig. 2. Electron micrographs of the control chloroplast (*A*) and 90 ppm fusilade-treated mesophyll cell (*B*) of peanut leaves (*Arachis hypogaea* L. cv. Giza 5). Scale bars: 0.5 (*A*) or 1 µm (*B*). C – chloroplast; G – granum; P – peroxisome; Pg – plastoglobuli; V – vacuoles; * – vesicles.

Discussion

Application of herbicides for weed killing affects physiological processes and cell organelle structure, and enhances the intracellular formation of ROS in crop plants (Peixoto *et al*. 2006,Yin *et al*. 2008, Jiang and Yang 2009). Fusilade at a low concentration caused phytotoxic activity in green alga, *Chlorella vulgaris* (Ma *et al*. 2002), and in maize plants (Horbowicz *et al*. 2013). ROS-dependent cell damage is controlled by the ratio of its production to scavenging by the antioxidant system (Banaś *et al*. 1993, Kawano 2003, Krieger-Liszkay 2005, Das and Bagchi 2010). Herbicides of ACCase inhibitors can cause over-production of ROS within plant cells (Banaś *et al*. 1993,

Table 1. The influence of two-week fusilade application (30, 60, and 90 ppm) on photosynthetic pigment contents of peanut (*Arachis hypogaea* L. cv. Giza 5) leaves. Values are means of four replicates ± SD. * – significant at $P<0.05$; ** – significant at $P<0.01$.

Treatments	Chl *a* [mg g^{-1}(FM)]	[%]	Chl *b* [mg g^{-1}(FM)]	[%]	Car [mg g^{-1}(FM)]	[%]	Chl *a/b*	Total	[%]
Control	1.54 ± 0.02	100	0.62 ± 0.08	100	1.00 ± 0.08	100	2.48	3.16	100
30 ppm	0.95 ± 0.06*	61.7	0.35 ± 0.02**	56.4	0.49 ± 0.05**	49.0	2.71	1.79	56.6
60 ppm	0.67 ± 0.27**	43.5	0.27 ± 0.08**	43.5	0.37 ± 0.14**	37.0	2.48	1.31	41.5
90 ppm	0.56 ± 0.21**	36.4	0.27 ± 0.14**	43.5	0.35 ± 0.07**	35.0	2.07	1.18	37.3

Table 2. The influence of fusilade application (30, 60, and 90 ppm) for two weeks on H_2O_2 and MDA contents of peanut (*Arachis hypogaea* L. cv. Giza 5) leaves. Values are means of four replicates ± SD. * – significant at $P<0.05$; ** – significant at $P<0.01$.

Treatments	H_2O_2 [nmol g^{-1}(FM)]	[%]	MDA [μmol g^{-1}(FM)]	[%]
Control	52.64 ± 2.25	100	21.25 ± 4.14	100
30 ppm	48.77 ± 7.46	92.6	26.47 ± 5.13	124.5
60 ppm	56.89 ± 9.70	108.0	42.31 ± 2.39**	199.0
90 ppm	40.88 ± 4.37*	77.6	44.52 ± 3.43**	209.4

Radwan, 2012). In the present study, treatment of peanut leaves with fusilade caused leaf chlorosis and necrosis in some parts of the leaf. Degenerated thylakoids and a decrease in photosynthetic pigment contents of peanut leaves resulted from the fusilade treatments. These injuries could be due to enhanced production of ROS and oxidative stress that can lead to death of plant. MDA content increased with the increasing fusilade concentration in leaves. Recently, in rape leaves subjected to sethoxydim herbicide (ACCase inhibitor), a reduction in growth rate, Chl, and lipid contents has been observed (Belkebir and Benhassaine-Kesri 2013). Therefore, injuries visible on the peanut leaves due to the fusilade treatments seemed to be due to enhanced lipid peroxidation and photosynthetic pigment degradation. In bristly starbur seedlings, fluazifop-*p*-butyl increased the content of MDA (Luo *et al*. 2004). Our interpretation was based on the fact that excessive amount of herbicides and other toxic organic substances are able to induce intracellular overproduction of ROS and hence to damage plant cells (Yin *et al*. 2008). The cooperative action of antioxidant enzymes plays an important role in scavenging ROS and maintaining the physiological redox status of organisms (Fecht-Christoffers *et al*. 2003). Among antioxidant enzymes, the SOD is the most effective intracellular enzymatic anti-oxidant which has been proposed to be important in plant defense against the toxic effects of elevated ROS concentrations (Fridovich 1986, Gill and Tuteja 2010). Moreover, the balance among activities of antioxidant enzymes, such as SOD, POX, CAT, and APX, is crucial in cells for determining the steady-state concentration of O^{2-}

Table 3. The influence of fusilade application (30, 60, and 90 ppm) for two weeks on guaiacol peroxidase (POX), catalase (CAT), ascorbate peroxidase (APX), and superoxide dismutase (SOD) activities of peanut (*Arachis hypogaea* L. cv. Giza 5) leaves. Values are means of four replicates ± SD. * – significant at $P<0.05$; ** – significant at $P<0.01$.

Treatment	POX [μM (guaiacol oxidized) min^{-1} g^{-1}(FM)]	[%]	CAT [μM (H_2O_2 decomposed) min^{-1} g^{-1}(FM)]	[%]	APX [μM (ascorbate oxidized) min^{-1} g^{-1}(FM)]	[%]	SOD [μM (NBT reduced) min^{-1} g^{-1}(FM)]	[%]
Control	36.67 ± 2.57	100	15.67 ± 4.91	100	2.99 ± 1.43	100	74.96 ± 17.15	100
30 ppm	105.00 ± 13.23*	286.3	7.50 ± 1.50**	47.8	2.03 ± 0.50	67.8	59.08 ± 28.47	78.8
60 ppm	185.33 ± 14.78**	505.4	11.75 ± 1.77	75.0	3.11 ± 0.90	104.0	53.09 ± 17.21	70.8
90 ppm	200.00 ± 5.66**	545.4	13.00 ± 2.83	82.9	5.59 ± 1.02*	186.9	31.92 ± 6.78**	42.5

and H_2O_2 (Di Cagno 2001, Mittler 2002, Jung 2004). In the present study, a variable change in the activities of antioxidant enzymes was detected in the FT peanut. Previous study indicated a reduction in CAT activity that was associated with upregulation of guaiacol peroxidase and APX in *Vicia faba* seedlings affected by lead (Wang *et al.* 2008). The CAT and SOD activities were reduced in the FT leaves. In contrast, the POX activity was induced by all fusilade concentrations, while APX activity was only induced by the highest concentration (90 ppm). Enhancement of both enzyme activities was accompanied in FT leaves by the reduction of H_2O_2 concentration. Both enzymes minimize the content of H_2O_2 and hence prevent its toxicity. Within a cell, SOD constitutes the first line of defense against ROS (Alscher *et al*. 2002). In the present study, a reduction in the activity of SOD due to the fusilade treatment indicated the ROS were not scavenged sufficiently, and therefore they caused the oxidative stress in peanut leaves. Overproduction of SODs in plant chloroplasts has been found to increase protection against herbicides (van Camp *et al*. 1996, Iannelli *et al*. 1999). It is suggested that the fusilade-induced oxidative damages in peanut leaves were probably related to lowered SOD activity.

In conclusion, peanut was sensitive to the fusilade treatment; this was indicated by leaf chlorosis, decreased photosynthetic pigments, and increased MDA content. Oxidative stress was enhanced by the fusilade treatment. Peroxidases (POX, APX) controlled H_2O_2 concentration during the fusilade treatment. Due to the reduction in SOD activity after the fusilade treatment, other ROS caused oxidative stress in peanut leaves.

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