

PRETREATMENT WITH POLYAMINES ALLEVIATE THE DELETERIOUS EFFECTS OF DIURON IN MAIZE LEAVES

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The effects of diuron, a photosystem II inhibiting herbicide, on lipid peroxidation, photosynthetic pigments, soluble protein, proline contents and some antioxidant enzymes in maize leaves were studied and protective effects of polyamines against diuron toxicity were investigated. Diuron significantly increased lipid peroxidation, suggesting oxidative damage in the plants and proline content, while it decreased total chlorophyll, carotenoid and soluble protein levels in the leaves during the experiments. Pretreatment with polyamines statistically decreased lipid peroxidation induced by diuron and spermine (SPM) proved to be the most effective polyamine. Also, pretreatment with polyamines significantly prevented the losses of total chlorophyll, carotenoid and soluble protein induced by diuron. On the other hand, pretreatment with polyamines significantly increased proline contents of the leaves in comparison with the leaves treated with diuron. Superoxide dismutase (SOD), guaiacol peroxidase (GPX) and glutathione reductase (GR) activities increased in the leaves treated with diuron while catalase (CAT) activity decreased. Pretreatment with spermidine (SPD) did not change significantly SOD activity at 24 and 72 hrs of diuron treatment but prevented the increase in SOD activity induced by diuron at 48 h. However, pretreatment with SPD increased GPX activity at 24 h and GR activity at 48 and 72 hrs. CAT activity in the leaves pretreated with SPD was similar to that of the leaves treated with diuron. Pretreatment with SPM prevented the increase in SOD activity induced by diuron at 48 h but significantly increased it at 72 h of diuron treatment. However, pretreatment with SPM did not significantly change GPX and GR activities during the experiments but reversed the decrease in CAT activity induced by diuron at 72 h. Pretreatment with putrescine (PUT) prevented the increase in SOD activity induced by diuron at 48 and 72 hrs while it increased GPX and GR activities at 48 h of diuron treatment. Also, the decrease in CAT activity induced by diuron at 72 h was completely prevented by PUT. It can be concluded that pretreatment of maize leaves with polyamines reduced the damage produced by diuron and the protective effects of polyamines against diuron toxicity were closely associated with antioxidant system.

Keywords: Diuron – polyamines – antioxidant enzymes – proline – lipid peroxidation

INTRODUCTION

Diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea] is a broad-spectrum phenylurea herbicide and algaecide used for pre- and post-emergent control of both broadleaf and grass weeds in agriculture. It is a very specific and sensitive inhibitor of photosynthesis [31].

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It binds to the Q_B -binding niche on D1 protein of the photosystem II complex in chloroplast thylakoid membranes, thus blocking electron transport from Q_A to Q_B [19]. The inability to reoxidize Q_B promotes the formation of triplet state chlorophyll, which interacts with ground state oxygen to form singlet oxygen. Both triplet chlorophyll and singlet oxygen can extract hydrogen from unsaturated lipids, producing a lipid radical and initiating a chain reaction of lipid peroxidation [21].

Protective mechanisms against the production of reactive oxygen species do exist in plants. In the past years, a role for polyamines as antioxidants has been proposed [7]. It has been reported that polyamines inhibit lipid peroxidation [36, 41, 45]. Polyamines have also been described as direct free radical scavengers [15] or to function as scavengers by interacting with other molecules [10]. It is well known that polyamines are associated with the thylakoid membranes, especially, the LHCII and the PSII complex of spinach. The tetramine, spermine (SPM) was mainly found in considerable concentrations in highly purified PSII-core antenna and reaction center particles of PSII [27]. Also, Besford et al. [9] observed that exogenous polyamines supplied to osmotically stressed oat leaves retarded protein degradation, inhibited loss of chlorophyll and stabilized the thylakoid membranes. In plants, involvement of polyamines in various kinds of environmental stress has already been demonstrated [9, 16, 17]. The increase in polyamine contents of plants resulted in more tolerance to environmental stresses [43]. In recent years, some methods, such as transgenic approaches and exogenous polyamine application, have been directed towards the agricultural use of polyamines [16]. Exogenously applied polyamines have shown some protective effects in conditions of wounding [4], high temperature treatment [25], ozone damage [34], salinity [16, 42] and herbicide treatment [17]. In these studies, the relationship between polyamines and antioxidant systems has usually been determined. Although there has been little data on the action of diuron on antioxidant systems [21, 37], information about the role of exogenous polyamines on diuron action in plants are completely missing. Therefore, the aim of the present work was to study the action of diuron on some oxidative stress parameters and antioxidant system in maize leaves and to investigate the protective effects of exogenous polyamines against diuron toxicity.

MATERIALS AND METHODS

Plant material and treatments

Maize (*Zea mays* L. cv RX 788) seeds were provided by MayAgro Seed Corporation, Bursa, Turkey. The plants were grown in plastic pots (16 cm height, 18 cm top and 12 cm bottom diameter) containing soil and peat (5:1) in a greenhouse (temperature: 25 ± 2 °C and relative humidity: $65 \pm 5\%$) for 10 days. Then the pots were divided into three groups; the first group served as control, the second group were sprayed with diuron at the recommended field dose (1.8 kg ha^{-1}) and the other was treated with diuron after sprayed with polyamines (PUT, SPM and SPD) at 1 mM concentrations once daily for three days. Tween 20 (0.05%) was used as surfactant. Control plants were only

sprayed with 0.05% Tween 20 in distilled water. Foliar samples were harvested at 24, 48 and 72 h after diuron application and the following analyses were performed. All treatments were repeated at least three times on different days.

Lipid peroxidation

Lipid peroxidation was measured in the term of malondialdehyde content (MDA, $\epsilon = 155 \text{ mmol}^{-1} \text{ cm}^{-1}$), a product of lipid peroxidation following the method of Heath and Packer [22].

Chlorophyll and carotenoid contents

Chlorophyll and carotenoid contents were determined according to Arnon [5] and Jaspars [23], respectively.

Proline content

Proline content was determined by the ninhydrin method [6].

Antioxidant enzyme assays

Leaf samples (0.5 g) were homogenized in 5 ml of cold 50 mM potassium phosphate (K_2HPO_4) buffer (pH 7.0) containing 1 mM EDTA and 1% polyvinylpolypyrrolidone. The homogenate was centrifuged at $20,000 \times g$ for 20 min at 4 °C and the supernatant was used for the following enzyme assay. Soluble protein content was determined according to the method of Bradford [11] with BSA as a standard.

SOD (EC 1.15.1.1) activity was based on the method of Beauchamp and Fridovich [8] as modified by Dhindsa and Matowe [14], which measures the inhibition in the photochemical reduction of nitroblue tetrazolium (NBT).

GPX (EC 1.11.1.7) activity was determined according to Urbanek et al. [40].

CAT (EC 1.11.1.6) activity was determined by following the consumption of H_2O_2 ($\epsilon = 39.4 \text{ mM}^{-1} \text{ cm}^{-1}$) at 240 nm for 3 min [1].

GR (EC 1.6.4.2) activity assay was based on the method of Foyer and Halliwell [20].

Statistical analysis

Variance analysis of mean values was performed by Duncan multiple comparison test using SPSS for Microsoft Windows (Ver. 10.0, SPSS Inc., USA) and statistical significance was determined at the 5% level ($P < 0.05$).

RESULTS

Lipid peroxidation

Lipid peroxidation, measured by MDA content, was significantly increased by the treatment of the leaves with diuron (Fig. 1). For example, the increment in MDA contents of the leaves treated with diuron compared to the control were determined to be 14.01%, 51.24% and 79.63% at 24, 48 and 72 hrs, respectively. Polyamines significantly prevented lipid peroxidation increment induced by diuron. Especially, SPM was the most effective polyamine in preventing lipid peroxidation. However, SPD and PUT also significantly prevented lipid peroxidation compared to the untreated leaves (Fig. 1).

Photosynthetic pigment contents

Changes in photosynthetic pigment levels in maize leaves were determined for 24, 48 and 72 h after they were sprayed with diuron. Total chlorophyll content significantly decreased in the leaves treated with diuron (Table 1). A decrease in total chlorophyll level of the leaves pretreated with polyamines was less than that of the leaves treated

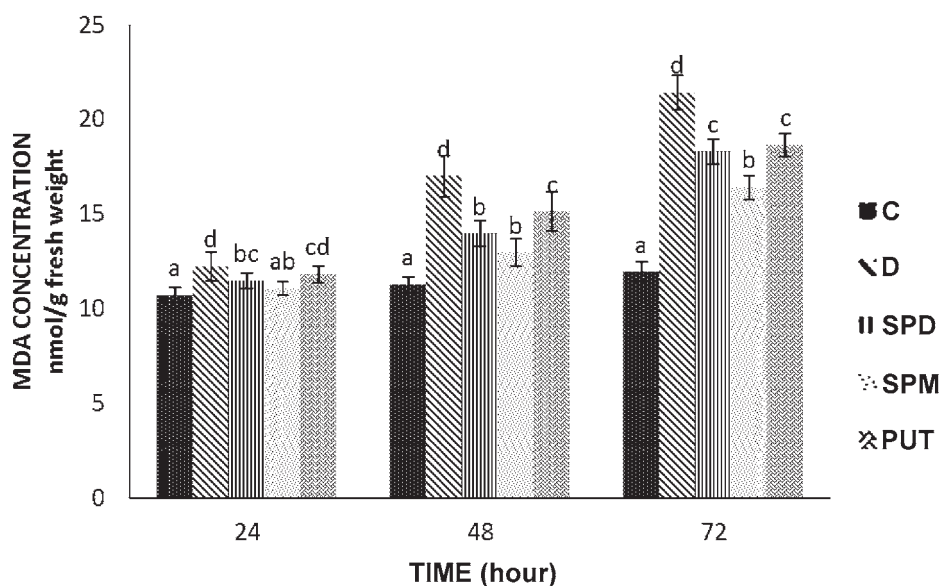


Fig. 1. Lipid peroxidation (malondialdehyde content) in maize leaves pretreated with polyamines and treated with diuron afterwards (C: Control, D: Diuron, SPD: Spermidine, SPM: Spermine, PUT: Putrescine). Vertical bars represent standard deviation of three samples. Within each hour, different letters indicate the differences among the means of three replicates. The differences between the means are significant at $P < 0.05$

Table 1

Total chlorophyll content of the leaves pretreated with polyamines and treated with diuron afterwards

Treatments	Total chlorophyll (mg/g fresh weight) Hours after diuron treatment		
	24	48	72
Control	2.78 ± 0.13* ^b	2.70 ± 0.04 ^c	2.36 ± 0.02 ^d
Diuron	2.40 ± 0.03 ^a	1.96 ± 0.01 ^a	1.43 ± 0.02 ^a
SPD	2.65 ± 0.04 ^{ab}	2.24 ± 0.02 ^d	1.91 ± 0.04 ^{bc}
SPM	2.57 ± 0.18 ^{ab}	2.16 ± 0.03 ^c	1.95 ± 0.02 ^c
PUT	2.46 ± 0.17 ^a	2.06 ± 0.04 ^b	1.86 ± 0.04 ^b

*Standard deviation of average of three replications. Values followed by different letters are significantly different from each other ($P < 0.05$) according to Duncan's test.

with only diuron. That is, polyamines significantly retarded chlorophyll degradation induced by diuron. SPM was found to be the most effective polyamine. After 72 h of treatment, total chlorophyll content decreased by 39.4% in the leaves treated with diuron compared to the control, while it was 17.37% in the leaves pretreated with SPM. Also, carotenoid content of the leaves treated with diuron decreased significantly (Table 2). After 72 h of treatment, the loss of carotenoid content was 46.15% in the leaves treated with diuron compared to the control. Carotenoid loss was significantly prevented by SPD and SPM pretreatments. It was found that carotenoid content of the leaves pretreated with SPD and SPM decreased by 23.07% and 26.92%, respectively. Also, carotenoid level of the leaves pretreated with PUT was higher than that of untreated leaves.

Table 2

Carotenoid content of the leaves pretreated with polyamines and treated with diuron afterwards

Treatments	Carotenoid content (mg/g fresh weight) Hours after diuron treatment		
	24	48	72
Control	0.28 ± 0.005* ^c	0.26 ± 0.005 ^c	0.26 ± 0.01 ^d
Diuron	0.20 ± 0.01 ^a	0.17 ± 0.005 ^a	0.14 ± 0.005 ^a
SPD	0.26 ± 0.01 ^{bc}	0.23 ± 0.02 ^b	0.20 ± 0.005 ^c
SPM	0.24 ± 0.005 ^b	0.22 ± 0.01 ^b	0.19 ± 0.005 ^c
PUT	0.21 ± 0.02 ^a	0.19 ± 0.005 ^a	0.17 ± 0.005 ^b

*Standard deviation of average of three replications. Values followed by different letters are significantly different from each other ($P < 0.05$) according to Duncan's test.

Soluble protein content

As can be seen in Table 3, soluble protein content significantly decreased after diuron treatment. A decrease in soluble protein level induced by diuron was observed after 24 h of treatment. The loss in soluble protein content was 27.39% in the leaves treated with diuron for 72 h compared to control. Pretreatment with SPD, SPM and PUT prevented the loss of soluble protein induced by diuron at 24 h of treatment. Pretreatment with SPD was not effective at 48 and 72 hrs of diuron treatment. However, pretreatment with SPM and PUT, respectively, was effective in preventing the loss of soluble protein at 48 and 72 hrs of diuron treatment.

Table 3

Soluble protein content of the leaves pretreated with polyamines and treated with diuron afterwards

Treatments	Soluble protein (mg/g fresh weight) Hours after diuron treatment		
	24	48	72
Control	6.49 ± 0.37 ^{*b}	6.24 ± 0.70 ^b	6.17 ± 0.24 ^c
Diuron	5.29 ± 0.24 ^a	5.20 ± 0.13 ^a	4.48 ± 0.17 ^a
SPD	5.97 ± 0.20 ^b	5.40 ± 0.42 ^a	4.94 ± 0.60 ^{ab}
SPM	6.25 ± 0.18 ^b	6.20 ± 0.12 ^b	5.26 ± 0.35 ^b
PUT	6.44 ± 0.35 ^b	6.41 ± 0.07 ^b	5.14 ± 0.19 ^{ab}

*Standard deviation of average of three replications. Values followed by different letters are significantly different from each other ($P < 0.05$) according to Duncan's test.

Proline content

Proline content significantly increased in the leaves treated with diuron (Table 4). For example, the increment in proline contents of the leaves treated with diuron, and compared to the control, were found to be 42.69%, 62.18% and 85.12% at 24, 48 and

Table 4

Proline content of the leaves pretreated with polyamines and treated with diuron afterwards

Treatments	Proline content (mg/g fresh weight) Hours after diuron treatment		
	24	48	72
Control	1.78 ± 0.09 ^{*a}	3.57 ± 0.41 ^a	3.90 ± 0.52 ^a
Diuron	2.54 ± 0.04 ^b	5.79 ± 0.53 ^{cd}	7.22 ± 0.50 ^b
SPD	3.51 ± 0.36 ^c	5.69 ± 0.44 ^c	9.54 ± 0.31 ^c
SPM	4.30 ± 0.24 ^d	4.56 ± 0.23 ^b	8.80 ± 1.04 ^c
PUT	5.33 ± 0.22 ^c	6.51 ± 0.44 ^d	10.66 ± 0.96 ^d

*Standard deviation of average of three replications. Values followed by different letters are significantly different from each other ($P < 0.05$) according to Duncan's test.

72 hrs, respectively. Pretreatment with polyamines also significantly increased proline level of the leaves. It was found that especially PUT significantly increased proline content, compared with the leaves treated with diuron. At the end of experiments, proline amount in the leaves treated with diuron was 7.22 mg per g fresh weight whereas in the PUT-pretreated leaves it was 10.66 mg per g fresh weight. Also, proline level of the leaves pretreated with SPD and SPM was higher than that of untreated leaves after 72 h of diuron treatment.

Enzyme activities

SOD activity gradually increased after the leaves were treated with diuron but statistically significant increases were recorded at 48 and 72 hrs. It was found that the increment in the SOD activity in the leaves treated with diuron was 12.37%, 31% and 44.72% at 24, 48 and 72 hrs of treatment, respectively, compared to the control. Pretreatment with polyamines did not change significantly SOD activity at 24 h of diuron treatment in comparison with the leaves pretreated with water. At 48 h of diuron treatment, SOD activity in the leaves pretreated with polyamines was the same as in the control. At 72 h of diuron treatment, the activity was found to be 28.01, 31.31 and 18.2 U per mg protein in the leaves pretreated with SPD, SPM and PUT, respectively, while it was 26.5 U per mg protein in the leaves treated with diuron (Fig. 2).

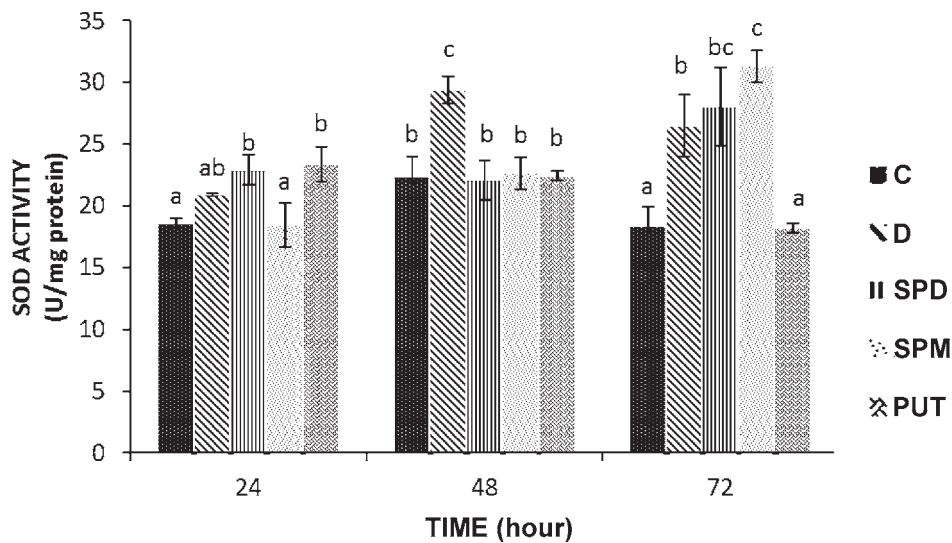


Fig. 2. SOD activity in maize leaves pretreated with polyamines and treated with diuron afterwards (C: Control, D: Diuron, SPD: Spermidine, SPM: Spermine, PUT: Putrescine). Vertical bars represent standard deviation of three samples. Within each hour, different letters indicate the differences among the means of three replicates. The differences between the means are significant at $P < 0.05$

GPX activity did not change significantly in the leaves treated with diuron, compared to the control, at 24 and 48 hrs of treatment, but a statistically significant increase was recorded at 72 h. Pretreatment with SPD increased GPX activity at 24 h of treatment but there was no significant change at 48 h and decreased at 72 h. The change in GPX activity of the leaves pretreated with SPM was not statistically significant either, in comparison with the leaves treated with diuron. However, the increase in GPX activity in the leaves pretreated with PUT at 48 h was found to be statistically significant (Fig. 3).

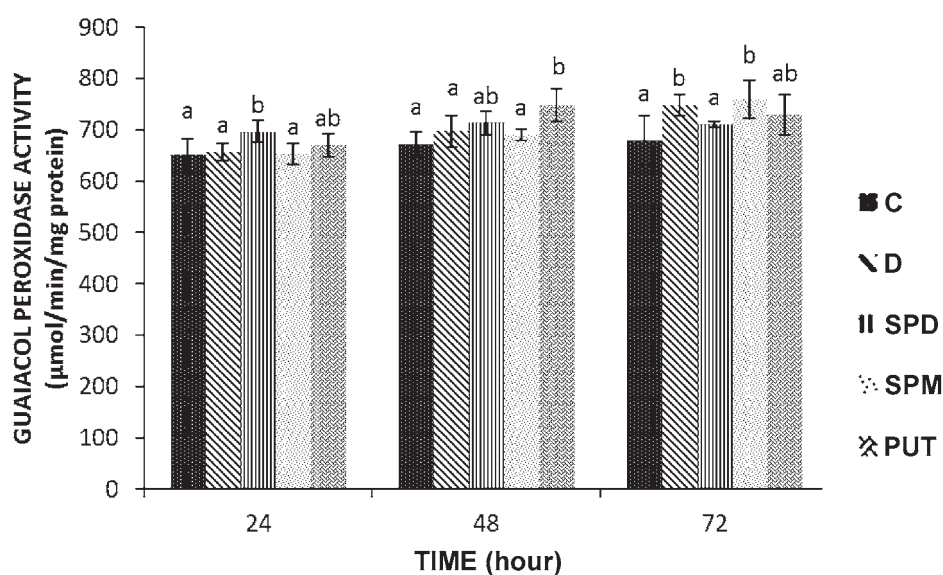


Fig. 3. Guaiacol peroxidase activity in maize leaves pretreated with polyamines and treated with diuron afterwards (C: Control, D: Diuron, SPD: Spermidine, SPM: Spermine, PUT: Putrescine). Vertical bars represent standard deviation of three samples. At each point of time, different letters indicate the differences among the means of three replicates. The differences between the means are significant at $P < 0.05$

After diuron treatment, GR activity gradually increased. It was found that GR activity increased by 60.4% at 72 h of diuron treatment. Pretreatment with SPD significantly increased GR activity. Also, GR activity in the leaves pretreated with PUT increased but a statistically significant increase was recorded at 48 h as compared to that in the leaves treated with diuron.

Pretreatment with SPM had no significant effect on GR activity following only diuron treatment (Fig. 4).

CAT activity was not changed significantly in the leaves treated with diuron at 24 and 48 hrs but it statistically decreased at 72 h. This loss was found to be 28.42%, compared to the control. Similarly, the same change was found in CAT activity of the leaves pretreated with SPD. Pretreatment with PUT and SPM significantly prevented the loss of CAT activity induced by diuron at 72 h (Fig. 5).

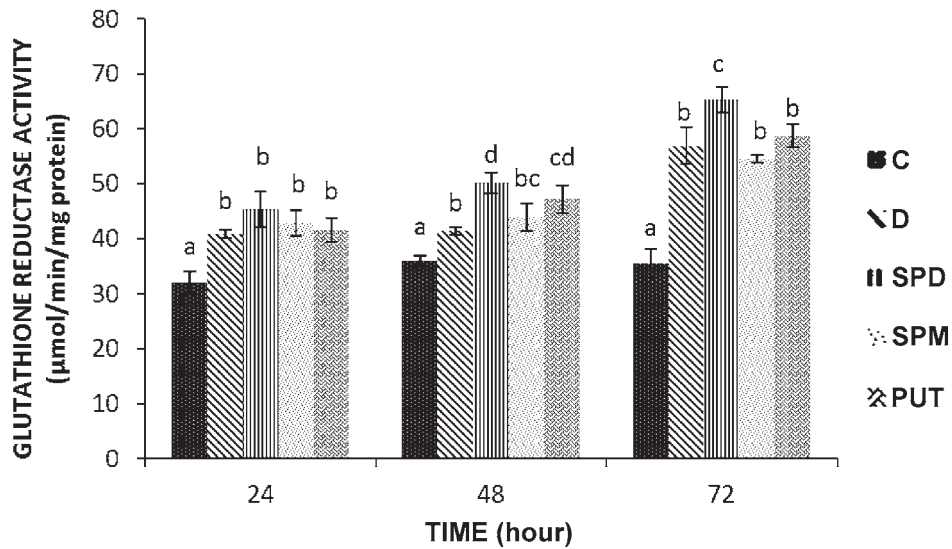


Fig. 4. Glutathione reductase activity in maize leaves pretreated with polyamines and treated with diuron afterwards (C: Control, D: Diuron, SPD: Spermidine, SPM: Spermine, PUT: Putrescine). Vertical bars represent standard deviation of three samples. Within each hour, different letters indicate the differences among the means of three replicates. The differences between the means are significant at $P < 0.05$

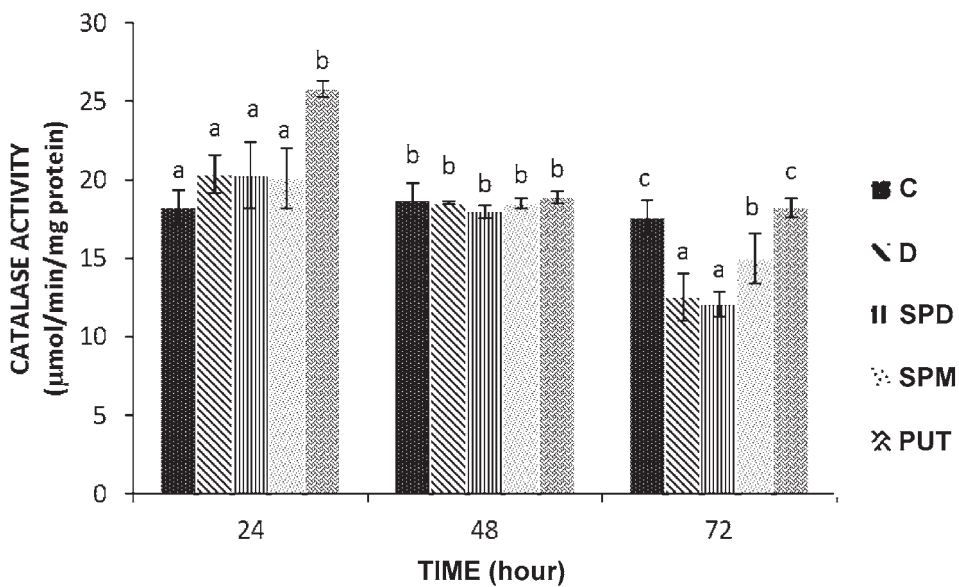


Fig. 5. Catalase activity in maize leaves pretreated with polyamines and treated with diuron afterwards (C: Control, D: Diuron, SPD: Spermidine, SPM: Spermine, PUT: Putrescine). Vertical bars represent standard deviation of three samples. Within each hour, different letters indicate the differences among the means of three replicates. The differences between the means are significant at $P < 0.05$

DISCUSSION

In response to diuron, lipid peroxidation and proline contents of maize leaves significantly increased while photosynthetic pigments and soluble protein levels decreased. The accumulation of MDA was measured as a sign of lipid peroxidation because MDA is one of products of lipid peroxidation and the result of cell membrane injury [2, 45]. The increase in MDA content of maize leaves may be due to the increase in ROS levels induced by diuron. However, it has been reported in several studies that diuron affected the production of ROS [31, 35]. In the present study, MDA levels in the leaves pretreated with polyamines were found to be lower than those of leaves treated only with diuron. SPM was the most effective in preventing to lipid peroxidation. Benavides et al. [7] described that lipid peroxidation induced by paraquat was almost completely prevented by SPM in sunflower leaf discs. Also, Kitada et al. [26] reported that SPM was the most effective in inhibiting lipid peroxidation in rat liver microsomes due to direct binding to microsomal phospholipids, and Tadolini et al. [36] attributed the inhibition of lipid peroxidation by SPM and SPD to the formation of a polycation/phospholipid vesicle complex. The more pronounced protective effect of SPM, in comparison with SPD and PUT, could be accounted for its longer chain and greater number of positive charges which result in a more effective neutralizing and membrane stabilizing ability [41].

Previous studies have demonstrated that the reduction of chlorophyll content might be a good indicator for monitoring damage of the plant growth and development [24]. In this study, it was shown that chlorophyll was rather sensitive to diuron. Total chlorophyll content in the leaves treated with diuron was significantly reduced, compared to the control. We have also found that the reduction in total chlorophyll content induced by diuron was significantly prevented in the leaves pretreated with polyamines. SPM and SPD were the most effective in preventing total chlorophyll loss in maize leaves. However, SPM has been reported as the very polyamine which preserved the most thylakoid integrity in oat leaves [38] and SPD was the most effective in inhibiting chlorophyll loss induced by paraquat in sunflower leaf discs [7].

Carotenoid levels also significantly decreased in the leaves treated with diuron, similar to total chlorophyll loss. Similarly, Fayez [19] reported that carotenoid and chlorophyll contents of soybean leaves treated with diuron were significantly reduced. In the present study, the reduction of carotenoid content induced by diuron was significantly prevented in the leaves pretreated with polyamines. SPM and SPD were the most effective in inhibiting the losses of carotenoids in maize leaves. The increment in carotenoid contents in the leaves pretreated with polyamines can reduce diuron toxicity because carotenoids act as efficient quenchers of triplet chlorophyll and singlet oxygen [13]. Also, polyamines can act directly as free radical scavengers and reduce the damage of diuron on photosynthetic pigments. Moreover, polyamines reverted the effect of diuron on the level of soluble protein almost completely at 24 h of treatment, SPM and PUT was the most effective at 48 h and the effect of only SPM was significant at 72 h in preventing of soluble protein losses. Similarly, Unal et al. [39] reported that exogenously applied SPM samples showed higher protein concentration than other exoge-

nously added SPD and PUT samples. Applications of SPD and SPM were effective in retarding the loss of D1, D2 and cytochrome f from the thylakoid membranes, as well as large Rubisco subunits and chlorophyll from the leaf tissue [9]. It was also reported that PUT, SPD and SPM are associated with light harvesting complex and PS II [27] and polyamines may protect photosystem II from UV-A radiation [39]. So, polyamines can protect PS II against diuron toxicity.

On the other hand, it was found that proline content significantly increased in the leaves treated with diuron. Similarly, proline content in the soybean leaves treated with diuron was recorded to be increased [19]. It is more probable that the proline increase reflected stress due to herbicide action. Due to its action as singlet-oxygen quencher and scavenger of OH radicals, proline is able to stabilize proteins, DNA and membranes [32]. In addition, it was reported that the damaging effects of singlet oxygen and hydroxyl radicals on PS II can be reduced by proline in isolated thylakoid membranes [3]. So, the increment in proline content of the leaves pretreated with polyamines can contribute to the role of free radical scavengers of polyamines and protect the plants against PS II inhibiting herbicide diuron.

SOD, GPX, GR and CAT play important roles in scavenging ROS and maintaining the physiological redox status of organisms [12]. In the present study, SOD activity significantly increased in the leaves treated with diuron for, respectively, 48 and 72 h. Similarly, increases in SOD activity have been observed in response to treatment with herbicides paraquat, diquat, diuron, atrazine and prometryne [17, 18, 24, 33]. In the present study, the effects of polyamines on SOD activity were different according to the polyamine tested and time after diuron treatment. Polyamines prevented the increase in SOD activity induced by diuron at 48 h of treatment but SPD and SPM increased the enzyme activity at 72 h of treatment. It was suggested that polyamines were implicated in direct scavenging of free radicals [30, 38], thereby SOD activity cannot be increased at 48 h of treatment.

In the present study, diuron caused significant changes in the activity of H₂O₂ scavenging enzymes. A decrease of CAT activity and an increase of GPX activity in the leaves treated with diuron at 72 h of the treatment as compared to the control were observed. Similarly; Kubis [30] reported that GPX activity increased in water stressed cucumber leaves while CAT activity decreased. Moreover, GPX activity was poorly stimulated in *Lemna minor* treated with diuron and even inhibited when exposure exceeded 48 h, while CAT activity was inhibited by the lowest concentration of diuron and at the shortest (6 h) time exposure [37]. On the other hand, no significant change of CAT activity was observed in *Scenedesmus obliquus* treated with diuron [21]. To test whether exogenously applied polyamines could protect the plants by affecting GPX and CAT activity against diuron toxicity, the change in activities of the enzymes was determined in the leaves pretreated with polyamines in the present study. Pretreatment with SPD increased GPX activity at 24 h of the treatment but decreased at 72 h compared with the leaves pretreated with water while significant change in CAT activity was not observed. Pretreatment with SPM did not change GPX activity during the whole series of experiments, in comparison with the leaves pretreated with water while it restored significantly the decrease in CAT activity induced by diuron. Pretreatment with PUT

increased GPX activity at 48 h of the treatment and CAT activity at 24 h of the treatment, while the decrease in CAT activity induced by diuron at 72 h of the treatment was completely prevented by PUT. There are both similar and different results in the literature referring to the effects of polyamines on the activities of both enzymes under various stress conditions. For example, pretreatment with SPD induced a significant decrease in the activity of both enzymes in barley leaves under water deficit [29], while the decrease of CAT activity was substantially reduced but a higher increase of GPX activity was induced by SPD in water-stressed cucumber leaves [30]. Zhang et al. [44] reported that exogenous application of PUT and SPD could effectively prevent cucumber seedlings from chilling injury by inducing activities of several antioxidant enzymes including SOD, GPX, APX and CAT.

In addition to SOD, GPX and CAT activities, a change of GR activity was also detected in the present study. It was found that GR activity gradually increased in the leaves treated with diuron during the whole experiment. This result was in accordance also with earlier results on *Lemna minor* and *Scenedesmus obliquus* treated with diuron [21, 37]. It is probable that the increase in GR activity under stress conditions is to provide GSH for ascorbate-glutathione pathway and to maintain a high ratio of GSH/GSSG. Pretreatment with SPD increased GR activity during the experiment, SPM did not change significantly GR activity and PUT increased the enzyme activity at 48 h of the treatment in comparison to the leaves pretreated with water. Similar to our results, Kubis [28] reported that GR activity increased in the barley leaves pretreated with SPD before water stress, and Verma & Mishra [42] recorded that pretreatment with PUT increased GR activity in salt stressed *Brassica juncea*. It is suggested that GR activity elevated by polyamines increase the ratio of NADP⁺/NADPH, thereby ensuring the availability of NADP⁺ to intercept electrons resulting in lower flow of electron to O₂ for generating ROS. As it is shown, polyamines are able to moderate the activities of scavenging system enzymes and thus influence the ROS level.

In conclusion, this is the first report examining the protective effect of polyamines against diuron damage in plants and our data show that pretreatment with polyamines reduce diuron toxicity at different degrees according to the polyamine and/or the tested parameter.

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