PLANTA DANINHA
SOCIEDADE BRASILEIRA DA CIÊNCIA DAS PLANTAS DANINHAS
<http://www.sbcpd.org>
ISSN 0100-8358 (print)
1806-9681 (online)

Article

Elimination of the Effect of Some Herbicides on the Growth of *Zea mays* and Accumulation in the Soil Using Urea

Eliminação do Efeito de Alguns Herbicidas no Crescimento de ***Zea mays*** *e Acumulação no Solo com Ureia*

ELHAKEM, A.H.[1]
ABD EL-SALAM, M.M.[1,2]*

ABSTRACT - This study evaluates the effect of urea on growth of herbicide-treated maize and its accumulation in the soil. When the seedlings were 15 days old, the plots were divided into five groups. One group served as control, two received treatments with metolachlor and isoproturon at the dose of 1.5 kg ha^{-1} and 2.5 L ha^{-1}, respectively. The two remaining groups received the same dose of herbicide along with urea added at a rate of 129 kg ha^{-1}. The application of the two herbicides appeared to cause a significant decrease in shoot dry weight, photosynthetic pigments, **d-**aminolevulinate dehydratase enzyme [ALA-D, EC 4.2.1.24] and total soluble carbohydrates of the maize plants. The application of urea appeared to alleviate the effects of both herbicides on maize growth. A statistical analysis demonstrated a significant association between the soil criteria and the accumulation of herbicides in it. This study clearly highlights the urgent need for use of urea to retract the effects of herbicides on maize growth.

Keywords: maize, soil accumulation, nitrogen fertilizer.

RESUMO - Neste estudo, foi avaliado o efeito da ureia sobre o crescimento do milho tratado com herbicida e sua acumulação no solo. Quando as plântulas tinham 15 dias de idade, as parcelas foram divididas em cinco grupos. Um grupo serviu como controle, e dois receberam tratamento com metolachlor e isoproturon nas doses de 1,5 kg ha^{-1} e 2,5 L ha^{-1}, respectivamente. Os dois grupos restantes receberam as mesmas doses de herbicida, juntamente com ureia adicionada a uma taxa de 129 kg ha^{-1}. A aplicação dos dois herbicidas parece ter causado diminuição significativa no peso seco da partícula, pigmentos fotossintéticos, enzima d-aminolevulinato desidratase [ALA-D, EC 4.2.1.24] e carboidratos solúveis totais das plantas de milho. A aplicação de ureia pareceu aliviar os efeitos de ambos os herbicidas no crescimento do milho. Uma análise estatística demonstrou associação significativa entre os critérios do solo e o acúmulo de herbicidas nele contidos. Este estudo evidencia claramente a necessidade urgente de utilização da ureia para retrair os efeitos dos herbicidas sobre o crescimento do milho.

Palavras-chave: *milho, acumulação de solo, fertilizante nitrogenado .*

*** Corresponding author:**
<mmagdy_hiph@yahoo.com>

Received: February 19, 2017
Approved: November 6, 2017

Planta Daninha 2018; v36:e018176075

INTRODUCTION

Maize (*Zea mays* L.) is one of the most important cereal crops in the world. Maize has a multipurpose position in the international economy as it is a

[1] Prince Sattam Bin Abdulaziz University, Kingdom of Saudi Arabia; [2] High Institute of Public Health, Alexandria University, Egypt.

Doi: 10.1590/S0100-83582018360100104

source of human food, animal and poultry feed and industrial products, such as starch. Maize grains contain protein (10.4%), fat (4.5%), starch (17.8%), vitamins and minerals (Khan et al., 2012a). Maize is highly susceptible to competition from weeds; yield losses of up to approximately 39.8% have been reported. Therefore, weed control is very essential in maize cultivation (Marshall, 2004; Amare et al., 2015).

Most Saudi Arabian soils contain a low N content and a considerable part of the applied N is volatized into the atmosphere, as well as lost into the soil through leaching, thus reducing the growth and yield of maize plants (Khan et al., 2012b). Several studies have found that the application of sufficient amounts of N was useful for promoting growth and for achieving higher yields of maize (Hassan et al., 2010; Khan et al., 2012b). In addition, the effectiveness of herbicides for controlling weeds and harvesting higher yields in maize crops has been documented in many research studies (Bibi et al., 2010; Khan et al., 2012a; Nadiger et al., 2013). Although herbicides are characterized as a highly effective tool for weed control, the use of herbicides has led to a change in the phytosociological composition of weeds and to a selection of biotypes that are resistant to herbicides, in addition to impact on the environment and on human health. When an herbicide is used to control weeds, most of it ends up in the environment - in the soil, water, and atmosphere - causing contamination in ecosystems or in harvested products. Moreover, herbicides can accumulate in the food chain and are toxic for human and animals; also, they have damaging negative effects on the biota (Marin-Morales et al., 2013).

Herbicides widely used for control of annual grasses and broadleaf weeds in maize plants include metolachlor and isoproturon, which belong to α-chloroacetamide and phenylurea groups of herbicides, respectively. Metolachlor {2-Chloro-N-(2-ethyl-6-methyl-phenyl)-N-(1-methoxypropan-2-yl) acetamide} affects protein, pigments, and gibberellic acid synthesis, cell division, minerals uptake and cell permeability (Stefanovic et al., 2010). Isoproturon (N, N-dimethyl- N-4 isopropyl phynylurea) affects photosynthesis, particularly the chloroplast electron-transport system (Gläβgen et al., 1999). Carbohydrates constitute most of the plant materials. Some herbicides induce decreases or increases in soluble sugars in plants (Hassan, 2000). Also, they found to be decreased the action of d-aminolevulinate dehydratase (ALD-D, EC 4.2.1.24) which control chlorophyll synthesis (Nemat Alla et al., 2001). Therefore, the aim of the present work was to achieve some physiological responses of maize plants to herbicides treatments with metolachlor or isoproturon and to evaluate the effectiveness of N fertilizer (urea) in removing the negative impact of herbicides on the soil and on maize growth.

MATERIAL AND METHODS

Plant material and growth conditions

A field experiment was carried out in different regions of Saudi Arabia (24.07° N, 47.58° E) during the summer of 2015. The region has hot desert climate. Precipitation varies throughout the year; the most common forms of precipitation are moderate rain, thunderstorms, and light rain. Over the course of a year, the temperature typically ranges from 10 °C to 44 °C. Maize seeds (*Zea mays*) were supplied by the Ministry of Agriculture of Saudi Arabia. After surface sterilization, the seeds were thoroughly washed several times and later soaked in tap water for eight hours. The maize variety was seeded at the rate of 40 kg ha^{-1} (recommended seeding rate) using a hand drilling method. The experimental site was properly monitored and irrigated whenever water was needed. When the seedlings were 15 days old, the plots were divided into five groups. One was left to serve as control, one received treatment with metolachlor at the recommended dose (1.5 kg ha^{-1}) (Amare et al., 2015) and another received isoproturon treatment also at the recommended dose (2.5 L ha^{-1}) (Nemat Alla et al., 2001). The other two groups of plots used the same doses of either herbicide with nitrogen fertilizer urea (46.5% N) which was added at a rate of 129 kg ha^{-1} (Nemat Alla et al., 2001). These quantities were mixed in a suitable amount of water to spray the surface area twice, in one direction and crosswise.

Soil sampling and analysis

Different types of soil samples were collected weekly for a period of one month from a depth of 0 - 20 cm close to the maize seeds to investigate the effects of soil properties on adsorption of herbicides. All of the soil samples were air-dried and sieved through a 2 mm sieve to remove debris and coarse gravel. Clay content was determined using the hydrometer method. A soil-water extract (1:5) was prepared. Soil pH was determined with a glass electrode pH meter. Soil organic matter (OM) was determined colorimetrically using the method described by Walinga et al. (1992). Herbicide residues were determined by gas chromatography-mass spectrometry (perkinElmer Clarus 500) analysis. The collection, preparation and chemical analyses of soil samples were performed according to the USDA (1994).

Measurement of shoot dry weight

The plants were removed from the soil and any loose soil was washed off. The shoots were separated from the roots. They were blotted to remove any free surface moisture and dried in an oven set to low heat (100 ^{o}F) for 48 hrs. Then the shoots were cooled in a desiccator and then weighed on a scale.

Determination of photosynthetic pigments

Plant photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) were determined using the methods of Dudek et al. (2014) for chlorophylls and carotenoids. This method was carried out at low light to protect the pigments. A known fresh weight (0.1 g m) of flag leaves was cut in an ice-cold porcelain mortar; some quartz sand and several mgs of Na_2C0_3 were added for reducing acidification. The leaves were ground with 1 mL of 80% acetone to squash. At the end of grinding, 3 mL of 80% acetone was added and the extract was stirred in the mortar; then the pigment solution was poured into a centrifuge tube. The extract was washed once or more times with 80% acetone, the remainder pigments from the mortar were transferred to a tube with cold 80% acetone, the volume was completed to 8 mL and well-mixed and the tube was covered with Parafilm to prevent evaporation of acetone during centrifugation. The extract was centrifuged for 3 min at 1000 rpm. After centrifugation, the sample color was measured within a short time and then the extract was stored in a cold (0-5 ^{o}C), dark place. The extract was measured against a blank of pure 80% aqueous acetone at 3 wave lengths of 480, 644 and 663 nm using a Spectronic 21 D spectrophotometer. Taking into consideration the dilutions made, the concentrations of the pigment fractions (chlorophyll a, chlorophyll b and carotenoids) could be determined as μg mL^{-1} using the following equations:

Chlorophyll a = 10.3 E663 - 0.918 E644 μg mL^{-1}

Chlorophyll b = 19.7 E644 - 3.87 E663 μg mL^{-1}

Carotenoids = 5.02 E480 = μg mL^{-1}

Subsequently, these concentrations were calculated on a fresh weight basis (as μg mL^{-1}), and as drought stress reduced leaf water content, the resulting values were corrected to a dry weight basis.

Determination of total soluble sugars

Total soluble sugars were determined by using the method of Riazi et al. (1985). Total soluble sugars were analyzed by reacting 0.1 mL of the alcoholic extract (1 g m^{-1} fresh leaves in 50 mL of 95% boiling ethanol) with 3.0 mL freshly prepared anthrone in a boiling water bath for 10 min and reading the cooled samples at 625 nm with a Spectronic 21 D spectrophotometer.

Assay of δ-aminolevulinate dehydratase [ALA-D, EC 4.2.1.24]

A known weight (0.5 g m) of fresh flag leaves was homogenized in chilled acetone. After centrifugation at 24000 rpm for 20 min, the residue (acetone-dried powder) was spread out on a filter paper and allowed to dry at room temperature. An aliquot of acetone-dried powder was extracted with a Tris-HCL buffer (0.05 M, pH 9) and then centrifuged at 24000 rpm for 15 min. The activity of ALA-D was assayed in a reaction mixer containing 1.8 mL of supernatant and

0.2 mL of a solute ion containing δ-aminolevulinate (ALA, 5 mg mL^{-1}) in a Tris-HCL buffer (0.05 M, pH 7). The reaction mixtures were allowed to react at 37° for an hour, and then the reaction was stopped by the addition of 1.0 mL M mercuric chloride in 10% trichloacetic acid followed by centrifugation. The quality of propholbilinogen was determined spectrophotometrically at 555 nm by the reaction with an equal volume of modified Ehrlich's reagent according to the procedure of Choiniere (2010).

Statistical analysis

The data were analyzed using the computer software package Statistical Package for Social Sciences (SPSS), version 11.0 (Daniel, 1995). The Pearson's correlation coefficient test was used to determine the correlations between the adsorption of herbicides and the physicochemical property parameters of the soil. A two-tailed p value < 0.05 was considered to be statistically significant. The paired samples t-test was used to compare the means before and after use of N fertilizer. A one-way analysis of variance (ANOVA) was used to find the significant differences in maize growth among the different treatments, with a level of significance of less than 5% ($p<0.05$).

RESULTS AND DISCUSSION

Persistence of herbicides in soil

The characteristics of soil collected from different regions in Saudi Arabia are shown in Table 1. All of the soil samples contained low percentages of OM, ranging between 0.02 and 0.68. Additionally, the soils were slightly alkaline with a pH range of 7.4 to 8.0. Similar results were found by Al-Falih (2000).

Table 2 shows that herbicides were more concentrated in soils with higher OM and clay contents compared with soils with lower OM and clay contents. Notably, there is a significant decrease in herbicide concentrations after the use of urea. There was a significantly positive correlation for absorption of herbicides in soil samples with clay and OM content ($p<0.01$) and a negative correlation was found with pH ($p<0.01$), as shown in Table 3.

The properties of different soil types were evaluated as predictors for adsorption and degradation processes. Soil composition is the most important factor affecting herbicide persistence through soil-herbicide binding (adsorption), leaching and vapour loss (volatilization).

Table 1 - Physico-chemical characteristics of soil samples collected from different regions in Saudi Arabia

Soil type n=12	pH	Organic matter content (%)	Soil texture		
			Sand (%)	Silt (%)	Clay (%)
Sandy	7.4	0.07	90.1	2.4	7.5
Loamy Sand	8.0	0.02	83.2	6.3	10.5
Sandy Loam	7.5	0.68	53.8	8.0	38.2

Table 2 - Metolachlor and Isoproturon concentrations in soil samples before and after addition of urea in Saudi Arabia

Soil type n=12	Unit	Herbicide concentrations				p-value Sig. (2-tailed)
		Metolachlor		Isoproturon		
		Before	After	Before	After	
		$\bar{x}$ ±SD	$\bar{x}$ ±SD	$\bar{x}$ ±SD	$\bar{x}$ ±SD	
Sandy	μg L^{-1}	0.63 ± 0.02	0.49 ± 0.02	0.90 ± 0.03	0.45 ± 0.02	.000*
Loamy Sand	μg L^{-1}	0.81 ± 0.09	0.37 ± 0.01	0.70 ± 0.04	0.31 ± 0.03	.000*
Sandy Loam	μg L^{-1}	1.79 ± 0.41	0.65 ± 0.06	0.94 ± 0.02	0.52 ± 0.01	.000**

* $p\leq0.05$; ** $p\leq0.01$.

Table 3 **-** Correlation between herbicide concentrations with clay, organic matter contents and pH of soil samples collected from different regions in Saudi Arabia

Herbicide concentrations n=12	Clay content		Organic matter content		pH	
	Correlation Coefficient	Sig. (2-tailed)	Correlation Coefficient	Sig. (2-tailed)	Correlation Coefficient	Sig. (2-tailed)
Metolachlor	0.692	0.012*	0.986	0.00**	- 0.923	0.061
Isoproturon	0.748	0.000**	0.841	0.023*	- 0.705	0.054

* Correlation is significant at 0.05 level (2-tailed); ** Correlation is significant at 0.01 level (2-tailed).

Soil composition is a physical factor determined by the relative amounts of sand, silt and clay in the soil (soil texture) as well as by OM content. Soil rich in clay and/or OM has a greater potential for carryover because of the increased binding of the herbicide to soil particles, with corresponding decreases in leaching and loss through volatilization. This reflects an increase in plant uptake and herbicidal activity. Medium- and fine-textured soils with OM content of more than 3% have the greatest potential to hold herbicides and injure sensitive rotation crops. Coarse- to medium-textured soils with lower OM content (less than 3%) are less likely to retain herbicides and have carryover issues. Under the right circumstances, however, herbicide carryover can occur in any type of soil (William, 2001). Morillo et al. (2004) determined the influence of different soil properties on herbicide adsorption. A percentage of herbicide eluted completely from soils with sand content > 80% and OM < 1%. In the present study, the variation of OM could be attributed to many factors, including sandy texture, good aeration and insufficient farming and management practices. All of these factors may be responsible for accelerating OM decomposition and, consequently, for low OM content in such soil. This finding is consistent with the results found by Akma et al. (2009), who evaluated the sorption and desorption of herbicides on soils with different ranges of OM content. According to the sorption and desorption results, OM and clay appeared to be the most important factor influencing the sorption capacity of 2,4-dichlorophenoxyacetic acid.

An important chemical property of soil that can influence herbicide persistence is pH. Low soil pH increases herbicide persistence. As soil pH drops below 6.0, herbicides become increasingly bound, or adsorbed, to soil particles. In higher-pH soils, smaller amounts of these herbicides are bound to soil particles (William, 2001). Herbicides were more strongly absorbed, less mobile, and less efficacious at a lower pH value. These observations were attributed to ionic bonding resulting from the protonation of basic functional groups on the herbicide molecules as pH decreases. The highest adsorption was found in the silty clay loam and the lowest, in the sandy loam soil. Conversely, the herbicides were more efficacious and mobile in the more coarsely textured soils (Stougaard et al., 1990). Ertli et al. (2004) found that the adsorbed amounts of isoproturon increased in the order of pH7 < pH8 < pH5. These findings can be explained by the formation of hydrogen bonds, typical at lower pH values, between the O, N and H atoms of the isoproturon and the surface groups.

According to the statistical analysis, there was a significant correlation (p<0.05) between herbicide concentrations and soil OM contents (Table 3). This suggests that OM was also a dominant parameter in the concentration of herbicide in soils with widely varying levels of OM, clay, sand, silt and pH. The increasing concentration of herbicide in soils with increasing OM content contradicts the results found by Rice et al. (2002), who attributed the increase in the degradation rate of herbicide in soils to the relatively high OM content. Yu et al. (2006) also showed that uptake of herbicide by soil decreased as the soil OM content increased. Tang et al. (2002) examined the availability of herbicide in different soil types and found that the greatest herbicide concentrations were in soil with the lowest OM content. Villaverde et al. (2008) investigated the influence of soil properties on herbicide adsorption and degradation. The study soils were characterized by low OM contents (0.3-1.0%) and varying clay contents (3-66%). Both OM and clay contents were found to be important in determining adsorption but the relative differences in clay content between soils were much larger than the differences in OM content and, thus, clay content was the main property for determining the extent of herbicide adsorption for these soils. Soil pH was negatively correlated with adsorption for all compounds, except metsulfuron-methyl. A clearly positive correlation was found between the degradation rate and the clay and OM content (p<0.01) and a negative correlation was found with pH (p<0.01).

Changes in maize shoot dry weight

Table 4 shows the response of shoot dry weight of maize plants to different treatments. Shoot dry weight increased in an orderly manner at all evolution stages (4 weeks after planting (WAP), 6 WAP, 8 WAP and 10 WAP) in the control as well as in the treated plants. The treatment with both herbicides caused a significant decrease ($p<0.05$) in shoot dry weight. The maximum values of 18.6, 40.2, 63.7 and 92.7 cm were recorded on metolachlor with urea-treated plants at stages 4WAP, 6WAP, 8WAP and 10WAP, respectively. On the other hand, the minimum values of 16.7, 34.7, 58.6 and 76.4 cm were recorded on isoproturon-treated plants at stages 4WAP, 6WAP, 8WAP and 10WAP, respectively.

Table 4 **-** Effect of metolachlor, isoproturon and their interaction with urea on shoot dry weight of maize at various growth stages

Treatment	Shoot dry weight (g)			
	4WAP	6WAP	8WAP	10WAP
	$\bar{x}$ ±SD	$\bar{x}$ ±SD	$\bar{x}$ ±SD	$\bar{x}$ ±SD
Control	20.5±3.52	42.6±4.05	67.6±5.52	95.3±6.13
Metolachlor	17.4±2.02	36.8±3.62	60.4±6.06	83.5±5.02
Isoproturon	16.7±1.68	34.7±3.08	58.6±5.07	76.4±7.08
Metolachlor + Urea	18.6±1.08	40.2±2.12	63.7±3.02	92.7±4.13
Isoproturon+ Urea	18.0±2.07	38.4±2.35	60.1±4.07	88.4±5.59
ANOVA-One Way F (p-value)	5.789 (0.001)*	11.905 (0.000)**	5.541 (0.001)*	22.530 (0.000)**

WAP: week after planting;. * $p\leq0.05$; ** $p\leq0.01$.

However, several investigations have reported that plant growth was greatly affected by different herbicides. Nemat Alla and Hassan (2014) recorded decreases in shoot height and fresh and dry weights of 7-d-old wheat seedlings after treatment with isoproturon. Nemat Alla et al. (2008) found that the growth parameters of maize seedlings were greatly reduced by metribuzin applications. Moreover, Stefanovic et al. (2010) reported a reduction in the growth of maize treated with metolachlor. Conversely, Hussein et al. (2007) reported a significant increase in various growth parameters of maize when supplemented with higher rates of N fertilizer. Khan et al. (2012b) demonstrated that the application of both a higher rate of N fertilizer and the use of herbicides (atrazine and Stomp) for controlling weeds in maize greatly increased various growth parameters of maize.

In addition, the reduction in growth caused by herbicides could be due to the inhibition of cell division and/or cell elongation. Kearney and Kaufman (1988) showed that herbicides inhibit cell division and cell elongation of treated seedlings. Moreover, changes occur in the amount of free amino acids and soluble protein, the inhibition of photosynthate transport and accumulation of neutral sugars, and the inhibition of mitosis and RNA synthesis.

On the other hand, the differing in the effects of metolachlor and isoproturon on maize plants might be due to the differential resistance to herbicides. However, the whole plant resistance to the herbicides was attributed to a lesser sensitivity of target site and/or increased herbicides degradation (Nemat Alla et al., 2001).

Changes in photosynthetic pigment content

It is clear from Tables 5 and 6 that the flag leaves of the control plants had higher pigments (Chl a, Chl b, carotenoids and total pigments) content at all evolution stages (4WAP, 6WAP, 8WAP and 10WAP). The treatment with the two herbicides resulted in a massive decrease ($p<0.05$) in the pigment content of the maize plants during the whole experimental period as compared with the untreated plants. The pattern of reduction in these pigments was more or less alike and metolachlor was more effective. The addition of urea to either herbicide generally retarded the effects of both herbicides to become mostly non-significant. The maximum values of Chl a, Chl b, carotenoids and total pigments were recorded on isoproturon with urea-treated plants at different growth stages.

Table 5 **-** Effect of metolachlor, isoproturon and their interaction with urea on chlorophyll a and chlorophyll b of maize plants at various growth stages

Treatment	Chl a (mg g^{-1} d wt)				Chl b (mg g^{-1} d wt)			
	4WAP	6WAP	8WAP	10WAP	4WAP	6WAP	8WAP	10WAP
	$\bar{x}$ ±SD	$\bar{x}$ ±SD	$\bar{x}$ ±SD	$\bar{x}$ ±SD	$\bar{x}$ ±SD	$\bar{x}$ ±SD	$\bar{x}$ ±SD	$\bar{x}$ ±SD
Control	4.36±1.22	5.73±1.82	7.35±2.41	8.65±2.36	1.86±0.23	2.13±0.35	2.64±0.67	2.97±0.56
Metolachlor	3.13±1.07	3.98±1.23	5.34±1.65	6.75±2.13	0.92±0.65	1.47±0.24	1.79±0.23	2.13±0.34
Isoproturon	3.46±1.25	4.24±1.57	6.46±2.24	7.43±2.55	1.35±0.45	1.87±0.56	2.34±0.55	2.45±0.75
Metolachlor + Urea	4.05±1.45	5.11±1.31	6.89±2.17	7.67±2.29	1.64±0.65	2.04±0.74	2.46±0.64	2.66±0.36
Isoproturon+ Urea	4.12±1.29	5.32±1.56	7.08±2.32	8.24±2.34	1.79±0.66	2.09±0.35	2.53±0.87	2.71±0.58
ANOVA-One Way F (p-value)	1.206 (0.000)**	2.937 (0.000)**	4.583 (0.000)**	5.144 (0.000)**	1.506 (0.000)**	0.487 (0.000)**	1.653 (0.003)*	1.901 (0.000)**

WAP: week after planting; * $p \leq 0.05$; ** $p \leq 0.01$.

Table 6 **-** Effect of metolachlor, isoproturon and their interaction with urea on carotenoids and total pigments of maize plants at various growth stages

Treatment	Carotenoids (mg g^{-1} d wt)				Total pigments (mg g^{-1} d wt)			
	4WAP	6WAP	8WAP	10WAP	4WAP	6WAP	8WAP	10WAP
	$\bar{x}$ ±SD	$\bar{x}$ ±SD	$\bar{x}$ ±SD	$\bar{x}$ ±SD	$\bar{x}$ ±SD	$\bar{x}$ ±SD	$\bar{x}$ ±SD	$\bar{x}$ ±SD
Control	1.43±0.32	1.98±0.54	2.43±0.65	2.94±0.75	7.65±2.12	9.84±2.12	12.42±3.21	14.56±3.43
Metolachlor	0.97±0.23	1.11±0.23	1.47±0.32	1.89±0.36	5.02±1.47	6.56±2.23	8.6±2.57	10.77±3.21
Isoproturon	1.08±0.45	1.32±0.51	1.86±0.54	2.32±0.43	5.89±1.53	7.43±2.1	10.66±3.56	12.2±2.66
Metolachlor + Urea	1.21±0.52	1.53±0.63	2.03±0.67	2.64±0.56	6.9±2.32	8.68±2.34	11.38±3.12	12.97±2.56
Isoproturon+ Urea	1.35±0.24	1.76±0.28	2.21±0.51	2.80±0.61	7.26±2.14	9.17±2.54	11.82±3.32	13.75±3.09
ANOVA-One Way F (p-value)	1.020 (0.000)*	0.789 (0.000)*	1.380 (0.001)*	0.486 (0.000)*	3.706 (0.000)**	5.590 (0.000)*	7.271 (0.000)*	9.053 (0.000)**

WAP: week after planting; * $p \leq 0.05$; ** $p \leq 0.01$.

The biosynthetic pathway of pigments is important plant- specific reactions accessible to herbicides interferences. In accordance with our results, Nemat Alla and Hassan (2014) found that treatment with isoproturon to 7-d-old wheat seedlings significantly decreased the contents of carotenoids, chlorophylls and anthocyanin. Moreover, decreases in contents of carotenoids, chlorophylls were also observed in wheat seedlings by butachlor and isoproturon (Nemat Alla et al., 2001) and maize by atrazine or fluometuron (Hassan, 2000).

Nevertheless, the reduction of chlorophyll by the herbicide was postulated not only to be the result of its degradation but also to the decrease of its synthesis (Kitchen et al., 1981). Also, Young et al. (1989) stated that chlorosis of plant tissue following application of herbicides may result as a consequence of either photooxidative destruction of existing pigments and/or inhibition of pigment biosynthesis. Nemat Alla et al. (2001) suggested that the induced photodestruction of chlorophylls in wheat seedling by butachlor and isoproturon was a result of the inhibition of carotenoids. Thus, an inhibition of carotenoid synthesis would subsequently lead to an interference with the protection of chlorophylls in the plant. However, the degradation of chlorophylls would result from the inhibition of carotenoid, which are chlorophyll protecting agents acting as chemical buffers to protect the chlorophylls and chloroplast from photooxidation by removing oxygen from the excited chlorophyll-oxygen complexes via a carotenoid-epoxide cycle.

Changes in ALA-D activity

Table 7 shows that ALA-D activity in flag leaves of maize plants was significantly reduced ($p<0.05$) as a result of herbicide treatments throughout experimental period (4WAP, 6WAP, 8WAP and 10WAP) as compared to controls. Isoproturon shows more reduction in ALA-D activity than metolachlor. It recorded 0.132, 0.164, 0.185 and 0.205 mg PBG released g^{-1} fresh wt h^{-1} at different evaluation stages 4WAP, 6WAP, 8WAP and 10WAP, respectively.

Table 7 **-** Effect of metolachlor, isoproturon and their interaction with urea on ALA-D activity of maize plants at various growth stages

Treatment	ALA-D activity (mg PBG released/g fresh wt/h)			
	4WAP	6WAP	8WAP	10WAP
	$\bar{x}$ ±SD	$\bar{x}$ ±SD	$\bar{x}$ ±SD	$\bar{x}$ ±SD
Control	0.203±0.02	0.235±0.05	0.275±0.06	0.297±0.05
Metolachlor	0.156±0.03	0.175±0.06	0.197±0.04	0.232±0.07
Isoproturon	0.132±0.02	0.164±0.04	0.185±0.03	0.205±0.04
Metolachlor + Urea	0.174±0.04	0.213±0.05	0.243±0.05	0.263±0.04
Isoproturon+ Urea	0.155±0.05	0.185±0.06	0.221±0.06	0.247±0.05
ANOVA-One Way F (p-value)	1.231 (0.000)*	0.051 (0.000)**	1.260 (0.000)*	0.182 (0.000)*

WAP: week after planting; * $p \leq 0.05$; ** $p \leq 0.01$.

On the other hand, the presence of urea with the herbicides raised the activity of ALA-D over the one detected in samples treated only with the herbicides though it still remained below the control values. The maximum values of the activity of ALA-D (0.174, 0.213, 0.243 and 0.263 mg PBG released g^{-1} fresh wt h^{-1}) were reported to metolachlor with urea-treated plants at 4WAP, 6WAP, 8WAP and 10WAP, respectively.

Inhibition of pigments biosynthesis was ascertained in relation to the changes in the activity of ALA-D, a key enzyme involved in chlorophyll biosynthesis. ALA-D activity is considered to be the rate-limiting step in chlorophyll synthesis. It was found that the ALA-D activity was significantly inhibited in wheat seedlings as a result of isoproturon application (Nemat Alla and Hassan, 2014). Similar results were found by Nemat Alla et al. (2001) in wheat seedlings inhibited by butachlor and isoproturon. They suggested that the inhibition of ALA-D activity might be due to the blocking of a step in the porphyrin synthesis pathway. Thus, a compound that blocks ALA-D synthesis would have far-reaching effects on biochemical and physiological systems in all plants. The reduction in enzyme activity appeared to be greatly correlated with the decrease of chlorophyll content in the treated tissues. The herbicide alone, which also greatly reduced chlorophyll, exhibited a great deal of inhibition of ALA-D activity. As a result of changes in this situation by the herbicidal treatments, an interference with plant metabolism is also expected with some disturbance in biochemical processes included in carbohydrates metabolism might lead to changes in their levels.

Changes in total soluble sugars

Data in Table 8 showed that total soluble sugars in flag leaves increased in control and treated plants throughout the exponential period (4WAP, 6WAP, 8WAP and 10WAP). As compared to control values, treatment with the two herbicides caused noticeable reduction ($p<0.05$) in

Table 8 **-** Effect of metolachlor, isoproturon and their interaction with urea on total soluble sugars of maize plants at various growth stages

Treatment	Total Soluble Sugars (mg g^{-1} d wt)			
	4 WAP	6 WAP	8 WAP	10 WAP
	$\bar{x}$ ±SD	$\bar{x}$ ±SD	$\bar{x}$ ±SD	$\bar{x}$ ±SD
Control	54.12±9.34	62.45±8.34	71.24±9.23	83.24±10.32
Metolachlor	40.34±8.76	47.63±9.3	54.73±7.46	64.05±9.54
Isoproturon	43.59±7.56	50.13±7.56	58.32±8.13	70.26±9.65
Metolachlor + Urea	47.46±7.54	55.43±8.34	64.02±9.02	76.45±8.03
Isoproturon+ Urea	50.59±8.64	58.23±7.26	66.13±8.24	79.31±10.21
ANOVA-One Way F (p-value)	16.146 (0.000)*	33.540 (0.000)*	29.094 (0.000)*	45.890 (0.000)**

WAP: week after planting; * $p \leq 0.05$; ** $p \leq 0.01$.

soluble sugars. Metolachlor shows the lowest values of total soluble sugars (40.34, 47.63, 54.73, and 64.05 mg g^{-1} d wt at 4WAP, 6WAP, 8WAP and 10WAP respectively).

The combination with urea to either herbicide slightly increased the amounts of total soluble sugars but the values remained lower than the control. On the other hand, isoproturon with the urea treatment shows the maximum values of total soluble sugars (50.59, 58.23, 66.13 and 79.31 mg g^{-1} d wt) at 4WAP, 6WAP, 8WAP and 10WAP, respectively.

The changes in the content of total soluble sugars might arise from an influence on the mechanism of their metabolism and/or disturbance in the rate of their transport out of the initial sources to the sinks in the plants. The decreases in soluble sugars found in these results might indicate a decrease in sugar biosynthesis as influenced by herbicides. Nemat Alla et al. (2001) found similar results in wheat seedlings influenced by butachlor and isoproturon. On the other hand, other findings claimed increases in the content of carbohydrates as a result of inhibition of transport of photosynthetes. Hassan (2000) reported that atrazine or fluometuron markedly increased sucrose content but significantly reduced glucose and polysaccharides. She claimed a reduction in photosynthesis activity and consequently in carbohydrate formation in spite of the accumulated sucrose, which might come from a decrease in its transport.

It is evident that the presence of urea with the two herbicides induced partial to complete recoveries of the tested parameters (shoot dry weight, pigments contents, total soluble sugars as well as ALA-D activity). Thus, urea addition was shown to counterbalance the toxic effect of metolachlor or isoproturon herbicides or to render them thoroughly ineffective. These effects of urea might be attributed to a restricted recovery in nitrogen metabolism. In this context, Nemat Alla et al. (2001) found a greater decrease in ammonia-N accumulation after treatment of the butachlor or isoproturon herbicides with (rather than without) the presence of nitrogen sources (potassium nitrate, urea or ammonium sulphate). In spite of the ammonia accumulated by the herbicides, which possibly reached toxic levels, a state of nitrogen starvation might therefore have occurred because its assimilation into organic forms was blocked. Moreover, they recorded an increase in soluble N as well as total N when nitrogen sources were applied with herbicides. This indicated that a great amount of ammonia was fixed as amino acids and, thus, total-N content was increased.

Furthermore, it was found that the effect of both herbicides on the other study parameters showed consequences to the altered nitrogen metabolism. Therefore, the application of urea appeared to equilibrate, to a great extent, the decreases in shoot dry weight, pigment contents, total soluble sugars as well as ALA-D activity induced by the herbicides. This finding indicated that urea induces maize plants to develop their physiological responses for increasing tolerance to herbicides.

ACKNOWLEDGEMENTS

This project was supported by the Deanship of Scientific Research at Prince Sattam Bin Abdulaziz University under the research project # 2015/01/3968.

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