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(Original Article)

Effect of Some Herbicides on the Total Seed Proteins Patterns of Maize and Sorghum Using Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS–PAGE)

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Abstract

Although herbicides are important in controlling weeds in corn and sorghum and increasing their yields, some of these chemicals have severe side effects on the environmental components. These negative effects may be extended to crop seed quality. This investigation was carried out with maize *(Zea mays* L.) and sorghum (*Sorghum bicolor* L.) in summer season of 2021 to study the effect of the recommended rate of the following herbicides: (carfentrazon 1.5% + florasulam 0.5% + flurxypyr-methyl 14%), (carfentrazon 1.5% + florasulam 0.5% + flurxypyr-meptyl 14% + halosulfuron-methyl 75%), (tribenuron-methyl 16% + carfentrazon ethyl 12%), (tribenuron methyl 16% + carfentrazon ethyl 12% + halosulfuron-methyl 75%), (bromoxynil 20% + MCPA sodium 20%), (bromoxynil 20% + MCPA sodium 20% + halosulfuron-methyl 75%), (halosulfuron-methyl 75%), (nicosulfuron 6%), nicosulfuron 6% + halosulfuron-methyl 75%), (foramsulfuron 2.25%), foramsulfuron 2.25% + halosulfuron-methyl 75%), comparing with the hand hoeing and the control on the seed proteins of commercial cultivars under Assiut field conditions. Results showed that all tested treatments gave different significant increases or decreases in seed protein profiles using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE). Data obtained showed that the bands with different molecular weights (KDa) of *Zea mays* and *Sorghum bicolor* seeds might be related to the mode of action of herbicides used to control associated weeds in these crops. The present results suggested the possibility of applying (SDS-PAGE) as supportive method to determine the sub-lethal effects of herbicides on total protein in sorghum and maize seeds.

Keywords: *Herbicides, Zea mays, Sorghum bicolor, SDS–PAGE, Electrophoresis*

Introduction

Mazie and sorghum are important crops in human food and livestock feed, so that protections of them from weed damages are very essential. Maize, (*Zea mays* L.) is an important cereal crop which ranks the third after wheat and rice (Ali

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and Abdelaal, 2020). Sorghum, (*Sorghum bicolor* L.) is ranked as the fifth leading cereal crop in Egypt and at the world level (Kobeasy *et al.,* 2005; Pandian *et al.,* 2022). The maize crop plant and weed species compete ruinously for nutrients, space, light, and water essential for their progress and advancement.

Among the different available weed control practices, chemical methods have become the new common tools in today's world (Sharma and Rayamajhi, (2022). Weed infestation, specifically grass weed species, pose a major problem in sorghum production and can reduce the crop yield up to 60% if left uncontrolled. Otherwise, the herbicides used in maize weed control, limited post-emergence herbicides are available to control broad-leaved weeds in sorghum due to its susceptibility to herbicides (Thompson *et al.,*2019; Dille *et al.,*2020). Weed control may be done either through manual eradication or herbicide application. Mechanical methods including hand weeding and hoeing are still useful but are getting expensive in laborious and time consuming.

Herbicides are a better conventional method used as integral part of the modern crop management system because it is cheaper, faster and it gives better control (Azza *et al.,* 2020). The continuous usage of the same herbicides or similar herbicide groups which have the same site of action successively over several years caused changing in weed flora, poor control, promoted the evolution of herbicide resistant weed biotypes, and had adverse effects on crops. To get the satisfactory control results, herbicide mixtures containing two or more active ingredients for weed control were applied (Pannacci *et al.,* 2007; Sulewska *et al*., 2012).

Balanced usage of herbicides should be considered in controlling weeds. The searching of the herbicide that had either no photoactivity effects or the adversely biochemical changes are essential to effectively control the sorghum weeds. Low herbicide concentrations may cause a biochemical change in proteins. The proteins may be increased or decreased with the herbicide treatments. These changes depend on several factors i.e., herbicide factors; concentration, site of action, systematic and environmental factors that are important in continues climate changes.

The use of available biochemical markers for the assessment of genetic diversity has received a great deal of attention in current years. SDS-PAGE is a practical and dependable method because seed storage proteins are extremely sovereign of environmental fluctuations (Akbar *et al.,* 2012). SDS-PAGE has been used as start point for using molecular techniques for studying the gene flow in crops. The aim of this work is studying the effects of some herbicides and their combinations on soluble seed total protein of maize and sorghum commercial cultivars using the SDS–PAGE protein profiles under Assiut field conditions.

Materials and Methods

Grain Cultivars: The crop cultivation, the herbicide treatments, seed samples and preparations were carried out during summer of 2021 in the Experimental Farm and Weed Science Laboratory of Plant Protection Department, Faculty of Agriculture, Assiut University. For maize, Watanya 6 cultivar was obtained from

Watanya Company for Seed Production, Giza, Egypt. Also, for sorghum, Ahmous cultivar was obtained from High Tech Company for Seed Production, Giza, Egypt.

Herbicides: One recommended concentration of each tested herbicide was applied on maize and sorghum crops in randomized complete block design (RCBD) experiment as post-emergence at 21-day post plantation using knapsack sprayer. The active ingredient and trade name and recommended rates of herbicides used in the experiments are shown in Table 1.

Table 1. The active ingredient, trade name, recommended rate, group, site of action of the tested herbicides

No.	Active ingredient	Trade name, Recommended rate /Feddan*	Group	Site of action
1	(Carfentrazon 1.5% + Florasulam 0.5% + Flurxypyr-methyl 14%)	Frosty16% SE,300 ml	Aryl triazolinone+Triazolopyrimidine + Aminopyridine	(Inhibition of Protoporphyrinogen Oxidase) + (Inhibition of Acetolactate Synthase) + (Auxin Mimics)
2	(Tribenuron methyl 16% + Carfentrazon Ethyl 12%)	Foldex28 % WP35 g	Sulfonylurea+ Aryl triazolinone	(Inhibition of Acetolactate Synthase) + (Inhibition of Protoporphyrinogen Oxidase)
3	(Bromoxynil 20% + MCPA Sodium 20%)	Rondo40% SP600 g	Phenoxyacetic acid+ Phenoxyacetic acid	(Inhbition of Photosynthesis at PSll - Histidine 215 Binders) + (Auxin Mimics)
4	Halosulfuron-methyl 75%	Inpul75% WG20 g	Sulfonylurea	Inhibition of Acetolactate Synthase
5	Nicosulfuron 6%	Active6% SC,400 ml	Sulfonylurea	Inhibition of Acetolactate Synthase
6	Foramsulfuron 2.25%	Equip2.25% OD, 750 ml	Sulfonylurea	Inhibition of Acetolactate Synthase

Protein extraction and electrophorus preparation

At harvest, one kilogram of maize and sorghum seeds was collected from each plot of the experiments. All chemicals and apparatus used were obtained from Molecular Biology Institute, Assiut University, Egypt.

Protein extraction

About 100 grams of whole seeds were crushed and ground with mortar and pestle to fine powder. 0.1-gram seed flour was put into 1.5ml micro-tube. Protein extraction buffer (400µl) was added to flour as an extraction liquid and mixed methodically in Eppendorf tube with a small glass rod. Bromophenol blue was added to extraction buffer as a dye to show the movement of protein in the gel. For cleaning the extraction, the homogenate samples were varied thoroughly by vertexing and centrifugation at 15,000 rpm for 10 minutes at room temperature, and then stored at - 4 ^{o}C until gel electrophoresis.

Preparation of electrophoretic gel

According to method of (Laemmli,1970) SDS-PAGE of total seed protein was carried out in 20% polyacrylamide slab gels in discontinuous buffer system. The gel was polymerized chemically by adding 15 microliters of tetramethylene-diamine and 10% ammonium per sulphate. The electrode buffer enclosed Tris glycine (9.0g Tris-HCl and 43.2g glycine per 3 liters buffer solution at pH 8.9)

with 3.0g SDS (0.1%). Ten microliters of sample were applied into the separation stacking gel sample wells.

Electrophoresis

Electrophoresis was carried out at 75V for around 3 hours until bromophenol blue marker reached bottom of the gel. The molecular weights of separated polypeptides were dogged by co-electrophoresis of molecular weight protein standards. The gels were stained with 2% commission blue solution for one hour. The gels were distained by washing with a solution containing water in the ratio of 5:20:75 (v/v) for about 2 hours.

Results and Discussion

Total protein analysis in maize seeds

Total protein analysis was performed for 13 treatments; 11 herbicide treatments (carfentrazon 1.5% + florasulam 0.5% + flurxypyr-methyl 14%), (carfentrazon 1.5% + florasulam 0.5% +flurxypyr-meptyl 14% + halosulfuron-methyl 75%), (tribenuron-methyl 16% + carfentrazon ethyl 12%), (tribenuron methyl 16% + carfentrazon ethyl 1% + halosulfuron-methyl 75%), (bromoxynil 20% + MCPA sodium 20%), (bromoxynil 20% + MCPA sodium 20% + halosulfuron-methyl 75%), (halosulfuron-methyl 75%) ,(nicosulfuron 6%), (nicosulfuron 6% + halosulfuron methyl 75%), (foramsulfuron 2.25%), (foramsulfuron 2.25% + halosulfuron-methyl 75%) , hoeing treatment and the control treatment.

Protein of purification fractions separated by SDS-PAGE (Figs. 1- 3) and analysis of the molecular weight in KDa (Table 1 & 2) showed that the total number of protein fractions produced by all treatments was 67 fragments. The number of protein fractions per treatment varied between 4 to 7 fractions.

These polypeptide fractions have a size ranged from 26.632 to 277.92 molecular weight in KDa of standard proteins. A total number of 67 polypeptide fractions were polymorphic in all treatments and the control samples. The three highest polypeptide fractions values were found at foramsulfuron 2.25%, (foramsulfuron 2.25% + halosulfuron-methyl 75%) and nicosulfuron 6% with 277.92, 162.73 and 146.36 KDa, respectively. However, the lowest polypeptide fractions values were found at halosulfuron-methyl 75%, (bromoxynil 20% + MCPA Sodium 20% + halosulfuron-methyl 75%) and (bromoxynil 20% + MCPA sodium 20%) with 26.632, 26.895 and 27 KDa, respectively.

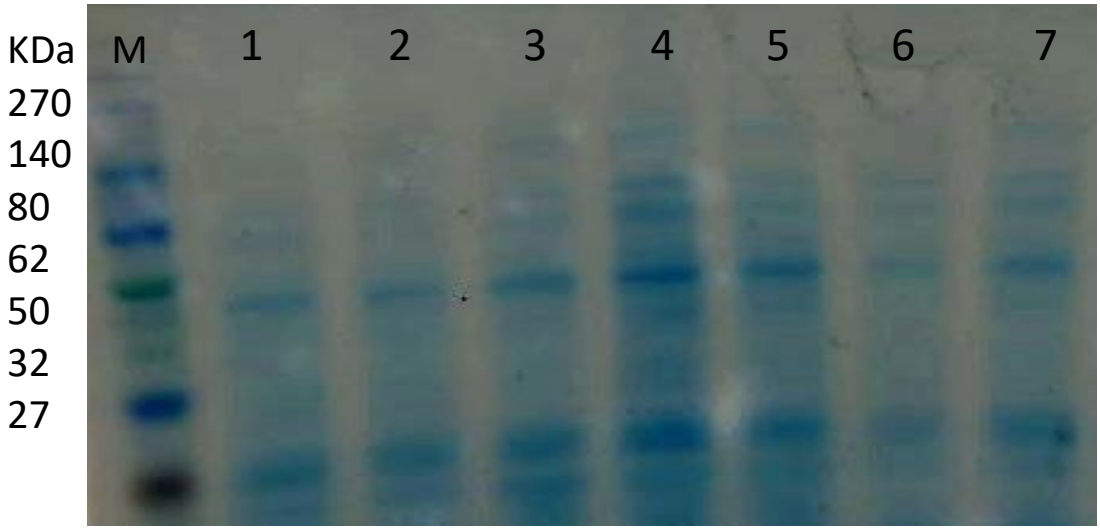

Fig1. Total protein of purification fractions separated. by SDS-PAGE Maize *(Zea mays*) seed samples with one herbicide recommended concentration. From left: marker then samples 1 = Control, 2 = Hand Hoeing, 3 = (Carfentrazon 1.5% + Florasulam 0.5% + Flurxypyr-methyl 14%), 4 = (Carfentrazon 1.5% + Florasulam 0.5% + Flurxypyr-meptyl 14% Halosulfuron-methyl 75%), 5 = (Tribenuron-methyl 16% + Carfentrazon Ethyl 12%), 6 = (Tribenuron methyl 16% + Carfentrazon Ethyl 12% + Halosulfuron-methyl 75%), 7 = (Bromoxynil 20% + MCPA Sodium 20%) , respectively

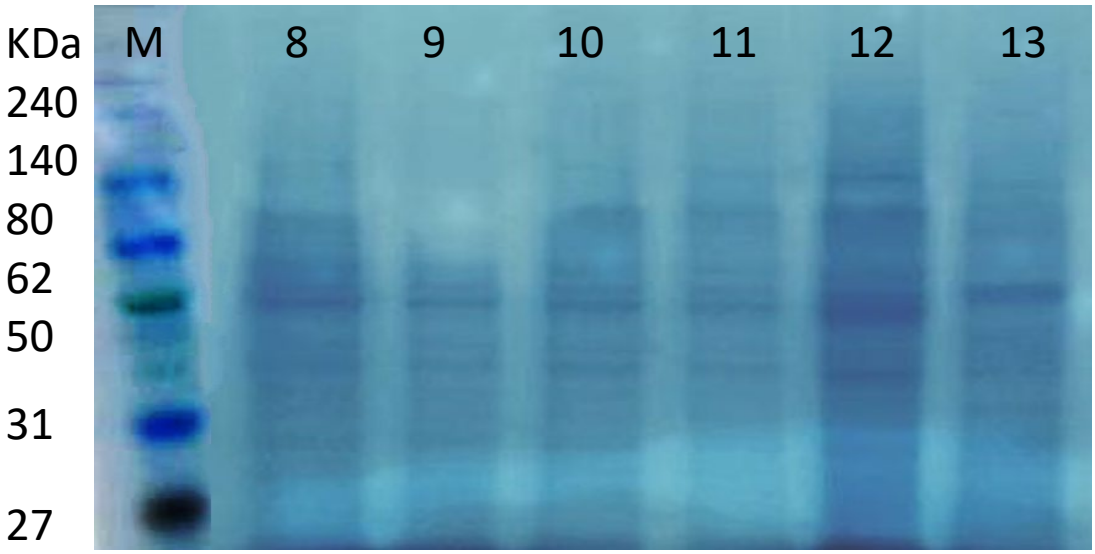

Fig. 2. Total protein of purification fractions separated by SDS-PAGE Maize (Zea mays) seed samples with one herbicide recommended concentration. From left: marker then samples, 8 = (Bromoxynil 20% + MCPA Sodium 20% + Halosulfuron-methyl 75%), 9 = (Halosulfuron-methyl 75%), 10 = (Nicosulfuron 6%), 11 = (Nicosulfuron 6% + Halosulfuron methyl 75%), 12 = (Foramsulfuron 2.25%), 13 = (Foramsulfuron 2.25%+Halosulfuron-methyl75%), respectively.

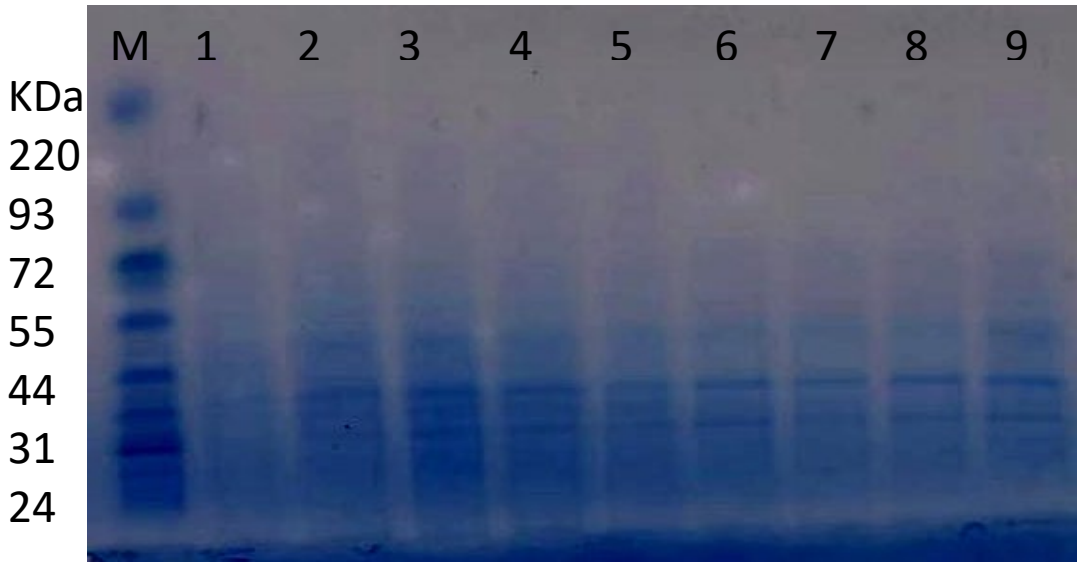

Fig 3. Total protein fractions separated by SDS-PAGE and the molecular weight in KDa of Sorghum (*Sorghum bicolor*) seed samples with one herbicide recommended concentration. From left: marker then samples, 1 = Control, 2 = Hand Hoeing, 3 = (Carfentrazon 1.5% + Florasulam 0.5% + Flurxypyr-methyl 14%), 4 = (Carfentrazon 1.5% + Florasulam 0.5% + Flurxypyr-meptyl 14% + Halosulfuron-methyl 75%), 5 = (Tribenuron-methyl 16% + Carfentrazon Ethyl 12%), 6 = (Tribenuron methyl 16% + Carfentrazon Ethyl 12% + Halosulfuron-methyl 75%), 7 = (Bromoxynil 20% + MCPA Sodium 20%), 8 = (Bromoxynil 20% + MCPA Sodium 20% + Halosulfuron-methyl 75%), 9 = (Halosulfuron-methyl 75%), respectively.

Table 2. Total protein fractions separated by SDS-PAGE and their molecular weight (mol.w.) in Kda of maize (*Zea mays*) seeds with different treatments.

Marker	Control	Hand Hoeing	Carfentrazon+ Florasulam +Flurxypyr-meptyl	Carfentrazon+Florasulam + Flurxypyr-meptyl +Halosulfuron-methyl	Tribenuron methyl +Carfentrazon Ethyl	Tribenuron methyl +Carfentrazon Ethyl+Halosulfuron-methyl	Bromoxynil+MCPA Sodium	Bromoxynil +MCPA Sodium + Halosulfuron-methyl	Halosulfuron-methyl	Nicosulfuron	Nicosulfuron+ Halosulfuron-methyl	Foramsulfuron	Foramsulfuron + Halosulfuron-methyl
-	1	2	3	4	5	6	7	8	9	10	11	12	13
(mol.w.)	(mol.w.)	(mol.w.)	(mol.w.)	(mol.w.)	(mol.w.)	(mol.w.)	(mol.w.)	(mol.w.)	(mol.w.)	(mol.w.)	(mol.w.)	(mol.w.)	(mol.w.)
												277.92	
240													
												175	
140							91.83						
			116.92	116.92									162.73
80					76.824	76.294	76.914			146.36			
	71.53			75.235			70.743				136.14	138.18	
		69.941	69.941	66.765	69.412	68.353		115.19					116.54
62	62.00	64.118	65.706							104.42	108.46		
				53	55.625	55.25	56.909					92	
50	49.00	50.75	51.5					88	87	85			85
				46.667	47.667						81		
31							30.692					75	
			30.273	29.545	29.909	29.909		62	60				
	28.00	27.727	27.2731		27.909					55	55		
27				27.455			27					49.143	49.5
								42.357					43.786
												38.429	
								35.895	26.632	35.526	35.158		

Table 3. Total protein fractions separated by SDS-PAGE and their molecular weight (mol.w.) in Kda of sorghum *(Sorghum bicolor*) seeds with different.

Marker	Control	Hand Hoeing	Carfentrazon+Florasulam + Flurxypyr-meptyl	Carfentrazon+Florasulam + Flurxypyr-meptyl +Halosulfuron-methyl	Tribenuron methyl +Carfentrazon Ethyl	Tribenuron methyl +Carfentrazon Ethyl+Halosulfuron-methyl	Bromoxynil+MCPA Sodium	Bromoxynil +MCPA Sodium + Halosulfuron-methyl	Halosulfuron-methyl
-	1	2	3	4	5	6	7	8	9
(mol.w.)	(mol.w.)	(mol.w.)	(mol.w.)	(mol.w.)	(mol.w.)	(mol.w.)	(mol.w.)	(mol.w.)	(mol.w.)
									369.55
220									
93	86.368								
72		81.947							
	67.714	65.571	63.429	64.857	62	61.286			
		58.429		56.423	56.423		58.429	57.714	59.857
55	52.385		55.269			53.538	52.385	51.231	51.808
44		47.769	48.346	46.038	47.192	43.731	43.154	43.154	46.038
	39.333	41.667							
		35.667	34.333	34.333					
31					32	32.667	32.667	32.333	33.333
24									
18									
15									

Total protein analysis in sorghum seeds

Total protein analysis in sorghum was performed on the same herbicide treatments tested on maize except 2 treatments. Protein of purification fractions separated by SDS-PAGE (Fig. 3) and analysis of the molecular weight in KDa (Table 3) showed that the total number of protein fractions produced by all samples was 39 fragments. The number of protein fractions per sample varied between 4 to 6 fractions per treatment. A total number of 39 polypeptide fractions. The total number of 39 fractions had 40 polypeptide fractions being monomorphic in control with 86.368 KDa, hand hoeing with 81.947 KDa and (halosulfuron-methyl 75%) with (369.55) KDa and the other 36 polypeptide fractions were polymorphic in treatments or untreated samples.

In the present study, nine from eleven herbicide maize cultivar treatments caused to add one, two, three peptide protein bands over the control/or hand hoeing. Sulfonylurea foramsulfuron showed 3 peptide protein bands and nicosulfuron added one band over the control, while halosulfuron-methyl from the same group decrease the peptide bands than the control. Otherwise, two additional peptide bands showed with halosulfuron-methyl as well as in the hand hoing of sorghum seeds. The main site effect of sulfonylurea herbicides is inhibition of acetolactate synthase. It seems that these effects differ from maize to sorghum species.

The additive bands in present results are compatible with (Kandil and Ibrahim, 2011; El-Rokiek *et al.,* 2012 and Elattar *et al.,* 2018). Who reported that the increasing protein contents percentage in grains was due to use of some herbicide treatments., suggesting that the synthesis of this polypeptide might be attributed to an increase in the amino acid content.

Decrease in the soluble protein content are well-known effects of herbicides inhibiting amino acid biosynthesis. Also, proteases might be involved in protein degradation to provide plants with amino acids that cannot otherwise be synthesized due to herbicide inhibition (Zulet *et al.,* 2013). In general, the alteration of active ingredients used in maize crop from triazine, acetanilide, thiocarbamate to sulfonylurea, resulted in changes of mechanism in related to weed suppression (Stefanović *et al.,* 2010). Halosulfuron-methyl is a sulfonylurea herbicide that inhibits acetolactate synthase (ALS), an enzyme involved in the biosynthesis of branched chain amino acids such as valine, leucine, and isoleucine (Senseman, 2007; Duggleby *et al*., 2008).

Soluble proteins of sorghum grains analysis of two low and high tannin varieties by polyacrylamide electrophoresis, were found 16-17 bands, but no bands have been detected in high tannin varieties (Chavan *et al.,* 1980). Treatments slightly affected electrophoretic protein patterns in sorghum grains compared with the control (Kobeasy *et al.,* 2005).

Crude protein percentages in wheat grains were significantly increased with flumetsulam + florasulam, pyroxsulam herbicide treatments compared with the unweed check (El-Rokiek *et al*. 2012; El-Metwally *et al*. 2013). (Sharaky and Ashour 1982) found that atrazine increased certain maize amino acids, i.e., leucine, cysteine, tyrosine, aspartic, alanine, isoleucine, arginine and histidine, while stomp increased valine, serine, tryptophan lysine and glycine.

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Hoeing treatment had a favorable effect on the content of glycine, alanine, leucine, isoleucine, serine, cystine, tyrosine, arginine and histidine, amino acids in comparison to unneeded and hand weeded treatments.

The present results disagree with (Abo El-Hassan and Hassanein, (2020) who found that applying herbicides (pendimethalin and linuron) with different doses had no effect on protein content or amino acids.

Sub-lethal concentrations of herbicides may possess growth regulators effects on the main crop metabolism Otherwise, herbicides may adversely affect the main crop by interfering with its essential biochemical processes such as protein metabolism and hydrolytic enzyme activity. Herbicide interference with biochemical pathways of treated plants varies according to the herbicide mode of actions, the degree of susceptibility or tolerance of the crop plant species, herbicide formulation, herbicide concentration, and environmental factors. The biochemical processes in herbicide treated plants might be affected, just as an herbicide contacts the site of action. As the herbicide concentration increases in plant tissue, additional sites of action may become involved.

In general, crops injury may be due to direct or indirect exposure to herbicide molecules (Pline and Hatzios, 2003; Essa *et al.,* 2018). Herbicides can cause negative effects on food crops as an unintended consequence of their use. Furthermore, these negative effects were genotoxic and morphological in nature (Hammok and Al-mandeel, 2020). Total proteins of low or high molecular weights are highly polymorphic and usually used for cultivar identification in many crops. Kobeasy *et al.,* -(2005) suggested that new bands which appear with the herbicides treatments might result from the over expression of genes, which might be involved in the resistance mechanisms. The seed storage protein pattern is considered as the genotypic fingerprint, therefore, used for several purposes such as plant variety protection, registration, certification, patents and as a breeding tool especially in flour quality breeding programs.

To sum up the results, from the eleven-herbicide tested, nine of them could increase the protein content. These observations may suggest that maize cultivar have herbicide tolerance/or resistance enzyme mechanisms over than found in sorghum cultivar where halosulfuron-methyl added one peptide protein band over than the control. These results suggest that although the herbicide tested has high efficacy in sorghum weeds, the low herbicide concentrates have adverse effects on seed quality. It may be due to sorghum cultivar has low herbicide defense. Halosulfuron-methyl is recommended for sorghum weed control without negative effect in protein. So that, the present results strongly suggest the possibility of applying (SDS-PAGE) as primary, supportive method to determine the sub-lethal effects of herbicides on total protein in sorghum and maize seeds.

Further studies about the resistant gene expression and enzymatic changes in sorghum cultivar with tested herbicide treatments are recommended to help breeders to find more sorghum resistant cultivars and introduce the suitable herbicides for controlling the weeds as halosulfuron-methyl. Also, herbicide residues in maize and sorghum seeds should be monitored. Moreover, specific molecular techniques should be applied for herbicide resistance.

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تأثير بعض مبيدات الحشـــائش على أنماط التفريد الكهربي للبروتينات في حبوب الذرة الشـــامية والذرة الرفيعة

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الملخص

تم إجراء تجربـة تفريـد كهربي على جيـل بولي أكريلاميـد دوديسـيـل كبريتـات الصـوديوم PAGE (SDS) باسـتخدام دقيق الذرة الشـامية (*Zea mays* L) ودقيق الذرة الرفيعة (*Sorghum bicolor* L) بعد الحصـاد لدراسـة تأثير معدل التطبيق الحقلي الموصـي به لمبيدات الحشـائش التالية: (كارفنترازون 1.5٪ + فلوراسـولام 0.5٪ + فلوروكسـيبير- ميبتيل 14٪)، (كارفنترازون 5.٪ + فلوراسولام 0.5 ٪ + فلوروكسيبير- ميبتيل 14٪ + هالوسولفورون- ميثيل 75٪)، (تريبينورون - ميثيـل 16٪ + كـارفنترازون إيثيـل 12٪)، (تريبينورون ميثيـل 16٪ + كـارفنترازون إيثيل 12٪ + هالوسـولفورون ميثيل 75٪)، (بروموكسـينيل 20٪ + MCPA صـوديوم 20٪)، (بروموكسينيل 20٪ + MCPA صـوديوم 20٪ + هالوسـولفورون ميثيل 75٪)، (هالوسولفورون ميثيل 75٪)، (نيكوسولفورون 6٪)، (نيكوسولفورون 6٪ + هالوسولفورون ميثيل 75٪)، (فورامسـولفورون 2.25٪)، (فورامسـولفورون 2.25٪ + هالوسـولفورون- ميثيل 75٪) بالإضـافة إلى العزيق اليدوي والكنترول. أظهرت النتائج أن جميع المعاملات عند وقت الحصـاد أعطت إما زيادة أو نقصـان في خصـائص البروتين SDS-PAGE، وأظهرت نطاقات ذات أوزان جزيئية مختلفة (كيلو دالتون) من دقيق الذرة الشـامية ودقيق الذرة الرفيعة، وكان ذلك مرتبطًا بطريقة عمل مبيدات الحشـائش المسـتخدمة في مكافحة الحشـائش المصـاحبة لهذه المحاصـيل. من هذه النتائج يقترح إمكانية تطبيق الترحيل الكهربائي على جيل بولي أكريلاميد دوديسـيل كبريتات الصـوديوم (SDS) كطريقة داعمة لتحديد فاعلية وتأثيرات الخواص الكيميائية لمبيدات الحشـائش ومخاليطها على البروتين الكلي القابل للذوبان في كل من دقيق الذرة الشامية ودقيق الذرة الرفيعة.

كلمات مفتاحية: *مبيدات الحشائش، الذرة الشامية، الذرة الرفيعة، الترحيل الكهربي، جيل بولي اكريلاميد*