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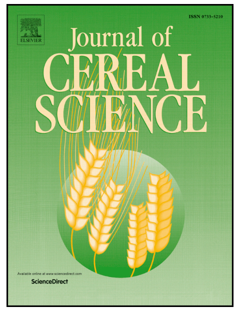

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EVALUATION OF THE NUTRITIONAL PROFILE OF SWEET MAIZE AFTER HERBICIDE AND FOLIAR FERTILIZER APPLICATION

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Abstract

Intensive weed management is required to meet the growing demands of sweet maize production. Herbicide application is inevitable in sweet maize production, while foliar fertilizer is commonly used in cropping in order to improve crop yield and quality. The effect of nicosulfuron and mesotrione, with and without foliar fertilizer, on the content of phytochemicals (i.e. carotenoids, tocopherols and free phenolic acids) in the kernels of three sweet maize hybrids was evaluated. Herbicides applied alone mainly improved the nutritive profile of the sweet maize kernel. The application of herbicides in combination with foliar fertilizer showed a high variability in the concentration of carotenoids, tocopherols and free phenolic acids. The significant change in the content of phytochemicals was induced by the applied treatments, but it is also genotype-dependent, which was also confirmed by the Principal Component Analysis.

Keywords: Phenolic acids; Tocopherols; Nicosulfuron; Foliar fertilizer.

1. Introduction

When consumed as a vegetable, sweet maize is mostly available as a frozen or preserved (canned) product due to the rapid conversion of water-soluble sugar into starch (Szymanek et al., 2006). In the past ten years, the total amount of exported frozen and preserved sweet maize products has increased by approximately 49% and 33%, respectively (FAOSTAT, 2016). This indicates that there is a demand for increased production worldwide. The application of herbicides in sweet maize crop is required in order to provide effective weed control. However, sweet maize is more sensitive to various stresses, including herbicides, than standard starchy maize, while it is considered to be a poor competitor to weeds, which is a limiting factor in the process of herbicide selection (O'Sullivan et al., 2000). Mesotrione, a member of the triketone group of herbicides, acts as an inhibitor of *p*-hydroxyphenylpyruvate dioxygenase (HPPD). HPPD catalyzes the bioconversion of tyrosine to plastoquinone and α-tocopherol (Mitchell et al., 2001). In sensitive plants, due to a decrease in the biosynthesis of carotenoids, bleaching of pigments can be noticed as a consequence of the HPPD inhibition. Nicosulfuron, a member of the sulfonylurea group of herbicides, inhibits acetolactate synthase (ALS), the key enzyme in the biosynthesis of the essential branched-chain amino acids: leucine, valine, and isoleucine (Schuster et al., 2007), thus affecting protein synthesis in plants. Both herbicides are registered for weed control in sweet maize and, when used at the recommended rate, they are rapidly metabolized to herbicidally inactive metabolites (O'Sullivan et al., 2000; Schuster et al., 2007; Kopsell et al., 2009). The first two decades of the twenty-first century were characterized by an increasing trend in the application of foliar fertilizer used as a supplement to soil fertilization in order to improve the crop yield and quality. Foliar fertilization provides crops with equally distributed and easily absorbable essential nutrients (micro- and macro-elements, amino acids, etc.) during plant development (Fageria et al., 2009; Silva Messias et al., 2013).

Sweet maize is an excellent source of health promoting phytochemicals such as carotenoids, tocopherols and phenolic acids (Ibrahim and Juvik, 2009; Das and Singh, 2016). Lutein and zeaxanthin protect ocular tissue against phototoxic damage by absorbing harmful high-energy blue light and prevent age-related macular degeneration (AMD) (Basu et al., 2001). The primary biological role of *β*-carotene is to enable provitamin A activity, but it can also act as a quencher of lipid radicals or singlet oxygen species (Grune et al., 2010). Tocopherols, the most powerful lipid-soluble antioxidants, protect the biological cell membranes by trapping peroxyl radicals and nitrogen oxide (Bramley et al., 2000). Phenolic acids are plant secondary metabolites which promote human health by quenching free radicals, scavenging singlet oxygen species, chelating metal ions or reacting with lipid alkoxyl radical (Das and Singh, 2016). Due to the benefits to human health, an attempt to obtain food of high nutritional quality has become a worldwide trend. The increase in the nutritional quality of sweet maize through herbicide application has been reported in only two papers (Kopsell et al., 2009; Cutulle et al., 2018).

The influence of herbicides and foliar fertilizers on the concentration of nutrients, of tocopherols and phenolic acids in particular, in sweet maize has not been published. These data are particularly important due to the continuous increase in the consumption of sweet maize worldwide. Therefore, the objective of this study was to assess the effects of herbicides from different groups with and without foliar fertilizer on the concentration of phytochemicals (i.e. carotenoids, tocopherols and free phenolic acids) in three different sweet maize hybrids. Furthermore, the principal component analysis was employed in order to evaluate the connection between the applied treatments and phytochemicals.

2. Material and methods

2.1. Field trial and treatments

In this research, three sweet maize hybrids – ZP504su (commercially available), ZP355su and ZP553su were sown in the first half of April 2017 in an experimental field at the Maize Research Institute Zemun Polje (44˚52´N, 20˚19´E). In the autumn (the beginning of November 2016) 100 kg/ha of mineral fertilizer (NPK 15-15-15) had been applied. In the spring (the beginning of March 2017) 200 kg/ha of urea fertilizer (46% N) had been incorporated into soil. A randomized block design with three replications was used for this experiment. Each hybrid was sown in three rows which were 5 meters long. Five treatments were investigated: C – control (without herbicide or foliar fertilizer (FF) application); M – mesotrione (120 g ai/ha); N – nicosulfuron (45 g ai/ha); M+FF – mesotrione + foliar fertilizer; N+FF – nicosulfuron + foliar fertilizer. Foliar fertilizer (FF) with the formulation: L amino acids – 6.5% w/w; total nitrogen – 3.0% w/w; total organic matter – 30.0% w/w, and seaweed extract – 4.0% w/w was applied at the recommended rate (1.5 L/ha). All treatments were applied at the 5-6 leaf stage by using a CO_2 pressurized sprayer (D-203S, R&D Sprayers Bellspray, Inc.) to deliver 200 L of water per hectare using a TeeJet 8002VS flat-flan nozzle.

Maize ears were hand harvested 21 days after pollination (technological maturity for sweet maize) and transferred to the laboratory. After desilking and dehusking, the undamaged kernels were collected and stored at -21ºC until analysis.

2.2. Chemical and HPLC analyses

For the determination of the concentration of tocopherols, carotenoids and free phenolic acids, approximately 1 g, 1.2 g and 1g of fresh kernel, respectively, was used. The extraction of tocopherols (α-T, β+γ-T and δ-T) was accomplished by using 10 mL of 2-propanol (Gliszczyńska-Swigło and Sikorska, 2004). The extraction of carotenoids (lutein + zeaxanthin (L+Z) and β-carotene) was performed by adding (2 × 6 mL) the mixture of methanol and ethyl acetate (6:4, v/v), (Rivera and Canela, 2012). The extraction of free phenolic acids (protocatechuic (PA), caffeic (CA), *p*-coumaric (*p*-CoumA), ferulic (FA) and cinnamic acid (CIN)) was achieved by using (2 × 5 mL) 80% methanol (Mesarović et al., 2017a). After homogenization in the ultrasound bath (30 min at 25 ºC) for all analyses, the extracts were centrifuged, filtered (0.45 μm nylon syringe filter) and directly injected into the Dionex UltiMate 3000 HPLC system (Thermo Scientific, Germany). For carotenoids only, prior to injection, the extracts were evaporated to the dryness under a stream of nitrogen and redissolved in the mobile phase. The same analytical column (Acclaim Polar Advantage II, C18 (150 × 4.6 mm, 3 μm) was used for the chromatographic separation of the tested phytochemicals. The mixture of acetonitrile and methanol (1:1, v/v) at isocratic program, 1 mL/min, was used as the mobile phase for the separation of tocopherols, while the mixture of methanol and acetonitrile, (90:10, v/v) at isocratic program, 1 mL/min, was employed for the separation of carotenoids. The detection of tocopherols and carotenoids was conducted by fluorescence (λ_{ex} 290 nm; λ_{em} 325 nm) and photodiode array (at 450 nm and 470 nm) detector, respectively. The mobile phase used for the separation of free phenolic acids and the wavelengths for detection were the same as reported by Mesarović et al., (2017a). The concentrations of the analyzed phytochemicals are expressed as μg per g of dry weight (DW) and reported as the mean value of three independent injections. The obtained value for DW was achieved by drying the fresh kernel (4 g) to constant weight in the ventilation dryer (105 ºC, 4h).

2.3. Data analysis

Two-factorial analysis of variance (ANOVA) for the randomized complete block design (RCBD) was conducted for the obtained results by using the M-STAT-C software (Michigan State University, 1989). For the determination of differences between hybrids (H), treatments (T) and the hybrid × treatment interaction (H × T), Fisher's least significant difference (LSD) test at 0.95 confidence level ($p \leq 0.05$) was employed. In order to interpret the data more easily, the obtained concentrations of the analyzed phytochemicals after all applied treatments were changed to percent difference from the control. Furthermore, the Principal Component Analysis (PCA) by using PLS Toolbox software package (v.6.2.1) within MATLAB (R2011a) was conducted. The tested data were mean-centered and auto-scaled and the singular value decomposition (SVD) algorithm was employed (95% confidence level) for Hotelling T2 limits.

3. Results

The tested hybrids, treatments and H × T interaction expressed significant impact on the concentration of analyzed phytochemicals (Table 1). The highest variability (3.66 %) between the tested factors was observed for *δ*-T content, while the lowest (0.91 %) was observed for FA content. The concentrations (µg/g DW) of all analysed phytochemicals after the applied treatments are given in Tables S1-S3 (Supplementary material).

Table 1. ANOVA and LSD value for the effect of hybrids, treatments and their interaction on the analyzed phytochemicals.

	Mean squares				$LSD_{0.05}$		
	H	T	H × T	CV (%)	H	T	H × T
L+Z	924.093**	32.17**	54.913**	3.62	0.76	0.98	1.70
β-carotene	3.176**	0.858**	0.431**	3.37	0.04	0.06	0.10
δ-T	0.069**	0.043**	0.193**	3.66	0.03	0.06	0.07
β+*γ*-T	60.664**	44.366**	50.54**	0.96	0.09	0.12	0.20
α-T	1.555**	0.504**	1.822**	2.05	0.04	0.06	0.10
PA	22.434**	227.37**	45.836**	2.70	0.91	1.18	2.04
CA	1.434**	0.36**	0.107**	0.95	0.00	0.01	0.00
p-coumA	132.291**	8.39**	29.564**	1.03	0.13	0.17	0.30
FA	34.65**	10.015**	7.663**	0.91	0.17	0.22	0.38
CIN	67.124**	2.691**	6.477**	1.42	0.06	0.08	0.14

**significant at 0.01 probability level; ~~df- degrees of freedom;~~ CV – coefficient of variation; LSD – Fisher's least significant difference test at 0.95 confidence level

3.1. Carotenoids

The obtained results revealed that all applied treatments significantly increased the concentration of lutein and zeaxanthin in all hybrids with the exceptions of mesotrione and nicosulfuron treatments for ZP355su and ZP553su, respectively, with regard to (w.r.t.) the control (Table 2). The combination of FF and mesotrione significantly increased the L+Z amount in ZP504su and ZP355su, as opposed to the nicosulfuron + FF treatment (Table S1). The content of *β*-carotene after all applied treatments was significantly higher compared to the control, except for ZP553su in the treatments with nicosulfuron and nicosulfuron + FF (Table 2). FF in combination with mesotrione and nicosulfuron had a greater impact on the increase of *β*-carotene in ZP504su and ZP553su (Table S1).

Table 2. Percent increase in the concentration of carotenoids in the sweet maize kernel after the applied treatments.

	% increase					
	ZP504su		ZP355su		ZP553su	
Treatment	L+Z	*β*-carotene	L+Z	*β*-carotene	L+Z	*β*-carotene
Control	0^{e}	0^{hi}	0^{h}	0^{n}	0^{f}	0^{gh}
Mesotrione	37.36^{b}	155.66^{b}	-40.99^{i}	69.00^{jk}	25.86^{e}	41.71^{e}
Nicosulfuron	19.73^{c}	33.69^{f}	81.15^{f}	126.32^{g}	-17.34^{g}	-35.74^{lm}
Mesotrione+FF	52.80^{a}	207.30^{a}	-3.12^{h}	31.50^{m}	32.28^{e}	67.20^{d}
Nicosulfuron+FF	11.29^{d}	103.29^{c}	40.37^{g}	87.13^{ij}	3.53^{f}	-30.01^{kl}

The percentages followed by a different letter are significantly different based on Fisher's least significant difference test at α = 0.05 level.

3.2. Tocopherols

All applied treatments significantly increased the amount of *δ*-tocopherol with the exception of mesotrione and mesotrione + FF treatments in ZP355su and nicosulfuron and nicosulfuron + FF treatments in ZP553su (Table 3). Significantly higher concentration of *β*+*γ*-tocopherols was noticed in ZP553su after all applied treatments compared to the control. The variability in *β*+*γ*-tocopherols was also observed for the other two hybrids after the applied treatments compared to the control. In ZP553su *α*-tocopherol content significantly decreased after all applied treatments compared to the control. The variability in *α*-tocopherol content was found in ZP504su and ZP355su after the applied treatments compared to the control. The combination of mesotrione + FF and nicosulfuron + FF significantly increased the content of *δ*- and *β*+*γ*-tocopherols in all hybrids, with the exception of nicosulfuron + FF treatment in ZP553su (Table S2). Furthermore, it was found in ZP355su and ZP553su that FF in combination with mesotrione significantly reduced the α-tocopherol content, as opposed to FF in combination with nicosulfuron.

Table 3. Percent increase in the concentration of tocopherols in the sweet maize kernel after the applied treatments.

	% increase								
	ZP504su			ZP355su			ZP553su		
Treatment	δ-T	$\beta+\gamma$-T	α-T	δ-T	$\beta+\gamma$-T	α-T	δ-T	$\beta+\gamma$-T	α-T
Control	0^{g}	0^{g}	0^{gh}	0^{d}	0^{i}	0^{f}	0^{d}	0^{k}	0^{b}
Mesotrione	131.80^{c}	38.77^{e}	57.96^{r}	-28.06^{e}	-35.85^{l}	-2.50^{f}	3.09^{d}	20.76^{h}	-5.41^{c}
Nicosulfuron	19.76^{fg}	-18.11^{j}	-32.29^{j}	27.11^{b}	26.76^{f}	38.32^{c}	-27.26^{e}	274.12^{a}	-61.66^{i}
Mesotrione+FF	165.89^{b}	71.01^{c}	77.16^{d}	-34.79^{ef}	-0.46^{i}	-25.87^{g}	42.84^{b}	43.37^{f}	-16.74^{d}
Nicosulfuron+FF	47.83^{e}	-4.47^{h}	-32.93^{j}	67.38^{a}	77.20^{d}	71.02^{a}	-42.55^{g}	195.65^{b}	-52.83^{h}

The percentages followed by a different letter are significantly different based on Fisher's least significant difference test at $\alpha = 0.05$ level. δ-T = δ-Tocopherol; $\beta+\gamma$-T = $\beta+\gamma$-Tocopherol; α-T = α-Tocopherol.

3.3. Free phenolic acids

Significantly higher concentration of free protocatechuic acid was found after all applied treatments compared to the control, with the exception of the treatments with nicosulfuron in ZP504su and ZP553su and nicosulfuron + FF in ZP553su (Table 4). Furthermore, the applied treatments significantly increased the free caffeic acid content with the exception of the mesotrione treatment in ZP355su and ZP553su, the nicosulfuron treatment in ZP504su and nicosulfuron + FF for ZP553su compared to the control. All applied treatments also increased the amount of free *p*-coumaric acid in ZP504su and ZP355su, with the exception of the nicosulfuron + FF treatment in ZP504su compared to the control. The significant accumulation of free ferulic acid in ZP355su and ZP553su was obtained after the applied treatments compared to the control, whereas the content of free ferulic acid in ZP504su was significantly lower compared to the control. The high variability in the concentration of free cinnamic acid was observed in all hybrids compared to the control after all applied treatments. It was noticed that the mesotrione + FF treatment and the nicosulfuron + FF treatment raised the concentration of free caffeic and cinnamic acid in ZP504su and ZP355su and free protocatechuic acid in ZP553su and free *p*-coumaric acid in ZP355su (Table S3).

Table 4. Percent increase in the concentration of free phenolic acids in the sweet maize kernel after the applied treatments.

	% increase														
			ZP504su					ZP355su					ZP553su		
Treatment	PA	CA	*p*-CoumA	FA	CIN	PA	CA	*p*-CoumA	FA	CIN	PA	CA	*p*-CoumA	FA	CIN
Control	0^{d}	0^{n}	0^{jk}	0^{f}	0^{m}	0^{fg}	0^{j}	0^{k}	0^{jk}	0^{e}	0^{gh}	0^{d}	0^{a}	0^{ij}	0^{g}
Mesotrione	-0.38^{d}	86.79^{l}	14.94^{gh}	-8.44^{i}	68.24^{k}	36.61^{c}	-2.56^{k}	15.07^{h}	30.19^{c}	-4.75^{f}	57.66^{b}	-0.72^{e}	-50.97^{g}	-2.53^{k}	-33.32^{j}
Nicosulfuron	-17.74^{f}	-26.67^{o}	7.37^{i}	-0.43^{f}	-72.50^{o}	12.25^{e}	6.16^{i}	3.36^{j}	37.54^{b}	28.22^{c}	-10.87^{i}	14.06^{c}	-17.32^{b}	10.36^{f}	-6.24^{h}
Mesotrione+FF	14.68^{b}	163.59^{g}	21.33^{f}	10.36^{d}	130.99^{i}	37.08^{c}	57.76^{a}	60.02^{c}	22.88^{e}	72.35^{a}	75.65^{a}	14.56^{b}	-46.58^{e}	7.98^{gh}	-55.63^{l}
Nicosulfuron+FF	2.39^{d}	46.05^{m}	-6.93^{l}	-1.58^{fg}	-34.41^{n}	-8.66^{h}	19.18^{f}	38.40^{d}	51.15^{a}	54.69^{b}	20.43^{e}	-6.13^{h}	-17.20^{b}	6.05^{h}	13.33^{d}

The percentages followed by a different letter are significantly different based on Fisher's least significant difference test at $\alpha = 0.05$ level. PA = protocatechuic acid; CA = caffeic acid; *p*-CoumA = *p*-coumaric acid; FA = ferulic acid; CIN = cinnamic acid.

3.4. *PCA*

In order to evaluate the connection between hybrids, applied treatments and analyzed phytochemicals, the PCA was applied and it resulted in the four-component model (85.32% of the overall data variance). PC1 and PC2 components explained 33.14% and 25.07% of the total data variance, respectively, and their mutual projections (factor scores and loadings) are shown in Figure 1a and Figure 1b. Interestingly, the PCA score (Figure 1a) revealed that the applied nicosulfuron and nicosulfuron + FF treatments influenced the concentration of *δ*- and *α*-tocopherol and free ferulic, caffeic, and cinnamic acid only in ZP355su. Similarly, the mesotrione and mesotrione + FF treatments influenced only the content of free protocatechuic acid, *β*-carotene, lutein and zeaxanthin in ZP504su and ZP553su. The variability of *β*+*γ*-tocopherol and *p*-coumaric acid was observed for the nicosulfuron and nicosulfuron + FF treatments in ZP553su and the mesotrione and mesotrione + FF treatments in ZP355su.

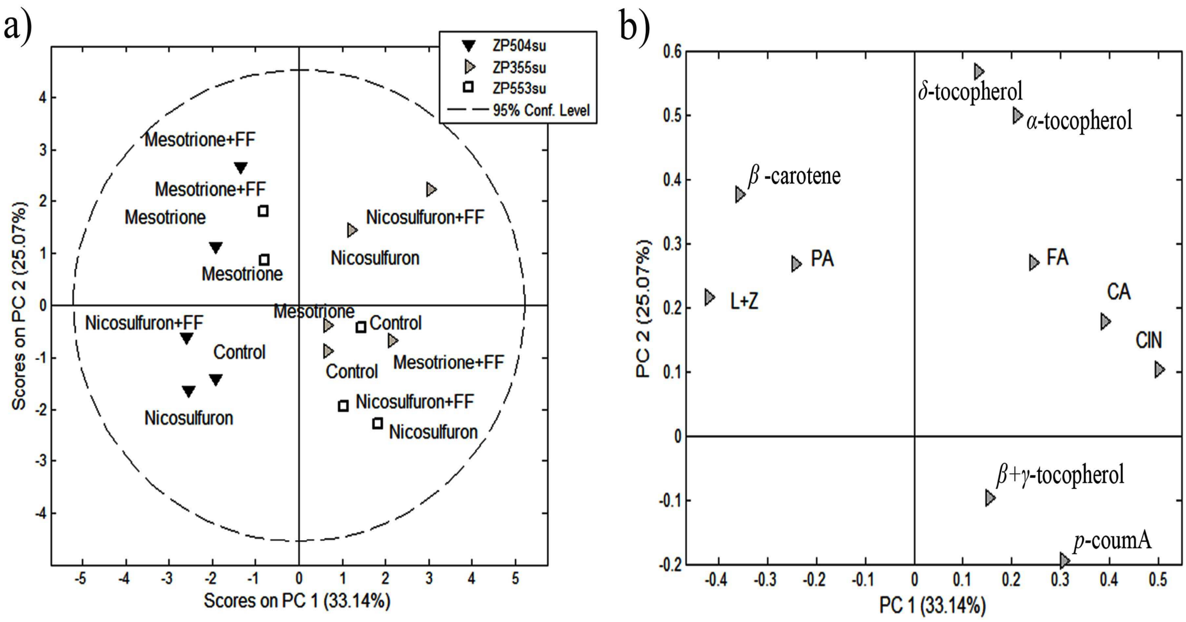

Figure 1. The obtained PCA score (a) and loading plot (b) for PC1 and PC2 components.

4. Discussion

The obtained concentration of carotenoids and tocopherols in the tested sweet maize hybrids was in agreement with Ibrahim and Juvik, (2009). However, the content of free phenolic acids obtained in our study was lower in comparison with the results obtained by Das and Singh, (2016). All applied treatments expressed significant variation in the concentration of phytochemicals in the tested hybrids. In line with our results, Kopsell et al.,

(2009) reported a significant increase in the content of carotenoids in a moderately sensitive sweet maize genotype. An increasing trend in the content of carotenoids after applying certain herbicides was also reported by Cutulle et al., (2018). The significant increase in the content of carotenoids in the sweet maize kernel could probably be explained by the formation of a large carotenoid pool as a result of mesotrione application (Kopsell et al., 2009). Mesotrione inhibits the HPPD enzyme and decreases the concentration of plastoquinone, which is a cofactor for phytoene desaturase (PDS). PDS is an important enzyme in carotenoid biosynthesis and its indirect inhibition could increase the concentrations of phytoene (Fritze et al., 2004). The accumulation of phytoene may continue for as long as the plant metabolizes mesotrione, after which the HPPD enzyme is reactivated. When the biosynthesis of plastoquinone starts again, PDS catalyzes the reaction and moves the substrate (a large pool of phytoene) into the carotenoid biosynthetic pathway, which further results into a higher concentration of carotenoids (Kopsell et al., 2009). McCurdy et al., (2008) reported that mesotrione suppressed PDS in leaf tissues, so it is possible that the same mechanism could take place in the kernel. It is possible that a similar mechanism could explain the tocopherol enrichment in the kernel after mesotrione application. The first reaction in the tocopherol biosynthesis starts with the conversion of *p*-hydroxyphenylpyruvic acid into homogentisic acid (HGA) by HPPD enzyme catalyzation (DellaPenna, 2005). HGA is then further subjected to various biochemical reactions and converted into all four forms of tocopherols. Due to HPPD inhibition after mesotrione application, as a consequence, a large pool of *p*-hydroxyphenylpyruvic acid could be formed. When mesotrione is metabolized in the plant and HPPD enzyme is reactivated, a high concentration of accumulated *p*-hydroxyphenylpyruvic acid moves as a substrate into the biochemical pathway, which results in a higher concentration of tocopherols. The variability in the concentration of tocopherols after mesotrione and nicosulfuron application obtained in our study was also reported by Mesarović et al., (2017b).

To the best of our knowledge, this is the first reported data on the influence of mesotrione and nicosulfuron, with and without FF, on the concentration of free phenolic acids in the sweet maize kernel. A trend in the accumulation of *p*-coumaric, cinnamic and ferulic acid after ALS inhibiting herbicides was reported by Orcaray et al., 2011, which is in line with our results. Furthermore, the variability in total phenolic compounds in the maize seedling after the application of herbicides belonging to different groups was observed

(Nemat Alla et al., 1995). Herbicides can modulate the secondary plant metabolites by affecting the shikimate pathway (Daniel et al., 1999; Orcaray et al., 2011). Nemat Alla et al., (1995) reported an increasing trend in the total hydroxyphenolic compounds and phenylalanine ammonia-lyase (PAL) activity after herbicide application. PAL catalyzes the reaction of the conversion of phenylalanine (one of the three final products of the shikimate pathway) into cinnamic acid, which is the common precursor for the synthesis of other phenolic derivatives. Furthermore, the conversion of cinnamic acid to coumaric acid is catalyzed by P450 monooxygenase (Daniel et al., 1999). The same enzyme is involved in phase I of herbicide metabolism, in which herbicide molecules are converted into less phytotoxic substances through chemical modification (De Carvalho et al., 2009). Furthermore, PAL can convert tyrosine directly into *p*-coumaric acid in grass, (Rösler et al., 1997). The observed changes in the PAL activity point out the diversity of herbicide effects, which results in huge variations in the secondary metabolites content. Some herbicides can reduce plant carbon fixation through photosynthesis, which can cause a reduced flow through the shikimate pathway and reduce the synthesis of phenols. Other herbicides can reduce the content of phenols by blocking the synthesis of aromatic amino acids (Daniel et al., 1999). The same authors reported that herbicides can both decrease and increase the total phenolic content in plants, which is in agreement with our study.

Another explanation for the higher content of antioxidants in the kernel is abiotic stress induced by herbicide application (Nemat Alla and Hassan, 2006). When the stress occurs, the plant responds with various biochemical reactions and *de novo* synthesis of both enzymatic and non-enzymatic antioxidants, such as carotenoids and tocopherols (Demidchik, 2015). Similarly, Kopsell et al., (2009) suggest that, after the diminution of metabolism induced by mesotrione and atrazine stress, plants respond by accumulating higher concentrations of carotenoids. Dragičević et al., (2010) reported the variability in the content of total phenolic compounds in maize shoots after herbicide application. A higher concentration of total phenolic compounds was found in maize leaves in the treatment with herbicides compared to the herbicide + FF treatment, which indicates that foliar fertilizer reduces herbicide stress (Brankov et al., 2017). Silva Messias et al., (2013) found that applied foliar fertilizer induced the improvement of secondary metabolites such as bound phenolic compounds and carotenoids, while our study showed a different trend in the content of phytochemicals in the treatments with foliar fertilizer. If foliar fertilizers improve the nutrient content (phytochemicals) in the crop, why do we observe a significant increase in carotenoids, tocopherols and free phenolic acids in treatments with mesotrione and nicosulfuron without foliar fertilizer (Table 2-4)? Perhaps such results indicate an incompatibility of the applied herbicides with the foliar fertilizer. Furthermore, it is known that nicosulfuron inhibits the biosynthesis of the essential branched-chain amino acids, but in what biochemical pathways does it affect carotenoids and tocopherols (Table 2-3)? The markedly different trend in the content of phytochemicals obtained in this study might indicate the variability in their susceptibility to herbicides and also the dependence on the genotype. The obtained variations in the content of phytochemicals indicate there is an alteration in the plant biochemical pathway in the presence of herbicides and foliar fertilizer and emphasize the complexity of the metabolic pathway that occurs (Cutulle et al., 2018). The performed PCA revealed that the variation in the content of phytochemicals depended both on the genotype and the applied treatments. Ibrahim and Juvik, (2009) reported differences in carotenoid and tocopherol contents between the sweet maize genotypes, indicating an allelic variation within gene loci regulating biosynthesis of these compounds.

4. Conclusion

HPPD and ALS inhibiting herbicides, with and without foliar fertilizer, modified the concentration of analyzed phytochemicals (i.e. carotenoids, tocopherols and free phenolic acids) in the sweet maize hybrids. Although the changes in the content of phytochemicals were different, the increasing trend occurs, at different rates, in the concentration of lutein, zeaxanthin, *β*-carotene, *δ*-tocopherol and free *p*-coumaric acid in ZP504su; of *β*-carotene, free *p*-coumaric and ferulic acid in ZP355su, and *β* + γ-tocopherol in ZP553su after the applied treatments when compared to the control. Significant decreases in the amount *α*-tocopherol and free cinnamic acid were observed in ZP553su after all treatments in comparison to the control. The PCA revealed that the content of phytochemicals was influenced by both the applied treatments and the sweet maize genotype. The variability in the alteration of phytochemical concentration which was observed in this study depended on both the applied treatment and the genotypes, which emphasizes the complexity of the biochemical pathways of plants and physiological mechanisms. The high variability and seemingly unfathomable plant processes after herbicide application with and without foliar fertilizer point out the need for further comprehensive studies in transcriptomics and metabolomics. Further research could include additional field experiments which would study the influence of some other combinations of herbicides, foliar fertilizers and safeners. The results obtained in this study highlight the potential of herbicide application, which is widely used in the agronomic practice, as a tool for improving the nutritive quality of the sweet maize and not only for weed control.

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Highlights

- First report of herbicides impact on free phenolic acids content in sweet maize kernel.
- Assessment of the effects of herbicides plus foliar fertilizer on eleven phytochemicals.
- Improved free ferulic acid and α- tocopherol content was noticed.
- Applied treatments gave sweet maize higher value in terms of functional foods.