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EFFECTS OF FERTILIZER TREATMENT ON THE POLYPHENOL CONTENT IN MAIZE AND VELVETLEAF COMPETITION

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Abstract: The aim of this research was to examine the effect of fertilizer treatment on the content of polyphenols in velvetleaf and maize, grown without use of herbicides under controlled conditions (12h/12h, T 25º/22ºC day/night). Plants were treated with organic - F1 and synthetic fertilizers - F2 and F3. The content of total phenols, individual polyphenolic acids and antioxidant activity of plant extracts were examined, as important indicators of the plant condition and its resistance to oxidative stress. The content of total phenols was determined spectrophotometrically (modified Folin-Ciocalteu method). The content of individual polyphenolic acids was determined by the high-performance liquid chromatography method, while measurement of polyphenols antioxidant activity was determined by the DPPH method. It was established that in maize, the application of fertilizers, neither in monoculture nor in competition with velvetleaf plants, caused statistically significant changes in the content of polyphenolic acids. However, in velvetleaf, the use of fertilizers led to an increase in the content of polyphenolic acids in conditions of competition vs monoculture. The content of chlorogenic, p-coumaric and trans-ferulic acids was statistically, significantly higher in plants of velvetleaf competition vs monoculture, and the content of chlorogenic, p-coumaric and cinnamic acids was lower in plants of maize competition vs monoculture. A statistically significant increase in the content of total polyphenols and their antioxidant activity in velvetleaf plants indicates the difficulty of removing weeds from crops, especially resistant populations. The research conducted and the results obtained confirm that weeds in competitive conditions manifest their natural competitive advantage, which is further enhanced by crop feeding.

Key words: polyphenols, velvetleaf, maize, competition.

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Introduction

The effects of fertilizer application on cultivated plants have been the subject of numerous studies. There is a significantly smaller number of investigations and studies related to their effects on the environment and the changes they cause in weed plants. The fact is that fertilizers are a source of biogenic elements, primarily nitrogen, for cultivated plants, but also for weed plants. Plants absorb about 50% of the nitrogen from applied fertilizers, and the remaining part ends up in groundwater and the atmosphere (Erisman et al., 2015).

The absorbed nitrogen and other biogenic elements in plants participate in various biochemical processes. Polyphenols represent an important group of metabolites (Marchiosi et al., 2020). They are products of the secondary metabolism of plants and their role in plants is diverse: assimilation of nutrients, photosynthesis, enzyme activity, protein synthesis, photoreceptors, protection from UV radiation, etc. (Wu et al., 2001; Liu et al., 2004; Ikhajiagbe and Ogwu 2021). They are especially necessary for the biosynthesis of aromatic amino acids (Waksmundzka, 1998) and in stressful conditions (competition, herbicide application, drought, etc.). As non-enzymatic antioxidants, polyphenols prevent the oxidation of lipids, carbohydrates, proteins and DNA (Shahidi and Zhong, 2010). Their activity is manifested in several ways: (1) direct binding of free oxygen and nitrogen radicals and hydrogen atom donation, (2) activation of antioxidant enzymes, (3) chelation of metal ions, (4) inhibition of some enzymes, etc. (Lobo et al., 2010). From the aspect of agricultural practice, applied fertilizers through polyphenols can affect the effectiveness of herbicides.

Phosphorus from applied fertilizers is incorporated into a multienzyme complex (Achary et al., 2017) that regulates the “Shikimic acid cycle” (Winkel-Shirley, 1999), i.e., the transformation of L-phenylalanine and L-tyrosine into different groups of polyphenolic compounds (Rodriguez et al., 2015). In addition, fertilizers with lower nitrogen concentrations increase the resistance of the weed, *Setaria viridis* (6x) to a.m. nicosulfuron and *Amaranthus retroflexus* on a.m. nicosulfuron, glyphosate and mesotrione (Cathcart et al., 2009). Heavy metals originating from fertilizers do not only accumulate in the plants, but they are usually deposited in the soil. Therefore, the aim of this research was to examine the effects of fertilizers on the content of polyphenols in velvetleaf (*Abutilon teophrasti*) and maize (*Zea mays*) grown in conditions without the use of herbicides, as well as the accumulation of heavy metals in the environment.

Material and Methods

The test plants *Abutilon teophrasti* (velvetleaf) and *Zea mays* (maize) were grown under controlled conditions (photoperiod 12h/12h, T av. 25º/22ºC day/night). Six seeds of each species were placed in 1-L pots in two systems: monoculture (velvetleaf or maize) and competition (maize and velvetleaf plants in the same pot). For each group of plants, the control is plants without fertilizer application. The plants were sown in soil certified for growing seedlings – Floragard TKS 1. The plants were treated with different fertilizers 25 days after germination. The effects of organic fertilizer – F1 (mixture of xydroxy acids: chlorogenic, caffeine and chichoric; 15 µl/100 ml of water), synthetic-organic – F2 (2% amino acids [alanine, isoleucine, histidine, etc.], 2% organic carbon, vitamin, 1.7% nitrogen and microelements [ferro, zinc, calcium, molybdenum, etc.]; 1.5 ml/100 ml of water) and synthetic F3 (nitrogen, phosphorus and potassium fertilizer; 1.5 ml/100 ml of water) were examined. The fertilizer was applied with a manual sprayer with a volume of 500 ml. The material samples for all analyses were taken 20 days after fertilizer application. The content of total polyphenols (plant material, soil) and the content of individual polyphenolic acids (plant material), their antioxidant activity (plant material) and the content of heavy metals in the soil were monitored.

The extraction of polyphenols from the plant material and the soil was carried out in 70% methanol in an ultrasonic bath (Asonic Pro 4P, 40 kHz) (2x for 30 minutes with a 15-minute break). For the analysis, 2.5 g of soil and 3 g of plant material crushed by liquid nitrogen in an oven were measured. The extracts were filtered through a 45-µm PTFE filter. The content of total phenols, individual polyphenolic acids and antioxidant activity were determined from the same extracts.

Determination of the total phenol content

The content of total phenols was determined spectrophotometrically according to the modified Folin-Ciocalteu method (Singleton et al., 1999). First, 50 µl of the plant extract was mixed with 250 µl of Folin's reagent (prepared in a 1:1 ratio with distilled water), 750 µl of 20% Na_2CO_3 and 3 ml of water and left at room temperature for 8 minutes. Then, 950 µl of water was added and incubated in the dark for 2 hours. The standard curve was defined by gallic acid in the concentration range 0–500 µg/ml. The content of total phenols was read spectrophotometrically at λ=765 nm (UV/Vis spectrophotometer UV-2100, Shimadzu, Japan), and their content was expressed as milligram equivalent of gallic acid per gram of dry matter (mgekvGK/g d.m.).

Determination of individual polyphenolic acid content

The content of individual polyphenolic acids was determined by high-performance liquid chromatography (HPLC) according to the method of Robbins (2003) with minor modifications. The elution mode was adapted to the length of the column used, Zorbax SB C18 4.6×250 mm, pore diameter of 5 µm, thermostated at 25°C. The Shimadzu Nexera-XR liquid chromatograph was used. The flow of the mobile phase was set to 1 ml/min, and the amount of injected sample was 10 μl. The injection was performed automatically, using an autosampler. A mixed solution of chlorogenic, p-coumaric, ferulic, and trans-cinnamic acids at a concentration of 1 mg/ml was prepared in 70% methanol and dilutions were made in the mobile phase at a 1:1 ratio. Calibration curves of the polyphenolic acids were constructed in the range of 25–250 μg/ml. Polyphenolic acid content was monitored at two wavelengths, 280 nm (cinnamon) and 325 nm (chlorogenic acid, p-coumaric acid and ferulic acid). The concentration of each polyphenolic acid in the samples was calculated using the LabSolutions software (Shimadzu) and expressed in μg/g s.m.

Monitoring the antioxidant activity of polyphenols

The antioxidant activity of the plant extracts was determined by the DPPH method (Gil et al., 2002). The prepared extracts were diluted 10 times, 200 μl of the diluted extract was pipetted out and 3.8 ml of the 0.1 mM DPPH reagent prepared and was added immediately before the analysis. The samples with the reagent were incubated in the dark for 30 minutes. The antioxidant activity reading was performed on a UV-2100 spectrophotometer (Shimadzu, Japan) at λ=517 nm and expressed as μmol TE/g s.m. A standard curve was generated from a solution of TROLOX (T) in the concentration interval 0–1000 μmol/l. The stock solution of TROLOX with a concentration of 1 mmol/l was prepared in 70% methanol and dilutions of different concentrations (0, 50, 100, 250, 500 and 750 μmol/l) were prepared.

Statistical analysis

All the results obtained were compared by the analysis of variance (LSD test) for the comparison between control and the fertilizer-treated plants and the t-test for independent samples to compare a given parameter between different treatments. The statistical package "Stat 7" was used to analyze the obtained values.

Results and Discussion

Research showed that there were no statistically significant changes in the content of individual polyphenolic acids in maize grown in monoculture and in competition with velvetleaf plants compared to untreated plants. However, in velvetleaf, the use of fertilizers (F1, F2 and F3) resulted in an increase in the content of individual polyphenolic acids in competitive conditions compared to monoculture conditions (Table 1), which indicates the stimulating effect of the fertilizers applied on the weed plants. The measured increase in ferulic acid content in maize plants (Table 1) cannot be clearly attributed to the effect of organic fertilizer (F1) due to the fact that this polyphenolic acid is naturally present in maize tissue (Adom and Liu, 2002). On the other hand, organic fertilizers are known to have a stimulating effect on plant growth through proteins in whose biosynthesis ferulic acid participates (He and Lin, 2001). All this leads to the conclusion that fertilizers enhance the natural feature of weeds – better competitiveness.

Table 1. Statistical analysis (LSD test) of the content of polyphenolic acids (µg/g fresh mass) in velvetleaf and maize leaves under competitive conditions after fertilizer application.

	velvetleaf competition				maize competition			
	chlorogenic	ferul	kumarn	cinnamon	chlorogenic	ferul	kumarn	cinnamon
K/F1	**	**	**	ns	ns	*	ns	ns
K/F2	**	**	**	ns	ns	ns	ns	ns
K/F3	**	**	**	ns	ns	ns	ns	ns
F1/F2	ns	ns	*	ns	ns	ns	ns	ns
F1/F3	ns	ns	ns	ns	ns	ns	ns	ns
F2/F3	ns	ns	ns	ns	ns	ns	ns	*
SD	6.62	9.75	7.18	12.97	6.626	1.121	1.712	3.350
mean	35.16	34.51	30.39	26.84	46.03	39.33	35.54	27.36

SD – standard error, K – control, ns – differences are not statistically significant, $p<0.05$*, $p<0.01$**, F1– organic, F2 – organic-synthetic and F3 – synthetic fertilizers.

The analysis of the obtained data revealed no difference in the effect between organic and synthetic fertilizers (except for the content of p-coumaric acid in velvetleaf in favor of F1, Table 1). In addition, a difference was found in the content of cinnamic acid in maize (Table 1) compared to synthetic fertilizers, although some studies showed a better effect of organic fertilizers (Stefanelli et al., 2010; Wendy et al., 2012; Ma et al., 2015). Hamouz et al. (2010) agree with this conclusion and explain that higher amounts of mineral fertilizers reduce the synthesis of polyphenols. The effect of fertilizers is mostly related to the amount of nitrogen present. Biesiada et al. (2010) believe that adding nitrogen reduces the content of polyphenolic acids in plants, and Ma et al. (2015) claim that reducing the amount of nitrogen reduces the amount of polyphenols in plant tissue. Similar changes in the content of polyphenols can be caused by fertilizers based on potassium and phosphorus, as well as sulfur. The study on the effect of sulfur fertilizers on the content of polyphenols in weeds (*Raphanus sativus*) showed a positive correlation of synthesis with an increase in the amount of sulfur (Zhou et al., 2013). Generally speaking, the uncontrolled application of fertilizers (especially nitrogen and phosphorus) poses numerous risks: accumulation of anion content in the soil (Kovacevic et al., 2010), salinization and alkalization of agricultural land, groundwater pollution, etc.

The working hypothesis was that fertilizers would affect the content of polyphenols in the cultivated plant due to the fact that maize plants belong to a group of C4 plants that better absorb available nitrogen from the soil (Bonifas et al., 2005). However, the competitive conditions and fertilizer application stimulated a greater competitive potential of the weeds (velvetleaf) compared to the cultivated plants (maize). Lindquist and Mortensen (1999) additionally indicate that the competitiveness of velvetleaf can be explained by the higher leaf area index of velvetleaf plants. Although both species are equally capable of absorbing the available nitrogen from fertilizers, the larger leaf area of velvetleaf plants (Barker et al., 2006) enables better utilization of available sunlight and the creation of a greater biomass. The addition of nitrogen provides a higher growth rate of velvetleaf plants (from 46 to 82%) than of maize plants (from 29 to 45%) (Bonifas et al., 2005). Similar to our research, the effect of nitrogen fertilizers on other weed species was also observed. Nitrogen stimulates the germination of *Avena fatua* seeds (76.1% vs. control, 21.6%; Agenbag and Villers, 2006) and their competitive advantage over wheat plants (Pourreza and Bahrani, 2015). The comparison of the content of each polyphenolic acid (processed t-test, Table 2) showed that the values were statistically significantly higher in the plants of the velvetleaf competition compared to the monoculture (except cinnamon) and lower in the plants of the maize competition compared to the monoculture (except ferul).

Table 2. Content of polyphenolic acids (µg/g fresh mass) in leaves of velvetleaf and maize grown in different systems after application of different fertilizers (t-test).

	velvetleaf				maize			
	chlorogenic	ferul	kumarn	cinnamon	chlorogenic	feruI	kumarn	cinnamon
F1 monoculture vs. competition								
t	54.79	98.865	116.981	-2.615	-4.887	42.374	-12.468	-15.968
p	**	**	**	ns	**	**	**	**
F2 monoculture vs. competition								
t	41.148	10.484	122.24	-2.181	-8.709	86.901	-66.225	-22.639
p	**	**	**	ns	**	**	**	**
F3 monoculture vs. competition								
t	15.481	40.743	63.611	-4.523	-8.452	219.28	-255.04	-14.368
p	**	**	**	*	**	**	**	**

ns - differences are not statistically significant, p<0.05*, p<0.01**, t-test value, F1 – organic, F2 – synthetic-organic and F3 – synthetic fertilizers.

In general, in addition to the content of polyphenols, the degree of their antioxidant activity is also important for plants as a mechanism for overcoming stress conditions (application of herbicides, drought, moisture, competition, etc.). The antioxidant potential of polyphenols occurs in two ways: donation of H atoms and electrons and neutralization of free radicals (Ksouri et al., 2008) and prevents the oxidation of proteins, lipids and carbohydrates (Shahidi and Zhong, 2010). It is considered that any disruption of the redox balance in the cells (exposure to free radicals) introduces the plant in a stateof oxidative stress (Vaya and Aviram, 2001). Free radicals trigger a chain reaction that destroys the cells by binding to the nearest stable molecules and taking over their electrons (Kaur and Kapoor, 2001). Therefore, plants/cells increase the synthesis of polyphenols (antioxidants) and antioxidant enzymes (Wu and Cederbaum, 2003). The measurement of the content of total polyphenols and their antioxidant activity was consistent with the changes in the content of individual polyphenolic acids compared to untreated plants in different growing systems (Tables 1, 3 and 4). The conditions of monoculture (conditions without stress) had no statistically significant effect on the change in the content of total polyphenols and their antioxidant activity in both tested species compared to the control, regardless of the application of fertilizers, (Table 3). Based on the results, it can be concluded that fertilizers (F2 and F3) influenced a slight increase in total polyphenols in the maize plants regardless of the level of significance (Table 3), which justifies the use of fertilizers.

Table 3. Statistical analysis and content of total polyphenols and antioxidant activity in leaves of velvetleaf and maize in monoculture after application of different fertilizers.

	velvetleaf				maize			
	UF (µg/g fresh mass)		AOA (µmol TE/g a.i)		UF (µg/g fresh mass)		AOA (µmol TE/g a.i)	
K/F1	1.33 1.20	ns	8.67 7.21	ns	0.51 0.69	ns	1.33 1.09	ns
K/F2	1.33 1.15	ns	8.67 7.76	ns	0.51 0.63	ns	1.33 1.43	ns
K/F3	1.33 1.44	ns	8.67 10.15	ns	0.51 0.82	ns	1.33 0.93	ns
F1/F2	1.20 1.15	ns	7.21 7.76	ns	0.69 0.63	ns	1.09 1.43	ns
F1/F3	1.20 1.44	ns	7.21 10.15	ns	0.69 0.82	ns	1.09 0.93	ns
F2/F3	1.15 1.44	ns	7.76 10.15	ns	0.63 0.82	ns	1.43 0.93	ns
SD	1.344		1.807		0.219		0.417	
mean	7.989		8.456		0.66		1.19	

SD – standard error, K – control, ns – differences not statistically significant, p<0.05*, p<0.01**, t-test value, UF – total polyphenols, AOA – antioxidant activity, F1 – organic, F2 – synthetic-organic and F3 – synthetic fertilizers.

On the other hand, the effect of the synthetic fertilizer F3 on the velvetleaf weeds points to the risk of using fertilizers and the effectiveness of herbicides in crops where there are resistant weed populations. However, the growing conditions in the competition clearly show the level of risk of fertilizer application. A statistically significant increase in the content of total polyphenols and their antioxidant activity in the velvetleaf plants indicates the difficulty of removing the weeds from the crops, especially resistant populations (Table 4). This fact is confirmed by the obtained content analysis in the cultivated maize plant (Table 4). The competition between crops and weedsis stressful and risky enough to achieve good yields. However, the expected effect of fertilizers on increasing the competitive advantage of the cultivated plant was significantly reduced based on the obtained results. The application of fertilizers statistically significantly reduced the content of total polyphenols and their antioxidant activity, in contrast to the effects on the weed species (Table 4). Some studies with other weed species partially support the results obtained in the studies conducted. In the case of the weed species *Vicia faba*, a lower content of polyphenols was found after the application of mineral fertilizers and increased after the combined application of mineral and organic fertilizers compared to the control (Cucci et al., 2019). There are also studies showing that *Helianthus tuberosus* plants without nitrogen application contain more chlorogenic acid and its antioxidant activity (Amarowicz et al., 2020).

Table 4. Statistical analysis and content of total polyphenols and antioxidant activity in leaves of velvetleaf and maize in competitive conditions after application of different fertilizers.

	velvetleaf				maize			
	UF (µg/g fresh mass)		AOA (µmol TE/g a.i)		UF (µg/g fresh mass)		AOA (µmol TE/g a.i)	
K/F1	0.59 0.92	0.001 **	2.12 5.63	0.000 **	1.24 0.95	0.008**	8.71 6.23	0.001**
K/F2	0.59 0.73	0.023 *	2.12 5.02	0.000 **	1.24 0.84	0.001**	8.71 4.76	0.000**
K/F3	0.59 1.38	0.000 **	2.12 9.29	0.000 **	1.24 1.20	ns	8.71 7.03	0.011*
F1/F2	0.92 0.73	0.004**	5.63 5.02	0.039*	0.95 0.84	ns	6.23 4.76	0.019*
F1/F3	0.92 1.38	0.000**	5.63 9.29	0.000**	0.95 1.20	0.018*	6.23 7.03	ns
F2/F3	0.73 1.38	0.000**	5.02 9.29	0.000**	0.84 1.20	0.000**	4.76 7.03	0.002**
SD	2.23		2.679		0.197		1.581	
mean	5.442		5.517		1.06		6.68	

SD – standard error, K – control, ns – differences not statistically significant, $p<0.05^*$, $p<0.01^{**}$, t-test value, UF – total polyphenols, AOA – antioxidant activity, F1– organic fertilizer, F2 and F3 – synthetic fertilizers.

The effect of the applied fertilizers on the content of total polyphenols in the rhizosphere of the roots of both cultivated species was also observed as part of the study. Under competitive conditions, the plants interact with each other through allelochemicals. Polyphenols as secondary metabolites also affect the level of competition through the soil. Studies on the allelopathic effects of maize chemicals on weed populations have shown that vanillic, ferulic, cinnamic and caffeic acids are responsible for competition (Jabran, 2017). During the research conducted in the rhizosphere of maize and velvetleaf under competitive conditions, only cinnamic polyphenolic acid was detected (out of 4 tested), regardless of the type of applied fertilizers (Figure 1). Based on the conducted research, it can be concluded that the examined species did not exhibit allelopathic action via polyphenols through the soil. However, the research conducted by Balah and Nassar (2011) showed that the water extract of velvetleaf inhibited maize seed germination by 44.4–74.0%, the growth of the root system by 70.51–80.76% and shoot by 53.60–75.94%. The results obtained by Nádasy et al. (2018) agree with these results.

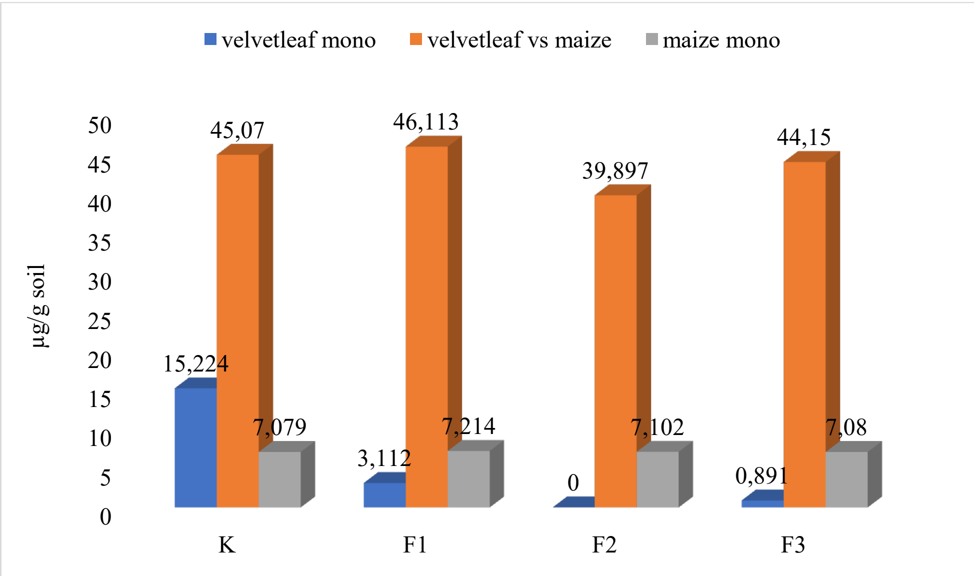

Figure 1. The content of cinnamic acid in the plots where maize and velvetleaf were grown in different cultivation systems.

Conclusion

In maize, the application of organic and synthetic fertilizers, neither in monoculture nor in competition with *Abutilon theophrasti* plants, caused statistically significant changes in the content of polyphenolic acids. However, in velvetleaf, the use of fertilizers resulted in an increase in the content of polyphenolic acids in competitive conditions compared to monoculture. The analysis of the obtained data did not reveal any difference in the effect between organic and synthetic fertilizers, except for the content of p-coumaric acid in velvetleaf and the content of cinnamic acid in maize when synthetic fertilizers were used. The content of individual polyphenolic acids was statistically significantly higher in velvetleaf plants grown with maize compared to plants grown in monoculture (except cinnamon) and lower in maize plants grown with velvetleaf compared with plants grown in monoculture (except ferul). Regardless of the level of significance, fertilizers influenced a slight increase in total polyphenols in maize plants, which justifies the use, but on the other hand, the effect of the synthetic fertilizer F3 on the velvetleaf weeds points to the risk of its use. A statistically significant increase in the content of total polyphenols and their antioxidant activity in velvetleaf plants indicates the difficulty of removing weeds from crops, especially resistant populations.

The research conducted and the results obtained confirm that weeds (velvetleaf) exhibit their natural competitive advantage in competitive conditions, which is further enhanced by crop feeding.

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EFEKAT PRIMENE ĐUBRIVA NA SADRŽAJ POLIFENOLA U KUKURUZU I ABUTILONU U USLOVIMA KOMPETICIJE

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R e z i m e

Cilj istraživanja bio je ispitivanje uticaja tretmana đubrivom na sadržaj polifenola u korovu abutilon i kukuruzu, gajenim bez primene herbicida u kontrolisanim uslovima ($12^h/12^h$, T 25º/22ºC dan/noć). Biljke su tretirane organskim – F1 i sintetičkim đubrivima – F2 i F3. Ispitan je sadržaj ukupnih fenola, pojedinačnih polifenolnih kiselina i antioksidativna aktivnost biljnih ekstrakata, kao važnih pokazatelja stanja biljke i njene otpornosti na oksidativni stres. Sadržaj ukupnih fenola je određivan spektrofotometrijski (modifikovana metoda Folin-Ciocalteu). Sadržaj pojedinačnih polifenolnih kiselina je određivan metodom tečne hromatografije visokih performansi, a antioksidativna aktivnost polifenola DPPH metodom. Ustanovljeno je da kod kukuruza, primena organskih i sintetičkih đubriva, ni u monokulturi ni u kompeticiji sa biljkama abutilona, nije uslovila statistički značajne promene sadržaja polifenolnih kiselina. Međutim kod abutilona, upotreba đubriva uslovila je porast sadržaja polifenolnih kiselina u uslovima kompeticije u poređenju sa monokulturom. Sadržaj hlorogene, p-kumarne i trans-ferulne kiseline bio je statistički značajno veći u biljkama abutilona gajenim sa biljkama kukuruza u poređenju sa biljkama gajenim u monokulturi, a sadržaj hlorogene, p-kumarne i cimetne kiseline manji u biljkama kukuruza gajenim u kompeticiji sa biljkama abutilona u poređenju sa biljkama gajenim u monokulturi. Statistički značajno povećanje sadržaja ukupnih polifenola i njihove antioksidativne aktivnosti u biljkama abutilona ukazuje na poteškoće uklanjanja korova iz useva, posebno rezistentnih populacija. Izvedena istraživanja i dobijeni rezultat potvrđuju da korovi u uslovima kompeticije ispoljavaju svoju prirodnu kompetetivnu prednost, koja se dodatno pojačava prihranom useva.

Ključne reči: polifenoli, abutilon, kukuruz, kompeticija.

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