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FOLIAR APPLICATION OF BIOSTIMULANTS AND THE ANTIOXIDANT PROPERTIES OF SOYBEAN SEEDS

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ABSTRACT
The study was carried out in 2014 - 2016 in Perespa, Poland. Soybean seeds Mavka cultivar were sown in the third decade of April. During the growing season four biostimulants: Kelpak SL (*Eclonia maxima* extract), Terra Sorb Complex (free amino acids), Atonik (phenolic compounds) and Tytanit (titanium) were used in four combinations each other, using lower or higher concentrations and single or double foliar spraying. All the results were compared to control (no biostimulant applied). After harvesting the plants, the antiradical activity of the seeds against ABTS •+cation radical was evaluated. Biostimulators enhance the yield quality without affecting the environment, thus they are recommended for use in sustainable agriculture. It was determined that the foliar application of Terra Sorb Complex had the most favorable influence on the studied property.

INTRODUCTION

Biostimulators aim at minimizing the influence of unfavorable stressors on crops, stimulating their growth, development, and enhancing the size and quality of the yield (Calvo, Nelson, & Kloepper, 2014; Matyjaszczyk, 2015). This particularly concerns abiotic factor–sensitive plants such as soybean or bean. Furthermore, the use of such agents appears to be valid in cases where traditional agronomic treatments are not sufficient to obtain yields that are higher and better in qualitative terms, such as proper cultivation, crop rotation, fertilization, or justified economic protective treatments (Słowiński, 2004).

Biostimulants may have natural or synthetic origin and contain free amino acids, humic compounds, extracts from algae and fruits, chitin, chitosan, efficient microorganisms (natural biostimulants) or growth regulators, phenolic compounds, inorganic salts, and nutrients (Al, Co, Na, Se, Ti, and Si) (synthetic biostimulants) (Calvo, Nelson, & Kloepper, 2014). The available publications demonstrate that legumes are rich in antioxidants (Amarowicz & Pegg, 2008; Marathe, Rajalakshmi, Jamdar, & Sharma, 2011; Gebrelibanos, Tesfaye, Raghavendra, & Sintayeyu, 2013). Moreover, different methods are available for improving antioxidant properties of fruits and vegetables, such as ozonization (Onopiuk et al., 2017), and use of biopreparations. However, the reports on the influence of biostimulators on the antioxidant activity of legume seeds are very few (A. Kocira, S. Kocira, Złotek, Kornas, & Świeca, 2015; A. Kocira et al., 2017). Therefore, the research on the influence of biostimulants on the antioxidant properties of soybean cultivar Mavka seeds appears to be desirable.

MATERIALS AND METHODS

The study was carried out in 2014-2016 in Perespa (50°66′N; 23°63′E), Poland. The soil type was characterized as Brown Rendzina belonging to the Rendzinas soil group. It is alkaline (pH in 1M KCl around 7.4–7.5) and rich in phosphorus, potassium, and magnesium. The experiment was established in a randomized block design in four replications with an elementary experimental plot area of 5 m^2. Seeds of soybean (*Glycine max* (L.) Merr.) cultivar Mavka were sown in the third 10-day period of April with the spacing of 30 x 3.5 cm. Over the growing season, four biostimulants: Kelpak SL (*Ecklonia maxima* extract), Terra Sorb Complex (free amino acids), Atonik (phenolic compounds), and Tytanit (titanium), were used in four combinations each other, using lower or higher concentrations and single or double foliar spraying (Table 1). The biostimulants were applied with a GARLAND FUM 12B battery field sprayer (Lecher LU 120–03) at a pressure of 0.30 MPa, using 300 L liquid per hectare. All the results were compared to control, where plants were treated with the same volume of water (no biostimulant applied). Tillage of plants was done using good agricultural practices. No pesticides were used (pest number did not exceed the thresholds of harmfulness).

Table 1. Scheme of biostimulants application.

Biostimulants	Number of applications Plant's stage	Concentration
Atonik	single spraying BBCH 13-15	0.1%
		0.2%
	double spraying BBCH 13-15 BBCH 61	0.1%
		0.2%
Tytanit	single spraying BBCH 13-15	0.07%
		0.13%
	double spraying BBCH 13-15 BBCH 61	0.07%
		0.13%
Kelpak SL	single spraying BBCH 13-15	0.7%
		1%
	double spraying BBCH 13-15 BBCH 61	0.7%
		1%
Terra Sorb Complex	single spraying BBCH 13-15	0.3%
		0.5%
	double spraying BBCH 13-15 BBCH 61	0.3%
		0.5%

After harvesting the plants, the antiradical activity of the seeds against ABTS •+cation radical was evaluated. The seeds were dried, ground in a laboratory grinder, and sieved (mesh size 0.310 mm). Flour was stored at a temperature of -20°C until analyzed. The antiradical activity of soybean seeds against ABTS•+ was determined using an acetone extract prepared acc. to a modified method by Kumar, Rani, Dixit, Pratap, & Bhatnagar (2010). Ground soybean seeds (100 g) were weighed into a conical flask with a ground glass stopper, poured with 2 mL of 70% acetone, and shaken at a room temperature of 2 h. Next, the samples were centrifuged at the speed of 10,000 x g and temperature of 20°C for 15 min. The supernatant was collected and stored in the dark at a temperature of -50°C until analyzed. The antiradical activity was determined according to the method developed by Re et al. (1999) ABTS (diammonium 2,2'-azino-bis (3-

ethylbenzothiazoline-6-sulphonic acid) was dissolved in distilled water until it has reached the concentration of 7mM. The ABTS•+ cation radical was obtained as a result of ABTS reaction with 2.45 mM potassium persulfate (K2S2O8). The reaction mixture was incubated at a room temperature for 12 h. The ABTS•+ solution was diluted with distilled water until its absorbance of 0.700 (±0.02) has reached at the wavelength of λ = 734. Determination of the antiradical activity consisted in the measurement of absorbance decrease during ABTS•+ cation radical reduction under the influence of antioxidants contained in the analyzed extracts, resulting in the formation of a colorless ABTS. To this end, 250 µL of the ABTS•+ solution was added to 5 µL of the extract, and the mixture was thoroughly mixed. Absorbance of the resultant solution was measured after 2h at the wavelength of λ = 734, using an EPOCH 2 microplate reader (BioTek, USA). The antiradical activity was expressed as Trolox equivalent (TE) w mg/g d.m.

Data on the antiradical activity of soybean seeds from four replicates of each combination were subjected to the statistical analysis. The Shapiro-Wilk test was performed for the normal distribution of data. The results were analyzed using one-way analysis of variance, ANOVA. The significance of differences between evaluated mean values was estimated by means of Turkey's test intervals of confidence at a significance level of $p < 0.05$. The statistical analysis was performed with Statistica 12 (StatSoft, Inc.).

RESULTS

Biostimulators and the methods of their usage influence the antiradical activity of soybeans (Table 2). In all the years of study of biostimulators this property was most favorably influenced by the foliar application of Terra Sorb Complex. In addition, in 2015 and 2016, similar effects were obtained using Atonik. Furthermore, increase of this property was obtained using Tytanit in 2014 and Kelpak SL in 2016. Only in the first year of study a significant influence of the application method on the studied property was determined. In that year, increased antioxidant properties in soybeans were obtained after a single and double application of lower concentration of the preparations. However, a tendency for increase of this property was observed in 2015 in the same biostimulator applications, and in 2016 after a single application of the preparation independent of the used concentration. In 2014, no significant differences in the studied property were observed between the control and combinations: single use of lower Tytanit concentration, double use of higher concentration of Atonik and Tytanit, single higher concentration of all preparations, and double higher concentration of Terra Sorb Complex. In the following year, a lack of significant differences in this property was observed between the control and the single application of lower Atonik concentration and higher Terra Sorb Complex concentration, as well as double use of Kelpak SL at higher concentration. In 2016, the antiradical activity of the seeds obtained from the control did not differ significantly from the combinations using Atonik, Kelpak SL, and Terra Sorb Complex. The interaction of biostimulators and their usage methods showed an increase of the property after the double use of lower Terra Sorb Complex concentration in 2014, as well as single higher concentration of the preparation in 2015 and 2016. In addition, significant increase in antioxidant properties was found in the last year of study after single application of lower Atonik concentration.

Table 2. Change in the antiradical activity of soybean cultivar Mavka effected by the application of biostimulants.

Year	Application (B)	Biostimulant (A)				Mean	Control
		Atonik	Tytanit	Kelpak SL	Terra Sorb Complex		
2014	B1	5.52 bc	5.66 efg	5.47 ab	5.58 cde	5.56 A	5.69 fg
	B2	5.69 fg	5.69 fg	5.57 cde	5.81 h	5.69 B	
	B3	5.70 fg	5.74 gh	5.62 def	5.64 ef	5.67 B	
	B4	5.45 ab	5.53 bcd	5.42 a	5.70 fg	5,52 A	
	Mean	5.59 B	5.66 C	5.52 A	5.68 C	5.61	
2015	B1	4.81 ghi	4.77 fgh	4.55 ab	4.65 b-e	4.69 A	4.91 ij
	B2	4.80 gh	4.75 e-h	4.63 bcd	4.73 d-h	4.73 A	
	B3	4.69 cf	4.60 abc	4.71 d-g	4.92 j	4.73 A	
	B4	4.80 gh	4.52 a	4.82 hij	4.65 b-e	4.70 A	
	Mean	4.78 B	4.66 A	4.68 A	4.74 B	4.71	
2016	B1	5.57 d	5.20 a	5.43 cd	5.46 cd	5.41 A	5.50 cd
	B2	5.38 bc	5.23 ab	5.39 c	5.49 cd	5.37 A	
	B3	5.51 cd	5.19 a	5.42 cd	5.56 d	5.42 A	
	B4	5.39 c	5.19 a	5.50 cd	5.40 c	5.37 A	
	Mean	5.46 B	5.20 A	5.44 B	5.48 B	5.39	

Abbreviations: B1- single spraying with low concentration of biostimulant; B2- double spraying with low concentration of biostimulant; B3 - single spraying with high concentration of biostimulant; B2- double spraying with high concentration of biostimulant. Different letters within the same year denote significant differences between the treatments at $p<0.05$.

The plants treated once with Atonik, in both concentrations as well as double treatment with lower concentration of the preparation, obtained similar values of antiradical activity of the seeds (Figure 1) which did not differ significantly. On the other hand, double application of higher concentration of this biostimulator reduced the studied property. The highest radical-scavenging activity was obtained after a single application of the higher concentration of Terra Sorb Complex, which slightly differs from the double application of a lower concentration of the biostimulator. In addition, these values do not significantly differ from the values obtained for the control. Both single use of lower and double use of higher concentration of Terra Sorb Complex resulted in a decrease of property, and the obtained values did not differ significantly. However, in the case of a double spray of the plants with higher Terra Sorb Complex concentration the obtained value of the property did not differ from the control. Low radical-scavenging activity was observed upon single application of lower Kelpak SL concentration. Furthermore, along with the increase of the number of applications and concentration of the preparation, the property was found to increase, and the highest value not differing from the control object was obtained after a single application of the higher concentration. On the other hand, an analysis of the influence of Tytanit on the property provided the highest radical-scavenging activity after the double application of the lower concentration, which differed slightly from the single use of the preparation at the same concentration. A decrease in the studied property was observed along with the increase of Tytanit concentration, and the lowest value was obtained after the single application of the higher concentration.

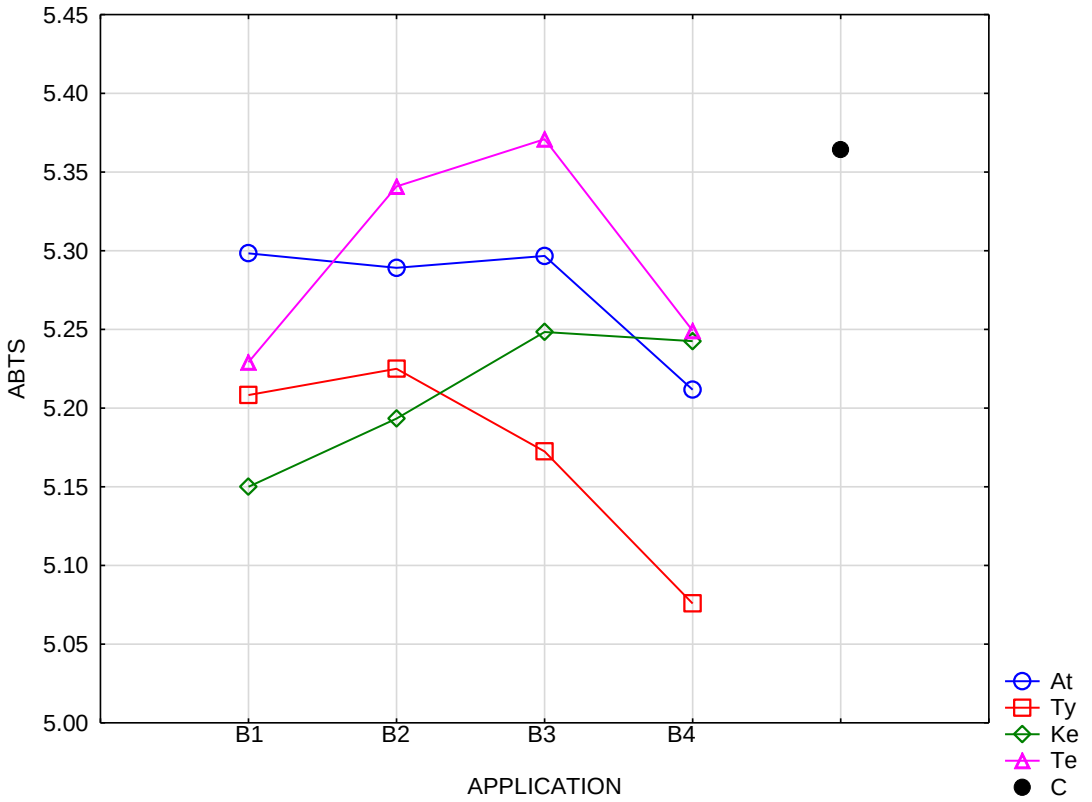

Figure 1. The influence of biostimulants on the antiradical activity of soybeans cultivar Mavka (mean from 2014–2016).

CONCLUSIONS

Biostimulators are safe for both humans and environment, and are recommended for use in sustainable agriculture. When used in crop cultivations, they contribute to enhance the quality of the yield. In the present study, biostimulators determined the quality of soybeans expressed as antioxidant properties. However, the use of these preparations did not result in the increase of the property in comparison to control in all cases. The most favorable influence on the antioxidant properties was the foliar application of the biostimulant based on free amino acids (Terra Sorb Complex) in the form of a single plant sprayed with a higher concentration or double with lower concentration of the biostimulant. Good effects were also obtained after single application of the biostimulator based on phenolic compounds (Atonik), irrespective of the concentration used, as well as after the double application of the lower concentration. In addition, the double use of higher Terra Sorb Complex or single use of higher Kelpak SL concentration had positive influence on the antioxidant properties of the seeds. The value of each tested character obtained in the above combinations did not differ significantly from the control object. In the remaining combinations, the antioxidant properties of the seeds differed clearly from the control by being significantly lower.

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