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Original Paper

Response of Sunflower (*Helianthus annuus* L.) to Application of Bioactive Humic Substances

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Sunflower is the second most cultivated oilseed crop in Slovakia. It remains an attractive crop in primary agricultural production because of the many benefits it provides. The study aimed to assess the impact of biological material (Subaro HTS, Sumiko HTS) and products containing bioactive substances (Humix® Universal, Humix Bór, Energen Fulhum Plus and Energen Stimul Plus) on sunflower production in the 2021–2022 growing seasons. A partial aim was to investigate the possible effect of adaptogens on a plant organism, specifically sunflower. The field trials were designed using the perpendicularly split block method with a randomized block complete design on an experimental basis of SAU in Nitra, located in a warm maize production area. The variability of weather conditions was statistically demonstrated on the achieved production parameters. Weather conditions were more favorable in 2022 regarding seed yield 3.25 ±0.31 tons per hectare (t.ha^{-1}) and oil content 47.14 ±0.90%. Evaluation of biological material confirmed that the hybrid Sumiko HTS achieved significantly higher yields of seeds 3.29 ±0.28 t.ha^{-1} and oil content 46.66 ±1.32% compared to Subaro HTS. The utilization of bioactive substance combinations exhibited significant impact on sunflower production. Specifically, superior values in the examined parameters, such as seed yield 3.30 ±0.26 t.ha^{-1} and oil content 46.99 ±1.12%, were observed in instances where Energen Fulhum Plus + Energen Stimul Plus preparations, inclusive of adaptogens, were applied during the growth stage BBCH 18.

Keywords: adaptogens, biological material, oil content, stimulators, yield of seeds

1 Introduction

Sunflower (*Helianthus annuus* L.) is cultivated in Slovakia on an area of about 73.60 thousand hectares with a production of 2.69 t.ha^{-1} (FAO, 2022). The oil content of the seeds obtained by the traditional extraction method ranges from 40–50% (Dunford et al., 2022). Sunflower oil is beneficial to health due to low saturated fat content, high content of polyunsaturated and monounsaturated fatty acids and vitamin E (Bhattacharya, 2023). High-oil varieties along with agronomic practices are two most important components of achieving higher production of sunflower (Beg et al., 2007).

The use of bioactive substances in various fields (agriculture, botany, and forestry) has the potential to produce results that cannot be achieved in other ways. Their use allows to better realize the genetic potential of crops, increase the resistance of plants to stress factors of biotic and abiotic nature and subsequently increase yields and improve quality (Chukhlantsev, 2010; Santini et al., 2021). Fertilization of sunflower is very important at the time of demand, so that if great attention is paid to determining the amount and duration of mineral fertilizers applied to it, a high quality and high yield can be achieved (Škarpa & Lošák, 2008). In addition to the essential nutrients in mineral fertilizers (Černý et al., 2010), substances that regulate plant growth and supplement them in the plant nutrition system are also supplied, thereby increasing the rate of soil and fertilizer utilization (Chukhlantsev, 2010; Lošák et al., 2022). Some products with stimulatory effects also include

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adaptogens, compounds that increase the ability of an organism to adapt to environmental factors and avoid damage from these factors (Panossian and Wagner, 2005). Its effect is thought to help the organism return to a state of equilibrium (Jawaid et al., 2011).

The objective of this paper was to assess the impact of biological material and products containing bioactive substances on sunflower production in the 2021–2022 growing seasons. Adaptogens contained in the used preparations are mentioned in the scientific literature only in the context of their effect on human or animal organisms. It was also interesting to investigate the possible effect of these substances on plants, specifically on sunflower.

2 Material and methods

2.1 Experimental location

The field experiment was carried out at the SPU research station in Nitra Dolná Malanta (E 18° 9′, N 48° 19′) in 2021 and 2022. The experimental base is in a maize growing region. It is characterized by a dry and warm climate. Precipitation and average temperatures during the growing seasons of the experimental years were recorded by the hydrometeorological station directly at the experimental base. The weather characteristics of the experimental years are shown in Figure 1 and 2. The soil type is Haplic Luvisol, silty loam (Tobiašová & Šimanský, 2009).

2.2 Experimental material

The soil samples were taken according to methodological instruction of The Central Control and Testing Institute in Agriculture (ÚKSÚP, 2022) in autumn and spring for the expected sunflower yield of 3.5 $t.ha^{-1}$ (Table 1). The winter wheat (*Triticum aestivum* L.) was used as forecrop according to rules of standard crop rotation system (Velasco et al., 2015).

2.2.1 Hybrids of sunflower

Sunflower hybrids Subaro HTS and Sumiko HTS (Syngenta Group, Basel, Switzerland) for EXPRESS® technology were used in the experiment. In the experiment was used twice-cross early hybrid (Sumiko HTS) and medium early (Subaro HTS), with oil contents of 48.5% (Sumiko HTS) and 47% (Subaro HTS). They are very resistant to lodging and have high resistance to *Sclerotinia sclerotiorum*, *Macrophomina phaseolina*, and very high resistance to *Plasmopara halstedii*.

2.2.2 Fertilizers with bioactive substances

Humix Universal® is special liquid foliar and soil fertilizer containing humic substances from Leonardite, macro-elements and micro-elements for the nutrition of field crops (Agrocultur Bio s.r.o., Nitra, Slovakia). Application to the leaf intensifies plant nutrition, promotes the growth of the root system and the whole plant, resulting in higher and better quality of yields. It contains humic substances minimum 3.0 weight percent (min. 3.0 wt. %)

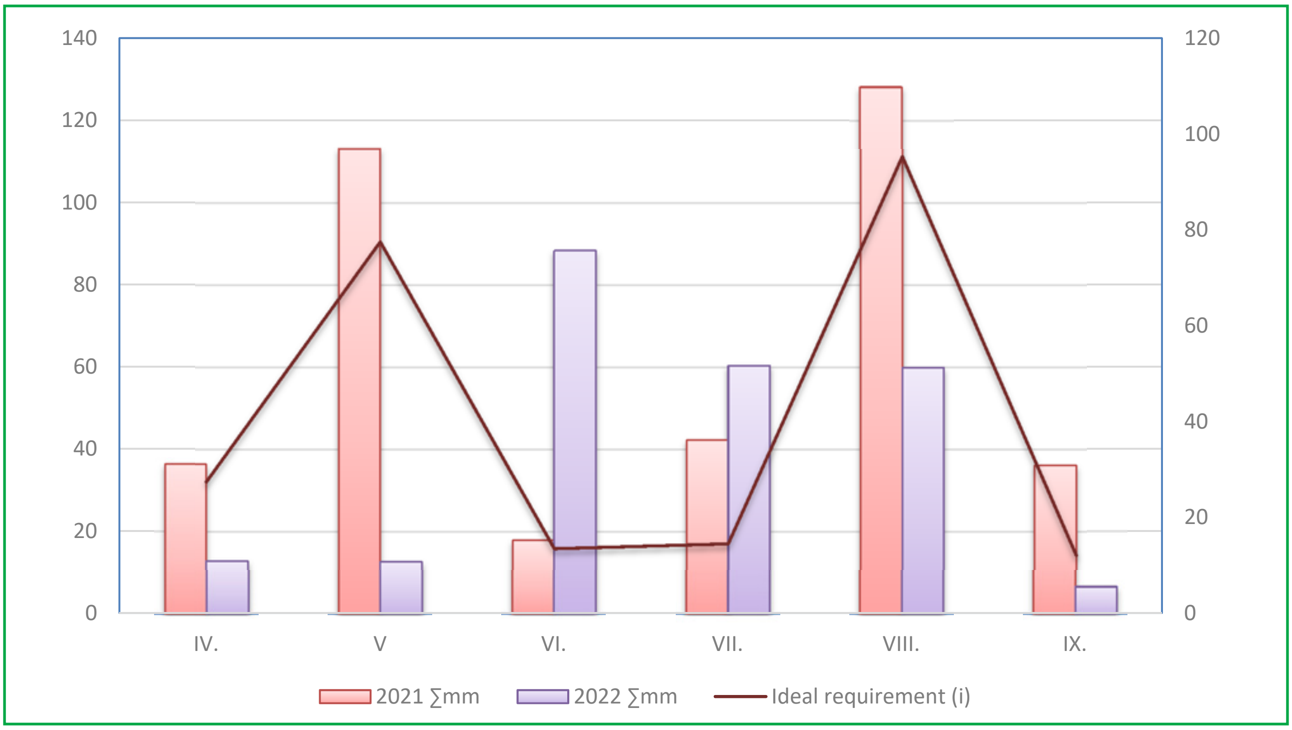

Figure 1 Average monthly precipitation (mm) in 2021 and 2022

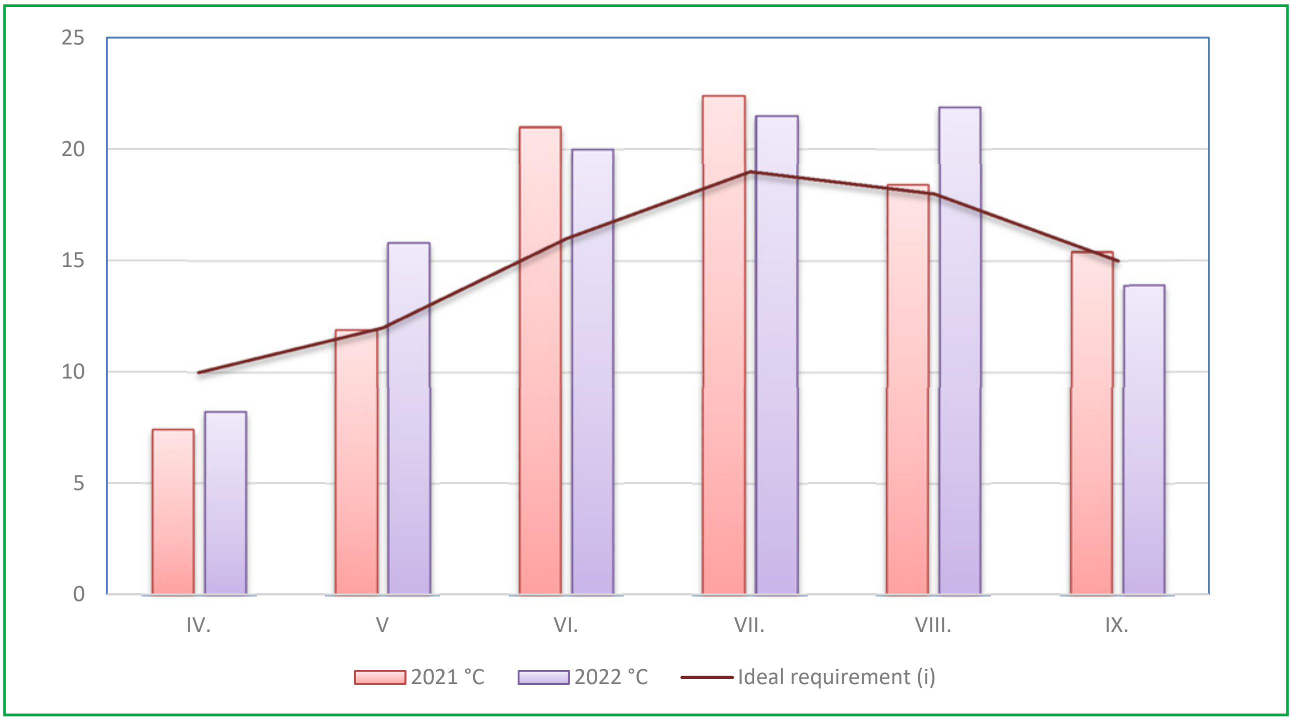

Figure 2 Average monthly air temperatures (°C) in 2021 and 2022

potassium (K_2O) (min. 2.5 wt. %), phosphorus (P_2O_5) (min. 1.0 wt. %), and Cu, Fe, B in trace amounts and bound in chelate form. The pH value is 9–10.

Humix Bór® (Boron) is a special liquid foliar and soil fertilizer designed to nutrition of a wide range of field crops with higher boron requirements (Agrocultur Bio s.r.o., Nitra, Slovakia). It contains bioactive natural substances based on humic substances from Leonardite (min. 8.0 wt. %), with (potassium K_2O in trace amount), boron (min. 2.5 wt. %) essential for plant growth, and trace elements (Cu, Zn, Fe) bound in chelate form. The pH value is 7–9.

Energen Fulhum Plus® (EGT System s.r.o., Otice, Czech Republic) is a treated and modified aqueous salt solution obtained by the original digestion of technical lignosulphate. The formulation is fortified with an ingredient acting on the formation of fine root hairs. It increases the energy of germination and significantly affects the speed and quality of germination, increasing the content of storage substances. It allows a balanced emergence of the crop and increases the power of photosynthesis. Contains humic substances and their salts (8%). Suitable for more arid areas.

Energen Stimul Plus (EGT System s.r.o., Otice, Czech Republic) contains seaweed extracts, adaptogens and other substances that promote growth, photosynthesis and increase seed size. Its main effect is in the main growth period when it promotes root formation, increases nitrate reductase activity and thus nitrogen uptake by 12–19% depending on the crop and variety, and protects crops from cold and drought until harvest. Contains humic substances and their salts (8%).

2.3 Experimental methods

Field experiments were established by split plot (60 m^2) method in three replications. Tillage (subsoil, autumn deep ploughing) and sowing (0.70 m distance between rows, and 0.22 m in the row) were carried out according to the principle of conventional cultivation technology of sunflower (Duflo & Benerjje, 2017). Industrial fertilizer ammonium nitrogen with dolomite LAD 27 (Duslo a.s., Šaľa, Slovakia) was used in doses 79 N $kg.ha^{-1}$ (2021) and 80 N $kg.ha^{-1}$ (2022) and calculated according to agrochemical soil analysis results and nutrient requirements of sunflower on expected yield 3.5 $t.ha^{-1}$

Table 1 Treatments of the used fertilizer, application doses and dates

Treatments with applied fertilizer	Dose	Growth stage
T0 – control treatment	–	–
T1 – Humix Universal® + Humix Bór®	5 $l.ha^{-1}$ (250 $l.ha^{-1}$ water)	BBCH 18(8 leaves unfolded)
T2 – Energen Fulhum Plus + Energen Stimul Plus	0.5 $l.ha^{-1}$ (250 $l.ha^{-1}$ water)	BBCH 18(8 leaves unfolded)

Table 2 Analysis of the nutritional soil status of the experimental area in 2021 and 2022

Autumn		
Nutrient and determination method	2020	2021
Phosphorus (mg.kg^{-1}) – colorimetrically by Mehlich III	103.00	94.00
Potassium (mg.kg^{-1}) – flame photometry by Mehlich III	335.00	329.00
Calcium (mg.kg^{-1}) – AAS by Mehlich III	2400.00	2450.00
Magnesium (mg.kg^{-1}) – AAS by Mehlich III	280.00	320.00
pH – by KCl (0.2 mol.dm^{-3} KCl) (pH units)	5.64	5.75
Spring		
Nutrient and determination method	2021	2022
Inorganic Nitrogen (mg.kg^{-1}) – sum of ammonium and nitrate nitrogen	18.30	17.50
NO_3^- – N (mg.kg^{-1}) – colorimetrically by phenol 2,4-disulfonic acid	11.20	9.30
NH_4^+- N (mg.kg^{-1}) – colorimetrically by Nessler's reagent	7.10	8.20

(Bujnovský & Ložek, 1996). The determination methods for individual nutrients and results are listed in Table 2. The oil content of sunflower seeds samples (weight of sample 200g) was determined by the extraction method (%) using a Soxhlet extraction apparatus according to Shahidi (2005).

2.4 Statistical analysis

The experimental results were processed and analysed using statistical software TIBCO Statistica®, Version 14.0 (TIBCO Software Inc., Palo Alto, California, USA). Multivariate analysis of variance (ANOVA) was used to determine the effect of the main experimental factors on the observed parameters of the sunflower. A post-hoc analysis using Tukey's HSD test was performed to determine any significant differences within factors with a significance level of $\alpha = 0.05$.

3 Results and discussion

3.1 Yield of seeds

The sunflower yield should be influenced by a set of various factors, which inherently include soil and habitat conditions, climatic and weather conditions, agrotechniques, biological material and bioactive agent application (Ion et al., 2015). The yield of sunflower is one of the main production parameters that is of most interest to the grower in terms of agricultural production (Ernst et al., 2022). As mentioned above, seed yield is closely related to the course of weather conditions (Lenárt, 2004). The obtained results confirmed the same statistically significant influence (P = 0.0125) of the year on the achieved yield of seeds (Table 3), where a statistically proven higher average yield of 3.25 ±0.31 t.ha^{-1} was obtained in the year 2022. This implies that the temperature and humidity tendency of the year 2022 was more suitable for sunflower cultivation in comparison with the year 2021. Another intensifying factor is the biological material, which was confirmed statistically highly significant (P = 0.0008). In comparing two hybrids with similar yield potential, the Sumiko HTS hybrid was statistically more productive with an average yield of 3.29 ±0.28 t.ha^{-1}. The same relationship was observed by Mrdja et al. (2012) where sunflower hybrids significantly influenced the yield of seeds. Due to the great diversity of genetic material available to the grower (Angeloni et al., 2017), it is very important to select a hybrid that will achieve stable and high seed and oil yields under different environmental conditions (Cvejić et al., 2019). One of the advanced technologies for sunflower cultivation is the use of mineral fertilizers in combination with bioactive substances, which is one of the main factors for increasing yields (Neshev et al., 2022). The results showed that the use of fertilizers containing bioactive substances had a statistically proven effect on the sunflower yield (Table 3). Statistically proven highest mean yield of 3.30 ±0.26 t.ha^{-1} was recorded on the treatments of Energen Fulhum Plus + Energen Stimul Plus formulation. The interaction relationships of the factors on the yield of seeds were statistically non-significant (Table 3).

3.2 Oil content

The oil formation process in the seeds was influenced by the year conditions statistically highly significant (P = 0.0001), where significantly higher oil content (47.14 ±0.90%) was recorded in 2022. Many studies agree that weather conditions play a key role in the production process of sunflower (García-López et al., 2014; Černý et al., 2018; Zapletalová et al., 2020; Vician et al., 2022). It is well known that cultivation practices are required to achieve the desired oil content with a specific composition, which represents the interactions between

Table 3 Effect of experimental factors on sunflower production in 2021 and 2022

Effect	Yield	Oil content
P value		
Year	0.0125*	0.0001**
Hybrid	0.0008**	0.0392*
Treatment	0.0017*	0.0149*
Year*Hybrid	0.3626	0.8655
Year*Treatment	0.2329	0.9911
Hybrid*Treatment	0.4346	0.3676

* statistically significant effect by 0.95 confidence intervals; ** statistically significant effect by 0.99 confidence intervals

Table 4 Multi-factor ANOVA of data of sunflower production parameters

	Yield (t.ha^{-1})	Oil content (%)	
Year	2021	3.09 ±0.21^{a}	45.47 ±1.24^{a}
	2022	3.25 ±0.31^{b}	47.14 ±0.90^{b}
Hybrid	Subaro HTS	3.06 ±0.21^{a}	45.95 ±1.35^{a}
	Sumiko HTS	3.29 ±0.28^{b}	46.66 ±1.32^{b}
Treatment	control	3.00 ±0.14^{a}	45.71 ±1.28^{a}
	T1	3.22 ±0.32^{b}	46.21 ±1.46^{ab}
	T2	3.30 ±0.26^{b}	46.99 ±1.12^{b}

different letters indicate significant differences (Tuckey HSD test, α = 0.05) between growing seasons, hybrids, and treatments

the growing environment, the appropriately selected hybrid and proper nutrition (Pereyra-Irujo & Aguirrezábal, 2007). The obtained results confirmed demonstrable influence of hybrids (Table 4), with a higher oil content of 46.66 ±1.32% observed by hybrid Sumiko HTS. Nutrition and fertilization included liquid fertilizers containing bioactive substances, which showed a statistically significant effect on the oil formation process. On average, the highest oil content of 46.99 ±1.12% was obtained on the variant treated with Energen Fulhum Plus + Energen Stimul Plus containing adaptogens. The effect of relationship interactions on oil content was statistically non-significant (Table 3).

4 Conclusions

The results obtained from field polyfactorial trials conducted in 2021 and 2022 unequivocally confirm a statistically significant impact on the production potential of sunflower. Notably, 2022 proved to be more favorable for both seed yield and oil content. In practical terms, it can be asserted that employing the Sumiko HTS hybrid resulted in higher values for production parameters, with a seed yield of 3.29 ±0.28 t.ha^{-1} and an oil content of 46.66 ±1.32%. An integral part of crop production is the correct choice of nutrition and fertilization of the crop grown. Can be concluded that of the used preparations the more effective (yield 3.30 ±0.26 t.ha^{-1}; oil content 46.99 ±1.12%) were the preparations in the combination of Energen Fulhum Plus + Energen Stimul Plus containing humic substances applied at the stage BBCH 18. The preparations with humic substances contribute to achieving higher production parameters of sunflower in conditions of western Slovakia.

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