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THE INFLUENCE OF THE APPLICATION OF DIFFERENT PLANT AQUEOUS EXTRACTS ON GRAIN AND PROTEIN YIELD IN SOYBEAN PRODUCTION

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Abstract: The aim of this study was to investigate the influence of aqueous extracts of different plant species on the grain and protein yield of soybean. The testing was conducted at the Institute of Field and Vegetable Crops in Novi Sad on the seeds of the NS Apolo variety. The aqueous extracts of the above-ground part of nettle, the above-ground part of nettle and comfrey, whole banana fruit, banana peel, onion bulbs leaves, the top parts of willow twigs and the top parts of soybean plants were foliarly applied. In addition to the untreated control variant, the experiment also included a distilled water control. Control with distilled water was to show whether the effect of aqueous plant extracts was due to plant material or just water. The results of the experiment showed that the use of aqueous extracts contributed to the increase in grain and protein yield. The increase in grain yield ranged from 9.48% to 15.34%, and the increase in protein yield from 9.31% to 16.16%. The best effect was achieved by applying the aqueous extract of the whole banana fruit and the aqueous extract of the mix of nettle and comfrey. By applying them each year, a significantly higher yield was achieved in relation to the control with distilled water.

Key words: aqueous extract, soybean, banana, nettle, comfrey.

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Introduction

Serbia is among the largest soybean producers in Europe. In the last ten years, soybean areas in Serbia have ranged from 144,000 to 202,000 hectares (Miladinov et al., 2020). In the last ten years, the average soybean yield has been 2.7 t/ha, with a minimum of 1.7 t/ha in 2012 and a maximum of 3.5 t/ha in 2014, which makes Serbia rank among the countries with the highest average yield in Europe (http://www.stat.gov.rs). Within soybean production, in addition to achieving high and stable yields, grain quality, i.e., the content of protein and oil in soybean grain, is also very important (Đukić et al., 2020). Most of the world's population uses meat, milk and eggs as sources of protein. However, with the increase in the number of inhabitants on Earth and the increase in the price of animal proteins, the need for a new source of plant proteins was created, so special attention was paid to the use of protein-oil crops, among which soybeans occupy the most significant place (Žilić et al., 2006). The increased acceptance of soybean proteins is due to manifold soybean qualities, good functional properties in food applications, high nutritional value, availability and low cost (Barać et al., 2004). Soybeans are used in the form of various products for human consumption. Therefore, it is necessary that part of soybean production be without the use of mineral fertilizers and pesticides (Dozet et al., 2019). Organic agriculture is a system of ecological production management that promotes biodiversity, organic matter, nutrient circulation and biological activity of land (Kovačević and Oljača, 2005). Organic production is based on a modern scientific understanding of ecology and agriculture and fully supports and monitors technological development and mechanization (Cvijanović et al., 2013). An increasing number of researches are aimed at finding the application of alternative measures in plant production in order to avoid unwanted consequences (Mamlić et al., 2021). In order to increase the yield and quality of products, foliar treatments with various plant extracts are increasingly applied (Dozet et al., 2017). Herbal extracts can be a significant source of various nutrients. The amount of nutrients depends on the plant species from which the aqueous extract is made, and the land on which the plant species grew. Also, herbal extracts are partially insecticides and fungicides due to bioactive chemicals found in the prepared treatment solution (Kim et al., 2005).

The aim of this study was to determine the influence of foliar fertilization with various plant extracts on the grain yield and soy protein yield per unit area, depending on the year, i.e., the amount of precipitation and temperature during the growing season.

Material and Methods

The object of research was the soybean cultivar 'NS Apolo' developed at the Institute of Field and Vegetable Crops in Novi Sad, Serbia. The field trials were conducted during three vegetative seasons (2019, 2020, and 2021) at the Rimski Šancevi experimental field (45°20′ N 19°51′ E, 80 m a.s.l.) near Novi Sad, Serbia. The trial was set up on chernozem soil type as a randomized complete block design (RCBD) with four replications under the conditions of dry farming. The plot size was 10 m^2. The inter-row spacing of 50 cm and the intra-row spacing of 4.5 cm were applied. Each year the plots were rotated with maize. Prior to sowing, soybean seeds were inoculated with the NS Nitragin microbiological fertilizer as a standard agrotechnical measure in soybean production.

During the three years of the experiment, neither significant disease occurrences nor insect occurrences were observed. Soybean seeds of the NS Apolo variety were sown in the first decade of April. Plants were treated twice. The first treatment was done in the V6 vegetative stage, and the second treatment was done in the R1 reproductive stage.

Treatments with the foliar application of aqueous extracts were as follows:

1. control – without any foliar spray;
2. distilled water (H_2O) only;
3. an aqueous extract of nettle (*Urtica dioica*) – prepared from above-ground parts, i.e., nettle tree and leaves;
4. an aqueous extract of nettle (*Urtica dioica*) and comfrey (*Symphytum officinale*) – prepared from the above-ground parts of nettle and comfrey in a ratio of 3:1;
5. an aqueous extract of the banana fruit (*Musa x paradisiaca*) – prepared from the ripe fruits of the banana, along with the peel;
6. an aqueous extract of banana peel (*Musa x paradisiaca*);
7. an aqueous extract of onion bulb leaves (*Allium cepa*);
8. an aqueous willow extract (*Salix matsudana*) – prepared from the tops of willow twigs;
9. an aqueous extract of soybean (*Glycine max*. (L.) Merr.) – prepared from the top parts of soybean plants.

For the preparation of plant extracts, one kilogram of plant material was used, which was cut into small pieces, the size of 1–4 cm. After cutting, the plant material was submerged in 10 l of rainwater and left to ferment with daily stirring. After fermentation, the obtained plant extract was filtered through gauze and left to stand at a temperature of 18–22 °C in glass bottles, hermetically sealed. Prior to the foliar treatment of soybean plants, the aqueous extract was diluted with distilled water in a ratio of 1:15. An application rate of 300 l per hectare was used.

Mechanical harvesting was conducted with a small harvester for plot trials (Wintersteiger elite). After basic plot harvesting, grain weight and moisture content were measured, and yield was calculated in kg ha^{-1}, with a moisture level of 14%. The protein content in the grain was measured by a spectrophotometer following the principle of the NIR technique. Based on the soybean grain yield and protein content, the protein yield per unit area was calculated.

Weather conditions. In 2019, the average daily air temperature was 19.5 °C, and the amount of precipitation during the growing season was 418.6 mm, with a deficit of 124 mm (Figures 1 and 2). From the date of sowing to the emergence of plants, 30.7 mm of precipitation was recorded, which was sufficient for uniform soybean emergence with the existing soil moisture. In the period from emergence to maturation, 310.7 mm of precipitation was recorded. The period of drought lasted from July 21 until the end of the vegetation period and amounted to 52 days. In 2019, during vegetation, there were 36 days with temperatures above 25 °C.

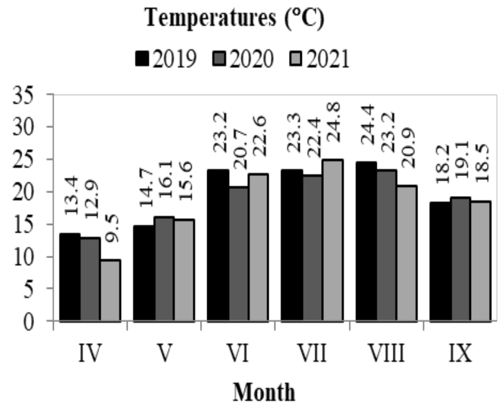

Figure 1. Average monthly temperatures during the vegetation season.

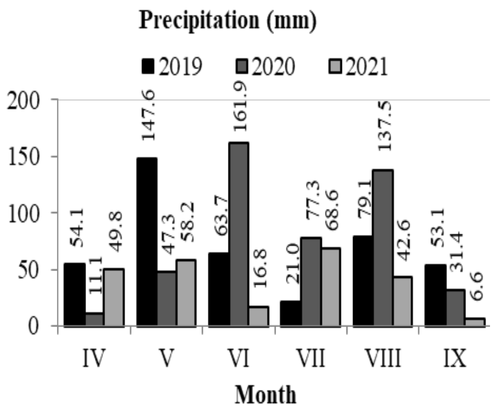

Figure 2. Average monthly precipitation during the vegetation season.

In 2020, the average daily air temperature was 19.1 °C, while the amount of precipitation was 466.5 mm during the growing season. In 2020, the most favourable conditions for soybean production were observed in the precipitation and air temperature distribution. There was no deficit of precipitation and drought that year, i.e., there was an excess of precipitation of 9.1 mm. During the soybean growing season, 17 days with temperatures above 25 °C were recorded.

In 2021, the average daily air temperature was 18.7 °C, while the amount of precipitation was 256 mm during the vegetation. The length of the vegetation period was significantly extended during 2021 due to the cold period. The vegetation period lasted for 142 days (Table 1). The precipitation deficit was 243.8

mm (Table 2). During the vegetation period of soybeans in 2021, the dry period began on June 19 and lasted until the plants matured, i.e. until September 19. The length of the dry period was 93 days, and 30 days with temperatures above 25 °C were recorded.

Table 1. Main dates for soybean vegetation from 2019 to 2021.

Year	Date of				Length of vegetation, days
	sowing	germination	maturation	harvest	
2019	15 April	02 May	10 September	12 September	132
2020	08 April	04 May	14 September	17 September	133
2021	24 April	01 May	19 September	20 September	142

Table 2. Potential and actual evapotranspiration during soybean vegetation from 2019 to 2021.

Year	SM	PV	PE	ET	PD	DS
2019	34.80	310.7	481.4	357.3	-124.1	21.07.
2020	42.31	415.4	462.1	471.2	+9.1	-
2021	46.01	190.2	480.1	236.2	-243.8	19.06.

Note. SM – soil moisture reserves during sowing time (mm), PV – precipitation during vegetation period (mm), ETP – potential evapotranspiration (mm), ETR – real (actual) evapotranspiration (mm), PD – precipitation deficit (mm), DS – drought start (day).

The results of the research were processed by analyzing the variance of the two-factorial experiment (Hadzivukovic, 1991), and the significance of the differences in treatment means was tested by the LSD test at 0.01 and 0.05 significance levels (statistical software “Statistica v.10.0”).

Results and Discussion

Soybean grain yield. Most aqueous extracts used as fertilizers or pesticides are largely the result of traditional knowledge, which is passed down from generation to generation. Plant material for the preparation of aqueous extracts is easily available, and their use does not economically burden plant production like foliar commercial fertilizers. There is scientific evidence that the use of certain aqueous extracts has a beneficial effect on plants (Lashin et al., 2013). However, for most aqueous extracts, scientific evidence does not exist even though they are traditionally used in agriculture. Some of the most commonly recommended preparations are aqueous plant extracts made from stinging nettle (Godlewska et al., 2020). The results of the research showed a significant influence of the year and weather conditions on the soybean grain yield. The highest yield was achieved in 2020 (3783.03 kg ha^{-1}), which was by 39.32% higher compared to 2021. A significantly higher yield can be explained by the fact that, in 2020, there was no deficit of precipitation, i.e. even a surplus of 9.1 mm was recorded, while in 2021, the deficit was 256 mm with a dry period of 93 days (Tables 1 and 2). Also, in 2020, during the vegetation, 17 days with temperatures above the optimal ones for normal growth and development of plants were recorded (Figure 1). In 2021, this period lasted significantly longer, as many as 30 days. Observing the influence of aqueous extracts on soybean grain yield during three years (Table 3), it can be concluded that, on average, the best effect was achieved by applying an aqueous extract of the whole banana fruit. The 3-year averaged yield was 3329.94 kg ha^{-1}, which is by 15.34% higher than in the control. The application of an aqueous extract of nettle and comfrey achieved a yield of 3300.20 kg ha^{-1}, which is by 14.58% higher than the control. Aqueous extracts of banana peel and nettle had a similar effect on soybean yield. The increase amounted to 13.07% and 12.99%, respectively. Aqueous extracts of soybean and onion bulb leaves had a similar effect. Their effect increased the yield by 11.87% and 11.46% (Table 3). The positive effect of the aqueous extract of the banana fruit, as well as the peel, can be attributed to the chemical composition of this fruit. Banana fruit is rich in potassium, phosphorus, calcium, manganese, magnesium, selenium, and it also contains vitamins C, B and A. Banana peel contains phosphorus which affects the flowering of the plant, then potassium which promotes plant development and growth, while calcium makes plants stronger (Sidhu and Zafar, 2018).

Dozet et al. (2017) also found a beneficial effect of the aqueous extract of nettle and comfrey on soybean grain yield. By applying the aqueous extract of these two plant species, the soybean yield was increased by 8.31%. The same authors also determined the beneficial effect of the aqueous nettle extract on soybean grain yield. Similar results of the positive and significant impact of the application of plant extracts, especially the nettle extract, are emphasized in the research of Ljubović (2015). The best results using the nettle extract were obtained with the average weight of fresh above-ground lettuce. Zavišić et al. (2015), in their research, found that the use of nettle extracts aims to protect crops from diseases and pests. Aqueous nettle extracts fermented for 14 and 21 days showed an inhibitory effect on the mycelium growth of the *Botrytis cinerea*. The aqueous nettle extract is rich in nitrogen, phosphorus, calcium, magnesium and iron and promotes plant growth (Rivera et al., 2012). If the effects of aqueous extracts are observed in relation to the second control (water only), it can be concluded that a significant increase in yield was achieved by using the aqueous extract of whole banana fruit (8.71%) and the aqueous extract of nettle and comfrey (7.95%). Observing the impact of aqueous extracts per year, it can be seen that the best effect was achieved by the foliar application of the aqueous extract of the whole banana fruit in 2019 (Table 3).

Table 3. The influence of aqueous extract application on the yield of soybean (kg ha^{-1}).

Treatment (B)	Years (A)			Average B
	2019	2020	2021	
Control	3032.50	3462.27	1962.49	2819.09
Water	3246.10^{ns}	3580.75^{ns}	2230.89^{ns}	3019.25^{ns}
Nettle	3477.65*	3872.40*	2369.96*	3240.00*
Nettle+comfrey	3524.73**	3963.50**	2412.37*	3300.20**
Banana fruit	3586.03**	3927.18**	2476.62**	3329.94**
Banana peel	3482.14*	3911.14*	2335.41*	3242.90*
Onion bulb leaves	3447.81*	3790.86^{ns}	2312.85*	3183.84*
Willow twigs	3365.42*	3715.11^{ns}	2262.11^{ns}	3114.21^{ns}
Soybean plant	3474.96*	3824.06*	2296.82*	3198.61*
Average A	3404.15**	3783.03**	2295.50	3172.00

*, ** Significant at 0.05 and 0.01 probability levels, respectively (LSD test); ns = not significant.

Factor	$LSD_{0.05}$	$LSD_{0.01}$
A	542.25	695.61
B	301.72	426.26
AxB	330.53	456.49
BxA	289.11	413.45

The yield was 3586.03 kg ha^{-1}, which is by 15.44% higher than in the control. Aqueous extracts of banana peel and nettle had a similar effect on soybean yield. The increase amounted to 12.91% and 12.80%, respectively. Aqueous extracts of soybean plants and onion bulb leaves also had a similar effect. Their effect increased the yield by 12.73% and 12.05%. If the effects of aqueous extracts in relation to water are observed, it can be concluded that a significant increase in yield was achieved by using an aqueous extract of the whole banana fruit (9.48%). In 2020, the best result was achieved by applying the aqueous extract of nettle and comfrey. The yield was 3963.50 kg ha^{-1}, which is by 12.65% higher than in the control. Using aqueous extracts of whole banana fruit and banana peel, the increase in yield was 11.84% and 11.48%, respectively, while using the nettle extract, the increase amounted to 11.59%. A similar effect was achieved by applying the aqueous soy extract. If the effects of aqueous extracts in relation to water are observed, it can be concluded that a better effect was achieved in 2020 than in 2019. A significant increase was achieved by applying an aqueous extract of nettle and comfrey (9.66%), whole banana fruit (8.82%) and banana peel (8.45%). A significant increase, but lower, was achieved by using aqueous extracts of nettle and soybean plants (7.53% and 6.36%). The effect of aqueous extracts in 2021 differs significantly from 2019 and 2020. Compared to the control, the increase in yield ranged from 13.24% to 20.76%. The best result was achieved by applying an aqueous extract of banana fruit. The yield was 2476.62 kg ha^{-1}, which is by 20.76%

higher than in the control. By applying aqueous extracts of nettle and comfrey and nettle, soybean grain yield was increased by 18.65% and 17.19%, respectively. An aqueous extract of banana peel increased the yield by 15.97%, an aqueous extract of onion peel by 15.15%, and an aqueous soy extract increased the yield by 14.56%. Unlike the previous two years, the application of water had a better effect than the aqueous extract of willow twigs. If the effects of aqueous extracts in relation to water are observed, it can be concluded that a significant increase in yield achieved only by using an aqueous extract of the whole banana fruit was 6.20%.

Protein yield. The results of the research showed a significant influence of the year and weather conditions on soybean protein yield. The highest yield was achieved in 2020 – 1461.56 kg ha^{-1}, which is by 38.59% higher compared to 2021 (Table 4).

Table 4. The influence of the aqueous extract application on the protein yield (kg ha^{-1}).

Treatments (B)	Years (A)			Average B
	2019	2020	2021	
Control	1178,68	1333,51	763,56	1091,92
Water	1257,65^{ns}	1367,99^{ns}	859,03^{ns}	1161,56^{ns}
Nettle	1353,41^{ns}	1502,99^{ns}	923,14^{ns}	1259,85^{ns}
Nettle+comfrey	1378,86*	1543,28*	954,64^{ns}	1292,26*
Banana fruit	1402,98*	1524,51^{ns}	979,45*	1302,31*
Banana peel	1358,82^{ns}	1506,74	908,73^{ns}	1258,10^{ns}
Onion bulb leaves	1346,54^{ns}	1469,12^{ns}	908,75^{ns}	1241,47^{ns}
Willow twigs	1301,54^{ns}	1429,57^{ns}	880,99^{ns}	1204,03^{ns}
Soybean plant	1357,92^{ns}	1476,30^{ns}	898,93^{ns}	1244,38^{ns}
Average A	1326,27	1461,56	897,47	

Factor	$LSD_{0.05}$	$LSD_{0.01}$
A	193,45	328,49
B	147,32	256,17
AxB	156,42	266,24
BxA	138,24	145,36

The best effect was achieved by applying an aqueous extract of the whole banana fruit. The achieved protein yield was 1302.31 kg ha^{-1}, which is by 16.16% higher than in the control. By applying the aqueous extract of nettle and comfrey, a protein yield of 1292.26 kg ha^{-1} was achieved, which is by 15.50% higher than in the control. The use of other extracts did not significantly affect protein yield. If the influence of aqueous extracts in relation to water treatment is observed, it can be concluded that no aqueous extract has led to a significant increase in protein yield. Observing the impact of aqueous extracts per year, it can be seen that, in 2019, the best effect was achieved by foliar application of an aqueous extract of the whole banana fruit. In this treatment, protein yield was 1402.98 kg ha^{-1}, which was by 15.99% higher than the control. When using the aqueous extract of nettle and comfrey, the increase was 14.52. In 2020, the use of the aqueous extract of nettle and comfrey significantly increased the protein yield by 13.59%. In 2021, a significant result was achieved by applying an aqueous extract of the whole banana fruit. Protein yield was increased by 22.04% compared to control. Weather conditions in some years have a huge impact on the variation of yield, protein and oil content in soybean grain (Dozet et al., 2019). The application of aqueous nettle extracts increased both the protein content and the oil content in the grain, i.e., there was an increase in the capacity for the accumulation of nutrients in the grain (Dozet et al., 2018).

Conclusion

The results show that foliar application of the different plant aqueous extracts can increase grain yield and protein yield. The increase in grain yield ranged from 9.48% to 15.34%, and the increase in protein yield from 9.31% to 16.16%. The best effect was achieved by applying the aqueous extract of the whole banana fruit and the aqueous extract of the mix of nettle and comfrey. By applying them each year, a significantly higher yield was achieved in relation to the control with distilled water. The best effect was achieved in the year with the largest precipitation deficit.

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UTICAJ PRIMENE VODENIH EKSTRAKATA RAZLIČITIH BILJAKA NA PRINOS ZRNA I PROTEINA U PROIZVODNJI SOJE

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R e z i m e

Cilj ovog rada je bio da se ispita uticaj vodenih biljnih ekstrakata na prinos zrna i proteina kod soje. Ispitivanje je obavljeno u Institutu za ratarstvo i povrtarstvo u Novom Sadu na sorti NS Apolo. Za folijarnu primenu korišćeni su vodeni ekstrakti: nadzemnog dela koprive, nadzemnog dela koprive i gaveza, ceo plod banane, kora banane, listovi lukovice crnog luka, vršni delovi grančica vrbe i vršni delovi biljaka soje. Pored kontrolne varijante, eksperiment je uključivao i kontrolu – destilovanu vodu. Kontrola destilovanom vodom će pokazati da li je efekat vodenih biljnih ekstrakata posledica biljnog materijala ili vode. Rezultati analize su pokazali da je upotreba vodenih ekstrakata doprinela povećanju prinosa zrna i prinosa proteina. Povećanje prinosa kretalo se od 9,48% do 15,34%, a povećanje prinosa proteina od 9,31% do 16,16%. Najbolji efekat je postignut primenom vodenog ekstrakta celog ploda banane i vodenog ekstrakta koprive i gaveza. Njihovom primenom svake godine postignut je znatno veći prinos u odnosu na kontrolu vodom.

Ključne reči: vodeni ekstrakt, soja, banana, kopriva, gavez.

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