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Effect of pulsed electromagnetic field on yield of grain, yield of protein and oil of soybean

Marija Bajagić[1], Vojin Đukić[2], Zlatica Miladinov Mamlić[2], Jovana Sekulić[3], Vojin Cvijanović[4], Nenad Đurić[5], Gorica Cvijanović[1]*

[1]*University of Bijeljina, Faculty of Agriculture – Bijeljina, Republic Srpska, Bosnia and Herzegovina*
[2]*Institute of Field and Vegetable Crops Novi Sad, Novi Sad, Serbia*
[3]*University of Kragujevac, Institute of Information Technologies Kragujevac, Kragujevac, Serbia*
[4]*Institute for Science Application in Agriculture, Belgrade, Serbia*
[5]*Institute for Vegetable Crops Smederevska Palanka, Smederevska Palanka, Serbia*
**Corresponding author: bajagicmarija@yahoo.com*

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Abstract: One of the latest environmentally friendly methods in soybean production technology is the pulsed electromagnetic field of low frequencies (PEMP). The paper presents the results of the influence of electromagnetic stimulation of soybean seeds on grain yield, protein and oil yield, depending on different agroclimatic conditions, exposure time and frequency. In the 2012–2017 research period, the soybean cv. Valjevka was used in the Institute of Field and Vegetable Agriculture experimental field, Novi Sad, Serbia. Immediately before sowing, the seeds were subjected to PEMP treatment, with a pulse generator and a tape applicator, in the following variants: electromagnetic field frequencies of 16, 24 and 30 Hz, and exposure time of 0, 30 and 60 min. The most successful variant of seed stimulation for all three examined parameters was at 16 Hz and 30 min, where the research results show that this measure can increase the examined parameters by more than 10%. The average yield of grain for all years of research with seed stimulation was 4.85% (3 338 kg/ha) compared to the control (3 203 kg/ha). The average grain protein yield in the treatments with PEMP was 1 315 kg/ha, which was 4.26% higher compared to the variant without PEMP (1 260 kg/ha), and the treatment was 4% higher in the average oil yield, 703 kg/ha compared to the control 676 kg/ha. Also, the analysis of the mutual dependence of the indicators is in a positive correlation, which is essential for plant breeding and the development of new technologies, which have economic justification, are safe for use and have a positive impact on adverse effects such as drought.

Keywords: *Glycine max* (L.) Merr.; nutrition; agroecolocical condition

Soybean (*Glycine max* (L.) Merr.) became an "absolute hit" on the world market, thanks to its economic importance. Oil and various products with a high percentage of protein are obtained by processing soybeans, which are used in the nutrition of domestic animals and human nutrition as raw materials in various industries. Namely, its importance is reflected in the composition of the grain, i.e. 45% protein, 20–22% oil, 20–26% carbohydrates, 5% minerals (phosphorus, potassium, calcium, sulphur, magnesium, etc.) and many vitamins (mainly A and B) (Yalçin 2018). Zhang et al. (2013) state that soy proteins contain high-quality amino acids, which, in terms of quality and importance, can be a substitute for meat proteins in the diet, especially for vegetarians. Soybean oils are multi-beneficial for diets lacking in omega-3 and omega-6 fatty acids. Soybean oil is characterised by a good ratio of fatty acids, namely: unsaturated linoleic, oleic and linolenic acids, and as representatives of saturated fatty acids: palmitic and stearic acids (Wang et al. 2019). Soybean as a plant crop was among the first to be

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used in genetic engineering (Maffei 2014), in plant breeding (Lewandowska et al. 2019), as well as the impact of various technologies considering its positive impact on the physical, chemical and biological properties of the soil (Đukić et al. 2017).

Planet Earth has a magnetic field that represents a natural component for all living organisms that influence biological processes (Joshi-Paneri et al. 2023). The intensity of the Earth's natural magnetic field is in the range of 30 to 70 mT (Maffei 2014). This phenomenon has interested many researchers in biology, medicine and agriculture, where many experiments related to the effects of electric and magnetic fields have been performed (Joshi-Paneri et al. 2023). Pietruszewski et al. (2007) explain that there are three main lines of experiments related to investigating the effects of electromagnetic fields (EMP) on plants: investigating the effects of extremely strong frequencies (Sarraf et al. 2021), examining the field in the absence of the Earth's magnetic field (Negishi et al. 1999), and the third and most represented research is related to the influence of EMP of low frequencies (Lewandowska et al. 2019, Sarraf et al. 2021). Many studies like Radhakrishnan (2019) and Abdel Latef et al. (2020) prove the effectiveness and changes caused by the action of EMP, such as increases in the electro potential of membranes, stimulation of protein and enzyme activity, a faster process of photosynthesis and a higher content of pigments, increased cell divisions, which affect the better accumulation of water and nutrients. The largest number of studies refer to treatments of seeds and plants in the initial stages of development, where the effects of seed germination morphological and productive properties of plants are examined (Himoud et al. 2022, Tirono and Hananto 2023). According to Bajagić et al. (2021), obtained results do not depend only on plant species (moisture content, growth rate, storage period) but also on climatic conditions (temperature, air and soil humidity) and on exposure time, intensity and nature of the field. Considering that these are physical actions on biological systems, these methods can be included in the domain of biophysical actions, better known as e-treatments or biostimulators for plants, whose mechanisms of action on plants are still unclear.

Climatic changes, which are increasingly unpredictable due to high temperatures and the occurrence of drought, adversely affect the achievement of high yields and stable production. In this direction, various researches on the use of EMP have shown positive changes in plants during unfavourable external conditions, such as drought (Bajagić et al. 2021), protection of plants from weeds (Stanković et al. 2016), diseases and pests (Abdollahi et al. 2012).

New world trends aimed at protecting the environment and producing health-safe food open up the possibility of finding new production technologies, which should be efficient and economical at the same time. Himoud et al. (2022), Tirono and Hananto (2023), and many other authors report on the beneficial effects of the pulsed electromagnetic field (PEMP) and that its use is introduced as one of the environmentally acceptable techniques that meet the requirements of organic agriculture. Many authors, such as Nair et al. (2018), and Radhakrishnan (2019), state that research is carried out both at the cell level and on whole organisms, where the mechanism of action of PEMP affects various biochemical processes of cells in plants, such as membrane electropotential, protein and enzyme activities, positive changes in the photosynthesis process and content pigments. Radhakrishnan (2019) states that the treatments affect increased cell division, and Nyakane et al. (2019) that it can affect the movement of charged particles through the cell membrane, which implies a faster absorption of water and nutrients, and plants grow faster and become more resistant to climatic conditions.

For EMP research to be more precise and successful, according to Maffei (2014), it would be desirable to collect data on the present static magnetic fields of the Earth, which are missing in the literature, as well as experimental research carried out in an open field under different climatic influences (Đukić et al. 2017, Bajagić et al. 2021).

Therefore, this research aims to examine the effect of stimulation of soybean seeds before sowing with different variants of pulsed electromagnetic fields of low frequency on grain yield, protein yield and oil yield in different agroecological conditions.

MATERIAL AND METHODS

Weather conditions. Data on temperature and precipitation, shown in Table 1, were collected at the meteorological station Rimski Šančevi, near Novi Sad. The sum of precipitation in the growing season for the multi-year average (1964–2017) was 375.3 mm. Compared to the multi-year average, the sum of precipitation was higher in 2013 (448.2 mm), 2014 (595.6 mm), 2015 (389.0 mm) and in 2016 (450.5 mm).

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Table 1. The sum of precipitation (mm) and average mean temperature (°C) for the research period 2012–2017 and the multi-year period 1964–2017

	2012	2013	2014	2015	2016	2017	1964–2017
The sum of precipitation							
April	82.8	35.8	51.2	15.0	74.5	57.0	47.6
May	52.2	118.1	202.1	192.0	85.0	82.9	67.7
June	27.5	125.7	38.2	28.0	143.18	65.7	87.2
July	47.7	34.1	141.1	2.0	68.4	12.0	66.4
August	3.5	26.7	78.7	99.0	45.8	17.4	58.2
September	13.1	107.8	84.3	53.0	33.7	81.5	48.2
Sum	226.8	448.2	595.6	389.0	450.5	316.5	375.3
Average mean temperature							
April	13.0	13.4	13.2	11.8	14.2	11.4	11.7
May	17.5	17.4	16.3	17.8	16.9	17.6	17.0
June	23.0	20.2	20.5	20.5	21.7	23.2	20.1
July	25.2	22.3	21.9	24.5	22.8	24.3	21.8
August	24.6	22.9	20.9	24.4	21.1	24.8	21.3
September	19.8	15.7	17.2	19.9	18.5	16.9	16.9
Average	20.5	18.6	18.3	19.8	19.2	19.7	18.1

In 2012 (226.8 mm) and 2017 (316.5 mm), the amount of precipitation was less. Also, it is important to point out that in addition to the amount of precipitation, the distribution of precipitation is extremely important, given that soybeans are demanding in terms of the amount of water in the phases of filling the grains (July–August). Therefore, the lack of precipitation is clearly visible in 2012 (August – 3.5 mm), 2015 (July – 2 mm), as well as in 2017 (July – 12.0 mm and August 17.4 mm). The average air temperature of the multi-year period was 18.0 °C, which is lower compared to all the examined years (2012 – 20.5 °C, 2013 – 18.6 °C; 2014 – 18.3 °C; 2015 – 19.8 °C, 2016 – 19.2 °C, 2017 – 19.7 °C).

Design of experimental research. The multi-year research was carried out on the experimental field in Rimski Šančevi (45°20'N, 19°51'E), near Novi Sad, region of Vojvodina – Serbia. The research period includes the vegetative season of 6 years (2012–2017). Each year, the area of the experimental plot was 10 m^2 (row spacing 50 cm × 5 cm). The experiment was set up according to randomised block design with four replications on the humus soil type of carbonate chernozem, class A-AC-C (humous – accumulative soils, arable + sub-arable (A), transitional (AC) and parent rock (C). The plant material used for this research is the soybean cv. Valjevka was selected by the Institute of Field and Vegetables in Novi Sad (0 ripening groups, length of the vegetation period up to 120 days, genetic potential for yield above 4 500 kg/ha). Standard agricultural practice was used, where all agrotechnical measures were carried out in optimal conditions. No significant attacks of diseases and insects were recorded. Seed stimulation with a pulsed electromagnetic field (PEMP) was treated in laboratory conditions using an apparatus with a specific spectral content. Five hundred grains were prepared for each subplot stimulated by a pulsating electromagnetic field. The device consists of a pulse generator and a strip applicator, through which the pulsating alternating movement of electric and magnetic fields takes place (Figure 1).

Figure 1. Apparatus of specific spectral content during seed stimulation immediately before sowing

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The pulse generator consists of a power supply that transforms the input network from 235 VAC to direct current, and then through a driver, controlled by a microcontroller for generating waveforms and frequencies, feeds the inductive circuit at the output that treats the desired mass. The device has controls for setting the frequency in the range from 1 Hz to 100 Hz with a potentiometer for the possibility of setting ± 1 Hz. The following command is to select the time duration in minutes from 1 min to 60 min. The seeds were exposed to low frequencies of PEMF (16, 24 and 30 Hz) for 0, 30 and 60 min immediately before sowing. Sowing was done immediately after the seeds were exposed to PEMF. In the stage of technological maturity, harvesting was carried out, and measurement of the weight of soybeans, grain moisture, and the yield per hectare with 14% moisture was calculated with the help of a Wintersteiger elite combine harvester, intended for trial plots with a small working area.

The yield of obtained soybean seeds is expressed in kg/ha at 14% moisture. In the laboratory of the Soybean Department, Institute of Agriculture and Vegetables, Serbia, the protein and oil content of the same seed was measured by the nuclear magnetic resonance spectroscopy (NMR) method, according to Granlund and Zimmerman (1975). Protein yield was calculated as the product of grain yield and seed protein content. At the same time, oil yield was calculated as the product of grain yield and seed oil content.

Statistical analysis. The research results were processed with descriptive statistics and analysis of the variance of the three-factorial experiment in the DSAASTAT program (Perugia, Italy). A three-way ANOVA was used to test for the effects of year, exposure time, and frequency strength. The correlations between the traits tested were also determined. The significance of the differences was tested with the least significant difference (*LSD*) test at the $P < 0.01$ and $P < 0.05$ significance levels. The results are presented in tabular form.

RESULTS AND DISCUSSION

The years of research (2012–2017), i.e. meteorological conditions in the vegetative period of soybean production, are of exceptional importance, given that soybean production takes place in an open field, without an additional irrigation system. Cvijanović et al. (2020) suggest that earlier sowing and seed stimulation, which affects faster germination and plant growth, are important measures of adaptation to climate change. According to Bajagić et al. (2021), lack of precipitation, high temperatures and occurrence of dry periods in the reproductive stages reduce the number of grains per plant, negatively affecting the total yield.

The effect of PEMF on soybean seed yield depends on years (Y), exposure time (T) and frequency (F), the interaction of exposure time and frequency (B × C) and the interaction of all three factors (Y × T × F) (Table 2). According to all examined factors, the total yield of soybeans is 3 269 kg/ha. The highest yield was determined in 2014 (4 945 kg/ha), given that in that year, there was enough rainfall, which had a regular schedule, as well as high temperatures that were suitable for normal soybean development. In the arid year 2015, the lowest yield was determined, only 2 004 kg/ha, 40.53% less than in 2014. Đukić et al. (2011) state that high temperatures lead to drying of the surface part of the soil, which negatively affects the germination of plants – the exposure time with PEMP lasting 60 min had the greatest effect on the increase in yield (3 268 kg/ha), with a statistical significance of $P < 0.01$ compared to the control variant. Seed stimulation had a statistically significant effect on the increase in yield, and the highest yield was achieved at a frequency of 24 Hz (3 327 kg/ha). The established results are in correlation with the studies of the treatment of soybean seeds with PEMP (frequency 16 Hz and 30 min), which positively influenced the germination of soybean seeds by 8%, which further influenced the increase in yield by 21% (Đukić et al. 2017). Similar results of increasing germination in three different soybean cultivars were recorded by Yalçin (2018). Various seed treatment studies suggest an increase in yield, depending on the intensity of the frequency and time of exposure (Maffei 2014, Sarraf et al. 2021). On average, for all years of research, the best combination for increasing yields by 11.30% is at 16 Hz and 30 min compared to the control. Similar results were obtained by Badiger and Hunje (2020) by examining the impact of pulsed electromagnetic field treatments on soybean seeds with strengths of 1, 10, 50 and 100 Hz for 5 h a day for 15 days. The highest yield of soybeans was at 50 Hz (21.70 q/ha) compared to the control (without stimulation, 20.23 q/ha), as well as the content of protein in the grain (37.85%) and seed oil (18.24%), in comparison to the control (37.17%, 17.62%). Radhakrishnan and Kumari (2012) state that seed stimulation with PEMP is important

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Table 2. The impact of pulsed electromagnetic field (PEMF) on soybean yield (kg/ha) in the period from 2012 to 2017

Year (Y)	Time (min) (T)	Frequency (Hz) (F) 16	24	30	Average Y × T	Average Y
2012	0	2 325	2 325	2 325	2 325	2 451
	30	2 769	2 405	2 284	2 486	
	60	2 363	2 652	2 613	2 543	
	average Y × F	2 485	2 461	2 407		
2013	0	3 430	3 430	3 430	3 430	3 569
	30	3 812	3 624	3 300	3 578	
	60	3 525	3 806	3 768	3 699	
	average Y × C	2 589	3 620	3 499		
2014	0	4 984	4 984	4 984	4 984	4 945
	30	5 302	5 003	4 327	4 877	
	60	4 718	5 144	5 061	4 974	
	average A × C	5 001	5 044	4 791		
2015	0	1 961	1 961	1 961	1 961	2 004
	30	2 174	2 047	1 701	1 974	
	60	1 897	2 166	2 170	2 078	
	average A × C	2 011	2 058	1 944		
2016	0	4 286	4 286	4 286	4 286	4 357
	30	4 736	4 340	4 069	4 381	
	60	4 174	4 681	4 351	4 402	
	average A × C	4 399	4 436	4 236		
2017	0	2 228	2 228	2 228	2 228	2 325
	30	2 595	2 292	2 046	2 311	
	60	2 120	2 509	2 359	2 329	
	average Y × F	2 314	2 343	2 211		
Average T × F	0	3 203	3 203	3 203	average T	3 203
	30	3 565	3 285	2 955		3 268
	60	3 133	3 493	3 387		3 338
	average F	3 300	3 327	3 181		
Average 2012–2017						3 269

	Y^{**}	T^{**}	F^{**}	YT^{ns}	AF^{ns}	TF	YTF^{**}
F-test	0.00	0.00	0.00	0.15	0.06	0.00	0.00
$LSD_{0.05}$	78.73	55.51	32.07	135.98	78.54	58.87	136.04
$LSD_{0.01}$	100.45	74.44	42.42	182.33	103.90	77.87	179.96

LSD – least significant difference; $^{**}P < 0.01$; ns – not significant

in improving soybean yield and productivity through faster mineral accumulation and enzyme activity, faster water uptake, germination and emergence, ultimately leading to increased yield.

The total average value of protein yield (1 288.02 kg/ha) was statistically very significant ($P < 0.01$) for all levels of the examined factors (Table 3), except for the year-frequency interaction, which was at the level of 5% and year-time exposure interactions where there was no statistical significance. The highest protein yield was determined in 2014 at 1 956 kg/ha, while the lowest yield was recorded in 2015 (792 kg/ha), correlated with the level of soybean yield. Large variations in protein yield per year are due to the

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Table 3. Impact of pulsed electromagnetic field (PEMF) on protein yield in soybean seeds (kg/ha) in the period from 2012 to 2017

Year (Y)	Time (min) (T)	Frequency (Hz) (F)			Average Y × T	Average Y
		16	24	30		
2012	0	908	908	908	908	962
	30	1 074	937	895	969	
	60	967	1 012	1 022	1 001	
	average Y × F	983	960	942		
2013	0	1 342	1 342	1 342	1 342	1 398
	30	1 482	1 423	1 300	1 402	
	60	1 387	1 486	1 476	1 450	
	average Y × F	1 404	1 417	1 373		
2014	0	1 964	1 964	1 964	1 964	1 956
	30	2 076	1 990	1 727	1 931	
	60	1 881	2 048	1 992	1 973	
	average Y × F	1 973	2 000	1 894		
2015	0	773	773	773	773	792
	30	856	810	673	779	
	60	755	856	857	823	
	average Y × F	794	813	768		
2016	0	1 699	1 699	1 699	1 699	1 736
	30	1 883	1 733	1 631	1 749	
	60	1 673	1 867	1 738	1 759	
	average Y × F	1 752	1 766	1 689		
2017	0	865	865	865	865	916
	30	1 002	894	797	897	
	60	826	961	871	886	
	average Y × F	908	932	908		
Average T × F	0	1 259	1 259	1 259	average T	1 260
	30	1 395	1 298	1 170		1 288
	60	1 248	1 372	1 326		1 315
	average F	1 301	1 310	1 252		
Average 2012–2017						1 288

	Y**	T**	F**	YT^{ns}	AF^{ns}	TF**	YTF**
F-test	0.00	0.00	0.00	0.20	0.06	0.00	0.00
$LSD_{0.05}$	31.36	22.42	13.87	54.92	33.99	24.03	58.87
$LSD_{0.01}$	40.01	30.06	18.35	73.64	44.96	31.79	77.87

LSD – least significant difference; ***P* < 0.01; ns – not significant

lack of moisture in a period that is very important for grain yield formation per plant and the occurrence of drought due to high temperatures. Different variants of the exposure time affected the protein yield variability at the statistical significance $P < 0.01$, where the highest yield of protein was observed at a duration of 60 min (1 315 kg/ha). The highest protein yield was recorded at 24 Hz (1 310 kg/ha). The highest protein average was achieved in 16 Hz and 60 min, by 10.87% compared to the control. Similar results were obtained by Đukić et al. (2020) in the study of seed treatment with an electromagnetic field of soybean seeds; on average for four years, the highest protein yield in the amount of 14.15% was in the treatment with 16 Hz × 30 min.

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Table 4. Impact of pulsed electromagnetic field (PEMF) on oil yield in soybean seeds (kg/ha) in the period from 2012 to 2017

Year (Y)	Time (min) (T)	Frequency (Hz) (F)			Average Y × T	Average Y
		16	24	30		
2012	0	195	495	495	495	523
	30	589	519	482	530	
	60	501	562	563	544	
	average Y × F	529	527	514		
2013	0	731	731	731	731	761
	30	809	780	697	762	
	60	746	810	809	788	
	average Y × F	762	774	745		
2014	0	1 049	1 049	1 049	1 049	1 045
	30	1 112	1 064	917	1 031	
	60	991	1 092	1 065	1 049	
	average Y × F	1 051	1 068	1 016		
2015	0	405	405	405	405	415
	30	444	427	372	414	
	60	384	448	448	427	
	average Y × F	411	426	409		
2016	0	876	876	876	876	882
	30	957	865	817	880	
	60	837	952	876	889	
	average Y × F	890	897	856		
2017	0	491	491	491	491	508
	30	576	514	452	514	
	60	467	562	527	519	
	average Y × F	511	523	591		
Average T x F	0	674	674	674	average T	674
	30	748	695	623		688
	60	654	739	715		703
	average F	693	703	672		
Average 2012–2017						689

	Y**	T**	F**	YT^{ns}	AF^{ns}	TF**	YTF**
F-test	0.00	0.00	0.00	0.07	0.09	0.00	0.00
$LSD_{0.05}$	17.16	11.17	7.18	27.35	17.59	12.44	30.46
$LSD_{0.01}$	21.89	14.97	9.50	36.67	23.26	16.45	40.29

LSD – least significant difference; ***P* < 0.01; ns – not significant

In Table 4, the average value of oil yield (689 kg/ha) was statistically very significantly different ($P < 0.01$) for all examined factors, except for the interaction A × B and A × C, where there was no statistical significance. The highest oil yield was determined in 2014 at 1 045 kg/ha, while the lowest yield was recorded in 2015 (415 kg/ha). Observing the influence of exposure time, it can be concluded that, on average, for all levels, oil yield increases by 1.93% at 30 min of exposure and 4.01% at 60 min of seed exposure compared to the control. Regarding the influence of frequency, the highest oil average was at 24 Hz (703 kg/ha). The highest oil yield of the examined combinations of seed stimulation is with 16 Hz and

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Table 5. Correlation between the tested traits

	Yield of grain	Yield of protein	Yield of oil
Yield of grain	1		
Yield of protein	0.999**	1	
Yield of oil	0.995**	0.992**	1

30 min (748 kg/ha). In pulsed electromagnetic field treatments on soybean seeds from 0 to 100 Hz, the greatest increase in seed oil content was at 50 Hz exposure (18.04%), as well as in seed protein content (37.65%) (Badiger et al. 2016).

It is known that protein and oil content are negatively correlated (Sobko et al. 2019). A strong negative correlation between protein and oil content has hindered efforts to improve soybean seed quality (Wang et al. 2019), given that soybean is an important source of both protein and oil for human consumption. The same authors state that soybean breeding should be focused on finding cultivars with increased protein and oil content. Unlike many studies, seed treatment with PEMP positively affected the correlation relationship. Table 5 shows the correlation dependences of the investigated traits, which are highly statistically significant. There is a positive correlation between grain yield and protein content (0.999**), between grain yield and oil yield (0.995**) and protein and oil yield (0.992**). These results imply that in addition to the influence of the genetic material of the cultivar, agrotechnics and climatic conditions, seed stimulation with PEMP simultaneously increases the amount of protein and oil, which is of great importance for the further technology of soybean production.

The following conclusions are drawn based on the obtained results: soybean yield, protein, and oil yield decrease in years with unfavourable climatic conditions and a characteristic water deficit in arid and semi-arid years. Seed stimulation with PEMP positively affects the increase of the examined parameters. The best combination of seed stimulation is with 16 Hz for 30 min in different agroclimatic conditions. Also, the effect of low frequencies of pulsating electromagnetic waves positively affects the yield of protein and oil, and there is no negative correlation. The introduction of new technologies, such as seed biophysical methods such as seed stimulation with PEMP, can influence the creation of high and stable yields, additionally under unfavourable agrometeorological conditions. The general importance of applying these treatments is considered an ecological, cheap and safe technique.

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