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TECHNOLOGIES FOR PRODUCING PLANT BIOSTIMULANTS USING COLD PLASMA AND LOW-PRESSURE MICROWAVE DISCHARGE

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ABSTRACT

In recent years, it has been realized that agriculture has become one of the economic sectors with a huge impact on the environment. There-

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ABSTRACT

fore, measures have been taken to reduce the negative impact of agricultural production on the environment. The use of biostimulants in agriculture, especially of plant origin, is part of this trend. However, obtaining suitable formulation of biostimulants requires the development of appropriate technologies for their production. Therefore, it was undertaken to investigate the possibility of using gliding arc cold plasma (GA) and low-pressure microwave (MW) discharges to produce water plant extracts with biostimulating potential. An increase in total polyphenol content and antioxidant activity was observed, indicating the high potential of using low-pressure microwave discharge to produce effective plant biostimulants. Also, low-pressure microwave discharge improved the extraction of elements such as Ca, K and Fe.

Introduction

In recent years, it has been realised that agriculture has become one of the economic sectors with a huge impact on the environment. The assumptions of the European Green Deal point to the need for a change of approach from plant cultivation using synthetic chemicals to technologies that use, among other things, natural biostimulant products. This change is contingent on preserving and, if possible, increasing crop yields, as a significant increase in demand for food and feed has been recorded worldwide in recent times, putting additional pressure on food producers.

According to EU Regulation 2019/1009, products based on plant extracts can be categorised as natural biostimulants, i.e. a group of products (other than fertilisers) that, when applied in small amounts, stimulate plant growth improve mineral uptake and increase plant tolerance to biotic and abiotic stresses. Nowadays, the use of biostimulants has been recognised as treatments that support physiological processes in plants and promote their growth and development most often under suboptimal conditions.

The agronomic potential of plant extracts is directly related to the total polyphenol and flavonoid content (Singh et al., 2015; Asparaga et al., 2023). In the literature, phenolic compounds at low concentrations are referred to as germination and plant growth stimulators (Ghareib et al., 2010) due to their ability to act as antioxidants, effective regulators of plant growth, soil processes and/or nutrients (Ertani et al., 2016).

However, generating new types of biostimulants from them requires a systematic approach to their design and further use. Such a comprehensive process must therefore start with the identification of raw materials, then define the extraction methods, and end with the development of a product that will effectively convert natural ingredients with potential biological activity into high-quality biostimulants. Methods to improve the efficiency of the extraction process are therefore constantly being sought.

One such method is gliding arc cold plasma discharge (GA), which has attracted the attention of plant physiologists for several years due to its potential to increase plant growth and resistance. It is considered an environmentally friendly method for improving crop production (Strejckova et al., 2016). Previous studies performed by our research group have shown that plasma secretion binds to the seed coat of *Metarhizium anisopliae* and *Trichoderma virens*, providing an alternative to chemical seed treatment. The results of this research have shown that the use of both plasma technologies, low-pressure microwave discharge and the combination of plasma and seed bio-treatment offers great potential in seed technology as value-added processes. Laboratory and field analyses have shown stimulation of seed germination in spring barley, winter oilseed rape and spring poppy, earlier seedling growth of these crops and positive effects on their yield (Strejckova et al., 2018; Kriz et al., 2021). Nowadays, interactions between liquids and plasma are widely used. Plasma is used to treat water by removing pesticides (Vanraes et al., 2017) or pharmaceuticals (Magureanu et al., 2015). However, there is still little research on this technology for its applications in agriculture (Kocira et al., 2022).

Another method that is becoming increasingly common is the use of microwaves in the extraction process including low-pressure microwave discharge (MW). Microwave discharges at low pressure are generated by ionizing a gas with microwave radiation (Barkhudarov et al., 2012). They can generate non-equilibrium plasma, which is advantageous for processes requiring low temperatures, such as organic synthesis and processing of heat-sensitive materials (Lebedev, 2015). Up till now the use of MW technology has had little application in agricultural science. Gorodecka et al. (2009) showed that MW treatment of seeds, including by microwave discharge, improves their germination. However, this technique is not yet well studied especially for agricultural applications.

Noting the potential of both GA and MW , we undertook to produce plant biostimulants using these technologies and based on an aqueous extract from a mixture of plant parts of field horsetail, rosehip and soapwort. Taking these considerations into account, we took as the aim of the study to evaluate the improvement of the biostimulant properties of the produced plant extracts MW and the improvement of biostimulant properties by GA-derived activation of aqueous extracts from plant parts of field horsetail, rosehip and soapwort.

Material and methods

Production of water plant extract using cold plasma (GA)

Plant extracts were prepared from mixture of dried and milled (powdered) field horsetail (*Equisetum arvense*) stems and branches, dog rose (*Rosa canina*) fruits (rosehips), and soapwort (*Saponaria officinalis*) roots. The hot water extraction method was used to obtain plant extracts. The extracts were prepared for a mixture of herbs. Specifically, an amount of 4757 mg horsetail, 238 mg of rosehip and 5 mg of soapwort was added to 100 mL of distilled water to obtain the mixture. The mixture was extracted for 30 minutes at 100°C. The obtained plant extract was subjected to cold plasma treatment for for 30 (GA 30s), 60 (GA 60s), 90 (GA 90s) and 120 (GA 120s) seconds, produced in a device comprised of the source of high voltage for generating a plasma discharge GVN1k-2011 by Radan Inc, Czech Republic. The main part of the source was a high-voltage transformer Resinblock 2000, which generated the electrical voltage, which was then applied to the electrodes in the plasma head. FL-2008 rotameter by Omega was integrated into the source, which allowed to adjust the working gas flow in the range of 0-100 SCFH (Standart Cubic Feet per Hour). The Gliding Arc plasma discharge was generated in the plasma head. The plasma head had cuboid shape form and was made of stainless steel. The inner space was equipped with an insulating layer of polytetrafluoroethylene (PTFE) to make it sufficiently electrically insulated from the surrounding environment. An electrical discharge was generated between a pair of divergent stainless-steel electrodes at a point with a minimum distance of 2 mm between the electrodes. Subsequently, the discharge channel was carried by the flowing air in the space between the electrodes, thereby blowing it out from the plasma nozzle area. The source of the working gas was provided by an air compressor Orlik, Czech Republic with minimum pressure of 600 kPa. The plant extract in the vessel was placed under the plasma head in the distance of 8 cm between the head and water surface. Working gas flow was maintained at 30 SCFH. The extract were then centrifuged at 4500 rpm for 10 minute and collected to tubes.

Production of water plant extract using low pressure microwave discharge (MW)

The plant mixture was prepared from of dried and milled (powdered) field horsetail (*Equisetum arvense*) stems and branches, rosehip (*Rosa canina*) fruits, and soapwort (*Saponaria officinalis*) roots. This mixture consisted of 4757 mg horsetail, 238 mg of rosehip and 5 mg of soapwort. The ground mixture was subjected to the low-pressure microwave discharge (MW) for 30 (MW 30s), 60 (MW 60s), 90 (MW 90s) and 120 (MW 120s) seconds generated in the apparatus Plasonic AR-550-M. The system was equipped with cylinder-form vacuum chamber with volume of 10 L. The plasma generator consisted of a mineral corundum tube, which was placed in the center of a microwave resonator. Microwave source MNG 1K-08 was supplied by Radan, spol. s.r.o., Czech Republic. The power supply enabled output power regulation from 100 to 800 W. Plasma beam was introduced into one end of the tube whereas the other led into the reactor chamber. The microwave discharge was generated at low pressure (100 Pa) and therefore it was necessary to drain gas from the vacuum chamber area. The extraction of the gas from the chamber was carried out using rotary oil pump Adixen (Pfeiffer Vacuum Inc, USA). The regulation of the internal environment in the vacuum chamber was realized using massflowmeter Bronkhorst FV 201-CV (Bronkhorst High-Tech B. V, Netherlands). The grounded mixture of plants was inserted in the stainless-steel cylindrical container with a diameter of 25 cm and a height of 15 cm. The container was placed in the bottom of the vacuum chamber. The operated plasma power was set to 500 W and the working pressure was set to 100 Pa. An aqueous extract was produced by adding 5000 mg of MW-activated mixture to 100 ml of distilled water and extracting for 30 minutes at 100°C. The extracts were then centrifuged at 4500 rpm for 10 minute and collected to centrifuge tubes.

Water plant extract - control combination

The control combination was an extract prepared as with GA except that the extract produced was not treated with cold plasma.

Total polyphenol content (TPC)

The total polyphenol content (TPC) in the water extracts was determined spectrophotometrically using Folin–Ciocalteu's reagent according to Bárta et al. (2021). Briefly, 10 µL of extract and 990 µL of distilled water was mixed with 50 µL of Folin-Ciocalteu's phenol reagent, followed by the addition of 150 µL of 20% (w/v) sodium carbonate. The absorbance was read at λ = 765 nm using BioMate 5 spectrophotometer (Thermo Scientific, USA) after

2 h of incubation at room temperature. Gallic acid was used for calibration. All measurements were repeated three times, and the results were expressed as milligrams of gallic acid equivalent (GAE) per litre of aqueous extract.

DPPH antioxidant assay

DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging assay was used to determine antioxidant activity, according to Bárta et al. (2021). An amount of 0.025 g radical was dissolved in 100 mL of methanol to obtain a stock solution. The working solution was further diluted to the concentration possessing absorbance 0,8 at 515 nm. The reaction mixture was prepared from 975 µL of 10% (v/v) DPPH stock solution and 25 µL of the analysed extract. The absorbance of the reaction mixture was measured at 515 nm after incubation at room temperature for 30 min using BioMate 5 spectrophotometer (Thermo Scientific, USA). Ascorbic acid was used for calibration. All measurements were repeated three times, and the results were expressed as milligrams of ascorbic acid equivalent (AAE) per litre of aqueous extract.

Elemental content in water plant extract (biostimulator)

An atomic absorption spectrometer (AAS) iCE 3500 series (Thermo Fisher Scientific, Cambridge, UK) operated in flame and ETA modes was used to determine the elements in individual samples. Extended lifetime graphite cuvettes (Thermo Fisher Scientific, Dreieich, Germany) were used for electrothermal atomization. AAS with a flame atomization was used in the case of Al, Ca, Cu, Fe, K, Mg, Mn, Na, and Zn determination. A N_2O/C_2H_2 flame served to determine Al. and Ca; otherwise, air was used as an oxidant. The burner length was 5 cm. Se was determined by a graphite furnace AAS. Peak areas served as signals in this case. Ten microliters of a Pd solution ($1.0\ g \cdot L^{-1}$) were injected as a matrix modifier during the determination of Se. The sample volume was 20 µL. A deuterium background correction was used in analyses performed in the flame while a Zeeman correction in the graphite furnace atomization modes. Analytical methods were carefully optimized and validated. Precisions (expressed as repeatability) were better than 5.0%. Accuracies were expressed as recoveries and determined using standard addition method. Their values lay in the interval from 95.0 to 105.0 %.

Statistical analysis

The data were analysed with Statistica 13.3 (TIBCO Software Inc., Palo Alto, CA, USA). Regularity of data distribution was checked with Shapiro-Wilk test. Significance of differences between the average values was verified with Tukey's test at the level of significance $p < 0.05$.

Results and discussion

Total polyphenol content of tested biostimulants (water extracts)

The biostimulation potential of plant extracts is directly related to the total polyphenol content, which represents one of the main parameters characterizing biostimulants. Total polyphenol content is closely related to antioxidants and other biological activities. Therefore, biostimulants should have as high a TPC as possible. The total polyphenol content (TPC) in the tested biostimulants ranged from 1077.5 to 1299.0 mg GAE/L of biostimulants (Fig. 1). The TPC levels in biostimulants produced using cold plasma (GA) were comparable to those in the control extract. A significantly higher TPC content was observed in the MW 60s biostimulant produced using low-pressure microwave discharges. A study by Neelam and Khan (2012) indicates that water extracts have a lower TPC content compared to extracts extracted using methanol. The use of methanol in producing plant biostimulants is not preferred due to environmental burden. Therefore, despite the lower TPC content of the aqueous extracts, this method appears to be preferable. As indicated by the study of Aremu et al. (2022), biostimulants with higher TPC content positively affect plant biometric traits such as plant height, stem diameter or leaf area. Therefore, as our study indicates that the application of low-pressure microwave discharge in the extraction process positively influenced the increase of TPC within the obtained plant biostimulants.

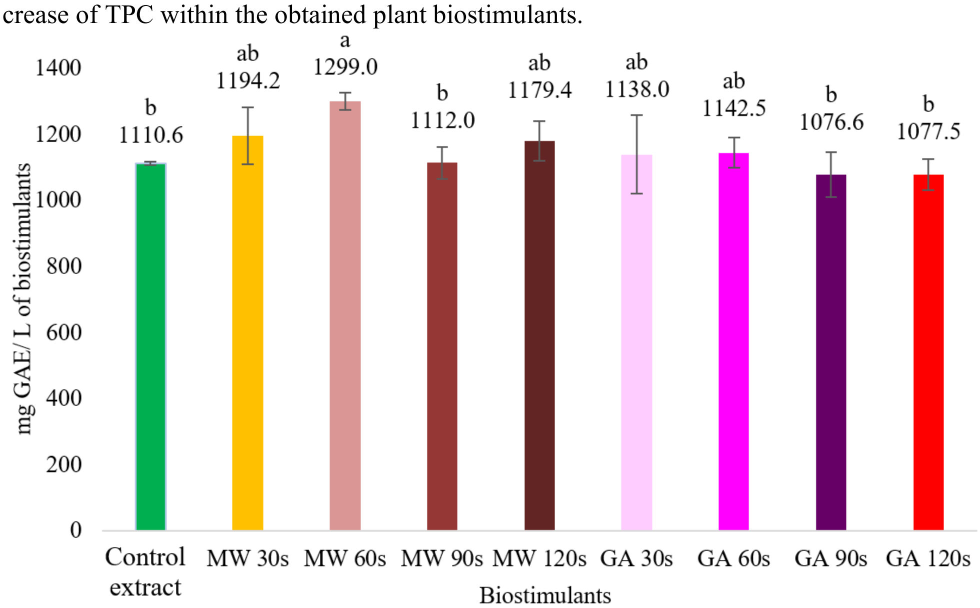

Figure 1. Total polyphenol content in tested biostimulants (water extracts). The figure presents the mean and SD values. Values not sharing a common letter indicate significant difference at $p \leq 0.05$.

Antioxidant activity DPPH of tested biostimulants (water plant extracts)

Another important parameter is the antioxidant activity (DPPH). In the tested biostimulators treated with cold plasma, a decrease in this potential was observed, but it was not significantly lower than in the control biostimulator (control extract). Also, the production of biostimulants using low-pressure microwave discharges did not result in a significant increase in antioxidant activity with one exception, which is the MW 60s biostimulator (Fig. 2). In this case, there was a 7.7% increase in antioxidant activity compared to the control extract.

Our study confirms the observations of Malenčić et al. (2008), Kolar et al. (2014) and Attanayake et al. (2015), who found a positive correlation between TPC content and antioxidant activity. The antioxidant activity, as reported by Neelam and Khan (2012), in plant extracts also depends on the used extraction agent. The water extracts had lower antioxidant activity compared to petroleum ether, chloroform and methanol extracts (Neelam and Khan, 2012).

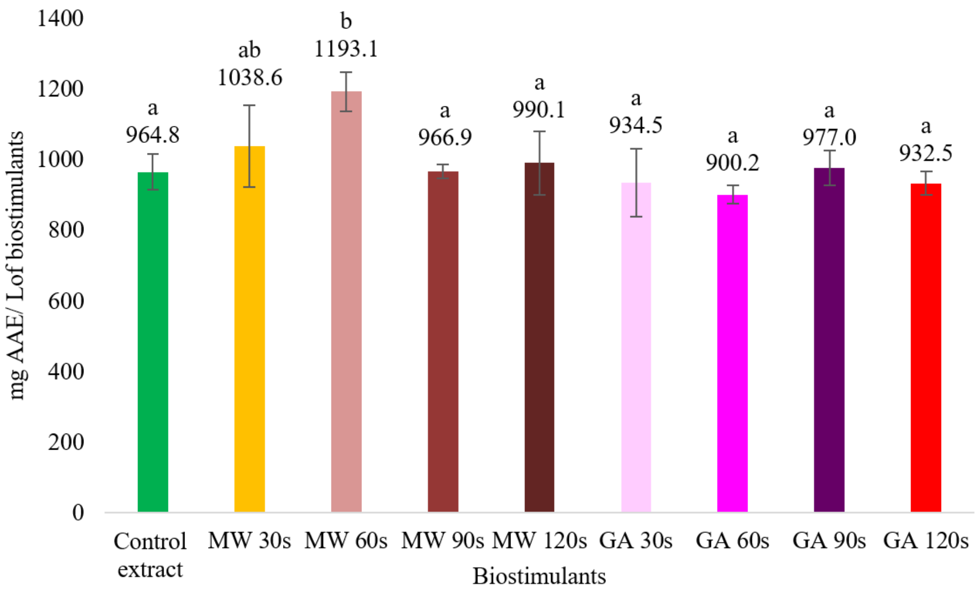

Figure 2. DPPH antioxidant activity determination of tested biostimulants (water extracts). The figure presents the mean and SD values. Values not sharing a common letter indicate significant difference at $p \leq 0.05$.

Table 1.
Elemental composition of tested biostimulants (mean ± SD)

Biostimulants		Ca	Cu	Fe	K	Mg	Mn	Zn	Na
		mg L^{-1}							
Control extract		792.33±20.59 ab	0.04±0.01 a	0.12±0.01 ab	2030.00±79.37 ab	366.67±4.73 b	1.73±0.04 a	0.47±0.02 a	5.83±0.61 a
GA	30s	791.33±17.24 ab	0.03±0.01 ab	0.11±0.01 abc	1827.67±50.33 de	359.33±2.89 b	1.63±0.02 bc	0.42±0.01 abc	5.34±0.21 a
	60s	777.33±15.57 ab	0.03±0.01 ab	0.11±0.02 abc	1853.33±60.28 cde	364.33±8.62 b	1.73±0.03 a	0.42±0.03 abc	5.37±0.27 a
	90s	791.67±13.32 ab	0.03±0.01 ab	0.09±0.01 c	1847.67±60.28 cde	363.33±0.58 b	1.69±0.02 ab	0.43±0.01 abc	5.38±0.17 a
	120s	774.67±22.59 ab	0.03±0.00 ab	0.09±0.01 bc	1700.00±72.11 e	355.67±9.29 b	1.65±0.06 abc	0.44±0.03 ab	5.57±0.34 a
MW	30s	813.67±22.59 a	0.02±0.00 b	0.11±0.01 abc	2143.33±55.08 a	398.67±6.51 a	1.73±0.02 ab	0.41±0.01 bc	5.56±0.17 a
	60s	813.00±14.18 a	0.02±0.00 b	0.12±0.02 a	2010.00±36.06 abc	397.67±6.03 a	1.73±0.03 a	0.40±0.02 bc	5.51±0.04 a
	90s	796.67±12.34 ab	0.02±0.00 b	0.12±0.01 a	1973.33±41.63 bcd	404.00±9.85 a	1.67±0.03 ab	0.41±0.01 bc	5.60±0.08 a
	120s	758.67±13.05 b	0.02±0.00 b	0.13±0.01 a	1963.33±11.55 bcd	393.67±9.02 a	1.57±0.02 c	0.39±0.02 c	5.29±0.15 a

Mean within a columns followed by the same letter are not significantly different at $p \leq 0.05$

Element content of tested biostimulants (water extracts)

In addition to the chemical content of the biostimulants, their mineral composition is important. In the preparations produced, the content of individual elements varied. These differences resulted from both the use of AG or MW and the time the extract was subjected to the action of cold plasma or a mixture of plants by low-pressure microwave discharge. The study showed that the preparations analysed contained the most potassium (K). The highest content of this element was found in the biostimulant MW 30s 2143.33 $mg \cdot L^{-1}$ and the lowest in the biostimulant GA 12s 1700.00 $mg \cdot L^{-1}$ (Table 1). It has been shown that the K content decreases with increasing time of application of GA or MW. A similar relationship was observed for Ca. The use of GA or MW in the production of biostimulants had a negative effect on Cu and Zn content. The use of MW in the extrusion process significantly increased the Mg content in the biostimulants. The Na content of the tested biostimulators was at a similar level and did not differ significantly. Summarizing the elemental content of the prepared biostimulants, it can be concluded that the use of low-pressure microwave irradiation has a positive effect on their content in relation to the control extract and the cold plasma-treated biostimulants.

Conclusions

This study proved the potential of using gliding arc cold plasma and low-pressure microwave discharge technologies to produce water plant extracts with biostimulant potential. The experiments confirmed the validity of using low-pressure microwave discharge, which caused a significant increase in the total polyphenol content in the biostimulant. Correspondingly, biostimulants produced using low-pressure microwave discharges also significantly increased antioxidant activity. Moreover, low-pressure microwave discharges improve the extraction of elements such as Ca, K, and Fe. Cold plasma technology also improved the extractibility of polyphenols, but their increased values were not statistically significant. Finally, the plasma treatment technologies represent a promising approach to increase the extractibility of bioactive compounds from plant materials for biostimulant production or similar applications.

Conflicts of Interest: The authors declare no conflict of interest.

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TECHNOLOGIE PRODUKCJI BIOSTYMULATORÓW ROŚLINNYCH Z WYKORZYSTANIEM ZIMNEJ PLAZMY I NISKOCIŚNIENIOWYCH WYŁADOWAŃ MIKROFALOWYCH

Streszczenie. W ostatnich latach zdano sobie sprawę, że rolnictwo stało się jednym z sektorów gospodarki o ogromnym wpływie na środowisko. W związku z tym podjęto działania mające na celu zmniejszenie negatywnego wpływu produkcji rolnej na środowisko. Wykorzystanie biostymulatorów w rolnictwie, zwłaszcza pochodzenia roślinnego, jest częścią tego trendu. Uzyskanie odpowiedniej formulacji biostymulatorów wymaga jednak opracowania odpowiednich technologii ich produkcji. W związku z tym podjęto się zbadania możliwości wykorzystania zimnej plazmy (GA) i niskociśnieniowych wyładowań mikrofalowych (MW) do produkcji wodnych ekstraktów roślinnych o potencjale biostymulującym. Zaobserwowano wzrost całkowitej zawartości polifenoli i aktywności przeciwutleniającej, co wskazuje na wysoki potencjał wykorzystania niskociśnieniowych wyładowań mikrofalowych do produkcji skutecznych biostymulatorów roślinnych. Ponadto, niskociśnieniowe wyładowanie mikrofalowe poprawiło ekstrakcję pierwiastków takich jak Ca, K i Fe.

Słowa kluczowe: zimna plazma, niskociśnieniowe wyładowanie mikrofalowe, ekstrakcja, wodny ekstrakt roślinny, skrzyp polny, dzika róża, mydlnica lekarska