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PHYSICAL PROPERTIES OF PLANT EXTRACTS WITH BIOSTIMULANT POTENTIAL PRODUCED USING COLD PLASMA AND LOW-PRESSURE MICROWAVE DISCHARGE

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ABSTRACT

The changing climate, causing more frequent abiotic stresses, has generated interest in products that can mitigate the negative impacts of these changes. One such group of preparations includes plant extracts with biostimulant potential, produced using various methods and techniques, including cold plasma and low-pressure microwave discharge. However, there is limited research on how production methods and techniques affect the physical properties of biostimulants, such as density, viscosity, and surface tension. This study aimed to evaluate the impact of the manufacturing process on these properties. The biostimulant was produced via hot water extraction using either cold plasma or low-pressure microwave discharge. Results showed that neither method reduced the surface tension of the biostimulants, but they significantly influenced the viscosity and density. Given these changes in physical properties, further research on droplet spectrum and plant coverage during application is necessary to determine the optimal technical parameters for effective spraying.

Introduction

The development of crop cultivation technologies aims to increase yields and improve food quality, driven by global population growth and shifting dietary preferences. Food security, especially amid rising geopolitical tensions, stands as one of the 21st century's crucial issues. Addressing food security challenges will require doubling plant production over the next 25–30 years, especially as initiatives to reduce animal production are pursued due to its environmental impact. Additionally, limited natural resources and the environmental damage caused by traditional agricultural practices have become pressing concerns. In response, new technological tools focused on sustainable agroecosystem development have emerged (Le Mire et al., 2016). Studies have shown that plant production methods such as those improving agricultural technology in areas like cultivation, fertilization, and irrigation may not fully exploit crop potential, particularly under climate related stresses and environmental threats. Furthermore, the increasing acceptance of sustainable and organic farming, in response to the rising food demand, opens avenues for new technological solutions that deliver high quality yields while minimizing the use of pesticides and mineral fertilizers, which harm ecosystems and human health. To mitigate the damage caused by abiotic and biotic stressors, plant production practices must incorporate measures to counteract these negative influences (Kocira et al., 2015; Posmyk and Szafrańska, 2016).

Gawrońska et al. (2011) noted that crop yield and quality depend both on the ability to counteract adverse environmental conditions and on the rapid regenerative capacity of plants. Biostimulants present a promising solution, aiding plant recovery from both biotic and abiotic stressors and enhancing yield (Kozak et al., 2016; Michałek et al., 2018).

Biostimulants can be derived from biological materials (e.g., humic substances, seaweed extracts, various plant parts, live microbial cultures, protein hydrolysates, peptides, amino acids, chitin, and chitosan) or synthesized compounds (e.g., growth regulators, phenolic compounds, inorganic salts, beneficial nutrients like sodium, aluminum, selenium, cobalt, silicon, and titanium) (Calvo et al., 2014; du Jardin, 2015; Kocira et al., 2020; Przybysz et al., 2014). However, as synthetic products are increasingly avoided, plant extracts with biostimulators potential are receiving more attention. These allelopathic preparations are based on bioactive compounds like phenols and flavonoids (Singh et al., 2015; Szparaga et al., 2021), and their active ingredients act at various metabolic levels to improve nutrient absorption, transport, and utilization. However, the results can vary widely depending on factors such as plant species, variety, growth stage, soil and climate conditions, and the type, dosage, timing, and application method of the biostimulant (Ertani et al., 2016; Kocira et al., 2017).

The physical characteristics of plant biostimulants are crucial, as they influence both the choice of technical parameters for application and the degree of plant coverage achieved. Key physical parameters include density, viscosity, and surface tension, each of which significantly impacts the quality and effectiveness of applications. These properties largely depend on the raw materials and extraction agents used in biostimulant production. However, no studies have explored the effects of microwave-assisted water extraction or cold plasma activation on these physical properties. This study, therefore, evaluates the impact of the production process on the density, viscosity, and surface tension of the biostimulant.

Materials and Methods

Biostimulant Production

The plant extracts with biostimulant potential (biostimulants) were made from a mixture of the aerial parts of field horsetail (*Equisetum arvense* L.), dog rose fruit (*Rosa canina* L.) and soapwort roots (*Saponaria officinalis* L.) at a ratio of (w/w), respectively: 95.3%, 4.6%, 0.1%. A hot water extraction method was used to obtain the biostimulant by extracting (w/v) 500 mg of the above mixture in 10 ml of distilled water. Three types of biostimulators were made. The first biostimulant (Control Extract) was hot extraction of the above mixture for 30 minutes at 100°C.

The second type of biostimulant was produced in four variants. The above mixture was subjected to low-pressure (100 Pa) microwave discharge (MW) of 500 W for 30 (MW 30), 60 (MW 60), 90 (MW 90), 120 seconds (MW 120) and then hot extraction for 30 min at 100°C. A Plasonic AR-550-M was used to generate the low-pressure microwave discharge. The system was equipped with a 10 liter cylinder shaped vacuum chamber. The generator consisted of a mineral alumina tube, which was placed in the center of the microwave resonator. The MNG 1K-08 microwave source was provided by Radan Inc, Czech Republic. The dried herb samples were placed in a cylindrical stainless steel container with a diameter of 25 cm and a height of 15 cm. The container was placed at the bottom of the vacuum chamber.

The third type of biostimulant was also made in 4 variants by generating the biostimulator by hot extraction of the above mixture for 30 min at 100°C and then, after cooling to 20°C, subjecting it to a Gliding Arc (GA) plasma discharge of 800 W for 30 (GE 30), 60 (GE 60), 90 (GE 90), 120 seconds (GE 120). The plasma discharge generation device consisted of a high-voltage discharge generation source GVN1k-2011 from Radan Inc, Czech Republic. The main part of the source was a high-voltage transformer Resinblock 2000, which generated electrical voltage, which was then applied to the electrodes in the plasma head. Integrated with the source was Omega's FL-2008 rotameter, which made it possible to regulate the flow of working gas in the range of 0-100 SCFH (Standart Cubic Feet per Hour). The Gliding Arc plasma discharge was generated in the plasma head. The source of the working gas was an Orlik, Czech Republic air compressor with a minimum pressure of 600 kPa. The herbal extract in the vessel was placed under the plasma head at a distance of 8 cm between the head and the water surface. The working gas flow rate was maintained at 30 SCFH.

All biostimulants were centrifuged at 4250×g for 5 min and filtered (Whatman quality filter paper, Grade 1)

Density Measurement of Biostimulants

The density of the produced biostimulants was determined using the gravimetric-volumetric method with a pycnometer. A calibrated pycnometer with a volume of 100 cm^3 and an accuracy of 0.001 cm^3, as well as a laboratory balance (Radwag, WRC 2000, Radom, Poland) were used for this purpose. The density (ρ_b) was calculated according to the formula:

$$\rho_b = \frac{m_b}{V} \quad (1)$$

where:

ρ_b – density of the biostimulant, $(g \cdot cm^{-3})$

m_b – mass of the biostimulant, (g)
V – volume of the biostimulant, (cm^3)

Surface Tension Measurement

Surface tension was measured using the Drop Shape Analyzer DSA30 (KRÜSS GmbH, Hamburg, Germany) with an automated measurement system based on the pendant drop method. Prior to measurements, essential input data, such as the dispensing needle diameter (outer diameter of 1.828 mm) and the density of the analyzed biostimulants, were entered into the device's control software. To ensure accuracy, the device manufacturer recommends using the largest possible drop volume that can remain suspended on the needle.

The automatic liquid dosing module of the goniometer allowed for precise application of measurement drops. For the Control Water (water that was used to prepare the extract) and Control Extract samples, 28 µl drops were used, while 12 µl drops were dispensed for the MW (microwave-treated) and GE (Gliding Arc plasma-treated) variants: MW 30, MW 60, MW 90, MW 120, GE 30, GE 60, GE 90, and GE 120.

The automated system analyzed the drop shape from captured images and calculated surface tension using the Young-Laplace equation. Each biostimulant and control sample was measured 30 times at a controlled temperature of 23°C, with results expressed in mN m^{-1}.

Method for Testing the Viscosity of Biostimulants

The viscosity of liquids was measured using a viscometer (Fungilab, Premium series, Barcelona, Spain) based on spindle torque measurement. An L1 spindle was used for this study. During viscosity measurement, the spindle was immersed in a vessel containing the selected liquid. An essential factor in the measurement was the volume of liquid in the vessel, as the rotating spindle needed to be submerged to a specific mark on the spindle shaft. For these tests, the vessel contained 400 ml of liquid. The spindle's rotation speed was consistent for all measurements at 250 RPM. Viscosity measurement for each liquid sample was conducted three times, and the average value was calculated. Each viscosity measurement lasted 15 seconds. Prior to each measurement, the liquid was stabilized.

Results and Discussion

The biostimulant produced using MW (microwave treatment) for 120 seconds exhibited the highest density compared to other biostimulants (Fig. 1). Other biostimulants produced with MW or GE (gelation treatment) had higher density than the biostimulant that was not subjected to MW or GE. Liquid density can impact the quality of agricultural spraying by affecting factors such as droplet size, spray range, and drift. The density of biostimulants influences liquid dispersion as it exits the spray nozzle, thereby affecting droplet size distribution. Higher density liquids tend to form larger droplets due to increased inertia, which may reduce drift but could impact uniform coverage (Spanoghe et al., 2007; Makhnenko et al., 2021; Makhnenko et al., 2024). Denser liquids, depending on the nozzles used, produce larger droplets that are less prone to drift. This is because larger droplets have higher terminal velocities, which limits their susceptibility to drift (Makhnenko et al., 2021; Nuyttens et al., 2009).

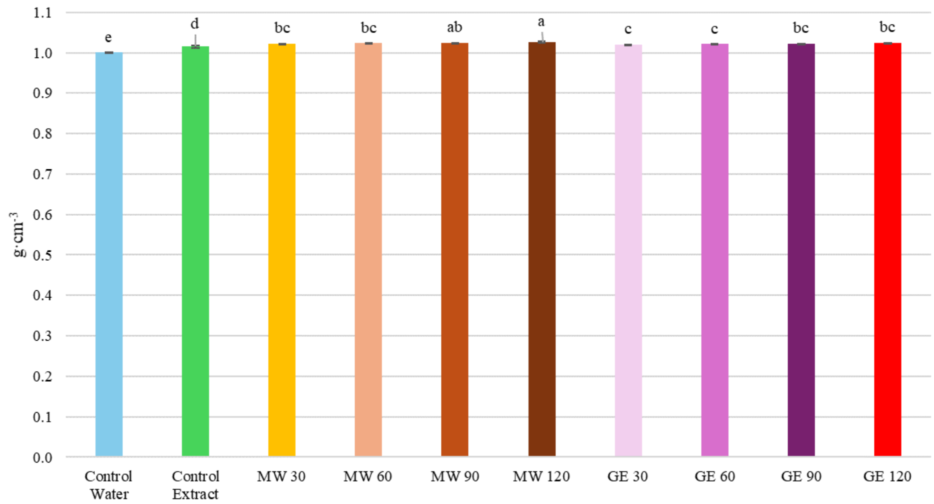

Figure 1. Density of biostimulants and water (mean + standard deviation). Values not sharing a common letter indicate significant difference at $p \leq 0.05$.

The produced biostimulants exhibited significantly lower surface tension compared to the surface tension of the water used in their production (Control Water) (Fig. 2). This reduction was due to the use of soapwort roots in the production process, which contain high amounts of saponins (Jo et al., 2024). Using low-pressure microwave discharge (MW) for 30 seconds (MW 30) or 60 seconds (MW 60) in the extraction process achieved the lowest surface tension, close to levels typically reached with adjuvants. It was observed that as the exposure time of the mixture used to produce the biostimulants increased, the surface tension values slightly rose. A similar trend occurred when the biostimulants were subjected to cold plasma (GE) (Fig. 2).

Lowering surface tension is crucial for the quality of biostimulant spraying and other agricultural applications. It leads to smaller droplet size and volume, improving the coverage of sprayed plants (Gong et al., 2022). However, smaller droplets are more prone to drift during application, emphasizing the importance of using suitable agricultural sprayers (Krawczuk et al., 2023; Krawczuk et al., 2021).

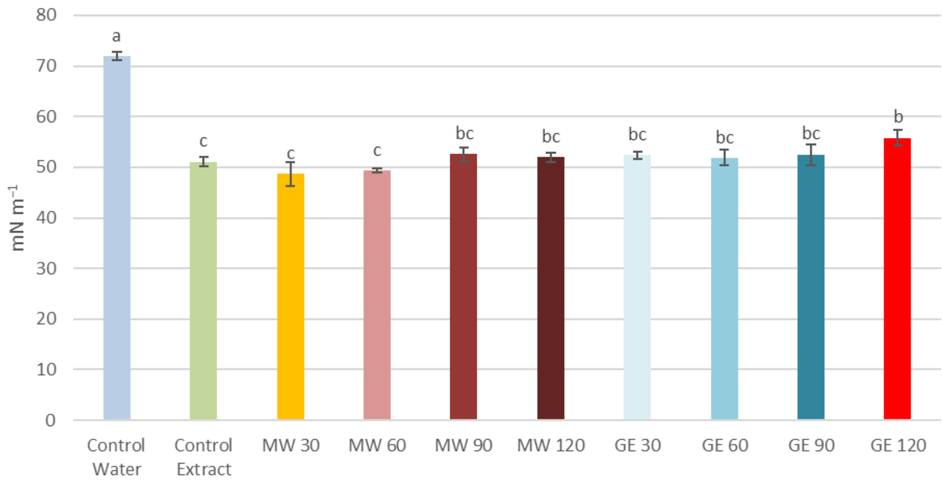

Figure 2. Surface tension of biostimulants and water (mean + standard deviation). Values not sharing a common letter indicate significant difference at $p \leq 0.05$.

The viscosity analysis of the produced biostimulants showed that increasing the treatment time of the mixture with low-pressure microwave discharge (MW) resulted in greater fluidity. The highest viscosity value was obtained at 120 seconds (MW 120) (Fig. 3). Treating the biostimulants with cold plasma (GE) also increased viscosity, though to a lesser extent than MW. The lowest viscosity was observed in the water used for biostimulant production and in the control biostimulant (Control Extract), which had not undergone MW or GE treatment. All tested biostimulants treated with MW or GE had significantly higher viscosity compared to the water used in their production and the untreated biostimulant.

Increased viscosity can lead to the formation of larger droplets during plant spraying (Gaillard et al., 2022). Higher viscosity in liquids can reduce wind-induced droplet drift and improve liquid retention on leaves, which may enhance the effectiveness of agrochemical applications (Zhang et al., 2021). Conversely, lower viscosity may increase the risk of droplet drift, reducing the effectiveness of agrochemical or biostimulant applications.

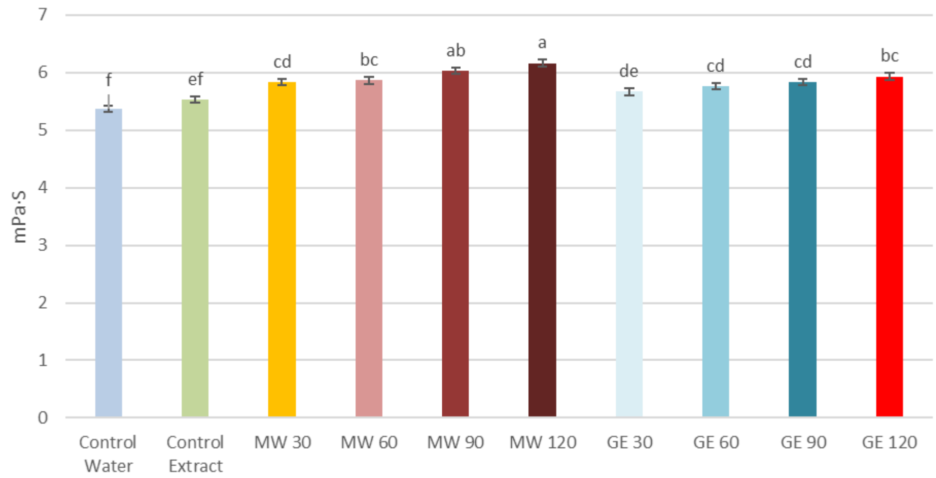

Figure 3. Viscosity of biostimulants and water (mean + standard deviation). Values not sharing a common letter indicate significant difference at p ≤ 0.05.

Summary

The conducted studies demonstrated a positive effect of using cold plasma (GE) and low-pressure microwave discharge (MW) in the production of plant-based biostimulants. Including soapwort root in the plant mixture significantly reduced the surface tension of the produced biostimulants. Although the use of cold plasma or low-pressure microwave discharge in production did not decrease surface tension, it notably affected viscosity. Application of MW or GE significantly increased the density of the biostimulants produced. Given these changes in physical properties, such as viscosity and density resulting from cold plasma or low-pressure microwave discharge, further research is necessary to examine droplet spectrum and plant coverage during application. This will aid in determining optimal technical parameters for effective spraying.

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

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WŁAŚCIWOŚCI FIZYCZNE EKSTRAKTÓW ROŚLINNYCH O POTENCJALE BIOSTYMULUJĄCYCH WYTWORZONYCH Z WYKORZYSTANIEM ZIMNEJ PLAZMY I NISKOCIŚNIENIOWEGO WYŁADOWANIA MIKROFALOWEGO

Streszczenie. Zmieniający się klimat powodujący coraz częściej występowanie stresów abiotycznych wpłyną na zwiększenie zainteresowania preparatami mogącymi niwelować negatywne skutki tych zmian. Jednymi z takich preparatów są ekstrakty roślinne o potencjale biostymulującym wytwarzane rożnymi metodami i technikami z wykorzystaniem m.in. zimnej plazmy czy niskociśnieniowego wyładowania mikrofalowego. Jest jednak niewiele doniesień dotyczących wpływu metody czy techniki wytwarzania biostymulatorów na ich właściwości fizyczne takie jak gęstość, lepkość i napięcie powierzchniowe. Dlatego też w pracy podjęto się oceny wpływu procesu wytwarzania biostymulatora na ww. cechy. Biostymulator wytwarzano metodą ekstrakcji wodnej na gorąco z zastosowaniem zimnej plazmy lub niskociśnieniowego wyładowania mikrofalowego. Badania wykazały, że zastosowanie w procesie wytwarzania zimnej plazmy lub niskociśnieniowego wyładowania mikrofalowego nie wpłynęło na zmniejszenie napięcia powierzchniowego biostymulatorów, ale wpłynęło istotnie na lepkość oraz gęstość wytwarzanych biostymulatorów. W związku ze zmianą właściwości fizycznych takich jak lepkość i gęstość w wyniku zastosowania zimnej plazmy lub niskociśnieniowego wyładowania mikrofalowego należy przeprowadzić dalsze badania dotyczące spektrum kropel oraz stopnia pokrycia roślin podczas zabiegu w celu doboru odpowiednich parametrów technicznych warunkujących jak najlepsze wykonanie oprysku.

Słowa kluczowe: biostymulator, ekstrakcja, skrzyp polny, lepkość, napięcie powierzchniowe, gęstość