

Article

Bioactive Compounds Extraction Using a Hybrid Ultrasound and High-Pressure Technology for Sustainable Farming Systems

Florin Nenciu ¹, Viorel Fatu ^{2,*}, Vlad Arsenoaia ^{3,*}, Catalin Persu ¹, Iulian Voicea ¹, Nicolae-Valentin Vladut ¹, Mihai Gabriel Matache ¹, Iuliana Gageanu ¹, Eugen Marin ¹, Sorin-Stefan Biris ⁴ and Nicoleta Ungureanu ^{4,*}

- ¹ Testing Department, National Institute of Research—Development for Machines and Installations Designed for Agriculture and Food Industry—INMA Bucharest, 013811 Bucharest, Romania; florin.nenciu@inma.ro (F.N.); persu@inma.ro (C.P.); voicea@inma.ro (I.V.); vladut@inma.ro (N.-V.V.); matache@inma.ro (M.G.M.); iuliadml@yahoo.com (I.G.); marineu61@yahoo.com (E.M.)
- ² Ecotoxicology and Bioresource Laboratory, Research and Development Institute for Plant Protection, 013813 Bucharest, Romania
- ³ Department of Pedotechnics, Faculty of Agriculture, Ion Ionescu de la Brad University of Agricultural Sciences and Veterinary Medicine, 700490 Iasi, Romania
- ⁴ Department of Biotechnical Systems, Faculty of Biotechnical Systems Engineering, University Politehnica of Bucharest, 006042 Bucharest, Romania; sorin.biris@upb.ro
- * Correspondence: viorel.fatu@icdpp.ro (V.F.); vnarsenoaia@uaiasi.ro (V.A.); nicoleta.ungureanu@upb.ro (N.U.)

Abstract: In the context of agricultural soil degradation caused by the extensive use of chemical amendments, ecological alternatives with minimal detrimental impact on ecosystems are gaining popularity. Recent advancements in processing technologies have improved the quality and extraction efficiency of bioactive compounds, particularly when multiple conventional or innovative techniques are being used to potentially overcome the most common limitations. This paper proposes the development and testing of a hybrid technology design that employs two extraction techniques, namely ultrasound and high pressure, that can be used either separately or in tandem. An initial assessment of the prototype potential for isolating the desired compounds was made, by testing three various working regimens for the processing of a mixture of onion, pea, and soybean. By incorporating the bioactive compounds produced during the experimental phase in the seedling transplantation holes, we were able to test the potential of stimulating the development rate of vegetables and reducing the attack of pests. The extracts obtained using the hybrid technology showed positive results when used to reduce pest attacks (decreasing average attack frequency by 7%), however had negative effects when used to promote biostimulation, when acted as an inhibitor. The hybrid extraction approach improved the mass transfer into solvent by 14% when compared to high-pressure processing and by 7% when compared to sonication.

Keywords: organic and sustainable agriculture; hybrid extraction; plant stimulation; pests attack



Citation: Nenciu, F.; Fatu, V.; Arsenoaia, V.; Persu, C.; Voicea, I.; Vladut, N.-V.; Matache, M.G.; Gageanu, I.; Marin, E.; Biris, S.-S.; et al. Bioactive Compounds Extraction Using a Hybrid Ultrasound and High-Pressure Technology for Sustainable Farming Systems. *Agriculture* **2023**, *13*, 899. <https://doi.org/10.3390/agriculture13040899>

Academic Editor: Alessio Cappelli

Received: 19 March 2023

Revised: 13 April 2023

Accepted: 17 April 2023

Published: 19 April 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Many communities around the world relied on nature to heal their crops; however, due to the development of complex and highly reliable synthetic chemicals, natural products have lost ground [1]. The widespread use of high-remanence chemical compounds in agriculture has lately become controversial, due to their potential toxicity and environmental pollution. Bioactive substances are potential substitutes for synthetic pesticides because they are easily accessible, quickly biodegradable, present a broad activity, and are not harmful to people or nontargeted species [2].

These ecological compounds typically refer to various secondary metabolites obtained from plants, fungi, microbes, and animals. A variety of new uses have been made possible by the recent research of bioactive products and technologies, and the new formulas

have additionally decreased manufacturing time and expenses. Ecofriendly novel extraction fluxes, based on unconventional methods of isolating bioactive compounds assisted by microwaves, pulsed electric fields, enzymes, ultrasound, or pressure, are replacing conventional extraction methods [3].

Recent developments in chemical characterization and the identification of symbiotic relationships at the microbiological level have supported the new approaches oriented towards organic agriculture [4]. Emerging technologies are expected to increase the economic feasibility of extractions, particularly from organic wastes, promoting a sustainable circular economy [5,6]. The emerging extraction techniques aim to meet the green process concept requirements [7], which include avoiding or minimizing the use of hazardous organic solvents, lowering extraction temperatures and energy use, increasing mass transfer and extraction yields, and maintaining the phytocomplex integrity, particularly when there are thermosensitive components.

The use of hybrid extraction is relatively new, but it has shown great potential to improve the selective separation of valuable compounds with applications in a variety of industries. A variety of bioactive compounds have been successfully extracted, including caffeine [8], capsaicinoids [9,10], apigenin [11], phenolics [12,13], and piceatannol [14]. The main classes of substances that prove bioactive effects are considered by many authors [15] to be polyphenols, terpenoids, polysaccharides, capsaicinoids, triterpenes and phytosterols, carotenoids and tocopherols, alkaloids, saponins, and glucosinolates. The extraction method may vary significantly even for the same technology used, because bioactive compounds can be produced from a variety of primary sources, such as agro-industrial residues, fungi, animals, and bacteria [16–19].

When developing or optimizing various extraction strategies, the elements usually considered are the source matrix, the relative solubility, the structure, and the chemical properties of the bioactive compound and the solvents used. In addition, a series of process parameters, such as temperature, pressure, pH, extraction period, and extraction selectivity, are also considered [20]. Conventional extraction techniques such as maceration and decoction present some important disadvantages, such as lengthy extraction periods, the need for large amounts of solvent, and lower efficiencies. When the primary solvent being used needs to have a high level of purity or is harmful during processing, difficulties become even more significant [21,22].

The most researched unconventional extraction technologies identified in the literature include ultrasound-assisted extraction (UAE), pressurized liquid extraction (PLE), supercritical fluid extraction (SCFE), microwave-assisted extraction (MAE), and pulsed-electric-field-assisted extraction (PEFAE) [23–25].

Ultrasound-assisted extraction (UAE) is one of the most effective nonthermal extraction techniques in terms of bioactive compounds' separation potential [6]. The ultrasonic waves are used to create cavitation, collisions, macroturbulence, and disturbance of the solid particulates, the pressure exceeding the attractive force joining the molecules together. This produces pores, which speed up mass transfer and allow the solvent to enter the material more easily [26]. It is widely acknowledged that using ultrasound in extraction methods improves extraction output and/or minimizes solvent usage. Furthermore, it was demonstrated that the composition of the collected bioactive compounds is unaffected by the use of ultrasound [27]. The separation of polyphenols, carotenoids, fragrances, and carbohydrates from plant matrices is effectively accomplished using ultrasound technology. Depending on the extraction requirements, the process can be configured differently and used with a wide range of solvents, including water, ethanol, methanol, or acetone. This method's potential to be applied at lower temperatures while still maintaining the stability of thermosensitive compounds is one of its primary benefits [28]. For optimal extraction, it is necessary to precisely regulate the functioning factors, such as frequency, power, pulse duration, temperature, time, solvent type, and liquid–solid ratio. Researchers have examined both the individual and combined impacts of these factors when attempting to extract

bioactive substances from fruits and vegetables, in order to maximize the production of one or more substances of interest [29].

The pressurized liquid extraction (PLE) method makes use of the mass transfer properties at increased pressures and temperatures, after immersion in a solvent. The temperature may play a significant role in the process, modifying the chemical characteristics of the solvent and improving extraction selectivity [30]. The desired component consequently desorbs from the sample and incorporates into the extraction solution. The setup for PLE could be static and dynamic or a combination of the two. Using the dynamic system, the fluid is constantly circulated in aliquots at a flow rate of 0.5 to 2.5 mL/min, while using the static method the solution is collected every 5 to 10 min [31].

The process of creating novel extraction methods takes into account a number of factors, including the choice of solvent, the impact of temperature and pH, specificity, the molecular structure of the product to be extracted, and process viability. By using optimization techniques specific to the procedure, one can reach at an appropriate set of parameters [32,33].

Further research into efficient and sustainable ways to filter, extract, characterize, process, and market high-quality bioactive substances is required in consideration of this increase in demand [34–36]. According to some studies, traditional chromatographic assessments do not accurately depict the efficacy of ecological crop management techniques due to the complex chemical and biological compounds in the soil and the synergies that are created between them [37–40].

The transition from laboratory to industrial-scale extract production, using a hybrid high-pressure and ultrasound system remains a difficult task. The goal of creating low-cost and high-concentrated extracts might present several technological challenges. Large-scale ultrasound-associated extraction is difficult to obtain, as a result of the demand for high ultrasound power and longer ultrasound irradiation periods. Since there are gauge restrictions on the ultrasound generator, applying the required power may be difficult or unaffordable. Among the potential ways to reduce expenses may be introducing new reactor designs, application of pulses, new types of extraction materials, or simultaneous operation of smaller reactors [7]. Given that the coupling of high-pressure systems with low-frequency ultrasound on the laboratory scale has significantly increased the extraction yield of the target compounds, the technique has a bright future for higher aggregated value items, such as pure chemicals or expensive raw materials.

The objective of the study is to develop an extractive technology that uses high-pressure processing and sonication and has a high degree of operational customization. Several field tests were conducted in order to determine whether the bioactive substances extracted using the equipment from various plant materials may serve as a biostimulator, repellent, or insecticide for vegetable crops. The research initial phase, which was documented in the current paper, involved processing three functional vegetables, onion, pea, and soybean, which are conventionally known to have the desired qualities. The biostimulation and antipest qualities of the produced compounds were assessed through field testing.

2. Materials and Methods

2.1. Research Approach and Expected Output

In order to produce efficient ecological and economic bioactive compounds, advanced treatment technologies have to be identified and tested in real conditions for an adequate configuration of the process parameters. The obtained compounds and their effect have to be tested and validated for various vegetable crops, in order to identify which recipe is the best for each species, for different development conditions. The key phases of the strategy development for producing ecological bioactive compounds that will help replace synthetic chemicals with harmful environmental effects are presented in Figure 1.

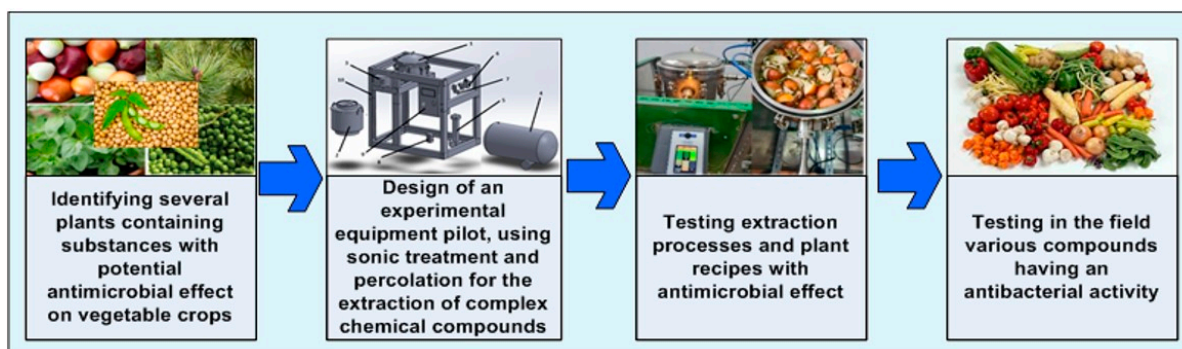


Figure 1. Phases in technology development for producing bioactive compounds applied to sustainable agriculture.

The obtained extracts were used for a series of experiments in the laboratory and in the field to evaluate the effect produced for three types of vegetables, namely cucumber (Ekol F1), bell pepper (Regal), and tomato (Florina 44). The seedling transplantation holes were treated with the bioproducts produced through the three extraction methods, and the results were evaluated comparatively according to several indicators, such as the number of leaves, the frequency of the attack, and the chlorophyll content index. According to specific research, plants such as onion (*Allium cepa* L.) contribute to the composition of the extract with antimicrobial and antioxidant activity [41,42], while peas (*Pisum sativum* L.) contribute to insecticidal [43] and antimicrobial action [44], and soy (*Glycine max* L.) as a product stabilizer and as a source of neurotoxins for insects [45].

2.2. Description of the New Pilot Equipment Designed for the Extraction of Bioactive Elements from Plants, Using Percolation and Sonication

The proposed bioactive extraction system has a new design that integrates two simultaneous processing operations: percolation and extraction in the ultrasound field. The operational parameters of the process are controlled using the programmable logic controller (PLC) as well as the ultrasound generator interface, allowing the extraction process to be managed sequentially or autonomously according to the specific needs. The percolation process involves creating a hydrostatic pressure in the extraction vessel, by means of a hydro-pneumatic cylinder, that receives compressed air from a 2.2 kW compressor. The ultrasonic extraction process involves the generation of an ultrasonic field with an amplitude of up to 200 μm , using a specialized equipment.

The functional diagram defining the operating principle designed to extract a wide range of concentrated bioactive compounds is presented in Figure 2.

As described in Figure 2, the operation of a concentrated bioactive extractor from vegetables and aromatic plants using ultrasounds and percolation (sequential or simultaneous extraction processes) is composed of a vessel for solvent storage (1), two pumps (2) and (4), an extraction vessel (3), ultrasonic generator (5) that produces a high and low amplitude sonic field, the solenoid valves 8, 9, 13, and 14 that actuate the hydro-pneumatic cylinder (10), the compressed air group (12), the pneumatic distributor (11) equipped with a pressure regulator, a pressure sensor (6), and the temperature sensor (7). The sensors are used to monitor the extraction process, while the input and operating data are displayed on a touchscreen interface.

The experimental model was designed in 3D using SOLIDWORKS software (3 D CAD 2016) and was constructed and tested within INMA Bucharest Institute.

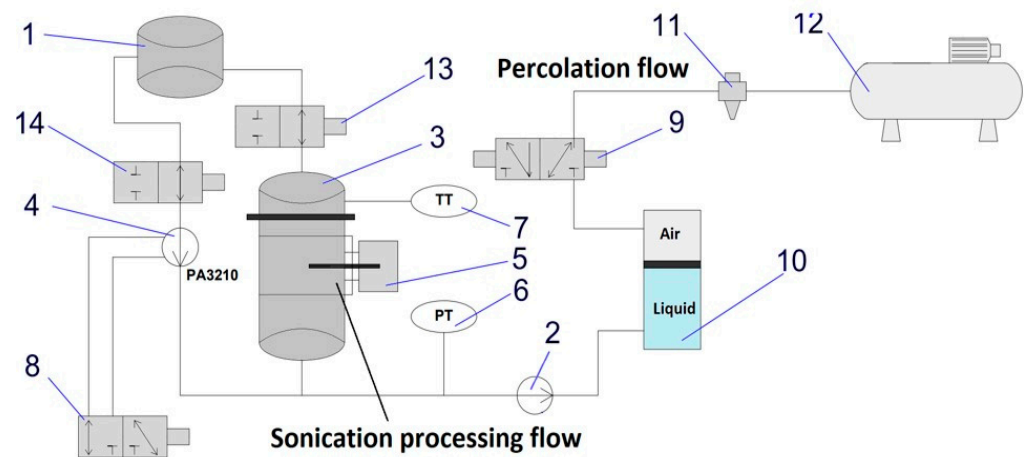


Figure 2. Functional schematic of a technology designed to produce bioactive compounds for agricultural use: (1) vessel for solvent storage, (2) first pump, (3) extraction vessel, (4) second pump, (5) ultrasonic generator, (6) pressure sensor (7) temperature sensor, (8, 9, 13, 14) solenoid valves, (10) hydro-pneumatic cylinder, (11) pneumatic distributor.

The main dimensional characteristics of the experimental model are the length, 1150 mm; width, 1050; height, 1500 mm; total mass, 140 kg; maximum extractive pressure, 10 bar; and useful volume processed on each batch, 20 L. Figure 3 depicts a simplified schematic and the experimental model, highlighting the key components. The technical details of each component are described in more detail in the following pictures.

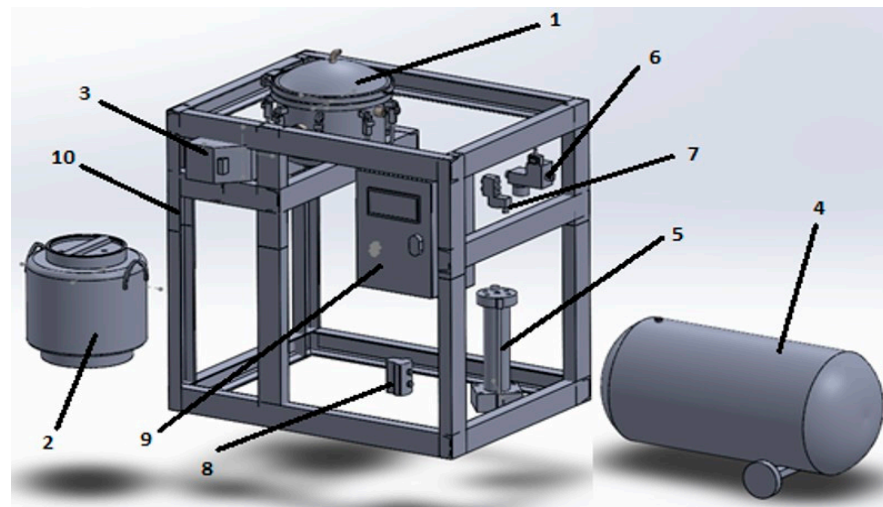


Figure 3. Simplified schematic and experimental model for the bioactive-compounds extraction equipment: 1, extraction vessel; 2, expansion and solvent storage tank; 3, ultrasonication system; 4, pressure generator; 5, hydro-pneumatic cylinder; 6, pressure regulator; 7, liquid filtration system; 8, liquid recirculation pump; 9, process monitoring and control system; 10, support structure.

All the main component parts of the technical equipment are mounted on a frame, made of u-type metal profiles, perforated with elongated holes— $35 \times 35 \times 1.25$ mm. The metallic frame was covered with 2 mm sheet metal, and laser cuts were made for the major component exits.

Figure 4a depicts the extraction vessel design, having a maximum capacity of 20 L. It is constructed of stainless steel and is made of three bodies that are connected together: upper cover (b), main body (c), and lower body (d), as well as two sieves for maintaining the processed material, the upper sieve (e) and the lower sieve (f).

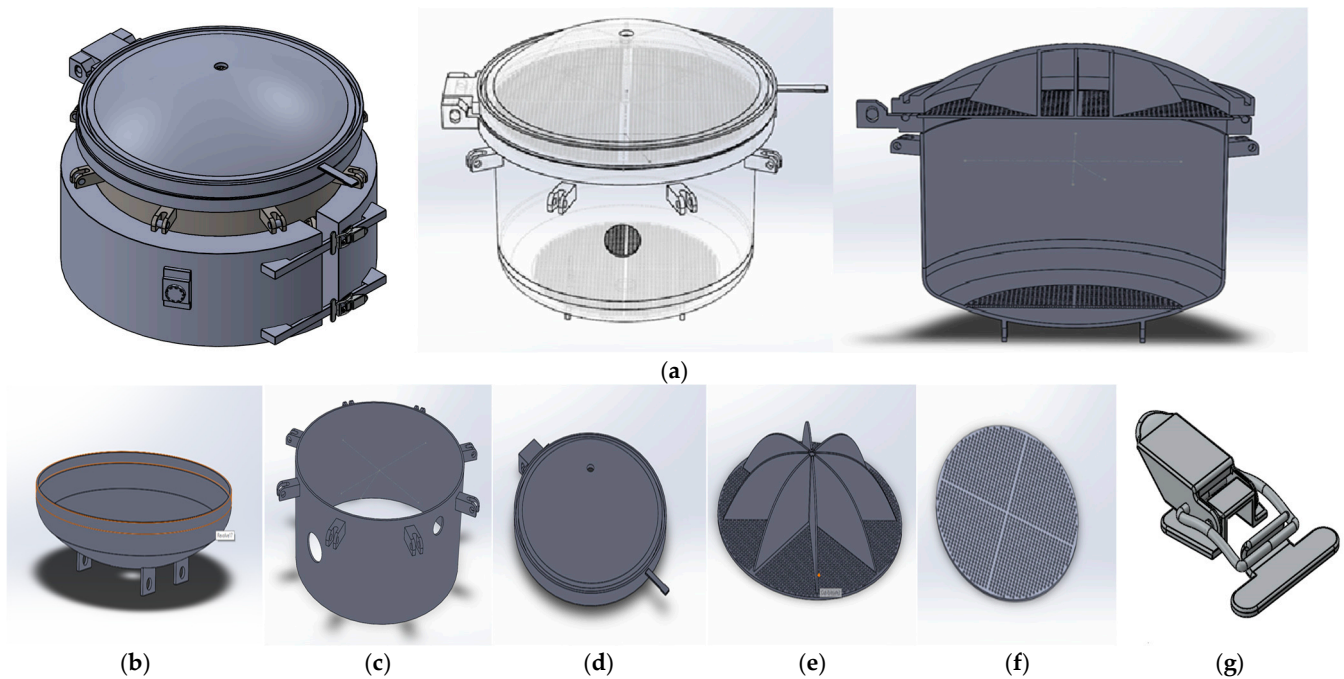


Figure 4. Extraction tank design, associated with the experimental pilot model: (a) overview of extraction vessel design, (b) upper cover, (c) main body, (d) lower body, (e) upper sieve, (f) lower sieve, and (g) locking clamp.

The bottom cover is welded to the main body and includes four mounting elements for secure attachment to the metal frame. The upper cover is fixed with a specific hinge-type mechanism. To ensure the tightness of the extraction vessel, it is designed with a sealing channel and a rubber seal at the junction of the two component parts. At the upper and lower ends of the vessel, as well as in the side wall, threaded holes are made for hydraulic connections. The maximum working pressure is 10 bar.

Figure 5 illustrates the most significant component parts of the pilot experimental model.

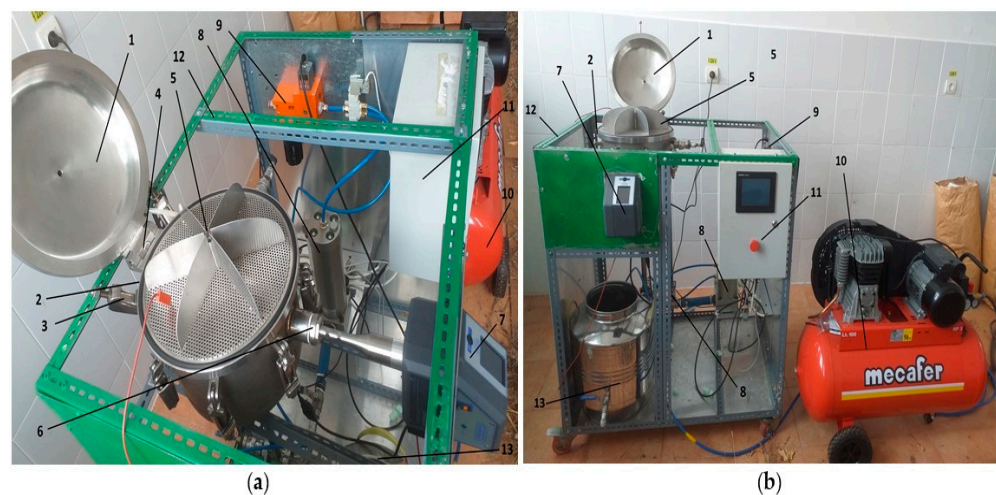


Figure 5. The final design of the pilot equipment used for the extraction of bioactive substances, using a hybrid ultrasound and high-pressure technology: (a) Experimental Extraction Pilot top view, (b) Experimental Extraction Pilot side view.

The main experimental pilot subassembly is the extraction vessel, which is provided with a high-pressure resistant cover (1), attached to the extractor body (2) by the locking

system (3) and the hinges (4). The extraction vessel contains two sieves that keep the processed material inside; however, in the case of finely grounded materials, an inner textile bag is additionally used. The upper sieve (5) is equipped with six stabilizing blades. The extraction vessel is provided with two openings for the recirculation of the solvent and a hole for the entrance of the sonication probe (6) of the ultrasonication equipment (7) (Hielscher UP400ST Ultrasonic Processor, Hielscher Ultrasonics, Teltow, Germany). The hydro-pneumatic cylinder (8) and the pressure regulator (9) have the role of maintaining a controlled pressure for the percolation phase. The compressed air generator (10) (Mecafer B3800B 200 L 10 bar, Mecafer, Valence, France) is used for providing the needed pressure for the equipment, while the programmable logic controller (11) has the purpose of controlling and monitoring processes. All subsystems are fixed on a metal frame (12), which forms the metal skeleton of the equipment, while the expansion vessel is made of stainless steel (13) and is equipped with a drain valve.

In order to circulate the solvent throughout the extraction process, a 30 L buffer tank (extraction tank) constructed of food-grade stainless steel with two holes in the upper part is used (Figure 6a). The vessel is also utilized to discharge the resulting extract. The expansion tank is made of DN300, stainless steel 304 L, with a thickness of 2 mm, $h = 465$ mm, and the inlet and outlet holes are 1.27 cm. Solvent recirculation processes during the extraction and discharge are carried out with a pump $h = 100$ mm, hole $\varnothing 15$ (Figure 6b). For the percolation process, the air pressure required for the operation is supplied from a 2.2 KW compressor, with an optimal output pressure of 10 bar (Figure 6c). The compressor's (Figure 6e) air supply is used to activate the hydro-pneumatic cylinder (Figure 6d). A distributor with a filter system and a pressure regulator is used to control the air volume.

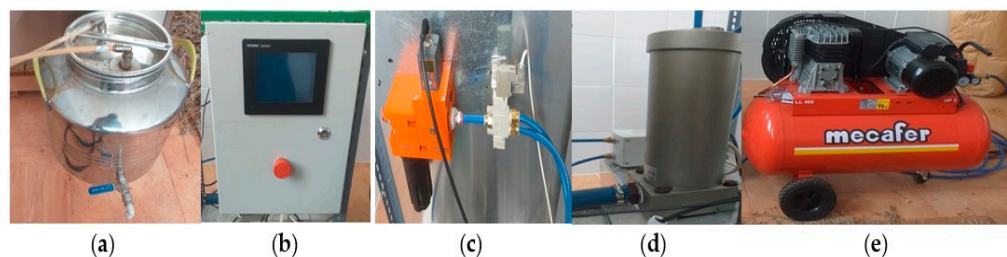


Figure 6. The main subassemblies of the experimental equipment for the extraction of bioactive compounds: (a) control panel, (b) recirculation pump, (c) recirculation pump, (d) hydro-pneumatic cylinder, (e) compressor.

The ultrasonication extraction is carried out with UP 400ST equipment (HIELSCHER, Bonn, Germany), with the following characteristics: working frequency 24 kHz, maximum amplitude 200 μm , maximum energy density 300 W/cm^2 , S2d22D sonotrode diameter 22 mm, power in water 180 W, and radiant surface 5.4 cm^2 . The ultrasonic subassembly was connected to the body of the extraction vessel using a bushing, which ensures tightness between the ultrasonic equipment's probe and the vessel, and a tightening screw.

The extraction process can be controlled simultaneously or separately for each individual process, using a programmable logic controller (PLC). The operations can therefore be customized for the percolation or sonication functioning, or the two processes can be carried out consecutively. The automation box consists of a 220 V/24 V switching voltage source, a programmable logic microcontroller ALPHA AL2-14MR-D, (Mitsubishi Electric, Tokyo, Japan) and a graphic interface (operating terminal) equipped with GOT 1000 touchscreen (Mitsubishi Electric, Tokyo, Japan).

2.3. Biofertilizer Production Technology Using Three Distinct Extraction Methods

The vegetable raw material was subject to the extraction process using three different methods: percolation, ultrasound treatment, and a hybrid processing method (using both percolation and sonication performed successively). Distilled water (8 L), yellow onion

(2.5 kg, sliced into 4 slices), green beans (1 kg), and dried soybeans (0.5 kg) were the ingredients used for the three experiments. The first phase of the methodology involves the preliminary preparation of the plant material subject to extraction, which includes the operation of weighing, cutting, washing, and mixing with the extraction solvent (in the presented case distilled water). The raw materials are added into a bag made of a special, very fine sieve to allow their expansion and compression during the process, then the upper sieve is placed in position, and the container is closed using the special safety locking system. Recipes that require ultrasonication involve an additional phase of connecting the UP400ST ultrasonic field generation system.

Figure 7 shows several images from the experimental work, using the experimental equipment.

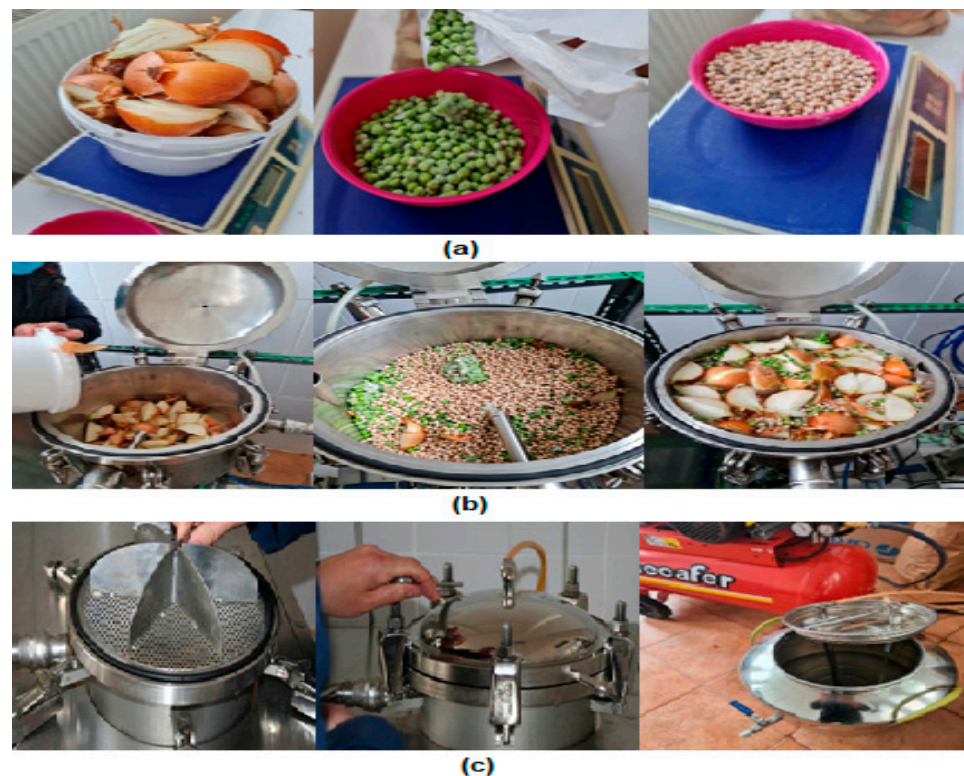


Figure 7. Description of the working method for the production of bioactive compounds: (a) weighing and shredding of the raw material, (b) adding the materials and the solvent to the extraction vessel, and (c) preparing the extraction and the expansion vessels.

2.3.1. Operation Mode No. 1: Extraction by High Pressure

The extraction process using high pressure had the following technical input data: extraction time, 7200 s; high extraction percolation pressure, 1.2–1.5 bar; low extraction percolation pressure, 0.2–0.6 bar; number of cycles at high pressure, 4 cycles; number of cycles at low pressure with quasi-dynamic pressure variation between 0.2 and 0.6 bar, 4 cycles; high-pressure cycle duration, 1200 s; low-pressure cycle duration, 600 s with a quasi-dynamic cycle duration of 60 s, a 60 s break before and after the high-pressure cycle and a 60 s break between low-pressure quasi-dynamic cycles; extraction temperature, 20–25 °C.

2.3.2. Operation Mode No. 2: Extraction Using the Ultrasound Process

The extraction operation using ultrasonication had the following technical input data: extraction time, 180 min; ultrasonic field amplitude, 0–46 μm ; low-amplitude duration of 0–15 μm , 600 s; duration of high amplitude of 20–40 μm , 600 s.

Figure 8 illustrates the workflow for the extraction equipment using the ultrasonication technique (integrating into the system an ultrasound generator).



Figure 8. The experimental operation for the ultrasonic extraction mode.

2.3.3. Operation Mode No. 3: Extraction Using Hybrid Process

In the hybrid processing, time was the single variable factor, and the other parameters remained similar as in the previously discussed cases. Therefore, the 180 min were split into 90 min intended for percolation and 90 min for sonication. Figure 9 shows the flowchart for the combined percolation–ultrasonic extraction method.

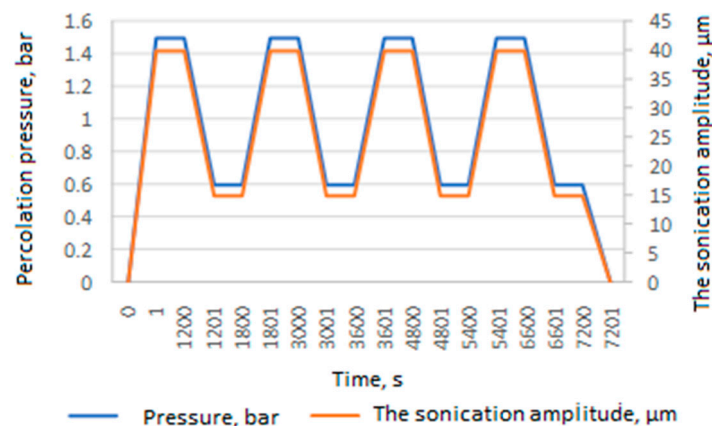


Figure 9. Diagram of the hybrid extraction process: percolation (compressed air)–ultrasonication (ultrasonic field generation).

Three extracts were produced as a result of the experimental activity, and they were examined for their potential as a biofertilizer and a bioinsecticide for three vegetable crops.

2.3.4. Testing the Bioactive Compounds for Vegetable Crops Established Outdoor

Plowing, soil fragmentation, irrigation, and fertilizer addition were the soil work-related tasks completed in the experiment's location, prior to the establishment of the crops. The seedlings were planted in soil openings, having 5 cm in circumference and 15 cm in depth, being positioned 1 m apart (both between rows and between plants) (Figure 10).

At the end of transplanting, each plant was irrigated with 1 L of water and then covered with a layer of dry soil to prevent evaporation. Ten days after transplanting, the first observations were made, considering the number of leaves on each plant, the intensity of the pest attack, and the chlorophyll content index. In the case of tomatoes, the chlorophyll content was not determined because the width of the leaf was smaller than the diameter of the evaluation window of the CCM 200 Plus chlorophyll meter (Opti-Science, Hudson, NY, USA).



Figure 10. Establishment of vegetable experimental cultures for evaluating the effectiveness of bioactive compounds.

Statistical analysis calculations were performed using GraphPad and Microsoft Excel 2014 software.

The selection of the plants used as sources of bioactive compound extraction was made after a review of the specialized literature and taking into account the experts' prior research and experiences. Table 1 lists a few research studies that had successful extractions from the same raw material and produced positive outcomes.

Table 1. The most important bioactive substances with a role in biostimulation, fertilization, and repellent against pests.

No.	Plant Species	Biological Activity	Compounds	Bibliographical References
1	<i>Allium cepa</i>	Antioxidant	Superoxide dismutase, catalase, glutathione peroxidase, peroxidase, glutathione reductase, glutathione S-transferase, glutathione, α -tocopherol, ascorbic acid, β -carotene	[41,42]
2	<i>Allium cepa</i>	Antimicrobial activity	Dipropyl disulphide, allicin	
3	<i>Allium cepa</i>	Insecticidal activity	Dimethyl disulphide	
4	<i>Pisum sativum</i>	Insecticidal activity	Protein PA1b	[43]
5	<i>Glycine max</i>	Insecticidal activity	Gm-TX	[44]
6	<i>Glycine max</i>	Extract stabilization	Lecithin	[45]

3. Results

The findings aimed at an evaluation of the experimental biofertilizer extraction equipment's capacity for producing viable components for the treatment of vegetable crops. At this phase, only the production of several solutions that could be tested in the field was pursued; no chemical characterization of the derived compounds was performed. After using the three operational methods (percolation, sonication, and mixed treatment), then the obtained solutions were tested for tomato, pepper, and cucumber crops.

The mass transfer from the raw material subjected to processing in the extraction solvent was 17% for the high-pressure processing, 24% for the ultrasonication process, and 31% for the combined percolation–ultrasonication process.

Bioactive Solutions Testing on Vegetable Crops Established in the Field

The three types of extracts have been used in 20%, where the solvent was the water obtained from the local irrigation wells. The evaluation was assessed according to the

number of leaves grown on each plant after 10 days from the treatment, normalizing according to the control sample. The stimulatory effect generated by the three solutions, measured as the plant growth rate stimulators, is depicted in Figure 11.

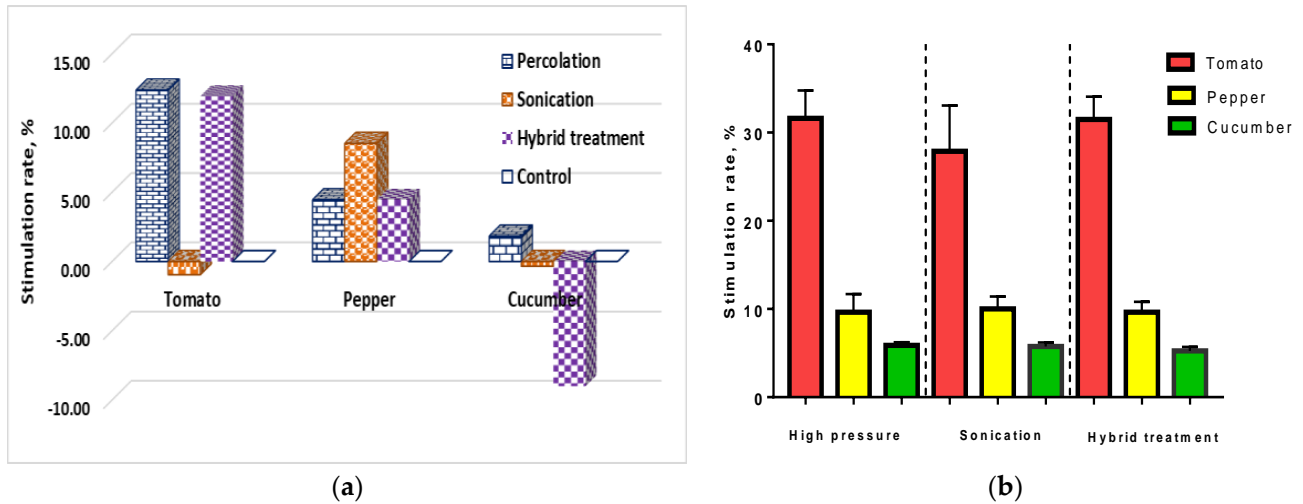


Figure 11. The influence of the obtained solutions on the tomato, pepper, and cucumber leguminous crops, in terms of seedling biostimulation: (a) stimulation rate measured according to the percentage of developed leaves (after 10 days) and (b) comparative evaluation of the extracts' impact on seedlings (median and standard deviation statistical processing).

The repellent or bioinsecticide effect for the three analyzed substances has been assessed by considering the number of attacked leaves and the attack frequency, within 10 days of establishing the culture. Figure 12 shows the number of attacked leaves, for each of the examined vegetable crops.

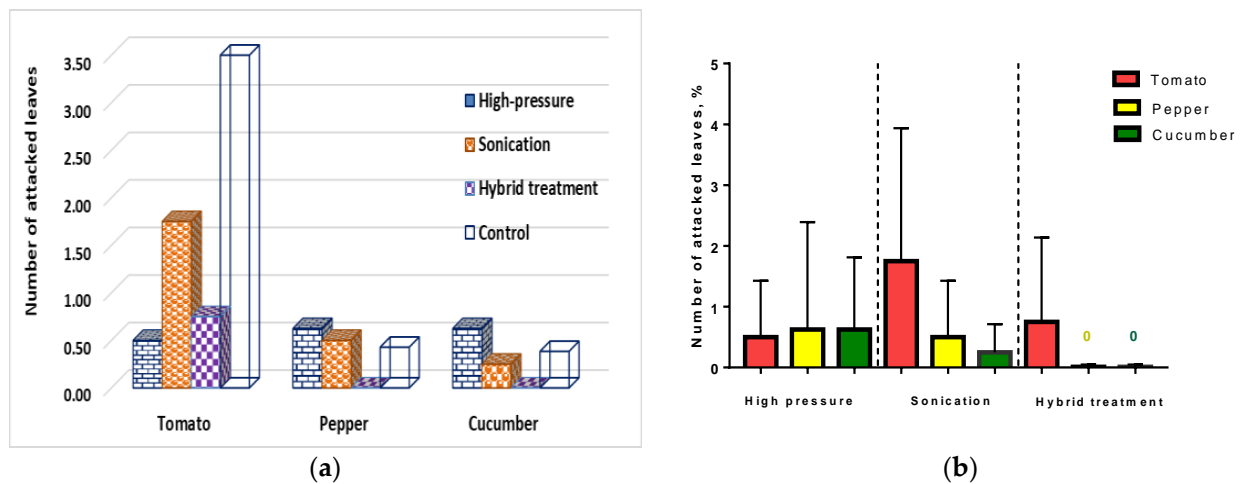


Figure 12. The influence of treatments on pest attack reduction: (a) rate of leaves attacked for tomato, pepper, and cucumber crops and (b) comparative evaluation of the extracts' impact on seedlings' resistance to pests (median and standard deviation statistical processing).

The frequency of pest attacks on vegetable leaves was also considered as a performance indicator of the obtained solutions. Figure 13 shows how often the seedlings were visited by pests.

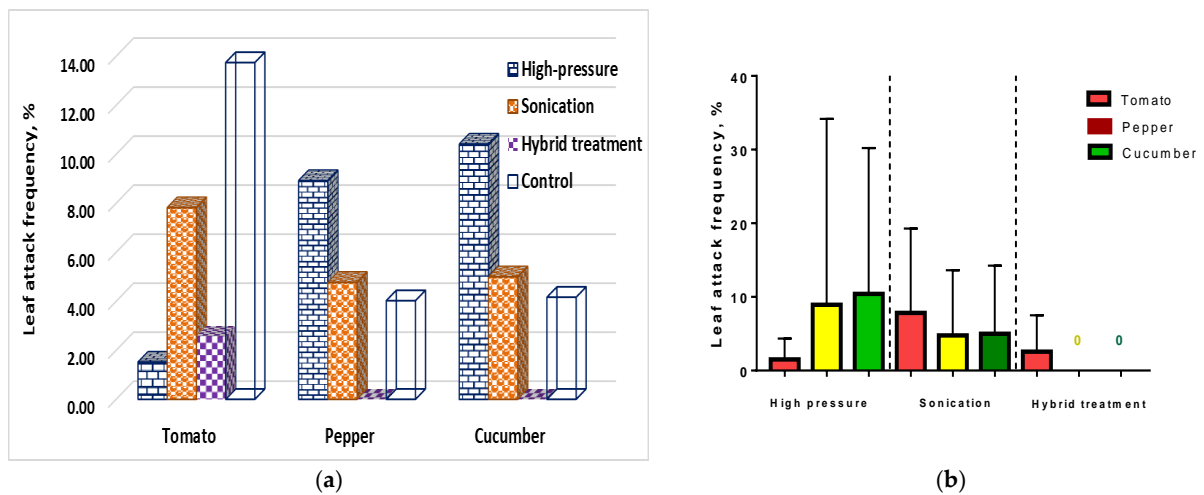


Figure 13. The influence of treatments on the pest attack reduction: (a) leaf attack frequency and (b) statistical evaluation.

The leaf chlorophyll content is a key indicator used to investigate leaf nutrient deficiencies and changes in chlorophyll. The chlorophyll content is influenced by fertilizer application (especially, nitrogen); therefore, the obtained solutions have an impact on this specific indicator, as can be seen in Figure 14.

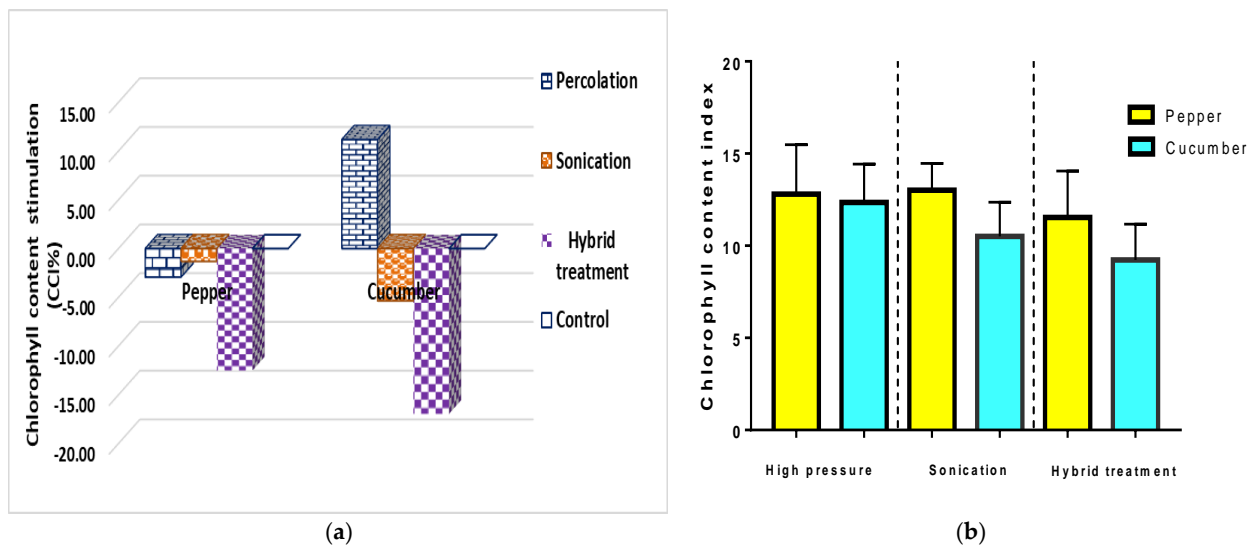


Figure 14. The influence of the obtained solutions as a biofertilizer, analyzed according to the chlorophyll content of the leaves: (a) chlorophyll content index and (b) statistical evaluation.

4. Discussion

4.1. Overview and Limitations of the Current Research

The central long-term objective is to develop an extraction technology and associate a suitable raw material in a way that allows for avoiding phytotoxic extraction solvents and very complicated extraction processes. After the development of the hybrid extraction equipment, it was intended to use water only as a solvent and to add certain synergistic compounds (such as soybean lecithin, which is amphiphilic and compatible with plant protection agents), to enhance the process.

This paper's primary goal was to provide an overview of the developed equipment design; however, at this stage does not include the characterization of the obtained compounds. The obtained solutions were used directly on cultures, where some indications

were obtained regarding extracts functionality, which will serve as a foundation for further investigations.

4.2. The Stimulation Rate Evaluation after 10 Days from the Application of the Extracts

The treatment of tomato roots with vegetable extracts obtained by percolation and hybrid treatment gave positive results, obtaining a 12% stimulation rate (compared to the control sample); however, the treatment with the extract obtained by sonication induced a decrease in the number of leaves. The pepper seedlings benefited from a 4–4.4% stimulation rate following the treatment with the extract obtained by percolation and hybrid extraction and by 8.5% in the case of extraction by sonication (compared to the control sample). Regarding cucumber seedlings, the treatment with vegetable extracts induced a modest increase in leaf growth of only 1.8% for the extract obtained by percolation and a negative effect in the case of the extracts made by sonication and hybrid extraction. Most likely, for the cucumber hybrid planted, an inhibitory effect was obtained by ultrasonication; therefore, only high-pressure percolation techniques will be considered for this plant. The statistical results (one-way ANOVA–Tukey’s multiple comparisons test) showed that there were small significant differences between the treatments, especially because the evaluation was carried out for a short period of time and a small number of measurements were made. The analysis of the positive and negative influences (six positives vs. three negatives) led, however, to the conclusion that this mixture of plant extracts offers a biostimulatory potential.

4.3. Evaluation of the Attack Frequency of Diseases and Pests after 10 Days from the Treatment with Plant Extracts

Plant extracts had a positive influence on the decrease in the frequency of attack on tomato leaves by *Septoria lycopersici*. The best results were obtained in the case of treating the seedlings with the extract made by the percolation method. The high values of the coefficient of variation from the statistical analysis indicate that the attack of *septoriosis* was sporadic, contrary to the meteorological conditions characterized by a high level of precipitation, which indicates the possibility of plant infection following the injury produced by insects. The attack on pepper leaves was uneven and caused by pests from the *limacidae* family, reaching frequency values of almost 9% in plants treated with the extract by percolation. Treating cucumbers with vegetable extracts processed by percolation and sonication favored both the presence of aphids and the appearance of foliar diseases. Due to the low values of the degree of attack for the cases where the hybrid treatment was used, it can be stated that there is a synergy between the two extraction methods and that the extraction of several active compounds requires complex processes.

4.4. The Influence of the Obtained Solutions to the Chlorophyll Content in the Leaves

Treatment with vegetable extracts obtained from onions, peas, and soybeans in the transplanting holes generally produced negative effects on the chlorophyll content of pepper and cucumber crops. The exception was only the treatment with the vegetable extract obtained by percolation for the cucumber crop. Since the values of the coefficients of variation related to the chlorophyll content were lower in the treated varieties than in the untreated control, it can be hypothesized that the plant extracts could interact with the magnesium ions in the soil in a way that limits the biological availability.

Through the simultaneous analysis of the defense effects against insects and diseases with the inhibition of chlorophyll accumulation, respectively the limitation of magnesium, it may be concluded that the plant extract could interact with living cells through mechanisms involving the functioning of ion pumping. This type of mechanism is the basis of modern pesticides.

4.5. Discussions Regarding the Hybrid Extraction Technology Compared to the Ultrasonication and High-Pressure Functioning

The hybrid ultrasound–high pressure assisted extraction process evaluated in the present research confirms that this technology allows improved performance in terms of extraction repellent or insecticidal compounds. The special feature of the equipment to work in different regimes (high pressure, sonication, or hybrid) and the potential to extensively vary the operating parameters confer very important advantages for further research. One of the enhancements that will be made to the equipment is the addition of an exterior cover designed to allow the heating or cooling agents, in order to have better control over the temperature.

This was only the first evaluation from a larger series of planned determinations for the equipment. We believe that it is required for the study to be extended over several batches and longer periods of time, for better relevance. Other medicinal plants with various compounds with a fertilizing or antimicrobial role will also be tested.

4.6. Comparative Evaluation of the Results with Other Similar Research

Research studies have shown that dipropyl disulphide can be obtained from *Allium cepa* (extraction in water, at room temperature for 3 h), obtaining a quantity of $27.54 \pm 3 \mu\text{g}$ per 100 g fresh tissue [46]; however, if the temperature is maintained at $100 \text{ }^\circ\text{C}$ for 2 h, then a quantity of $110.22 \pm 43.76 \mu\text{g}$ per 100 g of fresh tissue can be extracted [46]. The amount of Allicin extracted from the onion recorded values around $1168.09 \pm 149.60 \text{ mg/kg dw}$ Allicin when it was sonicated in a hydro-alcoholic solution for 30 min at 50 Hz [47]. During the extraction of Protein PA1b from *Pisum sativum* (in sodium acetate buffer, 0.1 M, pH 4.9, homogenization for 30 or 60 min at room temperature), 21.3% of pea flour was obtained [48]. When extracting Gm-TX from *Glycine max* (extraction in Tris–HC), $68.3 \pm 1 \text{ (mg/100 100 g)}$ defatted seed) was obtained, and while extracting Lecithin (in a solvent from a mixture of chloroform and methanol) $8.91 \pm 0.71 \text{ (mg/g soy flour)}$ was obtained [49].

Similar findings were observed when utilizing onion for reducing pest attacks [50]. The fact that the repellent effects persisted 10 days after the treatment shows that treating the transplant holes is an effective way to disperse volatile compounds gradually around crop plants. The product's ability to repel could be improved by adding garlic extract to the mixture.

5. Conclusions

There are several significant difficulties related to bioactive compounds used in organic agriculture, especially for those that have dual effects—insecticide and a biostimulator. The main challenges are the instability over time and the possible antagonistic effects.

In terms of their use as remedies at the time of the transplantation phase, the three different extraction techniques used to separate the compounds from onions, peas, and soybeans produced a range of mixed outcomes, most of which were favorable.

The more energetic extraction methods produce an accumulation of hydrophobic biochemical compounds in the mass of the bioproduct, compounds that have an inhibitory character for the development of pathogens but also have a phytotoxic effect at higher concentrations. For this reason, in-depth research will be carried out in the future studies on the component's characterization for more variations of the extraction parameters.

A series of experiments will be carried out to obtain several formulas aimed at vegetables' specific problems, regarding their biofertilizer and bioinsecticide activity.

Author Contributions: Conceptualization, F.N., V.F., V.A., C.P., I.V., N.-V.V., M.G.M., I.G., E.M., S.-S.B. and N.U.; methodology, F.N., V.F., V.A., C.P., I.V., N.-V.V., M.G.M., I.G., E.M., S.-S.B. and N.U.; software, F.N., V.F., C.P., N.-V.V., M.G.M., I.G. and E.M.; validation, F.N., V.F., C.P., I.V., N.-V.V. and M.G.M.; formal analysis, F.N., V.F., I.V., S.-S.B. and N.U.; investigation, V.F., I.V. and S.-S.B.; resources, N.U. and I.V.; data curation, N.U., V.F. and I.V.; writing—original draft preparation, F.N., V.F., C.P., I.V. and M.G.M.; writing—review and editing, F.N., V.F. and N.U.; visualization, V.A., I.V. and M.G.M.; supervision, F.N. and M.G.M.; project administration, F.N., V.F., N.U., I.V., N.-V.V., M.G.M. and S.-S.B.;

funding acquisition, F.N., V.A., C.P., N.-V.V., M.G.M. and N.U. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by The Romanian Ministry of Research, Innovation and Digitalization, Project PN 23 04 02 01 Contract no.: 9N/ 01.01.2023 SUSTAIN-DIGI-AGRI: Innovative biofertilizer production technology used to restore soil biodiversity and reduce the effects of drought on agricultural lands and Contract no. 760005/30.12.2022 cod.2 Establishment and operationalization of a Competence Center for Soil Health and Food Safety. The APC was funded by the University Politehnica of Bucharest, Romania, within the PubArt Program.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data were obtained in collaboration with several institutes and are not publicly accessible.

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

References

- Lengai, G.; Muthomi, J. Biopesticides and Their Role in Sustainable Agricultural Production. *J. Biosci. Med.* **2018**, *6*, 7–41. [[CrossRef](#)]
- Dini, I. Bio Discarded from Waste to Resource. *Foods* **2021**, *10*, 2652. [[CrossRef](#)]
- Wen, C.; Zhang, J.; Zhang, H.; Dzah, C.S.; Zandile, M.; Duan, Y.; Luo, X. Advances in ultra-sound assisted extraction of bioactive compounds from cash crops—A review. *Ultrason. Sonochem.* **2018**, *48*, 538–549. [[CrossRef](#)]
- Nenciu, F.; Oprescu, M.R.; Biris, S.-S. Improve the Constructive Design of a Furrow Diking Rotor Aimed at Increasing Water Consumption Efficiency in Sunflower Farming Systems. *Agriculture* **2022**, *12*, 846. [[CrossRef](#)]
- Nenciu, F.; Voicea, I.; Cocarta, D.M.; Vladut, V.N.; Matache, M.G.; Arsenoiaia, V.-N. “Zero-Waste” Food Production System Supporting the Synergic Interaction between Aquaculture and Horticulture. *Sustainability* **2022**, *14*, 13396. [[CrossRef](#)]
- Pai, S.; Hebbar, A.; Selvaraj, S. A critical look at challenges and future scopes of bioactive compounds and their incorporations in the food, energy, and pharmaceutical sector. *Environ. Sci. Pollut. Res.* **2022**, *29*, 35518–35541. [[CrossRef](#)] [[PubMed](#)]
- Dias, A.L.B.; de Aguiar, A.C.; Rostagno, M.A. Extraction of natural products using supercritical fluids and pressurized liquids assisted by ultrasound: Current status and trends. *Ultrason. Sonochem.* **2021**, *74*, 105584. [[CrossRef](#)] [[PubMed](#)]
- Tang, W.Q.; Li, D.C.; Lv, Y.X.; Jiang, J.G. Extraction and removal of caffeine from green tea by ultrasonic-enhanced supercritical fluid. *J. Food Sci.* **2010**, *75*, C363–C368. [[CrossRef](#)]
- Dias, A.L.B.; Arroio Sergio, C.S.; Santos, P.; Barbero, G.F.; Rezende, C.A.; Martínez, J. Effect of ultrasound on the supercritical CO₂ extraction of bioactive compounds from dedo de moça pepper (*Capsicum baccatum* L. var. *pendulum*). *Ultrason. Sonochem.* **2016**, *31*, 284–294. [[CrossRef](#)] [[PubMed](#)]
- Santos, P.A.C.; Aguiar, G.F.; Barbero, C.A.; Rezende, J. Martínez, Supercritical carbon dioxide extraction of capsaicinoids from malagueta pepper (*Capsicum frutescens* L.) assisted by ultrasound. *Ultrason. Sonochem.* **2015**, *22*, 78–88. [[CrossRef](#)]
- Yang, Y.-C.; Wei, M.-C. Ethanol solution-modified supercritical carbon dioxide extraction of triterpenic acids from *Hedyotis corymbosa* with ultrasound assistance and determination of their solubilities. *Sep. Purif. Technol.* **2015**, *150*, 204–214. [[CrossRef](#)]
- Sumere, B.R.; de Souza, M.C.; dos Santos, M.P.; Bezerra, R.M.N.; da Cunha, D.T.; Martinez, J.; Rostagno, M.A. Combining pressurized liquids with ultrasound to improve the extraction of phenolic compounds from pomegranate peel (*Punica granatum* L.). *Ultrason. Sonochem.* **2018**, *48*, 151–162. [[CrossRef](#)]
- Santos, M.P.; Souza, M.C.; Sumere, B.R.; da Silva, L.C.; Cunha, D.T.; Bezerra, R.M.N.; Rostagno, M.A. Extraction of bioactive compounds from pomegranate peel (*Punica granatum* L.) with pressurized liquids assisted by ultrasound combined with an expansion gas. *Ultrason. Sonochem.* **2019**, *54*, 11–17. [[CrossRef](#)] [[PubMed](#)]
- Vigano, J.; Assis, B.F.d.P.; athia-Neves, G.N.; dos Santos, P.; Meireles, M.A.A.; Veggi, P.C.; Martínez, J. Extraction of bioactive compounds from defatted passion fruit bagasse (*Passiflora edulis* sp.) applying pressurized liquids assisted by ultrasound. *Ultrason. Sonochem.* **2020**, *64*, 104999. [[CrossRef](#)]
- Makkar, R.; Behl, T.; Kumar, A.; Nijhawan, P.; Arora, S. Emerging therapeutic effects of herbal plants in rheumatoid arthritis. *Endocr. Metab. Immune Disord. Drug Targets* **2021**, *21*, 617–625. [[CrossRef](#)]
- Câmara, J.S.; Albuquerque, B.R.; Aguiar, J.; Corrêa, R.; Gonçalves, J.L.; Granato, D.; Pereira, J.; Barros, L.; Ferreira, I. Food bioactive compounds and emerging techniques for their extraction: Polyphenols as a case study. *Foods* **2020**, *10*, 37. [[CrossRef](#)] [[PubMed](#)]
- Shirahigue, L.; Antonini, S. Agro-industrial wastes as sources of bioactive compounds for food and fermentation industries. *Ciênc. Rural* **2020**, *50*, 1–17. [[CrossRef](#)]
- Lefebvre, T.; Destandau, E.; Lesellier, E. Selective extraction of bioactive compounds from plants using recent extraction techniques: A review. *J. Chromatogr. A* **2021**, *1635*, 461770. [[CrossRef](#)]
- Voicea, I.; Fatu, V.; Nenciu, F.; Persu, C.; Oprescu, R. Experimental research on bioinsecticide activity obtained by using an oleic extract from dwarf silver fir on some vegetable crops. *Sci. Pap.-Ser. B-Hortic.* **2022**, *66*, 575–580.

20. Azmir, J.; Sarker, M.Z.; Rahman, M.; Khan, M.S.; Awang, M.; Ferdosh, S.; Jahurul, M.H.A.; Ghafoor, K.; Norulaini, N.A.N.; Omar, A.K.M. Techniques for extraction of bioactive compounds from plant materials: A review. *J. Food Eng.* **2013**, *117*, 426–436. [[CrossRef](#)]
21. Li, J.; Niu, D.; Zhang, Y.; Zeng, X.A. Physicochemical properties, antioxidant and antiproliferative activities of polysaccharides from *Morinda citrifolia* L. (Noni) based on different extraction methods. *Int. J. Biol. Macromol.* **2020**, *150*, 114–121. [[CrossRef](#)] [[PubMed](#)]
22. Ciriminna, R.; Carnaroglio, D.; Delisi, R.; Arvati, S.; Tamburino, A.; Pagliaro, M. Industrial feasibility of natural products extraction with microwave technology. *Chem. Sel. Rev.* **2016**, *1*, 549–555. [[CrossRef](#)]
23. Ciobotaru, I.E.; Nenciu, F.; Vaireanu, D.I. The Electrochemical Generation of Ozone using an Autonomous Photovoltaic System. *Rev. Chim.* **2013**, *64*, 1339–1342.
24. Putnik, P.; Bursać Kovačević, D.; Režek Jambrak, A.; Barba, F.J.; Cravotto, G.; Binello, A.; Lorenzo, J.M.; Shpigelman, A. Innovative “Green” and Novel Strategies for the Extraction of Bioactive Added Value Compounds from Citrus Wastes—A Review. *Molecules* **2017**, *22*, 680. [[CrossRef](#)]
25. Gonzalez, M.; Barrios, S.; Budelli, E.; Pérez, N.; Lema, P.; Heinzen, H. Ultrasound assisted extraction of bioactive compounds in fresh and freeze-dried *Vitis vinifera* cv Tannat grape pomace. *Food Bioprod. Process* **2020**, *124*, 378–386. [[CrossRef](#)]
26. Rosello-Soto, E.; Koubaa, M.; Moubarik, A.; Lopes, R.P.; Saraiva, J.A.; Boussetta, N. Emerging opportunities for the effective valorization of wastes and by-products generated during olive oil production process: Non-conventional methods for the recovery of high-added value compounds. *Trends Food Sci. Technol.* **2015**, *45*, 296–310. [[CrossRef](#)]
27. Nayak, A.; Bhushanb, B.; Rosalesa, A.; Rodriguez Turienzoa, L.; Cortinaca, J.L. Valorisation potential of Cabernet grape pomace for the recovery of polyphenols: Process intensification, optimisation and study of kinetics. *Food Bioprod. Process.* **2018**, *109*, 74–85. [[CrossRef](#)]
28. Kumar, K.; Srivastav, S.; Sharanagat, V.S. Ultrasound assisted extraction (UAE) of bioactive compounds from fruit and vegetable processing by-products: A review. *Ultrason Sonochem.* **2021**, *70*, 105325. [[CrossRef](#)]
29. Anticono, M.; Blesa, J.; Frigola, A.; Esteve, M.J. High biological value compounds extraction from citrus waste with non-conventional methods. *Foods* **2020**, *9*, 811. [[CrossRef](#)] [[PubMed](#)]
30. Vazquez-Roig, P.; Picó, Y. Pressurized liquid extraction of organic contaminants in environmental and food samples. *Trends Anal. Chem.* **2015**, *71*, 55–64. [[CrossRef](#)]
31. Catarino, M.D.; Silva, A.M.S.; Mateus, N.; Cardoso, S.M. Optimization of phlorotannins extraction from *Fucus vesiculosus* and evaluation of their potential to prevent metabolic disorders. *Mar. Drugs* **2019**, *17*, 162. [[CrossRef](#)]
32. Jha, A.K.; Sit, N. Extraction of bioactive compounds from plant materials using combination of various novel methods: A review. *Trends Food Sci. Technol.* **2022**, *119*, 579–591. [[CrossRef](#)]
33. Nenciu, F.; Mircea, C.; Vladut, V.-N.; Belc, N.; Berca, M.L. Evaluation of wheat seed separation performances for new design of rotating cylindrical sieve, equipped with customizable homogenization coil. *Eng. Rural. Dev.* **2021**, *26*, 1478–1483. [[CrossRef](#)]
34. Fu, Y.; Luo, J.; Qin, J.; Yang, M. Screening techniques for the identification of bioactive compounds in natural products. *J. Pharm. Biomed. Anal.* **2019**, *168*, 189–200. [[CrossRef](#)]
35. Mahato, N.; Sinha, M.; Sharma, K.; Koteswararao, R.; Cho, M.H. Modern Extraction and Purification Techniques for Obtaining High Purity Food-Grade Bioactive Compounds and Value-Added Co-Products from Citrus Wastes. *Foods* **2019**, *8*, 523. [[CrossRef](#)]
36. Opreacu, M.R.; Biris, S.-S.; Nenciu, F. Novel Furrow Diking Equipment-Design Aimed at Increasing Water Consumption Efficiency in Vineyards. *Sustainability* **2023**, *15*, 2861. [[CrossRef](#)]
37. Nenciu, F.; Voicea, I.; Stefan, V.; Nae, G.; Matache, M.; Milian, G.; Arsenoia, V.N. Experimental research on a feed pelletizing equipment designed for small and medium-sized fish farms. *INMATEH-Agric. Eng.* **2022**, *67*, 374–383. [[CrossRef](#)]
38. Zhao, X.X.; Lin, F.J.; Li, H.; Li, H.B.; Wu, D.T.; Geng, F.; Ma, W.; Wang, Y.; Miao, B.H.; Gan, R.Y. Recent Advances in Bioactive Compounds, Health Functions, and Safety Concerns of Onion (*Allium cepa* L.). *Front. Nutr.* **2021**, *22*, 669805. [[CrossRef](#)]
39. Godlewska, K.; Ronga, D.; Michalak, I. Plant extracts—importance in sustainable agriculture. *Ital. J. Agron.* **2021**, *16*, 1851. [[CrossRef](#)]
40. Lin, Y.H.; Huang, H.W.; Wang, C.Y. Effects of High Pressure-Assisted Extraction on Yield, Antioxidant, Antimicrobial, and Anti-diabetic Properties of Chlorogenic Acid and Caffeine Extracted from Green Coffee Beans. *Food Bioprocess. Technol.* **2022**, *15*, 1529–1538. [[CrossRef](#)]
41. Benkeblia, N. Antimicrobial activity of essential oil extracts of various onions (*Allium cepa*) and garlic (*Allium sativum*). *LWT-Food Sci. Technol.* **2004**, *37*, 263–268. [[CrossRef](#)]
42. Shrestha, D.K.; Sapkota, H.; Baidya, P.; Basnet, S. Antioxidant and antibacterial activities of *Allium sativum* and *Allium cepa*. *Bull. Pharm. Res.* **2016**, *6*, 50–55.
43. Jouvensal, L.; Quillien, L.; Ferrasson, E.; Rahbé, Y.; Guéguen, J.; Vovelle, F. PA1b, an insecticidal protein extracted from pea seeds (*Pisum sativum*): 1H-2-D NMR study and molecular modeling. *Biochemistry* **2003**, *42*, 11915–11923. [[CrossRef](#)] [[PubMed](#)]
44. Hadrich, F.; El Arbi, M.; Boukhris, M.; Sayadi, S.; Cherif, S. Valorization of the peel of pea: *Pisum sativum* by evaluation of its antioxidant and antimicrobial activities. *J. Oleo Sci.* **2014**, *63*, 1177–1183. [[CrossRef](#)]
45. Oliveira, H.D.; Sousa, D.O.; Oliveira, J.T.; Carlini, C.R.; Oliveira, H.P.; Pereira, M.L.; Rocha, R.O.; Morais, K.S.J.; Gomez-Filho, E.; Vasconcelos, I.M. Gm-TX, a new toxic protein from soybean (*Glycine max*) seeds with potential for controlling insect pests. *Process Biochem.* **2010**, *45*, 634–640. [[CrossRef](#)]

46. Artacho Martín-Lagos, R.; Olea Serrano, M.F.; Ruiz-López, M.D. Comparative study by gas chromatography-mass spectrometry of methods for the extraction of sulfur compounds in *Allium cepa* L. *Food Chem.* **1992**, *44*, 305–308. [[CrossRef](#)]
47. Loredana, L.; Giuseppina, A.; Filomena, N. Biochemical, antioxidant properties and antimicrobial activity of different onion varieties in the Mediterranean area. *Food Meas.* **2019**, *13*, 1232–1241. [[CrossRef](#)]
48. Crévieu, I.; Bérot, S.; Guéguen, J. Large scale procedure for fractionation of albumins and globulins from pea seeds. *Mol. Nutr. Food Res.* **1996**, *40*, 237–244. [[CrossRef](#)] [[PubMed](#)]
49. Anshu, A.K.; Kumar, V.; Rani, A.; Tayalkar, T.; Parmar, H.S. Phosphatidylcholine content in soybean: Genetic variability and the effect of growing year. *Not. Sci. Biol.* **2022**, *14*, 10994. [[CrossRef](#)]
50. Debra, K.R.; Misheck, D. Onion (*Allium cepa*) and garlic (*Allium sativum*) as pest control intercrops in cabbage-based intercrop systems in Zimbabwe. *J. Agric. Vet. Sci. IOSR-JAVS* **2014**, *7*, 13–17. [[CrossRef](#)]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.