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RESEARCH ARTICLE

Effects of electromagnetic waves on parameters, hydration, and *in vitro* antimicrobial activity of the *Brassica oleracea* L. var. *italica Plenck.* and water

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Abstract

Our team has developed a device capable of emitting electromagnetic waves via a solenoid. Building on this breakthrough, our research endeavors to explore the effects of these waves on the weight and osmotic parameters of *Brassica oleracea* L. var. italic Plenck, specifically broccoli sprouts. These sprouts were subjected to various types of activated water infused with electromagnetic waves, with their weight measured in grams and their percentage composition analyzed. This study aims to elucidate the potential impact of electromagnetic waves on agricultural practices and nutritional outcomes. The findings present valuable insights into optimizing crop growth and enhancing nutritional value, thus holding promise for agricultural applications. In addition to nutritional aspects, contamination by pathogenic microorganisms poses a significant challenge in fruit and vegetable cultivation, particularly when organic fertilizers are employed. Mitigating microbial contamination in plant-based foods is paramount for preventing gastrointestinal and other infections, thereby safeguarding consumer health. In vitro studies were conducted to investigate the antimicrobial effects of electromagnetic waves across different frequencies. Water suspensions containing a density of 105 cells per milliliter of three microbial ATCC strains—*Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*—were utilized. These suspensions were exposed to electromagnetic radiation within the frequency ranges of 350-600 Hz (designated as frequencies F1) and 20-40 kHz (designated as frequencies F2), as well as combinations of both F1 and F2 frequencies, each lasting 15 minutes. Results indicated that the tested strains exhibited heightened sensitivity to exposures at 350-600 Hz and 20-40 kHz, leading to a decrease in cell viability by approximately 70% compared to untreated controls.

Keywords

electromagnetic waves, osmosis, antimicrobial action, *Brassica oleracea* L. var. *italica Plenck*

Introduction

Broccoli sprouts, scientifically classified as *Brassica oleracea* L. var. italic Plenck, are renowned for their dense biochemical profile, offering a plethora of essential nutrients. They serve as a notable source of macronutrients, encompassing carbohydrates, proteins, and fats. Rich in vitamins C, K, and various B-group vitamins, as well as essential elements including calcium (Ca^{2+}), iron in both ferrous (Fe^{2+}) and ferric (Fe^{3+}) forms, phosphorus (P^{2+}), and sulfur (S^{2-}), broccoli sprouts are a nutritional powerhouse (1). Of particular significance are the phytochemicals found abundantly in broccoli sprouts, such as sulforaphane, indoles, and glucosinolates. These compounds have been associated with antioxidant, anti-inflammatory, and potentially health-promoting properties (2). Extensive research underscores the manifold benefits of broccoli, including its antioxidant, antimicrobial, anti-inflammatory, anticancer, neuroprotective, and renoprotective effects (3). Broccoli and its derivatives enjoy widespread acceptance across diverse demographics, promising substantial nutritional and health advantages in combatting chronic diseases and metabolic disorders (3). Nonetheless, it is crucial to acknowledge that the composition of broccoli can exhibit variability due to factors like cultivation conditions and maturity stage. Hence, ensuring meticulous cultivation practices and exploring accessible, efficient methods to optimize yields are imperative for maximizing its nutritional potential.

Research endeavors have been directed towards harnessing electromagnetic waves to optimize seed germination, enhance germination rates, and expedite early plant development. Spectral analyses were conducted on water used for seed soaking, sprouting, and plant growth, utilizing electromagnetic wave parameters selected for their efficacy. The Non-equilibrium Energy Spectrum (NES) and Differential Non-equilibrium Energy Spectrum (DNES) methodologies were employed to analyze water influenced by various electromagnetic waves, with comparison to the spectral profile of broccoli [4–7]. Furthermore, NES and DNES analyses were applied to assess the effects on several plant species, including *Haberlea rhodopensis* Frv., *Moringa deifera* Lam., *Urtica diotica* L., *Malva sylvestris* L., and *Plantago major* L. [9]. Additionally, evidence of the anti-inflammatory properties of *Hypericum perforaticum* L. was demonstrated at specific energy levels (E=-0.1212 eV) and corresponding wavelengths (λ=10.23µm), along with vibrational frequencies ($\tilde{V}$=978cm^{-1}) [10]. *Rosa damascena* Mill. exhibited notable antioxidant effects at energy levels (E=-0.1387eV), wavelengths (λ=8.95µm), and vibrational frequencies ($\tilde{V}$=1117cm^{-1}), particularly renowned in Bulgaria [11]. Moreover, investigation into medicinal plants containing Ca^{2+} revealed significant results at energy levels (E=-0.1112eV), wavelengths (λ=11.15µm), and vibrational frequencies ($\tilde{V}$=897cm^{-1}) [12]. Basic physiological parameters of these plants were computed to comprehensively monitor their physiological responses to electromagnetic wave interventions. Analysis of electromagnetic wave parameters is conducted, particularly focusing on their stimulating effects in seed priming using non-ionizing electromagnetic waves (NIR-non-B ionizing radiation) (13). Positive impacts such as enhanced germination, accelerated seedling sprouting, and increased yield have been documented. Non-ionizing radiation, despite not inducing ion release processes, possesses adequate energy to elicit biological responses (14). The biological outcomes of NIR are found to be contingent upon various factors including thermodynamic parameters of the target, essential frequency characteristics, tissue water absorption spectra, bioparameters of the organism, and physical and chemical attributes of the surrounding environment (15). Cellular hydration has been identified as a biomarker for NIR effects (15), and the influence of osmosis on hydration has been elucidated (16). Hydration parameters were measured using osmosis/diffusion equipment (17). Leveraging this data, our investigation sought to examine the effects of electromagnetic frequencies on the growth and development of broccoli sprouts. Specifically, electromagnetic exposures at frequencies of 350-600 Hz (referred to as frequencies F1) and 20-40 kHz (referred to as frequencies F2), as well as combinations of F1 and F2 outcomes, were employed. This study endeavors to compare the impacts of electromagnetic waves on broccoli sprouts, thereby offering valuable insights for optimizing crop growth and nutritional quality.

Living organisms, encompassing microorganisms, are continuously subjected to electromagnetic vibrations. Notably, microorganisms exhibit heightened resilience to sound vibrations compared to other organisms (18). Nevertheless, the impact of electromagnetic vibrations on microorganisms remains largely unexplored. Given the escalating resistance of microorganisms to antimicrobial agents, this study endeavors to investigate the effects of electromagnetic waves with varying frequencies and durations on microorganisms across diverse taxonomic groups. The feasibility of manipulating this factor across different conditions and objectives further underscores its scrutiny. The objectives of the research, encompassing parameters such as broccoli mass, osmotic processes between water and broccoli extract, and the influence on bacteria under specific electromagnetic frequencies, aim to discern the differential effects of electromagnetic parameters.

Materials and Methods

Broccoli Sprout Harvest

Brassica oleracea L. var. *italic Plenck* (broccoli) sprouts were cultivated under controlled environmental conditions until reaching the desired maturity stage. Ordinance No. 16/28.05.2010 on the requirements for quality and control for conformity of fresh fruits and vegetables.

Preparation of Sprout Samples

A strainer removed the water from the broccoli sprouts, with care taken to avoid damaging them during this process.

Experimental setup

The broccoli sprouts were soaked in distilled water infused with 350-600 Hz (marked as frequencies F_1) and in the range 20-40 kHz (marked as frequencies F_2), as well as combinations of both F_1 and F_2 (350-600 Hz and 20-40 kHz). The various wave frequencies were applied for 30 seconds using a solenoid and a beaker (Fig. 1).

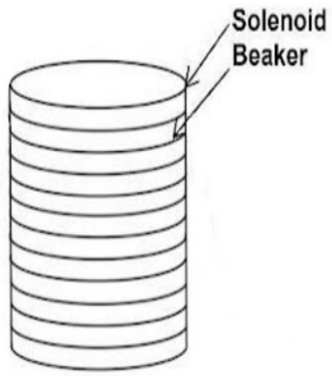

Fig. 1. Scheme of solenoid and beaker

We provided the same care conditions for broccoli sprouts in each group: F_1, F_2, and the combination F_1 and F_2. Distilled water was used as the watering medium for all samples. We controlled parameters like humidity, light, and soaking to observe the actual effect of water activation.

Weight measurement

A precision weight scale, meticulously calibrated in accordance with manufacturer specifications, was employed for the weight measurements. Prior to each measurement, the scale underwent a zeroing procedure to mitigate potential errors. Control samples of broccoli sprouts were meticulously positioned at the center of the scale to ensure precise measurement accuracy.

Water for soaking of broccoli

The examination of the waters sourced from the analyzed springs, concerning both their physicochemical composition and microbiological parameters, was meticulously executed in adherence to the protocols delineated in Ordinance No. 9/2001 as published in the Official State Gazette, issue 30, and decree No. 178/23.07 004. These regulations prescribe the criteria governing the quality standards applicable to water designated for consumption and domestic use.

Data Collection and Analysis

The weights of individual broccoli sprouts were meticulously documented in a standardized format. Subsequently, the gathered data underwent rigorous statistical analysis to ascertain various parameters such as mean weights, standard deviations, and other pertinent statistical measures. Comparative evaluations between distinct samples or experimental conditions were conducted employing suitable statistical tests.

Microorganisms

Pure cultures of three reference microbial strains were used - the Gram-negative bacterium *Escherichia coli* ATCC-8739, the Gram-positive bacterium *Staphylococcus aureus* ssp. *aureus* ATCC-6538, and the oval fungus *Candida albicans* ATCC-10231.

Nutrient media

Meat peptone agar and Mueller-Hinton agar (BUL BIO National Center of Infectious and Parasitic Diseases – Sofia, Bulgaria) were employed in the present study.

Experimental setup

Microbial suspensions were prepared in sterile distilled water at a density of 0.5 using the McFarland standard. Subsequent dilutions were made to achieve a cell density of 10^5 cells per milliliter. These suspensions underwent electromagnetic treatments at three distinct frequencies: 350-600 Hz, 20-40 kHz, and a combined frequency of 350-600 Hz and 20-40 kHz, each administered for a duration of 15 minutes. Following treatment, the cultures were plated onto Mueller-Hinton agar and then incubated at 37°C for 24 hours under aerobic conditions. The resulting colony counts were compared to untreated control samples.

Device for osmosis/diffusion

The device for osmosis/diffusion comprised a glass beaker with dimensions of h=12 cm, D=9 cm, and a volume 0.5 L (Fig. 2). The membrane used had dimension of h=12 cm and D=4 cm. This membrane, made from cellulose, had a width of 500 nm and contained pores measuring 100 nm in diameter.

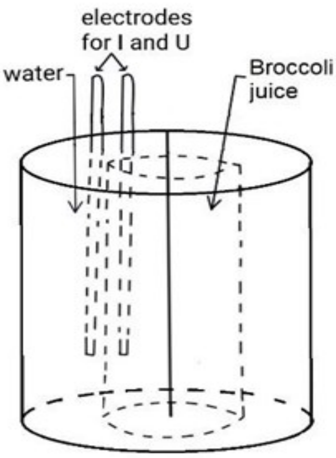

Fig. 2. Scheme of container for osmosis in glass beaker

Electrical device

The changes in electrical conductivity were measured with fixed AD 76309 probes of two identical ADWA AD330 EC meters.

Statistical analysis

The statistical analysis of the findings utilized the classical Student-Fisher method employing a t-test. Microsoft® Office Professional Plus Excel 2013 (version 15.0.4569.15060) was utilized for the computations, with appropriate permissions obtained from the respective authors' institutes, universities, and centers. Mean values, along with their correlation coefficients and t-statistics, were computed. The statistical dependence and reliability of the results were assessed using Student's t-test for independent samples, with each group consisting of 5 results. Significance levels were set at $p < 0.001$, $p < 0.01$, and $p < 0.05$ to ascertain the significance of observed differences.

Results and Discussion

Results of weighing the broccoli, soaked in waters, influenced with different EM waves

During our investigation into *B. oleracea* var. L. var. *italic Plenck*, our primary focus was on broccoli sprouts subjected to immersion in various water compositions. Figure 3 illustrates daily photographic documentation of each set of B. oleracea sprouts irrigated with distinct water types: those treated with water exposed to electromagnetic waves and control samples treated with regular water. All water variants were prepared utilizing distilled water. In the nomenclature, F1 and F2 denote the influence of specific electromagnetic frequencies, specifically 350-600 Hz and 20-40 kHz, respectively. F1 – 350-600 Hz, and F2 – 20-40 kHz, designate individual frequencies. The control group comprised sprouts soaked in water devoid of any electromagnetic wave exposure. Column 1 is designated for 350-600 Hz and 20 kHz- 40 kHz, column 2 for 350-600 Hz, and column 3 for 20-40 kHz. Column 4 represents the control samples.

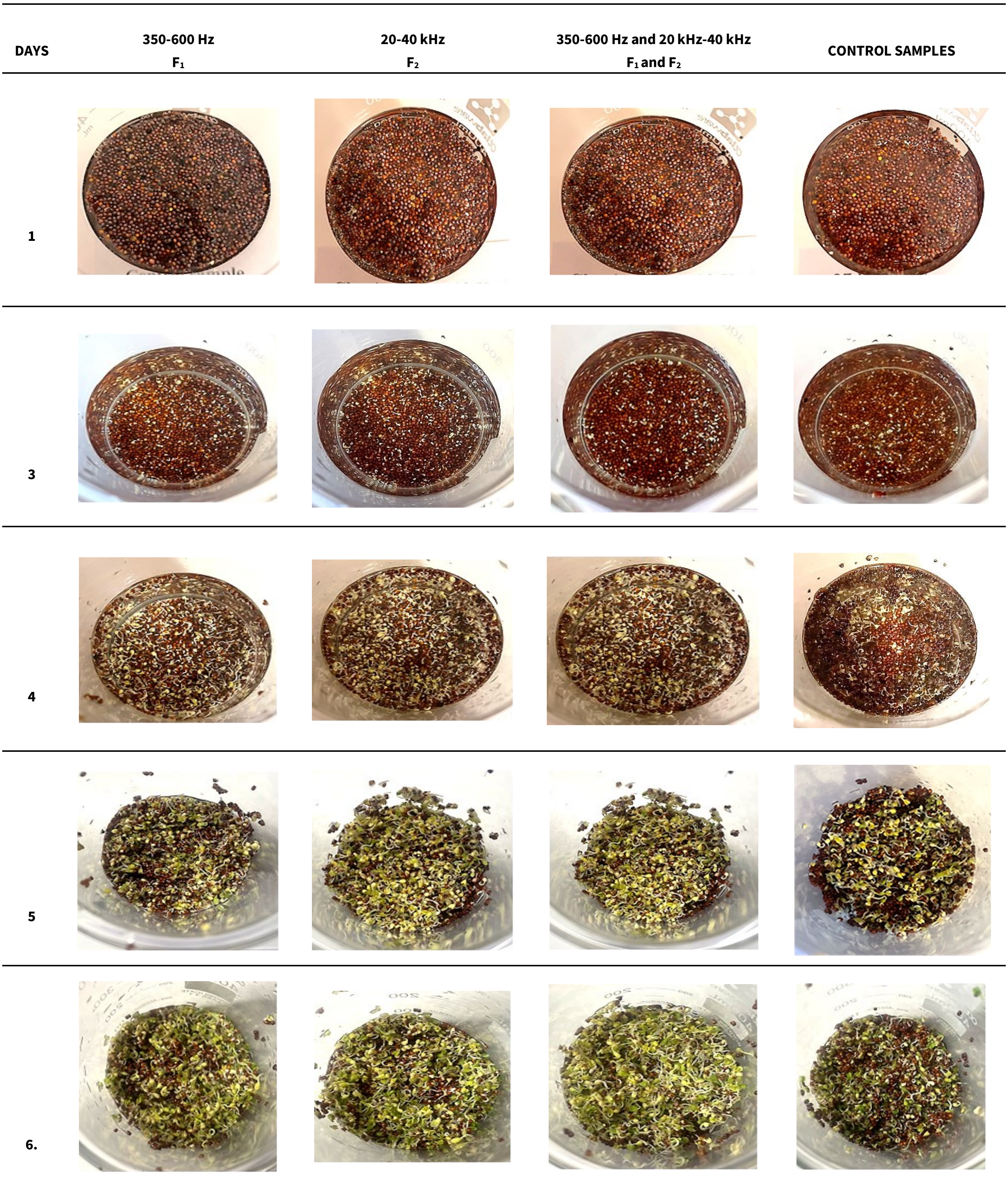

Fig. 3. Daily photos of *B. oleracea* L. var. *italica Plenck* sprouts watered with different types of water

The daily monitoring of broccoli sprout development provides valuable insights into the growth patterns of sprouted *B. oleracea.* Beginning from the second day of observation, discernible signs of germination are observed across all experimental groups. The photographic documentation enables a comprehensive comparison of broccoli sprout morphology over the duration of the study, facilitating the visual assessment of treatment effects on growth and development. Significantly, among the various *B. oleracea* groups examined, the F1 and F2 treatments exhibit the most pronounced levels of germination. Following closely behind is the F1 group subjected to frequencies ranging from 350 to 600 Hz. Conversely, the F2 group exposed to frequencies between 20 and 40 kHz, as well as the combination of F1 and F2 treatments, demonstrate slightly diminished germination rates. In contrast, the control group displays comparatively lower levels of *B. oleracea* germination compared to the experimental cohorts. These findings underscore the influence of different water treatments on the germination dynamics of broccoli sprouts and underscore the potential significance of electromagnetic wave exposure in stimulating growth processes. Table 1 demonstrate the changes in the weight in grams of *Brassica oleracea* L. var. *italica Plenck* sprouts watered with different types of water: F_1, F_2, and a combination of F_1 and F_2. The control was sprouts soaked with water without influence.

Table 1. Changes in the weight in grams of *B. oleracea* L. var. *italica Plenck* sprouts watered with different types of water: F_1, F_2, and a combination of F_1 and F_2.

Days	***B. oleracea* L. var. *italica Plenck* sprouts Weight (g)**			
	350-600 Hz (F_1)	**20-40 kHz (F_2)**	**350-600 Hz and 20-40kHz (F_1 and F_2)**	**Control group**
1.	20.0	20.0	20.0	20.0
2.	21.2	21.3	20.8	20.6
3.	25.8	26.1	26.4	23.5
4.	47.1	47.7	48.6	42.4
5.	63.2	65.6	67.4	56.8

The Student's t-test was applied to the group with the lowest results to analyze the effects of electromagnetic (EM) fields on broccoli. The broccoli was exposed to EM waves at 350-600 Hz. The data is as follows: Group: 350-600 Hz: (43.4, 43.0, 43.1, 43.3, 43.2); Control Group: (40.0, 36.6, 36.5, 40.1, 36.6). The results indicate significance at $p < 0.001$, with a t-statistic of 10.26 and a correlation coefficient of r: 0.9503. Similarly, for the groups with 20-40 kHz, 350-600 Hz and 20-40 kHz the results also showed significance at $p < 0.001$.

Similar results with $p < 0.001$ were obtained for groups exposed to both 20-40 kHz and 350-600 Hz frequencies.

Table 1 and Figure 3 depict the weight variation of broccoli sprouts, measured in grams, under distinct water treatments, including water treated with a combination of electromagnetic waves spanning frequencies of 350-600 Hz and 20-40 kHz. The control group comprised sprouts immersed in unaltered water. Our investigation aimed to quantitatively analyze the observed alterations induced by electromagnetic frequencies on broccoli seeds and their subsequent growth. The electromagnetic frequencies, delineated into two ranges—350-600 Hz (referred to as frequencies F1) and 20-40 kHz (referred to as frequencies F2)—were applied to influence the germination and growth of broccoli seeds. Daily weight measurements were conducted post-drying using an electronic scale to monitor the weight fluctuations of the sprouts. Initially, on the first day of observation, all experimental samples weighed precisely 20 grams per sample. Subsequent assessments revealed subtle changes by the third day, attributable to the initial stages of germination, thus rendering the differences negligible. However, by the fifth day, significant disparities in the weight of the broccoli sprouts became apparent. Broccoli sprouts treated with the combined electromagnetic waves (F1+F2) exhibited an average weight of 48.6 grams. In comparison, sprouts exposed solely to F1 registered an average weight of 47.7 grams, while those subjected solely to F2 weighed 47.1 grams. The control group of sprouts maintained in untreated water exhibited an average weight of 42.4 grams.

Results with bacteria in suspensions, influenced with different EM waves

The results of the experiment conducted with microorganisms are presented in Table 2. The data summarized in the table demonstrate that the tested electromagnetic waves exerted antimicrobial effects (19).

Table 2. Results of the conducted experiments with microorganisms

Microorganisms	**Percentage of colony counts compared to the control samples**			
	350-600 Hz (F_1)	**20-40 kHz (F_2)**	**350-600 Hz and 20-40 kHz (F_1 and F_2)**	**Untreated control samples**
E. coli	40.0	33.5	32.0	100
S. aureus	44.0	30.0	35.0	100
C. albicans	45.0	33.7	28.2	100
Average results	43.0	32.4	31.7	100

They were also sensitive to high-frequency exposures, with the most significant sensitivity observed at 20-40 kHz. The Gram-negative bacterium *E. coli* exhibited higher sensitivity to 350-600 Hz than the Gram-positive microorganisms. The oval fungus *C. albicans* showed slightly higher sensitivity to exposure at 20-40 kHz and 350-600 Hz than the tested bacteria.

There are statistically reliable differences ($p<0.001$) between untreated control samples and those influenced by 350-600 Hz, 20-40 kHz, 350-600 Hz, and 20-40 kHz.

The statistical results showed significant findings between F_1 and F_2, with $p<0.05$; $r=0.803$; t-statistic is 2.56. Additionally, significant findings were observed between F_1 and the group F_1 and F_2, with $p<0.05$; $r=0.9802$; t-statistic is 2.447.

The most substantial antimicrobial effect was obtained at 350-600 Hz and 20-40 kHz, followed by 20-40 kHz alone. The antimicrobial effect at 350-600 Hz was the lowest among these frequencies.

The results indicate that the increase in the frequency of applied electromagnetic exposure significantly decreases the number of examined *E. coli*, *S. aureus*, and *C. albicans*.

The electromagnetic effects observed in broccoli primarily manifest through alterations in the permeability of plant cell envelopes, thereby impacting the exchange of substances within the cells (20). The plant cell wall, a dynamic and intricate structure, undergoes changes in properties during its development, both locally along the cell wall and transversely. When subjected to electromagnetic fields, the treated system, notably water, absorbs energy, which facilitates the restoration of hydrogen bonds among water molecules. Analysis of certain spectral parameters enables the assessment of electromagnetic influences on water and the consequential alterations in various physicochemical parameters. The energy associated with hydrogen bonds exhibits a decrement from (-0.09) to (-0.1212) eV, leading to a reduction in surface tension. Consequently, fluctuations occur in the permeability of the cell membrane, with a decrease observed at (-0.1) eV and an increase at (-0.11) eV. Within the range spanning from (-0.1212) to (-0.1387) eV, there is an escalation in the energy of hydrogen bonds and, correspondingly, in surface tension. Living systems selectively absorb water, initiating processes from water sources exhibiting the lowest energies. These rearrangements significantly influence osmosis and diffusion, particularly as the energy of hydrogen bonds increases (17). Microorganisms exhibit sensitivity to electromagnetic waves within the frequency range of 2.104 to 2.10^8 Hz and beyond. Our findings align with prior studies such as that by Inhan-Garipet et al. (21), which reported diminished growth in both Gram-positive and Gram-negative strains following exposure to electromagnetic flux. Conversely, our research also indicates beneficial effects on the growth of *B. oleracea* L. var. *italic Plencken*, suggesting avenues for investigations reliant on bacterial and plant physiology. Osmosis, a fundamental process observed in aquatic environments, microorganisms, and plants, involves the movement of water across semi-permeable membranes in response to solute concentration gradients. When a bacterium is immersed in a hypotonic solution (characterized by lower solute concentration), water ingress occurs through the cell membrane via osmosis, potentially resulting in cell swelling due to turgor pressure. However, cells with thinner walls may be susceptible to rupture under such conditions (22).

The study explored the impact of acoustic sound and low-frequency electromagnetic fields on the growth and antibiotic susceptibility of various microorganisms, notably Escherichia coli and Staphylococcus aureus (23). Following exposure to a 5 mT electromagnetic flux for 15 minutes, E. coli exhibited significant inhibition, displaying a growth rate reduction of 0.08 log CFU.mL^{-1}. The findings suggest that environmental factors such as sound and electromagnetic flux have discernible effects on bacterial physiology. The study posits that the physical attributes of the magnetic field, including its frequency and exposure duration, prompt alterations in the structure of bacterial cell walls, primarily within their proteins. Consequently, these changes impact the composition and characteristics of peptidoglycan (24). Osmosis represents a fundamental process in plant biology, facilitating the transport of water and nutrients from the external environment to plant cells. Investigating osmotic responses under saline stress conditions in broccoli, researchers delved into the mechanisms underlying plant adaptation (25).

Osmosis processes between water and broccoli juice

Experiments were conducted involving osmosis, where broccoli juice was placed in a container with a semipermeable membrane. This container was immersed in a beaker filled with mountain spring water (26). The broccoli juice was then subjected to electromagnetic waves (Fig. 2). The results of osmosis between broccoli juice and water with different frequencies are shown in Table 3.

Table 3. Results of osmosis between broccoli juice and water with different frequencies

	Results on broccoli juice/ water µS.cm^{-1}			
	350-600 Hz (F_1) µS.cm^{-1}	20-40 kHz (F_2) µS.cm^{-1}	350-600 Hz and 20-40 kHz (F_1 and F_2) µS.cm^{-1}	Osmosis beginning 1 hour after µS.cm^{-1}
1.	182/691	187/697	194/707	173/687
	282/602	288/608	292/617	264/602
2.	181/690	186/699	192/709	175/688
	281/603	287/607	290/613	267/597
3.	183/688	185/695	195/705	176/691
	280/604	285/605	294/615	261/589
4.	184/694	185/698	194/703	179/693
	280/605	281/606	291/616	270/590
5.	185/691	187/695	198/704	181/687
	281/600	284/605	291/618	264/591

The following results were obtained with water in a semitransparent membrane one hour after osmosis. Valid statistical analyses were conducted for electric conductivity in µS.cm^{-1}:

Control group: 264, 267, 261, 270, 264 and Group after influence with 350-600 Hz: 282, 281, 280, 280, 281 µS.cm^{-1}. The results of the Student's t-test indicate significance at $p<0.01$, with a t-statistic of (-7.0030), and a correlation coefficient of $r=0.9899$.

Control group: 264, 267, 261, 270, 264 and Group after influence with 20-40 kHz – 288, 287, 285, 281, 284 µS.cm^{-1}.

The results of the Student's t-test indicate significance at p<0.01, with a t-statistic of (-6.7002), and correlation coefficient of r=0.9868.

Control group – 264, 267, 261, 270, 264 and Group after influence with 350-600 Hz and 20-40 kHz – 292, 290, 294, 291, 291 µS.cm^{-1}. The results of the Student's t-test indicate significance at p<0.01, with a t-statistic of (-4.3679), and a correlation coefficient of r=0.9895.

The three groups of results with frequencies F_1 (350-600 Hz), F_2 (20-40 kHz), and F_1 and F_2 (350-600 Hz and 20-40 kHz) after osmosis are statistically reliable with the Student's t-test at significance level of p<0.01.

A statistical analysis using the Pearson correlation coefficient was conducted to assess the influence of the osmosis process from Table 3 on the reduction of pathogenic bacteria count from Table 2.

The differences in electrical conductivity during osmosis from water to broccoli juice, subjected to electromagnetic waves with frequencies of 350-600 Hz and 20-40 kHz (F_1 and F_2), compared to the control samples, were 21, 17, 19, 15, and 17 µS.cm^{-1}. The percentage difference for the same frequencies in untreated bacteria was 68, 65, and 71.8%. The Pearson correlation coefficient was r=0.1886, indicating a weak positive correlation. This suggests that the osmotic process affects the reduction of *E. coli, S. aureus*, and *C. albicans* counts. This effect was more pronounced when exposed to electromagnetic waves.

The results in Fig. 4 illustrate reliable differences at 350-600 Hz, 20-40 kHz, 350-600 Hz and 20-40 kHz depending on increasing osmosis parameters in the osmosis process with mountain spring water and broccoli juice influenced by EM waves. The average result for 350-600 Hz was 26.7 min, for 20-40 kHz was 22.7 min, and for 350-600 Hz and 20-40 kHz was 17.7 min.

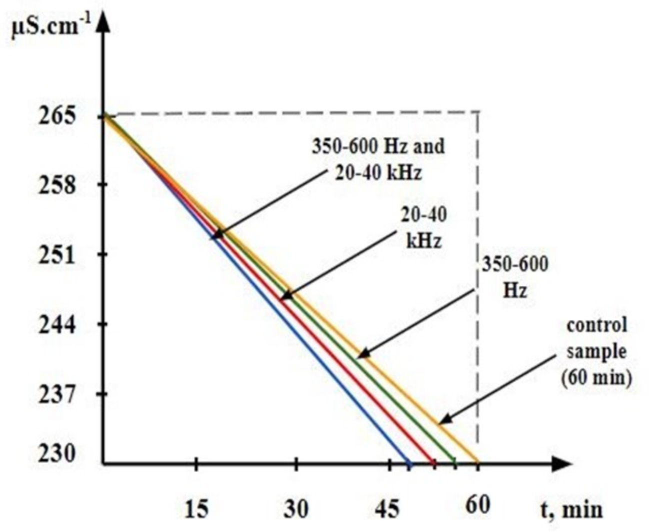

Fig. 4. Results at 350-600 Hz, 20-40 kHz, 350-600 Hz, and 20-40 kHz, with varying osmosis parameters in the osmosis process involving water and broccoli juice influenced by EM waves

Five measurements were conducted to assess the osmosis process. Control samples exhibited durations of 30.0, 29.8, 30.2, 30.3, and 29.8 minutes, yielding an average of 30.0 minutes. Conversely, samples exposed to frequencies of 350-600 Hz showed durations of 26.9, 26.3, 26.7, 27.1, and 26.3 minutes, with an average of 26.7 minutes. Statistical analysis utilizing Student's t-test, with a significance level of p < 0.05, indicated a Pearson correlation coefficient (r) of 0.963 and a t-statistic of 7.276. Results obtained with both 20-40 kHz and 350-600 Hz frequencies were found to be statistically significant. The presence of pathogenic microorganisms such as *E. coli, Staphylococci*, and fungi is common in the cultivation of fruits and vegetables, particularly when organic fertilizers are utilized. The mitigation of microbial contamination in plant-based foods is essential to mitigate gastrointestinal and other infections, thus ensuring consumer health. Further investigations are warranted to elucidate the underlying mechanisms and optimize the application of electromagnetic wave treatments in agricultural practices to enhance plant health and ensure food safety.

Table 4. Results of voltage in mV and electric current in A with different frequencies.

	Results on broccoli juice/ water mV/mA			
	350-600 Hz (F_1)	**20-40 kHz (F_2)**	**350-600 Hz and 20-40 kHz (F_1 and F_2)**	**Osmosis beginning 1 hour after**
1.	157/16	163/17	174/18	145/14
2.	158/16	164/17	175/18	147/15
3.	160/17	165/18	177/19	150/15
4.	159/16	166/18	180/20	143/13
5.	161/17	167/17	176/18	141/14

Table 4 illustrates the results of voltage in mV and electric current in A with different frequencies.

The following results were achieved with water in a semitransparent membrane one hour after osmosis. Valid statistical analyses were conducted for the electric voltage in mV:

For the control group: 145, 147, 150, 143, 141 mV and for the group influenced with 350-600 Hz: 157, 158, 160, 159, 161 mV. The results of the Student's t-test indicate significance with p < 0.001, with a t-statistic is (8.616), and a correlation coefficient of r = 0.9905. Similarly, for the groups subjected to 20-40 kHz, 350-600 Hz and 20-40 kHz, the results remain significant with p < 0.001.

Conclusion

The present study investigates the physiological responses of *B. oleracea* L. var. *italic Plenck* sprouts to various forms of water activation influenced by electromagnetic waves. The growth dynamics were documented through photographic evidence, while daily assessments of sprout mass were conducted. Comparative analyses were employed to discern distinctions among the different treatment modalities. Our findings reveal notable variations in weight distribution percentages across the treatment groups. On the final day of measurement, the weights were as follows: the 350-600 Hz and 20-40 kHz treatment cohort exhibited a mean mass of 67.4 grams, the 20-40 kHz treatment group registered 65.6 grams, the 350-600 Hz treatment group recorded 63.2 grams, and the control group attained a mass of 56.8 grams. Furthermore, the weight percentages on the concluding day demonstrated significant discrepancies: the 350-600 Hz and 20-40 kHz treatments achieved 100%, the 20-40 kHz treatment group reached 97.3%, the 350-600 Hz treatment group was at 93.8%, and the control group scored 84.3%. Remarkably, the disparity between the 350-600 Hz and 20-40 kHz treatment group and the control group amounted to 15.7%. Conversely, the disparities between the 20-40 kHz treatment group and the control group, and between the 350-600 Hz treatment group and the control group, were 8.8% and 6.4%, respectively.

The bacterial strains *E. coli, S. aureus*, and the oval-shaped fungus *C. albicans* were subjected to in vitro analysis to assess their sensitivity to electromagnetic vibrations at varying frequencies and durations of exposure (15 minutes). Frequencies within the range of 350-600 Hz, designated as F1, exhibited an antimicrobial effect against these microorganisms, resulting in a reduction of cell vitality by 57.0%. Similarly, frequencies ranging from 20-40 kHz, designated as F2, led to a reduction of viable microorganisms by 67.6%. Combining frequencies from both ranges, denoted as F1 and F2 respectively, resulted in a decrease of viable microorganisms by 68.3%. Based on these findings, it can be inferred that broccoli sprouts exposed to electromagnetic waves displayed notable differences in weight compared to control samples. These results suggest that the specific water treatments had a discernible impact on the growth and weight development of broccoli sprouts. Furthermore, the study demonstrated that the tested combinations of F1 (350-600 Hz), F2 (20-40 kHz), and the combined F1 and F2 (350-600 Hz and 20-40 kHz) electromagnetic waves exerted an additional positive effect on plants. These treatments not only stimulated plant development but also exhibited antimicrobial effects. Implementing effective measures to mitigate the presence of these pathogens is crucial for ensuring food safety and protecting consumer health. These findings underscore the potential of employing specific combinations of 350-600 Hz and 20-40 kHz electromagnetic waves as a novel approach to enhance plant growth and mitigate microbial contamination. The reduction in pathogenic bacteria likely influences osmosis processes, with the effects correlating positively with increasing electromagnetic frequencies. Osmosis positively impacts and corresponds with the physiological processes of broccoli as the frequencies increase.

Authors' contributions

FH, AII and ChS carried out the electromagnetic device experiments, while II and MTI performed the osmotic device experiments. They participated in sequence alignment and drafted the manuscript. TPP conducted the microbiological studies and also contributed to drafting the manuscript. II contributed to the study design and performed the statistical analysis. FH and AII conceived the study and participated in its design and coordination. All authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None.

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