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Study on the preparation and investigation of the effects of plasma activated water combining multiple minerals on the germination and early growth of *Lactuca sativa* L.

Major: Inorganic Chemistry

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THESIS OPENING

The urgency of the thesis

Crop yield has always been one of the most important issues in agriculture. Crop yield primarily depends on seed quality. In addition, there are other direct and decisive factors such as environmental conditions, fertilizer application, use of protective drugs against external influences, etc. To improve crop productivity, besides improving the variety, we also need to pay special attention to solutions that increase the germination, growth rates of seeds and factors that ensure the growth of plants during the growing process.

Traditional methods used to improve crop yield are increased fertilization, use of pesticides and emphasis on irrigation. However, all of the above methods have disadvantages such as increasing actual costs and polluting the environment.

The current trend is to apply new technologies to improve productivity without leaving chemical residues, without changing the composition of products and having adverse effects on human health. One of the current new research directions is to use physical techniques to treat seeds to improve and increase their germination capacity and rate such as irradiation, ultraviolet (UV), plasma... In which, plasma technology has received much attention because of its ability to support germination and growth, and provide more nutrients for plants. In the field of agriculture, there are few studies in the world that have shown the interaction of plasma activated water (PAW) with germination and growth for some crops, but these studies are still separate. discrete, not yet evaluated the impact of the plasma modulation process, the accompanying physico-chemical parameters. Therefore, research to systematize and clarify these issues is necessary. On the other hand, studies on the effects of using essential minerals nitrogen (N), phosphorus (P), potassium (K) in the early stages of plant growth have not been explored in depth and have not figured out whether or not N, P, and K should be used at this stage.

It is necessary and meaningful to gradually clarify, perfect and apply new technology, cold plasma technology, in Vietnam, a country with a specific agriculture. On that basis, the question is whether PAW can affect and change the germination - growth process in the early stages or not? In addition, does the addition of essential elements for plants such as minerals N, P, K in PAW makes the above processes better?

It is very important to complete the study of the above problems. On that basis, we carried out a thesis study with the topic **"Study on the preparation and investigation of the effects of plasma activated water combining multiple minerals on the germination and early growth of *Lactuca sativa* L."**

The success of the thesis will contribute to the development of a new research direction, with scientific and practical significance, safe for plants, humans, livestock and the environment. Besides, the thesis will also contribute as a basis for future practical implementation studies.

Objectives - Research content

Objective: To study the preparation and investigation of the effect of plasma activator in combination with macronutrients on seed germination and early growth of curly lettuce (*Lactuca sativa* L.).

Research Content

Content 1: Research on composition, concentration and properties of plasma activated water samples on two different discharge modes: Direct (corona) and indirect (Dielectric Barrier Discharge - DBD).

Content 2: Investigate the influence of H_2O_2 and minerals N, P, K on germination and early growth of lettuce.

Content 3: Investigate the effects of PAW and PAW combined with minerals N, P, K on the germination and early growth of lettuce.

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CHAPTER 1. OVERVIEW

1.1. Overview of plasma activated water for plants

1.1.1. Overview

Plasma is divided into two types: Thermal plasma and cold plasma (non-thermal plasma). For thermal plasma, the temperature of the electron is equal to the temperature of the ion and the temperature of the gas. Cold plasma is a form of plasma that is in thermal equilibrium, generating energy in free electrons, thus forming high-energy electrons (10,000 Kelvins), but neutral ions and atoms remain at room temperature. Since the experimental conditions of cold plasama are at low temperature (not much higher than room temperature) the experiments can be arranged for application on seeds. In addition, cold plasma systems often have low maintenance and energy costs [1, 2].

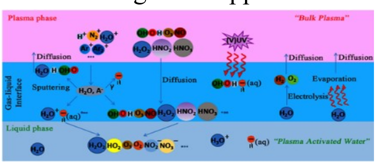

Figure 1.1. Description of the multiphase transfer of reactive oxygen species (ROS) and reactive nitrogen species (RNS) to water [1]

Depending on the medium, plasma can produce different substances. For example, in the atmosphere, plasma forms the following substances: UV rays, unstable compounds (O•, H•), highly stable compounds such as O_3, H_2O_2… In the gas plasma form – liquid, with the participation of a number of physicochemical phenomena involving collision of gas particles, mass transfer, sputtering and photolysis formed through UV photons. Accordingly, part of the active substances produced initially in the gas-phase plasma will reach the interface of the liquid plasma, enter the gas-liquid interface and then react with water molecules (Figure 1.1). PAW has high activity and can be applied in many different fields.

Liquid plasmas (in contact with liquids) are of great interest because of their wide applicability because they generate free radicals and free N radicals (RNS), which are effective agents against many biological agents. and chemistry, so they are suitable for broad applications such as biomedical, environmental, nanoscience and agriculture [1,3,4]. In agriculture, plasma gas is often used to act on seeds to enhance germination, plasma water is often used by seed osmosis to control the growth of seeds and plants [4].

Plasma-activated water is the product of the plasma jet's interaction with the liquid. Depending on the type of interaction, there can be several methods to generate PAW from different gases such as (i) discharge through the dielectric barrier, (ii) plasma discharge, (iii) corona plasma discharge, (iv) sliding arc discharge, etc. (Figure 1.2). Commonly used working gases include air, oxygen, nitrogen, helium, argon or mixture all of them [3].

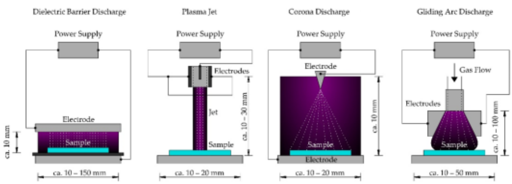

Figure 1.2. Diagram of PAW generation systems

1.1.2. Properties of PAW

1.1.2.1. pH value: In PAW, the acid value of water is formed from compounds of plasma gas with water.

1.1.2.2. Conductivity: In PAW, ionic compounds are produced that change electrical conductivity. These ions are ROS and RNS during plasma activation in solution which will contribute to the increase in conductivity of PAW.

1.1.2.3. Hydrogen peroxide: H_2O_2 is the ROS formed in PAW, which plays an important role in the antibacterial properties of PAW. Many studies have shown the chemical processes that take place with the formation of H_2O_2 and its importance for the antibacterial action of PAW.

1.1.2.4. Concentrations of nitrite ions (NO_2-) and nitrate (NO_3-): The detection of nitrite and nitrate ions.

1.1.3. Application of PAW in agriculture

PAW has received considerable attention from researchers in recent years due to its non-thermal, non-toxic, bactericidal... applications in biomedical, food, environmental treatment, and science. nano and specially to improve the issues of clean agricultural practice, an inevitable trend.

1.1.3.1 PAW for seed treatmentj

1.1.3.2. PAW promotes plant growth

1.1.3.3. PAW control diseases and pests

1.2. Research situation on plasma activated water for plants

1.2.1. In the world: In recent years, researchers from various fields have turned their attention to the study of using cold plasma technology on different materials in the field of biology and not biology. The presence of RONS is an important indicator of the effectiveness of cold plasma application. Many scientific papers related to cold plasma show that this technology is mainly applied to seeds and food. The effect of cold plasma on different particle morphology, physiology, and molecular properties is an area of research that has not been explored much, but is considered to have potential for high safety and efficacy.

1.2.2. In Vietnam: In our country, cold plasma technology is interested and researched in many different fields, mainly in health, food, water environment treatment such as: Research group of Bui group Nguyen Quoc Trinh has researched and manufactured food preservation equipment by creating a cold plasma source, controlling the temperature of the plasma generator. The generated device can inhibit or kill harmful bacteria. Compared to chemical preservation methods, high-temperature preservation, plasma method has been proved to be advantageous, leaving no unwanted chemical residues in processed foods [28]. Research group of Tran Ngoc Dam, Ho Chi Minh City University of Technical Education on plasma application in medical wastewater treatment, bottled drinking water [29]. In addition, there are many research results of other research groups such as the research team of the Institute of Physics of Ho Chi Minh City researching plasma applications for the medical field, the research group of Do Hoang Tung in Hanoi on the use of plasma. Using plasma to treat open wounds in medical…

In Institute of Applied Materials Science, our plasma research team, in the last 10 years, has successfully applied the fabrication and application of plasma gas and water to many fields, including agriculture. Our research also has many published works, projects, training masters and bachelors in the field of plasma for applications such as: environmental treatment, manufacturing new materials, consulting projects for provinces.

1.3. The role of oxidants and macronutrients for plants

1.3.1. Germination process

1.3.1.1. Principle of seed germination: The sprouting process begins with the absorption of water by the seed and ends with the emergence of the embryonic shaft.

1.3.1.2. Measurement of germination: The sign to determine the completion of germination and the beginning of seedling development is that the embryo is punctured with a seed that protrudes about 2 mm. The basic measure to evaluate the germination capacity of a seed population is the percentage of seeds that have completed germination at a given time [33,34].

1.3.1.3. Seed asorption: Absorbing water of seeds is the first necessary step in the germination process. Dried seeds usually have a moisture content (water content as a percentage of dry weight of the grain) in the range of 5–15% depending on the amount of seed oil and the surrounding environment, relative humidity in the air. [33, 35].

1.3.1.4. RNA and Protein Synthesis: Environmental factors, including light, temperature, soil water content, and nutrients, affect seed germination primarily through regulation of metabolism and growth. signaling pathways of GA and ABA [39].

1.3.2. Growth process

1.3.2.1. Principle of Growth: Plant growth can be defined as the increase in plant volume and/or weight with or without the formation of new structures such as organs, tissues, cells, and tissues. cells or cell organelles [41]. Growth is often associated with development (differentiation of cells and tissues) and reproduction (reproduction of new individuals). Several different criteria for determining plant growth rate: Height or width, plant weight (fresh, dry weight), cell count, protein content or other essential substances.

1.3.2.2. Wetting capacity, germination, seedling growth and enzyme activity

1.3.2. Role of H_2O_2: H_2O_2 is a strong oxidizing agent and was first known as an antibacterial. However, in recent times, the germination-stimulating effect of H_2O_2 has been demonstrated by different research groups around the world [60-62].

1.3.3. The role of macronutrients N, P, K: Elements play a number of important roles in plants and can be broadly classified into (1) the building blocks of plant cell walls, (2) aids in cell osmosis and maintenance of pressure, (3) energy transfer, (4) participates in enzyme-catalyzed reactions, (5) participates in reproduction [67].

1.4. Overview of *Lactuca savita* L.

1.4.1. General introduction: Lettuce (*Lactuca sativa* L.) belongs to the Asteraceae family, the largest family of flowering plants (Angiosperms), is one of the most popular garden vegetables in the world. world, especially known as "mixed salad". *Lactuca sativa* L. is the most important representative member of the genus Lactuca, which is consumed in increasing quantities, as it is considered a healthy food and carries many medicinal values [83, 84].

Figure 1.7. Seeds, flowers, curly lettuce plants

In recent years, the sprouting stage of curly lettuce, the pre-mature harvest stage, just 7 to 21 days after germination, has become a culinary trend because of growing conditions and harvesting curly lettuce is relatively easy and it is possible during this period, the amount of nutrients (ascorbic acid, β-carotene, α-tocopherol and phylloquinone) vitamins and minerals (Ca, Mg, Fe, Mn, Zn, Se and Mo) are 4 to 40 times higher than the adult stage [85, 86]. In addition, with the production and investment in a short period and the ability to withstand harsh weather conditions, curly lettuce is the object of recent research attention [87].

1.4.2. Characteristics of curly lettuce: Curly lettuce has a single taproot that can grow up to 60-70 cm long. Many other roots grow horizontally, up to 10-15 cm long. Curly lettuce has a short growing period, can be harvested after about 7-15 days and after about 120 days, the seeds can be harvested. Curly lettuce can be stored in a cold room, at 0- 1°C and 95% humidity, for up to 10-12 days. Lettuce is highly adaptive, so their phenotypic, morphological and physiological expression is also significantly affected by growing conditions.

1.4.3. Main pests and diseases of lettuce: The major threat to the formation and development of lettuce in general is *Pythium* and *Fusarium* [88, 94, 95]. Besides, recent studies have also shown that *Xanthomonas* is also the main causative agent of curly lettuce leaves with typical lesions of bacterial leaf spot. The leaf lesions are irregular, small, light green to black in color and 2 to 5 mm in diameter.

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CHAPTER II. MATERIALS AND EXPERIMENTAL METHODS

2.1. Materials

2.1.1. Chemicals

The chemicals used in the study were analytical grade which is listed below: Calcium phosphate monobasic – Sigma- Adrich (>95%); Potassium sulfate– Sigma- Adrich (>99%); Ammonium nitrate- Sigma- Adrich (>98%); Hydrogen peroxide solution (Sigma- Adrich, >34%); Distilled Water (from the Analytical Center - Institute of Applied Materials Science); Nitrogen gaz – Sovigaz (99,99%); Oxygen gaz – Sovigaz (99,99%); Air – 99,99%; Argon gaz – Sovigaz (99,99%)

Seeds of lettuce (Scientific name: *Lactuca sativa* L.) were collected in an orchard in Cai Rang District, Can Tho Province, Vietnam. Untreated coir substrate (by growers in Ben Tre Province).

2.1.2. Equipments

List of used equipment: Plasma generation; The pH and conductivity measurements HI-99130 (Hanna Instruments HI991300/ Romania); Device for measuring leaf area: CI- 202 (CID Bio-Science, Inc./ USA); Hi-Speed Refrigerated centrifuge capable of 20000 rpm: Z32 HK (Hermle/ Germany); High-resolution spectrophotometer: UV-1800 (Shimadzu/ Japan) Scanning electron microscope: S4800 S4800 (Hitachi/ Japan); Electronic scale with 4 odd numbers: DV214C (Ohaus/ USA); Stereo Microscope SZX12 (Olympus – Japan; Double Distiller Aquatron A4000D (Bibby Scientific/ England); Current Monitor Model 4100 (Pearson Electronics, USA); Oscilloscope GW Instek GDS-1102-U (Instek/ Taiwan); High-pressure probe HVP-28HF (Pinteck/ Taiwan); Power consumption meter - CAT II (China); Biological safety cabinets: AHC-4D8 (Esco/ Singapore); Kit test: NO3- Sera test kit, Germany; H2O2, Hanna Instruments HI3844 test kit, Romania; O3, Hanna Instruments HI38054 test kit, Romania…

2.2.4. Electrical characteristics of the plasma system

2.2.4.1 General introduction: The plasma generator system is a special electrical device, characterized by high voltage, low current, and variable frequency according to usage requirements. Discharge capacity, which is a measure of the efficiency of a plasma generating system, is regulated by the value of voltage or current, depending on the type of discharge.

2.2.4.2. Method of measuring the electrical characteristics of the plasma system: Measuring devices include an Oscilloscope task of displaying the graph of signal time voltage and luminous intensity. The voltage information is converted to digital information for reconstruction into a waveform displayed on the screen. The current meter and high voltage probe are connected directly to the channels on the oscilloscope.

2.3. Preparation and physicochemical characteristics of PAW samples

2.3.1. DBD plasma. Fixed parameters: Total air flow rate, flow pump, total solution volume. Variable parameters: Gas mixture flows.

2.3.2. Corona plasma. Fixed parameters: Total air flow rate, flow pump, total solution volume. Variable parameters: Gas mixture flows.

2.3.3. Investigation of physicochemical characteristics of PAW samples

In this experiment, the parameters of PAW are determined by physicochemical characteristics including pH, electrical conductivity (EC), NO_2^- ion, NO_3^- ion, H_2O_2, and ozone.

2.4. Preparations of samples containing H_2O_2, N, P, K, PAW, and PAW combined N, P, K

H_2O_2 samples; N, P, K samples; PAW sample; PAW samples combining N, P, K

2.5. Parameters evaluation for seed germination and early-stage growth of Lettuce

2.5.1. Germination assessment parameters: *Weight of a thousand seeds; Embryo's length; Seed germination rate.*

2.5.2. Assessment parameters of the growth process in the early stages: *Stem height and root length; Chlorophyll content;. Dry/fresh weight ratio; Leaf area; Morphology images.*

2.6. Effects of H_2O_2, N, P, and K on the germination and the growth of Lettuce seed

2.7. Effects of PAW and PAW in combination with macronutrients N, P, and K on germination and growth

2.8. Activity of antibacterial and antifungal

2.8.1. Minimum inhibitory concentration (MIC) method

2.8.2. Antibacterial ring diameter measurement method

2.9. Calculating and processing data

Data were collected and aggregated on 32-bit Microsoft® Excel® 2019 MSO (16.0.14131.20278) software. Data were statistically analyzed using SPSS 17.0 for Windows (SPSS, Chicago, IL). The standard deviation was calculated and analyzed to determine the smallest significant difference between the treatment methods with significance level $p \leq 0.05$. All data are the mean of four replicates ($n = 4$) and the errors (± SE). In the same row/column of values followed by different letters, the difference is statistically significant ($P \leq 0.05$) according to Duncan's test.

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CHAPTER 1. RESULTS AND DISCUSSION

3.1. Evaluation of the electrical characteristics of the plasma system

In this thesis, the plasma generator system consists of two parts: The plasma generator system with a fixed voltage of 24 kV, and a frequency of 1.5 kHz. With the above system, the electrical characteristics of the plasma were evaluated by analyzing an oscilloscope monitoring the plasma discharge according to the time. The results on the electrical characteristics of the plasma system are shown in Figure 3.1

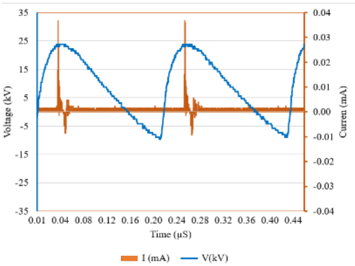

Figure 3.1. The voltage and current waveforms measured by probes during the discharge

From Figure 3.1, we can be seen that plasma discharges in the form of a pulse, with the highest voltage of 24 kV and the corresponding current value of 0,038 mA for each pulse. From the determination of voltage and current, we can calculate the power consumed by the plasma system.

Figure 3.2 shows an instantaneous power and energy based on the voltage and current calculated from Figure 3.1. The results show that although the instantaneous power of the pulse peak is 401,5 kW, the energy generated per pulse is only 31,3 mJ/pulse. The power consumption of the plasma system is quite low, calculated by the following formula:

P(W) = E pulse. F with f: Frequency of the device (1.5 kHz)

E pulse = 31.3 mJ. Thus, the power consumption of the plasma discharge system calculated according to the above theory is 47 W. The same result is also shown when using the CAT II power consumption meter to check the actual power consumption.

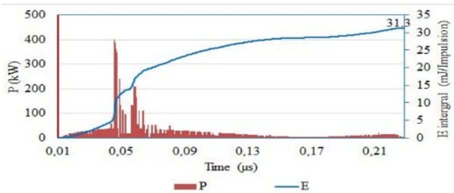

Figure 3.2. Time evolution of the instantaneous power and energy deposited

3.2. The relationship between the physicochemical properties of the plasma water sample and the discharge reactor types

3.2.1. pH value

Comparing the two cases, the pH value of the sample always gives a lower value in the case of corona plasma for the same discharge time. From the results, with 02 types of discharge, to form PAW, the pH value tends to decrease with increasing treatment time. The decrease in pH value associated with the formation of acidic compounds in PAW (H_2O_2, dissolved ozone, and HNO_x acid) is caused by plasma designs (DBD, corona...), voltage and current, and input gas.

3.2.2. EC concentration and temperature

EC values of all samples are increasing gradually. The highest conductivity matches the lowest pH for itself, as the both of these parameters are correlated.

However, from the research results, over time, in the first 10 minutes, the pH value in section 3.2.1 decreased sharply (corresponding to a sharp increase in EC value), then tended to decrease slightly; while for the EC value, a strong upward trend persisted, indicating the possibility that besides acidic compounds produced in PAW over time, other compounds were still present acidic is produced. This result is also similar to the studies on conductivity and pH value of PAW [10,11,17].

Regarding the temperature parameters of the PAW samples: the results of the study showed that the temperature of all the samples increased slightly during the plasma treatment for 30 min (4 ^{0}C). This suggests that the plasma generating system in this case is suitable for use in the life sciences, especially agricultural research, where the temperature is a sensitive parameter. In the publication of group L. Wang [104], and J. Li [105] also showed the same judgment about the temperature of the plasma generator system for agriculture.

3.2.3. *NO_3^-, NO_2^-, H_2O_2, O_3*

Both NO_2-, and NO_3- ions increase with the treatment time of 30 minutes. However, NO_3- content is always higher than NO_2- consistently. The low NO_2- content can be explained by the conversion of NO_2- to NO_3- in an oxidizing environment. Research by the group M.J. Traylor has also shown the same thing [106]. When argon was added at a 1:1 ratio to other input gases, the NO_3- content showed a significant increase, 2 to 3 times higher than that of samples with a mere air input.

The concentration of H_2O_2 tends to be similar to that of NO_3- as the plasma treatment time is increased: From 0 mg/L in the control sample to the highest value near 90 mg/ L at the plasma treatment time of 30 minutes. The formation of H_2O_2 in water mainly originates from the reaction between moisture in the air or water in the solution with oxidizing agents such as O• and OH•.

There is not much difference in from the amount of ozone dissolved in the sample. This response that dissolved ozone has little absorption in water. The highest measured concentrations of dissolved ozone for samples that did not exceed 0.4 mg/L during 30 min of treatment.

3.3. Selection of PAW samples for seed germination and plant growth

Based on studies and preliminary experimental values with survey results on 0_2 PAW generation systems, it shows that:

• Assessment of temperature: The measured temperature threshold achieved in practice between the DBD plasma system and the corona plasma shows that the temperature generated in the DBD system does not change, much different from the ambient temperature.

• Evaluation of the NO_3-, NO_2-, and H_2O_2 values: These parameters of the water samples generated from 2 types of discharge are suitable for further studies of the thesis.

• Evaluation of pH value: In the two types of plasma discharge in water, acidic compounds are produced, the most for corona discharge. The presence of argon at the rate of 50% (remaining N_2 and O_2), the maximum pH value after 30 minutes for 2 types of corona plasma and DBD plasma discharge was 4.68 and 5.21, respectively, compared with the control of 6, 9.

• Evaluation of ingredients and ratios when mixing: In general, for DBD, the gas mixture is air, and the air: argon mixture (O_2, N_2, and Ar with the ratio of 1, 4, and 5 respectively) are suitable for further study.

From the above evaluation, it shows that DBD plasma discharge type is more suitable for further studies of this thesis.

3.4. Effects of H_2O_2, N, P and K on germination and growth

3.4.1. Effect of H_2O_2

3.4.1.1. Germination process

For all cases, the weight of seeds soaked with H_2O_2 is always higher than that of the control sample and when compared to the original dry seed sample (increased from 58% to 95%). That said, the process absorbs a lot of water to create the best conditions for the seeds to germinate. Research by A. Paez-Garcia also shows that for plants to grow, seeds need to absorb a certain amount of water before they begin to germinate [107].

Besides direct measurement, the length of the workpiece was also observed by stereo microscope, the results are shown in Figure 3.15.

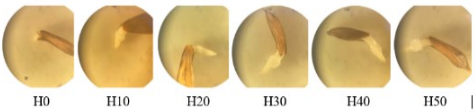

Figure 3.15. The embryo length of samples with different H_2O_2 content after 24 hours

The results showed that with H_2O_2 at different concentrations, there was an impact on the seeds, helping to increase the germination rate compared to the control sample. With only 10 ppm H_2O2 used, the seed germination rate increased continuously in the first 3 days and reached the highest of all the treated samples, reaching 67%. The results also showed that a decreasing tendency of seed germination was observed when increasing H_2O_2 content greater than 10 ppm. This idea once again confirms the role of H_2O_2 in having positive effects on plants. H_2O_2 has a strong oxidizing capacity that makes it able to interact with most biological molecules (including nucleic acids, proteins, and lipids), thereby leading to oxidative stress, and causing damage to plant cells.

3.4.1.2. Early-stage growth

• Stem height and root length

The results from Figure 3.19 show that under the influence of H_2O_2, the body height has changed compared to the control sample; Only 10 ppm of H_2O_2 contributes to a slightly 3% increase in the body height. However, with concentrations greater than 10 ppm, stem height tends to decrease gradually. Specially at the H_2O_2 content varying from 30 ppm to 50 ppm, the body height decreased from 8.1% to 29.1% respectively. For root length, the control sample showed a higher value than the other samples. Next, the H_2O_2 content of 10 ppm gave the best root length among the rest of the samples.

From the above observations, the root length tended to decrease with increasing H_2O_2 content in the experimental samples. This fact is consistent with statements from similar studies: At high concentrations, H_2O_2 negatively affects plants leading to biological stress on plant cells, but in moderate amounts, the role of H_2O_2 is quite beneficial [60,100].

Figure 3.19. Image of stem and root length of seeds after 5 days at different H_2O_2 concentrations

- The dry-to-fresh leaf weight ratio

The results of the dry-to-fresh leaf weight ratio of the aboveground part and the roots of the lettuce showed that when using H_2O_2 concentrations from 10 to 40 ppm, the results were higher than those of the control, especially in the H10 sample best, 34.6% higher. However, with the amount of H_2O_2 used above 10 ppm, this ratio is lower than that of the H10 sample. At 50 ppm, this ratio was reduced by about 4% compared to the control sample. Thus, H_2O_2 may be involved in weight gain through tissue chemotaxis. The use of exogenous H_2O_2 may also have made the root system stronger, thereby increasing the growth of seedlings, allowing the seedling to absorb nutrients and thus increasing biomass in addition to its ability to promote the activity of enzymes. Indeed, the studies of the group Ł. Wojtyla [108], S.Z. Ismail [109], and D. Li, [110] also showed that H_2O_2 supplementation resulted in stronger root systems, increased seedling growth, and increased salt tolerance.

- *Chlorophyll content*

Chlorophyll content increased by 200% when there was an increase in the function. The amount of H_2O_2, however, the higher the H_2O_2 content, the tendency to decrease the chlorophyll content. This issue suggests that although H_2O_2 is a strong oxidizing agent that causes cell damage and photosynthetic damage at high concentrations, at low concentrations it acts as a signaling molecule. This statement is also reinforced by the studies of A. Wahid [111], R. Aroca [112], R. Desikan [113]. Further, S. Neill et al. [114] also observed that H_2O_2 is an essential component of abscisic acid-induced stomatal closure signaling through the activation of calcium permeable channels in the plasma membrane.

3.4.1.3. Anti-bacterial and anti-fungal activity

It seems that at the concentration threshold above 50 ppm, H_2O_2 begins to show its bactericidal ability. However, to completely kill bacteria, the concentration needs to be above 1.000 ppm.

The use of H_2O_2 is effective in bactericidal and fungicidal processes, but at high concentrations it is not suitable for the growth of plants. Although not effective for supplementing the germination and growth stages, this result shows that we can use H_2O_2 as a disinfectant, quickly treating seeds before starting the germination process.

These results are also similar to the results of other studies that show that H_2O_2 can only kill fungi and bacteria at high concentrations, usually from high concentrations (> 35%) [116-118).

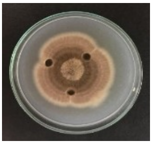

Figure 3.23. Results against the fungus *Fusarium* spp. of H2O2 at concentrations of 50 ppm, 200 ppm, 1.000 ppm, after 24 hours

3.4.2. Effects of macronutrients N, P, K on germination and early growth

3.4.2.1. Germination process

Specifically: Increases from 9.49% to 35.34% (at 100 ppm) for N, 7.22% to 28.52% (at 80 ppm) for P, and 11.79% to 20.15% (at 20). ppm) for K. Overall, this upward trend is not linear. At high content addition, the trend decreased gradually in the thousand grain weight for all 3 cases. The results also showed that, contrasted with K and P supplementation at the same concentration, the addition of N showed the highest efficiency (difference 7%, 15%).

For the billet length, the trend is similar to that for the thousand-grain weight. Specifically: Increase from 25% to 81.12% (at 100 ppm) for N; 35.20% to 88.78% (at 80 ppm) for P; and 37.76% to 78.57% (at 20 ppm) for K. Compared in 3 cases under the same conditions, the trends in embryo length development were as follows P > N > K.

Figure 3.25. Image of embryo length when adding concentration of N from 0 ppm to 200 ppm after 24 hours. (Directive from left: 0 ppm, 60 ppm, 80 ppm, 100 ppm, 200 ppm).

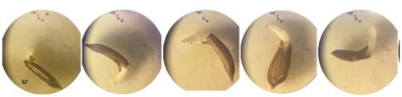

Figure 3.25. Image of embryo length when adding concentration of P from 0 ppm to 200 ppm after 24 hours. (Directive from left: 0 ppm, 60 ppm, 80 ppm, 100 ppm, 200 ppm).

Figure 3.27. Image of embryo length when adding concentration of K from 0 ppm to 200 ppm after 24 hours. (Directive from left: 0 ppm, 60 ppm, 80 ppm, 100 ppm, 200 ppm).

The results showed that the addition of N could increase the germination of seeds, from day 1 to day 3, especially on the first day of the N200 sample showed a 75% increase in germination compared to the control, up to day 2. Third, the germination rate of seeds increased by 59.8%. The trend of increasing germination rate appeared from samples N10 to N200.

From the survey results on embryo length, the weight of thousands of seeds, and seed germination rate, it can be seen that although there is a reserve of N in seeds (4.25 g/ 100 g of seeds), the addition of N in the germination stage still has a significant effect, helping to support the process of storing nutrients, preparing for the next stage of plant development. The amount of additional N provided in the early stages has a great impact on embryo formation, increasing seed weight. This result is also similar to some observations from the studies of M. Stitt [119] and C. Diaz [120].

In the case of P addition: The trend of gradually increasing germination rate was similar in the samples as for N. Figure 3.23, 3.25. 3.28 shows that: Although the amount of P stored in seeds is 0.97 g/100 g of seeds (Annex analysis results), the addition of P to the appropriate threshold for the germination stage will have a significant impact, helps support the process of storing nutrients, preparing for the next stage of plant growth. Therefore, an adequate supply of P is essential from the outset.

The case of K addition: The results also show a tendency to gradually increase the germination rate as N, P. Figures 3.23, 3.27. 3.28 showed that although the amount of K still exists in the seeds (0.983 g/100g), the extra absorption of exogenous K in lettuce seed still takes place, affecting the germination process. The previous study of group M. Farooq [133], H. Marschner [134], N. K. Fageria [136] also showed the same conclusion.

In general, the results obtained when supplementing with macronutrients show that: Low levels of N, P, and K in seeds can be detrimental to germination. Therefore, determining the right amount of minerals when supplementing will help increase the best germination efficiency.

3.4.2.2. Early-stage growth

• Stem and root height

For mineral N: The results showed that the added N content and root length had an inverse relationship. With the addition of N, the trend of gradually decreasing root length continued to occur. About body height: This shows that when N is supplemented at appropriate levels, it can stimulate the growth of plants, and vice versa, it can inhibit the growth of roots and stems.

For mineral P: Different from the influence of N, when P content was increased to 200 ppm, a trend of rapid decrease in stem height and root length appeared. These results are also consistent with previous studies of the authors M. Razaq [138], J. Hong [139] when they said that P is an essential substance for plant growth and especially affects significantly.

For mineral K: Different from the influence of N, the stem height and root length of curly lettuce tended to increase gradually from 0 ppm to 100 ppm, increasing 17.76% for stem height in sample P80 and 29.74% for root length in sample P100. When P content was increased to 200 ppm, a trend of rapid decrease in stem height and root length appeared. Visual observation (Figure 3.31) also shows similar results. These results are also consistent with previous studies of the authors M. Razaq [138], J. Hong [139] specially when they say that P is essential for plant growth significantly.

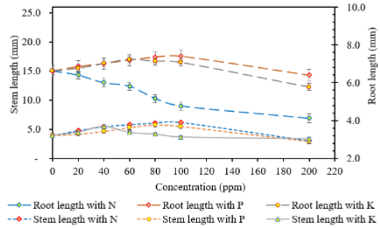

Figure 3.30. The values of stem height and root length of seeds after 7 days at different concentrations of N.

• Leaf area and chlorophyll content

The results shows that there is a similarity between chlorophyll content and leaf area. When increasing the content of different minerals, they all showed a tendency to increase to the appropriate level, then the indexes decreased respectively, including the indicators of germination as shown above. This shows that each mineral indicates a suitable content value for early growth.

A comparison between the 3 minerals shows that: To achieve the best growth, it is necessary to add N rather than add K, P. This indicates that the investigated seeds do not contain enough N for optimal growth. However, the amount of P. K required is not much. It can be recognized that the addition of N, combined with the amount of N, P, and K available in the seeds (4.25 g/ 100 g seeds, 0.97 g/100 g seeds, 0.98 g/100 g seeds according to respectively, the appendix to check the content of substances in the seeds) created the above significant difference. This shows the need to adjust the indicators of substance content accordingly for the germination, growth, and development of plants.

• The dry/ fresh weight ratio

The dry matter content through the dry/fresh weight ratio of the leaves is further investigated, which is also an important parameter in assessing plant growth efficiency.

This once again confirms the importance of determining the appropriate concentration threshold and the role of N, P, K supplementation in the early development of seedlings.

3.4.2.3. Conclusion

From the research results, it is shown that the important role of H_2O_2, N, P, K agents in germination as well as seed growth in the early stages:

In the case of H_2O_2: Besides its ability to improve the germination and growth of plants at low concentrations, H_2O_2 can also be used in bactericidal-fungal, seed washing before germination, or preservation and storage of seed…
In the case of N, P, K: It is necessary to add these reagents in the first stage. On each type of seed, determining the optimal threshold for adding macronutrients N, P, K to improve germination and growth is a matter of concern. Indeed, usually, the composition and content of substances in seeds are greatly influenced by geographical factors. Therefore, the study of supplementing minerals to achieve the best results during germination and growth is quite important in research to improve crop yield.

3.5. Effects of PAW and PAW in combination with macronutrients N, P, and K on germination and growth

3.5.1. Germination process

3.5.1.1. Effects of PAW and PAW in combination with macronutrients N, P, K on embryo length and grain weight

To understand the role of PAW and PAW in combined with minerals for germination, studies on embryo length and thousand seed weight were conducted. For all cases, the seed weight soaked with PAW was always higher than that of the Ctrl sample (increased from 24% to 147%). Although the weight of seeds soaked with PAW-5 to PAW-20 increased slowly from 1,62 g to 1,79 g, there was a significant increase in the PAW-25 and PAW-30 conditions, reaching 2,15 g and 3,24 g, respectively. Thus, the presence of ROS or seeds supplied with ROS-containing water induce compounds that enhance germination by disrupting seed dormancy. The presence of H_2O_2 in PAW can activate signaling molecules as demonstrated in the test with H_2O_2 [23-25]. In addition, when the whole particle was exposed to the plasma, only the outer surface of the particle was hydrolyzed by the cold plasma, so it can be seen that the wetting ability of the beads was improved by PAW, which increased the ability to seed water absorption. This is also mentioned in the same way as in previous studies [144, 145].

The length of the embryo is an indirect indicator of the rate of seed development. This demonstrates that PAW has a significant effect on seed germination and development. Bead embryo lengths increased from 3,92 mm to 7,02 mm for samples Ctrl to PAW-20, respectively, and then decreased to 6,24 mm and 5,06 mm for PAW-25 and PAW-20 and 30 respectively. The reduction in embryo length may be due to the excessive absorption of water leading to inappropriate re-formation of polysaccharide bridges between cellulose fibers in the cell wall, resulting in cell damage, breakdown, and death [194].

The results of the research and evaluation showed that using a 15-minute PAW had the most suitable parameters compared to the rest of the cases. Hence, in subsequent studies, PAW 15 was selected in combination with minerals at suitable concentrations.

Thus, under the influence of PAW-15, the absorption of additional N, P, and K increases as their content increases, becoming a condition for better embryo development, and creating a premise for later development of the seed.

3.5.1.2. Effect of PAW and PAW in combination with macronutrients N, P, K on germination rate

Except for sample PAW-25 and PAW-30, the use of PAW increases seed germination and germination rates are different between the samples the day. Finally, after 3 days, the germination rate increased steadily for samples CTRL to PAW-15 to reach the highest value of about 85% for samples PAW-15.

Figure 3.44. Germination and growth of Lettuce seeds after 7th days

When considering the germination rate of PAW-15 with different concentrations of P, and K added from 0 to 200 ppm, the trend was similar to that of N addition (Table 2, 3 Appendix). This response once again demonstrates the value of PAW in activating the germination process to achieve the highest value. Therefore, it can be concluded that the mechanism of germination under the influence of PAW is the activation of metabolism, especially the mobilization of nutrients. In addition, it is the active substances produced by PAW that regulate the signaling network and gene expression to help the seed germination process be best activated. With the observations and experimental results, it can be seen that the mechanism of action of PAW, during the germination stage of curly lettuce, PAW has activated the germination ability quite well. However, to achieve the best value, it is necessary to add minerals N, P, and K at moderate concentrations. This is very meaningful in controlling and increasing the germination rate of seeds.

3.5.1.3. The effect of PAW on the morphological change of seeds

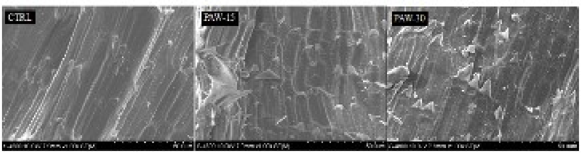

Figure 3.46. Scanning electron microscopy (SEM) images of the grain surface after 2 h

The images show that the surface structure of the particles changes drastically due to the plasma-activated conditions in the water. Specifically, in the PAW-soaked seed sample, the surface structure of the seeds was deformed and partially destroyed, the edges on the seed cuticle gradually disappeared, and peeling marks appeared. This observation is similar to that of Bafoil et al. [155] investigated the modification particle surface by plasma.

3.5.2. Seed growth process

3.5.2.1 Growth of stem height - root length

When compared the effect of PAW on stem height measured 3 days after sowing was highest in the PAW-15 sample, which increased by about 25% compared to the control sample. For the other treatment conditions, there was no significant difference in height except for the PAW-30 model, which was reduced by 36%.

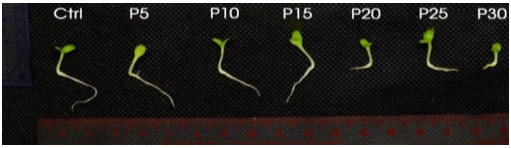

Figure 3.49. Seedlings were harvested on the 5th day

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The seedling image is observed in Figure 3.49, root morphology is heterogeneous and some roots appear as side branches, difficult to conclude the effect of PAW on root length. When comparing the root shape by the eye, the roots of sample PAW-15 were thin, less branched and longer than the roots of the other samples. The roots of samples PAW-10 to PAW-30 were shorter, thicker, and had more accessory or hairy roots, especially for PAW-15. Root length is not a determining factor in the growth and quality of leafy plants. During germination, the seed has enough nutrients from the nutrients stored inside the seed for the embryo to develop. After the germination period, the seedling needs additional external nutrients for its growth. In PAW, NO_3^- is not only a signaling molecule but also a substance that can indirectly add nutrients to seedlings. Thereforre, seedlings in all PAW samples had higher growth indicer than control samples.

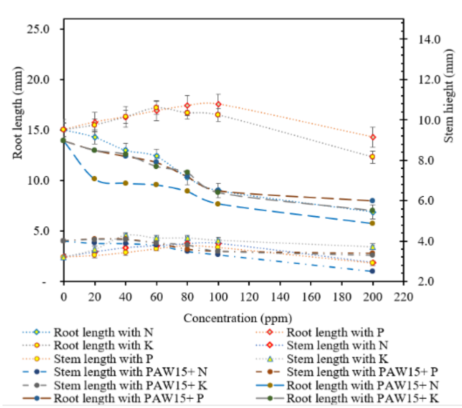

Figure 3.50. The stem height and root length for conditions without and with PAW-15 in combination with N, P, and K from 0 to 200 ppm

At present, there is no research to investigate the effect of coordination ability among them on seed germination and early growth by adding N, P, and K to plasma-activated water. However, recent studies have also investigated differences between the concentrations of active ingredients used for germination and growth in PAW samples. It shows that the quantity and content of active ingredients play an important role in improving the quality of germination and growth.

Therefore, it is necessary to further survey and measure other growth indicators in curly lettuce in the later period to form a basis for the conclusion that adding N, P, and K to PAW15 is really necessary for the growth growth in the early stages or not.

3.5.2.2. Leaf area and chlorophyll content

When the treatment time was increased, the chlorophyll content in leaves increased slightly compared to the corresponding control samples (> 20%), except PAW-15 and PAW-20 min with chlorophyll content significantly higher, 4.03 µg/L and 3.43 µg/L respectively, while the control sample was only 1.26 µg/L. This clearly demonstrated the positive effect of RONS species, especially NO_3^- and H_2O_2, on the chlorophyll-producing enzyme that interacts with the substrate during plant growth. Nitrate is an important component of photosynthesis, sustains plant life, and produces oxygen and nutrients. Without nitrate, the chlorophyll content in leaves is reduced, causing leaves to turn pale green or yellow [163]. Due to the highest chlorophyll content, the largest leaf area, the darkest color, the samples treated with PAW-15 had better quality than other samples. This proves that the chlorophyll content in leaves in green vegetables is an important quality indicator because when degraded, the color of the leaves is changed, reducing product quality. Thus, RONS species (NO_3^-, NO_2^-) promote the formation of photosynthetic active pigments by increasing the number of buffers and thylakoid proteins in leaves as well as by increasing chloroplast formation during growth leaf development [164-167].

The chlorophyll content of lettuce leaves is strongly correlated with theleaf area. It is an important component in photosynthesis, sustains plant life, and produces oxygen and nutrients. The effect of PAW treatment on the area and chlorophyll content of curly lettuce leaves ont day 7 after sowing is shown in Figure 3.54. At this early stage of development, leaf shape can be approximated using an ellipse. Compared with other samples, PAW-15 had the largest area and darkest leaf color. The leaf area in sample PAW-15 was nearly twice larger as that of the control samples (19.86 mm^2) This value is one of the criteria to determine the quality of vegetables. Leaves are photosynthetic organs that contain the pigment chlorophyll along with chloroplasts. Light energy is absorbed by their pigments and transferred to carbon fixation reactions (often called dark reactions) to produce organic matter for plants. In addition, chloroplasts perform several other functions, including fatty acid synthesis, synthesis of many amino acids, and immune responses in plants. Therefore, the increased leaf area demonstrates an increased photosynthetic rate of light absorption, leading to a significant increase in dry matter accumulation rate and crop yield.

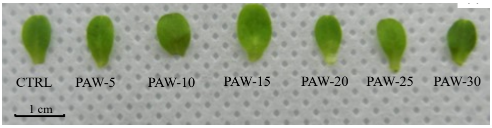

Figure 3.55. Effect of PAW on leaf area of curly lettuce

The results showed that there was a similarity between chlorophyll content and leaf area. When increasing the content of different minerals, there is a slight tendency to increase to the appropriate level, and then the indicators decrease accordingly, even though the germination indicators are as shown above. Under the influence of PAW-15, the leaf area was almost doubled and the chlorophyll content increased by 3.2 times compared with the control. In this investigation, both chlorophyll content and leaf area were slightly increased compared to PAW-15 and PAW-15 with added minerals, while chlorophyll content and leaf area were slightly increased, specifically.

3.5.2.3. Dry/fresh weight ratio

The results found that for all samples surveyed, the measured values for the samples were high. The increase or decrease in this ratio of curly lettuce is strongly correlated with the leaf area. The increase in dry/fresh weight ratio in samples treated with N-conjugated PAW at different concentrations was not much compared to the control sample with only PAW15. When the additional N content is above 60 ppm, this indicator tends to decrease gradually. This is consistent with previous studies by S. Padureanu et al. [153] who showed that PAW increased the dry/fresh weight ratio in plants by enhancing nutrient uptake and utilization. However, it may be due to the negative effects of high N content on plant growth and development, leading to reduced biomass accumulation, reduced nutrient utilization efficiency, oxidative stress and causing carbon-nitrogen imbalance inside the plant [167, 168].

For the addition of P, only a small amount of added concentration (20-40 ppm), there can be a combination of P and substances present in PAW (NO_3^-, H_2O_2) to increase bioaccumulation mass led to a slight increase in dry/fresh weight ratio, then when higher P was added, this index tended to decrease. This can be explained as the plant's ability to absorb nutrients decreases at a higher threshold of mineral supplementation.

For the addition of K: K also plays an important role in the regulation of N- exchange and fixation, in which chlorophyll is the major N storage site of the plant. Therefore, the addition of K at a high concentration did not cause a significant change in the dry/fresh ratio investigated.

3.5.3. Conclusion

Through the study, in the PAW samples over time, the PAW-15 sample showed the best early growth and germination in curly lettuce in 7 days of the study. The decisive factor for this is that PAW contains compounds of RONS and RNS at the right concentrations, which cause morphological changes in curly lettuce seeds resulting in better water and nutrient uptake. To evaluate whether PAW-15 promotes maximum germination and growth, the addition of minerals N, P, and K to PAW-15 The survey showed that the germination rate and growth index still tended to increase slightly under low addition amount. This supplement will have a negative effect if minerals N, P, and K are used in high concentrations. From the above results, it is necessary to use PAW in combination with macronutrients N, P, K to improve germination rate and seed development.

3.5.4. Antibacterial - antifungal ability of PAW

3.5.4.1. Antimicrobial

The solution made by cold plasma at intervals of less than 25 min did not affect the growth of *Xanthomonas* spp. This result suggests that solutions made from cold plasma are highly resistant to the bacteria cause leaf spots. After 1 day of bacterial culture, the colony density decreased as the plasma activation time increased, in which the highest number of colonies was in the control sample.

The increased resistance effect over time is due to the increase in ROS and RNS content formed during the solution action by cold plasma jets.

In the case of determining the antibacterial effect of the solution made by cold plasma based on the radius of the antibacterial ring in Table 3.2, it is clear that the plasma-formed solution in a period of 15 minutes, the antibacterial ring begins to appear with half a radius the glass is 02mm. The longer the action time of the plasma beam, the larger the diameter of the antibacterial ring. This idea indicates that the cold plasma solution is completely resistant to *Xanthomonas* spp.

3.5.4.2. Anti-fungal

The method of determining the minimum inhibitory content (MIC) was used to evaluate the antifungal activity of cold plasma samples. The results of determining the minimum inhibitory content of MIC of the solution made by cold plasma to *Fusarium* spp.

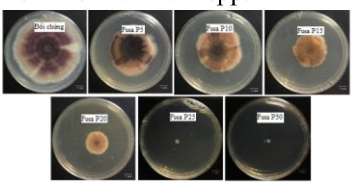

Figure 3.63. Photographs of agar plates containing control samples and solutions made by cold plasma at different times with *Fusarium* spp.

The figure above shows that the solution formed by cold plasma at different time intervals affects the growth of mycelium *Fusarium* spp.

dissimilarity. Therefore, we can conclude that the MIC point of the solution formed by cold plasma is at 25 min. This is consistent with previous studies, the solution formed by cold plasma at a longer time of 25 and 30 min has a larger antifungal ring, this is due to the antifungal compounds present in the solution created by a higher concentration of cold plasma [175].

With the ability to generate RNS and ROS from cold plasma discharge into water formed during the solution's impact by cold plasma jets, PAW clearly exhibits antibacterial and fungal activity. The increased resistance effect over time is due to the increase in the content of the compounds produced.

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CONCLUSIONS AND RECOMMENDATIONS

Conclusions

In this thesis, plasma activated water has been studied and prepared by gas-liquid plasma discharge technique on two types of discharge, DBD and Corona. The electrical characteristics of the plasma generating system as well as the composition and properties of the PAWs were also analyzed and evaluated. In addition, the study also evaluated the activity to improve the development of curly lettuce (*Lactuca sativa* L.) at the early stage through the weight index of thousand seeds, embryo length, germination rate for the plant. germination and stem height index, root length, chlorophyll content, dry/fresh weight ratio, leaf area for growth on the agents in the following cases: H_2O_2, N, P, K, PAW and the combination of PAW with N, P, K. The obtained results are as follows:

- ✓ Successfully built 02 systems to generate PAW from the discharge of 2 types of Corona plasma and DBD plasma on the same self-made source. These systems have also been electrically characterized on oscilloscopes with voltage, current and frequency parameters. On that basis, determine the electrical characteristics of the plasma discharge type and the power consumption of the plasma system. The results show that the source generates a pulsed discharge pattern, a characteristic of cold plasma, with a low power consumption of only 47 W. From the results, the system can be deployed effectively when applied on a large scale. big.
- ✓ The composition and properties of all PAW samples were determined from 2 types of discharge. Thereby, a suitable PAW sample was selected to be applied to the research on germination and early growth stage of lettuce. The results show that the PAW sample with a launch time of 15 minutes by the DBD plasma system is the most suitable for the study.
- ✓ The role of oxidizing agent H_2O_2 has been shown for germination and growth as well as antibacterial and antifungal activity. The results show that, at low concentrations, H_2O_2 at <10 ppm has the role of a signal molecule, a good

stimulant for seed germination and growth. On the other hand, with high H_2O_2 content (> 1,000 ppm), it will inactivate pathogenic bacteria and fungi present on seeds, which has a good effect on the process of seed washing and sample cleaning to limit the generation of pathogens in the early stage.

- ✓ The role of inorganic minerals N, P, K has been shown in the germination and early growth stages of curly lettuce. The results have determined the appropriate content of the above minerals for the good growth of plants in the early stages of development. The suitable concentrations for this process are 100 ppm for N, 80 - 100 ppm for P and 20 - 40 ppm for K. The results again show the need for nutritional supplementation of N, P, and K in the initial stage of seed.
- ✓ High activity of PAW was shown for the germination and growth of curly lettuce. Yes, water activated by cold plasma with DBD discharge for 10 to 20 minutes will contain RONs and RNSs at suitable concentrations, which have positive and effective impacts on seed germination and seedling development. In addition, the study also showed the antibacterial and antifungal ability of PAW, which inhibits the growth of microorganisms.
- ✓ It has been shown that combining PAW-15 with inorganic minerals such as N, P, K in the early stages for germination and growth on curly lettuce is necessary. Yes, the addition of minerals N, P, K at small doses: 40-60 ppm with N, 20-40 ppm with P and less than 10 ppm with K into PAW-15 solution showed that there are increasement for both germination and plant growth when compared with the control and PAW-15 samples. This result has found the optimal growth threshold of plants in this case.

Recommendations

Further study of the interaction of the mixture of N, P, K with PAW for sprouting and growth of curly lettuce. At the same time, learn more deeply about the mechanism of interaction between the composition, the content of substances created from PAW and the added elements to serve as a more complete basis for the physiological processes and mechanisms of food. thereby confirming the role and possibility of using plasma-activated water in agriculture and industry.
Investigate the growth indices of PAW applied on curly lettuce in the later period to demonstrate the effectiveness of PAW and the combination of N, P, K with PAW.

Test PAW on other vegetables in the same family to examine the interaction, growth and development of plants as a basis for further studies.
Further evaluate the difference in efficiency between PAW and plasma gas for the growth and development of curly lettuce.

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NEW CONTRIBUTIONS OF THE THESIS

The thesis has successfully researched the design and construction of two different plasma discharge systems to prepare plasma activated water samples. The composition, content and properties of these water samples were determined in order to aim at improving the quality of growth and development process in the early stages of Lactuca sativa L. The thesis also figure out that the addition with inorganic minerals such as N, P, K in the early stages of seeds is necessary because the contents of endogenous substances of the seeds are deficient, possibly due to geographical factors.

The thesis has completely assess the role of compounds from plasma activated water in the process of improving seed germination quality and early growth of seeds. The study also clarifies the impact mechanism of each compound and mixture on this process. The study also pointed out the necessity of combining plasma activated water with inorganic minerals N, P, K for germination and growth.

Besides, the thesis also shows the overall relationship between the preparation technique of the composition, properties and content of substances and the plant physiological processes. The obtained results are the basis for applying new techniques to plant development research field.

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LIST OF PUBLICATIONS

1. Than, H.A.Q., Pham, T.H., Nguyen, D.K.V., Pham, T.H., Ahmed Khacef, 2022, *Non-thermal Plasma Activated Water for Increasing Germination and Plant Growth of Lactuca sativa* L.*,* Plasma Chemistry and Plasma Processing, 42, pages73–89. *(SCI, IF = 3,148).*

2. Than, H.A.Q., Nguyen, D.K.V, Dinh, H.Q., Nguyen, T.T., Pham, T.H., Pham, T.H., 2021, *Effect of H_2O_2 Concentrations on The Germination and Seedling Growth at Early Stage of Lettuce (Lactuca savita var capitata* L.*),* Journal of Plant Protection, 4, 297 (ISSN 2354-0710)

3. Than, H.A.Q., Dinh, H.Q., Nguyen, D.K.V., Pham, T.H., Pham, T.H., 2021, *Evaluation of invitro antimicrobial efficacy of the cold plasma against Xanthomonas spp. and Fusarium spp.*, The 20th National Conference of Phytopathological Society of Vietnam V.P.S., page 345-354. (ISBN: 978-604-60-3373-8).