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Non-Thermal Plasma as an Alternative to Enhance the Early Growth Structures in Lentil Plants

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Abstract: The scarcity and contamination of water, aggravated by the effects of Climate Change, endanger the food supply, cause health problems to humans, and are a critical concern. New research has been carried out to improve the quality of water used in the agricultural sector. One of them is the technology of non-thermal plasma (NTP) generated by corona discharges using air as a working gas. In this study, the NTP is applied directly and causing the activation to three water sources: potable, wastewater from poultry farming, and rain, on the legume "lentil." The results show that the NTP applied to the different water conditions modifies the legume structure, obtaining a better germination and growth rate. In particular, it found that the best condition to stimulate the plant structure growth is using wastewater from poultry activities, which NTP activates. Likewise, it identified the internalization of pathogenic microorganisms such as *Escherichia coli* and *Salmonella typhimurium* since the early development of the plant. The bacteria reduction after NTP application is detected due to the effect of the reactive species generated by the NTP. The NTP application for water activation can represent an alternative to solve the demand for food since the development of the structures of legumes, particularly of lentils, is promoted.

Keywords: agriculture; bacteria-internalization; non-thermal plasma; growth-plant; pathogen

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1. Introduction

Agriculture is one of the most important productive sectors around the world. The priority needs to supply food for human, its strong relationships with cattle raising, and the development of new technologies for energy production. Together provide a source of employment in countries, enhancing their economic position and, reducing poverty and undernourishment in the population [1–4]. According to the Food and Agriculture Organization of the United Nations [5], agriculture demand tripled in the period between 1960–2015, and it is expected by 2050, more than 9 billion people and a particular population than 11 billion by the end of this century, will demand a significant accomplishment on this sector.

Today, food safety is threatened by an alarming scenario, a powerful and rising pressure on environmental resources [6–8]. It is reflected in the adverse effects generated because of anthropogenic activities associated with urbanization, industrialization, and pollution and the intensified conditions produced due to Climate Change [9,10]. Warming, modifications in precipitation regimes, unpredictable rainfalls, and droughts have caused severe alterations. Degradation of physical and chemical properties as well as damage of microbiota in non-renewable source, the soil, has carried out its declination of nutrients and hence, a stagnating of food production. With this source, water plays a vital role because its availability is essential to supply requirements for the irrigation of crops. Worldwide, some regions have been affected by water scarcity and the absence of safe effluents; then, technological alternatives to increase efficiency in the use and tolerance to the lack of this vital source to benefit the germination and development of crops are required [11].

The most widely used method to significantly enhance plant germination and growth is applying chemical substances such as fertilizers, pesticides, and plaguicides. Nevertheless, potential adverse environmental and human health effects have been identified and remain a critical concern [12,13]. In the last years, different technologies have been proposed to reduce the usage of chemical substances. However, there are disadvantages such as the high cost of physical installations and equipment, modifications in seed properties, changes in its behavior due to light, temperature, and oxygen, as well as significant periods required during their implementation and adverse effects generated for by-products [14–18].

Recently, novel green technology has been developed to contribute to agriculture applications: non-thermal plasma (NTP). This is generated by an intense electric field between two conductors and gas, resulting in a partially ionized gas containing ions, electrons, reactive chemical species, and UV radiation [19]. Some research works have demonstrated significant efficiencies incrementing of seedling development of NTP that is applied to activated water or directly on seeds, implying low environmental impact. These effects are attributed to the stimulation of sources and the change of their absorption properties, generated by the production of hydrogen peroxide, nitrate, and free radicals, which benefit the efficiency of water use [20–23]. Additionally, it is detected a protective effect associated with the inactivation of microorganisms without causing any damage to the organic structures or altering the physicochemical and organoleptic properties of food. Some treated seeds correspond to black soybean, tomato, pepper, maize, lettuce, radish, rapeseed, spinach, rice, and lentils [24–28].

Many studies used tap water to carry out experiments for agronomy applications [29,30]; unfortunately, in many countries, wastewater is required to irrigate crops due to water scarcity or unavailability in some places with the required quality parameters. It is worth mentioning that irrigation with polluted water significantly increases the risk of food contamination; hence, it represents a severe risk to human health. According to estimations by the Centers for Disease Control and Prevention [31], 60% of infectious diseases in people are extended from animals by different routes, which include: direct and indirect contact, vector-borne, foodborne, and waterborne. Hirneisen et al. [32] and Wright et al. [33] highlight the importance of the knowledge of bacteria-root relation because its interaction could represent a source of pollution and a potential risk for the human being, a product of bacteria migration through the structures of the plant. Furthermore, some research works focused on the germination rate under the influence of NTP. Nevertheless, they exclude important and specific parameters such as those involved in the growth of root, stem, and development of leaves, which are structures that enable anchorage of the plant to the soil, provide support, and absorb the nutrients and water that it requires in photosynthesis as well as in biochemical processes. The addition of these factors makes it possible to tolerate adverse conditions resulting from Climate Change enabling their internal flow for the development of the plant and establishing a beneficial relationship with the microbiota and environment [34,35].

This study considered the lentil as a seed high in protein, fiber, and micronutrients. This has importance recognized by the Food and Agriculture Organization of the United Nations [36] as one of the most promising legumes to help solve problems in the world of hunger and malnutrition. Evaluated the effects of the NTP generated by corona discharge under atmospheric pressure conditions in the early growth phase of the lentil plant. This was carried out in two stages with a plasma reactor configuration, which allows it to be adapted to the plant structures. The first treatment stage involves activating potable water, rainwater, and residual water from a real effluent from poultry farming by NTP before irrigation. In the second stage, the seed/plant receives direct application by NTP after its irrigation stage with the same water sources. Both treatments were applied six times on average for 15 days and compared with experiments used as patterns, with seeds irrigated with potable water, wastewater, and rainwater, without any treatment. Also, in the case of seeds irrigated with wastewater from poultry farming, carried out the internalization of *Escherichia coli* (*E. coli*) and *Salmonella enterica serotype typhimurium* (*S. typhimurium*) bacteria in the roots and stem of lentils.

2. Materials and Methods

2.1. Lentil Seeds for Experimental Tests

Lentil seeds were separated from solid wastes and washed with sterile water. Then, they were dried and cleaned with ethyl alcohol, and moisture was removed by exposure to environmental conditions (average temperature and pressure: 300 K, ~1 atm).

Lately, forty lentil seeds were placed in a sterile porous surface, a cotton layer (5 mm thickness) integrated in the bottom of each glass vessel (480 cm^3 volume), to simulate soil conditions. We do not consider this last source to avoid interferences attributed to minerals and microorganisms in the development and growth of plants. Then, irrigation with 25 cm^3 volume of liquid was carried out according to the following considerations:

- Experimental test 1: Potable water (PW).
- Experimental test 2: Activation of potable water by non-thermal plasma (ANTP-PW) for subsequent irrigation of lentils.
- Experimental test 3: Direct application of non-thermal plasma on lentils irrigated previously with potable water (DNTP-PW).
- Experimental test 4: Wastewater from poultry farming according to the physical and microbiological conditions generated within 24 h. Water could contain wastes from chicken feed, which has 12.0% raw protein, 3.5% crude fiber, 12.0% moisture, and pH = 6.4 because of a mixture of ground cereals such as sorghum, corn, and wheat (FW).
- Experimental test 5: Activation of wastewater from poultry farming by non-thermal plasma (ANTP-FW) for watering the lentils afterward.
- Experimental test 6: Direct application of non-thermal plasma in lentils irrigated previously with wastewater from poultry farming (DNTP-FW).
- Experimental test 7: Rainwater, harvested in the rainy season, 30 min after the rain started (RW).
- Experimental test 8: Activation of rainwater by non-thermal plasma (ANTP-RW).

The time of experimental evaluation for lentil growth was 15 days, in which tests 2, 3, 5, 6, and 8 received six times the proposed treatment by NTP. The seeds were grown under exposure to environmental conditions (average temperature and pressure: 300 K, ~1 atm). Morphological parameters concerning their length and thickness for the stem and the primary root were determined, whereas the leaves were counted. In cases: 4, 5, and 6, microbiological analysis was carried out to determine the internalization of *E. coli* and *S. typhimurium* bacteria in stem and root.

2.2. Configuration of the Non-Thermal Plasma Reactor

To carry out the experiments, the configuration of the reactor, according to Figure 1, was implemented. The negative electrode is a truncated cone shape manufactured with a mesh of stainless steel 304 with 38.5 cm^2 in surface area. It can be placed inside/outside of a square glass vessel of 480 cm^3, according to the plant size, to prevent damage to its structures.

The reactor has a gap of 1.0 cm; the anode was a cylindrical pin of 0.2 cm^2 surface area of stainless steel. It was located at the superior side of the experimental setup, concentrically to the cathode. The power supply to create NTP and devices for measurement of electrical parameters are shown in Figure 2. The first consists of an oscillator with a single transistor, which receives an input signal of 5 V of direct current and generates a pulsed signal with a high frequency in the primary coil of an inverter transformer to be amplified in the secondary winding of it. Lately, a voltage multiplier operates to obtain an output periodical and under-damping signal with an average voltage of 347 kV, a pulse width of 100 µs, and a frequency of 10 kHz. Voltage and current parameters were monitored by an oscilloscope

Tektronix TBS 1152B-EDU through a high voltage divider and a current probe Hantek CC-65, respectively.

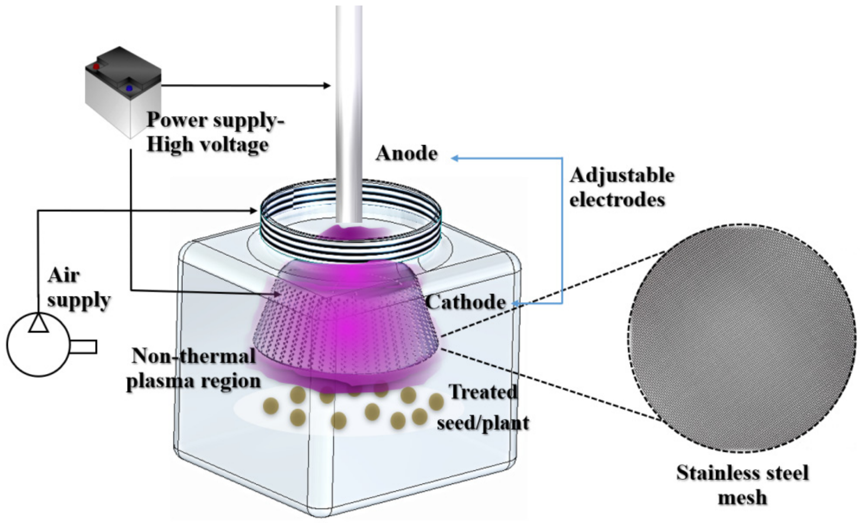


Figure 1. Experimental configuration of NTP plasma reactor.

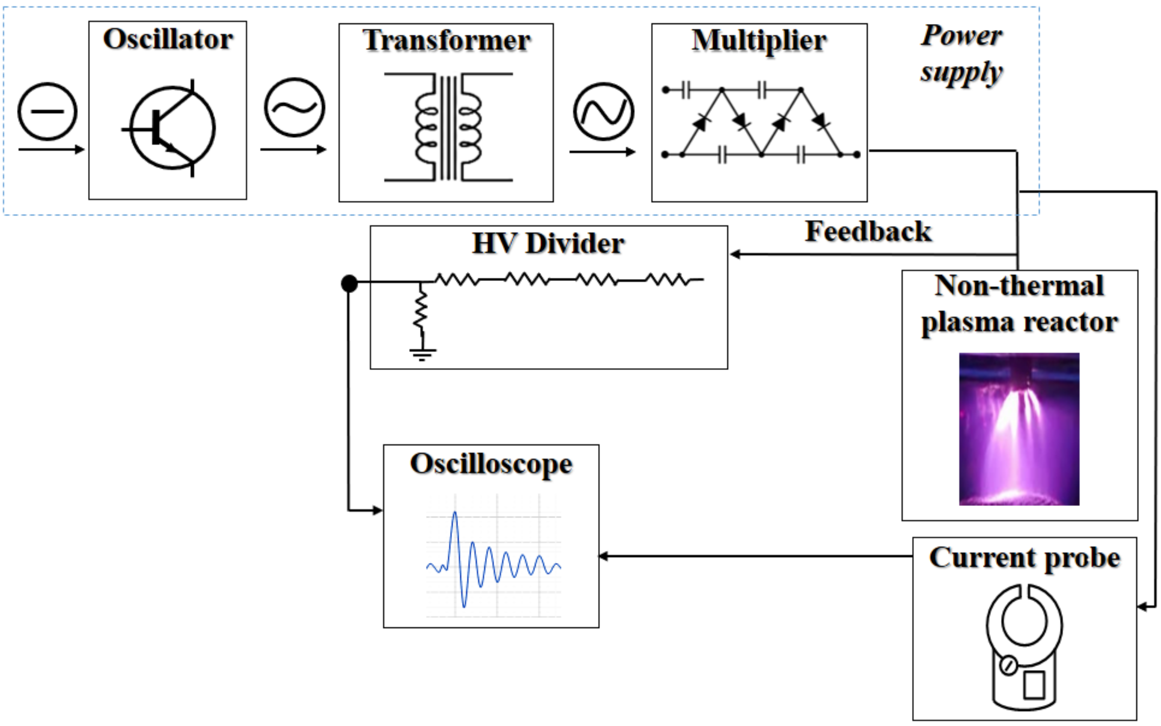


Figure 2. Experimental configuration of NTP system proposed in this study.

2.3. Activation of Hydric Supply by NTP and Irrigation Stage

For experimental tests, 2, 5, and 8, activation of a 25 cm^3 volume of the hydric source was carried out in a cleaned and sterile glass vessel. Cathode and anode were inserted into the vessel, and NTP was applied continuously for 120 s, maintaining air injection in the electrical discharge area. When the activation time finished, the vessel was closed and shaken by 120 s to be allowed to stand undisturbed for 1800 s. It was established according to the range value of the half-life time of ozone reported by Hoigné [37], Langlais, et al. [38], and Epelle, et al. [39]. Later, lentils were irrigated homogeneously using the activated water produced previously. A similar activation process for water generated from poultry activities was carried out, requiring an additional stage to remove particles of large size before starting the treatment. This process was repeated every two days, according to the hydric requirements of lentils, until complete six applications of the treatment.

2.4. Direct Application of NTP

For experimental tests 3 and 6, direct application of NTP required emptying homogeneously 25 cm^3 volume of potable water directly in a glass vessel containing lentil seeds in germination and, lately, growth. When the cotton base absorbed water, the corona reactor was adapted to apply NTP. The anode of the reactor can be able to be adjusted according to the size of the plant. As long as lentils did not have an ideal size of 50 mm, the positive electrode could be inserted into the glass vessel to carry out the treatment. When length had a superior value of 50 mm, the anode was placed outside of the container, maintaining a gap to promote contact between the synergistic effects produced by applying NTP and seeds/plants. At the final stage of direct implementation of plasma, vessels containing seeds/plants were closed for 1800 s. It was carried out every two days on average, considering the needs of lentils.

2.5. Measurement of Structures in Lentil Plants

At the end of the established period (15 days), lentil plants were carefully extracted to determine the dimensions of the root and stem using a Vernier measuring device. Then, the average length (L) of these structures was determined according to Gao et al. [40]:

$$L\,[\mathrm{cm}] = TL\,[\mathrm{cm}]/TP \quad (1)$$

where TL corresponds to the measured length of plants analyzed according to each structure and TP, the total number of plants.

In the case of the determination of the number of leaves, it was determined by counting the units of these structures per plant. Results in this study are presented in accordance to the statistical analyses indicated by Riley [41].

2.6. Microbiological Analysis of Water from Poultry Farming

After the remotion of particles of considerable size, took a homogeneous sample of poultry farming for microbiological analysis.

An aliquot of 0.1 mL was taken to be inoculated in Petri dishes containing 25 mL of Violet Red Bile agar (VRB agar, NEOGEN), used as a culture medium, by the spread plate technique. Serial dilutions were prepared and inoculated by triplicate; incubated in an inverted position at 310 K during 18–24 h. Later, bacteria counting was carried out to determine bacteria concentration in water by:

$$BC\,[\mathrm{CFU/mL}] = AB \cdot DF/VI \quad (2)$$

where BC is the bacteria concentration, AB is the average bacteria, DF is the dilution factor, and VI is the volume of inoculum plated.

For each analyzed sample, it is essential to have a control Petri dish with VRB agar without inoculum as a reference to ensure the sterility of the culture medium used in the experiments.

2.7. Microbiological Analysis for Internalization Bacteria

After treatments (experimental tests 4, 5, and 6) were carried out, when the established period for growing seeds irrigated with wastewater from poultry farming finished, the surface of the lentil was cleaned with ethyl alcohol to be divided into two sections: stem and root. Then, each segment (1 g) was mixed and ground in 10 mL of sterile water. Past 10 min, a pattern sample of liquid was available to achieve serial dilutions (1:10^1 to 1:10^6) and start the microbiological analysis to determine the bacteria concentrations which were internalized in the plant.

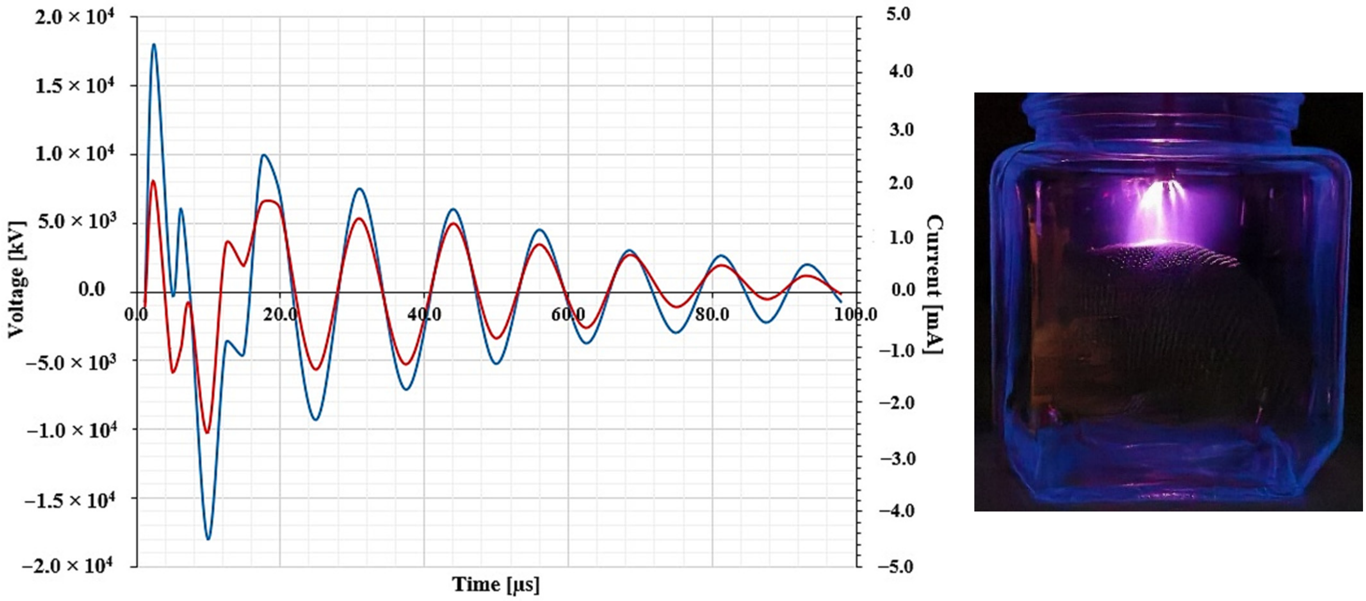
The microbiological growth medium used to determine bacteria concentration inside the structures of lentils was VRB agar. A volume of 25 cm^3 of the microbiological medium at 318 K was emptied in Petri dishes (by triplicate); once solidified, an aliquot of 0.1 mL of each pattern sample, and dilutions, were obtained from stems and roots was distributed by spread plate technique. Then, inoculated Petri dishes were incubated at 310 K for 18–24 h, and quantitative internalization of *E. coli* and *S. typhimurium* in the plant was carried out by:

$$log_{10} \text{ internalization} = log_{10} (C_0 - C_1) \quad (3)$$

where C_0 is the bacteria concentration of reference (pattern sample or without the application of treatment) and C_1 the is bacteria concentration detected after the treatment application by NTP. Bacteria concentration is determined according to (Equation (2)).

3. Results

- The experimental conditions established in the tests of this work allowed the identification of effects generated in different hydric supplies by the application of NTP. Figure 3a shows the voltage and current waveforms detected in corona discharge with the injection of air (Figure 3b), in the early development of structures of lentils.



(**a**) (**b**)

Figure 3. Waveform registered experimentally (**a**) as a result of corona discharge (**b**) to stimulate the growth of lentil structures.

In the following sections, the effects generated in lentils are described:

Main root: According to Table 1, lentil plants irrigated with PW reach a mean length of 33.96, *s.e.* 0.73 mm. The application of NTP stimulates the growth of this structure; plants irrigated with ANTP-PW are 44.5% taller than PW, while by DNTP-PW, lentils achieve an additional percentage of the root growth (74.8%).

The thickness of the main root (1.00 with *s.e.* 0.04 mm; Table 2, PW) is another modified parameter in plants due to its interaction with NTP. Both treatments using this technology, by activation (Table 2, ANTP-PW) or direct application (Table 2, DNTP-PW) on the plant after its irrigation, indicate that thickness of the root shows a thickening of at least 38% after 15 days of treatment.

Table 1. Length of the root of lentil plants in experimental tests.

Experimental Test	Mean Value Length of the Root [mm]
PW	33.96 with *C.I.* (32.43, 35.50)
ANTP-PW	49.06 with *C.I.* (46.19, 51.93)
DNTP-PW	59.39 with *C.I.* (55.50, 62.73)
FW	43.37 with *C.I.* (41.40, 45.34)
ANTP-FW	62.59 with *C.I.* (59.99, 65.19)
DNTP-FW	52.28 with *C.I.* (50.34, 54.22)
RW	65.12 with *C.I.* (62.48, 67.77)
ANTP-RW	42.80 with *C.I.* (39.90, 45.69)

Table 2. The thickness of the root of lentil plants in experimental tests.

Experimental Test	Mean Value Thickness of the Root [mm]
PW	1.00 with *C.I.* (0.92, 1.08)
ANTP-PW	1.38 with *C.I.* (1.23, 1.53)
DNTP-PW	1.43 with *C.I.* (1.32, 1.55)
FW	1.02 with *C.I.* (0.95, 1.08)
ANTP-FW	1.43 with *C.I.* (1.16, 1.71)
DNTP-FW	1.03 with *C.I.* (0.91, 1.15)
RW	1.41 with *C.I.* (1.29, 1.52)
ANTP-RW	1.42 with *C.I.* (1.28, 1.55)

Irrigation with the effluent of FW (Table 1) enables a mean growth in the root of 43.37 with *s.e.* 0.94 mm, which is 27.7% higher than the hydric supply using PW. About the thickness of the main root, it is 1.02 *s.e.* 0.03 mm (Table 2, FW). When water from poultry farming is activated (Tables 1 and 2, ANTP-FW), the length of the root reaches 44.3% additional, and 40.2% in its thickness compared with the usage of the same hydric source, but without the application of any treatment (FW). Considering the same reference, the main root increases 20.5% in length, and ~1.0% in its thickness by direct application of NTP on the irrigated plant (Tables 1 and 2, DNTP-FW).

By using RW to satisfy the hydric requirements of lentils, it is possible obtaining the highest mean length in the root (65.12, *s.e.* 1.24 mm; Table 1), and a mean thickness of 1.41 *s.e.* 0.05 mm (Table 2). With respect to RW, the closest performance is achieved through the use of ANTP-FW, which it is ~4% slightly lower in its mean length, but it is ~1% superior in its mean thickness.

When NTP is used to activate rainwater (Tables 1 and 2, ANTP-RW), lentil plants are 34.3% lower in mean length of the root than RW, but 26.0% superior in the same parameter to PW. In its thickness, an increment of 0.7% for RW used for irrigation is detected.

Stem: Hydric supply for lentils using PW achieves a mean height of 31.83 with *s.e.* 0.86 mm (Table 3) and a mean thickness of 0.95 with *s.e.* 0.03 mm (Table 4). By the application of NTP on PW, an increment of 54.2% in height structure is registered when this hydric source is activated (ANTP-PW) and, 22.6% in the case of direct treatment on the irrigated plant is carried out (DNTP-PW). Relative to the thickness of the stem (Table 4), no significant differences are detected in the implementation of treatments by NTP using potable water.

By the employment of FW, a mean height of 88.68 with *s.e.* 1.24 mm is generated, it reflects a significant mean difference (~178%) with respect to the irrigation with PW without the influence of NTP. In relation to FW, the performance in the development of the height of the stem enhances by more than 13.0% due to both treatments applying NTP.

In the case of RW, the height of the stem reaches 45.87 with *s.e.* 1.04 mm, which is 44.0% higher than PW but, 93.3% lower than FW. When RW is activated, a considerable increase is detected in the stem of height (39.0%).

Table 3. Height of the stem according to the applied treatment after fifteen days.

Experimental Test	Mean Value Height of the Stem [mm]
PW	31.83 with *C.I.* (29.96, 33.70)
ANTP-PW	49.08 with *C.I.* (46.34, 51.81)
DNTP-PW	39.03 with *C.I.* (35.53, 42.53)
FW	88.68 with *C.I.* (86.10, 91.27)
ANTP-FW	102.40 with *C.I.* (98.65, 106.16)
DNTP-FW	100.64 with *C.I.* (97.96, 103.31)
RW	45.87 with *C.I.* (43.64, 48.09)
ANTP-RW	63.75 with *C.I.* (60.17, 67.34)

Table 4. The thickness of the stem registered as a result of different treatments.

Experimental Test	Mean Value Thickness of the Stem [mm]
PW	0.95 with *C.I.* (0.89, 1.01)
ANTP-PW	1.07 with *C.I.* (0.94, 1.20)
DNTP-PW	0.99 with *C.I.* (0.88, 1.09)
FW	0.95 with *C.I.* (0.87, 1.03)
ANTP-FW	0.98 with *C.I.* (0.85, 1.11)
DNTP-FW	0.96 with *C.I.* (0.89, 1.04)
RW	1.00 with *C.I.* (0.95, 1.06)
ANTP-RW	1.03 with *C.I.* (0.94, 1.12)

Opposite to the root case, NTP does not have a substantial influence in the development of a more robust structure in the stem when any of the hydric sources evaluated in this work are used.

Leaves: Features derived from hydric supply have influenced the development of leaves in lentil plants. Table 5 is observed that, when PW is used for the irrigation of plants, it is detected the lowest mean number of leaves (0.67 with *s.e.* 0.21) in the experiments carried out. Likewise, this parameter is increased by the application of NTP in PW: 6.5-fold by activation (ANTP-PW) and, 5.8-fold by direct implementation (DNTP-PW) on the irrigated plant.

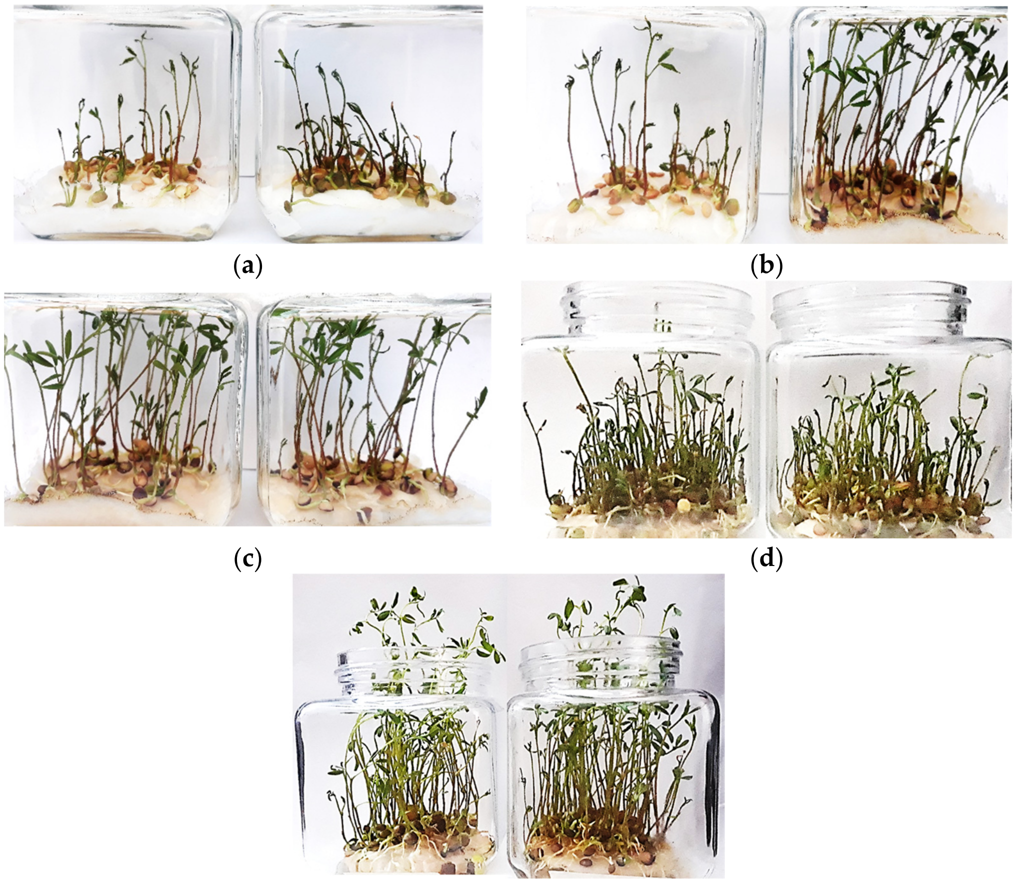
Table 5. The number of leaves in lentil plants irrigated with the hydric sources proposed in this work.

Experimental Test	Mean Value Number of Leaves
PW	0.67 with *C.I.* (0.22, 1.12)
ANTP-PW	5.00 with *C.I.* (3.76, 6.24)
DNTP-PW	4.55 with *C.I.* (4.08, 5.01)
FW	7.50 with *C.I.* (6.22, 8.78)
ANTP-FW	9.45 with *C.I.* (7.32, 11.59)
DNTP-FW	6.17 with *C.I.* (5.12, 7.18)
RW	5.25 with *C.I.* (4.26, 6.24)
ANTP-RW	3.67 with *C.I.* (2.89, 4.44)

Modification of aqueous source to FW in order to satisfy the requirements of plants, benefit the development of leaves. In this regard, the mean value of leaves corresponds to 7.50 with *s.e.* 0.60 mm. The application of NTP has a positive influence (>25%) when it is applied to activate the FW.

In the case of RW, the number of leaves increases 6.8-fold with respect to the use of PW; but when ANTP-RW, its positive influence is reduced ~30% to contribute to the efficiency in the development of these structures.

Figure 4 presents the final stages of treatments carried out using potable water, wastewater from poultry farming, and rainwater.



(**a**) (**b**) (**c**) (**d**) (**e**)

Figure 4. Comparative images of experimental tests after the fourth time of irrigation: (**a**) PW (**left**) vs. ANTP-PW (**right**), (**b**) PW (**left**) vs. FW (**right**), both without treatment, (**c**) ANTP-FW (**left**) vs. DNTP-FW (**right**), (**d**) RW (**left**) vs. ANTP-RW (**right**); in fifth time of irrigation; (**e**) FW (**left**) vs. ANTP-FW (**right**).

In the case of wastewater from poultry farming, microbiological analysis indicates a reduction in the concentration of *S. typhimurium* and *E. coli* bacteria at the final implementation of treatments by NTP.

Internalization of *S. typhimurium* bacteria. In experimental tests 4, 5, and 6 (Figure 5) FW was used to irrigate lentil seeds. For *S. typhimurium* bacteria, the initial average concentration in raw wastewater was 1.41×10^7 CFU/mL (mean 7.14-$\log_{10}$ with *s.d.* 0.15), and the identified structure with a higher concentration in the plant was the root. When wastewater is applied to lentil plants, *S. thypimurium* bacteria is internalized in 54.2% and 93.8% in stem and root, respectively, concerning the reported initial concentration. When NTP treatment is performed, applying it directly to the plant after its supply with wastewater from poultry farming, the internalization of bacteria is decreased by 1.22 (~94.0%) for the stem. At the same time, the root is detected 0.24-$\log_{10}$ (42.0%) reduction in relation to the test in which raw wastewater is used for hydric supply. Considering the same reference, when wastewater was activated by NTP before the irrigation stage, the highest reduction of bacteria internalized in the root is detected in this case (0.45-$\log_{10,}$ 65.0%), while in the stem, the concentration reaches a decrease in 0.66-$\log_{10}$ (59.5%).

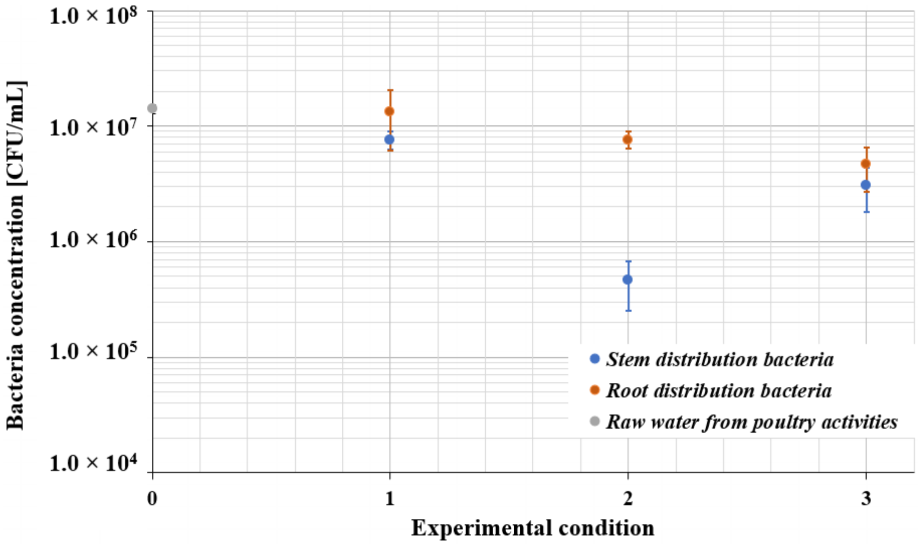


Figure 5. Internalization of *S. typhimurium* bacteria in lentil structures. Experimental condition: (0) raw wastewater from poultry activities, (1) without application of a treatment, (2) direct application of NTP to plants irrigated previously with wastewater from poultry activities, and (3) activation of raw wastewater used for irrigation of seeds.

Internalization of *E. coli* bacteria. The concentration of *E. coli* bacteria in raw wastewater is 7.00×10^6 CFU/mL (mean 6.84-$\log_{10}$ with *s.d.* 0.90; Figure 6); internalization of this microorganism in lentil plants is superior in root than in stem to the pattern sample. When the plant is irrigated with raw wastewater without any treatment, bacteria penetrate the stem at 62.4% and root at 84.0%. Once the NTP is applied directly to lentil plants after they have been irrigated, registered a reduction of 46.7% in stem and 21.8% in root in relation to the internalization registered using raw wastewater. On the other hand, when NTP is used to activate wastewater to be supplied to the plant, before the irrigation process, bacteria reach a decrease of 1.03-$\log_{10}$ (90.7%) in stem, and 0.26-$\log_{10}$ (44.7%) reduction in the root, with respect to the supply with raw wastewater from poultry farming.

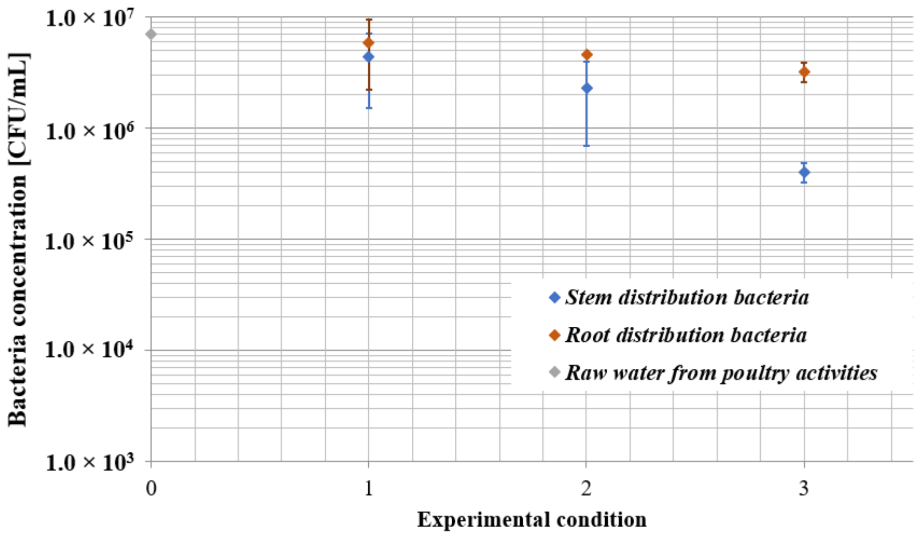


Figure 6. Internalization of *E. coli* bacteria in lentil structures. Experimental conditions: (0) raw wastewater from poultry activities, (1) without treatment application, (2) direct application of NTP to plants irrigated previously with wastewater from poultry activities, and (3) activation of raw wastewater used for irrigation of seeds.

4. Discussion

The growth of the organic structures in lentils depends on water. The unavailability of this vital source causes stomata closure and a reduction in the development of structures, deriving in the declination of the photosynthesis process. Under these conditions, production is affected, and the plant's susceptibility to being damaged by pathogens can cause infections, prompting affection in the end-consumer [42–45]. In response to the current situation of scarcity and contamination of water, our research focuses on the application of water sources of different characteristics. In this situation, is the NTP applied directly or by activation.

Lentil crop belongs to the legumes (*Fabaceae* family), which is considered dormant, so it needs a minimum stimulus to initiate its germination and growth stage [46]. In this regard, NTP has a positive influence as a stimulus agent in the development of lentils, whether applied directly or by activation of hydric supply. In this study, it is registered for both treatments, the main stimulus activity for germination within the first two days of the testing stage. Performance reaches ~6.0% superior to patterns (potable water or wastewater). In addition to this, the selected period of 120 s for the application of treatments is in accordance with those established for different leguminous plants [47]; hence, lentils could be considered as a seed capable of receiving NTP without it representing a risk or cause affectation in its growth.

Properties acquired by water and wastewater during NTP treatments, with the proposed configuration reactor, contribute to increasing lengths [29] and thickness of structures. Root and stem structures as well as the development of leaves are essential in the photosynthesis process. According to the results, the most effective treatment to benefit the growth of the primary root using potable water is the direct application of plasma. At the same time, this source is replaced by wastewater from poultry farming, and activation is the suggested treatment for developing the structures: root, stem and leaves. During the implementation of treatments, potable water and wastewater from poultry farming undergo a slight decline in pH (0.2) [30]. Zhou et al. [23] detected the reactive nitrogen species as substances able to partially acidify the aqueous medium, which could be associated with an increment in nitrogen fixation reported in legumes due to the implementation of NTP process [48]. It is known that a basic nutrient for plant growth is nitrogen [49,50]; plants can absorb it as nitrate (NO_3^-) or ammonium (NH_4^+). This absorption varies for each vegetable [51,52]. Two routes of ammonium assimilation have been identified. First, it is reduced to nitrite (NO_2^-) by the activity of the enzyme nitrate reductase. Then it is converted to NH_4^+ by the action of nitrite reductase to finally incorporate the latter into amino acids by glutamine synthetase and glutamate synthase [53].

In addition to this mechanism, researchers state that due to the production of reactive radicals and the synergistic effects of UV radiation, shock waves, and electric fields, the irreversible erosion of seed occurs [54] by the oxidation process of complex organic structures. This causes surface modification and, therefore, hydrophilicity, incrementing efficiency in the use of water used for the growth of plants [46,55]. These beneficial effects are higher when the ambient air is supplied to generate NTP [56,57].

On the other hand, rainwater generates better results for the main root and leaves without the application of any treatment. However, in relation to its stem, its activation enhances the height of this structure. It is attributed to the properties of rainwater, acquired during the interaction water-atmosphere [58], which confers to it, features such as a resource that is difficult to substitute. Nevertheless, in this regard, NTP confers to water and wastewater through the implementation of NTP, similar behavioral trends to rainwater. Some studies report that rainwater contains nitrogen compounds such as NO_2^-, NO_3^-, and NH_4^+ [59,60]. And that livestock and poultry waste have significant amounts of N, P, K, and other micronutrients; in such a way that high concentrations of NH_3 (ammonia), NO_3^- y NO_2^- have been determined [61].

The biochemical activity of NTP discharges into water, known as Plasma Activated Water (PAW), derives from synergistic effects between highly reactive oxygen and nitrogen species (RONS). RONS in PAW, typically include long-lived species such as NO_3^-, NO_2^-, hydrogen peroxide (H_2O_2), and ozone (O_3), with typical half-lives of several minutes, several days, and years, and other short-lived species such as hydroxyl radicals ($OH^\bullet$), nitric oxide ($NO^\bullet$), superoxide (O_2^-), peroxynitrate ($OONO_2^-$) and peroxynitrites ($ONOO^-$) [51]. These RONS act over potable water, wastewater from poultry farming, and rainwater in a different form. Furthermore, long-lived species have been shown to possess bactericidal properties [51]. The results in Figure 4 indicate the beneficial interaction of the RONS generated with the NTP. Implying a more significant growth of the lentil with the Wastewater from poultry farming because this type of water has a higher content of nitrogen compounds.

We consider that the concentrations of NO_3^- and NH_4^+ contained in each water type influence the lentil plant growth in the root and the stem. However, the mechanisms under which these compounds act need to be clarified; we are initiating the understanding to better use complex molecular mechanisms for the benefit of plant resources and to improve the yield of agricultural plants. For this reason, it is necessary to carry out further studies to establish the reasons for the changes induced by NTP in the physiological and biochemical processes of plants and, in this way, determine the fundamental facts in basic and applied research in this area of study. It is possible to highlight that the treatment using the NTP of the polluting industrial effluents deposited in the water has indicated that they do not cause harmful effects on the environment and the health of living beings [62,63].

In addition to the positive influence of NTP in improving physical parameters in the development of seeds, this study determines the internalization caused by the pathogen microorganisms *E. coli* and *S. typhimurium* under the influence of the applied treatments. According to the wastewater source, *S. typhimurium* bacteria achieve a higher concentration (1.41×10^7 CFU/mL) than *E. coli* bacteria (7.00×10^6 CFU/mL). In the internal structures of lentils, both bacteria are in higher concentration in the primary root than in the case of the stem, it could be attributed to the roughness and the contact time between the hydric supply and the root under environmental conditions, which allow bacterial penetration since the early stage of plant development. Nevertheless, when treatments by NTP are applied, an influence is detectable in bacteria concentration in the analyzed organic structures. *S. typhimurium* bacteria significantly reduce stem structure when receiving direct treatment. In this case, it is hypothesized that synergistic effects generated by the direct application of NTP interact heavily with bacteria retained in the stem, complicating its internalization motility, and making it more vulnerable than *E. coli* bacteria in this structure. On their behalf, *E. coli* bacteria show higher vulnerability through the activation of wastewater both in the stem and in the root than direct application of NTP, which these bacteria can resist.

The weak layer of Gram-negative bacteria is an influencing factor in the reduction of both pathogen microorganisms considered in this study [64]. This condition makes them more susceptible not only to the reactive radicals and chemical compounds of long short-term (hydrogen peroxide, nitric acid [65]), but also to stress mechanisms that involve synergistic exposure to UV radiation [55] and electric fields [64], even though NTP treatments do not cause significant DNA damage [66].

5. Conclusions

Non-thermal plasma (NTP) technology is currently being carried out for application in different areas such as materials, health, food processing, and agriculture, with the advantage that the process is carried out at a low temperature, making it very suitable for heat-sensitive materials. With the nature of NTP, the versatility of the design, how inexpensive it is to implement, and being environmentally friendly, NTP offers unique advantages over traditional processing technologies. This manuscript reports the effect of potable water, wastewater from poultry farming, and rainwater treated with NTP directly and indirectly (PAW) on the legume lentil's development. The results show that the different types of water used and treated with NTP in the legume (lentil) are promising because its growth quality is improved, which positively affects this legume. In the conditions where the experiments were carried out, in the first instance, the activation using the NTP of the wastewater from poultry farming was better. This is because it presents a more significant legume growth and a reduction in the bacteria *E. coli* and *S. typhimurium*. The latter is expected since it avoids pathogens' adverse effects due to NTP's properties. In other words, it can use the NTP advantageously in the decontamination of legume surfaces and without using chemical products that pollute the environment. This result is interesting since the wastewater from poultry farming can be activated before the irrigation of the plant, taking advantage of the bioactivity of PAW.

On the other hand, rainwater is a vital source of crops. It was determined that the non-thermal plasma confers properties to this type of water also cause positive effects on the development of lentil plants. Based on the results, it is found that the composition of the water has an essential role in the germination, development, and growth of the legume and could be expected in plants in general. This is because germination begins with water absorption, and the action of NTP could significantly influence its absorption capacity.

Based on the studies carried out in the present investigation, it is observed that there is an excellent potential for the application of the NTP for the activation of water. With more research and development, given how approached these studies. The proposed PAW could be promoted for the realization of a small prototype for the use of domestic crops and, in the future, for industrial use. In other words, significant ideas have been identified to carry out future studies in the area.

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