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Reactive nitrogen species produced in water by non-equilibrium plasma increase plant growth rate and nutritional yield

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ABSTRACT

Water quality, mineralization, and chemical composition, particularly pH and nitrogen compounds each, play a crucial role in plant development and growth. Treatment of water with non-equilibrium discharges results in the change of its properties and chemical composition, which in turn may affect plant growth process and subsequently agriculture produce quality. Both thermal and non-thermal discharges generated in air or in water produce a number of reactive neutral and charged species, electric fields, and ultraviolet radiation. Plasma treatment of water results in significant change of its properties like pH, oxidation–reduction potential (ORP), conductivity, and concentration of reactive oxygen and reactive nitrogen species (ROS and RNS). Here we report the results of an experimental study of the effect of water treated with different atmospheric plasmas on germination, growth rates, and overall nutritional value of various plants. In the study we have used three types of plasmas: thermal spark discharge, gliding arc discharge, and transferred arc discharge. It is shown that the effects of these plasmas on chemical composition of various types of water are qualitatively different. Non-thermal gliding arc discharge plasma results in lower (acidic) pH, and production of significant amount of oxidizing species (e.g. H_2O_2). Gliding arc discharge also causes significant acidification of water, but it is accompanied by production of reactive nitrogen species (NO, NO_2^- and NO_3^-). Spark discharge treatment results in neutral or higher (basic) pH depending on initial water composition, and production of RNS.

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1. Introduction

It is frequently stipulated that electric discharges in the atmosphere – lightning – are partially responsible for the formation of life on earth. In the 1950s Stanley Miller and Harold Urey performed a set of experiments where they attempted to recreate the conditions of early Earth to show the rise of the building blocks of life as we know it [1]. Miller used a closed loop of heated water with admixtures of hydrogen, ammonia, and methane, which were believed to be the main components in Earth's atmosphere at the time. They then treated this mixture with lab-scale lightning similar to the spark discharge used in the work presented here. After a few days of treatment, Miller's mixture turned brown and later they detected the presence of amino acids in it. Indeed, igniting plasma in oxygen–nitrogen–water mixture will produce various reactive species much needed for plant growth. Plasmas are beginning to enter into this arena [2]. Various plasmas are now used to increase wettability and germination of seeds [3–7], for pollution control, and disinfection of seeds or the water used to treat the plants [8–11]. Mostly corona and electrospray systems are used today, primarily due to their ease of use [12,13].

Discharges commonly used in plant, seed, or water treatment include dielectric barrier discharges [14,15], gliding arcs [16–18], DC, AC, or pulsed coronas [9,13,19], and various direct discharges in liquid [20–23] (Fig. 1). All of these discharges produce a mixture of important reactive oxygen and reactive nitrogen species which, when mixed with water, are able to significantly influence plant lifecycle and have potential to add plasma as a valuable modality in agriculture.

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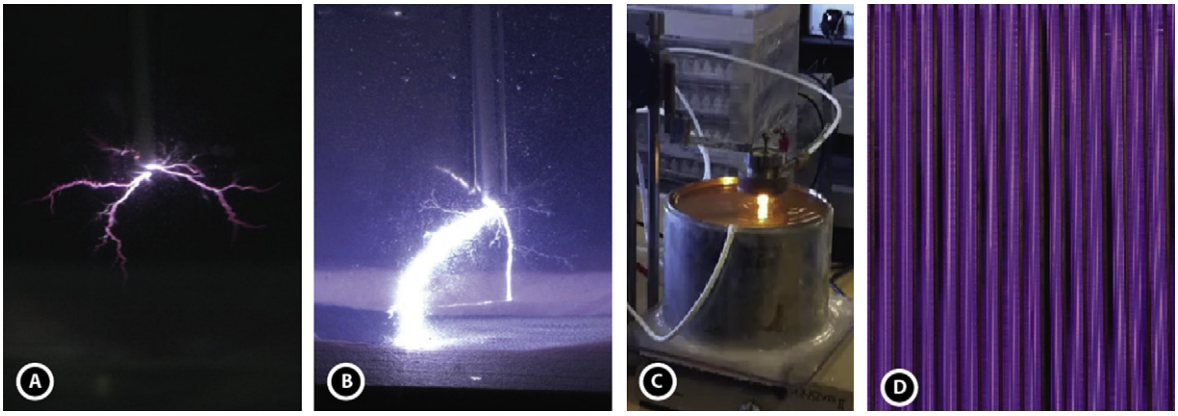


Fig. 1. Examples of discharges commonly used for liquid treatment: (A) pulsed corona inside water; (B) pulsed arc or spark inside water; (C) gliding arc plasmatron on top of water; (D) dielectric barrier discharge.

When we discuss agriculture, specifically treatment of plants, we need to realize that the lifecycle of fresh produce is complex (Fig. 2) [24]. Plasma may be used to:

- Sterilize seeds while in storage;
- Enhance seed germination;
- Capture atmospheric nitrogen in water to be used as fertilizer;
- Add reactive oxygen species and other oxidizers, combined with lowered pH, to reduce pathogen invasion of soils;
- Air cleaning, sterilization, and removal of volatile organic compounds in greenhouse facilities;
- Treatment, sterilization, and cleaning of water used for produce wash after harvest;
- Disinfection of produce before packaging;
- Air cleaning, sterilization, and removal of volatile organic compounds in the packaged produce storage facility and transportation vehicles;
- Control of pests and pathogens at the in-store display case and in-store storage;
- Removal of ethylene from air to reduce rate of aging;
- Sterilization of cutting boards, knives, and other food processing equipment both at home and in food processing facilities or grocery stores;
- Finally, plasma-assisted destruction of hazardous waste and/or waste-to-energy conversion of the non-hazardous food wastes.

In this manuscript we focus specifically on plasma treatment of water to initially improve germination rate of produce and then improve growth rate and nutritional content of the product.

2. Materials and methods

In this work we have studied the effects of three different types of plasmas: underwater spark discharge, transferred arc discharge and gliding arc discharge, on plant growth. These plasmas were used to treat either tap, spring or distilled water which was then applied to: watermelon (*Citrullus lanatus*), zinnia (*Zinnia peruviana*), alfalfa (*Medicago sativa*), polebeans (*Phaseolus coccineus*), and shade champ grass. Changes in water chemistry following the plasma treatment were analyzed. After preliminary results were gathered from the plasma systems and plants listed above, another set of experiments was conducted using the gliding arc discharge (plasmatron). The plasmatron system was used to treat spring water which was then applied to: radishes (*Raphanus sativus*), tomatoes (*Solanum lycopersicum*), and banana peppers (*Capsicum annuum*).

2.1. Plasma systems

2.1.1. Submerged spark discharge

The first plasma system used in our experiment is the underwater spark discharge. The electrode system made of a high voltage copper rod with a diameter of 3 mm concentrically fixed with a Teflon dielectric in a copper 2 cm diameter tube was submerged into the center of 500 mL of untreated water, which was held in a metal cup (Fig. 3). Only tap and spring water were used. We were unable to obtain a stable discharge generation in distilled water (probably due to low conductivity), and therefore distilled water was not used with the spark discharge. The discharge was

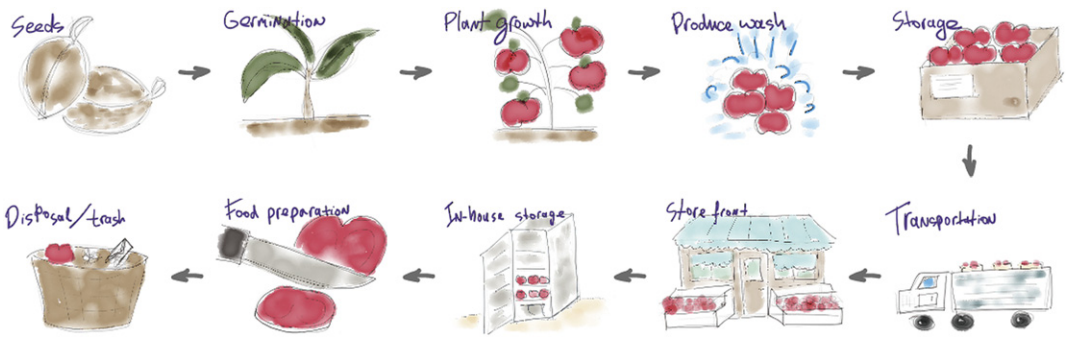


Fig. 2. Fresh produce lifecycle involves many steps and plasmas can be beneficial in most of them.

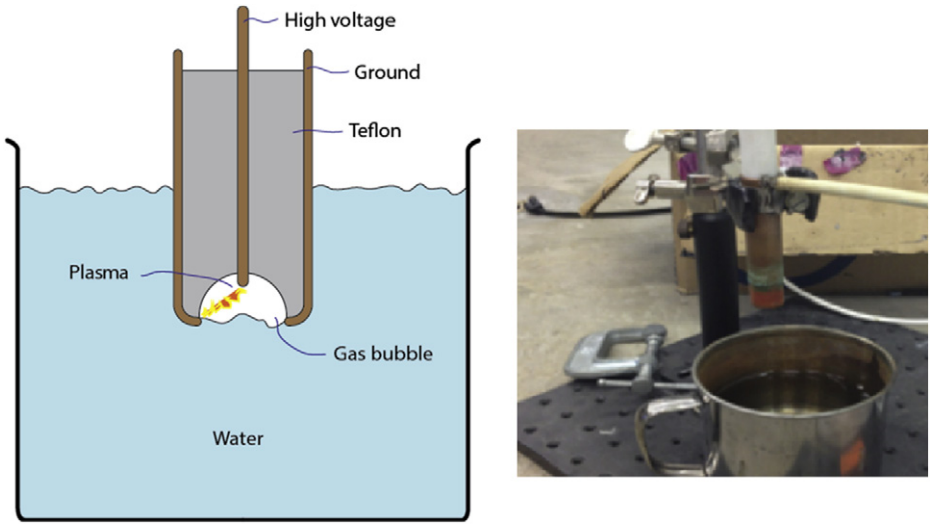


Fig. 3. Spark discharge system: schematic (left) and photograph (right).

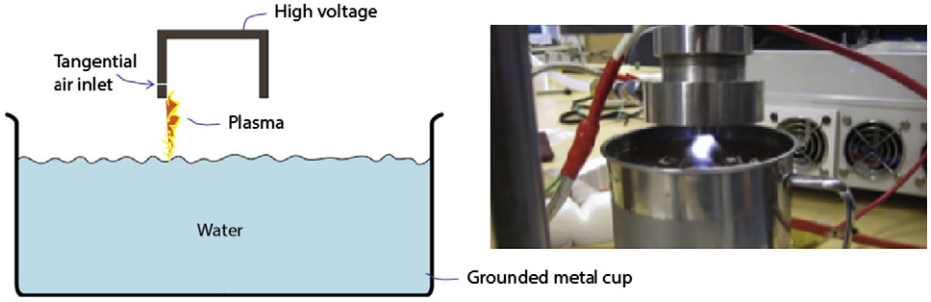


Fig. 4. Transferred arc system: setup schematic (left) and a photograph of the discharge in operation (right).

generated at 2 kV and 600 mA at an average frequency of 5 Hz. Each sample was treated for 2 min.

2.1.2. *Transferred gliding arc discharge*

The second plasma system used in our experiment is the transferred arc discharge. The discharge treats the surface of the untreated water. It is ignited in a vortex air flow (30 l/min at 55 kPa) between the high voltage electrode and the water surface serves as a second grounded electrode (Fig. 4). The treated sample was mixed periodically using a plastic spoon. Only tap and spring water were used. Distilled water was not used with the transferred arc discharge due to the lack of conductivity of the water. The discharge was ignited at 1.6 kV and 300 mA. Each sample was treated for 2 min.

2.1.3. *Gliding arc plasmatron*

The third plasma system used in our experiment is the gliding arc discharge (plasmatron) assembled as previously reposted by Gallagher et al. [25]. The plasmatron system requires condensed air flow (30 l/min at 70 kPa). This setup used a water pump to push the untreated water sample through clear plastic tube, up to a flow meter (Fig. 5). The water was fed through the flow meter at 25 mL/min and into a syringe needle. The syringe needle slowly

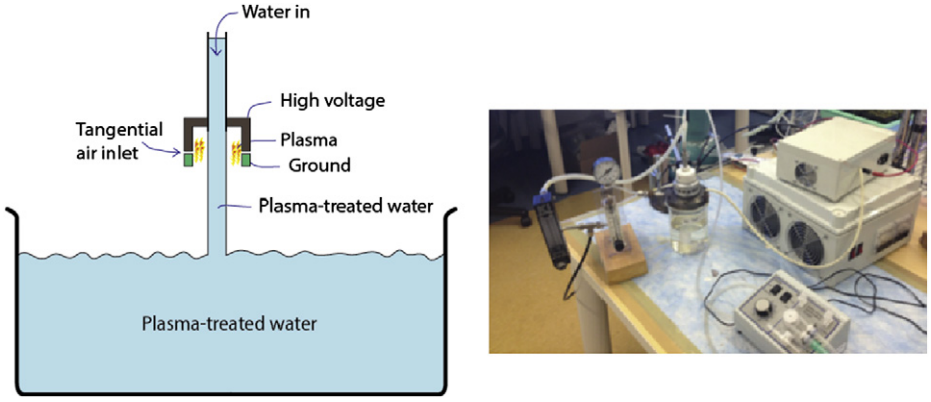


Fig. 5. Gliding arc system (plasmatron): setup schematic (left) and a photograph of the system (right).

Table 1
Plants used in the experiments.

Plant	# of seeds per pot	Notes
Watermelon	3	Large seeds
Zinnia	6–10	Small, thin seeds
Alfalfa Sprout	1 teaspoon	Soaked in distilled water prior to planting
Pole Bean	5	Large seeds
Shade Champ Grass	1 teaspoon	Small, thin seeds
Tomato	12 (3 seeds each in 4 holes)	Small seeds
Banana pepper	12 (3 seeds each in 4 holes)	Small, flat seeds
Radish	12 (3 seeds each in 4 holes)	Large seeds

Table 2
Results of water treatment with transferred arc for 2 min.

	Nitrate, mg/L	Nitrite, mg/L	Hardness ($CaCO_3$), mg/L	Chloride (Cl^-), mg/L	H_2O_2, mg/L
Tap water	56	12	>70	0	3
Spring water	56	12	>125	0	1

pumps the water above the electrode where it is mixed with the air flow. Tap, spring, and distilled water were used. The discharge was ignited at 800 V, 300 mA.

2.2. Plants

The seeds were planted in 4 × 4 starter pots with approximately 0.2 kg of soil (Table 1). Each plant type had 3 pot samples per treatment type: control and plasma treated. All plant samples were given their respective water types 30 mL every Monday and Wednesday, and given 40 mL every Friday to compensate for the weekend. Lights were placed above all of the plants on an automatic schedule from 8am to 6pm. The plant samples were cut, measured, and weighed after approximately 3 weeks. Plant samples were taken by removing the plant from its starter pot. Excess soil was shaken off of the roots and rinsed to ensure dirt removal. Roots were then patted dry with paper towels. The plants were cut to separate the roots from the stems. The root and stem lengths were measured, and the weight of both root and stems were taken. The stems were then ground using a garlic press to extract liquid, and that liquid was used as a sample for a BRIX refractometer (RFH113ATC, Omega).

Pesticide (Fungicide, Garden Safe) and fertilizer (Flower and Vegetable 10-10-10, Scotts) were used to compare against the sterilization and fertilizer-like properties of plasma-treated water. Pesticide was added to one group of plants after germination began, with each group given one full spray once per month. Fertilizer was added to the plants only once throughout the experiment. Each pot was given 2.5 g of the fertilizer.

2.3. Water and soil analysis

Here we analyzed the following chemical properties of water after plasma treatment: total concentration of chloride, nitrate, nitrite, and hydrogen peroxide, and total hardness. These were measured using test strips (Fischer Sci.). Each test strip was dipped into a sample of the treated water and allowed to rest for the recommended amount of time. The test strips were analyzed by color indication. Results were taken visually and compared to a color chart provided on each test strip container. The oxidation–reduction potential of the soil was measured with an electronic ORP meter (ORP-5041, Omega). The meter was placed into the soil 30 min after watering. Measurements were taken from both sides where the stem met the roots, and also from the center of the pot as well. The meter was held in place until the reading became stable and the number shown was recorded.

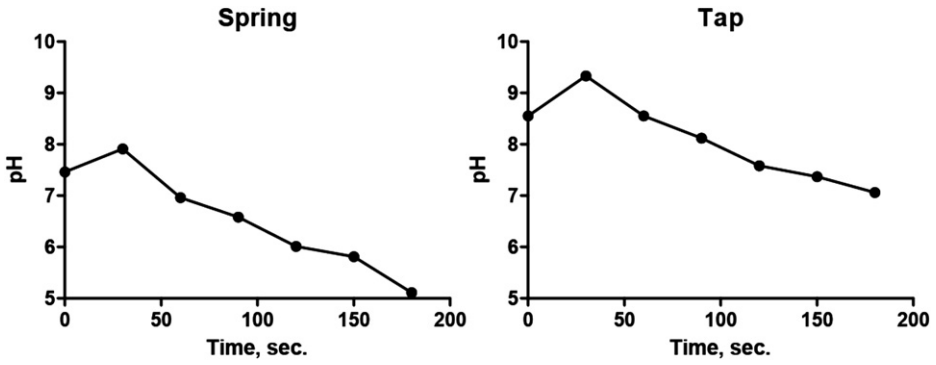


Fig. 6. Results of pH measurement of tap (right) and spring (left) water.

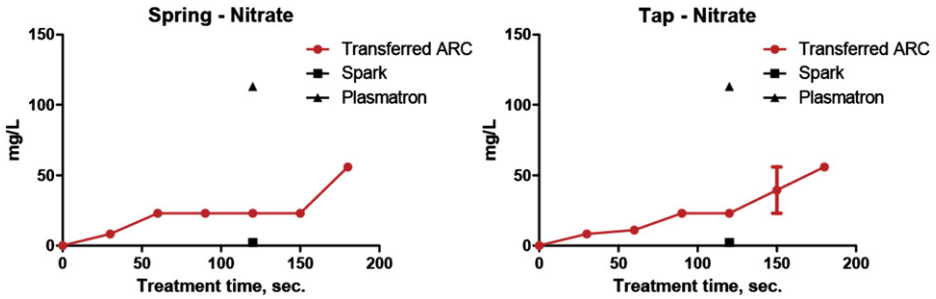


Fig. 7. Results of nitrate measurement of spring (left) and tap (right) water.

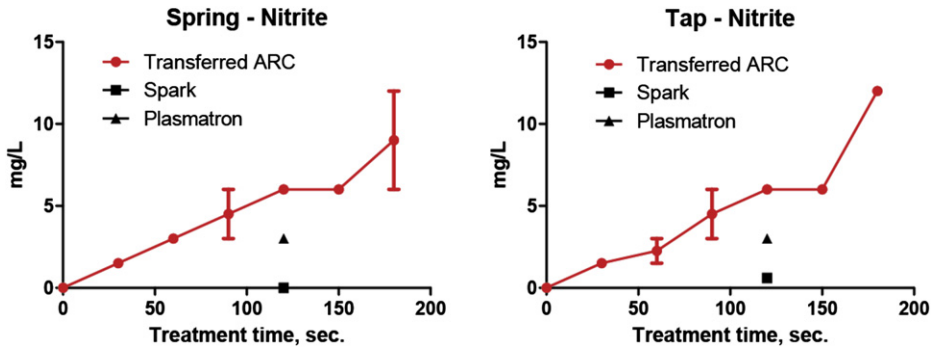


Fig. 8. Results of nitrite measurement of spring (left) and tap (right) water.

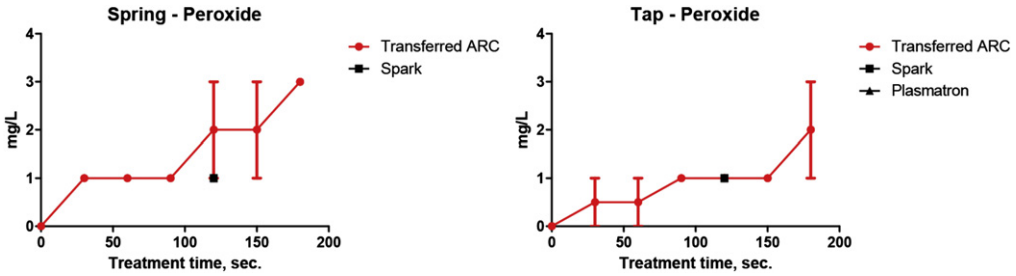


Fig. 9. Results of hydrogen peroxide measurement of spring (left) and tap (right) water.

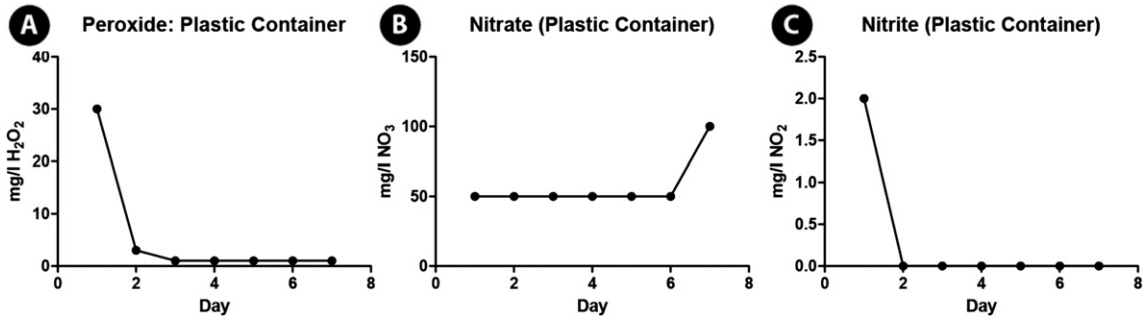


Fig. 10. The stability of hydrogen peroxide (a), nitrite (b) and nitrate (c) in plasma-treated water contained in either glass or plastic containers for several days. Water sample treated with the plasmatron 25 mL/min, cooled to room temperature before measuring.

3. Results

3.1. Water and soil analysis

Here we present the results of water analysis following the treatment with transferred arc (Table 2). The nitrate and nitrite concentrations after 2 min of treatment were measured to be 56 mg/L and 12 mg/L respectively, and were the same for both tap water and spring water. Total hardness concentrations were lower for tap water, at 70 mg/L, than with spring water, which was measured to be approximately 125 mg/L. In both tap and spring water no traces of chloride were detected by the test strips. Peroxide concentrations were higher in tap water than in spring water, which were measured to be 3 mg/L and 1 mg/L, respectively.

Transferred arc treated water pH measurements were taken with an electronic pH meter, calibrated before each use. Water was treated for 30, 60, 90, 120, 150, and 180 s, with the initial pH marked at 0 s. The readings were taken immediately after treatment. As seen with both spring and tap water, the pH increases at 30 s of treatment, followed by a steady decline over the course of the treatment time (Fig. 6).

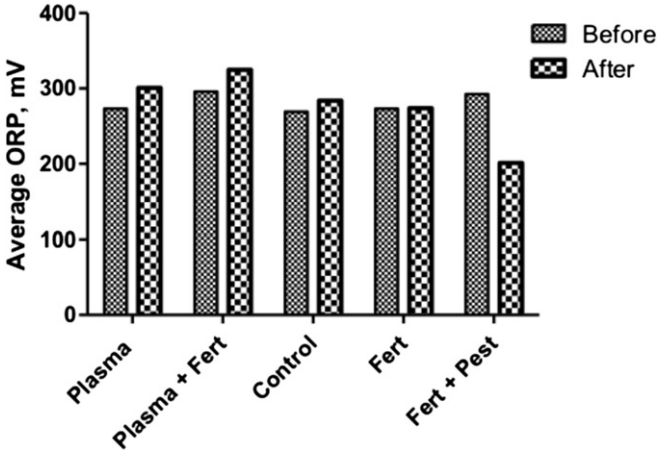


Fig. 11. Oxidation–reduction potential of the soil before and after watering.

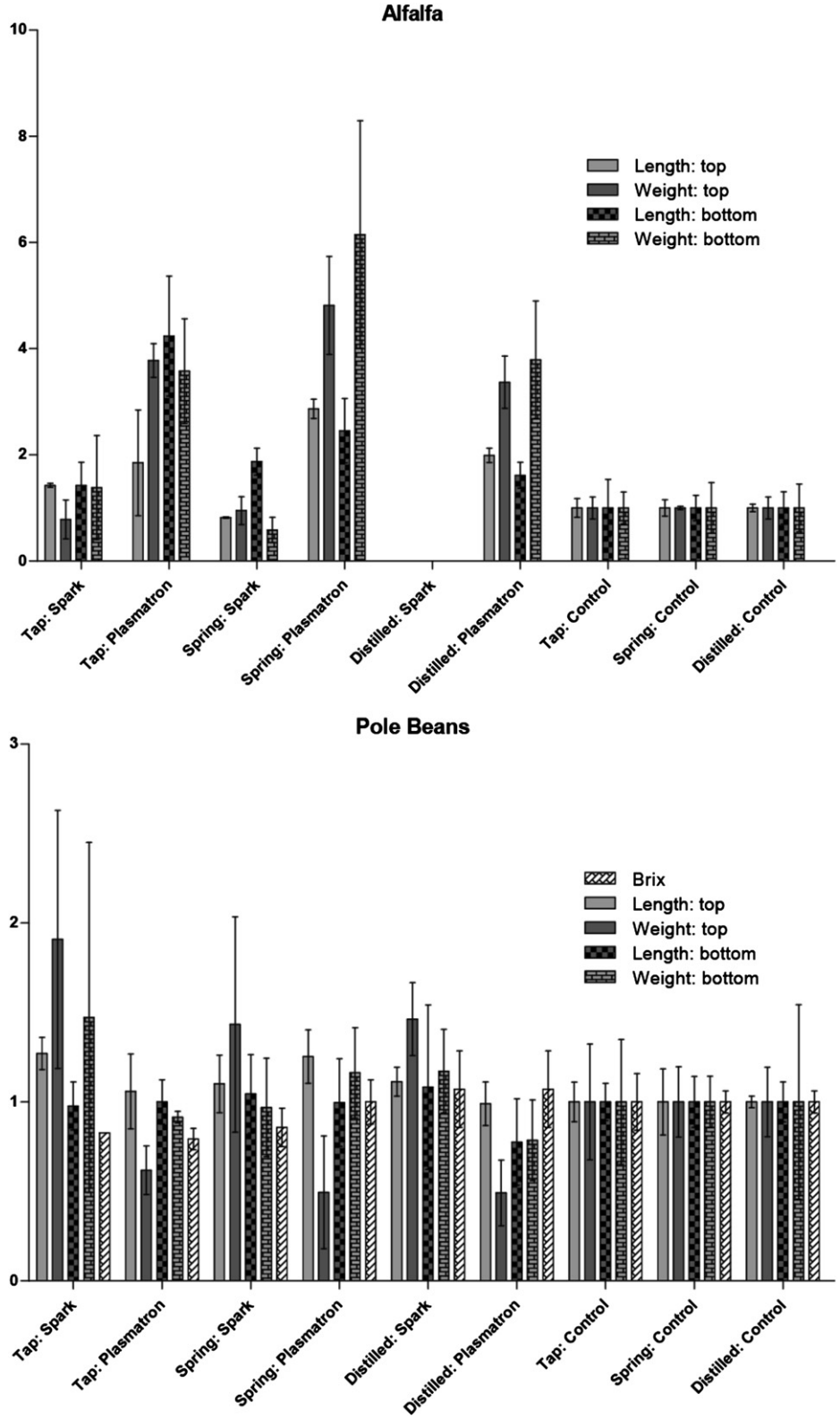


Fig. 12. Measurement results of weight and height of top and bottom of the plants as well as BRIX reading for alfalfa, polebeans, watermelon, and zinnia after plasma treatment. All measurements are in percent, normalized to control value (1 = 100%).

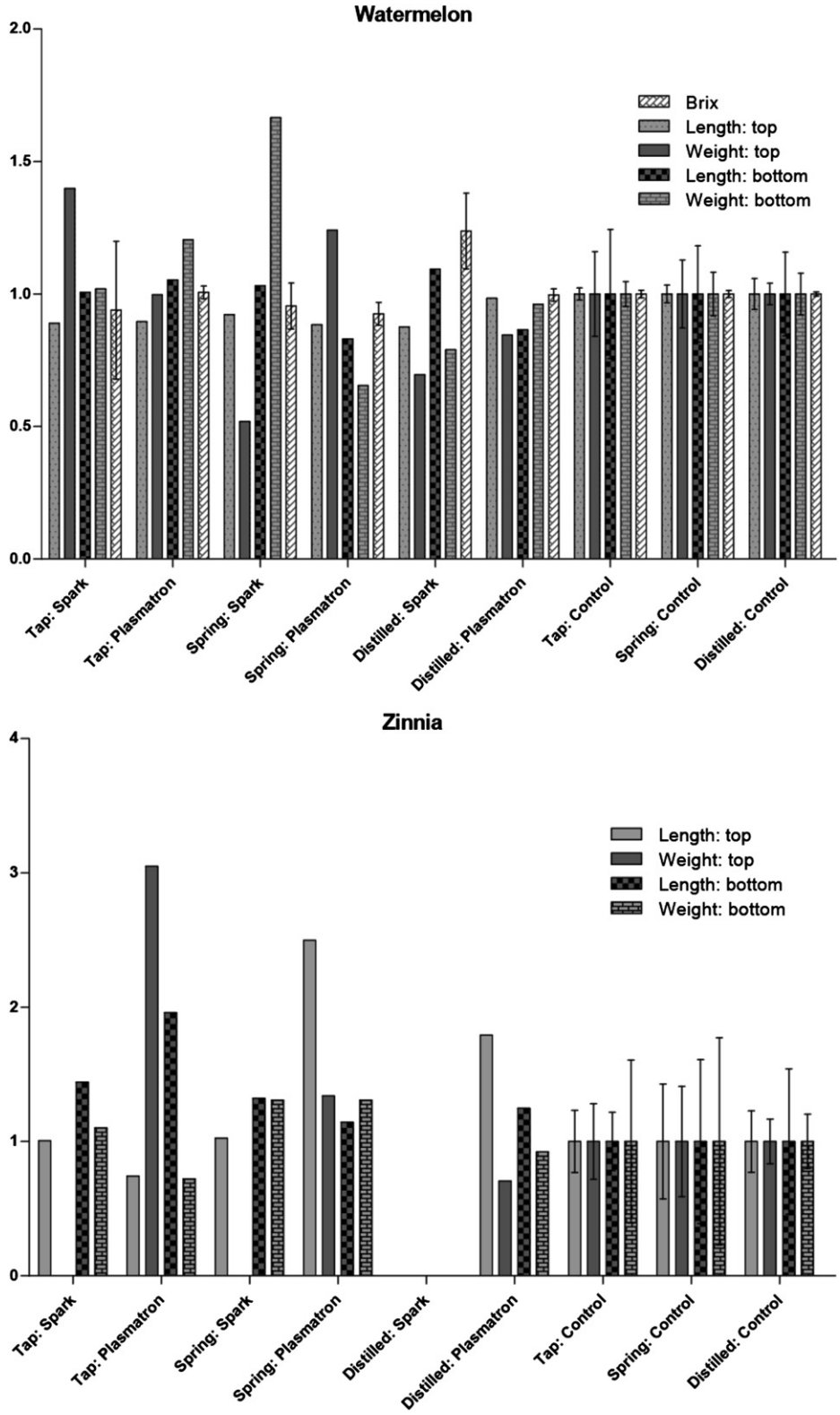


Fig. 12. (*continued*).

Transferred arc treated water nitrate and nitrite measurements were taken with test strips (Fischer Sci.) and compared to results from spark and plasmatron systems. Water was treated for 30, 60, 90, 120, 150, and 180 s, with the initial value marked at 0 s, and repeated with all plasma systems. The readings were taken immediately after treatment. As seen with both spring and tap water, there was a steady increase of both nitrate (Fig. 7) and nitrite (Fig. 8) concentration in the water treated with the transferred arc. The results were compared to the spark and plasmatron at 120 s. The spark system produced a nitrate concentration much lower

than the transferred arc at 120 s. The plasmatron system produced a nitrate concentration significantly higher than that of the same treatment time. Both the spark and plasmatron systems produced a nitrite concentration lower than the transferred arc at 120 s.

Transferred arc treated water peroxide measurements were taken with test strips (Fischer Sci.) and compared to results from spark and plasmatron systems. Water was treated for 30, 60, 90, 120, 150, and 180 s, with the initial pH marked at 0 s, and repeated with all plasma systems. The readings were taken immediately after treatment. With tap water, concentrations of peroxide were measured to be relatively stable with no major increases (Fig. 9). Increases in peroxide concentration were seen in spring water, however, large errors are seen in the measurement. Tap water measurements show the plasmatron produced a significant amount of peroxide compared to transferred arc, compared to the spark system which produced the same concentration at 120 s. With spring water, peroxide measurements from the spark system are about the same, considering significant error, as with the transferred arc.

We have also measured the stability of these compounds in water stored in either plastic or glass containers for several days (Fig. 10). Measurements were made using test strips, as above. Using the transferred arc plasma system with tap water, ran for at least a minute before collecting from the faucet, there is a small increase of peroxide concentration seen at the 3-min mark. Samples were taken from the spark and plasmatron systems at 2 min of treatment. The plasmatron demonstrates the biggest increase in peroxide concentration, measured at approximately 10 mg/L. At 2 min, the peroxide concentration was approximately the same 1 mg/L as seen with the transferred arc. The change of concentration of hydrogen peroxide, nitrate and nitrite was found to be independent of the type of container used and was identical for both glass and plastic containers. The results for spring water varied much more than that with tap. At 30 s, the concentration of peroxide stabilizes at 1 mg/L, and then jumps to 2 mg/L from 2 min to 2½ min, continuing to rise. All of the measurements were taken on the same day with the exception of

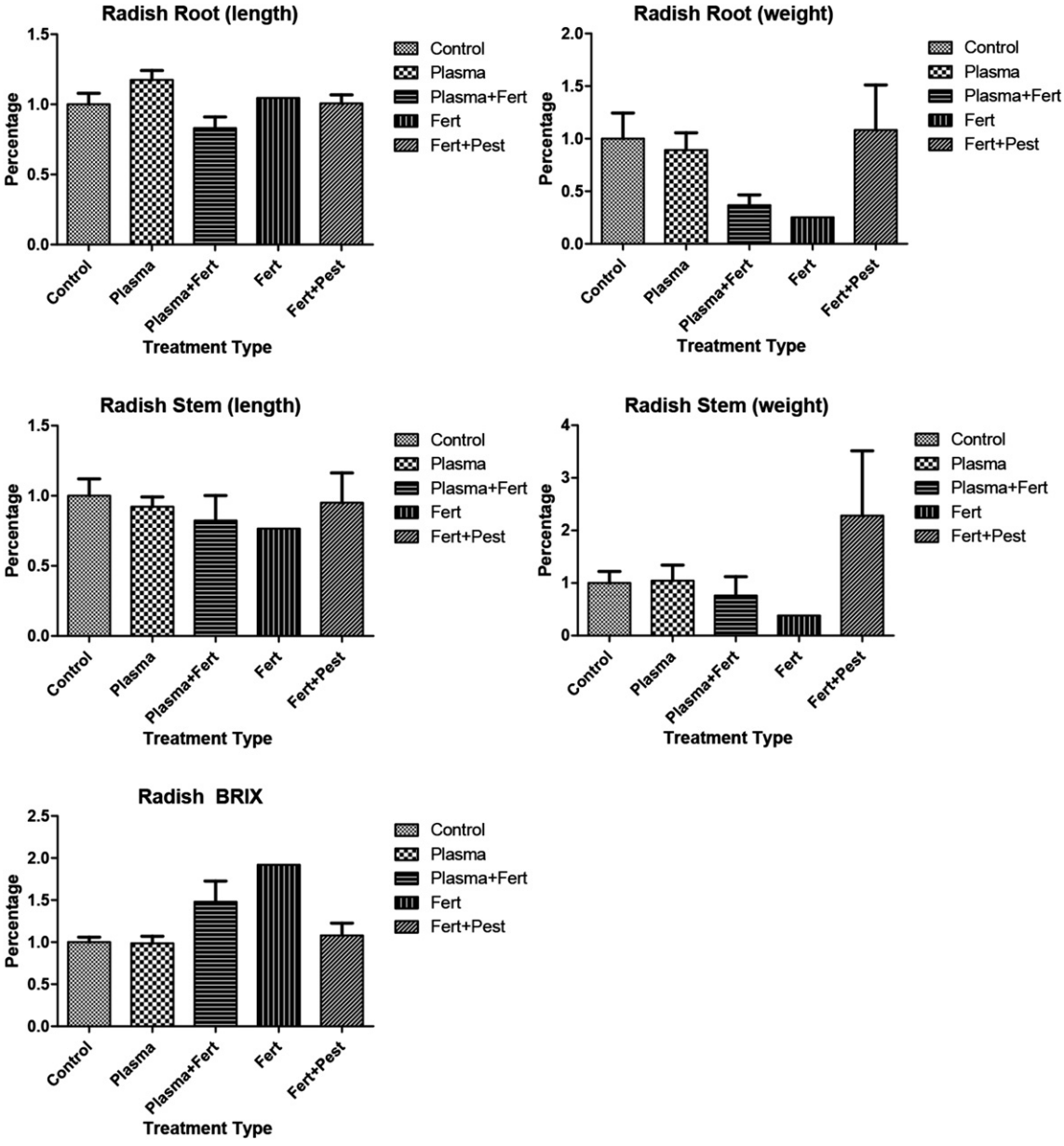


Fig. 13. Radish (*Raphanus sativus*) treatment results.

the plasmatron, and therefore have been omitted from the graph in Fig. 10.

Fig. 11 shows the results of an experiment where the oxidation–reduction potential of the soil of the pots housing the plants was measured. Using 7 holes in each pot consisting of one to the left and right of each of the three plants as well as one in the center. Measurements were taken before the plants were watered as well as after. One hour elapsed following the watering before the "after" measurements were taken.

3.2. Plant results

Fig. 12 shows the results for the plant samples. The measured variables are the length and weight of the stem (from the start of the root, upward, marked "top") and the length and weight of the roots (marked "bottom"). The data are normalized to the values of the control plants. The data for zinnia plants show that the plasmatron system achieved the best results in the categories for plasmatron (tap water), weight of the stem and length of the root, plasmatron (spring water) length of the stem. Less but still significant results were found for plasmatron (distilled) length of the stem. The best results for the alfalfa weight of the stem and roots were achieved with the plasmatron system while used with spring water, and less but still significant results were found for plasmatron fed with tap water (weight and length of the root) and with distilled water (weight of the stem and roots). On the contrary, watermelon plants grew best with the spark system ignited in tap and spring water where weight of the stem and root were measured to be the greatest compared to other treatment methods. Less but still significant results were found with the plasmatron working with tap and spring water (weight of the roots and stem). Lastly, pole bean plants were found to grow best with water treated with spark (both tap and spring water) and plasmatron (spring water) systems based on weight of the roots measurements.

Shade champ grass was also grown using plasma-treated water. No good method of comparison could be found since samples were grown too small. Visually there was virtually no difference between controls and plasma-treated water. BRIX measurements could not be taken due to the sample size and the lack of moisture in the sample. These experiments (not shown here) will be repeated in the future.

Based on the obtained results where the plasmatron system was shown to provide most promising effect on plants' growth and development, the next set of experiments was done using this system operated with spring water. Here we have used three plants: radish (*R. sativus*), banana pepper (*C. annuum*) and tomato (*S. lycopersicum*). The data presented in Figs. 13–15 below are normalized to the values of the control plants that we watered with untreated spring water.

Fig. 13 shows the results for radishes grown using spring water and the plasmatron system only. The measured length of the stem (from the start of the root, upward) shows that the fertilizer and pesticide combination provide the results equal to that of the control, with plasma, plasma and fertilizer, and fertilizer-treatment types showing lower values compared to the control set. Stem weight of plants (from the start of the root, upward) shows significant improvement which may be attributed to one of the plants significantly overgrowing for reasons unknown to us. Root weight shows that the plasma and fertilizer–pesticide combination treatment types allow achievement of values approximately equal to that of the control set. The plasma–fertilizer combination and fertilizer-treatment types result in root weight values less than that of the controls. The length of roots of the radishes watered with the plasma-treated water achieved values higher than that of the control set, and the fertilizer–pesticide combination and

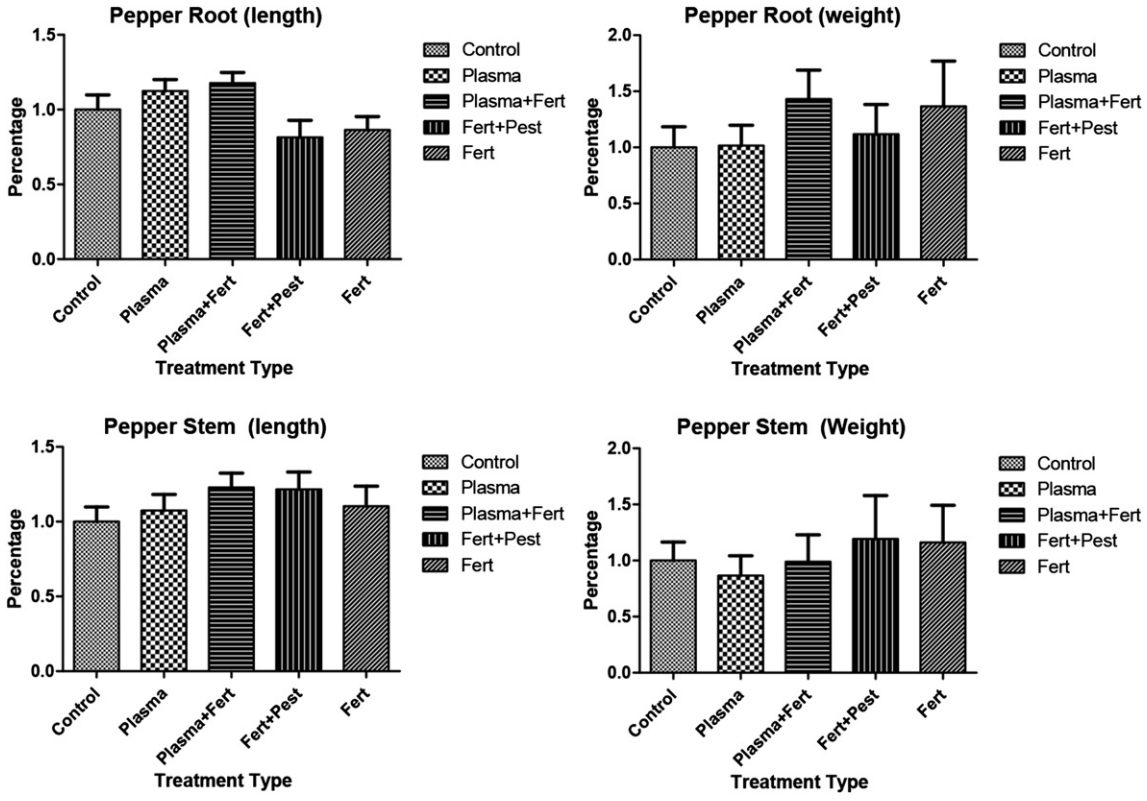


Fig. 14. Banana pepper (*Capsicum annuum*) treatment results.

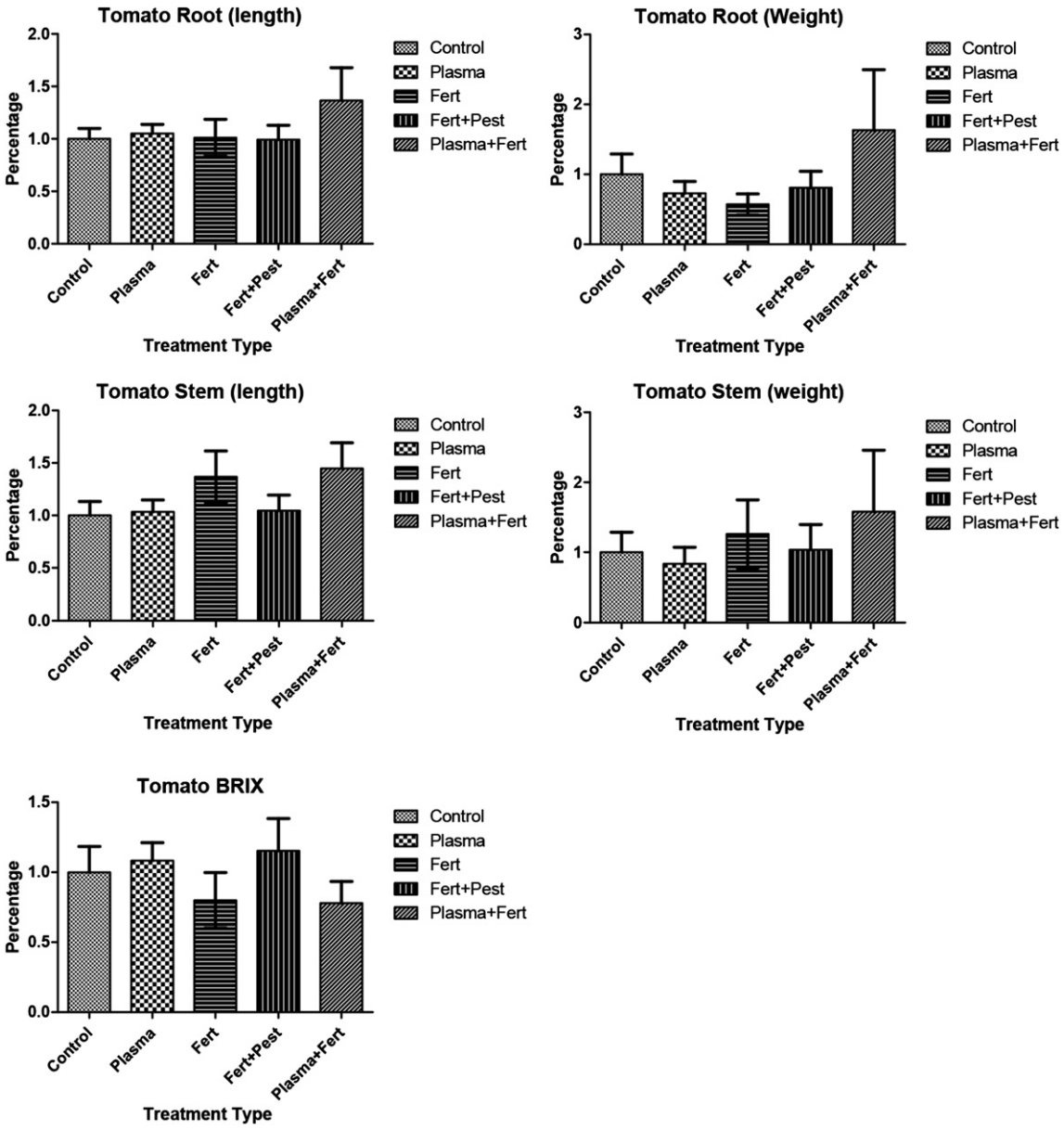


Fig. 15. Tomato (*Solanum lycopersicum*) treatment results.

fertilizer-treatment types achieved values equal to the controls. The plasma–fertilizer combination achieved values lower than the control set.

Fig. 14 shows the results for banana peppers. All treatment types were shown to result in the same stem weight of all the plants, and equal to the control set. However, stems were longer in the case of plasma, plasma–fertilizer, and fertilizer–pesticide treatment types than that of the control and fertilizer-treatment type sets. At the same time, the plasma and plasma–fertilizer-treatment types have achieved root parameters (length and weight) results higher than that of the control set, while the fertilizer and fertilizer–pesticide treatment types have values lower or equal to the control.

Fig. 15 shows the results for tomatoes grown using spring water, plasmatron-treated spring water alone or in combination with fertilizer and/or pesticide. As seen in the case of peppers, the stems of the tomato plants were measured to be about the same for all groups. The root weight and length data show that the plasma, fertilizer, and fertilizer–pesticide treatment types are approximately equal to the controls, while the values of the plasma–fertilizer-treatment type were higher than that of the controls.

4. Discussion and conclusions

In this manuscript we report on effect of plasma-treated water on plant development. Water composition plays an important, perhaps key, role in plant germination, development, and growth. Fig. 6 shows a significant drop in pH following plasma treatment while Figs. 7–9 show increase in nitrate, nitrite, and hydrogen peroxide concentration in the treated water. As expected, and reported on by the authors earlier [26], Fig. 10 shows that this effect is unstable and water loses hydrogen peroxide and nitrite concentration quickly. For this reason, if plasma-treated water is to be stored, some stabilizing agents would need to be developed; at least for the plasmas reported on in this manuscript.

The results of plasma treatmented water on plants may be summarized as “promising”. Clearly there is difference in the effect

of plasma-treated water on different plants [27,28] (Fig. 12, for example). These effects would need to be studied in further detail to develop plant treatment protocols for each type of plant if this application is to be adopted in an industrial setting. For small farms and/or homes, the use of plasma-treated water as a fertilizer needs to be assessed critically and recommendations for specific plants need to be made, similar to the powder and liquid fertilizers available in the stores. While it is known that hydrogen peroxide at lower pH levels shows high antimicrobial activity [29], it was not investigated in the reported on trial. We plan to develop this model and investigate plasma-treated water effect on plant microflora in the future.

Ethical statement

All the research in the presented work was done by the authors. No parts of this work have been published elsewhere and this manuscript presents 100% original work by the authors. To the best of our knowledge all previous work by us and by other groups has been properly references and attributed. Authors have no conflict of interest.

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