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The effects of three plasma-activated water generation systems on lettuce seed germination

Rouzbeh Abbaszadeh[1], Pegah Khosravi Nia[2], Mahsa Fattahi[3], Hamidreza Ghomi Marzdashti[4]*

[1]*Agriculture Research Institute, Iranian Research Organization for Science and Technology, Tehran, Iran*
[2]*College of Agriculture & Natural Resources, University of Tehran, Karaj, Iran*
[3]*College of Aburaihan, University of Tehran, Varamin, Iran*
[4]*Laser and Plasma Research Institute, University of Shahid Beheshti, Tehran, Iran*

**Corresponding author: Abbaszadeh@irost.ir*

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Abstract: Irrigation water treatment using plasma technology is a new approach to improve productivity. In this study the generation of plasma-activated water (PAW) as a novel subject in agriculture is investigated. Three water treatment systems were designed and evaluated: 1st PAW generation from vapor and injection into the water, 2nd PAW generation using electrical discharge on the water surface and 3rd PAW generation in the aeration path into the water. The lettuce seeds were irrigated with PAW. The germination rate and mean germination time were measured after 6 days. The seedlings weight and the number of seedlings with a length of more than 3 cm were also recorded after 8 days. The results showed that PAW had a significant effect on the germination rate and length of seedlings. Electrical discharge on the water surface had the best results. Germination rate and the number of seedlings with a length of more than 3 cm increased by 11 and 36% respectively. While injection of plasma-treated air into the water significantly reduced the number of seedlings longer than 3 cm, compared to the control. In conclusion, the PAW application can improve some attributes of lettuce germination depending on the PAW system.

Keywords: electrical discharge; irrigation; plasma; water treatment

Lettuce, (*Lactuca sativa* Linnaeus), belongs to the Asteraceae family. It is one of the most consumed vegetables all over the world. Lettuce production has grown dramatically, recently. Improvement of seed germination can potentially increase the yield of the plants.

Recently, cold plasma-activated water (PAW) has been used to improve the germination rate of different seeds (Ling et al. 2014; Boushehri and Abbaszadeh 2019) Cold plasma is a growing technology which is free of chemicals and environmentally friendly. Plasma-treated water, which is also known as PAW, has an acidic pH level which causes changes in oxidation-reduction potential (ORP) and conductivity. Additionally, PAW forms reactive oxygen species (ROS) and reactive nitrogen species (RNS). PAW has special chemical properties compared to untreated water which makes it a practical substitute for microbial disinfection (Thirumdas et al. 2018).

Researchers are focused on design and optimization of different PAW systems. Recently utilization of PAW in improving agricultural products growth has opened a new field for researchers in this area. Lightning is an example of plasma in nature, which causes plant growth.

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Lightening has the same impact as PAW on plant growth. The effect of electrical discharge in air and injection of treated air to water on the growth rate of strawberry, spinach and radish was investigated and the growth rate was observed. Creation of the nitrogen ions in the water, which were absorbed by the root of the plants, mainly caused the increase in the plant growth.

Reducing the microbial load of water was another achievement of this method (Takahata et al. 2015). In another study, the combination of 2 methods of PAW and plasma treated seed was studied in tomato, radish and pepper cultivation and positive impacts on plant growth were reported; however, optimization of plasma treatment was recommended (Sivachandiran and Khacef 2017). The treatment of *Arabidopsis thaliana* (L.) Heynh. seeds with cold plasma and application of plasma-activated water were studied and the growth of the plant was recorded. Meanwhile, the increase in nitrate and hydrogen peroxide concentrations in water were observed (Bafoil et al. 2018). The effects of PAW (generated using transient spark discharge) on wheat cultivated in the soil and a laboratory condition has been studied. The water uptake, seed germination, seedling, and plant growth parameters were investigated. In addition the content of photosynthetic pigments, soluble proteins and antioxidant enzyme activity with reactive oxygen concentration and nitrogen species in water were studied. As a result of these studies, it was reported that PAW utilization Improves germination, early seedling growth, photosynthetic pigment content in leaves, and soluble protein in roots; It stops antioxidant enzyme activity; and affects seedling metabolism, especially in soil with low nutrient content (Kučerová et al. 2019).

The purpose of this study was to study the effects of water activated by plasma generation systems on lettuce seed germination.

MATERIAL AND METHODS

In this study, V 260 lettuce seeds were used. Seeds were chosen to be approximately the same size, healthy and uniform. Each sterile petri dish (with a diameter of 9 cm), contained 25 lettuce seeds which were placed on a layer of sterile filter paper. Three replications were considered for each treatment. Distilled water was used in all treatments. Three systems were designed and evaluated for plasma application, which are described below. 200 mL water was used in each treatment which took for 20 minutes.

The high voltage power supply used in the generation of plasma had a maximum power of 30 W and a voltage of 12 kV. During the test, the pulse generator frequency was set on approximately 6 kHz. Control specimens were cultured without applying PAW. The schematic of the systems is shown in Figure 1

The system used in the treatment No. 1 was made of a glass tube, an air pump, a rubber tube, an electrode wire, and aluminum sheet as a ground electrode. The aluminum plate connected to the ground through a wire was pasted on the glass tube (5 mm in diameter). The copper wire (1 mm in diameter) connected to the high voltage power supply, was located inside the glass tube, 2.5 mm away from the aluminum plate. The air was pumped through the tube and plasma was generated between the two electrodes. The distilled water container was placed on the heater to evaporate it.

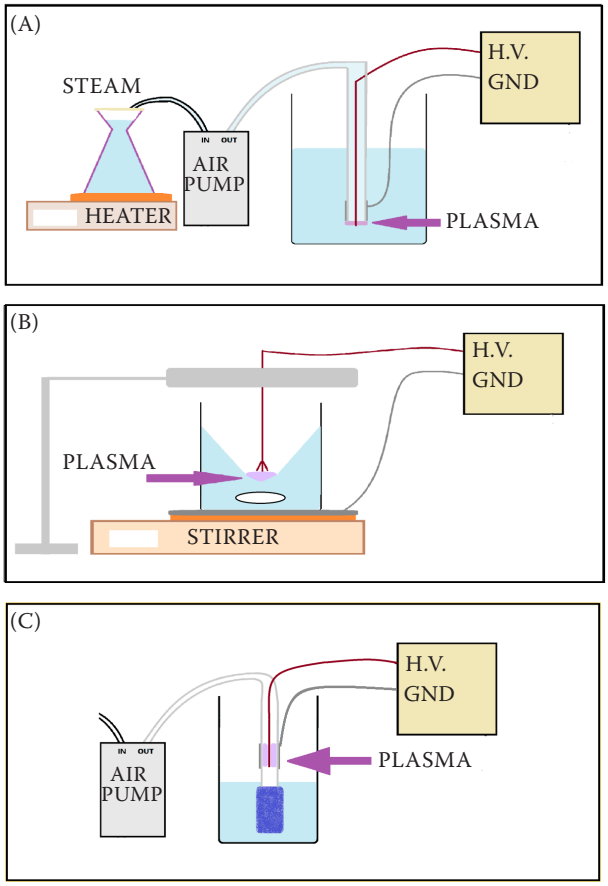

Figure 1. Water treatment systems through plasma: (A) Plasma production from water vapor and injection into the water, (B) plasma application by an electric discharge on the water surface and (C) creating plasma in aeration path to water with an air stone
H.V. – high voltage; GND –ground

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The pump air intake is provided by evaporating the distilled water and connecting the container duct to the air pump inlet. The steam was injected into the water right at the time when the steam was plasma activated.

The system used in treatment No. 2 was made of a glass beaker, a magnetic stirrer, a wire as an electrode, wire holder. The water was stirred due to magnet rotation and a small whirlpool was created in the middle of the container. The metal plate under the glass container works as an electrode connected to the ground, and the wire that was fixed by the wire holder in the middle of the whirlpool works as the high voltage electrode. Plasma is generated between water and the wire.

The system of treatment No. 3 was based on the dielectric barrier discharge (DBD) method and made of a glass tube, an air pump, a rubber tube, an electrode wire, an air stone, and an aluminum sheet. The aluminum sheet, which was connecting to the ground, was mounted on the outer part of the glass tube as a dielectric barrier. There was a non-insulated wire in a 1.2 mm diameter connected to high voltage power. This electrode was inside the tube which is adjacent to the aluminum plate. Plasma is formed between the internal electrode and dielectric, which is in the air passing way. The glass tube head was connected to the air pump by a rubber tube. At thevend of the tube, the air stone was inserted to minimize air bubbles size and inject the plasma-treated air into the water.

Electrical conductivity (EC) and acidity level (pH) of treated water were measured by portable EC meter (8306AZ model, AZ Instrument, Taiwan) and benchtop pH meter (Starter3100 model, OHAUS USA Inc., USA) respectively.

A slight increase in temperature was observed in each treatment. All seeds were given 3 mL of water produced by plasma treatment after the water was equilibrated with ambient temperature.

The petri dishes' lids were closed and sealed to prevent moisture evaporation. 2 mL of water was added to each specimen on a daily basis. The plates were all placed at the room temperature.

Germinated seeds were counted every day. Germination data were completed in 6 days. The seedling's weight and number of those one with a length of more than 3 cm were recorded after 8 days.

The germination percentage (*GP*), germination rate (*Rs*), and mean germination time (*MGT*) were calculated based on the following equations:

$$GP = 100 \times \frac{N_G}{N_T} \quad (1)$$

where: N_G – the number of germinated seeds; N_T – the total number of seeds (Hosseini and Rezvani 2009).

$$GR = \sum_{i=1}^{n} \frac{S_i}{D_i} \quad (2)$$

where: S_i – the number of germinated seeds per count; D_i – the number of days and n is the number of days that have been counted (Maguire 1962).

$$MGT = \frac{\sum_{i=1}^{n} A_i D_i}{\sum_{i=1}^{n} A_i} \quad (3)$$

where: A_i – the number of new germinated seeds on day; D_i, n – the number of whole days counted from beginning of germination (Wang and Chang 2003).

The experiment was conducted using a random design and the data were analyzed using SPSS software (version 16). The ANOVA was conducted first and then Duncan's test (0.05) was used to compare means.

RESULTS AND DISCUSSION

The measured values of EC, pH, and water temperature before and after the plasma application for each of the treatments are as shown in Table 1.

The PAW systems were named as: (*i*) Plasma production from steam, and injection into the water; (*ii*) applying plasma by an electric discharge on the water surface; (*iii*) plasma generation in the aeration path to the water with an air stone; and (*iv*) control treatment representative.

Results showed a significant increase in EC value in all plasma-treated water in comparison with the controlled treatment. pH level was decreased in all of the treatments. Variation of EC is attributed to presence of ions and reactive species formed in water. These products are generated during plasma treatment and dissolve in water. The decrease in the pH is related to formation of hydrogen peroxides, nitric acid, and peroxynitrous acid in the PAW and change from system to system (Thirumdas et al. 2018). It is possible that one of the main reasons for the greater effectiveness of system 2 is the larger volume of air around the position of plasma formation and the movement of water. Water temperature

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Table 1. Electrical conductivity (EC), acidity, and temperature of water treated using plasma systems

Treatments	Initial EC (μS·cm^{-1})		Acidity (pH)		Temperature (°C)	
	initial	final	initial	final	initial	final
System 1	4.82	69.7	4.98	3.53	27.9	37.9
System 2	4.82	168.7	4.98	3.31	27.9	37.7
System 3	4.82	50.0	4.98	3.98	27.9	26.7
Control	4.82	4.82	4.98	4.98	27.9	27.9

was increased in systems 1 and 2; however, it was decreased in system 3. Aeration and the lack of close contact of electrodes with water could be the reason of this slight decrease in temperature. The changes of germination percentage due to different treated waters were presented in Figure 2.

Utilization of packaged modified seeds with high viability may cause this non-significant difference. The highest germination percentage of lettuce seeds were observed in systems 2 and 3.

The effect of water treatments using plasma on lettuce seed germination rate was illustrated in Figure 3. A significant effect on the rate of lettuce seed germination was observed. The system 2 showed to have the highest impact on germination speed. Plasma discharges generate the reactive oxygen and nitrogen species and therefore acidity, electrical conductivity, and oxidation-reduction potential are changed. These new solutions affect the rate of seed germination (Attri et al. 2020).

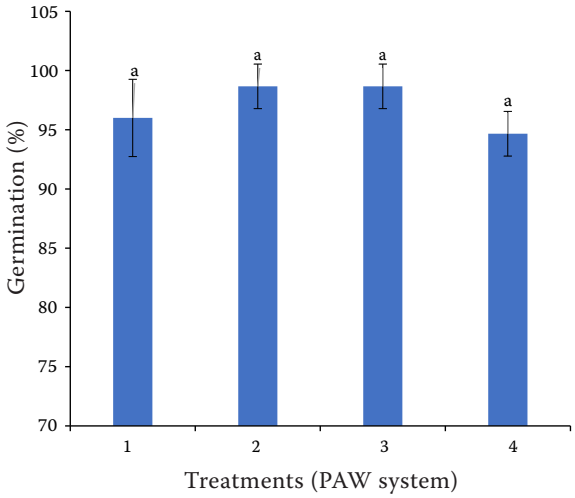

Figure 2. The effect of water treatments using plasma on germination percentage of lettuce seeds (*P*-value = 0.287)
Columns with the same letter do not significantly differ according to Duncan's multiple range test; PAW – plasma-activated water

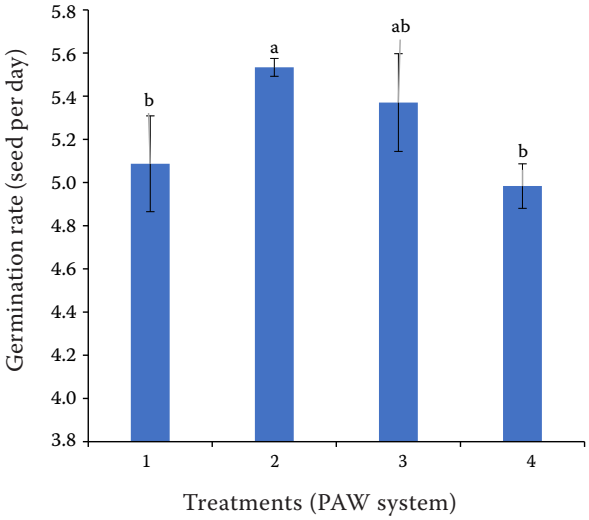

Figure 3. The effect of water treatments using plasma on lettuce seed germination rate (*P*-value = 0.038)
Columns with the same letter do not significantly differ according to Duncan's multiple range test; PAW – plasma-activated water

According Figure 4, there was no significant difference observed in germination time seeds. While systems 2 and 3 reduced this period.

The changes of lettuce seedling weight due to different PAW generation systems were presented in Figure 5. There was a significant diversity observed in the weights of seeds in different treatments. In this study, electrical discharge on the water surface increased seedling weight by 18%. Increasing the treatment time may lead to better results

The effect of water treatments using plasma on the height of lettuce seedlings was shown in Figure 6. The system 2 showed to have the highest impact on the number of seedlings which is longer than 3 cm. The higher growth in seeds treated with this system might be because of higher nitrate content, higher EC, or lower pH. This plasma exposer to water results in the production of a mixture of reactive oxygen and nitrogen species (RONS) that have a long

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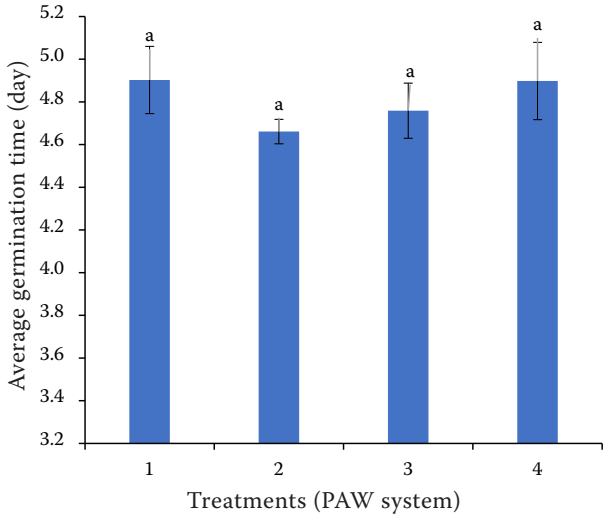

Figure 4. The effect of water treatments using plasma on average germination time of lettuce seeds (*P*-value = 0.31)
Columns with the same letter do not significantly differ according to Duncan's multiple range test; PAW – plasma-activated water

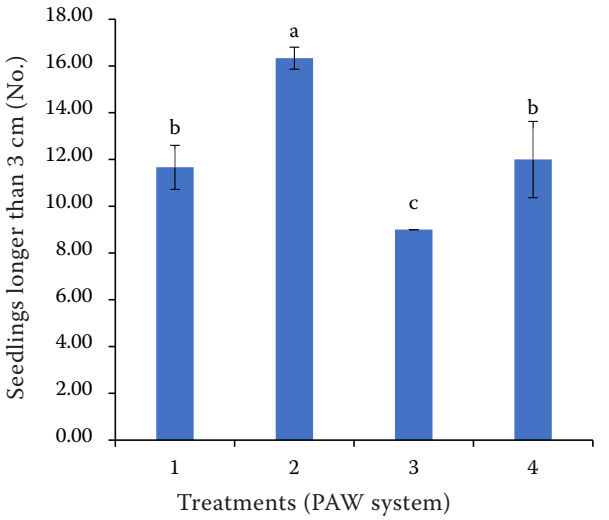

Figure 6. The effect of water treatments using plasma on the height of lettuce seedlings (No. of seedlings longer than 3 cm) (*P*-value = 0.0005)
Columns with the same letter do not significantly differ according to Duncan's multiple range test; PAW – plasma-activated water

lifetime, e.g. NO_3 (Attri et al. 2020). During treatment with system 3, some ozone as one of the products of plasma generation, is also injected into the water. Although ozone has the effect of inhibiting pathogens, this gas can reduce germination (Takashima et al. 2020). Therefore, it seems necessary to adjust the appropriate dose of ozone in this system.

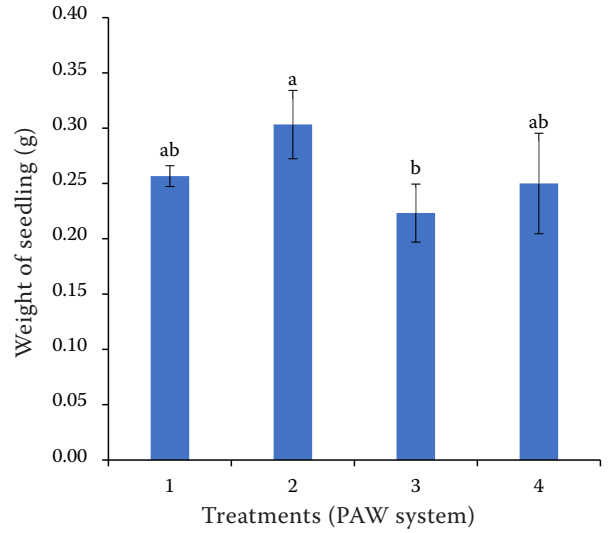

Figure 5. The effect of water treatments using plasma on lettuce seedling weight (*P*-value = 0.151)
Columns with the same letter do not significantly differ according to Duncan's multiple range test; PAW – plasma-activated water

Activation of deionized water by plasma and its use for wheat seed increased seedling weight. However, there was no significant effect on seed germination percentage and seedling length. The positive effect of plasma was likely because of increasing water intake and therefore, faster absorption of nutrients. In the early stages of seed growth (i.e. intake and germination) the hydrogen peroxide, nitrite and nitrate ions have the most important role in seed germination. Meanwhile, nitrates and other reactive nitrogen and oxygen species, might send a signal and cause growth in addition to their nutritional role (Kučerová et al. 2019).

Germination rate improvement and seedling length increment in lentil seeds were observed when it was irrigated with PAW. The hydrogen peroxide and nitrate, interaction terminated seed dormancy by the endogenous production of NO radicals (Zhang et al. 2017; Judée et al. 2018)

Although the positive effects of PAW on seed germination of different plants have been previously studied; but in this study, the effect of water treatment with different plasma systems was explored. A comparison of applied systems showed that the application of cold plasma through electric discharge to the water surface had the best results among all. The direct contact of plasma and electric discharge on water, and the greater volume of air

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around the plasma production area, and the presence of stirrers might be the main reasons for the higher achieved results of this method. This is likely caused by the formation of the filament in the air and the subsequent formation of the channel on the water surface. It is thought that germination improvement affected by factors such as an increase in nitrate and other nitrogen ions, hydrogen peroxide, and also variation in water acidity. The increase in these factors due to increased plasma treatment time is associated with enhanced seed germination (Sivachandiran and Khacef 2017).

In another study, the application of thermal spark discharge, gliding arc discharge, and transferred arc discharge were investigated. The promising effects of plasma-treated water were considered probable on plants. But for different plants, there are different effects that, like chemical fertilizers, reveal the necessity of determining a specific protocol for each plant (Park et al. 2013). In order to obtain better results, it is recommended to consume more energy by increasing the power or duration of treatment. Investigating other methods of plasma water activation could help researchers to find more efficient PAW systems. In the case of electrical discharge on the water surface, the distance between the electrode and the water surface, the rate of water circulation, and the method of connecting to the ground are some of the factors that can be improved in the future.

In this study, the possibility of water treated with plasma technology for improving the lettuce germination was observed.

CONCLUSION

The effect of water treatment by cold plasma application was investigated using three different systems, in which is described in details. Comparison of these systems showed that the generation of cold plasma by electrical discharge on the water surface has the best results in terms of EC elevation and pH decrease. The water activated by plasma was studied for irrigating lettuce seeds. The electrical discharge between high voltage electrode and water showed the best effects on germination. This is thought to be due to increased nitrates and other nitrogen ions, hydrogen peroxide, and changes in electrical conductivity and water acidity. The PAW generation from vapor and injecting into the water did not cause significant differences compared to the untreated water. Also, due to the negative changes observed from the formation of plasma in the aeration path to water, more attention is needed in using this treatment because the ozone produced can have destructive effects.

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