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Research Article

Impact of Plasma-Activated Water (PAW) on Seed Germination of Soybean

Rajesh Prakash Guragain [1], Suman Prakash Pradhan [2], Hom Bahadur Baniya [3], Bishnu Prasad Pandey [4], Niroj Basnet [1], Binita Sedhai [1], Santosh Dhungana [1], Ganesh Kuwar Chhetri [1], Ujjwal Man Joshi [1], and Deepak Prasad Subedi [1]

[1]*Department of Physics, Kathmandu University, Dhulikhel, Kavre, Nepal*
[2]*Department of Environmental Science and Engineering, Kathmandu University, Dhulikhel, Kavre, Nepal*
[3]*Department of Physics, Tri Chandra Multiple Campus, Tribhuvan University, Ghantaghar, Kathmandu, Nepal*
[4]*Department of Chemical Science and Engineering, Kathmandu University, Dhulikhel, Kavre, Nepal*

Correspondence should be addressed to Rajesh Prakash Guragain; rayessprakash@gmail.com, Hom Bahadur Baniya; hombaniya@gmail.com, and Deepak Prasad Subedi; deepaksubedi2001@yahoo.com

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The present study reports the generation of plasma-activated water (PAW) using dielectric barrier discharge (DBD), its physicochemical properties, and its potential impact on the seed germination and seedling growth of soybean. The results revealed significant changes in physical parameters, such as pH, total dissolved solids, total suspended solids, turbidity, conductivity, dissolved oxygen, and chemical parameters, such as calcium, chromium, sodium, manganese, nitrate, nitrites, phosphorus, and sulfur and biological parameter such as *E. coli* in water after plasma treatment. The concentration of dissolved oxygen, conductivity, nitrate, nitrite, and sulfur was increased with an increase in water treatment time, and the amounts of the other analyzed parameters decreased with the increase in water treatment time. The effects of untreated water and plasma-activated water treated for 20 minutes on soybean germination and growth were studied. The germination rate was found to be higher with plasma-treated water. Shoot lengths, seedlings length, vigor index, and germination rates were increased as compared to those germinated by normal water irrigation. The seedlings irrigated with PAW responded to the abundance of nitrogen by producing intensely green leaves because of their increased chlorophyll a as compared to seedlings irrigated with normal water. However, the content of chlorophyll b and carotenoids was found to decrease in the case of seedlings irrigated with PAW. Based on this report, we conclude that PAW could be used to substantially enhance seed germination and seedling growth.

1. Introduction

Soybean (*Glycine max*) is a globally important crop that is commonly grown for its edible bean, which can be consumed raw, defatted to produce a proteic animal feed, processed to produce meat and dairy substitutes, and fermented to produce items such as soy sauce [1, 2]. Food and nutritional security are some of the growing challenges worldwide.

In the last decade, a variety of physical, chemical, and combination intervention methods have been used to promote seed germination and sprout development, including gamma irradiation, electromagnetic fields, and UV treatment [3–5]. However, these technologies are linked to several major negative consequences, including expensive costs, long processing times, chemical residues in the environment, and environmental concerns. As a result, developing cost-effective and environment friendly solutions to boost sprout production is indeed necessary. In the context of climate change, crops worldwide are facing diverse environmental stresses. To overcome such challenges, plasma technologies can play an important role in early seedling growth through sterilization and preservation of seeds and fruits, improving seed germination and reducing pathogens in soil [6–12].

Plasma agriculture technology is being used in the sector of agriculture to enhance productivity and optimize resource loss [1, 13–15]. The novel plasma technology promotes sustainable and economic development by providing maximum efficiency at the lowest possible environmental cost [16–18]. Enhancement of seed germination and growth of plants can be achieved either by direct treatment of seeds by plasma or using plasma-activated water for irrigation [18–27].

Furthermore, the recent application of plasma-activated water (PAW) has increased in popularity among plant physiologists due to its appealing characteristics [28–30]. PAW has been proven to promote seed and seedling germination and plant development, limit the proliferation of plant-related pathogenic bacteria, and treat fungus-infected seedling [31–34]. Sivachandiran and Khacef examined the effects of PAW on seed germination and development rates in radish, tomato, and sweet pepper plants and found a significant impact on seed germination and plant growth on all these seeds [14]. Liumin Fan reported that PAW may significantly boost the germination and development of mung bean seeds. The adverse effects of PAW on mung bean seed germination were discovered when the plasma discharge time was increased [3]. Sajib et al. investigated the effect of PAW on black Gram seed germination and reported that the germination rate increased by 10–15% [35]. Naumona et al. reported that PAW treatment of rye seeds for 5 minutes boosted germination rates by 50% [36]. Chiara et al. discovered that PAW may increase the germination of soybean seeds, with the germination rate reaching 100% on the third day [1]. Loganathan et al. and Andreev et al. also discovered that PAW might increase drought tolerance and seed germination rate in radish and other crop seeds [14, 37].

These positive effects of plasma-activated water in agricultural applications are believed to be derived from the synergistic effects of various reactive species (particularly oxygen and nitrogen species) generated during the water treatment by plasma [27, 38, 39]. Moreover, the participation of reactive species in various signaling pathways in plants regulates the metabolic processes, plant development, and response to various stresses, which ultimately lead to enhanced germination and increased plant growth [2, 40, 41]. To fulfill the increased demand for food, it is vital to implement and acquire sustainable modern agriculture technologies. So, in this study, we investigated the potential application of PAW generated by using a low-cost handmade atmospheric pressure dielectric barrier discharge (APDBD) generated at 50 Hz line frequency, utilizing air as a carrier gas, on the proliferation effect of seed germination and growth of soybeans.

2. Materials and Methods

The experimental arrangement used in the present study is shown in Figure 1. The reactor consists of a transparent polycarbonate cylinder with a height of 10 cm, a diameter of 10 cm, and a 0.5 cm thickness. Brass electrodes with dimensions of 5.1 cm × 5.1 cm × 1.0 cm were used in the present study. The interelectrode separation was maintained at 20 mm for all treatments. PINTEX HVP-28HF (1000 : 1) and an oscilloscope probe (Siglent PB470, 70 MHz; 10 : 1) were used for the measurement of voltage and current waveforms. Tektronix TDS 2002, 60 MHz, was used for the analysis of the voltage and current signals. An optical fiber was inserted into an aperture in the cylinder to receive the signal from the discharge. A spectrometer from Ocean Optics Inc. (USB 2000+) was used for the measurement of emission spectra. In this work, a high voltage AC supply of 11.8 kV (r.m.s) operating at 50 Hz was used. A water sample was collected from the water source of Kathmandu University, Dhulikhel, Nepal (27°37′06.486″N and 85°32′23.245″E). For this experiment, 60 ml of water was kept in a borosilicate Petri dish and was inserted into the active plasma region on the dielectric barrier to ensure uniform treatment. Water was treated with plasma during the experiments, with exposure periods ranging from 1 to 20 minutes. After treatment, the water was preserved in sterilized reagent bottles prior to physical, chemical, and biological experiments, and analysis. In this study, pH, conductivity, dissolved oxygen, and total dissolved solids were quantified, using a standard multiparameter probe (Lutron, WA-2015), turbidity was determined by a turbidimeter (Hanna, HI88703) and total suspended solids by the gravimetric technique [42]. Similarly, heavy metals such as chromium, manganese, and sodium were quantified by atomic absorption spectrometry (SavantAA AAS, GBC), and other remaining chemical parameters were analyzed by the UV-visible spectrophotometric method (UV-1800, Shimadzu) following standard protocols [42].

2.1. Growing Condition. Initially, cocopeat was washed with distilled water and cooled at room temperature. After that, a germination tray (pot) with two compartments was taken, and the upper region of the tray (pot) was filled with cocopeat. About 180 ml of untreated water and plasma-activated water (treated for 20 min) were kept in the lower compartment of each tray, and a self-irrigation system was maintained on each tray through capillary action. Seeds of soybean were obtained from the Nepal Agricultural Research Council, Lalitpur, Nepal. Five replicates of 6 soybean seeds were sown in each tray (pot), and various germination parameters, such as final germination percentage (FGP), mean germination time (MGT), mean germination rate (MGR), coefficient of variation of germination time (CV_t), coefficient of velocity of germination (CVG), germination index (GI), and germination (G) value, were studied using the formula reported by various researchers. The seeds were irrigated with normal and treated water (PAW) at regular intervals. The images of the seedlings germinated using normal and plasma-activated water are shown in Figure 2.

2.2. Analysis of Germination Characteristics. Germination percentage (G%) is a measure of the germinability of the seeds [26, 43].

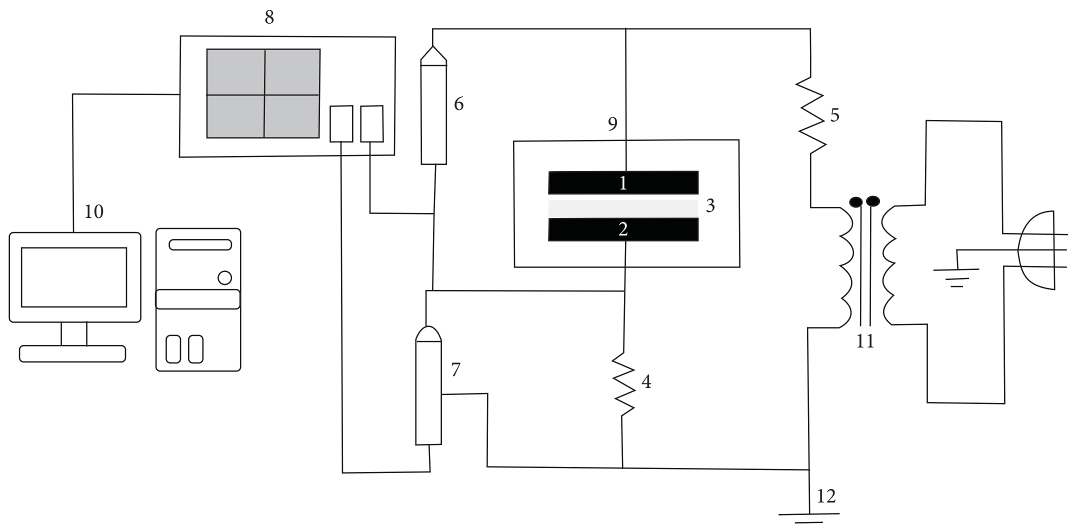

FIGURE 1: Schematic of the atmospheric pressure DBD system. (1, 2) Electrodes, (3) Petri dish, (4) ballast resistor, (5) shunt resistor, (6) high voltage probe, (7) oscilloscope probe, (8) oscilloscope, (9) reaction chamber, (10) computer interfacing, (11) high voltage transformer, and (12) ground.

FIGURE 2: Images of the seedlings germinated using normal and PAW.

$$G\% = \frac{\text{number of seedlings germinated}}{\text{total number of seeds used}} \times 100. \quad (1)$$

Five replicates of 6 seeds were taken, and the final germination percentage was measured using equation (1) on the 19th day of germination. $G\%$ can be relativized using the following equation [44].

$$R\% = \frac{\text{actual percentage}}{\text{highest percentage amongst group of data}} \times 100. \quad (2)$$

Mean germination time (MGT) is a measure of the rate and time spread of germination. It denotes the amount of time spent waiting for the sprout to germinate or emerge. Mean germination rate (MGR) is defined as the reciprocal of the mean germination time. MGT and MGR are calculated using equations (3) and (4) [45].

$$\text{MGT} = \frac{\sum Ey}{\sum E}, \quad (3)$$

where E is the number of seeds germinated on day y.

Similarly,

$$\text{MGR} = \frac{1}{\text{MGT}}. \quad (4)$$

The coefficient of variation of the germination time is estimated using the following equation [46].

$$\text{CV}_t = \frac{\text{standard deviation of germination time}}{\text{MGT}}. \quad (5)$$

Similarly, the coefficient of velocity of germination (CVG) indicates the rapidity of germination. Its value increases when the number of germinated seeds increases, and the time required for germination decreases [47].

$$\mathrm{CVG} = \frac{\sum_{i=1}^{k} n_i t_i}{\sum_{i=1}^{k} n_i} \times 100, \quad (6)$$

where n_i is the number of seeds germinated in the i^{th} time, and t_i is the time taken for seeds to germinate at i^{th} count.

The average daily germination rate (MGD) indicates the average number of seeds germinated per day.

$$\mathrm{MDG} = \frac{\text{final cumulative germination percentage}}{\text{total number of intervals required for final germination}}. \quad (7)$$

Similarly, germination (G) value is obtained by combining both the speed and completeness of germination into a composite score [48].

$$G = \mathrm{MDG} \times \mathrm{PV}, \quad (8)$$

where PV is the maximum or maximum quotient obtained when all accumulated germination percentages are divided by the corresponding time interval.

2.3. Statistical Analysis. All the experiments were performed with five repetitions, and the findings were expressed as mean ± standard error (SE). The significant difference in the mean of the physicochemical parameters of water was analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison analysis using GraphPad Prism 8.0.2. Similarly, the significant difference in the mean of the germination parameters was analyzed by an independent sample t-test followed by a two-tailed analysis using GraphPad Prism 8.0.2. Different letters in the same column and graph indicate the significant difference in mean values at $p < 0.05$.

3. Results and Discussion

3.1. Electrical Characterization of the Discharge. From Figure 3, it is evident that the current waveform consists of a large number of filamentary microdischarges. The existence of such microdischarge is a typical feature of atmospheric pressure dielectric barrier discharge (APDBD) [49].

Lissajous figures are the charge $Q(t)$-applied voltage $V(t)$ plots, during one period of the applied voltage. The energy dissipated per cycle and the average power dissipated can be calculated from Figure 4 [50].

For volume discharge, the capacitance of the dielectric-air combination C_{total} is given by the following equation [51].

$$\frac{1}{C_{\mathrm{total}}} = \frac{1}{C_{\mathrm{gas}}} + \frac{1}{C_{\mathrm{dielectric}}}, \quad (9)$$

where C_{gas} is the capacitance of air space and $C_{\mathrm{dielectric}}$ is the capacitance of dielectric.

The energy dissipated per cycle E is given by [52]

$$E = 4C_{\mathrm{dielectric}} \frac{1}{1 + \left(C_{\mathrm{gas}}/C_{\mathrm{dielectric}}\right)} U_{\mathrm{min}} \left(U_{\mathrm{max}} - U_{\mathrm{min}}\right), \quad (10)$$

and the average power dissipated is given by [52, 53].

$$P = 4fC_{\mathrm{dielectric}} \frac{1}{1 + \left(C_{\mathrm{gas}}/C_{\mathrm{dielectric}}\right)} U_{\mathrm{min}} \left(U_{\mathrm{max}} - U_{\mathrm{min}}\right), \quad (11)$$

where U_{min} is the minimum amount of applied voltage necessary to ignite the discharge, U_{max} is the maximum value of applied voltage, and f is the frequency of input voltage.

From the Lissajous figure, the energy dissipated per cycle is found to be 2.07 mJ, and the average power dissipated (P) is 103.38 mW.

Now, the value of discharge voltage (U_D) is estimated using the following equation [52–54].

$$U_D = U_{\mathrm{min}} \frac{1}{1 + \left(C_{\mathrm{gas}}/C_{\mathrm{dielectric}}\right)}. \quad (12)$$

In our case, the value of discharge voltage was found to be 2.46 kV. Using this value, the electric field in the discharge region was found to be 4.92 kV/cm.

Similarly, the estimation of average current was further used for calculating average current density [50].

$$I_{av} = \frac{P}{U_{\mathrm{min}}}. \quad (13)$$

The value of the average current was estimated to be 0.027 mA.

Now, electron density (n_e) can be obtained using the following equation [55].

$$n_e = \frac{J}{e\mu_e E}, \quad (14)$$

where J is the electron conduction current density, e is the electronic charge, μ_e is the electron mobility, and E is the electric field in the discharge region.

In this work, the electron conduction current density for discharge area of 20.41 cm^2 is 1.34×10^{-6} $\mathrm{A/cm}^2$, the electric field (E) in the discharge region is 4.92×10^3 V/cm, the electron mobility (μ_e) estimated form BOLSIG+ is 600 $\mathrm{cm}^2/\mathrm{Vs}$, and the electronic charge (e) is 1.6×10^{-19} C. Using these values in equation (6), the electron density (n_e) is estimated to be 1.07×10^9 cm^{-3}.

3.2. Optical Characterization of the Discharge. Figure 5 shows the OES from DBD generated in air at atmospheric pressure.

The line intensity ratio method was used for the estimation of electron temperature [56–58].

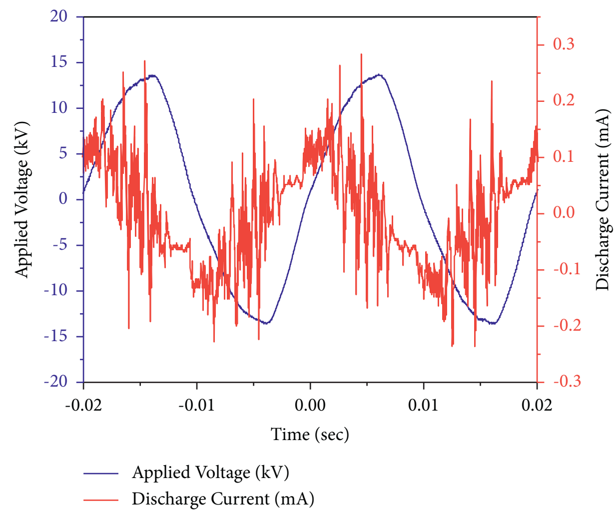

FIGURE 3: Current-voltage waveform of the plasma discharge.

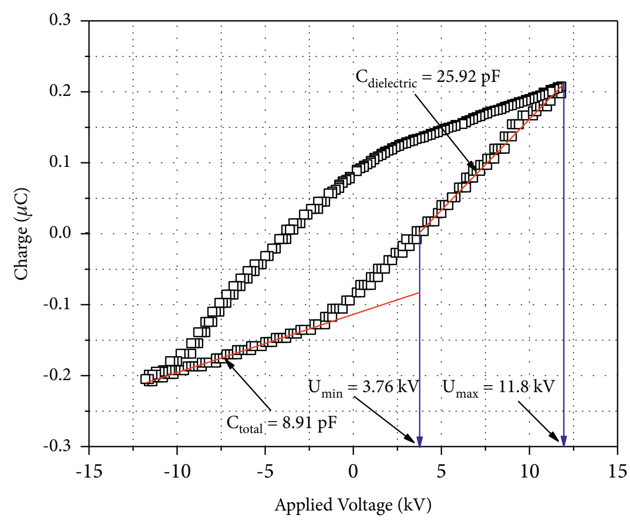

FIGURE 4: A typical Lissajous figure (Q-V plot).

$$\frac{R_1}{R_2} = \frac{I_1/I_2}{I_3/I_4} = \left(\frac{A_{pq}}{A_{rs}}\right)\left(\frac{g_p}{g_r}\right)\left(\frac{\lambda_{rs}}{\lambda_{pq}}\right)\left(\frac{A_{uv}}{A_{xy}}\right)\left(\frac{g_u}{g_x}\right)\left(\frac{\lambda_{xy}}{\lambda_{uv}}\right)\exp\left[-\frac{E_p - E_r - E_x + E_u}{kT_e}\right], \tag{15}$$

where R and I are the ratio and intensity of the spectral lines, A_{ji}, g_i, λ, E_i are the transition probability, statistical weight, wavelength, and energy of the spectral lines, respectively, and k is the Boltzmann constant.

From the spectrum, two lines of N I (493.51 nm, 410.99 nm) and two of N II (478.81 nm, 343.71 nm) were chosen. The corresponding values of A_{ji}, g_i, λ, E_i were taken from the NIST Atomic Spectra Database [59], and λ and I were taken from the discharge spectrum to estimate the electron temperature (T_e).

From Figure 6, the electron temperature (T_e) was estimated to be 1.37 eV.

3.3. Physical and Biological Parameters. Physical and biological parameters of DBD-treated water were compared with the control. Several physical parameters such as pH, turbidity, conductivity, and dissolved oxygen, which play an important role in seed dormancy and germination, were measured using a standard protocol described in the Material and Methods sections.

The pH scale is used to measure the acidity and alkalinity of any solution and directly related to the corrosivity of water and changing the shape and structure of proteins in living beings [60]. As given in Table 1, we observed a gradual decrease in pH with the increase in plasma treatment time.

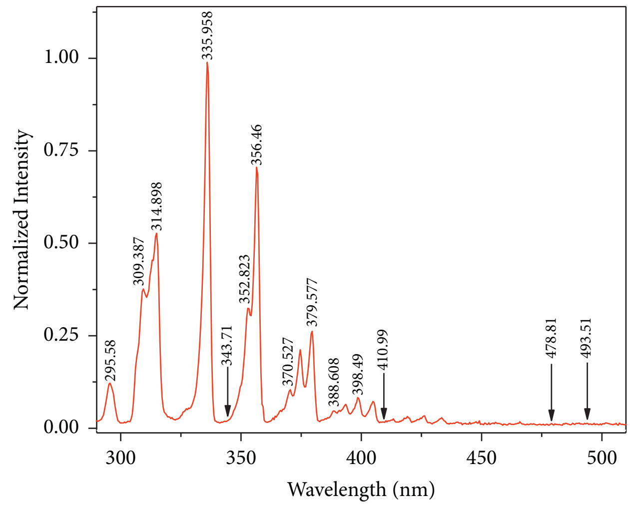

FIGURE 5: Optical emission of the discharge.

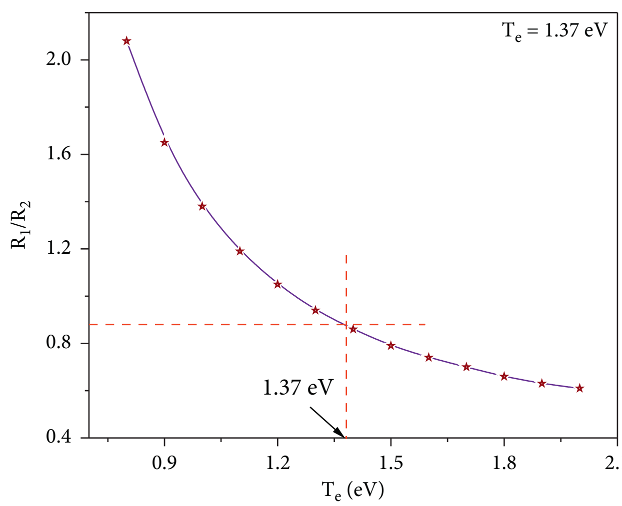

FIGURE 6: Plot of R_1/R_2 as a function of T_e.

TABLE 1: Physical and biological parameters of control and plasma-activated water (PAW).

Physical and biological parameters	Treatment time (minutes)				
	0	5	10	15	20
pH	8.91 ± 0.00^{a}	8.81 ± 0.00^{b}	8.72 ± 0.00^{c}	8.6 ± 0.00^{d}	8.5 ± 0.00^{e}
Turbidity (NTU)	1.4 ± 0.00^{a}	1.3 ± 0.00^{b}	0.8 ± 0.00^{c}	0.7 ± 0.00^{d}	0.6 ± 0.00^{e}
Total dissolved solid (mg/L)	152 ± 0.05^{a}	52 ± 0.01^{b}	27 ± 0.01^{c}	2 ± 0.05^{d}	0 ± 0.00^{e}
Total suspended solid (mg/L)	77 ± 0.05^{a}	52 ± 0.00^{b}	27 ± 0.00^{c}	2 ± 0.00^{d}	0 ± 0.00^{e}
Conductivity (μS/cm)	262 ± 0.00^{e}	265 ± 0.00^{d}	267 ± 0.00^{c}	269 ± 0.00^{b}	271 ± 0.00^{a}
Dissolved oxygen (mg/L)	6.51 ± 0.00^{e}	6.62 ± 0.00^{d}	6.73 ± 0.01^{c}	6.96 ± 0.02^{b}	7 ± 0.01^{a}
E. coli (CFU/100 mL)	100 ± 0.00^{a}	50 ± 0.00^{b}	0 ± 0.00^{c}	0 ± 0.00^{c}	0 ± 0.00^{c}

All the experiments were performed with five repetitions, and the findings were expressed as mean ± standard error (SE). The significant difference in the mean of the physical and biological parameters of water was analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison analysis using GraphPad Prism 8.0.2. Different letters in the same row indicate the significant difference in mean values at $p < 0.05$.

Furthermore, DBD-treated water revealed a decrease in solid particles, both dissolved and suspended, that are responsible for the turbidity of water.

With 20 minutes of treatment time, total dissolved solids decreased from 152.00 ± 0.05 to 0.00 ± 0.00 mg/L, total suspended solids decreased from 77.00 ± 0.05 to 0.00 ± 0.00 mg/L, and turbidity decreased from 1.4 to 0.6 NTU (Table 1). Next, we investigated the electrical conductivity of DBD-treated water. Electrical conductivity is an index of salt concentration that is responsible for the availability and movement of ions, which in turn increases the uptake of ions and dissolved oxygen for the overall growth of plant species [61, 62]. From Table 1, it was noticed that as the pH drops, the electrical conductivity of the solution increases, which is due to the higher mobility of H^+ relative to OH^-. The introduction of oxygen and other reactive species and ions into water by plasma sources might be responsible for the increase in total dissolved oxygen in PAW [63]. Furthermore, *E. coli* content in water was evaluated, and the results revealed that the number of colony counts of *E. coli* was significantly dropped, initially from 100 CFU to 0 CFU with 10 min of water treatment time (Table 1). The formation of reactive oxygen and nitrogen species (RON/ROS) in PAW might be responsible for the decrease in pH, which could be the prominent cause of its antimicrobial properties [64].

3.4. Chemical Parameters. The main principle behind the chemical reactivity of plasma-treated water is the formation of various reactive species of nitrogen and oxygen. Excess nitrate in drinking water, on the other hand, produces a variety of metabolic and physiological problems in humans.

However, because nitrate and nitrite ions are the primary sources of nitrogen for plants, this rise in nitrogen species might be beneficial in agricultural applications [65]. As given in Table 2, nitrate ion concentration was increased from 6.20 ± 0.20 to 9.00 ± 0.20 mg/L and nitrite ion concentration was increased initially from 0.00 ± 0.00 to 1.40 ± 0.03 mg/L. Similarly, the sulfur ion concentration is crucial for plant growth and metabolism. The shortage of sulfur may lead to chlorosis, a decrease in chlorophyll content, and inhibition of protein synthesis [66]. In our study, the concentration of sulfur increased gradually from an initial 0.088 mg/L to a final 0.11 mg/L. Moreover, calcium, sodium, phosphorus, and chromium concentrations decreased significantly with the increase in the water treatment time (Table 2). Calcium is considered a central regulator of plant growth and development as it is crucial for determining the structural rigidity of the cell wall and also serves as a messenger in many developmental and physiological processes of plants [67, 68]. The higher concentration of sodium leads to the inhibition of plant growth [69]. Furthermore, phosphorus is an essential component of nucleic acids, phospholipids, and energy-rich phosphate compounds and plays a crucial role in root growth, fruit and seed development, and disease resistance. However, overapplication of phosphorus will increase the risk of phosphorus losses from soil to water resources and impair water quality through eutrophication [70]. On the same note, chromium negatively impacts plant growth by impairing their essential metabolism. The toxic effects of chromium are correlated with the generation of reactive oxygen species, which cause oxidative stress in plants [71]. Calcium ion concentration decreased from 0.15 ± 0.00 to 0.13 ± 0.00 mg/L. Sodium-ion concentration initially decreased from 48.00 ± 0.00 to 35.00 ± 0.50 mg/L. The phosphorus ion concentration decreased slightly from 0.14 ± 0.02 to 0.11 ± 0.01 mg/L. The chromium ion concentration decreased from 0.95 ± 0.00 to 0.00 ± 0.00 mg/L. Nevertheless, although manganese is essential for all living beings as it acts as an enzyme cofactor in the biological cluster [72], the concentration of manganese remains constant at 4.4 mg/L in our study, as given in Table 2. RONS induced by PAW might cause seed coat cracking and make the seed coat thin, which improves its assimilation of water and nutrients, resulting in enhanced germination rate, germination index, and vigor index [3, 27, 73, 74].

3.5. Analysis of Growth Parameters. Tables 3 and 4 provide the variation of various germination parameters, such as final germination percentage (FGP), relativized percentage (R%), mean germination time (MGT), mean germination rate (MGR), coefficient of variation of germination time (CV_t), coefficient of velocity of germination (CVG), germination index (GI), and germination (G) value between the soybean seeds irrigated using control and plasma-activated water.

From Table 3, it was observed that the final germination percentage and the relativized percentage were found to increase by 11.32% and 18.86%, respectively, in the case of seedlings being irrigated with plasma-activated water. However, there was no significant change in the MGT and MGR of the seedlings germinated using control and plasma-activated water.

In the same way, CV_t decreases by 29.02% when seedlings are irrigated with plasma-activated water, as given in Table 4. In the case of seedlings irrigated by PAW, GI and MDG values both rise by 23.07% and 20.66%, respectively. A significant difference in the G value was observed between the seeds irrigated by control and PAW water. However, no significant change in the CVG was observed in the case of seedlings germinated using PAW.

Figure 7(a) shows that the number of leaves on seedlings irrigated by normal and PAW was the same when counted on the 30th and 37th days after germination. However, on the 44th day following germination, the number of leaves increased by 20% in seedlings that were irrigated with PAW. Also, as shown in Figure 7(b), the fresh weight of seedlings irrigated with PAW is greater than the fresh weight of seedlings irrigated with normal water on the 30th, 37th, and 44th days. On the 37th–44th days, however, no significant changes in the fresh weight of control-control or treated-treated seedlings were identified.

PAW activity and chemical composition, i.e., RONS concentrations, were linked to the influence of PAW on germination. Reactive species (RONs) produced in PAW may function as positive signal molecules to ease seed dormancy and accelerate seed germination by involvement in abscisic acid/gibberellic acid signaling pathways, resulting in increased seed germination. These reactive species are thought to play an important role in seed dormancy and germination control [3, 23, 27–29].

From Figure 8, it was seen that there was an increase in the shoot length and seedling length that were irrigated by PAW. As measured on the 30th, 37th, and 44th days after germination, TSL was found to increase by 21.75%, 22.92%, and 30.78%, respectively, than CSL. However, the root

Table 2: Chemical parameters of control and plasma-activated water (PAW).

Chemical parameters	Treatment time (minutes) 0	5	10	15	20
Nitrate (mg/L)	6.2 ± 0.2^{e}	7.5 ± 0.2^{d}	8 ± 0.1^{c}	8.5 ± 0.1^{b}	9 ± 0.2^{a}
Nitrite (mg/L)	0 ± 0.00^{d}	0 ± 0.00^{d}	0.5 ± 0.02^{c}	1 ± 0.02^{b}	1.4 ± 0.03^{a}
Sulfur (mg/L)	0.080 ± 0.000^{b}	0.088 ± 0.005^{b}	0.09 ± 0.005^{ab}	0.1 ± 0.004^{a}	0.11 ± 0.002^{a}
Calcium (mg/L)	0.15 ± 0.02^{a}	0.14 ± 0.01^{a}	0.13 ± 0.01^{a}	0.13 ± 0.01^{a}	0.13 ± 0.01^{a}
Sodium (mg/L)	48 ± 0.0^{a}	45 ± 0.3^{b}	42 ± 0.0^{c}	38 ± 0.5^{d}	35 ± 0.5^{e}
Phosphorus (mg/L)	0.17 ± 0.03^{a}	0.14 ± 0.02^{ab}	0.13 ± 0.01^{b}	0.12 ± 0.00^{c}	0.11 ± 0.00^{d}
Chromium (mg/L)	0.95 ± 0.04^{a}	0.86 ± 0.03^{b}	0.05 ± 0.04^{c}	0 ± 0.00^{c}	0 ± 0.00^{c}
Manganese (mg/L)	4.4 ± 0.02^{a}	4.4 ± 0.05^{a}	4.4 ± 0.04^{a}	4.4 ± 0.04^{a}	4.4 ± 0.03^{a}

All the experiments were performed with five repetitions, and the findings were expressed as mean ± standard error (SE). The significant difference in the mean of the physical and biological parameters of water was analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison analysis using GraphPad Prism 8.0.2. Different letters in the same row indicate the significant difference in mean values at $p < 0.05$.

Table 3: Estimation of final germination percentage, relativized percentage, mean germination time, and mean germination rate of soybean seeds irrigated using control and plasma-activated water.

Water	Final germination percentage (%)	Relativized percentage (%)	Mean germination time (MGT) (day)	Mean germination rate (MGR) $(\text{day})^{-1}$
Control	83.33 ± 6.14^{b}	80 ± 7.33^{a}	15.38 ± 0.22^{a}	0.0650 ± 0.0008^{a}
PAW	93.33 ± 4.66^{a}	96.67 ± 3.33^{b}	15.04 ± 0.17^{a}	0.0660 ± 0.0007^{a}

All the experiments were performed with five repetitions, and the findings were expressed as mean ± standard error (SE). The significant difference in the mean of the germination parameters was analyzed by an independent sample t-test followed by a two-tailed analysis. Different letters in the same column indicate the significant difference in mean values at $p < 0.05$.

Table 4: Estimation of coefficient of variation of germination time, coefficient of velocity of germination, germination index, mean daily germination percentage, and germination value of soybean seeds irrigated using control and plasma-activated water.

Water	Coefficient of variation of germination time (CV_t) (%)	Coefficient of velocity of germination (CVG) (%)	Germination index (GI) (day)	Mean daily germination percent (MDG) (%)	Germination value (G value)
Control	8.37 ± 1.43^{a}	6.5 ± 0.09^{a}	0.26 ± 0.02^{b}	4.21 ± 0.38^{b}	20.73 ± 3.8^{b}
PAW	5.94 ± 0.82^{b}	6.6 ± 0.08^{a}	0.32 ± 0.01^{a}	5.08 ± 0.17^{a}	30.96 ± 2.05^{a}

All the experiments were performed with five repetitions, and the findings were expressed as mean ± standard error (SE). The significant difference in the mean of the germination parameters was analyzed by an independent sample t-test followed by a two-tailed analysis. Different letters in the same column indicate the significant difference in mean values at $p < 0.05$.

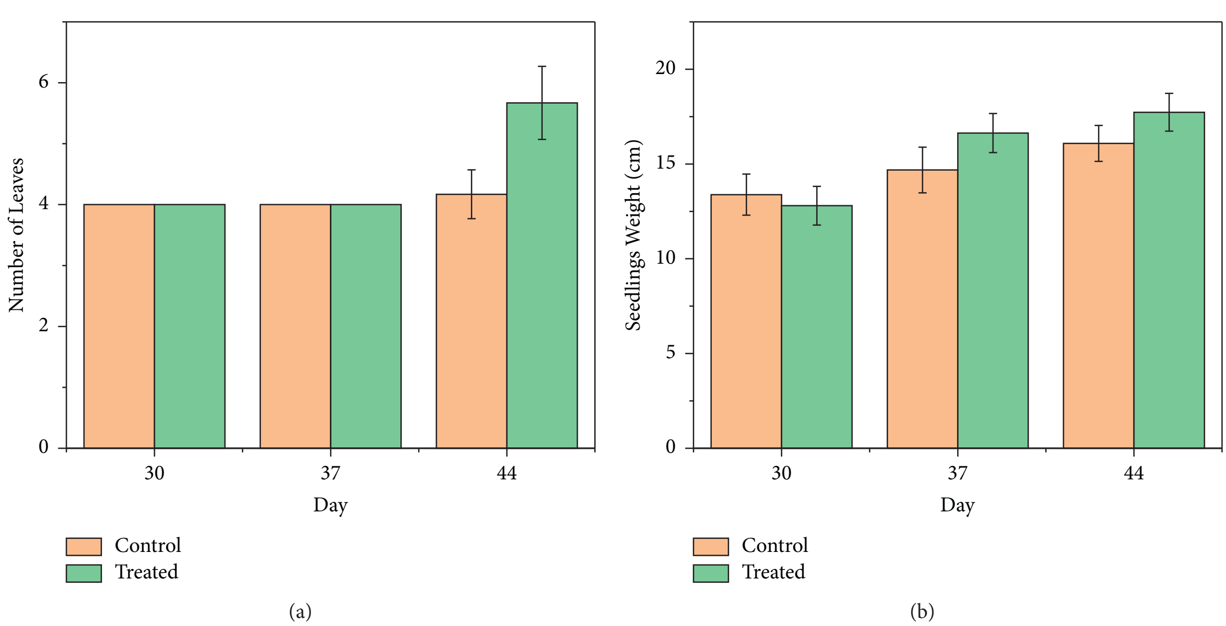

Figure 7: Estimation of (a) number of leaves and (b) seedlings fresh weight.

length of the seedings irrigated by PAW is found to decrease by 9.61% and 7.33% as measured on the 30th and 37th days after germination. But TRL was found to increase on the 44th day by 12% as compared to CRL. It was further noticed that the overall length of the seedlings was found to be greater in the case of TPL as compared to CPL by 1.69% and 16.01% as measured on the 37th and 44th days after germination. It has been reported by various research that the nitrate present in PAW absorbed through the roots of the seedlings is mainly responsible for the plant growth as it acts as a plant growth enhancer [23, 27, 33].

The sum of those properties of the seed that determine the potential level of activity and performance of the seed during germination and seedling emergence is known as "seed vigor." The vigor of seedlings was calculated using the following equation [2].

$$\text{Vigor index I (VI I)} = \text{mean seedling length (cm)} \times \text{germination (\%)}. \tag{16}$$

It was noticed that the VI I of seedings irrigated by PAW was found to be increased by 26.92% as compared to the seedlings irrigated by normal water. This increment positively correlates with the seedling length and fresh weight, which agrees with the results documented by various researchers. The changes in germination and early seedling growth observed in the experiment might be explained by the stimulation of certain natural signals, hormones, and enzyme activities [2].

3.6. Estimation of Chlorophyll and Carotenoids Contents. The chlorophyll a and b contents, as well as the total carotenoids content, were measured spectrophotometrically using the acetone extraction method and equation developed by Wellburn in 1994 [75]. Leaves contain chlorophyll, carotenoid, and anthocyanin and important pigments which play a critical role in plant growth and contribute greatly to the appearance of plants. Chlorophyll is the pigment that gives plants their characteristic green color and occupies a unique role in the physiology and productivity of green plants [76]. Chlorophyll is essential for the conversion of light into chemical energy, which helps in determining the photosynthetic rate and primary productivity in plants The amount of chlorophyll in the environment changes as a result of various external environmental factors. Therefore, it has been extensively used as an indicator for plant growth studies [77]. Although, leaf color and chlorophyll content play a critical role in plant growth and its appearance, very few studies have been carried out in the examination of the effects of plasma-activated water on plant growth activity concerning chlorophyll content.

In this study, we analyzed the change in chlorophyll and carotenoid contents of plants grown with 20 min treated water with plasma. As shown in Figure 9, our results indicate the increase in chlorophyll a content with the treatment time. But chlorophyll b and total carotenoid levels decreased slightly with the treated water supply. Chlorophyll a content increased from 24.55 ± 0.06 mg/g to 43.59 ± 0.04 mg/g. In contrast, chlorophyll b was dropped from 30.83 ± 0.35 mg/g to 7.01 ± 0.08 mg/g and total carotenoids also decreased from 30.05 ± 0.17 mg/g to 12.48 ± 0.13 mg/g. As carotenoid indicates the yellow color during chlorophyll degradation, our findings point to a higher accumulation of chlorophyll content in plant leaves, which may aid in increasing plant growth rate.

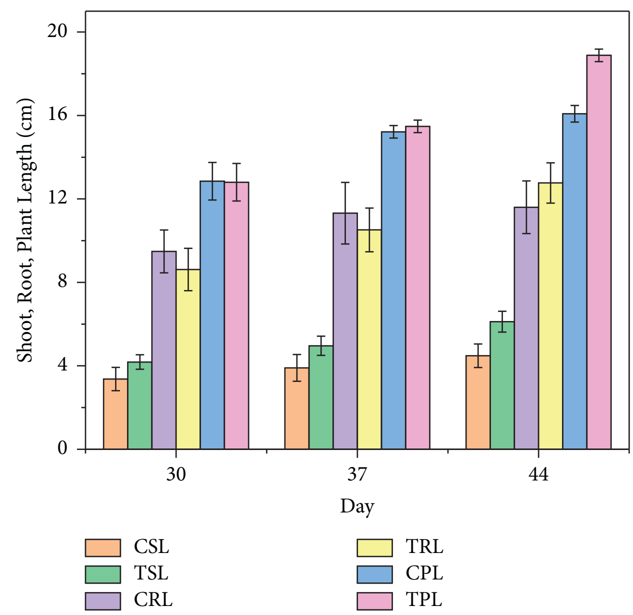

Figure 8: Estimation of control shoot length (CSL), treated shoot length (TSL), control root length (CRL), treated root length (TRL), control plant length (CPL), and treated plant length (TPL).

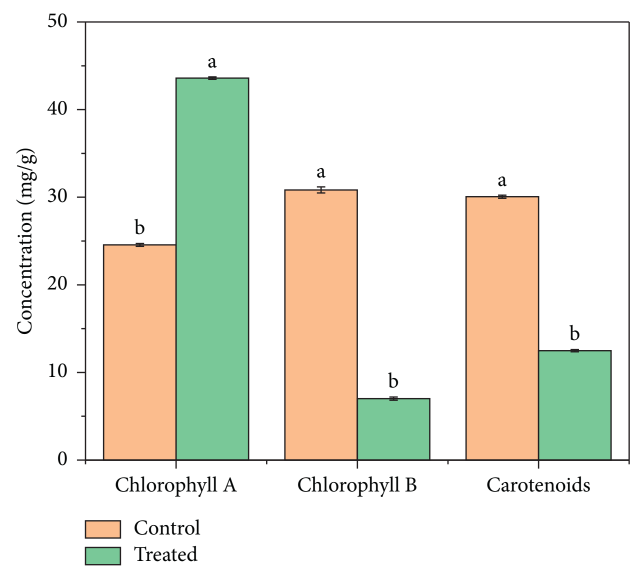

Figure 9: Estimation of chlorophyll and carotenoid content. Different letters denote notable difference between groups ($p < 0.05$).

4. Conclusion

In this study, reactive nitrogen and oxygen species were generated in well water employing a dielectric barrier discharge operating at 50 Hz. Here, the electron temperature and density were found to be 1.37 eV and $1.07 \times 10^9\ cm^{-3}$, respectively. The energy dissipated and power consumed by the discharge was estimated to be 2.07 mJ and 103.38 mW. Compared with ordinary water, this plasma-activated water (PAW) improved the germination rate, vigor index, and plant development of soybean. The use of PAW to irrigate seedlings resulted in positive physical and chemical changes in the plants. These plants respond to nitrogen availability by producing a strong green color in their leaves, which is consistent with the increase in chlorophyll content. Based on our findings, much greater yield could be achieved in a short time with the assistance of irrigation using PAW.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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