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Water absorption and seedling growth enhancement of Timur seeds (*Zanthoxylum armatum*) by using cold atmospheric pressure plasma

Samjhana Dahal[1], **Roshan Chalise**[1,2,3,*], **Raju Khanal**[1]

[1]*Central Department of Physics, Tribhuvan University, Kirtipur, Kathmandu 44613, Nepal*
[2]*Amrit Campus, Department of Physics, Tribhuvan University, Thamel, Kathmandu 46600, Nepal*
[3]*Department of Physics, St. Xavier's College, Tribhuvan University, Maitighar, Kathmandu 44600, Nepal*

*Corresponding author. Email: plasma.roshan@gmail.com

Abstract

Plasma, often called the fourth state of matter, is an ionized gas with wide applications in physics ranging from astrophysical phenomena and nuclear fusion research to material processing, biomedical technologies, and modern agricultural practices. This study investigates the application of cold atmospheric pressure plasma (CAPP) for improving the wettability, seedling growth, and chlorophyll content in the leaves of Timur seeds (Zanthoxylum armatum), a medicinally and culinarily valuable Himalayan plant. We have employed gliding arc discharge for direct seed treatment while the plasma-activated water (PAW) was generated using dielectric barrier discharge. The results reveal that direct plasma exposure had water absorption enhancement, as well as PAW significantly improved seedling growth, chlorophyll, and root–shoot development under laboratory conditions. Plasma diagnostics confirmed the formation of reactive oxygen and nitrogen species, with favorable physicochemical changes in PAW. The findings demonstrate the promising potential of CAPP for sustainable agrotechnological applications.

Keywords
Plasma-activated water, Cold atmospheric pressure plasma, Cylindrical dielectric barrier discharge, Gliding arc discharge.

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1 Introduction

Zanthoxylum armatum DC., commonly known as Timur, is a small deciduous shrub or tree native to the Himalayan region and widely used as a spice and traditional medicine [1, 2]. Its aromatic fruits and seeds contribute to the distinctive flavor of regional cuisines, while the plant contains bioactive compounds with analgesic, anti-inflammatory, and antimicrobial properties [3]. Given its economic and ethnobotanical importance, efforts to cultivate Timur sustainably are critical to reduce the pressure on wild populations [4]. Seed propagation remains the primary method for Timur multiplication. However, seed dormancy and low germination rates (often below 65%) pose constraints for large-scale production [5]. The dormancy of the seeds in Timur is mainly due to the hard seed coat that restricts water uptake and gas exchange [6]. Conventional methods such as hot water treatment, acid scarification, and chemical soaking are used to overcome dormancy, but can be time-consuming, potentially harmful, or costly [7].

Recent advances have introduced cold atmospheric pressure plasma (CAPP) as a new physical seed treatment capable of improving wettability and seedling growth enhancement without chemical residues [8, 9]. CAPP is generated by applying high voltage to the gas at atmospheric pressure, producing a partially ionized gas with reactive oxygen and nitrogen species (RONS), ultraviolet (UV) photons, charged particles, and electric fields [10, 11]. These reactive species interact with seed coats, causing physical and chemical modifications that promote water uptake and activate biochemical pathways within seeds [12]. Studies on diverse crops such as wheat [13], cauliflower [14], mushroom [15], and rice [16], green leafy [17] have shown that plasma treatment enhances nutrient uptake [18], reduces microbial contamination [19], and stimulates seedling growth [20]. However, investigations on Himalayan spices like Timur are limited [21]. In the present work, we study the effects of cold plasma treatment on Timur seed's wettability, seedling growth, and chlorophyll content in the plant's leaves.

2 Materials and Methods

2.1 Plasma Generation Setup

In this work, the experimental setup of cylindrical DBD was used to prepare PAW, and gliding arc discharge was used for direct seed treatment. The atmospheric pressure gliding arc discharge requires a high-voltage power source, which is applied between two curved electrodes with an air blower. The schematic diagram for the generation of atmospheric pressure air gliding arc and dielectric barrier discharge, and its characterization are shown in Figure 1 and 2 respectively. We used a gliding arc for direct treatment of seeds at different times, that is, 0 (control), 2, 4, 6, and 8 minutes. Similarly, the same treatment time of PAW is carried out by DBD. We used a 3.36 kV DC discharge voltage for discharge generation with a natural air flow of 15.0 LPM. The electrode gap is 14.0 mm, and the gap from the lower point to the seed surface is 4.0 mm. 20 seeds are treated at a time. The setup is shown schematically in Figure 1 for direct seed treatments and Figure 2 for water treatment to irrigate the plants during growth time.

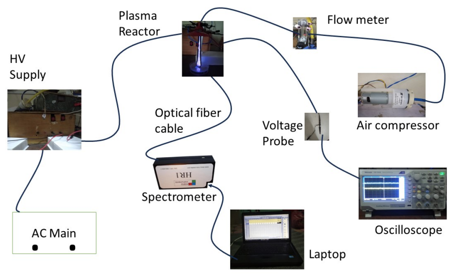

Figure 1: Schematic diagram of the gliding arc discharge plasma setup used for direct seed treatment.

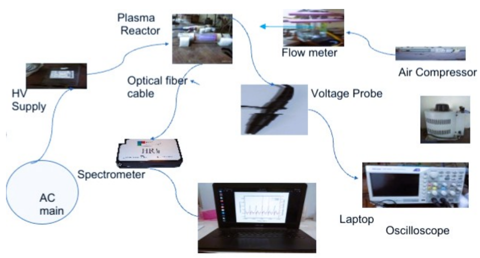

Figure 2: Schematic diagram of the cylindrical dielectric barrier discharge plasma setup used for water treatment.

2.2 Plasma Diagnostics

Producing plasma is not sufficient; it must also be characterized before it can be utilized in various fields. There are numerous ways to diagnose plasma. In this work, we characterize the discharge via electrical and optical methods. The produced plasma is optically characterized by using an HR-1 spectrometer. The spectrometer measures the intensity as a function of wavelength. It is used to estimate plasma properties, including electron temperature and electron density [22]. The intensity of the spectral line is given by [23];

$$I_{ij} = \frac{hcA_{ij}g_jn}{\lambda_{ij}U(T)} \exp\left(\frac{-E_j}{k_BT}\right) \quad (1)$$

where I and λ are the intensity and wavelength corresponding to the transition from i to j, respectively. h is the Planck's constant, c is the speed of light, n is number density of emitting species, $U(T)$ is partition function, A is the transition probability, k_B is Boltzmann's constant, T_e is electron excitation temperature, g_j is the statistical weight of upper energy level and E_j is upper energy level. Now, taking the ratio of intensity of two spectral lines from equation (1), we get,

$$\frac{I_1}{I_2} = \Big(\frac{g_1 A_1 \lambda_2}{g_2 A_2 \lambda_1}\Big) \exp\Big[-\frac{E_1 - E_2}{k_B T_e}\Big] \tag{2}$$

The subscripts 1 and 2 refer to the spectral lines of the same element selected.

Determination of electron density

The electron density of the discharge can be determined by using the Boltzmann-Saha equation [24]:

$$n_e = 2\Big(\frac{I_1}{I_2}\Big)\Big(\frac{\lambda_1}{\lambda_2}\Big)\Big(\frac{A_2}{A_1}\Big)\Big(\frac{g_2}{g_1}\Big)\Big[\frac{2\pi m_e k_B T_e}{h^2}\Big]^{\frac{3}{2}} \exp\Big[-\frac{E_1 - E_2 + E_i}{k_B T_e}\Big] \tag{3}$$

where I_1 and I_2 are the intensities of N (III) lines, λ_1, λ_2 are wavelength, A_1, A_2 are the transition probabilities, g_1, g_2 are the statistical weight of two different observations and E_i is the ionization energy of neutral atom.

2.3 Plasma-Activated Water

In our lab (Plasma Research Laboratory of CDP, TU, Kirtipur), the de-ionized water was treated with a cylindrical DBD to generate PAW. The temperature of the seeds was measured using an infrared thermometer. This instrument can measure temperatures ranging from -50.0 to 550.0°C. The seeds were treated directly, and then their temperature was recorded. The parameters of potential hydrogen (pH), total dissolved solids (TDS), electrical conductivity (EC), and oxidation reduction potential (ORP) were assessed using the RCYAGO 7-in-1 water quality tester. To calibrate the tester, it should be immersed in a buffer solution with a pH of 9.18. Reactive nitrogen species (RNS), nitrite, and nitrate concentrations were measured using test strips (Changchun MDC Medical Co., Ltd) as depicted. The test strips were dipped in PAW for two seconds, then removed, and left for more than 30 seconds to observe the color change.

2.4 Seed Collection and Preparation

The mature Timur (*Zanthoxylum armatum*) seeds were provided by Ms. Sunita Gurung of Ashok Medicinal and Aromatic Plants Center, Banepa. Also, during the harvest season, Mr. Sangat Sharma and Mr. Num Prasad Acharya provided seeds from local farmers in Kirtipur and Dang. Seeds were manually extracted from fruit pericarps, washed thoroughly in distilled water to remove the adhering pulp, and dried at ambient room temperature (25±2)°C for two weeks before experiments to achieve uniform moisture content. Seeds were evenly spread in a single layer on the grounded electrode and exposed to plasma for 0 (control), 2, 4, 6, and 8 minutes. Temperature during treatment was monitored and maintained below 45°C to avoid thermal damage. After treatment, seeds were immediately used for wettability tests. Initially, the wettability of Timur seeds was first conducted under controlled laboratory conditions (in a plant growth chamber at a temperature of around 25°C, limited light, and constant humidity) for about three months. The experiments were observed for another three months in a greenhouse-like setup near the laboratory, covered with transparent plastic to allow natural sunlight while maintaining moderate humidity and temperature. Additionally, we decided to investigate the effects of plasma treatment on younger plants instead. Therefore, young *Zanthoxylum armatum* seedlings were collected from a nursery and used for subsequent plasma exposure and growth analysis. Final wettability and seedling growth data were recorded under controlled laboratory conditions. So, we performed multiple tests using various methods.

2.5 Wettability

We divided 100 seeds into five groups at a time, each group containing 20 seeds, and repeated 5 times. For each treatment, 20 seeds were placed on moist laboratory filter paper in nine cm diameter Petri dishes and incubated in a growth chamber at 25 ± 2°C with 12 h light/dark cycles. We divided seeds into five groups and seed treatment (T) with different plasma time exposure (min): control, 2min T, 4min T, 6min T, and 8min T. 200.0 ml of untreated and treated de-ionized water was dipped for each section once a week and repeated it 5 times in the same procedure. The measurement of wettability using PAW by cylindrical dielectric barrier discharge (indirect method) and via direct treatment method by using gliding arc discharge are depicted in Figures 3. Seeds were moistened, and water absorption was recorded daily for 30 days. The experiment was conducted continuously for 10 hours from 8:00 a.m. to 6:00 p.m., with each batch of seeds receiving the same procedure. Wettability % is determined by using relation [25]:

$$\text{Wettability (\%)} = \frac{m_1 - m_0}{m_0} \times 100 \tag{4}$$

Where m_0 is the weight of the seed before dipping in water and m_1 is the weight of the seed after being dropped and taken out.

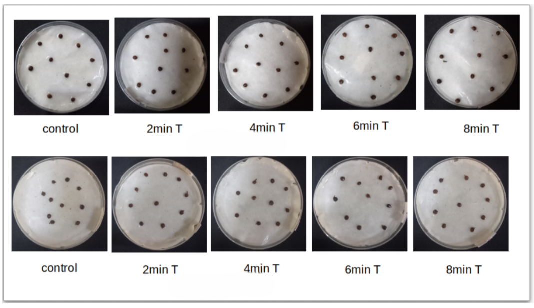

Figure 3: Seeds dipped in water for wettability through the direct treatment method using air gliding arc discharge.

2.6 Growth Parameter

The Timur plants were brought from farmer Ramkumari Shen, Kapurkot-3, Mulpani, Salyan. We divided 12 plants into four groups for a comparative study of cylindrical dielectric barrier discharge for PAW. Three morphologically similar plants were chosen for each group to maintain uniformity among samples. One of the limitations in this process. The plant provider does not provide more than 25 plants, so large samples could not able to taken. Also at this time, no other farm had succeeded in germinating the Timur plant. At first, we filled a glass with 40.15 g vermicompost soil up to a height of 4.0 cm. Subsequently, we planted a Timur plant after making a small hole. We added soil and noted the weight of the glass with plants by using a balance weighing machine. We placed the sample in a growth chamber maintaining temperature (25.0 ± 1.0)°C and humidity (75 ±15)%. Also, we used 15.0 ml of untreated and treated water to irrigate plants with an interval of two days. We observed the condition of the plant regularly and observed changes in the plant. Additionally, chlorophyll content was measured using the device TYS-B Chlorophyll meter, a small, handy, non-destructive device, the readings of which are expressed in non-metric SPAD units ranging from 0 to 200. Each plants from each section, with one leaf in each plant, were selected for the chlorophyll content test.

3 Results and Discussion

3.1 Electrical and Optical Characterization

From Figure 4 (a), the peak-to-peak discharge voltage of cylindrical DBD is 38.88 kV, and the discharge current is 0.58 mA. Similarly, from Figure 4 (b), the peak-to-peak discharge voltage of the gliding arc is 3.36 kV and the discharge current 31.80 mA. Figure 5 (a) and (b) show the OES of cylindrical DBD and gliding arc, the wavelength range 300 to 500nm and 200 to 500 nm, respectively. To know the exact species, we used the NIST database [26]. The nearest wavelength is searched, and the corresponding species are noted down.

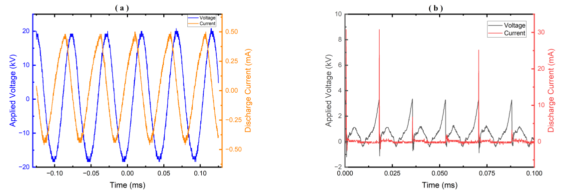

Figure 4: Current-voltage characteristics of atmospheric pressure air (a) DBD and (b) GAD.

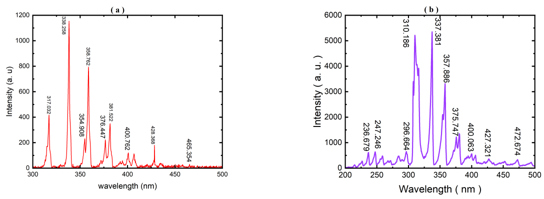

Figure 5: Optical emission spectra of atmospheric pressure air (a) DBD and (b) GAD.

Boltzmann plot method

To estimate the electron excitation temperature using the Boltzmann plot method, Equation 1 is employed. The necessary parameters are obtained from the NIST database: the wavelength, intensity, statistical weight, transition probability, and energy for the Boltzmann plot of the gliding arc discharge. By plotting the quantity, $ln\,(\lambda_{ji}I_{ji}/hCA_{ji}g_j)$ against the energy level (E_k), Figure 6 was obtained and the *slope* = *-1/k_B T* was found to be 1.13 eV. The temperature agrees with the temperature obtained from the line intensity ratio method and falls within the range suggested by [27].

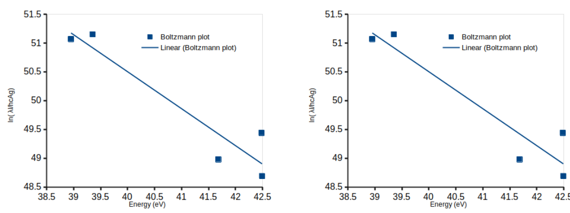

Figure 6: Boltzmann plot for estimating electron temperature of GAD and DBD.

We have selected two lines with the highest and lowest upper energy levels to determine the electron excitation temperature and density of DBD and gliding discharge plasma from identified peaks and corresponding necessary parameters. For gliding arc discharge:

$$\frac{576.18}{364.28} = \left(\frac{4 \times 9.12 \times 10^7 \times 400.06}{8 \times 1.88 \times 10^8 \times 236.66}\right) \exp\left[-\frac{41.68 - 42.49}{k_B T_e}\right]$$

which yields;

$$T_e = 1.66\text{eV}$$

For cylindrical dielectric barrier discharge:

$$\frac{388.15}{50.57} = \left(\frac{2 \times 1.41 \times 10^7 \times 489.91}{8 \times 4.29 \times 10^7 \times 312.56}\right) \exp\left[-\frac{39.02 - 41.92}{k_B T_e}\right]$$

which yields;

$$T_e = 1.41\text{eV}$$

Hence, the numerical estimation of electron temperature for the first spectrum is found to be 1.66 eV and 1.41 eV for gliding arc discharge and DBD, respectively.

Plasma density

The electron density (plasma density) in the discharges can be determined by the Boltzmann-Saha equation from equation (3). For air discharges, the ionization energy of nitrogen species is 14.53 eV. For the cylindrical DBD and gliding arc discharge, the electron densities are found to be 8.17×10^{18} cm^{-3} and 5.48×10^{17} cm^{-3}, respectively.

3.2 Effect of Plasma Treatment on Wettability Percentage

The wettability of seeds is directly related to their water uptake capacity. As the wettability increases, the water uptake absorption rate increases. Our focus was to observe the seed's water absorption and determine their wettability for inactivated and plasma-activated seeds. As water uptake is a function of activation time, we noticed changes in wettability for different activation times. From the graph, it can be seen that the wettability of seeds increases every hour up to some value and then saturates. The wettability of seeds for various treatment times by gliding arc discharge is shown in Figure 7. The graph shows that the wettability of seeds increases every two hours up to a certain value before becoming saturated. After eight hours, the seeds, in this case, have solidified. The improvement in wettability and seedling growth enhancement observed here is consistent with prior studies on plasma seed treatments in other species. The reactive oxygen and nitrogen species produced during plasma exposure etch the seed coat surface, increasing its permeability and facilitating water uptake, crucial step in breaking dormancy.

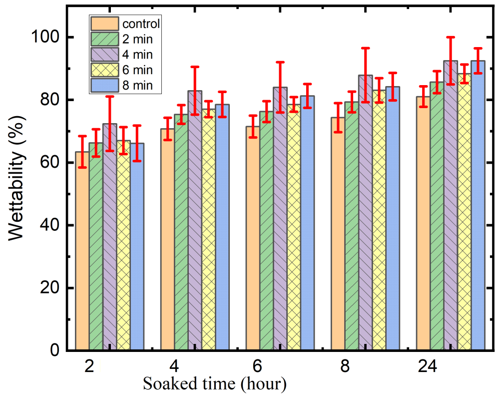

Figure 7: Wettability of direct treated Timur seeds by using gliding arc discharge.

3.3 Physico-Chemical Properties of Plasma-Activated Water

The atmospheric cylindrical DBD is used to treat the water to make PAW. The treatment of the water with the cylindrical DBD changes its physico-chemical properties. The addition of various ions like nitrates, nitrites, etc., affects other properties such as pH, electrical conductivity (EC), total dissolved solids (TDS), oxidation-reduction potential (ORP), as well as its temperature of PAW for different plasma exposure times, which are shown in Figure 8. The pH value of PAW is decreased from 7.95 ± 0.80 to 3.70 ± 0.50 when de-ionized water treated for control to 8 minutes is displayed in Figure 8(a). In this and all subsequent values are represented as the mean values ± standard deviation. The results are achieved by averaging the five different sets of data. The pH of water decreases with the increase in plasma discharge time, resulting in its acidic nature. It is dropped due to the production of reactive oxygen and nitrogen species (RONS) in PAW. From Figure 8(a), we noticed that the number of H^+ ions increases with an increase in plasma discharge time. The ability of ions to move is one of the crucial factors for the rapid wettability and growth of plants, which, in turn, enhances the intake of ions and dissolved oxygen.

Figure 8(b) shows how the electrical conductivity of the water increased from 0.0 to 2140.0 ± 16.0 μS/cm when de-ionized water was treated for

0 to 8 minutes. The movement of ions increases as the plasma discharge time increases because of the plasma's energy, which also serves to enhance the water's conductivity and growth processes. The electrical conductivity of the solution rises as the pH falls because the H^+ ion has more mobility than that of the OH^- ion. An increase in conductivity indicates a larger solute concentration dissolved in the water, which may impact the surrounding osmotic potential of the seed. Figure 8(c) shows that the variation of total dissolved solids in PAW increased from 0.0 to 1020.0 ± 1.0 ppm when de-ionized water was treated for control up to 8 minutes. As the plasma discharge time increases, total dissolved solids increase due to the formation of reactive oxygen and nitrogen species after the application of plasma in water. Water's osmotic potential rises in parallel with TDS concentration.

Figure 8(d) shows that the variation of oxidation and reduction potential(ORP) increased from 298.00 ± 0.26 to 460.00 ± 0.19 mV when deionized water was treated for 0 to 8 minutes. The oxidation-reduction potential is found to be maximum for 8 minutes of plasma-activated water. Higher ORP values indicate a more oxidative environment, which can have antimicrobial effects. We have measured the temperature of PAW, which increased from 22.1 ± 0.6 to 25.0 ± 0.9C when deionized water was treated for control for 8 minutes, as shown in Figure 8(e). The temperature of water is likely to increase when energy is applied in the form of sparks. This is mostly due to the average molecular kinetic energy continuing to rise, which also raises the thermal energy of water. We have used the testing strips to measure the concentrations of nitrate and nitrite in the PAW. The concentration of nitrate and nitrite solutions affects the color of the test strip. The concentration of nitrite and nitrate in water at various plasma discharge times increased from (0 - 10) mg/L and (0 - 100) mg/L when treated for control to 8 min, as shown in Figure 8(f). Compared to nitrite, the concentration shift was more noticeable. The absence of nitrite in comparison to nitrate can be explained by the fact that the conversion of nitrite to nitrate is enhanced under acidic conditions.

Hence, we found that electrical conductivity, total dissolved solids, and oxidation-reduction potential are significantly increased with increasing plasma discharge time with water, while pH is significantly decreased. This increment is due to the formation of oxidizing species and active ions. Most of the active ions produced during plasma exposure were H^+ ions, $NO_2{}^-$, $NO_3{}^-$ions, etc. As the plasma is treated with water for a longer period of time, the H^+ ion concentration in the water rises, resulting in a continuous decrement of pH value. Expanding the plasma treatment period increases the concentration of active ions (H^+, $NO_2{}^-$, and $NO_3{}^-$), which produces higher values of EC and TDS of PAW.

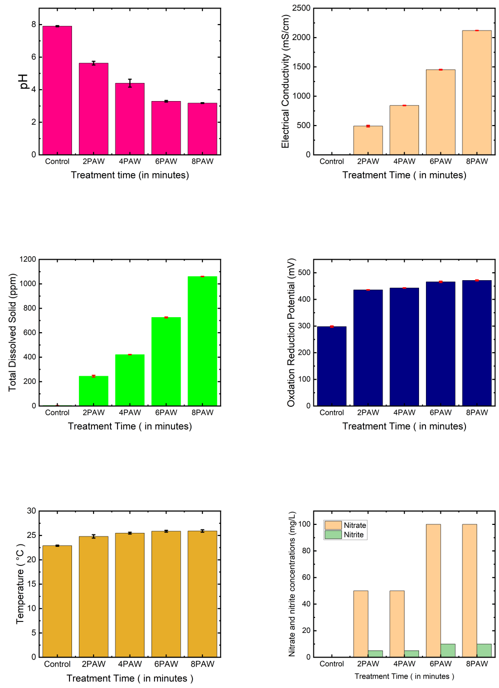

Figure 8: The physical and chemical parameters of PAW by using gliding arc discharge for different plasma exposure time (a) pH, (b) electrical conductivity, (c) total dissolved solids, (d) oxidation-reduction potential, (e) temperature, and (f) nitrite and nitrate concentrations.

3.4 Growth Parameter

We observed the condition of the plant regularly and observed changes in the plant. First, we noted the data of leaves before the plant; after some days, the leaves fell and started forming new leaves, as shown in Figure 9.

Figure 9: Growth of Timur plants by irrigating PAW made by cylindrical DBD after 19 days.

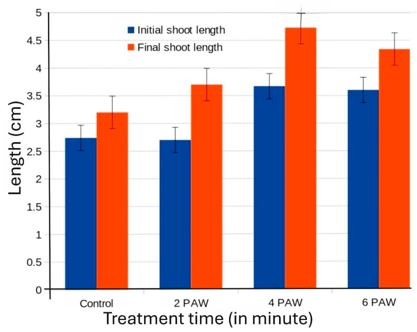

Figure 10: Shoot length of Timur plants comparison between before and after using PAW of different treatment times by cylindrical DBD.

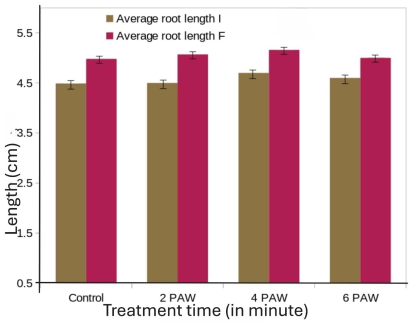

Figure 11: Root length of Timur plants comparison between before and after using PAW of different treatment times by cylindrical DBD

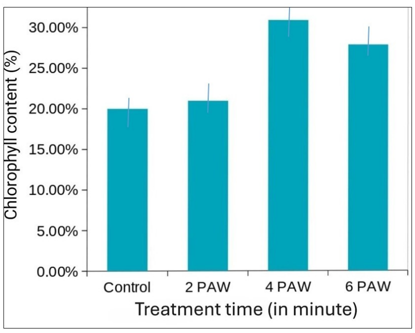

Figure 12: Chlorophyll content of Timur plant leaves with different plasma-activation time by cylindrical DBD.

Figure 10 and Figure 11 show average shoot length and average root length of Timur plantings after treatment for variable periods. Figure 10 showed a significant rise with PAW treatment, reaching (4.7 ± 0.3) cm at 4 PAW compared to (2.8 ± 0.2) cm in the control. The effect declined slightly at 6 PAW, suggesting an optimal treatment duration of 4 min. Figure 11 describes that root length increased from (4.3 ± 0.2) cm (control) to (4.9 ± 0.1) cm (4 PAW). Specific reactive oxygen and nitrogen species (RONS) are found in PAW. Due to its impacts on nutrient intake, hormone signaling, stress responses, water absorption, cell division, defense systems, and soil microbial populations, PAW can have a complex impact on plant growth, affecting both the length of shoots and roots. The proper application of PAW can improve plant growth and productivity in a sustainable and eco-friendly manner. Furthermore, each plant from each group (control, 2 PAW, 4 PAW, and 6 PAW), with one leaf of each plant, was selected for the chlorophyll content test, which was measured in each leaf, and the average was calculated. Leaf chlorophyll concentrations can rise as a result of enhanced nutrient availability, which can promote chlorophyll production. The 4 PAW treatment showed the highest chlorophyll content (30.2 ± 1.4)% while extending the treatment to 6 PAW slightly reduced performance. From Figure 12, it is evident that leaves from the plants treated with 4 PAW by cylindrical DBD exhibit higher chlorophyll content as compared to other PAWs.

4 Conclusion

We studied the effects of direct plasma treatment and plasma-activated water (PAW) on Timur (*Zanthoxylum armatum*). Cylindrical dielectric barrier discharge (DBD) and gliding arc discharge (GAD) plasmas were characterized, with discharge voltages/currents of 38.88 kV/0.58 mA (DBD) and 3.36 kV/31.80 mA (GAD). Spectroscopic analysis indicated electron temperatures of 1.41 eV (DBD) and 1.66 eV (GAD), and electron densities of 8.17×10^{18} cm^{-3} and 5.48×10^{17} cm^{-3}, respectively. PAW exhibited increased conductivity, total dissolved solids (TDS), oxidation-reduction potential (ORP), temperature, and decreased pH as activation time increased. Nitrate and nitrite levels both increased, with nitrate rising more significantly. Direct plasma improved seed wettability and water uptake, especially at 4 minutes. Additionally, PAW enhanced root and shoot growth and leaf greenness, with the highest chlorophyll retention using a 4-minute PAW treatment. These findings highlight the potential of cold atmospheric plasma technology to enhance Timur's growth and medicinal value, thereby supporting its wider cultivation and contribution to agronomy.

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