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Research Article

CHARACTERIZATION OF PLASMA ACTIVATED WATER GENERATED FROM GLIDING ARC DISCHARGE AND ITS APPLICATION ON ENHANCEMENT OF SEED GERMINATION OF RADISH (*Raphanus sativus var. longipinnatus*)

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ABSTRACT

In recent decades, generation of plasma activated water (PAW) from non-thermal atmospheric pressure plasma sources has received enormous attention due to their diverse applications. The research described in this paper is mainly focused on the preparation and characterisations of PAW produced from gliding arc discharge (GAD) operated with high voltages (9.7 kV, 50 Hz) power supply system and its use in the enhancement of seed germination of radish. The physical and chemical parameters of the PAW are investigated using a multi-parameter probe and UV-visible spectrometer. There are significant differences in physical parameters like pH and conductivity, and chemical parameters like concentration of nitrates, nitrites, ammonia in untreated and PAW. But no significant differences in temperature and total dissolved oxygen (TDO) are found. In order to determine the effects of PAW on seed germination, different germination parameters are calculated on radish (*Raphanus sativus*) which indicates that PAW can enhance the seed germination of radish.

Keywords: Germination parameters, plasma activated water (PAW), non-thermal plasma, reactive species

INTRODUCTION

It is always challenging for the whole world to fulfill the demands of the growing population on food supply from the limited cultivable land. In order to meet this demand, the only way is to increase the crop yields from the limited land. Escalating the seed germination rate and developing drought-resistant seeds could be the alternate way to improve crop production from limited land (Thirumdas *et al*., 2017). Chemical methods have been practicing as the best and quickest method to enhance seed germination since several years. But these types of methods not only give adverse effects on human beings but also on other living creatures including different self-running cycles in nature. The chemical residues coming from these methods as outcomes are harmful to human health and the environment. Therefore, it is obligatory to generate new alternative and clean technologies to improve seed germination and plant growth to meet the pace of developing population. Plasma acts as a standalone technology that provides innovative solutions for enhancing agricultural productivity. Application of non-thermal plasma developed at atmospheric pressure, could be the solution for increasing the germination rate and plant growth which do not leave any harmful residue to the surroundings and can produce quality and safety of food and other agricultural products (Brandenburg *et al*., 2019). Preliminary investigations so far have confirmed that pre-treatment of seeds of vital agricultural crops by low-temperature plasma is an effective method for the improvement of germination rate as well as shoot and root growth (Joshi *et al*., 2018). Generally, there are two ways to apply non-thermal plasma on seed germination and plant growth: direct method and indirect method. In the first method plasma directly applies to seeds which corrodes the seed coats and increases the water uptake capacity of the seeds (Ling *et al*., 2015, Hayashi *et al*., 2015). In the second method, instead of direct plasma, PAW is used for seed treatment. In our experiment gliding arc discharge (GAD) is used to produce PAW.

Plasma activated water

Water treated with plasma above or under the surface of it, is termed as plasma activated water (PAW) which is well known by reactive species present in it. PAW has unique chemical composition than water as the formation of reactive nitrogen species (RNS) and reactive oxygen species (ROS) during the discharge have the ability to modify the important properties of water like pH, oxidation-reduction potential, conductivity, H_2O_2 concentration, nitrate and nitrites concentration, etc.

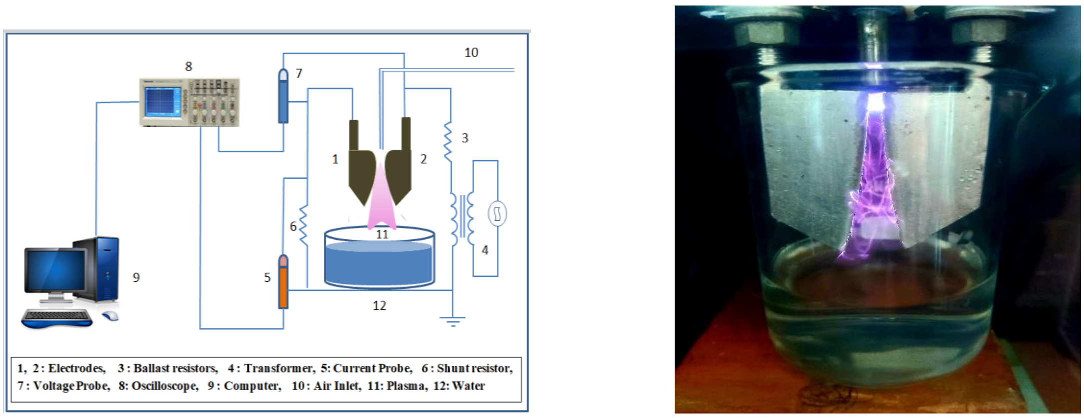
(Šimečková *et al*., 2020; Baniya *et al*., 2021). It can be generated by two major approaches. Either it can be prepared by direct contact of the non-thermal atmospheric pressure plasma streaming with the water or by inducing the plasma directly into the water. The chemical properties of PAW developed above the surface of water are different from that of the developed directly in the water (Thirumdas *et al*., 2018). In recent decades, the generation of PAW from non-thermal atmospheric pressure (NTP) plasma sources has received enormous attention due to its diverse applications (Brisset & Pawlat, 2016). Mostly, it used in the biomedical and agricultural field with applications in the treatment of cancer (Lu *et al.*, 2017), inactivation of bacteria (Traylor *et al.*, 2011; Yusupov *et al.*, 2013), medical device sterilization (Abuzairi *et al.*, 2018) as well as to increase the germination and growth rate of crops (Park *et al.*, 2013; Judee *et al.*, 2021) and food sterilization (Ma *et al.*, 2015; Lin *et al.*, 2019), etc. Prior to implementing PAW in different prospective domains, it is essential to thoroughly assess and characterize it in order to achieve optimal efficiency. Thus, in the first section of this work, a few parameters of the PAW are studied and compared with control water samples (distilled water).

Role of plasma activated water on seed germination

The term germination in the seeds of higher plants, especially in angiosperms refers to the projection of a root or shoots from the seed coat (Kader, 2005). Germination of seeds begins when dry seeds take water and it ends with the elongation of the radicle (Šírová *et al.*, 2011). Germination of seed is regulated by many factors including ambient conditions and intrinsic hormonal pathways. The RNS present in PAW, avails in several stimulation pathways along with the plant hormones and some ROS helps to break the seed dormancy and trigger seed germination. It has been suggested that nitrous oxide (NO) can break seed dormancy and is considered as an active agent in improving germination (Batak *et al.*, 2002). Further it has been also reported that H_2O_2 plays a significant role in activating the Catalase (CAT) genes, thereby facilitating the synthesis of new proteins that enhance the germination of *Paulownia tomentosa* seeds (Puač *et al.*, 2018). The primary focus of the research outlined in this paper lies in the process of generating plasma-activated water using GAD and its application for improving the germination of radish. The study encompasses an investigation into the physical and chemical characteristics of the PAW. This involves the measurement of parameters such as pH, conductivity, temperature, as well as the concentrations of nitrates, nitrites, and so forth. The presence of highly reactive nitrogen and oxygen species are affirmed over the interface between air and water surfaces by the optical method which are necessary to make water rich in reactive species. The role of PAW on seed germination of radish is studied by determining the various germination parameters.

EXPERIMENTAL SECTION

The diagrammatic representation of the experimental setup for the generation of discharge is shown in Fig. 1(a). The system consisted of two diverging aluminum electrodes which were fixed to a rectangular polycarbonate chamber having dimensions (15 cm × 15 cm × 15 cm). A small hole in the overlying face of the chamber was used to vent the required gas. The minimum space of 3 mm was maintained between the electrodes. The discharge was produced by supplying high voltages (9.7 kV) and line frequency 50 Hz through a Ballast resistor of 1.7 MΩ. Digital oscilloscope (Tektronix TDS2000) was used to determine the current-voltage waveform to estimate power per cycle. In order to determine the ROS and RNS present in the discharge, spectra were captured by using optical fibre with a Digital Spectrometer (Ocean Optics, USB 2000+). The spectra were measured by passing an optical fiber through a hole keeping the fibre at a distance of 4 cm from the discharge.



(a) (b)

Figure 1. (a) Diagrammatic representation of experimental arrangement of GAD system (b) photograph of water treatment.

A beaker having diameter of 10 cm was placed underneath the discharge which contained 40 mL of distilled water as shown in Fig. 1(b), for the treatment. The separation between electrodes and water was maintained at 5 mm. The treatments were done for different time periods, viz., 5 minutes, 10 minutes, and 15 minutes. Physical and chemical parameters of the untreated and treated water were analyzed with the help of different methods and instruments. Physical parameters like pH, conductivity, dissolved oxygen, and temperature were tested by multi-parameter probe (Lutron, 2015). Chemical parameters like concentration of nitrate, nitrite, and ammonia were determined from a UV-visible spectrometer.

To study the germination rate of the seeds, 100 radish seeds (*Raphanus sativus var. longipinnatus*) were taken in two petri dishes having a diameter 9.5 cm of each. Among two dishes, one was irrigated using distilled water and another was irrigated using PAW. Germinated seeds were started to count 24 hours after sowing. The readings were taken for three days when the average maximum and minimum temperature of the days were 20^{0}C to 28^{0}C. The process was replicated three times. Four different germination parameters were studied for the first three days of cultivation. All the parameters were calculated using the formula reported by Kader and Ranal in their reports (Ranal & De Santana, 2006; Kader, 2015).

i) Final germination percentage (FGP) $= \frac{\sum n_i}{N} \times 100\ \%$ [1]

Where n_i represents the total numbers of seeds germinated in ith day and N represents the total number of seeds

ii) Germination rate index (GRI) $= \frac{G1}{1} + \frac{G2}{2} + \cdots + \frac{Gx}{x}$ [2]

Where, G_1 indicates the germination percentage on day 1, G_2 indicates the germination percentage in day 2 and so on.

iii) Coefficient of velocity of germination (CVG) $= \frac{N1+N2+N3+\cdots+Ni}{N1T1+N2T2+N3T3+\cdots+NiTi} \times 100$ [3]

Where, N represents the number of seeds germinated every day and T reprensts the number of days from seedling corresponding to N seeds.

iv) Mean germination time (MGT) $= \frac{\sum fx}{\sum f}$ [4]

Where, *f* is the number of seeds germinated on day *x*.

Electrical and optical properties:

Electrical power:

Power dissipated per cycle in the discharge can be calculated by integrating the discharge voltage and current, as given in equation:

$$\text{Power (P)} = \frac{1}{T}\int_0^T V(t)\, I(t)dt \qquad [5]$$

here V(t) and I(t) are the voltage and current developed during the discharge and T be the time period of alternating supply (Lu *et al.*, 2012).

Electron density and electron temperature:

Electron density is calculated by electrical method given by equation (Baniya *et al.*, 2020):

$$n_e = \frac{j}{eE\mu_e} \qquad [6]$$

Where, j, e , E and μ_e represent current density, electronic charge, electric field strength and electron mobility respectively.

Electron temperature of the dischare is estimated by Boltzmann plot method given by the equation (Ohno *et al.*, 2006):

$$ln\left[\frac{I_{ji}\lambda_{ij}}{A_{ji}g_j}\right] = -\frac{E_j}{kT_e} + C \qquad [7]$$

Where, I_{ji}, λ_{ji}, A_{ji} and g_j represent intensity of emitted ligtht, wavelength of emitted light, transition porobability and statistical weight of upper level. Similarly, E_j, k, T_e represnts upper energry level, Boltzmann constant, electron temperatrue respectively and *C* is constant.

RESULTS AND DISCUSSION:

Electrical Properties

Fig. 2 (a) shows the waveform of discharge current and voltage of GAD discharge. The current voltage wave form shows that peak voltage and peak current are found to be 7 kV and 7 mA respectively at the applied voltage of 9.7 kV. The power dissipated per cycle of the discharge using Equation 5 is found to be 4.59 watt.

Electron density and electron temperature

For the electron density Equation 6 is used and in our condition, cross sectional area of the plasma = 0.60 cm^2, discharge voltage = 3.5 kV, discharge current = 3.65 mA minimum distance between the electrodes = 0.3 cm. The electron mobility of air is 592.1 cm^2/V.s (Raĭzer, 1991). With these values the electron density is estimated to be 5.49×10^9 cm^{-3}.

For the estimation of electron temperature nitrogen III (NIII) lines having wavelengths 335.87 nm, 374.59 nm, 379.29 nm, 393.29 nm and 433.29 nm and their respective intensities are used in Equation 7. Using the Boltzmann plot method electron temperature is determined as 1.24 eV. The calculated value of electron temperature and electron temperature confirms that the plasma generated using GAD system is non-thermal and applicable in agriculture.

Detection of Reactive species

Optical emission spectroscopy (OES) is used to detect the reactive oxygen and nitrogen species emitted by the discharge. OES enables us to obtain useful information on excited atomic and molecular states of gases. The typical optical emission spectrum of a gliding arc discharge operated at 9.7 kV with air as the feeding gas can be seen in Fig. 3. Reactive nitrogen and oxygen species constitute the majority of the emission profile from 200 to 450 nm. The interaction of energetic electrons with nitrogen and oxygen molecules in the ambient air results in the creation of these species (Abdelaziz *et al.*, 2018). The emission spectrum depicts that there is formation of NO_X(200-280 nm), OH (309 nm), N_2 second positive system (SPS) (311-380 nm), N_2 first negative system (FNS) between 300 nm - 440 nm wavelength range (Chen *et al.*, 2019). This specifies the presence of excited particles of nitrogen atoms as well as a large number of nitrogen-containing molecules and ionic reactive particles, such as NO (Akishev *et al*., 2010). The presence of these excited particles affirms the formation of reactive nitrogen and oxygen species (RNOS) above the water to be treated. With the aid of airflow, the RNOS produced in this process can be transferred into water, where they combine with the water to form reactive radicals. These transformations can be assessed through physicochemical testing of the resulting reactive water. OH radicals emitted at 309 nm are seemed to be produced from the collision of electrons, or metastable nitrogen atoms, with the molecules of water whereas the excited nitrogen species considered to be originated from the dissociation of nitrogen molecules present in the normal environment or gas used (Zhou *et al*., 2018; Adhikari *et al*., 2019; Liu *et al*., 2019).

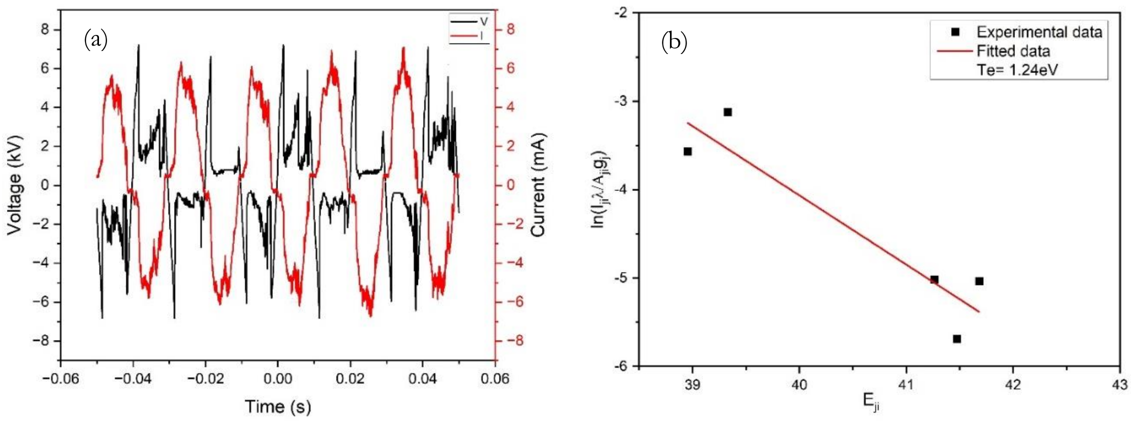


Figure 2. (a) Current voltage waveform of the discharge, (b) Determination of T_e using Boltzmann distribution plot.

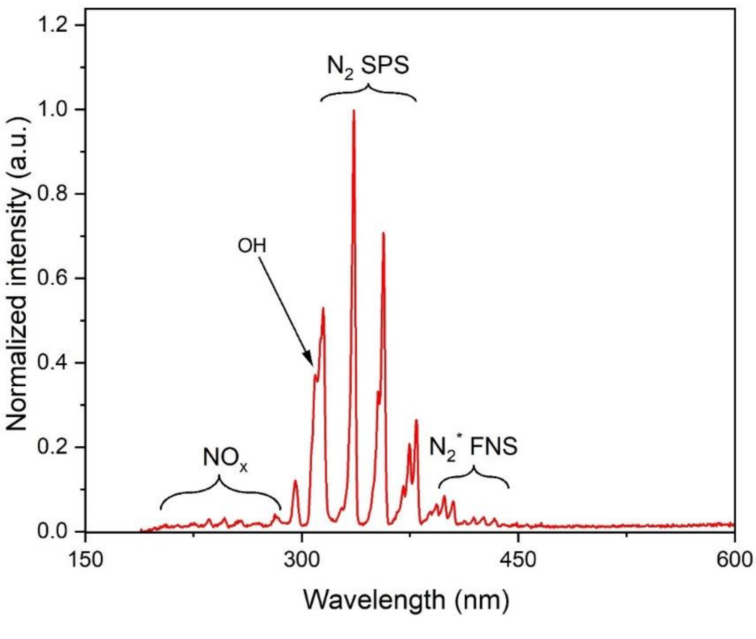
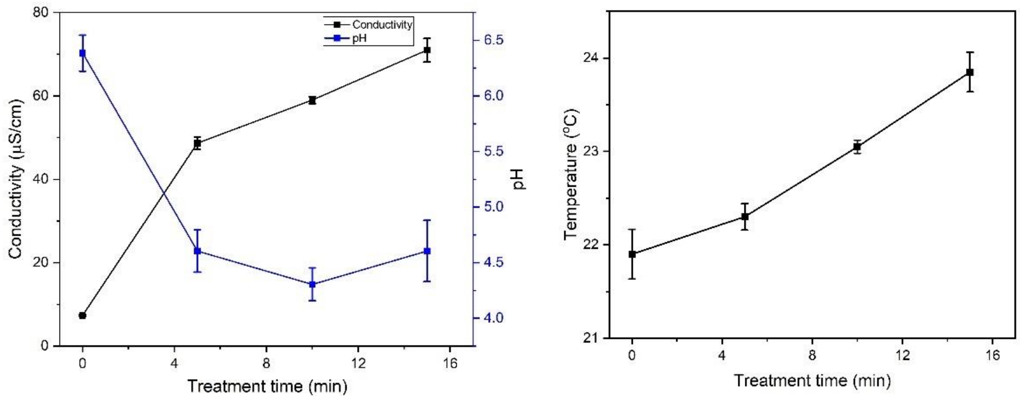


Figure 3. OES of GAD produce in air plasma at atmospheric pressure.

Physiochemical characteristics of PAW Physical parameter

Three physical parameters pH, conductivity and temperature of the distilled water are measured and their variation with time is studied. Fig. 4(a) shows the graphical representation of the variation of pH value and conductivity of distilled water with different treatment times. Initially, the untreated distilled water has a pH value of 6.4, after 5 minutes of treatment time it decreases to 4.6. Up to 10 minutes of treatment time, it decreases gradually and after 15 minutes of treatment time it reaches to again 4.6. The results depict that the discharge generated from GAD increases the acidity of distilled water.

Fig. 4(a) also depicts the linear relation between electric conductivity and treatment time. Initially, the untreated distilled water has electric conductivity of the value of 6 μS/cm but after 5 minutes of treatment time its value increases up to 51 μS/cm. After just 15 minutes of treatment time, its value increases eleven times than the initial value and reaches 70 μS/cm. This increase in conductivity is due to the formation of RNOS during the preparation of PAW. During plasma treatment, the reactive species and ions formed readily dissolve in water, which evidently modify the conductivity.



(a) (b)

Figure 4. Variation of (a) pH and conductivity (b) temperature with treatment time.

The variation of change in temperature with treatment time is plotted in Fig. 4(b). The graph shows that there are no significant changes in temperature with treatment time. Initially, the untreated water has a temperature of about 22 ^{0}C. After 10 minutes of treatment, its value increases by 1 ^{0}C. After 15 minutes of treatment, the temperature increases just by 2 ^{0}C. Since we are using non-thermal plasma having higher electron temperature and less ion and neutral species temperature, the temperature of the sample does not increase significantly.

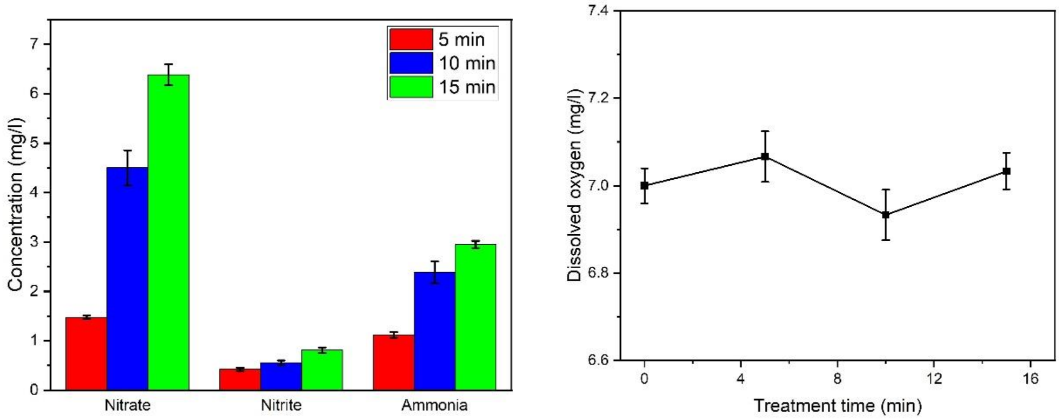
Chemical parameters

Four chemical parameters, viz. concentration of nitrate, nitrite, ammonia, and dissolved oxygen in the treated and untreated water were measured by UV-visible spectrophotometer. Fig. 5(a) represents the graphical representation of change in concentration of nitrate, nitrite and ammonia formation with treatment time in water respectively. It shows that as the treatment time increases the concentration of nitrite and nitrate formation increases. Initially, the nitrite and nitrate concentration of distilled water is found to be zero for both cases but after 5 minutes of treatment their values become 0.45 mg/l and 1.3 mg/l and after 15 minutes of treatment the concentration reaches 0.75 mg/l and 6.5 mg/l respectively. In PAW, the formation of nitrite ions take place when oxides of nitrogen react with water. Finally, when these nitrite ions react with hydrogen peroxide, the formation of nitrate ions takes place. As the treatment time increases there is a high chance of formation of oxides of nitrogen as a result, the concentration of nitrate ions and then nitrite ions increase.

The NH_3 concentration increases to 1.08 mg/l within 5 minutes of treatment time. The amount of NH_3 concentration increases almost linearly with the increase in plasma exposer time. After 15 minutes of treatment the value of concentration is obtained 3 mg/l. In plasma activate water, formation of ammonia takes place when hydrogen evolved from the excitation or dissociation of water combine with excited nitrogen. The concentration of ammonia in water depends on treatment time. As the treatment time increases, the rate of formation of excited nitrogen and hydrogen increases and finally concentration of ammonia increases. It has been suggested that higher concentrations of these RNOS species aid in accelerating seed germination (Guragain *et al*., 2021). The concentration of dissolved oxygen (DO) of untreated and treated water is measured by the Multi-parameter Probe. Fig. 5(b) demonstrates that GAD does not affect the concentration of dissolved oxygen significantly. Initially, the untreated distilled water has a concentration of DO of 7.1 gm/l. After 5 minutes of treatment, it increases slightly and at 10 minutes of treatment time, it slightly decreases and again increases to 7.1 gm/l after 15 minutes of treatment.

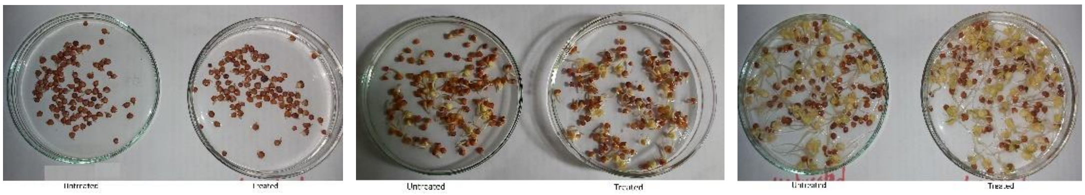
Seed germination

To study the effects of PAW on seed germination, four germination parameters are studied for the first three days of cultivation according to the Equations (1, 2, 3 and 4) mentioned above. The photographs of seed sprouting for the first three days are shown in Fig. 6.



(a) **(b)**

Figure 5. Change in concentration of (a) nitrate, nitrite, and ammonia (b) dissolved oxygen with treatment time.

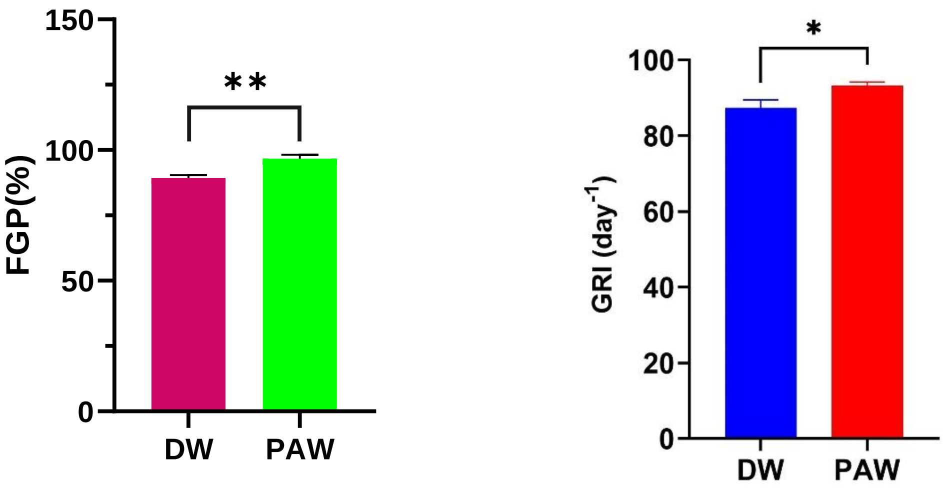


(a) **(b)** **(c)**

Figure 6. Images illustrating the progression of seed germination on the (a) first, (b) second, and (c) third days after sowing.

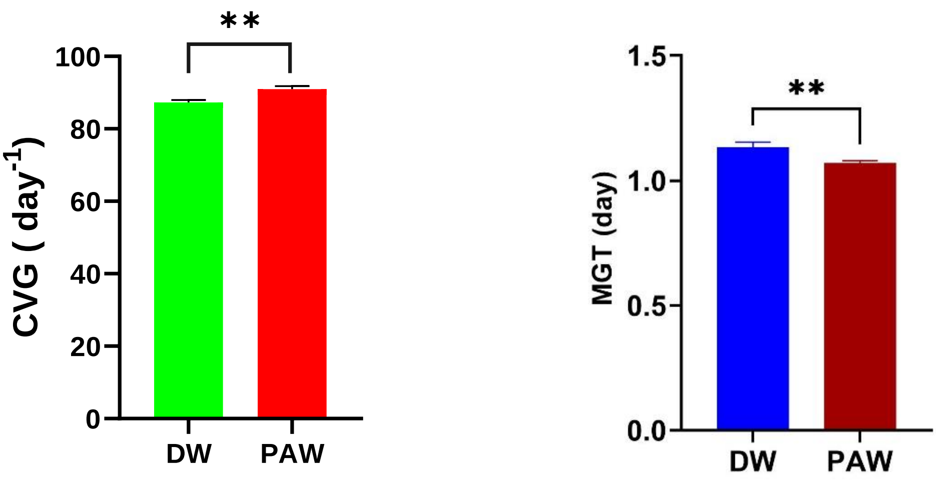
Fig. 7(a) shows the final germination percentage for three different seed lots. For every seed lot, the final germination percentage (FGP) is found to be higher in the vessel that is irrigated by plasma activated water than that of the distilled water. For the seeds irrigated by PAW, final germination percentages are found to be increased by 5.43%, 5.20% and 7.8% than that of DW for 1st, 2nd and 3rd seed lots respectively. Finally, the average FGP is 6.14% higher PAW environment than that of DW. The higher value of FGP indicates that there is greater germination of a seed population.

Fig. 7(b) represents the graphical representation germination rate index (GRI) of three different samples of radish seeds. There is higher GRI in the seeds which are irrigated by PAW than in DW. The average GRIs of the three lots of seeds is found to be 8.95% higher than that of seeds irrigated in DW. The GRI indicates the percentage of germination on each day of the germination cycle and higher value of germination shows higher and faster germination.



(a) **(b)**

Figure 7. Comparison of (a) FGP and (b) GRI in PAW and DW irrigated seeds. The asterisks denote notable difference between groups ($p < 0.05$).



(a) (b)

Figure 8. Comparison of (a) CVG and (b) MGT in PAW and DW for three seed lots. The asterisks denote notable difference between groups ($p < 0.05$).

Fig. 8(a) shows that the coefficient of velocity of germination (CVG)s are higher in all three seed lots which are irrigated by PAW. For PAW irrigated seeds, the average value of CVGs are found to be 5.93%, higher in comparison to DW irrigated seeds. The CVG indicates the speed at which seeds germinate. It rises as the number of germinated seeds rises and the time it takes for germination falls. Thus, the result shows that for all three seed lots, the number of germinated seeds is higher in petri dishes irrigated by PAW. In order to study the rapidity of germination time, mean germination time (MGT) is also calculated. Fig. 8(b) depicts the MGT for three different seed lots irrigated by DW and PAW. The MGT values for seed irrigated by PAW are found to be less than that of DW. The average value of MGTs for the PAW irrigated pot is 5.28%, less than that of DW. The lower value of MGT indicates that there is faster germination in seeds lots soaked in PAW.

CONCLUSIONS

In this study, it is found that the discharge produced from GAD is non-thermal plasma which is confirmed by studying electrical and optical properties. Physiochemical properties of PAW show that plasma is able to increase acidity and conductivity of distilled water but there is no significant increase in temperature after plasma treatment. Similarly, chemical parameters like concentration of nitrate, nitrite and ammonia all are found to be increased with treatment time. But there is no remarkable difference in the amount of dissolved oxygen for control and plasma treated water samples. Germination parameters like FGP, GRI, CVG and MGT are calculated for seeds (*Raphanus sativus*) irrigated by PAW and compared with seeds irrigated by untreated water. It is observed that FGP, GRI and CVG values for seeds sowed in PAW were 5% to 6% greater than that sowed in untreated water but the value of MGT is found to be less in seeds lots irrigated by PAW by 5.28% than that of untreated water. These findings indicate there was higher and faster germination in seed lots containing PAW. Thus, PAW generated from GAD operating at an industrial frequency of 50 Hz has the ability to change the physiochemical properties of water and the activated water has the potential to enhance the germination rate of the seeds.

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AUTHOR CONTRIBUTIONS

Santosh Dhungana: Conceptualization, investigation, software, data curation, writing-original draft, methodology. Rajesh Prakash Guragain: Optical characterization, revision, and editing. Dipak Prasad Subedi: acquisition, project administration, supervision. Hom Bahadur Baniya: resources, validation, visualization, revision, and editing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATUS

Upon reasonable request, the data that supports this study will be made available by the authors.

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