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# From seed to sprout: Unveiling the potential of non-thermal plasma for optimizing cucumber growth

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A R T I C L E I N F O

*Keywords:*
Non-thermal plasma (NTP)
Germination performance
Cucumber seeds
Dielectric barrier discharge
Reactive species

A B S T R A C T

*Background and aims:* Numerous strategies for enhancing seed germination and growth have been employed over the decades. Despite these advancements, there continues to be a demand for more effective techniques, driven by the growing global population. Recently, various forms of non-thermal atmospheric pressure plasma have garnered attention as environmentally friendly, safe, and cost-effective methods to enhance the agricultural and food sectors. This study explores the remarkable impact of non-thermal plasma (NTP) treatment on cucumber (Cucumis sativus L.) seed germination.
*Methods:* A cost-effective, custom-designed power supply operating at line frequency was used for treating seeds, with exposure times ranging from 1 to 7 min. Various germination parameters, including water contact angle measurements, mass loss, water imbibition rate, and seedling length, were evaluated to assess the impact of plasma treatment on seed germination.
*Results:* Cucumber seeds exposed to NTP treatment for 3 min and 5 min durations showed significant germination improvements, notably a 57.9 $\pm$ 4.25 % higher final germination percentage, 14.5 $\pm$ 3.75 % reduced mean germination time, and a remarkable 90.6 $\pm$ 4.64 % increase in germination index compared to the control. These results suggest that NTP treatment enhanced seed coat permeability, triggered essential biochemical processes, and expedited water absorption and nutrient assimilation, ultimately fostering faster and more synchronized germination.
*Conclusions:* Our findings underscore the potential of NTP as an innovative approach to improving seed germination in agricultural practices.

## 1. Introduction

Addressing the imperative of agricultural transformation is of paramount importance to significantly boost yield, enhance global food security, and tackle the pressing challenges faced by farming sector. The predominant reliance on subsistence agriculture has

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perpetuated a cycle of low productivity, emphasizing the urgent need to revamp and modernize agricultural practices in the country [1,2]. With two-thirds of Nepal's population still dependent on agriculture for their livelihoods, a comprehensive agricultural transformation becomes an indispensable endeavor to uplift rural communities, foster sustainable growth, and ensure a prosperous future for the nation [3].

In the realm of achieving a circular green economy in agriculture, diverse avenues are being explored as alternatives. Notably, direct treatment of seed surfaces through Dielectric Barrier Discharge (DBD) technology has emerged as a subject of growing interest within the agricultural domain [4,5]. Numerous studies have shown the benefits of non-thermal processing techniques (NTPs) in managing biological substances linked to seed contamination [6,7], surface characteristic alteration [8,9], metabolomic pathways [7], and enzymatic activity [10], improving seed germination and the early development [11,12]. This environmentally benign practice presents itself as a viable substitute for traditional farming methods, thus garnering considerable attention as a means to foster sustainable agriculture.

Numerous studies have shown that applying a suitable dose of plasma treatment to seeds can lead to significant improvements in various aspects of seed germination and seedling growth [13–15]. This treatment notably enhances the germination rate, speed, water uptake, seed vigor, as well as key characteristics of shoot and root development across a range of seeds, including cotton [16], soybean [17] centipede grass [12] radish [18], papaya [19] maize [20], etc. Furthermore, simultaneous treatment of 150 spinach seeds with Rolling DBD increased water uptake and germination rates after a 5-min treatment, while the RDBD device improved germination rates and reduced standard errors by treating seeds from all directions, representing a significant advancement in non-thermal atmospheric pressure plasma devices that can enhance the potential applications of plasma in agriculture [21]. Similarly, Florescu et al. treated sunflower seeds with plasma and found that the treated seeds grew taller, had greater total mass, and resulted in increased crop yield. The plasma exposure positively affected capitulum size, number of seeds per capitulum, and mass per thousand seeds [22].

Cucumber holds a prominent position as a vital vegetable crop globally, widely recognized for its consumption in fresh form. Renowned for its nutritional value, cucumbers serve as an abundant source of essential vitamins, minerals, and antioxidants [23]. Notably, cucumber holds an esteemed position among the top ten most significant vegetable crops cultivated globally [24]. The aim of this study was to explore the effects of non-thermal plasma (NTP) treatment on cucumber (*Cucumis sativus* L.) seeds and their growth-related characteristics. The seeds were treated with a custom-designed dielectric barrier discharge plasma using argon gas in an air environment. The study sought to understand the underlying mechanisms of plasma action on seeds and lay the groundwork for future field experiments.

## 2. Methods

### 2.1. Experimental design

Fig. 1 illustrates the experimental setup employed in the study. The setup comprises a polycarbonate reactor chamber with a rectangular shape, measuring 357.0 mm in length, 200.0 mm in width, and 150.0 mm in height. Within the reactor chamber, two copper electrodes are positioned in a rectangular shape, with a separation gap of 5 mm. The dimensions of the electrodes are 75.4 mm in length, 49.9 mm in width, and 10.0 mm in thickness. The lower electrode is covered with a polycarbonate dielectric layer, which has measurements of 150 mm in length, 120 mm in breadth, and 2 mm in thickness.

The flow rate of argon gas in the reactor chamber was kept constant at 5 L per minute. The rate of Ar gas flow within the compartment was quantified using a gas flow meter (Yamato Scientific, Japan). To generate a discharge, a sinusoidal voltage with a frequency of 50 Hz and a magnitude of 13.8 kV was applied to the electrodes. A Pintek HVP-28HF High Voltage Probe (manufactured by Pintek Electronics Co., Ltd, Taiwan), which could sense voltages up to 28 kV with a division ratio of 1000:1, was employed to measure the voltage across the electrodes. To measure the current, an oscilloscope probe (Kenwood PC-53 50 MHz Attenuator, Japan) was connected to a 10 kΩ shunt resistor. Both these probes were connected to a Tektronix digital oscilloscope model TDS 2002 (Tektronix, Inc. USA). Similarly, the spectra emitted within the plasma was captured using Ocean Optics USB2000+ spectrometer (Ocean Optics, Florida, USA). This spectrometer featured a slit size of 25 μm, a grating with 800 lines per millimeter, an optical resolution of 0.3 nm, and a wavelength range spanning from 200 to 1100 nm. These measurements were utilized for the identification

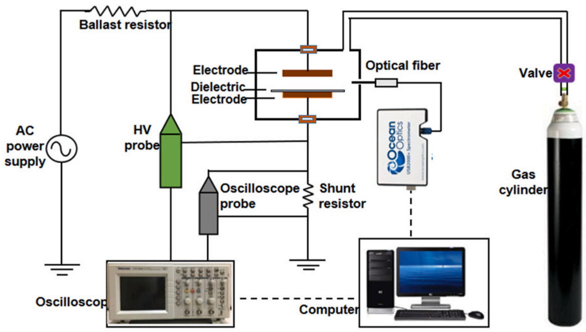



**Fig. 1.** Schematic diagram of the DBD plasma setup for seed treatment.

of the reactive species present in the discharge. Throughout the experiment, three sets of 50 dry cucumber seeds were placed within the active discharge zone of non-thermal plasma (NTP), positioned above the dielectric, and exposed for a duration ranging from 1 to 7 min.

### 2.2. Seed germination and growth

A locally cultivated cucumber seed variety ("Bhaktapur local") with an estimated germination rate of around 60 % was procured in partnership with the "Nepal Agriculture Research Council (NARC)" and indigenous farmers specifically for the purpose of this research. Only healthy and undamaged seeds were collected for the experiment. Cocopeat, a versatile coconut husk-based growth medium, was carefully prepared by washing, air-drying, sieving, and removing contaminants. It was chosen for seed germination due to its high water-holding capacity (7–8 times its weight) and aeration. Prior to seed sowing, cocopeat and vermicompost were meticulously mixed in a 4:1 ratio – four parts cocopeat to one part vermicompost. This blend was then placed in plastic bags, ensuring even distribution. The purpose was to provide seeds with balanced access to nutrients from both cocopeat and vermicompost for optimal growth. The use of plastic bags may have also helped to maintain moisture levels and prevent the mixture from drying out. Overall, the goal was likely to create a suitable growing medium for the cucumber seeds. The seedlings were grown under controlled conditions: 26 °C in light and 18 °C in the dark, with a 12-h light/12-h dark cycle, and a photon flux density of 100 μmol/m$^2$/s, along with a relative humidity range of 60–70 %. For evaluating the rate of germination and growth, three replicates of 50 cucumber seeds were taken. The seeds were considered to have germinated when a seedling emerged from its seed coat. To ensure that the growing medium remained moist and to compensate for evaporation loss, an equal volume of water was added on a regular basis. Several germination characteristics were investigated, including final germination percentage (FGP), coefficient of germination velocity (CVG), germination index (GI), and germination (G)-value [Eqs. (1)–(8)] [25–28].

$$\text{Final Germination percentage (FGP)} = \frac{\sum_{i=1}^{15} n_i}{N} \times 100 \tag{1}$$

Here, $n_i$ = number of seeds germinated in ith time and N = total number of seeds used.

$$\text{Mean Germination Time, MGT}(\bar{t}) = \frac{\sum_{i=1}^{15} n_i t_i}{\sum_{i=1}^{15} n_i} \tag{2}$$

where, $n_i t_i$ is the number of seeds germinated at an ith time interval, and $n_i$ is the number of seed germinated on the ith time.

$$\text{Coefficient of variation of germination time } (CV_t) = \frac{\sqrt{\frac{\sum_{i=1}^{15} n_i (t_i - \bar{t})^2}{\left(\sum_{i=1}^{15} n_i - 1\right)}}}{\bar{t}} \times 100 \tag{3}$$

$$\text{Coefficient of Velocity of Germination (CVG)} = \frac{\sum_{i=1}^{15} n_i}{\sum_{i=1}^{15} n_i t_i} \times 100 \tag{4}$$

$$\text{Germination Index (GI)} = \frac{\sum_{i=1}^{15} n_i}{t_i} \tag{5}$$

Here, $n_i$ = the number of seeds that sprouted in the $i^{th}$ time
$t_i$ = the time taken for seeds to sprout at $i^{th}$ count

$$\text{Uncertainty of Germination (U)} = \sum_{i=1}^{15} f_i \log_2 f_i \tag{6}$$

Here, $f_i = \frac{n_i}{\sum_{i=1}^{15} n_i}$, Where; $f_i$ is the relative frequency of germination.

$$\text{Synchronization Index (Z)} = \frac{\sum_{i=1}^{15} C_{n_{i,2}} t_i}{C_{\sum n_{i,2}}} \tag{7}$$

$$C_{n_{i,2}} = \frac{n_i(n_i - 1)}{2}$$

Where; $C_{n_{i,2}}$ = combination of seeds germinated in the $i^{th}$ time, and $n_i$ is the number of seed germinated on $i^{th}$ time.

$$\text{Germination value (GV)} = \text{Mean daily germination (MDG)} \times \text{Peak value (PV)} \tag{8}$$

Here,

$$\text{Mean Daily Germination (MDG)} = \frac{\text{Final cumnulative germination (\%)}}{\text{Period of germination}}$$

and

$$\text{Peak value (PV)} = \max\left[\frac{G_1}{T_1}, \frac{G_2}{T_2}, \ldots\ldots.., \frac{G_k}{T_k}\right]$$

Here, $T_i$ is the time from the start of the experiment to the ith interval, $G_i$ is the cumulative germination percentage in the ith time interval, and k is the total number of time intervals.

### 2.3. Seed surface wettability

The assessment of seed surface wettability and permeability involved measuring water contact angle to determine the level of hydrophilicity. The ability of a seed to initiate germination depends on its capacity to absorb water, with hydrophilic surfaces being more conducive to this process compared to hydrophobic surfaces. Thus, enhancing seed germination requires an increase in hydrophilicity, which is indicated by a decrease in water contact angle (WCA) on the seed surface [29]. In our investigation, the static water contact angle (WCA) was assessed utilizing the Ramé-Hart Goniometer Model 200 (Ramé-Hart Instrument Co. Succasunna, USA). This measurement was conducted employing the sessile drop method, and the analysis of droplet images was facilitated by the DROP image software. A droplet of distilled water, approximately V = 1 μL in volume, was carefully placed onto the surface of cucumber seeds. These measurements were conducted under ambient conditions, immediately following the plasma treatment. To ensure accuracy, the experiment was conducted using 10 seed samples for each treatment to determine the average contact angle value.

### 2.4. Mass loss

The weighing machine (MG124Ai, Bel Engineering, Italy) was utilized to determine the weight of a total of 25 cucumber seeds in three repetitions. The seeds were then treated individually for specified durations of 1, 3, 5, and 7 min using NTP. Immediately after the NTP treatment, the mass of each seed was measured again. Subsequently, the percentage of mass loss was determined by comparing the initial mass with the mass measured after treatment using Eq. (9) [26].

$$\text{Mass loss (\%)} = \frac{m_i - m_f}{m_i} \times 100 \tag{9}$$

Here, $m_i$ and $m_f$ are the initial and the final mass after treatment.

### 2.5. Imbibition rate

In this study, 25 seeds were used for each treated and untreated group, with three replicates for each group. The seeds were carefully placed in an individual Petri dish containing 50 mL of distilled water. Following the initial wetting, the seeds were dried by blotting, and their weights were measured using a precise electronic balance (MG124Ai, Bel Engineering, Italy) with an accuracy of 0.1 mg at regular intervals of every 2 h for a total duration of 10 h using Eq. (10) [26]. This allowed us for the measurement of the imbibition rate, which indicates the rate at which the seeds take in water during the soaking process.

$$\text{Imbibition rate (\%)} = \frac{\text{Final mass after being immersed in water for a specific time} - \text{Initial mass before soaked in water}}{\text{Initial mass before soaked in water}} \times 100 \tag{10}$$

### 2.6. Seedling length and vigor

A few normal seedlings were randomly selected from each replication on days 10, 20, 30, and 40 after sowing, and their seedling lengths were measured using a meter scale. These seedlings were carefully extracted from the germination tray to ensure the preservation of their root systems. Additionally, the vigor, "which encompasses all of the seed's characteristics influencing its potential for activity and performance during the processes of germination and seedling emergence", was estimated using Eq. (11) [30].

$$\text{Vigor Index (I)} = \text{Seedling length} \times \text{Final germination percentage} \tag{11}$$

### 2.7. Physico-chemical properties of water

To investigate the potential impact of non-thermal plasma treatment on seeds, a total of 25 seeds were utilized for each group: control and NTP treated seeds. The seeds were immersed in distilled water for a duration of 24 h to investigate the potential impact of plasma treatment on their properties. Subsequently, various parameters including pH, electrical conductivity, resistivity, total dissolved solids, and total dissolved oxygen were measured using a reliable and widely used XS Revio multi-parameter probe (XS Instruments, Italy), and turbidity was measured by a standard Lutron TU-2016 turbidity meter (Lutron Instruments, Taiwan).

### 2.8. Statistical analysis

The findings of three replicated tests were represented by the mean ± standard deviation. One-way ANOVA and Tukey's multiple comparison were used with GraphPad Prism 8.0.2 to evaluate the significant difference in mean germination parameters. The values labeled with different letters (a-e) indicate statistical significance at $p < 0.05$.

## 3. Results

### 3.1. Electrical characterization of the discharge

Fig. 2 illustrates the nature of the voltage applied and the discharge current when utilizing an AC power source with high voltage (13.8 kV). Within a single pulse of sine voltage, multiple instances of micro-discharge regimes characterized by filamentary streamers are observed. These filamentary streamer-based micro-discharges are the distinguishing characteristic of atmospheric dielectric barrier discharge (DBD) [31]. At an electrical potential of 13.8 kV, our investigations revealed a power consumption (P) of 26.7 W and an electron density of $8.10 \times 10^{11}$ $cm^{-3}$. Notably, we observed a discernible alteration in power consumption upon introducing seeds into the air gap.

### 3.2. Optical characterization of the discharge

Fig. 3 illustrates the spectrum of the argon non - thermal plasma (NTP) discharge. Within the spectrum, several argon species with high intensity are observed. Additionally, the presence of the OH emission band at 308 nm and weak atomic oxygen emissions at 844 nm and 926 nm confirm the generation of reactive oxygen species (ROS) in the produced discharge. Furthermore, the spectrum shows the presence of nitrogen first negative system, nitrogen second positive system, and argon species. The existence of reactive oxygen and nitrogen species (RONS) signifies the possibility of chemical reactions and the development of reactive species that could have an impact on the seed coat.

### 3.3. Determination of germination performances

On the sixteenth day after sowing, a comprehensive evaluation of various germination parameters of the seeds was conducted.

#### 3.3.1. Final germination percentage (FGP) and mean germination time (MGT)

Fig. 4 (a) illustrates the final germination percentage of the cucumber seeds assessed on the sixteenth day after planting. In comparison to the untreated one, seeds subjected to NTPs for 1 and 7 min showed an increase in germination percentage of 13 % and 20.5 % respectively. Additionally, the seeds treated for 3 and 5 min exhibited even higher germination percentages. However, there

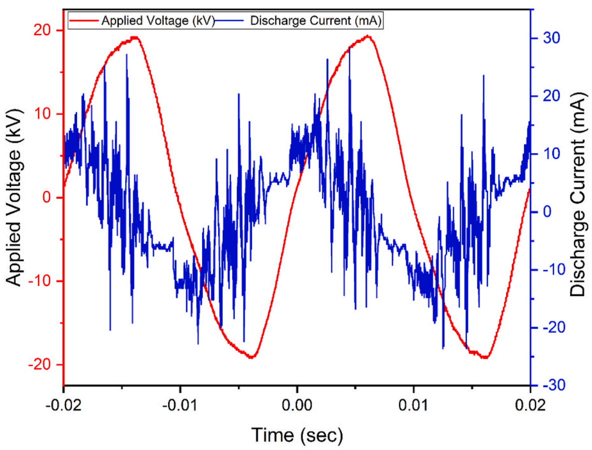



**Fig. 2.** Current-voltage waveform of the of argon discharge with voltage and frequency of 13.8 kV and 50 Hz at atmospheric pressure.

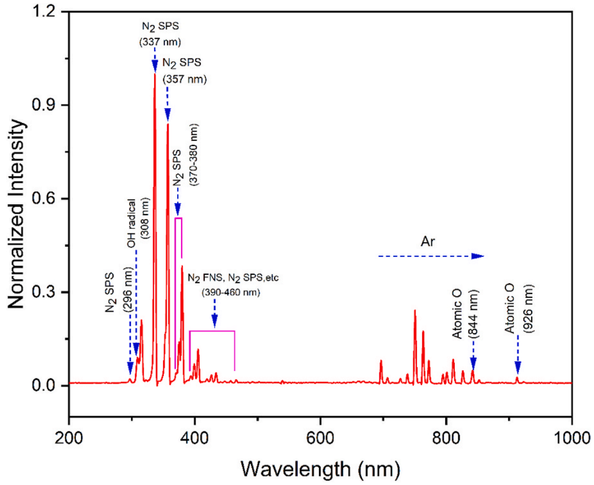



**Fig. 3.** Optical emission spectra of argon discharge with voltage and frequency of 13.8 kV and 50 Hz at atmospheric pressure.

were no significant differences in germination percentage observed between the seeds exposed to NTP for 3 and 5 min.

Similarly, mean germination time (MGT) indicates the average duration for seeds to sprout after being planted [32]. Plasma treatment has been shown to have a positive impact on seed germination by reducing the mean germination time [Fig. 4 (b)]. Specifically, the MGT of seeds treated for 1 min was reduced by 8.5 %. Furthermore, the MGT of seeds treated for 3, 5, and 7 min showed even greater reductions, with decreases of 10.9 %, 18.6 %, and 28.2 % respectively, compared to the control seeds.

### *3.3.2. Coefficient of variation of germination time ($CV_t$) and coefficient of velocity of germination (CVG)*

$CV_t$ is a crucial tool in research, offering insights into the consistency and reliability of germination under specific conditions [25]. When compared to the control group, the $CV_t$ increased by 17.6 % in seeds exposed to plasma for 1, 3, and 5 min. However, there was a notable 9.8 % decrease in the coefficient of variation of germination time $CV_t$ values compared to the control group [Fig. 5(a)]. It is worth noting that no significant variation in the $CV_t$ values was observed among the seeds exposed to plasma for 1, 3, and 5 min.

CVG is an indicator of germination speed [25]. The CVG values exhibited an increase of 9.2 % and 12 % when seeds were exposed to NTP for 1 and 3 min, respectively, as compared to the control groups. Additionally, it was observed that seeds treated for 5 and 7 min displayed even higher CVG values, with an increase of 21.8 % and 39.2 % respectively, in comparison to the control group [Fig. 5(b)].

### *3.3.3. Germination index (GI)*

Germination index (GI) measures the number of days needed for a certain proportion of seeds to germinate [33]. In the case of seeds treated with NTP, there is a notable increase in GI values compared to control group. GI of seeds subjected to NTP for 1, 3, 5, and 7 min increased by 25.5 %, 86.2 %, 92.8 %, and 63.4 % respectively, in comparison to the control group, as shown in Fig. 6. Among the seeds treated with NTP, those exposed for three and 5 min exhibited significantly higher GI values.

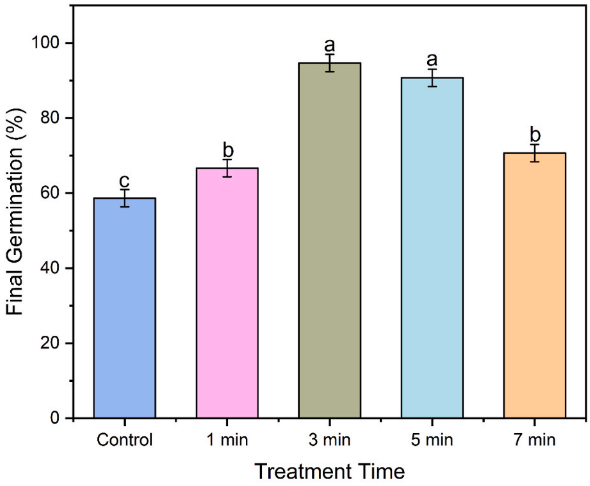
### *3.3.4. Uncertainty in germination process and synchronization index*

The uncertainty of the germination process (U) is a fundamental metric used to evaluate the levels of variability and consistency in seed germination outcomes. It offers valuable insights into the predictability and reliability of germination results within a defined treatment group [25]. The uncertainty in germination for seeds treated for 1, 3, and 7 min was found to decrease by 1.8 %, 1.8 %, and 13.21 % respectively [Fig. 7 (a)]. However, there was a slight increase in the uncertainty (U) value for seeds treated for 5 min compared to the control group. Conversely, the Z value, which measures how uniformly and consistently seeds germinate in a group, increased by 20 %, 30 %, 10 %, and 50 % for seeds subjected to NTPs for 1, 3, 5, and 7 min respectively, in comparison to the control group [Fig. 7 (b)]. These results indicate that treating seeds with NTPs leads to a significant reduction in U- values, while promoting synchronization (z). Among the treated seeds, those exposed for 7 min exhibited a comparatively reduced level of uncertainty and a higher degree of synchronization in terms of germination.

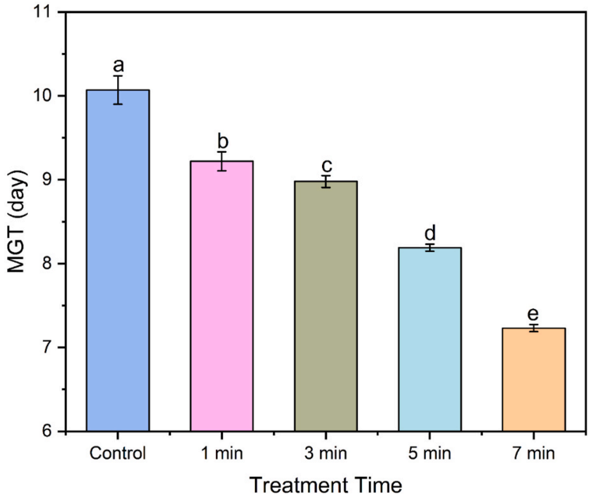
### *3.3.5. Germination value (GV)*

Germination Value (GV) is a metric that takes into account both the swiftness and thoroughness of seed germination. It combines the ratio of seeds that have successfully germinated with the duration required for germination [34]. Fig. 8 clearly illustrates the notable alterations in the G-value (Germination Value) of both the control group and the seeds subjected to NTP. Compared to the control group, the G-value of seeds exposed to NTP for 1, 3, 5, and 7 -minutes increased by 42 %, 217 %, 217 %, and 113 % respectively.

The time required for 10 %, 25 %, 50 %, 75 %, and 90 % of the total sown seeds to germinate are denoted as $T_{10}$, $T_{25}$, $T_{50}$, $T_{75}$, and $T_{90}$, respectively [27]. As seen from Table 1, the proportion of NTP exposed seeds germinated faster than the control one.




(a)




(b)

**Fig. 4.** Effect of non-thermal plasma on (a) final germination percentage, (b) mean germination time of cucumber seeds. Error bars represent standard deviation (n = 3). Different lowercase letters denote statistical differences between groups (Tukey's multiple comparison test, $P < 0.05$).

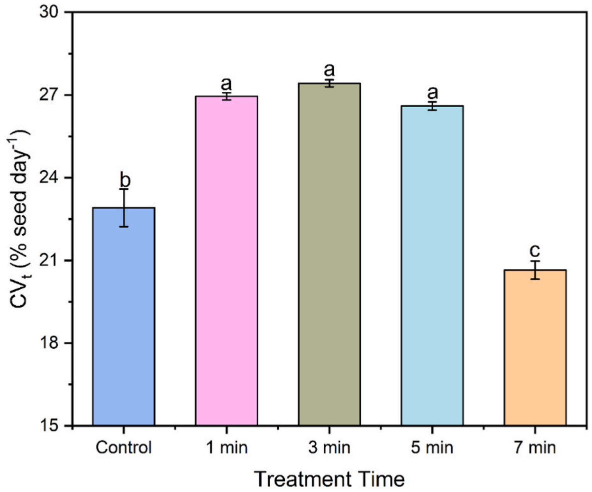
### *3.4. Estimation of seed surface wettability*

In Fig. 9, we observe the changes in water contact angle (WCA) on both untreated seeds and seeds exposed to NTP. The average water contact angle (WCA) and surface free energy (SFE) of the untreated seed was measured to be $72.05^\circ \pm 1.05^\circ$ and $40.44 \pm 0.65$ mJ/m$^2$. However, after subjecting the seeds to 10 s, the WCA and SFE gradually decreased to $30.46^\circ \pm 7.16^\circ$ and $64.26 \pm 3.61$ mJ/m$^2$, respectively. Further treatment above 10 s resulted in perfect wettability.

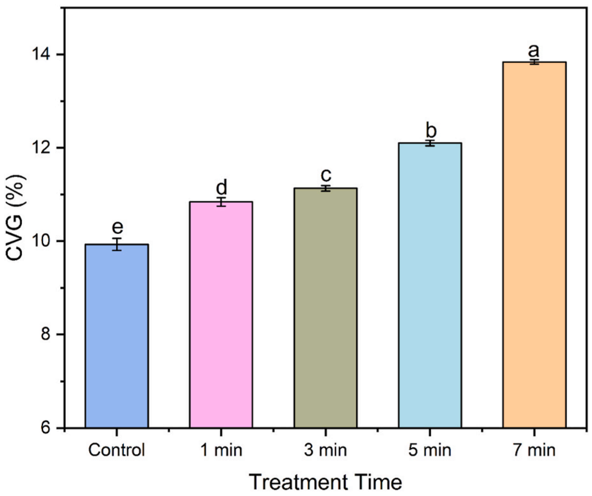
### *3.5. Mass loss (%) and water imbibition rate due to NTP treatment*

According to our investigation, it was observed that seeds subjected to NTP for 7 min exhibited the greatest mass loss compared to the control group, indicating a significant difference among the treatments [Fig. 10 (a)]. Here, the observed mass loss ranged from 7.8 % to 14.6 %. However, there were no significant variations in mass loss observed among the seeds exposed to NTP for 5 and 7 min.

Water imbibition plays a crucial role in breaking the dormant stage of the embryo and influences the germination rate of seeds. According to Fig. 10 (b), the water absorption rate in seeds treated with NTP for 3 min was significantly higher compared to other treatment times. Even after 4 h of immersion, the 3-min NTP-treated seeds absorbed approximately 36 % more water than the untreated seeds. However, the 5-min and 7-min treated seeds exhibited a lower imbibition rate compared to the control group.




(a)




(b)

**Fig. 5.** Effect of non-thermal plasma on (a) coefficient of variation of germination time (b) coefficient of velocity of germination of cucumber seeds. Error bars represent standard deviation (n = 3). Different lowercase letters denote statistical differences between groups (Tukey's multiple comparison test, $P < 0.05$).

### *3.6. Influence of NTP treatment on seedling length and vigor*

A few seedlings were carefully removed from the tray on days 10, 20, 30, and 40 after sowing the seeds. Using a meter scale, the length of the seedlings was estimated [Fig. 11 (a)]. During the first 30 days, no noticeable variations in seedling length among the control group and the seeds exposed to NTP were observed. However, after 40 days, it became evident that the seeds exposed to NTP for 5 and 7 min exhibited a notable increase in growth compared to the control group.

Vigor represents a measure of enhanced plant growth, reflecting the seed's capacity for successful germination and subsequent development into a healthy seedling [35]. A significant difference in vigor index (I) was observed between the control group and the NTP-treated seeds (excluding the 1-min treatment), which is also visually depicted in Fig. 11 (b). When assessing vigor through seedling length, the NTP-treated seeds (excluding the 1-min treatment) demonstrated a considerably higher level of vigor compared to the untreated seeds. Specifically, the seeds treated with NTP for 3, 5, and 7 min exhibited approximately 75 %, 97 %, and 59 % greater vigor, respectively, in comparison to the untreated seeds.

### *3.7. Influence of NTP-treated seeds on the physicochemical parameters of water*

Based on Table 2, it was noted that the pH, ORP, and resistivity exhibited a decrease in the treated samples compared to the control group. Conversely, the electrical conductivity and turbidity of distilled water increased in samples containing NTP treated seeds.

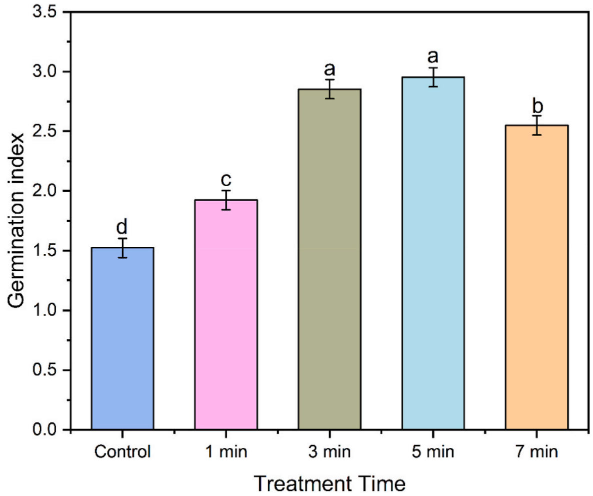



**Fig. 6.** Effect of non-thermal plasma on germination index of cucumber seeds. Error bars represent standard deviation (n = 3). Different lowercase letters denote statistical differences between groups (Tukey's multiple comparison test, $P < 0.05$).

Notably, there was no noticeable difference in the dissolved oxygen levels between the control and NTP treated groups.

## 4. Discussion

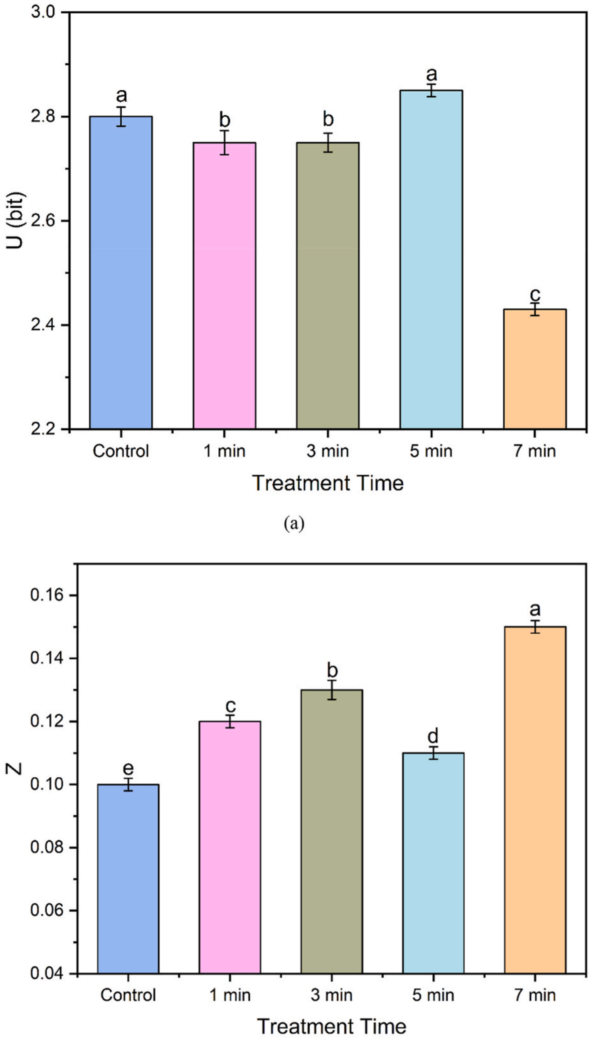
The results of the present study indicated that NTP can be used to improve the germination and early growth of cucumber seeds, with the most favorable outcomes observed at 3 min and 5 min of exposure. Beyond this range, particularly at 7 min, the positive effects diminish, emphasizing the importance of optimizing NTP exposure duration for specific agricultural applications. The observed effects of exposing seeds to plasma and their subsequent germination characteristics can be attributed to various potential factors. These include the stimulation of seed dormancy breaking, activation of growth-promoting substances, induction of a stress response, generation of reactive species, and modulation of hormone levels and signaling pathways [36,37]. These factors can influence the synchronization of germination, the speed of germination, and the overall variability in germination patterns, as reflected by the coefficient of variation of germination time ($CV_t$) and the coefficient of velocity of germination (CVG) values [Fig. 5].

Similarly, the production of reactive oxygen and nitrogen species (RONS) and nitric oxide during plasma discharges [Fig. 3] has been shown to eliminate seed dormancy, increase germination probability i.e., stimulates metabolic activities, such as enzyme production and hormone synthesis, and enhance biomass [38,39]. These compounds penetrate into the seeds, modifying the water uptake rate and improving wettability, thereby resulting in decreased germination time in the case of NTP exposed seeds. Additionally, the mild stress induced by plasma treatment prompts the activation of protective mechanisms within the seed, leading to improved viability and faster germination [8]. Further, the observed increase in GI values for plasma-treated seeds can be attributed to a combination of enhanced seed coat permeability, activation of enzymes and hormones, increased metabolic activity, antimicrobial properties, and induced stress response [40–42]. These mechanisms collectively contribute to improved germination rates and reduced MGT in plasma-treated seeds compared to untreated seeds.

Our investigation also indicated that exposing seeds to NTP can modify their surface properties, influencing factors like wettability and contact angle, which, in turn, affect aspects of plant outcomes, such as germination rates, early growth, and nutrient absorption. This can be attributed to the chemical mechanisms of seed surface modification, resulting in increased hydrophilicity. This modification might occur through the adsorption of reactive radicals such as hydroxyl free radicals (OH), ionized molecules of N2, ozone (O3), electrons, and UV radiation from the discharge [Fig. 3]. Interaction between these species and organic surfaces, like seeds, causes partial breakage of surface polymeric chains, resulting in the formation of oxygen and nitrogen-containing functional groups [43,44]. The incorporation of new hydrophilic functional groups or an increase in surface porosity can alter the seed surface. This alteration promotes the formation of pores or cavities in the seed integument, facilitating water penetration [43,45]. So, treating seeds with plasma enhances the interaction between the seed surface and water, there improving wettability Plasma treatment oxidizes surface fibers and creates covalent cross-links between them, further reducing the hydrophobic nature of the seeds and enhancing their wettability. However, the precise impact varies depending on seed type, NTP treatment parameters, and environmental factors, necessitating further research for a comprehensive understanding across diverse plant species and conditions.

In addition, we also observed significant mass loss in seeds subjected to NTP treatment compared to the control group. These can be attributed to potential reasons such as erosion of the seed coat, chemical reactions induced by the reactive species generated during NTP, surface modifications caused by the treatment, and possible vaporization or desorption of volatile compounds due to increased temperature [37,46]. However, as the NTP exposure duration increased, the mass loss was relatively smaller. This could be attributed to the deposition of reactive species on the seed coat during the treatment, resulting in less mass loss [8,18].

Numerous research studies have emphasized the importance of water absorption in triggering the end of the embryo's dormancy




(b)

**Fig. 7.** Effect of non-thermal plasma on (a) uncertainty of germination process and (b) synchronization index of cucumber seeds. Error bars represent standard deviation (n = 3). Different lowercase letters denote statistical differences between groups (Tukey's multiple comparison test, $P < 0.05$).

phase and significantly influencing seed germination rates [47,48]. The observed increase in water imbibition rate in the 3-min NTP-treated seeds can be attributed to the functionalization and modification of the protective surface layers of the seeds [49,50]. In contrast, the seeds treated for 5 min, and 7 min exhibited a significantly lower imbibition rate compared to the control group. This phenomenon can likely be attributed to the seed coat's susceptibility to rupture due to exposure to the reactive oxygen and nitrogen species (RONS) generated during the argon discharge treatment process [8,51]. The higher water absorption rate observed in the 3-min NTP-treated seeds in our case may be linked to their higher final germination percentage.

Numerous studies have also reported that an appropriate dosage of non-thermal plasma treatment has a significant positive impact on seedling length and vigor [9,52]. The results obtained from the present study suggest that non-thermal plasma treatment increased the seedling vigor of cucumber seeds, with the most significant effects observed for the 3-min and 5-min NTP treatments. However, when the seeds underwent lower-duration treatments, there was not a substantial effect on the vigor index. The significant increase in growth observed in seedlings exposed to NTP treatment for 5 and 7 min compared to the control group after 40 days can be attributed to various factors, including the stimulation of physiological processes, modulation of hormonal regulation, induction of a stress response, and improved nutrient availability [7,52–54]. Similarly, these enhancements in vigor observed in the seeds treated with NTP can be attributed to a combination of factors, including enhanced germination, activation of metabolic processes, hormonal regulation, improved nutrient uptake, and the induction of a stress response and defense mechanisms [26,55,56]. These findings highlight the potential of NTP treatment in promoting successful germination and robust seedling development, providing valuable insights for applications in agriculture and seed quality enhancement. In addition, the immersion of non-thermal plasma-treated seeds leads to

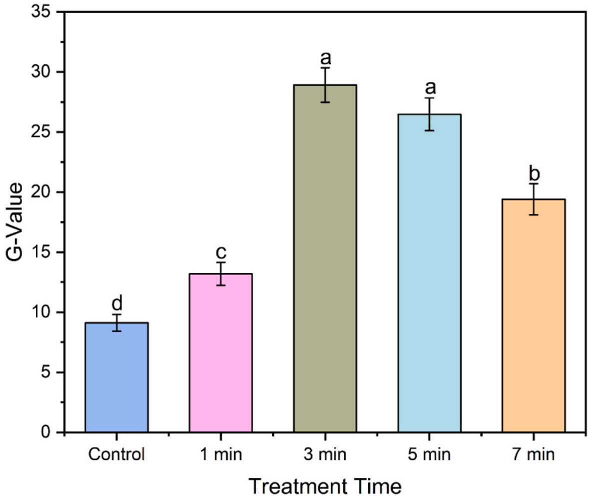



**Fig. 8.** Effect of non-thermal plasma on germination value of cucumber seeds. Error bars represent standard deviation (n = 3). Different lowercase letters denote statistical differences between groups (Tukey's multiple comparison test, P < 0.05).

**Table 1**
Time required for a certain percentage of seeds to germinate.

| Treatment Time | $T_{10}$ (days) | $T_{25}$ (days) | $T_{50}$ (days) | $T_{75}$ (days) | $T_{90}$ (days) |
|---|---|---|---|---|---|
| Untreated | $6.9 \pm 0.26^a$ | $7.96 \pm 0.13^a$ | $9.03 \pm 0.05^a$ | $11.3 \pm 0.14^a$ | $13.3 \pm 0.03^a$ |
| 1-min | $5.8 \pm 0.03^b$ | $7.16 \pm 0.14^b$ | $8.79 \pm 0.07^a$ | $10.3 \pm 0.05^b$ | $13.2 \pm 0.08^a$ |
| 3-min | $5.1 \pm 0.02^c$ | $6.34 \pm 0.03^c$ | $8.33 \pm 0.05^b$ | $9.73 \pm 0.03^c$ | $10.8 \pm 0.02^b$ |
| 5-min | $5.1 \pm 0.02^c$ | $5.92 \pm 0.03^d$ | $7.67 \pm 0.07^c$ | $8.86 \pm 0.03^d$ | $10.4 \pm 0.01^c$ |
| 7-min | $4.9 \pm 0.03^c$ | $5.48 \pm 0.03^e$ | $6.69 \pm 0.05^d$ | $7.90 \pm 0.04^e$ | $8.74 \pm 0.02^d$ |

Different letters (a-e) in the same column denote a notable difference among the groups (Tukey's multiple comparison test, P < 0.05).

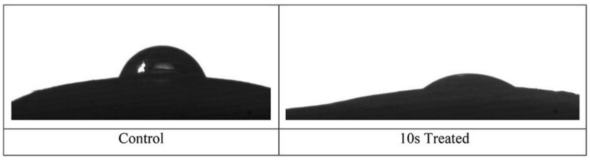
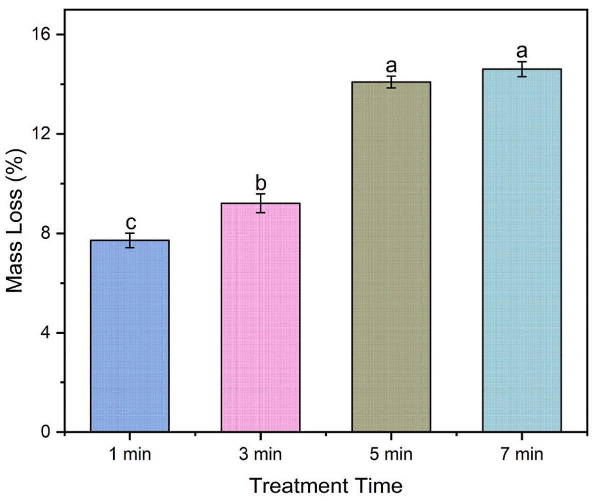


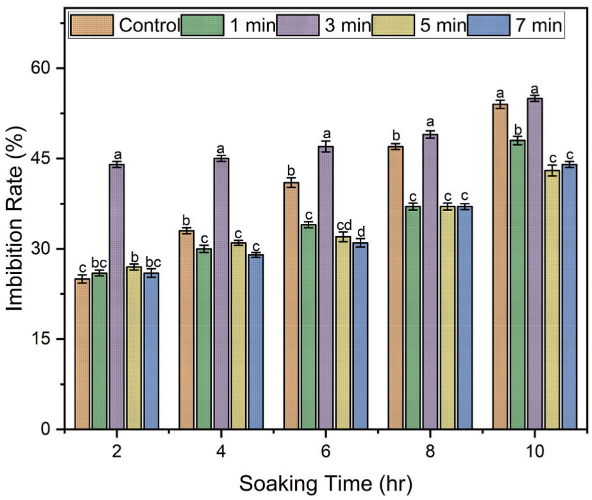

**Fig. 9.** Water droplets deposited on control (left) and NTP treated (right) cucumber seeds.

several favorable effects on the germination process. Firstly, the decline in water pH following the treatment can be attributed to the generation of various reactive species, including ozone (O3), hydrogen peroxide (H2O2), and nitric oxide (NO). These reactive species contribute to improved nutrient availability, enhanced conductivity within the seeds, and potentially favorable osmotic conditions for water absorption, ultimately supporting germination enhancement. Furthermore, when non-thermal plasma-treated seeds are immersed, the resistivity of water decreases due to the discharge of ions and other dissolved substances from the treated seeds. This is a consequence of the interaction between the seeds and the reactive species generated by the non-thermal plasma treatment. The modified seed surface promotes better contact with water and aids in the dissolution of substances, creating an environment conducive to germination enhancement. Additionally, the increase in water's electrical conductivity (EC) observed after immersing non-thermal plasma-treated seeds is a result of multiple factors. These factors include the enhanced release of ions from the seed surface, activation of metabolic processes leading to the production of charged species, chemical reactions generating new soluble compounds, and modifications on the seed surface that promote interactions with water. The increased conductivity further facilitates nutrient uptake and metabolic processes, reinforcing the positive impact on germination. Based on our investigation, we can affirm that suitable dose of non-thermal plasma treatment on seed changes water pH, electrical conductivity, resistivity, etc. And may demonstrate its positive influence on germination. The treatment improves nutrient availability, enhances conductivity within the seeds, and creates favorable conditions for water absorption and metabolic processes, ultimately supporting and promoting germination enhancement.

Thus, the observed improvement in germination parameters following plasma treatment of seeds for varying durations could be attributed to the potential effects of pathogen inactivation [57,58], activation of biochemical processes [37,59], surface modification [8,11], and the presence of an optimal treatment duration [7,60], which collectively may contribute to enhanced seed viability and




(a)



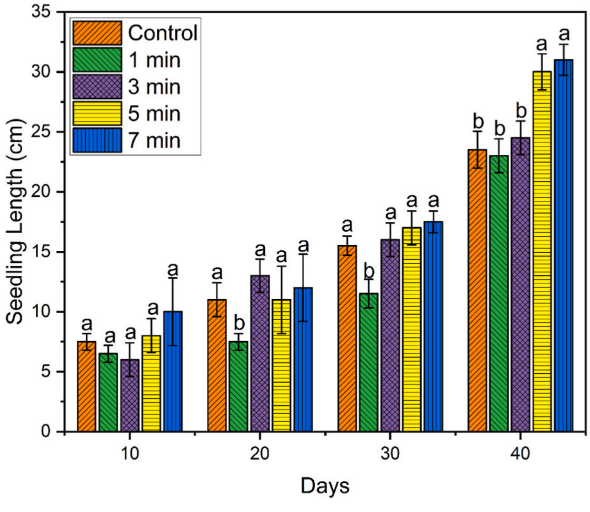

(b)

**Fig. 10.** Estimation of (a) mass loss, (b) water uptake capacity in control and NTP treated cucumber seeds. Error bars represent standard deviation (n = 3). Different lowercase letters denote statistical differences between groups (Tukey's multiple comparison test, $P < 0.05$).

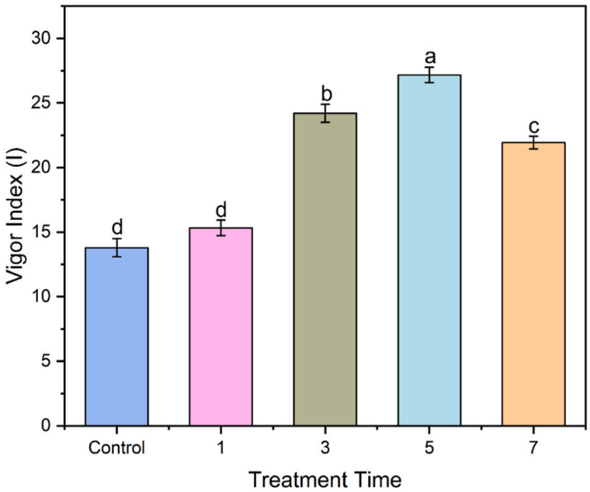
germination potential.

## 5. Conclusion

The findings of this study provide compelling evidence for the positive effects of non-thermal plasma (NTP) treatment on cucumber seed germination and subsequent plant growth. The electrical characterization of the NTP discharge confirms the presence of filamentary streamers while the optical analysis reveals the generation of reactive species. The results demonstrate that NTP treatment enhances germination performance by increasing germination percentages, reducing mean germination time, and improving synchronization. These improvements are attributed to enhanced seed coat permeability, stimulated biochemical reactions, and accelerated water uptake and nutrient absorption facilitated by NTP treatment. Additionally, NTP-treated seeds exhibit significant growth enhancement in terms of seedling length and vigor. The acceleration of cucumber seed germination exhibited a notable correlation with the presence of reactive oxygen species (ROS) in the discharge. This correlation may facilitate the integration of oxygen radicals within the seed coat, thereby augmenting the process of water absorption. Consequently, it was observed that the appropriate dosage of argon discharge treatment resulted in a more significant acceleration of germination when compared to the control group. These findings highlight the potential of NTP as a promising technology for improving seed germination and promoting plant growth in cucumber cultivation. However, further research is needed to unravel the underlying mechanisms and optimize NTP treatment parameters for different seed types and environmental conditions, ultimately maximizing the desired effects on crop performance.




(a)




(b)

**Fig. 11.** Effect of non-thermal plasma on the (a) seedling length, (b) vigor of cucumber seeds. Error bars represent standard deviation (n = 3). Different lowercase letters denote statistical differences between groups (Tukey's multiple comparison test, P < 0.05).

**Table 2**
Alterations in the physico-chemical properties of distilled water due to the insertion of NTP exposed seeds.

| Treatment Time | pH | Electrical Conductivity (μS/cm) | Resistivity (kΩ cm) | Total Dissolved Solid (mg/L) | Oxidation Reduction Potential (mV) | Dissolved Oxygen (mg/L) | Turbidity (NTU) |
|---|---|---|---|---|---|---|---|
| **Untreated** | $6.41 \pm 0.01^a$ | $25.77 \pm 0.15^e$ | $41.63 \pm 0.12^a$ | $17.17 \pm 0.12^e$ | $149.33 \pm 0.58^b$ | $3.16 \pm 0.01^b$ | $22.13 \pm 0.06^e$ |
| **1-min** | $6.31 \pm 0.01^b$ | $27.70 \pm 0.17^d$ | $37.30 \pm 0.17^b$ | $19.37 \pm 0.06^d$ | $151.33 \pm 0.58^a$ | $3.10 \pm 0.01^c$ | $32.77 \pm 0.15^c$ |
| **3-min** | $6.28 \pm 0.01^c$ | $30.60 \pm 0.10^b$ | $32.80 \pm 0.18^d$ | $21.57 \pm 0.06^b$ | $139.10 \pm 0.20^c$ | $3.06 \pm 0.01^d$ | $35.37 \pm 0.06^b$ |
| **5-min** | $6.26 \pm 0.01^d$ | $31.83 \pm 0.12^a$ | $31.23 \pm 0.06^e$ | $22.83 \pm 0.12^a$ | $137.67 \pm 0.58^c$ | $2.87 \pm 0.01^e$ | $40.17 \pm 0.06^a$ |
| **7-min** | $6.23 \pm 0.01^e$ | $28.73 \pm 0.21^c$ | $35.27 \pm 0.12^c$ | $20.57 \pm 0.06^c$ | $133.33 \pm 1.15^d$ | $3.31 \pm 0.01^a$ | $30.73 \pm 0.12^d$ |

Different letters (a-e) in the superscript represent significant difference in mean value, One-way ANOVA, Post-hoc Tukey Multiple comparison test (P < 0.05).

## Declarations

### *Data availability*

"The data that support the findings of this study are available from the corresponding authors upon reasonable request".

## CRediT authorship contribution statement

**Rajesh Prakash Guragain:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **Hom Bahadur Baniya:** Data curation, Investigation. **Deepesh Prakash Guragain:** Data curation, Investigation, Writing – review & editing. **Suman Prakash Pradhan:** Resources. **Deepak Prasad Subedi:** Conceptualization, Supervision.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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