Impact of non-thermal plasma treatment on the seed germination and seedling development of carrot (*Daucus carota sativus L.*)

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Abstract
Seed germination is a complicated physiological process that starts with the seed absorbing water and concludes with the radicle emerging. The kinetics and amount of water uptake by seeds are known to be influenced by both seed surface properties and the surrounding environment. As a result, altering seed surface features are linked to seed medium and is a valuable strategy for controlling seed germination. In the agricultural field, non-thermal plasma surface activation of seeds is currently being investigated as an efficient pre-sowing treatment for modifying seed germination. The impact of non-thermal plasma (NTP) on the germination and seedling growth of carrot seeds at room temperature and atmospheric pressure for varied treatment times was investigated in this study. Seed’s germination properties and growth parameters were examined for both control and NTP-treated seeds. Germination-related parameters such as germination percentage, vigor index, and chlorophyll content were all improved by NTP treatment. However, no significant changes were seen in the carotenoid content. Similarly, the \textit{in-vitro} radical scavenging activities, total phenol, and total flavonoid contents in the seedlings were altered by NTP treatment. Our results indicate that NTP treatment has a favorable effect on carrots germination and seedling development.

1. Introduction

United Nations Food and Agriculture Organization (FAO) reported that global grain output is at 2.216 billion tons, while demand is around 2.254 billion tons, and 9 million people are still in hunger [1]. In the past few decades, the demand for fresh fruit and vegetable consumption has increased due to global population growth which has led to food scarcity. To overcome this scarcity, the production in limited land should be increased and food should be stored for a long time without deteriorating the quality. Worldwide food shortage has been increased with increasing industrialization, urbanization, infrastructure, and insufficient land structure and natural resources. And for that, the main challenge is to search for a single viable and cost-effective solution that can be applied to overcome the challenge of modern food scarcity [2, 3]. In contemporary research, the focus is given to the development of plasma-treated seeds and to meet the challenges to increase food production and ensure food safety. Plasma originally being searched as fusion energy has been currently used in medicine, agriculture, surface modifications, pollution control, electronics, water sterilization, etc [4–10]. Plasma treatment has resulted in the establishment of a distinct, quickly expanding area known as ‘plasma agriculture’ in today’s world. In agriculture and food, the use of plasma will concentrate on each step of this agriculture-food chain from seed germination-plant growth-plant yield-food safety [11, 12]. Plasma agriculture is an emerging technology that has been commercialized and contributed to agricultural products more effectively. As a result
of controlling plant diseases and increasing crop yields, plasma agriculture may be able to boost productivity with less impact on the environment [8, 13, 14].

Plants are subjected to a variety of stresses on a regular basis, including water scarcity, waterlogging, toxicity, high salinity, and extreme temperatures. Crop yields are reduced because of these pressures. Modern agriculture technology intervention can revolutionize agriculture, which can be assessed by the aggregate degree of application of a specific technology within a given geographic area or population. Promoting seed germination and plant growth is the most direct way to increase crop yield [15]. Cold plasma treatment has been recognized as a suitable technology to improve the germination efficiency of seeds and subsequent plant growth. Improving the germination rate of seeds through cold plasma technology is not only beneficial to agricultural applications, but also in the food processing sector [16–18]. The plasma can be generated under mild conditions with simple plasma sources, including corona discharges, glow discharges, dielectric barrier discharges, and plasma jets [19, 20]. It is well established non-thermal plasma (NTP) can be used in augmenting the potentiality of plant cultivation. Pre-sowing treatment by the NTP technique can boost seed activities, including faster germination, better germination rate, quicker growth, enzymatic activities, and the yield of plants [6, 21–23]. The non-thermal plasma also can be used indirectly in agriculture by generating plasma-activated water. The plasma has the ability to produce reactive oxygen and nitrogen species (RONS), as well as has the capacity to alter the pH, electrical conductivity, and oxidation-reduction potential of the water. The rate of seed germination, plant growth, and agricultural yields are all affected by these solutions [24, 25]. In plasma agriculture, previously, experiments were performed on the seeds of radish, pea, tomatoes, carrot, and wheat which showed a high increase in the germination percentage and seeding length after plasma treatment [24–33].

In a developing country like Nepal, agriculture is the main source of food, income, and employment for the majority. But agricultural development is not satisfactory due to the traditional method of farming which results in low productivity. The use of science and modern technology can be effective to cover food scarcity at a faster pace. For this, plasma technology is one of the new ways to make farming more efficient and grow more food. Implementation of plasma-treated seeds for agriculture purposes reduces health hazards, i.e., crops yield increases that facilitate harmful chemicals. Among the important crops of the Apiaceae family, Carrot (Daucus carota sativus L.), is the most main root vegetable that contains carotenoids, flavonoids, polycyclic, vitamins, and minerals. Carrots were first used for medical purposes and act as a gold mine of antioxidants, anticarcinogens, and immune enhancers [31, 34]. In this study, we examined the effect of non-thermal plasma on seed germination and seedling growth of carrots.

2. Materials and methods

2.1. Experimental setup

Figure 1(a) depicts the laboratory setup and figure 1(b) shows the nature of the discharge used in the investigation. The reactor chamber consists of a transparent polycarbonate cylinder (35.7 cm × 20.0 cm × 15.0 cm). The discharge between two rectangular parallel copper electrodes (7.54 cm × 4.98 cm × 1.0 cm) was generated using a sinusoidal voltage of 12.5 kV and a frequency of 50 Hz. The distance between the two copper electrodes is maintained at 5 mm for all treatments. A polycarbonate plate (15.0 cm × 12.0 cm × 0.2 cm) serves as a dielectric. The seeds’ surfaces were treated with a high voltage alternating current administered between the two electrodes. The discharge voltage was measured using a high voltage probe (PINTEK HVP–28 HF; 1000:1), and the discharge current was measured with an oscilloscope probe over a 10 kΩ shunt resistance. The current and voltage signals were monitored and analyzed using a digital oscilloscope (Tektronix TDS 2002, 60 MHz). The emission spectra were measured using a spectrometer from Ocean Optics, Inc. (USB 2000+). The Ar gas flow rate is regulated by a flow meter at 4 l min⁻¹ to assure the discharge’s stability. To ensure uniform treatment, 250 carrot seeds were physically inserted within the dielectric barrier’s active plasma zone. During the experiments, seeds were exposed to NTP for 1 to 4 min. Seeds were sterilized and kept in sterile jars until biological research could be conducted.

2.2. Seed collection and growing condition

Seeds of carrot were received from the Nepal Agricultural Research Council (NARC), Lalitpur, Nepal. For the investigation, only healthy seeds with no visible flaws were chosen. A cocopeat was collected and cleaned with distilled water before seeding. It was then dried at room temperature. After that, a germination tray (pot) was filled with cocopeat. Three replicates of 250 carrot seeds were sown in each tray, and germination parameters were measured on various germination days. The moisture level of the cocopeat was controlled every two days with a water solution to minimize variations due to evaporation.
2.3. Plant collection and extract preparation
On day 70 of germination, leaf samples were taken for biochemical examination. 5 g of room dried carrot samples were macerated with 50 ml of 80% Methanol and 50% Ethanol for 24 h to assess the change in flavonoids and total phenolics. The macerated extract was filtered the next day with Whatman filter paper, and the sample was dried with a vacuum evaporator (Hanil/Modul 4080 C). The extracted crude samples were stored at 4 °C until further analysis.

2.4. Analysis of growth characteristics
2.4.1. Estimation of germination percentage
Germination percentage was calculated using equation (1) [35].

\[
\text{Germination percentage} = \frac{\sum_{i=1}^{j} n_i}{N} \times 100
\]  

Where, \( n_i \) is the number of seeds germinated on the \( i \)th day and \( N \) is the total number of seeds used.

2.4.2. Relativized percentage of germination
Equation (2) can be used to relativize the germination percentage [36].

\[
R\% = \frac{\text{Actual percentage}}{\text{The highest percentage among the group of data}} \times 100
\]  

This normalization enables comparisons between treatments that are equivalent when the quantity of dormancy disrupts varies.
2.4.3. The mean germination time (MGT)
The mean germination time is an indicator and spread of germination over time. It indicates the amount of time it takes for a seed to germinate or emerge \[36, 37\]. The mean germination time was determined using equation (3).

\[ \bar{t} = \frac{\sum_{i=1}^{l} n_i t_i}{\sum_{i=1}^{l} n_i} \]  

(3)

Where, \( n_i t_i \) is the number of seeds germinated at an \( i \)th time interval, and \( n_i \) is the number of seed germinated on the \( i \)th time.

2.4.4. The mean germination rate (MGR)
Mean germination rate was calculated as the reciprocal of the mean germination time \[37\].

\[ \varphi = \frac{1}{\bar{t}} \]  

(4)

Where, \( \bar{t} \) is the mean germination time.

2.4.5. Uncertainty of the germination process
The level of uncertainty related to the relative frequency of germination distribution is indicated by the germination process uncertainty \[38\]. Equation (5) was used to calculate uncertainty.

\[ U = \sum_{i=1}^{l} f_i \log_2 f_i \]  

(5)

Here, \( f_i = \frac{n_i}{\sum_{i=1}^{l} n_i} \)

Where, \( f_i \) is the relative frequency of germination.

2.4.6. Synchrony of the germination process
It determines the extent to which individuals in a particular population overlap. The synchronization index generates a number if and only if two seeds complete the germination process at the same time \[38\]. It was calculated using the formula below.

\[ Z = \frac{\sum_{i=1}^{l} C_{n_i,2} t_i}{C_{\sum n_i,2}} \]  

\[ C_{n_i,2} = \frac{n_i(n_i - 1)}{2} \]  

(6)

Where; \( C_{n_i,2} = \) combination of seeds germinated in the \( i \)th time, two by two and \( n_i \) is the number of seed germinated on \( i \)th time. When all seeds germinate at the same time, the number \( Z \) equals one. And, at least two seeds can sprout at the same time when \( Z \) is zero.

2.4.7. Coefficient of variation of germination time (CVt)
The following expression was used to compute the coefficient of variation germination time \[38\].

\[ CV_t = \frac{S_t}{\bar{t}} \times 100 \]  

(7)

\[ S_t = \sqrt{\frac{\sum_{i=1}^{l} n_i (t_i - \bar{t})^2}{\sum_{i=1}^{l} (n_i - 1)}} \]

Where \( S_t \) denotes the standard deviation of the germination time and \( \bar{t} \) denotes the mean germination time.

2.4.8. Germination index (GI)
The germination index is a measurement of how long it takes for a specific percentage of seeds to germinate (in days) \[39\]. The following expression was used to calculate the germination index:

\[ GI = \frac{\sum_{i=1}^{l} n_i}{t_i} \]  

(8)

Where,

\( n_i = \) the number of seeds that sprouted in the \( i \)th time
\[ t_i = \text{the time taken for seeds to sprout at } i\text{th count} \]

### 2.4.9. Coefficient of velocity of germination (CVG)

Equation (9) was used to estimate the coefficient of germination velocity [40].

\[ CVG = \frac{\sum_{i=1}^{\ell} n_i t_i}{\sum_{i=1}^{\ell} n_i} \times 100 \]  
\[ \text{Equation (9)} \]

### 2.4.10. Time to 50% germination (T50)

\[ T_{50} = \frac{t_i + \left( \frac{\sum_{i=1}^{\ell} n_i}{2} - n_i \right)}{n_j - n_i} (t_j - t_i) \]

To get the values of \( n_i \) and \( n_j \) in the preceding equation, we need to look at the total number of seeds germinated, which is given below as a condition.

\[ n_i < \frac{\sum_{i=1}^{\ell} n_i}{2} < n_j \]

Where,

- \( n_i = \) nearest cumulative number of seeds germinated \( C_{n_i} < \frac{\sum_{i=1}^{\ell} n_i}{2} \)
- \( n_j = \) nearest cumulative number of seeds germinated \( C_{n_j} > \frac{\sum_{i=1}^{\ell} n_i}{2} \)
- \( t_i = \) the time span that corresponds to \( n_i \)
- \( t_j = \) the time span that corresponds to \( n_j \)

Various time-related germination characteristics, such as \( T_{10}, T_{25}, T_{75} \) and \( T_{90} \) were calculated using the same above formula by replacing \( \sum_{i=1}^{\ell} n_i \) with \( \frac{\sum_{i=1}^{\ell} n_i}{10}, \frac{\sum_{i=1}^{\ell} n_i}{4}, \frac{3\sum_{i=1}^{\ell} n_i}{4} \) and \( \frac{9\sum_{i=1}^{\ell} n_i}{10} \) respectively.

### 2.4.11. Mean daily germination (MGD) percent

It is the daily average of how many seeds germinate. The ratio of the number of seeds germinating every day to the total number of seeds germinated is another way to look at it [41]. It was estimated using equation (11).

\[ \overline{G} = \frac{GP}{T_n} \]

Where, \( GP \) denotes the final cumulative germination percentage and \( T_n \) denotes the total number of intervals needed for final germination.

### 2.4.12. Germination value

As mentioned by Czabator (1962), ‘germination value is calculated by integrating the speed and completeness of germination into a composite score’ [42].

\[ GV = MDG \times PV \]

Where, \( MDG = \) mean daily germination and \( PV = \) total number of seeds that have germinated at the point on the germination curve where the rate of germination starts to slow down.

### 2.4.13. Estimation of mass loss due to plasma treatment

50 seeds in three replicates were subjected to non-thermal plasma for 0, 1, 2, 3, and 4 min, respectively. The seeds were then weighed in an electronic balance (MG124Ai, Bel instruments) immediately after the plasma treatment. The level of deterioration of seed was evaluated by mass loss (W), using equation (13) [43].

\[ W = \frac{W_o - W_f}{W_o} \times 100\% \]

where \( W_o \) denotes the seed’s starting mass and \( W_f \) denotes the seed’s mass after the treatment.

### 2.4.14. Estimation of water uptake capacity of seed

Seeds were subjected to low-temperature plasma for 0, 1, 2, 3, and 4 min, respectively. In three repeats, 50 seeds were utilized for each variety. The seeds were then weighed in an electronic balance (MG124Ai, Bel instruments).
immediately after the plasma treatment (t) and then every 1.5 h (t+1.5, t+3, t+4.5, t+6, t+7.5, t+9, t+10.5, t+12).

The wettability of the treated and untreated seeds was calculated using equation (14) [32, 44].

\[ \text{Wettability} = \frac{m_i - m_0}{m_0} \times 100\% \] (14)

Where, \( m_0 \) is the mass of the seeds before they are soaked in water, and \( m_i \) is the mass after they have been soaked in water.

2.4.15. Estimation of the vigor index

'Vigor refers to the sum of a seed’s characteristics that determine its potential level of activity and performance during germination and seedling emergence'. The vigor of seedlings was estimated using equations (15), (16) [35].

\[ \text{Vigor Index I (VI I)} = \frac{\text{Mean seedling length (cm)} \times \text{Germination (\%)} }{100} \] (15)

\[ \text{Vigor Index II (VI II)} = \frac{\text{Dry weight of the seedling length (g)} \times \text{GR(\%)} }{100} \] (16)

2.4.16. Determination of change in chlorophyll content and total carotenoids

Chlorophyll-a, chlorophyll-b, and total carotenoid contents were determined in acetone solvent by standard protocols as used by various investigators [45].

2.4.17. Determination of changes in total flavonoids and total phenolics

An aluminum chloride colorimetric test was used to evaluate the change in total flavonoid content of root extracts for various seedlings germinated from plasma-treated seeds [46]. The results were reported as milligrams of quercetin equivalents per gram of dry weight of extract (mg QE/g DW) using a standard curve prepared using quercetin. Furthermore, the changes in total phenolic content of leaf extracts with plasma treatment time were quantified using Folin-Ciocalteu’s [46]. Gallic acid was used to find an appropriate calibration curve, and the results were expressed as milligrams of gallic acid equivalent per gram dry weight of extract (mg GAE/g DW).

2.4.18. Determination of change in radical scavenging potential

The changes in free radical scavenging potential of root extracts with plasma treatment were determined by ABTS and DPPH assay as described by previous investigators [47].

\[ \% \text{ABTS/DDPH Scavenging} = \frac{A - B}{A} \times 100\% \] (17)

Where A and B are the absorbances of the control and test sample, respectively.

3. Statistical tools

All experiments were performed at least triplicates and the findings were expressed as mean ± standard deviation. The significant difference in mean of the seedlings growth was analyzed by one-way analysis of variance (ANOVA) followed by Tukey’s multiple comparison analysis using Graph Pad Prism 8.0.2. Differences were considered significant at \( p < 0.05 \). The values marked by different letters (a-e) and different asterisks (“*”-“***”) are statistically significant at \( p < 0.05 \) according to One-way ANOVA, post hoc Tukey, multiple comparison test.

4. Results and discussion

4.1. Electrical characterization of the discharge

Figure 2 depicts the discharge’s typical voltage and current waveforms. The current waveform clearly comprises of many discharge pulses corresponding to micro discharges. The existence of micro-discharge identifies atmospheric pressure DBD. Lissajous figures are plots of charge Q (t) versus applied voltage V (t) over a single period of applied voltage. From the Lissajous figure (figure 3), the power consumed, and the energy dissipated per cycle was found to be 100.50 mW and 2.01 mJ respectively.
Now, electron density \( (n_e) \) can be obtained using equation (18) \([48, 49]\)

\[
n_e = \frac{J}{e \mu_e E}
\]  

Here, \( J \) is the average current density, \( e \) is the electronic charge, \( \mu_e \) is the electron mobility and, \( E \) is the electric field in the discharge region.

The electron mobility was estimated to be 368.04 \( \text{cm}^2 \text{V}^{-1} \text{s}^{-1} \) using Bolsig + software. Using these values in equation (18), the electron density \( (n_e) \) is estimated to be \( 8.44 \times 10^8 \text{ cm}^{-3} \)
4.2. Influence of NTP on germination parameters

Three replicates of 250 healthy seeds were taken on different trays and the final germination rate was calculated on the 25th day. From figure 4, we noticed that plasma-treated seeds were found to have more germination potential as compared to the control seeds. The final germination percentage for 1, 2, and 3 min seedlings increased by 13.69%, 22.22%, 26%, and 9%, respectively, compared to control seedlings.

According to these findings, plasma treatment has a positive effect on the germination rate. However, treatment for a longer time results in a decrease in the germination rate.

Table 2 shows that the relativized percentage, mean germination time (MGT), mean germination rate (MGR), uncertainty of germination process (U), and synchrony germination process (Z), for carrot seeds.

![Figure 4. Effect of NTP treatment on germination of carrot.](image-url)

### Table 1. Equations used for the estimation of chlorophyll-a, chlorophyll-b, and total carotenoids with extracting solvent 100% Acetone.

| Extracting solvent (100% Acetone) | Chlorophyll-a | Chlorophyll-b | Total Carotenoids |
|------------------------------------|--------------|--------------|-----------------|
|                                    | 12.70Absorbance_{663 nm} - 2.69Absorbance_{645 nm} | 22.90Absorbance_{645 nm} - 4.68Absorbance_{663 nm} | 1800Absorbance_{490 nm} - 1.82[Chl-a] - 83.02[Chl-b] |

### Table 2. Effect of NTP on the relativized percentage of germination, mean germination time (MGT), mean germination rate (MGR), uncertainty of germination process (U), and synchrony germination process (Z), for carrot seeds.

| Treatment time | Relativized percentage (%) | Mean germination time (MGT) (day) | Mean germination rate (MGR) (day)^{-1} | Uncertainty of germination process (U) (bit) | Synchrony of germination process (Z) |
|----------------|---------------------------|----------------------------------|--------------------------------------|-------------------------------------------|--------------------------------------|
| Control        | 78.33 ± 1.52^a            | 10.79 ± 0.03^a                   | 0.092 ± 0.0003^a                      | 2.26 ± 0.05^a                             | 0.24 ± 0.005^b                       |
| 1 min          | 89.0 ± 1.0^c              | 10.77 ± 0.02^c                   | 0.093 ± 0.00^c                        | 2.44 ± 0.01^b                             | 0.21 ± 0.00^a                        |
| 2 min          | 96.67 ± 0.57^b            | 10.71 ± 0.02^b                   | 0.093 ± 0.00^b                        | 2.63 ± 0.01^a                             | 0.17 ± 0.005^a                       |
| 3 min          | 99.0 ± 0.57^b             | 10.66 ± 0.03^b                   | 0.094 ± 0.00^b                        | 2.62 ± 0.02^b                             | 0.19 ± 0.00^a                        |
| 4 min          | 85.670 ± 0.33^d           | 10.15 ± 0.05^c                   | 0.098 ± 0.0003^a                      | 2.01 ± 0.02^d                             | 0.30 ± 0.005^a                       |

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Table 3. Effect of NTP on the coefficient of variation of germination time, germination index, coefficient of velocity of germination, mean daily germination percent, and germination value of carrot seeds.

| Treatment time | Coefficient of variation of germination time (CVt)(%) | Coefficient of velocity of germination (CVG)(%) | Germination Index (GI) (day) | Mean daily germination percent (MDG)(%) | Germination value (G-Value) |
|----------------|-----------------------------------------------|------------------------------------------|-----------------------------|-------------------------------------|-----------------------------|
| Control        | 14.95 ± 0.71<sup>a</sup>                      | 9.26 ± 0.03<sup>c</sup>                  | 6.93 ± 0.1<sup>a</sup>     | 2.70 ± 0.01<sup>c</sup>            | 15.70 ± 0.33<sup>d</sup>   |
| 1 min          | 17.39 ± 0.21<sup>c</sup>                      | 9.28 ± 0.01<sup>cd</sup>                 | 7.96 ± 0.1<sup>c</sup>     | 3.07 ± 0.01<sup>c</sup>            | 19.98 ± 0.49<sup>c</sup>   |
| 2 min          | 18.50 ± 0.19<sup>b</sup>                      | 9.33 ± 0.02<sup>c</sup>                  | 8.67 ± 0.08<sup>b</sup>   | 3.32 ± 0.01<sup>b</sup>            | 21.12 ± 0.28<sup>b</sup>   |
| 3 min          | 25.03 ± 0.24<sup>c</sup>                      | 9.37 ± 0.02<sup>b</sup>                  | 9.06 ± 0.1<sup>c</sup>     | 3.40 ± 0.01<sup>c</sup>            | 22.72 ± 0.38<sup>b</sup>   |
| 4 min          | 15.03 ± 0.58<sup>d</sup>                      | 9.85 ± 0.03<sup>c</sup>                  | 8.02 ± 0.07<sup>c</sup>   | 2.93 ± 0.01<sup>d</sup>            | 19.94 ± 0.26<sup>c</sup>   |

Table 4. Variation of time to T<sub>10</sub>, T<sub>25</sub>, T<sub>75</sub> and T<sub>90</sub> germination between untreated and plasma treated seeds.

| Treatment time | T<sub>10</sub> (%) | T<sub>25</sub> (%) | T<sub>75</sub> (%) | T<sub>90</sub> (%) |
|----------------|-------------------|-------------------|-------------------|-------------------|
| Control        | 7.61 ± 0.08       | 9.45 ± 0.02       | 10.56 ± 0.02      | 11.44 ± 0.01      | 11.85 ± 0.01      |
| 1 min          | 7.36 ± 0.04       | 9.40 ± 0.02       | 10.51 ± 0.02      | 11.37 ± 0.01      | 11.86 ± 0.00      |
| 2 min          | 7.39 ± 0.03       | 9.15 ± 0.01       | 10.08 ± 0.02      | 11.53 ± 0.01      | 12.71 ± 0.01      |
| 3 min          | 7.13 ± 0.04       | 9.12 ± 0.01       | 9.97 ± 0.01       | 10.89 ± 0.01      | 12.51 ± 0.06      |
| 4 min          | 7.43 ± 0.04       | 9.13 ± 0.01       | 9.7 ± 0.01        | 10.39 ± 0.02      | 10.89 ± 0.02      |

seedlings. Low uncertainty levels indicate that germination will be concentrated over time. A low value (around zero) indicates that germination is well-coordinated [36, 38]. As shown in table 2, there is a considerable synchronization of germination between control and plasma-treated seedlings.

As seen from table 3, the coefficient of the velocity of germination time between control and plasma treated seeds differ significantly. CVt was found to increase by 16.32%, 23.75%, 69.2%, and 0.5% in the case of seed being treated by plasma for 1 min, 2 min, 3 min, and 4 min respectively as compared to untreated seeds. The coefficient of germination velocity (CVG) is a measure of how quickly seeds germinate. ts value rises as the number of germinated seeds increases and the germination period decreases [40]. When seeds are exposed to NTP for 1 min, 2 min, 3 min, and 4 min, respectively, CVG rises by 0.2%, 0.75%, 1.18%, and 6.37%, as compared to the untreated seed (table 3).

When seeds were exposed to NTP, their GI value increased by 14.86%, 25.10%, 30.73%, and 15.72%, respectively, compared to untreated seeds. In the case of seeds treated for 1 min and 4 min, however, no significant changes were detected. A higher GI score corresponds to a higher germination percentage and rate [29]. We noticed a significant change in MDG between control and plasma treated seed. We further noticed that the germination value increased by 27.26%, 34.52%, 44.71%, and 27% for 1 min, 2 min, 3 min, and 4 min. NTP treated seed compared to untreated seed.

We further found that plasma-treated seed germinates quicker than untreated seed as indicated in table 4. RONS are involved in a variety of processes in seeds, including maturation, ripening, aging, and germination [50]. Many signaling mechanisms in the plant life cycle, such as the breaking of dormancy during seed germination, rely on hydrogen peroxide [51]. It has been reported that exogenous H<sub>2</sub>O<sub>2</sub> promotes maize (Zea mays L.), and sunflower seed germination by stimulating gibberellic acid (GA) biosynthesis and abscisic acid (ABA) catabolism [52]. Additional evidence has suggested that GA promotes the germination of latent caryopses via modulating the amount of ABA and the ROS-antioxidant state [53]. So, our findings also shows that RONS generated in the discharge might trigger GA to enhance seed germination. The interaction of the RONS in plasma with the seed coat may result in more effective water and nutrient uptake, as well as enhanced germination percentage, GI, and vigor index, as well as improved seed growth [11, 54, 55].

4.3. Estimation of mass loss due to plasma treatment

Figure 5 shows that for control, 1, 2, 3 min, and 4 min treatments for 50 seeds, the 4 min treatment resulted in the greatest mass loss, and the difference between treatments was significant. However, no significant variations in the mass loss were found across plasma exposure times of 2 and 3 min. The etching of the seed surface by plasma treatment may be responsible for the reduction in mass of the treated carrot in this case [28, 56].
4.4. Estimation of water uptake capacity of seed

The results revealed that plasma treatment accelerates the water absorption of carrot seeds (figure 6). The increased exposure period of low-temperature plasma was found to be positively correlated with greater and more intense water absorption. Plasma treatment markedly hydrophilized the surface of seeds and increased the water imbibition (absorption). The most noticeable difference in the case of 3 min was visible after 24 h when the seed absorbed 38.37% more water than control. Because a hydrophilic surface absorbs more water to initiate germination than a hydrophobic surface, an increase in hydrophilicity, on the seed surface, is crucial in enhancing seed germination [27, 57].
Figure 7. (A) Photograph of control and plasma-treated seedlings, (B) Effect of NTP exposure at different time interval on growth parameters of carrot seedlings on various days, (C): Shoot and Root Length [CS = control shoot length, CR = control root length, $S_i$ = shoot length for $i$th minute, $i = 1, 2, 3$; $R_i$ = Root length for $i$th minute] taken on various days after germination.
4.5. Estimation of the seedling length
Seedlings were gently taken from the germination tray and the cocopeat from the roots was removed without inflicting any physical damage to the plants to estimate their length. A measuring scale was used to measure the length of the seedlings, shoots, and roots from the 30th to the 58th day after germination.

Figure 7 (A) depicts a seedling with its root intact, whereas figures 7 (B), (C) depict the comparable measurements of the root and shoot lengths. On the 30th day after germination, seedling length rose by 25.73% for 4 min plasma treated seeds, compared to untreated seeds. No significant differences in the shoot length were observed between control and 1 min, 2 min, and 3 min plasma-treated seeds. On the 58th day following germination, seedling length rose by 19.57% and 21.41% for 2- and 3 min plasma-treated seeds, respectively, as compared to control seeds. In the case of seeds treated with NTP for 4 min, however, there was a 4.86% reduction in shoot length.

Similarly, on the 58th day, NTP treatments for 1, 2, 3, and 4 min increased root length by 2.72%, 6%, 19.09%, and 29.09%, respectively, as compared to the untreated one. According to our observations, exposing the seed to non-thermal plasma for the appropriate amount of time greatly improves the seedling length.

4.6. Estimation of the vigor index
The vigor index I was considerably enhanced after plasma treatment (figure 8). The VI I of treated seeds for 1 min, 2 min, and 3 min improved by 1.15, 1.35, and 1.52 times, respectively, as compared to control seeds. Seed treated for 4 min, on the other hand, resulted in a 1.13 reduction in VI I compared to control. These increases were positively related to seedling length, fresh weight, and higher germination index which is consistent with the findings of various researchers [43, 58]. The interaction of non-thermal plasma (NTP) and vegetal cells might lead to the activation of natural signals, hormones, and enzyme activities, which could explain the differences in germination and early seedling development reported in our experiment [59].

We noticed that, when NTP was applied to seed for 1 to 4 min, VI II increased by 2%, 2.24%, 3.26%, and 2.71% compared to control (figure 9). This is due to the increase in the total dry weight of seedlings.

It has been observed by various researchers that exposing seeds to plasma enhances water absorption, reduces the contact angle of the seed surface, and functionalizes the seed coat by forming new chemical bonds on the surface. It is proposed that interactions of plasma reactive species with seeds change not only seed coat properties but also the physiology of germinating seeds for various plant species [26]. Because of these properties, the plasma can cause minor surface etching (through ion bombardment) or even enrich the seeds’ surface with oxygen-containing functional groups [60]. These groups can significantly increase the surface

![Figure 8](image-url)
wettability, which has a very positive effect on seed metabolism and germination, as well as surface permeability for nutrients [1, 43]. So, RONS generated in the discharge might be transformed to hydrogen peroxide through additional interactions with water vapor molecules, and taken up during the imbibition process, and positively affect metabolic activities. From our observation, we noticed that in comparison to untreated seeds, plasma-treated seeds resulted in a significant improvement in wettability and might result in quicker germination.

4.7. Determination of change in chlorophyll content and total carotenoids
Chlorophyll and carotenoid content in plants are some of the prominent indicators for plant health. Chlorophylls come in several different forms. The most frequent forms found in nature are chlorophyll a and chlorophyll b. They can be present in green algae as well as in photosynthesizing plants. The intensity of a plant’s
color is determined by chlorophyll pigments. Pigment content has an impact on plant chemical composition and is used in numerous statistical relationships involving their physiology and chemistry. Carotenoid is a family of lipophilic chemicals that range in color from yellow to orange to red. Because carotene pigments are covered by chlorophyll, they have little impact on plant color. Carotenoids are pigments that help with photosynthesis. They also shield chlorophyll from overexposure to light. So, we analyzed the change in chlorophyll-a, chlorophyll-b, and total carotenoid content of plant extracts with reference to the plasma treatment time. Our results revealed the gradual increase in chlorophyll-a and chlorophyll-b content with plasma treatment time till 3 min and decreased in 4 min of treatment. Similarly, total carotenoid was gradually decreased with an increase in plasma treatment time (figure 10).

4.8. Determination of changes in total flavonoids and total phenolics and radical scavenging capacity

Plants’ defensive mechanisms rely heavily on phenolic and flavonoid compounds, which are linked to a variety of health advantages. Antioxidants are chemicals that supply electrons to damaged cells in order to prevent and stabilize damage caused by free radicals. The ability of antioxidants to donate hydrogen is assumed to be the reason for their action on DPPH. Free radical scavenging actions are critical for preventing the harmful effects of free radicals in various diseases. The DPPH free radical scavenging method is widely used to test the antioxidant properties of plant extracts. The addition of the extract to a violet-colored DPPH solution reduces it to a yellow-colored product, diphenylpicryl hydrazine, in a concentration-dependent manner in the

![Figure 11. (A) Change in total phenolic content, (B) Change in flavonoids content, (C) Change in DPPH scavenging potential, and (D) Change in ABTS scavenging potential between control and plasma-treated seedlings extract.](image-url)
DPPH assay [63]. Because of the short time necessary for analysis, this approach has been widely utilized to predict antioxidant activity. In this study, we analyzed the change in flavonoids, total phenolics, and DPPH radical scavenging activity as effect of plasma treatment. Our results revealed that the phenolics and flavonoids were increased with the increase in plasma treatment time till 3 min of treatment (figures 11(A), (B)). This could be beneficial in improving the defensive response against unfavorable conditions [64]. Similarly, the DPPH scavenging was changed positively with the plasma treatment of 3 min and decreased sharply in 4 min of treatment time (figure 11(C)). Changes in these activities disclose the positive impacts of plasma treatment on plant materials for their biochemical activities [65, 66].

5. Conclusions

In our study, we found that the NTP treatment is an effective approach to enhance germination potential without causing serious damage to the seeds. According to our findings, plasma treatment significantly enhanced germination characteristics, vigor index, and seedling length. After plasma treatment, reactive species may degrade the seed coat, making it susceptible to moisture imbibition as measured by water absorption, encouraging sprouting. Spectrophotometric analysis indicated that plasma treatment significantly enhanced Chlorophyll levels. However, no significant difference in carotenoid contents was observed. Our findings revealed that seeds treated with plasma for three minutes had a greater quantity of flavonoids and phenolics. Similarly, plant extracts had a higher radical scavenging capacity against both DPPH and ABTS free radicals up to 3 min of plasma treatment time, but this dropped dramatically at 4 min. Using this technique as a regular practice for seed preparation before planting might help to reduce pesticide usage during the crop cycle, increasing agricultural production while minimizing environmental degradation.

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Data availability statement

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

CRediT authorship contribution statement

RPG: Preliminary works, laboratory works, data generation, data analysis and authorization, prepared, revised and edited the manuscript. SPP: laboratory works, data generation, prepared, revised and edited the manuscript. HBB: Preliminary works, laboratory works. BPP: supervision of works, data verification, revised and edited the manuscript. DPS: supervision of works, data verification, revised and edited the manuscript.

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