

Effects of Cold Atmospheric Plasma Jet Treatment on the Seed Germination and Enhancement Growth of Watermelon

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Abstract

The current study was carried out to reveal the possible impacts of non-thermal plasma on water uptake by seeds, seed germination and vigor of seedlings of watermelon seeds using cold plasma jet at atmospheric pressure and room temperature. Cold atmospheric plasma jet with nitrogen gas sources was employed in this study. Five treatment doses and one control were used to conduct germination parameters. The effects of the different duration time of cold atmospheric plasma on the germination of treated watermelon seeds were studied. The cold plasma operated at 3 Kv and 14 l/min as a fixed input voltage and a fixed flow rate respectively. Cold atmospheric plasma increased the germination percentage of watermelon as well as the growth parameters (root and shoot length, dry weight), and the vigor of seedlings. The effects of cold plasma during this study depended on exposure time. The operation time of 4 min considered as an appropriate plasma dose to promote the germination parameters.

Keywords

Non-Thermal Plasma, Atmospheric Plasma, Germination Rate, Vigor Index

1. Introduction

Physical methods like radiation, high electromagnetic field, microwave irradiation and cold atmospheric pressure plasma play a vital role solely in medication for microorganism decontamination [1], however also are helpful for industrial purposes, e.g. for increasing surface hydrophilicity and material surface modification [2]. However, a promising physical technique that gives a broad vary of

interesting industrial applications is cold atmospheric plasma which generated at low temperature [3]. Cold atmospheric plasma devices are a basic tool in a wide range of technological and bio-medical applications. [4] [5] [6]. On the other hand, in last two decades, several cold atmospheric plasma devices were investigated, as have their use with thermally sensitive materials and medical applications [7]-[13]. The plasma treatment creates a complex mixture of surface functionalities that influence surface physical and chemical properties and leads to a dramatic modification of wetting behavior of the surface [14] [15] [16]. Not solely the chemical structure but additionally the roughness of the surface is affected by the plasma treatment; this also might change the wettability of the surface [17]. It has also been demonstrated that wetting of biological tissue might be changed by low-temperature radio frequency plasma [18] [19] [20]. Cold (non-equilibrium) radiofrequency plasma treatment of biological objects becomes a very important tool for modification of their chemical and physical properties [18] [19] [20] [21].

One of the potential areas of cold plasma research is plant cultivation, particularly pre-sowing treatment by cold atmospheric plasma technique [22]. Promising results have been reported with regard to increasing seed germination rate, activity and reduction in germination time [23] [24] [25], increasing wettability and, implicitly, water absorption [26], and for decontamination of grains and vegetables seeds [27] [28], enzyme activity [29] [30], and also the yield of plants [31]. There are still several open problems with respect to the mechanisms of plasma action on cells and tissues [21]. As an example, physical and chemical mechanisms of the interaction of cold plasmas with tissues and therefore the actual roles of various plasma constituents in tissue treatment remain enigmatic. On the other hand, cold atmospheric plasma was considered as an economical and safe approach for seeds treatment with regard to the normal chemical methods. It's known that, the traditional chemical methods were usually uneconomic as results of large amounts of chemicals were required, and some residual chemicals on the seeds coat would bring soil pollution. For the cold plasma treatment, its damage to the seeds was quite weak as a result of the active substances might solely enter the seeds approximately several nanometers [32] [33], and therefore the residual environmental pollutants were quite few.

Melon is a very important horticultural crop cultivated mostly in arid and semi-arid region of the world where salinity, high temperature, fast soil drying, and crust formation are barriers to good melon crop establishment. In several seeds, germination can be inhibited by mechanical restriction exerted by the seed coat. Permeability limitation of water and gases is typical attributable to hard seed coat. However, the large seed cavity in the watermelon and imbibed coat serves as a continuous wet layer around the embryo by which the oxygen must transverse.

Moreover, no study has been previously reported on the effects of cold atmospheric plasma on melon seeds germination and early seedling growth. In

this paper, the cold atmospheric plasma jets (CAPJ) application for seeds surface modification and changes in the dynamics of water uptake into the watermelon seeds is reported. The authors also investigate the optimization of the operating conditions in cold atmospheric plasma to increase the percentage germination and total vigor of seedlings. Also, the current study aims to prove that the seeds germination and early growth of watermelon change after seeds pretreatment by cold atmospheric plasma jet, and seeks a beneficial treatment dosage for the seeds.

2. Materials and Methods

2.1. Plant Material

The popular Niagara watermelon seeds were obtained locally from Saudi Arabia market. Two hundred randomly selected fresh seeds and stored at 25°C in the dark until reached dried stage.

2.2. Characteristics of Plasma Source

The plasma treatment of melon seeds was performed by cold atmospheric plasma jet. The cold atmospheric plasma jet system is mainly composed of electrodes, dielectrics, voltage controller, nitrogen gas and a high-voltage power supply. The high-voltage power supply is a commercially available transformer for neon light. This power supply is utilized in the cold atmospheric plasma to generate the plasma jet in order to cut the overall cost of the device by replacing the expensive RF power supply representing the major cost. The power supply has an output of 10 kV, 30 mA and 20 kHz which gives an output in the range of very low frequency of RF. This power supply has an overload, open circuit, earth leakage and short circuit protection. The input of this power supply is connected to 220 V, 12 A voltage controller. The voltage controller regulates the primary voltage of the high-voltage transformer. The electrode system of the plasma jet consists of two parallel stainless steel disks separated by an insulator. The outer electrode (Cathode) and the inner electrode (anode) have the same thickness and diameter 15 mm and 2 mm respectively. The two electrodes are separated by an insulator material, which made of Teflon. The Teflon disk has a 1 mm thickness and 15 mm in diameter. The two electrodes and Teflon disk have center hole of 1 mm and 1.2 mm diameter respectively, through center hole nitrogen gas is flowing. The output terminals of the power supply are connected to the cathode and anode terminals of the plasma jet via a 1 mm single copper isolated cable as can be seen in **Figure 1**. The gas flow system is responsible for delivering the gas to the plasma jet at the appropriate flow-rate. It consists of the gas storage cylinder, dual-stage gas flow regulator, and gas connection rubber hose. In this study Nitrogen gas was used to test the plasma jet operation. The nitrogen gas is stored in a high-pressure gas cylinder [13].

Once nitrogen is introduced through the inner electrode and high-voltage ac power is applied, the plasma generation between the two electrodes. Sufficient

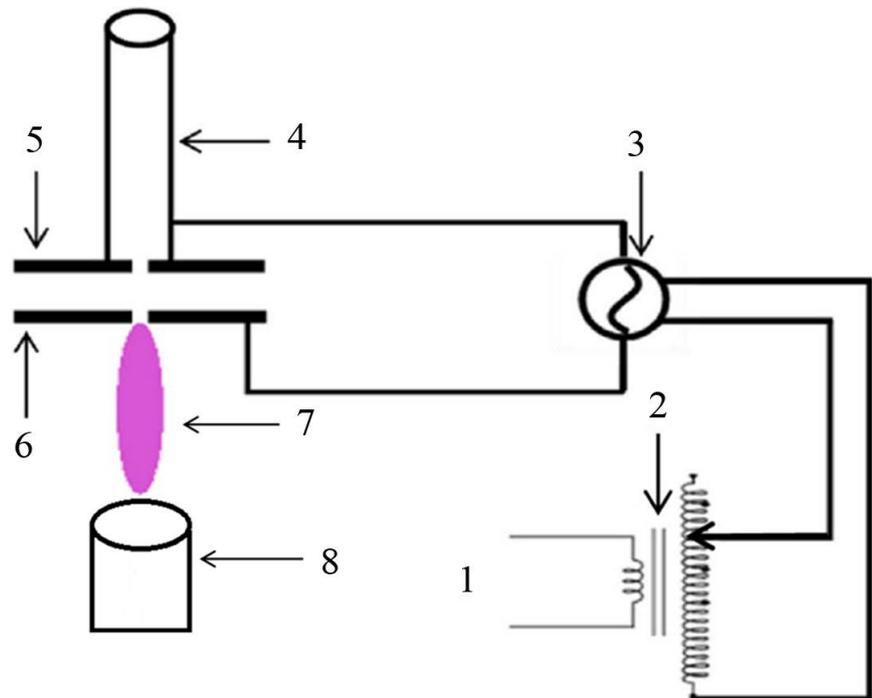


Figure 1. The electric circuit of cold atmospheric plasma jet device, 1 input electric source 220 V, 2 voltage controllers, 3 neon power supply, 4 gas feeding through copper tube, 5 anode, 6 cathode, 7 plasma jet and 8 acrylic box.

voltage applied to nitrogen gas ionizes nitrogen atoms by driving off electrons. Free electrons can trigger further ionization of neighboring nitrogen species by a collision. This series of reactions convert the nitrogen gas to the plasma state, which is distinctly different from solid, liquid, and gas states. The device generates a room temperature plasma jet that passes through the hole of the inner electrode and extends up to 7 mm beyond the end of the Teflon evolve.

2.3. Seeds Treatment

The plasma jet ejected to the acrylic box which contain a melon seeds. From experimental results, it shows that 14 l/min of nitrogen gas and at 3 Kv as a fixed input voltage was most suitable for plasma generation for our system. Plasma irradiation time was optimized to obtain the suitable experimental condition for the germination enhancement of melon seeds. The seeds were distributed uniformly and exposed to plasma for durations of 2, 4, 6, 8 and 10 minutes, each run contain twenty seeds. All treatments were conducted at least three replicates. The data in this study were recorded as the mean value \pm standard deviation. A GM1150 Infra-Red thermometer is employed to measure the temperature of a melon seeds to be treated. This thermometer has a measuring temperature range from -50 to 1150°C . For the wettability measurements, the dry seeds of treated and non-treated were first weighed and recorded as m_1 ; afterward, they were soaked in water for 12 h and also the seeds weighted each 2 h and recorded as m_2 after absorbing the surface water on the seeds. The water uptake by seeds is

called imbibition, which leads to the swelling and the breaking of the seed coat. The water uptake was calculated using the following equation:

$$\text{Water uptake\%} = \frac{m_2 - m_1}{m_1} \times 100 \quad (1)$$

Seeds were transferred to transparent plastic cup containing one layer of filter-paper, each cup contained twenty seeds. Seeds germination was carried out at 25°C in the dark. The seeds were monitored for duration of 10 days. In the 10th day the germination percentage, the length and mass of roots, shoots and sprouts were measured. Subsequently, roots, shoots and sprouts were dried until a constant mass was reached, for weight determination. Plant material for dry weight was dried at 90°C for 96 h. Germination characteristics were calculated using the following equations [34]:

$$\text{Germination rate} = \frac{\text{Number of seeds germinated in 4 days}}{\text{Total number of seeds}} \times 100\% \quad (2)$$

$$\text{Final germination percentage} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds planted}} \quad (3)$$

The seed vigor I and II were calculated in modification according to [35], using the following equation:

$$\text{Seed vigor I} = \text{Shoot length (cm)} + \text{Root length (cm)} \times \text{germination (\%)} / 100 \quad (4)$$

$$\text{Seed vigor II} = \text{Dry weight of seedling (root + shoot)} \times \text{germination (\%)} / 100 \quad (5)$$

3. Result and Discussion

3.1. Seeds Temperature Measurement

Figure 2 shows the temperature of melon seeds after plasma treatment. Infra-Red thermometer is used to measure the temperature of seeds. From this figure, it can be seen that, the temperature increase continuously by increase the expose time of nitrogen plasma. At 2min of operation time the seeds temperature equal room temperature (25°C). Even the operation time reach 10 min, the seeds temperature is as low as 28°C. It mean that, cold atmospheric plasma jet present a suitable method to enhancement germination without harmful effects.

3.2. Influence of CAPJ on Water Uptake

The findings suggest acceleration in the water uptake of watermelon seeds treated by cold atmospheric plasma jet. From **Figure 3**, it can be shows that, higher and more intensive water uptake positively correlates with exposure time of cold atmospheric plasma. In the case of 4min cold atmospheric plasma application dose, the most striking difference was visible after the first 2 h, when the seeds absorbed 52% more water compared to the control (37%). In contrast, longer imbibition time causes a decrease in the dynamics of water uptake in plasma treated seeds. These findings suggest that plasma treated seeds reached water-saturation significantly faster compared to untreated seeds. After 12 h the

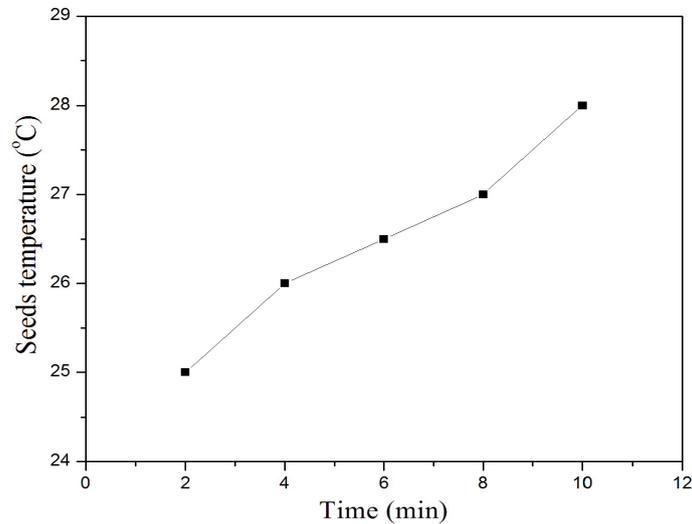


Figure 2. The temperature of melon seeds after plasma treatment.

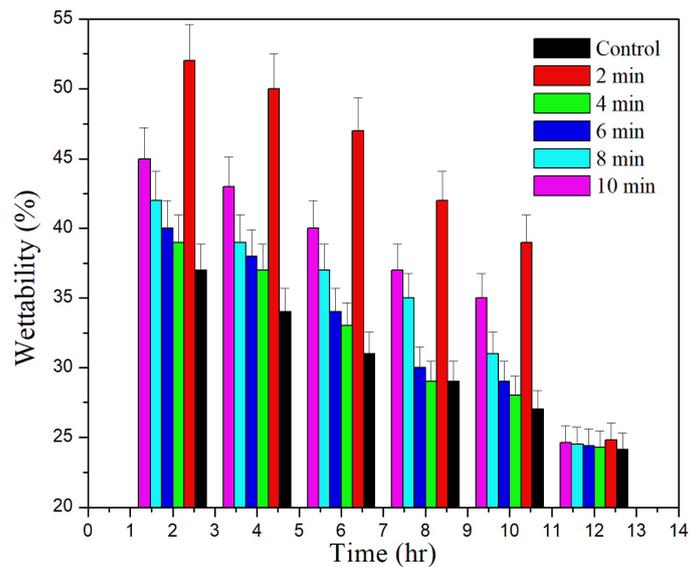


Figure 3. The wettability of treated and untreated melon seeds through 12 h soaked in water.

seeds were mostly well-watered in all variants, and there were no significant differences among the variants [36].

3.3. Germination Rate

The results of melon seed germination rates after the cold atmospheric plasma treatments were shown in **Figure 4**. On the 4th day of plant, the germination rate was 5% for untreated seeds. On the other hand, it can be seen that the plasma treatment has a dramatic effect on melon seeds. The germination rate was 10%, 60%, 40% 30% and 35% at 2, 4, 6, 8 and 10min operation time of CAPJ treatment. After 4 days of cultivation, it can be seen that, the cotyledon could be observed only for 4, 6, 8 and 10 min expose plasma irradiation (**Figure 5**). As

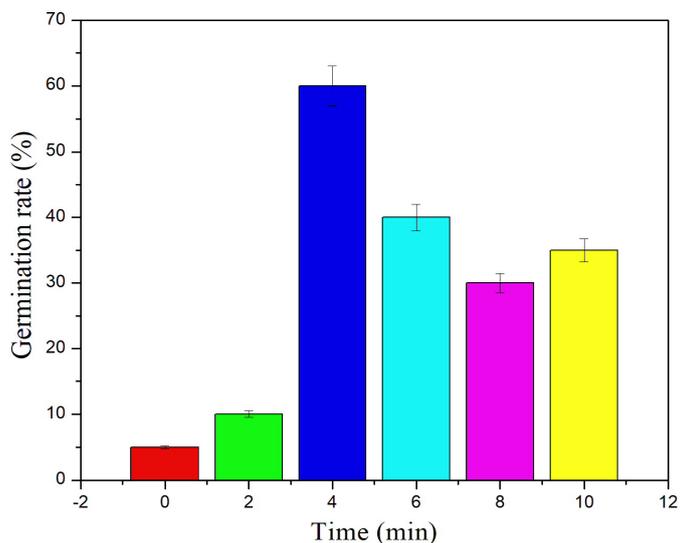


Figure 4. Germination rates of treated and untreated melon seeds.



Figure 5. The treated and untreated melon seeds after 4 days of cultivation; (1) untreated; (2) 2 min irradiated; (3) 4 min irradiated; (4) 6 min irradiated; (5) 8 min irradiated and (6) 10 min irradiated.

compared to untreated seeds, the cold atmospheric plasma jet has enhanced the seeds germination rate and the seedling growth. It is demonstrated that the reactive oxygen and nitrogen species (RONS) are the main signaling molecules regulating many developmental processes in mammalian, fungi, and plants. The required quantity of reactive species in plants can play an important role on the regulation of growth and development [37]. However, RNS and ROS species have positive effect on abscisic acid production, which is the pivotal hormone responsible for ignition and maintenance of the seed dormancy [38]. However, **Figure 6** shows the effect of cold atmospheric plasma on germination percentage after 10 days. From this figure, it can be observed that, the germination percentage of watermelon seeds were also different after cold atmospheric plasma treatment. The germination percentage increased by 72%, 93.5%, 75%, 87% and 85%

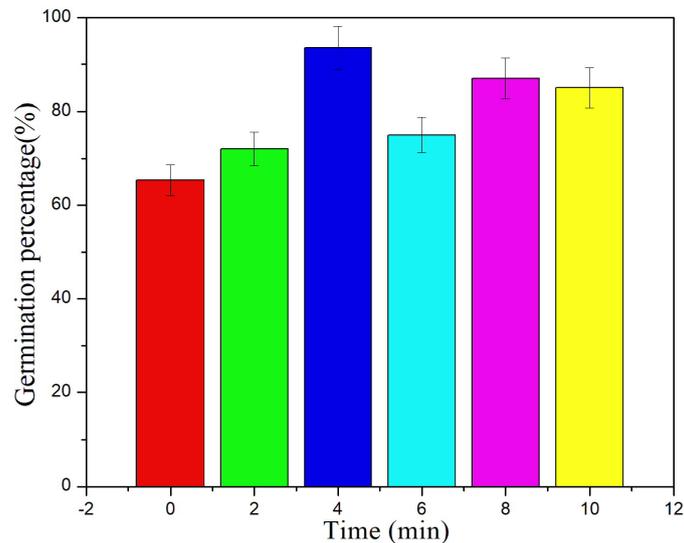


Figure 6. Germination percentage of treated and untreated melon seeds after 10 days of cultivation.

at 2, 4, 6, 8 and 10 min operation time of cold plasma treatment respectively. But, the germination percentage increased by 65.5% for untreated watermelon seeds. These results indicated that the cold atmospheric plasma has a significant effect in enhancement the germination percentage for watermelon seeds. Moreover, the active particles generated by the cold plasma could penetrate through the seeds coat and directly influenced cells inside the seeds; while the seeds coat operated like a partially permeable membrane, which only allowed passage of certain small ions or particles [24].

3.4. Seedling Growth: Shoot and Root Length

The positive effect of cold atmospheric plasma is observed between untreated and treated melon seeds on the short term germination and seedling growth. Therefore, in order to understand the long term combined effect of plasma treated melon seeds, the shoot and root lengths of melon seeds were measured after 10 days of agriculture. **Figure 7** shows the effects of plasma treatment seedling growth of melon seeds. It can be observed the shoot + root length of seeds which treated by plasma longer than control on after 10 days. The root length was 6cm for untreated melon, but increased to 12.5, 14, 10, 9.1 and 8.4cm for 2, 4, 6, 8 and 10 min expose plasma irradiation respectively. Similarly, the shoot length was 13 cm for untreated melon, while it was significantly enhanced to 21, 24.5, 18.5, 16, and 14.5 cm for 2, 4, 6, 8 and 10 min expose plasma irradiation respectively. It can notice that, the operation time of 4 min considered as an appropriate plasma dose to promote the root and shoot growth. Many researches have demonstrated that, the cold plasma treatment could promote seedling growth of plants; Dhayal *et al.* [39] reported that the seedling growth of *Carthamustinctorius* L. was significantly enhanced by a cold plasma treatment; Zhou *et al.* [40] observed that the tomato seedling growth was improved by an

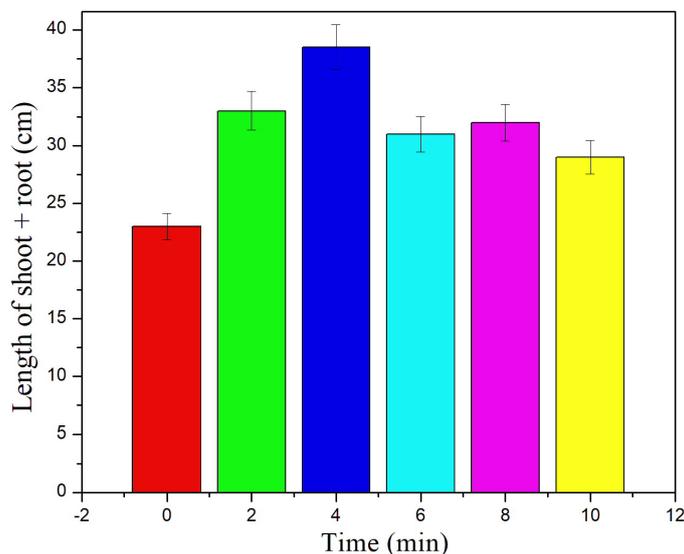


Figure 7. Seedling growth (shoot and root length) of treated and untreated melon seeds.

atmospheric pressure plasma treatment; Sera *et al.* [24] also found that wheat and oat seedling growth was enhanced by a cold plasma treatment. Moreover, Li *et al.* reported that a cold plasma treatment with an appropriate energy level promoted the soybean seedling growth, while much lower or higher energy levels did not show any promoting effects [33].

3.5. Dry Weight of Seedling

Under influence of CAPJ in the time range of 0 - 10 min the seedling dry weight (Figure 8) significantly increased. The weight of dry matter accumulations in the plants grown from the nitrogen plasma treated seeds were approximately 68.35 mg under treatment duration of 4 min and 10 days after sowing, while it was slightly lower in case control seeds (55.52 mg). Overall, nitrogen plasma treatment were produced the most favourable condition of plant growth activity and dry matter accumulation with respect to control seeds. The seedling emergence test showed that nitrogen plasma pretreatment changed the sprouting speed of watermelon seeds, resulting in alteration of the strong seedling percentage. The first sprout occurred from the fourth day after sowing for plasma treatment seeds while occurred after six days for control one (Figure 5). These results indicate that, the emergence acceleration of sprout by plasma treatment enhancement the weight of seedling.

It known that, in the N_2 atmosphere, N_2 molecules could be excited and ionized by high-energy electrons when the discharge plasma occurred, generating. N radicals and N_2^+ , as shown in the following reactions [41].



Previous research showed that plasma-induced actions on the seed coat could

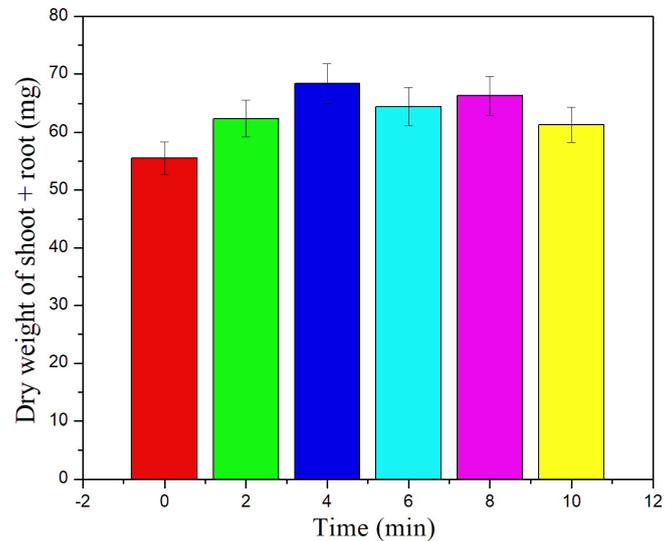


Figure 8. Dry weight of seedling of treated and untreated melon seeds.

lead to the penetration of active species (such as reactive ions) and UV into seeds, which probably affect the physiological reactions, seed germination and growth [42] [43].

3.6. Vigor Index

All treated seeds have higher value of vigor index I and II as can be seen in **Figure 9**. The highest positive responses were found when the seeds were treated for 4min. An intensity of 4 min cold atmospheric plasma produced increases in vigor index I and II, 17% and 25% respectively compared to the control seeds. However, there has been approximately a 5% - 40% decrease in yield for seeds with low vigor compared with seeds with high vigor [44]. From our results, cold atmospheric plasma jet leads to significance enhancement seed vigor so that high-quality yield in agricultural production will be produced. Finally, due to the higher root and shoot length for treated seeds with respect control one; the vigor indexes of treated seeds were significantly higher than that of the control. So that, appropriate nitrogen cold atmospheric plasma treatment can enhance seed vigor index in laboratory conditions.

3.7. Dry Sprouts Weight

The growth enhancement of watermelon measured by brought sprouts to dehydrate after measuring the length of root. The melon sprouts were dehydrated at 90°C for 96h and was weighted by digital balance. The total mass was calculated in the percentages from the ratio of the weight of sprout between pre and post dehydration process. From result, as can be seen in **Figure 10**, it could be observed that, all treated seeds had a greater mass compared control one. This could be proved that cold atmospheric plasma has a positive effect on germination enhancement. Previous research showed that the dry mas of radish sprouts

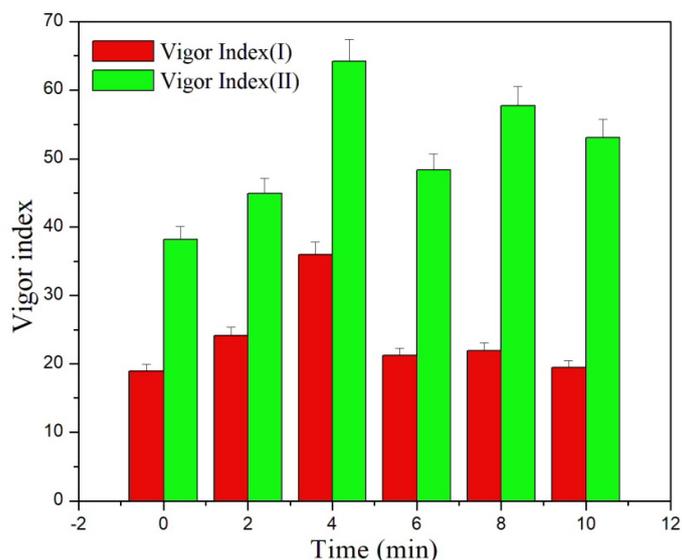


Figure 9. Vigor index of treated and untreated melon seeds.

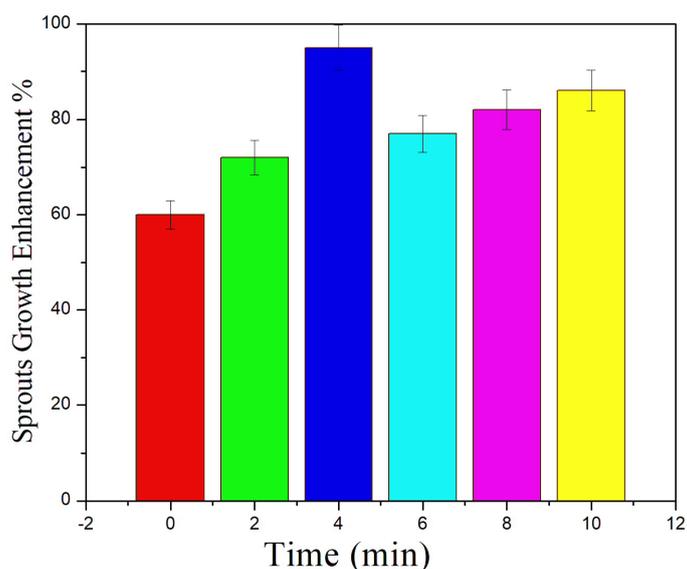


Figure 10. Dry sprouts weight of treated and untreated melon seeds.

which treated by non-thermal plasma recorded higher value with respect to untreated one [45].

4. Conclusion

The important advantage of the cold atmospheric plasma jet treatment of seeds is that the plasma source is capable working in wet and dusty environments and continuous mode. Nitrogen cold atmospheric plasma jet induced germination and growth of melon seeds was investigated in this study. The water uptake by seeds, seed germination and vigor of seedlings all improved after plasma treatment with an appropriate plasma dose, as well as the seedlings length and

weight. Cold atmospheric plasma treatments exhibited significant differences compared with the control seeds. Cold atmospheric plasma provided a dramatic solution of slow germination rate due to hard seed coat of watermelon, which prevents penetration of water and gases. The fast emergence of treated seeds with respect to untreated one can be attributed to the dramatic increase of wettability which leads to the enhancement of Seedling.

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