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Effect of Dielectric Barrier Discharge Plasma Jet on Germination and Activation of Squash Seeds (*Cucurbita pepo L.*)

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Abstract

In this Experiment, the impact of "Dielectric Barrier Discharge (DBD) Plasma jet" (5.47 watt) on activation and germination of Squash seeds *(Cucurbita pepo L.)* is studied at various periods. The seeds are divided into (soaked seeds and dry seeds). After exposing the seeds, measurements are taken for three germination characteristics (root length, shoot length, and number of leaves). The results show that exposing the plasma jet on the seeds soaked with water is better than dry seeds. Results of plasma exposure on soaked plant seeds at root length at exposure time (5 minutes) show superiority to control by 15.80% while there is no effect at (0.5 minutes) on dry seeds is shown during different exposure times (0.5, 1, 3, 5 min), and a clear increase is shown in shoot length and number of leaves compared to the control group. This indicates that the discharge of the plasma dielectric barrier contributes to stimulating the seeds and increasing their germination rate.

Keywords: DBD, germination rate, plasma jet, RONS, squash seed.

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تأثير نافث البلازما لتفريغ الحاجز العازل على إنبات وتنشيط بذور قرع الكوسا (*Cucurbita pepo L.*)

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الملخص:

في هذا العمل، تمت دراسة تأثير "نافث بلازما تفريغ حاجز العازل (DBD)" ذات قدرة (٥,٤٧watt) على تنشيط وإنبات بذور قرع الكوسا (*Cucurbita pepo L.*) في فترات مختلفة. حيث تم تقسم البذور إلى (بذور منقوعة وبذور جافة). بعد تعريض البذور، تم أخذ القياسات لثلاث خصائص إنبات (طول الجذر، طول الساق، عدد الأوراق). اذ أظهرت النتائج أن تعريض البذور المنقوعة بالماء لنافث البلازما أفضل من البذور الجافة. تظهر نتائج التعرض بالبلازما على بذور نبات منقوع بطول الجذر في وقت التعريض (٥ دقائق) تفوقاً على الكونترول بنسبة ١٥,٨٠٪ بينما لم يكن هناك تأثير عند (٠,٥ دقيقة) على البذور الجافة كما تظهر النتائج خلال أوقات التعرض المختلفة (٠,٥، ١، ٣، ٥ دقائق) زيادة ملحوظة في طول الساق وعدد الأوراق مقارنة بمجموعة الكونترول. وهذا يؤكد أن البلازما تفريغ حاجز العازل يساهم في تحفيز البذور وزيادة معدل إنباتها.

1. Introduction

The fourth state of matter is plasma, first described by the American chemist Irving Langmuir in 1922. In general, it refers to ionized gases and reactive species, free radicals, charged particles, and atoms in both their ground and excited states [1-4]. Plasma is classified into thermal and non-thermal; non-thermal plasma is distinguished from thermal plasma because there are no side effects in it and because of its frequent use in scientific fields, because non-thermal plasma arises from several types of discharges, including "the dielectric barrier discharge (DBD) plasma". The reason for using this type of plasma discharge is due to its ease of use and low cost, as it has wide applications in medicine and heat-sensitive materials [5-8], and this type of discharge is considered one of the most important and safest and healthiest technologies in plant productivity and food supply operations [9-11]. It has many functions in food applications due to its ease of use and efficiency, such as microbiological cleaning, improvement of food and quality preservation [12, 13].

The creation of reactive atom species, such as reactive oxygen and nitrogen species (RONS), is a crucial characteristic of plasma[14-16]. Since nitrogen and oxygen are the two main components of air, reactive oxygen species (ROS) and reactive nitrogen species (RNS) both include nitrogen oxides. The atmospheric pressure plasma treats and modifies surfaces and water and the decontamination effect when materials are exposed to it [17, 18].

This work depended on the seeds of the squash plant, which is the most important crop in the cucurbit family, due to its health benefits, including its impact on the prostate glands, blood sugar, cholesterol, immunity, parasite liver, the bladder, the prevention of parasites, and treatment of depression, as well as its nutritional and medical advantages[19-21]. It has been proven that DBD plasma disinfects and sterilizes seed surfaces by RONS and UV rays, which contributes to increasing the percentage of seed germination and seed germination rate and stimulating plant growth. By increasing its ability to absorb water well [2].

This paper has appeared the effect of plasma (DBD) on Squash seeds and the mechanism of interaction between the plasma jet and plant seeds, noting its effect on the processes of stimulating squash seeds and increasing their growth rate, in addition to exploring their economic importance in agriculture and medicine.

2. Materials and working methods:

2.1.Plasma system

A plasma discharge barrier dielectric system manufactured by the researchers was used [22]. The plasma system consists as illustrated figure (1) of the following components: a plasma needle, a high-voltage power supply, helium gas, and a gas flowmeter with a flow rate of 3L/min as in figure (1) and by the dielectric barrier discharge (DBD), the plasma jet is produced using two electrodes (cathode, anode) and they are isolated from each other and connected to the discharge tube, where the non-thermal plasma originates inside the discharge tube and exits through the plasma jet [23, 24]. The system operates at a high voltage 9 kV in order to ionize the gas molecules, with a power (5.67 watts), which makes it simple and cheap.

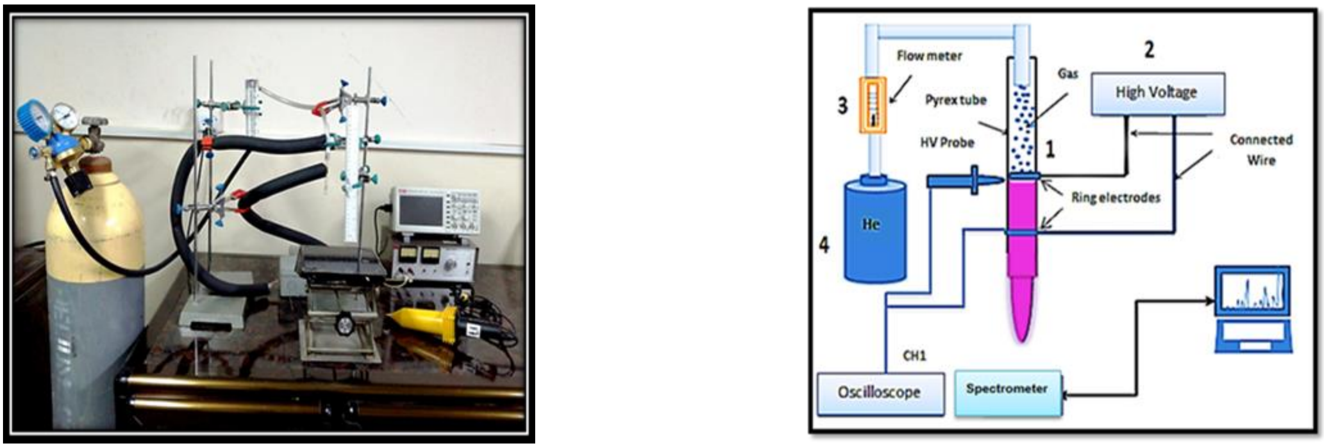


Fig. 1. The plasma system that was designed by the researcher [12] (a picture inside the scientific laboratory, diagram)

The system used to produce non-thermal plasma was relied upon at different times (0.5, 1, 3, and 5) minutes in order to expose the seeds of the squash plant.

2.2.The seeds

Squash (Cucurbita pepo L.) seeds produced by (HOGER) were used in 2022, with an 80% germination rate and a 98% purity and were used as two group (soaked seeds, dry seeds). The seeds were irradiated with a non-thermal plasma in four groups and were grown hydroponically and compared to the control. According to the characteristics of the seeds listed by the producer, the seeds have a short germination period of no more than 45 days. Ten seedlings (per Petri dish; 9 cm) exposed to plasma and ten seeds (not exposed to plasma) were prepared using the cardboard method [25].

Fig.2. Seeds used: a- dried *(Cucurbita pepo L.) seeds*, b- soaked (Cucurbita pepo L.) seeds (right).

3. Statistical analysis:

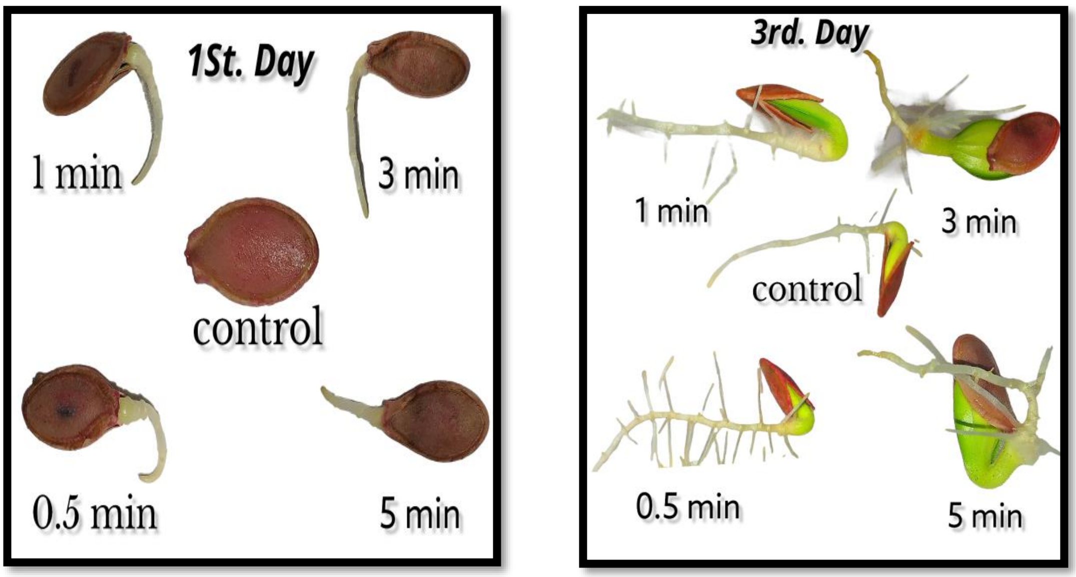
To examine the experiment's data, a Complete Randomized Design (C.R. D.) utilizing a 2 x 5 factorial experiment (similar to a seed treatment exposure time) was utilized and version 9 of the "Statistic Analysis System (SAS)" was used to run Duncan's multiple range test to assess whether there have been any significant differences between the treated seeds at the level of probability ($P < 0.05$) [26, 27].

4. Photos

The attached photos in this study have been taken with a high-resolution camera (108 mega pixels) and edited and modified using version 4.2.3 of Photo Room Pro.

5. Results and Discussion

Dielectric barrier discharge plasma (DBD) was used to expose the seeds of squash (Cucurbita pepo L.) at different times (0.5, 1, 3 and 5 min.) with control seeds in this study because the dielectric barrier discharge (DBD) plasma jet activates enzymes that promote breaking of dormancy in seeds and initiating the germination process [28].



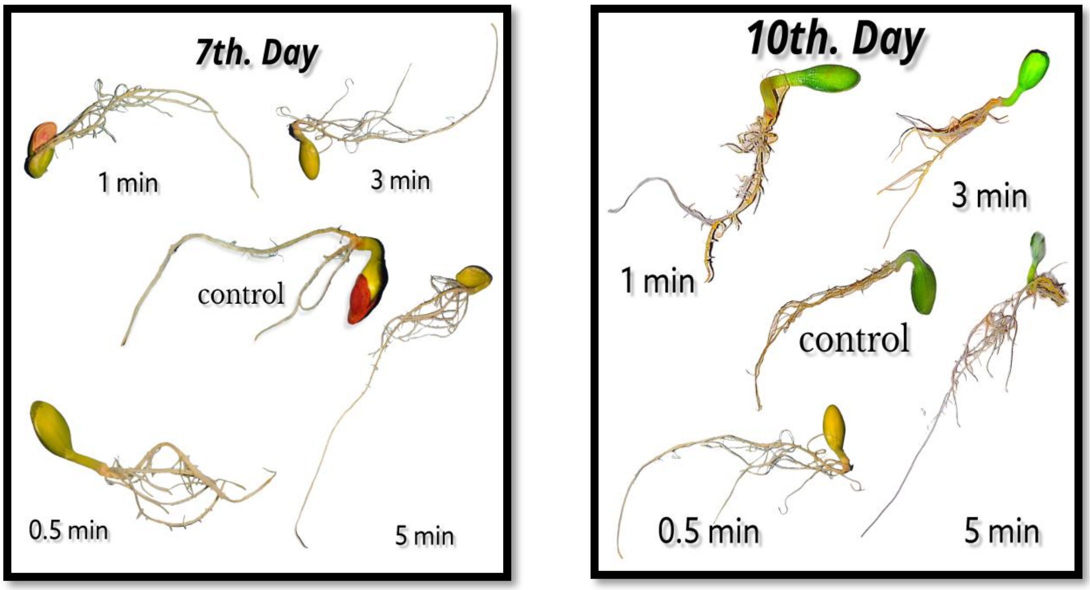


Fig. 3. Growth stages of seeds *(Cucurbita pepo L.)* exposed to (DBD) plasma jet at various intervals, for ten days and compared with control seeds.

It is necessary to break the dormant phase in plant seeds to increase germination, the seed ripening phase, undeveloped embryo, impermeability of the seed coat to oxygen and water, and hormonal imbalances are all problems for seeds. Gibberellic acid (GA) is responsible for ending the dormant phase, while abscisic acid (ABA) is responsible for maintaining dormancy. These acids and enzymes are affected by physical factors, as they activate enzymes that promote the breaking of dormancy in seeds and the start of the germination process[9, 29]. There are some experiments that used "Scanning Electron Microscopy (SEM)" to find out the effect of the resulting plasma jet on the seeds of the plant, where it is found that it works on scratches and polishes the seed layer, which leads to an increase in the process to enhance the absorption of water in the seeds during the drinking process and increase the growth and germination of seeds as shown in Fig(4) [30, 31] .

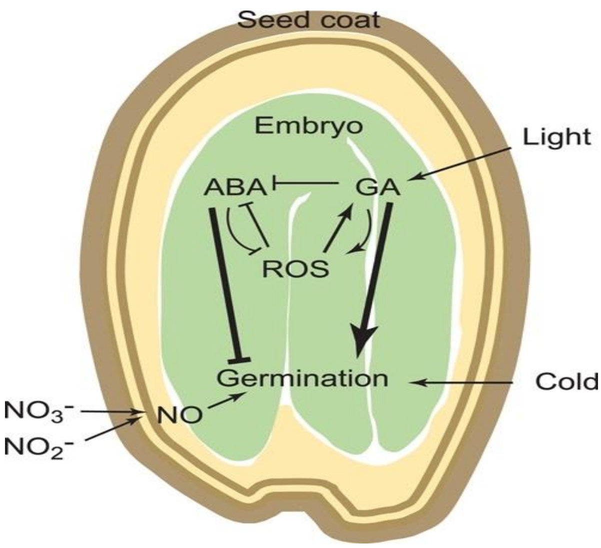


Fig. 4. Effect of plasma and reactive species on seedlings of *(Cucurbita pepo L.)* seeds[9]. ROS: Reactive oxygen species, GA: Gibberellic acid, ABA: Abscisic acid.

By using its own equation, the germination rate was determined[32].

$$R.G = \frac{n_g}{n_t} \times 100\% \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots..(1)$$

Figure (5) displays the percentages of the germination rates of dry seeds, soaked seeds, and control seeds, demonstrating that soaked seeds clearly outperform dry seeds when exposed to the non-thermal plasma jet.

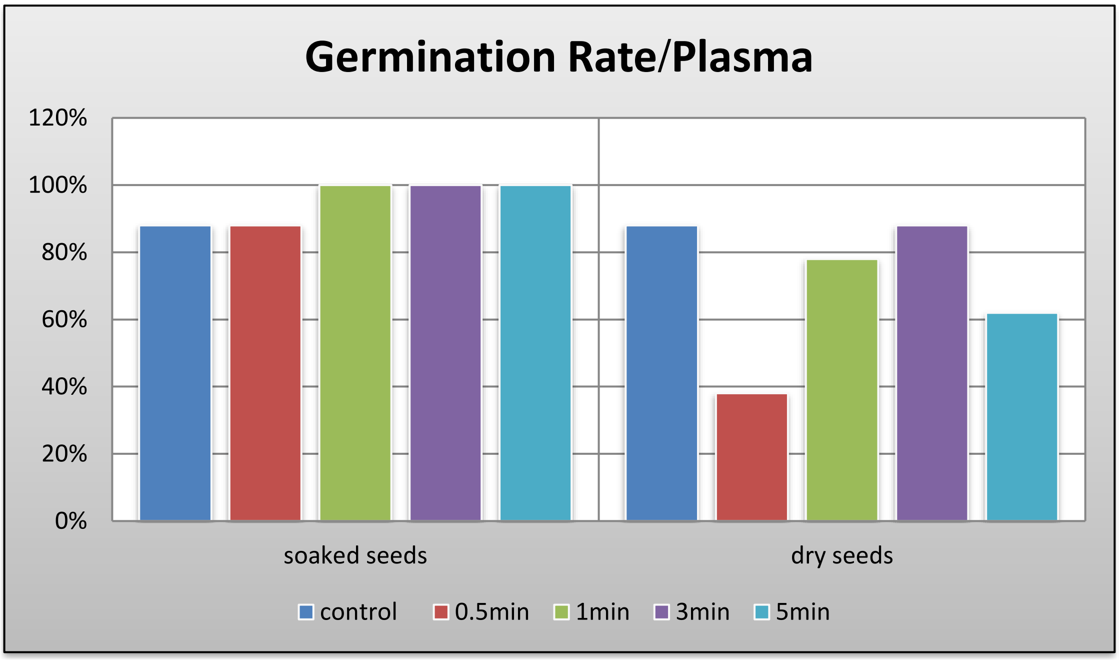


Figure (5) compares the plasma jet-exposed Squash plant seeds' germination rates to those of the control group.

The interaction of the plasma jet and the reactive species produced in the air with the water molecules present in the soaked seeds causes an increase in the germination rate because water plays a significant and significant role in growth rates, which explains the superiority and superiority of the barrier plasma treatment of soaked seeds over dry seeds[33].

It comes out that the percentage of germination in the soaked seeds exposed to the plasma jet was accomplished for the times (1,3,5) at a rate of 100%, although there was no difference in the percentage of germination between the control and the exposure time (0.5 minutes) and water[34]. Additionally, it was demonstrated in Figure (5) that the percentage of germination decreased when dry seeds were exposed to the plasma jet due to the absence of the reaction medium (water), as the reactive species interact with the liquid medium in this instance[35], this makes plasma a germination inhibitor in this situation.

The effects of non-thermal plasma on the root length of soaked and dry seeds are depicted in figure (6), where the average values for ten seeds have been taken, showing a preference for soaked seeds over dry seeds on day [36].

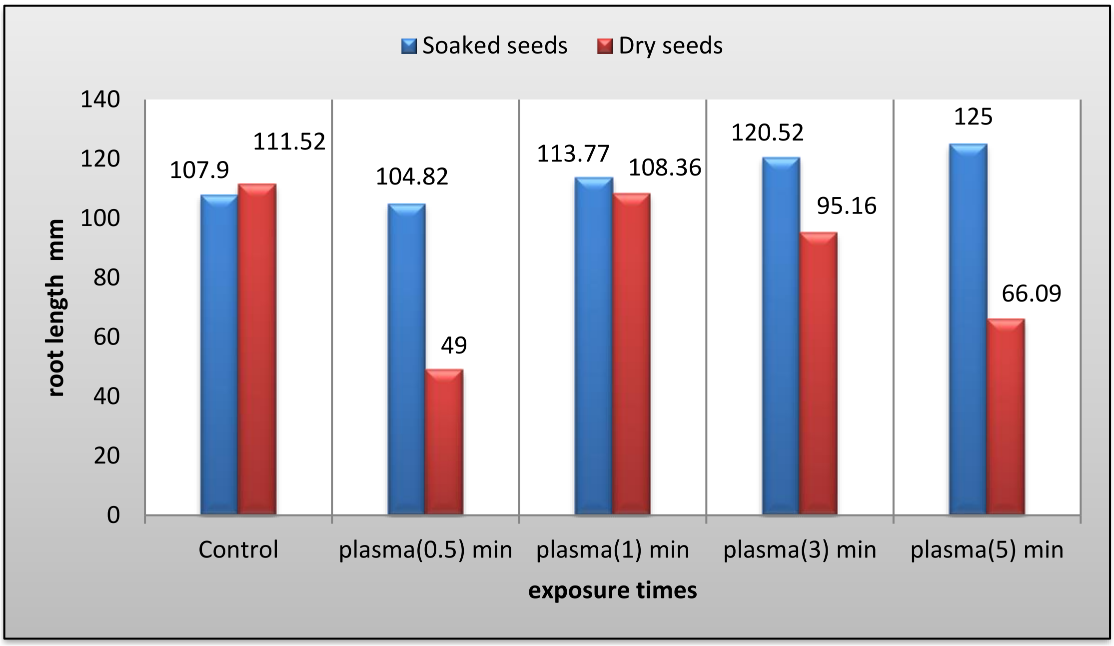


Fig. 6. Rate germination for root length of Squash (Cucurbita pepo L.) seed for each of the plasma treatment of soaked and dry seeds at various exposure times of 0.5 min, 1 min, 3min, 5min with control on day ten.

The results of (DBD) plasma jet exposure on soaked and the root length after ten days was (125 mm) superior to the average of the seeds that were not exposed by 15.80% for dry seeds by figure (6), which showed the advantage of a 5-minute exposure time for seeds soaked with the highest average. The average root length was then exposed for 3 minutes, outperforming the control by 11.7%. Where the non-thermal plasma works to make holes and defects in the pores of the outer shell, and this means that at the exposure time of 5 minutes, the seed coat becomes more defective and perforated, which helps the cause of the water absorption process through the process of imbibition. This has also been proven by the researchers[2], who showed that the plasma jet (DBD) reduces the apparent angle of contact between the seed surface and the water droplet. However, it was discovered that the plasma's detrimental impact on dry seeds caused an increased the proportion of growth inhibitors in the seeds, a case that prevented (DBD) plasma jet from breaking the dormancy of the seed. This is due to the interaction of the plasma with the inhibitors present on the seeds of the dry *(Cucurbita pepo L.)* plant, and this was not the case in the seeds soaked with water. For 24 hours, the seed coat's inhibitors were removed after soaking. *(Cucurbita pepo L.)* seeds are affected by non -thermal plasma when the charged molecules collided with the plasma plane with them. In the plasma production process, ions, electrons, free radicals, ozone, nitrogen oxides and hydroxy peroxide are formed in the plasma. It also shows physical factors such as ultraviolet radiation, infrared radiation and helium flow effects[37].

The effects of non-thermal plasma on seeds that were dry and submerged in water for 24 hours are shown in figure (7). Average values for ten seeds have been taken, which showed that in day tenth the seeds that were placed in water for 24 hours it was better than dry seeds[38].

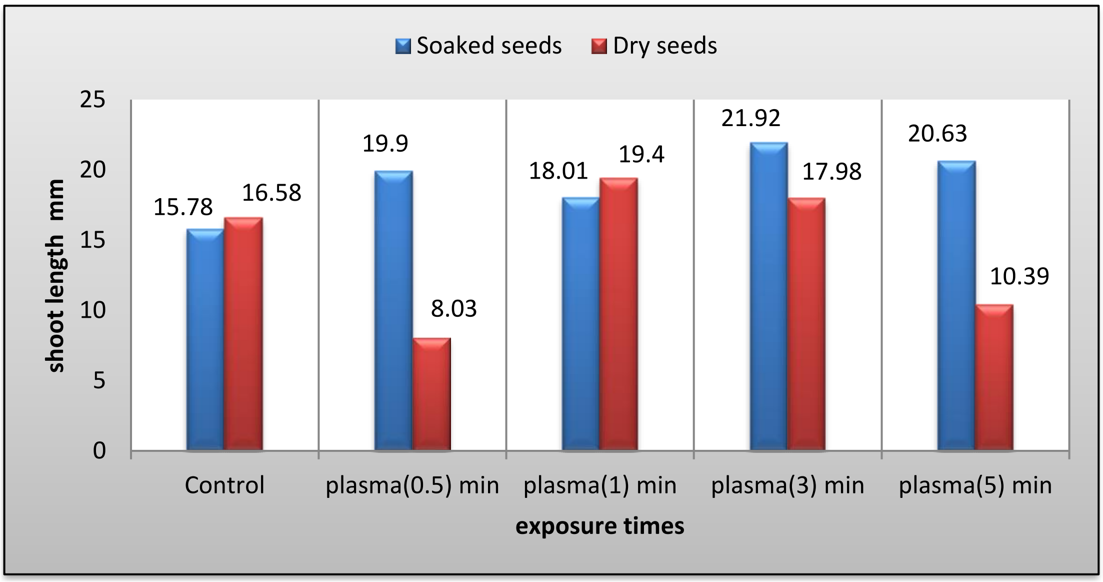


Fig. 7. Rate germination for shoot length of Squash (Cucurbita pepo L.) seed for each of the seeds soaked and dry seeds plasma treatment at various exposure times of 0.5 min, 1 min, 3min, 5min with control in day ten.

After obtaining the average values for 10 seeds, the findings displayed in figure (7) reveal the impact of (DBD) plasma on the shoot length of soaked seeds. The average shoot length germination at 3 min (21.92mm) performed 39% better than the control seedlings. An average growth of shoot length of 20.63 mm was observed after exposure of the adult for 5 minutes, exceeding the control by 30%. Then comes the time followed by the adult (0.5 min), who recorded an average leg length of (19.9 mm), outperforming the control by 26.5%, and occupied the exposure time (1 min) from the exposure times, recording (18.01 mm), outperforming the control also by 14.5%. The discussion has shown the relationship of the (DBD) plasma jet with according to the researcher's findings, the water content in a plant's shoots, which aids in its growth, triggers the activation of germination as a plant's shoot length increases [39, 40]. While in dry seeds, Seeds exposed to plasma have a detrimental effect, resulting in lower seed germination rates than control seeds.

The findings in figure (8) illustrate how non-thermal plasma affected the number of leaves on the soaking seeds. The preference for soaked seeds over dry seeds was demonstrated by the treatment of seeds, where the average values for 10 seeds were taken.

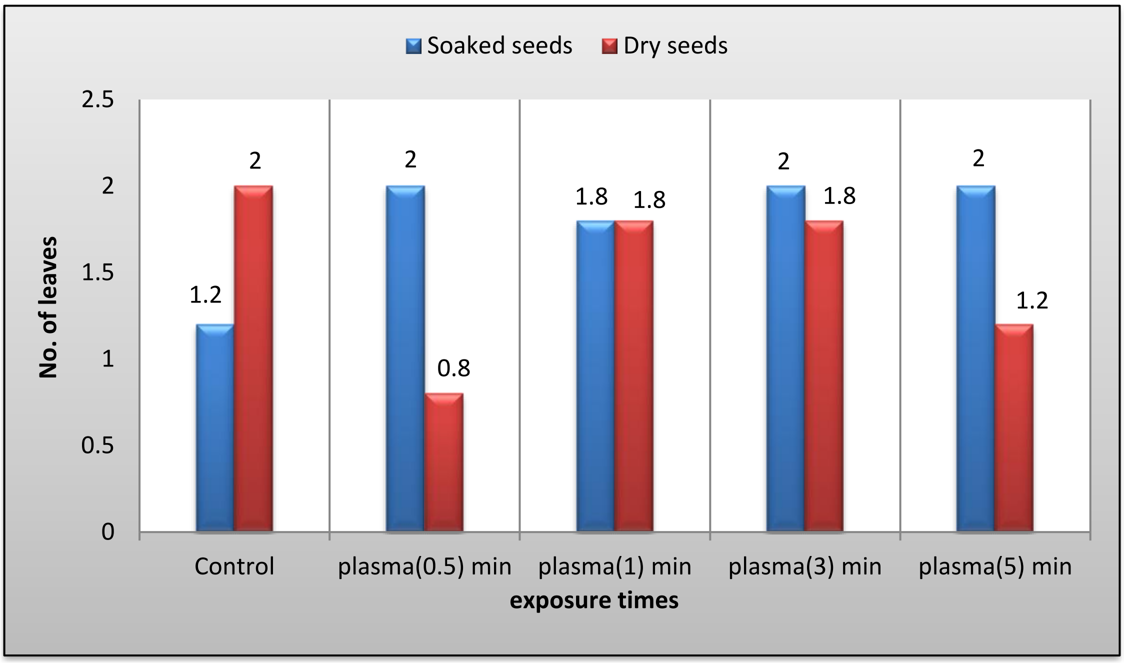


Fig. 8. Rate germination for No. of leaves of Squash (Cucurbita pepo L.) seed for each of the seeds soaked and dry plasma treatment at various exposure times of 0.5 min, 1 min, 3min, 5min with control.

According to the findings in figure (8), non-thermal plasma had an impact based on how many of the seeds' leaves had been soaked. It was discovered that there were significant differences between exposure times (0.5, 3, and 5) when compared to the control seeds. The soaked seeds responded favorably to plasma after one hour, exceeding the exposure times of (0.5, 3, 5) min by determining the ten seeds with the highest average number of leaves (2), exceeding ten control seeds on average had 67% fewer leaves, while (1.2) average leaves per seed after one minute of plasma exposure exceeding the control by 50%. The (DBD) plasma jet had a beneficial effect on the germination process by increasing the number of leaves, which aids the plant in improving the process of photosynthesis, which is compatible with the findings of the researchers[41]. All of them saw how the plant's photosynthesis was progressing thanks to the growing number of leaves.

The results indicate different activities of growth and catalytic enzymes to break the dormancy barrier of exposed seeds and the response of seeds exposed to plasma to the activity of a group of different enzymes, for example Malondialdehyde (MDA) as a sign of membrane damage and oxidised membrane lipids [42]. The research has shown that seeds exposed to non-thermal plasma (DBD) indicate lower concentrations of MDA with an increase in germination and seed growth, which was consistent with the researcher's study [43].

6. Conclusions

The impact of (DBD) plasma jet on soaked seeds and dry seeds at different times (0.5, 1, 3, 5 min). DBDJ appears to scratch the surfaces of seed coats and creating structural defects that lead to an increase in the imbibition process that helps the seeds to activate and grow. As the exposure time (5 minutes) is the best in terms of activation and increasing the size of the roots, shoots, and for the soaked seeds, the size and number of leaves., which will improve the rate of germination. The plasma jet produces reactive species that interact with media that contain water molecules, and because of this, the treatment of the plasma jet on the soaked seeds became better than on the dry seeds.

Disclosure of potential conflicts of interest

The authors declare that their financial interests are not in conflict.

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