Effect of RF plasma on Moringa seeds germination and growth

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ABSTRACT
The paper presents the effect of cold plasma (RF-Ar low-pressure plasma) on Moringa oleifera seeds. Five groups of seeds characterized by the different exposure times (1, 5, 10 and 15 mints) as well as untreated seeds. The highest germination parameters were obtained for seeds treated for the exposure times of 1 min. Analysis of the data showed a statistically significant impact of RF plasma on the seeds germination parameters. The surface properties of the plasma-treated samples are examined using scanning electron microscopy (SEM). Improvements appeared in the germination percentage and germination potential of the treated seeds and increase in the length of the plant for seeds exposed to the RF plasma at treatment time 1 and 5 min. from 4.32% to 26.40% for roots and from 15.91 % to 18.62 % for shoots, respectively. Also, it is observed that the weight of seeds increased for the samples treated at 1 and 5 min from 4.89% to 6.22%, and the weight of the shoots is increased from 6.19% to 6.93%.

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1. Introduction

Moringa oleifera trees are one of the world’s most useful and nutritious plants grow in tropical and subtropical areas, and they are a food resource, the leaves are rich in protein, calcium and phosphorous and it has many medical uses [1].

Germination of M. oleifera seed occurs within 5–30 days, there are many treatments for seed treatment to reduce the time of germination which might include: cracking the shells, soaking seeds, dehulling seeds, and plasma treatment. M. oleifera trees grow in tropical and subtropical areas, and they are a food resource. The leaves are rich in protein. Biodiesel fuel could be produced from a seed and used to purify water [2].

RF-plasma at low pressure has many technological applications, and the treatment by plasma is one of the applications which are used to improve germination and growth of seeds. This treatment can be used to induce desirable changes in developmental and physiological processes in plants, improve seed resistance to stress and diseases, modify seed coat structures, increase the permeability of seed coats and stimulate seed germination, seedling growth in surface disinfection, decontamination and biotechnology [3–5].

The effects of atmospheric-pressure N2, He, air, and O2 micro plasma on seed germination and seedling growth of mung beans was studied by Zhou et al. [6]. The germination percentage of air plasma for treated samples reached approximately 95%, whereas the corresponding value for O2 plasma for treated seeds ascended to 72%. Seeds treated by He and N2 plasma had the lowest germination rate of 30%, almost the same as that for control samples.

Effect of cold plasma on soybean seeds was studied at plasma powers 0, 60, 80, 100 and 120 W for 15 s. Treatments had positive effects on seed germination and seedling growth; the highest stimulatory effect was at 80 W [7].

Urva et al. [8] studied the effect of low power continuous wave laser on germination, seedling growth and mineral profile in M. oleifera. The laser energy levels used for seed irradiation showed variable effects on germination, seedling growth and mineral profile. The mineral contents were recorded to be higher in seedling raised from laser-treated seeds, which were higher in roots versus shoots and leaves.

The aim of the present work is to investigate the effect of RF-low pressure low power plasma on germination of moringa seeds, involving investigation of the effect of exposure time of RF-plasma on seeds germination.

2. Material and method

The plasma generating chamber is cylindrical shaped and made of stainless-steel, with a 25 cm diameter and a 36 cm height and contains two parallel plates of stainless-steel electrodes with a diameter of 5 cm and a distance of 5 cm between them. The two electrodes are placed inside the dielectric ceramics. The electrodes
are connected with an RF power supply and Z-matching network (13.56 MHz and 0–200 W). The chamber is vacuumed by a rotary pumping system to a base pressure of $10^{-4}$ torr. High purity Ar gas is used as a working gas and is pumped into the chamber through a needle valve. The pressure of the Ar gas is fixed at 2 torr.

Moringa seeds are a Saudi production of Indian origin. The number of treated seeds was 100 seeds, divided into 5 groups; every sample groups were treated at 1, 5, 10, and 15 min by RF plasma power of 100 W and Ar gas pressure of 2 torr. The morphological characteristics of the treated seeds have been examined by scanning electron microscopy (JSEM 7400F, Joel, Japan).

The seeds were put in the Petri dishes on filter paper, 24 h after exposure to plasma, 5 ml of water was added to the petri dish every 48 h, and the seeds were covered with filter paper. The Petri dishes were placed in a dark place at a temperature of $26 \pm 0.5^\circ$C to germinate. The measurement time of germination and seed growth was 10 days. The seeds germination started on the third day. The measurements of roots and shoots were performed by a ruler on the third day after the germination began. The statistical characteristics of the seeds are described by the following parameters [9].

Germination of percentage (%)  
$$= \frac{\text{total number of germinated seeds}}{\text{total number of seeds}} \times 100$$  \hspace{1cm} (1)

Germination potential (%)  
$$= \frac{\text{total number of germinated in 3 day}}{\text{total number of seeds}}$$  \hspace{1cm} (2)

After 10 days of germination, when the leaf was grown, the roots and shoots were separated from the seed and their weights were measured.

Water absorption was measured to investigate the imbibition of water into 5 g of seeds for two samples, a control sample (untreated sample), and a sample treated by plasma at 1 min. The seeds were immersed in 25 ml of water and measured the weight of seeds after draining the water every 12 h. The weight gain percentage was given by the following formula [10]:

$$\text{weight gain} (%) = \frac{W_{\text{after}} - W_{\text{before}}}{W_{\text{before}}} \times 100$$

where $W_{\text{before}}$ and $W_{\text{after}}$ are the weight of seeds before and after water absorption, respectively.

3. Results and discussion

The seeds were placed between the cathode and the anode. The plasma treatment of the seeds has fixed at RF power of 100 W and Ar gas pressure of 2 torr, while the treatment times are varied between 1–15 min.

Figure 1 shows the experimental setup used to generate the RF-plasma.

3.1. SEM imaging of seeds

Figure 2 shows the morphological change in the seed coat surface using SEM for untreated and treated seed at 1, 5 and 10 min.

After Ar plasma treatment, the surface becomes more wrinkled and roughness indicating grafting of functional groups into the seeds surface. On the other hand, there was a more etching on the surface of the plasma-treated seed compared with the untreated sample. This can facilitate the more efficient absorption of water. Also, RF plasma leads to a change in the wetting properties of seeds due to oxidation in the surface of seeds and a decrease in the apparent contact angle [11–14].

![Figure 1. The experimental setup.](image-url)
Figure 2. SEM images of Moringa seed (a) untreated and treated by plasma at (b) 1 min (c) 5 min and (d) 10 min.

Figure 3. Germination percentage of seeds treated with RF plasma at power 100 W.
3.2. Effect of treated plasma on seed germination and growth

Figure 3 shows a comparison of the germination percentage or germination rate for untreated seeds and those treated with RF plasma at different treatment times (1, 5, 10 and 15 min).

The germination percentage for untreated seeds (control sample) was 83.33%, the germination percentage for seeds treated at 1 min 100% started off the second day of germination, the fifth day of planting seeds. While the seeds were treated at 5 min, the germination rate was increased gradually to reach 100% on the sixth day of germination and decreased to 66.67% for seeds treated at 10 min, and 0.0% for seeds treated at 15 min. This indicates that an increase in plasma treatment time might be deleterious for seeds. It can cause the death of the seed.

Figure 4 shows the germination potential for a control sample and treated samples. The germination potential for seeds treated at 1 min is maximum (100%), and then less until it reaches the minimum value (0%) at 15 min.

Figure 5(a,b) shows the length of the roots and shoots for all seeds which measured at the third, fifth, and seventh days after germination. The results show an increase in the length of for treated seeds at 1 and 5 min
Figure 6. The weight of roots and shoots of seeds treated with RF plasma at power 100 W.

Figure 7. Comparison between the water absorption by the plasma-treated seeds with the control sample.

compared to the untreated seeds and those treated at 10 min.

Figure 6 shows the relationship between the weights of roots and shoots with RF plasma exposure times. The roots weight of the treated sample at 1 and 5 min is increased from 4.89% to 6.22% and the shoots weight was increased from 6.19% to 6.93%, respectively. Moreover, the weight decreased when seeds were treated by plasma at 10 min compared with control samples.

A comparison between the water absorption by seeds treated with Ar plasma at plasma power 100 W at 1 min and the untreated (control) sample is shown in Figure 7. This increase in water absorption can be due to etching in the outer layer of treated seeds. The Moringa seeds have a hard coat, and the seed treatment by plasma creates clefts in the seed coat, an increase of uptake water, and the germination becomes faster.

4. Conclusions

*M. oleifera* (Moringaceae) is a tree currently used by traditional medicine in tropical Africa, America, and Asia to treat systemic arterial hypertension.
The plasma treatment of the seeds has fixed at RF power of 100 W and Ar gas pressure of 2 torr, while the treatment times are varied between 1–15 min.

The RF plasma treatment used for seed treatment showed variable effects on germination and seedling growth.

The plant length and weight for plasma-treated seeds at 1 and 5 min is larger than that of the untreated sample. The study found that increasing the plasma exposure times above 5 min brings about damage to the seeds.

However, this technique, which has low gas pressure and low power rather than atmospheric plasma, could possibly be used to improve the *M. oleifera* germination, seedling and growth.

Further study is required for the effect of the plasma input power and the gas pressure on germination and growth of seeds.

**Disclosure statement**

No potential conflict of interest was reported by the author.

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