Plasma Medicine, 6(3–4): 389–396 (2016)

Effect of Low-Pressure Radio Frequency Plasma on Ajwain Seed Germination

A. Gholami,[a] N. Navab Safa,[a,*] M. Khoram,[b] J. Hadian,[c] & H. Ghomi[a]

[a]Laser and Plasma Research Institute, Shahid Beheshti University, Tehran 19839 63113, Iran; [b]Department of Physics, Borujerd Branch, Islamic Azad University, Borujerd, Iran; [c]Department of Agriculture, Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Tehran, Iran

*Address all correspondence to: Nasrin Navab Safa, Laser and Plasma Research Institute, Shahid Beheshti University, Tehran 19839 63113, Iran; Tel.: +98 21 2990 4006; Fax: +98 21 2243 1775, E-mail: n_navabsafa@sbu.ac.ir

ABSTRACT: In this article, we study the effect of low-pressure radio frequency (RF) plasma on seed germination and growth of the ajwain plant. Ajwain seed is a medicinal plant that possesses antibacterial and antioxidant properties. Results show that RF plasma treatment of ajwain seed improves seed germination rate by ~11%. The best result was observed at a plasma power of 50 W. Moreover, plasma treatment of ajwain seed leads to a significant change in seed wettability and plant growth parameters including shoot and root length improvement after plasma treatment.

KEY WORDS: radio frequency, low-pressure plasma, seed germination, ajwain seed, medicinal plant, wettability

I. INTRODUCTION

From ancient times to the present day, medicinal plants have had an important role in health promotion and disease control in human societies. Currently, 25% of existing drugs have herbal origins.[1] Due to the devastating impacts and side effects of chemical medicines, public regard of herbal remedies has increased in recent years.[2] *Trachyspermum ammi*, commonly known as ajwain, is a medicinal plant that grows in some areas of Egypt, Iran, Pakistan, Afghanistan, and India. It is considered to be a valuable drug plant due to its various chemical constituents containing phenolic compounds, especially thymol, which is reported to possess antibacterial and antioxidant activities.[3]

A big challenge to grow plants, especially medicinal plants, is herbal dormancy. Even after placement in suitable conditions, many kinds of seeds are not able to germinate and thus remain in a dormant mode. Some morphological changes must occur to break the seeds' dormancy. Two types of seed dormancy are endogenous and exogenous. Exogenous dormancy such as physical, mechanical, and chemical dormancy is constrained by external factors of the embryo. Endogenous dormancy such as morphological, physiological, and double dormancy is constrained by internal factors of the embryo.[4]

Cold-moist stratification is a common method used to break herbal plant dormancy, but it is a time-consuming process.[5] In some cases, hormones such as gibberellin and chemical components can be used as substitutes for stratification to break dormancy,[6] but the relative cost of these chemical methods is a drawback. Moreover, using chemical methods for the long term leads to unwanted physiological effects on human health and causes environmental problems.[7]

Cold plasma is a recently proposed technology for different applications in agriculture and food industry.[8–10] It is suggested to decrease the time of dormancy and increase crop yield. Bormashenko et al. reported that cold RF air plasma treatment may modify the surface wetting properties of lentils, beans, and wheat seeds.[11] Jiayun et al. showed that cold plasma treatment promotes *Andrographis paniculata* germination and improves seed permeability significantly, resulting in accelerated seed germination and seedling emergence.[12] Koga et al. studied the effects of air nonthermal plasma irradiation on *Arabidopsis thaliana* seeds and reported that plasma irradiation led to a 12% increase in seed weight and a 39% increase in seed number.[13] Ling et al. suggested that cold plasma treatment improves oilseed rape drought tolerance by improving antioxidant enzyme activities, increasing osmotic-adjustment products, and reducing lipid peroxidation, especially in drought-sensitive cultivars.[14] Será et al. applied low-pressure discharge to stimulate germination of seeds using a microwave power supply.[15]

In this article, we describe the effect of cold plasma on germination and growth parameters of the ajwain medicinal plant. Cold plasma can be generated in atmospheric or low-pressure conditions. We use low-pressure RF plasma to investigate the plasma effect on ajwain seeds. Some of the main aims of your research include studying of the effects of RF plasma power on seed growth and seed surface modifications.

II. MATERIALS AND METHODS

In our experiments, we used RF capacitively coupled plasma at a frequency of 13.56 MHz. The experimental setup was comprised of three main components: RF power supply, plasma reactor, and vacuum system. The plasma reactor was a chamber comprised of a Pyrex cylindrical tube with a diameter of 100 mm, thickness of 3 mm, and length of 350 mm. Two metal mesh electrodes were included, which the central flat electrode was connected to the RF generator, and the other grounded electrode was wrapped around the chamber. Chamber pressure was kept at 7.5 x 10^{-2} Torr using a rotary vacuum pump. Figure 1 schematically shows the experimental setup used for ajwain seed plasma irradiation.

Germination tests were carried out under laboratory conditions on seeds supplied by the Medicinal Plants and Drugs Research Institute of Shahid Beheshti University, Tehran, Iran. Ajwain seeds were exposed to 50 W air RF plasma for 2 min. The total number of seeds for each control (untreated) and treated sample was 75 seeds, which were cultivated in three Petri dishes of 9-cm diameter. Each Petri dish was watered with 2 mL of distilled water and closed using parafilm to control evaporation rate. The closed Petri dishes were then placed in a germinator for 8–16 hours (radiation-darkness) for 12 d at a temperature of 22 ± 0.5°C and relative humidity of 75%. Germination began on the third day and reached a maximum value on the seventh day. Root and shoot length of the ajwain sprout was measured after 12 d. We repeated the experiment three times. We counted the germinated seeds daily and the germination percentage (GP) and germination index (GI) were calculated using following formulas:

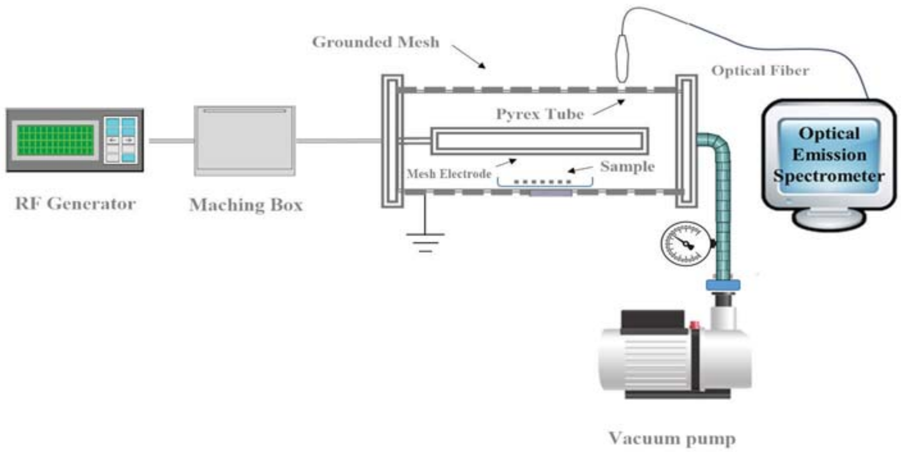

FIG. 1: Schematic diagram of experimental setup for irradiation of ajwain seeds by low pressure RF plasma

$$GP_i = \frac{n_i}{N} \times 100 \quad (1)$$

and

$$GI = \sum_i \frac{n_i}{i}, \quad (2)$$

where n_i represents the number of germinated seeds on the *i*th day, and *N* represents the total number of planted seeds.

To study the effect of plasma power on ajwain seed germination, some experiments were performed at plasma powers of 50, 80, and 100 W. Optimum treatment time was adjusted to 2 min, as in previous experiments. Besides studying seed germination parameters, seed surface wettability and plasma characteristics were also used to explain results. Plasma emission spectra at different conditions were registered with the use of a spectrometer (Ocean Optics HR2000 + ES) to determine plasma reactive species concentration. The spectrometer was able to measure the wavelength interval of 200 to 1100 nm.

III. RESULTS AND DISCUSSION

Figure 2 provides a visual comparison of the treated (right) and control (left) samples of the ajwain seed germination after 7 d. The figure clearly shows that both the number and size of sprouts in the treated sample were greater than those of the control. Figure 3 displays the seed GP for different RF plasma powers of 50, 80, and 100 W at a treatment time of 2 min. It can be seen that increasing the plasma power does not always lead to improvement in seed germination. According to Fig. 3, GP is best at an RF

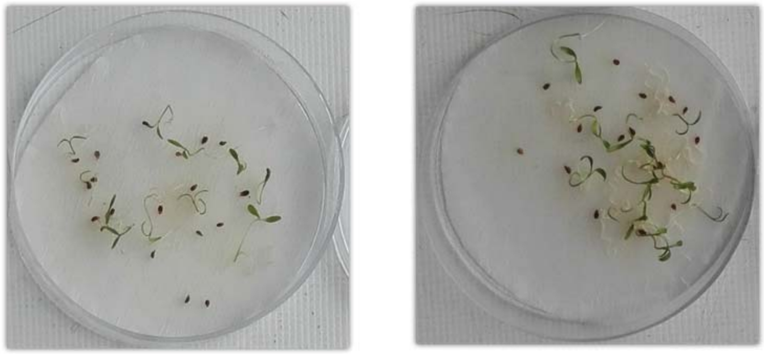

FIG. 2: Ajwain sprouts after 7 d. (Left) control sample; (right) treated sample

plasma power of 50 W. An increment of ~11.1% in GP, compared to the control, was obtained after applying RF plasma on ajwain seeds. Although applying an RF plasma power of 80 W increases GP compared to that of untreated seeds, observations indicate that increased plasma power may lead to damaging effects to the seeds. Figure 3 shows that the seed GP decreases after increasing RF plasma power beyond 50 W, and the seed germination rate is even lower than that of the control sample when plasma power increases to 100 W.

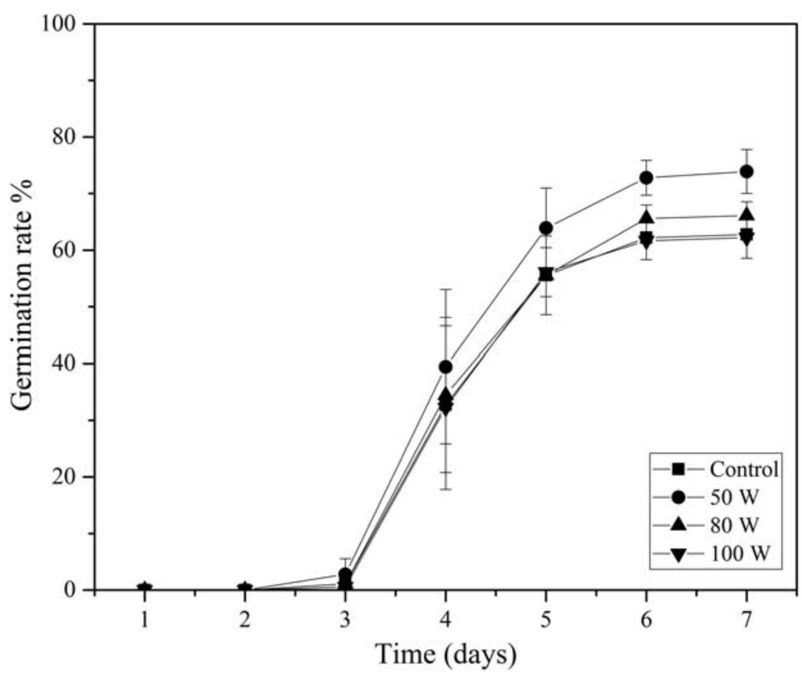

FIG. 3: Ajwain seeds germination at different RF plasma powers

Table 1 shows GP and GI at different conditions based on the definitions presented in in Eqs. (1) and (2). According to the table, GP and GI for ajwain seeds treated at the plasma power of 50 W increase by ~11.1% and 1.22, respectively. However, they show some decrease when seeds are treated at the plasma power of 100 W. Figure 4 presents shoot and root length of ajwain plants 12 d after seed planting. It can be seen in this figure that sprout length differs with various plasma powers. Samples treated with 50 W RF plasma power have the tallest root, and the best germination rate occurred at this plasma power as well. The root length of the ajwain sprout increased by ~34% at RF plasma power of 50 W, relative to control samples. However, root length increments are not considerable for RF plasma powers of 80 and 100 W (~2% and 10% for plasma powers 80 and 100 W, respectively). According to Fig. 4, the maximum increment in shoot length takes place when ajwain seeds are treated at RF plasma power of 100 W

TABLE 1: Germination rate and germination index of ajwain seeds in treated seeds and control seeds

Plasma power (W)	GP	GI
Control	62/77	8/41
50	73/88	9/63
80	66/11	8/80
100	62/22	8/33

GI, Germination index; GP, germination percentage.

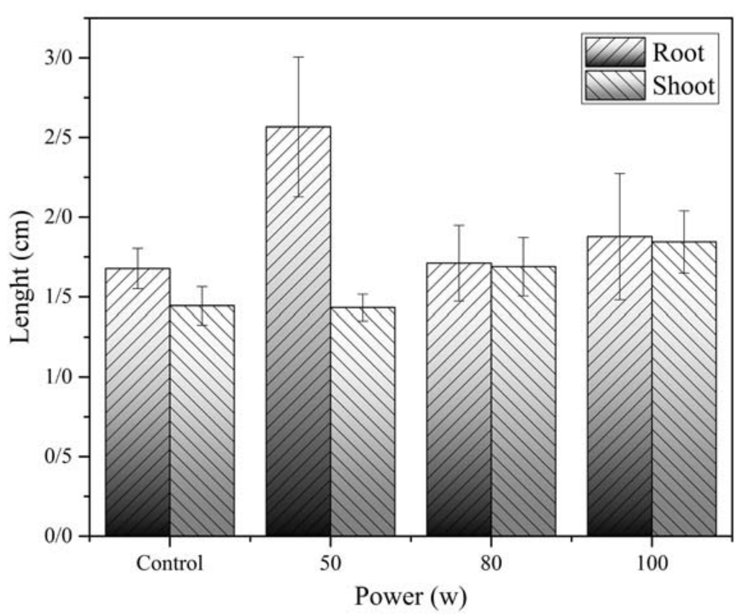

FIG. 4: Root and shoot length of ajwain sprouts at different RF plasma powers

(10% compared to the control). No considerable growth in the shoot length of sprouts is observed at a plasma power of 50 W.

Figure 5 displays the contact angle of water droplets on the untreated and treated ajwain seeds. The treated seeds were exposed for 2 min to an RF plasma power of 50 W. The contact angle test was performed by dropping 1.5 μL of deionized water onto the seed surface. As is apparent from Fig. 5, seed surface wettability significantly changed under the influence of RF plasma treatment. Reduction in the water contact angle from 122° to ~10° under the effect of RF plasma treatment suggests a strong change in seed

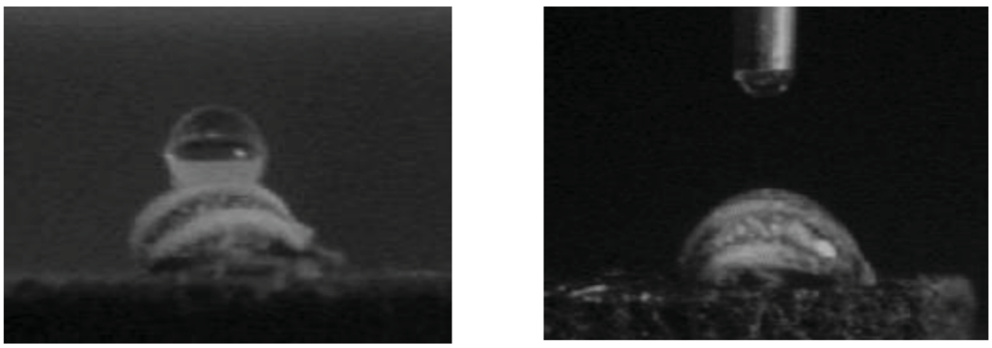

FIG. 5: The contact angle of water droplet on the ajwain seed, left; untreated, and right; treated samples

surface characteristics and wettability. The obvious increment in seed surface wettability is one of the most important reasons for increasing the seed germination rate.

Figure 6, which displays the RF plasma spectra at different plasma powers, implicitly identifies the involved chemical species and the main cause of the seed–plasma effect. It is apparent that plasma emission is intensified with increasing plasma power.

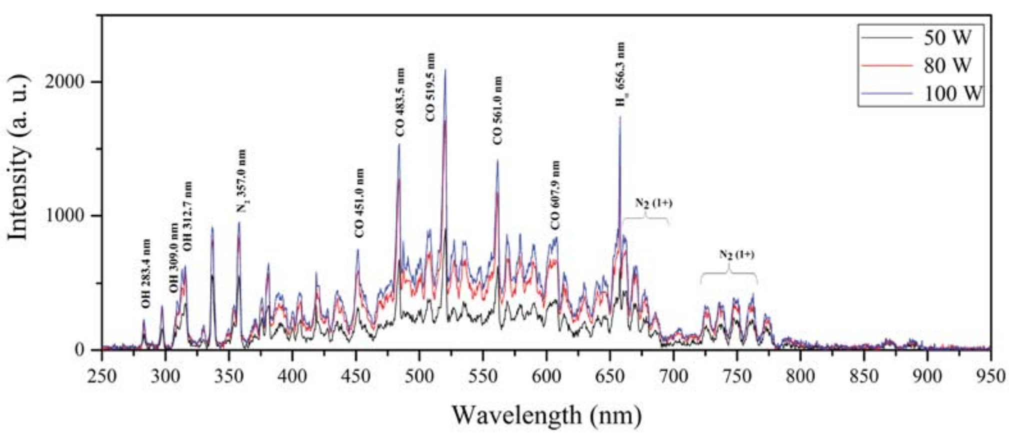

FIG. 6: Optical spectra emitted from RF air plasma for powers of 50, 80, and 100 W

Main peaks were monitored in the spectra in the range of 450–650 nm, which originate from CO and hydrogen species. CO emission lines at 451.0, 483.5, 519.5, 561.0, and 607.9 nm and an H emission line at 656.3 nm may represent products of the seed surface etching process. Several peaks belonging to OH and nitrogen species can also be observed in the spectral range of 250–400 nm. Spectral emissions at 283.4, 309.0, and 312.7 nm indicate the presence of OH species in the RF plasma. The peak of 357.0 nm also corresponds to the second-order emission of molecular nitrogen.

Clearly, plasma interaction with material surfaces leads to chemical changes on the surface, resulting in modification of surface properties. The obvious conclusion is that increasing seed surface hydrophilicity under the influence of plasma can affect seed germination and growth.

IV. CONCLUSIONS

In this article, we investigated the effect of low-pressure RF cold plasma on germination and growth of ajwain. Improvement in germination and growth of ajwain seeds is important because of the resulting benefits for medicinal applications. Results show that plasma can promote ajwain seed germination and growth, and best results were observed at a plasma power of 50 W. Primary findings show that seed surface is modified under the influence of plasma species, although more research is required to understand the main mechanisms of seed germination under the influence of RF plasma. Chemical species, charged particles, ions, and UV light generated by nonthermal plasma can have important roles in cellular processes because these species interact with many biomolecules as activators or inhibitors.

REFERENCES

1. Rates SM. Plants as source of drugs. Toxicon. 2001 May 31;39(5):603–13.
2. Benzie IF, Wachtel-Galor S, editors. Herbal medicine: biomolecular and clinical aspects. Boca Raton, FL: CRC Press; 2011.
3. Bairwa R, Sodha RS, Rajawat BS. Trachyspermum ammi. Pharmacog Rev. 2012 Jan 1;6(11):56.
4. Baskin CC, Baskin JM. Seeds: ecology, biogeography, and evolution of dormancy and germination. Philadelphia: Elsevier; 1998.
5. Emery DE. Seed propagation of native California plants. Santa Barbara: Santa Barbara Botanic Garden; 1988.
6. Hilhorst HW, Karssen CM. Seed dormancy and germination: the role of abscisic acid and gibberellins and the importance of hormone mutants. Plant Growth Reg. 1992 Aug 1;11(3):225–38.
7. Struik PC, Siert G. Wiersema. Seed potato technology. Wageningen, The Netherlands: Wageningen Academic Publishers; 1999.
8. Dorraki N, Mahdavi V, Ghomi H, Ghasempour A. Elimination of diazinon insecticide from cucumber surface by atmospheric pressure air-dielectric barrier discharge plasma. Biointerphases. 2016 Dec 1;11(4):041007.
9. Thirumdas R, Sarangapani C, Annapure US. Cold plasma: a novel non-thermal technology for food processing. Food Biophys. 2015 Mar 1;10(1):1–11.
10. Misra NN, Schlüter O, Cullen PJ. Cold plasma in food and agriculture. 1st ed. San Diego: Academic Press; 2016.

11. Bormashenko E, Grynyov R, Bormashenko Y, Drori E. Cold radiofrequency plasma treatment modifies wettability and germination speed of plant seeds. Sci Rep. 2012 Oct 17;2:741.
12. Jiayun T, Rui H, Xiaoli Z, Ruoting Z, Weiwen C, Size Y. Effects of atmospheric pressure air plasma pretreatment on the seed germination and early growth of Andrographis paniculata. Plasma Sci Technol. 2014 Mar;16(3):260.
13. Koga K, Thapanut S, Amano T, Seo H, Itagaki N, Hayashi N, Shiratani M. Simple method of improving harvest by nonthermal air plasma irradiation of seeds of Arabidopsis thaliana (L.). Appl Phys Express. 2015 Dec 8;9(1):016201.
14. Ling L, Jiangang L, Minchong S, Chunlei Z, Yuanhua D. Cold plasma treatment enhances oilseed rape seed germination under drought stress. Sci Rep. 2015 Aug 12;5:13033.
15. Será B, Stranák V, Serý M, Tichý M, Spatenka P. Germination of Chenopodium album in response to microwave plasma treatment. Plasma Sci Technol. 2008 Aug;10(4):506.