Plasma seed priming in green cumin: physiological and developmental study

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Abstract

Green cumin is an herbaceous, annual, delicate, and aromatic plant in the family Apiaceae, also known as kammun and senut, which is among the oldest, the most economical, and the most popular medicinal plants. In this study, pretreatment of cumin seeds was studied with plasma technology without using chemicals through damage to seed for germination. To this end, cumin seeds were examined in a control group (no treatment) and treatment with cold plasma for 5 and 10 minutes in other groups. The physiological activities of catalase (CAT), peroxidase (POX), and superoxide dismutase (SOD), as well as malondialdehyde (MDA) and soluble sugars were studied in the samples. According to the results, mean growth of seeds in 5 min plasma treatment was higher than the control and 10 min exposure groups. Mean activities of CAT, POX, SOD, and soluble sugars were higher in plants obtained from 5 min plasma irradiation than the control and 10 min plasma treatment. It can be concluded that treatment of cumin seeds with plasma increases SOD and CAT antioxidant enzymes activities. Besides, seed treatment for 5 minutes led to the best results.

Keywords: *Seed priming; green cumin; plant growth; plasma; antioxidant enzymes*

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Introduction

Cumin cyminum L. is a plant in the Apiaceae family that has many characteristics such as low germination, poor vigor reserves, and poor establishment in soil. Cumin height varies from 5 to 10 cm depending on the environmental conditions. Green cumin contains oil (7%), resin (13%), and essential oil and aluron (4-2.5%). This plant is grown globally in Iran, Tajikistan, Uzbekistan, Morocco, Turkey, India, Egypt, Syria, Cyprus, Mexico, Bulgaria, and Chile (Bharti et al., 2018). Cumin is a plant with antioxidant, antibacterial, and antifungal properties, which is widely used in the pharmaceutical, food, and cosmetic industries.

There are a variety of technologies for seed enhancement that increase seed moisture during the treatment. In this study, a dry seed treatment, namely plasma treatment, was used to increase the seed coat permeability without increasing seed moisture content. Plasma is referred to as an ionized gas whose atoms are disintegrated into negative electrons and positive

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ions. The use of cold plasma for plant seed pretreatment is a practical method compatible with agricultural environment that can increase crop yields (Jiafeng et al., 2014). Seed pretreatment with plasma stimulates seed germination and suppresses plant fungal and bacterial pathogens (Griesser, 2011). Additionally, plasma can improve plant physiological metabolism including dehydrogenase, superoxide dismutase, and peroxidase activities, photosynthetic pigments, photosynthetic efficiency, and nitrate reductase activity (Jiafeng et al., 2014). Li Ling et al. (2014) studied the effect of different plasma powers on soybean and found the best plasma power of 80 watts for soybean growth. The effect of plasma on okra seeds was examined at different times and the best seed yield was observed under plasma irradiation for 5 minutes (Randeniya and de Groot, 2015). Moreover, the effect of plasma was studied on the growth and yields of various plants such as *Oryza sativa* (D. Chen et al., 2019), *Solanum melongena* L. (Zhou et al, 2012), *Lycopersicon esculentum* (Yin et al, 2005), and *Triticum aestivum* (Sera et al., 2010).

Since plasma is a fast process, it is highly capable of inactivating microorganisms in food and plants and requires relatively simple equipment. Therefore, establishment of this technology is very cost-effective on an industrial scale. In this study, therefore, some physiological and developmental characteristics of green cumin were investigated following the influence of plasma.

Materials and Methods

Seed preparation and treatment

Quality cumin seeds obtained from Pakan Seed Company (Isfahan, Iran) were subjected to atmospheric pressure and room temperature by cold plasma using a surface discharge reactor, and then irradiated by plasma for 5 and 10 minutes.

The plasma reactor consisted of two electrodes placed on either side of a glass plate (length 121 mm, width 31 mm, thickness 1.1 mm). The voltage electrode was a set of 19 copper wires, each wire with a diameter of 111 micrometers and a length of 11 mm. The distance between adjacent wires was 6 mm and the ground electrode was an aluminum and copper strip (length 31 mm, width 13 mm).

The seeds were evenly distributed on each wire. The plasma reactor was placed in a chamber. The rectangular section flew at a speed of 1 liter per minute. Discharge was generated in AC. We used a high-voltage transformer (Electroputere TIRB 0-20), which provided a sinusoidal voltage of 11 Hz to measure the discharge voltage and was connected to a high-voltage probe (Tektronix, USA) with a current-carrying shunt resistor measuring 9 mm.

Environmental method and seed sowing

According to the time of seed exposure to plasma, cumin seeds were sown in three plastic pots (20 × 15 cm) containing a mixture of clay, perlite, and pit moss (1:1:1). A control sample without plasma irradiation was sown and labeled with the same conditions. Samples were transferred to a greenhouse at 22 ± 2 ℃ and 70% relative humidity, and irrigated once every two days. After seed growth, seedlings were collected to prepare cross sections.

Biochemical and physiological studies

Extraction of antioxidant enzymes

Enzymes were extracted from fresh plant tissue by 0.1 M sodium phosphate buffer (pH = 7) containing 2% polyvinylpyrrolidone (PVP).

Evaluation of SOD activity

SOD is a byproduct of oxygen metabolism and, if not controlled, causes the incidence of different cellular damages (Hayyan et al., 2016). SOD activity was measured based on photochemical inhibition of nitro boletra zolium reduction and the absorbance was then read at 560 nm.

Assessment of POD activity

POD activity was measured based on the catalysis of guaiacol polymerization using increasing adsorption at 470 nm and an extinction coefficient of 26.16 $cm^{-1}.M^{-1}$ for guaiacol.

Table1
The results of biochemical and physiological analyses

	sod (IU/gr.Fw)			cat (IU/gr.Fw)			Peroxidase (IU/gr.Fw)			MDA (nmol/gr.Fw)			soluble sugars (mgr/gr.Dw)			total flavonoids (mgr/gr Fw)		
	R3	R2	R1	R3	R2	R1	R3	R2	R1	R3	R2	R1	R3	R2	R1	R3	R2	R1
Control (untreated)	0.58	0.60	0.70	1.09	1.14	1.16	0.97	0.93	0.89	8.24	8.53	8.69	5.07	5.03	5.23	3.71	3.44	3.50
5min	1.22	1.41	1.36	1.83	1.67	1.72	1.75	1.73	1.59	5.49	5.82	5.55	7.30	7.79	7.63	3.76	4.08	4.00
10min	0.88	0.94	0.84	1.60	1.65	1.43	1.23	1.11	1.08	6.76	7.10	7.51	6.60	6.51	6.69	4.69	4.31	4.38

Assessment of CAT activity

CAT activity was calculated by following H_2O_2 decomposition at 240 nm and applying an extinction coefficient of 39.4 $cm^{-1}.M^{-1}$.

MDA assay

Physiological activity of MDA was assessed using an extinction coefficient of 1.55 $cm^{-1}.M^{-1}$ with nM. g^{-1}. FW^{-1} unit. It is necessary to measure MDA because it indicates lipid peroxidation, determines cellular oxidative stress levels, creates an enhanced lipoxidated end product by binding to proteins, and induces mutation by binding to purine bases.

Concentration of soluble sugars

The modified method of Schlegel (1986) was used to measure the concentrations of soluble sugars. The absorbance of samples was read by a spectrophotometer at 485 nm. Glucose concentrations (mg/g dry weight, DW) of samples were calculated using standard glucose solutions.

Measurement of total flavonoids

The concentration of total flavonoids was measured using aluminum chloride dyeing method. Sampling was done by a spectrophotometer at a wavelength of 510 nm. The standard quercetin curve made by Merck Company with concentrations of 20-80 mg/l was used to calculate the concentration of flavonoid compounds.

Developmental examination of the sample

After cutting the seedlings in 1:1 glycerol-alcohol solution, the prepared sections were stained by hand using Zaji Carmen and methyl green. Colored specimens were examined using an optical microscope (ZEISS model) equipped with a calibrated lens to identify the number of cells and the related results. In addition, photos of the specimens were taken using a Sony digital camera (DSC-W310) installed on a microscope.

Results

Seed sowing

In this study, an increase was observed in the germination rate of cumin seeds under the influence of plasma. The growth rate of plasma-irradiated seeds for 5 min was higher than those exposed to plasma for 10 min.

Biochemical and physiological tests

The results of SOD, CAT, and POX enzymes, besides MDA and soluble sugars assays are presented in Table 1. These results indicate increased amounts of SOD, CAT, POD, and soluble sugars, but decreased MDA under 5 min of plasma irradiation, which resulted in more favorable growth of cumin seeds than the control and 10

Table 2
Results of biochemical and physiological studies

LSD			Multiple Comparisons				
Dependent Variable	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
total flavonoids (mgr/gr Fw)	Control	5min	-.39667*	.14042	.030	-.7403	-.0531
		10min	-.91000*	.14042	.001	-1.2536	-.5664
	5min	Control	.39667*	.14042	.030	.0531	.7403
		10min	-.51333*	.14042	.011	-.8569	-.1697
	10min	Control	.91000*	.14042	.001	.5664	1.2536
		5min	.51333*	.14042	.011	.1697	.8569
soluble sugars (mgr/gr.Dw)	Control	5min	-2.46333*	.13477	.000	-2.7931	-2.1336
		10min	-1.49000*	.13477	.000	-1.8198	-1.1602
	5min	Control	2.46333*	.13477	.000	2.1336	2.7931
		10min	.97333*	.13477	.000	.6436	1.3031
	10min	Control	1.49000*	.13477	.000	1.1602	1.8198
		5min	-.97333*	.13477	.000	-1.3031	-.6436
MDA (nmol/gr.Fw)	Control	5min	2.86667*	.22309	.000	2.3208	3.4126
		10min	1.36333*	.22309	.001	.8174	1.9092
	5min	Control	-2.86667*	.22309	.000	-3.4126	-2.3208
		10min	-1.50333*	.22309	.001	-2.0492	-.9574
	10min	Control	-1.36333*	.22309	.001	-1.9092	-.8174
		5min	1.50333*	.22309	.001	.9574	2.0492
Peroxidase (IU/gr.Fw)	Control	5min	-.76000*	.08861	.000	-.9768	-.5432
		10min	-.32667*	.08861	.010	-.5435	-.1098
	5min	Control	.76000*	.08861	.000	.5432	.9768
		10min	.43333*	.08861	.003	.2165	.6502
	10min	Control	.32667*	.08861	.010	.1098	.5435
		5min	-.43333*	.08861	.003	-.6502	-.2165
CAT (IU/gr.Fw)	Control	5min	-.61000*	.06880	.000	-.7783	-.4417
		10min	-.43000*	.06880	.001	-.5983	-.2617
	5min	Control	.61000*	.06880	.000	.4417	.7783
		10min	.18000*	.06880	.040	.0117	.3483
	10min	Control	.43000*	.06880	.001	.2617	.5983
		5min	-.18000*	.06880	.040	-.3483	-.0117
SOD (IU/gr.Fw)	Control	5min	-.70333*	.06031	.000	-.8509	-.5558
		10min	-.26000*	.06031	.005	-.4076	-.1124
	5min	Control	.70333*	.06031	.000	.5558	.8509
		10min	.44333*	.06031	.000	.2958	.5909
	10min	Control	.26000*	.06031	.005	.1124	.4076
		5min	-.44333*	.06031	.000	-.5909	-.2958

*: the mean difference is significant at the 0.05 level

min plasma exposure. Accordingly, it can be concluded that the levels of enzymes were better in green cumin under 5 min plasma exposure, which can be attributed to higher cellular protein synthesis. The enzymes, however, dropped in cumin irradiated with plasma for 10 min, suggesting that a detrimental effect occurs due to the collision of more ions to cells over a longer period, thereby preventing better activity of ribosomes in the protein synthesis. Moreover, the highest phenol content in 10-minute plasma treatment indicates the presence of antioxidants to stabilize the two-layer phospholipid membrane.

According to the biochemical and physiological results obtained with three repetitions, the mean levels of antioxidant enzymes are reported presently.

Physiological activities of CAT in cumin were 1.13, 1.74, and 1.56 in the control, 5 min, and 10 min plasma treatments, respectively, indicating that the enzyme content was higher in cumin exposed to plasma for 5 minutes than those of the control and 10 min plasma exposure.

Physiological activities of SOD in cumin were 0.62, 1.33, and 0.88 in the control, 5 min plasma, and 10 min plasma treatments, respectively. Therefore, SOD level in cumin seeds was better following irradiation by plasma for 5 minutes than the other two treatments.

Physiological activities of 8.48, 5.62, and 7.12 were obtained for cumin seed MOD in the control, 5 min plasma, and 10 min plasma treatments, which was again more favorable in 5 min treatment.

For the physiological activity of POD, values of 0.93, 1.69, and 1.14 were recorded for the control, 5 min plasma, and 10 min plasma exposures, respectively. The concentrations of soluble sugars (mg/g DW) in cumin seeds calculated using standard glucose solutions were 5.11, 7.57, and 6.6, respectively, for the control, 5 min, and 10 min plasma irradiations, respectively. These imply that it can improve the activity of photosynthetic pigments, photosynthetic efficiency, and nitrate reductase activity.

The amount of the available phenol obtained in three repetitions was found as following: 3.55 (mg/g FW) in control specimens, 3.94 (mg / gr Fw) in 5-minute plasma treatment, and 4.46 (mg/g FW) in 10-minute plasma treatment, indicating a higher phenol content in the seeds treated under 10-minute plasma (Table 2).

Close examination of cross-sections of several plasma-treated buds did not reveal any damage to the primary bud within the compound buds (Fig. I). Majority of the plant body, such as the brain, the bulk of the stem and root skin, peripheral circle, leaf mesophyll, and fleshy parts of the fruit included parenchyma, and most of the plant tissues contained epidermis, metroplasia, parenchyma, and the cellulose drainage vessel (Fig I. b). The cross section of the stem and root shown in Figs. II and III also did not show any signs of damage.

Discussion

Plants exposed to high concentrations of metal ions in the soil, caused by industrial activities, can produce reactive oxygen radicals

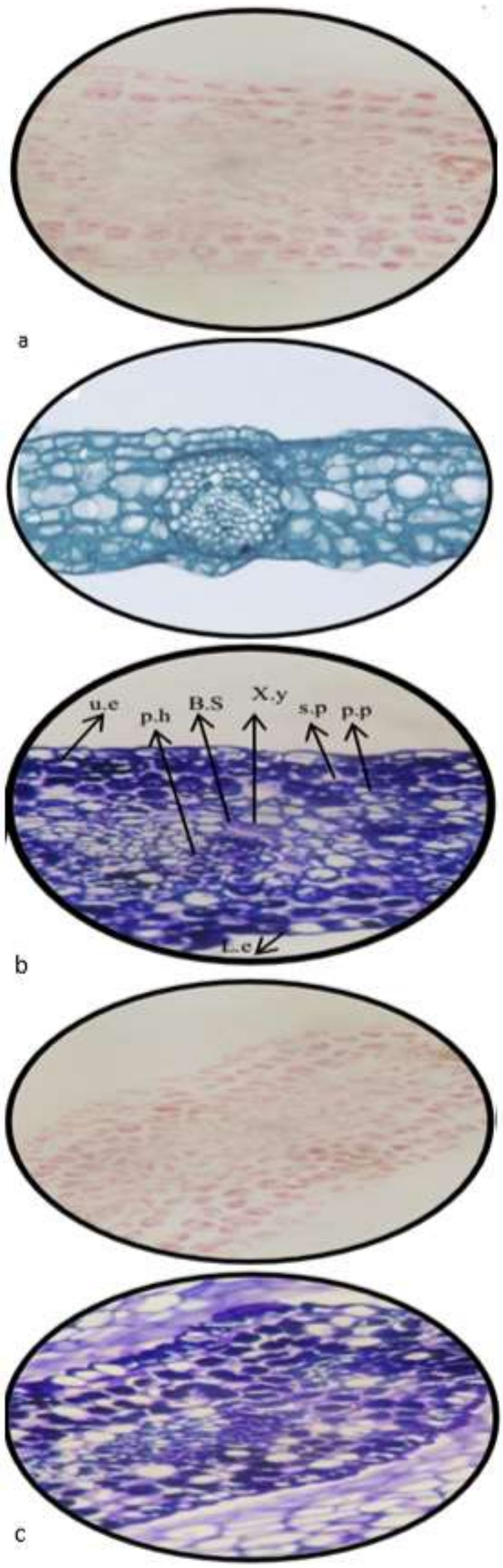
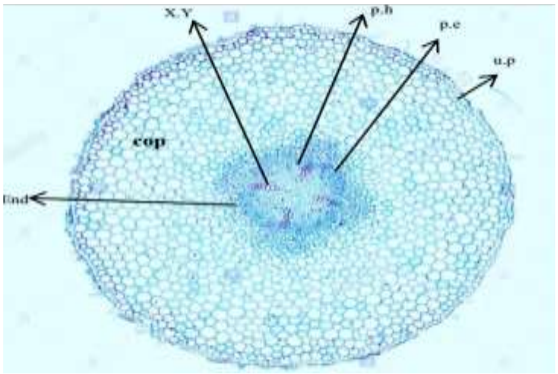
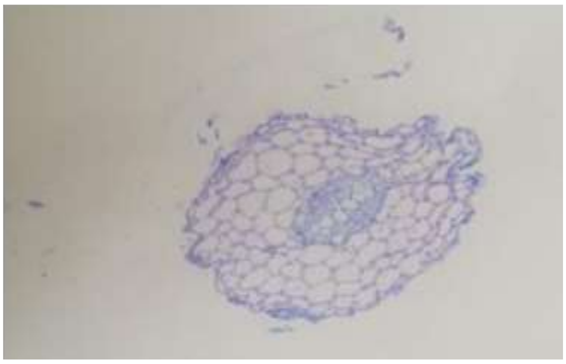


Fig. I. Cross section of green cumin leaf; a: control, b: 5 min, c: 10 min



a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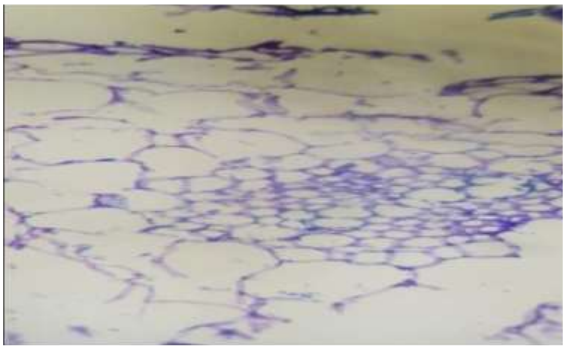
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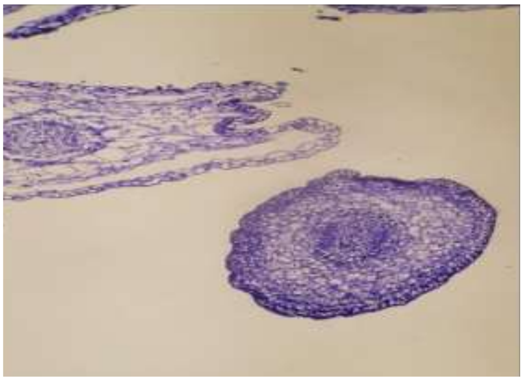
Fig. II. Cross section of green cumin root; a: control, b: 5 min, c: 10 min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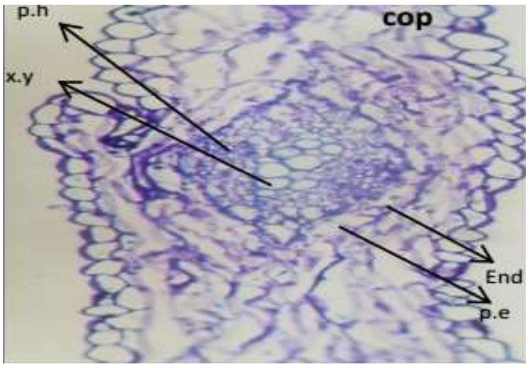

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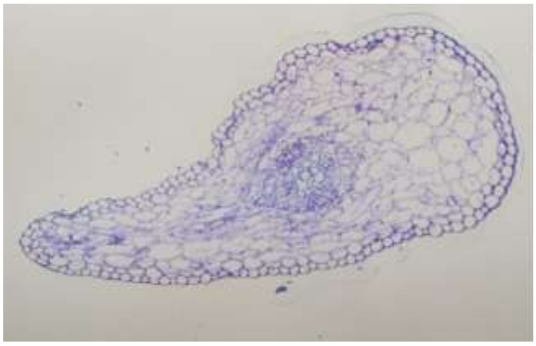
c

Fig. III. Cross section of green cumin stem; a: control, b: 5 min, c: 10 min

(ROS) such as -O^{2-}, O, and -OH. Studies have shown that ROS is seriously harmful to the cell due to oxidative damage to cellular structure and function. Plants are equipped with antioxidant systems to reduce oxidative damage, counteract pathogens, and prevent ROS production, among which such antioxidant enzymes as SOD, ascorbate peroxidase (APX), and CAT present more activity.

SOD is one of the major factors preventing O^{-2} extrusion, resulting in the formation of O_2 or H_2O_2 molecules. However, H_2O_2 is toxic to the cell and is excreted by the CAT or POD to O_2 or H_2O. Therefore, preserving the activity of antioxidant enzymes plays an important role in inhibiting ROS-induced metal ions.

One of the various treatments performed on green cumin was salinity stress in which the antioxidant and potential oil contents were investigated at different salt concentrations and the best yield was obtained at the highest salt concentration (Rebey et al., 2012). Cumin exposed to hormone pretreatment (gibberellin and quinine) had better yield at low concentrations for 24 hours than at higher concentrations for shorter periods.

Reduction of germination because of salinity and drought stress results in reductions in water uptake rate, hormone secretions, and enzyme activities, as well as negative effects of low osmotic potential on the biochemical processes of different germination stages, leading to the production of such compounds as antioxidants that play important roles in reduction of stress effects and better seedling growth (Bitarafan et al., 2019).

In this study, 5 min plasma exposure led to increased amounts of SOD, CAT, POX, and soluble sugars, but it decreased MDA levels, which resulted in a more favorable growth of green cumin seed in the control and 10 min plasma treatment. It can, therefore, be concluded that higher enzyme levels of cumin in 5 min plasma irradiation is because of extra cellular protein synthesis. The decreased levels of enzymes in plasma treatment for 10 min results from increased collision of ions to cells over a longer period, leading to a detrimental effect and inhibition of better ribosomal function in protein synthesis. Likewise, the best plasma impact time was found to be 5 minutes in okra.

Although an increase in ROS initially acts as a secondary messenger in the induction of resistance, their accumulation at high concentrations incurs extensive damages to plant tissue (Yadarar et al., 2015). Phenolic compounds are one of the major groups of chemicals that can be found in certain plants. Phenolic compounds are strong antioxidants, scavenging free radicals and performing as hydrogen donors, reducing agents, metal chelate, and single oxygen extinguishers. They act as quercetin which has been confirmed to be effective in stabilizing two-layer phospholipids against the peroxidation induced by reactive oxygen species (ROS).

Our results suggest that the increases in antioxidant enzymes (SOD, CAT, and POX) can neutralize these free oxygen radicals and minimize damage to the plant tissue.

The increase in germination rate probably results from the fact that the very thin lipid layer, which discharges seed water, is eliminated by the plasma impact and reduces the biopolymer chain length forming the seed, thereby increasing seed water uptake (Bormashenko et al., 2012).

Conclusion

Altogether, the results showed that the average growth of seeds was higher in 5 min plasma treatment than the control and 10 min plasma exposure. Moreover, the average physiological activities of catalase, peroxidase, superoxide dismutase, and soluble sugars were increased in plants from 5 min plasma treatment than the control and 10 min plasma irradiation.

References

Bharti, R., S. Kumar and **M. J. Parekh**.2018. 'Development of genomic simple sequence repeat (gSSR) markers in cumin and their application in diversity analyses and cross-transferability'. *Industrial Crops and Products, 111*, 158-164.

Bitarafan, F., M. Khodaeian, O. Tabatabaei-Malazy and **M. M. Amoli**.2019. 'Influence of antioxidants' gene variants on risk of diabetes mellitus and its complications: a systematic review'. *Minerva endocrinologica, 44*(3), 310- 325.

Bormashenko, E., R. Grynyov, Y. Bormashenko and **E. Drori**.2012. 'Cold radiofrequency plasma treatment modifies wettability and germination speed of plant seeds'. *Scientific reports, 2*(1), 1-8.

Chen, D., P. Chen, Y. Cheng, P. Peng, J. Liu, Y. Ma, Y., . . . and **R. Ruan**.2019. 'Deoxynivalenol decontamination in raw and germinating barley treated by plasma-activated water and intense pulsed light'. *Food and Bioprocess Technology, 12*(2), 246-254.

Chen, H. H., Y. K.Chen and **H. C. Chang**. 2012. 'Evaluation of physicochemical properties of plasma treated brown rice'. *Food Chemistry, 135*(1), 74-79.

Griesser, S. S., S. Prakas and **H. J. Griesser**. .2011. 'Plasma discharge treatment for improved germination of seeds and killing of fungal spores on seed coats'. *Final project report to the Australian Flora Foundation. Ian Wark Research Institute, University of South Australia, Mawson Lakes, SA, 5095*.

Haghirossadat, F., F. Bernard, M. Kalantar, M. Sheikhha, F. Hokmollahi, M. Azimzadeh and **M. Hoori.** 2010. '*Bunium persicum* (Black

Caraway) of Yazd province: chemical assessment and Evaluation of its antioxidant effects'. *SSU_Journals, 18*(3), 284-291.

Hayyan, M., M. A. Hashim and **I. M. AlNashef** .2016. 'Superoxide ion: generation and chemical implications'. *Chemical reviews, 116*(5), 3029-3085.

Hou, P., Z. Xie, L. Zhang, Z. Song, J. Mi, Y. He and **Y. Li**. 2011. 'Comparison of three different methods for total RNA extraction from *Fritillaria unibracteata*, a rare Chinese medicinal plant'. *Journal of Medicinal Plants Research, 5*(13), 2835-2839.

Jiafeng, J., H. Xin, L. Ling, L. Jiangang, S. Hanliang, X. Qilai, . . . and **D. Yuanhua**.2014. 'Effect of cold plasma treatment on seed germination and growth of wheat'. *Plasma Science and Technology, 16*(1), 54-60.

Kanani, P., Y. M. Shukla, A. R. Modi, N. Subhash and **S. Kumar**.2019. 'Standardization of an efficient protocol for isolation of RNA from Cuminum cyminum'. *Journal of King Saud University-Science, 31*(4), 1202-1207.

Kumar, R., A. K. Thakur, A. Vikram, A. Vaid and **R. Rane**. 2019. 'Effect of cold plasma treatment of seeds on quality of seed crop of Okra'. *International Journal of Economic Plants, 6*(2), 73-77.

Ling, L.,J. Jiafeng. L. Jiangang,S. Minchong, H. Xin, S. Hanliang and **D. Yuanhua**.2014. 'Effects of cold plasma treatment on seed germination and seedling growth of soybean'. *Scientific reports: 4*, 5859.

Randeniya, L. K. and **G. J. de Groot**. 2015. 'Non-thermal plasma treatment of agricultural seeds for stimulation of germination, removal of surface contamination and other

Saberi, M., M. Sanavy, R. Zare and **H. Ghomi**.2019. 'Improvement of photosynthesis and photosynthetic productivity of winter wheat by cold plasma treatment under haze condition'. *Journal of Agricultural Science and Technology, 21*(7), 1889-1904.

Scholtz, V., B. Šerá, J. Khun, M. Šerý and **J. Julák**.2019.' Effects of nonthermal plasma on wheat grains and products'. *Journal of Food Quality, 2019*. |Article ID 7917825 | https://doi.org/10.1155/2019/7917825

Sera, B., P. Spatenka, M. Šerý, N. Vrchotova and I. Hruskova.2010 . 'Influence of plasma treatment on wheat and oat germination and early growth'. *IEEE Transactions on Plasma Science, 38*(10), 2963-2968.

Sivachandiran, L. and **A. Khacef.**2017. 'Enhanced seed germination and plant growth by atmospheric pressure cold air plasma: combined effect of seed and water treatment'. *RSC advances, 7*(4), 1822-1832.