

Effect of Pretreatment of Seed with Cold Plasma on Some Physiological and Quality Traits of Sunflower (*Helianthus annuus* L.) Plant in Completion with Weeds [†]

Mojgan Khakian ¹, Hassan Makarian ^{2,*}, Mehdi Baradaran Firouz Abadi ², Hossein Mirzaei Moghadam ², Mehdi Momeni ³ and Maziar Farzinbeh ¹

¹ M.Sc. Student, Department of Agronomy and Plant Breeding, Faculty of Agriculture, Shahrood University of Technology, Shahrood, Iran; mojgankhakian@yahoo.com (M.K.); email1@com.cn (M.F.)

² Faculty of Agriculture, Shahrood University of Technology, Shahrood, Iran; email2@com.cn (M.B.F.A.); email3@com.cn (H.M.M.)

³ Assistant Prof., School of Physics and Nuclear Engineering, Shahrood University of Technology, Shahrood, Iran; mehdy_momeny@yahoo.com

* Correspondence: h.makarian@yahoo.com

[†] Presented at the 1st International Online Conference on Agriculture—Advances in Agricultural Science and Technology (IOCAG2022), 10–25 February 2022; Available online: <https://iocag2022.sciforum.net/>.

Abstract: In order to improve competitiveness of crops with weeds an experiment was carried at Shahrood University of Technology in 2017. Experimental factors included cold plasma at six levels: control, hydro-priming of seeds for 10 h, pretreatment of seeds with cold plasma radiation for 15 and 30 s, hydro-priming of seeds for 10 h + cold plasma radiation for 15 and 30 s and weed control at three levels: control (no weeding), weeding all season and application of trifluralin (1200 g. ai. ha⁻¹). Dielectric barrier discharge plasma jet was operated in ambient air under sterile conditions. Results showed that membrane stability index and carotenoid increased by cold plasma and hydro-priming treatments in weed free than weeds infested conditions. Total chlorophyll content increased by 8.87% and 7.74% in weeding and herbicide application, respectively, compared with no weeding treatments. Sunflower seeds protein percentage increased significantly by application of hydro-priming + cold plasma radiation for 30 s compared with herbicide application treatment. Sunflower seed oil percent also increased by using cold plasma radiation for 15 and 30 s in weed infested treatments compared with weed free and herbicide application condition. Based on our results, pre-treatment of seeds by cold plasma and hydro-priming could significantly improve some physiological and quality traits of sunflower through increasing of the crop competitive ability with weeds.

Keywords: germination; integrated weed management

Citation: Khakian, M.; Makarian, H.; Abadi, M.B.F.; Moghadam, H.M.; Momeni, M.; Farzinbeh, M. Effect of Pretreatment of Seed with Cold Plasma on Some Physiological and Quality Traits of Sunflower (*Helianthus annuus* L.) Plant in Completion with Weeds. *2022*, *4*, x. <https://doi.org/10.3390/xxxxx>

Academic Editor:

Published: date

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Sunflower (*Helianthus annuus* L.) is the fifth most important plant in the world for edible oil production and its oil plays an important role in nutrition due to its special properties such as high oxidation stability and presence of dual bond fatty acids such as linoleic acid (Moschner and Biskupek-Korell 2006). Sunflower in the early growing season has little competitive potential with weeds and its yield significantly reduce by weeds, so it must be fully protected against weeds (Knezevic et al., 2013). Weeds are the most important limiting factor in agricultural systems and if they are not fully controlled in the fields, they will reduce crop yield by 10 to 100% depending on the competitive ability of weeds (Auskarniene et al., 2010). Today, the use of herbicides is the main method for controlling weeds, but due to the high price of these chemicals and environmental concerns,

the agricultural community has tended to use non-chemical management methods to control weeds. (Hiltbrunner et al., 2007). One of the practical management methods to increase crop growth and competitiveness with weeds is the use of priming and other relevant methods which improve seed vigor and seedling establishment in the field (Basra et al., 2004). Hydropriming is one of the seed pre-treatment methods to enhance seed vigor and establishment, which involves soaking the seed in water for a certain period of time before planting (Santini et al., 2017). It seems that, accelerated germination of pretreatment seeds may be due to increased activity of degrading enzymes such as alpha-amylase, increased level of bioenergetic charge in the form of increased numbers of mitochondrial function, increased DNA, RNA and ATP synthesis (Afzal et al., 2002). In a study, hydroprimed soybean seeds significantly increased 1000-grain weight, grain yield and protein percentage compared to control (Bayat & Sepehry 2010). Suitable establishment of plants affected by seed pre-treatment methods has so far the seed germination been effective in reducing weed damages.

Plasma technology has been used in agricultural researches in recent years and its positive effect on seed germination has been proved (Thirumdas, 2018). Cold plasma can enhance plant physiological processes such as dehydrogenase activity, superoxide dismutase, peroxidase activity, photosynthetic pigments, photosynthetic efficiency, and nitrate reductase activities (Mildažienė et al., 2019). The positive effect of cold plasma on seed germination properties of plants such as *Chenopodium album* (Sera et al., 2010) and soybean (Ling et al., 2014) has been previously reported. Hussain et al. (2017) using gamma irradiation on sunflower seeds reported that this technique can increase germination, growth and subsequently improved sunflower yield. According to the results of the present study, it seems that hydropriming and plasma irradiation can improve the competitiveness of sunflower against weeds by improving seedling establishment. Therefore, this study was conducted to investigate the effect of cold plasma seed pre-treatments on the growth and yield of sunflower in the presence of weeds.

2. Materials and Methods

This study was arranged as a factorial experiment in a Randomized Complete Block Design (RCBD) with three replications at research farm of Shahrood University of Technology, Shahrood, Iran on Haysan-33 cultivar of sunflower plant during 2017 growth season. The farm is geographically located at 55°4' east longitude and 36°29' north latitude with an average elevation of 1420 m above sea level. The first factor consists of cold plasma at six levels: control (no pretreatment), seed hydroprime with distilled water for 10 h, seed pretreatment with plasma irradiation for 15 s, seed pretreatment with plasma irradiation for 30 s, seed hydroprime for 10 h + plasma irradiation for 15 s, Hydroprim seeds for 10 h + plasma irradiation for 30 s (plasma discharge power was 80 W). The second factor was weed control at three levels: Trifluralin 2500 mL (1200 g ai ha⁻¹), control (no-weeding), and full-season weeding. The field was fallowed in the year before the test, so, after a deep plowing in the autumn of the previous year, the field preparation practices were conducted in favor of weather conditions in early June 2016. Initially, the field was tilled by a moldboard plow. Then, using two disks the field leveling was performed. The sowing was performed in the second half of June 2017. The experimental field consisted of 54 plots and each plot consist of four planting lines, 14 m length with a distance between rows of 60 cm and on rows 25 cm. The soil texture of the experimental field was clay loam. Weed control was applied by hand in plots should be treated with weeding. Pre-treatment with cold plasma seeds was performed in the plasma laboratory of Shahrood University of Technology. The seeds were treated using argon gas by cold plasma devise (Plasma Supply Model BK 9401, Made in Iran). Cold plasma radiation consists of a stream of electrons and protons produced in a small flame by the device described above. Treflan 48% EC herbicide at a dose of 1200 g. ai. ha⁻¹ was sprayed using Spanish-made Matabee sprayer with a volume of 280.27 L of water per hectare and a pressure of 2.8 bar on the plots. Then, immediately mixed with soil to a depth of 5 to 10 cm. The chlorophyll content of each

plant was measured using a chlorophyll meter (SPAD-502, Minolta Konica). In each layer of canopy, the chlorophyll content was measured for three leaves and its mean was considered as the SPAD number of that layer. Photosynthetic pigments were also obtained by the Arnon method (1949). The amount of carotenoids and total chlorophyll was calculated using Equations (1) and (2).

$$\text{Carotenoid} = 100 (A_{470}) - 3.27 (\text{mg chl. a}) - 104(\text{mg chl b})/227 \quad (1)$$

$$\text{Total Chlorophyll} = \text{Chlorophyll a} + \text{Chlorophyll b} \quad (2)$$

The percentage of grain protein was also measured by Kjeldahl method.

Plasma membrane stability was calculated from Equation (3).

$$\text{MSI} = 1 - (C_1/C_2) \times 100 \quad (3)$$

They transferred to the laboratory and after drying in oven for 48 h at 70 °C, weighed with a precise scale. The percentage of oil in the oil was measured using the device and Soxhlet method. Statistical analysis was performed using MSTAT-C software (1.42) and graphs were extracted using Excel software and LSD test at 5% level was used to compare the means.

3. Results

3.1. SPAD Number

Results showed that leaf chlorophyll content (SPAD number) significantly affected by control treatments at the level of 5% (Table 1). But the simple effect of cold plasma and the interaction of cold plasma × control treatments on this trait were not significant.

SPAD number (leaf chlorophyll content) in the presence of weeds showed a significant decrease of 4.85 and 4.34% compared to weeding and herbicide application respectively (Figure 1).

Table 2. Analysis of variance (mean squares) Effect of treatments on some sunflower traits.

Sources of change	DF	SPAD	Carotenoid	Total chlorophyll	Membrane Stability	Protein	Oil
Repeat	2	6.191 ^{ns}	0.008 [*]	1.453 ^{ns}	284.708 [*]	1.954 ^{ns}	20.590 ^{**}
Control	2	21.636 [*]	0.064 ^{**}	2.345 [*]	1741.945 ^{**}	122.817 [*]	21.011 ^{**}
The plasma	5	2.30 ^{ns}	0.039 ^{**}	0.827 ^{ns}	257.304 ^{**}	66.658 ^{ns}	38.876 ^{**}
plasma × Control	10	7.242 ^{ns}	0.028 ^{**}	0.653 ^{ns}	430.425 ^{**}	121.183 ^{**}	32.942 ^{**}
Error	34	5.367	0.002	0.669	59.071	32.942	2.617
CV%		5.75	12.83	11.27	16.75	18.13	3.77

ns: non-significant, *, **: significant in $\alpha = 0.05$ and 0.01 respectively.

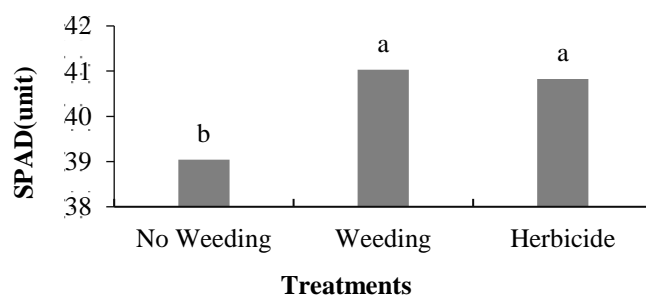


Figure 1. Effect of control treatments on leaf chlorophyll content (SPAD).

3.2. Carotenoid

The results showed that the simple and interaction effects of cold plasma and weed control treatments on carotenoids were significant at the level of 1% probability. The amount of carotenoids in weeding conditions showed a significant increase compared to non-weeding conditions (Figure 2). Pretreatment of seeds by cold plasma for 15 and 30 s increased leaf carotenoids compared to hydro-priming treatment.

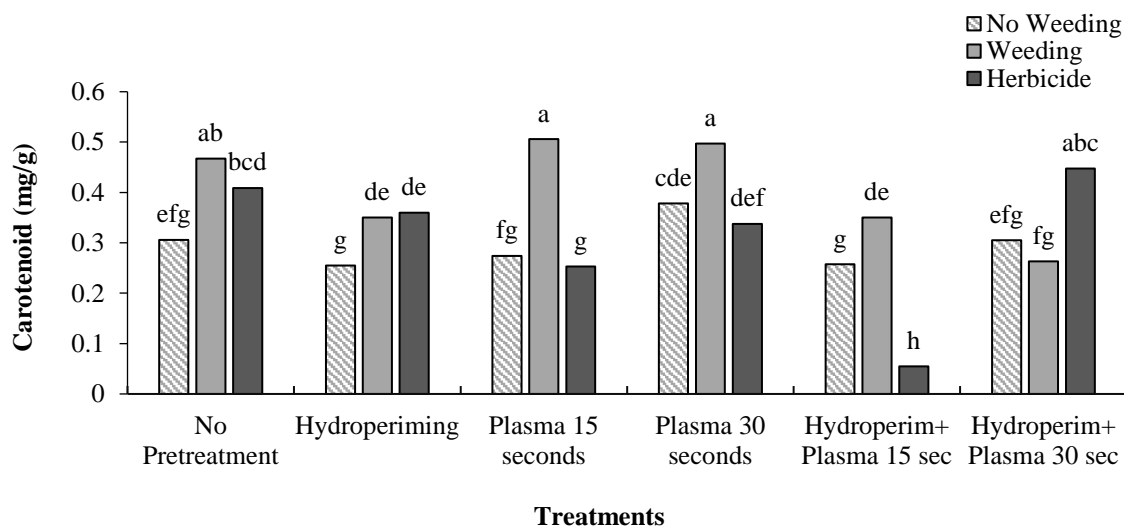


Figure 2. Interaction effects of treatments on the level of carotenoids.

3.3. Total Chlorophyll

The results of analysis of variance showed that only the simple effect of control treatments on total chlorophyll were significant at 5% level (Table 1). Results showed that total chlorophyll decreased by 8.87 and 7.74% in the conditions of no weeding compared to weeding and herbicide application respectively (Figure 3).

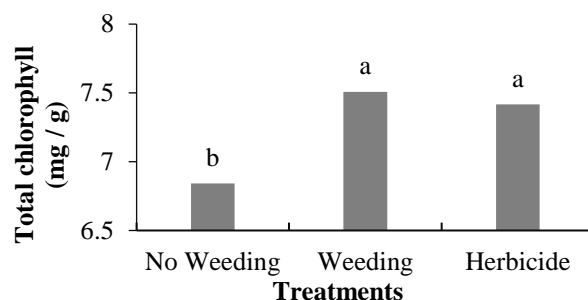


Figure 3. Effect of control treatments on total chlorophyll content.

3.4. Membrane Stability Index

Simple effects of weed control and plasma treatments and bilateral effects of treatments significantly (at the level of 1% probability) affected membrane stability index (Table 1). Membrane stability in priming treatments along with plasma radiation levels in the presence of weeds was significantly higher than hydroprime, control and plasma alone treatment in the presence of weeds (Figure 4).

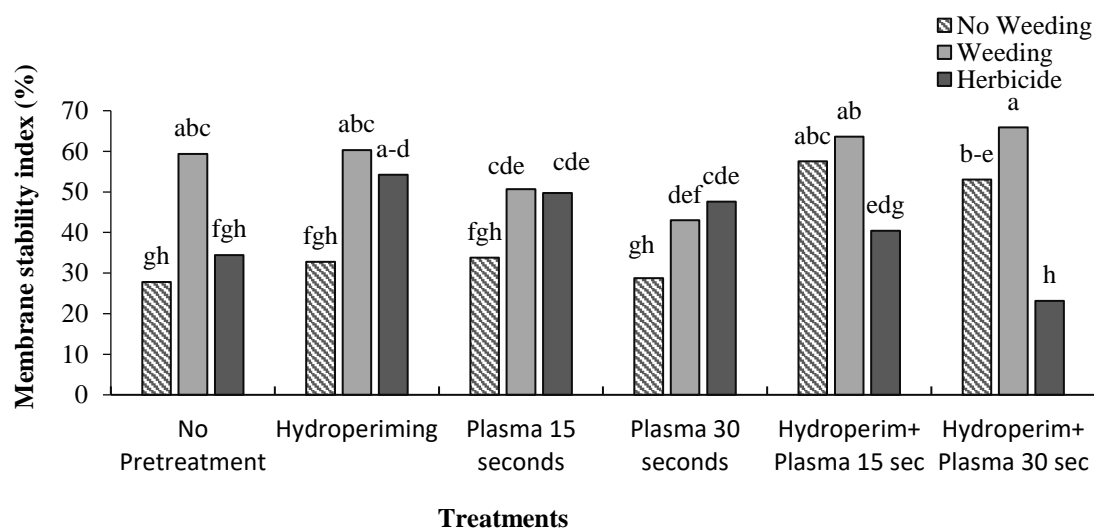


Figure 4. Interaction effects of treatments on the membrane stability index.

3.5. Percentage of Protein

The simple effect of control and bilateral effects of treatments at the 5% level was effective on the protein percentage. In case of plasma application for 30 s alone or with priming protein percentage increased significantly in the presence of weeds (Figure 5).

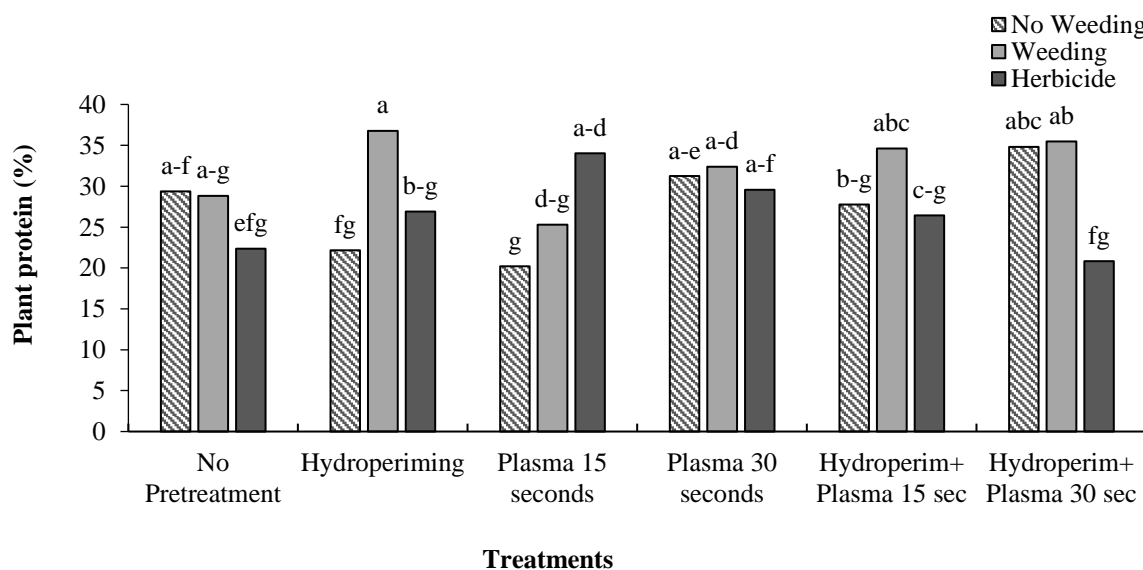


Figure 5. Interaction effects of treatments on soybean protein.

3.6. Percentage of Oil

As shown in Table 1, the simple effects of control and plasma on oil percentage were effective ($p \leq 0.01$). Also, the interaction effect of treatments on the soybean oil was significant ($p \leq 0.01$). Planting hydro-primed seeds under weeding produced the highest percentage of oil, although this treatment didn't have any significant difference than cold plasma exposure time for 15 s in non-weeding condition but showed an increase of 7.6% compared to the weed-infected control (no pretreatment).

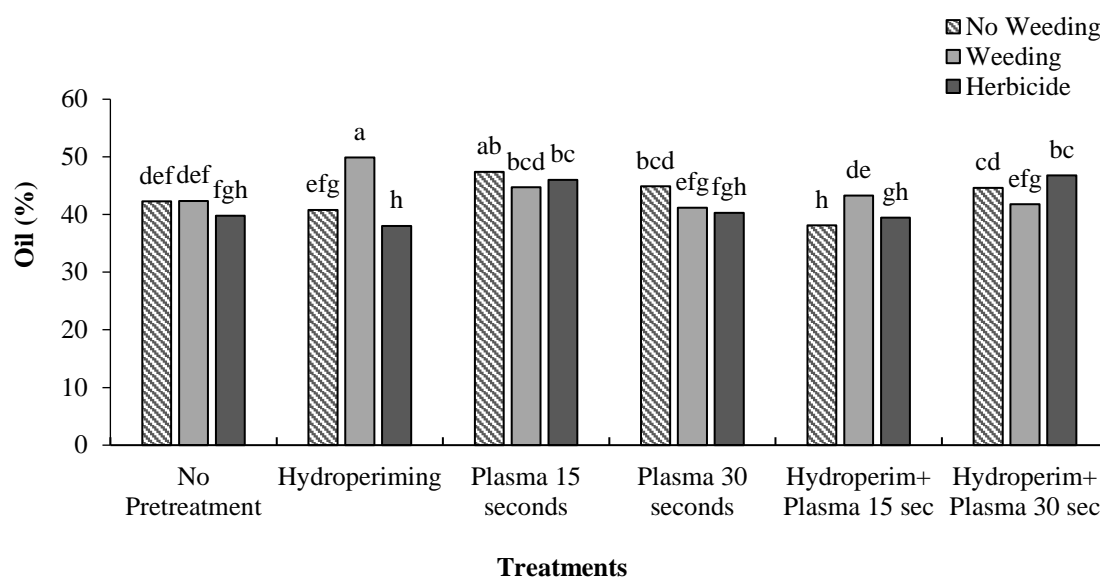


Figure 6. Interaction effects of t treatments on soybean oil.

4. Discussion

Based on our results SPAD value decreased in weedy treatments (Figure 1). SPAD value gradually increased in response to increasing weed-free period as compared with the weedy control. The SPAD value can be regarded as a suitable criterion in soybean because it represents nutritional conditions (Shafagh-Kolvanagh et al., 2008). The direct relationship between the SPAD number and nutrients show that weeds reduce sunflower pigments by consuming soil nutrients. Chlorophyll content in plants depends on soil N availability and crop N uptake, which are important management factors in arable farming. Crop N uptake is important, as N is needed for chlorophyll formation, which is important for photosynthesis, i.e., the conversion of absorbed radiance into plant biomass (Jongschaap, and Booij, 2004). In our study, membrane stability index decreased by weeds but cold plasma + priming increased this index significantly than control treatment (Figure 4). It has been reported that biotic and abiotic stress can decrease membrane stability index (Mamnabi et al., 2017). It seems that exposure of hydro-primed seeds on cold plasma can through improving seed germination and plant growth can enhance membrane stability index. Protein percentage increased by exposure of seed to cold plasma for 30 s in weedy condition. Similar to protein percentage, interaction effects of treatments had a significant effect on seed oil.

Most studies on cold plasma and seed germination have been under laboratory conditions, but field-based investigations have also been reported. Filatova et al. [14] observed a 10–20% increase in the field germination capacity of honey clover, and catgut seeds with cold plasma treatment, whereas Ahn et al. [44] did not observe any changes in the germination percentage. The mechanisms of improved seed germination by plasma have been thoroughly investigated. The most frequently reported factors are changes in the physical and chemical properties of the seed coat or surface. Physical and chemical changes to the seed surface can result in elevated hydrophobicity and water permeability that enhances water imbibition, which is required for seed germination. Increased hydrophobicity and water permeability of the seed surface after plasma treatment has been observed (Wang et al., 2017).

In conclusion, application of cold plasma and hydro priming on sunflower seeds can improve seed germination and some physiological traits of sunflower plants that are effective on plant growth and competitive ability with weeds.

Institutional Review Board Statement:

Informed Consent Statement:**Data Availability Statement:****Conflicts of Interest:** The authors declare no conflict of interest.**References**

1. Afzal, I.; Ahmad, N.; Basra, S.M.A.; Ahmad, R.; Iqbal, A. Effect of different seed vigour enhancement techniques on hybrid maize (*Zea mays* L.). *Pak. J. Agri. Sci* **2002**, *39*, 109–112.
2. Ahn, C.; Gill, J.; Ruzic, D.N. Growth of plasma-treated corn seeds under realistic conditions. *Sci. Rep.* **2019**, *9*, 4355.
3. Auskarniene, O.; Psibisauskiene, G.; Auskalnis, A.; kadzys, A. Cultivar and plant density influence on weediness in spring barely crops. *Zemdirb. Agric.* **2010**, *97*, 53–60.
4. B.A.; Rojas-Aréchiga, M.; García Morales, E. Priming effect on seed germination: Is it always positive for cacti species? *J. Arid Environ.* **2017**, *147*, 155–158.
5. Basra, S.; Ashraf, M.; Iqbal, N.; Khaliq, A.; Ahmad, R. hysiological and biochemical aspects of presowing heat stress on cotton-seed. *Seed Sci. Technol.* **2004**, *32*, 765–774.
6. Bayat, S.; Sepehry, A. Paclobutrazol and salicylic acid application ameliorates the negative effect of water stress on growth and yield of maize plants. *Int. J. Res. Agric. Sci.* **2012**, *8*, 127–139. (In Persian with English abstract).
7. Filatova, I.I.; Azharonok, V.V.; Kadyrov, M.A.; Beljavsky, V.; Gvozdo, A.; Shik, A.; Antonuk, A.E.; Belarus, N. The effect of plasma treatment of seeds of some grain and legumes on their sowing quality and productivity. *Rom. Rep. Phys.* **2011**, *56*, 139–143.
8. Hiltbrunner, J.; Jeanneret, P.; Liedgens, M.; Stamp, P.; Streit, B. Response of weed communities to legume living mulches in winter wheat. *J. Agron Crop. Sci.* **2007**, *193*, 93–102.
9. Hussain, F.; Iqbal, M.; Shah, S.Z.; Qamar, M.A.; Bokhari, T.H.; Abbas, M.; Younus, M. Sunflower germination and growth behavior under various gamma radiation absorbed doses. *Acta Ecol. Sin.* **2017**, *37*, 48–52.
10. Jongschaap, R.E.E.; Booij, R. Spectral measurements at different spatial scales in potato: Relating leaf, plant and canopy nitrogen status. *Int. J. Appl. Earth Obs. Geoinf.* **2004**, *5*, 205–218.
11. Knezevic, S.; Elezovic, Z.I.; Datta, A.; Vrbnicanin, S.; Glamoclija, D.; Simic, M.; Malidza, Goran. Delay in the critical time for weed removal in imidazolinone-resistant sunflower (*Helianthus annuus*) caused by application of pre-emergence herbicide. *Int. J. Pest. Manag.* **2013**, *59*, 229–235, doi:10.1080/09670874.2013.830797.
12. Ling, L.; Jiafeng, J.; Jiangang, L.; Minchong, S.; Xin, H.; Hanliang, S.; Yuanhua, D. Effects of cold plasma treatment on seed germination and seedling growth of soybean. *Sci. Rep.* **2014**, *4*, 5859.
13. Mamnabi, S.; Nasrollahzadeh, S.; Ghassemi-Golezani, K.; Raei, Y. Improving yield-related physiological characteristics of spring rapeseed by integrated fertilizer management under water deficit conditions. *Saudi J. Biol. Sci.* **2020**, *27*, 797–804.
14. Mildažienė, V.; Aleknavičiūtė, V.; Žūkienė, R.; Paužaitė, G.; Naučienė, Z.; Filatova, I.; Lyushkevich, V.; Haimi, P.; Tamošiūnė, I.; Baniulis, D. Treatment of common sunflower (*Helianthus annuus* L.) seeds with radio-frequency electromagnetic field and cold plasma induces changes in seed phytohormone balance, seedling development and leaf protein expression. *Sci. Rep.* **2019**, *9*, 6437.
15. Moschner, C.R.; Biskupek-Korell, B. Estimating the content of free fatty acids in high-oleic sunflower seeds by near- infrared spectroscopy. *Eur. J. Lipid Sci. Technol.* **2006**, *108*, 606–613.
16. Santini.
17. Sera, B.; Spatenka, P.; Sery, M.; Vrchotova, N.; Hruskova, I. Influence of plasma treatment on wheat and oat germination and early growth. *IEEE Trans. Plasma Sci.* **2010**, *38*, 2963–2968.
18. Sera, B.; Spatenka, P.; Sery, M.; Vrchotova, N.; Hruskova, I. Influence of plasma treatment on wheat and oat germination and early growth. *IEEE Trans. Plasma Sci.* **2010**, *38*, 2963–2968.
19. Shafagh-Kolvanagh, J.; Zehtab-Salmasi, S.; Javanshir, A.; Moghaddam, M.; Dabbagh Mohammady Nasab, A. Effects of nitrogen and duration of weed interference on grain yield and SPAD (chlorophyll) value of soybean (*Glycine max* (L.) Merrill.). *J. Food Agric. Environ.* **2008**, *6*.
20. Thirumdas, R. Exploitation of cold plasma technology for enhancement of seed germination. *J. Agric. Sci. Technol* **2018**, *13*, 1-5.
21. Wang, X.-Q.; Zhou, R.-W.; de Groot, G.; Bazaka, K.; Murphy, A.B.; Ostrikov, K. Spectral characteristics of cotton seeds treated by a dielectric barrier discharge plasma. *Sci. Rep.* **2017**, *7*, 5601.
22. Wang, X.-Q.; Zhou, R.-W.; de Groot, G.; Bazaka, K.; Murphy, A.B.; Ostrikov, K. Spectral characteristics of cotton seeds treated by a dielectric barrier discharge plasma. *Sci. Rep.* **2017**, *7*, 5601.