

Effect of physical energy on germination and seedling vigor of alfalfa seeds (*Medicago sativa* L.)

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Abstract: Recently, non-thermal technologies have emerged as a means to ensure the safety of agricultural products while also promoting plant growth and reducing pathogenic and chemical contamination of seeds. An experiment was conducted to investigate the effect of various treatments on the germination characteristics of alfalfa seeds. The experiment utilised a completely randomised design with five treatments and three replications, including cold plasma exposure, direct current (DC) electromagnetic field, magnetic field, and a combination of plasma exposure with magnetic and electromagnetic fields. The treated seeds were compared to the control seeds (without exposure) in terms of seedling length, germination rate index (GRI), vigor index, and seed germination. The results indicated that cold plasma treatment and a combination of plasma and magnetic field treatment significantly increased the germination rate compared to the control and other treatments. Furthermore, the combined treatment of plasma and electromagnetic fields, as well as the individual treatment with magnetic fields, resulted in a significant increase in root length and, consequently, the allometric coefficient. Non-thermal technologies are a promising approach to enhancing seed performance, particularly in terms of the rate of germination and seedling length.

Keywords: early growth; electromagnetic field exposure; magnetic field exposure; plasma exposure

A successful agricultural system relies on the production of suitable and high-quality seeds. This is because seeds are the starting point of the production cycle in agricultural and horticultural systems. To have a thriving and sustainable seed industry, it is essential to conduct regular and precise studies that align with long-term goals. This will enable us to make significant progress in this field (Ghaderi-Far et al. 2014). Seed is the most important part of the plant that plays an essential role in the regeneration, preservation, and transfer of plant genetic

material. Seed germination is a vital step in the plant life cycle and growth. The seeds must grow rapidly to maximize their potential yield. One of the main causes of reduced seed germination of different plants is often due to contact of the seed surface with various sources of contamination, including bacterial contamination of soil, microorganisms, and fungi (Baskin and Baskin 2003; Morison et al. 2008).

Non-thermal technologies are designed to ensure the safety of agricultural products and longer shelf life (Ebadi et al. 2009). At the same time, it does not

hurt their quality characteristics (Černák et al. 2009). Cold plasma technology is a new non-thermal technology for processing agricultural products that can inactivate pathogens at room temperature, while minimising damage to heat-sensitive compounds, increasing the shelf life of agricultural products (Ebadi et al. 2019). Recently, innovative non-thermal plasma (NTP) technology has attracted much attention in agriculture as an alternative to stimulate plant growth and reduce pathogenic and chemical contamination of seeds (Gholami et al. 2016).

The first attempts to improve seed quality began with the application of magnetic and electromagnetic fields in the 1930s. Many scientists have shown increased seed vigor and germination of different species by treatment with electromagnetic and magnetic fields. Vashisth and Nagarajan (2008), Reported that the magnetic field increased the vigor of chickpea seeds by 46–71%, improved the seed length of seedlings by 58–90%, and the dry weight of seedlings by 25–47%. Also, Aladjadian (2002) showed that applying a magnetic field of 0.15 tesla strongly increases germination, fresh weight, stem length, and stem development of the maize seeds.

The objective of this study was to examine the potential impact of physical energy exposure on the germination and seedling vigour characteristics of alfalfa seeds.

MATERIALS AND METHODS

This study was done in the post-harvest laboratory of the Iranian Research Organization for Science and Technology to investigate the effect of vacuum plasma, electromagnetic field, magnetic field, and their combined treatments on the germination of alfalfa seeds (*Medicago sativa* L.), which prepared from a local store. To perform this experiment, alfalfa seeds were first washed with water for 30 minutes. Then they were disinfected with 1% sodium hypochlorite solution for 3 min and washed well with distilled water three times for 2, 3, and 5 minutes. Then, under the laminar hood, with the help of pence, 20 seeds were placed in each petri dish on a layer of Whatman No. 1 filter paper and 5 mL of water was added to them. To disinfect the petri dishes, they were exposed to UV rays for 30 min before placing the seeds in them. Pretests were performed on seed samples to obtain the optimal time and intensity of cold plasma, electromagnetic field, and magnetic field. Finally, the optimal treat-

ments include applying cold plasma to the seed under vacuum conditions with a pressure of 15 PSI and duty cycle 16 for 30 s, applying direct current electromagnetic field treatment (dc) with 10 m tesla intensity for 80 min (Figure 4), and applying magnetic field treatment by two strong magnets with a field intensity of 200 m tesla for 70 minutes. In addition, for combined treatments, first plasma treatment and then electromagnetic field and magnetic field treatments were applied to the seeds. During the experiment, the number of germinated seeds was counted daily. The criterion for seed germination was root germination of at least 3 mm. At the end of the 21st day (three weeks), germination percentage, germination rate, vigor index, root length, stem length, and seedling weight were measured. Figure 1 shows the different components that were used to apply the cold plasma to the seed under vacuum conditions.

Alfalfa seeds were placed in a plasma chamber on an aluminum support as shown in Figure 2. As shown in Figure 3, the rate of seedling growth during 21 days at plasma treatment and control, shows a significant difference.

Germination percentage (GP) is approximate of the feasibility of a population of seeds that are germinated (Singh et al. 2019), and calculated as:



Figure 1. Vacuum plasma setup used in the experiment
1 – vacuum pump; 2 – plasma chamber; 3 – high voltage generator



Figure 2. Seed placement location in the vacuum plasma chamber
arrow shows embedded location for seeds

$$GP = \frac{N}{N_1} \times 100 \quad (1)$$

where: N – the number of germinated seeds; N_1 – the number of seeds used.

Germination rate (GR) is the number of seeds germinated as per the total number of seeds planted from the day of germination and calculated as:

$$GR = \sum_{i=1}^d \frac{N_i}{D_i} \quad (2)$$

where: N_i – the number of germinated seeds per count; D_i – the number of days; d – the number of days that have been counted (Maguire 1962).

The seedling length was defined as the sum of the root and shoot length. Seedling vigor was calculated following as (Kumar et al. 2005):

$$VI = GP \times \text{seedling length (root + shoot)} \quad (3)$$

Allometric coefficient (AC) or seedling growth rate calculated by obtaining the ratio of root length to stem length (Hassan Nouriyani 2019):

$$AC = \frac{RL}{SL} \quad (4)$$

where: RL – the root length; SL – the shoot length.

At the end of the test, the length of the stem and the root were measured. To measure these indices, 10 seedlings of each treatment were randomly selected and the length of their roots and stems were measured and recorded. The obtained results were statistically analysed using SPSS statistical software (version 25) and Excel software (version 2018) was used to draw the relevant graphs. One-way ANOVA at a 95 % confidence level was done to analyse the level of significance. Also, the mean comparison test was performed using the Duncan test at a 5% probability level.

Table 1 defines the treatments that used in this research.

Table 1. Definition of single and combined treatments used in this study

Treatments	Description
Control	–
EL+P	Direct current electromagnetic field treatment (dc) with 10 m tesla intensity for 80 min+ cold plasma under vacuum conditions with a pressure of 15 PSI and duty cycle 16 for 30 second.
MG	Magnetic field with an intensity of 200 m tesla for 70 minutes.
EL	Direct current electromagnetic field treatment (dc) with 10 m tesla intensity for 80 minutes.
P	Cold plasma under vacuum conditions with a pressure of 15 PSI and duty cycle 16 for 30 second.
MG+P	Magnetic field with an intensity of 200 m tesla for 70 min + cold plasma under vacuum conditions with a pressure of 15 PSI and duty cycle 16 for 30 second.

EL – electromagnetic field treatment; P – vacuum cold plasma treatment; MG – magnetic field treatment

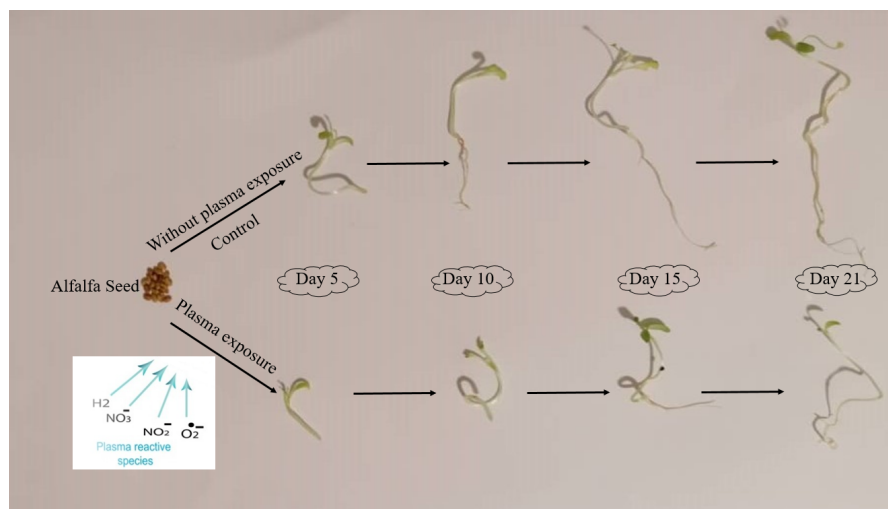


Figure 3. Seedling growth under different conditions



Figure 4. Electromagnetic setup used in the experiment
1 – Tesla meter; 2 – embedded location for seed; 3 – variac;
4 – Helmholtz coil

RESULTS AND DISCUSSION

Germination rate. The results of statistical analysis showed that cold plasma treatment had a significant effect on germination rate ($P < 0.05$). The highest germination rates 6.49 and 6.58, were observed in the combined treatments of cold plasma and magnetic field and cold plasma treatment at vacuum conditions with a time of exposure of 30 s, which was significantly different from all other treatments and control (Figure 5). This finding is in line with Tang et al. (2016) discovery that non thermal plasma (NTP) stimulation significantly enhanced the germination rate of alfalfa seeds after 20 s of treatment. Results showed that control and combined treatment of cold plasma and electromagnetic field had the lowest germination rate.

Germination percentage. The results of statistical analysis showed that cold plasma treatment had no significant effect on germination percentage ($P < 0.05$). The highest germination percentage of (92.5%) was observed in the combined treat-

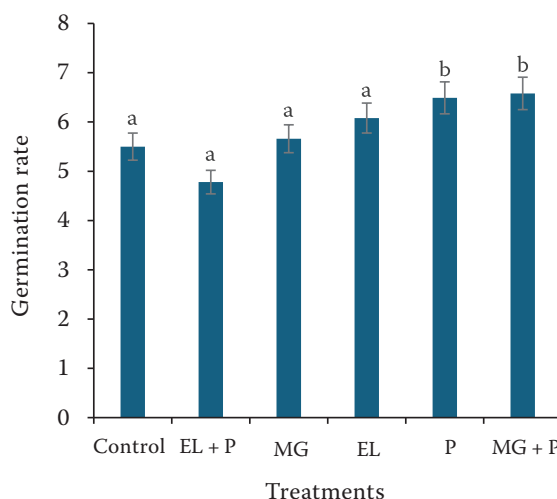


Figure 5. The germination rate of alfalfa seeds in different treatments

Letters in the columns indicate no significant difference at the level of 5%; EL – electromagnetic field treatment; P – vacuum cold plasma treatment; MG – magnetic field treatment

ment of cold plasma and magnetic field and the lowest germination percentage was related to the control treatment (Figure 6). This finding is consistent with Nelson et al. (1977) discovery that electrical treatment does not have a significant effect on the germination percentage of alfalfa seeds.

In general, under the studied treatments, the combination of cold plasma and magnetic field treatments increases the germination percentage compared to the magnetic field and control treatments, while the cold plasma treatment, when combined with the electromagnetic field, reduces the germination percentage compared to Electromagnetic field and control treatment.

Vigor index. The results of statistical analysis showed that cold plasma treatment had no significant effect the vigor index ($P < 0.05$). The highest vigor index 578.125 was observed in the combined treatment of cold plasma and magnetic field and

Table 2. Analysis of variance for effect of treatments on germination rate

Sources of variation	SS	df	MS	F-value	P-value
Between groups	6.897	5	1.379	29.462	0.00*
Within groups	0.562	12	0.047		
Total	7.458	17			

*Significant at level of $P < 0.05$; *df* – degree of freedom; SS – sum of squares; MS – mean square

Table 3. Analysis of variance for effect of treatments on germination percentage

Sources of variation	SS	df	MS	F-value	P-value
Between groups	378.625	5	75.725	6.756	0.003 ^{ns}
Within groups	134.5	12	11.208		
Total	513.125	17			

^{ns} – not significant at $P > 0.05$; *df* – degree of freedom; SS – sum of squares; MS – mean square

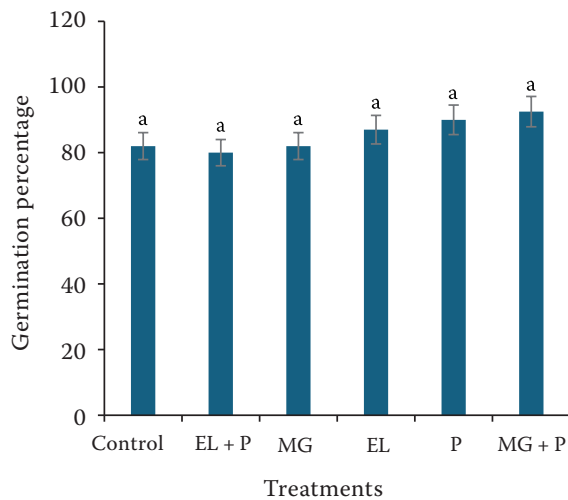


Figure 6. Germination percentage of alfalfa seeds in different treatments

Letters in the columns indicate no significant difference at the level of 5%; EL – electromagnetic field treatment; P – vacuum cold plasma treatment; MG – magnetic field treatment

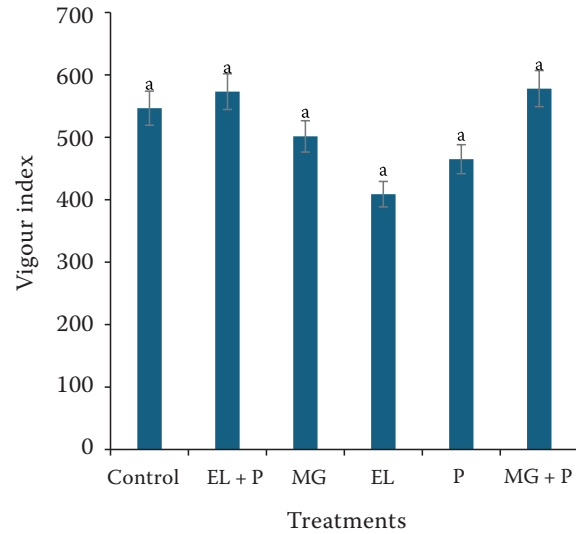


Figure 7. Vigor index of alfalfa seeds in different treatments

Letters in the columns indicate no significant difference at the level of 5%; EL – electromagnetic field treatment; P – vacuum cold plasma treatment; MG – magnetic field treatment

the lowest vigor index was related to the electromagnetic field treatment (Figure 7). This finding is consistent with Tang et al.'s (2016) discovery that using the low temperature plasma (LTP) technique with a seed processing machine is effective and practical for stimulating crop seed germination.

Overall, the combination of cold plasma and magnetic field treatments resulted in a higher vigor index compared to the magnetic field and control treatments.

Allometric coefficient. The results of statistical analysis showed that cold plasma treatment had a significant effect on Allometric coefficient ($P < 0.05$). The allometric coefficients of 0.61674 and 0.53024 were observed for the magnetic field and combination of cold plasma and electromagnetic field treatments, respectively. These values were significantly different from those of all other treatments and the control (Figure 8).

Table 4. Analysis of variance for effect of treatments on vigor index

Sources of variation	SS	df	MS	F-value	P-value
Between gr.	66 772.954	5	13 354.591	1.536	0.251 ^{ns}
Within gr	104 359.91	12	8 696.659		
Total	171 132.863	17			

ns – not significant at $P > 0.05$; df – degree of freedom; gr – group; MS – mean square SS – sum of squares

Table 5. Analysis of variance for effect of treatments on allometric coefficient

Sources of variation	SS	df	MS	F-value	P-value
Between groups	0.562	5	0.112	8.17	0.001*
Within groups	0.165	12	0.014		
Total	0.727	17			

*Significant at level of $P < 0.05$; df – degree of freedom; SS – sum of squares; MS – mean square

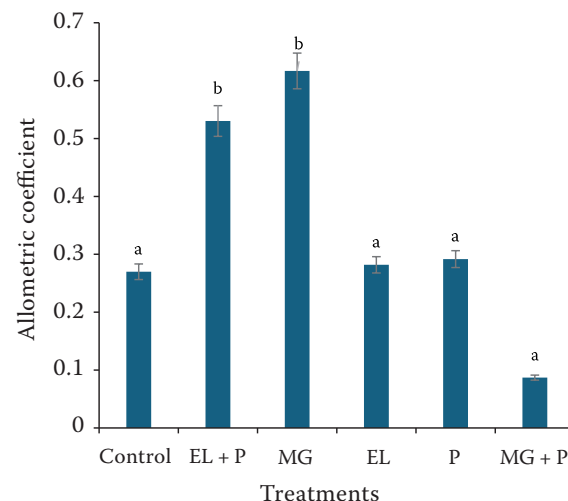


Figure 8. Allometric coefficient of alfalfa seeds in different treatments

Letters in the columns indicate no significant difference at the level of 5%; EL – electromagnetic field treatment; P – vacuum cold plasma treatment; MG – magnetic field treatment

Overall, compared to the magnetic field and control treatments, the combination of cold plasma and magnetic field treatments resulted in a decrease in the allometric coefficient.

According to our findings, in terms of germination rate, a significant increase was observed in treated samples compared to control samples. This was in agreement with the finding of Nedyalkova et al. (2019) that mentioned germination rates were increased by plasma exposure.

Nevertheless, cold plasma treatment had no significant effect on vigor index, which was in contrast to findings in previous studies by Zahoranová et al. (2016).

The highest germination percentage was recorded in plasma-treated seeds with magnetic radiation for 200 mT. According to the obtained results, plasma treatment positively affected the germination rate. It may be due to the modification in seed coat morphology, improved penetrability, and water uptake through seeds which promoted germination rate. The results are also in strong agreement with Ling et al. (2014).

CONCLUSION

The field of physical seed treatment represents an innovative area of research with the potential to enhance crop yield. Despite the promising results observed in laboratory settings, the full commercial potential of this approach remains untapped. Over the past two decades, significant efforts have been made to enhance yield through pre-sowing treatments.

The results of this study indicate that the combined application of cold plasma and a magnetic field significantly enhances germination rates of seeds in comparison to the control. Additionally, the use of the magnetic field significantly enhances the allometric coefficient.

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