Agriculture, Environment & Society (2022) Volume 2, Issue 1, P. 7-13

Agriculture, Environment & Society

Journal homepage: www.aes.uoz.ac.ir

# Ultrasonic waves can have an effect on some of the physiological traits of cowpea when trifluralin is added to the soil

Abbas Nasiri Dehsorkhi [*a], Hassan Makarian [b], Mehrdad Mahlooji [c], Seyed Hassan Mirhashemi [d], Siavash Bardehji [e], Sima Sadat Seyedi [a], Navid Kargar Dehbidi [f]

[a] M.Sc. Graduate of Agroecology, Department of Agronomy and Plant Breeding, Faculty of Agriculture, Shahrood University of Technology, Shahrood, Iran
[b] Department of Agronomy and Plant Breeding, Faculty of Agriculture, Shahrood University of Technology, Shahrood, Iran
[c] Horticulture Crops Research Department, Isfahan Agricultural and Natural Resources Research and Education Center, AREEO, Isfahan, Iran
[d] M.Sc. Graduate of Water Engineering, Faculty of agriculture, University of Zanjan, Zanjan, Iran
[e] Ph.D Graduate, Department of Agronomy and Plant Breeding, College of Agriculture, Isfahan University of Technology, Isfahan, Iran
[f] Ph.D Candidate of Natural Resources and Environmental Economics, Department of Agricultural Economics, School of Agriculture, Shiraz University, Iran

ARTICLE INFO

*Article history:*

Received: 21 February 2022

Accepted: 24 March 2022

Available online: 31 March 2022

*Keywords:*

Carotenoid

Herbicide

Hydro-priming

Leaf nitrogen

Treflan

ABSTRACT

An experiment was conducted at the Faculty of Agriculture, Shahrood University, as a randomized complete block design with four replications to investigate the effect of ultrasonic waves and seed priming on some quality traits of cowpea under soil application of trifluralin. Nine treatments were: T1: control, T2: ultrasonic waves, T3: ultrasonic waves + reduced herbicide dose (1 L $ha^{-1}$), T4: ultrasonic waves + recommended herbicide dose (2 L $ha^{-1}$), T5: hydro-priming, T6: hydro-priming + reduced herbicide dose, T7: hydro-priming + recommended herbicide dose, T8: reduced herbicide dose, T9: recommended herbicide dose. The results showed that the effect of treatments was significant on all traits except leaf phosphorus. The maximum chlorophyll a (1.30 mg $g^{-1}$ FW), carotenoid (1.82 mg $g^{-1}$ FW), leaf relative water content (79.9 %), and leaf nitrogen (3.97%) were obtained in ultrasonic treatment, which resulted in a significant increase of 28.7, 22.1, 7.9, and 18.5 percent, respectively, in comparison to the control. In comparison to the ultrasonic treatment, ultrasonic waves + recommended herbicide dose reduced chlorophyll b, RWC, and leaf nitrogen by 29.3, 21.1, and 35.3 percent, respectively. In comparison to herbicide application alone, the combination of ultrasonic waves and the recommended herbicide dose reduced chlorophyll a and total chlorophyll by 29.7 and 22.2 percent, respectively. Overall, the results of the present study showed that pretreating cowpea seeds with ultrasonic waves could increase photosynthesis pigments, relative water content, and leaf N (in the absence of herbicide use).

## Highlights

- An experiment was conducted at the Faculty of Agriculture, Shahrood University, as a randomized complete block design with four replications to investigate the effect of ultrasonic waves and seed priming on some quality traits of cowpea under soil application of trifluralin.
- The results showed that the effect of treatments was significant on all traits except leaf P.
- The ultrasonic treatment increased chlorophyll a, carotenoid, RWC, and leaf N by 28.7, 22.1, 7.9, and 18.5 percent, respectively, compared to the control.
- Overall, the results of the present study showed that pretreating cowpea seeds with ultrasonic waves could increase photosynthesis pigments, relative water content, and leaf N (in the absence of herbicide use).

## 1. Introduction

Cowpea (*Vigna sinensis* L.) is mostly grown in India and West Africa, as well as in warmer South and North America and throughout the tropics and subtropics. The grain of cowpea is very rich and delicious and contains about 22% protein (up to 35%), 1.3 to 2% fat, and 60% to 67% carbohydrates. It has a significant energy value and 340 calories per 100 g of the food. The ratio of protein, carbohydrate, and B

* Corresponding author
E-mail address: abasnasiri110@yahoo.com (A. Nasiri Dehsorkhi)
http://dx.doi.org/10.22034/aes.2022.331872.1025

vitamin varies considerably depending on the variety and seed origin (Fallah, 2010).

One of the biophysical methods of seed priming is the pretreatment of seeds with ultrasonic waves. Continuous shocks of ultrasonic waves increase the permeability of the seed coat, accelerate water absorption, and increase tissue temperature. Increased inflammation rate and tissue temperature in ultrasonic waves-treated seed may be linked to accelerated changes in seed metabolism (Rasouli et al., 2020a). Under the influence of ultrasonic waves, due to heat production, many biochemical changes occur in tissues, including increasing the rate of chemical reactions, increasing the rate of diffusion of substances, and breaking down compounds such as enzymes and killing microorganisms, which results in an increase in rootlet length and weight (Ashnagar et al., 2019). By applying ultrasonic waves to the seeds, the mechanical resistance to the root outlet is removed. These seeds have more time to grow compared to control seeds, so the root length of treated seeds is longer than control seeds (Eisvand and Latifinia, 2020).

The researchers found that pretreatment with ultrasonic waves increased the activity of alpha-amylase, protease, alcohol dehydrogenase, glucose-6-phosphate dehydrogenase, total and soluble proteins, and total sugar concentrations in most treatments compared to the control. Increasing the activity of alpha-amylase, protease, alcohol dehydrogenase, arginase, and glucose-6-phosphate dehydrogenase enzymes increases germination percentage, germination rate, and dry weight of seedlings (Rasouli et al., 2020a). Improvement of seed germination characteristics due to the use of ultrasonic waves has been reported in several studies (Nazari et al., 2014; Ciu and Sung, 2014; Sharififar et al., 2015; Machikowa et al., 2013; Toth, 2012; Miyoshi and Mii, 1988; Wang et al., 2012; Aladjadjiyan, 2011). In another study, the results showed that irradiation of cowpea seeds with ultrasonic waves at two time periods of 4 and 8 minutes led to increased growth characteristics, yield, yield components, and quality characteristics of the plant (Jamshidi, 2016).

Trifluralin (treflan), which is used in soil and before planting, belongs to the family of dinitroanilines and acts by preventing the division and elongation of cells. It is used to control a wide range of broadleaf weeds and weeds of the Gramineae family (Esmaeilnejad Khiavi, 2019). However, this herbicide can have adverse effects on sensitive crops. For example, research has shown that the application of higher concentrations of the herbicide trifluralin reduces leaf chlorophyll content and nitrogen content in cowpea and soybean plants (Behran et al., 1979). The results of another study showed that the application of Treflan herbicide significantly reduces plant height, leaf number, shoot dry weight, and fresh weight of chickpea pods (Rasooli, 2012). In a study of soybean cultivars, the researchers said that with increasing trifluralin consumption, height, leaf area, shoot dry weight, root dry weight, and nodal dry weight decreased significantly, and that the lowest measured value of these traits was obtained with the use of the highest amount of herbicide (Roodi et al., 2009). In sunflower plants treated with trifluralin herbicide, root and stem length, fresh weight, and dry weight of shoots and roots were reduced, which was significant at high concentrations of herbicide (Moradbeigi and Khara, 2011). In another study, the results showed that with increasing the concentration of trifluralin, the content of photosynthetic pigments in plants inoculated with mycorrhizal fungi and not inoculated plants decreased (Esmaeilnejad Khiavi, 2019).

In the results of studies, it looks like priming and ultrasonic waves can be used to help plants grow better. This will help improve the plant's quality characteristics. On the other hand, it seems necessary to evaluate the effect of trifluralin herbicide in soil on primed seeds. Therefore, the aim of this study is to investigate the effects of ultrasonic waves and hydro-priming on photosynthetic pigments, relative leaf water content, nitrogen, and phosphorus of cowpea leaves.

## 2. Materials and methods

The experiment was performed as a randomized complete block design with four replications in greenhouse conditions in the Faculty of Agriculture, Shahrood University of Technology. Nine treatments were: T1: control, T2: ultrasonic waves, T3: ultrasonic waves + reduced herbicide dose (1 L. $ha^{-1}$), T4: ultrasonic waves + recommended herbicide dose (2 L. $ha^{-1}$), T5: hydro-priming, T6: hydro-priming + reduced herbicide dose, T7: hydro-priming + recommended herbicide dose, T8: reduced herbicide dose, T9: recommended herbicide dose.

**Table 1. Soil physical and chemical characteristics**

| Soil texture | Clay | Sand | Silt | Total nitrogen | pH | EC | Potassium | Phosphorus |
|---|---|---|---|---|---|---|---|---|
| | | | % | | | dS/m | ppm | |
| Clay loam | 30.7 | 20.1 | 49.2 | 0.105 | 7.79 | 1.34 | 181.4 | 14.4 |

Seven seeds were sown in pots with a diameter of 25 cm and a height of 30 cm. To create drainage, holes were made in the bottom of the pot, and then coarse-grained sand was poured into the bottom of the pot to a height of 2 cm, and the rest of the pot was filled with field soil. Seeds related to hydro-priming treatment were soaked in water for 7 hours before sowing and then planted in appropriate pots. The seeds were also soaked in water for 7 hours before using ultrasonic waves. To apply ultrasonic waves, an ultrasonic bath (Digital Ultrasonic Model 4820_CD) with a constant frequency of 24 kHz for 6 minutes at ambient temperature in distilled water was used.

The studied traits included chlorophyll a and b, total chlorophyll, carotenoids, relative water content, nitrogen, and phosphorus of the leaf. Finally, after collecting the data, they were analyzed using MSTATC software, and

the means were compared using the LSD test at a probability level of 5%. Also, the correlation coefficients of the traits were estimated by SPSS software.

## 3. Results and discussion

The results of analysis of variance showed that the effect of experimental treatments on chlorophyll a, total chlorophyll, carotenoids, relative leaf water content, and leaf N was significant at one percent probability level and on chlorophyll b at five percent probability level. However, leaf phosphorus was not affected by the treatments (Table 2).

### 3.1. Chlorophyll a

The highest amount of chlorophyll a (1.3 mg $g^{-1}$ FW) was related to ultrasonic waves treatment, which showed a significant difference with all treatments and compared to the control, it increased by 28.7% (Table 3). By applying ultrasonic waves to the seeds, the mechanical resistance to radicle emergence was removed. These seeds had a long time to grow compared to the control seeds, so the radicle length of the treated seeds was longer than the control seeds (Eisvand and Latifinia, 2020). It seems that using ultrasonic waves to stimulate and increase the germination rate and rapid establishment of the plant can increase the activity of the plant root in the absorption of water and nutrients, which will have a direct effect on chlorophyll content, photosynthesis, and finally plant yield. In this regard, increasing the germination rate due to the use of ultrasonic waves has been reported in several studies (Ciu and Sung, 2014; Goussous et al., 2010; Yaldagard et al., 2008).

**Table 2. Analysis of variance for investigated traits of cowpea as affected by trial treatments**

| SOV | df | Mean Square | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Chlorophyll a | Chlorophyll b | Total chlorophyll | Carotenoid | Relative water content | Leaf N | Leaf P |
| Block | 3 | 0.110 | 0.012 | 0.066 | 0.190 | 49.028 | 0.519 | 0.145 |
| Treatment | 8 | 0.225 ** | 0.028 * | 0.403 ** | 0.239 ** | 109.817 ** | 0.759 ** | 0.159 ns |
| Error | 24 | 0.006 | 0.010 | 0.011 | 0.003 | 1.238 | 0.027 | 0.137 |
| CV (%) | - | 8.3 | 13.9 | 6.4 | 3.7 | 1.5 | 4.9 | 18.7 |

ns, * and ** are not significant, significant at the 5% and 1% probability levels, respectively.

The lowest amount of chlorophyll a was related to the ultrasonic waves + recommended herbicide dose and hydro-priming + recommended herbicide dose treatments, which were in a statistically significant group (Table 3). The results showed that the cultivation of primed seeds in soil containing a lower concentration of herbicide (reduced dose) produced a higher amount of chlorophyll a compared to the reduced herbicide dose treatment (in the absence of seed priming). However, the cultivation of primed seeds in soil containing a higher concentration of herbicide (recommended dose) resulted in lower chlorophyll production compared to the recommended herbicide dose treatment alone (Table 3). Using ultrasonic waves with the recommended herbicide and hydro-priming with the recommended herbicide significantly cut chlorophyll a by 29.7% and 19.8%. This is compared to the recommended herbicide dose application without pretreatment of seeds (Table 3).

**Table 3. Mean comparison for investigated traits of cowpea as affected by trial treatments**

| Treatments | Chlorophyll a | Chlorophyll b | Total chlorophyll | Carotenoid | RWC | Leaf N |
|---|---|---|---|---|---|---|
| | (mg $g^{-1}$ FW) | | | | (%) | |
| T1 | 1.01 c | 0.71 abc | 1.73 b | 1.49 c | 74.0 c | 3.35 cd |
| T2 | 1.30 a | 0.82 a | 2.13 a | 1.82 a | 79.9 a | 3.97 a |
| T3 | 1.07 bc | 0.73 abc | 1.80 b | 1.66 b | 73.2 cd | 3.62 b |
| T4 | 0.57 e | 0.58 c | 1.16 d | 1.09 f | 63.1 g | 2.57 g |
| T5 | 1.16 b | 0.84 a | 2.00 a | 1.72 b | 76.2 b | 3.65 b |
| T6 | 1.05 bc | 0.74 ab | 1.79 b | 1.65 b | 72.2 d | 3.55 bc |
| T7 | 0.65 e | 0.64 bc | 1.29 d | 1.20 e | 65.3 f | 2.87 f |
| T8 | 0.89 d | 0.65 bc | 1.54 c | 1.47 c | 69.8 e | 3.17 de |
| T9 | 0.81 d | 0.67 bc | 1.49 c | 1.35 d | 69.1 e | 3.07 ef |

Means within each column followed by the same letter are not at 5% level according to least significance difference (LSD) test.
T1: control, T2: ultrasonic waves, T3: ultrasonic waves + reduced herbicide dose (1 L $ha^{-1}$), T4: ultrasonic waves + recommended herbicide dose (2 L $ha^{-1}$), T5: hydro-priming, T6: hydro-priming + reduced herbicide dose, T7: hydro-priming + recommended herbicide dose, T8: reduced herbicide dose, T9: recommended herbicide dose

Under the influence of ultrasonic waves due to heat production, many biochemical changes occur in tissues, including increasing the rate of chemical reactions, increasing the rate of diffusion of materials, breaking down materials such as enzymes, and the destruction of microorganisms, which implies an increase in root length and weight (Ashnagar et al., 2019). It seems that the seeds treated with ultrasonic waves by increasing the root length use the resources in the soil more quickly, which ultimately improves the chlorophyll content of the leaves. There are also more conditions in place to make the herbicide work better on the plant, and this trait has been lessened in the herbicide treatments that have this trait. In this regard, the researchers stated that the main effect of trifluralin is to prevent root growth and the formation of lateral roots. Decreased root growth reduces

the absorption of water and nutrients required by the plant and affects the growth of shoots (Roodi et al., 2009). Results showed that when the concentration of trifluralin was increased, the amount of photosynthetic pigments in plants that had been inoculated with mycorrhizal fungi and plants that had not been inoculated with mycorrhizal fungi both decreased (Esmaeilnejad Khiavi, 2019).

### 3.2. Chlorophyll b

The results showed that the highest amount of chlorophyll b was related to hydro-priming (0.84 mg $g^{-1}$ FW) and ultrasonic waves (0.82 mg $g^{-1}$ FW) (Table 3). In this regard, in a study conducted on cowpea, the results of the use of ultrasonic waves showed that the highest amount of chlorophyll b was obtained in 4 minutes of wave treatment (Jamshidi, 2016). In another study, the results showed that ultrasonic waves and magnetic fields had a statistically significant effect on the amount of chlorophyll in the leaves at a probability level of 1% (Marghaeizadeh et al., 2014).

The results of comparing the means of the treatments showed that the combined application of primed seeds (sonicated and primed with water) with herbicides (especially higher concentrations) significantly reduced chlorophyll b. The combined application of ultrasonic waves and recommended herbicide (2 L $ha^{-1}$) reduced chlorophyll b compared to ultrasonic waves treatment by 29.3%. Also, the use of hydro-priming with the recommended herbicide (2 L $ha^{-1}$) cut chlorophyll b by 23.9% when compared to the hydro-priming treatment (Table 3). Chlorophyll content may be down because trifluralin makes plants stop taking iron from their roots to their shoots, which stops chlorophyll synthesis and makes leaves look yellow. In plants that have been treated with trifluralin, a lack of chlorophyll can make them less able to use sunlight and thus less able to store carbon (Amiri et al., 2010). A decrease in wheat chlorophyll b content due to the use of herbicides has also been found (Hana et al., 2015).

### 3.3. Total chlorophyll

The results showed that the highest chlorophyll content of leaves was related to ultrasonic waves and hydro-priming treatments, which were in a statistically significant group and increased by 23.1% and 15.6%, respectively, compared to the control (no treatment) (Table 3). In this regard, the results showed that the highest amount of total chlorophyll in cowpea was related to hydro-priming and pretreatment of seeds with plasma radiation for 15 seconds, which were in a statistically significant group (Vaziri, 2018).

The lowest total chlorophyll content was related to the combined treatments of ultrasonic waves + recommended herbicide and hydro-priming + recommended herbicide, which were in a statistically significant group and had a significant difference with other treatments. Ultrasonic waves + recommended herbicide dose and hydro-priming + recommended herbicide dose reduced the total chlorophyll by 22.2% and 13.5% relative to only applying the recommended herbicide dose treatment. However, the combined application of these pretreatments with a reduced amount (1 L $ha^{-1}$) compared to the application of reduced herbicides alone showed a significant increase in total chlorophyll (Table 3). This result can be attributed to the greater effectiveness of the herbicide at higher concentrations in the pretreated seeds. It seems that the use of ultrasonic waves and hydro-priming accelerates the germination of seeds and that increasing the root length causes the plant to use more soil nutrients, thus increasing plant growth. On the other hand, elongation of root length exposes the plant to more herbicides, which reduces the absorption of water and nutrients and consequently reduces the chlorophyll content in herbicide treatments with treated seeds is not unexpected. In this regard, a decrease in chlorophyll content due to the application of higher concentrations of herbicide in sunflower (Sameni et al., 1976) and cowpea and soybeans (Behran et al., 1979) has also been reported.

### 3.4. Carotenoid

The highest content of carotenoid (1.82 mg $g^{-1}$ FW) was obtained in ultrasonic waves treatment, which showed a significant difference with all treatments and increased by 22.1% compared to the control (no treatment). However, the lowest leaf carotenoid content (1.09 mg $g^{-1}$ FW) was observed in the ultrasonic waves + recommended herbicide dose treatment, which had a significant difference with all treatments (Table 3). The results showed that the combined application of primed seeds (hydro-priming and ultrasonic waves) with a reduced dose of herbicide could significantly increase carotenoid compared to the control. Ultrasonic waves + reduced herbicide dose and hydro-priming + reduced herbicide dose treatments significantly increased carotenoid by 11.4% and 10.7%, respectively, compared to the control (Table 3). The treatment with a recommended dose of herbicide (2 L $ha^{-1}$) significantly reduced carotenoid by 8.2% compared to the treatment with a reduced dose of herbicide (1 L $ha^{-1}$). Also, the results showed that ultrasonic waves + recommended herbicide dose treatment significantly reduced carotenoid by 9.2% compared to hydro-priming + recommended herbicide dose treatment (Table 3).

In the present study, seeds were soaked in water for the same period in hydro-priming and ultrasonic waves treatments. Seeds that were exposed to ultrasonic waves in addition to soaking, due to their more permeable shells compared to hydro-primed seeds, were exposed to higher amounts of herbicides in a shorter time, and herbicide with faster penetration into these seeds caused adverse effects on the plant. In this regard, studies have shown that with increasing the concentration of trifluralin, the content of carotenoids in plants inoculated and not inoculated with mycorrhizal fungi decreased (Esmaeilnejad Khiavi, 2019). The use of higher doses of herbicide also reduced the total chlorophyll and carotenoid in chickpea (Khan et al., 2006).

### 3.5. Leaf relative water content (RWC)

The results showed that the highest RWC by 79.9% was related to ultrasonic waves treatment, which in comparison with hydro-priming and control treatments, had a significant increase of 4.8 and 7.9%, respectively (Table 3). In this regard, research has shown that the hydro-priming of cowpea seeds causes a significant increase in the RWC in the presence of weeds (Vaziri, 2018). Therefore, it can be concluded that primed seeds grow longer roots than untreated plants, which leads to better root efficiency, more water uptake, and more relative water content of the leaves.

The lowest RWC (63.1%) was observed in the ultrasonic waves + recommended herbicide dose treatment, which had a significant difference with all other treatments. The combined application of ultrasonic waves and recommended herbicide dose treatment (2 L $ha^{-1}$) reduced the relative water content compared to ultrasonic waves and control treatments by 21.1% and 14.8%, respectively. Also, the results showed that the ultrasonic waves + recommended herbicide dose treatment compared to the hydro-priming + recommended herbicide dose treatment caused a significant reduction in the RWC by 3.4% (Table 3). Based on the results, it is possible that the positive effects of hydro-priming and ultrasonic waves on seed germination and the increase in root length caused the seedlings to be exposed to more herbicide, and subsequently, in the later stages, the rootlet longitudinal growth and thus its access to soil moisture decreased. In this regard, research results have shown that with increasing trifluralin concentration, the relative water content in both inoculated and non-inoculated plants decreased, although mycorrhizal treatments had a higher relative water content than non-mycorrhizal plants (Esmaeilnejad Khiavi, 2019). The results of estimating the correlation coefficients of the traits showed a significant positive correlation between the RWC and chlorophyll a ($0.92^{**}$), chlorophyll b ($0.40^{*}$), total chlorophyll ($0.87^{**}$), and carotenoid ($0.90^{**}$) (Table 4). Considering the significant positive correlation between chlorophyll content and relative water content, it can be inferred that pretreatment of cowpea seeds will increase plant water uptake by improving root growth, which will ultimately improve growth and photosynthetic pigments. In this regard, a significant positive correlation has been reported between leaf chlorophyll and RWC in cantaloupe (Nasiri Dehsorkhi et al., 2020).

### 3.6. Leaf nitrogen

The results showed that the highest leaf N (3.97%) was related to ultrasonic waves treatment, which in comparison with hydro-priming and control treatments, had a significant increase of 8.7% and 18.5%, respectively. The lowest leaf N (2.57%) was observed in the ultrasonic waves + recommended herbicide dose treatment, which had a significant difference with all treatments (Table 3). Ultrasonic waves increase growth indices, especially root length in seedlings, which can play an important role in obtaining moisture and nutrients from the environment (Rasouli et al., 2020b). Jamshidi (2016) found that the best way to get the most protein from cowpea seeds was to not stress them out and use ultrasonic waves for 4 minutes.

The combined application of ultrasonic waves with the reduced dose of herbicide could significantly increase leaf N compared to the control treatment. However, the combination of primed seeds with the recommended dose of herbicide significantly reduced leaf N. The combined application of ultrasonic waves and recommended herbicide dose (2 L $ha^{-1}$) reduced leaf N compared to ultrasonic and control treatments by 35.3% and 23.3%, respectively. The ultrasonic waves and recommended herbicide dose treatment in comparison with hydro-priming and recommended herbicide dose treatment caused a significant reduction of leaf N by 10.5% (Table 3).

In this case, research results have shown that the application of higher concentrations of trifluralin herbicide reduced leaf chlorophyll content and nitrogen content in cowpea and soybean (Behran et al., 1979). The application of higher doses of herbicide reduced the nitrogen content of chickpea (Khan et al., 2006). With the application of Lentagran, Persoit, and Treflan herbicides, the nitrogen content of chickpea shoots decreased by 6%, 50%, and 78%, respectively (Rasooli, 2012). The results of trait correlation showed that leaf N had the highest significant positive correlation ($0.85^{**}$) with total leaf chlorophyll (Table 4). Due to the fact that nitrogen is involved in the structure of chlorophyll, the observed correlation is not far from what one would expect.

**Table 4. Correlation coefficients for the investigated traits of cowpea**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| **1- Chlorophyll a** | 1 | | | | | | |
| **2- Chlorophyll b** | 0.42 ** | 1 | | | | | |
| **3- Total chlorophyll** | 0.94 ** | 0.70 ** | 1 | | | | |
| **4- Carotenoid** | 0.88 ** | 0.43 ** | 0.85 ** | 1 | | | |
| **5- RWC** | 0.92 ** | 0.40 * | 0.87 ** | 0.90 ** | 1 | | |
| **6- Leaf N** | 0.83 ** | 0.54 ** | 0.85 ** | 0.79 ** | 0.77 ** | 1 | |
| **7- Leaf P** | 0.31 ns | 0.49 ** | 0.42 ** | 0.45 ** | 0.35 * | 0.22 ns | 1 |

ns, * and ** are not significant, significant at the 5% and 1% probability levels, respectively.

## 4. Conclusion

Overall, the results of the present study showed that the cultivation of cowpea seeds pretreated with ultrasonic waves and hydro-priming in soil treated with trifluralin herbicide reduced photosynthetic pigments, relative leaf water content, and leaf N content. Primed seeds appear to initiate enzymatic activities for germination. Therefore,

after cultivation in the soil, they receive trifluralin more quickly and in a shorter time. The herbicide immediately affects the germination processes and stops or reduces germination. On the other hand, unprimed seeds have barriers, like shell and embryonic coatings, which stop or slow down the herbicide from getting into the seed and getting into the seedling.

## References


Aladjadjiyan, A., 2011. Ultrasonic stimulation of the development of lentils and wheat seedlings. Romanian Journal of Biophysics, 21(3), 179-187.

Amiri, S., Kazemitabaar, S.K., Ranjbar, G., Azadbakht, M., 2010. The effect of trifluralin and colchicine treatments on morphological characteristics of jimsonweed (*Datura stramonium* L.). Trakia Journal of Sciences, 8(4), 47-61.

Ashnagar, N., Mir Mahmoodi, T., Jalilnejhad, N., 2019. Effects of electromagnetic field and ultrasonic waves on seed germination, seedling characteristics and essence percent of Thymes (*Thymus vulgaris* L.). Journal of Crop Ecophysiology, 13(1), 57-72. (In Persian).

Behran, Sh., Maftoun, M., Sheibany, B., Hojjati, S.M., 1979. Effect of fertilizer-N and herbicides on the growth and N content of soybeans and cowpeas. Agronomy Journal, 71, 533-538.

Ciu, K.Y., Sung, J.M., 2014. Use of ultrasonication to enhance pea seed germination and microbial quality of pea sprouts. International Journal of Food Science and Technology, 49, 1699-1706.

Eisvand, H.R., Latifinia, E., 2020. Effects of hydropriming and ultrasonic waves on seed leakage and germination of Artemisia (*Artemisia aucheri* L.). Iranian Journal of Seed Science and Research, 7(2), 179-190. (In Persian).

Esmaeilnejad Khiavi, N., 2019. Protective effects of mycorrhizal fungi, gamma amino butyric acid (GABA) and magnetic water on squash (*Cucurbita pepo*) morphological, physiological and biochemical charachterics under herbicide trifluralin induced to toxicity. PhD thesis in Plant Physiology, Department of Biology, Urmia University. (In Persian).

Fallah, S., 2010. Agronomy (General and Special). Shahrekord University Press. (Translated). (In Persian).

Goussous, S.J., Samarah, N.H., Alqudah, A.M., Othman, M.O., 2010. Enhancing seed germination of four crops species using an ultrasonic technique. Experimental Agriculture, 46, 231-242.

Hana, S., Leila, M.A., Nedjoud, G., Reda, D.M., 2015. Physiology and biochemistry effects of herbicides Sekator and Zoom on two varieties of wheat (Waha and HD) in semi-arid region. Annual Research & Review in Biology, 5(5), 449-459.

Jamshidi, M., 2016. Interaction of ultrasonic waves and salicylic acid on drought-stressed cowpea. MSc thesis, Faculty of Agriculture, Shahrood University of Technology. (In Persian).

Khan, M.S., Zaidi, A., Rizvi, P.Q., 2006. Biotoxic effects of herbicides on growth, nodulation, nitrogenase activity, and seed production in chickpeas. Communications in Soil Science and Plant Analysis, 37, 1783–1793.

Machikowa, T., Kulrattanarak, T., Wonprasaid, S., 2013. Effects of ultrasonic treatment on germination of synthetic sunflower seeds. International Journal of Biological, Biomolecular, Agricultural Food and Biotechnological Engineering, 7(1), 1-3.

Marghaeizadeh, Gh., Gharineh, M.H., Fathi, Gh., Abdali, A.R., Farbod, M., 2014. Effect of ultrasound waves and magnetic field on germination, growth and yield of *Carum copticum* (L.) C. B. Clarke) in lab and field conditions. Iranian Journal of Medicinal and Aromatic Plants, 30(4), 539-560. (In Persian).

Miyoshi, K., Mii, M., 1988. Ultrasonic treatment for enhancing seed germination of terrestrial orchid, Calanthediscolor, in a symbiotic culture. Scientia Horticulturae, 35, 127-130.

Moradbeigi, H., Khara, J., 2011. An evaluation of some physiological and biochemical parameters resulting from interaction of herbicide trifluralin and mychorrizal colonization by Glomus versiforme in sunflower plants (cv. Lakomka). Journal of Plant Biology, 3(10), 59-70. (In Persian).

Nasiri Dehsorkhi, A., Varnaseri Ghandali, V., Makarian, H., Ramezan, D., Estekhdami, P., 2020. The effect of poultry manure and zinc sulfate on growth and yield of cantaloupe (*Cucumis melo* L.) in competition with weeds. Horticultural Plants Nutrition, 2(2), 46-69. (In Persian).

Nazari, M., Sharififar, A., Asghari, H.R., 2014. *Medicago scutellata* seed dormancy breaking by ultrasonic waves. Plant Breeding and Seed Science, 69(1), 15-24.

Rasooli, R., 2012. The effect of Lentagran, Persoit and Treflan herbicides on growth, nodulation and nitrogen fixation of three varieties of chickpea (*Cicer aritinium* L.). MSc Thesis, Ferdowsi University of Mashhad. (In Persian).

Rasouli, F., Gholipour, M., Jahanbin, K., Asghari, H.R., 2020a. Effect of ultra-sonic waves on germination, some germination enzymes and biochemical parameters of *Echinacea purpurea* L. seed. Iranian Journal of Seed Science and Research, 7(1), 41-54. (In Persian).

Rasouli, F., Gholipour, M., Asghari, H.R., Hojjati, M., 2020b. The effect of imbibition time and ultrasound pretreatment on germination and biochemical feature of *Echinacea purpurea* L. Iranian Journal of Seed Science and Research, 7(2), 205-215. (In Persian).

Roodi, D., Ghadiri, H., Maftoon, M., 2009. Effect of trifluralin and iron on morphological characteristics and iron absorption of soybeans. Weed Research Journal, 1(1), 25-36. (In Persian).

Sameni, A.M., Maftoun, M., Hojjati, S.M., Sheibany, B., 1976. Effect of fertilizer-N and herbicides on the growth and N content of sunflower. Agronomy Journal, 68, 285-288.

Sharififar, A., Nazari, M., Asghari, H.R., 2015. Effect of ultrasonic waves on seed germination of *Atriplex*

*lentiformis*, *Cuminum cyminum*, and *Zygophyllum eurypterum*. Journal of Applied Research on Medicinal and Aromatic Plants, 2(3), 102-104.

Toth I., 2012. The effects of ultrasound exposure on the germination capacity of birds foot trefoil (*Lotus corniculatus* L.) seeds. Romanian Journal of Biophysics, 22(1), 13-20.

Vaziri, R., 2018. Effect of seed pre-treatment with cold plasma on the growth and yield of cowpea (*Vigna sinensis* L.) in competition with weeds. MSc Thesis, Faculty of Agriculture, University of Shahrood. (In Persian).

Wang, Q., Chen, G., Yersaiyiti, H., Liu, Y., Cui, J., Wu, C, Zhang, Y., He, X., 2012. Modeling analysis on germination and seedling growth using ultrasound seed pretreatment in switchgrass. PLoS ONE, 7(10), e47204.

Yaldagard, M., Mortazavi, S.A., Tabatabaie, F., 2008. Application of ultrasonic waves as a priming technique for accelerating and enhancing the germination of barley seed: optimization of method by the Taguchi approach. Journal of Institute of Brewing, 114(1), 14-21.