Available online at http://bjas.bajas.edu.iq
https://doi.org/10.37077/25200860.2024.37.2.2
College of Agriculture, University of Basrah

Basrah Journal of Agricultural Sciences

ISSN 1814 – 5868 Basrah J. Agric. Sci., 37(2), 17-26, 2024 E-ISSN: 2520-0860

Sonication Assisted Callus Growth, Protein Content, and Plant Regeneration of Silybum *marianum* L.

Shifa M. Salih

Department of Biology, College of Education for Pure Sciences, University of Mosul, Iraq

***Corresponding author email**: dr.shifasalih@uomosul.edu.iq

Received 28th March 2024; Accepted 11th June 2024; Available online 31st December 2024

Abstract: *Silybum marianum* L. is considered one of the most extensively used medicinal plants worldwide due to its therapeutic benefits. While ultrasound waves were used to enhance the properties of numerous plant species. However, no study was investigated on applying ultrasonic waves to this particular plant. Therefore, the study amid to assess the impact of different exposure periods (0,10, 20, 25, 30, 35, and 40 minutes) of a frequency of 47.6 KHz on callus induction, protein content, and plant regeneration in *S. marianum*. The effect of ultrasonication was distinctive in accelerating callus induction of *S. marianum*, especially in short exposing periods (10 and 20 minutes). The percentage of callus formation reached 100%, 83.3% for cotyledons and stem respectively, when exposed to 20 minutes of ultrasound. Additionally, growth and total protein content were increased at 40 days and 80 days post- treatments. In contrast, long exposing periods (30, 35, and 40 minutes) had a negative impact on callus induction from all explants, as well as on callus growth and protein content. Moreover, ultrasonication stimulated one-step shoot regeneration during callus induction. The percentage of this phenomenon reached 100% for cotyledon at 10 and 20 minutes exposing period. This study confirmed the advantages of applying ultrasonic waves, particularly during shorter period to enhance the cultivation of *S. marianum in vitro*.

Keywords: Explants, Milk thistle, Shoot regeneration, Ultrasonic waves.

Introduction

Milk thistle or *Silybum marianum*, often known as cardus marianus, blessed thistle, and other names, is an essential medical plant belongs to Asteraceae family (Abbasi *et al*., 2010). Its fruits have been used for medicinal purposes, particularly to treat liver diseases (Sadowska *et al*., 2023). Milk thistle was discovered in the Mediterranean region, it is now widespread around the world. The main constituent of *S. marianum* is silymarin, which has promising anti-carcinogenic, anti-inflammatory, and anti-fibrotic properties. In addition to its cytoprotective, neuroprotective, and cardioprotective (Pickova *et al*., 2020). Recently, researchers reported that milk thistle extract may serve as an antioxidant, immunological booster, and growth stimulator (Abdel-Latif *et al*., 2023). Plant tissue culture is the most commonly employed biotechnology technique in the fields of primary and practical sciences (Abebaw *et al*., 2021**).**

In vitro culture of medicinal plants has become a reliable technique for the production of high amounts of plant material (Khan *et al*., 2021). Enhancing callus

induction such as in *Dianthus caryophyllus* (Mahood, 2021) and *Cuminum cyminum* (Salih & Al-Jirjees, 2023), plant regeneration, and the production of natural plant compounds is achieved through the application of various biotic or non-biotic stimulants. Consequently, numerous techniques have been tried to increase and improve the response of plant cells and secondary metabolites production (Tůmová *et al*., 2014).

Ultrasonic wave, characterized frequency is above 20 kHz. It is a physical stimulus that affects various types of living organisms. The impact of ultrasonic waves varies depending on cell type, response to this stimulator on the one hand, and intensity of the waves (Quarato *et al*., 2023). Using the frequency domain, two sets of these waves were distinguished:

1- More than twenty to one hundred kHz (low energy and intensity).

2-100 kHz and above which are called high-intensity and high-energy waves (Nowacka & Wedzik, 2016). The first set of ultrasound has received a lot of attentions due to its possible applications in biotechnology, biology, and agriculture (Rajewska & Mierzwa, 2017; Huang *et al*., 2022). Several plants have been reported to have increased cell and callus proliferation under the influence of ultrasonic waves such as *Corylus avellana* (Safari *et al*., 2013; Ghanati *et al*., 2015) and Soybean (Yang *et al*., 2015). Therefore, this study was conducted to confirm the stimulatory impact of sonication on callus biomass, shoot regeneration, and protein content of milk thistle. applying multi-drops and cross-section techniques.

Materials & Methods

Seed germination

This study was performed in the Laboratory of Plant Tissue culture, Research Unit, Biology Department, College of Education for Pure Sciences. Seeds *of S. marianum* were washed with tap water for about 15 minutes. Then, seed surface sterilization was performed according to Eari *et al*. (2017) with a few modulations. The seeds were submerged in a 3% sodium hypochlorite (NaOCl) commercial bleach solution for 20 minutes. Afterward, rinse in sterile distilled water three to four times. Then, sterile seeds were transferred to 100 ml glass jars containing 20 ml MS medium (Murashige & Skoog, 1962) medium free from growth regulators. For the first three days, the specimens were incubated in a culture room under completely dark circumstances. Then, they were transferred to photoperiod (light-dark) (16-8 hours) at 25±2 °C. The axenic seedlings were used as a source of explants.

Callus initiation

Thirty-day-old seedling explants including cotyledonary leaves, stems and leaves of *S. marianum* were used for callus induction. According to Al-Mashhadani (2018), the combination of (MS+1.0 $mg.L^{-1}$ naphthaleneacetic acid (NAA) and 0.5 $mg.L^{-1}$ 6-benzyl adenine BA) was applied for callus initiation from leave explants as the best medium. Thus, explants of cotyledons and stems were placed on the combination of MS medium + 2.0 $mg.L^{-1}$ NAA and 1.0 $mg.L^{-1}$ BA. All samples were incubated under 16/8 h (light/dark) photoperiod at 25 ± 2 °C in the growth room. The callus was re-cultured every 20 days at the same combinations.

Ultrasonic treatment

Different explants from thirty-day-old seedlings (true leaves, stems and cotyledonary leaves) of *S.marianum* were exposed to ultrasonic waves at a frequency of 47.6 KHz for 10, 20, 25,30, 35, and 40 minutes. The treated explants were cultured on the same

combinations as mentioned previously, and incubated in the culture room under the same circumstances. The callus fresh weight for both treated explants and control (untreated), was determined after 40 and 80 days from treatment.

Protein estimation

The method of Lowry *et al.* (1951) was used to estimate the total protein content of the 40- and 80-day-old callus initiating from ultrasonicated explants and the control.

Statistical analysis

The number of replicates was four for each treatment. Duncan's Multiple Range Test (DMRT) was used to separate means at significant level P≤0.05 (Duncan, 1955). SPSS software was used to analyze data.

Results & Discussion

It was pronounced from the results of this work that exposure of various parts of *S. marianum* to a 47.6 KHz frequency of ultrasound has an accelerated effect on callus induction. The time needed to begin callus initiation was seven days for all explants, compared to 12 days for the control (Table 1).

Table (1): Callus induction from explants of *S. marianum* exposed to a frequency of 47.6 KHz ultrasonic waves.

Exposure period (min)	*Callus initiation (%)			Beginning of callus induction (day)			P *value* (P≤0.05)
	Cotyle-dons	Stem	leaves	Cotyled-ons	Stem	Leaves	
10	91.6	83.3	83.3	7	7	7	0.7796^{NS}
20	100	83.3	83.3	7	7	7	0.3374^{NS}
25	75.0	66.6	66.6	9	10	9	0.6762^{NS}
30	33.3^{*}	58.3	66.6	10	11	11	0.0035^{*}
35	25.0^{*}	58.3	41.6	13	14	13	0.0014^{*}
40	25.0^{*}	41.6^{*}	25.0^{*}	13	14	13	0.0600^{NS}
Control	83.3	75.0	66.6	10	12	12	0.3796^{NS}
P *value* (P≤0.05)	0.0001^{*}	0.0016^{*}	0.0001^{*}				

* Significant difference at the 0.05 level by chi-square test NS: Non-significant

This reduction in time caused by ultrasound may be explained by the idea that sound is a catalyst, initiating phytohormone synthesis, particularly auxins (Kim *et al*., 2021).

Additionally, ultrasonication may facilitate the transfer of molecules within the cell such as water and oxygen. Moreover, hydrolytic enzymes could be activated by ultrasonic waves (Patero & Augusto, 2015). Furthermore, the percentage of callus formation increased to reach 100% and 83.3% from cotyledonary leaves, stem, and leaves exposed to 20 minutes respectively, which was the best treatment besides 10 minutes treatment. Furthermore, increasing the time of exposure to 30, 35, and 40 minutes had a negative effect through the reduction of callus induction ratio from all the explants. This may be due to damage of cell membrane and consequently loss of vital biological molecules. Peng *et al*. (2020) referred that the use of severe waves could have detrimental repercussions, of which disruption of plasma membranes. Koochani *et al*. (2020) reported that using the same intensity of ultrasound waves at various time intervals has a variety of outcomes either positively or negatively affecting the development and well-being of the somatic embryos of cucumber. As shown in table (2), the positive effect of short-term exposure to ultrasonic waves represented by 10 minutes and 20 minutes, had reflected on the fresh weight of callus initiated from all parts of the plant after 40 days. The highest values recorded in these periods were 10.9 g and 9.8g for stem and cotyledons callus respectively.

Table (2): Impact of ultrasonic waves on callus fresh weight after 40 days of exposure.

Exposure period (min)	Fresh weight (g) ±SD		
	Cotyledons	**Stem**	**leaves**
10	8.700 ± .0816 a	10.100 ± .1414 a	7.300 ± .1414 a
20	9.800 ±.1154 a	10.900 ± .1633 a	8.100 ± .4242 a
25	7.400 ±.1414 a	8.800 ± .2943 a	6.600 ± .2943 a
30	6.300 ±.1633 a	7.600 ± .1414 a	4.500 ± .3651 c
35	6.100 ± .11547 a	7.200 ± .0816 a	4.100 ± .3162 c
40	4.200 ± .0816 *b	6.100 ± .2160* b	3.200 ± .5944* b
Control	8.200 ± .1825 a	9.100 ± .2309 a	5.600 ± .5477 a
P *value*(P≤0.05)	.000*	.000*	.000*

* Significant difference at the 0.05 level by One-Way ANOVA table
Different small letters refer to significant differences, similar letters refer to non-significant differences

According to research, ultrasonic waves primarily affect plants mechanically via acoustic cavitation, which increases water uptake (Carrillo-Lopez *et al*., 2021). On the other hand, the long-term exposure period (30, 35, and 40 minutes) had a negative impact on the biomass of the callus, as the fresh weight of the callus decreased for all explants particularly the callus of leaves that reached 3.2 g (Table 2). Dobránszki *et al*. (2020) considered sonication a form of non-biotic stress. Therefore, long-lasting exposure to this stress causes growth inhibition by interfering with the metabolic activities caused by high microstreaming within the cells (Delran *et al*., 2023).

It was found that the effect of sonication is determined by the time of exposure, frequency and intensity of ultrasonic irradiation. Moreover, as a result of sonication treatment cellular ultrastructures, enzyme stability, cell development characteristics, the breakdown of extracellular polymers, DNA release from the nucleus, and a reduction in cell stability are all altered (Rokhina *et al*., 2009).

Similarly, the fresh weight of the callus 80 days post- treatment was increased at 10 and 20 minutes of exposure time. The best values were 14.1g and 12.7g for stem and cotyledon respectively compared to 11.3 and 10.3 for the control (Table 3).

Table (3): Callus fresh weight of *S. marianum* after 80 day of exposure to 47.6 KHz

Exposure period (min.)	Fresh weight (g) ±SD		
	Cotyledons	Stem	leaves
10	11.200 ± .1414 a	13.300 ± .2160 a	9.700 ± .18257 a
20	12.700 ± .1825 a	14.100 ± .0816 a	10.300 ± .1414 a
25	8.200 ± .1414 a	10.900 ± .1414 a	9.600 ± .2943 a
30	7.100 ± .2160 a	8.400 ± .1825 a	7.900 ± .0816 a
35	5.700 ± .0816 a	6.200 ± .2160 a	5.400 ± .2160 b
40	5.400 ± .1825* b	5.900 ± .1825* b	5.100 ± .1154* b
Control	10.300 ± .1825 a	11.300 ± .2160 a	8.600 ± .4242 a
P value(P≤0.05)	.000*	.000*	.000*

* Significant difference at the 0.05 level by One-Way ANOVA table
Different small letters refer to significant differences within-patients comparison, similar letters refer to non-significant differences

Conversely, the high period exposure time decreased callus fresh weight. Interestingly, ultrasonication had a positive effect on the total protein content of callus. The highest value recorded at 40 days post-treatment was (0.92 $mg.g^{-1}$) for leaves callus at 25 minutes (Fig. 1).

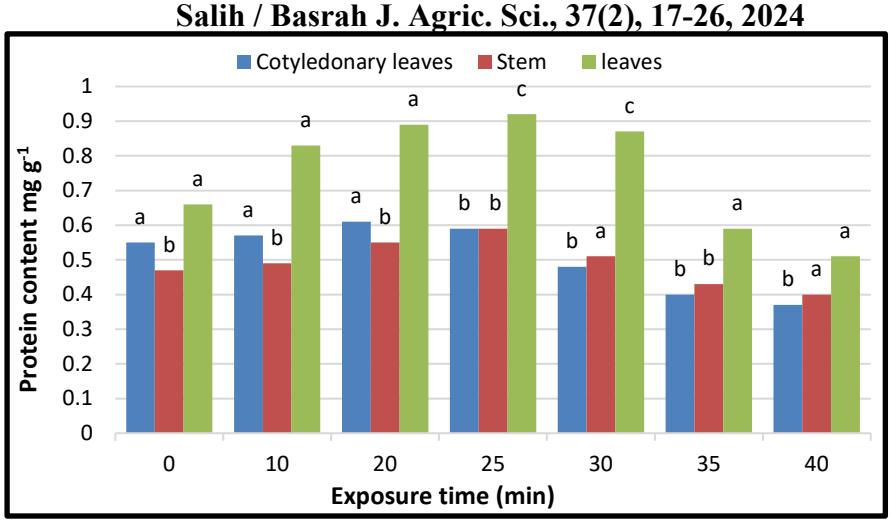

Fig. (1): Effect of ultrasonication on callus total protein content of *S. marianum* after 40 days of exposure.

On the other hand, even 30 minutes exposure treatment stimulated protein content especially for callus of stems and leaves, which reached 0.51 mg g^{-1} and 0.87 mg g^{-1} respectively compared to 0.47 mg.g^{-1} and 0.66 mg.g^{-1} for control. This increase may be due to the synthesis of stress proteins. Ultrasonic waves represent thermal and mechanical stresses for plants. Zhou *et al*. (2023) referred that different plant species have been found to contain a wide variety of stress proteins. This is because of the induction of heat shock proteins (HSPs) which accumulate as protective proteins. This suggestion is confirmed by other researchers (Bourgine & Guihur, 2021; González-Gordo *et al*., 2023). However, the results indicated that at 80 days post-treatment, the same manner was shown (Fig. 2).

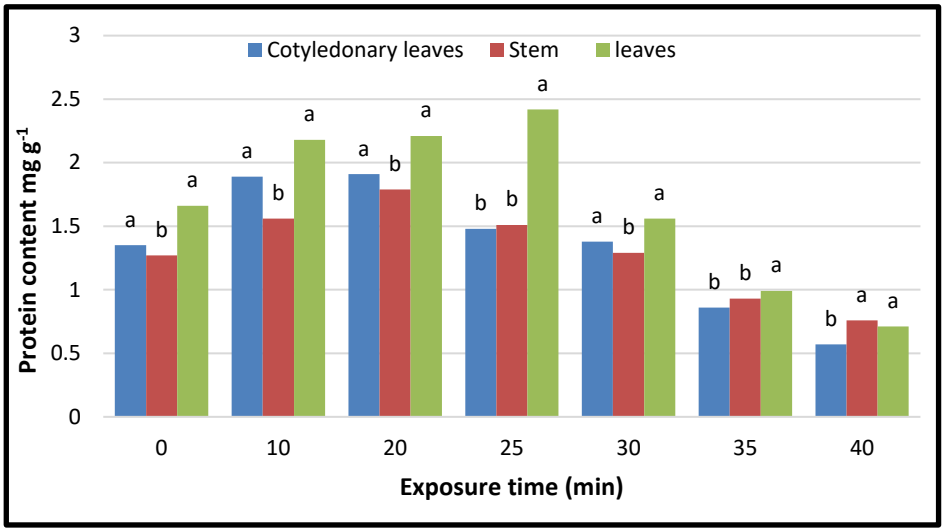

Fig. (2): The total protein content of callus derived from *S. marianum* explants after 80 days of exposure to 47. 6 KHz.

Increasing the total protein content was recorded in the callus of *Dendranthema morifolium* exposed to ultrasonics (Zhao *et al*., 2003). It is clear that there is a wide range of studies that used ultrasounic waves for various applications in different biological systems. However, only a few studies focused on applying this physical factor to stimulate *in vitro* shoot regeneration. In this work, ultrasonication of *S. marianum* explants induced shoot regeneration during callus induction (Table 4). Both 10 minute and 20 minute exposure time were suitable for all explants. However, the percentage was 100% for cotyledons and leaves at 10 minutes exposure time compared to 87.5% and 66.5% for control respectively. Organogenesis began after 20 days compared to 30 days for the control explant at the same medium of callus induction (Fig. 3a-c). The impact of ultrasonication on shoot regeneration also was confirmed in other plants such as *Cucurbita pepo* (Ananthakrishnan *et al*., 2007) and *Prunus armeniaca* (Pérez-Caselles *et al*., 2021). The regenerated shoots of milk thistle were rooted successfully in MS medium free from growth regulators (Fig. 3d).

Table (4): Effect of ultrasonic waves on shoot regeneration from callus of *S. marianum*

Exposure period (min)	Shoot regeneration (%)		
	Cotyledons	Stem	leaves
10	100	75	87.5
20	100	87.5	100*
25	87.5	50.0	87.5
30	37.5	50.0	75.0
35	0.0*	0.0*	0.0*
40	0.0*	0.0*	0.0*
Control	87.5	75	62.5
P *value* (P≤0.05)	0.0001*	0.0001*	0.0001*

* Significant difference at the 0.05 level by chi-square test

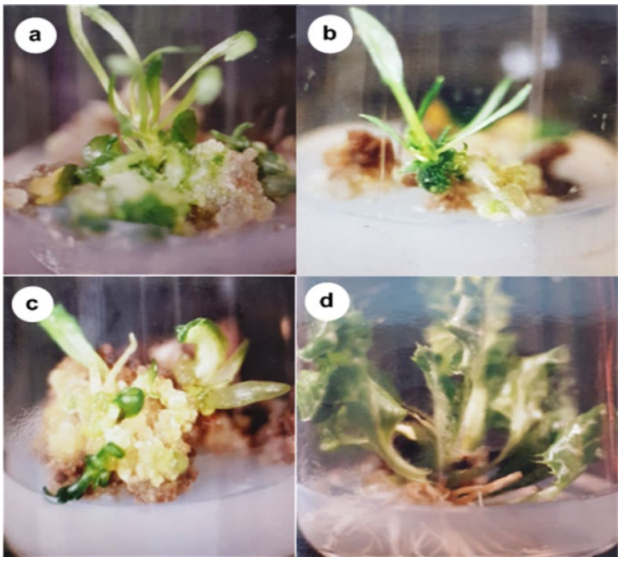

Fig. (3): Effect of ultrasonication on plant regeneration of *S. marianum*: (a) shoot regeneration from cotyledon explants exposed for 20 minutes (b) Shoot regeneration from stem explants exposed for 20 minutes (c) shoot regeneration from untreated cotyledon explants (control) after 30 days of cultured (d) regenerated *S. marianum* plant.

Conclusion

This work clearly showed that short-term exposure to ultrasonic waves can accelerate callus induction, growth, and increase total protein content, in addition to increasing shoot regeneration and consequently, plantlets formation. Therefore, treatment using ultrasonication can be employed as a novel and easy technique to enhance callus initiation and plant regeneration. Generally, using ultrasonic treatment in agriculture and biotechnology can be a safe and effective method. However further research on various culture media and sample exposure times to ultrasound are still needed.

Acknowledgment

I would like to express my gratitude to College of Education for Pure Sciences, University of Mosul for contributing resources that improved this research.

Contributions of Authors

Percent of contribution 100% by the author.

ORCID:

S.M.S.: https://orcid.org/0000-0001-6119-6631

Conflict of interests

The author declares that there is no conflict of interest concerning the publication of this paper.

References

Abbasi, B. H., Khan, M. A., Mahmood, T., Ahmad, M., Chaudhary, M. F., & Khan, M. A. (2010). Shoot regeneration and free-radical scavenging activity in *Silybum marianum* L. *Plant Cell, Tissue and Organ Culture*, *101*(3), 371-376. https://doi.org/10.1007/s11240-010-9692-x

Abdel-Latif, H. M., Shukry, M., Noreldin, A. E., Ahmed, H. A., El-Bahrawy, A., Ghetas, H. A., & Khalifa, E. (2023). Milk thistle (*Silybum marianum*) extract improves growth, immunity, serum biochemical indices, antioxidant state, hepatic histoarchitecture, and intestinal histomorphometry of striped catfish, *Pangasianodon hypophthalmus*. *Aquaculture, 562*, 738761. https://doi.org/10.1016/j.aquaculture.2022.738761

Abebaw, Y. M., Tobiaw, D. C., Abate, B. A., Eshete, B. K., Seymour, S. K., & Tesfaye, K. (2021). Plant tissue culture research and development in Ethiopia: a case study on current status, opportunities, and challenges. *Advances in Agriculture*, *2021*, 1-12. https://doi.org/10.1155/2021/9979549

Al-Mashhadani, A. R. M. (2018). *Supporting callus initiation and differentiation of Silybum marianum L. plant by exposing to two types of laser.* M. Sc. Thesis, University of Mosul. 89pp. (In Arabic).

Ananthakrishnan, G., Xia, X., Amutha, S., Singer, S., Muruganantham, M., Yablonsky, S., & Gaba, V. (2007). Ultrasonic treatment stimulates multiple shoot regeneration and explant enlargement in recalcitrant squash cotyledon explants *in vitro*. *Plant Cell Reports, 26*(3), 267-276. https://doi.org/10.1007/s00299-006-0235-1

Bourgine, B., & Guihur, A. (2021). Heat shock signaling in land plants: From plasma membrane sensing to the transcription of small heat shock proteins. *Frontiers in plant Science*, *12*,710801. https://doi.org/10.3389/fpls.2021.710801

Carrillo-Lopez, L. M., Garcia-Galicia, I. A., Tirado-Gallegos, J. M., Sanchez-Vega, R., Huerta-Jimenez, M., Ashokkumar, M., & Alarcon-Rojo, A. D. (2021). Recent advances in the application of ultrasound in dairy products: Effect on functional, physical, chemical, microbiological and sensory properties. *Ultrasonics Sonochemistry, 73*, 105467. https://doi.org/10.1016/j.ultsonch.2021.105467

Delran, P., Frances, C., Peydecastaing, J., Pontalier, P. Y., Guihéneuf, F., & Barthe, L. (2023). Cell destruction level and metabolites green-extraction of *Tetraselmis suecica* by low and intermediate frequency ultrasound. *Ultrasonics Sonochemistry, 98*, 106492.https://doi.org/10.1016/j.ultsonch.2023.106492

Dobránszki, J., Hidvégi, N., Gulyás, A., Tóth, B., & Da Silva, J. A. T. (2020). Abiotic stress elements in in vitro potato (*Solanum tuberosum* L.) exposed to air-based and liquid-based ultrasound: A comparative transcriptomic assessment. *Progress in Biophysics and Molecular Biology, 158*, 47-56. https://doi.org/10.1016/j.pbiomolbio.2020.09.001

Duncan, D. B. (1955). Multiple Range and Multiple F-Test. *Biometrics, 11*, 1-5.

Eari, S., Aghdasi, M., Ahmadzadeh, E., & Mianabadi, M. (2017). Influence of plant growth regulators on callus induction, silymarin production and antioxidant activity in Milk Thistle (*Silybum marianum* L. Gaertn.) under tissue culture medium. *Journal of Medicinal plants and By-product, 6*(1), 59-69.https://jmpb.areeo.ac.ir/article_113151.html

Ghanati, F., Safari, M., & Hajnorouzi, A. (2015). Partial clarification of signaling pathway of taxanes increase biosynthesis by low intensity ultrasound treatment in hazel (*Corylus avellana*) cells. *South African Journal of Botany, 96*, 65-70. https://doi.org/10.1016/j.sajb.2014.10.012

González-Gordo, S., Palma, J. M., & Corpas, F. J. (2023). Small Heat Shock Protein (sHSP) gene family from sweet pepper (*Capsicum annuum* L.) fruits: Involvement in ripening and modulation by nitric oxide (NO). *Plants, 12*(2), 389. https://doi.org/10.3390/plants12020389

Huang, Y., Mei, G., Fu, X., Wang, Y., Ruan, X., & Cao, D. (2022). Ultrasonic waves regulate antioxidant defense and gluconeogenesis to improve germination from naturally aged soybean seeds. *Frontiers in Plant Science, 13*, 833858. https://doi.org/10.3389/fpls.2022.833858

Khan, A., Shah, A. H., and Ali, N. (2021). *In-vitro* propagation and phytochemical profiling of a highly medicinal and endemic plant species of the Himalayan region (*Saussurea costus*). *Scientific Reports, 11*(1), 23575.https://doi.org/10.1038/s41598-021-03032-1

Kim, J. Y., Lee, H. J., Kim, J. A., & Jeong, M. J. (2021). Sound waves promote *Arabidopsis thaliana* root growth by regulating root phytohormone content. *International Journal of Molecular Sciences, 22*(11), 5739. https://doi.org/10.3390/ijms22115739

Koochani, M., Ahmad, M. A. J. D., Arbabian, S., Ghanati, F., & Marandi, S. J. (2020). A comparative study on the effects of ultrasound and some growth factors on somatic embryogenesis and artificial seed production in cucumber (*Cucumis sativus* L.). *Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 48*(4), 1915-1928.https://doi.org/10.15835/nbha48411669

Lowry, O. H., Rosebrough, N. J., Farr, A., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry, 193*(1), 265-275.https://www.jbc.org/article/S0021-9258(19)52451-6/fulltext

Mahood, H. E. (2021). Effect of plant growth regulators and explant source on the induction of callus of *Dianthus caryophyllus* L. *Basrah Journal of Agricultural Sciences*, *34*(2), 100-106. https://doi.org/10.37077/25200860.2021.34.2.08

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia plantarum, 15*(3), 473-497.https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

Nowacka, M., & Wedzik, M. (2016). Effect of ultrasound treatment on microstructure, colour and carotenoid content in fresh and dried carrot tissue. *Applied Acoustics*, *103*, 163-171. https://doi.org/10.1016/j.apacoust.2015.06.011

Patero, T., & Augusto, P. E. (2015). Ultrasound (US) enhances the hydration of sorghum (*Sorghum bicolor*) grains. *Ultrasonics Sonochemistry*, *23*, 11-15. https://www.sciencedirect.com/science/article/pii/S1350417714003277?via%3Dihub

Peng, Y., Zhang, Z., Kong, Y., Li, Y., Zhou, Y., Shi, X., & Shi, X. (2020). Effects of ultrasound on *Microcystis aeruginosa* cell destruction and release of intracellular organic matter. *Ultrasonics sonochemistry, 63*, 104909. https://doi.org/10.1016/j.ultsonch.2019.104909

Pérez-Caselles, C., Faize, L., Burgos, L., & Alburquerque, N. (2021).Improving adventitious shoot regeneration and transient Agrobacterium-mediated transformation of apricot (*Prunus armeniaca* L.) hypocotyl sections. *Agronomy, 11*(7), 1338.https://doi.org/10.3390/agronomy11071338

Pickova, D., Ostry, V., Toman, J., & Malir, F. (2020). Presence of mycotoxins in milk thistle (*Silybum marianum*) food supplements: A review. *Toxins*, *12*(12), 782. https://doi.org/10.3390/toxins12120782

Quarato, C. M. I., Lacedonia, D., Salvemini, M., Tuccari, G., Mastrodonato, G., Villani, R., & Sperandeo, M. (2023). A review on biological effects of ultrasounds: key messages for clinicians. *Diagnostics*, *13*(5), 855.https://doi.org/10.3390/diagnostics13050855

Rajewska, K., & Mierzwa, D. (2017). Influence of ultrasound on the microstructure of plant tissue.

Innovative food science and emerging technologies, 43, 117-129. https://doi.org/10.1016/j.ifset.2017.07.034

Rokhina, E. V., Lens, P., & Virkutyte, J. (2009). Low-frequency ultrasound in biotechnology: state of the art. *Trends in biotechnology, 27*(5), 298-306.https://doi.org/10.1016/j.tibtech.2009.02.001

Sadowska, K., Andrzejewska, J., Ligocka, A., Korczyk-Szabo, J., & Haban, M. (2023). Bioactive substances and microbiological quality of milk thistle fruits from organic and conventional Farming. *Applied Sciences, 13*(14), 8536. https://doi.org/10.3390/app13148536

Safari, M., Ghanati, F., Behmanesh, M., Hajnorouzi, A., Nahidian, B., & Mina, G. (2013). Enhancement of antioxidant enzymes activity and expression of CAT and PAL genes in hazel (*Corylus avellana* L.) cells in response to low-intensity ultrasound. *Acta Physiologiae Plantarum, 35*, 2847-2855. https://doi.org/10.1007/s11738-013-1318-6

Salih, S. M., & Al-Jirjees, R. F. (2023). The multi-drops and cross-sections, efficient methods for establishing cell suspension culture of *Cuminum cyminum* L. and plant regeneration. *Basrah Journal of Agricultural Sciences*, *36*(2), 47-58. https://doi.org/10.37077/25200860.2023.36.2.04

Tůmová, L., Tůma, J., & Hendrychova, H. (2014). Effect of ultrasound on the isoflavonoid production in *Genista tinctoria* L. suspension cultures. *Pharmacognosy Magazine*, *10* (Supplementary 2), S425.https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4078351/

Yang, H., Gao, J., Yang, A., & Chen, H. (2015). The ultrasound-treated soybean seeds improve edibility and nutritional quality of soybean sprouts. *Food research international, 77*, 704-710. https://doi.org/10.1016/j.foodres.2015.01.011

Zhao, H. C., Wu, J., Zheng, L., Zhu, T., Xi, B. S., Wang, B., & Younian, W. (2003). Effect of sound stimulation on *Dendranthema morifolium* callus growth. *Colloids and Surfaces B: Biointerfaces, 29*(2-3), 143-147. https://doi.org/10.1016/s0927-7765(02)00184-4

Zhou, P., Graether, S. P., Hu, L., & Zhang, W. (2023). The role of stress proteins in plants under abiotic stress. *Frontiers in Plant Science, 14*, 1193542. https://doi.org/10.3389/fpls.2023.1193542

تعزيز نمو الكالس، المحتوى البروتيني واخلاف نباتات *Silybum marianum L.* بالامواج فوق الصوتية

شفاء مهدي صالح

جامعة الموصل ، كلية التربية للعلوم الصرفة، قسم علوم الحياة

المستخلص: يعد نبات شوك الحليب أو الكلغان *Silybum marianum* L. من أكثر النباتات الطبية استخداماً في العالم نظراً لفوائده العلاجية. أستُخدمت الامواج فوق الصوتية لتحسين العديد من الانواع النباتية، ومع ذلك لاتوجد اي دراسة تناولت تطبيق الامواج فوق الصوتية على هذا النبات. وعليه تناول هذا العمل اختبار مدات تعريض مختلفة (10 ،20، 25، 30 ،35 و40 دقيقة) بتردد 47.6 KHz على استحداث الكالس، المحتوى البروتيني واخلاف نبات الكلغان. أظهرت النتائج بوضوح التأثير التحفيزي للأمواج فوق الصوتية في تسريع استحثاث الكالس لنبات *S. marianum* ولاسيما في مدات التعريض القصيرة (10 و20 دقيقة). وبلغت نسبة استحداث الكالس لقطع الاوراق الفلقية والسيقان 100 % و 83.3 % على الترتيب عند مدة التعريض 20 دقيقة. فضلاً عن ذلك نمو الكالس والمحتوى البروتيني الكلي ازداد بعد 40 يوماً و80 يوماً من التعريض للامواج فوق الصوتية. كذلك كان التاثير التحفيزي لهذه المعاملات واضحاً في تكون الافرع الخضرية بخطوة واحدة اثناء استحداث الكالس. أكد هذا العمل ايجابيات استخدام الأمواج فوق الصوتية في تحسين استجابة نبات *S. marianum* خارج الجسم الحي والتي ربما تؤثر على محتواه من المركبات الفعالة.

الكلمات المفتاحية: الجزء النباتي، شوك الحليب، انتاج الافرع الخضرية، الأمواج فوق الصوتية.