



ULTRASOUND IMPACT ON GREENHOUSE LETTUCE PRODUCTIVITY

Nasr G.E.M.¹; E.M. Darwish²; Y.F. Sharobeem³ and S.H. Abd-Elrahman^{*4}

¹Department of Agriculture Engineering, Faculty of Agriculture, Cairo University, Egypt.

²Department of Plant Physiology, Faculty of Agriculture, Cairo University Egypt.

^{3,4}Agriculture Engineering Research Institute (AEnRI) Research Center Egypt.

Email: hebaabedalrhman@yahoo.com

Abstract

This research aim is study the effect of ultrasound waves on the some marketing properties of lettuce plant. The experiment was carried out using hydroponic Nutrient Film Technique system (NFT) A- shape, in Giza governorate, through three economic period during the agricultural seasons of 2016/2017 under greenhouse growing conditions. Ultrasound device designed and assembled locally to generate different frequencies, with constant power ≈ 40 W. The present study seeks to the impact of ultrasound wave with different frequencies "F" of 20, 30 and 40 kHz, and exposure periods "EP" of 0, 60, 300 and 600 sec, on some marketing properties for lettuce (*Lactuca sativa L.*) i.e., fresh vegetative mass "V_m" (g), average leaves number "L_n", average leaf area "L_a" (mm²), stem firmness "S_F" (kg cm⁻²), and chlorophyll ratio "C_r" using randomize complete block design (RCB). Results affirmed that, using ultrasound frequency 30 kHz, with "EP" 60 sec recorded max. values of marketing properties of V_m (555.25g), L_n (27.75), and L_a (417.06 mm²). Meanwhile, S_F, recorded max. value of 7.01 kg cm⁻² and C_r of 30.73% at zero sec.

Key words: Ultrasound, hydroponic, greenhouse, lettuce plants.

Introduction

Application of biophysical methods i.e., microwave and laser radiation, magnetic field and ultrasound treatments, increased root mass up 24%, vegetative mass from 10 to 45%, yield from 10 to 50%, and increased resistance to pests and diseases, better qualitative characteristics of products (protein, sugar, vitamins etc), decreasing mineral fertilizers from 10 to 15%, decreasing application of pesticides (Vasilevski, 2003). Schulze *et al.* (2005) explained that stress factors on plant (stressors) can shared to two classes: biotic and/or abiotic factors. The abiotic stressors embrace the physical face as; wind, radiation, electrical, magnetic, water, temperature, soil movement, mineral salts, pollutants submergence, and gaseous toxins. Sound is a mechanical wave that travels in a medium, longitudinal and straight line fashion. There are three types of sound; A) Infrasound (frequencies less than 20 Hz), is not hearable by human ears, B) Acoustic or audible sound (frequency ranged between 20 Hz and 20kHz) is what human beings hear, C) Ultrasound or ultrasonic (frequencies higher than 20 kHz) cannot be heard by humans also. Sound and ultrasound can interact with biological tissues by thermal and mechanical processes (O'Brien, 2007; Whittingham *et al.*, 2007; Rokhina *et al.*, 2009; Hassanen *et al.*, 2013; Abuhamade *et al.*, 2014; and El-Nokrashy, 2015). Ultrasonic could be generate by; Piezo-electric generator and/or Magneto-striction generator (Don, 2010). It can affect the growth and developmental functions of plants at different levels by their characteristics i.e., frequency, period, amplitude, power, intensity, wavelength, propagation speed (Teixeira and Dobranszki, 2014). Ultrasonic application on plant had enhanced effects, where, on rice (*Oryza sativa L.*) cell number ml⁻¹, increased by 14 and 34.2 %, and the fresh weight by 28 and 55.5 %, and cultures were enhanced when exposed by ultrasound about 28 kHz, for 2 and 5 sec, (Liu *et al.*, 2003). Meanwhile, production yield of a secondary metabolite, and valepotriate, could be doubled in liquid culture, after sonicated by ultrasound 40 kHz, for 180 to 300 sec, (Stratuet *et al.*, 2012; and Russowski *et al.*, 2013). Soilless culture, including hydroponics, aquaponics, and aeroponics, is considered one

of the more innovative agricultural strategies to produce more from less, (Lal, 2016). Hydroponics offer many advantages for commercial agriculture. Where, arid lands, such as deserts, can be transformed into productive lands in greenhouses using limited amounts of water (reused and less is lost through evaporation and run-off), by allowing it and nutrient application directly in appropriate levels to the crop growth, to each plant roots. Likewise, the control over the wind, rain and sunlight, as well as, decrease the losses in yield and increase the quality (Furlani *et al.*, 1999, and Sorenson *et al.*, 2009). There are several design in greenhouse lettuce production i.e., Nutrient Film Technique (NFT), floating raft method, vertical tower method, and a final model is the ground/landscape pot or upright plastic bag model (Natalie *et al.*, 2018). Lettuce is a member of the Compositae (sunflower or daisy family). It is one of the oldest known vegetables. It is rich in minerals contents like potassium (very necessary to maintain appropriate levels of liquids in the body), manganese, magnesium, iron (required for the formation of red blood cells and the transportation of oxygen to different parts of the body), calcium and phosphorus together (for the correct well-being of the bones). These nutritional benefits of lettuce can help prevent anemia and aid in protecting the body from indigestive agents. Lettuce is an excellent source of vitamins A (which helps protect the eye), K, E, C, B₆ and thiamine (Bunning and Kendall, 2012). Also, it contains an antioxidant that has a medicinal property to prevent of certain type of (cancers, colon, prostate and lungs). It contains amino acids (necessary for the construction of muscular and nervous tissues) (Romani *et al.*, 2002).

The aim of research was to study the effect of ultrasound waves on the some marketing properties of lettuce plant i.e., vegetative fresh mass "V_m", leaves No., leaf surface area "L_a", stem firmness "S_F" and chlorophyll ratio "C_r".

Materials and Methods

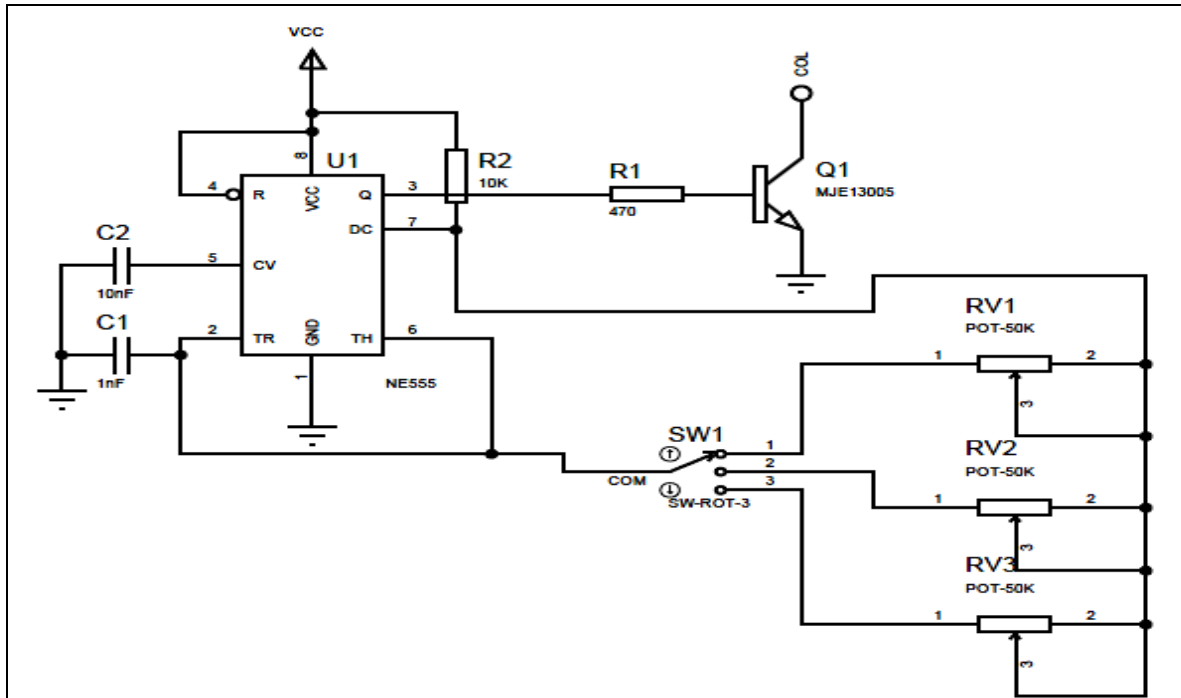
The research experiment was carried out using hydroponic Nutrient Film Technique system (NFT). The experiments include three economic periods in the

agricultural seasons of 2016/2017 under greenhouse growing conditions, using the following materials;

Ultrasonic generator circuit, It was designed and assembled locally have different frequencies "F" of 20, 30 and 40kHz, ($\pm 15\%$), with adjustable power 40W. Their components were shown in Fig. 1.

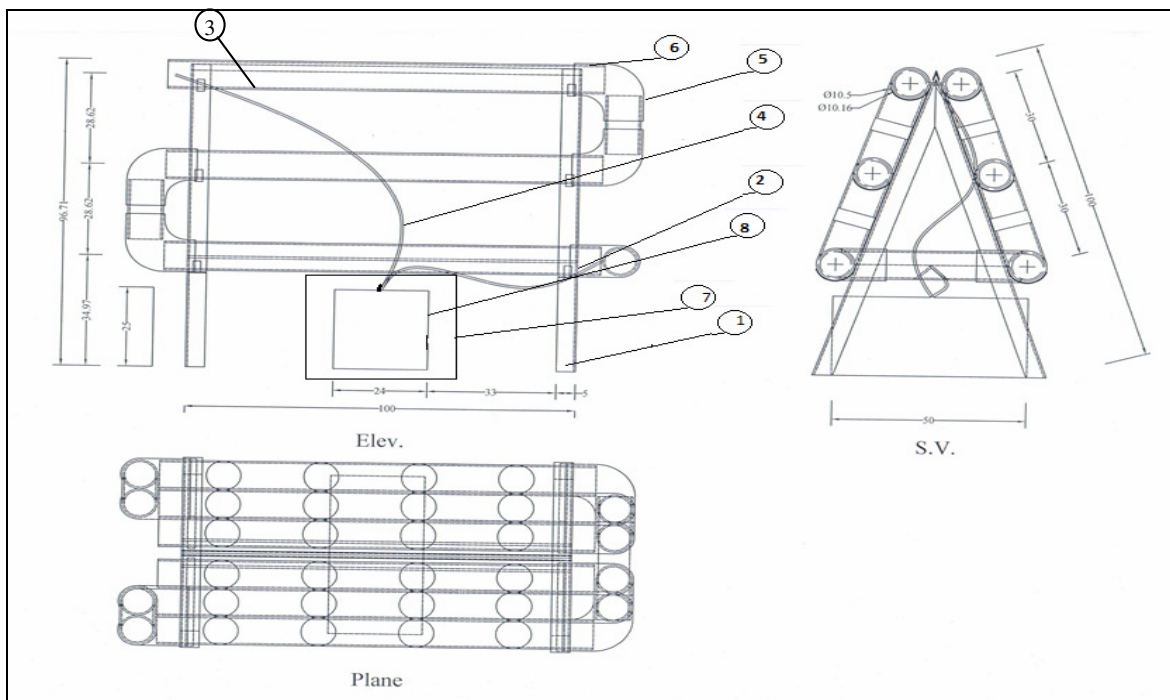
Greenhouse, The greenhouse dimensions were 9x3x2m for length, width, and height respectively. It covered with white cloth "seran" (200 μm , thickness, and 0.96 $\text{g}\cdot\text{cm}^{-3}$, density), used in the experimental to increase height temperature in summer.

Hydroponic unit: The NFT system "A-shape system" structure as illustrated in Figs. 2 and 3 mainly consists of; triangle steel frame has forming 60°, with dimensions of 100 x 100 x 3 cm for length, height and thickness, respectively. PVC are 6 tubes with 100 cm length, and 10.16 cm, (≈ 4 inch) diameter as hydroponic unit, fixed on frames at three levels, 40, 75, and 100cm, from ground. Tank with capacity of 30 L. submerged pump with 2.4 m, head, 1800 L. h⁻¹, discharge, AC 220-240V, and 50Hz, timer and nutrient solution composition was described by (El-Behiary, 2001) as shown in Table (1).



C1: Capacitor 1 μf ., R1: Resistor 450 Ω ., RV1; Resistor covariance 20kHz.
 C2: Capacitor 10 μf ., R2: Resistor 10K., RV2: Resistor covariance 30kHz.
 U1: Ultrasonic microphones EFROSB40K65 RV3: Resistor covariance 40kHz.
 IC1: NE 555 SW1: key switch.

Fig. 1 : Ultrasonic generator translator circuit components



1: Frame 2: Holder 3: Pipes 4: Tubes
 5: Stylized 6: Elbow 7: Pump 8: Tank

Fig. 2. : Main parts of hydroponic system.



1: Ultrasonic device 2: Frame 3: Tubes 4: Pipes

Fig. 3 : Hydroponic system and device

Table 1 : Element concentrations in the used nutrient solution

Element	Concentration (ppm)	Element	Concentration (ppm)
N	200	Mn	1.0
P	70	Cu	0.039
K	300	Zn	0.044
Ca	190	B	0.17
Mg	50	Mo	0.1
Fe	5.0		

Soilless media with peatmoss and perlite with ratios of 1:3 were used as, artificial media for supporting lettuce plants inside the hydroponics net through polyethylene pots (20 and 20 cm for diameter and length).

Lettuce plant (*Lactuca sativa L.*), variety Limor Hyp., supplied by private company, were allowed to grow till maturity.

Marketing parameters affected by ultrasound in plants i.e., fresh vegetative mass " V_m " were recorded by digital electrical balance with an accuracy of 0.001g. Leaves No " L_n " were counted and recorded. Leaf surface area " L_a " (mm^2) was determined using portable digital area meter. Penetrometer, ST 308 with accuracy $\pm 1\%$, was used to measuring stem strength (firmness). Chlorophyll meter was used to measuring chlorophyll ratio " C_r ".

After 70 days from transplanting, samples were harvested and collected from each period. Each period exposed to different ultrasound frequency individually (20, 30, 40 kHz), with three exposure periods (0, 30, 300, 600 sec). The number of lettuce plants used for each the experiment was 96 plants which potted in the same day.

The experimental design is randomized complete block design (RCB), with three replicates and two factors. Main factor: Effects of exposed frequency (F), and second factor: exposed period (EP), and data generated from the experiments were subjected separately to the proper statistical analyses of variance according to Snedecor and

Cochran (1976). Mean comparison between treatments and their interactions was determined using least significant difference (LSD) test at a 0.05 probability significance level.

Results and Discussion

From Figs. (4 through 6) it is clearly shown that exposed periods "EP" did not have any significant effects on " V_m " and " L_a ". Also, it was found that the highest " L_n " (26 and 25.25) with "EP" (60 and 300) sec. Where results, show clearly that increase "EP" only from 0 to 60 sec, significantly increased " L_n " by 7.22%. Meanwhile, the largest " L_a " were 413.97 and 405.73 mm^2 were obtained at "EP" 300 and 600 sec. Max and min values of " S_F " were 8.93 and 6.93 kg cm^{-2} recorded at "EP" 300 sec, and control (0 sec). The best " C_r " treatment at 600 sec and max. value was 33.69 and min value was 31.21% at zero sec and was non-significant with all "EP".

Generally, it was notable to annotation however, that V_m , L_n , and L_a , followed similar patterns, when treated plants with 30 kHz of ultrasonic waves. Where, V_m , L_n , and L_a has direct proportion with "EP" from 0 to 60 sec., thereafter the relation has negative proportion till 600 sec. (Figs. 4 through 6). Their values were improved slightly by increasing "EP" from 0 to 60 sec, about 12.62, 5.71, and 3.6%, respectively, as well as, their values decline sharply by increasing "EP" from 0 to 600 sec about 47.28, 22.53, and 6.83%, with the same arrangement. Plants with "EP" 60 sec, ranked last in terms of " S_F " (6.11 kg cm^{-2}), meanwhile, plants with "EP" 0 sec has the heights " S_F " (7.01 kg cm^{-2}). The best treatment to chlorophyll ratio zero sec time and max value was 30.73% and min value was 29.64% at 60 sec and was non-significant with all "EP".

At plants exposed by 40 kHz, plants with "EP" 600 sec., ranked last in terms of " V_m " (245.25g), meanwhile, plants with "EP" 60 sec., has the heights " V_m " (479.5g). Plants " V_m " value was improved slightly from "EP" 0 to 60 sec (0.52%), as well as, its value decline sharply about 30.4% from "EP" 60 to 300 sec, and 26.52%, from "EP" 300 to 600 sec. Although, there were negative significant differences in " V_m " between control and "EP" 300 and 600 sec. There were negative

exponential correlations between "L_n" and "EP", where, L_n = 29.822 e^{-0.75EP}, with R² ≈ 0.748. "EP" did not have any significant effects on "L_a". Fig. (7) revealed that "S_F" ranged between 8.23 and 5.20 kg cm⁻² "Ep" with control recorded the largest "S_F" followed by 60, 300, and 600 sec. Fig. (8) showed that best treatment to "C_r" zero sec time and max value was 32.18% and min value is 26.27% at 600 sec and was non-significant with all "EP".

The above results clear that the best stress on lettuce plant were ranging from 60 to 300 sec at 20 to 30 kHz to

obtain the highest L_a at 60 sec and C_r at 0 to 60 sec and 20 kHz, but for highest V_m, L_a and lowest S_f at 60 sec and 20 kHz that may due to the stress revealed that optimum values can be attributed to the elimination of cells that have increased the rate of growth. These were results agreement with Schulze *et al.* (2005). Then Similar results were demonstrated by Wu and Wu, 2007; Stratu *et al.*, 2012; Russowski *et al.*, 2013; Hassanen *et al.*, 2013; Jaime *et al.*, 2014; Teixeira and Dobranszki, 2014; Cao *et al.*, 2015; Ran *et al.*, 2015; Nazari *et al.*, 2017; Ozkurt and Altuntas 2018.

Frequency of ultrasound waves:

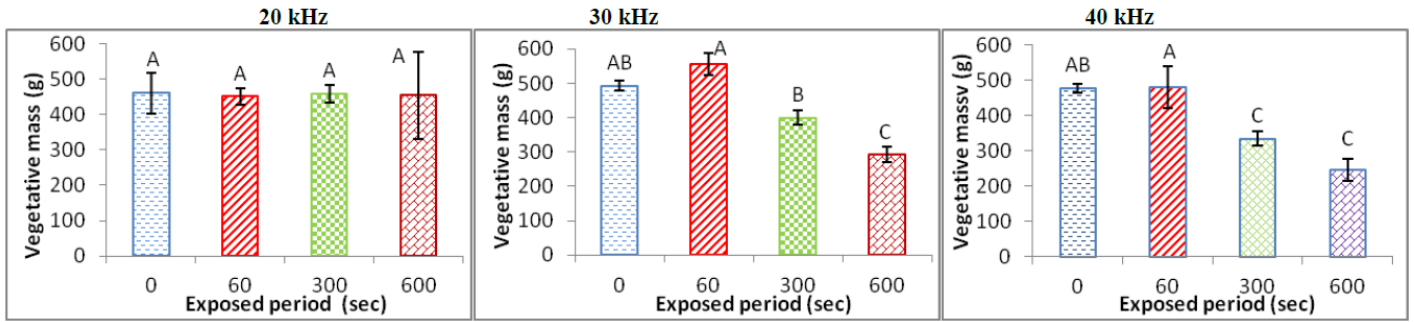


Fig. 4 : Effect of exposed period on vegetative mass at different ultrasound waves.

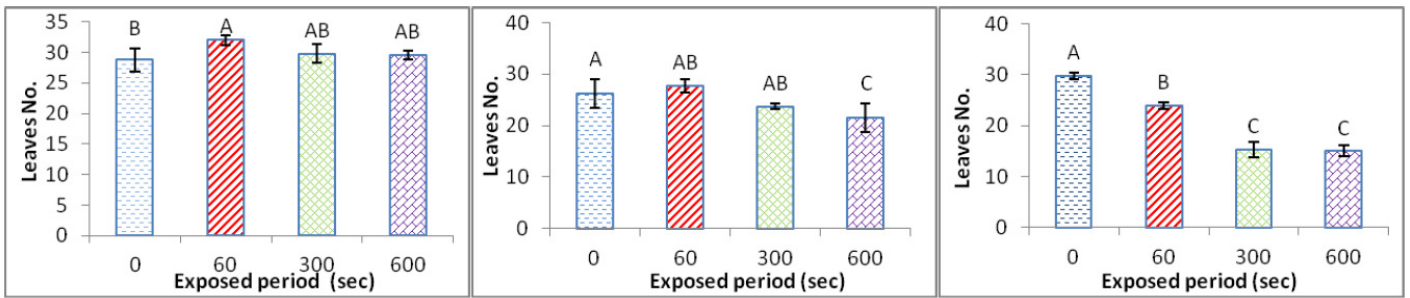


Fig. 5 : Effect of exposed period on leaves No at different ultrasound waves.

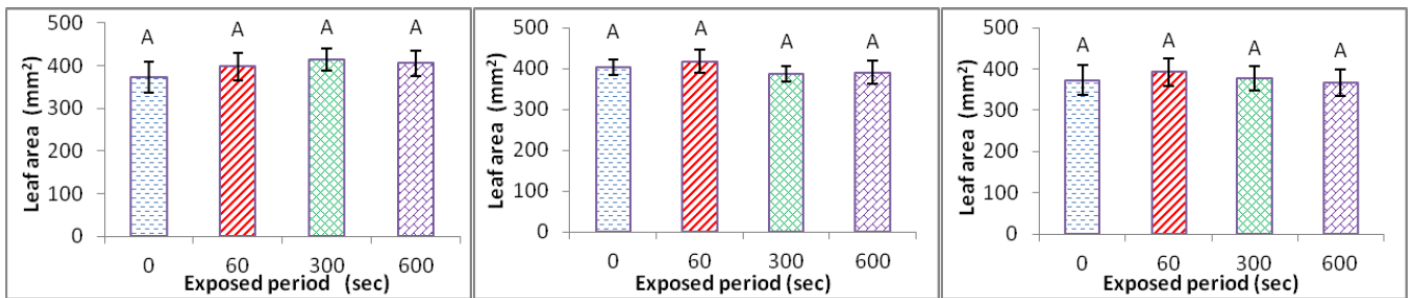


Fig. 6 : Effect of exposed period on leaf area at different ultrasound waves.

Frequency of ultrasound waves:

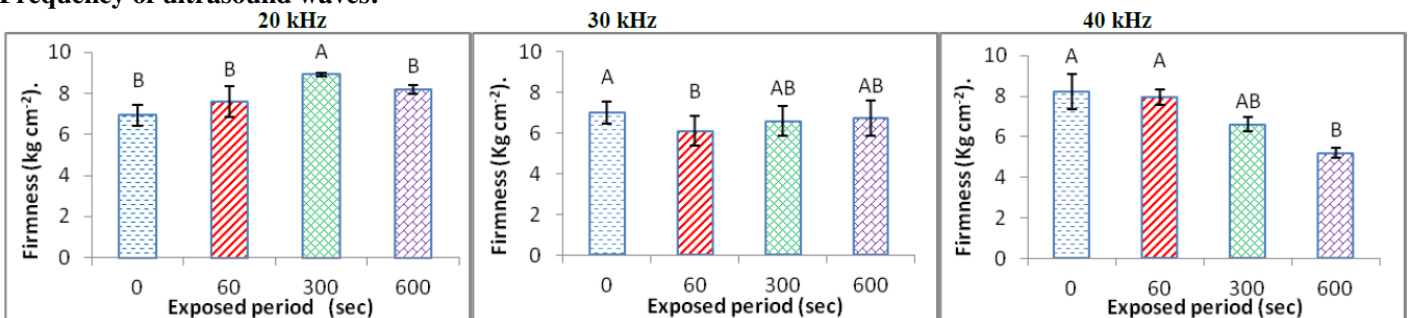


Fig. 7 : Effect of exposed period on firmness at different ultrasound waves.

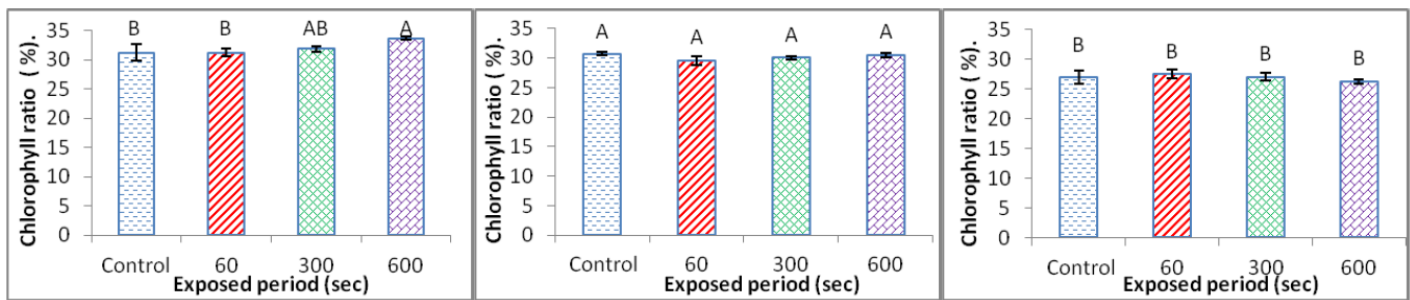


Fig. 8 : Effect of exposed period on chlorophyll ratio at different ultrasound waves.

Conclusions

The obtained results showed that the use of ultrasound waves is suitable to improve plant growth for lettuce in most properties especially when used at time 60sec, and frequency 20kHz, which gave an increase of fresh vegetative mass " V_m " (g), average leaves number " L_n ", average leaf area " L_a " (mm^2), stem firmness " S_F " (kg cm^{-2}), and chlorophyll ratio using randomize complete block design (RCB). Results affirmed that, using ultrasound frequency 30 kHz, with "EP" 60 sec recorded max. values of marketing properties of V_m (555.25g), L_n (27.75), and L_a (417.06 mm^2). Meanwhile, S_F , recorded max. value at (7.01 kg cm^{-2}), with 0 sec, and C_r (30.73%) at zero sec. Ultrasonic treatment significantly influence the growth of plants and the ultrasound treatment influences positively the chlorophyll pigment synthesis and pest the best effect being obtained when the time of exposure was short.

References

- Abuhamade, A.; Chaoui, R.; Jeanty, P.; and Paladini, D. (2014). Ultrasound in obstetrics and gynecology: A practical approach. Free E-Book "society of ultrasound in medical education".
- Bunning, M. and Kendall, P. (2012). Health Benefits and Safe Handling of Salad Greens. Colorado State University Extension Fact Sheet No. 9.373. <http://www.ext.colostate.edu/pubs/foodnut/09373.html>.
- Cao, H.; Huang, W., Qiao, J.; Wang, Y.P. and Zhao, H.J. (2015). Research on vibration mechanism of plant cell membrane with ultrasonic irradiation. Chinese Physical LETT. Vol.32, No. 3.
- Don, W.D. (2010). Acoustic Waves, Book. ISBN 978-953-307-111-4, pp. 466, Sciyo, Croatia, downloaded from SCIYO.COM.
- El-Behiary, U.A.; El-Shinawy, M.Z.; Medany, M.A. and Abou-Hadid, A.F. (2001). Utilization of "A-shape" system of nutrient film technique (NFT) as a method for producing some vegetable crops intensively. 5th IS Protect. Cult. Mild Winter Climate. Eds. Fernandez, Martinez & Castilla Acta Hort. 559: 581-586.
- El-Nokraschy, H.M. (2015). Infrasound under control nokraschy engineering. An de Masch 24: D-25488 Holm, Germany. p6.
- Furlani, P.R.; Silveira, L.C.P.; Bolonhesi, D. and Faquin, V. (1999). Cultivo hidropônico de plantas. Campinas: iac, 52p (boletim técnico 180).
- Hassanen, R.H.E.; Tian-zhen, H.; Ya-Feng, L. and Bao-ming, L. (2013). Advances in effects of sound waves on plants. Journal of Integrative Agriculture. 10.1016/S2095-3119 (13).
- Jaime, A.; Silva, T. and Dobranszki, J. (2014). Sonication and ultrasound: impact on plant growth and development. Plant Cell Tiss Organ Cult (2014) 117:131–143. Springer Science + Business Media Dordrecht.
- Lal, R. (2016). Feeding 11 billion on 0.5 billion hectare of area under cereal crops. Food Energy Secure. 5: 239–251.
- Liu, Y.Y.; Yoshikoshi, A.; Wang, B.C. and Sakanishi, A. (2003). Influence of ultrasonic stimulation on the growth and proliferation of *Oryza sativa* Nipponbare callus. Coll Surf B: Biointerfaces 27: 287–293.
- Meisam, N. and Mohammad, E. (2017). Impacts of ultrasonic waves on seeds: A Mini-Review. Agri Res & Tech: Open Access J 6(3): ARTOAJ. MS.ID.555688.
- Natalie, P.B.; Robert, C.; Hochmuth, W. and Laughli, L. (2018). An Overview of Lettuce Production Systems and Cultivars Used in Hydroponics and Protected Culture in Florida. Florida Univ.
- Nazari, M. and Eteghadipour, M. (2017) Impacts of Ultrasonic Waves on Seeds: A Mini-Review. Agri Res & Tech: Open Access J 6(3): ARTOAJ. MS.ID.555688.
- O'Brien, Jr W.D. (2007). Ultrasound-biophysics mechanisms. Progress in Biophysics and Molecular Biology, 93: 212-255.
- Ozkurt, H. and Altuntas, O. (2018). Quality Parameter Levels of Strawberry Fruit in Response to Different Sound Waves at 1000 Hz with Different dB Values (95, 100, 105 dB). Article. Agronomy, 8, 127. 10 of 13.
- Ran, H., Yang, L. and Cao, Y. (2015). Ultrasound on Seedling Growth of Wheat under Drought Stress Effects. Agricultural Sciences, 6: 670-675.
- Romani, A., Pinelli, P.; Galardi, C.; Sani, G.; Cimato, A. and Heimler, D. (2002). Polyphenols in Greenhouse and Open-Air-Grown Lettuce. Food Chem. 79: 337–342.
- Russowski, D.; Maurmann, N.; Rech, S.B. and Fett-Neto, A.G. (2013). Improved production of bioactive valepotriates in whole-plant liquid cultures of *Valerianaglechomifolia*. Ind Crops Prod 46: 253–257.
- Schulze, E-D; Beck, E. and Muller-Hohenstein, K. (2005). Environment as Stress Factor: Stress Physiology of Plants. In: Schulze E-D, Beck E, Muller-Hohenstein K, editors. Plant Ecology. New York, NY: Springer. p 7-11.
- Snedecor, G.A. and Cochran, W.G. (1976). Statistical Method. Iowa State Univ. Press, Ames.

- Sorenson, R.; Technician, E. and Relf, D. (2009). Extension Specialist, Horticulture, Virginia Tech.Home Hydroponics. Virginia cooperative extension.426-084.
- Stratu, A.; Peptanariu, M. and Anghelache, O. (2012). Aspects regarding the behavior of the *Satureja Hortensis* l. Species to the ultrasound treatment. *Analele Științifice ale Universității, Al. I. Cuza” Iași s. II a. Biologievegetală*, 58(1): 59-64.
- Teixeira, J.A. and Dobranszki, J. (2014). Sonication and ultrasound: impact on plant growth and development. *Plant Cell Tiss Organ Cult* (2014) 117:131–143.
- Vasilevski, G. (2003). Perspectives of the application of biophysical methods in sustainable agriculture. *bulg. J. Plant Physiol.* 179–186.
- Whittingham, T.A. (2007). Medical diagnostic applications and sources. *Progress in Biophysics and Molecular Biology*, 93: 84-110.
- Wu, M.Y. and Wu, J. (2007). In-vitro investigations on ultrasonic control of water chestnut. *Journal of Aquatic Plant Management* 45: 76-83.