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# Ultrasonic Waves Stimulate the Activity of Thymine Nucleotide Biosynthetic Enzymes, Nucleic Acids and Proteins Content of Sesamum Indicum L. Stem Calli

*Nihal E. Al-Taee, Sajida A. Abood and Mozahim K. Al-Mallah* *

## ABSTRACT

The present study revealed that the effect of ultrasonic waves in stimulating the activity of the enzymes thymidylate synthase (TS), dihydrofolate reductase (DHFR) and serine hydroxy methyl transeferase (SHMT), the amount of nucleic acids (DNA and RNA) and the protein content. In addition, the changes in fresh weight of seedling stem-derived callus of Sesamum indicum L. grown in initiation medium Murashige and Skoog (MS) + naphthalene acetic acid (NAA 1.0 mg L-1) + benzyl adenine (BA 2.0 mg L-1) which was previously exposed to 50 KHz of different periods (5, 10, 15, 20, 30, 40, 60 and 70 min). The period 40 min exposure had sustainable effect through enhancement the specific activity of TS, DHFR, and SHMT enzymes which recorded 2.08, 0.95 and 0.28 µmol/min/mg protein respectively compared with 1.48, 0.41 and 0.13 in control. The amounts of DNA and RNA were increased from 56, 530 to 75, 710 µg/g callus fresh weight respectively compared to control. On the other hand, the protein content and the fresh weight of callus were increased from 1.43 to 2.25 mg/g and 3.45 to 10.88 g respectively compared to control. Unfortunately the exposure period 70 min decreased the specific activity of the above enzymes, nucleic acids, as well as the fresh weight of callus.

**Keywords**: Thymine Nucleotide Biosynthetic Enzymes, Ultrasonic Waves, Callus, Sesame.

## 1. INTRODUCTION

Plant tissue culture is an important tool of plant biotechnology. Recently more uses have been found for ultrasound in plant tissue culture (Lin 2003), that deal with the applications of sound frequen- cies in the inaudible range generally from 20-100 kHz, although special applications occur outside that range (Gupta and Ibaraki 2008). One of its potential applications is studying the activity of nucleotide biosynthesized thymine enzymes. This is an important step in the de novo pathway of DNA synthesis and repair to produce deoxy thymine monophosphate (dTMP) from deoxy uridine monophosphate (dUMP) by methylation cycle (Montgmery *et al*., 2004). This pathway is catalyzed by three enzymes: thymidylate synthase (TS), dihydrofolate reductase (DHFR) and serine hydroxy methyl transeferase (SHMT). Using any inhibitors of the above enzymes change their activities and blocked DNA biosynthesis. Therefore, these enzymes are an important target for chemo- therapeutic drugs of tumor cells as anticancer treatments. Consequently, the biological effects of ultrasound primarily included mechanical effect, thermal effect and cavitations effect (Shi *et al*., 2002). Recent studies (Xie and Liu, 2010) have been shown that low intensity ultrasound increases enzyme activities or promotes the cell growth by improving the mass transfer and stimulating physio- logical activities of cells (Barton *et al.*, 1996). The frequency and the energy amount required for the influence of ultrasonic treatment appear to vary widely between species and cultivars. Mild ultra-sonic waves stimulate protein syn-thesis in plant cells and protoplasts significantly (Joersbo and Brunstedt, 1990) and affect plasma membrane permeability (Peng *et al*., 2012). Ultrasonic treatment was reported to cause reversible inhibition of DNA, RNA and protein synthesis

* Department of Biology, College of Science; and College of Education, University of Mosul, Iraq. Received on 5/9/2012 and Accepted for Publication on 12/2/2013.

in *Pisum sativum* root meristem cells (Miller *et al*., 1976).

It seems that limited infor- mation are available about ultra- sound effect on plant tissue culture. The aim of this research is to find the effect of ultrasonic waves on the activity of dTMP biosynthetic enzymes, the amount of nucleic acid and the protein content in callus cultures of *Sesamum indicum* L., a plant belonging to the family Pedialaceae.

## 2. MATERIALS AND METHODS

**Initiation cultures of stems callus:**

Seeds of *Sesamum indicum* L., used as a source of plant material, were obtained locally from Mosul-Iraq. They were surface sterilized with 96% ethanol for 2 min, immersed in 3% sodium hypochlorite (NaOCl) solution for 5 min and washed thoroughly with sterilized distilled water. The sterilized seeds were cultured on agar-solidified Arnon and Hoagland medium (Arnon and Hoagland, 1944) and incubated in culture room at 23± 2°C for germination. At age of 12-15 days, seedling stems segments were excised at 1.5 cm and cultured on agar-solidified Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) containing 1.0 $mgL^{-1}$ NAA and 2.0 $mgL^{-1}$ BA. All cultures were incubated under fluorescent tube light (2000 Lux) at 23± 2°C, 8/16 respectively.

- **Exposure stems callus to ultrasonic waves:**

A set of callus samples taken at weight of 1.0 gm each were placed in 100 mL glass tubes. They were exposed to ultrasonic waves at frequency of 50 kHz for 5, 10, 15, 20, 30, 40, 60 and 70 min (Salih and Al–Obaedi 2011). The exposed samples were then cultured on agar solidified MS medium.

- **Assay of thymine nucleotide biosynthetic enzymes activity in aqueous extract of callus:**

Samples (3.0 gm) each of callus cultures grown in initiation medium at 60 days old were exposed to Ultrasonic waves as described above. They were crushed in glass mortar at 4°C in the presence of 30 mL of 50 mM potassium phosphate buffer pH 7 containing 0.56% of N-acetyl cysteine. The homogenate mixture was sonicated by probe soniphire at 20 kHz/second for 0.5 min (Romany, PG-1545). The mixture was centrifuged at 9000 rpm for 1 hr at 4°C and the supernatant was stored in deep freeze for further experiments.

- **Thymidylate synthase (TS) (EC 2.1.1.45):**

Activity measurements of TS was estimated spectrophotometrically by following the increase in the absorbance at 340 nm in the presence of dUMP as a substrate and THF as a cofactor (Friedkin, 1963).

- **Dihydrofolate Reductase (DHFR) (EC 1.5.1.3):**

The activity of DHFR was estimated by spectrophotometric method (Osborne and Huennekens, 1958) which based on the decrease of absorbance at 340 nm in the presence of DHF as a substrate and NADPH as hydrogen donor.

- **Serine hydroxyl methyl transferase enzyme SHMT (EC 2.1.2.1):**

The activity of SHMT was assayed spectrophotometrically by following the decrease in the absorbance at 298 nm in the presence of serine as substrate and THF as a cofactor (Uyeda and Rabinowitz, 1968).

- **Assessment of nucleic acids content:**

Nucleic acids extracted from sesame callus were assayed spectrophotometrically which depends on inhibiting nuclease enzymes activity and precipitating both DNA and RNA. Their concentrations were estimated by comparison with the standard curve prepared from pure yeast cells (Cherry, 1962). The amount of DNA was determined from standard curve using calf thymus DNA (Giles and Mayer, 1967).

- **Total protein and fresh weight determinations of callus tissue:**

Total protein extracted from callus tissues was estimated according to the modified method of Lowery using bovine albumin as a standard (Schacterle and Pollack, 1973). The fresh weight of callus was also determined.

## 3. RESULTS

- **Ultrasonic waves affect the specific activity of (TS, DHFR, SHMT) enzymes and DNA and RNA content:**

The results proved the stimulating effect of ultrasonic waves in both specific activity of enzymes TS, DHFR, SHMT and the nucleic acids (DNA and RNA) content in callus tissue of sesame previously exposed to 50 KHz

frequency used in this study. The period 40 min encouraged the specific activity of TS, DHFR, SHMT enzymes which reached 2.08, 0.95, 0.28 µmol\min\mg protein respectively, compared to 1.48, 0.41 and 0.13 µmol\min\mg in the control (Table 1). Again, in the same table, an increase in the DNA level to 75 µg/gm when compared to 56 µg/gm in the control specimen. Also, RNA content reached to 710 µg/gm compared to 530 µg/gm in the control. Whereas, 70 min used for callus exposure reduced nucleic acids amount and the specific activity of above enzymes.

**Table (1): Exposure Effect of Sesame Stem Callus to 50 KHz Ultrasonic Waves in Specific Activity of Biosynthetic Thymine Enzymes and Nucleic Acids Content**

| Treatments min | Specific activity of enzymes µmol\min\mg protein ∓ (SE) | | | Nucleic acid content (µg\gm) | |
|---|---|---|---|---|---|
| | TS* | DHFR** | SHMT*** | DNA | RNA |
| Control | **0.432**1.470 ∓ | **0.002**0.412 ∓ | **0.011**0.130 ∓ | 56 b | 530 e |
| 5 | **0.123**1.082 ∓ | **0.031**0.621 ∓ | **0.011**0.164 ∓ | 57 b | 554 d |
| 10 | **0.081**1.181 ∓ | **0.042**0.643 ∓ | **0.002**0.142 ∓ | 59 b | 566 c |
| 15 | **0.092**1.161 ∓ | **0.070**0.542 ∓ | **0.010**0.145 ∓ | 41 d | 460 g |
| 20 | **0.095**1.210 ∓ | **0.051**0.681 ∓ | **0.004**0.188 ∓ | 58 b | 581 b |
| 30 | **0.085**1.194 ∓ | **0.007**0.586 ∓ | **0.003**0.198 ∓ | 45 d | 420 h |
| 40 | **0.161**2.081 ∓ | **0.601**0.951 ∓ | **0.031**0.289 ∓ | 75 a | 710 a |
| 60 | **0.090**1.372 ∓ | **0.012**0.375 ∓ | **0.004**0.110 ∓ | 52 c | 510 f |
| 70 | **0.032**0.650 ∓ | **0.002**0.121 ∓ | **0.001**0.098 ∓ | 35 e | 210 i |

***TS**: The amount of enzyme required to produce 1 µmol of DHF/min/mg protein. ** **DHFR**: The amount of enzyme required to oxidize 1 µmol of NADPH/min/mg protein., ***SHMT:** The amount of enzyme required to convert 1 µmol of THF to methylene THF/min/mg protein. No. of replicates 3/ treatment.
∓ SE : ∓ Standard Error, Values carry different letters refer to significant variations at $p < 0.0001$.

- **Effect of Ultrasonic waves on the total protein and callus fresh weight :**

The results showed a limited increase in the protein content of stem-derived callus which subjected to ultrasonic waves. The highest level of protein was 2.15 mg/gm fresh weight obtained with the exposing period of 40 min compared to 1.46 mg/gm in the control. Whereas, the expose period of 70 min decreased clearly the protein content of callus (Fig 1.B). Data in Fig (1.A) showed that ultrasonic waves enhanced the fresh weight of callus that exposed to 50 KHz. The 40 min exposing period was so good for callus treatment where 1.0 gm fresh weight of treated callus increased to 10.88 gm (Fig 2.B). Interestingly, all selected periods were successful except the 70 min period (Fig 2.C) as the fresh weight reduced to 2.71 gm compared to 3.45 gm in the control (Fig 2.A).

## 4. DISCUSSION

In the present study, the efficient stimulation of the activity of TS, DHFR, SHMT enzymes and the enhancement of nucleic acids (DNA and RNA) content by exposure to ultrasound waves might due to both mechanical and stable cavitations effects of 50 KHz ultrasound waves. This accelerates the convection transport and improve the membrane permeab- ility, supporting the uptake of foreign substances and the release of intracellular products in the cells. However, this expected effects are widely believed to be the main factors explaining the ultrasonic enhancement of biochemical reac-reactions (Xie and Liu 2011). Recently, they found that this might be due to the defense responses

of cells to the damage evoked by the ultrasound as demonstrated by investigator (Lin and Wu 2002; Lin *et al.,* 2001). It is most likely that through the function of Ultrasound, cells might be damaged in some ways, inducing the mechanism defense of these cells (Lin and Wu, 2002). It was well established in plant sciences that plants can react to biotic and a biotic stimuli with an array of defense responses, such as the production of active oxygen species, the cross linking of cell- wall proteins, and the accumulation of secondary metabolites. Some of these responses have been induced by various physical and mechanical agitation, wounding, and hydro-static pressure have demonstrated the defense responses evoked by ultrasound in plant cell suspension cultures (Lin *et al*., 2001).

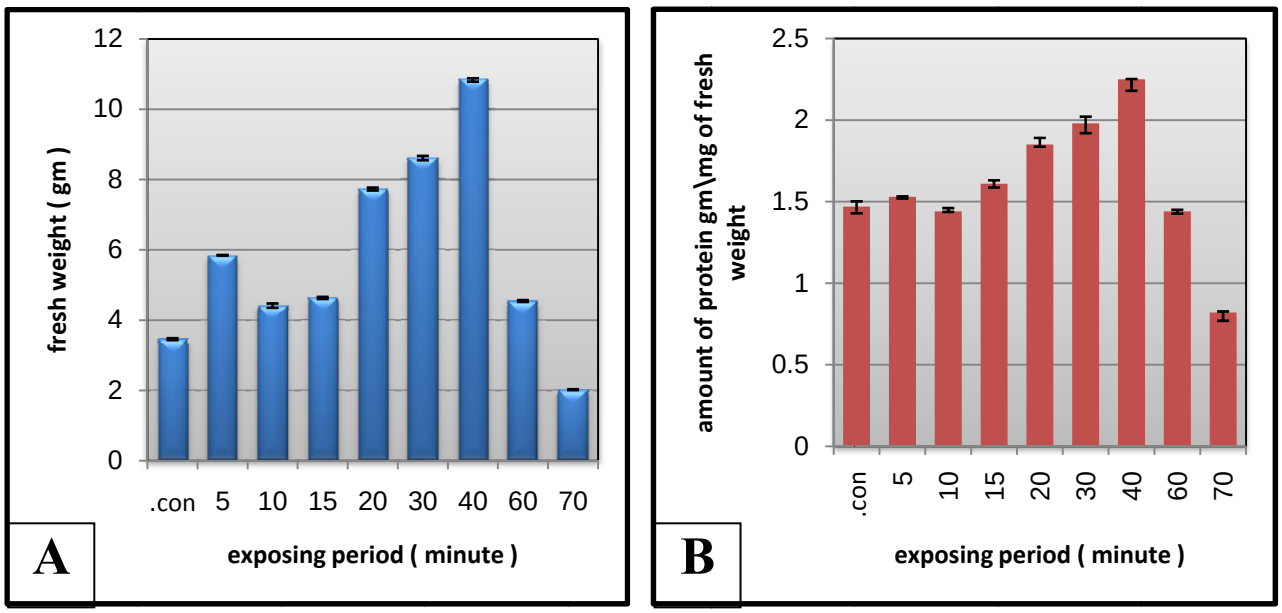


**Fig. (1) : Effect of exposure *Sesamum indicum* L. callus o 50 KHz ultrasonic waves in fresh weight (A) and protein content (B) after 30 days. ⊤ : represent Standard deviation**

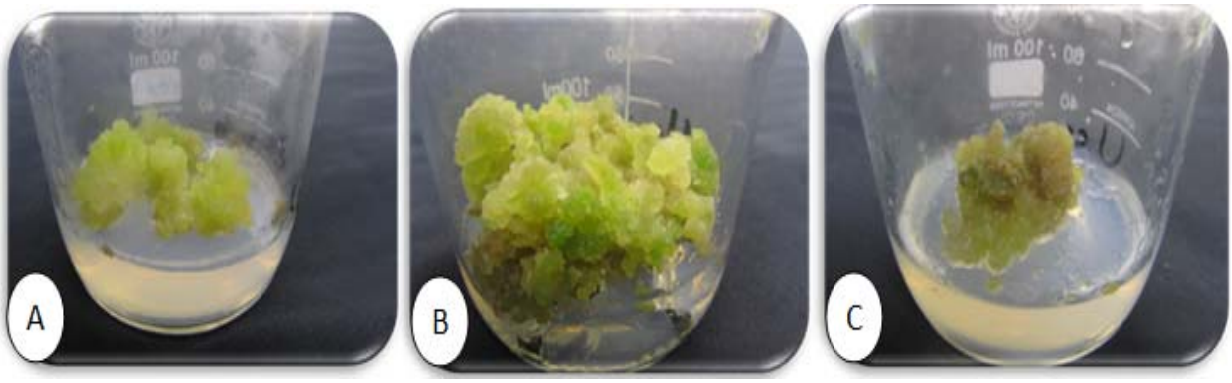


**Fig. (2) : Effect of exposure to 50 KHz ultrasonic waves on growth of *Sesamum indicum* L. callus after 30 days.**

**(A): Control (B): At 40 min period. (C): At 70 min period**

Low intensity ultrasound might stimulate enzyme activity. Barton *et al.,* (1996) compared the activities of some glycosidase enzyme in the presence and absence of ultrasound. Other studies showed an increase of invertase activity toward sucrose in using ultra- sound waves. They concluded that ultrasonication could produce effi- cient homogeneous reaction mixture and facilitate diffusion to the active sites of the enzymes, reducing the depression

of secondary metabolites to enzyme activity. Few studies have observed a similar mechanism of enzyme activity stimulation. A study by other investigators (Lin *et al.,* 1997) reported that the activity of inulinase could get 60% rise in the presence of ultrasound of 20W. It was observed a more significant increase in activity in the presence of ultrasound for immobilized enzyme than for free enzyme (Zong *et al*., 2000; Vercet *et al*., 2002). Ultrasound waves influence the enzyme inactivation through series of effects including thermal effect, generation of free radicals by water sonolysis, the mechanical forces caused by micro- streaming and shock waves (Price 1992; Ayatollah *et al*., 2011) alone or incombi- nation, can damage the enzyme protein structure integrity resulting in reducing enzyme activity (Vercet *et al*., 1998).

Generally, the stimulating effect of ultrasonic waves of fresh weight and protein content in *Sesamum* callus is likely due to the formation of small pores or cavities known as acoustic cavitations in cells, enhancing the uptake of ions, nutrients, growth regulators and amino acids from medium to the cell (Lamberova and Kosolapova 2008; Salih and Al-Obaedi, 2011). On the other hand, the decreases of both fresh weight and protein content when this callus exposed to 50 KHz waves for 70 min might be attributed to the mechanical effect of ultrasonic waves, which in sometimes, leads to degeneration and death of cells (Lui *et al*., 2005). Several investigators pointed out that these waves inhibited DNA, RNA synthesis and protein product in *Pisum sativum* (Suslik, 1988).

## 5. CONCLUSIONS

From the results of the present study it was clear that ultrasonic waves express a positive effect on the activity of thymine nucleotide biosynthetic enzymes, nucleic acids content and proteins of *S. indicum*. The suggestion that this technique is potential to be used in plant biotechnology to explain this steps in other plants.

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# الموجات فوق السمعية تحفز فعالية إنزيمات بناء نيوكليوتيد الثايمين، محتوى الأحماض النووية والبروتينات لكالس سيقان السمسم *(Sesamum Indicum L.)*

***نهال عزت الطائي، وساجدة عزيز عبود، ومزاحم قاسم الملاح****

## ملخص

أظهرت الدراسة الحالية تأثير الموجات فوق السمعية في تحفيز نشاط مجموعة إنزيمات الثايميدليت سنثيز (TS) والداي هيدروفوليت ريدكتيز (DHFR) والسيرين هيدروكسي مثيل ترانسفيريز (SHMT). كما أنها أدت إلى زيادة كمية الأحماض النووية (DNA و RNA) ومحتوى البروتينات فضلاً عن التغييرات الحاصلة في الوزن الطري للكالس المشتق من سيقان بادرات السمسم (Sesamum indicum L.) النامية في ظروف معقمة على وسط MS الصلب الحاوي نفثالين حامض الخليك (1.0 ملغم لتر−1) وبنزيل أدنين (2.0 ملغم لتر−1) التي سبق تعريضها بتردد 50 كيلو هيرتز ولمدد (5، 10، 15، 20، 30، 40، 60، 70 دقيقة). وتفوقت مدة التعريض 40 دقيقة في زيادة الفعالية النوعية لإنزيمات (TS) و(DHFR) و(SHMT) لتسجل 2.08، 0.95، 0.28 مايكرو مول/ دقيقة/ ملغم بروتين على التوالي قياساً بـ 1.48، 0.41، 0.13 مايكرومول/ دقيقة/ ملغم بروتين لعينة المقارنة على التوالي. وبلغت كمية الحامض النووي DNA 75 مايكروغرام/غرام وزن طري والحامض النووي RNA 710 مايكروغرام/غرام وزن طري قياساً بمحتواها في عينة المقارنة البالغة 56 مايكروغرام DNA/غرام وزن طري و530 مايكروغرام RNA/ غرام وزن طري. وسجل المحتوى البروتيني 2.25 ملغم/غرام من وزن الكالس قياساً بـ 1.43 ملغم /غرام في عينة المقارنة وتحفز الوزن الطري للكالس إلى 10.88 غرام قياساً بـ 3.45 غرام في عينة المقارنة. وأدت مدة التعريض 70 دقيقة إلى خفض الفعالية النوعية للإنزيمات المشار أليها وكمية الأحماض النووية وكذلك الوزن الطري للكالس.

**الكلمات الدالة:** إنزيمات بناء نيوكليوتيد الثايمين، الأمواج فوق السمعية، الكالس، السمسم.

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