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Studies of the Effect of Ultrasonic Waves on: IV – Germination, Growth Regulators and Nucleic Acid Contents of Peanut Seedlings

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Abstract. The effect of ultrasonic waves on seed germination, nucleic acid contents as well as growth regulators of peanut seedlings were carried out. The data obtained showed that, ultrasonification has affected seed germination as well as water content of peanut, that lowest percentage of germination and water content were obtained at short exposure to ultrasonic waves (15 minutes).

The DNA and RNA contents were decreased by ultrasonic waves especially at long exposure (60 minutes). Ultrasonification of peanut seedlings induced a marked reduction in cytokinin activity. At long exposure (60 minutes), auxin content of peanut seedlings showed marked increasing in treated seedlings as compared with untreated until 72 hours. However, gibberellin contents showed verse manner as that of auxin, thus short exposure (15 minutes) recorded the highest values until 72 hours.

Introduction

Investigations of the biological effects of ultrasound dated back to 1928 by Harvey and Loomis[1]. Krishnamurthy *et al.*[2] observed that the ultrasonication has sharply reduced the germination of *Vigna sinensis* L. and percentage of germination decreased gradually with increasing time of ultrasonication. The decrease of the percentage of germination may be due to the oscillations produced by ultrasound that causes force and stress on the cell membrane, which further becomes the reason for the loss of osmotic integrity of the cell. Such loss directly leads to the cell death Hedges and Leeman[3]. Yamada and Ueda[4] observed the effects of ultrasonication on root tips of *Vicia faba* and reported chromosome breaks, vacuolization of nuclei and necrosis. The effects of ultrasonic

waves on the karyology were also observed by different workers by employing *Allium cepa* (Selman[5]), maize, cucumber and melon (Geogrgieva *et al.*[6]) and *Vicia faba* root tips (Slotova *et al.*[7], Gregory *et al.*[8] and Miller *et al.*[9]).

Newcomer[10] showed that ultrasonication is able to cause changes in the chromosomes of plant cells, throughout breaking up by the vibrations and the resulting pieces observed to recombine in regular ways, or if the radiation is strong enough they may be destroyed. He also added that ultrasonic affected the growth pattern of cells by depolymerizing action on products within the cell, such as deoxyribonucleic acid (DNA), and starch. These effects are not due to localized heating or cavitations, but are due to mechanical effects resulting from ultrasonic vibrations directly.

Elpiner[11] used needles vibrating at 25 KHZ for treating *Nitella* cells. He observed mechanical perturbations such as eddying of the cell contents at low amplitudes and destruction of the cell in violent and chaotic fluid motions at high amplitudes. Hedges and Leeman[3] recorded nuclear psychosis and fragmentation of nuclei in human lymphocytes exposed to ultrasonication.

Ravi Meher *et al.*[12], Weinberger and Piener[13] and Krishnamurthy *et al.*[2] observed that the seedling growth were gradually decreased by ultrasonication except at short time (5 minutes) and attributed that the ultrasonication increased the activity of indol oxidase enzyme which controls the oxidation of auxin production (responsible for growth).

Abhayavardhani and Bhalla[14] recorded various types of chromosomal aberrations in *Nigella sativa* with ultrasonic exposure. They added that the percentage of chromosomal aberrations were directly proportional to the increase in the dose levels. Riesz and Takashi[15] showed that the deleterious effects of ultrasonic were due to mechanical effects in DNA degradation, inactivation of enzyme lipid peroxidation and cell killing.

The present work is an attempt to elucidate effect of ultrasonication on the seed germination, DNA, RNA and growth regulators of peanut seedlings.

Material and Methods

1 – Seed germination and ultrasonication

Seeds of peanut (Arachis hypogea linn. Cultivar Giza 4) were used. Pure strains of seeds were supplied by Egyptian Agriculture Research Center, Giza. Seeds were presoaked in distilled water for 13 hours. Ultrasonication was carried out (using ultrasonic apparatus. Bkansonic, Mod. B-5200. HF – out power = w. working frequency = 47. KHZ + 6%) for 15 and 60 minutes, at a time 200 seeds taken 500 ml of distilled water. The level of water was kept uniform dur-

ing exposure. Control seeds were kept without ultrasonication. The percentage of germination, fresh and dry weight as well as water contents were investigated in treated and untreated seedlings after 24, 48 and 72 hours.

Another lot of seeds were pre-soaked in distilled water for 13 hours, then germinated until radical become 2-3 cm. Ultrasonication was carried for 15 and 60 minutes. Control seedlings were kept without ultrasonication. Nucleic acid and growth regulators were investigated in treated and untreated seedlings after 3,24,48 and 72 hours.

2 – Extraction, fractionation and bioassay cytokinins

a – Extraction

Extraction of plant material was carried out according to the method described by Ghazi[16] using ethyl alcohol (80%) for extraction, then ground with 50% ethyl alcohol for three times during the extraction, period for 12 hours using an electric stirrer. After filtrations, the extracts were combined together and concentrated under vacuum to few ml. The residue was dried at 100ºC till constant weight.

b – Fractionation

Fractionation of plant extract was carried out according to the method described by Nitsch and Nitsch[17]. The concentrated extract was brought to pH 9, the alkaline aqueous solution was shaken four time with an equal quantities of petroleum ether (60-80). The aqueous fractions were acidified to pH 2.5 and extracted four times with an equal quantity of ethyl acetate, aqueous containing cytokinins were dried, the residue was dissolved in few ml of ethyl alcohol (80%) and loaded on paper chromatography (15 × 30 cm) with volume equal to 0.1 g dry weight. Loaded chromatograms were developed in a descending manner using isopropanol: ammonia 25% : water (10:1:1 v/v) as a running solvent. After development, each chromatogram was cut transversely into 10 equal strips, each strip was divided into small pieces and being eluted overnight at 5ºC with 5 ml of distilled water in a small petri-dish, in which the solutions were ready for bioassay. The method used in this investigation for the bioassay of cytokinins was essentially similar to that of Esashi and Leopold[18].

3 – Extraction, separation and quantitative analysis of auxins and gibberellins

Extraction and separation were carried out according to the method described by Hegazy[19]. Analysis of auxins and gibberellins using GLC analysis Vogel[20].

4 – Determination of nucleic acid contents

The method of determination of RNA and DNA was carried out according to Schmidt and Thannhuser[21] and its modification described by Morse and Carter[22].

a – Extraction

A known weight of air-dried powdered plant materials was extracted with 5% TCA, then it was three times with 5 ml methanol chloroform in the ratio of 1:2. The delipidated material was dissolved in 2 ml of 1N KOH at 37ºC for 16-20 hours and precipitated with 0.4 ml of 6N HCl, then it was centrifuged. The precipitate contains the DNA fraction, while the supernatants contains RNA. TCA was added to the supernatant to give final concentration of 5% TCA. Then it was centrifuged, the supernatant constituted the RNA fraction. The precipitate was hydrolyzed in 5 ml of 5% TCA at 90ºC for 30 minutes, cooled and then centrifuged and the supernatant constituted the DNA fraction.

b – Estimation

Quantitative determination of RNA was described by Dische[23] and by Burton[24].

Results

1 – Effect of ultrasonication on the germination and water content of peanut seedlings

Figure (1) shows that ultrasonication affected percentage of germination at short exposure (15 minutes) and brought about stimulatory action at long exposure (60 minutes).

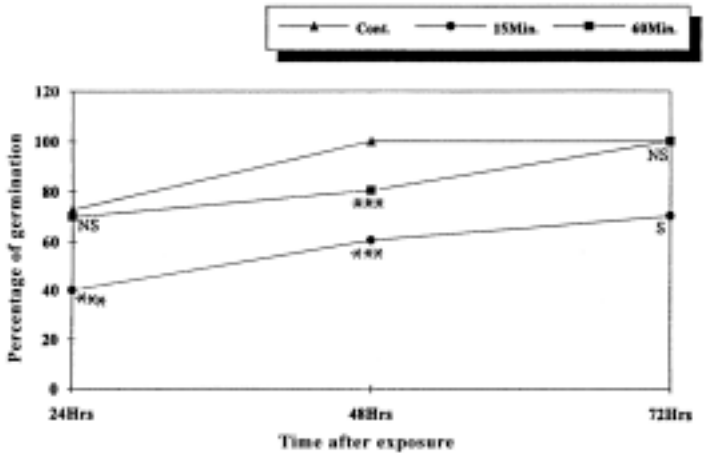

FIG. 1. Effect of ultrasonication of germination of peanut seeds.

Figure (2) shows that percentage of water contents increased with time after exposure for 24, 48 and 72 hours respectively. The results reveal that short exposure to ultrasonic waves (15 minutes) has retarded germination more than long exposure (60 minutes).

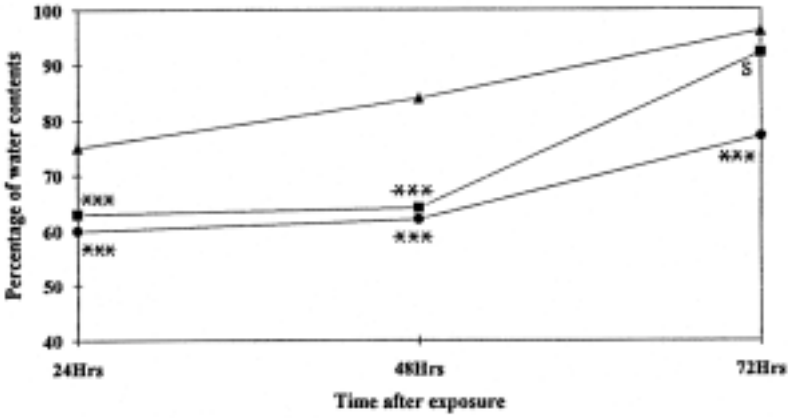

FIG. 2. Effect of ultrasonication of water contents of peanut seedlings.

2 – Effect of ultrasonication of nucleic acid contents of peanut seedlings

It was shown from Fig. (3) that DNA and RNA contents detected in the treated and untreated seedlings were gradually increased with lapse of experiments.

Ultrasonication specifically long exposure (60 minutes) was more effective on DNA and RNA contents than short exposure (15 minutes).

3 – Effect of ultrasonication on the growth regulators of peanut seedlings

The results obtained in Fig. (4a&b) reveal that there was a marked increase in the activity of cytokinin during the experimental period. Ultrasonication of peanut seedlings induced a marked reduction in cytokinin activity as being compared with control. After 3 hours of exposure, the untreated seedlings contained three significant promoting zones having cytokinin activity, with R_F value fallling between: 0.0-0.1, 0.2-0.3 and 0.8-0.9 respectively. Short exposure to ultrasonic waves decreased the activity level and only one significant promoting zone having cytokinin activity with R_F value falling between: 0.2-0.3 at the same period was noticed, while with long exposure no activity of cytokinin substance was detected (Fig. 4a). After 24 hours of exposure, there were about 5 significant promoting zones having cytokinin activity with R_F values falling between: 0.0-0.1, 0.3-0.4, 0.4-0.5, 0.6-0.7 and 0.8-1.0 in untreated seedlings, four significant zones having cytokinin activity their R_F values falling between: 0.0-0.1, 0.3-0.4, 0.6-0.7, and 0.8-0.9 at short exposure and two significant zones

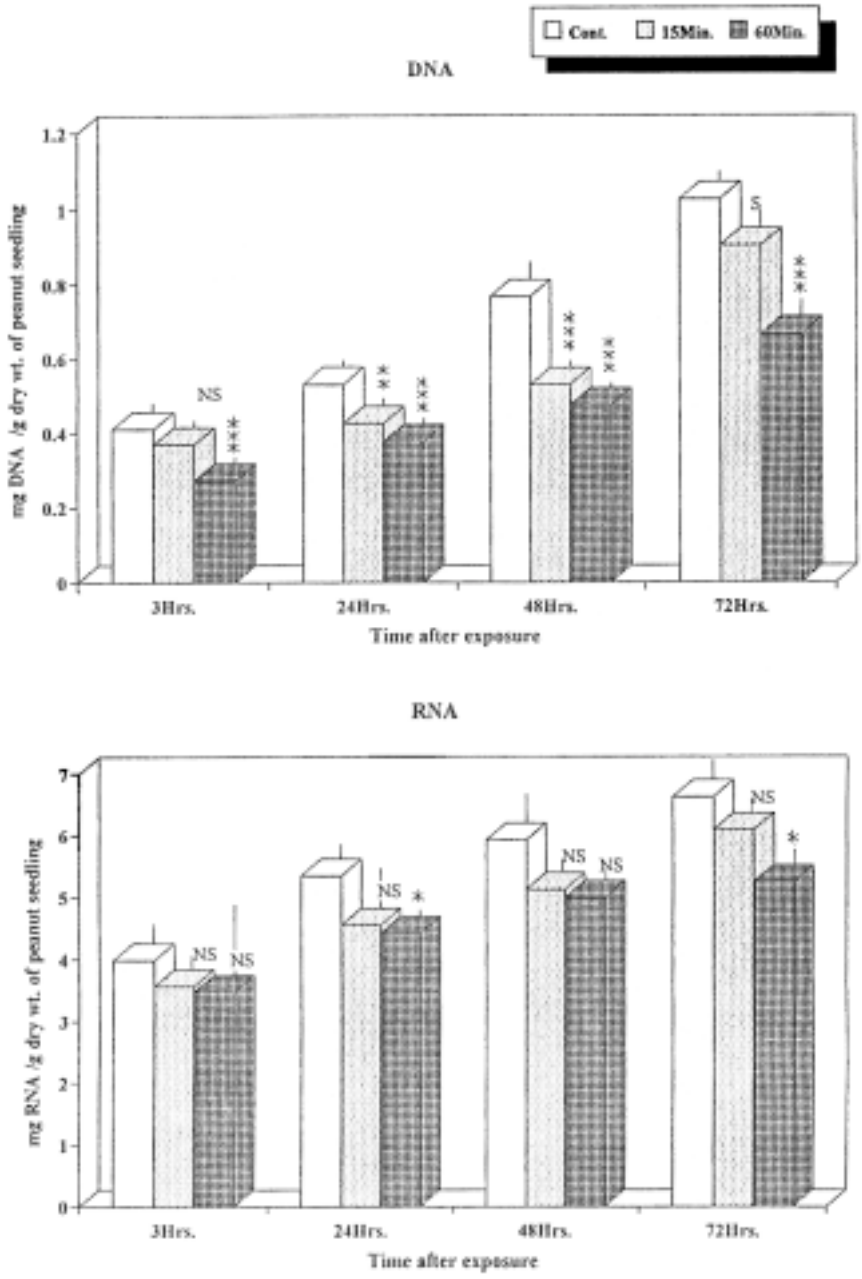

FIG. 3. Effect of ultrasonication on DNA and RNA contents of peanut seedlings.

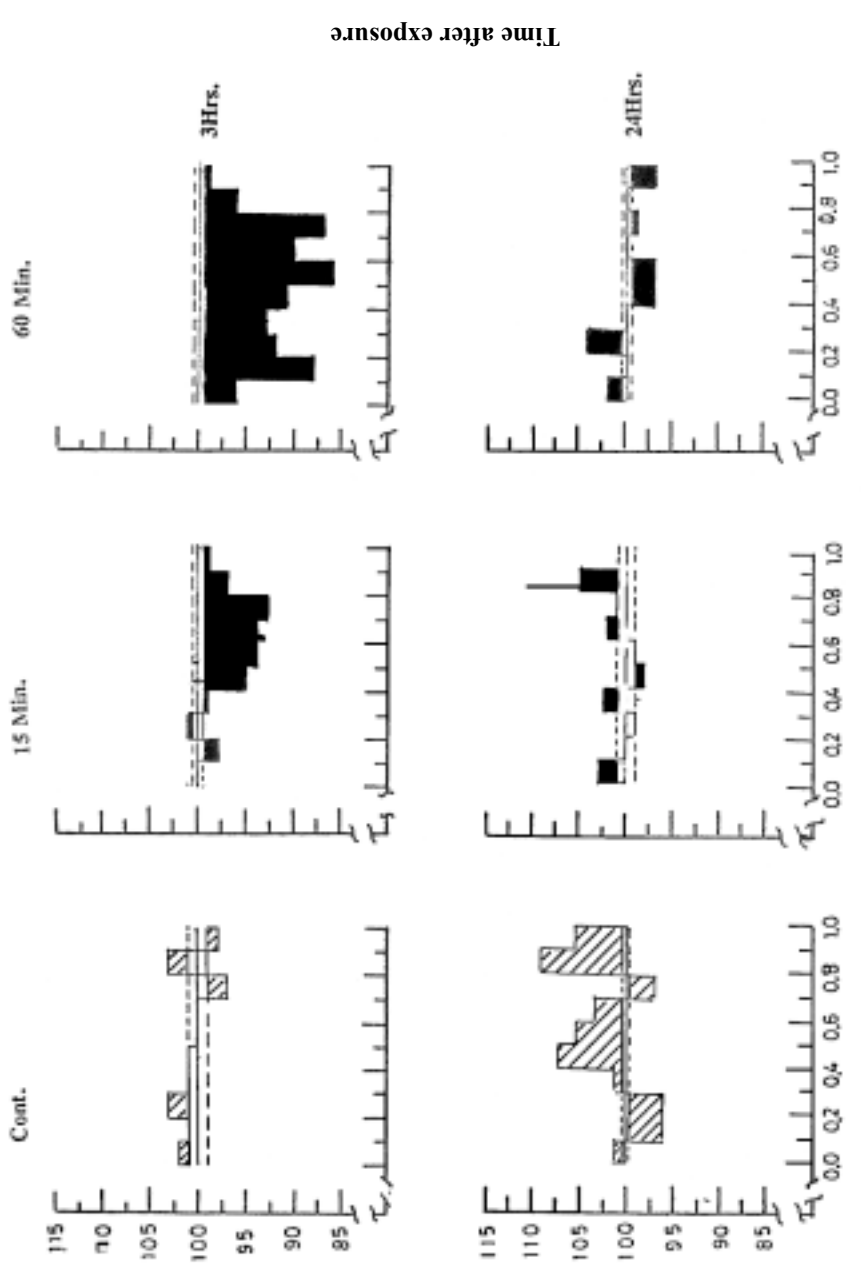

FIG. 4a. Effect of ultrasonication on cytokinin substances content in the fractionated extract of peanut seedlings.

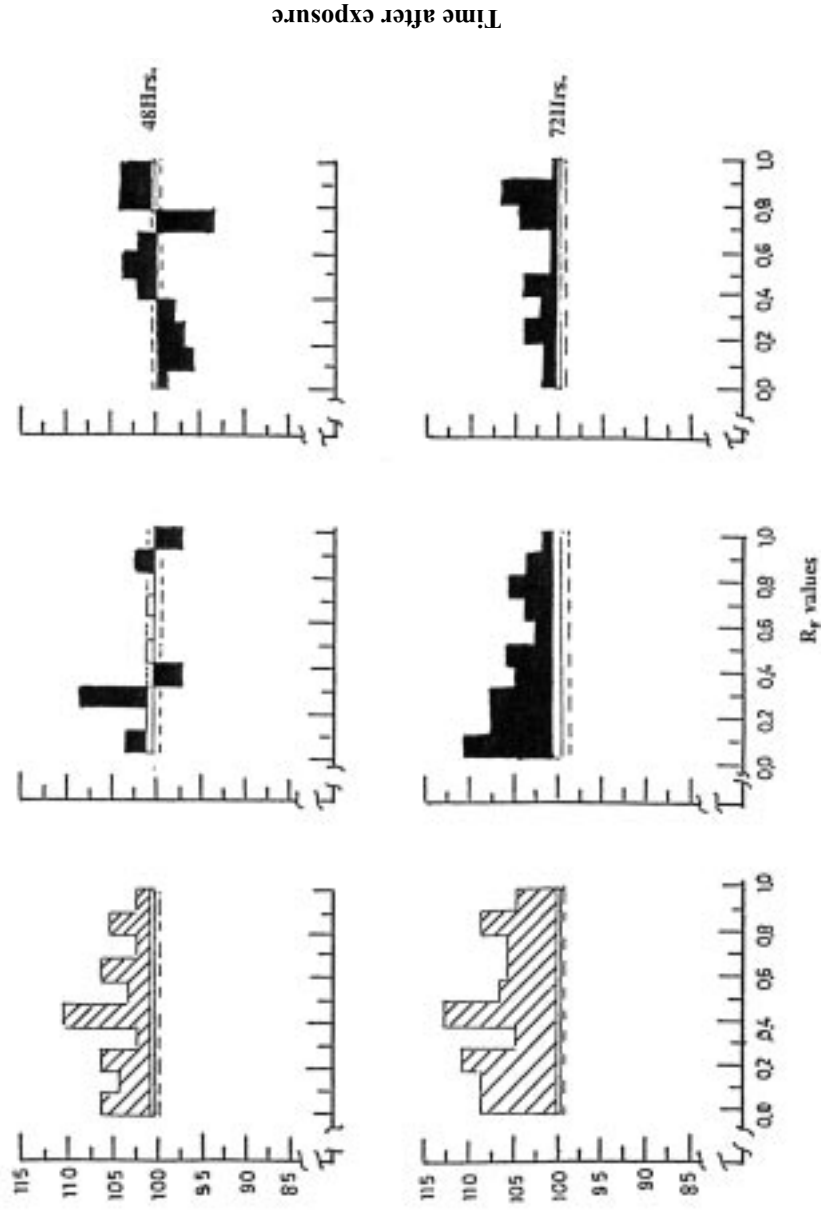

FIG. 4b. Effect of ultrasonication on cytokinin substances content in the fractionated extract of peanut seedlings.

only of cytokinin activity with R_F values 0.0-0.1 and 0.2-0.3 at long exposure in the same period (Fig. 4a). After 48 and 72 hours of ultrasonication the number and the activity levels of cytokinin substances were increased in either the treated (15 and 60 minutes) or the untreated seedlings. The activity levels were obviously more impaired in the extracts than those of untreated ones (Fig. 4b).

At short exposure to ultrasonic waves (15 minutes) the auxin contents of treated seedlings as well as the untreated increased with lapse of time of germination throughout the experimental period. In seedlings treated with long exposure (60 minutes) auxin increased suddenly after 3 and 24 hours of exposure and then decreased (Fig. 5). Gibberellin contents increased with lapse of time germination throughout the experimental period (Fig. 6). In the treated seedlings, gibberellin contents increased with time of germination until 48 hours with short exposure to ultrasonic waves (15 minutes). At long exposure (60 minutes), gibberellin contents decreased with lapse of time of germination as being compared with untreated.

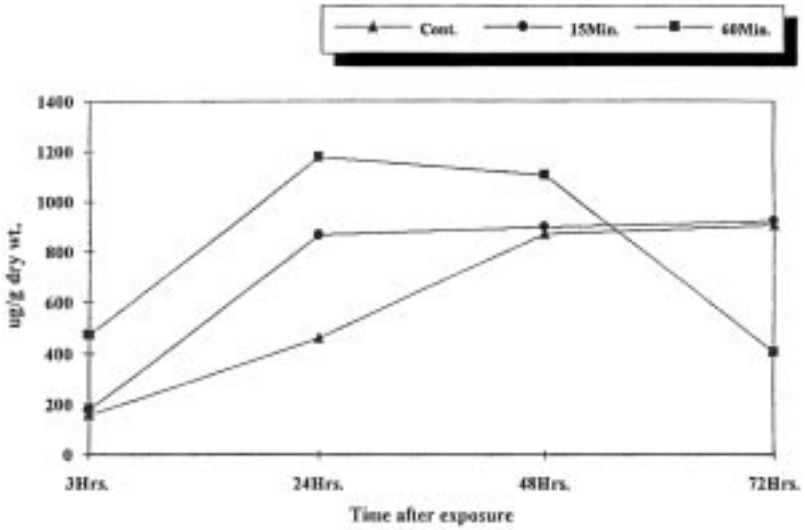

FIG. 5. Effect of ultrasonication on auxin contents of peanut seedlings.

Discussion

The results presented in Fig. (1) are in accordance with the observation reported by many studies that ultrasonic waves are extremely active as a germination inhibitor for a very wide variety of seeds (Ravi Meher *et al.*[12] and Weinberger and Piener[13]. Results showed that the decrease in the percentage of germination of peanut seeds at short exposure than lone ones might be attributed to:

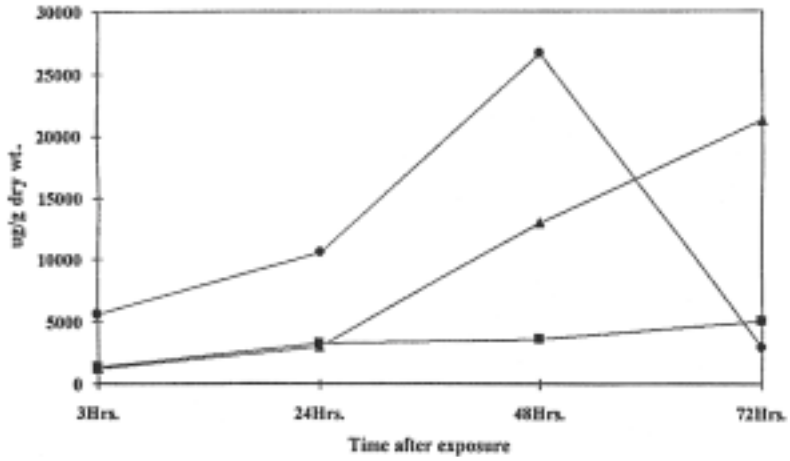

FIG. 6. Effect of ultrasonification on gibberellin contents of peanut seedlings.

a) Microrupture, chemical changes of cell organelles and accelerates the diffusion of material through membranes by increasing the concentration gradients in their vicinity Richards[25].

b) The oscillations produced by ultrasound causes force stress on the cell membrane, which further becomes the reason for the loss of osmotic integrity of the cell Hedges and Vidyavati[26].

c) Reduction of auxin production by increasing the activity of indole oxidase enzymes Krishnamurthy *et al.*[2].

The water content of peanut seedlings was declined by ultrasonic waves (Fig. 2). The reduction in water content may be supported by the studies of Hedges and Leeman[3], who stated that ultrasonic waves cause stress on the cell membrane. The stress increased the dry matter per cell in expense of its water content Soeder *et al.*[27].

Data recorded in the present work revealed that, both DNA and RNA gradually increased in the ultrasonic treated and untreated seedlings (Fig. 3). Street and Opik[28] recorded that the dry seeds have low nucleic acid contents and both RNA and DNA levels in each seedling increased sharply in the early stage of germination. Ultrasonic treated seedlings showed reduction in DNA and RNA contents as compared with untreated ones. Newcomer[10] stated that, ultrasonic have depolymerizing action on the cell product, such as deoxyribonucleic acid (DNA). He added that these effects were not due to localized heating or cavitation but were due to mechanical effects resulting from ultrasonic vibrations directly. The DNA and RNA contents decreased with time of exposure to ultrasonic waves (from 15 to 60 minutes). In this connection Gor-

don[29] demonstrated that the effect of ultrasonic waves on the nucleic acids were increased with increasing time of exposure.

In the present work, cytokinin contents were increased with lapse of time of germination in both ultrasonic treated and untreated seedlings (Fig. 4a&b). Cytokinins appeared to be important in the plant growth and development Shoog and Schmitz[30] and Horgan[31]. The inhibiting effect of ultrasonication increased with increasing time of exposure from 15 to 60 minutes, this reduction may be attributed to the destruction of cytokinins already present, or due to its effect on the biosynthesis of cytokinins already present, or due to its effect on the biosynthesis of cytokinins or its effect on the conversion of cytokinins to inactive bound forms. Gas liquid chromatography results (Fig. 5) revealed that the increase in IAA level in seedlings under ultrasonic exposure may be attributed to slow reduction in the level of auxin biosynthesis Sideris *et al.*[32] or decrease of peroxidase activity Reddy *et al.*[33]. In this connection Sembdner *et al.*[34] stated that the decarboxylation reaction (one of IAA catabolism) is catalyzed by peroxidase for numerous plant species. Conversely, the reduction of IAA levels in seedlings after 72 hours at long exposure may be due to inhibition in the enzyme system which converted indole acetaldehyde to indole acetic acid Kelly[35]. IAA contents were increased with long exposure (60 minutes) after 3, 4 and 48 hours than short exposure (15 minutes) and untreated one, such increase may be attributed to the increase in tryptophane. Libbert[36] showed that the increase of IAA was associated with increase of tryptophane.

The ultrasonic waves increased in gibberellic acid content with short exposure (15 minutes) while they were reduced with long exposure (60 minutes) (Fig. 6). In this regard, ultrasonic waves were gradually decreased the seedlings growth by increasing time of exposure due to reduction in gibberellic acid contents with long exposure Weinberger and Piener[13].

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دراسات عن تأثير الموجات فوق الصوتية على : ٤- الإنبات ومنظمات النمو والأحماض النووية لبادرات الفول السوداني

حجازي صادق حجازي و صفية محمد غازي و حنان السيد ضيف
قسم النبات ، كلية العلوم ، جامعة الزقازيق

المستخلص . يستهدف هذا البحث دراسة تأثير الموجات فوق الصوتية على إنبات بذور الفول السوداني وكذلك محتويات البادرات لكل من منظمات النمو والأحماض النووية .

بينت النتائج أن للموجات فوق الصوتية تأثيراً مثبطًا لإنبات الفول السوداني ومحتواها المائي وأقل نسبة إنبات وكذلك أقل كمية ماء وجدت عند تعريض البذور للموجات فوق الصوتية لمدة ١٥ دقيقة .

أظهرت النتائج نقصانًا في كمية RNA, DNA لبادرات الفول السوداني نتيجة التعرض للموجات فوق الصوتية وهذا النقصان يزداد بزيادة فترة التعرض لهذه الموجات .

أوضحت النتائج أيضًا أن كمية السيتوكينين تقل عند تعريض البادرات للموجات فوق الصوتية بينما تزداد كمية الأوكسينات نتيجة التعرض حتى ٧٢ ساعة بعد التعريض . أبانت النتائج أيضًا أن المحتوى الجبريليني للبادرات المعرضة تسلك مسار المحتوى الأوكسيني حيث تشمل أعلى القيم في حالة التعرض للموجات فوق الصوتية لمدة ١٥ دقيقة .