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STUDIES ON THE EFFECT OF ULTRASONIC WAVES ON: LIPIDS STRUCTURE AND ACTIVITY OF LIPASE IN PEANUT SEEDLINGS AND CELL CULTURES

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ABSTRACT

In the present work, the effect of ultrasonic waves on lipid metabolism and lipase activity in seedlings and cell culture of peanut was carried out. The neutral lipid contents of peanut seedlings were slightly increased by ultrasonication, but the total lipid as well as phospholipids content decreased. The reduction of phospholipids and total lipid contents of peanut seedlings was accelerated by ultrasonication exposure time from 15 to 60 minutes. Lipase activity of peanut seedlings increased also with time of exposure to ultrasonic waves. Cultured cells of peanut total lipids, diacylglycerols (DG), sterols (S) and free fatty acids (neutral lipid fractions), phosphatidylinositol (PI) as well as lipase activity were increased ultrasonication, while triacylglycerols (TG) and sterol esters (SE) as well as phosphatidylethanolamine (PE), phosphatidylcholine (PC) and phosphatic acid (PA) were decreased.

INTRODUCTION

Various workers made successful efforts to elucidate the biological effects of ultrasonic waves on several of higher plants particularly on their seed germination, pollen germination and other physiological processes. A number of reports were concerned with higher plants but very little attention has been paid towards the effect of ultrasonic waves on microorganisms.

Zvyagintsev[1] recorded that ultrasonic treatment of soil may damage and kill microorganisms, and ultrasonic disintegrator of low frequency is recommended.

Riesz and Takashi[2] stated that, the chemical effects of ultrasonic waves in aqueous solutions are due to acoustic cavitation, which refers gas bubbles in liquids. Exposing the cell wall to cavitation forces either inside or outside the cell, ruptures the wall contents. They also observed that the deleterious effects of ultrasonic waves were due to the DNA degradation, inactivation of enzyme, lipid peroxidation and hence cell killing.

Phosphatidylcholine as a fraction of phospholipids was associated with defatted soybean protein to produce multiform complexes by sonication[3]**.** They added that as the ultrasonic power increase the protein-phosphatidylcholine complexes sediment. Oil was on the other hand, associated with the protein-phosphatidylcholine complexes to form major complexes.

Lamellar structure of phospholipid vesicles were obtained by short time ultrasonication[4]**.** These vesicles were formed in order to take a mechanically stable lamellar structure.

The present work is an attempt to elucidate variations in lipids structure of peanut seedlings and cultured cells by ultrasonic waves.

MATERIAL AND METHODS

(I) Seed germination and ultrasonication:

Germination of seeds and exposure of seeds to ultrasonic waves were carried out as mentioned in[5].

(II) Cell suspension culture and ultrasonication:

Initiation of peanut callus, cell suspension culture preparation and exposure of cultured cells to ultrasonication were carried out as mentioned in[5].

(1) Lipid extraction:

Extraction of lipids from plant seedling and cultured cells were carried out according to the method of[6]. A known weight of fresh plant material and cultured cells (1g) were placed in isopropanol and left overnight at 15°C before being ground in 20 volumes of this solvent and extracted. After further extraction with chloroform: methanol (2:1 followed by 1:2), the combined extracts were reduced to near dryness with a rotary evaporator, taken up in a small volume of Folch lower phase solvent[6] and washed twice with upper phase solvent. The lipid extracts in the lower phase were evaporated to a small volume, dried with sodium sulphate and centrifuged. The supernatant was transferred to small vials, dried with a rotary evaporator and made to a convenient volume corresponding to a known weight of fresh plant materials or cultured cells in chloroform (0.5 ml/g fresh weight material or cultured cells).

(2) Separation of lipids using thin-layer chromatography

For one dimensional separation 10-50 µl extract were spotted on 20 x 20cm glass plates of 250 µm thick silica gel, (Merk G-60). Neutral lipids were separated in the following double solvent system of[7]**:** (1) diethyl ether: acetic acid (24:1 V/V), run to 13cm from the base-line; (2) petroleum ether: diethyl ether: acetic acid (90: 10: 1) run to 1 cm from the top of the plate. Polar lipids were generally separated in the solvent system of[8]**,** i.e. chloroform: methanol: acetic acid: water (170: 30: 20: 7).

(3) Identification of lipids on thin-layer plates:

Lipids were visualized in iodine vapour or by spraying with 50% sulphuric acid and heating at 100°C. Identification was carried out by comparison with published diagrams for available standard lipids with the used solvent systems or by reaction of specific classes of lipids to various reagents.

(4) Quantitative estimation of total lipids:

Total lipids were determined by the sulphuric acid charring method of[9]. Neutral lipid spots separated by thin-layer chromatography were estimated in the presence of silica gel by the method of[10] while phospholipids were estimated by the procedure of[11].

(5) Assay of lipase:

(i) Extraction of crude enzyme:

Extraction of crude lipase from peanut seedlings and cultured cells was the same as in the extraction of amylase enzyme carried out by[5].

(ii) Quantitative determination of lipase activity:

Determination was carried out according to the method adopted by[12]. Samples of 0.1 ml crude enzyme extract were added to 1 ml of tris-HCl buffer (pH 8) and 1ml of oil emulsion. Blanks were made by using 0.1 ml of distilled water instead of enzyme extract. Both of sample and blank were incubated at 37°C for one hour, then boiled to stop the reaction and completed to a known volume. Titrated against 0.05M NaOH solution using thymolphthaline as indicator.

The enzyme activity was calculated according to the following equation:

1ml of 0.05M NaOH = 0.045 mg of fatty acids

RESULTS

(1) Effect of ultrasonication on lipids structure:

Figure (3) showed that the total lipid contents of treated and untreated seedlings were markedly decreased during the experimental period. The neutral lipid fractions (Fig. 1) showed that the ultrasonication has little effect on diacylglycerides than the other determined neutral lipid fractions. The longest exposure time (60 minutes) increased this fraction than the shortest one (15 minutes) except after 72 hours after exposure. The long exposure caused marked increase in sterols as compared with the short one and control treatments. Also, long exposure led to the highest values of free fatty acids as compared with short exposure and control treatments except after 3 hours of exposure. Ultrasonication has strong effect on triacylglycerides and sterol esters and the long exposure to it was more effective than short one.

The studied phospholipids fractions, phosphatidylcholine (PC) and phosphatidylethanolamine (PE) (Fig. 2) showed that ultrasonication reduced their amounts in treated as compared with untreated seedlings. The long was more effective than short exposure in reducing the amount of PC and PE except after 72 hours of exposure.

In the cultured cells of peanut the total lipid contents were markedly increased by ultrasonication (Fig. 5). The average values of the neutral lipid fractions in the cultured cells varied significantly after ultrasonication. In the neutral lipid fractions the triacylglycerides and sterol esters content decreased in treated cultured cells as compared with untreated control. On the opposite, diacylglycerides, sterols and free fatty acids were increased in the treated ones. The phospholipid contents of ultrasonication treated cultured cells of peanut were significantly lower than that of the untreated samples except phosphatidylinositol (PI).

(2) Effect of ultrasonication on lipase activity:

Figure (5) indicated that ultrasonication led to significant increase in lipase activity in the treated as compared with untreated seedlings. The short exposure time (15 hours) had less effect on the lipase activity as compared with long exposure. In the cultured cells (Fig. 5) ultrasonication markedly increased the activity of lipase.

DISCUSSION

The changes in lipid metabolism in ultrasonic treated and untreated seedlings should be accompanied side by side with changes in sugars, into which the lipids may be converted. As normal germination of peanut seedlings proceeds, the lipid contents (total lipids, neutral lipids and phospholipids) decrease sharply except some soluble neutral fractions such as sterols and diacylglycerides which increase. In this connection [13]recorded that most of the stored lipids were converted to carbohydrate for seedling growth.

In the present study, ultrasonic waves treatments of peanut seedlings decreased total lipid contents as well as phospholipids fractions. This was attributed to the measured high activity of lipase enzymes caused by ultrasonication of peanut seedlings. These results

were confirmed by[14]. Similarly, [3]recorded reduction in oil contents of soybean as a result of the increase in ultrasonic power.

Neutral lipids, sterol and diacylglyceride were gradually increased with lapse of germination time after the ultrasonic treatment in the plant seedlings. However, in case of treated seedlings they were found in high values in comparison with the control. Free fatty acids, sterol esters and triacylglycerides were on the opposite gradually decreased by the ultrasonic waves. The obtained results were in agreement with those of[3], who stated that reduction in oil contents of soybean was a result of the increase in ultrasonic power.

The results revealed that PC, PE and total phospholipids were decreased in ultrasonic treated and untreated peanut seedlings. **Strack**[15] stated that phospholipids fractions stored in the seeds were metabolized releasing choline during seeds germination and seedlings developments. **Wharfe and Harwood**[16] referred this to choline kinase and ethanolamine kinase which increased in soybean seeds by germination.

Ultrasonication led to more decrease in phospholipids and this may be attributed to the:

(a) Reduction of phosphatidylcholine content by forming protein-phosphatidylcholine complexes sedimented with ultrasonication[3].
(b) Aggregation of phospholipids in vasicles of lamellar structure as a result of the mechanical effects of ultrasonication[4].
(c) Activation of lipase and other enzymes and lipid peroxidation[14 & 2].

In the cultured cells, triacylglycerols were significantly decreased in response to ultrasonic waves resulting mainly from reduction in the biosynthesis as well as breakdown of triacylglycerols. This was accompanied by a significant increase in the diacylglycerols and free fatty acids. The results obtained agree with those of[17].

Significant increases in the sterols were detected in the ultrasonication treated cells, while their amounts of sterol esters were greatly decreased as compared with untreated cells. This suggested that part of the sterol content have become esterified by fatty acids[18 & 17].

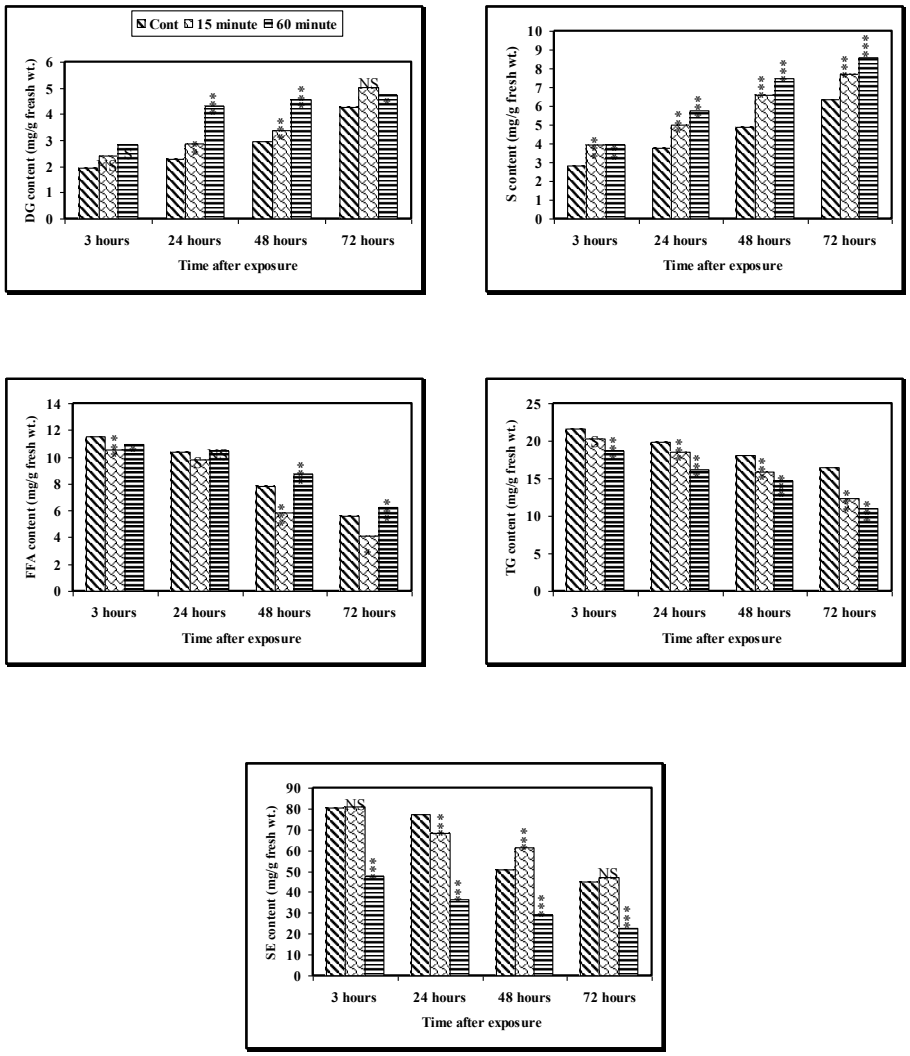


Figure (1): Effect of ultrasonication on neutral lipid fractions (diacylglycerides "DG", sterols "S", free fatty acids "FFA", triaylglycerides "TG" and sterol esters "SE") of peanut seedlings

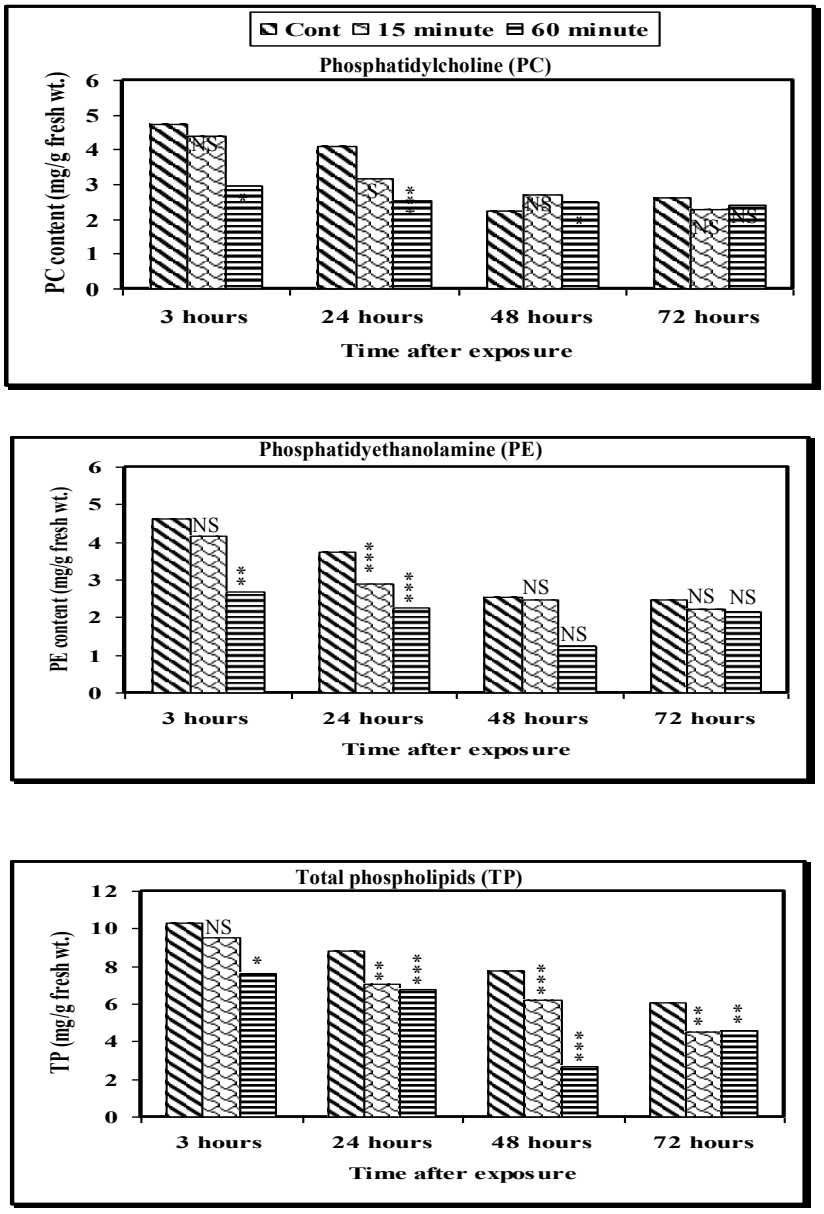


Figure (2): Effect of ultrasonication on phospholipid fractions of peanut seedlings

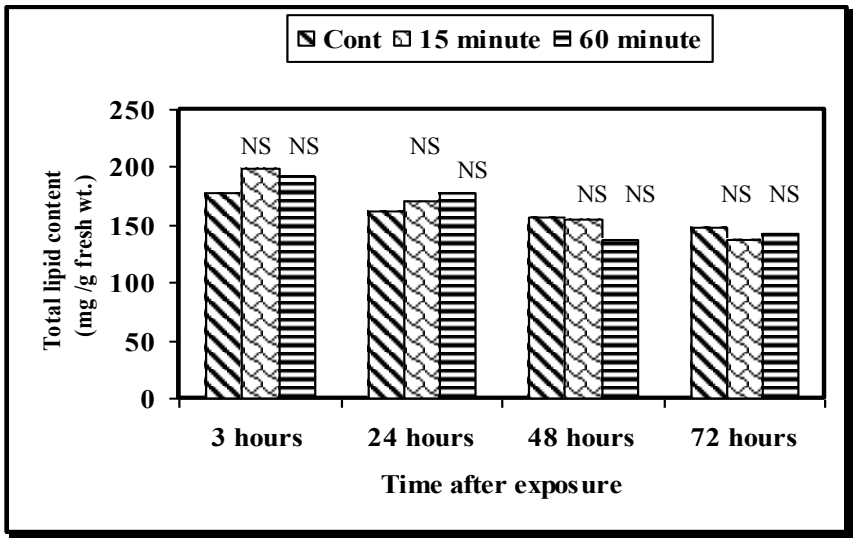


Figure (3): Effect of ultrasonication on total lipid contents of peanut seedlings

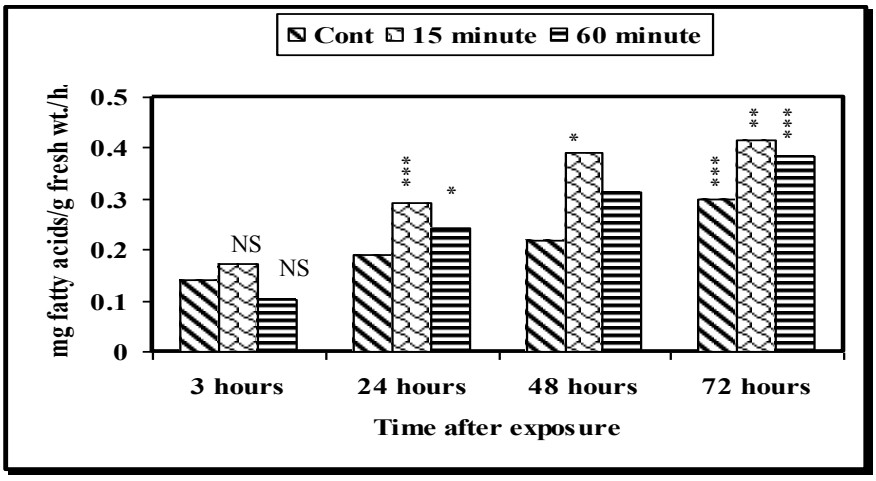
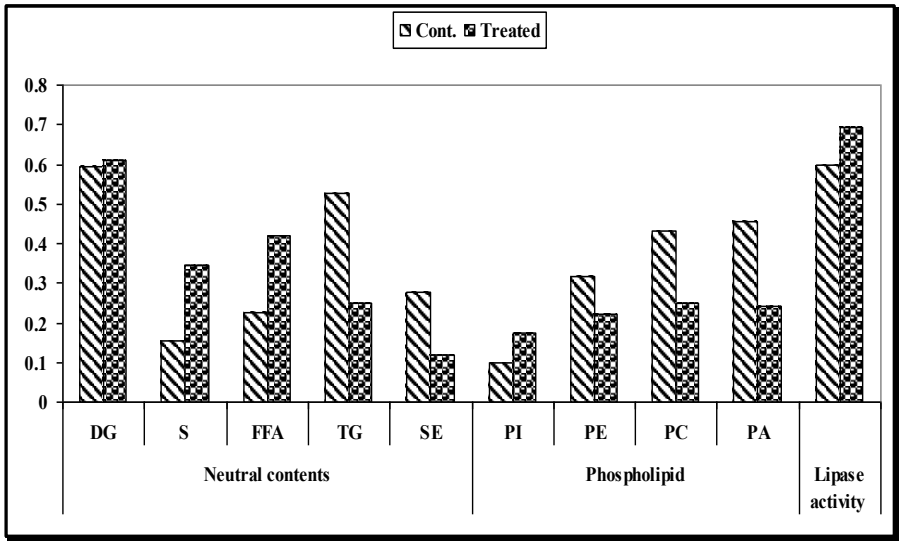


Figure (4): Effect of ultrasonication on lipase activity of peanut seedlings



NS = P > 0.05 S = P < 0.05 * P > 0.02 = P > 0.01*** - P > 0.001**

Figure (5): Effect of ultrasonication on lipid contents (mg/g fresh weight) and lipase activity (mg fatty acid/g fresh weight) of peanut cultured cells

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(الملخص العربي)

دراسات عن تأثير الموجات فوق الصوتية علي: أيض الدهون ونشاط إنزيم الليبيز في كل من البادرات ومزارع أنسجة الفول السوداني

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في هذا البحث تم دراسة تأثير الموجات فوق الصوتية علي أيض الدهون في كل من البادرات ومزارع أنسجة الفول السوداني. أظهرت النتائج أن كمية الدهون المتعادلة تزداد بالموجات فوق الصوتية بينما المحتوي الكلي لليبيدات والفوسفولبيدات قد نقص في بادرات الفول السوداني، وقد وضح أن النقص في الليبيدات والفسفولبيدات تزداد بزيادة فترة التعرض للموجات فوق الصوتية في البادرات.

أما في مزارع الأنسجة أبانت أن كمية الدهون الكلية، FFA, S, DG وكذلك PI تزداد بالموجات فوق الصوتية بينما تقل SE, TG وكذلك PA, PC.

أوضحت النتائج زيادة ملحوظة في نشاط إنزيم الليبيز في كل من بادرات ومزارع أنسجة الفول السوداني.