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The potential of ultrasonic waves for sustainable management of cotton leafworm *Spodoptera littoralis* (Lepidoptera:Noctuidae)

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Abstract

Physical pest control is effective in integrated pest management, as chemical treatments can be extremely hazardous and cause environmental pollution. One of these physical methods is the use of ultrasound waves in insect pest control. Cotton leafworm *Spodoptera littoralis (*Boisd.) (Lepidoptera:Noctuidae) causes great harm to a widespread diversity of crops in the Middle East and is one of the main economic pests of Egyptian cotton. Under laboratory conditions, the effect of ultrasound waves on the larval weight gain, food consumption and feces of second and fourth stage of cotton leafworm larvae were recorded, also total protein, total carbohydrate, total lipids and hepatic enzymes glutamate oxaloacetate transferase (GOT/AST), glutamate pyruvate transferase (GPT/ALT) and alkaline phosphatase after 9 days of exposure to ultrasound waves was determined for the 6th instar larvae compared with untreated control larvae. A commercial ultrasonic device was used with a frequency of 50000 HZ. Within an enclosed chamber contains the second and fourth cotton leaf worm larvae are at a distance 20 cm from the device another, chamber in another place contains the untreated larvae (Control). All larvae allow feeding on castor leaf oil. The results show that the larval weight gain, food consumption and feces of the treated larvae were less than the untreated control. The larval weight gain for the second stage larvae were 0.30gm compared with 0.60gm for control along exposure days and 0.47gm for the 4th instar compared with 0.76gm for 4th instar of control food consumption were 0.30gm for the treated second and fourth instar compared with 0.50gm compared with the second and fourth instar control, feces 0.28gm for the second instar compared with 0.42gm feces weight of control and 0.21gm feces for the 4th instar compared with 0.34 gm of control, there is significance disturbance in the tested biochemical parameters and hepatic enzymes show a significant increase as hepatic cells degeneration as compared with untreated control which assures the useful of used of ultrasonic waves as pesticide alternative.

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Introduction

Spodoptera littoralis (Boisd.) (Lepidoptera: Noctuidae) is recognized as a major agricultural pest, inflicting significant damage to cotton crops globally. Conventional pest control strategies largely depend on synthetic chemical insecticides, which pose serious threats to environmental safety, human health, and non-target beneficial organisms. Consequently, the search for eco-friendly alternatives has intensified in recent years, including the application of physical control methods such as ultrasonic waves. Ultrasound has emerged as a novel technique for pest suppression, influencing various insect behaviors and physiological processes According to Adhikari (2022) studies have demonstrated that ultrasonic waves can interfere with insect communications, navigations and feeding behavior, ultimately leading to reduced populations and crop damage. recent research has investigated the efficacy of ultrasonic waves against various insect's pest, including the cotton leaf worm (Agee and Webb, 1969a and Gallo *et al.*, 2018). Research has shown that ultrasonic waves can alter the mating behavior of certain insect species, leading to reduced reproduction and population growth (Zhang *et al.*, 2019) . Recent research has investigated the efficacy of ultrasonic waves against various insect pests, including the cotton leafworm (Ammar *et al.*, 2024a and Agee and Webb, 1969b). These studies have reported promising results, including reduced population density and improved crop yields (Saranya *et al.*, 2019). Additionally ultrasonic waves have been shown to be effective against other pests, such as aphids and whiteflies, without harming beneficial insects (Urairi *et al.,* 2022).

The use of ultrasonic waves for pest control offers several advantages over traditional methods, including reduced environmental impact and potential for targeted control (Ibrahim *et al.*, 2016). However, further research is needed to optimize the use of ultrasonic waves for cotton leafworm control and to understand the underlying mechanism of action (Bhairavi *et al.*, 2016).

Materials and methods

The current study was conducted in 2024 at the Plant Protection Institute, Sids Agricultural Research Station, Beni-Suef Governorate, Egypt, to assess the effects of ultrasonic waves on larval development, food intake, excretion, and key biochemical parameters in *S. littoralis,* aiming for a safe, non-chemical pest control method.

1. Ultrasonic generator:

A commercial ultrasonic generator (Model DATA#SS65030 – Germany) was employed. This device emits siren-like sounds in the range of 30,000–65,000 Hz with a sound pressure level of 160 dB, effective over an area of 2000–2500 square feet.

2. Digital balance:

A high-precision balance (accuracy 0.001g) was used for measuring leaf weight before and after exposure, along with larval body mass and fecal matter.

3. Tested pest:

A laboratory-reared colony of *S. littoralis,* maintained without pesticide exposure following Ghoneim (1985), was used. Second and fourth instar larvae were selected and fed castor leaves.

4. Methods:

Larvae of the 2^{nd} and 4^{th} instars were separated into treated and control groups, each comprising 20 larvae (four replicates of five larvae). Treated groups were exposed to continuous ultrasonic waves for about nine days (until pre-pupation). Control groups were housed in a separate laboratory (>2500 sq. ft. away) to prevent any exposure.Daily assessments included measurements of larval weight, leaf consumption, and feces. At the 6th instar stage, biochemical and enzymatic analyses were conducted for previously treated individuals. Data analysis was performed using paired t-tests in Microsoft Excel as per Jones *et al.* (1989).

5. Biochemical bioassays:

5.1. Sample preparation:

Three 6^{th}-instar larvae per treatment group were frozen at -17 ±1°C until analysis. Homogenates were prepared in phosphate buffer (pH 7.0) using a Teflon homogenizer and centrifuged at 8000 rpm for 15 minutes at 5°C. Supernatants were collected for test in.

5.2. Determination of total protein content:

Protein levels were quantified following Raheja and Snedecor (1970), using bovine serum albumin as a standard.

5.3. Determination of total carbohydrate content:

Total carbohydrate content was assessed using the anthrone method (Singh *et al*., 1981).

5.4. Determination of total lipid content:

Lipid content was estimated with the phospho-vanillin reagent method (Knight *et al.,* 1972).

5.5. Determination of glutamate oxaloacetate transferase (GOT/AST), glutamate pyruvate transferase (GPT/ALT) and alkaline phosphatase:

Activities of GOT (AST), GPT (ALT), and alkaline phosphatase were determined using the protocols of Reitman and Frankel (1957) and Laufer and Schin (1971), respectively).

Results and discussion

1. Effect of ultrasonic waves on total larvae weight gain, consumed food and daily feces:

The data presented in Figures (1) and (2) clearly indicate that exposure to ultrasonic waves significantly reduced the daily weight gain of the 2nd and 4th instar larvae of *Spodoptera littoralis*. The treated larvae exhibited weight gain percentages of 30% and 27%, respectively, compared to 60% and 47% in the untreated controls. (Figures 3 and 4). Regarding fecal output, Figures (5) and (6) reveal that treated larvae produced less waste of 0.28 g and 0.21 g for 2nd and 4th instars, respectively compared to 0.42 g and 0.34 g in the control groups (5 and 6). These findings are consistent with earlier studies. For example, Pyykkö and Starck (1985) demonstrated that mechanical energy from sound waves could cause structural disturbances in insect tissues. Similarly, Kiruba *et al*. (2006) suggested that when the frequency of the sound matches the tissue's natural frequency, it may lead to damage. Additionally, Mullick (2002) and Jinham *et al*. (2012) reported that exposure to gamma radiation and mechanical waves negatively affected the longevity and reproductive capacity of *Tribolium castaneum*. Sistanizadeh-Aghdam *et al*. (2020) also confirmed the larvicidal impact of ultrasonic waves on immature mosquito stages under laboratory Conditions.

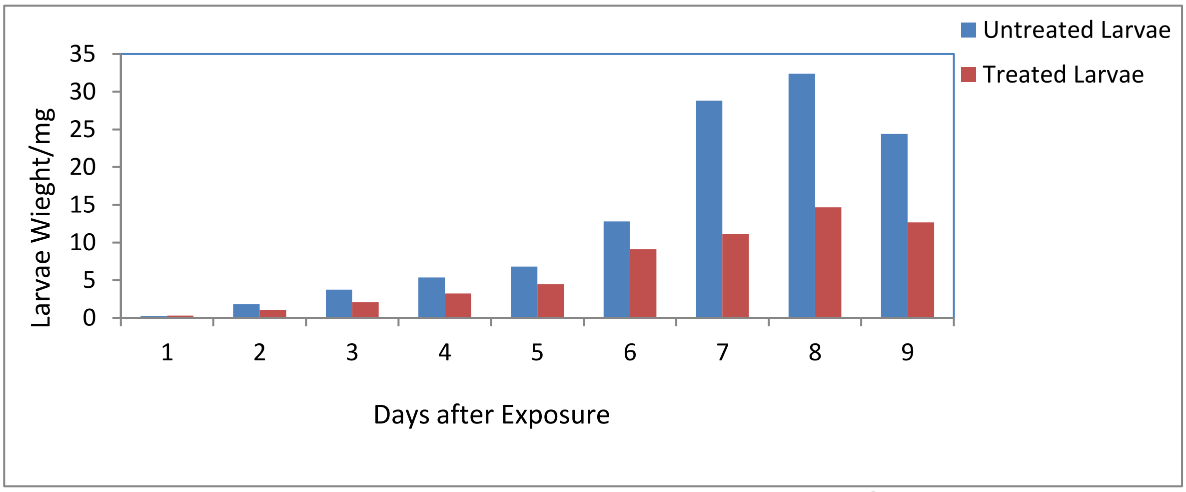

Figure (1): Daily larval weight gain in (mg) for the treated and untreated 2nd instar larvae of *Spodoptera littoralis*.

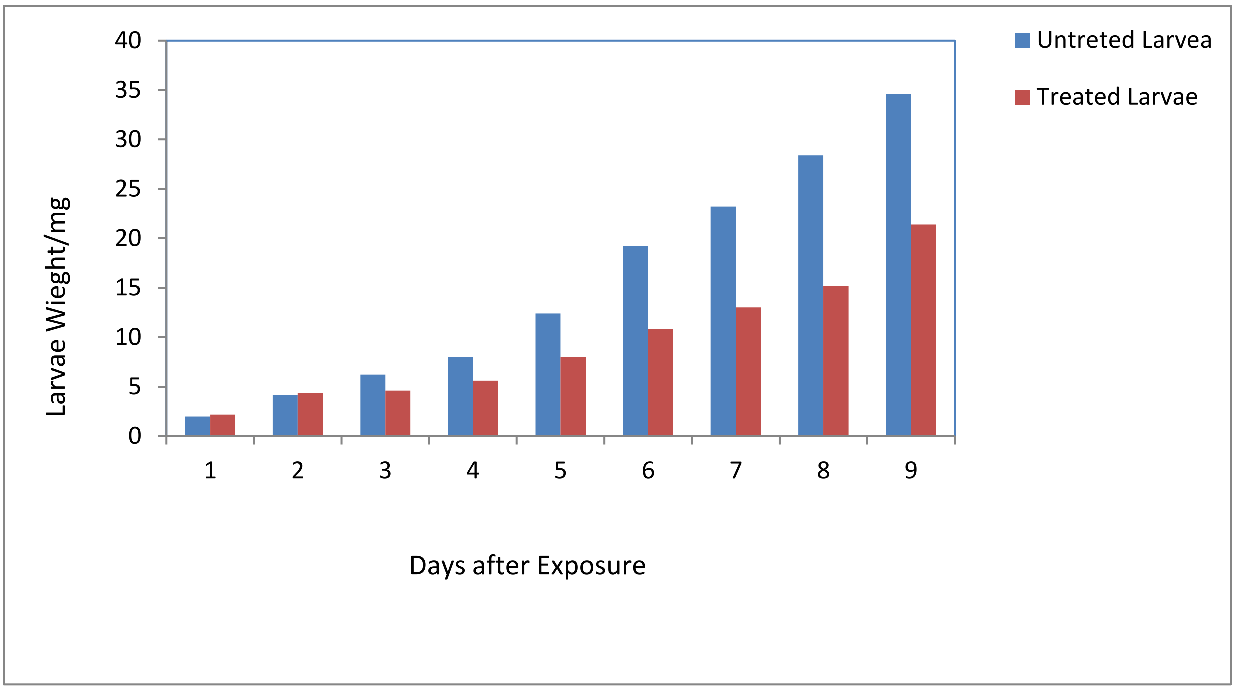

Figure (2): Daily larval weight gain in (mg) for the treated and untreated 4th instar larvae of *Spodoptera littoralis* .

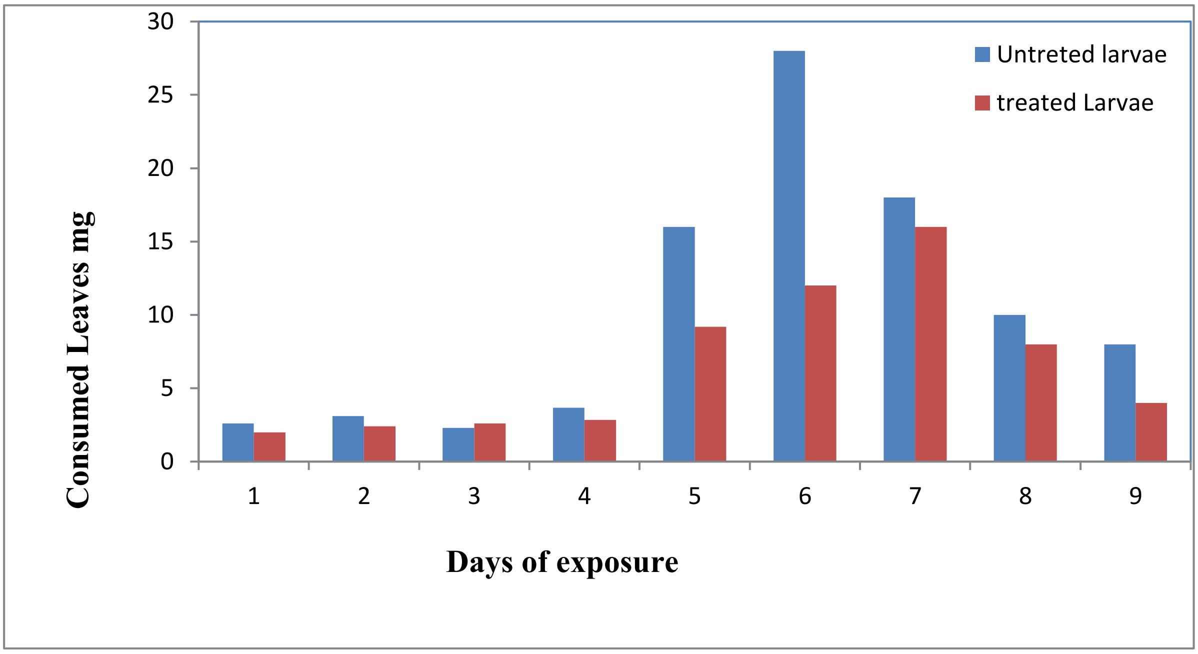

Figure (3): Wight of consumed leaves (mg) for treated and untreated 2nd instar larvae of *Spodoptera littoralis.*

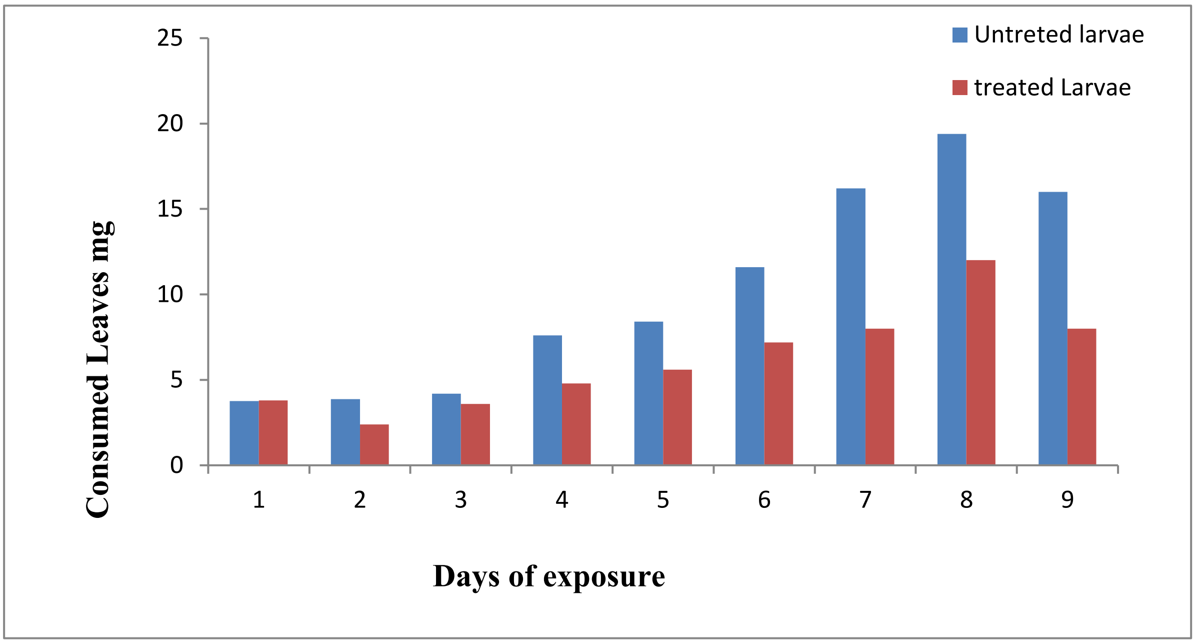

Figure (4): Wight of consumed leaves (mg) for treated and untreated 4th instar larvae of *Spodoptera littoralis.*

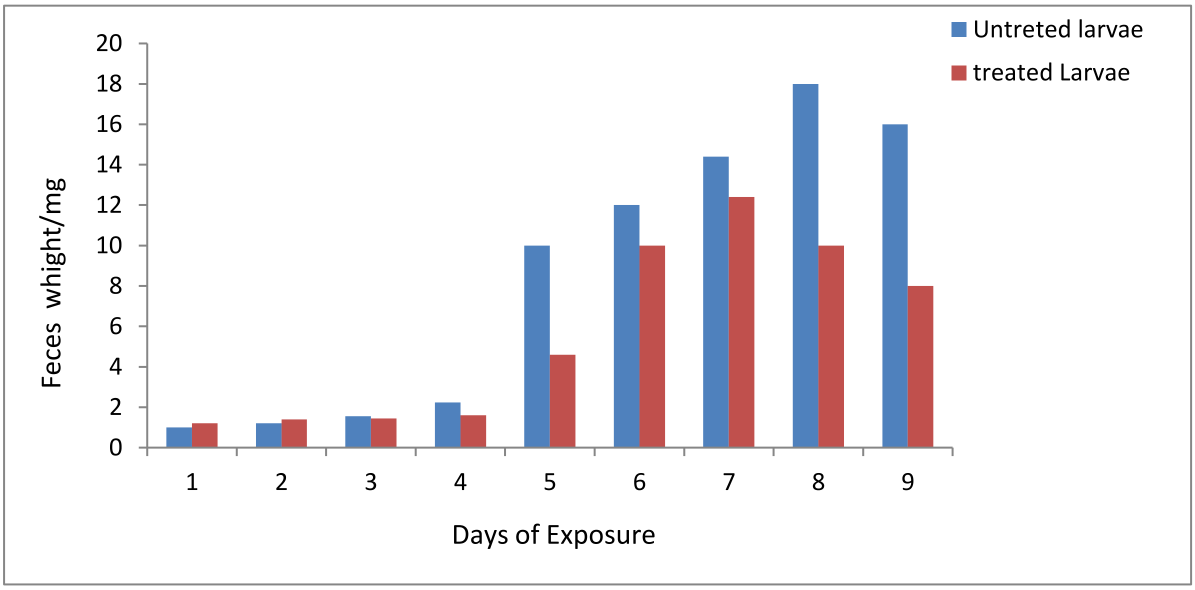

Figure (5): Wight of daily feces (mg) for treated and untreated 2nd instar larvae of *Spodoptera littoralis.*

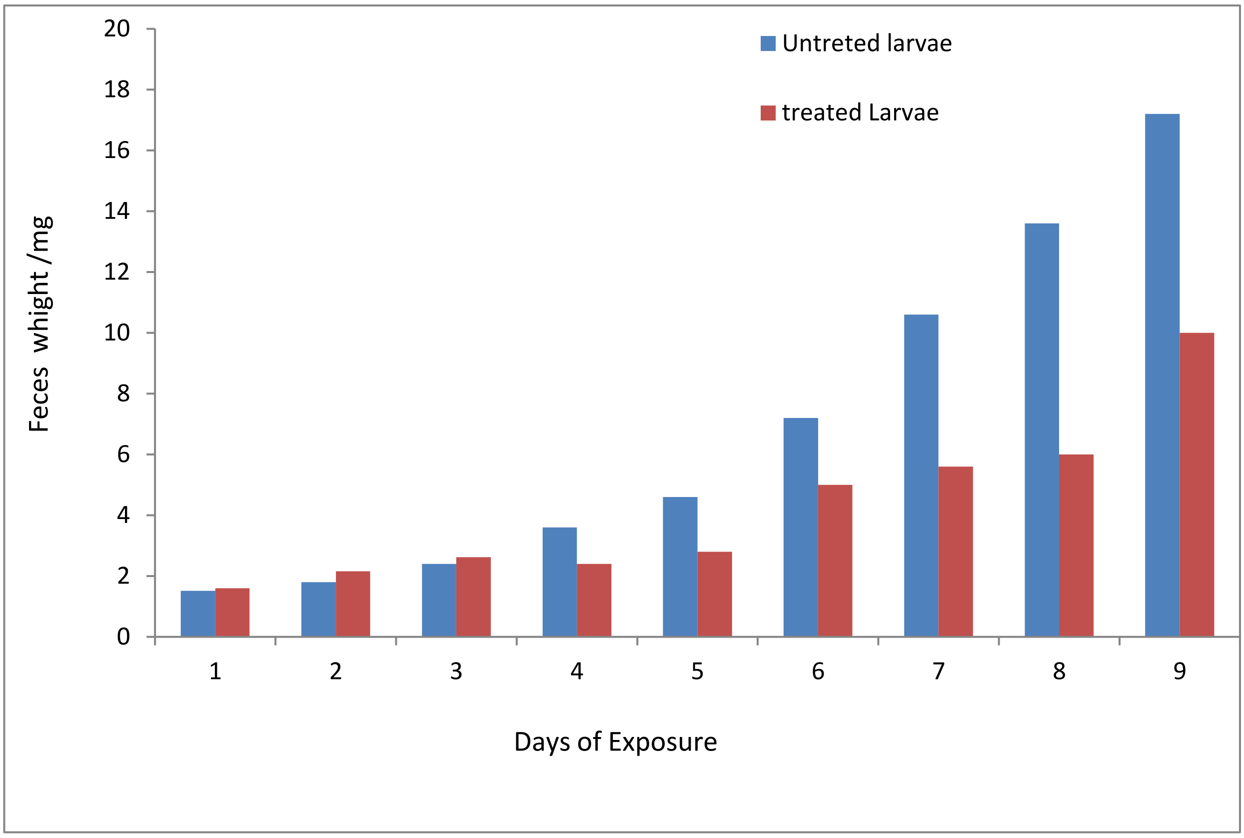

Figure (6): Wight of daily feces (mg) for treated and untreated 4th instar larvae of *Spodoptera littoralis.*

2. Effect of ultrasonic waves on total protein, total carbohydrate, total lipids of *Spodoptera littoralis* larvae:

Table (1) shows that exposure to ultrasonic waves caused a marked reduction in essential biochemical components of the larvae. The treated group exhibited a significant decline in total carbohydrate levels, measuring 30.4 ± 0.64 µg/g b.w., compared to 39.8 ± 0.81 µg/g b.w. in the untreated group. Similarly, the total protein content was reduced to 32.4 ± 1.26 µg/g b.w. in treated larvae, in contrast to 41.1 ± 0.72 µg/g b.w. in the control group. A comparable pattern was observed in lipid content, which decreased from 37.3 ± 0.51 µg/g b.w. in the control to 29.8 ± 0.90 µg/g b.w. in the treated larvae. These reductions may be attributed to physiological disruptions caused by ultrasonic exposure. According to Sistanizadeh-Aghdam *et al.* (2020), ultrasound can damage the insect cuticle, thereby affecting internal biochemical balance. Wang *et al.* (2024) noted that high-intensity ultrasound alters protein conformation, which impacts digestibility and functional properties such as emulsification and gelation. Furthermore, Du *et al.* (2022) and Alshabka *et al.* (2020) emphasized that ultrasound-induced cavitation can break cell walls, enhance the release of intracellular polysaccharides and modify their molecular weight and biological activity.

Table (1): Effect of ultrasonic waves on total protein, total carbohydrate, total lipids of *Spodoptera littoralis* larvae .

Total carbohydrate (µg/g b.w.) (Mean ± S.E.)				
	C1	C2	C3	Mean±SE
Control	38.4	39.8	41.2	39.8±0.81
Treated	31.6	29.4	30.3	30.4±0.64
Total proteins (µg/g b.w.) (Mean ± S.E.)				
	C1	C2	C3	Mean±SE
Control	39.8	41.2	42.3	41.1±0.72
Treated	31.2	32.4	33.7	32.4±1.26
Total lipids (µg/g b.w.) (Mean ± S.E.				
	C1	C2	C3	Mean±SE
Control	36.8	38.4	36.9	37.3±0.51
Treated	29.7	31.5	28.4	29.8±0.90

3. Effect of ultrasonic waves on SGPT, SGOT and alkaline phosphatase of *Spodoptera littoralis:*

Table (2) illustrates that larvae exposed to ultrasonic waves exhibited significantly elevated enzyme activities. The SGOT (AST) level increased to 49.0 ± 1.55 IU/L in the treated group, compared to 27.3 ± 0.69 IU/L in the control. Similarly, SGPT (ALT) activity rose to 41.8 ± 0.67 IU/L from 30.7 ± 1.14 IU/L, while alkaline phosphatase increased to 124.5 ± 0.92 IU/L from 116.4 ± 1.04 IU/L. These elevations in hepatic enzyme activities suggest cellular stress or tissue damage resulting from ultrasonic exposure. Mawson *et al.* (2010) and Nadar & Rathod (2017) discussed how ultrasonic frequency, and intensity can modulate enzyme functionality, leading to either activation or inhibition. Zha *et al.* (2008) reported similar findings, indicating that ultrasound stress can alter acetylcholinesterase activity in insects. Other studies, such as Ammar *et al.* (2024b), observed significant increases in detoxification-related enzymes in insects exposed to insecticides, suggesting a broader stress response mechanism. Likewise, Amin and Ibraheim (2021) found that ultrasound exposure in mammals led to elevated liver enzymes, implying hepatic tissue degeneration, while El Senosi *et al.* (2015) confirmed biochemical alterations in liver enzyme levels due to ultrasonic waves in animal

Table (2): Effect of ultrasonic waves on SGPT, SGOT and alkaline phosphatase of *Spodoptera littoralis.*

SGPT				
	C1	C2	C3	Mean±SE
Control	28.9	32.8	30.4	30.7±1.14
Treated	43.1	40.8	41.7	41.8±0.67
SGOT				
Control	28.7	26.4	27.0	27.3±0.69
Treated	46.8	52.0	48.2	49.0±1.55
Alkaline Phosphatase				
Control	124.1	126.3	123.2	124.5±0.92
Treated	118.3	114.7	116.2	116.4±1.04

The results of this study demonstrate that ultrasonic waves, emitted from a commercially available device, exert significant biological effects on Spodoptera littoralis larvae. Exposure to ultrasonic frequencies led to substantial reductions in larval weight gain, food intake, and fecal output across both the

2nd and 4th instars. Furthermore, significant biochemical alterations were observed, including notable decreases in total protein, carbohydrate, and lipid levels, alongside marked elevations in hepatic enzyme activities (SGPT, SGOT, and alkaline phosphatase). These physiological and biochemical disruptions suggest that ultrasonic waves interfere with the normal metabolic functions and cellular integrity of the larvae. Given these outcomes, ultrasonic waves may serve as a promising alternative to conventional chemical insecticides in integrated pest management programs. Their use offers a safer, non-chemical method for pest control that reduces environmental impact and potential harm to non-target organisms. However, further investigations under field conditions are recommended to validate the long-term efficacy and optimize the practical application of ultrasonic wave technology in agricultural pest management.

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