

### Original Research Article

## Efficacy of Audible Sound Waves in Inflicting Tissue Damage and Mortality in *Tribolium castaneum* (Coleoptera: Tenebrionidae) Larvae

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### Abstract

Physical control methods are preferred in post-harvest management of insect pests because chemical intervention is extremely hazardous. Use of audible sound waves in insect pest intervention is a novel idea which has not been popular hitherto. An attempt has been made in this study, to understand the mechanical impact of sine sound waves on red flour beetle, *Tribolium castaneum* (Herbst), a common secondary pest of flour and other stored products. Last instar *T. castaneum* larvae (n = 50 and number of replicates = 4) were bombarded with 12 different frequencies of sine sound waves (900 to 2 000 Hz) by placing them directly on a matted polymer surface facilitating maximum sound absorption. The LD<sub>50</sub> value of sine sound waves for the larvae was 665.14 and the LD<sub>90</sub> 1339.43 Hz. The dorsal pronotum of adult beetles, developing from bombarded larvae were studied using scanning electron microscope (SEM) to look for mechanical damage of the dorsal cuticle. The setae were lost or damaged, the epicuticle was obliterated and the sockets became obscure. The results of the study would throw light on the applications of sound waves in the interventional management of stored products pests.

**Keywords:** Physical control; last instar larvae; sine sound waves; mechanical damage; scanning electron micrograph.

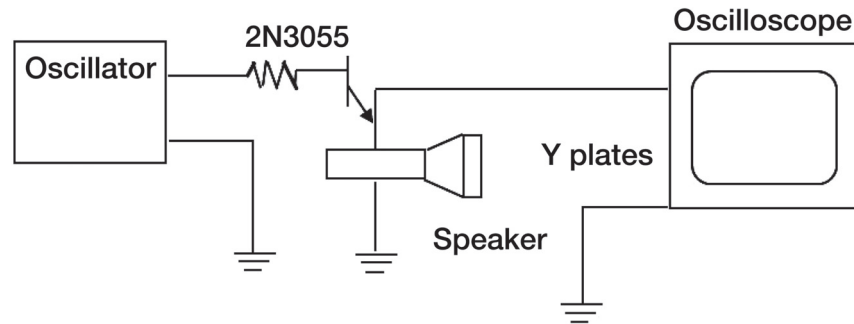
### INTRODUCTION

Pests of stored products are exceptionally complicated to control with chemicals (Thorpe, 1987), since serious problems of genetic resistance by insect species, pest resurgence, residual toxicity, photo toxicity, vertebrate toxicity, widespread environmental hazards and increasing costs of synthetic pesticides have directed the need for effective alternatives (Prakash et al., 1987; Scholler and Flin, 2000; Rahman and Talukder, 2006). Most stored products are in the final state and treatments may seriously affect their table quality and render them unfit for human consumption (Daft, 1991). Hence the strategies to manage pests of stored products would primarily involve physical treatment methods, which normally do not leave behind harmful residues or impair the table quality of the produce. Post harvest conditions are better suited to physical control methods because the environment is rather confined and the material is of high economic value (Vincent et al., 2003). For example, Diatomaceous Earths, which have a physical mode of insecticidal action, can provide long-term protection against stored product insects (Athanassiou et al., 2005; Vayias et al., 2006). Furthermore, it has been reported that physical control methods, such as cold, heat, carbon dioxide, microwaves and ionizing radiation are used extensively as post harvest quarantine treatments (Hallman, 2001; Jinham and Das, 2004; Wu et al., 2006; Wang et al., 2003) where disinfestations of a given pest at a predetermined level of control must be achieved.

Varieties of such management techniques like ultrasonic waves (Nelson, 1966; Salam and Abdel, 1987; Locatelli and Traversa, 1989; Bedi and Singh, 1992; Nelson et al., 1998), ultraviolet, gamma and X-rays (Sokoloff, 1961; Dennis et al., 1962; Calderon et al., 1985; Hasaballa and Boshra, 1992; Singh et al., 1994; Aldryhim and Adam, 1999) have been tried against insects and obtained considerable success. According to Kirkpatrick and Harein (1965) audible sound waves drastically reduced the progeny and fitness of *Plodia interpunctella*. Kiruba et al. (2009) reported that triangular audible waves affect the moulting of *Corcyra cephalonica* Stainton significantly. However, very little is known on the use of sound waves in the management of coleopteran pests of stored products. Therefore an attempt is made in this study, to understand the mechanical impact of sine sound waves on red flour beetle *Tribolium castaneum* (Herbst), a common secondary pest of flour and other stored products.

### MATERIALS AND METHODS

Sine sound waves are generated in an audio assembly consisting of an audio amplifier connected to an audio oscillator. The electromechanical transducer consists of a loud speaker (Kiruba et al., 2009). The amplitude of the electrical signal fed to the transducer is maintained at 2 volt peak to peak by monitoring it continuously using an oscilloscope (Fig. 1).



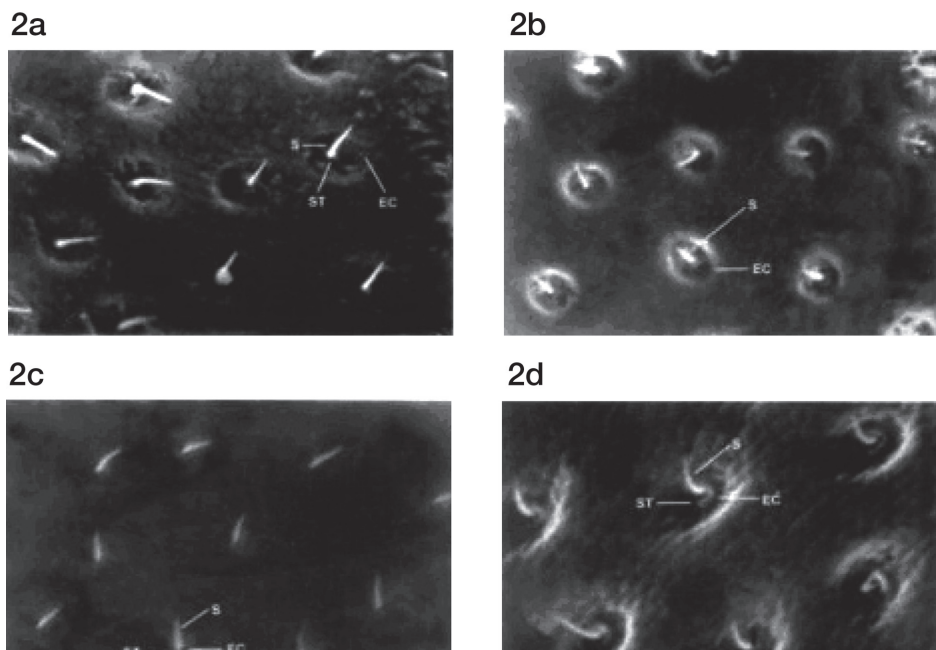
**Figure 1:** Schematic representation of circuit diagram

Last instar *T. castaneum* larvae (n = 50 and number of replicates = 4) were bombarded with 12 different frequencies of sine sound waves (900, 1 000, 1 100, 1 200, 1 300, 1 400, 1 500, 1 600, 1 700, 1 800, 1 900 and 2 000 Hz) by placing them directly on a matted polymer surface facilitating maximum sound absorption. No floor cover was provided to the larvae. The sound waves from the transducer were directed on the larvae from a distance of 10cm for a period of 10 minutes (Xiaozhou et al., 2000). Even though heavy mortality of larvae, pupae and adults was recorded, a few exposed larvae developed into adults. The larvae showed variation in their susceptibility to sound waves. Such variations in larval susceptibility have already been reported in *T. castaneum* exposed to gamma rays (Hasan and Khan, 1998). The LD<sub>50</sub> value of sine sound waves for the larvae was 665.14 and the LD<sub>90</sub> 1339.43 Hz. The dorsal pronotum of adult beetles, developing from bombarded

larvae were studied using scanning electron microscope (SEM) to look for mechanical damage of the dorsal cuticle. The specimens were dried and mounted on stubs by means of double adhesive tape and coated with a 100-200 Å thick film of gold in an IB ion coater from EIKO, Japan for 2-4 minutes. They were then subjected to scanning in a HITACHI-450A SEM Japan, using 10 and 15 KV emission of current. Electron micrographs were taken at 100 ×, 400 × and 1 000 × magnifications. Morphological details of the dorsal pronotum were studied by analysing these micrographs.

**RESULTS**

Development to adult stage was found to be lowest at 1 100 Hz exposure (3.12 ± 0.23%) and slightly higher



**Figure 2:** Scanning Electron Micrograph of a section of the dorsal pronotum of *T. castaneum* (1000 X). S – Seta EC – Epicuticle ST – Socket. 2a, Control S – Long EC –Normal ST – Socket with tromogen cell beneath. 2b, 900 Hz exposure S – Shorter EC - Damaged architecture. 2c, 1 000 Hz exposure S – Bent EC – Heavy damage ST Borders confluent. 2d, 1 100 Hz exposure S – Damaged tips EC – Heavy damage ST – Obscure.

**Table 1:** Probit analysis of sound frequency and mortality.

Frequency	Log (ln) frequency	Larval mortality percentage
900	6.802	58.6 ± 6.8
1 000	6.9077	63.5 ± 6.21
1 100	7.003	85.5 ± 8.45
1 200	7.090	84.5 ± 7.99
1 300	7.17	79.1 ± 6.08

a = -411.29, b = 69.42, r = 0.81, LD<sub>50</sub> log frequency 6.5 = 665.14 and LD<sub>90</sub> log frequency 7.2 = 1 339.43 Hz

**Table 2:** One-way Analysis of Variance and Tukey comparison of mortality in *T. castaneum* larvae exposed to different frequencies of audible sound (n = 10)

Frequency	Mean	SD	SE
900	58.6 a	3.78	1.19
1 000	63.6 b	4.09	1.29
1 100	85.6 c	2.07	0.65
1 200	84.6 c	1.9	0.6
1 300	79.2 d	3.68	1.16

Note: Means marked with different letters vary significantly (Tukey Test) [F value: 147.73 - significant at P < 0.01]

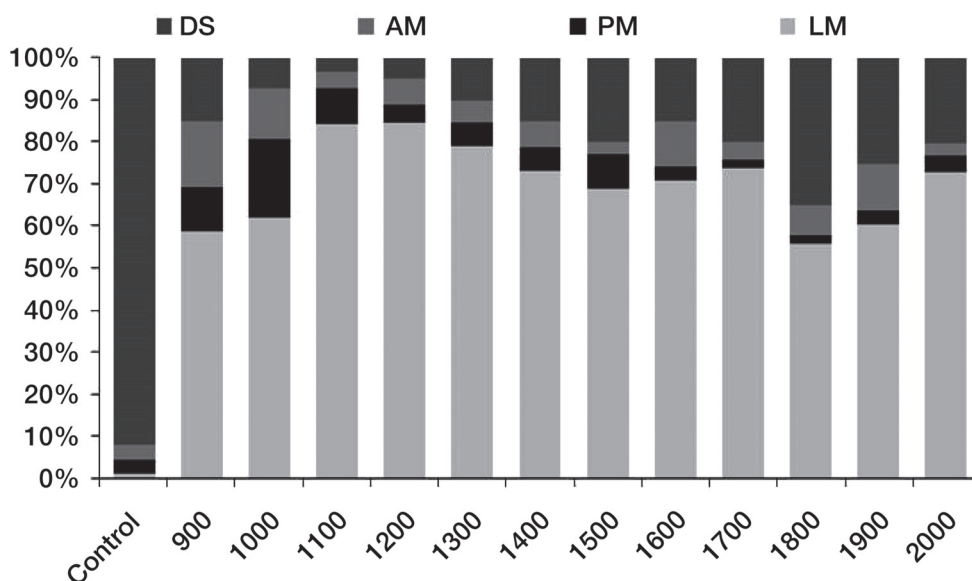
at 1 000 (7.33 ± 0.93%) and 1 200 Hz (5.03 ± 0.56%) exposures (Table 1). The resonant frequency of *T. castaneum* larvae was between 900-1 200 Hz. Beyond 1 200 Hz, the mortality of larvae was more or less uniform. But the developmental success percentage was significantly lower compared to that of control beetles (Table 1 and 2). This could be explained based on observations made in a

previous study when adults from irradiated larvae reach eclosion, the damage in the imaginal discs is probably greatly amplified so that *T. castaneum* would die either at eclosion or shortly thereafter (Hasan and Khan, 1998). The three high impact levels (1 000, 1 100 and 1 200 Hz) were selected for electron micrograph studies. The mechanical injury to insects treated with sound waves has been clearly illustrated with electron micrograph studies (Fig. 2). The cuticle has been badly damaged by sound waves. The setae are lost or damaged, epicuticle obliterated and sockets became obscure.

**DISCUSSION**

Audible sound waves have mechanical energy, which can effect vibrational changes in molecules (Pyykko and Starck, 1985). When the frequency of the sound waves equals the frequency of the organ there is possibility for total damage of the resonant tissue (Kiruba et al., 2009). Sound waves make molecules oscillate and it is normal for them to damage any system that oscillates at its frequency (Acton, 1974). The cracking of glass at its resonant frequency is a classical example (Norton and Karczub, 2003). *T. castaneum* larvae exposed to sine sound waves in the frequency range of 900 – 1 100 Hz experienced mechanical damage. Pradzynska (1982) reported that ultrasonic waves successfully controlled *Sitophilus granarius* adults inside a wheat grain mass. All insects contain microscopic stable gas bodies that can oscillate under the influence of ultrasound (Vincent et al., 2003).

Even though most of the severely damaged larvae could not complete metamorphosis, some bombarded larvae



**Figure 3:** Percentage of mortality and developmental success of the last instar larvae of *T. Castaneum* exposed to narrow spectrum of sine sound waves. (DS – Developmental success %, AM – Adult mortality %, PM- Pupal mortality % and LM- Larval mortality %).

metamorphosed into adult beetles, with perceptible injuries in the dorsal region where the sound waves hit the insect in the grub stage.

The beetles so severely damaged externally, still survived. But one cannot rule out damage to internal organs, especially to the reproductive system. It has been shown in *Tribolium* sp. adults that the histological damage associated with ionizing radiations was the cause for post-radiation mortality. Furthermore, the mechanical strength of the elytra of beetles irradiated as 14-day-old larvae gradually diminished, as the dose was increased (Hasan and Khan, 1998). If such damage has occurred, the fecundity and fitness of the surviving beetle is likely to be reduced and sound waves can be considered to be on par with other physical and chemical interventional strategies. Sound wave intervention of insect pests could be done on a large scale, in the management of both field and stored grain pests.

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