Electromagnetic wave and Microwave heating: an eco-compatible solution to control *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae)

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ABSTRACT

Rhynchophorus ferrugineus (Oliver) (Coleoptera: Curculionidae) is the primary pest of palms. Although several methods are used for its control, there is concern that insecticidal treatments can cause substantial environmental pollution. Therefore, the use of electromagnetic waves (EMWs) or microwaves (MWs) to control *R. ferrugineus* is an attractive alternative option. Microwave heating is increasingly used to manage a wide variety of agricultural and forest pests because it induces thermal death in insects that have a thermal tolerance lower than that of the host matrix. In this study, EMWs (360 Hz, 100 W) applied for 60 min caused 100% mortality of adult RPWs but had no effects on larvae. Microwave heating (2.45 GHz, 500 W) caused 100% mortality of adults in 4 min, whereas larvae required MW heating for only 40 s to achieve 100% mortality. The brain of adult *R. ferrugineus* was the most damaged region following EMW exposure, with severe damage to the neural cells after 60 min. The neural bundles were broken, and the granular cells were scattered. Microwave exposure affected the cuticle and muscles in adult *R. ferrugineus* after 4 min and the midgut, cuticle, and muscles in larvae after 40 s. The gut epithelium separated from the basement membrane and became highly vacuolated, almost disintegrating. The cuticle was reduced with separated layers, and muscle fibers were also affected. This work confirms that EMWs and MW heating are promising, ecocompatible methods to fight the spread of *R. ferrugineus*.

Keywords: Control, Electromagnetic waves, Microwaves, *Rhynchophorus ferrugineus*, Histopathology.

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INTRODUCTION

R. ferrugineus Olivier, is the primary pest of palms and causes considerable economic damage. The RPW appeared first in the 1980s in the Middle East and has since spread slowly during the mid-1990s and quickly there after that in many countries especially in the Asia, Africa, Mediterranean area, Caribbean, Europe, and North America during the last two decades (Rmili *et al*., 2020). *R. ferrugineus* is widely distributed in Egypt, over 261,000 palm trees were infested from 1992 to 2000, and of which approximately 23% were removed (El-Sebay, 2007, Sauvard *et al*., 2010). The most damaging life stage is larva because it causes frond deformity, which leads to palm death (Idris *et al*., 2015). *R. ferrugineus* larvae create cavities and tunnels through the palm tree trunk and reduce the transfer of nutrients and water between the root system and the crown that deteriorate the mechanical structure . However, the detection of weevil larvae at an early stage is an important challenge (Rach *et al*., 2013, Rmili *et al*., 2020).

Different biological, chemical, mechanical, and physical techniques have been developed for use against *R. ferrugineus*. Physical techniques to suppress *R. ferrugineus* and protect palms are based especially on the use of gamma radiation and x-ray (Ramachandran 1991) and electromagnetic energy at microwave frequencies (Massa *et al*., 2011).The physical techniques are combined with other control methods within an integrated pest management strategy (Gliblin-Davis *et al*., 2013), including insect traps, plant extracts, synthetic insecticides, and biological control (Faleiro, 2006, EPPO, 2008, El-Shafie *et al*., 2011, Di Ilio *et al*., 2018, El Namaky *et al*., 2020, El Sadawy *et al*., 2020). However, to date, an economic and effective method has not been adopted for the total extermination of RPW, and chemical applications remain the most widely used method of control (Massa *et al*., 2019). Electromagnetic waves (EMWs) and microwaves (MWs) have been used in many applications, including remote sensing, imaging, quality sensing, and dielectric heating in a pre- or post-harvest environment (Mahabadi *et al*., 2013).The use of MWs against the *R. ferrugineus* is based on the radiation inducing a thermal increase in the pest (Massa *et al*., 2019). The goal is to heat the *R. ferrugineus* to a lethal temperature, or to affect the reproduction ability of survivors, without harming the plant tissues (Hamid 1968, Massa *et al*., 2019, Rmili *et al*., 2020). (Hoffmann, 1985) demonstrated that the rates of growth, development, reproduction and the maintenance of daily activities of insect were affected by temperature. Generally, insects avoid very hot environments that may increase water loss due to heat stress (Henry, 1996). High temperatures are associated with increasing epidermal water permeability and melting of the epicuticular wax layer in Locust amigratoria Loveridge (1968). Also, elevated temperature is associated with cytoskeleton collapse and cell death in Drosophila melanogaster embryos. Additionally, changes in the phospholipid composition of cell membranes cause a decrease in membrane fluidity (Lu *et al*., 2010). The ability of MWs to heat some materials is due to the interactions between the electromagnetic field and the molecules constituting the materials. In the EM spectrum, MWs are in the frequency range 300 MHz to 300 GHz. Additionally, weevils are unlikely to develop resistance to radiation, as often occurs with repeated use of chemical insecticides. (Massa *et al*., 2019) applied MWs to the external layers of infested *Phoenix canariensis* palms, and lethal temperatures to insects were reached without affecting internal palm tissues. Moreover, the MW treatment affected the reproductive ability of the surviving weevils. (Lu *et al*., 2010) used conductive heating and MW radiation to kill *Tribolium castaneum* (Coleoptera: Tenebrionidae) and protect the flour during storage without significantly affecting its quality. In this work, the efficacy of EMWs and MW heating were tested against *R. ferrugineus* adults and larvae in vitro. In addition to measuring mortality, the histological alterations in the cuticle, muscles, and midgut were examined to better understand the mechanisms of EMW- and MW-induced death.

MATERIALS AND METHODS

Insect collection

R. ferrugineus adults and larvae were collected from naturally infected date palm trees from various parts in Abu Rawash, the Giza Governorate, Egypt. Which is located in the western edge of the Nile delta located in northwest of Greater Cairo, the topography of the land is relatively flat (altitude ranging from 10 to 20 m) and the area slopes gently towards the Nile River. The field-collected R. ferrugineus were maintained and established according to methods of (Nassar and Abdullah, 2001).

Application of electromagnetic wave and microwave heating

Electromagnetic waves (360 Hz and 100 W) were produced by an electromagnetic palm rescue device (Fig. 1A). The MW heating was applied to adults and larvae using a previously described method (Lu et al., 2010). Microwave characterization of the materials was performed by the Electronics Research Institute, Cairo, Egypt, and was conducted using the truncated coaxial cable technique (Gabriel and Peyman, 2006) and parameters at 2.45 GHz and 500 W, as shown in (Fig. 2A).

Direct exposure to R. ferrugineus adults and larvae

This technique was designed to simulate R. ferrugineus habitat. Clean palm bark was placed in plastic pots (size 7 × 7 × 5 cm); then, 10 late instar larvae (4–5 gm) and adult (0.60–0.76 gm) individuals were placed in each pot. R. ferrugineus adults and larvae were divided into five groups of 30 insects for each group, and the procedures were repeated three times for each group. The five groups were the following: Group I, control R. ferrugineus that received sugarcane as food and were not exposed to any waves; Group II, adults exposed to EMWs (360 Hz and 100 W for 15, 30, 45 and 60 min); Group III, larvae exposed to EMWs (360 Hz and 100 W for 15, 30, 45 and 60 min) (Fig. 1B); Group IV, adults exposed to MWs (2.45 GHz and 500 W for 1, 2, 3 and 4 min); and Group V, larvae exposed to MWs (2.45 GHz and 500 W for10, 20, 30 and 40 s) (Fig. 2B). Treatment was repeated three times with 10 insects per replicate.

Light microscopy

Samples were taken from control adults and larvae as well as from those exposed to EMWs for 60 min or MWs for 4 min (adults) or 40 s (larvae). The brain and body wall of both control and exposed weevils were fixed in 10% formalin buffer solution for 24 h and then serially dehydrated in an ethanol series and embedded in wax. Transverse sections of 1-μm thickness were cut, dewaxed, and stained with hematoxylin and eosin. The staining was conducted according to (Volkman and Peters, 1989).

Calculations and statistical analysis

The data recorded in different experiments were analyzed by the standard statistical tools.

Analysis of variance (ANOVA) was used to check the significance of differences between means at $P \leq 0.05$. The percent mortality of R. ferrugineus was calculated by the following formula:

Percent mortality = (Number of dead insect ÷ Total number of initial insect) × 100

Corrected percent mortality was worked out after adjusting with the mortality in control using formula given by Abbott and subjected to Probit analysis (1925). Also, data was generated for estimation of Lt50 and Lt90 values. The corrected percent mortality was calculated by using the following formula:

Corrected Percent Mortality = (Pt - Pc ÷100 – Pc) × 100

Where, Pt= Observed mortality in treatment; Pc= Observed mortality in control.

RESULTS

R. ferrugineus adults and larvae

After exposure to EMWs, adult was extremely susceptible compared with larvae, indicating larvae were more resistant to EMWs than adults (Table 1). The efficacy of EMWs increased as the exposure time at 360 Hz increased, and adult mortality reached 90% to 100% after 60 min. The mortality rates were significantly variable (20% to 30%) after 15, 30, and 45 min of EMW exposure. However, larvae didn't exhibit mortality after EMW exposure at the same interval time. Also, mortality was not observed in the control group. A probit analysis of the time-mortality of adult is used and the L_t50 and L_t90 values were 29.8 and 80.5 min, respectively (Table 1).

Table 1. Mortality percentages (mean± SE) of adult and larvae *Rhynchorus ferrugineus* after exposure to electromagnetic waves at various time intervals

Electromagnetic waves (360 Hz, 100 W)			
		95% CI	
Tim (min)	**Adult**	**Lower**	**Upper**
15	23±3.33	8.9	37.7
30	43.3±3.3	28.9	57.7
45	63.3±3.3	48.9	77.7
60	90±5.7	65.15	114.84
F	48.6		
P value	0.00**		
Slop ± SE	2.97±0.32		
Lt_{50} (min)	29.8		
Lt_{90} (min)	80.5		

SE: standard error of the mean (n=30). In each treatment, 30 adults were exposed. **highly significant (P <0.05), Lt50: time that kills 50% of adult, Lt90: Lethal time that kills 90% of adult, CI: Confidence interval limits for mean.

R. ferrugineus adults and larvae

The effects of MWs on the mortality of *R. ferrugineus* adults and larvae are presented in (Tables 2), respectively. All adults were dead after 4 min of exposure, and most stopped moving in a few seconds. The mortality rate ranged from 70-90% after 3 min of exposure, 50-70% after 2 min, and 40-60% after 1 min. As shown in (Table 2), the mortality rates of larvae were also affected by exposure time to MWs.

Mortality reached 100% at 40 s. larval mortality ranged from 50% to 60% at 10 s, 60% to 70% at 20 s, and 80% to 90% at 30 s. Therefore, there was a positive relation between MW exposure time and mortality rate, with increasing MW exposure time causing a significant increase in *R. ferrugineus* mortality. Also, a probit analysis of the time-mortality are used and the L_t50 and L_t90 values of adult were 1.2 and 4 sec, respectively while the L_t50 and L_t90 values of larvae were 1 and 3.6 sec , respectively (Table 2).

Table 2. Mortality percentages (Mean± SE) of adult and larvae *Rhynchorus ferrugineus* after exposure to microwaves at various times interval.

Microwaves							
2.45 GHz, 500 W							
Time (sec)	Adult	95% CI		Time (sec)	Larvae	95% CI	
		Lower	Upper			Lower	Upper
1	50±5077	25.15	74.84	1	53.33±3.33	38.99	67.67
2	60±35.15	35.15	84.84	2	66.66±3.33	52.32	81.00
3	80±5.77	55.15	104.84	3	83.33±3.33	68.99	97.67
4	96.6±3.33	82.32	111.0	4	96.66±3.33	82.32	111.0
F	15.6				32.33		
P value	0.001**				0.00**		
Slop ± SE	2.35±0.31				2.33±0.31		
Lt_{50} (sec)	1.2				1		
Lt_{90} (sec)	4				3.6		

SE: standard error of the mean (n=30). In each treatment, 30 adults and larvae were exposed. **highly significant (P <0.05), Lt50: time that kills 50% of adult, Lt90: Lethal time that kills 90% of adult, CI: Confidence interval limits for mean.

Light microscope observations

The brain was composed of nerve cells and associations of connective tissue and blood vessels (Fig. 1a). The neural cells of adult *R. ferrugineus* brains were severely damaged after 60 min of *R. ferrugineus* exposure (Fig. 1b). In contrast to the control, the neural bundles were broken, and the granular cells were scattered. Many areas of the brain showed many empty spaces without any neural cells.

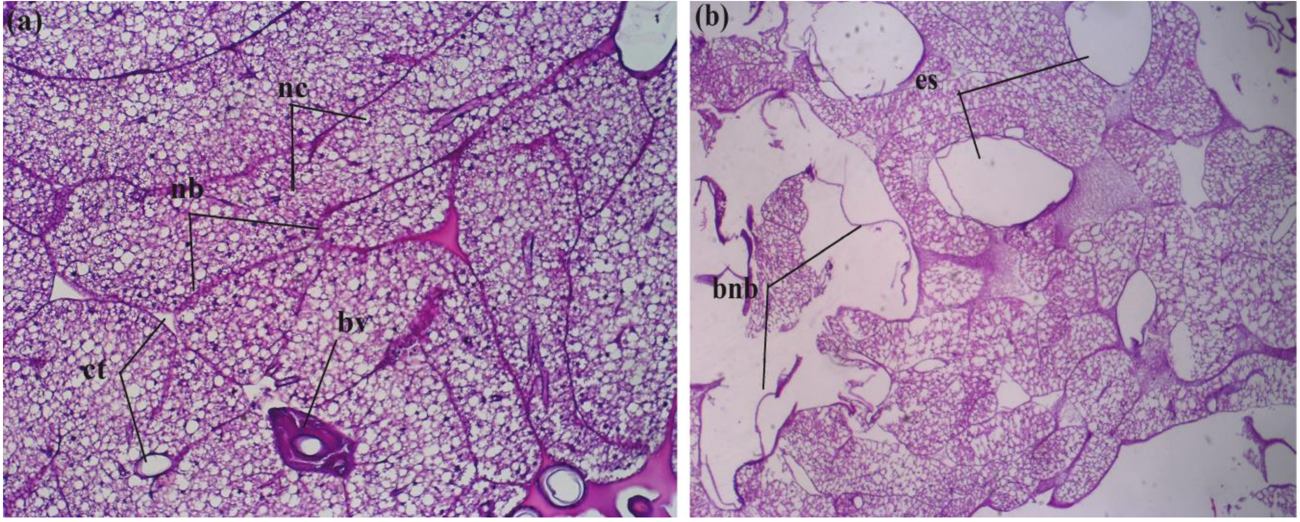

Fig. 1. Brain tissue of *Rhynchorus ferrugineus* adult. (a) Control brain showing nerve cells (nc), neural bundles (nb), connective tissue (ct), and blood vessels (bv).(b) Treated brain showing broken neural bundles (bnb) and empty spaces (es) without any nerve cells (10×).

After 4 min of MW exposure, severe cuticular changes occurred in adults. In contrast to the control, the epicuticle was severely corrugated, and the epidermal layer was completely split. By contrast, the normal cuticle of adult *R. ferrugineus* consisted of thin layer, the epicuticle, followed by a dark, longer layer, and the exocuticle. The endocuticle was the thickest layer of the cuticle (Fig. 2a).

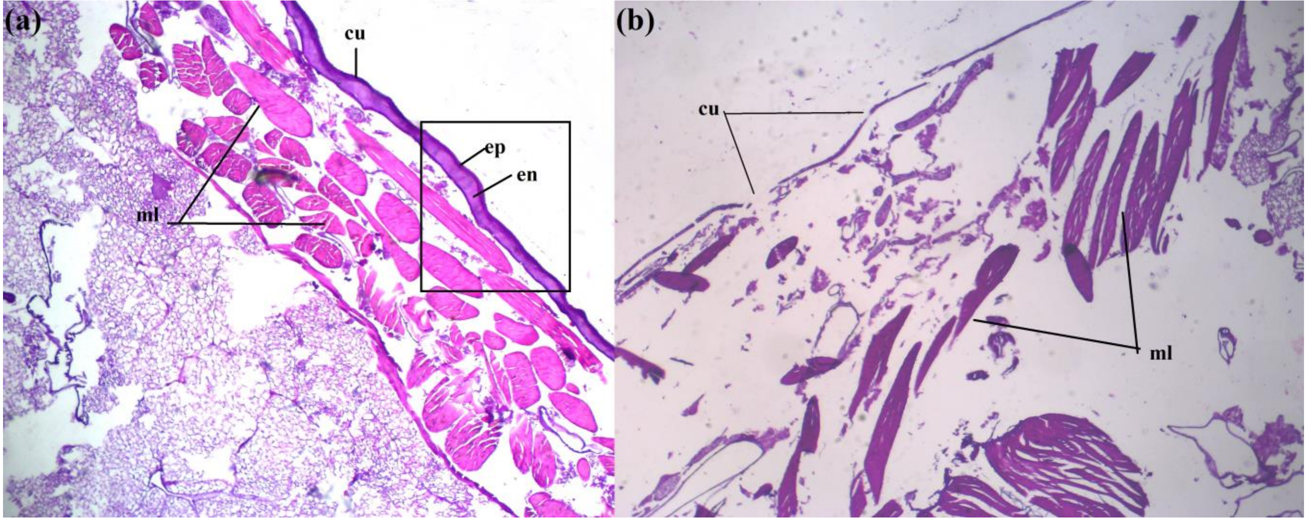

Fig. 2. Transverse hematoxylin and eosin-stained sections of (a) the cuticle and muscles of a normal control adult of the red palm weevil (epicuticle (ep), endocuticle (en), muscle layers (ml)) and(b)the cuticle and muscles after 4 min of exposure to microwaves(cuticle (cu), muscle layer (ml)) (10×)

The effects of MWs on *R. ferrugineus* larvae are presented in (Figs. 3 and 4). The cuticle, muscles and midgut were damaged after 40 s of exposure. The gut epithelium separated from the basement membrane and became highly vacuolated, almost disintegrating. The cuticle was reduced and its layers separated, and the normal arrangements of muscle fibers completely disappeared with many interspersed spaces.

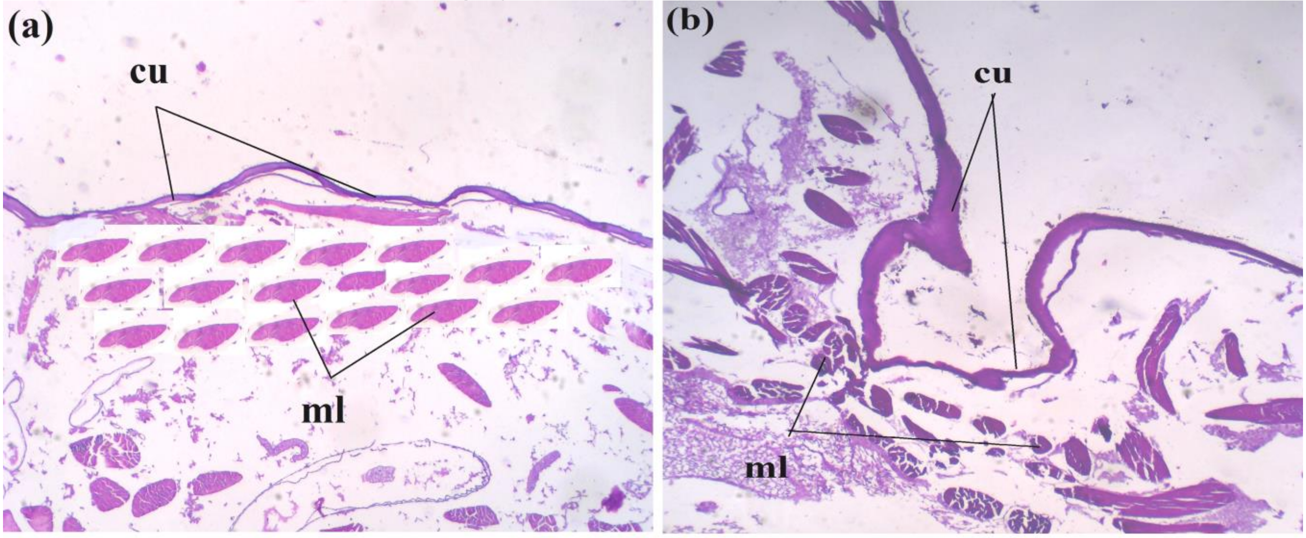

Fig. 3. Transverse hematoxylin and eosin-stained sections of (a) the cuticle (cu) and muscle layers (ml) of a normal control last instar larva of *Rhynchorus ferrugineus* and (b) the cuticle and muscles after 40 s of exposure to microwaves(10×).

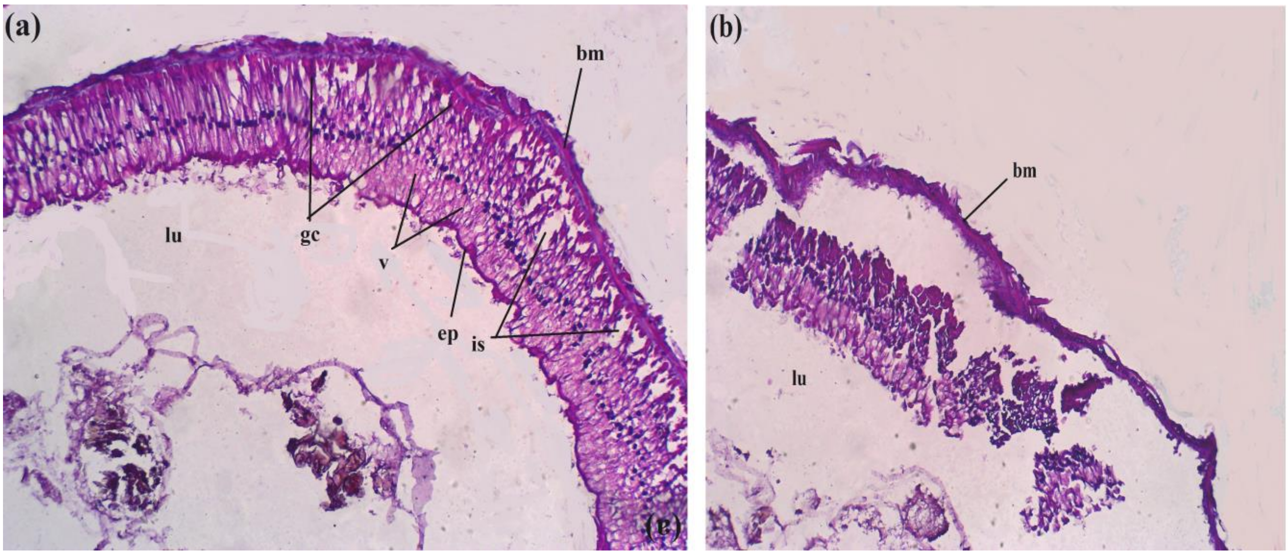

Fig. 4. Transverse sections through (a) the midgut of a normal control last instar larvae of *Rhynchorus ferrugineus* (basement membrane (bm), goblet cells (gc), villi (v), epithelium cells (ep), interspace (is), and lumen (lu) (b) the midgut after 40 s of exposure to microwaves, showing complete separation of epithelial cells from the basement membrane (10×).

DISCUSSION

The mortality of *R. ferrugineus* adults and larvae was significantly different after exposure to EMWs and the thermal effects of MWs. The effects of *R. ferrugineus* on adults were greater than those on larvae at 45 and 60 min, indicating larvae were more resistant to EMWs than adults. In general, the traveling EMWs seemed to simply penetrate the insect body. This outcome most likely occurred because the EMW energy was radiant, and the radiation did not heat the weevil body. In addition, the insect bodies were too small to act as containers of electrons. (Thielens *et al*., 2018) exposed four different types of insects to continuous electromagnetic fields at various frequencies from 2 to 120 GHz. (Sanborn, 2008) and (Nakai *et al*., 2009) suggest that such absorption causes dielectric heating that affects insect behavior, physiology, and morphology. In the present work, the non-thermal effects of EMWs on adult *R. ferrugineus* produced significant histopathological alterations and mortality. The histological observations were only recorded in the brain after 60 min of EMW exposure. Such observations have not been previously reported. Therefore, the results were discussed with those for other insects. The damage in the brain was extensive with broken neural bundles and scattered granular cells. Many areas of the brain showed many empty spaces without any neural cells. After 30 s in irradiated female and male *R. ferrugineus*, with necrosis and vacuolar degeneration of germinal cells in the ovary and degenerative changes in the precursors of the seminal epithelium and spermatids (Massa *et al*., 2019).

Diba *et al*. (2013) demonstrated that EMWs can be used as a nondestructive method for termite control, which is very useful because EMWs are odourless and application is easy, noise-free, and environmentally friendly. In this study, and the R. ferrugineus in this study died immediately. Adult *R. ferrugineus* mortality reached 100% after 4 min, whereas 100% mortality was reached in larvae after 40 s. Thus, RPW larvae were much more sensitive to MWs than the adults. Rmili *et al*. (2016) analyzed temperature distribution and concluded that small larvae are much more sensitive to MW heating than adults. Massa *et al*. (2019) reported the same result. However, according to Rmili *et al*. (2020), the higher water content in larvae than in adults strongly limits the penetration of MWs inside larvae, compared with that in adults. The histological analysis showed that cuticle and muscles were the first sites damaged after adult *R. ferrugineus* were exposed to MWs for 4 min. The cells directly exposed to the exterior when the insect cortex is damaged and may suffer oxidation reactions that lead to death (Woo *et al*., 2000). Following MW exposure in this study, the cuticle layers were thinner with blurred boundaries. Similarly, Lu *et al*. (2010) and Martano *et al*. (2018) found that the boundaries between layers of the cuticle are unclear after heat treatment of RPW male and female. They investigated the histological and morphological features of RPW male and female reproductive systems irradiated at three different exposure times (5, 15, and 30 s) with MWs (2.45 GHz and 500 W). The lesions in exposed female and male *R. ferrugineus* increase with the increase in exposure time, and necrosis are observed in all female samples at 30 s and in most male samples (75%). In the current work, the cuticle, muscles, and midgut of *R. ferrugineus* larvae were damaged after 40s of MW exposure. Similarly (Lu *et al*., 2010) examined the effects of MW irradiation and conductive heating on the midgut of adult *T. castaneum*. The midgut cells were smaller than control midgut cells and the nucleus appeared hollow as well as the nuclear matrix was unevenly distributed.

In conclusion, it is hoped that the results in this paper will help to better understand the responses of *R. ferrugineus*. Adults and larvae to EMWs especially and also to MW heating and support the fight against the spread of the *R. ferrugineus*.

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