Evaluation of extremely low-frequency electromagnetic resonance designed pulses for controlling red palm weevils, *Rhynchophorus ferrugineus,* by estimating the metabolic and enzyme activity responses

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Abstract

A study aims to investigate the effect of extremely low-frequency electromagnetic field (ELF-EMF) designed pulses on the biological and physiological systems of *Rhynchophorus ferrugineus* (Red Palm Weevils) reared insects under specific conditions for a specified period. About 300 RPW insects at three stages (larvae, pupae, and adults) are evenly divided. Each stage is similarly categorized into two groups: a tested group exposed to ELF-EMF with a strength of 30 mGauss at a frequency of 20 Hz for 86 minutes, and a control group not exposed. A magnetic field is generated and induced at the centre of the rearing holder via an electric current using a current generator device; the magnetic field strength is measured in Gauss with a gauss meter. During rearing, a predetermined number of insects from each group (control and tested) are isolated or taken for biochemical analysis tests that assess the core influence of the ELF-EMF designed pulses on the exposed insects compared to the unexposed, and the results are recorded. The biochemical tests involve measuring and comparing parameters such as total proteins, total carbohydrates, total lipids, invertase, and alkaline phosphatase enzymes. Additionally, the body weight of the insects is recorded before and after exposure, and the change in body weight and growth rate are calculated for comparison. The results indicate that body weight, growth rate, and the biological components of R. ferrugineus are negatively affected by exposure to ELF-EMF designed pulses.

Keywords: *Rhynchophorus ferrugineus,* ELF-EMF, Arecaceae, Egypt

Introduction

Rhynchophorus ferrugineus has become known as the most predominant and lethal pest or insect that infests palm species *(*Arecaceae*)* and invades their tissues worldwide; its host palms are notably increased tenfold from only four host palms to more than 40 host palms currently. The main common and most preferred hosts of palm trees that are infected with *R. ferrugineus* are coconut, date palm, canary palm and oil palms [2, 3, 6]. *R. ferrugineus* has huge substantial economic influence on the host palm industry; through the financial losses and economic impact as a result of the high loss rate, the yield reduction, the infested palm removal, and the high cost of the usage of pesticide treatments as a protective process of the host palms from the pest infestations and the control or management programs. It was first named the red palm weevil (RPW) by Oliver in 1970. *R. ferrugineus* originates from the *Rhynchophorus* genus, *Coleoptera* order, *Dryophthoridae* subfamily, and *Curculionidae* family. RPW has a wide and highly expanded distribution around the world. It is considered one of the five main species that originated from tropical and Southeast Asia; it is normally distributed in Southeast Asia and Melanesia. *R. ferrugineus* is a sexually dimorphic pest that goes through a life cycle inside the palm trunk. This life cycle contains four consequent developmental phases or stages; this life cycle starts with egg, larva, pupa, and ends with the adult as clarified in figure (1). This life cycle lasts between 45 and 298 days [1, 6].

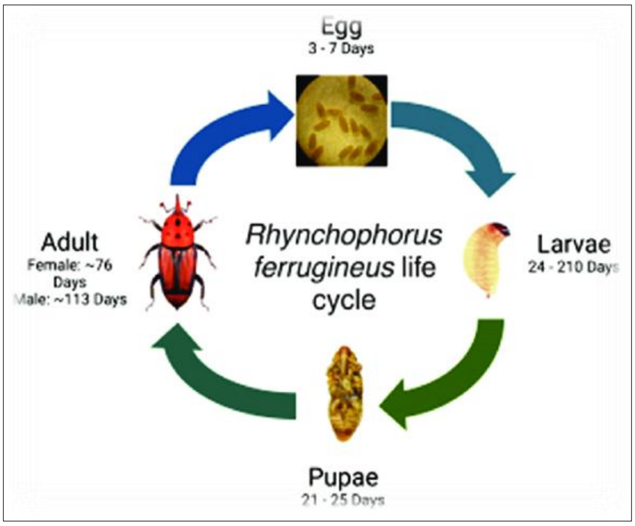

Fig 1: The four developmental stages of *R. ferrugineus* (1)

Although many researches have been conducted regarding RPW control or detection, it is not that easy to detect it unless the palm tree is cut and investigated; due to its masked life style, its ability to live and feed inside the plant (larva stage) which protects it from risky external conditions, insecticides, natural enemies, in addition to the high locations of detection; as it hides in the top of tall trees that can be hardly observed or investigated [4, 7]. *R. ferrugineus* can infest the stems and the growing points in a way that can be hardly detected in the early stages of infestation; through some methods such as visual inspection, larva's grubbing sound detection, detection of the chemical volatile signatures as a result of the palm infestation, detecting temperature by thermal imaging. Meanwhile, it can be detected in the severe damage phase of the host palm in which some signs or symptoms can be observed indicating the presence of the insects; such as deep holes or tunnels and dryness of the palm trunk tissues or the external leaves or the fruits of the tree, fiber damage and extrusion, in addition to the oozing of viscous brown fluid with a chewed fibers accompanied with a fermented odour [5-7].

Red Palm Weevil detection can also be carried out using some developed techniques, such as using infrared cameras, thermal imaging, chemical and acoustic detection through distinguishing the RPW sound and vibrations among the other sounds. There are many studies have been conducted regarding its detection and management; some control guidelines or programs for *R. ferrugineus* control conducted by (FAO 2020) involve some management techniques; the chemical control either for the treatment in the early stages of pest infection by insecticides or as a protective precautionary measure before the pest infestation, using biological control solutions using microorganisms such as some nematodes, fungi, viruses and bacteria. In addition to using food-baited or synthetic lures for pheromones-based trapping techniques for the insect attraction and control or killing that depends on the aggregation pheromones, sterile insect techniques and the application of integrated pest management programs on broad scales that uses a mixture of the previously mentioned tools for the pest control [2, 3, 7]. Despite all these detection or management methods, they have limited success; as a result of evolving resistance against the insecticides' usage and overdependence, which leads to many environmental hazards. Therefore, there is a huge and urgent need to develop a sustainable and innovative way for Red Palm Weevils control to reduce and mitigate the environmental hazards and the resistance consequences by mitigating the dependence on insecticides or pesticides [9].

Electromagnetic fields have become hardly separated from daily life or ordinary living or working environments; it can be easily generated by many ordinarily usable devices within a frequency range of 50 or 60 Hz. Although it lies in the range of extremely low-frequency, non-ionizing waves of the whole spectrum, it is proven later that it can affect the biological systems and the physiology of tissues, generating some electrical and metabolic changes in the cell [11]. Extremely low-frequency electromagnetic fields (ELF-EMF) are normally lies within the range up to 100 Hz or 300 Hz that can be generated through a power of high voltage line of transmission that can produce this field inside the biological systems; therefore, in the recent decades, there is a lot of attention has been paid towards the impact of ELF-EMF on the biological systems [12]. There is a promising strategy or technique that involves using ELF-EMF in the pest control through affecting the physiological aspects of the pests involving metabolic activity rates and enzymatic processes through the change in the main biological components and enzymes of the insect that influence and alter its metabolic and cellular activities and processes as a result of the stress [8]. Electro-Magnetic field (EMF) usage as a stress source has been of great interest to investigate its effect on the biological systems and their metabolic processes. This study focuses on the investigation of the influence of electro-magnetic field (EMF) designed pulses on the physiological activities including the metabolic and enzymatic aspects of Red Palm Weevils *(R. ferrugineus)* by measuring 1- total proteins, 2- total carbohydrates, 3- total lipids as determined comparative parameters between the treated and untreated insects with ELF-EMF in addition to the measurement of the activity of some enzymes as invertase, alkaline phosphatase before and after the exposure, as well.

Materials and Methods

a) Insect isolation, preparation and rearing

The experiment started with the isolation of about 300 reared insects of R. ferrugineus and separating them into two groups; 150 insects as the control group (not exposed to EMF designed pulses) and kept away from the tested group and the source of the EMF pulses, and 150 insects as tested group (to be exposed to EMF designed pulses). Each group of them includes the 3 developmental stages of R. ferrugineus; 50 larvae, 50 pupae, and 50 adults were involved in both the groups (control group and the tested group. Under laboratory circumstances, the two groups were reared on a stem of sugarcane. All the body weights of the stages of the two groups are measured in milligrams and recorded to follow and recognize the gain in body weight and compare the body weight before and after exposure, in addition to the estimation of the growth rate.

b) Production, inducing, and adjustment of ELF-EMF designed pulses

This step involved the exposure of a number of red palm weevil larvae to extremely-low frequency electromagnetic field (ELF-EMF) designed pulses. It was mandatory before the exposure process for studying the physiological effects of the ELF-EMF pulses on these exposed larvae to be investigated, the strength or the potency of the EMF pulses had to be calibrated and adjusted. Electromagnetic Field creation is carried out using an electric current inducing device that generates an electric current, which consequently aids in the generation of a magnetic field, forming these pulses of ELF-EMF into a plastic rearing holder. The strength of EMF is assessed in milligauss (mG) through a gauss meter device, and the optimum flux potencies were set to be suitable for *R. ferrugineus* is about 30 milligauss, with a frequency of about 20 Hz for about 86 min. Every value resembles several actual readings taken at the rearing holder central point until the exact suitable value of MF potency and frequency has been chosen.

c) Insect sampling and preparing for the biochemical analysis

Samples of the larvae, pupae, and adults of the control and tested groups were prepared through their homogenization in distilled water and put in a cool centrifuge at 8000 rpm at 2 °C for 15 minutes.

d) Carrying out the biochemical analysis

All the stages (larvae, pupae, and adults) of the control and tested groups underwent specific biochemical tests; total proteins, total carbohydrates, total lipids, invertase, and alkaline phosphatase enzymes activity of both the groups to calculate the actual change value and subsequently, the percentage of change in their values in exposed and unexposed groups to compare between the values of the two experimental groups of all the developmental stages.

e) Examining the influence of the EMF pulses on the body weight and growth rate

That step includes examining the body weight of each developmental stage of RPW used in the experiment before and after the exposure to EMF pulses in a table to examine the following:

- The change in body weight of the exposed insects after the exposure process, as compared to their weight before the exposure process, across all the developmental stages.
- The growth rate of the insects of the two experimental groups (control group & tested group) across all the developmental stages to evaluate the effect of EMF designed pulses on the growth rate of the exposed insects (tested group) and compare it with the normal growth rate of the unexposed insects (control group).

Results and Discussion

Effect of Electro-Magnetic Field (EMF) on body weight of red palm weevil *(R. ferrugineus)* across developmental stages

Results in Table 1 show the body weight (mg) of 50 larvae, pupae, and adults of *R. ferrugineus* reared under ELF-EMF designed pulses exposure compared to a control group without electromagnetic field (EMF) treatment. The larvae were reared for 20 days, pupae for 41 days, and adults for 20 days under standard laboratory conditions. The body weight of individuals in the treatment group was significantly lower than that of the control group across all stages. The growth rate per larva per day and the gain in weight during the rearing period were recorded, showing substantial reductions in the treated group. The larval weight significantly decreased in the treatment group compared to the control. The control group recorded a mean weight of 69.98 mg/larva, while the treatment group averaged 55.22 mg/larva. The growth rate *I* day also dropped from 3.50 mg/day (control) to 2.76 mg/day (treatment), resulting in a lower gain in weight (0.35 mg vs. 0.28 mg). These findings indicate that exposure to EMF pulses negatively impacted larval growth and weight accumulation.

Table 1: Effect of ELF-EMF designed pulses on body weight and weight gain of red palm weevil (*Rhynchophorus ferrugineus*) across developmental stages"

Instar	Duration (Days)	No. of Larvae (Control)	Total Weight (Control, mg)	Weight/Larva (Control, mg)	Growth Rate (Control, mg/day)	Gain Weight (Control, mg)	No. of Larvae (Treatment)	Total Weight (Treatment, mg)	Weight/Larva (Treatment, mg)	Growth Rate (Treatment, mg/day)	Gain Weight (Treatment, mg)
larvae	20	50	3499	69.98	3.50	0.35	50	2761	55.22	2.76	0.28
pupae	41	50	6199	123.98	3.02	1.23	50	2944	58.88	1.44	0.61
adult	20	50	7729	154.58	7.73	3.86	50	2222	44.44	2.22	1.12
total	81	150	17,427	116.65	4.09	5.44	150	7927	52.82	2.14	2.01

Gain Weight (mg) = (Growth Rate x Durat10n). Growth Rate= (Weight/Larva + Duration)

Results in Table (2) summarize the body weight and gain weight of *R. ferrugineus* across its developmental stages under EMF exposure compared to the control group. A total of 150 individuals (50 larvae, 50 pupae, and 50 adults) were reared under standard laboratory conditions, with significant differences observed in weight, growth rate, and gain weight between the control and treatment groups. The larvae reared under EMF exposure exhibited a significant reduction in weight compared to the control group. The average weight per larva in the control group was 69.98 mg, while the treated group recorded 55.22 mg. The growth rate per larva per day decreased from 3.50 mg/day (control) to 2.76 mg/day (treatment), resulting in a lower gain weight of 0.28 mg in the treatment group compared to 0.35 mg in the control group. These findings indicate that EMF field exposure significantly impeded larval growth. The pupal stage demonstrated the most substantial reductions under EMF exposure.

The averages weight per pupa was 123.98 mg in the control group, compared to 58.88 mg in the treatment group. Similarly, the growth rate per larva per day dropped from 3.02 mg/day in the control group to 1.44 mg/day in the treated group. The gain weight of treated pupae (0.61 mg) was less than half of that observed in the control group (1.23 mg). These results highlight the pupal stage's heightened susceptibility to EMF exposure, significantly impairing its development. The adult stage also showed significant reductions in weight and growth parameters. The average weight per adult was 154.58 mg in the control group, while the treated group recorded 44.44 mg. The growth rate per day decreased from 7.73 mg/day (control) to 2.22 mg/day (treatment), with a corresponding reduction in gain weight from 3.86 mg in the control group to 1.12 mg in the treatment group. These reductions suggest a profound impact of EMF exposure on adult robustness and potential reproductive capacity.

The total weight and gain weight across all stages were significantly lower in the treatment group compared to the control group. The cumulative weight dropped from 17,427 mg in the control group to 7,927 mg in the treatment group, and the total gain in weight decreased from 5.44 mg to 2.01 mg. Among the stages, the pupal stage experienced the most pronounced reductions, emphasizing its vulnerability to EMF exposure.

Table 2: Effect of EMF exposure on weight across different life stages of the red palm weevil *(Rhynchophorus ferrugineus)*

Stage	Control Weight (Mean ± SE)	Treated Weight (Mean ± SE)	Absolute Change (A)	% Change	Significance *(p*-value*)*
Larvae	3499 ± 118.55	2761 ± 88.54	754	33.43%	p < 0.05
Pupae	6199 ± 118.55	2944 ± 88.54	544	66.43%	p < 0.05
Adults	7729 ± 118.55	2222 ± 88.54	422	46.30%	p < 0.05

Control weight (mean±SE): Average weight with standard error for the control group. **Treated weight (mean± SE):** Average weight with standard error for the group exposed to the EMF. **Absolute change (A):** The difference between control and treated weights =control - treated. % **Change:** The percentage change due to the treatment =absolute Change/ control) * 100.

Table 3: Biochemical analysis of red palm weevil larvae *(Rhynchophorus ferrugineus)* in response to exposure to EMF

Parameter	Control mean± SE	Treatment Mean± SE	Absolute Change (A)	% Change
Invertase (µgGiucose/min/g b.wt)	3499 ± 118.55	2761 ± 88.54	754	33.43%
Alkaline Phosphatase (U x 103 g b.wt)	1775±88.6	1725 ± 54.73	66	2.42%
Total Lipids (mg/g b.wt)	15.32	14.11	0.33	3.01%
Total Carbohydrates (mg/g b.wt)	70.33	66.17	5.54	7.66%
Total Protein (mg/g b.wt)	90.22	88.01 ± 3.24	1.22	1.43%

Control mean ± SE and Treatment mean ± SE columns show the average and standard error for each parameter in both control and treatment groups. Absolute change (.6.) represents the absolute difference between control and treatment.

Table 4: Biochemical analysis of red palm weevil pupa e *(Rhynchophorus ferrugineus)* in response to EMF exposure

Parameter	Control mean ± SE	Treatment Mean ± SE	Absolute Change (A)	% Change
Invertase (µg Gluco se/min /g b.wt)	6199 ± 118.55	2944 ± 88.54	544	66.43%
Alkaline Phosphatase (U X } 03 g b.wt)	1865 ± 88.6	1885 ± 54.73	74	1.42%
Total Lipids (mg/g b.wt)	17.6	16.22	0.53	4.22%
Total Car bohydrates (mg/g b.wt)	65.34	77.66	5.54	7.66%
Total Protein (mg/g b.wt)	88.43 ± 5.03	81.21 ± 3.24	4.25	3.22%

Control mean ± SE and Treatment mean ± SE columns show the average and standard error for each parameter in both control and treatment groups. Absolute change (.6.) represents the absolute difference between control and treatment.

Table 5: Biochemical analysis of red palm weevil adults *(Rhynchophorus ferrugineus)* in response to EMF exposure

Parameter	Control mean±SE	Treatment Mean± SE	Absolute Change (Li)	% Change
ln vertase(µgGlucose/min/g b.wt)	7729 ± 118.55	2222 ± 88.54	422	46.3%
Alkaline Phosphatase (U x 103 g b.wt)	1775 ± 88.6	1455 ± 54.73	46	0.41%
Total Lipids (mg/g b.wt)	13.3	15.42	0.13	2.43%
Total Carbohydrates (mg/g b.w t)	44.55	64.11	3.12	5.42%
Total Protein (mg/g b.wt)	89.33 ± 5.03	75.22 ± 3.24	2.25	4.22%

Control mean ± SE and Treatment mean ± SE columns show the average and standard error for each parameter in both control and treatment groups. Absolute change (.6.) represents the absolute difference between control and treatment.

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