



Research Article

Efficiently Controlled *Callosobruchus maculatus* (F.) (Fam. Bruchidae) by Ultraviolet Ray or Microwave Energy in Conjugation with Pirimiphos-Methyl

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Abstract: This study aimed to explore novel approaches for effectively managing *Callosobruchus maculatus* while ensuring minimal impact on human health and the environment. The investigation involved exposing the insects to ultraviolet rays (254 nm) for 24 hours, which did not directly cause mortality in adult *C. maculatus* but led to a significant reduction in their progeny by 10%. The reduction in progeny further increased when insects were pre-exposed to a sublethal dose of actellic at LC₂₅, resulting in a reduction of 39%. Additionally, ultraviolet rays exhibited a significant ovicidal effect, with hatchability reduced to 26% compared to 73% in the control group. Microwave exposure at a low energy level for 4-6 minutes demonstrated a significant acute lethal effect, reduced progeny, and ovicidal action. Combining microwaves with actellic at LC₂₅ slightly enhanced the lethal effect. In conclusion, this study unveils promising strategies for the effective management of *Callosobruchus maculatus*, highlighting the potential of ultraviolet rays and microwave energy, either alone or in combination with actellic, while emphasizing the importance of minimizing adverse impacts on both human health and the environment.

Keywords: pest management, actellic, progeny reduction, ultraviolet ray, low-energy microwave, ecological impact

Abbreviation

LC ₂₅	Lethac concentration which kills 25% of population
UV	Ultraviolet
MHz	Mega hertz
LDP line	Dose Probit line
ANOVA	Analysis of variation
Nm	Nano meter

1. Introduction

Legumes, also known as pulses, rank as the second most crucial category of crops globally due to their rich content of proteins, minerals, and vitamins. Consequently, they face a threat from various beetles that invade and damage seeds, leading to weight loss, as noted by El-Gedwy et al.¹ Typically, infestation begins in the field, where eggs are laid on mature pods.²⁻³ Over 150 insect pests pose a threat to pulse crops, with *Callosobruchus chinensis* (Linn.), *C. maculatus* (Fab.), and *C. analis* (Fab.) causing both quantitative and qualitative damage to stored grains.⁴ The predominant methods for controlling stored grain pests involve insecticides and fumigants. However, prolonged use of these chemicals has led to environmental hazards and the development of resistance in stored product pests, prompting increasingly restrictive policies.⁵⁻⁶

In response to these challenges, environmentally friendly alternatives are gaining traction. Methods such as inert dusts, plant extracts, oils, leaf powders, pressurized carbon dioxide, and temperature management techniques (both low and high temperature) are being explored as substitutes for synthetic pesticides.⁷⁻⁸ Microwave radiation is emerging as a particularly promising eco-friendly approach, causing no hazardous impacts on humans.⁹ The physiological processes of insects are adversely affected by microwave radiation, leading to a significant reduction in their reproduction and survival.¹⁰⁻¹⁴ Microwave technology demonstrates potential applications in pest management, offering a rapid and nondestructive means of targeting incipient insect infestations. This approach holds the potential to benefit both producers and consumers of packaged foods.¹⁵

Recently, there has been a growing interest in employing high-energy ionizing radiation for insect pest control, partially driven by an increased awareness of the hazards and limitations associated with traditional insecticides.¹⁶ Investigations into the use of ionizing radiation to combat insect pests affecting stored products have taken place in various regions worldwide. The appeal of irradiation lies in its numerous advantages as a disinfestation treatment for stored products, most notably the absence of chemical residues linked to insecticides or fumigants. In parallel, researchers have explored the potential use of ultraviolet (UV) rays to control or, at the very least, suppress the development of various species of stored-product insects.¹⁶ The impact of different irradiation durations of UV (0, 10, 20, 30, 40, and 50 minutes) significantly influences the mortality of immature stages of *C. maculatus*.¹⁷ Evaluation of the effect of UV on the egg hatching of *C. maculatus* revealed that all exposure periods (2, 4, 8, 16, 24, 32, and 40 minutes) reduced egg hatching compared to the control.¹⁸ The mortality of stored-product pests *T. confusum* and *Plodia interpunctella* exposed to microwave radiation (2,450 Megahertz (MHz)), either intermittently or continuously, was assessed, taking into account exposure time, power, and different growth stages.¹⁹ Effective control with ultraviolet radiations requires a 12-hour exposure for *Sitophilus oryzae* and a 24-hour exposure for *Tribolium castaneum*.²⁰ A 24-hour exposure resulted in a 95.24% and 89.72% reduction in progeny and a preventive efficiency of 94.25% and 93.37%, respectively.²⁰ All stages of *C. maculatus* and cowpea seeds exposed to the lowest energy level of microwave (17% of 800 W) for 2, 3, 4, 5, and 6 minutes exhibited highly significant effects on adult mortality, hatchability, reduction of adult progeny, eggs, larvae, and pupae, particularly at the 5-minute mark.²¹

Microwave radiations serve as an alternative to traditional chemical methods for eradicating insects and mites infesting stored grains, including adult insects and larvae of *S. granarius* (L.), *S. oryzae* (L.) (Coleoptera: Curculionidae), and *Rhyzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae).²² The mortality of these pests' increases with both power and exposure time, and complete reduction in F₁ (new generation progeny)-progeny of all insects was achieved at medium power during a 4-minute exposure.²¹ Irradiation, encompassing methods like UVC and microwaving, is employed to suppress pest populations in stored products, providing environmentally friendly alternatives to protect stored products from pest attacks for ensuring healthy, safe food.²³ Electromagnetic waves of super-high frequency and microwave bands stand out as efficient methods for food pest control.²⁴ Exposing insects such as *S. oryzae* and *T. castaneum* to microwave irradiation (2,450 MHz) for 25 seconds resulted in preventive efficiency for stored material reaching 97.68% and 99.02%, respectively. The exposure periods needed to kill 50% of some coleopteran adults (LT₅₀%) (Lethal concentration which kills 50% of the population) ranged from 13 to 14 seconds.²⁰ The differential or selective absorption of energy from radio frequency and microwave fields holds promise for stored-grain insect control.²⁵ Microwave energy, explored as an alternative to fumigation, was applied for controlling the dried fig (*Ficus carica*) moth (*Ephestia cautella*) *Cadra cautella*, there study investigated the physical, microbiological, and sensory properties of figs, determining dielectric coefficients, loss factors, and calculating microwave energy penetration

depths.²⁶

The objective of the planned study is as follows: assess the lethal impact and the effect on reduction of microwave radiation and ultraviolet (UV) exposure, individually and in conjunction with low doses of actellic, on *Callosobruchus maculatus* adults and their immature stages.

2. Materials and methods

2.1 Tested insects

The original strain of the tested insect cowpea beetle, *Callosobruchus maculatus* (F.) (Fam: Bruchidae) was obtained from the Department of Stored Grain and Product Pests Research, Plant Protection Research Institute, Sakha Branch Agriculture Research Station. Insect species were tested and their life cycle was identified according to Badawy and Doraeham.²⁷ The cowpea seeds used for insect culture and experiments were previously sterilized by freezing at -18 °C for one week to kill any prior insect infestation. Then stored in sealed polyethylene bags at 5 °C until required for experiments. Newly emerged adults (0-24 hrs. old) were used. In order to obtain the adults, the culture medium was sieved to remove the old beetles therein and the emerged insects were collected for experiments. Glass jars containing adults were transferred to an incubation 26 ± 1 °C, 65 ± 5% R.H. Daily observation of the laid eggs were followed to get the needed stages.

2.2 Preparation of seeds

Cowpea seeds, *Vigna unguiculata* (L.) Walp. var. Azmerly, were used as a medium, carrying the tested insects. The moisture contents of these seeds were measured by oven-drying duplicate samples each of 5 gm, at 130 °C for one hour, then calculated from the following formula:

$$\text{Moisture content \%} = \frac{\text{Initial seeds weight} - \text{Final seeds weight}}{\text{Initial seeds weight}} \times 100$$

The seeds were stored in sealed polyethylene bags in a refrigerator at 5 °C until further investigations.

Ultraviolet rays (UV) and microwaves were tested alone or in combination with actellic (Pirimiphos-methyl) for their toxicity against *C. maculatus*.

2.3 Ultraviolet (UV) rays

2.3.1 Effect on mortality

An ultraviolet lamp (UVGL-55, UPLAND, CA. USA) was used in the tests, the long wave was 365 nm (nano meter) and the short wave was 254 nm were adopted, and ten pairs of adults of cowpea beetle were placed in petri dishes with a diameter of 9 cm containing cowpea seeds treated with actellic, pirimiphos-methyl (at LC₂₅, according to El-Zun et al.)²¹ or control ones. Three replications were made for each case. Petri dishes were covered with transparent metal gauze and placed under a UV lamp static at a vertical distance of 14 cm. The ultraviolet lamp and the dishes were placed inside a glass jar with dimensions, 25 in height × 35 × 50 cm. Then jar was covered with a glass lid. Exposure to ultraviolet radiation continued for different periods (12, 18 and 24 hours). At the end of each specified period, the mortality number of insects for each treatment was recorded.

2.3.2 Effect on progeny

Insects still alive after 24 h of exposure (survivals) were stored under laboratory conditions to allow insects to complete their life cycle. After ten days, the parent insects were removed. At the end of the life cycle period, adults emerged were counted and the % reduction of progeny was calculated as follows:

$$\% \text{ Reduction} = \frac{\text{No. of adults emerged in control} - \text{No. of adults emerged in treatment}}{\text{No. of adults emerged in control}} \times 100$$

Two types of control were done, seeds carrying insects without being subject to UV exposure, and insects confined to seeds treated with actellic at LC₂₅.

2.3.3 Ovicidal effect

For studying the ovicidal action of UV rays, 20 g of cowpea seeds carrying 200-250 eggs were exposed to UV light for 24 h, at the same vertical distance mentioned above and this was replicated three times. The same treatment without subjecting to UV light was considered as a control. Egg hatching was examined and % hatchability was calculated.

2.4 Microwave

2.4.1 Effect on adult mortality

A microwave oven (EM-280 M, Electra, Japan, Capacity 28 L and cavity dimensions 21.9 × 35 × 35 cm). Twenty adults of *C. maculatus* were confined to 9 cm diameter petri-dish containing actellic-treated cowpea seeds (at LC₂₅) or non-treated ones. Three replicates were performed for each treatment. Dishes were covered and placed inside the oven. The oven was operated at a low energy level *i.e.* 17% of power out (output: 800 W). The operating frequency of the oven was 2,450 MHz. Exposure periods were 2, 3, 4, 5 and 6 minutes. For each period the temperature inside the oven was recorded by inserting the bulb of a thermometer inside the seeds. At the end of each specified period, mortality counts were recorded.

2.4.2 Effect on progeny

To study the effect on progeny, similar treatments were carried out except that each dish carried 40 insects. Following each exposure period, survival insects were stored under laboratory conditions to allow insects to complete their life cycle. After 10 days of storage, parent adults were removed. At the end of the life cycle period, adults emerged were counted and the % reduction of progeny was calculated.

2.4.3 Ovicidal action

For testing the ovicidal action, an initial No. of 200 eggs was placed in a dish containing 20 gm of cowpea seeds, and this was replicated three times. Dishes containing eggs were exposed to the microwaves inside the oven for different periods. At the end of each specified period, eggs were left to hatch and % hatchability was calculated.

2.4.4 Data analysis

Normality and homoscedasticity of dependent variables were checked, and results showed heterogeneity of the data. Mortality data were corrected for control mortality using Abbott's formula.²⁸ Statistical analysis of the toxicity data was performed using probit analysis to estimate the LC₅₀ (Dose Probit line (LDP line)). One-way Analysis of Variation (ANOVA), followed by Duncan's Multiple Comparisons Test (Graph Pad Software, San Diego, California, USA), was performed to compare between means of the tested parameters at a 5% confidence level.

3. Results

3.1 Ultraviolet irradiation

3.1.1 Effect on mortality

Results presented in Table 1, showed that ultraviolet rays have no direct mortality to adults of *C. maculatus*. Insects exposed to UV for 24 h showed no mortality even at the highest tested energy (*i.e.* the frequency, 254 nm). Actellic

alone caused 15 % mortality, while joint action of actellic and UV produced 20 % mortality.

3.1.2 Effect on progeny

It seemed that exposure to these rays for the same period resulted in latent action, significantly appeared as a reduction in No. of F₁ offspring (reduction of adult progeny = 10%). 24 h simultaneous exposure to actellic-treated seeds and UV short waves showed significant joint action against adults of *C. maculatus*. The insecticide alone caused a 28% reduction of F₁ adult progeny whereas the interaction between actellic and UV rays resulted in a 39% reduction. Also, the result obtained exhibited that the long wave has not any effect with all treatments at all exposure periods.

Table 1. Lethal and latent effects of ultraviolet rays and their interaction with actellic on adults of *Callosobruchus maculatus* and their progeny

Treatment	Duration (h)						% reduction of adult progeny [†]
	12		18		24		
	Short [‡]	Long [§]	Short	Long	Short	Long	
UV	0	0	0	0	0	0	10 ^c
UV + Actellic (LC ₂₅)	0	0	0	0	20	20	39 ^a
Actellic (LC ₂₅)	0	0	0	0	15	15	28 ^b
Control	0	0	0	0	0	0	0 ^d

In the same column, values followed by a common letter are not significantly different

[‡] short wave, 254 nm

[§] long wave, 365 nm

[†] Parent adults were exposed to UV for 24 h (short waves)

3.1.3 Ovicidal effect

Eggs of *C. maculatus* exposed to UV short waves for 24 h showed 26% hatchability versus 73% for control eggs, Table 2. This result supports findings for other insect species. In the present study prolongation the exposure period alongside using of UV-long wave (thought to be less harmful to humans) might improve the insecticidal activity of this ray.

Table 2. Ovicidal action of ultraviolet rays on eggs^{*} of *Callosobruchus maculatus*

Treatment	% hatchability
UV	26 ^b
Control	73 ^a

In the same column, values followed by a common letter are not significantly different

* Eggs were exposed to UV for 24 h (short waves)

3.2 Microwave radiation

Eggs or adults of *C. maculatus* confined to cowpea seeds of 12.5% moisture content were subject to microwave heating. The operating frequency of the radiation source (an oven) was 2,450 MHz but the oven was operated at the lowest energy level, i.e. 17% of 800 W. cowpea seeds used were treated or non-treated with pirimiphos methyl (actellic)

at LC₂₅. Insects or eggs were exposed to microwave heating for different periods, *i.e.* 2, 3, 4, 5 and 6 minutes.

3.2.1 Effect on adult mortality

Results in Table 3 revealed that exposure for 4, 5 or 6 minutes caused 55, 65 and 100% mortality of adult insects. At each of the tested exposure periods, adults exposed to microwaves alone were significantly less susceptible than those exposed simultaneously to microwaves plus pirimiphos methyl.

Table 3. Lethal effects of microwaves[†] and their interaction with actellic on adults of *Callosobruchus maculatus*

Treatment	% mortality following exposure time (minutes)				
	2	3	4	5	6
Microwaves	5.0 ^b	10.0 ^b	55.0 ^b	65.0 ^b	100.0 ^a
Microwaves + Actellic (LC ₂₅)	10.0 ^a	26.0 ^a	71.0 ^a	80.0 ^a	100.0 ^a
Actellic (LC ₂₅)	0.0 ^c	0.0 ^c	0.0 ^c	0.0 ^c	0.0 ^b
Control	0.0 ^c	0.0 ^c	0.0 ^c	0.0 ^c	0.0 ^b

In the same column, values followed by a common letter are not significantly different

[†] Operating frequency 2,450 MHz, low energy level (17% of power output, 800 W)

N.B. Temperatures of heated seeds were found to be 47, 53, 60, 71 and 77 °C for the exposure periods, 2, 3, 4, 5 and 6 minutes, respectively

3.2.2 Effect on progeny

At each exposure period, survivals were stored to study the latent effect on their progeny. At the end of the life span, F₁ progeny of parent survival adults were significantly affected. Survival, preexposed to microwave for 5 minutes showed 25% reduction of their adult progeny (Table 4). The effect was more pronounced for insects simultaneously exposed to microwaves plus to pirimiphos methyl (% of reduction of progeny at the same exposure period was 31%).

Table 4. Effect of microwaves and their interaction with actellic on adults of *Callosobruchus maculatus*

Treatment	% mortality following exposure time (minutes)			
	2	3	4	5
Microwaves	11.0 ^a	15.0 ^b	23.0 ^a	25.0 ^b
Microwaves + Actellic (LC ₂₅)	15.0 ^a	20.0 ^a	24.0 ^a	31.0 ^a
Control	0.0 ^b	0.0 ^c	0.0 ^b	0.0 ^c

In the same column, values followed by a common letter are not significantly different

3.2.3 Ovicidal effect

Microwaves showed also, great ovicidal action against *C. maculatus*. Hatchability following exposure periods of 2, 3, 4, 5 or 6 minutes were 26, 18, 11, 4 and 0%, respectively, versus 74% of control eggs (Table 5).

Table 5. Effect of microwaves and their interactions with actellic on adults of *Callosobruchus maculatus*

Treatment	% hatchability following exposure time (minutes)				
	2	3	4	5	6
Microwaves	26.0 ^B	18.0 ^B	11.0 ^B	4.0 ^B	0.0 ^B
Control	74.0 ^A	74.0 ^A	74.0 ^A	74.0 ^A	74.0 ^A

In the same column, values followed by a common letter are not significantly different

4. Discussion

The present study seeks alternative approaches to chemical synthetic insecticides, aiming for methods that are relatively safe for humans and the environment while exhibiting effective control against the stored product insect, *C. maculatus*. Two physical methods, namely ultraviolet (UV) rays and microwave radiation, were assessed for their efficacy in managing the tested insect. The findings revealed that ultraviolet rays did not exhibit direct toxicity to adults of *C. maculatus*. However, exposure to these rays appeared to have a latent effect, notably in the form of a significant reduction in the number of F₁ offspring (a 10% reduction in adult progeny). Furthermore, a 24 h simultaneous exposure to actellic-treated seeds and UV short waves demonstrated a significant synergistic effect against *C. maculatus* adults. While the insecticide alone caused a 28% reduction in F₁ adult progeny, the combined action of actellic and UV rays resulted in a more pronounced 39% reduction in adult progeny. This suggests a cooperative impact of actellic and UV rays in enhancing the control of *C. maculatus* adults. Additionally, there was a 26% hatchability observed compared to the 73% hatchability in control eggs. These findings align with the studies of Heidari et al.,¹⁷ which reported significant effects on the mortality of immature stages of *C. maculatus* based on different UV irradiation times (0, 10, 20, 30, 40, and 50 minutes). Similarly, Sedaghat et al.¹⁸ noted that various exposure periods (2, 4, 8, 16, 24, 32, and 40 minutes) of UV irradiation reduced the egg hatching of *C. maculatus* in comparison to the control. In line with the findings of Draz et al.,²⁰ the exposure of *S. oryzae* and *T. castaneum* to ultraviolet radiations for 24 hours resulted in a significant reduction in progeny (95.24% and 89.72%) and demonstrated preventive efficiency of 94.25% and 93.37%, respectively. Our study indicates that exposing eggs or adults of *C. maculatus* to microwave heating for varying periods (4, 5, and 6 minutes) caused mortality rates of 55%, 65%, and 100% in adult insects, respectively. The hatchability percentages following exposure periods of 2, 3, 4, 5, and 6 minutes were 26%, 18%, 11%, 4%, and 0%, respectively, compared to the 74% hatchability observed in control eggs. These outcomes align with findings of El-Zun et al.,²¹ where all stages of *C. maculatus* and cowpea seeds exposed to microwaves at the lowest energy level (17% of 800 W) for 2, 3, 4, 5, and 6 minutes resulted in highly significant effects on adult mortality, hatchability, and the reduction of progeny (adult, egg, larvae, and pupa), particularly at the 5-minute mark.

In 2019, Al-Akhdar et al.²² clarified that the mortality of pests demonstrated an increase with both power and exposure time or a combination of both factors. Microwave radiation sensitivity was notably higher in *R. dominica* and *R. echinopus*, with LT₅₀ values of 3.07, 2.07, 1.17, and 1.96 minutes at low and medium-low powers. Complete reduction in F₁-progeny for all insects was achieved at medium power during a 4-minute exposure. In a study by Draz et al.,²⁰ they illustrated that exposing *S. oryzae* and *T. castaneum* to microwave irradiation (2,450 MHz) for 25 seconds resulted in a preventive efficiency for stored material reaching 97.68% and 99.02%, respectively. The adequate exposure periods to achieve a 50% mortality rate in coleopteran adults (LT₅₀%) were found to be 13 and 14 seconds, respectively. Furthermore, a study by Baysal et al.²⁶ revealed that microwave energy was applied to control the dried fig moth *C. cautella*. It was determined that heating for 90 seconds in a 900 W microwave oven at 2,450 MHz was sufficient to eliminate most stages of *C. cautella*.

In 2008, Bhalla et al.²⁹ disclosed that microwave radiation effectively controlled all stages of *C. maculatus*, with pest mortality increasing proportionally to the extended exposure period. Halverson et al.³⁰ observed a decrease in germination percentages with prolonged exposure to microwave radiation. Similarly, Vadivambal et al.⁹ noted a reduction in corn germination associated with increased microwave power or exposure time, or a combination of both. Microwaves, characterized as electromagnetic waves within the range of 300 to 3,000 MHz, exhibit substantial potential

as an alternative method for pest control in stored cereals and their derivatives. The lethal effect is attributed partially to the generated heat within the irradiated materials and partly to the direct absorption of microwaves.¹⁹ Irradiation involves the use of specific wavelengths and energy of electromagnetic radiation to control pests on commodities, with ionizing radiation sourced from radioisotopes like cesium and cobalt.³¹ Insect disinfestation in stored applications (less than 1 kg) is a documented application of this process. Das et al.³² found no significant differences in the quality of grain flour protein and flour yield in samples treated with microwave energy that resulted in 100% mortality. Microwave radiation was effective in eliminating mites without impacting the quality characteristics of the product, with the added advantage that it can be applied to packaged products.³³ Various radiations, including ultraviolet light, microwaves, infrared light, and radiofrequency waves, can be utilized for disinfestation, with studies reporting positive results.³⁴ Srivastava and Mishra³⁵ explored the application of microwave, ultraviolet light, and vacuum, both individually and in combination, for controlling the adult stage of *R. dominica* in rice grains. The combined treatments yielded superior results while minimizing changes in rice quality attributes.

5. Conclusion

Microwave and UV can be used in cowpea protection, and also can be used to reduce the use of synthetic pesticides. Microwave and UV affected on progeny of *C. maculatus* and can be used in the Integrated Pest Management (IPM) program. Ultraviolet rays did not exhibit direct toxicity to adults of *C. maculatus*. However, exposure to these rays appeared to have a latent effect, notably in the form of a significant reduction in the number of F₁ offspring (a 10% reduction in adult progeny). After 24 h simultaneous exposure to actellic-treated seeds and UV short waves demonstrated a significant synergistic effect against *C. maculatus* adults. While the insecticide alone caused a 28% reduction in F₁ adult progeny, the combined action of actellic and UV rays resulted in a more pronounced 39% reduction in adult progeny. The study suggests a cooperative impact of actellic and UV rays in enhancing the control of *C. maculatus* adults. Additionally, there was a 26% hatchability observed compared to the 73% hatchability in control eggs.

Authors contribution

Eslam A. Negm, Walaa M. Alkot and Fatma M. A. Khalil designed the experiments, collected, and analyzed the data; Ahmed M. Abouelatta wrote the manuscript draft; Ahmed M. Abouelatta and Alzahraa Abdelaty Elmadawy analyzed and discussed the results.

Conflict of interest

The authors declare that they have no competing interest.

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