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Effect of microwave radiation on *Tribolium confusum* Jaquelin du Val (Coleoptera: Tenebrionidae) and *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae: Bruchidae)

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Abstract
A household microwave system was used to study the effect of microwave radiations on two stored products insects: *Tribolium confusum* and *Callosobruchus maculatus* in wheat flour and cowpea seed respectively. All experiments were conducted at five exposure times of 5, 10, 15, 20 and 25sec at power level of 400 W. Mortality was significantly increased at LSD < 0.05 with increase of exposure times. Complete mortality of *T. confusum* adults was obtained at 25 sec, whereas 98.8% mortality was obtained the same exposure time for *C. maculatus* adults. The emergence percentage, loss percentage of wheat grain infested with *T. confusum,* egg laying and germination of microwave treated cowpea seed were significantly decreased with increase in microwave exposure periods, which concluded that microwave could be used as alternative method for controlling insects in stored grain area

Keywords: Control, microwave, emergence, stored-products insects, germination

1. Introduction

Stored product pests result in intensive losses in quality and quantity of post-harvest food grain. The serious damage of stored products is caused either by environmental factors or by biological agents [1]. Post-harvest losses caused by insects, mites, rodents and microbes in stored grains have been estimated at 10% throughout the world. These losses have been reported around 1% and 10–30% in developed and developing nations respectively [2]. The biggest problem in yield, storage and marketing of the cereals and legumes is the infestation by the insects. Stored grain insect infestations, occurrence and numbers are directly related to geographical area and climatic condition [3]. During one season, significantly high rates of reproduction was seen and 10-15% of the stored grain are destroyed while the remaining is contaminated with unfavorable odors and flavors. They also have a substantial role in transportation of storage fungi [4]. Stored-product of agriculture and animal origin are attacked by approximately 355 species of mites, 70 species of moths and more than 600 species of beetle resulting in high economic losses. Among all of the storage-pests, two of the most destructive beetle species in stored grain are the *T. confusum* and *C. maculatus* [2].

Flour beetles (*Tribolium. spp*) are the most extensive pest in the world. The beetles feed and cause damage resulting quantitative and qualitative losses [5]. *T. confusum* is associated with human stored products. The association between human and *Tribolium* may be as old as 4000 years. These beetles are incapable to infest whole grain and therefore it is secondary pests of grains such as wheat, barley, maize, rice, oats, and rye. Their typical habitat is in stored flour of these grains, therefor they are commonly called flour beetle. These insects may also be found in other types of stored foods materials such as pulses, chocolate, nuts and even spices like red pepper and ginger [6].

The brunched beetles are the only significant agricultural pest insects of stored pulses in Africa and Asia that presently ranged in tropical and sub-tropical area of the world. *C. maculatus* is one of the most distractive storage pests of *Vigna* spp. such ass cowpea, green gram, mung been, black gram and adzuki in the tropics. Pods of cowpea attacked by *C. maculatus* in storage for 8 months could have as much as 50% of seeds damages [7]. Therefore, control of stored-product pests to prevent economic losses caused by insects is important from the yield to the consumer's [1].

Different insect control methods have been evaluated to save the grain and use of the chemical insecticides is the common method associated with the major problem that residues remain in the food grain which potentially affecting the health of the consumers. The chemical control methods also have negative affect on environment including water and air [8]. Hence, there is search for non-chemical alternative method for the control of insects in the post-harvest stored products. Microwave disinfestation could be used as an alternative method of killing insect in storage [9].

Microwaves are electromagnetic waves which lie between infrared radiation and radio waves in the electromagnetic spectrum. Their frequency ranges from 300 MHz to 300 GHz which corresponds to the wave length 1-1000 mm [10]. The principle behind killing of insects using microwaves is the dielectric heating of insects which depend on its electrical properties [10, 11]. Biological material exposed to microwave radiation absorbs an amount of energy which depends on their dielectric characteristics.

Several studies based on microwave radiation were conducted to control *T. castaneum* [12-14, 9], *Callosobruchus chinenesis* [15] and pulses beetle [16]. However, there is chance for the radically control method to solve the problem [17]. Previous studies were shown that the microwave radiation hade insecticidal effect, therefore, the present study was conducted to investigate the effectiveness of microwave radiation for the control of stored product insects.

The objectives of this paper were to determine the mortality, egg laying, emergence and storage loss percentages of two stored grain insects: *T. confusum*, and *C. maculatus* in related stored products at one microwave power levels and different exposure times. Also to determine the germination potential of microwave treated cowpea.

2. Materials and methods

This study was conducted from Sep 20, 2015 to Jan 20, 2016.

2.1 Samples used

The flour of wheat variety (*Triticum vulgare*) Sakha 69 and cowpea seeds variety (*Vigna sinensis*) Kareem 9 obtained from the market were selected for the study. The whole flour of wheat and cowpea seeds were sieved and cleaned from husks, dust or any inert materials. The conditioned samples were then stored at room temperature in sealed bags in the Laboratory of plant protection Department of Arid Land Agriculture King Adbulaziz University until used for the experiments.

2.2 Stored grain insects and culture preparation

Two major stored-grain insects *T. confusum* Jaquelin du Val (Coleoptera: Tenebrionidae) and *C. maculatus* (F.) (Coleoptera: Chrysomelidae: Bruchidae) were selected for the study. For culture preparation cleaned and sterilized (heating at 70 ºC for 1hr) wheat flour and cowpea samples were placed in glass jars separately to reabsorb moisture. Then, transfer amounts 300 gm cowpea beans and 400 gm wheat flour to depth of 5 cm to separately sterilized culture jars. Adults of flour beetle and cowpea beetle (200-300 and 200-250) from previous culture were added to each jar and sealed with muslin and placed at 30 ºC ±2 and 75±5 RH. Respectively after two weeks and one week, the insects were sieved out, discarded or transferred to another jar. Adult of flour beetle (10-15) and cowpea beetle (2-4) days after emergence ware used for experiment work, according to [27].

2.3 Microwave and irradiation of samples with microwave

For the microwave treatment, samples were irradiated with microwave energy using a household microwave system (Model No: 565.8962781) made in Singapore. The effective height, weight and length of the conveyor were 196, 290 and 300 mm, respectively (1280 W capacity; frequency 2450 MHz).

Samples were exposed to different exposure times and one level power of microwave for determination of mortality, egg laying, reduction of emerged progeny adults, loss percentage of infested wheat grain by *T. confusum*, after storage and microwave treated cowpea seeds germination.

2.4 Determination of mortality and reduction of emerged progeny adults

The experiments were conducted with wheat flour and cowpea seed samples. Batches of 10 pairs (10-15 days old of *T. confusum* adults) and 5 pairs (2-4 days old of *C. maculatus* adults) were placed in Petri dishes (9 cm in diameter containing 20 gm wheat flour and 10 gm cowpea seed respectively). The samples were exposed to microwave radiation at 400 w power level and different exposure periods (5, 10, 15, 20 and 25 seconds) on adults. After one hour from treatment, the percentages of live and dead insects were counted. The samples were held in laboratory for three hours at room temperature and the insects were checked for mortality again. The samples with adult survivors were transferred to small glass jar (volume, 0,4L) the jar was sealed with the muslin and placed under laboratory conditions. For *T. confusum* number of adult's emergence was counted after 5-6 weeks. After two weeks, all adults of *C. maculatus* were removed. The number of laid eggs was counted for each treatment. After three weeks of treatment, the number of emerged adults and emergence percentages were calculated from the following equation:-

$$\text{Emergence (\%)} = E/G \times 100$$

Where:

E = No. of emerged adults.

G = No. of laid eggs.

2.5 Effect of microwave radiation on population density in infested samples and the loss percentages after storage:

Batches of 400 adults of reed flour beetles (10-14 days old), and 250-300 adults of cowpea beetles (2-4 days old) were placed on sterilized related sample (400 gm of wheat flour and 250 gm cowpea seeds respectively) in separately glass jar. After one week, all adult insects were removed. The infested culture of flour and cowpea seeds were divided in samples (20gm of each and 10 gm of each respectively), and placed in Petri dishes. The samples were treated with microwave at five different exposure periods as above mentioned. Samples were cooled to room temperature and were transferred to glass jars (0,4L), covered with muslin and placed under laboratory conditions. For the reed flour beetle after 5-6 weeks, number of F_1 emerged adults were counted. After three months, the means of emerged adults were counted and the loss of the wheat flour weight was calculated as a below:

$$\text{Loss (\%)} = W_1 - W_2 / W_1 \times 100$$

Where:

W_1: weight of the sample before treatment and the storage.

W_2: weight of the sample after treatment and the storage.

The reduction percentage in number was calculated by the following equation:

$$\text{Reduction (\%)} = E_C - E_T / E_C \times 100$$

Where:

E_C: mean of number emerged adult in control.

E_T: mean of number emerged adult in treatment.

In cowpea beetle before that to transfer the sample to class jars, the laid eggs for each treatment were counted. After three weeks, the number of emerged adults, emergence (%) and reduction (%) were calculated as above.

2.6 Determination of germination and swelling tests

A. Germination

For germination test the cowpea seeds were exposed to different microwave exposure periods, by plating 20 seeds. Three filter papers in 9 cm diameter Petri dish added with 5.5 ml of distilled water [18]. The plates were covered in plastic bag to prevent the desiccation of filter paper and held at 25 ^{0}C for 5 days. On fifth day the numbers of germinated seeds were recorded and germination percentage was calculated

B. Swelling test

Three grams of treated cowpea seeds, by microwave radiation were weighed and submerged by tap water in Petri dish. After 1 and 4 hours, the seeds were dried with tissue paper and reweighted calculation the absorbed water. Three replicates were done for each treatment and control. The above-mentioned method was carried out according to [18].

2.7 Statistical analysis

All obtained data were statistically analyzed using Finney (1971) [19] software. Comparisons among the means of the various treatments were performed, using the revised least significant different (L. S. D) at < 0.05 level. Angular transformation was done for the percentage values.

3. Results

3.1 Mortality and reduction of emerged progeny adults of *Tribolium confusum*

The adult mortality and reduction of emerged adults of *T. confusum* are given in Table 1. The mortality of adult insects in control was zero. The mortality rate of adult at different microwave exposure periods (5, 10, 15, 20 and 25sec) was 0.0, 35.0, 43.3, 86.6 and 100 percent, respectively. There was no significant difference in the mortality in the mortality of adult between 5sec exposure time and control at P<0.05 (LSD <0.05 = 4.69). The mortality was significantly increased with increase of microwave exposure time. 100% mortality was achieved at 25 sec exposure time.

The numbers of emerged adult after 5-6 weeks of exposed to different exposure time (5, 10, 15, 20 and 25 sec) were 151.0, 94.2, 54.0, 9.3 and 0.0, respectively. The number of emerged adults in the control was 157.0. There was no significant different in the mean number of emerged adult between control and 5, 20 and 25 sec of exposure time at P<0.05 (LSD <0.05 = 11.8). The number of emerged adult was significantly decreased (P<0.05) with increase of exposure time; it was achieved up to zero at 25 sec exposure time. Reduction percentages were increased as mortalities with increased of exposure time up to one hundred percent at 25 sec of exposure time.

Table 1: Effect of Microwave radiation on mortality, emergence of *Tribolium confusum* adults at different exposure period.

Exposure periods (sec)	Mortality of adults % ± SD	Mean No. of emerged adults ± SD	Reduction %
Control	$0.0^a \pm 0.0$	$157.0^a \pm 8.6$	--------
5	$0.0^a \pm 0.0$	$151.0^a \pm 7.4$	3.8
10	$35.0^b \pm 5.0$	$94.2^b \pm 8.5$	40.0
15	$43.3^c \pm 2.9$	$54.0^c \pm 7.5$	65.6
20	$86.6^d \pm 2.8$	$9.3^d \pm 0.5$	94.1
25	$100^e \pm 0.0$	$0.0^d \pm 0.0$	100.00
LSD <0.05	4.69	11.8	

*The mean followed by the same letters are not significantly different at $P \leq 0.05$

*Angular transformation was done for the percentage values

3.2 Mortality, egg laying and emergence of *Callosobruchus maculatus*

The mortalities rate of *C. maculatus* adult at 400 W and different microwave exposure times (5, 10, 15, 20 and 25sec) were 18.5, 30.7, 58.6, 75.6 and 98.6 percent, respectively. The numbers of total laid eggs and numbers of laid eggs per female, counted after two weeks of exposed to microwave, for each exposure time were {(458, 320, 232, 90 and 0.0) and (37.2, 31.5, 28.1, 21.1 and 0.0) respectively}. After three week of treatment numbers of emerged adults at mentioned exposure periods were 265, 168, 108, 36 and 0.0 and emergence percentages were 57.8, 52.5, 46.5, 40.0 and 0.0, respectively. The reduction percentages (8.1, 16.5, 26.7, 36.4 and 100.0 percent, respectively) were increased as mortalities with increased of exposure time. The control mortality, numbers of total laid eggs, numbers of laid eggs per female, numbers of emerged adults, emerged percentages and reduction percentages were 0.0, 516, 34.0, 325, 62.9 and 0.0, respectively (Table. 2). The mortality was significantly increased with increase of exposure time at P<0.05 (LSD <0.05 = 5.31). The emergence percentages were significantly decreased with increased of exposure time up to zero percent at 25 sec exposure time at P<0.05 (LSD <0.05 = 3.80), and the reduction percentage of emerged adults were increased as mortalities. One hundred percent reduction was achieved at 25 sec exposure time.

Table 2: Effect of microwave radiation on mortality, egg laying and emergence of *C. maculatus* beetle.

Exposure period (sec)	Mortality of adults (%) ± SE	No. of laid eggs		No. of emerged adults	Emergence (%) +SE	Reduction (%)
		Total	Per female			
Control	$0.0^a \pm 0.0$	516	34.0	325	$62.9^a \pm 3.1$	0.0
5	$18.5^b \pm 4.2$	458	37.2	265	$57.8^b \pm 2.9$	18.4
10	$30.7^c \pm 4.1$	320	31.5	168	$52.5^c \pm 3.2$	48.3
15	$58.6^d \pm 5.3$	232	28.1	108	$46.5^d \pm 2.4$	66.7
20	$75.6^e \pm 4.5$	90	21.1	36	$40.0^e \pm 2.2$	88.9
25	$98.6^f \pm 4.3$	0.0	0.0	0.0	$0.0^f \pm 0.0$	100.0
LSD<0.05	5.31				3.80	

*The mean followed by the same letters are not significantly different at $P \leq 0.05$

*Angular transformation was done for the percentage values.

3.3 Emergence of *T. confusum* beetles population from infested wheat flour and the percentage of loss after storage

The emergence rate and loss percentage of wheat flour were directly related to the time of emergence and exposure of microwave radiation (table. 3.) Mean number of emerged adults from infested wheat flour at different exposure periods (5, 10, 15, 20 and 25 sec), after two months were 120.0, 88.1, 2.1, 0.0 and 0.0, respectively, and after three months were 615.0, 511.3, 99.0, 0.0 and 0.0, respectively. Whereas the meant of emerged adults of two months and three months were 370.0, 311.0, 52.2, 0.0 and 0.0 respectively. Number of emerged adult significantly decreased (*P*<0.05) with increasing of exposure time at used power of microwave energy. Completely stopped of emergence was achieved at the longest exposure times (20 and 25 s).

Loss of wheat flour % after 3 months at the same exposure times were 72.0, 59.0, 9.5, 1.0 and 0.0 percent, respectively. Percentage of wheat flour was significantly decreased *P*<0.05 (LSD <0.05 = 3.2) with increased of exposure times. It was achieved to zero percent at the longest exposure period (25 sec). The reduction percentage was increased (5.7, 22.7, 87.5, 100.0 and 100.0, respectively) with increasing of exposure time. The mean of emerged adults, loss of wheat flour percentage and reduction percentage in control were 386.5, 76.4 and 0.0 percent, respectively.

Table 3: Effect of microwave radiation on *T. confusum* beetles population from infested wheat grains and the percentage of loss after storage.

Exposure periods (sec)	Mean of emerged adults from infested grains after			Loss of wheat flour % after 3 months ± SD	Reduction % ± SD
	2 months ± SD	3 months ± SD	mean		
Control	140.3 ± 4.1	630 ± 9.0	386.5	76.4 ± 2.2	------
5	120.0 ± 4.1	615.0 ± 8.7	370.0	72.0 ± 5.9	5.7
10	88.1 ± 12.0	511.3 ± 14.2	311.2	59.0 ± 2.1	22.7
15	2.1 ± 0.2	99.0 ± 4.3	52.2	9.5 ± 0.4	87.5
20	0.0 ± 0.0	0.0 ± 0.0	0.0	1.0 ± 0.1	100.0
25	--------	--------	---------	-------	100.0
LSD <0.05	A B	30.5 26.7		3.2	

A: for the effect of exposure periods comparisons
B: for the emergence time comparisons (2, 3 months)
Angular transformation was done for the percentage values

3.4 Emergence of *Callosobruchus maculatus* from infested cowpea seed

After treated of the infested cowpea samples with different microwave exposure periods (5, 10, 15, 20, 25 and 30sec) the numbers of laid eggs for each treatment were 172.4, 152.3, 160.2, 181.3, 175.1 and 192.3, respectively. After three weeks, the numbers of emerged adults were 137.0, 110.0, 45.1, 30.1, 8.2, and 0.0 and the emergences (%) were 79.4, 72.2, 28.1, 16.6, 4.6 and 0.0, respectively. The mean number of emerged adults and emergence percentage were significantly decreased at *P*<0.05 (LSD <0.05 = 12.1 and 5.2 respectively) with increasing of microwave exposure times. The reduction (%) were increased (14.4, 31.3, 71.8, 81.2, 94.8 and 100.0, respectively) with increased of exposure periods. It was achieved to zero percent at the 30 sec of microwave exposure period.

The mean number of laid eggs, number of emerged adults, emergence percentage and reduction % at the control were 185.0, 160.2, 86.5 and 0.0, respectively. (Table. 4)

Table 4: Effect of microwave radiation on emergence of *C. maculatus* from infested cowpea seed

Exposure period (sec)	Mean N0. Of laid egg ± SD	Mean No. of emerged adults ± SD	Emergence (%) ± SD	Reduction (%)
Control	185.0± 7.3	160.2^{a}± 4.2	86.5^{a} ± 3.9	0.0
5	172.4± 9.2	137.0^{b}± 5.6	79.4^{b} ± 5.5	14.4
10	152.3± 8.1	110.0^{c}± 4.3	72.2^{c} ± 0.5	31.3
15	160.2± 3.2	45.1^{d}± 5.2	28.1^{d} ± 2.1	71.8
20	181.3± 12.1	30.1^{e}± 1.3	16.6^{e} ± 0.6	81.2
25	175.1± 8.3	8.2^{f}± 2.5	4.6^{f} ± 2.4	94.8
30	192.3± 5.6	0.0^{f}± 0.0	0.0^{f} ± 0.0	100.00
LSD <0.05		12.1	5.2	

*The mean followed by the same letters are not significantly different at *P*≤0.05
*Angular transformation was done for the percentage values.

3.5 Swelling of cowpea seeds and germination:

The results of swelling and germination of cowpea seeds subjected to microwave radiation for 5, 10, 15, 20 and 25 sec are presented in Table. 5. The control swelling and germination were 73.2 and 98.5%, respectively. The mean of swelling % of cowpea seeds after 1 and 5 days, at different microwave exposure periods (5, 10, 15, 20 and 25sec), were 72.8, 75.7, 75.1, 71.3 and 65.0 respectively. Mean followed by the same later were not significantly different at *P*<0.05 (LSD <0.05 = 3.33), (Table. 5). The germination percentages were 85.42, 76.02, 63.42, 49.0 and 5.0, respectively. It was significantly reduced at *P*<0.05 (LSD <0.05 = 5.71) with increasing of exposure times.

Table 5: Effect of microwave radiation on swelling of cowpea seeds and germination percentage.

Exposure period (sec)	Swelling time (hr)	Swelling %		Germination after 5 days (%) ± SD
		(%) ± SD	Mean	
Control	1 5	42.4 ± 71.4 104.3 ± 2.2	73.2[b]	98.51[a] ± 3.1
5	1 5	46.4 ± 3.2 99.1 ± 2.9	72.8[b]	85.42[b] ± 4.1
10	1 5	48.2 ± 4.6 103.3 ± 5.1	75.7[bc]	76.02[c] ± 5.3
15	1 5	49.2 ± 3.8 101.1 ± 1.3	75.1[bc]	63.42[d] ± 6.5
20	1 5	44.9 ± 8.1 97.8 ± 2.2	71.3[b]	49.0[e] ± 5.1
25	1 5	38.1 ± 6.4 92.0 ± 2.4	65.0[a]	5.0[f] ± 0.0
LSD <0.05			3.33	5.71

*The mean followed by the same letters are not significantly different at $P \leq 0.05$

*Angular transformation was done for the percentage values.

Fig. 1 shows that microwaves have potential to kill and control insects in stored products (wheat flour and cowpea seed). Highest rate of mortality percentage and lowest rate of emergence percentage of *T. confusum* and *C. maculatus* adults were obtained at high level (25 sec) exposure time corresponding to a microwave irradiation. It was significantly affected with increase in microwave exposure times. Loss percentage of infested wheat grains after storage and germination of cowpea seeds were also decreased with increase of exposure time.

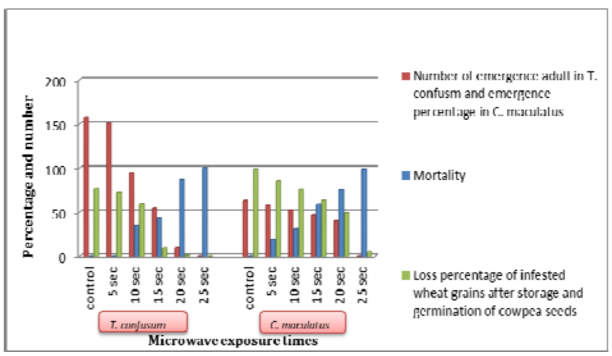

Fig 1: Effect of microwave radiation on stored product pests (*T. confusum* and *C. maculatus*).

4. Discussion

Microwave radiation treatment extensively has been investigated by several researchers as alternative method of killing insects and seems to have a greater potential of controlling insects in stored grain because of several advantages such as having no chemical residues on the food products, control of all developmental stages of storage pests and having minimal impact on the environment [20-22]. Insects are unlikely to take resistance to this treatment [23]. There are a lot of studies that shows the importance of microwave exposure for grains and seeds preservation, in order to control it from *Tribolium confusum, Plodia interpunctella* [24], *T. castaneum* [13], *Sitophilus zeamais, Tribolium castaneum,* and *Plodia interpunctella.* [9], Pulse beetle [16], *S. granarium* [14], Maize weevil [25]. It was the evident that increasing power and exposure times caused increasing mortality on the eggs of *E. kuehniella* [26]. There has been a lot of researcher on microwave disinfestation of cereals especially wheat [27-33] and of some food materials such as cherries [34]. Several studies based on microwave radiation were tested to control *T. castaneum* [12, 9], *Callosobruchus chinenesis* [15].

In the present study, we used one power of microwave radiation (400 W) at different exposure times (5, 10, 15, 20 and 25sec), on stored products pest of *T. confusum* and *C. maculatus* adults. We found the adults mortality was increased with increased of microwave exposure times. This may be because of water present in the body fluid that has high frequency oscillation dielectric molecules [24].

Microwave radiation with good penetrability can control pests available inside or outside grain kernels [35]. Exposure to microwave energy not only kills the insects but also could cause physical injuries and reduced reproduction rates in surviving insects [36, 27]. This paper brings the research initiatives especially on microwave radiation for their potential use for disinfestation of stored food products. In the present research we evaluated the different exposure periods to microwave radiation on two stored produces insects such as *T. confusum and C. maculatus* in infested wheat flour and been respectively. The rate of reproduction in surviving insects and the rate of new emerged adult from microwave treated infested culture ware reduced with increasing microwave exposure times.

Earlier studies showed that eradication of the insects increased with the increase in microwave energy, but the seed viability, germination capacity, and seedling vigor decreased by exposure to microwave energy [37, 38]. Singh *et al.* also concluded that germination capacity and seed viability of chickpea, pigeon pea, and green gram were reported to be affected by microwave exposure time and power level [16]. Similar results, that is, poor germination rate in cowpea seeds, were also reported in the present research due to loss of moisture content of cowpea seeds with increase in microwave exposure times.

5. Conclusion

The result of the present study shows that microwaves have a potential to kill and control insects in stored products that the highest rate of mortality and lowest rate of emergence percentage of *T. confusum* and *C. maculatus* adults were obtained at high level exposure time (25 sec). Microwave treatment is a potential means of replacing other methods and techniques for the control of storage pests because of pollution free environment, selective radiation and heating, energy minimization, equivalent or better quality retention.

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