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MICROWAVE RADIATION EFFECTS ON THE DIFFERENT STAGES OF *Sitophilus oryzae* (Linné, 1763) (COLEOPTERA, CURCULIONIDAE) EVOLUTIVE CYCLE IN RICE, FOCUSING ITS CONTROL.

José G. Franco[1], Caio H. Franco[2], Suely S. H. Franco [1], Paula B. Arthur[2], Valter Arthur[2],

[1]Instituto de Pesquisas Energéticas e Nucleares (IPEN / CNEN - SP)
Universidade de São Paulo
Av. Professor Lineu Prestes 2242
05508-000 São Paulo, SP, Brazil
gilmita@uol.com.br
zegilmar60@gmail.com

[2]Laboratório de Radiobiologia e Ambiente – Centro de Energia Nuclear na Agricultura
Universidade de São Paulo
P.O Box 96
13400-970 Piracicaba, SP, Brazil
caiohaddadfranco@lnbio.cnpem.com.br
arthur@cena.usp.br

ABSTRACT

As insects increase in radiotolerance as they develop and usually several developmental stages of pest may present in grain shipped commodity, it is important to know the microwave radiation susceptibility of stages of the target insect before the establishment of microwave radiation quarantine treatments. The current research had the aim to evaluate the microwave radiation effects on several phases of the rice weevil evolution cycle (*S. oryzae*), focusing its control. This specie is considered as on of the most serious worldwide pests for stored grains. The tests have been done in glass vials with 250 grams of whole grain (brown) rice and the irradiation was done in a 2,450 MHz commercial microwave oven, model Carousel II (potency of 800W). It was determined the exposure time needed to each phase control for the insect evolutive cycle, concluding that the immature phases (larvae and pupae), contained inside the rice, are more sensitive, requiring only 100 seconds to obtain 100% control while the egg phase requires a longer exposure (130 seconds). Referring to the grown phase, the time required to attain the lethal dose was 160 seconds. All the exposure time have been irradiated with a low potency (240 W). It also displayed that to greater quantities of rice (1.0 kg), with egg presence and forming a 2.0-centimeter layer on the microwave plate surface, it required an exposure time of 180 seconds. Therefore, in a more effective way, we can recommend these 180 seconds exposure time to the control of all phases concerning the insect evolutive cycle.

1. INTRODUCTION

The problems caused by insects in agriculture, both in the field and inside warehouses, are quite concerning, consisting of a very high diversity of important pest-insects that damage storage products. Weevils and moths are insects identified as the main responsible for the huge losses caused in stored grains, specially those considered of higher consumption, like beans, rice, coffee, wheat, corn and byproducts (flour, bran and corn-flour). In Brazil, *Sitophilus oryzae* [1] is included among one of the seven insects that cause major losses in stored grains.

Regarding grains and its byproducts conservation and storage process, various types of radiation are still being studied: infrared radiation, gamma rays, X rays, accelerated electron, among others; chemical addition is yet the most widely used method for conservation process.

There is a need for a better conservation process with advanced technology, higher efficacy, lower cost and absence of side effects; therefore the irradiation process has been considered an obvious solution. Consisting of grains disinfestation with a given dose of radiation by inhibiting insect's reproduction, or killing infesting population, the irradiation process presents good features and desired control qualities. Irradiation is an effective method, which can be easily embedded in modern practice of grains handling, and its recommended for its absolute safety and full effectiveness [2] [3].

Although overall efficiency, products treated with gamma radiation, X rays and accelerated electrons may present problem of reinfestation, precisely because they leave no residue, unlike the chemical treatment that leave residues, but these treatment never reaches a 100% level of insect's control; usually these two types of methodology are used in large quantities of products with these two control methods are applied to large quantities of products. Due to this process limitation, it is not rare that insects can be found inside few-days-stocked products, which were commercially acquired.

Because of the limitation presented by the current methods of conservation, this study researched the effects of microwave radiation at all stages of *S. oryzae* life cycle, which can be considered as an alternative control method for small amounts of grains, where the final consumer can assure the disinfestation of rice before consumption; this method of control can be incorporated at domestic level (final consumer residence) since the ordinary microwave oven, available in the market since 1967, became an essential home small appliance. Microwave oven is present in residences in almost all parts of the world, being found in over 90% of households in the United States of America. In Brazil, there was large increase in the number of units in recent years; when commercialization of microwave were 209,500 in 1990; 250,000 in 1992; 600,000 in 1994 and number over 900,000 in 1995 [4].

The possible use of microwave heating was explored seeking control of *S. oryzae*. It was obtained 100% mortality for adults with an exposure time of 180 seconds. However, complete mortality of larvae inside and outside of the grain was only achieved at exposures of 180 and 240 seconds respectively. The temperature of the rice mass with these exposure times was slightly above 60 ° C in the upper layer (0 - 3.0 cm) and 90 ° C in the lower layer (3.0 – 6.0 cm). Overall it was observed that for each time of exposure, mortality increased proportional to the depth of the irradiated rice container [5].

Studies performed in the United Kingdom have shown that microwaves of 896 MHz, with potency of 800W, controlled only 30% of insects (*Oryzaephilus surinamensis*, *Sitophilus granarius* and *Tribolium costaneum*) in beans. It was also concluded that *Ephestia cautella* mortality was 80% [6].

Another study used physical methods to measure hot air and microwave irradiation in the control of *S. oryzae*, has shown that the microwaves have the advantages of rapid increase in temperature and the heat selectivity at exposures of 60 seconds to approximately 800W radiation, the beans are heated to 65 ° C, destroying insects in samples of 500 grams of wheat [7].

The combination of microwave irradiation (exposure for 10 minutes) with vacuum control was studied as a control mechanism for four insects: *Sitotroga cerealella* (100% of control in rye and corn; 96.8% of control in wheat), *Rhyzopertha dominica* (100% of control in corn; 99.4% in rye and 95.6% in wheat), *Sitophilus oryzae* (100% of control of rye and 99.2% of control in wheat) and *Sitophilus zeamais* (100% of control in corn) [8].

2. METHODOLOGY

The survey was conducted in the Laboratories of Entomology, College of Agronomy Escola Superior de Agronomia de Paraguaçu Paulista (Paraguaçu Paulista - SP), with the insect specie *Sitophilus oryzae* (Linné, 1763) (Coleoptera, Curculionidae) in controlled environmental conditions of 25 ± 2 ° C temperature and 70 ± 5% relative humidity.

The initial colony of *Sitophilus oryzae* was kindly provided by the Entomology Section of CENA / USP (Piracicaba – SP), where it has been cultivated in whole grain (brown) rice substrate for several generations. During the development of the experiment, insect cultivation was maintained in clear glass bottles with eight inches diameter aperture and 3,000 ml volume capacity, containing 500 grams of commercial whole grain (brown) rice. On the bottle cover there was an adapted metal-coated cap with tissue paper, type "Yes" in order to allow gas exchange and prevent penetration from dust, mites and natural enemies.

The substrate used was rice *Oryza sativa cv*. IAC-201. To avoid interference from other insects, which could occur within the grains, these were kept at low temperatures (-15 ° C) for a period of approximately 30 days, in order to eliminate any kind of possible latent infestation.

The experiment was divided into the following steps: testing egg, larvae, pupae and adults. The division was kept based on the determined period in days of all the stages of the life cycle of the insect under the cited climate conditions.

A Sharp brand microwave oven was used, model Corousel II, with frequency 2,450 MHz, yield power of 800 W, but used in low power (30%), corresponding to 240 W, with revolving plate, which provides an improved temperature distribution in irradiated rice. The distance from the radiation source to the rice was 17 cm.

2.1 Test with eggs

The insects were placed on the grains and left for a period of 24 hours, so that female performed the oviposition; later, the insects were removed and the grains irradiated with microwaves with the following exposure times: first with 0 (control), 15, 30, 45 and 60 seconds, and then with 0 (control), 75, 100, 115, and 130 seconds, both at low power (30%). Each treatment consisted of five replicates with 50 grams of grains infested with a total of 250 grams of grains for treatment, which were placed in plastic pots measuring 10 cm high and 5 cm in diameter.

After irradiation, the rice mass temperature was measured through a mercury thermometer and the grains were placed in a climatic chamber where the detection of adult's emergence was performed.

2.2 Test with larvae

The insects were placed on the grains and left for a period of 24 hours, so that females performed the oviposition; later, the insects were removed and the grains left in climatic chamber until larvae hatching occurred; after the larvae reached full development within grains during 21 days, it was performed irradiation with exposure times: first with 0 (control), 130, 160, 190 and 210 seconds, and then with 0 (control), 85, 100, 115 and 130 seconds, both at low power (30%). Each treatment consisted of five replicates with 50 grams of grains by treatment, totalizing 250 grams, which were placed in plastics pots.

After irradiation, the rice mass temperature was measured through a mercury thermometer and the grains were placed in a climatic chamber where the detection of adult's emergence was performed.

2.3 Test with pupae

The insects were placed on the grains and left for a period of 24 hours, so that female performed the oviposition; later, the insects were removed and the grains were left in climatic chamber until hatching of larvae

and pupae completed transformation within the grains (32 days); it was performed irradiation with exposure times: first with 0 (control), 160, 190, 210 and 240 seconds, and subsequently with 0 (control), 80, 100, 120 and 140 seconds in both low power (30%). Each treatment consisted of five replicates with 50 grams of grains by treatment, totalizing 250 grams, which were placed in plastics pots.
After irradiation, the rice mass temperature was measured through a mercury thermometer and the grains were placed in a climatic chamber where the detection of adult's emergence was performed.

2.4 Test with adults

Insects with variable age were placed on the grains and left for 24 hours, so that there was adaptation of the population; it was performed irradiation with an exposure time of 0 (control), 40, 80, 140 and 160 seconds at low power (30%). Each treatment consisted of five replicates with 50 grams of grains by treatment, totalizing 250 grams, which were placed in plastics pots.
After irradiation, the rice mass temperature was measured through a mercury thermometer and the grains were placed in a climatic chamber where the detection of adult's mortality and filial generation emergence was performed.

In each test, it was used randomized design with five replicates per treatment.
The original data was subjected to exploratory analysis (outliers observation and normality) test by Harley (homogeneity of variances); when necessary, the original data was subjected to the linear regression analysis to verify the existence of a relationship between the media and the variance, indicating the suitable transformation for data normalization.
The transformation used was the square root, and then it was analyzed that the variance was continuous with the Tukey test (a = 0.05) when the F test was significant at the 5% level.

3. RESULTS AND DISCUSSION

Shown in Table 1 the total number of emerged insects per replicate in each treatment and their mean value, when the eggs of *S. oryzae* were irradiated with increasing doses of microwave radiation.
From the results of Table 1, we observe that with doses of up to 30 seconds of exposure to microwave irradiation does not induce deleterious effects to eggs inside the grains of rice, thus not affecting the emergence of adult insects when compared with the control. With a dose of 60 seconds, the microwave radiation increased the viability of eggs by 42% compared to control. This is probably related, in effect, stimulating microwave radiation, or radioestimulaçao part of an area of study called "Hormesis", defined as the positive stimulation of a biological system by non-harmful doses of a physical, chemical or biological agent. With these doses, it was not possible to achieve the lethality of eggs, which was achieved in *Rhyzopertha dominica* experiments [9], (despite being another kind of insect, determined that dose / time above 1 minute on high and medium potential was sufficient to eliminate 100% of the population); yet for *S. oryzae*, the changes did not reached damaging levels, being necessary to redifine the ideal lethal dose / time, following the same methodology.

Table 1 - Number and average adult insects *Sitophilus oryzae L.* from eggs inside grains of rice, irradiated with increasing microwave radiation doses (time) and rice temperature for each dose.

Time (seconds)	Rice Temperature (^oC)	RP	Number of Emerged Insects
0	25	1	21
		2	22
		3	23

		4	21
		5	19
		Mean	**23.2 b**
15	36	1	18
		2	23
		3	18
		4	23
		5	28
		Mean	**22.0 b**
30	37	1	23
		2	23
		3	18
		4	24
		5	18
		Mean	**21.2 b**
45	50	1	17
		2	22
		3	26
		4	23
		5	35
		Mean	**24.6 ab**
60	53	1	25
		2	23
		3	35
		4	32
		5	36
		Mean	**30.2 a**

Averages followed by the same letter do not differ by Tukey test at 5% probability.

From Table 2, with dose / time 75 seconds, we observe that also increased the viability of eggs by 38% compared to control, with a significant difference at the level of 5%. This is probably also related to radiostimulation (Hormesis). The dose / time of 115 seconds irradiation induced deleterious effects to eggs inside the grains of rice, affecting the emergence of adult insects in more than 50% when compared with the control. But only the dose of 130 seconds which was the overall lethality induced in eggs irradiated inside the grains of rice. These results being similar to the observed in litterature [8] [5] [6] [7].

Table 2 - Number and average adult insects *Sitophilus oryzae L.* from eggs inside grains of rice, irradiated with increasing microwave radiation doses (time) and rice temperature for each dose.

Time (seconds)	Rice Temperature (^{o}C)	RP	Number of Emerged Insects
0	25	1	27
		2	22
		3	25
		4	24
		5	20
		Mean	**22.6 b**
75	62	1	26
		2	25

		3	32
		4	36
		5	38
		Mean	**31.4 a**
100	70	1	22
		2	23
		3	19
		4	18
		5	15
		Mean	**19.4 b**
115	74	1	10
		2	11
		3	09
		4	08
		5	07
		Mean	**9.4 c**
130	77	1	0
		2	0
		3	0
		4	0
		5	0
		Mean	**0 d**

Averages followed by the same letter do not differ by Tukey test at 5% probability.

Listed in Tables 3 and 4, the total number of emerged insects per replicate in each treatment and their mean values when larvae and pupae of *S. oryzae* were irradiated with increasing doses of microwave radiation inside the grains of rice.

The results of Tables, we can see that already with doses of 130 and 160 seconds of exposure to microwave radiation induced lethal deleterious effects for these phases of the life cycle of the insect, killing 100% of the population, with significant differences between control and the other treatments. These results were similar to those observed in the litterature [8] [5] [6] [7].

As we can see, the experiments were conducted with doses / times greater than 120 and 150 seconds; this results represents that, based on results obtained with eggs, the larval and pupal stages were more resistant, which in fact did not occur, then, These tests were repeated dose / exposure times less than 160 seconds and 130 to determine the lethal dose for these phases.

Table 3 - Number and average adult insects *Sitophilus oryzae L.* from larvae inside grains of rice, irradiated with increasing microwave radiation doses (time) and rice temperature for each dose.

Time (seconds)	Rice Temperature (^oC)	RP	Number of Emerged Insects
0	25	1	23
		2	24
		3	21
		4	29
		5	25
		Mean	**24.4 a**
130	77	1	0
		2	0
		3	0

		4	0
		5	0
		Mean	**0 b**
160	93	1	0
		2	0
		3	0
		4	0
		5	0
		Mean	**0 b**
190	109	1	0
		2	0
		3	0
		4	0
		5	0
		Mean	**0 b**
210	121	1	0
		2	0
		3	0
		4	0
		5	0
		Mean	**0 b**

Averages followed by the same letter do not differ by Tukey test at 5% probability.

Table 4 - Number and average adult insects *Sitophilus oryzae L.* from pulpae inside grains of rice, irradiated with increasing microwave radiation doses (time) and rice temperature for each dose.

Time (seconds)	Rice Temperature (°C)	RP	Number of Emerged Insects
0	25	1	16
		2	23
		3	20
		4	25
		5	15
		Mean	**19.8 a**
160	93	1	0
		2	0
		3	0
		4	0
		5	0
		Mean	**0 b**
190	109	1	0
		2	0
		3	0
		4	0
		5	0
		Mean	**0 b**
210	121	1	0
		2	0
		3	0
		4	0
		5	0
		Mean	**0 b**

240	136	1	0
		2	0
		3	0
		4	0
		5	0
		Mean	**0 b**

Averages followed by the same letter do not differ by Tukey test at 5% probability.

Listed in Tables 5 and 6 the numbers of emerged insects per replicate in each treatment and their mean value when larvae and pupae were irradiated with increasing doses of microwave radiation inside the grains of rice, previously infested.

Analysing the results of these tables, we can observe that the dose (time) 100 seconds of microwave radiation exposure induced lethality for both stages of the life cycle of the insect, controlling 100% population and significant differences between the control and the other treatments. In previous tests higher doses (greater time) were used for these two phases, starting tests at doses greater than 120 to 150 seconds of microwave radiation, because this was taken as reference the results obtained with irradiation of eggs, where the lethal dose determined experimentally was 130 seconds. Given this result, we found that the larval and pupal stages were more resistant, which in fact did not occur. Usually with gamma radiation from Cobalt-60, which is also an electro-magnetic wave, the radioresistence increases gradually in the stages of egg, larvae, pupae and adult. Probably, this increased resistance of eggs in contrast to larval and pupal stages, is related to a loss of moisture or dissection of the same, this is because the contact of microwave radiation on rice triggers a process in the vibrating molecules, causing friction and hence generating heat, losing moisture, but as the eggs are placed inside the grains of rice and covered with a gelatinous substance excreted by the female for protection, it probably makes them a slighly tougher. On the contrary, it happens to the larvae and pupae, this because after hatching, the larvae begin to feed on the grains and the more developed, is practically the only layer, facilitating moisture loss (desiccation) and when they are to be transformed pupae, construct the outlet orifice of the adults after emerging, turning these phases then more vulnerable to desiccation. These results are also similar to those observed in the litterature [8] [5] [6] [7].

Table 5 - Number and average adult insects *Sitophilus oryzae L.* from larvae inside grains of rice, irradiated with increasing microwave radiation doses (time) and rice temperature for each dose.

Time (seconds)	Rice Temperature (^oC)	RP	Number of Emerged Insects
0	25	1	12
		2	18
		3	36
		4	16
		5	11
		Mean	**18.6 a**
85	70	1	11
		2	05
		3	00
		4	00
		5	01
		Mean	**3.4 b**
100	72	1	0
		2	0
		3	0

		4	0
		5	0
		Mean	**0 b**
115	74	1	0
		2	0
		3	0
		4	0
		5	0
		Mean	**0 b**
130	77	1	0
		2	0
		3	0
		4	0
		5	0
		Mean	**0 b**

Averages followed by the same letter do not differ by Tukey test at 5% probability.

Table 6 - Number and average adult insects *Sitophilus oryzae L.* from pulpae inside grains of rice, irradiated with increasing microwave radiation doses (time) and rice temperature for each dose.

Time (seconds)	Rice Temperature (^oC)	RP	Number of Emerged Insects
0	25	1	31
		2	25
		3	32
		4	31
		5	27
		Mean	**29.2 a**
80	68	1	06
		2	0
		3	0
		4	0
		5	0
		Mean	**1.2 b**
100	70	1	0
		2	0
		3	0
		4	0
		5	0
		Mean	**0 b**
120	76	1	0
		2	0
		3	0
		4	0
		5	0
		Mean	**0 b**
140	81	1	0
		2	0
		3	0
		4	0
		5	0
		Mean	**0 b**

Averages followed by the same letter do not differ by Tukey test at 5% probability.

The number of emerged insects and longevity by repetition within each treatment and their averages are shown in Table 7, when adult insects *Sitophilus oryzae* were irradiated with increasing doses of microwave radiation. By the results presented on this table, it can be observed that with the exposure time of 160 seconds to complete radiation induced lethality in adult insects immediately after irradiation. It can also be observed that the effects of radiation were proportional with increasing dose, and already with a dose of 80 seconds, the radiation induced a very drastic effect on longevity and reproduction of insects, decreasing by approximately 50% and 90% longevity. Thus proving that indeed the toughest stage of the life cycle of the insect microwave radiation is the adult phase, with significant differences between the control and the other treatments. These results being similar to those observed in the litterature [8] [5] [6] [7].

Table 6 - Number and average of adult insects longevity *Sitophilus oryzae L.* emerged in grains of rice, irradiated with increasing microwave radiation doses (time) and rice temperature for each dose.

Time (seconds)	Rice Temperature (^oC)	RP	Longevity	F1 Emerging Generation
0	25	1	68	70
		2	105	94
		3	87	125
		4	70	191
		5	85	119
		Mean	**83**	**123.8 a**
40	48	1	66	108
		2	71	55
		3	69	68
		4	86	83
		5	103	59
		Mean	**79**	**74.6 b**
80	68	1	54	44
		2	58	01
		3	72	23
		4	15	0
		5	0	0
		Mean	**39.8**	**13.6 c**
120	76	1	10	0
		2	12	0
		3	13	0
		4	09	0
		5	05	0
		Mean	**9.8**	**0 d**
160	85	1	0	0
		2	0	0
		3	0	0
		4	0	0
		5	0	0
		Mean	**0**	**0 d**

Averages followed by the same letter do not differ by Tukey test at 5% probability.

In the case of larger quantities (1.0 kg), which should be commonly used at the level of consumer household, as a safety measure, the dose should be 180 seconds because with this amount layer of rice of about 2.0 cm is formed per entire length of the plate from the microwave, requiring a longer time, since the irradiation is kept continuous and not intermittent, and therefore not allowing it to blend the product at the time of irradiation, occurring a few points where the temperature can be lower and the uneven extension of the dish from the microwave.
In spite of all the findings, at the moment, the immediate application of this method for insect-control has t be questioned by the economic point of view. Large scale researche for optimization of this method (aiming the uniformization of the heating by microwaves) must be performed.
Also additional studies with temperature and frequency of the dielectric properties of grains and insects are needed for improvement of each method of control.

4. CONCLUSIONS

With the results obtained in this study we can conclude that:
- The larval and pupal stages are the most sensitive, requiring only 100 seconds of exposure at low power to make a control of 100%.

- The egg stage requires an exposure time of 130 seconds also in low power.

- Finally, adult stage needs 160 seconds exposure in low power to cause total mortality of the insect's population in rice.

As a safety precaution, we recommend a time of 180 seconds at low power to control all phases of the life cycle of *S. oryzae* on rice.

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