THE IMPACT OF MICROWAVE RADIATION AND COLD STORAGE ON *CALLOSOBRUCHUS MACULATUS* (F.) (COLEOPTERA: BRUCHIDAE) UNDER LABORATORY CONDITIONS

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ABSTRACT: The feasibility of using different types of microwave radiation and cold storage to control the *Callosobruchus maculatus* (F) in stored grains was evaluated. The mortality rate of adult insects at 200 W power level, different exposure times (0, 2, 4, 6 and 8 min) and 48 h cold storage period was determined. Moreover, different age groups of *C. maculatus´*s eggs were exposed to 100 W power level and 0, 2, 4, 6 and 8 min exposure periods at 48 h cold storage period. There was a direct relationship between mortality rate and exposure times. Furthermore, the results imply a significant difference in susceptibility of adult insects in using radiation priori or post cold storage treatment. Likewise, a direct positive relationship between mortality rates and microwave radiation exposure times was observed. At the power level of 100 W and 48 h cold storage period, 3-4 days-old eggs were more tolerant than the other age groups, followed by 4-5, 1-2, 2-3 and 0-1 days-old eggs.

KEY WORDS: Disinfestations, Heat manipulation, Microwave Power level, Stored- grain insects.

Members of the family leguminoseae are amongst the largest plant famillies world wide. They have a large economic value as their grains contain large amount of protein that are useful in food and fodder productions (Dasbak et al., 2009). Cowpea, *Vigna unguiculata* (L.) Walp, is a major source of dietary protein in tropical and subtropical regions of the world especially where availability and consumption of animal protein is low (Echezona, 2006).

Storage of cowpea grain over long periods, especially at small scale farming levels, is limited due to cowpea *Callosobruchus maculatus* (F.). This pest causes loss of weight, nutritional value and viability of stored grains (Swella & Mushobozy, 2007).

C. maculates is an agricultural pest insect of Africa and Asia that presently range throughout the tropical and subtropical world (Beck and Blumer, 2009). The larvae feed on the pulse seed contents reducing their degree of usefulness and making them unfit either for planting or for human consumption (Ali et al., 2004).

Temperature is one of the principal factors delimitating survival and reproduction of insects. Lethal temperatures are those above or below the suboptimum which will eventually kill the organism. Pest management through temperature manipulation is receiving renewed interest as a non-chemical method with lack of residue problem (Hallman & Denlinger, 1999).

Insects in stored grain can be controlled by manipulating the physical environment or by applying physical treatments to the grain and insect species. Microwave heating is based on the transformation of alternating electromagnetic field energy into thermal energy by affecting polar molecules of a material

(Mullin, 1995). Microwaves energy is no persistent in the environment and does not hazardous impacts or damage to foodstuff (Vadivambal et al., 2007).

Insects under microwaves radiation are prone to some types of stress such as controlled atmosphere and cold ambient (Wang and Tang, 2001). Cold storage treatments have also been developed for quarantine purposes and for use against exotic fruit flies and other insects (Gould, 1994). In order to more clarify the lethality of microwave radiation and cold storage on different age groups of *C. maculatus* eggs and sensitivity of adult insects, the present investigation was undertaken. In this study, the aim was to identify the susceptibility of eggs and adult insects of *C. maculatus* to different types of radiation and cold storage.

MATERIALS AND METHODS

Rearing of experimental insects

Stock culture of *C. macullatus* was established from infested cowpea seeds of the Urmia (37.39° N 45.4°E, a town in west Azarbijian Province, Iran) central market in 2009. The insects were reared on healthy seeds in 1-liter jars which covered with a perforated muslin cloth held in place with two tight rubber bands and maintained under controlled conditions of temperature 27±2° C and Relative Humidity 60 ± 5. The insects were reared for 2 generations before initiation of the experiments.

Preparation of eggs for experiments

In each experiment, 1 to 5 days-old eggs were used. To obtain known age groups of eggs unsexed adults were collected from stock cultures and introduced on healthy seeds, the resultant eggs was kept under same conditions. In each trial, 20 infested seeds was selected and placed in a Petri dish.

Preparation of adult insects for experiments

Using a fine sable brush mixed sex of 1-7 days old adult insects were counted out in batches of 15 on to Petri dishes containing 2 g of rearing medium.

Microwave system

All the experiments were conducted using a kitchen type, 2450 MH_z microwaves oven (Butane, BC 320 W) with capability of producing 100 through 1000 W microwave power.

Bioassays

Preliminary power level tests were carried out prior to each experiment to determine a range of power that would produce nearly 25-75% mortality at the lowest and the highest levels, respectively (Robertson et al., 2007).

The experiments using microwave power and cold storage duration was conducted according the following two fashions, 1: the insects exposed to radiation and then after kept at cold storage, 2: the test procedure was vice versa of the previous method.

For eggs we employed the first fashion procedure at 100 W power level and 0, 2, 4, 6 and 8 min exposure times.

In the case of adult insects, each Petri dish containing 15 insects and 2 g of rearing medium was placed in the microwaves generator oven. The insects were exposed to 200 W power level for 0, 2, 4, 6 and 8 min. prior and post treatment with microwave radiation the samples were kept under cold storage conditions (3 ± 1°C) for 48 h. At the end of each experiment insects were maintained on their rearing medium under rearing conditions to recover. In each bioassay, mortality was recorded after recovery period. Those insects that did not move when lightly probed or shaken in the light and mild heat were considered dead. After 24 h of incubation, the mortality data were recorded.

For eggs, after preparation of 1 to 5 days old eggs 20 infested seeds was selected and placed in a Petri dish and then exposed to 100 W power level for 0, 2, 4, 6 and 8 min exposure times. Afterward, samples were kept under cold storage conditions (3 ± 1°C) for 48 h. After treatment, each experimental unit was inspected daily to remove emerged adults. Inspection of samples was continued until no adult emergence was observed for three consecutive days. At the termination of experiment the number of emerged adults and unhatched eggs was recorded.

The experimental units and bioassay procedures were identical in all trials. In each trial, the control Petri dish was treated identically except that no microwaves radiation and cold storage treatment was employed. Each test was replicated four times.

Data analysis

Mortality data were subjected (if necessary) to appropriate transformation before analysis. LT_{50} and LT_{95} values were estimated by subjecting mortality data to the maximum likelihood program of probit analysis (Robertson et al., 2007) using SPSS 15 software.

RESULTS AND DISCUSSION

Present results display that in all experiments microwaves power showed lethal effects to the tested insects. According to results a direct positive relationship between mortality rates and microwaves exposure times was observed. In adult insects at power 200 W at 2, 4, 6 and 8 min exposure times then 48 h cold storage the observed mortalities were 10, 33.33, 68.33 and 90% respectively. But, when cold storage period was 48 h and then after the insects exposed to radiation the mortalities were decreased to 1.66, 16.66, 50 and 78.33%, respectively (Fig. 1). Statistical data shows the comparison of adult insects of *C.macullatus* in two different usages of microwave exposure times and 48 h cold storage (Table 1). These results imply that there was a significant difference in susceptibility of adult insects in using radiation before or after the cold storage.

Analysis of T-test revealed that, the main effect of microwaves exposure times before and after 48 h cold storage were highly significant (Table 2). Therefore, there was a significant difference between mortality rates due to radiation before and after cold storage of treatments.

LT_{50} and LT_{95} values were estimated from the probit analysis of different age groups of egg mortality and are given in Table 3. In all experiments, microwaves power showed lethal effects to the tested eggs also a direct positive relationship between mortality rates and microwave exposure times was observed. At the power level of 100W and 48 h cold storage period, 3-4 days-old eggs were more tolerant than the other age groups, followed by 4-5, 1-2, 2-3 and 0-1 days-old eggs.

Stored-product insects are serious pests of dried, stored, durable agricultural commodities and of many food stuffs worldwide. In recent decades there is much interest in alternatives to conventional insecticides for controlling stored-product due to of increasing consumer demand for product that is free of insects and insecticide residues. In current study, microwaves radiation was lethal to test insects. The mechanisms involved in the lethal action of microwaves radiation are previously understood. The hazardous impacts could be due to the high frequency oscillation of the water molecules in the body of the insects. Microwave radiation has deleterious effects on insects such as reduction of reproductive rate, losing body weight and malformation as well (Nelson, 1996). In all treatments, the percentage of mortality increased with an increase in duration of radiation period. Among two types of radiation adult insects were susceptible to using microwave radiation before the 48 h cold storage. Low temperatures have been used widely to manage pest population in bulk stored grain. Typically, development of stored product insects slows or stops when the temperature drops below 14°C (Banks and Fields, 1995). Therefore, thermal treatments using temperatures higher and lower than the range have been exploited for pest control. Disinfestations of stored-products with physical control methods such as using microwaves energy coupled with cold storage treatment can be an alternative measure to pesticides in killing insects. Combined application of microwaves power with cold storage treatment could be considered as a potential measure, which helps to reduce stored-products insects´ populations in IPM programs. Using microwave radiation and cold storage in these trials significantly effect survival and adult emergence of *C. macullatus*. Based on the data collected in current study it could be concluded that using microwave radiation along with cold storage is merit to be considered as a useful tool in stored-products insects control systems.

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Table 1. Susceptibility comparison of adult *C.macullatus* in two different usages of microwave exposure times and 48 h cold storage period.

Type of radiation	**LT_{50}**	**LT_{95}**	**Confidence limits 95%**	**Intercept**	**Slope**	**$^2\chi$ (df=1)**	**Sig.**
Before 48 h cold storage treatment	4.532	10.314	(4.114 - 4.978)	1.978	4.532	6.710	0.000
After 48 h cold storage treatment	5.926	13.488	(5.388 - 6.546)	1.441	5.926		

Table 2. Mortality (mean ± Standard error) and T value of insects exposed to microwave radiation and 48 h cold storage at power 200W.

Exposure times of radiation(min)	**Radiation**	**Mean ± SE**	**T value**
2	Before 48 h cold storage	1.50 ± .289	3.273
	After 48 h cold storage	0.25 ± 0.250	
4	Before 48 h cold storage	5 ± 0.408	5
	After 48 h cold storage	2.5 ± 0.289	
6	Before 48 h cold storage	10.25 ± 0.479	3.422
	After 48 h cold storage	7.50 ± 0.645	
8	Before 48 h cold storage	13.50 ± 0.289	3.130
	After 48 h cold storage	11.75 ± 0.479	

Table 3- Susceptibility of different age groups of *C.macullatu´s* eggs at 100 W power level for 2, 4, 6 and 8 min exposure times and 48 h cold storage period.

Different age groups of eggs	Total eggs	LT_{50}	LT_{95}	Confidence limits 95%	$^2\chi$ (df=2)	Sig.
0-1 days-old	962	0.608	318.028	(0.223 -0.988)	1.108	0.002
1-2 days-old	313	1.714	24.288	(0.778 - 2.079)	0.498	0.000
2-3 days-old	787	1.081	1.740	(0.112 - 0.914)	1.805	0.012
3-4 days-old	170	2.146	44.862	(0.442 - 2.050)	0.100	0.002
4-5 days-old	250	2.090	13.420	(1.272 -2.801)	3.513	0.000

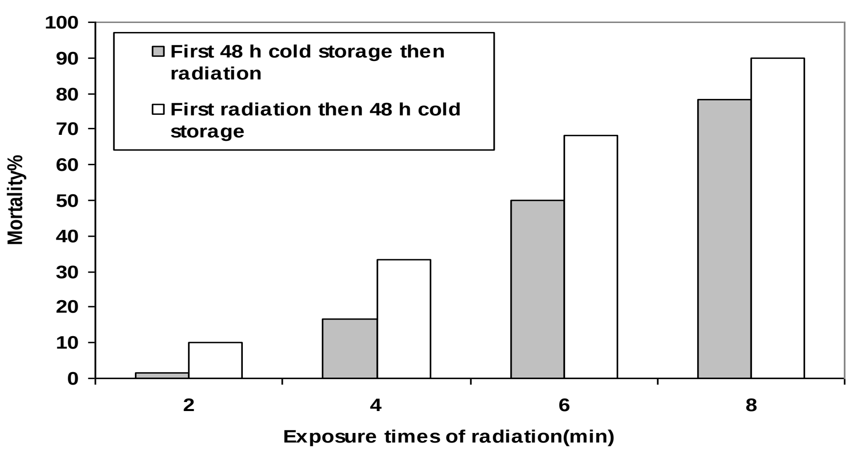

Figure 1. Percent mortality of adult *C.maculatus* at 200W power and different exposure times.