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Full Length Research Paper

The impact of microwaves irradiation and temperature manipulation for control of stored-products insects

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The impact of microwaves irradiation and temperature manipulation against adults of saw-toothed grain beetle, *Oryzaephlius surinamensis* (L.) and cigarette beetle, *Lasioderma serricorne* (F.), over various exposure times and cold storage period was evaluated. The insects were exposed to 2450 MHZ at five different power levels of 0, 100, 200, 300 and 400 W for five exposure times of 0, 3, 6, 9 and 12 min. A complete control was achieved for tested insects at 400 W power levels for exposure time of 12 min and 72 h cold storage period. At a given time, a direct positive relationship between the mortality rates and microwaves irradiation power levels was obtained. For *O. surinamensis* at 100, 200, 300 and 400 W power levels and 24 h cold storage for 3 min exposure period, the mortality rates were 40, 50, 55 and 72%, respectively. Similar trend was also observed for *L. serricorne*. Substantiate variation in the susceptibility of tested insects to microwaves energy and cold storage period was apparent in the overlapping confidence interval of the LD_{50} values. In the analysis of variance (ANOVA) the R-squared value revealed that 90.8% of variability in the susceptibility of *O. surinamensis* could be explained by the microwaves power, cold storage period and exposure duration. This criterion was 93.2% for cigarette beetle. As expected, all of the first and second order interactions were significant. Moreover, there was sufficient evidence that the most important factor was the exposure period, followed by power level and cold storage time. The exposure period showed a higher omega-squared (ω^2) value, which implies substantial contribution to explaining insect mortality variations. Combinations of microwaves radiation and cold storage were found highly compatible and synergistic. This was more significant for the insects which were exposed to the highest level of the microwaves irradiation and cold storage period. Synergistic interaction indicates that, microwave irradiation can be used with cold storage for management of the insects in question. This treatment could provide an effective and friendly environmental treatment technique in integrated pest management (IPM) program.

Key words: Cold storage, microwaves, saw-toothed grain beetle, cigarette beetle.

INTRODUCTION

Control of stored-products pests has been one of the major tasks for conservators because the damage inflicted to foodstuff is irreversible. A number of insect species pose a potential threat to a variety of stored-products. *Oryzaephlius surinamensis* and *Lasioderma serricorne* have a widespread distribution in the most part of the world. These species are recognized as cosmopolitan pests attacking stored-products and cause serious losses both in quantity through feeding damage and quality by contaminating the product with cast skin and frass.

Fumigants are commonly applied for control of stored-products pests. Two of the commonly used fumigants are methyl bromide and phosphine. Methyl bromide is now under threat of withdrawal because it apparently depletes the earth's ozone layer (Leesch et al., 2000). Phosphine has been used in a variety of habitats for a long time (Rajendran and Muralidharan, 2001). Conventional use of phosphine has been frequent failure to control insects and certain insects have developed resistance to phosphine (Bell and Wilson, 1995). Moreover, concerns about the further development of resistance to phosphine have made the search for new alternatives imperative.

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Any compound that can reduce the insecticide load in a particular storehouse with adequate effectiveness to control insects may be of outmost importance in stored-product insect control programs. The main challenge is now for alternative substances and methods which are inexpensive, convenient to use and without substantial disruption of the environment. According to these criteria, physical control methods could be of paramount importance. Some physical control methods such as microwaves energy and temperature manipulation have been used for treatment alone earlier (Johnson et al., 2003; Wang et al., 2003).

Microwaves energy is not persistent in the environment and does not have hazardous impacts or damage to foodstuff (Vadivambal et al., 2007; Warchalewski et al., 2000; Halverson et al., 1996). Exposure to microwaves energy could cause physical injuries and reduced reproduction rates in surviving insects. For instance, treated larvae may develop into adults with deformed or missing legs and although, surviving insects were capable of reproduction, however, the reproduction rate decreased considerably (Nelson, 1996). Microwaves utilize very high frequencies; this enables rapid heating to be achieved with much lower field intensities. The penetration depth is an important factor, as the microwaves intensity diminishes with increased penetration. With retrospect, due to limited penetration of microwaves energy into foodstuff mass, it seems likely that employment of microwaves radiation alone could not be considered as a promising insect control measure under field conditions.

Insects under microwaves irradiation are prone to some types of stress such as controlled atmosphere and cold ambient (Wang and Tang, 2001). The storehouse environment is usually one that is enclosed, allowing for the manipulation of temperature. Thus, the use of temperature to restrict insect population is an excellent tool for the stored-product industry. Exposure to temperatures only 5°C above the optimum are capable of slowing or stopping insect activity and development and depending on the species, are capable of causing death. Exposures to temperatures between 42 to 50°C for short periods of time have produced over 90% mortality (Fields, 1992).

A review of the literature revealed the scarcity of information concern over optimal power levels of microwaves radiation combined with cold storage period in insect killing programs. To clarify the combined impact of these insect control measures the present investigation was undertaken.

MATERIALS AND METHODS

Test insect

O. surinamensis and *L. serricorne* samples were collected from local stores and shops, in Urmia (37.39°N 45.4°E), a town in West Azarbijian Province (Iran) in 2008. These insects were selected due to their economic importance, the most part of the world including Iran. Stock cultures were established and maintained on heat-sterilized oat and wheat flour at 27 ± 2°C, 65 ± 5% relative humidity (RH) and 14 h photoperiod in wide-mounted glass jars covered with pieces of muslin cloth fixed by rubber bands. All insects were cultured under moderately crowded conditions to ensure proper development of the resultant insects. Insects were reared for two generations before initiation of experiments.

Preparation of insects for experiments

Before each treatment run, using a fine sable brush mixed sex of 5 days old adult insects was counted out in batches of 60 on to Petri-dishes containing 20 g of rearing medium.

Bioassays

The bioassay experiments using microwaves power and cold storage duration (solely and in combination) were conducted under rearing conditions. The experiment units and bioassay procedures were identical in all trials. Preliminary power level tests were carried out prior to each experiment to determine a range of power that would produce ≈ 25 to 75% mortality at the lowest and the highest levels, respectively (Robertson et al., 2007). In each experiment after termination of cold storage duration insects were allowed to recover on their usual media under rearing conditions. In each bioassay, mortality was recorded after exposure to cold storage and recovery period. Those insects that did not move when lightly probed or shaken in the light and mild heat were considered dead.
To commence microwaves irradiation each Petri dish containing 60 insect and 20 g of rearing medium was placed in a kitchen type, 2450 MHz microwaves oven (Butane, BC320W) with capability of producing 100 through 1000 W microwaves power. For microwaves irradiation five power outputs of generator was set at 0, 100, 200, 300 and 400 W. The exposure times were 0, 3, 6, 9 and 12 min. At the termination of treatment, the samples along with their respective control groups were maintained under cold storage conditions (4 ± 1°C) for 0, 24, 48, and 72 h. In each trial, the control Petri dish was treated identically except that no microwaves irradiation and cold storage treatment was employed. At the termination of cold storage period, insects were transferred to clean jars containing rearing medium and maintained under rearing conditions. After 24 h of incubation, the data were recorded in term of the number of live and dead adults. Each test was replicated three times. Mortality data from the replicates were pooled and mortality response was determined. In order to evaluate the combined impact of the microwaves power and cold storage, the estimated LD's of either treatment were separately combined and employed in the trials.

Data analysis

The median lethal dosage (LD_{50}) and LD_{95} of microwaves irradiation and LT50 values was estimated by subjecting mortality data to the maximum likelihood program of probit analysis (Robertson et al., 2007) using SPSS software. This program has a provision for control mortality. Two insects group were considered significantly different in their susceptibility to a treatment, if a confidence interval of relative median potency does not include the value of 1 (Norušis, 2008). The synergistic, antagonistic and additive effect of each combination was calculated by using the formula proposed by Ahmed and Khaique (2005). The value of joint action ratio > 1.05 will indicate synergism and between 0 and 0.95, the antagonistic action and those between 0.95 and 1.05 will indicate additive effect. Mortality data were normalized by an arc-square-root transformation, analyzed by a one-way ANOVA through factorial

Table 1. Summary of regression of probit analysis of *O. surinamensis* and *L. serricorne* exposed to microwaves radiation for 3 min.

Species	Treatment	LD_{50}	LD_{95}	Slope	SR[a]	Type of action
A	Power	204 (131-286)	3890 (1305-26603)	1.29		
	Cold storage[b]	15.07 (9.3 - 58.6)	103 (34-2405 -----) [c]	1.01		
	Power + 24 h Cold storage	196 (142-250)	1081 (901-11715)	1.71	1.04	Additive
	Power + 48 h Cold storage	120 (96-139)	342 (284-459)	3.61	1.70	Synergism
	Power + 72 h Cold storage	107 (----------)[c]	1514 (----------)[c]	1.42	1.90	Synergism
B	Power	360				
	Cold storage[b]	305.8(220-629)	4851(1418- 20235)	1.73	1.17	Synergism
	Power + 24 h Cold storage	245.9 (182-298)	3646 (----------)[c]	1.41	1.46	Synergism
	Power + 48 h Cold storage	170.2 (---------)[c]	22510 (----------)[c]	0.78	2.11	Synergism
	Power + 72 h Cold storage					

a, Synergistic ratio; b, treatment of cold storage alone caused negligible mortality; c, could not be calculated with reasonable accuracy. (A) *O. surinamensis;* (B) *L. serricorne.*

Table 2. Summary of regression of probit analysis of *O. surinamensis* and *L. serricorne* exposed to microwaves radiation for 6 min.

Species	Treatment	LD_{50}	LD_{95}	Slope	SR[a]	Type of action
A	Power	204 (131 - 286)	3890 (1305-26603)	1.29		
	Cold storage[b]	15.07(9.1 –58.6)	103 (34 -2405 ----)[c]	1.01		
	Power + 24 h Cold storage	111 (0.13 - 178)	378 (277 - 63203)	3.11	1.83	Synergism
	Power + 48 h Cold storage	113 (89 - 124)	327 (271- 441)	3.57	1.79	Synergism
	Power + 72 h Cold storage	89 (68 -105)	203 (172 - 271)	4.60	2.28	Synergism
B	Power	145				
	Cold storage[b]	149 (129 – 184)	475 (381.9 – 690.2)	3.46	0.97	Additive
	Power + 24 h Cold storage	127 (101.- 149)	356.2 (294 - 484.8)	3.69	1.13	Synergism
	Power + 48 h Cold storage	118.3 (0.3 - 185)	309.4 (195 –16975)	3.94	1.22	Synergism
	Power + 72 h Cold storage					

a, Synergistic ratio; b, treatment of cold storage alone caused negligible mortality; c, could not be calculated with reasonable accuracy. (A) *O. surinamensis;* (B) *L. serricorne.*

experiments and followed by Tukey's test to compare differences among the various treatments at the $\alpha = 0.05$ level.

RESULTS

Lethality of microwave energy, cold storage and exposure period

Dosage-mortality values estimated from the probit analyses of different insect groups are given in Tables 1 to 4. These tables display that, in all experiments microwaves power showed lethal effects to the tested insects. In some cases considerable overlap in 95% confidence interval of relative median potency was observed (Tables 1 to 4). Therefore, no statistically significant difference between the estimated LD_{50} values was secured. Treatment of cold storage alone caused negligible mortality and estimated LT_{50} value was 15.07 and ds (Tables 1 to 4). The lethality of microwaves irradiation enhanced greatly at higher power level and an inverse relationship between microwaves power level and estimated LT_{50} values in a given cold storage period was obtained (Table 5). This effect was more striking at the highest level of either treatment. Almost always the combined effect of microwaves irradiation and cold storage period was synergistic. This effect was more pronounced at the highest period of cold storage (Tables 1 to 4). Analysis of variance revealed that, the main effect of microwaves irradiation level, exposure time and cold storage period was highly significant (Table 6). Therefore, there was a significant difference between levels of these treatments. For instance, mortalities at 100 and 400 W power levels for *O. surinamensis* at a given cold storage period and exposure time were significantly different. Similar conclusions from separation of means were secured in the case of cold storage and exposure period. All interactions among microwaves radiation level, exposure period and

Table 3. Summery of regression of probit analysis of *O. surinamensis* and *L. serricorne* exposed to microwaves radiation for 9 min.

Species	Treatment	LD_{50}	LD_{95}	Slope	SR[a]	Type of action
A	Power	204 (131 - 286)	3890 (1305-2660)	1.29		
	Cold storage[b]	15.0 (9.3 – 58.6)	103 (34-2405 ---)[c]	1.01		
	Power + 24 h Cold storage	89 (75 -116)	260 (217-347)	3.9	2.29	Synergism
	Power + 48 h Cold storage	87 (64 - 104)	211 (178-286)	4.3	2.34	Synergism
	Power + 72 h Cold storage	77 (49 - 95)	208 (172-289)	3.8	2.68	Synergism
B	Power	117				
	Cold storage[b]	112(90.8 – 129)	263.6 (223 – 342.7)	4.43	1.04	Additive
	Power + 24 h Cold storage	102 (78 – 120.9)	265 (221.2 – 355.9)	3.97	1.14	Synergism
	Power + 48 h Cold storage	80.9(51.5 – 102)	257 (209.0 – 370.4)	3.28	1.44	Synergism
	Power + 72 h Cold storage					

a, Synergistic ratio; b, treatment of cold storage alone caused negligible mortality; c, could not be calculated with reasonable accuracy. (A) *O. surinamensis;* (B) *L. serricorne.*

Table 4. Summary of regression of probit analysis of *O. surinamensis* and *L. serricorne* exposed to microwaves radiation for 12 min.

Species	Treatment	LD_{50}	LD_{95}	Slope	SR[a]	Type of action
A	Power	204 (131-286)	3890 (1305-9660)	1.29		
	Cold storage[b]	15.07(9.3-58.6)	103 (34- --------)[c]	1.01		
	Power + 24 h Cold storage	88 (---------)[c]	136 (----------)[c]	8.7	2.31	Synergism
	Power + 48 h Cold storage	78 (41-92)	146 (124-276)	6	2.62	Synergism
	Power + 72 h Cold storage	72 (15-88)	135 (115-419)	65	2.83	Synergism
B	Power	100				
	Cold storage[b]	92.8 (57.9– 101)	142.9 (120.9– 655)	6.8	1.07	Synergism
	Power + 24 h Cold storage	76.0 (48 – 93.3)	176.5(147.2– 254)	4.5	1.31	Synergism
	Power + 48 h Cold storage	79.1(43 – 93.07)	147.7(124 – 281)	6.1	1.26	Synergism
	Power + 72 h Cold storage					

a, Synergistic ratio; b, treatment of cold storage alone caused negligible mortality; c, could not be calculated with reasonable accuracy. (A) *O. surinamensis;* (B) *L. serricorne.*

and cold storage duration was highly significant (Table 6). This table displays that, interactions involving two factors, for example power level with cold storage period and interaction three factors (power level × cold storage period × exposure time) are highly significant. The significant interaction indicates that, the factors are not independent; in other words, the difference between simple effects of microwaves power level for different levels of cold storage is significant, conversely, the difference in simple effects of cold storage at the different levels of microwave power is significant. Thus, any simple effect is dependent upon the level of the other factor in the experiment.

The significant interaction of three factors implies that the power level with cold storage period interaction differs with the level of exposure period. The adjusted R-squared value revealed that 90.8% of variability in the susceptibility of *O. surinamensis* could be explained by the microwaves power, cold storage duration and exposure period. Moreover, R-squared value revealed that, the analysis of variance as a statistical model does fit the data well. In the case of *L. serricorne,* a similar fashion of interpretation also could be expressed. To determine unambiguously the relative importance of each factor, omega squared, ω^2, is calculated. This measure indicates what proportion of the variation in the dependent variable is related to a particular variable or factor. Omega squared values for *O. surinamensis* and *L. serricorne* were calculated and is shown in Table 6.

Synergistic effect between microwaves energy, cold storage and exposure period

At the 12 min exposure period, microwaves energy in combination with cold storage produced the highest

Table 5. LT_{50} values (min) for *O. surinamensis* and *L. serricorne* exposed to microwaves irradiation and cold storage.

Insect	Power (W)				Cold Storage (h)
	400	300	200	100	
A	2.43 (1.03-2.8)	3.3 (0.03-4.6)	3.47 (2.57-4.2)	5.35 (3.16-7.2)	24
	2.31 (------)[a]	2.96 (2.50-3.3)	3.75 (0.22-5.7)	4.63 (2.97-5.9)	48
	2.29(------)[a]	2.73 (2.17-3.2)	2.83 (1.48-3.1)	3.50 (1.70-4.7)	72
B	3 (291 - 376)	4(0.44 - 6.16)	4.23 (0 - 6.63)	8.07 (5.7-13.8)	24
	3 (261 - 4.36)	3 (2.5 - 3.50)	3.8(2.8 - 4.6)	5.4 (3.2 - 7.33)	48
	(----------)[b]	2.8(2.2 - 3.35)	2.9 (1.9 - 3.7)	4.3 (2.0 – 5.9)	72

a, The confidence interval could not be calculated with reasonable accuracy; b, the LT_{50} value and confidence interval could not be calculated with reasonable accuracy. (A) *O. surinamensis;* (B) *L. serricorne.*

Table 6. Significance levels for ANOVA on mortality of *O. surinamensis* and *L. serricorne* exposed to microwaves radiation, cold storage at different exposure periods.

Insect	Source	df	F	Significance
A	Corrected model[a]	63	30.7	0.00
	Intercept	1	23866	0.00
	Microwave power level	3	223.1	0.00
	Cold storage period	3	240.8	0.00
	Exposure time	3	23.3	0.00
	Microwave power level * Cold storage	9	13.1	0.00
	Microwaves power * Exposure time	9	8.7	0.00
	Cold storage period * Exposure time	9	7.0	0.00
	Microwave power * Cold storage * Exposure time	27	7.9	0.00
	Error	128		
	Total	192		
	Corrected Total	191		
B	Corrected model[b]	63	22.5	0.00
	Intercept	1	15892	0.00
	Microwave power level	3	178.5	0.00
	Cold storage period	3	152.2	0.00
	Exposure time	3	13.7	0.00
	Microwave power level × Cold storage	9	8.2	0.00
	Microwaves power × Exposure time	9	5.5	0.00
	Cold storage period × ×Exposure time	9	4.9	0.00
	Microwave power × Cold storage × Exposure time	27	5.2	0.00
	Error	128		
	Total	192		
	Corrected Total	191		

a, R squared = .938 for *O. surinamensis;* b, R squared = 88% for *L. serricorne.* (A) *O. surinamensis* and ω^2 value for power level, exposure time and cold storage period is 0.40, 0.41 and 0.03, respectively; (B) *L. serricorne* and ω^2 value for power level, exposure time and cold storage period is 0.31, 0.36 and 0.02, respectively.

synergistic effect (Table 4). However, at the 3 min exposure time with combined effect of microwave power at 24 h cold storage period, only an additive type of action was secured (Table 1). Therefore, to obtain the best synergistic effect prolongation of the cold storage period is imperative. From Tables 1 through 4 it could be concluded that, a direct relationship between the exposure period and enhanced impact do exist. For *O.* surinamensis the synergistic ratio at 3, 6, 9 and 12 min exposure time combined with 72 h cold storage period

was 1.90, 2.28, 2.68, and 2.83, respectively (Tables 1 to 4). In the case of *L. serricorne*, these Tables display irrespective values for the period of exposure time in question, 2.11, 1.22, 1.44, and 1.26 in the same order.

DISCUSSION

Insects within stored foodstuffs cause numerous quality and health issues. Because of this, International organizations such as FDA (1997) and FGIS (1999) set tolerances and grade standards regulating the number of insects and insect fragments above specified tolerances to make the product illegal for human consumption. The saw-toothed grain beetle, *O. surinamensis* and *L. serricorne* are two cosmopolitan and destructive invaders of foodstuffs. Control of stored-products pest insects is essential wherever foodstuffs quality is to be maintained.

Fumigation is one of the most successful methods of rapidly controlling insect's infesting stored-products. A good fumigant should have some characteristics consistent with the fumigation protocol, which ensures an appropriate level of insect control and produces the minimum of hazardous side effects. Unfortunately, the two available fumigants, methyl bromide and phosphine, fall short of this ideal (Collins et al., 2002).

A new approach in insect control research could be the use of less hazardous substances or control methods, which are more compatible with environment. Method for the control strategies that are environmentally sustainable and avoid the use of conventional pesticide is of paramount important. 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, but little attention has been paid to this issue earlier.

In the current study, microwaves radiation was lethal to test insect. 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). However, application of microwaves radiation in insect killing programs could be limited due to insufficient penetration depth. Zhu et al. (1995) reported that, microwaves attenuate exponentially in penetration to foodstuffs.

Cold storage can affect the insects in various ways. Ayvaz and Karabörklü (2008) reported that, reproductive ability and number of living adults of *Ephestia kuehniella* decreased depending on the length of the cold storage period. Similar results have been reported for the other insects (Johnson et al., 1997; Özder, 2004; Larentzaki et al., 2007).

The major advantage of cold storage is that, it can easily be coupled with other method of pest control measures, such as microwaves radiation. In general, the reduction of temperature in the environment stresses the insect (Ikediala et al., 1999), thereby, making it more susceptible to other control measures (Wang and Tang, 2001). Almost in all trials there was sufficient indication that longer microwaves energy exposure and cold storage duration could achieve better kill than shorter ones of similar power level. From this point of view, the results were in agreement with the findings of Neven (1994) who studied the combined effects of heat treatment and cold storage on mortality of fifth-instars' codling moth.

It is well established that a good control agent must kill the target insect with acceptable level of the agent in a short period of time. Since microwaves power combined with cold storage is lethal to the stored-products insects and because methyl bromide may not be available for use as a fumigant in immediate future, combined application of microwaves power with cold storage treatment could be considered as a potential measure which can help reduce stored-products insects' populations in integrated pest management (IPM) programs.

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