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Combination of Microwave Radiation and Cold Storage for Control of *Oryzaephilus surinamensis* (Col.: Silvanidae) and *Sitophilus oryzae* (Col.:Curculionidae).

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ABSTRACT

The impact of microwave radiation in conjunction with cold storage on *Oryzaephilus surinsmensis* (Herbst) and *Sitophilus oryzae* (L.) adults either continuously or intermittently was evaluated under laboratory conditions. The insects were exposed to 2450 MHz at power level of 100 W for exposure time of 10 min continuously and intermittently. In all experiments, the highest mortality rate was achieved for intermittent exposure time of 10 min and 72 h cold storage duration. Intermittent exposures were generally more effective in killing insects of both species compared with those of continuous irradiation. Combinations of microwave radiation and cold storage were found highly compatible and synergistic. The synergistic interaction indicates that microwave radiation can be used with cold storage for management of *Oryzaephilus surinsmensis* and *Sitophilus oryzae* adults. This treatment could pave the way for an effective and friendly environmental treatment technique under field conditions.

Key word: Microwave irradiation, Heat treatment, Stored products pests, Synergistic impact

INTRODUCTION

Over the past decades, the stored-products pests control measures 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 variety of stored-products. *Oryzaephilus surinsmensis* and *Sitophilus oryzae* have a wide spread distribution worldwide. This species is recognized as one of the most cosmopolition pests attacking stored-products and causes serious losses both in quantity through feeding damage and quality by contaminating the product with cast skin and frass (Ja Hyun and Ryoo, 2000). Wheat and rice are two of staple cereal products in the world. It is estimated that annual losses of cereal grains due to insects and rodents are about 10 % in North American and 30 % in Africa and Asia, but higher losses and contamination often occur locally (Hill, 1990).

Based on previous experiences, 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 the 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 failed frequently to control insects and certain insects have developed resistance to phisphine (Bell and Wilson, 1995). Moreover, concerns about the further development of resistance to phosphine have made the search for new alternatives imperative (Halverson *et al*., 1999).

Any compound that can reduce the insecticide load in a particular storehouse with adequate effectiveness to control insects may be of outmost importance in store-product insect control programs. The main challenge is now for alternative substances and methods, which are expensive in, 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 (Johnson *et al*., 2003; Wang *et al*., 2003).

Microwave energy is not persistent in the environment and is not hazardous or impact or damage foodstuffs and hence there are no adverse effects on human beings (Vadivambal *et al*., 2007; Warchalewski *et al*., 2000). Exposure to microwave 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). Microwave radiation, with good penetrability, can kill pests existing inside or outside grain kernels (Halverson *et al*., 1999). The penetration depth is an important factor, as the microwave's intensity diminishes with increased penetration. Due to the limited penetration of microwave energy into foodstuff mass, it seems likely that employment of microwave radiation alone could not be considered as a promising insect control measure under field condition.

The test insects under microwave irradiation are prone to some types of stress such as controlled atmosphere and ambient cold (Wang and Tang, 2001). The warehouse environment is usually one that is enclosed, allowing the manipulation of its 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 is capable of slowing or stopping insect activity and development and depending on the species, capable of causing death (Field, 1992). Exposures to temperature above 55˚C for short periods of time have produced 100 percent mortality (Zhao *et al*., 2007).

The review of the literature revealed the scarcity of information over optimal levels of microwave radiation combined with cold storage period in the insects killing programs. The objectives of this research are as followed:

1- To determine the combined effect of microwave radiation and cold storage on *Oryzaephilus surinsmensis* and *Sitophilus oryzae* adults.

2- To evaluate the impact of microwave power on wheat germination.

MATERIALS AND METHODS

Test insects: *Oryzaephilus surinsmensis* and *Sitophilus oryzae* samples were collected from local stores and shops, in Urmia (37.39˚N 45.4˚E), a town in West Azarbijan Province (Iran) in 2008. These insects were selected due to their economic importance throughout the world including Iran. Stock cultures of *O. surinsmensis* was established and maintained on heat-sterilized rolled oats containing small amounts of wheat germ and brewers' yeast. Soft rice was used for *S. oryzae*. All the insects were kept at 27 ± 2˚C, 65 ± 5 % Relative Humidity (RH) and 14/ 10 h photoperiod (lighty dark) 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 1-7 days old adult insects wer counted out in batches of 20 on to Petri-dishes containing 10 g of rearing medium.

Bioassay: The combined bioassay experiments using microwaves power and cold storage duration were conducted. The experiment units and bioassay procedures were identical in all trials. In experiments after termination of cold storage duration, the 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 mild heat were considered dead.

To commence microwave irradiation each Petri-dish containing 20 insects and 10 g of rearing medium was placed in a kitchen type, 2450 MHz microwave oven (LG, BC320W) with capability of producing 100 through 1000 W. For microwave radiation one power output of the generator was set at 0 and 100 W. The exposure time was 10 min continuously and intermitenttly (5 min under the radiation, 2 min rest and out of the oven and under radiation for 5 min again). At the termination of treatment, the samples along with their respective control were maintained under cold storage condition (6 ± 1˚C) for 0, 48 and 72 h. In each trial, the control Petri-dish was treated identically except that no microwaves radiation and cold storage treatment was employed. At the termination of cold storage period, insects were allowed to recover and maintained under rearing conditions. After 48 h of incubation, the data were recorded in terms of the number of dead adults. Each test was replicated twelve times. The bioassay was conducted at 25 ± 2˚C, Relative Humidity (RH) 65 ± 5 % and 14 h photoperiod.

Determination of germination: Germination tests were conducted according to the principles stated in International Seed Testing Association (ISTA, 1999) methods with minor modification. Seeds of wheat were exposed to microwave radiation at one power level of 100 W for nine exposure times of 0, 2, 4, 6, 10, 12, 14, 16 and 20 min. For each compound hundred treated seeds were soaked with 100 mL of distilled water for 10 min. Pretreated seeds were spaced uniformly on sheet paper and placed in a germination cabinet for 10 d at 20˚C. Non-irradiated seeds were treated identically and served as control standards for comparison. Each experiment was replicated four times on four different days. The number of germinated seeds was counted after 2, 4, 6, 8 and 10 days.

Statistical analysis: Analysis of variance was used to check the significance of differences between the mortality of insects for different exposures, and also differences in the mortality of different insect species. In bioassay and germination tests, the data were normalized an arc-square-root and square-root transformation respectively, analyzed by one-way analysis of variance (ANOVA) followed by Tukey's test to compare differences among the various treatments at the $\alpha = 0.05$ level. The student t-test was used for comparing the mean of the two groups.

RESULTS

Present results display that in all experiments the microwave power showed lethal effects to the tested insects. The mortality percentages for *O. surinsmensis* and *S. oryzae* after continuously irradiated or with alternative irradiation and both with exposure times of 48 and 72 h cold storage are shown in Table 1.

At 100 W power level for exposure time of 10 min continuously, the mortality of *O. surinsmensis* was 37.98 % and 45.98 % respectively for exposure times of 48 and 72 h cold storage period. As irradiation exposure was changed to alternatively (5.5min), the mortality was increased to 49.85 % and 71.98 % respectively. Results of ANOVA showed that mortality varied significantly with exposure irradiation and exposure times of cold storage ($F = 168.334$; $df = 3$; $P < 0.05$). Tukey's test shows that mortality of *O. surinsmensis* was significantly higher with intermittent exposure for exposure time of 72 h cold storage period. For *S. oryzae*, the mortality was 32.06 % and 39.19 % at 100 W power level, respectively, for exposure time 10 min continuously and for exposure times of 48 and 72 h cold storage period. When the irradiation exposure was changed to alternativ**es**, the mortality increased to 42.37% and 68.46 % respectively. The effect of irradiation exposure and exposure times of cold storage on mortality was the same for *S. oryzae* as for *o. surinamensis*. Results of ANOVA showed that mortality of *S. oryzae* differed significantly with exposure irradiation and exposure times of cold storage ($F = 159.484$; $df = 3$; $P < 0.05$). Tukey's test shows that mortality of *S. oryzae* was significantly higher with intermittent microwave exposure for exposure time of 72 h cold storage period.

Table 1: Mortality (Mean ± SE) of insects exposed to microwave radiation and cold storage.

Insect	Power (W)	Irradiation			
		Continuously		Intermitenttly	
		10 min		5-5 min	
		Exposure times of cold storage (h)		Exposure times of cold storage (h)	
		48	72	48	72
O. surinamensis	100	37.94±1.24[d]	45.98 ±1.02[b]	49.85±0.97[b]	71.98±1.21[a]
S. oryzae	100	32.06±1.12[c]	39.19±1.14[b]	42.37±1.03[b]	68.46±1.63[a]

Means within column with similar letter(s) are not significantly different ($P \geq 0.05$) according to Tukey's test. Data were transformed using square root prior to analysis.

The t-test showed a significant difference between the mortality rates of insects for continuously and intermittent irradiation treatment Table 2.

Table 2: T value and relevant statistics of insects exposed to microwave radiation and cold storage period

Insect	Sources	df	T-value	Sig.
O. surinamensis	Continuously[a] and Intermitenttly[b] irradiation×48 h cold storage	22	-7.6525	0.00
	Continuously and Intermitenttly irradiation×72 h cold storage	22	-17.7353	0.00
S. oryzae	Continuously and Intermitenttly irradiation×48 h cold storage	22	-6.7737	0.00
	Continuously and Intermetenttly irradiation×72 h cold storage	22	-16.7219	0.00

[a]: 10 min, [b]: 5-5 min

The t-test revealed a significant difference between mortality rate of the insects after 48 and 72 h cold storage periods (Table 3).

Comparing the susceptibility of adult insects of *O. surinsmensis* and *S. oryzae*, however, there was no significant difference between the mortality of these two species at exposure to intermittent irradiation, for an exposure time of 72 h cold storage period.

Table 3: T value and Sig. of insects exposed to microwave radiation and cold storage period

Insect	Sources	df	T-value	Sig.
O. surinamensis	Continuously[a] irradiation×48 and 72 h cold storage	22	-5.0218	0.00
	Intermitenttly[b] irradiation×48 and 72 h cold storage	22	-15.2836	0.00
S. oryzae	Continuously irradiation×48 and 72 h cold storage	22	-4.4272	0.00
	Intermitenttly irradiation×48 and 72 h cold storage	22	-15.3545	0.00

[a]: 10 min, [b]: 5-5 min

The t-test showed significantly higher (P < 0.01) mortality of *S. oryzae* then that of *O. surinsmensis* (Table 4). This may be due to the larger size of *O. surinamensis* which may favor a high probability of direct microwave absorption.

Table 4: T value and Sig. of insects exposed to microwave radiation and cold storage.

Insect	Power (W)	Irradiation							
		Continuously				Intermitenttly			
		10 min				5-5 min			
		Exposure times of cold storage (h)				Exposure times of cold storage (h)			
		48		72		48		72	
		t value	Sig.	t value	Sig.	t value	Sig.	t value	Sig.
O. surinamensis and *S. oryzae*	100	-3.50	0.002	-4.42	0.000	-5.287	0.000	-1.82	0.082

Germination tests: The germination rate of wheat after exposure to microwave radiation is shown in Table 5.

Means within column with similar letter(s) are not significantly different ($P \geq 0.05$) according to Tukey's test.

The standard error from four replicates of 100 seeds each was less than 7% of the mean value in all cases. Times ranging from 4 to 20 min significantly decreased the germination potential in comparison with unirradiated seed. Hence, we can conclude that with increasing exposure time, the germination of the seeds was lowered significantly. The decrease in germination at exposure time was due to the increase in temperature of the sample. High temperature affects the germination capacity of the seed. Results of ANOVA showed that germination differed significantly with exposure time (F = 09/4586; df = 8; $P < 0.05$).

Table 5: Percentage germinability (Mean ± SE) of wheat radiated with microwave energy at different times

Time (min)	Viability Germination rate (%)
0	9.85±.02 a
2	9.85±0.03a
4	9.77±0.02 ab
6	9.73±0.06 abc
10	9.70±0.07 abc
12	9.69±0.06 abc
14	9.60±0.05 bc
16	9.54±0.03 c
20	0.35±0.02 d

DISCUSSION

The insect pests of stored grain very often enter a bin after it is filled and cause numerous quality and health issues. Because of this, International organizations such as FAD (1997) and FGIS (1999) set tolerances and grade standards regulating the number of insects and insects fragments above specified tolerances which make the product illegal for human consumption. The *O. surinsmensis* and *S. oryzae* are one of the cosmopolitan and destructive invaders of foodstuff. Control of stored-products pest insects is essential wherever foodstuff 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 use of fewer hazardous substances or control methods, which are more environment compatible. 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, microwave radiation was lethal to test insects. The mechanisms involved in the lethal action of microwave 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 microwave 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 methods of pest control measures, such as microwave 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). Our results showed the use of intermittent power to be more effective in killing insects than continuous irradiation. The results were in agreement with the findings of Shayesteh and Barthakur (1996), who studied the effects of microwave radiation on mortality of *T. confusum* and *plodia interpuctella*. Almost in all trials, there was sufficient indication that microwaves energy exposure and longer cold storage duration could achieve better kill than shorter of similar cold storage period . These results were in agreement with the findings of Ikediala (1999), who studied the combined effects of microwaves power and cold storage on mortality of *Cydia pomonella*.

There is a general aggreement that a control agent must kill the target insect with an acceptable level of the agent in a short period of time. Since, microwave 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 the immediate future, combined application of microwave power with cold storage treatment could be considered as a potential measure which helps to reduce stored-products insect populations in a given control program.

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