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EFFECT OF MICROWAVE RADIATION AND COLD STORAGE ON *TRIBOLIUM CASTANEUM* HERBST (COLEOPTERA: TENEBRIONIDAE) AND *SITOPHILUS ORYZAE* L. (COLEOPTERA: CURCULIONIDAE)

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Abstract: The combined impact of microwave radiation and cold storage on *Tribolium castaneum* Herbst and *Sitophilus oryzae* L. adults either continuously or intermittently was evaluated. The insects were exposed to 2 450 MHz at power level of 100 W for exposure time 10 min, continuously and intermittently. In all experiments, the highest rate of mortality was achieved for exposure time 10 min intermittently 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 *T. castaneum* and *S. oryzae* adult developmental stage. This treatment could provide an effective and friendly environmental treatment technique in Integrated Pest Management (IPM) program.

Key words: microwave irradiation, temperature manipulation, stored products pests

INTRODUCTION

Control of stored products pests was 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 (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 America and 30% in Africa and Asia, but higher losses and contamination often occur locally (Hill 1990).

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 was frequent failure to control insects and certain insects developed resistance to phosphine (Bell and Wilson 1995). Moreover, concerns about a further development of resistance to phosphine contributed to the search for new alternative 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 most importance in store 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 microwave energy and temperature manipulation were used alone for treatment earlier (Johnson *et al.* 2003; Wang *et al.* 2003).

Microwave energy is not persistent in the environment and does not hazardous impacts or damage to foodstuff and hence there are no adverse effects on human beings (Warchalewski *et al.* 2000; Vadivambal *et al.* 2007). Exposure to microwave energy could cause physical injuries and reduce reproduction rates in surviving insects. For instance, treated larvae may develop into adults with deformed or missing legs, 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 1999). The penetration depth is an important factor, as the microwave's intensity diminishes with increased penetration. With retrospect, due to 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 conditions.

Insects under microwave irradiation are prone to some types of stress such as controlled atmosphere and cold (Wang and Tang 2001).The warehouse environment is usually one that is enclosed, allowing for the manipu-

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lation the 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 can slow or stop insect activity development and depending on the species, and is capable of causing death (Field 1992). Exposure to temperature above 55°C for short periods of time caused 100 per cent mortality (Zhao 2007).

A review of the literature revealed the scarcity of information on optimal levels of microwave radiation combined with cold storage period in insects killing programs. The objectives of this research were as follows:

1 – to determine a combined effect of microwave radiation and cold storage on *T. castaneum* and *S. oryzae* adults
2 – to evaluate the impact of microwave power on wheat germination.

MATERIALS AND METHODS

Test insects

T. castaneum and *S. 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 chosen due to their economic importance throughout the world including Iran. Stock cultures were established and maintained on heat-sterilized wheat flour and rice at 27±2°C, 65±5% relative humidity (RH) and 14 h photoperiod were reforded 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 their proper development. Insects were reared for two generations before initiation the experiments.

Preparation of insects for experiments

Before each treatment, using a fine sable brush mixed sex of 1–7 days old adult insects were counted out in 20 Petri dishes containing 10 g of rearing medium.

Bioassay

The combined bioassay experiments using microwave power and cold storage duration were conducted. The experiment units and bioassay procedures were identical in all trials. In the 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 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, 2 450 MHz microwave oven (LG, BC320 W) with capability of producing 100 to 1 000 W microwave power. For microwave radiation one power output of generator was set at 0 and 100 W. The exposure time was 10 min continuously and intermitenttly (5 min under radiation, 2 min rest and out of the oven and under radiation for 5 min again). At termination of the treatment, the samples along with their respective control were maintained under cold storage conditions (6±1°C) for 0, 48 and 72 h. In each trial, the control Petri dish was treated identically except microwave irradiation and cold storage treatment was employed. At the termination of cold storage period, insects were allowed to recover and were 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 one hundred treated seeds were soaked in 100 ml of distilled water for 10 min. Pretreated seeds were spaced uniformly on a sheet of 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 in four different days. The number of germinated seeds was counted after 2, 4, 6, 8 and 10 days.

Statical analysis

Analysis of variance was used to check the significance of differences between mortality of insects at different irradiation exposure, cold storage and exposure time, and also differences in the mortality of different insect species. In bioassay and germination tests, the data were normalized by 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 various treatments at $\alpha = 0.05$ level. Student's t-test was used for comparing the means of two groups.

RESULTS

Present results show that in all experiments microwave power had lethal effects to the tested insects. The mortality percentages for *T. castaneum* and *S. oryzae* at continuous irradiation and alternative radiation for exposure times of 48 and 72 h cold storage period is shown in table 1. At 100 W power level for exposure time of 10 min continuously, mortality of *T. castaneum* was 25.86% and 31.94% respectively, for exposure times of 48 and 72 h cold storage period. As irradiation exposure was changed alternatively (5.5 min), mortality was increased to 37.95% and 65.22%. Results of ANOVA showed that mortality varied significantly with irradiation exposure and exposure times of cold storage ($F = 909/117$; $df = 3$; $p < 0.05$). Tukey's test shows that mortality of *T. castaneum* was significantly higher at intermittent exposure for exposure time of 72 h of cold storage period. For *S. oryzae*, mortality was 32.06% and 39.19% at 100 W power level, respectively, for continuous exposure time 10 min and for exposure times of 48 and 72 h cold storage period. When the irradiation exposure was changed to alternatively, mor-

Table 1. Mortality (mean ±SE) of insects exposed to microwave irradiation and cold storage

Insect	Power [W]	Irradiation			
		continuously		intermitently	
		10 min		5–5 min	
		exposure times of cold storage [h]		exposure times of cold storage [h]	
		48	72	48	72
T. castane	100	25.86±1.65 c	31.94±.59 b	37.95±1.28 b	65.22±1.81 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 the same letter(s) are not significantly different (p ≥ 0.05) according to Tukey's test. Data were transformed using square root prior to analysis

Table 2. T-value and significance of insects exposed to microwave irradiation and cold storage period

Insect	Sources	df	T-value	Significance
T. castaneum	continuously[a] and intermitenttly[b] irradiation x 48 h cold storage	22	–5.8513	0.00
	continuously and intermitenttly irradiation x 72 h cold storage	22	–15.6483	0.00
S. oryzae	continuously and intermitenttly irradiation x 48 h cold storage	22	–6.7737	0.00
	continuously and intermetenttl*y* irradiation x 72 h cold storage	22	–16.7219	0.00

[a] : 10 min, [b] : 5–5 min

Table 3. T-value and significance of insects exposed to microwave radiation and cold storage period

Insect	Sources	df	T-value	Significance
T. castaneum	continuous irradiation x 48 and 72 h cold storage	22	–2.6048	0.00
	intermitent[b] irradiation x 48 and 72 h cold storage	22	–13.7335	0.00
S. oryzae	continuous irradiation x 48 and 72 h cold storage	22	–4.4272	0.00
	intermitent irradiation x 48 and 72 h cold storage	22	–15.3545	0.00

[a] : 10 min, [b] : 5–5 min

tality was 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 *T. castaneum*. Results of ANOVA showed that mortality of *S. oryzae* differed significantly with irradiation exposure and exposure times of cold storage (F = 484/159; df = 3; $p < 0.05$). Tukey's test shows that mortality of *S. oryzae* was significantly higher at intermittent exposure for exposure time of 72 h cold storage period.

The t-test showed significant difference between mortality of insects for continuous and alternative irradiation (Table 2). Likewise, 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 *T. castaneum* and *S. oryzae* however, there was no significant difference between mortality of these two species at exposure to intermittent irradiation, for exposure time 72 h cold storage period but t-test showed significantly higher ($p < 0.01$) mortality of *S. oryzae* then that of *T. castaneum* (Table 4). This may be due to the larger size of *T. castaneum*, which may favour a high probability of direct microwave absorption.

Germination tests

The germination rate of wheat after exposure to microwave irradiation is shown in table 5. 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 non-irradiated seeds. Hence, we can conclude that with increasing exposure time, germination of the seeds was lowered significantly. The decrease in germination at exposure time was due to the increase in temperature of a sample. High temperature affects the germination capacity of the seeds. Results of ANOVA showed that germination differed significantly with exposure time (F = 09/4586; df = 8; $p < 0.05$).

Table 4. T-value and significance of insects exposed to microwave irradiation and cold storage

Insect	Power [W]	Irradiation							
		continuously				intermitently			
		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.
T. castane and *S. oryzae*	100	–3.046	0.006	–3.694	0.001	–2.665	0.014	–1.377	0.182

sig. – significance

Table 5. Percentage germinability (mean±SE) of wheat irradiation with microwave energy for different times

Time [min]	Viability
	germination rate [%]
0	9.85±02 a
2	9.85±0.03 a
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

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

DISCUSSION

Insects within stored foodstuffs cause much harm to their quality and health issues. Because of this, International organizations such as FAD (1997) and FGIS (1999) set up tolerances and grade standards regulating the number of insects and insects fragments above specified values making the product illegal for human consumption. *T. castaneum* and *S. oryzae* are ones 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 insects 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 of use to minimize hazardous substances or to improve-control methods, which are more compatible with environment. A method for the control strategies that are environmentally sustainable and avoids the use of conventional pesticides is of paramount important. Disinfestation of stored products with physical control methods such as using microwave 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 irradiation was lethal to the test insect. Mechanisms involved in the lethal action of microwave irradiation were previously understood. Hazardous impacts could be due to a high oscillation frequency of water molecules in the body of insects. Microwave irradiation has deleterious effects on insects such as reduction of reproductive rate, losing body weight and malformation as well (Nelson 1996). However, the application of microwave irradiation in insect killing programs could be limited due to insufficient penetration depth. Zhu *et al.* (1995) reported that microwaves attenuate exponentially 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 cold storage period. Similar results were reported for other insects (Johnson *et al.* 1997; Özder 2004; Larentzaki *et al.* 2007).

A major advantage of cold storage is that it can easily be coupled with other methods of pest control measures, such as microwave irradiation. In general, a 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. From these point of view results were in agreement with 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 microwave energy exposure and longer cold storage duration could achieve better killing than shorter of similar cold storage periods. These results were in agreement with the findings of Ikediala (1999), who studied combined effects of microwave power and cold storage on mortality of *Cydia pomonella*.

It is well established 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 the use as a fumigant in 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 IPM programs.

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REFERENCES

Ayvaz A., Karabörklü S. 2008. Effect of cold storage and different diets on *Ephestia kuehniella* Zeller (Lep. Pyralidae). J. Pest Sci. 81: 57–62.

Bell C.H., Wilson S.M. 1995. Phosphine tolerance and resistance in *Trogoderma granarium* Everts (Coleoptera: Dermestidae). J. Stored Products Res. 31: 199–205.

Collins P.J., Daglish G.H., Bengston M., Lambkin T.M., Pavic H. 2002. Genetics of resistance to phosphine in *Rhyzopertha dominica*. J. Econ. Entomol. 95: 862–869.

Federal Grain Inspection Service. 1999. Official United States standards for grain subpart m: United States standards for wheat. www.usda.gov/gipsa/reference- library/standards/810101. pdf

Field P.G. 1992. The control of stored-product insects and mites with extreme Temperatures. J. Stored Products Res. 28: 89–118.

FAD (Food and Drug Administration) 1997. Wheat flour-adulteration with insect fragments and rodent hairs (CPG 7104.06). Compliance Policy Guides Manual, Chapter 5: "Foods, Color and Cosmetics", Sub Chapter 578-Processed Grain. http://www.fda.gov/ora/compliaance_ref/cpg/cpgfod/cpg578-450. htm

Halverson S.L., Phillips T.W., Bigelow T.S., Mbata G.N., Payton M.E. 1999. The control of various species of stored-product insects with EHF energy. Available in: http://www.epa.gov/spdpublc/mbr/airc/1999/54 halver.pdf

Hill D.S. 1990. Pests of Stored Products and their Control. CRC Press, Boca Raton, FL, 274 pp.

Ikediala J.N., Tang J., Neven L.G., Drake S.R. 1999. Quarantine treatment of cherries using 915 MHz microwaves: temperature mapping, codling moth mortality and fruit quality. Postharvest Biol. Technol. 16: 127–137.

ISTA (International Seed Testing Association) 1999. International rules for seed testing. Annex to Chapter 5: "The Germination Test". Seed Sci. Technol. (Supplement) 27: 27–32.

Ja Hyun N.A., Ryoo M.L. 2000. The influence of temperature on development of *Sitophilus oryzae* (*Coleoptera: Curculionidae*) on dried vegetable commodities. J. Stored Products Res. 36: 125–129.

Johnson J.A., Valero K.A., Hannel M.M. 1997. Effect of low temperature storage on survival and reproduction of Indian meal moth (*Lepidoptera: Pyralidae*). Crop Protect. 16: 519–523.

Johnson J.A., Wang S., Tang J. 2003. Thermal death kinetics of fifth instar *Plodia interpunctella* (*Lepidoptera: Pyraidae*). J. Econ. Entomol. 96: 519–524.

Larentzaki E., Powell G., Copland M.J.W. 2007. Effects of cold storage on survival, reproduction and development of adults and eggs of *Franklinothrips vespiformis* (Crawford). Biol. Control 43: 265–270.

Leesch J.G., Knapp G.F., Mackey B.E. 2000. Methyl bromide adsorption on activated carbon to control emissions from commodity fumigations. J. Stored Products Res. 36: 65–74.

Nelson S.O. 1996. Review and assessment of radio-frequency and microwaves energy for stored-grain insect control. Trans. ASAE 39 (4): 1475–1484.

Özder N. 2004. Effect of different cold storage periods on parasitization performance of *Trichogramma cacoeciae* (Hymenoptera: Trichogrammatidae) on eggs of *Ephestia kuehniella* (Lepidoptera: Pyralidae). Biocontrol Sci. Technol. 14: 441–447.

Rajendran S., Muralidharan N. 2001. Performance of phosphine fumigation of bagged paddy rice in indoor and outdoor stores. J. Stored Products Res. 37: 351–358.

Shayesteh N., Barthakur N.N. 1996. Mortality and behavior of two stored-product insect species during microwave irradiation. J. Stored Products Res. 32: 239–246.

Vadivambal R., Jayas D.S., White N.D.G. 207. Wheat disinfestations using microwave energy. J. Stored Products Res. 43: 508–514.

Wang S., Tang J. 2001. Radio frequency and microwaves alternative treatments for insect control in nuts. J. Agric. Eng. 10: 105–120.

Wang S., Tang J., Johnson J.A., Hassan E., Hallman G., Drake S.R., Wang Y. 2003. Dielectric properties of fruits and insect pests as related to radio frequency and microwave treatments. Biosyst. Eng. 85: 201–212.

Warchalewski J.R., Pradzynska A., Gralik J., Nawrot J. 2000. The effect of gamma and microwave irradiation of wheat grain on development parameters of some stored grain pests. Nahrung 44: 4111–414.

Zhao S., Qiu Ch., Xiong Sh., Cheng X. 2007. A thermal lethal model of rice weevils subjected to microwave irradiation. J. Stored Products Res. 43: 430–434.

Zhu S., Zhang Y., Marchant T.R. 1995. ADRBEM model for microwave heating problems. Appl. Math. Modeling 19: 287–304.

POLISH SUMMARY

EFEKT NAŚWIETLANIA MIKROFALAMI I WARUNKÓW ZIMNEJ PRZECHOWALNI NA *TRIBOLIUM CASTANEUM* HERBST. (COLEOPTERA: TENEBRIONIDAE) I *SITOPHILUS ORYZAE* L. (COLEOPTERA: CURCULIONIDAE)

Oceniano łączne działanie naświetlania mikrofalami i zimnej przechowalni, stosowane w trybie ciągłym lub z przerwami, na *T. castaneum* Herbst. i na dorosłe osobniki *S. oryzae* L. Owady były eksponowane na 2 450 MHz, przy poziomie mocy 100 W i czasie 10 minut. We wszystkich doświadczeniach najwyższe tempo śmiertelności uzyskano dla czasu 10-minutowej ekspozycji przerywanej i 72 godzin zimnego przechowywania. W porównaniu do ciągłego naświetlania, przerywana ekspozycja była bardziej efektywna w zabijaniu owadów obydwóch gatunków. Kombinacje naświetlania mikrofalami i zimnej przechowalni były kompatybilne i synergistyczne. Synergistyczne współdziałanie wskazuje, że naświetlanie mikrofalami może być wykorzystane łącznie z zimnym przechowywaniem, w celu zwalczania dorosłych osobników *T. castaneum* i *S. oryzae.* Ten zabieg może być efektywną i przyjazną środowisku techniką w programie integrowanej ochrony roślin.