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Disinfestation of life stages of *Tribolium castaneum* in wheat using microwave energy

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Abstract Insecticide residues have adverse effects on humans, and insects develop resistance to insecticides. Hence there is a need for an alternate method for disinfestation of grain. Disinfestation of grains using microwaves can be an alternate method to insecticides for killing insects. A method to control *Tribolium castaneum* (Herbst) in wheat using microwave energy is described in this manuscript. An industrial microwave system operating at 2.45 GHz was used to determine the mortality of life stages of *T. castaneum*. Wheat samples of 50 g each at 14, 16, and 18% moisture content (wet basis) were infested with 5 and 10 insects. The infested samples were then exposed to microwaves at four different power levels of 250, 300, 400, and 500 W for two exposure times of 28 and 56 s. One hundred percent mortality was achieved at 400 W for eggs and for larvae, pupae and adult at 500 W. The mortality rates were lower at the lower power levels and shorter exposure time. Among the life stages, eggs were most susceptible followed by larvae, adult and pupae. Germination of wheat kernels was lowered after treatment with microwave energy.
Key words: disinfestation, stored-grain insects, wheat, *Tribolium castaneum*, microwaves, mortality.

INTRODUCTION

Canada is the largest producer of high protein milling wheat in the world although it is only the seventh largest wheat producing country (Agriculture and Agri-Food Canada 2004). Maintaining quality and quantity are the main criteria for safe storage. Canada has a zero tolerance for insects in stored-grain for human consumption (Canada Grain Act 1975). 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). Since losses of grain due to insect infestation are very high, disinfestation of grain is important for its safe storage.

The various methods of insect control can be grouped as physical, biological or chemical methods. Physical treatments include different types of traps, temperature control, mechanical impact, physical removal, abrasive and inert dusts, and ionizing radiation (Sinha and Watters 1985; Muir and Fields 2001). Microwave disinfestation is a physical method to control insects in grain. The biological method is to use living beneficial organisms as natural enemies, to control pests but they are not suitable for dealing with heavy infestations (Subramanyam and Hagstrum 2000). The chemical method uses insecticides to kill the insects. For the past several decades, efforts have been devoted to the study of possible alternative insect control methods that might be helpful in minimizing the environmental hazards associated with chemical insecticides and residues in food (Nelson and Stetson 1974).

Tribolium castaneum (Herbst) is a secondary grain feeder and feeds on grain germ, broken kernels, grain products, and grain flour (Lhaloui et al. 1988). It is found across Canada, mainly in bins where grain is stored for long periods, such as farm silos and country elevators. It prefers damaged grain, but will attack whole wheat, feeding first on the germ and then on the endosperm. The red flour beetle lays eggs in the grain bulk and it spends its entire life cycle outside the grain kernels. Each female lays 300 to 400 eggs and egg-laying occurs when the temperature is over 20^{o}C. Development from egg to adult takes 15 to 20 d under optimum conditions (35^{o}C and r.h between 70 and 90%). The red flour beetle will fly when the temperature is 25^{o}C or higher, so the infestations can spread quickly (Agriculture Canada 1981 Sheet No.75).

Microwaves can be used to control insects in stored cereals and cereal products. Microwaves are electromagnetic waves with frequencies ranging from about 300 MHz to 300 GHz and corresponding wavelengths from 1 to 0.001 m (Decareau 1985). Microwave heating is based on the transformation of alternating electromagnetic field energy into thermal energy by affecting polar molecules of a material (Brygidyr 1976).

The use of microwaves to kill insects is based on the dielectric heating effect produced in grain which is a relatively poor conductor of electricity. Since this heating depends upon the electrical properties of the material, there is a possibility of advantageous selective heating in mixtures of different substances (Hamid et al. 1968). In a mixture of dry food stuffs and insects, it is possible to heat the insects to a lethal temperature because they have high moisture content while leaving the drier foodstuff unaffected or slightly warm (Hurlock et al. 1979). The major advantage of using microwave energy is that no chemical residues are left in the food and hence no adverse effects on human beings (Hurlock et al. 1979). Microwave energy has no adverse effect on the environment as do chemicals. Insects are unlikely to develop resistance to this treatment (Watters 1976). High frequency radiation may not only kill insects by the dielectric heat induced within them but may also affect the reproduction of the survivors (Hamid et al. 1968).

The objectives of this research were:

1. To determine the mortality of egg, larval, pupal and adult stages of *Tribolium castaneum*, in 50 g wheat at three different moisture content (14,16, and 18%) four different power levels (250, 300, 400, and 500 W), and at two exposure times (28 and 56 s)
2. To conduct a germination test on the wheat subjected to microwave energy.

MATERIALS AND METHODS

Grain samples

The CWHRS (Canada Western Hard Red Spring) wheat (*Triticum aestivum L.*) was selected for the experimental study due its excellent milling and baking qualities with minimal protein loss during milling. Initial moisture content was determined by drying 10 g of unground grain at 130 ± 2^{o}C for 19 h (ASAE 2001) and was expressed on a wet mass basis. After determining the initial moisture content of the sample (11%), the grain was then conditioned to 14, 16 and 18% by adding a calculated quantity of distilled water and rotating the grain mixture for about 1 h. The moisturized grain was then kept in air tight plastic bags until used for the experiments.

Tribolium castaneum

The *T. castaneum* were obtained from Landmark (MB) in 1998, and grown in the laboratory on whole wheat flour and brewers yeast at 30^{o}C and 70% r.h.

Industrial microwave dryer

All the experiments were conducted in an Industrial microwave dryer (Model No: P24YKA03, Industrial Microwave Systems, Morrisville, NC). The microwave dryer consists of a belt assembly, an applicator, fan and heater assembly and control panel. The maximum speed of the conveyor is 3 m/min. The power output of the generator is adjustable from 0 – 2000 W. A plastic, microwavable rectangular box 30 cm x 3 cm x 1 cm was made to hold a 50 g sample of wheat. All the experiments were conducted by placing the sample in this box and allowing it to pass on the conveyor belt.

Experimental design

The experiments were conducted with wheat samples at 14, 16 and 18% moisture content and at two infestation levels of 5 and 10 insects per 50 g of sample. The experiments were carried out at two different exposure times. At the maximum speed (3 m/min) of the conveyor it takes 28 s for the sample to pass the applicator and at the speed of 1.5 m/min the sample is exposed to microwave energy for 56 s. The power is adjustable and the various power levels selected for the study were 250, 300, 400 and 500 W.

Determination of mortality

Fifty grams of sample were placed in the box and adult insects were added to the sample. The conveyor was switched on and ensured that it was running at its maximum speed. The power was adjusted to the desired level. The grain, along with the insects, was then kept on the conveyor belt and the sample was subjected to microwave energy. When the box came out of the conveyor it was gently taken out and the sample was spread on a sheet of paper. The numbers of alive and dead insects were counted. The adult insects were considered dead if they failed to respond to gentle rubbing with a small brush. The sample was allowed to cool and the insects were checked for mortality again after 15 min. When the number of insects observed was not 100%, the same experiment was repeated until 100% recovery was achieved. Three replicates were done for all the mortality experiments.

To determine the mortality of the egg stage, the grain along with the eggs were treated with microwave energy and then returned to favorable environment at 35^{o}C and 70% relative humidity, where the eggs can develop into larvae. After about a week, the numbers of larvae that emerged from the eggs were counted. For the larval stage, the experiments were conducted in the same way as for the adult insects. The pupae were subjected to microwave energy and returned to a favorable environment (35^{o}C and 70% r.h). After one week the total number of adults that emerged from pupae was counted.

Determination of germination

Germination of the wheat seeds subjected to different levels of microwave power was assessed by plating 25 seeds on Whatman no. 3 filter paper in a 9-cm diameter Petri-dish saturated with 5.5 mL of distilled water (Wallace and Sinha 1962). The plates were placed in a plastic bag to prevent desiccation of the filter paper and kept at 25^{o}C for 7 d. On the seventh day the germinated seeds were counted and the germination percentage was calculated.

Statistical analysis

Analysis of variance ($p < 0.05$) was done to check the significance between the mortality of life stages of insects at different grain moisture contents, microwave power levels and exposure times (SAS version 9.1, Statistical Analysis Systems, Cary, NC).

RESULTS AND DISCUSSION

Mortality of *Tribolium castaneum* eggs

The mortality percentages for *Tribolium castaneum* eggs at various power levels and for 14, 16 and 18% m.c. wheat are shown in Table 1. At a power of 250 W and an exposure time of 28 s, the mortality was 64, 58 and 68 % for 14, 16, and 18% m.c. wheat respectively. At 300W and 28 s, the mortality increased to 81, 85, and 88% for 14, 16, and 18% m.c. wheat respectively. One hundred percent mortality was achieved at 400 W for both the exposure times. Analysis of Variance showed that there was no significant difference in the mortality of eggs of *T. castaneum* at different moisture contents. But the mortality was significantly higher at higher power levels and greater exposure times.

Mortality of *Tribolium castaneum* larvae

The mortality percentage for *T. castaneum* larvae at 14, 16 and 18% moisture content wheat is shown in Table 2. The mortality increased as power level and exposure times were increased. One hundred percent mortality was achieved at 500 W for an exposure time of 28 s and at 400 W for an exposure time of 56 s, similar to the adult insects. Analysis of Variance showed that mortality of larvae was not significantly different at 14 and 16% m.c. wheat and at 16 and 18% m.c. wheat but there was a significant difference between 14 and 18% m.c. wheat. Analysis of variance also showed that mortality of larvae is significantly higher than pupae and adults.

Table 1. Mortality (mean ± standard error) of *Tribolium castaneum* eggs exposed to microwave radiation in wheat at 14, 16 and 18% moisture contents

Power, W	Moisture content, %					
	14		16		18	
	Exposure time, s		Exposure time, s		Exposure time, s	
	28	56	28	56	28	56
250	64 ±12.2	84±9.0	58±13.8	84±9.2	68±16.5	88±6.5
300	81±6.2	93±6.3	85±8.1	100	88±7.0	96±7.2
400	100	100	100	100	100	100

Since 100% mortality was achieved at 400 W, experiments were not performed at 500 W for both exposure times.

Table 2. Mortality (mean ± standard error) of *Tribolium castaneum* larvae exposed to microwave radiation in wheat at 14, 16 and 18% moisture contents

Power, W	Moisture content, %					
	14		16		18	
	Exposure time, s		Exposure time, s		Exposure time, s	
	28	56	28	56	28	56
250	53±3.6	79±2.2	61±5.1	77±4.2	63±0.57	78±1.0
300	72±4.4	93±1.3	74±5.6	95±.7.0	78±4.04	96±1.7
400	91±4.6	100	93±6.3	100	95±3.7	100
500	100	*	100	*	100	*

* Since 100% mortality was achieved at 28 s exposure time, experiments were not performed at 56 s exposure time.

Mortality of *Tribolium castaneum* pupae

The mortality percentage for *T. castaneum* pupae at 14, 16 and 18% moisture content wheat are shown in Table 3. At a power level of 250 W and an exposure time of 28 s, the mortality percentage for *T. castaneum* pupae was 43, 44 and 59% and at an exposure time of 56 s, mortality was 74, 78 and 78% respectively for 14, 16 and 18% m.c. wheat. Analysis of variance shows that mortality of pupae is significantly different at 14, 16 and 18% m.c. wheat.

Table 3. Mortality (mean ± standard error) of *Tribolium castaneum* pupae exposed to microwave radiation in wheat at 14, 16 and 18% moisture contents

Power, W	Moisture content, %					
	14		16		18	
	Exposure time, s		Exposure time, s		Exposure time, s	
	28	56	28	56	28	56
250	43±1.1	74±3.4	44±1.7	78±5.1	59±4.7	78±4.7
300	55±9.4	86±2.3	67±1.7	94±2.8	72±2.5	91±0.5
400	76±3.0	100	78±4.5	100	87±3.6	100
500	100	*	100	*	100	*

* Since 100% mortality was achieved at 28 s exposure time, experiments were not performed at 56 s exposure time.

Mortality of *Tribolium castaneum* adult

The mortality percentages for adult *Tribolium castaneum* at various power levels and for 14, 16 and 18% m.c. wheat are shown in Table 4. At a power level of 250 W and an exposure time of 28 s, the mortality percentage for adult *T. castaneum* was 45% at a moisture content of 14%. As the power was increased to 300, 400 and 500 W, the mortality also increased to 58, 85 and 100%, respectively. At the same power level, higher mortality was obtained for longer exposure times. As exposure time was increased, higher mortality was achieved at lower power levels. For example, at 500 W, 100% mortality was obtained at an exposure time of 28 s. When the exposure time was increased to 56 s, 100% mortality was obtained at a power of 400 W.

Results of ANOVA showed that mortality of *T. castaneum* was significantly higher in 16% m.c. wheat than in 14% m.c. wheat ($p<0.05$) whereas there was no significant difference in the mortality between 16 and 18% m.c. wheat. The mortality was significantly different at different power levels and exposure times. The mortality increased with an increase in either power level or exposure time or both.

Comparing the mortality of life stages of *T. castaneum*, the eggs were most susceptible followed by larvae, adult and pupae. This result differs from the results of Mahroof et al. (2003a, 2003b). Mahroof et al. (2003a) reported that during heat treatment of mills using gas heaters to 50-60^{o} C, old instars and pupae appeared relatively heat tolerant compared to other life stages. Mahroof et al. (2003 b) conducted experiments to study time-mortality relationships for life stages of *T. castaneum* exposed to elevated temperature of 50-60^{o}C. They concluded that young larvae were the most heat–tolerant stage. Hamid and Boulanger (1969) concluded that the mortality of larvae was the same as the adult *T. confusum* at different temperatures.

Table 4. Mortality (mean ± standard error) of *Tribolium castaneum* adults exposed to microwave radiation in wheat at 14, 16 and 18% moisture contents

Power, W	Moisture content, %					
	14		16		18	
	Exposure time, s		Exposure time, s		Exposure time, s	
	28	56	28	56	28	56
250	45±11.6	77±2.9	56±2.9	81±4.9	55±11.2	73±9.1
300	58±1.1	90±1.7	68±7.2	95±4.2	66±6.1	93±3.9
400	85±5.0	100	86±2.5	100	90±2.3	100
500	100	*	100	*	100	*

* Since 100% mortality was achieved at 28 s exposure time, experiments were not performed at 56 s exposure time.

Germination

The results of the germination test conducted for 14, 16 and 18% moisture content wheat at various power levels and at exposure times of 28 and 56 s are shown in Figs. 1 and 2, respectively. At 250 W, the seed germination percentage was 81, 77 and 81% at 14, 16 and 18% m.c. wheat respectively. As the power was increased the germination percentage was lowered. At 500 W, the germination was zero for an exposure time of 56 s. The germination of the control sample was around 96-97%. As the power and exposure time were increased, the germination was lowered significantly. Results of ANOVA show that germination at 14 and 16% m.c. was significantly different and there was no significant difference in the germination between 16 and 18% m.c. wheat.

Campana et al. (1993) studied the physical, chemical and baking properties of wheat dried with microwave energy. They concluded that germination capacity was affected by exposure to microwave energy. The decrease in germination capacity was related to the final temperature and the initial moisture content of the grains. Bhaskara et al. (1998) studied the effect of microwave treatment on quality of wheat seeds infected with *Fusarium graminearum.* Their results showed that eradication of the pathogen increased with the total microwave energy, but the seed viability and seedling vigour decreased accordingly.

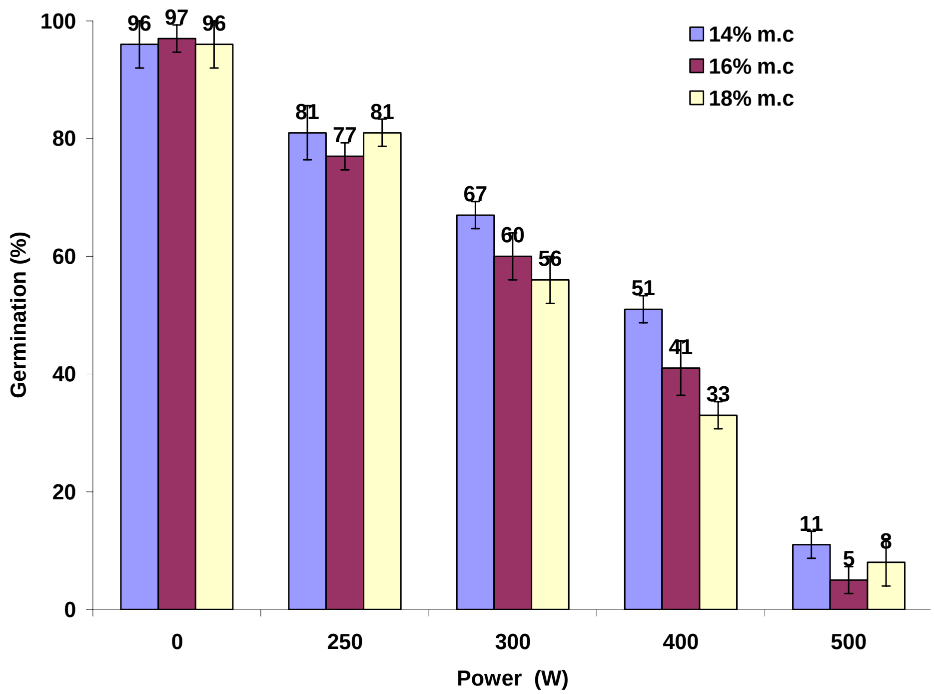

Fig 1: Germination of 14, 16 and 18% m.c wheat at an exposure time of 28 s for different power levels.

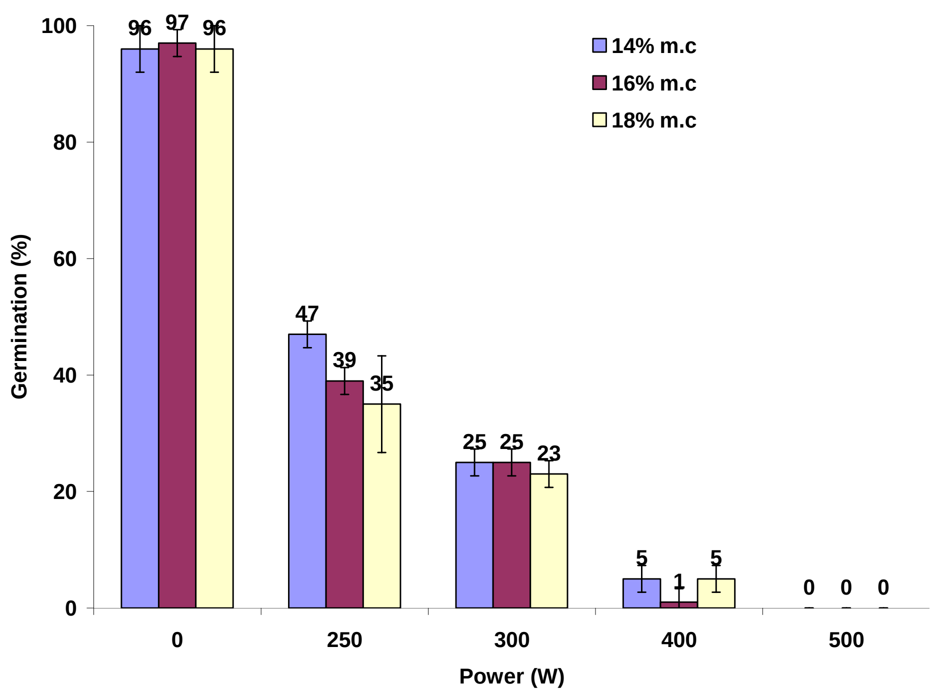

Fig 2: Germination of 14, 16 and 18% m.c. wheat at an exposure time of 56 s for different power levels.

Conclusions

For the egg stage, 100% mortality was achieved at 400 W for both exposure times and for larvae, pupae, and adult 100% mortality was achieved at 500 W for 28 s, and at 400 W for 56 s. There was no significant difference in the mortality of eggs and larvae for the 14, 16, and 18% m.c. wheat but for pupae and adults there was significant difference for different m.c. wheat. The *T. castaneum* eggs are most susceptible followed by larvae, adults and pupae. Germination was lowered as power level and exposure times were increased. One hundred percent mortality can be achieved using microwave energy but the major limitation is that the germination of seed is significantly affected.

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