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DETERMINATION OF MORTALITY OF LIFE STAGES OF *TRIBOLIUM CASTANEUM* IN RYE USING MICROWAVE ENERGY

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Abstract

Rye is the only cereal grain other than wheat to have the necessary qualities to make bread. Due to food safety issues and environmental concerns arising out of use of chemicals to control insects in grain, there is a need for an alternative method of killing insects. Disinfestation of grains using microwave energy could be an alternative to chemical methods. A pilot scale industrial microwave dryer operating at 2450 MHz was used to disinfest the life stages of *Tribolium castaneum* in rye. Rye was conditioned to 14, 16, and 18% moisture contents and 50 g samples were used for the experiments. Mortality experiments were performed at four power levels 200, 300, 400, and 500 W and at two exposure times of 28 and 56 s. The results of this study showed that *T. castaneum* eggs were the most susceptible and *T. castaneum* adults were the least susceptible to microwave energy. There was no significant difference between the mortality of life stages of *T. castaneum* in 14, 16, and 18% m.c. rye. Germination of rye was lowered after treatment with microwave energy.

Key words: microwave, disinfestation, mortality, rye, grain.

INTRODUCTION

Rye (*Secale cereale* L.), a member of grass family, is second to wheat among the grains most commonly used in the production of bread. Rye flour is inferior to wheat in production of high volume pan breads, because it lacks essential elasticity and gas-retention properties. Rye is also used in the production of alcoholic beverages and Rye is the acknowledged trademark of Canadian Whiskey (Bushuk 2001). Rye is extremely winter hardy and can grow in sandy soils with low fertility. It can be cultivated in areas that are generally not suitable for other cereal crops (Bushuk 2001). The world rye production for the year 2005-2006 was 14.5 million tonnes and Canada produced around 0.36 million tonnes (359,000 tonnes) of rye (USDA 2007). The European Union produces 25-30% of the global rye crop and the Russian Federation produces another 25-30% (Agriculture Statistics 2001).

As livestock feed, rye has a feeding value of about 85-90% of corn and has more digestible protein and nutrients than oats or barley (Oelke et al. 1990). Rye straw is fibrous and tough and hence it is not extensively used in livestock feed but usually used as livestock bedding (Bushuk 2001).

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). Post harvest losses of grains due to insects are estimated to be around 5 to 10% or about 1.4 to 2.8 billion dollars per year in 2006 (USDA 2006). Grains harvested and stored in the hottest part of the year have a great chance of becoming infested and hence wheat, rye, barley or oats are more likely to have insect problems than corn or soybeans which are harvested during the cooler months of the year (Chappell et al. 2000). Since losses of grain due to insect infestation can be very high, disinfestation of grain is very important for the safe storage of grain.

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 can attack whole wheat, feeding first on the germ and then on the endosperm. The red flour beetle lays eggs in the grain bulk and 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°C. Development from egg to adult takes 15 to 20 d under optimum conditions (35°C and RH between 70 and 90%). The red flour beetle will fly when the temperature is 25°C or higher, so the infestations can spread quickly (Agriculture Canada 1981).

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 (Mullin 1995).

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; Ikediala et al. 1999; Wang et al. 2003). 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; Ikediala et al. 1999; Wang et al. 2003). 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 rye samples at three different moisture content (14, 16, and 18%) four different power levels (200, 300, 400, and 500 W), and at two exposure times (28 and 56 s).
2. To conduct a germination test on the rye subjected to microwave energy.

MATERIALS AND METHODS

Grain samples

The rye (*Secale cereale* L.) cultivar ‘Musketeer’ was used for the experimental study. Initial moisture content was determined by drying 10 g of unground grain, in triplicate, at 130 ± 2°C for 16 h (ASABE 2006) in a hot air oven (Model No. 130D, Thelco Laboratory Oven, Winchester, VA) and was expressed on a wet mass basis. After determining the initial moisture content of the rye (11%), the grain was then conditioned to 14, 16, and 18% by adding the calculated quantity of distilled water and rotating for about 1 h. The samples were then kept in polythene bags and stored in a refrigerator for 72 h for uniform moisture distribution. The moisture content was then verified by drying 10 g of sample, in triplicate, and the moisturized grain was then kept in air-tight plastic bags until used for the experiments.

Tribolium castaneum

Tribolium castaneum were obtained from Landmark (MB) in 1998, and grown in the laboratory on whole wheat flour and brewers yeast at 30°C and 70% RH. To obtain different life stages, adults were mixed with wheat flour and brewers yeast and kept for different periods in environmental chambers (CONVIRON, Controlled Environments Limited, Winnipeg, MB) at 30°C and 70% RH.

Eggs were collected by sifting the flour after two days with a 425 μm aperture sieve and separated the eggs with a soft bristle brush using a Nikon SMZ 1000 microscope. The age of the eggs collected were between 0-48 h old. The larval period varies between 12-15.3 days at 30-37.5°C and 90% RH and the pupal period requires an additional 3.9-5.5 days at 30-37.5°C (Sinha and Watters 1985). Hence to obtain larval and pupal stage, adults mixed with wheat flour and brewers yeast were left for two and three weeks, respectively and larvae and pupae were collected manually using a soft bristle brush. The fifth instar larvae were collected for the experiments so that handling was easier compared to the smaller larvae.

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 was 3 m/min. The power output of the generator was adjustable from 0 – 2000 W. A microwavable rectangular box 30 cm x 3 cm x 1 cm was made to hold a 50 g sample of grain. 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 rye 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 took 28 s for the sample to pass the applicator and at the speed of 1.5 m/min the sample was exposed to microwave energy for 56 s. The power is adjustable and the various power levels selected for the study were 200, 300, 400, and 500 W.

Determination of mortality

Fifty grams of sample were placed in the sample holder and adults 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 live 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 recovered was not 100%, the same experiment was repeated until 100% recovery was achieved. A minimum of three replicates were used for all the mortality experiments. Larval mortality was determined in the same manner as the adults. Control mortality was determined by placing the insects in the sample box, which was allowed to pass on the conveyor at zero power.

To determine the mortality of the eggs and pupae, the grain along with the eggs and pupae, treated with microwave energy was mixed with wheat flour and brewer's yeast and then returned to favorable environment at 35^{o}C and 70% RH, for two weeks and ten days, respectively for the eggs and pupae to develop into larvae and adults. After ten days, the numbers of adults that emerged from pupae were counted and after two weeks, the numbers of larvae that emerged from the eggs were counted. Control mortality was determined by allowing the eggs and pupae to pass on the conveyor with zero power and returned to favorable environment. The mortalities of eggs and pupae were corrected for control mortality using Abbott's formula (Abbott 1925)

Mortality percent = $(x\text{-}y)/x * 100$

where x is the percent living in control and y is the percent living in treated sample.

Determination of germination

Germination of the rye 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°C for 7 d. On the seventh day the germinated seeds were counted and the germination percentage was calculated.

Statistical analysis

A factorial experiment design of three factors (moisture content, power level, exposure time) was performed. Three replications were done for mortality and germination experiments with control samples for all the experiments. 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 2002). Comparison of means and grouping was done using Scheffe's test ($P < 0.05$) and the results were tabulated.

RESULTS AND DISCUSSION

Mortality of *Tribolium castaneum* life stages

Mortality of *Tribolium castaneum* eggs. The mortality percentages for *T. castaneum* eggs, larvae, pupae and adults at four power levels, two exposure times and three moisture contents of rye are listed in Table 1. The control mortality of the eggs at 14% m.c. at 28 and 56 s were 18 and 20%, respectively. The mortality of *T. castaneum* eggs were calculated using Abbot's formula because the control mortality was higher than 10% (Abbott 1925). The abbot's corrected mortality for *T. castaneum* eggs when treated with microwaves at 200, 300, and 400 W, were 31, 94, 100, and 100%, respectively, for 14% m.c. rye at 28 s exposure time and the mortality was 98 and 100%, at 200 and 300 W, respectively at 56 s exposure time. One hundred percent mortality was achieved at 400 W for 28 s and at 300 W for 56 s exposure time. Analysis of variance showed that moisture content was not a significant factor in the mortality of eggs.

Mortality of *Tribolium castaneum* larvae. The mortality of control larvae for 14% m.c. rye was zero for all the other control samples. The mortality at 200, 300, 400, and 500 W for 28 s exposure time were 42, 77, 100, and 100 %, respectively for 14% m.c. rye. The mortality at 200, 300, and 400 W for 56 s exposure times were 77, 98, and 100%, respectively. Analysis of variance showed that moisture content was not a significant factor in determining the mortality of larvae. Comparison of means (Table 2) explains that the mortality was not significantly different at 14, 16, and 18% m.c. by single grouping. The mortality was significantly higher at higher power levels with no significant difference in the mortality between 400 and 500 W and the mortality was significant at 28 and 56 s exposure times.

Mortality of *Tribolium castaneum* pupae. The control mortality of pupae at 14% m.c. for 28 and 56 s were 8 and 13 %, respectively. The mortality of *T. castaneum* pupae were calculated using abbot's formula (Abbott 1925). The mortality of *T. castaneum* pupae in 14% m.c. rye at 200, 300, and 400 W were 42, 82, and 100 %, respectively for 28 s exposure time and 75, 100, and 100% at 56 s exposure time showing that mortality was significantly higher at higher power levels and exposure times. There was no significant difference in the mortality of pupae at different moisture content.

Comparison of means indicates that mortality means were grouped as one for all the moisture content whereas the mortality of pupae was significantly higher at higher power levels and exposure times.

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

Tribolium castaneum life stage	Power, (W)	Moisture content, (%)					
		14		16		18	
		Exposure time, (s)					
		28	56	28	56	28	56
Eggs	200	31 ± 16.7	98 ± 4.9	36 ± 9.8	96 ± 6.2	27 ± 13.4	98 ± 4.9
	300	94 ± 10.0	100	94 ± 6.6	100	94 ± 10.9	100
	400	100	100	100	100	100	100
	500	100	#	100	#	100	#
Larvae	200	42 ± 9.8	77 ± 10.3	37 ± 10.3	80 ± 6.3	40 ± 11	63 ± 10.3
	300	77 ± 10.3	98 ± 4.1	75 ± 12.2	97 ± 8.2	73±15.1	100
	400	100	100	100	100	100	100
	500	100	#	100	#	100	#
Pupae	200	42 ± 9.0	75 ± 11.4	46 ± 11.3	77 ± 7.3	50 ± 9.4	70 ± 9.3
	300	82 ± 11.4	100	74 ± 11.4	100	83 ± 9.7	100
	400	100	100	100	100	100	100
	500	100	#	100	#	100	#
Adults	200	35 ± 8.4	75 ± 8.4	32 ± 9.8	67 ± 12.1	32 ± 9.8	68 ± 9.8
	300	63 ± 10.3	100	62 ± 9.8	100	63 ± 10.3	100
	400	98 ± 4.1	100	97 ± 8.2	100	93 ± 8.2	100
	500	100	#	100	#	100	#

since 100% mortality was achieved at 400 W, experiments were not performed at 500 W for 56 s.
* Average of six replicates (three replicates for five insects per 50 g and three replicates for 10 insects per 50 g of sample).

Mortality of *Tribolium castaneum* adults. The control mortality of adult was 0 for all the m.c. rye. The mortality at 200, 300, 400, and 500 W for 28 s exposure times were 35, 63, 98, and 100 %, respectively for 14% m.c. rye. The mortality at 200, 300, and 400 W for 56 s exposure times were 75, 100, and 100%, respectively. The mortality increased with increasing power levels and exposure times at all the moisture contents. One hundred percent mortality was achieved at 500 W for 28 s exposure time and at 300 W for 56 s exposure time. The results of ANOVA showed that there was no significant difference in the mortality of adults at different moisture contents. The mortality was significantly higher at higher power levels and exposure times.

Table 2. Comparison of mortality means among moisture content for life stages of *T. castaneum* in rye.

Tribolium castaneum life stage	Moisture content (%)		
	14	16	18
Egg	90.4a	90.8a	89.6a
Larva	86.7a	86.0a	84.6a
Pupa	87.4a	87.2a	87.8a
Adult	84.4a	82.1a	82.3a

Means within the same row followed by the same letter are not significantly different (P < 0.05).

Table 3. Comparison of mortality means among power levels for *T. castaneum* life stages in rye.

Tribolium castaneum life stage	Power (W)			
	200	300	400	500
Egg	64.4b	96.9a	100.0a	100.0a
Larva	56.4c	86.7b	100.0a	100.0a
Pupa	60.1c	89.8b	100.0a	100.0a
Adult	51.4c	81.9b	98.3a	100a

Means within the same row followed by the same letter are not significantly different ($P < 0.05$).

The comparison of means among moisture contents for the mortality of life stages are shown in Table 2. The comparison of means using Scheffe's test, grouped mortality at all the moisture content in one group showed that there was no significant difference in the mortality between various moisture contents. The comparison of means among power levels are shown in Table 3. The mortality increases with increase in power level and there was no significant difference in the mortality between 400 and 500 W.

When the mortality of all the life stages were analyzed, the most susceptible was *T. castaneum* egg and the least susceptible was the adult stage. The mortality of larval and pupal stage was in between egg and adult with no significant difference between the two. These results are similar to the results of Tilton and Brower (1983) who stated that the embryonic stage of an insect is a time of extreme radio sensitivity and adult insects are more radio resistant than the other stages (Hasan and Khan 1998). Hamid and Boulanger (1969) studied the mortality of larvae and adults of *T. confusum* and stated that the mortality of larvae was the same as the adult at different temperatures.

Shayesteh and Barthakur (1996) and Watters (1976) determined that eggs are the most susceptible to microwave energy followed by pupae, adults and larvae. Halverson et al. (2003) reported that eggs and young larvae are less susceptible and the most vulnerable stage was pupae. 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. (2003b) conducted experiments to study the 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. Menon and Subramanyam (2000) studied the effect of high temperatures on life stages of *T. castaneum* during steam heat treatment. Their study reported that degree of heat tolerance was the highest in pupae, followed by late instar larvae, adults, early instar larvae, and eggs. Differences in larval and adult susceptibility seem to vary among species (Nelson 1996). Lethal temperatures for mortality of insects vary not only with the species but also with the developmental stage of insects (Fields 1992). Arthur (2006) studied the initial and delayed mortality of late instar larvae, pupae and adults of *T. castaneum* and *T. confusum*. They stated that there is a possible shift in susceptibility of life stages at different temperatures. At 46^{o}C pupae were the most heat tolerant life stage but at temperatures of 50^{o}C or higher early instars were the most heat tolerant life stage showing that there is an interaction between temperature and life stage of the species.

The effect of radiation on insects is related to their constituent cells. Cell division and tissue differentiation occur during embryonic development in eggs. The dividing cells are very sensitive to radiation and hence eggs are highly susceptible to radiation whereas the adult stage is more resistant (Ahmed 2001).

Moisture loss

When the rye was treated with microwave energy at different power and exposure time, there was a moisture loss corresponding to the exposed power and time. The moisture loss of rye at different moisture content, power and exposure time are given in Table 4.

Table 4. Average moisture loss (in percentage points) ± standard error of rye treated with microwave energy at different moisture content, power level, and exposure time.

Moisture content (%)	Power (W)						
	200		300		400		500
	Exposure time (s)						
	28	56	28	56	28	56	28
14	0.8 ± 0.2	1.4 ± 0.1	1.2 ± 0.1	2.5 ± 0.1	1.7 ± 0.1	3.9 ± 0.1	2. 2 ± 0.1
16	0.9 ± 0	1.8 ± 0	1.3 ± 0	2.7 ± 0.1	1.9 ± 0	4.3 ± 0.2	2.5 ± 0.1
18	1.0 ± 0	2.0 ± 0	1.6 ± 0.1	3.1 ± 0	2.1 ± 0.1	4.6 ± 0.1	2.8 ± 0.1

The moisture loss at 200, 300, 400, and 500 W for 28 s exposure times were 0.8, 1.2, 1.7 and 2.2 percentage points, respectively for 14% m.c. rye. As the exposure time was increased to 56 s, the moisture loss increased to 1.4, 2.5, and 3.9 percentage points for 200, 300, and 400 W, respectively. Similar range of moisture loss was observed for 16 and 18% m.c. rye. The moisture loss was significantly higher at 18% followed by 16 and 14% m.c. rye.

Germination results

The germination percentage of rye at 14, 16, and 18% m.c. exposed to 28 and 56 s at different power levels are shown in Figures 1 and 2, respectively.

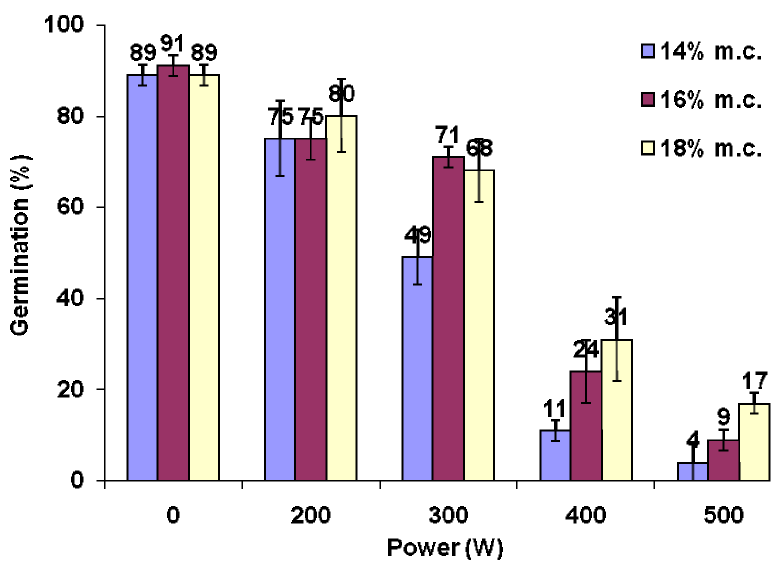

Figure 1. Germination percentage of 14, 16, and 18% m.c. rye at 28 s exposure time.

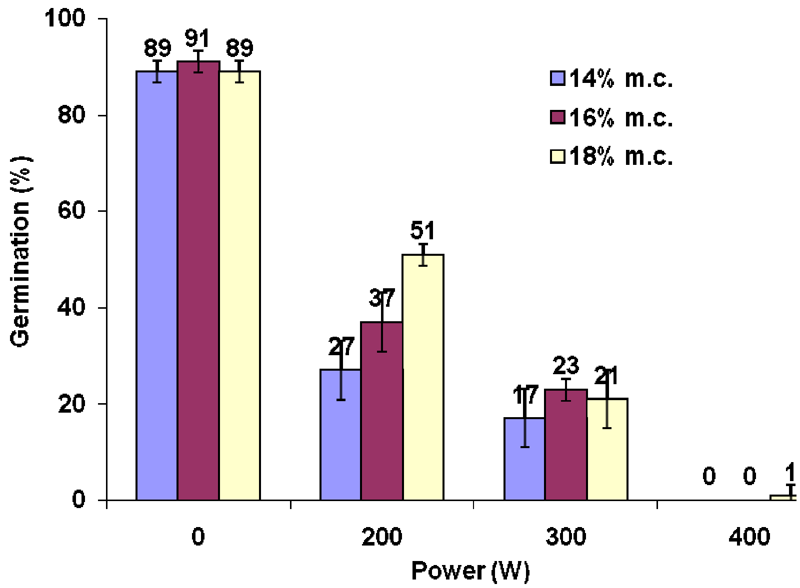

Figure 2. Germination percentage of 14, 16, and 18% m.c. rye at 56 s exposure time.

The germination decreased with an increase in power or exposure time or both. Analysis of variance showed that moisture content, power, and exposure time had a significant effect on the germination of rye. Statistical analysis showed that germination was significantly higher at 18%, followed by germination at 16% and the lowest at 14% m.c. This germination trend can be related to the temperature effect. The temperature was the lowest at 18% m.c. and hence the germination was the highest.

Tran (1979) studied the effect of microwave energy on *Acacia* seeds and determined that germination of *Acacia longifolia* and *Acacia sophorae* was enhanced by microwave energy at 2450 MHz. Thuery (1992) has stated that exposure of seeds to 650 W, 2.45 GHz microwaves for about 30 s is sufficient to ensure a high rate of germination. The microwaves act on the strophiola, a sensitive part located on the ventral side of the seed, which may thus become more water permeable. The effect of the radiation varies according to the species: clover, peas, beans, and spinach respond favorably whereas wheat, corn, and cotton are less sensitive. Though some of the studies have shown that exposure to microwave enhances germination, Campana et al. (1993) studied the physical, chemical and baking properties of wheat dried with microwave energy and concluded that germination capacity was decreased by exposure to microwave energy. The decrease in germination capacity was related to the final temperature and the initial moisture content of the grains.

The results of the current study have shown that complete mortality of life stages of *T. castaneum* in rye can be achieved using microwave energy. Among the various life stages, eggs were the most susceptible followed by larvae and pupae with no significant difference between the two. Adult stages of *T. castaneum* were the least susceptible to microwave energy. There was no significant difference in the mortality of egg, larval, pupal and adult stages of *T. castaneum* at various moisture contents.

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