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Microwave energy as an alternative control method for stored grain pests

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Abstract:

Chemicals are widely used to kill pests in stored grain even though these affect the environment and the consumers, which make it necessary to seek safer methods of pest management. As an alternative for traditional chemical methods, microwave radiations are used to kill insects and mites infest stored grains. Samples of wheat grain were infested with adult insects and larvae of *Sitophilus granarius* (L.), *Sitophilus oryzae* (L.) (Coleoptera:Curculionidae) and *Rhyzopertha dominica* (Fabricius) (Coleoptera : Bostrichidae). Crushed wheat was used with adult mites, *Dermatophagoides farinae* Hughes (Acari: Pyroglyphidae) , *Tyrophagus putrescentiae* (Schrank) and *Rhizoglyphus echinopus* (Fumouze and Robin) (Acari: Acaridae). Wheat kernels were subjected to microwave treatment at three powers; low, medium-low at 6 exposure times and medium at 5 times. The mortality of pests increased with either power as well as exposure time or both. *R. dominica* and *R. echinopus* were more sensitive to microwave radiation where LT_{50} values were 3.07, 2.067, 1.17 and 1.96 minutes at low and medium-low powers, respectively. Complete reduction in F_1-progeny of all insects was obtained at medium power for 4 min. of exposure. *D. farinae* was the most tolerant mite to microwave radiation with LT_{50} values 2.57, 2.47 and 2.22 min. for the three powers, respectively. Biochemical analyses of wheat grains at the maximum time at which high mortality was obtained showed no detectable changes in the quality of protein, hardness, moisture and color. Germination of microwave treated wheat decreased, whereas exposure time, power or both increased. Managing stored grains requires the use of various techniques to ensure that the quality of the grain entering the storage facility does not deteriorate over time. Using microwaves opens up a new field to get rid of insects and mites in stored grains without any chemical pollution.

Introduction

Wheat is a major source of food for both humans and animals. It occupies a central position in the agriculture sector and the national economy. Pest infestation may start from the field and continues through storage until the grain is processed for consumption. Stored grain pests cause high economic losses by feeding on stored grains and harm public health by contamination of food.

A loss due to insect infestations is a great problem as they feed, bore and ruin grains and speed up the process of decay, so they may account for 20-30% production loss and, in severe cases, they cause a total loss. Mites are important pests of stored grains, seeds, and other stored foods. Contamination with it causes both the qualitative and quantitative losses. The mites consumed up to 3% by weight of the grain. Mites feed on germ and destroy it completely but consume very little of the remaining material. The infested seeds look healthy but not capable of germination,which results in less plant population and the low yield (Al-Akhdar *et al.,* 2015; Islam *et al.,* 2004; Singh and Kaur, 2018 and Mahmood *et al.*, 2011).

Historically, the managementof stored-product pests has depended on the application of chemical pesticides, but increasing attentiveness of the risks these chemicals pose to environmental quality and human health has made it necessary to seek safer methods. The evolution of insecticide resistance has aggravated the problem and increased the need to develop pest management programs that are less chemical-dependent.

Hence, an alternative technique of killi ng insects in stored grain is searched. As anal ternative technique of killing pests in stored c ereals, particularly wheat, microwave disinfestations appear to have excellent poten tial. In the agriculture and food industry, the utilization of microwave energy and drying already has a position (Hamid *et al.,* 1968; Hamid and Boulanger, 1969; Kirkpatrick and Roberts, 1970; Nelson and Stetson, 1974; Watters, 1976; Tilton and Brower, 1987; Shayesteh and Barthakur, 1996 and Vadivambal *et al.,* 2007 and 2010).

The focus of recent research has been on

1.The effect of microwave use on the populat ion of six of the most abundant grain pests st ored, three insects; lesser grain borer *Rhyzopertha dominica* (Fabricius) (Coleoptera:Bostrichidae), wheat weevil *Sitophilus granarius* (L.) and rice weevil *Sitophilus oryzae* (L.) (Coleoptera:Curculionidae) and three mites:house dust mites *Dermatophagoides farinae* Hughes (Acari: Pyroglyphidae), mould mite *Tyrophagus putrescentiae* (Schrank) and *Rhizoglyphus echinopus* (Fumouze and Robin) (Acari: Acaridae), on wheat at various microwave power levels and exposure times.

2.The quality of the treated grains:

2.1. Sodium dodecyl sulphate (SDS) -Protein electrophoresis.

2.2. Physical properties (moisture, hardness and color).

2.3.Germination potential of microwave treated wheat.

Materials and methods

1. Microwaves:

The microwave frequency used in the study is a standard 2450 MHz. A microwave oven, EM-280 M, Electra, Japan, capacity 28 L and cavity dimensions 21.9 × 35 × 35 was used.The oven operated at three energy levels (low, medium-low and medium) with 17%, 44% and 66% of power output (output: 800W).

2. Insects:

Adults of S. *granarius*, *S. oryzae* and *R. dominica* reared on wheat seeds in glass jars (each of approximately 200 ml) and each jar was covered with muslin cloths and fixed with rubber bands. To have an initial population of *insect* adults homogenous in age, about 200 adults were introduced into jars containing seeds for egg-laying and then kept in an incubator at 28±2°c and 65±5 % R.H. After three days, all insects were removed from the media and the jars were kept again at controlled conditions. Adults of (1-2 weeks old) and larvae of (10-15 days old) were used for the experiment.Low and medium-low powers were tested after 1, 2, 4, 6, 8 and 10 mintsof exposureand 1, 2, 3, 4 and 5 mintsfor medium power, on adults and larvae of three insects. Mortality percentages were recorded after 24 hrs.of treatment for adults and were left till F_1-progeny appeared,while treated larvae stage left to estimate percentages of decrease.Reduction percentages in the progeny offspring were calculated by the following equation (El-Lakwah *et al.*, 1996).

$$\% \text{ Reduction} = \frac{\text{No. of progeny of control} - \text{No. of progeny of treat}}{\text{No. of progeny of control}} \times 100$$

3. Mites:

Strains of *T. putrescentiae*, *D. farinae* and *R. echinopus* were collected from infested grain samples. To obtain a pure culture, adults were placed in rearing plastic rings containing crushed wheat at 30±2°c and 70±5 % RH. Likewise, the three microwave powers were tested for 1,2,3,4 and 5 minutes, adult stages only were evaluated.The tested pests were identified and reared in the laboratories of Plant Protection Research Institute, Agriculture Research Centre, Dokki, Giza, Egypt.

4. Quality of the treated grains:

4.1. Sodium dodecyl sulfate (SDS) -Protein electrophoresis:

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) is used by an analytical method in biochemistry to study banding patterns of treatments to separate protein mixtures by their molecular mass. Separation of charged molecules in an electric field under study were evaluated according to the method of Laemmli (1970) and Studier (1973).

4.2. Physical eroperties of treated wheat:

All test quality was carried out in the Agricultural Engineering Research Institute

4.2.1. Hardness: hardness testing was carried out by the Penetrometer system (Digital Force Gauge Model FGN-20G).Nidec-Shimpo Corporation, Japan.

4.2.2. Moisture: humidity of seed testing were carried out by grain moisture meter (Draminski electronics in agriculture SN: 24435).

4.2.3. Color: color testing was carried out by Color Analyzer (model RGB-1002). The resulted data were changed to images by website:https://htmlcolors.com/color-picker.

5. Determination of germination:

Germination of microwave-subjected wheat seeds was evaluated by placing 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). To prevent filter paper from being desiccated, the plates were placed in a plastic bag and kept at 20°C for 7 days. The germinated seeds were counted on the seventh day and the percentage of germination was calculated.

6.Statistical analysis:

Statistical analysis of data was carried out according to Duncan's multiple range test (Duncan, 1955).Lethal effects of microwave energy were evaluated as percentages of cumulative mortality due to powers; corrected for mortality in the control variant according to Abbott's formula (Abbott, 1925). Virulence of the isolates was estimated by median lethal time (LT_{50}) which wes calculated by the probit analysis (Finney, 1971) for the variants treated with different microwave powers. Confidence intervals of varying LT_{50} values were calculated at p-level < 0.05.

Toxicity index (Ti) was calculated using Sun (1950) equation as follow:

$$Ti = \frac{LT_{50} \text{ of the most effective power}}{LT_{50} \text{ of less effective power}} \times 100$$

Results and discussion:

1. Mortality of insects:

Mortality of *S.oryzae*, S. *granarius* and *R. dominica* adult stages at different temperatures and exposure times is shown in Table (1). For the three insects, the control mortality was zero. LT_{50} values at the low power were 3.07, 3.17 and 4.31 mints for *R. dominica*, *S. oryzae* and *S. granarius*, respectively. *R. dominica* was more sensitive to medium-low power with Lt_{90} 3.95 min.

followed by *S. oryzae*, while *S. granarius* was more tolerant to heat to 10.42 min. *S. granaries* appeared relatively heat tolerant compared with the other two species.

Table (1): Mortality of adult insects after exposure to microwave radiations in min.

Power	Insect	LT90	Slope	RR	Index	LT50	Lower limit	Upper limit
Low	*Rhyzopertha dominica*	8.49	2.9	1	100	3.07	2.66	3.5
	Sitophilus oryzae	11.97	2.22	1.03	96.99	3.17	1.53	5
	Sitophilus granarius	17.58	2.04	1.36	73.74	4.31	2.3	7.91
Medium-low	*Rhyzopertha dominica*	3.95	1.82	1	100	1.17	0.79	1.52
	Sitophilus oryzae	5.91	2.65	1.11	90.1	1.3	1.02	1.56
	Sitophilus granarius	10.42	1.52	1.28	78.22	1.5	0.42	2.06
Medium	*Sitophilus oryzae*	1.05	0.79	1	100	0.03	-	-
	Rhyzopertha dominica	2.41	1.78	15.69	6.37	0.41	0.07	0.72
	Sitophilus granarius	4.31	2.1	40.69	2.46	1.06	0.703	1.35

Complete reduction in F_1-progeny was found to *S. oryzae* after 8 min. of exposure to low power and to the three insects after 4 min. of medium power. On the other hand, there was100% reduction in F_1-progeny to larvae of *S. granarius* which was exposed to low and medium-low power after 4 min. (Table , 2).

Table (2): Reduction % in F_1-progony of infected wheat with adults and larvae of insects after treatment with microwave radiation.

F1-progeny	Time	Low			Medium-low			Time	Medium		
Adults		***Rhyzopertha dominica***	***Sitophilus oryzae***	***Sitophilus granarius***	***Rhyzopertha dominica***	***Sitophilus oryzae***	***Sitophilus granarius***		***Rhyzopertha dominica***	***Sitophilus oryzae***	***Sitophilus granarius***
	1	7.933	0	50	29.7	62.8	65.38	1	92.5	79.6	85.88
	2	21.66	16.66	51.27	67.82	81.3	78.19	2	96.66	98.33	96.15
	4	24.66	61.75	69.23	72.52	89.1	93.58	3	98.33	100	100
	6	41.15	75.94	78.19	87.98	93	100	4	100	100	100
	8	89.27	100	97.42	96.66	100	100	5	100	100	100
	10	100	100	100	100	100	100	-	-	-	-
Larvae		***Rhyzopertha dominica***	***Sitophilus oryzae***	***Sitophilus granarius***	***Rhyzopertha dominica***	***Sitophilus oryzae***	***Sitophilus granarius***		***Rhyzopertha dominica***	***Sitophilus oryzae***	***Sitophilus granarius***
	1	0	31.1	93.81	73.33	78.2	89.44	1	98.3	65.5	95.24
	2	20	80.5	94.3	93.33	83.3	97.08	2	100	96.11	96
	4	40	84.4	100	95	85	100	3	100	98.33	97.7
	6	55	86.6	100	100	93.3	100	4	100	100	100
	8	75	91.6	100	100	100	100	5	100	100	100
	10	96.67	100	100	100	100	100	-	-	-	-

These results are in agreement with those obtained by Vadivambal *et al.* (2007) who reported that the mortality of *S. granarius* at 250, 300, 400 and 500W was 41%, 64%, 84%, and 100%, respectively, for 28 Sec. exposure time,while at an exposure time of 56 Sec., 100% mortality was obtained at 300W. as compared to 400W. for *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) and *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae), respectively. Bedi and Singh (1992) also studied the effects of microwaves on three stored-grain insect species: larvae of *Corcyra cephalonica* (Stainton) (Lepidoptera Pyralidae), adults of *Callosobruchus chinenesis* (L.) (Coleoptera : Chrysomelidae) and *R. dominica*. The experiments were conducted at varying frequencies between 12 and 18 GHz and with exposure times of 2, 5, and 10 min. Their results suggested that mortality of insects increased significantly with an increase in both the frequency and the exposure times. At the same trend, Microwave radiation was used to control *Oryzaephilus surinamensis* (L.) (Coleoptera:

Silvanidae) adult beetles on dried stored figs. At 900 W. and 50 sec., complete mortality was accomplished (Reza Sadeghi *et al*., 2019).

2. Mortality of mites:

The mortality data for *T. putrescentiae*, *D. farinae* and *R. echinopus* at various thermal levels and exposure times are shown in Table (3). There were not any mortality observed in the controls. At a low power and exposure time of 2.75, 3.59 and 7.51 min. there was 50% mortality of *R. echinopus, T. putrescentiae* and *D. farinae*, respectively. As the temperature was increased to medium power, the mortality of *T. putrescentiae, R. echinopus* and *D. farinae* after a 1.44, 1.66 and 2.22-m exposure increased to 50%, respectively. Similar results for *D. pteronyssinu s*and *D. farinae* were reported by Ernieenor and Ho (2010) who found that the mortalities of adult mites exposed to 2,450 MHz microwave radiation produced by 3 ovens at various exposure times and power settings were 99.4, 84.1 and 44.8, respectively at 3 power settings. At high and medium powers, there was 100% mortality in both species when exposed for 300 seconds. The mean mortality rates at low power were 10.8 ± 0.7% for *D. pteronyssinus* and 9.7 ± 2.6% for *D. farinae*. Due to the heat produced by high-frequency oscillation of dielectric molecules, such as water and body fluid of mites, direct absorption of microwaves is extremely efficient in killing mites.

Table (3): Mortality of adult mites after exposure to microwave radiations in min.

Power	Mite	LT_{90}	Slope	RR	Index	LT_{50}	Lower limit	Upper limit
Low	***Rhizoglyphus echinopus***	9.39	1.99	1	100	2.067	2.75	1.52
	Tyrophagus putrescentiae	8.1	2.95	1.4	71.34	2.078	3.59	2.49
	Dermatophagoides farinae	16.32	2.38	2.22	44.86	2.57	7.51	3.74
Medium low	***Rhizoglyphus echinopus***	7.76	2.23	1	100	2.06	1.52	2.57
	Tyrophagus putrescentiae	7.73	2.24	1.01	99.47	2.07	1.8	2.34
	Dermatophagoides farinae	5.76	3.48	1.2	83.62	2.47	2.06	2.87
Medium	***Tyrophagus putrescentiae***	4.48	2.58	1	100	1.44	0.99	1.79
	Rhizoglyphus echinopus	3.6	3.79	1.15	86.55	1.66	1.35	1.95
	Dermatophagoides farinae	5.19	3.48	1.55	64.64	2.22	1.85	2.28

3. Quality of the treated grains:

To maintain the quality of the treated wheat that was exposed to microwave power, the lowest time that causes 100% mortality to one of the three insects at each power was tested for the following evaluations.

3.1. Protein electrophoresis:

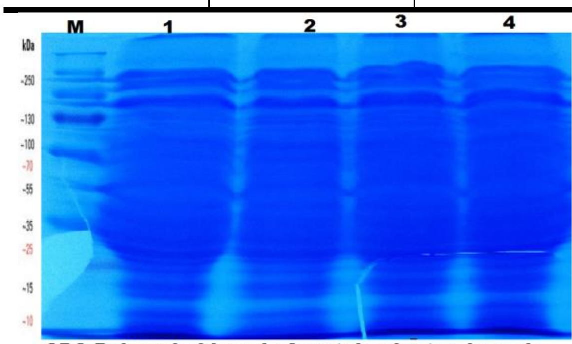
Data represented in Table (4) and illustrated in Figure (1) showed that due to the exposure of wheat grains to different powers of the microwave, some bands of electrophoresis of protein contents were generated and some disappeared. In low treatment, the band with mw 79 pb generated and the band with mw was 94 pb disappeared, but in low medium treatment two bands with mw 145 and 85 pbwere generated while the band with mw 35 disappeared. Also for medium treatment the band with mw 85 pbwas generated and the band with mw 35 pb disappeared compared tocontrol treatment. On the other hand, total the polymorphic bands for microwaves treatments were 2, 3, 2 with polymorphism ratios of 11.76 %, 17.65 % and 11.76 % for low, medium-low and medium power, respectively, as shown in Table (5). Kasarada *et al.* (1998) and Jaramillo *et al.* (1999) reported that SDS-PAGE was widely used to separate proteins related to genetic background and can be used to certify the genetic make-up of wild, cultivars, or newly derived cereal plants. Campana *et al*. (1993) cleared the physical and chemical properties of wheat dried with microwave energy and reported that the protein content was not affected but the functionality of gluten was altered gradually with increasing the time of exposure.

Table (4): Effect of microwaves on protein electrophoresis of wheat grains.

	Control	Low	Low medium	Medium
Total bands	17	17	18	17
Generated bands	-	1	2	1
Disappeared bands	-	1	1	1
Polymorphic bands	-	2	3	2
Polymorphisms %	-	11.76	17.65	11.76

Table (5): Effect of microwaves on generated bands and polymorphisms of wheat grains.

Bands No.	M.W.(k.da)	Control	Low	Low medium	Medium
1	145	-	-	+	-
2	133	+	+	+	+
3	121	+	+	+	+
4	105	+	+	+	+
5	94	+	-	+	+
6	88	+	+	+	+
7	85	-	-	+	+
8	79	-	+	-	-
9	71	+	+	+	+
10	50	+	+	+	+
11	41	+	+	+	+
12	35	+	+	-	-
13	30	+	+	+	+
14	26	+	+	+	+
15	25	+	+	+	+
16	23	+	+	+	+
17	21	+	+	+	+
18	19	+	+	+	+
19	16	+	+	+	+
20	14	+	+	+	+



SDS-Polycrylmide gel of protein electrophorasis of wheat treated with microwave

Figure (1): Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS – PAGE) protein banding patterns of tested wheat seeds as affected by microwave treatment. Where: M = Standard marker 1= control 2= low 3= medium low 4=medium.

3.2. Physical properties of treated wheat:

3.2.1. Moisture loss:

The initial moisture content (IMC) of untreated wheat kernels was found to be 7.23%. Table (6) indicated that with an increase in power level, there was a non-significant decrease in moisture content from 7.12% to 7.06 %, and 6.93% at low, mid-low and medium, respectively.

3.2.2.Hardness:

The values of hardness attributes of wheat grains were tested after exposure to microwave radiation in periods that 100% mortality was achieved. However, hardness of wheat did not get affected by treatment (Table, 6).

Table (6): Effect of microwaves on hardness and moisture of wheat grains.

	Low	Mid-low	medium	control	LSD 0.05	F-value	*p*-value
Moisture	7.12^{a}	7.06^{a}	6.93^{a}	7.23^{a}	0.326	1.2015	.323ns
Hardness	48.64^{a}	49.06^{a}	54.95^{a}	48.61^{a}	9.98	0.792	.506ns

3.2.3.Color:

As demonstrated in Figure (2), the color of treatedgrainswith mid-low and medium powers showed more significant changes than control. For all colors, there were no changes between low power treated grains and control. Red, blue and green colorsshowed 237, 192 and 99 degrees in control and low power increase to 238, 196 and 1.6 with mid-low and 239, 199 and 114 with medium powers, respectively.

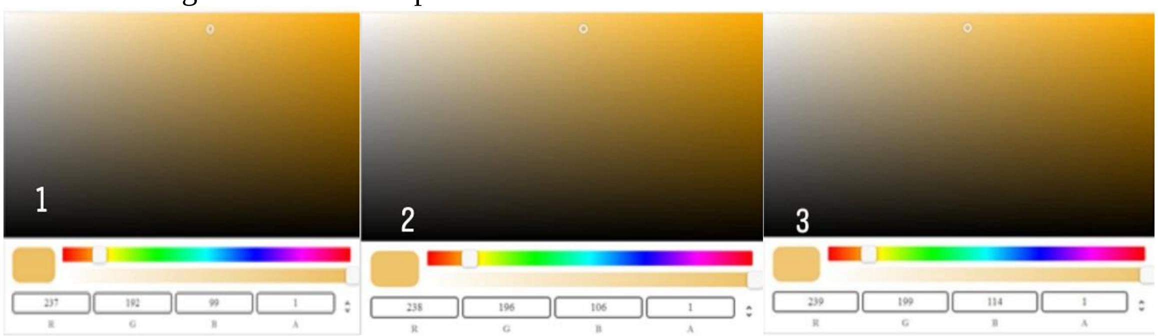


Figure (2): Effect of microwave on wheat colors. 1.Control and low power 2. Medium-low power 3-Medium power.

As for moisture content of wheat kernels that were subjected to microwave exposure, there were no significant differences between power levels, while it was significant in both time and periods and interaction between power and time levels (Abd El-Raheem and Saadiya , 2016). Manickavasagan *et al.* (2013) examined the date fruits which were not get affected by microwave treatment. But the gumminess of the dates treated at 300, 600 and 800 W. was significantly lower than the untreated and treated at 180 W. dates.

4. Determination of germination:

The control germination for wheat seeds was tested. Exposure times were 10, 6 and 4 for the three powers low, mid-low and medium, respectively. The germination decreased at high exposure time or power was due to the increase in temperature of the sample confirming that power (F=37.65; df=12, 60; P<0.0001) whereas moisture content had no significant effect (F= 1.2; df= 36; P=.3231 ns) on germination (Table, 7).

Table (7): Effect of microwave exposure on wheat germination.

	Low	Mid-low	Medium	control	LSD 0.05	F-value	*p*-value
Germination	14.75[b]	8.25[c]	3[d]	22.25[a]	4.178	37.65	.0000
Percentage	59%[b]	33%[c]	12%[d]	89%[a]	16.71	37.65	.000

Our results recommended that the microwave is a good application to get rid of insects and mites from stored grains and products that will be used in feeding. But it is not recommendeded to utilize with seeds because of microwave radiations and high temperature which were supposed to affect the germination capacity of the seeds. These results are at the same line with Vadivambal *et al.* (2007) who reported that germination of wheat kernels was lower after treatment with microwave energy of 500W. for an exposure time 56 sec. Campana *et al.* (1993) studied the chemical, physical and baking properties of wheat heated and dried with microwave energy. They founded that germination capacity was affected by exposure to microwave energy. The final temperature and the initial moisture content of the grains are responsible for the decrease in germination capacity. Bhaskara *et al.* (1998) studied the effect of microwave treatment on the quality of wheat seeds infected with *Fusarium graminearum* (Schwabe). Their results showed that seed viability and seedling vigor decreased accordingly. Similar results were obtained by Aladjadjiyan (2002) who studied the influence of microwave radiation on germination of ornamental perennial crops. Their results proved that at 850 W. the seed germination decreased.

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