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Use of Microwave Heating Technology for Disinfestation of Stored Green Gram (*Vigna radiata*) against Cowpea Bruchid *Callosobruchus maculatus* (Fabricius)

R. Kooner[1], D.K. Sharma[1], K.S. Suri[1]

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ABSTRACT

Background: Pulses are an integral component of food in India which acts as major source of protein required for growth and maintenance of body. But during their storage they suffer heavy losses resulting in depletion of their quality and edibility. The present studies focused on disinfestation of green gram grains using microwaves as an alternative approach to chemical methods for controlling insects in grains and pulses.

Methods: In this laboratory studies during 2017-18, the grains of green gram were infested with different life stages of the cowpea weevil and exposed to 200, 400, 600 and 800 W microwave power levels, each at an exposure period of 10, 20, 30 and 40 s.

Result: Eggs were the most susceptible, while pupal and adults were the least susceptible life stages to microwave treatments. Complete mortality of eggs was achieved with microwave treatments (400, 600 and 800 W), each at an exposure period of 30 and 40 s. Complete inhibition of larval stage was attained with 400 and 600 W (30 and 40 s) and 800 W (20, 30 and 40 s exposure) while for pupal stage it was observed at the higher wattages of 600 (40 s exposure) and 800 (30 and 40 s exposure). Microwave wattage of 600 W for 40 sand 800 W for 30 and 40 s exposure caused 100% mortality of 2d old adults of cowpea bruchid.

Key words: Bruchid, Cowpea weevil, Exposure period, Life stages, Microwave.

INTRODUCTION

The increasing alertness about human health and environmental issues relating to agrochemicals use in agriculture has led to interest in alternate forms of pest management (Aulakh and Ravisankar, 2017). Synthetic pesticides are currently the method of choice to protect stored grains from the losses caused by insect-pests (Mahfuz and Khalequzzaman, 2007). But continuous use of these chemicals has created serious issues such as direct toxicity to consumers besides development of resistance and resurgence in insect-pests (Zettler, 1991). Finding safe alternatives to synthetic insecticides to protect stored grains and grain products from insect infestations are highly desirable (Sharma *et al.,* 2018). High temperature has been used extensively against stored grain insect-pests using different methods like fluidized beds, radio frequencies, microwaves and hot air (Fields, 1992; Beckett *et al*., 2007). Amongst non-chemical approaches, application of microwave energy has gathered momentum in the recent times (Vadivambal and Jayas, 2007; Yadav *et al.,* 2012) which provides safe and hygienic mode of disinfestation. Insects die when exposed to high temperatures because of their limited physiological capacity to thermo regulate (Fields, 1992). Cowpea weevil eggs, larvae, and pupae are trapped within the seed and therefore, they are targets for management using elevated temperatures (Murdock *et al*., 1997). Differential heating during microwave exposure seems to be a better option for food grain disinfestation (Vadivambal *et al*., 2010a). Microwaves cause dielectric heating of water soluble molecules when they pass through biological materials containing water (Das *et al*., 2013). Since both grains and insects infesting food materials have different dielectric properties, they are heated to different degrees on exposure to microwave radiations (Wang *et al*., 2003).

Pulse bruchids are the most damage causing insects in stored legumes and amongst them, *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae), is a major pest of legume crops and is cosmopolitan in nature (Benzi *et al*., 2009; Halder *et al*., 2010 ; Bhalla *et al*., 2008) and the damage due to this pest affects the germinative ability and nutritive value of the seed (Divya *et al.,* 2018). Ovipositing females glue eggs to the seed coat and upon hatching, larvae chew into seeds directly below the egg (Shunmugadevi and Radhika, 2020). Their life cycle is completed inside a single kernel and a visible window appears where larvae pupate (Radha and Susheela, 2014). After pupation, adults

[1]Department of Entomology, Punjab Agricultural University, Ludhiana-141 004, Punjab, India.

Corresponding Author: R. Kooner, Department of Entomology, Punjab Agricultural University, Ludhiana-141 004, Punjab, India. Email: rubalsidhu05@pau.edu

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emerge from the kernel by making a circular emergence hole. *C. maculatus* has a short generation time of 22 to 30 d and adults live for about 7 d under laboratory conditions (25°C) (Fox, 1993; Rees, 2004; Farrell, 2010). In this experiment, use of microwave energy for disinfesting green gram grains against cowpea bruchid was evaluated.

MATERIALS AND METHODS

The experiment was conducted at room temperature in Post-harvest Technology Laboratory, Department of Processing and Food Engineering, PAU, Ludhiana during 2017-18. The adults of *C. maculatus* were maintained on sound and fresh grains of green gram variety, SML-668 (Punjab Agricultural University recommended variety). Females start laying eggs within 24-48 hours after mating and fresh eggs are transparent. To obtain grains with freshly laid eggs of *C. maculatus*, petri plates containing 100 g grains were placed in insect culture jars maintained at laboratory conditions. These petri plates were drawn out of the culture jars after 24 hours and the grains with single egg were segregated and these eggs were then kept for exposure studies to different microwave frequencies against egg, larval and pupal stages of *C. maculatus.* For exposure of egg stage, a sample of 100 g fresh grains mixed with 100 grains each containing one freshly laid egg was taken for each treatment and exposed to different microwave treatments. Another set of grains with eggs was kept under laboratory conditions and they were exposed after 9th and 17th d to evaluate the effect of different microwave frequencies against larval and pupal stages of the insect, respectively. To evaluate the effectiveness of microwave frequencies against adult stage of the insect, the adults were directly exposed to various microwave frequencies. For this purpose, ten adults (mixed sex) were released in 100 g grains and exposed and the observations on % mortality were recorded. Different life stages of *C. maculatus i.e.* egg, larva, pupa and adult were exposed to different wattages and different exposure periods in a LG Microwave model (MC 7648 WSH). The sample holder used was a microwaveable container (9.5 × 9.5 × 5.0 cm) with lid to hold a 100 g sample. Experiments were conducted by keeping samples in this container. Green gram grains with moisture content of 9.00% were used for the experiment. The experimental treatments were four exposure times, 10, 20, 30 and 40s and four power levels, 200, 400, 600 and 800W. Each treatment was replicated thrice in completely randomized design.

The observations on number of exit holes, adult emergence, per cent adult mortality, per cent weight loss, per cent grain damage as well as seed germination (%) were recorded. A representative sample of 100 grains (average weight 3.826 g) was taken out of the total sample (100 g) and the number of exit holes and adult emergence was counted from whole of the sample. The weight loss (%) was calculated using count and weight method given by Adams and Schulten (1978). One thousand grains were taken randomly from the sample. The number of insect damaged and undamaged grains was counted and their weight was taken on an electronic weighing balance.

$$\text{Weight loss (\%)} = \frac{(UNd) - DNu}{U\,(Nd + Nu)} \times 100$$

Where,

U= Weight of undamaged grains.

Nu= Number of undamaged grains.

D= Weight of damaged grains.

Nd= Number of damaged grains.

The grain damage (%) was calculated from healthy (without holes) and insect damaged grains separated on thousand grain count basis and the following formula was used.

$$\text{Grain damage (\%)} = \frac{\text{Number of insect damaged grains}}{\text{Total number of grains}} \times 100$$

Germination of mungbean grains was tested using 'paper towel method'. Hundred grains were taken at random from each treatment and were kept between blotting papers, which was wrapped in wax papers and tied with rubber bands at both the ends. These paper towels were kept in an incubator at 20-22°C temperature (ISTA, 1985). On the 7th day after start of test, paper towels were opened to record germination of seeds by using the following formula:

$$\text{Per cent germination} = \frac{\text{Germination grains}}{\text{Total number of grains}} \times 100$$

Data pertaining to different observations were subjected to analysis of variance using statistical software SPSS v 20.0 (SPSS, 2011). The comparison of means was done using Duncan's multiple range test (DMRT).

RESULTS AND DISCUSSION

Egg stage

Green gram grains treated with microwave wattages of 400, 600 and 800W, each at an exposure period of 30 and 40 s completely inhibited the appearance of exit holes as these treatments caused complete kill of the eggs. All the microwave treatments were significantly different from untreated control which recorded maximum exit holes (25.33/ 100 grains) (Table 1). Maximum adult emergence was observed with treatment 200W and 10 s exposure period (58.00 adults/100g) but it was significantly lower than the untreated control (85.00 adults/100 g).

No weight loss of green gram grains was observed in treatments with 400W, 600W and 800W, each at an exposure period of 30 and 40 s (Fig 1). Maximum grain damage of 12.40% was recorded in 200W at 10 s exposure period which was significantly lower than the untreated control (15.72%). Treatments with 400, 600 and 800W, each at exposure period of 30 and 40 s, recorded zero grain damage and they were significantly better than rest of the treatments (Fig 2).

Larval stage

Microwave wattages of 400 W and 600 W (each at 30 and 40 s exposure) and 800 W (20, 30 and 40 s exposure) recorded zero exit holes. Thus no adult emergence and weight loss of green gram grains was observed in these treatments (Table 2 and Fig 1). Maximum adult emergence (48.00 adults/100g) was recorded in 200 W (10 s exposure). Further, among the treatments, significantly maximum grain damage (10.12%) was recorded in 200W (10 s exposure) but it was significantly better than the untreated control (14.59%) (Fig 2).

Table 1: Effect of different microwave wattages and exposure periods on egg stage of *C. maculatus* in stored green gram.

Treatment	Exposure period (s)	No. of exit holes/100 grains (Mean±SE*)	Total no. of adults emerged (Mean±SE*)
200 W	10	9.33±0.14^{c}	58.00±0.86^{e}
200 W	20	8.67±0.14^{c}	30.00±0.35^{d}
200 W	30	4.00±0.13^{ab}	18.67±0.43^{c}
200 W	40	2.33±0.09^{ab}	10.67±0.31^{abc}
400 W	10	6.00±0.11^{bc}	38.67±0.39^{d}
400 W	20	3.33±0.17^{ab}	17.33±0.28^{c}
400 W	30	0.00±0.00^{a}	0.00±0.00^{a}
400 W	40	0.00±0.00^{a}	0.00±0.00^{a}
600 W	10	3.33±0.21^{ab}	20.67±0.28^{c}
600 W	20	1.67±0.11^{b}	6.67±0.38^{ab}
600 W	30	0.00±0.00^{a}	0.00±0.00^{a}
600 W	40	0.00±0.00^{a}	0.00±0.00^{a}
800 W	10	1.33±0.11^{a}	12.33±0.17^{bc}
800 W	20	0.33±0.14^{a}	3.67±0.21^{ab}
800 W	30	0.00±0.00^{a}	0.00±0.00^{a}
800 W	40	0.00±0.00^{a}	0.00±0.00^{a}
Untreated control	-	25.33±0.46^{d}	85.00±0.44^{f}

*Means within same column followed by same letter are not significantly different (Duncan's MRT, P<0.05).

Pupal stage

Zero exit holes/100 grains were observed with microwave wattages of 600W (at 40 s exposure) and 800 W (at 30 and 40 s exposure). These treatments were statistically at par with 400W (40 s), 600W (20, 30 and 40 s) and 800W (10 s) with 2.00, 2.00, 0.67, 0.00 and 1.67 exit holes/100 grains, respectively. Treatments with microwave wattages 600 W (40 s exposure) and 800 W (30 and 40 s exposure) completely inhibited adult emergence (Table 3).

Among different treatments, no weight loss and grain damage (Fig 1 and 2) was observed in microwave treatments with 600 W (40 s exposure) and 800 W (30 and 40 s exposure). Maximum grain damage (18.09%) was observed in untreated control.

Adult stage

Complete kill of 2 d old adults was observed when the beetles were exposed to 600 W (40 s) and 800W (30 and 40 s exposure) microwave treatments (Table 4).The least effective treatment was with 200W at all its exposure periods and they were also statistically at par with untreated control.

For complete kill of egg, larva, pupa and adult, microwave wattage and exposure time was inversely related like at 400 W, 30 s were required for complete kill of larva whereas 20 s exposures was enough to kill the larva at 800W. Egg and larval stage were the most susceptible as compared to pupal and adult stage. It was in line with the findings of Purohit *et al.* (2013), who reported 100% mortality of all life stages (egg, young larva, old larva, pupa and adult) with exposure to 400 W power level for 28 s, with surface temperature of green gram (68.1°C). Similar results were found by Loganathan *et al.* (2011) and Vadivambal *et al.* (2010b). Molins (2001) also stated that an increase in

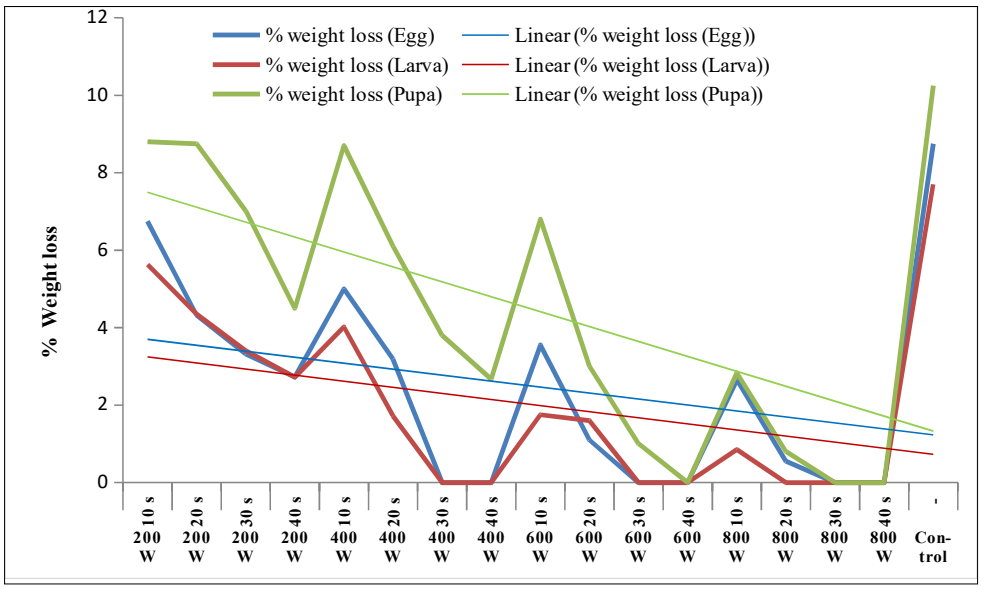

Fig 1: Effect of different microwave treatments on weight loss of green gram grains.

microwave power level leads to temperature rise, which is lethal to the organisms in terms of reduced fecundity, delay in development, reduced locomotion and insect respiration arrest. Ahmady *et al.* (2016) studied the effect of different exposure times of 5, 10, 15, 20 and 25 s at power level of 400W and observed 98.80% mortality of *C. maculatus* at an exposure time of 25 s in stored cowpea. Elzun *et al.* (2012) reported complete mortality of all life stages of *C. maculatus* after exposing the samples of infested cowpea seeds to microwave power level of 136W for 360 s exposure time. Similarly in studies conducted by Singh *et al.* (2012),

Table 2: Effect of different microwave wattages and exposure periods on larval stage of *C. maculatus* in stored green gram.

Treatment	Exposure period (s)	No. of exit holes/100 grains (Mean±SE*)	Total no. of adults emerged (Mean±SE*)
200 W	10	5.67±0.18^{d}	48.00±0.38^{e}
200 W	20	5.00±0.48^{cd}	32.67±0.27^{d}
200 W	30	4.00±0.13^{bcd}	18.67±0.13^{c}
200 W	40	2.00±0.17^{abcd}	14.00±0.23^{bc}
400 W	10	3.67±0.15^{abcd}	26.33±0.17^{d}
400 W	20	0.67±0.14^{ab}	7.33±0.43^{ab}
400 W	30	0.00±0.00^{a}	0.00±0.00^{a}
400 W	40	0.00±0.00^{a}	0.00±0.00^{a}
600 W	10	2.33±0.24^{abcd}	6.67±0.48^{ab}
600 W	20	1.67±0.30^{abc}	5.33±0.17^{a}
600 W	30	0.00±0.00^{a}	0.00±0.00^{a}
600 W	40	0.00±0.00^{a}	0.00±0.00^{a}
800 W	10	1.67±0.20^{abc}	4.00±0.13^{b}
800 W	20	0.00±0.00^{a}	0.00±0.00^{a}
800 W	30	0.00±0.00^{a}	0.00±0.00^{a}
800 W	40	0.00±0.00^{a}	0.00±0.00^{a}
Untreated control	-	24.00±0.39^{e}	73.33±0.37^{f}

*Means within same column followed by same letter are not significantly different (Duncan's MRT, P<0.05).

the different microwave exposure times and power level combinations for 100% mortality of *C. chinensis* were found at 100 s at 700 W, followed by 160 s at 560 W, 200 s at 420 W, 240 s at 280 W and 300 s at 140 W power levels and the grain damage was significantly affected.

Microwave energy at power level of 300, 400, 500 and 600 W against larval and adult stages of stored grain insects recorded complete mortality at 500 W for 14 s or at 400 W for 28 s as per the results obtained by Johnson *et al.* (2011). According to Barbosa *et al.* (2016) cowpea grains (two cultivars taken) infested with larvae of *C. maculatus* were exposed to 240 W microwave power level for 0 (control), 30, 60, 90, 120 and 150 s. For both cultivars, there was a significant reduction in number of insects emerged per grain and in number of insects emerged per treatment. The microwave exposure periods lethal to *C. maculatus* larvae were 120 and 150 s. Karabulut and Baykal (2002) also recorded similar observations and stated that treatment with microwave radiations effectively killed all developmental stages of storage pests with minimal impact on the environment. Azizoglu *et al.* (2011) noticed similar observations for *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) where increasing power level and exposure times in microwave caused increased mortality and further prevented grain damage by this storage pest. Ismaeel and Alsenjari (2008) recorded similar results with microwave radiations. The mortality among the adults of *C. maculatus* that have hatched from eggs exposed to microwave radiations, was 96.6, 96.6, 90.0, 100.0 and 100.0% for cowpea, chickpea, green pea, lentil and broad beans, respectively at the high levels of radiation energy 780 W for 90 s as compared with a control treatments of 6.6, 50.0, 33.3, 76.6 and 86.6%, respectively.

Seed germination

The data on seed germination presented in Table 5 revealed that non-significant differences were observed among

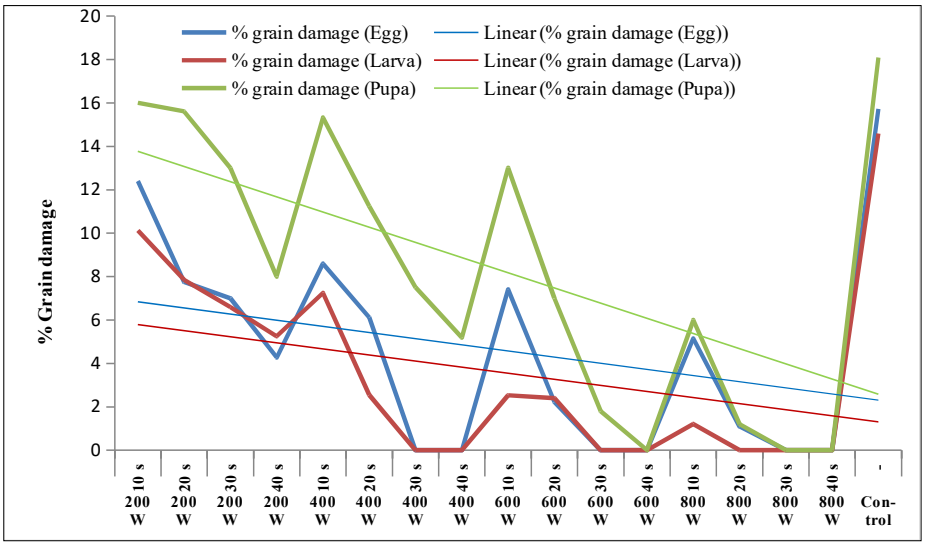

Fig 2: Effect of different microwave treatments on grain damage of green gram grains.

different treatments in respect of seed germination. Seed germination ranged from 80.67 to 88.67 per cent among different treatments. Similar results were reported by Mohapatra *et al.* (2014) and Wang *et al.* (2010), who both reported non-significant differences in germination after treatment with microwave energy.

Table 3: Effect of different microwave wattages and exposure periods on pupal stage of *C. maculatus* in stored green gram.

Treatment	Exposure period (s)	No. of exit holes/100 grains (Mean±SE*)	Total no. of adults emerged (Mean±SE*)
200 W	10	10.00±0.09 ^{f}	88.33±0.21^{h}
200 W	20	9.33±0.11^{f}	66.67±0.30^{g}
200 W	30	6.67±0.06^{de}	66.00±0.23^{g}
200 W	40	6.33±0.06^{de}	34.00±0.37^{e}
400 W	10	8.00±0.36^{ef}	87.33±0.05^{h}
400 W	20	5.67±0.13^{de}	51.00±0.41^{f}
400 W	30	3.00±0.15^{bc}	24.33±0.29^{d}
400 W	40	2.00±0.00^{ab}	12.67±0.29^{bc}
600 W	10	5.00±0.24^{cd}	65.67±0.11^{g}
600 W	20	2.00±0.17^{ab}	21.00±0.55^{cd}
600 W	30	0.67±0.14^{ab}	6.00±0.11^{ab}
600 W	40	0.00±0.00^{a}	0.00±0.00^{a}
800 W	10	1.67±0.20^{ab}	16.00±0.33^{cd}
800 W	20	0.33±0.14^{a}	4.00±0.13^{ab}
800 W	30	0.00±0.00^{a}	0.00±0.00^{a}
800 W	40	0.00±0.00^{a}	0.00±0.00^{a}
Untreated control	-	16.00±0.18^{g}	90.67±0.24^{h}

*Means within same column followed by same letter are not significantly different (Duncan's MRT, P<0.05).

Table 4: Effect of different microwave wattages and exposure periods on adult mortality of *C. maculatus* in stored green gram.

Treatment	Exposure period (s)	Adult mortality (%) (Mean±SE*)
200 W	10	3.33±6.14^{a}
200 W	20	6.67±6.14^{ab}
200 W	30	13.33±2.71^{ab}
200 W	40	13.33±8.85^{ab}
400 W	10	20.00±4.27^{bc}
400 W	20	20.00±11.07^{bc}
400 W	30	30.00±0.00^{c}
400 W	40	30.00±3.66 ^{c}
600 W	10	60.00±3.40^{d}
600 W	20	73.33±2.21^{e}
600 W	30	93.33±6.16^{fg}
600 W	40	100.00±0.00^{g}
800 W	10	83.33±2.71^{ef}
800 W	20	90.00±0.00^{fg}
800 W	30	100.00±0.00^{g}
800 W	40	100.00±0.00^{g}
Untreated control	-	0.00±0.00^{a}

*Means within same column followed by same letter are not significantly different (Duncan's MRT, P<0.05).

Table 5: Effect of different microwave wattages and exposure periods on germination of mungbean.

Treatment	Exposure period (s)	Per cent seed germination (Mean±SE*)
200 W	10	86.00±0.96
200 W	20	87.33±1.27
200 W	30	84.00±1.18
200 W	40	86.33±1.14
400 W	10	82.67±1.70
400 W	20	86.00±1.24
400 W	30	85.67±2.39
400 W	40	85.67±1.00
600 W	10	84.00±1.67
600 W	20	86.67±2.33
600 W	30	88.67±0.61
600 W	40	88.00±0.51
800 W	10	85.33±2.02
800 W	20	84.33±1.83
800 W	30	82.67±1.29
800 W	40	85.33±1.19
Untreated control	-	80.67±1.72

*Means within same column are non-significant (Duncan's MRT, P<0.05).

CONCLUSION

Mortality of *C. maculatus* all life stages was significantly higher at higher exposure time and power levels. Among the life stages of this pulse beetle, eggs were the most susceptible to microwave energy followed by larval, pupal and adult stages. It can be concluded that microwave energy may serve as a potential means of replacing other methods and techniques for the control of stored grain insect pests as their application do not leave any undesirable residues.

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Declaration of competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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