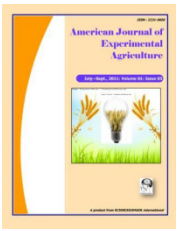

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Efficacy of Microwave Irradiation on the Postharvest Control of Cowpea Bruchid, (*Callosobruchus maculatus*) Coleoptera: Bruchidae on Stored Cowpea

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Authors' contributions

This work was carried out in collaboration between all authors. All the authors were involved in designing the experiment, laboratory studies, statistical analysis and managing the literature search. Author COE wrote the first draft of the manuscript while the other authors edited it. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To investigate the efficacy of microwave irradiation against cowpea bruchid
Study Design: Completely Randomized Design (CRD) replicated five times.
Place and Duration of Study: Crop Protection Research Centre Laboratory, St Xavier's College (Autonomous), Palayamkottai India between May to November, 2012.
Methodology: Mortality, damage assessment, germination test and proximate composition assays were assessed under laboratory conditions (28±2ºC, 70–75% RH and 11:13h photoperiod). Five pairs of 2 day old *C. maculatus* collected from stock culture were kept in a Petri-dish containing 50 healthy seeds (Ife brown variety) and exposed to

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microwave irradiation at 100 power level for 0,4,6,8, and 10 minutes.
Results: The study showed that microwave irradiation significantly (P≤0.05) increased the mortality of *C. maculatus* when compared with control without adversely affecting the viability and proximate composition of the seeds. The potential use of microwave irradiation as alternative seed protectants against *C. maculatus* on stored cowpea is recommended.

Keywords: Cowpea; cowpea bruchid; efficacy; microwave irradiation.

1. INTRODUCTION

Cowpea (*Vigna unguiculata* L. (Walp) is a major source of dietary protein and also used as green vegetables in forms of pod and leaves [1,2]. Nigeria is the World's largest producer with an annual production of 2.1 million tons [3].

With it's high protein, cowpea is a natural supplement to cereal, root and tuber supplies in African diet [3]. Insect pests are major constraints to cowpea production [4,5] and post-harvest losses related to insect pest damage are a major problem for smallholder farmers in Nigeria. *Callosobruchus maculatus* is a serious insect pest of stored cowpea. Synthetic insecticides have been used to control this insect pest. However, the problems of many synthetic insecticides which include: High persistence, poor knowledge of application by resource-poor-farmers, high cost, non availability, genetic resistance and hazards to environment and human health have necessitated the search for alternative environmentally safe and sustainable control measures [6].

The use of irradiation, heat treatments, biopesticides, integrated pest management, use of insect hormones [7,8] have been employed as alternative control strategies [9] recommended disinfestation method of control against *C. maculatus*. The objectives of this research are to ascertain the effect of microwave irradiation on the mortality of *C. maculatus*, viability of cowpea seeds and proximate compositions of irradiated cowpea seeds.

2. MATERIALS AND METHODS

2.1 Insect Culture

Stock culture of *Callosobruchus maculatus* was established from infested cowpea seeds purchased from World bank market, New Owerri, Imo State. Insects were reared on susceptible healthy seeds (Ife Brown) kept in plastic containers covered with perforated muslin cloth held in place with tight rubber bands. The culture was maintained under laboratory conditions (28±2ºC, 70–75% RH and 11:13h photoperiod) at crop protection Research Centre, St Xavier's College, Palayamkottai, Tamil Nadu, India. Normal, healthy 1-2 day old male and female *C. maculatus* were used for the experiment. Sexing was done following the methods of [10].

2.2 Microwave Irradiation Bioassay

Five pairs of 1- 2 day old *C. maculatus* collected from stock culture were kept into Petri-dish containing 50 healthy cowpea seeds (Ife Brown) exposed to microwave irradiation at 100 watts power level for 0, 2, 4, 6, 8 and 10 minutes. The experiment was conducted using

Completely Randomized Design (CRD) replicated five times. Mortality was recorded at 24, 48, 72 and 96 hours after treatments. Mortality data were corrected using the method of [11]. LD_{50} values were estimated by subjecting mortality data to the maximum likelihood program of probit analysis [12] using SPSS software. Weevil Perforation Index (WPI) was determined using the procedure of [13].

$$\text{Weevil Perforation Index} = \frac{\%\text{ of treated grains perforated} \times 100}{\%\text{ of control grains perforated} + \%\text{ of treated grains perforated}}$$

2.2 Effect of Irradiations on the Viability of Cowpea Seeds

2.2.1 Germination test

This was carried out in the screen house using seedling Trays. Two seeds from each treatments were randomly selected and planted in each hole and watered daily. The experimental design was Completely Randomized Design (CRD) replicated ten times.

Data were collected on seedling emergence at 4, 5, 6 and 7 days after planting. Percentage

emergence was determined using the formular: $\frac{\text{Number of emerged seeds} \times 100}{\text{Number of seeds planted}}$

2.2.2 Proximate composition

After three months of storage, irradiated and unirradiated cowpea seeds were analyzed in triplicate for protein, moisture, ash content, crude fibre and carbohydrate using the official and standard methods of analysis [14].

3. RESULTS AND DISCUSSION

3.1 Mortality Test

Results on the effect of microwave irradiations (100 watts) on the control of the *C. maculatus* are presented in Table 1. The results showed that all the exposure periods 2, 4, 6, 8 and 10 minutes reduced the population of *C. maculatus* 24 hours after exposure with insects exposed to 100 watts power level for 8 minutes having the highest mortality level (24.05±5.04) while control had the least value (0.00). All the exposure periods significantly ($P<0.05$) differed from control except insects exposed to 2 minutes and 4 minutes. There was no significant difference ($P>0.05$) among *C. maculatus* exposed for 6, 8 and 10 minutes.

At 48 hours after exposure to microwave irradiation, the mortality level followed the same trend with *C. maculatus* exposed for 8 minutes having a percentage mortality level of (43.89±7.16) while control had the least value (0.00±0.00). However, insects exposed for 2 and 4 minutes did not significantly ($P>0.05$) differ from control. Insect exposed for 6, 8 and 10 minutes did not differ from one another but significantly ($P<0.05$) differ from other exposure periods.

At 72 hours after exposure, *C. maculatus* exposed for 10 minutes had the highest mortality level (67.54±6.05) while control had the least value (0.00±0.00). The results showed that all the exposure periods except insects exposed for 2 minutes significantly ($P<0.05$) differed from control. However, there was no significant difference ($P>0.05$) between insects exposed for 6, 8 and 10 minutes.

At 96 hours after exposure, the percentage mortality level was in the following order: 10 minutes exposure (74.41±3.53) > 8 minutes (77.26±4.05) > 6 minutes (55.00±5.49) >4 minutes (33.09±2.00) > 2 minutes (16.55±2.21) > control (0.00±0.00).

Results showed that mortality of *C. maculatus* increased with increase in the time of exposure to microwave irradiation. The mortality of insects could be due heating up of water in insects thereby causing death by cellular disruption [15]. Similar observation was made by [16] who reported that application of high energy microwaves in the flowing grain of a pilot scale trial.

Table 1. Effect of microwave irradiation (100 watts) on the mortality of *C. Maculates*

Treatment	Mortality			
	24 hrs	48 hrs	72 hrs	96 hrs
Control (0 Minutes)	0.00^{c} ±0.00	0.00^{c} ±0.00	0.00^{c}±0.00	0.00^{e}±0.00
2 Minutes exposure	4.22^{c} ±2.59	4.22^{c} ±2.59	14.09^{bc}±7.46	16.55^{d}±2.21
4 Minutes exposure	8.17^{bc} ±6.48	15.83^{bc}±4.38	27.02^{b}±5.62	33.09^{c}±2.00
6 Minutes exposure	19.89^{ab} ±3.79	31.94^{ab}±6.09	54.41^{a}±4.41	55.00^{b}±5.49
8 Minutes exposure	24.05^{a} ±5.04	43.89^{a}±7.16	66.98^{a}±3.99	77.26^{a}±4.05
10 Minutes exposure	22.11^{ab} ±3.10	32.50^{ab}±7.12	67.54^{a}±6.05	74.41^{a}±3.53

Means with the same letters within the same column are not significantly different (P≥0.05)

Was effective in killing insect pests without any negative effect on the quality of the grains. [17] who worked on disinfestations of stored corn using microwave energy reported that microwave irradiation was effective against 3 stored grain insects: *Sitophilus zeamais*, *Tribolium castaneum* and *Plodia interpunctella*. They reported that microwave irradiation with good penetrability can kill pests existing inside or outside grain kernels.

3.2 Lethal Time (LT_{50})

This was determined using the maximum likelihood of probit analysis and the results are as presented in Table 2. The results showed that mortality increased with time after exposure to microwave irradiation. Ninety six (96) hours after exposure had the least LT_{50} value of 5.2 minutes when compared with other exposure periods. The LT_{50} value is in the following order: 96 hours after exposure (5.2 minutes) < 72 hours < (5.8 minutes) < 48 hours (12.2 minutes) <24 hours (29.1 minutes).

The result implies that exposure of *C. maculatus* to microwave irradiation (100 power level) for 29.1 killed 50% of the pest population within 24 hours. Exposure for 12.2 minutes killed 50% of pest population within 48 hours, exposure for 5.8 killed 50% of pest population within 72 hours while exposure for 5.2 minutes killed 50% of pest population within 96 hours. This corroborates the report of [18] who worked on a thermal lethal model of rice weevils subjected to microwave irradiation and reported that lower power with longer treatment time is more effective at killing insect pests.

Table 2. LT_{50} values of microwave irradiations (100 watts)

Time (hours)	LT_{50}
24	29.1±5.6
48	12.2±0.9
72	5.8±0.5
96	5.2±0.4

3.3 Damage Assessment

The effect of microwave irradiation on the percentage perforation of cowpea seeds, number of seeds perforated and Weevil Perforation Index (WPI) is shown in Table 3. Results showed that there were significant differences (P≤0.05) among the exposure periods. Control had the highest percentage seed perforation value (81.20±2.42) while seeds exposed to microwave irradiation (100 watts) for 10 minutes had the least value (36.80±2.42). Similarly, control had the highest number of seeds perforated (40.60±1.21) while seeds exposed to microwave irradiation for 10 minutes had the least value (18.40±1.21).

The least Weevil Perforation Index (45.3) was recorded in cowpea seeds exposed to microwave irradiation for 10 minutes. Exposure to microwave irradiation for 2 minutes had had WPI (68.5), exposure for 4 minutes had WPI (55.7), exposure for 6 minutes had WPI (55.2) while exposure for 6 minutes had WPI (51.7).

Results on the assessment of damage to cowpea seeds exposed to microwave irradiation (100 watts power level) shows that the different exposure periods gave varied degree of protection when compared with control. The different exposure periods did not effectively protect the cowpea seeds after 3 months of storage as seen in the Weevil Perforation Index values which are above 50. However, cowpea seeds exposed to microwave irradiation for 10 minutes which have a Weevil Perforation Index of (45.3) offered better protection against *C. maculatus* when compared with other exposure periods. The reduction in the percentage perforation of cowpea seeds exposed for 10 minutes could be due to reduction in moisture content and the hardening of seed coat as a result of longer time of exposure to microwave radiation which makes it difficult to perforate the seeds.

3.4 Germination Test

The results of the effect of microwave irradiation on the seed viability are as shown in Table 4. Emergence count was taken at 4, 5, 6 and 7 days after planting. At 4 days after planting, cowpea seeds exposure for 4 minutes had the highest value (35.00±10.67). However, there was no significant difference (P≤0.05) among all the exposure periods At 5 days after planting, cowpea seeds exposed for 2 minutes had the highest value (55.00±11.67) while cowpea seeds exposed for 10 minutes had the least value (10.00±6.67). All the exposure periods significantly (P≤0.05) differed from cowpea seeds exposed for 10 minutes but did not differ significantly (P≥0.05) from one another.

At 6 days after planting, cowpea seeds exposed for 2 and 4 minutes had the highest values (65.00) while those exposed for 10 minutes had the least value (25.00±11.18). Though there was no significant difference (P≥0.05) among all the exposure periods.

At 7 days after planting, their performances were in the following order: 2 minutes exposure (70.00±11.06) > 4 minutes (65.00±13.02) > 6 minutes (60.00±12.47) = 8 minutes (60.00±12.47) > control (40.00±14.53) > 10 minutes (30.00±11.06). Though, there was no significant difference.

The results showed that the germination and emergence of cowpea seeds exposed to microwave irradiation decreased with increase in exposure time. However, there was no significant difference (P≥0.05) among all the exposure periods. Similarly observation was made by [17] who reported that germination of corn exposed to microwave irradiation decreased as power level and exposure time increased. [19] Who studied the physical, chemical and baking properties of wheat dried with microwave energy concluded that germination capacity was decreased by microwave energy. The decrease in germination capacity was related to the final temperature and the moisture contents of the grain.

Table 3. Damage assessment of cowpea seeds exposed to microwave irradiation (100 watts)

Treatments	Perforated seeds	Unperforated seeds	% seed Perforation	WPI
Control	40.60^{a}±1.21	9.40^{c}±1.21	81.20^{a}±2.42	-
2 minutes	27.80^{b}±1.83	22.20^{b}±1.83	55.60^{b}±3.66	68.5
4 minutes	22.60^{c}±1.81	27.40^{a}±1.81	45.20^{c}±3.61	55.7
6 minutes	22.40^{c}±1.08	27.60^{a}±1.08	44.80^{c}±2.15	55.2
8 minutes	21.00^{c}±0.71	29.00^{a}±0.71	42.00^{c}±1.41	51.7
10 minutes	18.40^{c}±1.21	31.60^{a}±1.21	36.80^{c}±2.42	45.3

Means with the same letters within the same column are not significantly different (P≥0.05)

Table 4. Effect of microwave irradiation (100 watts) on the percentage grmination of cowpea seeds

Treatments	4 DAP	5 DAP	6 DAP	7 DAP
Control	20.00^{a}±8.17	20.00^{b}±18.17	40.00^{a}±14.53	40.00^{a}±14.53
2 Minutes exposure	25.00^{a} ±11.18	55.00^{a} ±11.67	65.00^{a}±10.67	70.00^{a}±11.06
4 Minutes exposure	35.00^{a} ±10.67	50.00^{ab}±10.54	65.00^{a}±13.02	65.00^{a}±13.02
6 Minutes exposure	15.00^{a} ±7.64	40.00^{ab} ±12.47	55.00^{a}±11.67	60.00^{a}±12.47
8 Minutes exposure	10.00^{a} ±6.67	25.00^{ab}±8.33	60.00^{a}±12.47	60.00^{a}±12.47
10 Minutes exposure	10.00^{a}±6.67	10.00^{c}±6.67	25.00^{a}±11.18	30.00^{a}±11.06

DAT – Days after planting. Means with the same letters within the same column are not significantly different (P≥0.05)

3.5 Proximate Composition

The results on the effect of microwave irradiation (100 watts) on the proximate composition of cowpea seeds are presented in Tables 4 and 5. The proximate compositions determined are protein, fat, fibre, ash, moisture and carbohydrates. The results showed that control had the highest protein value (24.07±0.34) while cowpea seeds exposed for 10 minutes had the least value (21.00±0.21). Control significantly (P≤0.05) differed from other treatments. There was no significant difference (P≥0.05) between cowpea seeds exposed for 4 and 6 minutes. Similarly, there was no significant difference between cowpea seeds exposed for 8 and 10 minutes. The range of protein content is between 21.00 and 24.07. The reduction in protein content noticed in this experiment could be as a result of denaturing of protein by microwave irradiation that involves heat. The protein range between 21.00 and 24.07% observed in this

experiment is similarly to the report of [20]. The results from their experiment on Lipids and other Constituents of *V. unguiculata* and *Phaseolus vulgaris* grown in Northern Nigeria showed protein content value between 20.5 and 31.7%. [21] Who worked on influence of mutation induction on the chemical composition of cowpea, *V. unguiculata* reported a protein content value between 16.04 and 31.06%. Similarly, [22] who researched on Physico-chemical and functional properties of bean flours of three cowpea (*V. unguiculata* varieties in Ghana reported a protein content between 26.53 and 29.00%.

The result on the effect of microwave irradiation on the fat content of cowpea seeds showed that control (unexposed) seeds had the highest value of 3.07±0.29 while cowpea seeds exposed for 2 minutes had the least value 2.20±0.12. Control significantly (P≤0.05) differed from other exposure periods. However, there is no significant difference among other exposure periods. The significant difference observed is an indication that the difference was induced by microwave irradiation. The reduction in crude fat content is healthy as most fatty foods are associated with high cholesterols that are injurious to human health. [22] who worked on compositional evaluation of some cowpea varieties and some under-utilized edible legumes in Nigeria reported an average crude fat content of 5.9%. [21] reported a crude fat content of 2.40 – 4.33%, [23] reported a crude fat content of 2.50–3.99% while [20] observed a crude fat content of 1.14–3.03%.

Cowpea seeds exposed for 2 minutes had the highest fibre content (2.23±0.03) while the seeds exposed for 10 minutes had the least value (1.73±0.03). There was no significant difference (P≥0.05) among cowpea seeds exposed for 2, 4 and 6 minutes but they significantly differ from others. Similarly, there was no significant difference among seeds exposed for 8, 10 minutes and control. [21] Reported a crude fibre content of 1.03–3.95% while [20] reported a crude fibre content of 1.7–4.5%. High crude fibre content could be useful in providing bulk to foods to aid digestibility and to minimize constipation [23].

Microwave irradiation (100 watts) did not significantly affect the ash content of cowpea seeds. However, control had the highest value (3.53±0.27) while cowpea seeds exposed for 10 minutes had the least value (2.93±0.09). [23] Reported ash content of 2.95–3.22% while [24] who worked on the Varietal Differences in Physical, Chemical and Proximate composition of cowpea (*V. unguiculata*) reported ash content of 3–4%. High ash content could be important source of minerals for consumers [25].

Moisture content of cowpea seeds was significantly (P≤0.05) affected by microwave irradiation. Cowpea seeds exposed for 2 minutes had the highest value (10.67±0.24) while the ones exposed for 10 minutes had the least (8.20±0.20). There was no significant difference among all the exposure periods except cowpea seeds exposed for 10 minutes. Reduction in moisture content could be as a result of heat generated by microwave irradiation. Low moisture content will enhance storability of cowpea seeds and could minimize seed perforation by *C. maculatus* owing to hardness of seed coat.

Carbohydrate content of cowpea seeds was significantly affected by microwave irradiation. Cowpea seeds exposed for 10 minutes had the highest value (63.80±0.15) while the control had the least value (56.97±0.90). Cowpea seeds exposed for 10 minutes significantly differed from other exposure periods. There was no significant difference among cowpea seeds exposed for 2, 4 and 6 minutes. [23] Reported carbohydrate content of 50.22-53.98%. High carbohydrate and ash contents indicate that cowpea could be important sources of minerals and energy for consumers [25].

Table 5. Effect of microwave irradiation (100 watts) on the proximate composition of cowpea seeds

Treatments	Protein	Fat	Fibre	Ash	Moisture	Carbohydrate
Control	22.97^{b}±0.20	2.20^{b}±0.12	2.23^{a}±0.03	3.27±0.03	10.67^{a}±0.24	58.67^{c}±0.24
2 Min. exposure	23.00^{b} ±0.15	2.53^{b}±0.07	2.13^{ab}±0.12	3.10±0.06	10.10^{a}±0.06	59.13^{c}±0.27
4 Min. exposure	22.73^{b}±0.18	2.57^{b}±0.03	2.07^{ab}±0.09	3.07±0.03	9.53^{ab}±0.54	60.03^{c}±0.49
6 Min. exposure	21.20^{c}±0.12	2.43^{b}±0.03	1.83^{bc}±0.03	3.10±0.12	9.33^{ab}±0.41	62.10^{b} ±0.26
8 Min. exposure	21.00^{c} ±0.21	2.33^{b}±0.07	1.73^{c}±0.03	2.93±0.09	8.20^{b}±0.20	63.80^{a}±0.15
10 Min. exposure	24.07^{a}±0.34	3.07^{a}±0.29	1.87^{bc}±0.09	3.53±0.27	10.50^{a}±0.45	56.97^{d}±0.90

4. CONCLUSION

The study showed that microwave irradiation significantly (P≤0.05) increased the mortality of *C. maculatus* when compared with control without adversely affecting the viability and proximate composition of the seeds. The potential use of microwave irradiation as alternative seed protectants against *C. maculatus* on stored cowpea is recommended.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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