Efficiency of Cold Plasma and Microwave Power on Different Stages of *Callosobruchus maculatus*

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Abstract

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This study investigated the effectiveness of cold plasma (CP) technology and microwave (MW) treatments on various stages of *Callosobruchus maculatus*. Beetle stages were exposed to three voltage levels of CP (150, 200 and 250V) for seven different exposure periods (1-20 minutes) and MW at two power levels (100 and 180W) for seven exposure periods (0.5-10 minutes). The results obtained showed that the effects of CP and MW on the beetle stages were independent of voltage, power level, and exposure time. For CP, mortality rates of *C. maculatus* stages were 51.2, 55.0, 56.9 and 50.5% after 5 minutes of exposure at 150V for adults, eggs, larvae, and pupae, respectively. Complete kill of all stages was achieved after 5 minutes of exposure at 250V. For MW, at 100W power level and 1 minute of exposure, mortality rates were 71.0, 67.7, 75.5 and 56.7% for the above-mentioned stages, respectively. Complete kill of all stages was achieved after 6 minutes of exposure at 180W. The larval stage was the most sensitive to both CP and MW, while the pupal stage was the most tolerant. Additionally, exposure to CP voltages enhanced cowpea seed germination, whereas microwave treatment decreased germination by 24%. CP voltages also reduced the cooking time of cowpea seeds.

Keywords: Cold plasma, microwave, *Callosobruchus maculatus*, germination.

Introduction

Cowpea, with its protein content ranging from 23% to 25%, serves as a valuable protein source suitable for the diets of many people in developing countries. However, small-scale cowpea production faces significant challenges, as farmers often experience substantial losses in weight, nutritional value, and grain viability due to various insect pests both in the field and during storage (Kaliramesh *et al*., 2013; Mkenda & Ndakidemi 2014).

Callosobruchus maculatus (Coleoptera: Bruchidae) is a major pest of pulses, causing storage losses of 10–20% (Phillips & Throne, 2009), and losses can reach 100% of stored cowpea beans within a few months (Kang *et al*., 2013; Melo *et al*., 2015). Infested seeds cannot be planted or consumed due to the holes caused by Bruchids (Adedire *et al*., 2011).

The use of chemical insecticides for the control of beetles may lead to unintended side effects, such as adverse effects on human and animal health, development of insecticide resistance, and environmental pollution (Ayub, 2021).

Phosphine (PH_3) fumigation is globally recognized as the most effective method for disinfecting large quantities of cowpeas. However, the significant drawback is the development of high resistance of treated insects to PH_3 (Venkidusamy *et al*., 2017). Therefore, it is essential to develop safe substitute methods that offer the same benefits as phosphine while minimizing its risks.

Cold plasma (CP) technology is employed in food processing to boost antimicrobial activity, modify structure, decontaminate surfaces, and disinfect food-processing equipment and saving stored grain from insect infestation.

Such technology has many benefits, including minimal heat, quick release time and quick processing time. Pathan *et al*. (2021) showed that cold plasma exhibits strong effect against *C. chinensis* and has the potential to effectively control a broad spectrum of storage pests. Additionally, it is recommended as a promising technique for protecting dry food against stored product pests without any effect on food quality (Nasr, 2020; Sayed *et al*., 2021; Zinhoum, 2020). Microwaves, a form of electromagnetic energy, offer swift heating for disinfestation purposes. This method relies on the dielectric heating effect within the grain, which, being a relatively poor conductor of electricity, facilitates the process. A key benefit of microwave energy is its lack of chemical residue in food, along with no adverse effects on human health (Abed *et al*., 2023).

Microwave treatment is considered a safe and competitive alternative method to fumigation for the control of insect pests (Sadeghi *et al*., 2018). It has no adverse effects on food safety and environmental damage.

The objectives of this study were to examine the efficacy of cold plasma technology and microwave treatment against *C. maculatus* stages as well as the impact of each method on cowpeas seed gemination, hardness and cooking time.

Materials and Methods

Insect culture

The initial culture of *C. maculatus* was obtained from a laboratory culture maintained for several generations on dry cowpea seeds in the Stored Grain Pests Department, Plant Protection Research Institute.

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C. maculatus beetles were bred on sterilized and conditioned cowpea seeds in 500 ml glass jars. Each jar contained 300-400 adult beetles (male and female) aged 0-2 days, for egg-laying purposes. To prevent insect escape, the jars were covered with muslin cloth secured by a rubber band. The insect-containing jars were then kept in an incubator at 26±2°C and 60±5% relative humidity for one week. Afterward, the parent adults were sieved out and discarded. The jars were maintained in the laboratory under the same conditions to allow for the development of different beetle stages.

Cold plasma source

Cold plasma is produced through a process called Corona discharge, which involves a corona discharge head connected to a high-voltage power supply and a fan. When the voltage exceeds the air breakdown value, an electrical arc is created. This arc is expelled from a small aluminum dielectric enclosure made of medium-thickness bare aluminum by a stream of air. While the input voltages ranged from 0 to 250V, the output voltage reached 15 kv.

Microwave source

The microwave oven used was Samsung Model MS23K3513AK. 230v-50Hz, frequency as 2450 MHz, with power input of 1150W and power output of 800 W.

Bioassay treatment

Firstly, biological tests were performed in order to determine the duration of the various developmental stages at 26±2°C and 60±5% RH. Batches of 30 adults or 20g of food containing immature stages (eggs, larvae or pupae) were introduced in a petri dish and regularly distributed in one layer.

Petri dishes were exposed to various cold plasma voltage levels of 150, 200 and 250 V for different exposure periods (1-20 minutes) inside a foam chamber at a fixed distance of 4 cm from the cold plasma nozzle, and other Petri dishes were placed in a microwave device and exposed to MW power of 100 and 180 W for different exposure periods (0.5 -10 minutes).

All procedures were repeated five times. 24 hours after exposure, adult mortality was determined. In the case of immature stages, all replicates poured in jars covered with a muslin cloth and fixed with a rubber band. Jars were incubated under rearing conditions until adult emergence which were counted. The effect of treatments on the immature stages was estimated based on the reduction in adults emergence rate using the following equation:

$$\text{Reduction rate (\%)} = \frac{\text{No. of emerged adults in control} - \text{No. of emerged adults in treatment}}{\text{No. of emerged adults in control}} \times 100$$

Cowpea seed hardness

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

Seed germination rate (%)

Groups of 25 cowpea seeds treated with 250V of CP for 5 min, 180 W of MW for 6 min and control) were placed into each dish on top of a moist cotton layer. Four replicates of treated and untreated seeds were used. After 14 days, the numbers of germinated seeds were counted and expressed as percent germination rate.

Cooking time

Two hundred grams (treated with 250 V of CP for 5 min, 180 W of MW for 6 min and control) of cowpea seeds were soaked for 1 h in tap water then cooked in 1250 ml of water. The average cooking time (min) for each replicate was determined.

Results and Discussion

Effect of Cold plasma (CP)

The effect of CP at three input voltage levels 150, 200 and 250V at different exposure periods was investigated against *C. maculatus* stages (Table 1). Results obtained showed that, adult mortality rate and reduction in adult emergence were increased with increasing CP power and exposure time.

At 150 V and 5 mins of exposure, the mortality rate of *C. maculatus* stages were 51.2, 55.0, 56.9 and 52.5% for adults, eggs, larvae and pupae, respectively. These values increased when the exposure time was prolonged to 10 min to reach 76.5, 81.7, 82.5 and 75.3% for the aforementioned stages, respectively. Meanwhile, all stages needed 20 min of exposure at 150 V to be completely killed.

The mortality rate increased with CP voltage increase, and reached at 200 V and 5 min of exposure 95.0, 96.1, 97.8 and 90.1%, for the above-mentioned stages, respectively. Complete mortality was obtained for adults, eggs and larvae after 7 min of exposure and for pupae after 10 min of exposure. 250 V and 5 min of exposure was sufficient to completely kill all beetle stages.

Results showed that mortality rate and reduction in adult emergence were increased with increasing voltage and exposure time. Anbarasan *et al*. (2022) indicated that the decrease in adult emergence increased with higher voltages and exposure periods when cold plasma was used at low pressure (<2 mbar) and different voltages (1.0–2.0 kv) when applied against *C. maculatus* beetle stages. Maximum mortalities of 100.0%, 98.89%, and 37.33% were achieved after 24 minutes of extended CP exposure at 2.0 kv in the adult, pupal, and egg stages, respectively.

Furthermore, the larval stage of *C. maculatus* exhibited high sensitivity to CP, whereas the pupal stage was the most tolerant. Nasr *et al.* (2020) revealed that the larval stage of both *Oryzeaphilus surinamensis* and *Plodia interpunctella* was highly sensitive to cold plasma, with complete kill achieved at the lowest voltage (150V) after 20 and 25 minutes of exposure, for the two insects, respectively. In contrast, the pupal stage of these insects exhibited the highest tolerance. Conversely, the pupal stage of *Sitophilus granaries*, *Rhizopertha dominica*, and *Tribolium castaneum* showed the highest tolerance to cold plasma (Nasr *et al*., 2020).

Table 1. Effect of cold plasma treatment against *C. maculatus* different developmental stages.

Voltage (V)	Stage	Mortality rate (%) ± standard error after different exposure periods (min)						
		1	2	5	7	10	15	20
150	Adult	20.0±0.0	30.0±2.9	51.2±1.4	66.7±2.6	76.5±1.7	100.0±0.0	100.0±0.0
	Egg	22.2±2.9	33.3±1.9	55.0±3.0	68.3±1.7	81.7±3.3	100.0±0.0	100.0±0.0
	Larva	27.5±2.0	36.3±1.0	56.9±1.0	70.5±2.0	82.5±2.3	100.0±0.0	100.0±0.0
	Pupa	19.8±1.7	29.7±2.6	50.5±0.0	63.4±1.0	75.3 ±1.7	100.0±0.0	100.0±0.0
200	Adult	55.5±0.0	76.2±1.7	95.0±2.9	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
	Egg	56.7±1.7	77.5±3.4	96.1±1.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
	Larva	67.7±1.7	78.4±1.0	97.8±0.3	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
	Pupa	50.0±2.9	71.7±1.7	90.1±1.0	99.0±1.0	100.0±0.0	100.0±0.0	100.0±0.0
250	Adult	88.3±1.7	95.1±1.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
	Egg	89.9±4.8	96.7±2.1	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
	Larva	91.2±0.0	98.3±1.7	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
	Pupa	83.2±1.0	94.1±1.7	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0

Effect of Microwave (MW)

The data obtained when using MW power levels of 100 and 180 W for different exposure periods against *C. maculatus* stages is illustrated in Table 2. Results showed that adult mortality rate and reduction in adult emergence were increased with the increase of MW power and exposure period, with complete adult mortality achieved when exposed to MW at 100 W for 8 min and 4 min of exposure at 180 W. Adult emergence reduction rates were 67.7, 75.5 and 56.7% for eggs, larvae and pupae exposed for 1min at the lowest power 100W, respectively, whereas, a complete reduction in adult emergence was achieved after 6 min for larvae and 8 min of exposure for egg and pupal stages.

At 180 W power level, all larvae were totally killed after 2 min of exposure only, while eggs and pupae needed 6 min of exposure to reach 100% kill. Exposure at 180 W power level for 6 min was enough to kill all stages of *C. maculatus,* which is in agreement with previous report (Abd El-Raheem & Said, 2016). Complete mortality of *C. maculatus* was immediately achieved at an air temperature of 60 °C with microwave (MW) exposure for a 6 min exposure period to hot air at a power level of 2900 W (Mohapatra *et al*., 2019).

Effect of treatments on cowpea seeds characteristics

The effect of CP and MW treatments on cowpea seeds germination rate, hardness and cooking time compared with untreated seeds are summarized in Table 3. The results obtained showed that germination rate of cowpea seeds were 88%, 60% and 84 % for treated seeds with CP, MW and untreated seeds, respectively.

These results obtained indicated that CP treatment increased cowpea seed germination rate compared with untreated seeds. Whereas, the MW treatment decreased the germination rate by only 24 %, which is in agreement with a previous report on wheat seeds (Abotaleb *et al*., 2021). The average seed hardness (texture) values obtained were 61.7, 73.7 and 58.7 (N) of the CP, MW and control treatments, respectively. Results also showed that there were insignificant changes in the hardness of cowpea seeds treated with CP. Furthermore, the seed cooking time reached 39.7, 47.0 and 41.7 min in response to CP, MW and control treatments, respectively. CP treatment decreased cooking time compared with microwave and control treatments. Likewise, Sarangapani *et al*. (2017) reported earlier that exposure to Radio Frequency plasma for 5-15 min decreased black gram seeds hardness, cooking time, ash and moisture content.

It can be concluded from this study that CP treatment at 250 V for 5 min and MW power of 100 W for 6 min caused complete mortality to all stages of *C. maculatus*. Thus, it can be recommended that the use of cold plasma and microwave treatments can be considered as a safe alternative technique for the control of stored cowpea pests.

Table 2. Effect of Microwave treatment against *C. maculatus* developmental stages.

Power (W)	Stage	Mortality % ± standard error after different exposure periods (min)						
		0.5	1	2	4	6	8	10
100	Adult	63.8±1.6	71.0±1.6	81.9±1.1	90.4±1.5	99.3±1.0	100.0±0.0	100.0±0.0
	Egg	68.5±2.5	67.7±1.7	75.0±1.4	94.4±1.1	98.6±1.0	100.0±0.0	100.0±0.0
	Larva	71.7±1.7	75.5±1.1	86.0±1.9	93.5±0.0	100.0±0.0	100.0±0.0	100.0±0.0
	pupa	52.0±1.2	56.7±1.7	73.0±2.0	88.3±1.7	95.0±1.6	100.0±0.0	100.0±0.0
180	Adult	79.7±1.3	83.2±1.0	98.6±1.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
	Egg	73.9±1.8	86.0±2.9	94.2±1.0	99.3±1.0	100.0±0.0	100.0±0.0	100.0±0.0
	Larva	86.0±1.4	94.1±1.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
	pupa	56.0±1.9	76.2±1.7	89.5±1.1	98.6±1.0	100.0±0.0	100.0±0.0	100.0±0.0

Table 3. Effect of different treatments on some properties of cowpea seed.

Parameters	Treatments		
	Cold plasma	Microwave	Control
Germination rate (%)	88.0±0.6	60.0±1.0	84.0±0.6
Hardness (N)	61.7±1.2	73.7±2.0	58.7±0.9
Cooking time (min)	39.7±0.9	47.0±1.5	41.7±1.5

الملخص

زينهم، رشا وم. نصر. 2025. تأثير معاملات البلازما الباردة وموجات الميكرويف على الأطوار المختلفة لخنفساء اللوبياء (*Callosobruchus maculatus*). مجلة وقاية النبات العربية، 43(2): 215–219. https://doi.org/10.22268/AJPP-001322

هدفت هذه الدراسة إلى التحقق من فعالية كلٍّ من البلازما الباردة وموجات الميكرويف على الأطوار المختلفة لخنفساء اللوبياء (*Callosobruchus maculatus*). تمّ تعريض الأطوار المختلفة لثلاث مستويات من فروق الجهد (150، 200 و250 فولت) من البلازما الباردة على 7 فترات تعريض تراوحت من 1 حتى–20 دقيقة؛ ومستويين طاقة لموجات الميكرويف (100 و 180 وات) و 7 فترات تعريض تراوحت من 0.5 حتى – 10 دقائق. أظهرت النتائج أن تأثير كلٍّ من البلازما الباردة وموجات الميكرويف على الأطوار المختلفة لخنفساء اللوبياء يعتمد على فرق الجهد (الفولت) ومستوى الطاقة ومدّة التعريض. بالنسبة للبلازما الباردة، بلغت نسب موت أطوار خنفساء اللوبياء 51.2، 55.0، 56.9 و 50.5% بعد 5 دقائق من التعريض عند على 150 فولت لكلٍّ من الحشرة الكاملة، البيض، اليرقات والعذارى، على التوالي. تم الحصول على موت كامل للحشرة بعد التعريض لمدة 5 دقائق عند 250 فولت. أمّا بالنسبة لموجات الميكرويف، وعند استخدام طاقة 100 وات ومدّة تعريض لدقيقة واحدة، فقد بلغت معدلات الموت 71.0، 67.7، 75.5، و 56.7% لكلٍّ من الحشرة الكاملة، البيض، اليرقات والعذارى، على التوالي. كما حصل موت كامل للحشرة بعد التعريض لمدة 6 دقائق عند طاقة 180 وات. كان طور اليرقة هو أكثر الأطوار حساسية، بينما كانت العذارى أكثرها تحملاً. كما أن التعريض للبلازما الباردة أدى إلى زيادة نسبة إنبات بذور اللوبياء في حين أن معاملة موجات الميكرويف الموجات الدقيقة قد خفَّضتها بنسبة 24% مقارنةً بالشاهد. وعلاوةً على ذلك، فقد خفّضت البلازما الباردة الوقت اللازم لطهي بذور اللوبياء.

كلمات مفتاحية: بلازما باردة، ميكروويف، خنفساء اللوبياء، إنبات.

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