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Susceptibility of The Saw-Toothed Grain Beetle, *Oryzaephlius surinamensis* (L.) and The Indian Meal Moth, *Plodia interpunctella* (Hübner) Infested Stored Products to Cold Plasma

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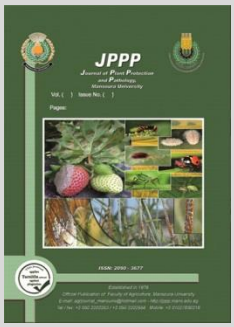

ABSTRACT

The response of the saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.) stages and immature stages of the Indian meal moth, *Plodia interpunctella* (Hübner) to cold plasma generated from corona discharge was investigated. Three voltage levels of cold plasma (150, 200 and 250 V) in a combination of six exposure times between (1-25 minutes) were used. Results showed clearly that, the effect of cold plasma against two insect stages was power and exposure time–independent. In addition, the effect of cold plasma was higher on *O. surinamensis* stages than immature stages of *P. interpunctella* and the larval stage of each insect species was most sensitive to cold plasma, which completely killed at the lowest voltage (150 V) after 20 and 25 min of exposure for *O. surinamensis* and *P. interpunctella* larvae, respectively. While, the pupal stage of each insect was the most tolerant one. Mortality percentages at the highest voltage (250 V) and the longest time of exposure (25 min) were 98.9 and 99.0% for *P. interpunctella* and *O. surinamensis* pupae, respectively. Thus, cold plasma is a promoting technique for stored product insects control in the near future with further studies to know the best method for product exposure without any effect on product quality.

Keywords: *Oryzaephilus surinamensis, Plodia interpunctella*, cold plasma, voltage, exposure time.

INTRODUCTION

The Saw-toothed grain beetle, *Oryzaephlius surinamensis* (L.) and the Indian meal moth, *Plodia interpunctella* (Hübner) are a widespread and known as the most important pests attacking stored-products within the food industry, food manufacturing stores and retail facilities (Trematerra and Thron, 2012). Insect species infest stored products such as a cereal products, dried fruits, chocolate, cocoa and nuts and causing a serious loss in both the quantity through feeding damage and quality by contaminating the product with cast skin feces and a silken webbing of *P. interpunctella* (Phillips *et al.*, 2000); (Rossi *et al*., 2010). The use of methyl bromide to fumigate food commodities and facilities must be phased out in accordance with the Montreal protocol due to its effect on the ozone layer with the exception of certain critical use exemptions for quarantine and pre-shipment purposes (EPA, 2004). So, alternative environmentally friendly techniques are clearly needed. Plasma is the 4th state of substance according to a scheme expressing an increase in the energy level from solid to liquid to gas and ultimately to plasma (Misra *et al.*, 2011).

Recently, an emerging novel technology called Cold Atmospheric Plasma (CAP), a specific type of plasma that is less than 40°C at the point of application, it was applied to bacteria, plant cells and animal cells (Bermudez *et al.*, 2013). However, it has been proven that, the effect is different in the three organisms (Dobrynin *et al*., 2009). It is demonstrated that the reactive oxygen and nitrogen species (RONS) are the essential signaling molecules organizing many growth processes in mammalian, microorganisms and plants. In addition, it was used in sterilization for medical applications (Laroussi, 2005). The dose value of (RONS) can play an essential role in the control of pest control techniques (Kwon *et al*., 2019). Many investigators studied the effect of cold plasma against various food contaminant microorganisms on different materials, (Ohkawa, 2006; Gweon, 2009 and Moisan *et al*., 2001). Mishenko *et al.* (2000) evaluated the effect of a plasma jet on *Sitophilus granaries* (L.). Keever *et al.* (2001) examined it on cigarette beetles, *Lasioderma serricorne* (F.). The aim of this study was to evaluate the effect of cold plasma against different stages of *O. surinamensis* and immature stages of *P. interpunctella*.

MATERIALS AND METHODS

Rearing of insects:

Adults of *O. surinamensis* were carefully isolated from the infested dates collected from storehouses, then introduced to a glass jar (approx. 250 cc) contains 150 g of sterilized and conditioned whole-wheat flour with 5% of yeast. In the case of *P. interpuntellca*, larvae were collected from the fallen date fruits at Faculty of Agriculture farm (Cairo University) then reared on a diet consisting of ground wheat, fine sugar, dry yeast and glycerol (65: 10: 10:15 by weight, respectively). Jars of two insect species were covered with pieces of muslin cloth and fixed with rubber bands. The jars of two tested insects were placed in an incubator at 28 ± 1^{0}C and 65 ± 5% RH.

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The obtaining of insect stages:

***O. surinamensis*:**

Adults (a week old) of *O. surinamensis* were separated from the culture. In the case of immature stages, biological tests were performed in order to determine the duration of the various developmental stages at rearing conditions, 100 couples of newly emerged adults were introduced to food in a glass jar for 24 hours then separated from the jars.

***P. interpuntellca*:**

For eggs isolation**,** five couples of newly emerged moth was put in a glass cage with gauze lid (mesh width 2 mm). The cage was covered with a muslin cloth and fixed with a rubber band. Eggs failed through the gauze lids on a glass petri dish were collected daily. Larvae and pupae for tests were taken from the media.

Characteristics of plasma source:

Corona discharge head connected to a high voltage power supply and a fan. When a high voltage exceeds the air breakdown value an electrical arc occurs. It is puffed out from the small dielectric enclosure which made of medium thickness bare aluminum by the stream of air and is usually several inches long and one millimeter diameter. The input voltages vary from 0 to 250 V, but the output voltage reaches 15 kV (Figure l).

Bioassay:

Batches of thirty *O. surinamensis* adults were introduced to petri dish contains one layer of 20 g insect food or 20 g of food contains separated immature stages (egg, larva and pupa) were introduced in a petri dish and regularly distributed in one layer.

On the other hand, batches of thirty eggs, larvae and pupae of *P. interpunctella* were introduced to petri dish contains one layer of 20 g insect diet. All petri dishes were exposed to various cold plasma voltage levels of 150, 200 and 250V for exposure times (1, 5, 10, 15, 20 and 25 min) inside a foam chamber, (Figure 2). The distance between the nozzle of discharge plasma and insect food was fixed at 10 cm. Exposure to cold plasma had been repeated three times. Adult and immature stages mortalities inspected after 24 hrs from exposure ended. In the case of immature stages, replicates poured into jars, then covered with a muslin cloth and fixed with a rubber band then incubated in rearing conditions until adult emergence.

Fig.1. Corona discharge plasma

Fig.2. Cold plasma device and foam chamber

Data analysis:

The lethal concentrations of cold plasma of the insect stages were statistically analyzed according to Finney (1971).

RESULTS AND DISCUSSION

The effect of cold plasma at three input voltage levels, 150, 200 and 250 V in combination of six exposure times was investigated against different stages of *O. surinamensis* and the immature stages of ***P. interpunctella*** insects infested stored products (Tables 1and 2).

***O. surinamensis*:**

Data given in Table (1) indicated the impact of cold plasma against *O. surinamensis* stages. At the lowest voltage 150 V and 5 min of exposure, mortality percentages were 53.3, 44.4, 61.1 and 25.4% of adults, eggs, larvae and pupae, respectively. Meanwhile, these values were increased when the exposure time extended to 15 min to record 81.1, 69.6, 95.6 and 64.4% of above-mentioned insect stages, respectively. Adults and larvae were also completely killed when exposed to cold plasma at 150 V for 25 and 20 min of exposure, respectively. Two hundreds voltage of cold plasma and 5 min of exposure caused 44.4, 39.4, 74.6 and 35.4% mortality of aforementioned insect stages, respectively. Furthermore, complete kill was achieved at 200 V after 15 and 10 min of exposure for adults and larvae, respectively.

At the highest voltage (250 V), mortalities were 40.0, 45.1, 57.7 and 40.5% of adults, eggs, larvae and pupae after one min of exposure, respectively. In addition, complete mortality of adults, eggs and larvae was achieved after 10, 15 and 5 min of exposure.

***P. interpunctella*:**

Data of *P. interpunctella* immature stages are summarized in Table (2). At 150 V, mortality percentages were 27.8, 60.0 and 22.2% for eggs, larvae and pupae after 5 min of exposure, respectively. These values were increased to reach 81.1, 96.7 and 76.7% when the exposure time prolonged to 20 min. Meanwhile, complete larval mortality was obtained at this voltage after 25 min of exposure.

The mortality percentages of *P. interpunctella* stages exposed to 200 V of cold plasma for 5min were 35.6, 64.4 and 31.1 % of eggs, larvae and pupae, respectively. The corresponding mortalities values were increased to 85.6, 97.8 and 80.0 % after 15 min, respectively. Complete mortality of larvae was also achieved after 20 min of exposure.

While, the highest mortalities were obtained against eggs, larvae and pupae of *P. interpunctella* at the highest

voltage (250 V). Mortalities were 61.1, 77.8 and 48.9% and 86.7, 96.7and 71.1% after 5 and 10 min of exposure, respectively. Moreover, a complete kill of larvae was achieved after 15 min of exposure only.

Lethal concentrations of cold plasma at 150 and 200 V to immature stages of *P. interpunctella*:

The lethal time (LT) values and parameters of mortality regression line for different stages of *P. interpunctella* exposed to cold plasma at 150 and 200 V are presented in Table (3). At 150 V, the mean values of LT_{50}, LT_{90}, LT_{95} and LT_{99} were 8.0, 4.1 and 9.5; 36.3, 17.4 and 40.9; 55.7, 26.2 and 61.8 and 124.6, 56.8 and 143.1 min for eggs, larvae and pupae, respectively.

The corresponding values at 200 V were 5.9, 26.3, 40.2 and 89.0 and 6.1, 26.4, 40.1 and 87.5 min for eggs and pupae, respectively. The results of mortality percentages for larvae of *P. interpunctella* exposed to cold plasma at 200 V and mortality percentages for different stages of *O. surinamensis* exposed to all power of cold plasma not subjected to statistical analysis. All results showed clearly that, the effect of cold plasma against two insect stages was power and exposure time–independent. The larval stage of each insect species was most sensitive to cold plasma while, the pupal stage of each insect was the most tolerant one. In addition, the effect of cold plasm on *O. surinamensis* was higher than *P. interpunctella*. The *P. interpunctella* larvae were sensitive to plasma treatment than pupae because they showed a significant increase in lipid peroxide levels and protein contents, which indicate the oxidizing effects of such treatment but, sclerotized cuticle protecting the pupa, (Abd El-Aziz *et al.*, 2014). The mortality of *Tribolium castaneum* adults also was significant increased with an increase in voltage of cold plasma, exposure time and decrease in the distance between the electrodes (Mahendran, 2016).

Table 1. Response of *O. surinamensis* stages treated with cold plasma

Power (V)	Stage	Mortality % ± S.E. after indicated exposure time (min)					
		1	5	10	15	20	25
150	Adults	33.3 ±1.9	53.3 ± 1.1	60.0 ± 1.9	81.1 ± 2.9	95.6 ± 1.1	100.0 ± 0.0
	Eggs	37.0 ± 1.9	44.4 ± 0.7	53.4 ±1.8	69.6 ± 1.2	78.1 ± 0.7	92.2 ± 0.6
	Larvae	46.7 ± 0.7	61.6 ± 0.9	90.3 ± 1.7	95.6 ± 0.6	100.0 ± 0.0	100.0 ± 0.0
	Pupae	13.0 ± 1.6	25.4 ± 2.9	34.3 ± 2.5	64.4 ± 1.6	69.9 ± 0.9	86.0 ± 1.6
200	Adults	35.6 ± 1.1	44.4 ± 2.2	91.1 ± 1.1	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	Eggs	29.0 ± 1.5	39.4 ± 0.7	44.1 ± 1.2	69.4 ± 0.7	80.8 ± 1.2	95.0 ± 0.6
	Larvae	56.1 ± 1.6	74.6 ± 0.7	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	Pupae	15.8 ± 1.2	35.4 ± 0.9	40.4 ± 1.5	71.7 ± 0.6	77.8 ± 0.6	91.6 ± 0.9
250	Adults	40.0 ± 1.9	81.1 ± 1.1	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	Eggs	45.1 ± 0.9	77.5 ± 1.3	91.6 ± 0.9	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	Larvae	57.7 ± 1.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	Pupae	40.5 ± 2.1	65.7 ± 1.5	74.4 ± 0.7	87.6 ± 0.9	97.6 ± 0.3	99.0 ± 0.0

Table 2. Response of *P. interpunctella* stages treated with cold plasma

Power (V)	Stage	Mortality % ± S.E. after indicated exposure time (min)					
		1	5	10	15	20	25
150	Eggs	8.9 ± 1.1	27.8 ± 1.1	45.6 ± 2.2	67.8 ± 1.1	81.1 ± 1.1	93.3 ± 1.9
	Larvae	13.3 ± 1.1	60.0 ± 1.9	72.2 ± 2.9	85.6 ± 2.2	96.7 ± 1.9	100.0 ± 0.0
	Pupae	6.7 ± 1.9	22.2 ± 2.9	40.0 ± 1.9	65.6 ± 2.2	76.7 ± 1.9	88.9 ± 1.9
200	Eggs	12.2 ± 1.1	35.6 ± 2.9	51.1 ± 1.1	85.6 ± 1.1	88.9 ± 2.9	94.4 ± 1.1
	Larvae	14.4 ± 1.1	64.4 ± 1.1	75.6 ± 2.2	97.8 ± 1.1	100.0 ± 0.0	100.0 ± 0.0
	Pupae	11.1 ± 1.1	31.1 ± 2.9	61.1 ± 2.2	80.0 ± 1.9	87.8 ± 2.9	92.2 ± 2.2
250	Eggs	41.1 ± 2.2	61.1 ± 1.1	86.7 ± 1.9	88.9 ± 1.1	97.8 ± 1.1	100.0 ± 0.0
	Larvae	47.8 ± 1.1	77.8 ± 1.1	96.7 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	Pupae	38.9 ± 2.2	48.9 ± 1.1	71.1 ±1.1	86.7 ± 1.9	96.7 ± 0.0	98.9 ± 1.1

Table 3. Lethal concentrations of cold plasma at 150 and 200 V to the immature stages of *P. interpunctella*

Stage	Voltage (V)	Lethal time (min) and their 95% confidence limits				Slope± S.E.	R
		LT_{50}	LT_{90}	LT_{95}	LT_{99}		
Eggs	150	8.0 (4.1-12.3)	36.3 (31.0 -124.7)	55.7 (51.8 -255.4)	124.6 (131.3-1012.7)	2.00 ± 0.17	0.95
	200	5.9 (2.5-9.1)	26.3 (21.2-88.0)	40.2 (36.5-177.7)	89.0 (97.4-690.0)	2.00 ± 0.16	0.95
Larvae	150	4.1 (3.4 - 4.8)	17.4 (14.2-22.4)	26.2 (20.2-36.1)	56.8 (40.5-89.5)	2.03 ± 0.17	0.98
Pupae	150	9.5 (5.4-14.6)	40.9 (35.7-139.0)	61.8 (57.7-279.5)	143.1 (137.6-1064.8)	2.02 ± 0.19	0.96
	200	6.1 (3.6-8.5)	26.4 (3.6-8.5)	40.1 (30.9-99.3)	87.5 (68.2-305.5)	2.01± 0.16	0.97

R = Correlation coefficient of regression line. S.E. = Standard error of regression line

In addition, Nasr *et al*. (2020) studied the effect of cold plasma at voltage levels (150, 200 and 250 V) for nine exposure times between (1-25 min) on different stages of *Sitophilus granaries* (L.), *Rhyzopertha dominica* (F.) and *Tribolium castaneum* (Herbst). Results showed that, the adult stage of each insect species was the most susceptible to cold plasma while, the pupal stage of was the most tolerant. Meanwhile, *S. granarius* was the most sensitive to cold plasma compared with *R. dominica* and *T. castaneum* which were the most tolerant insects. Further studies are

needed to understand how treating insects on various organic materials will alter the efficacy of plasma for insect control and damage or modification to the substrate will occur. Thus, cold plasma is a promoting technique to control stored product insects.

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حساسية خنفساء الحبوب المنشارية وفراشة جريش الذرة الهندية التى تصيب المواد المخزونة للبلازما

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تم دراسة مدى إستجابة الأطوارالمختلفة لخنفساء الحبوب المنشارية *Oryzaephilus surinamensis* (L.) والأطوار الغير كاملة لفراشة جريش الذرة الهندية *Plodia interpunctella* (Hübner) للبلازما الناتجة من جهاز الكورونا، وفى هذه الدراسة تم إستخدام فروق جهد (150، 200و 250 فولت) من البلازما مع ست فترات زمنية للتعريض (1 - 25 دقيقة). وأظهرت النتائج بوضوح أن تأثير البلازما على أطوار الحشرتين قد توقف على فرق الجهد ووقت التعريض. وكان أيضاً تأثير البلازما على أطوار خنفساء الحبوب المنشارية أعلى من الأطوار الغير كاملة لفراشة جريش الذرة الهندية وكان الطور اليرقى لكلتا الحشرتين أكثر حساسية للبلازما حيث أنه بتعريضها على أقل فرق جهد (150 فولت) قد ماتت كلياً بعد فترة 20، 25 دقيقة من التعريض ليرقات خنفساء الحبوب المنشارية وفراشة جريش الذرة الهندية على التوالى. بينما كان طور العذراء لكل حشرة هو الأكثر تحملاً للبلازما وكانت نسب الموت على أعلى فرق جهد (250 فولت) وأطول فترة تعريض (25 دقيقة) هى 98,9، 99,0٪ لعذارى فراشة جريش الذرة الهندية و خنفساء الحبوب المنشارية على التوالى. ولذلك تعتبر البلازما من الطرق الواعدة فى المستقبل القريب لمكافحة حشرات المواد المخزونة مع دراسات ملحقية لمعرفة الطريقة المثلى لتعريض المادة الغذائية بدون أى ضرر علي جودتها.