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Efficacy of cold plasma against three of stored grain insects

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Abstract
The effect of cold plasma generated from corona discharge on different stages of *Sitophilus granaries* (L.), *Rhizopertha dominica* (F.) and *Tribolium castaneum* (Herbst.) were investigated, insect species stages exposed to three voltage levels (150, 200 and 250V) of the cold plasma for nine exposure times between (1-25 minutes). Results showed that, adult stage of each insect species was most sensitive to cold plasma while, the pupal stage of each insect was the most tolerant one. Also, *S. granarius* were the most sensitive to cold plasma followed by *R. dominica* and *T. castaneum* was the most tolerant insect, a complete kill of *S. granaries* adults was obtained after 10min only at 250V but eggs and larvae need 15min of exposure. Only 67.3% of Pupa was killed after the longest exposure period (25min). Meanwhile, cold plasma at 250V and 25min of exposure was sufficient to kill adults and larvae of *R. dominica* completely. While, the lowest effect of cold plasma was achieved against ***T.*** *castaneum* stages, the highest voltage (250V) and 25min of exposure gave 80.0, 78.5, 79.2 and 50.6% mortality of adults, eggs, larvae and pupae, resp. Also, a significant enhancement in germination indices of wheat seeds was achieved after exposing to cold plasma.

Keywords: *Sitophilus granarius*, *Rhizopertha dominica*, *Tribolum castaneum*, cold plasma-Germination

1. Introduction

Wheat is the third most important field crop produced in the world after maize and rice. The high quality flour produced from wheat forms the major ingredient of various food products [4]. Stored grains are infested by many insect pests causing an extensive loss in weight and quality. The management of 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. Several insects now resistant to organophosphorus insecticides, the lesser grain borer *R. dominica* (F.) adults were resistant to both Malathion, pirimiphos-methyl [7]. As of January 1, 2005, methyl bromide fumigation has been banned except for critical use exemptions that were granted by the Parties of the Protocol [2]. Cold Atmospheric Plasma CAP is a specific type of plasma that is less than 40°C at the point of application. Its effect on various food contaminant microorganisms such as *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Saccharomyces cerevisiae*, *Candida albicans*, *Aspergillus Niger*, *A. flavus*, *Fusarium* spp. and other microorganisms has been studied on different materials [15, 9, 3, 14]. [13] Evaluated the effect of a plasma jet on *Sitophilus granarius* (L.) insect followed by [10] whom examined it on cigarette beetles *Lasioderma serricorne* (F.).
The interaction of plasma with living cells is still very complicated due to the complex structure of both plasma and living cells. The plasma was applied to bacteria, plant cells, and animal cells. However, it has been proven that the effect is different in the three organisms [5]. It is illustrated that the reactive oxygen and nitrogen species (RONS) are the essential signaling molecules organizing many growth processes in mammalian, microorganisms, and plants. The dose value of RONS can play an essential role in the control of pest control techniques [6]. The aims of this study to evaluate the efficacy of cold plasma against *Sitophilus granarius* (L.), *Rhizopertha dominica* (F.) and *Tribolium castaneum* and wheat seeds germination.

2. Materials and Methods

2.1. Insects:

The grain weevil *Sitophilus granarius* (L.), the lesser grain borer *Rhizopertha dominica* (F.) and the red flour beetle *Tribolium castaneum* (Herbst.) stages were investigated. All species were reared and maintained under laboratory conditions for several generations at 26 ± 2°C and 60 ± 5% RH.

2.2. Characteristics of Plasma Source

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

2.3. Bioassay

Labortory strains of *S. granaries* (L.), *R. dominica* (F.) and *T. castaneum* (Herbst) were used in the experiment. 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 thirty adults of each tested insect species were introduced to Petri dish contains one layer of 20g wheat kernels (variety Gemmiza 11) in case of *S.*

granaries and *R. dominica* or wheat flour in case of *T. castaneum*. Also, 20 g of food contains separated immature stages (egg, larva and pupa) 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 250Vfor exposure times between (1 - 25 minutes) inside foam chamber Fig. (2). the distance between the nozzle of discharge plasma and wheat seeds or flour was fixed at 4 cm. All procedures had been repeated three times. Adult and immature stages mortalities inspected after 24hrs from exposure end. In the case of immature stages, replicates poured in jars then covered with a muslin cloth and fixed with a rubber band then incubated in rearing conditions until adult emergence.

2.4. Germination tests

Twenty wheat seeds were distributed regularly and spread flat out on the bottom of the acrylic box and subjected at 250V for 10 and 15 min. The distance between the nozzle of discharge plasma and wheat seeds was fixed at 4 cm. Test carried out at 25ºC in the dark. The seeds were monitored for duration of 10 days. In the 10th day the germination percentage, the length and mass of roots, shoots and sprouts were measured. Subsequently, roots, shoots and sprouts were dried until a constant mass was reached, for weight determination. Plant material for dry weight was dried at 90ºC for 96 hrs. Germination indices and the vigor indexes were calculated using the following equations[17, 16]:

$$\text{Germination potential} = \frac{\text{Number of seeds germiated in 1 days}}{\text{Total number of seeds}} \times 100\% \quad (1)$$

$$\text{Germination rate} = \frac{\text{Number of seeds germiated in 4 days}}{\text{Total number of seeds}} \times 100\% \quad (2)$$

$$\text{Germination percentage} = \frac{\text{Number of seeds germiated in 10 days}}{\text{Total number of seeds planted}} \quad (3)$$

$$\text{Vigor Index I} = \frac{\text{fresh seedling weight in mg} * \text{germination percentage}}{100} \quad (4)$$

$$\text{Vigor Index II} = \frac{\text{dry seedling weight in mg} * \text{germination perce}}{100}$$

2.5. Data analysis

The lethal concentrations of cold plasma to the adults of the three stored grain insects were statistically analyzed [8]. The obtained data were analyzed by ANOVA test and significant means were separated by Duncan's multiple range tests using a computer program of SPSS 14.0.

3. Results and Discussion

The effect of cold plasma at three input voltage levels, 150, 200 and 250V and nine exposure times was investigated against three insects of stored grains *i.e., S. granaries* (L.), *R. dominica* (F.) and *T. castaneum* (Herbst).

3.1. *S. granaries*

Data of *S. granaries* in Table (1) showed that, the effect of cold plasma was power and exposure time –independent. Mortality percentages of *S. granaries* stages exposed to cold plasma at 150V for 5min were low and recorded 17.8, 13.5, 15.6 and 00.0% for adults, eggs, larvae and pupae, respectively, these values were increased at 15min to reach 88.9, 70.1, 76.9and 25.5%. Meanwhile, 100% kill of adults and larvae was achieved after exposing to 150 V for 20 and 25 min, resp.

After 5 min from exposing to cold plasma at 200V, the mortality percentages were 48.9, 19.5, 20.0 and 00.0% for adults, eggs, larvae and pupae, resp. Complete mortality was achieved of adults, larvae and eggs after 15, 20 and 25min of exposure, resp. The highest mortality percentages against *S. granaries* stages were observed at 250V and different exposure time; these were at 5min 77.8, 56.3, 66.4, and 0.0% of adults, eggs, larvae and pupae, resp. Also, complete kill of adults was obtained after 10min only at 250V but eggs and larvae need 15min of exposure. Pupa was tolerant to cold plasma and affected by 67.3 after 25min of exposure.

3.2. *R. dominica*

Data given in table (2) indicated the impact of cold plasma against *R. dominica* stages. Results revealed that the effect of plasma on *R. dominica* was lower than *S. granaries*, no mortality percentages of adults, eggs, larvae and pupae were observed at 150V up to 5m of exposure, resp. meanwhile the mortality percentages were increased gradually to reach 82.5, 80.0, 81.0, and 30% after 25min of exposure of above-mentioned insect stages, resp. The corresponding mortalities at 200V were 95.6, 84.4, 91.1and 45.2%. While, the highest mortalities were obtained against *R. dominica* at the highest voltage (250V), mortalities after 7and 20min of exposure were 66.6, 48.9, 56.7and 0.0% and 97.8, 93.0, 96.7and 46.6% of adults, eggs, larvae and pupae, resp. Also, complete mortality against adults and larvae was achieved after 25min of exposure.

3.3. *T. castaneum*

The lowest effect of cold plasma at different input voltage levels and time of exposure was observed against *T. castaneum* stages Table (3), no mortality achieved of tested stages at 150V up to 10min of exposure and the mortality percentages were less than 50% at the longest exposure time (25min) 43.3, 39.6, 39.7 and 25.6% of tested stages, resp.

200Vand 25min of exposure caused mortality values 74.1, 69.0, 70.5and 45.2 % of tested stages, resp. The corresponding values at the highest voltage (250V) were 80.0,7 8.5, 79.2 and50.6%.

3.4. Lethal time of cold plasma at 200V to different stages of the tested insect species

The lethal time (LT) values and parameters of mortality regression line for different stages of the tested insect species exposed to cold plasma at 200V are presented in Table (4). The lethal time values required to kill 50% of adult, egg, larva and pupa were 4.6, 8.5, 8.2 and 23.0; 12.6, 16.4, 12.1 and 26.7 and19.9, 18.9, 17.4and26.8min for *S. granaries*, *R. dominica* and *T. castaneum*, resp. The same trend could be applied for LT_{95} level with different stages of tested insect species. The results of mortality percentages for different stages of the tested insect species exposed to 150 and 250V not subjected to statistical analysis.

All results showed clearly that the adult stage of each insect species was most sensitive to cold plasma while, the pupal stage of each insect was the most tolerant one. Also, *S. granarius* were the most sensitive to cold plasma followed by *R. dominica* and *T. castaneum* was the most tolerant

insect
Adults of *T. castaneum* were exposed in groups of 5 insects with flour to 20 different treatment combinations, distance between the electrodes (3, 4 or 5 cm), total exposure time (1, 2, 3, 4 or 5 min) and applied voltage (1,000, 1,750 or 2,500 V), mortality assessed after 24 h of incubation at 35°C and analyzed by multiple regression analysis revealed that, a significant increase in mortality of *Tribolium castaneum* adults was observed with increase in applied voltage, exposure time and decrease in the distance between the electrodes. No significant color change was observed on refined wheat flour [11]. On the other hand [1] found that, the *P. interpunctella* larvae were sensitive to plasma treatment than pupae because they showed a significant increases in lipid peroxide levels and reductions in glutathione and protein contents, which indicate the oxidizing effects of such treatment but, sclerotized cuticle protecting the pupa.

3.5. Germination Indices

Results of the influence of cold plasma treatment on the growth, germination and other activities of wheat seeds are shown in Table (5). After one day of the plant, the germination Potential% increased slightly as a result of plasma exposure from 18% for the control seeds to 65% for the seeds treated with plasma for 10 min. while, it increased vastly to 90.3% after15min of exposure. In the case of the germination rate, it enhanced to 79% and 95.1% for the seeds treated with plasma for 10 and 15 min, resp. compared with 35.1% in untreated seeds, Fig. (3).
These results indicated that, the germination potential and germination rate for gliding arc plasma exhibited significant difference compared with untreated one. In conclusion, the enhancement of the wheat seeds germination depends on the cold plasma dose; the dose value of RONS can play an essential role in the control of growth and improvement of plants [12]. It can be concluded that, cold plasma enhancement the germination potential and germination rate of wheat seeds. The positive effect cold plasma is observed between untreated and treated wheat seeds on the short term germination and seedling growth. The mean root length for the seeds treated at (10and 15min) was significantly enhanced to record (1422.13 and 1632.12 mm) compared with untreated seeds (1121.12 mm), the same effect of cold plasma was achieved in the shoot length and the corresponding values were (510.01 and 692.02 mm) compared with control (298.2mm).
Similarly, the mean fresh and dry weight of the seedlings for the seeds treated at 10min was higher than that of the untreated seeds 55.6 and 60.0%, resp. meanwhile, the fresh and dry weight of the seedlings produced from seeds exposed for 15min was increased by 74.1 % and 78.4 %, resp. Table (6). Also, the vigor indexes of treated seeds were significantly higher than that of the control, so that high-quality yield in agricultural production will be produced.
Finally, due to the higher root and shoot length for treated seeds with respect to control one; the vigor indexes (I and II) of treated seeds were significantly higher than that of the control. So that, appropriate cold atmospheric plasma treatment can enhance seed vigor index in laboratory conditions. The wheat sprouts were dehydrated at 90˚C for 96h and weighted by digital balance indicated that, all treated seeds had a greater mass compared control one. Previous research showed that the dry mas of radish sprouts which treated by non-thermal plasma recorded higher value with respect to untreated one [18].

4. Tables and Figures

Fig 1: Corona discharge plasma

Fig 2: Exposing of tested insect stages to cold plasma

Fig 3: The treated and untreated Wheat seeds after 4 days of cultivation.

Table 1: Effect of cold plasma against *S. granarius* stages infested stored wheat grains

Voltage V	stage	% Mortality % ±S.E. after indicated exposure time(min)								
		1	2	5	7	10	12	15	20	25
150	adult	00.0±0.0	00.0±0.0	17.8±0.3	40.0±1.2	61.1±0.3	71.1±0.9	88.9±0.7	100.0±0.0	100.0±0.0
	Egg	00.0±0.0	00.0±0.0	13.5±0.6	32.6±1.7	48.2±1.7	63.5±2.7	70.1±0.9	85.5±2.3	94.2±3.8
	larva	00.0±0.0	4.4±0.3	15.6±0.7	32.2±0.3	51.3±2.2	66.7±2.5	76.9±2.1	95.6±2.6	100.0±0.0
	pupa	00.0±0.0	00.0±0.0	00.0±0.0	00.0±0.0	6.7±0.5	12.0±1.6	25.5±1.6	29.6±0.9	35.5±1.2
200	adult	00.0±0.0	17.8±0.7	48.9±0.3	70.0±0.6	82.2±0.3	93.3±0.6	100.0±0.0	100.0±0.00	100.0±0.00
	Egg	00.0±0.0	00.0±0.0	19.5±0.3	41.2±1.4	55.1±1.8	68.2±0.9	84.4±2.0	95.7±0.3	100.0±0.00
	larva	00.0±0.0	00.0±0.0	20.0±1.0	42.0±3.0	57.5±5.7	74.6±2.7	84.6±3.0	100.0±0.00	100.0±0.00
	pupa	00.0±0.0	00.0±0.0	00.0±0.0	3.3±0.3	9.0±1.0	15.5±0.9	29.8±0.9	38.9±2.0	55.0±3.0
250	adult	6.7±0.0	33.3±0.6	77.8±0.7	84.4±0.9	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
	Egg	0.0±0.0	10.5±0.5	56.3±3.1	70.4±1.1	77.3±1.4	85.6±2.8	100.0±0.00	100.0±0.00	100.0±0.00

	larva	6.7±1.9	15.5±0.7	66.4±1.5	89.6±1.4	91.3±1.5	95.5±2.8	100.0±0.00	100.0±0.00	100.0±0.00
	pupa	0.0±0.0	0.0±0.0	0.0±0.0	8.9±0.7	10.5±0.5	25.6±0.8	37.6±1.4	48.5±0.6	67.3±3.0

S.E. = Standard error.

Table 2: Effect of cold plasma against of *R. dominica* stages infested stored wheat grains

Voltage V	stage	% Mortality % +S.E. after indicated exposure time(min)								
		1	2	5	7	10	12	15	20	25
150	adult	00.0±0.0	00.0±0.0	00.0±0.0	31.1±1.5	41.5±1.5	52.1±2.0	64.4±3.3	75.8±1.3	82.5±2.3
	Egg	00.0±0.0	00.0±0.0	0.0±0.0	0.0±0.0	6.7±0.6	16.7±0.6	46.7±0.6	68.9±0.7	80.0±0.6
	larva	00.0±0.0	00.0±0.0	00.0±0.0	27.6±1.0	38.5±1.5	48.9±2.0	60.0±2.7	77.5±3.4	81.0±3.0
	pupa	00.0±0.0	00.0±0.0	00.0±0.0	00.0±0.0	00.0±0.0	10.0±1.0	21.5±0.7	25.9±0.6	30.0±1.8
200	adult	00.0±0.0	00.0±0.0	11.1±0.3	38.9±1.2	51.2±1.4	57.8±3.3	70.4±1.8	85.3±1.6	95.6±0.3
	Egg	00.0±0.0	00.0±0.0	00.0±0.0	3.4±0.8	13.3±0.7	25.6±0.6	35.6±1.4	70.0±0.8	84.4±1.8
	larva	00.0±0.0	00.0±0.0	00.0±0.0	18.9±0.5	44.4±1.2	46.7±0.7	55.6±2.2	78.9±1.1	91.1±2.8
	pupa	00.0±0.0	00.0±0.0	00.0±0.0	00.0±0.0	00.0±0.0	12.0±0.6	20.5±1.1	34.8±1.3	45.2±0.8
250	adult	00.0±0.0	15.6±0.6	18.5±0.3	66.6±2.7	72.6±2.2	78.8±1.5	89.9±4.8	97.8±0.3	100.0±0.0
	Egg	00.0±0.0	15.0±0.8	45.6±1.5	48.9±0.3	62.5±1.3	73.6±1.8	84.9±2.1	93.0±1.5	98.6±0.7
	larva	00.0±0.0	15.0±0.8	33.7±1.9	56.7±2.5	63.3±0.6	76.7±1.2	87.8±0.7	96.7±2.1	100.0±0.00
	pupa	00.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	6.7±0.7	22.2±0.6	33.5±1.5	46.6±1.5	55.7±2.6

S.E. = Standard error.

Table 3: Effect of cold plasma against of *T. castaneum* stages infested stored wheat grains

Voltage v	Stage	% Mortality % +S.E. after indicated exposure time(min)								
		1	2	5	7	10	12	15	20	25
150	Adult	00.0±0.0	00.0±0.0	0.0±0.0	0.0±0.0	00.0±0.0	14.4±1.4	20.0±0.6	35.6±0.9	43.3±0.6
	Egg	00.0±0.0	00.0±0.0	00.0±0.0	00.0±0.0	00.0±0.0	10.7±0.6	17.3±0.5	30.0±1.2	39.6±0.3
	Larva	00.0±0.0	00.0±0.0	00.0±0.0	00.0±0.0	00.0±0.0	12.8±0.4	18.7±0.6	32.8±1.0	39.7±1.6
	Pupa	00.0±0.0	00.0±0.0	00.0±0.0	00.0±0.0	00.0±0.0	9.0±0.3	16.0±0.5	22.2±1.4	25.6±1.5
200	Adult	00.0±0.0	00.0±0.0	00.0±0.0	00.0±0.0	00.0±0.0	20.0±0.6	46.7±0.6	65.6±0.7	74.1±1.3
	Egg	00.0±0.0	00.0±0.0	00.0±0.0	00.0±0.0	00.0±0.0	18.6±0.3	40.9±1.6	60.5±1.3	69.0±1.6
	Larva	00.0±0.0	00.0±0.0	00.0±0.0	00.0±0.0	00.0±0.0	19.6±0.4	44.8±1.4	64.5±1.0	70.5±2.0
	Pupa	00.0±0.0	00.0±0.0	00.0±0.0	00.0±0.0	00.0±0.0	12.0±1.0	20.5±1.0	34.8±2.1	45.2±2.5
250	Adult	00.0±0.0	00.0±0.0	10.0±0.6	33.3±0.6	50.0±1.0	55.6±0.9	68.9±0.9	76.7±0.6	80.0±1.2
	Egg	00.0±0.0	00.0±0.0	9.0±0.6	27.6±1.2	40.5±1.8	51.5±1.3	63.5±2.0	71.6±1.6	78.5±2.6
	Larva	0.0±0.0	00.0±0.0	9.0±0.3	30.0±1.5	47.2±1.6	52.5±1.6	64.7±1.6	72.8±2.0	79.2±3.4
	Pupa	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	3.3±0.5	20.0±1.0	31.6±1.5	42.2±1.4	50.6±1.3

S.E. = Standard error.

Table 4: LT_{50} and LT_{99} values with their confidence limits for different insect stages exposed to 200V of cold plasma.

Insect	stage	Lethal time(m)		95% confidence limits				Slope ± SE	R
		LT_{50}	LT_{95}	LT_{50}		LT_{95}			
				Lower	Upper	Lower	Upper		
S. granarius	Adult	4.6	16.8	4.0	5.1	13.9	21.8	2.91 ± 0.27	0.98
	Egg	8.5	22.0	7.6	9.3	20.1	27.3	3.91 ±0.18	0.99
	Larva	8.2	21.4	7.9	8.6	19.9	23.3	3.97 ± 0.18	0.99
	Pupa	23.0	67.4	21.7	24.7	57.5	82.4	3.52 ± 0.2	0.99
R. dominica	Adult	12.6	29.0	11.8	13.4	25.7	34.2	4.55 ± 0.37	0.98
	Egg	16.4	33.7	15.4	16.9	31.4	36.5	5.24 ± 0.23	0.99
	Larva	12.1	33.9	10.6	13.6	30.6	44.7	3.69 ± 0.19	0.98
	Pupa	26.7	85.6	24.8	30.2	65.6	127.3	3.27 ± 0.33	0.99
T. castaneam	Adult	19.9	39.0	15.7	18.2	32.6	52.2	4.5 ± 0.6	0.97
	Egg	18.9	44.2	16.9	19.5	36.1	61.6	4.2 ± 0.6	0.98
	Larva	17.4	42.2	16.2	18.7	34.9	58.0	4.3 ± 0.6	0.98
	Pupa	26.8	84.8	23.5	33.9	56.4	192.6	3.6 ± 0.6	0.98

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

Table 5: Effect of plasma at 250V for different exposure times on the Germination characteristics and Seedling growth.

Exposure Time (min)	G. Potential% ± S.D.	G. Rate% ±S.D.	G. % ±S.D.	Root Length (mm) ±S.D.	Shoot Length (mm) ±S.D.
0	18.4±1.0 a	35.0 ±1.3 a	41.0 ±1.1a	1121.1±0.0a	298.2±2.1a
10	65.2 ±1.0 b	79.0 ±1.1 b	86.0 ±1.3b	1422.1±0.0b	510.0±2.3b
15	90.3 ±1.1c	95.1 ±1.8 c	99.0 ±1.0c	1632.1±1.2c	692.0±2.6c

G. = Germination; S.D. = Standard deviation; the different letters in the same column mean significant difference among various treatments at P < 0.05 level.

Table 6: Effect of cold plasma at 250V for different exposure times on vigor index and the weight of fresh and dry sprout.

Exposure Time (min)	Fresh Weight (mg) ±S.D.	Dry Weight(mg) ±S.D.	Vigor Index I ±S.D.	Vigor index II ±S.D.	Fresh sprout weight (mg)±S.D.	Dry sprout weight(mg)±S.D.
0	1088.2 ±2.1a	450.6±1.2a	1025.6± 1.1a	341.3±1.0a	786.8±1.2a	298.2±1.3a
10	1693.5±2.3b	720.8±1.1b	1735.4±1.2b	753.5±1.2b	1125.6±1.2b	503.2±1.5b
15	1894.8 ±2.6c	803.9±1.4c	1887.8±1.3c	923.1±1.0c	1402.5±1.0c	620.1±1.2c

S.D. = Standard deviation; the different letters in the same column mean significant difference among various treatments at P < 0.05 level.

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