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EFFECT OF COLD PLASMA ON MORTALITY OF *Tribolium Castaneum* ON MAIDA FLOUR

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Abstract: Cold plasma is an emerging non thermal processing technology and is one of the novel promising methods for insect mortality. In order to study whether cold plasma could be suitable for control and to investigate the effect of plasma stress on mortality of *Tribolium castaneum*, three treatment variables were used: electrode gap (3 to 5cm), exposure time (1 to 5min) and applied voltage (1000 to 2500V). Mortality rate of an adult was examined after 24h of incubation at 35°C with twenty different treatment combinations as per CCD of RSM. Significant increase in mortality rate of an adult was observed with increase in applied voltage, exposure time and decrease in electrode distance. No significant color change was observed due to plasma exposure on Maida flour using L, a, b color values. This study will pave the way for an effective low temperature treatment technique in stored food product insect management.

Key words*: Non thermal plasma, Tribolium castaneum, mortality, colour*

INTRODUCTION

The red flour beetle, *Tribolium castaneum* is a worldwide insect pest of stored products and processed food commodities. It can infest a variety of products and is perhaps the most economically important insect pest of processed food. Many attempts have been devoted to explore alternative non- toxic pest control methods and one such novel method is cold plasma as it does not leave any chemical residue on the treated

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food products. Atmospheric cold plasma (ACP) is a relatively new technology for microbiological decontamination and sterilization of foods. Due to its unique properties plasma is often referred to as the fourth state of matter according to a scheme expressing an increase in the energy level from solid to liquid to gas and ultimately to plasma [1]. It consists of highly energetic species in permanent interaction including photons, electrons, positive and negative ions, free radicals, and excited or non excited molecules and atoms [2][3][4]. CP can efficiently kill or inactivate bacteria, yeasts, and molds and other hazardous microorganisms, as well as spores and biofilms that are generally very difficult to inactivate [5]. Non thermal antimicrobial treatments of fruits, vegetables and other food produce have been the subject of much research. In the last decade, the atmospheric pressure cold plasma research has greatly increased due to finding important applications in various fields. It is a promising technology that is simple to setup, easy to operate and economical [6].

The non thermal plasma treatment could provide an effective and environmentally friendly treatment in integrated pest management program. The effect of plasma on insects were studied by Bures *et al*. (2005) [7] on green peach aphids and Donohue *et al*. (2006) [8] on western flower thrips, *Frankliniella occidentalis* (pergande); tobacco thrips, *Frankliniella fusca* (Hinds); Asian tiger mosquito, *Aedes albopictus* (Skuse); German cockroach, *Blattella germanica* L. and the two spotted spider mite, *Tetranychus urticae* Koch. Recently Abd El-Aziz *et al.* 2014 [9] also studied the effect of NTP on control of Indian meal moth *Plodia interpunctella* (Lepidoptera: Pyralidae). NTP causes oxidative damage in P. *interpunctella* larvae by generating reactive oxygen stress in their bodies. The recent advances in cold plasma have allowed scientists to successfully develop many different systems, with parameters that can be adjusted for the development of uniform discharge of plasma, such as voltage, electrode distance and exposure time [10]. The objective of this study was to investigate whether cold plasma could be useful for control of *Tribolium Castaneum*, to investigate the effect of plasma stress on mortality of *Tribolium Castaneum* and also to study the colour changes that occur after the treatment on the maida flour.

MATERIAL AND METHODS

The plasma system (Fig. 1) used for the study has been designed and developed indigenously at Indian Institute of Crop Processing Technology consists of two planar electrodes, made of metallic plates and separated by variable gas or air gap [11]. A stainless steel chamber with size of 350x350x350mm and this reactor allows working pressure in the range of near atm to vacuum (under 1 mbar). The distance gap between the two electrodes is mechanically adjusted and reactor is also provided with view glass to see the discharge. The electrodes are covered with Teflon sheets and energized by a high voltage power in the range of 1-40kV and frequency of 50Hz. One of the electrodes is also covered with dielectric barrier, in order to avoid arc between the electrodes.

Experimental Design. Central Composite Design (CCD) of Response Surface Methodology (RSM) were used for the experimental design and plan with independent variables voltage, time and distance between the electrodes. The CCD consisted of three factors with three levels i.e. applied voltage (1000 - 2500 V), exposure time (1 - 5 min) and distance between the electrodes (3 – 5 cm). Table. 1. shows the experimental range and level for the treatment as used by Markovic *et al.*, [12] and the number of experiments required (N) is given by the expression: 2^k (2^3 = 8; star points) + 2 k (2 × 3 = 6; axial points) + 6 (center points; 6 replications). Accordingly, the CCD matrixes of 20 experiments covering the full design of five factors were used for building quadratic models as shown in Table. 2. The experimental data obtained from the CCD model experiments can be represented in the form of the following equation:

$$y = a_0 + a_1 d + a_2 v + a_3 t + a_4 dv + a_5 dt + a_6 vt + a_7 d^2 + a_8 v^2 + a_9 t^2 \quad (1)$$

Where y represents the response function, a_0 is an intercept and d, v, t are independent variables, where a_1 to a_3, a_7 to a_9 and a_4 to a_6 are the coefficients of the linear, quadratic and interactive terms, respectively.

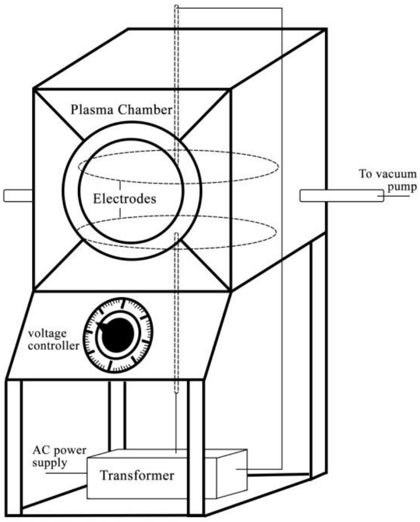

Figure 1. A representation of plasma chamber and electrode connection to the transformer

Table 1. Experimental range and levels of the independent variables

Sl. No	*Independent variable*	*Factor*	*Experimental range and level*				
			-1.68	*-1*	*0*	*1*	*1.68*
1	*Electrode gap (cm)*	*d*	*2*	*3*	*4*	*5*	*6*
2	*Voltage (volts)*	*v*	*500*	*1000*	*1750*	*2500*	*3000*
3	*Exposure time (sec)*	*t*	*0.5*	*1*	*3*	*5*	*7*

Mortality assay. Adult *Tribolium castaneum* was treated inside a plasma chamber as per the experiment designed using CCD. Five insects were taken in 9 x 9 cm LDPE packaging material with 1 g feed and the insects were exposed to plasma with various combinations. It includes distance between the electrode as 2, 3, 4, 5 or 6, exposure time as 1, 3, 5, 7 or 30 s and applied voltage as 500 V, 1000 V, 1750 V, 2500 V or 3000 V. After treatment the packaging material containing insect and maida flour was stored at 35°C for 24 h to assess the mortality.

Table 2. Experimental design and experimental plan

Run	Experimental Design			Experimental Plan		
	Electrode distance (cm)	Voltage (volts)	Exposure Time (Sec)	D	v	t
1	-1	-1	-1	3	1000	1
2	+1	-1	-1	5	1000	1
3	-1	+1	-1	3	2500	1
4	+1	+1	-1	5	2500	1
5	-1	-1	+1	3	1000	5
6	+1	-1	+1	5	1000	5
7	-1	+1	+1	3	2500	5
8	+1	+1	+1	5	2500	5
9	-1.68	0	0	2	1750	3
10	+1.68	0	0	6	1750	3
11	0	-1.68	0	4	500	3
12	0	+1.68	0	4	3000	3
13	0	0	-1.68	4	1750	0.5
14	0	0	+1.68	4	1750	7
15	0	0	0	4	1750	3
16	0	0	0	4	1750	3
17	0	0	0	4	1750	3
18	0	0	0	4	1750	3
19	0	0	0	4	1750	3
20	0	0	0	4	1750	3

Colorimeter test. Hunter lab ColorFlex EZ, 45/00 Color Spectrophotometer (Hunter Associates Laboratory, Inc., Reston, Virgina, USA) was used for the measurement of colour of plasma treated maida flour. Initially the colorimeter was calibrated with the black and white tiles. All the 20 treated maida flour and control sample were analysed and L, a, b values were recorded.

RESULTS AND DISCUSSION

RSM was used for obtaining the relationship between independent variables and the response. The 20 combinations of experiments were carried out and the response for mortality and colour value were observed (Tab. 3).

The multiple regression analysis of the experimental data using RSM revealed that mortality of insect, color of treated maida flour are related by the following second order polynomial equations:

$$y_1 = 30.29 + 21.15A + 23.21B - 5.00C + 15.00AB + 7.27E^{-15}AC + 1.07E^{-14}BC - 2.26A^2 - 2.8B^2 + 7.91C^2 \quad (2)$$

$$y_2 = 90.11 - 0.36A - 0.10B + 0.18C - 0.53AB - 0.45A - 0.53BC - 0.097A^2 + 0.24B^2 + 0.073C^2 \quad (3)$$

Where A, B, C are the corresponding coded factors of the applied voltage, exposure time, distance between electrodes respectively. The diagnostic plots given in Fig. 2. was used for estimating the adequacy of the regression model and it shows that the data points indicate that neither response transformation was required nor there was any apparent problem with normality. The general perception of straight line is quite clear in the normal probability supporting the hypothesis of normal distribution.

Table 3. Experimental Response

	Factor 1	*Factor 2*	*Factor 3*	*Response 1*	*Response 2*
Run	*A: Voltage*	*B: Exposure time*	*C: Distance between electrodes*	*Mortality of insect*	*Colorimeter L value of flour*
	V	min	Cm	%	
1	*2500*	*1*	*5*	*0*	*91*
2	*1750*	*3*	*4*	*20*	*89.5*
3	*1750*	*3*	*4*	*60*	*90.61*
4	*2500*	*5*	*5*	*60*	*88.53*
5	*1000*	*5*	*5*	*0*	*91.47*
6	*1000*	*5*	*3*	*0*	*90.85*
7	*1750*	*3*	*4*	*60*	*89.64*
8	*1750*	*3*	*4*	*20*	*90.27*
9	*1750*	*3*	*6*	*60*	*90.51*
10	*1750*	*3*	*4*	*20*	*89.82*
11	*2500*	*5*	*3*	*60*	*89.98*
12	*1000*	*1*	*3*	*0*	*89.07*
13	*1000*	*1*	*5*	*0*	*91.52*
14	*2500*	*1*	*3*	*0*	*90.06*
15	*500*	*3*	*4*	*0*	*90.33*
16	*1750*	*3*	*4*	*20*	*90.82*
17	*1750*	*7*	*4*	*100*	*90.88*
18	*1750*	*0.5*	*4*	*0*	*90.65*
19	*1750*	*3*	*2*	*100*	*90.33*
20	*3000*	*3*	*4*	*100*	*89.4*

Interactive effect of processes of independent variables. Using multiple nonlinear regression model (Eqn 2 & 3) three dimensional contour plots were drawn to show the effects of binary combinations of independent variables on the predicted mortality. These plots were shown in Figs. 3, 4 and 5. Fig. 3 shows the integrated effect of distance between electrodes and the applied voltage on mortality. It indicates that at constant distance between electrodes, the increasing applied voltage increases the Mortality of insects and the mortality also increases with the increase in the total exposure time. The combined effect of voltage and electrode gap on mortality is shown in Fig. 4. From the graph it is interpreted that at constant exposure time, the mortality of insects increases with the decrease in the distance between electrodes and the voltage. Fig. 5 shows the interactive effect of exposure time and electrode gap on mortality. It was estimated that at constant voltage, the mortality of insects started increasing with increase in the exposure time and decrease in the distance between electrodes. It was also found that there is no significant difference on the color of plasma treated maida on twenty different treatment combinations and is shown in Tab. 3.

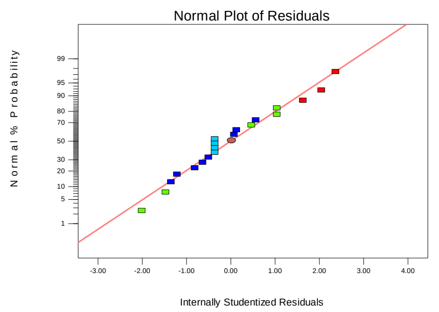

Figure 2. Internally studentized and normal % probability plot of death of Tribolium castaneum

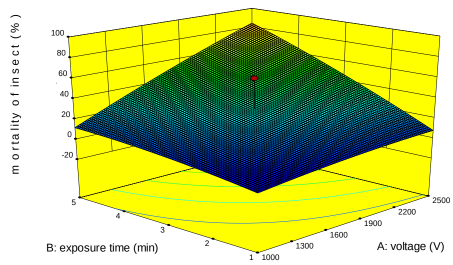

Figure 3. Effect of voltage and exposure time on mortality of insects

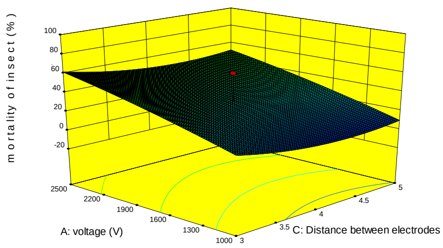

Figure 4. Effect of voltage and electrode gap on mortality of insects

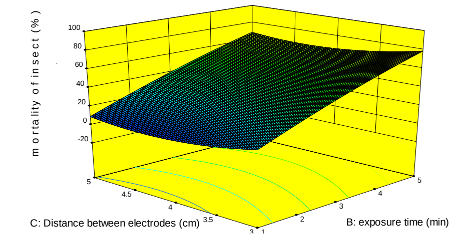

Figure 5. Effect of electrode gap and exposure time on mortality of insect

CONCLUSIONS

Mortality of *Tribolium castaneum* with maida feed was investigated with independent variables like applied voltage, exposure time and electrode gap distance. The results obtained from the experiments clearly indicated that to increase the mortality of insects, either the applied voltage or exposure time have to be increased or distance between the electrodes should be decreased. As cold plasma is a non thermal technique, there is no change in the quality of stored food products. This method is cost effective and can be an alternative for the traditional fumigation technique without any chemical residues. This research showed that cold plasma is an efficient tool for control of *Tribolium castaneum*, however further studies are required in design and development of commercial and continuous cold plasma treatment.

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UTICAJ HLADNE PLZME NA MORTALITET *Tribolium Castaneum* U BRAŠNU

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Sažetak: Hladna plazma je nova tehnologija hladne prerade koja utiče na uginuće insekata . U cilju ispitivanja pogodnosti hladne plazme za kontrolu *Tribolium castaneum* korišćena su tri varijabilna tretmana: zazor elektroda (3 do 5 cm), vreme izlaganja (1 do 5 min) i napon (1000 do 2500V) . Stopa smrtnosti je ispitivana nakon 24h inkubacije na

35°C sa dvadeset različitih kombinacija tretmana kao prema CCD od RSM. Značajno povećanje stope smrtnosti primećena je sa porastom napona, vremena izloženosti i smanjenja zazora elektroda. Nema značajne promene boje zbog izlaganja brašna plazmi. Ova studija će otvoriti put za efikasnu tehniku tretmana niskim temperaturama u skladištu prehrambenih proizvoda radi kontrole insekata.

Ključne reči: *hladna plazma, Tribolium castaneum, mortalitet, boja*

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