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Effect of cold plasma on mortality of *Tribolium castaneum* on refined wheat flour

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ABSTRACT

Non-thermal (cold) plasma treatment is one of the novel promising methods for insect control in stored products. In order to study whether this technology could be useful for control of *Tribolium castaneum* (Herbst) and to investigate the effect of plasma stress on mortality of *Tribolium castaneum*, three treatment variables were used for the generation of the cold plasma: 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). Adult *T. castaneum* were exposed in groups of 5 insects with flour to 20 different treatment combinations and mortality assessed after 24 h of incubation at35°C. Results were analysed by multiple regression analysis. Significant increase in mortality was observed with increase in applied voltage, exposure time and decrease in the distance between the electrodes. No significant colour change was observed on refined wheat flour, a bleached wheat (*Tribolium castaneum* L.) flour, due to plasma exposure (maximum of 5 min) as assessed by Hunter colour lab L, a, b values. This study will pave the way for an effective and friendly environmental treatment technique in stored food product insect management.

Key words: Colour, Mortality percentage, Non-thermal plasma, *Tribolium castaneum*

The red flour beetle [*Tribolium castaneum* (Herbst)] is a worldwide insect pest of stored products and 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 residue-free non-toxic pest control methods. One such novel method is cold plasma treatment. Cold plasma (CP) or non-thermal plasma treatment 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 (Misra et al. 2011). A plasma 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; (Laroussi, 2005 (Bardos and Barankova 2010; Fernandez et al., 2013).

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Cold plasma 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 (Niemira, 2012). 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 owing to finding important applications in various fields. It is a promising technology that is simple to setup, easy to operate and economical (Tyata et al. 2012).

Cold plasma treatment has potential to provide an effective and environmentally friendly treatment in an integrated pest management programme. The CP causes oxidative damage in *Plodia interpunctella* (Hubner) larvae by generating reactive oxygen stress in their bodies. The effect of plasma on insects were studied by Bures et al. (2005) on green peach aphids and Donohue et al. (2006) on western flower thrips, *Frankliniella occidentalis* (pergande); tobacco thrips, *Frankliniella fusca* (Hinds); Asian tiger mosquito, *Aedes albopictus* (Skuse); German cockroach, *Blattella*

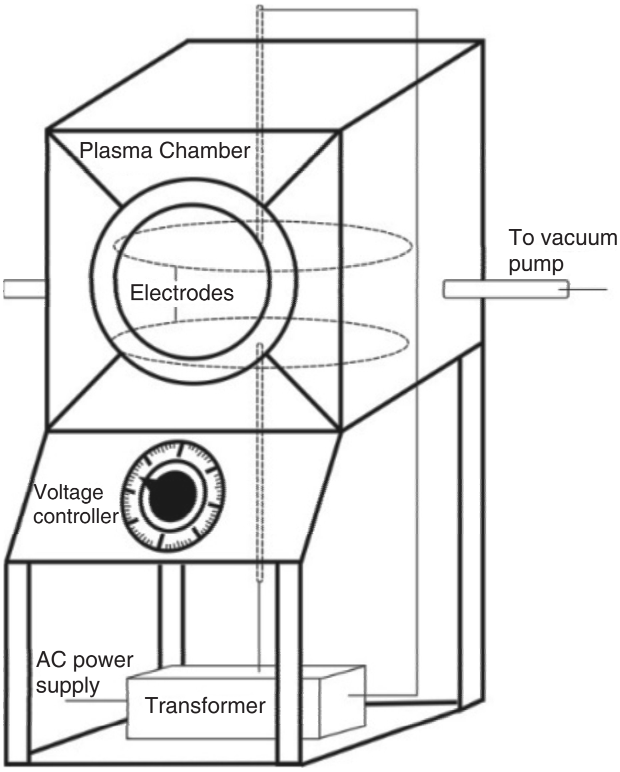

Fig. 1. Plasma chamber and electrode connection to the transformer

germanica L. and the two spotted spider mite, *Tetranychus urticae* Koch. Recently, Abd El-Aziz et al. (2014) also studied the effect of CP on control of Indian meal moth *Plodia interpunctella* (Lepidoptera: Pyralidae). Recent advances in cold plasma technology 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 (Mahendranand Alagusundaram, 2015).

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 refined (wheat) flour.

MATERIALS AND METHODS

The plasma generating system (Fig. 1) used for the study has been designed and developed at the IICPT, Thanjavur. It consists of two planar electrodes, made of metallic plates and separated by variable gas or air gap [Mahendran and Alagusundaram (2015b)], a stainless steel chamber (350 mm×350 mm×350 mm) and a 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 1-40 kV and frequency of 50 Hz. One of the electrodes is also covered with dielectric barrier, in order to avoid arcing between the electrodes.

Experimental design

The experimental design and plan was set up according to the system ofCentral Composite Design (CCD) of Response Surface Methodology (RSM), with independent variables: voltage, time and distance between the electrodes. The CCD consisted of three factors with three levels, i.e. applied voltage (1,000 – 2,500 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 following the system used by Markovic et al. (2012) wherethe 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 (centre 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:

$$y = a_0 + a_1d + a_2v + a_3t + a_4dv + a_5dt + a_6vt + a_7d^2 + a_8v^2 + a_9t^2 \quad (1)$$

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.

Colorimeter test

Hunter lab ColorFlex EZ, 45/00 Color

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

Table 2 Experimental design and its 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	2,500	1	5	0	91
2	1,750	3	4	20	89.5
3	1,750	3	4	60	90.61
4	2,500	5	5	60	88.53
5	1,000	5	5	0	91.47
6	1,000	5	3	0	90.85
7	1,750	3	4	60	89.64
8	1,750	3	4	20	90.27
9	1,750	3	6	60	90.51
10	1,750	3	4	20	89.82
11	2,500	5	3	60	89.98
12	1,000	1	3	0	89.07
13	1,000	1	5	0	91.52
14	2,500	1	3	0	90.06
15	500	3	4	0	90.33
16	1,750	3	4	20	90.82
17	1,750	7	4	100	90.88
18	1,750	0.5	4	0	90.65
19	1,750	3	2	100	90.33
20	3,000	3	4	100	89.4

Spectrophotometer (Hunter Associates Laboratory, Inc., Reston, Virgina, USA) was used for the measurement of colour of plasma-treated refined wheat flour. Initially the colorimeter was calibrated with the black and white tiles. All the 20 treated refined wheat flour and control sample were analysed and L, a, b values were recorded.

Mortality assay

Adult *Tribolium castaneum* were treated inside a plasma chamber as per the experiment designed using CCD. Five insects were exposed in a 9 cm × 9 cm LDPE packaging material with 1 g feed. Combinations of values used were: distance between the electrode as 2, 3, 4, 5 or 6 cm, 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 refined wheat flour was stored at 35°C for 24 h to assess the mortality.

RESULTS AND DISCUSSION

Response surface methodology (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 (Table 2).

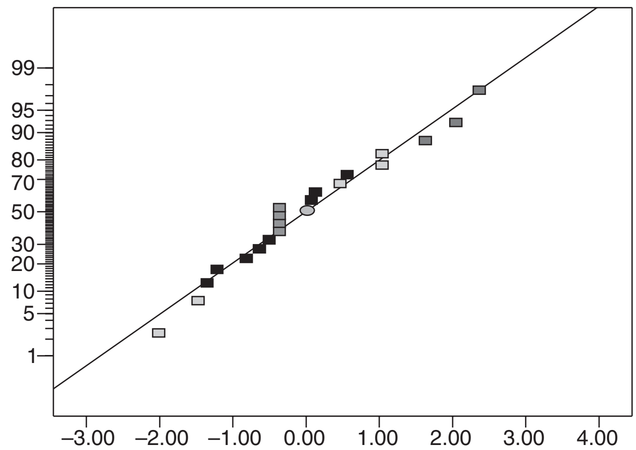

Fig. 2. Internally studentized and normal % probability plot of death of *Tribolium castaneum*

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

$$y_1 = 30.29 + 21.5A + 23.21B - 5.00\,C + 15.00\,AB + 7.27E^{-15}AC + 1.07E{-}14\,BC - 2.26\,A^2 - 2.8B^2 + 7.91C^2 \quad (2)$$

$$y_2 = 90.11 - 0.36A - 0.10\,B + 0.18C - 0.53AB - 0.45\,AC - 0.53BC - 0.97A^2 + 0.24B^2 + 0.073C^2 \quad (3)$$

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 show that the data points indicated that neither response transformation was required nor there was any apparent problem with normality. The general perception of straight line was quite clear in the normal probability supporting the hypothesis of normal distribution.

Interactive effect of processes of independent variables

Using multiple nonlinear regression model (Eqn 2 and 3), three dimensional contour plots were drawn to show the effects of binary combinations of independent variables on the predicted mortality. These plots are shown in Figs. 3, 4 and 5. Fig. 3 shows the integrated effect of distance between electrodes and the applied voltage on mortality. It indicated that at constant distance between electrodes, the increasing applied voltage increased the mortality of insects and the mortality also increased 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 increased 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.

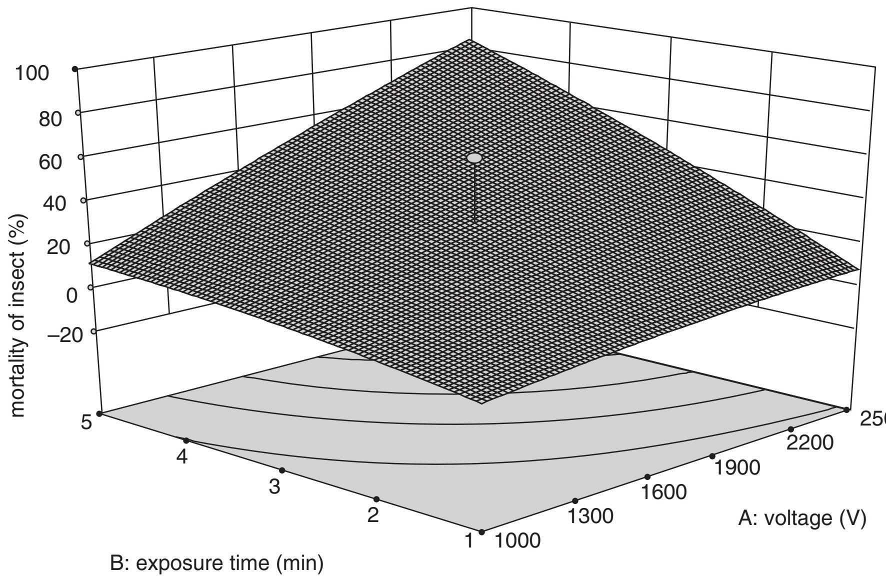

Fig. 3. Effect of voltage and exposure time on mortality of insects

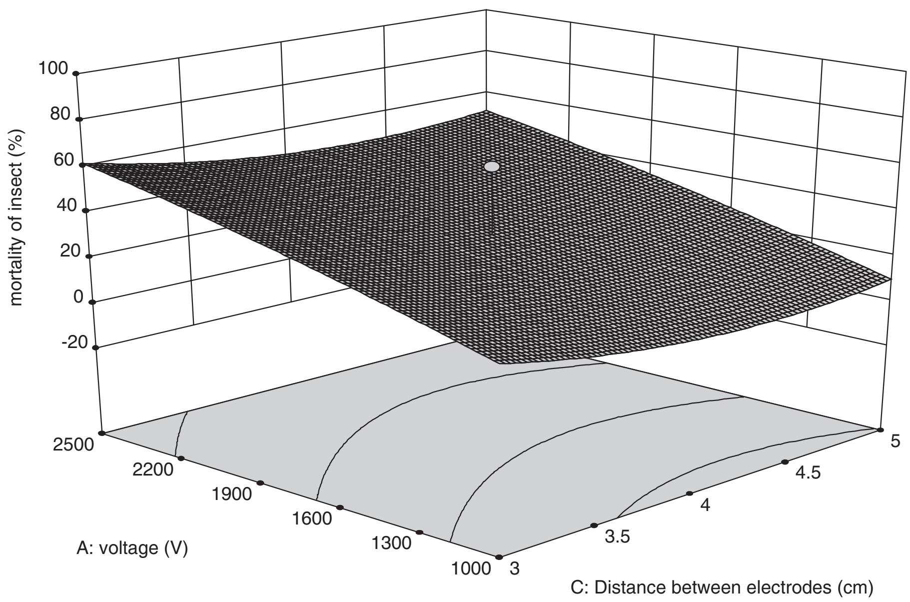

Fig. 4. Effect of voltage and electrode gap on mortality of insects

It was also found that there was no significant difference on the colour of plasma treated refined wheat flour on 20 different treatment combinations. Results are shown in Table 2.

CONCLUSION

Cold plasma-induced mortality of *Tribolium castaneum*, fed on refined wheat flour,was investigated with independent variables: 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

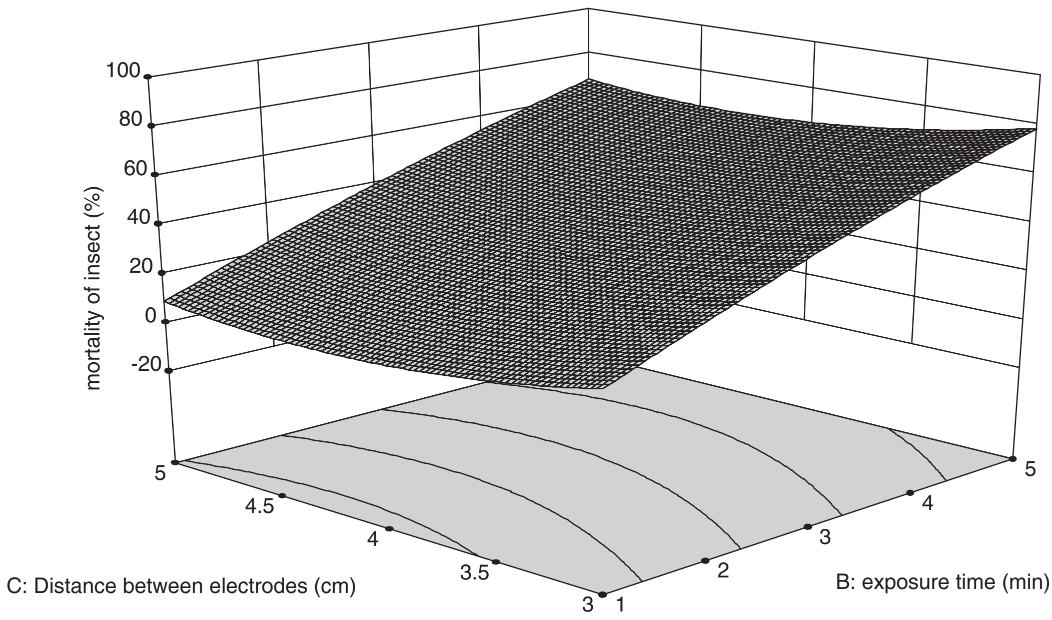

Fig. 5. Effect of electrode gap and exposure time on mortality of insect

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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