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Influence of cold plasma on mortality rate of different life stages of *Tribolium castaneum* on refined wheat flour

K. Ratish Ramanan, R. Sarumathi, R. Mahendran*

Department of Food Engineering, Indian Institute of Food Processing Technology, Thanjavur, Tamilnadu, India

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ABSTRACT

Red flour beetles, *Tribolium castaneum* (Herbst) are a major problem in packed refined wheat flour. Cold plasma is an emerging method which can be used to control the insects in stored food products. The effect of plasma on egg, larva and adult stages of *T. castaneum* was studied via three variables i.e. voltage, exposure time and distance between plasma electrodes. An experiment was designed and optimization of treatment conditions was carried out by response surface methodology. Twenty combinations of variables at different levels were experimented to analyse the response. Predictive equation for the mortality of insect stages were derived as a second order polynomial equation by quadratic model fitting at 95% confidence level. Interactive effects between independent variables were studied using 3D surface plot graphs. In all flour beetles' stages, 100% mortality can be achieved depending on plasma exposure time and plasma intensity. Optimum effect of plasma in controlling all stages of *T. castaneum* was found to be at 2500 V for 15 min at 3.7 cm distance between electrode. It could be a promising nonchemical, nonthermal method for integrated pest management in stored food products.

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 (Diekmann, 2009). Mostly, the refined wheat flour reaches the consumer in packed form. Stored packages which contains any life stage of an insect, (e.g. egg which is hard to differentiate from flour particles) can form as a source of quality deterioration and weight loss by further development and reproduction of insects. It can also create favourable conditions for growth of microorganism in the flour which leads to major food safety issues (Yar et al., 2017).

Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae) commonly known as red flour beetle, is the major pest rapidly capable of causing extensive damage to the stored grain and its products. *T. castaneum* majorly feed and affect the flour and milled products and also observed in seeds, pulses, millets, nuts, dried fruits, forest products, spices and animal matter. The presence of *T. castaneum* can cause greyish discoloration and produce pungent off-odour by scent glands, making flour unfit for consumption. Due to their activities, a relative weight loss of 328 mg in flour was observed during lifetime of a single insect (Good, 1936; Hagstrum and Subramanyam, 2006).

For the last three decades, fumigation using various gaseous toxic insecticides like phosphine and methyl bromide have been the most common pest management for stored grain and grain products globally. Due to repeated fumigation, *Tribolium* genus have developed insecticide resistance all over the world. *T. castaneum* have developed insecticide resistance against fumigants like phosphine (Gautam et al., 2016), methyl bromide (Rajendran, 1992) and pyrethroids like deltamethrin (Singh and Prakash, 2013), cypermethrin, cyfluthrin (Collins, 1990).

Research on other techniques in the control of *T. castaneum* include ozone (McDonough et al., 2011; Xinyi et al., 2017), radio frequency waves (Yu et al., 2016), microwaves (Lu et al., 2010), soft electrons (Imamura et al., 2004), ultraviolet radiation (Faruki et al., 2007), gamma irradiation (Ahmadi et al., 2013; Awang, 1984) and essential oils of *Carum copticum* L. (C. B. Clarke) (Sahaf et al., 2007), *Thymus persicus* (Ronniger ex Rech. f.) (Saroukolai et al., 2010), mustard, coconut, sesame and rocket

* Corresponding author.
E-mail address: mahendran@iifpt.edu.in (R. Mahendran).

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seed (Khanzada et al., 2015).

Cold plasma is a nonthermal and nonchemical method of insect control which serves in various industrial application (Donohue et al., 2006). Plasma is a fourth state of matter which is either wholly or partially ionized gas. The composition of plasma includes various reactive species such as gas atoms, molecules at ground and excited state, electrons, positive and negative ions, free radicals, quanta of electromagnetic radiation like UV photons and visible light. As there is no thermodynamic equilibrium between these reactive species leads to cooling of ions and more effectiveness from neutral species than energy transfer from electrons, thus the gas can be maintained at low temperature. Hence it is also known as nonthermal plasma. Cold plasma can be produced in gases at atmospheric or vacuum pressures by electrical discharges with less power input (Misra et al., 2016). Plasma was experimented over various insect species like Indian meal moths (*Plodia interpunctella* (Hübner)), vinegar fruit fly (*Drosophila melanogaster* (Meigen)), body lice (*Pediculus humanus humanus* L.), citrus mealybugs (*Planococcus citri* (Risso)), green peach aphids (*Myzus persicae* (Sulzer)), german cockroaches (*Blattella germanica* L.), western flower thrips (*Frankliniella occidentalis* (Pergande)), tobacco thrips (*Frankliniella fusca* (Hinds)), asian tiger mosquitoes (*Aedes albopictus* (Skuse)), twospotted spider mites (*Tetranychus urticae* (Koch)) and edible insect flour from yellow mealworms (*Tenebrio molitor* L.) (Abd El-Aziz, et al., 2014; Bures et al., 2006; Buβler et al., 2016; Donohue et al., 2008; Ferreira et al., 2016; Rumpold et al., 2014). No research work has been found on the impact of plasma over life stages of *T. castaneum* mortality in a food medium.

A study on the feasibility of cold plasma for control of adult *T. castaneum* was studied and obtained 100% mortality without change in colour quality of the refined wheat flour (Mahendran et al., 2016). The current study was focused on the effect of nonthermal plasma and its primary and secondary site of action on egg, larva and adult stages of *T. castaneum* on refined wheat flour. The objective was to analyse the influence of parameters like voltage, exposure time and electrode gap, on the mortality of insects and to optimize the conditions for higher mortality due to nonthermal plasma to further develop integrated pest management of refined wheat flour.

2. Materials and methods

2.1. Refined wheat flour

Refined wheat flour was purchased from local market and used for treatment and rearing of *T. castaneum* culture. Three control flour samples with insect stages were incubated to check the quality of flour affecting mortality of insects and it was ensured that quality of flour did not affect the insect stages. Flour was sieved using 300 μm mesh for collecting uniform particle size and separation of eggs after incubation of insects.

2.2. Rearing of different stages of *T. castaneum*

All stages of *T. castaneum* were reared using the pre-sieved refined wheat flour. Adult insects used for the rearing of insect cultures were obtained from previously infested flour. 10 adult insects were allowed to mate in 10 g of pre-sifted flour inside a plastic container with minute holes in the lid for aeration. Similarly, 8 cultures were incubated separately. All the cultures were kept inside a dark area at 28 °C and 70% relative humidity (RH) which falls in the suitable temperature range of 22.5–35 °C and 70% RH for prolonged adult life (Howe, 1962). After 48 h, 1–2 d old *T. castaneum* eggs were separated from the cultures using 300 μm sieve and verified under stereomicroscope. These eggs were used in experimental treatments and adults were allowed to incubate in the same cultures. After 25 d, the cultures were sieved to obtain 20–22 d old larvae (old instars) using 300 and 500 μm sieves and used in treatment samples. Further, the culture with remaining larvae and eggs (without previously included adults) were incubated for 20 d and the newly emerged adults were separated using 500 μm sieve and used for the cold plasma treatment.

2.3. Cold plasma treatment

Samples of 10 g of pre-sifted flour consisted of 10 eggs (1–2 d old), 5 larvae (20–22 d old), 5 adults (28–30 d old) separately for each treatment in 9 cm diameter glass petri plates with lid. The samples were treated with cold plasma system developed at Indian Institute of Food Processing Technology and consists of chamber of 350 × 350 × 350 mm which is operated at about 1 mbar under high voltage in the range of 1–10 kV with 50 Hz frequency supplied to the electrodes with adjustable clearance. The samples were exposed to plasma produced between two electrodes by dielectric barrier discharge (DBD) method.

2.4. Mortality determination

Three control samples containing same number of insects without cold plasma treatments were also incubated along with the treated sample at room condition (similar to packed flour storage condition). Further 20 d later, the number of larvae emerged from the eggs were observed and counted. Similarly, after treating larvae and adult insects the samples were kept at room temperature for 24 h and number of individuals which showed no response to stimuli and deformed were counted as dead insects. The mortality was calculated using Abbott's formula (Abbott, 1925).

2.5. Experimental design

20 combinations of experiments were conducted for each stage of *T. castaneum* based on the central composite design (CCD) of response surface methodology (RSM) considering three independent variables: voltage (V), exposure time (min), distance between electrodes (cm). The experimental range and level of the factors were tabulated (Table 1). The relationship between insect mortality and factors were found using a second order polynomial equation (Eq. (1).):

$$y = \beta_0 + \Sigma\beta_i x_i + \Sigma\beta_{ii} x_i^2 + \Sigma\beta_{ij} x_i x_j \quad (1)$$

where y is the predicted response value, x is independent variables and the coefficient of functions (i.e.) constant, linear, quadratic and interactive effects are represented as β_0, β_i, β_{ii} and β_{ij} respectively.

Table 1
Independent variables and their experimental level in central composite design.

Independent variables	Unit	Experimental Level				
		−1.68	−1	0	1	1.68
Voltage (V)	V	500	1000	2000	3000	3500
Exposure time (t)	min	2	5	10	15	20
Distance between electrodes (d)	cm	2	3	4	5	6