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RESEARCH PAPER

**Journal of Biodiversity and Environmental Sciences (JBES)**

ISSN: 2220-6663 (Print) 2222-3045 (Online)

Vol. 7, No. 5, p. 150-156, 2015

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## The effect of non-thermal plasma to control of stored product pests and changes in some characters of wheat materials

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Article published on November 30, 2015

**Key words:** Non-thermal Plasma, Stored product pests, wheat characters, Dielectric-barrier discharge.

### Abstract

Pesticides greatly reduce the damages caused by pests but they entail some risks. The point of the study was to examine the effect of non-thermal plasma (NTP) on *Tribolium confusum* (Coleoptera, Tenebrionidae) and *Ephestiakuehniella* (Lepidoptera, Pyralidae) and possible changes in some characters of treated wheat. For each pest, Three hundred and sixty 2-3 instar larvae among wheat at 5 separate groups were used. Fat, protein, ash and moisture content of wheat were measured. Experiments were done by a completely randomized design in triplicates. The results indicated that NTP has sufficient insecticidal effect. Mean percentage of mortality reached to 100%±0 after 20 s for both pests. A positive correlation was observed between exposure time and mean percentage of mortality. Non-significant difference was found between the treated and untreated groups in case of wheat traits. It is concluded that application of NTP can be considered as an appropriate alternative.

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## Introduction

*Tribolium confusum* (Coleoptera, Tenebrionidae) and *Ephesiakuehniella* (Lepidoptera, Pyralidae) feed on various stored products such as flour and cereals (Shafique *et al.*, 2006). *T. confusum* is attracted to grains containing high moisture and drastically reduce the quality and quantity of the infected products (Ahmed and Ahmed, 2002). The beetles emit unpleasant odor and can expedite the growth of the fungus in the contaminated products (Rustamani, 2014). *E. kuehniella* populations will increase rapidly if there is suitable condition for larval development. Silky webs produced by the larvae in density leads to increased humidity and the formation of moulds, which can produce toxins.

Application of fumigants is one of the main strategies to control of stored product pests (Pinniger and Harmon, 1999). Methyl bromide was successful fumigant in controlling of insect pest in post-harvest for a long time (Schneider *et al.*, 2003). But after recognizing the destructive effect of methyl bromide on the ozone layer, its consumption was prohibited (EPA, 2004), and trying to find suitable replacements has been started. Methyl bromide alternatives due to some limitations including high cost, impact on the commodity, difficulty of performance and resistance to them have not been developed yet (Lebedev *et al.*, 2013, Kevin *et al.*, 2006, Visalakshy and Krishnamoorthy, 2012).

A favored substitute might be the application of Non-thermal Plasma (NTP) (Bures *et al.*, 2006). NTP frequently was used for industrial purposes such as air filtration (Kelly-Wintenberg *et al.*, 2000), treatment of hazardous waste (Eliasson and Kogelschatz, 1991) and microbial decontamination (Efremov *et al.*, 2000, Laroussi *et al.*, 2003, Montie *et al.*, 2000, Moreira *et al.*, 2004) and promising results have been obtained. NTP is formed at atmospheric pressure, thus its application on thermally sensitive products is possible. There is likely that surface modification can be done without changes on properties (Kevin *et al.*, 2006).

Following the successful results of NTP in microbial decontamination (Efremov *et al.*, 2000, Montie *et al.*, 2000). Mishenko *et al.*, (2000) applied a combination of radiation and plasma at entomology branch against *Sitophilus granaries*, for the first time. Keever *et al.*, (2001) affected *Lasioderma serricornis* by NTP and counted mortality of the adults.

Donohue *et al.*, (2006) tested the effect of plasma (HTA-250A power) on *Frankliniella occidentalis*, *F. fusca*, *Aedes albopictus*, *Tetranychus urticae* and *Blattella germanica* for 60, 90, 120 and 180 s. Indian meal Moth, *Plodia interpunctella* larvae and pupae were exposed to atmospheric pressure plasma jet (APPJ). The number of APPJ pulses and distance from nozzle of APPJ were variable. Mortality, malformation and the emergence of offspring were observed. (Abd El-Aziz *et al.*, 2014). Bures *et al.*, (2006) recorded the mortality of *Myzus persicae*, *Planococcus citri* and *Pediculus humanus* treated by NTP after 1, 3, 5 and 24 h.

Given that NTP was applied in various fields and promising results have been obtained (Birmingham and Hammerstorm, 2000, Mizuno, 2007) it can be considered as an eco-friendly alternative for chemical methods. But limited studies have been done on the use of NTP for pest control in warehouse. To suggest application of NTP, more studies on its insecticidal effect and impacts on treated commodities should be tested. So the study was done for assessing the effect of NTP on *T. confusum* and *E. kuehniella*. The study was concerned with possible changes in some characters of wheat including Fat, protein, ash and moisture content between treated and control groups.

## Material and method

### Insect

*T. confusum* and *E. kuehniella* were collected from contaminated flour and transferred to Entomology laboratory. Adults were kept in plastic bottles and fed by wheat, wheat flour, yeast and bran. Adults were removed after 24 h and their offspring were reared in incubator at 26±2 °C and 65±5 r.h.

*Dielectric Barrier Discharge (DBD) Device*

APPD was generated between parallel electrodes by using the device in fig1. The discharge is taking place in normal atmospheric air, at frequency about 13 kHz. To generate DBD plasma a modified AC high voltage power supply was used. During all experiments, the applied voltage of the device was 10 kV. The room air was used and no separate gases were added.

*Mortality assays*

For each pest, Three hundred and sixty 2-3 instar larvae at separate five groups were used for bioassay tests. In any test, insects among wheat were placed between two electrodes and exposed to NTP for various times (2, 5, 10, 20 and 30 sec). The experiment was carried out in triplicates. For every stage, one group with sufficient replicates was left untreated as control.

*Biochemical tests*

To measure fat, ash and moisture contents AACC 30-25.01, AACC 08-01.01 and 44-16A respectively was used. Calculation of protein values were carried out according to the AOAC (1980).

*Statistical analysis*

Experiments were done by a completely randomized design. Obtained results were analyzed by the help of SPSS program ver.18. Independent T-test was used to determine the differences between the mortality of *T. confusum* and *E. kuehniella* and the treated and

untreated groups in terms of wheat traits groups. Results were reported as Mean ± SEM. The level of significance for each experiment was set at  $P < 0.05$ . excel software used for Drawing curves and estimating of R square and equation of regression line.

**Results**

*Effect of plasma on insect mortality*

The insects were exposed by plasma for various times and the mean percent mortality of treated bio tests are shown in table 1. It is apparent from this table that the maximum mortality (100±0) for *T. confusum* was found in 20 s and the minimum (38.88±1.66) observed in the treatment 2 s. Mean percentage mortality for *E. kuehniella* reached to 100% at 20 s treatment and 2 s resulted in the lowest mortality (28.57±0.57). Data for *T. confusum* can be compared with *E. kuehniella* which shows similarity. Mortality was increased, with gaining the time. Slopes of the regression line were 20.26 and 21.12 for *T. confusum* and *E. kuehniella* respectively (table.1) that confirmed a positive correlation between exposure time and mortality. It is noteworthy that, Mortality for 45, 60 and 75 s was 100 % that the results are not mentioned in the table. NTP had sufficient insecticidal effects on the both bio tests and statistical tests revealed no significant differences between mortality of *T. confusum* and *E. kuehniella* ( $p=.89$ ).

**Table 1.** Mean percent mortality of *T. confusum* and *E. kuehniella* larvae exposed to non-thermal Plasma for various time periods.

Pest	Exposure time(S)	Mean mortality (%)	Slop of regression line	R square
<i>T. confusum</i>	0	0±0	20.26	88%
	2	38.88±1.66		
	5	66.66±1.1		
	10	92.7±1.92		
	20	100±0		
	30	100±0		
	0	0±0		
<i>E. kuehniella</i>	2	28.57±0.57	21.12	91%
	5	62.50±1.15		
	10	87.50±0.88		
	20	100±0		
	30	100±0		

*Lethal time values*

Table 2 illustrates lethal exposure times at the 50, 90% level for the bio tests. As can be seen from the table (below), LT<sub>50</sub> are 2.83 and 3.53(s) for *T. confusum* and *E. kuehniella* respectively. Slopes of the probit curves are estimated 2.64 and 2.78 for *T. confusum* and *E. kuehniella*.

*Effect of plasma on wheat characters*

Some factors of treated and untreated wheat including Fat, protein, ash and moisture contents were listed in table 3. Measured fat for control and treatment was 1.12±0.01 and 1.14 ±0.02 respectively. Protein values were calculated 7.85±0.02 for control and 7.85±0.01 for treatment. Ash content of control (1.50±0.02) and treatment (1.50±0.14) was the same. Moisture content was recorded 5.80±0.28 and 5.63±0.35 for treated and untreated wheat.

**Table 2.** Lethal exposure times at the 50, 90% level for *T. confusum* and *E. kuehniella*.

Pest	LT <sub>50</sub> (s)	LT <sub>90</sub> (s)	Slope	Chi-square	df	P value
<i>T. confusum</i>	2.83 (1.77-3.82)	8.63 (6.22-15.54)	2.64	5.32	3	.15
<i>E. kuehniella</i>	3.53 (3.04-4.02)	10.20 (8.70-12.50)	2.78	4.47	3	.21

**Discussion**

Various insects cause quantitative and qualitative losses in stored products per year. Chemical pesticides widely are used to control stored product pests (Konradsen *et al.*, 2003). With respect to the negative effect of pesticides on the environment, trying to find suitable alternatives, is the subject of many studies. (Coronado *et al.*, 2004). Ozone or essential oil introduced as

replacements, but due to high cost, negative environmental effects and other weaknesses have not developed yet (Bures, 2004, Bures *et al.*, 2006). NTP was applied in various biological researches like sterilization and chemical decontamination and hopeful findings were recorded (Laroussi, 2002, Montie *et al.*, 2000). Lethal effect of NTP on pests has been tested in limited studies.

**Table 3.** Mean values of fat, protein, ash and moisture contents of the tested wheat.

Factor	Control (%)	Treatment (%)	t	df	pv	Significant difference
Fat	1.12±0.01	1.14±0.02	-0.80	7	.46	-
Protein	7.85±0.02	7.85±0.01	0.45	7	.67	-
Ash	1.50±0.02	1.50±0.14	-0.019	7	.98	-
moisture	5.80±0.28	5.63±0.35	0.55	7	.61	-

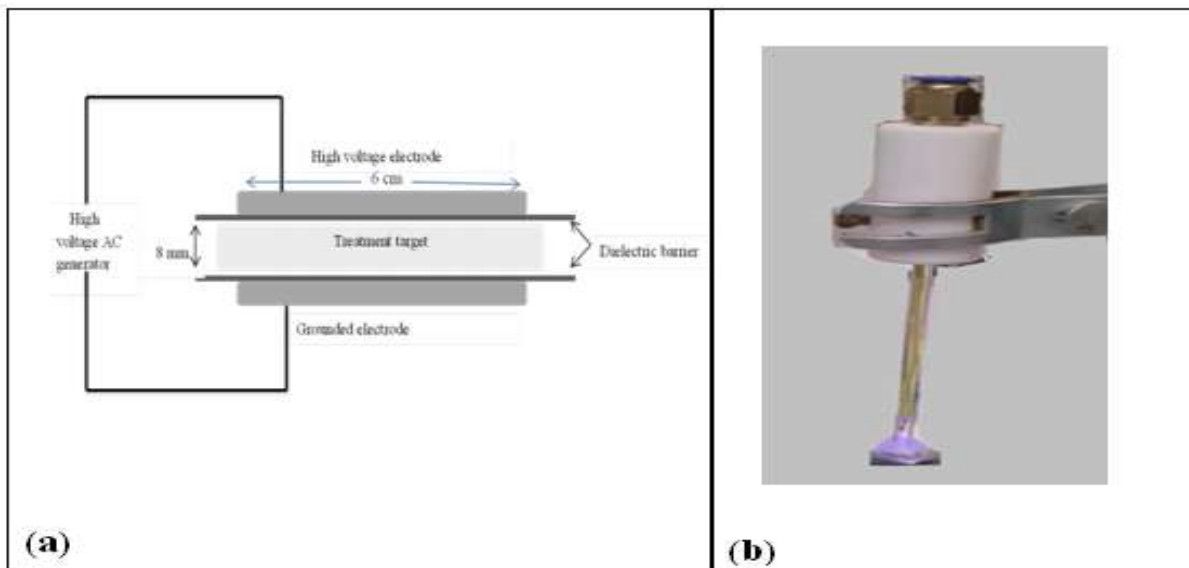
Obtained results proved insecticidal effect of NTP against *T. confusum* and *E. kuehniella*. In both cases, Mortality reached to 100% after 20 s. Statistical test showed that there is no significant difference between mortality of *T. confusum* and *E. kuehniella* (p=.89). At first study on insecticidal effect of NTP 100% mortality was counted (Mishenko *et al.*, 2000) which proved the results of the present study. Ninety-two percent of mortality was reported in exposed *Lasiodermaserricornes* with NTP. The results similar

to our findings confirmed that NTP can control various pests successfully. (Keever *et al.*, 2001).

By changing some variables such as time, can change the insecticidal effect of plasma. In accordance with Donohue *et al.*, (2006) our results showed a positive correlation between exposure time and mortality for both pests. Our results confirmed by Abd El-Aziz *et al.*, (2014), who found that NTP has effect on *Plodia interpunctella* survival. Larval and pupal mortality

was gained with increasing of APPJ pulses and decreased by reduction of distance from the jet. Mode of action plasma on various living tissues like bacteria, mammalian cells and insects has been studied, but significantly different mechanisms were proposed ( Dobrynin *et al.*, 2009). In the case of gram-positive bacteria, damage to the cell membrane has been introduced as the cause of death (Mendis *et al.*, 2002). While, Donohue *et al.*, (2006) observed

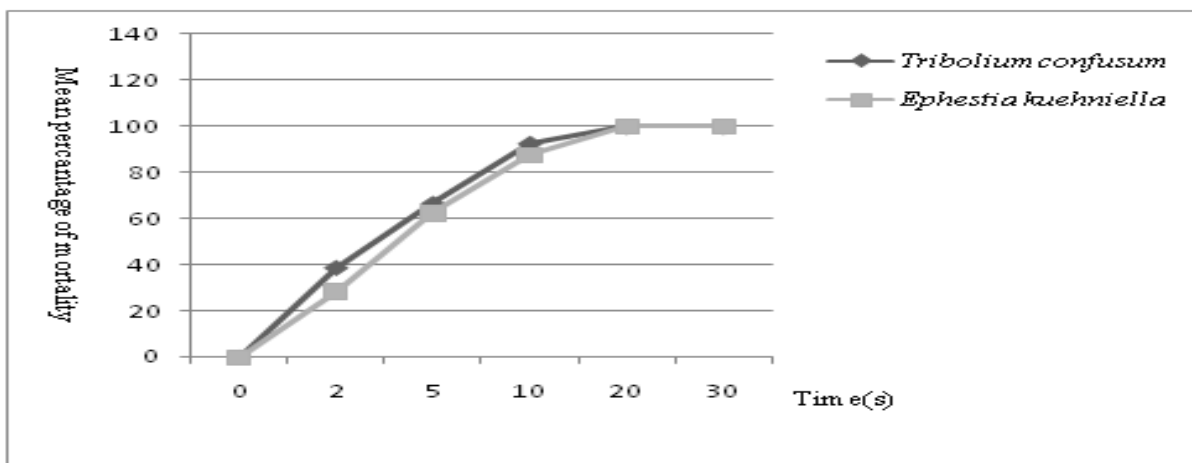
some behavioral changes in treated cockroaches and they recommended that NTP affect nervous and neuromuscular system. Abd El-Aziz *et al.*, (2014) said that Larvae are more susceptible compared with pupa that can be due to sclerotized cuticle of pupa. Different mechanisms can be related to different methods of plasma generation and differences in the structure of organisms.



**Fig. 1.** A schematic of the DBD Device (a) and a schematic of the plasma jet Device (b).

NTP has a couple of advantages. It can be generated at ambient temperature with low electrical power and has no harmful effect on appearance of the treated products. In the study, non-significant changes were observed between treated and untreated wheat in

point of measured characters. This accords with Rehn and Viol (2003) observations, which found no considerable changes in *Hordeum vulgare* seeds exposed to plasma.



**Fig. 2.** Comparison of mortality rate for *T. confusum* and *E. kuehniella* larvae.

Not only the negative effect of plasma on the product has not been reported, Dobinov *et al.*, (2000), stated that germination rate of *Avena sativa* seeds was increased after treating by plasma.

In a nutshell, the possibility of using NTP for pest control is considerable. Short time of treatment, flexibility and lack of harmful residues are the advantages of this method which can be noted. Physics experts are hopeful to use of NTP for cereal and other stored products during transport to the silos. Although, further studies are required to use a new technology and various aspects of its application must be examined. The mechanism of plasma and histological changes at treated insects can be part of future studies, as well.

#### Acknowledgment

The authors would like to thank Mr. Ostadi and Mrs. Hoseini for their technical assistance and Mr. Nikmaram and Mrs. Amini for their helpful comments.

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