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Ozone Fumigation as Safe Method for Controlling *Stegobium paniceum* (Linnaeus, 1758) (Coleoptera: Anobiidae) Infesting *Capsicum minimum* (Solanales: Solanaceae) Fruits in Storage

[1]Amira Ahmed Kamel Hassan Negm, [1]Rasha Ahmed Zinhoum,
[2]Samia Mohmed Abd El-Hameed and [3]Amira Afify

[1]Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt
[2]Horticulture Research Institute, Agricultural Research Center, Dokki, Giza, Egypt
[3]Entomology Department & Biotechnology Department,
Faculty of Science, Cairo University, Giza, Egypt

Abstract: The drugstore beetle *Stegobium paniceum* (L.) (Coleoptera: Anobiidae,) infests a variety of dried herbs and medicinal plants as red hot pepper plants. It's a quarantine dangerous pest which there are restrictions on it when exporting many stored products like red hot pepper. Alternatives for insecticides in management of insects in the grain handling and packing units are essential for maintenance the quality of the products and decline the environmental impact. Ozone fumigation was investigated as a safe way for controlling *S. paniceum* before exporting or in case of infestation in the stores. Results indicated that treating adults of *S. paniceum* with 4800 ppm of ozone gas resulted 100% mortality. The percentage of reduction was significantly increased with the increscent of concentration dose for ozone gas. Adult emergence was completely inhibited at 4800 ppm after treatment of egg stage. The results revealed presence of DNA damage for the cells of the whole-body of both sexes of *S. paniceum* after exposing to LC_{50} of ozone. The results also indicated that the dose of ozone that caused 100% mortality did not alter the chemical characteristics of red pepper (*Capsicum mnimum* Mill.).

Key words: Ozone · Comet Assay · DNA Damage · Red Pepper

INTRODUCTION

Stegobium paniceum (L.) infests different dried foods, like biscuits, chocolate, flour, dried bread, spices, drugs [1]. It's the largely wide spread storage pest can be found in botanicals.

Capsicum minimum Mill. (red pepper) fruit is considered as a vital medicinal fruit in Egypt. Its medicinal importance includes phenolic compounds, carotenoids and ascorbic acid that play an important protection role against many human diseases [2].

Using alternatives to insecticides is very important including the use of biological control, like pathogens, predators and parasites or physical control methods by using ozone gas [3].

Many studies state that using ozone gas against larvae of some stored product pests such as *Ephestia cautella, plodia interpunctella, Trogoderma granarium and Tribolium castaneum* is very effective as it caused many biochemical and ultrastructural changes in the previously mentioned pests [4]. Using of ozone gas considered as an attractive unconventional method for controlling the stored product insects because it decomposes naturally into molecular oxygen and may be generated at the usage site. So, there are no ozone residues in the grains [5].

Ozone is a strong oxidant, reactive to bio molecules. Ozone decreases the activation of both viruses and bacteria and causes cellular DNA damage. Ozone used for many purposes such as an antimicrobial agent, storage food without compromising quality or causing any harm to human, control insect and food sanitizer [6].

There is a damage for DNA of *S. paniceum* was estimated after application of ozone, using the single cell gel electrophoresis (SCGE) (Alkaline comet assay) measure, normally called the comet assay [7, 8].

Corresponding Author: Amira Ahmed Kamel Hassan Negm, Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt.

As a outcome of the deficiency in DNA damage response Genomic instability and cancer formation may occur [9]. For the period of electrophoresis, the DNA fragments move out of the single cells, forming a tail toward the anode and give the damaged cells the shape of comet. The remain nucleus form the head of the comet, whereas the tail is formed by the fragments. The damage greatness is closely related to the extension of the tail [10]. Consequently, this study intends to assess the genotoxic effect of using the ozone gas for controlling of *S. paniceum* and for estimating its impact on the chemical composition of *C. minimum* (hot pepper) fruit.

MATERIALS AND METHODS

Insect: *Stegobium paniceum* beetles used in ozone experiment were reared in Stored Grain and Product Pests Department, Plant Protection Research Institute, whereas they were kept at 28°C and 65 R.H. on red pepper for at least three months.

Red pepper Fruits of *Capsicum minimum* were obtained from the Medicinal and Aromatic Plants Research Department, Horticulture Research Institute, Agricultural Research Centre. The chemical analysis was done at the Central Laboratory of the Horticulture Research Institute, Agricultural Research Centre. Red pepper was ground in a home grinder to a powder for chemical analysis

Ozone Generator and Treatment of Insects with Ozone: The generation of ozone took place through a controlled flow of oxygen, through a corona discharge in the ozone generator from purified extra-dry oxygen providing gas at the laboratory of Food Technology Research Institute, Agriculture Research Centre.

Ozone fumigation was carried out inside different volumes of glass containers (⅛, ¼, ½ and 1 L capacity). The ozone cylinder was connected to the inlet tube of the flask with a short hose. fifteen pairs of *S. paniceum* were kept separately into mesh bags (4 × 8 cm) containing 10 g of red pepper and in case of egg, the numbers of eggs of *S. paniceum* which resulted from the 15 pairs of *S. paniceum* adults for 24 h were put into a same bag which conation 10 g of red pepper. Then closed with rubber bands and then introduced into a glass container. Four replicates were used for each tested concentration of 1200, 2400, 3600 and 4800 ppm for 1 h. Four untreated bags with ozone were kept as control for each stage. Mortality percentage was calculated after 24 h from the end of the exposure period for adult and adult emergency for eggs stage after 5- 6 weeks. The percentage of reduction in the adult emergence was calculated according to Henderson and Tilton [11].

Reduction % = Control- treated/ Control x 100

Examination the Damage of DNA by Alkaline SCG Assay: After treating adult of *S. paniceum* with LC_{50} of ozone gas, the whole-body of male and female adults were crushed in 1 ml cold phosphate buffer solution (Ca^{2+} free). Alkaline SCG Assay was used to inspect the single strand breaks of DNA (incomplete excision repair sites and single strand breaks), cross-linking and alkali-labile sites [12]. The comet assay analyses DNA damage level in the whole-body cells of *S. paniceum* for evaluating the genotoxic impacts of ozone. Three replicates for each sample were prepared. A barrier filter (605 nm) and an excitation filter (524 nm) of an Axio fluorescence microscope (Carl Zeiss, Germany) were used for comets analysis.

Evaluation of DNA Damage: DNA damage was exposed to ethidium bromide stain, using a fluorescent microscope. The percentage of migrated DNA (DNA tail %) and the length of migrated DNA were measured (tail length) (TL), as well as the product of both, called the tail moment (TM), using comet analysis software 4.0 made by Kinetic Imaging, Ltd (Liverpool, U.K.), connected to a CCD camera. every sample contained 50-100 chosen cells (minimum of 25 cells/slide and 3 slides/treatment were estimated).

Study of Effect of Ozone Gas on Chemical Characterisation of *Capsicum minimum*: For each sample, 50 g of *C. minimum* was exposed to 4800 ppm for 1 h of ozone gas (O_3) that caused 100% mortality for *S. paniceum*.

Determination of Capsaicin: Capsaicin in the samples of *C. minimum* fruit were measured with HPLC technique [13].

Alkaloids: The absorbance of the alkaloid complex was measured according to [14] .

Total Flavonoids: Total flavonoids were determined by using the method of [15].

Total Phenols Compounds: For the extraction of phenolic compounds, it was conducted according to [16].

Antioxidant: The activity of antioxidant for the extracts was studied through the determination was evaluated by using the method reported by [17].

Ascorbic Acid: Ascorbic acid was estimated according to [18].

Acidity: Total acidity was measured as mentioned in [19].

Determination of Total Free Amino Acids (gm. /100gm Dry Weight): Total free amino acids were measured according to [20].

Determination of Total Anthocyanin: Total anthocyanin was estimated by using mg/100 g dry weight of hot pepper according to[21].

Pigments Content: Pigments (chlorophyll a, b and carotenoids, (mg/100 g dry weight) were measured according to[22].

Data Analysis: Probit analysis was used for estimating the values of LC_{50} and LC_{99} [23]. Data are presented as mean ± SD and was statistically analysed, of the one way analysis of variance (ANOVA), (Tukey) to compare the effect of different concentrations of ozone gas on adult mortality and reduction percentages in adults emerged from eggs ($P \leq 0.05$) using SPSS software (version 15; SPSS, Chicago, IL, U.S.A.). Students t-test was performed to determine the damage that took place in DNA and the chemical changes in red pepper after treating with ozone.

RESULTS

Effect of Ozone Gas on *S. paniceum*: Under laboratory conditions, ozone fumigation treatments of adult *S. paniceum* at 1200, 2400, 3600 and 4800 ppm, for 1 h caused gradual significant increase mortality, comparing those in control (Table 1). High concentration of ozone gas (up to 4800 ppm) resulted complete mortality in adults. The reduction percentage in adulte emergence was significantly increased with the increscent of concentration dose for ozone gas. Adult emergence was completely inhibited at 4800 ppm.

The LC_{50} and LC_{99} values of ozone gas fumigation impact against eggs and adults of *S. paniceum* were shown in Table 2.

Comet Assay: Single-cell Gel Electrophoresis: The data shows that there is DNA damages in the whole-body cells of *S. paniceum* males and females exposed to ozone (Fig. 1 A–D). There is a major variation in percentage of tailed cells, compared to intact cells after ozone exposure. The data of the comet assay indicates considerable increase in the mean TM and % T at the exposed adults (female) using ozone (Table 3). The mean data of TL shows that there is no impact increment between the different sexes of exposed adults using ozone (Table 3).

Evaluation the Effect of Ozone Gas on Chemical Properties of *Capsicum minimum*: The effect of the high concentration (4800 ppm) of ozone fumigation that caused 100% mortality of *S. paniceum* on *C. minimum* is

Table 1: Mortality percentages of *S. paniceum* adults and reduction percentages in adults emerged from eggs exposed to ozone fumigation

Ozone concentration (ppm)	Corrected mortality % of adults (Mean± SD)	Reduction % of adults (Mean± SD)
1200	22.5 ± 1.59^{d}	27.19 ± 1.45^{d}
2400	48.33 ± 3.67^{c}	45.80 ± 1.2^{c}
3600	69.17 ± 4.79^{b}	73.72 ± 1.53^{b}
4800	100 ± 0.0^{a}	100 ± 0.0^{a}
F(df)	339(3)	2070(3)
P value	<0.001	<0.001

Means within the same column followed by different small letters are significant ($P< 0.05$)

Table 2: Lethal concentration values, confidence limits, slope and correlation (R) of *S. paniceum* eggs and adults exposed to ozone

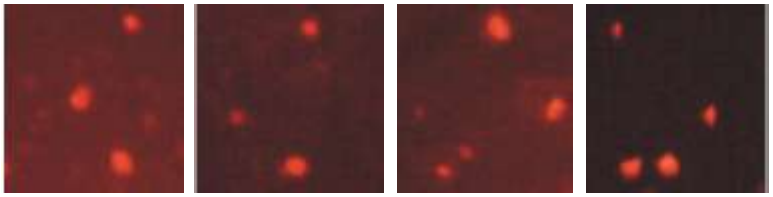
			Confidence limits (ppm)					
			LC_{50}		LC_{99}			
Stage	LC_{50} (ppm)	LC_{99} (ppm)	Lower	Upper	Lower	Upper	Slope ± SE	*R*
Egg	2235.19	4554.06	1530.09	3265.20	3974.96	5688.44	2.51 ± 0.39	0.93
Adult	2021.09	4177.72	1452.44	2812.37	3367.71	6457.22	2.78 ± 0.35	0.92

R = correlation coefficient, S.E. = standard error of regression line

Table 3: DNA damaging activity of ozone in male and female adults of *S. paniceum*, mean tail length (TL) (im), mean tail DNA (% T) and mean tail moment (TM)

	TL		% T		TM	
Treatment	Male	Female	Male	Female	Male	Female
Control	2.3 ± 0.31[a]	2.3 ± 0.044[a]	2.97 ± 0.16[a]	2.9 ± 2.5[a]	0.069 ± 0.02[a]	0.07 ± 0.05[a]
Ozone	3.9 ± 0.35[b]	3.4 ± 0.11[b]	8.17 ± 0.7[b]	16.2 ± 2.7[b]	0.34 ± 0.05[b]	0.7 ± 0.12[b]
t(df)	-5.9(4)	-16.3(4)	-12.5(4)	-6.3(4)	-8.7(4)	-2.2(4)
P value	<0.004	<0.001	<0.001	<0.003	<0.001	<0.091

Means within the same column followed by different small letters are significant ($P< 0.05$)



A B C D

Fig. 1: Effect of ozone on DNA of the whole body cells of male and female adults of *S. paniceum*(A, B - Control male and female respectively, C, D -ozone treated male and female respectively)

Table 4: The effect of ozone gas on chemical properties of *Capsicum minimum*

Treatment	Capsaicin (mg/100 g)	Alkaloids (mg/100 g)	Flavonoids (mg/100 g)	Total phenols (mg/g)	Antioxidant (%)	Ascorbic acid (ASA)	Total acidity (%)	Total free amino acids (g/100 g)	Total anthocyanin (mg/100 g)	Chlorophyll A (mg/g)	Chlorophyll B (mg/g)	Carotene (mg/g)
Control	0.307±0.0[a]	0.70±0.0[a]	0.61±0.0[a]	0.21±0.0[a]	59.9±0.0[a]	9.16±0.0[a]	0.041±0.0[a]	0.073±0.0[a]	0.15±0.0[a]	0.26±0.0[a]	0.29±0.0[a]	29.29±0.0[a]
Ozone	0.310±0.0[a]	0.68±0.0 [a]	0.63±0.0 [a]	0.22 ±0 .0[a]	53.4±0.0[b]	8.56±0.0[b]	0.042±0.0[a]	0.106±0.0[b]	0.14±0.0[a]	0.30±0.0[a]	0.42±0.0[a]	28.67±0.0[b]

Means within the same column followed by different small letters are significant ($P< 0.05$)

presented in Table 4. No significant differences at $P< 0.05$ in amounts of capsaicin, alkaloids, amounts of flavonoids, total phenols, total acidity, total anthocyanin, chlorophyll A and chlorophyll B between the control and ozone treated samples, whereas there were significant differences at $P< 0.05$ between the control and ozone treatments in the amount of antioxidant (more in control), ascorbic acid (more in control), total free amino acids (more in ozone) and carotene (more in control).

DISCUSSION

Ozone is a promising safe way for controlling stored-grain insects and microorganisms and to decrease myco-toxins without leavening any residues. ozone insecticidal activity involves the insect's respiratory system, which is likely to be the main cause for mortality after exposure to ozone [24] (Tiwari *et al*., 2010). In the present study, increasing the concentration of ozone fumigation significantly increased the mortality of adult *S. paniceum* with different tested concentrations. exposing the pest to 4800 ppm of ozone gas 100% adult mortality took place and adult emergence was completely inhibited. Also, it was found that the egg stage of *S. paniceum* is more tolerant to ozone than adult stage. This might be because of the chemical nature of the egg chorion that might prevent entry of the ozone. These data are in concord with [25] who proved that high concentrations (4800ppm) of ozone gas was extremely toxic ($p < 0.05$) for adults of *S. paniceum* and *Gibbium psylloides*. [4] stated that increasing ozone gas concentration or exposing periods increased the larval mortality of *Plodiainter punctella , Ephestia cautella, Tribolium castaneum* and *Trogoderma granarium*. They stated that 100% mortality for all species was reached at 400 ppm for 8 h.

In vitro ozone is genotoxic to plants, cell cultures and microorganisms. The result of *in vivo* cytogenetic examinations on research animals after inhalation exposure are incompatible. It was confirmed that there are chromosome aberrations in the lymphocytes of the Chinese hamsters and not found in mice. Exposing for ozone gas has the ability to induce chromatid deletion in pulmonary macrophages in rats. For human, there are a small number of studies and experiments that didn't give a conclusion on the cytogenetic effects of ozone gas exposure in lymphocytes in humans. On the other hand, ozone induces lung adenomas in strain A/J mice, not in Swiss-Webster mice after four to six months of inhalation exposure [26]. In the immunostaining study, the results of

the comet assay indicated a significant enlarge in the mean tail moment and percentage of DNA tail only of female adults after exposing to ozone comparing to the untreated group. It can be explained by the previous conflict of the results of ozone.

According to the obtained results, ozone fumigation had proven to be effective and did not harm the quality of red pepper. [27] proved that the content of capsaicin of the dried chillies decreases when exposure time increases without statistical differences. Treated red chili pepper with different ozone concentrations 0.5, 1, 1.5 and 2 mg /L had no significant impact on capsaicin, total carotenoid and flavonoid [28].

In this experiment, there are a great differences between total phenolic content and total flavonoids contents. The total phenolic content in red pepper treated with ozone increased compared with un treated sample while total flavonoids content decreased after exposure, this result is in agreement with [28] who reported that espousing the red pepper to 2ppm ozone increased total phenolic content.

The activity of Antioxidant was found to be reduced. The result of ozone exposure on antioxidant of fruit is wholly based on ozone dosage[29].

The recent findings are in harmony with those of [30] who indicated the higher efficiency of ascorbic acid (vitamin C), which play a big role in extending the shelf-life of fresh products.

There are insignificant differences of Chlorophyll A and Chlorophyll B between ozone and the control. The data of this study are similar to the previous work reported by[27] who found that the colour of dried chillies does not change after ozone exposure for 0, 5, 10, 15, 20, 25 and 30 min.

CONCLUSION

Ozone treatment is a safe way for controlling *S. paniceum* and can be considered as a good alternative to pesticides. It was proved that treating the pest with ozone gas caused great dame for DNA. It was also proven that Ozone gas didn't affect the major chemical properties of *C. minimum*. hence, it can be used for protecting herbs during storage without any harm for their quality

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