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Effect of ozone gas against life stages of Ephestia kuehniella Zeller (Lepidoptera: Pyralidae) in laboratory and a storehouse

Moraglio S. T., Bosco L., Pogolotti C., Tavella L.

Dipartimento di Scienze Agrarie, Forestali e Alimentari (DISAFA), University of Torino, Largo Paolo Braccini 2, 10095 Grugliasco (TO), Italy

Corresponding author: Luciana Tavella
tel. +39 011 6708533 – fax +39 011 6708535
e-mail luciana.tavella@unito.it

Abstract

Effects of gaseous ozone on *Ephestia kuehniella* eggs, larvae, pupae and adults were evaluated in laboratory and storehouse trials. In laboratory, all life stages were freely exposed in a test chamber to five ozone concentrations (5, 10, 20, 50 and 100 ppm) for 24 h to assess mortality, and also development for the treated eggs, larvae and pupae, and the number of eggs laid by the treated adults. In a storehouse, the effect of ozone at 50 ppm on adults was evaluated in comparison with routine treatment (pyrethrin application) and untreated control. In laboratory, all tested ozone concentrations significantly increased mortality of all *E. kuehniella* stages, in comparison with untreated control; however, complete mortality was observed only for adults starting from ozone concentration of 50 ppm. Time needed for the eggs to hatch and the larvae to pupate was delayed by ozone treatments, starting from ozone concentration of 10 or 5 ppm, respectively. Moreover, ozone affected also the number of eggs laid by surviving adults in the 24 h following ozone treatment: egg hatching rate was significantly lower at all concentrations. In the storehouse trials, the ozone treatment at 50 ppm killed almost all exposed adults as the pyrethrin treatment. Therefore, the results from this study suggest that ozone can be considered as an effective alternative to the current routine spray of pyrethrins during spring and summer.

Highlights

- Ozone application increased mortality for all life stages compared with the control
- Complete mortality was observed only for adults, at concentrations ≥ 50 ppm
- Mortality of eggs laid by treated adults increased with the increase of ozone concentration
- Ozone concentration of 50 ppm killed 99% of exposed adults in a storehouse trials
- Ozone can be considered as an effective alternative to pyrethrin application

Key words: Mediterranean flour moth; CT value; mortality; development time; hazelnut storehouse.

1. Introduction

Stored-product insects can cause serious postharvest losses, and they also contribute to contamination of foodstuffs through the presence of live insects, chemical excretions from their body, production of silk from mandibular glands, dead insects, and cast skins, general infestation of buildings and other storage structures (Hubert et al., 2018; Phillips and Throne, 2010). Chemical control is becoming increasingly difficult due to the development of insect resistance to insecticides (Boyer et al., 2012; Pimentel et al., 2007), the phase out of methyl bromide in 2005 as a fumigant, the environmental and safety concerns, and the consumers' demand for organic and pesticide residue-free food (Hansen et al., 2013; Işikber and Athanassiou, 2014; Jian et al., 2013; Phillips and Throne, 2010; Tiwari et al., 2010). Consequently, with the aim at implementing alternative measures to control stored-product insects, research has been recently focused on the application of ozone (O_3) as an effective fumigant (Hansen et al., 2013, 2012; Işikber and Athanassiou, 2014; Jian et al., 2013; McDonough et al., 2011; Tiwari et al., 2010). Actually, applications of ozone are environmentally friendly because ozone can be easily generated with electricity and dried air on site, and it decomposes rapidly to molecular oxygen without leaving any residue on the commodity (Işikber and Athanassiou, 2014; Jian et al., 2013; Kells et al., 2001).

Ozone is a strong oxidant with a long history of safe use in many fields and mostly used in the industries of pharmaceuticals, synthetic lubricants, and many other organic compounds, and for various other environmental applications (Jian et al., 2013; Tiwari et al., 2010). It has been already widely applied as a powerful disinfectant in water treatment, for controlling microorganisms in water and air sources, eradicating water-borne parasites, and at present, many municipal water treatment systems control bacteria with ozone instead of chlorine oxide (Jian et al., 2013). Moreover, ozone has been successfully used as a sterilant or disinfectant and sanitizer for the treatment of agriculture products and equipment, such as fruit and vegetables, dairy and swine effluent, meat, gelatin, manufacturing equipment, packaging materials; it prevents microorganism activity on food surfaces, is effective on degradation of mycotoxins and pesticide residues on foods, and extends the shelf life of fruit and vegetables (Jian et al., 2013; Kamber et al., 2017; Pandiselvam et al., 2017; Tiwari et al., 2010; Trombete et al., 2016).

Application of ozone in stored-products is effective in controlling insects, microorganisms such as fungi and bacteria, and degrade pesticide residues (Al-Ahmadi et al., 2009; Işikber and Athanassiou, 2014; Jian et al., 2013; Savi et al., 2016, 2015; Tiwari et al., 2010). Among stored-product insect pests, ozone is effective to control weevils, such as *Sitophilus* spp. (Coleoptera: Curculionidae), and other beetles, such as the lesser grain borer *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae), the drugstore beetle *Stegobium paniceum* (L.) (Coleoptera: Anobiidae), the merchant grain beetle *Oryzaephilus mercator* (Fauvel), the sawtoothed grain beetle *O. surinamensis* (L.) (Coleoptera:

Silvanidae), *Tribolium* spp. (Coleoptera: Tenebrionidae), the rusty grain beetle *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae), and moths, such as the Angoumois grain moth *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae), the Mediterranean flour moth *Ephestia kuehniella* Zeller and the Indian meal moth *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) (Bonjour et al., 2011; Hansen et al., 2013, 2012; Işikber and Athanassiou, 2014; Jian et al., 2013; Mahroof et al., 2018; McDonough et al., 2011; Sadeghi et al., 2017; Sousa et al., 2016; Subramanyam et al., 2017; Tiwari et al., 2010). At different concentrations and exposure times, ozone is effective to control both external and internal insect feeders (Hansen et al., 2012).

For the control of stored-product moth pests, the effects of ozone application have been largely investigated on the Indian meal moth *P. interpunctella* (Bonjour et al., 2011; Hansen et al., 2013; Keivanloo et al., 2014, 2013; Kells et al., 2001; McDonough et al., 2011; Shaghaghian et al., 2014; Tiwari et al., 2010), whereas fewer studies are available for the genus *Ephestia*, including the Mediterranean flour moth *E. kuehniella*, which is one of the most important stored-product pests worldwide (Athanassiou et al., 2008; Yaman et al., 2015). Moreover, in these studies experimental conditions, such as exposure time and ozone concentration, modalities of exposure (i.e., specimens exposed freely or in presence of commodities), vary highly (Abo-El-Saad and Elshafie, 2011; Hansen et al., 2012; Husain et al., 2015; Işikber and Öztekin, 2009; Işikber et al., 2007; Sadeghi et al., 2017). Since the effectiveness of ozone depends on its application amount (i.e., concentrations and exposure times) but in a greater extent on its reaction with insects after the substrate organic matter oxidation (Jian et al., 2013), different effects of ozone on insects assessed in these studies might be caused by the presence or absence of the organic substrate (Jian et al., 2013).

Therefore, this study was designed at improving the knowledge on the effects of ozone on all life stages of *E. kuehniella* (i.e., eggs, larvae, pupae and adults) that were freely exposed at different ozone concentrations in laboratory conditions to evaluate the lethal effects (i.e., direct mortality), and also sublethal effects such as developmental times of eggs, larvae and pupae, and hatching of eggs laid by the treated adults. Moreover, since in Piedmont, northwestern Italy, *E. kuehniella* is one of the main pests of stored hazelnuts in processing hazelnut factories, the study aimed at implementing alternative control measures; therefore, an application trial was conducted in a hazelnut storehouse to compare the efficacy of the routine treatment (weekly sprays of pyrethrins during spring and summer) with ozone treatment at the lowest lethal concentration on *E. kuehniella* adults evaluated under laboratory conditions.

1. Materials and methods

2.1. Insect supply

Eggs and larvae (2nd- and 4th-instars) of *E. kuehniella*, provided by Biotop (Livron-sur-Drôme, France), were used to start insect cultures. Colonies were reared on durum wheat flour plus dried brewer yeast (2%), and kept in climatic chambers under controlled conditions (25±1°C; 60±5% RH; L:D 16:8). Larvae were confined in glass containers (length×width×height 20×10×10 cm), opened on the top and covered with an insect-proof fine gauze (mesh size 0.3×0.4 mm), until adult emergence. Every 24 h, the new emerged adults were collected with a pneumatic aspirator (Vacuum Pump mod. 707, Asal s.r.l., Cernusco s/N, Milano, Italy); then, they were grouped in around 100 specimens and placed in a Plexiglas cylinder to allow oviposition. Cylinders (height 20 cm, diameter 10 cm) were closed by an insect-proof net (mesh size 0.6×1 mm) at both extremities, and placed inverted over a Petri dish (diameter 13.5 cm), containing a black cardboard on the bottom to facilitate counting of the egg that fell on it. Every 24 h, eggs were collected with a fine brush, and stored in cold climatic chambers (5±1°C) no longer than one week. In fact, they were weekly used to start new colonies, in order to continuously obtain cohorts of the same age of larvae, pupae and adults.

2.2. Ozone generator and gas monitoring

In the trials, the ozone gas was produced using the mobile combined system (ozone and ozonated water production) COMBI.O3 (model C100-AG, O3Technology S.r.l., Pisogne, Brescia, Italy), ozone generator with corona discharge technology and nominal capacity 100 g/h. The oxygen supply for ozone generator was provided by two molecular sieves (model ATF-23, Chart Sequal Technologies Inc., Ball Ground, Georgia, USA) fed with surrounding air by a compressor (model 2750CGHI60, Gardner Denver Thomas GmbH, Fürstenfeldbruck, Germany), and the power supply for ozone generator was provided by two power transformers 5.0-0-5.0 kV, 100mA. The ozone gas was released into a test chamber, which was different for the laboratory and storehouse trials, as below described. The ozone concentration was measured by means of a digital monitor-controller, and it was continuously monitored into the test chamber by the recirculation of ozone-enriched air from the chamber through a BMT 964 UV-photometric ozone analyzer (BMT Messtechnik Gmbh, Berlin, Germany) that controlled the ozone generator output. The recirculation of ozone enriched air was assured by a ventilator (model MF401MF0.85M, P 0.85 KW). The movable machinery (length×width×height 128×98×110 cm) was also equipped with an electrochemical ozone detector (model EV49303PL, 0-5 ppmv, EDS, San Giovanni Lupatoto, Verona, Italy) to measure the ozone concentration in the environment.

2.3. Laboratory trials

The laboratory trials were carried out in a test chamber consisting of a Plexiglas (thickness 1 cm) chamber (length×width×height 150×60×120 cm, volume 1080 L), hermetically sealed and equipped, at one of the longer sides, with two openings for handling (diameter 11 cm), closable with

lids and gaskets. In one of the shorter sides, there were two openings (diameter 6 cm), in which the inlet and outlet flexible PVC pipes (diameter 6 cm, length 2.0m and 1.5m, respectively), connected with the ozone generator, were sealed with a multi-purpose silicone sealant. The inlet pipe was placed 15 cm above the outlet pipe, and inserted for 50 cm of the length into the test chamber in order to provide and maintain the recirculation of ozone-enriched air flow (pressure of 0.3 bar) inside the chamber. The following ozone concentrations were tested for 24 h of exposure time: 0 (only airflow, no ozone), 5, 10, 20, 50, and 100 ppm. The ozone concentration (in ppm) was also multiplied by the exposure time (in minutes) to calculate the dosage [CT, concentration (ppm) × time (minutes)] values (McDonough et al., 2011). The temperature inside the test chamber was measured through a column thermometer placed inside the test chamber during all the trials, and it always increased from the initial 25 °C-38 °C as the functioning of the machinery caused a heating of the output airflow, independently of ozone addition.

In laboratory trials, cohorts of eggs, larvae, pupae and adults of *E. kuehniella* were tested. Eggs were daily collected from adult rearing, and eggs not older than 24 h were used. Larvae (aged from 2^{nd}-to 3^{rd}-instar) and pupae were collected from cohorts of the same age, and adults not older than 24 h were used. Groups of 10 specimens of the same life stage were placed each one into an opened glass container (height 4 cm, 10.5 cm diameter), covered with an insect-proof net (mesh size 0.6×1 mm) tightly sealed with a PVC tape. Eggs, larvae and pupae were transferred into their containers by means of a brush. In the containers with eggs a black filter paper was laid down on the bottom to facilitate the egg counting, while in the containers with larvae or pupae a small amount (5 ml) of durum wheat flour was added. Before using them, all eggs were observed by a stereomicroscope, and those ones showing alterations in colour (from light brown to black) and/or shape, or wrinkled looking were discarded, while all larvae and pupae were gently touched with a brush to check their vitality. For each life stage and treatment, overall 90 specimens were used as follows: 10 specimens were inserted into each container, three containers were arranged for each experiment (for a total of 30 eggs, 30 2^{nd}-3^{rd}-instar larvae, 30 pupae and 30 adults), and three experiments were performed for each ozone concentration (for a total of 90 eggs, 90 2^{nd}-3^{rd}-instar larvae, 90 pupae and 90 adults).

After the ozone exposure, eggs, larvae, pupae and adults were transferred to Petri dishes (diameter 13.5 cm) containing the standard diet described above, and reared under controlled conditions (25±1°C; 60±5% RH; L:D 16:8). Newly emerged larvae were periodically transferred to other Petri dishes to avoid any cannibalism on eggs. Eggs, larvae and pupae were examined 1, 3, 7 and 10 days after the ozone treatment, when all vital eggs, larvae and pupae in the control treatment hatched, pupated or moulted to adults, respectively. Eggs showing alterations in colour, larvae that were immobile, and larvae and pupae changing colour and/or having stiff shrunken bodies were

considered as dead. Adults were checked for their mortality immediately and 24 h after the ozone treatment. Moreover, eggs laid by treated adults in the 24 h-period following the end of the treatment were counted; then, they were collected with a fine brush, transferred to Petri dishes and reared until their hatching.

2.4. Storehouse trials

From late June to mid-July, the storehouse trials were conducted in a hazelnut processing factory located in Cortemilia (Cuneo, Piedmont, NW Italy). In the storehouse, three separate chambers (length×width×height 21×21×5m, volume 220 L) hermetically sealed were used as test chambers to compare the following treatments: ozone treatment; pyrethrin treatment (as routine treatment); no application (as untreated control). For ozone treatment, the lowest concentration which killed 100% of adults in the laboratory trialswas chosen and applied in the chamber for 24 h, by using the ozone generator described above. For routine treatment, pyrethrins synergised with piperonyl butoxide (AquaPy®, Bayer CropScience srl, Environmental Science, Milano, Italy, containing 3 g of pyrethrins, 13.5 g of piperonyl butoxide, coformulants q.s. for 100 g) was nebulized using a ULV cold fogger (Vector Fog C20, Vectornate, Englewood Cliffs, NJ, USA) at 100 mL/L following the standard procedures.

In the storehouse trials, *E. kuehniella* adults were tested to compare the effectiveness of the ozone and pyrethrin treatments. Newly emerged adults (<1 day-old) were collected from laboratory mass rearing culture and transferred into Plexiglas opened cylinders (height 20 cm, diameter 10 cm), closed at both extremities with an insect-proof net (mesh size 0.6×1 mm). Overall 540 *E. kuehniella* adults were used as follows: 10 adults were released into each cylinder, six cylinders were placed in each test chamber for each experiment (for a total of 60 adults per treatment and experiment), and the experiment was repeated three times, on June 26, July 3 and July 10 (for a total of 180 adults per treatment). In each chamber, the six cylinders were placed two at each of three heights, i.e., 0.1 m, 1.5 m and 2.0 m from the ground. Mortality of adults in each cylinder was checked 24 h after the ozone and pyrethrin treatments.

During all trials, temperature in the three chambers was recorded using temperature data loggers (Hobo® Pro Series, Onset Computer Corporation, Bourne, MA, USA), and it was similar ranging from 25 °C to 28 °C in the three replicates.

2.5. Statistical analyses

In laboratory trials, proportions of dead specimens or unhatched eggs in each container were compared for each stage among ozone concentration via a binomial distribution model with a logit link function, using the general linear model (GLM) procedure of the software IBM SPSS® Statistics 24 (IBM Corp., NY, USA). Means were then separated at $P<0.05$ using the Bonferroni test under the GLM procedure. Mean times needed for eggs to hatch, for larvae to pupate, and for adults

to emerge from pupae after the treatment were compared among ozone concentrations with the Log-Rank test (IBM SPSS® Statistics 24, IBM Corp., NY, USA).

In storehouse trials, percentage mortality of *E. kuehniella* adults in the three treatments and at different heights in the chambers was compared via a binomial distribution model with a logit link function, using the GLM procedure of the software IBM SPSS® Statistics 24 (IBM Corp., NY, USA). Means were then separated at $P<0.05$ using the Bonferroni test under the GLM procedure.

3. Results

3.1. Laboratory trials

Mean mortality of *E. kuehniella* eggs, larvae, pupae and adults after 24 h-exposure to ozone under laboratory conditions was significantly different among ozone concentrations for all tested stages (Tables 1 and 2). However, eggs, larvae, pupae and adults showed a variable susceptibility at the tested ozone concentrations, in terms of both mortality (Tables 1 and 2), and time needed for egg hatching and larval pupation (Fig. 1 and 2 and Table 3) or vitality of laid eggs for adults (Table 2). At 5 ppm ozone concentration (CT value = 7200 ppm min), mortality of all stages was significantly higher than that in the untreated control, even if not immediately but 24 h after the ozone treatment for adults (Tables 1 and 2). Then, for eggs, mortality did not differ significantly by increasing ozone concentration from 10 ppm (CT value = 14400 ppm min) to 100 ppm (CT value = 144000 ppm min), fluctuating from 52.2% to 62.2%, respectively (Table 1). However, there was a significant delay in hatching for eggs exposed to 10, 20 and 50 ppm, reaching an average of 5.5±0.2 days after the treatment, in comparison with eggs in the untreated control, which hatched on average 3.2±0.2 days after the treatment (Fig. 1 and Table 3). Hatching time of eggs exposed to 100 ppm (3.6±0.5 days after the treatment) did not differ significantly from that of eggs in the untreated control and of eggs exposed to 50 ppm (Table 3). For larvae, mortality increased significantly lower than that at 20 ppm, but significantly higher than that in the untreated control (Table 2). There was significant difference in hatching rate of eggs laid by females in the 24 h period after the ozone exposure between treatments, except for ozone concentration of 100 ppm in which at the end of ozone exposure all adults were dead and consequently no eggs were found. Also egg mortality was significantly lower in the untreated control than that at all ozone treatments; with some exceptions, there were significant differences in mortality among concentrations (Table 2).

3.2. Storehouse trials

Since adult mortality did not significantly differ at the three tested heights (GLM: Wald $\chi^2 = 1.40$; df = 2; P = 0.497) and in the three dates of experiment replication (GLM: Wald $\chi^2 = 1.40$; df = 2; P = 0.497), all the data obtained at different heights and in different dates were pooled. Almost all adults

were found dead after both ozone and pyrethrin treatments, while mortality of the adults was significantly lower with values below 10% in the untreated control (Table 4).

4. Discussion

Although higher mortalities at the lowest ozone concentrations were observed for pupae, in the present study complete mortality was obtained only for adults, therefore showing a higher susceptibility to ozone exposure compared to the other stages. In fact, for adults, complete mortality was achieved at the CT value of 72000 (50 ppm for 24 h), whereas for the other stages it was not achieved even at the CT of 144000 (100 ppm for 24 h). These results are consistent with those obtained by Hansen et al. (2012); in their study, all *E. kuehniella* adults died at the lowest tested CT of above 150000 (20-21 ppm for 5 days), while eggs, larvae and pupae needed CT values higher than 300000 (35-36 ppm for 6 days) for complete mortality. On the other hand, complete mortality of adults, larvae and pupae, and high mortality of *E. kuehniella* eggs (above 85%) were obtained with ozone application at CT values just of 36000 (300 ppm for 2 h) in the study published by Işikber et al. (2007), but the brief pressure drop to 100 mm Hg applied before ozone fumigation could have affected the results.

Results are indeed more variable when considering studies conducted on insects exposed to ozone not freely, but in the presence of commodities. Işikber and Öztekin (2009) reported that higher values of CT of more than 800000 (13.88 mg/L, about 7000 ppm, for 2 h) were required for complete mortality of larvae, pupae and adults of *E. kuehniella* placed in 2 kg of wheat, but eggs, showing mean mortality of about 60%, proved to be the least susceptible stage to ozone gas. Differently, a lower value of CT of 450 was enough to cause complete mortality of larvae placed in 50 g of dried figs (Sadeghi et al., 2017). However, the presence of different commodities makes these results not comparable. Further CT should be evaluated on freely exposed each moth stage to establish their lethal exposure.

Similarly to the tested species, also adults and eggs of the almond moth *Ephestia cautella* (Walker) showed to be the most and least susceptible stage with mortality of 85% and 10% at the highest CT evaluated of 1440 (2 ppm for 12 h) when freely exposed to ozone, respectively (Abo-El-Saad and Elshafie, 2011). In the same family Pyralidae, also freely exposed stages of *P. interpunctella* showed similar susceptibility: complete mortality for adults and eggs required the lowest (30000, 1800 ppm for 60 min) and the highest (324000, 1800 ppm for 180 min) CT values, respectively (McDonough et al., 2011).

In our study, ozone exposure did not cause only *E. kuehniella* mortality, but it also affected the time needed for the exposed eggs and larvae to hatch and pupate, respectively. In fact, eggs exposed at 10, 20 and 50 ppm hatched later than eggs exposed at 0 and 100 ppm, and larvae exposed at 5, 10,

20, and 50 ppm pupated later than larvae exposed at 0 and 100 ppm. Larvae surviving after ozone treatment were therefore able to pupate, but later, perhaps due to a reduction of their feeding activity after ozone exposure. Our results showed that not only lethal, but also sublethal effects of ozone at sublethal concentrations can be effective in stored-product pest control, reducing both feeding damage by larvae and adult fecundity, as mortality of eggs laid by treated adults increased at increasing ozone concentration. Reduction in walking activity under sublethal ozone exposure was observed for populations of the maize weevil *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) (Sousa et al., 2017). Such effects might limit the insect ability to escape the ozone exposure, implicating in a low risk of resistance evolution in a short term (Sousa et al., 2017), and these aspects should be further evaluated also for *E. kuehniella* populations.

Currently, control of *E. kuehniella* infestations in storehouses is based on the use of conventional insecticides, depending on trap captures for decision-making; some alternative and biorational management tools such as temperature management, controlled and modified atmospheres, and use of natural enemies exist (Phillips and Throne, 2010). In order to respond to the consumers' demand for organic and pesticide residue-free food, ozone gas can be also an efficient substitute of chemical treatments. In the present study, the storehouse trials demonstrated that ozone gas was effective against adults of *E. kuehniella*, the most susceptible and the only freely exposable stage, and indirectly against eggs laid by treated adults. However, both hazelnuts and storehouse equipment could be damaged by excessive ozone exposure. Ozone is actually a powerful oxidant, second only to the hydroxyl-free radical. Therefore, it is capable of oxidizing many inorganic and organic compounds in air and water, and even if the effect of ozone on quality of grain and derived products is highly debated in the literature (Jian et al., 2013), lipid oxidation can affect kernel quality of shelled hazelnuts (Wang et al., 2018). Furthermore, ozone oxidizes most metals and reacts directly with some hydrocarbons such as aldehydes, polymers, or double bonds within its chain structure such as in natural rubber, wool, protein, and paint (Jian et al., 2013). Corrosion by ozone to materials used by the stored product industry is a potential disadvantage for ozone application and should be carefully evaluated. Ozone must be continuously supplied and might increase corrosion rates on metal components and degrade equipment such as rubber seals, and electrical equipment at unacceptable rates. Therefore, to reduce the corrosion, ozone should be used at low concentration, and contact between ozone and equipment should be minimized (Jian et al., 2013). Application of ozone at 50 ppm is the recommended concentration to be used in the fumigation of empty facilities and grain storage bins to suppress insect and microorganism populations and reduce mycotoxins (Abo-El-Saad and Elshafie, 2011; Jian et al., 2013). This concentration demonstrated to be effective on *E. kuehniella* adults in both laboratory and storehouse conditions.

In the present study, *E. kuehniella* adults resulted susceptible to the recommended concentration of 50 ppm of ozone application in both laboratory and storehouse conditions. Furthermore, also eggs laid by adults survived in the following 24 h showed a mortality of more than 30%. Based on the results on lethal and sublethal effects of ozone gas on *E. kuehniella* in the present study, ozone can be considered as an effective alternative to routine application of synergised pyrethrin during spring and summer; however, further studies are required to test effect of ozone gas on quality parameters of the tested commodity.

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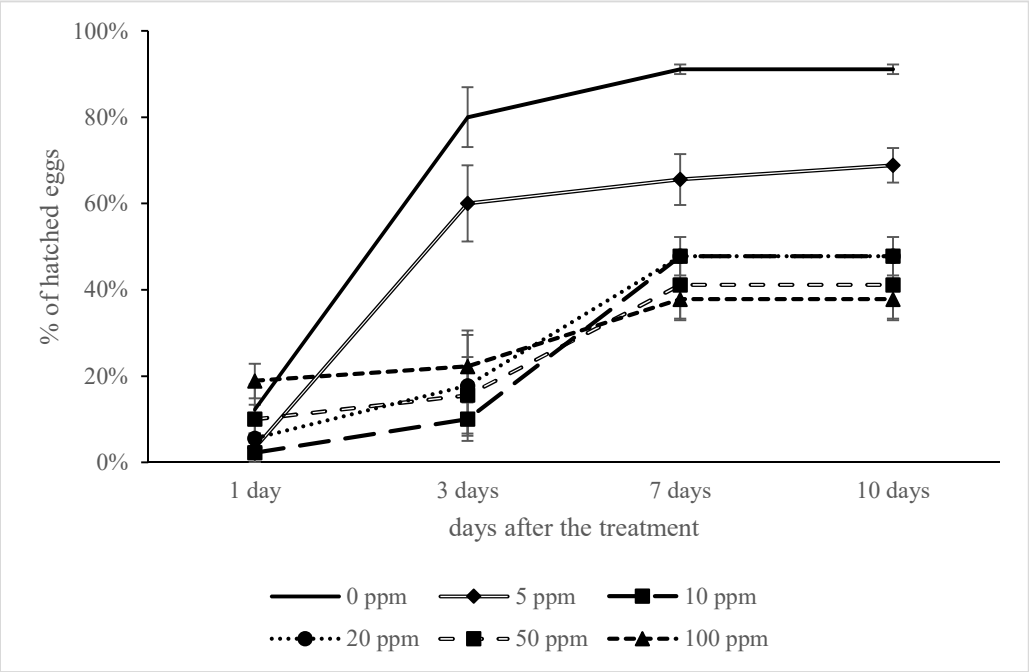

Figure 1. Cumulative mean percentages (±SE) of *Ephestia kuehniella* eggs hatched 1, 3, 7 and 10 days after ozone treatment in the test chamber.

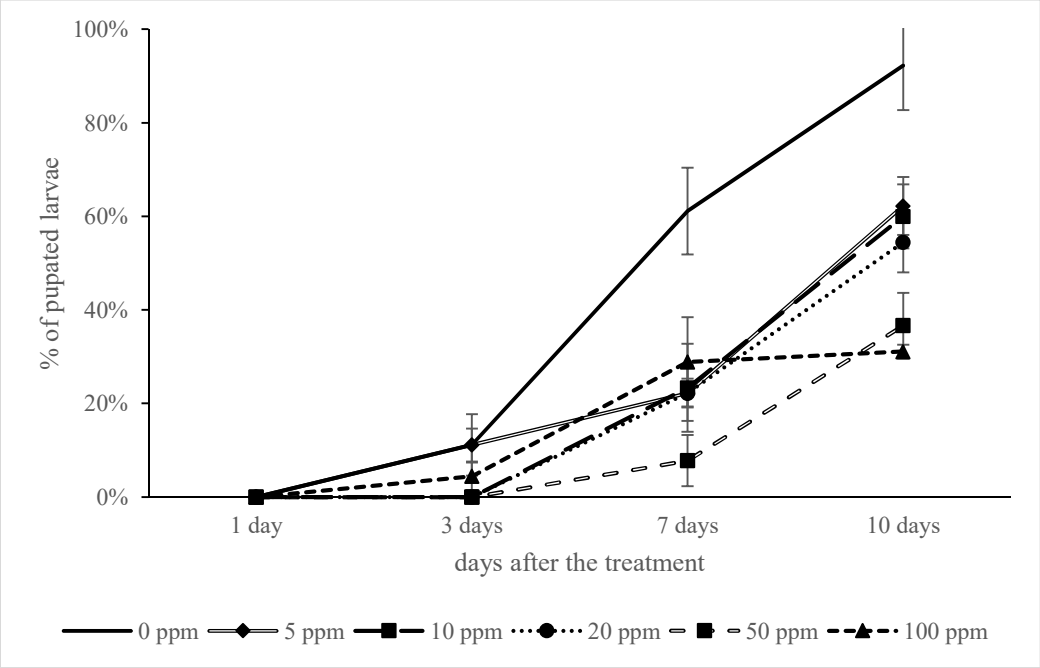

Figure 2. Cumulative mean percentages (±SE) of *Ephestia kuehniella* larvae pupated 1, 3, 7 and 10 days after ozone treatment in the test chamber.

Table 1. Mean percentages (±SE) of mortality of *Ephestia kuehniella* eggs, larvae and pupae freely exposed to different gaseous ozone concentrations (ppm) for 24 hours in laboratory conditions. Mortality was assessed 10 days after treatment, when no more eggs hatched, no more larvae pupate, and no more adults emerged. In each column, values followed by the same letter are not significantly different (Bonferroni test, $P<0.05$, under GLM procedure with binomial distribution and logit link).

Dose (ppm)	CT product (ppm-min)	mortality (%)		
		eggs	larvae	pupae
0	0	8.33±2.41 c	9.17±2.60 c	8.33±2.41 d
5	7200	31.11±4.55 b	37.78±3.64 b	61.11±5.88 c
10	14400	52.22±4.34 a	40.00±10.67 b	64.44±6.26 bc
20	28800	52.22±2.78 a	45.56±4.12 b	74.44±2.42 b
50	72000	58.89±4.84 a	63.33±8.33 ab	76.67±3.73 ab
100	144000	62.22±5.47 a	68.89±8.57 a	85.56±6.69 a
Wald χ^2		71.416	77.232	107.676
df		5	5	5
P		<0.001	<0.001	<0.001

449 **Table 2.** Mean percentages (±SE) of mortality of *Ephestia kuehniella* adults freely exposed to
450 different gaseous ozone concentrations (ppm) for 24 hours in laboratory conditions, immediately and
451 24 hours after the end of the trial, and mean percentages (±SE) of mortality of eggs laid in the 24
452 hour-period following the end of the treatment. In column, values followed by the same letter are not
453 significantly different (Bonferroni test, $P<0.05$, under GLM procedure with binomial distribution and
454 logit link).

Dose	CT product	adult mortality (%)		laid egg	
(ppm)	(ppm-min)	immediately	after 24 h	no.	mortality (%)
0	0	6.67±1.42 d	11.67±1.12 d	2830	5.28±0.71 d
5	7200	17.78±4.01 c	35.56±6.48 c	1413	14.22±3.15 c
10	14400	28.89±7.16 c	41.11±7.90 bc	1938	13.01±2.21 c
20	28800	53.33±5.53 b	61.11±6.33 b	2235	20.84±2.83 b
50	72000	94.44±2.94 a	100.00±0.00 a	470	33.01±4.24 a
100	144000	100.00±0.00 a			
Wald χ^2		4540.645	14750.860		396.011
df		4	4		4
P		<0.001	<0.001		<0.001

455

456

Table 3. Mean time (days±SE) needed for *Ephestia kuehniella* eggs and larvae to hatch and pupate, respectively, after the exposure to different gaseous ozone concentrations (ppm) for 24 hours in laboratory conditions. In column, values followed by the same letter are not significantly different (Log-Rank test, P<0.05).

Dose (ppm)	CT product (ppm-min)	Time (days) from the treatment to	
		Hatching for the exposed eggs	Pupation for the exposed larvae
0	0	3.22±0.17 c	7.53±0.24 b
5	7200	3.11±0.14 c	8.21±0.36 a
10	14400	6.07±0.29 a	8.83±0.20 a
20	28800	5.28±0.36 a	8.78±0.21 a
50	72000	5.00±0.44 ab	9.36±0.22 a
100	144000	3.65±0.50 bc	6.64±0.32 b
χ^2		67.678	48.655
df		5	5
P		<0.001	<0.001

463 **Table 4.** Mean percentages (±SE) of mortality of *Ephestia kuehniella* adults freely exposed to
464 different treatments for 24 hours in a hazelnut storehouse. Values followed by the same letter are not
465 significantly different (Bonferroni test, $P<0.05$, under GLM procedure with binomial distribution
466 and logit link).

Treatment	mortality (%)
Untreated	9.44±2.21 b
Pyrethrum (100mL/L)	98.89±0.76 a
Ozone (50 ppm)	99.44±0.56 a
Wald χ^2	118.772
df	2
P	<0.001

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