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# Efficiency of ozone gas treatment against *Plodia interpunctella* (Hübner) (Lepidoptera:Pyralidae) (Indianmeal Moth) in hazelnut

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# Abstract

In this study, ozone gas at different concentrations (16.7, 33.3 and 66.6 mg/L) were exposed to all biological stages (egg, larva, pupa and adult) placed at top and bottom of the hazelnut for various exposure periods (2, 4 and 6 hours). In biological tests conducted in presence of hazelnuts, 100% mortalities of all biological stages of *P. interpunctella* placed at top of the commodity were obtained at tested ozone concentrations and exposure periods while it was easier to kill the adult and pupa stages that the larva and egg stages. While it was possible to kill 100% of the adults and pupae placed at bottom of the commodity at tested ozone concentrations and exposure periods, 100% mortalities of all life stages of *P. interpunctella* placed at any of the ozone treatments. Generally, the mortalities of all life stages of *P. interpunctella* placed at bottom of the commodity. It was easy to kill the pupae and adults of *P. interpunctella* placed at bottom of the commodity. It was easy to kill the pupae and adults of *P. interpunctella* placed at bottom of the commodity. It was easy to kill the pupae and adults of *P. interpunctella* placed at bottom of the commodity. It was easy to kill the pupae and adults of *P. interpunctella* placed at bottom of the commodity. It was easy to kill the pupae and adults of *P. interpunctella* placed at bottom of the commodity. Just as 100% mortalities of the larva and adult stages were not obtained even at the highest ozone concentration for the longest exposure period. In conclusion, in this study, it was observed that ozone gas only at high concentrations can control all biological stages of *P. interpunctella* in hazelnut and therefore could have an alternative potential for methyl bromide in quarantine applications in short application period.

Keywords: Plodia interpunctella, ozone gas, hazelnut, fumigation

# Introduction

Hazelnut production is an important agricultural activity in Turkey. Hazelnut cultivation is mainly performed on steeplands in Black Sea region of Turkey and it is being an important source of income for a large number of family farms (Dikmen, 1999). Turkey produces 73% of world production and exports 84% of its production, which accounts for around 20% of total agricultural exports from Turkey (Fiskobirlik, 2003). Storage pests infesting hazelnut especially during drying and storage period may cause significant problems in hazelnut sector. The Indianmeal moth (*Plodia intepunctella* (Hubner)) reduces fruit quality by feeding and damaging the fruit and contaminating by leaving its excretions and other residues as silky net weaves (Damarlı et al., 1997). Large populations can develop before being detected and severe damage may occur rapidly (Jarratt, 2001). Moreover, from a phytosanitary point of view, during export, the presence of insects, or their fragments, has cost inestimable losses due to cargo returns.

Ozone is a triatomic form of oxygen (O<sub>3</sub>) and is referred to as activated oxygen, allotropic oxygen or pure air. It is an unstable gas and its life span lasts about 20 minute, depending on the temperature. Electrical generation of ozone eliminates the handling, storage, and disposal problems of conventionally used post-harvest pesticides. Attractive aspect of ozone is that it decomposes rapidly (half-life of 20-50 min) to molecular oxygen without leaving a residue. These attributes make ozone an attractive candidate for controlling insects and fungi in stored products. Ozone in its gaseous form has been also considered to have potential to kill insect pests in commodities and was subjected of several research studies (Erdman, 1980; Mason et al., 1997; Kells et al., 2001). High mortality was achieved for adults of the maize weevil, *Sitophilus zeamais* (Mostsch.), and the red flour beetle *Tribolium confusum* (Jacqueline de Val), and the larval stage of the Indian meal moth, *Plodia interpunctella* (Hübner) exposed to lower ozone concentrations ranging from to 5 to 45 ppm (Erdman, 1980; Kells et al., 2001).

Methyl bromide has frequently used as a fumigant for disinfestations of other stored agricultural commodities such as nuts, cereals and fruits since it kills the insects rapidly, has a wide spectrum of activity and relatively low-cost (Fields and White, 2002). However it had been banned in developed countries since 2005 and scheduled for worldwide withdrawal from routine use as a fumigant in 2015 under the directive of the Montreal Protocol on Substances that Deplete Ozone Layer (Schneider et al., 2003) except quarantine, laboratory and pre-shipment purposes. As a consequence, there is a critical need to develop new fumigants for quarantine purposes, where rapid insect mortality is required (exposure time less than 1 day). Thus, the objective of present study was to determine toxicity of ozone at high concentrations and short exposure time against all life stages of *Plodia interpunctella* (Hübner) in hazelnut under laboratory conditions.

### **Material and Methods**

#### Commodity

In-shell hazelnuts with m.c. of 10.5%±0.5 were used in the tests. In order to minimize the reaction of microbial loads in the commodity with ozone the hazelnuts used in the tests were sterilized under high pressurized stem.

### **Fumigation chambers**

Test chambers consisted of 3 liter glass jar, each capped with a metal stopper equipped with entry and exit tubing. Two pieces of rubber tubing, 5 cm long, 6.2 mm ID, were attached to the tubing and sealed with pinch-clamps. The desiccators were sealed with silicone vacuum grease.

### Test insects

Tests were carried out on all life stages (adult, larva, pupa and egg) of *P. interpunctella*. All life stages of *P. interpunctella* were obtained from cultures reared at  $26 \pm 1^{\circ}$ C and  $65 \pm 5\%$  r.h. on a diet of a 10:2:1 mixture of wheat flour: wheat germ: dried Brewers yeast using standard culture techniques. Eggs aged 1-2 days in 9 cm Petri dishes were placed in 3 litter jars and then were exposed to the treatments. Larvae were removed from culture jars and exposed to the treatments 21 days after oviposition. Two days old pupae were obtained by daily separation from culture jars and were exposed to the treatments. Newly emerged, aged 0-1 day, adults were placed in culture jars and then were exposed to the treatments.

### Ozone fumigation procedures

Ozone generator in laboratory scale was provided from the company Ozomax Inc., Ozone gas was generated using a laboratory corona discharge ozone generator (Model OZO-1VTT) from purified extra dry oxygen feed gas. Ozone was introduced as gaseous into the exposure jars using an ozone generator. Pressure in each jar was measured using a 0 to 800 mm Hg vacuum digital gauge. The 100 mm Hg measure referred to herein is absolute pressure, with 760 mm Hg considered as atmospheric pressure. Prior to each test, twenty larvae, pupae or adults were confined, separately, inside 3 cm diameter by 8 cm long wire-mesh cages. For eggs mortality evaluation, fifty eggs placed in opened Petri dishes were used per fumigation.

For intermittently repeated ozone treatment in presence of commodity, each desiccators was loaded separately with 1.3 kg of in-shell hazelnut, and then 50 eggs, 25 pupae, adults and larvae were confined inside the wire-mesh cages and inserted into top and bottom position of the commodity, and the desiccators were briefly evacuated to 760 mm Hg. Afterwards, ozone gaseous at concentrations of 16.7, 33.3 and 66.6 mg/L was flushed into exposure jar until reaching atmospheric pressure and it was repeated every half an hour for 2, 4 and 6 hours. Untreated control insects were exposed to atmospheric conditions. Each test was replicated at three times. For all ozone fumigations, r.h. and temperature were maintained at  $65\pm5\%$  at atmospheric pressure and  $26\pm1^{\circ}$ C, respectively.

# Data processing and analysis

After each treatment, larvae, pupae, and adults were transferred to 250-mL jars containing standard diets and were held at  $26 \pm 1^{\circ}$ C and  $65 \pm 5\%$  r.h. until examined for mortality. The eggs in their Perspex slides were held under the same conditions until the oviposition sites were examined for egg hatch. Mortality data was subjected to Arcsin transformation and then, were analyzed using one-way analysis of variance (ANOVA). The means were separated using the LSD method at 5% level.

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# **Results and Discussion**

Toxicity data for empty space ozone treatments indicated a remarkable difference in susceptibility between the life stages of *P. interpunctella*. Adult and pupae stages mortality rates were found to be high at low ozone concentrations (8.4 and 16.7 mg/L) and short application periods (30 at 60 minute), whereas egg and larval mortality rates were very low. High mortality rates were obtained from the larvae and egg stages with the increase in the application period, but 100% mortality of the larvae and egg was achieved at the highest exposure times (240 and 360 minutes) and ozone gas concentration (66.6 mg/L). In empty space fumigation of ozone, susceptibility of the adult and pupae stages was similar to ozone gas while they were sensitive to ozone gas than the larvae and egg. As a result, it was easy to kill the adults and pupas at low ozone concentrations for short exposure times while higher ozone concentrations and longer exposure periods were required to obtain complete mortality of the larvae and eggs.

In biological tests conducted in presence of hazelnuts, 100% mortalities of all biological stages of *P. interpunctella* placed at top of the commodity were obtained at tested ozone concentrations and exposure periods while it was easier to kill the adult and pupae that the larvae and egg stages. While it was possible to kill 100% of the adults and pupas placed at bottom of the commodity at tested ozone concentrations and exposure periods, 100% mortality of the larvae and egg were not obtained. Similarly to the results obtained from empty space ozone fumigation, there was significant difference in susceptibility of biological stages of *P. interpunctella* to ozone treatments. The mortalities of the adults and pupas was higher than those of the larvae and eggs. Generally, the mortalities of all life stages of *P. interpunctella* placed at bottom of the commodity for ozone treatments were lower than those placed at top of the commodity, while the ozone treatments resulted in low mortalities of the egg and larvae placed at bottom of the commodity. Complete (100%) mortalities of the larvae and adult stages were not obtained, even with the combination of the highest ozone concentration and the longest exposure period.

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