Journal of Plant Protection and Pathology

Journal homepage & Available online at: www.jppp.journals.ekb.eg

Efficacy of Ozone Gas on the Greater Wax Moth, *Galleria mellonella* Larvae

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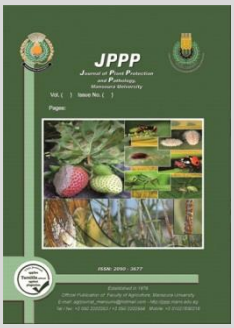

ABSTRACT

The greater wax moth, *Galleria mellonella* was reported to be one of the most destructive insect pests of honeybee wax and wood hives. Addition to hindering and reduction of honey bee activities particularly in the weak colonies. In comparison to untreated larvae, fourth instar larvae of *G. mellonella* were treated with ozone as a gas at various concentrations (250, 500, and 1000 ppm) for four different times (0.5, 1, 1.5, and 2 h). The present study clearly shows that both exposure time to ozone and the gas concentrations have obvious influence on larval mortality of *G. mellonella*. The lowest larval mortality (40%) were recorded after 7 days at 0.5 h exposure time when concentration of ozone were 1000 ppm comparing with control. The larval mortality was significantly increased as ozone concentration and exposure time increased and resulting complete mortality after 7 days of treatment at 1000 ppm and exposure time 1 h. The complete larval mortality was achieved after 3 days at 1000 ppm and exposure time 1.5 h. While the two concentrations of ozone (250 and 500 ppm) were caused lower mortality of *G. mellonella* larvae and the highest mortality was 20 and 50% at exposure time 2.0 h after 7 days, respectively. According to our findings, ozone may be a useful fumigant in the management of *G. mellonella*. Ozone breaks down quickly into oxygen and has very little environmental effect and highly safe insect control method without any residual and possibility for application.

***Keywords*:** ozone, greater wax moth, larval mortality, *Galleria mellonella*

INTRODUCTION

The greater wax moth, *Galleria mellonella* (L.) (Lepidoptera: Pyralidae), consumes pollen and wax that is kept in the combs of active honey bee colonies (Milam, 1970). Rather than attacking mature bees, it destroys the combs of weak colonies by chewing on the comb and weaving tunnels lined with silk through the cell wall and across the comb's face. This keeps the bees from being able to exit their cell by their abdomen, which causes them to starve to death. Additionally, they search for a spot in the bee hive's soft wood to spin their cocoons. Stored honey combs can also be destroyed by *G. mellonella*. As a result, it is regarded as a significant problem for honeybees. The extent of the infestation and the amount of time that has passed since the infestation started will determine the type of damage. A mass of hard, silky web may eventually fill the frames and combs of preserved combs, destroying them entirely. A box (super) of combs can become ineffective in around a week under ideal wax moth growth circumstances (Owayss and Abd-Elgayed, 2007). Wax moths are most active during the warm, sunny months of the year, which is when damage mostly happens. The larger wax moth can produce a significant amount of metabolic heat, which can boost the immediate temperature surrounding them by up to 25° over the average environment temperature. Nevertheless, significant damage can still occur during the cool part of late fall and early spring. Even combs that don't appear to have any wax moth eggs at the time of storage could nonetheless have eggs that will eventually hatch. Unless they are treated, they should be checked often for indications of a moth infestation (Charrière and Imdorf, 2004).

To reduce its harm, a number of pest control strategies have been used; humans have worked to maintain clean, debris-free beehives. Prior to being stored, combs were additionally fumigated (Caron, 1992). Fumigants and other synthetic insecticides are currently the principal tools used in the management of the larger wax moth. However, the widespread application of these substances in *G. mellonella* control initiatives has resulted in serious issues, including increased resistance to the insecticides used, detrimental effects on the environment, and harm to human health (Ross, 1999). Thus, different methods for managing *G. mellonella* have been assessed and implemented, including freezing, ozone gas, and changed atmospheres (James, 2011; Zhu *et al.*, 2016; Falah *et al.*, 2017).

Ozone (O_3) gas has been suggested as an excellent alternative for methyl bromide since it leaves no residue in treated food, does not significantly deplete nutrients in commodities, and does not induce insect resistance. According to Tiwari *et al*. (2010), ozone is regarded as an efficient, cost-effective, and environmentally benign fumigant. Ozone is an unstable gas that forms when high voltage electric discharge is applied to oxygen (Kim *et al.*, 1999). Due to its short half-life (20–50 min) in room temperature, quick breakdown into oxygen, and lack of residue on treated, stored goods, ozone is gaining a lot of interest as a fumigant (Mendez *et al.*, 2003). The US Food and Drug Administration (FDA) has authorized the use of ozone in the purification of bottled water and food products (FDA, 2001). Several reports demonstrated the successful application of ozone on stored product against various insect pests (Zakladnoy *et al.*, 2003; Niakousari *et al*., 2010; Isikber

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DOI: 10.21608/jppp.2024.274017.1222

and Athanassiou, 2015; Abd El-Ghaffar *et al.*, 2016, 2017; Gad *et al*., 2021a, b; Mahmoud *et al*., 2023; Sitoe *et al.*, 2024). However, there are a few reports on effect of ozone on *G. mellonella*. Hence, we focus in the current investigation on examining the susceptibility of larval stage of *G. mellonella* to ozone gas.

MATERIALS AND METHODS

Insect rearing and ozone generation

A laboratory strain of the greater wax moth, *G. mellonella* was obtained from larvae collected from infested beehives. The experiments were run on fourth instar larvae obtained from the laboratory culture after being reared for several generations under constant temperature and relative humidity (26±2°C) and (65±5% r.h). Larvae were reared on natural diet on bee wax. While the ozone gas was produced from a generator developed at the Center of Plasma Technology, Al-Azhar University, Nasr City, Cairo, Egypt by methods described by Gad *et al.* (2021b).

Exposure of *G. mellonella* larvae to ozone gas

Ten fourth instar larvae of *G. mellonella* were exposed to three different ozone gas concentrations, 250, 500, and 1000 ppm for 0.5, 1, 1.5, and 2 h. The experiments were conducted in four replicates. After the exposure times were assessed, the treated larvae were incubated at 26± 2°C and 65± 5% relative humidity. The treated larvae were placed in glass jars with a diameter of five cm and a depth of one cm. The jars were covered with muslin cloth and incubated under the same conditions. The percentage of larval mortality was recorded every day for a period of seven days and analyzed using ANOVA. At a significance threshold of < 0.05, mean separations were computed using Tukey's HSD test. The SPSS 21.0 programmer was used to do the statistical analysis (SPSS, Chicago, IL, USA).

RESULTS AND DISCUSSION

The data in figures (1-4) clearly show that both exposure time to ozone and the gas concentrations have obvious influence on larval mortality of *G. mellonella*. The lowest larval mortality (40%) were recorded after 7 days at 0.5 h exposure time when concentration of ozone were 1000 ppm comparing with control ($P < 0.01$: $F = 39.9$; $df = 3, 12$) (Figure 1). The larval mortality was significantly increased as ozone concentration and exposure time increased and resulting complete mortality after 7 days of treatment at 1000 ppm and exposure time 1 h ($P < 0.01$: $F = 2.0$; $df = 3, 12$) (Figure 2). The complete larval mortality was achieved after 3 days at 1000 ppm and exposure time 1.5 h ($P < 0.01$: $F = 5.6$; $df = 3, 12$) (Figure 3). While the two concentrations of ozone (250 and 500 ppm) were caused lower mortality of *G. mellonella* larvae and the highest mortality was 20 and 50% at exposure time 2.0 h after 7 days, respectively ($P < 0.01$: $F = 5.0$; $df = 3, 12$) (Figure 4). Obtained results from the present study showed that the larvae of *G. mellonella* were more sensitive to ozone gas particularly at the highest concentration (1000 ppm). This concentration was enough to reach complete mortality at 3 h exposure time after 3 days of treatment. Similar results were obtained by Kells *et al.* (2001) observed a greater rate of mortality for *Plodia interpunctella* larvae exposed to 50 ppm of ozone for three days. According to research by Isikber and Oztekin (2009), the larval stage of *Tribolium confusum* is more vulnerable to ozone than other life stages, with a mortality rate of 86.3%. Osman (2009) investigated the impact of 1 g/m^3 of ozone on *Ephestia kuehniella* for 5 h and found that the full impact of ozone exposure on the mortality rate did not become apparent in larvae until at least six days had passed. Al-Ahmadi *et al.* (2009) demonstrated that after 6 h of exposure, 30 ppm of ozone was sufficient to cause *Oryzaephilus surinamensis* to completely die as an adult. Additionally, *P. interpunctella* adults died entirely after being exposed to 500 ppm of ozone for 60 minutes (McDonough *et al.*, 2011). According to Hussain (2014), the mortalities percentage of *E. Cautella* increased gradually as the exposure time to ozone gas and the time after treatment increased. Larvae treated with 80 ppm caused 38.92% of the mortality at 1 h of exposure, and the complete mortality was obtained after 5 h of exposure.

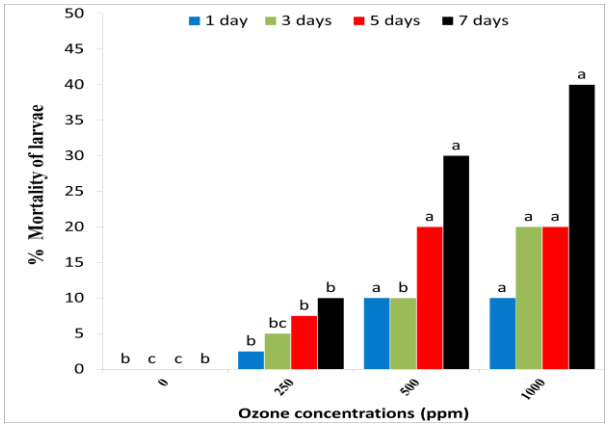

Figure 1. Mean larval mortality (%±SE) of *Galleria mellonella* after exposure for 0.5 h by ozone different concentrations for 1, 3, 5 and 7 days post treatment

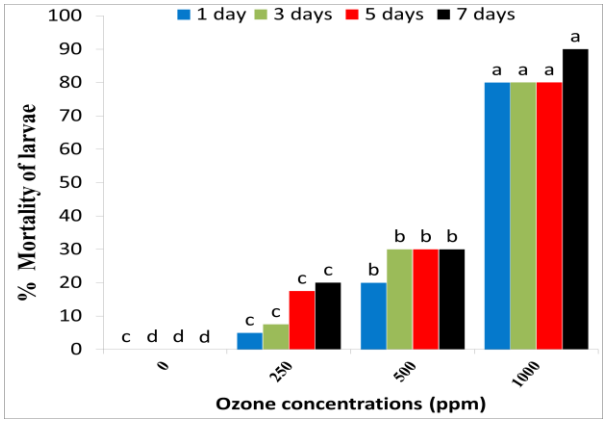

Figure 2. Mean larval mortality (%±SE) of *Galleria mellonella* after exposure for 1 h by ozone different concentrations for 1, 3, 5 and 7 days post treatment

According to Keivanloo *et al.* (2014), the greatest mortality rate of 85.0% was observed at an ozone concentration of 5 ppm over a 2 h exposure time. It was anticipated that the sensitivity to ozone would rise with the size and contact surface area of *P. interpunctella* larvae. According to Subramanyam *et al.* (2014), adult *Rhyzopertha dominica* was very vulnerable to ozone. Regarding *Trogoderma granarium*, our findings concurred with those of Taha *et al.* (2017) and Mahmoud *et al*. (2023). The strong

oxidizing capabilities of ozone may be the reason for its substantial toxicity against *G. mellonella* larvae identified in this experiment. Consequently, it might interact with double bonds present in DNA, proteins, and polyunsaturated fatty acids. Reactive oxygen species (ROS) are created when ozone breaks down into dioxygen. These free radicals have the power to oxidase polyunsaturated fatty acids and cause substantial changes to DNA and protein molecules (Hermes-Lima, 2004). Holmstrup *et al.* (2011) state that as a result, these impacts may cause cell damage and insect death.

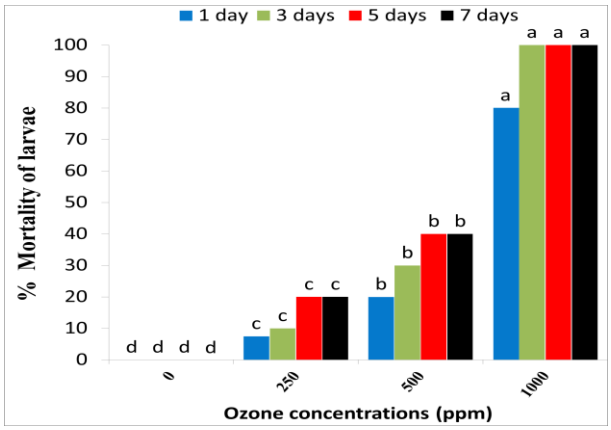

Figure 3. Mean larval mortality (%±SE) of *Galleria mellonella* after exposure for 1.5 h by ozone different concentrations for 1, 3, 5 and 7 days post treatment

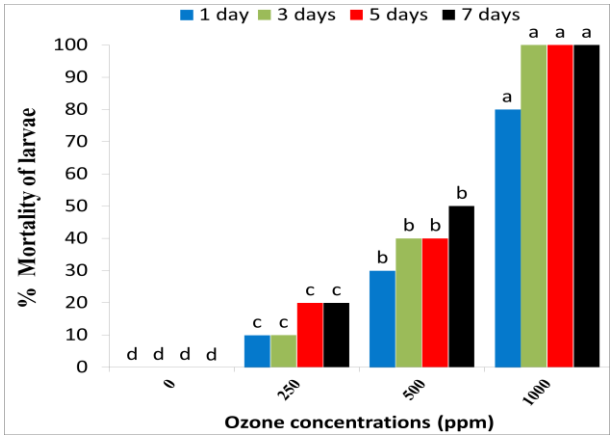

Figure 4. Mean larval mortality (%±SE) of *Galleria mellonella* after exposure for 2.0 h by ozone different concentrations for 1, 3, 5 and 7 days post treatment

CONCLUSION

Our finding sheds light on how effective ozone is at suppressing *G. mellonella* larvae. Given that the greater wax moth killed completely after a brief exposure, it was evident that ozone treatments were extremely toxic. These findings suggest that ozone may be a useful fumigant in the management of *G. mellonella*. Ozone breaks down quickly into oxygen and has very little environmental effect, making it a potential green fumigant. Ozone gas is highly safe insect control method without any residual and possibility for application.

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كفاءة غاز الاوزون على يرقات دودة الشمع الكبرى

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الملخص

تعتبر دودة الشمع الكبرى ، واحدة من أكثر الآفات الحشرية تدميراً لشمع نحل العسل وخشب الخلايا. بالإضافة إلى إعاقة وتقليل نشاط نحل العسل خاصة في الطوائف الضعيفة. بالمقارنة مع اليرقات غير المعالجة، تمت معالجة يرقات عمر رابع بغاز الاوزون بتركيزات مختلفة (250، 500، و1000 جزء في المليون) لأربع مرات مختلفة (0.5، 1، 1.5، و2 ساعة). توضح الدراسة الحالية بوضوح أن كلا من وقت التعرض للأوزون وتركيزات الغاز لهما تأثير واضح على موت يرقات دودة الشمع. تم تسجيل أدنى معدل لوفيات اليرقات (40%) بعد 7 أيام عند 0.5 ساعة من وقت التعرض عندما كان تركيز الأوزون 1000 جزء في المليون مقارنة مع الكنترول. زاد معدل وفيات اليرقات بشكل ملحوظ مع زيادة تركيز الأوزون ووقت التعرض مما أدى إلى موت كامل بعد 7 أيام من العلاج عند 1000 جزء في المليون ووقت التعرض ساعة واحدة. تم تحقيق معدل الوفيات الكاملة لليرقات بعد 3 أيام عند 1000 جزء في المليون ووقت التعرض 1.5 ساعة. بينما تسبب التركيزان للأوزون (250 و500 جزء في المليون) في انخفاض معدل وفيات يرقات دودة الشمع وكانت أعلى نسبة وفيات 20 و50% عند وقت التعرض 2.0 ساعة بعد 7 أيام، على التوالي. وفقا للنتائج التي توصلنا إليها، قد يكون الأوزون مدخن قوى في مكافحة دودة الشمع الكبرى, ويتحلل الأوزون بسرعة إلى أكسجين وله تأثير بيئي قليل جدًا وطريقة آمنة للغاية لمكافحة الحشرات دون أي بقايا وقابل للتطبيق.