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Impact of Ozone Gas on Cutworm, *Agrotis Ipsilon* (Lepidoptera: Noctuidae)

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ABSTRACT

Agrotis ipsilon (Hufnagel) (Lepidoptera: Noctuidae) is considered one of the most destructive pests worldwide. It attacks most agriculture crops, causing high yield loss due to its nutrition on roots. So, management of this pest is essential, especially with other new methods of management, this is because of the disadvantages and risks of chemical insecticides. Ozon (O_3) is one of the alternative methods for pest management which is caused by non-thermal atmospheric pressure plasma created by dielectric barrier discharge (APPD) and oxygen gas fed into an ozone generator. Ozon application is becoming more popular, especially because it doesn't leave any residue and doesn't require aeration to exclude the gas. In this study, four ozone concentrations were applied to larvae and pupae of *A. ipsilon* to detect larval mortality and follow the remaining live larvae and pupae until the end of the pest life. The concentrations were, 250, 500, 1000 & 1500 ppmv for 15 min. The results proved the effectiveness of ozone on larvae from both mortality and denaturation of the rest larvae after seven days of the beginning of the experiment. The mortality rate increases with increasing ozone concentration. As soon as all treated pupae couldn't emerge the adult, caused different malformations and failure of emerging moths.

INTRODUCTION

The black cutworm, *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae), represents a significant agricultural threat as a cosmopolitan polyphagous pest. Its economic importance stems from its extensive host range, encompassing numerous economically vital crops including wheat, corn, cotton, and various vegetable species (Wang *et al.*, 2021). The larval feeding behavior is particularly destructive during early plant developmental stages, characterized by foliar consumption and stem severance at ground level (Mesbah *et al.*, 2020). Falin *et al.*, (2019) emphasized that a critical challenge in implementing integrated pest management strategies lies in identifying both highly efficacious insecticides and optimal application methodologies for black cutworm control.

While chemical control methods have demonstrated considerable efficacy against *A. ipsilon* populations (Joshi *et al.*, 2020; Ismail, 2021; Siebert *et al.*, 2012), the widespread application of conventional insecticides has generated mounting concerns regarding their ecological impact, including detrimental effects on beneficial organisms, environmental contamination, and the evolution of insecticide resistance [Pathak *et al.*, 2022; Philippe *et al.*, 2021]. This situation necessitates the development of alternative control strategies that maintain efficacy while minimizing environmental and human health risks. In this context, ozone emerges as a potential sustainable alternative to conventional pesticide applications.

Ozone is useful against insect pests (Metwally *et al.,* 2023; Metwally *et al.,* 2024). United States Environmental Protection Agency (USEPA) and the US Food and Drug Administration (FDA) have designated ozone (O_3) as "GRAS,". Generally, it was recognized as safe, due to high reactivity and potent oxidizing power. Ozone has been employed worldwide to reduce aflatoxin contamination, kill microorganisms, purify drinking water, and sterilize food (Gad *et al.,* 2021).

This study aims to evaluate using ozone gas to control larvae and pupae of cutworm, *Agrotis ipsilon*.

MATERIALS AND METHODS

1-Rearing the Pest:

A laboratory colony of *Agrotis ipsilon* was established using specimens obtained from the Agriculture College, Cairo University, following the rearing methodology described by Moustafa *et al.*, (2021). The colony was maintained under controlled environmental conditions at 25 ± 2°C temperature, 70 ± 5% relative humidity, and a photoperiod regime of 12:12 hours (light: dark). To mitigate larval cannibalistic behaviour, individual larvae were isolated in cylindrical containers (3.5 cm height × 7.0 cm diameter) containing sawdust substrate for moisture regulation (He *et al.,* 2019). Larvae were provided with fresh *Ricinus communis* L. (castor bean) foliage daily until pupation. Upon adult emergence, moths were transferred to 5L glass containers and provisioned with 10% sucrose solution as a nutritional source (Kandil *et al.,* 2020).

2-Ozone Production:

Ozone gas was prepared from a generator developed by Dr Khaled Hussein Metwaly at the Centre of Plasma Technology, Al-Azhar University, Nasr City, Cairo, Egypt. A refined extra dry oxygen feed gas was used to produce ozone from oxygen. Adjusting the concentration within a set range is made possible by a belt pan in the monitor controller. A plug-in sensor on the monitor controller can be adjusted to measure ozone levels within a range. Through a dielectric barrier discharge (DBD) reactor, oxygen was pumped, which, as a consequence, created ozone gas. A discharge voltage is applied between two coaxial electrodes that are spaced apart by an open gap that allows oxygen to pass through and a glass dielectric to produce this type of discharge. This vacant area creates a filament discharge, and the electrons generated there are energetic enough to break up the oxygen molecules that makeup ozone. The amount of ozone contained in the containers was measured using an ozone analyser (Model H1-AFX - Instrumentation, USA). 220 V at 50 Hz was the input voltage of the variable high voltage transformer used in the AC test set. A space existed between the two electrodes, the inner and outer ones being linked to a voltage transformer.

As seen in Figure (1), the voltages required to produce ozone were controlled using a transformer control box, sometimes known as a variable AC power transformer regulator. When an oscillating voltage is applied across a gap that is isolated by a dielectric substance, a substantial amount of ozone is created in a silent AC discharge. The dielectric's function is to put an end to micro-discharges. The discharge electrons' simple dissociation of oxygen molecules is the fundamental mechanism. When the produced atomic oxygen interacts with oxygen molecules, ozone is created. Before ozone forms in the discharge, the oxygen molecules dissociate.

If the electric field in the gap is strong enough to induce breakdown, oxygen passing through the discharge device is partially transformed into ozone (Šimek *et al.*, 2012, Nassour *et al.*, 2016].

1) The main processes that produce oxygen atoms in pure oxygen are:

$$e + O_2 \rightarrow e + O + O \quad [1]$$

2) The primary process that forms ozone from oxygen atoms is:

$$O + O_2 + M \rightarrow O_3^* + M \rightarrow O_3 + M \quad [2]$$

Where M is a partner in a third collision (O, O_2, 0_3).

A significant amount of the discharge energy is consumed by ions when the micro-discharge is too weak, which prevents ozone formation conversely, there is a maximum strength to the micro-discharge due to the chemistry involved in ozone generation. According to the reaction, at a very weak micro-discharge, each oxygen atom forms one ozone molecule **(Homola *et al.*, 2019).** When oxygen atom concentrations rise and more reactions involving oxygen atoms become significant, this statement becomes inaccurate. The primary rival responses are:

$$O + O_3 + M \rightarrow 2O_2 \quad [3]$$

$$O + O_3^* \rightarrow 2O_2 \quad [4]$$

O_3^* represents an excited transient ozone species, the reaction's original result.

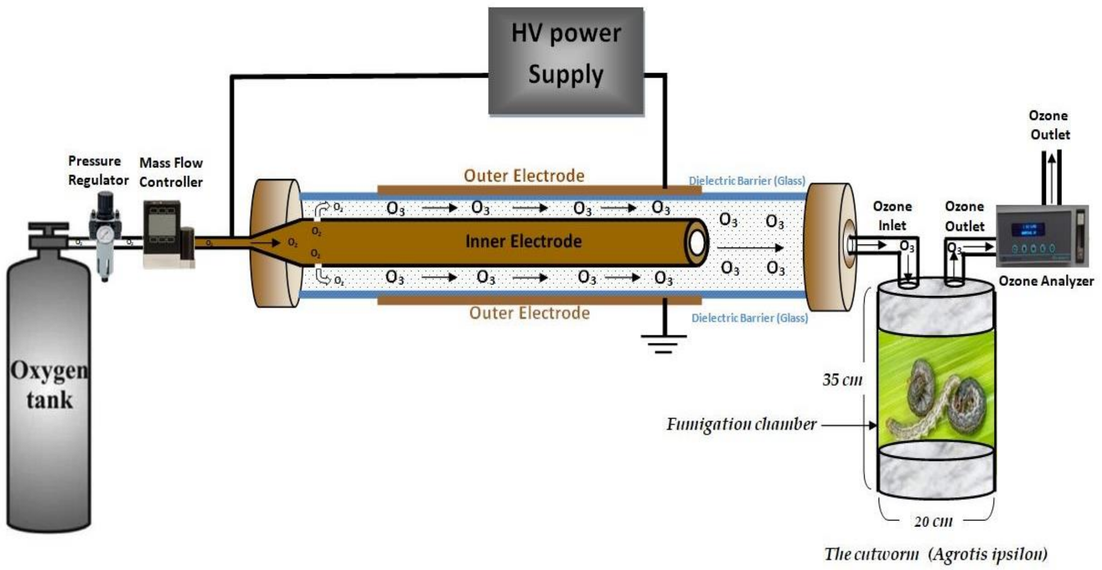

Fig.1: Schematic diagram of ozone generation and fumigation setup.

Characterization of Electrical Properties:

At room temperature and atmospheric pressure, the coaxial Dielectric Barrier Discharge (DBD) Ozone reactor system's current voltage oscillogram is displayed in Figure (2A). The filamentary modes in DBD release current when the applied voltage is higher than the breakdown voltage. As seen in Figure (2A) at different ozone concentrations of 250, 500, 1000, and 1500 ppmv, at an oxygen flow rate of 0.1 (L/min), the coaxial DBD reactor's applied voltage waveforms and corresponding current waveforms were measured. These values are 3.3, 3.8, 6.4and 8 kV, respectively.

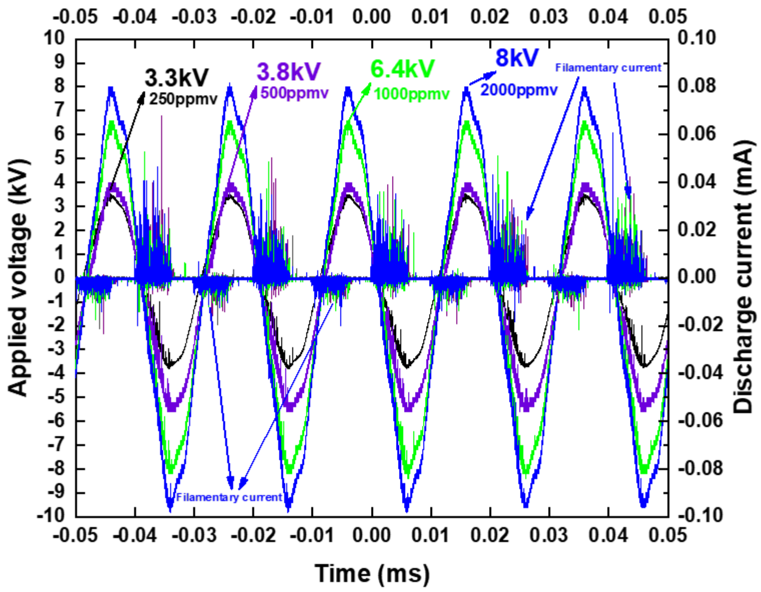

Fig. 2(A): Waveforms of the applied voltage to the reactor and associated current measured for Ozone concentration (250, 500, 1000, 1500ppmv) at oxygen flow rate 0.1 l/min.

Power Measurement Method:

Lissajous **(**Fig. 2B**)** was used to calculate the power consumption of the coaxial DBD Ozone producing system using power measurements and their relevance.

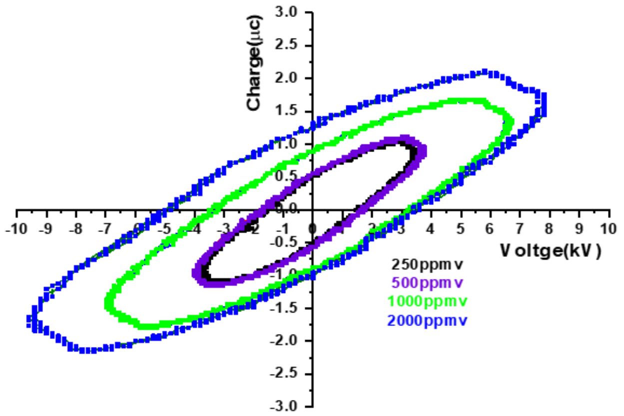

Fig. 2 (B): Lissajous diagrams measured for ozone concentration (250, 500, 1000, 1500ppmv) at a flow rate of 0.1 (l/min).

Using the (Q–V) curve of the Lissajous figure, the accumulated charge and the voltage across the transformer's output windings were used to calculate the energy input during a single cycle of the coaxial dielectric barrier discharge Ozone generator. Generally, the instantaneous current I(t) multiplied by the applied voltage V(t) yields the plasma discharge power P(t).

$$P(t) = I(t)V(t) \tag{1}$$

Therefore, electric energy (E_{el}) multiplied by "2" (i.e., the region bounded by the charge-voltage curves in the Lissajous diagram) gives the input energy of the coaxial dielectric barrier discharge Ozone generator for a single cycle. The Lissajous diagram can be used to determine the minimum external voltage (V_{min}) at which ignition occurs, the electric power (P_{el}), and the electric energy consumed per voltage cycle (E_{el}). (Wagner *et al.,* 2003, Okazaki *et al.,* 1993, Mahmoud *et al.,* 2023 and Salem *et al.*, 2024)

$$E_{el} = 2(V_{\max}Q_0 - Q_{\max}V_0) \equiv \text{area of (Q - V) diagram}$$

$$P_{el} = \frac{E_{el}}{T} = fE_{el} \qquad (2)$$

Where f is the frequency of 50 Hz.

The consumed power in this investigation was found to be 0.52 watts at an applied voltage of 3.3kV, 0.65 watts at 3.8kV, 1.65 watts at 6.4kV, and 2.8 watts at 8kV.

Method of Application:

Ozonation was conducted in a specially modified plastic container (20 × 15) cm with two openings, one of them for gas entering and the other for gas exiting **(**Fig. 1**)**. Each container contained 10 larvae of the 4th instar with three replicates for each concentration. Four ozone concentrations (250, 500, 1000, and 1500 ppmv) were utilized with exposure of 15 min., and the mortality of larvae after exposure was calculated for 7 days. Then the effect of ozone was followed for the remained larvae. Similar conditions were prepared for the control groups but without using ozone**.**

Such an experiment was carried out against black cutworm pupae and for the time of exposure and ozone concentrations.

Statistical Analysis:

Mortality data were estimated by using probit analysis Ldp line (Abbott, 1925) and LC_{50} values were determined using probit analysis statistical method by Finney, 1971. L.S.D. values were calculated by the Costat program (Costat software, 1990).

RESULTS

1-Effect of Ozone on Larvae of *Agrotis ipsilon*:

1.1-Effect on Mortality of Larvae:

Results in Table (1), demonstrated that mortality of 4th instar cutworm larvae increased by increasing ozone concentrations. The lowest concentration was 250 ppmv and caused 20% total mortality after 7 days of the beginning of the experiment. Then, the second tested concentration of ozone was 500 ppmv which caused 33.33% total mortality. As soon as, the next tested concentration was 1000 ppmv which caused 46.67% mortality. However, the highest ozone concentration was 1500 ppmv which caused total mortality to 73.33%.

Also, the table eliminated that the effect of mortality at 250 ppmv appeared after 5 days after the beginning of the experiment, while the beginning mortality appeared after three days at 500 ppm. In contrast, mortality at higher concentrations of 1000 & 1500 ppmv appeared after one day of larvae treatment.

Moreover, Table (2) and Fig. (3) illustrated that LC_{50} was 849.34 ppm and LC_{90} was 4620.44 ppm and the slope was 1.74.

Table 1: Mortality % of 4th instar larvae of *Agrotis ipsilon* treated with ozone.

Treatment	Conc. ppmv	Mortality after treatments %				Total Mortality %
		One day	Three days	Five days	Seven days	
Ozone	250	0	0	6.67	13.33	20
	500	0	6.67	13.33	13.33	33.33
	1000	6.67	6.67	13.33	20	46.67
	1500	20	13.33	26.67	13.33	73.33

Table 2: Lethal concentrations of *Agrotis ipsilon* larvae treated with ozone.

Treatment	Conc. (ppmv)	Corrected mortality %	LC_{50}	LC_{90}	Slope± S.D.	LC_{90}/ LC_{50}
Ozone	250	20	849.34	4620.44	1.74 ± 0.23	5.44
	500	33.33				
	1000	46.67				
	1500	73.33				

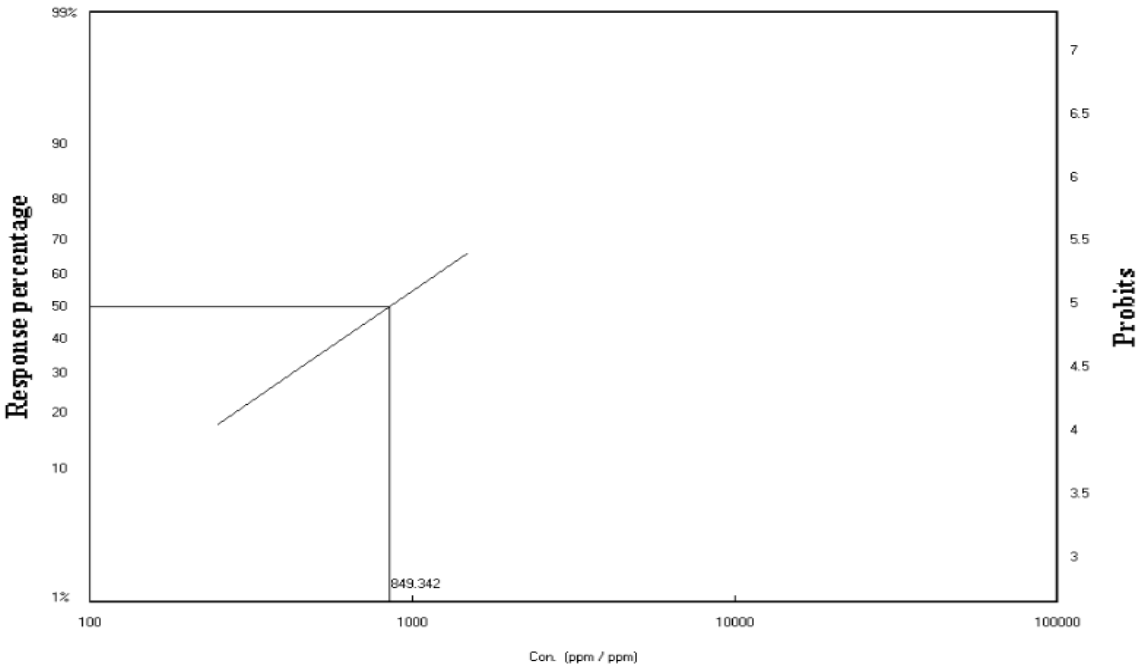

Fig. 3: LC-P line for ozone of *A. ipsilon* larvae.

1.2-Effect on Biological Aspects of *A. ipsilon* Larvae:

Results in Table (3) and Fig. (3) indicated that larvae treated with 250 ppm of ozone caused a decrease in durations of 4th, 5th, 6th, pre-pupa, pupa, and adult male durations. However, these mentioned durations increased with 500, 1000& 1500 ppmv durations compared with control, except for males because these ozone concentrations caused mortality of larvae inside cocoons.

The least significant difference (L.S.D.) for all values was significant.

Table 3: Biological aspects of *A. ipsilon* larvae treated with ozone different concentration

Biological aspects	control	250 ppmv	500 ppmv	1000 ppmv	1500 ppmv	L.S.D
4th instar	5±0.1	4.5±0.05	5.3± 0.07	5.6±0.1	6±0.1	0.975
5th instar	4.8±0.1	3.7±0.05	4.8± 0.07	5.1±0.1	5.3±0.1	0.241
6th instar	4±0.1	3.9±0.05	4.8± 0.08	5.3±0.1	5.7±0.1	0.211
Pre-pupa	1.5±0.1	1.4±0.08	1.7± 0.08	1.9±0.2	2.1±0.2	0.221
Pupa	7.5±0.1	7±0.1	8.2± 0.1	9±0.2	11±0.2	1.161
Adult male	7±0.1	5±0.1	---	----	----	0.944

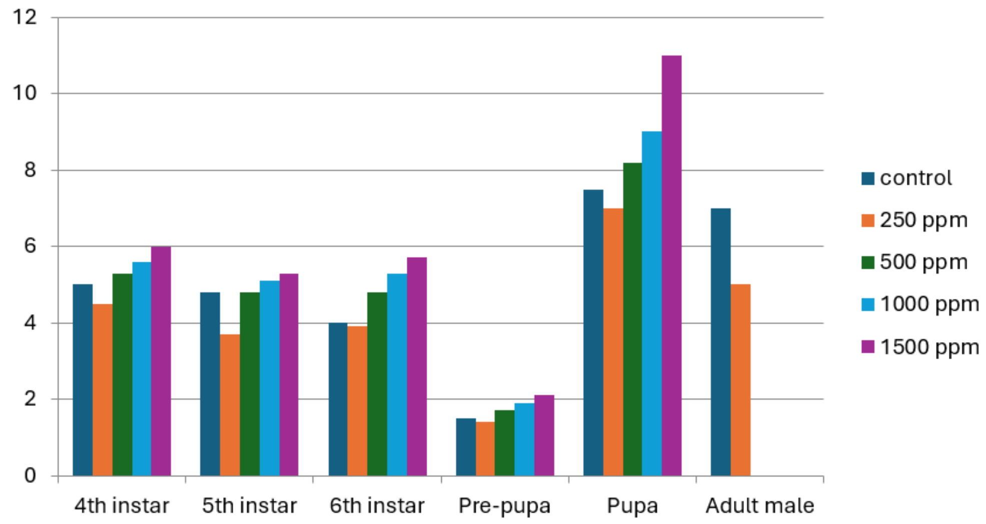

Fig. 4: Biological aspects of larvae treated with ozone.

1.3-Malformations of Treated Cutworm Larvae with Ozone:

In Figure (5), malformations to followed larvae appeared when treated with different concentrations of ozone. When the larvae treated with different concentrations of ozone mortality percentages were detected for 7 days, after that, larvae were followed until pupation, so malformations appeared during pupation or during trials of moth to emerge from the cocoon, these malformations were appeared in Figure (6).

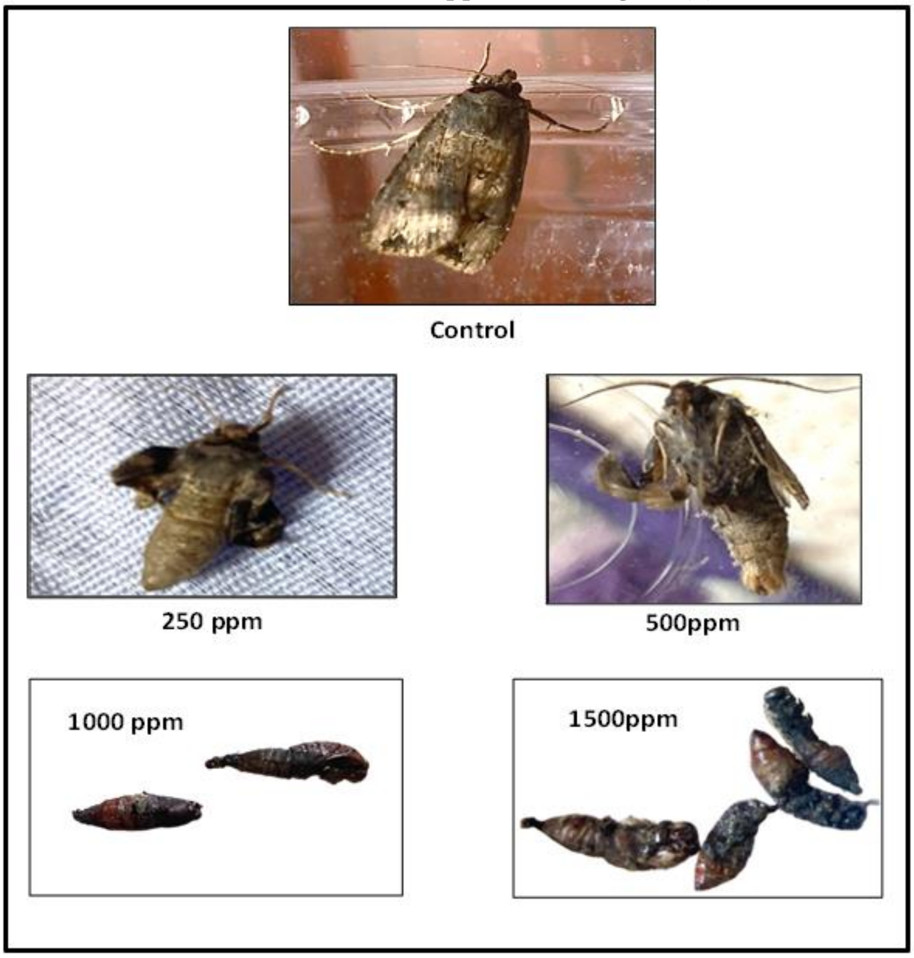

Fig. 5: Denaturation of pupae due to treatment of larvae with different concentrations of ozone.

2-Effect of Ozone on Pupae of *Agrotis ipsilon:*

Treatment of cutworm pupae with different concentrations of ozone 250, 500, 1000& 1500 ppmv led to the failure of the emergence of moths causing malformations of pupae (Fig.6).

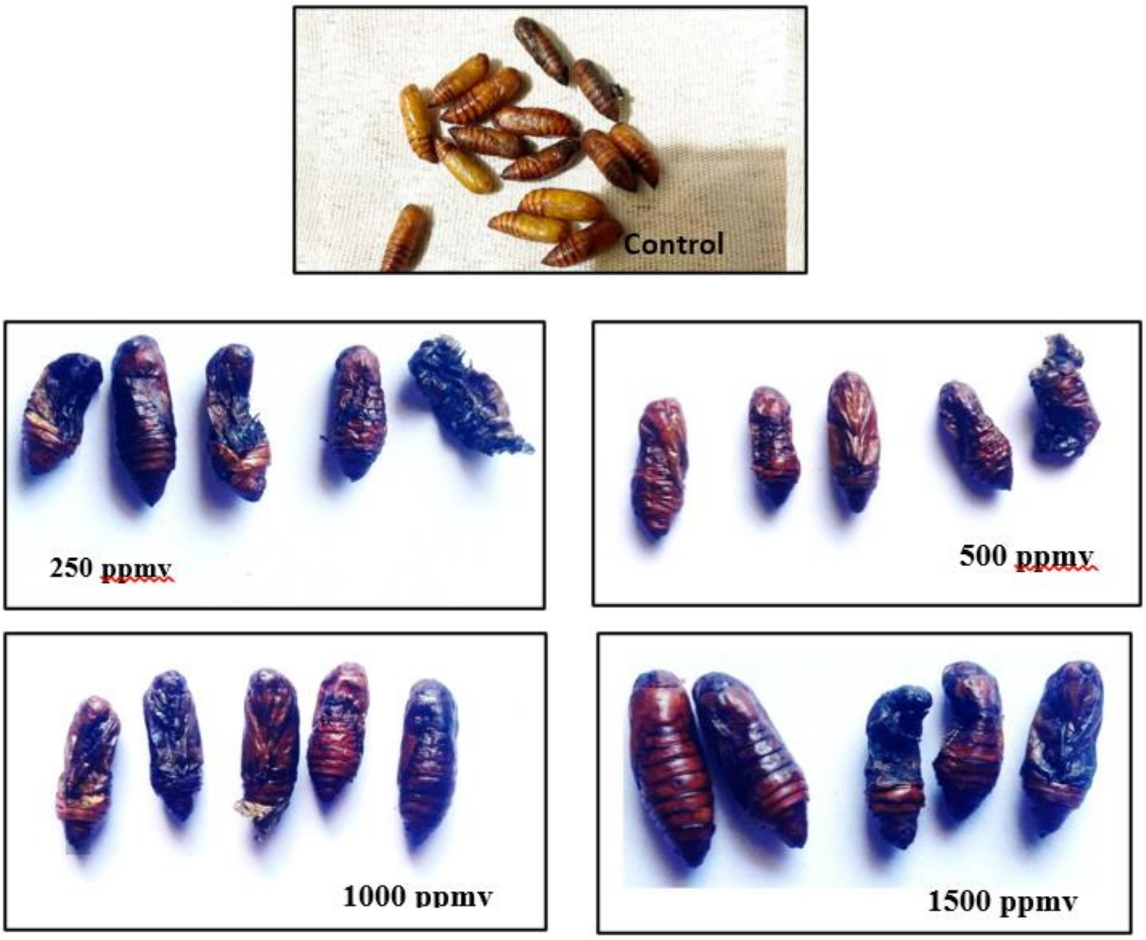

Fig. (6): Malformations of treated pupae with different concentrations of ozone.

DISCUSSION

The black cutworm *Agrotis ipsilon* is a dangerous pest all over the world that play a negative role in many crops damage, so its management especially with safe methods was very necessary (Wang *et al*., 2021; Nashwa and Abeer, 2022). Ozone O_3 is regarded as an eco-friendly agent than traditional insecticides (Moustafa *et al.* 2021; Hend *et al*., 2024).

Our findings proved the effectiveness of ozone on the mortality of cutworm larvae when larvae are exposed to different concentrations of ozone for 15 min. of exposure. The same results were obtained from Keivanloo *et al.* (2014) who observed that the greatest mortality rate of *Plodia interpunctella* larvae was 85.0% with ozone concentration of 5 ppm over a 2 h exposure time. Taha *et al.* (2017) and Mahmoud *et al.* (2023) verified the effectiveness of ozone on the mortality of *Trogoderma granarium*.

In addition, the following treated larvae of *A. ipsilon* with ozone couldn't complete the life cycle and it's worth noting that the resulting pupae from treated larvae were deformed. Hermes-Lima, (2004) and Holmstrup *et al.*, (2011) established that the reason for the malformation of *G. mellonella* larvae is exposure to high ozone concentrations that can cause oxidative stress, leading to various physiological and biochemical changes and These may damage cellular membranes, proteins, and DNA, that can result in reduced growth, development abnormalities, or mortality.

Furthermore, Nofal *et al.,* (2024) reported that *G. mellonella* larvae treated with different concentrations of ozone showed different mortality percentages that increased with increasing ozone concentrations.

Additionally, exposure of *A. ipsilon* pupae to different concentrations of ozone caused malformations to pupae without exposure to moths or during trial of moths' exposure. Ghada and Amal (2015) evaluated the effect of some plant extracts against the pupae of *Spodoptera littoralis* causing malformation also to pupae. This malformation may be due to the sensitive stage of pupae that can't tolerate some influences during the metamorphosis process.

According to Al-Ahmadi *et al.* (2009), *Oryzaephilus surinamensis* completely dies as an adult after 6 h of exposure to 30 ppm of ozone. Was sufficient to cause. As well as Osman (2009) and Ghada *et al.* (2024) demonstrated the effect of 1 g/m3 of ozone on *Ephestia kuehniella* for 5 h of exposure and investigated the full impact of ozone on the mortality rate that did not become apparent in larvae till at least six days had passed. Besides, *Plodia interpunctella* adults died entirely after exposure to 500 ppm of ozone for 60 minutes (McDonough *et al.,* 2011). Moreover, Hussain (2014), investigated that the mortality percentage of *E. cautella* increased gradually as increasing the exposure time to ozone gas and the time after treatment.

These findings align with studies by Palou *et al.* 2002; Forney *et al.* 2007 and Wei *et al.* 2007. Additionally, (Ibrahim *et al.* 2014) observed differential susceptibility among life stages of the potato tuber moth (*Phthorimaea operculella*), with eggs, pupae, adults, and larvae showing varying responses to different ozone concentrations and exposure times.

Additionally, Rasha and El-Shafei, 2019 tested the effect of 100, 300 and 5 of ozone gas on eggs, larvae, and pupae of the Indian meal moth, *Plodia interpunctella* for 15 to 15 min. and explored that life stages increased by increasing the exposure time in each ozone concentration. The same results were concluded by Niakousari *et al.,* (2010); Keivanloo *et. al*., (2013); Hassan (2014); Shaghaghian, *et al.,* (2014) Abd El-Aziz *et al.,* (2017) on different insects.

Conclusion:

The results of this study demonstrate the effectiveness of ozone treatment generated through non-thermal atmospheric pressure plasma (APPD) in managing the destructive pest Agrotis ipsilon. Key findings include:

- Ozone exposure caused significant direct mortality in *A. ipsilon* larvae, with mortality rates increasing at higher ozone concentrations.
- Surviving larvae exhibited physiological disruption and developmental abnormalities over the 7-day post-treatment period.
- All treated *A. ipsilon* pupae failed to successfully emerge as adult moths, displaying various morphological malformations.

These findings indicate that ozone generated through APPD technology represents a promising non-chemical pest management approach for *A. ipsilon*. The residue-free and aeration-free nature of this method makes it an attractive alternative to traditional insecticides, particularly given the pest's widespread resistance to many chemical control agents. Further research is warranted to optimize ozone exposure parameters and evaluate impacts on other life stages and target pests.

Declarations

Ethics Approval and Consent to Participate: Ethics committee approval was not required for this study as the experimental work was conducted exclusively on invertebrate pest species (*Agrotis ipsilon*). All authors have verified that this work represents original research and has not been previously published elsewhere.

Competing Interests: The authors declare that they have no competing interests that could have influenced the work reported in this paper.

Author Contributions: W.H.A., G.E.A., H.M.I., L.A.A., and Kh.H.M. contributed equally to this work. Their contributions encompassed conceptualization, experimental design, data analysis, manuscript preparation, and final manuscript review. All authors have read and approved the final version of the manuscript.

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