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The Effects of Propylene oxide Fumigation on the Mortality of *Plodia interpunctella* (Hubner) (Lepidoptera: Pyralidae)*

Propilen Oksit Fumigasyonunun *Plodia interpunctella* (Hubner) (Lepidoptera: Pyralidae) Ölümleri Üzerine Etkileri

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Abstract: This study was carried out in 2014-2015 at Kahramanmaraş Sütçü İmam University, Faculty of Agriculture, Department of Plant Protection, Entomology Laboratory. This study aims to evaluate the viability of propylene oxide (PPO) as an alternative fumigant to methyl bromide (MeBr) for the efficient control of *Plodia interpunctella* (Hubner) (Lepidoptera: Pyralidae) and to model the mortality rates of *P. interpunctella* insects using different regression techniques. The biological effectiveness of PPO was assessed across all life stages of *P. interpunctella* during a brief exposure period (4 hours) under three conditions: normal atmospheric pressure (PPO alone), low pressure (PPO+Vacuum), and an atmosphere enriched with carbon dioxide (CO_2) (PPO+CO_2). For all PPO treatments, PPO was directly introduced into the fumigation chamber at seven or more concentrations using different micro-syringes of different volume ranges: 2.5-25 µl l-1, 1.5-45 µl l-1, 1.5-30 µl l-1, and 0.5-15 µl l-1 for eggs, larvae, pupae, and adults, respectively. The results indicate that 100% mortality was observed in eggs at concentrations of 25 µl l^{-1}, 20 µl l^{-1} and 20 µl l^{-1}; in larvae at concentrations of 45 µl l^{-1}, 30 µl l^{-1} and 40 µl l^{-1}; in pupae at concentrations of 30 µl l^{-1}, 15 µl l^{-1} and 25 µl l^{-1}; and in adults at concentrations of 15 µl l^{-1}, 10 µl l^{-1} and 10 µl l^{-1} for the PPO alone, PPO+Vacuum, and PPO+CO_2 treatments, respectively. The results obtained from the developed regression models for insect mortality reveal that these models generally exhibited a better fit when described by exponential and third-order polynomial functions. In summary, this study indicates that PPO treatments hold significant promise for rapid insect control, particularly in the case of the Indian meal moth, rendering them invaluable for quarantine purposes.

Keywords: Indian meal moth, dried figs, fumigant, modelling, mortality

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Öz: Bu çalışma 2014-2015 yıllarında Kahramanmaraş Sütçü İmam Üniversitesi Ziraat Fakültesi Bitki Koruma Bölüöü Entomoloji Labaratuvarı'nda gerçekleştirilmiştir. Bu çalışmanın amacı, *Plodia interpunctella* (Hubner) (Lepidoptera: Pyralidae)'nın etkin kontrolü için metil bromüre (MeBr) alternatif bir fumigant olarak propilen oksidin (PPO) uygulanabilirliğini değerlendirmek ve farklı regresyon teknikleri kullanarak *P. interpunctella* böceklerinin ölüm oranlarını modellemektir. Yapılan çalışmada; PPO'nun biyolojik etkinliği, kısa maruz kalma süresi (4 saat) altında üç farklı koşulda test edilmiştir: normal atmosferik basınçta (yalnızca PPO), düşük basınçta (PPO+vakum) ve karbon dioksit (CO_2) ile zenginleştirilmiş bir atmosferde (PPO+CO_2). PPO'ya yönelik tüm uygulamalarda, PPO; farklı hacim aralıklarına sahip farklı mikro şırıngalar kullanılarak yedi veya daha fazla konsantrasyonda doğrudan fümigasyon çemberine uygulanmıştır: sırasıyla yumurtalar için 2.5-25 µl l-1, larvalar için 1.5-45 µl l-1, pupalar için 1.5-30 µl l-1 ve erginler için 0.5-15 µl l-1. Elde edilen sonuçlar, tek başına PPO, PPO+vakum ve PPO+CO_2 uygulamalarının sırasıyla yumurtalarda 25 µl l^{-1}, 20 µl l^{-1} ve 20 µl l^{-1} konsantrasyonlarında; larvalarda 45 µl l^{-1}, 30 µl l^{-1} ve 40 µl l^{-1} konsantrasyonlarında; pupalarda 30 µl l^{-1}, 15 µl l^{-1} ve 25 µl l^{-1} konsantrasyonlarında; ve erginlerde 15 µl l^{-1}, 10 µl l^{-1} ve 10 µl l^{-1} konsantrasyonlarında %100 ölüm sağladığını göstermektedir. Böcek ölümleri için geliştirilen regresyon modelleri, genellikle üstel ve üçüncü dereceden polinom fonksiyonlarıyla tanımlandığında daha iyi bir uyum gösterdiğini ortaya koymaktadır. Özetle, yapılan bu çalışma PPO uygulamalarının hızlı böcek kontrolünde özellikle Kuru meyve güvesinde umut vadeden bir potansiyel olduğunu ve karantina amaçları için büyük öneme sahip olduğunu göstermektedir.

Anahtar Kelimeler: Fumigant, kuru incir, kuru meyve güvesi, modelleme, ölüm orani

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*This study contains data from the master's thesis.

INTRODUCTION

The Indian meal moth, scientifically known as *Plodia interpunctella* (Hubner) (Lepidoptera: Pyralidae), is a highly destructive pest and poses a significant threat to stored products on a global scale (Mohandass et al., 2007; Nansen and Phillips, 2004; Razazzian et al., 2015). The larvae of *P. interpunctella* feed on the fruit reduce fruit quality and display a distinctive behavior by constructing a continuous silken web, both internally within the infested food and on its surface. Within this intricate web, they actively feed on the stored food, leading to the creation of an unsightly and unsanitary environment. The web itself serves as a habitat for the larvae, offering protection as well as a means for facilitating their feeding activities. This silk-encased environment is not only aesthetically unappealing but also contributes to the degradation of the infested produce. The infestation is further compounded by the inclusion of larval excreta and cast skins within the web, contributing to its undesirable characteristics (Tripathi, 2018). Additionally, the accumulation of these materials may contribute to an undesirable odor, further emphasizing the detrimental impact of *P. interpunctella* infestations on stored products. Infestation with of *P. interpunctella* may result in direct losses of infested produce. In addition, the economic impact extends to indirect costs, including expenses for pest control measures, reduced product quality, and customer losses (Phillips et al., 2000).

Fumigants play a crucial role in pest control within stored products, encompassing large commodities, packaged materials, and various structures (Rajendran and Sriranjini, 2008); hence, methyl bromide (MeBr) finds extensive application as a fumigant (Bell, 2000). Nevertheless, its categorization as an ozone-depleting substance took place in 1992, falling under the regulations of the Montreal Protocol on Substances that Deplete the Ozone Layer (Delpage, 2020). Consequently, developed countries began phasing out the use of MeBr in 2005, while developing countries completed their phase-out by 2015 (Gareau, 2010). The phase-out of MeBr could significantly negatively impact global agriculture, primarily because there are no readily available alternatives that can rapidly disinfect commodities. This circumstance emphasizes the pressing requirement for the development of novel fumigants specifically designed for quarantine purposes, aiming to guarantee swift and comprehensive control measures.

Therefore, efforts have been made worldwide to find suitable substitutes for postharvest commodity treatments. Alternative fumigants have been explored, with options such as ethyl formate (Park et al., 2021), carbon disulfide (Wright, 2003), nitric oxide (Liu et al., 2021), and sulfuryl fluoride (Zettler et al., 1998) being considered. However, it's important to note that these alternatives come with various drawbacks and limitations (Fields and White, 2002; Rajendran, 2001). For example; carbon disulfide, has been shown to be highly toxic to crops or nurseries and has not been approved in many countries due to possible toxic residues (Fields and White, 2002). Ethyl formate has been identified as an explosive, flammable and corrosive substance (Bond and Monro, 1984), and has been found to produce carcinogenic compounds when used on food (Wesley et al., 1965). Sulfuryl fluoride is chemically very promising, but cannot be used on food due to the established tolerances for food residues (Fields and White, 2002).

Propylene oxide (PPO) stands as a liquid fumigant acknowledged for its safety in the context of food sterilization. With a boiling point of 35°C, it emits an ether-like odor under normal temperature and pressure conditions (Navarro and Navarro, 2016). PPO is known for its efficacy in reducing microflora and microfauna, making it effective in sterilizing food products (Maille et al., 2023). One of the reasons PPO is considered safe for food sterilization (Dhulipalla et al., 2023) is its rapid conversion to non-toxic propylene glycols, both in the soil and the human stomach. This minimizes any potential harm to the environment and human health.

Compared to MeBr, PPO presents reduced environmental risks and does not contribute to ozone depletion, making it a more environmentally friendly fumigant (Meylan et al., 1986). The use of PPO as a food sterilant dates to 1958, and since then, it has gained popularity in fumigation studies for its effectiveness against stored-product insects. Studies have shown that PPO can successfully eliminate eggs and larvae of various

stored-product insect pests, making it a valuable tool for pest control (Isikber et al., 2017; Myers et al., 2021; Navarro et al., 2004).

The use of PPO as a fumigant comes with the disadvantage of flammability, as it can ignite when its concentration in the air falls within the range of 3% to 37% (Navarro et al., 2004). To minimize the risk of combustion, it is advisable to apply PPO under low-pressure (LP) conditions or in an atmosphere enriched with carbon dioxide (CO_2). The combination of PPO with low pressure (PPO+Vacuum) or carbon dioxide (PPO+CO_2) demonstrates potential as an alternative to MeBr. Previous research has established that PPO displays outstanding insecticidal properties in vacuum conditions, efficiently eliminating all life stages of diverse stored-product insects within an exceptionally brief exposure time of 4 hours (Creasy and Hartsell, 1999; Isikber et al., 2006; Navarro et al., 2004; Zettler et al., 2003). In their studies, Isikber et al. (2006) and Navarro et al. (2004) conducted a comparative analysis to assess the efficacy of PPO alone, PPO+Vacuum, and PPO+CO_2. Their objective was to ascertain the required dosages essential for achieving significant mortalities across all life stages of *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae), a stored-product insect species. This investigation was conducted within a concise exposure time of 4 hours. Their research findings demonstrated a synergistic effect on the toxicity of PPO to *T. castaneum* when combined with vacuum (PPO+Vacuum) and carbon dioxide (PPO+CO_2). Based on these published results, it is suggested that PPO warrants further evaluation as a potential substitute for MeBr, especially in specific commodities and under certain conditions. Numerous reports on insect toxicity consistently highlight the potential of PPO as a viable alternative to MeBr in particular postharvest scenarios (Creasy and Hartsell, 1999; Isikber et al., 2006; Navarro et al., 2004; Zettler et al., 2003). These insect toxicity studies establish an initial database supporting the idea that PPO could effectively replace MeBr in various post-harvest situations.

To date, no comprehensive studies have been conducted on the interaction between various doses of PPO applications and their effects on mortality rates at different life stages of *P. interpunctella*. Thus, given the lack of fundamental knowledge about the relationship between PPO applications and insect mortality, there is an urgent need to investigate whether regression models can serve as valuable tools for modeling insect mortality. Regression techniques are a fundamental set of statistical methods used in data analysis to model the relation between a dependent variable and one or more independent variables (Nunez et al., 2011). These techniques are widely employed in various fields, including economics (Busu and Trica, 2019), finance (Zhao et al., 2018), healthcare (Buselli et al., 2020), and engineering (Cemek et al., 2020), to make predictions and understand the underlying patterns in data. Nevertheless, it is noteworthy that a significant gap exists in the field of entomology, particularly concerning the utilization of regression techniques to model insect mortality.

Based on the above, the objectives of the study are twofold: (1) To investigate the potential of PPO as an alternative fumigant to MeBr for the rapid control of *P. interpunctella* and (2) To model the mortality rates of *P. interpunctella* using different regression techniques.

MATERIAL AND METHOD

This study was carried out in 2014-2015 at Kahramanmaraş Sütçü İmam University, Faculty of Agriculture, Department of Plant Protection, Entomology Laboratory.

Cultivation of Insect Culture Used in Biological Tests

The nutrient mixture used for the cultivation of *P. interpunctella* consists of wheat bran (2 kg), corn flour (350 g), glucose syrup (400-500 mL), glycerol (350-400 mL), and inactive yeast (20-25 g). The wheat bran and corn flour were subjected to a protective measure against insect infestation by placing them in a freezer at -20°C for 3-4 days. First, the ingredients were carefully mixed by hand according to the specified proportions and then processed in a mixer. To obtain the adult eggs of *P. interpunctella*, the adults were transferred from vacuum-sealed culture vessels to a 3-liter glass container. The opening of the container was covered with a net and the container with the adult eggs was left in an air-conditioned environment for 24 hours. A net, allowing only the eggs to pass through, was then attached to the opening of the container, which was then inverted to facilitate the deposition of the eggs on a paper surface. The collected

eggs were then placed in 3-liter jars, each containing 350-400 grams of food, with the openings of the jars covered with a net to facilitate air circulation. The culture jars were then kept in a dark incubator at a constant temperature of 30±1°C and a relative humidity of 65±5%, with insect growth monitored daily. These procedures were strictly maintained throughout the research period to ensure the continuity of the cultures (Küçüktopcu, 2023).

Fumigant and Carbon Dioxide (CO_2) Gas

The fumigant PPO was acquired from SERVA Electrophoresis GmbH (Heidelberg, Germany) with a purity of >%99 (CAS No. 75569, Cat. No. 33715). Subsequently, the PPO was transferred to a 100 ml glass bottle and securely sealed with a septum. During the treatment phase, the predetermined amount of PPO was drawn up from the glass bottle using a gas-tight micro syringe (Hamilton, Switzerland).

Carbon dioxide (CO_2) employed in the biological experiments was sourced by Linde Gas Company (Ankara, Türkiye) in a pressurized steel cylinder with a purity of 99%.

Fumigation Chamber

The fumigation chamber consists of 3-L glass jars with metal lids, each with a metal tube that has an inlet and an outlet. Two flexible silicone tubes, each 5 cm long and 0.62 cm in diameter, were placed over the inlet and outlet metal tubes. These silicone tubes were securely fastened with metal clamps to create a gas-tight environment. To ensure no gas escapes, silicone is carefully applied around the edges of the metal lids before they are sealed. This gas-tight system allows the safe use of vacuum and PPO without any leakage.

Biological Tests

In all PPO treatments, 20 adults (1-2 days old), 20 pupae (1-2 days old), 20 late larvae (28-32 days old), and 50 eggs (1-2 days old) of *P. interpunctella* were used. To accommodate these stages, each developmental group was carefully placed in separate 50 ml glass vials. Sun-dried Sarılop (Calimyrna) (Ficus carica L.) dried fig varieties with a moisture content of 21±1% were used for the biological tests. This food medium was added to the vials to meet the larvae's dietary needs, filling approximately 1/3 of their volume (equivalent to 10 g for 50 ml vials).

In order to ease the introduction of PPO gas and to ensure the containment of insects, the vial openings were covered with a fine muslin mesh and tightly secured with plastic rubber. These prepared insect vials were then positioned within 3-L glass jars, each equipped with a metal lid, forming a sealed fumigation chamber. This arrangement ensured a gas-tight environment for both the test insect and the PPO gas.

For the application of PPO under a LP condition, a vacuum pump (KNF N026 1.2.AN 18 Diaphragm Vacuum Pump) was employed to evacuate the air from the 3-liter fumigation chamber, leading to a reduced pressure of 100 mm Hg. To precisely monitor the LP within the chamber, a Celesco vacuum gauge (SE-2000) was employed. Once the desired pressure was achieved, PPO was introduced into the chamber using a gas-tight micro-syringe (Hamilton Company, Bonaduz, Switzerland).

To conduct PPO treatment under a CO_2 atmosphere, the fumigation chamber's pressure was lowered to 60.8 mm Hg and then reestablished to normal atmospheric pressure by introducing CO_2 gas. The CO_2 concentration was accurately gauged using a CO_2 O_2^{-1} measurement device (CheckPoint, PBI-Dansensor, Denmark). Once the desired CO_2 level of 92% was attained, PPO was added to the fumigation chamber using a microsyringe.

For all PPO treatments, PPO was directly introduced into the fumigation chamber at seven or more concentrations using different micro-syringes of different volume ranges: 2.5-25 µl l^{-1}, 1.5-45 µl l^{-1}, 1.5-30 µl l^{-1}, and 0.5-15 µl l^{-1} for eggs, larvae, pupae, and adults, respectively.

After completing the biological tests, the fumigation chamber lids were promptly closed, and the chambers were placed within a dark climate chamber for 4 hours, maintaining a temperature of 26±1°C and a relative

humidity of 65±5%. To ensure the validity of the results, each PPO treatment was replicated four times, and four control groups were included for each treatment condition.

Regression Techniques

Regression techniques involve establishing a mathematical relationship between measurements of two variables, typically denoted as 'y' and 'x.' This relationship is designed to enable the prediction of the value of one variable,'y', based on measurements of the other variable, 'x.' When assessing the appropriateness of measured data for the application of regression techniques, creating a graphical representation of the data is recommended. This approach is often the most effective way to identify data characteristics that might render them unsuitable for regression analysis. Plotting the data on a graph can visually inspect whether data points exhibit anomalies or errors. Such anomalies may suggest the influence of human error or instrument malfunction on these specific data points. In such cases, it is advisable to investigate and verify the accuracy of these data points before proceeding with regression analysis.

If a linear relation exists between the variables, it can be expressed as:

$$y = ax + b \qquad (1)$$

In cases where the relation between two variables, 'y' and 'x', is not initially linear but follows a power-law relation such as $y = ax^c$, it is possible to transform the data to create a linear relation. By taking the logarithm of both sides of the equation, a linear relation can be derived as follows:

$$\log(y) = \log(a) + c\log(x) \qquad (2)$$

Any quadratic and higher-order relations between one variable, y, and another variable, x, can be expressed using a power series in the following form:

$$y = ax + bx^2 + cbx^3 + \cdots + dx^p \qquad (3)$$

When the appropriate form of the relation between variables in measurement data sets is not readily discernible through visual inspection or grounded in established physical laws, a practical approach involves employing a trial-and-error method. This approach entails iteratively testing various functional forms or equations that may potentially elucidate the connection between the variables 'y' and 'x.'

RESULTS

PPO Applications and Their Effects on Mortality

As a result of a two-way analysis of variance, it was determined that both the PPO treatments and the biological stages of *P. interpunctella* had a statistically significant impact on mortality rates ($F_{3,569}$=213.29, P=0.0000 for PPO treatments; $F_{3,569}$=3.23, P=0.0022 for biological stages). Significant differences were observed in mortality rates among the biological stages of *P. interpunctella* when exposed to varying concentrations of PPO ($F_{3,108}$=49.73; P=0.000 for eggs; $F_{3,172}$=48.43; P=0.000 for larvae; $F_{3,140}$=59.59; P=0.000 for pupae; and $F_{3,140}$=68.61; P=0.000 for adults). Table 1 presents the average mortality rates of all life stages of *P. interpunctella* for PPO alone, PPO+Vacuum, and PPO+CO_2 treatments over 4 hours. The percentage mortality data for *P. interpunctella* eggs, larvae, pupae, and adults after exposure to PPO alone, PPO+Vacuum, and PPO+CO_2 treatments for 4 hours are presented in Table 2-5, respectively.

Focusing solely on the comparison between the three different treatments and ignoring the differences in the biological stages of *P. interpunctella*, we found an average mortality rate of 56.63% for the PPO treatment alone, 74.92% for the PPO+Vacuum treatment and 70.59% for the PPO+CO_2 treatment. While there were no statistical differences between the mean mortality rates of *P. interpunctella* exposed to the PPO+Vacuum and PPO+CO_2 combination, both combinations had statistically significantly higher mean mortality rates than the PPO treatment alone. In addition, all PPO treatments showed statistically significant differences compared to the control group ($F_{3,140}$=63.00; P=0.000) (Table 1).

Table 1. Average percentage mortality rates of all life stages of *Plodia interpunctella* exposed to different PPO application for 4 hours.

Çizelge 1. Farklı PPO uygulamalarına 4 saat boyunca maruz kalan Plodia interpunctella'nın tüm yaşam evrelerinin ortalama yüzde ölüm oranları.

Applications	N	Average Mortality (%) ± SE	F and P Value
PPO	3720	56.63±5.23 **B***	$F_{3,140}$=2.30; P=0.080
PPO+Vacuum	3720	74.92±4.39 **A**	$F_{3,140}$=1.46; P=0.228
PPO+CO_2	3720	70.59±4.55 **A**	$F_{3,140}$=1.27; P=0.286
Control	3720	8.47±0.47 **C**	$F_{3,140}$=63.00; P=0.000

*Differences between means were analyzed by the Tukey test at a 5% significance level. Different capital letters in the same column are statistically different from each other.

Table 2. Average mortality rates (%) *Plodia interpunctella* eggs were exposed to various concentrations of PPO alone, PPO+Vacuum, and PPO+CO_2 for 4 hours.

Çizelge 2. Tek başına PPO, PPO+Vakum ve PPO+CO_2'nin çeşitli konsantrasyonlarına 4 saat boyunca maruz bırakılan Plodia interpunctella yumurtalarının ortalama ölüm oranları (%).

PPO Concentration (µl l^{-1})	Mortality (%) ± SE				F and P value	Average mortality (%)
	PPO	PPO+Vacuum	PPO+CO_2	Control		
2.5	10.50±1.26 **Gc***	34.50±0.96 **Fb***	39.00±0.58 **Fa***	5.00±0.58 **Bd***	$F_{3,12}$=364.74 P=0.000	22.25±3.82 **D***
5	28.00±0.82 **Fb**	45.50±0.96 **Ea**	49.50±0.50 **Ea**	6.00±1.42 **ABc**	$F_{3,12}$=410.52 P=0.000	32.25±4.46 **CD**
7.5	34.50±0.96 **Eb**	61.00±1.29 **Da**	63.50±1.71 **Da**	10.50±0.50 **Ac**	$F_{3,12}$=433.90 P=0.000	42.38±5.61 **BCD**
10	57.50±1.50 **Db**	71.00±1.29 **Ca**	73.00±1.00 **Ca**	10.50±1.26 **Ac**	$F_{3,12}$=523.18 P=0.000	53.00±6.55 **ABCD**
15	69.00±1.29 **Cc**	95.00±0.58 **Ba**	87.50±0.96 **Bb**	5.00±0.58 **Bc**	$F_{3,12}$=2059.15 P=0.000	64.13±9.16 **ABC**
20	89.00±1.29 **Bb**	100.00±0.00 **Aa**	100.00±0.00 **Aa**	6.00±1.42 **ABc**	$F_{3,12}$=2254.82 P=0.000	73.80±10.18 **AB**
25	100.00±0.00 **Aa**	100.00±0.00 **Aa**	100.00±0.00 **Aa**	10.50±0.50 **Ab**	$F_{3,12}$=32041.00 P=0.000	77.62±10.00 **A**
F and P value	$F_{6,21}$=868.13 P=0.000	$F_{6,21}$=917.68 P=0.000	$F_{6,21}$=746.62 P=0.000	$F_{6,21}$=7.58 P=0.000	For applications: $F_{3,108}$=49.73, P=0.000 For dosage periods: $F_{6,105}$=7.79, P=0.000 For applications*dosage periods $F_{18,84}$=221.80, P=0.000	
Average mortality (%)	55.50±5.88 **b***	72.43±4.79 **a**	73.21±4.29 **a**	7.64±0.58 **c**		

*One-way ANOVA was applied to mortality data for PPO concentrations in each column and PPO treatments in each row. Means within a row with the same lowercase letter and within a column with the same uppercase letter were not significantly different (Tukey's HSD test at the 5% level).

A PPO concentration of 25 µl l^{-1} resulted in 100% mortality of *P. interpunctella* eggs when exposed to PPO alone, while a PPO concentration of 20 µl l^{-1} was effective for both PPO+Vacuum and PPO+CO_2. Focusing only on the comparison between the three different treatments, ignoring PPO concentrations, no statistically significant difference in toxicity was observed between PPO+vacuum and PPO+CO_2 treatments against the egg stage of *P. interpunctella*. Thus, it was determined that the PPO+vacuum and PPO+CO_2 were the most effective PPO treatments tested for mortality of the egg stage (Table 2).

Table 3. Average mortality rates (%) of *Plodia interpunctella* larvae exposed to various concentrations of PPO alone, PPO+Vacuum, and PPO+CO_2 for 4 hours.

Çizelge 3. Tek başına PPO, PPO+vakum ve PPO+CO_2'nin çeşitli konsantrasyonlarına 4 saat boyunca maruz kalan Plodia interpunctella larvalarının ortalama ölüm oranları (%).

PPO Concentration (µl l^{-1})	Mortality (%) ± SE				F and P value	Average mortality (%)
	PPO	PPO+Vacuum	PPO+CO_2	Control		
1.5	3.75±1.25 **Ib***	15.00±2.04 **Ha***	7.50±1.45 **Ib***	5.00±2.04 **Ab***	$F_{3,12}$=8.48 P=0.003	7.81±1.37 **F***
2.5	13.75±1.25 **Hb**	27.50±1.45 **Ga**	23.75±1.25 **Ha**	6.25±1.25 **Ac**	$F_{3,12}$=55.00 P=0.000	17.81±2.24 **EF**
5	23.75±1.25 **Gb**	37.50±1.45 **Fa**	32.50±1.45 **Ga**	6.25±2.40 **Ac**	$F_{3,12}$=65.82 P=0.000	25.00±3.16 **E**
7.5	33.75±1.25 **Fc**	52.50±1.45 **Ea**	42.50±1.45 **Fb**	5.00±2.04 **Ad**	$F_{3,12}$=169.00 P=0.000	33.43±4.63 **DE**
10	50.00±2.04 **Eb**	63.75±2.40 **Da**	57.50±1.45 **Eab**	6.25±1.25 **Ac**	$F_{3,12}$=200.05 P=0.000	44.37±5.88 **CD**
15	65.50±0.00 **Db**	73.75±1.25 **Ca**	68.75±1.25 **Dab**	6.25±2.40 **Ac**	$F_{3,12}$=452.88 P=0.000	53.43±7.11 **BC**
20	76.25±1.25 **Cb**	88.75±1.25 **Ba**	82.50±1.45 **Cab**	5.00±2.04 **Ac**	$F_{3,12}$=651.78 P=0.000	63.12±8.77 **AB**
25	81.25±1.25 **Cc**	98.75±1.25 **Aa**	88.75±1.25 **Bb**	6.25±1.25 **Ad**	$F_{3,12}$=1144.00 P=0.000	68.75±9.47 **AB**
30	87.50±1.45 **Bb**	100.00±0.00 **Aa**	97.50±1.45 **Aa**	5.00±0.00 **Ac**	$F_{3,12}$=1972.00 P=0.000	72.50±10.15 **A**
40	97.50±1.45 **Aa**	100.00±0.00 **Aa**	100.00±0.00 **Aa**	6.25±1.25 **Ab**	$F_{3,12}$=2369.57 P=0.000	75.93±10.40 **A**
45	100.00±0.00 **Aa**	100.00±0.00 **Aa**	100.00±0.00 **Aa**	6.25±1.25 **Ab**	$F_{3,12}$=5625.00 P=0.000	76.56±10.48 **A**
F and P value	$F_{10,33}$=735.51 P=0.000	$F_{10,33}$=533.85 P=0.000	$F_{10,33}$=704.00 P=0.000	$F_{10,33}$=0.14 P=0.999	For applications: $F_{3,172}$=48.43, P=0.000 For dosage periods: $F_{10,165}$=11.08, P=0.000 For applications*dosage periods $F_{30,132}$=139.68, P=0.000	
Average mortality (%)	57.50±5.01 **b***	68.86±4.64 **a**	63.75±4.83 **ab**	5.79±0.46 **c**		

*One-way ANOVA was applied to mortality data for PPO concentrations in each column and PPO treatments in each row. Means within a row with the same lowercase letter and within a column with the same uppercase letter were not significantly different (Tukey's HSD test at the 5% level).

A PPO concentration of 45 µl l^{-1} was necessary to achieve 100% mortality of *P. interpunctella* larvae when exposed to PPO alone, while PPO+Vacuum and PPO+CO_2 required 30 µl l^{-1} and 40 µl l^{-1} PPO concentrations, respectively. The percentage mortality data showed that the most toxic treatment for the larvae was the PPO+vacuum, followed by the PPO+CO_2 and PPO alone (Table 3).

When exposed to PPO alone, a concentration of 30 µl l^{-1} was needed to achieve 100% mortality of *P. interpunctella* pupae. In contrast, PPO+ Vacuum and PPO+CO_2 treatments required lower concentrations of 15 µl l^{-1} and 25 µl l^{-1} PPO, respectively, to achieve similar results. The percentage mortality data analysis revealed that the most toxic treatment for pupae was the PPO+Vacuum treatment, followed by the PPO+CO_2, and finally, PPO alone (Table 4).

Table 4. Average mortality rates (%) of *Plodia interpunctella* pupae exposed to various concentrations of PPO alone, PPO+Vacuum, and PPO+CO_2 for 4 hours.
Çizelge 4. Tek başına PPO, PPO+Vakum ve PPO+CO_2'nin çeşitli konsantrasyonlarına 4 saat boyunca maruz bırakılan Plodia interpunctella pupalarının ortalama ölüm oranları (%).

PPO Concentration (µl l^{-1})	Mortality (%) ± SE				F and P value	Average mortality (%)
	PPO	PPO+Vacuum	PPO+CO_2	Control		
1.5	17.50±1.45 **Hb***	27.50±1.45 **Fa***	21.25±1.25 **Gb***	11.25±1.25 **Ac***	$F_{3,12}$=25.43 P=0.000	19.37±1.64 **F***
2.5	32.50±1.45 **Gc**	47.50±1.45 **Ea**	38.75±1.25 **Fb**	15.00±0.00 **Ad**	$F_{3,12}$=131.91 P=0.000	33.43±3.12 **E**
5	48.75±1.25 **Fc**	66.25±1.25 **Da**	57.50±1.45 **Eb**	13.75±1.25 **Ad**	$F_{3,12}$=312.85 P=0.000	46.56±5.18 **DE**
7.5	60.00±2.04 **Ec**	78.75±1.25 **Ca**	67.50±1.45 **Db**	15.00±0.00 **Ad**	$F_{3,12}$=400.20 P=0.000	55.31±6.28 **CD**
10	71.25±1.25 **Db**	92.50±1.45 **Ba**	76.25±1.25 **Cb**	13.75±1.25 **Ac**	$F_{3,12}$=696.85 P=0.000	63.43±7.70 **BC**
15	82.50±1.45 **Cb**	100.00±0.00 **Aa**	87.50±1.45 **Bb**	11.25±1.25 **Ac**	$F_{3,12}$=1120.27 P=0.000	70.31±8.97 **AB**
20	87.50±1.45 **BCb**	100.00±0.00 **Aa**	97.50±1.45 **Aa**	15.00±0.00 **Ac**	$F_{3,12}$=1564.00 P=0.000	75.00±9.04 **AB**
25	92.50±1.45 **Bb**	100.00±0.00 **Aa**	100.00±0.00 **Aa**	13.75±1.25 **Ac**	$F_{3,12}$=1937.57 P=0.000	76.56±9.41 **AB**
30	100.00±0.00 **Ac**	100.00±0.00 **Ab**	100.00±0.00 **Aa**	15.00±0.00 **Ad**	$F_{3,12}$=----- P=---	78.75±9.50 **A**
F and P value	$F_{8,27}$=408.38 P=0.000	$F_{8,27}$=690.38 P=0.000	$F_{8,27}$=551.91 P=0.000	$F_{8,27}$=2.70 P=0.025	For applications: $F_{3,140}$=59.59, P=0.000 For dosage periods: $F_{8,135}$=8.17, P=0.000 For applications*dosage periods $F_{24,108}$=146.45, P=0.000	
Average mortality (%)	65.83±4.54 **b***	79.16±4.28 **a**	71.80±4.52 **ab**	13.75±0.37 **c**		

*One-way ANOVA was applied to mortality data for PPO concentrations in each column and PPO treatments in each row. Means within a row with the same lowercase letter and within a column with the same uppercase letter were not significantly different (Tukey's HSD test at the 5% level).

To achieve 100% mortality of *P. interpunctella* adults when exposed to PPO alone treatment, a concentration of 15 µl l^{-1} PPO was necessary. However, for PPO+Vacuum and PPO+CO_2, lower concentrations of 10 µl l^{-1} and 7.5 µl l^{-1} PPO were sufficient to achieve the same mortality rate. Analysis of the percentage mortality data indicated no significant difference in the toxicity of PPO+Vacuum and PPO+CO_2 treatments against adults, and both combination treatments were more toxic to adults than PPO alone treatment (Table 5).

Table 5. Average mortality rates (%) *Plodia interpunctella* adults exposed to various concentrations of PPO alone, PPO+Vacuum, and PPO+CO_2 for 4 hours.

Çizelge 5. Tek başına PPO, PPO+Vakum ve PPO+CO_2'nin çeşitli konsantrasyonlarına 4 saat boyunca maruz kalan Plodia interpunctella erginlerinin ortalama ölüm oranları (%).

PPO Concentration (µl l^{-1})	Mortality (%) ± SE				F and P value	Average mortality (%)
	PPO	PPO+Vacuum	PPO+CO_2	Control		
0.5	11.25±1.25 **Fc***	36.25±2.40 **Fa***	26.25±2.40 **Fb***	5.10±2.04 **Ac***	$F_{3,12}$=46.88 P=0.000	19.68±3.31 **D***
1	16.25±1.25 **Fc**	51.25±1.25 **Ea**	42.50±1.45 **Eb**	5.00±2.04 **Ad**	$F_{3,12}$=201.33 P=0.000	28.75±4.91 **CD**
1.5	18.75±1.25 **Fc**	62.50±1.45 **Da**	53.75±1.25 **Db**	7.50±1.45 **Ad**	$F_{3,12}$=388.86 P=0.000	35.63±5.99 **BCD**
2.5	18.75±2.40 **Fb**	77.50±1.45 **Ca**	70.00±2.04 **Ca**	7.50±1.45 **Ac**	$F_{3,12}$=357.15 P=0.000	43.44±7.97 **ABCD**
5	33.75±1.25 **Eb**	88.75±2.40 **Ba**	82.50±1.45 **Ba**	7.50±1.45 **Ac**	$F_{3,12}$=534.00 P=0.000	53.12±8.78 **ABCD**
7.5	58.75±2.40 **Db**	97.50±1.45 **Aa**	100.00±0.00 **Aa**	5.00±00 **Ac**	$F_{3,12}$=1010.33 P=0.000	65.31±9.95 **ABC**
10	71.25±1.25 **Cb**	100.00±0.00 **Aa**	100.00±0.00 **Aa**	7.50±1.45 **Ac**	$F_{3,12}$=2087.29 P=0.000	69.68±9.76 **AB**
12.5	90.00±2.04 **Bb**	100.00±0.00 **Aa**	100.00±0.00 **Aa**	7.50±1.45 **Ac**	$F_{3,12}$=1286.33 P=0.000	74.37±10.05 **A**
15	100.00±0.00 **Ac**	100.00±0.00 **Ab**	100.00±0.00 **Aa**	5.00±00 **Ad**	$F_{3,12}$=----- P=---	76.25±10.63 **A**
F and P value	$F_{8,27}$=450.12 P=0.000	$F_{8,27}$=270.39 P=0.000	$F_{8,27}$=465.52 P=0.000	$F_{8,27}$=0.83 P=0.581	For applications: $F_{3,140}$=68.61, P=0.000 For dosage periods: $F_{8,135}$=6.37, P=0.000 For applications*dosage periods $F_{24,108}$=125.81, P=0.000	
Average mortality (%)	46.52±5.48 **b***	79.30±3.86 **a**	75.00±4.55 **a**	6.38±0.47 **c**		

*One-way ANOVA was applied to mortality data for PPO concentrations in each column and PPO treatments in each row. Means within a row with the same lowercase letter and within a column with the same uppercase letter were not significantly different (Tukey's HSD test at the 5% level).

For three different applications (PPO alone, PPO+Vacuum, and PPO+CO_2), the mortality rates (%) of *P. interpunctella* at different developmental stages (eggs, larvae, pupae, and adults) were plotted against the PPO concentration, as shown in the Figure 1a-d.

In all cases, mortality rates showed a general upward trend with increasing PPO concentrations. These observed averages were statistically different from the control group, indicating a significant impact of PPO concentration on the mortality rates of *P. interpunctella* at various developmental stages across the three distinct applications.

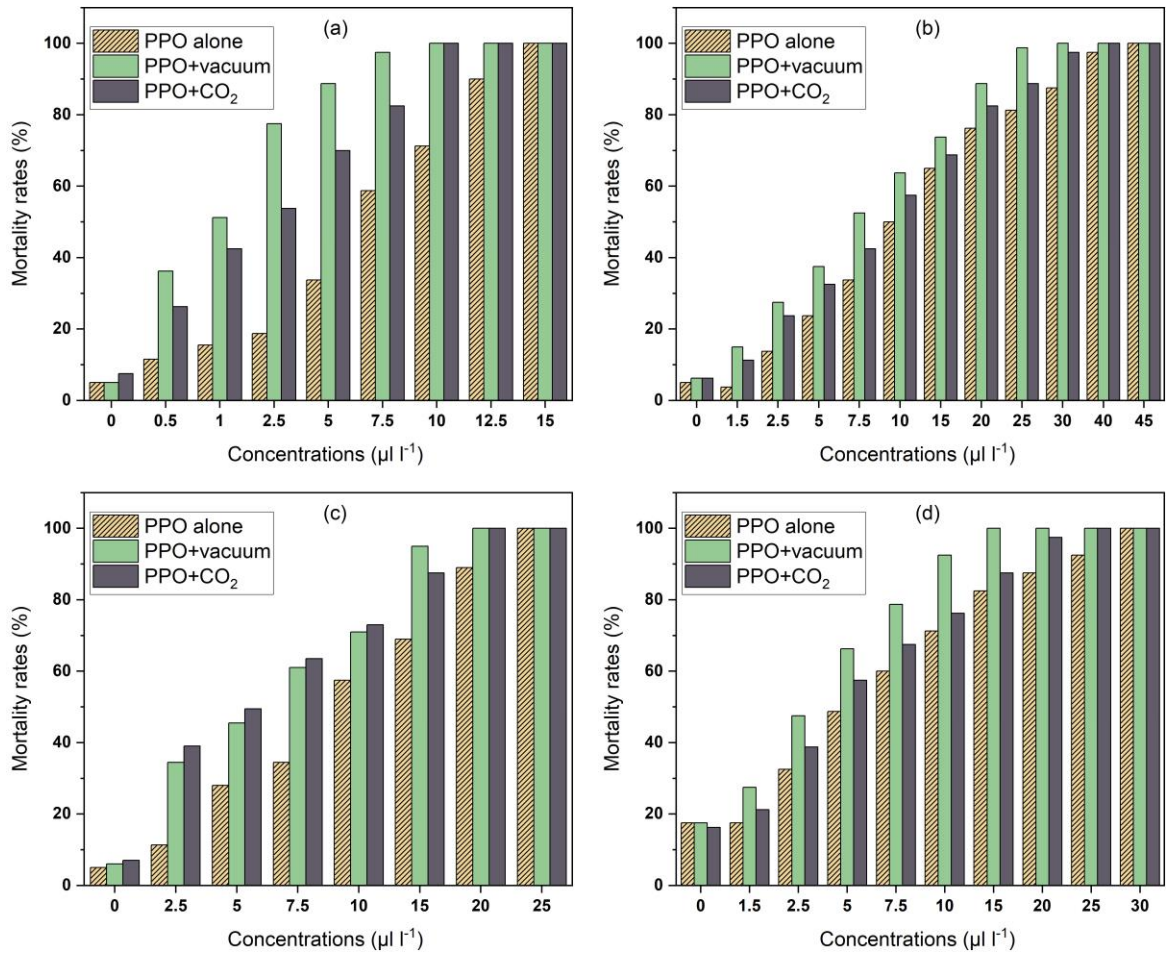

Figure 1. The mortality rates versus PPO concentrations for (a) adults, (b) larvae, (c) eggs, and (d) pupae of *Plodia interpunctella* for different PPO applications.

Şekil 1. Farklı PPO uygulamaları için Plodia interpunctella'nın (a) erginleri, (b) larvaları, (c) yumurtaları ve (d) pupaları için PPO konsantrasyonlarına karşı ölüm oranları.

Fitted Regression Models

To investigate the relationships between PPO applications and mortality of *P. interpunctella*, different regression models (linear, logarithmic, exponential, and polynomial) were used. The most appropriate model was selected by trial and error, focusing on the highest coefficient of determination (R^2), lowest root mean square error (RMSE), and the sum of squares error (SSE). The strategies for finding an appropriate model include the forward method, starting with a straightforward model, such as a straight line. If this basic model does not adequately fit the data, more complex models are considered, such as a 2nd-degree polynomial, and continuing this process is necessary (Alexopoulos, 2010).

The fitted regression models for each application are displayed in Figure 2a-m. The blue curves represent the fitted models in these figures, while the black points correspond to the training data. Upon examination of the developed models, it becomes evident that only one model (for adults under PPO-alone treatment) exhibits a linear relationship (y=6.455x+6.213). In contrast, the remaining models are generally best fitted with exponential and 3rd-degree polynomial functions. For example, the exponential model is the most suitable for describing the relationship for larvae under PPO+Vacuum treatment, as indicated by its high R^2 value of 0.996, low RMSE of 2.576, and SSE of 53.09. Meanwhile, the 3rd-degree polynomial model proves to be the most appropriate choice for characterizing the relationship observed in eggs subjected to the PPO+CO_2 treatment. This conclusion is supported by its notably high R^2 (0.990), along with the low RMSE (4.378) and SSE (76.65).

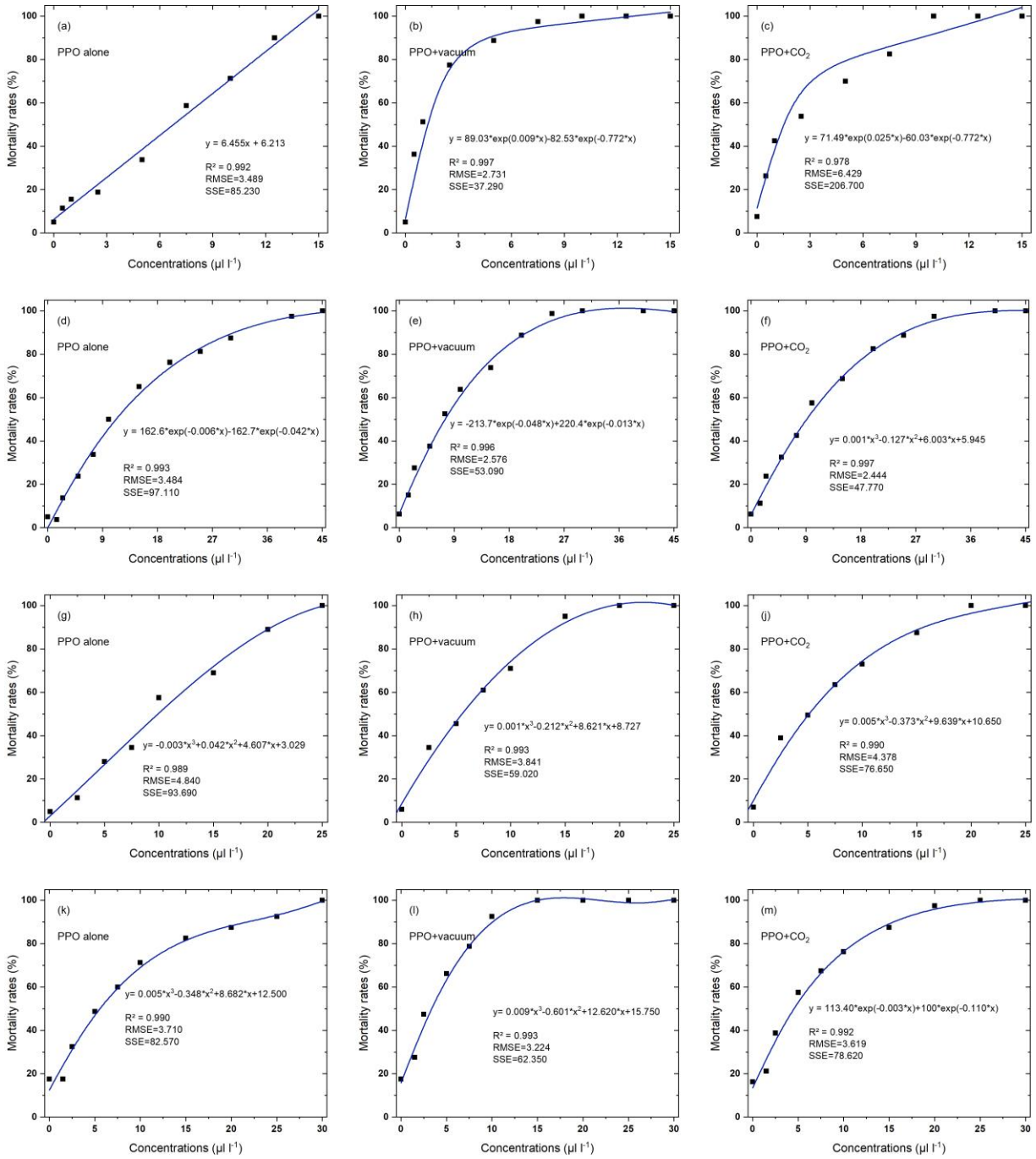

Figure 2. The fitted regression models for (a-c) adults, (d-f) larvae, (g-j) eggs, and (k-m) pupae of *Plodia interpunctella* for different PPO applications.

Şekil 2. Farklı PPO uygulamaları için Plodia interpunctella'nın (a-c) erginleri, (d-f) larvaları, (g-j) yumurtaları ve (k-m) pupaları için uygun regresyon modelleri.

DISCUSSION AND CONCLUSION

It is well known that PPO has shown promising results in controlling stored-product insect pests (Isikber et al., 2017; Navarro et al., 2004). Nevertheless, it is crucial to note that the effectiveness of commercial fumigation is subject to various factors, encompassing environmental conditions during treatment, the nature of the treated product, and the performance of the equipment involved. These factors play a pivotal role in determining the overall success of the fumigation process. Therefore, it is essential to evaluate the insecticidal efficacy of PPO and its ability to thoroughly penetrate the product, as this information is of

great importance for the wide commercial application of PPO gas in the food industry. In this context, this study focuses on investigating the insecticidal efficacy of PPO in three different treatments (PPO alone, PPO+Vacuum, and PPO+CO_2) against all biological stages of *P. interpunctella*, using dried figs as the test substrate.

Statistically significant differences were observed in the mortality rates between different biological stages of *P. interpunctella* when exposed to PPO alone, PPO+Vacuum, and PPO+CO_2 treatments. The test results showed clear trends: the highest mortality rates were observed at concentrations of 15 µl l^{-1} for adults, 45 µl l^{-1} for larvae, 25 µl l^{-1} for eggs, and 30 µl l^{-1} for pupae. The results suggest that *P. interpunctella* larvae and pupae exhibit greater resistance to PPO treatments overall, whereas *P. interpunctella* adults and eggs show higher sensitivity to these treatments. The order of tolerance from the most resistant to the most susceptible life stage for PPO treatments was as follows: larvae > pupae > eggs > adults. These results are consistent with previous research by Isikber et al. (2012), who also reported that *P. interpunctella* eggs and larvae had greater resistance to PPO+Vacuum treatments, underscoring the consistency of these results. Also, Isikber et al. (2017) reported similar trends in *T. castaneum*, where eggs exhibited the highest sensitivity to PPO with an LD_{99} value of 30.1 mg L^{-1} for a 4-hour exposure, while pupae were the most tolerant, with an LD_{99} value of 146.5 mg L^{-1}. These findings highlight the exceptional efficacy of PPO against *P. interpunctella* eggs, particularly within very brief exposure periods. This bears significance due to the inherent difficulty in managing stored product insect eggs using conventional fumigants and contact insecticides, often necessitating prolonged exposure durations for effective control.

Based on the findings from all bioassays of this study, it was determined that the PPO+Vacuum and PPO+CO_2 resulted in a pronounced increase in toxicity against all life stages of *P. interpunctella*. Isikber et al. (2001) assessed the efficacy of PPO alone, PPO+Vacuum, and PPO+CO_2, during a brief exposure period of 4 hours on all life stages of *T. castaneum*. Their research revealed that PPO+Vacuum and PPO+CO_2 exhibited heightened insecticidal activity across all life stages of *T. castaneum*, except for eggs, as compared to the application of PPO alone. Zettler et al. (2003) observed that the use of PPO in combination with CO_2 demonstrated effectiveness against seven distinct stored-product insect pests, which encompassed *P. interpunctella*, *T. castaneum*, *Tribolium confusum* Duv. (Coleoptera: Tenebrionidae), *Trogoderma variable* Ballion (Coleoptera: Dermestidae), *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae), *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae), and *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae), thus affirming its broad-spectrum utility.

This study is noteworthy for its pioneering use of regression methods to model insect mortality, and it paves the way for a better understanding of the relationship between mortality and PPO concentrations. These findings provide valuable insights that can empower scientists and practitioners to potentially develop more effective and targeted strategies for pest management and quarantine procedures.

In the test results, the recommended and effective doses were found to be 15 µl l-1 for adults, 45 µl l-1 for larvae, 25 µl l-1 for eggs and 30 µl l-1 for pupae. The results of this study indicate that PPO is a promising substitute for MeBr to rapidly eliminate insect infestations in dried figs. This study is a basic study and it is clear that with the involvement of different disciplines, this study will gain importance and provide practical results. In this way, the potential of the fumigant used in our study will be fully revealed and the possibilities of its use will be better understood. To determine the insecticidal efficacy of this fumigant against stored dried fig pests, studies on different types of food and different pests should be supported. In addition, it is undoubtedly important to develop and apply this study in large-scale commercial trials under warehouse and grain silo conditions

CONFLICT OF INTEREST

The authors assert that they have no conflicts of interest related to the content presented in the article.

DECLARATION OF AUTHOR CONTRIBUTION

Conceptualization, Y.K. and A.A.I.; methodology, Y.K.; software, Y.K.; validation, Y.K.; formal analysis, Y.K.; research, Y.K.; sources, Y.K. and A.A.I.; data curation, Y.K.; writing-original drafting, Y. K.; writing-review and editing, A.A.I.; visualization, Y.K.; supervision, A.A.I.; project management, Y.K. and A.A.I. All authors have reviewed and approved the final published version of the article.

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