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Optimizing phosphine fumigation efficiency in hazelnut industry: Determining optimal exposure time for stored product pest control



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Abstract

Hazelnut, as with many other stored products, are susceptible to infestation by a variety of stored insect pests. Phosphine fumigation is a widely used method to control pests in stored products, including hazelnut kernels. This study aimed to determine the optimal exposure time for phosphine fumigation for management of stored product pests in hazelnuts. Four treatments with different exposure times (3, 4, 5, and 6 days) were conducted using various development stages of Oryzaephilus surinamensis (L.) (Coleoptera: Silvanidae), Tribolium confusum Jagcquelin du Val, (Coleoptera: Tenebrionidae), Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae), Ephestia kuehniella Zeller (Lepidoptera: Pyralidae), and Plodia interpunctella (Hübner) (Lepidoptera: Pyralidae) together with the control group. The trials were conducted in a commercial chamber of a hazelnut processing facility. The insects were placed in plastic containers within jute sacks filled with hazelnuts, fumigations were done under gasproof sheet and the survival rate was assessed after treatments. The results showed that a 3-day exposure period was sufficient to fully eradicate the pupal and adult stages of O. surinamensis. For T. castaneum, 100% mortality was achieved in both larvae and adults from 3 days of exposure, but the pupal stage required at least 5 days. For T. confusum, all larvae and adults died in all exposure periods, but the pupal stage required at least 4 days. In the case of the moth species, a 100% mortality rate was achieved in the larval and pupal stages of both E. kuehniella and P. interpunctella at all exposure periods. The mortality rate of E. kuehniella eggs was 99% after 3 and 4 days of exposure, and a fumigation period of 5 days was required to control the entire population. However, only 67% of P. interpunctella eggs were controlled after 3 days of exposure. The time and stage factors were found to be significant in the egg stage of P. interpunctella. The results suggest that a 5-day exposure period is the most effective for controlling tested stored product pests in hazelnuts.

Keywords: Hazelnut, Phosphine, Exposure period, Stored product pests

INTRODUCTION

Phosphine is a common utilized fumigant for the control of stored product pests, which can cause significant economic losses by contaminating and damaging stored grains, dry fruits, nuts, and other harvested commodities. The use of phosphine is in demand for stored product pest control, due to its low cost, versatility, high efficiency, free from toxic residue, easy accessible and use. However, the effectiveness of phosphine in controlling stored product pests dependent upon a number of factors, including the concentration of phosphine, the temperature and humidity of the storage environment, and the durations of exposure (Daglish et al., 2002).

Exposure time is a crucial factor that can influence the effectiveness of phosphine in pest control. Long-term application periods are generally more effective for controlling stored product pests (Hole et al., 1976), as enables the phosphine to reach all areas of the storage facility and ensure that pests are exposed to lethal concentrations of the fumigant. Previous studies have also demonstrated that longer exposure durations are more effective than the application of high concentrations over shorter periods (Bell, 1976; Ahmedani et al., 2007; Fukazawa & Takahashi, 2017; Agrafioti et al., 2020). This is because high concentrations may induce narcosis in pests, which can reduce the toxic effect of phosphine by decreasing metabolism (Bell, 1979; Winks, 1985; Chaudhry, 2000). In addition, one of the important reason for development of phosphine resistance is short exposure time and the cost of the treatment increased with longer exposure times. Therefore, it is important to balance the need for prolonged exposure with the need to minimize the risk of phosphine resistance and the cost of treatment (Athanassiou et al., 2016; Chadda, 2016). In the hazelnut processing industry, where time is a limited factor, the goal is to achieve a pest-free product in the shortest possible time due to their intensive production plans.

Studies have examined the effect of various exposure times on the mortality of stored product pests treated with phosphine. For instance, Ahmedani et al. (2007) found that increasing the exposure time of phosphine from 1 to 5 days resulted in a significant increase in the mortality of Trogoderma granarium Everts (Coleoptera: Dermestidae) larvae, with 100% mortality achieved at the 5-day exposure duration. Another study by Bell et al. (1984) found that complete mortality of diapausing T. granarium larvae was achieved in a 4-day exposure period with phosphine, with the exposure time required for 100% mortality increasing to 7 days in proportion to the age of the diapausing larvae. Collins et al. (2005) determined that different exposure times were necessary to reach 99.9% mortality (LT_{99.9}) of Rhyzopertha dominica (Coleoptera: Bostrichidae) adults at varying phosphine concentrations. Minimum and maximum LT_{99.9} were determined to be between 5 and 8.8 days, depending on the concentrations of phosphine in susceptible strains, while $LT_{99,9}$ was between 3.7 and 14 days in strong resistant strains.

Effective fumigation requires adherence to established practices and procedures to ensure that pests are fully eliminated. Even a mortality rate of 90% after fumigation is considered a failure and a mortality rate of over 99.9% is generally required (Hole et al., 1976). Poor fumigation methods may only kill visible pests, but leave behind stages that can survive and quickly reproduce, leading to a new infestation (Chadda, 2016). Additionally, the efficacy of fumigation depends on the developmental stage of the species. Immobile stages exibiting reduced

metabolism may experience an increased likelihood of survival during phosphine fumigation (Hole et al., 1976; Rajendran et al., 2001; Chadda, 2016). As a result, the optimal and successful fumigation procedure should provide complete mortality in all stages of the species within the planned exposure period.

Hazelnuts, like many other stored commodities, are prone to pest infestations during the postharvest period (Hagstrum et al., 2013). Several insect species, including Cadra cautella (Walker), Ephestia kuehniella Zeller, Plodia interpunctella Hübner (Lepidoptera: Pyralidae), Tribolium castaneum (Herbst), Tribolium confusum Jacqueline du Val (Coleoptera: Tenebrionidae), Oryzaephilus surinamensis (L.), and Oryzaephilus mercator (Fauvel) (Coleoptera: Silvanidae), commonly infest stored hazelnuts as well as other dried fruits and nuts (Alkan, 1959; Yasan & Kiper, 1972; Ozman-Sullivan et al., 2009; Hagstrum et al., 2013; Moraglio et al., 2018). The control of these stored product pests in the hazelnut industry is crucial for preserving the quality and value of the nuts. One of the primary objectives of the hazelnut industry is to identify the most efficient and cost-effective method that achieves the desired level of mortality. Hence, there is a necessity to optimize fumigation operations in order to strike the perfect balance between effectiveness and duration. This study aimed to establish the most time-efficient duration for phosphine fumigation for the hazelnut industry. To accomplish this, trials were conducted involving five prevalent stored hazelnut pests and various developmental stages of these pests.

MATERIALS AND METHODS

Insect rearing and handling of stages

A total of five species including Oryzaephilus surinamensis, Tribolium confusum, Tribolium castaneum, Ephestia kuehniella, and Plodia interpunctella were used in the experiments. The coleopteran species were reared in 1-liter glass jars with different food sources. A diet consisting of five parts oatmeal, five parts cracked wheat, and one part dry yeast was provided to O. surinamensis, while T. confusum and T. castaneum were given a diet of ten parts wheat flour and one part dry yeast. The jars were covered with fine muslin cloth to allow for aeration. Plastic containers (1.2 liter) used for mass rearing of the lepidopteran species. E. kuehniella was provided with a diet of ten parts wheat bran, half a part wheat flour, and a quarter part corn flour, while P. interpunctella was reared on mixture of wheat bran (90 g), dry yeast (10 g), honey and water mixture (18 g), glycerol (46 g), sunflower oil (3 g), and water (15 ml). Once adults emerged, they were daily removed to copulation cages. All jars and containers were kept in a growth chamber at a temperature of 26 °C, 65%±5 relative humidity, and a 16:8 photoperiod (light:dark). Mass production of the species has been continuously ongoing for more than three years in the Laboratory of Entomology, Department of Plant

Protection, at Ordu University in Türkiye.

In the experiments, the pupae (0-24 hours old) and adults (0-15 days old) of *O. surinamensis* were collected for use. To obtain young pupae, mature larvae were sieved and separated from the stock culture, and the pupae were collected the following day. In the case of *T. confusum* and *T. castaneum*, mature larvae (5-6th stage), pupae (0-24 hours old), and adults (0-15 days old) were collected by sieving, and the age of the stages was equalized. In the lepidopteran species, eggs (0-24 hours old), mature larvae (4-5th stage), and pupae (0-24 hours old) were collected for the trials. All stages of individuals were handled with a fine brush.

Chemical and fumigation chamber

Fumigation trials were conducted under gas-tight polyethylene sheeting in chamber of a commercial hazelnut processing facility in Ordu (Sagra Grup Gıda Üretim ve Tic. A.Ş., Ordu, Türkiye). Aluminum phosphide (Detia Gas EX-B, 57% DP, bag) was used to generate phosphine. An average of circa 20 tons of raw (shelled) hazelnuts, placed inside jute sacks, were transferred to the chamber on pallets. One bag of aluminum phosphide (34 g) per 11 m³ was applied in all treatments as a registered dose. A total of 230-260 jute sacks of hazelnuts were fumigated under sheeting in a heated chamber, with a calculated volume of 85-88 m³ (eight bags, 272 g aluminum phosphide used). In all trials, temperature and humidity were recorded as 20-25 °C, 60%±15 relative humidity, using a Hobo data logger (Onset, USA).

Fumigation trials

The studies consisted of four treatments in which fumigations were conducted at four different exposure times including 3, 4, 5 and 6 days. We used five replications for different stages of insects. Then, a total of 50 pupae and 100 adults of O. surinamensis; 50 larvae, 50 pupae and 100 adults of T. confusum and T. castaneum; 150 eggs, 100 larvae, and 50 pupae of E. kuehniella and P. interpunctella were exposed in each time unit. The insects were placed with 30 cc cylindrical plastic containers inside the jute sacks. Three holes (1 cm diameter) were opened on the lid of the containers and covered with muslin cloth. Additionally, pinholes were opened on the sides of the containers to maximize the penetration of gas. For the feeding stages of the insects, a food supply (identical to the rearing diet) was included in the containers. To ensure the same exposure conditions in each treatment, the containers of replications were placed in same pattern. The gas-proof sheet was closed and surrounded by sandbags to prevent air escape. After reaching the targeted exposure time, the chamber and sheeting were opened and ventilated for 24 hours. The plastic containers were then transferred to the laboratory and kept in test cabinets with the same conditions as previously mentioned. The hole procedure was implemented for the control group, which was not exposed to phosphine in same conditions. After 24 h, the survival of the insects was evaluated by disturbing them with a brush. Adults and larvae that exhibited no observable movement were recorded as dead. Eggs and pupae that failed to hatch after 15 days were also classified as dead.

Statistical analysis

Statistical analysis was performed by generalized linear model (GLM) with a binomial distribution (i.e., dead or alive) with a logit link function. The Wald Chi-squared test was used to assess statistical significance of the fixed factors i.e. time (length of exposure), pest species and their stages. If significant, post-hoc pairwise comparisons based on estimated marginal means were performed using the least significant difference (LSD) test (P<0.05). All analysis was performed using the R software (R Core Team, 2022) with the following packages: brgIm2 (Kosmidis et al., 2020) for bias reduction in GLM, car (Fox & Weisberg, 2011) for analysis of GLM, and emmeans (Lenth, 2022) for pairwise comparisons.

RESULTS

The mortality rate of control groups in all species was below 5% (Table 1), therefore, there was no need to correct the mortality rate. Mortality increased with the length of exposure and varied among the insect species and development stages. [species (Wald X^2 =13.76, d.f.=4, P=0.0081); time (Wald X^2 =34.80, d.f.=3, P<0.0001); stage (Wald X^2 =8.60, d.f.=3, P=0.0351)].

Both the pupal and adult stages of *O. surinamensis* were fully eradicated in fumigations even after just 3 days of exposure (Figure 1).

In *T. castaneum*, there were no significant differences found for either time (Wald X^2 =2.37, d.f.=3, *P*=0.4984) or stage (Wald X^2 =4.21, d.f.=2, *P*=0.1220). The mortality rate of both larvae and adults reached 100% starting from 3 days of phosphine exposure, while mortality rates were 96% in 3-day exposure and 98% in 4-day fumigation in the pupal stage (Figure 2). Total mortality (100%) were observed after 5-day exposure (Figure 2). There was no significant difference between mortality rates in pupal stages of *T. castaneum* within the exposure times (Wald X^2 =1.99, d.f.=3, *P*=0.5743).

Similarly, no significant differences were found in either exposure times (Wald X^2 =3.15, d.f.=3, P=0.3686) or developmental stages (Wald X^2 =3.14, d.f.=2, P=0.2083) for *T. confusum*. Complete mortality was observed in all larvae and adults of *T. confusum* across all exposure periods (Figure 3). However, 3-day exposure duration was found to be insufficient for complete control of the pupal stage. Achieving 100% mortality required a 4-day exposure time (Figure 3). No significant difference was observed in the mortality rates of *T. confusum* pupal stages across the different exposure times (Wald X^2 =2.71, d.f.=3, P=0.4384).

Species	Duration (d)	Mortality (%)			
		Egg	Larvae	Pupae	Adult
Tribolium confusum	3	-	0	0	0
	4	-	0	0	1
	5	-	0	0	0
	6	-	0	2	0
Tribolium castaneum	3	-	2	0	0
	4	-	4	2	0
	5	-	0	4	0
	6	-	2	0	0
Oryzaephilus surinamensis	3	-	-	2	2
	4	-	-	4	2
	5	-	-	2	4
	6	-	-	0	2
Ephestia kuehniella	3	4	0	4	-
	4	1.33	0	2	-
	5	2	0	2	-
	6	1.33	0	2	-
Plodia interpunctella	3	2	0	0	-
	4	0.44	0	4	-
	5	2.67	0	0	-
	6	1.33	0	2	-

Table 1. The mean percentage mortality of the control group of the treatments.

Oryzaephilus surinamensis



Figure 1. Mortality rate (%) (mean ± S.E.) of the pupae and adult stages of the *Oryzaephilus surinamensis* within the different phosphine exposure times



Tribolium castaneum





Tribolium confusum

Figure 3. Mortality rate (%) (mean ± S.E.) of the larvae, pupae and adult stages of the *Tribolium confusum* within the different phosphine exposure times



Figure 4. Mortality rate (%) (mean ± S.E.) of the egg, larvae and pupae stages of the *Ephestia kuehniella* within the different phosphine exposure times



Plodia interpunctella

Figure 5. Mortality rate (%) (mean \pm S.E.) of the egg, larvae and pupae stages of the *Plodia interpunctella* within the different phosphine exposure times. (GLM, binomial distribution, Wald χ^2 test followed by LSD post hoc multiple comparison test, * *P*<0.05)

Fumigation trials resulted in a 100% mortality rate in the larval and pupal stages of *E. kuehniella* all exposure periods (Figure 4). There were no significant differences identified in either time (Wald X^2 =1.19, d.f.=3, *P*=0.7543) or stages (Wald X^2 =0.89, d.f.=2, *P*=0.6415) for *E. kuehniella*. The mortality rate of eggs of *E. kuehniella* was 99% after 3 and 4 days of exposure, and no significant difference were found in mortality of eggs among the exposure periods (Wald X^2 =1.03, d.f.=3, *P*=0.7949). A fumigation period of 5 days was required to control the entire population (Figure 4).

Significant differences were found between exposure times (Wald X^2 =80.93, d.f.=3, P<0.0001), and developmental stages of *P. interpunctella* (Wald X^2 =9.65, d.f.=2, P=0.0080). Throughout all exposure periods, complete mortality was observed in all larvae and pupae of *P. interpunctella* (Figure 5). However, the mortality rate of *P. interpunctella* (Eigure 5). However, the mortality rate of exposure. Subsequently, the mortality rate increased to 97% after 4 days of exposure, and reached 100% after 5 days of fumigation, demonstrating a statistically significant difference between exposure periods (Wald X^2 =80.74, d.f.=3, P<0.0001).

DISCUSSION

Phosphine fumigation was effective in controlling the entire population of O. surinamensis at all exposure periods. Both the pupal and adult stages were affected equally. In line with our findings, Hole et al. (1976) also reported that at 25 °C, O. surinamensis was the most susceptible species to phosphine fumigation in all exposure periods (2, 4, and 7 days) when compared to other twelve stored product insect species. They found that at the 7-day exposure period, much lower concentrations were needed to kill 100% of the insect species compared to shorter exposure periods. At this point, the needed phosphine concentration for absolute control of O. surinamensis was 0.013 mg/l, while it was 0.32 for the weevil species. In the ambient temperatures similar with our study (20-25 °C), Ferizli et al. (2007) found that 5 days of exposure duration is required for complete control of the egg, larvae, pupae, and adult stages of O. surinamensis and C. cautella. In another study, Athanassiou et al. (2016) also found that in trials of phosphine fumigation at low pressure and 48 hours of exposure, 100% mortality was only observed in O. surinamensis adults among the seven species of stored product pests. The species with the next highest mortality was T. confusum larvae, with 99.8% mortality in the 48 hours of exposure to low-pressure fumigation. They found that the maximum mortality was 75.6% in the pupal stage of *T. confusum* at all exposure periods. Although the fumigation was performed under normal air pressure in our study, we were unable to achieve 100% control at 3 days of exposure and required at least 4 days of exposure. We found 100% mortality in other stages such as larvae and adults in 3 days of exposure

period and above. This finding supports the work of Aulicky et al. (2015) in which they also reported the absolute effectiveness of 3 days of exposure on adults and larvae. Our results for *T. castaneum* were similar to those for *T. confusum* for adults and larvae. Additionally, the pupal stage could not be 100% controlled at 3 days of exposure period in *T. castaneum*, and a longer (5 days) exposure duration was needed to kill all the pupae. Winks, (1984) also indicated that the eggs and pupal stages of *T. castaneum* are more tolerant and absolute control requires longer exposure times.

The biological stage of an insect is recognized as a crucial factor influencing the effectiveness of phosphine fumigation. Previous studies have indicated that low metabolism stages, such as early eggs and pupae, tend to enter a state of inactivity or "switch off" during exposure to phosphine for certain periods (Bell, 1976; Winks, 1984; Chaudhry, 1997). However, the development of tolerant stages continues, and they soon advance to the next susceptible stage (Bell, 1979; Nakakita & Winks, 1981; Winks & Waterford, 1986; Chadda, 2016). Therefore, longer exposure periods are required to target these insect stages. Bell (1976) reported that eggs of *E. kuehniella* and *P. interpunctella* were tolerant to phosphine fumigation. The study revealed that to achieve complete control of E. kuehniella eggs, 3 and 4 days of exposure were necessary at 25 °C and 20 °C, respectively. Similarly, at these same temperature conditions, 3 and 5 days of exposure were required for *P. interpunctella* eggs. This finding aligns with our own research, where we also observed that a 5-day exposure to phosphine at 20-25 °C resulted in 100% control of both E. kuehniella and P. interpunctella eggs. Temperature plays a crucial role in reducing exposure times. For instance, Phillips et al. (1999) reported that P. interpunctella eggs achieved 100% mortality after only 2 days of exposure at 32 °C, whereas it took 6 days of exposure at 5 °C to achieve the same mortality rate. However, exposing higher temperatures can influence the quality of the hazelnuts (Guiné et al., 2015).

The age of the eggs of moths during exposure to phosphine is important, as older eggs are more susceptible due to metabolism during embryonal development (Bell, 1976)., young eggs (1 day old) of E. kuehniella and P. interpunctella survived until they were older (4 days old). However, when experiments were conducted with older (2-4 days) eggs, 100% mortality was achieved even at a 24-hour exposure period (Bell, 1976). Additionally, Mbata et al. (2004) also found that in the low pressure applications the required exposure time decreases as the egg age of P. interpunctella increases. Based on these findings, we suppose that, as we used 0-24 hour old eggs of P. interpunctella in our trials, they were much more tolerant to phosphine until they reached nearly hatching age, which is around 4-7 days (Mbata & Osuji, 1983).

The optimum exposure time also depends on the

susceptibility of the pest strain to phosphine. In the control of resistant strains, exposure duration is more critical than the concentration of phosphine (Chaudhry, 1997; Daglish, 2004). Based on earlier studies, the required exposure period increases when resistant populations are present (Rajendran et al., 2001; Collins et al., 2005; Fukazawa & Takahashi, 2017). Even under high concentrations (3000 ppm) of phosphine, the percentage of immobilized adults of resistant R. dominica, O. surinamensis, and T. castaneum strains increases with exposure duration, while susceptible populations are 100% affected after the shortest exposure time (Lampiri et al., 2021). In our study, we did not determine the phosphine-resistant conditions of the population used in the trials. However, there was no indication of resistance based on the mortality data of the species. But, we determined the endurance of the immobile stages. Only the eggs and pupae stages of the species were not fully controlled at the shortest exposure periods, and longer times were required.

CONCLUSION

In conclusion, the study found that phosphine fumigation is an effective method for controlling stored hazelnut pests, with a mortality rate of 100% in all species tested. O. surinamensis was fully eradicated in fumigations even after just 3 days of exposure, with no significant differences found for either time or stage factors. Similarly, T. castaneum and T. confusum also had a high mortality rate, but longer exposure periods (min. 5 days) were required to completely control the pupal stage. In the case of moth species, the study found that fumigation resulted in a 100% mortality rate in the larval and pupal stages of both E. kuehniella and P. interpunctella at all exposure periods. However, in the egg stage of P. interpunctella, the time and stage factors were found to be significant and 5 days of exposure period was required to control the entire population.

Hazelnuts are commonly fumigated prior to transfer to warehouses or before processing. The fumigation implementations must be fully successful due to the phosphine doesn't have an insecticidal residual effect. Our results indicate that the most effective phosphine fumigation duration for jute sack-stored hazelnuts is 5 days of exposure. We believe that, this optimization of hazelnut fumigation duration is crucial for hazelnut processing facilities and will enhance their ability of management with stored product pests. However, phosphine resistance should be investigated before the management to ensure the effectiveness.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

ESY is presently employed as Food Engineer at Sagra Grup Gida Üretim ve Tic. A.Ş. The remaining authors declare no apparent conflicting financial interests or personal associations with the aforementioned company that could potentially influence the findings presented within this manuscript.

Author contribution

AG: conceptualization, data curation, formal analysis, resources, writing - review & editing, YEA: conceptualization, investigation, data curation, formal analysis, writing draft - review & editing, \$K: investigation, TNB: investigation, ESY: conceptualization, resources

Ethical approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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