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THE EFFECT OF PHOSPHINE APPLICATIONS ON MORTALITY OF THE SAWTOOTH GRAIN BEETLE, *ORYZAEPHILUS SURINAMENSIS* (L) (COLEOPTERA: SILVANIDAE)

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ABSTRACT

In this research, the toxicity of phosphine (PH_3) at 200 ppm was evaluated for different exposure periods against eggs (0-24, 24-48, 48-72 h old), larvae (15 d), pupae (0-24, 24-48 h) and adults (7-14 d) of the saw tooth grain beetle, *Oryzaephilus surinamensis*. All experiments were carried out at 30ºC and at 65% r.h. Mortality rates for different exposure periods were subjected to probit analysis and LT_{99} values were calculated. The results showed that the LT_{99} values for 0-24, 24-48, 48-72 h old eggs were 13.282, 9.216 and 8.536 h respectively at the 200 PH_3 ppm concentration. The LT_{99} values for 0-24 and 24-48 h pupal stages were 13.401 and 18.837 h respectively, while the LT_{99} value for the adult stage was 3.876 h. A 45 min exposure period was found sufficient to obtain complete mortality of the larval stage in both applications. The study showed that the larval stage was the most sensitive stage of *O. surinamensis* to PH_3 and the pupal stage was the least sensitive.

INTRODUCTION

Turkey is one of the most important dried fruit and nut producing and exporting countries (figs, raisins, apricots, hazelnut, pistachio etc.). The saw-tooth grain beetle *Oryzaephilus surinamensis* is one of the main pests of dried fruits in Turkey. Because of their small size, they easily enter packaged foods and can cause major infestation problems in stores and in dried fruit processing plants. Both adults and larvae are considered pests since they both live on the same food source. Most of the Turkish processing and packinghouses are located in the Izmir and Aydin provinces of the Aegean Region. Insect infestation of dried fruits poses a constant problem since the Aegean region experiences a warm and moist climate conducive to insect population growth.

Methyl bromide (MB) had been used for many years in Turkey to disinfest the storage pests which infest dried fruits in the processing and storage houses. However,

MB is already banned from use in post harvest sector in Turkey. The only chemical alternative to MB presently available in Turkey is phosphine. But MB takes a short time to disinfest the product, whereas, phosphine is characterised as a slow acting fumigant to which insects can develop resistance. The aim of this study with phosphine, was to determine the exposure times needed to give an acceptable level of control for all the life stages of *O. surinamensis*. Consequently, the temperature and concentration in the experiments were chosen to mirror the climate of the region and the practice of the storage authorities.

MATERIALS AND METHODS

Culture and preparation of test insects

A laboratory colony of *O. surinamensis* maintained in a rearing room at 25±1ºC and 65±5% relative humidity (r.h.) was used for these experiments. This species was reared on a mixture of 90% broken wheat, 5% yeast and 5% glycerol (by weight). For the experiment, 0-1, 1-2, and 2-3 day-old eggs were used. Eggs were obtained by placing 500–1000 adults beetles in 100 g of wheat flour containing 5 g of brewers' yeasts. After 24 h the eggs were separated from the flour and the adults by sieving using US standard sieves mesh #35 and #100. The study was replicated three times on 35 eggs per application and exposed in 20 ml glass vials covered with fine-mesh tops.. Mortality was determined 8 days after the gas application.

Larvae were separated from the rearing medium at the 15th day after hatching. For this stage, 20 larvae were placed in a vial containing 2 g rearing diet. Experiments were replicated 5 times. Mortality was determined 3 days after application.

Pupae were separated from rearing media on a daily basis. For the experiment, 0-1 and 1-2 day-old pupae were used. For this stage, 15 pupae were placed in vials containing 2 g rearing diet. Experiments were replicated 3 times. Mortality was determined 8 days after application.

For the adult stage, 7-14 day-old individuals were separated from the rearing medium. In the experiments, 25 adults were placed in a vial containing 2 g rearing diet. Experiments were replicated 5 times. Mortality was determined 5 days after application.

Fumigation procedure

The fumigation studies were conducted at a fixed concentration of 200-ppm phosphine. All tests were carried out at 30±1ºC and 65±5% relative humidity. Eggs (0-1, 1-2, and 2-3 d-old), larvae (15 d-old), pupae (0-1 and 1-2 d-old), and adults (7-14 d-old) of *O. surinamensis* were exposed to a fixed gas concentration (200 ppm) of phosphine in a recirculatory apparatus consisting of four flasks, over a range of exposure periods, from 0 min (control) to 18 h. The recirculation apparatus was set

up to provide small fumigation chambers for one concentration and four exposure periods. The flasks were connected together to a small electric pump, which was set up to re-circulate the gas evenly throughout the apparatus (Hasan and Reichmuth, 2004). Phosphine gas was generated in a gas burette by reacting a 0.6 g pellet of aluminium phosphide formulation with 5% sulphuric acid (Anonymous, 1975). The required quantity was drawn from a gas burette using a gas-tight syringe. After dosing, the phosphine concentrations were checked in the re-circulatory apparatus at start of an exposure using a Bedfont phosphine monitor fitted with a built-in suction pump for sampling the gas. The sample gas was circulated back into the chambers. At the end of the exposure, insect vials were taken out and transferred to a desiccator kept at 30±1ºC and 65±5% relative humidity until observations of mortality were carried out.

Mortality data were corrected using Abbott's formula (1925), and then subjected to probit analysis using the POLO PC program (LeOra software, 1994) and LT_{50} and LT_{50} values were calculated.

RESULTS AND DISCUSSION

Phosphine Toxicity to eggs

Figure 1 shows that various ages of the egg stage of *O. surinamensis* responded differently to phosphine. Mortality increased with extended exposure period. Eggs aged 0-1 days were found to be more tolerant than 1-2 or 2-3 day-old eggs. Complete mortality was achieved in an 18 h application of PH_3 for 0-1 d-old eggs, in 12 h for 1-2 d-old eggs and in 9 h for 2-3 d-old eggs. Results of probit analysis are given in Table 1. Calculated LT_{50} levels for 0-1, 1-2 and 2-3 d-old eggs were found to be 8.60, 4.13, and 3.43-h respectively. These findings indicated that 0-1 d-old eggs were more resistant to PH_3 than 1-2 and 2-3 d-old eggs.

TABLE 1
The effect of phosphine (200 ppm) on mortality values for the different egg stages of *Oryzaephilus surinamensis* at 30±1ºC and 65±5% r.h

Age	χ^2	D.f.	Slope ±SE	LT_{50}	LT_{99}
0 -24	11.6	7	12.34±2.14	8.60 (7.50-9.20)*	13.28 (11.65-19.74)
24-48	3.1	6	6.67±0.68	4.13 (3.75-4.44)	9.22 (8.28-10.73)
48-72	1.9	5	5.87±0.60	3.43 (3.08-3.71)	8.54 (7.57-10.12)

*Fiducial limit were calculated at P≤0.05

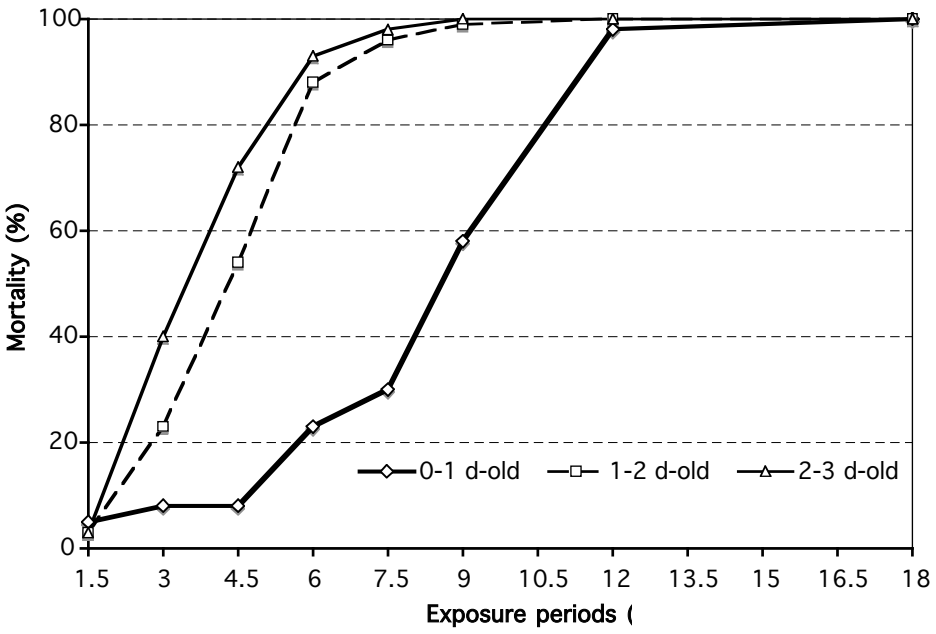

Figure 1. Mortality of 0-1, 1-2 and 2-3 d-old eggs of O.surinamensis following application of 200 ppm of phosphine for different exposure periods at 30±1°C and 65±5% r.h.

Toxicity on larvae

Figure 2 shows the response of 15 d-old larvae of *O. surinamensis* to phosphine. Results presented in the figure show that larvae were very susceptible to phosphine. Complete mortality was achieved in 45 min. application of PH_3 (Fig. 2). The series of exposure periods–response data obtained from these experiments were not suitable for probit analysis so that LT_{50} values could not be calculated.

Toxicity on Pupae

Results presented in Fig. 3 show the relative toxicity of phosphine to pupae of *O. surinamensis*. Mortality increased with the extended exposure period. There were no clear differences between 0-1 and 1-2 d-old pupae in their response to phosphine. Complete mortality was achieved after 12 h application of PH_3 for 0-1 and 1-2 d-old pupae. Results of probit analysis are given in Table 2. Calculated LT_{50} levels for 0-1 and 1-2 d-old pupae were found to be 2.41 and 3.05-h respectively.

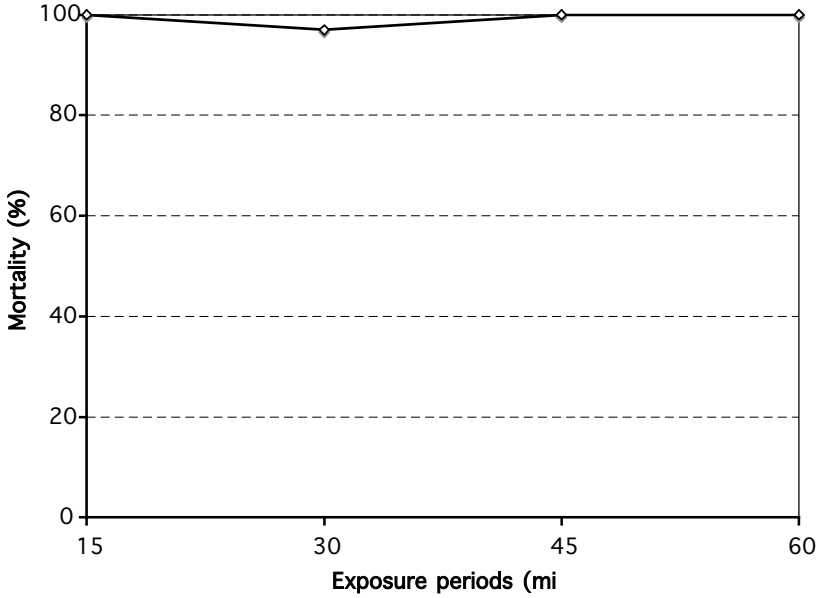

Figure 2. Mortality of larvae (15 d-old) of *O. surinamensis* following application of 200 ppm of PH_3 for different exposure periods at 30±1°C and 65±5% r.h.

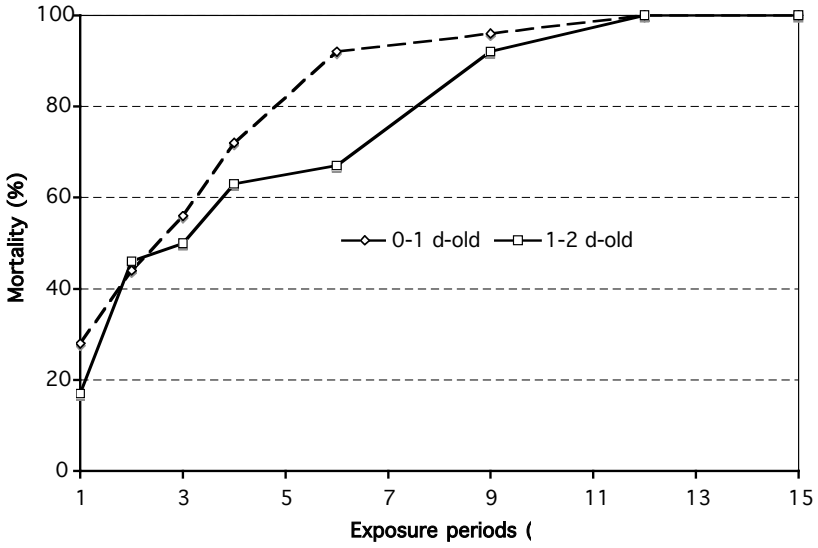

Figure 3. Mortality of 0-24 and 24-48 h old pupae of *O. surinamensis* following application of 200 ppm of PH_3 for different exposure periods at 30±1°C and 65±5% r.h.

TABLE 2
The effect of phosphine (200 ppm) on mortality as expressed in LT (hours to obtain % of mortality) values for for 0-1 and 1-2 d-old pupae of *Oryzaephilus surinamensis* at 30±1ºC and 65±5% r.h

Age	_^{2}	D.f.	Slope ±SE	LT_{50}	LT_{99}
0 -24	3.3	8	3.12 ±0.68	2.41 (1.25-3.29)*	13.40 (9.41-29.29)
24-48	7.0	8	2.94 ±0.60	3.05 (1.65-4.18)	18.84 (13.08-39.85)

*Fiducial limit were calculated at P≤0.05

Toxicity on Adults

Results presented in Fig. 4 show the relative toxicity of phosphine to the adult stage of *O. surinamensis*. Mortality increased with the extended exposure period. Complete mortality was achieved after 4 h application of PH_3. Results of probit analysis are given in Table 3. Calculated LT_{50} level for the adult stage was found to be 1.36-h.

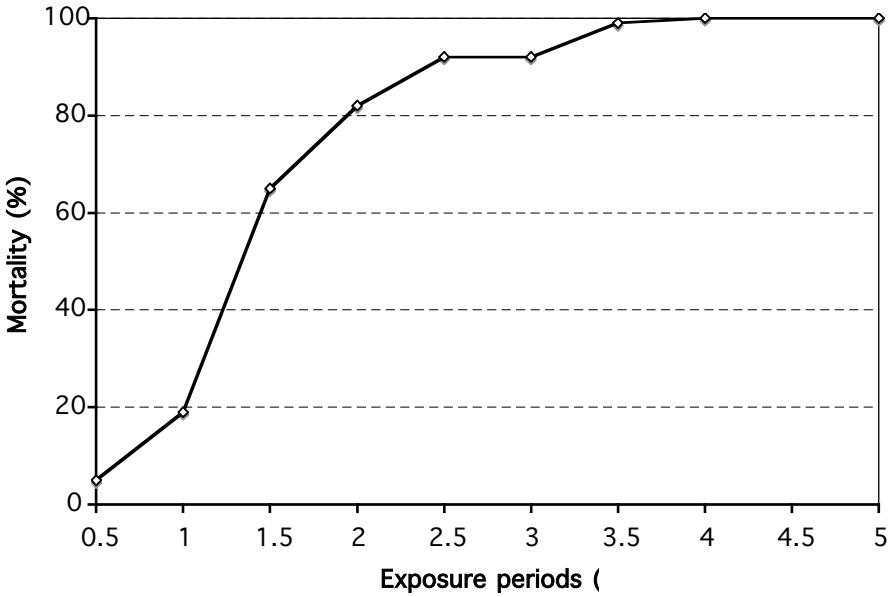

Figure 4. Mortality of 7-14 d-old adults of *O. surinamensis* following application of 200 ppm of PH_3 for different exposure periods at 30±1ºC and 65±5% r.h.

TABLE 3
The effect of phosphine (200 ppm) on mortality as expressed in LT (hours to obtain % of mortality) values for 7-14 d-old adults of *Oryzaephilus surinamensis* at 30±1ºC and 65±5% r.h

χ^2	D.f.	Slope ±SE	LT_{50}	LT_{99}
11.7	7	5.10±0.32	1.36 (1.21-1.49)*	3.88 (3.30-4.83)

*Fiducial limit were calculated at P≤0.05

DISSCUSSION

Although the passive life stages were considerably more tolerant to the fumigant than the active ones, it was found in our laboratory tests that a 18-h exposure at 30±1ºC and 65±5% r.h. with phosphine effectively controlled all life stages of *O. surinamensis*. In a comprehensive survey of PH_3 toxicity to all life stages of 13 species of stored product beetles in their culture media, Hole *et al.* (1976) found that the most tolerant life stages were either eggs or pupae, confirming earlier work by Lindgren and Vincent (1966) and Bell (1976).

In the present test, both adults and larvae of *O. surinamensis* were the most susceptible satage compared to eggs and pupae. The most tolerant life stage was the pupa followed by the egg. Similar results for fumigant gases were reported in previous studies by other researchers. The egg and pupal stages of stored-product insects are generally more tolerant than larvae or adults to the fumigants phosphine (Lindgren and Vincent, 1966; Howe, 1973, 1974; Bell, 1976), methyl bromide (Hole, 1981), and carbonyl sulphide (Plarre and Reichmuth, 1997; Zettler *et al.*, 1997, 1999). Pratt (2005) also reported that relative susceptibility of life stages is generally in the order: eggs ≈ pupae < larvae ≈ adults. The usual explanation for this is that larvae and adults are more metabolically active, and hence have a higher metabolic rate, than eggs or pupae (Sun, 1947). All of these findings emphasize the importance of metabolic rate in PH_3 toxicity. The metabolic rate of an insect is dependant on several factors, and phosphine toxicity to members of an insect species also varies with changes in these factors. The major factors are: insect life stage, temperature, humidity, and feeding status (Sun, 1947).

It is known that the metabolic rate, and fumigant tolerance vary within life stages. In the present tests, the results showed that young eggs (0-1 d-old) are most tolerant when compared to older ones. Similar results were reported in previous studies by other researchers. Day-old eggs of the confused flour beetle *Tribolium confusum* have a lower metabolic rate, and a higher tolerance to fumigants, such as

carbon disulfide, than 3-5 day-old eggs, which in turn are less metabolically active and more tolerant to CS_2 than 7 day-old eggs (Pratt, 2005).

The present study was carried out at a high temperature that reflected the region's climate where *O. surinamensis* is the important pest of dried fruit in Turkey. The only reason that could explain how the insect colud be killed in a 18-h exposure period would be the high temperature. Several published reports document increased mortality of stored-product beetles exposed to fumigants at high temperatures. Because the fumigant gases are mainly absorbed by the exposed animals through their respiratory systems, factors that influence respiratory activity in insects, for example changes in temperature, could also affect the uptake of a fumigant (May, 1989). The toxicity of phosphine to insects has been shown to increase with a rise in temperature (Sato *et al*., 1973), probably due to an increase in metabolic rate and oxygen consumption that also stimulates uptake of the fumigant. Thus, a decrease in respiration rate at low temperatures, and a consequent decrease in the uptake of a fumigant, could jeopardize the effectiveness of a fumigation operation.

In conclusion, this research was undertaken to provide background information for the exposure period needed to kill the insect under laboratory conditions. For pest control using phosphine, further background information for the other dried fruit pests is needed under both laboratory and field conditions in Turkey.

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