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Efficacy of ECO_2 Fume® under low pressures against the egg stages of dried fruit pests

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ABSTRACT

Dried fruits in Turkey may be infested by dried fruit beetles [mainly *Carpophilus hemipterus* (L.)], almond moth [*Ephestia cautella* (Walker)] and the sawtoothed grain beetle [*Oryzaephilus surinamensis* (L.)]. While the dried fruit beetles and almond moth infestations occur in orchards, the latter pest only infests the dried fruits in processing plants. Our previous studies showed that all stages except egg stages of dried fruit pests were completely killed by phosphine applications alone. In this study, egg stages of *Carpophylus hemipterus*, *Ephestia cautella*, *Oryzaephilus surinamensis, Tribolium castaneum* (Herbst), and *T. confusum* Jacquelin du Val were subjected to high phosphine concentrations up to 3,000 ppm at different low pressures at 20, 25, and 30°C in the laboratory. The results showed that high phosphine concentrations under low pressures were found very effective in controlling egg stages of dried fruit pests

Key words: Almond moth, Dried fruits, Dried fruit beetles, ECO_2 Fume, Egg mortality, Low pressure, Phosphine, Sawtoothed grain beetle

Turkish dried fruit and nuts' exports dominate the World markets and were valued at US$15 billion in 2014. International trade in horticultural products is highly dependent upon effective pest control measures that both preserve the produce quality and prevent spread of pests across international borders Postharvest pest control activities performed either pre-shipment, in-transit, or on arrival play an important role of this process. Dried fruits in Turkey are subject to infestestations by dried fruit beetles *Carpophilus hemipterus* (L.), almond moth [*Ephestia cautella* (Walker)] and the sawtoothed grain beetle [*Oryzaephilus surinamensis*(L.)]. Dried fruit beetles and almond moth infestations take place in orchards, whereas sawtoothed grain beetle only infests the dried fruits in processing plants. Specis of *Tribolium* are found occasionally in dried fruit processing plants.

Use of methyl bromide (MeBr) in Turkey has been phased-out since 2005 under control measures of the Montreal Protocol. Before its ban, MeBr was the only fumigant used for disinfestation treatments of dried fruits with 2–3 treatments throughout the whole production process in any season.

The MeBr was an excellent fumigant providing satisfactory pest control with a 24 h exposure treatment. So far there has not been any other alternative fulfulling the requirements needed by Turkish dried fruit producers. Although sulfuryl fluoride (SO_2F_2), a registered MeBr alternative, has been proven to have similar efficacy, it has not yet been marketed in Turkey.

Among non-toxic alternatives, only controlled atmospheres are in use in Turkey. However, their use is more or less restricted to organic procuduction. Other alternatives that have been studied to date include PH_3 fumigation, high presssure CO_2, irradiation and ozonation applications by various researchers in Turkey (Tutuncu et al., 2004; Cetinkaya et al., 2006; Uslu et al., 2006, Sen et al., 2009; Tutuncu and Emekci, 2014).

Thus, the aim of this study was to provide data on the efficacy of dual combinations of various low pressures and PH_3 concentrations at various temperatures againts the egg stages of dried fruit pests.

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MATERIALS AND METHODS

Rearing test insects

Test insects were cultured in a constant room temperature 25°C and 65% r.h. All insects were reared in 1L glass jars whose mouths were covered with twice-folded polyester cloth. *Carpophilus hemipterus* was reared on an artificial diet including corn (*Zea mays* L.) flour, glucose, sugar, yeast, distilled water and agar, as described by Donahaye and Navarro (1989). *Ephestia cautella* and *Oryzaephilus surinamensis* were reared on a diet composed of broken wheat, 5% yeast and 5% glycerol (by weight), whereas *Tribolium castaneum* and *T. confusum* were reared on a mixture of broken wheat (*Triticum aestivum* L.) and yeast 5% (by weight).

Egg collection

Eggs of *C. hemipterus* were collected by means of a fine brush from the inner surface of polyester cloth that was used to cover the mouth of the jars, whereas the eggs of other coleopteran insects were obtained simply by sieving the adults from the culture medium [wheat flour plus dried yeast 5% (by weight)]. Daily emerged *E. cautella* adults were transferred in a PVC cage provided with a bottom mesh that allowed the eggs to drop into a PVC tray. Daily collected 1-day-old eggs of test insects were held in the rearing room to obtain 2- or 3-day-old eggs of each species

Experimental protocol

Eggs of test insects confined in 25 mL ventilated PVC vials were placed into 28 L vacuum chambers (Labconco®, Model No 5530000) equipped with two 3-way ball valves. One ball-valve of the chamber was connected to the vacuum gauge (Digi-Vac®), whereas the other ball-valve was connected to the vacuum pump (Becker®, U420 model). All the connections were made using vacuum hose. The air in the chamber was evacuated with the vacuum pump to the target level. Once the target low pressure level was attained, an inflatable rubber tube containing phosphine taken from the ECO_2 Fume® cylinder was connected to the vacuum chamber through the ball-valve which was previously connected to the vacuum pump. By opening the valve very slightly, phosphine gas was let into the chamber, so as the pressure inside the chamber was increased to a desired level. By doing this, eggs were exposed to known PH_3 levels in known low-pressure levels. Then the vacuum chambers were placed in 700-L incubator (Binder® Model, KB 720) adjusted at a given temperature for a specified length of time. After treatment, the vacuum chambers were vented to ambient pressure, vials containing the eggs were removed from vacuum chambers and placed in insect rearing room at 25°C for an appropriate period of time to allow egg to hatch. Experiments were conducted at 25, 30°C and 75% rh using vacuum levels of 50, 100, 150, 200, 250, 760 mm Hg and PH_3 levels of 0, 1,000, 1,500, 2,000, 3,000 ppm in dual combinations.

Statistical analysis

Results were investigated by GLM procedures Data were analysed through ANOVA with the JMP 60 Statistical Software, and statistically significant differences between treatments were computed using the LSD test (P<005)

RESULTS AND DISCUSSION

Complete mortality of tested pest species was recorded after 24 h of exposure to dual combinations of various low pressures and PH_3 concentrations at different temperatures (Table 1). The main pests of dried figs *C. hemipterus* and *E. cautella*, which infest the fruits in the orchards (Erakay and Ozar, 1976; Turanli, 2003), can be completely controlled after 24

Table 1 Age and number of eggs of test species used in the experiments

Species	Age of eggs (d)	Number of test individuals
Carpophilus hemipterus	1-2	100
Ephestia cautella	1-3	100
Tribolium castaneum	1-3	100
Tribolium confusum	1-3	100
Oryzaephilus surinamensis	1-3	100

Table 2 Vacuum level and PH_3 concentrations combinations that gave complete mortality in the eggs of tested species after 24 h of exposure at different temperatures

Species	Age (day)	Temperature (°C)	Vacuum level (mm Hg)	PH_3 concentration (ppm)		
				1000	1500	2000
Carpophilus hemipterus	1-2	20	250	100	100	100
Orzaephilus surinamensis	1-3	25	250	100	100	100
Ephestia cautella	1-3	30	050	-	100	100

h of exposure. Eggs of *Tribolium* spp. could not be controlled under current experimental conditions with 24 h of exposure.

After the complete ban of MeBr in 2005, phosphine became the only toxic fumigant marketed in Turkey. Other non-toxic alternatives such as modified atmospheres or vacuum applications have not adopted well enough by fruit processing plants, since these alternatives need longer exposures, exceeding 24 h, to be effective. Mbata (2004) found that LT_{99} value for one-day-old eggs of *E. cautella* that were exposed to 50 mm Hg at 25°C was calculated as 5945 h and increased to 33345 h by increasing the low pressure to 300 mm Hg. Bagci et al. (2006) reported that complete mortality in the eggs of *O. surinamensis* was achieved by 57 h of exposure to either 36 or 721 mm Hg low pressures at 25°C. Similarly, Finkelman et al. (2003, 2004) estimated the LT_{99} values for 1-d-old eggs of *E. cautella* exposed to ≥ 50 mm Hg at 20–30°C as ≥ 35 h.

On the other hand, long gas release time of up to 18 h of solid $MgPH_3$ formulations (e.g., FUMI-CELL^{TM}) cause time loss during the fumigation applications and thus prevent the use of solid formulations in large scale in dried fruit sector (Ferizli et al., 2003). Instead of solid PH_3 formulations, Muhareb et al. (2003) proposed to the use of ECO_2FUME, a ready-to-use, non-flammable mixture of PH_3 and CO_2, to shorten the exposure time at normal atmospheric conditions. They found that increasing the PH_3 concentration from 500 to 1000 ppm resulted in increased egg mortality in *C. hemipterus* from 9.05% to 98% at 267°C after 24 h of exposure.

In this study, we found that ECO_2FUME applied at the concentrations of 1000 or 1500 ppm at 20–25°C for 24 h did not cause complete egg mortality in *Ephestia cautella*, whereas increase in PH_3 concentration and decrease in pressure level caused increased mortality rates. Complete egg mortality in *E. cautella* could only be possible by increasing the temperature to 30°C. Complete mortality in eggs of *Tribolium* spp. were not achieved by 24 h exposures. However, these are very rarely found in dried fruit processing plants.

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