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Research Article

Efficacy of Phosphine Fumigation under Cold Temperature against *Frankliniella occidentalis* (Pergande, 1895) (Thysanoptera: Thripidae) on Carnation

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Abstract

Many pests cause serious economic losses in the carnation cultivation areas in Türkiye. Western flower thrips, *Frankliniella occidentalis* (Pergande, 1895) (Thysanoptera: Thripidae) is harmful on carnation by feeding on both leaves and flowers and is listed as a quarantine pest in some exporting countries. Due to invasions of western flower thrips some chemical control measures are traditionally used as a phytosanitary measure for control of western flower thrips on cut carnation, however, traditional insecticides do not provide its satisfactory control. Phosphine has been investigated as an alternative to traditional insecticides for pest control on cut flowers. In this study the toxic effect of the phosphine gas on egg, larva, pupa, and adult stages of western flower thrips were determined by using the QuickPHlo-R® aluminium phosphide formulation (77.5%) and QuickPHlo-R® quick phosphine generator in a cold storage facility in the Korkuteli district of Antalya province in Türkiye in 2018. The doses of 1.1, 2.2 and 3.3 g m^{-3} of QuickPHlo-R® granules were used for 48 hours exposure time under low temperature conditions (6 ºC). Adult stage of the *F. occidentalis* at 1.1 g m^{-3} has the lowest mortality rate with 75.71% while the highest toxic effect of phosphine gas was observed at 3.3 g m^{-3} for 48 h exposure time with a 100% mortality rate of all biological stages of *F. occidentalis*. It is concluded that QuickPHlo-R® fumigation technology against the western flower thrips under low temperature conditions could be used easily, especially to meet quarantine conditions of the exporter country of the cut flower.

Keywords: *Frankliniella occidentalis*, Carnation, Cold temperature, Phosphine, Phytosanitary.

Düşük Sıcaklık Altında Fosfin Fümigasyonunun Karanfil Üzerinde Bulunan *Frankliniella occidentalis* (Pergande, 1895) (Thysanoptera: Thripidae)'e Karşı Etkinliği

Öz

Türkiye'de karanfil ekim alanlarında birçok zararlı, ciddi ekonomik kayıplara neden olmaktadır. Batı çiçek thripsi, *Frankliniella occidentalis* (Pergande, 1895) (Thysanoptera: Thripidae) hem yaprak hem de çiçeklerle beslenerek karanfil üzerinde zararlı olup bazı ihracat yapan ülkelerde karantina zararlısı olarak listelenmektedir. Batı çiçek thripslerinin istilası nedeniyle, bazı kimyasal kontrol önlemleri geleneksel olarak kesme karanfil üzerindeki batı çiçek thripslerinin kontrolü için bir bitki sağlığı önlemi olarak kullanılmaktadır, ancak geleneksel böcek öldürücüler tatmin edici kontrolünü sağlamamaktadır. Fosfin, kesme çiçeklerde haşere kontrolü için geleneksel insektisitlere alternatif olarak araştırılmıştır. Bu çalışmada batı çiçek tripslerinin yumurta, larva, pupa ve ergin

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dönemleri üzerine fosfin gazının toksik etkisi QuickPHlo-R® alüminyum fosfit formülasyonu (%77.5) ve QuickPHlo-R® hızlı fosfin üreteci kullanılarak belirlenmiştir. 2018 yılında Antalya ili Korkuteli ilçesindeki soğuk hava deposu tesisinde 1.1, 2.2 ve 3.3 g m^{-3} dozlarında QuickPHlo-R® granülleri düşük sıcaklık koşullarında (6 ºC) 48 saat maruziyet süresi boyunca kullanılmıştır. *Frankliniella occidentalis*'in 1.1 g m^{-3}' teki ergin aşaması, %75.71 ile en düşük ölüm oranına sahipken, fosfin gazının en yüksek toksik etkisi, 48 saatlik maruz kalma süresi boyunca 3.3 g m^{-3}'te tüm dönemlerinde ölüm oranları %100 olarak gözlenmiştir. *Frankliniella occidentalis*'in biyolojik evreleri için düşük sıcaklık koşullarında batı çiçek thripslerine karşı QuickPHlo-R® fümigasyon teknolojisinin özellikle kesme çiçeğin ihracatçısı ülkenin karantina koşullarını karşılamak için kullanılabileceği kanısına varılmıştır.

Anahtar Kelimeler: Bitki sağılığı, Düşük Sıcaklık, Fosfin, *Frankliniella occidentalis*, Karanfil.

1. Introduction

There are many pests that cause serious economic losses in the carnation cultivation in Türkiye. Thrips are an important problem for carnation flowers during growing season and after harvest. One of the major thrips pests of carnation is western flower thrips [*Frankliniella occidentalis* (Pergande, 1895) (Thysanoptera: Thripidae)] which is harmful on carnation by feeding on both leaves and flowers. As a result of this damage, the plant can dry completely. Cut flowers are one of the major revenue-generating products in the global market (Teixeira da Silva, 2003). The importation of plants and plant parts, including cut flowers, poses a biosecurity risk to plant industries, the environment, and the economy of the importer country. Besides that, ornamental plants, due to their positive effects on people due to their aesthetic properties, provide a livelihood for hundreds of thousands of people in our country as well as all over the world. The concept of cut flower refers to the fresh, dried, dyed or bleached state of flowers, buds, leaves, and leaves that are often used in bouquets, baskets, wreaths, and arrangements (Teixeira da Silva, 2003).

Frankliniella occidentalis is not only harmful by feeding on plant tissue but also damages it by carrying plant viruses such as impatiens necrotic spot virus and tomato spotted wilt virus (Allen and Broadbent, 1986; Daughtrey et al., 1997). Due to invasions of western flower thrips on exported products, carnations are rejected from time to time, this is especially true for countries with very strict quarantine regulations (Seaton and Joyce, 2010). On the other hand, western flower thrips is a serious quarantine pest organism and listed in EPPO A2 list of pests recommended for regulation as quarantine pests (Anonymous, 2020a). On this basis, exporters and producers of the cut flowers must get some precautions for control of this pest at least before the shipment. For control of this insect at the postharvest stage there are some chemical control measures that can be taken in the cut flower without lowering the quality criteria. To control the thrips population among carnation flowers, producers regularly spray insecticides, which is ineffective as all thrips are not completely killed (Bumroongsook and Kilaso, 2018). Therefore, thrips need to be eliminated after harvest in accordance with the standard of export of agricultural materials to foreign countries.

Fumigation has been the main method of post-harvest protection either to disinfest cut flowers and agricultural products, or to protect them from insect infestation to meet export requirements. As a postharvest insect control agent, phosphine (PH_3) is usually used as an alternative to methyl bromide. Methyl bromide depletes the ozone layer and allows increased ultraviolet radiation to reach the earth's surface. Therefore, methyl bromide is a Class I ozone-depleting substance defined by the Montreal Protocol. In Türkiye, methyl bromide has been banned since 2008 (Ertürk et al., 2018). Metal phosphide such as aluminium or magnesium phosphide has been the primary choice of commercial fumigators since the 1930s. Phosphine is usually generated from metal phosphide and contains ammonia, which is generated from carbamate concurrently with phosphine to prevent generating phosphine too fast. Nevertheless, ammonia is phytotoxic. Recent advances in phosphine technology have resulted in the ability to place phosphine gas formulation a mixture of 2% phosphine and 98% (w/w) carbon dioxide in compressed cylinders (ECO2FUME®) for safe and rapid fumigation. The ECO2FUME® has been tested and used on a variety of fresh fruits and vegetables for postharvest insect control at low temperatures in many countries. The ECO2FUME® is also registered ready to use phosphine gas formulation for cut flowers against *F. occidentalis* in Türkiye (Anonymous, 2020b). Moreover, the QuickPHlo - R aluminium phosphide formulation and the QuickPHlo phosphine generator series are developed to generate rapid and continuous phosphine gas on-site during the fumigation. There is a lack of published data on toxicity of low temperature phosphine fumigation to the life stages of western fower thrips. In this study, it was evaluated that toxic effect of the phosphine gas on eggs, larvae, pupae and adults of western flower thrips. QuickPHlo-R® aluminium phosphide formulation that has an active ingredient content of 77.5% aluminium phosphide was used, by using the QuickPHlo-R® quick phosphine generator. This study was carried out to create an alternative to ECO2FUME® formulation which is unique in its field.

2. Material and Method

2.1. Insect Culture

Western flower thrips collected from the greenhouses in the provinces of Antalya and Muğla in Türkiye. The insect was reared under 25±1ºC, 65±5% relative humidity (r.h.) and long day (16:8 h L:D) photoperiod conditions at the Entomology Laboratory of the Biology Department of the Faculty of Science, Muğla Sıtkı Koçman University. For this purpose, the insects were transferred ventilated glass jars (1 L, 18 cm height x 10 cm diameter). Each of the jar lids was drilled with a 9 cm hole and covered with thrips-proof cloth. For feeding of adult insects, the bean [*Phaseolus vulgaris* (L.) (Fabaceae)] pods and an amount of vermiculite were introduced in the jars. Cattail, *Typha* sp. (Typhaceae), pollens were added as additional food source for adults. Vermiculite was used as a suitable pupation site. First instar larvae emerged in a few days and started feeding with bean pods and pollens. The bean pods were changed every second day with fresh bean pods. Dry pods were removed when needed. Mature second instar larvae turned into pupae into vermiculite media and adults emerged in a few days (Ertürk et al., 2018).

2.2. Fumigation Procedure

To perform the fumigation experiments a small scale QuickPHlo-R® rapid phosphine generator from United

Phosphorus Ltd (Mumbai, India) was used. The generator liberates the phosphine gas from the aluminium phosphine granules (QuickPHlo-R). The QuickPHlo-R® rapid phosphine generator has a stainless-steel reaction chamber where the reaction takes place. The rapid generator has a deactivation system and scrubber for eliminating the residue of aluminium phosphide formulation. Thus, the generator greatly reduces to safe levels for the disposal of the active ingredients. The generator has inlet and outlet connections pipes and the blower fan to provide gas circulation to the fumigation unit. Thus, the phosphine gas produced in the generator was transmitted to the fumigation chamber by the circulating airflow (Figure 1).

QuickPHlo-R® aluminium phosphide (77.5%), in the form of granules from a sealed foil pouch, was added to the reaction chamber and then the reaction chamber was hermetically sealed with gasket and clamp. The water container of the QuickPHlo-R® phosphine generator was filled with water. The QuickPHlo-R® aluminium phosphide granules, which encounter water in the container, started to release phosphine gas. The phosphine gas level in the fumigation chamber was monitored periodically using the portable gas analyzer, Dräger X-AM® 5000 (Drägerwerk AG & Co., Lübeck, Germany), during the fumigation process. The fumigation process was started by operating the phosphine generator and phosphine gas formed for 1.5 hours was delivered to the fumigation unit by circulating airflow method. After the production of phosphine gas from the granules was completed, the generator was turned off, and the connections were removed and the fumigation units were kept closed for 48 hours. At the end of the two days, fumigation chambers were opened and allowed at least 3 hours of ventilation and fumigation were terminated in this way. The experiments were carried out at an average temperature of 6 °C.

The trials were set up in specially prepared one cubic meter gas-tight polyethylene tanks. The tanks were used as a fumigation chambers. Carnations were placed in fumigation chambers in 50 x 17 x 35 cm plastic crates with at least 20 cut carnations inside. Larvae, pupae and adults of the *F. occidentalis* were put in the 100 ml plastic sample containers. Since the western flower thrips lay their eggs in the plant tissue, the French bean pods were used for oviposition sites at least two days. In this way, the effect of fumigant on hatching was evaluated. The containers specially prepared for insects were covered with 80 mesh screen printing silk. Thus, an environment that did not allow insects to escape and provides ventilation was created. Plastic crates with carnations were placed on top of each other and left inside the fumigation chamber. These plastic sample containers were distributed among carnations in the plastic crates. Fumigation chambers were sealed with very impermeable film (VIF) (Plastika Kritis, ORGASUN®) using insulating tape. For the efficacy evaluation, each fumigation chambers were exposed different PH_3 concentration. In the experiment 1.1, 2.2 and 3.3 g m^{-3} QuickPHlo-R® granules were used. 2.2 g QuickPHlo-R® granules liberates approximately 1 g PH_3 gas which is equates 716 ppm. For the positive control ECO2FUME® cylindered gas formulation with 70 g m^{-3} was used. After the fumigation process was completed, life stages of the test insects were transferred to a climate chamber and kept at 25±1°C, % 60±5 r.h. and 16:8 h L:D photoperiod. Before being scored for mortality of adults, larva and pupa mortalities were assessed after 2 d. and the toxic effect of the PH_3 on the egg stages of F. occidentalis was determined after incubation for 7 d.

2.2. Statistical analyses

This experiment was conducted in a completely randomized design with four replications. Variance analysis was applied to the mortality percentage according to corrected Abbott's formula by using IBM SPSS Statistics Version 20 Software (Erper et al., 2022). The differences between the treatments were analyzed by means of Duncan's multiple range test ($P < 0.05$).

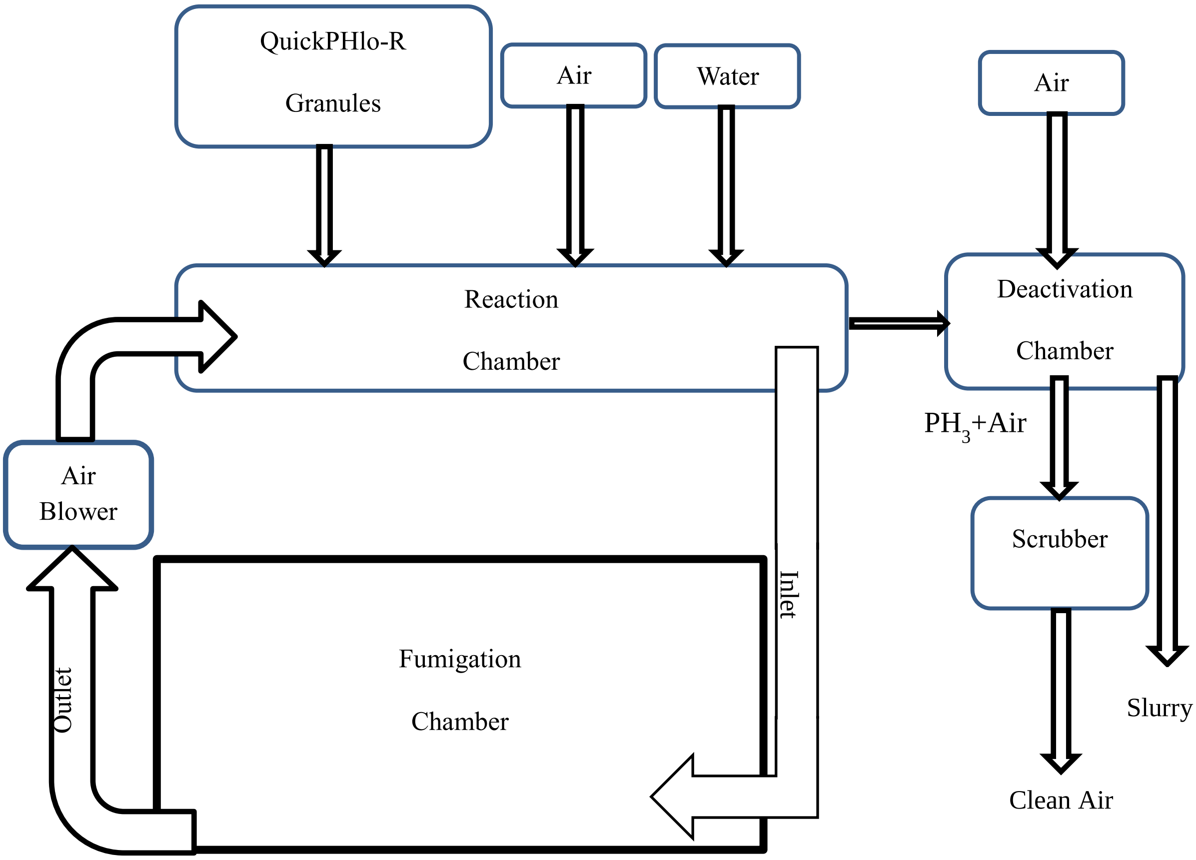

Figure 1. Block diagram of phosphine gas generation from the QuickPHlo-R® rapid phosphine generator
(Şekil 1. QuickPHlo-R® hızlı fosfin üretecinden fosfin gazı üretiminin blok şeması)

3. Results and Discussion

The mortality of adults, larvae, pupae and hatching rates of the eggs exposed to different PH_3 fumigation regimes were given (Figure 2-5). Adult stage of the F. occidentalis at dose of 1.1 g m^{-3} had the lowest mortality rate with 75.71%. The highest toxic effect of phosphine gas was observed at the dose of 3.3 g m^{-3} with 100% mortality rate, similar to positive control group. The dose of 2.2 g m^{-3} was resulted in 87.17% mortality. Except the positive control group, all phosphine treatments has a different statistical group ($F= 505,134$; $df= 3$; $P < 0.05$) (Figure 2).

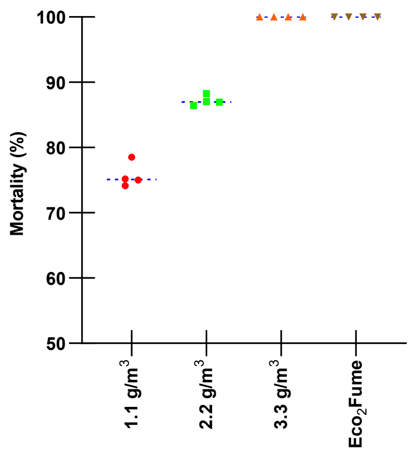

Figure 2. Toxic effect of the different dosage of PH_3 on the adults of *Frankliniella occidentalis* after 48 hours fumigation period in the carnation

(Şekil 2. Karanfilde 48 saatlik fümigasyon periyodundan sonra Frankliniella occidentalis'in erginlerinde PH3'ün farklı dozlarının toksik etkisi)

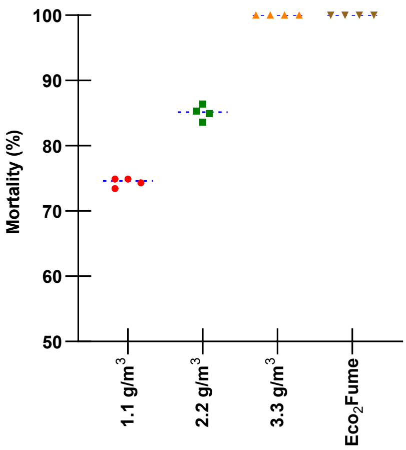

Figure 3. Toxic effect of the different dosage of PH_3 on the larvae of *Frankliniella occidentalis* after 48 hours fumigation period in the carnation

(Şekil 3. Farklı dozlarda PH_3'ün karanfilde 48 saatlik fümigasyon periyodundan sonra *Frankliniella occidentalis* larvaları üzerindeki toksik etkisi)

Several publications reported that western flower thrips adults were very susceptible to PH_3 fumigation (Karunaratne et al., 1997; Ertürk et al., 2018). Besides, Karunaratne et al., (1997) revealed that *Heliothrips haemorrhoidalis* (Thysanoptera: Thripidae) were completely killed after phosphine fumigation at the dose of 300 µl L^{-1} for 18 h of exposure time at 24 °C. Similarly, Emekci et al., (2014) found that 1000 ppm ECO2FUME® for 24-h exposure time was resulted in 100% mortality of *F. occidentalis* adults at 4 ºC. To eliminate the negative effect of low temperature on the mortality of *F. occidentalis* adults, the short exposure time of fumigation for 48 h, which resulted in 100% adult mortality was performed. In the case of the larval stage insect mortalities varied between 74.39-100.00% (Figure 3).

The lowest larval mortality observed at the lowest dose of 1.1 g m^{-3}. The highest mortality rate was 85.06% at the dose of 2.2 g m^{-3}, which did not achieve complete mortality of the larvae. Phosphine fumigation at the dose of 3.3 g m^{-3} gave 100% mortality of the larvae. Tunç et al., (2004) reported that for 99% mortality of the larvae of *F. occidentalis* was achieved at 80 µl acetone L^{-1} air for 34.3 h exposure time. It can be said that insecticides with fumigant action have been most toxic to the thrips, because of especially those that hide inside crevices within the plant. Lethal effects of phosphine gas fumigation against F. occidentalis egg and pupa stages were different (Figure 4-5).

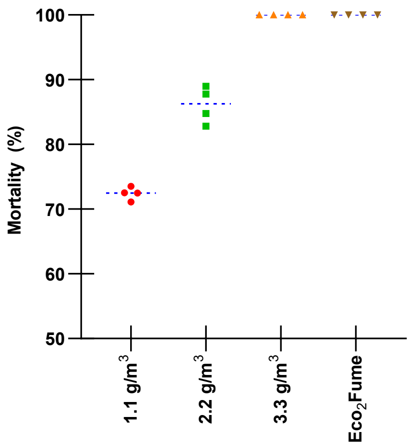

Figure 4. Toxic effect of the different dosage of PH_3 on the eggs of *Frankliniella occidentalis* after 48 hours fumigation period in the carnation

(Şekil 4. Farklı dozlarda PH_3'ün karanfilde 48 saatlik fümigasyon periyodundan sonra *Frankliniella occidentalis* yumurtaları üzerindeki toksik etkisi)

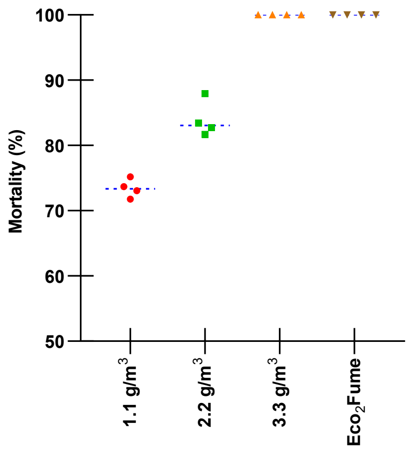

Figure 5. Toxic effect of the different dosage of PH_3 on the pupae of *Frankliniella occidentalis* after 48 hours fumigation period in the carnation

(Şekil 5. Farklı dozlarda PH_3'ün karanfilde 48 saatlik fümigasyon periyodundan sonra *Frankliniella occidentalis* pupaları üzerindeki toksik etkisi)

At the dose of 1.1 g m^{-3} egg and pupae, the mortality was 72.40% and 73.41%, respectively. The mortality rates of *F. occidentalis* eggs and pupae were determined for 86.08% and 83.94% at the dose of 2.2 g m^{-3}, respectively. Exposure of phosphine gas at the highest dose of 3.3 g m^{-3} resulted in 100% mortality for both pupae and eggs of *F. occidentalis*. It is known that embryonic stage such as eggs and postembryonic stages such as pupae of the insect species are more tolerant to phosphine than the adults and larval stages (Hole et al., 1976).

In a previous study, the order of tolerance to phosphine fumigation for biological stages of Indian meal moth, Plodia interpunctella (Hübner) ranged eggs> pupae> 5 weeks old larvae> 2 weeks old larvae (Storey, 1973). It is also important that the western flower thrips insert their eggs in the plant tissue; therefore, it is difficult to kill with the conventional insecticide. Because of its specific gravity of 1.21, similar in density to air, phosphine has a good penetration ability to all kinds of commodities and structures.

4. Conclusions and Recommendations

The phosphine generator QuickPHlo-R® generates pure phosphine gas from aluminium phosphide granules without ammonia, which is not a detrimental effect on cut flowers or fresh commodities. Also, when compared to conventional aluminium or magnesium phosphide formulations, QuickPHlo-R® technology has some advantages, such as more user-friendly because of less gas releasing time, independent from environmental conditions, gas circulation with forced air to the entire structure, not applied directly to the treated commodity and on-site application.

Present study indicated that QuickPHlo-R® fumigation against the western flower thrips could be used, especially to meet quarantine conditions of the exporter country of the cut flower. Also, it is an advantage that the QuickPHlo-R® can operate independently from the ambient temperature, which is thought to provide convenience to the fumigator. Further research on the thrips is needed to determine the efficacy of the PH_3 toxicity in shorter exposure periods, different dosage and temperature conditions, which has not a negative effect on the quality criteria of cut carnation.

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