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Original article (Orijinal araştırma)

Effect of different phosphine gas concentrations against *Frankliniella occidentalis* (Pergande, 1895) (Thysanoptera: Thripidae) on tomato and green pepper fruit, and determination of fruit quality after application under low-temperature storage conditions[1]

Farklı fosfin gaz konsantrasyonlarının düşük sıcaklık depolama koşullarında domates ve yeşil biberde karantina zararlısı *Frankliniella occidentalis* (Pergande, 1895) (Thysanoptera: Thripidae)'e karşı etkileri ile uygulama sonrası meyve kalitesi üzerine etkisinin belirlenmesi

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Abstract

In this study, we investigated the control of western flower thrips [*Frankliniella occidentalis* (Pergande, 1895) (Thysanoptera: Thripidae)], which is an important pest in tomato and green pepper growing areas in Turkey, under cold storage conditions with phosphine fumigation. The trials were conducted in Mersin in 2015, due to infestations of *F. occidentalis* damaging the export quality of tomatoes and green peppers. The experiments were conducted by using ECO$_2$FUME® with different phosphine gas concentrations including 500, 1000 and 2000 ppm. Fumigation chambers were kept at 4°C. Lowest mortality was seen at 500 ppm gas concentration with 72% for larval stage on tomatoes and 73.75% mortality for pupal stage on green pepper. The data demonstrate that the minimum requirement of phosphine gas concentration 100% mortality of all stages of *F. occidentalis* was at 2000 ppm. The quality of treated fruit was investigated by analyzing weight loss, sensory quality, fruit firmness, fruit skin color, total soluble solids, titratable acidity contents, physiological and pathological disorders. After treatment, there were no changes in physiologic, pathological and shelf-life properties of the products. The results indicated that, phosphine is a suitable fumigant at low temperature for disinfestion of *F. occidentalis* from tomatoes and green peppers before shipment.

Keywords: Fumigation, phosphine, quality analyze, quarantine, storage

Öz

Bu çalışma ile Türkiye'de domates ve yeşil biber alanlarında önemli bir zararlı olan batı çiçek tripsi [*Frankliniella occidentalis* (Pergande, 1895) (Thysanoptera: Thripidae)]'nin soğuk hava depolama koşullarında fosfin fümigasyonu ile kontrolü araştırılmıştır. Domates ve yeşil biberin ihracat kalitesine zarar yapan *F. occidentalis* bulaşmasına bağlı olarak, denemeler 2015 yılında Mersin'de gerçekleştirilmiştir. Denemeler ECO$_2$FUME® preparatı ile 500, 1000 ve 2000 ppm'lik farklı fosfin gaz konsantrasyonunda test edilmiştir. Fümigasyon birimleri 4°C tutulmuştur. En düşük ölüm oranı 500 ppm gaz konsantrasyonunda %72 ile domateste larva döneminde, yeşil biberde ise %73.75 ölüm oranı ile pupa döneminde görülmüştür. Çalışmadan elde edilen verilere göre batı çiçek tripsi'nin bütün dönemlerinin ölümü için en az 2000 ppm'lik gaz konsantrasyonunun gerekli olduğu ortaya konmuştur. Uygulama yapılan meyvenin kalitesi, ağırlık kaybı, duyusal kalite, meyve sıkılığı, meyve rengi, toplam çözünebilir katı madde, titrasyon asitliği içeriği, fizyolojik ve patolojik bozukluklar analiz edilerek incelenmiştir. Fosfin uygulamasından sonra ürünlerde fizyolojik, patolojik ve raf ömrü üzerinde herhangi bir değişim olmamıştır. Sonuçlar, taşıma öncesinde düşük sıcaklıkta *F. occidentalis*'in domates ve yeşil biberden dezenfekte edilmesi için fosfinin uygulanabileceğini ortaya koymuştur.

Anahtar sözcükler: Fümigasyon, fosfin, kalite analizi, karantina, depolama

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Introduction

Fresh vegetables such as tomatoes (*Solanum lycopersicum* L., Solanaceae) and green pepper (*Capsicum annuum* L., Solanaceae) are important agricultural export products in Turkey with about 12.6 kt of tomatoes and 2.3 kt of green pepper exported annually (Anonymous, 2016). The major pest of these vegetables in Turkey is western flower thrips, *Frankliniella occidentalis* (Pergande, 1895) (Thysanoptera: Thripidae) (Kütük et al., 2016).

Treatment of fruit with aluminum phosphide formulation has a negative effect on fruit quality, including discoloration, softening of the fruit flesh and shortened shelf life under low-temperature storage conditions (Desmarchelier et al., 1998; Ferizli et al., 2004). Therefore, it is also important to determine the quality of the applied product as well as the effect of the application of the phosphine gas on the target pest. For agricultural products to be exported, in addition to freedom from quarantine pests, fruit quality is another important parameter. For fumigant applications, the effects of fumigant to the pests are mostly investigated, while quality studies are done less frequently. If quality is affected, the market value of treated products falls and causes the exporter economic loss.

Frankliniella occidentalis is a cosmopolitan and polyphagous pest (Do Bae et al., 2015). The damage this pest either directly by feeding on plant tissue or indirectly by transmitting plant viruses, for example, tomato spotted wilt virus (Allen & Broadbent, 1986) and impatiens necrotic spot virus (Daughtrey et al., 1997). According to Tunç et al. (2012), *F. occidentalis* was reported for the first time in the Mediterranean Region (Burdur Province in 1992 and Antalya Province in 1993) of Turkey. The first appearance of western flower thrips in the Aegean region was observed on glasshouse tomatoes in Izmir at 1995. The presence of *F. occidentalis* on tomatoes and green pepper is a problem which negatively impacts international trade of fresh vegetables. Due to infestations of western flower thrips in exported products, tomatoes and green peppers are rejected from time to time, this is particularly the case for countries whose quarantine regulations are very strict (Seaton & Joyce, 2010). Also, chemical control of this pest under field conditions is very difficult because it is a polyphagous and multivoltine pest. For the last few decades, either pure phosphine gas alone or combination with CO_2, has been tested for postharvest fumigation of fresh food products such as apple, broccoli, cut flowers, lettuce, grape, peach, pear, plum, paprika, and white chrysanthemum under cold weather conditions (Liu, 2008; Zhang et al., 2012; Rogers et al., 2013; Emekci et al., 2014).

Adults of this pest are most commonly located in the flowers of the host plants, and may also enter closed buds. Eggs are laid in the parenchymatous tissues of leaves, fruit and flower parts. Therefore, fumigation is very important for control of thrips, especially when fresh fruit and vegetables are stored in warehouses. As a postharvest insect control agent, phosphine is usually used as an alternative to methyl bromide. In the atmosphere, methyl bromide depletes the ozone layer and allows increased ultraviolet radiation to reach the earth's surface. Methyl bromide is a Class I ozone-depleting substance defined by the Montreal Protocol. In Turkey, methyl bromide has been banned since 2008. $ECO_2FUME^{®}$ (2% phosphine and 98% CO_2 w/w) cylindered gas formulation of phosphine is safe for commercial use, effective and ready to apply with user-friendly fumigation of food and other commodities. It is used to a relatively limited extent for quarantine treatment of imported grain, flour, fresh fruit and vegetables, oil seeds and nuts that come in shipping containers. For this reason, researchers are searching for alternative methods for postharvest pest management.

There little research on the effect of low-temperature phosphine fumigation for against western flower thrips on tomatoes and green pepper. The aim of this work is to evaluate the efficiency of different phosphine gas concentration under cold storage conditions on the mortality of western flower thrips eggs, larvae, pupae and adults. Also, the effect of phosphine on the quality of the treated tomatoes and green pepper were investigated in vitro.

Material and Methods

Mass rearing of insects

Initial cultures of western flower thrips were established with *F. occidentalis* adults originally collected from greenhouses in Mersin and Adana Districts of Turkey between March and June in 2015. About 50 female and 10 male western flower thrips were collected with an aspirator and transferred to ventilated jars. Glass jars (1 L, 18 cm height x 10 cm diameter) were used to mass rear the thrips mass. For ventilation, the center of the jar lids was drilled with a 7-cm hole and covered with thrips-proof cloth. Adult insects, french bean [*Phaseolus vulgaris* (L.) (Fabaceae)] pods and an amount of vermiculite were put in the jars. French bean pods served as food and oviposition sites for the thrips. Cattail, *Typha* sp. (Typhaceae), pollens were added as additional food source for adults. Vermiculite was used as a suitable pupation site. First instar larvae (L_1) emerged in a few days and started feeding with bean pods and pollens. Every second day fresh bean pods were added to jars and dry pods were removed when needed. Mature second instar larvae (L_2) turned into pupae into vermiculite media and adults emerged in a few days. Insect rearing was done in climate-controlled rooms under 25±1ºC, 60±10% RH and long day (16:8 h L:D photoperiod) conditions Biological Control Research Institute, Adana.

Experimental design

Experiments were performed at the premises of PackErman Logistics Centre (Mersin, Turkey) at 4°C and 75±5% RH in June 2015. With tomato and green pepper fruit, the fumigation treatments against eggs, larvae, pupae and mixed-sex adults of *F. occidentalis* were performed as three (500, 1000 and 2000 ppm) phosphine concentrations ($ECO_2FUME^®$, Cytec Industries B.V., Netherlands) on the same day. All stages of western flower thrips (except for eggs) were placed between tomato and green pepper fruit in a special container. In the trials, four replicates were used and each replicate had 25 individual larvae, pupae and mixed-sex adults. Untreated tomato and green pepper fruit were used as controls. In the experiment, we could not use positive control because there was no registered fumigant that has same formulation and properties as $ECO_2FUME^®$ in Turkey. Three kg of tomato and green pepper fruit were separated for post-treatment quality analyses and the rest of tomato and green pepper fruit were stored for 7 d at 5±0.5ºC and 7±0.5ºC, respectively, at 85-90% RH. In addition to the post storage period, shelf-life samples were kept for 2 d at 20±1ºC and 65-70% RH. Some quality analyses were performed on samples taken both after storage and shelf life. The quality analyses were conducted according to randomized block experimental design with three replicates each of 3 kg fruit.

Fumigation procedure

For the experiments, a special 1.55 m^3 (1.55 x 1 x 1 m) volume fumigation chamber was made from PVC. Plastic boxes (40 x 25 x 30 cm) containing tomatoe and green pepper fruit were put into each fumigation chamber and all life stages of *F. occidentalis* (eggs, larvae, pupae and adults) were placed at the bottom, center and upper level of the chamber. The chambers were kept in the cold rooms (4°C) and sealed with virtually impermeable film (VIF) (Plastika Kritis, ORGASUN®) using insulation duct tape. Phosphine gas was delivered to each chamber via Parker-Parflex tube attached to a $ECO_2FUME^®$ cylinder. Tested phosphine concentrations were 500, 1000, 2000 ppm, which are equivalent to 34.8, 69.6 and 139.2 g/m^3 $ECO_2FUME^®$, respectively. Each experimental unit was fumigated for 24 h. During the trial, phosphine concentrations in the chamber were determined using a CertiPH_3os 2240 gas monitor (Messtechnik GmbH, Kirchseeon, Germany) and gas leakage in the room was controlled by the Dräger Pac 7000 (Drägerwerk AG & Co. KGaA, Lübeck, Germany). Temperature of the room was continuously recorded with a Dixell CoolMate XLH260 (Pieve d'Alpago, Italy) during the experiments. Desired phosphine gas concentration was calculated as follows.

$$ECO_2FUME(g) = \frac{50g \text{ x desired phosphine gas concentration } (ppm)}{718\, ppm}$$

After fumigation, the cold room was ventilated and test insects were taken out from each fumigation unit. For mortality assessment, the insects were transferred to a climate chamber and kept at 25±1ºC, 60±5% RH and 16:8 h L:D photoperiod. After 2 d, adults, larvae and pupae mortality were assessed, and mortality of the eggs were determined after incubation for 7 d.

Quality analysis

Tomato and green pepper samples were weighed before and after storage and shelf life on an electronic scale (XB 12100; Presica Instruments Ltd., Switzerland, 0.05 g accuracy), and percentage weight loss determined. Tomato fruit firmness was determined using a penetrometer (Effegi FT 011, Fujihira. Industry Co., Ltd., Tokyo, Japan) with a 7.9-mm diameter head and conical-shaped spear; the results are expressed in Newton (N).

The external skin color was measured at the equatorial area on both sides of 15 fruit using a colorimeter (CR-300; Minolta Co., Osaka, Japan), and the average scores were recorded in terms of CIE-L* a* b* values. These values were then used to calculate chroma ($C^* = [a^{*2} + b^{*2}]^{1/2}$), which indicates the intensity or color saturation and hue angle ($h^o = \tan^{-1} [b^*/a^*]$) that is expressed in degrees and represents: 0° (red-purple), 90º (yellow), 180º (bluish-green), and 270º (blue) (McGuire, 1992).

The total soluble solid (TSS) content of the tomato and green pepper fruit juice was determined by using a digital reflectometer (PR-1; Atago, Tokyo, Japan) and expressed as a percentage. The titratable acidity (TA) was determined by titrating 10 ml of juice with 0.1 N NaOH up to pH 8.1. The results are expressed as g malic acid/100 ml of fruit juice, in accordance with the AOAC standards (1984).

Physiological and pathological disorders over fruit surfaces were examined and their rates were expressed as a percentage. Sensory quality evaluation of appearance was conducted by an experienced six-member panel and a five-point hedonic scale: overall appearance scale (1, extremely poor; 3, poor; 5, moderate and limit of marketability; 7, good; and 9, excellent). Each assessor tasted and evaluated three fruit per replicate. The procedures for sensory evaluation of horticultural crops described by Heintz & Kader (1983) were follow by the panel.

Statistical analysis

Fumigation assays were performed with randomized block experimental design with four replicates. Variance analysis was performed with transformed data. In addition, the differences among treatments were analyzed by means of Tukey multiple comparison test ($P < 0.05$). All statistical analyses were conducted with MINITAB® Release 16 package program. Data obtained from quality analyses were subjected to analysis of variance (ANOVA) by using the IBM® SPSS® Statistics ver. 19 statistical software (IBM, New York, NY, USA). Significant differences between the means for each vegetable (tomato and green pepper) were determined by Duncan’s multiple range tests at $P < 0.05$. Standard deviation of the mean was also calculated from the replicates.

Results and Discussion

The lowest effect after 24 h of fumigation was seen in the larval stage at 500 ppm phosphine. At 2000 ppm, 100% mortality was recorded from all stages of western flower thrips after 24 h at 4ºC (Table 1). All treatments were statistically different ($F = 11.57$; df = 2, 11; $P < 0.05$).

Table 1. Mortality effects of different phosphine concentrations on different stages of *Frankliniella occidentalis* in tomatoes

Phosphine concentration (ppm)	Mortality (%)±SE*							
	Egg		Larvae		Pupae		Adult	
500	77.29±8.75	b**	72.50±15.00	b	78.75±2.50	b	82.99±14.18	a
1000	86.39±7.68	b	100.00±0.00	a	100.00±0.00	a	97.22±5.56	a
2000	100.00±0.00	a	100.00±0.00	a	100.00±0.00	a	100.00±0.00	a

* SE: Standard error;
** Same letters within columns are not significantly different according to Tukey’s multiple range test ($P < 0.05$).

In green pepper there was a linear relationship between the concentration of phosphine gas and its effect. The lowest mortality of 74% was at 500 ppm for the pupal stage. The highest mortality of 100% rate was recorded for all stages of *F. occidentalis* (Table 2) at 2000 ppm.

Table 2. Mortality effects of different phosphine concentrations on different stages of *Frankliniella occidentalis* in green pepper

Phosphine concentration (ppm)	Mortality (%)±SE*							
	Egg		Larvae		Pupae		Adult	
500	90.00±7.98	b**	85.00±12.91	a	73.75±9.46	b	77.08±11.55	b
1000	100.00±0.00	a	95.00±5.77	a	95.00±10.00	a	100.00±0.00	a
2000	100.00±0.00	a	100.00±0.00	a	100.00±0.00	a	100.00±0.00	a

* SE: Standard error;
** same lower-case letters within columns are not significantly different according to Tukey's multiple range test ($P < 0.05$).

Liu (2008) reported 100% mortality of the *F. occidentalis* was after 18-h exposure period with ≥250 ppm phosphine. In a previous study, cooled iceberg lettuce (3ºC) fumigated with phosphine gas (484 ppm) at room temperature for 18 h, bioassay resulted in complete control of this pest (Liu, 2011). Similarly, Emekci et al. (2014) reported total mortality of all stages of *F. occidentalis* and mites, *Tetranychus cinnabarinus* (Boisduval, 1867), at minimum of 1000 ppm (70 g ECO_2FUME/m^3) after 24 h at 4ºC. Pre-adult insect stages are known to be more tolerant to fumigants because their respiration rates are much lower than adult insects. The results of this study showed that insects collected from different regions have different responses to different doses of the same chemical. Therefore, it is thought that the differences in the mortality rates of the green pepper and tomato were caused by this. Eggs and pupae of several species are reported as the most phosphine tolerant stages (Chaudhry, 1997). Shorter fumigation period with lower phosphine gas level would also help prevent injury to vegetables. The treated fruit were compared with untreated controls to determine potential injury from the fumigant. No negative effects of the fumigation treatments were found. Phosphine fumigation applications at low temperature could be used effectively to control *F. occidentalis* on fresh tomato and green pepper fruit for export. The effect of different phosphine doses on weight, firmness and color (hº) of the tomato fruit is shown in Table 3. The weight loss was similar after 7 d of storage and 3 d of shelf life, and ranged from 1.09-1.20% and 2.37-2.57%, respectively. The no significant effect of the different doses of phosphine on tomato firmness of fruit was found and after shelf life, firmness varied between 18.43 and 20.23 N, respectively. The effect of phosphine treatments on color (hº) of tomato fruit was found to be minimal and after shelf life varied between 44.1 and 49.2.

Table 3. The effect of different concentrations of phosphine on weight, color and firmness of tomato fruit

Phosphine concentration (ppm)	Weight loss (%)			Firmness (N)			h° value	
	AS	ASL	AT	AS	ASL	AT	AS	ASL
Control	1.20^{ns}	2.37^{ns}	21.05^{ns}	20.95^{n}	18.43^{ns}	47.44^{ns}	44.89^{ns}	44.07^{ns}
100	1.12	2.46	21.61	21.82	20.08	47.06	45.82	48.40
200	1.09	2.57	22.27	22.38	20.23	46.44	48.94	45.09
300	1.11	2.40	21.36	21.37	18.98	43.97	47.52	49.24

^{ns}: Not significant; AS: after storage; ASL: after shelf life; AT: after treatment.

The variation of TSS, TA and sensory evaluation of tomato fruit according to phosphine treatments is given in Table 4. The effect of the phosphine doses on TSS, TA and sensory evaluation of tomato fruit were similar. After shelf life, TSS, TA and sensory evaluation ranged between 4.20-4.60%, 0.29-0.37 g/100 ml and 4.2-4.6, respectively. No negative effect of phosphine treatments was observed for these parameters.

Table 4. Effects of different concentrations of phosphine on TSS (%), TA content (g citric acid/100 ml) and sensory analysis of tomato fruit after treatment, storage and shelf life

Phosphine concentration (ppm)	TSS content			TA content			Sensory analysis	
	AT	AS	ASL	AT	AS	ASL	AS	ASL
Control	4.45^{ns}	4.45^{ns}	4.20^{ns}	0.33^{ns}	0.28^{ns}	0.29^{ns}	4.8^{ns}	4.4^{ns}
100	4.15	4.25	4.50	0.29	0.30	0.37	5.0	4.6
200	4.50	4.35	4.50	0.31	0.27	0.31	4.8	4.2
300	4.45	4.35	4.60	0,29	0.29	0.31	4.8	4.4

^{ns} : Not significant; AS: after storage; ASL: after shelf life; AT: after treatment.

The variation in weight loss and color values (C* and hº) of green pepper fruit according to phosphine treatments is given in Table 5. The effect of phosphine treatments on weight and color of green pepper fruit were similar. After 7 d of storage followed by 2 d of shelf life the weight loss ranged between 1.62-1.76%, C* and hº color values ranged between 37.18-40.84 and 112.63-113.47, respectively.

Table 5. The effect of different concentrations of phosphine treatments to weight loss and color of green pepper fruit

Phosphine concentration (ppm)	Weight loss (%)		C* value			hº value		
	AS	ASL	AT	AS	ASL	AT	AS	ASL
Control	0.71^{ns}	1.76^{ns}	39.54^{ns}	39.70^{ns}	38.35^{ns}	112.71^{ns}	112.93^{ns}	112.73^{ns}
100	0.80	1.62	39.59	39.08	40.84	112.66	112.71	112.63
200	0.67	1.63	39.16	41.00	38.79	113.02	112.66	112.97
300	0.87	1.72	37.29	40.46	37.18	113.06	112.28	113.47

^{ns}: Not significant; AS: after storage; ASL: after shelf life; AT: after treatment.

The effect of the phosphine treatments to TSS, TA and sensory evaluation of green pepper fruit were similar. After shelf life the TSS, TA and sensory evaluation of green pepper samples were on average 4.53%, 0.12 g/100 ml and 4.7, respectively (Table 6).

Table 6. Effects of different concentrations of phosphine on TSS (%), TA content (g citric acid/100 ml) and sensory analysis of green pepper fruit after treatment, storage and shelf life

Phosphine concentration (ppm)	TSS content			TA content			Sensory	
	AT	AS	ASL	AT	AS	ASL	AS	ASL
Control	4.23^{ns}	4.50^{ns}	4.60^{ns}	0.13^{ns}	0.14^{ns}	0.13^{ns}	5.0^{ns}	4.8^{ns}
100	4.30	4.45	4.60	0.12	0.14	0.11	5.0	4.6
200	4.20	4.40	4.45	0.12	0.13	0.12	5.0	4.6
300	4.17	4.50	4.45	0.13	0.14	0.10	5.0	4.8

^{ns}: Not significant; AS: after storage; ASL: after shelf life; AT: after treatment.

After the storage and shelf life, physiological and pathological changes were not determined for the phosphine treated tomato and green pepper fruit. When using this fumigant or any other product, the efficiency in controlling the pests and toxicity should be determined along with and effects on product quality (Desmarchelier et al., 1998). After storage and shelf life, no physiological and pathological disorders were found in the phosphine treated tomato and green pepper fruit and the reason for this was there was not skin damage. Skin damage causes both decay and color alteration. For example, long exposure to a high phosphine dose (2.0 g/t) in dry fig caused darkening due to skin damage, but phosphine treatment had limited effect on the chemical composition of fruit according with previous studies (Meyvaci & Sen, 2007; Sen et al., 2009, 2015). Consequently, considering the physical, chemical and sensory analysis, no negative effects were identified to fruit quality after the treatment, storage and shelf life in green pepper fruit treated with phosphine (ECO$_2$FUME®) for 24 h at 500, 1000 and 2000 ppm.

The results indicate that ECO$_2$FUME® is a suitable fumigant for *F. occidentalis* disinfestation of tomatoes and green peppers at low temperature before shipment, with no negative effects on quality parameters.

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