

## Reciprocal effect of ethyl formate and phosphine gas on two quarantine pests, *Tetranychus urticae* (Acari: Tetranychidae) and *Myzus persicae* (Hemiptera: Aphididae)

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Received: 25 August 2021

Revised: 9 September 2021

Revision accepted: 10 September 2021

**Abstract:** Fumigation of fruits and vegetables during quarantine and pre-shipment (QPS) treatment should be effective with a shorter fumigation time to minimize phytotoxicity. In this research study, a shorter fumigation time, 2 hours exposure which is shorter than that of the current commercial fumigation procedures using a lower dose of ethyl formate (EF) mixed with phosphine (PH<sub>3</sub>) on strawberry was investigated. The reciprocal effect between EF and PH<sub>3</sub> against nymphs and adult *Myzus persicae* (Sulzer) and *Tetranychus urticae* (Koch) was evaluated. In addition, L(Ct)<sub>50</sub> and L(Ct)<sub>99</sub> of EF only and EF mixed with PH<sub>3</sub> were analyzed at 5°C and 20°C. The synergistic ratio (SR) of L(Ct)<sub>50</sub> and L(Ct)<sub>99</sub> for the nymph and adult stages of *M. persicae* were >1.0, which indicated a synergistic effect between EF and PH<sub>3</sub>. However, the SR values of L(Ct)<sub>50</sub> and L(Ct)<sub>99</sub> of the nymph and adult stages of *T. urticae* were ≤1.0 indicating that there was no synergistic effect between the two fumigants against *T. urticae*. Our results showed that the reciprocal effect between EF and PH<sub>3</sub> has different effects on *M. persicae* and *T. urticae*. This could be attributed to the biological and physical differences between the class Arachnida and Insecta. The synergistic effect between EF and PH<sub>3</sub> against *M. persicae* within a shorter exposure period and without phytotoxicity on fruits and vegetables will significantly benefit the horticultural industry.

**Keywords:** strawberry fumigation, ethyl formate, phosphine, reciprocal effect, synergistic effect

## INTRODUCTION

There were many negative impacts on methyl bromide (MeBr) reported such as high level of human acute and chronic toxicity, phytotoxic damage to perishable commodities as well as an ozone depletion property. Based on pha-

sing out schedule of MeBr, it has been used only critical use exemption (CUE) purpose, but it has not decreased in quarantine and pre-shipment (QPS) use (Mellouki *et al.* 1992; Kim *et al.* 2021). By the International Plant Protection Convention (IPPC), it was recommended to replace with technically feasible alternatives in QPS use and it is scheduled

for phasing out (Gareau 2015). However, there was a few possible chemical and physical options are under research and developed, particularly for perishable commodities.

Ethyl formate (EF), originally has been developed to use for cereal grain and dried fruit fumigation in Australia, is one of the potential options to alternative of MeBr on perishable commodities because of its fast kill, which is almost the same as MeBr, and safe for fumigators and customers (Ren and Mahon 2006; Ren *et al.* 2008). However, EF shows relatively high absorptive on commodities in high humidity conditions, such as strawberry and low penetration into commodities such as cut flowers (Weller and Graver 1998; Simpson *et al.* 2004). Therefore, for a successful fumigation, it is important to ascertain proper  $L(Ct)_{99\%}$  (99% lethal concentration  $\times$  time) on target pests and commodities (Lee *et al.* 2016).

Phosphine (PH<sub>3</sub>) gas is broadly used as cereal and grain fumigant, was developed certain fruit and vegetable fumigation because it can penetrate quickly and deeply into the commodities and relatively less phytotoxic damage on commodities (Lee *et al.* 2016). However, its insecticidal action relies on longer holding period of fumigation time to control the target pests. This causes increasing fumigation cost overall and phytotoxic damages to certain perishable commodities (Kim *et al.* 2019). Therefore, reducing fumigation time is required to extend application on various types of commodities in QPS use.

Green peach aphid, *Myzus persicae* (Sulzer) is a common insect pest in various perishable commodities including strawberry cultivation. *M. persicae* sucks juice of plants and is a vector of various plant viruses which can cause leaf curling and decreasing production. It can be found at harvested products and causes a quarantine problem if not properly controlled (Aharoni *et al.* 1979). Several chemicals such as acetaldehyde, ethyl formate, and natural volatiles have been evaluated as alternatives of MeBr to control green peach aphid for QPS purpose, but only ethyl formate (EF) has been registered for commercial purpose (Aharoni *et al.* 1979; Stewart and Mon 1984; Hammond *et al.* 2000).

Two-spotted spider mite, *Tetranychus urticae* (Koch), is an important agricultural insect pest in perishable commodities. *T. urticae* attacks the surface of plant leaves and intake chlorophyll cell, which cause withering of plant leaves, defoliation, and hindering photosynthesis. Postharvest disinfestation of two-spotted spider mite was estimated as physical and chemical methods, such like hot water and cool storage (Lester *et al.* 1997), essential oil (Lim *et al.* 2012), and methyl iodide (Waggoner *et al.* 2000).

Generally, insect pests were known as hard to kill when exposed to low concentration of EF and short fumigation time of PH<sub>3</sub> (Karunaratne *et al.* 1997). Moreover, high concentration of EF treatment can cause phytotoxic damage on some perishable commodities such as strawberry when applied at low temperature (Lee *et al.* 2013). Stewart and Mon (1984) reported that higher dosage (0.9%, v/v) of EF caused phytotoxic damage on lettuce than lower dosage (0.5%). Lee *et al.* (2012) reported that 16.7 g m<sup>-3</sup> of EF for 2 h at 13°C showed complete control to *T. urticae* on exported paprika without any phytotoxic damage but same fumigation conditions showed not suitable to strawberries because high sorption at low temperature was caused to phytotoxic damage. Regarding PH<sub>3</sub>, Weller and Graver (1998) reported that PH<sub>3</sub> was the most effective fumigant to control several insect pests which include two-spotted spider mite without phytotoxic damage on cut flower at 13°C, but it required to be combined with other treatments to achieve complete control of pests. Li *et al.* (2014) reported that PH<sub>3</sub> exposure time is more important than PH<sub>3</sub> concentration. Based on recent study, EF mixed with PH<sub>3</sub> showed a synergistic effect on cotton aphid (*Aphis gossypii*) (Lee *et al.* 2016). The purpose of this study is to identify that EF with PH<sub>3</sub> have reciprocal effect on common infested insects of *M. persicae*, and *T. urticae* on exported strawberries without phytotoxicity.

## MATERIALS AND METHODS

### 1. Insects and chemicals

All insects were reared at 25  $\pm$  1°C and 55–60% relative humidity (RH) with a 16 : 8 (L : D) h photo period in a laboratory at the APQA (Animal & Plant Quarantine Agency) Kimchon, Republic of Korea and collected in breeding dishes for fumigation tests. Green peach aphid (*M. persicae*) was reared on hot pepper plants, and two-spotted mite (*T. urticae*) was reared on bean plants. Nymph and adult stages of each insect were used 2–3 old from eggs and nymphs stage, respectively. Ethyl formate was provided from Safe-fume Co Ltd. and 2% phosphine (balanced with carbon dioxide) was provided by Cytec (Sydney, Australia).

### 2. Fumigation

Insect samples were placed in desiccators (6.9 L) and sealed with a glass stopper equipped with a septum (Alltech Associates Australia, Cat. No.15419). The volume of each

desiccator was measured by weighing a maximum water capacity of the desiccators at 20°C. A filter paper (Watman No.1) was inserted into the glass stopper to provide a liquid evaporation surface for the injected ethyl formate (EF). A mini fan with a magnetic bar was placed at the bottom of each desiccator for entire circulation of inner air. Prior to fumigation treatment, all chambers were unsealed and left overnight at 5 and 20°C and 70% RH. Fumigation bioassays of EF and PH<sub>3</sub> alone, as well as EF mixed with PH<sub>3</sub> were carried out with adults and nymphs of green peach aphid (*M. persicae*) and two-spotted mite (*T. urticae*). For efficacy test on *M. persicae*, the dose range of EF was from 1.00 to 18.00 and PH<sub>3</sub> was four doses (1.00, 2.00, 3.00, and 4.00). In case of efficacy on *T. urticae*, the dose ranged of EF was from 2.00 to 20.00 and PH<sub>3</sub> was from 0.01 to 4.00. To evaluate the reciprocal effect, EF ranged from 1.67 to 33.40 g m<sup>-3</sup> mixed with 1.00 g m<sup>-3</sup> of PH<sub>3</sub> for *M. persicae* assay and 0.05 g m<sup>-3</sup> of PH<sub>3</sub> for *T. urticae* assay which is dose of PH<sub>3</sub> to minimize the phytotoxic damages of strawberry at 5°C. The scheduled dosage for each desiccator including the required volume for PH<sub>3</sub> and EF concentrations was calculated based on methods reported by Ren *et al.* (2011) and Lee *et al.* (2013). All treatments and control were replicated three times. After 2 h fumigation, desiccators were aerated for one hour and the treated insect samples were removed and reared at 25°C and 60% RH. The end-point mortality was determined 72 h after fumigation.

### 3. Measurement of fumigant concentration

During fumigation, the concentrations of ethyl formate (EF) and phosphine (PH<sub>3</sub>) were measured at set time intervals. EF was determined using an Agilent portable GC 7890A equipped with a flame ionization detector (FID) after separation on a HP-5 Column (J&W Sci. 19091J-413). The oven temperature was 150°C. Injector and detector temperatures were 240°C. Phosphine was also determined using an Agilent portable GC 7890A, but equipped with a nitrogen phosphine detector (NPD). The oven temperature was 240°C. Injector and detector temperatures were 320°C. The concentration of EF and PH<sub>3</sub> was calculated based on the GC peak area against external EF and PH<sub>3</sub> gas standards.

### 4. Determination of concentration time (Ct) product of fumigant in a fumigation desiccator

The concentrations of fumigant were monitored at time intervals of 0.5 and 2 h over 2 h exposure periods, respec-

tively and were used to calculate the Ct product. The Ct products were calculated from Eq. 1.

$$Ct = \Sigma(C_i + C_{i+1}) (t_{i+1} - t_i) / 2 \quad (\text{Eq. 1})$$

Where: C is fumigant concentration (g m<sup>-3</sup>)

t is the time of exposure (h)

i is the order of measurement

Ct is concentration × time products (g h m<sup>-3</sup>)

### 5. Statistical analysis

The toxicological dose response to ethyl formate (EF) and phosphine (PH<sub>3</sub>) alone, and EF with PH<sub>3</sub> by *M. persicae* and *T. urticae* was analyzed by probit analysis based on a computer program written by Ge Le Pattourel, Imperial College, London, as adopted by Don-Pedro (1989). As part of the analysis, the slopes of the probit transformations were determined as well as Chi-square tests of data homogeneity for different treatments and development stages. The indices of toxicity measurement derived from this analysis were L(Ct)<sub>50</sub> = median lethal concentration that causes 50% response (mortality) and L(Ct)<sub>99</sub> = lethal concentration that causes 99% response (mortality) of exposed the stages of nymph and adult of *M. persicae* and *T. urticae* determined from a range of at least 10 different Ct products to ensure that the observed data covered mortality from 0% to 100% and adequately covered the intermediate range.

### 6. Determination of the combined toxicity of ethyl formate and phosphine mixtures

A measurement of combined toxicity was calculated by the formula of synergistic ratios (SRs). Synergistic ratios were defined by Hewlett and Plackett (1959) and calculated from Eq. 2.

$$SR = L(\text{ct}) \text{ of ethyl formate alone} / L(\text{Ct}) \text{ of ethyl formate with PHs (mixture)} \quad (\text{Eq. 2})$$

Where: SR = 1 describes an additive action

SR < 1 describes an antagonism

SR > 1 describes a synergism

## RESULTS AND DISCUSSION

### 1. Efficacy on green peach aphids

The L(Ct)<sub>50</sub> and L(Ct)<sub>99</sub> values of ethyl formate (EF) for the nymph stage of green peach aphid (*M. persicae*) were

**Table 1.** The efficacy of a 2 h exposure of the adult and nymph stages of *Myzus persicae* to ethyl formate at two temperatures conditions in terms of L(Ct)<sub>50</sub> and L(Ct)<sub>99</sub>

Temp. (°C)	Developmental stage	Ethyl formate				
		L(Ct) <sub>50</sub> (g h m <sup>-3</sup> ) (95% CL)	L(Ct) <sub>99</sub> (g h m <sup>-3</sup> ) (95% CL)	Slope ± SE	DF	χ <sup>2</sup>
5	Nymph	2.55 (1.57–3.30)	6.95 (6.08–8.08)	5.34 ± 0.75	12	118.49
	Adult	4.04 (3.57–4.40)	7.66 (7.32–8.13)	8.35 ± 0.73	12	30.54
20	Nymph	1.88 (1.69–2.05)	4.69 (4.33–5.20)	5.87 ± 0.42	13	33.02
	Adult	2.16 (2.07–2.24)	4.45 (4.33–4.59)	7.39 ± 0.24	13	2.71

χ<sup>2</sup> is based on pooling of data with low expectation

**Table 2.** The efficacy of a 2 h exposure of the adult and nymph stages of *Myzus persicae* to PH<sub>3</sub> at two temperature conditions

Temperature (°C)	Developmental stage	Phosphine			
		Dose (g m <sup>-3</sup> )	C × t (g h m <sup>-3</sup> )	Mortality (%)	Total no. tested/ Total no. dead
5	Nymph	1.00	1.51	0.0	54/0
		2.00	3.78	0.0	87/0
		3.00	5.44	0.0	35/0
		4.00	7.32	0.0	73/0
	Adult	1.00	1.51	0.0	57/0
		2.00	3.78	0.0	62/0
		3.00	5.44	0.0	46/0
		4.00	7.32	0.0	69/0
20	Nymph	1.00	1.42	0.0	51/0
		2.00	3.65	0.0	75/0
		3.00	5.32	0.0	81/0
		4.00	7.60	0.0	58/0
	Adult	1.00	1.42	0.0	56/0
		2.00	3.65	0.0	71/0
		3.00	5.32	0.0	60/0
		4.00	7.60	0.0	49/0

2.55 and 6.95 g h m<sup>-3</sup> at 5°C, and 1.88 and 4.69 g h m<sup>-3</sup> at 20°C, respectively, determined from the fitted slopes, 5.34 and 5.87. The L(Ct)<sub>50</sub> and L(Ct)<sub>99</sub> values of EF for the adult stage of *M. persicae* were 4.04 and 7.66 g h m<sup>-3</sup> at 5°C, and 2.16 and 4.45 g h m<sup>-3</sup> at 20°C, respectively, determined from the fitted slopes, 8.35 and 7.39 (Table 1). The Chi-square result at 20°C was not significant ( $P < 0.05$ ) for the adult data, but were significant for the nymph data in both temperature and adult data at 5°C. These results are relatively consistent with other reports. Lee *et al.* (2012) reported that *M. persicae* was completely controlled at 16.70 g m<sup>-3</sup> of EF fumigation for 2 h at 13°C. However, none of the doses

of PH<sub>3</sub> over the same range of concentrations applied for 2 h had an observed effect on the adult and nymph stages at 5 and 20°C (Table 2). This means that fumigation of PH<sub>3</sub> only cannot control *M. persicae* within short fumigation time, and other report represented that 24 h fumigation of PH<sub>3</sub> also requires high concentration of PH<sub>3</sub> to control *M. persicae* (Kim *et al.* 2019).

L(Ct)<sub>50</sub> and L(Ct)<sub>99</sub> values of EF with 1.00 g m<sup>-3</sup> of phosphine (PH<sub>3</sub>) for the nymph stage of *M. persicae* were 1.51 and 4.71 g m<sup>-3</sup> at 5°C, and 0.61 and 3.93 g h m<sup>-3</sup> at 20°C, respectively, determined from the fitted slopes, 4.71 and 2.87. The L(Ct)<sub>50</sub> and L(Ct)<sub>99</sub> values of EF with 1.00

**Table 3.** The efficacy of a 2 h exposure of the adult and nymph stages of *Myzus persicae* to ethyl formate mixed with 1.00 g m<sup>-3</sup> of phosphine at two temperature conditions in terms of L(Ct)<sub>50</sub> and L(Ct)<sub>99</sub>

Temp. (°C)	Developmental stage	Ethyl formate mixed with phosphine				
		L(Ct) <sub>50</sub> (g h m <sup>-3</sup> ) (95% CL)	L(Ct) <sub>99</sub> (g h m <sup>-3</sup> ) (95% CL)	Slope ± SE	DF	χ <sup>2</sup>
5	Nymph	1.51 (1.19–1.78)	4.71 (3.92–6.20)	4.71 ± 0.58	17	438.93
	Adult	1.72 (1.49–2.04)	5.01 (3.82–8.29)	4.92 ± 0.80	17	267.98
20	Nymph	0.61 (0.46–0.74)	3.93 (3.12–5.43)	2.87 ± 0.27	15	127.79
	Adult	0.88 (0.71–1.04)	3.53 (2.81–4.98)	3.85 ± 0.42	15	197.55

χ<sup>2</sup> is based on pooling of data with low expectation.

**Table 4.** The efficacy of a 2 h exposure of the nymph and adult stages of *Tetranychus urticae* to ethyl formate at two temperature conditions in terms of L(Ct)<sub>50</sub> and L(Ct)<sub>99</sub>

Temp. (°C)	Developmental stage	Ethyl formate				
		L(Ct) <sub>50</sub> (g h m <sup>-3</sup> ) (95% CL)	L(Ct) <sub>99</sub> (g h m <sup>-3</sup> ) (95% CL)	Slope ± SE	DF	χ <sup>2</sup>
5	Nymph	12.18 (11.00–13.37)	32.20 (26.98–41.81)	5.51 ± 0.54	14	107.95
	Adult	12.02 (10.33–13.70)	41.00 (31.93–61.26)	4.37 ± 0.51	14	243.14
20	Nymph	7.06 (4.66–9.03)	27.05 (17.72–44.46)	4.37 ± 0.79	12	115.96
	Adult	8.55 (6.84–10.70)	24.06 (17.15–48.44)	5.18 ± 0.94	12	218.23

DF is Degree of Freedom. χ<sup>2</sup> is based on pooling of data with low expectation.

g m<sup>-3</sup> of PH<sub>3</sub> for the adult stage of *M. persicae* were 1.72 and 5.01 g h m<sup>-3</sup> at 5°C, and 0.88 and 3.53 g h m<sup>-3</sup> at 20°C, respectively, determined from the fitted slopes, 4.92 and 3.85 (Table 3). The Chi-square results of nymph and adult at 5°C and 20°C were significant ( $P < 0.05$ ).

## 2. Efficacy on two-spotted spider mite

The L(Ct)<sub>50</sub> and L(Ct)<sub>99</sub> values of EF for the nymph stage of two-spotted mite (*T. urticae*) were 12.18 and 32.20 g h m<sup>-3</sup> at 5°C, and 7.06 and 27.05 g h m<sup>-3</sup> at 20°C, respectively, determined from the fitted slopes, 5.51 and 4.37. The L(Ct)<sub>50</sub> and L(Ct)<sub>99</sub> values of EF for the adult stage of *T. urticae* were 12.02 and 41.00 g h m<sup>-3</sup> at 5°C, and 8.55 and 24.06 g h m<sup>-3</sup> at 20°C, respectively, determined from the fitted slopes, 4.37 and 5.18 (Table 4). The Chi-square results of nymph and adult at 5°C and 20°C were significant ( $P < 0.05$ ).

The L(Ct)<sub>50</sub> and L(Ct)<sub>99</sub> values of PH<sub>3</sub> for the nymph stage of two-spotted mite (*T. urticae*) were 0.34 and 4.33 g h m<sup>-3</sup> at 5°C, and 0.32 and 2.30 g h m<sup>-3</sup> at 20°C, respec-

tively, determined from the fitted slopes, 2.12 and 2.74. The L(Ct)<sub>50</sub> and L(Ct)<sub>99</sub> values of PH<sub>3</sub> for the adult stage of *T. urticae* were 0.10 and 2.30 g h m<sup>-3</sup> at 5°C, and 0.21 and 0.60 g h m<sup>-3</sup> at 20°C, respectively, determined from the fitted slopes, 2.12 and 5.11 (Table 5). The Chi-square results of nymph and adult at 5°C and 20°C were significant ( $P < 0.05$ ). These results indicate that PH<sub>3</sub> is better to control *T. urticae* than EF but requires high concentration. However, fumigation with high concentration of phosphine could cause damage on strawberry. Soma *et al.* (2007) reported that 0.50 and 2.0 g m<sup>-3</sup> fumigation for 24 h caused phytotoxic damage on strawberry at 15°C. Also, Lee *et al.* (2013) reported that 1.0 g m<sup>-3</sup> of phosphine fumigation for 24 h at 5°C caused phytotoxic damage in terms of discoloration of calyx of strawberry. Meanwhile, Liu (2008) reported that 0.70 g m<sup>-3</sup> and 1.40 g m<sup>-3</sup> of phosphine fumigated for 24 h were not induced phytotoxic damage on strawberry at 2°C. It is assumed that these differences could be originated in difference of variety of strawberry between Asia and America.

The L(Ct)<sub>50</sub> and L(Ct)<sub>99</sub> values of EF with 0.05 g m<sup>-3</sup> of

**Table 5.** The efficacy of a 2 h exposure of the nymph and adult stages of *Tetranychus urticae* to PH<sub>3</sub> at two temperature conditions in terms of L(Ct)<sub>50</sub> and L(Ct)<sub>99</sub>

Temp. (°C)	Developmental stage	Phosphine				
		L(Ct) <sub>50</sub> (g h m <sup>-3</sup> ) (95% CL)	L(Ct) <sub>99</sub> (g h m <sup>-3</sup> ) (95% CL)	Slope ± SE	DF	χ <sup>2</sup>
5	Nymph	0.34 (0.26–0.45)	4.33 (2.44–10.99)	2.12 ± 0.26	19	241.61
	Adult	0.32 (0.27–0.39)	2.30 (1.55–4.10)	2.74 ± 0.27	18	101.08
20	Nymph	0.10 (0.06–0.14)	1.30 (0.71–5.27)	2.12 ± 0.36	14	125.89
	Adult	0.21 (0.17–0.31)	0.60 (0.36–8.77)	5.11 ± 1.55	9	310.31

DF is Degree of Freedom. χ<sup>2</sup> is based on pooling of data with low expectation.

**Table 6.** The efficacy of a 2 h exposure of the nymph and adult stages of *Tetranychus urticae* to ethyl formate mixed with 0.05 g m<sup>-3</sup> of phosphine at two temperature conditions in terms of L(Ct)<sub>50</sub> and L(Ct)<sub>99</sub>

Temp. (°C)	Developmental stage	Ethyl formate mixed with phosphine				
		L(Ct) <sub>50</sub> (g h m <sup>-3</sup> ) (95% CL)	L(Ct) <sub>99</sub> (g h m <sup>-3</sup> ) (95% CL)	Slope ± SE	DF	χ <sup>2</sup>
5	Nymph	12.01 (9.60–13.97)	28.57 (22.37–49.40)	6.18 ± 1.07	10	95.91
	Adult	11.47 (8.59–13.97)	44.06 (32.51–77.85)	3.98 ± 0.63	10	402.96
20	Nymph	9.42 (8.56–10.16)	23.35 (20.42–28.44)	5.90 ± 0.55	12	72.23
	Adult	9.89 (9.19–10.52)	23.73 (21.06–28.00)	6.12 ± 0.49	12	109.94

DF is Degree of Freedom. χ<sup>2</sup> is based on pooling of data with low expectation.

**Table 7.** Comparative efficacy of ethyl formate and ethyl formate mixed with phosphine against *Myzus persicae* and *Tetranychus urticae*

Temperature (°C)	Target insect pest	Developmental stage	<sup>a</sup> Synergistic ratio of L(Ct) <sub>50</sub>	<sup>b</sup> Synergistic ratio of L(Ct) <sub>99</sub>
5	<i>M. persicae</i>	Nymph	1.69	1.48
		Adult	2.35	1.53
20		Nymph	3.08	1.19
		Adult	2.45	1.26
5	<i>T. urticae</i>	Nymph	1.01	1.13
		Adult	1.05	0.90
20		Nymph	0.75	1.16
		Adult	0.86	1.01

<sup>a</sup>Synergistic ratio (SR): L(Ct)<sub>50</sub> of ethyl formate alone/L(Ct)<sub>50</sub> of ethyl formate with phosphine. <sup>b</sup>Synergistic ratio (SR): L(Ct)<sub>99</sub> of ethyl formate alone/L(Ct)<sub>99</sub> of ethyl formate with phosphine.

PH<sub>3</sub> for the nymph stage of *T. urticae* were 12.01 and 28.57 g h m<sup>-3</sup> at 5°C, and 9.42 and 23.35 g h m<sup>-3</sup> at 20°C, respectively, determined from the fitted slopes, 6.18 and 5.90. The L(Ct)<sub>50</sub> and L(Ct)<sub>99</sub> values of EF with 0.05 g m<sup>-3</sup> of PH<sub>3</sub> for the adult stage of *T. urticae* were 11.47 and 44.06

g h m<sup>-3</sup> at 5°C, and 9.89 and 23.73 g h m<sup>-3</sup> at 20°C, respectively, determined from the fitted slopes, 3.98 and 6.12 (Table 6). The Chi-square results of nymph and adult at 5°C and 20°C were significant ( $P < 0.05$ ).

Synergistic effect of ethyl formate (EF) with phosphine

(PH<sub>3</sub>) on green peach aphid (*M. persicae*) and two-spotted mite (*T. urticae*) was analyzed by Hewlett and Plackett model (Eq. 2), and the synergistic ratio (SR) values was > 1.0 (Table 7) which means that the EF mixed with PH<sub>3</sub> had synergism. These results are comparable to other researches. The SR values of L(Ct)<sub>50</sub> and L(Ct)<sub>99</sub> for adult *Aphis gossypii* at 5°C were 4.55 and 2.33, and at 20°C, they were 2.22 and 1.45, respectively (Lee *et al.* 2016). In case of nymph stages, the SR values of L(Ct)<sub>50</sub> and L(Ct)<sub>99</sub> at 5°C were 3.03 and 1.89, and at 20°C were 1.28 and 1.23, respectively (Lee *et al.* 2016).

However, SR values were lower or similar to 1.0 (Table 7) which means that the synergistic effect was slight or nothing on *T. urticae*. Though this difference of synergism between *M. persicae* and *T. urticae* is not clearly proved, but other reports showed possibility that there could be differences of chemical susceptibility by arthropod species. Simpson *et al.* (2004) reported that thrips and mealybugs were susceptible to EF while it is least susceptible on pacific spider mite, *Tetranychus pacificus*. Karunaratne *et al.* (1997) reported that greenhouse thrips, *Heliothrips haemorrhoidalis* (Bouch), were controlled at relatively low concentration (0.78 g m<sup>-3</sup>) for 2 h fumigation of PH<sub>3</sub>, but *M. persicae* was not controlled at high concentration (6.37 g m<sup>-3</sup>) of PH<sub>3</sub>. From these results, we might estimate that synergistic effect of EF and PH<sub>3</sub> could affect differently to arthropods by species.

In conclusion, to reduce fumigation time and dosage of fumigant to meet the QPS requirement of exported strawberry, this research obtained data demonstrated that the synergistic toxicity of ethyl formate with phosphine on *M. persicae*. This could be beneficial in terms of reducing fumigation time for this pest, which is known to be hard to control under low temperatures conditions without certain phytotoxic damages. For *T. urticae*, however, we could not obtain a synergistic effect. It is probably because of biological and physical differences between class Arachnida and Insecta. There should be more researches about mode of action of synergistic effect of EF and PH<sub>3</sub> need to be done on different insect pests. We strongly suggest that 2 h fumigation at low dose of EF mixed with PH<sub>3</sub> apply to the exported strawberry could also be extended to control other quarantine pests and offers alternative to methyl bromide for fruit and vegetable applications.

## ACKNOWLEDGEMENTS

This study was supported by grant from the Animal

and Plant Quarantine Agency of the Ministry of Agriculture, Food, and Rural Affairs of the Republic of Korea (Z-1543086-2017-19-01).

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