applied sciences

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A New Approach: Ethyl Formate Fumigation to Control *Bemisia tabaci* (Hemiptera: Aleyrodidae) in a Yellow Melon Vinyl House

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Featured Application: A new fumigation approach of ethyl formate in a vinyl house to control *B. tabaci*.

Abstract: Due to concerns over the resistance development to existing pesticides, chemical poisoning among farmers, and chemical residue on crops, sprung up a growing need to develop new pest control strategies for utilization in protected houses in Korea. A series of experiments tested a new technology using a fumigant, ethyl formate, in growing crops in the protected houses. It was revealed that the glasshouse was inadequate for the fumigation system using the fumigant since ethyl formate gas sharply decreased due to gas leaking through the gaps between the glass frames. On the other hand, the gas concentration was stable during the fumigation process. Experiments were also conducted to evaluate its phytotoxicity on cucurbits crops (yellow melon). The crops were fumigated at 20 °C in three fumigation schedules (2, 4, and 12 h). The results revealed that the developmental stages of yellow melon showed no sign of phytotoxicity in all conditions. However, the fumigation damaged the shoots of red pepper in higher humidity and at a longer duration. Interestingly, *Bemisia tabaci* were (100%) completely killed in all these conditions. Based on the results of the above experiments on the high efficacy on the control of *Bemisia tabaci* and zero phytotoxic effects of the ethyl formate fumigation on yellow melon, verification experiments for the effectiveness were conducted thrice in farmer's yellow melon vinyl houses and once in a farmer's cucumber vinyl house. Results demonstrated that ethyl formate fumigation for 2 h at 2 g m^{-3} concentration could 100% kill the adults of *Bemisia tabaci* with no phytotoxic effect on the crops. Therefore, we could conclude that the 2-h fumigation system with 2 g m^{-3} ethyl formate would be a new alternative to the existing chemical spraying methods.

Keywords: *Bemisia tabaci*; cucurbits; ethyl formate; fumigation; vinyl house

Citation: Kwon, T.H.; Park, C.G.; Lee, B.-H.; Jeong, I.-H.; Lee, S.-E. A New Approach: Ethyl Formate Fumigation to Control *Bemisia tabaci* (Hemiptera: Aleyrodidae) in a Yellow Melon Vinyl House. *Appl. Sci.* **2022**, *12*, 5173. https://doi.org/10.3390/app12105173

Academic Editor: Alexios Polidoros

Received: 8 April 2022
Accepted: 17 May 2022
Published: 20 May 2022

1. Introduction

Sweet-potato whitefly (SPW, *Bemisia tabaci*) was an insect pest with more than 900 host species, including various fruit vegetables globally [1]. SPW has more than 24 biotypes, including B and Q biotypes (B-ty and Q-ty), which were the most harmful and hard to control among the biotypes in vinyl house cultivation [2]. Since the first occurrence of B-ty in Korea in 1998 [3,4], it has spread throughout the southern area of Korea in 2005 [5].

Yellow melon (YM) is one of the major fruit vegetables grown in a vinyl house in Korea [6], cultivated in about 7633 ha of vinyl house area and occupying 14% of the total

domestic vinyl house acreage [7]. In YM cultivation in vinyl houses, SPW is one of the virus vectors that damage plants by sucking the sap from plant leaves, thus making the leaves shiny or with blackened sooty mold by producing honeydew. The income loss by the infestation of SPW was estimated as 5% (30 million USD annually) of the total income of farmers (612 million USD/7633 ha) in Korea, while farmers used 28 million USD for its control annually [7,8].

Although many insecticides, such as organophosphates, carbamates, pyrethroids, insect growth regulators, and neonicotinoids have been developed to control SPW, it experienced difficulty in becoming resistant due to indiscriminate use and the short generation time of the species [9,10]. Furthermore, current pesticides such as spinosad and imidacloprid to control SPW in vinyl houses are restricted during the fertilization season due to pollinators, such as honeybees [11]. Moreover, the application of pesticides was restricted due to the enforcement of maximum residue limits (MRLs) for registered pesticides and the introduction of a positive list system that regulated residues of 0.01 ppm for unregistered pesticides on various crops [12].

Another difficulty in applying insecticides for controlling SPW is the even distribution of the chemicals to every niche of the SPW [13]. As a result, the insecticides sprayed cannot reach every SPW target, especially those living on the undersides of leaves. Therefore, new pesticides and new application techniques must ensure effective and strategic pest management with consumer safety in controlling SPW in vinyl house farming. Through our previous studies, ethyl formate (EF) was currently recognized with the possibility to control insect pests on fruit vegetables in terms of having less issues, with no residues in final production [6].

EF is a naturally occurring substance in cheese, orange, and soil [14]. It is currently used in quarantine as an alternative to methyl bromide (MB) due to its insecticidal effect against stored insects [15], aphids [16], and flies [17]. EF was also used as an alternative to phosphine, which requires long usage in controlling stored grain insect pests due to its valuable attributes, such as its total control of insects [18]. Additionally, there are no regulations concerning residues using EF since it has been used as food additives such as flavoring agents for a long time and classified as "Generally Recognized as Safe" by the Food and Drug Administration of the USA [19]. Due to post-fumigation customer safety on numerous commodities, including fruits and vegetables, EF and its application have been adopted as alternative options in the chemical disinfestation method in quarantine and the post-harvest section.

Thus, we first evaluated the possibility of agricultural use of EF, especially during the growing season of YM in vinyl houses. In this research, we conducted (1) a preliminary study on insecticidal efficacy of EF fumigation to SPW adults in desiccators and phytotoxicity assessment of EF fumigation on yellow melon plants in small chambers, (2) a scaled-up study on insecticidal activity and phytotoxicity assessment of EF fumigation in a medium-size vinyl house, and (3) a field study using practical field trials on the efficacy of EF fumigation in farmers vinyl houses.

2. Materials and Methods

2.1. Insects and Yellow Melon Plants

The colony of SPW obtained from the National Institute of Agricultural Sciences, RDA, Korea, in 2019 was reared on a laboratory-grown tobacco plant without any insecticide selection until use in experiments at 25 ± 1 °C with 60 ± 10% RH and 16:8 h L:D. During the assessment of the phytotoxicity impact of EF, yellow melon (*Cucumis melo*) was seeded in flowerpots containing a horticultural substrate. Then, the yellow melon was grown for eight weeks from the seedling stage to the fruiting stage in the laboratory (27 ± 1 °C, 70 ± 10% RH, and 16:8 h L:D).

2.2. Fumigant (Ethyl Formate)

Liquid ethyl formate (Fumate™, 99%) was supplied from Safefume Inc. (Gangwondo, Korea). For the chamber (0.275 m^3) application, the Fumate™ was vaporized with natural air in a Tedlar® gas sampling bag (1l, SKC Inc., Covington, GA, USA). In the field trials, a portable vaporizer attached to a modified fan was used for vaporizing the EF gas into natural air.

2.3. Measurement of EF Concentration and Determination of CT (Concentration × Time) Products

To calculate *Ct* product, the concentrations of EF in fumigation desiccators and chambers were determined at 0.1, 1, 2, and 4 h after exposure using a gas chromatograph (Shimadzu GC 17A, Kyoto, Japan) equipped with a flame ionization detector (FID) after separation on a DB5-MS column (30 m × 0.25 mm i.d., 0.25 µm film thickness; J&W Scientific, Folsom, CA, USA). The oven temperature was constant at 100 °C, and injector and detector temperatures were 250 and 280 °C, respectively. Helium was used as a carrier gas at a 1.5 mL/min flow rate. The concentrations of EF were calculated based on the peak area against external EF standards. The calibration curve standards were prepared by spiking a known volume of liquid EF into a 1l Tedlar® gas sampling bag. The *Ct* products were calculated based on the following equation [20]:

$$Ct = \sum \frac{(C_i + C_{i+1})(t_{i+1} - t_i)}{2}$$

where C = concentration of fumigant (mg/L), t = time of exposure (h), i = order of measurement, and Ct = concentration × time product (g·h m^{-3}).

The efficacy trials were conducted using 6.8 L desiccators, sealed with a glass stopper equipped with a septum (Alltech Associates Australia, Cat. No. 15419, Sydney, Australia) [16]. The specific volume of each desiccator was measured by the weight of the water it held at 21 °C. A filter paper (Whatman No. 1) was inserted into the glass stopper to provide an evaporation surface for the injected liquid EF. The desiccators were tightly sealed with high vacuum grease. A magnetic stirrer was placed at the bottom of each desiccator to ensure the even distribution of fumigant. The phytotoxicity experiments for plants were carried out in 0.275 m^3 chambers, and the scheduled dose of EF was calculated using the equation:

$$V_f = \left(1 - \frac{T}{273}\right)\left(\frac{1.7 \times 10^4 \times C \times V}{P \times M \times N}\right)$$

where V_f = volume of fumigant at a specific dose (mL); T = temperature (°C); C = intended concentration of fumigant (g m^{-1}); V = volume of fumigation chamber (L); p = pressure (mm Hg); M = molecular weight of fumigant; N = purity of gas (%).

2.4. Insecticidal Efficacy of EF Fumigation to B. tabaci Adults in Desiccators

The efficacy of EF to adult SPW was evaluated in fumigation desiccators (6.8 L), where a magnetic bar was placed at the bottom for air circulation. The insect breeding dish (1 × 5.5 cm), inoculated with 20 SPW adults, was placed at the bottom of the desiccator. After sealing the desiccators, different doses of EF (0.1, 0.3, 0.5, 0.7, 0.9, 1.2, 1.5, 2.0 and 2.3 g m^{-3}) were vaporized into the respective desiccator. The desiccators vaporized with EF were placed in incubators at 29 ± 1 °C for 2, 4, and 12 h. The application doses were calculated using the equation reported [20]. After fumigation, the desiccators were opened and aerated for 1 h in a fume hood, whereas the control desiccators were not fumigated. The insect breeding dishes treated with EF were removed from the desiccators and kept in an incubating chamber (25 ± 1 °C, 60 ± 10% RH) until the mortality check. The mortality of SPW adults was determined 24 h after removal from the desiccators. All treatments, including the control, were replicated in triplicate.

2.5. Phytotoxicity Assessment of EF Fumigation on Yellow Melon Plants in Small Chambers

Phytotoxic damage to the seedlings stage (2 weeks after seeding), the flowering stage (4 weeks after seeding), and the fruiting stage (8 weeks after seeding) of yellow melon plants were assessed through an index of visible damage, chlorophyll content, and leaf color, until one week after fumigation in the laboratory (27 ± 1 °C, 70 ± 10% RH). Three potted yellow melon plants were transferred to a 0.275 m^3 chamber in which a data logger (TR-72U, T&D Co., Ltd., Matsumoto, Japan) was placed. Each chamber was fumigated with 2.0, 1.5, or 1.0 g m^{-3} of EF under 28 ± 2 °C and 90 ± 10% RH for 2, 4, and 12 h. After fumigation, the chambers were opened and aerated for 1 h naturally. Then, the yellow melon plants were moved from the chambers to the rearing room (27 ± 1 °C, 70 ± 10% RH). Phytotoxicity was determined over seven days after the 4 h fumigation. The overall phytotoxic index was measured using the following scale: 0 (no leaf damage), 1 (<5% leaves affected), 2 (5–25% leaves affected), 3 (25–50% leaves affected), and 4 (>50% leaves affected). The chlorophyll content and colors of five leaves per plant were measured using a chlorophyll meter (SPAD-502 Plus, Spectrum Technologies Inc., Bridgend, UK) and a colorimeter (TES 135A, Electrical & Electronic Corp., Taipei, Taiwan), showing Color L, Color a and Color b values, and then hue values calculated using the formula: [Color L × 2 + Color a × 2 + Color b × 2]/2, respectively. All measurements, including the control, were repeated in triplicate.

2.6. Insecticidal Activity and Phytotoxicity Assessment of EF Fumigation in a Middle Size Vinyl House

Insecticidal activity on SPW adults and phytotoxicity to yellow melon plants were evaluated in a 350 m^3 vinyl house (23 m × 3.8 m × 4.0 m) at Gyeongsang National University, Jinju, South Korea. Ninety-six yellow melon plants were transplanted 2 weeks after seeding into the vinyl house. Eight weeks after transplanting, the yellow melon plants were fumigated with EF 2.0 and 1.5 g m^{-3} using portable vaporizers for 2 and 4 h under 29 ± 1 °C, 90 ± 10% RH. During this fumigation, we used four vaporizers because each vaporizer can cover only 100 m^3. Moreover, to measure the EF concentration, sample lines for EF gas sampling were placed onto 6 spots (3.0, 1.5, and 0.8 m above soil surface at two midpoints) in the vinyl house. After vaporization, the concentration was determined at 0.5, 1.0, 2.0, and 4.0 h by collecting the EF gas using an air pump (DOA-P704-AC, Gast Manufacturing, Inc., Benton Harbor, MI, USA) from each sampling spot into a Tedlar® bag. Next, the amount of EF gas was then measured using the same gas chromatography mentioned above. At the end of fumigation, the vinyl house was opened and then aerated for 1 h naturally.

The effect of EF fumigation on SPW was evaluated. Twenty SPW adults were inoculated on a tobacco leaf disk in a breeding dish (4.5 cm diameter). In addition, six breeding dishes containing the whitefly adults were placed on the leaves of yellow melon at the six spots in the vinyl house before EF vaporization. The mortality of SPW adults was determined over 24 h after fumigation. It was considered dead if SPW adults did not move their appendages after touching a fine brush.

Assessments of phytotoxic damage to the three different growth stages of yellow melon plants were done for up to 1 week after vaporization under 27 ± 1 °C, 70 ± 10% RH. The overall visible damage index, chlorophyll contents, and colors of leaves were measured using the same methods mentioned above. After the 2 and 4 h EF fumigation, the gates of the vinyl house were opened and ventilated. To check safety for workers (fumigators), the EF concentrations in the air of the entrance and exit gates of the vinyl house were monitored at 5, 10, 20, 30, and 60 min after ventilation. All measurements were repeated three times.

2.7. Practical Field Trials on the Efficacy of EF Fumigation in Farmers' Vinyl Houses

Based on the promising results from the laboratory and scale-up vinyl house evaluations on insecticidal activity and phytotoxicity of EF fumigation, more practical assessments

on the EF fumigation were conducted in farmers' vinyl houses in triplicate. The first field trial was conducted in the vinyl house (580 m^3, 55 m × 3.5 m × 3.0 m) in Seongju county, South Korea, where the harvest of yellow melon fruits was completed with SPW occurring naturally on the leaves. EF fumigation was conducted for 2 h with a dose of 2.0 g m^{-3} using a portable vaporizer from 19:00 p.m. (21–32 °C, 80 ± 10% RH). EF sample lines were placed at 12 spots (4 spots each at 3.0, 1.5, and 0.8 m above the soil surface) to check the uniformity of EF concentrations in the vinyl house. Air collections from the sampling spots were made at 0.5, 2.0, 4.0, and 12.0 h after exposure. The EF concentrations in the vinyl house were determined by analyzing the air with GC-FID. The GC conditions used in this experiment were mentioned in the "Measurement of EF concentration and determination of CT (concentration × time) products" above. The effectiveness of EF fumigation to SPW adults was evaluated in two ways: (1) by comparing the number of SPW occurring naturally on leaves of the YM plant before and after treatments, and (2) by checking the mortality of SPW inoculated onto tobacco leaf disks in 9 breeding dishes. After completing the 12 h fumigation, an assessment of phytotoxicity of the yellow melon plants was made in the same way mentioned above. Again, all measurements were repeated.

The second field trial was carried out in the same vinyl house described in the first trial, except for the EF dose, fumigation time, and exposure time. EF was fumigated with a dose of 1.5 g m^{-3} for 4 h from 19:00 p.m. (27–30 °C, 90 ± 10% RH). After vaporization, the EF concentration was determined at 0.5, 2.0, and 4.0 h.

The third field trial was conducted in the larger vinyl house (1140 m^3, 100 m × 3.8 m × 3.0 m) than the first and second trials at Chilgok-gun county, South Korea, in which the harvest of yellow melon fruits was completed, and where SPW was occurring naturally on yellow melon leaves as in the cases of the prior trials. EF was fumigated for 2 h with the dose of 2.0 g m^{-3} by vaporizing the EF using a portable vaporizer from 19:00 p.m. (29–31 °C, 85 ± 15% RH. EF concentrations inside the vinyl house were determined by the same methods previously indicated at trials 0.5, 1.0, and 2.0 h after vaporization. In addition, the mortality of SPW adults and phytotoxicity to YM plants were assessed by the same methods in the prior trials.

2.8. Statistical Analyses

Mortalities of SPW were analyzed using ANOVA, and the means were separated based on LSD tests at a significance level of $p = 0.05$. Phytotoxicities to yellow melon plants were compared between EF-fumigated and non-fumigated control plants using *t*-tests at $p = 0.05$. All statistical analyses were carried out using SAS (ver. 9.4; SAS Institute Inc., Cary, NC, USA, 1998).

3. Results

3.1. Insecticidal Efficacy of EF Fumigation to B. tabaci Adults in Desiccators

Table 1 shows the effect of EF fumigation time against SPW adults for three different fumigation times (12, 4, and 2 h) at 29 ± 1 °C. The LCT values increased with fumigation time, from 1.67 g·h m^{-3} in 2 h fumigation to 7.65 in 12 h fumigation. This was equally the same in the cases of LCT_{50} and LCT_{70}.

Table 1. Effect of time of ethyl formate fumigation against *B. tabaci* at 29 ± 1 °C.

Fumigation Time (h)	LCt_{50} (95% CL, g·h m^{-3})	LCt_{70} (95% CL, g·h m^{-3})	LCt_{90} (95% CL, g·h m^{-3})	Slope ± SE	*df*	χ^2
2	0.41 (0.29–0.51)	0.63 (0.47–0.79)	1.67 (1.33–2.58)	3.24 ± 0.60	11	21.63
4	0.50 (0.18–0.79)	0.89 (0.47–1.23)	2.08 (1.60–2.71)	2.06 ± 0.39	18	31.06
12	1.89 (1.18–2.46)	3.35 (2.58–4.75)	7.65 (5.23–18.65)	2.11 ± 0.46	19	86.26

3.2. Phytotoxicity Assessment of EF Fumigation on Yellow Melon Plants in Small Chambers

The phytotoxicity assessment results for 12, 4, and 2 h EF fumigation to the different stages (seedling, flowering, and fruiting) of yellow melon plants are shown in Table 2.

Table 2. Phytotoxicity on yellow melon according to different fumigation times with ethyl formate in chambers in a laboratory.

Fumigation Time (h)	*Ct* Value (g·h m^{-3})	Growth Stage of Yellow Melon	Damage Index [a] (Mean ± SE)			Chlorophyll Content (Mean ± SE)			Hue Value [b] (Mean ± SE)		
			Control	Treatment	*p* Value [c]	Control	Treatment	*p* Value	Control	Treatment	*p* Value
2	1.9 ± 0.3	Seedling	0.0 ± 0.0	0.0 ± 0.0	-	29.7 ± 0.5	30.0 ± 0.2	0.56	38.8 ± 0.4	40.0 ± 0.4	0.38
		Flowering	0.0 ± 0.0	0.0 ± 0.0	-	28.8 ± 1.1	30.8 ± 0.4	0.12	39.8 ± 0.3	40.2 ± 0.5	0.63
		Fruiting	0.0 ± 0.0	0.0 ± 0.0	-	29.9 ± 0.3	30.8 ± 0.1	0.16	39.0 ± 0.2	40.9 ± 0.1	0.60
4	2.4 ± 0.3	Seedling	0.0 ± 0.0	0.0 ± 0.0	-	31.8 ± 1.2	32.3 ± 0.7	0.20	39.6 ± 1.3	41.8 ± 0.5	0.27
		Flowering	0.0 ± 0.0	0.0 ± 0.0	-	32.4 ± 0.7	32.6 ± 1.3	0.53	39.1 ± 1.3	41.8 ± 0.1	0.16
		Fruiting	0.0 ± 0.0	0.0 ± 0.0	-	36.6 ± 1.3	37.1 ± 1.1	0.48	40.3 ± 0.3	41.5 ± 0.2	0.53
12	8.9 ± 0.1	Seedling	0.0 ± 0.0	1.0 ± 0.0	0.02	22.5 ± 0.7	22.4 ± 1.3	0.61	40.5 ± 0.7	40.7 ± 0.3	0.60
		Flowering	0.0 ± 0.0	1.0 ± 0.0	0.02	31.4 ± 2.3	33.3 ± 0.7	0.13	40.0 ± 1.2	42.1 ± 1.1	0.53
		Fruiting	0.0 ± 0.0	1.0 ± 0.0	0.02	35.8 ± 1.7	36.7 ± 0.3	0.53	40.9 ± 0.3	41.8 ± 0.7	0.48

[a]: [Color L × 2 + Color a × 2 + Color b × 2]1/2. [b]: Damage index: 0 (no leaf damage), 1 (<5% leaves affected), 2 (5–25% leaves affected), 3 (25–50% leaves affected), 4 (>50% leaves affected). [c]: *t*-test. Fumigation temperature: 28 ± 2 °C, relative humidity: 90 ± 10%.

When the plants were fumigated with EF for 2 and 4 h, no significantly different phytotoxicity between the control and treatment existed in the three different developmental stages of yellow melon in terms of damage indices, chlorophyll contents, and hue values. The eventual *Ct* values were 1.9 and 2.4 g·h m^{-3} at 2 and 4 h fumigation at that time, respectively. However, when fumigated for 12 h (with an eventual *Ct* of 8.9 g·h m^{-3}), the damage index increased to one, showing burning symptoms on new young leaves of the yellow melon plants. In these cases, the chlorophyll content and hue values were not significantly different between the control and treatment in all developmental stages of yellow melon. This phytotoxicity result means that 12 h EF fumigation to control SPW can not be applied in vinyl house cultivation on yellow melon plants. Thus, we conducted only 2 and 4 h fumigations to follow scaled-up experiments.

3.3. Insecticidal Activity and Phytotoxicity Assessment of EF Fumigation in a Middle Size Vinyl House

To confirm the no phytotoxicity of EF fumigation on yellow melon plants under lab assessment, the phytotoxicity was evaluated again in a 350 m^3 vinyl house (Table 3). The EF *Ct* values were 2.0 ± 0.1 and 3.3 ± 0.7 g h m^{-3} in 2 and 4 h fumigation, respectively, which are slightly higher than the lab assessment (Table 3). No visible damage was observed in all growth stages of EF-fumigated plants in both fumigation times.

Table 3. Phytotoxicity to the three different growth stages of yellow melon by 2 and 4 h ethyl formate fumigation in a 350 m^3 vinyl house.

Fumigation Time (h)	*Ct* Value (g·h m^{-3})	Growth Stage of Yellow Melon	Damage Index [a] (Mean ± SE)			Chlorophyll Content (Mean ± SE)			Hue Value [b] (Mean ± SE)		
			Control	Treatment	*p* Value [c]	Control	Treatment	*p* Value	Control	Treatment	*p* Value
2	2.0 ± 0.1	Seedling	0.0 ± 0.0	0.0 ± 0.0	-	28.5 ± 0.8	29.0 ± 0.7	0.48	35.4 ± 0.3	36.1 ± 0.7	0.37
		Flowering	0.0 ± 0.0	0.0 ± 0.0	-	29.4 ± 1.1	31.6 ± 0.4	0.53	36.7 ± 0.7	39.6 ± 0.1	0.53
		Fruiting	0.0 ± 0.0	0.0 ± 0.0	-	29.2 ± 0.3	33.8 ± 0.1	0.43	39.0 ± 0.2	41.9 ± 0.1	0.47
4	3.3 ± 0.7	Seedling	0.0 ± 0.0	0.0 ± 0.0	-	29.8 ± 0.3	31.4 ± 1.1	0.53	37.2 ± 0.4	39.8 ± 1.1	0.48
		Flowering	0.0 ± 0.0	0.0 ± 0.0	-	32.8 ± 0.3	33.1 ± 0.7	0.53	39.6 ± 0.1	41.3 ± 0.6	0.56
		Fruiting	0.0 ± 0.0	0.0 ± 0.0	-	36.6 ± 1.3	37.1 ± 1.1	0.53	41.3 ± 0.3	42.5 ± 0.2	0.53

[a]: [Color L × 2 + Color a × 2 + Color b × 2]1/2. [b]: Damage index: 0 (no leaf damage), 1 (<5% leaves affected), 2 (5–25% leaves affected), 3 (25–50% leaves affected), 4 (>50% leaves affected). [c]: *t*-test. Fumigation temperature: 29 ± 1 °C, relative humidity: 90 ± 10%.

No significant difference existed in chlorophyll content and hue values between the control and fumigated yellow plants of all three growth stages. The EF concentration in the air of the entrance and exit gates was monitored according to the ventilation times to check EF safety levels. After fumigation with EF, the concentration was sharply decreased along with the ventilation time from 160 to 10 ppm at the entrance gate and from 123 to 10 ppm at the exit gate (Table 4). However, the concentration was less than 10 ppm from the start of ventilation when the house was fumigated for 4 h.

Table 4. Assessment of standby safety of ethyl formate levels * (mg/L) at entrance and exit gates of the vinyl house during 1 h ventilation after 2 and 4 h fumigations.

Time after Ventilation (min)	2 h Fumigation		4 h Fumigation	
	Entrance	Exit	Entrance	Exit
0	0.530 ± 0.053	0.407 ± 0.030	<0.033	<0.033
5	0.218 ± 0.026	0.242 ± 0.020	<0.033	<0.033
10	0.086 ± 0.009	0.119 ± 0.007	<0.033	<0.033
20	<0.033	<0.033	<0.033	<0.033
30	<0.033	<0.033	<0.033	<0.033
60	<0.033	<0.033	<0.033	<0.033

*: EF TLV: <0.331 mg/L.

3.4. Practical Field Trials on the Efficacy of EF Fumigation in Farmers' Vinyl Houses

We tested the efficacy of EF fumigation on the mortality of SPW and the phytotoxicity effect on the fruiting stage of yellow melon in farmers' vinyl houses three times with the respective fumigation times of 12, 4, and 2 h (Tables 5–7).

Table 5. The first vinyl house trial on the ethyl formate concentration and *Ct* values at the gas sampling sites in an EF-fumigated vinyl house, mortality of *B. tabaci*, and phytotoxicity to the fruiting stage of yellow melon plants.

Fumigation Time (h)		Bottom (0.8 m above Soil)	Middle (1.5 m from Soil)	Top (3.0 m from Soil)
0.5		1.5 ± 0.0	1.8 ± 0.3	1.6 ± 0.1
2.0		1.7 ± 0.0	1.9 ± 0.2	1.8 ± 0.1
4.0		1.1 ± 0.0	1.2 ± 0.1	1.1 ± 0.0
12.0		0.5 ± 0.0	0.7 ± 0.1	0.6 ± 0.0
Ct values (g h m^{-3})		8.2 ± 0.5	9.2 ± 1.8	9.7 ± 1.0
Mortality (%, Mean ± SE)	Control	0.0 ± 0.0	*p* value = 0.001	
	Breeding dish	100.0 ± 0.0		
	Control	0/10	-	
	Natural occurrence	11/11		
Phyto-toxicity (Mean ± SE)	Control	0.0 ± 0.0	*p* value = 0.02	
	Damage index [a]	1.0 ± 0.0		
	Control	38.3 ± 1.0	*p* value = 0.48	
	Chlorophyll content	39.7 ± 0.7		
	Control	36.8 ± 1.1	*p* value = 0.51	
	Hue value [b]	37.3 ± 0.7		

[a]: Damage index: 0 (no leaf damage), 1 (<5% leaves affected), 2 (5–25% leaves affected), 3 (25–50% leaves affected), 4 (>50% leaves affected). [b]: [Color L × 2 + Color a × 2 + Color b × 2]1/2. -: impossible to check. Fumigation temperature: 21–32 °C, relative humidity: 80 ± 10%.

In the first vinyl house trial (Table 5), the EF concentrations at the gas sampling spots tend to be decreased by the time after fumigation.

The EF concentrations at the bottom of the vinyl house were 1.5 ± 0.2, 1.7 ± 0.7, 1.1 ± 0.5, and 0.5 ± 0.3 g m^{-3} by 0.5, 2.0, 4.0, and 12.0 h EF fumigation, respectively; finally producing the *Ct* value of 8.2 ± 0.5 g h m^{-3}. Over time, the decrease in EF concentrations

was also shown at the middle and top spots inside the vinyl house. The *Ct* values were highest at the top spots (9.7 g h m^{-3}), followed by the middle (9.2 g h m^{-3}) and bottom spots (8.2 g h m^{-3}). With these EF concentrations (or *Ct* values), 100% of the SPW adults were killed, whether inoculated from laboratory-reared colonies or naturally occurring colonies.

Table 6. Ethyl formate concentration and *Ct* values of second field trial which effect *B. tabaci* and phytotoxicity to fruiting stage of YM plants inside a vinyl house by fumigation times.

Fumigation Time (h)		Bottom (0.8 m above Soil)	Middle (1.5 m from Soil)	Top (3.0 m from Soil)
0.5		1.6 ± 0.0	1.6 ± 0.0	1.6 ± 0.0
1.0		1.3 ± 0.2	1.3 ± 0.1	1.4 ± 0.3
2.0		1.2 ± 0.1	1.2 ± 0.1	1.4 ± 0.3
4.0		1.0 ± 0.2	1.3 ± 0.2	1.3 ± 0.2
Ct products (g h m^{-3})		4.8 ± 0.1	4.9 ± 0.1	5.1 ± 0.2
Mortality (%, Mean ± SE)	Control	0.0 ± 0.0	*p* value = 0.001	
	Breeding dish	100.0 ± 0.0		
	Control	0/11	*p* value = 0.001	
	Natural occurrence	10/10		
Phyto-toxicity (Mean ± SE)	Control	0.0 ± 0.0	-	
	Damage index [a]	0.0 ± 0.0		
	Control	37.2 ± 1.3	*p* value = 0.53	
	Chlorophyll content	37.1 ± 0.4		
	Control	40.5 ± 2.1	*p* value = 0.46	
	Hue value [b]	40.3 ± 1.3		

[a]: Damage index: 0 (no leaf damage), 1 (<5% leaves affected), 2 (5–25% leaves affected), 3 (25–50% leaves affected), 4 (>50% leaves affected). [b]: [Color L × 2 + Color a × 2 + Color b × 2]1/2. -: impossible to check. Fumigation temperature: 21–32 °C, relative humidity: 80 ± 10%.

Table 7. Ethyl formate concentration and *Ct* values of third field trial which effect *B. tabaci* and phytotoxicity to fruiting stage of YM plants inside a vinyl house by fumigation times.

Fumigation Time (h)		Bottom (0.8 m above Soil)	Middle (1.5 m from Soil)	Top (3.0 m from Soil)
0.5		1.3 ± 0.2	1.6 ± 0.1	1.6 ± 0.3
1.0		1.3 ± 0.0	1.3 ± 0.0	1.4 ± 0.0
2.0		1.2 ± 0.0	1.3 ± 0.0	1.3 ± 0.0
Ct products (g h m^{-3})		1.9 ± 0.3	2.1 ± 0.1	2.1 ± 0.3
Mortality (%, Mean ± SE)	Control	0.0 ± 0.0	*p* value = 0.001	
	Breeding dish	100.0 ± 0.0		
	Control	0/6	*p* value = 0.001	
	Natural occurrence	6/7		
Phyto-toxicity (Mean ± SE)	Control	0.0 ± 0.0	-	
	Damage index [a]	0.0 ± 0.0		
	Control	38.1 ± 0.7	*p* value = 0.53	
	Chlorophyll content	39.6 ± 0.3		
	Control	40.8 ± 1.3	*p* value = 0.36	
	Hue value [b]	42.1 ± 1.1		

[a]: Damage index: 0 (no leaf damage), 1 (<5% leaves affected), 2 (5–25% leaves affected), 3 (25–50% leaves affected), 4 (>50% leaves affected). [b]: [Color L × 2 + Color a × 2 + Color b × 2]1/2. -: impossible to check. Fumigation temperature: 21–32 °C, relative humidity: 80 ± 10%.

There was negligible visible damage to new leaves of yellow melon plants. but no significant difference in the chlorophyll content and hue values between control and fumigated plants.

In the other two trials (Tables 6 and 7), the EF concentration after a fumigation at the gas sampling spots showed a similar decreasing tendency to the first trial. However, the

final *Ct* values in the second and third trials were lower than those in the first trial, possibly because of the shorter fumigation times of 4 and 2 h in both problems, respectively. The *Ct* values at the bottom, middle, and top spots of the vinyl houses were 4.8 ± 0.1, 4.9 ± 0.1, and 5.1 ± 0.2 g h m^{-3} in the second trial and 1.9 ± 0.3, 2.1 ± 0.1, and 2.1 ± 0.3 g h m^{-3} in the third trial, respectively. SPW adults from two colonies were 100% killed by the fumigations in both trials. No phytotoxicity in visible damage, chlorophyll content, and hue value were shown in both trials.

4. Discussion

Fumigation is a gas-type substance that quarantine pests in enclosed spaces and EF has been studied as quarantine fumigants to control various quarantine insect pests of commodities, such as fruits [21–23], vegetables [24], nursery plants [25], and mushrooms [17]. However, this fumigant EF was first studied to control general agricultural pests in vinyl houses instead of quarantining pests in quarantine containers. It is also known to have low mammalian toxicity and breaks down rapidly, thereby leaving no residues in the environment [18].

As a result, it was confirmed that the insecticide rate of more than 90% for SPW was established with a weak amount of 2 g m^{-3} of ethyl formate in the YM vinyl house of the crop, and the YM had no phytotoxic damage. Therefore, our results showed EF fumigation has the potential to control SPW in vinyl houses cultivating YM plants. The fumigant has an insecticidal effect when vaporized in a space sealed and exposed to insect pests in gaseous form, which means that the longer the exposure time, the higher the insecticidal effect [26]. Furthermore, the fumigant uses the concept of CT (Concentration × time) products to check the mortality relation against insect pests. Our result of EF efficacy to SPW showed $LCt_{90\%}$ of 2 h fumigation at a high concentration of EF was lower than the 12 h fumigation at a low concentration of EF.

EF is a fumigant that is usually used on perishable commodities, such as fruits and vegetables alternative to MB [27], whose schedule was phased out due to the ozone-depleting effect under the Montreal Protocol [28], its phytotoxic damage to perishable commodities [29], and chronic toxicity to human [30]. EF is also used on stored grain to control stored grain pests, such as *Sitophilus oryzae*, *Tribolium castaneum*, and *Rhyzopertha dominica* [31] and is an alternative to phosphine (PH_3) due to resistance causing the need for long exposure times [32]. Our experiment showed the first attempt to use EF for agricultural purposes, not just quarantine.

Frequent use of insecticides or acaricides accelerates the development of resistance to these chemicals. For example, neonicotinoid insecticides, such as thiamethoxam and imidacloprid, are commonly used to control whitefly and aphids, and resistance against these insecticides has been reported [33]. In addition, resistance to spinosad, frequently used to control thrips, has been reported [34]. However, insect resistance to EF has not yet been reported, which highlights the possibility of using EF as a greenhouse insect pest disinfectant in the future. Conventional insecticides are evenly sprayed throughout the vinyl house. However, these conventional methods cannot reach secluded spots, cracks, and crevices in the vinyl house or sometimes at the ventral leaf surfaces. On the contrary, EF fumigation can reach every out-of-the-way spot and every pest inside the fumigated vinyl house. Fumigation may also be safer for workers because they can avoid direct exposure to the gas.

Thus, more studies are needed to evaluate the efficiency of EF fumigation at different developmental stages of various agricultural insect pests and to elucidate the phytotoxic effects of EF fumigation on various agricultural crop plants. To conclude, the results of this study offer new insight into using EF fumigation to totally or partially replace currently used spray insecticides or acaricides in protected farming of YM. In a previous study reported by Jeong et al. (2020) [35], ethyl formate did not show any phytotoxicity when they performed a fumigation study using a small chamber of 0.275 m^3. They also determined phytotoxicities on other agricultural crops, such as eggplant, crop peppers,

and tomatoes, under the treatment of ethyl formate with two different exposure times of 2 and 4 h and complete control conditions on B. tabaci adults with 2.0 g EF/m^3 and 1.5 g EF/m^3, respectively [35]. In this report, the authors found that temperature and humidity conditions induced different phytotoxicities according to plant species when ethyl formate was applied [35].

This study is the first attempt, globally, to examine the efficacy and weakness of the drug through indoor experiments to confirm the applicability of liquid fumigants to facility pests. Since the fumigant is treated in gaseous form, it can increase its control efficiency than insecticide sprayed in powdered form to control micro-pesticides, such as beetles and aphids hidden in narrow micro-spaces between smuggled crops. Additionally, if a fumigation treatment system is introduced, its application to smart facility cultivation can reduce the time and cost. Ethyl formate, a fumigant used for quarantine, is considered a relatively safe substance in terms of residual problems. When these ethyl formates were applied to live crops, it was found that pests could be controlled without weakening the crops. Based on the results of this study, it is necessary to evaluate its effectiveness by applying it to facilities where the actual crops are planted, considering various factors, such as facility conditions and cultivation environment. On the other hand, it was also found that ethyl formate treatment may cause weakness under specific environmental conditions [35]. Given the multiple drugs having excellent control effects but have not overcome this weakness, such a problem in the ethyl formate drug must be mitigated.

5. Conclusions

It was necessary to develop new, proper strategies for controlling insect pests in agricultural facilities due to a dramatic increase in insecticide resistance in major insect pests, toxicological issues to workers, and residual properties of pesticides in crops. With this regard, we studied a new application of ethyl formate, a safe fumigant, to control *B. tabaci*, which has severely damaged yellow melon (YM) in vinyl houses. The LCT_{90} values of 2, 4, and 12 h EF fumigation against *B. tabaci* were 1.67, 2.08, and 7.65 g h m^{-3}, respectively. As for the LCT_{90} values of *B. tabaci* by fumigation times on YM, 2 and 4 h EF fumigation treatments had no phytotoxic effects on YM. Based on these results, high efficacy of ethyl formate was found to control *B. tabaci* adults and had no phytotoxic effects on YM in a vinyl house. Therefore, EF fumigation for 2 h with a 2 g m^{-3} concentration level could control against over 90% *B. tabaci* with no phytotoxic effects on YM. It would be a new alternative tool for the currently used pesticides.

Author Contributions: Conceptualization, T.H.K., B.-H.L. and S.-E.L.; methodology, T.H.K., B.-H.L. and I.-H.J.; software, T.H.K.; validation, B.-H.L., I.-H.J. and S.-E.L.; formal analysis, T.H.K. and B.-H.L.; investigation, C.G.P., B.-H.L. and S.-E.L.; resources, T.H.K. and C.G.P.; data curation, T.H.K., B.-H.L., I.-H.J. and S.-E.L.; writing—original draft preparation, T.H.K., B.-H.L. and S.-E.L.; writing—review and editing, T.H.K., C.G.P., B.-H.L., I.-H.J. and S.-E.L.; visualization, T.H.K. and S.-E.L.; supervision, B.-H.L. and S.-E.L.; project administration, S.-E.L.; funding acquisition, B.-H.L. and S.-E.L. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET) through the Crop Viruses and Pests Response Industry Technology Development Program, funded by the Ministry of Agriculture, Food and Rural Affairs (MAFRA) (321098-3).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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