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RESEARCH

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Optimizing ethyl formate fumigation in greenhouse cucurbit crops for efficient control of major agricultural pests, *Myzus persicae* and *Thrips palmi*

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Abstract

Background Ethyl formate (EF) is naturally occurring volatile compound used as quarantine fumigant for pest control. Recently, conversion of uses of EF was tried from quarantine to agricultural field due to its promising efficacy. However, there is a lack of studies on the residue pattern on crops and soil and the phytotoxic mechanism of EF in greenhouse environment. This study aimed to evaluate the efficacy, residue analysis, and phytotoxicity of EF fumigation in controlling *Myzus persicae* and *Thrips palmi*, on cucurbit crops and establish an optimized fumigation strategy for use in greenhouses.

Results The results showed that EF was more effective against *M. persicae* than against *T. palmi*. Residue analysis indicated that EF rapidly decomposed and was not retained after 30 min in leaves and 2 h in soil after fumigation, suggesting the potential for residue-free pest control. Phytotoxicity test revealed that watermelon was the most sensitive crop to EF, and H_2O_2 accumulation was observed above a concentration of 7.5 g/m^3. A strategy to reduce phytotoxicity with sodium bicarbonate during fumigation showed promising results in reducing phytotoxic effects on the crops. The optimized EF fumigation with 6 g/m^3 was applied in a greenhouse, resulting in 100% and 40% mortality of *M. persicae* and *T. palmi*, respectively, with no notable phytotoxicity and EF residue in the treated crops and soil.

Conclusion This study demonstrates that optimized EF fumigation can be an environmentally sustainable method for controlling pests in greenhouses, paving the way for improved pest management practices and sustainable agriculture. Further research is needed to validate these findings and explore the potential of EF fumigation for other crops and pests.

Keywords Fumigant, Ethyl formate, Residue analysis, Phytotoxicity mechanism, Sodium bicarbonate

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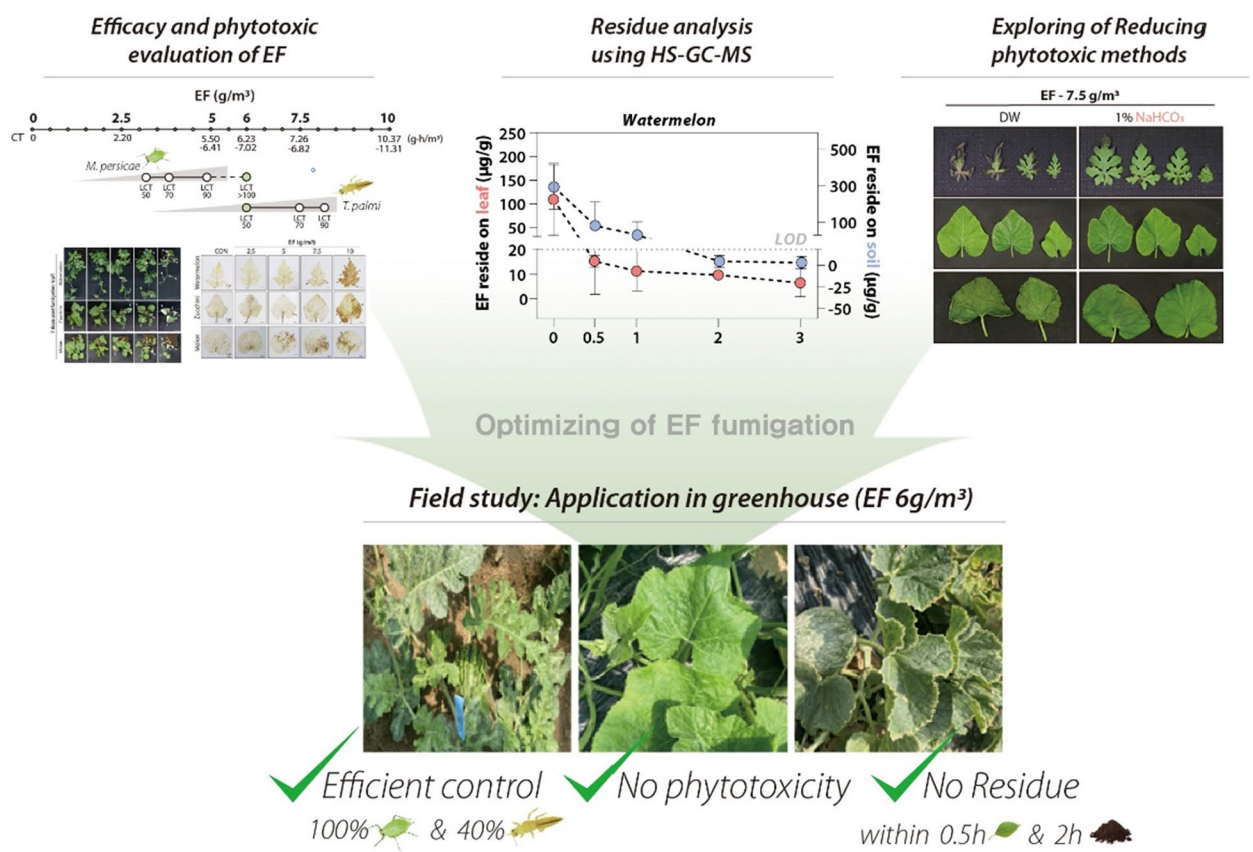
Graphical Abstract



Background

Greenhouses provide an artificially closed agricultural system for continuous crop production, mitigating environmental issues such as low sunlight in some seasons, humidity, and unpredictable weather conditions caused by climate change [1–3]. However, the extensive use of pesticides in greenhouses, essential for controlling pests such as aphids, thrips, whiteflies, and mites [4], can have negative impacts such as pesticide residues, development of pesticide resistance, and contamination of the soil and aquatic environments [5–7].

As environmental concerns in agriculture have become increasingly important worldwide, it is crucial to consider more sustainable methods for greenhouse pest control [6–8]. One promising approach is using alternative fumigants with less environmental impact than traditional pesticides. Ethyl formate (EF) is an alternative method that has shown potential for effectively controlling pests while minimizing environmental contamination [9]. EF is a naturally occurring substance that is safe for use as a flavoring agent in the food industry and is easy to decompose to formic acid and ethanol by water [9, 10]. Besides its natural properties, EF has been identified as a promising alternative to methyl bromide (MB), a worldwide quarantine fumigant, in quarantine applications [11, 12]. The use of EF as a phytosanitary fumigant is highly effective against a wide range of pests, including *Bemisia tabaci*, *Curculio sikkimensis, Drosophila suzukii*, *Pseudococcus comstocki*, and *Planococcus citri* [11, 13–16].

Although EF has many advantages, a remarkable disadvantage is its potential to cause phytotoxicity in plant leaves, rather than in fruit products [12, 17–20]. Leaf damage caused by EF can directly affect crop quality and quantity, making it a major issue in greenhouse pest control. It is essential to understand the factors affecting efficacy, phytotoxicity, chemical stability, and decomposition properties of EF in greenhouse environments. These factors include the concentration of EF used in fumigation, exposure duration, crop species, the growth stage

of the plant, and environmental conditions such as temperature and humidity. The first application trials of EF in an agricultural environment were conducted recently for controlling whiteflies in a yellow melon vinyl house [14]. However, further studies are needed to understand the use of EF in greenhouse pest control and minimize its phytotoxicity and environmental impact.

Therefore, this study aimed to evaluate the optimized EF fumigation method in the greenhouse environment for controlling two major insect pests, *Myzus persicae* (Sulzer) and *Thrips palmi* (Karny), in major cucurbit crops, including watermelon, zucchini, and melon. We investigated (1) EF residue using headspace gas chromatography–mass spectrometry (HS–GC–MS) for prediction of environmental impact, (2) phytotoxicity of EF to three cucurbit crops (watermelon, zucchini, and melon) depending on the developmental stage at laboratory and field scales, and (3) strategies for reducing the phytotoxicity of EF for broadening the scope of its use at high concentrations. Our findings provide valuable insights into how efficient EF fumigation can enhance environmental sustainability in protected farming.

Methods

Chemicals

In this study, EF (97% purity, analytical grade) and 3,3'-diaminobezidine (DAB) were purchased from Sigma–Aldrich Co. (St. Louis, MO). Sodium bicarbonate (Extra pure grade), ethanol (Guaranteed reagent grade), and acetonitrile (HPLC grade) was purchased from Duksan (Seoul, Republic of Korea). *n*-hexane (Guaranteed reagent grade) was purchased from DAEJUNG Chemical&Metals (Siheung, Republic of Korea). For field experiments, Fumate (liquid EF 99% purity) was supplied by SAFEFUME (Hoengseong, Republic of Korea).

Insect and plant materials and growth conditions

The green peach aphid, *Myzus persicae* Sulzer (Insecta: Hemiptera: Aphididae) was reared on *Nicotiana tabacum* (L.), and *Thrips palmi* Karny (Insecta: Thysanoptera: Thripidae) was raised on *Cucumis sativus* as host plants without any insecticide use under laboratory conditions. Watermelon (*Citrullus lanatus* Schrad.), Korean zucchini (*Cucurbita moschata* Duch.), and melon (*Cucumis melo* L.) were purchased from Nongwoo Bio Co., Ltd. (Suwon, Republic of Korea), ASIA SEED Co., Ltd. (Seoul, Republic of Korea), and the Kiban Co. Ltd. (Ansung, Republic of Korea), respectively. The three crops were cultivated from seeds to fruiting stages under laboratory conditions. The growing conditions of insects and plants were as follows: growth room under 60% relative humidity, 25 ± 1 ℃ with photoperiod conditions of 16:8 (light: dark). After fumigation, the plants were maintained under the same conditions during the phytotoxicity evaluation period.

EF fumigation against the test insects and evaluation of phytotoxic effects

Efficacy of EF was evaluated using adult *M. persicae* and *T. Palmi.* The insect breeding dish (1 × 5.5 cm), was inoculated with 30 mixed-sex-age adults of each insect and *N. tabacum* leaves in triplicate. In 2 h fumigation, different doses of EF were fumigated at concentrations of 0.1–10 g/m^3 in a 6.8 L-desiccator. For phytotoxic evaluation, EF fumigation was conducted at concentrations of 2.5, 5, 7.5, 8, and 10 g/m^3 for 2 h at 25 ± 1 °C, using three individuals per crop for each EF fumigation condition. Fumigations were conducted in a 0.275 m^3 fumigation chamber. Each desiccator was equipped with fans to ensure good mixing of the fumigant. The control group was not exposed to EF. To measure the concentration of EF in the atmosphere, the gas in the 0.275 m^3 fumigation chamber was sampled in a 1 L-Tedlar bag at time intervals of 0.5, 1, and 2 h after the onset of fumigation treatment and analyzed using gas chromatography with a flame ionization detector (GC-FID, Agilent 6890N, Agilent Technologies Inc., Santa Clara, CA) and an HP-1 column (30 m length × 250 μm internal diameter × 0.25 μm film). The oven, injector, and detector temperatures were maintained at 100 °C, 250 °C, and 290 °C, respectively. The EF concentration of the sampled gas was calculated based on the peak area against external standards. Eq. (1) was used to calculate the concentration–time (CT) values [21]. LCT (Lethal concentration × time) values were calculated using SAS (ver. 9.4; SAS Institute Inc., 1998).

$$\mathrm{CT} = \sum (C_i + C_{i+1})(t_{i+1} - t_i)/2 \quad (1)$$

where C is the fumigant concentration (g/m^3); t is the time of fumigation (h); i is the order of measurement; CT is the concentration × time product (g h/m^3).

Evaluation of post-fumigation EF-residue

To determine the analytical condition of EF using gas chromatography–mass spectrometry (GC–MS, Agilent 5973N, Agilent Technologies Inc., Santa Clara, CA), Scan mode was performed using the standard chemical in organic solvents (*n*-hexane or acetonitrile), and selected *n*-hexane as a suitable organic solvent for confirming the retention time (RT) of EF (RT: 1.44 min) and predictable decomposition product of ethanol (RT: 1.36 min) (Additional file 1: Fig. S1). The analysis conditions of GC–MS included maintaining the oven temperature at 60 °C for 3 min, followed by an increase of temperature by 45 °C per minute up to 230 °C and maintaining the temperature for 1 min. Helium was used as the carrier gas, and

the total flow rate was set at 17.2 mL/min. The product ions of EF were set at 45.2 for quantitative ions and 46.1 for qualitative ions (Additional file 1: Fig. S1). The SIM mode analysis was conducted to determine the residual concentration of EF using the set ion values. EF residue analysis on leaves and the soil was performed by connecting Headspace Autosampler (Agilent's G1888, Agilent Technologies Inc., Santa Clara, CA) to GC–MS (HS–GC–MS). The HS analysis was carried out at an oven temperature of 70 °C, a loop temperature of 75 °C, a transfer line temperature of 80 °C, an equilibration time of 0.2 min, and an injection time of 0.5 min. A mass of 1 g of each leaf or soil sample was placed in a headspace vial and analysis was conducted immediately without a preparation process for minimization of the effects of EF hydrolysis. In this regard, no recovery test was performed. A standard curve reflecting the matrix effect was established for each 1 g of crop and soil adding the range of 10, 50, 100, 250, 500, 1000, and 2000 μg EF in n-hexane using a 10 μL syringe. The limit of detection (LOD) and limit of quantification (LOQ) of each are shown in Additional file 1: Table S1.

For laboratory-scale EF residue evaluation, three individuals of each crop at the flowering stage were individually placed in a separate 0.275 m^3 fumigation chamber and fumigated for 2 h at a high concentration (8 g/m^3 EF for reaching the CT value of over 10 g h/m^3, same with > LCT_{90} for *T. Palmi*). After opening, 1 g of soil and leaf was immediately sampled into the headspace vial before the vial cap was sealed using a crimper. The residual amount of EF was determined by HS–GC–MS at 0 h, 0.5 h, 1 h, and 2 h post-fumigation (hpf).

Post-fumigation EF-phytotoxicity evaluation

Phytotoxic effects of EF on the crops were evaluated at different developmental stages (seedling, flowering, and fruiting) or EF concentration over a period of 2 h at 25 ± 1 °C. The phytotoxicity of EF was evaluated at 0 (Control), 2.5, 5.0, 7.5, and 10 g/m^3 using individuals of each crop at the flowering stage. After EF fumigation, the desiccator was opened and ventilated for 30 min. Crop individuals were transferred to the growth room (25 ± 1 °C) and maintained until 21 days post-fumigation (dpf), the end of observation. Phytotoxicity was evaluated by measuring the amount of chlorophyll and weight and recording visual observations. Photographs were taken at 3 and 7 dpf, and chlorophyll was measured at 7 and 21 dpf using SPAD-502 plus (Konica Minolta Inc., Tokyo, Japan). At 21 dpf, to compare the growth of individuals after EF fumigation, the shoot system of each plant was cut, and its weight was measured. Phytotoxicity was evaluated using the following phytotoxic evaluation criteria: 0 (no leaf damage), 1 (< 5% leaves affected), 2 (5%–25% leaves affected), 3 (25–50% leaves affected), 4 (> 50% leaves affected), and 5 (> 90%, severe phytotoxicity). Chromaticity was calculated with [{(Color L^2) + (Color a^2) + (Color b^{*2})}/2]. To confirm reactive oxygen species (ROS) in EF concentration-dependent responses, the leaves of the fumigated individuals were stained with DAB to detect H_2O_2. The DAB staining solution contained 10 mM Na_2HPO_4, 0.05% Tween-20, and 1 mg per ml DAB solution was filtered overnight (about 16 h) in the dark. Leaves were washed in a bleaching solution (ethanol:acetic acid:glycerol = 3:1:1) at 90 °C for 30 min to remove chlorophyll before they were photographed.

Evaluation of pre-treatment with sodium bicarbonate sprays on phytotoxicity after EF fumigation

Crops were pre-treated with sodium bicarbonate ($NaHCO_3$) to reduce phytotoxicity damage caused by EF fumigation. Using distilled water (DW) for dilution, 0.5% (pH 8.2) and 1% (pH 8.2) $NaHCO_3$ solutions were made and sprayed on the leaves of crops before fumigation. After that, the plants were air-dried for 1 h to remove moisture. One control group was sprayed with DW, and the other control group was treated with 0.5% or 1% $NaHCO_3$ only to check for the side effects of $NaHCO_3$. Crops were fumigated with EF at concentrations of 7.5 and 10 g/m^3, which caused mild and severe phytotoxicity, respectively.

Practical field study for optimizing EF fumigation in greenhouse

A farmer's greenhouse (L = 25 m × B = 4 m × H = 3.4 m = 3 40 m^3) was rented at Sancheong of the Republic of Korea (35°21′ 06.8" N 127° 56′ 18.1" E, and 87 m a.s.l.) and the crops were sown at an interval of two weeks to acquire the three developmental stages during fumigation exposure. The greenhouse study was conducted for 4 months (May–August) of 2022 summer. Based on the efficacy and phytotoxic effect of EF, a concentration of 6.0 g/m^3 EF was chosen for obtaining the CT value of 6.0 g h/m^3 (same with > LCT_{90} value for *M. persicae* and > LCT_{50} for *T. palmi*) for low phytotoxicity. EF fumigation was initially conducted at 25 ± 1 °C and 70% humidity, and the temperature and humidity during the experiment were recorded. EF sampling points were placed at 9 spots (3 spots each at 0.5 m (bottom), 1.5 m (middle), and 3.0 m (Top) above the soil surface) for sampling using a 1 L-Tedlar bag with a vacuum pump. Air was sampled at 0.5, 1, and 2 h after fumigation and analyzed by GC-FID following the method described in "EF fumigation against the test insects and evaluation of phytotoxic effects" sections. Leaf and soil sampling was conducted at 1 hpf and analyzed following the method described in "Evaluation of post-fumigation EF-residue" section. At 7 dpf,

phytotoxic evaluation was performed using SPAD-502 plus (Konica Minolta Inc., Tokyo, Japan) and a colorimeter (TES-135A, TES Electrical Electronic Corp., Taiwan) to determine the phytotoxic index as described in "Post-fumigation EF-phytotoxicity evaluation" section.

Results

Efficacy and phytotoxicity of EF against two major agricultural pests and three cucurbit crops

The LCT_{50}, LCT_{70}, and LCT_{90} values of EF on *M. persicae* were 3.04, 3.87, and 5.48 g h/m^3, respectively, for the adult stage with fitted slopes of 5.00 ± 0.62 for 2 h fumigation (Table 1). The LCT_{50}, $LCT_{70,}$ and LCT_{90} values of EF on *T. palmi* were 6.23, 8.06, and 9.89 g h/m^3, respectively, for the adult stage fitted slopes of 2.30 ± 0.40. EF was about two times more effective for controlling *M. persicae* than for controlling *T. palmi* (Table 1). Phytotoxicity evaluation of EF was conducted at a laboratory scale based on the concentration and LCT_{90} value of each pest to predict maximum phytotoxicity (Table 1 and Fig. 1).

At the concentration of 5 g/m^3 (CT value of 6.41 g h/m^3), controlling over 90% *M. persicae*, there was no difference in three indices including phytotoxic index, chlorophyll content, and chromaticity in all developmental stages of the three crops (Fig. 1a, Additional file 1: Fig. S2 and Table S2). However, a CT value of 10.14 g h/m^3 for controlling over 90% *T. palmi* highly affected leaves causing them to wither in all three developmental stages of the crops (Fig. 1b, Additional file 1: Fig. S3 and Table S2). Among the three cucurbit crops, watermelon was the most sensitive to EF fumigation, regardless of the stage of development. Individuals died completely with no new leaves regenerated in the

Table 1 Lethal concentration–time (LCT) of ethyl formate (EF) on *Myzus persicae* and *Thrips palmi* at 25 ℃

Insects	Stage	LCT_{50} (95% CI)	LCT_{70} (95% CI)	LCT_{90} (95% CI)	Slope ± SE	*df*	x^2
Myzus persicae	Adult	3.04 (2.67–3.46)	3.87 (3.40–4.55)	5.48 (4.64–7.03)	5.00 ± 0.62	7	27.37
Thrips palmi	Adult	6.23 (5.71–7.02)	8.06 (7.76–8.31)	9.89 (8.27–12.14)	2.30 ± 0.40	8	26.42

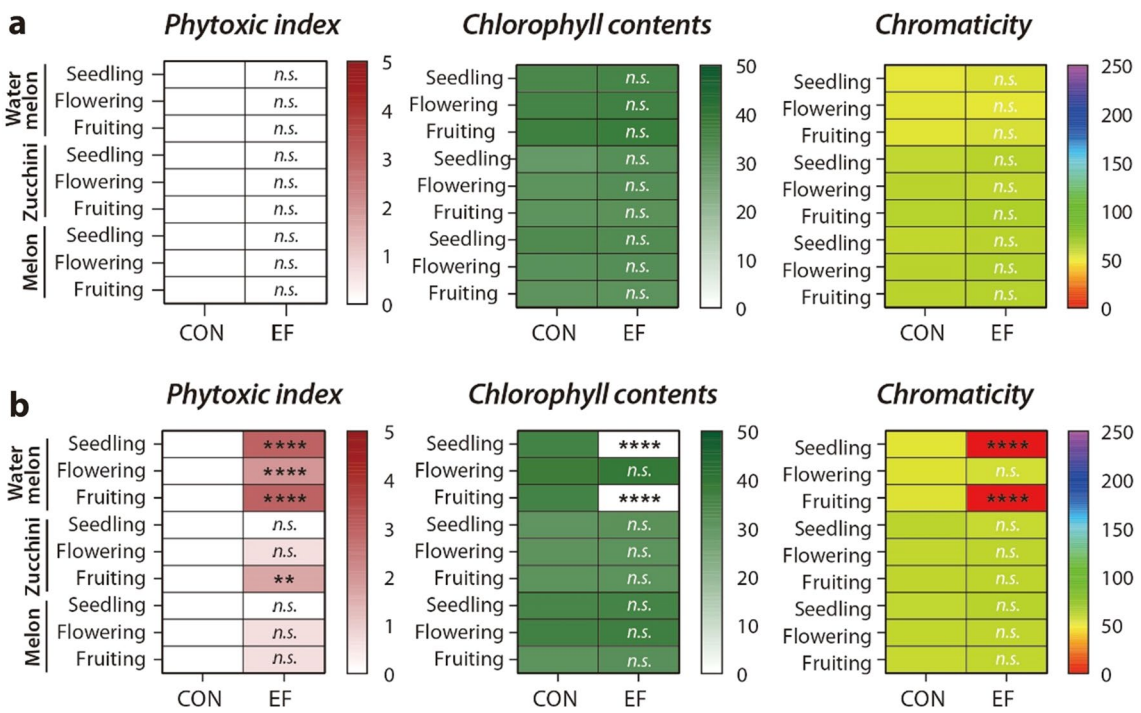


Fig. 1 Phytotoxic effect screening of ethyl formate (EF) towards three cucurbit crops according to developmental stages. EF fumigation was conducted using 0.275 m^3 fumigation chamber in Lab-scale with 5.5% loading ratio (w/v). Phytotoxic index, chlorophyll contents, and chromaticity at 7 days post-fumigation [depending on the target pest, (**a**) *Myzus persicae* (EF conc. 5 g/m^3, CT value 6.41 g h/m^3) (**b**) *Thrips palmi* (EF conc. 8 g/m^3, CT value 10.14 g h/m^3)]. Phytotoxic index = 0 indicates no leaf damage; 1 indicates < 5% leaves affected; 2 indicates 5–25% leaves affected; 3 indicates 25–50% leaves affected; 4 indicates > 50% leaves affected; and 5 indicates > 90%, severe phytotoxicity. Statistical differences were analyzed with Student's *t* test between control (CON) and EF ($n = 3$). ** < 0.01, **** < 0.001, and *n.s.*, not significant

seedling and fruiting stages of the watermelon (Additional file 1: Fig. S3).

Establishing analytical method and residue evaluation of EF on crop leaves and soil using HS–GC–MS

EF easily and rapidly hydrolyzes into formic acid and ethanol under humid conditions (Fig. 2a) [10]. Therefore, it is necessary to determine both EF and ethanol in residue analysis. For quantitative residue analysis using GC–MS, *n*-hexane was a more suitable organic solvent than acetonitrile for good separation and peak shape of EF and ethanol (Fig. 2b). The average basal EF content without EF fumigation was 30.8 μg/g for watermelon, 120.5 μg/g for zucchini, 18.5 μg/g for melon leaf, and undetectable in the soil (Fig. 2c, d and Additional file 1: Fig. S4). Therefore, a standard curve of EF for HS–GC–MS was established with 1 g of leaf or soil to reflect the matrix effect. Linearity was expressed as R^2, and the values for soil, watermelon, zucchini, and melon leaf, were 0.9938, 0.9945, 0.9932, and 0.9939, respectively (Fig. 2e), and the results mean it is suitable for determining EF in the leaf and soil samples.

Determination of EF residual pattern on leaf and soil in lab-scale experiment

Due to the rapid decomposition of EF [10, 22], understanding the residual pattern of EF in early timing after ventilation is essential for evaluating the residue of EF in the leaf or soil in laboratory-scale experiments (Fig. 3a). We selected the concentration of 8 g/m^3 for 2 h of EF for residue pattern evaluation under the condition reached LCT_{90} for *T. palmi* (Table 1). Soon after ventilation, the EF residue in the leaves of the three crops (watermelon, zucchini, and melon) were 109, 90.7, and 49.2 μg/g, respectively. The residue quickly decreased to below LOD after 0.5 h after ventilation (Fig. 3b, d, and f). On the other hand, EF residue was 3–20 times higher in each crop's soil than in the crop leaf, and the values of residue in the soil were 309.7 μg/g for watermelon soil, 937.43 μg/g for melon

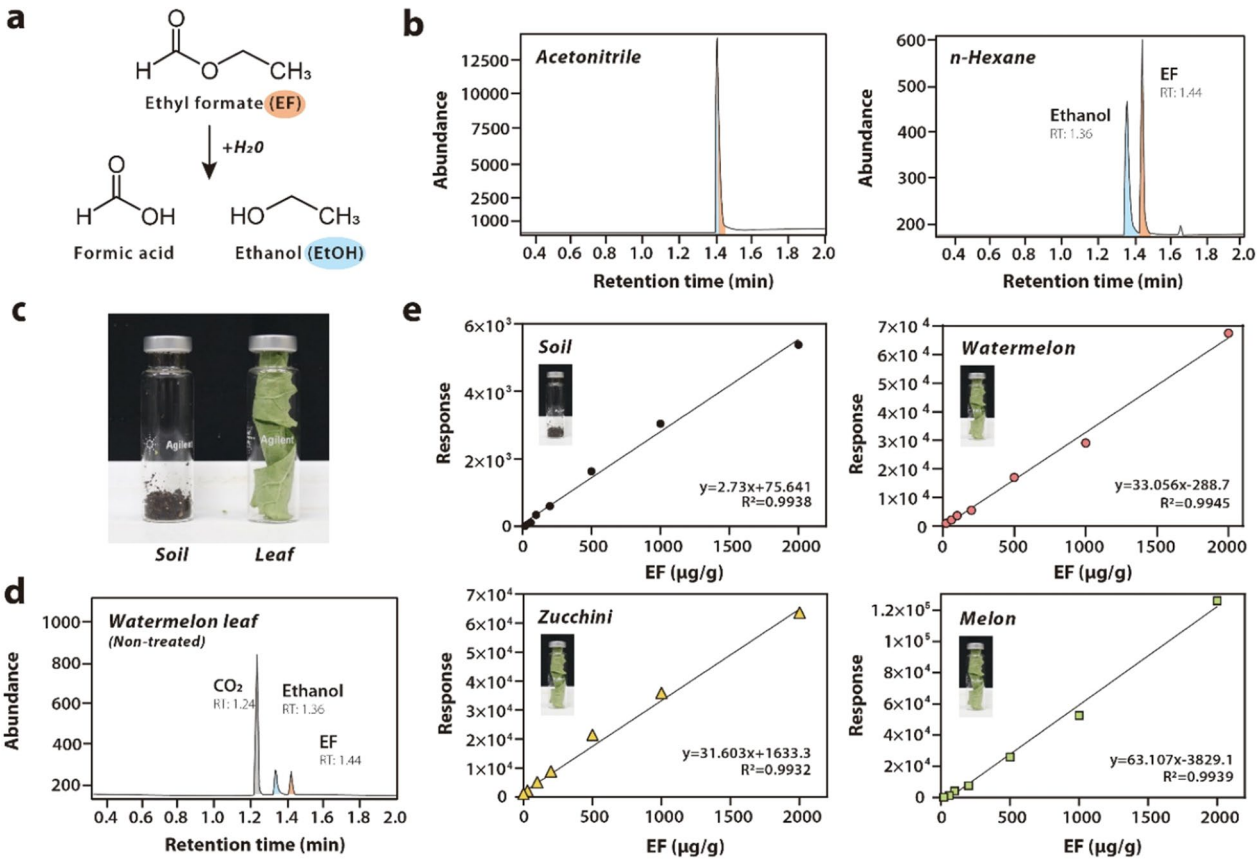


Fig. 2 Establishment of residue evaluation of ethyl formate (EF) using headspace gas chromatography–mass spectrometry (HS–GC–MS). **a** Hydrolysis reaction of EF, **b** solvent optimization for separation between ethanol (EtOH) and EF using acetonitrile or n-Hexane. **c** 1 g of soil or leaf in headspace vial for autosampler. **d** Evaluation of basal content in ethanol and EF in 1 g of watermelon leaf. Retention time (RT) of three chemicals was 1.24 min for carbon dioxide (CO_2), 1.35 min for EtOH, and 1.44 min for EF, respectively. **e** Standard curve of EF with 1 g of soil and each crops leaf for consideration of basal content

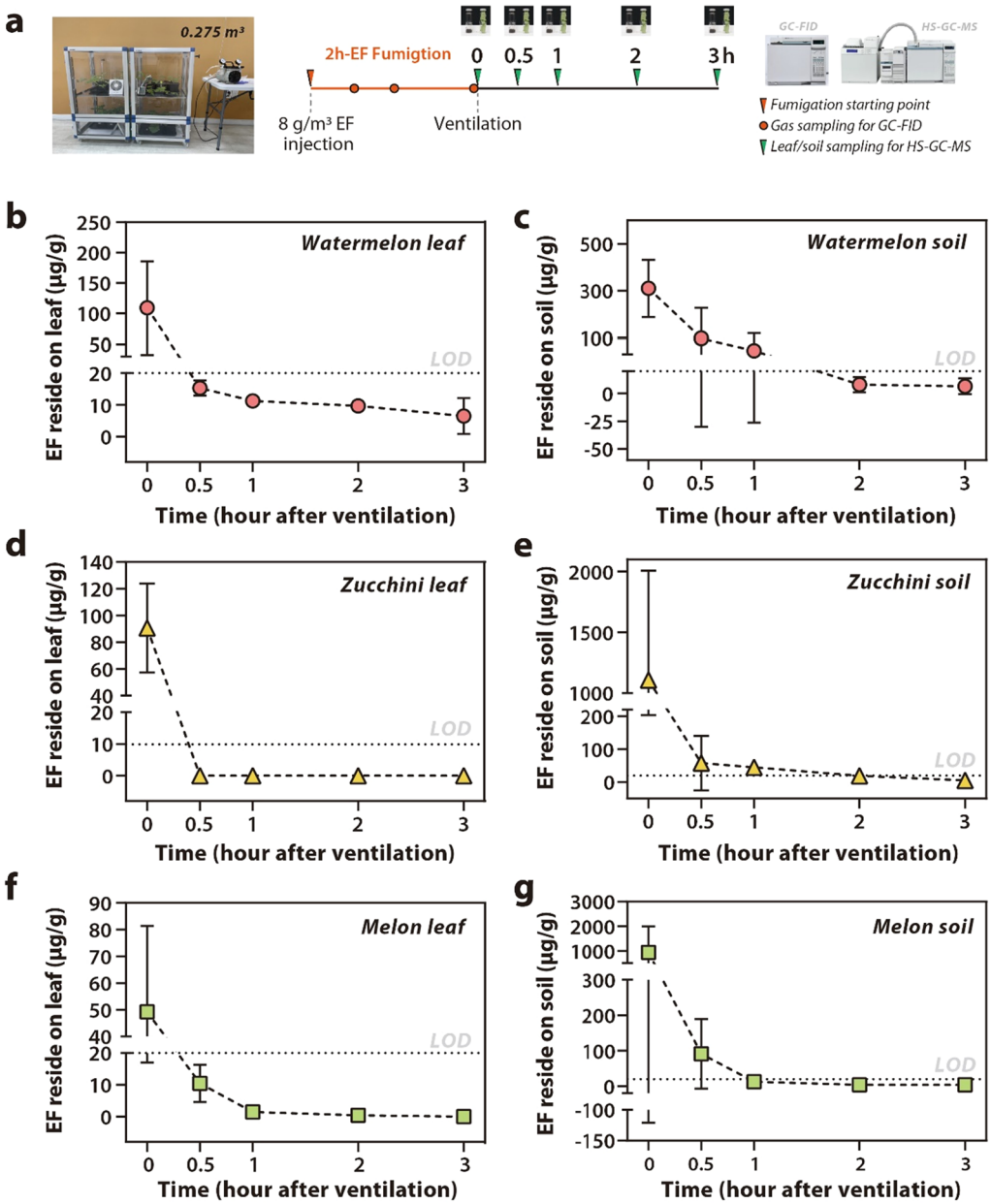


Fig. 3 Time-dependent residue evaluation of ethyl formate (EF) on three crop leaves and soil at lab-scale (0.275 m^3) experiment. **a** Experimental scheme with the time point of gas, leaf, and soil sampling. The fumigation conditions were as follow: 8 g/m^3 EF for 2 h controlling *Thrips palmi* at lab scale (0.275 m^3). Each batch was conducted in three independent three repetitions, and 1 g of leaf or soil was collected at 0 (immediately), 0.5, 1, 2, and 3 h after ventilation. EF residue evaluation was conducted separately (**b**, **c**) watermelon, (**d**, **e**) zucchini, and (**f**–**g**) melon leaf or soil. The limit of detection (LOD) is represented with a dotted line (Additional file 1: Table S1)

soil, and 1106.54 μg/g for zucchini at 0 h after ventilation (Fig. 3c, e, and g). After 1 h, the EF levels were below LOD in the melon soil at 44.98 μg/g (Fig. 3g). EF levels in the watermelon and zucchini soil decreased to below LOD at only 2 h after ventilation (Fig. 3c, e). There was low EF residue in all soils and crops in the environment at only 2 h after ventilation (Fig. 3 and Additional file 1: Fig. S5). Thus, our results suggested that EF fumigation could be a potent residue-free method for pest control in greenhouses, and it would enhance environmental sustainability.

EF-phytotoxicity and method to reduce the phytotoxic effect on the EF-treated crops

Phytotoxicity of EF was observed at the CT value of 10.14 g h/m^3 (8 g/m^3 for 2 h) in all crops and developmental stages (Fig. 1b). The phytotoxic effect was observed from a concentration of 7.5 g/m^3 or higher in all crops (Fig. 4a and Additional file 1: Table S3). Accumulation of H_2O_2 was also observed, using DAB staining, from over 7.5 g/m^3 in watermelon and zucchini, and from over 5 g/m^3 in melon (Fig. 4b). Interestingly, the appearance of brown dots in DAB staining was localized at the edge of a leaf in the 7.5 g/m^3 EF treatment group in watermelon and zucchini, whereas the dots were sparse in the melon. The location of the spots after DAB staining matched well with the part that withers at 7 dpf (Fig. 4). Chlorophyll content of watermelon was not significantly different at 7 dpf. Similar to what was observed under visual assessment and DAB staining, chlorophyll content slightly decreased above 7.5 g/m^3 at 21 dpf (Fig. 4c). In zucchini, the chlorophyll content in the range of 5–10 g/m^3 significantly decreased at 7 dpf relative to that of the control group, but recovered at 21 dpf (Fig. 4c). For the melon, the chlorophyll content was affected in the 10 g/m^3 EF-treated group without recovery (Fig. 4). Finally, the fresh weight of the shoot system was evaluated for recovery and inhibition of growth after fumigation (Fig. 4). All crops were affected at a concentration of 10 g/m^3 EF. This result suggested that the maximum concentration of EF for application in a greenhouse is about 7.5 g/m^3 with slight phytotoxicity and 5 g/m^3 with no phytotoxicity.

Overall, high oxidative stress was induced by EF fumigation on the leaves of all crops in the range of 7.5–10 g/m^3. During fumigation, the humidity in the closed system, such as a fumigation chamber or greenhouse, reached over 70–99% (Additional file 1: Table S4). Thus, there was a possibility of hydrolysis of EF to formic acid

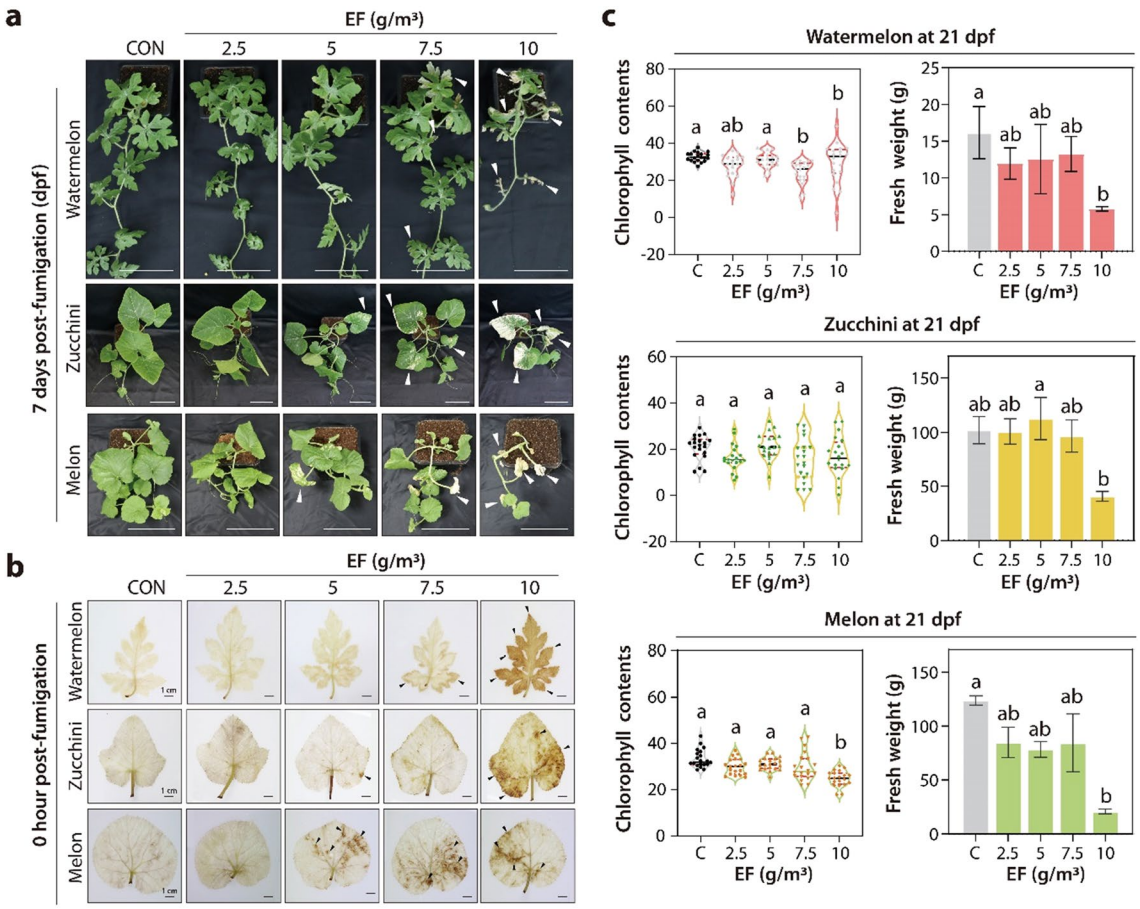


Fig. 4 Phytotoxic effect of ethyl formate (EF) on the tested crops each at flowering stage under laboratory condition. **a** Photographs of growing plants after EF fumigation at 7 days post-fumigation (dpf) with a loading ratio (w/v) of 1%. Each CT value was shown in Additional file 1: Table S3. **b** DAB (3, 3′-diaminobenzidine) staining for localization of production of H_2O_2 by EF at 0 (immediately) hour post-fumigation. Triangle (▲) indicated an observed phytotoxic effect point. **c** Chlorophyll contents (SPAD unit) and fresh weight of each group with 20 different points at 21 dpf. Different letters above symbols indicate significant differences ($p < 0.05$) as determined using a one-way ANOVA with post hoc Tukey's test ($a > b$)

and ethanol in the leaves. Therefore, we hypothesized that formic acid, one of the products from hydrolysis of EF, can cause phytotoxicity in the leaf because we observed that EF had not been retained in the leaves since 1 hpf (Figs. 2a, 3).

Phytotoxicity was reduced by neutralizing formic acid with sodium bicarbonate ($NaHCO_3$) during fumigation. Pre-treatment with 0.5% or 1% $NaHCO_3$ was performed with independent treatment or half treatment with DW treatment on the same watermelon (Fig. 5). When EF fumigation was conducted at the concentrations of 7.5 and 10 g/m^3 for 2 h, phytotoxic effect was observed in watermelon (Fig. 4). First, 0.5 or 1% $NaHCO_3$ did not cause phytotoxicity itself, but only white spots on the leaf (Fig. 5a and Additional file 1: Fig. S6). Reduction of phytotoxicity was observed at 7.5 g/m^3 in both $NaHCO_3$ treatment groups, but pre-treatment with 1% $NaHCO_3$ was more effective in reducing phytotoxicity than pre-treatment with 0.5% $NaHCO_3$ (Fig. 5a). At the concentration of 10 g/m^3 EF, the effectiveness of $NaHCO_3$ in reducing phytotoxicity was diminished, and the phytotoxic damage at 10 g/m^3 for 2 h was so severe that the watermelon plants were not able to recover from it (Fig. 5a and Additional file 1: Fig. S7). This pattern of reducing phytotoxic damage was observed in both the independent treatment and half treatment with DW treatment groups on the same watermelon (Fig. 5a and Additional file 1: Fig. S7). Interestingly, the white spot on the leaf after pre-treatment with $NaHCO_3$ disappeared after EF fumigation (Fig. 5b). A pattern of phytotoxic reduction similar to that of the watermelon leaf was observed in the zucchini leaf but not in the melon leaf (Additional file 1: Fig. S7).

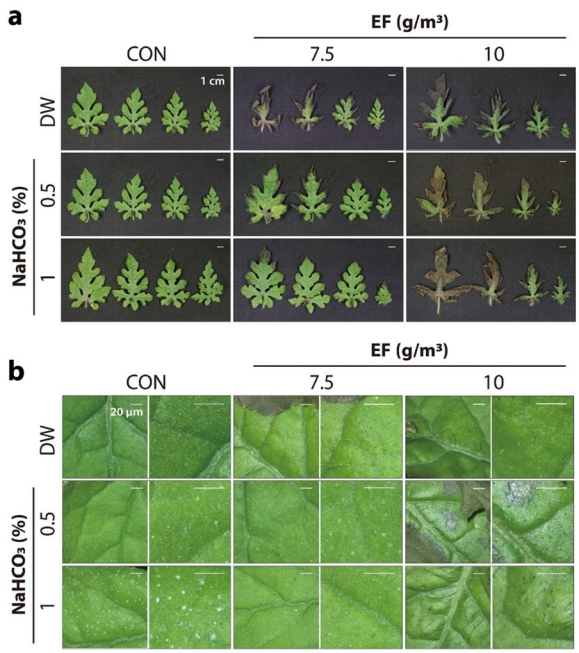


Fig. 5 Sodium bicarbonate ($NaHCO_3$) mediated reduction of ethyl formate (EF) induced-phytotoxicity on watermelon leaves. Pre-treatment of 0.5% or 1% $NaHCO_3$ was conducted before 2 h EF fumigation at a concentration of 7.5 or 10 g/m^3 in (**a**) independent treatment. Photographs were pictured at 3 days post-fumigation. **b** Microscope image after pre-treatment of sodium bicarbonate ($NaHCO_3$) with and without EF fumigation on watermelon leaves

Optimization EF fumigation for greenhouse environment to control pests with lower phytotoxicity

Based on the results of residue evaluation and phytotoxic damage reduction, we selected the concentration of 6 g/m^3 EF for controlling *M. persicae* (> LCT_{90}) and *T. palmi* (> LCT_{50}) for application in greenhouses (Fig. 6). For the fumigation of EF in the greenhouse, gas sampling lines for collecting gas were installed at three different heights and points (Fig. 6a). During 2 h EF fumigation, the EF concentration in the greenhouse decreased faster than in the laboratory-scale set up. This result could be due to the high humidity in the greenhouse.

Optimized EF fumigation at 6 g/m^3 for 2 h had an average CT value of 6.23 g h/m^3 for three different heights and points implying the same CT with the expected LCT value (Fig. 6b). At this CT value, there was no significant difference in the phytotoxicity evaluation indices, including phytotoxic index, chlorophyll content, and chromaticity before and after EF fumigation at 7 dpf, and leaf damage was also not observed in

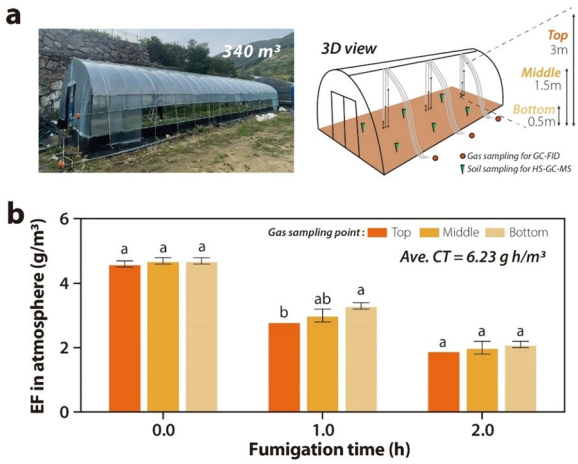


Fig. 6 Field application of ethyl formate (EF) fumigation technique in a vinyl house environment (340 m^3). **a** Photograph of the greenhouse with a 3D view diagram for gas and soil sampling. **b** EF concentration–time (g/m^3) in the air of the greenhouse during fumigation with 6 g/m^3 EF for 2 h with average concentration–time (CT). Measured temperature and humidity during fumigation were 26.8–34.2 ℃ and 70–99%, respectively. Different letters above symbols indicate significant differences ($p < 0.05$) as determined using a one-way ANOVA with post hoc Tukey's test ($a > b$)

the visual assessment (Fig. 7a–c and Additional file 1: Table S4). And mortality rates for *M. persicae* and *T. palmi* reached 100% and 40%, respectively, as a result of reaching the targeted CT (6.23 g h/m^3) (Fig. 7d).

Additionally, the EF residue in the soil and all crops at various developmental stages were below LOD after fumigation with 6 g/m^3 EF for 2 h (Table 2). Overall, an optimal EF fumigation of 6 g/m^3 for 2 h in the greenhouse was determined with 100% mortality of *M. persicae* and 40% mortality of *T. palmi* without phytotoxicity and residue in 340 m^3 greenhouse environment.

Discussion

Comparative efficacy of EF fumigation across pest species

In this study, the efficacy of EF fumigation against two major agricultural pests, *M. persicae* (Hemiptera) and *T. palmi* (Thysanoptera), was evaluated, with the results showing LCT_{50} values of 3.04 g h/m^3 and 6.23 g h/m^3, respectively (Table 1). Comparing these findings with those of other studies on the EF efficacy against various quarantine pests reveals differences in susceptibility among orders and species. For instance, the mushroom sciarid fly (*Lycoriella mali* Diptera) and the two-spotted spider mite (*Tetranychus urticae* Arachnida) exhibited

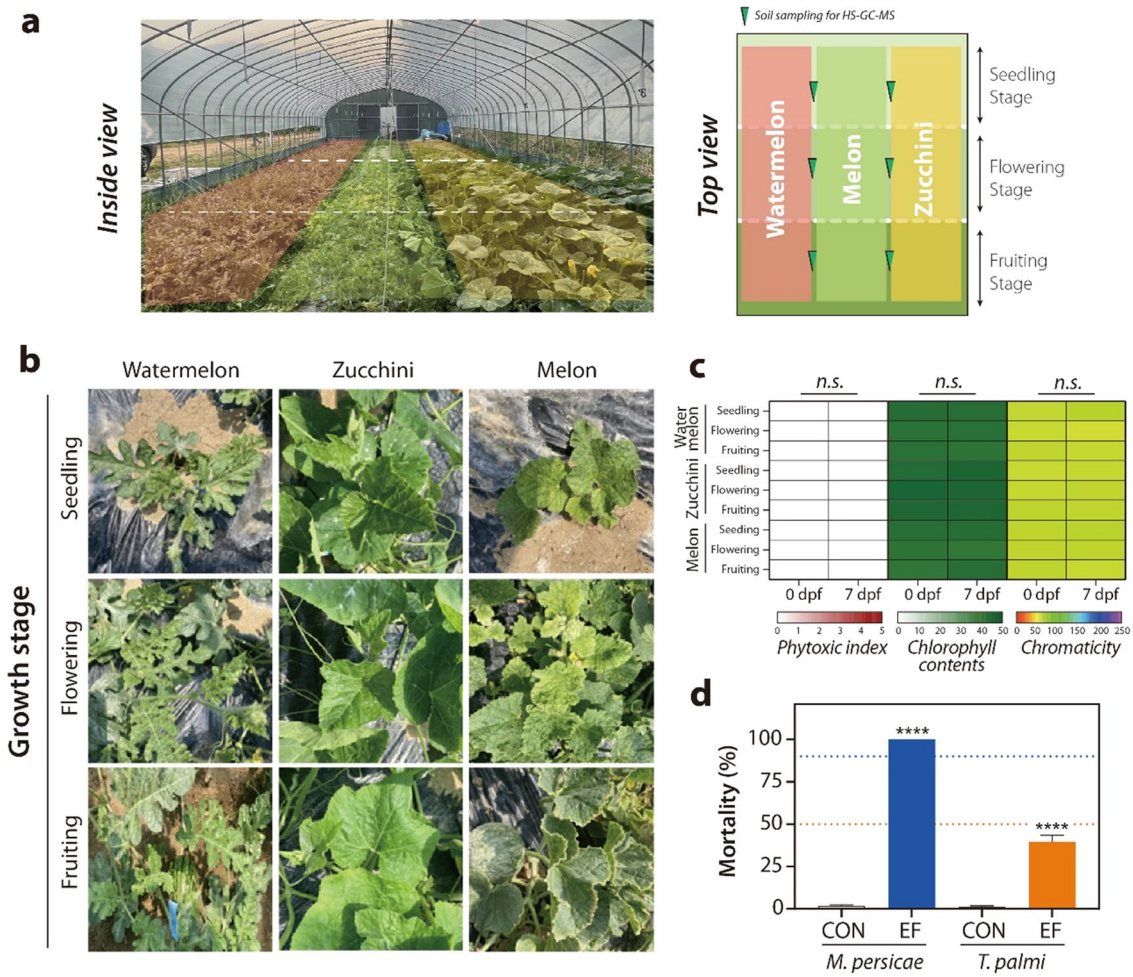


Fig. 7 Phytotoxicity evaluation and efficacy test of ethyl formate (EF) fumigation in a vinyl house environment (340 m^3). **a** Cultivation photograph and diagram of three different crops by plant developmental stages. **b**, **c** Photographs and phytotoxic indices of each developmental stage of each crop at 7 days post-fumigation. *n.s.,* Not significant. **d** Mortality of *Myzus persicae* and *Thrips palmi* after 6 g/m^3 EF fumigation for 2 h (CT = 6.2 ± 0.1 g h/m^3, n = 50 in 5 replicates, Temp.: 26.8–34.2 ℃, Humidity: 70–99%). Dot lines represent the target concentration–time (CT) value of each pest (> LCT_{90} value for *M. persicae* and > LCT_{50} for *T. palmi*)

Table 2 Residue evaluation of ethyl formate (EF) on crop leaves and soil after EF fumigation for 2 h with 6 g/m^3 EF in 340 m^3 greenhouse in field scale ($n = 3$ for crop, n = 6 for soil, temperature: 26.8–34.2 ℃, Humidity: 70–99%)

Crop/soil	Stage	Control		1 hpf		3 hpf	
		Sample (g)	EF (μg/g)	Sample (g)	EF (μg/g)	Sample (g)	EF (μg/g)
Watermelon	Seedling	1.02 ± 0.01	< LOD[a]	1.07	< LOD	1.04	< LOD
	Flowering			1.06	< LOD	1.05	< LOD
	Fruiting			1.05	< LOD	1.05	< LOD
Zucchini	Seedling	1.03 ± 0.01	< LOD	1.05	< LOD	1.06	< LOD
	Flowering			1.08	< LOD	1.03	< LOD
	Fruiting			1.05	< LOD	1.06	< LOD
Melon	Seedling	1.02 ± 0.02	< LOD	1.07	< LOD	1.04	< LOD
	Flowering			1.06	< LOD	1.05	< LOD
	Fruiting			1.05	< LOD	1.05	< LOD
Soil		1.03 ± 0.03	< LOD	1.06	< LOD	1.03	< LOD

[a] < LOD: below LOD (limit of detection), The LOD of each crop and soil was shown in Additional file 1: Table S1

similar LCT_{50} values of 7.8 g h/m^3 and 8.55 g h/m^3 to those for *T. palmi* [23, 24], whereas two Homoptera species, citrus mealybug (*P. citri*) and comstock mealybug (*P. comstocki*), displayed higher LCT_{50} values of 11.93 g h/m^3 and 29.41 g h/m^3, respectively [18, 20]. Other notable results include an LCT_{50} value of 0.41 g h/m^3 for the silverleaf whitefly (*B. tabaci*) showing high susceptibility to EF and an LCT_{50} value of 530.3 g h/m^3 for the chestnut weevil (*C. sikkimensis*), showing high insensitivity [14, 15]. The observed variations in EF efficacy across the pest species might be attributed to their differences in physiology. For example, differences in respiratory systems or cuticle thickness could influence the rate of fumigant penetration and its overall efficacy (Subramanyam and Hagstrum, 1996). Understanding the species-specific responses to EF fumigation is crucial for developing effective pest control strategies in agriculture. Overall, greenhouse insect pests were relatively susceptible to EF, indicating its potential as a pesticide for agricultural use.

Effectiveness of EF fumigation with reduced environmental hazard

EF hydrolyzes easily in humid environments as shown in Eq. (2) [10]. This property influences two main aspects of EF usage, including maintaining concentration during fumigation and promoting agricultural sustainability.

$$\mathrm{HCOOEt}\ (\text{Ethyl formate, EF}) + \mathrm{H_2O} \rightarrow \underline{\mathrm{HCOOH}} + \mathrm{EtOH} \quad (2)$$

During fumigation, the CT value of EF decreases more rapidly (Fig. 6b) than that of other fumigants such as MB and PH_3 [25–27]. For most fumigants, a slight decrease in concentration occurs due to adsorption or leakage. However, for EF, an additional decrease in concentration occurs due to decomposition, particularly under humid conditions [14]. This makes achieving the target CT value challenging. There was a gap between the CT values obtained in laboratory-scale and field-scale experiments (Table 1 and Fig. 6b). The CT value of 6.41 reached with 5 g/m^3 EF for 2 h in a 0.275 m^3 fumigation chamber, was barely reached in the greenhouse field site treated with 6 g/m^3 of EF (Table 1 and Fig. 6b).

Also, EF is a natural product that comes from the biosynthesis of the plants [28] and the naturally produced EF can inflate the determined residue value of EF after EF fumigation. Therefore, it is important to determine the basal content of EF in the plants being tested to obtain accurate residue values after fumigation (Fig. 2e). Due to the hydrolytic property of EF, we conducted a direct residual analysis without a preparation, using HS–GC–MS at a lab scale (Fig. 3). Any preparation steps such as extraction and concentration with vaporization could compromise the accuracy of the analysis. In this regard, a relatively high LOD, ranging from 10–20 μg/g, was observed across the three crops and soil (Additional file 1: Table S1), compared to HS–GC–MS analysis of nonpolar and volatile chemicals [29]. As such, the direct approach with HS–GC–MS for EF residue analysis provided crucial insights into the time-dependent analysis of EF on leaves and soil (Fig. 3).

The degradability of EF contributes to its sustainability in agricultural settings. After fumigation, EF rapidly disappears through air dilution via ventilation and reacting with water present in leaves and soil in the greenhouse as shown in Eq. (2). As phytotoxicity does not occur up to reaching the target CT value in a greenhouse environment, EF provides significant sustainability benefits in agriculture.

Potentiality of reducing EF-phytotoxicity with sodium bicarbonate

Phytotoxicity induced by EF was restricted only to the affected leaves immediately after fumigation (Fig. 4). New leaf growth and overall growth of crops in EF-treated groups displayed normal chlorophyll content and fresh weights relative to the control, except for the highest concentration of 10 g/m^3 for 2 h (Fig. 4). We hypothesized that if formic acid contributed to phytotoxicity, neutralizing it with $NaHCO_3$ Eq. (3) should be able to prevent EF-induced phytotoxicity. Indeed, pre-treatment with $NaHCO_3$ effectively reduced EF-induced phytotoxic damage on the leaves at concentrations of 7.5–10 g/m^3 for watermelon and zucchini (Fig. 5 and Additional file 1: Fig. S7). The disappearance of white spots ($NaHCO_3$) on the leaf after EF fumigation also strongly supported our hypothesis (Additional file 1: Fig. S7).

$$\underline{HCOOH} + NaHCO_3 \rightarrow HCOONa + CO_2 + H_2O \quad (3)$$

Overall, a possible mechanism of EF-induced phytotoxicity is proposed in which formic acid is one of the major contributors to EF-induced phytotoxic effects on crop leaves. $NaHCO_3$ was effective in reducing phytotoxicity through neutralization reactions. Furthermore, $NaHCO_3$ is already being used as an alternative fungicide in plant gardening [30, 31] and has no adverse effects on humans and the environment (Fact sheet, EPA). Therefore, although it was not tried in the field in this study, pre-treatment with $NaHCO_3$ has the potential to be used as an EF-phytotoxicity mitigation agent for higher target CT values.

Optimizing the concentration based on phytotoxicity and convenience of EF fumigation

It's crucial to monitor the fumigant's concentration during fumigation, with CT values affected by various factors like leakage, absorption, and loading ratios. Typically, higher loading ratios lead to lower CT values because of absorption [32, 33]. A CT value exceeding 8 g h/m^3 caused significant phytotoxicity in three crops at varying developmental stages (Figs. 1 and 4). Interestingly, the melon's flowering stage exhibited a marginally different phytotoxic response at the same EF concentration of 5 g/m^3 (Figs. 1 and 4). Under the same conditions of 5 g/m^3 EF, the CT value difference between 6.41 g h/m^3 in the screening phytotoxicity (Fig. 1) and 5.49 ± 1.64 g h/m^3 in the concentration-dependent phytotoxicity evaluation was negligible (Additional file 1: Table S3). Thus, selecting the appropriate EF concentration for the desired CT value is pivotal for fine-tuning EF fumigation in greenhouses.

Generally, there is a difference in target mortality between quarantine and agricultural pest control purposes. For quarantine purposes, 100% mortality is essential for the international trade in plants due to biosecurity concerns, whereas it is not necessary for the agricultural purposes. In this regard, severe phytotoxicity was observed at the CT value of 10.14 g h/m^3 for controlling 90% of *T. palmi* (Fig. 1 and Additional file 1: Table S2). Although pre-treatment with $NaHCO_3$ can reduce EF-phytotoxicity, such additional procedures should be excluded from general use unless necessary. Therefore, we decreased the target mortality (LCT_{50}) for *T. palmi* to a concentration of 6 g/m^3 EF, which guaranteed 100% mortality of *M. persicae* when applied in the field study (Table 1 and Fig. 6).

Adults of *T. palmi* are thin-bodied and hide between the veins on the underside of leaves and live in the soil during the pupal stage, making them very difficult to control with traditional insecticides. However, fumigants are gas-type pesticides that can spread evenly in a closed system (Fig. 6b), making them effective in controlling thrips. Therefore, fumigants are effective in maintaining low populations of *T. palmi* and 100% mortality of *M. persicae* through continuous application at an optimized concentration of 6 g/m^3 EF in a greenhouse. Additionally, at this concentration, no residue of EF occurs despite its constant use because it is highly biodegradable (Fig. 3 and Additional file 1: Table S4), and phytotoxicity can be minimized (Fig. 6d and Additional file 1: Table S4), making it a suitable pest control method. Furthermore, there have been no reports of pest resistance to EF over the past few decades. EF can effectively control phosphine-resistant individuals, known to have similar target sites, at comparable LCT values [34]. The concentration of EF in greenhouse air rapidly dropped below the permissible airborne exposure limit (100 ppm, equivalent to 0.3 g/m^3) within 10 min of ventilation [14, 35]. Prior research indicated that achieving a zero EF concentration through ventilation takes over 40 min, with more than an hour recommended to ensure a reduced inhalation risk in 340 m^3 greenhouse [35].

Overall, this study demonstrated the potential of EF fumigation as a sustainable and effective pest management tool for controlling thrips and aphids infesting cucurbit crops in greenhouses. Through investigating its efficacy, chemical residual properties, and phytotoxicity, we were able to optimize the application of EF to balance pest control and environmental sustainability. Future research should focus on scaling up and applying phytotoxicity mitigation methods to various plants under field conditions. This will help refine and optimize EF application strategies, ensuring efficient and sustainable pest management across a broader range of crops and pests.

Conclusion

This study investigated the efficacy, phytotoxicity, and pesticide residue of EF fumigation for the control of two major agricultural pests (*M. persicae* and *T. palmi*) in three cucurbit crops: watermelon, zucchini, and melon. The results showed that EF was more effective in controlling *M. persicae* than in controlling *T. palmi*. The EF fumigation concentration was optimized to minimize phytotoxicity and residue levels in the greenhouse. An analytical method using HS–GC–MS was established to evaluate the residue of EF on crop leaves and the soil. It was found that EF residues quickly decreased to below the LOD within 0.5 h in the leaf and 2 h in the soil after ventilation, suggesting that EF fumigation could be a residue-free method for pest control in greenhouses, thus enhancing environmental sustainability. A concentration of 6 g/m^3 EF was found to be the optimal EF concentration, being 100% and 40% effective against *M. persicae* and *T. palmi,* respectively, without causing significant phytotoxicity or leaving residues on the plants and soil. Additionally, a strategy to reduce phytotoxicity by neutralizing formic acid with $NaHCO_3$ during fumigation showed promising results in reducing the phytotoxic effects of EF on the watermelon and zucchini. Through this, higher EF concentrations could be applied without causing harm to plants, allowing for effective control of *T. palmi*, a challenging pest insect to manage in greenhouses. In summary, this study demonstrates that EF fumigation can be a potent, residue-free, and environmentally sustainable method for controlling major agricultural pests in greenhouses when applied at the optimized concentration. The optimized EF fumigation strategy has the potential to improve pest management practices in various greenhouse settings and crops, promoting environmentally friendly and sustainable agricultural practices.

Abbreviations

EF	Ethyl formate
MB	Methyl bromide
DAB	3,3′-Diaminobezidine
GC-FID	Gas chromatography with a flame ionization detector
CT	Concentration–time
LCT	Lethal concentration–time
GC–MS	Gas chromatography–mass spectrometry
RT	Retention time
HS	Headspace autosampler
LOD	Limit of detection
LOQ	Limit of quantification
hpf	Hours post-fumigation
dpf	Days post-fumigation
ROS	Reactive oxygen species
DW	Distilled water
EtOH	Ethanol

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40538-023-00486-5.

Additional file 1: Fig. S1. Scan mode for identification of product ion to establish ethyl formate mode analysis using gas chromatography-mass spectrometry (GC–MS).**Fig. S2.** Photographs of phytotoxicity evaluation of ethyl formate (5 g/m^3 for 2 h, CT = 6.41 g•h/m^3) for controlling *Myzus persicae* depending on developmental stages towards three crops using 0.275 m^3 fumigation chamber in Lab scale. **Fig. S3.** Photographs of phytotoxicity evaluation of ethyl formate (8 g/m^3 for 2 h, CT = 10.14 g·h/m^3) for controlling *Thrips palmi* depending on developmental stages towards three crops using 0.275 m^3 fumigation chamber in Lab scale. **Fig. S4.** Additional chromatograms and standard curves using Headspace Gas Chromatography-Mass Spectrometry (HS-GC–MS). (a) Evaluation of basal content in ethanol and EF in 1 g of zucchini, melon leaf, and soil. Retention time (RT) of three chemicals was 1.24 min for carbon dioxide (CO_2), 1.35 min for EtOH, and 1.44 min for EF, respectively. (b) Standard curve of EF and ethanol in a headspace sampling vials without leaf or soil for quantification of EF and ethanol basal contents. **Fig. S5.** Chromatogram of time-dependent residue analysis of ethyl formate (EF, red line). **Fig. S6.** Reducing EF induced-phytotoxicity strategy with sodium bicarbonate ($NaHCO_3$). Pre-treatment of 0.5% or 1% $NaHCO_3$ was conducted before 2 h EF-fumigation at a concentration of 7.5 or 10 g/m^3 in half treatment with distilled water (DW) treatment on the same watermelon. Photographs were pictured at 3 days post-fumigation. **Fig. S7**. Photographs of phytotoxicity of EF towards zucchini and melon leaves with microscope image after pre-treatment of sodium bicarbonate ($NaHCO_3$) with and without ethyl formate (EF) fumigation. **Table S1**. Limit of detection (LOD) and limit of quantification (LOQ), and standard curve information for ethyl formate (EF) residue analysis. **Table S2**. Phytotoxicity evaluation of ethyl formate (EF) at 7 days post-fumigation depending on target pest and developmental stages towards three crops using 0.275 m^3 fumigation chamber in Lab-scale (CON: Control, EF: EF-treated group). **Table S3.** CT values for ethyl formate (EF) in three crops phytotoxicity assessments. **Table S4**. Optimal ethyl formate (EF) fumigation condition with 6 g/m^3 EF for 2 h with phytotoxicity evaluation at 7 days post-fumigation (dpf) depending on developmental stages towards three crops in 340 m^3 greenhouse in field scale (Temp.: 26.8–34.2℃, Humidity: 70–99%).

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Author contributions
KK: conceptualization, data curation, investigation, visualization, writing—original draft, writing—review & editing; CK: investigation, validation, writing—original draft, writing—review & editing; THK: formal analysis, investigation. HJ: formal analysis, methodology; YK: investigation, visualization; YC: investigation, software; DK: formal analysis, investigation; YL: formal analysis, investigation; DK: formal analysis, investigation; BL: conceptualization, funding acquisition; SL: conceptualization, funding acquisition, supervision, writing—original draft, writing—review & editing. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that there are no conflicts of interest.

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