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A New Disinfestation Approach Against Some Greenhouse Pests Using Ethyl Formate Fumigation

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훈증제 에틸포메이트를 이용한 몇 가지 시설하우스 해충에 대한 새로운 방제 전략

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ABSTRACT: Ethyl formate (EF) is a rapid kill, environmentally safe, and low mammalian toxicity fumigant, registered to disinfest quarantine insect pests from imported agricultural products. A new concept for controlling insect pests of agricultural crops was tested in a fumigation chamber with EF. Control efficacy of and phyto-toxicity due to EF fumigation were evaluated against four pests (*Thrips palmi*, *Bemisia tabaci*, *Myzus persicae*, and *Tetranychus urticae*) and on seedlings of four fruit vegetables (FVs; yellow melon, cucumber, tomato, and pepper). Ethyl formate fumigation at a dose of 1.5 g m^{-3} for 12 h produced >93.3% mortality in *T. palmi*, *B. tabaci*, and *M. persicae*. However, *T. urticae* was tolerant to fumigation, showing only 20% mortality at 2.0 g m^{-3}. In terms of concentration × time (CT) products, at least 8.9 g·h m^{-3} CT at 20 ± 1.5℃ was needed to achieve > 90% mortality against the three susceptible insect pests. Fumigation at 1.5 g m^{-3} for 12 h caused no phyto-toxicity to any of the four FV seedlings. Ethyl formate application, as a new disinfestation method in greenhouses, could be an alternative to reduce the use of conventional insecticides. However, further studies are needed to determine the efficacy of this method at different pest developmental stages and in different greenhouse environments. Additionally, research is needed to elucidate the phyto-toxicity of EF application at different growing stages of a wide variety of crops.

Key words: Ethyl formate, Greenhouse, *Bemisia tabaci*, *Myzus persicae*, *Thrips palmi*

초 록: 에틸포메이트는 살충 효과가 빠르고 환경에 무해하며 특히 인축독성이 낮아 검역용 훈증제로 등록되어 있다. 이러한 에틸포메이트를 이용하여 훈증 챔버 (0.275 m^3) 내에서 4종의 농업 해충 (오이총채벌레, 담배가루이, 복숭아혹진딧물, 점박이응애)과 4종의 작물 (참외, 오이, 토마토, 고추) 유묘기를 대상으로 12시간 훈증 처리하여 약효와 약해를 평가하였다. 에틸포메이트 1.5 g/m^3의 약량으로 12시간 훈증 했을 때 담배가루이와 복숭아혹진딧물은 93.3% 이상의 높은 살충력를 나타내었으나, 점박이응애는 2.0 g/m^3 약량에서도 20% 미만의 낮은 살충력을 나타내었다. 그리고 에틸포메이트의 CT값(농도 x 시간)이 훈증 처리 온도 20 ± 1.5℃에서 8.9 g·h/m^3 이상일 때 점박이응애를 제외한 나머지 3종의 해충에 대해서 90% 이상의 살충 효과를 얻을 수 있었다. 에틸포메이트 1.5 g/m^3의 약량으로 상기 4작물을 12시간 훈증했을 때 약해 증상은 나타나지 않았다. 따라서 에틸포메이트 훈증기술은 시설 내 해충방제를 위한 새로운 방식이 될 수 있으며 관행적인 살충제의 사용을 줄일 수 있을 것으로 생각된다. 그러나 해충의 충태별, 작물의 생육 시기별 에틸포메이트 약효 및 약해에 대한 많은 연구가 이루어져야 할 것으로 생각된다.

검색어: 에틸포메이트, 시설하우스, 담배가루이, 복숭아혹진딧물, 오이총채벌레

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The total area under fruit vegetable (FV) cultivation in Korea is 49,270 ha, of which 78% (38,623 ha) is occupied by greenhouses. The top four Korean FVs are yellow melon, cucumber, tomato, and pepper, up to 45% of which are grown in greenhouses (MAFRA, 2018). However, controlling insect pests is more complicated in greenhouse cultivation than in outdoor stable environments because of the rapid development of insecticide resistance and frequent resurgences (Douglas, 2018).

The most common but hard-to-control insect and mite pests in greenhouse cultivation are palm thrips (*Thrips palmi*; Thysanoptera: Thripidae), sweet potato whitefly (*Bemisia tabaci*; Hemiptera: Aleyrodidae), cotton aphids (*Myzus pericae*; Hemiptera: Aphididae), and two-spotted spider mites (*Tetranychus urticae*; Trombidiformes: Tetranychidae). The damage caused by these pests in greenhouses is continuously increasing (Bass et al., 2015). Pest control in greenhouses relies primarily on the application of conventional insecticides or acaricides, but their repeated use has induced resistance against these pesticides (Bao et al., 2014, Hamada et al., 2019). Pesticide application in greenhouses is restricted during the fertilization season in order to protect pollinators such as honey bees. Pesticide use is further limited owing to the established maximum residue limits (MRLs) of the registered pesticides, and due to the recent enforcement of the positive list system (PLS), which allows residues of 0.01 ppm for unregistered pesticides (MFDS, 2019). Owing to these reasons, new approaches to insect pest management in greenhouses are needed for pesticide residue-free farming of FVs.

Fumigation is an inevitable and important pest control method, particularly in quarantine situations, which involves the release of gaseous fumigants into the air in a confined space (Rajendran, 2004). Fumigation is a common process in which relatively high doses of toxic gases, such as methyl bromide (MB), are used to meet quarantine guidelines that demand a proven efficacy (>99.9996%) at targeting quarantine pests (Couey and Chew, 1986). However, MB is being phased-out because of its ozone-depleting characteristics and its acute and chronic inhalation toxicity in the workplace.

Recent studies have highlighted the possibility of using ethyl formate (EF), which is currently used for traded fruits and vegetables. Ethyl formate is a flavoring agent that has been characterized as 'Generally Recognized as Safe' (GRAS) by the Food and Drug Administration (FDA) of the USA (Agarwal et al., 2015, Lee et al., 2016). Interestingly, our preliminary studies (unpublished) have indicated that EF was quite effective against some minute and flying insect pests that infest fruit surface and leaves, even at relatively low temperatures (>15℃).

In order to reduce the use of conventional pesticides, and to take steps towards the pesticide-free farming of FVs in greenhouses, we tested the possibility of using EF to control greenhouse insect pests. We evaluated EF for its phyto-toxicity to four FV crop plants, and for its efficacy against three dominant insect pests and a spider mite, which are pests of major concern because of their potential to develop pesticide resistance.

Materials and methods

Insects and Plants

Colonies of *B. tabaci*, *T. palmi*, *M. persicae*, and *T. urticae* were reared on laboratory-grown tobacco, cucumber, and bean plants, respectively at 25 ± 1°C with 60 ± 10% RH and a light-dark cycle of 16:8 h (L:D). *B. tabaci*, *T. palmi*, and *M. persicae* were obtained from the National Institute of Agricultural Sciences, RDA, Korea, in 2019. The acaricide-susceptible *T. urticae* was obtained from the Gueongnam Agricultural Research & Extension Service in 2007. From the initial rearing of the pest colonies up to the start of the experiment, no chemicals had been applied. The four FVs of yellow melon (*Cucumis melo*), cucumber (*Cucumis sativus*), tomato (*Solanum lycopersicum*), and pepper (*Capsicum frutescens*), were grown up to the seedling stage in the laboratory (27 ± 1°C, 70 ± 10% RH, and 16:8 h L: D).

Chemicals for fumigation

Liquid ethyl formate (Fumate^{TM}, 99%) was supplied from Safefume Inc., Gangwon-do, Korea. For chamber (280 L) application, liquid ethyl formate (EF) was vaporized with nitrogen gas through a heater and was discharged into the chamber.

Efficacy of ethyl formate against four studied pests

Ethyl formate fumigation on the four abovementioned pests were performed in fumigation chambers (280 L) with a mini fan, which was placed at the bottom for inner air circulation.

Four insect breeding dishes (1 × 5.5 cm), each of which was inoculated with 20–30 adults of *T. urticae* or the other test insects, were placed at the bottom of each fumigation chamber. After sealing the fumigation chambers, different doses of liquid EF (1.0, 1.5, or 2.0 g m^{-3}) were vaporized with nitrogen gas through heaters and were discharged into the respective chambers. The fumigation chambers treated with EF were placed in incubators at 20 ± 1℃ for 12 h. The application doses were calculated using the equation reported by Ren et al. (2011). After 12 h fumigation, the chambers were opened and aerated for 1 h in a fume hood system. The control chambers were not fumigated. The treated pest samples were removed from the chamber and kept at 25 ± 1℃ and 60 ± 10% RH until mortality recording. Mortality of adults of the four pests was determined 24 h after they were released from the fumigation chambers. All treatments, including the control, were replicated three times.

Phyto-toxicity to the four FVs seedlings

Assessments of phytotoxic damage to the four crop plant seedlings, through estimation of variables such as chlorophyll content and leaf color, were evaluated after the seedlings were maintained under the above mentioned conditions for one week. 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), 4 (>50% leaves affected). The chlorophyll content and colors of five leaves of one plant per crop were measured using a chlorophyll meter (SPAD-502 Plus, Spectrum Technologies Inc., Bridgend, UK) and a colorimeter (TES 135A, Electrical & Electronic Corp., Taipei, Taiwan), respectively. All measurements were repeated three times.

Measurement of fumigant concentration and determination of CT (concentration × time) products

During fumigation, the concentration of EF was measured using a gas chromatographer (GC 17A, Shimadzu, 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 Inc., Folsom, CA). The oven temperature was 100℃. The injector and detector temperatures were 250 and 280℃, respectively. Helium was used as a carrier gas at a flow rate of 1.5 mL/min. The concentration of EF was calculated based on the peak area against the external EF gas standard. The concentration of the EF fumigant was monitored at time intervals of 0.5, 1, 2, 4, and 12 h. These concentrations were used to calculate the CT products (Ren et al., 2011).

Statistical analyses

Mortalities of the four pests were analyzed using ANOVA, and the means were separated based on LSD tests at a significance level of $P = 0.05$. Phyto-toxicities to the four crops were compared between EF-fumigated and non-fumigated control seedlings using *t*-tests at $P = 0.05$. All statistical analyses were carried out using SAS (ver. 9.4; SAS Institute Inc., 1998).

Results and Discussions

Table 1 shows the efficacies of three doses of EF (1.0, 1.5, and 2.0 g m^{-3}) against four pests (*B. tabaci*, *T. palmi, M. persicae*, and *T. urticae*), which are dominant in greenhouses and readily develop pesticide resistance. *B. tabaci* seemed to be most susceptible to EF fumigation, followed by *M. persicae* and *T. palmi,* while *T. urticae* was quite tolerant to EF fumigation. The mortality of *B. tabaci* reached 100% even at the lowest dose of 1 g m^{-3}. The mortalities of *T. palmi* and *M. persicae* were >93% at the dose of 1.5 g m^{-3}. *T. urticae* mortality only reached 20% at the highest dose of 2 g m^{-3}, at which the mortalities of the other three insect pests were >94.4%. Concentration × time (CT) products shows that at least 8.9 g·h m^{-3} at 20 ± 1.5℃ is needed to acquire > 90% mortality in *B. tabaci*, *T. palmi,* and *M. persicae*.

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 (Hamada et al., 2019). In addition, resistance to spinosad, which is frequently used to control thrips, has been reported

Table 1. Efficacy of ethyl formate against *Thrips palmi*, *Bemisia tabaci*, *Myzus persicae*, and *Tetranychus urticae* in 280 L chambers following 12 h-exposure at 20 ± 1.5℃

Dose ($g\ m^{-3}$)	CT value ($g\cdot h\ m^{-3}$)	*Thrips palmi*	*Bemisia tabaci*	*Myzus persicae*	*Tetranychus urticae*
0	0.0	0.0 ± 0.0 c	0.0 ± 0.0 b	0.0 ± 0.0 c	0.0 ± 0.0 c
1	4.4	57.5 ± 3.8 b	100.0 ± 0.0 a	76.7 ± 5.1 b	0.0 ± 0.0 c
1.5	8.9	93.3 ± 3.3 a	100.0 ± 0.0 a	93.3 ± 6.7 a	13.3 ± 3.3 b
2.0	9.3	94.4 ± 5.6 a	100.0 ± 0.0 a	100.0 ± 0.0 a	20.0 ± 0.0 a
Statistics	-	$F_{3,11}$ = 138.7 P < 0.001	$F_{3,11}$ = Infinity P < 0.001	$F_{3,11}$ = 120.9 P < 0.001	$F_{3,11}$ = 36.0 P < 0.001

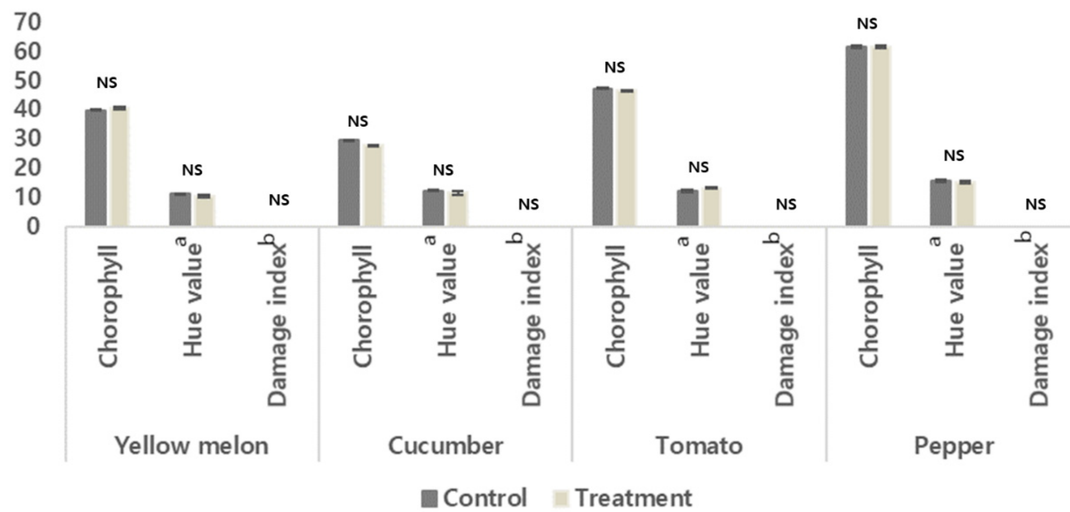

Fig. 1. Phyto-toxicity of 1.5 g m^{-3} ethyl formate on yellow melon, cucumber, tomato, and pepper at 20 ± 1.5℃ for 12 h. [a][Color L × 2 + Color a × 2 + Color b × 2]/2, [b]Damage index: 0 (no leaf damage), 1 (<5% leaves affected), 2 (5-25% leaves affected), 3 (25-50% leaves affected), 4 (>50% leaves affected). "NS" represents non-significant differences in the phytotoxic damage indices between the treatment and the control by *t*-test at P = 0.05.

(Bao et al., 2014). However, insect resistance to EF has not yet been reported, which highlights the possibility of using EF as a green-house insect pest disinfectant in the future.

Conventional insecticides are evenly sprayed throughout the greenhouse. However, insecticides sprayed by conventional methods cannot reach secluded spots, cracks, and crevices in the greenhouse 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 greenhouse. Fumigation may also be safer to workers because they are able to avoid direct exposure to the gas.

The results of the phyto-toxicity assessments of EF fumigation to the seedlings of four FVs are shown in Fig. 1. There were no significant differences between untreated and EF-treated (1.5 g m^{-3} for 12 hours) seedlings in terms of chlorophyll content, color changes, and overall damage. In this experiment, we did not evaluate the phyto-toxicity of EF at different developmental stages (such as blooming or harvesting time) of the four FVs. Thus, further studies are needed to determine the phyto-toxicity at different developmental stages of the tested crops.

Ethyl formate was firstly investigated as a grain fumigant for boxcars in Australia (Neifert et al., 1925). Owing to the valuable characteristics of EF, such as a quick knockdown effect comparable to that of methyl bromide, it is used to disinfest different kinds of fruits and vegetables for quarantine purposes (Lee et al., 2016, 2018; Yang et al., 2016). It is also known to have low mammalian toxicity and to break down rapidly, thereby leaving no residues in the environment (Haritos et al., 2003; Lee et al., 2007).

Although this study was done on a small scale in a fumigation chamber, the results exhibited that EF fumigation is applicable for disinfestation of greenhouse insect pests such as thrips, aphids, and whitefly. The greenhouse environment is spatio-physically complicated, showing distinct fluctuations in temperature and humidity. Thus, more studies are needed to evaluate the efficiency of EF fumigation at different developmental stages of pests, and to elucidate the phytotoxic effects of EF fumigation on different phonological stages of FVs. 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 FVs.

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