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COMPARATIVE TOXICITY OF ETHYL FORMATE FOR CONTROL OF PESTS OF SWEET PERSIMMONS FOR EXPORT

Jin-Hoon Cho[1], Chung Gyoo Park[1], Max Yoo[2], Bong-Su Kim[3], Byung-Ho Lee[3*]

[1.] Division of Applied Life Science, Gyeongsang Nat'l. Univ., South Korea
[2.] The Linde group, South Korea
[3.] Dongbu ARI, Dongbu Hannong Co. Ltd, South Korea
Corresponding author's e-mail: *byungholee@dongbu.com*

ABSTRACT

Vapormate™, a new gas formulation of ethyl formate (EF) with carbon dioxide, is an alternative fumigant to methyl bromide (MB). For applying export persimmons in Korea, the efficacy of EF was evaluated on an overwintering and summer type of *Tetranychus urticae* adults and egg stage of *Asiacornococcus kaki* which are pests in sweet (non-astringent) persimmon in Korea. Fumigated for 6 h at 5°C, the concentration × time (*Ct*) products of EF to overwintering and summer type of *T. urticae* was LC_{99}=147.9 and 18.8 g h m^{-3} and that was LT_{99} > 39.3 and 52.5 g h m^{-3} to *A. kaki*, respectively. We found sensitivity on EF is completely different from seasonal types of the mite as well as insect species.

Key words: Ethyl formate, Vapormate, methyl bromide chemical alternatives, export persimmon, quarantine, *Tetranychus urticae*, *Asiacornococcus kaki*, overwintering type, summer type.

INTRODUCTION

The current treatment for postharvest sweet persimmon disinfestation of pests such as overwintering two spotted spider mite [*Tetranychus urticae* Koch], grape-myrtle scale [*Asiacornococcus kaki* (Kuwana)] and persimmon fruit moth [*Stathmopoda masinissa* Meyrick] is methyl bromide fumigation (Jang, 2010). An alternative treatment is required as a result of its phase out in 2005 for all uses except quarantine treatments (UNEP, 2005) In addition, effects of human poisoning by methyl bromide are attributed as the fumigant causing great number of fatalities and injuries (EPA, 1986). Plant volatiles such as ethyl formate (EF) have been shown to have insecticidal properties (Rohitha *et al*. 1993). One important advantage of using volatiles such as EF for fumigation is that only trace residues were found on treated products (Desmarchelier and Ren, 2009). The Food and Drug Administration (FDA, 2004) has reviewed the use of EF as a flavoring agent and has characterized this compound as generally recognized as safe.

Ethyl formate has been used for disinfestation of pests in stored dried fruit since 1927 (Simmons and Gertler, 1945). More recently, EF has been tested for use in some fresh commodities. For example, packaged head lettuce infested with green peach aphid was exposed to 0.5-1.5% EF at 15℃ under vacuum for up to 2 h (Stewart and Mon, 1984). EF at 35g/m^3 with CO_2 has been showed the complete effectiveness on egg, lymph and adult stages of citrus mealybugs, *Planococcus citri,* for applying banana and orange without any phytotoxic damages (Sung et al, 2008, 2009).

For the treatment of persimmons to be exported from Korea, the efficacy of EF was evaluated on an overwintering and summer type of adult stage of *Tetranychus urticae* and egg stage of *Asiacornococcus kaki* which are the main pest in sweet persimmons in Korea.

MATERIALS AND METHODS

Test insects

Adults of overwintering and summer type of *Tetranychus urticae* were tested against a range of EF concentrations to ascertain the effect of fumigation on mortality. The overwintering type was collected from a farm in the Geochang, Gyeongsangnamdo Korea in 2011, while the summer type obtained from Gyeongsangnamdo Agricultural Research and Extension Services (Jinju, Korea) in 2006. We maintained the colony at 24±2℃, 60~70% RH, and a photoperiod of 16:8 (L:D) h on kidney beans, *Phaseolus vulgaris* L. For exposure to EF, a section of kidney bean with 30 adult mites was placed in Petri dish (6 cm i.d.). After treatment, adults of overwintering and summer type of *T. urticae* were held for 24 h at 25℃ and 50~60% RH, and then evaluated for mortality.

Grape-myrtle scale, were field collected from persimmon trees in Gyeongsang National University (Jinju, Korea). For exposure to EF, a sweet persimmon infested with 8~10 protonymph, deutonymph, or adult grape-myrtle scales were placed in desiccators (3-4 sweet persimmons per desiccator). For the egg stage, eggs were gently removed from adult female grape-myrtle scale with a small paint brush and placed in Petri dish. After treatment, the egg stage was held for 3 d at 25℃ and 50~60% RH, before evaluating for mortality. Petri dishes containing eggs were held for 8 d at 25℃ and ≥80% RH to ensure eggs survival and evaluation for mortality.

Measurement of ethyl formate

Concentrations of EF were monitored at 0.5, 2, 6 h after the injection of EF. The gas samples were stored in Tedlar® gas sampling bags using a gas-tight, 25 mL syringe, The *Ct* products were calculated from the arithmetic average of EF concentration readings during the 6-h exposure period.

Fumigant concentrations were determined using a gas chromatograph (GC-17A, Shimadzu co., Japan) fitted with a DB-WAX, flame ionization detector at 250°, injection port was at 100° (He served as carrier gas) and oven temperature was 100°.

Fumigant and fumigation

The fumigant used was an analytical grade (99.7%) liquid formulation of EF supplied by Aldrich Chemical Company Inc.,

Each target pest was exposed to at least nine concentrations (three replications per concentration) of EF between 0.3 and 66.8 mg/L, resulting in a range of mortality between 0 and 100%. All target pests were exposed to EF for 6 h at 5℃.

The fumigation chambers were 6.7-L desiccators, each equipped with a ground glass stopper fitted with a rubber septum (Schott Duran, German). A filter paper (55 mm i.d.) was inserted into the glass stopper to provide a liquid evaporation surface for the injected EF. Each experimental run consisted of four fumigated desiccators of different EF concentrations and one non-fumigated control desiccator.

For exposure to EF, a replicate consisted of Petri dishes or sweet persimmons containing target pests placed inside desiccators sealed with glass stopper. A partial vacuum was pulled with a syringe, and reagent grade (99.7% purity) liquid EF was injected through a rubber septum covering an inlet port in the glass stopper onto filter paper affixed to the underside of the stopper. At the completion of the 6 h fumigation, the desiccators were opened and aired for 1 h in a fume cupboard.

Data analysis

Lethal concentration estimates were performed using Polo Plus software program (LeOra Software, 2003). For grape-myrtle scale eggs, the LC_{99} was determined from estimated mortality, which was based on subtracting the number of hatched insects in each treatment from hatched insects in the control.

RESULTS

The observed and fitted data relating mortality to Ct products are shown Fig. 1, 2 and the results of probit analysis of the data are summarized in Table 1. The overwintering type *T. urticae* was more resistant to EF than the summer type. The Ct products for LC_{50} of EF were 66.25 and 11.46 mg h L^{-1}, and those for LC_{99} were 147.98 and 18.82 mg h L^{-1} for overwintering and summer type *T. urticae*, respectively, at 5℃, 6 h exposure.

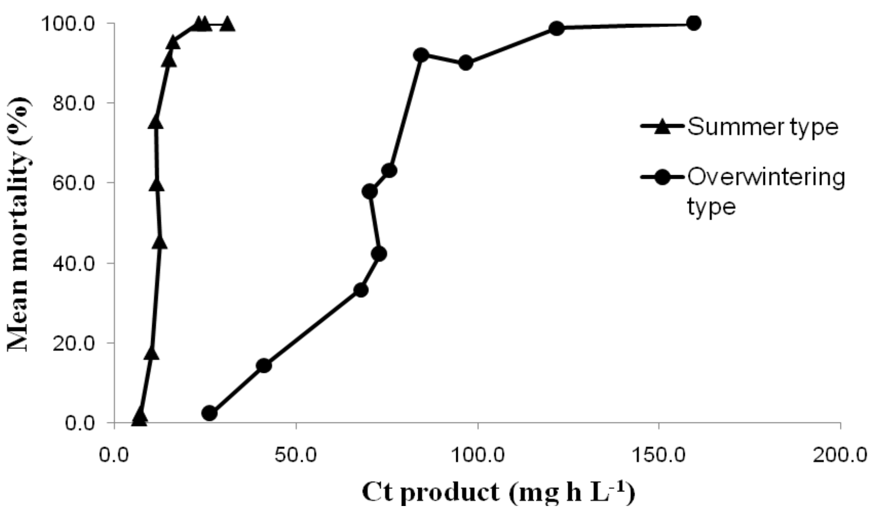

Fig. 1- Mortality of summer and overwintering type of *T. urticae* exposed to a range of Ct product for 6 h at 5°C.

The fumigant toxicity of EF to live stages of *A. kaki* was determined using a serial range of EF concentrations. The Ct products for the LC_{99} for egg, nymph and adult was 25.18, 41.1, 15.17 mg h L^{-1}, respectively. On the basis of LC_{99} values, tolerance of the live stages of *A. kak*i was in descending order: egg < nymph < adult.

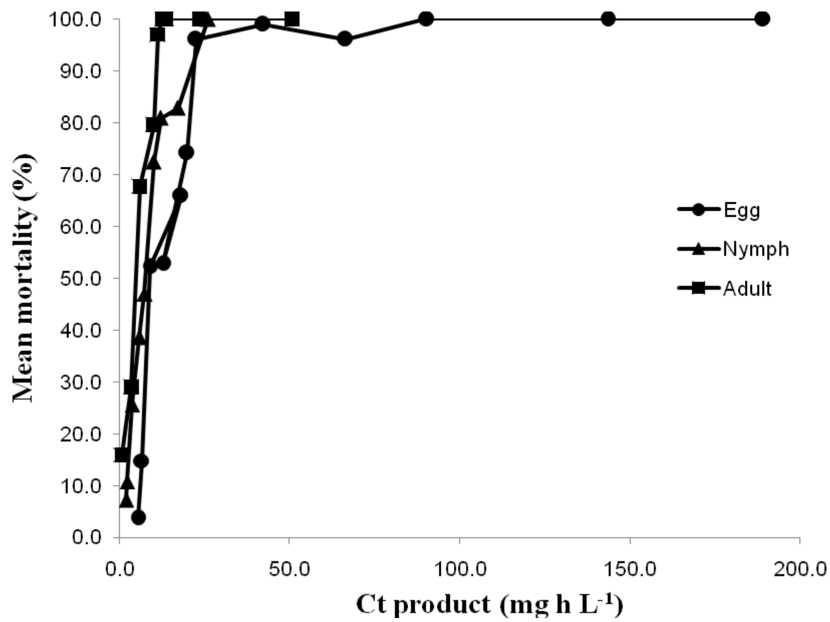

Fig. 2- Mortality of various life stages of *A. kaki* exposed to a range of Ct product for 6 h at 5℃.

Table 1. Dosage (measured as Ct product) estimates and parameters of regression of Probit mortality for exposure of the *T. urticae* and *A. kaki* to ethyl formate for the 6 h fumigation at 5±1℃.

Target pest	Type	Life stage	n[a]	Slope ± SE	LC_{50} (mg h L^{-1}± 95% CL)	LC_{99} (mg h L^{-1}± 95% CL)
Tetranychus urticae	Overwintering	Adult	1080	6.67 ± 0.78	66.25 (55.64~74.96)	147.98 (115.82~275.49)
	Summer	Adult	990	10.79 ±1.31	11.46 (10.53~12.32)	18.82 (16.31~25.68)
Asiacornococcus kaki	Summer	Egg	1519	3.685 ±0.370	12.20 (9.54~15.02)	52.18 (35.35~112.43)
		Nymph	624	2.944 ±0.221	6.66 (5.89~7.52)	41.12 (30.61~62.38)
		Adult	740	5.234 ±1.001	5.45 (3.90~6.59)	15.17 (12.24~22.73)

[a] Total number treated over three replications

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