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Ethyl Formate Plus Methyl Isothiocyanate Is A Potential Liquid Fumigant for Stored Grains

Yonglin. Ren*, T. Van Emmerik, D. A. Mahon, ByungHo Lee and B. Padovan

Abstract: A new fumigant designed for the grains industry consists of 95% ethyl formate (EF) and 5% methyl isothiocyanate (MITC) as a synergistic additive. The formulation is stable at 25, 30 and 45℃ during 2, 3 and 4 months storage. The formulation shows a high level of efficacy in controlling the major grain pests at all life stages of the insects. The dose to kill insects at all stages of *Rhyzopertha dominica*, *Sitophilus granerium*, *Sitophilus oryzae* and *Tribolium castaneum* in infested wheat and barley is 80 mg L^{-1} for 5 days and 25℃. After 8 days holding period, residues of EF and MITC were marginal or below the experimental permit level of 0.2 mg kg^{-1} and 0.1 mg kg^{-1} respectively, without the need for forced aeration of the wheat.

Introduction

Ethyl formate (EF) is an old fumigant which had been successfully used for individual package fumigation of dry fruits since 1929 (Simmons and Fisher 1945) and evaluated for grain protection in the 1980s (Muthu 1984). For the past few years, CSIRO Entomology has evaluated the use of EF as a fumigant for control of stored product insects. In previous commercial-scale trials with EF (90 gm^{-3}) on wheat (Desmarchelier *et al.* 1998), complete kill of mixed aged cultures of *Rhyzopertha dominica*, and adults and larvae of *Tribolium castaneum*, was achieved. Complete control of adult *S. oryzae* and 99% of progeny were also killed relative to the control. Results from all previous laboratory and commercial – scale trials with EF on wheat, barley, oats, field peas and canola (Desmarchelier *et al.* 1998; Ren & Mahon 2003; Mahon *et al*, 2003) have shown that the internal larval stages of *Sitophilus oryzae* are difficult to control. The dosage of EF required to completely kill the mixed aged cultures of *S. oryzae* was > 160 gm^{-3}, which is well above the flammable threshold of 84 – 91 gm^{-3}.

Methyl isothiocyanate (MITC) is colourless solid which is slightly soluble in water. It has a high boiling point (117 – 180℃) and a density of 1.069 g/mL. MITC occurs naturally at levels of 0.001 – 0.05 mg/kg in brassicas (Sarwar and Kirkeaard 1998a, b & c) where it has an important role in protecting these crops against pests (Sarwar and Kirkeaard 1998c). MITC is used as a soil fumigant for nematodes, fungi, and other diseases in vegetables and fruit (Gaetano & Matta 1992). Metam sodium degrades in the soil to MITC which is play the role of fumigant function (Ajwa, *et al.* 2003).

Previous research has shown that MITC can significantly reduce the dosage of EF to below the flammable level. In addition, MITC can synergise the toxicity of EF. Ren *et al.* (2005) reported that adults of *S. oryzae* were unaffected by 5.9 mg/L of EF for 24 hours at 25℃, but the addition of 5% MITC resulted in a 99% mortality of *S. oryzae* adults at the same EF dose, indicating significant synergism between EF and MITC.

This paper reports on an evaluation of a new formulation of EF consisting of 95% EF plus 5% MITC, which was developed by CSIRO Entomology, as a candidate fumigant for use on stored commodities.

Materials and Methods

Materials

The wheat used in this study was Australian Standard White (ASW). The moisture content of the wheat was 10.5, 12.5 and 14.5% determined by using a Graintec HE 50 electronic moisture meter and verified by use of the oven method. The results obtained were expressed as a percentage calculated from replicates.

The EF used was the formulation Eranol, supplied by Orica Australia and has an active ingredient of 97.1% EF. The MITC was supplied by Aldrich Chemical Company Inc. The formulation used was 95% EF + 5% MITC, v/w.

Measuring Stability of Formulation

The stability of the formulation was deter-

CSIRO Entomology, GPO Box 1700, *Canberra ACT*, 2601, *Australia*
* *Corresponding author*: *yonglin. ren@ csiro. au*

mined during storage at 25, 30, and 45℃ for 2, 3 and 4 months. Confirmation of identity was obtained by Gas Chromatography/Mass Spectroscopy (GC/MS) on a Finnigan Ion Trap, after separation on a capillary DB – 624 column (J & W, 122 – 1334). The formulation was determined on a Tracor 220 GC, equipped with a flame photometric detector, after separation on a 1m glass column packed with HayeSep Q (Alltech, 2801). A Bruker Tensor 37 FTIR spectrometer equipped with a Deuterated L – α – Alanine doped Triglycine Sulphate, DLATGS detector, was used to collect all spectra. Samples were analysed using a diamond with ZnSe lens single reflection ATR MIRacle accessory (Pike Technologies). For both the background and samples, 32 scans were collected over the wavelength range 600 – 4000 cm^{-1} at a spectral resolution of 4 cm^{-1}. Two drops of the liquid formulation were placed on the ATR crystal and the sample covered with a volatiles cover (Pike Technologies). ATR – FTIR spectra were collected from the liquid formulation during storage at different times and temperatures in 10ml glass micro flasks (Alltech) sealed with mininert valves (Alltech).

Measuring Concentrations of EF and MITC

The concentration of the EF and MITC components was determined using a Varian STAR 3400CX gas chromatograph (GC) equipped with a flame ionisation detector (FID) after isothermal separation on a 30m (0.53mm (i. d.) megabore capillary column ZBWAX (B13844) at the oven temperature of 95 or 140℃. The concentrations of EF were calculated on the basis of peak areas against external standards, prepared by dilution in 250 ml bottles with a Mininert valve equipped with septa (Alltech Australia, Cat. No. 95326). A sample volume of 50 – 100 l was injected into the GC – FID.

Insects and Bioassays

Toxicity studies of EF alone compared with EF + MITC were conducted on adult and mixed aged cultures (an unknown quantity of eggs, larvae and pupae from four cultures of different start dates). Wheat and barley columns (1.5m tall (24.5cm inside diameter (i. d.)) with a capacity of 52kg and 95% full were used for bioassays. Steel cylindrical cages (50mm (30mm i. d.) containing mixed aged cultures of *T. castaneum*, *S. granerium*, *S. oryzae* and *R. dominica* where placed at various points within the grain bulk. The application rates used were 80 and 2 × 80 = 160 g/t (double injection of 80 g/t, after 4 hours, the second dose was injected) for EF and 80 g/t for EF + MITC was applied to the top of column and subjected to a low rate of recirculated air (1 gas exchange/hour) at 25℃ and for 5 days exposure. Bioassay samples were retrieved at the end of the fumigation period, the adult insects were counted and removed and the remaining mixed – age cultures incubated at 25℃ and 70% RH. Subsequent emerging adult insects were counted weekly for a period of 6 weeks, with live and dead adults removed at each count.

Measuring Sorption/Desorption of Formulation on/from Wheat

The sorption of the formulation on wheat was measured as disappearance of EF and MITC in the headspace of grain filled Erlenmeyer flasks. The concentration of EF and MITC was measured by GC at timed intervals. Ethyl formate and MITC standards were prepared for calibration of the GC and calculation of the concentration of EF and MITC in the fumigation flasks. At the end of the fumigation, the Erlenmeyer Flasks were opened and the grain was placed in 2.5L glass jars in a fume cupboard for initial aeration, but later removed to a constant temperature room and humidified where necessary. For the desorption study, a fumigated sample was taken immediately after opening the flask (called 0 day aeration) and on the following 1, 3, 8 and 14 days after the start of aeration. The desorption sample was placed into a 100mL flask equipped with a ground-glass joint and a septum, using a 90% fill ratio. As with the sorption procedure, the headspace readings were taken as soon as possible, and following readings were taken on 1, 3, 8 and 14 days after sampling for desorption, until the headspace concentration of EF and MITC no longer increased or decreased. Residue samples were taken at the same time as the desorption study (immediate after opening called Day 0, and on Day 1, 3, 8 and 14 of aeration), and where possible analysed immediately. If they were not able to be analysed immediately they were stored in a – 20℃ freezer and thawed to room temperature during analyses.

Analysis of EF and MITC Residues in Wheat

Residues of EF in wheat were analysed following the procedure as described by Vu and Ren (2004). A wheat sample of 100g and 100mL of 70% (w/w) ammonium nitrate were

placed in a sealed 250 mL flask and the levels of the residue were determined by sampling the headspace of the flask. Levels of EF residue were determined against spiked standards, prepared by adding EF to untreated commodity plus extraction solvent. The MITC residues were analysed by headspace Solid Phase Micro-extraction (HS – SPME). The SPME fibre used was an 85 m Polyacrylate (PA) (Sigma – Aldrich Australia, Cat. 57304). A wheat sample of 50g in a 250 ml flask fitted with a sample port was immersed in a heated oil bath for 45m. The SPME fibre was inserted through the septa into the sample and exposed for 5m and then taken to the GC. Levels of MITC residue were determined against spiked standards, prepared by adding MITC to methanol. MITC was determined by a Varian *CP*-3800 gas chromatograph (GC) equipped with a flame ionisation detector (FID) after isothermal separation on a 25m (0.53 mm i. d. Varin Capillary column CP – Pora-BOND Q at the oven temperature of 150℃.

Results and Discussion

Stability of Formulation

The stability of EF and MITC in a ratio of 95:5 v/w % (EF:MITC) after storage for 2, 3 and 4 months at 25, 30 and 45℃ was determined using GC/FID, with the peak areas compared with those of a standard formulation. The percentage of MITC and EF present was then calculated.

No significant decrease in the level of MITC or EF was found at any of the temperatures and times investigated. IR GC/MS spectra of the liquid formulation were collected by FTIR and GC/MS. There was no presence of interconversion, or breakdown products (e. g. methylamine, formic acid, and ethanol) (Fig. 1 and 2) whose bands are associated with EF, and MITC (aliphatic, carboxylic, and MITC functionalities) at the temperatures and times studied. The formulation therefore appears stable at the times and temperatures tested.

Table 1. Percentage of MITC/EF at different storage times and temperatures

emperature (℃)	MITC/EF at different storage time (months)		
	2	3	4
25	100/100	100/100	109/97
30	97/99	102/103	97/98
45	101/98	100/92	93/94

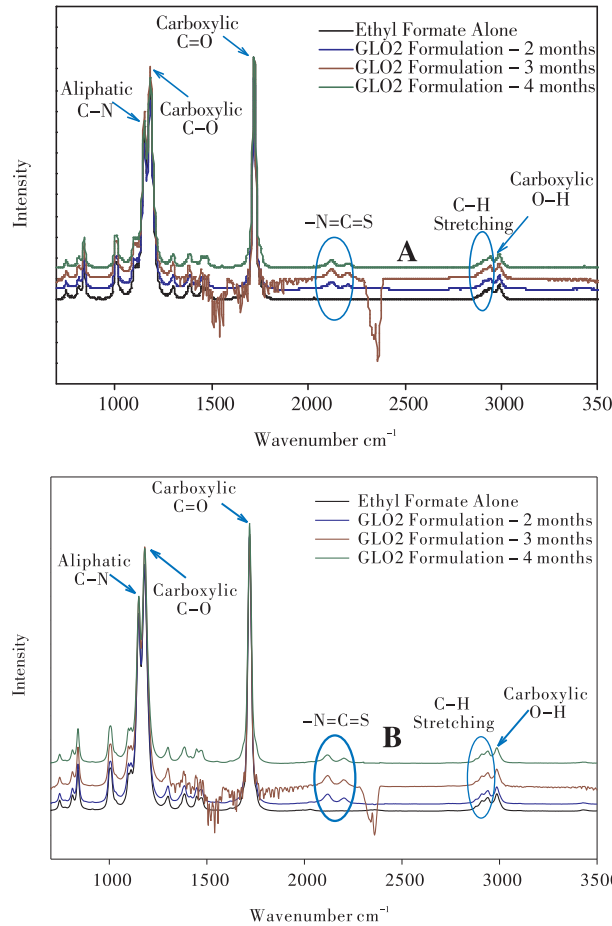

Fig. 1 IR spectra of the liquid formulation stored for 3m at 25℃(A) & 45℃(B)

Bioassays

The bioassay results from a 52kg wheat and barley column showed that all stages of the four insect species (*T. castaneum*, *S. granerium*, *S. oryzae* and *R. dominica*) and adults of three insect species (*T. castaneum*, *S. oryzae* and *R. dominica*) were completely killed by EF + MITC of 80 g/t and 160 g/t of EF alone respectively.

A dose of 80 g/t of EF without MITC killed all adult *T. castaneum* and *R. dominica*, but only 98% – 100% of *S. oryzae* adults. These results are consistent with previous commercial-scale trials that showed EF used alone controlled adult *T. castaneum* and *R. dominica*, but not *S. oryzae* adults, fumigated in wheat, split faba beans and sorghum (Mahon *et al.* 2003; Ren *et al.* 2003).

Sorption and Desorption

The sorption of the formulation (for both EF and MITC) on wheat decreased with an increase in the moisture content of wheat (Fig. 3 and Fig. 4). Within the first 3h after application, almost 70% of the formulation was ab-

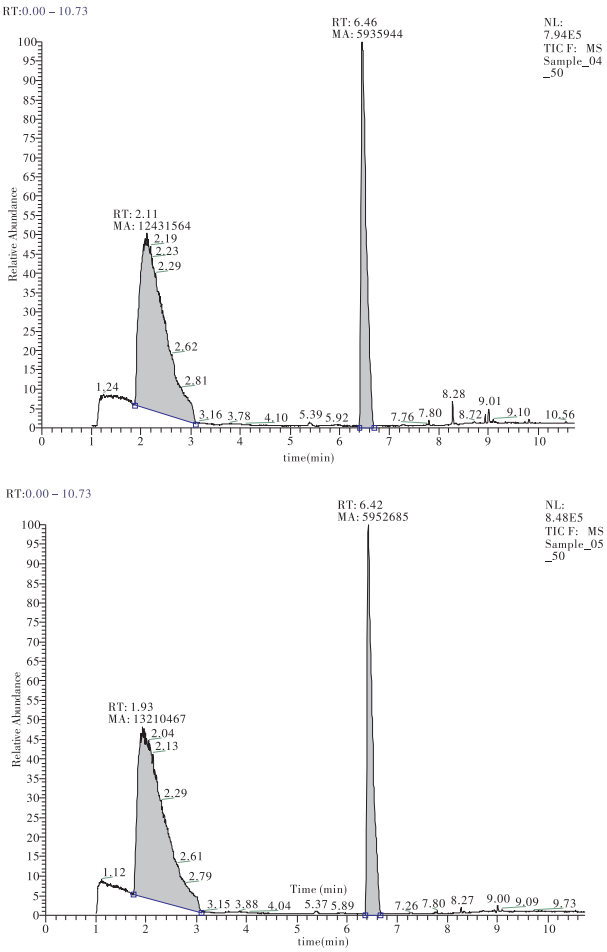

Fig. 2 GC/MS spectra of the liquid for mulation stored for 3m at 25℃(A) and 45℃(B)

sorbed by the wheat. Desorption rate of EF and MITC from the wheat was affected by the moisture content, exposure time and holding period. The desorption rate decreased with increasing moisture content, e. g. more EF and MITC were desorbed from 10.5% mc wheat than from 12.5 and 14.5% mc wheat (Fig. 5). More EF and MITC were desorbed from wheat which was treated with a short exposure period. The first day holding period removed 70% – 80% of the EF and MITC from wheat. The levels of EF and MITC which were desorbed from wheat at different moisture content and exposure time were significantly below the TLV of 300ppm EF and 0.1 ppm MITC.

Residues of EF and MITC in Wheat

During the period of exposure, both EF and MITC declined, e. g. MITC residue levels of 0.6, 0.5, 0.2 and 0.1mg/kg in wheat (12.5% mc, at 25℃) after 1, 3, 8 and 14 days fumigation (Fig. 6). During the holding period, both EF and MITC were further reduced, e. g. 12.5% mc wheat fumigated for 1 day, MITC

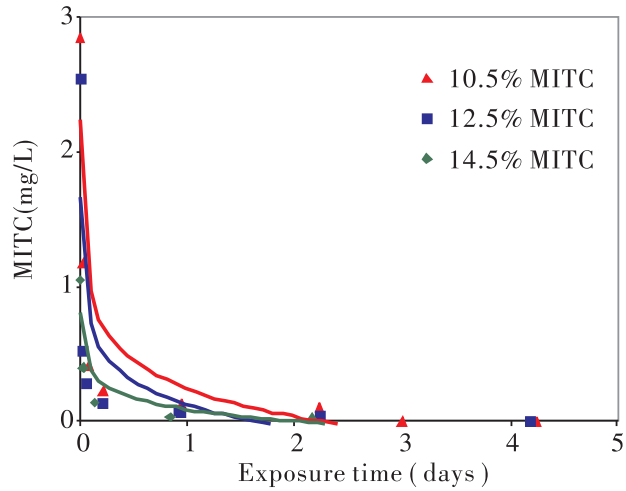

Fig. 3 Sorption of MITC on wheat at 25℃

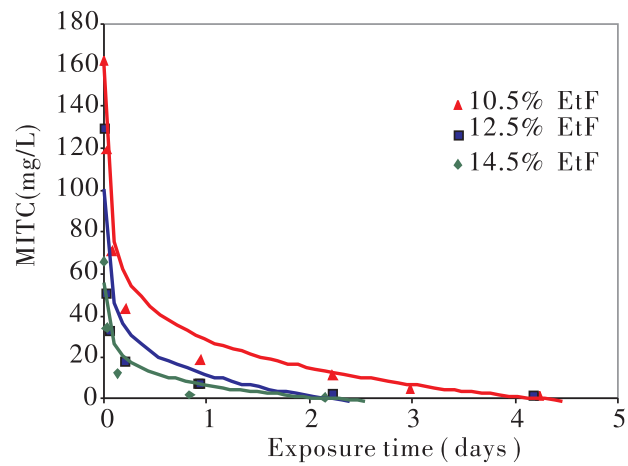

Fig. 4 Sorption of EF on wheat at 25℃

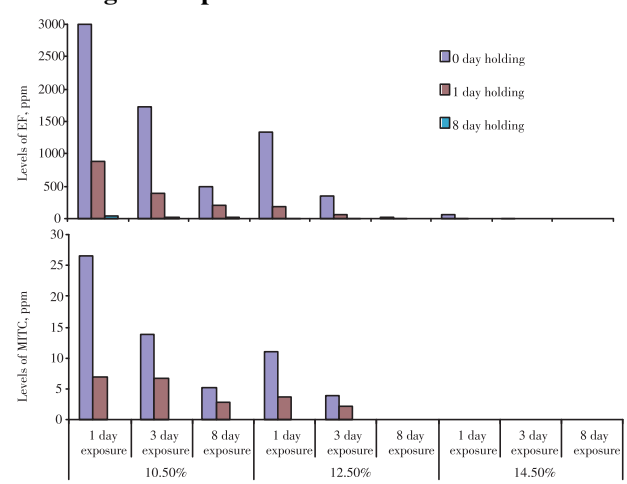

Fig. 5 Desorption of EF and MITC from wheat at 25℃

residue levels were 0.5, 0.1 and 0.07 mg/kg after 1, 8 and 14 days holding. The increasing moisture content appeared to accelerate the decrease in both EF and MITC residues. After 8 days holding period, residues of EF were above those in the control grain sample, but below the experimental permit level of 0.2 mg/kg, without

the need for forced aeration. The levels of the MITC in the same wheat after 8 days holding period had also declined to marginal or below the experimental permit level of 0.1 mg/kg without the need to use forced aeration. The EF results are consistent with previous commercial-scale trials with EF on wheat, barley, oats and peas (Desmarchelier *et al.* 1998; Mahon *et al.* 2003; Ren *et al.* 2003).

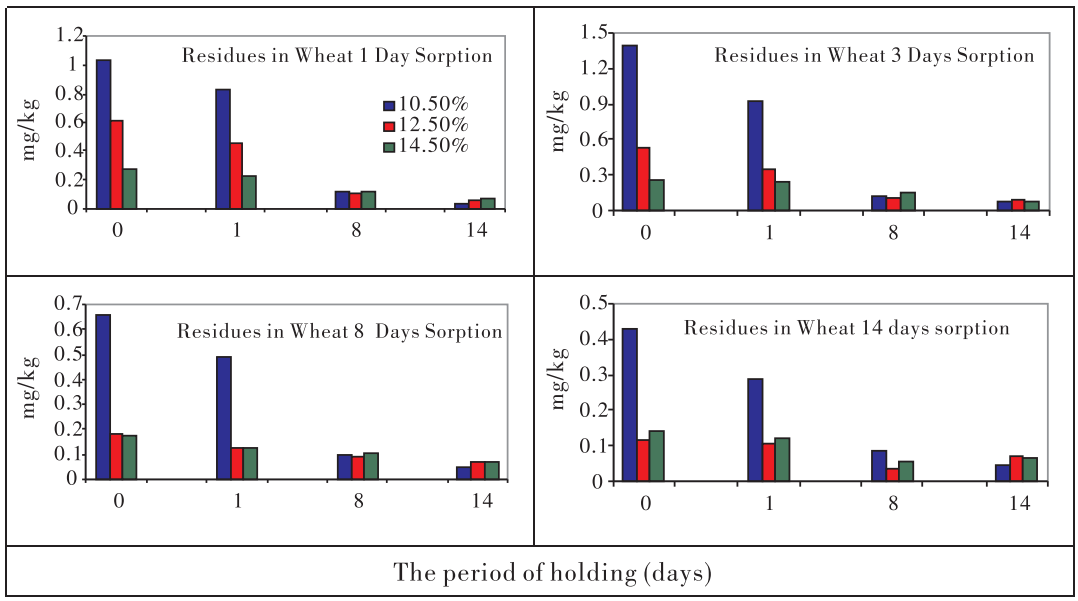

Fig 6. MITC residues in wheat treated with increasing exposure periods and with varying moisture content and holding periods (days).

Conclusions

All stages of the four insect species (*T. castaneum*, *S. granerium*, *S. oryzae* and *R. dominica*) and adults of three insect species (*T. castaneum*, *S. oryzae* and *R. dominica*) were completely killed by EF + MITC of 80 g/t, compared to EF alone which failed to achieve complete mortality of *S. oryzae* adults. EF + MITC desorbed over an 8 day period to levels that were within the experimental permit level for residues of both fumigants. MITC appears to have a synergistic effect against pests allowing EF to be effective at lower doses than EF used alone. We conclude that EF + MITC formulation has potential as a fumigant for controlling stored product pests in commodities.

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