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THE FUMIGANT BEHAVIOUR OF ETHYL FORMATE MIXED WITH NATURAL PLANT PRODUCTS

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ABSTRACT

The ethyl formate (EF) was tested in mixtures with various natural plant products (NP) as a fumigant against internal stages of *Sitophilus oryzae* in grain. Novel formulations of EF (EF: NP=9:1, (v/v)) were shown to be chemically stable for 1 month after first mixing. EF acts as a good solvent for various NP having insecticidal activity.

Toxicity of EF itself and various NP formulations of EF to mixed age cultures (MAC) of S. oryzae was compared in 6 and 24-hr exposures. EF plus carvone and EF plus thujone showed slightly more toxicity than EF alone for 6-hr exposures at 67.5mg/L and significantly more toxicity for 24-hr exposures at 33.8mg/L to pupal stages of S. oryzae. EF plus monoterpene formulations showed no significantly enhanced toxicity compared to EF itself, especially in these short term exposures.

Continuing research is aimed at finding NP formulations having enhanced toxicity with respect to internal stages of stored grain insects as well as low mammalian toxicity.

INTRODUCTION

Ethyl formate (EF) as a fumigant was first found effective for grain fumigation on box-cars in Australia (Neifert et al., 1925). Field trials of surface treatment of wheat were reported in 1946 but EF was ineffective because of inappropriate dose and application method (Wilson and Mills, 1946). Use of EF on grain has been proposed several times during the past 30-40 years, but the disadvantage of lower insecticidal toxicity compared to other fumigants and higher flammability hindered further research. Current chemical strategies involving safer, environmentally friendly chemicals make it appropriate to carry out further tests on EF as a potential alternative fumigant. EF is currently registered as a fumigant on dried fruit in Australia (Hilton and Banks, 1997) and is used as a food additive for various reasons worldwide (FDA, 1979). Recently there has been more research into use of EF for durable commodities because of its valuable attributes such as fast kill (<2 days), its safe use for grains and cereals, rapid breakdown and possible protectant as well as fumigant properties (Annis, 2000). Recent field trials showed success in terms of rapid fall off to below the maximum residue level (MRL) without forced aeration,

keeping within the threshold limit value (TLV) (<100ppm for fumigation period), flammability limit and maintenance of quality (germination and colour etc). However, there is some concern that there was incomplete control of the internal stages of *Sitophilus oryzae* (particularly larvae and pupae) due to loss of EF during application, low doses (85g/t) to avoid exceeding flammability limit and low temperature conditions for the fumigation period.

Herein, the present work describes the screening of EF mixed with other natural plant products with respect to chemical stability and enhanced toxicity of EF to internal stages of *S. oryzae*.

EXPERIMENTAL PROCEDURE

Preparation of formulations

Formulations are listed in Table 1. Each formulation was made in a foil-wrapped vial to protect against photo-breakdown. Selection of natural products for this research was based on their fumigant and/or grain protectant toxicity (Lee et al., 2001a, 2001b, Lee 2003, Lee et al., 2004a, 2004b).

Chemical stability of formulations

Chemical stability of each formulation was determined within 2 hr and then 1 month after preparation. Analyses were performed on a Varian Star 3400 GC with ZB-Wax column (30m_ 0.53mm ID _ 1.00μm) with FID operated in conjunction with Star chromatography workstation program. The instrument oven was programmed as follows: start at 70 °C held for 2 min, 70-80°C at 1°C /min, 80-190°C at 10°C/min, 190-250°C at 30°C/min. Injector and detector temperature was 250°C.

Fumigant bioassays

Mixed age treatment: Mixed-age cultures of S. oryzae were set up by adding adults (1g) to media (wheat 800g; variety Rosella, m.c 11.3-12.1%) at 25°C and 60-70% relative humidity (r.h.). They were left 4-5 weeks, at which time there were representative numbers of each stage (egg, larvae, pupae and adults) based on known development rates (Howe, 1952). After this time, the wheat was mixed and divided using a Böerner divider, to give 16 samples of 49.5-50.5 g and each sample spilt 5 times (10 g each). This procedure resulted in, on average, 129 emergent adults in 10 g non-fumigated grain within 4 weeks. Fumigation of mixed-age cultures (10 g) was carried out in 100 ml glass containers (Schott Bottle) with modified airtight sealed lids fitted with a cylindrical septum. Doses of 67.4 and 101.0 mg/L of each formulation were tested for 6 hr and this treatment was replicated at least 3 times. No chemical was added to the flasks used for control mortality. After treatment, the

cultures were held at 25± 0.5 °C in a constant temperature room under normal rearing conditions. The concentrations of atmospheric/respiratory gases in representative bottles were measured using a Fisher Gas Partitioner (Model 1200, Column Temp.; 48 ºC, integrator; Hewlett Packard HP 3394A) with TCD. CO_2 concentrations in control bottles after 6 hr were found to be 6.1-10.7 %. On completion of fumigation the flasks were aired in a fume hood for 48 hr. The wheat was then transferred to culture jars and incubated at 25 ± 0.5 °C, 60-70 % r.h. for 8 weeks. Adult emergence was recorded every week until the next generation would have emerged (Howe, 1952). Statistics were based on total emergence in the treated sample against total emergence in the control sample.

Pupal stage treatment: Pupae stages were obtained by placing approximately 50 adults on 200 g of wheat and incubating at 25 °C for 3 days prior to fumigation when adults were removed, and the wheat incubated for 4 weeks at 25°C, 60-70% rh. The wheat was then mixed and divided using a Böerner divider, to give 4 samples of 49.5-50.5g and each sample was divided 5 times (10 g each). Two of them were kept as controls and three were used for treatment. Controls gave on average 12.2 emergent adults in non-fumigated grain after 3-4 weeks.

Fumigation of pupae was carried out by the same method in mixed-age culture experiments using different doses (33.8 and 67.5 mg/L) and different exposure times (6, 24hr). CO_2 concentrations in pupal stage controls at 6hr and 24hr were found to be 0.25-0.38 and 0.37-1.05 %, respectively. Adult emergence was recorded at 2, 3 and 4 weeks post-fumigation.

RESULTS

The stability of formulated EF is shown in Table 1. Two components (EF and NPs) in new liquid formulations were shown to have no degradation and no reaction during 1 month.

The toxicity of EF itself and EF formulations to mixed age cultures of *S. oryzae* for 6-hr exposure is shown in Table 2. EF at 67.4 mg/L after 6hr exposure showed 57-69% and at 101 mg/L showed 80% of inhibition of mixed aged cultures of *S. oryzae*. There was no significant inhibition found with other formulations. Most formulations at 67.4 mg/L were similar or less toxic compared to EF itself. EF plus p-cymene was significantly less toxic than EF itself.

The toxicity of EF and EF formulations to pupal stage of *S. oryzae* for 6 and 24hr exposures was shown in Table 3. EF at 33.8 mg/L resulted in 55.6 and 59.3% inhibition for a 24hr exposure. At 67.5 mg/L, inhibition was 61.1% for 6hr exposure and 79.7% for a 24hr exposure. For 6-hr exposure, EF plus carvone, EF plus menthone and EF plus thujone at 67.5 mg/L showed slightly more toxicity to pupal stages of *S. oryzae*. Other formulations at 67.5 mg/L were of similar or lower toxicity

compared to EF itself. However, with a 24-hr exposure with half the concentration (33.8 mg/L), EF plus thujone and EF plus carvone were more toxic and other formulations were similar or slightly less toxic than same concentrations of EF to pupal stages of *S. oryzae*.

TABLE 1

List of EF formulations and their chemical stability

New formulated candidate chemicals	Mixing ratio, EF: Chemicals (V/V)	Chemical stability	
		< 2hr	1 month
1,8-cineole	9:1	S	S
Limonene	9:1	S	S
Benzaldehyde	9:1	S	S
Thymol	9:0.5	S	S
Eugenol	9:1	S	S
Menthone	9:1	S	S
Terpin-4-ol	9:1	S	S
p-Cymene	9:1	S	S
_-Pinene	9:1	S	S
Thujone	9:1	S	S
Carvone	9:1	S	S
Estragole	9:1	S	S

S (Stable): liquid mixed formulations showed no change in composition or ratio by GC analysis (FID)

DISCUSSION

Many natural products have fumigant or/and grain protectant properties. For example, limonene to *Sitophilus oryzae* (Karr and Coat, 1988) and *Callosobruchus maculatus (*Don-Perdro, 1996), linalool to *Acanthoscelides obtectus, Rhyzopertha dominica* and *S. oryzae (*Weaver and Subramanyan., 2000), terpineol, carvacrol, thymol and eugenol to *A. obtectus* (Regnault-Roger *et al*., 1993), anethole and cinnamaldehyde to *Tribolium confusum* and *S. zeamais* (Ho et al., 1997; Huang and

TABLE 2

Toxicity of EF and formulated EF to mixed age cultures of *S. oryzae* (6hr exposure, 25°C)

EF & EF + chemicals	Applied Dose (mg/L)	Average number Untreated	Treated	Percent (%) inhibition
EF	67.4	133	41.3	68.9
EF	67.4	124	53.3	57.0
EF	101	124	24.3	80.4
EF + 1,8-Cineole	67.4	133	52.5	60.5
EF + 1,8-Cineole	67.4	124	49.5	60.1
EF + 1,8-Cineole	101	124	34	72.6
EF + Limonene	67.4	133	36.5	72.6
EF + Limonene	67.4	124	61	50.8
EF + Limonene	101	124	31.5	74.6
EF + Benzaldehyde	67.4	133	50.5	62.0
EF + Benzaldehyde	67.4	124	49	60.5
EF + Benzaldehyde	101	124	32.5	73.8
EF + Thymol	67.4	133	51.5	61.3
EF + Thymol	67.4	124	45.5	63.3
EF + Thymol	101	124	18.5	85.1
EF + Eugenol	67.4	133	50.5	62.0
EF + Eugenol	67.4	124	41.5	66.5
EF + Eugenol	101	124	19.5	84.3
EF + Menthone	67.4	133	53.5	59.8
EF + Terpin-4-ol	67.4	133	45.5	65.8
EF + p-Cymene	67.4	133	89	33.1
EF + _-Pinene	67.4	133	56	57.9
EF + (-)-Thujone	67.4	133	48.5	63.5
EF + Carvone	67.4	133	40	69.9
EF + Estragole	67.4	133	52	60.9

EF: Ethyl formate - Each formulation : 9:1 (EF: Chemicals ,v/v) except thymol 9.5:0.5(v/v)

Inhibition is based on percent of untreated emerged adults

Average number of emerging adults is based on more than 3 replicates

TABLE 3

Toxicity of EF and formulated EF to pupal stages of *S. oryzae* (6 and 24hr exposure, 25°C)

EF & EF formulation	Applied Dose (mg/L)	Exposure Time(hr)	Av. No. of emerging adults Untreated	Treated	Percent (%)
Control			15		0
EF	33.8	24		6	59.3
EF	67.4	24		3	79.7
Control			12.0		0
EF	33.8	24		5.3	55.6
EF	67.4	6		4.7	61.1
EF+1,8-Cineole	33.8	24		6	50
EF+1,8-Cineole	67.4	6		4.7	61.1
Control			12.0		0
EF+Limonene	33.8	24		6.7	44.4
EF+Limonene	67.4	6		6	50
Control			14		0
EF+ Benzaldehyde	33.8	24		13.4	4.8
EF+ Benzaldehyde	67.4	6		10.6	23.8
EF+ Thymol	33.8	24		5.4	61.9
EF+ Thymol	67.4	6		12.0	14.3
Control			8.8		0
EF+ Eugenol	33.8	24		3.3	61.9
EF+ Eugenol	67.4	6		6	31.4
Control			11		0
EF+ Menthone	33.8	24		9.4	15.2
EF+ Menthone	67.4	6		2.6	75.8
EF+Terpin-4-ol	33.8	24		4	63.6
EF+Terpin-4-ol	67.4	6		13.4	-
Control			10.6		0
EF+ p-Cymene	33.8	24		10.6	-
EF+ p-Cymene	67.4	6		13.4	-
EF+_-Pinene	33.8	24		1.4	87.3
EF+_-Pinene	67.4	6		4	61.9
Control			16.8		0
EF+ Thujone	33.8	24		3.3	80.1
EF+ Thujone	67.4	6		2.7	84.1
EF+Carvone	33.8	24		3.3	80.1
EF+Carvone	67.4	6		4.7	72.1
Control			9.5		0
EF+ Estragole	33.8	24		6.7	29.8
EF+ Estragole	67.4	6		7.3	22.8

EF: Ethyl formate,

Each formulation: 9:1 (EF: Chemicals, v/v) except thymol 9.5:0.5(v/v)

Inhibition is based on percent of untreated emerged adults

Average number of emerging adults is based on 3 replicates.

- : No inhibition

Ho, 1998); 1,8-cineole and limonene to *R. dominica* (Dunkel and Sears, 1998) and *T. confusum* (Prates et al., 1998), camphor, 1,8-cineole and eugenol to *T. confusum, S. granarius, S. zeamais* and *Prostephanus truncatus* (Obeng-Ofori and Reichmuth, 1997,1999), estrogole and anethole to *S. oryzae*, *Callosobruchus chinensis* and *Lasioderma serricorne* (Kim and Ahn, 2001), 1,8-cineole, linalool, p-cymene, terpinen-4-ol, thujone to *S. oryzae* (Lee *et al*., 2001a), menthone, linalool, _-pinene to *S. oryzae* (Lee *et al*., 2001b), eugenol, isoeugenol and methyl eugenol to *T. confusum, S. zeamais* (Huang *et al*. ,2002), 1,8-cineole to *T. castaneum* and Phosphine-resistant *T. castaneum (*Lee *et al*., 2002), 1,8-cineole to internal stage of *S. oryzae* (Lee *et al*., 2004a), 1,8-cineole and other candidate monoterpenes to *T. castaneum, R. dominca* and *S. oryzae* (Lee *et al*., 2004b). Although comparison of fumigant toxicities between fumigants is difficult, the above mentioned chemicals, mostly monoterpenes, are of relatively low toxicity (Lee *et al*., 2004b).

Toxicity of EF to insects has been reviewed (Adu and Muthu., 1985, Muthu *et al*., 1984; Hilton and Banks, 1997). Muthu *et al*. (1984) reported that eggs of *S. oryzae* were the most susceptible and pupae were the most tolerant stages for EF fumigation. For wheat fumigation with EF, *S. oryzae* adults with short exposure times (< 3 hr) showed a wide range of responses for a given Concentration × Time product (CT product), but high concentrations appeared much more effective than low ones (Annis, 1998). Wright et al. (2001) reported that the CT product for *S. oryzae* (late larvae/early pupae stage) was at least 660 mg h/L at 25°C, 30-50% r.h, for complete control. The CT product for *S. oryzae* was 1500 mg h/L at 25 and 30°C and >2000 mg h/L at 15°C. Young pupae of *S. oryzae* are the most tolerant stage (>2200 mg h/L) (K. Damcevski, personal communication). In current research, EF showed fumigant toxicity against *S. oryzae* adults with 50% filling ratio of wheat; exposure time of 12min and 2h achieved 100% mortality for concentration of 340 and 210 mg/L, respectively. Also, an exposure time of 3h achieved 94 % mortality for a concentration of 130 mg/L. All *S. oryzae* larvae were killed at 109, 130 and 155 mg/L of EF on 500, 1000 and 1500g wheat, respectively, in a 48-hr exposure (Damcevski and Annis, 2001). However, this higher dose is not applicable to field trials because of flammability limits for EF (85g/t).

In testing mixtures of candidate natural products and EF, our results showed no significant enhanced toxicity, especially in terms of short-term exposure (6 and 24-hr exposure). There are some experimental limitations for fumigations longer than 48 hr because of the relatively high CO_2 concentration (5-15%) generated in sealed systems. CO_2 concentration greater than 3% synergises the activity of EF (G. Dojchinov, personal communication). The significant result is the potential use of EF as a solvent for application of other various natural materials as fumigants and/or protectants. None of the chemicals in these experiments reacted or degraded. Future work is aimed at finding EF formulations enhancing the potential synergistic action.

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