A synergistic mixture of diatomaceous earth and deltamethrin to control stored grain insects

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Abstract

In order to mitigate the negative effect of diatomaceous earth (DE) on bulk density and grain flowability, DE was mixed with other insecticides. This paper investigates the efficacy of a mixture of DE and deltamethrin against Sitophilus zeamais, the maize weevil, *Rhyzopertha dominica*, the lesser grain borer, and *Tribolium castaneum*, the red flour beetle. Five mixtures of DE and deltamethrin were prepared in the laboratory containing the same quantity of DE and different concentrations of the active ingredient of deltamethrin. The ratio of DE and deltamethrin in formulations were: DE 1 part: deltamethrin 0.00025, 0.00050, 0.00075, 0.0010 and 0.00125 parts. Co-toxicity and Co-efficient values higher than 100 (S. zeamais 170–386, R. dominica 188–601, and *T. castaneum* 157–285) indicated synergism between DE and deltamethrin.

Keywords: Diatomaceous earth, Deltamethrin, Ready to use mixture, Co-toxicity, Co-efficient, Synergism, Grain insect pests

1. Introduction

Insects infesting grain after harvest cause economic loss to producers and the grain and food industry. During the past few decades application of synthetic pesticides to control agricultural stored-products insect pests has been a standard practice. However, with the growing evidence regarding detrimental effects of many of synthetic pesticides on health and environment, the grain industry wants to reduce the use of synthetic pesticides because of insecticide deregulation, resistant populations and consumer concerns over insecticide residues. Therefore, there is a need to evaluate alternatives to conventional synthetic pesticides.

Diatomaceous earth (DE) is a natural product registered in some countries for use directly on stored grain and in empty grain stores to control insects (Korunic, 1998; Subramanyam and Roesli, 2000). The main advantages of DEs are its low-toxicity to mammals and its stability. Amorphous SiO_2, the main active ingredient in natural DE, is considered Generally Recognized as Safe (GRAS), and is a registered food additive (21 CFR 182.90, 182.1711) in the United States. Several tests with DE have shown there were no effects on end use quality in baking, malting or pasta production (Aldryhim, 1990; Korunic et al., 1996).

However, DE does have some disadvantages that hinder its widespread use (Fields, 1999). DE significantly reduces the bulk density (test weight) and flowability of grain. Because of high concentrations needed to control insect pests it is dusty to apply and it has a low efficacy against some insect species. High grain moisture contents and high air relative humidity significantly reduce its efficacy (Fields and Korunic, 2000). Currently used DEs are applied between 100 (only against *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae), the most sensitive insect against DE) and 1000 ppm (Subramanyam and Roesli, 2000). Rates above 300 ppm cause a considerable bulk density reduction (test weight) and significant reduction in grain flowability (Korunic et al., 1998; Korunic, 1998). Also, the presence of high DE concentration with increased quantity of crystalline silica may cause respiration problems (silicosis) to workers after long exposure. Hence, it is essential for researches to evaluate the use of novel DE formulations that are effective against insects at lower dose rates.

Deltamethrin is used in stored-grain protection, plant protection, livestock protection and public health. Deltamethrin has a very broad spectrum of control, and is effective in controlling *Rhyzopertha dominica*

(F.) (Coleoptera: Bostrichidae) resistant to organophosphate (OP) insecticides, but not *Sitophilus* spp. (Coleoptera: Curculionidae) and *Tribolium* spp. (Coleoptera: Tenebrionidae) (Daglish et al., 1995). Deltamethrin resistance has also been reported for *R. dominica* (Lorini and Galley, 1996).

Several studies using insects of stored products have shown synergism between OPs and pyrethroids (Daglish eta al., 1995; Dalglish, 1998). To date there are no published data regarding synergism between DE and deltamethrin. The mixture of DE and deltamethrin was developed to mitigate the disadvantages of DE and reduce deltamethrin residues in grains. It addition to efficacy benefits, the combination of different modes of action could also reduce the development of resistance. The objective of this research was to determine if mixtures of DE and deltamethrin acted synergistically against three storage insects, *Sitophilus zeamais* Motschulski, *R. dominica* and *Tribolium castanum* (Herbst).

2. Materials and methods

Mixed-sex adult *S. zeamais*, *R. dominica* and *T. castaneum*, 7 to 21 d old, were used for all experiments. *Sitophilus zeamais* and *R. dominica* were cultured on wheat with approximately 14% m.c. *Tribolium castaneum* was cultured on white flour with 5% brewer's un-activated yeast. Rearing was conducted at 30 ±1^{0}C and 70±5% r.h.

Uninfested clean Canadian Western Hard wheat with 14.4% m.c. was used in the experiment. In each replication there was 100 g of clean whole wheat kernels in 500 ml glass jars sealed with filter paper. The DE (Celite Corporation, USA) contained 89% SiO_2 with median particle size of 10 µm and specific gravity of 2.2. Moisture (as shipped) was 3% and crystalline silica was less than 0.1%. It contained Al_2O_3 4%, Fe_2O_3 1.7%, CaO 1.4%, Na_2O 1.2%, MgO 0.6% and K_2O 0.5% (Technical Data, Celite Corporation, USA). Delta tech (AgrEvo USA Company, Wilmington, DE, USA) is a powdered product contained 99% deltamethrin as the active ingredient. A determined quantity of powder was dissolved in a solvent and emulsifier (EPA List of Other Pesticide Ingredients Inert – List 4) and in piperonyl butoxide (PBO). The ratio of deltamethrin and PBO was 1:4. These concentrated emulsions contained different quantities of deltamethrin were mixed with DE for grain dusting and used alone for grain spraying.

Five formulations of mixtures of DE and deltamethrin emulsion were prepared in the laboratory containing one part of DE and different quantities of deltamethrin (0.00025, 0.00050, 0.00076, 0.0010 and 0.00125 parts). Deltamethrin alone was used in the following concentrations (ppm): 0.05, 0.1, 0.2, 0.3 and 0.4; DE alone (ppm): 100, 200, 300, 400 and 500; and mixtures of DE and deltamethrin (ppm): 50, 75, 100, 125 and 150. The various formulations at different concentrations were added to grain in each of the large jars containing 300 g of wheat. Jars were tightly closed with lids and thoroughly shaken by hand for one min. The grain from each jar was divided into three jars (sub-replications) with 100 g each. Untreated grain serves as control (0 ppm). Then 50 unsexed 7- to 21-d-old adults of either *S. zeamais*, *R. dominica* or *T. castaneum* were added into each jar. There was only one species per jar.

To test deltamethrin alone, deltamethrin at various doses was sprayed with a 0.6 ml emulsion on 300 g of wheat and thoroughly mixed in a tightly closed jar. After mixing, grain from each jar (300 g) was divided into three jars (sub-replications) with 100 g each. Grain sprayed with water served as control (0 ppm). After 6 d the entire contents of each jar was sieved to separate insects from the grain. The number of dead and live adult insects was counted. The corrected mortality was calculated according to Abbott's formula (Abbott 1925), and LD_{50} and fiducial limits (with 95% CIs) and X^2 analysis according to probit software.

The joint action of DE and deltamethrin individually in the insecticide mixture was determined on the basis of LC_{50} values of each insecticide and was determined on the basis of Co-toxicity Co-efficient (CTC) values of the mixture (Sun and Johnson, 1960). DE served as a standard insecticide. If the values of the CTC are higher than 100, this is an indication that the substances in the mixture have a synergistic action (Sun and Johnson, 1960; Ramasubramanian and Regupathy 2003).

3. Results

All mixtures with all insects show synergism (Tables 1-3). The dosages of both substances in the mixture are significantly reduced in a comparison with dosages when the substances are used alone. For example, based on the LD_{50} results, deltamethrin dosages in formulation D are reduced for seven, nine and four times and dosages of DE are reduced for 10, 20 and 11 times in controlling *S. zeamais*, *R. dominica* and

T. castaneum, respectively, in the comparison with dosages of DE and deltamethrin applied alone (Tables 1-3).The LD_{50} for *S. zeamais* for DE combined with deltamethrin is from 40 to 167 ppm compared to 376 ppm when DE is used alone. Similarly for the same species, the LD_{50} for deltamethrin combined with DE is 0.042 to 0.062 ppm compared to 0.28 ppm of deltamethrin used alone (Table 1).

Table 1 Co-toxicity Co-efficient (CTC) of ready to use insecticide mixture of DE and deltamethrin against *Sitophilus zeamais.*

Formulation	Ratio DE: deltamethrin	LC_{50} (ppm)	Fiducial limit 95% (ppm)	Deltamethrin alone and in mixture LC_{50} (ppm)	DE alone and in mixture LC_{50} (ppm)	Regression equation* m	b	r	X^2	CTC
DE	1:0	376	353-404	-	376	39005	-5.05	0.98	17	-
deltamethrin	0:1	0.29	0.25-0.33	0.29	-	2.04	+6.11	0.95	18	-
Formulation A.	1:0.00025	167	160-176	0.042	150	5.08	-6.31	0.97	19	170
Formulation B.	1:0.00050	125	118-133	0.062	113	3.79	-2.96	0.94	20	182
Formulation C.	1:0.00075	66	61-71	0.049	59	3.15	-0.74	0.97	20	288
Formulation D.	1:00100	42	39-46	0.042	38	3.24	-0.27	0.96	18	386
Formulation E.	1:00125	40	37-43	0.050	36	3.68	-0.92	0.96	6	353

*Y = mx + b

Table 2 Co-toxicity Co-efficient (CTC) of ready to use insecticide mixtures of DE and deltamethrin against *Rhyzopertha dominica.*

Formulation	Ratio DE: deltamethrin	LC_{50} (ppm)	Fiducial limit 95% (ppm)	Deltamethrin alone and in mixture LC_{50} (ppm)	DE alone and in mixture LC_{50} (ppm)	Regression equation* m	b	r	X^2	CTC
DE	1:0	468	442-495	-	468	3.88	-5.37	0.93	19	-
deltamethrin	0:1	0.23	0.20-0.28	0.23	-	1.40	+5.88	0.28	18	-
Formulation A.	1:0.00025	166	151-190	0.041	149	1.40	-0.01	0.94	6	188
Formulation B.	1:0.00050	166	151-190	0.083	149	2.25	-0.01	0.94	6	270
Formulation C.	1:0.00075	45	38-54	0.033	40	1.25	+2.94	0.96	9	419
Formulation D.	1:00100	26	19-32	0.026	23	1.02	+3.55	0.99	6	601
Formulation E.	1:00125	31	26-36	0.038	28	1.34	+3.00	0.99	5	433

*Y = mx + b

Table 3 Co-toxicity Co-efficient (CTC) of ready to use insecticide mixtures of DE and deltmethrin against *Tribolium castaneum.*

Formulation	Ratio DE: deltamethrin	LC_{50} (ppm)	Fiducial limit 95% (ppm)	Deltamethrin alone and in mixture LC_{50} (ppm)	DE alone and in mixture LC_{50} (ppm)	Regression equation* m	b	r	X^2	CTC
DE	1:0	1142	1076-1222	-	1142	3.85	-6.78	0.99	7	-
deltamethrin	0:1	0.47	0.45-0.49	0.47	-	5.33	+6.72	0.99	8	-
Formulation A.	1:0.00025	453	426-487	0.11	408	3.58	-4.51	0.97	5	157
Formulation B.	1:0.00050	183	176-196	0.09	165	6.14	-8.89	0.99	3	283
Formulation C.	1:0.00075	180	173-186	0.13	162	6.55	-9.77	0.97	4	227
Formulation D.	1:00100	118	112-124	0.12	106	4.13	-3.56	0.95	21	285
Formulation E.	1:00125	132	123-142	0.13	119	3.23	-1.86	0.96	12	217

*Y = mx + b

The LD_{50} of *R. dominica* for DE combined with deltamethrin is from 26 to 166 ppm compared to 468 ppm when DE is used alone. Similarly for the same species, the LD_{50} for deltamethrin combined with DE is 0.026 to 0.082 ppm compared to 0.23 ppm of deltamethrin used alone (Table 2).The LD_{50} of *T. castaneum* for DE combined with deltamethrin is from 118 to 453 ppm compared to 1142 ppm when DE is used alone. Similarly for the same species, the LD_{50} for deltamethrin combined with DE is 0.09 to 0.13 ppm compared to 0.47 ppm of deltamethrin used alone (Table 3).

4. Discussion

Several DE formulations are now commercially available, and many studies document that their effective action against a wide range of stored-product insect species (Subramanyam and Roesli, 2000). In order to reduce the dosages of DE it can be mixed with other compounds such as silica gel, dry honey, un-activated yeast and sugar to increase the efficacy (Korunic and Fields, 1995; Subramanyam and Roesli, 2000). However, high doses of these mixtures still have a significant negative effect on grain bulk density and flowability (Korunic et al., 1998). One of the possible solutions to the implications that are caused by the use of DE in high doses is the combined use of DE with other reduced-risk methods. Such methods include extreme temperatures (Fields et al., 1997), grain cooling with a DE surface treatment (Nickson et al., 1994), in a mixture with entomopathogenic fungi (Michalaki et al., 2007) or with synthetic insecticides (Arthur, 2004), or in a mixture with plant extracts and a bacterial metabolite (Korunic, 2007; Athanassiou and Korunic, 2007). Experimentation with these components often revealed a synergistic or enhanced effectiveness effect (Lord, 2001; Korunic, 2007; Athanassiou and Korunic, 2007).

The combination of insecticides from different groups may overcome resistance and the weak effectiveness of pyrethroids against some stored-grain insect species. Several OPs have been mixed with pyrethroids (Arthur, 1994; Daglish, 1998). However, concentrations used in these mixtures were still high and often half the recommended concentration of each compound was used in the mixture. Despite the increased efficacy and lower concentrations in some cases, high concentrations of both OP and pyrethroids in mixtures could cause residue issues with consumers.

The main advantage of a DE + deltamethrin mixture is similar effectiveness against tested insect species which is not the case when DE and deltamethrin are applied alone. The new mixture generated 95 to 100% mortality of all species exposed to approximately 100 ppm containing 0.1 ppm of deltamethrin and 90 ppm of DE (Korunic, unpublished data). This was well below the concentrations of DE + pyrethroids or OPs + pyrethroids. One probable cause of this synergism is the combination of different modes of action: physical (desiccation) and chemical (toxicity). Insects desiccate from the DE and could become weaker and less resistant to the toxic action of deltamethrin. Therefore, lower concentrations of deltamethrin in combination with DE are needed for control. Deltamethrin and PBO are used at approximately 0.5 to 1 ppm and 2 ppm, respectively, for long-term protection of stored grain. In most cases, DE formulations are registered at concentrations of 500 to 1000 ppm. Another advantage of using the new developed mixture of DE and deltamethrin is acceptable efficacy in grain treated with very low doses of both active ingredients. These concentrations are much lower in comparison with concentrations required for effective control when these two insecticides are applied either alone or as mixtures with other insecticides (Daglish, 1998; Athanassiou, 2006), in a mixture with entomopathogenic fungi (Michalaki et al., 2007). These concentrations are similar in efficacy to concentrations of the mixture of DE + chlorpyrifos-methyl + deltamethrin + PBO (Arthur, 2004), DE + abamectin (Athanassiou and Korunic, 2007) or DE + plant extract (Korunic, 2007). However, the substances in the mentioned mixtures are not yet registered as grain protectants in any country or perhaps only a few. Deltamethrin and DE are registered as grain protectants in many countries, which could simplify the process of the adoption of this mixture as a grain protectant.

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