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ORIGINAL ARTICLE

Toxicity and combined action of some insecticides and clove oil against *Rhyzopertha dominica* in wheat grain

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Abstract

Concerns about food quality and environmental protection have led to the search for effective and safe insect control measures. This study was carried out to evaluate the efficacy of some insecticides (malathion, alpha-cypermethrin, lambda-cyhalothrin) and clove oil, alone and in combinations, to protect wheat grain against *Rhyzopertha dominica.* Adult mortality, progeny emergence and weight loss of treated grain were examined. The results revealed that the tested insecticides and clove oil alone showed high efficiency to *R. dominica* with respect to mortality, progeny of the adults and weight loss of wheat grain. The mixing of lambda-cyhalothrin and clove oil with the most effective insecticide (alpha-cypermethrin) enhanced its efficacy to *R. dominica.* It was more efficient against *R. dominica* than when used alone with respect to mortality and progeny of the adults. However, mixing alpha-cypermethrin with malathion reduced the efficacy of alpha-cypermethrin against *R. dominica* with respect to mortality and progeny of the adults. Combinations of alpha-cypermethrin and clove oil reduced wheat grain loss more than using them alone. Mixing lambda-cyhalothrin and clove oil with low concentrations of alpha-cypermethrin improved its efficacy against *R. dominica* and therefore may reduce environmental pollution, lower risks to human health, and delay insect resistance development.

Key words: alpha-cypermethrin, clove oil, insecticides, lambda-cyhalothrin, malathion, *Rhyzopertha dominica*, wheat

Introduction

The lesser grain borer, *Rhyzopertha dominica* (L.) (Coleoptera: Bostrychidae), is the major pest of wheat in most of the world, and has developed resistance to some insecticides used for its control (Arthur 1996, 2004; Daglish 2008). It causes significant damage to stored grains mainly: wheat, barley, sorghum, maize and rice (Adams 1995). This borer is a primary pest which attacks undamaged whole grain covered with the hard husks, causing the damaged grains to become susceptible to attack by secondary pests (Ahmed *et al.* 2013).

The use of insecticides as grain protectants and for seed treatment is a common strategy for stored-product protection against insect pests (Arthur 1996; Athanassiou *et al.* 2005; Kavallieratos *et al.* 2011; Derbalah 2012; Derbalah *et al.* 2012). However, increasing awareness that in addition to health risks, there are environmental issues and other problems such as overall resistance of insects to chemical insecticides. Researchers are therefore trying to find other, safer alternatives. There several ways to solve the problems involved with chemical insecticides. One solution is to use chemical insecticides that are relatively safe or have low mammalian toxicity such as pyrethroids. Another solution is using insecticides of natural origin that are eco-friendly and safer than traditional ones such as natural oils (Ileke *et al.* 2014). Moreover, to decrease the hazards of synthetic insecticides on human health and the environment, chemical insecticides at low concentrations can be combined with other safe control agents (Obeng-Ofori and Amiteye 2005; Athanassiou *et al.* 2009; Singh *et al.* 2012).

Therefore, this study attempted to evaluate the efficacy of some insecticides (alpha-cypermethrin, lambda-cyhalothrin and malathion) that are relatively safe compared to other chemical insecticides against *R. dominica* in wheat grain with respect to mortality of adults, progeny and weight loss of treated wheat grain.

We also evaluated the efficacy of clove oil (*Syzygium aromaticum*) as a safe alternative to chemical insecticides against *R. dominica* and identified chemical components that may be responsible for its insecticidal activity using gas chromatography-mass spectrometry (GC-MS). Our final aim was to evaluate the combined effect of examined chemical insecticides and clove oil against *R. dominica* in wheat grain as a way to reduce the side effects of chemical insecticides on human health and to overcome insect resistance towards these insecticides.

Materials and Methods

Test insect

A colony of *R. dominica* was reared in our laboratory for over 12 years without exposure to insecticides. The insect was reared on sterilized whole wheat at 26°C (±1°C) and 65% (±5%) relative humidity (RH) under complete darkness. The unsexed adults, 14 days old, were used in insecticidal studies. All experiments were carried out under the same environmental conditions.

Chemicals

Four treatments were used in this study: malathion with a trade name of Malathion 57% EC and lambada-cyhalothrin with a trade name of Lambda 5% EC produced by Kafr El Zayat for Pesticides and Chemicals, Kafr El Zayat, Egypt; alpha-cypermethrin with a trade name of Super Alpha 10 EC produced by ElHelb for Pesticides and Chemicals, Demiatea, Egypt and clove oil was obtained from Elcaptain Company for Extracting Natural Oils, Plants & Cosmetics, Al Azhar, Cairo, Egypt. Clove oil was analyzed using GC-MS analysis as described by Ayoola *et al.* (2008) and the results showed the presence of some fatty acids and their derivatives such as 1,8-cineole, linalool, butanoic acid, eugenol and caryophyllene.

Treatments

A series of test concentrations of the examined insecticides (malathion, alpha-cypermethrin, lambada-cyhalothrin) and clove oil were prepared in acetone. Twenty grams of uninfected wheat grain were introduced into 300 ml glass jars. The grains were treated by adding 2 ml of the test concentrations prepared in acetone of the examined insecticides and clove oil. Control jars were treated with acetone only and each treatment replicated three times. All jars were shaken manually for approximately 5 min to achieve equal distribution of the insecticides and clove oil through the entire grain mass. The jars were left for 30 min for complete evaporation of the solvent. The test concentrations for alpha-cypermethrin were 1, 5, 10, 20 and 30 mg a.i. · l^{-1} which equal 0.002, 0.010, 0.020, and 0.030 mg · 20 g^{-1} grain after acetone evaporation and 50, 100, 200 and 300 mg a.i. · l^{-1} (equal 0.10, 0.20, 0.040 and 0.60 mg · 20 g^{-1} grain) for lambda-cyhalothrin while for malathion the test concentrations were: 100, 200, 300 and 400 mg a.i. · l^{-1} (equal 0.20, 0.40, 0.60 and 0.80 mg a.i. · 20 g^{-1} grain). However, for clove oil the test concentrations were: 10,000, 20,000, 40,000 and 80,000 mg a.i. · l^{-1} (equal 20, 40, 80, and 160 mg a.i. · 20 g^{-1} grain). Twenty *R. dominica* adults were introduced into each jar. The jars were covered with cheese cloth and fastened by rubber bands to prevent escape of insects and to ensure proper ventilation. The adult mortality was recorded at 24, 48 and 72 h after the initial treatment and the dead insects were removed. The adults were considered dead if they were unable to move after gentle prodding with a fine-haired brush or/and unable to move or walk during a 2-min observation period. After the last mortality count, all jars were held under similar conditions and after 12 weeks the numbers of emerged adults of *R. domminca* were counted and expressed as progeny production.

The following formula was used to determine the reduction percentage in the number of progeny after 12 weeks:

$$\% \text{ reduction} = 1 - x/y \times 100,$$

where: x – the number of adults emerging in the treatment, y – the number of adults emerging in the control (El-Lakwah *et al.* 1992). The grain was sieved and the powder was discarded. The weight of remaining grain in treatments and control was recorded. The weight loss percentage was calculated with the following formula:

$$\% \text{ weight loss} = Wu - Wi/Wu \times 100,$$

where: Wu – weight of uninfected grain, Wi – weight of infested grain (El-Lakwah *et al.* 1992).

Efficacy of the tested insecticide combinations

The combined effect of the examined insecticides and clove oil was carried out by mixing the most effective insecticide (alpha-cypermethrin) at a concentration of 10 mg · l^{-1} with other insecticides and clove oil at mixing ratios of 1 : 1, 1 : 2 and 1 : 3. Test concentrations for each mixture were prepared by further diluting the mixture with acetone. Wheat grain was treated with 2 ml of test concentrations of different mixtures and *R. dominica* adults were introduced as mentioned before. The adult mortality was recorded at 24, 48 and 72 h after the initial treatment and the dead insects were removed. The co-toxicity coefficient (Sarup *et al.* 1980) and synergistic factor (Kalayanadundaram and Das 1985) for the mixtures were also calculated after calculating the LC_{50} for each combination as shown in the following equation:

$$\text{Co-toxicity coefficient} = LC_{50} \text{ of insecticide (alone)}/ LC_{50} \text{ of combination} \times 100,$$

$$\text{Synergistic factor (SF)} = LC_{50} \text{ of insecticide (alone)}/ LC_{50} \text{ of combination},$$

A value of SF >1 indicates synergism and SF <1 indicates antagonism.

Data analysis

Statistical analysis was conducted using the statistical package SPSS version 12.0 (Levesque 2007). The mortality percentages in contact toxicity assay were calculated after 24 h of treatment as the mean of three replicates. The mortality percentages were subjected to probit analysis (Finney 1971) to obtain the LC_{50} using SPSS 12.0 (SPSS, Chicago, IL, USA). The progeny and weight loss data in seed protectant experiments were submitted to a one-way analysis of variance (ANOVA). Mean separations were performed by Student-Newman-Keuls (SNK) test.

Results

Toxicity of individual insecticides on adult mortality of *Rhyzopertha dominica*

The efficacy of the examined insecticides and clove oil alone against *R. dominica* with respect to adult mortality is presented in Table 1. Alpha-cypermethrin was the most effective treatment against *R. dominica* followed by lambda-cyhalothrin, malathion and clove oil with LC_{50} values of 15.04, 18.40, 230.60 and 40,429 mg a.i. · l^{-1} after 24 h of treatment, respectively. The mortality of *R. dominica* adults significantly increased with time for all tested insecticides with LC_{50} values of 10.50, 12.0, 179.50 and 30,844 mg a.i. · l^{-1} after 48 h of treatment as well as 1.87, 9.69, 134.7 and 23,239 mg a.i. · l^{-1} after 72 h of treatment for alpha-cypermethrin, lambda-cyhalothrin, malathion and clove oil, respectively.

Toxicity of insecticide mixtures against adult mortality of *Rhyzopertha dominica*

The combined effect by mixing the most effective insecticide (alpha-cypermethrin) with other tested

Table 1. Efficacy of evaluated insecticides and clove oil on mortality of *Rhyzopertha dominica* adults

Treatments	Time [h]	LC_{50} [mg a.i. · l^{-1}]	95% confidence limits		Slope ±SE
			upper	lower	
Alpha-cypermethrin	24	15.04	21.50	11.50	1.07 ± 0.10
	48	10.50	11.10	6.31	0.96 ± 0.10
	72	1.87	2.00	0.20	1.32 ± 0.11
Lambda-cyhalothrin	24	18.40	23.70	13.19	1.10 ± 0.10
	48	12.0	15.20	8.40	1.50 ± 0.12
	72	9.69	12.06	7.04	1.90 ± 0.13
Malathion	24	230.60	276.72	192.16	2.00 ± 0.14
	48	179.50	215.40	149.58	2.50 ± 0.15
	72	134.70	161.64	112.25	2.80 ± 0.17
Clove oil	24	40,429.00	46,319.00	35,325.00	2.10 ± 0.16
	48	30,844.00	35,293.00	26,736.00	2.10 ± 0.17
	72	23,239.00	26,791.00	19,783.00	2.00 ± 0.18

insecticides and clove oil against *R. dominica* with respect to adult mortality is shown in Tables 2–4.

The LC_{50} values of alpha-cypermethrin mixed with lambda-cyhalothrin, at a ratio of 1 : 1 were: 14.60, 7.45, 1.40 mg a.i. · l^{-1}, after 24, 48 and 72 h of treatment, respectively (Table 2). At the mixing ratio of 1 : 2, the LC_{50} values were: 12.20, 5.05 and 1.26 mg a.i. · l^{-1}, after 24, 48 and 72 h of treatment, respectively. Finally, the LC_{50} values at the mixing ratio of 1 : 3 were: 9.89, 3.53, and 1.24 mg a.i. · l^{-1} after 24, 48 and 72 h of treatment, respectively. Significant synergism of alpha-cypermethrin toxicity was observed as the result of mixing it with lambda-cyhalothrin. The synergistic factors for alpha-cypermethrin were: 1.03, 1.40 and 1.33, after 24, 48 and 72 h of treatment, respectively at the mixing level of 1 : 1. However, the synergistic factors of alpha-cypermethrin at the mixing level of 1 : 2 were: 1.23, 2.07 and 1.48 after 24, 48 and 72 h of treatment, respectively. Finally, the synergistic factors of alpha-cypermethrin at the mixing level of 1 : 3 were: 1.52, 2.97 and 1.50 after 24, 48 and 72 h of treatment, respectively (Table 2).

The effect of mixing alpha-cypermethrin with malathion on its efficacy against *R. dominica* adults is shown in Table 3. The LC_{50} values of alpha-cypermethrin mixed with malathion at the mixing ratio of 1 : 1 were: 51.70, 11.50 and 6.00 mg a.i. · l^{-1} after 24, 48 and 72 h of treatment, respectively. At the mixing

Table 2. Effect of lambada-cyhalothrin on the toxicity of cypermethrin against *Rhyzopertha dominica* adults

Treatments	Time [h]	LC_{50} [mg a.i. · l^{-1}]	Co-toxicity coefficient [%]	Synergistic factor	Type of action
Alpha-cypermethrin	24	15.04	–	–	–
	48	10.30	–	–	–
	72	1.87	–	–	–
Alpha-cypermethrin + lambda-cyhalothrin at ratio of 1 : 1	24	14.60	103.0	1.03	synergistic
	48	7.45	140.90	1.409	synergistic
	72	1.40	133.5	1.335	synergistic
Alpha-cypermethrin + lambda-cyhalothrin at ratio of 1 : 2	24	12.20	123.2	1.230	synergistic
	48	5.05	207.90	2.079	synergistic
	72	1.26	148.4	1.484	synergistic
Alpha-cypermethrin + lambda-cyhalothrin at ratio of 1 : 3	24	9.89	152.0	1.520	synergistic
	48	3.53	297.4	2.974	synergistic
	72	1.24	150.8	1.508	synergistic

Table 3. Effect of malathion on the toxicity of cypermethrin against *Rhyzopertha dominica* adults

Treatments	Time [h]	LC_{50} [mg a.i. · l^{-1}]	Co-toxicity coefficient [%]	Antagonistic factor	Type of action
Alpha-cypermethrin	24	15.04	–	–	–
	48	10.30	–	–	–
	72	1.87	–	–	–
Alpha-cypermethrin + malathion at ratio of 1 : 1	24	51.70	29.90	0.299	antagonistic
	48	11.50	91.30	0.913	antagonistic
	72	6.00	31.16	0.311	antagonistic
Alpha-cypermethrin + malathion at ratio of 1 : 2	24	64.4	23.35	0.233	antagonistic
	48	14.4	72.91	0.729	antagonistic
	72	6.80	27.50	0.275	antagonistic
Alpha-cypermethrin + malathion at ratio of 1 : 3	24	69.20	21.73	0.217	antagonistic
	48	14.59	71.96	0.719	antagonistic
	72	7.30	25.61	0.256	antagonistic

Table 4. Effect of clove oil on the toxicity of cypermethrin against *Rhyzopertha dominica* adults

Treatments	Time [h]	LC_{50} [mg a.i. · l^{-1}]	Co-toxicity coefficient [%]	Synergistic or antagonistic factor	Type of action
Alpha-cypermethrin	24	15.04	–	–	–
	48	10.30	–	–	–
	72	1.87	–	–	–
Alpha-cypermethrin + clove oil at ratio of 1 : 1	24	14.90	100.90	1.009	synergistic
	48	9.50	110.50	1.105	synergistic
	72	1.71	109.30	1.093	synergistic
Alpha-cypermethrin + clove oil at ratio of 1 : 2	24	14.0	107.40	1.074	synergistic
	48	6.15	170.63	1.706	synergistic
	72	1.42	131.69	1.316	synergistic
Alpha-cypermethrin + clove oil at ratio of 1 : 3	24	13.84	108.60	1.080	synergistic
	48	7.04	149.14	1.491	synergistic
	72	1.30	143.84	1.438	synergistic

ratio of 1 : 2, the LC_{50} values were: 64.4, 14.4 and 6.80 mg a.i. · l^{-1} after 24, 48 and 72 h of treatment, respectively. Finally, the LC_{50} values at the mixing ratio of 1 : 3 were: 69.20, 14.59 and 7.30 mg a.i. · l^{-1} after 24, 48 and 72 h of treatment, respectively. Significant antagonism of alpha-cypermethrin toxicity was observed as the result of mixing it with malathion. The antagonistic factors for alpha-cypermethrin were: 0.29, 0.91 and 0.31 after 24, 48 and 72 h of treatment, respectively at the mixing level of 1 : 1. However, the antagonistic factors of alpha-cypermethrin at the mixing level of 1 : 2 were: 0.23, 0.72 and 0.27 after 24, 48 and 72 h of treatment, respectively. Finally, the antagonistic factors of alpha-cypermethrin at the mixing level of 1 : 3 were: 0.21, 0.71 and 0.25 after 24, 48 and 72 h of treatment, respectively (Table 3).

The effect of mixing alpha-cypermethrin with clove oil on its efficacy against *R. dominica* adults is shown in Table 4. The LC_{50} values of alpha-cypermethrin mixed with clove oil at a ratio of 1 : 1 were: 14.90, 9.50 and 1.71 mg a.i. · l^{-1} after 24, 48 and 72 h of treatment, respectively. At the mixing ratio of 1 : 2, the LC_{50} values were: 14, 6.15, and 1.42 mg a.i. · l^{-1} after 24, 48 and 72 h of treatment, respectively. Finally, the LC_{50} values at the mixing ratio of 1 : 3 were: 13.84, 7.04 and 1.30 mg a.i. · l^{-1} after 24, 48 and 72 h of treatment, respectively. Significant synergism of alpha-cypermethrin toxicity was observed as the result of mixing clove oil at different mixing levels. The synergistic factors for alpha-cypermethrin were: 1.009, 1.10 and 1.09 after 24, 48 and 72 h of treatment, respectively, at the mixing level of 1 : 1. However, at the mixing level of 1 : 2, the synergistic factors for alpha-cypermethrin were recorded with values of 1.074, 1.706, and 1.316 after 24, 48 and 72 h of treatment, respectively. Also, the synergistic effect for alpha-cypermethrin at the mixing level of 1 : 3 with synergistic factors of 1.08, 1.49 and 1.43 after 24, 48 and 72 h of treatment, respectively, was recorded (Table 4).

The results showed that the mixing of alpha-cypermethrin with lambda-cyhalothrin and clove oil improved its efficacy against *R. dominica* with respect to adult mortality (Tables 2, 4). On the other hand, the mixing of alpha-cypermethrin with malathion reduced its efficacy against *R. dominica* with respect to adult mortality (Table 3). Among examined mixing ratios in this study, the mixing ratio of 1 : 2 between alpha-cypermethrin and other tested insecticides showed the highest synergism or antagonism of alpha-cypermethrin against *R. dominica* adults.

Effect of individual insecticides on progeny of *Rhyzopertha dominica* and weight loss of wheat grain

The numbers of emerged *R. dominica* adults significantly decreased when treated with the examined insecticides and clove oil alone compared to the untreated control (Table 5). Furthermore, the reduction in progeny of *R. dominica* increased by increasing the concentration levels of the examined insecticides and clove oil. The highest reduction in *R. dominica* progeny was recorded in wheat grain treated with lambda-cyhalothrin followed by malathion, alpha-cypermethrin and finally clove oil. The results also showed that the examined insecticides and clove oil significantly reduce the weight loss of wheat grain compared to untreated control. The highest reduction in the grain weight loss was recorded in wheat treated by lambda-cyhalothrin (0.05%) followed by malathion (0.10%), alpha-cypermethrin (0.10%) and clove oil (0.20%), respectively (Table 5).

Table 5. Effect of the tested insecticides and clove oil on progeny of *Rhyzopertha dominica* and weight loss of wheat grain after 12 weeks of treatment

Treatments	Concentration [mg a.i. · l^{-1}]	Mean number of progeny	Reduction in progeny [%]	Mean grain weight [g]	% of grain weight loss
Alpha-cypermethrin	1	5.33 ± 0.02 bc	94.28	19.26 ± 0.12 ab	3.70
	5	3.00 ± 0.04 bc	96.78	19.69 ± 0.14 ab	1.50
	10	0.60 ± 0.01 bc	99.35	19.72 ± 0.17 a	1.40
	20	0.00	100	19.98 ± 0.15 a	0.10
Lambda-cyhalothrin	10	4.0 ± 0.45 bc	95.71	19.51 ± 0.16 ab	2.45
	20	2.66 ± 0.12 bc	97.14	19.67 ± 1.10 ab	1.65
	30	0.00	100	19.97 ± 1.10 a	0.15
	40	0.00	100	19.99 ± 0.45 a	0.05
Malathion	100	3.0 ± 0.07 bc	96.78	19.59 ± 0.23 ab	2.05
	200	2.3 ± 0.02 bc	97.53	19.89 ± 0.89 a	0.55
	400	0.00	100	19.94 ± 1.2 a	0.30
	600	0.00	100	19.98 ± 1.10 a	0.10
Clove oil	10	12.66 ± 0.98 b	86.43	19.00 ± 0.58 b	5.00
	20	10.0 ± 0.11 bc	89.28	19.59 ± 0.36 ab	2.05
	40	2.30 ± 0.02 bc	97.53	19.84 ± 0.87 a	0.80
	80	1.30 ± 0.01 c	98.60	19.96 ± 0.78 a	0.20
Control	0.00	96.30 ± 1.020 a	0.00	14.43 ± 0.37 c	27.85

The different letters represent significant differences between the means using Student-Newman-Keuls (SNK) test; initial number of adults was 20 individuals/jar; initial weight of wheat grain was 20 g

Effect of insecticide mixtures on progeny of *Rhyzopertha dominica* and weight loss of wheat grain

The numbers of emerged *R. dominica* adults and weight loss of treated wheat grain significantly decreased when treated with the examined insecticide mixtures (1 : 1, 1 : 2 and 1 : 3) compared to untreated control (Tables 6–8). The highest reduction in the progeny of adults and weight loss of wheat grain was recorded for alpha-cypermethrin mixed with lambda-cyhalothrin followed by clove oil and malathion with different mixing ratios (Tables 6–8). Of the different mixing ratios, the highest reduction in the progeny of *R. dominica* adults and weight loss of wheat grain was recorded with the mixing ratio of 1 : 3 (Tables 6–8). The results showed that the effect of alpha-cypermethrin mixtures with lambda-cyhalothrin, clove oil and malathion at different ratios on the progeny of *R. dominica* adults and weight loss in wheat grain was improved with different mixing ratios (Tables 6–8) compared to its effect alone (Table 5).

Discussion

The use of insecticides as grain protectants for control of insects in stored products is common. However, using chemical insecticides that are safer for human health and the environment like pyrethroids and some malathion are a source of major concern. The results of this study indicate that the examined insecticides (malathion, alpha-cypermethrin, lambda-cyhalothrin) alone can achieve considerable levels of mortality of *R. dominica* adults in stored wheat. Also, increasing the exposure time of treated grain to the examined insecticides increased the efficacy against *R. dominica* adults (Derbalah 2012; Derbalah *et al.* 2012). Our results support those reported by other researchers concerning the use of insecticides as grain protectants (Athanassiou *et al.* 2005; Derbalah 2012; Derbalah *et al.* 2012). In our study malathion showed lower efficacy against *R. dominica* compared to the two examined pyrethroids. This agrees with the findings of Singh *et al.* (2012) who reported that malathion is not very effective against the lesser grain borer (*R. dominica*) while pyrethroid compounds are very effective against the same insect.

Another way to ensure human health is by using eco-friendly control agents of natural origin such as plant oils. The results of this study showed that clove oil had considerable efficacy against the controlled insect. A few reviews have shown the potential effect of various plant oils in protecting grains against major stored-product insects (Don-Pedro 1989; Obeng-Ofori 1995; Shaaya *et al.* 1997; Derbalah and Ahmed 2010).

Table 6. Effect of cypermethrin and lambda-cyhalothrin mixtures on progeny of *Rhyzopertha dominica* adults and weight loss of wheat grain after 12 weeks of treatment

Treatments	Concentration [mg a.i. · l⁻¹]	Mean number of progeny	Reduction in progeny [%]	Mean grain weight [g]	% of grain weight loss
Alpha-cypermethrin	1	5.33 ± 0.02	94.28	19.26 ± 0.12	3.70
	5	3.00 ± 0.04	96.78	19.69 ± 0.14	1.50
	10	0.60 ± 0.01	99.35	19.72 ± 0.17	1.40
	20	0.00	100.00	19.98 ± 0.15	0.10
Alpha-cypermethrin + lambda-cyhalothrin at ratio of 1 : 1	5	1.00 ± 0.01	98.95	19.97 ± 0.25	0.15
	10	0.00	100	20.00	0.00
	15	0.00	100	20.00	0.00
	20	0.00	100	20.00	0.00
Alpha-cypermethrin + lambda-cyhalothrin at ratio of 1 : 2	5	0.00	100	20.00	0.00
	10	0.00	100	20.00	0.00
	20	0.00	100	20.00	0.00
	30	0.00	100	20.00	0.00
Alpha-cypermethrin + lambda-cyhalothrin at ratio of 1 : 3	10	0.00	100	20.00	0.00
	20	0.00	100	20.00	0.00
	30	0.00	100	20.00	0.00
	40	0.00	100	20.00	0.00
Control	0.00	96.0 ± 1.2	0.00	14.43 ± 0.36	27.85

Table 7. Effect of cypermethrin and malathion mixtures on progeny of *Rhyzopertha dominica* adults and weight loss of wheat grain after 12 weeks of treatment

Treatments	Concentration [mg a.i. · l⁻¹]	Mean number of progeny	Reduction in progeny [%]	Mean grain weight [g]	% of grain weight loss
Alpha-cypermethrin	1	5.33 ± 0.02	94.28	19.26 ± 0.12	3.70
	5	3.00 ± 0.04	96.78	19.69 ± 0.14	1.50
	10	0.60 ± 0.01	99.35	19.72 ± 0.17	1.40
	20	0.00	100	19.98 ± 0.15	0.10
Alpha-cypermethrin + malathion at ratio of 1 : 1	5	2.30 ± 0.12	97.60	19.64 ± 0.54	1.80
	10	0.00	100	20.00	0.00
	15	0.00	100	20.00	0.00
	20	0.00	100	20.00	0.00
Alpha-cypermethrin + malathion at ratio of 1 : 2	5	4.00 ± 0.37	95.83	18.57 ± 1.10	7.15
	10	1.00 ± 0.01	98.95	19.47 ± 0.98	2.65
	20	0.00	100	20.00	0.00
	30	0.00	100	20.00	0.00
Alpha-cypermethrin + malathion at ratio of 1 : 3	10	5.00 ± 0.29	94.79	19.82 ± 1.14	0.90
	20	0.00	100	20.00	0.00
	30	0.00	100	20.00	0.00
	40	0.00	100	20.00	0.00
Control	0.00	96.00 ± 1.2	0.00	14.43 ± 0.36	27.85

The insecticidal activity of clove oil against stored-product insects may be due to the presence of fatty acids and their derivatives such as 1,8-cineole, linalool, butanoic acid, eugenol and caryophyllene that are known for their insecticidal activity (Park *et al.* 2003; Negahban *et al.* 2006; Rozman *et al.* 2007; Lopez *et al.*

Table 8. Effect of cypermethrin and clove oil mixtures on progeny of *Rhyzopertha dominica* adults and weight loss of wheat grain after 12 weeks of treatment

Treatments	Concentration [mg a.i. · l^{-1}]	Mean number of progeny	Reduction in progeny [%]	Mean grain weight [g]	% of grain weight loss
Alpha-cypermethrin	1	5.33 ± 0.02	94.28	19.26 ± 0.12	3.70
	5	3.00 ± 0.04	96.78	19.69 ± 0.14	1.50
	10	0.60 ± 0.01	99.35	19.72 ± 0.17	1.40
	20	0.00	100	19.98 ± 0.15	0.10
Alpha-cypermethrin + clove oil at ratio of 1 : 1	5	3.00 ± 0.14	96.87	19.23 ± 1.21	3.85
	10	0.00	100	20.00	0.00
	15	0.00	100	20.00	0.00
	20	0.00	100	20.00	0.00
Alpha-cypermethrin + clove oil at ratio of 1 : 2	5	1.00 ± 0.01	98.95	19.55 ± 1.36	2.25
	10	0.00	100	20.00	0.00
	20	0.00	100	20.00	0.00
	30	0.00	100	20.00	0.00
Alpha-cypermethrin + clove oil at ratio of 1 : 3	10	0.00	100	20.00	0.00
	20	0.00	100	20.00	0.00
	30	0.00	100	20.00	0.00
	40	0.00	100	20.00	0.00
Control	0.00	96.00 ± 1.2	0.00	14.43 ± 0.36	27.85

2008; Ogendo *et al.* 2008; Derbalah and Ahmed 2010; Derbalah 2012; Derbalah *et al.* 2012). The high toxicity of linalool, linalyl acetate and 1.8-cineole was reported against the rice weevil *Sitophilus oryzae* and *R. dominica* (Rozman *et al.* 2007). Even though it is not entirely understood how plant oils work, the mode of action of the bioactive natural monoterpenoids isolated from plant extract oils such as that identified in this study may be due to inhibition of acetylcholinesterase (Lee *et al.* 2000; Rozman *et al.* 2007; Derbalah 2012).

Stored grain insects can coexist within a storage bin, and hence a binary combination of insecticides may give more complete control than the application of a single insecticide, especially when mortality from the individual components is not complete or progeny production occurs even when parental adults are killed (Fang *et al.* 2002; Athanassiou *et al.* 2008; Chintzoglou *et al.* 2008). Also, combinations of insecticides with each other or with other safe control agents such as natural oil allow for a reduction in insecticide concentration, improve efficacy and could decrease hazardous risks on human health and the environment. In this study the mixing of clove oil and lambda-cyhalothrin with the most effective insecticide (alpha-cypermethrin), compared to being used alone, enhanced its efficacy against *R. dominica* with respect to adult mortality and progeny. Our results are in agreement with Obeng-Ofori and Amiteye (2005) who found that the mixing of plant oils with pirimiphos-methyl improved its toxicity persistence and subsequently resulted in higher mortality of rice weevil in maize grains treated with the mixture. Moreover, Singh *et al.* (2012) reported that the formulation of deltamethrin in vegetable oil has dangerously high toxicity against the lesser grain borer. Insecticide combinations have several benefits. They minimize the amounts of the ingredients and make the application of both more effective and economical. Furthermore, they are comparatively less hazardous to the environment and are considered to be an ideal eco-friendly option for the management of stored-product insects. The synergistic effect of alpha-cypermethrin combinations with clove oil may be due to the natural oil inhibiting some factors, such as detoxifying enzymes in *R. dominica*, which can act against synthetic chemicals (Thangam and Kathiresan 1991), or the oils may help in the unique distribution of the insecticide over the grain surfaces which increases its chances of being picked up by insects (Obeng-Ofori and Reichmuth 1999). Moreover, the synergistic effect on the toxicity of alpha-cypermethrin combinations with lambda-cyhalothrin may result from the inhibition of the detoxification process in insects (Metcalf 1955) by inhibition of naturally detoxifying oxidation reactions (Sun and Johnson 1960). Since the synergistic action is due to the inhibition of the enzyme system within the body of the insect, which is responsible for the destruction of insecticides inside the body of the insect to reduce toxicity. Inhibition of this enzyme system reduces the chances and rate of insecticides in the body of the insect and thus increases toxicity (Faraone *et al.* 2015). The antagonistic effect of malathion on the toxicity of alpha-cypermethrin may result from the chemical structure/properties or mode of action of the chemicals. Therefore, chemical/physical properties of malathion and alpha-cypermethrin probably play an important role in their observed antagonistic effect against *R. dominica* when mixed together (Faraone *et al.* 2015).

The reduction in progeny of controlled insects contributes to the success of controlling processes and reduces the loss in grain quality and quantity. In this study the evaluated insecticides and clove oil alone significantly reduced the progeny of *R. dominica*. This agrees with the findings of Derbalah *et al.* (2012) and Derbalah (2012) who reported that the use of natural plant extracts and malathion significantly reduced the progeny of stored-product insects. The reduction in the progeny of *R. dominica* adults by insecticide mixtures was higher than when insecticides were used alone and may be due to the synergistic effect on the toxicity of insecticide mixtures relative to its toxicity alone. This agrees with the findings of Athanassiou *et al.* (2009) who noted that complete mortality and progeny production suppression of *Liposcelis entomophila* was achieved by the application of chlorpyriphos-methyl with deltamethrin. The mechanism by which oil protects the seeds is not completely clear, but it appears that vegetable oils affect egg laying as well as embryo and larvae development on the surface of the seed (Singh *et al.* 2012).

The reduction in weight loss of wheat grain treated with lambda-cyhalothrin and alpha-cypermethrin mixture was higher than other combinations. This is may be due to the higher synergism of this mixture's toxicity against *R. dominica* relative to the toxicity of other mixtures. The high toxicity of this mixture led to higher mortality of insect adults and lower damage of treated grain and subsequently lower loss of grain weight relative to other mixtures. These results are in agreement with previous studies of Torres *et al.* (2014) and Tapondjou *et al.* (2005) who reported that the essential oils reduced weight loss of maize grain caused by *R. dominica*.

Conclusions

In conclusion the specific combinations of alpha-cypermethrin with lambda-cyhalothrin and clove oil evaluated in our study would be better than using these insecticides alone for controlling *R. dominica* with respect to mortality and progeny of the adults as well as wheat grain losses. However, the combinations of malathion with alpha-cypermethrin improved the efficacy of malathion and reduced the efficacy of alpha-cypermethrin when used alone against *R. dominica* insects. So, it may be advisable to further evaluate combinations of malathion with other insecticides that are suitable for control of *R. dominica* insects. Mixing chemical insecticides at low concentrations especially with plant oils may be a way to lower levels of environmental pollution, reduce risks to human health and insect resistance. These combinations could be used to purge the storage areas of stored-grain insects before storage of grain. It can also be used to evaporate storage areas and also to clean empty grain containers.

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