Evaluation of the effectiveness of essential oils blends with the pesticide methomyl in controlling third and fifth instar larvae of *Tribolium castaneum*

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Abstract Essential oils are widely used in various industries due to their chemical composition. Despite their effectiveness, chemical pesticides are harmful to the environment due to their high transmission potential and difficulty in degrading. Biological pesticides, on the other hand, degrade rapidly. A series of laboratory studies were performed to identify the lowest concentration of the insecticide methomyl that causes more significant mortality in *Tribolium castaneum* third and fifth-stage larvae. The concentration of 0.5 ml/L resulted in the highest mortality rate of 87.31 and 86.48 percent and the lowest mortality rate of 72.13 and 72.13 percent with 0.125ml/L, compared to the control treatment, which resulted in a mortality rate of 0.83 and 1.67 percent of third and fifth-stage larvae, respectively. For the fifth and third instar larvae, the treatment (0.25 + basil oil) resulted in the highest mortality rates of (88.33 and 86.67) percent, respectively. The treatment (0.25 + harmal oil) resulted in a mortality rate of 83.33 percent of both larva stages, compared to the control treatment, which resulted in 1.67 and 5.55 percent of fifth and third instar larvae, respectively. It resulted in reducing the use of chemical pesticides while increasing their efficacy, which might have a favorable influence on the environment.

Keywords: Harmal Oils, Basil Oils, Insecticide, Repellent effect, *Tribolium castaneum* Larvae Stage

Introduction

Tribolium castaneum is a rusty red flour beetle (Herbst). This insect belongs to the Coleoptera order and Tenebrionidae family with sheath wings (Anwar *et al.*, 2005). In most of the world's locations, notably the tropics, T. castaneum is the principal vector of insect dispersal. The bug spreads via grain and its products in general and flour in particular; the insect is distributed through grinders and flour (Duke *et al.*, 2000). They account for 80 percent or more mill insects (Bariş *et al.*, 2006). Uric acid produced by the bug causes the

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materials infected with this insect to acquire a strong and unpleasant smell, as well as a reduction in the viscosity of the dough generated from the contaminated flour, making it unsuitable for bread manufacture and lowering wheat's commercial value (Aslan and Sahln, 2004).

Bakeries and pastry shops face a problem with a beetle bug called Red rusty flour (*T. castaneum*) (Herbst), infesting their storage grains and their derivatives, which has been a problem for mill and commercial facility owners (Jbilou *et al.*, 2006). Physical control methods such as centrifugation, heat, adhesives, and radiation (Sana *et al.*, 2000) and biological control methods such as parasites and pathogens are all used to control this insect. Essential oils can be safely used to control stored pests due to their aromatic and volatile components obtained from various plant parts (Perricone *et al.*, 2015).

EOs is blends of spice-derived chemicals that can impart taste and scent. Essential oil refers to hydrophobic oils or fatty acids derived from plants. Due to their aromatic characteristics, EOs are used in various industries. For instance, EOs creates fragrances, toiletries, and soaps (S ão Pedro *et al.*, 2013) and repels insects (McDonald *et al.*, 1970). Plants do not consistently create EOs across all components. Flowers, leaves, roots, seeds, stems, and other plant components may store various substances in varying amounts and compositions. Identifying and quantifying the components need susceptible tools (Chamorro *et al.*, 2012). In one study, the EOs of a single basil plant had over 40 component compounds (Raut and Karuppayil, 2014).

Various insecticide-manufactured chemicals, including bromide methyl and diazinon, are also used in pest management (Lohar, 2001). Incorrect application of these pesticides has resulted in several issues, including the contamination of flour with chemical residues and an unpleasant odor from certain pesticides. It also kills pests' natural enemies. Consequently, it disrupts the ecological equilibrium, resulting in insect epidemics, environmental contamination, and high toxicity to people (Mondal, 2006). Also, many insects have become resistant to the pesticides used to get rid of them (Peterson and Ems-Wilson, 2003). This research was performed to avoid the harmful effects of chemical pesticides or reduce their toxicity by using natural products derived from plants, such as essential oils, which have important components and are effective against harmful stored pests. This study aimed to examine the impact of methomyl insecticide in different concentrations on the mortality rate of flour beetle larvae in their third and fifth stages. Furthermore, the effect of EOs (basil and harmal oils) on the mortality rate of flour beetle larvae when combined with methomyl pesticide concentrations; the effect of these EOs alone on the mortality rate of flour beetle larvae in their third and fifth stages, and finally, the repellent and attractant effect of EOs on the third and fifth stage flour beetle larvae were tested.

Materials and methods

The study was conducted on 25-4-2021 at the Department of Ecology, College of Science. The third and fifth instar larvae of *T. castaneum* were obtained from the lab that had been raised for several generations under lab conditions. A permanent colony was made by placing insects with wheat seeds inside glass containers 13 cm in diameter and 5 cm in height. Harmal and basil oil were received from the General Company for Essential Oils for the experiment. The trials used 500 mL of these essential oils. A commercial preparation package of the synthetic carbamate (Methomyl) was bought from (Syngenta) Swiss Agricultural Agency / Iraq Branch / Baghdad to conduct the study.

Three concentrations of the pesticide (Methomyl) were prepared as the recommended concentration (0.5), then made 2-fold serial dilution to 0.25 and 0.125 ml/L; in addition to the control that contained only water. Essential oils were dissolved in pure acetone (1:2) ml /L (Oil: acetone). They were put in a particular container to use in the experiments, with a bit of detergent added to the bottle to distribute and combine well with pesticide (Rahmat *et al.*, 2006).

The impact of Methomyl concentrations, EOs, and the blends of pesticide and EOs oils on T. castaneum

Third and fifth instar larvae of T. castaneum were collected from the colony formed and put in Petri dishes with a diameter of 9 cm and three replications, with ten larvae per duplicate and three replications. The uninfected wheat seeds were soaked in 1 ml of pesticide from each prepared concentration, harmal and basil oils alone, and the Eos oils combined with pesticide methomyl for 30 seconds, then placed on drying paper and finally placed in the dishes containing the (third and fifth instar larvae) with 1 g of wheat every repetition. The mortality percentage was calculated after 24 hours of treatment.

The impact of EOs alone in attracting and expelling the fifth stage larvae of flour beetles

Clean Petri dishes 15 cm in diameter and 2 cm in height were used, dividing the plate into two equal sections in the middle with a marker pen, the first (A) and second (B) with a tiny circle (2 cm) in the center of the plate to

deposit insects. The region (B) of the plate was cleaned with a cotton ball soaked in (1 ml) of harmal and basil oils, while portion (A) was left untreated. Then, ten adults were put in the center of the plate, and then 15 ml/L minutes later, the percentage of repellent and attraction was determined, and the experiment was repeated. The same procedure was repeated during the third and final larval stages. The percentages of repellent and attraction were computed using the method (Mc Donald *et al.*, 1970; Abdul Sattar and Mayser, 2000). PR = 2 (C - 50), Where PR = expulsion percenta, C = the proportion of insects in the untreated fraction.

Statistical analysis

The data were analyzed using Complete Randomized Design (CRD), and the Least Significant Differences test (LSD) was used at the 0.05 probability level to determine the differences between the transactions (McDonald *et al.*, 1970). The Abbott Formula corrected the percentage of deaths (Abbott, 1925; McDonald, 1968).

Results

The effect of methomyl on the mortality rate of third-stage larvae

The higher percentage of mortality with 0.5 ml /L concentration, with a significant difference from the other treatments, resulted in the highest mortality rate of 87.31 percent and the lowest mortality rate of 72.13 percent with 0.125ml/L, compared to the control treatment, which resulted in a mortality rate of 0.83 percent (Table 1). The treatment with 0.5 ml /L resulted in the most significant death rate, and the mortality rate rose with increasing duration time for all treatments. The treatment of 0.5 resulted in a 100% kill rate. In contrast, the treatment of 0.125ml/L resulted in a 95.55 percent mortality rate after 72 hours of treatment. Time was a substantial influence on raising the mortality rate. The most effective rate occurred over the 96 hours, reaching 75.55 percent, while the lowest occurred during the 24 hours, reaching 35.55 percent, as in table 1.

The effect of methomyl concentrations on the mortality rate of fifth-stage larvae of flour beetles

The concentration of 0.5 ml/L outperformed the other treatments. In the concentration of 0.5 ml/L, the maximum mortality rate was 86.48 percent,

while the lowest was 72.13 percent at 0.125ml/L, compared to 1.67 percent in the control treatment (Table 2). The duration time resulted in the most significant mortality rate of 60.0 percent of concentration of 0.5 ml. The lowest mortality rate by 0.125 ml/L, reaching 40.0 percent after 24 hours of treatment, compared to the control treatment, which had a mortality rate of 5.55 percent after 24 hours of treatment, and the mortality rates of all concentrations increased with time; the mortality rates in the concentrations (0.5 and 0.25) ml/L reached 100 percent. After 72 hours of treatment, the mortality rate in the treatment of 0.125 ml was 85.92 percent compared to the control treatment, which had a mortality rate of 3.33 percent. After 96 hours of treatment, the mortality rate of 0.125ml/L reached 100%. The preceding table also showed that 96 hours had the most effective mortality rate of 75.0 percent, much higher than the other treatments. In 24 hours, the mortality rate was the lowest.

Table 1. Effect of Methomyl on the mortality rate of third-stage larvae of flour beetles

Treatments (ml/L)	Third s Time (stage lar hour)	Rate Treatments		
	24	48	72	96	
0.5	63.33	85.92	100	100	87.31
0.25	40.00	61.85	86.66	100	72.13
0.125	36.66	61.85	90.00	100	72.13
Control	0.00	3.33	0.00	0.00	0.83
Time modified	35.00	53.24	69.17	75.00	
Overlap =4.513 ,	Time =2,167	, 0.05	L.S.D Tr	eatments	=2.167

*The treatments are statistically significant (p <0.05).

Table 2. The effect of methomyl concentrations on the mortality rate of fifthstage larvae of flour beetles

Treatments (ml/L)	last-stage larvae Time (hours)				Treatments Rate			
	24	48	72	96				
0.5	60.00	85.92	100	100	86.48			
0.25	43.33	72.59	100	100	78.98			
0.125	40.00	62.59	85.92	100	72.13			
Control	0.00	3.33	3.33	0.00	1.67			
Average time	35.83	56.11	72.31	75.00				
L.S.D. 0.05 overlap= 3.648, time = 1.226								

*The treatments are statistically significant (p < 0.05).

The effect of adding EOs in a ratio (1: 1) with the methomyl in the mortality rate of third and fifth stage larvae of flour beetles

The results showed that when EOs (1:1) was added to the pesticide Methomyl concentration, the fifth instar larvae had a greater incidence of death than the third instar larvae (Table 3). Basil oil outperforms harmal oil in the mortality of third and fifth instar larvae of beetle. The highest mortality rates (88.33 and 86.67 percent) were seen in the fifth and third instar larvae, respectively, when treated with (0.25 + basil oil). In comparison, the treatment (0.25 + harmal oil) resulted in a mortality rate of 83.33 percent of both larva stages. In contrast, the control treatment resulted in (1.67 and 5.55) percent of fifth and third instar larvae, respectively.

Treatments (ml/L)	Third larvae	instar	Treatments Rate	Fifth stag	Treatments Rate	
	24 hour	48 hour		24 hour	48 hour	
0.25+ basil oil	73.33	100	86.67	76.66	100	88.33
0.125+basil Oil	70.00	100	85.00	70.00	100	85.00
0.25+harmal oil	66.66	100	83.33	66.66	100	83.33
0.125+harmal oil	63.33	100	81.67	63.33	100	81.67
Control	0.00	0.00	0.00	0.00	3.33	1.67
Average time	54.66	80.00		55.33	80.67	
L. S. D. 0.05 for treatments =4.658 Interference =8.612, time = 6.551					reatments =4 21, time = 5.4	

Table 3. The effect of adding EOs (1: 1) with methomyl insecticide concentrations on the mortality rate of third and fifth larvae stages of flour beetles

*The treatments are statistically significant (p < 0.05).

The effect of EOs alone on the mortality rate of third-stage larvae of flour beetles

Basil oil has a considerable mortality rate over harmal oil, as seen in Table 4. Basil oil resulted in a 53.05 percent mortality rate. In contrast, the harmal oil treatment resulted in a 45.29 percent mortality rate, compared to the control treatment, which resulted in no mortality of 0.83 percent. After 24 hours

of treatment, the mortality rate of basil and harmal oils was 33.33 and 26.00 percent, respectively, compared to the control, which was 00.00 percent. After 96 hours of treatment, the percentage of mortality rates increased in all EOs treatments. The basil and the harmal oils achieved a mortality rate of approximately 75.55 and 65.18 percent, respectively, compared to the control treatment of 3.33 percent. The 96-hour period resulted in the most significant mortality rate of 48.02 percent, while the 24-hour period resulted in the lowest mortality rate of 19.78 percent, a considerable difference (Table 4).

Treatments	Time (h	Treatments			
	24	48	72	96	Rate
Basil oil	33.33	43.33	60.00	75.55	53.05
Harmal oil	26.00	36.66	53.33	65.18	45.29
Control	0.00	0.00	0.00	3.33	0.83
Time Rate	19.78	26.66	37.78	48.02	

Table 4. The effect of EOs alone on the mortality rate of third-stage larvae of flour beetles

*The treatments are statistically significant (p < 0.05).

The effect of EOs alone on the mortality rate of fifth-stage larvae of flour beetles

Results revealed that basil oil outperforms harmal oil in mortality rates, with a substantial difference. Basil and harmal oils had a mortality rate of 72.50 and 66.66 percent, respectively, compared to the control treatment, which had a mortality rate of 1.67 percent (Table 5). After 24 hours, the basil and harmal oils had a mortality rate of 40.00 and 36.66 percent, respectively, compared to the control, which was 0.00. The mortality rate rose with time, reaching 100% for all EOs treatments compared to the control treatment, which had a mortality rate of 3.33 percent after 96 hours. The 96-hour period resulted in the highest mortality rate of 67.78 percent, while the 24-hour period resulted in the lowest mortality rate, with a difference of 25.55 percent; the results revealed an increase in mortality rates in the fifth-stage larvae compared to the third-stage larvae (Table 5).

Treatments	time (h	ours)		Treatments Rate	
	24	48	72	96	_
Basil oil	40.00	66.66	83.33	100	72.50
Harmal oil	36.66	56.66	73.33	100	66.66
Control	0.00	0.00	3.33	3.33	1.67
Average time	25.55	41.11	53.33	67.78	
Interformer 7.24			-		

Table 5 The effect of EOs alone on the mortality rate of fifth-stage larvae of moss beetles

Interference =7.264 , time = 6.153, L.S.D. 0.05 Treatments = 4.992

*The treatments are statistically significant (p <0.05).

The effect of EOs alone in attracting and repelling the fifth stage larvae of flour beetles

The Basil and harmal oils were repellents to the fifth-stage larvae of flour beetles, as shown in Tables 6 and 7. Harmal oil had the most excellent repelling rate of 74.50 percent, followed by basil oil at 59.50 percent. The results show that harmal oil had a 24.50 percent appeal ratio compared to basil oil, with a 39.50 percent attractiveness ratio as in Table 6.

Table 6. The effect	of EOs alone in	attracting the fifth	stage larvae of flour
beetles			

		Treatments Rate
15	30	
36.00	43.00	39.50
23.00	26.00	24.50
29.50	34.50	
	36.00 23.00	36.00 43.00 23.00 26.00

Interference = 10.026, time = 5.615, L.S.D. coefficients. 0.05= 5.615

*The treatments are statistically significant (p < 0.05).

The percentage of repelling insects							
Treatments	Time (minute)		Treatments Rate				
	15	30					
Basil Oil	63.00	56.00	59.50				
Harmal Oil	76.00	73.00	74.50				
The time average	69.50	64.50					
Interference = 10.957, time = 5.499, L.S.D. coefficients. 0.05= 5.499							

Table 7. The effect of EOs alone in repelling the fifth stage larvae of flour beetles

*The treatments are statistically significant (p <0.05).

Discussion

Methomyl insecticide had a mortality rate of (86.48 and 87.31) against the flour beetle's third and fifth stage larvae at a concentration of 0.5 ml/L. These results were comparable to other that shows that nutmeg oil and the essential oil of garlic was more harmful to *T. castaneum* by contact than S. zeamais (Lee *et al.*, 2004). When 0.25 ml/L of methomyl was combined with basil oil in a 1: 1 ratio, it had the most significant mortality rates (86.67%, 88.33%) against the third and fifth stage larvae of the flour beetle insect compared to the concentrate 0.25 ml/l with harmal oil. Perveen and Zaib (2013) investigated the toxicity of essential oil. They discovered that adults of *S. zeamais* and *T. castaneum* were similarly vulnerable to the harmal oil's contact toxicity, with LD50 values of 56 and 52 mg mg-1 insect, respectively. Furthermore, 12-day-old *T. castaneum* larvae were more resistant to the oil's contact toxicity than adults, but 14- and 16-day-old larvae were just as vulnerable as adults

Using basil oil and harmal oil alone has affected the flour beetle's third and fifth stage larvae. Basil oil surpassed harmal oil in mortality percentages (72.50 percent vs. 53.05 percent) against flour beetle larvae in the third and fifth stages, respectively. The EOs harmal oil and basil oil demonstrated a repelling effect on the flour beetle insect's third and fifth larva stages. The repellant effect of harmal oil was 74.50 percent greater than that of basil oil. The results were equivalent to others. According to the research conducted by Elnabawy (2021), they studied the repellency and effectiveness of eight essential oils. They found that EOs exhibited high mortality, and Syzygium aromaticum can be used as a repellent against *T. castaneum*. In the experiment carried out by Epidi and Odili (2009), the effectiveness of plant powders from Telferia occidentalis (fluted pumpkin), Zingiber officinale (ginger), Vitex grandifolia (Vitex), and Dracaena Arborea (dragon tree) at 5g, 10g, and 15g/500g seeds against the storage pest *T. castaneum* (Herbst) in groundnut was investigated; The findings indicated that these plants exhibited high efficiency in controlling *T. castaneum*. The study performed by Adak *et al.* (2020) shows that EO nanoemulsions can be recommended as alternate management of rice storage insects, and eucalyptus oil (EO) is one of the most effective options against *Sitophilus oryzae* and *T. castaneum*. According to research conducted by Lee *et al.* (2004), six of the essential oils that were isolated from members of the family Myrtaceae that are present in Australia exhibited effective biocides toxic effects against three main stored-grain insects: *S. oryzae*, *T. castaneum*, and *Rhyzopertha Dominica*.

The results concluded that the insecticide methomyl at 0.5 ml/L resulted in a mortality rate of 100%.; In contrast, the treatment of 0.125 ml/L resulted in a mortality rate of 95.55 percent, compared to the control treatment, which resulted in a 5.55 percent mortality rate after 72 hours of treatment. Mixing Methomyl 0.25 ml/L with essential oils in a 1:1 ratio produced the highest mortality rate for the third and fifth larval stages. Basil oil outperforms harmal oil in the mortality of third and fifth instar larvae of beetle. The treatment (0.25 + basil oil) resulted in the highest mortality rates of (88.33 and 86.67) percent for the fifth and third instar larvae, respectively. In comparison, the treatment (0.25 + harmal oil) resulted in a mortality rate of 83.33 percent for both stages of larva, compared to the treatment of 0.25 ml/L alone, which is (72.13,78.98) percent for the fifth and third instar larvae. That study concluded that chemical pesticides may be reduced while their efficacy was enhanced, reducing the negative influence on the environment. The basil and harmal oils were repellents to the fifth-stage larvae of flour beetles. Harmal oil had an excellent repelling rate of 74.50 percent, followed by basil oil, 59.50 percent. The study proposed that essential oils may be applied to minimize the harmful effects of the chemical pesticide methomyl, which is presently used to control stored grain pests and to discover additional active essential oils that are efficient at repelling insects in storage.

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