

Evaluation of some plant oils against larvae of Khapra beetle (*Trogoderma granarium* E.) (Coleoptera: Dermestidae)

Dr. Fatehia Nasser Gharsan

Saudi Arabia/ Albaha university/Science college/Biology

Abstract: The repellent effect of six plant essential oils against Khapra beetle larvae was studied. Furthermore, the efficacy of the most potent repellent oil in protecting wheat seeds against infestation was evaluated. Results showed Clove oil as the most potent repellent against the insects (-60%), While Fennel oil showed an attractive effect (20%). The essential oils can be organized in descending order according to the repellent effect: Clove > Camphor > Cardamon > Onion, Fennel Clove oil showed an efficacy in protecting wheat seeds against infestation, as mortality percentage increased with increasing concentrations and contact time period; it reached 91.67% at concentration 4 for 48 hrs exposure. High concentrations also protected seeds from damage; protection was 100% at 4 concentration. The seeds damage percentage decreased with increased oil concentrations. The study also investigated the effect of larvae on wheat seed germination; as germination increased with higher Clove oil concentration. The study showed the high efficacy of Clove oil in protecting wheat seeds against pest infestation.

Keywords: Khapra beetle, plant oils, Toxicity, *Trogoderma granarium*, Stored product insects.

1. INTRODUCTION

Khapra beetle is a very destructive pest of stored grain and cereal in hot and dry climates of the world. During storage, grains are destroyed by many stored-grain insects that are responsible for world wide loss up to 10 – 40% annually (1). Khapra beetle is the major threat to stored wheat in Saudi Arabia Currently, intensive use of synthetic insecticides for the control of stored products pests has resulted in serious problems including insecticide resistance, environment contamination, unacceptable pesticide residues in food, lethal effects on non target organisms, and so on (2, 3, and 4). Botanical insecticides have long been used as attractive alternatives to synthetic insecticides for insect pest management because botanicals cause little threat to the environment or to human health (5, 6 , and 1)

In the meantime, the repellent, deterrent, and biological effects of some plant materials against stored product insects have been studied by many researches (7 , 4, 8 ,9 and 10)

Many studies have been conducted to evaluate the effectiveness of different types of plant oils and extracts against Khapra beetle (11 ,12, 13, 14)

In this present study the effects some plant oils on larvae of Khapra beetle (*Trogoderma granarium* E.) (Coleoptera: Dermestidae) were evaluated.

2. MATERIALS AND METHODS

Insect culture:

The larvae of Khapra beetle were obtained from stock culture maintained under controlled conditions of 27± 2C and 70%RH. The insects were kept with whole wheat in plastic jars (400ml), covered with muslin cloth and tightened with rubber bands to prevent the escape of insects.

Plant oils:

Six commercially available essential oils were tested in this study (*Clove, Syzygium aromaticum; Camphor, Eucalyptus camaldulensis; Cardamom, Elettaria cardamomum; Fennel, Foeniculum vulgare and Onion, Allium cepa L* . These oils were assayed on larvae of Khapra beetle.

Repellency:

The repellency of the oils was assessed using an olfactometer. The olfactometer design allows insects to choose one of four arms in response to different odor stimuli. In the experiment six larvae of Khapra beetle were used; eight replicates were done for each oil. 1 µL of each oil was on a small piece of filter paper and placed in two arms while other arms remained empty. Percentage repellency values were computed using the formula by (15):

$$\text{Intensity of reaction} = \frac{S-C}{S+C} \times 100$$

S= treated arm, C= control arm

Contact toxicity:

For bioassay tests, the thin film technique described by (16) was used. Five concentrations of the Clove oil (0.1, 1, 2, 3, 4 µL/ml of acetone) were selected. One ml of each concentration was evenly spread on the bottom of the Petri-dish. The Petri-dishes used as control were treated with 1 ml acetone only. Ten mature larvae were exposed to the thin film of clove oil for 48 hours. Larval mortality counts were recorded in (6, 12, 24, 48) hours after treatment, according to the formula of (17).

Percentage grain damage:

Larvae were treated with different concentrations of Clove oil. Then completed their development on clean grains. Percentage grain damage was calculated on 49th days after treatment. Numbers of perforated holes were recorded and the following general formula was used to determine the percentage of grain damage (12):

$$(\%) \text{ Grain damage} = \frac{\text{No. of perforated grains}}{\text{Total no. of grains counted}} \times 100$$

Percentage of germination:

Germination test was carried out to ascertain the effect of these treatments on the germination of the wheat grain infested with larvae after treatment with clove oil. Thirty grains from each treatment were placed in petri-dish containing Cotton. After 10 days, the germination percentage was calculated using the formula (12):

$$(\%) \text{ Germination percentage} = \frac{\text{No. of germinated grains}}{\text{Total no. of grains in Petri-dish}} \times 100$$

3. RESULTES AND DISCUSSIONS**1 - Repellency**

The repellent action of (Clove, Camphor, Cardamom, Fennel, Onion and Caraway oils) was studied against larvae of Khapra beetle. Data in (table 1) showed that Clove oil had the lead in repellent action where repellent percentage reached (-60%). Fennel and Onion oils had less repellent action (-30%) against larvae of Khapra beetle. The rest of the essential oils had a moderate repellent action. While, Caraway oil showed an attractive effect, Table(1). Similar data were obtained by (18, 19, 20 and 21). They proved that Clove oil had repellent effect on some stored products insects.

Table (1): List of plant oils and repellency effects against larvae of Khapra beetle

Plant oils	The scientific name	Part of the plant	(%)Repellency
Clove	<i>Syzygium aromaticum</i>	Seeds	-60%
Camphor	(<i>Eucalyptus camaldulensis</i>)	Leaves	-50%
Cardamom	<i>Elettaria cardamomum</i>	Seeds	-50%
Fennel	<i>Foeniculum vulgare</i>	Seeds	-30%
Onion	<i>Allium cepa L</i>	Onion	-30%
Caraway	<i>Carum carvi</i>	Seeds	20%

Contact toxicity:

The toxic effects of Clove oil against larvae of Khapra beetle are shown in table (2). The toxicity was increased by increasing concentrations and time of exposure. At 4 μ /ml concentration after 48h, highest larvae mortality was (91.7%). Treatment of larvae with Clove oil at concentration 0.1, 1, 2, 3 and 4 μ /ml after 48h exposure caused mortality (5.6, 16.7, 50, 61.1 and 91.7%) respectively. Many investigators recorded the larvicidal activity of some plant extracts and oils against many stored grain insects (22, 23, 24 and 25)

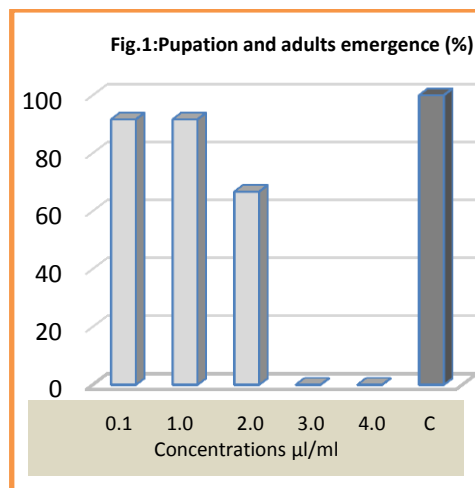
Table (2): Toxicity of Clove oil to the 3rd instar larvae of Khapra beetle

Concentrations μ L/ml	6h	12h	24h	48h
0	0	0	0	0a
0.1	0	0	0	5.6b
1	0	0	5.6	16.7c
2	0	0	16.7	50d*
3	0	0	22.2	61.1e*
4	0	5.6	58.3	91.7f*
LC50	0	-	-	2.7 μ L/M
LC95	0	-	-	3.97 μ L/M

Each column followed by the same letter is not significantly different from another (One-way ANOVA; Tukey test at, $P < 0.05$)- *mean high significant difference

Biological activity of Clove oil:

Fig. 1 shows the percent pupation and adults emergence decrease from 100% in control to 91.67, 91.67, 66.67, 0 and 0% when larvae were treated with Clove oil at concentrations 0.1, 1, 2, 3 and 4 μ /ml respectively. These results are in agreement with (22) who found that black seed oil and sesame oil reduced the larval pupation and adults emergence of *Tribolium confusum*. These results are in agreement also with (26, 27 and 28)

Fig.1: Effect of Clove oil on the development of 3rd larvae instar of Khapra beetle

Percentage grain damage and germination

and germination of wheat infested by larvae which were treated with Clove oil. The percentage of grain damage and germination decreased with increasing concentrations. Fig2. (29, 30 and 12) reported that on application of some powders against Khapra beetle, the results revealed that the powders were very effective at controlling damage and positively affected seed germination of groundnut seed.

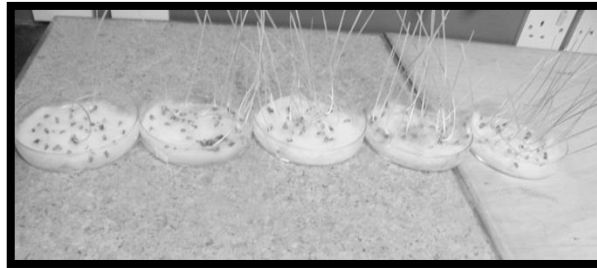


Fig.2: Percentage of germination of wheat grain infested with insect larvae treated with different concentrations of Clove oil.

During the study it was observed that fungal growth occurred in petri dishes certain seeds untreated with oil and those treated with low oil concentrations. Due to damage caused by larval infestation. No fungal growth occurred in seeds infested with larvae treated with high oil concentration. Table.3; fig.3a and b.



Fig.3a: Grains infected with fungal growth due to insect infestation

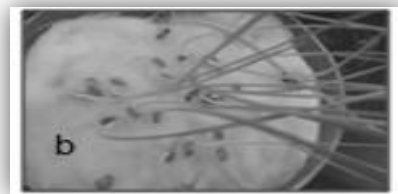


Fig.3b: Using Clove oil prevented fungal growth.

Table.3: Percentage of germination and seed damage of wheat grain when larvae were treated with Clove oil

Con. μL/ml	(%) Germination	Grain damage	Fungal growth
0	20%a	93a	<i>Aspergillus niger</i> <i>Aspergillus flavus</i> <i>Penicilium sp</i>
0.1	33.33%b*	83b	<i>Aspergillus niger</i> <i>Aspergillus flavus</i> <i>Penicilium sp</i>
1	40%c*	67	<i>Penicilium sp</i> <i>Rhizopus sp</i>
2	78.6d*	40d*	<i>Rhizopus sp</i>
3	96.7e*	6.67e*	No fungal growth
4	100f*	0f	No fungal growth

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