Effect of essential oil of colocynth, *Citrullus colocynthis* and spearmint, *Mentha spicata* against the khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae)

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Abstract

Khapra beetle *Trogoderma granarium* is considered to be the most severe primary pests, affected both the quality and quantity of wheat during storage. Therefore, khapra beetle is a serious problem in developing and developed countries. A laboratory study was performed to evaluate the influence of the essential oil rate of spearmint *Mentha spicata* and colocynth *Citrullus colocynthis* against khapra beetle *T. granarium* to determine the mortality rate of egg, larvae, and adults. Throughout the experiment, increasing the doses of essential oil of spearmint resulted in the highest rate of mortality of the khapra beetle egg, larvae, and pupa compared with increasing the rate of essential oil of colocynth. Thus, a linear relationship was found between the mortality rate of khapra egg, larvae, and pupa with the essential oil rate of spearmint. The highest mortality rate of adults (94%) was obtained by applying 50 µL of spearmint. In contrast, the highest concentrations (40 and 50 µL) of essential oil of colocynth showed the lowest mortality rate of khapra beetle adults. This study suggested that the essential oil of spearmint could be utilized as potential control agents for stored-product insects in wheat.

Keywords: *Trogoderma granarium*, colocynthis, spearmint, essential oil, wheat, grain storage insects.

1. Introduction

Insects and pests cause approximately 11 to 25% damage to the storage food and 8% to the grain production of the world [1]. Moreover, different of the stored grain products can be infected by insects during shipping and transportation. Pests also cause approximately 10 to 40% damage to the storage food in granaries houses [2]. Insects also cause a lower germination percentage to the stored grain products through the influence on the embryos of stored grain; moreover, the protein content will be reduced [3]. There is a pressing need to use the friendly environmental techniques to control these insects without causing any damage to the storage of food products because the damage of storage food and grain products is a serious problem in the developing countries [4]. Among different stored grain insects, *T. granarium* commonly called the Khapra beetle, which is a member of the family Dermestidae. Khapra is originated in India and then spread to different regions worldwide except Antarctica [1]. Khapra is the most invasive insects in the world that influence the quality and quantity of wheat *Triticum aestivum* L. during the storage period because this insect is capable of surviving during a long period in a dormant stage [5]. It is being calculated that approximately 0.3 to 2.6% of the wheat grain damage caused by Khapra beetle during the period of storage, which is about 1 to 11 months [4]. Depending on the environmental conditions (i.e., temperature, light, and moisture), season, and the species of host, khapra differs in the rate of development and survival stage. For example, this insect can generate 1 to 9 or more within one year under high humidity conditions. Moreover, the development of the egg, pupa, and adult can take one week; however, larvae may take one month to develop into adults of khapra [1].

Liquid pesticides and gases such as methyl bromide and phosphine have been widely utilized to protect wheat grain from different insects including Khapra because these insecticides are cost-effective and simple method [6]. However, the constant utilize of these insecticides has caused environmental pollution and insect resistance [7, 8]. Thus, botanical insecticides have been
utilized as alternatives ways to control and management of insects (i.e., khapra) because these kinds of insects cause little damage to the human health and the environment [9-13]. In developing countries, botanical insecticides have been used to control a wide range of insects because these insecticides are more effective, safe, easy to apply, and cheap than synthetic pesticides [14, 15, 9]. Fixed oil of clove buds Syzygium aromaticum and spearmint leaves inhibit the population (larvae and adults) of khapra beetle [16]. Different types of plant essential oil were used to control various grain insects, including T. granarium [17]. Several works have been carried out to manage stored-product insects in wheat by utilizing the essential oil of different medicinal and aromatic plants [18, 19]. Therefore, this present study was carried out to evaluate the pesticidal activity of different doses of essential oil of coloynth and spearmint against the khapra beetle T. granarium Everts under laboratory conditions.

2. Material and Methods

2.1 Insect culture

Different populations of khapra beetle (Trogoderma granarium Everts) were collected from the various grain storehouses of Al-Qadisiyah province for getting on the heterogeneous sample and rearing under laboratory condition. Moreover, these samples were identified depending on the taxonomy keys of insects. The sterilized jar was utilized for keeping the insect sample for two months and getting the homogenous sample of the khapra beetle. The adults of khapra beetle were sieved and saved into the sterilized jar containing the uninfected wheat grains that were sterilized at 60°C for 60-90 minutes. The samples were kept in an incubator at 30± 2°C and 65± 5% relative humidity for 72 hours for egg-laying. Then, the insects were removed from jars to new containers by utilizing sieve and a fine brush. The adult samples were initially kept for another 72 hours to get enough eggs. The first larvae appeared after four days in these samples, and the highest number of larvae appeared after one week. Three days old egg, two days old larva, two days old pupa and four days old adults of Trogoderma granarium were utilized for all bioassays.

2.2 Collection of plant materials and essential oil extracts

Samples were collected from plants, including seeds of C. colocynthis and leaves of M. spicata from different locations in Al-Diwaniyah city, Al-Qadisiyah province, Iraq. The plant samples were dried through placing them within room conditions for three weeks. Then, the dried materials were ground and powdered. The ground samples (leaves of spearmint and seeds of bitter apple) were hydrodistillied for three hours to get the essential oil by using the Clevenger-type apparatus hydrodistillation. After extraction, anhydrous sodium sulfate was utilized to remove water from the essential oil samples. The stock solution (spearmint or bitter apple essential oil) was kept into the refrigerator at 4°C until preparing the different concentrations of essential oil (10, 20, 30, 40, and 50 µL).

2.3 Fumigant test

Glass jars were utilized (capacity 70 ml) for testing the influence of different concentrations of essential oil (spearmint or bitter apple) against the mortality of Trogoderma granarium. Filter papers (Whatman No.1) were placed under the surface of screw caps and treated with an appropriate concentration of the essential oil for each plant species. Muslin cloths were placed under the glass jar caps to avoid insect contact with the essential oil. For spearmint concentrations of 10, 20, 30, 40, and 50 µL and bitter apple concentrations of 10, 20, 30, 40, and 50 µL were applied for the lab study. The empty glass jar was filled with wheat grain only (control treatment). Each concentration and control treatment were replicated three times. The mortality of insect was recorded after 48 hours of treatment by utilizing Abbott’s formula [20]. Corrected mortality (PT) was calculated using the following formula:

\[ PT = \frac{(Po - Pc)(100 - Pc)}{Po} \times 100 \]

Where Po = observed mortality and Pc = controlled mortality.

Data obtained from each rate of essential oil of spearmint or bitter-apple were subjected to regression analysis by using the R software program.

3. Result and Discussion

For applying different doses of essential oil of spearmint, the linear regression analysis suggested that the mean values of the mortality rate of T. granarium egg were significantly increased by increasing the essential oil (EO) doses of spearmint. However, a nonlinear regression analysis suggested that the essential oil doses significantly increased the mortality rate of T. granarium egg until using a 40 µL of bitter-apple. The mortality rate of T. granarium egg was significantly decreased when the rate of 50 µL of colocynth applied (Fig. 1). The mortality rate of T. granarium larvae was significantly increased by the spearmint essential oil rate (mortality rate of khapra beetle larvae = [3.667 ln (EORate) + 0.66]). In contrast, the predicted mortality rate of T. granarium pupa was significantly increased (non-linearly) with EO of colocynth (Fig. 2). A strongly significant linear relationship was
apparent for the mortality rate of *T. granarium* pupa vs. EO rate of spearmint (Fig. 3). In contrast, the mortality rate of *T. granarium* pupa was also significantly increased by the essential oil rate of colocynth (Fig. 3). However, using the EO of spearmint was highest effect on the mortality of *T. granarium* pupa than using the EO of colocynth. The mortality rate of *T. granarium* adult was significantly increased (non-linearly) by EO of spearmint (mortality rate of khapra beetle adult = $[0.5954 \ln(EORate)^2 + 13.477 \ln(EORate) + 17.996]$. Similarly, the mortality rate of *T. granarium* adult was significantly increased (non-linearly) by EO of colocynth (Fig. 4). For better management, the results might be useful to control the khapra beetle (*T. granarium*) insect in open and closed area. However, applying the high rate of essential oil of spearmint (above 50 µL) may leave a persistent smell on the grain treated. Moreover, a high rate applied to control *T. granarium* may cause a strong smell and unpleasant teste to the grain treated, which leads to reducing the quality. Thus, this aspect has to be kept in mind when trying to use a higher rate for effective results.

In the present study, among all doses of EO of *M. spicata* or *C. colocynthis* are most efficient against the egg, larvae, pupa, and adults of khapra beetle. Panezai *et al.*, [1] also reported that the essential oil of different plant species (peppermint, thyme, rosemary, and lemon balm) were proved to be the most efficient fumigant, against tow pest *T. granarium* and *Tribolium castaneum* adults, and using EO of rosemary caused 80% mortality in *T. granarium* and 59% mortality in *T. castaneum*. Younes *et al.*, [21] reported that the applying different doses of plant oil for different plant species (garlic, onion, olive, rosemary, sunflower, peppermint, and camphor) showed a significant effect on the mortality rate of *T. granarium* adult, and applying low doses of peppermint and camphor showed a high impact in the mortality of *T. granarium* adult compared with other plant species. Laznik *et al.*, [22] conducted a lab study in which four essential oil of plant species were used, and rosemary caused 60% mortality rate on another pest granary weevil adults. However, these authors reported that peppermint gave a satisfactory influence of about 97% mortality rate on granary weevil adults under high-temperature conditions.

![Figure 1. Effect of treatments on mortality rate of *T. granarium* egg.](image)
**Figure 2.** Effect of treatments on mortality rate of *T. granarium* larvae.

**Figure 3.** Effect of treatments on mortality rate of *T. granarium* pupa.
Figure 4. Effect of treatments on mortality rate of *T. granarium* adult.

**Conclusion**

The study revealed significant variations in insecticidal influences of different doses of essential oil for spearmint and colocynth on khapra beetle egg, larvae, pupa, and adult. Using essential oil of spearmint showed the highest mortality rate than utilizing essential oil of colocynth. The present study suggested that using spearmint essential oil could become a viable alternative to conventional chemical control management. Notwithstanding, further laboratory studies need to be carried out to evaluate the safety of this essential oil before practical utilize in stored-product khapra beetle control.

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