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Effect of Seed Oils of *Trigonella foenum-graecum* and *Peganum harmala* on the Life Cycle of *Trogoderma granarium*

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Abstract

The khapra beetle, *Trogoderma granarium* (Everts), is among the most economically important insect pests of stored grains and grain products around the world, resulting in extensive quantitative and qualitative losses in the process of storage. This study was carried out to evaluate the insecticidal effect of seed oils of *Trigonella foenum-graecum* (fenugreek) and *Peganum harmala* (harmal) on *T. granarium* at the fourth instar larval and adult stages. The three concentrations of each oil used (2%, 4%, and 8%) were investigated. Mortality of larvae was measured after 24 hours and 48 hours of exposure, while mortality of adults was measured after 24 hours, 48 hours, and 72 hours. It was found that the mortality increased considerably with an increase in the concentration and exposure period of the oil. The maximum larval mortality occurred at 8% concentration after 48 hours, which reached 96.6% and 71.6% for *T. foenum-graecum* and *P. harmala* oils, respectively. However, maximum mortality of the adult insects also occurred at 8% concentration after 72 hours, which reached 94.6% and 89.6% for *T. foenum-graecum* and *P. harmala* oils, respectively. The results demonstrate that both plant oils have insecticidal potential against *T. granarium*, with the oil from fenugreek seeds showing more activity compared to the oil from harmal seeds. The oils from these plants can be used as green substitutes to chemical insecticides for managing stored grain pests.

Keywords: *Trogoderma granarium*; *Trigonella foenum-graecum*; *Peganum harmala*; Plant oils; Stored grain pests; Mortality

1. Introduction

Stored grains and their products provide one of the most important sources of food for the world's population. However, considerable quantity and quality losses take place during storage owing to insect infestations. Khapra beetle, *Trogoderma granarium* (Everts) (Coleoptera: Dermestidae) is one of the most harmful and economically important stored product pests of the world. This beetle infests a variety of stored products such as wheat, rice, barley, maize, sorghum, pulses, and processed grain products. Infestation of this beetle adversely affects the quality and economic value of the grains and leads to considerable economic losses during storage, especially in hot and dry conditions [1–3].

The *Trogoderma granarium* is among the most important quarantine pests owing to its ability to thrive in harsh conditions. The larvae enter into diapause, survive without feeding for a long time, and thrive in areas with low moisture. Its elimination becomes extremely difficult because of this characteristic. In addition to this, it is easy for it to travel internationally in stored products [1, 4].

It is thought that the larval stage is the most destructive developmental life cycle stage of *T. granarium* due to the fact that the larvae have a high habit of feeding on the grain seeds and milled products, which causes weight loss in addition to contaminating the storage material with larval hair, molted skin, feces, and dead larvae [2,5].

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The control of storage insects has been achieved using synthetic insecticides and fumigants. However, the continued application of such chemicals has resulted in many complications, such as resistance to insecticides, environmental contamination, pesticide residues in food products, side effects on nontarget organisms, and increasing consumer worries regarding food safety. Thus, there is a need for the development of environmentally safe alternatives [1, 6].

Botanical insecticides represent potential alternatives since they possess a variety of biologically active substances, such as alkaloids, terpenoids, flavonoids, phenols, and essential oils. The modes of action of these phytochemicals are diverse: contact poisoning, fumigation, deterring feeding, inhibiting egg laying, regulating growth, and acting as repellents. Unlike chemical insecticides, botanical insecticides demonstrate low mammalian toxicity, fast biodegradability, and short environmental persistence [6, 7].

Of all medicinal plants, *Trigonella foenum-graecum* L., which is commonly referred to as fenugreek, is an annual herb within the legume family, commonly cultivated in Asia, North Africa, and Mediterranean areas. Fenugreek seeds are known to be rich in various bioactive compounds such as alkaloids, saponins, flavonoids, fixed oils, fatty acids, and phenolic compounds, which have various properties, including being antioxidants, antimicrobial, anti-inflammatory, and insecticidal in nature. Fenugreek seeds oil and extract are highly toxic to various stored-product insects [8].

Similarly, another such species that falls under this category is *Peganum harmala* L. (Syrian rue), which belongs to the Zygophyllaceae family. This perennial medicinal plant is found abundantly across the regions of Middle East, North Africa and Central Asia. The seeds of *Peganum harmala* L. contain significant amounts of β-carbolines, namely, harmine and harmaline along with flavonoids, phenolics and oils. These compounds exhibit biological activity in terms of their insecticidal, antimicrobial, antifungal, antioxidant and repellent properties [6,7].

In recent times, growing interest has been accorded to assessing the use of oils extracted from plants as environmentally-friendly means for protecting grains in storage. Oils that are essential and fixed, obtained from medicinal plants, have been found to be very effective in combating insects that infest grains due to their qualities like toxicity, repellency, inhibition of feeding, growth, and development of the insects [3,6].

Hence, the current study was carried out to investigate the effectiveness of various doses of *Trigonella foenum-graecum* and *Peganum harmala* seed oils against the fourth instar larvae and adults of *Trogoderma granarium* in the laboratory environment, in order to test their possible application as eco-friendly botanicals for grain storage protection.

2. Materials and Methods

2.1. Insect Culture

An insect colony of *Trogoderma granarium* (Everts) (Coleoptera: Dermestidae) was set up through collection of naturally-infested grains of wheat from local grain stores. Identification of the species was done by use of conventional taxonomic keys for stored product insects [9]. Insects were kept in glass containers (15 cm × 8 cm), which contained sterilized wheat grains as feeding medium. The containers were covered with muslin cloth held in place with rubber bands to enable insects to breathe in while preventing escape of the insects from the jars. Rearing of the insects took place under controlled laboratory conditions at temperatures of 30 ± 2°C and 70 ± 5% humidity [10,11].

2.2. Collection of Experimental Insects

Fourth instar larvae and adult beetles that just emerged from pupation were collected from the laboratory stock culture. Larvae were categorized based on the body size and morphology using stereomicroscopy, while only healthy adults were considered for the bioassay [9].

2.3. Plant Oils

Pure commercial seeds oil of fenugreek (*Trigonella foenum-graecum*) and harmal (*Peganum harmala*) with purity percentage of 100% was supplied by Al-Emad Herbal Oil Factory, Mosul, Iraq. The oils were kept in amber color bottles at 4 degrees centigrade until using [12].

2.4. Preparation of Oil Concentrations

Three levels (2%, 4%, and 8%) of each seed oil were prepared by dissolving in sterile distilled water, which contained 0.1% Tween 80. This is because Tween 80 acts as an emulsifier to help dissolve the oil [12].

2.5. Bioassay on Fourth-Instar Larvae

Ten fourth-instar larvae were placed in each Petri dish (9 cm diameter), with three replications per concentration. Each replication was treated with 3 mL of the respective oil concentration evenly. The control insects were treated with sterile distilled water that contained Tween-80. About 5 g of sterilized wheat grains were offered to them as food. All Petri dishes were kept under the environmental conditions mentioned before. After 24 h and 48 h, the number of mortalities was noted. The larvae that were not responsive when stimulated gently with a small brush were regarded as dead [10, 12].

2.6. Bioassay on Adults

Trogoderma granarium adults emerging from their pupal stage were introduced into the Petri dishes at 10 insects per dish, and each set of treatments had three replications. Adults were exposed to each of the concentrations of oils (2%, 4% and 8%) using 3 ml per insect, while the control set was treated with sterile distilled water mixed with Tween-80. The mortality rate was determined after 24 hours, 48 hours and 72 hours [10,12].

2.7. Statistical Analysis

The study design employed was CRD. The percent mortality values underwent analysis of variance (ANOVA), and the treatment means were analyzed using Duncan's multiple range test, with a level of significance of $P \leq 0.05$. All statistical analysis was done using SAS, version 9.4 [13].

3. Results

3.1. Effect of *Trigonella foenum-graecum* and *Peganum harmala* Seed Oils on Fourth-Instar Larvae of *Trogoderma granarium*

The bioactivity of the seed oils of *Trigonella foenum-graecum* and *Peganum harmala* against fourth-instar larvae of *Trogoderma granarium* is depicted in Table 1 and 2. It was observed that both the essential oils were effective against larval mortality, whereby mortalities increased gradually with an increase in concentration and time of exposure to the oils ($P \leq 0.05$).

Of all the tested concentrations, 8% of *T. foenum-graecum* seed oil displayed maximum insecticidal activity with 56.6% and 96.6% larval mortalities after 24 and 48 hours, respectively. On the other hand, the lowest mortality was recorded at 2% concentration after 24 and 48 hours at 36.6% and 66.6%, respectively. The intermediate concentration gave 46.6% and 86.6% after 24 and 48 hours, respectively. No mortality was recorded in the control group without treatment (Table 1).

Table 1 Effect of different concentrations of *Trigonella foenum-graecum* seed oil on the mortality (%) of fourth-instar larvae of *Trogoderma granarium* after 24 and 48 h of exposure

Concentration	Time / hour		Average concentration
	24	**48**	
2 %	36.6	66.6	51.6 c
4 %	46.6	86.6	66.6 b
8 %	56.6	96.6	76.6 a
Average time	46.6 B	83.2 A	

Means followed by the same uppercase letter in the same row and the same lowercase letter in the same column are not significantly different according to Duncan's Multiple Range Test at $P \leq 0.05$.

Likewise, the mortality rate of fourth-instar larvae in relation to *P. harmala* seed oil is dependent on the concentration and time of exposure. The maximum percentage mortality (71.6%) was recorded for the concentration of 8% after 48 h of exposure, while mortality at the same concentration was observed to be 35.6% after 24 h of exposure. The minimum percentage mortality (15.6%) was recorded at the 2% concentration after 24 h of exposure, and 45.6% mortality was observed at the same concentration after 48 h of exposure (Table 2).

Table 2 Effect of different concentrations of *Peganum harmala* seed oil on the mortality (%) of fourth-instar larvae of *Trogoderma granarium* after 24 and 48 h of exposure

Concentration	**Time / hour**		**Average concentration**
	24	**48**	
2 %	15.6	45.6	30.6 c
4 %	21.6	64.6	43.1 b
8 %	35.6	71.6	53.6 a
Average time	24.2B	60.6A	

Means followed by the same uppercase letter in the same row and the same lowercase letter in the same column are not significantly different according to Duncan's Multiple Range Test at P ≤ 0.05.

3.2. Effect of *Trigonella foenum-graecum* and *Peganum harmala* Seed Oils on Adults of *Trogoderma granarium*

The insecticidal effects of *Trigonella foenum-graecum* and *Peganum harmala* seed oil against *Trogoderma granarium* adults were presented in Table 3 and 4. It was shown that there was a significant effect of oil concentration and exposure time on adult mortality (P ≤ 0.05). Mortality was observed to increase steadily with increasing oil concentration and exposure time.

Of all the different concentrations evaluated, the insecticidal effect of *T. foenum-graecum* seed oil was at its best. In the highest concentration level, there was 24.6%, 52.0%, and 94.6% mortality after 24, 48, and 72 h of exposure, respectively. On the other hand, the lowest concentration (2%) had a mortality percentage of 14.6%, 21.2%, and 74.6% after 24, 48, and 72 h, respectively. There was 21.2%, 34.6%, and 84.6% mortality after 24.

Likewise, adults exposed to *P. harmala* seed oil experienced an increase in mortality in a dose-dependent manner. The highest dose of 8% caused mortality of 25.6%, 49.0%, and 89.6% at the end of 24, 48, and 72 hours, respectively. The lowest dose of 2% caused mortality of 12.6%, 21.6%, and 64.6% in the same intervals, while the middle dose of 4% caused mortality of 19.2%, 38.6%, and 79.6%.

There were statistically significant variations between the concentrations and periods of exposure (P ≤ 0.05). The period of 72 hours was observed to have the highest mortality in all treatments. This indicates that increased duration increases the toxicity of the two oil-based botanical products. This shows a clear dose and time dependency, which is a common feature in botanical insecticides for storage insects.

Table 3 Effect of different concentrations of *Trigonella foenum-graecum* seed oil on the mortality (%) of adult *Trogoderma granarium* after 24, 48 and 72 h of exposure

Concentration	**Time / hour**			**Average concentration**
	24	**48**	**72**	
2 %	14.6	21.2	74.6	36.8 c
4 %	21.2	34.6	84.6	46.8 b
8 %	24.6	52.0	94.6	56.8 a
Average time	20.1C	35.9B	84.6A	

Means followed by the same uppercase letter in the same row and the same lowercase letter in the same column are not significantly different according to Duncan's Multiple Range Test at P ≤ 0.05.

Table 4 Effect of different concentrations of *Peganum harmala* seed oil on the mortality (%) of adult *Trogoderma granarium* after 24, 48 and 72 h of exposure

Concentration	Time / hour			Average concentration
	24	48	72	
2 %	12.6	21.6	64.6	32.9 c
4 %	19.2	38.6	79.6	45.8 b
8 %	25.6	49.0	89.6	54.7 a
Average time	19.1 C	36.4 B	77.9 A	

Means followed by the same uppercase letter in the same row and the same lowercase letter in the same column are not significantly different according to Duncan's Multiple Range Test at P ≤ 0.05.

4. Discussion

It has been shown in the current research that the oils isolated from seeds of *Trigonella foenum-graecum* and *Peganum harmala* have a relatively high insecticidal efficacy towards the fourth instar larvae as well as adult forms of *Trogoderma granarium*. Mortality in both stages is found to be significantly higher with an increase for time. It indicates a dose-dependent and time-dependent effect. Increased exposure increases the penetration of the active substances into the insects' tissues [14-16].

Of the two botanical oils used in the study, *T. foenum-graecum* was found to be more effective as compared to *P. harmala* for its insecticidal properties. While at the highest concentration, (8%) the fenugreek seed oil caused mortality of 96.6% in fourth-instar larvae within 48 hours and in adults within 72 hours, *P. harmala* gave 71.6% and 89.6% mortality rates. This enhanced effectiveness could be due to the different qualities and quantities of their phytocomponents. The phytocomponents present in the fenugreek seeds include steroidal saponins, flavonoids, alkaloids, phenolic compounds, and unsaturated fatty acids [17,18].

A number of modes of action have been identified in regard to insecticidal properties of plant oils. Essential oils and fixed plant oils could penetrate the insect integument; affect cell membrane integrity; inhibit acetylcholinesterase activity; affect neurotransmission; cause inhibition of mitochondrial respiration; and decrease the insect feeding activity. Moreover, the volatile components could produce fumigation effect on insects by penetrating their spiracles and causing paralysis that results in death. Having multiple biologically active components in plant oils increases the chances of insects becoming resistant to them than to artificial insecticides with single component [14,15,19].

The action of *P. harmala* seed oil against insects seems to be related primarily to the presence of β-carboline alkaloids such as harmine and harmaline, along with flavonoids and phenolics. Such bioactive secondary metabolites are known for their neurotoxic, antifeedant, growth-inhibiting effects as well as interference with metabolic enzymes. The increase in mortality rates with an increase in exposure time is indicative of the fact that these bioactives need some time to produce their maximal action [18,20].

The results presented above are consistent with studies conducted by Isman [6], whose research showed that botanical insecticides possess insecticidal properties through diverse modes of action such as neurotoxic, anti-feedant, growth-inhibitory and physiologic activities. The same was noted by Pavela [7], who found out that the higher doses and increased time of exposure to plant extracts caused increased mortality of stored-product insect pests. Similarly, Papachristos and Stamopoulos [17] revealed that essential oils had both contact and fumigation toxicities against stored-product beetles and caused high mortality rate and inhibited insect development. Also, Regnault-Roger *et al*. [16] noted that essential oils contained complex blends of terpenoids and phenolics and therefore acted on diverse physiological targets, which decreased the probability of resistance development. Other authors, such as Campolo *et al*. [11] and Pérez *et al*. [8], have shown similar insecticidal effect of botanical oils against *Trogoderma granarium* and other stored-product insects.

The increased susceptibility at 48 h in larvae and 72 h in adults indicates that constant exposure results in enhanced permeation through both cuticle and respiratory system of active substances. Such time-dependent response has been noted in many botanical oils tested against stored-product pests, whereby death increased gradually with both increasing concentration and increasing time of exposure. Essential oils can also influence insects development via interference with molting process, inhibition of feeding activity, reduction in reproduction ability, and histological destruction of digestive and nervous systems [15,16,19].

Earlier studies had proven the excellent efficiency of plant oils for the control of *T. granarium* and other storage insects. Essential oils of many aromatic plants have been found to be effective in terms of contact toxicity, fumigation properties, repellency, deterrence, and growth inhibiting. Recent studies have found some essential oils to cause 90% or more mortality rates for larvae and adults of *T. granarium* [14,16,17,20].

The growing apprehension about resistance to pesticides, environmental pollution, and food safety has resulted in a push towards the development of more environmentally friendly methods for protecting stored grains. The use of botanical oils has been of interest since these are biodegradable, sustainable, safer for mammals, and display a number of bioactive actions against insect pests. Unlike traditional insecticides, botanical oils demonstrate reduced environmental persistence along with being toxicants, repellents, feeding deterrents, oviposition inhibitors, and growth regulators [14–16,19].

In conclusion, the findings of this current study show that both *Trigonella foenum-graecum* and *Peganum harmala* seed oils can be considered potential botanical insecticides against *Trogoderma granarium*. However, fenugreek seed oil demonstrated better performance during the entire testing period and is therefore more likely to be used in IPM systems of stored grain. It is recommended that further research be aimed at the investigation of these botanical oils in commercial storage conditions, their identification of major active compounds, determination of their effects on grain quality and seed germination, as well as the development of stable formulations (nano-emulsion or micro-encapsulation) [14,15,17,19,20].

5. Conclusion

The present research has found that the oils from *Trigonella foenum-graecum* and *Peganum harmala* seeds have substantial insecticidal activity towards the fourth instar larvae and adults of *Trogoderma granarium*. It is evident from the results that mortality increased with an increase in the oil concentration and time period.

From these findings, one can infer that both types of seed oil are viable eco-friendly substitutes for synthetic insecticides used traditionally for controlling khapra beetle pests in grain stores. They are viable options for inclusion in IPM strategies because of their natural sources, biodegradability, and various modes of action.

Additional research is necessary in order to determine the active components that contribute to this insecticidal action, study the effectiveness of these compounds in commercial grain storage systems, examine their effects on grain quality and seed germination, and formulate stable products through methods such as nano-emulsions and microencapsulation.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

Statement of ethical approval

This study did not involve human participants or vertebrate animals. Therefore, ethical approval was not required.

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