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The efficiency of some plant powders in the mortality of the red flour beetle *Tribolium castaneum* (Herbst)

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Abstract

A laboratory study was conducted to evaluate the efficacy of various plant powders, including fenugreek (*Trigonella foenum-graecum*), pomegranate (*Punica granatum*), anise (*Pimpinella anisum*), and harmel (*Peganum harmala*), at doses of 2g, 4g, and 8g. The larval and adult stages mortality of the red flour beetle *Tribolium castaneum* (Herbst) was recorded after 24, 48, and 96 hours. The results revealed that the plant powder and the concentration-dependent had a significant effectiveness after 24 h and 48 h for both larva and adult stages mortality, respectively. Moreover, all plant powders and their concentrations, in addition to their interactions between them, had a crucial effectiveness after 96 h as well. The harmel powder scored the most effective, achieving the highest mortality rates of both the larval and adult stages. After 24 h was the highest mortality rates were 29.67% and 68.22% of the larval and adult stages, respectively. Additionally, the results demonstrated that powder concentrations increased the mortality from 37.28% at 2g to 45.59% at 8g and 62.58% at 2g to 70.14% at 8g after 96 h of exposure of the larval and adult stages, respectively, reinforcing a concentration-dependent increase; the mortality rates also increased.

Key words: rusty flour beetle, stored products insects, plant-derived insecticides, harmel powder, post-harvest pests.

I. Introduction

Wheat and its products are among the most important foods in Iraq and the world due to their connection to the food security of nations, as they provide the world's population with about 20% of total calorie intake (Juárez *et al*., 2021; Mahmood *et al*., 2024). Additionally, they contain fibers, antioxidants, vitamins, starch, essential amino acids, and minerals, making them a primary source of carbohydrates and protein (Khalid *et al*., 2023). The Food and Agriculture Organization (FAO) estimated post-harvest losses in agricultural production at about 10% in developed countries and 20% in developing countries (Liu *et al*., 2021; Papanikolaou *et al*., 2022). Annually, 25-30% post-harvest losses were recorded in cereal crops worldwide (Cala *et al*., 2021). Whereas, the FAO estimated that losses caused by pests in stored grains and oilseeds account for 40% of global production (FAO, 2023).
Stored crops and their products are attacked by insect pests belonging to different orders, particularly the Coleoptera, which includes more than 600 species that are classified as pests of stored products (Huang *et al*., 2020). The rusty flour beetle *T. castaneum* (Herbst) is one of the most important species that attack stored grains and their products with both its larvae and adults, causing significant economic damage (Abdullah *et al*., 2025).
Infestation by the rusty-red flour beetle leads to damage food materials, either directly due to the feeding of larvae and adults on stored materials using their chewing mandibles, or indirectly by contaminating them with highly volatile

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toxic secretions "benzoquinones", and other chemicals secreted in the infested food from glands located on the prothorax and posterior abdomen (Papanikolaou *et al*., 2022). Furthermore, the infested grains become unfit for cultivation or human consumption due to damage to the protein content within the grains (FAO, 2023). Also, the adults have the ability to fly, which allows them to easily move between different parts of the storage as well as between the field and the storage (Liu *et al*., 2021).

The proper protection of stored grains and their products after harvest contributes to achieving global food security (Papanikolaou *et al*., 2022). Despite the chemical insecticides' effectiveness against most insect species, they had many negative effects on the environment and human health (Shah & Parveen, 2023).

Stored product insects have developed resistance to the main classes of insecticides, such as organophosphorus and pyrethroids (Fan *et al*., 2025). Thus, the increased awareness of the negative effects of synthetic insecticides has led to research and focus on alternatives from natural products as insecticides to control post-harvest pests (Kumar *et al.*, 2024).

Non-industrial products derived from natural sources have proven their efficacy as a promising tool in plant protection (Gvozdenac, 2021; Al-Hayali & Qader, 2024). These products are characterized by many positive features when used in the environment, as they act as a quick deterrent to insect feeding and have lower stability in nature (Korunić *et al*., 2020). Natural materials, especially botanical, have low toxicity to non-target organisms and rapid effectiveness, resulting in a lesser negative impact on beneficial organisms compared with synthetic insecticides (Boate and Abalis, 2020). Also, they can be used in conjunction with biocontrol agents to be safer for humans and fish (Daraban *et al*., 2023). Nevertheless, their rapid degradation in the environment and the frequent usage may limit the use of this safe tool (Deb & Kumar, 2020).

Several studies have listed the efficacy of botanical powders in the red flour beetle control. Ahmed *et al*. (2017) found that treating with Sweetsop powder (*Annona squamosa* L.) at a rate of 7.5g/25g (30% w/w) provides the best protection for stored millet seeds against infestation with the red flour beetle *T. castaneum*. Powders of neem seeds (*Azadirachta indica* Juss), turmeric roots (*Curcuma longa* L.), and sweet flag (*Acorus calamus* L.) are used to control the red flour beetle *T. castaneum* (Iqbal *et al*., 2015). Naimi *et al*. (2022) confirmed in their study, conducted to demonstrate the repellent effect of powders and essential oils (EOs) from several plant species on adult *T. castaneum* (Herbst), that the fenugreek seed powder (*T. foenum-graecum*) had a repellent effect of 34.67%, while the harmel seed powder (*Peganum harmala*) recorded a negative repellent effect of 16.10%.

Materials and Methods:

The experiment was conducted in the laboratories of the biology department-College of Education, Pure Science/University of Thi–Qar, during March-April/2025.

1-Preparation of the laboratory culture of *T. castaneum*:-

The red flour beetle, *T. castaneum* insects was obtained from infested flour. The insect was identified by standard classification keys. Every 30 pairs (1:1) (female: male) were placed in 500 ml plastic containers (sterilized with 70% ethanol, washed with distilled water, and dried). The containers were covered with gauze and held on with rubber bands. Each container was filled with 50g of sterilized artificial medium (wheat flour+5% yeast powder) (Sial *et al*., 2017). They were kept in an incubator (28±2ºC, 65±5%) (Al-Emara *et al*., 2021). The larval stage was obtained by placing the eggs in the plastic dishes in the incubator at the previously mentioned temperature and humidity. Then the eggs were monitored daily to obtain the larvae. The adult stage was obtained by raising the pupae in the incubator, after which the flour was sifted using a sieve (25-30 mesh) to obtain the adults (Mohamed, 2023).

2- Preparation of plant powders:

The Parts of plants were grinding (obtained from the local markets of Nasiriyah city), as shown in the table below:

Local name	**Scientific name**	**Family**	**Used plant part**
Fenugreek	*Trigonella foenum-graecum*	Fabaceae	Seed
Pomegranate	*Punica granatum*	Lythraceae	Fruit peel

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Anise	*Pimpinella anisum*	Apiaceae	Seed
Harmel	*Peganum harmala*	Nitrariaceae	Seed

Then, the powders were weighed to obtain 2g, 4g, and 8g of each plant. A 96 sterilized plastic containers were prepared, then 50 g of wheat flour was placed in each.

3- Testing the powder's effect on the larval stage mortality rates of *T. castaneum*:

48 sterile plastic containers were prepared, with 50g of sterilized wheat flour filling each. The containers were divided into four groups (12 containers for each). Each group was treated with a plant powder (fenugreek, pomegranate, anise, and harmel). Each group was further divided into four sub-groups (3 containers for each). Each sub-group was treated with 0 (control treatment), 2g, 4g, and 8g of each plant powder. Then, larvae were added (10 larvae per container), each container was covered with gauze and held on with rubber bands, and kept in an incubator (28±2°C, 65±5%) **(Saxena, 2016)**. Mortality rates were recorded after 24, 48, and 96 h, corrected according to Orell and Schneider equations **(Püntener, 1981):**

$$\%\ \text{Corrected mortality} = \frac{(\%\ \text{mortality in treatment} - \%\ \text{mortality in control})}{100 - \%\ \text{mortality in control}} \times 100$$

4- Testing the powder's effect on the adult stage mortality rates of *T. castaneum*:

48 sterile plastic containers are prepared and divided, and mortality rates are recorded as adopted in the previous paragraph (taking into account that the adults are transported instead of larvae).

Statistical analysis:

The data is analyzed statistically using precision factorial experiments (two factors) in a complete randomized design (CRD), compared Averages are compared by the least significant difference (LSD) of 0.05 by program the GeneStat statistical analysis program.

II. Results

1- Effect of plant powders in the larval stage of *T. castaneum* after 24 h exposure:

The result of Table 1) investigates the effect of different plant powders (fenugreek, pomegranate, anise, and harmel) on the larval stage of the red flour beetle (*T. castaneum*) after 24 h of exposure, using varying concentrations (2g, 4g, and 8g). The results reveal that Harmel powder was significant, with the most effective maintaining a high an average effect of 29.67%, indicating strong insecticidal properties. In contrast, pomegranate powder was the least effective, with the lowest mortality, with an overall mean of 19.4%.

A strong, significant, effective concentration-dependent trend is observed, where increasing the quantity of powder enhances mortality rates, as the overall mean mortality rises from 18.26% at 2g to 28.45% at 8g. Notably, the interaction effect between powder type and concentration quantity (A×B) was not statistically significant (NS).

Table 1: Effect of plant powders in the larval stage of *T. castaneum* after 24 h exposure:

% corrected mortality				
Conc.(g) / plant	2	4	8	Av. plant
Fenugreek	20	21.3	25.5	22.27
Pomegranate	8.9	20	29.3	19.4
Anise	15	25	29	23
Harmel	29	30	30	29.67

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Av. Conc..	18.26	24.08	28.45	
LSD (0.05)	Plant(A)= 2.9*	Conc.(B)=3.2*	A×B= NS	

The result study (Table 2) evaluated the insecticidal effects of plant powders (fenugreek, pomegranate, anise, and harmel) on the larval stage of the red flour beetle (*T. castaneum*) after 48 h of exposure, using three different concentrations (2g, 4g, and 8g). The results indicated that harmel powder was the most potent, consistently achieving the highest mortality rates with an average effectiveness of 48.29%.
A clear and significant concentration-dependent trend is observed, where increasing the amount of plant powder significantly enhances mortality rates. The overall mean mortality rises from 32.39% at 2g to 38.85% at 8g. Additionally, the interaction effect (A×B) was not significant (NS).

Table 2: Effect of plant powders in the larval stage of *T. castaneum* after 48 h exposure:

% corrected mortality				
Conc.(g) / plant	**2**	**4**	**8**	**Av. plant**
Fenugreek	30.33	35.08	33.33	32.91
Pomegranate	20.22	20.25	35.08	25.18
Anise	33	35	36.98	.3499
Harmel	45.99	48.88	50	48.29
Av. Conc..	32.39	34.8	38.85	
LSD (0.05)	Plant (A)= 4.3*	Conc.(B)=1.6*	A×B= NS	

The result in Table 3) investigated the insecticidal effects of plant powders (fenugreek, pomegranate, anise, and harmel) on the red flour beetle (*T. castaneum*) larval stage after 96 h of exposure, using three different concentrations (2g, 4g, and 8g). The result confirmed that the plant powder, the concentration-dependent, as well as the interaction between them, had a significant effect on the larval stage mortality of the red flour beetle.
The results also indicated that harmel powder remained the most effective, achieving the highest mortality rates across all concentrations (48.99% at 2g, 50.55% at 4g, and 58% at 8g), with an average effectiveness of 52.51%. This confirms Harmel's strong and persistent insecticidal activity, proving to be the most lethal plant powder against *T. castaneum* larvae over an extended period.
Pomegranate powder remained the least effective overall, showing the lowest mortality at 2g (20.23%) and 4g (25%), but improving significantly at 8g (35.35%), leading to an average effectiveness of 26.86%. This trend suggests that pomegranate requires higher concentrations to achieve significant insecticidal effects.
A concentration-dependent trend was observed, where increasing the amount of plant powder significantly enhances mortality rates. The overall mean mortality increases from 37.28% at 2g to 45.59% at 8g, further confirming that higher concentrations improve the insecticidal efficacy of plant powders. Suggesting that the mortality increase due to higher concentrations remained consistent across all plant powders, without significant variation based on specific powder type.

Table 3: Effect of plant powders in the larval stage of *T. castaneum* after 96 h exposure:

% corrected mortality				
Conc.(g) / plant	**2**	**4**	**8**	**Av. plant**

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Fenugreek	39.89	39.89	45	41.59
Pomegranate	20.23	25	35.35	26.86
Anise	39.99	40.79	44	41.59
Harmel	48.99	50.55	58	52.51
Av. Conc.	37.28	39.06	45.59	
LSD (0.05)	Plant(A)= 5.5*	Conc.(B)=4.07*		A×B= 1.7*

Table 4 examined the insecticidal effects examination for different powders (fenugreek, pomegranate, anise, and harmel) on the red flour beetle (*T. castaneum*) adult stage after 24 h of exposure, using three different concentrations (2g, 4g, and 8g). The statistical analysis results confirmed that the plant powder and the concentration-dependent factor had a significant effect on the larval stage mortality of the red flour beetle. The results revealed that Harmel powder was the most effective, achieving the highest mortality rates with an average effectiveness of 68.22%. This indicates that harmel maintains strong and consistent insecticidal activity, making it the most lethal plant powder against adult *T. castaneum* within a short exposure period.
Anise powder exhibits the lowest insecticidal effects, with anise showing an average mortality of 55.36%.
A concentration-dependent trend was evident, where increasing the plant powder concentration enhances insecticidal activity. The overall mortality rate rises from 59.08% at 2g to 65.93% at 8g, confirming that higher concentrations improve the powder's effectiveness. Additionally, the interaction effect (A×B)is not significant (NS).

Table 4: Effect of plant powders in the adult stage of *T. castaneum* after 24 h exposure:

% corrected mortality				
Conc.(g) / plant	**2**	**4**	**8**	**Av. plant**
Fenugreek	65.66	67	69.99	67.55
Pomegranate	50	54.66	63.65	56.1
Anise	53	53	60.07	55.36
Harmel	67.66	67	70	68.22
Av. Conc..	59.08	60.42	65.93	
LSD (0.05)	Plant(A)= 9.5*	Conc.(B)=5*		A×B= NS

The outcomes in Table 5) show the insecticidal effectiveness of four plant powders (fenugreek, pomegranate, anise, and harmel) against the *T castaneum* (red flour beetle)adult stage after 48 h of exposure at different concentrations (2g, 4g, and 8g). The statistical analysis confirmed that the plant powder and the concentration-dependent factor had a significant effect on the larval stage mortality of the red flour beetle. The findings indicated that harmel powder was the most effective botanical insecticide, with an average effectiveness of 71.01%. This suggests that harmel remained highly toxic to *T. castaneum* over time, making it a promising natural alternative for pest control in stored grains.
On the other hand, anise powder exhibits the lowest insecticidal activity, with an average effectiveness of 57.2%.
The overall mortality rate across concentrations increases from 60.09% at 2g to 67.43% at 8g, further confirming a concentration-dependent trend, where increasing the concentration of plant powder enhances insecticidal activity.

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However, the Least Significant Difference (LSD) test at the 0.05 level showed that the interaction effect (A×B) was not significant (NS).

Table 5: Effect of plant powders in the adult stage of *T. castaneum* after 48 h exposure:

% corrected mortality				
Conc.(g) / plant	**2**	**4**	**8**	**Av. plant**
Fenugreek	64.5	67	67	66.17
Pomegranate	53.88	58.99	65.65	59.51
Anise	52.99	55.55	63.07	57.2
Harmel	69	70.02	74.01	71.01
Av. Conc..	60.09	.6289	67.43	
LSD (0.05)	Plant(A)= 5.8*	Conc.(B)=1.06*		A×B= NS

Table 6 shows the evaluation of the four botanical powders (fenugreek, pomegranate, anise, and harmel) insecticidal activity against the *T. castaneum* (red flour beetle) adult stage after 96 h of exposure at different concentrations (2g, 4g, and 8g). The result confirmed that the plant powder, the concentration-dependent, as well as the interaction between them, had a significant effect on the adult stage mortality of the red flour beetle. The data showed that harmel powder remained the most effective botanical insecticide, achieving 73.65%. On the other hand, pomegranate and anise powders exhibit the lowest insecticidal activity, with an average mortality of 60.08%.

The overall mortality rate across all powders and concentrations increases from 62.58% at 2g to 70.14% at 8g, reinforcing a concentration-dependent trend, where increasing the concentration of plant powder enhances insecticidal activity.

The interaction between the type of powder and the applied concentration in treatment harmel**X**8g was the highest mortality at 77.22%. On the contrary, treatment with anise is the lowest, with an average mortality of 55% at 2g.

These findings confirmed that harmel powder had strong and persistent insecticidal activity over time, making it a reliable natural alternative for pest management in stored grains.

Table 6: Effect of plant powders in the adult stage of *T. castaneum* after 96 h exposure:

% corrected mortality				
Conc.(g) / plant	**2**	**4**	**8**	**Av. plant**
Fenugreek	69	70.01	72	70.34
Pomegranate	55.59	58.99	65.65	60.08
Anise	55	59.54	65.69	60.08
Harmel	70.72	73.02	77.22	73.65
Av. Conc..	62.58	65.39	70.14	
LSD (0.05)	Plant(A)= 8.2*	Conc.(B)=2.06*		A×B= 3.8*

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III. Discussions

The results of the current study, in light of previous research on the insecticidal effects of botanical powders against *T. castaneum* (red flour beetle), Comparing the findings with other studies that have investigated plant-derived insecticides for stored-product pest control. Several studies have examined the effectiveness of various botanical powders, essential oils, and plant extracts, revealing their potential as eco-friendly alternatives to synthetic insecticides:

1. The Insecticidal Effect of Botanical Powders on Different Life Stages of *T. castaneum*:

The results in the seven tables indicate that botanical powders of harmel (*Peganum harmala*), fenugreek (*Trigonella foenum-graecum*), anise (*Pimpinella anisum*), and pomegranate (*Punica granatum*) demonstrate varying degrees of toxicity against both the larval and adult stages of *T. castaneum*. Consistently, harmel powder exhibited the highest mortality rates, followed by fenugreek on larvae and anise on adults, while pomegranate powder was the least effective.

The Harmel plant has high effectiveness against *T. castaneum*. Harmel extract could cause significant reductions in *T. castaneum* populations due to their potent fumigant and contact toxicity **(Hassani *et al*., 2020)**. *P. harmala* seeds extract showed the highest efficacy, significantly inhibiting larval growth and disrupting the insect's developmental cycle, leading to reduced progeny production **(Jbilou, 2022)**.

The reason for the harmal (*P. harmala*) efficiency against the red flour beetle may be due to its content of alkaloids such as harmaline and harmine, known for their neurotoxic effects on insects. These compounds interfere with neurotransmission in insect pests, leading to high mortality (**Zhang *et al*., 2023)**.

In the current study's results, fenugreek powder as well as anise powder have moderate insecticidal activity. Additionally, the fenugreek extracts may cause high mortality in stored-product pests (**El-Nadi *et al.*, 2020)**. Therefore, this suggests that fenugreek can be a sustainable alternative for grain protection.

The essential oils from anise seeds exhibited fumigant toxicity against stored-product insects **(Kumar *et al*., 2015)**. However, since the current study used powdered forms instead of oils, the observed effects might be attributed to contact toxicity rather than fumigant action. Or anise-based formulations may require higher concentrations to achieve significant insect mortality **(Negahban *et al*., 2007)**.

The result of the current study confirmed that Pomegranate powder has the lowest insecticidal activity among the tested botanicals. This may be attributed to the fact that pomegranate extracts had repellent rather than toxic effects on *T. castaneum* **(Chaudhry, 2017)**. Additionally, pomegranate's insecticidal properties were less effective than other plant-based insecticides, possibly due to the lower presence of toxic alkaloids and phenolic compounds **(Rajashekar *et al*., 2013)**

2. Concentration-Dependent Effects:

The results demonstrate a clear concentration-dependent effect, where higher concentrations (8g) consistently led to increased mortality rates. This may be due to botanical powders exhibiting greater toxicity at increased concentrations due to enhanced exposure and ingestion by insects **(Sharififard *et al*., 2019)**. This trend is particularly evident in harmel and fenugreek powders, suggesting that their bioactive compounds require sufficient concentrations to disrupt insect physiological processes.

Besides, the current study revealed that the larval stage of *T. castaneum* is more susceptible to fenugreek powder than the adult stage. This may be because younger insect stages are more vulnerable to plant-based insecticides due to their underdeveloped detoxification enzymes **(Hikal *et al*., 2021)**. The results suggest that botanical powders could be more effective as preventive treatments targeting immature pest stages before they mature into reproductively active adults. Besides, the effectiveness of botanical powders against stored-product pests varies significantly based on powder type and applied concentration **(Abbasipour *et al*., 2022)**.

3. Long-Term Persistence and Relevance to Integrated Pest Management (IPM):

The current study also highlights the persistence of botanical insecticides over time, as mortality rates remained high even after 96 h of exposure. Certain plant-derived insecticides exhibit residual effects, prolonging their efficacy **(Isman, 2020)**. The result of the current study emphasized that the strong performance of Harmel powder over time suggests that it has the potential for sustained pest control without requiring frequent reapplication.

The findings of this study contribute to the growing body of evidence supporting the use of botanical powders as eco-friendly insecticides for stored-product pest control. Compared to synthetic chemical insecticides, botanical insecticides offer a safer alternative, reducing the risk of pesticide residues in food while maintaining high efficacy. The high

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effectiveness of harmel makes it a particularly promising candidate for use in Integrated Pest Management (IPM) programs, where botanical powders can be applied alongside other control methods such as proper grain storage techniques, physical barriers, and biological control.

IV. Conclusion

Harmel powder is the most effective plant-based treatment against the larval and adult stages of *T. castaneum*; fenugreek (on adults) and anise (on larvae) show moderate effectiveness, with similar levels of toxicity, while pomegranate was the weakest insecticidal agent. The higher concentrations improve the insecticidal efficacy of plant powders against *T. castaneum***.** These findings confirm that harmel powder is the most effective natural insecticide, maintaining the highest and most stable mortality rates even after 96 h of exposure. The results emphasize that prolonged exposure enhances the insecticidal potential of plant powders, with concentration-dependent effects playing a crucial role in their efficacy. These insights support Harmel's potential as a reliable botanical alternative to synthetic insecticides, with potential applications in integrated pest management strategies for stored grain protection.

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