



Green Insecticidal Approach: Toxicity of *Peganum harmala* Fixed Oil against *Tribolium castaneum*

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Abstract: This study investigated the insecticidal effect of the fixed oil of *Peganum harmala* L., collected from the Reguiba region in Oued Souf Province, against the stored-product pest *Tribolium castaneum* (Herbst, 1797). The fixed oil was extracted from the seeds using a Soxhlet apparatus, yielding 24.33%, whereas mechanical cold pressing produced an exceptionally higher yield of 30.13%. Bioassays were conducted using two methods: direct contact and fumigation. Three concentrations (2.5, 5, and 10 $\mu\text{l mL}^{-1}$) were tested, with three replicates for each. In direct contact tests, mortality reached 100% after 24 h at 2.5 and 5 $\mu\text{l mL}^{-1}$, and 90% after 6 h at 10 $\mu\text{l mL}^{-1}$, with a median lethal concentration (LC_{50}) of 2.34 $\mu\text{l mL}^{-1}$ after 12 h. During fumigation, 100% mortality was observed after 48 h at 5 $\mu\text{l mL}^{-1}$ and after 24 h at 10 $\mu\text{l mL}^{-1}$, with an LC_{50} of 7.52 $\mu\text{l mL}^{-1}$ after 12 h. Mortality data were analyzed using probit analysis and corrected with Abbott's formula. These results demonstrate that the toxicity of *P. harmala* fixed oil is both dose- and time-dependent, as adult *T. castaneum* mortality increased progressively with higher concentrations and longer exposure periods. Overall, the study highlights the potential of *P. harmala* fixed oil as a natural biopesticide for the management of major stored-product pests.

Key words: Fixed oil, *Peganum harmala*, *Tribolium castaneum*, stored-product pest, contact toxicity, insecticidal activity.

Stored-product pests are among the main causes of significant losses in cereal grains during post-harvest storage, due to direct feeding, contamination, and quality deterioration, which negatively affect food security (Lustig *et al.*, 1977a;

Ahmad *et al.*, 2019; Madni *et al.*, 2025; Ouaraous *et al.*, 2025). Grain storage remains an important tool for food and seed retention, ensuring global food security. It is, therefore, essential to have knowledge of proper grain storage as the ultimate determinant of the survival of millions of people (Gill and Sharma, 2021). Inherently, grain stored in poor environments remains one of the first to fall victim to insect infestation. Insect infestation results in substantial losses of quantity evidenced by the loss of as many as 3% to 40% of the stored commodity mass in traditional grain storage (Rajendran, 2002; Shafique *et al.*, 2006). In addition to the mass losses of the product, the most quantifiable loss occurs as it declines in value due to the loss of protein content (Lü and Huang, 2021). Additionally, the insects' metabolism promotes the development of toxic microorganisms within the commodity due to the microclimate produced, making it no longer safe for human consumption (Pai, 2010; Nath *et al.*, 2024).

From this category of insect pests, the red flour beetle, *Tribolium castaneum* (Herbst, 1797), stands out as one of the most recognized species with the capacity to infest all forms of stored cereal and legume grains (Lustig *et al.*, 1977b; Pai, 2010; Dia *et al.*, 2018). This global insect beetle results in significant quantitative and qualitative losses to these grains with changes in coloration to grey and an offensive pungent smell. This could be attributed to the excretion of benzoquinones (Lis *et al.*, 2011; Majeed *et al.*, 2016; Atta *et al.*, 2020), which are responsible for the changes not only making the grains unfit for human consumption due to the development of an unpleasant or rancid taste but are also responsible for significant allergenic or carcinogenic properties for human consumption (Lis *et al.*, 2011; Badran *et al.*, 2025). Although this species is more common in tropical environments, it thrives best in the Oued Souf region (Southeastern Algeria). This region, one of Algeria's principal agricultural centers for cereals, has extreme temperature variations (20° to 40°) (Barkat *et al.*, 2023). Further, traditional practices, such as mass usage of natural fertilizers (poultry excrements), contribute to increasing residual moisture levels, thereby creating conditions conducive to optimal development of *T. castaneum* populations for wheat as well as flour (Lü and Huang 2021). The temperature

levels as well as the moisture content can affect feeding behavior, translating into commodity weight loss. A 4° increase from 28° to 32° of temperature enhanced whole grain sorghum's weight loss by 2-fold, then another 3° increment from 32° to 35° reduced weight loss by 1.76-fold (Majeed *et al.*, 2016). These combined factors exacerbate storage difficulties and threaten local food security, making the search for control methods adapted to this Saharan context imperative.

While chemical pesticides have long been the predominant solution, their intensive use has led to major environmental and health crises, including food chain contamination and the emergence of pest resistance (Nicolopoulou-Stamati *et al.*, 2016; Mishra *et al.*, 2020). Faced with these challenges, the transition toward sustainable agriculture has become essential, placing biological control and bio-based alternatives at the core of crop protection strategies (Azaizeh *et al.*, 2002; Mouden *et al.*, 2017). Among these alternatives, plant-derived products, rich in secondary metabolites such as terpenes, flavonoids, and alkaloids, offer a promising pathway due to their biodegradability and low ecotoxicological impact (Negahban *et al.*, 2006; Souto *et al.*, 2021). While essential oils have been widely documented as pillars of integrated pest management (IPM) (Lahlou, 2004; Regnault-Roger *et al.*, 2008), the exploration of other extracts, such as fixed oils, represents a crucial opportunity to diversify modes of action against stored-product insects, particularly in regions where these resources are locally available.

Peganum harmala L. (Zygophyllaceae), commonly known as 'Harmal', occupies a privileged place in traditional Algerian phytotherapy for treating various ailments (Baba Aissa, 1999; Benbott *et al.*, 2022). Its seeds are recognized for a broad spectrum of pharmacological properties, including antibacterial, anticancer, vasorelaxant, and antiprotozoal activities (Apostolico *et al.*, 2016; Ait Abderrahim *et al.*, 2019; Jalali *et al.*, 2021). This therapeutic efficacy is intrinsically linked to its exceptional richness in secondary metabolites, particularly beta-carboline alkaloids such as harmine and harmaline (Apostolico *et al.*, 2016). Although alkaloid extracts and essential oils of this plant have been extensively documented, the insecticidal potential of its fixed oil remains

poorly explored against stored-product pests (Apostolico *et al.*, 2016; Bitchagno *et al.*, 2022). Yet, the unique physicochemical characteristics of this oil could significantly influence insect physiology and behavior. It is also established that the biochemical profile of *P. harmala* is strongly influenced by edapho-climatic factors. In this regard, the population from Oued Souf, subjected to extreme thermal and hydric stress, might offer an original and particularly bioactive composition.

The present study aimed to systematically evaluate, for the first time, the insecticidal efficacy of fixed oil derived from locally sourced *Peganum harmala* against adult *Tribolium castaneum* through both contact and fumigation bioassays. The influence of application dose and exposure duration on mortality responses was quantified to characterize toxicity dynamics and determine dose–time relationships to assess toxicity patterns and application efficiency. The findings are intended to establish a scientific basis for the valorization of this Saharan botanical resource as a sustainable candidate for integration into stored-grain pest management strategies.

Materials and Methods

The region of Reguiba, one of the municipalities of the Wilaya of Oued Souf, is located 30 km north of the provincial capital. It is characterized by its strategic geographical location, bordered to the north by the commune of El Hamraia, to the south by the commune of Teghzout, to the east by the communes of Qamar and Sidi Aoun, and to the west by the communes of Sidi Khelil, Tendla, and Jamaa. Reguiba covers an area of 144.7 km² and is inhabited by approximately 20,000 people according to the 2014 census. Agriculture is the main economic activity in the region, where cereals, legumes, vegetables, fruits, and date palms are cultivated.

Tribolium castaneum, the red flour beetle, is a cosmopolitan Tenebrionid. For experimental purpose it was in the biology laboratory using appropriate equipment. Rearing containers included glass jars and plastic boxes with perforated lids to allow for ventilation, filled to a depth of 2 to 3 cm. The insects were fed whole wheat flour mixed with 5% dry yeast to enrich the diet with proteins. An incubator was used to maintain a constant temperature.

For handling the insects and cleaning the substrate, tweezers, soft brushes, and sieves were employed. Healthy adult beetles were introduced into the containers in groups of 50 to 100 individuals to avoid overcrowding.

Peganum harmala was collected in November 2024 from the Reguiba region. A voucher specimen (N° AB-65, 2018) was identified by a botanist Pr. Boudjelal Amel, and was deposited in the Herbarium of the Department of Nature and Life Sciences, University of Mohamed Boudiaf, M'Sila, Algeria. The seeds were carefully cleaned to remove all impurities, rinsed with tap water, and then dried in a dark, moisture-free environment for several days to preserve their active properties. Once completely dried, the seeds were ground into a fine powder using an electric grinder. This powder was subsequently used for the extraction of fixed oil.

Cold pressing, a mechanical extraction method was used to obtain oils from oilseeds and nuts. In cold pressing, the seeds of *Peganum harmala* are continuously pressed using a mechanical press. They are then placed in a container and subjected to hydraulic pressure without the application of heat, as heat can reduce the nutritional value and alter the flavor of the resulting oil. This traditional process yields a crude oil (<https://mooriing.com>).

Contact bioassays

The insects were separated using a 0.5 mm sieve and gently handled with a brush. Twenty adult beetles were placed individually into plastic Petri dishes (8 cm in diameter). These adults were treated with three concentrations (2.5 $\mu\text{L mL}^{-1}$; 5 $\mu\text{L mL}^{-1}$; and 10 $\mu\text{L mL}^{-1}$) of fixed oil, diluted in Tween 80 (used as a solvent), based on preliminary test results. Each concentration was tested in triplicate, along with a control group. After 5 minutes of shaking to ensure homogenization and complete evaporation of the solvent, all Petri dishes were placed in a controlled chamber at an average temperature of $26.25 \pm 1.5^\circ\text{C}$ and relative humidity of $53 \pm 1.41\%$. Mortality rates were recorded hourly for all tested concentrations over a 72-hour period.

Fumigation bioassay

The fumigant toxicity of *P. harmala* fixed oil against *T. castaneum* adults was evaluated using airtight glass jars (1 L capacity). To ensure strict

exposure to the vapor phase and eliminate any physical contact, the calculated doses (2.5, 5, and 10 $\mu\text{L mL}^{-1}$ air) were applied to a cotton carrier. This carrier was suspended in the upper third of the jar using a fine thread secured by the screw cap, maintaining a clear distance from the insects at the bottom. Batches of twenty (20) adults were introduced into each jar before it was hermetically sealed. Mortality was monitored periodically over a 72-hour period, and each treatment was replicated three times.

The mortality rate for both control and treated groups was recorded at specific time intervals. The observed mortality percentage was calculated using the following equation:

$$M_o (\%) = (N_d/N_t) \times 100$$

where, M_o is the observed mortality rate (%); N_d is the number of dead individuals and N_t is the total number of individuals in the bioassay. However, the number of individuals counted as dead in a treated population does not necessarily reflect the true number killed by the toxic agent, as a certain degree of natural mortality exists in all populations. Therefore, observed mortality rates were corrected using Abbott's formula (1925) to account for this natural background mortality:

$$M_c (\%) = (M_t - M_o / 100 - M_o) \times 100$$

where, M_c = corrected mortality (%), M_t = mortality in treated group (%), M_o = mortality in control group (%). Corrected mortality rates were then transformed into probits using Bliss Cavalier's table (1976). These probits were plotted against the natural logarithm of the tested concentrations to estimate the median lethal dose (CL_{50}). The CL_{50} value was calculated from the regression equation generated using Microsoft Excel. In addition, all statistical analyses were conducted using IBM SPSS Statistics V27.0.1, with a significance level set at $p < 0.05$. One-way ANOVA was applied to the corrected mortality percentages, followed by Tukey's HSD test to compare the toxic effects among different concentrations. Probit analysis was used to estimate CL_{50} , along with its 95% confidence intervals, slope, and chi-square (χ^2) values, following the method of Sakuma (1998).

Results and Discussion

Oil extraction from the seeds was carried out using the Soxhlet method with hexane as a

solvent. After extraction and removal of solvent residues, the obtained yield was 30.13%. The oil yield was lower than the 44.21% reported by Dahamna and Chergui (2021) and also differs from values obtained by Kuşmenoğlu *et al.* (1996) in Turkey and Lalla *et al.* (1999) in Morocco. These differences may be attributed to geographic origin, environmental conditions, and extraction methods.

Both contact and fumigation tests demonstrated that the fixed oil from *Peganum harmala* exhibited varying degrees of toxicity against *T. castaneum* (Fig. 1). ANOVA analysis indicated that the tested oil concentrations (2.5, 5, and 10 $\mu\text{L mL}^{-1}$) had highly significant toxicity effects ($p < 0.01$) in the contact test and very highly significant effects ($p < 0.001$) in the fumigation test across the exposure times of 6 h, 12 h, 18 h, and 24 h. However, during the 24 h contact exposure, the oil concentrations showed no difference, as they all resulted in 100% mortality. Tukey's post-hoc test showed that the highest mortality was observed at the 10 $\mu\text{L mL}^{-1}$ concentration compared to 2.5 and 5 $\mu\text{L mL}^{-1}$ across all exposure periods in both tests. Exceptionally, after 24 h in the contact test, all three concentrations reached 100% mortality. Moreover, the highest dose (10 $\mu\text{L mL}^{-1}$) achieved over 90% mortality within the first 6 hours of contact exposure. In contrast, for the fumigation test, a mortality rate of 92.15% was only reached after 24 hours. The lowest concentration (2.5 $\mu\text{L mL}^{-1}$) led to 100% mortality after 24 hours in the contact test, while it resulted in only 41.17% mortality in the fumigation test (Fig. 1).

The probit analysis data in Table 1 showed that the contact method was significantly more effective against *T. castaneum* than the fumigation method under laboratory conditions. The fixed oil of *P. harmala* exhibited lower CL_{50} values of 4.165, 2.343, and 0.934 $\mu\text{L mL}^{-1}$ at 6, 12, and 18 hours of exposure in the contact test, respectively. In comparison, CL_{50} values for the fumigation test were 7.746, 7.526, and 4.558 $\mu\text{L mL}^{-1}$, respectively.

Furthermore, Table 1 indicates that the highest levels of response homogeneity in *T. castaneum* individuals were recorded in the contact test, with slope values of 2.800, 2.623, and 2.173 at 6 h, 12 h, and 18 h post-treatment, respectively. Conversely, the fumigation test

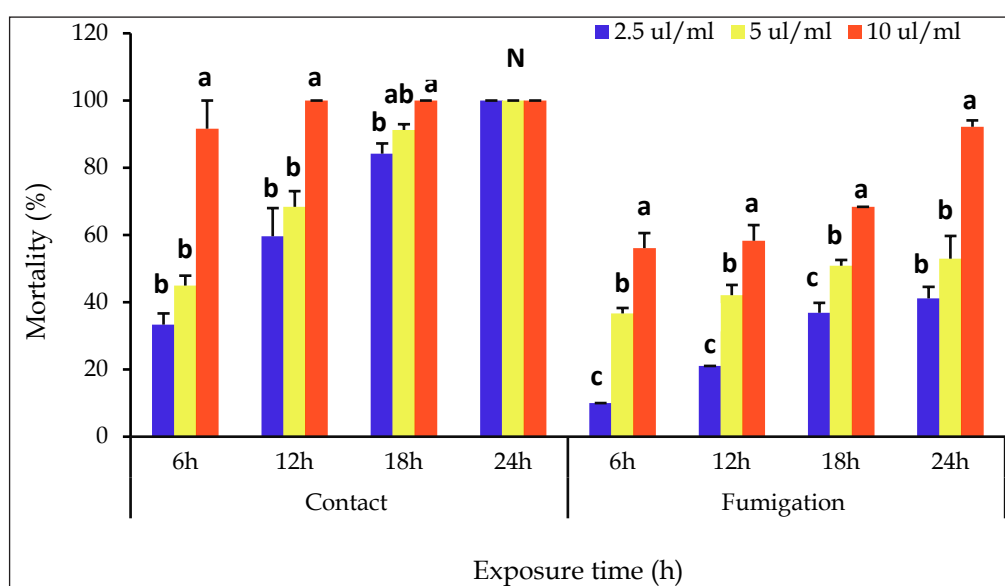


Fig. 1. Corrected mortality of *Tribolium castaneum* expressed as mean \pm SEM of three replicates ($n = 3$). Bars sharing the same letter within the same period are not significantly different. N: not significant. Differences were tested using Tukey's test at $p = 0.05$.

displayed lower slope values of 2.407, 1.577 and 1.353, highlighting the differences in individual response to the tested concentrations and application methods.

The fixed oil demonstrated high toxicity against adult *T. castaneum*. All tested concentrations (2.5, 5, and 10 $\mu\text{l mL}^{-1}$) caused 100% mortality after 24 hours in direct contact assays, while fumigation led to 92.15% mortality after the same exposure time. The highest dose (10 $\mu\text{l mL}^{-1}$) induced over 90% mortality within the first 6 hours of contact. The median lethal concentrations (CL_{50}) were estimated at 2.34 $\mu\text{l mL}^{-1}$ for direct contact and 7.52 $\mu\text{l mL}^{-1}$ for fumigation after 12 hours of exposure. These values are lower than the CL_{50} reported for

Artemisia sieberi oil (16.76 $\mu\text{l L}^{-1}$) by Neghaban *et al.* (2006), indicating greater potency.

Significant mortality recorded in our fumigation assays, despite this inherent limitation, can be attributed to the presence of a 'volatile bioactive fraction' dissolved within the lipid matrix. This is physically evidenced by the oil's intense and pungent aromatic profile. In the confined environment of the sealed bottles, these volatile compounds (notably terpenoids and alkaloids identified in our phytochemical screening) accumulate in the headspace over 12 to 48 hours. Once a toxic threshold is reached, these vapors penetrate the respiratory system of *T. castaneum* via the spiracles. This 'reservoir effect' of fixed oils is supported by recent literature; for instance, Khemira *et al.* (2020)

Table 1. Estimates of the lethal doses of *Peganum harmala* fixed oil against *T. castaneum*

Bioassay	Exposure time (h)	LD_{50} ($\mu\text{l/mL}$)	95% confidence limits		Slope \pm SE	χ^2	P-value
			Lower	Upper			
Contact	6	4.165	-	-	2.8 \pm 0.343	13.007	< 0.001
	12	2.343	-	-	2.623 \pm 0.404	16.468	< 0.001
	18	0.934	-	-	2.173 \pm 0.563	3.317	0.069
	24	-	-	-	-	-	-
Fumigation	6	7.746	6.627	10.689	2.407 \pm 0.341	1.377	0.241
	12	7.526	6.026	9.562	1.577 \pm 0.312	0.638	0.424
	18	4.558	3.383	5.899	1.353 \pm 0.304	0.104	0.748
	24	3.536	-	-	2.502 \pm 0.341	9.821	0.002

LD_{50} : lethal dose that kills 50% of insects, χ^2 : chi-square value, SE: standard error, p -value: probability.

demonstrated that various botanical oils exert significant insecticidal activity through vapor-phase toxicity. Furthermore, our findings align with the methodology of Kifle *et al.* (2025) in *Heliyon*, which validated the use of botanical fractions and oils as effective fumigants in closed-system bioassays. Given that the essential oil yield from this Saharan ecotype was negligible, these results confirm that the aromatic fixed oil serves as a potent and more accessible biopesticide alternative.

Compared to other studies, *P. harmala* oil exhibited superior efficacy. For example, *Matricaria chamomilla* oil had a CL_{50} of $2.789 \mu\text{L mL}^{-1}$ (Aimene and Djanet, 2021), *Artemisia arborescens* recorded $1.346 \mu\text{L mL}^{-1}$ (Said *et al.*, 2016), and *Pinus pinaster* oil achieved only 18% mortality at $50 \mu\text{L mL}^{-1}$ after 24 h (Amitouche and Rakem, 2017). This confirms the high effectiveness of *P. harmala* oil even at low concentrations. This is further supported by the findings of Kemassi *et al.* (2013), who reported strong toxicity of the essential oil from *P. harmala* leaves against *Schistocerca gregaria* larvae (L5), with noticeable neurotoxic symptoms such as motor disturbances, convulsions, and loss of balance. Similarly, (Eltahir and Dahab (2019) demonstrated the oil's effectiveness against *Trogoderma granarium* larvae, with a CL_{50} of $23.5 \mu\text{g mL}^{-1}$ by ingestion and $49.7 \mu\text{g mL}^{-1}$ by contact.

Phytochemical analysis shows that *P. harmala* extracts are rich in alkaloids, flavonoids, triterpenes, coumarins, and tannins. Flavonoids, in particular, play a central role in plant defense against insect herbivores (Acheuk and Doumandji-Mitiche, 2013). Additionally, *P. harmala* oils from the Mediterranean region exhibited antibacterial activity against both Gram-positive and Gram-negative bacteria such as *S. aureus* and *E. coli* (Apostolico *et al.*, 2016), which supports its traditional medicinal use.

From a toxicological perspective, the fixed oil of *P. harmala* presents moderate acute toxicity with a CL_{50} of $501.47 \text{ mg kg}^{-1}$ (Dehiri *et al.*, 2021) and appears safe at doses below 100 mg kg^{-1} . Furthermore, the methanolic extract showed no signs of toxicity up to 3000 mg kg^{-1} (Abbas *et al.*, 2021), with no behavioral effects or mortality observed over 14-28 days of acute and subacute testing. However,

Benbott *et al.* (2022) highlighted potential dose-dependent hepatotoxicity at high alkaloid concentrations, evidenced by elevated liver enzymes, histopathological abnormalities, and weight loss emphasizing the need for careful use.

In light of these findings, *P. harmala* fixed oil appears to be a promising natural biopesticide with strong, rapid, and dose-dependent insecticidal activity. Nevertheless, practical application requires further studies addressing extract standardization, formulation stability, field effectiveness, impact on non-target species, and comprehensive toxicological evaluations to ensure safe and sustainable use.

Conclusions

The results of this study highlight the strong insecticidal potential of fixed oil extracted from *Peganum harmala* seeds, a plant native to Algerian Saharan ecosystems, against *Tribolium castaneum* under both contact and fumigation exposure. Complete mortality (100%) was recorded, along with very low LC_{50} values ($2.34 \mu\text{L mL}^{-1}$ for contact and $7.52 \mu\text{L mL}^{-1}$ for fumigation after 12 h of exposure), reflecting the high toxicity and rapid insecticidal action of this natural product. These findings emphasize the considerable promise of *P. harmala* oil as an effective botanical insecticide derived from local Algerian plant resources. To consolidate and expand upon these outcomes, further investigations are required to identify the bioactive compounds responsible for the observed insecticidal activity and to evaluate their efficacy against other major stored-product pests prevalent in North African and Mediterranean regions. In conclusion, *P. harmala* represents a valuable candidate for the development of eco-friendly biopesticides adapted to arid and semi-arid agroecosystems. Nevertheless, its large-scale application will depend on in-depth studies addressing formulation optimization, ecotoxicological safety, chronic toxicity, and rigorous validation under real storage and field conditions typical of Algerian grain protection systems.

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