

ADULTICIDAL ACTIVITY OF AQUEOUS EXTRACTS OF *Pergularia tomentosa* L. (Asclepiadaceae) ON *Tribolium* BEETLES (Coleoptera: Tenebrionedeae)

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Abstract. Beetles of the species *Tribolium* are serious pests in cereal storage, causing economic losses and impairing the quality of the grain during storage. Plant extracts, such as aqueous extracts of *Pergularia tomentosa* L., are currently being studied as possible alternatives to synthetic insecticides for controlling these pests. Phytochemical screening of *Pergularia tomentosa* revealed a richness in bioactive secondary metabolites, in particular flavonoids, saponosides, terpenes, sterols, alkaloids and free quinones. Bioassays carried out on two species of beetle (*Tribolium castaneum* and *T. confusum*) revealed strong insecticidal activity, with efficacy varying depending on the dose and method of application (ingestion or contact). The aqueous extract of *P. tomentosa* exhibited pronounced insecticidal activity when applied directly, producing mortality rates of 63.33–86.67% through ingestion and 86.67–100% through contact exposure. Mortality generally appeared after 48 h at high concentrations. Evaluation using traditional toxicological parameters (mortality rate, LD₅₀ and LT₅₀) confirmed the efficacy of the extracts, in particular the acetone and methanol fractions, which induced up to 90% mortality after 72 hours. These results confirm the strong biocidal potential of *P. tomentosa* against *Tribolium* imagos.

Keywords: aqueous extracts; insecticidal activity; *Pergularia tomentosa*; phytochemical screening; Sahara.

INTRODUCTION

Ensuring food security for an estimated nine billion people by 2050 without compromising environmental sustainability represents a major challenge for global agriculture [43].

According to the Food and Agriculture Organization of the United Nations, agricultural production must increase substantially to meet the demands of a continuously expanding global population. However, this challenge is compounded by significant post-harvest losses, with over 30% of agricultural products being compromised during extended storage periods and under varying climatic conditions [10, 20, 38]. These environmental factors create favorable conditions for the proliferation of insect pests, particularly storage beetles, which pose a substantial threat to food security and agricultural sustainability [15, 17, 18].

In Algeria, cereal products, particularly wheat, are indispensable for the food system and national economy. However, the only way to ensure the connection between annual harvest and continuous consumption is through post-harvest preservation [7]. Post-harvest losses are always an indicator of food insecurity. The FAO estimates that the quantity of grain destroyed by insects each year could feed 100 million people [6, 20]. Among these insects is *Tribolium castaneum* (Herbst, 1797) and *Tribolium confusum* (Jacquelin du Val, 1863). These confused beetles (Insecta: Coleoptera) are considered a strict secondary pest that significantly damages stocks of various starchy commodities, particularly cereal flours [15, 18, 19, 27, 37, 44].

T. castaneum causes considerable economic damage [27, 47]. From the age of three days, adult females lay around ten eggs per day, up to a total of 500 to 800 eggs during their lifetime. At 30°C, hatching generally occurs after five days [15]. Although *T. castaneum* and *T.*

confusum are very similar in terms of morphology and ecology [31], they differ in certain morphological and histological characteristics, as well as in the chemical composition of their wings [49]. As for *Tribolium confusum*, the adults, which are dark reddish-brown in color, measure approximately 3.4 mm in length, are oval in shape and have a flattened body. The larvae, which are worm-like and whitish to yellowish in color, go through 7 to 8 larval stages before attaining maturity in about 22 days at 30°C. The nymphal phase lasts approximately 10 days under the same conditions [14].

The principle means of controlling these insects is the use of chemicals pesticides. Actually, they represented the most practical techniques to protect the stored products against these insects, since they are effective and facilitators in their application. Though, their global influences on the ecosystems are unknown [7, 16, 28, 36].

The intensive use of synthetic pesticides has disrupted ecosystem functioning and affected ecological equilibrium, prompting growing interest in botanical alternatives rich in bioactive secondary metabolites, plants offer sustainable, biodegradable, and locally available resources for the development of natural biopesticides. From early uses such as nicotine in the 17th century to modern experimental advances, research has increasingly highlighted their potential [26, 29, 46, 38].

Today, the scientific community regards plant-derived pesticides as a promising and environmentally friendly alternative to synthetic chemicals. To highlight the insecticidal potential of certain endogenous plants from the Algerian Sahara, Although many species are already known to have these characteristics, further studies are still needed to better understand their potential. This current study seeks to advance and expand upon existing research regarding Saharan flora with insecticidal properties [13, 17, 25, 26, 29, 34, 43].

The family Asclepiadaceae comprises 200 genera and 2500 species of perennial shrubs and herbs distributed throughout the tropics and temperate areas of the world, including the Sahara region such as in Saudi Arabia, Egypt and very common in the Algerian desert. *Pergularia tomentosa* is a member of the Asclepiadaceae family [1, 4, 5, 21, 22, 39]. can reach one meter, and the perennial shrub about 50-60 cm. The leaves are opposite, simple, petiole of 0.5 to 1.5 cm long. The fruits are oblong, globulous follicles. The seeds are dark brown in color, ovate of 7 to 9 mm [4, 5, 8, 41]. A white sticky fluid from the leaves and fruits are secreted when break of the plant. It is characterized by a cross-latex that has a inflammatory effect on the skin [30, 32, 39].

This part of the study highlights the insecticidal potential of Saharan plants against *Tribolium* beetles, a major pest of stored foodstuffs. The innovative nature of this work lies in the fact that no previous research has focused on the use of these plant species in this context, either nationally or internationally. The originality of this scientific contribution is evident on several levels. On one side, the plant studied is endemic to the Ghardaïa region, which gives the research a unique and localised dimension. On the other hand, the processes applied-both for the extraction of bioactive compounds and for the experimental treatment methods-are different from the conventional approaches reported in the literature.

MATERIALS AND METHODS

Insect test. In this study, two species of beetles of the genus *Tribolium*; *T. castaneum* and *T. confusum*, were used to evaluate the insecticidal activity of aqueous extracts of *P. tomentosa*. Among them, *T. castaneum* is the most commonly found beetle in food stocks [9, 31].

In order to obtain a homogeneous and abundant population, intensive breeding was implemented at the research laboratory. The adult individuals were initially sourced from a domestic semolina stock. Breeding was carried out in glass jars (18 cm high, 11 cm in diameter) containing 500 g of commercial semolina. The bottles were kept in a dark room at 32 ± 2 °C and 70% relative humidity [26]. After one month of incubation, a sufficient number of individuals were obtained for the experiments.

Plant material. *Pergularia tomentosa* L. (Asclepiadaceae) usually is called Kalga in traditional medicine for many purposes [17].

The aerial parts of *Pergularia tomentosa* were harvested during the flowering period. The harvesting area is located upstream the Metlili Valley, in the province of Ghardaïa located at 32°11'49" N, 03°46'54" E (Ghardaïa region, Algeria). These leaves are used to prepare extracts.

Extraction of secondary metabolites. The aerial parts of *P. tomentosa* were collected and properly rinsed with the water to remove dust and any other matter stick to the leaves. They are separated and put it on pack, in a vacuum room. When the leaves are completely dried,

they are crushed by using an electric grinder to obtain a thin and homogeneous powder. The matter is passed a sieve through a mesh smaller than 0.5 mm than is stored in jars and they kept away from light and humidity. Each box has the name of the species, the part used, the date and the place of harvest. The powder leaves is the final material used for the preparation of the aqueous extracts [17, 25, 26, 29].

Two different protocols were used to extract the secondary metabolites from the plant studied.

The first protocol, reflux extraction is used, consisted of introducing 100 g of leaves powder into a 1000 mL flask containing a solvent mixture composed of 2/3 methanol and 1/3 distilled water. The flask was equipped with a condenser to condense the volatile fractions during extraction. The mixture was heated to 50°C using a heating flask for 6 hours, while ensuring homogeneous stirring. After cooling, the mixture was filtered using Whatman filter paper. The filtrate separated from the solvent (methanol) was evaporated using a HeiVAP (Heidolph) rotary evaporator, equipped with a water bath overheating protection system, set at a temperature of 50 °C and a rotation speed of 100 rotations/min. The final extract, in aqueous form, was recovered for further analysis [17, 29].

The second protocol is commonly adapted [43], with some modifications. It involved cold maceration extraction 50 g of plant powder was macerated in 250 mL of acetone at room temperature for 24 hours under continuous shaking using a (Precise Shaking Incubator WIS-10, Korea). After maceration, the mixture was filtered three times using Whatman filter paper. The combined organic filtrates were evaporated to dryness at 45°C using a rotary evaporator to obtain a dry extract. This extract was then weighed, labeled, and stored in amber bottles at 4°C.

Phytochemical screening. The phytochemical screening of the aqueous extracts of this plant studied is carried out by the standard characterization methods described by Harbone (1977, 1998) [23, 24]. These detection tests focused on the chemical compound: terpenes sterols, saponosides, flavonoids, tannins and phenolic compounds and alkaloids.

Biological tests. Two treatment modes were adopted in this study: ingestion and contact.

The ingestion protocol consists of administering 5g of semolina to ten adult *T. confusum* beetles, placed individually in sterile Petri dishes. The insects were treated by spraying them with 4 mL of methanolic aqueous extract, applied at different concentrations which are 100%, 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20% and 10%. At the same time, two controls were set up: a positive control, consisting of a reference insecticide from the synthetic pyrethroid family (Decis® EC50, trade name: HILAC), and a negative control, consisting of distilled water. Each concentration was tested in three independent replicates. Mortality was registried every 24 hours over a period of ten days, with dead individuals being counted and preserved. The

Kinetic evaluation of the mortality was carried out in regular periods of 10 days [17].

For the contact test, the acetone extract of *P. tomentosa* was applied at four different doses: 50 mg/mL, 30 mg/mL, 10 mg/mL and 5 mg/mL. Each dose was sprayed directly on 10 adult of *T. castaneum* beetles placed in a petri dish lined with filter paper. The negative control was treated with a mixture of DMSO and distilled water (v/v). For this test, the sprayed volume was determined after calculating the surface coverage of the filter paper. Three sprays were applied to ensure uniform coverage of the entire surface. The volume corresponding to a single spray was measured and then multiplied by three, resulting in a total volume of 1.75 mL. Each dish received 1.75 mL of either the tested product or the control solution. The positive control was treated with an insecticide formulation of DécisExpert® EC100, a synthetic pyrethroid based on deltamethrin (100 g/L), used at its approved application dose (1.75 mL). The entire experiment was organized into six batches, each containing 30 individuals divided into three Petri dishes. The rearing conditions were kept identical to those described above to ensure the homogeneity of the tests. Insect mortality was recorded over a period of 2 hours with an observation period of 72 hours [25, 26].

Mortality rate. Mortality is the first critic for judging the effectiveness of chemical or biological treatment. The percentage of mortality observed in control and treated adults of the beetles of *Tribolium* imagos is estimated by applying the following formula [29, 33, 38]:

$$\text{Observed Mortality} = [N_D / N_t] \times 100$$

where, N_D : Number of deaths; N_t : Number total of beetles;

Statistical analysis. The experimental results were interpreted and analysed statistically using ANOVA via MINITAB software (version 15). This method compares inter- and intra-group variances in order to determine the significance of the differences observed.

RESULTS

Phyto-chemical screening. The phytochemical characterization tests of aqueous extracts found in *P. tomentosa* allow the presence or the absence of bioactive molecules.

Table 1. Phyto-chemical screening of aqueous extracts of *Pergularia tomentosa*.

Chemical groups	Aqueous extracts	
	Acetonic	Méthanolic
Total alkaloids	++	+++
Flavonoids	+++	++
Saponosids	-	++
Tannin Catechic	+++	+
Tanin Gallic	-	-
Free quinons	++++	++++
Terpénoides	+	+++
Sterols	+++	++
Stéroïdes	+++	++

(-): absence, (+): weak presence, (++) : medium presence, (+++) : strong presence

From the results including on the table 1, it remarked that the acetone and methanol aqueous extracts of *P. tomentosa* have a rich and diverse phytochemical profile, revealing extraction efficiency that varies depending on the solvent used. Both extracts show a high free quinone content. The acetone extract is distinguished by its abundance of flavonoids and sterols, steroids, while the methanol extract is characterised by a richness in total alkaloids and terpenoids. The absence of gallic tannins and the low content of catechinic tannins, which was particularly noticeable in the methanolic extract, reflect a specificity in the extractable compounds. Furthermore, the exclusive presence of saponosides in the methanolic extract highlights the importance of solvent choice in targeting certain groups of metabolites. In general, this screening highlights the pharmacological potential of the plant, with the methanolic extract appearing to be the most complete due to the diversity of secondary metabolites.

Effect of aqueous extracts on the mortality of *Tribolium* beetles. The evolution of the cumulative mortalities percentages of different doses of extracts of *P. tomentosa* is presented in Figure 1. For the adults of confused beetle (*T. confusum*) treated with aqueous-methanolic extract of *P. tomentosa*, the mortality rate is a few to those reported on the adults treated with methanolic extract. They are to $86.67 \pm 0.58\%$, $63.33 \pm 0.58\%$, $36.67 \pm 0.58\%$, $33.33 \pm 0.58\%$, $16.67 \pm 0.58\%$, $13.33 \pm 0.58\%$, $13.33 \pm 0.58\%$, $10.0 \pm 0.00\%$, $6.67 \pm 0.58\%$ and $3.33 \pm 0.58\%$ for 100%, 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20% and 10% respectively. Since, the mortality the adults of *T. confusum* started after the 4th day (96 hours) of treatment for the most and higher doses testes, with the exception of the lower doses (30%, 20% and 10%) where the mortality begins from of the 8th day of exposure.

Analysis of the results showed that the mortality rates observed in insects treated with the hydro-methanolic extract were statistically similar to those registered in the positive control group, which suggests that the extract produced a comparable effect. However, a significant difference was observed when these mortality rates were compared with those of adults in the negative control group, indicating that the treatment had a clear effect compared with treated insects.

Compared to the negative control values, the adults of *T. confusum* treated with the aqueous extract of aqueous-methanolic extract at doses 100%, 90%, 80% and 70%, is a significant difference was maintained ($P < 0.001$). In this period, none of these adults died exposed by the negative control (distilled water). Unlike, for confused beetle treated with positive control (insecticidal product), the mortality rate is 100% after 48 hours.

Indeed, from this results is suggested that the both of these extracts show the possibilities of use as a bio-insecticidal treatment. The extracts applied to high doses (100%, 90% and 80%) gave a significant mortality rates, can be compared to those noted by the insecticide

synthetic treatment used as a positive control. However, some adults exposed to extracts at the lowest concentration (10%), the mating times, egg-laying had seen. It may be reflects the weak or limited effect of extracts at these doses on the adults of *Tribolium confusum*.

For the acetone extract of *P. tomentosa* L., the different doses (50 mg/mL, 30 mg/mL, 10 mg/mL and 5 mg/mL) had varied effects on the mortality rates of *T. castaneum*. After 2 hours, mortality rates were very low

for all doses except for the positive control. However, after 14 hours, a significant increase in mortality rates was observed for all doses. After 72 hours, mortality rates reached 96.67% for the 50 mg/mL dose and gradually decreased for the other doses, including 30 mg/mL, 10 mg/mL and 5 mg/mL, with mortality rates of 86.67%, 76.66% and 60% respectively. These results showed a clear dose-dependent effect: increasing the concentration of the acetone extract of *P. tomentosa* L. caused more rapid and more intense toxicity.

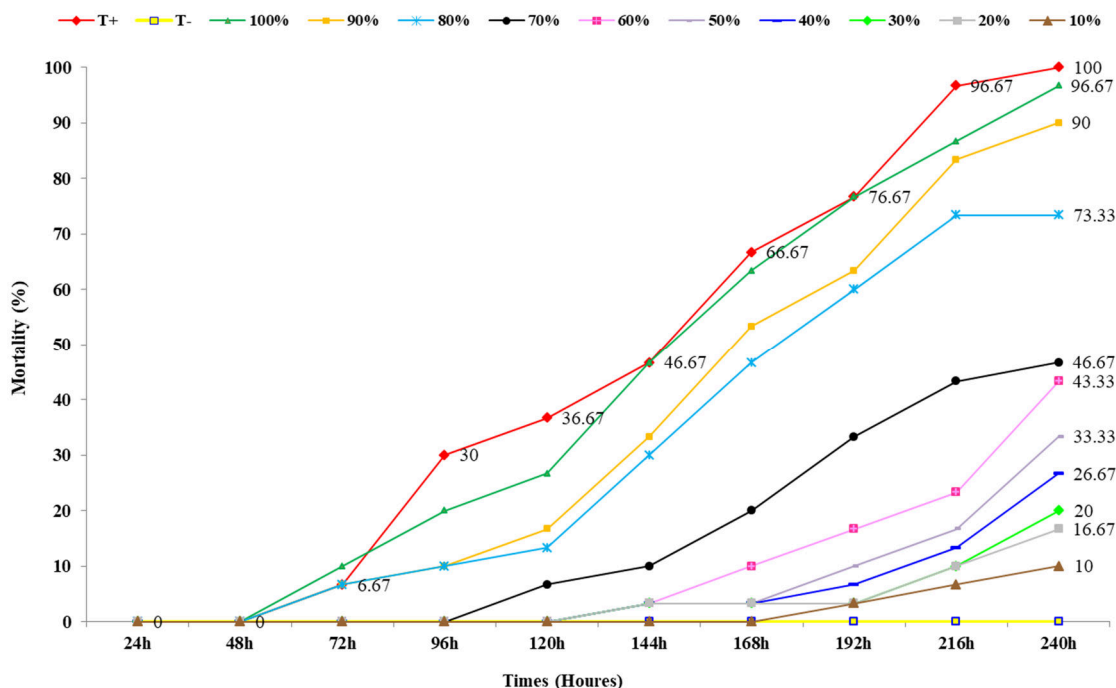


Figure 1. Variation over time of mortality rate observed in different controls and treated groups with methanolic aqueous extract of *Pergularia tomentosa*. (C100 = [0.0315 mg/mL]; C90% = [0.0284 mg/mL]; C80% = [0.0252 mg/mL]; C70% = [0.022 mg/mL]; C60% = [0.0189 mg/mL]; C50% = [0.0157 mg/mL]; C40% = [0.0126 mg/mL]; C30% = [0.00945 mg/mL]; C20% = [0.0063 mg/mL]; C10% = [0.00315 mg/mL]; T(+) = HILAC[®]EC50; T(-) = Distilled Water).

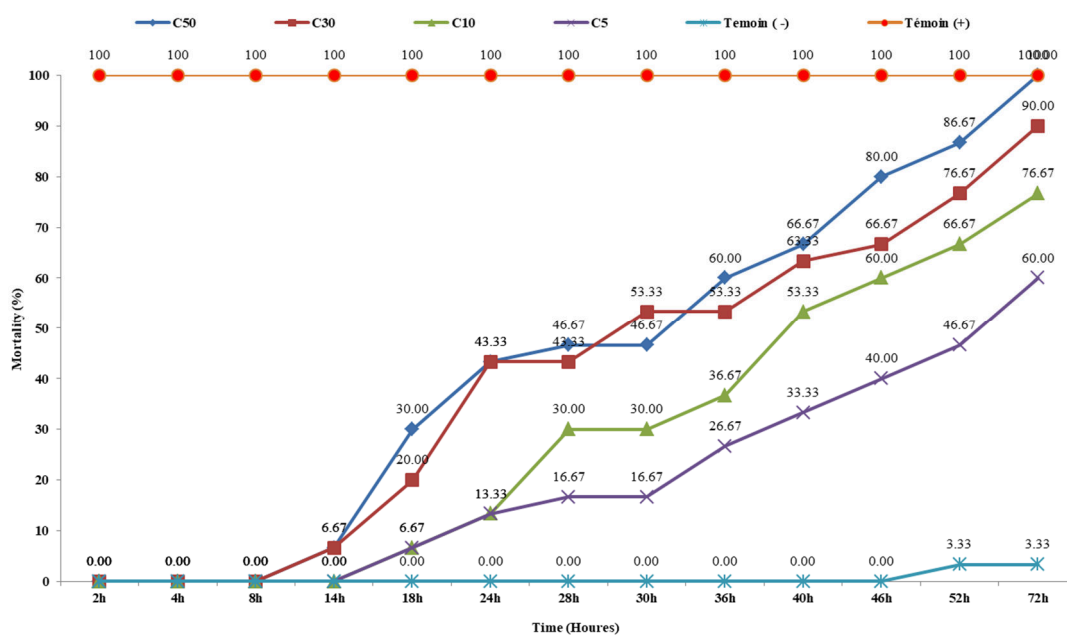


Figure 2. Variation over time of mortality rate observed in different controls and treated groups with acetonic aqueous extract of *Pergularia tomentosa*. (C50=[1 mL/mL]; C40=[0.67 mL/mL]; C30=[0.43 mL/mL]; C20=[0.25 mL/mL]; C10=[0.11 mL/mL]; C5=[0.05 mL/mL]; T(+) = Decis[®]EC50; T(-) = Water +DMSO (v/v).



Figure 3. Imagos of *Tribolium* beetles before treatment (living insects)



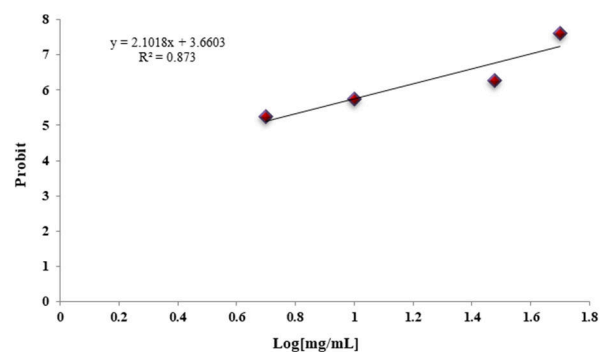
Figure 4. Imagos of *Tribolium* beetles after treatment with aqueous extract of *Pergularia tomentosa* (dead insects)

Direct spraying of the extracts selected onto *Tribolium* beetle imagoes caused a number of symptoms characteristic of acute poisoning. A gradual decrease in locomotory activity was observed, followed by convulsive movements and an inability to remain on the walls of the experimental devices, indicating an alteration of the neuromuscular system. In the most severe cases, exposure caused the death of the insects, usually preceded by a gradual darkening of the cuticle a few hours after treatment. This change in coloration could result from denaturation of cuticular proteins or internal oxidative processes associated with tissue degradation. The mechanisms responsible involve disruption of synaptic transmission, inhibition of cellular respiration and alteration of cuticle integrity, leading to convulsions, energy failure and fatal dehydration.

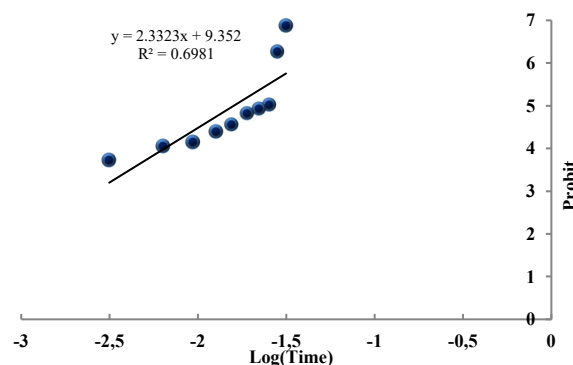
Biocidal efficacy (LD) of the aqueous extract on *Tribolium*. The Lethal doses LD_{50} and LD_{90} for both the methanolic and acetone extracts of *P. tomentosa* were estimated using regression equations (Fig. 1 A, B). The results are grouped in the Table 2, showed the values of lethal doses, which are defined by the high ingestion and contact toxicity of these aqueous extracts against the adults of *Tribolium* beetles. The methanolic extract has lethal doses (50, 90) of 0.14 mg/mL and 0.048 mg/mL respectively. For the acetone extract, LD_{50} and LD_{90} are reported into 4.340 to 17.67 mg/mL depending on the exposure time, highlighting the importance of considering exposure duration when evaluating the efficacy of natural insecticides.

Table 2. Estimated lethal doses of *Pergularia tomentosa* (LD_{50} & LD_{90})

Aqueous extract	Regression equation	Regression coefficient	LD_{50} (mg/mL)	LD_{90} (mg/mL)
Acetonic	$y = 2.1018x + 3.6603$	$R^2 = 0.873$	4.340	17.67
Methanolic	$y = 2.3323x + 9.6075$	$R^2 = 0.6981$	0.014	0.048



A. Effect the concentration of acetic aqueous extract of *P. tomentosa* on the mortality of *T. castaneum* imagoes after 70h.



B. Effect of the concentration of methanolic aqueous extract of *P. tomentosa* on the mortality of *T. confusum* imagoes after 10 days of exposure.

Figure 5. Probits regression lines with the logarithms of doses aqueous extracts of *Pergularia tomentosa* on the adults of *Tribolium* beetles

Lethal times of the aqueous extract on *Tribolium* imagoes. The values of lethal times (LT_{50} LT_{90}) are presented in Tables 4 and 5. The lethal time is greater than 23.82 days for the lower doses of 30%, 20% and 10%. The lethal time is 6.94 days, 8.67 days and 10.94 days for adults exposed to 100%, 90% and 80% respectively. For doses of 70%, 60%, 50% and 40%, the lethal time is between 14.04 and 59.15 days. For doses of 30%, 20% and 10%, the lethal time is greater than 213.81 days. These results, explained by the highest doses (100%, 90% and 80%), appear to be a notable toxicity and activity; these results remain comparable to those (LT_{90}) where the LT_{90} is 10.32 days, 13.69 days and 18.29 days for adults treated with 100%, 90% and 80% of *P. tomentosa* respectively. For the lowest doses, the LT_{90} was exceeded by 23.9 days.

DISCUSSION

Phyto-chemical screening. Phytochemical screening results revealed that the aqueous, acetonic, and methanolic extracts are rich in secondary metabolites, including alkaloids, flavonoids, terpenes, sterols and tannins. Previous studies have consistently reported the presence of alkaloids in extracts from this plant.

The phytochemical analysis of *P. tomentosa* leaves conducted by Cherif (2020) [17], revealed the abundant presence of secondary metabolites, including flavonoids, saponins, glycosides, terpenes, sterols and total alkaloids. Similarly, Boulouvar (2011) and Tlili *et*

Table 3. Estimated lethal times of *Pergularia tomentosa* methanolic extract (LT_{50%} and LT_{90%})

(%)	Equation	Coefficient	LT ₅₀ (hour)	LT _{50%} (day)	LT ₉₀ (hour)	LT ₉₀ (day)
100 %	y = 7.435x - 11.52	R ² = 0.849	166.69	6.94	247.78	10.32
90 %	y = 6.454x - 9.963	R ² = 0.839	208.16	8.67	328.65	13.69
80%	y = 5.734x - 8.872	R ² = 0.845	262.57	10.94	439.01	18.29
70 %	y = 5.536x - 8.993	R ² = 0.767	337.00	14.04	573.91	23.91
60 %	y = 4.699x - 7.809	R ² = 0.645	531.98	22.16	996.08	41.50
50%	y = 4.276x - 7.285	R ² = 0.543	746.46	31.10	1487.15	61.96
40 %	y = 3.521x - 6.099	R ² = 0.417	1419.80	59.15	3279.15	136.63
30%	y = 2.554x - 4.476	R ² = 0.283	5131.66	213.81	16271.70	677.98
20%	y = 1.327x - 2.351	R ² = 0.144	346388.06	14432.83	3192601.31	133025.05
10%	y = 1.176x - 2.084	R ² = 0.144	1056354.10	44014.75	12949258.4	539552.43

Table 4. Estimated lethal times of *Pergularia tomentosa* acetonic extract (LT_{50%} and LT_{90%})

Doses (%)	Équations	Coefficient R ²	LT ₅₀ (Hour)	LT ₉₀ (Hour)
50%	Y=4.471x+1.373	R ² =0.979	26.63	51.55
30%	Y=3.994x-1.008	R ² =0.991	31.93	66.85
10%	Y=3.733x-1.018	R ² =0.992	40.94	90.24
5%	Y=3.378x-0.822	R ² =0.989	52.91	126.72

al. (2015) [12, 48] also identified bioactive compounds such as flavonoids, tannins, coumarins, and alkaloids in the foliage of *P. tomentosa* (Asclepiadaceae).

Whereas these last two studies, reported the presence of flavonoids, tannins, coumarins and alkaloids in the leaf part of *P. tomentosa* (Asclepiadaceae).

Effect of aqueous extracts on the mortality of *Tribolium* beetles. The insecticidal effect of aqueous extracts of *P. tomentosa* on the mortality of insects is depending on the dose tested. Indeed, these results can be added the possibility the used as the bio-insecticidal product where the higher doses induce a notable mortality rate. In contrast, the adults exposed to the lowest concentrations, the mortality rates are few, that may be illustrated to the presence rigid structural of confused beetles has prevented the effect of extracts aqueous. Some the adults of *Tribolium* beetles are seen on the movement (locomotion activity) were exposed to the lowest doses. It was explained that the treatment applied has less effect by contact. Previous works [34], found that latex of *Pergularia tomentosa* has a significant mortality rate $96.49 \pm 6.07\%$ against *Locusta migratoria* (Orthoptera-Acrididae). Many studies on latex of *P. tomentosa* (Asclepiadaceae), have described that latex is mostly rich in terpenoids, alkaloids and cardenolides, as well as various proteins and enzymes, such as proteases, chitinases and glucosidases [3, 22, 30].

Our study demonstrates that aqueous extracts of *P. tomentosa* exhibit insecticidal properties, even at low concentrations. Notably, insects exposed to these plant extracts displayed signs of intoxication, particularly movement disorders, indicating a neurotoxic effect of the aqueous extract. These findings suggest that *P. tomentosa* represents a promising source of bioactive compounds that could be developed into novel insecticidal products, with particular consideration for their potential applications in natural ecosystem management.

Biocidal efficacy (LD) of the aqueous extract on *Tribolium*. The lethal doses revealed that *P. tomentosa* methanolic extract is more toxic than extract against

T.confusum. That which is evidenced by the studies on the insecticidal activity of *Melia azedarach* (Meliaceae) on adults *T. castaneum* is due to the presence of Triterpenoids that have an anti-nutritional effect and inhibit [13]. Also, they cause death and malformations in offspring [16]. Tannins have insecticidal properties, larvicides and repellents; they influence the growth, development and fertility of several phytophagous insects [2]. After treatment, at the end of the second day, these insects saw collectively on the group in Petri dishes and the marginalized formations were observed on the confused beetles, such as reduced locomotion activity and the mating times in the lowest doses. These manifestations are perhaps suggested the presence of toxic substances in the studied extracts. In this context at the under-conditions with temperature 29.7 °C and 73% relative humidity can be more influenced by ecological changes, in relation to its duration, than any of the other instar of development of the confused beetles insects [14]. Moreover, the mortality rate is 97.5% in *Acanthoscelides obtectus* Say (Coleoptera-Bruchidae) at the end of the 4th day after treatment with the high dose 0.40 µL/g of leaves of *Callistemon viminalis* (Myrtaceae) [36].

Lethal times of the aqueous extract on *Tribolium* imagos. Several research studies focus on finding alternative methods to traditional insecticides by using products of biological origin. These studies aim to estimate the lethal doses and times 50% (LT₅₀) that would allow determining the toxicity of natural active molecules as well as pest tolerance to these substances. *T. castaneum* showed sensitivity to thyme essential oils, with estimated LT₅₀ values ranging from 32.80 to 69.37 hours for the essential oil of *Thymus lepto botrys* (Lamiaceae), native to southwestern Morocco [35].

Furthermore the application of chemical insecticides on *Ceratitis capitata* (Tephritidae) by contact is effective, with LT₅₀ varying according to the nature and dose of the active substances. Chlorpyrifos (96 g/100 L of water) appears to be the most effective, with a duration of less than 5 minutes, compared to other active ingredients tested, including Deltamethrin [41].

According to Herouini (2021), *Pergularia tomentosa* seed oil exhibits intermediate toxicity compared to the two other oils tested (*Citrullus colocynthis* L and *Datura stramonium* L). Less toxic than *C. colocynthis* oil, it nevertheless remains significantly more active than *D. stramonium* oil. At high concentrations (80% and 70%), it causes rapid mortality with relatively short LT_{50} values (4.53 and 6.64 days), indicating notable efficacy. Conversely, at lower concentrations ($\leq 60\%$), the LT_{50} values extend to approximately 16 days, indicating a significant reduction in its insecticidal potency. The LT_{90} analysis corroborates these results, showing that *P. tomentosa* oil maintains good efficacy only at high doses, while its activity decreases markedly at low concentrations, although it remains superior to that of *D. stramonium* [25].

The results of the present study are consistent with previous findings on the insecticidal activity of essential oils. These essential oils act through multiple mechanisms, including neurotoxic effects, enzyme inhibition, disruption of insect development, and cuticular alteration. Moreover, their efficacy is strongly influenced by formulation and application technology. These observations support and confirm the results obtained in the present study [40].

This study demonstrates that *P. tomentosa*, a Saharan species, is a potentially promising source of natural biopesticides, exhibiting dose-dependent insecticidal activity and an effective spectrum of action against beetle pests of stored foodstuffs. The richness of this plant in bioactive secondary metabolites, correlated with the toxicological performances observed, suggests a high potential for application in integrated biological control. It should also be noted that insects exposed to aqueous extracts of *P. tomentosa* show signs of intoxication, including movement disorders, indicating a neurotoxic effect of these extracts. Therefore, *P. tomentosa* can be considered a valuable source of bioactive molecules. In addition, its status as spontaneous species of the northern Sahara gives this study an original botanical dimension, highlighting the value of exploring the local Saharan flora for the development of new insecticides that respect natural ecosystems. The novelty of this research is further highlighted by the originality of the extraction methods and experimental treatments, which differ from conventional approaches reported in the literature. Furthermore, the absence of comparable studies at both national and international level underscores the innovative nature and scientific importance of this research.

Acknowledgements. The authors gratefully acknowledge that this study is part of the ongoing research conducted by the first and second authors on endemic Saharan plants, representing their dedicated long-term commitment to understanding this unique botanical heritage.

Conflict of interest. There is no actual or potential conflict of interest in relation to this article.

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Received: September 12, 2025

Accepted: July 1, 2026

Published Online: July 7, 2026

Analele Universității din Oradea, Fascicula Biologie

Print-ISSN: 1224-5119

e-ISSN: 1844-7589

CD-ISSN: 1842-6433

Oradea University Press

<https://www.bioresearch.ro/revistaen.html>

<https://doi.org/10.61215/auofb.2026.2.02>

