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Chemical Profile and Insecticidal Activity of Ruta Graveolens Leaf Extracts against Bruchus Rufimanus (Cleopetera: Bruchidea)

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ABSTRACT: Plant-derived insecticides are being investigated as potential alternatives to conventional synthetic insecticides for agricultural pest management. This study aimed to evaluate the chemical composition and contact insecticidal activity of *Ruta graveolens* leaf extracts against adult *Bruchus rufimanus*.

Leaves collected in the Zelfana region of Ghardaïa Province, Algeria, were extracted by maceration using ethyl acetate and ethanol. Preliminary phytochemical screening was performed, and the chemical profile of the ethyl acetate extract was analysed by high-performance liquid chromatography (HPLC). Contact bioassays were conducted to assess insect mortality at different concentrations and exposure durations, while molecular docking was used to investigate the predicted interactions of selected compounds with insect-related enzymes.

Phytochemical screening revealed the presence of flavonoids, tannins, steroids, and alkaloids. Insecticidal activity varied with concentration and exposure duration, with the ethyl acetate extract exhibiting pronounced toxicity against adult *B. rufimanus* (LC_{50} = 7.65 mg/mL after 72 h). HPLC analysis enabled the tentative identification of chlorogenic acid, tannic acid, quercetin, adonitol, gallic acid, coumaric acid, caffeic acid, and ascorbic acid. Molecular docking indicated favourable predicted interactions of chlorogenic acid, quercetin, and tannic acid with α-amylase, procathepsin L2, and acetylcholinesterase.

These findings suggest that *R. graveolens* leaf extracts, particularly the ethyl acetate extract, warrant further investigation as potential botanical insecticides for integrated pest management.

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1. INTRODUCTION

Globally, faba bean (*Vicia faba* L.) is one of the major cultivated legume crops. Its high protein content makes it an important source of nutrition for both humans, through its seeds, and livestock, through its seeds and pods. However, increased faba bean production may also favour the development of associated insect pests [1].

Climate change can influence pest dispersal and insect population growth rates. It may alter overwintering patterns, favour the emergence of additional generations, and affect the activity of both insect pests and their natural enemies. Several studies have demonstrated that temperature plays an important role in the initiation and termination of diapause. When specific environmental thresholds, particularly temperature thresholds, are reached, obligatory diapause may terminate. Climate change has also affected species distribution and dispersal patterns, with many species migrating earlier in recent years than in the past [2].

In many regions worldwide, including Europe, the broad bean weevil, *Bruchus rufimanus* Boheman, 1833 (Coleoptera: Chrysomelidae), represents a major biotic threat to faba bean (*Vicia faba* L.) seed quality, with considerable economic implications [3].

Adult weevils are highly mobile and can fly several kilometres, facilitating the spread of infestations across regions. In addition, the ability of larvae to survive for extended periods inside seeds increases the risk of pest dissemination when infested seed lots are sown. The species can also spread nationally and internationally through the commercial trade of seeds [1, 4].

Bruchus rufimanus has a univoltine life cycle [5], and adults emerge when temperatures reach approximately 15°C [2]. At emergence, adults are sexually immature and feed on pollen from various flowering plant species [1]. They subsequently colonise faba bean fields during the vegetative and flowering stages of the crop [2, 5].

Consumption of faba bean pollen promotes reproductive organ development, allowing adults to reach sexual maturity and initiate mating behaviour. During pod formation, females lay their eggs on the pod surfaces. Oviposition continues until no green pods remain or until the female dies. The larvae then bore through the pod wall to reach the developing seeds [5].

Larval development takes place entirely within a single seed [5]. During this stage, larvae feed on the internal seed structures, particularly the endosperm and embryo [6]. This feeding activity reduces seed germination and can cause yield losses of up to 70% compared with healthy seeds [7, 1]. Upon completing development, the adult emerges from the seed, leaving a characteristic round exit hole.

These exit holes significantly affect the visual, organoleptic, and nutritional quality of the seeds and reduce their storability. Such damage can have substantial economic consequences because infested seed lots may be rejected from human and animal food markets[1, 3, 5, 6].

Because the larvae of *B. rufimanus* develop inside the seeds, they are difficult to control using insecticides. Control strategies therefore primarily target adults. To prevent oviposition, insecticide treatments must be applied during full bloom and early pod formation [2].

Various management strategies have been investigated for controlling this pest, including adjustments to sowing dates, plant density, and cultivar selection. These factors have been shown to significantly influence the level of damage caused by *B. rufimanus* [2].

Other approaches include semiochemical-based strategies, such as pheromone traps [5, 8]; biopesticides, including entomopathogenic fungi [3]; plant-derived insecticides, such as essential oils [9]; and host-plant resistance through the selection of partially or fully resistant faba bean varieties [10, 11].

Despite these advances, the management of *B. rufimanus* in most European countries continues to rely heavily on chemical insecticides, particularly pyrethroids [3, 5].

However, the intensive use of chemical insecticides has been associated with environmental pollution, increased operational costs, and the development of insecticide resistance. These challenges are further compounded by the reported tolerance of the insect to certain active ingredients. Consequently, increasing application rates to overcome resistance may not restore

treatment efficacy and can further increase environmental contamination, economic costs, and potential risks to human health [12].

Furthermore, chemical control may adversely affect beneficial non-target insects through exposure to insecticides applied to flowering crops [3].

The development of new insecticides with reduced environmental and health impacts is therefore an important research priority. Owing to their diverse biological activities, plant-derived phytochemicals represent potential alternatives to conventional insecticides and may contribute to more sustainable pest management strategies, provided that their efficacy against target pests is maintained [12].

Botanical extracts and plant-derived essential oils are increasingly being investigated as potential alternatives to conventional pesticides because of their biodegradability, potentially lower mammalian toxicity, and diverse modes of action against insect pests. These products often contain complex mixtures of bioactive phytochemicals, including phenolic compounds, terpenoids, and aldehydes, which may exert toxic, repellent, antifeedant, and growth-inhibitory effects on insects [13, 14].

Several plant species, including *Ruta graveolens* (Rutaceae), have demonstrated insecticidal properties. Extracts from the aerial parts of these plants have shown activity against fleas, sugar beet cyst nematodes, and other agriculturally important pests [15]. *Ruta graveolens* is also traditionally used as a food flavouring agent and as a medicinal plant for treating headaches, overeating, and renal insufficiency [16]. However, the contact insecticidal activity of *R. graveolens* leaf extracts, as well as that of their isolated active compounds, has not been investigated against adult *Bruchus rufimanus*.

Consequently, this study aimed to characterize the chemical profile of *Ruta graveolens* leaf extracts collected in the Zelfana region of Ghardaïa Province, Algeria, and to evaluate their contact insecticidal activity against adult *Bruchus rufimanus* under controlled laboratory conditions. Mortality was assessed at different extract concentrations and exposure durations, and lethal concentrations (LC_{50}) were estimated. In addition, molecular docking using AutoDock Vina was performed to investigate the predicted interactions of selected phytochemicals with insect-related molecular targets. This approach provides preliminary computational insights into possible ligand–target interactions that may warrant further experimental investigation.

2. MATERIALS AND METHODS

2.1 Sample collection

Freshly harvested leaves of *Ruta graveolens* (locally known as "Fidjel") were collected in February in the Zelfana area (latitude: 32° 23' N, longitude: 4° 13' E, altitude: 355 m).This area is located 65 km northeast of Ghardaïa, a city in southern Algeria. The taxonomic confirmation of the collected material was carried out by Prof. Bensemaoune Youcef (University of Ghardaïa, Algeria).The plant material was air-dried in the shade at room temperature for 15 days, then ground and stored in paper bags until use.

2.2 Preparation of extracts

We macerated 50 g of leaf powder in 250 mL of one of two solvents—ethanol or ethyl acetate—for 24 hours at room temperature, protected from light, and under constant agitation. Following maceration, the mixture was filtered, and this filtration process was repeated twice. Once filtration was complete, each solution was evaporated to dryness using a rotary evaporator (Heidolph). Finally, the crude extracts were stored at +4 °C in hermetically sealed, labeled vials wrapped in aluminum foil to prevent any degradation of the product prior to subsequent use.

2.3 Insect rearing

Immature stages of *Bruchus rufimanus* were collected in February from an infested domestic stock of fava beans. To obtain a homogeneous and abundant population, a laboratory rearing program was established at the Department of Agronomic Sciences—part of the Faculty of Nature, Life, and Earth Sciences at the University of Ghardaïa (Algeria)—under controlled environmental conditions (standardized diets and hosts, with daily monitoring). The rearing environment was maintained with a photoperiod of 12 hours of light followed by 12 hours of darkness, at a temperature of 27 ± 2 °C and a relative humidity of 65 ± 5%. The fava beans served as both the rearing medium and the primary food source. After one month of incubation, a sufficient number of individuals was obtained for the experiments. Adults of uniform age were collected and used for biological assays.

2.4 Phytochemical screening

Phytochemical screening was performed using a qualitative analytical method, employing techniques to identify the presence or absence of secondary metabolites in plant species through color changes or precipitation for each organic substance [17]. The tests focused on chemical compounds such as flavonoids, tannins, terpenes, coumarins, free quinones, alkaloids, reducing compounds, saponins, steroids, and anthocyanins.

2.5 Toxicity test

The contact toxicity of the ethanol and ethyl acetate extracts against adult *Bruchus rufimanus* was evaluated using contact toxicity bioassays. For each extract, three concentrations were selected following several preliminary toxicity tests (9, 12, and 15 mg/mL) were tested, together with a negative control consisting of 5% DMSO (diluted in distilled water). Each treatment, including the negative control, was performed in triplicate. For each replicate, 1 mL of the extract solution or the corresponding control solution was applied to a filter paper placed inside a Petri dish, ensuring uniform coverage of the entire surface. Once the filter papers were treated, 20 adult insects were introduced into each dish. The dishes were then closed with a lid to prevent the insects from escaping. Rearing conditions were kept identical to those described previously, ensuring consistency across the assays. Insect mortality was recorded at predefined intervals, ranging from 2 to 72 hours post-treatment. During each assessment, an insect was considered dead if it showed no spontaneous movement and failed to react to gentle mechanical stimulation of its body or appendages.

The following formula is used to assess the proportion of mortality seen in adult of the beetles of *B. rufimanus* [18].

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

Were, N_D: Number of deaths; N_t: Number total of beetles.

Lethal concentrations (LC_{50} and LC_{90}), corresponding to the concentrations of *R. graveolens* leaf extracts required to cause 50% and 90% mortality, respectively, were estimated using dose-response analysis based on the Probit model. Mortality data were converted into Probit values, and extract concentrations were log-transformed to establish the dose-response relationship and determine the LC_{50} and LC_{90} values.

2.6 HPLC analysis

The bioactive compounds present in the ethyl acetate extract were analysed by high-performance liquid chromatography (HPLC) using a Thermo Scientific Vanquish UHPLC system at the Faculty of Science and Technology, University of Ghardaïa, Algeria. The extract was prepared by dissolving 0.5 mg of the sample in 5 mL of HPLC-grade methanol, followed by filtration before injection. Chromatographic separation was performed using an Ultra-Force C18 column (2.5 × 100 mm, 1.8 µm) maintained at 25 °C. Isocratic elution was carried out using a mobile phase consisting of methanol, acetonitrile, and purified water (40:15:45, v/v/v), at a flow rate of 0.80 mL/min. The injection volume for the reference standards was 20 µL, and UV detection was performed at 254 nm. The values obtained were then compared against data from the laboratory's own reference databases. Furthermore, whenever authentic reference standards were available, the identification of selected compounds was confirmed by co-injection, using retention indices under identical HPLC conditions.

2.7 In silico study and molecular docking

Molecular docking simulations were performed to evaluate the potential interactions between the bioactive compounds of the ethyl acetate extract of *Ruta graveolens* leaves and insect target enzymes related to the insecticidal activity against *Bruchus rufimanus*. Since no experimentally resolved crystal structures are available for *B. rufimanus*, homologous insect enzymes were selected as predictive models. The crystal structures of *Tenebrio molitor* larval α-amylase (PDB ID: 1JAE), digestive procathepsin L2 proteinase (PDB ID: 3QJ3), and native acetylcholinesterase from *Drosophila melanogaster* (PDB ID: 6XYS) were obtained from the RCSB Protein Data Bank (accessed July 24, 2026). These enzymes were selected due to their roles in insect digestion, nutrient assimilation, development, and neurophysiological regulation [19-21].

The major constituents of the ethyl acetate extract of *R. graveolens* leaves were identified by HPLC analysis, and chlorogenic acid, tannic acid, quercetin, adonitol, gallic acid, coumaric acid, caffeic acid, and ascorbic acid were selected for docking

evaluation. Their three-dimensional structures were retrieved from the PubChem and DrugBank databases and converted into PDB format prior to molecular docking analysis [22].

Temephos and deltamethrin were used as reference insecticides. Protein structures were prepared using MGL AutoDock Tools by removing water molecules, adding polar hydrogens, and assigning atomic charges, while ligands were optimized before docking. Molecular docking was performed using Auto Dock Vina to predict binding affinities within the active sites of the selected enzymes [23]. Docking poses were visualized using BIOVIA Discovery Studio Visualizer v21, and interactions were analyzed. The docking protocol was validated by redocking native co-crystallized ligands using identical parameters, and RMSD analysis was used to confirm the reliability of the docking procedure. Based on binding affinities compared with the reference insecticides (temephos and deltamethrin), chlorogenic acid, tannic acid, quercetin, adonitol, and deltamethrin were selected for molecular interaction analysis.

2.8 Data analysis

Statistical analyses were performed using R 4.5.1. Extract type, concentration, and exposure time were considered the main experimental factors. Their main effects and interaction effects were evaluated using a repeated ANOVA test as a factorial statistical model, with statistical significance assessed at $\alpha = 0.05$. Where appropriate, post hoc comparisons were conducted to identify differences between treatment levels, with adjustment for multiple comparisons. Model assumptions and the experimental unit were considered when selecting the statistical procedure. Results were summarized using appropriate descriptive statistics and test statistics, including degrees of freedom, F-values, and p-values.

3. RESULTS AND DISCUSSION

3.1 Extraction yield

Bioactive molecules are extracted from *Ruta graveolens* leaves via cold maceration using two solvents (ethanol and ethyl acetate), yielding two crude extracts (Table 1).

Table 1. Extraction yield and organoleptic characterization of extracts of *R. graveolens*.

Organ	Extracts	Appearance	Color	Odor	Yield (%)
leaves	Ethanol	Pastey	Dark green	Unpleasant	2.16
	ethyl acetate	Pastey	Darker green	Désagréable	1.24

The ethyl acetate extract yielded the lowest result (1.24%).However, the ethanolic extract provided the highest yield (2.16%).This can be attributed to the high solubility of the leaves' bioactive molecules in ethanol, which allows for the extraction of the maximum amount of these compounds of interest. Another study by [24] reported yields of 0.36% and 0.21% for the leaves and flowers of *R. graveolens*, respectively.

3.2 Phytochemical analysis

Phytochemical analysis of *R. graveolens* (Table 2) reveals the presence of alkaloids, flavonoids, tannins, and steroids. Furthermore, the ethyl acetate extract contains coumarins and free quinones, whereas the ethanol extract is poor in these compounds.

Table 2. Phytochemical constituents of extracts of *R. graveolens*.

Compound	Ethanolic extract	Ethyl acetate extract
Tannins	(++)	(+)
Flavonoids	(++)	(++)
Free quinones	(-)	(++)
Coumarins	(-)	(+)
Steroids	(+)	(+)
Terpenoids	(-)	(-)

Reducing compounds	(-)	(-)
Alkaloids	(+)	(+++)
Saponins	(-)	(-)
Anthocyanins	(-)	(-)

- negative; + weakly positive; ++ moderately positive; +++ strongly positive.

According to [25], *Ruta graveolens* is well known for its aromatic and medicinal uses. The active ingredients of the plant are glycosides (such as rutin, a flavonoid), alkaloids (quinolones), furocoumarins (psoralens), essential oil of methyl ketones, alcohols and some other compounds.

Alkaloids have biological and pharmacological applications; these natural phytochemicals protect the plant, possess antiviral properties, and serve as important sources of drugs with diverse medicinal properties, including antitumor, anticancer, antimalarial, antiarrhythmic, and antiviral compounds [26].

Tannins are used in cosmetics and pharmaceuticals for their biological activities, including antioxidant, antibacterial, antifungal, antimalarial, anticancer, antimicrobial, and anti-inflammatory effects, as well as for their dermatological properties. Coumarins, for their part, exhibit antioxidant, bactericidal, and fungistatic properties, along with effective insecticidal activity due to their toxicity.

Flavonoids possess a range of biological properties—including anti-inflammatory, antioxidant, antiviral, antimicrobial, anticancer, cardioprotective, neuroprotective, and antifungal effects—serving biological functions in plants (defense against herbivores, ultraviolet radiation, and pathogens) and exhibiting countless pharmacological activities [26].

3.2 Insecticidal effect

Bioassays were conducted to evaluate the insecticidal activity of extracts of *R. graveolens* against the pest *Bruchus rufimanus*. Adult bruchid mortality was measured at various extract concentrations and exposure durations. The insecticidal response increased progressively with the dose and duration of treatment (Figures 1). The negative control showed no mortality.

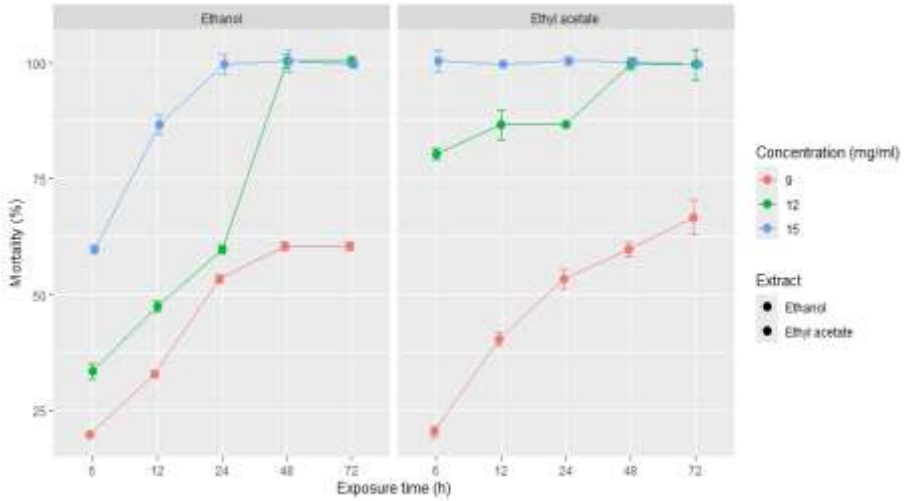

Figure 1. Variation over time of mortality rate observed in different treated groups of *Bruchus rufimanus* with the ethanolic and the ethyl acetate extract of *Ruta graveolens*.

At a concentration of 9 mg/mL, both extracts resulted in mortality rates not exceeding 40% during the first 12 hours of exposure. However, mortality reached approximately 60% after 48 hours of exposure, indicating a moderate insecticidal effect at this dosage.

Furthermore, the mortality rates observed with the 12 mg/mL concentration of the ethyl acetate extract were higher than those obtained with the ethanolic extract. Indeed, mortality of nearly 90% was recorded within 12 to 24 hours of exposure to

the ethyl acetate extract, reaching approximately 100% after 48 hours. In contrast, the ethanolic extract resulted in mortality rates that did not exceed 60% during the first 24 hours of exposure.

However, at a concentration of 15 mg/mL, the ethyl acetate extract caused approximately 100% mortality in adult bruchid beetles after an exposure time of 12 hours. The ethanolic extract resulted in a 100% mortality rate after an exposure time of 24 hours. The results confirm that *R. graveolens* extracts exert a significant insecticidal effect on the adult *B. rufimanus* tested, with marked sensitivity to the ethyl acetate extract compared to the ethanol extract.

The contact insecticidal toxicity of different extracts of *R. graveolens* against adult of *B. rufimanus* has not been studied. However, the total mortality rates observed with various concentrations of *R. graveolens* extracts on adult of *B. rufimanus* were similar to those reported by [27], who demonstrated the efficacy of *Artemisia campestris* essential oil against *B. rufimanus*, achieving a mortality rate of 100%. Similarly, [28] demonstrated a highly significant toxic effect on the survival of *B. rufimanus* in fumigation tests using *Mentha pulegium* L. essential oil; he recorded total mortality of both male and female *B. rufimanus* after 24 hours of exposure to the highest oil dosage.

Furthermore, [29] also demonstrated that the essential oil of *Ruta graveolens* exhibits larvicidal activity against fourth-instar larvae of *Culiseta longiareolata*: at an extract concentration of 25 ppm, the larval mortality rate reaches 100%.

A factorial analysis was used to investigate the effects of extract type, concentration, and exposure time on adult *Bruchus rufimanus* mortality. According to the current ANOVA output, significant effects were detected for extract type ($F = 319.346$, $p = 0.003$), concentration ($F = 6657.233$, $p = 9.02 \times 10^{-8}$), and exposure time ($F= 706.588$, $p = 3.17 \times 10^{-1}$). Significant interactions were also reported for extract type × concentration ($F = 81.295$, $p = 5.77 \times 10^{-4}$), extract type × exposure time ($F = 46.377$, $p = 1.41 \times 10^{-5}$), concentration × exposure time ($F = 42.554$, $p = 2.41 \times 10^{-9}$), and the three-way interaction ($F = 50.483$, $p = 6.62 \times 10^{-1}$). These findings suggest that the mortality response varied according to the combined effects of extract identity, concentration, and exposure duration.

To quantify insecticidal efficacy, mortality rates, adjusted against the logarithms of the applied concentrations, enabled the straightforward determination of effective concentrations, specifically the lethal concentrations (LC_{50} and LC_{90}). The LC value reflects the sample's acute toxicity and short-term toxicity potential, with the mortality rate varying as a function of the logarithms of the applied concentrations. Lower LC values indicate higher insecticidal activity, as a smaller quantity of active compounds is required to induce mortality, thereby facilitating more effective pest control. The LC_{50} and LC_{90} values were calculated and are presented in Table 3.

Table 3. Lethal dose values of extracts of *Ruta graveolens*.

Extract	Exposure time (h)	Regression equation	R^2	LC_{50} (mg/mL)	LC_{90} (mg/mL)
Ethanol	6	y= -0.537+4.855x	0.950	13.82 ± 0.84	25.38 ± 4.64
	12	y= -2.139+6.864x	0.868	10.97 ± 0.94	16.86 ± 2.75
	24	y= -5.758+10.972x	0.741	9.56 ± 1.66	12.51 ± 1.61
	48	y= -4.975+11.032x	0.810	8.02 ± 1.69	10.48 ± 1.21
	72	y= -4.975+11.032x	0.810	8.02 ± 1.69	10.48 ± 1.21
Ethyl acetate	6	y= -10.689+15.479x	0.992	10.32 ± 0.22	12.48 ± 0.24
	12	y= -7.568+12.834x	0.991	9.53 ± 0.27	12.00 ± 0.24
	24	y= -5.819+11.302x	0.971	9.06 ± 0.52	11.77 ± 0.43
	48	y= -4.975+11.032x	0.810	8.02 ± 1.69	10.48 ± 1.21
	72	y= -3.978+10.158x	0.810	7.65 ± 1.77	10.23 ± 1.24

LC_{50}: lethal concentration (mg mL^{-1}) that kills 50% of the exposed *Bruchus rufimanus*; LC_{90} (mg mL^{-1}): lethal concentration that kills 90% of the exposed *Bruchus rufimanus*; R^2: Coefficient of determination; Y: Probits of percentage mortality X: logarithm of concentration.

Table 3 presents the values obtained for the lethal concentrations of *R. graveolens* ethanolic and ethyl acetate extracts against *B. rufimanus* individuals. LC_{50} values for the ethyl acetate extract ranged from 10.32 to 7.65 mg/mL. It exhibited higher toxicity

than the *R. graveolens* ethanolic extract (LC_{50} values ranging from 13.82 to 8.02 mg/mL).The ethyl acetate extract proved more toxic to *B. rufimanus* than the ethanolic extract. These differences reflect variations in relative toxic efficacy and highlight the toxic action of the ethanolic extract, which was slower but dependent on the duration of exposure. These data highlight a strong inverse relationship between the lethal concentration and the duration of treatment. These results indicate that these extracts could contribute to alternative approaches for controlling adult bruchid beetles, particularly as a complementary component within integrated pest management strategies.

However, there does not appear to have been any other work examining the insecticidal effects of ethanolic and ethyl acetate extracts of *R. graveolens* on individuals of *B. rufimanus*. Furthermore, a previous study on the essential oilof *R. graveolens* demonstrated strong larvicidal activity against *Culiseta longiareolata*, with LC_{25} and LC_{50} values of 6.96 and 10.11 ppm, respectively. Consequently, these two lethal concentrations lead to a significant reduction in the weight, volume, biochemical composition, and caloric indices of third- and fourth-instar *Culiseta longiareolata* larvae [29]. In addition, the toxicological parameters of essential oils from *M. pulegium* harvested in the Loukkos region show higher toxicity toward adult *B. rufimanus* twenty-four hours after fumigation; the LC_{50} values are 7.15 and 11.99 µl/l of air for males and females, respectively [28].

However, the insecticidal activity of other *Ruta* species has been documented. For example, insecticidal assays conducted with the essential oil of *Ruta chalepensis* against *Aedesaegypti* and *Anopheles quadrimaculatus* yielded LC_{50} values of 22.2 and 14.9 ppm, respectively. Thus, they demonstrated significant repellent activity against the larvae [30].

Furthermore, [31] observed that fourth-instar *C. longiareolata* larvae are susceptible to aqueous extracts of *R. chalepensis* leaves, resulting in varying mortality rates depending on the concentrations used and, above all, the duration of exposure to the extracts. To achieve 50% larval mortality after the first day, the *R. chalepensis* extracts concentration must be 22.17 mg/mL, while a concentration of 46 mg/mL ensures 90% mortality. By the second day, the LC_{50} and LC_{90} values were 8.82 mg/mL and 14.31 mg/mL, respectively.

The bioactivity of *R. graveolens* extracts is primarily linked to their chemical composition. Plant secondary metabolites are increasingly recognized as eco-friendly, bio-insecticidal alternatives to synthetic products for pest and mosquito control. The increased toxicity of *R. graveolens* extracts could be associated with the presence of phenolic compounds, specifically flavonoids and tannins (Table 1) which are widely recognized for their role in plant defense mechanisms against herbivorous insects [32, 33].This study highlights the efficacy of *R. graveolens* extracts against adult *B. rufimanus*. Their composition, rich in phenolic compounds, and their biological activity (which depends on dose and exposure duration) confirm their suitability for use in integrated and ecologically sustainable biological control strategies.

Although promising insecticidal activity has been observed against adult *B. rufimanus*, the potential impact of *R. graveolens* extracts on non-target organisms remains to be determined. It is therefore essential to conduct further ecotoxicological studies to assess their safety with regard to beneficial insects—particularly pollinators and predators—before considering their application in agricultural ecosystems.

3.4 HPLC analysis

HPLC analysis of the crude *R. graveolens* extract revealed a rich phytochemical profile. A total of twelve phenolic compounds were detected, eight of which were tentatively identified by comparison with authentic standards (Figure 2). The identified compounds include chlorogenic acid (RT = 1.830 min), tannic acid (RT = 4.515 min), quercetin (RT = 5.203 min), adonitol (RT = 9.628 min), gallic acid (RT = 19.215 min), coumaric acid (RT = 21.937 min), caffeic acid (RT = 23.665 min), and ascorbic acid (RT = 27.262 min). However, four peaks remained unidentified by this analysis.

Although eight compounds were provisionally identified by comparing them to authentic reference standards based on their retention times, this method does not provide definitive structural confirmation. Further analysis, using complementary techniques—such as LC-MS/MS or NMR—is required to confirm the identity and chemical structure of these compounds. Furthermore, four chromatographic peaks remained unidentified, highlighting the importance of continued research in phytochemistry.

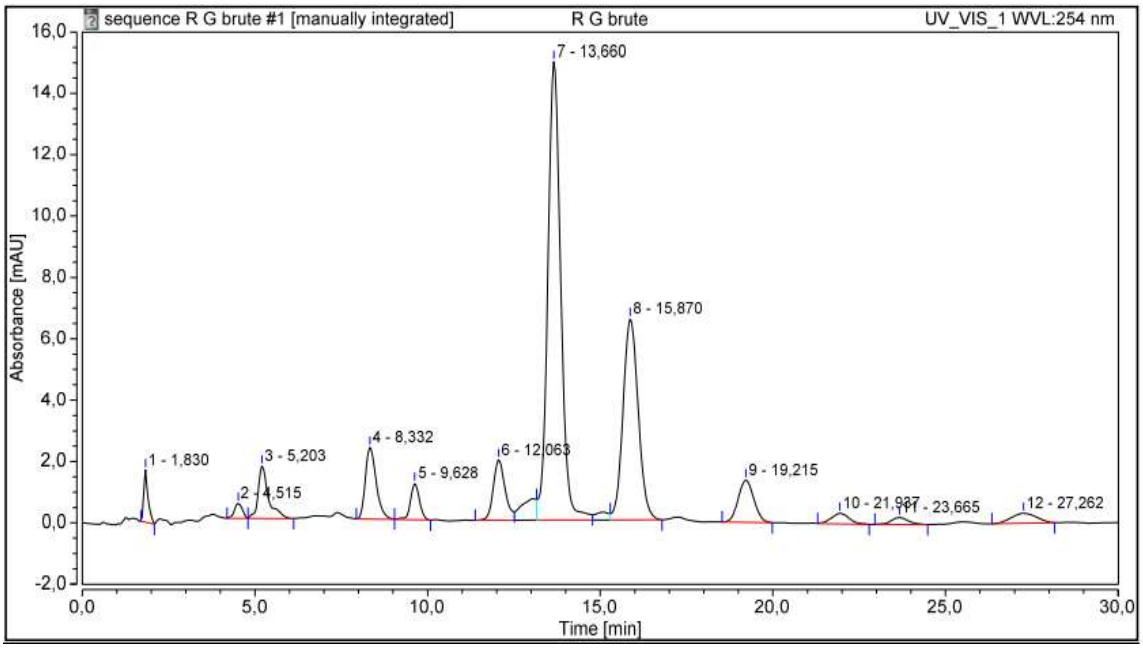

Figure 2. HPLC Chromatogram of ethyl acetate extract of *Ruta graveolens*.

These results are consistent with previous studies that also highlighted the abundance of these secondary metabolites in *R. graveolens*. A study by [34] identified several phenolic acids in the *R. graveolens* extract, such as caffeic acid, chlorogenic acid, p-coumaric acid, p-hydroxybenzoic acid, protocatechuic acid, and syringic acid.

Similarly, [35] confirmed that the ethanol extract of *R. graveolens* is a rich source of catechol, coumarin, and hydroquinone, whereas resorcinol and coumarin characterize the ethyl acetate extracts.

Furthermore, the phenolic compounds identified in the extract of *R. graveolens* could contribute to its insecticidal activity. [36] has been shown that quercetin influences the behavior and life history of herbivores, natural enemies and pollinators. Thus, the study by [37] suggests that gallic acid could act as an elicitor triggering a direct defense response in the tea plant against geometrid larvae.

Furthermore, the insecticidal potential of plant-derived phenolic compounds, such as caffeic acid, has been extensively documented [38]. The lethal effect of caffeic acid on insects has also been confirmed in the tobacco cutworm, *Spodoptera litura* (Fabricius) [39], and in the tomato fruit borer [40]. In addition to causing mortality, various studies have demonstrated that caffeic acid and other plant-derived phenolic compounds can exert negative effects on insect feeding, larval growth rates, and reproduction [41, 42].

The results of [43] support the claim that tannic acid present in seaweed exerts time- and dose-dependent insecticidal activity against *A. devastans*. According to their findings, seaweed tannins disrupted the normal physiological metabolism of adults, causing numerous adverse changes in several key total body proteins.

Nevertheless, the available data do not allow for the specific attribution of the insecticidal effects observed in this study to these compounds. Further biochemical and biological investigations, both *in vitro* and *in silico*, are required to confirm these predicted contributions.

3.5 *In silico* study and molecular docking

The molecular docking results revealed distinct binding behaviors among the tested *Ruta graveolens* phytochemicals toward the selected insect enzymes. The calculated binding affinities of chlorogenic acid, quercetin, tannic acid, and deltamethrin against *Tenebrio molitor* larvalα-amylase (PDB ID: 1JAE), digestive procathepsin L2 proteinase (PDB ID: 3QJ3), and *Drosophila*

melanogaster acetylcholinesterase (PDB ID: 6XYS) are presented in Table 4. All compounds showed negative binding energies, indicating favorable predicted interactions with the investigated targets.

Table 4: Molecular docking scores of major compounds of *Ruta graveolens* against *Tenebrio molitor* larvalα-amylase (PDB ID: 1JAE)

Compounds	**BindingAffinity (kcal/mol)**		
	Alpha-Amylase (PDB = 1JAE)	**Digestive procathepsin L2 proteinase (PDB = 3QJ3)**	**Acetylcholinesterase (PDB = 6XYS)**
Ascorbic acid	-6.5	-5.3	-5.7
Caffeic acid	-6.5	-5.4	-7.0
Chlorogenic acid	**-8.8**	**-6.5**	**-9.4**
Coumaric acid	-6.1	-5.5	-6.6
Gallic acid	-6.5	-5.6	-6.4
Quercetin	**-8.1**	**-5.9**	**-8.8**
Adonitol	-5.7	-4.5	-5.7
Tannic acid	**-9.7**	**-11.4**	**-11.0**
Temephos	-7.0	-5.3	-6.5
Delthamethrin	**-7.3**	**-5.7**	**-8.3**

Tannic acid exhibited the strongest and most consistent binding affinity among the tested compounds, reaching −9.7 kcal/mol for α-amylase, −11.4 kcal/mol for digestive procathepsin L2 proteinase, and −11.0 kcal/mol for acetylcholinesterase. These values were higher than those obtained for the reference insecticides, suggesting a strong predicted stability of the tannic acid–enzyme complexes. This behavior may be related to the polyphenolic structure of tannic acid, which contains multiple hydroxyl groups capable of forming hydrogen bonds and electrostatic interactions with amino acid residues within enzyme active sites. Similar effects have been reported for tannic acid, where its phenolic structure contributed to insecticidal activity through interference with physiological and enzymatic processes [44].

Chlorogenic acid and quercetin also displayed strong binding performances, particularly toward α-amylase and acetylcholinesterase. Chlorogenic acid showed affinities of −8.8 and −9.4 kcal/mol against α-amylase and acetylcholinesterase, respectively, whereas quercetin exhibited affinities of −8.1 and −8.8 kcal/mol. Previous studies have demonstrated the insecticidal potential of these compounds. Chlorogenic acid reduced survival, development, pupation, adult emergence, and reproduction in *Mythimna separata* and affected detoxification-related mechanisms [45]. Quercetin was also reported to affect survival, growth, feeding behavior, and digestive physiology of *Spodoptera litura* [46, 47]. These reports support the possible contribution of chlorogenic acid and quercetin to the insecticidal activity of *R. graveolens* extract.

The interaction profiles further supported the docking results and provided details on the molecular contacts established within each enzyme active site. The two- and three-dimensional interaction patterns of chlorogenic acid, quercetin, tannic acid, and deltamethrin with α-amylase, digestive procathepsin L2 proteinase, and acetylcholinesterase are illustrated in Figures 3, 4 and 5respectively

For α-amylase (PDB ID: 1JAE) (Table 5), chlorogenic acid formed attractive charge interactions with ASP185 and GLU222, together with hydrogen bonds involving LYS188 and TYR139. Quercetin displayed a broader interaction network through hydrogen bonds with ALA4, ARG374, ASP3, and PRO316, accompanied by π-anion and π-donor interactions involving GLU378 and THR319. Tannic acid interacted with ASP287, whereas deltamethrin formed hydrogen bonds with SER12 and THR320 and hydrophobic contacts with ARG374 and LEU277 (Figure 3). These interactions suggest favorable stabilization of the compounds within the α-amylase binding region.

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Table 5: Molecular docking results of Chlorogenic Acid, Quercetin, Tannic Acid, and Deltamethrin with *Tenebrio molitor* alpha-amylase (PDB ID: 1JAE)

Complexe	Residues	Interaction type	Distances (Å)
1JAE - Chlorogenicacid	ASP185	Attractive charge	4.11
	GLU222	Attractive charge	3.20
	LYS188	Conventionalhydrogen bond	2.78
	TYR139	Conventionalhydrogen bond	3.07
1JAE – Quercetin	ALA4	Conventionalhydrogen bond	2.28
	ARG374	Conventionalhydrogen bond	2.02
	ASP3	Conventionalhydrogen bond	3.39
	PRO316	Conventionalhydrogen bond	3.34
	GLU378	Pi-anion	4.53
	THR319	Pi-donorhydrogen bond	2.93
1JAE - Tannic Acid	ASP287	Carbon hydrogen bond	3.54
1JAE – Delthamethrin	SER12	Conventionalhydrogen bond	2.19
	THR320	Conventionalhydrogen bond	2.13
	ARG374	Alkyl	5.22
	LEU277	Alkyl	4.74

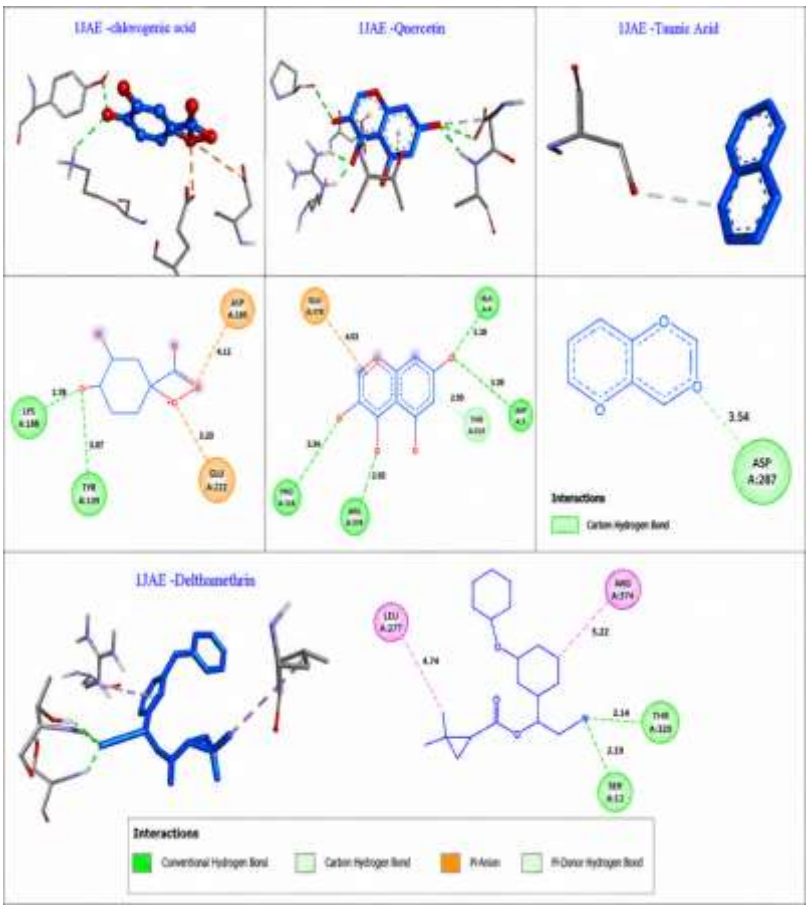

Figure 3. 2D and 3D molecular interactions of Chlorogenic Acid, Quercetin, Tannic Acid, and Deltamethrin with the active site of *Tenebrio molitor* alpha-amylase.

For digestive procathepsin L2 proteinase (PDB ID: 3QJ3) (Table 6), chlorogenic acid established multiple stabilizing interactions involving ARG303, ASN304, ALA107, and ASP281 through hydrogen bonding, together with an attractive charge interaction with ASP229. Quercetin interacted with ASN225, ASN307, GLU224, and ASP223 through hydrogen bonding and π-anion interaction. Tannic acid formed a hydrogen bond with SER102, while deltamethrin interacted with ASP110, SER108, PHE109, and PRO106 through hydrogen bonding and π/alkyl interactions (Figure 4). These molecular contacts suggest that the tested phenolic compounds may occupy important regions within the protease binding cavity.

Table 6. Molecular docking results of Chlorogenic Acid, Quercetin, Tannic Acid, and Deltamethrin with digestive procathepsin L2 proteinase from *Tenebrio molitor* (PDB ID: 3QJ3)

Complexe	Residues	Interaction type	Distances (Å)
3QJ3 - Chlorogenicacid	ASP229	Attractive charge	4.73
	ARG303	Conventionalhydrogen bond	2.22
	ASN304	Conventionalhydrogen bond	2.56
	ALA107	Conventionalhydrogen bond	3.20
	ASP281	Conventionalhydrogen bond	3.24
3QJ3 –Quercetin	ASN225	Conventionalhydrogen bond	2.16
	ASN307	Conventionalhydrogen bond	2.81
	GLU224	Conventionalhydrogen bond	3.40
	ASP223	Pi-anion	3.47
3QJ3 - Tannicacid	SER102	Conventionalhydrogen bond	2.55
3QJ3 –Delthamethrin	ASP110	Conventionalhydrogen bond	1.97
	SER108	Carbon hydrogen bond	3.39
	PHE109	Pi-donorhydrogen bond	3.93
	PRO106	Alkyl	5.06

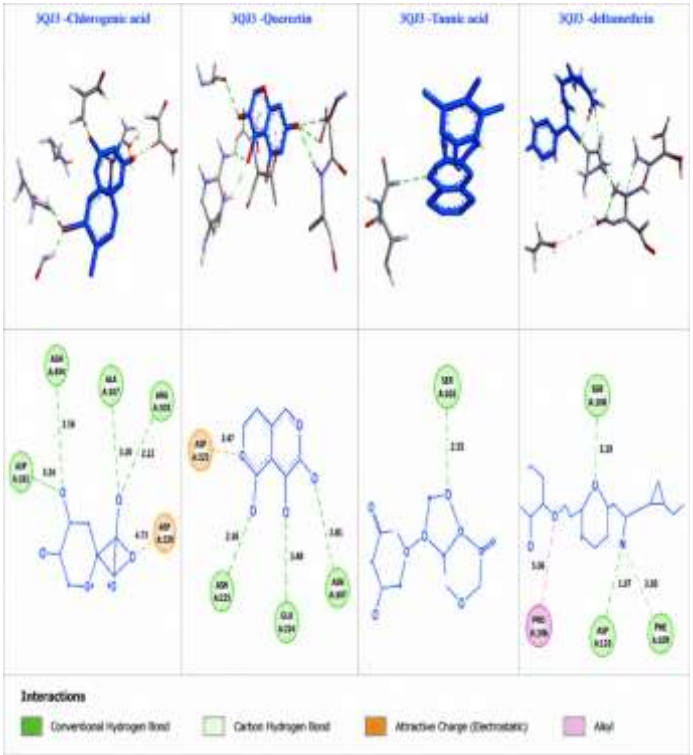

Figure 4. 2D and 3D molecular interactions of Chlorogenic Acid, Quercetin, Tannic Acid, and Deltamethrin with the active site of *digestive* procathepsin L2 proteinase from*Tenebrio molitor*.

The acetylcholinesterase interaction analysis revealed strong binding profiles for the selected compounds (Table 5 and Figure 5). Chlorogenic acid formed several hydrogen bonds involving ASP160, ILE161, GLU81, and ILE82, while quercetin established contacts with ARG17, ALA157, ASN163, and LEU62 through hydrogen bonding and π-interactions. Tannic acid showed strong interactions with LYS278 and LYS403 through hydrogen bonding, π-cation, and π-alkyl contacts, consistent with its high predicted affinity toward this enzyme. Deltamethrin interacted with ARG70, TRP321, and TYR71 through hydrogen bonding and π-interactions. These interactions support the stable binding of the selected phenolic compounds within the acetylcholinesterase active site. This finding agrees with [48], who identified acetylcholinesterase as a relevant target of plant-derived insecticidal compounds from *Seriphidium herba-album*.

Table 7. Molecular docking results of Chlorogenic Acid, Quercetin, Tannic Acid, and Deltamethrin with the active site of acetylcholinesterase from *Drosophila Melanogaster*

Complexe	Residues	Interaction type	Distances (Å)
6XYS-Chlorogenic acid	ASP160	Attractive charge	4.95
	ASP160	Conventionalhydrogen bond	2.44
	ILE161	Conventionalhydrogen bond	1.78
	ILE161	Conventionalhydrogen bond	3.10
	GLU81	Conventionalhydrogen bond	3.34
	ILE82	Conventionalhydrogen bond	3.32
6XYS-Quercetin	ARG17	Conventionalhydrogen bond	1.92
	ALA157	Conventionalhydrogen bond	2.84
	THR158	Carbon hydrogen bond	3.23
	ASN163	Pi-donorhydrogen bond	2.80
	LEU62	Pi-Alkyl	4.65
6XYS-Tannic acid	LYS278	Conventionalhydrogen bond	2.13
	LYS278	Pi-Alkyl	5.05
	LYS403	Pi-Cation	3.96
	LYS403	Pi-Alkyl	4.56
6XYS-Delthamethrin	ARG70	Conventionalhydrogen bond	2.99
	ARG70	Conventionalhydrogen bond	3.09
	TRP321	Pi-donorhydrogen bond	3.76
	TYR71	Pi-Alkyl	4.36
	TRP321	Pi-Alkyl	4.17

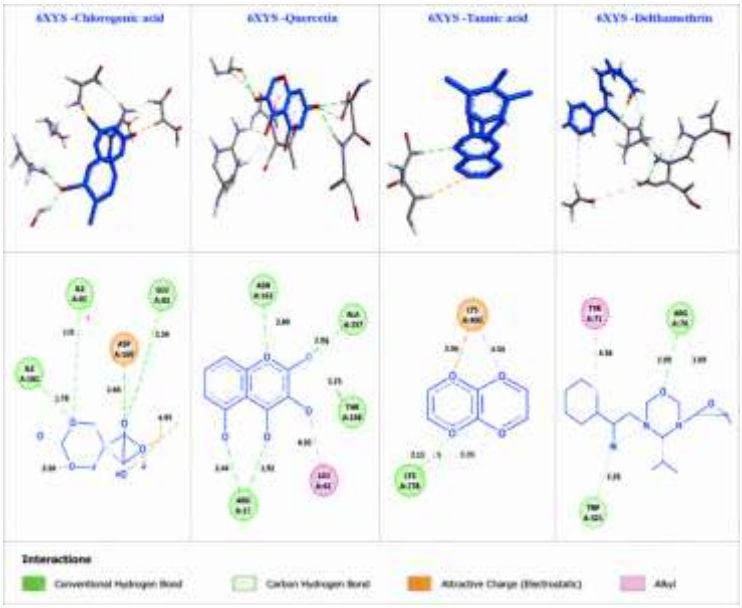

Figure 5. 2D and 3D molecular interactions of Chlorogenic Acid, Quercetin, Tannic Acid, and Deltamethrin with the active site of acetylcholinesterase from *Drosophila Melanogaster*

Overall, tannic acid, chlorogenic acid, and quercetin exhibited the most promising docking profiles among the tested compounds, showing strong binding affinities and extensive interaction networks within the investigated enzyme targets. These findings suggest that the phenolic constituents of *R. graveolens* may contribute to insecticidal activity against *Bruchus rufimanus* through possible interference with digestive enzymes and acetylcholinesterase function. Further biochemical and biological investigations—focusing on chlorogenic acid, tannic acid, and quercetin—are necessary to determine their respective insecticidal activities against *Bruchus rufimanus*. Such studies would allow for the assessment of each compound's contribution and the identification of potential synergistic or antagonistic effects among the constituents of the crude extracts.

4. CONCLUSION

The drawbacks associated with the use of various categories of synthetic pesticides to control insect pests in stored commodities are becoming an increasing concern. Given the promising results obtained through the application of plant extracts, it appears that these could be used effectively to combat destructive insects, such as *Bruchus rufimanus*. This study demonstrates that *R. graveolens* extracts exhibit significant contact toxicity against adult *Bruchus rufimanus* under laboratory conditions, with mortality increasing as a function of both concentration and duration of exposure. Thus, phytochemical analysis by HPLC made it possible to identify several compounds, notably tannic acid, chlorogenic acid and quercetin, which revealed strong affinities within the enzymatic targets studied. These results suggest that the phenolic constituents of *R. graveolens* may contribute to insecticidal activity against *Bruchus rufimanus*, potentially by interfering with digestive enzymes and the functioning of acetylcholinesterase.

Consequently, as part of this work, we advocate for the evaluation of a wide range of plant-derived substances intended to combat the insect species in question, while promoting health and preserving the environment. The practical application of *R. graveolens* extracts also requires in-depth research into formulation stability, storage conditions, persistence, and residual insecticidal activity in various environments. Furthermore, field trials are essential to validate the efficacy of these extracts under actual agricultural conditions and to determine their potential integration into sustainable pest management programs.

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AUTHOR CONTRIBUTIONS

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Supervision: Wafa Bensania.

All authors read and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ETHICS STATEMENT

This study involved the use of *Bruchus* insects as an experimental model for evaluating the biological activity of plant extracts. No human participants or vertebrate animals were involved in this research. Ethical approval was not required for this study, in accordance with the applicable institutional requirements and regulations.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

[1] Pichon, A., McNamara, L., Nolan, E., & Alves, S., 2026. Implications of in-seed mortality of Bruchus rufimanus and its associated natural enemies for pest management under temperate oceanic climate conditions. Frontiers in Agronomy, 8, 1888691.

[2] Almogdad, M., Semaškienė, R., & Tamošiūnas, K., 2023. Optimizing insecticide application timing for broad bean weevil control and minimizing crop damage in broad bean (Vicia faba Linn.). Plants, 12(9), 1839.

[3] Lugendo, A. R., Ben Fekih, I., Caparros Megido, R., Pierreux, J., Francis, F., & Segers, A., 2025. Efficacy of entomopathogenic fungi against Bruchus rufimanus (Coleoptera: Chrysomelidae) in laboratory and field trials using dropleg spraying technique. Agriculture, 15(4), 376.

[4] Kaniuczak, Z., 2004. Seed damage of field bean (Vicia faba L. var. minor Harz.) caused by bean weevils (Bruchus rufimanus Boh.) (Coleoptera: Bruchidae). Journal of Plant Protection Research, 44(2), 125–129.

[5] Segers, A., Megido, R. C., Lognay, G., & Francis, F., 2021. Overview of Bruchus rufimanus Boheman 1833 (Coleoptera: Chrysomelidae): Biology, chemical ecology and semiochemical opportunities in integrated pest management programs. Crop Protection, 140, 105411.

[6] Pichon, A., Byrne, S., Alves, S., Nolan, E., & McNamara, L., 2025. Evidence of Pyrethroid Resistance in the bruchid beetle, Bruchus rufimanus, in Ireland. Crop Protection, 107407.

[7] Huber, J., Chaluppa, N., Voit, B., Steinkellner, S., & Killermann, B., 2023. Damage potential of the broad bean beetle (Bruchus rufimanus Boh.) on seed quality and yield of faba beans (Vicia faba L.). Crop Protection, 168, 106227.

[8] Raderschall, C. A., Johansson, Y., & Lundin, O., 2025. Semiochemically assisted trap cropping to reduce broad bean beetle (Bruchus rufimanus) infestation in faba bean. Agriculture, Ecosystems & Environment, 388, 109669.

[9] Ainane, T., Abdoul-Latif, F. M., Baghouz, A., Montassir, Z. E., Attahar, W., & Ainane, A., 2023. Essential oils rich in pulegone for insecticide purpose against legume bruchus species: Case of Ziziphora hispanica L. and Mentha pulegium L. AIMS Agriculture and Food, 8, 105–118.

[10] Dell'Aglio, D. D., & Tayeh, N., 2023. Responsiveness of the broad bean weevil, Bruchus rufimanus, to Vicia faba genotypes. Entomologia Experimentalis et Applicata, 171(4), 312–322.

[11] Segers, A., Dumoulin, L., Megido, R. C., Jacquet, N., Cartrysse, C., Kamba, P. M., Pierreux J., Richel A., Blecker C., & Francis F., 2022. Varietal and environmental effects on the production of faba bean (Vicia faba L.) seeds for the food industry by confrontation of agricultural and nutritional traits with resistance against Bruchus spp. (Coleoptera: Chrysomelidae, Bruchinae). Agriculture, Ecosystems & Environment, 327, 107831.

[12] Al-Sayed, S. E., Eltalawy, H. M., Abuelsaad, A. S., Zaky, M. Y., & Ebaid, H., 2026. Efficacy of Lavandula dentata essential oil against Tribolium castaneum: Insecticidal activity, behavioral deterrence, and molecular docking insights. Journal of Stored Products Research, 119, 103204.

[13] Jyotsna, B., Patil, S., Prakash, Y. S., Rathnagiri, P., Kishor, P. K., & Jalaja, N., 2024. Essential oils from plant resources as potent insecticides and repellents: Current status and future perspectives. Biocatalysis and Agricultural Biotechnology, 61, 103395.

[14] Al-Ansi, A. N., Al-Quraishy, S., Murshed, M., & Ibrahim, K. E., 2026. Evaluation of the insecticidal activity of Cinnamomum verum bark extract against the red flour beetle, Tribolium castaneum (Coleoptera: Tenebrionidae). Journal of Entomological Science, 1, AOP.

[15] Jeon, J. H., Lee, S. G., & Lee, H. S., 2015. Isolation of insecticidal constituent from Ruta graveolens and structure-activity relationship studies against stored-food pests (Coleoptera). Journal of Food Protection, 78(8), 1536–1540.

[16] Luo, P., Feng, X., Liu, S., & Jiang, Y., 2024. Traditional uses, phytochemistry, pharmacology and toxicology of Ruta graveolens L.: A critical review and future perspectives. Drug Design, Development and Therapy, 6459–6485.

[17] Rouari, L., Gouzi, H., Ghermaoui, M., Benaceur, F., Kemassi, A., Merabti, B., & Chaibi, R., 2022. First study of larvicidal activity of Algerian Oudneya africana extracts against Culex pipiens larvae. Ukrainian Journal of Ecology, 12(1), 65–70. https://doi.org/10.15421/2022_337

[18] Kemassi, A., Herouini, A., Hadjseyd, A., Cherif, R., & Ould El Hadj, M. D., 2019. Effet insecticide des extraits aqueux d'Euphorbia guyoniana (Euphorbiaceae) récoltée dans Oued Sebseb (Sahara algérien) sur le Tribolium castaneum. Lebanese Science Journal, 20(1), 55–70.

[19] Strobl, S., Maskos, K., Betz, M., Wiegand, G., Huber, R., Gomis-Rüth, F. X., & Glockshuber, R., 1998. Crystal structure of yellow meal worm α-amylase at 1.64 Å resolution. Journal of Molecular Biology, 278(3), 617–628. https://doi.org/10.1006/jmbi.1998.1667

[20] Rawlings, N. D., Barrett, A. J., & Bateman, A., 2024. MEROPS: The database of proteolytic enzymes, their substrates and inhibitors. Nucleic Acids Research, 52(D1), D400–D407.

[21] Benaceur, K., Youcefi, M. N., & Benaceur, F., 2026a. Chemical profile and insecticidal properties of essential oil obtained from Algerian Artemisia herba-alba leaves. Acta Parasitologica, 71, 171. https://doi.org/10.1007/s11686-026-01363-5

[22] Kim, S., Chen, J., Cheng, T., Gindulyte, A., He, J., He, S., Li, Q., Shoemaker, B. A., Thiessen, P. A., Yu, B., Zaslavsky, L., Zhang, J., & Bolton, E. E., 2023. PubChem 2023 update. Nucleic Acids Research, 51(D1), D1373–D1380. https://doi.org/10.1093/nar/gkac956

[23] Trott, O., & Olson, A. J., 2010. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. Journal of Computational Chemistry, 31(2), 455–461. https://doi.org/10.1002/jcc.21334

[24] Attia, E. Z., Abd El-Baky, R. M., Desoukey, S. Y., El Hakeem Mohamed, M. A., Bishr, M. M., & Kamel, M. S., 2018. Chemical composition and antimicrobial activities of essential oils of Ruta graveolens plants treated with salicylic acid under drought stress conditions. Future Journal of Pharmaceutical Sciences, 4(2), 254–264.

[25] Malik, A. A., Mir, S. R., & Ahmad, J., 2013. Ruta graveolens L. essential oil composition under different nutritional treatments. American-Eurasian Journal of Agricultural & Environmental Sciences, 13, 1390–1395.

[26] Álvarez, E. C., Recalde-Luna, S., Molina, M. P., Torres, D. Q., Alvarado, J. R., & Moreno, W. Q., 2025. Chemical composition and biological action of the Ruta graveolens L. and Ambrosia arborescens: Sociocultural incidence. Afinidad. Journal of Chemical Engineering Theoretical and Applied Chemistry, 82(605), 431–439.

[27] Titouhi, F., Amri, M., Messaoud, C., Haouel, S., Youssfi, S., Cherif, A., & Jemâa, J. M. B., 2017. Protective effects of three Artemisia essential oils against Callosobruchus maculatus and Bruchus rufimanus (Coleoptera: Chrysomelidae) and the extended side-effects on their natural enemies. Journal of Stored Products Research, 72, 11–20.

[28] Amzouar, S., Boughdad, A., Maatoui, A., & Allam, L., 2016. Comparison of the chemical composition and the insecticidal activity of essential oils of Mentha pulegium L. collected from two different regions of Morocco, against Bruchus rufimanus (Bohman) (Coleoptera: Chrysomelidae). Advances in Environmental Biology, 10(7), 199–208.

[29] Bouabida, H., & Dris, D., 2020. Effect of rue (Ruta graveolens) essential oil on mortality, development, biochemical and biomarkers of Culiseta longiareolata. South African Journal of Botany, 133, 139–143.

[30] Ali, A., Demirci, B., Kiyan, H. T., Bernier, U. R., Tsikolia, M., Wedge, D. E., Khan, I. A., Can Başer, K. H., & Tabanca, N., 2013. Biting deterrence, repellency, and larvicidal activity of Ruta chalepensis (Sapindales: Rutaceae) essential oil and its major individual constituents against mosquitoes. Journal of Medical Entomology, 50(6), 1267–1274.

[31] Benhissen, S., Habbachi, W., Rebbas, K., & Masna, F., 2019. Bioactivité des extraits foliaires de Ruta chalepensis L. (Rutaceae) sur la mortalité des larves de Culiseta longiareolata (Diptera, Culicidae). Lebanese Science Journal, 20(1), 1.

[32] Chauhan, N. S., Punia, A., & Sohal, S. K., 2020. Insecticidal effects of phenolic compounds on the development of polyphagous Spodoptera litura larvae. Allelopathy Journal, 50(2), 185–194.

[33] Elqady, E. M., El-Said, E., Moustafa, M. A., Almozini, R. N., Badr, N. F., Ismail, F. S., Ahmed W. H. & Abd El-Sadek M. E., 2026. Enhanced polyphenolic production in tissue culture-derived Opuntia ficus-indica increases larvicidal activity against Spodoptera exigua: Biochemical, histological and molecular docking evidence. Scientific Reports, 16(1), 25102.

[34] Ekiert, H., Piekoszewska, A., Muszyńska, B., & Baczyńska, S., 2014. Accumulation of P-coumaric acid and other bioactive phenolic acids in in vitro culture of Ruta graveolens SSP. divaricata (Tenore) Gams. Medicina Internacia Revuo, 102(26), 24–31.

[35] Saeed, Y. S., Ali, J. F., & Mohammed, M. A., 2023. Chemical composition, antioxidant, and antibacterial activity of Ruta graveolens (Rutaceae). Biodiversitas Journal of Biological Diversity, 24(6).
[36] Riddick, E. W., 2021. Potential of quercetin to reduce herbivory. Agriculture, 11(6), 476.
[37] Zhang, X., Ran, W., Li, X., Zhang, J., Ye, M., Lin, S., Liu, M., & Sun, X., 2022. Exogenous application of gallic acid induces direct defense of tea plant against Ectropis obliqua caterpillars. Frontiers in Plant Science, 13, 833489. https://doi.org/10.3389/fpls.2022.833489
[38] Zarmakoupi, C., Mpistiolis, K., Pantazis, G., Psatha, P., Dimitriadi, D., Kitsiou, F., Eliopoulos P., Patakioutas G., & Mantzoukas S., 2023. Caffeic acid and biopesticides interactions for the control of storage beetles. Applied Biosciences, 2(2), 211–221.
[39] Punia, A., Singh, V., Thakur, A., & Chauhan, N. S., 2023. Impact of caffeic acid on growth, development and biochemical physiology of insect pest, Spodoptera litura (Fabricius). Heliyon, 9, e14593.
[40] Joshi, R. S., Wagh, T. P., Sharma, N., Mulani, F. A., Sonavane, U., Thulasiram, H. V., Joshi, R., Gupta, V. S., & Giri, A. P., 2014. Way toward "dietary pesticides": Molecular investigation of insecticidal action of caffeic acid against Helicoverpa armigera. Journal of Agricultural and Food Chemistry, 62, 10847–10854.
[41] Nakhaie, B. M., Mikani, A., & Moharramipour, S., 2018. Effect of caffeic acid on feeding, α-amylase and protease activities and allatostatin—A content of Egyptian cotton leafworm, Spodoptera littoralis (Lepidoptera: Noctuidae). Journal of Pest Science, 43, 73–78.
[42] Punia, A., Chauhan, N., Singh, R., Kaur, S., & Sohal, S., 2022. Growth disruptive effects of ferulic acid against Spodoptera litura (Fabricius) and its parasitoid Bracon hebetor (Say). Allelopathy Journal, 55, 79–92.
[43] Petchidurai, G., Sahayaraj, K., Al-Shuraym, L. A., Albogami, B. Z., & Sayed, S. M., 2023. Insecticidal activity of tannins from selected brown macroalgae against the cotton leafhopper Amrasca devastans. Plants, 12(18), 3188.
[44] Tan, M., Wu, H., Yan, S., & Jiang, D., 2022. Evaluating the toxic effects of tannic acid treatment on Hyphantria cunea larvae. Insects, 13(10), 872. https://doi.org/10.3390/insects13100872
[45] Lin, D. J., Fang, Y., Li, L. Y., Zhang, L. Z., Gao, S. J., Wang, R., & Wang, J. D., 2022. The insecticidal effect of the botanical insecticide chlorogenic acid on Mythimna separata (Walker) is related to changes in MsCYP450 gene expression. Frontiers in Plant Science, 13, 1015095. https://doi.org/10.3389/fpls.2022.1015095
[46] Selin-Rani, S., Senthil-Nathan, S., & Thanigaivel, A., 2016. Toxicity and physiological effect of quercetin on generalist herbivore, Spodoptera litura Fab., and a non-target earthworm, Eisenia fetida Savigny. Chemosphere, 165, 257–267. https://doi.org/10.1016/j.chemosphere.2016.08.136
[47] Herrera-Mayorga, V., Guerrero-Sánchez, J. A., Méndez-Álvarez, D., Paredes-Sánchez, F. A., Rodríguez-Duran, L. V., Niño-García, N., Paz-González A.D., & Gildardo Rivera G., 2022. Insecticidal activity of organic extracts of Solidago graminifolia and its main metabolites (quercetin and chlorogenic acid) against Spodoptera frugiperda: An in vitro and in silico approach. Molecules, 27(10), 3325.
[48] Benaceur, K., Benaceur, F., Youcefi, M. N., Boufissiou, A., Kadi, I., & Chaibi, R., 2025. Multifunctional bioactivities of Algerian Seriphidium herba-album (Asso) essential oil: Allelopathic, insecticidal, and antibacterial insights. Journal of Essential Oil Bearing Plants, 28(6), 1242–1259. https://doi.org/10.1080/0972060X.2025.2588661