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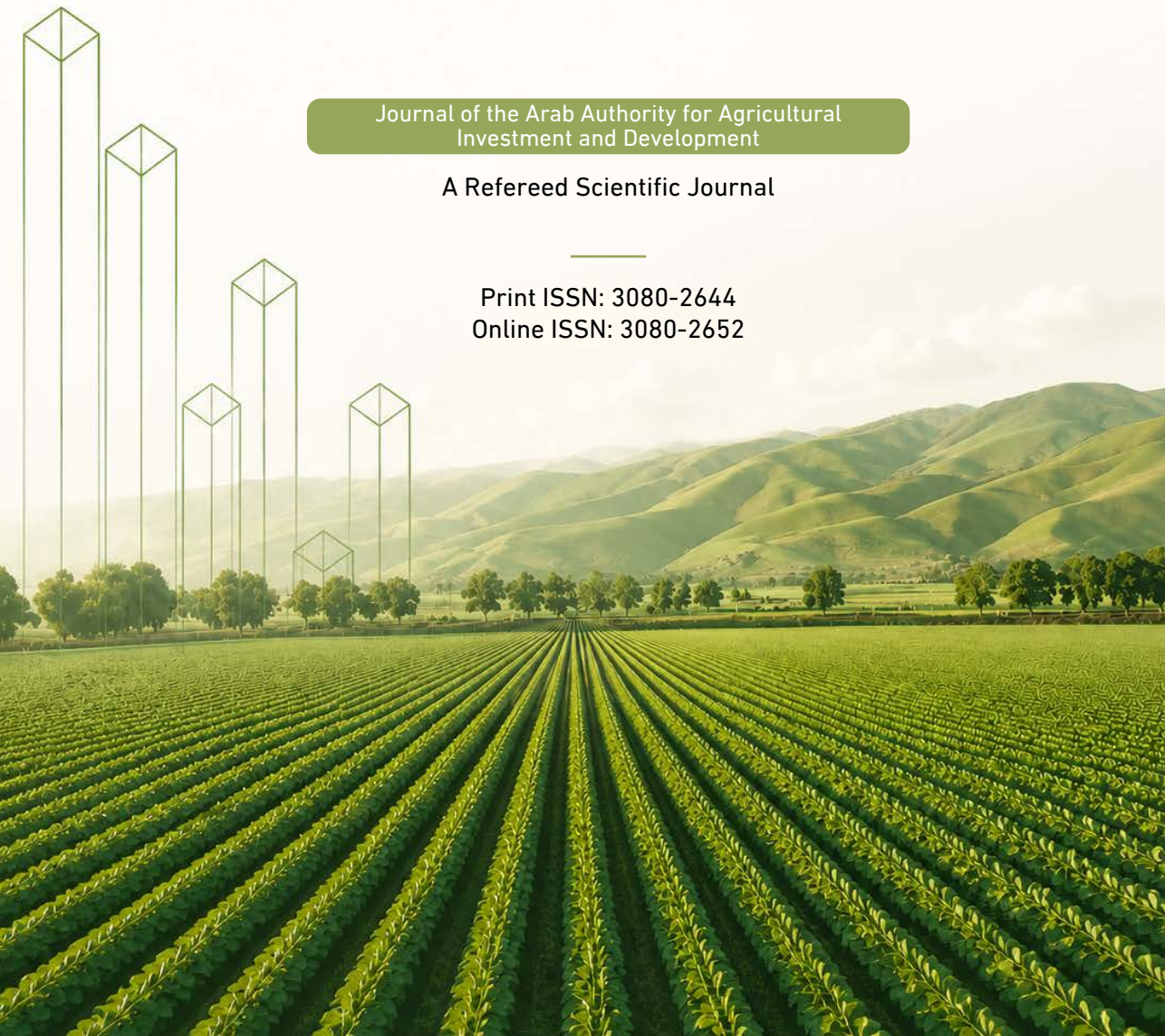
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Rosmarinic Acid as a Promising Biopesticide Against Rhynchophorus Ferrugineus in Date Palm

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Abstract:

The red palm weevil (*R. ferrugineus*) is one of the most destructive pests of date palm (*P. dactylifera* L.), causing substantial economic losses and threatening the sustainability of date palm production worldwide. The extensive use of synthetic insecticides has accelerated the evolution of insecticide resistance while raising serious concerns regarding environmental contamination, non-target effects, and food safety. Consequently, the development of effective botanical alternatives has become a priority for sustainable pest management. This study evaluated the insecticidal potential of Rosmarinic acid, a naturally occurring plant phenolic compound, against *R. ferrugineus* under laboratory and field conditions. Laboratory bioassays assessed acute toxicity and sublethal physiological effects using an artificial diet incorporation method. Toxicity was determined by estimating LC_{50} values, whereas nutritional indices, including the efficacy of conversion of ingested food (ECI) and digested food (ECD), were used to evaluate physiological disruption. In addition, the molecular mechanism of action was investigated by analyzing the expression of key detoxification genes, including cytochrome P450, glutathione S-transferase (GST), and general esterases. Field trials conducted during 2022–2023 in Baltim, Kafr El-Sheikh, Egypt, evaluated trunk injection of Rosmarinic acid at 3, 4, and 5 mL L^{-1}, with Deltamethrin (0.25 mL L^{-1}) serving as the reference insecticide. Treatment performance was assessed through destructive sampling, Palmear® acoustic detection, and residue analysis of palm tissues and fruits. Rosmarinic acid exhibited clear dose-dependent toxicity, with LC_{50} values of 2.041, 2.230, and 2.575 mL L^{-1} against fourth-instar larvae, eighth-instar larvae, and adults, respectively. It also significantly reduced nutritional efficiency and suppressed detoxification-related gene expression, indicating a multi-target mode of action. The highest field recovery rate (93.33%) was achieved with

Rosmarinic acid at 5 mL L^{-1}, exceeding both Rosmarinic acid at 4 mL L^{-1} and Deltamethrin (86.67%). Furthermore, no Rosmarinic acid residues were detected in palm tissues or fruits, whereas Deltamethrin residues remained detectable. These findings highlight Rosmarinic acid as an effective, environmentally compatible, residue-free botanical insecticide suitable for integration into sustainable management programs against the red palm weevil.

Keywords: Rosmarinic acid, Red palm weevil, *Rhynchophorus ferrugineus*, Date palm, Sustainable pest management.

Introduction:

Date palm (*Phoenix dactylifera* L.) is one of the most important fruit crops cultivated in arid and semi-arid regions worldwide. Global date production reached approximately 9.66 million metric tons, harvested from around 1.29 million hectares worldwide, with an average productivity of 6.84 tons ha^{-1} (FAO, 2023). Egypt is the leading global producer of dates, contributing approximately 1.71 million metric tons annually from nearly 14.63 million productive palms cultivated over 78.21 thousand hectares, with an average productivity of 116.38 kg $palm^{-1}$ (MALR, 2021). The economic importance of date palm production makes the protection of this crop from destructive pests a major priority.

The red palm weevil (*Rhynchophorus ferrugineus* Olivier) represents one of the most damaging insect pests affecting date palm production worldwide. The cryptic feeding behavior of larvae inside the trunk often results in delayed detection, extensive tissue destruction, and, in severe cases, palm death (Al-Dosary et al., 2016). Pest-related losses contribute substantially to reductions in date palm productivity, with *R. ferrugineus* considered among the primary biological constraints affecting date palm cultivation (El-Juhany, 2010). In Egypt, economic losses associated with red palm weevil infestations have been estimated at approximately 4 billion Egyptian pounds between 1992 and 2000 (Abbas, 2019), highlighting the urgent need for effective and sustainable management approaches.

Management of *R. ferrugineus* has traditionally depended on repeated applications of synthetic insecticides. However, intensive reliance on chemical control has raised concerns related to environmental contamination, effects on beneficial

organisms, pesticide residues, and the development of insecticide resistance (Al-Ayedh et al., 2016). Reports of reduced susceptibility to commonly used insecticides, including pyrethroids, further emphasize the limitations of conventional control strategies and the need for alternative approaches compatible with integrated pest management (IPM) programs.

Plant-derived bioactive compounds have received increasing attention as potential alternatives to synthetic insecticides because of their diverse biological activities and generally favorable environmental characteristics. Essential oils, botanical extracts, and plant secondary metabolites have demonstrated insecticidal, antifeedant, and growth-regulating effects against several agricultural pests (Piri et al., 2020; Darrag et al., 2022). Among these compounds, phenolic metabolites have attracted considerable interest due to their antioxidant, antimicrobial, and insecticidal properties (Darrag et al., 2022; Guan et al., 2022).

Rosmarinic acid ($C_{18}H_{16}O_8$) is a naturally occurring phenolic ester widely distributed in numerous plant species, particularly members of the Lamiaceae family (Guan et al., 2022). Owing to its broad spectrum of biological activities, Rosmarinic acid has attracted increasing attention as a plant-derived bioactive compound with potential applications in sustainable pest management. Previous studies have demonstrated that purified Rosmarinic acid, as well as Rosmarinic acid-enriched plant fractions, can adversely affect insect survival, development, feeding behavior, and physiological performance. Insecticidal activity has been reported against several economically important insect pests, including the pea aphid (*Acyrthosiphon pisum*), lesser grain borer (*Rhyzopertha dominica*), citrus mealybug (*Planococcus citri*), and oriental fruit fly (*Bactrocera dorsalis*) (Khan et al., 2016; Mughees et al., 2021). Moreover, evidence from lepidopteran and hemipteran insects suggests that Rosmarinic acid may disrupt oxidative homeostasis and alter detoxification-related pathways, including cytochrome P450 monooxygenases and glutathione S-transferases, thereby contributing to its insecticidal activity (Khan et al., 2019; Kashyap and Reddy, 2025).

Despite these promising findings, current knowledge remains largely limited to laboratory-based evaluations involving a relatively narrow range of insect species.

Information regarding the efficacy of purified Rosmarinic acid against the red palm weevil (*R. ferrugineus*), one of the most destructive pests of date palm, remains scarce, particularly under field conditions. Furthermore, the relationships among insecticidal activity, physiological disruption, detoxification responses, field performance, and residue behavior in treated palms have not yet been comprehensively investigated. Recent advances in formulation technologies, including nanoemulsions and biodegradable delivery systems, have further highlighted the potential of Rosmarinic acid as a botanical insecticidal candidate while emphasizing the need for comprehensive biological evaluation before practical application (Naveed et al., 2023).

Therefore, the objective of the present study was to comprehensively evaluate the insecticidal potential of purified Rosmarinic acid against *R. ferrugineus* using an integrated laboratory-to-field approach. Specifically, the study aimed to (1) evaluate the insecticidal activity of Rosmarinic acid against different developmental stages of *R. ferrugineus* under laboratory conditions by determining lethal concentrations (LC_{50}); (2) assess the sublethal effects of Rosmarinic acid on larval feeding performance and nutritional utilization through determination of nutritional indices, including approximate digestibility (AD), efficiency of conversion of ingested food (ECI), and efficiency of conversion of digested food (ECD); (3) investigate potential molecular responses associated with Rosmarinic acid exposure by analyzing the expression patterns of major detoxification-related genes, including cytochrome P450 monooxygenases, glutathione S-transferases (GSTs), and esterases; (4) evaluate the field efficacy of Rosmarinic acid applied through trunk injection against naturally infested date palms and compare its performance with Deltamethrin using destructive assessment and Palmear® acoustic detection technology; (5) characterize the residue behavior of Rosmarinic acid and Deltamethrin in palm tissues and date fruits following trunk injection to provide preliminary information regarding residue persistence and food safety considerations.

Materials and Methods:

Rosmarinic acid isolation, purification, and quantification

Fresh aerial parts of Origanum vulgare L., Melissa officinalis L., Salvia officinalis L., Rosmarinus officinalis L., and Thymus vulgaris L. were collected during the 2022

growing season. Plant materials were authenticated, shade-dried at room temperature (25 ± 2 °C), and finely ground. The powdered samples were subjected to Soxhlet extraction using n-butanol as the extraction solvent for 6 h, following the procedure described by Sik et al. (2020). The obtained extracts were concentrated under reduced pressure using a rotary evaporator at 40 °C and stored at 4 °C until further analysis.

Rosmarinic acid was subsequently isolated from the obtained extracts using appropriate purification procedures. The isolated fractions were subjected to chromatographic and spectrometric analyses to confirm the identity of the target compound. High-performance liquid chromatography coupled with mass spectrometry (HPLC–MS) was used for qualitative and quantitative determination of Rosmarinic acid. Compound identification was based on comparison of retention time and mass spectral fragmentation patterns with those of an authentic Rosmarinic acid standard.

Chromatographic separation was achieved using a C18 reversed-phase column (250 mm × 4.6 mm, 5 µm) with a gradient mobile phase consisting of water containing 0.1% formic acid and acetonitrile at a flow rate of 1.0 mL min^{-1}. Detection was performed at 330 nm. Quantification was carried out using an external calibration curve generated from authentic Rosmarinic acid standards.

The Rosmarinic acid content was calculated based on the amount of compound recovered relative to the initial dry plant material used for extraction and expressed as mg g^{-1} dry weight (DW). This approach enabled accurate comparison of Rosmarinic acid yield among the evaluated plant species. For subsequent biological assays, the isolated Rosmarinic acid fraction was used as the tested active compound, rather than crude plant extracts, to minimize the potential influence of other co-extracted phytochemicals on biological responses.

Insects

The red palm weevils (*R. ferrugineus*) used in this study were obtained from laboratory colonies originally established from adults collected in date palm–infested fields between October and December 2022. The adults were maintained in mesh cages (57.5 × 29 × 58 cm) containing pineapples to facilitate mating and oviposition under controlled environmental conditions of 30 ± 1°C, 75 ± 5% relative humidity (RH), and a 16 h light:8 h dark photoperiod. Newly hatched larvae were initially reared on pineapples, while second-instar larvae were transferred individually to an artificial diet and kept in 400 mL perforated plastic cups under the same temperature, humidity, and photoperiod conditions (Hussain et al., 2016).

Laboratory Bioassays

Laboratory bioassays were conducted during 2022–2023 at the Plant Protection Research Laboratory, Agricultural Research Center (ARC), Dakahlia, Egypt. Laboratory colonies of *R. ferrugineus* were maintained under controlled environmental conditions (30 ± 1 °C, 75 ± 5% relative humidity, and a 16:8 h light:dark photoperiod). Fourth-instar larvae, eighth-instar larvae, and adults were used for toxicity evaluation.

Rosmarinic acid obtained from the isolated active fraction and confirmed by HPLC–MS analysis was used in all biological assays. Insects were exposed to artificial diets supplemented with Rosmarinic acid at concentrations of 3, 4, and 5 mL L^{-1}. Deltamethrin (Decis® 25 EC) at 0.25 mL L^{-1} was used as the reference insecticide, while untreated insects receiving the same artificial diet without Rosmarinic acid or insecticide served as the control.

For each developmental stage, 100 individuals were evaluated per treatment. The experiment consisted of five independent replicates, with 20 insects per replicate. Larvae and adults were continuously exposed to treated diets for 10 days. Mortality was recorded daily, and mortality percentages were corrected for control mortality using Abbott's formula (Abbott, 1925).

Corrected mortality data were subjected to probit analysis to estimate median lethal concentrations (LC_{50}) using POLO-PC software (LeOra Software LLC, Berkeley, CA, USA) according to Russell et al. (1977). Cumulative mortality data at different exposure periods were analyzed using repeated-measures ANOVA after angular transformation. Treatment means were separated using Fisher's least significant difference (LSD) test (SAS Institute, 2017).

Growth Inhibition and Nutritional Indices

The effects of Rosmarinic acid on larval feeding performance and nutritional utilization were evaluated using fourth- and eighth-instar larvae of *R. ferrugineus*. These developmental stages were selected because they allow accurate measurement of larval biomass gain, food consumption, and frass production.

Artificial diets were prepared by incorporating Rosmarinic acid at the previously determined LC_{50} concentrations for each larval instar. The untreated control diet contained no Rosmarinic acid. Twenty-five larvae of each instar were individually placed in aerated plastic containers containing treated or untreated diets. Five independent

biological replicates were established for each treatment, with five larvae considered as one replicate.

Larvae were maintained under controlled laboratory conditions (30 ± 1 °C, 75 ± 5% relative humidity, and a 16:8 h light:dark photoperiod). After three days of continuous feeding, larval weight gain, food consumption, and frass production were measured using an analytical balance.

Nutritional indices were calculated according to standard procedures:

- Approximate digestibility (AD):

AD (%) = [(food ingested – frass weight) / food ingested] × 100

- Efficiency of conversion of ingested food (ECI):

ECI (%) = [weight gained / food consumed] × 100

- Efficiency of conversion of digested food (ECD):

ECD (%) = weight gained / (food ingested – frass weight)

Data were analyzed using one-way ANOVA, and treatment means were compared using Fisher's LSD test (SAS Institute, 2017).

Detoxification-Related Gene Expression Analysis

The molecular response of *R. ferrugineus* larvae to Rosmarinic acid exposure was investigated by analyzing the expression patterns of major detoxification-related gene families, including cytochrome P450 monooxygenases, glutathione S-transferases (GSTs), and esterases.

Fourth- and eighth-instar larvae were fed artificial diets containing Rosmarinic acid at their respective LC_{50} concentrations. Control larvae received untreated diets. Five biological replicates were established for each treatment, with five larvae pooled per replicate.

After 72 h of exposure, larvae were dissected under sterile conditions in ice-cold physiological saline. Midgut tissues were collected, immediately frozen in liquid nitrogen, and stored at –80 °C until RNA extraction.

Total RNA was extracted using the RNeasy® Universal Mini Kit (Qiagen, Hilden, Germany). Complementary DNA synthesis was performed using the PrimeScript First Strand cDNA Synthesis Kit (TaKaRa Clontech, Paris, France). Quantitative real-time PCR was conducted using a CFX96 Touch™ Real-Time PCR System (Bio-Rad, UK) with SYBR® Premix Ex Taq™ II.

The expression levels were normalized using β-actin (GenBank accession no. KM438516) as an internal reference gene. Relative expression changes were calculated using the $2^{-}\Delta\Delta Ct$ method (Livak and Schmittgen, 2001). Statistical differences among treatments were determined using one-way ANOVA followed by Fisher's LSD test.

Field Evaluation:

Field experiments were conducted during the 2023 growing season in a commercial date palm orchard naturally infested with *R. ferrugineus* in Baltim, Kafr El-Sheikh Governorate, Egypt. Two economically important cultivars, 'Barhi' and 'Hayani', were selected for evaluation.

Experimental Design and Trunk Injection Application

The experiment was arranged as a completely randomized design (CRD). Treatments included Rosmarinic acid at 3, 4, and 5 mL L^{-1} and Deltamethrin (Decis® 25 EC) at 0.25 mL L^{-1}. Water-injected palms served as untreated controls.

Each treatment consisted of three replicates, with each replicate represented by one naturally infested palm. Palms were selected based on comparable infestation symptoms and similar infestation severity before treatment.

Trunk injection was performed using a Syngenta® injection device following a standardized procedure. Four downward-angled holes (30 cm depth and 1.2 cm diameter) were drilled uniformly around the trunk above the soil surface. Rosmarinic acid solution was injected at 15 mL per hole (60 mL $palm^{-1}$ total), whereas Deltamethrin solution was applied at 25 mL per hole (100 mL $palm^{-1}$ total). After injection, holes were sealed with moist clay to reduce leakage and facilitate systemic movement of the injected solutions.

Evaluation of Treatment Efficacy

The efficacy of the insecticide treatments was evaluated 60 days after trunk injection using three complementary assessment methods to improve the reliability of field evaluation.

1) Visual assessment of external symptoms: Each treated palm was visually inspected for the presence of characteristic symptoms of *Rhynchophorus ferrugineus* infestation, particularly the exudation of yellowish-brown viscous sap from trunk tissues. The absence of these symptoms was considered an initial indication of palm recovery.

2) Destructive assessment: Following visual inspection, the infested trunk tissues were carefully dissected to determine the survival status of *R. ferrugineus* individuals. Palms were scored according to the presence of living insects as follows:
    - 0 = No living *R. ferrugineus* individuals detected (recovered palm).
    - 1 = One or more living *R. ferrugineus* individuals detected (non-recovered palm).

3) Acoustic assessment: Trunk infestation was also evaluated using the Palmear® acoustic detection system, which integrates acoustic sensing technology with artificial intelligence to detect internal feeding activity of *R. ferrugineus*. Detection results were interpreted according to the manufacturer's recommendations and converted into numerical scores as follows:
    - 0 = Green signal (no detectable infestation).
    - 0.5 = Orange signal (uncertain detection).
    - 1 = Red signal (active infestation detected).

The final assessment of treatment efficacy was based primarily on the destructive examination, while visual symptom assessment and acoustic detection were used as complementary methods to support the diagnosis and improve the accuracy of field evaluation.

Collection of Plant Samples for Residue Analysis:

To evaluate residue persistence and systemic distribution, palm frond bases and fruits were collected after treatment. Frond bases were collected one month after trunk injection. Four fronds from different directions around each palm were sampled, and basal sections were combined to form one composite sample per palm.

Fruit samples were collected at two developmental stages: Bisr (early ripening) one month after treatment and Tamr (fully ripe stage) two months after treatment. Approximately 250 g of fruits were collected from each direction of the palm, pooled into a composite sample (approximately 1 kg per palm), and transported under refrigerated conditions for residue analysis.

Rosmarinic Acid and Deltamethrin Residue Determination

Residue analyses were performed at the Central Laboratory for Analysis of Pesticide Residues and Heavy Metals in Food (QCAP), Agricultural Research Center, Giza, Egypt. The laboratory is accredited according to ISO/IEC 17025:2017 by the Egyptian Accreditation Council (EGAC) and the American Association for Laboratory Accreditation (A2LA).

Sample extraction was performed using the validated QuEChERS procedure. Rosmarinic acid residues were quantified using LC–MS/MS, whereas Deltamethrin residues were analyzed using GC–MS according to validated analytical protocols.

The analytical method showed a limit of quantification (LOQ) of 0.01 mg kg^{-1}. Quality control procedures, including calibration, recovery tests, and analytical validation, were performed according to laboratory accreditation requirements.

Field data were analyzed using analysis of variance (ANOVA). Treatment effects were considered significant at $p \leq 0.05$. Mean comparisons were performed using Fisher's LSD test (SAS Institute, 2017).

Results and Discussion:

Purification and quantitative profiling of rosmarinic acid from Lamiaceae plant sources.

Rosmarinic acid was isolated and purified from the aerial parts of *Origanum vulgare* L., *Melissa officinalis* L., *Salvia officinalis* L., *Mentha piperita* L., *Thymus vulgaris* L., and *Rosmarinus officinalis* L. using Soxhlet extraction followed by purification procedures. The extracted compound was obtained separately from each plant source and was not applied as a crude botanical extract in subsequent bioassays. Instead, purified Rosmarinic acid was used as the active compound in all toxicity, physiological, biochemical, and molecular experiments.

The extraction process was performed using different solvents, and n-butanol exhibited the highest recovery efficiency, which is consistent with its suitable polarity for extracting phenolic compounds (Sik et al., 2020). The identity and purity of the isolated compound were confirmed by high-performance liquid chromatography coupled with mass spectrometry (HPLC–MS), based on the characteristic retention time and mass spectral profile of authentic Rosmarinic acid. The quantitative content of Rosmarinic acid was calculated based on the amount of compound recovered per gram of dry plant material (mg g^{-1} dry weight), allowing direct comparison among different plant sources.

Quantitative analysis revealed significant variation in Rosmarinic acid content among the examined species. *O. vulgare* showed the highest concentration (46.7 ± 2.0 mg g^{-1} dry weight), followed by *M. officinalis* (32.0 ± 0.1 mg g^{-1} dry weight), whereas lower concentrations were detected in *S. officinalis*, *M. piperita*, *T. vulgaris*, and *R. officinalis* (Table 1). These differences may reflect species-specific variation in genetic background, enzymatic regulation, and environmental factors affecting the phenylpropanoid biosynthetic pathway (Sik et al., 2020). The obtained results indicate that *O. vulgare* and *M. officinalis* represent efficient natural sources for Rosmarinic acid production and provide suitable starting materials for further development of botanical insecticidal formulations.

Table 1. Yield and quantitative profiling of purified rosmarinic acid isolated from different Lamiaceae plant species based on HPLC–MS analysis.

Plant species	*Rosmarinic acid content (mg g^{-1} DW ± SE)
Origanum vulgare L.	46.7 ± 2.0
Melissa officinalis L.	32.0 ± 0.1
Salvia officinalis L.	20.6 ± 0.1
Mentha piperita L.	18.2 ± 0.3
Thymus vulgaris L.	16.1 ± 1.1
Rosmarinus officinalis L.	10.3 ± 0.3

*Values are expressed as mean ± standard error (SE) of three independent extractions. DW = Dry Weight.

Laboratory Toxicity of Rosmarinic Acid Against Adults and Larvae of the Red Palm Weevil

The mortality responses of different developmental stages of *R. ferrugineus* exposed to purified Rosmarinic acid and Deltamethrin are presented in Figure 1 (a–d).

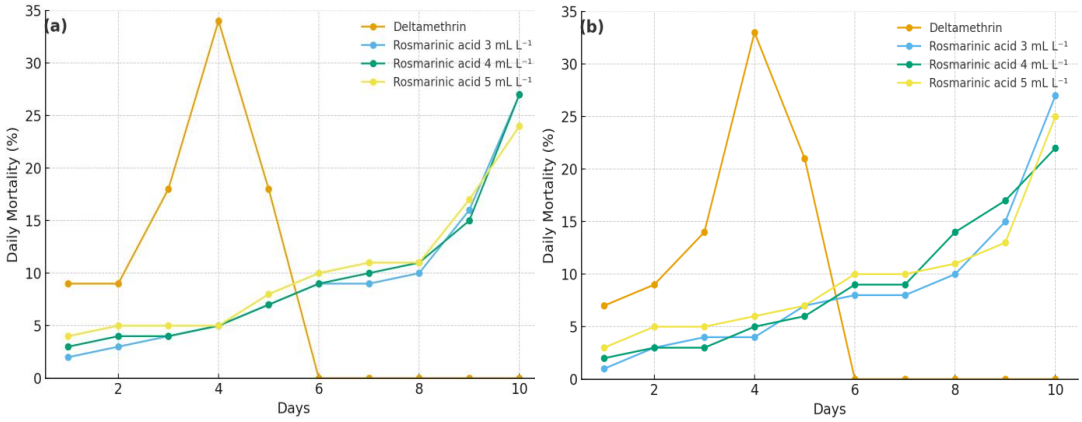
Both compounds induced detectable toxic effects under laboratory conditions, with mortality increasing progressively throughout the exposure period, indicating a time-dependent response to treatment.

The susceptibility of *R. ferrugineus* varied among developmental stages. Fourth-instar larvae exhibited the highest sensitivity to Rosmarinic acid, followed by eighth-instar larvae, whereas adults showed relatively lower susceptibility. In addition, Rosmarinic acid produced a concentration-dependent response, as increasing the concentration from 3 to 5 mL L^{-1} resulted in increased mortality across all tested stages.

The cumulative mortality results (Figure 1d) showed that Rosmarinic acid at 5 mL L^{-1} resulted in 100% mortality of fourth-instar larvae. For eighth-instar larvae, mortality reached 95% and 90% following exposure to Rosmarinic acid at 5 and 4 mL L^{-1}, respectively. In comparison, Deltamethrin at 0.25 mL L^{-1} caused mortality rates of 87%, 84%, and 77% in fourth-instar larvae, eighth-instar larvae, and adults, respectively. These results demonstrate that Rosmarinic acid exhibited significant insecticidal activity against different developmental stages of *R. ferrugineus*, with greater effectiveness observed at higher concentrations and against larval stages.

The differences in susceptibility among developmental stages may be related to developmental variation in physiological characteristics, feeding behavior, or other stage-specific factors that influence insect response to toxic compounds. Similar variations in susceptibility among insect stages have been reported for several botanical compounds and synthetic insecticides, where differences in insect development and metabolic capacity may affect toxicity outcomes.

Previous studies have also reported the insecticidal activity of Rosmarinic acid



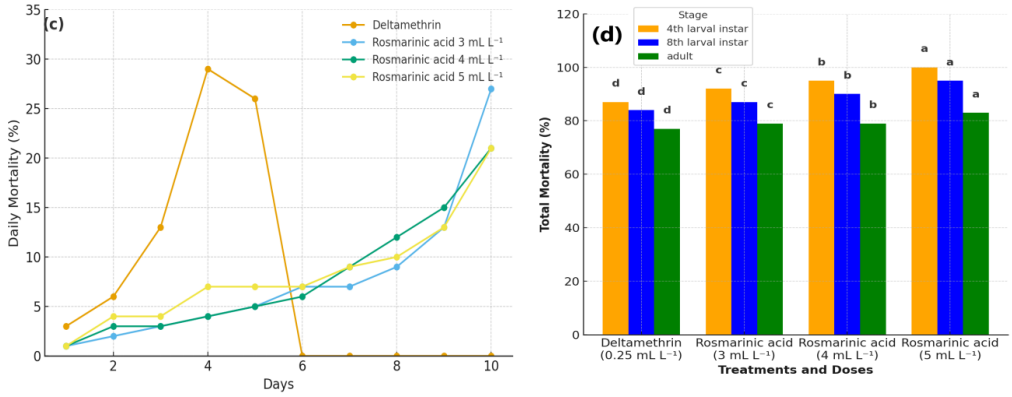


Figure 1. Mortality responses of *R. ferrugineus* to Deltamethrin (Decis® 25 EC) at 0.25 mL L^{-1} and Rosmarinic acid at three concentrations of 3, 4, and 5 mL L^{-1} under laboratory conditions: (a) fourth-instar larvae, (b) eighth-instar larvae, (c) adults, and (d) cumulative mortality of the three developmental stages.

The toxicity of purified Rosmarinic acid differed among the developmental stages of *R. ferrugineus*, although all tested stages exhibited susceptibility to the compound (Table 2). Fourth-instar larvae were the most sensitive stage, with an LC_{50} value of 2.041 mL L^{-1} (95% CI: 1.487–2.594 mL L^{-1}), followed by eighth-instar larvae (LC_{50} = 2.230 mL L^{-1}; 95% CI: 1.717–2.743 mL L^{-1}). Adults exhibited the lowest susceptibility, with an LC_{50} value of 2.575 mL L^{-1} (95% CI: 2.030–3.119 mL L^{-1}). The approximately 26.2% increase in LC_{50} from fourth-instar larvae to adults indicates a reduction in sensitivity with advancing developmental stage under the experimental conditions.

The probit slope values varied among developmental stages, with values of 1.16 ± 0.24, 0.81 ± 0.13, and 0.60 ± 0.10 for fourth-instar larvae, eighth-instar larvae, and adults, respectively. The higher slope value observed in younger larvae suggests a more consistent concentration–mortality response within this developmental stage, whereas the lower slope values in older larvae and adults indicate greater variability in individual responses to Rosmarinic acid exposure. These differences may reflect developmental

variation in physiological characteristics affecting insect susceptibility; however, further investigations are required to clarify the underlying mechanisms.

The goodness-of-fit analysis indicated that the probit model adequately described the concentration–mortality relationships for all tested stages. The calculated χ^2 values were 0.3250, 1.6652, and 3.3362 for fourth-instar larvae, eighth-instar larvae, and adults, respectively, with no significant deviations between observed and expected mortality values ($p > 0.05$). These results confirm the suitability of the fitted probit models and support the reliability of the estimated LC_{50} values.

Previous studies have reported insecticidal activity of Rosmarinic acid against other insect species. For example, Rosmarinic acid exhibited toxicity against the stored-product pest *Rhyzopertha dominica*, with an LC_{50} value of 42.4 µg mL^{-1} (Mughees et al., 2021). Additionally, Rosmarinic acid has shown biological activity comparable to some conventional insecticides in laboratory assays against different insect pests, including *Bactrocera dorsalis*, suggesting its potential as a botanical insecticide candidate. However, further studies addressing formulation development, non-target effects, and field-scale performance are required before practical implementation.

Table 2. Dose mortality and probit analysis of different developmental stages of *R. ferrugineus* treated with pure Rosmarinic acid.

Stage	LC_{50} (mL L^{-1})	95% CI (low – high)	Slope ± SE	χ^2
4th larval instar	2.041	1.487 – 2.594	1.16 ± 0.24	0.3250
8th larval instar	2.230	1.717 – 2.743	0.81 ± 0.13	1.6652
Adult	2.575	2.030 – 3.119	0.60 ± 0.10	3.3362

LD_{50} values were calculated after three days of feeding. LD_{50} values were not determined at 10 days because all treatments produced mortality exceeding 50% before the end of the experimental period.

Larval Growth- and Development-Retarding Effects of Rosmarinic Acid at LC_{50} Concentrations

The effects of Rosmarinic acid on larval nutritional performance and growth-related parameters of *R. ferrugineus* were evaluated using LC_{50} concentrations previously estimated for the fourth- and eighth-instar larvae (2.041 and 2.230 mL L^{-1},

respectively). After 72 h of continuous feeding on Rosmarinic acid-treated artificial diets, significant differences were observed in the nutritional indices, including efficiency of conversion of ingested food (ECI), efficiency of conversion of digested food (ECD), and approximate digestibility (AD) (Table 3).

Fourth-instar larvae exposed to Rosmarinic acid at their corresponding LC_{50} concentration (2.041 mL L^{-1}) exhibited a significant reduction in ECD compared with eighth-instar larvae treated with 2.230 mL L^{-1} ($p < 0.0001$). Similarly, ECI values were significantly reduced in fourth-instar larvae, indicating a lower efficiency in converting ingested food into larval biomass following Rosmarinic acid exposure. These results suggest that Rosmarinic acid affected food utilization efficiency, particularly in younger larvae, under the tested experimental conditions.

In contrast, AD values showed a different response pattern, with significant increases observed following Rosmarinic acid exposure in both larval stages ($p < 0.0001$). The fourth-instar larvae treated with 2.041 mL L^{-1} exhibited a 25.39% higher AD value compared with eighth-instar larvae treated with 2.230 mL L^{-1}. The increase in AD, accompanied by reductions in ECI and ECD, indicates that although larvae were able to digest a greater proportion of consumed food, the conversion of digested nutrients into body biomass was reduced.

The observed alterations in nutritional indices demonstrate that Rosmarinic acid exposure influenced larval feeding efficiency and nutrient utilization, with stronger effects recorded in fourth-instar larvae. Differences between larval stages may be related to developmental variation in feeding behavior, metabolic activity, or physiological requirements; however, further biochemical investigations are required to clarify the mechanisms underlying these responses.

Similar effects of Rosmarinic acid on insect growth and nutritional performance have been reported in other insect species. Khan et al. (2019) observed growth inhibition in *Acyrthosiphon pisum* following exposure to Rosmarinic acid, suggesting that this phenolic compound may interfere with physiological processes involved in insect development. Together with the present findings, these results support the potential role of Rosmarinic acid in disrupting insect growth and feeding performance and highlight its relevance for further evaluation as a botanical insecticidal compound.

Table 3. Nutritional indices (mean ± SE) of 4th and 8th larval instars of R. ferrugineus fed on artificial diets incorporated with Rosmarinic acid at LD_{50} (2.041 mL L^{-1} and 2.230 mL L^{-1}, respectively) compared with control diets.

Treatment	Larval instar	*AD	*ECI	*ECD
Control (untreated)	4th	65.02 ± 0.15b	19.03 ± 0.30a	35.04 ± 0.74a
	8th	63.45 ± 0.28b	17.91 ± 0.10b	32.19 ± 0.31b
Rosmarinic acid (2.041 mL L^{-1})	4th	74.01 ± 0.35a	5.75 ± 0.53d	7.76 ± 0.71d
Rosmarinic acid (2.230 mL L^{-1})	8th	59.02 ± 0.44c	16.01 ± 0.11c	27.13 ± 0.20c

AD = approximate digestibility; ECI = efficacy of conversion of ingested food; ECD = efficacy of conversion of digested food. Means ± SE values having the same letter(s) within the column are not significantly different (Fisher's LSD test, α = 0.05).

Physiological Impacts of Rosmarinic acid at LC_{50} on the Host Detoxification Defense Mechanism

Figure 2 illustrates the relative expression of three major detoxification gene families, namely Cytochrome P450, Glutathione S-transferase (GST), and Esterase, in the 4th- and 8th-instar larvae of *R. ferrugineus* following exposure to Rosmarinic acid at their respective LC_{50} concentrations (2.041 and 2.230 mL L^{-1}). Detoxification-related gene expression was generally higher in the 8th instar than in the 4th instar. The greatest transcriptional response was observed for GST, followed by Cytochrome P450, indicating that these enzymes constitute the primary metabolic response to Rosmarinic acid exposure. In contrast, Esterase expression remained relatively low and showed no significant variation between larval instars, suggesting a limited contribution to the detoxification process under the experimental conditions (Figure 2).

Cytochrome P450 monooxygenases, glutathione S-transferases (GSTs), and esterases represent the principal detoxification enzyme families in arthropods and play fundamental roles in the metabolism of xenobiotics and insecticide resistance. GSTs catalyze the conjugation of reduced glutathione with electrophilic compounds, producing more water-soluble metabolites that can be readily eliminated (Xu et al., 2015). Esterases hydrolyze ester-containing compounds into their corresponding acids and alcohols, thereby contributing to the detoxification of a wide range of xenobiotics (Bhatt et al., 2021). Cytochrome P450 enzymes catalyze oxidative reactions that facilitate the

metabolism and clearance of both endogenous metabolites and foreign chemicals (Scott et al., 1998). Increased expression of these detoxification genes is widely recognized as an adaptive response that enhances insect tolerance to toxic compounds.

Despite the induction of detoxification-related genes, Rosmarinic acid produced high mortality in both larval instars, indicating that the induced detoxification response was insufficient to counteract its toxic effects. These findings suggest that Rosmarinic acid disrupts essential metabolic defense pathways in *R. ferrugineus*, thereby contributing to its pronounced insecticidal activity.

The precise mode of action of Rosmarinic acid in insects has not yet been fully elucidated. However, plant-derived phenolic compounds are known to interfere with multiple physiological targets involved in insect survival. Previous studies have demonstrated that these compounds can inhibit detoxification enzymes, including GSTs and acetylcholinesterase, and modulate key neurophysiological targets such as γ-aminobutyric acid (GABA)-gated chloride channels and octopamine receptors, ultimately leading to neurotoxic effects (Yu and Abo-Elghar, 2000; Enan, 2005). Therefore, further investigations integrating biochemical, molecular, and neurophysiological approaches are required to elucidate the precise mechanism by which Rosmarinic acid exerts its insecticidal activity against *R. ferrugineus*.

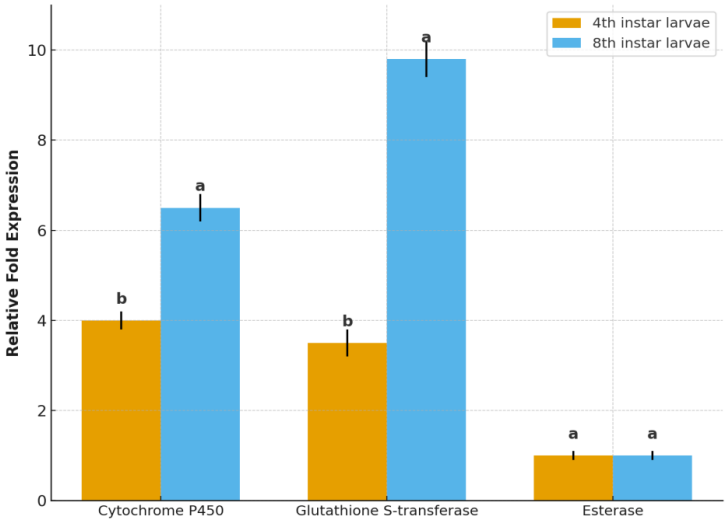


Figure 2. Expression pattern of detoxification genes of 4th- and 8th-instar red palm weevil larval midguts in response to Rosmarinic acid LD_{50} (2.041 mL L^{-1} and 2.230 mL L^{-1}). Bars (means ± SE) followed by different letters are significantly different (Fisher's LSD test, α = 0.05).

Trunk infestation experiment

Trunk injection treatments significantly improved the recovery of date palms naturally infested with *R. ferrugineus* 30 days after application (Table 4). All Rosmarinic acid treatments and the reference insecticide, Deltamethrin (Decis® 25 EC), resulted in significantly higher recovery rates than the water-injected control ($p \leq 0.0001$), in which no palm recovery was observed and all weevils remained alive. The highest recovery rate (93.33%) was achieved with Rosmarinic acid at 5 mL L^{-1}, where 14 out of 15 treated palms showed complete recovery. Rosmarinic acid at 4 mL L^{-1} and Deltamethrin at 0.25 mL L^{-1} each produced an identical recovery rate of 86.67% (13 of 15 palms), whereas the lowest Rosmarinic acid concentration (3 mL L^{-1}) resulted in 80.0% recovery (12 of 15 palms).

The efficacy of Rosmarinic acid increased with concentration, demonstrating a clear dose-dependent response under field conditions. The highest concentration (5 mL L^{-1}) produced the greatest recovery among all treatments and numerically exceeded

that obtained with Deltamethrin. However, this difference should be interpreted cautiously unless supported by a direct statistical comparison between treatments. Overall, these findings indicate that Rosmarinic acid is highly effective in suppressing red palm weevil infestations and has strong potential as a botanical component of integrated pest management programs.

The slightly higher recovery observed with Rosmarinic acid than with Deltamethrin may be associated with the increasing occurrence of pyrethroid resistance in some *R. ferrugineus* populations, including reduced susceptibility to deltamethrin, as previously reported (Wakil et al., 2018). Although resistance was not directly evaluated in the present study, this explanation is consistent with previous reports and further supports the need to develop alternative control strategies that reduce reliance on conventional synthetic insecticides.

In addition to its insecticidal activity, Rosmarinic acid possesses well-documented antioxidant and antimicrobial properties (Petersen and Simmonds, 2003). These biological activities may contribute to limiting secondary microbial infections and promoting tissue recovery following larval damage. Although these mechanisms were not investigated in the present study, they may partially explain the favorable field performance of Rosmarinic acid and warrant further investigation under commercial production conditions.

Table 4. Effect of Deltamethrin and Rosmarinic acid injected into date palm trunks on the number of trees recovering from red palm weevil infestation.

Treatment	Concentration (mL L^{-1})	Number of palm recovery	Palm recovery (%) after 30 days
Control (distilled water)	0	0	0.0^{d}
Deltamethrin (Decis® 25 EC)	0.25	13	86.67^{b}
Rosmarinic acid	3	12	80.0^{c}
	4	13	86.67^{b}
	5	14	93.33^{a}

*Values having the same letter(s) within the column are not significantly different (Fisher's LSD test, $\alpha = 0.05$).

Residue Analysis of Rosmarinic Acid and Deltamethrin in frond bases and date palm fruits

Residue analysis was conducted using palm frond bases collected one month after trunk injection and date fruits harvested at the Bisr (early ripening) and Tamr (fully ripe) stages two months after treatment. No detectable residues of Rosmarinic acid were found in any of the analyzed tissues collected from Rosmarinic acid-treated palms. Likewise, no pesticide residues were detected in samples obtained from the water-injected control. In contrast, residues of the reference insecticide Deltamethrin were detected in all analyzed tissues from treated palms, with mean concentrations of 0.55 mg kg^{-1} in palm frond bases, 0.27 mg kg^{-1} in Bisr fruits, and 0.15 mg kg^{-1} in Tamr fruits (Table 5).

These findings indicate clear differences in the persistence of the two compounds following trunk injection. Under the sampling conditions of the present study, Rosmarinic acid declined to concentrations below the analytical detection limit within one month in palm frond bases and within two months in date fruits, whereas measurable Deltamethrin residues remained detectable in both vegetative and reproductive tissues. This behavior is consistent with the reported fate of many plant-derived phenolic compounds, which are readily metabolized by endogenous plant enzymes and soil microorganisms into naturally occurring metabolites, resulting in relatively low environmental persistence (Isman, 2020; Lengai et al., 2020). Consequently, botanical pesticides generally exhibit shorter persistence and lower residue burdens than many conventional synthetic insecticides, including pyrethroids (Isman, 2020).

Although the absence of detectable Rosmarinic acid residues suggests a low potential for residue persistence under the conditions evaluated in the present study, these findings should be interpreted within the limits of the experimental design and the analytical detection method employed. Residue dissipation may vary according to formulation, application rate, environmental conditions, and plant physiological status. Therefore, additional studies investigating residue dissipation kinetics, metabolite identification, and multi-location field validation are required to comprehensively characterize the environmental fate and food safety of Rosmarinic acid under commercial production conditions. Nevertheless, the present results indicate that

Rosmarinic acid exhibits a favorable residue profile compared with the reference synthetic insecticide Deltamethrin, supporting its potential as a botanical insecticide for integration into sustainable integrated pest management (IPM) programs.

Table 5. Rosmarinic Acid and Deltamethrin residues in frond bases and fruits (Bisr and Tamr) of injected date palms.

Treatment	Concentration (mL L^{-1})	Residue concentration (mg kg^{-1})		
		Palm frond bases	Bisr (early ripening)	Tamr (fully ripe)
Control (distilled water)	0	NO	NO	NO
Deltamethrin (Decis® 25 EC)	0.25	0.55	0.27	0.15
Rosmarinic acid	3	NO	NO	NO
	4	NO	NO	NO
	5	NO	NO	NO

Conclusions:

Rosmarinic acid was effectively extracted from *O. vulgare* and *M. officinalis*, which exhibited the highest yields. Laboratory and field evaluations demonstrated strong insecticidal activity of Rosmarinic acid against *R. ferrugineus*, with the highest concentration providing efficacy comparable to or exceeding that of Deltamethrin. The fourth larval instar was the most susceptible developmental stage (LC_{50} = 2.041 mL L^{-1}). Rosmarinic acid significantly impaired larval growth and nutritional performance and modulated the expression of detoxification-related genes, particularly GST and Cytochrome P450, indicating disruption of the insect's metabolic defense mechanisms. Field application resulted in 93.33% recovery of infested date palms. Moreover, residue analysis revealed no detectable Rosmarinic acid residues in palm frond bases one month after trunk injection or in Bisr and Tamr fruits two months after treatment, whereas Deltamethrin residues remained detectable, confirming the superior environmental compatibility and food safety profile of Rosmarinic acid. Collectively, these findings demonstrate that Rosmarinic acid is a promising botanical insecticide and support its future commercial development as an eco-friendly component of integrated management programs against the red palm weevil.

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حمض الروزمارينيك كمبيد حيوي واعد ضد سوسة النخيل الحمراء في نخيل التمر

حمض الروزمارينيك كمبيد حيوي واعد لمكافحة سوسة النخيل الحمراء (*Rhynchophorus ferrugineus*) في نخيل التمر

إبراهيم الدسوقي عرفات

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الملخص:

تُعد سوسة النخيل الحمراء (*Rhynchophorus ferrugineus* Olivier) من أخطر الآفات التي تهدد زراعة نخيل التمر (*Phoenix dactylifera* L.) على مستوى العالم، لما تسببه من خسائر اقتصادية كبيرة وتأثيرات سلبية على استدامة الإنتاج. وقد أدى الاعتماد المكثف على المبيدات الكيميائية إلى ظهور سلالات مقاومة، إضافة إلى زيادة مخاطر التلوث البيئي وبقايا المبيدات في الأغذية، مما يستدعي البحث عن بدائل نباتية آمنة وفعالة ضمن برامج المكافحة المتكاملة للآفات. هدفت هذه الدراسة إلى تقييم الكفاءة الإبادية لحمض الروزمارينيك، وهو مركب فينولي طبيعي مشتق من النباتات، ضد سوسة النخيل الحمراء تحت الظروف المعملية والحقلية. أُجريت اختبارات السمية باستخدام الغذاء الصناعي المحتوي على تراكيز مختلفة من حمض الروزمارينيك، مع تقدير قيم LC_{50}، ودراسة التأثيرات الفسيولوجية من خلال مؤشرات كفاءة تحويل الغذاء المأكول (ECI) والمهضوم (ECD). كما تم تقييم آلية التأثير الجزيئية بقياس مستوى التعبير الجيني لإنزيمات إزالة السمية، وهي السيتوكروم P450، وجلوتاثيون إس-ترانسفيراز (GST)، والإستريزات العامة. ونُفذت التجارب الحقلية خلال عامي 2022–2023 في بلطيم بمحافظة كفر الشيخ باستخدام تقنية الحقن في جذع النخيل بتركيزات 3 و4 و5 مل/لتر، مع استخدام الدلتامثرين (0.25 مل/لتر) كمبيد مرجعي. وتم تقييم كفاءة المعاملات من خلال الفحص الإتلافي، وجهاز Palmear® للكشف الصوتي المبكر، وتحليل متبقيات المبيدات في قواعد السعف والثمار. أظهرت النتائج علاقة واضحة بين الجرعة والاستجابة، حيث بلغت قيم LC_{50} نحو 2.041 و2.230 و2.575 مل/لتر لليرقات في العمرين الرابع والثامن والحشرات الكاملة على التوالي. كما أدى حمض الروزمارينيك إلى خفض كفاءة الاستفادة من الغذاء وتثبيط واضح للتعبير الجيني لإنزيمات إزالة السمية، مما يشير إلى آلية تأثير متعددة الأهداف. وحقق التركيز 5 مل/لتر أعلى معدل تعافٍ للأشجار (93.33%) مقارنة بكلٍ من تركيز 4 مل/لتر والدلتامثرين (86.67%). ولم تُسجل أي متبقيات لحمض الروزمارينيك في قواعد السعف أو الثمار، بينما استمرت متبقيات الدلتامثرين في الظهور، مما يؤكد التوافق البيئي العالي وسلامة الغذاء المرتبطة باستخدام

حمض الروزمارينيك، ويدعم إمكاناته كمبيد نباتي مستدام ضمن برامج المكافحة المتكاملة لسوسة النخيل الحمراء.

الكلمات المفتاحية: حمض الروزمارينيك، سوسة النخيل الحمراء، *Rhynchophorus ferrugineus*، نخيل التمر، الإدارة المستدامة للآفات.