OPEN ACCESS Plant Protection, 10 (03) 2026. 475-483 DOI:10.33804/pp.010.03.6089

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Available Online at EScience Press
Plant Protection
ISSN: 2617-1287 (Online), 2617-1279 (Print)
http://esciencepress.net/journals/PP

Research Article

Insecticidal Potential of Rosemary (*Rosmarinus officinalis*) Essential Oil and Extracts against the Southern Cowpea Beetle (*Callosobruchus maculatus*)

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ARTICLE INFO

Article history
Received: 23rd February, 2026
Revised: 19h May, 2026
Accepted: 24th May, 2026

Keywords
Rosmarinus officinalis
Botanical insecticides
Callosobruchus maculatus
Essential oil toxicity
Stored grain protection

ABSTRACT

Stored-product beetles cause substantial quantitative and qualitative losses in stored legumes worldwide, necessitating the development of safer and environmentally sustainable alternatives to synthetic insecticides. The present study evaluated the insecticidal potential of *Rosmarinus officinalis* essential oil and extracts against larval and adult stages of beetles under laboratory conditions. Different concentrations (0.05, 0.10, 0.15, and 0.20 mg/mL) were tested at exposure periods of 24, 48, and 72 h to determine mortality responses and toxicity levels. The results demonstrated that rosemary essential oil and extracts significantly ($P \leq 0.05$) increased mortality of both larvae and adults in a concentration- and time-dependent manner. The essential oil exhibited greater toxicity than the solvent extracts. Maximum larval mortality (100%) was achieved with essential oil at 0.20 mg/mL after 72 h, whereas rosemary ethyl extract caused 89.58% mortality under the same conditions. Adult mortality also increased markedly at higher concentrations, reaching 93% after 72 h exposure to essential oil at 0.20 mg/mL. Probit regression analysis revealed strong linear dose-response relationships, with high coefficients of determination (R^2 = 0.89-0.95). Furthermore, LC_{50} values decreased with increasing exposure duration, indicating enhanced toxicity over time. The observed insecticidal activity may be attributed to the presence of bioactive compounds, particularly 1,8-cineole and other monoterpenes, which likely interfere with respiratory and neurological functions of insects. In conclusion, rosemary essential oil and extracts demonstrated strong bioefficacy against beetle pests and may serve as promising eco-friendly alternatives to synthetic insecticides for the protection of stored legumes.

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Introduction

Cowpea (*Vigna unguiculata* (L.) is a highly nutritious legume crop that is used for its high protein content (around 17-43%), carbohydrates, lipids, vitamins and minerals including calcium, phosphorus and iron (Howieson et al., 2008; Boukar et al., 2019). It is a key component of food and nutritional security, especially in arid and semi-arid areas of Africa and Asia where it is well known to tolerate drought and sub-optimal soil conditions (Boukar et al., 2019; Hall, 2021).

Cowpea is an important pulse crop in Iraq and is grown in different agro-ecological zones. According to CSO (2021), the cultivated area under this crop was about 29 thousand dunams (equal to 7,166 acres) and the total production was amounted to 197 thousand tons during 2021. In spite of its economic and nutritive value, cowpea production and storage are badly affected by insect pests, especially cowpea beetle (*Callosobruchus maculatus*) which is known as one of the most destructive storage pests of legumes throughout the world (Abi et al., 2022).

Cowpea beetle is a destructive stored grain insect pest throughout the tropical and subtropical countries of the world including Iraq (Ouaarous et al., 2025). The larvae of the beetle enter into seeds, cause infection and destroy endosperm and embryo tissues leading to significant quantitative and qualitative losses. The germination potential and nutritional quality of such seeds is greatly reduced (Bhalla et al., 2008; Odeyemi et al., 2009). This damage directly impacts food security and seed viability (Singal and Singh, 1990; Singh, 2016). In addition, *C. maculatus* is known to cause losses of 5 percent to more than 30 percent after harvest, depending on the infestation level and the storage conditions (FAO, 2022).

The routine and unselective use of synthetic insecticides for the management of stored grain insect pests has resulted in grave concerns regarding pesticide resistance, toxic residues in food stuffs and contamination of natural environments and ecosystems (Sparks and Nauen, 2015; Isman, 2020; Katopodi and Detsi, 2021). These health and environmental threats have accentuated the search for alternative, innocuous, environmentally-benign and sustainable pest management solutions (Talukder and Howse, 1993).

Botanical insecticides and essential oils have received greater and wider recognition, acceptance and interest in the recent past as a source of potential substitutes to these deleterious synthetic pesticides. Many secondary metabolites produced naturally by plants are insecticidal, repellent, antifeedant and growth-regulating. These naturally occurring compounds are crucial in defense mechanisms against insect herbivores and pathogens (Regnault-Roger et al., 2012; Isman, 2020).

Among plant-derived products, rosemary (*Rosmarinus officinalis*) essential oil has been extensively investigated against a large number of storage pests for its high bioactivity and insecticidal potential (Nathan et al., 2005; Pavela and Benelli, 2016).

Rosemary essential oil is mainly composed of monoterpenes and oxygenated monoterpenes, such as 1,8-cineole, α-pinene, borneol, and camphor with proven antimicrobial and insecticidal properties (Bakkali et al., 2008; Houzi et al., 2024). These compounds have exhibited diverse modes of action including impairment of nervous systems, disruption of respiration, and inhibition of different enzymatic activities of insects (Pavela and Benelli, 2016).

Essential oils are gaining more and more interest and recognition for their wide-spectrum biopesticidal properties such as antimicrobial, fungicidal, and insecticidal effects, in addition to their biodegradability and environmental safety relative to traditional pesticides (Isman, 2020; Raveau et al., 2020). They have a low persistence in the environment which decreases the associated environmental risks of residue accumulation and make them appropriate for post-harvest protection systems (Regnault-Roger et al., 2012).

The use of plant essential oils as sustainable grain protectants, especially for stored product pest management, has been highlighted by recent studies. This is owing to their ability to degrade rapidly and have several modes of action which make them promising alternatives to grain protectants that are less prone to resistance development (Pavela and Benelli, 2016; Isman, 2020). Moreover, they have a relatively short residual activity of about 7-15 days under storage conditions which also makes them good candidates for food safety application (Chaudhari et al., 2021).

In recent years, there has been an increased interest in biopesticides since several researchers have reported higher utilization of essential oils for IPM programs in stored grain protection (Douiri et al., 2014; Isman, 2020). As cowpea is directly consumed in Iraq both as food and seed, there is a dire need to design strategies for the management of cowpea beetle which should be innoxious, residue-free, and eco-friendly.

Hence the present study was undertaken to assess the efficacy of plant-based essential oil for the control of *C. maculatus* as a replacement for the detrimental synthetic insecticides in stored cowpea in the laboratory.

Materials and Methods

Insect culture

Adult insects were collected from naturally infested cowpea seeds and reared under controlled conditions in the laboratory using an incubator set at 26 ± 2°C, with 60-70% relative humidity (RH). The insects were reared on sterilized cowpea seeds (200 g) contained in glass jars. Mouths of each of the jars were wrapped with muslin cloth/cotton wool, tied with rubber bands to permit aeration and to prevent insect from escaping. In order to ensure nonstop supply of insect progenies, fresh sterilized cowpea seeds were supplied to the jars at regular intervals. Eggs were then carefully removed and freshly emerged adults were picked up and utilized in subsequent bioassays. Standardization of cultures in the laboratory provided uniformity of age and physiological state of test insects and hence making the experimental results more reliable.

Collection and preparation of plant material

Rosemary (*Rosmarinus officinalis* L.) fresh plant materials were collected from local nurseries. The plant samples were carefully cleaned with soft brush to eliminate any dirt and impurities adhered to the plant surface. To reduce moisture and retain volatile constituents, the plant materials were dried under the shade in a properly aerated laboratory at room temperature until the attainment of a constant weight. The samples were turned at regular intervals during drying to prevent contamination and decomposition from fungi. After drying, the plant material was pulverized using an electric grinder and stored in sterile, labeled air-tight containers at low temperature for further use in subsequent bioassays.

Essential oil extraction

Dried *R. officinalis* leaves and flowering buds were used for essential oils extraction by hydro-distillation technique in a Clevenger type apparatus. In brief, 100 g of the dried plant materials was hydro-distilled for 3 h. Extraction procedure was repeated three times to guarantee reproducibility. The essential oil thus obtained was separated from the aqueous phase and dried with anhydrous sodium sulfate to remove the traces of moisture. The purified oil was then added to dark glass vials and kept at 4°C for further use in experiments (Ladd Jr et al., 1978). The average essential oil yielded was 2.1% (v/w). The efficiency and reliability of maintaining phytochemical integrity of aromatic plants in hydro-distillation with a Clevenger apparatus made it one of the most widely used methods for extraction of volatiles (Bakkali et al., 2008; Dhifi et al., 2016).

Preparation of ethanolic extract

To prepare the ethanolic extract, 10 g of ground rosemary plant material was added to 200 mL of ethanol and shaken for 2 h using an electric blender for facilitation of the extraction of bioactive constituents. The resultant mixture was filtered and concentrated as a crude extract in an electric oven at 45-50°C (Abbott, 1987). The extract was then preserved in sterile containers in a refrigerator until subsequently used in bioassays. Ethanol is extensively used as an extraction solvent due to its ability to extract flavonoids, phenolics, terpenoids and other secondary metabolites with insecticidal activity.

Evaluation of insecticidal activity of essential oil and rosemary extract against adult insects

Contact toxicity bioassays were used to assess the insecticidal activities of rosemary essential oil and ethanolic extract against adults of cowpea beetle. The two highest concentrations (0.15 mg/mL and 0.20 mg/mL) were chosen for evaluation of adult mortality on the basis of preliminary screening tests. Clean and insect free cowpea seeds (30 g) were put into Petri dishes and sprayed with the test concentrations using a hand sprayer of 500 mL capacity for uniform application. Treated seeds were placed on sterile filter paper for air-drying for about 5 min prior to use.

In each Petri dish, 10 adult insects (5 males and 5 females) were placed and there were 5 replications for each treatment. The dishes were wrapped in muslin cloth and tied with rubber bands to allow aeration. In the control treatments, only distilled water was sprayed. The experimental units were maintained at a temperature of 30°C and a RH of 60%. Data on adult mortality was recorded after an exposure period of 48 h and 72 h (Ferrero et al., 2006). Insects were deemed dead if they did not move when gently prodded with a fine brush. The experiment was carried out using Completely Randomized Design (CRD) to eliminate the experimental error and bias due to treatments.

Effect of essential oil and ethanolic extract on larval mortality

Four concentrations viz., 0.05, 0.10, 0.15 and 0.20 mg/mL, of rosemary essential oil and ethanolic extract were used to assess the larval mortality of the beetle. Each treatment was replicated 5 times and there were 10 larvae in each replicate. Larvae were transferred to sterile Petri dishes and sprayed directly with various concentrations of essential oil and ethanolic extract using a hand sprayer. Distilled water was used to spray the control larvae.

After the treatment, all the Petri dishes were kept for incubation at 30°C and 60% RH. Larval mortality was recorded after exposure of 24, 48 and 72 h (Ferrero et al., 2006). Larvae were considered dead if they did not move when gently prodded with a fine needle. Progressive assessment intervals allowed the assessment of immediate and delayed toxic effects of the botanical treatments.

Statistical analysis

The experimental design used in the bioassays was CRD. The data collected were analyzed statistically using SPSS software. The Least Significant Difference (LSD) test was used to compare treatment means at $P \leq 0.05$. Abbott's formula (Abbott, 1925) was used to calculate corrected mortalities.

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

Results and Discussion

Insecticidal potential of rosemary essential oil and extracts against beetle larvae

In the present study, the insecticidal activity of *R. officinalis* was found to rise significantly with the rise in concentration and time of exposure. Larval mortality showed highly significant difference ($P \leq 0.05$) when treated with rosemary essential oil at all tested concentrations (from 0.05 to 0.20 mg/mL) and exposure periods (24, 48 and 72 h). Mortality was found to be dose and time dependent, suggesting a strong dose and time dependence response to toxicity. The larval mortality rate was the highest (100%) at 0.20 mg/mL after 72 h of exposure while the lowest mortality (18%) was recorded at 0.05 mg/mL after 24 h of exposure (Figure 1; Table 1). The statistical analysis also showed that concentration and exposure time had significant effects with the $LSD_{0.05}$ values being 9.00 and 7.80 respectively whereas the interaction between these two factors was not significant.

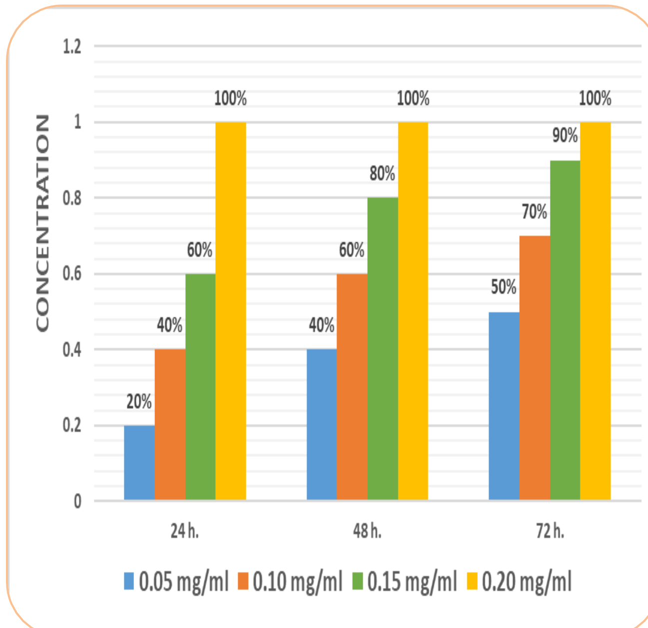

Figure 1. Mortality of beetle larvae treated with rosemary essential oil.

Table 1. Mortality of beetle larvae exposed to rosemary essential oil.

Concentration (mg/mL)	%Mortality			Mean
	Time (h)			
	24h	48h	72h	
0.05	18.00	36.73	45.83	33.52 d
0.1	42.00	59.18	70.83	57.34 c
0.15	64.00	77.55	87.50	76.35 b
0.2	88.00	95.92	100.00	94.64 a
Mean	53.00 c	67.35 b	76.04 a	
LSD 0.05	Concentration = 9.00, Time = 7.80, concentration × time = $15.59^{N.S}$			

The extracts of rosemary also showed significant insecticidal activity against the larvae of beetles, but the activity level was relatively low as compared to the essential oil. All the tested concentrations showed significant increase in mortality rate as compared with the untreated control. The highest mortality was recorded with 0.20 mg/mL concentration, showing 68%, 84%, and 90% mortalities after 24, 48, and 72 h respectively

(Figure 2). The results suggest that the toxicity of extracts towards beetle larvae is attributable to the bioactive components contained in rosemary extracts.

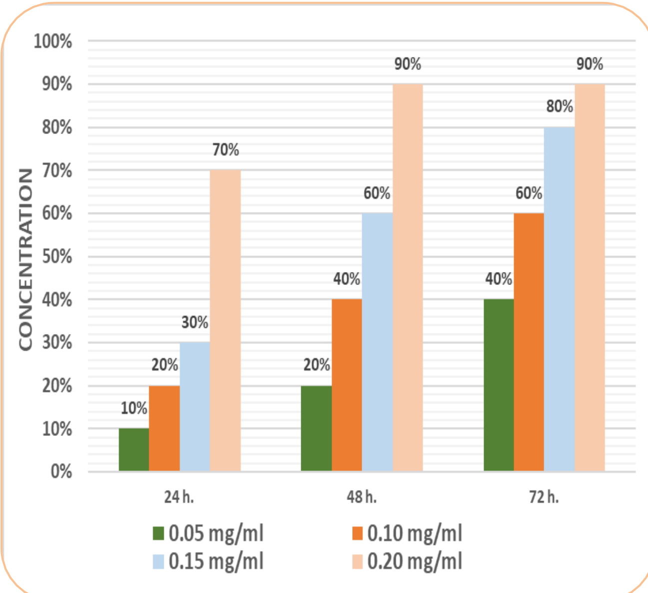

Figure 2. Mortality of beetle larvae treated with rosemary ethyl extract.

In the same way, the rosemary ethyl extract gave significant mortality of larvae in a dose and time dependent manner (Table 2). The highest mortality of 89.58% was observed at a concentration of 0.20 mg/mL after 72 h followed by 83.33% after 48 h at the same concentration. However, the minimum mortality of 13.06% was recorded at the concentration of 0.05 mg/mL after 24 h. The overall mean mortality ranged from 23.10% with the lowest concentration to 80.09% with the highest concentration. The ethyl extract of rosemary showed an increase in average mortality rate from 36.53% after 24 h to 64.06% after 72 h, indicating that the extract is toxic to beetle larvae in concentration and time dependent manner.

Probit analysis of the toxicity of rosemary essential oil

Probit regression analysis also revealed the toxic efficacy of rosemary essential oil and extracts against beetle larvae (Figure 3). There was a clear dose-response relationship as mortality was observed to be linearly related to the $\log_{10}$ concentration at all exposure periods. The regression models showed strong goodness-of-fit values (R^2 from 0.89 to 0.95), showing the reliability of the probit models in predicting larval mortality. It was found that mortality increased with increasing exposure time giving the highest toxicity after 72 h. Furthermore, LC_{50} values were found to lower gradually as the exposure time increased, indicating increased toxicity and efficacy of treatments as exposure time increased.

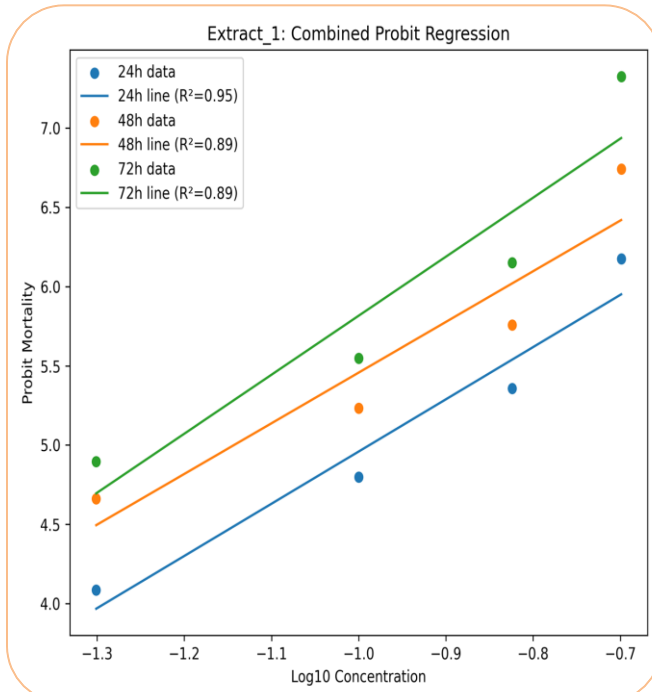

Figure 3. Combined probit regression graphs of the lethal concentration (LC_{50}) of beetle larvae exposed to rosemary essential oil extract.

Table 2. Mortality of beetle larvae exposed to rosemary ethyl extract.

Concentration (mg/mL)	%Mortality Time (h)			Mean
	24 h	48 h	72 h	
0.05	13.06	20.83	35.42	23.10 d
0.1	24.90	35.42	54.17	38.16 c
0.15	40.82	58.33	77.08	58.74 b
0.2	67.35	83.33	89.58	80.09 a
Mean	36.53 c	49.48 b	64.06 a	
LSD0.05	Concentration = 10.94, Time = 9.47 concentration × time = $18.95^{N.S}$			

Probit analysis of the toxicity of rosemary ethyl extract

Similarly, probit analysis of rosemary ethyl extract confirmed that there was significant positive correlation between concentrations and larval mortality (Figure 4). Increased mortality was observed with increasing exposure time and the lowest LC_{50} value was recorded after 72 h which shows the maximum toxicity as exposure durations prolonged. The high R^2 values between the regression models and the observed data (0.90-0.95) provided an additional confirmation of the consistency and predictive ability of the regression models.

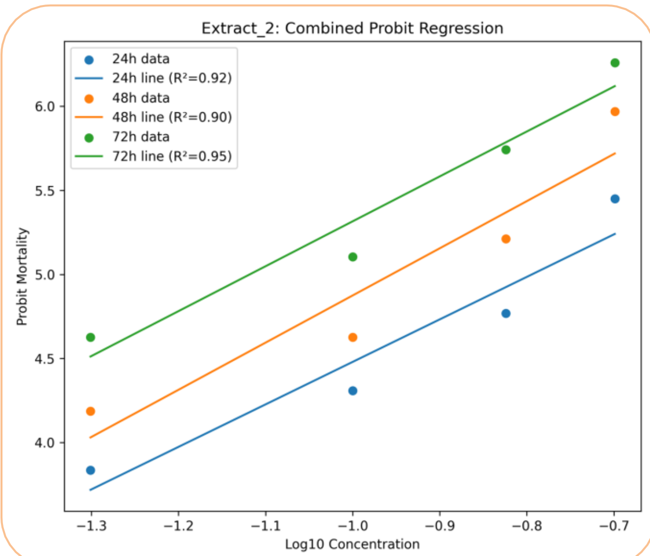

Figure 4. Combined probit regression graphs showing the LC_{50} of beetle larvae exposed to rosemary ethyl extract.

The results of the current investigation are consistent with earlier studies reporting the insecticidal effect of rosemary and other plants extracts against stored-product insects. Abbas et al. (2022) found that rosemary extract significantly enhanced the mortality rate of red rust mealy bugs and the effect was more pronounced as the concentration of rosemary extract increased. Likewise, Jassim et al. (2024) showed that the survival and life-table parameters of *C. maculatus* were negatively impacted when *Moringa oleifera* extracts were applied leading to a decrease in the number of insect populations. Khoter (2002) also reported that the mortality of southern cowpea beetle significantly increased with the successive increase of concentrations of the extracts and with longer exposure time. He also found that garlic extract and the rue extract at high concentrations showed mortality rates up to 92% and 60% respectively.

The insecticidal properties of rosemary can be explained by its high amount of bioactive phytochemicals such as glycosides, monoterpenes, oxygenated compounds, and phenolics. These phytochemicals can infiltrate the insect cuticle or permeate through the respiratory spiracles and result in paralysis, deranged physiological function and finally the death. Moreover, some plant-based compounds have antifeedant, repellent, growth inhibitory and oviposition deterrent properties which disrupt egg development, larval molting and adult emergence (Halawa et al., 1998).

Insecticidal potential of rosemary essential oil and extracts against beetle adults

Essential oil and extracts from rosemary also showed substantial toxicity against adults of cowpea beetle. Highly significant mortalities ($P \leq 0.05$) occurred at high concentrations (0.15 and 0.20 mg/mL) of the essential oil. The mortality after 48 h of exposure was 46% and 83% at concentrations of 0.15 and 0.20 mg/mL respectively, which further rose to 66% and 93% after 72 h at the same concentrations (Figure 5).

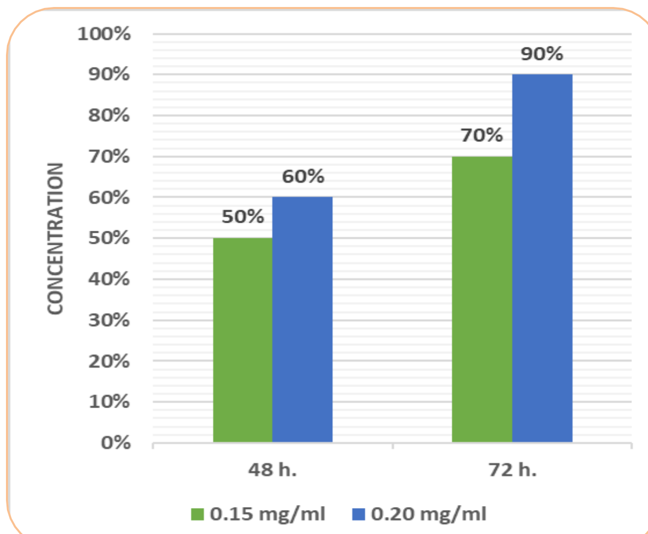

Figure 5. Mortality rate of adult beetles treated with rosemary essential oil extract.

Likewise, rosemary extract at higher concentrations, greatly enhanced mortality of adults of cowpea beetle. Mortality rate was 23% at 0.15 mg/mL and 56% at 0.20 mg/mL after 48 h which increased to 36% and 70% respectively after 72 h (Figure 6). These findings showed that the rosemary-based products were also highly effective against adult beetles, especially when exposed for a longer time.

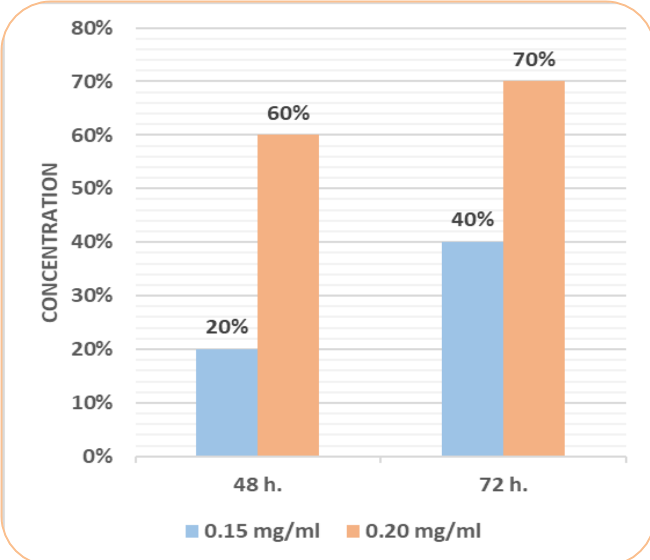

Figure 6. Mortality rate of adult beetles treated with rosemary ethyl extract.

The present results are in agreement with previous reports which prove the protective potential of rosemary against stored-product pests. El-Kareim et al. (2023) reported that crushed leaves of *R. officinalis* had a significant effect in reducing adult emergence of *C. maculatus* and provided effective protection to stored cowpea seeds. Rosemary powder was also found to cause 80.63% mortality of cowpea weevils after 96 h of exposure (Abuelnnor, 2023). Mortality increased progressively with increasing treatment duration which corroborated the present results.

Conclusions

The results of the present study demonstrate that *Rosmarinus officinalis* essential oil and extracts possess strong insecticidal activity against both larval and adult stages of beetle pests. Toxicity increased significantly with increasing concentration and exposure duration, with the essential oil exhibiting greater efficacy than solvent extracts. The observed bioactivity may be associated with the high abundance of 1,8-cineole and other biologically active monoterpenes present in rosemary essential oil, classifying the plant within the cineoliferum chemotype.

The findings suggest that rosemary-derived products could serve as promising eco-friendly alternatives to synthetic insecticides for the protection of stored legumes. Their biodegradability, short environmental persistence, and reduced risk to consumers make them suitable candidates for integration into sustainable pest management programs. The insecticidal action may involve disruption of respiratory spiracles, induction of anoxia, neurotoxic interference with acetylcholinesterase activity, and impairment of other vital physiological functions in insects. Overall, rosemary essential oil and extracts offer considerable potential for the development of safer botanical insecticides for stored-product pest management.

Authors' Contributions

AAAHAH and SSA conceived and designed the study on the insecticidal potential of rosemary (*Rosmarinus officinalis*) essential oil and extracts against the southern cowpea beetle, *Callosobruchus maculatus*. AAAHAH conducted the laboratory experiments, performed bioassays, collected and analyzed the data, and carried out the experimental investigations. AAAH provided overall supervision and ensured methodological rigor and analytical consistency throughout the research. AAAH and AAAHAH further contributed as co-supervisors by providing critical guidance on experimental procedures, data interpretation, and refinement of the study at different stages. SSA and AAAHAH prepared the initial draft of the manuscript, including data compilation, result interpretation, and organization of the scientific content. All authors subsequently reviewed and revised the manuscript critically for important intellectual content, validated the analyses and findings, and approved the final version for publication.

Research Funding

This research did not receive any grant from funding agencies.

Conflict of Interest

The authors declare no conflict of interest.

Sustainable Development Goals Targeted

SDG 2: Zero Hunger

SDG 12: Responsible Consumption and Production

SDG 15: Life on Land

Policy Addressed

The study supports sustainable pest management through the use of rosemary essential oil as an eco-friendly alternative to synthetic insecticides, consistent with Integrated Pest Management (IPM) principles and the FAO Code of Conduct on Pesticide Management.

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