Article

Insecticidal Activities of *Atriplex halimus* L., *Salvia rosmarinus* Spenn. and *Cuminum cyminum* L. against *Dactylopius opuntiae* (Cockerell) under Laboratory and Greenhouse Conditions

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Abstract: The wild scale insect *Dactylopius opuntiae* is one of the major insect pests of prickly pear *Opuntia ficus-indica* (L.) in Morocco. The present study investigated the insecticidal potential of the aqueous and hydroalcoholic extracts of *Atriplex halimus* (leaves), *Salvia rosmarinus* (leaves), and *Cuminum cyminum* (seeds) to control nymphs and adult females of *D. opuntiae* under laboratory and greenhouse conditions. Among the tested samples, the aqueous extract of *A. halimus* showed the highest activity on nymphs and adult females of *D. opuntiae* in the laboratory and when combined with black soap under greenhouse conditions. The difference in toxicity of the plant species in the study was correlated with their saponin content. These results provide evidence that the aqueous extract of *A. halimus* leaves could be incorporated into the management of the wild scale insect as an alternative to chemical insecticides.

Keywords: *Dactylopius opuntiae*; *Opuntia ficus-indica* L.; bioinsecticides; *Atriplex halimus*; saponins
1. Introduction

The Cactus pear, *Opuntia ficus-indica* (L.) Mill. (Caryophyllales: Cactaceae), is native to Mexico’s arid and semi-arid regions [1]. It was introduced to Morocco in 1770 as a good crop for years of low levels of rainfall, and for its multiple uses and applications [2]. The Cactus pear is widely represented in the Moroccan rural landscape because it requires low maintenance, and it is a natural boundary of crop fields. The cactus has experienced a resurgence of interest in several countries during the last decade because of its ecological, environmental, and socio-economic role. In most Mediterranean countries, cactus cultivation has become an intensive crop [3]. In Morocco, prickly pear plantations have been strongly encouraged by the Green Morocco Plan strategy (PMV-2008) as an alternative crop in less economically and culturally advantaged regions. The area of cacti has increased from 45,000 ha in the early 1990s to more than 150,000 ha in 2017 [4]. Several companies and cooperatives have been created to process the prickly pear, producing various products and by-products for different industrial, medicinal, pharmaceutical, and cosmetic sectors. This crop has suffered from the attack of the wild cochineal *Dactylopius opuntiae* (Cockerell) (Hemiptera: Dactylidiidae) in many countries, especially in the Mediterranean basin (Spain, Portugal, Morocco, Tunisia, Egypt, Israel, Lebanon, Turkey, and Greece), where it has become a serious pest of prickly pear crops [5]. This wild cochineal was first detected in the Sidi Bennour area, 70 km from El Jadida (western central Morocco) in September 2014 [6]. Due to the Mediterranean climate, *D. opuntiae* has spread rapidly, causing severe damage in most of the cactus-growing areas of Morocco, where farmers and cooperatives lost thousands of hectares of cactus, causing substantial socio-economic and environmental losses [6,7]. The females of this insect develop a white waxy layer that protects them from predators and parasitoids and provides them with perfect humidity; when they are crushed, they produce carminic acid, a natural dye used in the food, textile, and cosmetic industries [8]. Nymphs and adult females of *D. opuntiae* feed on plants by sucking sap from the cladode, leading to the destruction of the plant, and eventually killing it in the case of severe infestation.

In Morocco, significant progress has been made in developing integrated pest management (IPM) options to control this devastating insect [4]. Morocco’s National Office of Food Safety (ONSSA) has approved several pesticides to protect cactus crops and limit new infestations, including chlorpyrifos, pyriproxyfen, and mineral oils [9]. In addition to the resistance problem, these insecticides can severely affect human health and contaminate the environment, mainly plants and soil, and negatively impact natural enemies [6]. Therefore, the use of alternative approaches to control this pest (host plant resistance, natural enemies, plant extracts, vegetable and essential oils, and mycoinsecticides) has become a priority in the research field, as it is economically feasible and environmentally friendly [2].

Different botanical extracts are used as eco-friendly insecticides to control and manage different crop pests and plant diseases, due to the secondary compounds they contain, which have insecticidal properties against different insect pests [10]. In this context, Vigeras et al., (2009) [11] evaluated the efficacy of different plant extracts against *D. opuntiae* under laboratory conditions. They reported a high insecticidal effect of *Chenopodium ambrosioides* L., *Mentha piperita* L, *Mentha viridis* L., *Tagetes erecta* L., and *Tagetes florida* L. against second instars of *D. opuntiae* with 82, 92, 95, 98, and 99% of mortality at 72 h after treatments, respectively. Recently, Ramdani et al., (2021) [4] found that *Capsicum annuum* L. fruit extract at 200 g/L in combination with black soap at 60 g/L could be used as one IPM component to control *D. opuntiae*. Goncalves Diniz et al., (2020) [12] evaluated the effectiveness of the combinations of *Fusarium catingaense* L. isolates with extracts of *Nicotiana tabacum* L. and the mortality of the wild cochineal, and recorded 98.7% mortality in the greenhouse using 10% of aqueous extract of *N. tabacum* (w/v).

In this study, we selected three aromatic and medicinal plants, namely *Atriplex halimus* L. (leaves), *Salvia rosmarinus* Spenn. (leaves), and *Cuminum cyminum* L. (seeds), that grow or are cultivated in the region of Rhamna for their socio-economic importance for the habitats and their richness in different types and classes of secondary metabolites that can have a toxic
impact on insect pests and scale insects. The major phytochemicals in *R. officinalis* and *C. cymimum* are phenolic metabolites, which are categorized into phenolic acids, flavonoids, and non-flavonoids [13–16], which include the major acids that characterize *S. rosmarinus* (syn. *R. officinalis*), rosmarinic, chlorogenic, and caffeic acids [13,14] and those of *C. cymimum*, chlorogenic, neochlorogenic, salicylic, cinnamic, and coumaric acids and coumarins [15,16]. However, the presence of these constituents depends on environmental conditions and extraction methods [17]. Likewise, in flavonoids, phenolic acids, and saponins, especially syringetin derivatives and atriplaxeside A and B are the main constituent of *A. halimus* [18–20]. In this context, the objective of this work was to evaluate the toxicity effect of the selected botanical extracts applied alone or in combination with detergent as alternative biopesticides to control nymphs and adult females of *D. opuntiae* under laboratory and greenhouse conditions.

2. Material and Methods

2.1. Collection and Preparation of Plant Extracts

*Atriplex halimus* (Leaves), *S. rosmarinus* Spenn (leaves), and *C. cymimum* (seeds) were collected in Benguerir-Rhamna, Morocco (32°14′34.82″ N, 7°57′34.54″ W, Altitude 449 m). The materials were sorted, dried in a well-ventilated and dark place at room temperature, and ground using a laboratory mill (Retsch GM 200). The ground material was extracted by maceration at 50 °C for 30 min in 20/80 (v/v) MeOH/H$_2$O and 100% water. Next, the extractive solutions were filtered twice on Whatman filter sheets, evaporated under reduced pressure till dryness and then lyophilized (Labconco FreeZone Legacy 2.5 L) to afford respective crude extracts. The extraction yields were 26.94, 14.89, and 15.92% (for 100 g of dry plant) for the aqueous extracts and 24.58, 10.12, and 11.36% (for 100 g of dry plant) for the 20/80 (v/v) MeOH/H$_2$O extracts of *A. halimus*, *S. rosmarinus*, and *C. cymimum*, respectively.

2.2. Determination of Secondary Metabolites

2.2.1. Total Polyphenol

The total phenol contents (TPC) of the extracts were estimated following the Folin–Ciocalteu (FC) method with slight modification [21,22]. A total of 200 µL of each extract (1 mg/mL) was mixed with 1 mL of FC reagent (Fresh diluted 1:10 with distilled water), allowed to react for 5 min at room temperature in the dark, and then 800 µL of 7.5% (w/v) of (Na$_2$CO$_3$) was added. After 30 min standing in the dark, the absorbance was measured at 760 nm against a blank. TPC were calculated based on the calibration curve of gallic acid and expressed as gallic acid equivalents (GAE) in micrograms per gram of the dried extract.

2.2.2. Total Flavonoids

The total flavonoid contents (TFC) of extracts were determined using the aluminum chloride (AlCl$_3$) method [23]. A total of 1 mL of extract at 1 mg/mL in methanol was added to 0.5 mL of AlCl$_3$ (1.2%) and 0.5 mL of potassium acetate (120 mM) and allowed to react for 30 min at room temperature. The absorbance of each reaction mixture was measured at 415 nm to express the TFC as micrograms of quercetin equivalent per g of dry weight extract.

2.2.3. Total Saponins

Saponins analysis was performed according to the method applied by Irigoyen et al., (2018) [24] by adding 250 µL of the tested extract (2.5 mg/mL) to 875 µL of Lieberman–Buchard reagent (16.7% acetic acid in 83.3% concentrated sulfuric acid). The absorbance of the vortexed solution was determined at 528 min after 30 min. The total saponin content of the extracts was evaluated as mg of Quillaja saponin per g of dry extract.

2.2.4. UHPLC-MS Characterizations

The chemical profiling of extracts was conducted at Rothamsted Research (Harpenden, UK) on an Ultra-High-Performance Liquid Chromatography-Mass Spectrometry (UHPLC-MS) system equipped with a Dionex UltiMate 3000 RS UHPLC system coupled
Samples were injected (10 µL) onto a reversed-phase Hypersil GOLD C18 selectivity HPLC column (3 µm, 30 × 2.1 mm i.d. Thermo Fisher Scientific) maintained at 35 °C. The elution consisted of a gradient of water/0.1% formic acid (A) and acetonitrile/0.1% formic acid (B) (0 min, 5% B; 0.1–20 min, 5–100% B; 20–25 min, 100% B; 25–26 min, 100–5% B; 26–31 min, 5% B) using a flow rate of 0.3 mL/min. Mass spectra were collected using an LTQ Orbitrap Elite with a heated ESI source (Thermo Scientific, Bremen, Germany), acquired in negative mode with a resolution of 120,000 over m/z 50–1500. The source voltage, sheath gas, auxiliary gas, sweep gas and capillary temperature were set to 2.5 kV, 35 (arbitrary units), 10 (arbitrary units), 0.0 (arbitrary units), and 350 °C, respectively. Default values were used for other acquisition parameters. Automatic MS–MS fragmentation was performed on the top four ions using an isolation width of m/z. Ions were fragmented using high-energy C-trap dissociation with a normalized collision energy of 65 and an activation time of 0.1 ms. Data were collected and inspected using Xcalibur v. 2.2 (Thermo Fisher Scientific). UV spectra were derived from PDA in LC-MS analyzes.

2.3. Insect Rearing

Healthy young cladodes of prickly pear cactus were planted in plastic pots (27 cm in diameter by 24 cm in height) filled with a mixture of soil, sand, and peat at greenhouse temperature of 30 ± 2 °C. They were exposed to heavily infested cladodes with D. opuntiae collected from Marchouch region (33°56′10″ N 6°69′21″ W). Each infested cladode was placed between two pots of healthy ones for 20 days to allow the development of mature pest females, which were carefully selected for further bioassays.

2.4. Laboratory Bioassays

The toxicity of plant extracts against nymphs and adult females of D. opuntiae was assessed at a temperature of 26 ± 2 °C, a relative humidity of 75% and a photoperiod of 14/10 h of light/darkness. A preliminary test was performed for all extracts at 5% against adult females of D. opuntiae by direct contact application to select the most effective extracts. Further bioassays were conducted at four concentrations (0.625, 1.25, 2.5, and 5%) of each plant extract using a 1 L hand sprayer (0.5 mL of the tested extract per petri dish) in a completely randomized design with five replications per concentration for each treatment. Ten females of the same age and ten first instar nymphs of D. opuntiae were deposited on pieces of cladodes of the same size with an entomological brush and placed in glass Petri dishes (90 mm × 15 mm). Control nymphs and female cochineal were treated with water. The mortality of adult females was evaluated every 24 h within the eight days of treatments using a binocular microscope (Motic DM-143), whereas nymphs’ mortality was recorded 1, 3, 24, 48, 72, and 96 h after exposure. Dead females lost their natural color to dark brown and desiccation of their bodies, while dead nymphs showed no movement and severe color changes.

Mortality was calculated according to Abbott’s formula [25]:

\[
\text{Corrected Mortality (\%) = \left(\frac{\% \text{mortality in treatment} - \% \text{mortality in control}}{100 - \% \text{mortality in control}}\right) \times 100}
\]

2.5. Greenhouse Trials

Healthy young cladodes of O. ficus-indica were planted in pots (27 cm in diameter by 24 cm high) filled with a mixture of soil, sand, and peat at a temperature of 30 ± 2 °C. The cladodes were infested artificially using the method described above. Treatments were applied to selected cladodes at medium infestation levels (26–50%) using a modified rating scale from Silva (1991). In general, the approximate number of colonies ranged from 41 to 80 per cladode. The ratings for the infestation level were performed using a scale of 1–6 using: (1) 1 to 5 colonies, (2) 6 to 15 colonies, (3) 16 colonies up to 25% of the cladode covered by cochineal, (4) 26 to 50%, (5) 51 to 75%, (6) 76 to 100% of the cladode covered by cochineal.
Experiments were conducted in a randomized complete block design with four replications. Cladodes were sprayed beforehand with black soap at 10 g/L, which served to remove the cuticular wax and exposed the females and nymphs to tested plant extracts, and then the 5% treatments were applied twice using a 1 L hand sprayer (5 mL per cladode) at a seven day interval [4]. Cladodes treated with water alone and water mixed with black soap served as controls. Nymphs’ mortality was recorded at 1, 2, 3, and 4 days after the treatment and females’ mortality was assessed 3, 5, and 7 days after the first and second application, each.

2.6. Data Analysis
Mortality percentages were transformed into angular values (arcsine √P) before the statistical analysis. Under laboratory conditions, the transformed percentages were subjected to two-way analysis of variance (ANOVA) (concentration and extract source). Under greenhouse conditions, the transformed percentages were subjected to one-way analysis of variance. Lethal concentration for 50% and 90% mortality (LC50 and LC90, respectively) were determined using probit analysis [26]. The means were compared by Newman–Keuls tests at p < 0.05 using XLSTAT 2021.1 by Addinsoft.

3. Results
3.1. Laboratory Bioassays

3.1.1. Insecticidal Effects of Different Plant Extracts on D. opuntiae: Preliminary Bioassays
Initially, we evaluated the insecticidal activities of the six extracts at a concentration of 5% against adult females of D. opuntiae using foliar application under lab conditions. The results revealed a significant difference in mortality of females between the six tested extracts after 5 days of application, where the aqueous extracts were more effective than the hydroalcoholic extracts irrespective of the plant species; see Table 1. The corrected mortality rates significantly increased to 89.28% for both A. halimus aqueous and hydroalcoholic extracts 8 days after application (DF = 6, p < 0.0001). However, S. rosmarinus and C. cyminum extracts possessed the lowest insecticidal activity against D. opuntiae at 5%, with a corrected mortality rate not exceeding 17.85%.

Table 1. Mean percentage mortality ± SE of D. opuntiae adult females after exposure to six plant extracts at 5%.

| Treatments                  | 1 d   | 2 d   | 3 d   | 4 d   | 5 d   | 6 d   | 7 d   | 8 d   |
|-----------------------------|-------|-------|-------|-------|-------|-------|-------|-------|
| A. halimus aqueous extract  | 6.67 ± 3.33 | 13.33 ± 3.33 | 20.00 ± 5.77 | 30.00 ± 11.55 | 40.00 ± 5.77 | 53.33 ± 3.33 | 60.00 ± 10.00 | 90.00 ± 5.77 (89.29) |
| A. halimus hydroalcoholic extract | 0.00 b | 0.00 b | 0.00 b | 13.33 ± 8.82 | 27.33 ± 6.36 b | 34.33 ± 8.69 b | 55.67 ± 17.95 b | 90.00 ± 10.00 (89.29) b |
| C. cyminum aqueous extract  | 3.33 ± 3.33 | 3.33 ± 3.33 ab | 3.33 ± 3.33 b | 3.33 ± 3.33 b | 3.33 ± 3.33 c | 10.00 ± 5.77 b | 16.67 ± 12.02 (10.71) b |
| C. cyminum hydroalcoholic extract | 0.00 b | 0.00 b | 0.00 b | 3.33 ± 3.33 b | 3.33 ± 3.33 c | 16.67 ± 8.82 b | 23.33 ± 12.02 (17.85) b |
| S. rosmarinus aqueous extract | 0.00 ± 7.67 ± 3.84 ab | 15.33 ± 3.33 a | 19.00 ± 7.00 | 22.67 ± 10.67 ab | 22.67 ± 10.67 b | 22.67 ± 10.67 b | 22.67 ± 10.67 (17.14) b |
| S. rosmarinus hydroalcoholic extract | 0.00 ± 6.67 ± 3.33 ab | 10.00 ± 0.00 ab | 10.00 ± 0.00 b | 10.00 ± 0.00 b | 13.33 ± 3.33 b | 16.67 ± 3.33 (10.71) b |
| Check (water)               | 0.00 b | 0.00 b | 0.00 b | 3.33 ± 5.77 b | 3.33 ± 5.77 c | 3.33 ± 5.77 b | 6.67 ± 5.77 b |
| p-value                     | 0.109 | 0.019 | 0.000 | 0.036 | 0.002 | 0.000 | 0.005 | <0.0001 |

Means in the same column followed by different letter(s) are significantly different based on Newman–Keuls test (p < 0.05). Percentages in parentheses represent the corrected mortality of the last day.

3.1.2. Effect of A. halimus Extracts on D. opuntiae Nymphs and Adult Females
Based on the preliminary results, the insecticidal activities of the most toxic extracts (A. halimus, aqueous and hydroalcoholic) against the nymphs and adult females were tested using four different concentrations; see Tables 2 and 3. The results showed a significant
difference in mortality of nymphs from 24 h after treatment in a dose- and time-dependent manner; see Table 2. The ANOVA analysis showed a highly significant difference (DF = 2, \( p < 0.0001 \)) in mortality of \( D. \) opuntiae nymphs 4 days after the spray. The methanolic extract of \( A. \) halimus leaves and the aqueous extract at 5% concentration recorded the highest corrected mortality rates with 77.27% and 67.04%, respectively.

### Table 2. Mean percentage mortality ± SE of \( D. \) opuntiae nymphs after exposure to aqueous and methanolic extracts of \( A. \) halimus.

| Treatments                      | Concentrations (mg/L) | 1 h  | 3 h  | 1 d  | 2 d  | 3 d  | 4 d  | Mortality (%)               |
|---------------------------------|-----------------------|------|------|------|------|------|------|-----------------------------|
| \( A. \) halimus aqueous extract | 0.625                 | 0.00 | 4.00 | 4.00 | 4.00 | 7.20 | 12.00 | 14.00 ± 7.35<sup>b</sup>   |
|                                 | 1.25                  | 0.00 | 4.00 | 6.00 | 10.00| 16.00| 26.00 | 6.00 ± 4.00<sup>b</sup>   |
|                                 | 2.5                   | 0.00 | 5.20 | 7.20 | 11.20| 17.20| 35.00 | 4.09 (26.14)<sup>b</sup>  |
|                                 | 5                     | 2.00 | 5.20 | 8.00 | 20.00| 33.00| 71.00 | 8.43 (67.04)<sup>a</sup>  |
| \( A. \) halimus hydroalcoholic extract | 0.625             | 0.00 | 4.00 | 6.00 | 10.00| 14.00| 22.00 | 8.00 (11.36)<sup>a</sup>  |
|                                 | 1.25                  | 0.00 | 6.00 | 8.00 | 10.00| 16.00| 22.00 | 5.83 (11.36)<sup>a</sup>  |
|                                 | 2.5                   | 0.00 | 8.00 | 10.00| 12.00| 16.00| 34.00 | 7.31 (25.00)<sup>b</sup>  |
|                                 | 5                     | 2.00 | 14.00| 36.00| 52.00| 71.00| 80.20 | 8.43 (77.27)<sup>a</sup>  |
| Check (Water)                   | 0.00<sup>c</sup>      | 0.00 | 0.00 | 4.00 | 4.00 | 4.00 | 12.00 | 2.00 (2.00)                |
| \( p \)-Value (Model)           | 0.613                 | 0.483| 0.031| 0.000| 0.000| 0.09 | 0.702|                            |
| \( p \)-Value (Concentration × Treatments) | 0.530     | 0.702| 0.092| 0.035| 0.009| 0.702| 0.02 |                            |

Means in the same column followed by a different letter(s) are significantly different based on Newman–Keuls test (\( p < 0.05 \)). Percentages in parentheses represent the corrected mortality of the last day.

### Table 3. Mean percentage mortality ± SE of \( D. \) opuntiae females after exposure to aqueous and methanolic extracts of \( A. \) halimus.

| Treatments                      | Concentration (mg/L) | 3 d  | 5 d  | 6 d  | 7 d  | 8 d  | Mortality (%)               |
|---------------------------------|----------------------|------|------|------|------|------|-----------------------------|
| \( A. \) halimus aqueous extract | 0.625                | 6.00 | 16.00| 16.00| 20.00| 22.00 | 9.70 (2.50)<sup>cd</sup>  |
|                                 | 1.25                 | 12.00| 28.00| 34.00| 42.00| 44.00 | 5.83 (27.50)<sup>bcd</sup>|
|                                 | 2.5                  | 14.00| 30.00| 34.00| 44.00| 44.00 | 6.78 (30.00)<sup>bcd</sup>|
|                                 | 5                    | 26.00| 76.00| 78.00| 88.00| 88.00 | 5.47% (85.00)<sup>a</sup>  |
| \( A. \) halimus hydroalcoholic extract | 0.625           | 6.00 | 12.00| 26.00| 26.00| 26.00 | 7.50 (7.50)<sup>ed</sup>  |
|                                 | 1.25                 | 12.00| 26.00| 32.00| 42.00| 42.00 | 7.35 (27.50)<sup>bcd</sup>|
|                                 | 2.5                  | 14.00| 30.00| 34.00| 44.00| 44.00 | 5.10 (30.00)<sup>bcd</sup>|
|                                 | 5                    | 26.00| 76.00| 78.00| 88.00| 88.00 | 5.47% (85.00)<sup>a</sup>  |
| Check (Water)                   | 0.00<sup>c</sup>      | 0.00 | 0.00 | 0.00 | <0.001 | 0.00 |                            |
| \( p \)-Value (Model)           | 0.014                 | 0.092| 0.000| 0.000| <0.0001| 0.000 |                            |
| \( p \)-Value (Concentration × Extract) | 0.234     | 0.716| 0.203| 0.150| 0.294 | 0.096|                            |

Means in the same column followed by a different letter(s) are significantly different based on Newman–Keuls test (\( p < 0.05 \)). Percentages in parentheses represent the corrected mortality of the last day.

Data analysis showed a highly significant difference in the insecticidal effect of both \( A. \) halimus extracts on \( D. \) opuntiae females at different exposure times (\( p < 0.0001 \)) (Table 3). The mortality of adult females increased significantly after 5 days of application (DF = 3, \( p < 0.0001 \)). The increasing concentrations of the extracts significantly increased the mortality of females for different periods. At 8 days after treatment, the highest corrected mortality, 85.00% of females, was recorded for the aqueous extract of \( A. \) halimus at 5% concentration followed by hydromethanolic extract with 52.50% mortality for the same concentration (DF = 3, \( p < 0.0001 \)).

Lethal concentrations LC<sub>50</sub> and LC<sub>90</sub> varied between the tested plant extracts. The lethal concentration values of plant extracts tested against \( D. \) opuntiae nymphs showed that \( A. \) halimus aqueous extract recorded LC<sub>50</sub> = 3.25% and LC<sub>90</sub> = 5.47%, and \( A. \) halimus extract 20% methanol recorded LC<sub>30</sub> = 3.06% and LC<sub>90</sub> = 5.20% (Table 4) four days after treatment.
The lethal concentration values of plant extracts tested against *D. opuntiae* females indicated that the *A. halimus* aqueous extract (LC$_{50}$ = 2.99% and LC$_{90}$ = 5.29%) were more effective than *A. halimus* extract 20% methanol (LC$_{50}$ = 3.43% and LC$_{90}$ = 7.13%) seven days after treatment (Table 5).

### Table 4. Lethal concentrations (LC$_{50}$ and LC$_{90}$) of *A. halimus* extract, against *D. opuntiae* nymphs (at $p < 0.05$) after exposure to different times.

| Extract                  | Time | LC$_{50}$ | LC$_{90}$ | Coefficient of Determination | Graph Equation  |
|--------------------------|------|-----------|-----------|-----------------------------|-----------------|
| *A. halimus* extract in water | 4 days | 3.25      | 5.47      | $R^2 = 0.8955$              | $y = 18x - 8.5$ |
|                          | 3 days | 7.24      | 13.47     | $R^2 = 0.8049$              | $y = 6.42x + 3.5$ |
|                          | 2 days | 12.07     | 22.17     | $R^2 = 0.8556$              | $y = 3.96x + 2.2$ |
|                          | 1 day  | -         | -         | -                           | -               |
|                          | 3 h    | -         | -         | -                           | -               |
| *A. halimus* extract 20% methanol | 4 days | 3.06      | 5.20      | $R^2 = 0.7572$              | $y = 18.66x - 7.1$ |
|                          | 3 days | 3.71      | 6.05      | $R^2 = 0.6284$              | $y = 17.1x - 13.5$ |
|                          | 2 days | 4.77      | 7.89      | $R^2 = 0.638$               | $y = 12.8x - 11$ |
|                          | 1 day  | 4.57      | 8.91      | $R^2 = 0.7101$              | $y = 9.2x - 8$   |
|                          | 3 h    | -         | -         | -                           | -               |

### Table 5. Lethal concentrations (LC$_{50}$ and LC$_{90}$) of *A. halimus* extracts, against *D. opuntiae* females (at $p < 0.05$) after exposure to different times.

| Extract                  | Days | LC$_{50}$ | LC$_{90}$ | Coefficient of Determination | Graph Equation  |
|--------------------------|------|-----------|-----------|-----------------------------|-----------------|
| *A. halimus* extract in water | 8    | 2.54      | 4.58      | $R^2 = 0.9728$              | $y = 19.667x$   |
|                          | 7    | 2.99      | 5.29      | $R^2 = 0.7938$              | $y = 17.4x - 2$ |
|                          | 6    | 3.19      | 5.38      | $R^2 = 0.7921$              | $y = 18.2x - 8$ |
|                          | 5    | 3.53      | 6.09      | $R^2 = 0.7567$              | $y = 15.6x - 5$ |
|                          | 4    | 5.78      | 10.22     | $R^2 = 0.8526$              | $y = 9x - 2$    |
|                          | 3    | 8.23      | 14.68     | $R^2 = 0.9109$              | $y = 6.2x - 1$  |
| *A. halimus* extract 20% methanol | 8    | 2.10      | 4.57      | $R^2 = 0.9847$              | $y = 11.8x + 16$|
|                          | 7    | 3.43      | 7.13      | $R^2 = 0.972$               | $y = 10.8x + 13$|
|                          | 6    | 3.75      | 6.88      | $R^2 = 0.9942$              | $y = 12.8x + 2$ |
|                          | 5    | 4.43      | 8.21      | $R^2 = 0.9839$              | $y = 10.6x + 3$ |
|                          | 4    | 6.22      | 10.67     | $R^2 = 0.931$               | $y = 9x - 6$    |
|                          | 3    | -         | -         | -                           | -               |

### 3.2. Greenhouse Trials

According to the results mentioned above, we further evaluated the insecticidal effects of *A. halimus* aqueous extract against *D. opuntiae* nymphs and adult females under greenhouse conditions. No evidence of phytotoxicity was observed (chlorosis, change in coloration, and necrosis lesions) in treated cladodes during and after treatment. The statistical analysis showed significant differences in mortality of adult females and nymphs caused by the aqueous extract of *A. halimus* (5%) in combination with black soap during the different exposure periods (Tables 6 and 7). At day 2 after treatment, *A. halimus* with the detergent induced high mortality of nymphs with 90.00%, which increased significantly up to 100% at day 4 after application (DF = 2, $p < 0.0001$) (Table 6).
Table 6. Mean percentage ± SE of *D. opuntiae* nymphs after exposure to *A. halimus* extract and its combination with black soap under greenhouse conditions.

| Treatments/Exposure Time | 1 d    | 2 d    | 3 d    | 4 d    |
|--------------------------|--------|--------|--------|--------|
| *A. halimus* extract in water (5%) + Black soap (10 g/L) | 57.50 ± 7.50 a | 90.00 ± 5.77 a | 90.00 ± 5.77 a | 100.00 ± 0.00 a |
| Black soap + water      | 14.00 ± 5.89 b | 21.25 ± 8.51 b | 35.00 ± 11.37 b | 41.25 ± 12.97 b |
| Water                   | 0.00 b | 0.00 c  | 0.00 c  | 0.00 c  |

*p*-Value: <0.0001 <0.0001 <0.0001 <0.0001

Means in the same column followed by a different letter(s) are significantly different based on Newman–Keuls test (*p* < 0.05).

Table 7. Mean percentage ± SE of *D. opuntiae* females after exposure to *A. halimus* extract using two sprays.

| Treatments/Exposure Time | % Mortality of Females after First Spray | % Mortality of Females after Second Spray |
|--------------------------|----------------------------------------|------------------------------------------|
|                          | 3 d         | 5 d         | 7 d         | 10 d        | 12 d        | 14 d        |
| *A. halimus* extract in water (5%) + Black soap (10 g/L) | 15.75 ± 6.66 a | 27.50 ± 7.98 a | 42.50 ± 6.08 a | 63.75 ± 14.90 a | 68.00 ± 14.67 a | 83.75 ± 8.37 a |
| Black soap + water      | 0.00 b      | 0.00 b      | 2.00 ± 0.82 b | 3.00 ± 1.73 b | 3.00 ± 1.73 b | 3.50 ± 2.22 b |
| Water                   | 0.00 b      | 0.00 b      | 0.00 b       | 0.00 b      | 0.00 b      | 0.00 b      |

*p*-Value: 0.012 0.001 <0.0001 0.000 <0.0001 <0.0001

Means in the same column followed by a different letter(s) are significantly different based on Newman–Keuls test (*p* < 0.05).

The ANOVA showed significant differences in mortality of adult females caused by different treatments and the control starting from day 7 after the first spray (Table 7). The mortality of adult females remained low and did not exceed 40% during the first week after application. The second application significantly increased the mortality rate of the females from 63.75% (day 10 after spraying) to 83.75%, (day 14 after application) (DF = 2, *p* < 0.0001), while the solution in water of black soap at 10 g/L used as a control induced a very low female mortality rate (3.50%) following the same treatment (Table 7).

3.3. Phytoconstituents Composition

3.3.1. Total Polyphenol, Flavonoid, and Saponin Contents

To correlate the observed activities with the phytoconstituents of the tested samples, we initially quantified their total phenols, flavonoids and saponins contents; see Table 8. Overall, aqueous extracts were shown to contain higher levels of phenolic compounds compared to hydroalcoholic extracts. Water extracts of *S. rosmarinus* featured the highest total polyphenol (170.03 ± 12.33 mg GAE/g DW) and flavonoid (33.46 ± 1.46 mg QE/g DW) contents compared to *C. cyminum* and *A. halimus*. However, both aerial part extracts of *A. halimus* showed high contents of saponins (24.09 ± 0.71 mg SSE/g DW) compared to the other plant extracts, which explains the foamy aspect of the extracts.

Table 8. The total contents ± SE of polyphenols, flavonoids, and saponins in the tested extracts.

| Assay                   | *C. cyminum* | *S. rosmarinus* | *A. halimus* |
|-------------------------|--------------|-----------------|--------------|
|                        | Aqueous      | Hydroalcoholic  | Aqueous      | Hydroalcoholic | Aqueous | Hydroalcoholic |
| TPC (mg GAE/g DW)       | 45.51 ± 1.45 | 42.81 ± 3.52    | 170.03 ± 2.33| 166.76 ± 4.95 | 36.29 ± 2.18| 33.28 ± 2.06  |
| TFC (mg QE/g DW)        | 22.63 ± 0.24 | 20.82 ± 0.28    | 33.46 ± 1.46 | 24.68 ± 1.02  | 8.11 ± 0.30  | 4.57 ± 0.45   |
| Saponines (mg SSE/g DW) | 2.73 ± 0.34  | 1.81 ± 0.47     | 4.36 ± 0.52  | 3.63 ± 0.31   | 24.09 ± 0.71 | 18.90 ± 1.15  |

*DW*: dry weight, *QE*: quercetin equivalent, *SSE*: standard saponin equivalent, *GAE*: gallic acid equivalent.
3.3.2. Phytochemical Profiling of *A. halimus* Extract

Annotation of the secondary metabolites of the most active aqueous extract, *A. halimus*, was carried out using LC-MS/MS (Figure 1). Inspection of the total ion chromatogram allowed a list of major components to be generated (Table 9) and use of the MSMS data suggested the identities of the observed metabolites. A total of 58 compounds were detected according to the following criteria: (i) the calculated mass was close to the experimental mass (error \( \leq 5 \text{ ppm} \)); (ii) fragmentation patterns (MS/MS) were in agreement with the proposed structure; (iii) Putative compounds belonged to a class of compounds previously listed to occur in the studied plant species (or genus).

![Figure 1. LC-MS profile of *A. halimus* aqueous extract. Data collected in negative ion mode following reversed phase separation. Numbers relate to retention time.](image)

Most of them were triterpenoid saponins and hydroxylated and glycosylated compounds derived mainly from olean-12-en-28-oic acid or oleanolic acid (37 compounds), in addition to phytoecdysones (e.g., Dimorphamide A derivative). Simple organic acids such as citric acid, succinic acid, hydroxymethylglutaric acid, and phenolic acids including protocatechuic acid were also identified. Flavonoid aglycones and sulphated derivatives, including isorhamnetin and isorhamnetin sulfate, atriplexoside A, narcissin, and quercetin derivatives were also detected.

Under the collision energies employed, the fragmentation pattern of the saponins showed the molecular ion followed by low intensities of ions resulting from the successive loss of sugar moieties starting from the external unit. A loss of \( m/z \) 162 was characteristic of an ether glucosidation (rather than an ester glucosidation which releases predominantly \( m/z \) 180). Glucuronic acid ether-type conjugation was characterized by a loss of \( m/z \) 176. The mass spectrum also showed ions from the aglycone (\( m/z \) 455, 469, 471, 515, etc.) depending...
on the oxidation pattern of the molecule. As usual in olean-12-en-28 oic acid, ions from the retro-Diels–Alder (RDA) degradation mechanism were often evidenced with very low intensities at m/z 206 and 248 for the aglycone at m/z 455 or at m/z 207 and 262 (eventually m/z 221 and 248) for the aglycone at m/z 469. Flavonoids were also identified based on their fragmentation patterns displaying the characteristic aglycone ion in the MS². Isorhamnetin was believed to constitute the aglycone of most of the flavonoids owing to characteristic peaks in the MS² at m/z 152 and 163 from the RDA in ring C. The number of substitutions on the isorhamnetin backbone was determined to be identical to the number of protons lost from its corresponding mass (m/z 315) when comparing to the aglycone ion of a particular flavonoid (e.g., monodesmodic with aglycone at m/z 315 and bidesmodic with aglycone at m/z 314). Sugars were identified based on the losses of their corresponding masses (m/z 162 for glucoside, m/z 146 for rhamnose, m/z 132 for arabinose-like sugar). Finally, a compound was putatively annotated when it has been previously reported from either the studied plant or one of the species of the genus [18,20,27–29] and its MS characteristics matched with fragmentation patterns listed above. The other compounds were annotated by comparison of their respective MS characteristics with reported MS data in the literature [30–33].

Table 9. Chemical profiling of the aqueous extract of A. halimus.

| RT   | Exp. MS | Calc. MS | ΔMS | MS/MS                          | Proposed Compounds                                                                 | Ref. |
|------|---------|----------|-----|--------------------------------|------------------------------------------------------------------------------------|------|
| 1.05 | 191.02  | 191.02   | 0.0020 | 173, 129, 111              | Citric acid                                                                        | [31] |
| 1.35 | 117.02  | 117.02   | 0.0006 | 99, 73                       | Succinic acid                                                                      | [30] |
| 1.39 | 161.05  | 161.05   | 0.0005 | 161, 143, 99                | Hydroxymethylglutaric acid                                                        |      |
| 2.01 | 315.07  | 315.07   | 0.0000 | 315, 152                     | Protocatechuic acid, O-glucoside ether                                             | [32] |
| 2.71 | 359.10  | 359.10   | 0.0000 | 197, 153                     | Syringic acid O-glucoside ether                                                    | [33] |
| 3.64 | 285.06  | 285.06   | 0.0000 | 285                           | Protocatechuic acid, arabinoside ether                                             |      |
| 5.91 | 903.24  | 903.24   | −0.0021 | 741, 314                     | Bidesmodic isorhamnetin, tetraglucoside ether                                      |      |
| 6.40 | 557.06  | 557.06   | −0.0011 | 477, 315                     | Isorhamnetin, glucoside monosulfate                                                |      |
| 7.32 | 741.19  | 741.19   | −0.0011 | 710, 314                     | Bidesmodic isorhamnetin, diarabinoglucoside ether                                 |      |
| 7.40 | 769.22  | 769.22   | −0.0017 | 769, 314                     | Bidesmodic Isorhamnetin, diharhamoglucoside ether                                 |      |
| 7.48 | 755.20  | 755.20   | −0.0011 | 314                           | Atriplexoside A                                                                    | [18] |
| 7.64 | 609.15  | 609.15   | −0.0009 | 609, 315                     | Isorhamnetin, glucoarabinoside ether                                               |      |
| 7.69 | 639.16  | 639.16   | 0.0002 | 345                           | Spinacetin, arabinoglucoiside ether                                                |      |
| 7.75 | 525.31  | 525.31   | −0.0004 | 479, 319, 159                | 20-Hydroxyecdysone or 22-epi-20-hydroxyecdysone                                   | [27] |
| 7.80 | 541.30  | 541.30   | −0.0007 | 495                           | 20-Hydroxyecdysone derivative                                                      |      |
| 7.87 | 623.16  | 623.16   | −0.0008 | 623, 315                     | Azaleatin rutinoside or narcissin                                                  | [20] |
| 8.19 | 395.01  | 395.01   | −0.0001 | 315                           | Isorhamnetin sulfate                                                              |      |
| 8.19 | 507.11  | 507.11   | −0.0004 | 507, 344                     | Spinacetin, glucoside ether                                                        |      |
| 8.55 | 733.11  | 733.11   | −0.0004 | 653, 477, 315                | Isorhamnetin, O-ferruloylglucoiside, monosulfate                                   |      |
| 8.58 | 825.43  | 825.43   | −0.0016 | 825, 663, 487, 113           | Olean-12-en-28-oic acid, dihydroxy-, glucogluconic acid ether                      |      |
| 8.59 | 971.48  | 971.49   | −0.0010 | 810, 748, 629, 585, 539      | Olean-12-endoic acid, diglucoside ether-, glucoside ester                         |      |
| 8.72 | 649.18  | 649.18   | −0.0028 | 649, 563, 429, 315, 219      | Dimorphamide A derivative                                                         | [28] |
| 8.72 | 633.18  | 633.19   | −0.0061 | 457, 439                     | Dimorphamide A, mono acetyl derivative                                             | [28] |
| 8.78 | 449.14  | 449.14   | 0.003  | 449                           | Unknown                                                                            |      |
| 8.94 | 823.41  | 823.41   | −0.0014 | 823, 661, 555                | Olean-12-en-28-oic acid, monohydroxy-, monooxo-, glucogluconic acid ether          |      |
| 9.27 | 795.42  | 795.42   | −0.0006 | 795, 675, 663                | Olean-12-en-28-oic acid saponin                                                   |      |
| 9.35 | 823.41  | 823.41   | −0.0014 | 823, 661                     | Olean-12-endoic, monooxo-, diglucoside ether                                      |      |
| 9.55 | 497.07  | 497.07   | 0.0005 | 497, 377, 215                | Unknown                                                                            |      |
| 9.82 | 853.42  | 853.42   | −0.0013 | 854, 691, 647, 629, 515      | Olean-12-en-28-oic acid, dihydroxy, monooxo-, glucogluconic acid ether-, methyl ester |      |
| 9.95 | 645.18  | 645.19   | −0.0033 | 645, 469, 425, 219, 193      | Dimorphamide A derivative                                                         | [28] |
Table 9. Cont.

| RT  | Exp. MS | Calc. MS | ∆MS  | MS/MS | Proposed Compounds                                                      | Ref. |
|-----|---------|----------|------|-------|----------------------------------------------------------------------|-----|
| 10.02 | 807.42  | 807.42  | −0.0012 | 807, 687, 645, 583 | Olean-12-en-28-oic acid, monooxo-, glucogluconic acid ether |     |
| 10.08 | 825.43  | 825.43  | −0.0015 | 825,770, 707, 663, 645 | Olean-12-en-28-oate monohydroxy, monooxo, glucoside ether, glucoside ester |     |
| 10.23 | 809.43  | 809.43  | −0.0014 | 809, 647, 585, 175 | Olean-12-endioic acid, glucurononic acid ether-, glucoside ester |     |
| 10.27 | 851.41  | 851.41  | −0.0009 | 851, 689, 627, 513, 113 | Olean-12-endioic acid, glucurononic acid ether-, methyl ester |     |
| 10.40 | 999.48  | 999.48  | −0.0008 | 999, 627, 657, 613 | Olean-12-endioic acid, diglucoside ether-, glucoside ester, methyl ester |     |
| 10.6  | 867.44  | 867.44  | −0.0018 | 821, 659, 690, 175 | Olean-12-en-28-oic acid, monooxo, glucoside ether, acetylglucoside ester |     |
| 10.6  | 659.34  | 659.34  | −0.0009 | 659, 583, 483, 175, 113 | Olean-12-en-28-oic acid, dioxo, glucurononic acid ether |     |
| 10.83 | 969.47  | 969.47  | −0.0003 | 969, 649, 807, 789, 763 | Olean-12-en-28-oate dioxo, glucoside ether, glucoside ester |     |
| 10.92 | 809.43  | 809.43  | −0.0010 | 809, 629, 585, 539 | Olean-12-endioic acid, monooxo, glucoside ether, glucoside ester |     |
| 11.03 | 837.43  | 837.43  | −0.0020 | 837, 717, 675 | Olean-12-en-28-oate glucoside ether, glucoside ester |     |
| 11.05 | 849.43  | 849.43  | −0.0018 | 849, 687, 627, 583 | Olean-12-en-28-oate glucoside ether, glucoside ester |     |
| 11.09 | 897.45  | 897.45  | −0.0017 | 897, 627, 583 | Olean-12-endioic acid saponin |     |
| 11.20 | 851.44  | 851.44  | −0.0018 | 851, 627, 583, 539 | Olean-12-en-28-oate dioxo, glucoside ether, acetylglucoside ester |     |
| 11.35 | 955.49  | 955.49  | −0.0016 | 955, 793, 731, 613, 569 | Olean-12-en-28-oic acid, monooxo-, diglucoside ether, glucoside ester |     |
| 11.36 | 661.36  | 661.36  | −0.0007 | 661, 585, 485, 113 | Olean-12-en-28-oic acid, dioxo, glucurononic acid ether |     |
| 11.49 | 879.43  | 879.44  | −0.0045 | 879, 627, 583 | Olean-12-en-28-oate monohydroxy, monooxo, glucoside ether, acetylglucoside ester |     |
| 11.57 | 925.48  | 925.48  | −0.0002 | 925, 717, 675 | Olean-12-endioic acid saponin |     |
| 11.64 | 689.35  | 689.35  | −0.3543 | 689, 513, 113 | Olean-12-endioic acid, monooxo, glucoside ether, methyl ester |     |
| 11.86 | 691.37  | 691.37  | −0.0044 | 691, 515, 113 | Olean-12-endioic acid, monohydroxy, monooxo, glucurononic acid ether |     |
| 11.95 | 793.44  | 793.44  | −0.0012 | 793, 631, 455, 175, 113 | Olean-12-en-28-oic acid, glucurononic acid ether |     |
| 12.07 | 679.37  | 679.37  | −0.0005 | 679, 399, 369, 191 | Olean-12-en-28-oic acid saponin |     |
| 12.29 | 853.46  | 853.46  | −0.0009 | 853, 775, 673, 613, 569 | Olean-12-en-28-oic acid, glucurononic acid ether |     |
| 12.51 | 835.45  | 835.45  | −0.0009 | 835, 775, 673, 613, 569 | Olean-12-en-28-oic acid, glucurononic acid ether |     |
| 12.93 | 645.36  | 645.36  | −0.0011 | 645, 469, 113 | Olean-12-en-28-oic acid, monooxo, glucurononic acid ether |     |
| 13.12 | 807.42  | 807.42  | −0.0013 | 807, 627, 583, 469, 99 | Olean-12-en-28-oate, dioxo, glucoside ether, glucurononic acid ether |     |
| 13.35 | 675.37  | 675.38  | −0.0033 | 675, 499, 113 | Olean-12-en-28-oic acid, monohydroxy, glucurononic acid ether |     |
| 13.47 | 647.38  | 647.38  | −0.0005 | 647, 471, 113 | Olean-12-en-28-oic acid, monooxo, glucurononic acid ether |     |
| 14.15 | 793.43  | 793.44  | −0.0049 | 793, 613, 569, 523, 113 | Olean-12-en-28-oic acid, glucurononic acid ether |     |
| 14.57 | 687.38  | 687.38  | 0.0031  | 687, 645, 627, 583, 565 | Olean-12-en-28-oic acid saponin |     |
| 15.05 | 631.38  | 631.38  | 0.0004  | 631, 455, 113 | Olean-12-en-28-oic acid, glucurononic acid ether |     |
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4. Discussion

The present study demonstrated the insecticidal activity of the aqueous and hydroalcoholic extracts of *A. halimus* against *D. opuntiae*, quantified their total phenol, flavonoid and saponin contents, and annotated their secondary metabolites. Both extracts were able to kill nymphs and adult females under laboratory conditions, while only the aqueous extract preserved this activity at the greenhouse scale where the mortality rate exceeded 70%. The *A. halimus* aqueous extract showed substantial activity and was able to kill the nymphs and adult females by 100% and 83.75%, respectively, when tested at 5% in a combination with 10 g/L black soap. The observed activities might be attributed to the high contents of saponins that amounted to 24.09 mg SSE/g DW at *A. halimus* aqueous extract. These in vitro results were in line with the LC-MS studies in which we identified 58 secondary metabolites, and among them 37 suggested olean-12-en-28-oic or oleanolic acids derivatives.

*A. halimus* is a rich source of phenolic compounds, such as various flavonol glycosides isolated from 60% methanic fraction, namely syringetin 3-O-beta-D-rutinoside, syringetin 3-O-beta-D-glucopyranoside, and isorhamnetin 3-O-beta-D-rutinoside or narcissin [20], which agree with our investigation. In addition, the investigation of bioactive chemical compounds conducted by Kabbash and Shoieb (2012) on aerial parts of the plant identified two flavonol glycosides, designated as atriplexoside A [3’-O-methylquercetin-4’-O-ß-apiofuranoside-3-O-(6”-O-ß-rhamnopyranosyl-ß-glucopyranoside)] that was present in our extract and atriplexoside B [3’-O-methylquercetin-4’-O-(5”-ß-xylopyranosyl-ß-apiofuranoside)-3-O-(6”-O-ß-rhamnopyranosyl-ß-glucoside)], as well as two phenolic glycosides, an ecdysteroid, a megastigman, and two methoxylated flavonoid glycosides [18]. The present study’s results corroborate the findings of Donia et al., (2012) that also isolated hederaugenin-3-O-ß-D-glucuronopyranoside and oleanolic acid-3-O-ß-D-glucuronopyranoside (calenduloside E) from *Atriplex farinosa* Forssk [34]. The oleanolic acid 3-O-ß-D-glucopyranosyl-28-O-ß-D-glucopyranoside was extracted by Kumarihamy et al., (2015) from the EtOH extract of *A. canescents* (Pursh) Nutt. leaves [35].

Plant secondary metabolites, such as alkaloids, tannins, saponins, lectins, terpenoids, phenolics, phenylpropanoids, and glycosides, have shown significant insecticidal activity against various crop pests including the wild cochineal, *D. opuntiae* [4,10,36–38]. Our findings are consistent with instances in the literature [39,40]. El-Gougary (1998) [39] has demonstrated the insecticidal activity of the ethanol extract of *A. halimus* against the cotton caterpillar *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) with an LD50 value of 5.6 mg/larva. The ethanolic extract and petroleum ether of *A. halimus* were also used in a ratio of 1:1 (v/v) with organo-phosphorus insecticide, pirimiphos-methyl (acetic) and chlorpyrifos-methyl (reldan), on the adult insect *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). The petroleum-ether extracts strongly furnished synergic effects with chlorpyrifos-ether, while the ethanol extracts displayed synergistic effects with pirimiphos-methyl with ratios of 2.8× and 1.4×, respectively [39]. Kamal et al., 2017 [40] studied the insecticidal activities of saponins and various solvent extracts of *Atriplex lacinia* against various pests. Crude saponins from *Atriplex lacinia* L. were most effective against *Heterotermes indicolus* (Wasmann) (Isoptera: Rhinotermitidae), *Rhizopertha dominica* F. (Coleoptera: Bostrichidae), and *T. castaneum* (Herbst), causing 90.36 ± 0.6, 80.1 ± 0.7, and 86.5 ± 0.6% mortalities, respectively. The saponin extract also showed the highest activity against *Monomorium pharaonic* (Linnaeus) (Hymenoptera: Formicidae), with an LD50 of 78 mg/MI.

Likewise, the low to moderate activity encountered for the two other plants of our study was consistent with a previous study by Idris et al., (2021) [41]. Indeed, the ethanolic extract (50%) of *S. rosmarinus* (syn. *R. officinalis*) leaves showed moderate toxicity on the adult females of the wild cochineal *D. opuntiae* after five days of application in both laboratory and field conditions. This mortality rate reached 38.7 ± 4.0% in laboratory conditions and 25.6 ± 5.1% in field treatments after 5 days of exposure. The wild cochineals were, however, sensitive to *Virginia tobacco* L. extract, which induced considerable toxicity
on nymphs and adult females after 5 days of application under laboratory conditions with a mortality rate of 91.7 ± 5.1% and 83.4 ± 3.1%, respectively. In addition, the mortality rates were 87.1 ± 3.8% and 78.5 ± 3.6% for nymphs and adult females, respectively, after 5 days of treatment in the field [41].

The insecticidal activity of C. cyminum extracts has been rarely studied. However, in a recent study by Khorrami et al., (2019), it was revealed that the Fe₃O₄ methanolic extract of C. cyminum was significantly more repellent against the potato insect pest Phthorimaea operculella (Zeller) [42].

Interesting studies have been published in recent years regarding the insecticidal activities of many plant extracts against D. opuntiae. Lopes et al., (2018) showed that the extracts of leaves and pods of Libidibia ferrea (Mart. ex Tul.) L. P. Queiroz were more effective against the wild cochineal, causing 81% mortality of nymph II and 97% of adult females, ten days after treatment, while Agave sisalana (Perrine) extracts controlled only adult females, causing 51–97% mortality. According to this study, the insecticidal activity of L. ferrea extracts can be explained by the toxicity presented by their secondary compounds such as flavonoids, saponins, tannins, gallic acid coumarins, steroids, and phenolic compounds [37]. Viguera et al. (2009) found similar results by assessing the extracts of C. ambrosioides, M. piperita, M. viridis, T. erecta, and T. florida Sweet on D. opuntiae, with mortality ranging from 35% of nymphs to 98% for adult females, suggesting that terpenoids in the extracts may be responsible for this toxicity [11].

On the other hand, Ramdani et al., (2021) reported that the application of Capsicum annum at 200 g/L in combination with black soap at 60 g/L showed excellent control of D. opuntiae females with 87.31% at 7 days after application under field conditions. However, the use of the C. annum extract alone at 200 g/L showed less mortality of females with only 18.40% [43]. The current study showed that the insecticidal effect of plant extracts against D. opuntiae nymphs and females was enhanced by applying the detergent black soap. However, further studies are necessary to establish an adequate formulation based on aqueous A. halimus extract or its active chemical compounds, compatibility with other biopesticides, and impact on natural enemies. Laboratory and greenhouse studies should be supported by field trials to confirm the effectiveness of this bioproduct against wild cochineal and determine the most effective method of the application under field conditions.

5. Conclusions

The findings of the current study indicate that an aqueous extract of A. halimus possessed a good insecticidal activity against D. opuntiae. Nymphs and adult females of D. opuntiae were more sensitive to the aqueous extract of A. halimus leaves compared to the 20% methanolic extract from the same plant and compared to the other tested plant extracts. Phytochemical determination revealed a high concentration of total saponin. Additionally, LC-MS characterization indicated the presence of valuable biologically active compounds and a large number of saponin derivatives, especially triterpenoid saponins, which could be responsible for insect mortality. However, further studies are required on individual purified components to determine those most effective for wild cochineal mortality, and in addition, to develop formulations based on the bioactive molecules with detergent to dissolve to wax as new and more effective biopesticides.

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