
Research Article

Comparative evaluation of the aphicidal activity from *Ziziphus spina-christi* leaf and stem bark extracts against *Aphis fabae* Scopoli

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Abstract

Recently, using plants as a renewable source of bioactive molecules has aroused worldwide interest in the search for environmentally friendly alternatives that are less toxic and less costly than chemical pesticides. The black aphid *Aphis fabae* Scopoli (Homoptera: Aphididae) is a major insect pest that considerably compromises the quality and productivity of bean crops. This study aimed to compare the aphicidal activity of extracts derived from the medicinal plant *Ziziphus spina-christi* (L.) (Rhamnaceae) against *Aphis fabae* to determine and select the most potent extracts in terms of toxicity. We treated aphid larvae and adults with various extracts (of hexane, dichloromethane, methane and water) from the leaves and stem bark of the plant using laboratory contact toxicity methods. The toxicity of the extracts was assessed based on the percentage of residual population (PR%) in aphid larvae and adults. Our results indicate that the methanolic extracts of *Z. spina-christi* leaves and bark had the lowest residual population percentages (RP less than 30%) compared with those recorded for the other extracts. The methanol extracts were more toxic to *Aphis fabae* larvae than adults. We conclude that methanol extracts of *Z. spina-christi*, particularly the stem bark methanol extract, can be considered an effective botanical aphicide harmless to the health and environment and therefore can be used to combat aphids as an alternative to chemical insecticides.

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1. Introduction

The aphids are major pests of agricultural and horticultural crops worldwide, causing direct harm by sucking plant sap, and also causing indirect harm by transmitting over 300 pathogenic viruses (Dedryver et al., 2010). The aphids excrete honeydew on the surface of the leaves, encouraging the growth of sooty moulds that reduce the photosynthetic surface area (Blackman & Eastop, 2017; Dedryver et al., 2010). The adults and nymphs feed on plant sap and attack almost every part of the plant: fruit, flowers, buds, and leaves. This causes yellow, rolled or withered leaves, deformed flowers and galls on the roots and stems of affected plants (Blackman & Eastop, 2017).

The *Aphis fabae* Scopoli (Homoptera: Aphididae) was one of the 14 most important aphid species for agriculture worldwide (Blackman & Eastop, 2017). It continues to be one of the main pests of a large number of crops and species of ornamental plants; their host includes over 200 species of host plants all over the world (Akca et al., 2015). *A. fabae* extends over a wide area, and species can be found in Western Asia, Europe, South America, and Africa (Blackman & Eastop, 2017). This complex species aspires to sap from the plant, reducing the efficiency of photosynthetic and respiratory gas exchange, causing lesions on the leaves and the honeydew excretion (Shannag, 2007). Additionally, they can transmit many viruses to plants (Blackman & Eastop, 2017). In affected tissues, it damages between 7 and 33% of the protein content (Shannag, 2007).

Chemical pesticides are known to cause resistance in pests, their resurgence, the death of non-target organisms and reduction in food resources for the beneficial arthropods (parasitoids, predators and pollinators), and environmental pollution, despite the best of intentions when used (Ndakidemi et al., 2016). The application of chemical insecticides worldwide as the primary means of aphid control on various crops has led to numerous problems, including failure in the control of pests, the negative effects on the environment and the appearance of aphid resistance to insecticides (Foster et al., 2017).

Aphids have been controlled with several organophosphate and carbamate pesticides, their harmful effects on human health and the environment have gradually led to a growing dependence on neonicotinoids and pyrethroids (Dewar & Denholm, 2017). These are the synthetic pesticides most commonly used against *A. fabae*, and their excessive use is causing grave problems in the environment and human health (Bennour et al., 2021). The negative impact of neonicotinoid pesticides on pollinators has led to their gradual banning and restriction, despite the effectiveness of several of these pesticides (Dewar & Denholm, 2017). Growing consumer concern about pesticides, and developing resistance to chemical pesticides in diverse aphid species, led to the need to find other control strategies (Foster et al., 2017).

These problems have led to using safe strategies to control *A. fabae*, in which plant extracts are attracting growing interest (Bennour et al., 2021). Using botanical extracts to combat pests is a strategy that is more respectful of the environment than chemical insecticides. Botanical products have little effect on other organisms non-target, human health, and the environment. Moreover, they are both easy to apply and inexpensive (Tarusikirwa et al., 2020).

Numerous plant species from diverse botanical families are used against *A. fabae*, showing the potential for toxicity, repellency, and distortion of the biological cycle against this pest (Bennour et al., 2021). Meradsi and Laamari (2016) tested the bioinsecticidal effect of methanol and water leaf extracts from *Vicia faba* (Fabaceae) as an aphid-resistant cultivar. Acheuk et al. (2017) found that the ethanol extract of the aerial part of *Artemisia judaica* L. (Asteraceae) has a significant insecticidal effect. In addition, Amin and Majeed (2018) evaluated the insecticidal activity of water extracts of *Eucalyptus* sp (Myrtaceae) and *Allium sativum* (Liliaceae), *Allium cepa* (Liliaceae), and *Cinnamomum* sp (Lauraceae). Chaieb et al. (2018) tested the essential oil of *Citrus aurantium* L. (Rutaceae) peel *in vivo* against black aphids. Moreover, Al-Jassani et al. (2020) studied the biocidal effect of water leaf extract from *Sonchus oleraceus* (Asteraceae) against black aphids.

Numerous previous studies suggest that the bioactivities of the medicinal plant *Ziziphus spina-christi* (L.) (Rhamnaceae) could be due to a high content of secondary metabolites. The identification of several bioactive metabolites from *Z. spina-christi* has been reported in previous studies (Tuenter et al., 2017). This species is identified as a rich source of various active compositions, including flavonoids, triterpenoids, alkaloids, sapogenins, saponins and sterols (-sitosterol) (Hussein, 2019). It contains many essential oils, tannins, betulinic acid, and phytosterols (Kadioglu et al., 2016). Alhassan et al. (2019) found that steroids, flavonoids, tannins, lipids, anthraquinones, saponins and alkaloids exist in leaf extracts of *Z. spina-christi*. The results from Ads et al. (2022) revealed the presence and identification of 36 phytochemical compounds on *Z. spina-christi* stem bark, belonging to different phytochemical classes such as organic acids, alkaloids, hydrocarbons, triterpenes, fatty acids, flavonoids and triterpenes.

Previous research has demonstrated various beneficial properties of *Z. spina-christi*. The leaves showed anticancer activity (Soliman et al., 2019), antibacterial activity (Mervat et al., 2018), and molluscicide activity (Yousef & El-Kassas, 2013). Stem bark also has antimicrobial and cytotoxic properties (Ads et al., 2017, 2018). Guezzoun et al. (2024) studied the insecticidal activity of *Z. spina-christi* leaves and stem bark by estimating the mortality rate and lethal dose (LD₅₀) of hexane, dichloromethane, methanol and water extracts at 48 hours. Leaf and stem bark methanolic extracts showed a very high mortality of *A. fabae*, while dichloromethane extracts showed the lowest mortality rate.

The present work aimed to study the toxicity effect of four extracts (hexane, chloromethane, methanol and water) as a function of time (after 24, 48 and 72 hours), the type of solvent used, the part of the plant extracted, the concentration of the treatments, and the stage of development of the *A. faba* aphid. The percentage of residual aphid population (PR%) was compared in order to select the most effective extracts as aphicides and determine the stage of aphid development most sensitive to these extracts.

2. Materials and methods

2.1 Collection and breeding of aphids

The aphids in this study are the adults and larvae of *Aphis fabae* Scopoli collected at the end of January 2024 from bean plants (*Vicia faba* L.) grown in fields in the Eloued region (south-east Algeria). Aphids were reared following the method of Salari et al. (2010) with a few modifications. The *A. fabae* aphid was maintained in the laboratory using infested faba bean plants as hosts were placed in 45×33×19 cm plastic storage bins, lid made of cling film with mesh holes to allow air exchange, was reared under a growth chamber at a temperature of 25 ± 1 °C, a relative humidity of 60 ± 10% and a photoperiod of 16:8 h of artificial light at an intensity of approximately 4000 lux.

2.2 Plant collection and extract preparation

In the present study, leaf and stem bark specimens of *Ziziphus spina-christi* were obtained in October 2022 from the Oued souf region, South East Algeria. Samples were cleaned of dust and dirt by washing with tap water, followed by distilled water. All plant material was then air-dried at 37 °C for 10 days. The dried plant material was ground separately into powder using an electric grinder and sieved through a 2 mm mesh to facilitate extraction, and stored in airtight containers. Each part of the plant was extracted by solvents with different polarity according to the protocol of Chaouche et al. (2015) with some modifications. The extraction was carried out by maceration of 10 g of plant material in 100 mL of hexane, 100 mL of dichloromethane, 100 mL of methanol and 100 mL of distilled water successively at room temperature with magnetic stirring. After 24 hours, the extracts were individually filtered using Whatman filter paper. The filtrates were then

concentrated under vacuum by evaporation at a temperature not exceeding 45 °C and stored at 4 °C until use.

2.3 Bioassay study

From the different extracts obtained, a range of experimental treatments of four doses of 60, 30, 15, and 7.5 mg mL⁻¹ of each extract was prepared with a 10 % dimethyl sulphoxide (DMSO) solution. The contact toxicity method was applied to examine the insecticidal activity of individual extracts against larvae and adults of *Aphis fabae* according to Pascual-Villalobos and Robledo Miras (1999) with some modifications. A 250 µL aliquot of each concentration of each extract was applied to separate filter paper discs in Petri plates (4.5 × 0.5 cm) and left for 3 to 5 minutes to absorb the aliquots. Ten healthy insects of the same age and size for both larvae and adults were placed on each plate using a clean brush. A negative control was prepared with (DMSO)10 % solution. Five replicates for each concentration and control treatment were included. The plates were then placed in a growth chamber at a temperature of 25 ± 1 °C, a relative humidity of 60 ± 10% and (L:D) of 16:8 h. The efficacy of the different extracts was evaluated by estimating the percentage of the residual aphid population. Aphid population movements were gently probed with a brush and residual individuals were counted after 24, 48 and 72 hours of exposure. The percentage of residual populations was determined according to (Baba-Aissa et al., 2017):

$$PR (\%) = \left[\frac{(NMF)_t}{(NMF)_c} \right] \times 100$$

Where:

PR (%): the percentage of residual populations.

(NMF)_t: number of mobile forms in the treatment.

(NMF)_c: number of mobile forms in the control.

Toxicity categories:

(PR > 60%): neutral or slightly toxic molecule.

(30% < PR < 60%): moderately toxic molecule.

(PR < 30%): toxic molecule.

2.4 Data analysis

The program (IBM SPSS Statistics V27.0.1) was used for all statistical tests at a significance level of 0.05. Residual population percentage (RP%) data were subjected to a one-way analysis of variance (ANOVA), followed by a multiple comparison test (Tukey) to compare the effect of toxicity caused by the different extracts. Data relating to residual population percentage (PR%) and exposure time were analysed using the non-parametric (Kruskal-Wallis) to determine the significance of exposure time on the insecticidal capacity of the extracts.

3. Result

The toxicity of *Z. spina-christi* leaf and stem bark extracts against the *A. fabae* aphid, as assessed by the percentage of the residual population (PR%) of larvae and adults, was highly variable depending on multiple factors (Figures 1 and 2).

Our studies have shown that PR% levels of *A. fabae* varied according to the type of solvent particularly in the lower concentrations, the results of the ANOVA test showed highly significant (p < 0.01: **) to very highly significant (p < 0.001: ***) differences between the four extracts of *Z. spina-christi* (leaf and stem bark) during exposure periods of 24 and 48 hours at doses of 60, 30 and 15 mg mL⁻¹. On the other hand, at the low dose of 7.5 mg mL⁻¹, a significant difference (p <

0.05: *) between the types of leaf extracts appeared after 72 hours of exposure in both larvae and adults, but for stem bark extracts were reported after 48 hours of exposure in larvae. In addition, the results of the Tukey test revealed the superiority of methanol leaf and stem bark extracts over water, hexane and dichloromethane extracts in terms of toxic efficacy. Furthermore, the aphicidal potential of methanol stem bark extract was slightly higher than that recorded in leaf extract, at lower PR% levels (Figures 1 and 2).

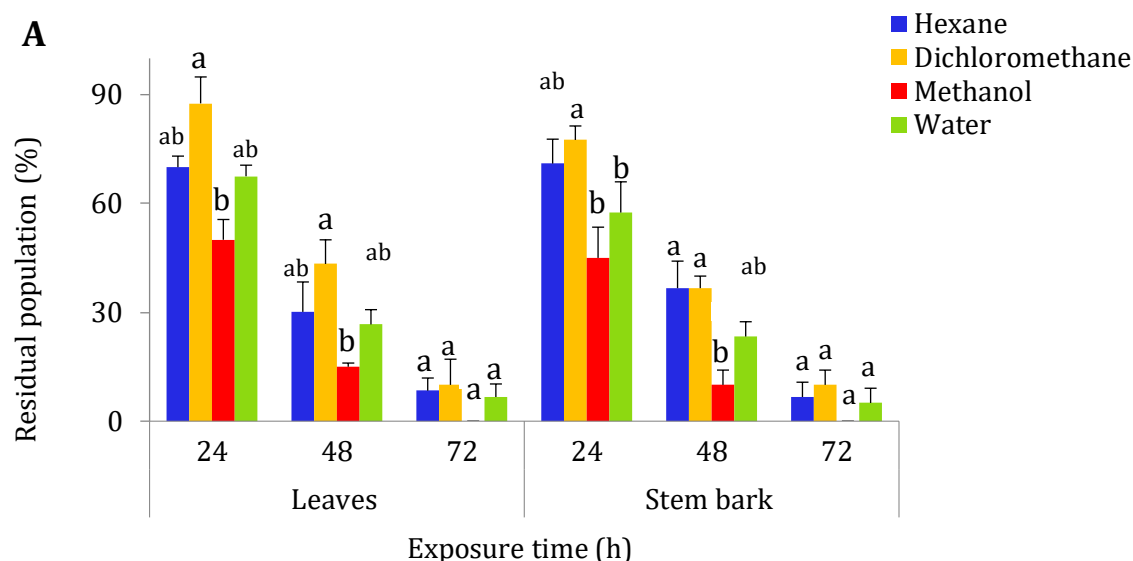
The results of our study indicated that PR% of *A. fabae* varied as a function of exposure time, with Kruskal-Wallis test data also showing at least highly significant differences ($p < 0.05$: *) between *A. fabae* PR% rates relating to exposure duration (24 h, 48 h and 72 h) to leaf and stem bark extracts in all concentrations.

The PR% of black aphids also varied depending on the development stage of the aphids. The insecticidal effect of the extracts was stronger in larvae than in adults. Moreover, the PR% of aphids varied according to the concentration of the extract. The toxic effects are reduced with decreasing doses. The highest concentration (60 mg mL^{-1}) proved to be the most efficient at achieving maximum toxic levels than lower concentrations (30 , 15 and 7.5 mg mL^{-1}) (Figures 1 and 2).

Considering that methanol extract is the most toxic extract, methanol extract of leaves at the high concentration (60 mg mL^{-1}), caused moderate toxicity ($30\% > \text{PR} > 60\%$) initially (after 24 h) and has evolved into a highly toxic ($\text{PR} < 30\%$) and early (after 48 h) and reached the null value after 72 h of exposure on larvae (Figure 1). However, for adults (Figure 2), methanol extract had almost the same effect as observed on larvae, but with slightly higher PR % levels. The 30 mg mL^{-1} concentration revealed low toxicity ($\text{PR} > 60\%$) initially (after 24 h) and progressed to high toxicity from 48 h of exposure in larvae (Figure 1). Whereas for adults, methanol extract only reached high toxicity at the last stage (after 72 h of exposure) (Figure 2).

The toxic effect of the 15 mg mL^{-1} concentration on larvae only reached high toxicity after 72 h of exposure (Figure 1). While for adults, it did not reach high toxicity, even after 72 h of exposure (Figure 2).

In addition, the toxicity effect of methanol extract from stem bark at concentrations (60 , 30 mg mL^{-1}) was almost similar to that observed with leaf extract. With a concentration of 15 mg mL^{-1} , methanol extract only reached high toxicity ($\text{PR} < 30\%$) after 72 h of exposure in larvae (Figure 1). However, in adults, the toxic effect remained the same as in larvae, but with a slightly higher PR% (Figure 2). For the low concentration (7.5 mg mL^{-1}), the toxicity effect was similar to that observed with the 15 mg mL^{-1} concentration in larvae (Figure 1). While for adults, no highly toxic effect was observed, even after 72 h of exposure (Figure 2).



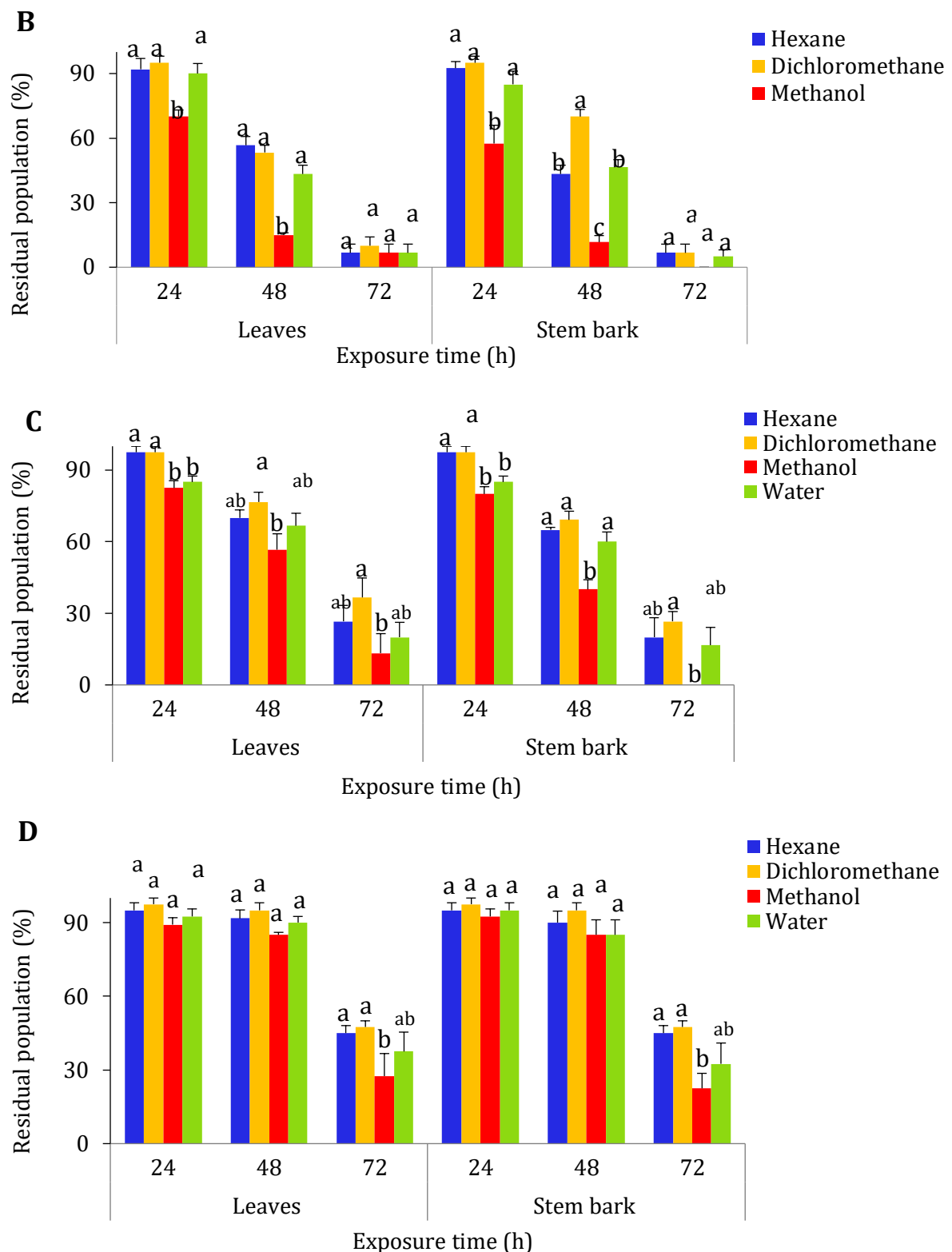
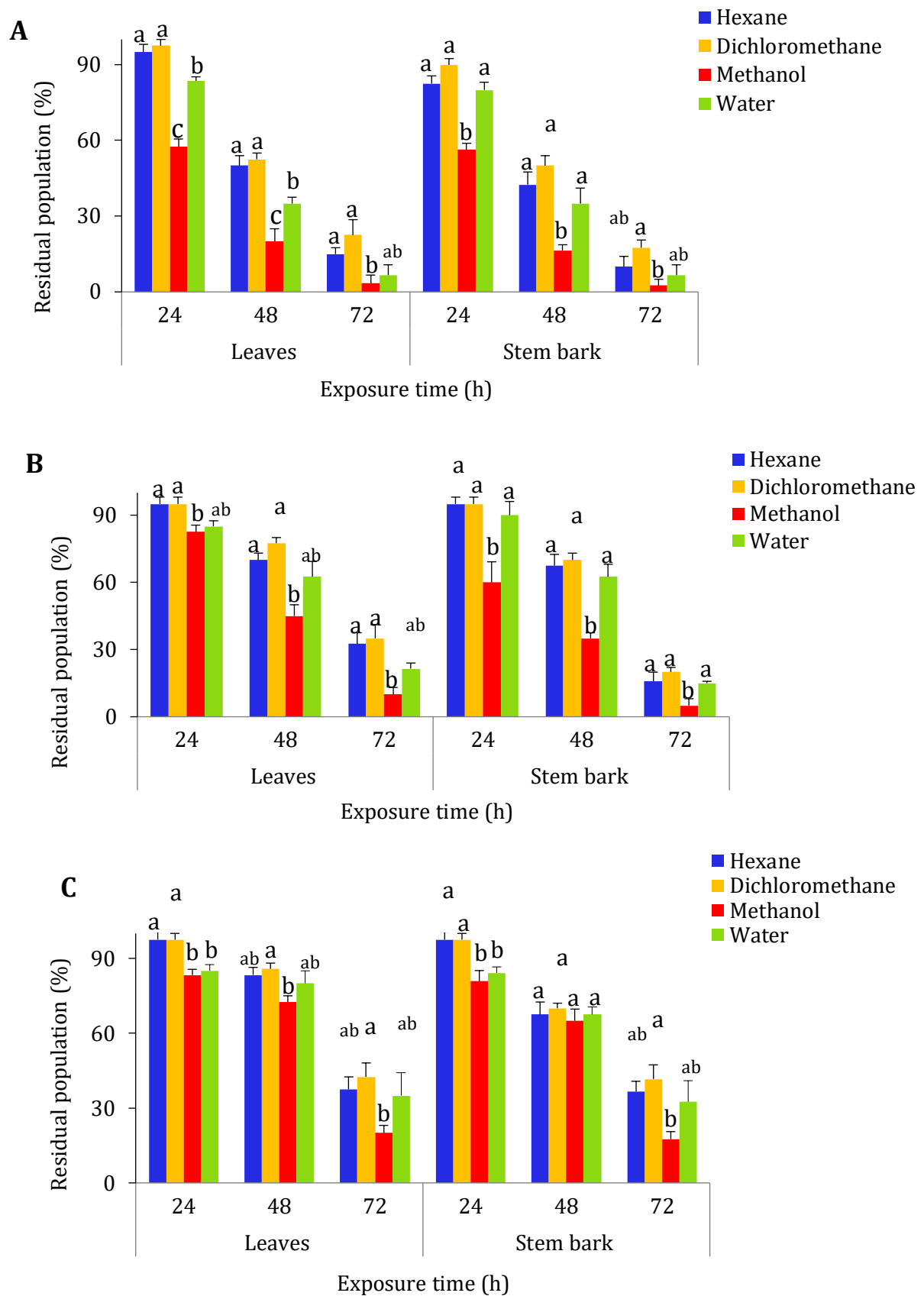


Figure 1. Comparison of residual population percentage of *Aphis fabae* larvae. Concentrations: 60 mg mL⁻¹ (A), 30 mg mL⁻¹ (B), 15 mg mL⁻¹ (C), 7.5 mg mL⁻¹ (D) of extracts from leaves and stem bark of *Z. spina-christi* after 24, 48 and 72 hours of exposure. All data is expressed as mean \pm SEM of five replicates. Bars with the same letter for the same period are not significantly different; Tukey test at $p = 0.05$.



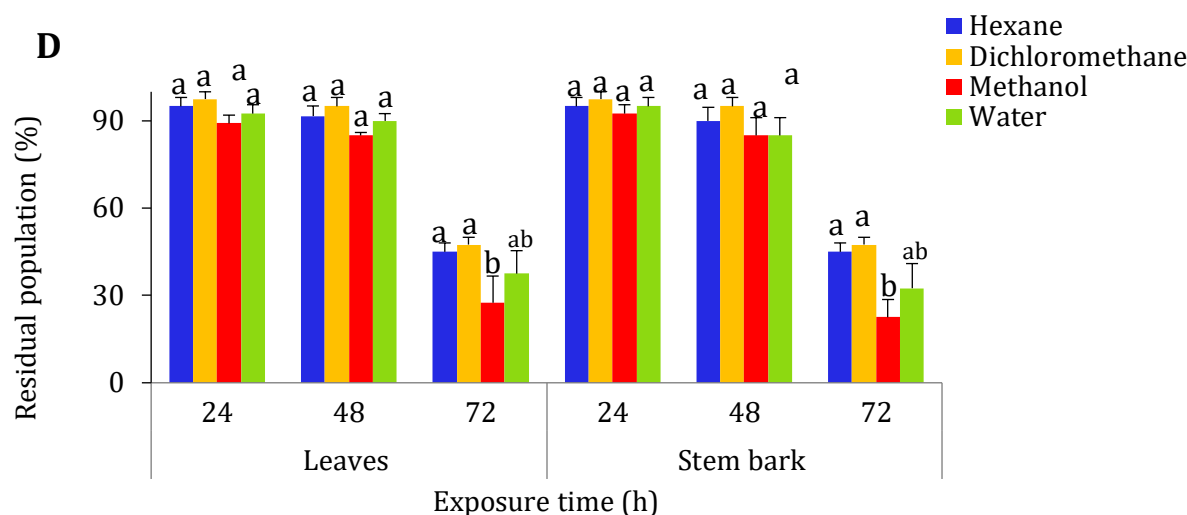


Figure 2. Comparison of residual population percentage of *Aphis fabae* adults.

Concentrations: 60 mg mL^{-1} (A), 30 mg mL^{-1} (B), 15 mg mL^{-1} (C), 7.5 mg mL^{-1} (D) of extracts from leaves and stem bark of *Z. spina-christi* after 24, 48 and 72 hours of exposure. All data is expressed as mean \pm SEM of five replicates. Bars with the same letter for the same period are not significantly different; Tukey test at $p = 0.05$.

4. Discussion

Because of problems resulting from using chemical products to control pests, it is urgent to introduce natural products, mainly of plant origin, to control insect pests, particularly *A. fabae*, one of the major problems in bean-growing in the world's regions. The activity of plant biopesticides is based on the action of secondary metabolites, and they are used as alternatives to protect plants from parasites (Benelli et al., 2016; Isman & Grieneisen, 2014), such as aphids pests (Smith et al., 2018). Generally, bioinsecticides are obtained from medicinal or industrial food plant extracts and products that present minimal risks to health and the environment (Isman, 2015; Pavela, 2017).

Our results showed that extracts of the medicinal plant *Z. spina-christi* have different toxicity effects on *A. fabae* depending on the type of solvent, exposure time, the aphid developmental stage, and the concentration.

Our results indicated that the type of solvent used significantly affected the PR% rate. This could be because the insecticidal activity of the plant extracts differs according to their degree of polarity concerning the content of active components. The solvents diffuse into the solid material of the plant during extraction, solubilising similarly polar compounds, they contain a mixture of secondary metabolites from medicinal plants, such as flavonoids, glycosides, alkaloids, terpenoids and lignans (Handa et al., 2008).

Our results demonstrate significant insecticidal potential in methanol extracts. The methanol extracts consistently show the highest toxicity effect compared to the other extracts (water, hexane and dichloromethane), in a dose-related manner. This indicated neither of the water, hexane, and dichloromethane extracts killed *A. fabae* more than the methanol-based extracts at all concentrations. Due to its high polarity, the insecticidal activity of methanol extracts can be due to the presence of major quantities of bioactive secondary metabolites. Therefore, the methanol leaf and stem bark extracts of *Z. spina-christi* are potential bioinsecticides against aphids.

In general, methanol extract is the most active, offering highly efficient extraction for a range of bioactive compounds (Darah et al., 2013). The superior aphicidal effect of methanolic extracts could be due to the greater solubility of secondary components or many volatile plant metabolites

in this solvent, therefore increasing the power of these extracts (Noureldeen et al., 2022). This is in line with previous studies which have shown the efficacy of methanol extracts of various plant species in controlling aphid populations. Shehawy et al. (2019) showed that methanol extract of *Citrullus colocynthis* resulted in the highest mortality of *Aphis craccivora*, then ethyl acetate and petroleum ether. The same result was also observed in Ahmed et al. (2020) which found that the mortality of methanol extracts of *Brevicoryne brassicae* L. (cabbage aphid), exceeded that of ethanol and chloroform extracts.

Guezzoun et al. (2024) demonstrated that the methanol extract of *Z. spina-christi* leaves was richer in polyphenols and saponins and that the methanolic extract of stem bark contained very high quantities of polyphenols, flavonoids, tannins and saponins compared with those of water, hexane and dichloromethane extracts. Polyphenols can also be toxic to herbivorous insects (Singh et al., 2021). Saponins have shown great potential with significant insecticidal activities such as food deterrence, growth inhibition, mortality and cytotoxicity against aphids, beetles, weevils, leafhoppers, worms and mites (Singh & Kaur, 2018). Plants' secondary metabolites compounds, including phenolic substances, flavonoids, alkaloids, anthocyanins, quinones, lignans, terpenoids, peptides and amines, are used primarily in biopesticides (Patil, 2020).

The methanol toxicity of stem bark extract was superior to that of the leaves against *A. fabae* based on the PR% values, indicating that insecticidal activity depends on the type of plant material. This activity could be attributed to the individual efficacy or synergistic action of the biological substances present in the extracts of these plant parts.

Our results show that insecticidal activity increases as exposure time increases. This could be attributed to the synergistic interaction of various bioactive substances as exposure time increases. Furthermore, the insecticidal action of the methanol extracts was stronger in larvae than in adults, suggesting that their sensitivity was higher than that of adults. The structure of the cuticular layers, particularly the epicuticular layer, is critical to insects because they control the surface properties and provide a barrier against water, ions, pesticides and pathogens (Neville, 2012). The chemical composition of the epicuticular layer, particularly lipids, varies greatly from larva to adult in certain cases (Richards, 1978). The sensitivity of larvae may be due to their high capacity for absorption and penetration of extracts through the cuticle.

It was evident from our study that the insecticidal activity decreased with decreasing the concentration of plant extracts. Imran et al. (2021) indicate that the mortality rate is directly related to the dose of extract, which means that when the dose of extract is reduced, the toxicity effect diminishes, translating into an increase in the residual population rate (PR%) of *A. fabae*.

5. Conclusion

It can be concluded that the leaves and bark of *Z. spina-christi* have an insecticidal capacity against *A. fabae*, particularly for larvae. According to our results, methanol extracts of leaves and stem bark have a greater toxicity effect than other extracts on larvae than on adults, followed by water extract, hexane and dichloromethane. Since *Z. spina-christi* is already used for medicinal purposes, it could be a safer alternative for human health and the environment than the chemical pesticides currently used to control aphids. Further in-depth studies are needed to assess the toxicity of methanol extracts from this plant on mammals and non-target organisms, including predators, parasites and insects that pollinate plants. These assessments ensure that any recommendations for using *Z. spina-christi* methanol extracts as insecticides are effective and ecologically safe.

Conflict of interest

The authors declare that he has no conflict of interest.

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Author contributions

Nassima Guezzoun: Conceptualisation; writing—review and editing; writing—original draft; investigation. Bachir Khezzani: Supervision; writing—reviewing and editing. Mehdi Selmane: Supervision; writing—review and editing; conceptualisation. Naoual Zemmouli: writing—review and editing. All authors read and approved the final manuscript.

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