RESEARCH ARTICLE Agricultural Science Digest, Volume 46 Issue 1: 72-80 (February 2026)

Synergistic Insecticidal Activity of *Cynanchum acutum* and *Sonchus maritimus* Extracts against Aphids

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10.18805/ag.DF-799

ABSTRACT

Background: This study evaluated the insecticidal effectiveness of aqueous extracts from *Cynanchum acutum* L. (climbing vine swallowworts), *Sonchus maritimus* L. (Sow Thistle) and their 1:1 mixture against aphids collected in El Oued Province.

Methods: Plant materials were prepared using standard extraction techniques and bioassays were conducted on adult and larval aphids at five different concentrations, with mortality noted after 24 and 48 hours.

Result: Probit analysis showed significant, dose- and time-dependent mortality for all extracts, with larvae generally more vulnerable than adults. The mixture was the most effective, showing the lowest LC_{50} values: from 0.73 mg/mL (adults, 24 h) to 0.56 mg/mL (adults, 48 h) and from 0.49 mg/mL to 0.12 mg/mL for larvae. *Sonchus maritimus* L. alone exhibited potent activity against larvae (LC_{50} = 0.025 mg/ml at 48 h), while *Cynanchum acutum* L. was less powerful. Nearly complete larval mortality was observed at concentrations above 0.666 mg/ml for all extracts. These findings suggest that mixed botanical extracts have great potential as sustainable, potent agents for aphid control, justifying further field testing and safety evaluations.

Key words: Aphids, Bio-insecticide, Botanical extract, Climbing vine swallowworts, Sow thistle.

INTRODUCTION

The primary reason aphids are considered pests is due to their economic damage and their ability to reproduce rapidly (Chandi and Gill, 2019; Kinley *et al*., 2021; Luo *et al*., 2022). Aphids are important vectors of plant viruses, converting plants into viral hosts and enabling virus transmission by various vector arthropods (Lee *et al*., 2022; Shah *et al*., 2022). Aphid-borne viruses present significant threats to crops in temperate and subtropical regions (Joni and Bishwajeet, 2017; Rabadán *et al*., 2025).

Aphids are a widespread global pest and thrive in a variety of environments (Hartl *et al*., 2024; Javed *et al*., 2025). Their presence on ornamentals and crops is especially problematic in Mediterranean and North African regions, including Algeria, where local medicinal plants are being explored for pest management (Hemmami *et al*., 2023).

The intensive use of chemical insecticides for aphid control has raised concerns about water and soil contamination, chemical residues in food products and the development of resistant aphid populations (Barakat *et al*., 2023; Dhuldhaj *et al*., 2023). These challenges have shifted research focus towards more environmentally friendly and sustainable strategies within integrated pest management (IPM) systems (Dwivedi and Singh, 2022; Sharifzadeh *et al*., 2025).

Plant extracts rich in bioactive secondary metabolites, such as alkaloids, flavonoids, phenolics and terpenoids, are among the most promising alternatives (Abbas *et al*., 2021; Altemimi *et al*., 2017; Maphetu *et al*., 2022). These compounds may serve toxic, antifeedant, or repellent functions, supporting their use in biological control (Tlak and Dar, 2021). Essential oils and botanical insecticides have shown particular promise for aphid control in recent studies (Assadpour *et al*., 2024; Wang *et al*., 2024).

This study evaluates the bioactivity of aqueous extracts from two Algerian wild species, *Cynanchum acutum* L. and *Sonchus maritimus* L., focusing on their impact on aphid survival, feeding and reproduction in controlled lab settings. It also explicitly tests whether combining these extracts produces synergistic effects greater than the sum of their individual actions or simply additive effects. Furthermore, the research explores the traditional uses of these plants

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How to cite this article: Alia, Z., Khechekhouche, E.A., Fatima, K., Cherrada, N., Guehef, Z.H., Amara, D.G. and Messaoudi, M. (2026). Synergistic Insecticidal Activity of *Cynanchum acutum* and *Sonchus maritimus* Extracts against Aphids. *Agricultural Science Digest.* **46(1):** 72-80. doi: 10.18805/ag.DF-799.

Submitted: 01-10-2025 **Accepted:** 29-12-2025 **Online:** 07-01-2026

and assesses their potential as sources for new insecticidal compounds. The ultimate goal is to develop eco-friendly, sustainable aphid control methods, addressing the rising demand for alternatives due to environmental concerns and increasing aphid resistance to conventional insecticides.

MATERIALS AND METHODS

This experiment investigates the impact of aqueous extracts from *Sonchus maritimus* L. and *Cynanchum acutum* L. on aphids. Their effectiveness in controlling aphids was assessed to evaluate their potential as natural, safe insecticides. The study was carried out during the rabi sessions of 2024-11 and 2025-05 at the Laboratory of Biology, Environment and Health, Department of Biology, Faculty of Life and Natural Sciences, University of El Oued, Algeria.

Region situation

El Oued region (33°23′53.124″N; 6°51′33.466″E) is located in the southeastern part of the country, with an area of 44,586.80 km^2. It is bordered to the northeast by Tébessa Province, to the north by the Khenchela region, to the northwest by the Biskra region, to the west by the Djelfa region and to the south by the Ouargla region. To the east, it shares a border of approximately 300 km with the Republic of Tunisia. El Oued comprises 12 districts, each with a total of 30 (Alia *et al*., 2025).

Methods outside the Laboratory (Biological material collection)

Cynanchum acutum was collected from the garden of Martyr Hamma Lakhdar University in El Oued Municipality. The sample of *Cynanchum acutum* L. was collected in January, during its dormant phase, as the plant was not in its active growing or flowering stage. A healthy and disease-free specimen was selected, exhibiting no visible signs of mineral deficiencies or pathological symptoms.

Sonchus maritimus L. was collected from Ghamra Station in late January or early February, during its active vegetative growth phase, before the peak flowering period. A healthy, disease-free specimen was selected, showing no signs of mineral deficiencies or pathology.

Aphid colonies (*Aphis nerii* Fonscolombe, 1841) were collected in early May from *Nerium oleander* (oleander) shrubs located in the University Garden of El Oued municipality.

Methods inside the Laboratory

Plants sample preparation

The aerial part of the plant was thoroughly washed with tap water to remove dust, then rinsed with distilled water. It was then spread in a thin layer and placed in a well-ventilated area, away from sunlight and dust, with occasional turning (Abubakar and Haque, 2020). The material was left for approximately 10 days until it was scorched. After the sample has thoroughly dried, the plant material is manually cut into small pieces. The sample is placed in clean, dry paper bags or plastic containers, then stored in a refrigerator at 4°C until use (Ahmad *et al*., 2022).

Preparation of aqueous plant extract

Sixty grams of plant material were soaked in 600 mL of water for 24 hours. After this period, the water was replaced with fresh water of the same volume, while the plant material was kept. The previous water was filtered and retained. This process was repeated three times, including the initial soaking. The collected extracts were stored in a refrigerator at 4-5°C after each water change, for a total of three days. The combined extract was then transferred to glass containers and incubated at 50°C until the water had completely evaporated. Once dried, the solid extract was scraped off using a sharp blade and stored in a glass vial covered with aluminium foil to protect it from light and contamination until use. For the mixed extract, 30 grams of each plant were used (Abubakar and Haque, 2020; Ahmad *et al*., 2022).We prepared three aqueous extracts as follows:

- Aqueous extract (1) of *Cynanchum acutum* L.
- Aqueous extract (2) of *Sonchus maritimus* L.
- Aqueous extract (3) of mixed plants (Mixed plant extract).

Biological control

After completing the preparation of the aqueous extract (Table 1) and to evaluate its effectiveness as an insecticide, it is tested on aphid insects as follows:

Aphid control using the aqueous extract

This experiment uses 90 mm Petri dishes, with the number depending on the number of concentrations being tested. Each dish contains 10 aphids. Three types of water-based plant extracts were used: one from *Sonchus maritimus* (L.) Hill, one from *Cynanchum acutum* L. and one from a mixture of both plants. The test conducted on both young (nymph) and adult aphids, with each group treated separately.

Statistical analysis

The study data were analysed using the Statistical Package for Social Sciences (SPSS) under Windows. The results obtained from the bioassay were analysed using Probit Analysis Software, with standard error and 95% confidence intervals.

The difference between Probit and Logit is that Probit assumes a normal distribution of the data, while Logit assumes a non-normal distribution.

Table 1: Concentrations of the aqueous extract.

Petri dishes	Concentrations (mg/ml)
Control	Only distilled water
C1	0.466
C2	0.533
C3	0.666
C4	1
C5	1.333

RESULTS AND DISCUSSION

General observation

Early insights from the study revealed a decrease in aphid numbers in the treatment groups with aqueous plant extracts, compared to the control group, which maintained a high infestation level. Although differences between treatment groups were minimal, *Cynanchum acutum* L. performed slightly better than *Sonchus maritimus* L. and the combination of both extracts. These findings support the idea that the selected plant extracts may be more effective in suppressing aphid reproduction. Additionally, treated aphids exhibited a gradual colour change from yellow to dark brown, ultimately leading to their death, suggesting a direct toxic effect of the extracts on the insects.

Results of aqueous extracts applied to aphids

Adult

Fig 1 presents data from an experiment in which different concentrations of three aqueous plant extracts were applied to 10 aphid individuals (adults), with mortality rates recorded after 24 and 48 hours of treatment (Extract 1: *Cynanchum acutum* L., Extract 2: *Sonchus maritimus* L. and Extract 3: mixed plant extract).

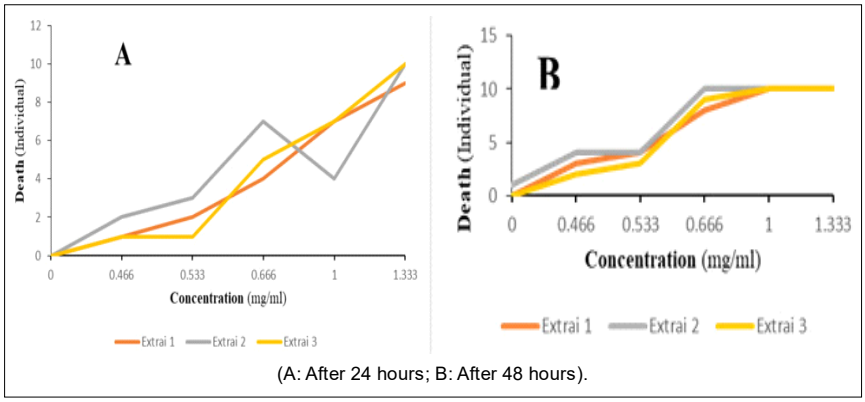
Fig 1 illustrates the dose-response relationship between three aqueous plant extracts (Extract 1, Extract 2 and Extract 3) and aphid mortality after 24 hours (Panel A) and 48 hours (Panel B). In both panels, the extract concentration (mg/ml) is plotted on the x-axis and the number of dead aphids on the y-axis.

Panel A shows a positive correlation between concentration and aphid mortality after 24 hours, with Extract 2 and Extract 3 causing a nearly linear increase in mortality at 1.33 mg/ml, resulting in 10 dead aphids. Extract 2 also showed a dose-dependent response but exhibited more variability at intermediate concentrations, possibly due to differences in the stability or uptake of the active compound.

Panel B (48 hours) shows that aphid mortality rates increased for all extracts with prolonged exposure, reaching 10 deaths at the highest concentrations (1 mg/ml). The mortality curves of the different extracts converged at higher concentrations after 48 hours, with initial variations in Extract 2 disappearing (0.66 mg/ml). These results confirm that the aqueous extracts exhibit a dose- and time-dependent effect, with mortality increasing at higher concentrations and over extended exposure periods.

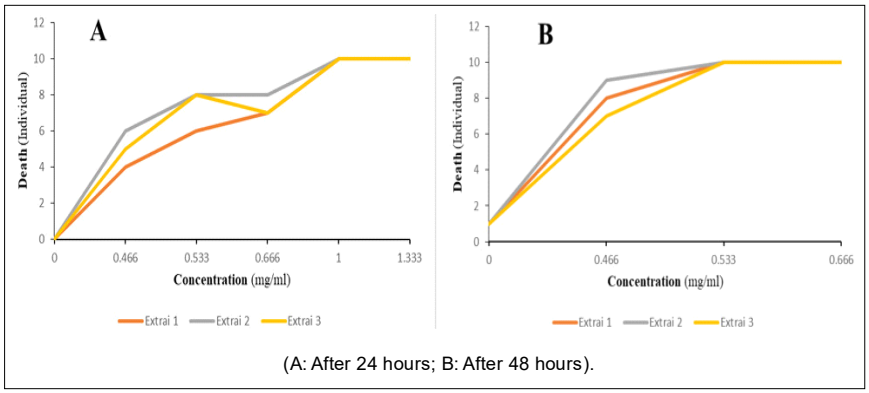
Larvae

The experiment shown in Fig 2 measured the insecticidal activity of three plant extracts when applied to aphid larvae. The experiment involved treating ten aphids at each



(A: After 24 hours; B: After 48 hours).

Fig 1: Death of Aphids adults after applying the different aqueous extracts.



(A: After 24 hours; B: After 48 hours).

Fig 2: Death of Aphid larvae after applying the different aqueous extracts.

concentration level and measuring their survival rate after 24 and 48 hours.

Fig 1 provides a quantitative assessment of aphid larval mortality following exposure to increasing concentrations of three aqueous plant extracts, measured at two time intervals: 24 hours (Panel A) and 48 hours (Panel B) post-treatment. Mortality is reported as the number of dead individuals out of 10 per group.

Panel A shows that at 0.46 mg/ml, all three extracts killed 4 to 6 larvae, with Extract 2 causing the most deaths at 0.66 mg/ml (8 larvae). At 1 mg/ml, all extracts nearly caused complete mortality with 10 larvae killed. After 48 hours, mortality increased for all extracts, reaching a plateau of 10 deaths at a concentration of 0.53 mg/ml, highlighting their dose-dependent efficacy and saturation at higher concentrations.

Modelling (Probit analysis)

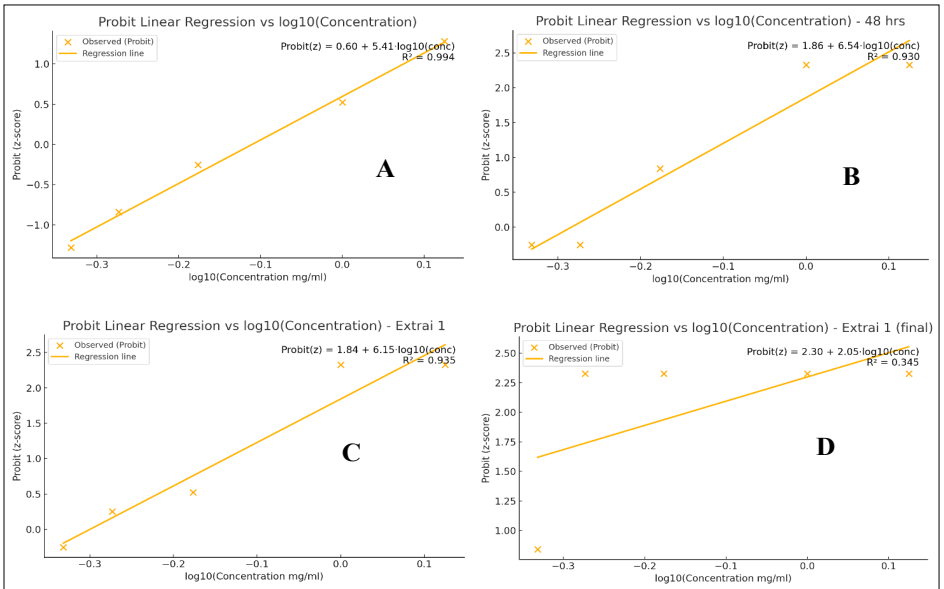
Fig 3 presents a toxicological analysis of *Cynanchum acutum* L. extract on aphid developmental stages, with Probit regression data in panels A-D. Panel A (adults at 24 hours) shows a sharp increase in mortality between 0.53 and 0.66 mg/ml, with a strong fit (R^2 = 0.99). Panel B (adults at 48 hours) also shows increased mortality with prolonged exposure (R^2 = 0.93). In panel C (larvae at 24 hours), mortality reaches 100% at higher doses (R^2 = 0.93), while panel D (larvae at 48 hours) shows a poor fit (R^2 = 0.34) due to a plateau effect. Overall, larvae are more sensitive to prolonged exposure, confirming the extract's high effectiveness.

The probit regression analysis of aphid mortality from exposure to *Sonchus maritimus* L. extract utilizes data from multiple developmental stages and periods, as illustrated in Fig 4.

Panel A's model exhibits moderate performance (R^2= 0.65) for adult mortality after 24 hours, with a steep increase in mortality between 0.53 and 0.66 mg/ml, although some fluctuations are observed. Panel B (48 hours) also demonstrates moderate performance (R^2 = 0.67), showing an increase in mortality with both dose and time, but individual sensitivity is evident. Panel C (larvae at 24 hours) shows strong performance (R^2 = 0.91), with a dramatic increase in mortality at 1 mg/mL and higher. Panel D exhibits poor regression performance due to near-total mortality across most concentrations, complicating dose differentiation. The data highlight a dose-dependent effect of *Sonchus maritimus* L., with increased toxicity over prolonged exposure, especially in larvae.

The probit regression analysis in Fig 5 assesses the impact of Extract 3 on aphid mortality in both adult and larval stages after 24 and 48 hours.

Panel A's model exhibits strong performance (R^2 = 0.93), characterized by a sharp dose-response curve, in which mortality increases significantly between 0.53 and 0.66 mg/mL, reaching full mortality at higher doses. Panel B (R^2 = 0.87) confirms consistent increases in mortality, indicating that prolonged exposure enhances effectiveness, particularly for adults. Panel C's 24-hour analysis (R^2 = 0.87) shows high larval sensitivity, while Panel D (48 hours) has a weaker fit due to near-total mortality at most concentrations. Across all panels, the extract demonstrates potent insecticidal effects, with mortality sharply rising between 0.53 and 0.66 mg/ml and larvae becoming more susceptible over time. Fig 3, 4 and 5 show that the mixed plant extract (Extract 3) exhibits the most predictable dose-response relationship, achieving near-total mortality at intermediate concentrations. *Cynanchum acutum* L. (Extract 1) shows high efficacy, especially in adults and



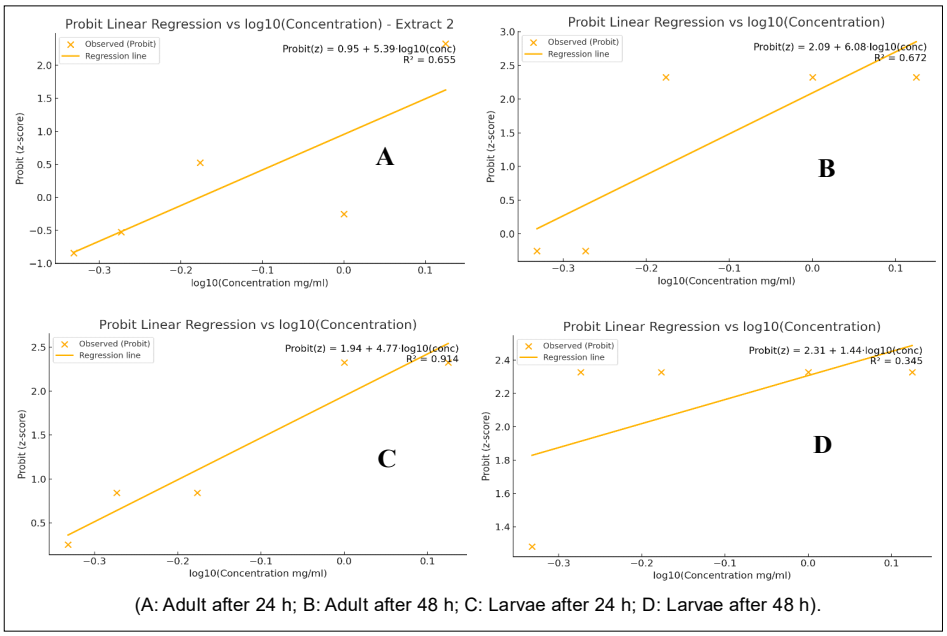
(A: Adult after 24h; B: Adult after 48h; C: Larvae after 24h; D: Larvae after 48h).

Fig 3: Modelling probit analyses of *Cynanchum acutum* L. extract (Extract 1) applied from aphids.

larvae after 24 hours, while *Sonchus maritimus* L. (Extract 2) shows more variable effects. Larvae are generally more sensitive than adults, particularly with prolonged exposure. The mixed extract is the fastest and most effective, followed by *Cynanchum acutum* L., while *Sonchus maritimus* L. is less potent. The critical concentration range for full mortality is between 0.53 and 0.66 mg/mL, highlighting the potential of mixed formulations for aphid control.

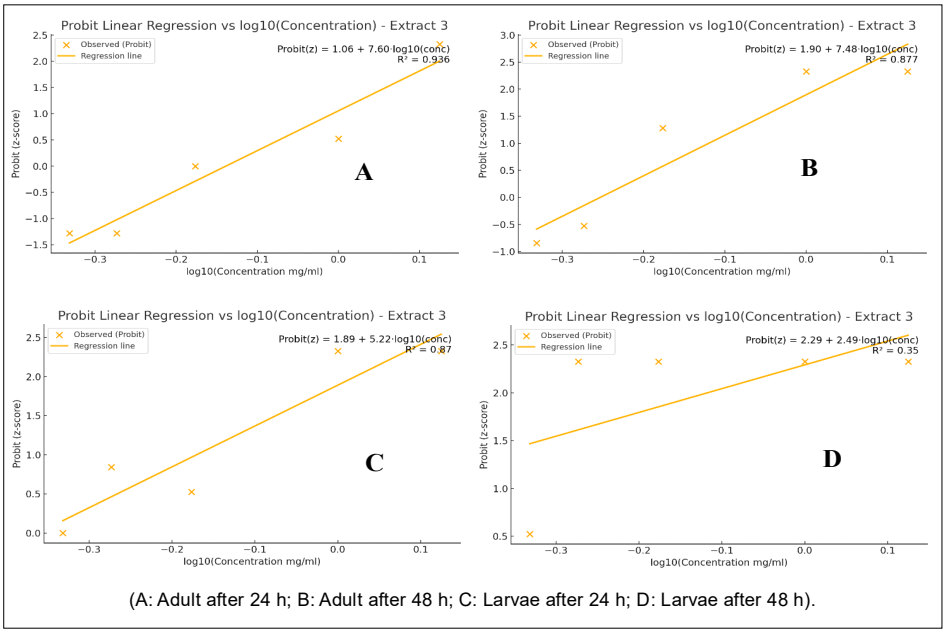
Lethal concentration (LC)

Table 2 shows the lethal concentration measurements (LC_{10}, LC_{50} and LC_{90}) required to cause 10%, 50% and 90% mortality in aphid adults and larvae after 24 and 48 hours of exposure to *Cynanchum acutum* L., *Sonchus maritimus* L. and their 50:50 combination. Lethal concentrations decrease with prolonged exposure, indicating the accumulation of pesticide effects over time.



(A: Adult after 24 h; B: Adult after 48 h; C: Larvae after 24 h; D: Larvae after 48 h).

Fig 4: Modelling probit analyses of *Sonchus maritimus* L. extract (Extract 2) applied from aphids.



(A: Adult after 24 h; B: Adult after 48 h; C: Larvae after 24 h; D: Larvae after 48 h).

Fig 5: Modelling probit analyses of mixed plant extract (Extract 3) applied from aphids.

Larvae are more sensitive than adults, with consistently lower LC values at 48 hours. The mixed extract, combining *Sonchus maritimus* L., yielded the lowest LC values, particularly for larvae, with an LC_{50} of 0.12 mg/ml at 48 hours. *Cynanchum acutum* L. was less effective, especially in adults, with higher LC values. All extracts-maintained LC_{90} values below 1.5 mg/ml, demonstrating strong insecticidal potential. Larvae's significantly lower LC_{50} and LC_{90} values confirm their greater vulnerability to botanical insecticides, particularly after 48 hours. The mixed extract and *Sonchus maritimus* L. outperform *Cynanchum acutum* L., making the mixed extract the most effective for rapid aphid control.

Statistical summary

The toxicological effectiveness of *Cynanchum acutum* L. and *Sonchus maritimus* L. aqueous extracts, used alone or combined (1:1), was assessed against adult and larval aphids by measuring LC_{50} at 24-and 48-hour post-exposure (Table 3). A clear trend emerged, with LC_{50} values decreasing significantly from 24 to 48 hours, indicating increased toxicity with prolonged exposure. In adults, *C. acutum* and *S. maritimus* showed a 33.3% and 32.8% reduction in LC_{50}, respectively, after 48 hours. Larvae showed a greater reduction in LC_{50} values, with 84.8% for *C. acutum* and 93.6% for *S. maritimus*, indicating greater susceptibility (Table 3). The mixture's LC_{50} at 24 hours was close to the expected value, indicating additivity; however, at 48 hours, it exceeded the expected value, suggesting mild antagonism. In larvae, the mixture showed significant antagonism, with the LC_{50} more than double the expected value at 48 hours (Table 3). The data indicate that while both extracts are effective, combining them may reduce larval toxicity due to antagonistic interactions.

This study reinforces the growing evidence that botanical extracts can serve as practical and environmentally sustainable alternatives to synthetic insecticides. Aqueous extracts of *Cynanchum acutum*, *Sonchus maritimus* and their 50:50 mix proved highly effective against adult and nymphal aphids, achieving complete mortality at higher concentrations and longer exposure times. This dose- and time-dependent toxicity aligns with previous studies, showing that plant-based compounds can match or even surpass the efficacy of conventional pesticides under laboratory conditions (Ahmed *et al*., 2021; Pavela, 2015).

A particularly significant outcome is the greater susceptibility of aphid nymphs compared to adults, a trend widely observed in aphid control research (Kinley *et al*., 2021; Paliwal *et al*., 2022). This heightened vulnerability is commonly attributed to the physiological characteristics of nymphs, including thinner cuticles, reduced detoxification enzyme systems and increased feeding rates, which amplify exposure to active compounds. The practical implication is that targeting early instars may enhance the efficacy of botanical insecticides and interrupt aphid population growth more efficiently. In this context, our results further corroborate recent reports by (Kumaraswamy and Huang, 2024)

Table 2: Lethal concentrations of the different extracts and the aphid stage.

Plants extract	LC (mg/ml)	Adult		Larvae	
		24 h	48 h	24 h	48 h
Cynanchum acutum L.	LC_{10}	0.45	0.33	0.31	0.018
	LC_{50}	0.78	0.52	0.50	0.076
	LC_{90}	1.34	0.82	0.81	0.319
Sonchus maritimus L.	LC_{10}	0.38	0.28	0.21	0.003
	LC_{50}	0.67	0.45	0.39	0.025
	LC_{90}	1.15	0.74	0.73	0.19
Mixed two plants (50:50)	LC_{10}	0.49	0.38	0.27	0.037
	LC_{50}	0.73	0.56	0.49	0.12
	LC_{90}	1.07	0.83	0.88	0.39

Table 3: Assessment of lethal concentration and interaction outcomes for *Cynanchum acutum* and *Sonchus maritimus* extracts against adults and larvae.

	Stage	LC_{50} 24 h	LC_{50} 48 h	% Reduction	Observed mixture LC_{50}	Expected additive LC_{50}	Interpretation
Cynanchum acutum	Adult	0.78	0.52	33.3%	0.73 (mix)	0.725	Additive
Sonchus maritimus	Adult	0.67	0.45	32.8%	-	0.67	Synergism
Mixture	Adult	0.73	0.56	23.3%	-	-	Slight antagonism
Cynanchum acutum	Larvae	0.50	0.076	84.8%	0.49 (mix)	0.445	Antagonism
Sonchus maritimus	Larvae	0.39	0.025	93.6%	-	-	Synergism
Mixture	Larvae	0.49	0.12	75.5%	-	-	Strong antagonism

and (Büchel *et al*., 2016), which indicates that flavonoids, phenolics and alkaloid-rich extracts disrupt aphid digestion and enzymatic processes, resulting in both acute mortality and developmental inhibition.

Among the tested extracts, the mixed formulation (combining *Cynanchum acutum* and *Sonchus maritimus*) frequently produced the lowest lethal concentration values, suggesting synergistic or additive effects among phytochemicals. Such synergy is increasingly recognized as a key advantage of using complex botanical mixtures, which can broaden the spectrum of bioactivity, delay the evolution of resistance and enhance the reliability of pest control (Ahmed *et al*., 2021; Pavela, 2015). Indeed, several secondary metabolites identified in these plants, including cardenolides, flavonoids (quercetin, rutin, kaempferol) and phenolics, are well-known for their neurotoxic, feeding deterrent and growth-regulating actions (Ikbal and Pavela, 2019; Rawat *et al*., 2019). The observed rapid, near-total mortality at concentrations between 0.533 and 0.666 mg/ml across all tested extracts underscores their practical utility as bioinsecticides.

From an ecological and agronomic perspective, using locally available plants for biopesticides helps reduce environmental contamination, protect beneficial insects and combat pesticide resistance. These extracts are biodegradable, pose minimal risk to non-target organisms (as determined by testing) and integrate well with pest management strategies. However, laboratory results may not always reflect field conditions, requiring field trials to assess environmental degradation, pest population variability and interactions with other biological control agents (Collinge *et al*., 2022; Colmenarez and Vasquez, 2024). Furthermore, comprehensive risk assessments, including impacts on beneficial arthropods, pollinators and crop yield, are necessary before recommending large-scale deployment (Dymond *et al*., 2025).

The decrease in LC_{50} values over time for both extracts supports previous findings on the cumulative toxicity of plant-based bioinsecticides (Şengül and Canpolat, 2022). The increased sensitivity of larvae confirms the developmental stage-dependent vulnerability in insects. Antagonistic effects observed in mixture treatments, especially at the larval stage, may result from chemical interactions that require further study. These results emphasize the need to evaluate both individual plant extracts and their combined interactions, as assumed synergism cannot be assumed to be present. These findings are crucial for developing integrated pest management strategies in Algeria, aligned with the NA 1648 biopesticide evaluation standard (Acheuk *et al*., 2022; Boukhatem *et al*., 2022).

Ultimately, this study emphasizes the importance of combining botanical diversity, traditional knowledge and modern techniques to enhance pest management tools. Advances in phytochemistry and molecular biology can reveal synergistic mechanisms and inform the design of bioinsecticides. Future research should focus on field validation, process optimization, economic viability and regulatory pathways for commercial adoption.

CONCLUSION

This study demonstrates that a 50:50 blend of *Cynanchum acutum* L. and *Sonchus maritimus* L. extracts is the most effective botanical insecticide for controlling aphids, showing the lowest LC_{50} and LC_{90} values, especially after 48 hours. *Sonchus maritimus* extract alone is also potent, particularly against larvae and can be used in resistance management. The mixed extract's quick action makes it ideal for integrated pest management (IPM). Further field trials are needed to confirm its effectiveness and safety. This approach could reduce reliance on synthetic pesticides and offer a sustainable solution for agriculture.

Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Conflict of interest

On behalf of all the authors of the aforementioned manuscript, I am writing to confirm that we have no conflicts of interest related to the research, authorship and publication of this article. None of the authors has any financial, personal, or institutional interests that could be perceived as influencing the outcomes or interpretations presented in the manuscript.

The findings and conclusions in our study are based solely on objective scientific data and analysis. We assure you that this work was conducted without any bias or external influence that could compromise its integrity.

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