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Effect of phenology, geographic location and extraction solvent on the insecticidal activity of *Artemisia campestris* L. against *Aphis fabae* Scop.

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ABSTRACT:

Aphids, *Aphis fabae* Scop., are one of the most widespread agricultural pests in the world. They target various ornamental and cultivated plant species, including *Vicia faba* L., causing significant damage to all plant parts, which reduces yield and affects seed quality. Using plant extracts might be considered as a novel environmentally alternative technique to control this insect. The insecticidal activity of methanolic and chloroformic extracts of *Artemisia campestris* L. against aphids was investigated in this study. *A. campestris* was collected from two different sites in which we carried out two samples. The first was during the vegetative stage of the plant, while the second was during its reproductive stage. Results of the lethal dose (LD50) showed a toxic effect of all extracts used on aphids. We found that extracts prepared from *A. campestris* of Chabka 2, had a higher insecticidal activity against aphids, especially samples collected during its vegetative phase. The results also indicated that insect mortality varied significantly depending on the extraction solvent, with methanol proving to be the most effective. Or study shows that *A. campestris* is an effective insecticide against aphids and suggests that botanical extracts may be well appropriate alternative to chemical insecticides for pest management. However, extraction solvent, biotic and abiotic conditions of the plant species can affect its insecticidal activity.

Keywords: *Artemisia campestris*, *Aphids fabae*, *Vicia faba*, insecticidal activity, phenological growth, extraction solvent, abiotic conditions.

Introduction

Aphids, *Aphis fabae* Scop. (Hemiptera: Aphididae) remain to be the most important pests of many cultivated and ornamental plant species. Their host plants encompass more than 2000 host plants worldwide (Akca*et al.,* 2015). Aphids target all parts of the plant, particularly favoring the newly growing tips for depositing nymphs. This leads to stunted growth and damage to the leaves (Stoddard *et al.,* 2010). The damage is, in fact, critical at all development stages of the plant growth since they feed on young leaves, flower buds and young pods. Aphids are also the main reason for the transmission of plant viruses. They are the primary vectors for over 30 plant viruses, including non-persistent viruses affecting faba beans, peas, beets, and potatoes, as well as persistent viruses like the potato leafroll virus (PLRV) and beet yellow net virus (BYNV) (Almogdad and Semaškienė, 2021).

The damage encountered due to pest pressure make controlling aphids inevitable and the common practice to manage them depends largely on synthetic insecticides, which has been used since 1970 (Aslan *et al.,* 2004). Pyrethroid insecticides, for example, are considered as the main class used for foliar application to manage aphids (Johnson *et al.,* 2009). Furthermore, Pyrethroid (e.gfenvalerate) and neonicotinoid pesticides (e.g thiacloprid) were highly effective against aphids under laboratory conditions (Purhematy*et al.,* 2013). Chlorpyrifos methyl plus cypermethrin, oxydemeton methyl, imidacloprid and thiometon present the most efficient effect against aphids (Ayala *et al.,* 1996). However, the excessive use of chemical insecticides is linked to various social and environmental concerns. In fact, these insecticides can adversely affect non-target species within the agricultural ecosystem. They can also disturb the ecological balance between pests and predators, escalate cost of production, lead to health risks to farmers and users, and are responsible on severe pollutions (Shah *et al.,* 2008; Stevenson and Belmain, 2016). In addition to all these constrains, a high resistance to chemical insecticides has been developed by *A. fabae* (Bennour *et al.,* 2021). These risk factors are leading more farmers to turn to aromatic and medicinal plants with insecticidal properties, a trend that is gaining traction in research programs worldwide (Bendifallah and Merrah, 2023), and is presenting multiple benefits. Basically, plants are less expensive and easily available because of their natural occurrence. They are also less harmful to beneficial and non-target insects and herbivores. Their extracts are biodegradable, making them environmentally friendly alternatives to synthetic insecticides (Kayange *et al.,* 2019).

The use of botanical insecticides is known to be widespread, especially in low-income countries, where farmers, based on their traditional knowledge, use homemade extracts from numerous plant species to provide insecticidal and repellency activities (Nascimento *et al.,* 2022). This is possible because plant species produce a wide range of bioactive compounds that serve as chemical and physical defences against pests. Several species of Asteraceae family have been reported to have insecticidal activity, namely *Artemisia judica* with an inhibitory effect of embryogenesis for eggs and

a good larvicide activity on the larvae of common mosquito (Acheuk et *al.,* 2017) and *Artemisia herba-alba* on *Culisera longiareolata* larvae (Nabti, 2020). The insecticidal activity of *Artemisia campestris* L. has also been proven on *Spodoptera littoralis, Bruchus obtectus* (Akrout *et al.,* 2007), *Culex quinquefasciatus* (Pavela, 2009), *Culex pipiens* (Masotti *et al.,* 2012), *Callosobruchus maculatus* and *Bruchus rufimanus* (Titouhi *et al.,* 2017), *Culex pipiens* larvae (Alami *et al.,* 2023) and *Tribolium castaneum* (Mokhfi *et al.,* 2024). However, the insecticidal potential of this plant species has been minimally studied, particularly regarding aphids, which cause significant losses in bean crops. *Vicia faba* is regarded as the most important food legume, covering 58,000 hectares, or 44.3% of the total area allocated for this crop category in Algeria (Acheuk*et al.,* 2017).

In the present study, the effect of methanolic and chloroformic extracts of *A. campestris* against *A. fabae* was tested to explore its insecticidal potential. Next, we examined whether the origin of the plant and its lifespan could significantly influence insecticidal activity, as the presence of bioactive compounds can vary based on factors such as geographical location and growth stage (Li *et al.,* 2020; Ciocan *et al.,* 2023; Ayada *et al.,* 2024).

Material and methods

Pest

Apterous adult aphids (*Aphis fabae)* were collected from private agricultural field which is located at 20 Km West of Boumerdes Department (North of Algeria) (Figure 1). Aphids used in this study measure around 2 mm, stocky, black, with three pairs of wax pots on their abdomen. In *Vicia faba*, aphids cause significant damage. By feeding on sap, aphids lead to reduced plant growth, lower reproductive success specifically in fruiting and the toxic effects of their saliva, which can result in the destruction, deformation, and discoloration of plant tissues. In addition, honeydew deposits on the leaf surfaces of plants, promoting the development of sooty would that reduces leaf photosynthesis and dirties consumable parts (leaves or fruits) (Rhino and Ryckewaert, 2017).

Plant material

The areal parts (leaves and stems) of *A. campestris* were used to prepare our extracts. This plant was collected randomly from two different sites, namely Mankeb Ben Hamed and Chabka 2, located at Djelfa Department (Algeria) at two phenological stages: the vegetative stage (May, 2021) and the reproductive stage (January, 2022) (Figure 1, Table 1). Samples were then dried at room temperature for 20 days. They were ground into a fine powder and stored in black glass jars until needed.

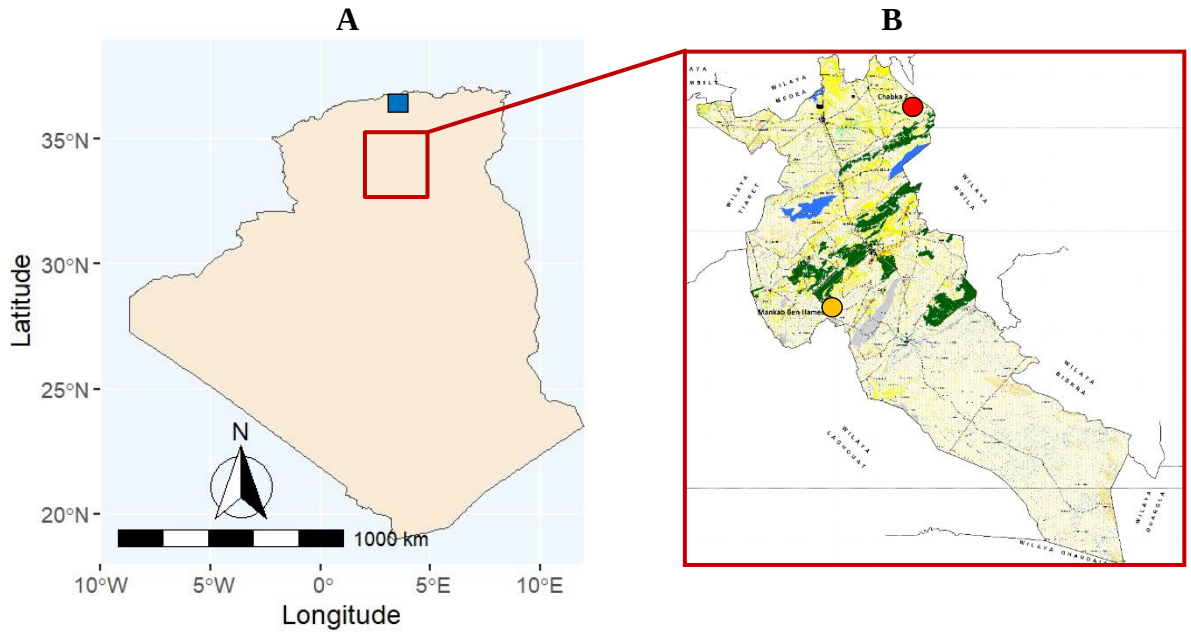

Figure 1. Geographical location of the study area and sites. A illustrates the location of Boumerdes Department where we collected *Aphis fabea* (blue square) and Djelfa Department used to collect *A. campestris* (red square). B illustrates the location of Chabka 2 (red circle) and Mankab Ben Hamed (orange circle) at Djelfa department.

Table 1. Characteristics of sampling sites.

Site	Latitude	Longitude	Altitude (m)	Sample	Phenological stage
Chabka 2	35°24'20.5" N	3°39'50.2" E	882	V_1	Végétative
				R_1	Reproductive
Mankab Ben Hamed	34°24'51.0" N	2°50'44.0" E	1282	V_2	Végétative
				R_2	Reproductive

Determination of moisture content

The water content was determined using the gravimetric method, which involves measuring the loss of plant mass after heating in a ventilated isothermal oven at 120 °C until a constant weight is achieved. The water content is calculated according to the following formula (Zhang *et al.*, 2012):

$$TH\ \% = [\ (M_0 - M_1) / M_0] \times 100$$

Where:

M_0: Mass in g before the oven
M_1: Mass in g after the oven

Phytochemical tests

The fine powder of *A. campestris* was qualitatively screened for the following compounds: flavonoids, coumarins, total tannins, alkaloids, anthocyanins, quinones, Starch, iridoids, saponins, sterols and Terpenes. These tests are based on color reactions and/or precipitation that permit to identify chemical compounds in plants or other biological materials. They were determined according to methods outlined by Ronchetti et Russo (1971), Wagner (1983) and Békro *et al.*, (2007). So, all experiments were conducted in triplicate.

Preparation of *A. Campestris* extracts

Two solvents are used for the maceration of the different samples of *A. campestris* (methanol and chloroform). From each sample, 10 g were added to 100 ml of solvent and macerated for 72 hours. During this period, the solutions were stored in a dark environment at room temperature. The macerate was then filtered and evaporated at 40°C. The dry residues thus obtained were weighed then recovered by adding 4 to 5 ml of each solvent and stored at 4°C until used (Ankwuru *et al.*, 2011).

Evaluation of yield extraction

The yield of each extract was determined according to the following formula (Ankwuru *et al*., 2011):

$$R\ \% = (W_1 / W_0) \times 100$$

Where:

W_1: Weight of extract residue
W_0: Weight of the dried aerial parts of the plant

Determination of insecticidal activity

A fresh leaf of *Vicia faba* was placed on hydrophilic wet cotton, which served as a support for the pests, and then positioned in the center of a Petri dish (the experimental unit). Ten adult aphids were selected and gently placed on the leaves. From each extract, we deposed 1 µl on aphids' thorax and we registered individual mortality every day (24h, 48h and 72h). Aphids considered as control received 1 µl of solvent only (methanol or chloroform). The Petri dishes were kept under laboratory conditions for 72h (temperature: 25°C and relative humidity: 80%). After that, three dilutions were prepared, namely 1/10, 1/25 and 1/50. They are considered respectively as D_2, D_3, D_4, whereas, the crude extract as D_1. All experiments were conducted in triplicate.

Determination of mortality rate

The mortality of aphids was unregistered after 24, 48 and 72h. The mortality rate was expressed by percentage and calculated as follows (Abbott, 1925):

$$M\% = [(P - T) / S] \times 100$$

Where:

M: Corrected Mortality expressed by percentage
P: Mortality caused by the methanolic extract substance
T: Mortality of control
S: Number of surviving individual control

The mortality rates for the different concentrations of our extracts are plotted on a toxicity curve, which allows for the determination of the LD50 (Abed and Ducos de Lahitte, 1993).

Statistical analysis

The statistical analysis was performed using the one-way repeated-measures ANOVA to determine whether aphids' mortality rates are significantly different between the three time points (24h, 48h and 72h). Pairwise paired t-test were performed between the levels of the within-subjects factor (different time points) to identify which time points are different. P-values are adjusted using the Bonferroni multiple testing correction method. Additionally, we conducted several two-way repeated measures ANOVAs to simultaneously assess the impact of two within-subject factors on a continuous outcome variable. They are used to determine whether there is a significant interaction between extracts dose (D1-D4) and time (24h, 48h and 72h) on aphids' mortality rate. Two-way repeated measures ANOVA are also performed to evaluate the differences effect of *(i)* geographical location, *(ii)* phenological stage of *A. campestris* used to prepare extracts and *(iii)* solvent used for extraction over time. In our study, the interaction between phenological stage of *A. campestris* and time was not statistically significant. This is why we analysed the effect of phenological stage on aphids' mortality at every time point. To enhance the reliability of the results from this test, we performed a Wilcoxon rank-sum test, which is appropriate when the factor variable has only two levels (reproductive stage and vegetative stage).

All statistical analyses were performed using RStudio version 4.3.2.

Results

Moisture contents

We noted that the amount of water content during the vegetative phase of *A. campestris*, in both sampling sites, was higher than in the reproductive phase. However, the weight loss at the Mankab Ben Hamed site was lower compared to the Chabka 2 site during the reproductive phase (Table 2). These results suggest that the moisture content of the aerial parts of *A. campestris* can be influenced by the intensity of abiotic conditions associated with different geographical locations.

Table 2. Water content and extraction yield of the two different solvents in *A. campestris.*

Samples	Sampling weight (g)		Water content	Extraction yield (Methanol)	Extraction yield (Chloroform)
	Wet	Dry			
V1	1000	365.26	63.47%	17.041 %	16.541 %
R1	1000	461	53.90 %	13.961 %	12.351 %
V2	1000	361.81	63.81%	15.819 %	13.985 %
R2	1000	518.58	48.14%	11.174 %	10.275 %

Yield extraction

After extracts evaporation, we obtained 15.819 % of crude extract at the vegetative phase and 11.174 % for the reproductive phase at Mankab Ben Hamed. However, the higher yield extraction was noted at Chabka 2, with 17.041% at the vegetative phase and 13.961 % at the reproductive phase,

when using methanol (Table 2). The results exhibit similar trends when chloroform is used as the extraction solvent. We propose that the differences in extraction yield may be influenced by the biotic and abiotic conditions to which *A. campestris* is exposed at various sites.

Phytochemical screening

The identification of the different classes of secondary metabolites constituting a plant species allows having an idea on its biological activities. Thus, the phytochemical screening carried out on *A. campestris* which is collected from the two sites -Mankab Ben Hamed and Chabka 2 – showed similarities regarding the nature of chemical compounds, but presented considerable differences in their amount. Results revealed the presence of saponins, flavonoids, coumarins, total tannins (Gallic and Catechin), alkaloids, quinones, sterols and terpenes during both the vegetative and the reproductive phases of *A. campestris* (Table 3). However, there is a complete absence of starch, anthocyanins, and iridoids at both sites and during both phenological phases. Furthermore, a low amount of coumarins and flavonoids was found during the reproductive phase, comparatively to the vegetative one in both sites. Phytochemical compounds such as saponins, flavonoids and terpenes were present in equal amounts across all four samples. Therefore, the comparison of the phytochemical results from the different samples showed that Chabka 2 was the site with the highest concentration of secondary metabolites.

Table 3. Phytochemical screening of *A. campestris.* (+++): Strong positive reaction; (++): Medium positive reaction; (+): Weak reaction; (-): Negative reaction.

Phytochemical Compounds	Samples				Results
	V1	R1	V2	R2	
Starch	-	-	-	-	Appearance of a purplish-blue color
Saponins	+	+++	+	+++	1-2cm of foam
Flavonoids	+++	+++	+++	+++	Appearance of a pink-red color
Coumarins	+++	+	+++	+	Appearance of intense fluorescence under the UV lamp (360 nm)
Tannins	+++	+++	++	+++	Appearance of a dark green color
Catechics	++	+	+	+	Green blue color
tannins	++	+	+++	+	Dark green color
Gallic tannins					
Alkaloïds	+++	+	+++	+	The formation of a white precipitate and a brown precipitate respectively
Anthocyans	-	-	-	-	Appearance of a red color
Quinons	+++	++	+++	+	No color change
Sterols	++	+++	+	+	The appearance of a fleeting purplish color turning green
Terpens	+++	+++	+++	+++	Appearance of a red-brown color
Iridoids	-	-	-	-	Appearance of a red-violet or blue color

Insecticidal activity

Aphids' mortality was statistically significantly different at the three time points ($F = 125.1$, $p <$

0.001). Post-hoc analyses with a Bonferroni adjustment revealed that all pairwise differences, between time points, were statistically different ($p < 0.0001$). The mortality rate was 67.9 ± 19.3 after 24 hours of extracts application against 75.8 ± 17.5 after 48 hours. However, the highest mortality rate (83.4 ± 15.3) was observed after 72 hours in all extracts.

To determine which of the four extracts is more effective, we performed one-way ANOVA at each level of time points. A significant difference was observed between extracts after 24 hours ($F = 4.279$, $p < 0.01$), 48 hours ($F = 4.268$, $p < 0.01$) and 72 hours ($F = 5.815$, $p < 0.01$) of aphids' treatment (Table 4). However, pairwise comparisons, at 24h and 48h, showed that the mortality rate was not different between the two extracts prepared from *A. campestris* of Chabka 2 (R1 and V1). The same result was noted for extracts R2 and V2 of Mankab Ben Hamed. After 72h of application, the extract V1 was the most effective on insect mortality with 92.2 ± 7.11 %, while R1 had a mortality rate was 85.7 ± 12.6 %. Finally, R2 and V2 presented the smallest mortality rate with, respectively, 78.9 ± 19.5 % and76.8 ± 14.8 % (Table 4, Figure 2). Based on these results, we can infer that extracts derived from Chabka 2 exhibited greater insecticidal activity against aphids, particularly those prepared from *A. campestris* collected during its vegetative phase.

Table 4. Results of one-way ANOVA applied at each level of time point. Means ± sd followed by the same letter within column are not significantly different at $p < 0.05$.

Sample	24h	48h	72h
R1	72.6 ± 12.8^{a}	79.9 ± 13.0^{a}	85.7 ± 12.6^{b}
V1	76.4 ± 13.2^{a}	83.4 ± 10.5^{a}	92.2 ± 7.11^{a}
R2	60.6 ± 22.0^{b}	68.9 ± 17.9^{b}	76.8 ± 14.8^{c}
V2	62.1 ± 23.1^{b}	70.8 ± 22.6^{b}	78.9 ± 19.5^{c}
Statistic F	4.279	4.268	5.815
p-value	0.007	0.007	0.001

Effects of dose, geographical location, phenology, and solvent on aphid mortality

Comparative bioassays of botanical extracts

A two-way repeated measures ANOVA was performed to evaluate the effect of different dose treatments over time on aphids' mortality. There was a significant difference on the mortality ($F = 3.13$, $p < 0.01$) between the different extract concentration (Table 5). Pairwise comparisons, using paired t-test, show that the mean insect mortality was significantly different between D1, D2, D3 and D4 at the different time point ($p < 0.001$). In all extracts, we observed that the mortality rate increases with higher concentrations of the respective doses over the duration of the bioassay. The highest mortality rate of aphids was observed when the crude extract of *A. campestris* was applied after 72 hours of the bioassay. However, the minimum mortality rate was observed after 24 hours when applicated the extract D1 (Figure 3).

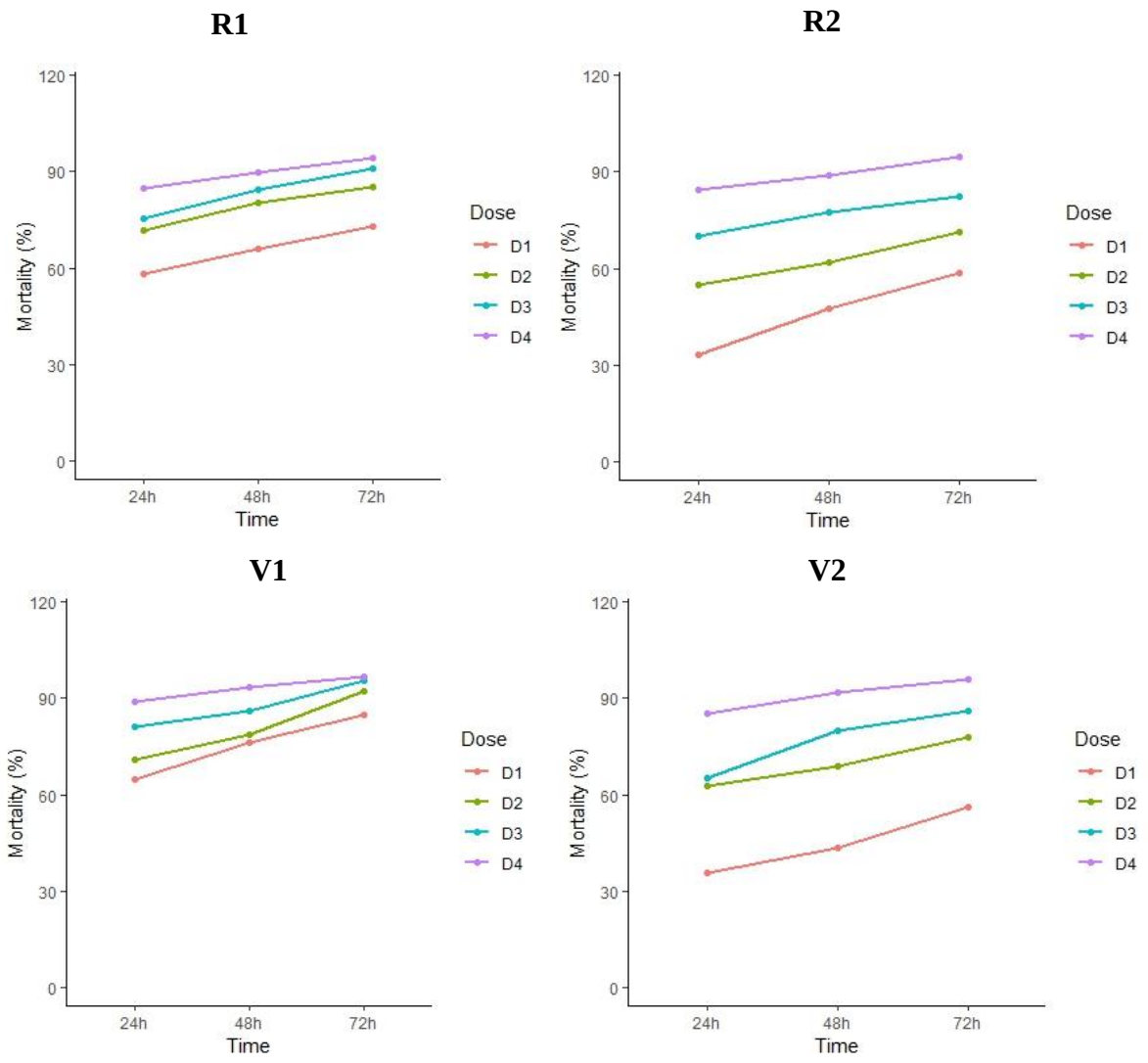

Figure 2. **Aphids' mortality rate along different time points.** The effect of different *A. campestris* extracts (V1 and V2 correspond to the vegetative stage of the plant from Chabka 2 and Mankab Ben Hamed, while R1 and R2 are samples carried out during the reproductive stage) and their respective doses applied on aphids at 24h, 48h and 72h.

Comparative bioassays among different sites

Result of the two-way repeated measures ANOVA revealed a significant interaction between site and time ($F= 7.13$, $p <0.05$) suggesting that the origin of *A. campestris* -Chabka 2 or Mankab Ben Hamed- had an impact on mortality rate which among different point times. Indeed, extracts prepared from *A. campestris,* collected at Chabka 2 present higher mortality rates comparatively to those collected at Mankab Ben Hamed over the bioassay experiment (Table 5, Figure 3). This result indicates that geographical location can influence the composition of extracts, significantly affecting the insecticidal activity of *A. campestris*.

Table 5. Two-way ANOVA results performed on repeated measurements. Means ± sd followed by the same letter within column are not significantly different at p = 0.05.

Factor		Statistic F	p-value	Time		
				24h	48h	72h
Dose	D1	F = 3.14	p<0.01	48.0±20.9^{a}	58.2±18.3^{a}	68.1±17.4^{a}
	D2			65.0±11.2^{b}	72.3±13.1^{b}	81.6±10.9^{b}
	D3			72.9±12.4^{c}	81.8±09.8^{c}	88.6±09.7^{c}
	D4			85.7±06.8^{d}	90.7±07.1^{d}	95.3±05.2^{d}
Site	Mankab Ben Hamed	F = 7.13	p<0.05	61.4±22.3^{b}	69.8±20.2^{b}	77.9±17.2^{b}
	Chabka 2			74.5±13.0^{a}	81.7±11.8^{a}	89.0±10.7^{a}
Phenology	Reproductive stage	F = 0.525	p>0.05	66.6±18.8^{a}	74.4±16.4^{a}	81.2±14.3^{a}
	Vegetative stage			69.3±20.0^{a}	77.1±18.6^{a}	85.6±16.0^{b}
Solvent	Chloroform	F = 6.41	p<0.01	63.8±12.2^{a}	72.4±18.6^{a}	82.4±16.5^{a}
	Methanol			72.1±16.4^{b}	79.1±15.8^{b}	84.4±14.1^{a}

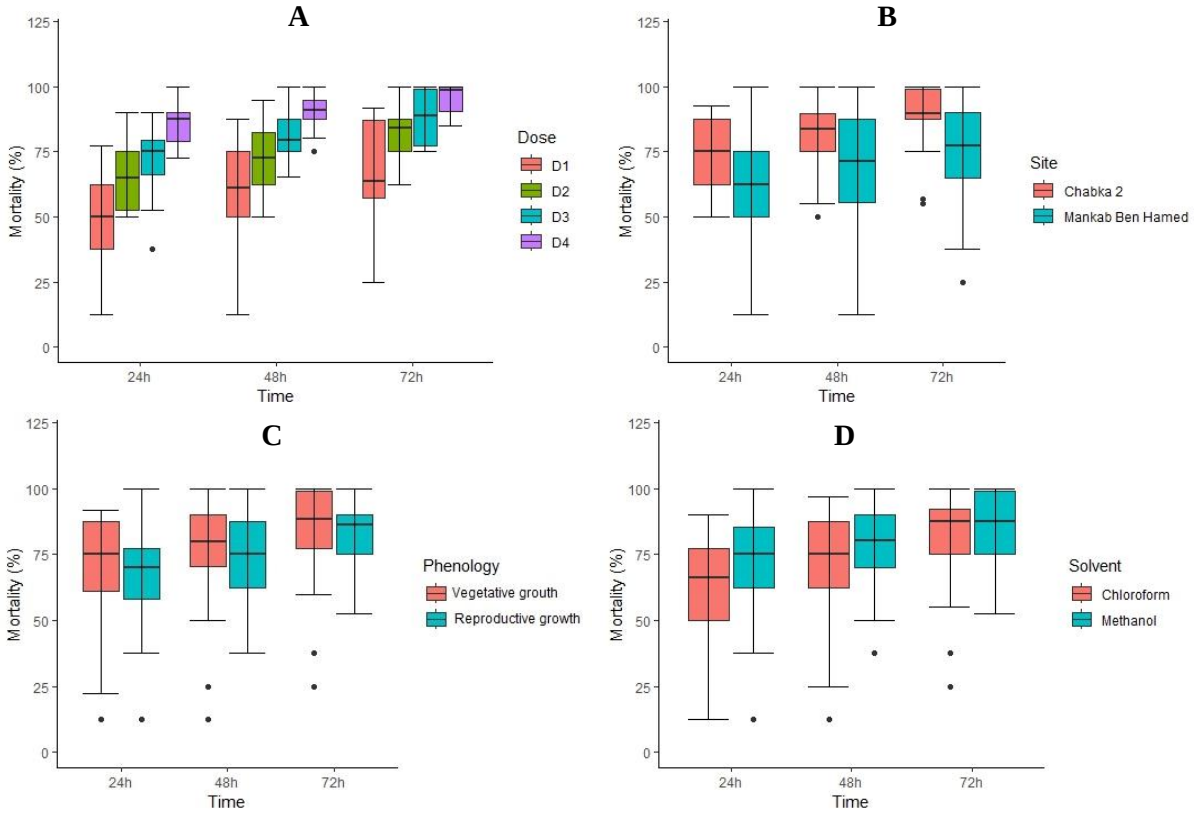

Figure 3. Insecticidal activity of *A. campestris* extracts. A represents the difference of aphids' mortality within different dose of extracts. B demonstrates the difference between sites. C indicates the difference in mortality rate of aphids between the two phenological stages, while D indicates this difference but between the two exaction solvents used in this study, namely chloroform and methanol.

Comparative bioassays using different solvents

Results showed a statistically significant interaction between solvent and time on insect mortality (F = 6.41, p < 0.01) (Table 5, Figure 3). Therefore, the effect of solvent variable was analysed at each time point. P-values were adjusted using the Bonferroni multiple testing correction method. The effect of solvent was significant at 24h and 48h, but not at 72h. Moreover, pairwise

comparisons show that the mean insect mortality was significantly different between chloroform and methanol at 24h (p = 0.001) and at 48h (p = 0.003), but not at 72h (p = 0.291).

Comparative bioassays between phenological stage

Results of the two-way repeated measures ANOVA did not reveal any interaction effect of phenology and time on mortality rate (p > 0.05) (Table 5). However, there was a statically significant main effects of time (as explained above) and phenology, but only after 72 hours of application. The *two-sample Wilcoxon test* confirm these results. It *showed* that the median mortality in vegetative stage of *A. campestris* was 88.2 (IQR = 21.6), whereas the median in reproductive stage was 86.2 (IQR = 15.5). *The* two-sample Wilcoxon test showed that the difference was significant (p < 0.05, effect size r = 0.20). Furthermore, any significant differences were noted after 24 and 48 hours of application (Table 6).

Table 6. Comparative results of the effect of *A. campestris* phenological stages used against aphids' mortality rate. IQR corresponds to the Interquartile Range.

Factor	Time					
	24 h		48h		72h	
	Median	IQR	Median	IQR	Median	IQR
Reproductive stage	70.0	19.0	77.0	25.0	86.2	15.5
Vegetative stage	75.0	26.2	80.0	19.6	88.2	21.6
p-value	p = 0.289		p = 0.234		p = 0.047	

Determination of lethal dose (DL50)

Results regarding the concentration values of various *A. campestris* extracts that induced 50% mortality in Aphis fabae indicate that all extracts were toxic to the insect based on LD50. However, there were notable differences in their levels of toxicity (Table 7). The extracts prepared from samples collected during the vegetative growth of *A. campestris* (V1 and V2), had smaller DL50 values then those collected in the reproductive growth (R1 and R2), regardless of the solvent used or the plant origin (Chabka 2 and Mankab Ben Hamed). These results suggest that lower LD50 values indicate higher toxicity of the extract to the insect.

Table 7. Lethal Dose (LD50) of Different *A. campestris* extracts on aphids. Mean ± SD (n = 3). The Lethal Dose 50 (LD50) refers to the dose that results in 50% mortality of *Aphis fabae*.

Sample	Solvent	Linear regression			DL50 (g/l)
		a	B	R^2	
R1	Chloroform	1.59	0.30	0.763	0.136 ± 0.110
	Methanol	1.08	0.21	0.548	0.133 ± 0.078
R2	Chloroform	0.97	0.19	0.507	0.159 ± 0.113
	Methanol	1.01	0.21	0.483	0.144 ± 0.108
V1	Chloroform	1.26	0.36	0.606	0.075 ± 0.096
	Methanol	1.24	0.38	0.613	0.071 ± 0.082
V2	Chloroform	1.10	0.36	0.481	0.087 ± 0.100
	Methanol	1.07	0.34	0.513	0.105 ± 0.099

Discussion

Insecticidal activity of *A. campestris* extracts against *A. fabae*

Our findings demonstrated a significant insecticidal effect of *A. campestris* extracts on *A. fabae*, a phytophagous insect that infests cultivated plants, particularly *Vicia faba*. Effectively, lethal dose (LD50) results showed the toxic effect of all extracts used on aphids (Table 7). In line with this study, previous research has reported the potential insecticidal effects of *A. campestris* on various insect species. It has been found that different larvicidal efficiency, represented mainly by the repellency effect on *Tribolium castaneum* larvae after 2-24 hours of exposure to both hexane and acetone extracts from the aerial part of the plant(Pascual-Villalobos and Robledo, 1998). Another study indicated that the methanolic extract exhibited the highest larvicidal activity, achieving 100% mortality of Culex quinquefasciatus (mosquito larvae), with an estimated LD50 value of approximately 23 parts per million (Pavela, 2009). However, the larval mortality induced by the ethanolic leaf extract of *A. campestris* was only 33.6% of the *Culex pipiens* L.; mosquito larvae. The lethal concentrations (LC_{50}), after 48 h of exposure were low; 9898 ppm (Masotti *et al.*, 2012). Furthermore, the lifespan of the insects *Spodoptera littoralis* and *Bruchus obtectus* were moderately reduced, in response to the treatment with the essential oil of *A. campestris*, with an average inhibition of 50% (Akrout *et al.*, 2007). Essential oil had also a strong fumigant-specific toxicity effects against *Callosobruchus maculatus* and *Bruchus rufimanus*, but presented lower side effects on their parasitoids wasps; *Dinarmus basalis* and *Triaspis luteipes* (Titouhi *et al.*, 2017). The antileishmanial and cytotoxic activities of essential oil of *A. campestris* was evaluated by Benmouffok *et al.* (2021), where they noted a considerable antileishmanial activity (IC50 of 14.02±0.18 µg/ml), but a low cytotoxicity (CC50 of 183±0,18 µg/ml). Another study tested the essential oils extracted from the aerial parts of both wild and cultivated *A. campestris*, against *Culex pipiens* larvae (Diptera: Culicidae), a pest mosquito widely suspected to be the vector responsible for West Nile virus transmission, noted that the cultivated *A. campestris* essential oil had the highest larvicide activity (LC_{50}, 9.79µg/ml), but no significant difference was noticed between wild and cultivated *A. campestris* essential oil in terms of LC_{90} (Alami *et al.*, 2023). Furthermore, a recent study aimed to evaluate the insecticidal potential of powders and methanolic extracts of *A. campestris* against adults of *Tribolium confusum* and *Tribolium castaneum* showed that adults of *T. castaneum* were more sensitive than those of *T. confusum*, with adults' mortality rate equal to 100%. However, larvae of both species were found to be sensitive to the powder with a mortality rate ranging from 80 to 90%. Furthermore, the methanolic extracts of A. campestris demonstrated remarkable efficacy against the larvae of both species, as well as the adults, achieving a 100% mortality rate after 7 days of treatment at various concentrations (Mokhfi *et al.*, 2024). Regarding all these results, we can conclude that such novel methods of producing biocides to control different disease vectors could offer a potential alternative to conventional chemical insecticides.

Geographical location of *A. campestris* causes shift in insect mortality

Our study reveals that the origin of *A. campestris* significantly impacts insect mortality by influencing the composition of the plant, particularly the quality and quantity of secondary metabolites, as shown by the phytochemical screening (Table 3). The substantial content of chemical compounds in *A. campestris* from Chabka 2, compared to Mankab Ben Hamed, may be influenced by both abiotic factors, such as environmental conditions, and biotic factors, which can act independently or in combination (Ruiz Rodríguez *et al.,* 2014; Maieves *et al.,* 2015) and lead the plant species to produce a higher quantity of secondary metabolites. Indeed, the phytochemical profile of methanolic extracts of *A. campestris* showed that ascorbic acid, isovanilic acid, cinnamic acid, di nitro salycilic acid, luteolin 8 C glucoside and luteolin 6 C glucoside presented higher content in *A. campestris* collected from Chabka 2, comparatively to Mankab Ben Hamed (Brahmi *et al.,* 2023). This difference in secondary metabolites between regions has already been noted in previous studies. According to Naili *et al.* (2010), *A. campestris*, collected from the south of Libya does not contain tannins and coumarins. Moreover, *A. campestris* grown at Boussaâda and Oum El Bouaghi, in Algeria, do not contain alkaloids (Boudjouref, 2019), while the plant originally from Hodhna region do not contain starch, anthocyans and iridoids (Bendifallah and Merrah, 2023). However, *A. campestris* collected from Ouergla region was rich in tannins (Ould El Hadj *et al.,* 2003) and the one from Portugal is known by its richness in flavonoids and alkaloids (Rocha *et al.,* 2021). Moreover, many studies have reported on how environmental factors such as temperature, humidity, precipitation, solar radiation, and soil characteristics affect the synthesis of various secondary metabolites in different plant species. For example, in *Catharanthus roseus* (L.) G. Don, temperatures of 40° C caused a significant increase in alkaloids, such as vinblastine and catharanthine as compared to exposure to 30° C (Guo *et al.,* 2007). At the same time, the content of terpene and polyphenolic compounds in *Centella asiatica* (L.) increased after exposure to sunlight for an entire day compared to plants exposed to 50% of light (Khatib *et al.,* 2011). Xu *et al.* (2022) showed that the difference in secondary metabolites and the biological activity of *Echinacea purpurea* depended on the geographical location (latitude and longitude), precipitation and soil pH. The content of polyphenols, flavonoids and tannins in *Juniperus thurifera* was strongly depended on the geographical, bioclimatic and edaphic conditions (Benhssaine *et al.,* 2023). Other studies have indicated that the synthesis of secondary metabolites can also be significantly influenced by additional environmental factors present at the sampling site, referred to as environmental stressors (Lia *et al*., 2020), such as chemical stress (Verma and Shukla, 2015), nutrients (Dar *et al.,* 2016) and metal ions (Ma *et al.,* 2018). However, Qaderi *et al.* (2023), in their study on plant responses to climate change, demonstrated that factors such as high temperatures, increased carbon dioxide levels, drought stress, and enhanced ultraviolet-B radiation also serve as regulatory mechanisms for secondary metabolite synthesis. Additionally, recent studies showed that the soil microbial community plays an important role in producing secondary metabolites (Xiao *et al.,* 2022; Lv *et al.,*

2024). Microorganisms play a crucial role in regulating hormone levels, nutrient absorption, the availability of precursor substances, and enzyme and gene expression, thereby promoting the synthesis and accumulation of secondary metabolites in plants (Lv *et al.,* 2024). Therefore, we can infer that the insecticidal activity of *A. campestris* is closely linked to both abiotic and biotic factors, as the expression of the plant's secondary metabolites is primarily a response to these influences.

Extraction solvent exhibits variation in insect mortality

Our study demonstrates that the extraction solvent plays a significant role in determining the insecticidal activity of *A. campestris*, with the chemical compounds extracted using methanol resulting in a higher mortality rate for aphids. This result could be due to the considerable capacity of methanol to separate bioactive compounds. For instance, Boudjouref *et al.* (2019) showed that the methanolic extract of *A. campestris,* originally from Boussaâda and Oum El Bouagui in Algeria, had a higher content of polyphenols, flavons and flavonols and tannins, comparatively to the water extracts. They vary from 88.61 to 82.84 µgGAE/mgDW in polyphenols, from 111.93 to 217.75 µgQE/mgDE in flavones and flavonols and from 36.88 to 70.59 µgCE/mgDE in tannins. However, flavonoids were lower than the water extract. Their content was from 12.91 to 13.72µgQE/mgDE in the methanolic extract and from 31.84 to 33.14 µgQE/mgDE in the water extract. Another study on *A. campestris* of Boussaâda showed that the ethyl acetate extract was rich in phenolic compounds with 481.25 ± 0.026 mgGAE/gDW, while the chloroform extract had the highest content of flavonoid with 34.37 ± 0.056 mgQE/gDE (Djidel and khennouf, 2014). Furthermore, a comparative study of four solvents (dichloromethane, ethyl acetate, butanol and water), prepared from *A. campestris* grown in Tunisia, showed that the best one for the extraction of total polyphenols (345.8 mgGAE/gWE) and total flavonoids (296.3 mgQE/gDE) when butanol and ethyl acetate were used as solvents, respectively (Metouia *et al.,* 2022). The extraction yield percentages that should be another cause reinforcing our results, they exhibited some similarities in the two solvents used as already shown in Table 3 and those tested by Metouia *et al.* (2022). In addition to the differences in polarity among various solvents, the extraction yield may not solely depend on this factor; it could also be influenced by extraction conditions such as temperature. These conditions affect several physical properties, including viscosity, solubility, diffusivity, and surface tension, which collectively contribute to a higher concentration of chemical compounds and, consequently, a greater bioactive effect (Latitha and Jayanthi, 2012). Our findings permit to suggest that the higher insecticidal activity of the methanolic extract (Figure 3, Table 5) can be related to its richness in bioactive compounds that cause a considerable insect mortality. Thus, testing other solvent and controlling extraction conditions may provide novel horizons on bioinsecticides.

Phenological phase drives shifts in insect mortality

The notable insecticidal activity of *A. campestris* collected during the vegetative phase may be attributed to its abundance of bioactive compounds, which are closely linked to a high concentration

of secondary metabolites. This richness can, in turn, be a result of the strong allelochemical effects that *A. campestris* exerts on its immediate environment. Effectively, at the approach of the dry season, coinciding with the peak of the vegetative phase, the *Artemisia* genus produces a high quantity of secondary metabolites that inhibit seed germination of plant species, which can be found around *Artemisa,* to avoid competition to water during the dry season (Lahmar-Zemiti, 2001). The inhibitory effect of *A. campestris* on mycorrhizal fungal colonization in roots of three sand-dune grasses, i.e. *Agropyron psammophilum, Elymus canadensis* and *Panicum virgatum* has been already shown by Yun and Maun (1997). Our result can also be related to the high yield extraction during the vegetative phase comparatively to the reproductive one (Tableau 3). In fact, Sefi *et al.* (2010) had reported that the yield extraction of *A. campestris*, collected from Mahdia (Tunisia) during the vegetative phase, was higher 19.36 ± 0.02. The variation in chemical composition between phenological phases was already noted in *A. campestris* grown from Tunisia (Akrout *et al.,* 2003) and from Djelfa region (Touil *et al.,* 2017), where the essential oil obtained during the vegetative phase, had a high oil content and/or chemical composition. Furthermore, the recent study of Brahmi *et al.,* (2023) showed that the methanolic extracts during the vegetative phase of *A. campestris*, the same extracts used in our study, presented higher content in polyphenols, flavonoids and tannins comparatively to the reproductive phase. In conclusion, *A. campestris* tends to produce a high concentration of chemical compounds, enabling it to effectively engage in biotic interactions and serve as an efficient bioinsecticide against aphids.

Conclusion

The present study has demonstrated the possible using of botanical extracts prepared from *A. campestris* as insecticide against *A. fabae*, infesting crop production of *Vicia faba*. Our results have showed that the origine of the plant play an important role on its potential insecticidal activity. The growth development of *A. campestris* significantly influences its insecticidal activity, particularly during the vegetative stage. We can suggest that both abiotic and biotic conditions exert pressure on the plant, prompting it to produce secondary metabolites not only for survival under challenging conditions but also to balance the trade-off between vegetative and reproductive growth. Thus, secondary metabolites produced constitute a veritable resource of bioactive compounds that lead to a high potential insecticidal activity of *A. campestris*. The extraction solvent had also a considerable impact on the efficiency of our botanical insecticides. However, further studies using other solvents and extraction processes such as decoction or infusion are strongly needed. Furthermore, testing the insecticidal effect of this plant in the field and on other pests is recommended. Further studies should be conducted *(i)* to identify the biotic and abiotic factors that regulate the synthesis and accumulation of secondary metabolites, and *(ii)* to determine which specific bioactive compounds are most effective against aphids.

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Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this research paper.

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