

Evaluation of biocidal activity of four Lamiaceae leaves on the black bean aphid *Aphis fabae* Scopoli, 1763 (Homoptera: Aphididae)

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Abstract: The objective of our work was the valorisation of four aromatic plants growing spontaneously in Kabylia (Algeria) by the evaluation of their insecticidal activity against the black bean aphid *Aphis fabae* Scopoli, 1763. These are oregano, thyme, rosemary and lavender. The plants were subjected to a phytochemical analysis to determine their secondary metabolites composition. The results obtained showed that the four extracts are toxic to *A. fabae* and can significantly reduce its populations. LD50s of 7.76 %; 8.91 %; 9.72 % and 12.88 % were recorded for extracts of oregano, rosemary, thyme and lavender respectively. In addition, the phytochemical screening shows the presence of flavonoids, tannins, saponins and polyphenols. The polyphenols extraction indicates that the oregano extract is the richest with a polyphenol content of 218.73 (\pm 0.22) μ g GAE ml⁻¹. This substance has significant biocidal power.

Key words: *Aphis fabae*; mortality rate; Lamiaceae; polyphenols; phytochemical screening

Ovrednotenje biocidne aktivnosti izvlečkov listov štirih vrst ustnatic (Lamiaceae) na črno fižolovo uš, *Aphis fabae* Scopoli, 1763 (Homoptera: Aphididae)

Izvleček: Predmet razikave je bilo ovrednotenje insekticidne aktivnosti štirih aromatičnih rastlin, ki rastejo samoniklo na območju Kabylije (Alžirija) na črno fižolovo uš *Aphis fabae* Scopoli, 1763. Izbrane rastline so bile dobra misel, timijan, rožmarin in sivka. V rastlinah so s fitokemičnimi analizami določili sestavo sekundarnih metabolitov. Rezultati so pokazali, da so bili vsi štirje izvlečki strupeni za črno fižolovo uš in so znatno zmanjšali njeno populacijo. Vrednosti LD50 so za izvlečke dobre misli, rožmarina, timijana in sivke znašale 7,76 %; 8,91 %; 9,72 % in 12,88 %. Fitokemične analize rastlin so pokazale prisotnost flavonoidov, taninov, saponinov in drugih polifenolov. Izvleček polifenolov je pokazal, da je bila na njih najbogatejša dobra misel z vsebnostjo 218,73 (\pm 0,22) μ g GAE ml⁻¹, kar kaže na njeno znatno biocidno moč.

Ključne besede: *Aphis fabae*; smrtnost; Lamiaceae; polifenoli; fitokemijska analiza

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1 INTRODUCTION

Herbivorous insects are responsible for significant losses to agriculture due to food damage, but also by carrying pathogens such as viruses (Kortbeek et al., 2019). They have implemented a range of strategies allowing them to discover their host plants and then reproduce and develop at their expense (Huignard, 2013). Aphids are considered among the most important pests of crops. They cause significant financial losses, and are responsible for yield losses in many cultivated plants (Tagu et al., 2004).

These pests cause direct damage by sucking sap, and indirectly by transmitting phytopathogenic viruses and by secreting honeydew on which sooty mold is deposited, which decreases photosynthesis (Blackman and Eastop, 2000; 2006). The use of insecticides is one of the first methods used against aphids (Esmaili-Vardanjani et al., 2013). However, as a result of excessive use of these products, some aphids have developed resistance to most of them. In addition, insecticides destroy beneficial organisms and disrupt ecosystems (Harmel et al., 2010; Sabahi et al., 2011).

It is therefore necessary to have an alternative control program and to search for new products, on the one hand, to ensure effective protection of agricultural production, and on the other hand, to contribute to sustainable management of the environment (Bouchelta et al., 2005). For this purpose, several control methods are recommended to limit the level of aphid outbreaks in crops (Harbaoui et al., 2008). Among these, the use of plant extracts endowed with insecticidal activities which offer a certain potential (Isman, 2000). In fact, plants have developed different mechanisms to defend themselves against insects, including the production of specialized metabolites that act as natural insecticides (Kortbeek et al., 2019).

According to Huignard (2013), plants are rich in phenolic compounds. The latter are of significant physiological and morphological importance in the plant kingdom; they are involved in growth, reproduction and give the plant some resistance against pathogens and pests (Maroun et al., 2013). The main objective behind this work is to study the insecticidal activity of four aqueous extracts of Lamiaceae: oregano (*Origanum floribundum* Munby), thyme (*Thymus numidicus* Poir.), rosemary (*Rosmarinus officinalis* L.) and lavender (*Lavandula stoechas* L.) on the black bean aphid *Aphis fabae* Scopoli, 1793 (Hemiptera: Aphididae) which can transmit 30 viral diseases to plants (Jahan et al., 2019), as well as the research of the main active elements and the content of polyphenols that exist in each plant.

2 MATERIALS AND METHODS

2.1 HARVESTING PLANT MATERIAL

During this experiment, four species of Lamiaceae were tested on the black bean aphid, these are rosemary (*Rosmarinus officinalis* L.), thyme (*Thymus numidicus* Poir.), oregano (*Origanum floribundum* Munby) and lavender (*Lavandula stoechas* L.). Rosemary and lavender were harvested in a private garden located in the region of Draâ El Mizan at an altitude of 432 m (36° 32'8"N. 3° 50'3"E.). Whereas the thyme came from the mountains of Ait Bouadou (Ouadia) at an altitude of 828 m (36° 30'0"N. 4° 1'0"E.), and oregano was harvested in a private plot located in the Makouda region at an altitude of 458 m (36° 47'27"N. 4° 4'1"E.).

2.2 PREPARATIONS OF AQUEOUS EXTRACTS

For each tested plant, leaves are dried and then crushed. 50 g of powder for each plant were macerated in 1 l of distilled water for 24 hours. Resulting solutions were filtered then stored in vials and kept in the dark. 11 doses (1 %, 2 %, 3 %, 4 %, 5 %, 10 %, 20 %, 30 %, 40 %, 50 % and 100 %) whose concentrations vary according to the dilution of the stock solution in the distilled water were prepared.

2.3 EXPERIMENTAL DESIGN

An experimental design formed from 144 jars of infested beans are treated with the four plant extracts by means of contact. 12 batches including a control batch have been used for each extract. Aphids were given one dose per batch, with three repetitions for each dose. The three control jars were treated with distilled water.

2.4 APPLICATION OF TREATMENTS

After sowing the bean seeds ('Hista') in plastic pots, small seedlings obtained were infested with 40 aphids; larvae of different stages and adults. After three days of infestation, the treatment was applied by spraying different doses of the four aqueous solutions on the aphid colonies. In order to follow the chronological evolution of aphids mortality subjected to different extracts at different concentrations, observations were made daily after spraying. A magnifying glass has been used to count the dead and alive aphids.

2.5 CHARACTERIZATION OF MAIN CHEMICAL COMPONENTS

Phytochemical study enables the detection of bioactive secondary metabolites existing in the leaves of the plants tested. It is based on coloring and precipitation reactions by specific chemical reagents. The method used is the approach adopted by Tona et al. (1998) and Longaga et al. (2000).

Besides, total polyphenol content is obtained by spectrophotometry according to Folin-Ciocalteu (FCR) method (Singleton et al., 1999).

2.6 DATA ANALYSIS

Mortalities in the treated batches (M_o) were expressed according to Abbott's formula (1925) in corrected mortality (M_c), taking into account natural mortalities observed in the control batches (M_t). Mortality is expressed as a percentage calculated using the following formula:

$$M_c = (M_o - M_t / 100 - M_t) \times 100$$

M_c : Corrected mortality rate

M_o : Mortality rate in the treated population

M_t : Mortality rate in the control population

2.7 KRUSKAL-WALLIS TEST

The Kruskal-Wallis non-parametric test has been used to compare the average mortalities obtained with different treatments. It is based on classification in ascending order of all observations (Legras and Kohler, 2007).

2.8 CALCULATION OF LETHAL DOSE (LD50) AND LETHAL TIME (TL50)

The method of Finney (1971) based on the regression of the probits of mortalities depending on the logarithms of the doses of solutions tested made it possible to determine the LD50. The 50 % lethal dose of each extract was estimated, after exposure of aphids to different concentrations tested. These values were determined from an experimental curve giving the variations in mortality according to increasing concentrations of the extracts. The 50 % lethal time (TL50) was also determined by the same method. The time is transformed into a logarithm and the percentage of mortality corrected into probit.

3 RESULTS

After spraying the leaf solutions of the four Lamiaceae on *A. fabae* populations, an increase in the percentage of mortality of the latter appeared as a function of time and dose (Fig. 1). Total mortality was observed after 12 days of treatment at the highest doses (50 % and 100 %) for oregano, thyme and rosemary extracts, and at the 100 % dose for lavender extract (Table 1).

3.1 KRUSKAL-WALLIS TEST

The mortality-dose boxplot shows that the mortality rate is dose dependent; as the dose increases, the mortality rate increases (Fig. 2). This is confirmed by the Kruskal Wallis test, with a p-value of 5.834e-14 for thyme, 3.891e-16 for oregano, 2.695e-12 for rosemary and 9.585e-14 for lavender. These values are less than 0.05. This means that there is a highly significant effect of dose on aphid mortality.

The Mortality-time boxplot shows that the mortality rate is higher at time T6 = 12 days after treatment (Fig. 3). This is confirmed by the Kruskal Wallis test with a p-value of 1.811e-14 for thyme, 9.912e-14 for oregano, 5.711e-16 for rosemary and 5.006e-16 for lavender. These values confirm that there is a very significant effect of time on aphid mortality.

3.2 DETERMINATION OF THE LD50 OF THE VARIOUS TREATMENTS TESTED ON *A. FABAE*

After spraying the aqueous extracts of the four plants tested on different batches of aphids, at doses ranging from 1 % to 100 %, the percentages of mortality transformed into probits were recorded and plotted in figure 4. The LD50 of each product confirms the results obtained in the tests. Indeed, the LD50 obtained showed that the solutions tested present a high degree of toxicity towards these insects. Oregano extract gave the lowest lethal dose (7.76 %). It is therefore the most toxic extract compared to the other biopesticides with LD50s of 8.91 %, 9.72 % and 12.88 % for rosemary, thyme and lavender extracts respectively.

3.3 DETERMINATION OF THE TL50 OF THE VARIOUS TREATMENTS TESTED ON *A. FABAE*

Calculation of the lethal time showed that the extracts tested on the black bean aphid had a fairly high

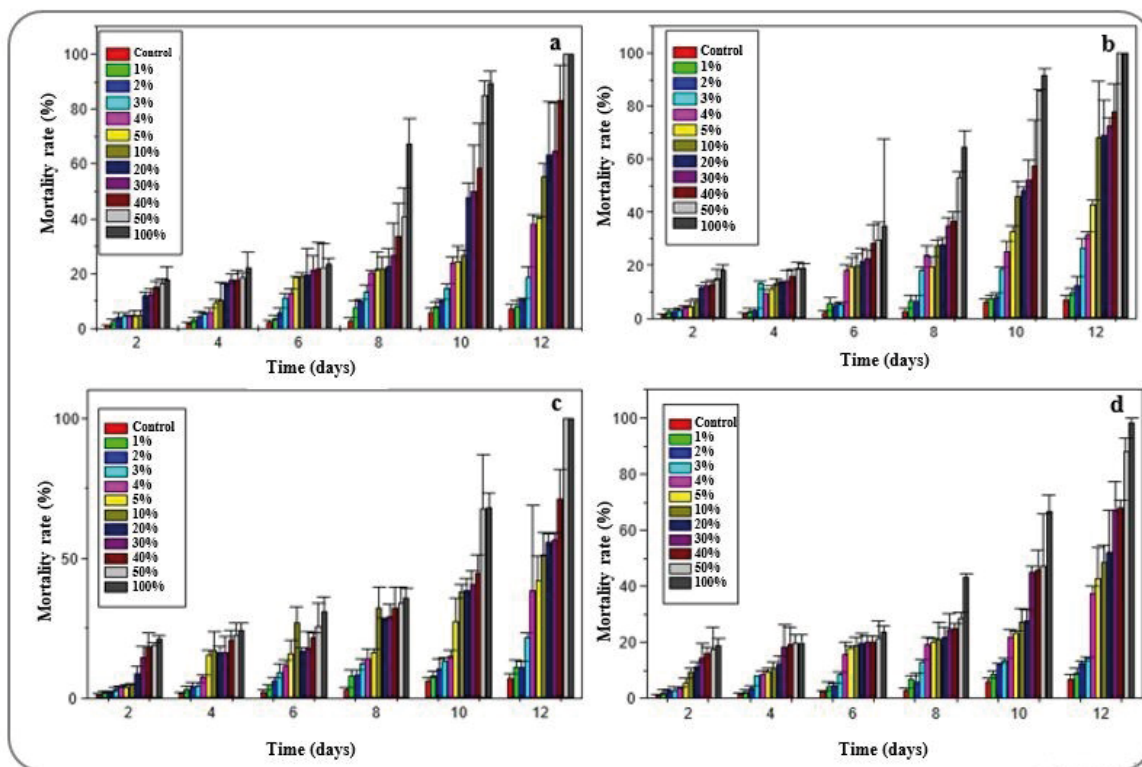


Figure 1: Aphid mortality rate (a: Thyme extract, b: Oregano extract, c: Rosemary extract, d: Lavender extract)

Table 1: Corrected mean mortality of populations of *Aphis fabae* after 12 days of treatment with the 4 aqueous extracts

Dose (%)	Corrected mean Mortality (%)			
	Oregano	Thyme	Rosemary	Lavender
1	3.03	0.88	2.58	1.76
2	5.61	2.95	4.13	5.46
3	20.7	12.61	15.51	7.81
4	26.1	33.63	33.57	32.86
5	38.42	35.66	37.34	38.57
10	65.79	51.83	47.68	44.93
20	66.53	60.64	52.25	48.09
30	70.29	61.81	53.44	64.81
40	76.06	82.25	69.18	65.94
50	100	100	100	87.29
100	100	100	100	100

biocidal activity that could reduce 50 % of the pest population on the 5th day after spraying the solutions based of oregano leaves (123.03 hours) and of thyme (128.82 hours) and after 6 days for those based on rosemary leaves (134.89 hours) and lavender (141.25 hours) leaf solutions (Fig. 5).

Table 2: Results of the chemical analysis of the four plants tested

	Thyme	Oregano	Rosemary	Lavender
Total tannins	+	+	+	+
Catechetical tannins	+	+	+	+
Gallic tannins	+	+	+	+
Anthocyanins	-	-	+	-
Saponoside	+	+	+	+
Mucilages	+	+	+	+
Reducing sugars	+	+	+	+
Flavonoids	+	+	+	+
Total Polyphenols	+++	+++	+++	+++
Glucosides	+	+	+	+
Starch	+	+	+	+
Protein	+	+	+	+

(-): absence of substance; (+): presence of substance; (+++): very high substance content

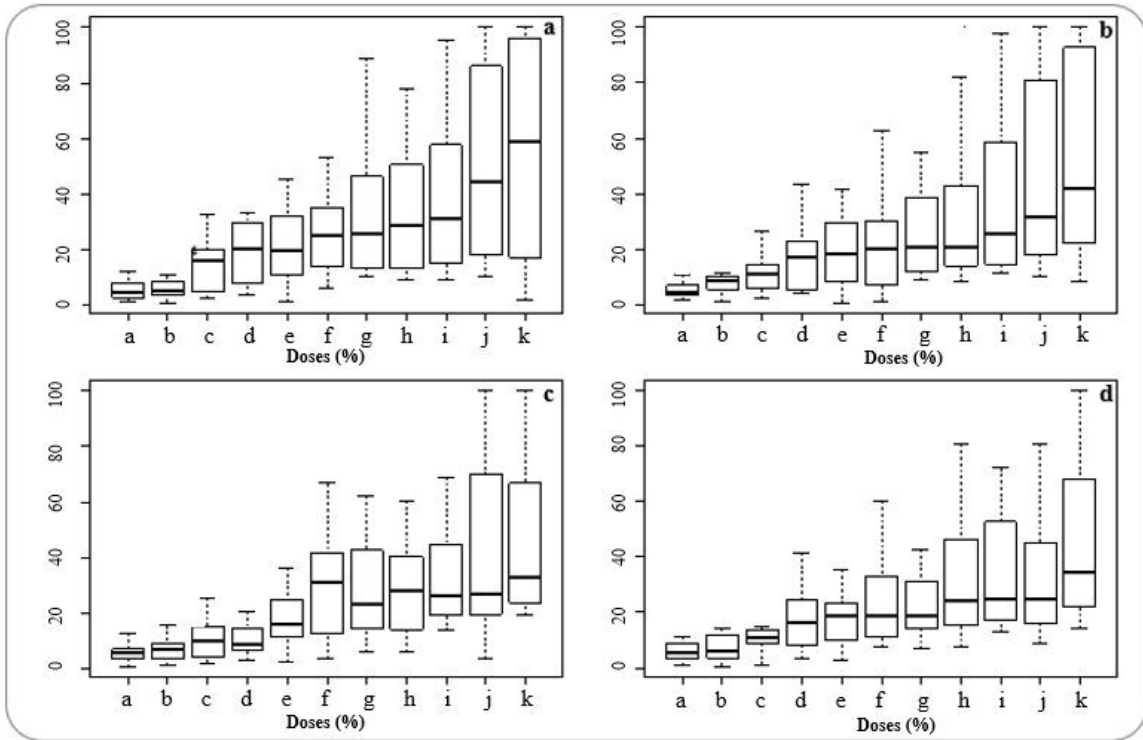


Figure 2: Mortality-dose boxplot for the four aqueous extracts (a: Oregano extract, b: Thyme extract, c: Rosemary extract, d: Lavender extract)

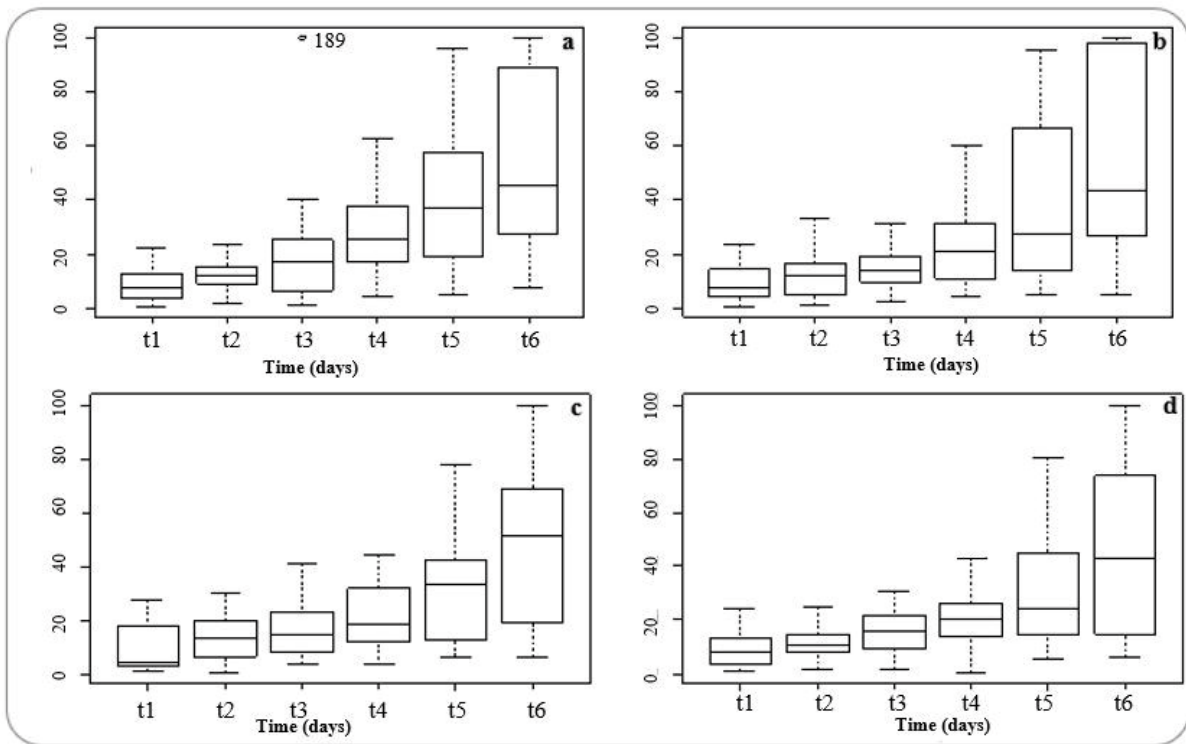


Figure 3: Mortality-Time boxplot for the four aqueous extracts (a: Oregano extract, b: Thyme extract, c: Rosemary extract, d: Lavender extract)

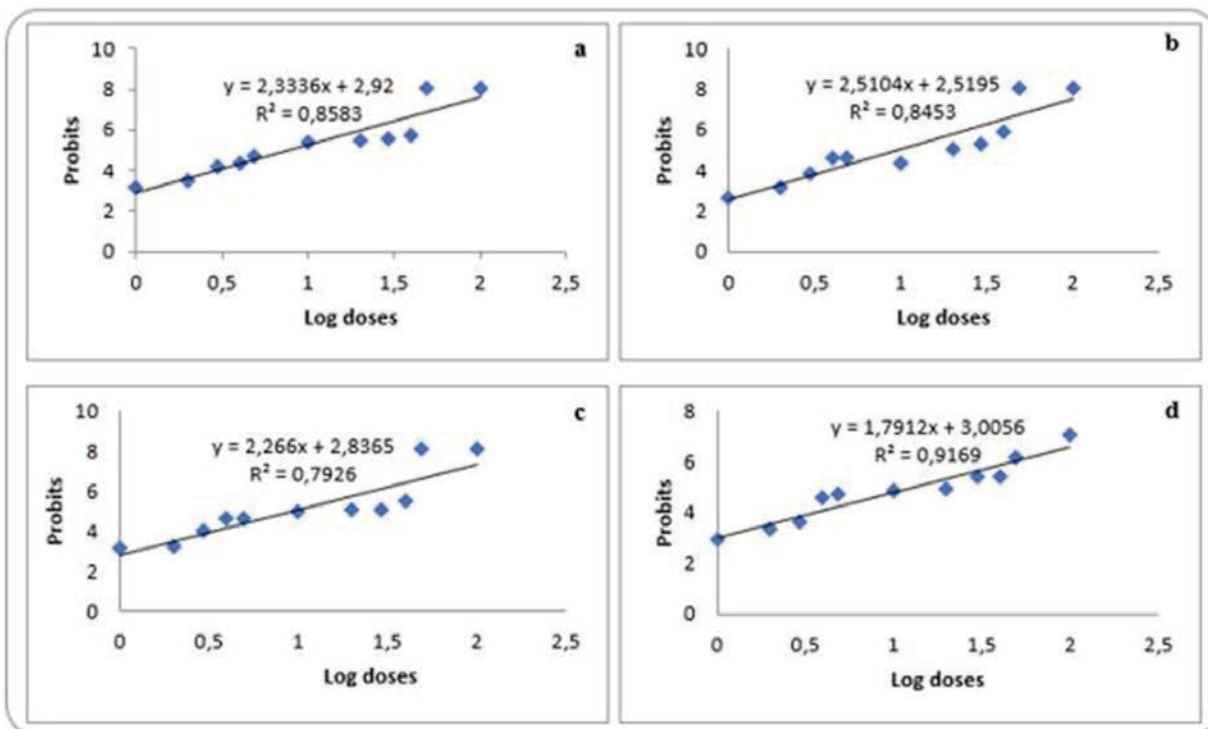


Figure 4: Aphid mortality rate as a function of the logarithm of the dose (a: Oregano extract, b: Thyme extract, c: Rosemary extract, d: Lavender extract)

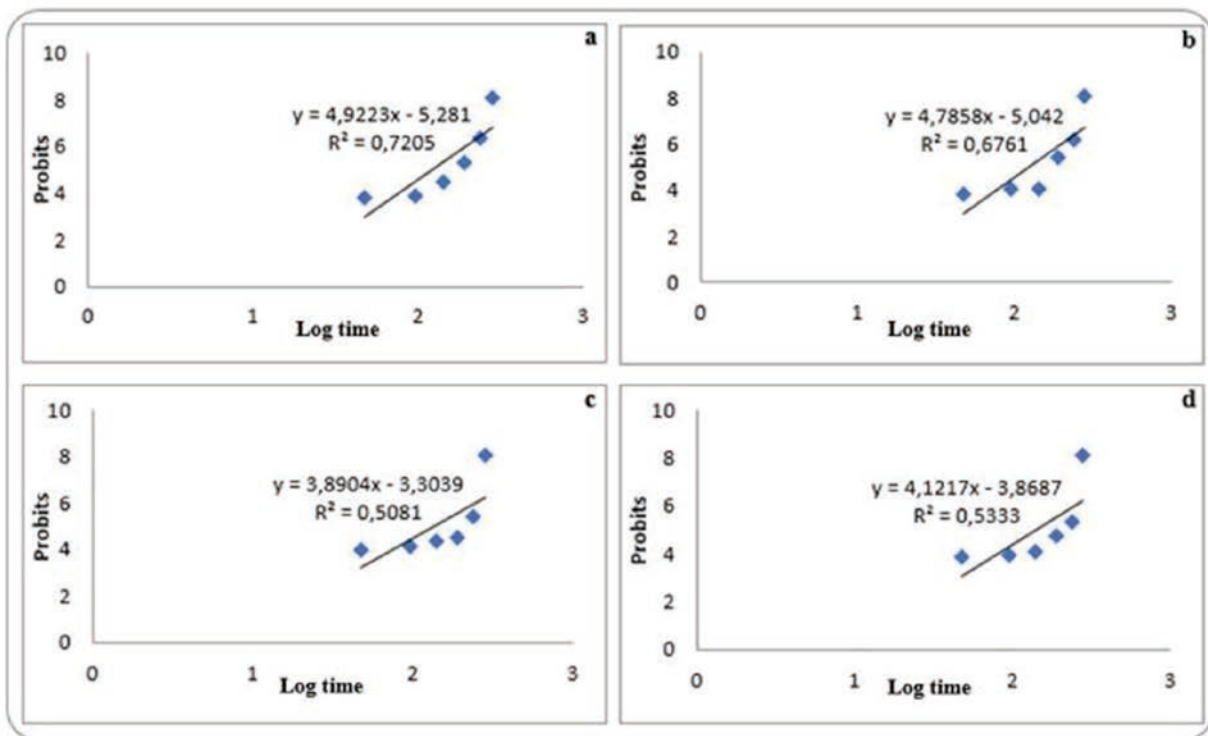


Figure 5: Aphid mortality rate as a function of the logarithm of time (a: Oregano extract, b: Thyme extract, c: Rosemary extract, d: Lavender extract)

3.4 CHEMICAL COMPOSITION OF THE EXTRACTS TESTED

The results of the phytochemical characterization tests for the four species made it possible to demonstrate the presence of several common compounds between them. These are polyphenols, flavonoids, tannins, saponosides, mucilages, reducing sugars, glucosides, starch and proteins (Table 2). The presence of anthocyanins was only observed in *Rosmarinus officinalis*. Our results also showed that all four plants have very high polyphenol content.

3.5 TOTAL PHENOL CONTENT

UV/Visible spectrophotometry made it possible to quantify the level of polyphenols present in the extracts prepared from the four plants. The results obtained are expressed in microgram equivalents of gallic acid standard used per ml of extract ($\mu\text{g GAE ml}^{-1}$ extract) and determined by the equation: $y = ax + b$. The results are reported in Figure 6.

The results obtained revealed that the four plants are rich in polyphenols with a total phenol content that varies between $64.2 \mu\text{g GAE ml}^{-1}$ and $218.73 \mu\text{g GAE ml}^{-1}$. From these results, it appears that oregano contains the

highest concentration of polyphenols compared to the other Lamiaceae species.

4 DISCUSSION

Under the conditions of this study, aqueous extracts obtained from Lamiaceae leaves had an effect on the mortality of *A. fabae* populations. The results obtained after the bioassays with the 4 biopesticides showed a direct relationship between aphid mortality rates on the one hand and product concentration and exposure time on the other hand.

Aphids were very sensitive to the bioassays as extract concentrations increased and time passed. In the range of eleven concentrations tested, the 50 % and 100 % doses induced high toxicity on aphids after 12 days of contact. The reference control caused low mortality rates compared to the biopesticides tested.

Several studies have shown that the toxic effect of plant extracts is related to the concentration of the extract and the period of exposure.

The work of Habou et al. (2011) showed that the biocidal effect of the essential oil of *Jatropha Jatropha curcas* L. on the black bean aphid increases with the increase of the dose. They obtained a mortality rate of 100 % for the 15 % dose after 96 h. Authors indicate that the number of dead aphids increases with the duration of treat-

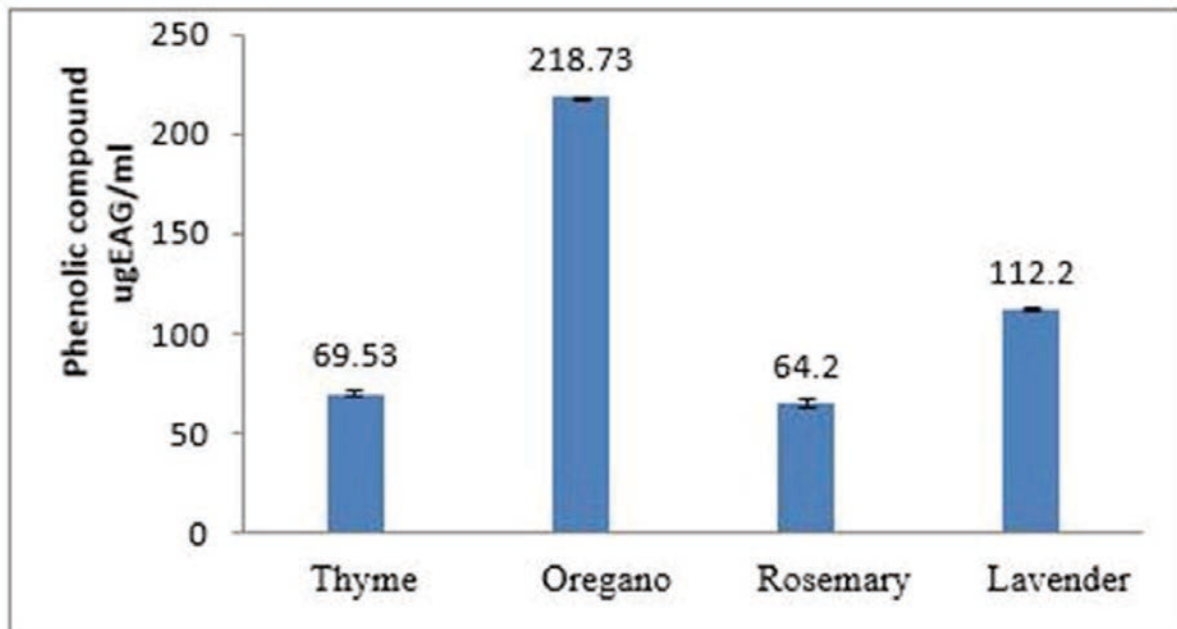


Figure 6: Polyphenol content of the four Lamiaceae expressed in micrograms of gallic acid equivalents per milliliter of extract ($\mu\text{g GAE ml}^{-1}$)

ment, it is after 72 hours that they observe a high mortality rate and which becomes stable between 96 and 120 hours. Besides, Akantetou et al. (2011) mentioned that the mortality rate of aphids subjected to different concentrations of oil of the whitish basil (*Ocimum canum* Sims) increased numerically in a linear manner according to the observation periods (1, 3, 5 and 24 hours). Similarly, Laznik et al. (2011) stated that the highest mortality rate of *Aphis pomi* populations occurred after the third day after treatment with *Symphytum officinale* and *Calendula officinalis* extract compared to the first and second day post-treatment.

The insecticide effect-dose relationship was also confirmed by the study conducted by Benoufella-Kitous et al. (2014) on *A. fabae*. According to these authors, the two aqueous extracts of nettle and fern showed a considerable insecticidal effect at the highest dose, i.e. 73.8 % and 75.1 % mortality respectively. The biocidal effect of nettle and fern on *A. fabae* increased during the days following the application of the aqueous extracts, reaching a maximum after 6 days. Our results are confirmed by those found by Baba-Aissa et al. (2017) who showed that the toxicity of the essential oil of sour orange is dependent on the dose, indicating that more the dose increases, more the formulation presents a greater biocidal effect which results in a reduction in the density of populations of *A. fabae*. In addition, Kulimushi's study (2014) showed that the degree of toxicity of the extracts is related to the dose used. After treatment of populations of *A. fabae* with aqueous extracts of garlic and papaya leaves and their combination, the average number of aphids decreased from 1 to 36 aphids and on average 86.92 % of aphids were controlled.

These results are in agreement with those Saïfi and Belhamra (2018) noted that the toxicity of the essential oil of *Thymus palleescens* de Noë depends on the concentration. These authors recorded on populations of *A. fabae*, after 24 h, a mortality rate of 34.75 % at the dose of 12 $\mu\text{l ml}^{-1}$. In a study on the insecticidal activity of sage against the black bean aphid, Benoufella-kitous et al. (2020) demonstrated that the effect of this plant with regard to this pest is the highest 9 days after treatment. Furthermore, Oulebsir-Mohand Kaci et al. (2015) reported that the mortality rate of *Myzus persicae* (Sulzer, 1767) treated with two plant extracts *Eucalyptus globulus* Labill. and *Thymus vulgaris* L. increases proportionally with increasing dose. The latter state that the D4 dose (8 $\mu\text{l ml}^{-1}$) shows a mortality rate of 65.4 % and 71.7 % respectively for the two extracts and that the aphid mortality rate is increasing over time. Likewise, in a study on the biological activity of santolina (*Santolina africana* Jord. & Fourr) against the aphid *Aphis craccivora* C.L.Koch, 1854], Lebbal et al. (2017) demonstrated that the toxic

effect of this plant with regard to this pest was proportional to the dose, with a mortality rate of 80 % at the dose of 15 % after 24 hours. According to Kumar and Patel (2017), the extract of *Cassia angustifolia* M.Vahl. showed a toxic effect on *Brevicoryne brassicae* (L., 1758) with a mortality rate of 100 % at doses of 7 % and 10 % after 72 hours of exposure.

On the other hand, Acheuk et al. (2017) mentioned that the crude ethanolic extract of *Artemisia judaica* L. revealed potent insecticidal effects against the black aphids, *A. fabae*. Total mortality (100 %) was reached 2 hours after treatment with the highest concentration. Besides, Lebbal et al. (2018) found that the extract obtained by maceration of *Thymus algeriensis* Bioss. & Reut. at a concentration of 25 % was the most effective, with a larval mortality rate of 70 % of *A. fabae* individuals after 24 h.

The toxic effects of the solutions could depend on their chemical composition and the sensitivity level of the insects. According to Saidj (2007), among the plants whose efficacy has been evaluated, aromatic plants of the Labiatae family were the most active as direct insecticides but also as inhibitors of oviposition and larval development of insects. Chiasson and Beloin (2007) suggested that biopesticides act directly on the cuticle of insects and mites, especially soft-bodied ones such as aphids.

The phytochemical study of the four aqueous extracts showed that these plants contain mainly flavonoids, tannins, saponosides and other polyphenols. These results confirm that plants of the Lamiaceae family are medicinal plants rich in secondary compounds that have a toxic effect against insect pests. Similarly, Asghari et al. (2017), in a phytochemical analysis study carried out on several plants of Lamiaceae used in medicine in Aligudarz region, in Iran, show that the three species of thyme: *Thymus daenesis* Celak, *Thymus ericalyx* (Roninger) J alas, and *Thymus lancifolius* Celak are rich in flavonoids and tannins.

According to Huignard (2013), secondary compounds in plants can cause the death of insects that try to consume them by disrupting the functioning of the nervous system, the digestive system or by preventing larval growth. For instance, phenolic compounds are toxic when ingested by phytophagous insects (Kortbeek et al., 2019). They are present in all parts of higher plants: roots, stems, leaves, flowers, fruits and seeds (Medic-Saric et al., 2003; Boizot and Charpentier, 2006). According to Galeotti et al. (2008), secondary metabolites such as alkaloids, saponosides, polyphenols (flavonoids and tannins) have pharmacological and toxicological activity. Some alkaloids, anthocyanins, flavonoids, quinines, lignans, steroids, and terpenoids have commercial application in the pharmaceutical and biomedical fields and are included in

drugs, dyes, flavours, fragrances and insecticides (Teixeira Da Silva, 2004).

The results obtained revealed that the four plants are rich in polyphenols with a total phenol content varied between 64.2 µg GAE ml⁻¹ and 218.73 µg GAE ml⁻¹. These results of the quantitative analysis of polyphenols are close to those of several authors. Celiktas et al. (2007) noted polyphenol concentrations for the crude extract of *Rosmarinus officinalis* ranging from 34.1 to 119 mg GAE g⁻¹. Cocan et al. (2018), in a study on the biological activity of rosemary officinale extracts, state that this plant has a polyphenol content that is 86.05 ± 0.40 mg GAE g⁻¹. Fadili et al. (2015) mentioned that *Rosmarinus officinalis* and *Thymus satureioides* Coss. are rich in polyphenols for all the fractions studied and the concentration of polyphenols varies between 21.66 ± 2mg GAE g⁻¹ to 185.71 ± 4 mg GAE g⁻¹. Kholkhal et al. (2013) reported that the polyphenol content of *Thymus ciliatus* Desf. is 64.23 mg GAE g⁻¹. In Morocco, Bachiri et al. (2016) noted that *Lavandula stoechas* and *L. dentata* L. are composed of polyphenol contents of 150.34 mg g⁻¹ and 184.02 mg g⁻¹ respectively.

In general, oregano extract was the most effective (LD50 = 7.76 %, TL50 = 123.03 hours) on aphid populations. Low LD50 values indicate strong insecticidal activity and low TL50 values indicate a rapid biocidal effect. The obtained results seem to show that the polyphenols with the highest concentration in this plant would be the active ingredient that plays a determining role in the biocidal activity of this plant. The richness of the aqueous extract in chemically active compounds could explain the traditional use of this plant in various fields. However, the synergistic additive effect of the different compounds may also be a factor explaining the remarkable activity revealed by the rosemary extract. The latter was found to be highly toxic to aphids (LD 50 = 8.91 %).

Lavender extract ranked last (LD50 = 12.88 %, TL50 = 141.25 hours) compared to the other solutions tested despite its high polyphenol content. This would suggest that the insecticidal activity of plants is not limited to some of their major constituents; it could also be due to some minority constituents. According to Akantetou et al. (2011), the difference in toxicity between the different extracts could be explained by the growing conditions, the harvesting period and the climatic and edaphic conditions. The distribution of secondary metabolites may change during plant growth. This may be related to conditions of high temperature, sun exposure, drought and salinity, which stimulate the biosynthesis of secondary metabolites such as polyphenols (Falleh et al., 2008; Zouali et al., 2010).

Some works has shown that the toxic effect of extracts depends on the nature of the plant. According

to Khalfi-Habes and Sellami (2010), oregano shows a stronger insecticidal action than rosemary and thyme. These results confirm those obtained in the present study. Similarly, Kumar and Patel (2017) state that *Curcuma angustifolia* Roxb. causes a higher toxic effect compared to the other plants tested (*Cercis gigantean* L., *Cannabis sativa* L., *Parthenium hysterophorus* L., *Lobelia chinensis* Lour., *Solanum nigrum* L. and *Ageratum conyzoides* L.). In a study on the biological activity of nettle and fern on the black bean aphid, Benoufella-Kitous et al. (2014) showed that the most significant toxic effect was recorded for the second species. Benoufella-Kitous (2015) notes that among 8 plant species tested against *A. fabae*, the most toxic extracts were those from the leaves of toothed lavender with an efficacy of 99.4 % at the 10 % dose, sage with an efficacy of 98.5 % at the 40 % dose and garlic with an efficacy of 97.9 % at the 10 % dose.

5 CONCLUSION

The present study showed the relative importance of the use of botanical pesticides, namely aqueous extracts of oregano, rosemary, thyme and lavender leaves, against the black bean aphid.

The phytochemical study revealed the presence of the main groups of active chemical compounds in these plants (polyphenols, gall and tannins, saponosides, flavonoids). Fractionation of these extracts will probably allow the isolation of the active principles responsible for their biological activities.

These aromatic plants being very commonly found in Algeria could open up interesting prospects for their use in the production of biopesticides. They therefore appear as potentially usable for an integrated management of aphids after field tests to confirm their aphicidal activity.

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