**Insecticidal Properties and Chemical Composition of “*Myrtus communis L*” and “*Rosmarinus officinalis* L” Essentials Oils against pests *Aphis faba* L. (Hemiptera: Aphididae)**

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**Abstract :** The black beans Aphid, Aphis faba L are very important pest species of many greenhouses and field plants in the world. which causes damages through feeding on sap and transmitting viral diseases. In this research, the essential oils of medicinal plants: *Myrtus communis L* (Myrtacées) and *Rosmarinus officinalis L* (Lamiacées) were investigated for their fumigant toxicity and repellent activity against aphis faba L, under laboratory conditions. LC50 values of essential oils after 23 hours for rosemary oil were 32,04 µL/cm3 and 34.48 µL/cm3 for basil oil and (35.20-35.23 µL/cm3). The composition **chimic** of the essentials oils was analysed by (GC-MS), showed a variety of chemical compounds that make up the essence of each oil, basil essential oil α-pinene, 1,8 cineole, α-terpinol, linalool. Rosemary oil consisting of: 1, 8-cineole, 2-pinene, D-camphor, β-Pinène, Camphene. Susceptibility tests are carried out under laboratory conditions against a pest aphid black, and this biological activity is mainly attributed to the richness of these essences in terpene compounds known to be effective against pests.

The obtained results indicated this study results suggested that the essential oils have a great potential to be used in agriculture against aphis faba L as an alternative to chemical pesticides for pest control in the near future.

**Key words:** chemical compounds, ficia faba L, huiles essentielle, pesticides, fumigant toxicity.

**Introduction**

The cultivation of legumes is one of the important economic crops in various countries of the North African region in general and Algeria in particular, as it is the largest among food legumes in Algeria due to the fact that its grains are a high-quality and low-cost source of protein for a large segment of the population. About 68 locally cultivars, have been identified by morphological and agronomic characterization (**Meradsi, 2016).** The production of green beans, according to the statistics of the Ministry of Agriculture and Rural Development for the year 2018 in Algeria, amounted to 3,086,891 quintals per hectare, with a yield of 94.6 quintals per hectare, recording an increase from the year 2017, when the production yield reached a ceiling of 88.9 quintals per hectare, with a production of 2,886,198 quintals. (**Agricultural Statistics, 2018**).

This culture is confronted with several biotic and abiotic constraints. Among these biotic agents are the aphids which have a very special place, from a depredation point of view, the aphid causes direct loss of yields and the deterioration of the quality of the vegetable. (**Mostafa *et al.,*., 2014**). It is accepted that Herbivorous insects are responsible for significant losses to agriculture due to food damage, but also by carrying pathogens such as viruses, and as agricultural production systems become more intensive, the number of pests also tends to increase, which corresponds to an increase in the severity of damage to crops. (**Mahani *et al.,*., 2021 ; Kortbeek *et al.,*., 2019)**

Aphids (Aphididae), are small (1-10 mm) soft-bodied insects. They present a cosmopolitan distribution with predominance in temperate regions*.* They have displayed a remarkable ability to develop resistance to almost every insecticide with which they have been treated (**Arthurs *et al.,*., 2016**). This small biting insect is one of the major economic pests in agriculture, causing huge losses associated with reduced crop production and quality. (**Will *et al.,*., 2014 ; Attia *et al.,*., 2016**), The black bean aphid, Aphis fabae L (Hemiptera: Aphididae), its wide host range includes more than 200 host plant species , exempl beans, tomatoes, Potatoes, and Tobacco,…ets. (**Akca *et al.,*., 2015**). They feed on imperfect sap, absorbing 1 liter during a few weeks and excrete In the form of honeydew bringing ants that feed on them in exchange for protection, the honeydew stimulates the synthesis of black mold fungus on infected plant surfaces, which impedes tissue metabolism and leaf fall, aborting flowers, drying out buds, which means exhausting them and weakening their productive ability (**Bowling et al.,., 2016**)

At the present time, chemicals are the single most effective method in terms of cost and effectiveness, but the regular and intensive use of them has given the latter resistance to these active molecules, in addition to its negative effects on soil, plants, animals and humans **(Nicoletta *et al.,*., 2015**). This prompted the interest in plant extracts and their use as natural alternatives, One of these alternatives may be the use of essential oils, which are often more selective, do not accumulate in the environment and show low mammalian toxicity (**Czerniewicz *et al.,.,* 2018**). It is necessary to search for environmentally friendly alternatives, and medicinal plants were the best alternative. Essential oils carry out insecticidal activities by physical and physiological mean (**Rahman, 2017**). Currently the use of insecticides is one of the first methods used against aphids. (**Vardanjani *et al.*, 2014)**. Several previous research studies have confirmed the effectiveness of essential oils, at least in part, against various insect pests, In this context, the toxicological activity of the essential oil of basil (*Myrtus communis* L) and rosemary (*Rosmarinus officinalis*) plants was highlighted, by determining its chemical composition and evaluating its insecticidal activity at different concentrations on black bean *Aphis fabae L*.

**MATERIALS AND METHODS**

**Plant material:** The level of effectiveness of two types of essentials oils (myrtle, rosemary) collected from the Tasalah region of the Mila State in March 2021, were tested on bean plants grown in a highly infected field, in the salty area of the Constantine State.

*Myrtus communis L* (*Myrtacées*)

*Rosmarinus officinalis* (*Lamiacées*)

**Animal material:** Adults of *Aphis faba* (Homopetra: Aphididae), sucking biting insects, polyphagie. Place of collection: Al-Malha area in the governorate of Constantine, Identified by Ben Kenana Naima using an 8x magnifying glass:

Size: 0.25mm - 2.4mm

Head color: dark brown to black.

Chest color: dark brown to black.

Belly color: black with white arcs

**Oil extraction:** The essential oil is extracted by water distillation method, using a Dean Starck distillation apparatus. The yield is determined by treating 100 g of the plant material in a stainless steel flask with a capacity of 500 ml. The volume of the essential oil obtained after extraction (h) and the dry mass (kV), and it is given by the following formula**:**

**MR (%) = (h x K / KV) 100**

**GC-MS chromatography:** The extracted oil samples are kept in microtubes at 4 °C. away from the light, The oils were analyzed by gas chromatography with mass spectrometry (GCMS-TQ8040 NX), separation column type RXi-5MSK, 30 m length, 0.25 mm diameter, 0.25 µm film thickness, injection volume of the sample 1µl, temperature oven 280°C,

In the chemistry lab of the Technical Platforms Laboratory in the physical and chemical analysis of CRAPC and Ouargla, the analysis was performed using a GCMS-TQ8040 NX chromatograph equipped with a flame ionization detector (FID), and an RXi-5MSK electrode (30 m diameter x 0.25 mm inside; 0.25 film thickness μm) and a Supelcowax 10 pole having the same characteristics as the previous ones.

The temperature programming for this analysis was as follows:

 - RXi-5MSK Column: 60°C (5 min), 60°C to 280°C at a gradient of 2°C/min

 - Supelcowax 10, at 50°C (5 min), 50°C to 200°C with a temperature gradient of 2°C

min-1 (°C.min-1), the injector and reagent respectively were maintained at temperatures 250°C and 300°C. The carrier gas was helium, the flow rate was set at 1.50 ml. Volume of 1 μl of essential oil sample

**Method used to determine toxicity**

The experiment was conducted according to a complete randomized design (CRD), the live moving individuals of a bean insect were monitored in all Petri dishes after 3 days (2 h, 4 h, 6 h, 19 h, 23 h, 34 h, 43 h) using a magnifying glass. The Henderson-Tilton equation (**Kütükoğlu *et al.,* 2012)**

in each plate we placed a healthy green bean leaf, then transferred 15 “bean” insects in each plate (without separating the sexes).

Then we add to it a cotton swab moistened with a specific concentration of oil, we left the dishes for an hour before closing the Petri dishes with gauze with fine holes that allow oxygen to pass through and do not allow insects to escape Each oil sample contained three concentrations (0,8 µL/cm3, 1,61 µL/cm3, 3,23 µL/cm3). and each concentration was 3 iterations with the presence of the control experiment.

**Determining death rates:**

A two-lens magnifying glass was used to count dead aphids. Mortality, expressed as a percentage, calculated according to Abbott's formula (**Abbott W.S., 1925**)

**Mc = (Me - Mt / 100 - Mt) 100**

**Mc** = percentage corrected death rate.

**Mt** = mortality in the untreated control.

**Me** = mortality rate for the tested sample

**Statistical analyzes of the data:**

Three replicates are performed for all samples and the obtained results are presented as mean ± standard deviation. An ANOVA (one-way analysis of variance) test is applied to the presented data and is performed using STATISTICA 7.1 software (Statsoft Corporation, Tulsa, Oklahoma, USA).

**RESULTS AND DISCUSSION**

**Results**

The yield of essential oil from the leaves of medicinal plants in Table (1)

**Chromatographic analysis of the studied essential oils**

The results obtained after chromatographic separation of the studied oils (*Myrtus communis* L, *Rosmarinus officinalis)* are shown in Tables (2, 3). Each sample contains 20 molecules, representing 90.0% of the total composition of each essential oil.

**Toxicological efficacy of essential oils tested on insects of *Aphis faba* L**

The mortality rate among aphids recorded during the observation hours, from the first hours after exposure to different doses of essential oils of the studied plants: C1 =0,8 µL/cm3, C2= 1,61 µL/cm3, C3= 3,23 µL/cm3, recorded in Tables (4) and Figures (1, 2).

 The mortality rate of aphids treated with *Myrtus communis l* oil was very low, not exceeding 40% in the first six hours of the three doses. In the period from 6 to 23 hours, the mortality rate of aphids increased with a preference for the third dose, which had a killing rate of 86.7%, to record 100% after 29 hours of exposure to 3,23 µL/cm3and 43 hours after exposure to 1,61 µL/cm3. (Figure1).

the first hours of exposure to doses of *Rosmarinus officinalis* oil, the killing rate among insects is low (30%), to rise to reach within 23 hours after exposure to high killing rates at the dose 1,61 µL/cm3 and 3,23 µL/cm3 recorded 70% and 80%, respectively While the first dose of 0,8 µL/cm3 was constantly rising until the end of the observation period (43 hours) to reach a percentage of 93.4%, while the second and third doses had percentage mortality rates. (figure3)

**Determination of CL50 for different essential oils tested on *Aphis faba***

The lethal doses of 50% (CL50) of the essential oils (*Myrtus communis L*, *Rosmarinus officinalis*) obtained (Table 5) are shown. The CL50 of each product supports the toxicity observed in the tests, The CL50s showed that *Rosmarinus officinalis* oil more toxic LD50 (1,14 µL/cm3 ), While *Myrtus communis L*. (2,31 µL/cm3).

**Statistical analysis results:**

Analysis of the interactions between the studied factors on aphids shows that the "treatments" and "interaction" were statistically significant (p < 0.05) that confirmed the increase in the average mortality rates of aphids with increasing the dose of the essential oil.(Table 4)

**Chromatographic analysis of samples:**

In the basil plant sample, a high percentage of oxygenated monoterpenes was observed, which is characterized by the presence of three monoterpene alcohol compounds, which are 1-8cinéole (31,29%), followed by α-terpinol (4,21%) linalool (3.90%). in addition to α-Pinene (36,10%), in a study similar to ours “Chemical composition and antifungal activity of the essential oil of *Myrtus communis L*. on strawberry fruits”, reported the presence of the monoterpene hydrocarbon Limonene (16,22%), α- Pinene15.93%), followed by 1-8 cinéole (9,12%), and the presence of monoterpene alcohol in this essential oil was mentioned as Linalool (7.49%), bergamiol (3.13%) and α-terpinéol (4.30%). (**Touaibia, 2014**).

GC/Ms analysis of the essential oil of *Rosmarinus officinalis* contains the main compounds: 1-8 cinéole

(36,94%), α-Pinene (12,70%), D-Camphor (9.90%), β-Pinene (7 ,76%), Camphene (5,50%), Borneol (4,30%), Caryophyllene (4.70%), α-Terpineol (3,18%), O-cymene (2,84%) (Table 3) According to previous studies, the essential oil of the rosemary plant often contains aromatic compounds such as borneol, camphene, camphor, eucalyptol, flavonoids such as épigénie, diosmine, tannins, acid. Rosmarinic acid, diterpènes, rosmarinicine and the rest of the components in proportions of less than 1%, and the results were identical and supported by some previous studies of this type (**AINANE, 2018**), (**BOUTABIA, 2016** ; **Mekonnen, 2016**; **J. H. Tak. 2016** **; J. Akbari. 2015**)

From these results it can be concluded that the chemical composition of the plant as a whole depends on the location of growth and harvest as well as the time of harvest in the vegetative cycle. (**R.V. De Barros Fernandes. 2014)**

**Toxicological activity of essential oils: (*M. Communis L, R. officinalis*).**

The results of the insecticidal activity tests conducted on *Myrtus communis* L oil (Fig. 1 and Table 4) showed that in experimental conditions there is a direct relationship between aphid mortality percentages and its concentration. Based on it, an increase in the mortality rate of aphids was recorded with high doses of essential oil (0,8 µL/cm3), the first and second doses (1,61 – 3,23 µL/cm3) gave a weak average mortality rate of (13.63 ± 0.32 %) after 6 hours of treatment, and (43.63±0.32%) after 23 hours of exposure, While the average mortality rate of insects exposed to a dose of (1,61 µL/cm3)of basil essential oil after 6 hours increased increasingly according to the observed periods (63.23 ± 0.1%), (72.80 ± 0.72 %),( 92.80 ± 0.72%) et (99.7 ± 0.06%) respectively for 19, 23, 29 et 34 hours of exposure to aphids on the treated leaves. The third dose (3,23 µL/cm3) was effective A high biocidal, which led to an increase in the mortality rate to (60.93±1.01%) after 19 hours, and complete extermination of insects (100%) after 34 hours of exposure. **Niroumand et al**., (**2018**) reported that the essential oil of the basil plant *Ocimum basilicum* conferred insecticidal activity against *Acyrthosiphon pisum* of pea. The study results confirmed **Russo et al**., (**2015**) the use of the essential oils of *eucalyptus* (Myrtacées) as a promising alternative for pest control in adults of Tribolium alum (Coleoptera:Tenebrionidae) Other result in previous work showed strong effects of E. globulus oil on Aphis. fabae. He reported on the exterminatory activity of insects (**Russo *et al.*, 2018**).

The analysis of the results (Figure 2 and Table4) show that the essential oils of *Rosmarinus officinalis* are highly effective against aphids after 23 hours of exposure, as the death rate ranged (70.83 ± 0.15%) at (3,23 µL/cm3).and (57.04 ± 0.57%) in The lowest dose of it (1,61 µL/cm3), while it amounted to (46.37 ± 0.42%) at the lowest dose (0,8 µL/cm3), while the death rate in the first six hours of contact was weak for the three doses where the lethal dose was at a concentration (3,23 µL/cm3) after 29 hours. When comparing this result with previous studies, we find a difference in the hours of reaching total annihilation with the study that he made (**AINANE. 2018**), Rosmarinus officinalis oils contain insecticidal properties that can be used as a pesticide for the flour beetle pest. "*Tribolium castaneum*" (**Ahsaei *et al*., 2019**), It also has the ability to exterminate cabbage looper insects (*Trichoplusia ni*) (**Tak *et al*., 2015**), **Shawer** et al., (2022) reported in a recent study that rosemary oil, Rosmarinus "officinalis", had significant toxicity against rice weevil and negative toxicity against red flour beetle insects.

It is possible that its effectiveness is due to the presence of other oils as a mixture with the oil of the Rosmarinus officinalis plant, and it is in line with what was found when studying the Bactericidal effect of the essential oils of *Ocimum basilicum* L. (Lamiacées) on cotton Aphis gossypii (**Akantetou *et al*., 2020**) "An increase in aphid mortality with high doses of essential oil. This means that the toxicity of the prepared solutions increases with the dose."

**Total discussion**

There is an increase interest in the use of biological products and essential oils in agriculture to provide alternative resources to treat pests and aphids.)**Charaabi *et al*., 2018**). Several studies reported the ability of essential oils to be a potential source of botanical pesticides. These essential oils possess a complex mixture of several compounds, which can exert their activities through neurotoxic effects and often-exhibiting mutual synergistic relationships (**Albouchi *et al.,* 2018**). However, this synergic effect can be efficient in preventing the development of resistant insect pest populations (**Shujie *et al.,* 2020**), The high toxicity of oils against aphids is due to their waxy nature. Studies have shown that oxygenated compounds act directly on the insects' skin. (**Gonzalez *et al.,* 2013**)

Although each essential oil contains its own molecules that work according to different mechanisms an overdose makes the oil effective against aphids since they are constantly feeding. The time after treatment will be proportional to the amount of treatment product given in the insect’s body and thus increase the rate of mortality. It is known that essential oils are toxic substances that have a severe effect on insect pests. This matter was clarified in some previous works that showed that some components of essential oils that Insect contact, works to prevent the synthesis of the hormone (hormone juvénile), which means the possibility of cutting a ring in the life cycle of the pest, and some components of the oils occupy the active water site of the enzyme acetyl-cholinestèrase, which leads to its inhibition.(**Amzouar *et al*., 2016**). The susceptibility of insect pests to essential oil depends on concentrations, on exposure time, on insect developmental stages on temperature. (**Pavela *et al.,* 2018**)

**Conclusion:**

Laboratory treatment of direct toxicity effect through contact (evaporation) with aphids confirmed that the essential oils subjected to the experiment according to the different chemical type, dose and duration of treatment have insecticidal activity of great importance. Can be used as alternatives to synthetic chemicals in the context of integrated pest management of beans and other crops and marketed as an environmentally friendly insecticide

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**Will, Torsten Dr, Andreas Vilcinskas, Rainer Fischer.** "Novel pest control methods

**Table 1: Extract yield and color of essential oils from the plant *Myrtus communis L, Rosmarinus officinalis.***

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Gender** | **Family** | **colour** | **smell** | **Yield (%)** |
| **M. Communis L** | Myrtacées | Pale yellow | fresh and resinous. | 1,71 |
| **R. Officinalis** | Lamiacées | Colourless | very camphor | 2,5 |

**Table 2. Chemical composition of *Myrtus communis L*. essential oil tested**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Hes.T** | **R.T** | **A%** | **CompName** | **Total** |
| ***Myrtus communis L*** | **11,904****16,444****21,568****18,915****26,278****26,750****16,194****27,196****23,269****25,538****29,256****27,932****21,770****21,187****19,064****15,593****17,494****14,058****15,460****27,986****20,049****25,896** | **36,10%****31,29%****4,21%****3,90%****3,89%****2,01%****1,94%****1.47%****1.43%****1,00%****0,70%****0,68%****0,66%****0,56%****0,53%****0,51%****0,47%****0,42%****0,41%****0,33%****0,32%****0,31%** | **2-pinene****Eucalyptol****α-terpinol****linalool****Gernyl acetate****Methyl eugénol****o-Cymene****Caryophyllene oxid****Linalyl acetate****α.-Terpinyl acetate****β-Guiaiene*****Z,Z,Z*-1,5,9,9-tetramethyl-1,4,7 cycloundecatriene****Estragole****Butanoic acid, 2-methyl-, 2-methylbutyl ester****3-Carène****Ɣ- terpinène****Β- pinene****Butanic acid,2 methyl****2-Methyl-2-(4-methyl-3oxopentyl)cyclohexane****L- Pinocarveol****Eugénol** | **92,96%** |

**Table 3. Chemical composition of *Rosmarinus officinalis* essential oil tested**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Hes.T** | **R.T** | **A%** | **CompName** | **Total** |
| ***Rosmarinus officinalis*** | 16,46911,87120,21214,06912,63320,83627,20121,56816,20514,93221,19124,40118,91427,93729,35826,20128,39811,54217,50329,14028,789 | 36,94%12,70%9,90%7,76%5,50%4,30%4,17%3,18%2,84%1,23%1,21%1,14%0,97%0,70%0,67%0,43%0,42%0,39%0,38%0,28%0,23% | Eucalyptol2-PineneD-Camphorβ.-PinèneCampheneBorneolCaryophyllene oxidα.-Terpineolo-Cymeneβ.-MyrcèneTerpinen-4-olβ-Menthanelinaloolα- Humuleneσ-cadinene α- copaeneƔ- muuroleneα- thujeneƔ- terpinèneƔ- cadinene10S, 11S –Himachal-3- (12),4-diene | **95,04%** |

**Table 4**. **Percentage of mortality (%) of *Myrtus communis, Rosmarinus officinalis* d’*Aphis faba* after exposure for 34 hours to essential oils.**

|  |  |  |
| --- | --- | --- |
|  | Dose(µL/cm3) | **mortality (%)** |
|  | **0 h** | **6 h** | **12 h** | **19 h** | **24 h** | **29 h** | **34 h** |
| **T-** |  | 0±0 **Aa** | 0±0 **Ea** | 0±0 **Fa** | 0±0 **Ja** | 0±0 **Ja** | 0±0 **Fa** | 0±0 **Ia** |
| ***Myrtus communis* L** | **0,8** | 0±0 **Ae** | 0±0 **Ee** | 0±0 **Fe** | 13,33±5,77 **Gd** | 26,67±5,77 **Hc** | 43,33±5,77 **Eb** | 56,67±5,77 **Ga** |
| **1,61** | 0±0 **Ad** | 0±0 **Ed** | 13,33±5,77 **Dc** | 20±0 **Ec** | 33,33±5,77 **Fb** | 36,67±5,77 **Eb** | 66,67±5,77 **Da** |
| **3,23** | 0±0 **Ag** | 6,67±5,77 **Df** | 26,67±5,77 **Ce** | 43,33±5,77 **Cd** | 63,33±5,77 **Dc** | 86,67±5,77 **Bb** | 100±0 **Aa** |
| ***Rosmarinus officinalis*** | **0,8** | 0±0 **Ad** | 0±0 **Ed** | 3,33±5,77 **EFd** | 6,67±11,55 **Hd** | 33,33±5,77 **Fc** | 56,67±5,77 **Db** | 76,67±5,77 **Ca** |
| **1,61** | 0±0 **Ag** | 16,67±5,77 **Cf** | 33,33±5,77 **BCe** | 46,67±11,55 **Cd** | 60±10 **Dc** | 70±10 **Cb** | 86,67±11,55 **Ba** |
| **3,23** | 0±0 **Ae** | 30±10 **Ad** | 53,33±5,77 **Ac** | 80±10 **Ab** | 96,67±5,77 **Aa** | 100±0 **Aa** | 100±0 **Aa** |

* The values are the mean ± AND three repetitions (n = 3).
* The different capital letters (A>B>C>D>E>F>G>H>I>J) indicate significant differences in mortality between the concentrations of the different plants tested at a given time (

columns)

* while the different lowercase letters (a>b>c >d>ee>f>g) indicate significant differences in mortality of each plant species at different times (rows),using Fisher's LSD post-hoc test (p<0.05

**Table 5 : Table LC50 of *Myrtus communis, Rosmarinus officinalis,* d’ Aphis *faba* after exposure for 23 hours to essential oils.**

|  |  |
| --- | --- |
| **Temps** | **LC50 (µL/cm3)** |
| ***Myrtus communis*** | ***Rosmarinus officinalis*** |
| 24 h | 2,31 | 1,14 |

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