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ANTIFUNGAL AND INSECTICIDAL ACTIVITIES OF ESSENTIAL OILS OF CHRYSANTHEMUM CORONARIUM AND ACHILLEA MILLEFOLIUM AGAINST CITRUS FRUITS PESTS AND DISEASES

ATIVIDADES ANTIFÚNGICA E INSETICIDA DOS ÓLEOS ESSENCIAIS DE CHRYSANTHEMUM CORONARIUM E ACHILLEA MILLEFOLIUM CONTRA PRAGAS E DOENÇAS DE FRUTOS CÍTRICOS

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Abstract
Of all the insect pests threatening fruit tree cultivation, the Mediterranean fruit fly, Ceratitis capitata, is considered one of the most damaging species, particularly for citrus and peach trees in Mediterranean countries.The main objective of this work is to evaluate the antifungal and insecticidal activity of the essential oils of Achillea millefolium (Asteraceae) and Chrysanthemum coronarium (Asteraceae) against the pests of orange, Citrus sinensis,namely the insect Ceratitis capitata and the pathogenic fungi that accompany it.The

Resumo
De todas as pragas de insetos que ameaçam o cultivo de árvores frutíferas, a mosca-das-frutas-do-mediterrâneo, Ceratitis capitata, é considerada uma das espécies mais prejudiciais, particularmente para as árvores cítricas e de pêssego nos países mediterrâneos. O principal objetivo deste trabalho é avaliar a atividade antifúngica e inseticida dos óleos essenciais de Achillea millefolium (Asteraceae) e Chrysanthemum coronarium (Asteraceae) contra as pragas da laranja, Citrus sinensis, nomeadamente o inseto Ceratitis capitata e os

essential oils of the two plants used were extracted using a Clevenger-type apparatus. Their antifungal activity was tested by radial culture and their insecticidal activity by fumigation.All essential oils exhibited antifungal activity against Aspergillus niger, Fusarium sp1, Penicillium sp, and Fusarium sp2 at the highest concentration (5 µL/mlair). Achillea millefolium essential oil induced 100% mortality after 24 hours of exposure in adult Ceratitis capitata pests. However, for Chrysanthemum coronarium essential oil, larvae were the most affected stage, reaching approximately 35% mortality at the dose (5 µL/ml air).These essential oils could be used as antifungal and fumigant agents against Ceratitis capitata by increasing the dose.

Keywords: Ceratitis Capitata. Antifungal Activity. Insecticidal Activity. *Achillea millefolium. Chrysanthemum coronarium*. Pest Control.

fungos patogénicos que o acompanham. Os óleos essenciais das duas plantas utilizadas foram extraídos utilizando um aparelho do tipo Clevenger. A sua atividade antifúngica foi testada por cultura radial e a sua atividade inseticida por fumigação. Todos os óleos essenciais exibiram atividade antifúngica contra Aspergillus niger, Fusarium sp1, Penicillium sp e Fusarium sp2 na concentração mais elevada (5 µL/mlair).O óleo essencial de Achillea millefolium induziu 100% de mortalidade após 24 horas de exposição em pragas adultas de Ceratitis capitata. No entanto, para o óleo essencial de Chrysanthemum coronarium, as larvas foram o estágio mais afetado, atingindo aproximadamente 35% de mortalidade na dose (5 µL/ml de ar). Esses óleos essenciais poderiam ser usados como agentes antifúngicos e fumigantes contra Ceratitis capitata, aumentando a dose.

Palavras-chave: *Ceratitis Capitata. Atividade Antifúngica. Atividade Inseticida. Achillea millefolium. Chrysanthemum coronarium. Controle de Pragas.*

1 INTRODUCTION

Fruit growing is an integral part of Algeria's economic and social life. This large country, thanks to its geographical location and diverse soil and climate conditions, has the privilege of cultivating several fruit species and producing fresh fruit year-round.

Of all the insect pests threatening fruit growing, the Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae), is considered one of the most damaging species Wiedemann (1829), particularly for citrus and peach trees in Mediterranean countries.

Ceratitis capitata is a major pest in Africa, having spread to virtually every other continent; it has undoubtedly become the most important pest in its family. This fly is highly polyphagous and causes damage to a large number of unrelated fruit crops.

It represents one of the most serious problems for agriculture in general and citrus growing in particular. Damage caused by the Mediterranean fruit fly is one of the main obstacles to the production of healthy fruit and its export. Annual damage is variable and can reach 10 to 20% or moreFéron (1962). It also transmits fungi that cause fruit rot.

Synthetic chemical pesticides used to control this pest have harmful effects on all organisms, increasing the risk to public health and the environment.

In biotechnological control, it is essential to identify host plant resistance, biological control methods, pheromones, cultural practices, and finally, biocidal and insect-repellent plants Iannacone and Lamas (2003).

Numerous studies have investigated the biocidal activity of plants, particularly aromatic plants, through their essential oils and hydrosols. The results are very encouraging, which prompted us to conduct this study to determine whether the selected plants have insecticidal and antifungal properties against citrus pests. Our study focused primarily on the two species *Chrysanthemum coronarium* and *Achillea millefolium.*

Achillea is an Asteraceae family comprising 115 species, commonly known as yarrow, native to Europe, Asia, and North Africa, and introduced to parts of the New World (Mohamed-Elamir and al. (2008).

Achillea millefolium is an easy-to-grow perennial with good drought tolerance. It thrives in rock gardens or as a border plant, except for the taller species. Its dense, deeply lobed, and often grayish foliage contrasts beautifully with its brightly colored umbel-shaped flowers.

Achillea species are among the oldest medicinal plants, used both for pharmaceutical purposes and in folk medicine. These plants contain a complex of different pharmacological compounds, for example, terpenes, flavonoids, alkaloids, tannins, lignans, etc.

They are diuretics, emmenagogue agents, used for wound healing, stomach aches, and diarrhea, and are antispasmodic, antiseptic, and also used to reduce perspiration and stop bleeding.Motavaliza dehkakhky et al. (2013)

Chrysanthemum is distributed in two main centers, one in the Mediterranean region, the other in China and Japan. In Algeria, the genus comprises 20 species, 8 of which are endemic. *Chrysanthemum coronarium* is an annual herbaceous plant, widely distributed in the Mediterranean region, Japan, China, and the Philippines. It has large flower heads, generally bicolored white and yellow. The species is an ornamental plant.

Chrysanthemum coronarium has medicinal properties; The leaves are expectorant and stomachic, while the flowers are stomachic; it is used against constipation, is effective in controlling nematodes, and protects plants against caterpillars.

The objective of this work is to evaluate the antifungal and insecticidal activities of essential oils from Chrysanthemum coronarium and Achillea millefolium against major citrus pests and diseases.

2 MATERIALS AND METHODS

The plants studied were harvested in the Maghnia region, Tlemcen province of Algeria, during April and May. The harvested parts consisted of leaves, stems, and flowers (aerial parts).

The harvested plants were air-dried in the shade at room temperature for 8 days.

The essential oils were extracted by hydrodistillation using a Clevenger-type apparatus. 400 g of plant material were placed in a round-bottom flask filled with distilled water, and the mixture was brought to a boil for 4 hours. The vapors, laden with volatile substances, passed through a condenser, condensed, and were collected in a separatory funnel. The aromatic water and the essential oil separated based on their difference in density.

The extracted oil was stored at a temperature of 4°C in opaque glass bottles, hermetically sealed to protect it from air, light, and temperature fluctuations, which are the main agents of degradation. Adegraded oil loses its biological activity.

The chemical composition of these oils was determined byBenomari (2018)for *Chrysanthemum coronarium* (Table 1) and Istamkulova et al.(2024) for *Achillea millefolium*, who indicated their main major components (Table 2).

Table 1

Main major components of essential oil ofC.coronarium(Benomari, 2018)

Major components	%min	%max
Myrcene	25.2	36.4
Camphor	5.5	15.2
Cis-chrysanthenylacetate	0.8	7.1
Trans-chrysanthenylacetate	1.5	5.2
Lyratylacetate	2.4	7.1
Z-B-ocimene	0.9	5.5
E-B-ocimene	0.2	4.5

Table 2

Main major components of essential oilof A.millefolium (Istamkulovaand al., 2024)

Major components	%
Terpinolene	6.78
m-cymene	18.82
Eucalyptol	1.94
(-)-therpinen-4-ol	2.96
Ascaridol	20.43

2.1 In vitro fungalactivity

To test the antifungal effect of essential oils, we used four fungal species responsible for rot, isolated from oranges collected from the studied orchard: *Aspergillus niger, Fusarium sp. 1, Penicillium sp.,* and *Fusarium sp. 2.*

The technique involves adding the oil at different concentrations (0.25, 0.5, and 1 µl) to the solid culture medium in 90 mm Petri dishes, which have been previously inoculated with 50 µl of the fungal suspensions. A Wattman paper disc, soaked in 10 µl of essential oil diluted in 10% DMSO (dimethyl sulfoxide), is placed in the center of the Petri dish. The culture is then incubated in the dark, lid down, for seven days at 25°C. Each trial is repeated three times. A control, containing the fungal suspension, is prepared under the same conditions using a paper disc soaked in distilled water.

After incubation at 28°C for 2 to 7 days, taking into account the growth of the control, the antifungal index is calculated using the following formula:

2.2 Fungal index (Inhibition percentage) = (Dc - Dt) / Dc x100

Dc: Diameter of the control mycelia;

Dt: Diameterof the treated mycelia.

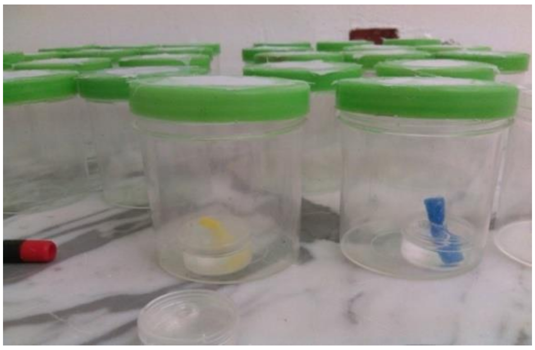
2.3 Insecticidal Activity

This test was performed by inhalation. Cotton soaked in the essential oils of the plants studied, *Chrysanthemum coronarium* and *Achillea millefolium,* was placed in jars at different concentrations (0, 0.5 µl, 2 µl, and 5 µl). Ten insects of the same generation were introduced into each hermetically sealed jar under favorable conditions (industrial honey and water for feeding, in a moderately warm environment) (Figure 1).

The same method was used to test L3 larvae and pupae with the addition of the essential oil at different concentrations (0; 0,5µl; 2µl et 5µl).

Figgure 1

Experimental jars



Mortality was monitored by counting dead insects (adults or larvae) starting on the first day of treatment (after 24 hours).

The trials were repeated three times for each dose, with control groups treated in parallel without exposure to the essential oil.

The experiments on the insecticidal activity of the essential oils of the studied plants, *Achillea millefolium* and *Chrysanthemum coronarium*, were conducted at the Sterile Insect Laboratory, Directorate of Research on the Environment and Living Organisms (DREV), National Center for Nuclear Science and Technology (CNSTN), Sidi Thabet Technopole, Tunis, Tunisia, under the supervision of Ms. Meriem M'Saad.

2.4 Statistical Analysis

Statistical analysis was performed using ANOVA and Python software.

3 RESULTS AND DISCUSSION

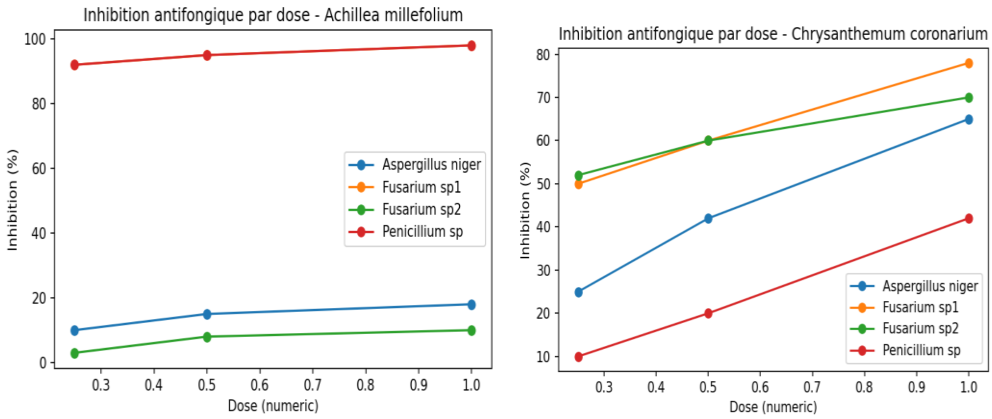
3.1 Antifungal activity of essential oils

We tested the toxicity of essential oils from the studied plants on the strains, using three different concentrations (1 µl/ml, 0.5 µl/ml, and 0.25 µl/ml).

The two graphs below (Figure 2) illustrate the percentages of growth inhibition of the four fungal strains (*Aspergillus niger, Fusarium sp1, Fusarium sp2,* and *Penicillium sp*) as a function of the dose of the two essential oils.

Figure 2

Percentages of growth inhibition of the four fungal strains (*Aspergillus niger, Fusarium sp1*, *Fusarium sp2, and Penicillium sp*) according to essential oil dose



(a) Inhibition rate of*Achillea millefolium* (b) Inhibition rate of *C.coronarium*

3.1.1 Inhibition rate - Achillea millefolium

— Efficacy: Activity was extremely high, but highly selective.

— Major Target: *Penicillium sp. and Fusarium sp. 1* were inhibited at rates very close to 100% even at the lowest dose (0.25 µl/ml), remaining constant thereafter.

— Resistance: The two other strains, *Aspergillus niger and Fusarium sp. 2*, showed strong resistance, with inhibition rates remaining below 15%.

3.1.2 Inhibition rate- Chrysanthemum coronarium

— Activity: This oil exhibited good fungicidal activity, achieving up to 75% inhibition.

— Targets: The two Fusarium strains (sp1 and sp2) were the most sensitive (approximately 75% and 70% at the 1.0 µl/ml dose). *Aspergillus niger* was also well inhibited (≈ 62%).

— Least sensitive: *Penicillium sp*. was the least sensitive to this oil, although its inhibition rate increased significantly with the dose, rising from approximately 10% to 42%.

The results presented showed a clear variability in antifungal activity depending on the essential oil and the fungus tested. *Achillea millefolium* stood out as the most effective, with inhibition rates exceeding 90% against *Fusarium sp1 and Penicillium sp. Chrysanthemum coronarium* exhibited moderate activity, particularly against *Fusarium sp2* (60.67%) and *Aspergillus niger* (44%).

These differences suggest variability in the bioactive compounds responsible for antifungal activity among plant species.

3.2 Estimation of the ED50 of the two tested essential oils

3.2.1 For Achillea millefolium

The perfect correlation ($\rho = 1.000$; $p < 0.001$) between the dose and the inhibition rate forall fungal strains indicates a response strongly dependent on the concentration of the essential oil (Table 3).

Table 3

Dose-inhibition relationship and ED50 estimation for Achillea millefolium

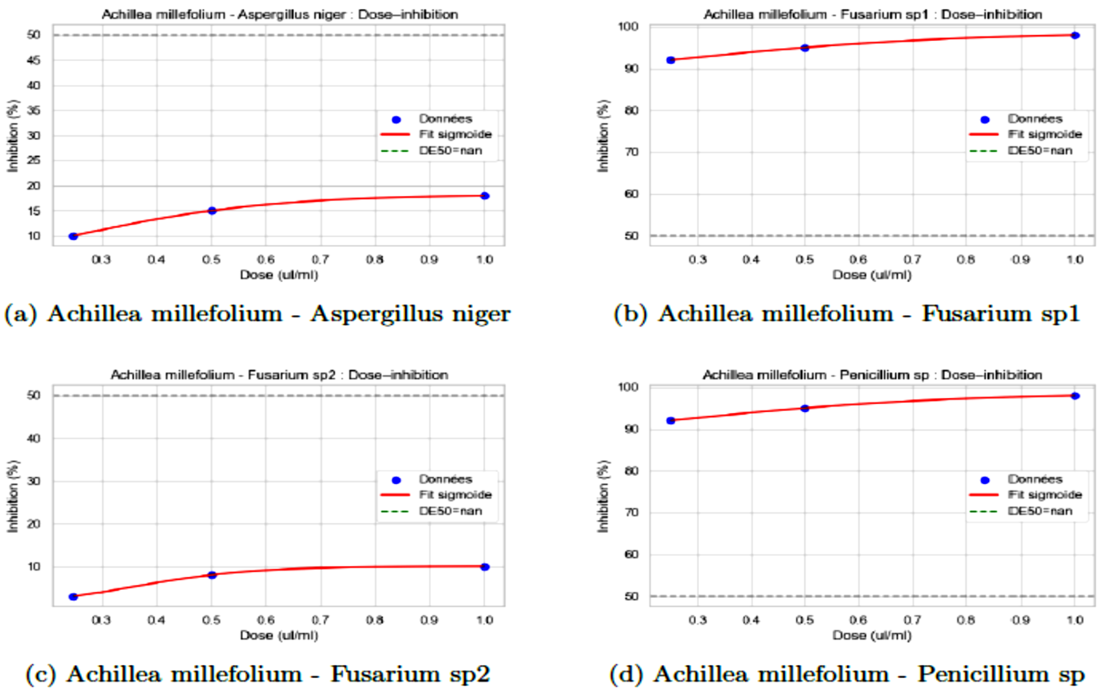
Fungus	Spearman ρ	p-value	DE50 (µl/ml)
Fusarium sp1	1.000	0.000	-
Aspergillus niger	1.000	0.000	-
Penicillium sp	1.000	0.000	-
Fusarium sp2	1.000	0.000	-

However, the lack of defined values for the effective dose 50 (ED50) indicates that the 50% inhibition threshold was not reached, suggesting that the tested concentrations of *Achillea millefolium* were insufficient to exert significant average inhibition on the studied fungi.

The following figure illustrates the relationship between the essential oil dose and the observed inhibition rate for the different fungal strains (Figure 3), thus allowing visualization of the general trend and estimation of the effective dose 50 (ED50) when it is reached.

Figure 3

Relationship between the dose of essential oil and the rate of inhibition observed for the different fungal strains.



(a) Achillea millefolium - Aspergillus niger (b) Achillea millefolium - Fusarium sp1

(c) Achillea millefolium - Fusarium sp2 (d) Achillea millefolium - Penicillium sp

3.2.2 For Chrysanthemum coronarium

The results indicate a perfect correlation between dose and inhibition rate for all tested fungi ($\rho = 1.000$; $p < 0.001$). The low values of the effective dose 50 (ED50) (between 0.19 and 0.62 µl/ml) reveal a strong antifungal activity of Chrysanthemum coronarium essential oil, particularly against *Fusarium sp2* and *Fusarium sp1* (Table 4).

Table 4

Dose-inhibition relationship and ED50 estimation for Chrysanthemum coronarium

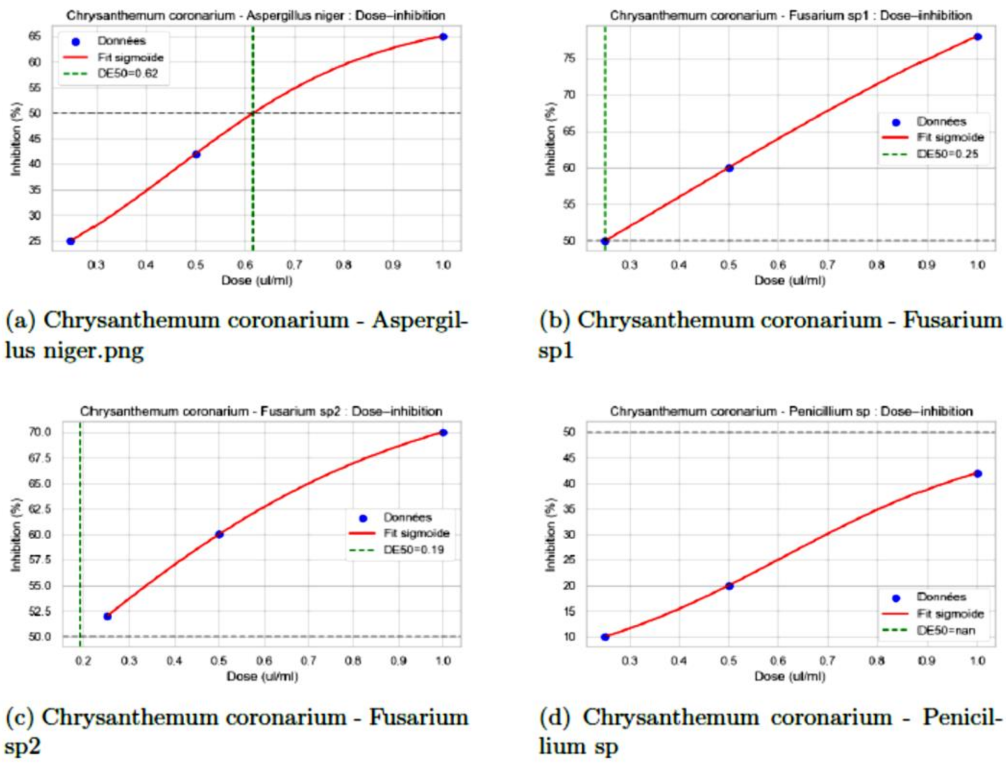
Fungus	Spearman ρ	p-value	DE50 (µl/ml)
Fusarium sp1	1.000	0.000	0.25
Aspergillus niger	1.000	0.000	0.62
Penicillium sp	1.000	0.000	-
Fusarium sp2	1.000	0.000	0.19

The absence of ED50 for *Penicillium sp*. suggests, however, a lower sensitivity of this fungus to this oil.

Figure 4 below illustrates the relationship between essential oil doses and inhibition levels observed for the different fungal strains, thus allowing visualization of the general trend and estimation of the effective dose 50 (ED50) when it is reached (Figure 5 and 6).

Figure 4

Relationship between essential oil doses and observed inhibition rates for different fungal strains.



(a) Chrysanthemum coronarium - Aspergillus niger.png

(b) Chrysanthemum coronarium - Fusarium sp1

(c) Chrysanthemum coronarium - Fusarium sp2

(d) Chrysanthemum coronarium - Penicillium sp

Figure 5

Essential oil inhibitory activity of C.coronarium on Aspergillus niger (D)and Fusarium sp1 (G) over time. The control strain (without H.E) is the one placed at the top of the photo.

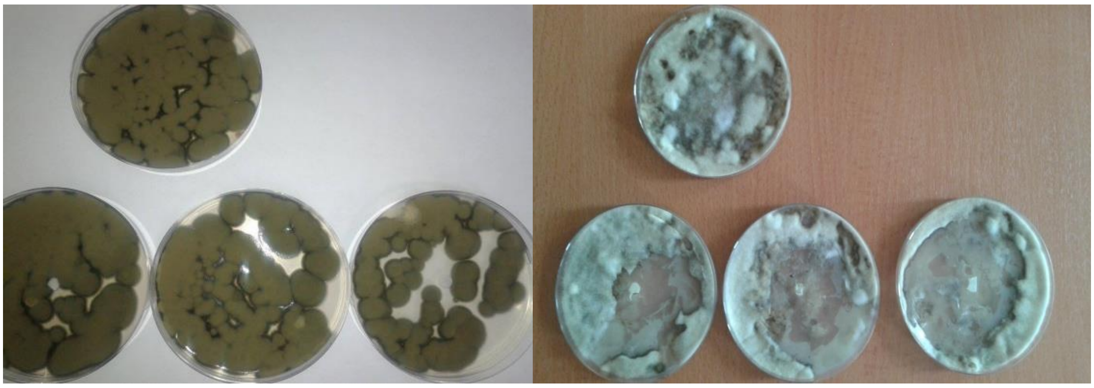


Figure 6

Inhibitory activity of A. millefolium essential oil on Aspergillus niger (G) and Penicillium sp. (D) over time.

Plant-based essential oils are attracting increasing interest as environmentally friendly alternatives to fungicides and insecticides.

This study showed that the essential oils of two plants, *Chrysanthemum coronarium and Achillea millefolium,* exhibit antifungal activity against *Penicillium sp., Fusarium sp. 1, Fusarium sp. 2, and Aspergillus niger.*

Essential oils are complex volatile mixtures. Monoterpenes and sesquiterpenes are generally the main groups of compounds responsible for much of their biological activity.

The antifungal activity and toxicity of the essential oils of *Chrysanthemum coronarium and Achillea millefolium* are due to their main components (Tables 1 and 2).

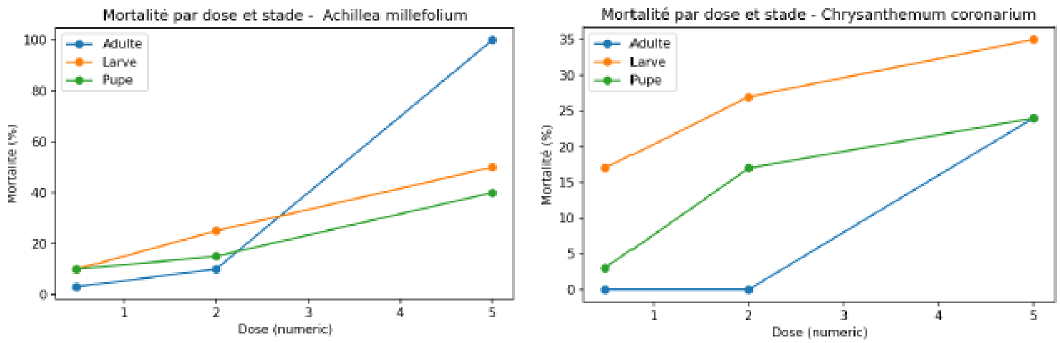
Achillea millefolium has a high content of ascaridole and m-cymene, making it promising for use in antimicrobial and antifungal control. Volatile compounds from *C. coronarium* have been the subject of several studies. The major compounds that characterized the essential oil of this species—myrcene, camphor, etc.—listed in Table 1, confirm thisBenomari (2018).

3.2.3 Insecticidal activity

The two graphs below (Figure 7) show the mortality rates (%) of the pest insect *Ceratitis capitata* at its three developmental stages (Adult, Larva, Pupa) as a function of the dose for the two tested essential oils.

Figure 7

Mortality rate (%) of the insect pest Ceratitis capitata at its three stages (Adult, Larva, Pupa) as a function of the dose for the essential oils A. millefolium and C. coronarium



Mortality rate *A. millefolium* *b)* Mortality rate *C. coronarium*

3.2.4 Mortality rate - Achillea millefolium

— Efficacy: This is the most effective essential oil, achieving a mortality rate close to 100% at a dose of 5 µl/ml.

— Most sensitive stage: Adults show the greatest sensitivity, with a very steep mortality curve.

— Less sensitive stages: Larvae and pupae also show an increase in mortality, but more moderately (approximately 50% and 40% respectively at a dose of 5 µl/ml).

3.2.5 Mortality rate - Chrysanthemum coronarium

—Tendency: with the dose of 5µl/mlmortality increase reached its maximum rate around 35− 40%.

— Most susceptible stage: Larvae were the most affected stage, reaching approximately 35% at the 5 µl/ml dose.

— Resistance: Pupae and adults were less affected; the mortality curve of adults increased significantly after the 3 µl/ml dose.

We can deduct from these results that the essential oil (EO) of *Chrysanthemum coronarium* is moderately effective as an insecticide against the Mediterranean fruit fly (Flaminiand al., 2003;Bardaweel and al., 2015). The essential oil of this species exhibits insecticidal properties against stored food pests (Perezandal., 1999).

Several studies have reported the antioxidant, antibacterial, and antiproliferative activities (Alvarez-Castellanos, 2001; Pérez and al., 2003and Bardaweel and al., 2015)of *C. coronarium* essential oil (Benomari, 2018).

In a previous study, the contact and fumigation activities of *Achillea vermicularis, A. teretifolia, and A. biebersteinii* oils against *Sitophilus granarius* showed that 1,8-cineole, piperitone, and camphor are the main components of these plants. The researchers also reported that these plant essential oils had no fumigation activity.Kimand al.(2003) andPolatoğlu and al.(2013)tested extracts of 30 aromatic plants and the essential oil of five plants for their contact and fumigation activities against*Lasiodermaserricorne.* They reported that the activity varies depending on the plant material and the exposure time.

In a toxicity test on *Leptinotarsa decemlineata*, the essential oils showed a toxic effect against this pest. Based on the results obtained, Achillea species could be used as alternative bio-insecticides and bio-herbicides **(**Cakiand al., 2015).

Previous research has investigated the chemical composition of Achillea essential oil, including its antibacterial (Barel and al., 1991; Magiatis and al., 2002) and insecticidal (Calmasurand al., 2006;Jovanovic and al., 2007; Magdy et Samir, 2008) properties. This work showed that the essential oil extracted from the leaves of *Achillea wilhelmsii (Asteraceae)* exhibited volatile toxicity to *Sitophilus granarius and Tribolium comfusum(*Calmasur et al., 2006).

The insecticidal activity of the essential oils we studied can be attributed to their high monoterpene content.

3.3 Estimation of lethal concentration values for the ceratitis larvae population

We calculated the lethal concentrations to know the concentrations of essential oil of *Chrysanthemum coronarium* and*Achilleamillefolium* that determine the mortalities for the Ceratitis larvae population (Table5).

The previous table allows us to conclude that the lethal effects of essential oil of *Chrysanthemum coronarium* and*Achillea millefolium on* the larvae, chrysalis and adult depended on the doses used.

Table 5

Estimated values of lethal concentration (CL) after essential oil of Chrysanthemum coronarium, Achillea millefolium treatment of larval Ceratitis capitata

DL	***Chrysanthemum coronarium***			***Achillea millefolium***		
	Larva	**Pupa**	**Adult**	**Larva**	**Pupa**	**Adult**
10	1,32	1,32	3,98	0,56	0,67	0,35
20	3,30	3,30	4,73	1,23	1,76	0,62
30	6,39	6,39	5,37	2,16	3,53	0,95
40	11,23	11,23	5,97	3,51	6,37	1,36
50	**19,01**	**19,01**	**6,60**	**5,50**	**11,07**	**1,90**
60	32,20	32,20	7,30	8,63	19,22	2,66
70	56,58	56,58	8,12	13,98	34,71	3,81
80	109,43	109,43	9,21	24,56	69,28	5,79
90	**273,14**	**273,14**	**10,95**	**53,69**	**180,70**	**10,35**

The oils from both plants showed activity against *Ceratitis capitata* (larva, pupa, and adult). According to Table 4, the LC50 values were 19.01 µl/L air for larvae and pupae and 6.60 µl/L air for adults for *Chrysanthemum coronarium*. For *Achillea millefolium,* the values were 5.50 µl/L air for larvae, 11.07 µl/L air for pupae, and 1.90 µl/L air for adults.

These results suggest that the essential oils of the tested plants could be used as control agents against the Mediterranean fruit fly.

The overall results obtained under our experimental conditions show that, after treatment with oils, we note average mortality rates, which can vary from 20 to 35% depending on the essential oil used and its concentration, except for adults which, treated with *Achillea millefolium* essential oil, have a mortality rate of 100%.

Studies on the contact effect have shown that the activity of plant essential oils varies depending on the insect species. This is thought to be due to the physiological structure of the insects and also depends on the plant's origin (Gökce et al., 2007; Kordali et al.,2007; Alkan et Gökçe, 2012).

Furthermore, it has been observed that the activity of the essential oil varies according to the application time. This can be explained by the exposure time, or by the ability of the active compound(s) to penetrate the organism.

Natural control methods against *Ceratitis capitata* have been studied worldwide**(**Khani et Asghari,2012;Rossi, 2012; Tabti,2015;Benelli, 2018; Benelli *and al.*, 2021**;** Bounouira, 2024).

4 CONCLUSION

Based on the results obtained, this study found that the essential oils of the plants studied have significant insecticidal and antifungal effects with increasing doses. Therefore, they can be used as effective and harmless biological and natural treatments against pests such as Mediterranean fruit fly and other pathogenic fungi, and can be used by farmers with complete peace of mind.

Integrate these oils into an integrated pest management strategy by reducing the use of chemical pesticides and continuing to optimize doses.

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Authors' Contribution

All authors contributed equally to the development of this article.

Data availability

All datasets relevant to this study's findings are fully available within the article.

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