Study of the Insecticidal Effect of Essential Oils and Extracts of Two Medicinal Plants and Evaluation of Their Synergistic Potential in Vivo

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Received: 07/2023, Published: 08/2023

Abstract:

Plants synthesize secondary metabolites, which are chemical substances (bioactive molecules) that play a vital role in chemical defense, fight against herbivores, and infectious agents.In this perspective, we conducted a study to evaluate the toxicity of essential oils formulated from the leaves of *Pistacialentiscus*L. and *Juniperusphoenicea* L. and their synergistic effect on the populations of citrus aphid (Aphis).The objective of our study was to compare the physicochemical properties of extracts from two medicinal plants, to evaluate their synergistic potentials in vivo, and to determine the insecticidal activity by using different treatments with essential oils and extracts, depending on the time and solvent used.

The recorded results gave promising values of mortality rates for their individual or synergistic use, which are of the order of 83.43% for the essential oil of *P.lantiscus* and 99.03% for the essential oil of *J.phoenicea.* The synergy of the essential oils was 88.63%, which is higher than the effect of the essential oil of *J .phoenicea* alone, indicating a synergistic effect between the two plants. A mortality rate of 97% was recorded for the acetone extract of *J.phoenicea* and 56.60% for the acetone extract of *P. lantiscus.* The synergy of the acetone extracts was 75.03%, which means that their effect was between the acetone extract of *J.phoenicea* and the acetone extract of *P.lantiscus*alone, indicating that certain molecules are partially synergistic between the two plants. The mortality rates for the methanolic extracts were 80% for the methanolic extract of *J.phoenicea* and 99% for the methanolic extract of *P.lantiscus.* The synergy of the methanolic extracts was

94.65%, which means that their effect was lower than the effect of the methanolic extract of *P.lantiscus* alone, indicating that certain molecules are partially synergistic between the two plants. A mortality rate of 87.47% was recorded for the aqueous extract of *J.phoenicea* and 91% for the aqueous extract of *P.lantiscus*. The synergy of the aqueous extracts was 93.27%, which means that their effect was higher than the effect of the aqueous extract of *P.lantiscus* alone, indicating that there is a positive effect between the two plants.The lethal times determined by probit analysis (TL20, TL50, TL90) showed that the treatment "synergistic essential oil" showed the shortest TL50 with 4.98 hours, while the treatment "methanolic extract of *P.lentiscus*" showed the shortest TL90 with 12.06 hours.These same results allowed us to say that the bioproducts based on essential oils have shown a very significant effect on the citrus aphid (Aphis).
Keywords: Secondary metabolites, Medicinal plants, Citrus aphids, Synergy, Bioproducts

Tob Regul Sci. ™ 2023;9(1): 3983-3997
DOI: doi.org/10.18001/TRS.9.280

1. Introduction

Algeria is well known for the importance of citrus cultivation, but also for low yields due in part to insect attacks. In this regard, it is important to study insect populations in citrus orchards in order to develop a strategy for crop protection, Loussert, (1989).

Of all the insect pests of citrus, aphids are the most problematic group. According to Lakhal, (2020),the biological and ethological characteristics of these insects, including their prodigious biotic potential and their extraordinary adaptation to the maximum exploitation of the environment through their polymorphism, make them major pests of crops.

When feeding on plant sap, aphids inject salivary toxins and phytopathogenic viruses Hullé*et al.*(2019). Their toxins can cause a characteristic leaf curling and stunted branch growth (Loussert,1989). Most crop pests have been controlled for many years by the use of pesticides of all kinds. However, we now know that pesticides can sometimes have harmful effects on non-target insects and ecosystems. The negative impact of pesticides is even greater when it affects populations of beneficial insects for crops (Collignon et *al.,* 2003).

The problems of resistance and toxicity of synthetic insecticides have led to the need to find more effective and healthier alternatives. Therefore, medicinal plants have been used for centuries for their therapeutic properties. They contain a variety of chemical compounds, some of which have biological activity against pathogens, insects and weeds. Nyegue,(2005) They are therefore an interesting source of new compounds for the search for bioactive molecules,(Pichon, 2016).

The objective of this study is to compare the physicochemical properties of extracts from two medicinal plants, *P. lantiscus* L and *J. phoenicea,* and to evaluate their synergistic potential on citrus aphid populations in vivo in order to reduce the damage caused by this destructive agent and reduce the use of chemicals and thus limit their harmful effects on the environment and human health.

2. Materials and Methods

2.1. Plant material

Two plant species, *J.phoenicea* and *P.lentiscus* were collected in the Medjadja region of Chlef province, Algeria, in November and December 2022, respectively.

The plant material was authenticated by Mr. Omar Nadji, a professor at Hassiba Benbouali University in Chlef, Algeria The geographical coordinates are latitude: 36.25, longitude: 1.4, or 36° 15′ 0″ North, 1° 24′ 0″ East. After collection, the leaves were washed, dried, and ground. The extracts were prepared by infusion and maceration. The physicochemical properties of the extracts were determined to assess the quality of the essential oils (EOs).

2.2. Animal material

To determine the *in vivo* insecticidal activity of our plants, we conducted our experiment in a Thomson citrus orchard. The orchard is a pilot farm "Si Yahi" located in the commune of Medjadja, which is located 18 km from Chlef at an altitude of 152 m.

2.3. Extraction of essential oil

The extraction of essential oil by hydrodistillation using a Clevenger-type apparatus was performed from the leaves of *J. phoenicea* and *P. lantiscus* by placing 100 g of dried plant material in 500 ml of distilled water at a temperature not exceeding 60°C.

The separation of the two components, the organic phase (essential oil) and water, is done by a separating funnel; the oil is weighed and stored at 4°C in dark bottles until use.

2.4. Chemical detection of secondary metabolites

This is a technique that allows determining the different chemical groups contained in a plant organ. According to Muanda in 2010, it is about physico-chemical reactions that identify the presence of chemical substances such as: alkaloids, polyphenols (flavonoids, tannins), saponosides, glycosides.

2.5. Experimentalprotocol

- **Preparation of the composition**

The protocol adopted in our experiment is a combination of several protocols already referenced in order to obtain a formulation suitable for our conditions.

In 1L of rainwater with acid pH, add 5ml of sunflower oil, 5ml of liquid soap (black soap) and 30g of clay. This constitutes the base solution to which 1ml of essential oils, extracts or blends are added. The preparations are then ready to be used on the *in vivo* field.

The positive control is a polyvalent systemic insecticide for foliar application and ensuring protection against a large number of harmful insects that attack fruit trees and citrus fruits. It contains 20% acetamipride.

- **Experimental device**

We randomly identified 43 citrus trees(figure1) infested by aphids, with three repetitions per treatment. On each tree, we labeled 5 branches in different directions (North, South, East, West, Center). Under each branch treated with the appropriate mixture, we placed an umbrella to collect the dead pests after spraying. A count of the living aphids (Nv) before spraying and the dead aphids (Nt) was performed every two hours (2h, 4h, 6h, 8h, 10h and 24h).

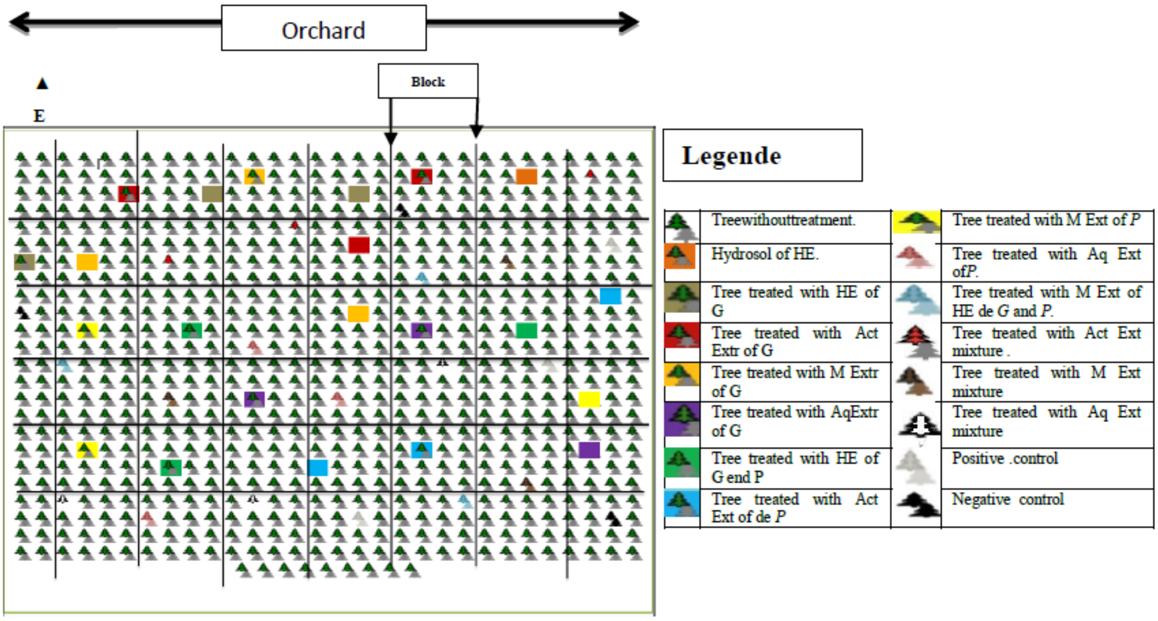

Figure1: experimental device in the citrus orchard.

2.6. Insecticide contact activity

Mortality was recorded every 2 hours. Mortality was calculated and corrected using Abbott's formula (1925), which is expressed as follows:

$$\boldsymbol{Mc} = \frac{\boldsymbol{Me - Mt}}{\boldsymbol{100 - Mt}} \times \mathbf{100}$$

Mc = mortality corrected in percentage.
Me = mortality of the tested sample.
Mt = mortality in the untreated control.
Each treatment was repeated 3 times and the TL50 and TL90 values were set by the regression lines of the tests used.

2.7. Statistical analyses

Our in vivo results of Nv and Nt were dependent on the choice of branches and the number of aphid infestations, so we deemed it useful to report our results in the form of rates in order to compare them. All statistical tests and graphs were performed using the RStudio software (version 2023.3.0.386) (Posit team, 2023). The chosen significance level was 0.1% ($p < 0.001$).

a. Kruskal-Wallis test: The Kruskal-Wallis test was used to determine if there is a significant difference between the aphid infestation rate and the different directions.

b. One-way ANOVA: The data are organized into several groups based on a single grouping variable, which is the mortality rate in our study (Millot, 2018).

c. Repeated measures ANOVA: Used to simultaneously evaluate the effect of two intra-subject factors, namely treatment type and time, on a continuous response variable, namely the aphid mortality rate.

d. Analysis by the Probit method: Used to determine the lethal times (TL) corresponding to different percentage efficacies for each treatment. The data were transformed into base 10 logarithm to satisfy the assumptions of the Probit analysis Sakuma, (1998).

3. Results and Discussion

3.1. Essential oil yield

The yield of extraction differs from plant to plant. It is higher in *J.phoenicea* (0.37%) followed by synergy (0.29%) and finally *P.lantiscus* (0.17%). The results found for the yield by comparison to the results in the literature are different. For *J.phoenicea,*Abdelli in 2017 found a yield of 0.21% and for *P.lantiscus,*Arabi (2018) found a yield of 0.4%, while Belhachat in 2019 found a yield similar to the one we found (0.17%). The essential oil of the mixture of the two plants gave an intermediate yield (0.29%) between that of *P.lentiscus* and *J.phoenicea*. The yield of essential oils is influenced by various factors, such as sunlight, the nature and components of the soil, temperature, altitude, climate, region of cultivation and the genetic composition of individuals (Achak, 2006).

3.2. Phytochemical screening of the studied species

The results of the phytochemical screening show that the two plants studied contain a variety of secondary metabolites (Table 1), including tannins, saponins, flavonoids, and polyphenols. These secondary metabolites are known for their antioxidant, anti-inflammatory, insecticide, and antimicrobial properties.

Table1:Secondary metabolites detected in the plants studied.

	Polyphenols	Tannins	Flavonoïds	Saponins
Aqueous extract of *P. lantiscus*	+	+	+	+
Méthanoïque Extract*P. lantiscus*	+	+	+	+
AcétoniqueExtract*P. lantiscus*	+	+	+	+
Aqueous extract of *J. phoenicea*	+	+	-	+
Méthanoïque Extract*J. phoenicea*	+	+	+	+
AcétoniqueExtract*J. phoenicea*	+	+	+	+
Aqueousextract of Synergie	+	+	-	+
Méthanoïque Extract Synergie	+	+	+	+
acétoniqueExtract Synergie	+	+	+	+

The results of the phytochemical analyses revealed the presence of most of the extracts studied, with the exception of the aqueous extract of *J.phoenicea* and the aqueous extract of synergy, which do not contain flavonoids.

In fact, the studies of Alzand et al. (2014), Latif et al. (2014) and Fadel et al. (2016) reported the presence of tannins and flavonoids in the extracts of *J. phoenicea,* while Makhloufi et al. (2014) and Amalich et al. (2016) reported the absence of tannins.

The analysis of the extracts of *P. Lentiscus* revealed the presence of tannins and saponins. Arab et al. (2014) and Merzougui (2015) in their studies on *P. Lentiscus* detected the presence of tannins and the absence of saponins. This same result was reported by Bammou et al. (2015), who detected a significant amount of saponins in the leaves of lentiscus.This difference in the parameters can be explained by the influence of external factors such as temperature, humidity, etc.

3.3. Evaluation of the insecticidal effect of essential oils and extracts of the plants (*J.phoenicea*, *P.lantiscus* and their synergistic potential).

These *in vivo* results (Figures 2, 3, 4 and 5) suggest that the OEs and extracts of *J. phoenicea* and *P. lantiscus* could be used as natural insecticides against aphids.

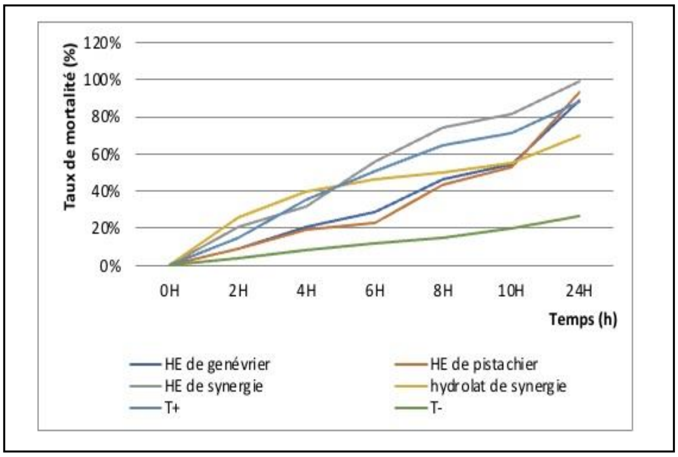

Figure 2: Mortality rate of aphids treated with essential oils over time

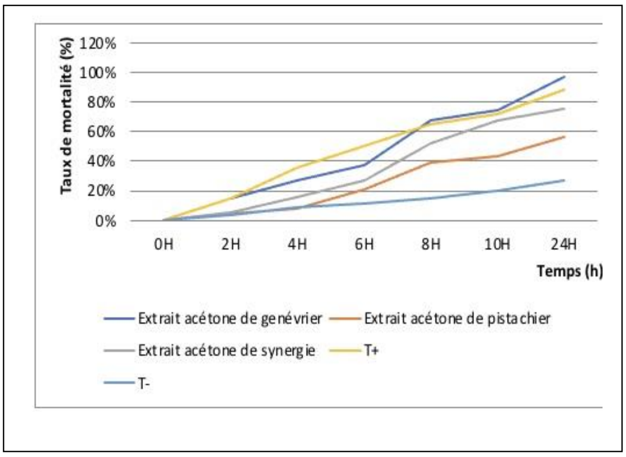

Figure 3: mortality rate of aphids treated with acetone extracts over time

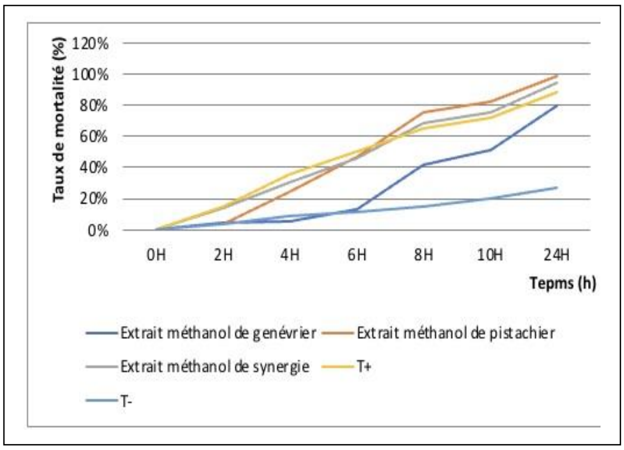

Figure 4: mortality rate of aphids treated with methanolic extracts over time

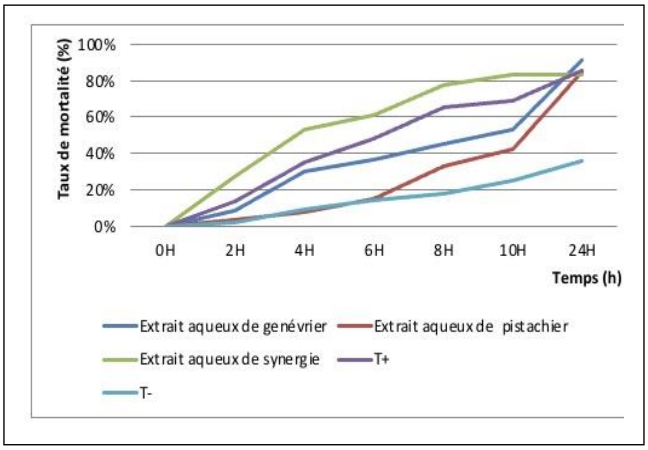

Figure 5: Mortality rate of aphids treated with aqueous extracts over time.

The mortality rates of aphids treated with essential oils of Pistacia (OEP), Juniperus (OEG), mixture (OES), Hydrosol (HS) and the different extracts of *P.lentiscus* (Ext ActP, Ext MP, Ext AqP) and *J.phoenicean* (Ext ActG, Ext MG, Ext AqG) and their mixtures (Ext ActS, Ext MS, Ext AqS) in vivo have shown a significant and positive insecticidal activity. The recorded mortalities are proportional to the exposure times (2h, 4h, 6h, 8h, 10h and 24h) and the treatments applied after 24h of exposure.

Mortality rates of 88.63%, 93.43%, 99.03%, 70%, 88.49%, and 26.50% were recorded respectively for (OEG, OEP, OES, HS, T+, and T-). The highest rate was recorded for OES followed by OEP, OEG, and HS. These results allowed us to conclude that the insecticidal effect of our biological treatment (OE) is effective compared to the positive control (chemical product) and even more important compared to the negative control (base preparation). This shows the effectiveness of the treatment applied *in vivo.*

The acetone extracts revealed mortality rates of 97%, 56.60%, 75.03%, 88.49%, and 26.50%, which were recorded respectively for Ext ActG, Ext ActP, Ext ActS, T+, and T-. The highest rate was observed for Ext ActG, followed by Ext ActS, Ext ActP, while the negative control, which represents the base preparation, showed a less effective effect from 6h.

The methanol extracts recorded mortality rates of 80%, 99%, 94.65%, 88.49%, and 26.50%, which were respectively for Ext MG, Ext MP, Ext MS, T+, and T-. The highest rate was that of Ext MP, followed by Ext MS, and Ext MG. While the positive control showed a less effective effect from 6h and even more important compared to the negative control, this shows the effectiveness of the treatment of Ext MP.

The aqueous extracts of the mixture recorded the highest mortality rate of 93.27%, followed by that of Ext AqP with a percentage of 91% and the lowest mortality was revealed by the treatment Ext AqG. The two control treatments (T+,T-), showed values of 88.49% and 26.50% respectively. Mortality rates of 87.47%, 91%, 93.27%, 88.49%, and 26.50% were recorded respectively for Ext AqG, Ext AqP, Ext AqS, T+, and T-. The highest rate was that of Ext AqS, followed by Ext AqP and Ext AqG.

Discussion

The treatments (OES, Ext ActG, Ext MP and Ext AqS) are more effective than the treatments (OE G, OE P, HS, Ext ActP, Ext ActS, Ext MG, Ext MS, Ext AqG, Ext AqP) but the overall results found are still significant in comparison to the controls.The mixtures of the two plants gave very satisfactory *in vivo* mortality rates, in fact the majority of the results showed a positive synergistic effect.

According to Riahi et al., (2013), the results of this biological treatment can be justified by the presence of certain chemical compounds such as β-phellandrène (47.14%) which is the main component of *J. phoenicea* from the region of Tabarka, Tunisia. Other studies conducted by Achak et al., (2008), on *J.phoenicea* from Tunisia and Morocco, found 31 monoterpene components, of which the essential oil from Tunisia is rich in α-pinene (35.46%), as well as 45 components in the essential oil from Morocco and α-pinene with a percentage of 38.2%. He concluded that the monoterpenes of OE are toxic to many insects.

The leaves of *P.lantiscus,* according to Belhachat (2019), are rich in monoterpenes, mainly α-pinene (6.91%) and hydrocarbon sesquiterpenes with a rate of 9.20%, of which the main constituent is β-caryophyllene (5.1%). Regnault-Roger et al., (2012) show that the insecticidal activity of monoterpenes contained in HEs can be due to several mechanisms that affect multiple targets by more effectively disrupting the cellular activity and biological processes of insects.

Ben Abdelkader (2012), moreover, showed that the insecticidal efficacy of an OE would be due to the nature and chemical structure of its terpenoid constituents.

From these different studies, a trend seems to emerge, namely that monoterpenes that affect insect cells represent the most widespread biochemical family in the essences extracted from the genus Juniperus with "alpha-pinene" as the main constituent, (Boufares, 2020).

3.4. Synergistic potential of different treatments

The antagonistic and synergistic effects of the different mixtures tested are represented in figures 6, 7, 8, and 9, and the results obtained are at satisfactory levels of efficiency.

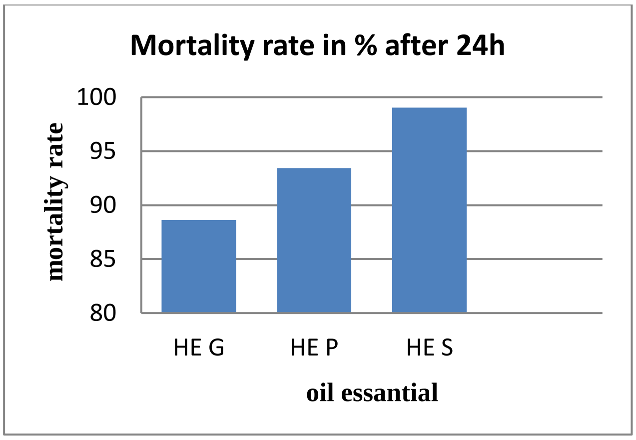

Figure 6: Aphids mortality rate as a function of essential oils after 24 hours

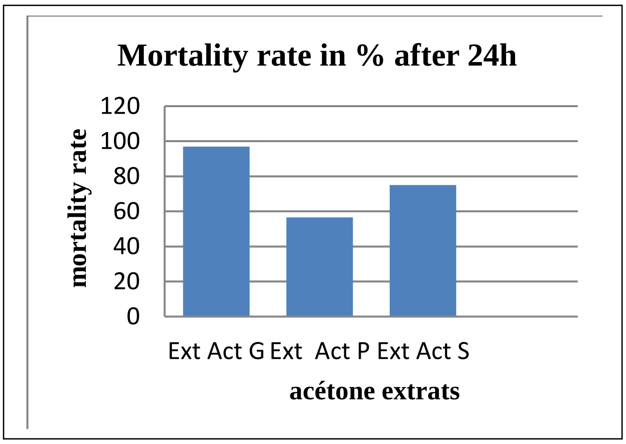

Figure 7: Aphids mortality rate as a function of acetone extrats after 24 hours

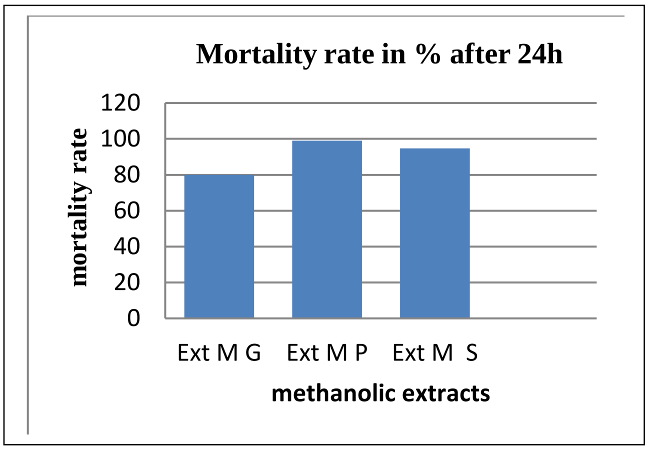

Figure 8: Aphids mortality rate as a function of methanolic extracts after 24 hours

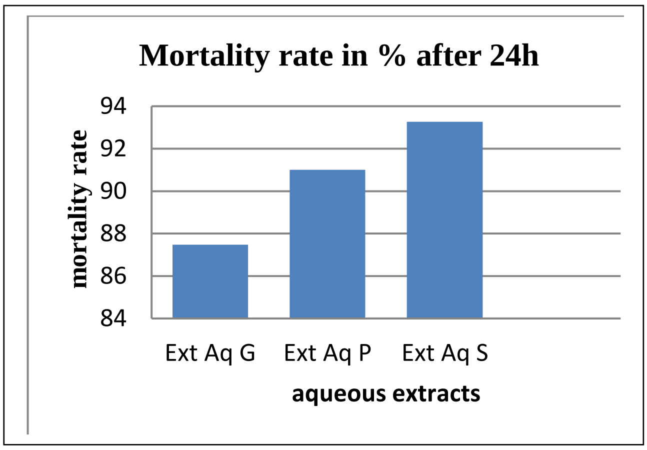

Figure 9: Aphids mortality rate as a function of aqueous extracts after 24 hours

The results showed that the essential oil mixture of the two plants was more effective than the essential oil of each plant separately (*J. phoenicea*and *P. lantiscus*). The mortality rates recorded were of the order of 88.63% for OE G and 93.43% for OE P, and OES was 99.03%. The OEs effect was superior to the two plants individually: OE S >OEP and OEG, from which we deduce a synergistic effect between the two plants. The acetone and methanol extracts have a partial fractional synergy effect between the two plants, in fact the values found are respectively of the order of 97% for Ext ActG and 56.60% for Ext ActP, and the mixture of the two acetone extracts was 75.03%. of the order of 80% for Ext MG and 99% for Ext MP, while those of the mixture were 94.65%.: Ext Act P<Ext Act S < Ext Act G and (Ext MG< Ext MS <Ext MP).
The aqueous extract revealed a synergistic effect of the mixture compared to the two individual plants Ext AqS> Ext AqP and Ext AqG. The mixture gave a rate of the order of 93.27% which is higher than that of Ext AqG which was of the order of 87.47% and that of Ext AqP which was of the order of 91%. To this effect, we conclude that the mixture of the two plants has favored a maximum extraction of the molecules.

Discussion

The results obtained for the mixture of essential oils and aqueous extracts of the two plants revealed a positive interaction between the essential oil of *J.phoenicea* and *P.lantiscus* on the citrus aphid. This observation is in agreement with those obtained by the work of Bassolé et al. (2012) who showed that most studies are focused on the interaction of phenolic monoterpenes (thymol, carvacrol) and phenylpropanoids (eugenol) with other groups of components, while hydrocarbon compounds are less used. Burt (2004) concluded that whole (raw) essential oils, have higher activity than mixtures of their main components, suggesting that minor essential oil components are critical for activity and may have synergistic and potentiating effects. Roman et al. (2008) reported that sublethal doses applied in binary mixtures showed a significant synergistic effect and thought that the synergistic effects of complex mixtures are important in plant defense against herbivores and also reported that the identification of such synergies within complex mixtures can allow the development of more effective control agents as well as the use of smaller absolute amounts in the mixture to obtain satisfactory levels of efficacy. They confirmed that among the more active compounds, we found that the hydrocarbons γ-terpinene and p-cymene and the ether 1,8-cineole proved to be the most important synergist for both application methods.

The results obtained for the mixture of acetone extracts and methanol extracts of the two plants show that certain molecules are in partial fractional synergy between the two plants of *J.phoenicea*and *P.lantiscus* on the citrus aphid. The work of Chouhan et al. (2017) confirmed that the combination of certain particular oils produced synergistic effects which can come from either the combined activity of two main components of essential oils, or from the interaction between several components.

The knowledge of the bioefficacy of terpenes and their synergistic relationships will help in the standardization of products and maximize its biological power.

3.5. Comparative effect of different treatments over time and mortality rates.

To compare the different treatments over time, we performed a statistical analysis based on boxplots of the aphid mortality rate according to the types of treatments at different exposure times (Figure 10).

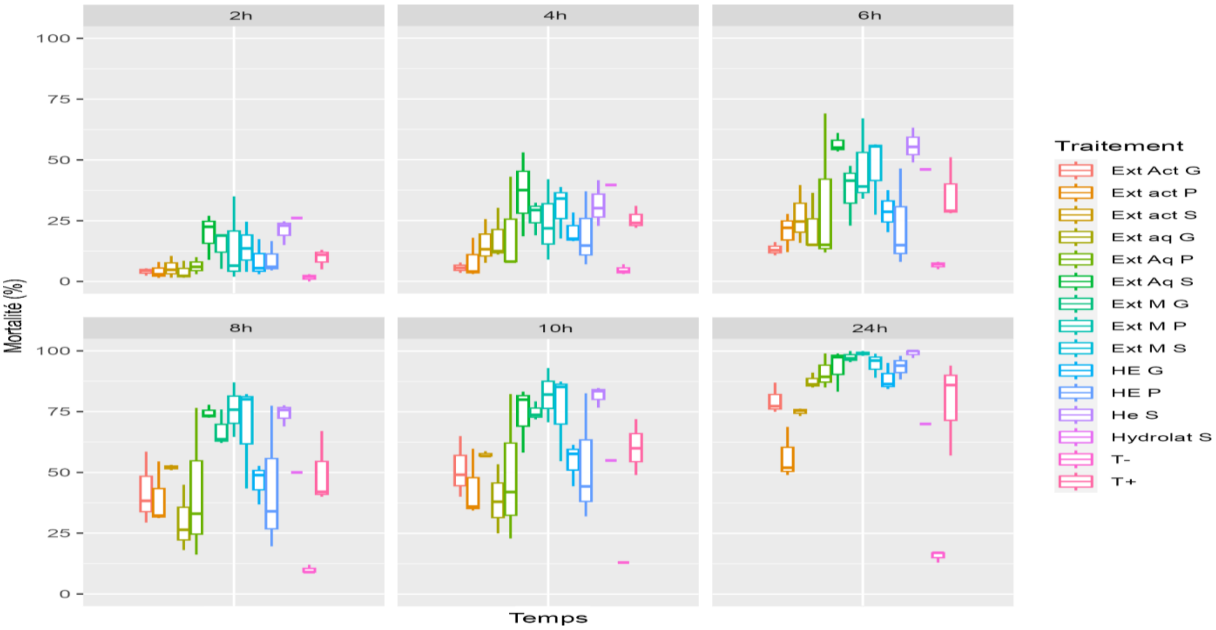

Figure 10: Boxplots of the aphid mortality rate according to the treatments at different exposure times

According to the figure 10, we conclude that no significant difference was observed between the different types of treatments after 2 and 4 hours of exposure, suggesting that the aphid mortality rate is not significantly influenced by the type of treatment during these exposure periods. However, it is important to note that these results do not allow to totally excluding the effectiveness of the treatments, as significant differences may still be observed with higher levels of significance.

A significant difference was observed after 6 hours of exposure between HS, T- and T+, with HS being the most effective treatment. After 8 hours of exposure, the same significant difference was observed between HS, T- and T+, as well as another significant difference between Ext AqS, T- and T+, with Ext AqS being the most effective. After 10 hours of exposure, the same significant difference was still observed between HS, T- and T+, as well as significant differences between Ext actS, T- and T+, with Ext actS being the most effective.

Finally, after 24 hours of exposure, several significant differences were observed, clearly showing the efficacy of HS, OEP, Ext MP and OES compared to T-. However, the efficacy of Ext MP was higher than that of HS. In addition, Ext MG and Ext MP were more effective than Ext actS.

3.6. Corrected mortality

The corrected mortality rates (Figure11) with respect to the negative control (base preparation) were deduced using the Abbott formula (1925).

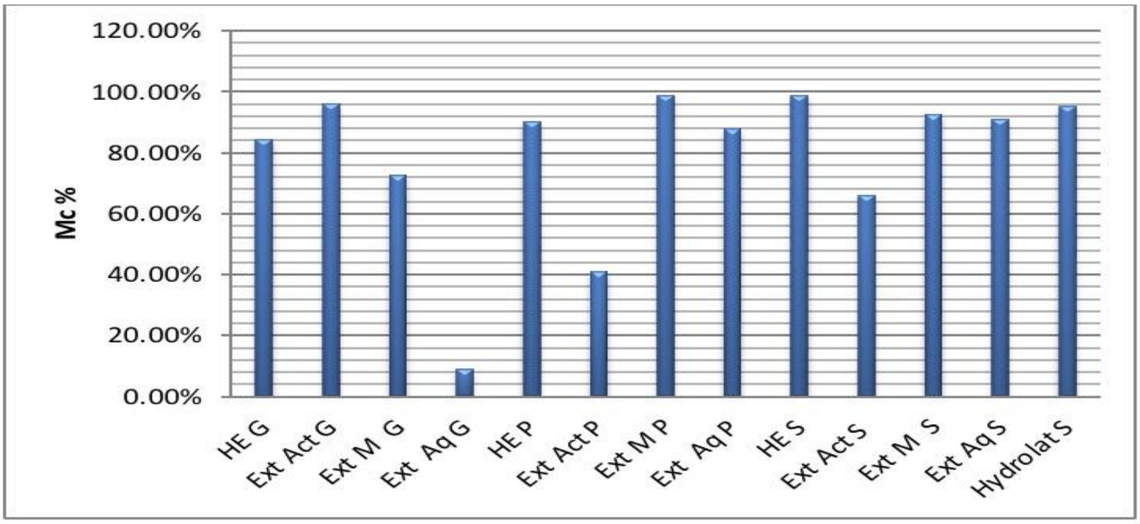

Figure 11: Corrected mortality(CM)

The corrected mortality rates of *J. phoenicea* were higher with the acetone extract treatment (95.91%) while the lowest rate was recorded by the aqueous extract (8.95%). *P. lentiscus* showed a corrected mortality of 98.63% for the methanolic extract and 40.95% for the acetone extract. Different mixtures, especially those that revealed synergy between the two plants, gave better corrected mortality rates than those that revealed partial synergy or even an antagonist. Synergy essential oil gave the best rate followed by synergy hydrolat, synergy methanolic extract, synergy aqueous extract and synergy acetone extract which are respectively 98.68%, 95.18%, 92.72%, 90.84%, 66.02%.

From these different results, we deduce that mixture-based treatments are more effective and they gave higher rates than those of each plant individually [OEG (84.53%), OEP (90.06%), OES (98.68%)].

3.7. Determination of lethal times

TL20, TL50, and TL90 are the times that cause the percentage of mortality in the citrus aphid population after 24 hours of application of the treatments "different essential oils and extracts and the two controls".

The negative control (T-) recorded the highest values, suggesting that it takes longer to eliminate the aphids. On the other hand, the HS treatment showed the shortest time to eliminate 20% of the aphids (1.23 hours). The HES treatment had the shortest TL50, with 4.98 hours, while the Ext MP treatment displayed the shortest TL90, with 12.06 hours. Figure 12 illustrates grouped histograms that allow a clear visualization of the differences between the treatments in terms of observed values.

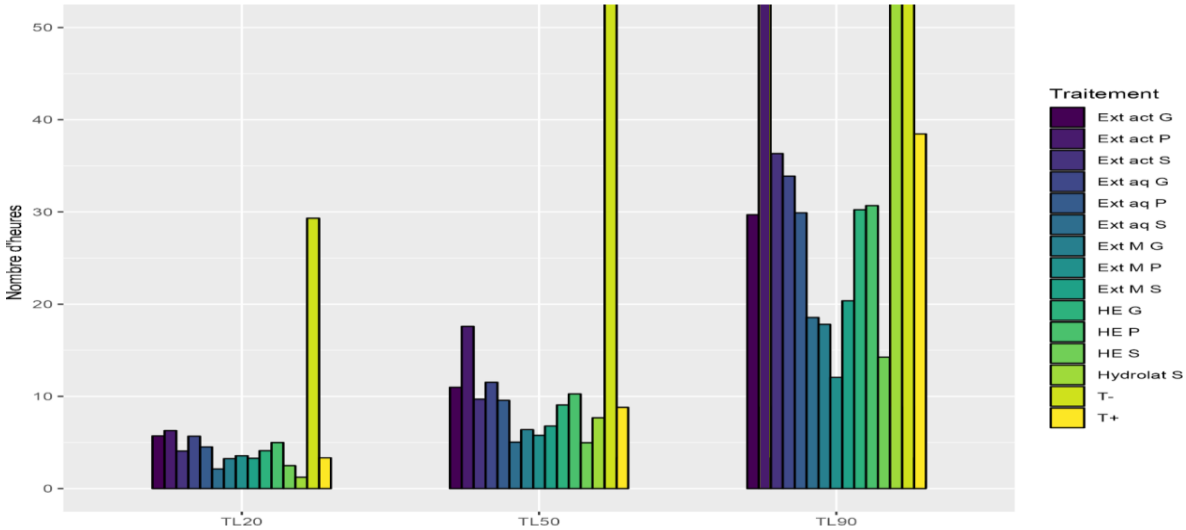

Figure 12: TL20, TL50, and TL90 in hours for each treatment used

After 5 hours, OES and Ext AqS killed 50% of the aphid population, followed by Ext MP which took 5 hours and 78 minutes. The longest time was recorded for Ext ActP, which was 17 hours and 60 minutes.

To eliminate 90% of the population, Ext MP took 12 hours and 6 minutes, followed by OES with 14 hours, and the longest time was recorded for HS.

4. Conclusion

The results of this study showed that the extracts of the two plants (*Pistacialantiscus L.*) and (*Juniperusphoenicea L.)* have different physico-chemical properties and the phytochemical characterization is generally positive.

The insecticidal activity was evaluated with different constituents: the essential oils and extracts (methanolic extract, acetone extract, and aqueous extract) of the plants *(P.lantiscus*) and (*J.phoenicea*) and their mixtures in order to determine their synergistic or antagonistic effect.

The recorded results gave promising values of mortality rates for their individual or synergistic use and that all the bioproducts applied recorded a reduction in terms of abundance of citrus aphids compared to the control, so the different extracts have an *in vivo* synergistic insecticidal activity.

Lethal times determined by probit analysis (TL20, TL50, TL90) showed that the "synergistic essential oil" treatment showed the shortest TL50 while the "methanolic extract of *P.lantiscus*" treatment showed the shortest TL90.

From these results obtained, we deduce that *P.lantiscus*and *J.phoenicea*and their synergistic effect have insecticidal effects that must be valued, thus we remedy the problems of persistence in the consumed product and of course the health of the consumer and respect the ecological balance.

It would therefore be interesting to extend the range of the insecticide test as well as the characterization of the active compounds in the different extracts in order to identify the different molecules responsible for the biological activity of this plant.

Reference:

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