SIFT DESK

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Biological control trials against aphid vector species using phytosyme, *Pistacia lentiscus* and *Vitex agnus-castus* oils

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Research

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CITATION

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ABSTRACT

Fruit tree crops in Tunisia suffer from different incurable viral diseases that are easily transmissible by aphis vectors. To face these natural enemies, abusive use of pesticides threatens permanently the public health throughout environmental pollution and the jam from residues. In the frame of this work, certification plants deriving from the sanitation process by *in vitro* culture have been diagnosed. Then, biological control trials against aphid vector species were carried out in vitro, meaning a synthetic enzyme, *Pistacia lentiscus*and*Vitex agnus-castus* oils. As results, ELISA serological analysis proved the effectiveness of shoot-tip-grafting in virus elimination and the production of "healthy" certified material. As well, the DL50 of phytosyme has been evaluated to 1% concentration and a TL50 to 24 hours. As for the essential oils used, DL50 of pistachio oil was assessed to 0.1% and a TL50 of 24h for *A. gossypii* and 48h for l'*A. spiraecola* and *M. persicae*. *Vitex agnus-castus* oilsreacted more effectively against *A. gossypii* with the same DL50 and TL50 values. However, the different assays were more effective by contact then by systemic treatment. These encouraging fundings have to be re-investigated *in vivo* to verify their efficiency, field stability and to determine their afterglow period which plays an important role in the success of the biocontrol.

Keywords: Viral Diseases, Aphis Vectors, Biological Control

1. INTRODUCTION

Tristeza disease caused by the phytovirus tristeza or Citrus tristeza virus (CTV) is known to be one of the most devastating diseases of citrus fruits worldwide. Most citrus species and some species of the Rosaceae family have been reported as susceptible hosts for CTV. The damage caused results in necrosis of the liberator's vessels, both in the underground part and in the aerial part. The virus begins its attacks on the peripheral root hair, then the necrosis gradually spreads to the large roots and the trunk (Klotz and Fawcet, 1952 ; Fawcet, 1952).

Aphids belong to the order of Homoptera, to the suborder of Sternorrhynchea, to the superfamily of Aphidoidae (Homoptera) (Alain, 2006). Citrus aphids in Tunisia are *Aphis gossypii*, *Aphis spireacola and Myzus persicae* (Deguine and Leclant, 1997).

According to the international organization for the biological control of animals and noxious plants, biological control was the use of living organisms (insects, bacteria, nematodes, etc.) or their derivatives to control pest populations and prevent or reduce the loss or damage caused to crops(Hautier, 2003; Lambert, 2005; Maisonhaute, 2009).

Phytozyme is one of the most important biocontrol agents of aphids in citrus orchards. Understanding the efficiency and host specificity of natural enemies can help improve their effectiveness as biological control agents of particular pests.

We hypothesize that the sensitivity of aphids, i.e. the mortality rate, differed according to (i) the stages (larvae or adults), (ii) the duration (between 24 and 48 hours), (iii) the product used (phytozyme , essential oil), (iv) the content of product used and (v) the species of aphids (*A. gossypii*, *A. spiraecola* and *M. persicae*). The highest mortality rate defined the most effective product for controlling aphids.

2. MATERIALS AND METHODS

2.1. Plant materials

The essential oils used during these experiments were extracted from the leaves and seeds, dried from pistachio (*Pistcia lentiscus*) andgattilier (*Vitex agnus-castus*).

2.1.1. Drying and storage

The freshly harvested leaves were dried in the shade in a dry and ventilated place for 15 days. Once dry, they were collected in clean bags for later extraction of their oil.

2.1.2. Oil extraction

The apparatus used for hydro-distillation is of the Clevenger type, it consists of a balloon heater which allows the homogeneous distribution of the heat in the balloon, then in pyrex glass where the dried leaves are placed, crushed and distilled water. A column of condensation of the vapour (refrigerant) which comes from the heating of the flask, a collector in pyrex glass also which receives the extracts from the distillation. 100 g of the dry and ground leaves (or seeds) were placed in 250 mL round bottom flask, supplemented with 1000 mL of distilled water. The whole is brought to a boil for about 3 hours. Then the extracted oil was entrained by the water vapour, then condensed through the condenser. The liquid collected results in a distillate with a thin layer of oil on the surface which will then be separated, after the liquid has rested.

2.2. The phytozyme

Enzyme-based products dedicated to the agricultural sector supplied by ECORAMA (French company specializing in the development and marketing of enzyme-based hygiene solutions and processes).

2.3. Certification

2.3.1. Sampling

The samples were taken from seed stock plants or "clone heads": this is the category of starting material in the certification process that will be at the origin of certified plants (we take samples from these plants which have undergone a micrografting of apex in the laboratory then acclimatization by over-grafting on a vigorous rootstock in a glass greenhouse The serological detection of the viral entities is carried out with the ELISA test (DAS).

2.3.2. Identification of virus-carrying aphids

The systematics of the various specimens of aphids collected was based on the identification key of Blackman and Eastop (1984) which rests on certain members of taxonomic interest such as antennae, cornicles, cauda, eyelashes.

2.4. Tests against aphid vectors of viruses

2.4.1. Phytozyme control

Contact treatment

In Petri dishes containing tender leaves, aphids of the species *Aphis spiraecola*, *Aphis gossypii* and *Mezus persicae* were placed separately. These sheets are placed on 3 layers of filter paper soaked in water to avoid dehydration.The application of phytozyme was carried out by spraying with 3 different doses 1%; 2% and 3% diluted in distilled water. The aphid mortality rate was counted after 24 hours and 48 hours.

Systemictreatment

Using Petri dishes containing filter papers soaked in water, on which have been placed leaves of plants (which have already absorbed the diluted phytozyme in 3 different doses (1%; 2% and 3%) and colonized by aphids of the three species studied: *Aphis spiraecola, Aphis gossypii* and *Mezus persicae*.The counts of aphid mortality were counted after 24 hours and 48 hours.

2.4.2. Oil controls

Contact treatment

Using Petri dishes containing filter papers soaked in water, on which were placed leaves of plants colonized by aphids of the three species studied: *Aphis spiraecola*, *Aphis gossypii* and *Mezus persicae*. Preparation consisted of dissolving 1g of essential oil in 100mL of absolute ethanol. The essential oil was applied by spraying at 5 different doses in the order of 0.1%; 0.2%; 0.5; 1% and 1.5%. The counts of aphid mortality were counted after 24 hours and 48 hours.

Systemic treatment

Preparation consisted of dissolving 1g of oil in 100mL of absolute ethanol. Using Petri dishes containing filter papers soaked in water, on which have been placed leaves of plants (which have already absorbed oil diluted in 5 different doses 0.1%; 0.2%; 0, 5; 1% and 1.5%) and colonized by aphids of the three species studied: *Aphis spiraecola*, *Aphis gossypii* and *Mezus persicae*. The reading of the results continued as mentioned above.

3. RESULTS

3.1. Serological screening for certification viruses

According to certification standards, a source/plant material was considered infected if the average value of its optical density (OD) exceeds 2.5 to 3 times that of healthy control (negative).

3.1.1. Citrus certification virus serological testing

The tested samples were found to be healthy, ie free from CTV after *in vitro* regeneration process by apex micrografting.

3.1.2. Serological screening for pome fruit tree certification viruses

All samples werenot infected with either the ACLSV virus or the ApMV virus.

3.1.3. Serological screening of certification viruses for stone fruit trees

All samples are healthy so were not infected with PNRSV, ApMV, or PPV; nor by ACLSV. This serological diagnosis of fruit tree viruses was considered obvious given the effectiveness of specific antibodies.

3.2. Identification of virus-carrying aphids in the orchard

The distinction between the predominant species *A. spiraecola* and *A. gossypii* was mainly based on the number of bristles on the cauda and the colour of the cornicles.

A. gossypii is recognized for its pale cauda, containing 2 to 3 pairs of bristles, which is not the case for *A. Spiraecola*, where the cauda is dark in color and coated with 4 to 6 pairs of eyelashes.

3.3. Tests for controlling aphid vectors of viruses

3.3.1. Phytozyme aphid control trials

In vitro* effect of phytozyme on *A. gossypii

The mortality rate of adults of *A. gossypii* after contact treatment by phytozyme: We founded that the mortality rate was higher after 48 hours than that after 24 hours and it is estimated that the optimal phytozyme content is 2% where the mortality rate reaches 80% for 24 hours and 100% for 48 hours and the minimum phytozyme content is 1% where the mortality rate is around 63% for 24 hours and 83% for 48 hours (Fig. 1A).

The mortality rate of adults of *A. gossypii* after systemic treatment by phytozyme: We noted that the lowest mortality rate corresponds to a phytozyme content of 2% (20% for 24 hours and 50% for 48 hours) while the highest mortality rate corresponds to 1% (43% for 24h and 70% for 48h) (Fig. 1B).

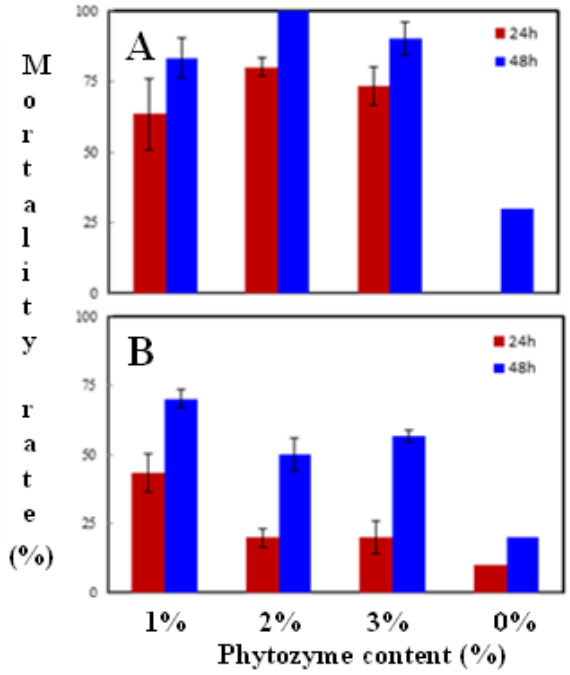

Figure 1. Mortality rate of adults of *Aphis gossypii* treated by phytozyme (A) contact and (B) systemic treatment. The values are the means of three independent experiments (+/- SE). Each experiment was carried out with ten individuals.

The mortality rate of larvae of *A. gossypii* after contact treatment by phytozyme: The mortality rate increased according to the phytozyme content.When the content was increased from 1% to 3% the mortality rate increased from 56% to 93% for 24 hours and from 73% to 100% for 48 hours (Fig. 2A).

The mortality rate of larvae of *A. gossypii* after systemic treatment by phytozyme: the mortality rate reached 46% for 24 hours and 70% for 48 hours for a concentration of 2% and decreased when the phytozyme content increased (Fig. 2B).

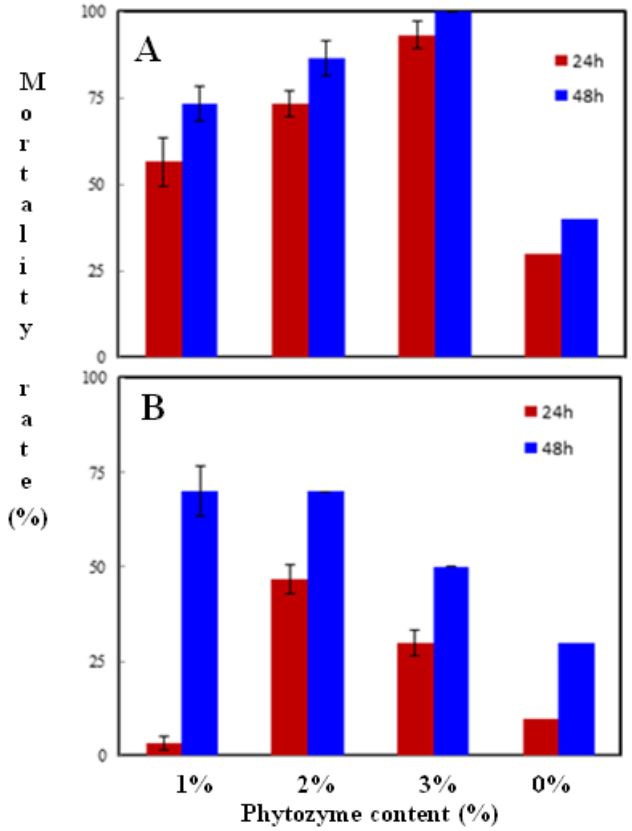

Figure 2. Mortality rate of larvae of *Aphis gossypii* treated by phytozyme (A) contact and (B) systemic treatment. The values are the means of three independent experiments (+/- SE). Each experiment was carried out with ten individuals.

In vitro* effect of phytozyme on *M. persicae

The mortality rate of adults of *M. persicae* after contact treatment by phytozyme: The mortality rate of *M. persicae* differed with time and the phytozyme content. When the content was low (1%), the mortality rate reached 93% for 48 hours and when the content was increased to 3% the mortality rate reached 100% (Fig. 3A).

The mortality rate of adults of *M. persicae* after systemic treatment by phytozyme: The highest mortality rate (83% at 24h and 90% at 48h) corresponded to the content of 3% and the low rate (50% at 24h and 73% at 48h) corresponded to the content of 2% (Fig. 3B).

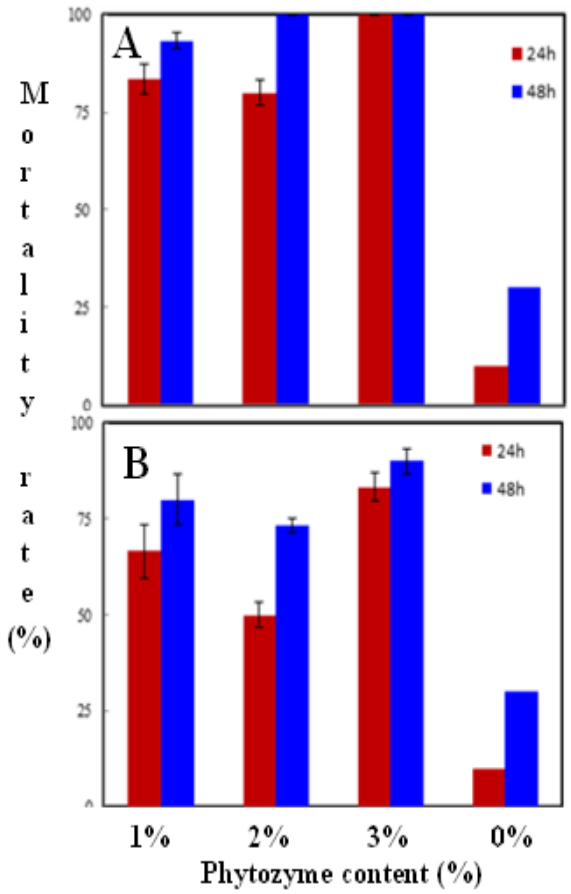

Figure 3. Mortality rate of adults of *Mezus persicae*-treated by phytozyme (A) contact and (B) systemic treatment. The values are the means of three independent experiments (+/- SE). Each experiment was carried out with ten individuals.

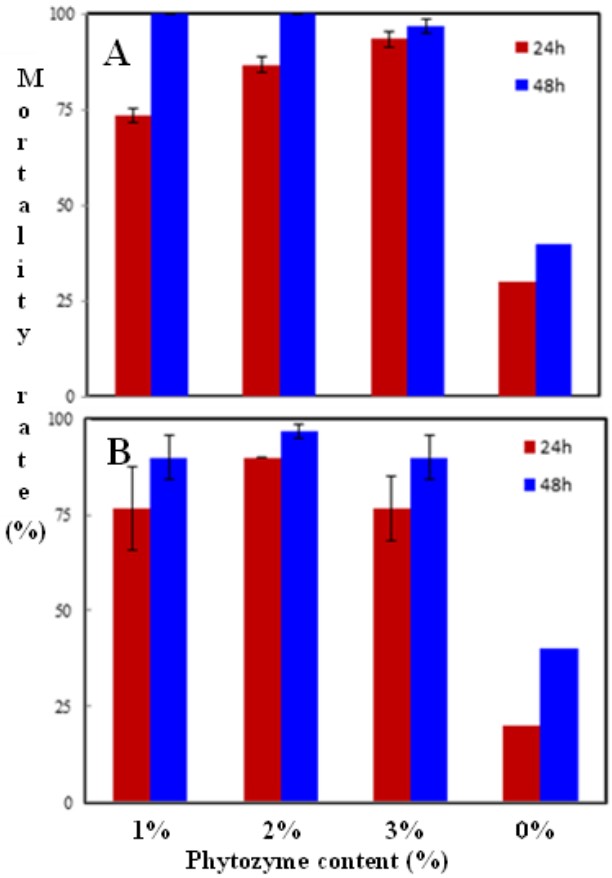

Figure 4. Mortality rate of larvae of *Mezus persicae*-treated by phytozyme (A) contact and (B) systemic treatment. The values are the means of three independent experiments (+/- SE). Each experiment was carried out with ten individuals.

The mortality rate of larvae of *M. persicae* after contact treatment by phytozyme: Acontent of 2% caused a mortality rate which reached 100% for 48 hours (Fig. 4A).

The mortality rate of larvae of *M. persicae* after systemic treatment by phytozyme: The highest mortality rate corresponded to a phytozyme content of 2% (90% at 24 h and 93% at 48 h) (Fig. 4B).

In vitro* effect of phytozyme on *A. spiraecola

The mortality rate of adults of *A. spiraecola*after contact treatment by phytozyme: Figure 5 showed that the highest mortality rate (30% for 24 hours and 66% for 48 hours) correspond to a phytozyme concentration of 3% (Fig. 5A).

The mortality rate of adults of *A. spiraecola*after systemic treatment by phytozyme: We noted that the higher or lower rate (6% for 24 hours and 36% for 48 hours) corresponded to a phytozyme content of 2% (Fig. 5B).

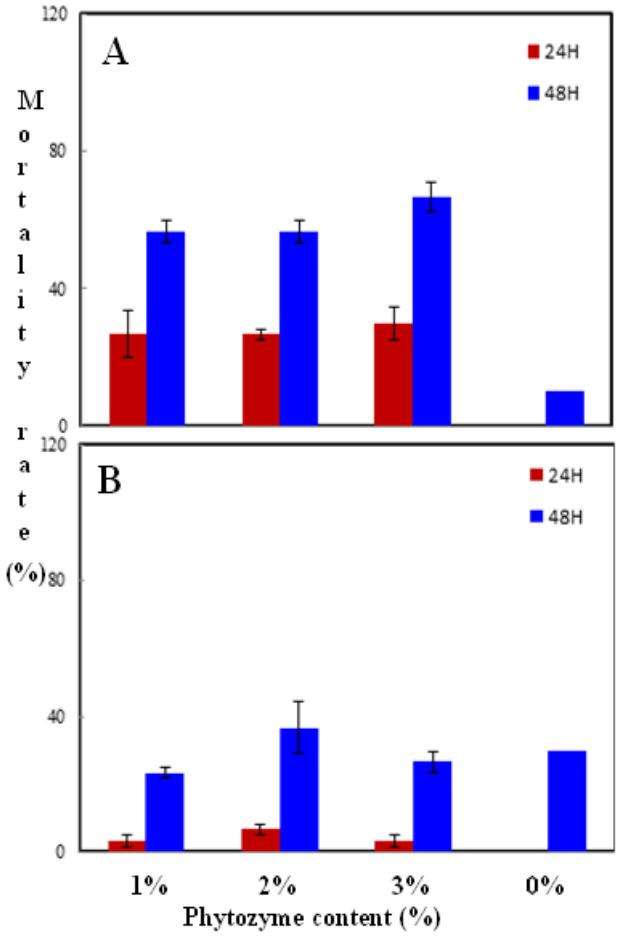

Figure 5. Mortality rate of adults of *Aphis spiraecola*treated by phytozyme (A) contact and (B) systemic treatment. The values are the means of three independent experiments (+/- SE). Each experiment was carried out with ten individuals.

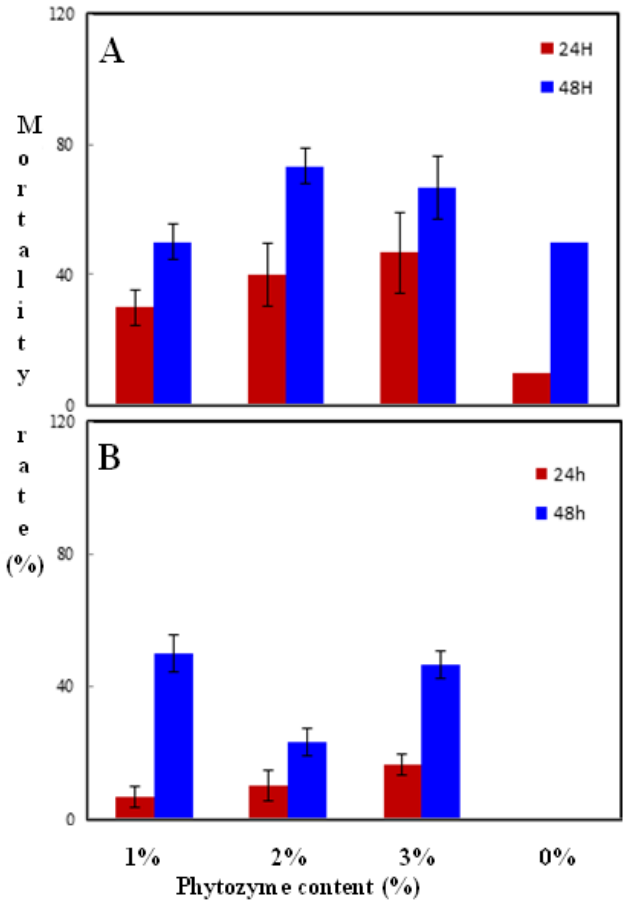

Figure 6. Mortality rate of larvae of *Aphis spiraecola*treated by phytozyme (A) contact and (B) systemic treatment. The values are the means of three independent experiments (+/- SE). Each experiment was carried out with ten individuals.

The mortality rate of larvae of *A. spiraecola* after contact treatment by phytozyme: As illustrated in Figure 16, the highest mortality rate corresponded to a phytozyme content of 2% and when the concentration was increased the mortality rate became low, especially at 48 hours (Fig. 6A).

The mortality rate of larvae of *A. spiraecola*after systemic treatment by phytozyme: The 1% of phytozyme content corresponded to the highest mortality rate, especially after 48 hours, when it reached 50% (Fig. 6B).

Treatment with phytozyme acted more effectively on *A.gossypii*, *A. Spiraecola* and *M. persicae* by contact compared to systemic treatment, but the effective doses differd depending on the species and evolutionary stages. All the previous results have shown that the phytozyme acted more strongly on the larval stages compared to the adult stages due to the higher sensitization of the larvae to the components of this product.

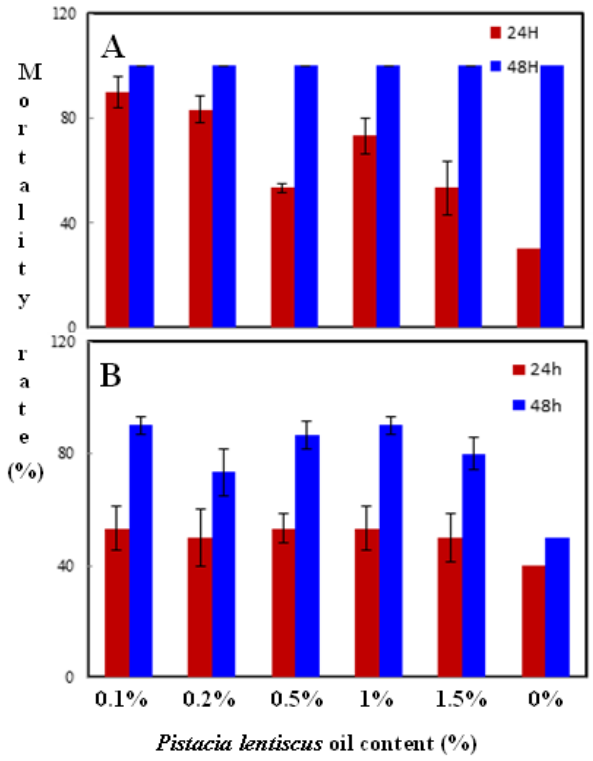

Figure 7. Mortality rate of adults of *Aphis gossypii* treated by *Pistacia lentiscus* oil (A) contact and (B) systemic treatment. The values are the means of three independent experiments (+/- SE). Each experiment was carried out with ten individuals.

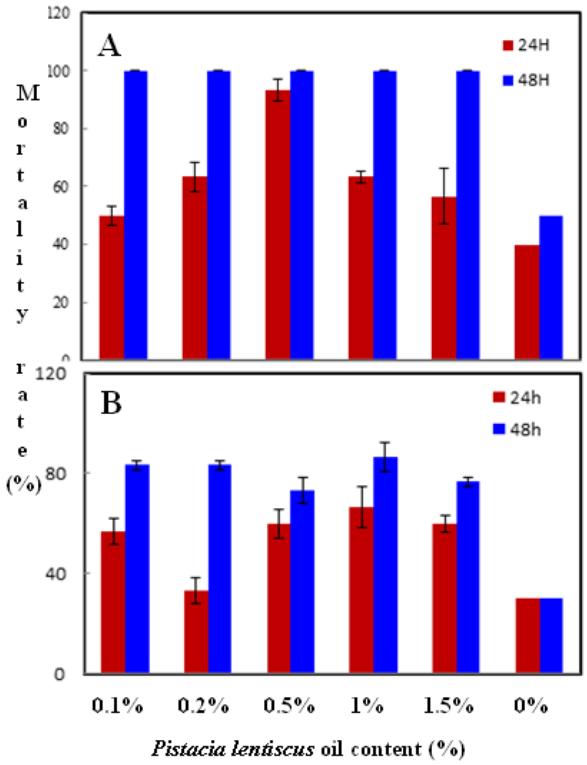

Figure 8. Mortality rate of larvae of *Aphis gossypii* treated by *Pistacia lentiscus* oil (A) contact and (B) systemic treatment. The values are the means of three independent experiments (+/- SE). Each experiment was carried out with ten individuals.

3.3.2. oils aphid control trials

In vitro* effect of *Pistacia lentiscus* oil on *A. gossypii

The mortality rate of adults of *A. gossypii* after contact treatment by *Pistacia lentiscus* oil:Under the effect of *Pistcia lentiscus* oil, the highest mortality rate from *A. gossypii* aphids corresponded to the concentration of 0.1% where it reached 90% for 24 hours and 100% for 48 hours. The mortality rate remained high for 48 hours by applying all the fixed concentrations (Fig. 7A).

The mortality rate of adults of *A. gossypii* after systemic treatment by *Pistacia lentiscus* oil:The aphid mortality rate reached 53% for 24 hours and 90% for 48 hours at a concentration of 0.1%. The one-day and two-day aphid mortality rate values were very closed despite the variability in essential oil content (Fig. 7B).

The mortality rate of larvae of *A. gossypii* after contact treatment by *Pistacia lentiscus* oil: It was found that the highest mortality rates were recorded after 48 hours than those after 24 hours. Indeed, the mortality rate reached 93% in 24 hours and 100% in 48 hours at a concentration of 0.5% (Fig. 8A).

The mortality rate of larvae of *A. gossypii* after systemic treatment by *Pistacia lentiscus* oil: The mortality rate reached 66% after 24 hours and 86% after 48 hours at a content of 1% (Fig. 8B).

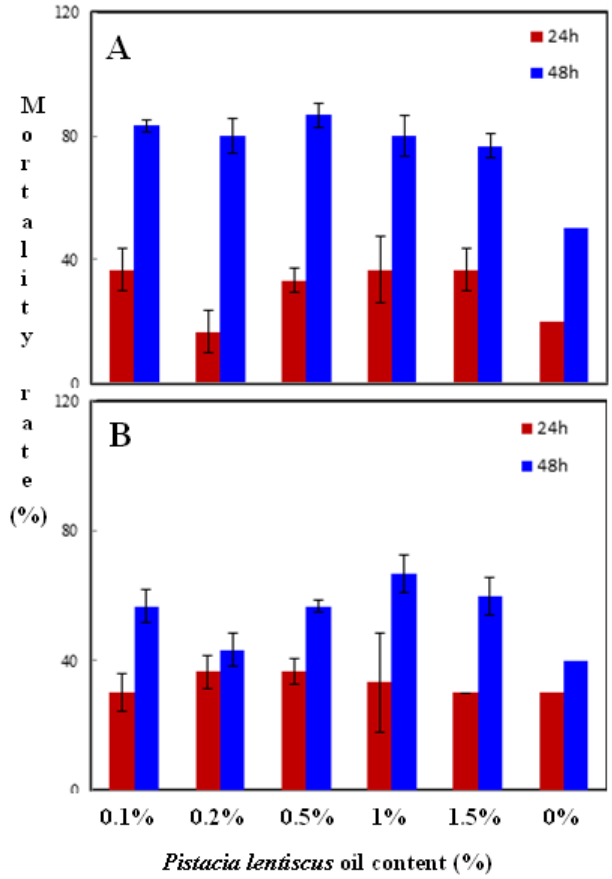

Figure 9. Mortality rate of adults of *Mezus persicae*treated by *Pistacia lentiscus* oil (A) contact and (B) systemic treatment. The values are the means of three independent experiments (+/- SE). Each experiment was carried out with ten individuals.

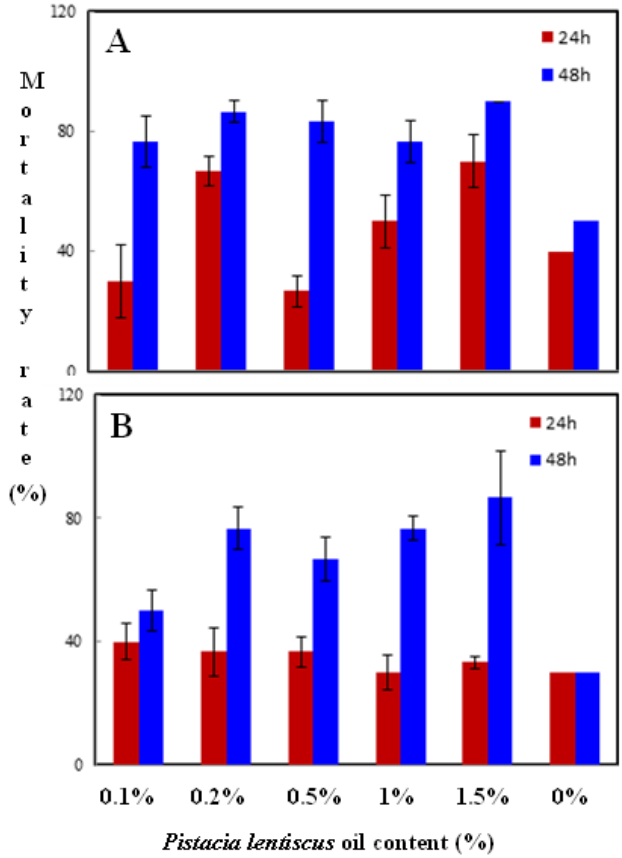

Figure 10. Mortality rate of larvae of *Mezus persicae*-treated by *Pistacia lentiscus* oil (A) contact and (B) systemic treatment. The values are the means of three independent experiments (+/- SE). Each experiment was carried out with ten individuals.

In vitro* effect of *Pistacia lentiscus* oil on *M. persicae

The mortality rate of adults of *M. persicae* after contact treatment by *Pistacia lentiscus* oil: The highest aphid mortality rates were those taken after 48 hours, since they reached 33% for one day, on the other hand they exceeded 86% after 2 days at a concentration of 0.5% (Fig. 9A).

The mortality rate of adults of *M. persicae*after systemic treatment by *Pistacia lentiscus* oil: Figure (19) showed that the highest mortality rate (33% for 24 hours and 66% for 48 hours) corresponded to the content of 1% and the lowest rate (43% for 24 hours and 36% for 48h) corresponded to the content of 0.2% (Fig. 9B).

The mortality rate of larvae of *M. persicae*after contact treatment by *Pistacia lentiscus* oil: The high mortality rates were obtained at a concentration of 1.5% for 48 hours, in fact, they reached 70% for one day and 90% for two days (Fig. 10A).

The mortality rate of larvae of *M. persicae* after systemic treatment by *Pistacia lentiscus* oil:At 1.5%, mortality rates reached 33% for 24 hours and 86% for 48 hours (Fig. 10B)

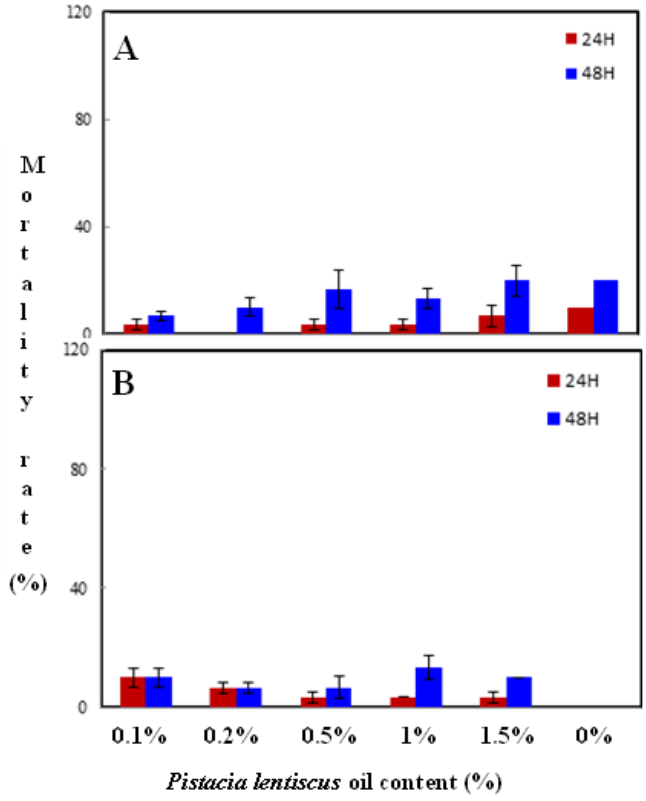

Figure 11. Mortality rate of adults of *Aphis spiraecola*by *Pistacia lentiscus* oil (A) contact and (B) systemic treatment. The values are the means of three independent experiments (+/- SE). Each experiment was carried out with ten individuals.

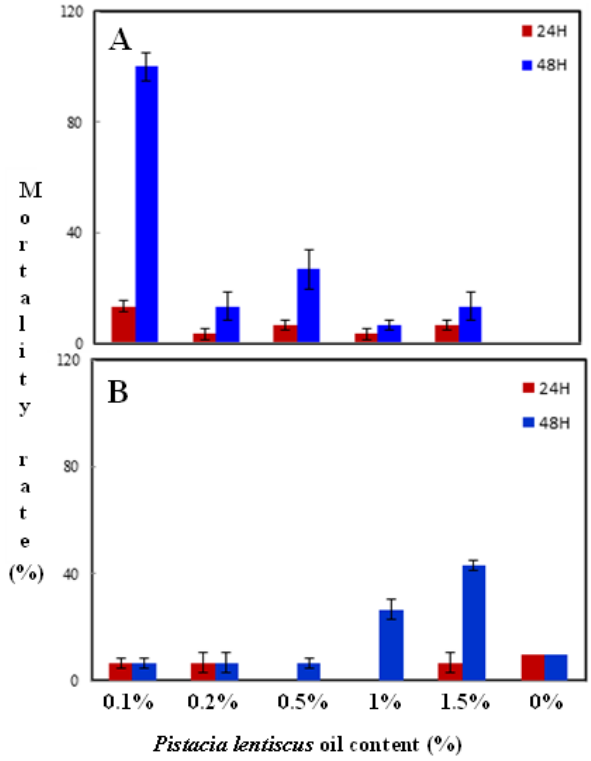

Figure 12. Mortality rate of larvae of *Aphis spiraecola*treated by *Pistacia lentiscus* oil (A) contact and (B) systemic treatment. The values are the means of three independent experiments (+/- SE). Each experiment was carried out with ten individuals.

In vitro* effect of *Pistacia lentiscus* oil on *A. spiraecola

The mortality rate of adults of *A. spiraecola* after contact treatment by *Pistacia lentiscus* oil: At 1.5%, mortality rates reached 6% for 24 hours and 20% for 48 hours (Fig. 11A)

The mortality rate of adults of *A. spiraecola* after systemic treatment by *Pistacia lentiscus* oil: Mortality rate equal to 3% for 24 hours and 13% for 48 hours with a content of 1% (Fig. 11B).

The mortality rate of larvae of *A. spiraecola* after contact treatment by *Pistacia lentiscus* oil: There was remarkable mortality rate of 100% for 48 hours, although it did not exceed a rate of 13% for 24 hours at a content of 0.1% (Fig. 12A).

The mortality rate of larvae of *A. spiraecola* after systemic treatment by *Pistacia lentiscus* oil: The high mortality rates were revealed after 48 hours despite that they were of the order of 6% for 24 hours and 43% for 48 hours with a content of 1.5% (Fig. 12B).

Treatment with *Pistacia lentiscus* oil showed that contact treatment gives higher effects compared to systemic treatment on the three species of aphids studied. The doses of oil used acted on the larvae because of their sensitization to this oil but for the species *A.gossypii*, the results obtained showed that the adults were rather more sensitive than the larvae.

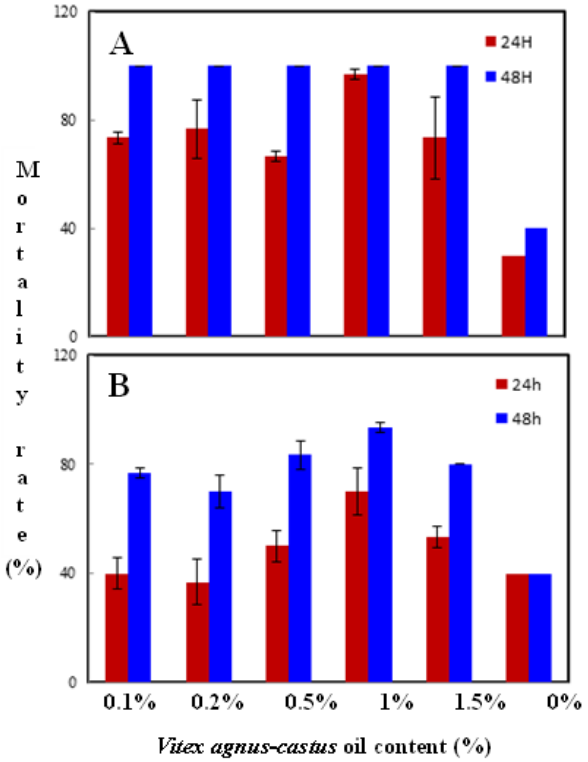

Figure 13. Mortality rate of adults of *Aphis gossypii* treated by *Vitex agnus-castus* oil (A) contact and (B) systemic treatment. The values are the means of three independent experiments (+/- SE). Each experiment was carried out with ten individuals.

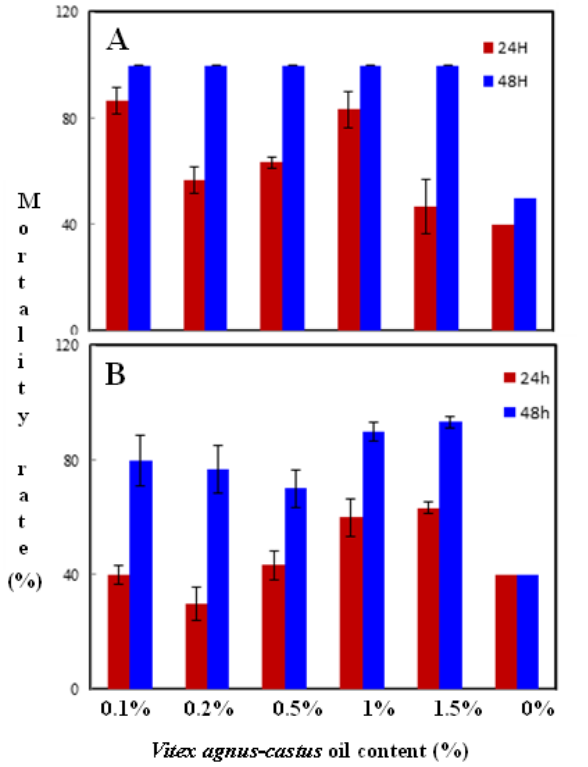

Figure 14. Mortality rate of larvae of *Aphis gossypii* treated by *Vitex agnus-castus* oil (A) contact and (B) systemic treatment. The values are the means of three independent experiments (+/- SE). Each experiment was carried out with ten individuals.

In vitro effect of *Vitex agnus-castus* oil on *A. gossypii*

The mortality rate of adults of *A. gossypii* after contact treatment by *Vitex agnus-castus* oil: When using rapidx oil for the adult aphid stage, the mortality rate reached 96% in 24 hours and 100% in 48 hours with a content of 1%, the latter rate remained stable with a content of 0.1% up to 1.5% (Fig. 13A).

The mortality rate of adults of *A. gossypii* after systemic treatment by *Vitex agnus-castus* oil: For the adult stage of aphids, we note that the highest values of mortality rate were those of 48 hours reaching up to 93% as against 70% in 24 hours with a content of 1% (Fig. 13B).

The mortality rate of larvae of *A. gossypii* after contact treatment by *Vitex agnus-castus* oil:0.1% was the most practical content of vitex oil; but this didnt prevent the effectiveness of vitex oil in different contents to fight against aphid larvae (Fig. 14A).

The mortality rate of larvae of *A. gossypii* after systemic treatment by *Vitex agnus-castus* oil: The mortality rate of aphids under the effect of vitex oil was not stable. It reached up to 63% for 24h and 93% for 24h at 1.5% (Fig. 14B)

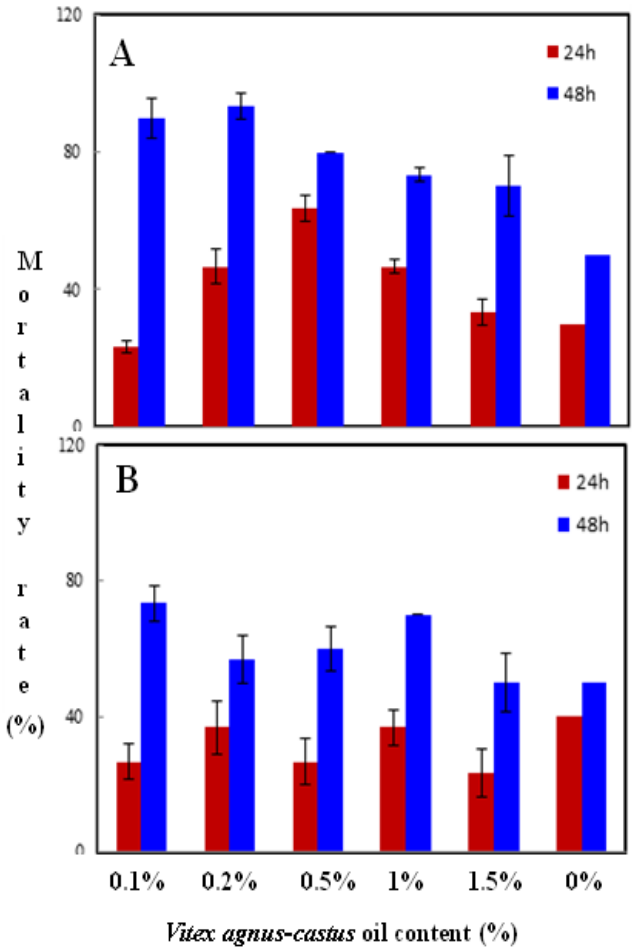

Figure 15. Mortality rate of adults of *Mezus perssicae*-treated by *Vitex agnus-castus* oil (A) contact and (B) systemic treatment. The values are the means of three independent experiments (+/- SE). Each experiment was carried out with ten individuals.

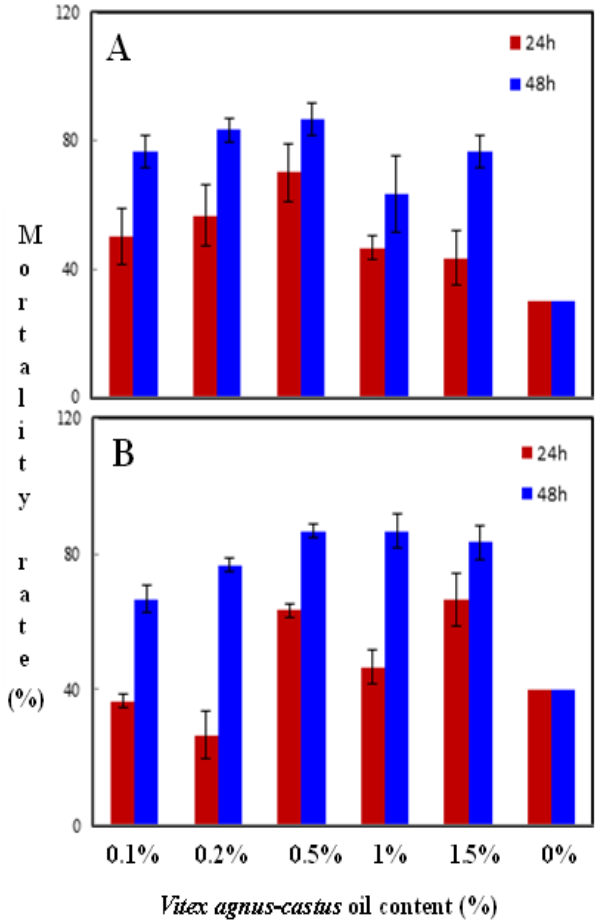

Figure 16. Mortality rate of larvae of *Mezus perssicae*treated by *Vitex agnus-castus* oil (A) contact and (B) systemic treatment. The values are the means of three independent experiments (+/- SE). Each experiment was carried out with ten individuals.

In vitro* effect of *Vitex agnus-castus* oil on *M. persicae

The mortality rate of adults of *M. persicae*after contact treatment by *Vitex agnus-castus* oil: We noted that unequal mortality rates were reaching up to 23% for 24 hours and 90% for 48 hours (Fig. 15A).

The mortality rate of adults of *M. persicae*after systemic treatment by *Vitex agnus-castus* oil: Mortality rates were more or less close to all levels, reaching up to 26% in 24 hours and 73% in 48 hours with a content of 0.1%. (Fig. 15B).

The mortality rate of larvae of *M. persicae* after contact treatment by *Vitex agnus-castus* oil: Mortality rate were 70% for 24 hours and 86% in 48 hours, these values corresponded to a content of 0.5% of vitex oil (Fig. 16A).

The mortality rate of larvae of *M. persicae* after systemic treatment by *Vitex agnus-castus* oil: Large values of mortality rate were noted for 48 hours, reaching up to 86%, on the other hand, they exceed 63% for 24 hours with a content of 0.5% (Fig. 16B).

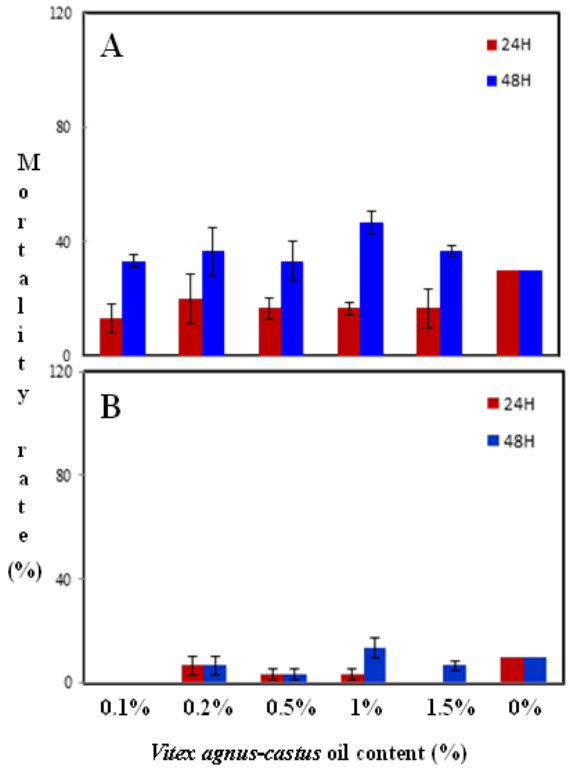

Figure 17. Mortality rate of adults of *Aphis spiraecola*treated by *Vitex agnus-castus* oil (A) contact and (B) systemic treatment. The values are the means of three independent experiments (+/- SE). Each experiment was carried out with ten individuals.

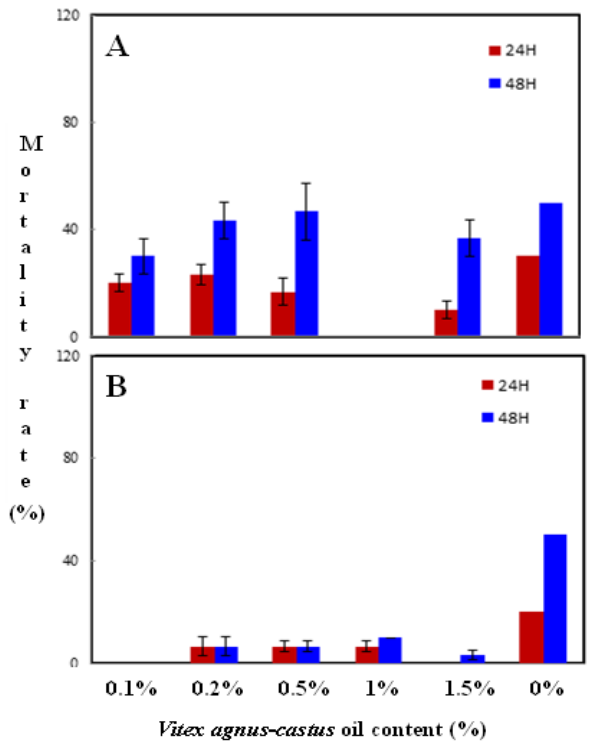

Figure 18. Mortality rate of larvae of *Aphis spiraecola*treated by *Vitex agnus-castus* oil (A) contact and (B) systemic treatment. The values are the means of three independent experiments (+/- SE). Each experiment was carried out with ten individuals.

In vitro* effect of *Vitex agnus-castus* oil on *A. spiraecola

The mortality rate of adults of *A. spiraecola*after contact treatment by *Vitex agnus-castus* oil: It is estimated that mortality rates more or less low, reaching up to 16% for 24 hours and 46% in 48 hours at 1%. By comparison with the control, the vitex oil was not effective for 24 hours, but it can be effective at a content of 1% for 48 hours (Fig. 17A).

The mortality rate of adults of *A. spiraecola*after systemic treatment by *Vitex agnus-castus* oil: Mortality rates were very low, the highest rates corresponded to 0.2% (6% for 24 hours and 6% for 48 hours) and the lowest rates corresponded to the 1.5% content (0% for 24 hours and 3% for 48 hours) (Fig. 17B).

The mortality rate of larvae of *A. spiraecola* after contact treatment by *Vitex agnus-castus* oil: The aphid mortality rate reached 16% for 24 hours and 46% for 48 hours with a content of 0.5% of HE and showed no effect at a concentration of 1% (Fig. 18A).

The mortality rate of larvae of *A. spiraecola* after systemic treatment by *Vitex agnus-castus* oil: It was found that the mortality rate was zero at a content of 0.1% of vitex oil, at a content of 0.5% the rate reached 16% during 24 hours, while the percentage of mortality after 48 hours was around 46% (Fig. 18B).

Treatment with vitex oil has shown that contact therapy gives more effective results than systemic treatment. The adult stages are the most sensitive to the components of this oil, especially for the species *A. gossypii*

Taking into account the results obtained during these tests, the different treatments made it possible to establish the lethal doses and the lethal times according to the type of product, its mode of action as well as the aphid species and their evolutionary stages (Table 1).

Table 1. Comparative study of the effectiveness of the tested products

Compound	Animal species	Treatment	Stage	DL50	TL 50	DL100	TL100
Phytozyme	*Aphis gossypii*	Contact	Adult	1%	24h	2%	48h
			Larva	1%	24h	3%	48h
		Systemic	Adult	1%	48h	Ø	Ø
			Larva	1%	48h	Ø	Ø
	Aphis spiraecola	Contact	Adult	1%	48h	Ø	Ø
			Larva	1%	48h	Ø	Ø
		Systemic	Adult	Ø	Ø	Ø	Ø
			Larva	1%	48h	Ø	Ø
	Mezus persicae	Contact	Adult	1%	24h	3%	24h
			Larva	1%	24h	1%	48h
		Systemic	Adult	1%	24h	Ø	Ø
			Larva	1%	24h	Ø	Ø
Pistacia lentiscus oil	*Aphis gossypii*	Contact	Adult	0.1%	24h	0.1%	48h
			Larva	0.1%	24h	0.1%	24h
		Systemic	Adult	0.1%	24h	Ø	Ø
			Larva	0.1%	24h	Ø	Ø
	Aphis spiraecola	Contact	Adult	Ø	Ø	Ø	Ø
			Larva	0.1%	48h	0.1%	48h
		Systemic	Adult	Ø	Ø	Ø	Ø
			Larva	Ø	Ø	Ø	Ø
	Mezus persicae	Contact	Adult	0.1%	48h	Ø	Ø
			Larva	0.1%	48h	Ø	Ø
		Systemic	Adult	0.1%	48h	Ø	Ø
			Larva	0.1%	48h	Ø	Ø
Vitex agnus-castus oil	*Aphis gossypii*	Contact	Adult	0.1%	24h	0.1%	48h
			Larva	0.1%	24h	0.1%	48h
		Systemic	Adult	0.1%	48h	Ø	Ø
			Larva	0.1%	48h	Ø	Ø
	Aphis spiraecola	Contact	Adult	Ø	Ø	0.1%	48h
			Larva	Ø	Ø	Ø	Ø
		Systemic	Adult	Ø	Ø	Ø	Ø
			Larva	Ø	Ø	Ø	Ø
	Mezus persicae	Contact	Adult	0.1%	48h	Ø	Ø
			Larva	0.1%	24h	Ø	Ø
		Systemic	Adult	0.1%	48h	Ø	Ø
			Larva	0.1%	48h	Ø	Ø

DL50: it is the lethal dose which affects 50% of individuals ; TL50: it's lethal time to affect 50% of individuals ; DL100: it is the lethal dose which affects 100% of individuals ; TL100: it's lethal time to affect 100% of individuals.Ø : not reached

Using phytozyme,the mortality of 50% of aphids was obtained at a dose of 1% which corresponded to the LD50 after 24 hours for the species *A. gossypii* for contact treatment but for systemic treatment, the LD50 requires 48 hours. For the species *A. spiraecola*, the LD50 was reached after 48 hours of treatment with a dose of 1% by the two tests. However, the adulticide systemic trial at various doses was not so effective even after 48 hours. Regarding the green peach aphid (*M. persicae*), the dose of 1% was satisfied to kill 50% of individuals after 24 hours.The 100% mortality of aphids was obtained at the 2% dose which corresponds to the LD100 after 48 hours for the species *A. gossypii* (adult) and at the 3% doses (larva) for contact treatment, but at other doses and for the other species there is no satisfying dose to kill 100% of individuals.

With *Pistacia lentiscus* oil,the mortality of 50% of aphids was obtained at a concentration of 0.1% which corresponds to the LD50 after 24 hours for the species *A. gossypii*, whether for contact or systemic treatment. For the species *A. spiraecola*, the LD50 was around 0.1% and requires 48 hours to act by contact at the larval stages which is not the case for the other tests. For the species, *M. persicae* the dose of 0.1% was satisfied to kill 50% of individuals (LD50) but after 48 hours.The 100% mortality of *A.gossypii*was obtained during the application of oil by contact at doses of 0.1% for 24 hours for the larvae and 48 hours for the adults. Likewise, the mortality of 100% of *M. persicae* larvae is obtained at the same concentration for 48 hours.

During *Vitex agnus-castus* oil treatment, the mortality of 50% of aphids of the species *A. gossypii*was obtained at a dose of 0.1% which corresponds to the LD50 after 24 hours for contact treatment but for systemic treatment, the LD50 requires 48 hours. For the species *A. spiraecola* there was no effective dose to kill 50% of individuals and for the species M. persicae, the dose 0.1% was an effective dose for the mortality of 50% of aphids at 48 hours except the larva stage of systemic treatment gives an effective result

at 24h.The 100% mortality of *A.gossypii*was obtained during the application of oil by contact at doses of 0.1% for 48 hours for larvae and adults and the 100% mortality of adults of *M. persicae*was obtained at a dose of 0.1% for 48h. 100% mortality of *A. gossypii*was obtained when applying oil by contact at a dose of 0.1% for 48h for larvae and adults and 100% mortality for adults of *M. persicae*was obtained at a dose of 0.1% for 48 hours.

4. DISCUSSION

Responses of aphids to phytoenzyme and plant oils werespecific and characteristic. They were divided into four categories of responses (1) Responses animal stage dependent:(dependent, please check it) The responses of animal species vary depending on the stage of development of the animal. The components used in the treatment act strongly during the larval stage and weakly during the adult stage. On the other hand, other substances show effects contrary to the first (Table 1). The response of *A. spiraecola* and *A. gossypii* were studied by Moradi et al. (2020). The results of the studies have shown variations in responses depending on the age of the tested individuals. (2) Responses animal treatment method dependent : We noticed during our study a divergence in the responses of the same animal and during the same stage of development for two different methods of treatment, either the application of contact treatment or systemic treatment (Table 1). Previous studies have mentioned the importance of the choice of method of treatment during application of component (Syngenta, 2007 ; Sánchez-Bayo et al.,2013). (3) Responses animal component dependent : We noticed different responses for the same animal during the same stage of development following the application of two different types of vegetable oils and comparing them with the phytozyme (Table 1). Effective control of aphids requires that the product applied with the proper method and at the correct time. The "active ingredient" refers to the compound in the product that is toxic to the insect (Herms et al. 2019). (4) Responses animal dependent : The obtained results showed different responses when the same compound was used during the same stage of development and for the same method of application on two different animals (Table 1). These results are in agreement with other previously published work (Herms et al. 2019 ; Moradi et al. 2020).

Biological control can be divided into three very distinct categories: conventional, incremental and protective. The classic method aims to implant an exotic antagonist in an environment where an exotic pest is rife (Cloutier and Cloutier, 1992). However, in the case of biological control by increase, the aim is to control an indigenous pest by increasing the occurrence of its natural enemy (s), naturally present but in insufficient quantity (Cloutier and Cloutier, 1992) or to introduce repetition of an enemy who would not survive, for example, winter conditions (US Congress, Office of Technology Assessment, 1995).Protective control aims to increase the occurrence of natural enemies by changing the environment and cultural practices. It is undoubtedly the most important and readily available mode of biological control because it often requires little effort and the enemies are adapted to the target environment (Weeden et al., 2007; US Congress, Office of Technology Assessment, 1995).

We confirmed the hypotheses cited in the introduction of this work which consists in proving the interaction between the efficacy of the product used for biological control with 5 important parameters, namely the animal species, the stage of development, method of treatment type and applied dose of product.

5. CONCLUSION

As part of research to limit the spread of viruses in fruit tree orchards, methods of monitoring aphids that carry these viruses are essential. Especially since the potential vectors of the disease *A. gossypii*, *A. spiraecola* and *M. persicae* are the most abundant in our orchards with a high percentage.Serological screening for citrus and fruit tree certification viruses has clearly shown the efficiency of viral sanitation by in vitro technology since almost all of the plants tested resulting from apex micrografting have negative or healthy results. This reflects the undoubted importance of biotechnology in the elimination of incurable

diseases in loccurance viruses. The aphid control test with the phytozyme has given encouraging results but other tests must be carried out to verify the effectiveness of this product, determine its period of persistence and the effect of the intervention period which plays an important role in the success of biological control.The sensitivity of aphids, that is to say the mortality rate differs according to the stages (larvae or adults), the duration (between 24 and 48 hours), the product used (phytozyme, Plant oils), the product content used and the species of aphids (*A. gossypii*, *A. spiraecola*, *M. persicae*).The highest mortality rate defines the most effective product for controlling aphids and it was obtained that the phytozyme was the most effective than the two oils used because its application results in high mortality of *A. Spiraecola* and *M. persicae* in a very short time and acts fairly on the larval and adult stages, but for the species *A. gossypii* the three products are effective.

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