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Evaluation of the Impact of Five Bio-insecticides of Plant Origin and a Chemical Insecticide on the Survival of Imagos of the Parasitoid *Aphidius colemani* under Laboratory Conditions

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Authors' contributions

This work was carried out in collaboration among all authors. Authors FK and BN designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors GN and BEH managed the analyses of the study. Author ZR managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The present study aims to evaluate the control pests without the harms of chemical pesticides as well as ensure the safety of bio-insecticides of plant origin vis a vis females of the parasitoid *Aphidius colemani*, important auxiliary in biological control.
Study Design: Experimental device was in a complete random block with three replications.

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Place and Duration of Study: Laboratory of Bio-insecticidal Entomology and Laboratory of weed science of Regional Center of Agricultural Research, Kenitra, INRA-Morocco and Laboratory of Nutrition, Health and Environment. Biodiversity and agro resources team Ibn Tofail University, Faculty of Sciences Kenitra Morocco. The experiment was conducted between the fall of 2018 (from September to December 2018) and the winter of 2019 (January-February 2019).
Methodology: To meet the objective of this work, four endemic plants were collected from the Gharb region of Morocco; these are the leaves of *Nerium oleander* that are available all year, the roots of *Mandragora autumnalis*, the fruits of *Capsicum frutescens*, and those of *Melia azedarach* these organs are well developed with complete maturity during the fall and winter. The fruits of *Melia azedarach* have been separated into seeds and pulps. Five concentrations of each aqueous extract were prepared (2; 5; 10; 15 and 20 g / 20 ml). In addition to control without any treatment (blanc) and a chemical insecticide (deltamethrin). All concentrations were administered by contact to females of *Aphidius colemani*.
Results: Comparing the bio-insecticides tested, the aqueous extract of the seed of *Melia azedarach* showed an insecticidal action that was the fastest and the most toxic. Thus, following exposure of 20 g/20 ml, the observation after 4 hours showed a minimum mortality rate of 3% and a maximum mortality rate of 50%, 96 hours after. This extract was followed by the aqueous extract of the leaves of *Nerium oleander* that showed a maximum mortality rate of 43%. However, their harmfulness remains much lower than that of deltamethrin. In addition, the aqueous extract of the roots of *Mandragora autumnalis* has proved to be safe for females of *Aphidius colemani*. On the other hand, the aqueous extract of the fruits of *Capsicum frutescens* and that of the pulp of *Melia azedarach* are moderately toxic.
Conclusion: By way of conclusion, the aqueous extract of the roots of *Mandragora autumnalis* could be used as an insecticide in parallel with the release of the parasitoid *Aphidius colemani*, in a biological control.

Keywords: Bio-insecticides; Aphidius colemani; plant extracts; Mandragora autumnalis; Capsicum frutescens; Nerium oleander; biological control.

1. INTRODUCTION

Considered a few years ago as a niche market, organic farming has continued to develop and today appears as a booming sector that is attracting more and more consumers. Indeed, the world area cultivated according to the organic mode was estimated at nearly 58 million hectares at the end of 2016. The global organic market is estimated at 87.3 billion US dollars (80.2 billion Euros) in 2016 with a census of more than 2.4 million organic certified farms in 2016 [1,2].

In Morocco, although the areas converted to organic farming remain low, this mode of production has developed in recent years. Policies supporting conversion, the organization of the sector and the proliferation of pesticides problems have made a significant contribution. Indeed, since 2011, the Moroccan government has affirmed its desire to boost organic farming, by signing, as part of the “Green Moroccan Planning”, a program to increase the cultivated area from 4,000 to 40,000 hectares (ha). However, as the deadline approaches, less than a quarter of this area is about 9,500 ha [3]. Also, it has been established as a law that meets the requirements of professionals in the organic farming sector in Morocco [4].

According to the Moroccan Association of the organic production sector created in 2010, organic farming concerned 800 000 ha in 2014, of which a majority of certified harvesting areas, more than 8000 hectares of crops and 1841 ha in conversion [5]. In terms of production, market gardening, citrus fruits and aromatic and medicinal plants account for more than 63% of total production, with respectively 21.680, 15.200 and 10.116 tonnes [3].

The Moroccan government has set a program contract for 2011-2020 for organic farming plans investments to the tune of 1.121 billion dirhams of which 286 million provided by the state. In 2020, it forecasts a 10-fold increase in production compared to 2009/10 to reach 400 000 tonnes, an increase in exports (60 000 tonnes in 2020, of which 80% is fresh, guaranteeing foreign exchange earnings equivalent to 800 Millions of Dirhams In terms of jobs, it is about creating around 9 million working days by 2020 against 1 million working days in 2012, the equivalent of 35,000 permanent jobs [6].

Based on the non-use of synthetic chemicals in the production process, organic farming involves a significant change in farming practices on the part of producers but also a reorientation of the methods of control and phytosanitary protection of crops. Among the most used methods in organic farming in Morocco, the parasitic hymenoptera of aphids especially *Aphidius colemani*. In biological control, the release of *Aphidius colemani* against aphids and bio-insecticides of plant origin could be controlled against another pest. In order to ensure the safety of the bio-insecticide found to be effective in the laboratory against the Mediterranean fruit fly, tests were carried out under laboratory conditions, the methodology and results are presented in this article.

2. MATERIALS AND METHODS

2.1 Entomological Equipment

The species *Aphidius colemani* used is marketed in the form of mummies. The target stage is the adult. Under laboratory conditions (T= 26ºC, HR= 75%), these emerging imagos of mummies inside round boxes 5 cm in diameter and 1.5cm in height. Inside each box, two pieces of cotton are deposited: one saturated with a solution of sucrose with a concentration of 500 g/l (food source) and another soaked with water (water and humidity source).

2.2 Bio-insecticide Tested

The selected aqueous extracts of plant origin are those that have proved highly effective on the various stages of the *Ceratitis capitata* in our laboratory conditions. These are roots of *Mandragora autumnalis*, fruits of *Capsicum frutescens*, leaves of *Nerium oleander*, and seeds and pulps of *Melia azedarach*. These plants were identified by weed science laboratory of the Regional Center of Agronomic Research of Kenitra, INRA-Morocco, and Laboratory of Nutrition, Health and Environment. Biodiversity and agro resources team. Ibn Tofail University, Faculty of Sciences Kenitra, Morocco.

The crude extracts (100 grams of dried powder in 0.5 liter distilled water) of these plants are diluted in distilled water in five concentrations: 2; 5; 10; 15; 20 g/20 ml. The positive control used is a Deltamethrin neurotoxin belonging to the pyrethroid family. The concentrations tested are the recommended concentration 30 cc/hl; its half ie 15 cc/hl and its double ie 60 cc/hl. The negative control consists of distilled water.

2.3 Mode of Administration of the Products

Each batch of 50 females was sprayed with 5 ml of each concentration.

2.4 Observation and Monitoring of Tests

Observations are made after each hour for the first 4 hours. After this period, the monitoring is daily for different concentrations of the products tested; the immobile useful insects are considered dead.

The observations are made in comparison with the untreated and those exposed to deltamethrin.

2.5 Experimental Design

The tests are conducted according to complete randomized design, two factors: Product, concentration. Three repetitions have been adopted.

To detect the effect of different concentrations, different aqueous extracts tested on adults of *Aphidius colemani*; an experimental unit consisting of 50 newly emerged females was adopted; they were placed in a rounded box 5.5 cm in diameter. They received the same food mentioned above. Each group of ten experimental units was treated with the same concentration.

The manipulations, experiments and observations are made in the day's light.

2.6 Data Processing and Statistical Analysis

2.6.1 Transformation of raw mortalities

Gross mortalities are corrected according to Abbott's formula [7]. This correction makes it possible to exclude the bias due to the natural mortality observed under the experimental conditions. Abbott's formula is expressed as:

Percent corrected mortality = (T-C) / (100-C) x 100

With:

- T: Percentage of deaths in the treated lot;
- C: Percentage of deaths in the untreated batch.

2.6.2 Comparison of treatments

To highlight any significant differences between the effects of different bio-insecticides on the imago, two-way analysis of variance was adopted. The Dunnett test was performed to compare the different treatments with the controls. Also, the Student-Newman-Keuls test was used to group all treatments that did not differ statistically. All tests are performed with a significance level of 5%. Statistical analyses are performed on raw data transformed into Arcsin (square root) using SPSS VERSION 17.

2.6.3 Calculation of toxicity parameters

The dose needed to kill half of an experimental lot (LC_{50}) is calculated by the EPA Probit Analysis Program Version 1.5. It allows modeling the effect of several increasing concentrations of an insecticide molecule on the mortality rate of insect batches (Concentration-effect relationship). For each product, the LC_{50} has been calculated by this software.

3. RESULTS AND DISCUSSION

3.1 Evaluation of the Effect of the Aqueous Root Extract of *Mandragora autumnalis* on the Survival of Adults of *Aphidius colemani*

Analysis of the variance indicates no significant effect of both factors: Concentration and duration of exposure (P> 0.05). The Dunnett test reflects the absence of a significant difference between the five concentrations applied and the controls. Indeed, throughout the experiment, no mortality was recorded in the batches exposed to the different concentrations of the aqueous extract tested compared to the controls.

3.2 Effect of the Aqueous Extract of *Capsicum frutescens* on the Adult Survival of *Aphidius colemani*

The results of the statistical analyses show that the responses of *Aphidius colemani* varied according to the concentration applied and the exposure time. The Dunnett test indicates that with the exception of lots treated with 2 g/20 ml concentration that did not differ significantly from the controls, the mortality rates in the lots treated with the different concentrations were significantly different from the untreated ones (controls). The Student-Newman-Keuls test showed that the mortalities registered four hours after treatments differ significantly from those recorded on the first day and the last three days.

According to Fig. 1, the harmful effect of the aqueous extract of *Capsicum frutescens* on the target is marked after 24 hours of exposure in the batches treated with concentrations greater than or equal to 5 g/20 ml, with a maximum mortality rate of about 17%, caused by the concentration 20 g/20 ml. Over time, this rate increased gradually to reach after 96 hours of exposure: 15; 20; 23; 27 and 33% for adults exposed to concentrations: 2; 5; 10; 15 and 20 g/20 ml. Moreover, after the same duration no mortality was recorded in the untreated lots.

The results found indicate that above 5 g/20 ml, the effect of the aqueous extract of *Capsicum frutescens* fruits on the target parasitoid is a slight effect that is observed from the first day of treatment. The maximum value remains below 35% mortality and according to the standards of the Institute of Organic and Biochemical Chemistry, the products causing a helper mortality rate of around 35% are considered moderately toxic. Indeed, hot pepper (*Capsicum frutescens*) macerations are used as a non-harmful insecticide in a wide range of crops [8]. The safety of the aqueous extract of hot pepper on different species of parasitoids has been noted by several authors. Thus, Rebek and Sadof showed that the different *Capsicum frutescens* extracts have no effect on the parasitoid *Encarsia citrine* [9]. Similarly, the alkaloids extracted from hot pepper fruits showed no significant effect on the parasitoid *Encarsia trans*ient regardless of the duration and concentration tested [10].

3.3 Evaluation of the Effect of the Aqueous Leaf Extracts of the *Nerium oleander* on the Adults of *Aphidius colemani*

Analysis of the variance reveals a highly significant effect of both factors: Concentration (P <0.001) and duration of exposure (P <0.001). Fig. 2 indicates that after four hours of treatment, a low mortality rate of about 3 and 7% was recorded in the sprayed lots by the concentrations 15 and 20 g/20 ml of the aqueous extract of *Nerium oleander*. This insecticidal activity was very marked by the time of exposure and the concentration applied. Thus, after three days of exposure, the maximum mortality rate was close to 43; 41; 33; 23 and 13% in the lots exposed respectively to: 20; 15; 10; 5; 2 g/20 ml.

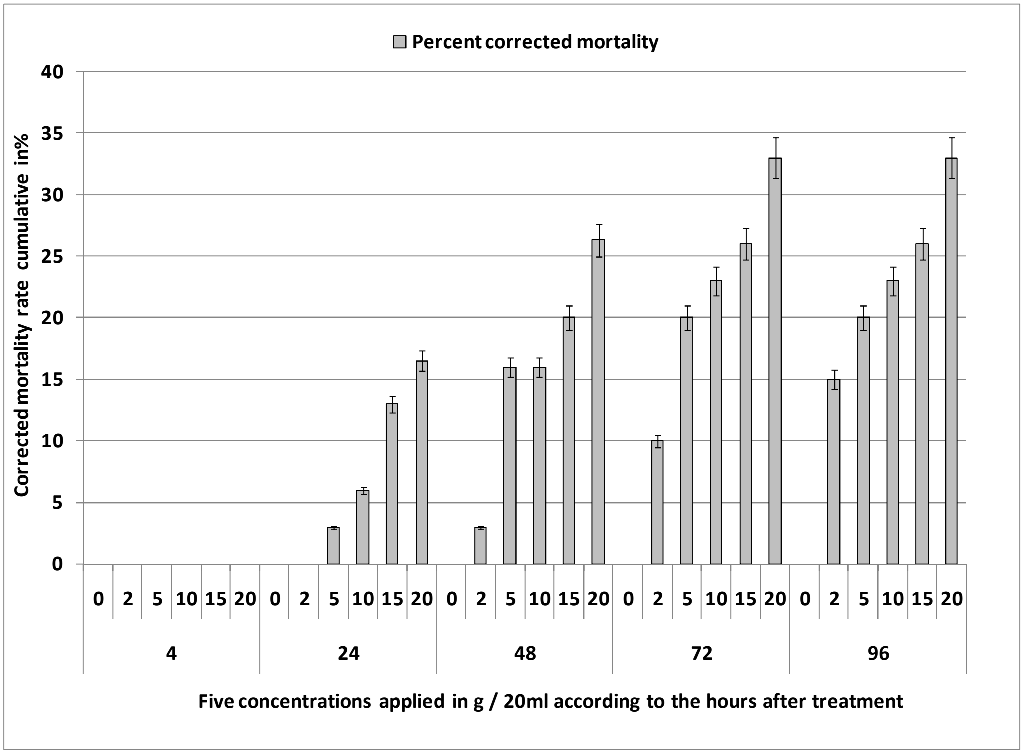

Fig. 1. ***Aphidius colemani*** **adult mortality chronology exposed to the five concentrations of** ***Capsicum frutescens*** **fruit extract**

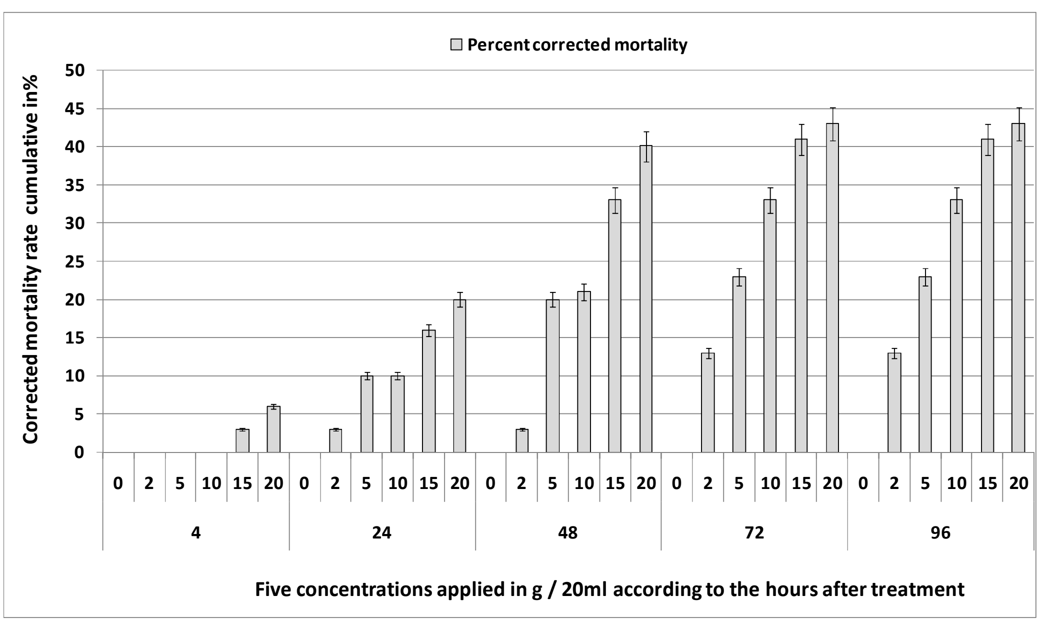

Fig. 2. Mortality chronology of newly emergent ***Aphidius colemani*** **adults exposed by exposure to the five concentrations of the aqueous extract of** ***Nerium oleander***

The rapidity of action of the *Nerium oleander* on the parasitoid's target imagos, described as a bio-insecticide of shock; the maximum mortality has exceeded 35%, it is a moderately toxic insecticide vis a vis auxiliaries. The mechanisms responsible for the toxicity of the *Nerium oleander* leaves are superimposed on those of the classical glycosides acting mainly on the inhibition of ATPase, membrane Na-K and by the elevation of the intracellular calcium [10].

3.4 Effect of the Aqueous Extract of the Fruit Pulp of *Melia azedarach* on the Imagos of *Aphidius colemani*

As with the previous extract, the analysis of variance reflects a highly significant effect of both factors: Concentration (P <0.001) and duration of exposure (P <0.001). In terms of concentration, the Student-Newman-Keuls test makes it possible to distinguish three homogeneous groups: the first group consists of the concentrations: 2 and 0 g/20 ml, the second is represented by the concentrations 10 and 5 g/20 ml and the third formed by the concentration 15 and 20 g/20 ml. The same test classifies the exposure times into three homogeneous groups: The first group exposure times 4 and 24 hours, the second represents 48 hours after treatment and the third groups the durations 72 and 96 hours of exposure.

Fig. 3 shows that the effect of the aqueous extract of *Melia azedarach* pulps on adults of *Aphidius colemani* was detectable after 24 hours of exposure for concentrations greater than or equal to 5 g / 20 ml, with corrected mortality rates very low estimated at: 1; 1.5; 3 and 3.7%, respectively in the lots treated with the concentrations: 5; 10; 15 and 20 g/20 ml. After four days of exposure the cumulative corrected mortality rates were approximate: 6; 8; 21 and 35%, successively in the batches treated with the concentrations: 5; 10; 15 and 20 g/20 ml.

3.5 Effect of the Aqueous Extract of the Seeds of *Melia azedarach* Fruits on the Imagos of *Aphidius. colemani*

The variance analysis showed a highly significant effect of both the concentration factor (P <0.001) and the duration after treatment (P <0.001). The Student-Newman-Keuls test indicates the presence of three groups of homogeneous exposure time: The first represented by the duration 4 hours, the second contains the durations 24 and 48 hours and the third groups the 72 and 96 hours after treatment.

The effect of this extract was rapid, observed just after 4 hours of exposure with very low corrected mortality rates, which increased gradually during the experiment and according to the concentration tested. Thus, after four days of exposure, the highest mortality rate was approximately 50% caused by the 20 g/20 ml concentration (Fig. 4).

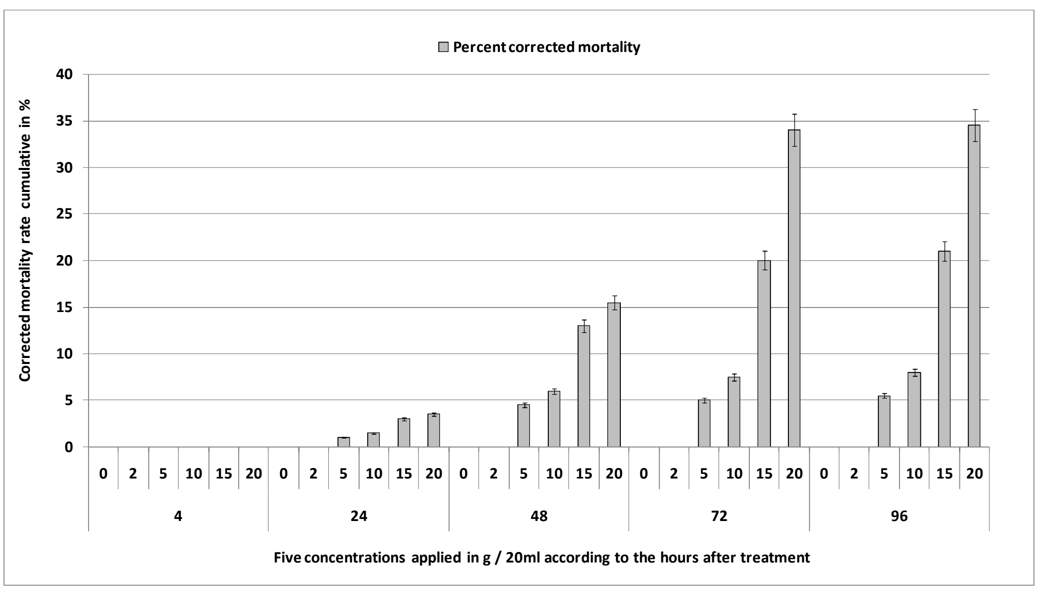

Fig. 3. Adult mortality chronology of *Aphidius colemani* following treatment with the five concentrations of the aqueous extract of *Melia azedarach* pulp

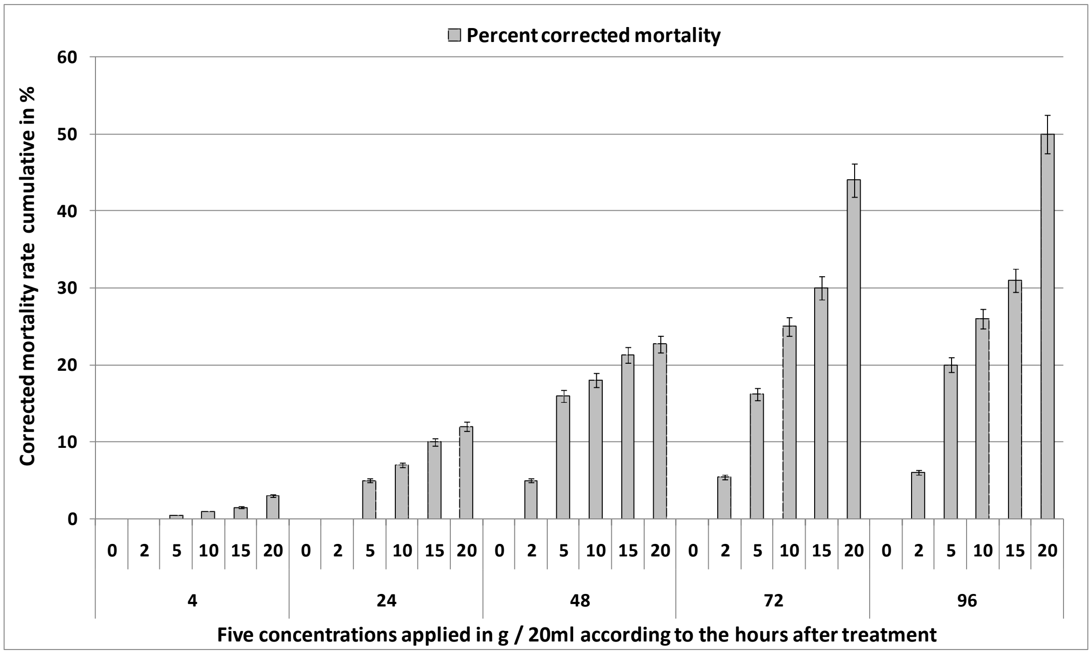

Fig. 4. Mortality chronology of newly emergent *Aphidius colemani* adults exposed by exposure to the five concentrations of the aqueous extract of *Melia azedarach* seeds

From the results indicated above, it is clear that both extracts having an insecticidal effect on the parasitoid, however, the extract from the seed has an action both faster and more toxic. Indeed, the aqueous extract of the pulps is observed only after one day of exposure; however, four hours after treatment was sufficient to notice the insecticidal impact of the aqueous extract of *Melia azedarach* seeds. Similarly, in the case of the aqueous extract of *Melia azedarach* pulp, the mortality rate did not exceed 35% which classifies it in the group of non-toxic bio-insecticides contrary to the aqueous extract of the seeds which caused 50% mortality is a bio-insecticide moderately toxic to *Aphidius colemani*.

These results could be explained by the fact that the biochemical composition of the pulp and seed of the fruit of *Melia azedarach* is different. The use of bio-insecticides based on *Melia azedarach* fruit extract without separation between seed and pulp at very low concentrations showed no adverse effect on the parasitoid *Encarsia* [11]. In addition, other species of plants of the same family Meliaceae especially *Azadirachta indica* whose fruits are rich in azadirachtin, are used with caution with moderate dosages given their danger to the brood [12].

3.6 Evaluation of the Effect of the Active Ingredient Deltamethrin on the Imagos of Parasitoid *Aphidius colemani*

Variance analysis reveals the presence of a very highly significant effect, both of the concentration factor (P <0, 0001) and the duration of exposure factor (P <0.0001).

For all durations of observation, the Student test reveals a significant difference between the mortality rates recorded in the different batches treated with the three concentrations of deltamethrin. The Dunnett test reveals a significant difference between the mortality rates of the three test concentrations and the mortality of untreated controls.

Fig. 5 shows that over time the mortality rate increases linearly in all experimental units treated. Thus, after four hours after treatment, deltamethrin caused 12; 15 and 38% mortality, to reach 50; 76; and 92% recorded on the fourth day of contact.

These recorded mortality rates reflect the threat of deltamethrin to parasitoid imagos of *Aphidius colemani*. Indeed, this active ingredient is among the most effective insecticides against a large number of pests, but it is harmful to the fauna useful in general. Its use causes a faunal imbalance in the treated agro-biotopes. In France, its use is only authorized as a bait for trapping *Ceratitis capitata* in organic crops [13]. The effects of deltamethrin are very toxic to the parasitoid *Diaretiella rapae* 24 hours after treatment [14]. Pest control with deltamethrin may be questioned due to its poor toxicity / ecotoxicity score. The substitution of this substance with type I (imiprothrin allethrin) or type II (including cycloprothrin) pyrethroids or with silafluofen, whose scores are less penalizing, is an option to maintain this family in control strategies [15]. Among the alternative routes of this family, the search for new natural formulas can be; among others, insecticidal plants. The latter must have a competitive efficiency to that of deltamethrin with a high lethal concentration (non-toxic).

3.7 Toxicity Parameters of the Products Tested: LC_{50} Lethal Concentration

This parameter makes it possible to classify the harmfulness of the aqueous extracts tested against the adults of *Aphidius colemani*. From Table 1, the aqueous extract of the seeds of *Melia azedarach* was the most toxic, followed by that from the leaves of *Nerium oleander*. In addition, the aqueous extract of *Melia azedarach* pulp and that of *Capsicum frutescens* fruit were the least toxic. The potential lethal concentration of the aqueous root extract of *Mandragora autumnalis* could not be calculated due to the lack of mortality caused by this extract, so it is a non-harmful extract for adults of *Aphidius colemani*.

The high toxicity of *Melia azedarach* seeds may be due to its high content of active chemical components, on the one hand, and the high solubility of its constituents in water, on the other hand.

Previous research has shown that aqueous extracts of *Azadirachta indica* seeds are not harmful to warm-blooded animals and bees but with very low concentrations; therefore their application must respect the same rules of caution as other insecticides used in biological control [8]. The aqueous extract of the roots of the mandrake is the least toxic the value too high of the LC_{50} could not be determined by the software used. It is a very effective insecticide on *Ceratitis capitata*, and not harmful, so it could substitute synthetic ceraticides in a biological control program.

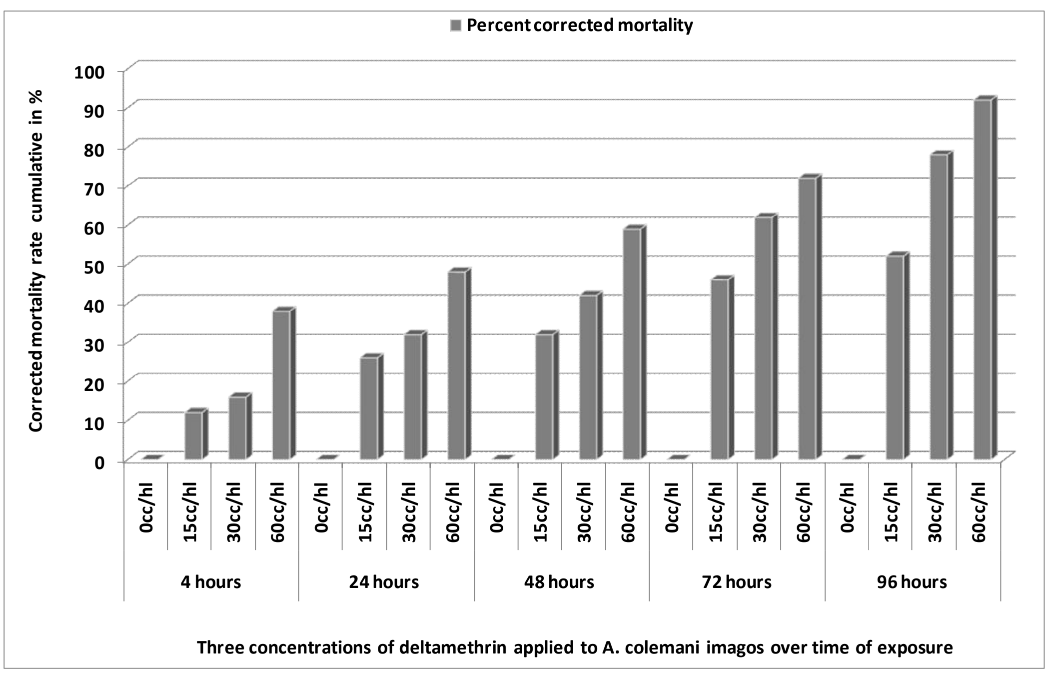

Fig. 5. Effect of different concentrations of deltamethrin on adults of *Aphidius colemani* compared to controls during the four days of treatment

Table 1. Lethal concentrations (LC_{50}) of aqueous extracts tested on the imagos of *Aphidius colemani*

Aqueous extract of	Plant part	CL_{50} (g/20 ml)	Confidence interval(g/20 ml)
Mandragora autumnalis	Roots	-----	-----
Melia azedarach	Pulp	25.211 (g/20 ml)	[20,011-30,230]
Capsicum frutescens	Fruits	30.645 (g/20 ml)	[27,341-38,379]
Melia azederach	Seeds	20.012 (g/20 ml)	[12,170-28,013]
Nerium oleander	Leaves	23.395 (g/20 ml)	[18,231-29,011]
déltaméthrine		15.506 (cc/hl)	-----

4. CONCLUSION

In light of these results, it is clear that the use of aqueous extracts from *Melia azedarach* seeds and *Nerium oleander* leaves is incompatible with the use of the parasitoid of *Aphidius colemani* in biological control. On the other hand, the effectiveness of the aqueous extract of the roots of the *Mandragora autumnalis* as an insecticide against the Mediterranean fly (*Ceratitis capitata*) and its safety against the females of *Aphidius colemani* places it as the bio-insecticide of vegetal origin the more compatible with biological control techniques in an organic farming program.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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