Evaluation of the Bio-insecticidal Effects of Three Extracts on the Larvae of the Green Lacewing *Chrysoperla carnea* (Stephen) under the 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 BH managed the analyses of the study. Author ZR managed the literature searches.
All authors read and approved the final manuscript.*

| Original Research Article |

ABSTRACT

Aims: To control aphids without the misdeeds of the chemical insecticides. To make sure of the
harmlessness of three bio-aphicides.
Study Design: Experimental device in complete random block with three replications.
Place and Duration of Study: Laboratory of bio-insecticidal entomology, Regional Center of
Agricultural Research of Kenitra, INRA- Morocco and the Biodiversity and Natural Resources

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Laboratory of Sciences Faculty, Ibn Tofail kenitra, morocco. The experiments were done between March and December 2017.

Methodology: The present study highlighted the lethal and sublethal effects of three bio-aphicides plants collected from the Gharb region of morocco: *Capsicum frutescens*, *Nerium oleander* and *Melia azedarach*, tested in five concentrations of their plant material against *Chrysoperla carnea* larvaes. Adding to these concentrations, two controls were used: one without any treatment (blanc) and another treated with a chemical insecticide (Deltamethrine).

Results: The obtained results indicated that the aqueous extract of the *Nerium oleander* was harmful to the larvae of the *Chrysoperla carnea*. Indeed, the insecticidal activity of different concentrations increased with the exposure time, reaching about 10; 16 and 20% of mortality, respectively by applying 10; 15 and 20 g/20 ml after three days of exposure. However, low concentrations of 2 and 5 g/20 ml caused no effect whatever the duration of exposure. Also, the corrected mortality rate caused by the aqueous extract of *Nerium oleander*, at the concentrations tested is much lower than that caused by the insecticide Deltamethrin.

Conclusion: *Nerium oleander* plant is harmful to the larvae of the *Chrysoperla carnea* at concentrations of 10; 16 and 20%. At these concentrations, the aqueous extracts of hot pepper and melia are not haurmful to the larvae of the green lacewing, so they can be recommended as a biological insecticide. Moreover, other works on these two extracts are outstanding in our laboratory.

1. INTRODUCTION

The citrus sector in Morocco is one of the pillars of the national economy with an area exceeding 118 000 ha [1] with an average production of 22 27 000 tonnes [2]. It is a major source of foreign exchange, exporting approximately 585 000 tonnes annually [3]. Despite this leading position in this sector, it still faces a number of obstacles that hinder its quantitative and qualitative improvement. Among which pest pressure and phytosanitary pest management occupies a place of choice.

Since the discovery of an outbreak of *Tresteza* disease in the citrus orchard of the Larache region (Morocco) [4], the potential aphids vectors of this disease constitute a serious threat to Moroccan citrus [4]. These species include: *Toxoptera aurantii, Aphis spiraecola, Aphis gossypii* the most abundant in Moroccan orchards [5]. To address the spread of viral disease nationally, industry stakeholders have adopted several means of protection; among which is the fight against vectors. In addition, in a citrus orchard, Moroccan citrus growers often resort to chemical control with insecticides harmful to useful wildlife, including predators and parasitoids of aphids [6,7,8]. In this context, a selection of effective bio-aphicides has been highlighted by our laboratory [8,9]. To ensure the safety of bio-aphicides deemed effective, a series of experiments on plant material and useful wildlife was conducted. Among the most active and abundant auxiliaries in citrus orchards, the predator *Chrysoperla carnea* (stephans, 1836) [4,10]. The methodology and results for biological bio-aphicide tests on larvae of this beneficial species are presented in the following article.

2. MATERIALS AND METHODS

2.1 Entomological Equipment

First-instar larvae of *Chrysoperla carnea* from eggs collected from citrus orchards in the Gharb region are used for laboratory testing. Leaves with chrysop eggs are brought back to the laboratory in moist boxes. They are kept under the ambient conditions (T= 26ºC; RH = 75%) of the laboratory inside the aerated cans of soaked cotton (source of water and moisture). After hatching, the first instar larvae are placed individually in Petri dishes (15 x 3 cm); each containing a piece of cotton soaked in water and several aphids of *Toxoptera aurantii* (source of food).

2.2 Bio-Aphicides Tested

Three bio-aphicides plants collected from the Gharb region of morocco: *Capsicum frutescens*, *Nerium oleander* and *Melia azedarach*. These plants were identified by the weed science laboratory of Regional Center of Agronomical

Research of Kenitra, INRA-morocco and Biodiversity and Natural Resources Laboratory of Sciences Faculty of Kenitra-Morocco.

The plant extracts tested on this auxiliary are bio-aphicides having proven high efficiency. It is the aqueous extract from: fruits of *Capsicum frutescens*, leaves of *Nerium oleander* and pulp of *Melia azedarach*. The crude extracts (100 gramme of dried powder in 0.5 litter 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

The different concentrations are administered by contact. They are applied directly and locally on the back of first instar larvae.

2.4 Observation and Monitoring of Tests

Observations are made after each hour for the first 8 hours. After this period, the monitoring is daily for four days of treatment of the larvae by the different concentrations of the products tested; the immobile useful insects are considered dead. After four days, the dead larvae are removed from the experimental boxes and the survivors remain under observation until the larvae in the distilled water-treated lots die.

2.5 Experimental Device

The tests were conducted according to a complete randomized design, two factors: product, concentration. Three repetitions have been adopted. The larvae are individually distributed in square-shaped plastic Petri dishes 15 cm wide and 3 cm deep each ten boxes receive the same concentration. The manipulations, experiments and observations are made in the light of day.

2.6 Data Processing and Statistical Analysis

2.6.1 Transformation of raw mortalities

Gross mortalities are corrected according to Abbott's formula [11]. 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-aphicides on the target, two-way analysis of variance was adopted. The Dunett 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 analyzes 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 (LD_{50}) is calculated by the EPA Probit Analysis Program Version 1.5. It allows to model the effect of several increasing concentrations of an insecticide molecule on the mortality rate of insect batches (dose-effect relationship). For each product the LD_{50} has been calculated by this software.

2.6.4 Evaluation of side effects

The calculation of lethal doses (LD) allows classifying the effectiveness of active ingredients, without giving information on the impacts and the potential harms of the products after the expiration of the duration of experimentation. Thus, the use of the LD50 as a primary criterion for determining the level of toxicity of a plant protection product to insects appears to be insufficient, should be supplemented by studies of the sublethal effects of products on physiological aspects (such as emergence of eggs, development time, periods) and / or that behaviour (such as locomotion, prey seeking, prey consumption, oviposition). Among the effects studied at the laboratory level of bio-insecticides / entomology, the longevity of surviving larvae after the duration of

experimentation and predation, compared with those untreated.

3. RESULTS AND DISCUSSION

3.1 Evaluation of the Lethal Effects of the Three Aqueous Extracts on *Chrysoperla carnea* Larvae

The overall statistical analysis of the effect of the three bio-aphicides on the survival of *C. carena* larvae shows that only the aqueous extract of the oleander has a significant effect. On the other hand, the two other extracts induced no mortality whatever the concentration applied or the exposure time. Indeed, several previous studies have confirmed the safety of fruit extracts of *Capsicum frutescens*. In 2003, studies showed that capsaicin had no effect on the auxiliary *Encarsia citrina* [12]. When the effect of the aqueous extract of *Capsicum frutescens* on the larvae of *Chrysoperla carnea* our results are similar to those of Bouchelta [13], which showed that, whatever the duration of exposure, the alkaloids extracted from the fruit of *Capsicum frutescens* used at concentrations equal to or less than 20 g/20 ml do not have a negative effect on first instar *Chrysoperla carnea* larvae. The harmless result of the two aqueous extracts is similar to that of *Lantana camara* and *Schimus* soft extracts at a concentration of 10%. In fact, the mortality of first instar larvae of *Chrysoperla externa* exposed to this concentration of each of the two extracts from these two plants and after 24 hours, 8 and 21 days are identical to those of controls without treatments [14].

The comparison of the average shows the absence of a significant difference between the effect of deltamethrin and that of the aqueous extract of the oleander; when it indicated a highly significant difference between this extract and the negative control. In addition, no significant difference was observed between the aqueous extract of hot pepper, that of melia and the Deltamethrin.

Concerning the analysis of the variance of the effect of the aqueous extract of the oleander on the target larvae, it reveals the presence of very highly significant effect, both of the concentration and the duration of exposure (P <0.0001). The Student-Newman-Keuls test grouped the concentrations into three homogeneous groups: the first encompasses 0; 2 and 5 g/20 ml, the second contains 10 and 15 g/20 ml and the third group contain the concentrations 15 and 20 g/20 ml.

Fig. 1 confirms the results of the statistical analyzes. Thus, the activity of the aqueous extract of *Nerium oleander* on first instar *Chrysoperla carnea* larvae is observed only after one day of exposure. Concentrations 2 and 5g/20 ml caused no effect whatever the duration of exposure. In addition, the insecticidal activity of the other three concentrations increased with the exposure time, reaching about 10; 16 and 20% respectively by applying 10; 15 and 20 g/20 ml after three days of exposure. Compared with water-only lots, these three concentrations have an insecticidal effect. These results are similar to those found by Leatemia and Murray [15], by applying the organic extracts of *Annona squamosa* seeds at concentrations of 30% and after two days of exposure of neonate larvae of *Chrysoperla carnea*, the corrected mortality is 22%. Fig. 1 also shows that the corrected mortality rate caused by the aqueous extract of oleander is much lower than that caused by Deltamethrin. This achievement highlights the mild effect of the aqueous extract of oleander on larvae of the first stage of the green lacewing. In contrast, previous studies have shown that a diflubenzuron-based synthetic insecticide causes 100% complete and rapid death of third-instar larvae of the same species studied [16]. The effects of Deltamethrin are detailed in the following section.

3.2 The Effects of Deltamethrin on the Neonate Larvae of the Green Lacewing

For the positive control (Deltamethrin), the analysis of the variance shows the very highly significant effect of both the applied concentration and the duration of exposure (P <0, 0001). The Student-Newman-Keuls test reveals the presence of three temporary groups, the first encompasses 48, 72 and 92 hours, the second contains 24 hours and the third consists of 4 hours after treatment, while each concentration constitutes a group to share.

Table 1 confirms the deleterious effect of Deltametrine on first-instar larvae of the lacewing. Indeed, the influence of the two concentrations 30 and 60 cc/hl is marked just after four hours of exposure. After this time, the corrected mortality rate gradually increased with the applied concentration and duration of exposure, reaching 33; 47 and 53% respectively with 15, 30 and 60 cc/hl after four days of treatment.
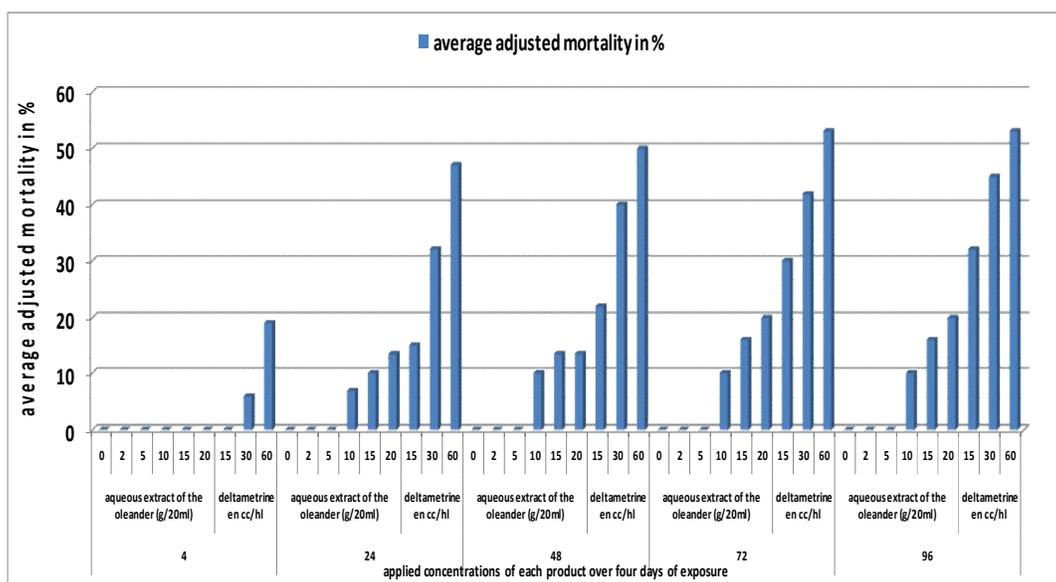
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Fig. 1. Evaluation of corrected mortality of the *C. carenae* larval after four days of exposure to different concentrations

Table 1. Summary of the LD$_{50}$ of the products tested on the larvae of the lacewing after 96 hours of exposure

Product	Right equation	LD$_{50}$
Capsicum frutescens	----------	---------- (g/20 ml)
Melia azedarach	----------	---------- (g/20 ml)
N. oleander	Y=2.324+1.114X	32.395 (g/20ml)
Deltamétrine	Y=5.127+1.691X	6.75 (cc/hl)

Table 2. Periods in days of *C. carena* larvae treated with different products

Product	Concentration (g/20 ml)	Minimum	Maximum	Mean ± SD		Coefficient of variation (%)
Control	0	8	11.38	9.69[*]±0.47		4.85
Capsicum frutescens	2	7	12.34	9.67[b*]±0.48		4.96
	5	6	13.00	9,50[b*]±0.65		6.84
	10	4	14.34	9.17[ab*]±0.74		8.07
	15	3	15.22	9.11[ab]±0.78		8.56
	20	1	16.28	8.64[a]±0.93		10.76
Melia azedarach	2	5	14.16	9.58[a*]±0.50		5.22
	5	6	13.00	9.50[a*]±0.74		7.79
	10	5	13.66	9.33[a*]±0.76		8.14
	15	3	15.22	9.11[a*]±0.98		10.75
	20	2	16.00	9.00[a]±0.68		7.55
N. oleander	2	5	13.66	9.33[c*]±0.89		9.54
	5	5	13.56	9.28[bc*]±1.06		11.42
	10	1	15.84	8.42[ab]±0.87		10.33
	15	1	14.44	7.72[ab]±1.06		13.73
	20	1	13.88	7.44[a]±1.11		14.91
Déltamethrine	15 cc/hl	2	9.78	5.89[c]	2.14	36.33
	30 cc/hl	1	7.94	4.47[b]	2.71	60.62
	60 cc/hl	0	7.26	3.63[a]	2.57	70.80

The rapidity of action of Deltamethrin on target larvae could be explained by the fact that this active ingredient is a pyrethroid; whose mode of action is the dysfunction of the sodium channels, it acts on both the central and peripheral nervous system; therefore, rapid paralyzing of targets. Also, Deltamethrin causes neurotoxic and neurohormonal effects added, certainly lead to ionic imbalances likely to modify the activities of membrane ATPases which eventually lead to rapid death [17]. Adding that, a study shows that spraying a Deltamethrin solution on caged bees has rapid lethal effects at a rate of 11.2 grams per hectare [18]. In addition, according to the collection of unintended effects of plant protection products written by the group "Side effects"; Deltamethrin weakens many auxiliary species, some of which regulate pest populations. In particular, Deltamethrin is toxic or highly toxic to pollinating insects and beneficial predators. It could be the cause of pest outbreaks, especially aphids [19,20].

3.3 The Degree of Toxicity of the Insecticides Used (LD$_{50}$)

Table 1 confirms the safety of the two aqueous extracts previously mentioned, and indicates that the toxicity of the aqueous extract of the oleander is much lower than that of Deltametrine. Several studies have shown that there are different phases of action of pyrethroids on insects, first there is a phase of intense excitation followed by a general paralysis (Knock-Down effect or KD). The insect can subsequently recover its motor skills or die depending on the dose used. In contrast, the toxicity of Deltamethrin to mammals is low. The Deltamethrin molecule is degraded rapidly even though it can sometimes be stored in fat and thus be eliminated more slowly [17].

3.4 Evaluation of the Side Effects of the Different Concentrations of the Three Aqueous Extracts Tested on *C. carena* 1st Larval Instar

Several factors are observed to evaluate the side effects of the three extracts tested; among the factors followed was the longevity of surviving treated larvae after four days of exposure.

Statistical tests indicate that for the same product applied there is a significant individual variability. Thus, the coefficients of variability in the batches treated with the aqueous extract of the *Capsicum frutescens* vary between 4.82 and 10.77% they are between 5.22 and 10.77% for the longevity of the surviving larvae after application of the aqueous extract *Melia azedarach*, the coefficient of variability in the lots exposed to treatment with the aqueous extract of *Nerium oleander* ranged from 9.58 to 14.87%. Similarly, the statistical comparison using the Student's test at the 5% threshold indicates that for the same concentration the period varies significantly depending on the product.

The shortest periods of the surviving larvae after treatment are those exposed to deltamethrin, however, the larvae exposed to the aqueous extract of hot pepper fruits at a concentration of 2 g/20 ml were able to survive the longest duration after treatment (approximately ten days). The S-N-K test shows no significant difference between the periods of surviving individuals after exposure to the three aqueous extracts, although *Nerium oleander* caused more mortality. The same test indicates that the higher the concentration the longevity of the survivors is shorter.

For the same product, the means affected by the same lowercase letter do not differ statistically from each other (Student's test at 5%), the averages followed by a star (*) do not differ statistically from the control.

4. CONCLUSIONS AND RECOMMENDA-TIONS

In light of these results, one might conclude that the aqueous extract of the oleander is slightly toxic. On the other hand, the two bio-aphicides from chili peppers and melia are not harmful to first-instar larvae of the green lacewing, regardless of the concentration applied. To be used in combination with biological control by first-instar larvae of the lacewing in an integrated citrus fruit orchard control program. In addition, additional tests should be carried out to evaluate their activities on the different stages of development of this entomophage and possibly the duration of persistence in the wild.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://sdiarticle4.com/review-history/51834