International Journal of Science and Research (IJSR)
ISSN: 2319-7064
ResearchGate Impact Factor (2018): 0.28 | SJIF (2018): 7.426

Evaluation of the Aphicide Activity of Two Essential Oils on the Survival of the Black Citrus Aphid *Toxoptera aurantii* (Boyer de Fonscolombe) (Homoptera: Aphididae)

Fahad Kaoutar[1; 2], Brhadda Najiba[2], Ziri Rabea[2], Benssallem El Hassan[3], Gmira Najib[2]

[1]Laboratory of Entomology / Bio-insectiocides, Plant Protection Research Unit, Regional Center for Agricultural Research of Kenitra. INRA-Morocco

[2] Laboratory of Nutrition, Health and Environment. Biodiversity and agro resources team
Ibn Tofail University, Faculty of Sciences Kenitra Morocco

[3]Weed Science Laboratory, Plant Protection Research Unit, Regional Center for Agricultural Research of Kenitra. INRA-Morocco

Abstract: ***In an attempt to elaborate a strategy of integrated pest management on citrus fruits in Morocco, the biocide activities of two essential oils on citrus black aphid Toxoptera aurantii infesting citrus and a potential vector of closterovirus, was studied in the laboratory. Both essential oils emulsified in 2% carboxymethylcellulose sodium water and 12.5 % of saccharose were applied to 10; 5 and 2.5 ml.l^{-1}. With regard to checks, both essential oils significantly affected the survival of the pest, during all the period of the treatment. As to the cedar's oil Cedrus atlantica, three concentrations were significantly different from the check. The concentration 10 ml.l^{-1} caused the mortality of nearly all individuals treated, after one day of exhibition. In the term of the experiment, the concentration 5 ml.l^{-1}, caused mortality estimated in: 50.25; 51; 52; 54.88 and 56.75 %, respectively, for the first, second, third, fourth stage larva and the adult stage; after the same duration, the concentration 2.5 ml.l^{-1} engendered a low mortality, but significantly different from the check. The values of the CL_{50} were comparable between the four larval stages. They were estimated at 6.14; 6.13; 6.05 and 6.00 ml.l^{-1}, for the first, second, third and fourth stage larvae. The TL_{50} of the concentration 5 ml.l^{-1}, was estimated at: 86; 82; 57; 56 and 23 hours, respectively, for the first, second, third, fourth larval stage and the adult. Concerning the efficiency of the Eucalyptus's oil on T. aurantii, it engendered a global average mortality of about 43 + 0.37%. As to the C. atlantica's oil, the concentration 10ml.l^{-1} provoked the mortality of most of the adults treated, which were comparable between five target stages. It bordered 93.63 %. The CL_{50} was estimated at 6.80; 6.62; 5.98; 5.93 and 4.92ml.l^{-1}, successively for the first, second, third, fourth larva stage and the adult. The values of Henry's rights and the CL_{90}, confirm the strong toxicity of Eucalyptus's oil on T. aurantii. The TL_{50} provoked by 5ml.l^{-1} concentration was estimated at 72; 69.14; 31.14; 30.20 and 19 hours, for the first, second, third, fourth larval stage and the imago. In spite of the efficiency of this bio-insecticide, all the concentrations applied, provoked the appearance of the reactions of phytotoxicity on the treated leaves.***

Keywords: biocide, *Toxoptera aurantii*, essential oils, *Cedrus atlantica*, *Eucalyptus grandis*, management, citrus, Morocco.

List of abbreviations cited in this article:
LC50: the concentration needed to kill half of a tested population.
CL90: the concentration needed to kill 90% of a tested population.
TL50: the time needed to kill half of a tested population.
P: the probability.
F: Fisher's F.
N: the number of individuals.

1. Introduction

The black citrus aphid *Toxoptera aurantii* (Boyer de Fonscolombe) has become a serious threat of concern to citrus growers worldwide ([18], [16] and [26]). The danger of this species lies mainly in its possible transmission of the virus responsible for Tristesza of Citrus disease. Until now, this semi-persistent disease is considered to be the most vulnerable in global citrus orchards ([18] and [16]).

The virus is expanding in several riparian countries, particularly Spain and Portugal ([18] and [26]). Given the mode of dispersion of both the virus and its vector, our country, Morocco, is threatened, even if, fortunately, until now, the presence of Tristeza disease is limited to a few trees in the Larach region [2]. On the other hand, *T. aurantii* exists everywhere in moroccan citrus orchards, in case of an accidental introduction of the virus to other zones, the disease will be rapidly disseminated. So, we have to be prepared for such a risk by fighting against the vector. Like other countries, the fight against *T. aurantii* is mainly chemical. However, this method has many disadvantages, in particular the elimination of auxiliary fauna, the phenomenon of secondary pest outbreaks, the problem of residues, accumulation of pesticides in the environment, the risk of poisoning of animals throughout the trophic chain and the resistance of insects to certain chemical families [10].

In order to control pests without the inconvenience of synthetic pesticides, the use of natural products is

continually sought, it gives great hope to Moroccan citrus growing. Indeed, new molecules are sought to, on the one hand, ensure effective protection of agricultural production, and on the other hand, contribute to sustainable management of the agro-ecosystem. However, no work on the use of essential oils as aphid killer has emerged, when the use of plant extracts with insecticidal activity offers some potential ([20]; [14]; 13]).

On our side, to look for alternative control methods to chemical control, compatible with the integrated management of *T. aurantii*, we tested two natural essential oils at different stages of the target. In this article, we present the results relative to the bio-insecticide effects of each product on the different eco-phases of this aphid.

2. Material and methods

2.1 Experimental conditions

The strain of *T. aurantii*, used for this study, was collected on citrus leaves infested with the biotope of El Menzeh and that of Sidi Allal Tazi (Kenitra, Morocco), during the spring period. The breeding was conducted in a thermo-adjustable air-conditioned growth chamber (Tmin = 20 ° C, Tmax = 25 ° C), under a photoperiod of 16/8 hours (Light: Darkness), favoring asexual reproduction, giving birth at apteres [17] and a relative humidity of 65 ± 5%. The citrus rootstocks (*Citrus aurantium* and *Citrange troyer*), 10 weeks old, were chosen as host plants. The feeding of the aphids under these conditions was assured by young leaves of both species of Citrus.

2.2 Essential oils to test

2.2.1 Eucalyptus essential oil (*Eucalyptus grandis*)

It is obtained by complete distillation, by steam distillation ([11] and [4]). The oil we used is a mobile liquid, clear, with a fresh smell, rich in eucalyptol (1.8-cineole).

2.2.2 Essential oil of cedar (*Cedrus atlantica*)

The extraction of cedar oil is done from branches, by hydro-distillation. The amount of oil produced is approximately 0.6 or 0.7% of the branch volume used for extraction [7]. The oil we used is colorless with a woody, powerful and sweet smell. This oil consists of sesquiterpenes, sesquiterpenols (atlantol) and sesquiterpenones, β-himachalene (47.1%), α-himachalene (17.3%).

2.3 Formulation

Both essential oils were tested as an emulsion (oil / water). Thus, by diluting both in water containing 2% sodium carboxymethylcellulose and 12.5% sucrose, three concentrations (emulsions) were prepared: 10; 5 and 2.5 ml / l. Each concentration of each product was tested on the five stages of *T. aurantii*. To meet the needs of the toxicity tests, several concentrations have been prepared.

2.4 Biological tests

Biological tests were applied to groups of 100 individuals from the same eco-phase of the same size. Each group of individuals was placed at the level of a Petri dish of 9 cm diameter. Experimental aphids were fed by young leaves of citrus previously wet with one of the test concentrations of each essential oil. The ends of the leaves were coated with cotton soaked in water. As a source of moisture, next to these treated leaves, cotton soaked in water was placed. This technique allowed the administration of the products by ingestion.

2.5 Experimental device

The tests were conducted according to a completely randomized device (CRD), with four factors: treatment (two levels), concentration (four levels: three concentrations plus control), duration of exposure (three levels: 24, 48 and 72 hours) and stage (five levels). We adopted four repetitions, repeated twice in time (4 * 2 = 8 repetitions).

2.6 Data Analysis

The mortalities were corrected according to the Abbott formula [1], and the different types of insecticidal activity were expressed in terms of efficacy, according to the same formula modified in degree of insecticidal efficacy by [22].

For statistical analyzes, the corrected mortalities were transformed into 2xArcsin (corrected mortality rate / 100) .The data were analyzed using Excel and SAS software. The determination of the remarkable concentrations was carried out according to the probits method. [12] by the software "EPA probit analysis program version 1.5" The lethal time, required for the death of 50% of the individuals studied (TL50), was deduced from the linear regression equations expressing the corrected mortality of the stage considered. according to the time.

3. Results and discussion

Dead individuals were easily spotted. They were motionless, withered with often a change of color and hidden paws.

3.1 Overall effect of the factors studied on the five target stages

In order to have a global vision of the impact of the two products on the five target stages, an overall statistical analysis was carried out. This first analysis reveals a very highly significant effect of the tested product ($F = 42249$, $P < 0.0001$), the concentration tested (dl = 3, $F = 512167$, $P < 0.0001$), the duration of exposure. (d1 = 2, $F = 11714.8$, $P < 0.0001$) and treated stage factor (d1 = 4, $F = 12636.8$, $P < 0.0001$).

3.2 Impact of *C. atlantica* cedar oil on *T. aurantii*

3.2.1 Effectiveness of *C. atlantica* oil on different ecophases of *T. aurantii*

After 24 hours of exposure, the responses of the five stages were comparable. Thus, compared to controls, the three applied concentrations strongly reduce the population of all stages (dl = 3, $F = 10665.4$, $P < 0.0001$). Concentration 10 $ml.l^{-1}$ caused the highest mortality rates, which were close to 99.63; 98.88; 99.00; 99.63 and 99.38%, respectively, for

larvae of the first, second, third, fourth larval and juvenile stage (Figure 1).

The Dunnett test reveals the presence, on the one hand, of a very highly significant difference between the 10 ml.l-1 concentration and the controls, and on the other hand, a significant difference between the two concentrations: 5 and 2, 5 ml.l-1 and the controls. Adding that for both concentrations of 5 and 2.5 ml.l^{-1}, the impact of the stage factor is very highly significant (dl = 4, F = 895.59, P <0.0001). Indeed, for the concentration 5ml.l^{-1}, the average corrected mortality rate recorded after 24 hours of exposure was: 24.75; 31.00; 38.63; 34.50 and 50.50% of deaths, respectively, for the first, second, third, fourth larval and imago stage. According to the same succession of age classes, the 2.5 ml.l^{-1} concentration caused mortality rates of approximately: 21.38; 22.25; 24.88; 25.13 and 32.50% of deaths (Figure 1).

At the end of the experiment, 72 hours after application of the treatments, the 5 ml.l^{-1} concentration caused mortality rates estimated at 50.25; 51; 52; 54.88 and 56.75%, respectively, for the first, second, third, fourth larval and adult stage. After this duration of exposure, the corrected mortality rates, which were recorded following application of the concentration: 2.5 ml.l^{-1}, were close to: 32.13; 31.88; 35.35; 43.25 and 39.50%, respectively, for the first, second, third, fourth and imago stage. However, the effectiveness of this concentration was very low, compared to the two previous ones (Figure 1).

These results show that after one day of exposure, the 10 ml.l^{-1} concentration of *C. atlantica* oil was very effective against *T. aurantii*. On the other hand, the same oil used by fumigation, at very low concentrations (10 and 20 µl/l) to control two species of aphids *Rhopalosiphum padi* (L) and *Shizaphis graminum* (Rondani) in the laboratory and for a very short time. duration of 24 hours, showed no aphicidal effect [9]. According to the same El-Miziani protocol [9], cedar oil has shown no insecticidal effect on a pest of the stored commodities *Tribolium castaneum* (Herbst) [24]. This could be explained by the fact that the essential oils are very volatile in the air and that the fumigation treatment is not effective whatever the duration of exposure. This oil caused no symptoms of phytotoxicity on the leaves that were treated. The absence of symptoms of phytotoxicity could be explained by the absence of impurities in the essential oil of *C. atlantica* used. In the literature, oils that do not produce symptoms of phytotoxicity are thinner oils, called narrow-range oils, or higher oils. Also, these pure essential oils have passed during their manufacture by a hydrogenation phase, which removes parts of the oil that could react with oxygen to form compounds damaging plants [8]. The aphicidal efficacy of this bio-insecticide could be explained by the fact that, probably, the administration of *C. atlantica* oil has brought about a rapid and definitive cessation of the nutrition of the different stages of the black aphid which caused the death of these insects, as a result of an anti-appetizing effect, or it disrupted the functioning of the digestive system of the pest, so its mode of action was achieved by ingestion. Indeed, oils can kill aphids in many ways. They can quickly asphyxiate and suffocate these pests, as they can also react with the fatty acids of the body of the insect, interfere with its metabolism and act as a poison, it is a mode of action by ingestion. They also have a repellent effect that discourages the feeding of insects that die from hunger that can be a mode of action of an anti-appetite [8].

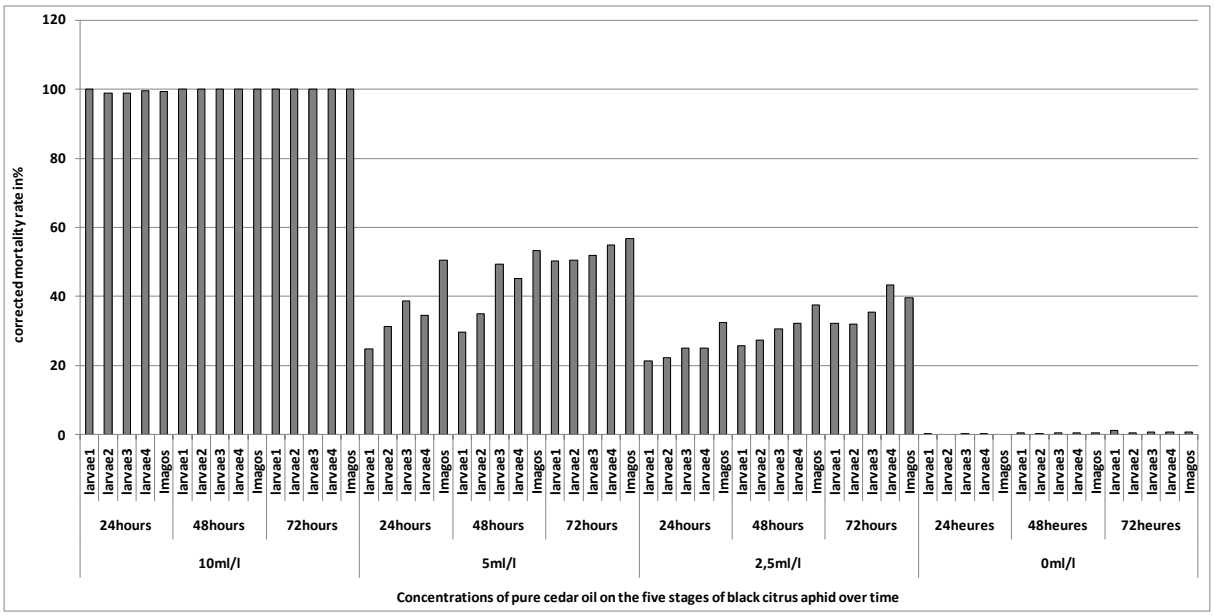

Figure 1: Cumulative effects of three concentrations of *C. atlantica* oil on the five stages of *T. aurantii* (N≥100 per stage and per concentration, the histograms affected by the same letter do not differ statistically between them (test Fischer and PPDS, P <0.0001)

3.2.2 Toxicity parameters of *C. atlantica* essential oil on *T. aurantii*

The lethal concentrations for each ecophase were calculated twenty-four hours after treatment. Thus, from Table 1, it appears that the LC_{50} values in ml.l^{-1} were comparable between the four larval ecophases. They were estimated at: 6.14; 6.13; 6.05 and 6.00 ml.l^{-1}, respectively, for the first, second, third and fourth larval stages.

On the other hand, the lowest LC_{50}, was recorded in the lot of juveniles stages, it was around 5.06 $ml.l^{-1}$. The CL_{90} values in $ml.l^{-1}$ and the slopes of Henry's straight lines confirm the high toxicity of *C. atlantica* oil against *T. aurantii* (Table 1). These results indicate that the older the insect, the more toxic this oil has been. This could be explained by the fact that, as the feeding frequency of the insects increases, as their trophic requirements increase, therefore, the older insects have ingested much more oil.

With regard to TL_{50}, this parameter could not be determined for the concentrations 2.5 and $10ml.l^{-1}$. For the first concentration, the corrected mortality rate of 50% was not reached, moreover, for the second concentration; all the insects were dead after one day of exposure (Figure 1). On the other hand, for the $5ml.l^{-1}$ concentration, the TL_{50} was estimated at: 86; 82; 57; 56 and 23 hours, respectively, for the first, second, third, fourth larval ecophase and adult stage. It has decreased as the age group has increased.

In case of a possible presence of Tristeza disease in a given orchard, this TL_{50}, does not seem to be short enough to kill the possibly virulent adults, before transmitting the virus responsible for Tristeza's disease ([21]; 6], [19] and [26]). Currently, this disease is declared present in a limited area in our country (Morocco), therefore, this oil of *C. atlantica* at a concentration of $10ml.l^{-1}$, could rapidly and significantly reduce the level of populations of *T. aurantii*, at a tolerable level in case of young planting. This oil appears as an alternative to control *T. aurantii*.

Table 1: Toxicity parameters of the oil of *C. atlantica* on the five ecophases of *T. aurantii* after 24 hours of exposure

Stage	Henry Curves	$\chi^2_{calculated} < \chi^2_{table\ (0,05\ ;1)} = 3,841$	CL_{90} in $ml.l^{-1}$	Confidence intervals of CL_{90}	CL_{50} in $ml.l^{-1}$	Confidence intervals of CL_{50}
Larvae of the first stage	Y=13,74x+42,14	0,01	7,82	6,95 - 9,50	6,14	5,50 - 6,90
Larvae of the second stage	Y=12,75x+33,15	2,78	8,14	7,37 - 9,40	6,13	5,61 - 6,70
Larvae of the third stage	Y=12,74x+33,36	1,53	8,07	7,27 - 9,41	6,05	5,51 - 6,64
Larvae of the fourth stage	Y=13,06x+35,24	1,70	7,72	6,89 - 9,40	6,00	5,48 - 6,69
imago	Y=12,42x+26,32	1,39	7,65	6,83 - 9,34	5,60	5,08 - 6,17

3.3 Impact of *Eucalyptus grandis* oil on *T. aurantii*

3.3.1 Efficiency of E. grown on the different ecophases of *T. aurantii*

In view of the results obtained by the global analysis, the oil of *E. grandis* caused an average mortality of 43 ± 0.37%, with variation in mortality rates depending on applied concentrations, target stage and duration of treatment.

After 24 hours of exposure, the corrected mortality rate varies between concentrations (dl = 3, F = 697.97, P <0.0001) and age classes (dl = 4, F = 279.46; P <0.0001). The comparison of means by the Dunnett test, shows a very highly significant difference between the three concentrations tested and the controls. As in the case of *C. atlantica* oil, the $10ml.l^{-1}$a concentration caused the highest corrected mortality rates and was comparable between the five stages (Figure 2).

On the other hand, the effect of the duration of exposure was striking for the 5 $ml.l^{-1}$ concentration. Thus, after the same duration of exposure, this concentration generated mortality rates of approximately: 26.25; 30.00; 40.38; 41.13 and 50.57%, respectively for the first, second, third, fourth larval and imago stage. Following the same succession of stages and after 48 hours of exposure, the same concentration produced higher cumulative mortality rates were estimated at: 32.00; 59.25; 58.75; 50.57 and 53.75%. Similarly, at the end of the experiment, cumulative mortality rates increased to 49.50; 51.00; 60.75; 60.88 and 62.00%, respectively for the first, second, third, fourth instar and imago stage. However, the $2.5ml.l^{-1}$ concentration was the least effective, for which the effect of duration of exposure was observed, only for the first and second larval ecophase and the adult stage.

From these results, we can say that, the oil of *E. grandis* at 10 $ml.l^{-1}$ concentration is a very potent aphicide. This insecticidal activity could be explained as previously, that is to say, that this oil has acted, either by ingestion by disturbing the digestive metabolism of the insect [8], or as anti-appetizing repellent [5].

3.3.2 Toxicity parameters of *E. grandis* grown on the different ecophases of *T. aurantii*

As with cedar oil, lethal concentrations were determined after one day of exposure of different ecophases to different treatments. LC_{50} were estimated at 6.80; 6.62; 5.98; 5.93 and $4.92ml.l^{-1}$, respectively for the first, second, third, fourth larval ecophase and adult stage (Table 2). Again, the toxicity increased as the stages were older. This could be explained, as previously, that is, the frequency of feeding was higher in older individuals, who accumulated much more oil in their bodies.

The values of the slopes of Henry's straight lines and the CL_{90} confirm the high toxicity of *E. grandis* against *T. aurantii* (Table 1).

For the last three stages, the toxicity of *E. grandis*, was higher than that of *C. atlantica* oil.

Here, too, the 2.5 $ml.l^{-1}$ concentration never caused a 50% mortality rate. While the concentration 10 $ml.l^{-1}$, has caused the death of almost all individuals after twenty four hours of exposure. Therefore, TL_{50} values could not be determined for these two concentrations. Moreover, in the case of application of the $5ml.l^{-1}$ concentration, the TL^{50} was estimated at 72.00; 69.14; 31.14; 30.20 and 19.00 hours, respectively, for the first, second, third, fourth larval and adult stages. As in the case of cedar oil, TL_{50} decreased with increasing insect age, but was not short enough to control potential vectors of Tristeza disease ([6]; [26]).

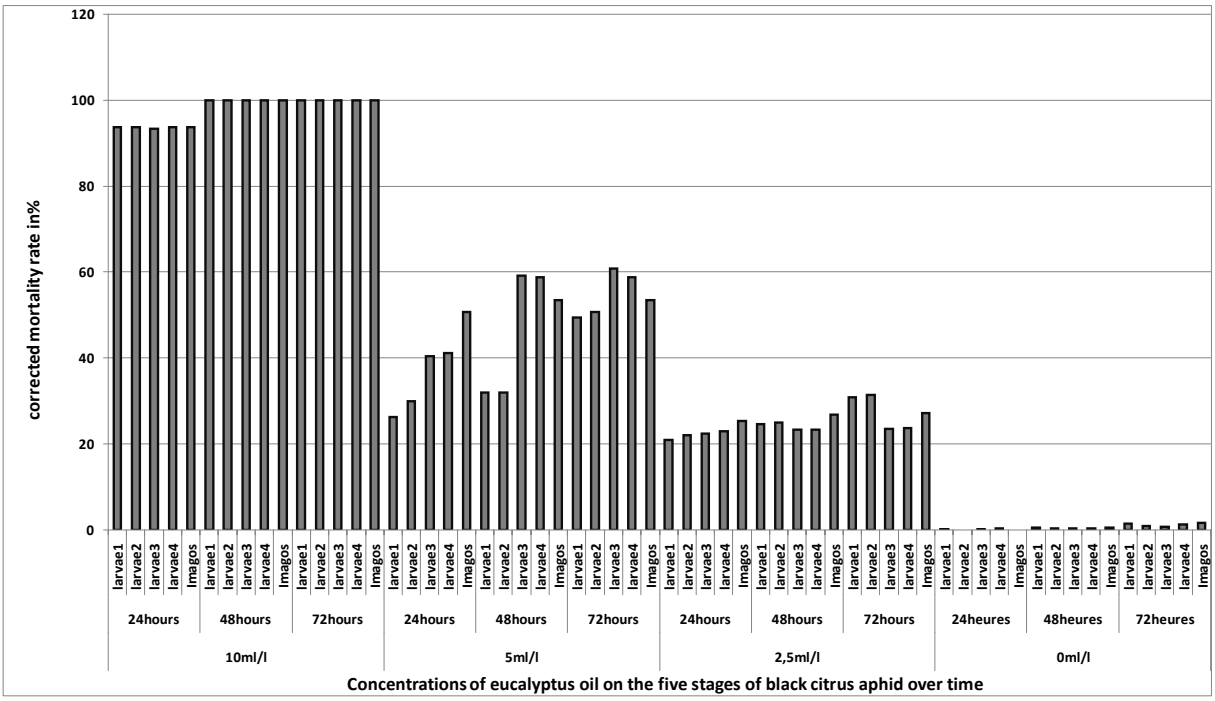

Figure 2: cumulative effects of the three concentrations of *E. grandis* on the five stages of the black aphid (N≥100 per stage and per concentration, the histograms affected by the same letter do not differ statistically from each other (Scheffé test, p ≤0.05).

Table 2: Toxicity parameters of the oil of *C. grandis* on the five ecophases of *T. aurantii* after 24 hours of exposure

Stage	Henry Curves	$\chi^2_{calculated} < \chi^2_{table\ (0,05\ ;1)}$ =3,841	CL_{90} in ml.l^{-1}	Confidence intervals of CL_{90}	CL_{50} in ml.l^{-1}	Confidence intervals of CL_{50}
Larvae of the first stage	Y=12,10x+33,5	2,31	9,59	8,75 - 10,81	6,80	6,09 - 7,45
Larvae of the second stage	Y=11,87x+30,40	2,89	9,58	8,70 - 10,92	6,62	5,94 - 7,25
Larvae of the third stage	Y=11,42x+23,23	3,00	9,58	8,54 - 11,35	5,98	5,30 - 6,60
Larvae of the fourth stage	Y=11,35x+22.38	3,20	9,60	8,54 - 11,42	5,93	5,24 - 6,56
imago	Y=10,19x+8,48	3,27	9,91	8,52 - 12,34	4,92	4,22 - 5,57

Despite the effectiveness of this bio-insecticide, all the concentrations that were applied, caused burns on the leaves. These symptoms could be attributed to phytotoxicity. The severity of these burns increased with the exposure time. Thus, after 72 hours of treatment, all the leaves were completely burned. The severity of these phytotoxicity symptoms was independent of the applied concentration. This could be explained by the great richness of this oil in cineole which is very reactive and very toxic following the double oxygen bond (O-O) [25].

4. Conclusion

The concentrations used in this study are significantly higher than those found in nature, tests conducted with low concentrations did not give results significantly different from the control groups. The results obtained show that the 10 ml.l^{-1} concentration is the most active for the two oils over the five stages of *T. aurantii*. Cedar oil at 10 ml.l^{-1} was very effective with a short TL_{50} and harmless on the treated leaves. Thus, in the short and medium term, this essential oil could be integrated in a management program against *T. aurantii*, in citrus orchards especially in a situation at risk of presence of Tristeza disease. However, it remains to evaluate their possible effects on natural auxiliaries (enemies and pollinators) associated or subordinate to citrus fruits. In fact, the most interesting biological products used in plant protection are those that have a minimal impact on all components of the agro-ecosystem except for the targeted pests [23]. The ideal would be to exploit the non-toxic anti-appetizing properties of the essential oils tested, as suggested by Akhtar and Isman [3]. Furthermore, knowing that plant extracts lose their biological activity under solar radiation [27], the mode and persistence of action, the application techniques and the impact of physical factors on the degradation of the botanical compounds tested should also be studied.

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