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INSECTICIDE ACTIVITY STUDY OF ESSENTIAL OILS OF DAUCUS CAROTA (L.) SSP. CAROTA AND CHENOPODIUM AMBROSIOÏDES (L.) ON WHITE LARVAE OF MELOLONTHA MELOLONTHA

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ABSTRACT: *In the goal of natural alternative research of insecticide properties and limited risks by environment, extracted oils from Daucus carota (L.) ssp. carota and Chenopodium ambrosioïdes (L.) have been tested on white larvae of Melolontha Melolontha. This study has two essays: the first has been realized in petri boxes where larvae haves been made in contact with different oil concentrations of Daucus carota (L.) ssp. carota and Chenopodium ambrosioïdes (L.). The second has been realized on the basis of the first essay so as only concentrations which have good results tested in pots containing soil issued of natural milieu. During all the period of exposition doses LC_{50} and LC_{99} have been determined as well as the lethal times, required for the death of 50% (LT_{50}) and 90% (LT_{99}) white larvae exposed to different concentrations of essential oils. We notice that essential oil of Chenopodium ambrosioïdes (L.) leads to very significative insecticide effect on Melolontha Melolontha larva rather than on Daucus carota (L.) ssp. carota.*

KEYWORDS*:* Daucus Carota (L.) ssp. Carota, Chenopodium Ambrosioïdes (L.), Melolontha Melolontha, Botanic Insecticide.

INTRODUCTION

Since long time damages, more and more frequently of white larvae, have been observed in prairies of woody environment, in ornamental nurseries and small trees fields and fruitful trees. Larvae of these insects destroy an important root systems, which highly reduce their growth, and in the worst cases cause their dryness, and their death. For the reason of this incidence synthesized insecticides have been used for larvae control, but potential damages for human health and environment by these insecticides are now considered as a potential problem.

Purpose of the work

This study has for objective to evaluate essential oils of D. carota (L.) ssp. carota and Chenopodium ambrosioïdes (L.) in order to develop alternatives to toxically chemic fight for the humans and the environment.

MATERIALS AND METHODS

Plant materials

D. carota (L.) ssp. carota flowers and air part of C. ambrosioïdes (L.) have been collected in April (2014) in Meknes region in the center of Morocco.

White larvae collect

In a field of avocado trees infested by white larvae of M. Melolontha which is situated in the domain of Ain El Orma-Meknes region, we have collected larvae M. Melolontha on the level of tree roots (**Fig. 1** and **2**).

Figure 1: Field of avocado of Ain El Orma

Figure 2: Presence of white larvae on the level of the roots of the avocado tree

Dryness of vegetal material

Collected organs are dried in the laboratory during 7 days. Raw material is spread on paper and turned frequently at ambient temperature of 25°C.

Essential oil extraction

The extraction of essential oil has been realized by hydrodistillation of 100g of this matter of the dried plant in 1,5L of water at 100°C in the machine Clevenger type (Clevenger J.F., 1928). The distillation takes 3 hours after recuperation of the first drop of the distillate. Essential oil is dried by anhydrous sodium sulfate and kept at 4°C in darkness. The yield of essential oil is expressed according to a dried matter (in mL/100g of dried matter).

Boutkhil et al., (2009) have permitted to identify 20 constituents representing 96,12% of the chemical compound of essential oils extracted from air plants of C. ambrosioïdes (L.). Also El Idrissi et al., (2013) have noticed the flowers essential oils of D. carota (L.) ssp. carota contain in majority: β-Asarone (46,47%), 10S,11S-Himachala-3(12),4-diene (29,90%) and β-Cubebene (4,50%).

Statistical analysis

To compare essential oils effects tested on the measured modalities, an analysis of variance followed by Scheffe test at 5% has been realized by means of soft wares excel 2003. The LC_{50} and LC_{99} have been determined by the Probit method according to Finney (Finney, 1971). Deaths have been corrected by the Abbott formuli (Abbott, 1925). Lethal times required for the death of 50% and 99% (LT_{50} and LT_{99}) of exposed larvae at different concentrations of essential oils have also been taken into consideration.

Toxicity tests

Essential oil concentration preparation: For essential oils of D. carota (L.) ssp. carota and C. ambrosioïdes (L.), five concentrations have been prepared 0,25; 0,5; 1; 1,25 and 2% diluted essential oil in sterile water by the aid of Tween 20 added at 5% compared to sterile water. The Tween plays a role of an emulsifier making essential oil soluble in water. The prepared solutions are put each one in volumetric flasks closed at 50ml in order to obtain enough quantity for the two tests. Their homogeneity is assured by the use of an agitator.

Petri boxes essays: White larvae have been taken from soil and put in recipient in order to get rid off soil particles attached to their bodies. Round pieces of filter paper Whathman type have been prepared to serve as support for white larvae in petri boxes made of glass and are imbibed by prepared treatment.

By the help of syringe, treatments, 1,5ml each, have been taken and applied to the center and to the edges of each petri box in order to imbibe the totality of piece of filter paper, and also to assure the contact of larvae with the applied treatment. Then 5 white larvae have been placed in each box (**Fig. 3**). The 3 repeated essays are accompanied each by is witness.

Figure 3: Five larvae put in a petri box of glass containing the applied treatment

Small pots essay: This essay has been led on the basis of obtained results during the first essay. Only the concentrations which have more deaths have been retained. In this essay treatments have been tested against white larvae placed in small pots containing soil from infected plot of white larvae. This soil has been taken at about 20 cm to 25 cm of the deep, at the level of the

roots. Used pots have the capacity of 1L full of soil. Then 5 larvae have been placed in each pot (**Fig. 4**).

Figure 4: Small pots full of soil containing white larvae

After the penetration of larvae in the deep (**Fig 5**) 5ml of each treatment has been spread, with the help of a syringe, on all the surface of the pots containing the soil. For the witness, we have applied only distilled water. The 3 essays are repeated, for each dose has been accompanied by its witness.

Figure 5: Small pots full of soil containing white larvae penetration in the soil

RESULTS AND DISCUSSION

Test results of toxicity in petri box

Essential oils of D. carota (L.) ssp. carota and C. ambrosioïdes (L.) have significantly affected the survival of white larvae. In the previous lots treated, the survival of white larvae varies from 1 and 7 days for the two essential oils, but in the witness lot this parameter oscillates from 2 and 9 days. The toxicity of the two essential oils depends on the concentration and the duration of exposition.

The survival of white larvae diminishes in accordance when the oil concentration and the duration of exposition increase (**Fig. 6** and **7**). The time of survival of 50% of white larvae exposed to different concentrations of essential oils varies from 2 and 5 days for D. carota (L.) ssp. carota and from 1 to about 4 days for C. ambrosioïdes (L.) depending on the concentration,

but in the witness lot, the white larvae live at the average of 6 days. The LT_{50} and LT_{99} negatively correlate to the concentration of the tested essential oils (**Table 1**).

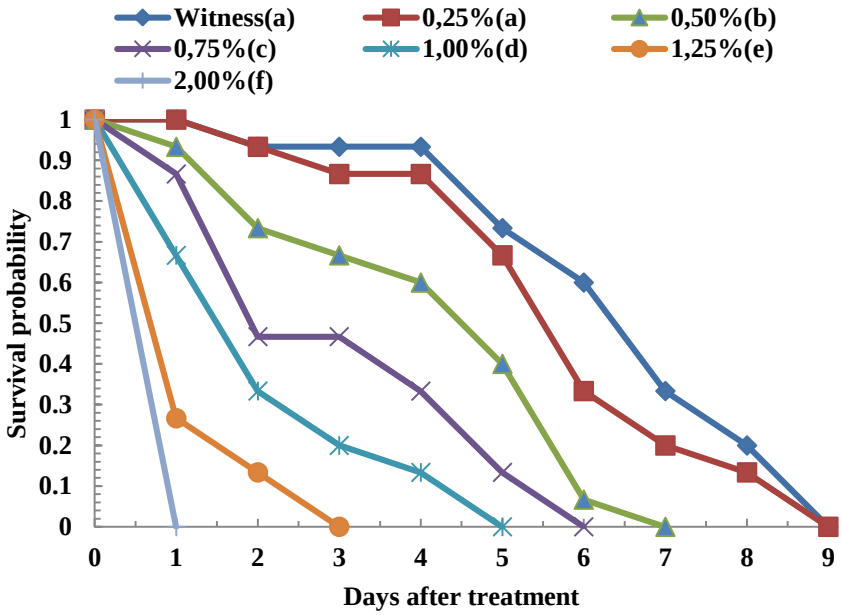

Figure 6: Survival of white larvae exposed to flower essential oil of D. carota (L.) ssp. carota in petri box

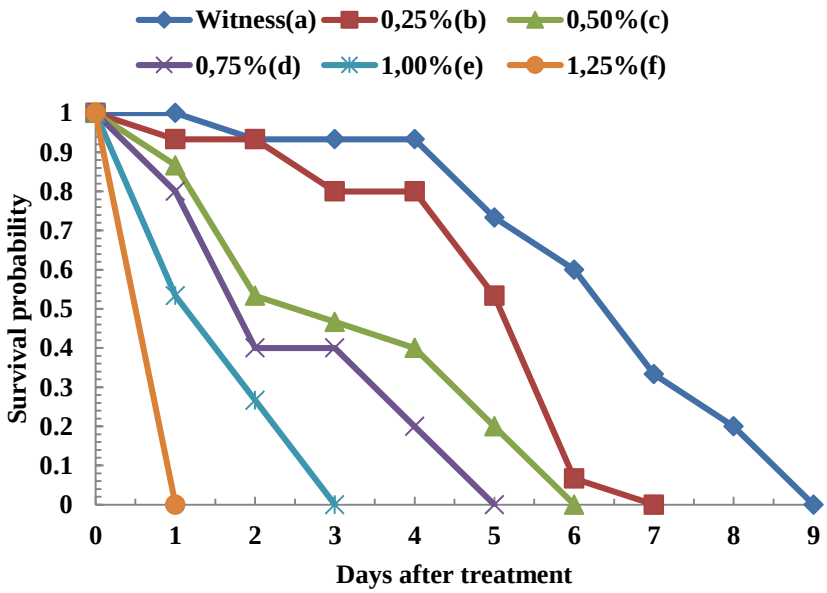

Figure 7: Survival of white larvae exposed to essential oil of C. ambrosioïdes (L.) in petri box

The survivals affected of the same tiny letter are not statistically different (Scheffe test, $p \leq 0,05$) meanwhile the others are neatly different.

Table 1: LT_{50} and LT_{99} (days) of white larvae exposed to essential oils of D. carota (L.) ssp. carota in petri box

Plants	Concentrations (%)	TL_{50}(%)	r>r(0,05; 2)	TL_{99}(%)	r>r(0,05; 2)
	0	5,82		11,52	
D. carota (L.) ssp. carota	0,25%	5,02	-0,99	9,95	-0,99
	0,50%	3,83		7,59	
	0,75%	2,99		5,94	
	1,00%	2,39		4,73	
C. ambrosioïdes (L.)	0,25%	4,42	-0,99	8,76	-0,99
	0,50%	3,18		6,30	
	0,75%	2,57		5,09	
	1,00%	1,63		3,22	

Toxicological parameters of tested essential oils have been gathered in **tables 2** and **3**. After 5 days of treatment, the values LC_{50} are: 0,55% and 0,36% respectively for D. carota (L.) ssp. carota and C. ambrosioïdes (L.). This shows that white larvae have been more sensible to essential oils of C. ambrosioïdes (L.) than to D. carota (L.) ssp. carota.

Table 2: Toxicity parameters of essential oil D. carota (L.) ssp. carota vis-à-vis white larvae in days after treatment in petri box

Plants	Days after treatment	Slope ±ES	$\chi^2_{calculé} < \chi^2_{(0,05; 2)} = 9,49$	CL_{50} (%) [Interval from confidence]	CL_{99} (%) [Interval from confidence]
D. carota (L.) ssp. carota	2	4,93±1,23	0,76	0,76 [0,56; 0,92]	2,26 [1,63; 5,53]
	3	6,33±1,66	2,04	0,71 [0,52; 0,83]	1,64 [1,27; 3,43]
	4	5,76±1,54	1,11	0,62 [0,43; 0,74]	1,56 [1,18; 3,46]
	5	8,00±2,94	0,83	0,55 [0,29; 0,68]	1,08 [0,84; 3,68]

SE: Standard error,

LC_{50} and LC_{99}: Lethal concentrations respectively 50% of used larvae.

Table 3: Toxicity parameters of essential oil C. ambrosioïdes (L.) vis-à-vis white larvae in days after treatment in petri box

Plants	Days after treatment	Slope ±ES	$\chi^2_{calculé} < \chi^2_{(0,05; 2)} = 7,82$	CL_{50} (%) [Interval From confidence]	CL_{99} (%) [Interval From confidence]
C. ambrosioïdes (L.)	2	4,52±1,25	3,38	0,62 [0,41; 0,77]	2,04 [1,41; 6,37]
	3	5,64±1,66	5,47	0,56 [0,35; 0,69]	1,45 [1,09; 3,76]
	4	5,16±1,32	1,71	0,46 [0,29; 0,57]	1,30 [0,97; 2,79]
	5	5,92±2,08	0,98	0,36 [0,14; 0,48]	0,89 [0,64; 3,15]

SE: Standard error,
LC_{50} and LC_{99}: Lethal concentrations respectively 50% of used larvae.

Toxicity tests results in tiny pots

Essential oils of D. carota (L.) ssp. carota and C. ambrosioïdes (L.) affects significantly the white larvae survival. In the treated lots the survival of white larvae varies from six to eleven day for the two essential oils, meanwhile in the witness lot this parameter equals 1 during all the period of exposition. The toxicity of these two essential oils depends on the concentration and the duration of the exposition. The survival of the white larvae diminishes in accordance when the concentration of the essential oils and the duration of the exposition increase. (**Fig. 8** and **9**). The time of survival of 50% of white larvae exposed to different concentrations of essential oils varies from 2 to 6 days for D. carota (L.) ssp. carota and from 3 to 5 days for C. ambrosioïdes (L.) according to concentration, but in the witness lot the white larvae live during all the exposition. The LT_{50} and LT_{99} negatively correlate to the tested essential oils concentrations (**Table 4**).

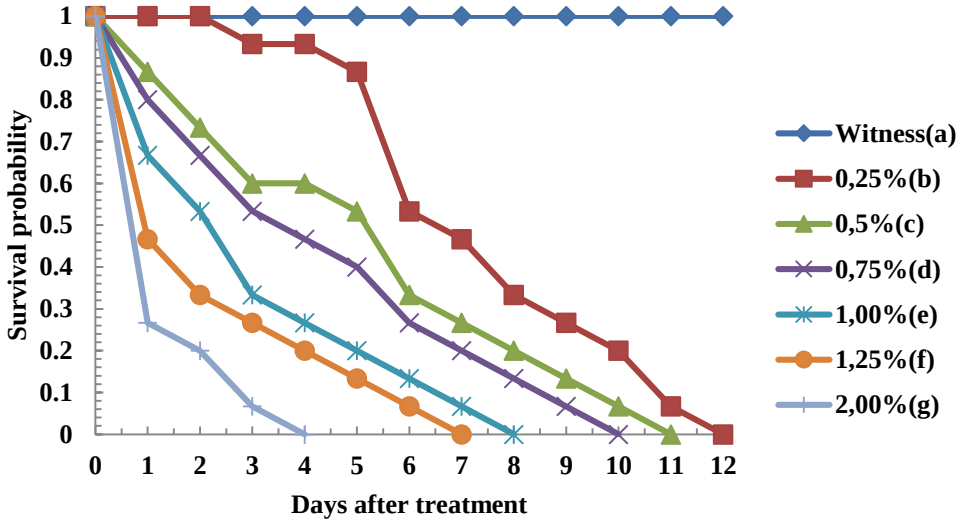

Figure 8: White larvae survival exposed to essential oil of D. carota (L.) ssp. carota in tiny pots

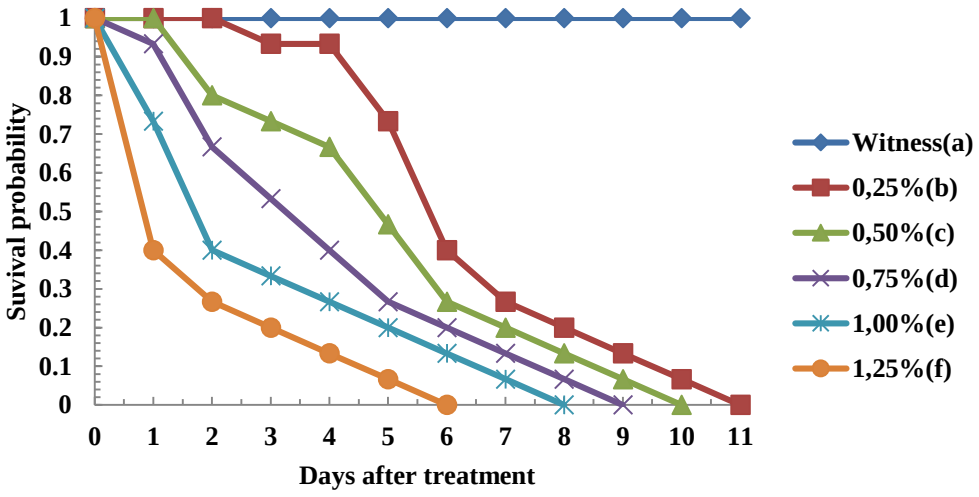

Figure 9: White larvae survival exposed to essential oil of C. ambrosioïdes (L.) in tiny pots

The survivals affected of the same tiny letter are not statistically different (Scheffe test, p≤0,05) meanwhile the others are neatly different.

Table 4: LT_{50} and LT_{99} (days) of white larvae exposed to essential oils of D. carota (L.) ssp. carota in tiny pot

Plants	Concentrations (%)	TL_{50}(%)	r>r(0,05; 3)	TL_{99}(%)	r>r(0,05; 3)
	0	-		-	
D. carota (L.) ssp. carota	0,25%	6,51	-0,98	12,88	-0,98
	0,50%	5,38		10,65	
	0,75%	4,79		9,48	
	1,00%	3,77		7,47	
	1,25%	3,50		6,93	
	2,00%	2,15		4,25	
C. ambrosioïdes (L.)	0,25%	5,60	-0,99	11,09	-0,99
	0,50%	4,74		9,38	
	0,75%	4,07		8,07	
	1,00%	3,63		7,18	
	1,25%	3,00		5,94	

Toxicology parameters of tested essential oils have been gathered in **tables 5** and **6**. After 5 days of treatment the values of LC_{50} are: 0,56% and 0,44% respectively for D. carota (L.) ssp. carota and C. ambrosioïdes (L.). This shows that white larvae were more sensible to essential of C. ambrosioïdes (L.) than to essential oils of D. carota (L.) ssp. carota.

Table 5: Toxicity parameters of essential oil D. carota (L.) ssp. carota vis-à-vis white larvae in days after treatment in tiny pot

Plants	Days after treatment	Slope ±ES	$\chi^2_{calculé} < \chi^2_{(0,05;\ 3)} = 9,49$	CL_{50} (%) [Interval from confidence]	CL_{99} (%) [Interval from confidence]
D. carota (L.) ssp. carota	2	3,06±0,65	1,56	0,99 [0,79; 1,29]	5,70 [3,22; 21,96]
	3	3,01±0,61	0,90	0,72 [0,55; 0,90]	4,25 [2,55; 13,24]
	4	3,60±0,68	1,30	0,65 [0,51; 0,80]	2,90 [1,94; 6,61]
	5	3,39±0,65	0,91	0,56 [0,42; 0,69]	2,71 [1,79; 6,39]

SE: Standard error,

LC_{50} and LC_{99}: Lethal concentrations respectively 50% of used larvae.

Table 6: Toxicity parameters of essential oil C. ambrosioïdes (L.) vis-à-vis white larvae in days after treatment in tiny pot

Plants	Days after treatment	Pente ±ES	$\chi^2_{calculé} < \chi^2_{(0,05;\ 3)} = 7,82$	CL_{50} (%) [Interval from confidence]	CL_{99} (%) [Interval from confidence]
C. ambrosioïdes (L.)	2	4,17±1,02	0,62	0,89 [0,73; 1,12]	3,19 [2,00; 11,40]
	3	3,38±0,80	0,19	0,75 [0,59; 0,96]	3,65 [2,13; 14,82]
	4	3,68±0,80	0,07	0,65 [0,51; 0,80]	2,79 [1,79; 7,96]
	5	2,76±0,68	0,46	0,44 [0,28; 0,58]	3,08 [1,74; 14,61]

SE: Standard error,

LC_{50} and LC_{99}: Lethal concentrations respectively 50% of used larvae.

In our study we notice that the essential oil of C. ambrosioïdes (L.) is richer than in monoterpenes (95,03%) in comparison to that of the oily phase of flowers of D. carota (L.) ssp. carota (0,98%). The essential oil of the two plants causes a very significantly reduction of the survival of white larvae of M. Melolontha. These effects can be linked to monoterpenes which are responsible of the toxicity of other insects. (Ngamo et al., 2007). In the treated insects these two essential oils can inhibit the acetylcholinesterase (López and Pascual-Villalobos, 2010) the actopamine (Price and Berry, 2006) or the cytochrome P450 of mono-oxygenasis (De-Oliveira et al., 1997).

From the point of view of physico-chemistry, the essential oils do not leave residues or wastes in the treated products, they are easily biodegradable (Claudia and Florian, 2013). Many of them act at the level of specific optamine neurotransmettor to invertebrates they are generally less toxic for mammals through oral. The LD_{50} are generally superior to 1000mg/kg of the weight of rates (Koul et al., 2008).

CONCLUSION

The use of essential oils apt to control harmful insects in the developing countries can constitute an alternative approach complementary to classical insecticide treatments. At the end of this study, the essential oils of C. ambrosioïdes (L.) are very effective against white larvae of M. Melolontha than essential oils of D. carota (L.) ssp. carota. To optimize effective doses, complementary experiments are necessary to precise the nature of responsible compounds of this activity. This approach can help to reduce the quantity of applied insecticides and then to reduce the negative impact of these synthetic compounds such as residues, resistance (Benhalima et al., 2004) and the environment pollution.

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