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Bio-insecticide effect of the extracts of *Urtica dioïca* and *Dryopteris filix-mas* on *Aphis fabae* (Homoptera: Aphididae)

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Research Article

Abstract : The evaluation of the nettle (*Urtica dioïca*) and the fern's (*Dryopteris filix-mas*) extracts' effectiveness on the black louse of the broad bean (*Aphis fabae*), shows that these extracts applied with a high dose (5 ml of extract/ 5ml of distilled water), present a toxic effect : 73.8% mortality with the nettle's extract and 75.1% with the fern's extract. It also emerges that the insecticide activity of these plants acts on long term. Finally, the comparison between the results obtained with the aqueous extracts and the results noted with application of the Mospilan 20sp, a chemical pesticide, are not very different.

Keywords: bioinsecticide effect, nettle, fern, *Aphis fabae*, dose

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1. Introduction

The synthetic organic pesticides have done enormous favors to humanity in fighting devastators, but their anarchical use during more than half a century engendered considerable harmful effects which incited scientists to search for fighting alternatives to replace synthetic organic pesticides with vegetal biodegradable and environmentally friendly bio-pesticides (Khalfi-Habes *et al.*, 2010).Literature mentions many examples of plants which have insecticide properties towards culture devastators . It seems a real potential of certain vegetal species whose insecticide effectiveness is proved (Toumnou *et al.,* 2012).

Some studies have been fulfilled in this direction regarding lice. Certain observations show the phytotherapeutic effect of *Artemesia vulgaris*, of *Mentha piperata*, of *Saponaria officinalis* and of *Tanacaetum annuum* (Ondet, 2007). Moreover, the effect of the plants Hyptis *spicigera, Azadirachta indica, Euphorbia balsamifera* (Bambara and Tiemtoré, 2008) and the orange rind extract (Jamar *et al.,* 2010) have been tested on lice. Some studies are being conducted in Algeria concerning the insecticide effect of essential oils of *Lavandula stoechas* and *Origanum glandulosum* on *Aphis pomi* Linnaeus, 1758 (Amirat *et al.,* 2011), the extracts of *Peganum harmala*, of *Melia azedarach* and of

Rosmarinus officinalis on *Aphis fabae* Scopoli, 1763 (Khalfi-Habes *et al.,* 2011 ; 2012). Sure enough, plants synthetize numerous secondary metabolites with potential insecticide effect. It's in this context that this work is registered. This article reports the results of an effectiveness test of aqueous extracts of two vegetal species on the black louse of the broad bean (*A. fabae*). It's about evaluating and comparing the effectiveness of the extracts compared with the Mospilan 20sp which is a pesticide of choice very used by agriculturists.

2. Materials and Methods

2.1. Plant Materials

Fern and nettle's leaves are collected from garden, University of Mouloud Mammeri of Tizi-Ouzou, Algeria

2.2 Extracts preparation

Once collected, the leaves of the two plants are cleaned with water in the laboratory, and then weighed. A quantity of 100gms of each fresh plant is immediately put in a traditional mortar and crushed until obtaining a doughy content which is filtered through a piece of fabric so that the ground leaves' remains are caught. The obtained liquid is collected in clean bottles. The retrieved filtrates represent the initial solutions.

2.3 Phyto preparations application

The different bio-trials are fulfilled in the laboratory room conditions. Dilutions of the initial solutions obtained from the two plants' extracts have been done. 6 treatment lots and a 7th for the chemical product are prepared for each extract. Each lot involves 5 pots; each one is made up of broad beans plants infested by different larva stages of *A. fabae*.

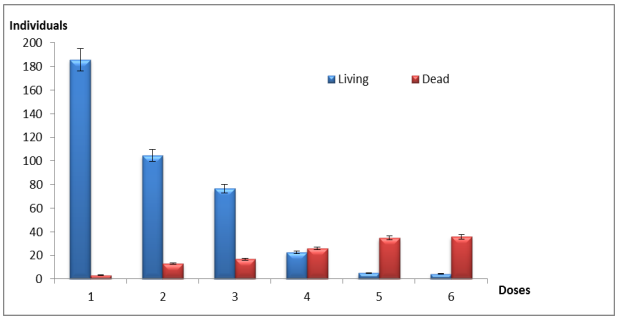
The first lot considered as a witness is treated with distilled water. The second lot's treatment is made by diluting one tenth of the initial solution (1 ml initial solution / 9 ml distilled water). The 3rd lot is treated by diluting 2 tenth of the initial solution (2 ml initial solution / 8 ml distilled water). For the 4th lot it is used a dilution of 3 tenth of the initial solution, or 3ml of initial solution / 7 ml distilled water. The 5th lot is treated with 4 tenth of the initial solution (4 ml initial solution / 6 ml distilled water). The 6th lot receives a treatment with half-dose of the initial solution (5 ml initial solution / 5 ml distilled water). The total individuals initially present before the different treatments are counted, then 3 and 6 days after treatment. Each respective lot receives only one spray of each phytopreparation so that the foliage gets well soaked. 5 repetitions have been done for each of the 5 tested doses.

2.4 Estimation of the treatments' toxicity

Totally, 140 observations constitute our data. The variables measured on the individuals are: mortality, dose and time. It is proposed to treat the data using the R language. To be able to study the mortality behaviour in detail according to the two variables, dose and time, we must first check whether the mortality data is Gaussian. This allows choosing the appropriate hypothesis test. The examination of the data corresponding to the mortality variable shows that it is not Gaussian. Besides, this is reinforced by Shapiro and Wilks' hypothesis test which gives a p-value of 2.518 x10^{-8}. In absence of data normality, the variance analysis isn't appropriate. Consequently we opt for the non parametric test of Kruskall-Wallis.

3. Results and Discussion

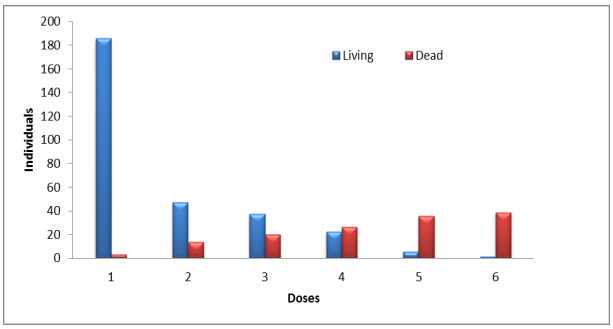
The dose's effect on the populations of *A.fabae.* The two experimented plants showed very toxic towards *Aphis fabae*. The mortality rate of the population of A. *fabae* treated with nettle and fern's extracts is clearly higher than the mortality rate of the untreated witness. The doses 10 % and 20 % present an effect on the populations of aphids. But this one remains negligible and the lice populations could, despite the treatments, swarm (Fig. 1). These results don't match with Ondet's results (2007) who points out that peppermint and wormwood infusions at 10 % allowed limiting satisfactorily the green lice number (*A. pomi*) on apple tree.



1 : Witness, 2 : Dose 10%, 3 : Dose 20%, 4 : dose 30%, 5 : Dose 40%, 6 : Dose 50%.

Fig. 1. Number of living and dead individuals after treatment with 5 doses of nettle extract

These vegetal extracts present a very high mortality rate starting from 40 % concentration. The dose 50 % represents the most toxic dose with a lethality rate of 89,5 % equivalent to an average of 35,8 dead lice for the nettle extract and of 97 % equivalent to an average of 38,8 dead lice for the fern extract (Fig. 2).

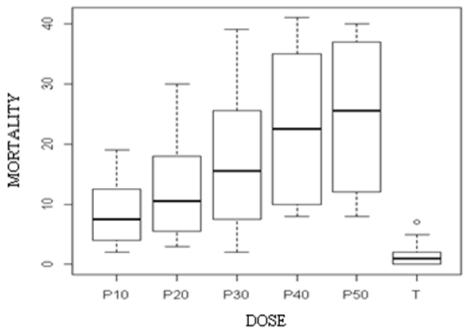


1 : Witness, 2 : Dose 10%, 3 : Dose 20%, 4 : dose 30%, 5 : Dose 40%, 6 : Dose 50%.

Fig. 2. Number of living and dead individuals after treatment with 5 doses of fern extract

Through these results, it appears that the insecticide activity is proportional to the doses whichever the tested extract is. The Mortality-Dosage boxplot presented on figure 3 shows that the mortality level as well as the variability increases according to the dosage. This is confirmed by Kruskall-wallis's test with a p-value of $5.826\ 10^{-12}$, very close to zero, what means that there is a very strong dependence between mortality and dosage.

These results are in agreement with those of Amirat *and al.* (2011) which signal that mortality rates of *A. pomi* treated with lavender and oregano (*Origanum glandulosum*) essential oils increase proportionally to the dose. Prisca *and al.* (2013) signal that a pure solution of absinthe wormwood extract presents a toxic activity towards *Myzus persicae* Sulzer, 1776 with an average number of 5,7 living lice per salad, 7 days after treatment. Likewise, pure solution of common wormwood extract presents a similar toxic effect on the lettuce louse (*Nasonovia ribisnigri* Mosley, 1841) with an average number of 4,8 living lice per salad plant (7 days post-treatment).



T : witness, P10 : 10%, P20 : 20%, P30 : 30%, P40 : 40%, P50 : 50%.

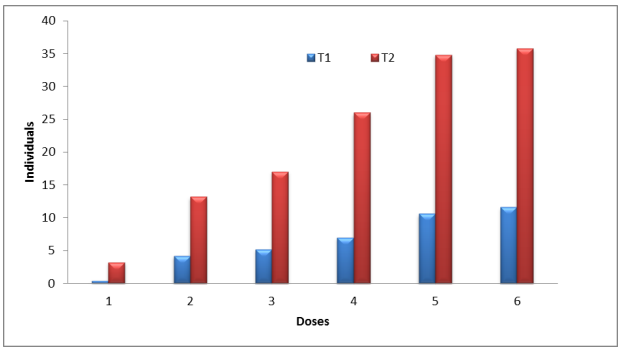
Fig. 3. Mortality-Dosage Boxplot

3.1 Time's effect on *A. fabae* populations

Our results show a slow bio-pesticide effect of the aqueous solutions applied on plants infested by the black louse. These extracts show a non negligible effect on A. *fabae* mortality at time T1, that is to say 3 days after treatment. However, the most toxic action is observed 6 days post-treatment (T2). Despite their weak effectiveness after 3 days of treatment, these extracts could engender mortality within the population of aphids. Thus at the dose 50%, the number of dead individuals moves from an average of 11,6 lice equivalent to a mortality rate of 29 % (1^{st} observation, T1) to 35,8 lice on average ,equivalent to 89,5% mortality (2^{nd} observation, T2) for the nettle extract (Fig. 4).

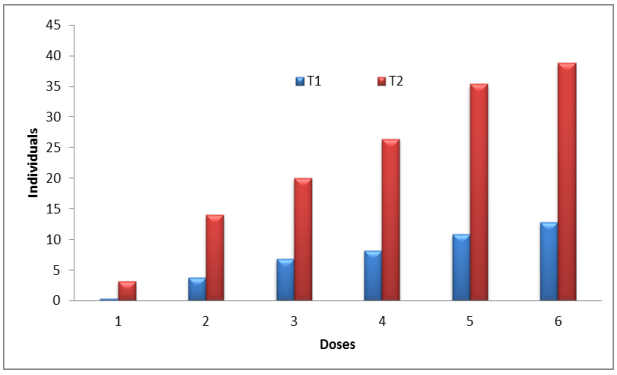
For the fern extract, an average of 12,8 dead individuals is registered at time T1 and an average of 38,8 dead lice corresponding to a mortality rate of 97 % is registered 6 days after application of the extract (T2) (Fig.5).

In the light of these results, it comes out that the time factor plays an important role regarding the treatment's effectiveness. The longer the action period of the bio-pesticide, the better its effectiveness is. Sure enough, the mortality-time boxplot presented in figure 6, shows that the mortality level variability is more important in the 6-day case (B and D) than in the 3-day case (A and C). This is confirmed by Kruskall-wallis test with a p-value very close to zero, so be it $9.154\ 10^{-10}$ which means that the dependence is high with time. Sure enough, there is a significant mortality variation according to the time.



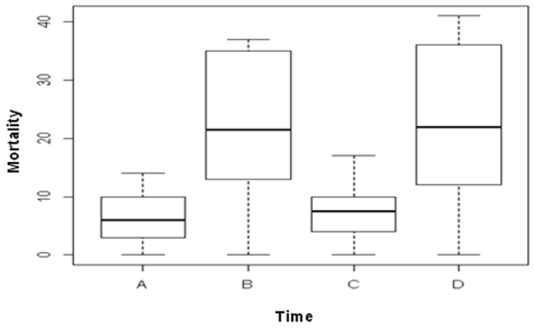
1 : Witness, 2 : Dose 10%, 3 : Dose 20%, 4 : dose 30%, 5 : Dose 40%, 6 : Dose 50%.

Fig. 4. Number of dead individuals 3 and 6 days after treatment with nettle extract



1 : Witness, 2 : Dose 10%, 3 : Dose 20%, 4 : dose 30%, 5 : Dose 40%, 6 : Dose 50%.

Fig. 5. Number of dead individuals 3 and 6 days after treatment with fern extract



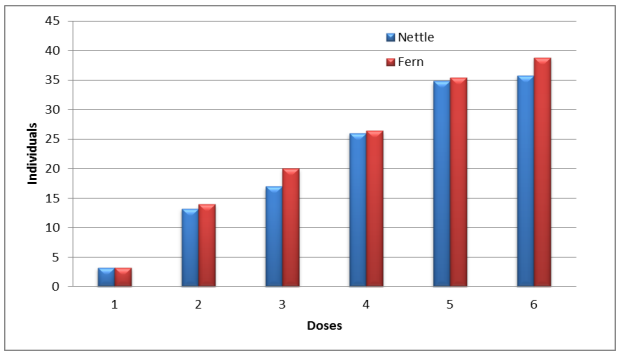
A : 3 days after treatment with nettle extract, B : 6 days after treatment with nettle extract,
C : 3 days after treatment with fern extract, D : 6 days after treatment with fern extract.

Fig. 6. Mortality-Time boxplot

Baroffio *and al.* (2009) signal that the effectiveness of the sesame oil-based treatment on the elder louse *Aphis sumbuci* Linnaeus, 1758 decreases with the passage of time. These authors note that the pourcentage of umbels attacked by this louse after 2, 10, 17 and 27 days treatment application is respectively: 5, 6, 29 and 36 %.

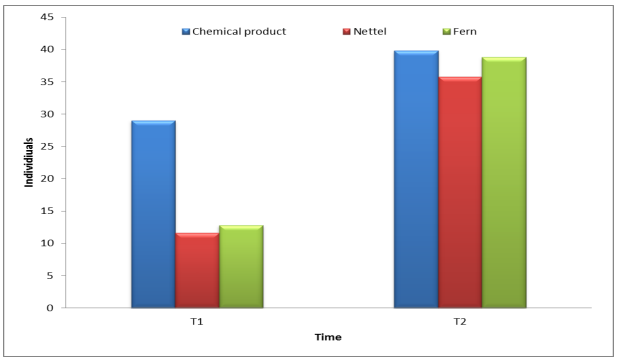
3.2 Effect of the extract's nature on the populations of *A. fabae*

The fern's extract presents a toxic effect slightly superior to the nettle's extract effect (Fig. 7). Picture1of the average and median mortalities given below shows that there is a slight improvement of the effectiveness regarding the fern's extract. However, this is not sufficient to conclude that there is a real difference of effectiveness. Sure enough, Kruskal Wallis's test which crosses mortality with the two variables gives a p-value of 0.659, largely superior to 5 %. That indicates that there is no significant difference between the 2 extracts. Bambara and Tiémoro (2008) mention a better effectiveness of the *Euphorbia balsamifera* based extract on the populations of lice compared with the d'*Hyptis spicigera* based extract. According to Khalfi-Habes *and al.* (2010), the plants' insecticide activity is probably attributed to the semiochemical substances contained in the vegetal. The study conducted by Ondet and Salva (2005), shows that the therapeutic effect of the vegetal extracts-based treatments towards stinging and sucking insects, differs according to the nature and the chemical composition of the used extract. The vegetal extracts effectiveness on the populations of aphids is highlighted by many authors. Thus the aqueous extract of H. *suaveolens* (Dansou *and al.*, 2007) as well as that of *Euphorbia balsamifera* and *Hyptis spicigera* (Bambara and Tiemtoré, 2008) and that of the absinthe wormwood and of the vulgar wormwood (Prisca *and al.*, 2013) provoke a high toxicity towards lice. Our study confirms the results of these earlier works since we also record a decrease in the number of lice within the treated populations with the studied extracts (Fig. 8).



1 : Witness, 2 : Dose 10%, 3 : Dose 20%, 4 : dose 30%, 5 : Dose 40%, 6 : Dose 50%.

Fig. 7. Number of dead and living individuals 6 days after treatment with nettle and fern's extracts



T1 : 3 days after treatment, T2 : 6 days after treatment

Fig. 9. Number of dead individuals after treatment with the two vegetal extracts and with the Mospilan 20sp

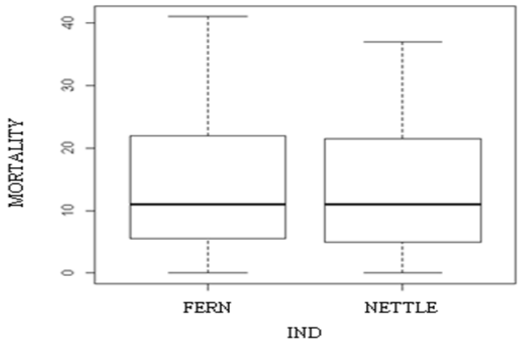
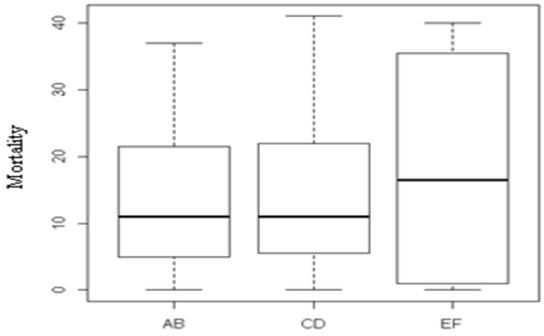


Fig. 8. Comparison of mortality between nettle and fern



(AB), fern (CD) and chemical product (EF)

Fig. 10. Mortality crossed with the variables nettle

Table- 1: Average and median mortality

			Average				
	Doses	10	20	30	40	50	T
AB	nettle	8,70	10,67	16,50	22,70	23,70	1,80
CD	fern	8,90	13,40	17,30	23,10	25,80	1,80
			Median				
	Doses	10	20	30	40	50	T
AB	nettle	7,50	10,50	16,50	23,00	24,00	1,00
CD	fern	9,00	10,50	20,00	22,50	37,00	1,00

3.3 Comparison between the vegetal extracts' effect and the insecticide's effect

To complete our study, we also compare the effectiveness of these two extracts with the effectiveness of the chemical product (Fig. 9). The latter, applied on populations of broad beans black lice, confirms that the nettle and the fern's extracts have a pesticide effect on *A. fabae* colonies, since we observe mortality rates very comparable to those obtained with the Mospilan 20sp (Fig. 10). Kruskal Wallis's test gives a p-value equal to 0, 9181, what means that there's no significant difference between the vegetal extracts and the chemical product. The comparison according to time shows a maximal effectiveness of the product at the end of 6 days. According to Attia *et al.,* (2011), certain studies show that many essential oils-based biopesticides are as effective as synthetic products. Likewise, the plants' natural extracts can be aids of choice in the programs aiming to manage the devastators' resistances to pesticides.

3.4 Conclusion

The obtained results prove that the insecticide virtues of *Urtica dioïca* and of *Dryopteris filix-mas* are obvious bearing in mind the high numbers of dead insects in the lots treated with these substances compared to the witness. Mortality rates increase proportionally to the dose and the insecticide activity of these plants acts on long term. These extracts have a toxic effect close to the chemical treatment's effect. In prospect, it would be appropriate to identify the active principles of these plants. In Parallel real environment tests are to be carried out to evaluate the practical effectiveness of these

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