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M. Rima, A. Chbani, Christine Roques, F. El Garah. Comparative study of the insecticidal activity of a high green plant (*Spinacia oleracea*) and a chlorophytae algae (*Ulva lactuca*) extracts against *Drosophila melanogaster* fruit fly. *Annales Pharmaceutiques Françaises*, 2021, 79 (1), pp.36-43. <10.1016/j.pharma.2020.08.005>. <hal-04217192>

HAL Id: hal-04217192

<https://hal.science/hal-04217192v1>

Submitted on 22 Jul 2024

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Comparative study of the insecticidal activity of a high green plant (*Spinacia oleracea*) and a chlorophytae algae (*Ulva lactuca*) extracts against *Drosophila melanogaster* fruit fly

Etude comparative de l'activité insecticide des extraits issus d'une plante verte (*Spinacia oleracea*) et d'une algue verte chlorophyte (*Ulva lactuca*) contre la mouche de fruit *Drosophila melanogaster*.

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Comparative study of the insecticidal activity of a high green plant (*Spinacia oleracea*) and a chlorophytae algae (*Ulva lactuca*) extracts against *Drosophila melanogaster* fruit fly

Objectives: Currently, the global interests tend to take advantage of the plant world as a renewable source of a natural and effective molecule, to find an eco-friendly, cost-effective, and less toxic alternative to the current synthetic pesticide. In this context, the present research was carried out in an attempt to study the insecticidal activity of extracts and pigments derived from the green plant *Spinacia oleracea* and the green alga *Ulva lactuca* against the fruit fly *Drosophila melanogaster* as an alternative to chemical insecticide. **Methods:** The toxicity of the aqueous, acetonic and ethanolic extracts as well as of the purified pigments (Chlorophylls and carotenoids) was determined by complementary *in vivo* tests (application by spraying oranges, toxicity by ingestion and repellent activity). Interestingly, each one of these methods corresponds to a specific insecticidal way of action.

Results: Results showed that acetone extracts which are rich in green pigments present the best insecticidal activities. On the other hand, the purified chlorophyllian pigments exhibited an interesting activity only by spraying method. Regarding the repellent activity, the aqueous extract of spinach displayed higher effectiveness.

Conclusion: Our study suggests the potential of tested plant and algal extracts, as well as of chlorophyllian pigments, to provide a safer alternative way to the use of synthetic pesticides.

Keywords : *Ulva lactuca*, *Spinacia oleracea*, pigments, extracts, insecticidal activity, *Drosophila melanogaster*

Etude comparative de l'activité insecticide des extraits issus d'une plante verte (*Spinacia oleracea*) et d'une algue verte chlorophyte (*Ulva lactuca*) contre la mouche de fruit *Drosophila melanogaster*

Objectifs : Actuellement, l'intérêt mondial tend à profiter du monde végétal comme étant une source renouvelable des molécules bioactives afin de chercher des alternatives écologiques, moins toxiques et rentables aux pesticides synthétiques. Dans ce contexte, cette étude est menée dans le but d'évaluer l'activité insecticide des extraits ainsi que des pigments issus de l'épinard (*Spinacia oleracea*) ainsi que d'une algue verte (*Ulva lactuca*) contre la mouche de fruit *Drosophila melanogaster*.

Méthodes : L'activité insecticide des extraits aqueux, acétoniques et éthanoliques ainsi que des pigments purifiés (Chlorophylles et caroténoïdes) a été déterminée par différents tests complémentaires (pulvérisation des oranges, toxicité par ingestion et activité répulsive). A noter que chacun de ces tests présente un mode d'action insecticide spécifique.

Résultats : Les résultats ont montré que les extraits acétoniques riche en pigments verts présentent la meilleure activité insecticide. D'autre part, les pigments chlorophylliens purifiés de l'épinard ainsi que de l'algue verte ont montré une activité intéressante uniquement par la méthode de la pulvérisation. Concernant l'activité répulsive, l'extrait aqueux issu de l'épinard a été le plus efficace.

Conclusion : Cette étude suggère la capacité des extraits évalués ainsi que des pigments chlorophylliens à fournir une alternative aux pesticides chimiques nocives.

Mots clés : *Ulva lactuca*, *Spinacia oleracea*, pigments, extraits, activité insecticide, *Drosophila melanogaster*

1. Introduction

Despite the ability of synthetic pesticides to eradicate pests, their harmful effects on human health and the environment and have generated worldwide interest in the development of alternative strategies [1]. Moreover, their massive use has contributed to the selection of resistant varieties of pests [2]. In this context, the search for natural, less toxic, cost-effective, and eco-friendly bio-pesticides has become a global challenge. Since terrestrial plants, as well as marine algae, are rich in bioactive substances that are naturally synthesized as a defense method against different types of infections, they have received attention as an effective alternative to chemical pesticides [1] [3].

Spinach (*Spinacia oleracea*) is a leafy green vegetable, native to south-western Asia. This plant is rich in vitamins, minerals, and in antioxidant agents [4]. In addition to its value in the medical field, an application in the agricultural field is possible. An earlier study demonstrated the capacity of spinach ethanol extract to inhibit the growth of *Aspergillus niger*, a fungus responsible for the black mold of fruits [5].

The seaweed *Ulva lactuca* or "sea lettuce" is a green macroalga, with a cosmopolitan distribution. Vitamins, minerals, proteins, lipids, and polyunsaturated fatty acids have been found in this alga [6]. Interestingly, several studies have demonstrated the potential of this seaweed to present an alternative to chemical pesticides. Abbassy *et al.*, 2014, proved an antifungal activity of this alga against some phytopathogenic fungi [7]. Also, they demonstrated an insecticidal activity against different stages of development of the cotton worm *Spodoptera littoralis*, a pest of legumes [7].

Among the bioactive molecules produced by terrestrial and marine plants, chlorophyllian pigments have found great interest. Indeed, these green pigments have a broad spectrum of

biological activities (antioxidant, anti-inflammatory, anticancer activity...) [8] [9]. Despite the scarcity of studies looking for the application of these pigments in the agriculture field, a potential antifungal activity of an organic extract rich in chlorophyll from the green alga *Ulva linza* against the phytopathogenic fungus *Penicillium digitatum* responsible for citrus green mold has been demonstrated [10].

Therefore, the purpose of the present study is to evaluate the insecticidal activity against the fruit fly *Drosophila melanogaster* of extracts and pigments (chlorophylls and carotenoids), from spinach leaves (*Spinacia oleracea*) and a seaweed (*Ulva lactuca*) collected from Lebanon. These two potential sources of bio-pesticides have been selected as they are abundant in Lebanon and inexpensive, which make their valorization possible even on a large scale. Moreover, they are rich in chlorophylls of interest.

In addition to the agricultural damage caused by *D. melanogaster* on sweet fruits and the economic impact on their storage [1], this fruit fly is considered the best model for the study of insecticidal activity. Indeed, it is easy to handle, requires a small breeding space and its life cycle is short enough to allow a large number of repetitions for a more accurate study [11]. The correlation between pigment contents and insecticidal activity was evaluated. *Spinacia oleracea* and *Ulva lactuca* have been selected as they are abundant in Lebanon and rich in chlorophylls of interest.

2. Materials / Methods

2.1 Plant material

To test their insecticidal activity, the green alga *Ulva lactuca* and the spinach leaves of *Spinacia oleracea* were used as algal and vegetal material, respectively. The green alga was freshly and manually harvested from a coastal area of the Mediterranean, El Mina – Tripoli –

Lebanon in July 2017. In order to eliminate the impurities that contaminate the seaweed samples (adhered sand particles, epiphytes, parasitic microorganisms), rigorous washing with seawater and then with distilled water was applied to the algae. They were transported in moistened plastic bags to the laboratory of the AZM research center at the Lebanese University, Tripoli-Lebanon. The algae samples were air-dried at room temperature and in the dark for several weeks, weighed continuously until complete drying. The same washing and drying procedures were applied to spinach leaves of *Spinacia oleracea*, purchased from a Tripoli local market in July 2017. Before extraction, the dried plant and algal materials were milled into fine particles in order to facilitate extraction.

2.2 Preparation of crude plant extracts

Dry samples of *Ulva Lactuca* alga and *Spinacia oleracea* leaves were macerated separately in distilled water, ethanol (96%, Sigma-Aldrich), and acetone (>99.5%, Sigma-Aldrich), with a ratio of 1:4 (m / v) at room temperature with regular shaking [3]. After 24 hours, the extracts were filtered off with a Whatman filter paper and stored at 4°C until use.

2.3 Quantification of pigments content

In order to study the correlation between the quantity of chlorophylls and carotenoids pigments and the insecticidal activity, the content of pigments in acetic and ethanolic extracts of the green alga and spinach was calculated. Note that pigments content was only quantified in these two extracts and not in aqueous extract which is unsuitable for pigments extraction. Absorbance was measured using an Evolution 60 UV-Visible spectrophotometer (Thermo Scientific). The formula used to calculate the level of pigments in each organic solvent is presented in Table 1 [12].

2.4 Purification of chlorophyll and carotenoid pigments

In order to separate the green pigments (Chl **a** and Chl **b**) and the carotenoid pigments (Carotene and xanthophyll) from *Spinacia oleracea* leaves and the *Ulva lactuca* alga, the method based on the differential solubility in organic solvents was used [13]. At the end of the purification, chlorophylls a and b were recovered separately in methanol (99.8%, Sigma-Aldrich). Their concentration of these green pigments in methanol was calculated using the corresponding formula (Table 1). On the other hand, carotene and xanthophyll were solubilized in diethyl ether (>99.0% Sigma-Aldrich). Noted that methanol and ether were used as negative control in the evaluation of the insecticidal activity of chlorophyll and carotenoid pigments, respectively.

2.5 Insect culture

Drosophila melanogaster fruit flies were obtained from infected orange containing larvae at different stages of development, brought from a field in Tripoli - Lebanon in February 2018. The damaged oranges were cut into pieces and deposited in a plastic jar. After two weeks, adult flies emerged. They correspond to the first generation of flies reared under laboratory conditions. Adults fruit flies were reared in plastic jars covered with a muslin cloth to allow the passage of air, with an orange cut into two pieces as a source of food, place of reproduction, and laying.

2.6 Insecticidal activity bioassay

The insecticidal activity against *D. melanogaster* adults was determined from various methods, each one having a specific insecticidal way of action.

We evaluated the activity of the aqueous, acetonetic and ethanolic extracts, as well as the purified pigments (Chlorophyll **a**, Chlorophyll **b**, Carotene, Xanthophyll) isolated from *Spinacia oleracea* leaves and the green alga *Ulva Lactuca*. Extraction solvents were used as negative control. Each test was repeated three times. The corrected efficacy of the extracts that nullifies any solvent

activity (negative control) was calculated by the following Sun-Shepard formula (Non-uniform population) [14].

$$\frac{\text{Mortality \% in treated plot} + \text{Change \% in control plot population} * 100}{100 + \text{Change \% in control plot population}}$$

With Change % in control plot population =

$$\frac{\text{Population in control plot after treatment} - \text{Population in control plot before treatment} * 100}{\text{Population in control plot before treatment}}$$

2.6.1 *Spraying oranges*

In order to mimic the natural conditions in which oranges are infected with the fruit fly, the method described by Chaieb *et al.*, 2010 was used with some modifications [15]. Oranges were washed superficially with distilled water and then each one was sprayed with 1 ml of extract. After drying, each orange was placed in a plastic jar with *D. melanogaster* adults (15 ± 5 flies). The mortality was recorded after 24h, 48h, and 72h. It should be noted that this method has two different modes of action. Indeed, the extract can penetrate into the insect by simple contact or by ingestion.

2.6.2 *Ingestion Toxicity*

The ability of an extract to kill the fruit flies by ingestion was determined through the method described by Aboussaid *et al.* (2009), with some modifications [16]. A small piece of orange impregnated with 1.0 ml of extract was placed in a plastic jar with *D. melanogaster* adults (15 ± 5 flies). The mortality of flies was recorded after 24, 48, and 72h.

2.6.3 *Repellent activity*

In order to study the repulsive activity of extracts against fruit flies, the method entitled "Choice Bioassays" was used with some modifications [17]. Briefly, a tube containing a piece of orange impregnated with 1.0 ml of extract was placed firstly in a plastic jar. On the other hand, and at a distance of 18 cm, a tube containing a piece of orange impregnated with the extraction solvent is placed in another jar (negative control). The two pots were connected by transparent paper with holes for breathing. To stop their movement and, therefore, facilitate their handling, adult *Drosophila* were anesthetized by placing them in the refrigerator at - 4°C for 5-7 minutes. The anesthetized flies (15 ± 5 flies) were then placed in the jar containing the extract and their location was recorded after 2h, 6h, and 24h.

- The repulsive activity of extract (%):

$$\frac{\text{Total number of flies} - \text{Number of flies remaining in the jar containing extract}}{\text{Total number of flies}} \times 100$$

2.7 Statistical analysis

After calculating percentage mortality, all values are expressed as mean \pm SEM of three parallel measurements using the SPSS statistical software (Version 22).

3. Results and discussion

3.1 Preparation of crude plant extracts

Aqueous, ethanolic, and acetonetic extracts of spinach and seaweed were prepared to evaluate their potential insecticidal activity. It should be noted that the solvents were chosen for health and safety reasons [18] as well as for the ability of ethanol and acetone solvents to extract the pigments [19].

3.2 Estimation of pigment contents

In order to study the correlation between pigment contents and insecticidal activity, the quantity of pigments present in acetone and ethanol extracts of spinach and alga was calculated from spectrophotometry measurements (Table 2).

The results show that the level of chlorophyllian pigments (Chl **a** and Chl **b**) and of carotenoids is significantly greater in spinach than in alga, in both acetonic and ethanolic extracts. These findings confirm that spinach is an essential source of pigments [20]. We notice also that acetone allows for better extraction of pigments than ethanol. This is in agreement with the study of Su *et al.* (2010) which revealed that the amount of chlorophylls extracted from aquatic plants by 90% acetone is greater than that extracted by 95% ethanol [21].

3.3 Insecticidal activity bioassay

3.3.1 Spraying oranges

The crude extracts of spinach and alga and the purified pigments were used in this test. The mortality rate of *D. melanogaster* was measured after 24, 48, and 72h of exposure (Figure 1).

Regarding aqueous extracts, only the spinach's shows an effect on flies resulting in $95.3 \pm 3.3\%$ of mortality after 72 hours (figure 1A). The activity of this extract may be due to its richness in phenolic compounds, which have shown significant insecticidal activity in several studies, although their mechanism of action has not been elucidated yet [22] [23]. Furthermore, ethanolic extracts of spinach and green alga show an insecticidal activity causing $82.5 \pm 5.5\%$ and $26.0 \pm 7.4\%$ of mortality respectively, after 72 hours. The most interesting results were obtained with both acetonic extracts as they led to the death of almost all the flies after 48h (figure 1A).

On the other hand, concerning the effect of the purified pigments, only the chlorophyllian pigments show an insecticidal activity (figure 1B) (no effect was recorded for carotenoids

pigments (data not shown)). The mortality rate of flies, in the presence of oranges sprayed with Chl **a**, derived from spinach and alga is respectively $95.6 \pm 2.8\%$ and $94.11 \pm 3.56\%$, after 72 hours. Moreover, the high activity of Chl **b** appears after 72 hours, reaching $91.6 \pm 5.6\%$ for spinach fraction and $93.2 \pm 5.1\%$ for alga one.

Considering our results on the amount of total chlorophylls in the most effective extracts (ethanolic extract of spinach and both acetonic extracts) (Table 2), one can attribute their activity to the presence of the green pigments. According to our knowledge, this is the first study demonstrating the insecticidal activity of green pigments. Surprisingly, the acetonic extract of *Ulva lactuca* shows a high insecticidal activity while a moderate level of total chlorophylls compared to the ethanolic extract of *Spinacia oleracea*.

3.3.2 Ingestion toxicity

In Figure 2 are presented the mortality rate of flies in the presence of a food source impregnated with the crude extracts after 24, 48h and 72h of exposure.

Interesting ingestion toxicity was observed for the acetonic extracts of plant and alga origin after only 24 hours, $46.2 \pm 4.3\%$ and $85.1 \pm 5.2\%$ of mortality, respectively. This percentage remains constant over time. Regarding aqueous and ethanolic extracts, low or no toxicity was observed against the *D. melanogaster* fruit fly. Note that all purified pigments had no effect in this assay (data not shown).

The difference of results with the previous test can be explained by the delivery method of the extract. Since green pigments have shown an effect only by spraying oranges this can be explained by the fact that they have a transcutaneous insecticidal mode of action. Indeed, the passage of an insect on a surface treated with an insecticide allows the penetration of this agent into the insect and then acts by targeting its specific site of action [24]. Moreover, the mode of

action by contact of the chlorophyllian pigments is confirmed by the presence of a correlation between its quantity in the extracts and its insecticidal activity only by the method of spraying oranges.

On the other hand, the difference in results between these two assays may have a chemical context. Indeed, a possible reactivity between the active compounds of extract and the natural acids (ascorbic and citric acid) present in the inner part of orange in the ingestion test could explain the loss of activity of the chlorophyllian pigments, as well as of the spinach aqueous extract, in comparison with the spraying test.

However, the high insecticidal activity of the acetonc extract of *Ulva lactuca* in both methods can be explained by the fact that algae contain unique bioactive compounds given the stressful marine environment [3] [25]. These bioactive molecules may present toxicity by contact as well as by ingestion (cumulative activity observed in the spraying method). Furthermore, the ingestion toxicity of acetonc extract of spinach suggests the presence of active compounds other than green pigments and which presents toxicity by feeding (cumulative activity observed in the spraying test). The insecticidal activity of both acetonc extracts is probably due to the presence of different active chemical compounds [23] [26]. Indeed, secondary metabolites (phenolic compounds, quinones, terpenoids, alkaloids...) have been reported to block metabolic pathways by interfering with vital enzymes or to act on the nervous system leading to permanent nerve stimulation and insect death [22] [27].

3.3.3 Repellent activity

Concerning the repulsive activity of extracts, the aqueous extract of spinach presented the best ability to repel *D. melanogaster* fruit fly until 24h with a mean repellence of $87.78 \pm 1.57\%$. (Table 3). The repellent activity of plants is due to the presence of certain chemicals that can irritate the olfactory sense of insects such as alkaloids, phenolics, terpenoids and flavonoids [28] [29]. In this sense, the richness of the aqueous extract of spinach in phenolic compounds, flavonoids, and terpenoids, can explain its important repulsive activity [30] [23]. Moreover, we should note that a recent study has demonstrated the ability of certain natural terpenoids to disturb the sense of *D. melanogaster* fruit fly thus exhibiting an antifeedant activity [31]. No interesting repellent activity was recorded for the purified pigments (data not shown).

Overall, our results reveal the capacity of natural plant-based insecticide to replace synthetic pesticides. Although natural products are not immune to insect pest resistance, it has been shown that plant extracts that contain several bioactive compounds are more complex than synthetic pesticides, which delays the acquisition of pest resistance. Indeed, natural extracts are mixtures of secondary metabolites that have different insecticidal modes of action, which makes the process of insect resistance more complicated [22].

4. Conclusion

In the present study, we assessed the benefit of using natural extracts of a Lebanese plant and alga as insecticidal and repulsive agents against *Drosophila* fruit flies. Several extracts showed good activity, according to the delivery methods. These encouraging results allow us to consider further experiments to elucidate the phytochemical composition of the most active extracts and the identification of the bioactive molecules. Overall, this work constitutes a first interesting step

in the development of Lebanese *Spinacia oleracea* and *Ulva lactuca* extracts as natural, efficient, and affordable alternatives to currently used synthetic pesticides.

Acknowledgments

This work was funded by the laboratory of applied biotechnology, AZM Center for Research in Biotechnology and its Applications, Doctoral School of Science and Technology, Lebanese University.

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Tableau 1 : Les formules utilisées dans le calcul du teneur des pigments (en $\mu\text{g} / \text{ml}$ d'extrait) (Lichtenthaler & Wellburn, 1985).

Table 1: The Formula used for calculating the content of pigments (in $\mu\text{g} / \text{ml}$ plant extract) (Lichtenthaler & Wellburn, 1985).

Solvent	Corresponding Formulas
Acetone (100%)	Chlorophyll a : $11.75 A_{662} - 2.35 A_{645}$ Chlorophyll b : $18.61 A_{645} - 3.96 A_{662}$ Carotenoids: $\frac{1000 A_{470} - 2.27 \text{ Chl a} - 81.4 \text{ Chl b}}{227}$
Ethanol (96%)	Chlorophyll a : $13.95 A_{665} - 6.88 A_{649}$ Chlorophyll b : $24.96 A_{649} - 7.32 A_{665}$ Carotenoids: $\frac{1000 A_{470} - 2.05 \text{ Chl a} - 114.8 \text{ Chl b}}{245}$
<ul style="list-style-type: none"> • To calculate in mg / g DW (Dry Weight) we multiplied by the volume of the extract in ml and then we divided by the dry mass of the sample in gram • A = Absorbance at a specific wavelength • Chl a: Chlorophyll a • Chl b: Chlorophyll b 	

Tableau 2 : La quantité des pigments présente dans les extraits acétoniques et éthanoliques d'épinard (*Spinacia oleracea*) et d'algue verte (*Ulva Lactuca*), en mg/g PS. Les valeurs sont exprimées en moyenne \pm SEM de trois mesures parallèles.

Table 2: The amount of pigments presents in the acetonic and the ethanolic extracts of spinach (*Spinacia oleracea*) and green alga (*Ulva Lactuca*), in mg/g DW. All values are expressed as mean \pm SEM of three parallel measurements

Nature and origin of the extract		Chl t	Chl a	Chl b	Carotenoids
Acetone	<i>Spinacia oleracea</i>	0.68 \pm 0.13	0.52 \pm 0.00	0.16 \pm 0.13	0.16 \pm 0.10
	<i>Ulva lactuca</i>	0.13 \pm 0.02	0.07 \pm 0.01	0.05 \pm 0.01	0.006 \pm 0.001
Ethanol	<i>Spinacia oleracea</i>	0.55 \pm 0.12	0.43 \pm 0.11	0.12 \pm 0.01	0.10 \pm 0.01
	<i>Ulva lactuca</i>	0.07 \pm 0.01	0.039 \pm 0.001	0.034 \pm 0.010	0.12 \pm 0.0011

- Chl a: Chlorophyll a ; Chl b: Chlorophyll b ; Chl t: Total Chlorophyll (Chl a + Chl b)

Tableau 3 : Activité répulsive des extraits aqueux, éthanoliques et acétoniques des extraits issus d'épinard (*Spinacia oleracea*) et d'algue verte (*Ulva lactuca*) après 2h, 6h et 24h d'exposition. La répulsion est exprimée en pourcentage. ND : non déterminé vu que les mouches sont anesthésiées à cause de l'accumulation des vapeurs d'acétone dans le récipient. Les valeurs sont exprimées en moyenne \pm SEM de deux mesures parallèles.

Table 3: Repulsive activity of the aqueous, ethanolic and acetonic extracts derived from the spinach (*Spinacia oleracea*) and the algae (*Ulva lactuca*) after 2h, 6h, and 24h of exposure. The repellence is expressed in percentage. ND: Note determined as the flies are anesthetized by the accumulation of acetone vapours in the jars. All values are expressed as mean \pm SEM of two parallel measurements.

Nature and origin of the extract		Repellence (mean \pm SEM)			Mean repellency (%)
		2h	6h	24h	
Aqueous	<i>Spinacia oleracea</i>	76.7 \pm 4.7	86.7 \pm 9.4	100.0 \pm 0.0	87.8 \pm 1.6
	<i>Ulva lactuca</i>	35.6 \pm 7.9	43.5 \pm 5.0	59.4 \pm 0.8	46.2 \pm 4.0
Ethanolic	<i>Spinacia oleracea</i>	32.3 \pm 1.5	45.8 \pm 5.9	60.4 \pm 2.9	46.2 \pm 2.5
	<i>Ulva lactuca</i>	32.4 \pm 7.2	36.9 \pm 0.8	63.1 \pm 0.8	44.1 \pm 2.4
Acetonic	<i>Spinacia oleracea</i>	51.2 \pm 1.7	58.6 \pm 2.0	ND	–
	<i>Ulva lactuca</i>	46.2 \pm 1.1	52.1 \pm 9.4	ND	–

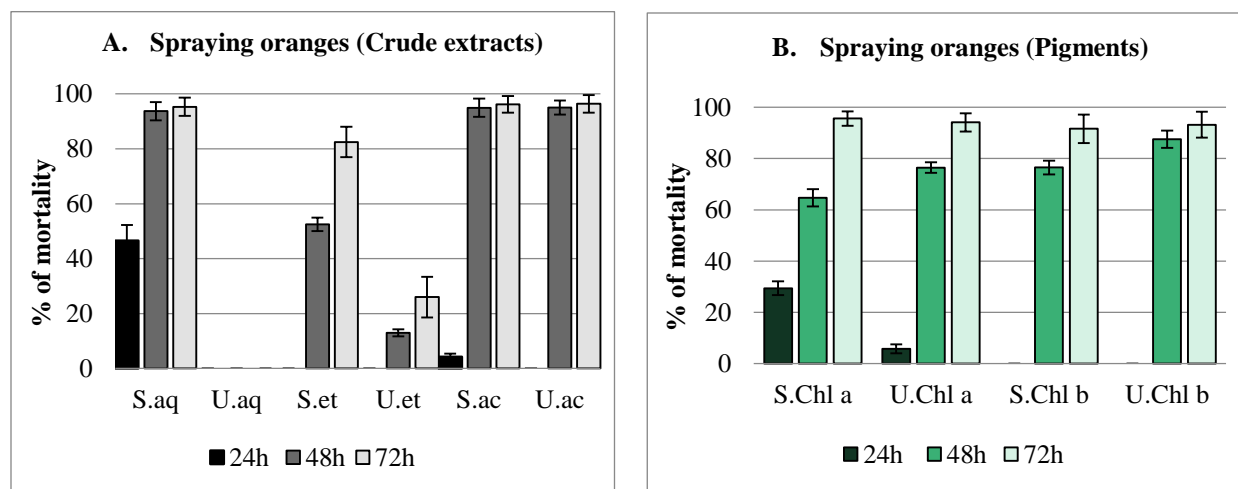


Figure 1: Histogram representing the variation of the mortality rate of flies with respect to the crude extracts' nature (A) and the pigments (B) sprayed on the oranges, originated from spinach (*Spinacia oleracea*) and green algae (*Ulva Lactuca*), after 24h, 48h and 72h of exposure. All values are expressed as mean \pm SEM of three parallel measurements. The percentage of mortality in negative control (acetone alone and methanol alone) was $10 \pm 5\%$. No mortality was recorded when the negative control was water or ethanol.

S.aq: Spinach aqueous extract, **U.aq:** Alga aqueous extract, **S.et:** Spinach ethanolic extract, **U.et:** Alga ethanolic extract, **S.ac:** Spinach acetonic extract, **U.ac:** Alga acetonic extract,

S.Chl a and **S. Chl b:** Chlorophyll **a** and Chlorophyll **b** purified from Spinach, **U.Chl a** and **U. Chl b:** Chlorophyll **a** and Chlorophyll **b** purified from alga.

Figure 1 : Histogramme représentant la variation du taux de mortalité des mouches en fonction de la nature des extraits bruts (A) et des pigments (B) pulvérisée sur les oranges, issus de l'épinard (*Spinacia oleracea*) et de l'algue verte (*Ulva lactuca*) après 24h, 48h et 72h d'exposition. Les valeurs sont exprimées en moyenne \pm SEM de trois mesures parallèles. Le pourcentage de mortalité dans le contrôle négatif (acétone et méthanol) était $10 \pm 5\%$. Aucune mortalité n'a été observée quand le contrôle négatif était l'eau ou l'éthanol.

S.aq : extrait aqueux de l'épinard, **U.aq :** extrait aqueux de l'algue verte, **S.et :** extrait éthanolique de l'épinard, **U.et :** extrait éthanolique de l'algue verte, **S.ac :** extrait acétonique de l'épinard, **U.ac :** extrait acétonique de l'algue.

S.Chl a and **S. Chl b:** Chlorophyll **a** et Chlorophyll **b** purifiés de l'épinard, **U.Chl a** and **U. Chl b:** Chlorophyll **a** et Chlorophyll **b** purifiés de l'algue verte.

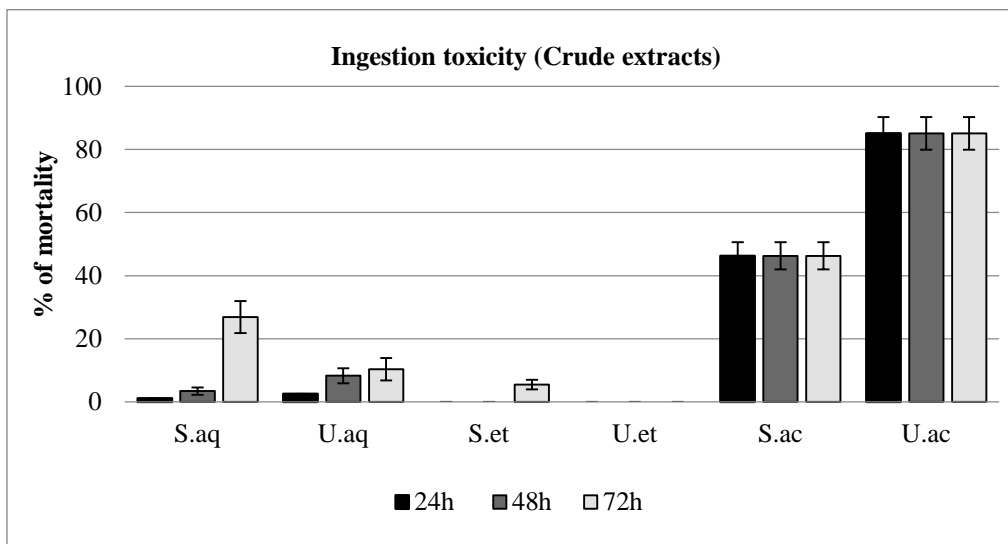


Figure 2: Histogram representing the variation of the mortality percentage of the flies with respect to the nature of the extract mixed with the food, after 24h, 48h and 72h of exposure. All values are expressed as mean \pm SEM of three parallel measurements. The percentage of mortality in acetone alone was $10 \pm$

5%. No mortality was recorded when the negative control was water or ethanol.

S.aq: Spinach aqueous extract, **U.aq:** Alga aqueous extract, **S.et:** Spinach ethanolic extract, **U.et:** Alga ethanolic extract, **S.ac:** Spinach acetonc extract, **U.ac:** Alga acetonc extract.

Purified pigments did not show activity in this test.

Figure 2 : Histogramme représentant la variation du taux de mortalité des mouches en fonction de la nature des extraits bruts (A) et des pigments (B) mélangés avec un morceau d'orange après 24h, 48h et 72h d'exposition. Les valeurs sont exprimées en moyenne + SEM de trois mesures parallèles. Le pourcentage de mortalité avec l'acétoe était $10 \pm 5\%$. Aucune mortalité n'a été enregistrée quand le contrôle négatif était l'eau ou l'éthanol.

S.aq : extrait aqueux de l'épinard, **U.aq :** extrait aqueux de l'algue verte, **S.et :** extrait éthanolique de l'épinard, **U.et :** extrait éthanolique de l'algue verte, **S.ac :** extrait acétonique de l'épinard, **U.ac :** extrait acétonique de l'algue. Les pigments purifiés n'ont pas montré d'activité insecticide par cette méthode d'évaluation.

Preparation of natural extracts and purified pigments



Green Seaweed
Ulva lactuca



Spinach
Spinacia oleracea

- **Extraction solvents:**

- Acetone
- Ethanol
- Water

- **Green pigments**

- **Carotenoids pigments**



Insecticidal activity



Fruit fly
Drosophila melanogaster

