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EVALUATION OF INSECTICIDE EFFECTS OF *Ruta chalepensis* ETHANOLIC EXTRACT ON MORTALITY, SEXUAL BEHAVIOUR AND OVIPOSITION OF *Drosophila melanogaster* (DIPTERA: DROSOPHILIDAE)

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ABSTRACT— The synthesis of pesticides has advanced significantly over the past century. Synthetic pesticides, on the other hand, have swiftly proven to be aggressive toward non-target creatures, such as helpful insects, animals, and people, in addition to their persistence in nature, which upsets ecological balances. This paper describes the toxic effects of *Ruta chalepensis* (*Rutaceae*) ethanolic extract on *Drosophila melanogaster* courtship and course of sexual behavior, mortality of *Drosophila melanogaster* larvae (determination of lethal concentrations and lethal times), and choice of oviposition environment and reproduction in this fly's adults. The plant's aqueous extract inhibits both mating and reproduction.

KEYWORDS: *Drosophila melanogaster*, *Ruta chalepensis*, ethanolic extract, mortality, sexual behavior, oviposition.

1. Introduction

Pesticides are a major factor affecting biodiversity, along with habitat loss and climate change. They can have short-term toxic effects on organisms directly exposed to them, or long-term effects. This has resulted in a marked decline in populations of many species living in agricultural areas [7]. Insecticides can enter the human food chain in many ways, but it is not only humans that are at risk, but also the environment and natural resources, such as soil and water [1].

Plants are therefore an interesting source of new compounds in the search for bioactive molecules. [13]. *Ruta chalepensis* is an aromatic plant, belonging to the Rutaceae family, commonly called by the local population "Fidjel". It is spontaneous, widely answered in North Africa, particularly in Algeria. It is frequently found in rock gardens, lawns and dry hillsides [8].

By examining immediate and delayed effects on D. melanogaster sexual behavior and oviposition, this study aims to assess the toxicity of R. chalepensis ethanolic extracts.

2. Materials and methods

2.1 Insect

It is an easy-to-grow, resilient, forgiving, and unobtrusive organism that can be utilized to address a variety of scientific questions. The wild strain was created from rotten apples that were found in the Algerian province of Annaba. Glass tubes of 9.5 cm in length and 2.25 cm in diameter are used for rearing, and they have a nutrient-rich agar media comprised of cornmeal and brewer's yeast within. The culture is maintained at a temperature of 25 1 °C, a humidity of 70 to 80 %, and a scotophase of 12 hours.

2.2 Ruta chalepensis

Ruta chalepensis is an aromatic plant, belonging to the *Rutaceae* family, commonly called by the local population "Fidjel". In North Africa, especially Algeria, it is frequently and spontaneously answered. It can frequently be found in arid hillsides, lawns, and rock gardens [8]. Pharmacological investigations have shown that the ethanoic extract of the plant of the aerial part of *Ruta chalepensis* has anti-inflammatory and antipyretic activity [21]. It contains essential oils, which are volatile chemicals with a distinct aroma that are made up of molecules generated by specific plants; the phrase "volatile" refers to how quickly essential oils evaporate. [23] particular specialized plant tissues, where it is generated and stored. They are in charge of the plant's distinctive odor [10].

2.3 Extraction

For the extraction of the leaves of R. chalpensis we macerated 84.70 g in powder in 200 ml of 70% ethanol for 24 hours at room temperature and in the shade. After filtration, the filtrate obtained was evaporated using a Rotavapor at a temperature of 50°C with speed number 2. A stirrer (a bar) was placed to eliminate the ethanol solvent. The paw recovered after maceration weighed 1.34 g of the paw, making a stock solution of 6.7 µg/ml which was kept at 4°C until use.

2.4 Treatment

The larvae were treated by ingesting medicine. 10 ml of the solution for each concentration was distributed over four tubes containing 40 g of food. From the bulk rearing, twenty early 2nd instar larvae (L2) were chosen at random and put into each tube. A fifth tube with no therapy is injected with twenty larvae as a control.

2.5 Effect on mortality

Five concentrations are examined after preliminary tests: 0.25/ 0.50/ 1/ 1,5/ 2 /and 2.5 g/ml. The larval development and death are observed throughout a 15-day period (time necessary to finish the development). Following that, fatal doses and timings (LC50, LC90, TL50, and TL90) were computed.

2.6 Effect on sexual behaviour and oviposition choice

80 pairs of control and sublethal-treated Drosophila (0.50 g/ml) that were isolated at emergence after four different crosses—control male x control female (20 pairs), treated male x control female (20 pairs), and treated male x treated female (20 pairs)—were used for sexual behavior assays (20 pairs).

These tests are conducted in a dry tube with ambient temperature and humidity in a room that is completely sealed off from any outside noise. We record which partner approaches the other, when the first contact occurs, and how many times it occurs. Additionally, the number of songs and licks used by the male to induce copulation is noted. The timing of several mating efforts that resulted in mating or did not result in mating, as well as the quantity and length of successful or unsuccessful courtships, are noted.

In plastic boxes with two culture media: control and treatment with 0.50 g/ml, placed at the bottom of each box, fertilized females are filtered out individually. These will be recovered after 48 hours so that the precision of the chosen medium may be used to count the eggs laid under a stereoscope.

2.7 Data analysis

Finney's mathematical formulas were used to calculate lethal concentrations (LC50% and LC90%) and lethal times (LT50% and LT90%) for the extract used in the toxicological investigation (Finney, 1971). We compared the variances of the "k" samples of the various parameters evaluated in this study using the statistical analysis application XLStat 2009. (Addinosoft NY).

3. Results

3.1 Effects on mortality

The results recorded in table (01) summarize the different toxicological parameters of the ethanolic extracts of *R. chalepensis*. These show that there is a strong positive correlation between the mortality rate and the exposure time to the ethanolic extract of *R. chalpensis* (R= 0.82 to 0.97). Our results indicate that the TL50% is 22.91 days for the low concentrations and 13.80 days for the highest concentration. The TL90% reaches 77.62 days for the 0.50 µg/ml concentration (TAB.1.A).

The results also show that there is a very weak positive correlation between the mortality rate and the concentrations of the ethanolic extract of R. chalepensis after 10 days of treatment (R=0.12). While a correlation between mortality and concentrations used from the 5th day until the end of the treatment was recorded. After 15 days of treatment the lethal concentration LC50% is 0.57 µg/ml while the LC90% is 208.93 µg/ml (TAB.1.B).

Table 1: Toxicological parameters of *R. chalepensis* towards *D. melanogaster*

A				
Concentrations	**Regression**	**R**	**LT50% (jours)**	**LT90% (jours)**
0,25 µg/ml	Y=-1,20+5,07X	0,97	16,59	*29,51*
0,50 µg/ml	Y=1,77+2,38X	0,84	*22,91*	*77,62*
1 µg/ml	Y=2,19+2,24X	0,82	*17,78*	*66,07*
1,50 µg/ml	Y=-1,23+5,43X	0,96	*13,80*	*13,99*
2 µg/ml	Y=-1,27+5,22X	0,95	*15,13*	*28,18*
2,50 µg/ml	Y=-1,26+5,51X	0,94	*13,80*	*23,44*
B				
Days	**Regression**	**R**	**LC50% (µg/ml)**	**LC90% (µg/ml)**
2	**Y=1,01-0,52X**	0,12	$2,1x10^{-8}$ µg/ml	$7,41x10^{-11}$ µg/ml
5	**Y=3,29+0,41 X**	0,78	$1,48x10^{4}$ µg/ml	$1,95x10^{7}$ µg/ml
10	**Y=3,82+0,89X**	0,42	20,89µg/ml	575,43 µg/ml
15	**Y=5,12+0,50X**	0,66	0,57µg/ml	208,93 µg/ml

3.2 Effect of R. chalepensis on sexual behaviour

The results obtained show that the longest time to first orientation is recorded in couples with control males and treated females (0.50µg/ml) with an average of 453.37 ±116.23 seconds, and the fastest mating time in the same couples with 476.50 ± 141.22 seconds. (Tab 2)

The results shown in table 2 show that in the treated male and female couples there is always a decrease in

the number of repetitions of the majority of the different sequences of sexual behavior (orientation 6.80 ± 1.37 touching 3.30 ± 053 vibration7.95 ± 1.25 licking 2.63 ± 0.49 and number 2.17 ± 0.36 of mating attempts). The average duration of mating in the control adults was recorded as 1999.88 ± 153.95 seconds (Tab. 2). The comparison of variances shows that there is no significant difference between the recorded times (Fobs: 0.745; p: 0.531).

Table 2: Effect of *R. chalepensis* (0,50 µg/ml) on courtship and different sequences of sexual behavior.

	First orientation time	First contact time	First vibration Time	First licking Time	First attempt Time	Coupling Time
♂C X♀C	**193,95 ± 66,96**	266,25 ± 68,78	273,55 ± 67,01	434,40 ± 83,77	442,60 ± 82,79	676,82 ± 107,17
♂*R.c* X♀*R.c*	**377,25 ± 92,37**	575,05 ± 113,96	433,60 ± 99,55	642,74 ± 122,58	672,56 ± 120,18	562,46 ± 135,17
♂ C X♀*R.c*	**453,37 ±116,23**	469,94 ± 107,91	479,53 ± 118,48	641,83 ± 117,27	639,75 ± 115,99	476,50 ± 141,22
♂*R.c* X♀C	**228,65 ± 62,19**	383,40 ± 95,14	281,75 ± 76,44	533,11 ± 99,67	638,00 ± 130,22	480,00 ± 145,87
F_{obs}	**3,503**	**2,681**	**3,086**	**1,482**	**1,641**	**0,234**
P	***0,019****	***0,053***	***0,032****	***0,227***	***0,188***	***0,872***

	Orientation number	Contacts number	Vibrations number	Licks number	Attempts number	Mating time
♂C X♀C	9,45 ± 1,30	4,85 ±0,57	17,30 ± 2,39	4,90 ± 1,17	3,80 ± 0,99	1999,88 ± 153,95
♂*R.c* X♀*R.c*	6,80 ± 1,37	3,30 ± 053	7,95 ± 1,25	2,63 ± 0,49	2,17 ± 0,36	1082,39 ± 133,66
♂ C X♀*R.c*	9,58 ± 1,31	6,22 ± 1,09	13,32 ± 2,18	2,77 ± 0,63	2,56 ± 0,56	948,00 ± 225,09
♂*R.c* X♀C	7,55 ± 1,77	3,75 ± 0,99	11,75 ± 2,66	2,74 ± 0,65	2,89 ± 0,98	1113,54 ± 129,13
F_{obs}	**0,579**	**2,932**	**3,952**	**1,411**	**1,207**	**0,745**
P	***0,630***	***0,039****	***0,011****	***0,247***	***0,314***	***0.531***

[♂C: Control Male**; ♂*R.c*:** Male treated with *R. chalepensis***; ♀ C:** Control female**; ♀ *R.c*:** Female treated with *R. chalepensis***]** (*: Significant, **: Highly significant, ***: Verry highly significant)

3.3 Effect on oviposition

For this part we kept the results of the crossbreeding of the control couples carried out in the previous part and we carried out three other crossbreeding: treated couples whose females are attracted by both environments (control and treated), however the statistical analysis gives an average of 37.80 ± 4.93 eggs laid by the females and 28.15 ± 2.33 larvae in the control environment, and in the treated environment we recorded an average of 28.35 ± 2.71 eggs and 22.10 ± 2.68 for the larvae (Tab 3).

While in the couples, where at least one of the two individuals was treated, a decrease in the number of larvae and eggs laid in the two environments was noted in comparison to that marked for the control couples. In the couples where the female was treated, 33.05 ± 4.08 eggs and 23.00 ± 2.72 larvae were counted in the control environment and 37.05 ± 4.23 eggs and larvae 23.25 ± 2.12. While for the couples where the male is treated 46.55 ± 4.62 eggs and 32.95 ± 3.87 larvae were recorded in the control medium and 28.05 ± 3.32 eggs and larvae 19.20 ± 2.18 (Tab 3).

Table 3: Effect of *R. chalpensis* (0,50µg/ml) on oviposition site selection in *D. melanogaster*.

Couples	The control eggs number	The treated eggs number	t_{obs}	*P*
♂C X♀C	**68,10 ± 6,63**	**46,75 ± 3,33**	***2,878***	***0,007****
♂*R.c* X♀*R.c*	**37,80 ± 4,93**	**28,35 ± 2,71**	***1,680***	***0,101***
♂ C X♀*R.c*	**33,05 ± 4,08**	**37,05 ± 4,23**	***-0,681***	***0,500***
♂*R.c* X♀C	**46,55 ± 4,62**	**28,05 ± 3,32**	***3,250***	***0,002****
Couples	**The control larvae number**	**The treated larvae number**	t_{obs}	*P*

♂C X♀C	**38,45 ± 3,03**	**26,80 ± 2,25**	***3,087***	***0,004***
♂***R.c*** X♀***R.c***	**28,15 ± 2,33**	**22,10 ± 2,68**	***1,700***	***0,097****
♂ C X♀***R.c***	**23,00 ± 2,72**	**23,25 ± 2,12**	***-0,072***	***0,943***
♂***R.c*** X♀C	**32,95 ± 3,87**	**19,20 ± 2,18**	***3,095***	***0,004****

[♂C: Control Male**; ♂*R.c*:** Male treated with *R. chalepensis***; ♀ C:** Control female**; ♀ *R.c*:** Female treated with *R. chalepensis***]** (*: Significant, **: Highly significant, ***: Verry highly significant)

4. Discussion

Insects play a variety of epidemiological roles, which makes them a serious public health issue [4], [14]. Generally speaking, neither D. melanogaster adults nor those of other drosophile species pose a threat to people [15]. Insecticide use has increased significantly over the past 50 years, causing significant environmental damage [19].

Significant advancements in the synthesis of pesticides were made last century. In addition to their persistence in nature, which disturbs ecological balances, synthetic pesticides have quickly demonstrated their aggressiveness toward organisms that are not targeted, including beneficial insects, mammals, and humans. Over the past few years, a new strategy has emerged. We have been evaluating alternative substances like biopesticides because of the environmental risks associated with the widespread use of conventional insecticides [24].

The focus of this study is the biological activity of aqueous and ethanolic extracts of the supposedly toxic plant in the context of the search for plant-derived bioactive molecules effective in the fight against the mouche of the vinaigre *D. melanogaster* and the *germanic leaf B. germanica*. Our goal in studying *R. chalpensis* is to ascertain both its toxic and differential effects on *D. melanogaster*. This ornamental plant is found in gardens; it is thought to be mellow and its presence keeps insects away. She pushes the insects away. [18] and is used to combat the tick parasites gale and the tick [9].

In this work, we tested the impact of the ethanol extract 0,25 µg/ml 0,50 µg/ml 1 µg/ml 1,50 µg/ml 2 µg/ml and 2,50 µg/ml Our ability to demonstrate that *D. melanogaster* larvae are sensitive to *R. chalpensis* extracts through eating on wine bottle corks allowed us to record an up to 100% larval mortality rate.

These results are consistent with previous research from several studies [16], [12]. The larvicide activity of the ethanolic extract of *R. chalepensis* is progressive since we see an increase in mortality as exposure time increases to a maximum level, and these mortality rates are strongly correlated with exposure time. These findings are similar to those reported by [3], [5] pertaining to plant effects *Nicotiana glauca* Graham (*Solanaceae*) on the mosquito *Culiseta longiareolata.* [16]. Indiquent une activité larvicide des extraits des plantes contre le moustique *Culex pipiens*. [20] have shown a toxic effect of P. harmala on mosquitoes.

The many behaviors of insects are primarily governed by a collection of nervous system-controlled motor actions in response to chemical cues from outside sources (food, sex pheromones, alarm pheromones, aphrodisiac secretions, gregarious pheromones,...) [17].

This study shown that the ethanolic extract of *R. chalepensis* has an impact on *D. melanogaster* fly mating and entirely alters adult *Drosophila* behavioral patterns. This might be caused by a change in the flies cuticular profile. Pairs treated with the ethanol extract of *Ruta chalpensis* in oviposition experiments showed a striking reduction in the quantity of eggs deposited.

Recently, several studies have been carried out indicating the effect of plants on sexual and oviposition behavior as well as on reproduction (fecundity and fertility) of insects; such as the inhibitory effect of *Peganum harmala* and *Daphne gnidium* on oviposition behavior and reproduction in *Culex pipiens* [2], [6]. The harmel plant is the most studied, studies indicate its effect on the sexual behavior of *B. germanica* [22] and feeding behavior, sexual behavior and oviposition behavior of *D. melanogaster* [15], [11].

5. Conclusion

We conducted a toxicological study using the ethanolic extract of *R. chalpensis* towards the larvae of the 2nd stage in order to determine the impact of this molecule on the vinegar fly. We demonstrated that treatment of the adults with the sublethal concentration (0,50 g/ml) of the plant extract disrupted the adult's ability to reproduce. The study demonstrates that treating adults disrupts the mating patterns of the species and the behavior of oviposition. It also demonstrates that treating adults causes a considerable drop in the quantity of eggs and larvae in the treated couples.

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