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Toxical Effect of Euphorbia Guyoniana Aqueous Extracts (Euphorbiaceae) on Mortality, Larval Development and Sexual Behavior of Drosophila Melanogaster (Diptera: Drosophilidae)

TOXICAL EFFECT OF *EUPHORBIA GUYONIANA* AQUEOUS EXTRACTS (*EUPHORBIACEAE*) ON MORTALITY, LARVAL DEVELOPMENT AND SEXUAL BEHAVIOR OF *DROSOPHILA MELANOGASTER* (DIPTERA: DROSOPHILIDAE)

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ABSTRACT

Euphorbia guyoniana (Boiss and Reut) is a plant of *Euphorbiaceae* family common throughout the northern Sahara and pre-desert regions. In traditional medicine, they are used in the treatment of various infections. *Euphorbiaceae* contain various chemical compounds families such as alkaloids, which give them a toxic effect. The toxic effects of seeds decoctions on mortality and development of 3rd instar larvae of *Drosophila melanogaster* are studied. A treatment by ingestion shows a good larvicidal activity of this extract. It was shown that the chemical compounds contained in the extract, act on the fly development cycle as most of pupae do not reach adulthood. We also studied the delayed effect of *E. guyoniana* aqueous extract on *D. melanogaster* sexual behavior. The ingestion of sublethal concentration (25µg/ml) affects nuptial courtship and different sexual behavior phases as well as mutual recognition of males and females.

Keywords*:* Dipetra, biopesticide, toxicity, development, sexual behavior.

INTRODUCTION

Biopesticides offer many advantages. They are ecologically much more compatible than chemicals and have increased specificity towards the species they are directed against (Deravel et al., 2014). These molecules are less persistent than their chemical counterparts and have lower toxicity to non-target species, supporting compatible use in integrated pest management programs (Deravel et al., 2014). In addition, biopesticides are often effective in low quantities and their bioactive molecules employ multiple modes of action, making them particularly attractive for limiting the emergence of resistant pests (Deravel et al., 2014). These products are gradually being employed in agriculture due to their low ecological impacts (Frost and Sullivan, 2009). Nevertheless, the market for biopesticides remains far below that of chemical plant protection products. Currently, all research on biological control is directed towards the use of plant extracts as bioinsecticides.

The Algerian flora is varied in the coastal regions, the mountainous massifs, the high plateaus, the steppe and the Saharan oases, of which more than 300 spontaneous plant species are found in the pre-Saharan region. A large part of these plant resources are found in a spontaneous state. The *Euphorbiaceae* family is among the five largest families of flowering plants with more than 6,000 species in 200-300 genera. In this study, the toxic effects of *Euphorbia guyoniana* were investigated, while determining the potential direct effects on larval mortality and development of *Drosophila* from the Annaba region (Algeria). *Drosophila melanogaster* is one of the harmful

cosmopolitan Diptera and widely used in scientific research. It is often visible throughout the year. The nuisance of this model comes from the fact that it can produce hundreds of insects on ripe fruits resistant to different living conditions in a more or less important time (Joly, 2006).

In this work, we search to test direct and indirect toxic effect of the *Euphorbia guyoniana* seeds extract (*Euphorbiaceae*; plant of Algerian Sahara) on the fruit fly *D. melanogaster*. The work objective is to evaluate the plant effect on mortality, development and sexual behavior of *D. melanogaster*.

MATERIALS AND METHODS

Insects

Drosophila melanogaster was described by Johann Wihelm Meigen in 1830. Its reproduction is very fast. Its life cycle is very short and includes three larval stages and a pupal stage from which emerges an adult that is able to fly and reproduce. A wild strain collected on rotten apples in the Annaba region (Algeria), is used. The rearing is performed in flasks (250 ml) stoppered with a foam pad and containing an agar-based nutrient medium made of cornmeal and brewer's yeast. The culture is maintained at 25±1°C, a humidity of 70 to 80% and 12 hours scotophase.

Euphorbia guyoniana

E. guyoniana is a laticious plant in the *Euphorbiaceae* family. It is common throughout the northern Sahara and pre-desert regions (Kemassi et al., 2013). In traditional medicine, they are used in many parts of the world in the treatment of several infections. It has caruncles-free, blackish seeds with gray longitudinal sides. The flowering is spread over the winter and spring seasons (Ozenda, 1991; Chehma, 2006). *Euphorbiaceae* contain various families of chemical compounds such as alkaloids, which give them a toxic effect (Kemassi et al., 2013). The plant was collected from the region of Ghardaia, Algeria (32° 29′ 00″ North, 3° 41′ 00″ East).

Preparation of E. guyoniana Seeds Extract

The plant used in this work is collected in the region of El M'Ghair (El-Oued, Algeria) (33° 57′ 02″ North latitude, 5° 55′ 27″ East longitude). The extraction of *E. guyoniana* seeds is done by decoction in distilled water and a stock concentration of (500 g/l) was prepared.

Effects on Mortality and Development

The insects were treated by ingestion.10 ml of the solution of the aqueous extract of *E. gyoniana* was put in 40 g of culture medium, which are then deposited in four tubes. In each tube, twenty larvae taken at random from the mass rearing, are immersed. Twenty other larvae are put in another tube containing only the control food, it is a control batch. We used 3 concentrations of *E. gyoniana* (25 µg/ml; 50 µg/ml; 200 µg/ml). The monitoring of mortality and development of the larvae is done during 15 days (time necessary to finish the development).

Effects on Sexual Behavior

We treated a group of larvae with the sublethal concentration of the aqueous extract of *E. guyoniana*, we established sexual behavior tests in order to measure the effect of these molecules on the sexual display of flies. For this study, the treatment was carried out with the use of a single concentration of *E. guyoniana* which is (25 µg/ml). The treatment with this sublethal concentration is done on third instar larvae and daily the adults are isolated as soon as they emerge. 48 hours after emergence these adults are used for sexual behavior tests where the time and

number of contacts, the time and number of wing vibrations, the time and number of licks, the time and number of mating attempts as well as the time and duration of mating if successful are noted. These tests are conducted in four types of crosses: control male X control female, treated male X treated female, control male X treated female and treated male X control female.

Data Analysis

Lethal concentrations and lethal times (LC16 %, LC50 %, LC90 %, TL16 %, TL50 %, and TL90 %), are calculated using the mathematical procedures of Finney in 1971. The data are transformed and normalized according to the Bliss tables. The calculations are performed on XLStat 2009.

RESULTS

Effects on Mortality

Table 1 shows the mortality rate evolution in *D. melanogaster* individuals treated with *Euphorbia* aqueous extracts in second larvae stage. Using 25 µg / ml of extract, the plant only causes 1.25% mortality after 5 days; this rate gradually increases until the end of treatment (Tab. 1). For 50 µg / ml concentration, the average larval mortality rate stabilizes at 2.5% for 5 and 10 days and increases from 23.75% to 15 days (Tab. 1). Mortality increases when the highest concentration (200 µg / ml) is used; 6.25% at 5 days and 28.75% at 10 days. More than 95% of insects die after 15 days (Tab. 1). We note that no mortality was observed in controls. At significance level $\alpha = 0.05$, the variances comparison shows that there are no significant differences between mortality recorded for the three concentrations used after 5, 10 and 15 days (Table 1).

The results recorded in Table 2 summarize the different toxicological parameters of aqueous extract of *E. guyoniana* seeds. These show that there is a strong correlation between the mortality rate and the exposure time of the flies to different extracts concentrations (the correlation coefficient "R" is from 0.86 to 0.90) (Table 2).

The lethal concentrations obtained after treatment, are 33.35 µg/ml, 67.61µg/ml, 144.54µg/ml and 165.96 µg/ml for LC16 %, LC50 % LC84 % and LC90%, respectively (Table 2). The lethal times, are 9.12 days for LT50% and 21.38 days for LT90 % (Table 2); For LT84 % and LT16 % it's between 18.62 and 4.68 days, respectively (Table 2).

Table 1: Corrected mortality rate in *D. melanogaster* after treatment with *E. guyoniana* seeds aqueous extract.

	25µg/ml	**50µg/ml**	**200µg/ml**	$\mathbf{F_{obs}}$	***p***
5 Days	1,25 %	2,50 %	6,25 %	***0,18***	***0,84***
10 Days	5 %	2,50 %	28,75 %	***0,096***	***0,91***
15 Days	11,25 %	23,75 %	95 %	***1,6***	***0,25***
$\mathbf{F_{obs}}$	***0,053***	***15,58***	***0,138***		
p	***0,983***	***0,0002******	***0,936***		

p < 0.05: significant; p < 0.01**: highly significant; p < .001***: very highly significant*

Table 2: Toxicological parameters of *E. guyoniana* seeds aqueous extract on *D. melanogaster*

Lethal Concentrations (µg/ml)					
	Regression	**LC 16%**	**LC 50%**	**LC 84%**	**LC 90%**
After 5 days	Y= 1,73 + 0,75 X ; **(R=0,998)**	***1071.52***	***22908,68***	***630957.34***	***1174897,55***
After 10 days	Y= 1,16 + 1,36 X ; **(R= 0,85)**	***123.03***	***660,69***	***4168.69***	***5754,40***
After 15 days	Y= -0,99 + 3,27 X ; **(R= 0,99)**	***33.35***	***67,61***	***144.54***	***165,96***
Lethal times (Days)					
	Regression	**LT 16%**	**LT 50%**	**LT 84%**	**LT 90%**
25 µg/ml	Y= 2,26 + 1,15 X ; **(R= 0,90)**	***33,11***	***239,88***	***2089,30***	***3162,28***
50 µg/ml	Y= - 0,97 + 4,48 X **(R= 0,94)**	***12,88***	***21,38***	***37,15***	***21,38***
200 µg/ml	Y= 1,68 + 3,47 X **(R= 0,86)**	***4,68***	***9,12***	***18,62***	***21,38***

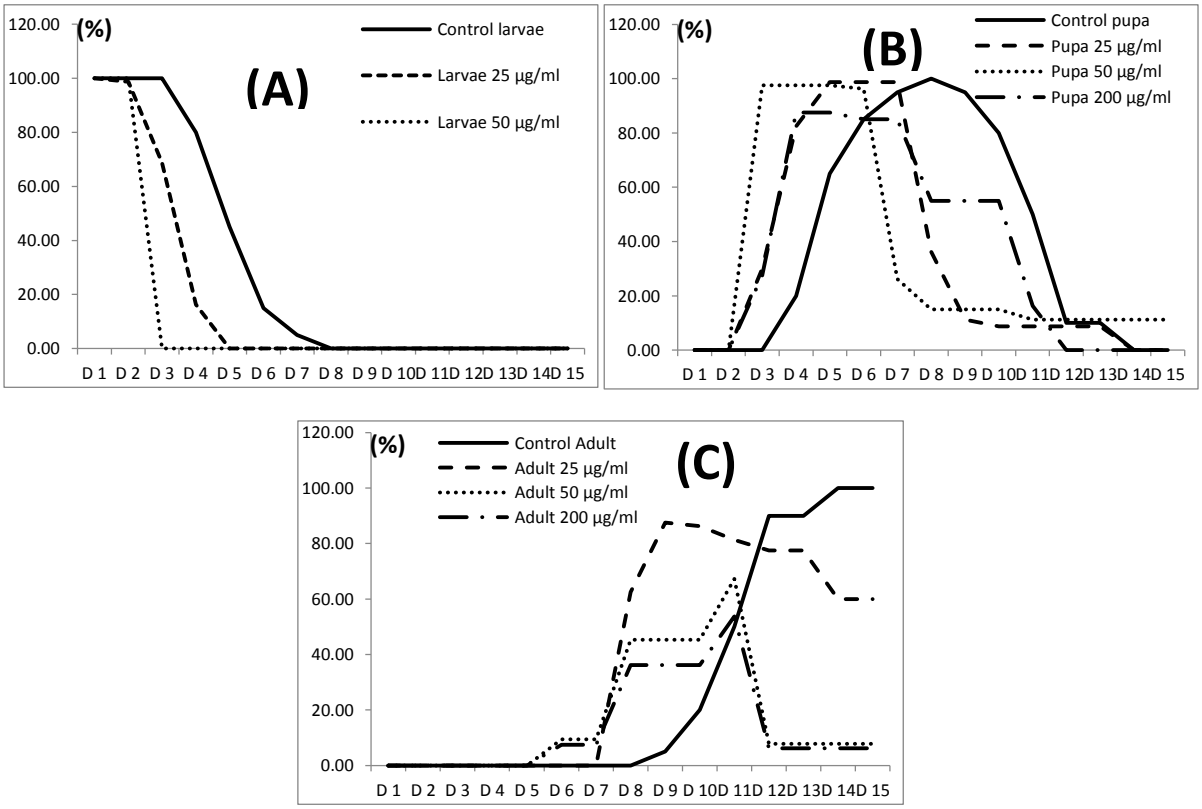

Figure 1: Effect of E. gyoniana seeds aqueous extracts on D. melanogaster development
***(A:** larval development**; B:** pupal development**; C:** adult development)*

Table 3: Effect of *E. gyoniana* seeds aqueous extracts on different sexual behavior sequences (Mean ±SEM).

	First contact time	First vibration Time	First licking Time	First attempt Time	Mating time
♂C X♀C	7.4±1.78	324.78±75.76	459.11±122.58	241.67±50.99	804.17±302.60
♂*E.m*X♀*E.m*	352.61±83.92	481±101.31	672.77±152.40	352.61±83.92	1336.5±261.5
♂CX♀*E.m*	4.10±1.21	625.60±168.90	727.11±198.14	659.60±173.54	946.86±224.73
♂*E.m*X♀C	9.90±1.86	221.33±148.46	576.62±195.58	292.60±157.14	818
F_{obs}	***0.81***	***2.21***	***1.18***	***4.54***	***2.11***
p	***0.49***	***0.101***	***0.33***	***0.008****	***0.15***
	Contacts Number	**vibrations Number**	**licks Number**	**attempts Number**	**Duration of mating**
♂C X♀C	241.67±50.99	11±8.07	8.44±2.66	8.56±2.42	852.60±53.42
♂*E.m*X♀*E.m*	352.61±83.92	9.4±2.52	8.30±2.40	10.17±2.94	9077.50±7471.50
♂CX♀*E.m*	659.60±173.54	5.20±1.22	4.33±1.46	4±1.27	1386±103.46
♂*E.m*X♀C	292.60±157.14	32.4±6.01	3.25±1.13	2.75±0.94	1702
F_{obs}	***4.54***	***6.18***	***7.01***	***9.72***	***2166.82***
p	***0.008***	***0.001****	***0.001****	***<0.0001******	***<0.0001******

[Mean: Average; SEM: Standard deviation of the mean; ♂Tm: Control male; ♂E.m: Male treated with E. gyoniana; ♀Tm: Control female; ♀ E.m: Female treated with E. gyoniana]

[p < 0.05: significant; p < 0.01**: highly significant; p < .001***: very highly significant]*

Effects on Development

E. gyoniana acts significantly on the fly development by inducing an acceleration in the larvae growth into pupae for all three concentrations. For adult stage, extract activity on development is recorded; only 80 % of the population treated with *E. gyoniana* seeds aqueous extracts reaches the adult stage (Figure 1).

Effect on Sexual Behavior

We recorded that treated couples (composed of treated males and females) take less time to establish the first contact between the two partners. The study shows that there are significant differences between the time of this mutual recognition F_{obs}= 0.81; *p*: 0.49 (Tab. 3). The same result is observed for the different times recorded during the sexual parade of *D. menlanogaster:* the first vibration time *p*: 0.101; the first licking time *p*: 0.33; for the first attempt time *p*: 0.008**; and mating duration *p*: <0.0001*** there are highly significant differences (Table 3).

The extract of the *E. gyoniana* plant has a highly significant influence on the contacts number between flies F_{obs}= 4.54; *p*: 0.008, on the wing vibrations number F_{obs}= 6.18; *p*: 0.001** as well as on the licks number F_{obs}=7.01; *p*: 0.001** (Table 3).

DISCUSSION

Insect control is entering a new "botanical" phase that provides molecules that are non-toxic to non-target organisms, biodegradable, less likely to cause resistance in target species, and appear to be one of the means in better harmony with the environment (Philogene, 1991). Natural plant extracts contain a wide variety of secondary metabolites to which

various biological and toxicological activities are attributed.

For our study on the effect in *D. melanogaster* mortality, we used larvae in the 3rd stage to evaluate the *E. gyoniana* seeds aqueous extract (with different concentrations and during different periods). The fly reveals a sensitivity which is indicated by mortality rates up to 95 % for high concentrations (25 µg/ml, 50 µg/ml and 200 µg/ml). The larvicidal activity of the *E. gyoniana* seeds aqueous extract is progressive since we record an increase in mortality as we advance in the exposure time to reach a maximum mortality rate, these mortality rates are strongly correlated to the exposure time. Other studies show that plant extracts can have an intense insecticidal activity on *Drosophila* such as Habbachi et al., (2013) when using *Peganum harmala* (*Zygophyllaceae*) extracts; where this work shows that the plant seeds cause more mortality compared to leaves. We also note, the works of Habbachi et al., (2019; 2020) when using the *Cleome arabica L.* extracts (*Capparidaceae*) against *D. melanogaster* larvae and Saadane et al., (2021) concerning the fly control using *Drimia maritima* extract.

Also, several studies have highlighted the toxic effect of North African aromatic plants on Diptera such as the effect of *Ricinus communis* and *Tetraclinis articulata* on mosquito larvae *Culex pipiens* (Linnaeus), *Aedes caspius* (Pallas), *Culiseta longiareolata* (Aitken) and *Anopheles maculipennis* (Meigen) (Aouinty et al., 2006) and those of Benhissen et al., 2018 when controlling *Culiseta longiareolata* with the plant *Nicotiana glauca*. Other studies show that plants from the Maghreb drylands are the most effective even against the most resistant insects such as locusts (Idrissi Hassani and Hermas, 2008; Idrissi et al., 1998; Idrissi Hassani., 2000; Lebouz., 2010; Kemassi et al., 2014) or domestic cockroaches (Masna, 2016). A recent study by Badalamenti et al., (2020) shows that the plant *Drimia pancration* (*Asparagaceae*) exhibits insecticidal activity against adults of *Stegobium paniceum* (Anobiidae).

In this study, treatment shows that the plant also influences this Diptera development since only 90 % of the treated larvae reach the adult stage. *E. gyoniana* causes acceleration in the larvae growth into pupae for the three concentrations chosen; it seems that the larvae do not consume the medium treated with this plant. Deepak et al., (2003) have also mentioned that the similar plant is not consumed by either humans or animals due to its toxicity. Hence, the *Drimia indica* oral administration in any form may be a matter of serious concern, and further toxicity studies are warranted before its use as an oral medicine (Aswal et al., 2019).

The plants may not kill the insects but they do block their reproduction. This effect type was observed in the fly *D. melanogaster* after treatment with *P. harmala* (El-Bah et al., 2016). The studies of Habbachi et al., (2019; 2020) show that aqueous and ethanolic extracts of *C. arabica* cause mortality up to 50 % in the fruit fly but acts significantly on the *Drosophila* sexual behavior (nuptial courtship). In a study Saadane et al., (2021), *D. maritima* bulbs disrupt the different sequences leading to mating. Our study shows that *E. gyoniana* seeds aqueous extract induces a blockage of sexual courtship and/or mating. This blockage is, especially, remarkable when one of the two partners is treated; this may be due to the mutual non-recognition between insects especially as the plant acts significantly on the contact sequence in the fly. *E. guyoniana* influences the sequence of mutual recognition between the two individuals (orientation and contacts), and consequently the plant also acts on several courtship sequences (singing, licking and attempting).The contacts role in partners mutual recognition has been proven in different insect species such as

cockroaches (Roth and Willis, 1952; Farine et al., 1993; Gropeaux, 1994).

Plant toxins are one of the most environmentally friendly control agents, some of which are involved in neuroendocrine regulation, metamorphosis and reproduction of insects (Philogene, 1991; Rembold, 1994). Male mate choice experience was not simply a measure of male preference; successful copulation also requires that the target female be receptive (Somashekar et al., 2011). Sexual behavior of both males and females is influenced by pheromones. The Gr68a gene is expressed in specific neurons in the forelegs of males and is recognized as a receptor for pheromones. Inactivation of the GR68a gene results in a reduction in male courtship performance (Bray and Amrein, 2003). A second candidate, Gr32a, is also involved in phenomenal recognition (Miyamoto. and Amrein, 2008). Recently a receptors family has been described (Flybase PPKs gene family). This family constitutes a new class of pheromone receptors (Thistle et al., 2012). Dickson and Delmir (2005), demonstrated that sexual behavior in Drosophila was conditioned by a single gene, fru (fruitless), a gene spliced differently depending on the individual sex. The two researchers, from the Institute of Molecular Biotechnology of the Austrian Academy of Sciences, recreated two artificial alleles, male and female, reproducing the spliced versions of the gene, and then implanted them in individuals of the opposite sex. The behavior of the flies was completely reversed.

The *E. gyoniana* toxic effects have also been demonstrated against other insect pests that show strong resistance to insecticides. We cite the work of Kemassi et al., 2013 when using *E. gyoniana* aqueous extracts against the *Tribolium castaneum* imagos (Coleoptera).

CONCLUSION

In this work, we initiated a protocol that has for objective to study the toxic effect of *E. guyoniana* aqueous extracts on *D. melanogaster* sexual behavior. We showed that treatment with the sublethal concentration 25 µg/ml disrupted fly sexual behavior. The study shows that adults' treatment disrupts all sequences leading to specie mating; these sequences are mainly orientation, contacts (recognition), wing vibrations, licking and mating attempts. We found that *E. guyoniana* affects mating; the mating success rate is higher in controls than in treated pairs. Chemical analysis of treated and control flies can tell us about any changes in the hydrocarbon profile of *D. melanogaster*.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

AUTHORS CONTRIBUTION

All authors contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript. All authors read and approved the final version.

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