

Toxic effects of *Cleome arabica* L. (Capparidaceae) aqueous extracts on mortality and sexual behavior of *Drosophila melanogaster* (Diptera: Drosophilidae)

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Abstract *Cleome arabica* is a spontaneous plant in arid zones, toxic and has hallucinogenic effects. In this work, we seek to evaluate the direct and delayed effect of the aqueous extracts on the vinegar fly *Drosophila melanogaster*, laboratory model par excellence. Treatment is by ingestion on second instar larvae (L2). The results show that mortality rates can reach 90% after 15 days of treatment as we recorded a disturbance of fly development. Observation of sexual behavior in treatment-emergent adults indicates that *C. arabica* acts negatively on the course of the different sequences leading to mating in *Drosophila*.

Keywords: courtship, mating, Saharan plant, toxicity, Vinegar fly

Introduction

Currently, a big effort is doing to control insects-pests by several methods (physical, biological or chemical method). In fact, the global production loss for arthropod pests is estimated at 15% (Pimentel and Williamson 2008); in addition, post-collect losses in sub-Saharan Africa would be \$ 4 billion per year for total annual production estimated at \$ 27 billion (2005–2007 annual average) (FAO 2011).

Chemical products used against insects have not been able to completely stop some harmful. In addition, they have increased the environmental record by animals and plants intoxication. The management of these problems has prompted researchers to move towards biological control in its various forms. New methods, as well as new products, are constantly being sought. In addition, to contribute to sustainable environmental management, the introduction of new alternatives for pest control is further encouraged (Louat 2013).

Despite this wide range of effective molecules, the main pests have developed more or less pronounced resistance towards them; this has led to more and more people turning to natural compounds from plants for the development of new insecticidal molecules. The insecticidal activity of aromatic plant extracts has also been confirmed by Jang et al 2002; Habbachi et al 2014; Mahmoudian et al 2002; Habbachi et al 2013; Idrissi-Hassani et al 1998; Abbasi et al 2003; Abbasi et al 2003b; Idrissi-Hassani and Hermas 2008; Abbasipour et al 2010; Masna et al 2016; Benhissen et al 2018).

Plant extracts have been used as insecticides by humans before Romans time, this practice continues to exist with many plants species known for their insecticidal properties (Balandrin et al 1985; Isman 2002). Plant products can be degraded more rapidly in the environment than synthetic compounds, and some may have increased specificity that may favor beneficial insects for the plant (Desneux et al 2007).

The Algerian Sahara has an exceptional floristic biodiversity, consisting of more than 500 species, of which there are 162 endemic species in the northern Sahara alone and to which is added a tradition of traditional pharmacopeia (Ozenda 1991). Several species are known for their remarkable therapeutic properties (Quezel 1978). Spontaneous plants in arid area are considered as phylogenetic resources of agronomic, economic, ecological and strategic interest (UNESCO 1960).

In this work, we search to test direct and indirect toxic effect of the *Cleome arabica* aqueous extract (Capparidaceae; plant of Algerian Sahara) on the fruit fly *Drosophila melanogaster*. The work objective is to evaluate the plant effect on mortality, development and sexual behavior of *D. melanogaster*. *Drosophila* is the best known and most studied in the laboratory among all beings (Tracqui et al 2003). It is

responsible for the fruits gray mold via the mushrooms that it transports them. Larvae can cause intestinal irritation or diarrhea if swallowed by eating infested fruit.

Materials and Methods

Insect

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 who is able to fly and reproduce.

Mass breeding

A wild strain recuperated on rotten apples in Annaba region (Algeria) was used. The culture is carried out in tubes (12 x 4 cm) clogged with a foam pad and containing a nutrient medium agar based cornmeal and brewer's yeast. The breeding was maintained at 25 ± 1 °C, a humidity of 70 to 80% and a scotophase of 12 hours.

Cleome arabica (Capparidaceae)

It is a toxic plant with foul smell and hallucinogenic effects (Gubb 1913; Ozenda 1991). *C. arabica* is used in traditional medicine as a diuretic and against rheumatism; it is also a therapeutic and anti-bacterial plant (Ladhari et al 2013).

Species effect has also been proven against different orders of insects (Doumandji-Mitiche and Doumandji 1993; Ozenda 1991; Koïta et al 2012; N'Guessan et al 2009). In this study, the aerial plant parts were collected in the Bousaada region (M'sila, Algeria, N 35 ° 23'28 ", E 04 ° 18'07", 406 m altitude). The plant was determined by Dr. Khellaf REBBAS (botanist at University of M'sila) using the Algerian flora of Quezel and Santa (1962-1963) (Figure 1).

C. arabica aqueous extract

For extraction, we put 250g fresh leaves in 500ml of distilled water and boiled for 30 minutes on hotplate (180 ° C). The mixture obtained is filtered using filter paper and 250 ml of the filtrate are recovered (1 g/L stock solution).

Treatment of larvae with *C. arabica* extract

We have prepared four different concentrations 35µg / ml, 70µg / ml, 100µg / ml and 200µg / ml. The extracts are ingested; each concentration is mixed with 40g of food that will be divided into four different tubes. In each tube, 20 second stage larvae are placed. A control tube contain 20 second stage larvae and it doesn't treated by *C. arabica* extracts. The monitoring of mortality and larvae development is done during 15 days (time necessary to finish the development).

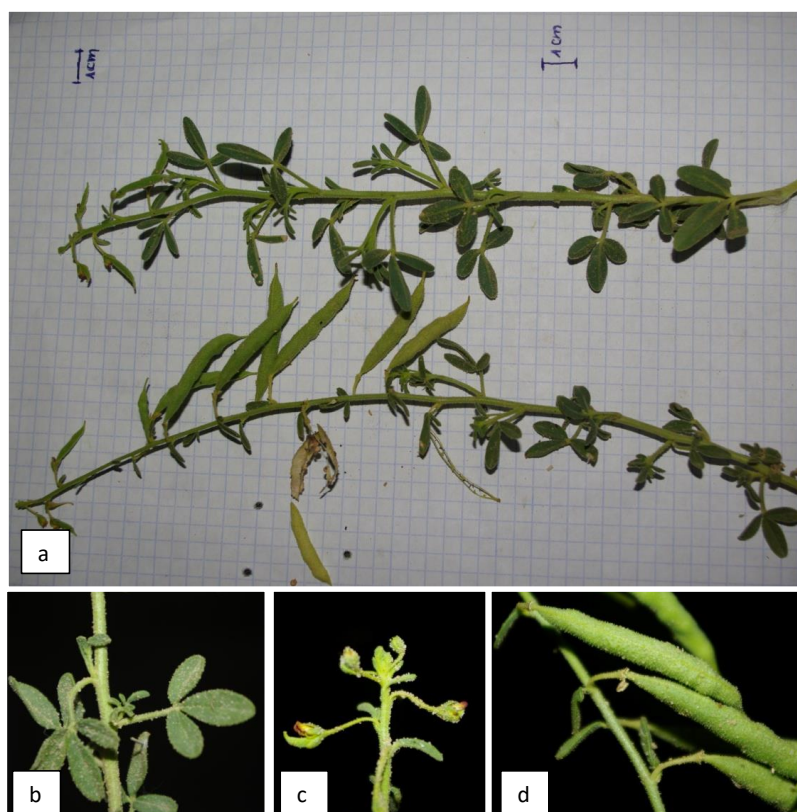


Figure 1 *Cleome arabica* L. (a. Plante, b. Leaf, c. Flowers, d. Fruit)
Bousaada, August 26, 2017, Source: Rebbas K

Effect of the plant extract on sexual behavior

Courtship behavior is a succession of predetermined and invariable actions (Clynen et al 2011; Chardonnet 2013). When a male encounters a virgin female, he orients his body in her direction and vibrates alternately each of his wings at a 45° angle to his main body axis. This vibration is a species-specific acoustic signal ('love song'; Bennet-Clark and Ewing 1970; von Schilcher 1976). When she is receptive, the female allows male to lick her genitalia with his proboscis. Then the male attempts copulation by thrusting his genital apparatus in an attempt to grasp the female's genitalia (Lasbleiz et al 2006; Revadi et al 2015).

In this work, we treated larvae with the sublethal concentration of *C. arabica* extract (35 µg/ml) and we recovered adults from their emergence. 48 hours later, these adults will be used for sexual behavioral tests and we note time and contacts number, time and vibrations number, time and licks number, time and attempts number as well as, time and mating duration if is success. These tests are carried out according to four crosses types: control male X control female, treated male X treated female, control male X treated female and treated male X control female.

Data analysis

The toxicological parameters (LC50%, LC90%, LT50% and LT90%) were calculated according to Finney's mathematical methods (Finney 1971). Regarding sexual behavior test, results were analyzed statistically by descriptive metric methods then a variances analysis (ANOVA) was performed on XLSTAT 2009 software (Addinsoft, New York, NY).

Results

Effect on mortality

The results show that *C. arabica* aqueous extracts act on larval development time and larval mortality as a function of applied concentration. The concentration 35 µg/ml has a low larvicidal activity. For the three concentrations (70, 100 and 200 µg/ml), 50% of the population is killed after 15 treatment days. There is a significant difference between mortality rate recorded for 35 µg/ml concentration ($P = 0.02$), while there is no time effect on mortality for the other concentrations (Table 1).

Table 1 Mortality rate caused by different concentrations of *C. arabica* extracts.

	35 µg/ml	70 µg/ml	100 µg/ml	200 µg/ml	F _{obs}	P
2 days	1.25	1.25	2.5	1.25	0.33	0.80
5 days	2.5	1.25	2.5	1.25	0.67	0.59
10 days	2.5	11.25	20	8.75	0.11	0.95
15 days	28.75	40	55	50	1.53	0.26
F _{obs}	4.98	0.71	0.58	0.47		
P	0.02*	0.56	0.64	0.71		

[($P < 0.05$) *: significant; ($P < 0.01$) **: highly significant; ($P < 0.001$) ***: very highly significant]

The larval mortality rates are weakly and positively correlated with extract concentrations used (Table 2A). The 50%lethal concentration reached 2818.38 µg / ml at 10 days and 131.83 µg / ml at 15 days exposure (Table 2A). 90% of larvae die with the 5011.87 µg / ml concentration after 15 days (Table 2A).

For lethal times, the results show that there is a strong positive correlation between the mortality rate and the exposure time of larvae to plant extracts (Table 2B). Our results indicate that LT50% is 81.28 days for low concentrations and 25.70 days for the highest concentration. The LT90% reaches 91.20 days for the highest concentration (Table 2B).

Effect on the sexual behavior of *D. melanogaster*

Effect on mating success rate

The results show that *C. arabica* aqueous extract administered at a sublethal concentration (15 µg / ml) delete fly mating, regardless of the sex treated in a couple (dyads) (Table 3). The mating success rate is 70% in controls, whereas it reaches 25% when males are treated with the Saharan plant (Table 3). The aborted mating rate (couples attempting to mate unsuccessfully) and null mating (no attempt or mating) is more important in couples composed by treated males and control females (Table 3).

Effect on the different sequences leading to mating

We recorded that the control and treated couples put, essentially, the same time to establish the first contact between partners. The same result is observed for the different times recorded during the *D. melanogaster* courtship (first vibration time $P=0.09$, first licking time $P=0.71$, first attempt time $P=0.55$, mating time $P=0.25$, mating duration $P=0.78$) (Table 4).

The extract of the *C. arabica* plant has a significant influence on the number of contacts between flies ($F_{\text{obs}} = 12.18$, $P<0.0001$), on the number of wing vibrations ($F=6.64$; $P=0.000$) and the number of licks ($F=6.350$, $P=0.001$) (Table 4).

Table 2 Mean values of thermographic records of body surface temperature ($^{\circ}\text{C}$) performed on the left and right sides of the crossbreed cows and calves, according to the times of day.

A									
	Right of regression	LC 50%	LC 90%	LC84%	LC16%	SLOPE	JLC50%	lim.Inf	lim.Sup
2 days	$Y=2.71+0.0649$ ($R=0.16$)	1.95E+35	1.02E+55	3.47E+50	1.07E+20	1.80E+15	230.31	8.47E+32	1.20E+22
5 days	$Y=3.38+0.25X$ ($R=0.55$)	3019951.72	3.98E+11	2.75E+10	331.13	9113.12	4.10	735945.29	12392372.74
10 days	$Y=1.93+0.89X$ ($R=0.59$)	2818.38	77624.71	36307.8	218.78	12.88	1.49	1897.22	4186.80
15 days	$Y=3.28+0.81X$ ($R=0.84$)	131.83	5011.87	2238.72	7.94	16.79	1.55	85.17	204.04
B									
	Right of regression	LT50%	LT90%	LT84%	LT16%	SLOPE	JLT50%	lim.Inf	lim.Sup
35 $\mu\text{g/ml}$	$Y=2.11+1.51X$ ($R=0.76$)	81.28	575.44	371.53	18.2	4.52	1.13	72.05	91.70
70 $\mu\text{g/ml}$	$Y=1.78+2.19X$ ($R=0.88$)	29.51	112.2	83.18	10.47	2.82	1.09	27.16	32.06
100 $\mu\text{g/ml}$	$Y=1.96+2.36X$ ($R=0.89$)	19.5	67.61	51.29	7.41	2.63	1.08	18.05	21.07
200 $\mu\text{g/ml}$	$Y=1.68+2.35X$ ($R=0.85$)	25.7	91.2	67.61	9.77	2.63	1.08	23.79	27.77

Means followed by the same letter do not differ statistically from each other: upper case in the row and lowercase in the column (Tukey's test; $P<0.05$).

CV: Coefficient of variation

Discussion

Insect control is entering a new botanical phase that provides non - toxic molecules for non - target organisms that are biodegradable and less likely to cause resistance in target species. Medicinal plants have an indefinite source of powerful bio-molecules, pharmacological, cosmetic and food (Duke et al 2008). In this study, we used the aqueous extract of *C. arabica* leaves as a bio-insecticide.

Our results show that *C. arabica* aqueous extracts cause mortality in *D. melanogaster* larvae. As well as the results of Habbachi et al (2013; 2014) and El-Bah et al (2016), in this *Drosophila* strain, the response to extracts of Algerian spontaneous plants (*Peganum harmala* and *Daphne gnidium*) is function of concentration and exposure time of the insects to the plant extract.

C. arabica showed its bioinsecticidal effect against *Ectomyelois ceratonia* Zeller (Lepidoptera) larvae using aqueous extracts; the effect of *C. arabica* aqueous extract at 15%, starts from the first treatment day, however the impact

of this extract at 5% appears 48 hours later (Korichi Almi et al 2016). Similar work demonstrates *C. arabica* insecticidal effect on third instar larvae of *Spodoptera littoralis* (Mediterranean brocade) (N'guessan et al 2009; Ladhari et al 2013).

Several works have highlighted the toxic effect of North African aromatic plants on Diptera such as the *Ricinus communis* and *Tetraclinis articulata* effect on larvae of mosquitoes: *Culex pipiens* (Linnaeus), *Aedes caspius* (Pallas), *Culiseta longiareolata* (Aitken) and *Anopheles maculipennis* (Meigen) (Aouinty et al 2006) and those of Benhissen et al 2018 in *Culiseta longiareolata* controlled by *Nicotiana glauca*. Other studies show that plants in Maghreb arid zones 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 and Oued El-Hadj 2014) or domestic cockroaches (Masna et al 2016).

Table 3 *C. arabica* effects (15 µg/ml) on the success rate of *D. melanogaster* mating.

	% Mating		
	Successful	Aborted	Nul
C♂ x C♀	70%	20%	10%
C♂ x C.a♀	55%	10%	35%
C.a♂ x C♀	25%	30%	45%
C.a♂ x C.a♀	55%	10%	35%

[C♂: Control male; C♀: Control female; C.a♂: treated male by *C. arabica* extracts; C.a♀: treated female by *C. arabica* extracts]

C. arabica with their secondary metabolites causes, also, physiological disturbances. Alkaloids dissuade phytophagous insects, affect the nervous system and cell division (Nabors 2008), and have a toxic and paralyzing effect on insects (Regnault-Roger et al 2005).

Plant-based toxicants are one of the most environmentally friendly means of control, some of which are involved in neuroendocrine regulation, metamorphosis, and insect reproduction (Philogene 1991; Rembold 1994). The experience of choosing the male partner was not simply a measure of male preference; Successful copulation also

requires the target female to be receptive (Somashekar et al 2011). Males and females sexual behavior is influenced by pheromones. Gr68a gene is expressed in specific neurons of males' anterior legs and is recognized as a pheromone receptor. Inactivation of 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 PPK" genes family). This family constitutes a new class of pheromone receptors (Thistle et al 2012).

Table 4 Effect of *C. arabica* aqueous extracts on different sequences of *D. melanogaster* sexual behavior (Mean ± SEM).

	First contact time	First vibration time	First licking time	First attempt time	Mating time
C♂ x C♀	118,70 ± 22,88	285,70 ± 73,59	332,10 ± 74,08	404,15 ± 80,70	333,35 ± 71,35
C.a♂ x C.a♀	80,10 ± 36,72	190,70 ± 58,75	262,20 ± 84,69	370,65 ± 105,22	324,65 ± 110,84
C♂ x C.a♀	79,85 ± 35,50	222,95 ± 54,00	323,40 ± 79,90	292,00 ± 80,87	322,15 ± 96,49
C.a♂ x C♀	112,95 ± 61,00	444,60 ± 186,56	250,55 ± 80,59	260,75 ± 99,76	169,50 ± 97,55
F _{obs}	0,41	2,287	0,459	0,701	1,395
P	0,75	0,09	0,71	0,55	0,251
	Contacts number	Vibrations number	Licking number	Attempts number	Mating duration
C♂ x C♀	8,50 ± 1,14	8,15 ± 1,83	5,65 ± 1,33	3,65 ± 1,09	1179,35 ± 185,56
C.a♂ x C.a♀	7,20 ± 7,20	7,45 ± 2,06	5,95 ± 1,96	3,15 ± 1,13	882,15 ± 208,50
C♂ x C.a♀	11,25 ± 1,94	11,10 ± 3,26	5,90 ± 1,60	2,60 ± 0,89	905,90 ± 216,79
C.a♂ x C♀	22,50 ± 4,48	19,75 ± 4,72	12,10 ± 3,11	4,50 ± 1,92	566,90 ± 294,78
F _{obs}	12,18	6,64	6,35	1,98	0,361
P	<0,0001***	0,000***	0,001**	0,12	0,78

[C♂: Control male; C♀: Control female; C.a♂: treated male by *C. arabica* extracts; C.a♀: treated female by *C. arabica* extracts] [(P<0.05)

*: significant; (P<0.01) **: highly significant; (P<0.001) ***: very highly significant]

In the present study, we noticed a disruption of different coupling sequences in *D. melanogaster* adults by the indirect effect of *C. arabica* aqueous extract. It was demonstrated that flies don't mate when couple is formed by treated males and control females, after some contact between partners, no courtship is observed this may be a change in the cuticular profile in flies. Contacts role in the partners mutual recognition has been proven in different insects species such as cockroaches (Roth and Willis 1952; Farine et al 1993; Gropeaux, 1994). In the goal of bioactive molecules research powerful against vinegar fly, *Spinosad*, *Bacillus thuringiensis var kurstaki*, *Azadiractine*, and *P. harmala* are bioinsecticides that have proven their activity on mortality and sexual behavior of *D. melanogaster* (Bensafi 2010; Bourbia 2012; El-Bah et al 2016). In this study, we show that control couples take longer than in treated couples; the study shows, also, that the aromatic plant extract acts (*C. arabica*) on key sequences leading to mating (vibration, licking and mating attempts).

Conclusions

This study indicates that *C. arabica* aqueous extract has a neurotoxic property, the sublethal concentration of the extract 15 µg / ml shows that treated individuals are incapable of presenting a complete sexual behaviour. A chemical analysis of treated and control flies can provide information on any changes in adult *D. melanogaster* courtship, pheromone secretions, mating time, and various males sexual behavior sequences. This work results suggest the presence of toxic substances in the studied extract which can lead to developing bioinsecticides based on *C. arabica* and which will be used in agriculture and sold on pesticide market.

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