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EVALUATION OF *CLEOME ARABICA* L. (*CAPPARIDACEAE*) TOXICITY: EFFECTS ON MORTALITY AND SEXUAL BEHAVIOUR OF *DROSOPHILA MELANOGASTER* (DIPTERA: DROSOPHILIDAE)

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Abstract
To control vinegar flies, insecticide trials were carried out using some extracts of Cleome arabica (Capparidaceae), a medicinal plant used for the treatment of inflammation, pyrexia and pain.
In this study, we evaluate the direct and delayed effect of the ethanolic plant extract on the vinegar fly Dorsophila melanogaster, the excellent laboratory model. The treatment was applied on 2nd larvae stage (L2), by ingestion. Several concentrations were used to determined fly mortality and sublethal concentration (0.5 µg / ml) was used to studied sexual behaviour of adults.
We record 50% morality rates after 15 days treatment and a disturbance of fly development. The results also show that C. arabica ethanolic extract has a negative effect on the Drosophila sexual behaviour.

Keywords: Cleome arabica, Drosophila melanogaster, mortality, sexual behavior.

1. INTRODUCTION

The English term "pest" comes from the Latin "pestis", which means "plague", it is defined according to the concise Oxford dictionary as being "a source of embarrassment or destruction, whether it is a person, an animal or something" (Badi, 2015). Pests are therefore all species whose activity makes them hostil to human well-being (McKay et al., 2013; Menasria et al., 2014; White et al., 2014; Badi., 2015; Govindarajan and Rajeswary, 2015).
There are different methods of pest control. Biological control, which consists in destroying harmful insects through the rational use of their natural enemies belonging either to the animal or plant kingdom, and chemical control, which uses different types of insecticides, each with their own physical and chemical characteristics (Strong et al., 2000).
Insecticides are classified according to their chemical structure or origin, as mineral or organic insecticides, natural or synthetic insecticides. Four main groups are distinguished: non-organic

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insecticides, organic insecticides of plant or synthetic origin and growth regulators (Bekhakheche, 2018).

Drosophila groups are known all over the world under various names: vinegar flies or small fruit flies. These very common insects often live close to human activities (Delbac et al., 2014) and reproduce very quickly (about 10 days at 25°C). A century of research has accumulated a wealth of information that is unparalleled among higher organisms, especially in the fields of genetics and developmental biology (Tracqui and Demongeot, 2003). Biological control remains the safest, most selective and most biodegradable. The objective of this work is to evaluate the mortality and development as well as the sexual behaviour of *D. melanogster*.

2. MATERIALS AND METHODS

2.1. Insect: *Drosophila melanogaster* was described by Johann Wihelm Meigen in 1830. It reproduces very quickly (200 to 300 eggs per female); it reproduces all year round, without interruption, with a new generation every 12 days at 25°C. Its life cycle is very short and includes three larval stages and a pupal stage from which an adult emerges who is able to fly and reproduce in less than 24 hours**.**

2.2. Mass breeding: A wild strain harvested from rotten apples in the Annaba region of Algeria is used. The breeding is carried out in plastic tubes (12cm long and 4cm in diameter) sealed with a foam pad and containing a nutritious agar medium based on corn semolina and brewer's yeast. The ageing is maintained at a temperature of 25±1°C, a humidity of 70 to 80% and a scotophase of 12 hours.

2.3. *Cleome arabica:* It is a spontaneous plant in arid areas with a foul odour, toxic and has hallucinogenic effects so it is a neurotoxic plant. In pharmacopoeia, some indigenous people of the Sahara use *C. arabica* as a diuretic and against rheumatism. The plant leaves used in this study come from the Bousaada region in the (M'sila, Algeria).

2.4. Preparation of the ethanolic extract of *C. arabica*: For the *C. arabica* leaves extraction we macerated 250g of dry powdered leaves in 500 ml ethanol for 24 hours at room temperature and in the shade. After filtration, the solution obtained was evaporated using a Rotavapor at 45°C to remove the ethanol solvent. The recovered leg was stored at 4°C until use.

2.5. Treatment of Drosophila L2 larvae by *C. arabica*: the larvicidal activity of the various extracts is studied by ingestion. The ethanolic extract according to the concentrations selected (0.25µg/ml, 0.5µg/ml, 1µg/ml, 2.5µg/ml and 5µg/ml) is mixed with 40g of larval food in four tubes. In each tube, 20 second-stage larvae from a mass culture are placed. In a fifth tube containing no treatment, 20 larvae are placed as a control. Mortality and larval development are monitored for 15 days (time required to complete development).

2.6. Effect of plant extract on sexual behaviour: nuptial parade in fruit flyers is a succession of predetermined and invariable actions **(**Clynen et al., 2011; Chardonnet, 2013). The male first moves towards a potential female and taps her on the cuticle with her front legs; if the female moves, the male follows and makes a wing vibrate. Then, when the female stops, the male runs in circles around her, licks her genitals with her proboscis and tries to mate **(**Revadi et al., 2015).

2.7. Data analysis: Lethal concentrations and lethal times (LC50%, LC90%, LT50% and LT90%) were calculated according to Finney's mathematical procedures (Finney, 1971). With regard to sexual behaviour test results were statistically analyzed by descriptive metric methods giving the mean, standard deviation of the mean, minimum and maximum and variance. Then the results were

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analyzed using a variance analysis (ANOVA) on XLSTAT 2014 software (Addinsoft, New York, NY).

3. RESULTS

3.1 Direct effect of ethanolic extracts of *C. arabica* on mortality and development of *D. melanogaster*:

The results show that the ethanolic extracts of *C. arabica* have an effect on larval development time and larval mortality depending on the concentration applied. The concentration 0.25 µg/ml has a low larvicidal activity. For concentrations (1, 2.5 and 5 µg/ml), 50% of the population was killed after 15 days of treatment. There is a significant difference between the recorded mortality rates and the 0.5µg/ml concentration (*p: 0.0001****), while there is no time effect on mortality at the other concentrations **(Tab. 1).**

Table 1. Mortality rates caused by different concentrations of C. arabica

	0.25 µg/ml	*0.5 µg/ml*	*1 µg/ml*	*2.5 µg/ml*	*5µg/ml*	F_{obs}	*P*
2 days	*0*	*0*	*36.25*	*0*	*25*	*5.72*	*0.005***
5 days	*1.25*	*3.75*	*38.75*	*57.5*	*31.25*	*2.32*	*0.104*
10 days	*22.5*	*25*	*43.75*	*40*	*35*	*3.469*	*0.034**
15 days	*25*	*37.5*	*48.75*	*50*	*50*	*2.651*	*0.074*
F_{obs}	*3.41*	*17.71*	*0.036*	*4,052*	*0.55*		
P	*0.53*	*0.0001****	*0.99*	*0,033*	*0.658*		

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

Larval mortality rates are weakly and positively correlated with the concentrations of the extract used **(Tab. 2A).** The lethal concentration of 50% reaches 23.99µg/ml at 10 days and 1.48 µg/ml of *C. arabica* extract at 15 days of exposure **(Tab. 2A).** 90% of larvae die with a concentration of 85.11µg/ml after 15 days **(Tab.2A).**

Table 2. Toxicological parameters of the larvicidal effect of ethanolic extracts of C. arabica on the L2 larvae of D. melanogaster

(A: larval exposure time, B: concentrations used, y: probits of mortality rates X: the decimal logarithm of concentrations and/or times)

A			
Time	***Right of regression***	$LC_{50\%}$	$LC_{90\%}$
2 days	*Y=1.67+2.48X (R=0.28)*	*21.88 µg/ml*	*79 µg/ml*
5 days	*Y=3.84+0.93X (R=0.36)*	*17.38 µg/ml*	*416.87 µg/ml*
10 days	*Y=4.53+0.34X (R=0.47)*	*23.99 µg/ml*	*100000 µg/ml*
15 days	*Y=4.87+0.73X (R=0.57)*	*1.48 µg/ml*	*85.11 µg/ml*
B			
Concentration	***Right of regression***	$LT_{50\%}$	$LT_{90\%}$
0.25 µg/ml	*Y=-1.32+5.25X (R=0.98)*	*15.85 j*	*27.5 j*
0.5 µg/ml	*Y=-1.21+5.36X (R=0.94)*	*14.12 j*	*24.5 j*
1 µg/ml	*Y=4.50+0.35X (R=0.90)*	*26.30 j*	*120226 j*
2.5 µg/ml	*Y= -1.55+6.34X (R=0.96)*	*10.71 j*	*16.98 j*
5 µg/ml	*Y= 4.06+0.68X (R=0.83)*	*23.9 j*	*1819.7 j*

For lethal times, the results show that there is a strong positive correlation between the mortality rate and the time of exposure of larvae to plant extracts **(Tab. 2B).** Our results indicate that the

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LT50% is 15.85 d for low concentrations and 26.30 d for sublethal concentration. The LT90% reaches 120226 days for the sublethal concentration **(Tab. 2B).**

3.2 Effect on the *D. melanogaster* sexual behaviour:

Effect on mating success rate:

The results show that the ethanolic extract of *C. arabica*, administered at a sublethal concentration of 0.5µg/ml, suppresses mating in flies, regardless of the sex treated within a couple (dyad) **(Tab. 3).** The mating success rate is 70% in controls, whereas it reaches 10% when males are treated with the Saharan plant **(Tab. 3).** The rate of aborted matings (couples attempting to mate unsuccessfully) and null matings (neither attempt nor mating) is higher in couples composed of treated males and control females **(Tab. 3).**

Table 3. Effects of C. arabica (0.5µg/ml) on the percentage of success of D. menlanogaster mating

	% coupling		
	successfully	aborted	Zero
♂C x ♀C	70	20	10
♂C.a x ♀C.a	0	0	100
♂Cx♀C.a	75	25	0
♂C.a x ♀C	10	55	35

[C: Control; C.a: C. arbica]

Effect on the different sequences leading to mating:

We have recorded that the control couples and treated couples take their sublime time to establish the first contact between the two partners. The same result is observed for the different times recorded during the sexual parade of *D. menlanogaster* (time of the first vibration *p:* 0.051; time of the first licking *p*: 0.001; time of the first attempt *p*: 0.844) but the mating is nil in treated couples **(Tab. 4).**

Table 4. Effect of ethanolic extracts of C. arabica on the different sequences of sexual behaviour of D. melanogaster (Mean ±SEM)

	Time of first contact	Time of the 1st vibration	Time of the 1st licking	Time of the 1st attempt	Time of coupling
♂C X♀C	118.700± 22.878	300.737± 75.932	349.579±75.886	449.056±83.076	476.214±73.463
♂C.aX♀C.a	92.250± 39.626	464.438±51.613	347.105±100.422	972.647± 91.441	/
♂CX♀C.a	52.000±18.406	156.650±30.091	201.750±33.015	311.850±85.569	1103.667±178.271
♂C.aX♀C	125 .556± 39.489	180.933± 30.265	274.933±53.438	417.923±118.962	1028.000±92.000
F_{obs}	1.118	2.724	6.524	0.274	3.754
p	0.347	0.051	0.001**	0.844	0.036*
	Number of contacts	Number of vibrations	Number of licks	Number of attempts	Mating time
♂C X♀C	8.500 ± 1.144	8.579±1.878	5.947 ± 1.367	4.056± 1.176	1684.786±79.943
♂C.aX♀C.a	10.300±1.257	15.750± 3.599	9.842± 2.266	5.118±0.977	/
♂CX♀C.a	17.000±2.307	32.850± 4.113	30.400± 3.168	21.800±2.447	1254.067± 363.385
♂C.aX♀C	13.944±1.800	18.200± 3.522	16.200± 3.559	11.923± 2.949	150.500±29.500
F_{obs}	1.632	3.768	4.597	4.899	4.381
p	0.189	0.015**	0.005**	0.004**	0.022*

[SEM: Standard deviation of the mean; ♂C: Control Male; ♂C.a: Male treated with C. arabica; ♀C: Control female; ♀ C.a: Female treated with C. arabica]

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The ethanolic extract of *C. arabica* significantly influences the number of attempts (Fobs= 4.899; *P*: 0.004) and the mating itself (mating time Fobs= 3.754; *p:* 0.036) (mating time Fobs =4.381; *p*: 0.022), most of the sexual parade sequences are not significantly affected by the plant extract **(Table 4).**

4. DISCUSSION

Currently, control in natural environments is increasingly moving towards the use of natural means to control different pests. This trend is driven by a major current concern to minimize the use of pesticides that pollute the environment and also lead to the emergence of resistant strains (Acheuk, 2012**).** Among these natural means is the use of plant extracts as bio-insecticides (Grethead et al., 1994**).** In this study, we used the ethanolic extract of the leaves of *C. arabica* as a bio-insecticide against *Drosophila melnogaster*.

Toxicological results show that larvae of *D. melanogaster* exposed to ethanolic extract of *C. arabica* to mortality have a function of dose and exposure time. The toxicity process is particularly important in case of ingestion it seems that the active substances attack digestion causing larval death, several studies have studied the toxicity of products generated from plants against *D. melanogaster* larvae, we include the work of **(**Idrissi et al., 1998; Idrissi Hassani L.M, 2000; Mahmoudian et *al*., 2002; Abbasi et *al*., 2003 a; Abbasi et al., 2003b ; Aouinty et al., 2006; Idrissi Hassani L.M et Hermas J, 2008; Lebouz I, 2010 ; Habbachi et al., 2013; Kemassi et Oueld El-Hadj, 2008; Habbachi et al., 2014; Merabti et al., 2015; El-Bah D, 2016; Masna F, 2016; Benhissen et al., 2018; Chabi et al., 2019; Habbachi et al., 2019).

The behaviour of insects and all animals is driven by interactions between neurons within their nervous systems. Insecticides have been chosen and sometimes designed for their remarkable ability to kill insects. Most attack specific sites in the insect's nervous system. So it is not surprising that insecticides at levels that do not lead to mortality can influence behaviour (Haynes, 1988; Rafalimanana, 2004).

Everyday, animals whose insects use many sources of information from their environment. These stimuli, particularly visual and olfactory, are used, among other things, to locate food sources and sexual partners (Pacaud, 2008; Louat, 2013).

Based on the origin of the emission and the recipient, chemical signals can be divided into two main groups; allelochemicals, signals that are emitted from an animal of one species to a member of a different species (Whittaker and Feeny, 1970) and pheromones, chemical signals that carry information from one individual to another member of the same species **(**Karlson and Lùscher, 1959). Intraspecific chemical communication plays an essential role in the sexual behaviour and social life of insects (Desneux et al., 2007; Badi, 2015).

In this study we recorded a disturbance of the adult parade sequences (Contact, Vibration, Licking, Attempt) of *D. melanogaster* that lead to mating with the ethanolic extract of *C. arabica*, this disturbance is remarkable when both partners are treated and when the treated male and the control female. Several studies have highlighted the different effects of aromatic plants such as *P. harmla*, *Daphne gnidium, colocynthis (L.), Spinosad*, *Bacillus thuringiensis var kurstaki*, *Azadiractine* on diptera (Bensafi H, 2010; Bourbia, 2012; Marbti et al., 2015; Benhissen, 2016; El-Bah, 2016; Marbti, 2016; Masna., 2016; Habbachi et al., 2019).

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ability to kill insects. Most attack specific sites in the insect's nervous system. So it is not surprising that insecticides at levels that do not lead to mortality can influence behaviour **(**Haynes, 1988; Rafalimanana, 2004).

5. CONCLUSIONS

In this work, we initiated a protocol to study the toxic effect of ethanolic extracts of *C. arabica* on larval mortality of *D. melanogaster*, on the one hand, and on adult sexual behaviour, on the other. Toxicological calculations (TL50%, TL90%) show the insecticidal effect of the extract on the fly. The results indicate that there is a strong positive correlation between mortality rates and concentrations used and, above all, between mortality and exposure times to extracts. Mortality rates for low concentrations can reach 50% by the 10th day of follow-up. The plant also causes acceleration in the fly development. We observed a complete disturbance in the sexual behaviour of flies; this shows the neurotoxic effect of the extract**.**

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