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ASSESSING THE TOXIC, BEHAVIORAL, AND REPRODUCTIVE IMPACTS OF *EUPHORBIA BUPLEUROIDES* ON *DROSOPHILA MELANOGASTER*

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Abstract: This study explores the insecticidal potential of the Saharan species *Euphorbia bupleuroides* by assessing the effects of its aqueous leaf extract on *Drosophila melanogaster*. The extract exhibited strong bioactivity, inducing complete mortality in larvae and males and high mortality in females. Feeding assays revealed a disruption of larval chemosensory responses, with treated larvae losing their ability to discriminate between nutritive substrates. At the behavioural level, the extract significantly altered courtship sequences and sharply reduced mating success, demonstrating an inhibitory effect on reproductive signalling. Oviposition experiments showed that females, whether treated or untreated, prefer the control substrate, confirming a marked repellent effect of the extract. Together, these findings highlight the potent insecticidal and behavioural-modulating properties of *E. bupleuroides* and support its potential use in environmentally sustainable, plant-based pest management strategies.

Keywords: *Drosophila melanogaster*, *Euphorbia bupleuroides*, toxicity, behaviour, biological control.

INTRODUCTION

Insects constitute one of the most ecologically influential groups on Earth, owing to their exceptional diversity, abundance, and ecological functions. They participate in pollination, nutrient recycling, food web dynamics, and ecosystem stability, while certain species can cause significant damage to agriculture, food storage, and public health (Benhissen 2016; Ngamo & Hance 2007). Among insect orders, Diptera includes numerous species of medical and agronomic importance. *Drosophila melanogaster*, although globally recognized as a model organism in genetics, physiology, and behaviour (Joly 2006; Apostolopoulou et al. 2013; Colombi 2021), can also behave as an opportunistic pest. It is attracted to fermenting substrates enriched with yeast (Barker et al. 1988; Becher et al. 2012) and can carry phytopathogenic microorganisms or microbial contaminants affecting crops and stored products (Jolivet 1980; Durisko et al. 2014).

The widespread and intensive use of chemical insecticides has generated major environmental and health concerns, including biodiversity loss, contamination of water and soils, and the development of insecticide resistance in pest populations (Hashimi et al. 2020; Ahmad et al. 2024; Zhou et al. 2025). Resistance mechanisms in insects metabolic, behavioural, genetic, or cuticular make chemical control increasingly ineffective and accelerate the search for safer alternatives (Al Naggar et al. 2025; Senthil-Nathan 2020). In this context, biological control and plant-derived biopesticides represent promising strategies. Phytochemicals and essential oils can act as neurotoxic, repellent, antifeedant, or growth-disrupting agents with reduced toxicity to non-target organisms and faster environmental degradation (George et al. 2014; Ayilara et al. 2023; Gupta et al. 2023). Several studies highlight the efficacy of plant extracts and essential oils against dipteran pests, including *D. melanogaster*, *Culex pipiens*, and *Aedes aegypti* (Sutthanont et al. 2010; Bouabida & Dris 2022; Baz et al. 2024; de Souza et al. 2020).

Algeria's rich Mediterranean flora constitutes an underexplored reservoir of bioactive compounds with insecticidal potential. Several native species used in traditional medicine, including *Ruta chalepensis*, *Solanum nigrum*, *Artemisia herba-alba* and *Lavandula angustifolia*, have demonstrated significant larvicidal, neurotoxic, or behavioural effects on insect pests (Belbachir & Tchenar 2019; Amrani et al. 2022; Rahat et al. 2021; Sayada et al. 2021). Despite this potential, many plants remain poorly investigated, particularly *Euphorbia bupleuroides*, a species traditionally used in North Africa but scarcely evaluated for insecticidal properties.

Given the ecological importance of *D. melanogaster* as a biological model and its relevance as a pest species, exploring plant-based alternatives is highly relevant. Therefore, the present study aims to evaluate the toxic and behavioural effects (mortality, feeding, mating, and oviposition) of an aqueous extract of *E. bupleuroides* on *D. melanogaster*, within the framework of an environmentally sustainable and targeted biological control strategy.

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MATERIAL & 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.

Euphorbia bupleuroides

Like many species of the genus Euphorbia, contains a toxic latex rich in bioactive compounds such as diterpenes, triterpenes and alkaloids, which underlie its medicinal and insecticidal properties. Euphorbia-derived natural products are well known for their antimicrobial, anti-inflammatory and cytotoxic activities (Salehi et al., 2019), while several species show highly diverse phytochemical profiles with strong biological effects (Benjamaa et al., 2022). Experimental studies have confirmed the insecticidal and larvicidal potential of *Euphorbia* extracts which effectively targets multiple insect pests (Younus et al., 2021).

Plant extraction

The plant material used in this study, *E. bupleuroides*, was collected from a mountainous region in north-central Algeria (Aflou, Laghouat province; 34°06′50″ N, 2°05′50″ E). Aqueous extraction was carried out by decoction. Fresh leaves (70 g) were immersed in 500 ml of distilled water and boiled for 30 minutes on a hot plate set at 180 °C. The resulting mixture was filtered through filter paper, yielding an aqueous extract with a concentration of 140 g/L, which was subsequently used for biological assays.

Effect on mortality

Treatment of larvae

We have prepared four different concentrations 10µg/mL, 35µg/mL, 50µg/mL and 70µg/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 contains 20 second stage larvae and it didn't treat by *E. bupleuroides* extracts. The monitoring of mortality and larvae development is done during 15 days (time necessary to finish the development).

Treatment of adults

In adult *Drosophila*, the assay involved oral administration of different concentrations of the aqueous extract of *E. bupleuroides* (70, 35, 10, and 5 µg/mL). For each concentration, 10 mL of the extract was mixed with 40 g of food and distributed into eight tubes (four for males and four for females), each containing 10 individuals isolated at emergence. Mortality was monitored over a 15-day period, with a control group included for each sex.

Effect on feeding behavior in larvae

Feeding behavior tests were conducted on *D. melanogaster* larvae at the third instar stage (L2) following exposure to treatments. Forty untreated larvae were used as controls. The assay is based on the olfactory ability of larvae, particularly at the second instar stage, to detect the presence of food, in order to better understand their attraction behavior. The experimental arena consisted of a plastic Petri dish (100 mm diameter) filled with 2% agar. The bottom was lined with a paper on which two circular zones (A and B), each representing 10% of the total surface area, were outlined in pencil. The materials used included: Whatman filter papers (15 mm diameter), fine forceps, needles (10 mm), stainless steel spatulas, stopwatches, and glass dishes. Filter papers were cut, rinsed three times with ethanol, and dried overnight at 90 °C. After sterilization, they were handled exclusively with sterile forceps. All materials were washed with tap water, rinsed three times with deionized water, followed by ethanol, and finally dried overnight at 90 °C.

Effect 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 (Bennet-Clark & 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 *E. bupleuroides* extract (1µg/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.

Effect on oviposition behaviour and reproduction

To determine the choice of the place or environment where the female Drosophila lays her eggs, in which she ensures the hatching of her eggs under optimal conditions, as well as their larval and pupal development until the adult stage. We realized a method consists in placing the pairs in plastic boxes containing two different media (control medium which is used for our daily mass rearing and medium treated with the aqueous extract of *E.bupleuroides*. at 1µg/ml). Adults must be virgin and sexually mature (separation

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of adults as soon as they emerge). 72 hours later, we recover the two media and observe with a magnifying glass the eggs laid and the number of larvae in each medium. At the end of this test, we calculated an oviposition preference index (OPI) for the different crosses tested. The OPI is calculated according to (Flaven-Pouchon *et al*., 2014) as follows: OPI = [a - b] / [a + b] when (a): number of eggs on treated medium; (b): number of eggs on control medium. Theoretically it varies between -1 (great aversion for the product) and +1 (great attraction for the product).

Data analysis

The toxicological parameters (LC50%, LC90%, TL50%, and TL90%) were calculated according to the mathematical procedures of Finney (Finney, 1971). Regarding the results of feeding behavior test; sexual and oviposition was statistically analyzed by descriptive metric methods and then an analysis of variances (ANOVA) was performed on XLSTAT 2009 software (Addinsoft, New York, NY).

RESULTS

Effect on mortality

Exposure of *D. melanogaster* larvae to aqueous extracts of *E. bupleuroides* revealed a clear dose-dependent toxic effect. The extract strongly affected both larval survival and development time.

At the lowest concentrations (5 and 10 µg/mL), mortality increased progressively, reaching 100% by day 10 (Table 1). A similar pattern was observed at 35 µg/mL, although mortality remained slightly lower at day 10 (80%) and reached 98.75% at day 15. In contrast, the highest concentration (70 µg/mL) exhibited delayed and moderate toxicity, causing only 28.75% mortality after 15 days.

Statistical analysis revealed a highly significant effect of concentration on mortality at days 5, 10, and 15 (Fobs = 8.71 to 24.87; $p < 0.001$), while no significant difference was observed at day 2. Within each concentration, mortality increased significantly over time for 5, 10, and 35 µg/mL ($p < 0.01$), whereas no time-dependent effect was detected at 70 µg/mL ($p = 0.47$), indicating reduced or slower action of the extract at high concentration (Table 1).

The results reveal an increasing mortality of males and females of *D. melanogaster* depending on the concentration and time of exposure to the tested extract. In males, mortality reaches 100% from 5 days at 35 µg/ml, reflecting a high sensitivity. In females, maximum mortality (95%) is observed at 15 days (Table.2). Statistical analyses show significant differences between concentrations, particularly at 5 and 15 days ($p<0.05$), confirming a dose- and time-dependent effect. Overall, males appear more sensitive than females to the toxicity of the extract. (Table.2).

The LC_{50} and LC_{90} values vary according to the stage and sex of *D.melanogaster.* In larvae, LC_{50} values increased slightly with exposure time (from 1.19 µg/ml to 5.16 µg/ml), indicating a high initial sensitivity that tended to stabilize after 10 days (Table. 3).

In males, LC_{50} values were significantly higher (from 10.47 µg/ml to 194.9 µg/ml), reflecting greater resistance compared to larvae. In contrast, females exhibit the lowest LC_{50} (0.18 µg/ml to 158.4 µg/ml), suggesting a marked sensitivity, particularly to low concentrations and short exposures. LT_{50} and LT_{90} (median and 90% lethal time) values show a dose-dependent trend: the higher the concentration, the more rapidly mortality occurs. At 70 µg/ml, the LT_{50} varies between 2.6 days (females) and 5.98 days (larvae), confirming a rapid toxic effect of the extract (Table. 3).

Table 1.

Mortality rate caused by the aqueous extract of *E. bupleuroides in larvae*

Larvae	5 µg/ml	10 µg/ml	35 µg/ml	70 µg/ml	F_{obs}	p
2 days	6.25%	5.00%	1.25%	6.25%	***1.92***	***0.15***
5 days	28.75%	36.25%	16.25%	6.25%	***8.71***	***0.001***
10 days	100.00%	100.00%	80.00%	13.75%	***24.87***	***<0.0001***
15 days	100.00%	100.00%	98.75%	28.75%	***13.87***	***<0.0001***
F_{ob}	***6.635***	***14.507***	***36.45***	***0,89***		
p	***0.007***	***0.001***	***< 0.0001***	***0.47***		

Table 2.

Mortality rate caused by the aqueous extract of *E. bupleuroides in adults*

Males	5 µg/ml	10 µg/ml	35 µg/ml	70 µg/ml	F_{obs}	p
2 days	5%	12.5%	60 %	12.5%	***0.182***	***0.907***
5 days	10%	27.5%	100 %	27.5%	***5.143***	***0.016***
10 days	22.5%	65%	100%	65%	***2.729***	***0.090***
15 days	55%	97.5%	100%	97.50%	***6.000***	***0.010***
F_{obs}	***1.500***	***4.271***	***3.000***	***2,000***		
p	***0.265***	***0.029***	***0.073***	***0,168***		
Females	***5 µg/ml***	***10 µg/ml***	***35 µg/ml***	***70 µg/ml***	F_{obs}	***p***
2 days	0 %	2.5 %	12.5 %	50 %	***3.474***	***0.051***
5 days	2.5 %	22.5 %	30 %	55 %	***4.261***	***0.029***
10 days	40 %	35 %	70 %	62.5 %	***0.786***	***0.525***
15 days	60 %	67.5 %	95 %	77.5 %	***3.857***	***0.038***
F_{obs}	***2.684***	***1.037***	***0.404***	***2.000***		

Males	5 µg/ml	10 µg/ml	35 µg/ml	70 µg/ml	F_{obs}	*p*
p	***0.094***	***0.411***	***0.753***	***0.168***		

Table 3.

Toxicological parameters of *E. bupleuroides* against *D. melanogaster*

	Larvae		Males		Females	
	LC 50% (µg/mL)	LC 90% (µg/mL)	LC 50% (µg/mL)	LC 90% (µg/mL)	LC 50% (µg/mL)	LC 90% (µg/mL)
2days	*1.19*	*3.80*	*10.47*	*6309.50*	*0.18*	*0.39*
5days	*0.21*	*3.72*	*25.70*	*5.62*	*5.75*	*0.89*
10days	*4.86*	*5.57*	*52.48*	*11.22*	*20.89*	*0.57*
15days	*5.16*	*5.70*	*194.90*	*46.77*	*158.40*	*3.98*
	LT 50% (day)	**LT 90% (day)**	**LT 50% (day)**	**LT 90% (day)**	**LT 50% (day)**	**LT 90% (day)**
70µg/mL	*5.98*	*9.92*	*5.62*	*13.80*	*2.60*	*125.80*
35µg/mL	*9.21*	*1.63*	*1.12*	*2.39*	*5.75*	*14.79*
10µg/mL	*1.87*	*2.31*	*5.62*	*13.80*	*69.18*	*36.30*
5µg/mL	*1.87*	*2.31*	*3.31*	*91.20*	*9.33*	*14.45*

Effect on the feeding behaviour

In control larvae: When simultaneously exposed to two different odor sources (control vs. treated), control larvae oriented slightly faster toward the treated medium (842.14 ± 167.56 s) than toward the control medium (576.92 ± 86.47 s), although this difference was not statistically significant ($F_{o\beta s}$ = 1.011; p = 0.881) (Table 4).

When confronted with identical odor sources (control vs. control or treated vs. treated), larvae showed a stronger attraction toward their native developmental medium (808.55 ± 93.69 s), whereas detection of the treated medium required considerably more time (588.33 ± 112.77 s). This contrast was highly significant ($F_{o\beta s}$ = 6.529; $p < 0.0001$), indicating that control larvae are more responsive to familiar odors and exhibit reduced attraction toward the extract-treated substrate.

In treated larvae: Treated larvae exposed to two identical odor sources also displayed no significant preference between control and treated media. They required on average 646.62 ± 91.22 s to detect the control odor and 360.43 ± 115.03 s to detect the treated odor, but the difference was not significant (**$F_{o\beta s}$** = 1.168; p = 0.896) (Table 4).

Table 4.

Detection time in response to different odor environments

Larvae	Environment	Number of larvae attracted	Detection time	F_{obs}	*p*
Control	**Control**	26	576.923 ± 86.470	***1.011***	***0.881***
	Treated	7	842.143 ± 167.561		
Treated (1µg/mL)	**Control**	23	572.522 ± 66.937	***1.254***	***0.627***
	Treated	11	658.182 ± 108.371		

Attraction index (AI)

The attraction index confirms the previously observed behavioural patterns. Control larvae exhibited positive AI values, indicating a clear attraction to the odor emitted by the treated medium. In contrast, treated larvae showed negative AI values, reflecting a pronounced aversion to the odor of *E. bupleuroides* extract. These opposite responses highlight a differential behavioural effect induced by the treatment (Table 5).

Table 5.

Attraction index of control and treated larvae towards *E.bupleuroides* extract

Control-Treated	
Control larvae	**Treated larvae**
1	- 0.71

Effect on sexual behavior

Effect on mating success

At the sublethal concentration of 1 µg/mL, the aqueous extract of *E. bupleuroides* markedly reduces mating success in *D. melanogaster*, regardless of which sex is treated. The mating success rate decreases from 70% in the control group to 25% when males are treated (Table 6). Moreover, unsuccessful or absent mating attempts are more frequent in pairs involving treated males and untreated females, indicating a strong inhibitory effect of the extract on courtship and copulation behaviour (Table 6).

Table 6.

Effects of aqueous extract of *E. bupleuroides* (1µg/mL) on the percentage of mating success of *D. melanogaster* [*C: control; E.b: E. bupleuroides*]

	Successful	Aborted	Nul
♂***C x*** ♀***C***	70%	20%	10%
♂***C x*** ♀***E.b***	15%	30%	55%
♂***E.b x*** ♀ ***C***	15%	65%	20%
♂***E.b x*** ♀***E.b***	25%	45%	25%

Effect on the courtship sequence

The aqueous extract of *Euphorbia bupleuroides* induces marked disruptions in both the timing and the structure of sexual behaviour in *D. melanogaster*, as shown in Tables 7 and 8.

According to Table 7, pairs involving treated males (♂E.b × ♀C) exhibit a dramatic reduction in mating duration (68.75 s) compared with control pairs (♂C × ♀C, 1179.35 s), demonstrating a strong inhibitory effect on copulatory ability. These treated males also show altered behavioural timing, with shorter latencies for first contact, vibration, licking and attempted copulation, indicating a disruption of the normal sequential progression of courtship.

Complementing these results, Table 8 shows that treated males perform significantly more behavioural events—particularly contacts, licking attempts, and unsuccessful mating attempts—than control males ($p < 0.0001$***). This overactivity reflects a loss of behavioural coordination, where males initiate courtship actions but fail to progress toward successful copulation.

Despite these profound impairments, vibration frequency does not differ significantly between treatments ($p = 0.344$; Table 8), suggesting that the earliest sensory and signalling components of the courtship ritual remain only marginally affected.

However, the later stages, especially licking, mounting attempts, and copulation, are severely disrupted according to both Table 7 (timing) and Table 8 (number of sequences).

Table 7.

Effect of aqueous extracts of *E. bupleuroides* (1µg/mL) on the timing of sequences of sexual behavior of *D. melanogaster* (Mean ±SEM) [*C: control; E.b: E. bupleuroides*]

Cross	*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	1179.35 ± 185.56
♂***C X*** ♀***E.b***	36.15 ± 7.80	71.55 ± 11.05	256.05 ± 75.13	192.35 ± 90.78	269.85 ± 147.93
♂***E.b X*** ♀ ***C***	52.55 ± 11.02	142.55 ± 18.73	306.45 ± 85.35	260.75 ± 99.76	68.75 ± 47.89
♂***E.b X*** ♀***E.b***	80.50 ± 22.53	114.80 ± 21.47	286.40 ± 58.91	370.65 ± 105.22	359.60 ± 132.04
F_{obs}	***10.326***	***9.634***	***5.555***	***6.745***	***18.454***
P	***<0.0001******	***<0.0001******	***0.000*****	***<0.0001******	***<0.0001******

(*Significant; **: Highly significant; ***: Very highly significant)

Table 8.

Effect of aqueous extracts of *E. bupleuroides* (1µg/ml) on the number of sequences of sexual behavior of *D. melanogaster* (Mean ±SEM) [*C: control; E.b: E. bupleuroides*]

Cross	Contacts number	Vibrations number	Licking number	Attempts number	Mating duration
♂***C*** × ♀***C***	5.65 ± 1.33	8.15 ± 1.83	5.65 ± 1.33	404.15 ± 80.70	1179.35±185.56
♂***C*** × ♀***E.b***	28.45 ± 1.89	14.70 ± 1.30	10.25 ± 3.13	192.35 ± 90.78	269.85 ± 147.93
♂***E.b*** × ♀***C***	36.80 ± 4.01	9.65 ± 2.04	18.15 ± 4.23	260.75 ± 99.76	68.75 ± 47.89
♂***E.b*** × ♀***E.b***	28.15 ± 2.91	11.40 ± 1.11	15.30 ± 3.56	370.65 ± 105.22	359.60 ± 132.04
F_{obs}	**14.895**	***1.136***	***8.987***	***6.745***	***18.454***
P	***<0.0001******	***0.344***	***<0.0001******	***<0.0001******	***<0,0001******

(*: Significant; **: Highly significant; ***: Very highly significant)

Effect on oviposition behaviour and reproduction

Effect on the number of eggs laid

The results displayed in Figure 1 show a clear reduction in oviposition when females are exposed to the aqueous extract of *E. bupleuroides*. In all four mating combinations, females laid substantially fewer eggs on the treated medium than on the control medium, demonstrating a strong oviposition avoidance response.

Control pairs (♂C × ♀C) produced the highest number of eggs overall, with a marked preference for the control substrate (Fig. 1). When only females were treated (♂C × ♀E.b), oviposition decreased sharply on both substrates, but females still preferentially avoided the treated medium (Fig. 1). Crosses involving treated males (♂E.b × ♀C) showed moderate egg production in the control medium, with minimal deposition in the treated medium (Fig. 1). The lowest fecundity was observed when both sexes were treated (♂E.b × ♀E.b),

confirming the strong inhibitory effect of the extract on reproductive output (Fig. 1).

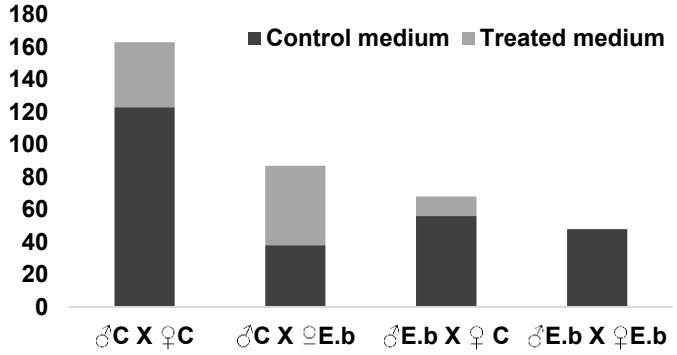

Fig. 1. Effect of aqueous extract of *E. bupleuroides* (1 µg/mL) on the total number of eggs in *D. melanogaster* [*C: control; E.b: E. bupleuroides*].

Effect on the number of larvae

Larval production varied markedly between crosses and environments, revealing a strong inhibitory effect of *E. bupleuroides* on post-oviposition development. In control pairs (♂C × ♀C), larval emergence was maximal in the control medium (142 larvae) but dropped drastically to only 5 larvae in the treated medium, indicating that the extract severely impairs embryonic and early larval viability.

In crosses involving treated females (♂C × ♀E.b), larval numbers remained extremely low in both environments (1 larva in control medium; 11 in treated medium), confirming that female exposure strongly compromises reproductive output. When only males were treated (♂E.b × ♀C), larval emergence also decreased sharply (9 larvae in control medium; 1 in treated medium), showing that paternal exposure negatively affects fertility, though to a lesser extent than maternal exposure.

Finally, in fully treated pairs (♂E.b × ♀E.b), larvae were produced exclusively in the control medium (30 larvae) and none in the treated medium, demonstrating complete inhibition of development when eggs are deposited on E. bupleuroides substrate.

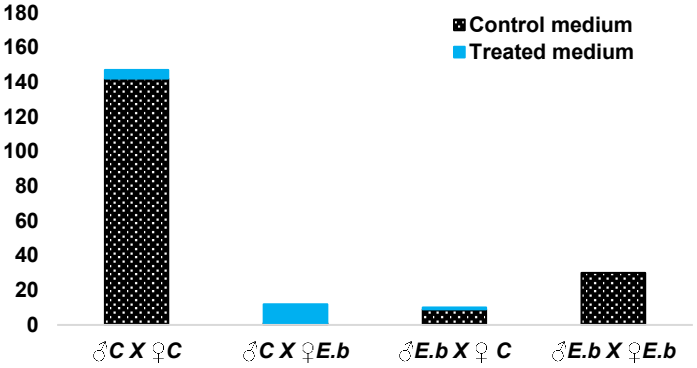

Fig. 2. Effect of aqueous extract of *E. bupleuroides* (1 µg/mL) on larval production in *D. melanogaster* [*C: control; E.b: E. bupleuroides*].

Oviposition preference index (OPI) of D. melanogaster females

The OPI values reveal clear and contrasting oviposition choices depending on the type of cross. Control females paired with control males (♂C × ♀C) strongly preferred the untreated medium (OPI = 0.93), and this preference was even absolute in fully treated couples (♂E.b × ♀E.b; OPI = 1.00), where no eggs were deposited on the treated substrate.

In contrast, females exposed to the extract (♂C × ♀E.b) showed a strong aversion to the control medium and instead preferred the treated environment (OPI = –0.83), suggesting that exposure alters their perception or tolerance to the plant-derived compounds. Meanwhile, when only males were treated (♂E.b × ♀C), females still favoured the control substrate (OPI = 0.80), indicating that paternal exposure alone does not override the innate avoidance of the toxic medium.

Table 9.

Oviposition preference index (OPI) of *D. melanogaster* females to aqueous extract of *E.bupleuroides* (1µg/ml) [*C: control; E.b: E. bupleuroides*]

Cross	*OPI*
♂*C* × ♀*C*	**0.93**
♂*C* × ♀*E.b*	**–0.83**

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Cross	OPI
♂*E.b* × ♀*C*	**0.80**
♂*E.b* × ♀*E.b*	**1.00**

DISCUSSION

Biological control seeks to suppress insect populations by targeting fundamental biological functions such as feeding, reproduction and dispersal (Pavela, 2016; Ramirez, 2020). In this context, our study demonstrates that the aqueous leaf extract of *E. bupleuroides*, a Saharan species rich in bioactive phytochemicals, exerts strong toxic, behavioural and reproductive disturbances on *D. melanogaster*. Such effects align with the growing interest in botanical insecticides as safer alternatives to synthetic chemicals, whose ecological and health impacts are now well documented (Ahmad et al., 2024; Muñoz-Bautista et al., 2025; Zhou et al., 2025).

All tested concentrations of *E. bupleuroides* extract led to complete larval mortality after 15 days, reflecting potent insecticidal activity. Similar toxicity levels have been reported for extracts from *Euphorbia guyoniana*, *Solanum nigrum*, *Drimia maritima*, and *Nicotiana glauca* against *D. melanogaster* (Chabi et al., 2022; Saadane et al., 2021; Rahat et al., 2021; Bouzar et al., 2021). Other works corroborate the broad insecticidal potential of plant-derived compounds against various pests (Bouayad et al., 2012; Kontsedalov et al., 2009; Ghanim & Kontsedalov, 2009). In particular, studies on *Peganum harmala* and *Cleome arabica* (Habbachi et al., 2013; Habbachi et al., 2019) highlight that desert plants often contain alkaloids, terpenoids and phenolics with strong larvicidal effects, consistent with the phytochemical richness of the *Euphorbiaceae*.

The efficacy of botanical extracts is generally attributed to their ability to interfere with neuromodulators (octopamine, acetylcholine) or endocrine pathways such as ecdysteroid signaling (Kostyukovsky et al., 2002; Spindler et al., 2009). Such mechanisms may explain the developmental delays and mortality observed in our study, comparable to effects reported for *Ruta chalepensis* (Bouabida & Dris, 2022; Amrani et al., 2022) and *Artemisia herba-alba* (Khlifi et al., 2013).

The disruption of larval olfactory and gustatory perception at 1 µg/mL reveals a strong neurobehavioural effect. Control larvae avoided the treated medium, whereas treated larvae showed reduced discriminatory ability. These observations suggest interference with the chemosensory system that regulates the detection of food sources (Ramaekers et al., 2005; Becher et al., 2012), consistent with reports showing that plant extracts can induce either attraction or repulsion depending on concentration and phytochemical composition.

Repellent activity similar to ours was described for *Urtica dioica* (Bouzar et al., 2022). Conversely, *C. arabica* extracts triggered attraction at sublethal doses (Habbachi 2020; Habbachi et al., 2020). Such variability aligns with the complexity of plant metabolites, which may modulate larval behavior through sensory masking, olfactory receptor interference, or disruption of fatty-acid-based attraction cues (Fourgeron, 2011).

Our results show that exposure to *E. bupleuroides* strongly disturbs the courtship sequence of adult flies. Treated males exhibited increased numbers of contacts, licking events and mating attempts, but significantly reduced mating duration and copulation success. These patterns indicate impaired sensory processing and locomotor coordination; an effect comparable to neurotoxic disruptions reported after sublethal exposure to other plant extracts (Habbachi et al., 2013; Habbachi et al., 2019; Saadane et al., 2021; Bouzar et al., 2021, Rahat et al., 2021) and synthetic insecticides (Young et al., 2020).

Plant-derived compounds can alter pheromone perception, nervous system activity or mating drive (George et al., 2014; Gupta et al., 2023), which explains the behavioral fragmentation observed here. The extract therefore appears to affect both motivational and motor components of courtship, ultimately reducing reproductive success and corroborating previous studies on *D. maritima*, *N. glauca*, *C. arabica* and *E. guyoniana* (Habbachi et al., 2019; Saadane et al., 2021; Bouzar et al., 2021; Chabi et al., 2022).

The reduction in egg laying and the sharply negative OPI values for most crosses indicate a strong repellent effect of *E. bupleuroides* on oviposition site selection. Both treated and untreated females consistently avoided the treated medium, an adaptive behaviour aimed at maximizing offspring survival (Curtsinger, 2019; CABI, 2021).

Similar avoidance has been reported for *D. maritima* and *U. dioica* extracts (Saadane et al., 2021; Bouzar et al., 2022), whereas *C. arabica* and *N. glauca* induced attraction to the treated substrate (Habbachi et al., 2019; Bouzar et al., 2021). This diversity reflects that oviposition responses depend on plant chemical profiles and may involve monoterpenes, alkaloids or phenolics known to modulate insect behavior (Ali et al., 2025; Qasim et al., 2024; de Souza et al., 2020).

Our findings also show that the treated medium completely inhibited larval emergence, confirming the dual toxic and repellent action of the extract. The strong reproductive inhibition observed when females were exposed aligns with the known sensitivity of reproductive pathways to phytochemicals and essential-oil constituents (Sharma et al., 2024; Gupta et al., 2023).

Plant-derived insecticides represent promising alternatives to synthetic pesticides, offering reduced environmental risks and slower resistance development (Ayilara et al., 2023; Al Naggar et al., 2025; Ngamo & Hance, 2007). The strong larvicidal, repellent and behavioural effects of *E. bupleuroides* observed here reinforce the potential of Saharan flora as a reservoir of natural pesticides, consistent with recent findings on

Ruta, *Artemisia*, *Euphorbia* and other aromatic plants (Günaydin & Savci, 2005; Majdoub et al., 2014; Lim et al., 2023).

CONCLUSION

This study shows that the aqueous extract of *E. bupleuroides* has a strong disruptive effect on *D. melanogaster*, acting simultaneously on survival, feeding behaviour, sexual activity, and reproduction. The extract caused marked toxicity in both larvae and adults, altered larval chemosensory responses, and significantly impaired courtship sequences and mating success. Females also avoided laying eggs on the treated substrate, revealing a clear repellent effect and reduced reproductive performance.

Overall, *E. bupleuroides* negatively affects multiple physiological and behavioural functions in *D. melanogaster*, highlighting its potential as a natural source of bioactive compounds for developing environmentally friendly insect control strategies.

AUTHORS CONTRIBUTION

All authors equally contributed to this study**.** Sarra HABBACHI, Nour El- imen BOUBLATA, Alina Iuliana TABIRCA, Reda DJAOUAHDOU, Wafa HABBACHI, Saliha BENHISSEN and Abdelkrim TAHRAOUI, designed and carried out the experimental study and writed the manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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