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IMPACTS OF *HYSCOMAUS ALBUS* AQUEOUS EXTRACTS ON MORTALITY, SEXUAL BEHAVIOR AND OVIPOSITION OF *DROSOPHILA MELANOGASTER* (DIPTERA; DROSOPHILIDAE)

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Abstract: Biological control is based on the use of plant extracts. *Hyscoymus albus* is a toxic hallucinogenic plant with insecticidal properties. In this study, we have shown the toxic effect of the plant with its aqueous extract with different concentrations on the *Drosophila melanogaster* fly which reaches up to 85%. A remarkable disturbance on sexual behavior sequences in adults when the sublethal concentration (5µg/ml) is used. A decrease in the number of eggs and larvae laid by females treated with the extract prepared by decoction shows the toxic and repellent effect of *H.albus* on *Drosophila melanogaster.*

Keywords: Insecticide, fruit fly, Saharan plant, mortality, reproduction**.**

INTRODUCTION

Insects, which constitute more than 50% of the diversity of the planet (Wilson, 1988) and nearly 60% of that of the animal kingdom (Pavan, 1986) are becoming increasingly important in research belonging to the phylum of arthropods insects play true epidemiological roles which makes it a major public health problem (Chandrashekar et al. 2003). Insects are widely studied because of their impact on human and animal health, as well as crops and habitats (Benhissen, 2016).

The botanical world has limited the use of chemical pesticides against these pests and true to the use of secondary compounds in plants to control these pests. *Hyoscyamus albus* belongs to the *Solanaceae* family, which includes around 20 species distributed in the Mediterranean basin, North Africa, and West Asia (Goullé et al., 2004; Jouzier, 2005). Species of the genus *Hyoscyamus* are important medicinal plants, represented by two main species: *Hyoscyamus niger* and *Hyoscyamus albus*. And they are very rich in tropanic alkaloids, mainly hyoscyamine and scopolamine (Nejadhabibvash et al., 2012).

The insect biological model in this study is the vinegar fly *Drosophila melanogaster*. It is one of the best-studied model organisms currently used for biological research, particularly in genetics and development (Tracqui et al., 2003). It is considered to be a dreaded nuisance both for the inconvenience caused by the parasitic diseases that it can inoculate (Jolivet, 1980; Joly, 2006; Habbachi et al., 2013a). It is also an important vector of various infectious microorganisms, including yeasts and phytopathogenic bacteria which attack not only vegetable and fruit crops but also cereal and sunflower crops (Corby-Harris et al., 1979; Kloepper et al., 1979; Corby-Harris et al., 2007; Nadarasah & Stavrinides, 2011; Becher et al., 2012). Adult fruit flies can become troublesome by their overgrowth. Larvae ingested with infested fruit or liquids stored in dirty containers cause diarrhea and sometimes intestinal irritations (Rageau, 1958, Joly, 2006; Delbac et al., 2014). It also remains a material of choice for bioassays.

This species is also used in the study of behavior, reproduction, relationships with other species, or even adaptation to the environment (Joly, 2006). Numerous behaviors of drosophila are based on the recognition of chemicals and in particular of pheromones representing intraspecific chemical signals, which play an important role in the attraction and discrimination of sexual partners (Symonds & Elgar, 2008).

Some studies have shown the sublethal-doses insecticides affects the behavior of harmful or beneficial insects (Haynes, 1988; Lûrning & Scheffer, 2007, Benhissen et al., 2018, Benhissen et al., 2019, Bekhakhche et al., 2018, Habbachi et al., 2019, Habbachi, 2020) in particular they induce a decrease in behavioral responses to sex pheromones in treated individuals (Wei & Du, 2004; Zhou et al., 2005).

This work aims to evaluate the direct and indirect effects of the *H. albus* leaves aqueous extracts. We search to determine the sublethal concentration (5µg / ml) activities on behavioral sequences leading to mating, fertility, and reproductive potential. Also, we evaluate its activities on olfactory attractiveness in the oviposition sites choice in *Drosophila*.

MATERIAL AND METHODS

Insect: *Drosophila melanogaster* was discovered by (Johann Wihelm Meigen, 1830), For the experiment, a stock of a wild strain, derived from fermented apples in the region of Annaba (Algeria),

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was kept in flasks containing a standard agar medium (cornmeal and brewer's yeast base), at a temperature of 25 ± 1 ° C, a humidity of 70% and a 12: 12 h light: dark cycle (Habbachi et al., 2013; Habbachi, 2020). It's a holometabolic insect that belongs to the Diptera order and Drosophilidae family. It's a prolific fly; a female can lay 200 to 300 eggs (Goudey-Perrière & Perrière, 1974; Colombani et al., 2006).

***Hyoscyamus albus*:** A fairly rare plant found in wasteland, poisonous and annual or biennial herbaceous, 30 to 90 cm high, branchy, slimy, and hairy, which gives off a strong odor. It is used for the treatment of bronchial asthma, spasmodic cough, and lung ailments and ailments. It is common in rubble, wasteland (Goullé et al., 2004). Aerial parts of *H. albus* were collected in the region of M'sila in November 2019 (south-central Algeria).

***H. albus aqueous extract' preparation*:** The extracts from the leaves are made by decoction in distilled water for 30 minutes, yielding a stock solution of 149 µg / ml. After preliminary tests, five concentrations are studied (0.5 µg / ml, 1 µg / ml, 2 µg / ml, 5 µg / ml and 10 µg / ml).

***H. albus aqueous extract' effects on mortality*:** Treatment by ingestion on 2nd instar larvae. 40 g of culture medium containing 10 ml of product are distributed in four tubes where 20 larvae are placed and a control tube. The mortality and development of the larvae are monitored for 15 days.

***H. albus aqueous extract' effects on sexual behavior and reproduction*:** We treated larvae with a sublethal concentration (5µg / ml) and the adults will be tested for the sexual display of flies (touching, vibration, licking, attempt, mating), the females which mate will be isolated and bet in a box containing two types of medium (control / treated) and we note female choices, the eggs number, and larvae are counted.

Data analysis

For the toxicological study, lethal concentrations (LC50% and LC90%) for the insecticide used were calculated according to Finney's mathematical procedures (Finney, 1971).

The various parameters measured in this study were statistically analyzed by descriptive metric methods, then we compared the "k" samples' variances of (ANOVA) on XLStat 2009 software (Addinosoft NY). The results of the choice tests are compared using the Monte-Carlo simulation, based on a Chi2 test at the threshold α = 0.05 (Vaillant & Derrij, 1992).

RESULTS

H. albus aqueous extracts' effects on mortality

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 (Tab. 1A).

Table 1.

Toxicological parameters of the larvicidal effect of *H. albus* aqueous extracts' on L2 larvae
(A: *larvae exposure time*, B: *concentrations*, y: *probits of mortality rates*, X: *the decimal logarithm of concentrations and/or times*)

A			
Lethal times (days)			
	regression	***LT 50%***	***LT 90%***
0.5µg/ml	Y= -1.31+2.59x (R= 0.66)	*263.02d*	*851.10d*
1µ g/ml	Y= 1.42+2.66x (R= 0.93)	*45.70d*	*66.06d*
2 µg/ml	Y=1.68+2.14x (R= 0.73)	*35.48d*	*138.03d*
5 µg/ml	Y= 1.97+1.57x (R= 0.76)	*83.17d*	*549.54d*
10 µg/ml	Y= -1.82+3.59x (R= 0.66)	*54.95d*	*177.80d*
B			
Lethal Concentration (µg/ml)			
Time	***regression***	***LC 50%***	***LC90%***
2 days	Y= 0.75-2.76 X (R= 0.02)	*33.88µg/ml*	*100.00µg/ml*
10 days	Y=1.89-012X (R= 0.03)	*25.91 µg/ml*	*32359.36µg/ml*
15 days	Y= 4.60-9.20 X (R= 0.11)	*1.09 µg/ml*	*1.52 µg/ml*

H. albus aqueous extract' (5 µg / ml) effects on sexual behavior

We recorded that control and treated couples put their sublime at the same time to establish the first contact between partners. The same result was observed for the different times recorded during the *D. melanogaster* sexual parade. Variances comparison shows that there is no significant difference between the recorded times (first contact time *p: 0.035*, first vibration time *p: 0.0385*, first licking time *p: 0.012*, first attempt time *p: 0.55*, mating time *p: 0.25*) (Tab.2).

Our results indicate that the LT50% is 263.02j for the low concentration and 83.17j for the sub-lethal concentration. The LT90% reached 549.54d for the sub-lethal concentration (Tab. 1A).

Larval mortality rates are weakly and positively correlated with the concentrations of the extract used (Tab. 1B). The lethal concentration of 50% reaches 25.91 µg / ml at 10 days and 1.09 µg / ml of the extract of *H. albus* at 15 days of exposure (Tab. 1B). 90% of the larvae die with the 1.52 µg / ml concentration after 15 days (Tab. 1B).

The *H. albus* aqueous extract has a significant influence on flies contacts number (Fobs = 12.18, *p: <0.0001*), on the wing vibrations number (F_{obs} = 6.776; *p: 0.000****) and the licks number (F_{obs} = 7.967, *p:*

*0.000****) as well as the attempts number (F_{obs}= 6.090; *p: 0.001***) (Tab.2).

Table 2.
H. albus aqueous extract' (5 µg/ml) effects on different sexual behavior sequences in *D. melanogaster* (Mean ± SEM)

	First contact time	First vibration Time	First licking Time	First attempt Time	Coupling Time
♂C X♀C	118,700±22,878	285,700 ±73,588	332,100± 74,083	404,150 ± 80,702	333,350 ± 71,353
♂*H.a* X♀*H.a*	184,710± 39,945	366,200 ± 89,951	341,530± 102,635	309,240 ± 80,258	239,580 ± 95,018
♂ C X♀*H.a*	229,960±62,430	311,130 ± 86,956	287,890 ± 80,081	363,180 ± 122,209	252,160 ± 109,988
♂*H.a* X♀C	162,750±42,955	259,450 ± 86,956	272,220 ± 77,612	232,620 ± 61,802	277,770 ± 90,324
F_{obs}	***3,024***	***1,028***	***0,791***	***3,869***	***0,730***
P	***0,035***	***0,385***	***0,503***	***0,012***	***0,537***
	Contacts number	**Vibrations number**	**Licks number**	**Attempts number**	**Mating time**
♂C X♀C	285,700 ± 73,588	8,150 ± 1,833	5,650 ± 1,330	3,650 ± 1,091	1179,350 ± 185,563
♂*H.a*X♀*H.a*	366,200 ± 89,951	25,950 ± 7,175	12,350 ± 3,605	7,850 ± 2,456	595,320 ± 151,652
♂CX♀*H.a*	311,130 ± 86,956	14,100 ± 4,978	3,650 ± 1,110	1,85 ± 0,634	332,940 ± 119,295
♂*H.a*X♀C	259,450 ± 86,956	30,300 ± 7,507	12,200 ± 3,082	8,250 ± 2,533	492,660 ± 128,636
F_{obs}	***1,028***	***6,776***	***7,967***	***6,090***	***4,615***
P	***0,385***	***0,000****	***0 ,000****	***0,001***	***0,005***

[♂**C:** Control Male**;** ♂***H.a*:** Male treated with *H. albus***;** ♀ **C:** Control female**;** ♀ ***H.a*:** Female treated with *H. albus*] (*: Significant, **: Highly significant, ***: Verry highly significant)

H. albus aqueous extract (5 µg / ml) effects on oviposition

The results show that control females (control and treated couples) prefer to lay their eggs and larvae in the control medium. Statistical analysis shows that there are very highly significant differences between the two choices (*p< 0,0001****) (Tab. 3. A. B).

Table 3.
H. albus aqueous extracts' (5 µg / ml) effect on *D. melanogaster* oviposition

	A			
	The control eggs number	**The treated eggs number**	t_{obs}	***P***
♂C X♀C	57,800 ± 2,855	/	36,251	<0,0001***
♂*H.a*X♀*H.a*	60,60 ± 09,92	5,050 ± 1,51	31,159	<0,0001***
♂C X♀*H.a*	9,500 ± 1,597	2,200 ± 0,687	13,22	0,001**
♂*H.a*X♀C	7,400 ± 1,241	3,800 ± 1,146	0,707	0,406
	B			
	The control larvae number	**The treated larvae number**	t_{obs}	***P***
♂C X♀C	41,75 ± 2,194	/	47,139	<0.0001***
♂*H.a*X♀*H.a*	36,65 ± 5,180	4,650 ± 1,653	15,827	0
♂C X♀*H.a*	1,100 ± 0,946	3,150 ± 1,314	2,173	0,149
♂*H.a*X♀C	4,300 ± 1,949	2,550 ± 1,293	1,665	0,205

[♂**C:** Control Male**;** ♂***H.a*:** Male treated with *H. albus***;** ♀ **C:** Control female**;** ♀ ***H.a*:** Female treated with *H. albus*]

When control males and treated females lay eggs and larvae in both environments (control, treated), the student t-test shows that there are highly significant differences between the two established choices (*p: 0.001***), while the control male and treated female pairs show that there are no significant differences between the two choices (Tab. 3. A. B).

DISCUSSION

The insecticides use aim is to reduce insect infestations below nuisance levels (Dent, 1991). However, their use has led to the long-term emergence of increasingly resistant strains (Georghiou & Lagunes-Tejeda, 1991). Phytosanitary products are used in the public health field to fight against human diseases vectors (malaria, yellow fever, denge fever etc.) (Louat, 2013). For decades, natural products use, especially, plant extracts, as a type of insect control. In Algeria, the research has started to develop, through a multitude of studies as (Aouinty et al., 2006; Habbachi et al., 2013; Habbachi et al., 2014; El-Bah et al., 2016; Bekhakheche et al., 2018; Chabi et al., 2018, Habbachi et al., 2019; Saadane et al., 2021).

In this study, we showed insecticidal activities of *H. albus* leaves aqueous extract on mortality, the toxicity process is particularly important in *D. melanogaster* larvae by ingestion. *H. albus* has a larvicidal effect; so, several studies have shown larvicidal activity of plantgenerated products against Dipterans (Aouinty et al., 2006, Habbachi et al., 2013; Habbachi et al., 2014, El-Bah et al., 2016 and those of Benhissen et al., 2018; Chabi et al., 2018, Habbachi et al., 2019, Kheroubi et al., 2020). We also found plants from arid areas of the Maghreb are the most effective, even against the most resistant insects such as locusts (Idrissi et al., 1998; Idrissi Hassani. 2000; Idrissi Hassani and Hermas, 2008; Lebouz, 2010; Abbasipour et al., 2010 and Kemassi and Oueld El-Hadj, 2014) or cockroaches (Masna et al., 2015). Our results work in with those of Habbachi et al. (2019) and Saadane et al. (2021), who showed that *Cleome arabica* (Saharian toxic plant) and *Drimia maritima* (Mediterranean plant) have a toxic effect on *Drosophila* mortality

The male partner selection experiment was not simply a measure of male preference; successful copulation also requires the target female to be receptive (Somashekar et al., 2011). We found that the aqueous extract (5 µg / ml) disrupts different parade sequences leading to mating in couples composed of one or both treated partners. The blockage of sexual parade or insect mating is especially noticeable when one of the two partners is treated; this may be due to the mutual non-recognition between the two insects especially as the plant acts significantly on the contact sequence in the fly. The contact's role in the mutual partners' recognition has been demonstrated in different insect species such as cockroaches (Roth & Willis 1952; Smyth, 1963; Farine et al., 1993; Gropeaux, 1994). One of the control means that works in better harmony with the environment are the use of plant-derived toxicants, some of which are involved in the neuroendocrine regulation, metamorphosis, and reproduction of insects (Philogene, 1991; Rembold, 1994). Our results are similar to the works of Bensafi, 2010; Bourbia, 2012; El- Bah et al., 2016; Habbachi et al., 2019) on Spinosad, *Bacillus thuringiensis var kurstaki*, Azadiractin, *Peganum harmala*, and *C. arabica* on the drosophila mating parade.

After mating, the second essential phase in insect reproduction is oviposition. Also, under neuronal control, leading to selective oviposition on adapted substrates (Becher et al. 2012). The repellent effect of insecticides can also induce a diet or a decrease in the insect's food supply, which can lead to a decrease in fertility (Li X et al., 2007; Louat, 2013).

Our study confirms that *H.albus* has a repulsive effect on Drosophila causing changes in its olfactory responses, concerning the anti-fertilizing and anti-dissolving effect of aqueous extracts, corresponding to the results of Khattak et al., 2006; Rehman et al., 2009a, 2009b on *Bactrocera zonata* and olive fly (*Bactrocera oleae*), (Amandeep & Meera, 2014) on *Tribolium castaneum* (Masna, 2016) on *Blattella germanica*, (Benhissen, 2016) and Benhissen et al., 2019 on *Culex pipiens*, and El-Bah, 2017 and Saadane et al., 2021 on *D. melanogaster*.

CONCLUSION

In this study, we indicate that *H. albus* aqueous extract has a toxic property; the sublethal concentration (5 µg / ml) disturbs the sexual behavior and oviposition of the flies. We show that the treated individuals are unable to present a complete nuptial courtship and that the control females prefer a control environment that offers the fly favorable conditions for its development and we noted the repulsive effect of *H. albus* aqueous extract. Chemical analysis of treated and control flies can provide information on any changes in adults' nuptial courtship, pheromone secretions, mating duration, and various sexual sequences for adults. This work results suggest the presence of toxic secondary metabolites in the extract studied, which can develop an insecticid based on *H. albus* leaves and can be used in agriculture because it will be cheaper.

AUTHORS CONTRIBUTION

All authors are equally contributed to this study. Sarra HABBACHI, Nour El Iméne BOUBLATA, Fatma Zohra SAADANE, Abir BOUZAR, Makarem RAHAT, Wafa HABBACHI, Saliha BENHISSEN, Khellaf REBBAS, Abedkrim TAHRAOUI, designed and carried out the experimental study and writing of the manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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