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NICOTIANA GLAUCA GRAHAM (*SOLANACEAE*) BIOACTIVITY AND TOXIC EFFECTS ON MORTALITY, FEEDING BEHAVIOR AND PUPATION CHOICE OF *DROSOPHILA MELANOGASTER* LARVAE (DIPTERA: DROSOPHILIDAE)

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ABSTRACT

Nicotiana glauca is a medicinal plant used by traditional healers as antibacterial, antifungal, antiviral and anti-inflammatory medicines. The leaves of *N. glauca* are very rich in indolic alkaloids which give it a larvicidal power which allows us to use it as a bio-insecticide.In the present study we were perfomed in the direct (mortality) and indirect (food attractiveness and pupation) toxic effects of the aqueous extract of *N. glauca* on the mortality and feeding behavior of the fruit fly *Drosophila* melanogaster. The treatment was administered to 2nd instar larvae (L2) and the mortality rate was monitored for 15 days. Similarly we treated the 2nd instar larvae (n=50) with a sub-lethal concentration of 25µg/ml. Two days after the treatment the third instar larvae of *D. melanogster* are exposed to two nutrient media (control and treated with *N. glauca*) and the choice of the larvae is noted during one hour of time.The findings show that after 15 days of treatment, mortality rates in *D. melanogaster* larvae can reach 50%, as we observed a disruption in olfactory and gustatory signals, with both control and treated larvae preferring the food preparation mixed with the aqueous extract of *N. glauca* Graham and losing their ability to smell their control medium. This indicates that the bioactive chemicals isolated from this poisonous plant are mostly appealing allelochemical substances.

Keywords: *Drosophila melanogaster*, *Nicotiana glauca*, mortality, feeding behavior, pupation.

INTRODUCTION

Today, the need for pesticides and the pesticide industry is changing there is an increasing need for more toxicologically and environmentally friendly pesticides and natural products. Natural products such as plants often meet this need (Duke et al., 2010).

Synthetic insecticides have resulted in the creation of insect resistance strains, ecological imbalance, and mammalian injury, among other issues. Because of the limitations of the insecticides that have been created thus far, researchers in the region are working hard to discover environmentally friendly alternatives. Botanical pesticides could one day replace synthetic insecticides since they are reasonably harmless, easily degradable, and widely available in many regions of the world (Sivagnaname and Klyanasundaram, 2004).

Biopesticides in general and those of plant origin in particular 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 towards non-target species, supporting compatible use in integrated pest management programs (Deravel et al., 2014). In addition, plant 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).

Researchers are interested in in-depth studies and analyses of the therapeutic efficacy as well as the toxicological aspect of plants (De Smet, 1993). For decades, the use of natural products, specifically aqueous or ethanolic plant extracts, as a type of insect control in Algeria has begun to develop, through a multitude of recent works (Aouinty et al., 2006; Kemassi et al., 2014; Habbachi et al., 2013; Aouinty et al., 2006, Benhissen, 2016; Merabti et al., 2015; Merabti, 2016, Masna, 2016; El-Bah et al.,2016; Bekhakheche,2018).

Insecticidal qualities are known in around 6,000 plant species, and many of these plants are employed by farmers in underdeveloped nations (Walia et al., 2008). Furthermore, only a small number of these plants have been investigated for insecticidal efficacy, with many of the studies being incomplete or using incorrect or insufficient bioassay methodologies (Isman, 2013).

The Solanaceae family belongs to the second plant group of the tubiflora which includes 6 families of about 2000 species each. They have some common characteristics: they are essentially herbs, well represented in temperate and cold areas, the organs richest in alkaloids are the roots, but all parts of the plant are toxic to varying degrees (Goullé et al., 2004).

The genus Nicotiana is well known for its insecticidal effects (Puripattanavong et al., 2013). Like other species of *Nicotiana*. Pyridine alkaloids are found in abundance in *N. glauca* Graham (Andersson et al., 2003). Pesticides containing alkaloids (nicotine and anabasine) have been frequently utilized. Nicotine is the most abundant alkaloid in *Nicotiana tabacum* (da Silva et al., 2013) and *Nicotiana rustica* (Lisko et al., 2013), but anabasine is most abundant in *Nicotiana glauca* (Lisko et al., 2013; Slyn'ko et al., 2013).

The objective of this work is to evaluate the direct (mortality) and indirect (food attractiveness and pupation) effect of the plant on the mortality and feeding behavior of *D. melanogster* in order to disrupt the olfaction and gustation of this fruit fly. Among all living things, the *Drosophila* is the most well-known and researched in the laboratory (Tracqui et al., 2003). It is the fly that causes grey rot in fruits due to the fungi it brings. If eaten while eating contaminated fruits, the larvae can cause intestinal discomfort or diarrhea.

MATERIALS AND METHODS

Insect

Johann Wihelm Meigen first described the Drosophila melanogaster in 1830. It reproduces at a breakneck speed. It has a short life cycle that involves three larval stages and a pupal stage before emerging as an adult capable of flying and reproducing (habbachi, 2020).

Mass Breeding

The wild strain was obtained from rotting apples in the Annaba (Algeria) region. Rearing is done in tubes (12 x 4 cm) containing cornmeal and brewer's yeast agar nutrient medium, which are plugged with a foam pad. Rearing is done at a temperature of 251°C, with a humidity of 70 to 80 % and a scotophase of 12 hours.

Nicotiana glauca Graham (Solanaceae)

It is an ornamental plant it has insecticidal properties (Benhissen et al., 2018). *N. glauca* evergreen (Asma et al., 2012), measuring 1 to 2 meters sometimes more, growing in the Mediterranean region, Anabasine is a nicotine-like alkaloid found in *N. glauca*, and its toxicity appears as a cholinergic syndrome characterized by weakness, hypertension, tachycardia, convulsions, and muscle fasciculations (Sercan et al., 2018). (Hoffman et al., 2015).

Plant Material

In spring 2019, leaves of *N. glauca* Graham were collected in Hodna (M'sila, central Algeria) for this investigation. PrRebbasKhellaf, Department of Biology, Faculty of Science, University of M'sila, identified the plant.

Extraction

We made the extract by boiling 146 g of fresh leaves in 2000 ml of distilled water on a hot plate at 180° C for 30 minutes. We recover 1000 ml of the filtrate (146 g/ml stock solution) after filtering the resultant combination via filter paper.

Toxic Effects of Aqueous Extracts of N. glauca in D. melanogaster

Toxicological Study

The toxicity test consists of exposing or administering (by ingestion) different concentrations (25 µg/ml, 75 µg/ml, and 100 µg/ml) of aqueous extracts of *N. glauca* to 2nd instar *Drosophila larvae* under well-controlled laboratory conditions. The mortality of the larvae is monitored for 15 days (time needed to finish development).

Olfactory Attractiveness and Pupation Test in D. melanogaster

Third instar larvae (L3) were tested for feeding behavior by attracting odors from the nutrient medium. The test matrix used was a plastic Petri dish (100 mm diameter) containing 2 % agar, with the bottom covered with a paper that was drawn in pencil. "A" and "B") arranged in a line; each of the areas represents 10% of the total surface of the arena (Figure 1). The artificial medium used is a smooth surface that facilitates the movement of the larvae.

The filter papers (already prepared) were introduced into tubes containing the control culture medium and mixed with the aqueous extract, 2 hours later the papers were put into petri dishes in zone A and zone B, then the tested larvae were put in and the choice of each larva and the time it took to reach the chosen zone was noted, 24 hours after the food attractiveness test the pupation zone of the larvae was noted.

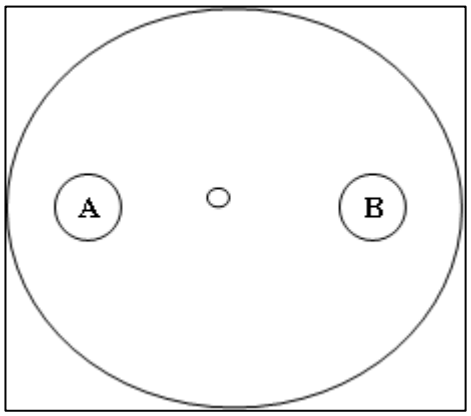

Figure 1: Diagram of the test arena (Petri dish, diameter 100 mm)

Data Analysis

The data from the tests of the larvae's eating behavior were examined using Monte Carlo simulation using a Chi^2 test with a non-significance threshold of 0.05. (Vaillant and Derrij, 1992). On XLstat, the results were also subjected to descriptive statistical analysis and variance comparison (2009).

RESULTS

Toxicity

The results of this work show that the aqueous extracts of *N. glauca* act on the mortality of the larvae according to the concentration applied. We manage to kill half of the population after 15 days of treatment with the concentration 100 µg/ml (P = 0.016*).On the other hand, the concentration25 µg/ml (p = 0.012*) presents low mortality rates, which we can consider as a sublethal concentration (25 µg/ml) (Figure 2).

Effects on the Feeding Behavior of Drosopihlla.melanogaster

Attraction of Control and Treated Larvae by the Aqueous Extract of N. glauca

During the first minutes of the test, we noticed that 44 % of the control larvae are attracted by the odor of the control medium and 56 % of these maggots are attracted by the odor of the treated medium. 52 % of the larvae treated with the extract are attracted by the odor of the control medium during the first minutes of the test and 42 % of the treated larvae are attracted by the odor of the treated medium, the rest of them do not make their choice (Table 1).

Selection of Control and Treated Larvae after 30 minutes of Observation

After 30 minutes of observation, we noticed that the larvae change their first choice or 18 % of the control larvae are attracted by the odor of the control medium and 54 % of these maggots are attracted by the odor of the treated medium (Table 2).

Choice of Control and Treated larvae after 60 minutes of observation

After 60 minutes of observation, we recorded that 14 % of the control larvae chose the control medium while 56 % of the latter chose the treated medium and 30 % of these maggots did not make their choice For the treated larvae after one hour of observation, 36 % of them chose the control medium and 30 % chose the treated medium, while 34 % of the latter did not make their choice (Table 3).

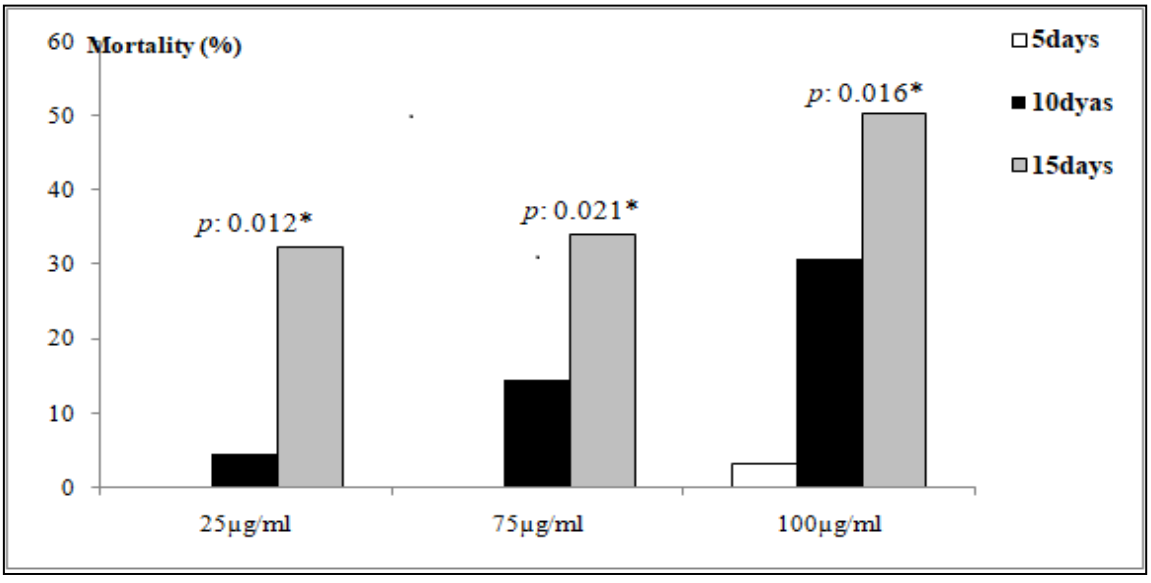

Figure 2: Effect of aqueous extract of *N. glauca* on mortality.

Table 1: The first choice of attractiveness of the control and treated larvae towards the different odors of the media tested by the aqueous extract of *N. glauca.*

	Control Larvae			Treated Larvae		
	C x C	**C x *N. g***	***N. g*x*N.g***	**C x C**	**C x *N. g***	***N. g* x *N. g***
Control	98 %	44 %	-	90 %	52 %	-
Treated	-	56 %	100 %	-	42 %	98 %
No choice	2 %	0 %	0 %	10 %	6 %	2 %

Detection Time

At the presence of two different odors (control vs. treated), control larvae move slower to the control medium with 344.960 ± 133719.672 seconds and faster to the medium treated with the aqueous extract of *N. glauca* average 267.540 ± 122055.968 seconds (F_{obs} = 1.096; p = 0.751). Regarding the treated larvae, the detection times recorded are on average 409.760 ± 64.809 and 432.180 ± 77.196 seconds to locate respectively, the papers soaked in the control medium and treated with the Aqueous extract of *N. glauca* (F_{obs} = 1.419 ; p= 0.224) (Table 4).

Time spent in each Medium

It is recorded that the control larvae spend 291.280 ± 65.733 seconds at the level of the soaked papers in the control media but these maggots spend more time of 704.280 ± 92.245 seconds on average in the treated media (F_{obs} = 1.969 ; p = 0.019*) thus the treated larvae spend 359.200 ± 74.770 seconds on average at the level of the soaked papers in the control media and 373.720 ± 76.905 seconds on average in the treated media in the presence of the two different odors (F_{obs} = 1.058 ; p = 0.845) (Table 5).

Pupation of Control and Treated Larvae

Concerning the observation of the pupation of the control larvae at the level of the arenas after the tests of feeding behavior, we marked that 10 % of the control larvae make their choice of pupation in the control medium and 28 % in the treated medium, On the other hand, in the presence of the control medium and the treated medium, the remainder of the latter do not make a decision. The follow-up of the chrysalis of the treated larvae after the tests showed that 28 % of the treated larvae make their choice for the control medium, and only 24 % for the treated medium, against 48 % of the latter do not make their choice when both media are present in the test box (Table 6).

Attraction Index

The calculation of the attraction index (AI) confirms the results obtained in our study and shows positive values that indicate that the larvae either control or treated present an attractive effect towards the odor of the environment treated by the aqueous extract of *N. glauca*. Insect control is entering a new "botanical" phase in which no-toxic compounds are used on non-target organisms, they are biodegradable, less likely to cause resistance in target species, and they appear to be one of the more environmentally friendly methods (Philogene,1991). Natural plant extracts contain a vast range of secondary metabolites, each of which has a different biological and toxicological activity (Saadane et al., 2021).

Table 2: Choice after 30 minutes of the control and treated larvae towards the different odors of the media tested by the aqueous extract of *N. glauca.*

	Control Larvae			Treated Larvae		
	C x C	**C x *N.g***	***N.g*x *N.g***	**C x C**	**C x *N.g***	***N.g*x *N.g***
Control	54 %	18 %	-	46 %	24 %	-
Treated	-	54 %	38 %	-	32 %	50 %
No choice	44 %	44 %	12 %	54 %	44 %	50 %

Table 3.Choice after 60 minutes of the control and treated larvae towards the different odors of the media tested by the aqueous extract of *N. glauca.*

	Control larvae			Treated Larvae		
	C x C	**C x *N.g***	***N.g*x*N.g***	**C x C**	**C x *N.g***	***N.g*x*N.g***
Control	52 %	14 %	-	38 %	36 %	-
Treated	-	56 %	28 %	-	30 %	52 %
No choice	48 %	30 %	72 %	62 %	34 %	48 %

Table 4: Detection time (seconds) of control and treated larvae in response to different odors of the tested medium control medium; medium treated with aqueous extract of *N. glauca*

Control larvae							
Environment	**Choice**	**N**	**Mean ±SEM**	**Min**	**Max**	**F_{obs}**	***P***
Control X Treated	**Control Environment**	22	344.960±133719.672	0	1353	1.096	0.751
	Treated Environment	28	267.540±122055.968	0	1439		
Control X Control	**A**	20	267.540±49.408	0	1439	1.762	0.751
	B	29	344.960±51.715	0	1353		
Treated X Treated	**A**	26	304.600±59.263	0	1593	1.678	0.073*
	B	24	222.940±45.748	0	1532		
TreatedLarvae							
Environment	**Choice**	**N**	**Mean ±SEM**	**Min**	**Max**	**F_{obs}**	***P***
Control X Treated	**Control Environment**	26	409.760±64.809	0	1597	1.419	0.224
	Treated Environment	21	432.180±77.196	0	1713		
Control X Control	**A**	19	238.340±53.939	0	1708	1.103	0.734
	B	26	651.471±89.096	0	1685		
Treated X Treated	**A**	26	280.760±54.406	0	1556	1.084	0.779
	B	23	323.240±56.639	0	1685		

[N : Average number of individuals from the different treatments, Mean : Average ; SEM : Standard deviation of the mean, Min : Minimum, Max : Maximum, A : Control environment, B : Treated environment]

P < 0.05: significant; P < 0.01**: highly significant P < 0.001***: very highly significant*

Table 5: The time spent (seconds) in each medium by control larvae and larvae treated with aqueous extract of *N. glauca.*

Control Larvae							
Environment	**Choice**	**N**	**Mean ±SEM**	**Min**	**Max**	**$F_{obs.}$**	***P***
Control X Treated	**Control Environment**	22	291.28±65.733	0	1547	1.97	0.019*
	Treated Environment	28	704.280±92.245	0	2468		
Control X Control	**A**	20	237.900±59.717	0	1572	1.67	0.077*
	B	29	436.120±77.085	0	1730		
Treated X Treated	**A**	26	310.980±59.273	0	1698	1.23	0.5
	B	24	394.440±65.843	0	1687		
Treated Larvae							
Environment	**Choice**	**N**	**Mean ± SEM**	**Min**	**Max**	**F_{obs}**	***P***
Control X Treated	**Control Environment**	26	359.200±74.770	0	1710	1.06	0.8
	Treated Environment	21	373.720±76.905	0	1736		
Control X Control	**A**	19	275.280±70.414	0	1700	1.03	0.9
	B	26	479.660±71.565	0	1683		
Treated X Treated	**A**	26	344.660±65.372	0	1501	1.09	0.8
	B	23	354.240±68.263	0	1735		

[*N : Average number of individuals from the different treatments, Mean : Average ; SEM : Standard deviation of the mean, Min : Minimum, Max : Maximum, A : Control environment, B : Treated environment****]***

P < 0.05: significant; P < 0.01**: highly significant P < 0.001***: very highly significant*

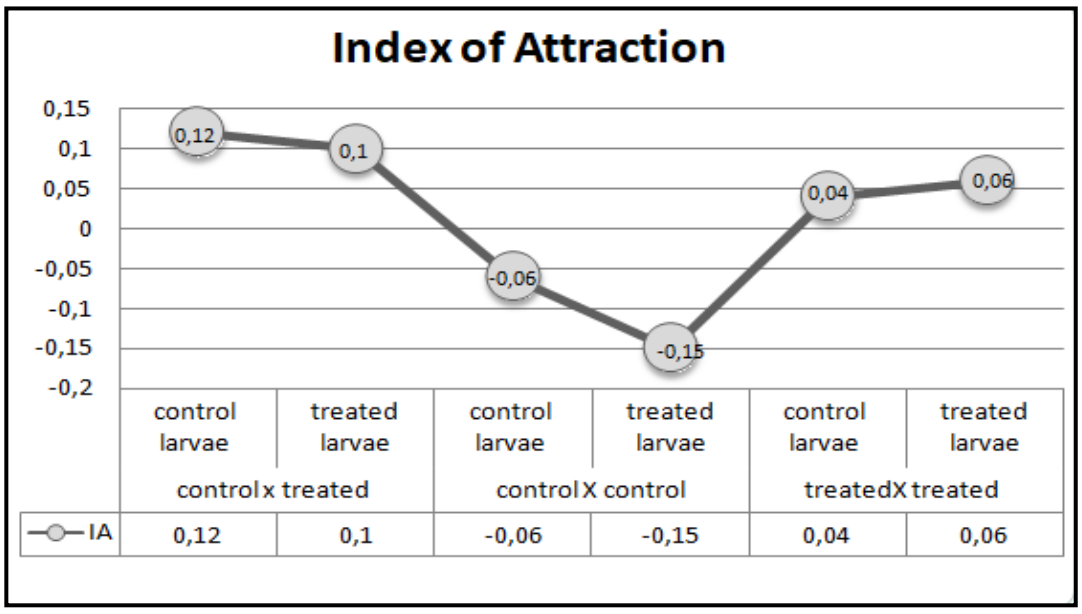

Figure 3: Attraction index of treated and control larvae towards the aqueous extract of *N. glauca.*

Table 6: Preoccupation of control and treated larvae with the different odors of the media tested by the aqueous extract of *N. glauca.*

	Control larvae			Treated Larvae		
	C x C	**C x *N.g***	***N.gxN.g***	**C x C**	**C x *N.g***	***N.gxN.g***
Control	48%	10%	-	36%	28%	-
Treated	-	28%	28%	-	24%	26%
No choice	52%	62%	62%	64%	48%	74%

DISCUSSION

In this study, we employed the aqueous extract of *N. glauca* to identify the plant's poisonous compounds, assess its neurotoxic potential, and determine whether it may be used as a bio-insecticide to control the spread of insect pests, The results of the present study show that the extracts of *N. glauca* cause significant mortality in *D. melanogaster* larvae. Similar work proves the insecticidal effect of *N. glauca* on *Culisetalongiareolata* (Benhissen et al., 2018) ,These results are similar to previous work on Diptera toxicity we note those reported in *Culexpipiens*with *Peganumharmala* (Habbachi et al., 2014; Benhissen, 2016) and *Blattellagermanica*(Masna, 2016), crop pests; on Lepidopteran larvae (Abdel-Rahman and Al-Mozini, 2008; Abbasipour et al., 2010) as well as on beetle larvae (Salari et al., 2012; Meera, 2014) and d'acridians (Idrissi-Hassani and Hermas, 2008; Kemassi et al., 2012). DhumadKadhim et al., 2015 reported the effects of different seed extracts of *P. harmala*on rice weevil *Sitophilusoryzae*L. at 1%, 2% and 3% concentration. After 24, 48, 72 hours, mortality rates of 98, 100 % were recorded with ethanolic extract and 64, 66, 74% with aqueous extract, respectively.

Indeed, some authors have been able to show the insecticidal property, towards a number of pests (Zeng et al., 2010; Bouayad et al., 2012; Kontsedalov et al., 2009; Ghanim and Kontsedalov, 2009; Marčić et al., 2009; Bretschneider et al., 2003), the action of *Daphne gniduim* has also been reported on *Spodopteralittoralis* (Pérez Izquierdoand Ocete, 1994), *Xanthogalerucaluteola* (Maistrello et al., 2005), *S.oryzae, Rhizoperthadominica* (Benayad, 2008) and *C.pipiens* (Habbachi et al., 2014). Dahchar et al., 2016 tested the larvicidal potential of aqueous extract of *D.gniduim*against two mosquito species, *C.pipiens* and *C.longiareolata*.

The chemosensory neural systems of *D. melanogaster* composed of olfactory and gustatory organs allow the guidance and location of food sources and/or sexual partners' remarkable fact in insects and in particular in *Drosophila* is that the gustatory organs are scattered over the body. The anterior edge of the wing is lined with gustatory sensilla alternating with mechanical sensilla .the functionality and role of taste cells in the *Drosophila* wing remains enigmatic and to date largely unknown (Stocker, 1994).

Chemical signals can be divided into two groups based on the source of emission and the receiver: allelochemicals, which are signals emitted from one animal to another (Whittaker &Feeny, 1970), and pheromones, which are chemical signals that carry information from one individual to another member of the same species (Karlson and Lùscher, 1959). Insect sexual behavior and social existence rely heavily on intraspecific chemical communication (Desneux et al., 2007; Badi, 2015).

Drosophila larvae possess on their anterior part, 3 external sensory organs: the dorsal organ (DO), the terminal organ (TO) and the ventral organ (VO). These

organs contain sensory sensilla allowing the larva to perceive the molecules present in its environment (Fourgeron, 2011). Only the dorsal organ (DO) is involved in olfactory perception. It is an organ consisting of a dome, containing sensilla with several pores, and six peripheral sensilla. The dome is innervated by 32 neurons including 21 ORNs (OlfactoryReceptorNeuron) (Oppliger et al., 2000; Kreher et al., 2005; Heimbeck et al., 1999). When these 21 neurons are inhibited, the larvae become anosmic, suggesting that these are the only olfactory neurons in the larvae (Larsson et al., 2004; Fishilevich et al., 2005).

When an odorant molecule reaches the OD, it will then bind to ORs, present on the dendrites of the ORNs. Twenty-five different ORs (OlfactoryReceptor) are expressed in larvae. Among these ORs, 14 are specific to the larval stage and 11 exist in both larvae and adults (Fishilevichet al., 2005). Larval ORs respond to aromatic and aliphatic compounds in the food (Kreheret al., 2005).

In the present study on the feeding behavior of *D. melanogaster* we observed that both control and treated larvae prefer the medium treated with the plant (an attractive effect). Some of the tested larvae lose the ability to detect, in our test a significant number of maggots fail to locate the control and/or treated odors and the study of Elbah 2017 showed that the extract of *P. harmala* seeds mixed with artificial medium seems to be attractive for maggots, nevertheless they take time to adapt the odor and arrive at the source. However, the leaves and flowers of *P. harmala* have a repulsive effect on the insect. It is likely that other factors, in addition to the repellent alkaloids, also determine the performance of control larvae relative to *P.harmala*.

In 2020 Habbachi also proved that *Cleome arabica* extracts disrupt the feeding behavior and attraction of the larvae or the larvae (control and treated) are attracted much more by the ethanolic extract (an attractive effect) of *C.arabica* with sub-lethal concentration (0.5µg/ml) in contrast to the aqueous extract which caused disruption of sensing of the nutrient medium and presents a repulsive effect.

Elbah (2017) also showed that control larvae are attracted by the odor of *D.gniduim* berries and not by its leaves, which may be due to the amount of attractive phenols in each part of the plant .In 2011, Fourgeron was able to show that the larvae of this fly are significantly attracted by the odor of their culture medium and particularly by the odor of unsaturated fatty acids.

The bioactive molecules of the aqueous extract of *N. glauca* significantly influence the place of pupation. When both media are used in the test arena the tested *Drosophila* larvae prefer to pupate away from the soaked papers of the culture media similarly the study of habbachi (2020) showed that larvae treated with aqueous and ethanolic extract of *C. arabica* prefer to pupate outside the two soaked papers in both culture media.

Therefore, there is a need to develop safe, biodegradable and inexpensive indigenous vector control methods (Kumar et al., 2012), with biologically active natural chemical components (Maheswaran et al., 2008; prabakar et al., 2004).Such as the attractive alkaloids of *N. glauca* that cause significant mortality and completely disrupt olfaction and gustation of *D. melanogaster*.

CONCLUSION

This study indicates that the aqueous extract of *N. glauca* has a neurotoxic property or it presents a significant mortality in the treated larvae although the sub-lethal concentration of the extract 25 µg/ml disturbs the olfactory and gustatory system of *D. melanogaster* or control larvae are unable to relate their control nutrient media and are always attracted to the treated nutrient preparation as well as the pupation which is always far from both

media which proves that the secondary molecules of this neurotoxic plant are mainly attractive allelochemical compounds. This could lead to the development of *N. glauca*-based bio-insecticides for agricultural usage and sale on the chemical market.

AUTHOR'S CONTRIBUTION

Each author contributed equally in writing this manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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