

Evaluation of the Toxic Effects of Aqueous Extracts of *Solanum Nigrum* L.(Solanaceae) on the Mortality and Development of *Drosophila Melanogaster* (Diptera : Drosophilidae).

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EVALUATION OF THE TOXIC EFFECTS OF AQUEOUS EXTRACTS OF *SOLANUM NIGRUM* L.(*SOLANACEAE*) ON THE MORTALITY AND DEVELOPMENT OF *DROSOPHILA MELANOGASTER* (DIPTERA : *DROSOPHILIDAE*).

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ABSTRACT

Solanum nigrum is a plant that has therapeutic properties of the *Solanaceae* family. It is used extensively in traditional medicine but also as a bio-insecticide to control insect pests. Some parts of this plant can be very toxic to livestock and humans. In the present study, the direct and delayed toxic effects of aqueous extracts of *S. nigrum* on the mortality and development of *Drosophila melanogaster* were investigated. For this study, an extraction method including decoction is adopted to extract the active principles from the leaves of *S. nigrum*. The treatment was carried out by ingestion on 2nd instar larvae (L2) and adults. The exposure to the aqueous extract shows that it has a good insecticidal activities, it acts on the larvae by inducing 96.25 % mortality rate at the end of treatment by 50g/l. The adults' mortality increases with the lengthening of the exposure time as well as with the dose increase. The maximum mortality was recorded is between 97.50% and 100% in males and females. We also recorded a disturbance in the flies development, which took less time to reach the adult stage when they were treated, and this for the five concentrations. Malformations were recorded in the adult wings after treatment with the different concentrations of the toxic plant. In addition, some flies showed a significant increase in size compared to controls.

Keywords: *Drosophila melanogaster*, *Solanum nigrum*, toxicity, development, mortality.

INTRODUCTION

In order to control insect pest populations, mankind is making considerable efforts, seeking new physical, chemical and biological control methods to limit their proliferation (Appel, 1990; Kim and al., 1995; Lyon, 1997). Chemical pesticides are the most widely adopted control method because of their effectiveness on the target (Cantrell and al., 2012; Casida & Durkin, 2013; Boulahbel, 2015; Habbachi, 2020).

Chemicals used in insect control have not been able to completely control some pests. In addition, they have

increased the environmental impact by poisoning living beings (Louat, 2013). Taking environmental and ecological problems seriously has prompted research organisations and institutions to look for alternative methods of controlling these pests. Indeed, in modern agriculture, thanks to the study of direct plant defenses and their mode of action, the focus is on the control of pests. Therefore, there is an increasing focus on natural compounds from plants for the development of new bio-insecticide molecules (Habbachi and al., 2013).

The possibilities of using secondary substances from plants against insect pests, has attracted much interest, some works have shown the wide variety of biological activities of plant-based preparations (Candan and al., 2003). Indeed, the botanical world provides means of control in better harmony with the environment. Many plants synthesise secondary metabolites, such as alkaloids, polyphenols, terpenes, steroids, and their essential oils have important biological properties against pests (Silva and al., 2002; Regnault-Roger and al., 2005).

Plant extracts have been used as pesticides for a long time as Pyrethrum, nicotine, and rotenone are all well-known compounds as insect agents of control (Crosby & Rucker, 1966). According to Jacobson (1989), more than 2000 plant species with insecticidal activity have already been identified. Algeria, thanks to its geographical situation, its relief, its great variety of climates and soils, has a varied flora in the coastal regions, the mountainous massifs, the high plateaus, the steppe and Saharan oases, containing more than 3000 plant species (Saad and al., 2005).

Studies have shown the toxic effect of bio-insecticide molecules by ingestion, and their activity on mortality and sexual behaviour of *D. melanogaster* as well as the feeding attractiveness of maggots before the pupal stage remains poorly understood. The latter act on the development of the flies also causing malformation in the wings (Habbachi and al., 2013 ; Elbah and al., 2016 ; Elbah., 2017 ; Habbachi and al., 2019 ; Habbachi and al., 2020 ; Habbachi., 2020 ; Saadane and al., 2021).

The *Solanaceae* family is a medium-sized one comprising about there are 96 genera and 3000-4000 species in the area worldwide (D'Arcy, 1979). Among the angiosperm families, *Solanaceae* is one of the most crucial families for individuals. Food is obtained from members of the family for food like potato (*Solanum*

tuberosum L.), tomato (*Solanum lycopersicum* L.) and aubergine (*Solanum melongena* L.) and as medicines like *Nicotiana tabacum* L. and *N. rustica* L., *Atropa belladonna* L., *Mandragora officinarum* L. and *Duboisia spp.* The *Solanaceae* family includes plants that range in size from trees to tiny annual herbs, and live in a variety of environments, including deserts and tropical climates rainforests; and in terms of morphology, with surprising variations in many cases and fruit characters (Knapp et al., 2004). Family members are well-known for the creation of a wide variety of secondary metabolites, e.g. alkaloids, flavonoids and terpenes (Evans, 1986). Recently, the ecological importance of *Solanum* alkaloids in the limitation of frugivores (Cipollini and Levey, 1997a; Albuquerque et al., 2006), as well as antifungal agents (Cipollini and Levey, 1997b).

The effects of *Solanum nigrum* extracts and solamargine alone were shown to be poisonous, although the effects differed between species. Solamargine and solasonine, for example, were ineffective against *Manduca sexta* but reduced the development of *Tribolium castaneum* larvae. (Weissenberg et al., 1998) . Solamargine has a detrimental effect on *Macrosiphum euphorbiae* reproduction. (Günter et al., 2000) and changed *Zophobas atratus'* cardiac activity (Ventrella et al., 2015). Surprisingly, *S. nigrum* extracts have a fatal toxicity against mosquitos. (Rawani et al., 2013) as well as beetles (Gokce et al., 2007) has been previously reported

In this work, we aim to test the direct and indirect toxic effect of the aqueous extract of *S. nigrum* (*Solanaceae*) on *Drosophila melanogaster*. A plant widely distributed throughout the world, ranging from tropical to temperate regions; in Europe, Asia and North America. and has been introduced in South America, Australia and Africa (Rizzo et al., 2019). In this work we evaluated the effect of the

plant on the mortality and development of *D. melanogaster*. *Drosophila* is the best known and most studied in the laboratory among all beings (Tracqui et al., 2003).

MATERIALS & METHODS

Insect

Drosophila melanogaster was described by Johann Wihelm Meigen in 1830. Its reproduction is very rapid. It has a very short life span and consists of three larval stages and a pupal stage from which an adult emerges that can fly and reproduce.

Mass Rearing

A wild strain collected from rotten apples in the Annaba region (Algeria) is used. The rearing is carried out in tubes (12 x 4 cm) plugged with a foam pad and containing an agar-based nutrient medium made of cornmeal and brewer's yeast. The culture is maintained at $25\pm 2^\circ$ C, a humidity of 70 to 80% and a scotophase of 12 hours.

Solanum nigrum (Solanaceae)

It's an herbaceous, annual, therophyte plant that can grow up to 70 cm; belonging to the *Solanaceae* family. Its habitat is cosmopolitan. Plants of the *S. nigrum* complex, analgesic, antispasmodic, and antiseptic have all been utilized in the past. antidyscentric, antinarcotic, emollient, diuretic, tonic, soporific, laxative, anticancer, antiulcer, and for diseases of the gastrointestinal tract of the neuro-vegetative system (Saijo et al., 1982; Akhtar & Muhammad, 1989; Schilling et al. 1992; Edmonds & Chweya, 1997; Manoko et al., 2007).

Preparation of the S. nigrum Aqueous Extract

It consists in taking the fresh leaves of the plant which were harvested in

November in the Boussaada region (M'sila, Algeria, N $35^\circ 23'28''$, E $04^\circ 18'07''$, 406 m altitude) then drying them well at an ambient temperature in the shade and in the open air for 7 days. A quantity of 116 g is boiled in one liter of distilled water for 30 minutes on a hot plate at 180° C. The solution obtained is filtered to obtain a stock solution of 116 g/l.

S. nigrum Aqueous Extract Effects on Larvae

For the treatment we prepared five different concentrations (10 g/l, 25 g/l, 50 g/l, 75 g/l, 100g/l). The toxicity test consists of exposing L₂ larvae to different concentrations of *S. nigrum* by ingestion. 10 ml of each concentration is added to 40 g of culture medium, which is then placed in four tubes. In each tube, twenty larvae taken at random from the mass rearing are placed. Another 20 larvae are placed in another control tube. The mortality and development of the larvae is monitored for 15 days.

S. nigrum Aqueous Extract Effects on Adults

The toxicity test consists of administering the different concentrations of the extract by ingestion to male and female *Drosophila* adults. 10ml of each concentration is mixed with 40 g of food which will be distributed in eight different tubes for each concentration (4 tubes for males, 4 tubes for females). In each tube, 20 adults (males or females) are placed and recovered from the emergence of the mass rearing. We monitored mortality for 15 days with a control preparation for each sex.

Data Analyses

The toxicological parameters (LC50%, LC16%, LC84% and LC90%, LT50%, LT16%, LT84% and LT90%) were calculated according to the

mathematical procedures of Finney (Finney, 1971).

RESULTS

Effects on Larvae Mortality

The results show that the aqueous extracts of *S. nigrum* act on the duration of larval development and on the mortality of the larvae according to the concentration applied. The 75 g/l concentration shows a

low larvicidal activity while the 50 g/l concentration manages to kill 95 % of the population at the end of the treatment. For the 3 concentrations (10; 25; 100 g/l), mortality varies between 30 % and 40 %, approximately. The analysis of the data indicates highly significant differences between the mortality rates of the larvae recorded during the 15 days of monitoring (p between 0.003 and 0.029) (Tab. 1).

Table 1: Mortality rates caused by the different concentrations of *S.nigrum* on L2 *D. melanogaster* larvae

	<i>10g/l</i>	<i>25g/l</i>	<i>50g/l</i>	<i>75g/l</i>	<i>100g/l</i>	<i>F_{obs}</i>	<i>p</i>
<i>2days</i>	0.00%	0.00%	0.00%	0.00%	1.25%	9.00	0.001 **
<i>5days</i>	0.00%	0.00%	0.00%	0.00%	1.25%	9.00	0.001 **
<i>10days</i>	2.50%	13.75%	11.25%	5.00%	5.00%	0.57	0.69
<i>15days</i>	38.75%	37.50%	96.25%	7.50%	30.00%	1.36	0.29
<i>F_{obs}</i>	4.23	0.01	8.76	5.27	0.032		
<i>P</i>	0.02 *	0.003 **	0.02 *	0.01 *	0.99		

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

The mortality rates of the larvae are weakly correlated to the concentrations of the extract used (Tab. 2A). The lethal concentration of 50% reached 645.65 g/l at 10 days and 11.22 g/l of the *S. nigrum* extract at 15 days of exposure (Tab. 2A). 90% of the larvae die with the 0.003 g/l concentration after 15 days (Tab. 2A).

For the lethal times, the results show that there is a strong positive correlation between the mortality rate and the larvae exposure time to the plant extracts (Tab. 2B). Our results indicate that the LT50% is 23.44 days for the low concentrations and 60.25 days for the highest concentration. The LT90% reaches 331.13 days for the highest concentration (Tab. 2B).

Effects on Adult Mortality

The results show that *S. nigrum* aqueous extracts also act on the adults mortality according to the concentration applied.

For females, all the concentrations used manage to kill 100% of the population after 15 days of treatment; there is a very highly significant difference between the mortality rates recorded for the concentration 75 g/l ($p < 0.0001$ ***), there is also an effect of time on mortality for the other concentrations (Tab. 3).

For males, there is a highly significant difference between the mortality rates recorded for the 25 g/l concentration ($p < 0.0001$ ***); there is a

Table 2: Toxicological parameters of *S. nigrum* aqueous extracts on *D. melanogaster* larvae L2

A									
Time	Regression	LC _{50%}	LC _{90%}	LC _{84%}	LC _{16%}	Slope	fcl50%	Low.lim	Up.lim
2days	Y=-2.05+1.61X (R=0.55)	23.44	147.9	97.72	5.75	4.115	1.20	19.53	28.128
5 days	Y=-2.05+1.61X (R=0.55)	23.44	147.9	97.72	5.75	4.115	1.20	19.53	28.128
10 days	Y=2.79+0.38X (R=0.39)	645.65	1.51	263.02	1.62	199.47	1.86	347.19	1200.90
15 days	Y=5.38-0.36X (R=0.123)	11.22	0.003	0.02	6.30	0.002	0.44	25.5	4.93
B									
[C]	Regression	LT _{50%}	LT _{90%}	LT _{84%}	LT _{16%}	Slope	fTI50%	Low.lim	Up.lim
10g/l	Y=-2.39+5.38X (R=0.898)	23.44	40.73	35.48	15.31	1.52	1.06	22.11	24.84
25g/l	Y=-2.50+5.85X (R=0.902)	19.05	31.62	28.18	12.88	1.47	1.05	18.14	20.00
50g/l	Y=-3.41+7.61X (R=0.893)	12.58	18.62	16.98	9.33	1.34	1.04	12.10	13.09
75g/l	Y=-2.06+4.82X (R=0.902)	28.84	53.70	46.77	17.78	1.62	1.07	26.95	30.85
100g/l	Y=1.91+1.73X (R=0.815)	60.25	331.13	223.87	16.21	3.71	1.20	50.21	72.30

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

(Up.lim= upper limit ; Low.lim = lower limit)

Table 3: Mortality rates caused by different concentrations of *S. nigrum* on *D.melanogaster* adults

Female	10 g/l	25 g/l	50 g/l	75 g/l	100 g/l	F _{obs}	p
2 days	10.00%	22.50%	6.25%	3.75%	5.00%	1.66	0.21
5 days	45.00%	71.25%	33.75%	47.50%	33.75%	11.29	< 0.0001***
10 days	90.00%	98.75%	96.25%	100%	87.50%	7.68	0.001**
15 days	98.75%	100%	100%	100%	100%	9	< 0.0001***
F _{obs}	9.96	16.93	3.37	57.54	4.9		
P	0.01*	< 0.0001***	0.05*	< 0.0001***	0.02*		
Male	10 g/l	25 g/l	50 g/l	75 g/l	100 g/l	F _{obs}	P
2 days	13.75%	32.50%	1.25%	6.25%	5.20%	8.62	0.001**
5 days	51.25%	76.25%	7.50%	33.75%	17.50%	4.58	0.013*
10 days	92.50%	98.75%	100%	98.75%	97.50%	3.2	0.043*
15 days	97.50%	100%	100%	98.75%	100%	7.07	0.002**
F _{obs}	5.44	69.55	3.79	8	10.44		
P	0.014*	< 0.0001***	0.06	0.003**	0.001**		

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

Table 4: Toxicological parameters of *S. nigrum* aqueous extracts on *D. melanogaster* females

A									
Time	Regression	LC _{50%}	LC _{90%}	LC _{84%}	LC _{16%}	Slope	∫cl50%	Low.lim	Up.lim
2 days	Y=4.66-0.72X (R=0.66)	0.33	0.006	0.01	7.94	0.035	0.64	0.51	0.21
5 days	Y=5.52-0.40X (R=0.4)	19.95	0.012	0.07	6.02	1.65	1.06	18.82	21.15
10 days	Y=5.79+0.78X (R=0.306)	0.09	4.16	1.77	0.005	18.43	1.46	0.07	0.13
15 days	Y=6.42+1.25X (R=0.826)	0.073	0.77	0.44	0.011	6.32	1.29	0.06	0.1
B									
[C]	Regression	LT _{50%}	LT _{90%}	LT _{84%}	LT _{16%}	Slope	∫T150%	Low.lim	Up.lim
10 g/l	Y=2.31+4.06X (R=0.988)	4.57	9.33	7.94	2.57	1.75	1.08	4.23	4.93
25 g/l	Y=2.38+5.16X (R=0.979)	3.16	5.92	4.89	2.04	1.54	1.06	2.98	3.35
50 g/l	Y=1.05+6.13X (R=0.943)	4.36	7.07	6.30	3.01	1.44	1.05	4.15	4.58
75 g/l	Y=0.31+7.87X (R=0.961)	3.89	5.62	5.24	2.95	1.33	1.04	3.74	4.04
100 g/l	Y=1.13+5.89X (R=0.901)	4.46	7.41	6.60	3.01	1.48	1.05	4.25	4.68

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

(Up.lim= upper limit ; Low.lim = lower limit)

Table 5: Toxicological parameters of *S. nigrum* aqueous extracts on *D. melanogaster* males

A									
Time	Regression	LC _{50%}	LC _{90%}	LC _{84%}	LC _{16%}	Slope	∫cl50%	Low.lim	Up.lim
2 days	Y=5.05-0.95X (R=0.559)	1.12	0.05	0.10	12.30	0.19	0.80	5.90	0.21
5 days	Y=6.59 1.27X (R=0.596)	17.78	1.73	3.01	107.15	0.18	0.80	22.22	14.22
10 days	Y=6.06+0.80X(R=0.387)	21.13	1.88	0.81	0.0028	3841.83	2.92	7.23	61.69
15 days	Y=6.40+1.03X(R=0.471)	0.04	0.77	0.04	0.0047	4.75	1.24	0.03	0.05
B									
[C]	Regression	LT _{50%}	LT _{90%}	LT _{84%}	LT _{16%}	Slope	∫T15%	Low.lim	Up.lim
10 g/l	Y=2.67+3.63X(R=0.994)	4.36	9.77	8.12	2.34	1.86	1.09	4.00	4.75
25 g/l	Y=2.81+4.76 X (R=0.975)	2.88	3.46	4.57	1.77	1.61	1.07	2.69	3.08
50 g/l	Y=-0.56+8.27X (R=0.886)	4.67	6.60	6.16	3.54	1.32	1.04	4.49	4.86
75 g/l	Y=1.66+5.00X (R=0.961)	4.57	8.39	7.34	2.95	1.57	1.06	4.31	4.84
100 g/l	Y=0.10+7.21X (R=0.925)	4.67	7.07	6.45	3.46	1.36	1.04	4.49	4.85

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

(Up.lim= upper limit; Low.lim = lower limit)

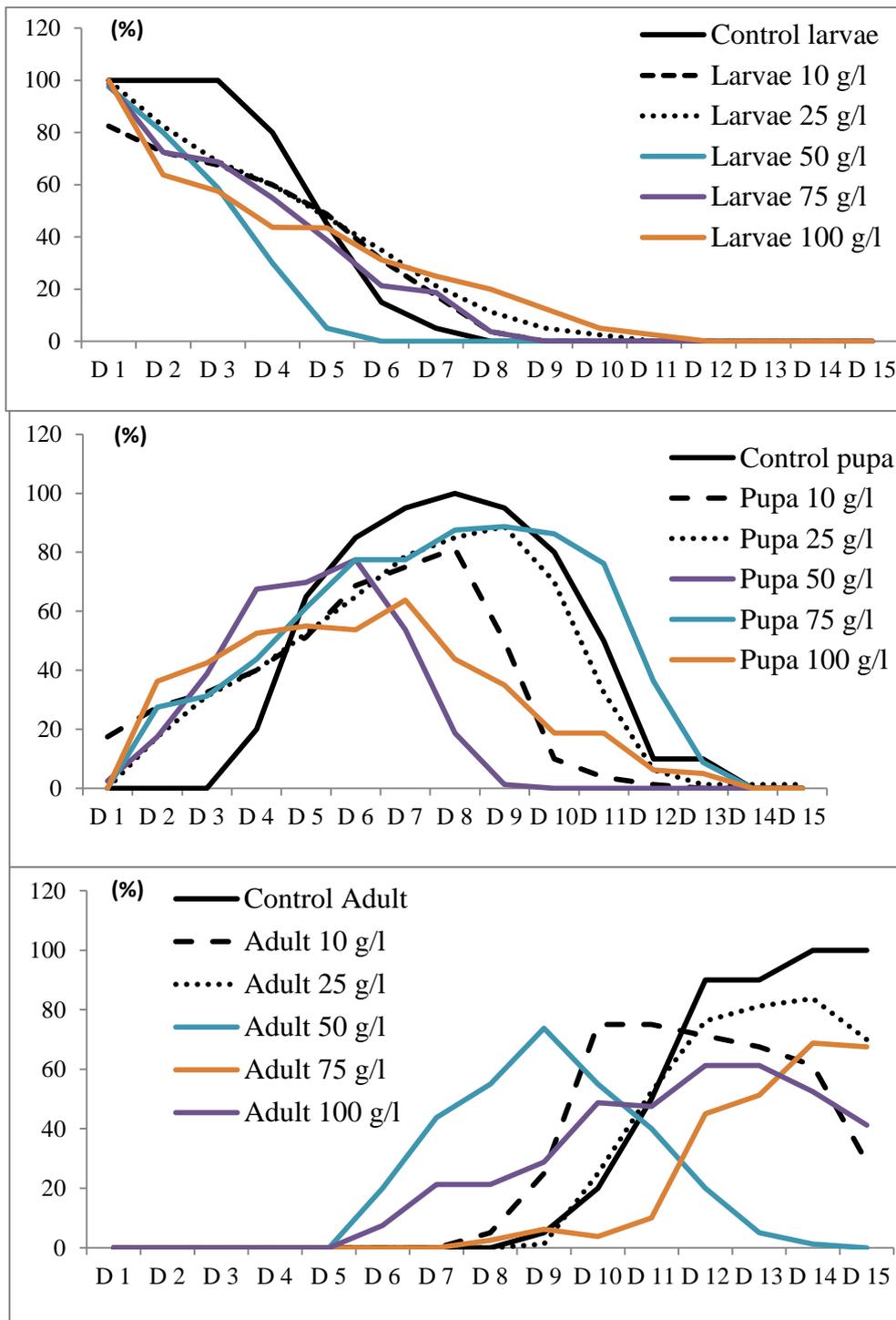


Figure 1. Effect of *S. nigrum* aqueous extract on the *D. melanogaster* development (A: Larval development; B: Pupal development; C: Adult development)

time effect on mortality for other concentrations (Tab. 3).

Mortality rates of adult females are strongly and positively correlated with the

concentrations of the extract used (Tab. 4A). The lethal concentration of 50 % reached 19.95 g/l at 5 days and 0.073 g/l of the *S. nigrum* extract at 15 days of

exposure (Tab. 4A). 90 % of the females died with the 0.77 g/l concentration after 15 days (Tab. 4A).

For the lethal times, the results show that there is a strong positive correlation between the mortality rate and the exposure time of *D. melanogaster* females to the plant extracts (Tab. 4B). Our results indicate that the LT50 % is 4.57 days for the low concentrations and 4.46 days for the highest concentration. The LT90 % reaches 7.41 days for the highest concentration (Tab. 4B).

The mortality rates of adult males are strongly and positively correlated with the concentrations of the extract used (Tab. 5A). The lethal concentration of 50 % reaches 17.78 g/l at 5 days and 0.04 g/l of the *S.nigrum* extract at 15 days of exposure (Tab. 5A). 90 % of the adults die with the concentration of 0.77 g/l after 15 days (Tab. 5A).

For lethal times, the results show that there is a strong positive correlation between the mortality rate and the exposure time of *D. melanogaster* males to the plant extracts (Tab. 5B). Our results indicate that the LT50 % is 4.36 days for low concentrations and 4.67 days for the highest concentration. The LT90 % reaches 7.07 days for the highest concentration (Tab. 5B).

Effects on D. melanogaster Development

S.nigrum acts significantly on the fly development by inducing acceleration in the larvae growth into pupae for all five concentrations. For the adult stage, we recorded an extract activity on the development, 100 % of the population treated with *S.nigrum* aqueous extracts reached the adult stage. We also noted that wing-level abnormalities and a change in morphology were present (Fig. 1).

DISCUSSION

The use of plant protection products is not without consequences for ecosystems. After application in the field,

these substances will be disseminated in the soil and in the air. Elements such as rain and wind will favour this dispersion, causing the contamination of soils, lakes, rivers, etc., sometimes very far from the site where the chemicals were applied. These substances have been found, for example, in ocean spray or in Arctic snow (Van der Werf, 1997).

At present, pest control in natural and man-made environments is increasingly moving towards the use of natural means to control the various pests. This trend is driven by a major concern to minimise 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).

Biomolecules belonging to the secondary metabolism of plants such as: terpenes, alkaloids, glycosides, polyphenols and essential oils, are easily biodegradable by enzymatic pathways, no biomagnification phenomenon having been described (Isman, 2002). These secondary metabolites develop little toxicity for vertebrates and are regularly consumed in food (Regnault-Roger and al., 2002).

Natural products are used, specifically plant extracts, as a type of insect control in Algeria has started to develop, through a multitude of recent works (Kemassi and Oueld Elhadj, 2008; Lebouz and Oueld Elhadj, 2010; Habbachi et al., 2013).

Mortality is the primary criterion for judging the chemical or biological efficacy of a treatment. Leaves of a Saharan test plant *S. nigrum* were presented to *Drosophila melanogaster* larvae and adults. To study and compare their efficacy, we chose the doses and time that caused the maximum mortality; i.e. the time of 15 days of exposure. In this study, we used the aqueous extract of *S. nigrum*, in order to identify the toxic substances of this plant and to see if the

plant, in addition to its medicinal capacities, can be used as a bio-insecticide to limit the proliferation of insect pests.

According to our results, the aqueous extract of *S. nigrum* administered by ingestion to 2nd instar larvae of *D. melanogaster* at different doses showed a strong insecticidal activity depending on the doses administered. The 50g/l concentration managed to kill 96.25% of the population at the end of the treatment which shows a strong positive correlation between mortality rates and exposure times of the larvae to the aqueous extract. Other studies have shown that plant extracts can have an intense insecticidal activity, such as the study by Benhissen et al (2019) on the aqueous extract of *Ruta chalepensis* (*Rutaceae*) and its direct effect on *Culiseta longiareolata* (*Culicidae*), this work showed that the mortality rate increases with the concentration of the extract and time. The highest mortality rate (100%) was obtained after 3 days of treatment for the highest concentration (33.2g/l). Further studies by Benhissen et al (2018) showed that the plant extract of *Nicotiana glauca* (*Solanaceae*) induced a mortality rate reaching 98.3% after 2 days of treatment when *Culiseta longiareolata* (*Culicidae*) larvae were exposed to the highest concentration (62.4g/l).

The application of aqueous extracts of *S. nigrum* on the 2nd instar larvae of *D. melanogaster* affected the survival of the adults. Indeed, this extract significantly reduced the longevity of males and females surviving the treatment. Female mortality increased with increasing exposure time as well as with increasing dose. . A maximum mortality percentage of 98.75 to 100% was recorded in females and 97.50 to 100% in males. In this regard, we cite the work of Bouzar et al (2021) who reported that the aqueous extract of *Nicotiana glauca* (*Solanaceae*) reduced the life span of the vinegar fly by causing significant mortality (100%) by treating the adults with the lowest concentration (10 µg/ml).

Insect structure and physiology vary during growth and development, with some developmental stages being more sensitive to insecticides than others (Hassan, 2009). Insect development can be disrupted by different insecticides, generally resulting in a lengthening of developmental duration; the appearance of morphological malformations and/or a reduction in longevity (Louat , 2013) .

In our experiments, the effect of *S. nigrum* on the development of the fly was also studied. The results show that its extracts influence the duration of embryonic life and the pupation and number of emerged adults causing an acceleration in the growth of treated larvae for all five concentrations. The results found for *S. nigrum* are in agreement with those of (Saadane et al., 2021) who proved that the plant extract of *Drimia maritima* (*Asparagaceae*) had a significant effect on the development of flies by also inducing an acceleration of the growth of larvae to pupae, at all three concentrations (0.12 µg/ml; 0.25 µg/ml; 0.5µg/ml). For the adult stage, developmental activity was recorded; only 80% of the population treated with *D. maritima* reached the adult stage.

Our results reveal that the aqueous extracts used, also induce various malformations in adults which are mainly located at the level of the wings (malformed or absent wings). These results agree with those of Elbah (2017) who also recorded malformations in the different stages of the vinegar fly following treatment with the aqueous extract of *Daphne gnidium* (*Thymelaeaceae*) leaves.

Several studies have highlighted the toxic effect of aromatic plants on Diptera such as (Idrissi et al., 1998 ; Idrissi Hassani L.M., 2000 ; Aouinty et al., 2006 ; Idrissi Hassani L.M. & Hermas J, 2008 ;; Lebouz I., 2010; Habbachi et al., 2013; Kemassi & Oueld El-Hadj, 2014; Habbachi et al., 2014; El-Bah D., 2016; Benhissen et al., 2018) .

CONCLUSION

The calculations of toxicological parameters (lethal concentrations and lethal times) indicate the differences. The observed mortality is positively correlated with the concentrations used and the exposure time. The toxicological tests showed the existence of toxicity towards the treated animals; these tests also allowed the determination of the LC50%, LC90%, and also and TL50%, TL90% for this product.

The results of this work suggest the presence of toxic substances in the studied extract which may lead to the development of bio-insecticides based on *S.nigrum* to be used in agriculture and sold on the pesticide market.

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