Research Article

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Azadirachtin effects on mating success, gametic abnormalities and progeny survival in *Drosophila melanogaster* (Diptera)

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Abstract

BACKGROUND: Azadirachtin is a prominent natural pesticide and represents an alternative to conventional insecticides. It has been successfully used against insect pests. However, its effects on reproduction require further analysis. Here we investigated lethal and sublethal effects of azadirachtin, on treated adults in a model insect, *Drosophila melanogaster* (Meigen). Dose–mortality relationships as well as several parameters of reproduction (mating, spermatogenesis, oogenesis and fertility) were examined.

RESULTS: Neem-Azal, a commercial formulation of azadirachtin, applied topically on newly emerged adults, increased mortality with a positive dose-dependent relationship. The LD_{50} (0.63 μg) was determined 24 h after treatment using a non-linear regression. Adults surviving this dose had a mating success that was divided by 3 and a progeny production reduced by half when males were treated, and even more when females were treated. When combining probability of survival, of mating and reduced progeny, it appeared that LD_{50} induced a 98% reduction in reproductive rates. Reduced progeny was partially explained by the effect of adult treatment on gametes number and abnormalities. The number of cysts and the apical nuclei positions within the cysts decreased by 29.7% and 20%, respectively, in males. In females, the number of oocytes per ovary and the volume of basal oocytes also decreased by 16.1% and 32.4%, respectively.

CONCLUSION: Azadirachtin causes significant toxic effects in both sexes and decreases the fecundity and fertility of *D. melanogaster*. Females are more sensitive to azadirachtin.

Supporting information may be found in the online version of this article.

Keywords: natural pesticide; azadirachtin; toxicity; spermatogenesis; oogenesis; *Drosophila*

1 INTRODUCTION

Crop losses caused by pests may represent the equivalent of the food needed to feed over 1 billion people.[1] The use of pesticides is therefore required to protect agrosystems. However, their side effects induce important health[2,3] and environmental problems.[4,5] Consequently, natural compounds with pesticidal properties (biopesticides) have been investigated for decades and offer a more sustainable solution to pest control than synthetic or conventional products.[6–8]

Azadirachtin, a tetranortriterpenoid derived from the seeds of the Indian neem tree (*Azadirachta indica* A. Juss, Meliaceae), is one of the main commercialised biopesticides and remains the most successful botanical pesticide in agricultural use worldwide.[9,10] It possesses a strong toxicity against insect pests of different orders.[9,11–14] Besides its insecticidal action, azadirachtin is also used in traditional medicine, in Asia and in Africa as an antidiabetic, immunostimulant, antimicrobial, antiviral, contraceptive and anticancer remedy.[15–17]

Azadirachtin is an insecticide with rapid biodegradability and without resistance problems due to its chemical complexity.[9] This pesticide is also reported to be relatively safer than most conventional insecticides.[18,19] It is non-toxic to humans and warm-blooded vertebrates[20] and without genotoxicity for mammals.[21,22] However, because azadirachtin acts as an insect growth disruptor,[23] negative side effects on beneficial arthropods are expected. Studies have reported various effects of neem products on non-target species, such as mites, parasitoids and

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bees.[24–29] For example, behaviour and morphological alterations have been highlighted in bees,[29] while Bernades *et al.*[27] did not detect any effect on worker bee mortality, flight, or respiration rate. The large panel of potentially affected functions and apparent contradictory conclusions are caused by the level of action of azadirachtin. This molecule interferes with the endocrine system by impairing juvenile hormone (JH) and 20-hydroxyecdysone (20E) pathways, which are involved in the regulation of many physiological functions.[9] Thus azadirachtin induces growth and moulting abnormalities and can cause significant alterations in the reproductive system of both male and female insects, including ovarian and testes development, fecundity, fertility, oviposition and egg viability.[9,12,30–32] Azadirachtin also shows moderate to strong cytotoxicity, neurotoxicity and antimitotic effects.[9,33,34] It acts on the expression of genes related to development in *Drosophila melanogaster*.[18] These studies provide knowledge about the range of functions potentially affected by this molecule, but the effects of azadirachtin on reproductive parameters would benefit from being investigated more deeply. The extensive use of *D. melanogaster*, as a model species for toxicological studies and the wide knowledge acquired about its physiology, endocrinology and genetics, could be instrumental in achieving a precise understanding of pesticide effects on reproduction. Therefore, the aim of this study was double: on the one hand, it quantified the side effects of azadirachtin on *D. melanogaster* flies using doses allowing 25% and 50% of survivors, i.e. mimicking possible exposure to the insecticide residues; on the other hand, it filled some gaps in evaluating azadirachtin reprotoxic effects in both sexes. Effects were assessed (1) following treatment on newly emerged adults, and (2) on various reproductive traits of adult males and females. Previous studies have already shown that azadirachtin impacts the reproduction of *D. melanogaster* treated at the larval[35] and pupal stages.[36] Thus, we considered its impact on the survival of progeny at various development stages and on fertility in both sexes. We quantified the mating process, fecundity of males and females (number of cysts and potential abnomalities of spermatocyte nucleus positions; number of oocytes and size of basal oocyte) and number of progeny produced by surviving adults (numbers of eggs, larvae, pupae and adults obtained at the F_1 generation). A toxicity study was first carried out to determine lethal doses for *D. melanogaster* adults in our experimental conditions.

2 MATERIAL AND METHODS

2.1 Insect rearing

Drosophila melanogaster (Canton-S) was reared on standard corn-meal medium at 25 ± 2°C, 70% relative humidity under a 12-h light/dark photoperiod. Flies were transferred every 3 days to avoid larval competition and to regularly provide abundant progeny for testing.

2.2 Insecticide and toxicity tests

The Neem Azal, commercial formulation (1%; TrifolioM GmbH; Lahnau, Germany), was used. It has the advantage of containing only azadirachtin as an active substance, but at an affordable price, and at a low concentration, ensuring safe use to the experimenters. Sundaram *et al.*[37] showed that pure azadirachtin and commercial formulations had similar effects in the spider mite, *Tetranychus urticae*.[37,38] Neem Azal was dissolved in acetone and applied topically on adults (sexes combined after screening and showing any difference between male and female) less than 6 h after ecdysis (1 μL per insect according to Di Prisco *et al.*).[39] Control insects were treated with solvent alone. Five doses (0.1, 0.2, 0.4, 0.6 and 1.2 μg of Neem Azal acetone solution) were tested,[36] considering three replicates per dose, each consisting of 300 insects. Adult mortality percentages were calculated between 24 and 96 h after treatment and corrected in accordance with Abbott.[40] A non-linear regression was used to determine the lethal doses (LD) LD_{25} and $LD_{50,}$ corresponding to 25% and 50% of adult mortality respectively, with their corresponding 95% fiducial limits (95% FL) and Hill slope.

2.3 Mating assays

Newly emerged male and female flies (< 6 h post-emergence) were separated and treated topically with azadirachtin. Then control (M_C, F_C) and treated (M_T, F_T) males and females were placed in vials according to sex (25 mm in diameter, 95 mm in height) containing a standard corn-meal medium. After 48 h, one male and one female were placed together in individual vials with food for the mating assay. Two conditions were tested: $M_C + F_C$ and $M_T + F_{T,}$ and matings were monitored for 3 h in the morning. The number of vials in which mating occurred was counted. Results are presented as a mating percentage in each group.

2.4 Cyst parameters in males

Developmental factors leading to male infertility were investigated by analysing post-meiotic abnormalities, i.e. cyst numbers and spermatocyte nucleus localisation, both parameters influencing the number of sperm produced.[41] Newly moulted male adults (< 6 h) were treated topically with azadirachtin at the LD_{50} (0.63 μg). Individuals from the control and treated series were placed in vials containing standard corn-meal medium. After 48 h, testes were dissected in a phosphate-buffered saline (PBS) solution with 4′,6-diamidino-2-phenylindole (DAPI) allowing nuclei staining.[42] Cysts were gently spread on the blade and covered with a cover slip. Cysts were then observed under a fluorescence microscope and the number of cysts per male and the position of spermatocyte nuclei within the cyst were counted. Thirty repetitions per condition were performed.

2.5 Ovarian parameters in females

To detect a possible impact of azadirachtin on female fecundity, classical ovarian parameters were investigated (number of oocytes and size of basal oocyte). Newly emerged female adults (< 6 h) were treated topically with the azadirachtin at the LD_{50} (0.63 μg). Individuals from the control and treated series (LD_{50}, 0.63 μg) were placed in vials containing standard corn-meal medium. After 48 h, ovaries were dissected out. After removal of the circumovarian fat body, the numbers of oocytes were scored together with the volume of the basal oocyte.[43]

2.6 Progeny output

Newly moulted (< 6 h) *D. melanogaster* adults were treated topically with azadirachtin at the LD_{25} and LD_{50}. Subsequently, one male and one female from the control and treated series were placed in a Petri dish (90 × 14.2 mm) containing standard corn-meal medium to obtain their progeny. Eight repetitions of different types of couples were realised (control male + control female; control male + female LD_{25}; male LD_{25} + control female; male $LD_{25,}$ + female $LD_{25;}$ control male + female LD_{50}; male

LD_{50} + control female, male LD_{50} + female LD_{50}). After 48 h, the adults were removed and generation 1 (F_1) was followed daily until all adults had emerged. The numbers of eggs, larvae, pupae and adults that emerged from each series of experiments were counted.

2.7 Statistical analysis

The mean ± standard deviations (SD) were calculated for each experimental group. Data from the toxicity assay were analysed using non-linear sigmoid curve fitting. The goodness of fit to the curve model was evaluated on the basis of R^2 values. The homogeneity of variances was checked using Bartlett's and Shapiro–Wilk tests. Analyses of variance (one-way and two-way) were performed and followed by Tukey's HSD test for multiple comparisons when significant. Repeated-measure ANOVA was also used for progeny output between stages for the same couple. Chi-squared was performed for anomalies of spermatocyte nucleus positions. Numbers of cysts and ovarian parameters were compared between the treated and control groups using Student's *t* test. In mating experiments, the treated group was compared to the control group using Fisher's exact test. All calculations were performed using GraphPad Prism (v6.01 for Windows; Available at: http://fr.freedownloadmanager.org/Windows-PC/GraphPad-Prism.html).

3 RESULTS

3.1 Insecticidal activity

Azadirachtin, applied topically on newly emerged adults of *D. melanogaster*, induces an insect mortality 24 h after treatment. Corrected mortality percentages were 13.92 ± 1.43% for the lowest dose (0.1 μg) and 71.80 ± 3.11% for the highest dose (1.2 μg). The mortality percentage recorded in untreated animals was 5.48 ± 0.53% (control mortality, Fig.1). Statistical analysis revealed a significant dose effect ($F_{4, 10} = 62.43$; $P < 0.0001$) and Tukey's HSD test showed a significant increase in mortality with increasing doses (Fig. 1). The lethal doses (LD) recorded with 95% fiducial limits (95% FL) were LD_{25} = 0.23 (0.13–0.38), LD_{50} = 0.63 (0.44–0.91) and LD_{90} = 4.85 (1.47–15.98) μg (Table 1). The non-linear regression fitted on these results indicated a Hill slope of 1.08 (0.54–1.62). No significant effect of the time elapsed after treatment (24 to 96 h) was observed ($F_{5, 60} = 1.05$; $P = 0.39$; Table 2).

3.2 Mating tests

In *D. melanogaster*, azadirachtin applied topically (LD_{50}) on the day of adult emergence induced, 48 h after treatment, clear effects on mating success (Fig. 2). In the treated group, the mating percentage significantly declined compared to controls ($M_T + F_T$: 13.8% vs $M_C + F_C$: 43.2%; Fisher's exact test, $P < 0.001$).

3.3 Cyst parameters

The effect of azadirachtin treatment was tested on male fecundity. Azadirachtin was applied topically at LD_{50} (0.63 μg) on male *D. melanogaster* on the day of emergence. The number of cysts per testis was counted 48 h after emergence. Control adults displayed 28.47 ± 2.16 cysts per testis. This number decreased significantly after topical application of azadirachtin and reached 20 ± 1.69 ($t_{58} = 3.08$; $P = 0.003$) (Fig. 3A). In addition, the percentage of spermatocyte nuclei in abnormal position significant increased in LD_{50} males ($\chi^2 = 5.30$, 1; $z = 2.30$, $P = 0.021$) (Fig. 3B).

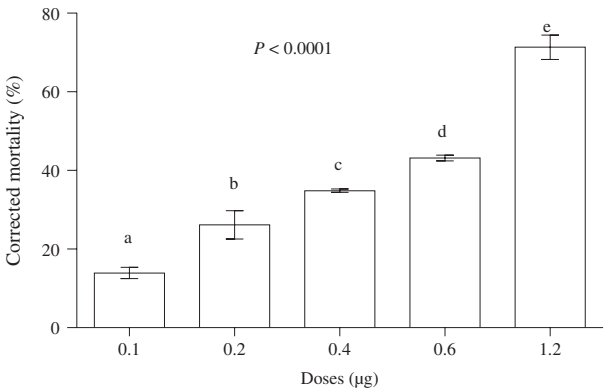

Figure 1. Toxicity of azadirachtin tested by topical application, at different doses, on the day of *Drosophila melanogaster* adult emergence: corrected mortality (%) at 24 h (mean ± SD; $n = 3$ replicates of 300 insects). Control mortality: 5.48 ± 0.53%. Different letters indicate a significant difference between control and treated series according to Tukey's HSD test.

Table 1. Toxicity of azadirachtin tested by topical application, at different doses, on the day of *Drosophila melanogaster* adult emergence: lethal doses and their fiducial limits

Parameter	Dose (μg)	Fiducial limits
LD 90	4.85	1.47–15.98
LD 50	0.63	0.44–0.91
LD 25	0.23	0.13–0.38
Hill slope	1.08 (0.54–1.61)	
R square	0.96	

Table 2. Toxicity of azadirachtin tested by topical application, at different doses, on the day of *Drosophila melanogaster* adult emergence: corrected mortality (%) from 24 to 96 h (mean ± SD; $n = 3$ replicates of 300 insects)

	Time (h)			
Dose (μg)	24	48	72	96
0.1	13.92 ± 1.43^{A}	15.96 ± 2.19^{A}	14.72 ± 2.24^{A}	14.65 ± 0.97^{A}
0.2	26.16 ± 3.58^{B}	23.67 ± 1.13^{B}	28.12 ± 1.22^{B}	27.77 ± 5.84^{B}
0.4	34.84 ± 0.44^{C}	33.85 ± 4.61^{C}	35.02 ± 5.92^{C}	34.89 ± 4.57^{C}
0.6	43.16 ± 0.74^{D}	42.46 ± 2.49^{D}	43.42 ± 2.47^{D}	43.87 ± 6.90^{D}
1.2	71.80 ± 3.11^{E}	69.88 ± 1.13^{E}	70.45 ± 6.20^{E}	69.88 ± 1.16^{E}

Means followed by the same uppercase letter do not differ significantly according to Tukey's HSD test at the level $P = 0.05$.

3.4 Ovarian parameters

The effect of azadirachtin treatment was tested on female fecundity. Azadirachtin was applied topically at LD_{50} (0.63 μg) on female *D. melanogaster* on the day of emergence. The number of oocytes was counted 48 h after emergence. We found a significant decrease in the number of oocytes in the treated series (13.90 ± 0.55) compared to controls (16.57 ± 0.53; $t_{58} = 3.43$; $P = 0.0011$, Fig. 4A). Furthermore, the volume of the basal oocyte showed a reduction in treated females (0.0048 ± 0.0003 mm^3) compared to controls (0.0071 ± 0.0005 mm^3) ($t_{58} = 3.87$; $P = 0.0003$, Fig. 4B).

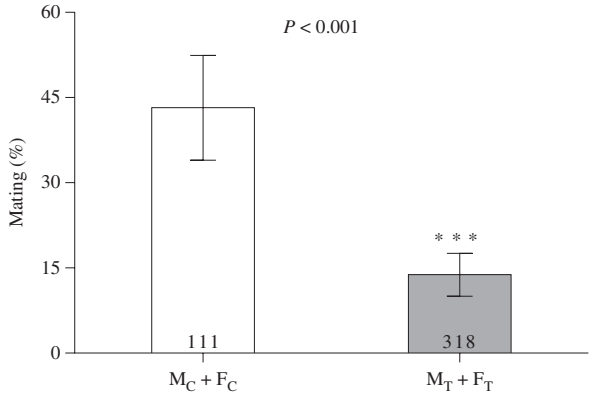

Figure 2. Effects of azadirachtin on mating percentages observed 48 h after treatment (0.63 μg) by topical application on the day of adult emergence of *D. melanogaster* (mean with 95% confidence intervals); M_C: male control; F_C: female control; numbers within each bar indicate the number of repetitions. *** Indicates significant differences, $P \leq 0.001$.

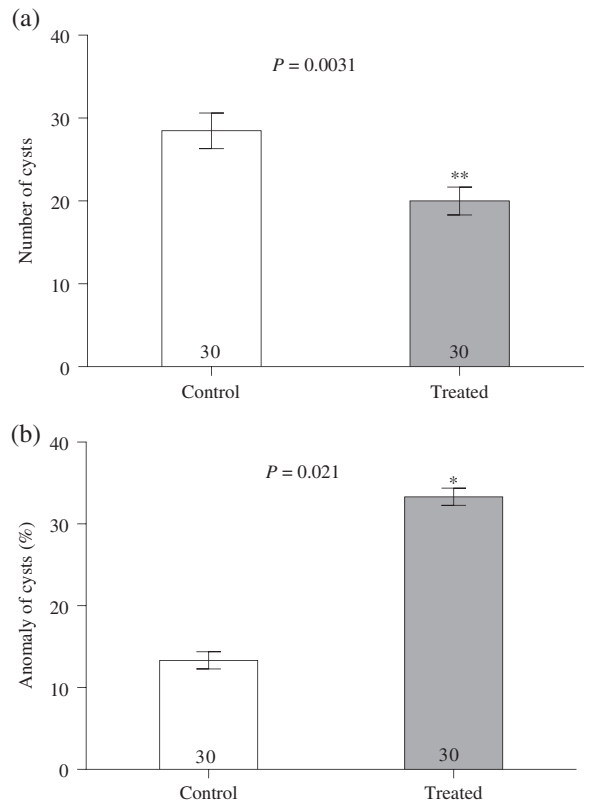

Figure 3. Effects of azadirachtin applied by topical application (0.63 μg) on day of *Drosophila melanogaster* adult emergence on male fecundity parameters (mean ± SD). (A) Number of cysts per testis 48 h after emergence. (B) Percentage of spermatocyte nuclei in abnormal position. Numbers within each bar indicate the number of repetitions. * Above bars indicates significant difference at $P \leq 0.05$. ** Above bars indicates significant differences at $P \leq 0.01$.

3.5 Progeny output

To evaluate the progeny from surviving treated adults, we monitored the different developmental stages (eggs, larvae, pupae and adults) in the progeny of couples formed with control animals (female, F_C; male, M_C) and/or animals treated with the LD_{50} (female, F_T, male M_T). Statistical analysis showed a significant decrease in

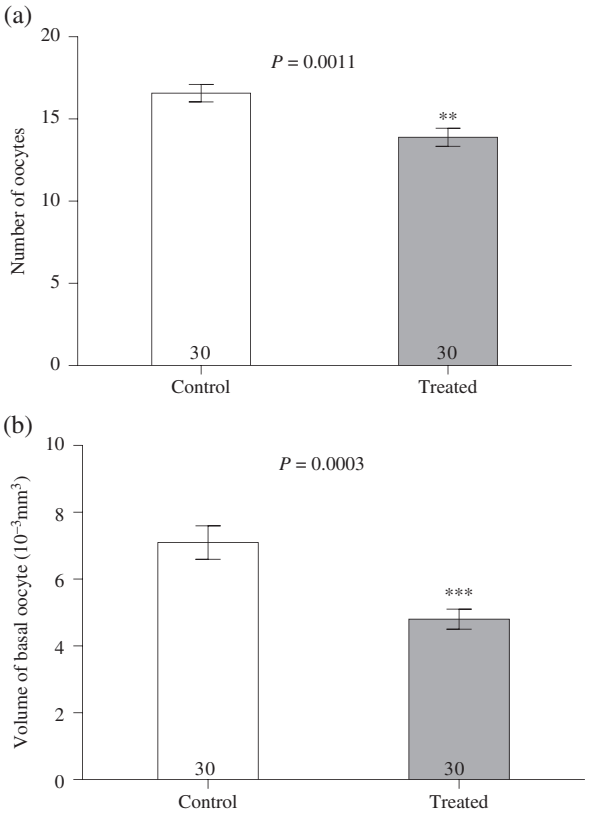

Figure 4. Effects of azadirachtin applied by topical application (0.63 μg) on the day of *Drosophila melanogaster* adult emergence on female fecundity parameters (mean ± SD). (A) Number of oocytes per ovary 48 h after emergence. (B) Volume of the basal oocyte 48 h after emergence. Numbers within bar indicate the number of repetitions. ** Above bars indicates significant differences $P \leq 0.01$. *** Above bars indicates significant differences, $P \leq 0.001$.

the numbers of eggs, larvae, pupae and adults in the F_1 generation for all treated series compared to couples from control individuals (repeated-measure ANOVA: treatment effect: $F_{6,42} = 241.90$, $P < 0.0001$; stage effect: $F_{3,21} = 15.94$, $P < 0.0001$; interaction: $F_{18,126} = 0.60$, $P = 0.89$; Fig. 5). The comparison of the different series revealed a significant dose effect. Irrespective of the developmental stage considered, Tukey's HSD test detailed three groups ($M_C + F_C > M_T + F_C > M_C + F_T = M_T + F_T$). This result reveals a greater sensitivity of females to azadirachtin.

In addition, for all stages evaluated, a dose-dependent effect was noted and a more significant reduction was recorded at the LD_{50} than at LD_{25} (Fig. S1, Supporting Information).

3.6 Reproductive rate

When taking into account the observed effects on survival, mating success (see section 3.2) and progeny survival (section 3.5), by multiplying the probabilities of survival and of mating by the mean progeny number per couple, it is possible to infer that a treated couple (with both sexes treated with LD_{50}) would produce a progeny of ~0.6 individuals [*P*(survival male) x *P*(survival female) x *P*(mating) × adult progeny = 0.5*0.5*0.138*17], while a control couple (neither sex treated) would produce ~31 individuals [*P*(mating) × adult progeny = 0.432*72]. Consequently, azadirachtin treatment at LD_{50} is evaluated to induce a 98% reduction in reproductive yield.

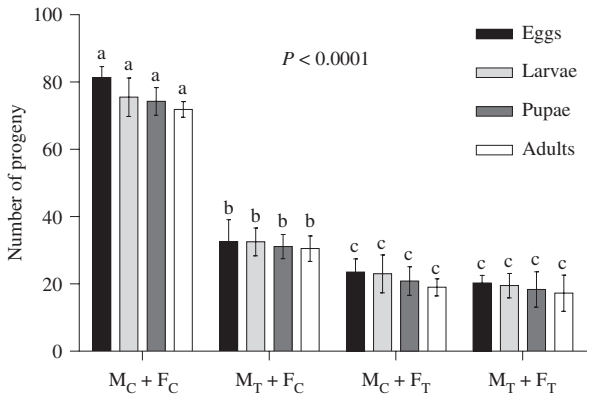

Figure 5. Effect of azadirachtin tested by topical application (0.63 μg), on the day of *Drosophila melanogaster* adult emergence, on the number of progeny (eggs, larvae L3, pupae, adults; M_C: male control; F_C: female control; M_T: male treated; F_T: female treated) (mean ± SD; $n = 8$ by couple). Different letters indicate a significant difference between the control and treated series of the same stage of development at level $P \leq 0.05$.

4 DISCUSSION

In this study, we investigated the effects of azadirachtin on adult toxicity and reproduction in a model insect *D. melanogaster*. Azadirachtin, applied topically on newly emerged adults, induced comparable mortality when evaluated at different times (24–96 h) after treatment. This result is in agreement with those of Andreazza *et al.*[44] on two other Drosophilidae, *Drosophila suzukii* (Matsumara) and *Zaprionus indianus* (Gupta). Azadirachtin acts with a dose–response relationship and the LD_{50} we obtained (0.63 μg or 630 ppm, evaluated 24 h after treatment) is close to that found for *D. melanogaster* last larval instar also by topical application.[35] Azadirachtin seems less toxic (1.17 μg) when applied on newly formed pupae of *D. melanogaster*.[36] Neem oil, another azadirachtin formulation applied topically at the same stage, induces similar toxicity in *Drosophila*[36] despite a different composition in the formulation. The most likely explanation for this effect is a lower susceptibility due to the metabolism or penetration through the cuticle (puparium) at this stage of development. Nevertheless, the pupal stage, a critical phase for adult formation, remains very responsive to azadirachtin[36] due to a drastic remodelling of most tissues and organs.[45]

Azadirachtin presents a stronger toxicity in *D. suzukii* and *Z. indianus* adults compared to *D. melanogaster*.[44] Indeed, a concentration of only 12 ppm causes 40% mortality in those species. Similar observations were reported by De Andrade-Coelho *et al.*[46] in female adults of another Diptera, *Lutzomyia longipalpis* (Lutz and Neiva). Previously, azadirachtin was reported to impair, with variable effects, the survival of different insect species.[9,11–14,44–51] Strong variations in insects' susceptibility to azadirachtin were noted depending on insect order, species, formulation, or the method of application.[9,11–14,25–29,44–51] Sensitivity of insects to insecticides varies also with the regulation of plasma membrane receptor[52] and/or ion channels,[34,52] penetration rate through the cuticle,[23] absorption by insects,[23] transport in tissues of the body[23] and metabolism.[23]

Several mechanisms may be at play in causing acute azadirachtin toxicity. The effects of azadirachtin on adult mortality may be linked to the cytotoxicity and induction of apoptosis via the impairment of insulin-signalling pathway[53] known to interact with JH and ecdysteroid[54,55] hormones inhibited by this insecticide. Azadirachtin-induced apoptosis causes general disruption in the organs of *D. melanogaster*[56] and in other species.[9,53,57] In particular, detrimental effects on various tissues, such as muscles (suppressed peristalsis), fat body and gut epithelial cells, have been reported.[9,58] A decrease of food intake and biochemical effects (decrease of α-amylase, chitinase, proteases and lipases), published recently[59] in *D. melanogaster*, may also contribute to azadirachtin acute toxicity. For instance, azadirachtin affects the chitin present[60] in the peritrophic membrane so that midgut protection against the mechanical damage and toxic compounds is impaired.[60]

Ecdysteroids, JH and insulin-signalling pathways play a crucial role in reproduction of *D. melanogaster*[54,55] and observations made in the current study indicate that fecundity and fertility in this species are adversely affected by azadirachtin treatment, resulting in a decreased progeny. First, we observed an impaired mating success after azadirachtin treatment. Then we found a reduced progeny. We also observed a reduction in cyst and oocyte numbers and also an increase in spermatocyte nucleus abnormalities and a decreased size of basal oocytes. All these gamete defects can explain the reduced progeny of treated adults. Together, reduced mating success and lower progeny suggest a strong deleterious impact (98% reduction) of LD_{50} azadirachtin treatment on reproductive yield.

Mating in *D. melanogaster* can be affected by azadirachtin because JH is required and plays an important role in this regulation processes in this species.[61] Moreover, ecdysone and JH seem to regulate the onset of female sex pheromone production,[62] mating[62] and some aspects of courtship in *D. melanogaster*.[61] In addition, the decision to engage courtship activities can be influenced by neuromodulators like dopamine (DA),[63] and recent studies show that the insulin-signalling pathway regulates JH and DA metabolism in *D. melanogaster*.[63]

Azadirachtin was found to inhibit oogenesis and spermiogenesis in several species.[9,64] Our observation of decreases in the number of oocytes and in the size of the basal oocyte can be explained by the severe degeneration of follicle cells, fragmentation in the germinal vesicle[9] and alterations of mitochondria caused by this insecticide.[65] Azadirachtin also alters or prevents the formation of new actin cytoskeleton resulting in the disruption of cell division and nutients transport.[9] This disruption may affect the process of dumping the cytoplasmic contents of nurse cells to the ovocyte and impact vitellogenesis.[66] Furthemore, azadirachtin interferes with ecdysteroid synthesis and vitellogenin synthesis (and/or its uptake), affecting oocyte development via JH[9,64] as noted in *D. melanogaster*.[36] The impact of azadirachtin on *D. melanogaster* adult females, as highlighted here, thus may be related to the fact that JH stimulates vitellogenesis for the developing oocytes and, together with 20E and insulin-signalling pathways,[53–55,66,67] controls the nutrient-sensitive checkpoint in oogenesis.[53–55] Reduction in the cyst number and the abnomalities observed can be explained by the impact of azadirachtin on the meiotic process responsible for sperm production and on histological and cellular structures.[9] Indeed, in *Mylabris indica* males (Coleoptera), Vivekananthan *et al.*[68] showed changes in the testes, such as vacuolation, shrinkage of testis cyst cells, clumped and fragmented chromatin materials, and disintegration and degeneration of germ cells, such as spermatogonia, spermatocytes, spermatids and spermatozoa. These effects of azadirachtin, in adult males, could be explained by the antagonistic action on ecdysone required for spermatogenesis; besides, JH is also needed for protein synthesis in male accessory glands.[54] Our last experiment, showing a reduced progeny in the treated adults, also suggested that

Drosophila females are more sensitive than males despite a similar recorded toxicity. This effect may be explained by the distinct physiologies of the two sexes.[69] For instance, Argue *et al.*[69] did indeed show a sexually dimorphic role of JH and presented DA as a candidate neuronal factor that differentially interacts with JH depending on the sex of the animal. Furthermore, the reduction of the egg layer can be explained by the neurotoxic effect[34] of azadirachtin that can interfere directly[34] or indirectly[54,55] on different step of the reproductive process in *D. melanogaster*[70] (e.g. follicular adrenergic signaling in ovulation or oviducte peristalsis).

5 CONCLUSION

Our study shows strong toxic effects of azadirachtin, from 24 h after topical treatment in *D. melanogaster*. We highlight a more drastic impact of azadirachtin on progeny output in treated females compared to males, even if this species is less sensitive than the targeted ones.[11,44,46] The impact of azadirachtin on *D. melanogaster* may be explained by the widely documented inhibition of JH and ecdysteroids,[9] and their interaction with insulin-signalling pathways.[54,55] Further investigations are now needed to achieve a better understanding of these effects and particulary the difference in sensitivity between the sexes. In addition, the molecular tools available when working with *D. melanogaster* should be instrumental for identifying the mechanisms of action of azadirachtin. On a more applied level, our results may provide interesting information for developing reproduction-control measures against the invasive *Drosophila* suzukii,[44] especially since azadirachtin is recommended in integrated pest-management programmes.[11]

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SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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