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Research Article

Delayed reproductive behavioral responses in *Drosophila melanogaster* (Dip., Drosophilidae) following exposure to aqueous extract of *Peganum harmala* and the insecticide Spinosad

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Abstract. This study aims to evaluate the delayed effects of two treatments Spinosad (a commercially available insecticide) and a natural bioinsecticide (aqueous extract of *Peganum harmala*) on the sexual behavior and oviposition of vinegar flies (*Drosophila melanogaster*). Sublethal concentrations (86 mg/ml *P. harmala*, 0.020 mg/ml Spinosad) of each product were administered via ingestion to second-instar (L2) larvae of *D. melanogaster*. The results indicated that exposure to Spinosad significantly reduced sexual activity in *D. melanogaster*, as evidenced by prolonged mating latency and a lower copulation rate. Moreover, its repellent properties alter female oviposition site preference and a marked decrease in the number of eggs laid. In contrast, the natural bioinsecticide (aqueous extract of *P. harmala*) exerted a milder effect: it reduced the frequency of courtship behaviors; it prevented mating in most individuals. Its moderate repellent effect also influenced oviposition site selection, leading to reduction in egg deposition.

Keywords: Natural insecticides, Sublethal concentration, Mating behavior, Oviposition, Botanical pesticide.

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Introduction

Harmful insects constitute a major threat to human health, animal welfare, and agricultural productivity, as they damage crops, transition of plant pathogens, and contribute to reduce yields and crop failures (Reckhaus, 2019).Chemical insecticides are frequently employed as a rapid and effective means of pest control, contributing to their widespread and growing use. However, the long-term and intensive application of synthetic insecticides has raised serious environmental concerns, including ecological imbalance, adverse health effects, and biodiversity loss, particularly through the decline of non-target species (Ahmed *et al.,* 2021). Although these compounds offer strong short-term efficacy, their sustained effectiveness is increasingly undermined by the emergence and dissemination of insect resistance. This resistance represents a critical obstacle to current and future pest management strategies (Nauen *et al.,* 2019; Sparks & Nauen, 2015). Plant-based pesticides offer a promising and environmentally sustainable alternative to synthetic chemical insecticides. These natural products are generally inexpensive, biodegradable, and eco-friendly. Their ability to act through multiple and often more selective mechanisms of action suggests that they pose lower risks to human health and non-target organisms (Sethuraman *et al.,* 2020). Among the most promising groups of plant secondary metabolites with potent antiparasitic and

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insecticidal properties are alkaloids (Wink, 1988; Hartmann, 1991). *P. harmala* (family Zygophyllaceae) is a well-known alkaloid-producing plant that synthesizes β-carboline and quinazoline derivatives, two classes of compounds recognized for their broad-spectrum biological activity (Kartal*et al.,* 2003).Biologically active compounds also represent a viable avenue for the microbial control of insect pests. In this context, several bioinsecticides have been developed and commercialized in recent years. One such example is spinosad discovered in the 1980s (Sparks *et al.,* 1998). Spinosad is a reduced-risk commercial insecticide derived from a bacterial fermentation product. It exhibits both contact and oral toxicity against insects (Begum & Islam, 2022). According to the Insecticide Resistance Action Committee (IRAC) classification, spinosad belongs to Group 5, which comprises allosteric modulators of nicotinic acetylcholine receptors in the nervous system (spinosyns) (in CSAN Niger 2017).

Owing to its genetic tractability, rapid life cycle, and ease of handling, the fruit fly *D. melanogaster* is widely recognized as an excellent model organism for evaluating insecticidal activity (Quiroz-Carreño *et al.,* 2020). It is also extensively used in reproductive toxicity testing, given its high fecundity and clearly defined courtship behaviors (Liu *et al.,* 2008). Moreover, sexual behavior in *D. melanogaster* serves as a sensitive bioindicator for assessing the sublethal and behavioral effects of plant-derived compounds (Elbah *et al.,* 2016).The present study aims to investigate the effects of a sublethal concentration of *P. harmala* aqueous leaf extract, and spinosad, on key reproductive behaviors in *D. melanogaster.* The study focused on courtship and mating sequences, reproductive output, and olfactory-substrateted oviposition site preference. By assessing these parameters, the study seeks to contribute to a better understanding of the potential of plant-based bioinsecticides and their behavioral impacts on insect models.

Materials and methods

Insects and Rearing Conditions

A wild-type strain of *D. melanogaster* was employed to investigate the effects of Spinosad and the aqueous leaf extract of *P. harmala* on specific sexual behavior and oviposition parameters. Adult flies were collected from ripe apples harvested in the Annaba region of Algeria. Cultures were established in the Ecology Laboratory of Marine and Coastal Environments (EMMAL), Faculty of Sciences, University Badji Mokhtar of Annaba (Algeria), and were maintained in 250 mL glass flasks containing a standardized artificial diet consisting of 33.33 g cornmeal, 7g agar-agar, and 33.33g yeast. This diet served as the control medium throughout the study. The rearing conditions were strictly controlled at a temperature of 24 ± 1 °C, relative humidity ranged from 65 % to 75 %, and a 12-hour dark photoperiod to simulate natural scotophase (Kihel *et al.,* 2022; Elbah *et al.,* 2016).

Spinosad

Spinosad is a pale gray to white solid compound. It constitutes the active ingredient of a phytosanitary agent exhibiting potent insecticidal properties. Chemically, Spinosad is a mixture of two macrocyclic lactones spinosyn A ($C_{41}H_{65}NO_{10}$) and spinosyn D ($C_{42}H_{67}NO_{10}$) naturally biosynthesized by the actinomycete bacterium *Saccharopolyspora spinosa* (Actinomycetales: Pseudonocardiaceae) (Mertz & Yao, 1990). In the present study, the insecticide employed was a commercial spinosad formulation (240 SC, a concentrated suspension), procured from Dow AgroScience (Italy) via its Algerian subsidiary. The product was diluted in distilled water to obtain sublethal concentrations appropriate for experimental application.

Preparation of the Aqueous Extract of *P. harmala*

P. harmala specimens were collected from the El Harmilia region, located in the Oum El-Bouaghi Province of Algeria (35°55′25″ N, 6°37′09″ E). An aqueous extract was prepared in the laboratory using 500 g of fresh *P. harmala* leaves, which were thoroughly rinsed and finely chopped before being boiled in 1.5 liters of distilled water. The decoction was maintained at a low simmer for 30 minutes to facilitate the extraction of bioactive compounds. Following cooling to room temperature, the mixture was filtered through a Whatman filter paper n°3 mm. To separate the liquid phase from the plant residues. The resulting aqueous extract, corresponding to a stock solution

with an initial concentration of 500 mg/mL, was collected in a sterile flask and stored at 4 °C in tightly sealed amber glass vials, protected from light.

Determination of the sublethal concentration

Preliminary toxicity assays were conducted in the laboratory using a range of bioinsecticide concentrations (Spinosad: 0.025 mg/mL, 0.050 mg/mL, 0.100 mg/mL, 0.200 mg/mL; *P. harmala*: 50 mg/mL, 100 mg/mL, 200 mg/mL, 300 mg/mL) to establish the dose–response relationship and to estimate the LC_{20} (86 mg/mL *P. harmala*, 0.020 mg/mL Spinosad), LC_{50} (163.16 mg/mL *P. harmala*, 0.060 mg/mL Spinosad), and LC_{90} (280.27 mg/mL *P. harmala*, 0.121 mg/mL Spinosad) values using probit regression (95% CI). These analyses highlighted the toxicity of the tested concentrations on the mortality of *D. melanogaster*, confirming a clear dose–effect relationship. For subsequent behavioral analyses, the LC_{20} (86 mg/mL *P. harmala*, 0.020 mg/mL Spinosad) was selected, as it induced measurable biological effects while maintaining mortality below 20 %, to provide reliable conditions for behavioral assessment.

Treatment

The treatment involved exposure to sublethal concentrations of Spinosad and the aqueous extract of *P. harmala*, administered via both contact and ingestion. Sublethal concentrations referred to doses below the lethal threshold that do not induce immediate mortality but may cause behavioral alterations, physiological disruptions, or morphological and developmental effects. The sublethal concentrations of Spinosad and *P. harmala* aqueous extract were incorporated into culture medium and were applied to second instar larvae (*D. melanogaster*).A control group was included in the experiment, in which individuals received distilled water only. Newly emerged adults were then maintained individually in glass rearing flasks containing the corresponding treated or controlled substrate. All procedures were carried out under controlled laboratory conditions (24 ± 1 °C, 65–75 % relative humidity), with strict adherence to standard experimental protocols (Elbah *et al.,* 2016; Kihel *et al.,* 2022).

Experimental Design

Sexual behaviour test

The sexual behavior test aimed to assess the impact of *P. harmala* extract and spinosad on courtship performance. Groups of 25 male and female flies aged 3 to 5 days, derived from control and treated cohorts (exposed to sublethal concentrations), were introduced into observation chambers in the laboratory to record courtship behaviors activity (Spieth, 1983; Greenspan, 1995). Observations were conducted between 9:00 a.m. and 1:00 p.m., corresponding to the peak period of sexual activity in *D. melanogaster* (Grillet *et al.,* 2005).Courtship behaviors were systematically recorded according to the criteria established by Hegde and Krishnamurthy (1979), encompassing orientation, tapping, wing vibration, licking, and attempted copulation (Liimatainen *et al.,* 1998). Latency to copulation and copulation duration were also precisely measured for each pair. Four mating combinations were tested: Control male × control female, treated male × treated female, control male × treated female, treated male × control female. An experiment to evaluate the effects of Spinosad and *P. harmala* aqueous extract on fecundity through ingestion and contact toxicity. Females that copulated during the behavioral assays were individually placed into plastic oviposition chambers containing two substrates: one with untreated culture medium (control) and one treated with the sublethal concentration of the test compound. After 48 hours, egg counts were performed under a CetiSteddy stereomicroscope (Renou *et al.,* 1997), with detailed characterization of oviposition site preference.

Statistical Analysis

Data normality was assessed using the Shapiro–Wilk test for variables related to time, sequence number, and egg deposition, while homogeneity of variances was evaluated using Levene's test. Sexual behavior data were analyzed by analysis of variance (ANOVA), and oviposition data were compared using Student's t-test. Differences were considered statistically significant at $p < 0.05$. Data from the oviposition site preference tests were compared using a Monte Carlo simulation based on a Chi-square test at a significance level of $\alpha = 0.05$ (Vaillant & Derrij, 1992).All statistical analyses and graphs were performed using XLSTAT software, version 2024.

Results

Effects of *P. harmala* on sexual courtship behaviour in *D. melanogaster*

Effects of *P. harmala* on the success rate of courtship sequences

The results indicated that the treatment significantly reduced the success of courtship sequences in *D. melanogaster*, resulting in a remarkable reduction in key sexual behaviors including wing vibration, licking, and mating attempts as well as a decline in copulation rates. Specifically, copulation was successful in only 56 % of pairs involving treated males and control females, 48 % of pairs with treated males and treated females, and just 32 % of fully treated pairs, in contrast to a 100 % success rate observed in control pairs (Table 1).

Effect of *P. harmala* on the duration of courtship sequences

The results indicated that treatment with a sublethal concentration of the aqueous extract of *P. harmala* significantly prolonged the duration of courtship behaviors in *D. melanogaster* compared to the control group ($p < 0.05$). In the control pairs, the initiation of sexual behavior was observed with a mean latency of 17.84 ± 1.03 seconds. Conversely, both treated pairs of males and females demonstrated substantially increased latencies in the onset of sexual activities. Specifically, orientation started at 72.32 ± 1.79 seconds, followed by the first contact at 140.68 ± 3.87 seconds, wing vibration at 201.39 ± 3.79 seconds, licking at 374.94 ± 6.63 seconds, and the attempt to copulate also at 487.00 ± 15.60 seconds. The latency to successful copulation was remarkably extended, reaching 585.12± 31.4 seconds. The differences were statistically significant, as indicated by the analysis of variance ($p < 0.05$) (Table 2).

Effect of *P. harmala* on copulation duration

The results showed that pairs in which only one partner was treated exhibited a moderate reduction in copulation duration compared to the 1271.04 ± 16.62 seconds. A more pronounced decrease was recorded when both partners were treated, with a mean duration of 896.50 ± 30.15 seconds. Nevertheless, this difference was not statistically significant ($p > 0.05$), indicating that the treatment did not exert a significant effect on this parameter (Fig. 1).

Effect of *P. harmala* on the frequency of courtship sequence repetitions

The data indicated that the frequency of repeated courtship behaviors varies significantly depending on the treatment applied to mating pairs. The group in which both males and females were treated with *P. harmala* extract showed the highest number of repetitions across all stages of the courtship sequence. Specifically, males performed orientation behaviors 11.40 ± 0.56 times, contact 12.20 ± 2.82 times, wing vibrations 15.40 ± 0.41 times, licking 9.94 ± 0.45 times, and copulation attempts 7.50 ± 0.18 times. In contrast, control pairs (untreated males and females) showed significantly fewer repetitions of each behavior. Analysis of variance confirmed that these differences were statistically significant across treatment groups ($p <0.05$) (Table 3).

Effects of *P. harmala* on the fecundity in *D. melanogaster*

Effects of *P. harmala* on oviposition site preference

Post-mating, fertilized females from the different crossing groups were monitored to assess oviposition site preference and fecundity. The results revealed remarkable variation in the distribution of eggs between treated and control substrates. In the control group, 25 females laid all their eggs exclusively on the control medium, whereas only 3 females deposited eggs on the treated medium. Conversely, in the experimental groups where either both sexes or only one partner was exposed to the treatment oviposition appeared random. In these cases, females laid eggs on both the treated and control substrates without clear preference (Table 4).

Number of eggs laid by *D. melanogaster*

According to the results, the average number of eggs laid by females from the control group was the highest, ranging from 40.96 ± 1.73 to 34.33 ± 4.25. In comparison, treated females laid an average of 22.60 ± 2.08 eggs, while those mated with treated males laid even to an average of 17.00 ± 1.12 eggs (Fig. 2).

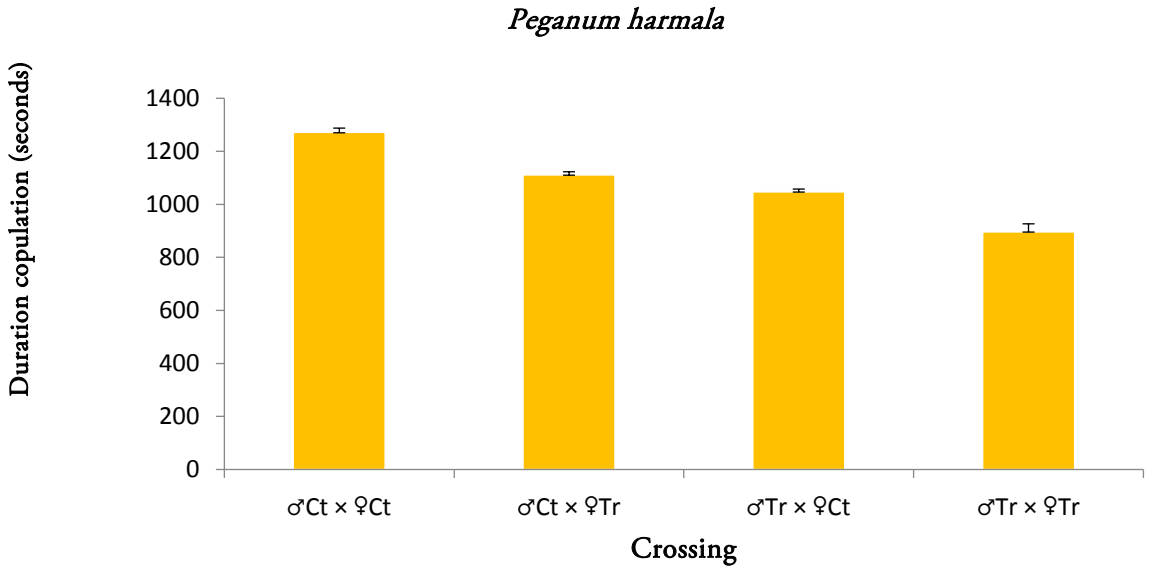

Fig 1. Copulation duration in *Drosophila melanogaster*. The bars represent mean and standard error of the (mean ± SEM) for different crossing (n = 25 pairs).

Table1. Success rates of courtship sequence in *Drosophila melanogaster* treated with aqueous extract of *Peganum harmala*

Crosses	Orientation	Contact	Vibration	Licking	Attempt	Copulation
♂Ct x ♀Ct	100 %	100 %	100 %	100 %	100 %	100 %
♂Ct x♀Tr	100 %	100 %	100 %	88 %	76 %	56 %
♂Tr x ♀Ct	100 %	100 %	100 %	80 %	72 %	48 %
♂Tr x ♀Tr	100 %	100 %	92 %	68 %	56 %	32 %

♂Ct: Control male; ♂Tr: Treated male; ♀Ct: Control female; ♀Tr: Treated female

Table 2. Duration (in seconds) of the courtship stages in *Drosophila melanogaster* (n = 25 pairs) (Mean ± SEM)

Crosses	Orientation	Contact	Vibration	Licking	Attempt	Copulation
♂Ct x ♀ Ct	17.84 ± 1.03	27.44 ± 0.74	39.32 ± 0.75	61.84 ± 4.28	72.88 ± 4.07	110.00 ± 4.56
♂Ct x ♀ Tr	30.52 ± 0.95	77.28 ± 2.40	102.56 ± 3.30	164.63 ± 3.63	234.77 ± 7.63	360.71 ± 13.23
♂Tr x ♀ Ct	53.40 ± 1.25	103.76 ± 3.16	160.32 ± 2.25	260.90 ± 8.53	350.44 ± 13.91	408.00 ± 13.88
♂Tr x ♀ Tr	72.32 ± 1.79	140.68 ± 3.87	201.39 ± 3.79	374.94 ± 6.63	487.00 ± 15.60	585.12 ± 31.45
F	4.165	13.486	16.717	7.093	6.492	15.39
p	< 0.008**	< 0.0001***	< 0.0001***	0.0002***	0.001**	< 0.0001***

♂Ct: Control male; ♂Tr: Treated male; ♀Ct: Control female; ♀Tr: Treated female; **: Highly significant; ***: Very highly significant; Mean: average; SEM: Standard error of the mean.

Table 3. Frequency of courtship sequence behaviors in *Drosophila melanogaster under* different treatment (n = 25 pairs) (Mean ± SEM)

Crosses	Orientation	Contact	Vibration	Licking	Attempt
♂Ct x ♀Ct	3.24 ± 0.17	2.44 ± 0.22	3.88 ± 0.88	3.40 ± 0.20	2.16 ± 0.12
♂Ct x ♀Tr	9.36 ± 0.34	9.16 ± 1.54	12.52 ± 0.37	7.04 ± 0.24	3.85 ± 0.29
♂Tr x ♀Ct	5.56 ± 0.23	5.56 ± 1.19	6.88 ± 0.23	4.45 ± 0.21	5.66 ± 0.31
♂Tr x ♀Tr	11.40 ± 0.56	12.20 ± 2.82	15.40 ± 0.41	9.94 ± 0.45	7.50 ± 0.18
F	8.652	10.837	2.154	3.566	3.674
P	< 0.0001***	< 0.0001***	0.099*	0.018*	0.017*

♂Ct: Control male; ♂Tr: Treated male; ♀Ct: Control female; ♀Tr: Treated female; *: Significant; **: Highly significant; ***: Very highly significant; Mean: average; SEM: Standard error of the mean.

Table 4. Oviposition site selection in *Drosophila melanogaster* cross different treatment groups

Crosses	N	Control substrate		*P*	Treatedsubstrate		*P*
		A	NA		A	NA	
♂Ct x ♀Ct	25	25	0	1S	3	22	< 0.828 NS
♂Tr x ♀Ct	16	16	0	1S	6	10	< 0.872 NS
♂ Ct x ♀Tr	12	10	2	0.978 NS	5	7	< 0.920 NS
♂Tr x ♀ Tr	8	4	4	< 0.887 NS	4	4	< 0.829 NS

N: Number of ovipositing females; A: Attracted; NA: Not Attracted; ♂Ct: Control male; ♂Tr: Treated male; ♀Ct: Control female; ♀Tr: Treated female, S: Significant; NS: Not significant.

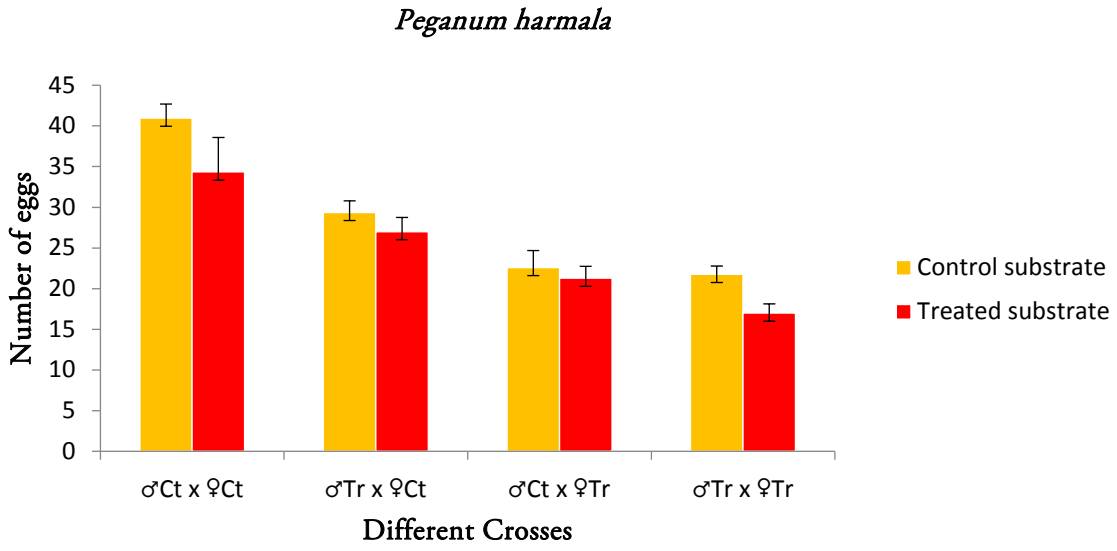

Fig. 2. Number of eggs laid by *Drosophila melanogaster.* The bars represent mean, standard error of the (mean ± SEM) for different substrate

Effects of Spinosad on courtship behaviour in *D. melanogaster*

Effects of Spinosad on the success rate of courtship sequences

The results demonstrated that control pairs consistently achieved a 100 % success rate in completing the full courtship sequence. In contrast, treated pairs exhibited significant disruptions in key courtship behaviors, including licking and mating attempts, which resulted in a marked decline in sequence completion rates. Furthermore, the proportion of successful copulations decreased substantially, reaching only 16 %, 24 %, and 52

% in pairs where both partners were treated, only the male was treated, or only the female was treated, respectively (Table 5).

Effect of Spinosad on the duration of courtship sequences

The results revealed that control pairs initiated the first courtship event significantly earlier, with the initial orientation occurring at 17.84 ± 1.03 seconds on average. In contrast, treated pairs exhibited a marked delay in initiating courtship behaviors. Specifically, sexual behaviors such as contact, wing vibration, licking, and attempted copulation occurred significantly later in treated pairs. The most pronounced delays were observed in the group where both males and females were exposed to Spinosad. Furthermore, copulation latency was substantially increased in treated pairs, ranging from 343.38 ± 11.82 seconds to 995.25 ± 48.52 seconds, depending on the treatment group. Statistical analysis indicated that these differences were highly significant ($p < 0.05$) across the various courtship stages (Table 6).

Effect of Spinosad on copulation duration

Results showed that the average copulation duration for control pairs was 1270.80 ± 59.11 seconds. Conversely, treated pairs exhibited a significant reduction in copulation time, ranging from 1034.33 ± 37.86 to 784.00 ± 117.808 seconds. Analysis of variances showed statistically very highly significant differences ($F = 11.647$, $p = 0.0001$) (Fig. 3).

Effect of Spinosad on the frequency of courtship sequence repetitions

The results indicated that treated pairs, where only females were exposed to Spinosad, showed the highest mean frequencies of courtship sequence repetitions, with values recorded as follows: orientation 9.80 ± 0.41, contact 10.00 ± 0.43, vibration 12.52 ± 0.37, licking 7.26 ± 0.45, and attempted copulation 12.31 ± 0.64. Conversely, control pairs displayed the lowest mean frequencies of sequence repetitions, namely orientation 3.16 ± 0.89, contact 2.44 ± 0.65, vibration 3.84 ± 0.68, licking 3.32 ± 1.14, and attempted copulation 2.08 ± 0.81. Analysis of variance revealed statistically very highly significant differences in the frequency of courtship sequence repetitions among the groups ($p < 0.05$) (Table 7).

Effects of Spinosad on the fecundity of *D. melanogaster*

Effects of Spinosad on egg-laying site preference

The results revealed notable variability in the distribution of eggs between treated and control substrates. In control pairs, all 25 females deposited their eggs in the untreated (control) medium with only two females laid their eggs in the treated substrate. However, in crosses where either both males and females or only one partner were treated, females exhibited a more random oviposition behavior, distributing their eggs across both treated and control substrates (Table 8).

Number of eggs laid by *D. melanogaster*

The results demonstrated a marked reduction in the number of eggs laid by treated pairs compared to controls, which averaged 43.32 ± 2.03 eggs in the untreated medium. This decline was especially pronounced when females were treated, with egg counts ranging from 23.33 ± 1.45 to 15.50 ± 1.70 eggs. In contrast, pairs with treated males exhibited an even greater decrease, with egg numbers falling between 15.25 ± 0.85 and 7.00 ± 2.00 eggs (Fig. 4).

Comparative effect of two bioinsecticides on the timing of the sexual display sequences in *D.melanogaster*

The result of courtship sequence durations in *D. melanogaster* showed significant differences between the two bioinsecticides ($p < 0.05$). Sequences occurred later in Spinosad treated individuals than in those treated with the aqueous extract of *P. harmala*, indicating a stronger disruptive effect of Spinosad on sexual behavior (Fig. 5).

Table 5. Success rates of courtship sequences in *Drosophila melanogaster* (N = 25)

Crosses	Orientation	Contact	Vibration	Licking	Attempt	Copulation
♂Ct x ♀Ct	100 %	100 %	100 %	100 %	100 %	100 %
♂Ct x♀Tr	100 %	100 %	100 %	88 %	76 %	52 %
♂Tr x ♀Ct	100 %	100 %	100 %	76 %	60 %	24 %
♂Tr x♀Tr	100 %	100 %	100 %	80 %	52 %	16 %

♂Ct: Control male; ♂Tr: Treated male; ♀Ct: Control female; ♀Tr: Treated female.

Table 6. Latency (in seconds) of courtship stages in *Drosophila melanogaster* following Spinosad exposure (Mean ± SEM)

Crosses	Orientation	Contact	Vibration	Licking	Attempt	Copulation
♂Ct x ♀Ct	17.84 ± 1.03	26.24 ± 2.26	39.32 ± 3.08	60.96 ± 4.33	72.04 ± 4.13	114.32 ± 7.52
♂Ct x ♀Tr	66.32 ± 2.69	82.16 ± 4.377	114.68 ± 4.24	192.36 ± 7.10	238.89 ± 8.97	343.38 ± 11.82
♂Tr x ♀Ct	110.84 ± 3.20	126.80 ± 5.20	139.16 ± 5.66	241.26 ± 7.83	335.20 ± 12.52	544.33 ± 35.99
♂Tr x ♀Tr	181.60 ± 5.58	201.52 ± 8.91	248.16 ± 10.21	402.10 ± 13.02	500.07 ± 19.90	995.25 ± 48.52
F	12.620	13.358	9.231	9.680	10.843	9.048
p	< 0.0001***	< 0.0001***	< 0.0001***	< 0.0001***	< 0.0001***	0.0001***

♂Ct: Control male; ♂Tr: Treated male; ♀Ct: Control female; ♀Tr: Treated female; *: Significant; **: Highly significant; ***: Very highly significant; Mean: average; SEM: Standard error of the mean.

Table 7. Number of repetitions of courtship steps in *Drosophila melanogaster* (n = 25 pairs) (Mean ± SEM)

Crosses	Orientation	Contact	Vibration	Licking	Attempt
♂Ct x ♀Ct	3.16 ± 0.89	2.44 ± 0.65	3.84 ± 0.68	3.32 ± 1.14	2.08 ± 0.81
♂Ct x♀Tr	9.80 ± 0.41	10.00 ± 0.43	12.52 ± 0.37	6.63 ± 0.31	4.26 ± 0.28
♂Tr x ♀Ct	7.28 ± 0.26	7.72 ± 0.26	7.32 ± 0.19	7.26 ± 0.45	12.31 ± 0.64
♂Tr x ♀Tr	6.00 ± 0.91	3.56 ± 0.22	9.48 ± 0.25	5.35 ± 0.13	7.00 ± 0.46
F	13.473	13.768	10.574	11.538	14.646
P	< 0.0001***	< 0.0001***	< 0.0001***	< 0.0001***	< 0.0001***

♂Ct: Control male; ♂Tr: Treated male; ♀Ct: Control female; ♀Tr: Treated female; ***: Very highly significant; Mean: average; SEM: Standard error of the mean.

Table 8. Oviposition site preference of *Drosophila melanogaster* females

Crosses	N	Control substrate		*p*	Treatedsubstrate		*P*
		A	NA		A	NA	
♂Ct x ♀Ct	25	25	0	1 S	2	23	1 S
♂Ct x♀Tr	13	11	2	< 0.828 NS	2	11	0.828 NS
♂Tr x ♀Ct	06	6	0	< 0.828 NS	6	0	0.828 NS
♂Tr x ♀Tr	04	4	0	< 0.828 NS	2	2	0.828 NS

N: Number of ovipositing females; A: Attracted; NA: Not Attracted; ♂Ct: Control male; ♂Tr: Treated male; ♀Ct: Control female; ♀Tr: Treated female, S: Significant; NS: Not significant.

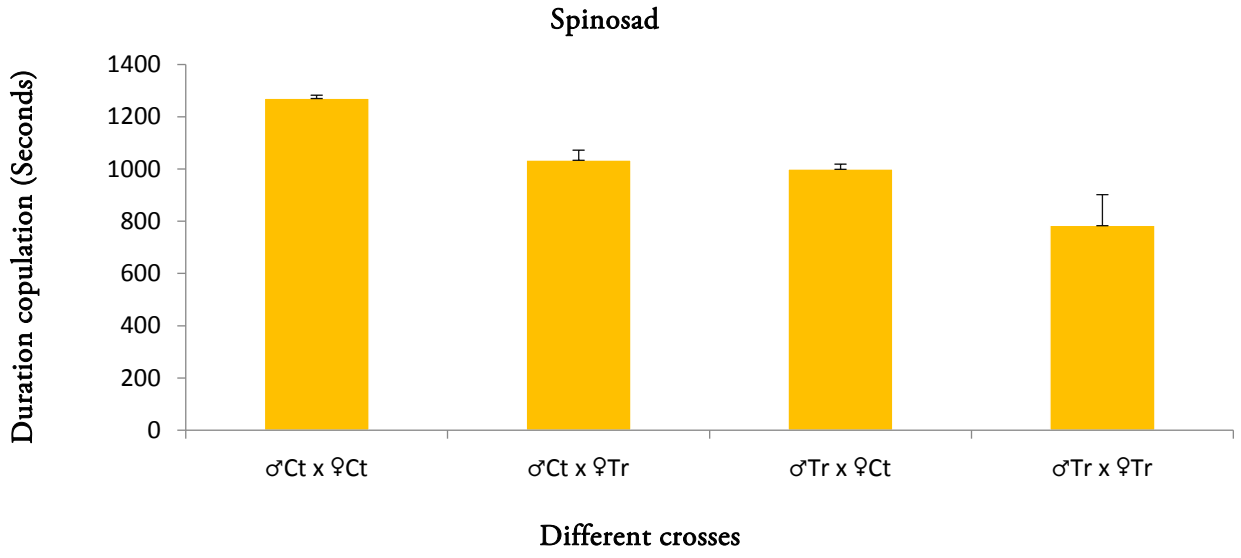

Fig. 3. Copulation duration in *Drosophila melanogaster*. The bars represent mean, standard error of the (mean ± SEM) for different crossing (n = 25 pairs)

Discussion

Sublethal concentrations of pesticides can disrupt insect physiology and behavior, notably by impairing reproduction, mobility, and pheromone detection (Bartling *et al.,* 2024). In *D. melanogaster*, mating activity is modulated by environmental cues such as food availability, and these signals are primarily processed through gustatory and olfactory chemosensory pathways (Gorter & Billeter, 2017). Owing to this sensitivity, *D. melanogaster* represents a valuable model for investigating the mechanisms regulating sexual behavior. Within this framework, we examined the effects of sublethal concentrations of an aqueous extract of *P. harmala* and of Spinosad on the sexual behavior of *D. melanogaster*, by analyzing the structured stages of courtship leading to copulation as well as the oviposition potential of females following mating. Both tested bioinsecticides significantly disrupted the courtship behavior and fecundity of *D. melanogaster*, with a stronger effect observed under Spinosad than with the aqueous extract of *P. harmala*. Pairs exposed to either bioinsecticide exhibited a marked reduction in the completion of courtship sequences as well as a notable decrease in copulation rate. This decline in receptivity may be attributed to an alteration in the perception or emission of chemical signals involved in sexual communication. In *Drosophila*, the mating ritual is largely regulated by gustatory and olfactory cues primarily cuticular pheromones that play a key role in social communication (Yew & Chung, 2015).

In addition, treated males required more time to orient toward females, accompanied by a decrease in the frequency of sequence repetitions. Taken together, these findings suggest that bioinsecticides may impair this orientation ability, making visual detection of females more difficult. In *D. melanogaster*, male orientation toward females relies heavily on visual information (Agrawal *et al.,* 2014; Coen *et al.,* 2016; Clemens *et al.,* 2018) ; Our findings demonstrate that exposure to bioinsecticides prolongs courtship duration in *D. melanogaster* compared with controls. While control males completed the sequence rapidly, exposure to sublethal concentrations markedly delayed the progression of sexual behaviors. This prolongation likely reflects disruptions in chemical communication and motor coordination between males and females, two processes essential for successful reproduction (Greenspan & Ferveur, 2000; Vosshall, 2008; McKinney *et al.,* 2015).The stereotyped sequence of courtship, in which the male approaches, taps, and licks the female to sample cuticular chemical cues prior to attempting copulation, is known to be highly conserved and innate. Indeed, even males reared in isolation perform the entire sequence when encountering a conspecific female (Greenspan & Ferveur, 2000). The observed delays in treated individuals therefore suggest that bioinsecticide exposure may interfere with the detection or processing of chemosensory cues, or with the motor outputs necessary for successful courtship. Such impairments are consistent with previous reports highlighting the central role of gustatory and olfactory pathways in regulating sexual communication in *Drosophila* (Vosshall, 2008).Under normal conditions, mated *Drosophila* females lay

several hundred eggs (David, 1963), The observed decrease in fecundity, accompanied by random oviposition, suggests that the treatment disrupts both reproduction and oviposition behavior in insects, even at sublethal concentrations. The behavior alalterations observed in pairs treated with the aqueous extract of *P. harmala* are likely attributable to the presence of β-carboline alkaloids, particularly harmaline and harmine, which are recognized for their neurotoxic properties and depressant effects on the central nervous system (Mahmoudian*et al.,* 2002).

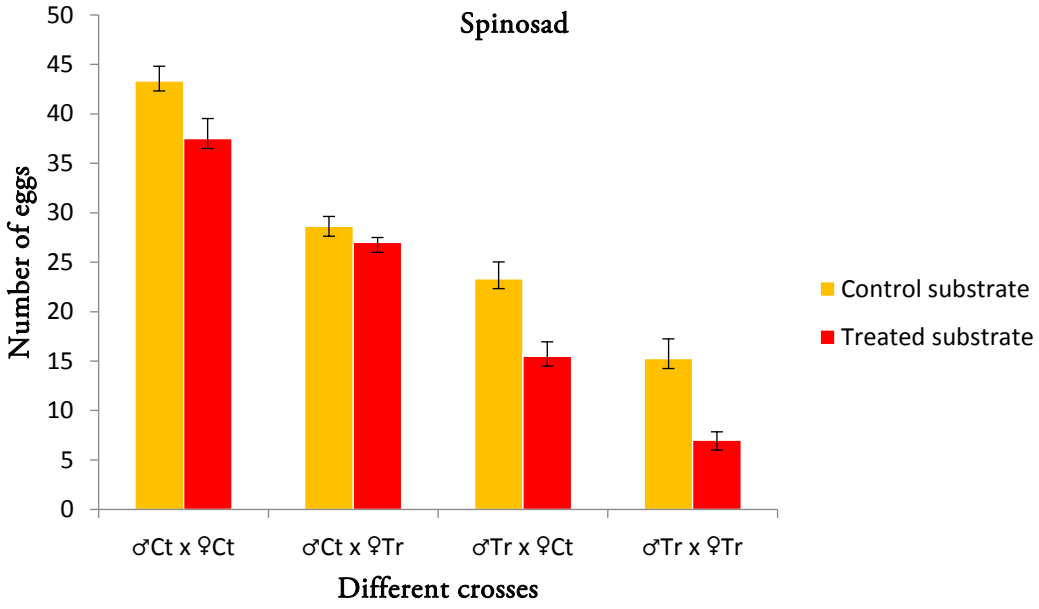

Fig. 4. Number of eggs laid by *Drosophila melanogaster*. The bars represent mean and standard error of the (mean ± SEM) for different substrates

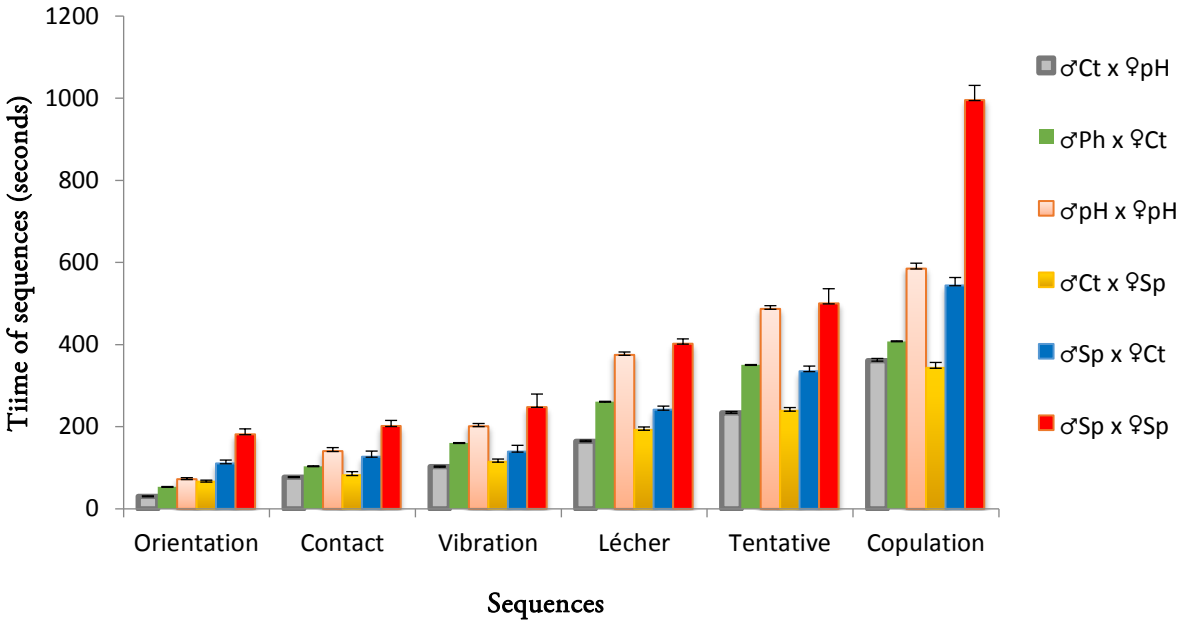

Fig. 5: Comparative effects of the two bioinsecticides on the timing of the sexual display sequences in *Drosophila melanogaster*. The bars represent mean and standard error of the (mean ± SEM) for different sequences

Such compounds can interferewith neural signaling pathways that govern sexual communication and reproductive behavior. Consistent with this hypothesis, Elbah *et al.,* (2016) demonstrated sublethal effects of *P. harmala* on *D. melanogaster*, including a pronounced reduction in courtship performance and egg deposition. Comparable disruptions have also been reported in other insect taxa, such as *Bactrocera oleae* (Rehman *et al.,* 2009), *Plutella xylostella* (Abbasipour *et al.,*2010), underscoring the broad spectrum of reproductive impairments caused by these alkaloids. More generally, growing evidence indicates that plant-derived secondary metabolites can significantly disrupt insect sexual communication. Extracts of *Drimia maritima* (Saadane *et al.,*2021), *Ruta chalepensis* (Amrani *et al.,*2022), *Citrullus colocynthis* (Kihel *et al.,*2022), *Ricinus communis* (Ai *et al.,*2021), *Curculigo orchioides* (Kushalan *et al.,*2022), as well as *Solanum nigrum* and *Armoracia rusticana* (Chowański *et al.,* 2018), have allbeen shown to impair mating signalsor fecundity.

The effects observed in treated pairs may be attributed to the mode of action of spinosad. This bioinsecticide primarily acts by activating nicotinic acetylcholine receptors, thereby disrupting neural transmission and leading to a range of behavioral impairments. Additional studies have suggested a potential interaction with GABAergic receptors, further enhancing its neurotoxic efficacy; (Sparks *et al*., 1998; Watson, 2001). This dual mechanism ofaction could explain why the observed effects were more pronounced compared to those induced by the *P. harmala* extract, reflecting a stronger disruption of courtship behavior and reproductive success.A growing body of research has documented the sublethal impacts of spinosad on insect sexual behavior across a variety of species. As a microbial-derived bioinsecticide, spinosad has been shown to interfere with courtship sequence dynamics and fecundity in several taxa, including *Tribolium confusum* and *Cryptolestes ferrugineus* (Vayias *et al.,* 2010), *Culex pipiens* (Benhissen *et al.,* 2023), *Aedes albopictus* and *Culex pipiens pallens* (Zhang *et al.,* 2025), *Glyphodes pyloalis* (Piri *et al.,* 2014), and *Spodoptera littoralis* (Pineda *et al.,* 2007).In *D. melanogaster*, additional investigations have explored the delayed effects of sublethal concentrations of various insecticides on reproductive behavior, including acephate (Mandi *et al.,* 2020), azadirachtin (Boulahbel *et al.,* 2015) andoberon (kissoum *et al.,* 2020). Collectively, these findings highlight the broad potential of spinosad and other insecticidal agents to interfere with sexual communication and reproductive processes in insects.Spinosad has been reported to affect oviposition behavior in a variety of insect species, including *Culex pipiens* (Michaelakis *et al.,* 2018), *Helicoverpa armigera* (Yao *et al.,* 2021), *Drosophila suzukii* (Pavlova *et al.,* 2017; Shaw *et al.,* 2019), and *Rhagoletis indifferens* (Yee, 2018).

Conclusion

In line with these findings, our study demonstrates that exposure to sublethal concentrations of both tested bioinsecticides significantly disrupts sexual behavior and oviposition in *D. melanogaster*, primarily by altering the temporal progression of courtship sequences. The commercial insecticide produces more pronounced effects than the plant extract, reflecting a higher efficacy in impairing reproductive behavior. Nevertheless, the natural extract of *P. harmala* also exerted significant effects, underscoring its potential as a promising bioinsecticidal alternative within sustainable pest management strategies. We would like to suggest, that valuable complement this study with histological and physiological analyses to gain deeper insights in to the mechanisms underlying the observed effects. Furthermore, to evaluate the potential impact on non-target insects to support the development of integrated pest management strategies that are both sustainable and environmentally responsible.

Author's Contributions

Khamsa Kermiche: methodology, investigation, draft preparation, visualization and Conceptualization; **Ismahane Lebbouz**: methodology, visualization; **Racha Benhacenne:** final review and editing; **Rachid Kechrid**: final review and editing; **Ines Kihel**: laboratory experiments and coordination; **Mounir Boumaza**: methodology, formal analysis, investigation; **Yasmine Adjami**: final review and editing; **Ayoub Hadjeb**: final review and editing; **Mohamed Laid Ouakid**: supervision, project administration and funding acquisition.

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Data Availability Statement

All data generated or analyzed during this study, including detailed methodology, are available from the corresponding author upon reasonable request.

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Ethics Approval and Consent to Participate

Insects were used in this study. All aplicable international, national, and institution alguidelines for the care and use of animals were followed. This article does not contain any studies with human participants performed by the authors.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Generative AI statement

The authors declare that no Gen AI was used in the creation of this manuscript.

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Research Article

تاثیر عصاره آبی گیاه اسپند (*Peganum harmala*) و مشره‌کش اسپینوساد در بروز پاسخ‌های رفتاری تولید مثلی با تأخیر در *Drosophila melanogaster* (Dip., Drosophilidae)

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چکیده: این مطالعه با هدف ارزیابی اثرات تأخیری دو تیمار اسپینوساد (حشره‌کش تجاری رایج) و یک حشره‌کش زیستی طبیعی (عصاره آبی *Peganum harmala*) بر رفتار جنسی و تخم‌گذاری مگس سرکه (*Drosophila melanogaster*) انجام شد. غلظت‌های زیرکشنده (۸۶ میلی‌گرم در میلی‌لیتر اسپینوساد، ۰.۰۲۰ میلی‌گرم در میلی‌لیتر اسپینوساد) از هر ترکیب از طریق بلع به لاروهای سن دوم *D. melanogaster* خورانده شد. نتایج نشان داد که قرار گرفتن در معرض اسپینوساد، فعالیت جنسی مگس سرکه *D. melanogaster* را بطور قابل توجهی کاهش داده و منجر به تأخیر طولانی مدت جفت‌گیری شده و میزان جفت‌گیری نیز کاش محسوسی داشت. علاوه بر این، خواص دفع‌کنندگی اسپینوزاد، روی ترجیح محل تخم‌گذاری ماده‌ها اثر گذاشته و منجر به کاهش قابل توجه تعداد تخم‌های گذاشته شده می‌گردد. در مقابل، حشره‌کش زیستی طبیعی (عصاره آبی اسپند) اثر ملایم‌تری داشت. به نحوی که فراوانی رفتارهای جفت‌خوانی را کاهش داد و در بیشتر افراد از جفت‌گیری جلوگیری کرد. اثر دورکنندگی متوسط این ترکیب نیز بر انتخاب محل تخم‌گذاری تأثیر گذاشت و منجر به کاهش تخم‌گذاری شد.

کلمات کلیدی: آفت‌کش‌های طبیعی، غلظت زیرکشنده، رفتارجفت‌گیری، تخم‌گذاری، آفت‌کش گیاهی

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