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Sublethal effects of *Ruta chalepensis* aqueous extract on sexual behavior and mate recognition in *Blattella germanica*

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Abstract. The German cockroach (*Blattella germanica*) is a resilient pest that exhibits a high reproductive capacity, posing significant risks to hygiene and human health. Recently, plant extracts have emerged as promising, environmentally safe alternatives for insect control. To mitigate the harmful effects of *Blattella germanica*, we conducted experiments using *Ruta chalepensis*. Preliminary trials involved administering aqueous plant extracts by ingestion, testing varying concentrations and exposure times to establish a sub-lethal dose (500 µg/mL). This dose was selected to study the plant's effects on the pest's behavior. Sub-lethal concentration (500 µg/mL) of the extract disrupted sexual attractiveness. Results confirmed that 86.66% of untreated control adults were attracted to extracts from untreated males and females. In contrast, adults treated with *R. chalepensis* leaf extracts (500 µg/mL) showed significantly reduced attraction (20–30%) to extracts from both treated and untreated individuals, regardless of sex. Additionally, sub-lethal concentrations altered the progression of sexual behavior phases. Treated partners exhibited no successful mating events, whereas untreated controls achieved an 80% success rate. These findings suggest that sub-lethal doses of this bio-insecticide interfere with chemical signals critical for mate recognition, impairing the cockroaches' ability to detect or interpret these cues.

Keywords: *Ruta chalepensis*, *Blattella germanica*, sexual activity, sexual attractiveness, sexual behavior.

Introduction

Cockroaches are medically significant pests, acting as both allergenic vectors and reservoirs of pathogenic organisms. Their presence exacerbates respiratory conditions like asthma and poses risks of food contamination, particularly in urban environments (Brenner & Kramer 2019, Hashemi-Aghdam & Oshaghi 2015, Donkor 2020). Chemical insecticides, while effective for acute infestations, have led to unintended consequences, including widespread resistance in pest populations and ecological disruption. The German cockroach (*Blattella germanica*), for example, exhibits resistance to 42 classes of synthetic insecticides, including organochlorides, neonicotinoids, and phenylpyrazoles (Naqqash et al. 2016, Zhu et al. 2016). These challenges underscore the urgent need for sustainable alternatives aligned with integrated pest management (IPM) principles (Demarque & Espindola 2021).

Plant-derived compounds offer a promising solution due to their bioactive properties and

historical use in traditional medicine (Farnsworth et al. 1986, Gul et al. 2022). Secondary metabolites can disrupt critical insect behaviors, including mating, feeding, and oviposition, while reducing fecundity and fertility (Veer & Gopalakrishnan 2016). Despite their potential, research on African entomofauna (particularly Orthopteroid diversity in North Africa) remains limited, with few studies targeting Dictyoptera (Yasri-Cheboubi et al. 2016, Deghiche-Diab et al. 2022).

In Algeria, recent efforts have prioritized plant-based urban pest management, with studies exploring aqueous extracts of indigenous species (Azoui 2017, Benhissen et al. 2018, Habbachi et al. 2019). Among these, the Rutaceae family stands out for its broad-spectrum bioactivity, including antifungal, antioxidant, and anti-inflammatory effects (Rao et al. 2021).

Ruta chalepensis L. (Rutaceae), locally termed "Fidjel," is a Mediterranean aromatic plant traditionally used to treat dermatological ailments, headaches, and inflammation (Merghache et al. 2009, Daoudi et al. 2016). Its rich repertoire of alkaloids and tannins suggests potential insecticidal properties, including repellency and growth disruption (Pelletier 1999, Vandenborre et al. 2011).

This study evaluates the sublethal effects of aqueous *R. chalepensis* leaf extracts on *B. germanica*, with two primary aims: i)to assess the extract's potential as a bioinsecticide targeting cockroach behavior and reproduction; ii) to determine its efficacy in suppressing population proliferation, offering insights for ecological pest management strategies.

Material and methods

Blattella germanica. Adult *B. germanica* has a body length of 13–16 mm and coloration ranging from light brown to yellowish-brown. Females typically display darker pigmentation compared to males. The pronotum bears two distinct longitudinal bands of variable length, spanning reddish-brown to black (Robinson 2005).

Ruta chalepensis is a perennial herbaceous plant characterized by a basal woody stem and a growth height of up to 1 m (Baba Aissa 1999). The aromatic leaves (6–12 cm long) are blue-green, ovate, and pinnately compound, divided into numerous oblong to lanceolate lobes. During the summer, it produces cup-shaped, dark yellow flowers (1–2 cm in diameter) with 4–5 petals, each fringed by long, ciliate margins (Brickell & Mioulane 2004).

Preparation of aqueous extract. The aqueous extract of *R. chalepensis* was prepared at a concentration of 500 µg/mL by boiling 500 g of fresh leaves in distilled water for 1 hour using a hot plate (maintained at 100°C to ensure continuous boiling). The mixture was filtered through Whatman No. 1 filter paper (pore size: 11 µm) to remove particulate matter, and the filtrate was stored at 4°C until use (Kasmi et al. 2017).

Sexual attractiveness test in an olfactometer. Five 8-day-old control or treated individuals of each sex and treatment were immersed in 1 mL of hexane for 5 minutes. An individual 8-day-old male or female was given a choice of two odors, 100 µl extract or 100 µl hexane (on a 0.5 cm^2 piece of filter paper), in a Y-maze olfactometer (1 cm i.d., branch=20 cm; side-pieces=15 cm; airflow=10 ml min^{-1}); which of the two odor sources the insect reached was recorded. The attractiveness of an extract (N= 10 insects) was tested by a Chi-square test, using the adjusted Pearson residuals. When insects were given two control stimuli, no choice was observed, with <5% of insects reaching the end of the tubes (Habbachi et al. 2009).

Sexual behavior test. Adults were collected daily following emergence from the pupal stage. These adults (males and females that had never been exposed to olfactory contact with each other) were separated by sex and reared under the same environmental conditions as described above until reaching sexual maturity at 8 days of age. Throughout these 8 days, the adults were administered an aqueous extract of *R. chalepensis* leaves at a sublethal concentration of 500 µg/mL. A control group of adults (n=10) that received

water instead of the extract was also maintained for comparative testing.

Statistical analysis. Data on sexual behavior durations were analyzed using descriptive statistics, including the mean, standard deviation, minimum, and maximum. Results were visualized as boxplots and analyzed using one-way analysis of variance (ANOVA) in XLSTAT software (version 2022.3; Addinsoft, New York, NY). Ethological test results from olfactometer experiments were compared using Monte Carlo simulations, with significance assessed via a Chi-square (χ^2) test at a threshold of $\alpha = 0.05$ (Vaillant & Derridj 1992).

Results

Effect on sexual attractiveness in the olfactometer

Effect of the aqueous leaf extract of *R. chalepensis* (500 µg/mL). The results reveal a strong attraction of untreated (control) individuals to *R. chalepensis* extracts. Control males (C♂) exhibited 80% attraction to both untreated males and females, but this attraction decreased to 60% toward treated males and 33.33% toward treated females (Table 1). Control females (C♀) showed high attraction to untreated individuals (86.67% to males and 93.33% to females), but 13.33% to treated females (Table 1).

Table 1. Attractiveness rates of *B. germanica* adults treated with *R. chalepensis* leaf extract (500 µg/mL) (C: Control; R: Extract of *R. chalepensis*; A: Attracted; NA: Not attracted; S: Significant; NS: Not significant)

Insects tested		Extracts			
		C♂	C♀	R♂	R♀
C♂	HAS	80.00%	80.00%	60.00%	33.33%
	NA	20.00%	20.00%	40.00%	66.67%
	P	0.978 (S)	0.978 (S)	<0.915 (NS)	<0.915 (NS)
C♀	HAS	86.67%	93.33%	66.67%	13.33%
	NA	13.33%	6.67%	33.33%	86.67%
	P	0.993 (S)	1.00 (S)	<0.915 (NS)	<0.915 (NS)
R♂	HAS	20.00%	40.00%	26.67%	33.33%
	NA	80.00%	60.00%	73.33%	66.67%
	P	<0.915 (NS)	<0.915 (NS)	<0.915 (NS)	<0.915 (NS)
R♀	HAS	40.00%	40.00%	20.00%	26.67%
	NA	60.00%	60.00%	80.00%	73.33%
	P	<0.915 (NS)	<0.915 (NS)	<0.915 (NS)	<0.915 (NS)

Statistically significant differences were observed in interactions between untreated individuals ($p > 0.978$ for control vs. control comparisons). In contrast, comparisons with treated individuals showed significantly lower attraction ($p < 0.05$), indicating a strong preference for untreated conspecifics (Table 1). In contrast, interactions involving treated individuals (R♂ and R♀) exhibited no significant differences ($p \leq 0.915$), suggesting that the *R. chalepensis* extract disrupts or masks natural pheromonal cues (Table 1).

Sexual behavior effects

Effect on mating success rate. The aqueous extract of *R. chalepensis* leaves (500 µg/mL) markedly reduced mating success in *B. germanica*. While 80% of control pairs (C♂ × C♀) achieved successful copulation, all combinations involving at least one treated individual (R♂ × R♀, R♂ × C♀, C♂ × R♀) showed 0% mating success (Table 2). Notably, 90–100% of these pairs

exhibited no mating behavior, and only a small proportion resulted in aborted attempts (Table 2).

Table 2. Effect of the aqueous leaf extract of *R. chalepensis* (500 µg/mL) on mating success rate in *B. germanica* (♂: Male; ♀: Female; C: Control; R: *R. chalepensis*)

Couples	Successful	Aborted	No mating
C♂ x C♀	80%	20%	0%
R♂ x R♀	0%	10%	90%
C♂ x R♀	0%	0%	100%
R♂ x C♀	0%	10%	90%

Effect of *R. chalepensis* extract on antennal contact behavior in *B. germanica*. Treated pairs of *B. germanica* (R♂ × R♀) exhibited a mean time to first antennal contact of 297.20 ± 69.13 seconds (range: 58–644 seconds) (Figure 1). In mixed pairs with treated females and control males (R♀ × C♂), the mean time increased to 386.00 ± 91.12 seconds (range: 147–1050 seconds) (Figure 1). By contrast, pairs involving control females (C♀ × C♂) showed the shortest mean time (249.30 ± 93.14 seconds; range: 16–983 seconds) (Figure 1). One-way ANOVA at α = 0.05 revealed significant differences in first antennal contact times across the tested groups (F = 3.94; $p \leq 0.01$) (Figure 1).

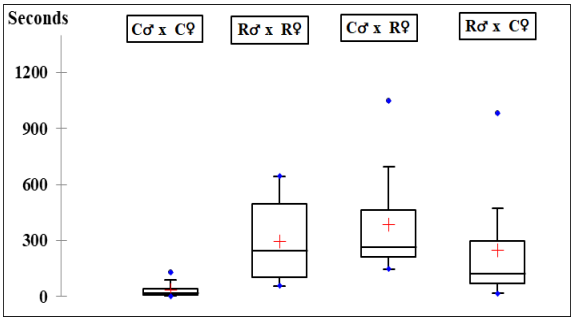

Figure 1. Extract effect on first antennal contact time in *B. germanica* (♂: Male; ♀: Female; C: Control; R: *R. chalepensis*, + Average ● Min / Max)

Effect on first wing-raising display (parade): Figure 2 demonstrates that *R. chalepensis* extract significantly alters the timing of the first wing-raising display (parade) in *B. germanica* males. In treated pairs (R♂ × R♀), males initiated wing-raising after 225.80 ± 128.00 seconds (range: 0–1145 seconds) (Figure 2). In pairs consisting of control males and treated females (C♂ × R♀), the behavior occurred after 284.10 ± 111.80 seconds (range: 0–833 seconds) (Figure 2). Interestingly, in pairs with treated males and control females (R♂ × C♀), males exhibited the fastest response, raising their wings to expose pheromone glands after 63.40 ± 43.11 seconds (range: 0–[maximum time not specified]) (Figure 2). One-way ANOVA revealed highly significant differences in parade initiation times across groups (F = 10.04; $p \leq 0.0001$), indicating that the extract disrupts the natural timing of this pheromone-releasing behavior (Figure 2).

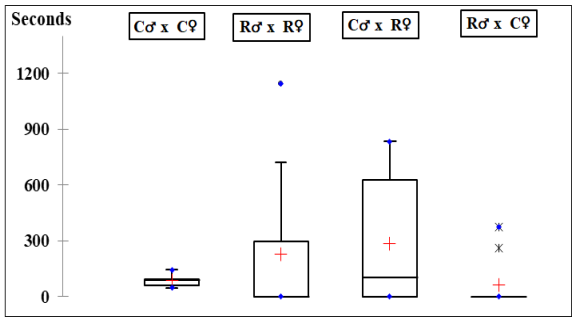

Figure 2: Extract effect on first wing-raising display in *B. germanica* (♂: Male; ♀: Female; C: Control; R: *R. chalepensis*, + Average ● Min / Max)

Effect on the number of wing-raising displays (parades). The aqueous *R. chalepensis* extract showed no significant impact on the frequency of wing-raising displays (parades) in *B. germanica* pairs. In treated pairs (R♂ × R♀), males performed an average of 1.10 ± 0.70 parades (range: 0–9) (Figure 3). In pairs composed of control males and treated females (C♂ × R♀), the frequency was slightly higher at 1.60 ± 0.77 parades (range: 0–7) (Figure 3). In contrast, treated males paired with control females (R♂ × C♀) exhibited 1.10 ± 0.90 parades (range: 0–9) (Figure 3). One-way ANOVA revealed no significant differences in the number of parades across the groups (F = 0.13; $p \leq 0.94$), indicating that the extract does not alter the frequency of this pheromone-releasing behavior

(Figure 3).

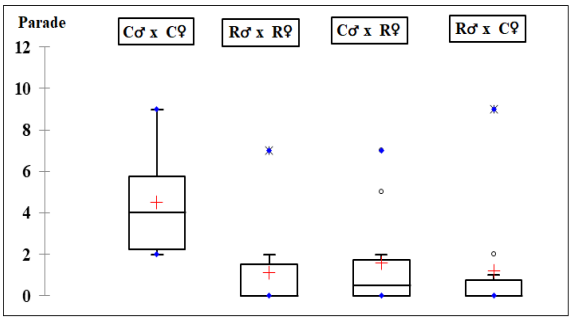

Figure 3. Extract effect on the number of wing-raising displays in *B. germanica* (♂: Male; ♀: Female; C: Control; R: *R. chalepensis*, + Average ● Min / Max)

Effect on the time to first licking behavior: Treated females (R♀ × Rσ) initiated feeding on the secretions of treated males after an average of 40.20 ± 40.20 seconds, with a time range between 0 and 402 seconds (Figure 4). In pairs composed of treated females and control males (R♀ × Cσ), the average time to the first lick was significantly longer at 218.00 ± 169.87 seconds, with a maximum of 1680 seconds (Figure 4). In the combination of control females with treated males (C♀ × Rσ), the average time to the first licking behavior was 115.60 ± 80.58 seconds, with a maximum of 736 seconds (Figure 4). One-way ANOVA revealed significant differences between the groups ($F = 4.11$; $p \leq 0.01$), indicating that the *R. chalepensis* extract significantly affects the timing of the first licking interaction during courtship (Figure 4).

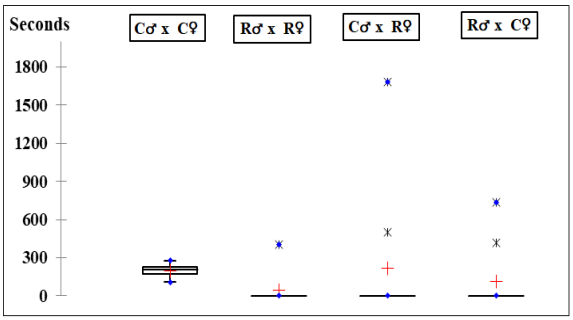

Figure 4. Extract effect on the time to first licking behavior in *B. germanica* (♂: Male; ♀: Female; C: Control; R: *R. chalepensis*) [+ Average ● Min / Max]

Effect on the number of licks. Figure 5 illustrates the impact of the aqueous *R. chalepensis* extract on the frequency of licking behavior in *B. germanica* pairs. In treated pairs (R♀ × Rσ), the average number of licks was 0.40 ± 0.40, with values ranging from 0 to 4 licks (Figure 5). In pairs composed of treated females and control males (R♀ × Cσ), the licking frequency ranged from 0 to 5, with a higher average of 0.70 ± 0.51 licks (Figure 5). Conversely, in pairs in which control females were exposed to secretions from treated males (C♀ × Rσ), licking behavior was observed only once during the trials (Figure 5). Statistical analysis using one-way ANOVA revealed significant differences between the groups ($F = 3.84$; $p \leq 0.01$), suggesting that the *R. chalepensis* extract alters the expression of this close-range courtship behavior (Figure 5).

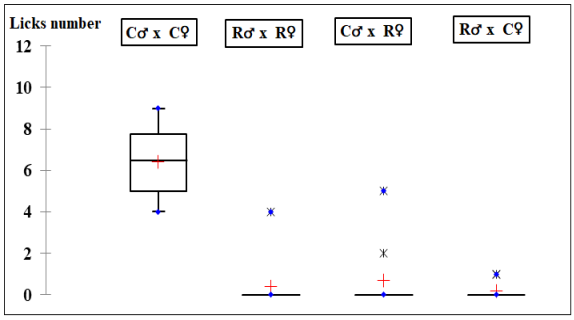

Figure 5. Extract effect on the number of licks in *B. germanica* (♂: Male; ♀: Female; C: Control; R: *R. chalepensis*, + Average ● Min / Max)

Effect on the time of the first mating attempt: As shown in Figure 6, only one pair attempted to mate in each of the following combinations: treated male × treated female (*R*♂ × *R*♀) and treated male × control female (*R*♂ × *C*♀) (Figure 6). In the former case, the mating attempt occurred after 430 seconds, whereas in the latter, it was significantly delayed, occurring only after 3600 seconds (Figure 6). In contrast, no mating attempts were observed in pairs composed of control males and treated females (Cσ × R♀), suggesting a possible inhibitory effect of the extract on female receptivity (Figure 6). One-way ANOVA revealed significant differences between the groups ($F = 4.29$; $p \leq 0.01$),

suggesting that treatment with *R. chalepensis* extract significantly affects the initiation of mating behavior in *B. germanica* (Figure 6).

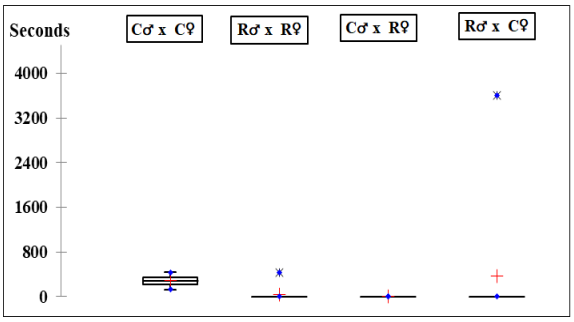

Figure 6. Extract effect on the time of the first mating attempt in *B. germanica* (♂: Male; ♀: Female; C: Control; R: *R. chalepensis*, + Average ● Min / Max)

Effect on the number of mating attempts. The treated pair attempted to mate twice during the test. In contrast, for the pair where the male was treated with a control female, this phenomenon was observed only once (Figure 7). It should be noted that no mating attempts were recorded for pairs in which the females were treated with control males (Figure 7). These results demonstrate the effect of the aqueous extract of *R. chalepensis* on the mating sequence. Statistical analysis of the data, based on variance comparison, indicates highly significant differences between the groups studied (Fobs = 14.64; $p < 0.0001$***) (Figure 7).

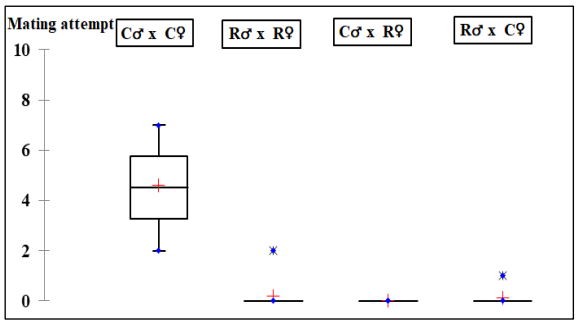

Figure 7. Extract effect on the number of mating attempts in *B. germanica* (♂: Male; ♀: Female; C: Control; R: *R. chalepensis*, + Average ● Min / Max)

Effect on mating time. Tests with different cockroach pairs indicate that the aqueous extract of *R. chalepensis* at a sub-lethal concentration (500 µg/mL) affects mating behavior (Figure 8). In all treated groups (whether both partners or only one were treated), no mating events were recorded. Mating was observed only in the control pairs, suggesting that the extract inhibits mating (Figure 8).

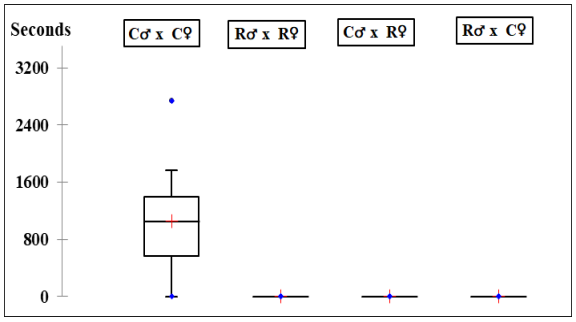

Figure 8: Extract effect on mating time in *B. germanica* (♂: Male; ♀: Female; C: Control; R: *R. chalepensis*) [+ Average ● Min / Max]

Discussion

Plant-derived compounds, including pheromones, extracts, and oils, interact with insect sensory systems to alter behavior, offering potential synergies with integrated pest management (IPM) strategies (Veer & Gopalakrishnan 2016, Rizvi et al. 2021). Insects, such as the gregarious German cockroach (*Blattella germanica*), rely on sophisticated olfactory mechanisms for essential behaviors, such as mate-finding and predator avoidance (He et al. 2021). In this study, we investigated the impact of aqueous *Ruta chalepensis* leaf extract on *B. germanica*'s gregarious and sexual behaviors. Our findings demonstrate that the extract significantly reduced the attractiveness of treated individuals to untreated conspecifics, suggesting either a repellent effect or interference with pheromonal communication. These results position *R. chalepensis* as a promising candidate for controlling ecological cockroach populations.

Sublethal concentrations of bio-insecticides can disrupt chemical signaling in cockroaches, as

evidenced by the reduced attractiveness of treated adults to conspecifics. This aligns with Visser's (1986) framework, where attractant efficacy depends on source concentration, diffusion dynamics, and internal insect orientation mechanisms. Secondary plant metabolites, such as those in *R. chalepensis*, are known to suppress mating, deter oviposition, and impair fecundity by disrupting neuroendocrine pathways (Divekar et al. 2022). In our study, treated pairs exhibited 90% null mating and 10% aborted mating, with complete mating failure in treated female–control male pairs. This finding mirrors those of Elbah (2017), who reported similar disruptions in *B. germanica* using *Peganum harmala* extracts.

Research on the impacts of insecticides on insect sexual behavior in the Mena region, particularly in Algeria, remains scarce (Amin et al. 2022). Our results corroborate limited regional studies: Masna (2016) observed 20% mating success in *P. harmala*-treated *B. germanica* pairs, while Habbachi (2013) linked *Bacillus thuringiensis* exposure to prolonged courtship latency. Neurotoxic effects of *R. chalepensis* essential oils, as reported in *Tribolium confusum* (Abdellaoui et al. 2018), likely extend to *B. germanica* through cuticular or tracheal penetration, disrupting neural coordination (Bessette et al. 2013). These findings collectively suggest that *R. chalepensis* interferes with pheromone-mediated mate recognition, critical for courtship initiation.

Beyond behavioral disruption, *R. chalepensis* exhibits antifungal, antimicrobial, and antioxidant properties (Raghav et al. 2006, Aouadhi et al. 2013). Its alkaloids and tannins may act as repellents or anti-feedants, impairing insect growth and fecundity (Pelletier 1999, Vandenborre et al. 2011). Such multitrophic effects underscore its potential in IPM frameworks targeting both pest behavior and population viability.

This study addresses a critical gap in Algerian entomological research, yet further work is needed to 1) elucidate the extract's specific bioactive compounds (e.g., via chromatographic analysis of cuticular hydrocarbons); 2) validate field efficacy under real-world conditions; 3) explore impacts on offspring viability and multigenerational resistance.

Our laboratory findings highlight *R. chalepensis*'s capacity to disrupt *B. germanica*'s sexual communication, likely through olfactory and contact pheromone interference. By impairing mate-finding and mating success, the extract could suppress population growth, offering a sustainable alternative to synthetic insecticides. This study demonstrates that aqueous leaf extracts of *R. chalepensis* significantly inhibit mating behavior in *B. germanica*, even at sublethal concentrations. These findings support its potential use as a botanical insecticide in integrated pest management strategies aimed at urban cockroach control.

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