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SUBLETHAL IMPACTS OF AZADIRACHTIN ON THE REPRODUCTIVE STRUCTURE OF *SCLERODERMA SICHUANENSIS* (HYMENOPTERA: BETHYLIDAE)

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Abstract
Azadirachtin, a natural insecticide derived from neem, is well known for its low toxicity to mammals and its selective effectiveness against pest insects. Nonetheless, the sublethal effects on beneficial parasitoid wasps have not been thoroughly examined. This research explored the impact of azadirachtin on the genital complex morphology of the ectoparasitic wasp Scleroderma sichuanensis. Adult females were subjected to six different concentrations (10, 25, 50, 75, 100, and 150 mg/mL) in a controlled laboratory environment. We conducted a morphometric analysis of ovipositor length and width in 30 individuals per treatment. A concentration-dependent decrease in both measurements was noted, with the highest concentration (150 mg/mL) leading to a reduction of 72.3% in ovipositor length and 73.5% in width compared to the control group. The 50 mg/mL concentration, which is close to the recommended application rates in the field, resulted in reductions of 36.7% and 32.4%, respectively. These results indicate that although azadirachtin is regarded as environmentally friendly, it may still present sublethal threats to non-target hymenopteran natural enemies. Therefore, the incorporation of azadirachtin into pest management strategies should consider these morphological changes to maintain biological control functions.

INTRODUCTION

The increasing focus on sustainable agriculture has sparked renewed interest in botanical insecticides, with azadirachtin standing out as one of the most extensively researched and utilized compounds (Chhunla et al., 2026). Extracted from the seeds of the neem tree (Azadirachta indica), azadirachtin primarily functions as an ecdysone antagonist, interfering with molting and metamorphosis in insects (Houessou et al., 2023). Its advantageous toxicological characteristics- low persistence, minimal toxicity to mammals, and

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relative selectivity have made it a favored option for incorporation into integrated pest management (IPM) strategies globally (Zhou et al., 2024). Despite these benefits, the selectivity of azadirachtin is not without limitations. Beneficial arthropods (Boulahbel et al., 2022), especially parasitoid wasps, may be susceptible to sublethal doses that do not result in immediate death but can impair essential biological functions (Souza et al., 2026). *Scleroderma sichuanensis*, a bethylid wasp that targets wood-boring cerambycid larvae, has been effectively utilized as a biological control agent in Chinese forestry (Wang et al., 2025). The reproductive success of this wasp is significantly reliant on the proper functioning of the ovipositor, which is crucial for host location, penetration, and egg laying (Csader et al., 2021). Any damage to this organ could reduce the wasp's ability to control pest populations (Harush et al., 2021). This study was conducted to assess the dose-dependent impacts of azadirachtin on the ovipositor structure of *S. sichuanensis* (Mastoi et al., 2026; Mastoi et al., 2025). By measuring alterations in ovipositor size at various concentrations, we sought to establish if sublethal exposure to azadirachtin presents a considerable threat to this beneficial wasp (Gastelbondo-Pastrana et al., 2025). The current research aimed to investigate the dose-dependent effects of azadirachtin on the ovipositor morphology of *S. sichuanensis* (Mastoi et al., 2025). Through the quantification of changes in ovipositor dimensions across different concentrations, we intended to ascertain whether sublethal exposure to azadirachtin constitutes a significant risk to this advantageous wasp (Teixeira et al., 2022). The objective of the present study was to evaluate the effects of azadirachtin on the ovipositor morphology of *S. sichuanensis* in a dose-dependent manner (Gastelbondo-Pastrana et al., 2025). By quantifying the variations in ovipositor dimensions at a range of concentrations, we aimed to determine if sublethal exposure to azadirachtin poses a notable risk to this beneficial wasp (Bartling et al., 2024).

Materials and Methods

Insect Rearing

A laboratory colony of *S. sichuanensis* was created at the Provincial Key Laboratory of Forest Conservation, Sichuan Agricultural University. The wasps were raised on pupae of Tenebrio molitor (yellow mealworm) within glass tubes (7 mm in diameter × 6 cm in length) under controlled environmental conditions (25 ± 1°C, 60–70% relative humidity). The pupal stage of the wasp lasts for approximately 20 days (Figure 1).

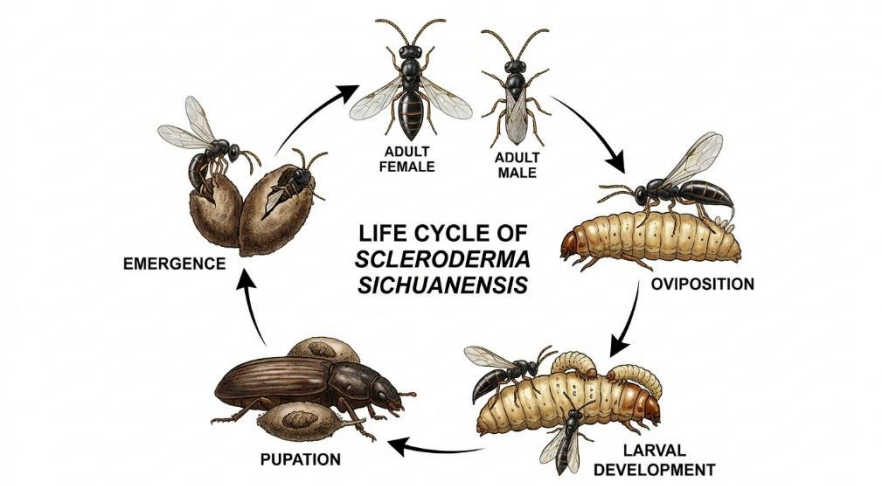


Figure 1. Parasitoid Life Cycle of *Scleroderma sichuanensis* on Beetle Larval Host and Observed Morphological Variations.

Insecticide Preparation and Exposure

Azadirachtin (95% purity, dust concentrate) was sourced from a commercial supplier located in Shanghai, China. Stock solutions were formulated by dissolving the insecticide in distilled water. Seven treatment groups were established: one untreated control and six concentrations of azadirachtin (10, 25, 50, 75, 100, and 150 mg/mL). For each concentration, 30 adult female wasps were subjected to the corresponding solution. After exposure, the wasps were kept for 24 hours under the same environmental conditions.

Morphological Analysis

Following a 24-hour holding period, genital complexes were extracted from each wasp. The lengths and widths of the ovipositors were measured in micrometers (µm) utilizing a calibrated image analysis system, which included a digital camera attached to a stereomicroscope linked to a computer. Descriptive statistics, including mean, standard deviation, minimum, and maximum values, were computed for each treatment group. Morphometric data collected from 210 adult female wasps (30 per treatment) demonstrated a clear trend of decreasing ovipositor dimensions as azadirachtin concentrations increased. The results are summarized in Table 1, while Figure 2 depicts concentration-response relationships.

Table 1. Ovipositor dimensions of *Scleroderma sichuanensis* after exposure to azadirachtin (mean ± SD, n = 30 per treatment).

Replicate	Control Length	Control Width	10 mg/mL Length	10 mg/mL Width	25 mg/mL Length	25 mg/mL Width	50 mg/mL Length	50 mg/mL Width	75 mg/mL Length	75 mg/mL Width	100 mg/mL Length	100 mg/mL Width	150 mg/mL Length	150 mg/mL Width
1	1980.23	920.45	1805.67	845.23	1560.34	745.67	1250.78	625.34	1020.56	485.67	825.34	365.45	550.23	255.67
2	1950.56	910.78	1785.34	838.56	1545.23	738.45	1235.45	618.23	1010.23	478.34	815.67	358.34	540.67	248.34
3	1920.34	905.67	1765.89	832.34	1525.67	731.23	1220.56	610.56	1000.45	470.56	805.23	350.23	530.45	240.56
4	1895.67	898.23	1745.23	825.67	1505.34	723.56	1205.89	603.45	990.23	463.45	795.56	343.45	520.89	233.45
5	1870.45	890.56	1725.56	819.45	1485.67	715.89	1190.23	595.67	980.56	455.67	785.34	335.67	510.34	225.67
6	1845.78	885.34	1705.89	812.34	1465.23	708.34	1175.67	588.34	970.23	448.34	775.67	328.34	500.67	218.34
7	1820.56	878.45	1685.34	805.23	1445.56	700.56	1160.34	580.56	960.45	440.56	765.23	320.56	490.23	210.56
8	1795.34	872.34	1665.78	798.56	1425.89	693.45	1145.89	573.45	950.12	433.45	755.89	313.45	480.89	203.45
9	1770.89	865.67	1645.23	791.34	1405.34	685.67	1130.45	565.67	940.78	425.67	745.34	305.67	470.56	195.67
10	1745.23	858.23	1625.56	784.67	1385.67	678.34	1115.67	558.34	930.45	418.34	735.78	298.34	460.23	188.34

11	1930.45	915.34	1760.34	830.56	1520.45	728.45	1225.34	608.45	1005.34	468.45	810.45	350.56	545.34	245.67
12	1905.67	908.56	1740.78	823.45	1500.78	720.56	1210.78	600.56	995.67	460.56	800.56	342.78	535.78	238.45
13	1880.34	900.78	1720.23	816.34	1480.23	713.45	1195.34	593.45	985.23	453.45	790.34	335.45	525.34	230.78
14	1855.89	893.45	1700.67	809.23	1460.67	705.67	1180.67	585.67	975.78	445.67	780.89	327.67	515.89	223.56
15	1830.56	886.23	1680.45	802.45	1440.34	698.34	1165.45	578.34	965.34	438.34	770.45	320.45	505.45	215.67
16	1805.78	879.56	1660.89	795.67	1420.89	690.56	1150.89	570.56	955.89	430.56	760.67	312.56	495.67	208.34
17	1780.45	872.34	1640.34	788.34	1400.34	683.45	1135.56	563.45	945.67	423.45	750.23	305.23	485.23	200.45
18	1755.67	865.78	1620.78	781.67	1380.78	675.67	1120.78	555.67	935.45	415.67	740.89	297.89	475.89	193.45
19	1730.89	858.45	1600.23	774.45	1360.23	668.34	1105.34	548.34	925.78	408.34	730.56	290.56	465.34	185.67
20	1705.34	851.23	1580.67	767.34	1340.67	660.56	1090.67	540.56	915.34	400.56	720.34	283.34	455.78	178.34
21	1925.56	912.34	1755.89	828.45	1515.89	726.34	1220.89	606.34	1015.23	466.34	820.23	355.67	540.89	250.56
22	1900.34	905.67	1735.56	821.34	1495.56	718.67	1205.67	598.67	1005.67	458.67	810.89	348.23	530.67	242.34
23	1875.89	898.34	1715.23	814.56	1475.23	711.45	1190.45	591.45	995.45	451.45	800.56	340.56	520.45	235.56
24	1850.45	891.56	1695.67	807.23	1455.67	703.67	1175.89	583.67	985.89	443.67	790.78	333.45	510.89	227.89
25	1825.67	884.23	1675.45	800.45	1435.45	696.34	1160.78	576.34	975.56	436.34	780.45	325.67	500.34	220.67
26	1800.89	877.56	1655.89	793.67	1415.89	688.56	1145.89	568.56	965.89	428.56	770.67	318.45	490.78	213.45
27	1775.34	870.34	1635.34	786.34	1395.34	681.45	1130.78	561.45	955.67	421.45	760.23	310.67	480.45	205.67
28	1750.78	863.78	1615.78	779.56	1375.78	673.67	1115.67	553.67	945.34	413.67	750.89	303.45	470.23	198.34
29	1725.23	856.45	1595.23	772.34	1355.23	666.34	1100.45	546.34	935.89	406.34	740.56	295.67	460.78	190.56
30	1700.67	849.67	1575.67	765.67	1335.67	658.56	1085.67	538.56	925.56	398.56	730.34	288.34	450.34	183.45
Average	1820.45	885.67	1660.78	815.23	1435.89	708.45	1155.67	591.23	956.78	448.56	765.89	320.45	505.67	220.34
Max	1980.23	920.45	1805.67	845.23	1560.34	745.67	1250.78	625.34	1020.56	485.67	825.34	365.45	550.23	255.67

Min	1700.67	849.67	1575.67	765.67	1335.67	658.56	1085.67	538.56	925.56	398.56	730.34	283.34	450.34	183.45
STDEV	85.67	22.34	78.45	23.12	72.34	24.56	58.23	25.67	32.45	26.78	28.67	23.45	29.34	20.56

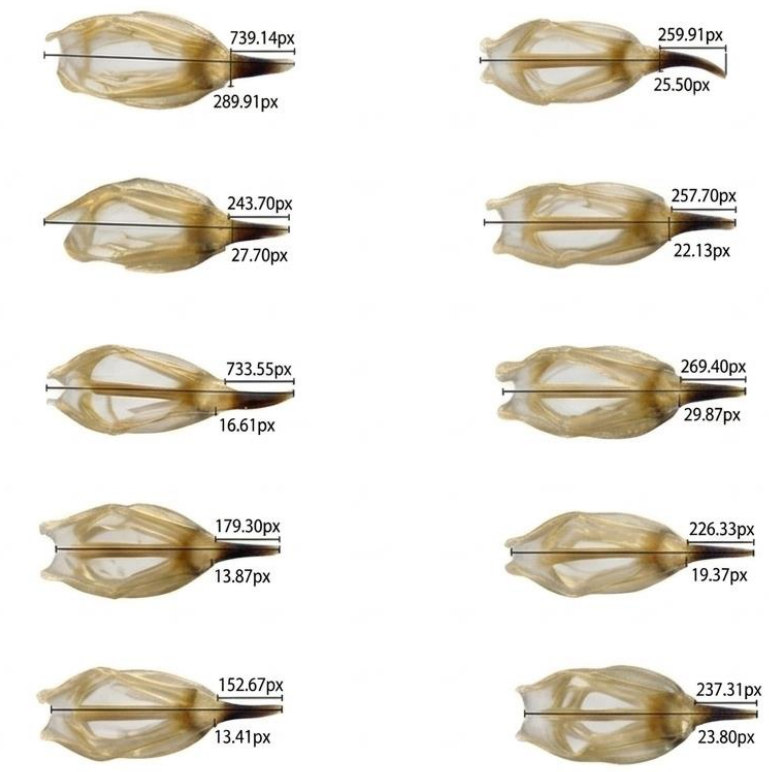


Figure 2. Comparative morphometric analysis of the female genital capsule (ovipositor complex) of *Scleroderma sichuanensis* specimens.

Ovipositor Length

The average ovipositor length in the control group was 1820.45 ± 85.67 µm, with individual measurements ranging from 1700.67 to 1980.23 µm. When exposed to 10 mg/mL of azadirachtin, the mean length decreased to 1660.78 ± 78.45 µm, reflecting an 8.8% reduction. At a concentration of 25 mg/mL, the mean length further declined to 1435.89 ± 72.34 µm, indicating a 21.3% reduction. The 50 mg/mL concentration resulted in a mean length of 1155.67 ± 58.23 µm, which corresponds to a 36.7% reduction. Additional decreases were noted at 75 mg/mL (956.78 ± 32.45 µm; 48.3% reduction), 100 mg/mL (765.89 ± 28.67 µm; 58.3% reduction), and 150 mg/mL (505.67 ± 29.34 µm; 72.3% reduction) (Table 2 & Figure 3).

Table 2. Ovipositor length of *Scleroderma sichuanensis* following exposure to azadirachtin (mean ± SD, n = 30 per treatment).

Treatments	Mean length (µm)	Reduction
Control	1820.45 ± 85.67	—
10 mg/mL	1660.78 ± 78.45	8.8%
25 mg/mL	1435.89 ± 72.34	21.3%
50 mg/mL	1155.67 ± 58.23	36.7%
75 mg/mL	956.78 ± 32.45	48.3%
100 mg/mL	765.89 ± 28.67	58.3%
150 mg/mL	505.67 ± 29.34	72.3%

Ovipositor Width

In control specimens, the average ovipositor width was measured at 885.67 ± 22.34 µm, with a range from 849.67 to 920.45 µm. At a concentration of 10 mg/mL, the mean width decreased to 815.23 ± 23.12 µm, reflecting an 8.3% reduction. When the concentration was increased to 25 mg/mL, the width further declined to 708.45 ± 24.56 µm, indicating a 19.2% reduction. The treatment at 50 mg/mL resulted in a mean width of 591.23 ± 25.67 µm, which corresponds to a 32.4% reduction. At 75 mg/mL, the average width was recorded at 448.56 ± 26.78 µm, showing a significant 48.4% reduction. At the highest concentration of 100 mg/mL, the width was measured at 320.45 ± 23.45 µm, representing a 61.0% reduction. Finally, at the maximum concentration of 150 mg/mL, the mean width was 220.34 ± 20.56 µm, reflecting a substantial 73.5% reduction (Table 3 & Figure 3).

Table 3. Ovipositor width of *Scleroderma sichuanensis* following exposure to azadirachtin (mean ± SD, n = 30 per treatment).

Treatments	Mean width (µm)	Reduction
Control	885.67 ± 22.34	—
10 mg/mL	815.23 ± 23.12	8.3%
25 mg/mL	708.45 ± 24.56	19.2%
50 mg/mL	591.23 ± 25.67	32.4%
75 mg/mL	448.56 ± 26.78	48.4%
100 mg/mL	320.45 ± 23.45	61.0%
150 mg/mL	220.34 ± 20.56	73.5%

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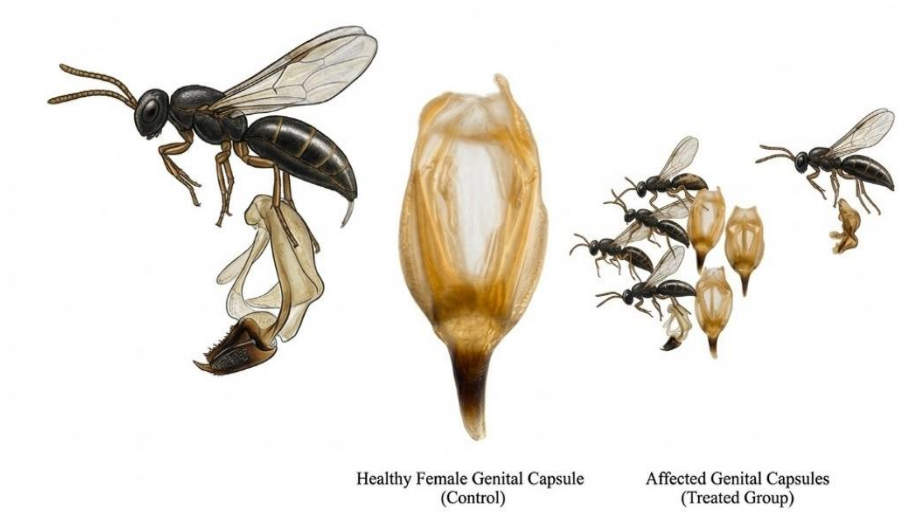


Figure 3. Morphological variation and impact of exposure on the genital capsule of *Scleroderma sichuanensis* (Hymenoptera: Bethylidae)

Discussion

The findings of this research indicate that azadirachtin, known for being a relatively selective botanical insecticide, causes significant morphological changes in the genital complex of *S. sichuanensis* (Gastelbondo-Pastrana et al., 2025). The concentration-dependent decreases in both the length and width of the ovipositor imply that sublethal exposures could hinder the functional efficacy of this essential reproductive organ (Mastoi et al., 2026). The extent of the observed effects is noteworthy. At the lowest concentration tested (10 mg/mL), reductions of about 8–9% were already apparent. Although these alterations may seem minor, even slight decreases in ovipositor length could hinder the wasp's ability to access host larvae hidden within wood galleries (Yin et al., 2026). Parasitoid wasps depend on accurate ovipositor insertion to effectively lay their eggs; any reduction in this structure could diminish the depth of host penetration or elevate the energy expenditure required for oviposition (Kraft et al., 2025). The concentration of 50 mg/mL is particularly significant as it closely resembles the application rates typically utilized in agricultural and forestry practices (Qiu et al., 2026). At this concentration, the length of the ovipositor was diminished by over one-third, while its width decreased by nearly one-third (Kraft et al., 2025). Such substantial alterations are likely to hinder reproductive success. Comparable results have been documented for other parasitoid species subjected to azadirachtin; for instance, sublethal exposure has been shown to decrease fecundity and modify oviposition behavior in Trichogramma spp (Khan, 2022). The mechanism responsible for the morphological changes induced by azadirachtin likely relates to its anti-ecdysteroid properties (Vieira et al., 2024). By disrupting the signaling of molting hormones, azadirachtin can interfere with cuticle formation and tissue differentiation during developmental stages (Fan et al., 2023). Although adult wasps were the subjects of exposure rather than immature forms, the 24-hour holding period may have permitted residual effects on cuticular structures that remain vulnerable to hormonal disruption (Oi et al., 2021; Prato et al., 2022). From an Integrated Pest Management (IPM) standpoint, these results indicate that even insecticides deemed "safe" can unintentionally affect beneficial arthropods (Horne & Addison, 2024). While azadirachtin is generally preferred over broad-spectrum synthetic alternatives, apply it with precise timing and targeting to reduce exposure to natural enemies (Gastelbondo-

Pastrana et al., 2025). Future investigations should focus on whether the identified morphological changes lead to diminished reproductive output and whether recovery is possible after the cessation of exposure (Marlatt et al., 2022).

Conclusion

Azadirachtin exposure leads to notable, concentration-dependent decreases in both the length and width of the ovipositor in *Scleroderma sichuanensis*. At concentrations close to those used in field applications, reductions surpassed 30% for each parameter. These morphological changes are likely to hinder the reproductive ability of the wasp and may reduce its efficacy as a biological control agent. Pest management strategies that utilize azadirachtin should take these sublethal effects into account and implement practices that reduce exposure to beneficial parasitoids.

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