



## Dose-Dependent Effects of Avermectin on the Reproductive Biology of *Scleroderma Sichuanensis*

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#### Authors' Contribution

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### ABSTRACT

*Scleroderma sichuanensis* is an important parasitoid used in the biological control of wood-boring pests. However, the extensive use of avermectin may negatively affect such beneficial insects. This study evaluated the impact of different concentrations of avermectin on the genital morphology of *S. sichuanensis* under controlled laboratory conditions. Seven concentrations (5, 20, 40, 60, 80, 100, and 120 mg/mL) were applied to adult females, while untreated individuals served as controls. Ovipositor length and width were measured in 30 wasps per treatment. The results demonstrated a clear concentration-dependent reduction in both parameters. At 5 mg/mL, ovipositor length and width decreased by 27.6% and 45.5%, respectively. At the highest concentration (120 mg/mL), reductions in length and width reached 96.8% and 95.6%, respectively. These findings indicate that sub-lethal exposure to avermectin induces significant morphological alterations in the reproductive structures of *S. sichuanensis*, potentially impairing its reproductive capacity and effectiveness as a biological control agent. These results show a clear dose-dependent effect of avermectin on reproductive morphology. The structural damage may reduce parasitoid fitness and threaten long-term population stability in agroecosystems. Therefore, integrating chemical control with conservation of beneficial parasitoids is essential for sustainable pest management.

### INTRODUCTION

Insects play an important role in the terrestrial ecosystem, so their services include nutrient cycling, soil formation, pollination, and natural pest control (Barragana-Fonseca and Gomez, 2025; Barragana-Fonseca et al., 2025; Billet, 2019; Wobale Birhanie, 2024). They are usually a sign of ecosystem health because the decline of insect species can often be an indicator of the broadening impairments of the environment (Ramola et al., 2024; Withaningsih et al., 2024). Even with their ecological significance, the population of insects is exposed to rising risks due to habitat destruction, altering climatic trends, and large-scale application of synthetic pesticides, which altogether affect the stability of biodiversity and agricultural performance (Regmi et al., 2025). Sub-lethal doses affect various insect physiological aspects such as the immune system, neurophysiology, biochemistry, sex ratio, fecundity, longevity, and weight (Afza et al., 2023; Bartling et al., 2024; De França et al., 2017). Moreover, synthetic

insecticides reduce the efficacy of predatory beetles and other biological agents of control when used in low doses (Cheng et al., 2022; Knapp et al., 2025). Nonetheless, their positive effect in integrated pest management (IPM) systems may be affected by pesticide sprays. Some of the studies investigated the acute toxicity levels of insecticides on parasitoids (Mata et al., 2024; Overton et al., 2023; Urbaneja-Bernat et al., 2025). To obtain an accurate assessment of the risk of insecticides on natural enemies, one should also consider sub-lethal effects (De Franca et al., 2017; Müller, 2018; Nozad-Bonab et al., 2021; Zhu et al., 2025). At the same time, adult longevity and fecundity, the rate of emergence, and the sex ratio of the offspring of parasitoids are the primary considerations of the insecticide sub-lethal risk assessment (Akhtar et al., 2022; Stanley and Preetha, 2016; Tonnga and Bayram, 2024). Insecticides have a physiological effect on the remaining insects (Gong et al., 2023). They can also affect their behaviour, though. The insect species *S. sichuanensis*

(Hymenoptera: Bethyridae), especially the Cerambycidae and buprestid beetles, are now among the most important natural predators of wood-boring insects in China (Mastoi et al., 2026; Mastoi et al., 2025; Yang et al., 2014). We used avermectin in a laboratory setting to assess the genital complex of *S. sichuanensis* in the current study. The genital complex of the parasitoid wasp is anatomically and functionally vital to reproduction, and any morphological injury may have a direct impact on reproductive effectiveness and, by extension, on the efficiency of biological control. The paper was therefore aimed at evaluating the dose-response of avermectin on genital complex morphology of *S. sichuanensis* in controlled conditions.

## MATERIAL AND METHODS

Pupa of *T. Molitor* (0.1-0.5 g) nourished with wheat bran and the vegetable food, which was free of contamination with pollutants, was given to adult female wasps as food. The rearing of the *S. sichuanensis* wasps was done in the provincial key laboratory of forest protection (College of Forestry, Sichuan Agriculture University, Sichuan, China). The concentrates of avermectin 97% dust were used (Shanghai, China).

### Insecticide Preparation

Insecticide was dissolved in distilled water to obtain stock solutions. Eight groups of treatments were developed, and an untreated control (0 mg/mL) and seven concentrations of avermectin: 5, 20, 40, 60, 80, 100, and 120mg/mL.

### Exposure Protocol

Thirty adult female wasps were used for each concentration treatment. The insects were exposed to the corresponding avermectin solutions under laboratory conditions. After treatment, the wasps were incubated for 24 h under controlled environmental conditions ( $25 \pm 1^\circ\text{C}$  and 60–70% relative humidity) to allow the expression of sub-lethal effects.

### Morphological Measurements

The genital complexes were carefully dissected from each wasp under a stereomicroscope. Ovipositor length and width were measured for all specimens. Measurements were recorded in micrometers ( $\mu\text{m}$ ) using a calibrated image analysis system.

### Statistical Analysis

The captured images were analysed using image analysis software to ensure precision and reproducibility of measurements. Descriptive statistics, including mean, minimum, maximum, and standard deviation (SD), were calculated for each treatment group. Statistical analyses were conducted using SPSS (version 27), while graphical representations were generated using GraphPad Prism.

## RESULTS

Morphometric data were obtained from adult female wasps ( $n = 30$  per treatment). A clear trend of decreasing ovipositor size was observed with increasing concentrations of avermectin. A concentration-dependent

reduction in both ovipositor length and width was recorded. The detailed measurements are summarized in Table 1, while the concentration–response relationship is illustrated in Fig. 1. The overall decline in ovipositor dimensions across treatments is further shown in Fig. 2. The mean length of the ovipositor in the control was 1937.46  $\mu\text{m}$  (SD = 118.28), and single scores were 1680.69  $\mu\text{m}$  and 222.65  $\mu\text{m}$ . The length was consistently reduced with increasing concentrations of exposure to avermectin. At 5mg/mL, the average length became 1403.37 (SD = 118.28)  $\mu\text{m}$  (SD = 109.45), which was 27.6% lower than the control. This was observed to be the same at 20mg/mL, with the average being 1380.59  $\mu\text{m}$  (SD = 62.49), representing a 28.7% decrease. At 40 mg/mL, a greater decrease was recorded, and the mean length decreased to 636.92  $\mu\text{m}$  (SD = 58.02), which is a reduction of 51.6%. The ovipositor mean length was found to be 466.06  $\mu\text{m}$  (SD = 46.07) at 60 mg/mL, a reduction of 75.9%. The mean was lower at 80 mg/mL, where it was 227.83  $\mu\text{m}$  (SD = 40.49), which is a drop of 88.2%. The worst effects were observed in the two highest concentrations: at 100mg/mL, the mean length was 87.98  $\mu\text{m}$  (SD = 22.31), 95% decrease; at 120mg/mL, the mean was 61.06  $\mu\text{m}$  (SD = 15.31), which represented a 96.8% decrease compared to the control.

A similar tendency was shown in the width of the ovipositor. The control group measurements are 912.68  $\mu\text{m}$  (SD = 57.1), and the measurement range is 760.20  $\mu\text{m}$  to 1000.05  $\mu\text{m}$ . The width values declined drastically with the concentrations of avermectin. The data was measured in 5 mg/mL, and the mean width was found to be 497.24  $\mu\text{m}$  (SD = 46.55), indicating a 45.5% reduction compared to the control population. It was found that there was a significant change at 20 mg/mL, with the mean width reduced to 132.14  $\mu\text{m}$  (SD = 40.05), representing an 85.5% reduction. The mean width at 40 mg /mL remained much lower (93.47  $\mu\text{m}$ , SD = 34.35) and 89.8% decrease. The mean width reduced to 64.33  $\mu\text{m}$  (SD = 22.450), and 93.0% of the width was decreased at 60 mg/mL. At 80mg/mL, the treatment led to a mean width of 54.96  $\mu\text{m}$  (SD = 16.66), equivalent to a 94.0% reduction. Further deterioration was noticed at 100mg/mL (48.81  $\mu\text{m}$ , SD = 16.04; 97.7% decrease) and 120mg/mL (39.77  $\mu\text{m}$ , SD = 12.93; 95.6% decrease).

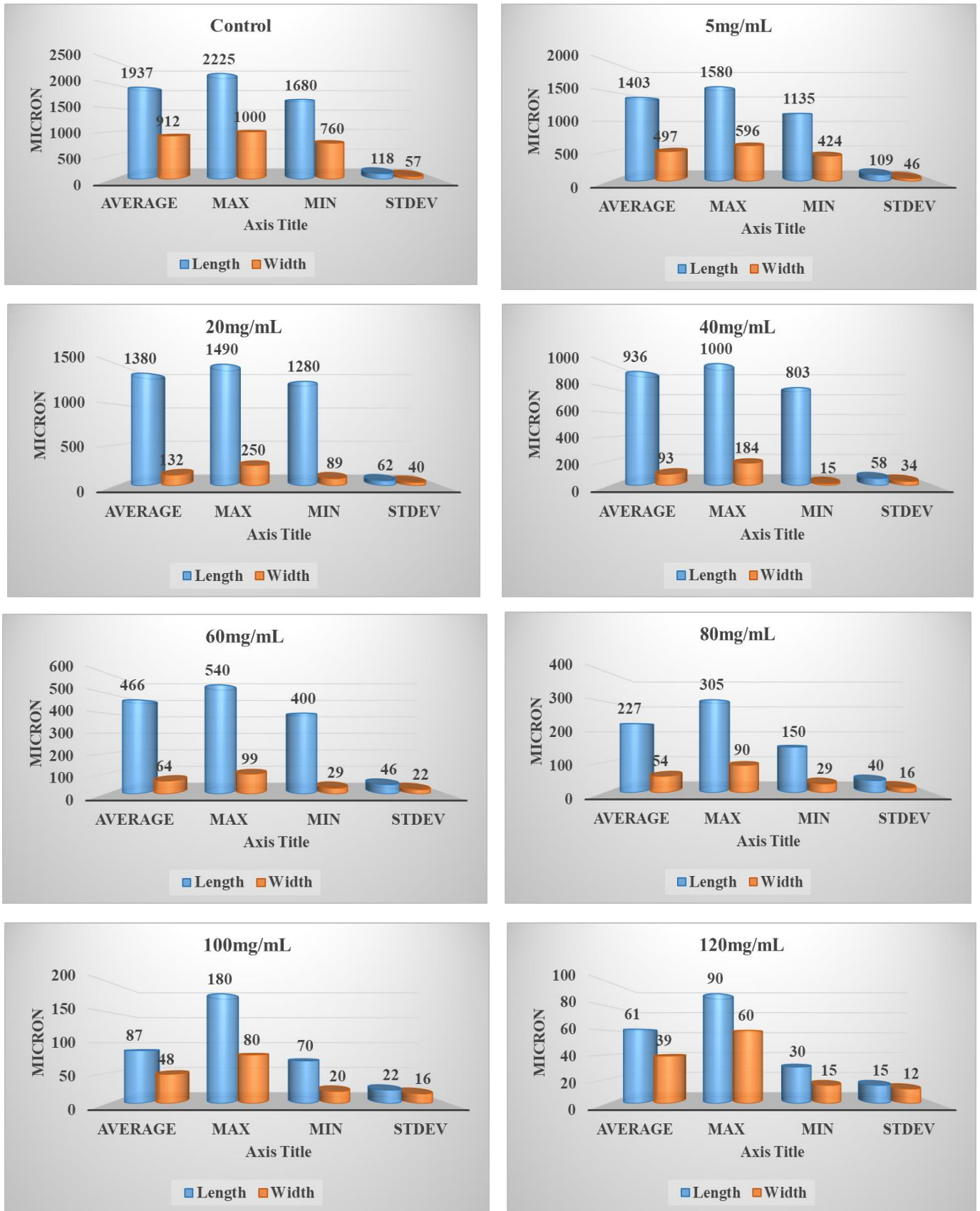
The use of insecticides to safeguard people's health is critical in regions with a high incidence of vector-borne diseases. Their ability to rapidly suppress vector populations and eliminate disease transmission has saved many lives. To ensure their efficacy, however, they must be used responsibly; their use must be controlled, and environmental consciousness and concerted control measures should be implemented. It is under the responsibility of the entomologists to promote balanced, sustainable, and scientifically guided approaches to managing vectors. In this way, we will be able to guarantee that insecticides remain useful and efficient in protecting environmental health without disrupting the ecological balance by conducting research, shaping the policy, and working together with the community.

**Table 1**

*Morphometric Measurements of Ovipositor Length and Width in Scleroderma Sichuanensis Following Exposure to Varying Concentrations of Avermectin*

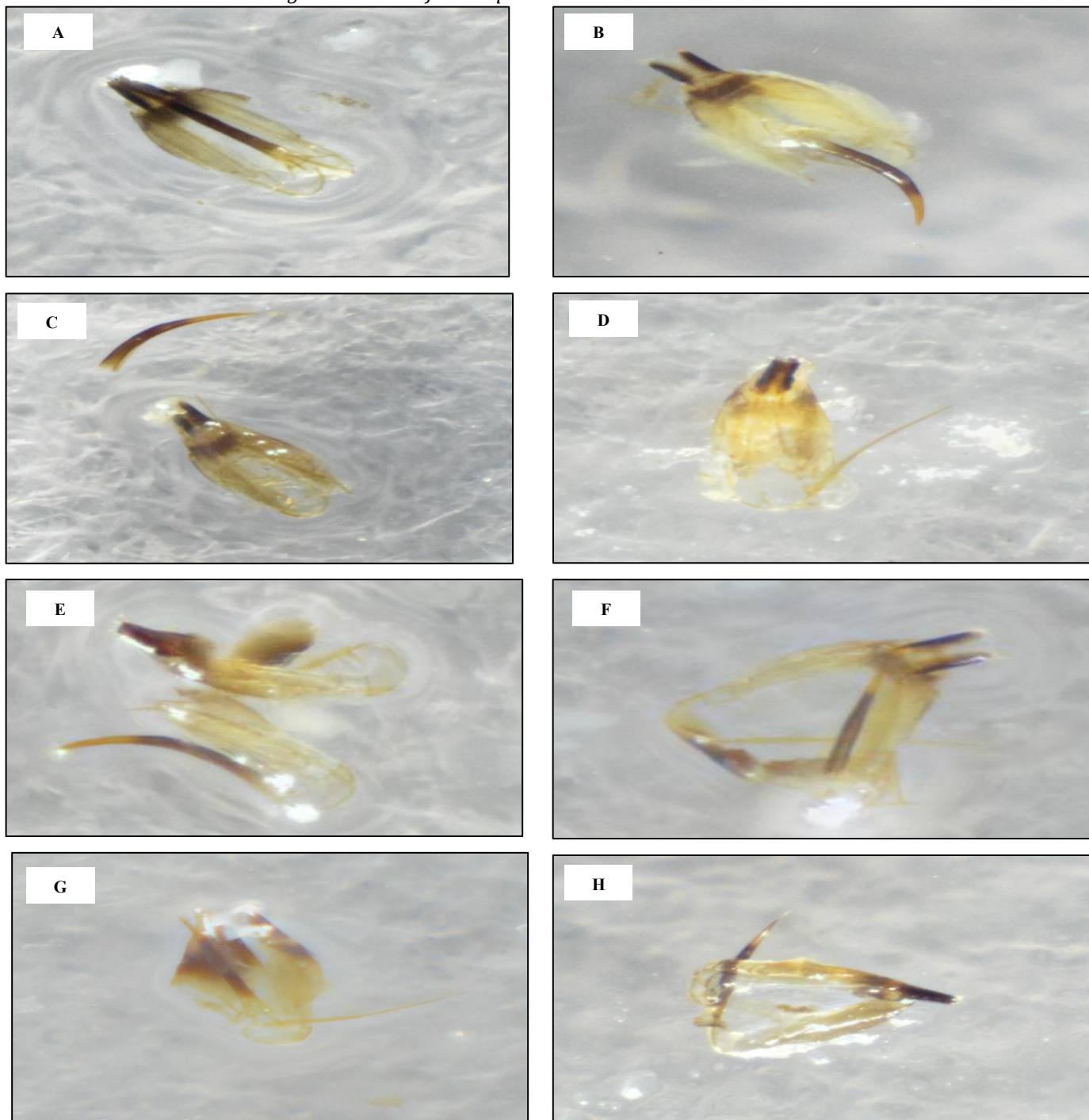
	Control		5mg		20mg		40mg		60mg		80mg		100mg		120mg	
	Length	Width	Length	Width	Length	Width	Length	Width	Length	Width	Length	Width	Length	Width	Length	Width
1	1950.25	920.5	1240.23	580.05	1280.58	150.2	1000.05	80.05	502.2	99.63	150.20	50.63	90.2	20.54	80.2	29.54
2	1900.25	950.58	1255.89	495.56	1380.56	105.56	990.25	95.56	403.54	59.45	200.54	59.45	80.56	80.22	56.54	49.22
3	1850.69	900.58	1225.89	470.2	1360.56	140.56	950.36	170.56	505.57	40.2	195.57	40.2	102.35	40.20	75.57	15.20
4	1820.58	850.58	1435.58	485.23	1290.56	105.56	960.36	95.05	405.05	35.05	305.05	35.05	72.05	50.05	50.05	15.05
5	1680.69	880.45	1320.56	475.05	1375.48	99.56	980.05	75.56	500.46	70.54	300.46	70.54	90.26	50.24	40.46	15.24
6	1820.69	820.25	1350.5	490.23	1305.05	105.00	890.05	90.56	500.36	40.65	200.36	40.65	80.48	55.25	30.36	25.25
7	1900.58	870.05	1135.56	480.56	1385.56	99.05	900.05	80.89	505.52	70.57	205.52	70.57	120.2	40.25	53.52	20.25
8	1950.88	900.05	1355.23	495.20	1305.2	165.05	985.75	95.45	510.56	49.58	300.56	49.58	70.56	30.25	50.56	30.25
9	2020.56	950.05	1325.56	470.52	1365.56	140.05	960.89	75.66	405.60	29.56	205.60	29.56	80.69	35.35	50.60	35.35
10	2200.26	1000.05	1328.23	458.20	1380.05	210.05	980.36	96.45	500.63	67.56	200.63	70.56	70.65	80.25	49.63	45.25
11	1870.05	950.05	1366.89	585.05	1350.05	150.05	970.66	81.05	500.56	94.88	200.56	40.88	70.56	28.15	50.56	35.15
12	1820.05	760.20	1200.86	430.36	1370.05	100.56	955.78	102.45	500.66	39.55	200.66	39.55	80.89	45.45	40.66	30.45
13	1870.23	910.63	1384.56	475.36	1300.89	250.56	980.24	99.65	400.66	90.05	200.66	70.05	70.21	72.05	58.66	45.05
14	1830.03	890.2	1432.56	493.56	1470.03	180.23	965.23	94.45	510.88	93.88	210.88	30.88	90.65	45.15	77.88	45.15
15	1890.22	970.69	1452.56	529.89	1400.02	100.56	985.75	48.58	500.89	50.56	200.89	50.56	80.84	29.45	78.89	29.45
16	1830.56	910.23	1435.63	574.05	1480.23	150.56	970.99	96.89	400.58	60.89	200.58	60.89	80.20	44.89	40.58	46.89
17	1910.58	860.56	1468.12	501.05	1300.23	100.56	981.05	120.89	540.56	50.89	240.56	50.89	80.52	45.75	79.56	45.75
18	1960.69	890.56	1524.1	464.56	1380.69	150.56	968.05	70.89	400.23	64.89	200.23	64.89	70.75	41.56	69.23	50.56
19	2030.89	960.56	1488.21	500.56	1400.25	150.50	982.05	80.12	500.27	92.56	300.27	42.56	90.75	50.65	70.27	30.65
20	2110.89	995.56	1400.65	554.56	1370.63	150.20	813.05	40.99	500.89	64.89	200.89	64.89	80.23	40.45	50.89	53.45
21	1996.12	815.78	1450.56	481.56	1350.56	170.56	858.25	155.56	470.69	90.56	270.69	70.56	80.20	50.63	70.69	50.63
22	1815.36	945.78	1480.56	435.05	1480.78	110.20	803.05	106.56	400.36	70.56	200.36	70.56	70.45	50.63	50.36	50.63
23	1965.78	905.56	1490.23	462.05	1490.89	96.63	857.05	90.90	450.65	30.69	250.65	30.69	80.20	50.2	90.65	50.2
24	1925.45	935.05	1500.23	488.56	1480.56	100.30	911.56	15.69	430.56	50.56	230.56	50.56	130.56	60.69	70.56	40.69
25	1985.23	965.05	1480.25	424.05	1400.76	89.30	947.05	79.77	410.45	50.58	210.45	50.58	90.85	20.89	80.45	50.89
26	1925.02	895.05	1490.2	570.05	1380.56	100.63	919.05	184.57	450.23	89.56	250.23	90.56	90.65	80.56	50.23	60.56
27	2005.09	895.96	1500.32	596.56	1400.15	100.89	864.20	117.52	500.56	40.56	200.56	40.56	80.85	50.56	66.56	50.56
28	1955.30	895.98	1580.11	451.69	1480.05	100.36	827.23	106.34	420.36	90.85	250.36	70.85	90.65	51.25	50.36	40.25
29	2225.65	985.54	1500.22	498.69	1400.05	99.36	969.23	55.63	450.26	50.58	250.26	50.58	90.63	52.56	77.26	52.56
30	2105.23	998.20	1501.12	499.25	1301.23	191.22	980.05	99.69	500.23	99.56	300.23	90.56	180.89	70.23	70.23	53.23
Avg	1937.461	912.677	1403.372	497.24	1380.594	132.14	936.924	93.466	466.06	64.329	227.83	54.963	87.984	48.811	61.06	39.77
Max	2225.65	1000.05	1580.11	596.56	1490.89	250.56	1000.05	184.57	540.56	99.63	305.05	90.56	180.89	80.56	90.65	60.56
Min	1680.69	760.20	1135.56	424.05	1280.58	89.3	803.05	15.69	400.23	29.56	150.2	29.56	70.21	20.54	30.36	15.05
STDEV	118.28	57.010	109.45	46.55	62.49	40.05	58.017	34.35	46.074	22.45	40.94	16.66	22.31	16.04	15.31	12.93

**Figure 1**  
 Concentration-Dependent Effect of Avermectin on Ovipositor Length and Width of *Scleroderma Sichuanensis*



**Figure 2**

Concentration-dependent impact of avermectin on the length and width of the ovipositor in *Scleroderma sichuanensis*. A (Control, 0mg) displays the normal ovipositor dimensions; B (5mg) shows a slight reduction in both length and width. C (20 mg) indicates a moderate decrease, D (40 mg) reflects a moderate to marked reduction, E (60 mg) presents a marked reduction, F (80 mg) reveals a severe reduction, G (100 mg) demonstrates a very severe reduction, and H (120 mg) illustrates an extreme reduction in both length and width of the ovipositor.

**DISCUSSION**

The result of the present study demonstrates that exposure to avermectin causes a concentration-dependent reduction in the genital complex size of *Scleroderma sichuanensis*. At higher concentrations, the effects were severe, with ovipositor length and width reduced by approximately 97% and 96%, respectively, compared to the control. Notably, even at a lower concentration (5 mg/mL), a significant reduction in ovipositor width

(45.5%) was observed. These findings raise concerns regarding the sub-lethal effects of avermectin in agricultural and forestry systems, where *S. sichuanensis* may function as a biological control agent (Gu et al., 2026; J. Li et al., 2025; Z. Li et al., 2025). The ovipositor is a structurally complex organ that is essential for the reproductive performance of parasitoids (Csader et al., 2021; De Franca et al., 2017; Eggs et al., 2018). It is involved in host search, substrate perforation, and egg

laying (Costain et al., 2018; Leather, 2018). Reproductive efficiency would be directly influenced in the case of any morphological abnormality of this structure (Leather, 2018). Our results agree with other studies that have reported sub-lethal morphological effects of insecticides on useful hymenopteran. An example is exposure to neonicotinoids that have been reported to reduce body size and alter wing morphology in *Aphidius* species, and macrocyclic lactones such as abamectin that have been reported to alter oviposition behaviour in some parasitoids (Ju et al., 2022). This paper is an extension of the previous work in that morphometric changes in the genital complex were directly measured over a broad range of concentration.

One such finding was a rather small increment in ovipositor length between 5 mg/mL (27.6) and 20 mg/mL (28.7), compared to the steep drop between 20 mg/mL and 40 mg/mL (between 28.7 and 51.6). The trend can reflect a particular concentration above which avermectin has proportionally dire effects on developing or adult reproductive tissues (El-Saber Batiha et al., 2020; Wang et al., 2026). These non-linear dose-response relationships have been reported in other insecticides and should be investigated further to identify the main critical concentration thresholds that would produce minimal harm to non-target organisms and at the same time retain the effectiveness of pests (Pisa et al., 2015). The decrease in ovipositor width was already considerable at 20 mg/mL (85.5%), indicating that the given parameter can be more susceptible to avermectin exposure compared to length. The ovipositor width can also be especially appropriate with the muscle-relaxant effect of avermectin that serves as a GABA agonist and results in the hyperpolarization of neurons and muscle cells (El-Saber Batiha et al., 2020). This physiological process may culminate in the flaccidity or atrophy of ovipositor muscles, which is manifested in the decreased width sensitivity between length and width, and possibly is due to variations in the composition of the tissue or the relative timing of the different structural elements (Eggs et al., 2018). Practically, the results have direct applications to the integrated pest management (IPM) (Ali and Zhuo, 2025; Zhou et al., 2024). Avermectin is also a popular forestry and agricultural insecticide applied to lepidopteran, mites, and leaf miners. Nevertheless, its application in the setting where *S. sichuanensis* occurs naturally or by augmentative release may prevent the delivery of biological control services accidentally (Khalil and Darwesh, 2019). The indicated morphological alterations suggest that even sub-lethal concentrations can impair the reproductive ability of this species, which can slow the population growth rates and the overall success when working with pests (Rawal et al.,

2025; Spence et al., 2025). Several limitations of this study should be identified. To begin with, the experiments that we did were carried out in a controlled laboratory environment that might not necessarily reflect the intricacies of the field environment, where other factors like pesticide decay, temperature changes, and availability of hosts affect the exposure results. Secondly, we determined morphological changes directly after a 24 hrs exposure. Thirdly, even though morphological alterations are strongly suggestive of functional deficiency, direct measures of reproduction outcome, including fecundity, egg durability, and larval survival, would contribute to the ecological importance of the findings (Li et al., 2024). Some gaps in this field will be addressed in the future by means of combining morphometric analysis with reproductive bioassays and by testing laboratory findings in semi-field or field environments (Tan et al., 2026). Besides, the exploration of the molecular mechanisms underlying the avermectin-induced morphological changes might bring illumination into the pathways that appear to be the most appropriate to break and help to design more selective pest management tools (Dong and Zhang, 2022; Villacis-Perez et al., 2022). The comparative research of different parasitic species would also help to identify whether the sensitivity found in *S. sichuanensis* is common to other useful hymenopteran species.

## CONCLUSION

The present study unequivocally demonstrates that avermectin induces significant, dose-dependent alterations in the reproductive morphology and physiology of *Scleroderma sichuanensis*. The highest tested concentration (120 mg/mL) resulted in a drastic suppression exceeding 95% in key reproductive parameters, accompanied by substantial reductions in body length (27.6%) and body width (45.5%) relative to the control. Notably, even sub-lethal exposure (5 mg/mL) elicited measurable morphological deformities, underscoring the high sensitivity of this parasitoid species to avermectin. These alterations are likely to impair fecundity, mating success, and population viability, thereby reducing the effectiveness of *Scleroderma sichuanensis* as a biological control agent. The findings highlight significant ecological risks associated with the non-target toxicity of avermectin, particularly under unregulated application. Therefore, its use in pest management must be carefully optimized in terms of dose and timing to ensure effective pest control while conserving beneficial arthropods. Further research is essential to establish safe threshold levels and assess long-term impacts for sustainable integrated pest management (IPM).

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