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DISRUPTION OF HOST-SEEKING RESPONSE OF FEMALE *SCLERODERMA SICHUANENSIS* TO Y-TUBE OLFACTOMETER INDUCED BY EMAMECTIN BENZOATE AND MATRINE

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Abstract

Scleroderma sichuanensis is an ectoparasitoid wasp used in the biological control of wood-boring beetles. This study examines the sublethal effects of three insecticides-emamectin benzoate and matrine on the host-seeking behavior of female *S. sichuanensis*. The wasps were exposed to LC_{20} concentrations of each insecticide and then evaluated in a Y-tube olfactometer to assess their olfactory responses, learning, and memory using synthetic host compounds from Monochamus alternatus at concentrations of 500, 100, and 10 mmol/L. Exposure to emamectin benzoate led to a reduction in host-selection rates to 61%, 51%, and 52% at concentrations of 500, 100, and 10 mmol/L, respectively. In contrast, matrine decreased selection rates to 57%, 33%, and 51%, respectively, when compared to the control. Regarding natural host cues, emamectin benzoate produced selection rates of 76% for

larval hemolymph and 60% for pupal odor, while matrine resulted in 67% and 77%, respectively. Both insecticides also influenced olfactory learning and memory; wasps exposed to emamectin benzoate exhibited selection rates of 60%, 62%, and 51% during the learning phase, and 56%, 37%, and 37% during memory evaluation. Conversely, matrine-exposed wasps demonstrated selection rates of 57%, 40%, and 50% during learning, and 60%, 40%, and 43% during memory assessment. These results suggest that sublethal exposure to emamectin benzoate and matrine can moderately impair host-seeking behavior, olfactory learning, and memory in *S. sichuanensis*, which may impact on its efficacy as a biological control agent. Consequently, it is essential to carefully evaluate the use of these insecticides in integrated pest management strategies that involve *S. sichuanensis* for biological control.

Introduction

The integration of biological control with chemical pest management requires careful selection of insecticides that minimize harm to beneficial organisms. Avermectin and botanical compounds like matrine are among the insecticides recognized for their lower environmental impact (Baker et al., 2020; Chen et al., 2026; Liu et al., 2026; Wahengbam et al., 2020). However, even these substances can have sublethal effects on non-target insects, including parasitoid wasps. *Scleroderma sichuanensis* is an important biological control agent for wood-boring beetles in forest ecosystems (Mastoi et al., 2025; Zheng et al., 2024). This wasp detects its host through olfactory signals, which include volatile compounds released by host larvae and their galleries (Gao et al., 2023; Mastoi, Bhatti, et al., 2026). Understanding how insecticides affect this host-seeking behavior is essential for developing integrated Pest Management (IPM) programs that effectively combine chemical and biological control strategies. Avermectin and emamectin benzoate are macrocyclic lactone insecticides that act as agonists of GABA-gated chloride channels, causing hyperpolarization of neurons and disrupting neurotransmission. These compounds are widely used in forestry and agriculture to manage lepidopteran and coleopteran pests. Matrine, a botanical alkaloid derived from *Sophora flavescens*, functions through various mechanisms, including sodium channel blockade and inhibition of acetylcholinesterase (Tao et al., 2020). It is considered a low-toxicity insecticide suitable for organic production systems. Previous studies have shown that avermectin can affect the behavior and reproduction of parasitoid wasps (Nozad-Bonab et al., 2021). While matrine is noted for its relatively low toxicity to natural enemies (Liu et al., 2023). However, the specific effects of these insecticides on the host-seeking behavior of *S. sichuanensis* have not yet been systematically evaluated (Li et al., 2025; Song et al., 2021; Yang et al., 2021). The current research aimed to evaluate the sublethal effects of emamectin benzoate and matrine on the olfactory responses, learning abilities, and memory retention of female *S. sichuanensis* (Chen et al., 2023). We hypothesized that these insecticides would cause moderate disruptions in host-seeking behavior, with effects less severe than those observed with neurotoxic insecticides such as pyrethroids and neonicotinoids.

Materials and Methods

Insect Reaping

The *Scleroderma sichuanensis* wasps were maintained in a laboratory colony at the Forest Conservation Laboratory, Sichuan Agriculture University, Chengdu, China. They were reared on pupae of Tenebrio molitor in glass tubes measuring 3 X 6 cm, kept at a temperature of 25 ±

1°C and a relative humidity of 60-70%. Only newly emerged adult female wasps were used for all experimental procedures (Mastoi, Bhatti, et al., 2026).

Insecticides and Sublethal Concentrations

Technical-grade insecticides were obtained from commercial suppliers in Shanghai, China: Emamectin benzoate (97% purity), and matrine (97% purity). Stock solutions were prepared by dissolving each insecticide in acetone. For toxicity testing, serial dilutions were created: Emamectin benzoate (40, 60, 120, 130, 200 mg\100 mL), and matrine (90, 100, 110, 120, 180 mg\100 mL). LC_{20} values were determined using the residual film method as described (Mastoi, Bhatti, et al., 2026). Probit analysis was performed to calculate the LC_{20} values used in behavioral assessments.

Host Cues Preparation

Synthetic compounds of *M. alternatus* at concentrations of 500, 100, and 10 mmo\L were prepared following the methodology described in (Mastoi et al., 2025)

Y-Tube Olfactometer

Behavioral assays were conducted using the Y-tube olfactometer, as detailed in Airflow was maintained at 0.5- 0.6 L/min, and each wasp was observed for five minutes (Mastoi et al., 2025).

Experimental Design

Assessments of olfactory response, learning, and memory were performed in accordance with the protocols outlined in (Mastoi et al., 2025). Wasps were exposed to LC_{20} concentrations of each insecticide.

2.1 Statistical Analysis

Statistical analyses were carried out as described in (Mastoi et al., 2025), utilizing chi-square tests, one-way ANOVA, and Duncan's multiple range test, with a significance level set at $P < 0.05$.

Results

Toxicity of Insecticides

Probit analysis revealed the following regression equations and LC_{20} values: for emamectin benzoate, $Y = 1.693X – 1.855$ (LC_{20} = 20.12 mg\L, 95% confidence limit: 2.978-2.524); and for matrine $Y = 2.545X – 1.358$ (LC_{20} = 3.55 mg\L, 95% confidence limit: 44.763-144.081). The correlation coefficients were 0.110 for emamectin benzoate and 0.713 for matrine (Table 1).

Table 1. Toxicology of Insecticides to *S. sichuanensis*

Insecticides	Regression equation of toxicity	Correlation coefficient (r)	LC20 (mg/L) Confidence Limits 95%
Emamectin benzoate	Y=1.693* -1.855	x2=0.810	20 (2.978-2.524)
Matrine	Y=2.545*-1.358	x2=0.713	355 (44.763-144.081)

Note:

- LC_{20} values indicate the sublethal doses needed to kill 20% of the females of the *S. sichuanensis* Population.
- The correlation coefficients evaluated the strength of the relationship between insecticides and toxicity.
- The confidence limit indicates the precision of the LC_{20} estimates.

Olfactory Response to Synthetic Host Cues

At the sublethal concentrations (LC_{20}) of emamectin benzoate and matrine, the selection rates attracted by the smells of 500, 100, and 10 mmol/L (synthetic compounds of *M. alternatus*) were as follows (Table 2 and Fig 1)

Table 2. Selection Rates of *Scleroderma sichuanensis* Under Different Insecticides

Insecticide	500 mmol/L	100 mmol/L	10 mmol/L
Emamectin Benzoate	61%	51%	52%
Matrine	57%	33%	51%
Control	78%	75%	72%

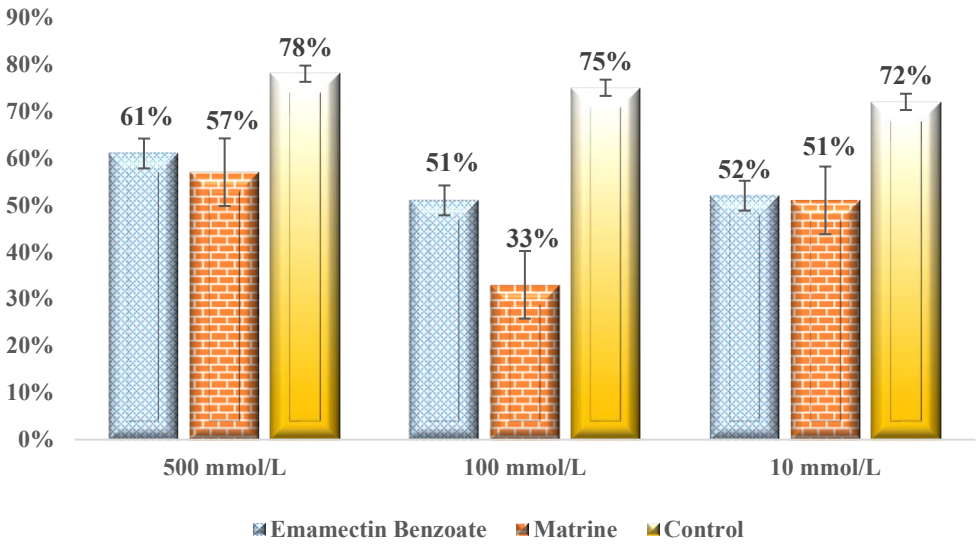


Figure 1. Selection Rates of *Scleroderma sichuanensis* Under Different Insecticides

The response rates (indicating active movement toward either olfactometer arm) for emamectin benzoate-treated wasps were 80%, 72%, and 71% at 500, 100, and 10 mmol/L, respectively. For matrine-treated wasps, the response rates were 87%, 87%, and 67%, respectively. Chi-square analysis revealed significant disruptions for emamectin benzoate at all three concentrations (500 mmol/L: $\chi^2 = 9.773$, P = 0.001; 100 mmol/L: $\chi^2 = 4.444$, P = 0.03; 10 mmol/L: $\chi^2 = 4.444$, P = 0.03). For matrine, significant effects were observed at 500 mmol/L ($\chi^2 = 9.773$, P = 0.001) and 10 mmol/L ($\chi^2 = 4.444$, P = 0.03), while at 100 mmol/L the effect was not significant ($\chi^2 = 0.267$, P = 0.604) when compared to the control group ($\chi^2 = 15.517$, P = 0.000) (Fig 2 and 3).

Table 3. Olfactory Response (Selection / Response Rates)

Host Cue Type	Concentration / Source	Parameter	Control (%)	Emamectin Benzoate (%)	Matrine (%)
Synthetic (*M. alternatus*)	500 mmol/L	Selection / Response	78 / 95	61*/ 80	57* / 87
	100 mmol/L	Selection / Response	75 / 93	51* / 72	33* / 87
	10 mmol/L	Selection / Response	72 / 92	52* / 71	51* / 67

Natural	Larval Hemolymph	Selection / Response	85 / 98	76* / 98	67* / 90
	Pupal Odor	Selection / Response	82 / 97	60* / 80	77* / 90

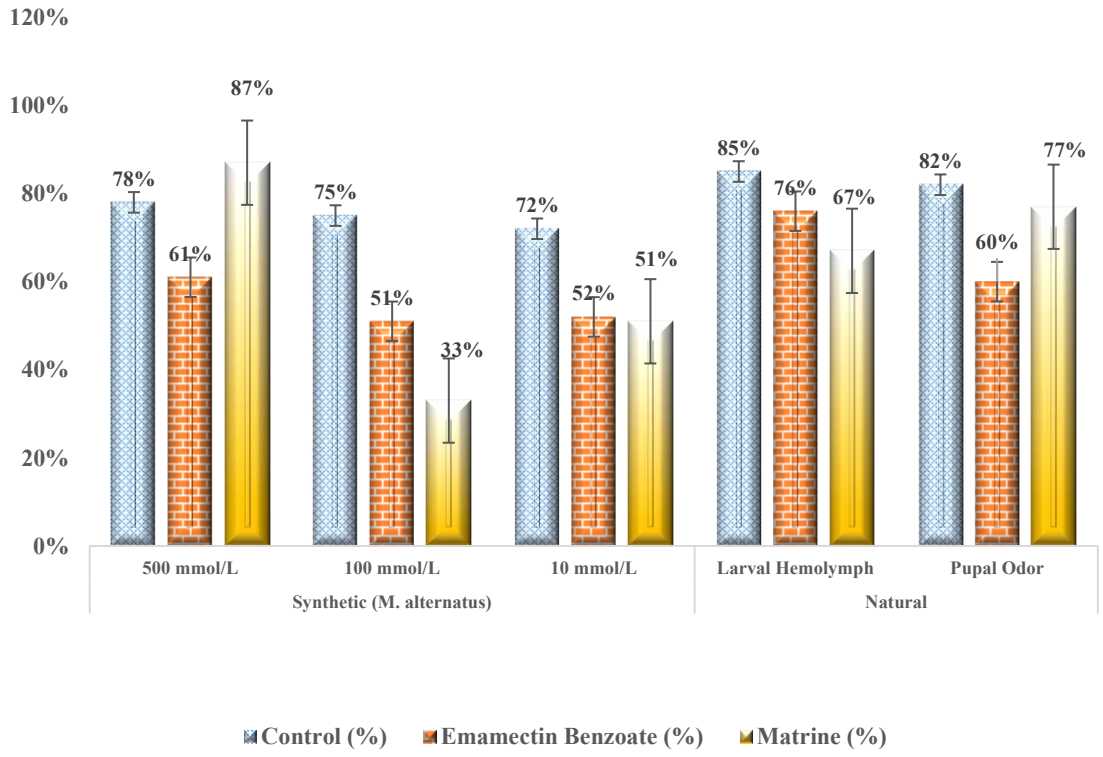


Figure 2. Selection rate (%) of female *S. sichuanensis* exposed to sublethal concentrations (LC_{20}) of emamectin benzoate and matrine in response to synthetic volatiles (500, 100, and 10 mmol/L) and natural cues (larval hemolymph and pupal odor) of host insects. Control (CK) was treated with acetone only. Asterisks indicate significant differences from the control (χ^2 test, $P < 0.05$). Data represent mean values.

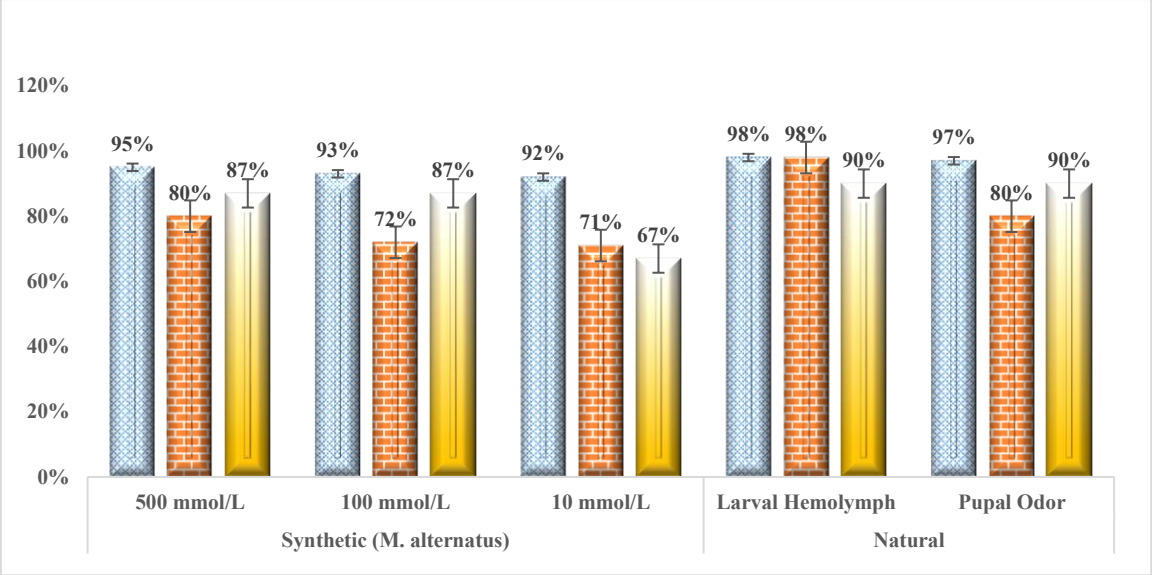


Figure 3. Response rate (%) of female *S. sichuanensis* exposed to sublethal concentrations (LC_{20}) of emamectin benzoate and matrine in response to synthetic volatiles (500, 100, and 10 mmol/L) and natural cues (larval hemolymph and pupal odor) of host insects. Control (CK) was treated with acetone only. Asterisks indicate significant differences from the control (χ^2 test, $P < 0.05$). Data represent mean values.

Olfactory Response to Natural Host Cues

For natural host cues, emamectin benzoate-treated wasps exhibited selection rates of 76% for larval hemolymph and 60% for pupal odor. Matrine-treated wasps showed selection rates of 67% for larval hemolymph and 77% for pupal odor, compared to the control rates of 85% and 82%, respectively. Response rates to natural cues remained relatively high. Emamectin benzoate-treated wasps showed 98% and 80% response rates for hemolymph and pupal odor, respectively, while matrine-treated wasps showed 90% and 93%, respectively (Table 3). Chi-square analysis confirmed significant disruptions for both insecticides against natural host cues. For emamectin benzoate, values were $\chi^2 = 17.066$ (P = 0.000) for hemolymph and $\chi^2 = 17.777$ (P = 0.000) for pupal odor. For matrine, values were $\chi^2 = 15.016$ (P = 0.000) for hemolymph and $\chi^2 = 19.288$ (P = 0.000) for pupal odor, all compared to the control group $\chi^2 = 21.600$, P = 0.000 for both cues (Table 4).

Table 4. Chi-square (χ^2) analysis summary comparing the olfactory selection frequencies of female *Scleroderma sichuanensis* between insecticide-treated groups (emamectin benzoate and matrine) and the control group across different host cues.

Host Cue	Emamectin Benzoate (χ^2, P)	Matrine (χ^2, P)	Control (χ^2, P)

500 mmol/L	$\chi^2 = 9.773$, $P = 0.001$	$\chi^2 = 9.773$, $P = 0.001$	$\chi^2 = 21.600$, $P = 0.000$
100 mmol/L	$\chi^2 = 4.444$, $P = 0.03$	$\chi^2 = 0.267$, $P = 0.604$	$\chi^2 = 15.517$, $P = 0.000$
10 mmol/L	$\chi^2 = 4.444$, $P = 0.03$	$\chi^2 = 4.444$, $P = 0.03$	$\chi^2 = 19.692$, $P = 0.000$
Larval Hemolymph	$\chi^2 = 17.066$, $P = 0.000$	$\chi^2 = 15.016$, $P = 0.000$	$\chi^2 = 21.600$, $P = 0.000$
Pupal Odor	$\chi^2 = 17.777$, $P = 0.000$	$\chi^2 = 19.288$, $P = 0.000$	$\chi^2 = 21.600$, $P = 0.000$

Sublethal exposure to emamectin benzoate and matrine resulted in moderate alterations in the host-seeking behavior of *Scleroderma sichuanensis*. Both insecticides diminished the olfactory response of female wasps to synthetic host cues of *M. alternatus* emamectin benzoate reduced selection rates to 61%, 51%, and 52% at 500, 100, and 10 mmol/L, respectively ($\chi^2 = 9.773$, 4.444, and 4.444; $P < 0.05$), while matrine produced 57%, 33%, and 51% ($\chi^2 = 9.773$ and 4.444 for 500 and 10 mmol/L; $P < 0.05$). Both insecticides also influenced learning and memory capabilities. Nevertheless, the relatively strong response to natural host cues (76% and 60% for emamectin benzoate; 67% and 77% for matrine) indicates that the wasps maintained a significant ability to identify host-related odors following insecticide exposure. These results suggest that while emamectin benzoate and matrine exert less severe effects compared to highly neurotoxic insecticides, their sublethal impacts on host-seeking behavior must still be considered when utilized in integrated pest management strategies involving *S. sichuanensis*. To mitigate potential risks, it is recommended to apply these insecticides during periods of low parasitoid activity, use the lowest effective concentrations, avoid mixing with other neurotoxic compounds, and monitor parasitoid populations regularly to ensure sustained biological control efficacy.

Discussion

The current research demonstrated that sublethal exposure to emamectin benzoate and matrine influenced the host-seeking behavior of *Scleroderma sichuanensis*. Both insecticides diminished female wasps' responses to the synthetic host cues of *Monochamus alternatus*, although the extent of this reduction varied between insecticides and across the concentrations tested. Emamectin benzoate produced selection rates of 61%, 51%, and 52% at concentrations of 500, 100, and 10 mmol/L, respectively, while matrine yielded rates of 57%, 33%, and 51%. These findings suggest that exposure to sublethal concentrations can impair female wasps' ability to respond appropriately to host-related odor cues (Se et al., 2026). Emamectin benzoate is a macrocyclic lactone insecticide that targets GABA-gated chloride channels, potentially

affecting neuronal activity (Moustafa et al., 2016; Pan et al., 2024). Such effects may alter sensory processing and, as a result, modify the behavioral responses of parasitoids to host odors. In this study, the observed reduction in selection rates following exposure to emamectin benzoate indicates that even sublethal concentrations can impact the olfactory response of *S. sichuanensis* (Rondoni et al., 2024). The reduction in response was not uniform across all concentrations, suggesting that the behavioral effect may not simply escalate with decreasing concentration. Rather, the response may be influenced by the interplay between insecticide exposure and the intensity of the odor stimulus (Andreazza et al., 2021). Matrine also influenced the host-seeking behavior of *S. sichuanensis* (Fang et al., 2024; Mastoi, Bhatti, et al., 2026; Mastoi et al., 2025). The selection rates recorded were 57%, 33%, and 51% at concentrations of 500, 100, and 10 mmol/L, respectively (Kang et al., 2025). Matrine is a botanical alkaloid known to operate through various mechanisms, including its impact on sodium channels and acetylcholinesterase activity (Kowah et al., 2022). These mechanisms may lead to alterations in nervous system function, thereby affecting the behavioral responses of parasitoids (Hughes & Libersat, 2018). The distinct responses observed across the three concentrations suggest that matrine's influence on host-seeking behavior may be contingent upon both insecticide exposure and the nature or concentration of the odor stimulus (Bloomquist et al., 2018). The response to natural host cues was less impacted than the reaction to synthetic compounds (Anton & Cortesero, 2022). After exposure to emamectin benzoate, the selection rate was 76% for larval hemolymph and 60% for pupal odor, while wasps treated with matrine exhibited selection rates of 67% and 77%, respectively. The relatively high responses to these natural cues imply that female *S. sichuanensis* may still possess some capacity to detect host-related odors even after being exposed to sublethal concentrations of insecticides. Natural host odors comprise a blend of volatile compounds rather than a singular or limited set of synthetic compounds, which may offer multiple odor signals for host identification (Chen et al., 2026; Qian et al., 2024). This could partially elucidate why the response to natural cues remained comparatively elevated. The learning and memory assessments further indicated that both insecticides affected the behavioral responses of *S. sichuanensis* (Nazari et al., 2025). In the learning assessment, wasps treated with emamectin benzoate exhibited selection rates of 60%, 62%, and 51%, while those treated with matrine displayed rates of 57%, 40%, and 50% at concentrations of 500, 100, and 10 mmol/L, respectively. A decrease in selection during the learning phase implies that exposure to these insecticides may disrupt the wasps' ability to associate host-related odors with the corresponding behavioral responses (Se et al., 2026). A comparable trend was noted in the memory assessment. Wasps treated with emamectin benzoate recorded selection rates of 56%, 37%, and 37%,

whereas matrine-treated wasps showed rates of 60%, 40%, and 43%. These findings suggest that the impacts of sublethal insecticide exposure may go beyond immediate odor detection, potentially affecting the retention or expression of learned responses related to hosts. The distinctions between synthetic and natural host cues hold significance from a biological control standpoint. Although both emamectin benzoate and matrine diminished the responses of *S. sichuanensis*, the wasps maintained the ability to respond to natural host odors at relatively high rates. This indicates that the intricate nature of natural odor blends may convey more information for host recognition compared to individual synthetic compounds (Portilla Pulido et al., 2022). Nevertheless, the observed reductions in learning and memory suggest that repeated or sublethal exposure to these insecticides could still impact the effectiveness of host searching over time (Kaur et al., 2025). From the standpoint of integrated pest management, these results indicate that emamectin benzoate and matrine might be less harmful to *S. sichuanensis* compared to highly neurotoxic insecticides; however, their impacts should not be overlooked (Aboelkassem et al., 2025; Mastoi, Memon, et al., 2026). Even slight alterations in host-seeking behavior, learning, and memory could diminish the effectiveness of parasitoids in finding appropriate hosts (Haverkamp & Smid, 2020). Consequently, when applying these insecticides in regions where *S. sichuanensis* plays a role in the biological control system, it is essential to thoughtfully consider the timing of application and exposure to mitigate potential effects on parasitoid activity.

Conclusion

Sublethal exposure to emamectin benzoate and matrine resulted in moderate alterations in the host-seeking behavior of *Scleroderma sichuanensis*. Both insecticides diminished the olfactory response of female wasps to synthetic host cues and influenced their learning and memory capabilities. Nevertheless, the relatively strong response to natural host cues indicates that the wasps maintained a significant ability to identify host-related odors following insecticide exposure. These results suggest that while emamectin benzoate and matrine may exert less severe effects compared to highly neurotoxic insecticides, their sublethal impacts on host-seeking behavior must still be considered when utilized in integrated pest management strategies involving *S. sichuanensis*. Careful timing and suitable application of these insecticides could mitigate their potential effects on the efficacy of this parasitoid as a biological control agent.

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