

Study on the Concentration-Dependent Toxic Effects of *Ailanthus altissima* and *Dysphania ambrosioides* Extracts on Red Imported Fire Ants

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ABSTRACT

This study utilized the water test-tube feeding method to assess the toxic effects and behavioral impacts of extracts from *Ailanthus altissima* and *Dysphania ambrosioides* at different concentrations (0%, 3%, 6%, 9%) on *Solenopsis invicta* (red imported fire ants). Single-factor analysis of variance (ANOVA) was employed to statistically analyze behavioral indices such as mortality rate, aggregation rate, pole-climbing rate, attachment rate, and walking rate. The results showed that both the toxicity and behavioral inhibitory effects of the two extracts increased with increasing concentrations, exhibiting a significant concentration-dependent pattern. Notably, the lethality rate of the *A. altissima* extract reached 96.67% at 9% concentration, while that of *D. ambrosioides* was 83.33%. These findings support the potential feasibility of using these extracts as biological control agents against *S. invicta* and provide experimental evidence for the development of plant-derived insecticides.

KEYWORDS

Red Imported Fire Ant; *Ailanthus altissima*; *Dysphania ambrosioides*; Plant extracts; Biological control

1. INTRODUCTION

The red imported fire ant, *Solenopsis invicta*, is an invasive and highly aggressive pest native to South America. It has spread to many countries and regions, posing a significant threat to ecosystems and agriculture worldwide. This species features a complex social structure, strong aggression, and remarkable adaptability. It can sting humans, causing severe pain and allergic reactions, and in extreme cases, it can be life-threatening. Additionally, it can damage local ecosystems, harming crops and infrastructure. Due to its large population and rapid spread, the red imported fire ant has been listed as one of the 100 most threatening invasive species globally.

Currently, the control of red imported fire ants primarily relies on chemical methods, such as baiting, nest flooding, and powder treatment. Although these methods are effective in the short term, long-term use can lead to increased resistance, harm non-target organisms, and cause environmental pollution. In contrast, biological control, an eco-friendly and sustainable approach, is gaining increasing attention. Natural products, particularly plant extracts, have become a focal point in current pest management research due to their low toxicity and minimal environmental impact.

Research has shown that various medicinal plant extracts exhibit significant insecticidal activity and behavioral interference effects on insects (Eva et al., 2017; Singhe et al., 2018). However, studies on the use of extracts from the Chinese flame tree (*Ailanthus altissima*) and the Chinese thorn

(*Dysphania ambrosioides*) in red imported fire ant control are still limited. Existing literature suggests that these plants are rich in active components such as alkaloid volatile oils, which have potential toxic and repellent effects. However, systematic experimental verification of their specific impacts on the behavior and survival of red imported fire ants is lacking. Therefore, this study aims to systematically evaluate the mortality rate and key behaviors, including aggregation, grasping, and crawling, of red imported fire ants at different concentrations of extracts from the Chinese flame tree and the Chinese thorn using the water test tube feeding method. The goal is to clarify the concentration-dependent patterns, provide theoretical support and data for the development of plant-based red imported fire ant control agents, and offer new insights for optimizing biological control strategies.

2. MATERIALS AND METHODS

The test pest red imported fire ant *Solenopsisinvicta* worker ants were collected from the wild community in Pu'er City, Yunnan Province. Medium-sized individuals with consistent physiological state were selected as experimental subjects and plant materials.

The root powder of *Ailanthusaltissima* was purchased from the local Chinese medicine market and ground into fine powder. The young stem and leaves of *Dysphaniaambrosioides* were freshly purchased from the local market, washed and dried at low temperature and crushed for future use.

The bait material, ham sausage king brand, was cut into small pieces of 1cm x 1cm and used as a poisoned bait.

2.1. Reagents and Equipment

Table1. Experimental reagents and materials

Reagent	Purity/Specification	Manufacturer
Wahaha Pure Water (Deionized Water)	1.5L	Hangzhou Wahaha Group Co., Ltd.
75% ethanol	500ml	Kunming Nantian Pharmaceutical Co., Ltd.
ethyl acetate	AR	Dangfang State-Owned Houqi Chemical Factory
petroleum ether	AR	Tianjin Damo Chemical Reagents Factory
Talcum powder	500g	Tianjin Huasheng Chemical Reagents Co., Ltd.

Table 2. Experimental equipment

Instrument	Model	Manufacturer
water bath	ZSXH-612	Shanghai Zhicheng Analytical Instrument Manufacturing Co., Ltd.
oven	DHG-9245A	Jiangsu Tongjun Instrument Technology Co., Ltd.
Vacuum filtration apparatus	SHZ-D(III)	Gongyi Yu Hua Instruments Co., Ltd.
pipette	200-1000ul	Dalong Xingchuang Experimental Instruments Co., Ltd.

2.2. Preparation Method of Plant Extract

Place 80g of stink tree root powder and 72g of dried *Schizonepeta tenuifolia* powder in a conical flask. Add deionized water, anhydrous ethanol, ethyl acetate, and petroleum ether in that order, with the total volume being 10 times the mass of the plant powder. Heat the mixture in a 50°C water bath for 2 hours. Filter the extract using a Buchner funnel and repeat this process three times. Combine the

filtrates, concentrate them in a water bath until dry, weigh the final product to calculate the extraction yield.

The extraction rate is the dry weight of the extract g and the dry weight of the plant powder g $\times 100$

The concentrated extract was sealed and stored in refrigerator 4°C for later use.

2.3. Configuration of Different Concentrations of Treatment Solutions

The two plant extracts were diluted with deionized water to three concentration gradients of 369. The clear water was used as the control group. All solutions were prepared and used in situ to ensure the stability and activity of the treatment solution.

2.4. Treatment and Experimental Methods of Red Imported Fire Ants

In the experiment, worker ants of the red imported fire ant were placed in 500mL plastic cups, with 30 ants per cup. Three concentrations of each plant extract were tested, and three control groups were set up using clear water. The inner walls of the plastic cups were evenly coated with talcum powder to prevent the ants from escaping. After a 3-hour starvation treatment, a 1cm x 1cm sausage was added to each cup as bait, and a wet cotton ball containing 400 mL of the treatment solution was inserted into the centrifuge tube. The treatment continued for 3 days.

Mortality: On the 3rd day, if no limbs or antennae are observed, the red imported fire ant is considered dead. Aggregation Rate: After 5 seconds of observation, check if at least three ants naturally cluster together. Climbing Ability: Observe if the red imported fire ant can climb over a 3cm bamboo stick and remain there for 5 seconds. Gripping Ability: After quickly flipping the cup, check if it remains attached to the inner wall or bottom. Locomotion: Check if the red imported fire ant can continuously crawl freely on the coordinate paper for more than 5 seconds.

2.5. Methods of Data Statistics and Analysis

The experimental data were statistically analyzed using SPSS250 software. The mortality rate and behavioral indicators were evaluated using one-way ANOVA to assess the significant differences among different concentrations. The significance level was set at $p < 0.05$. For comparing the differences between the two plant extracts, an independent samples t-test was used. The charts were created using Origin2023 software, presenting the mean and standard deviation (MeanSD).

3. DATA AND ANALYSIS

3.1. Extracting Effects of Two Kinds of Plants

$$\begin{aligned} \text{Extraction rate of Ailanthus altissima} &= \frac{\text{Dryweightoftheextract (g)}}{\text{Plantdrypowderweight (g)}} = \frac{15.606}{80} \times 100\% \\ &= 19.508\% \end{aligned}$$

$$\text{Extraction rate of soil catnip} = \frac{\text{Dry weight of the extract (g)}}{\text{Plant dry powder weight (g)}} = \frac{15.9757}{72} \times 100\% = 22.188\%$$

3.2. The Toxic Effect of Toon Extract on Red Fire Ants

The trend of mortality, climbing rate, attachment rate and walking rate of red fire ants after the application of distilled water and different concentrations of toon extract was shown in Figure 1.

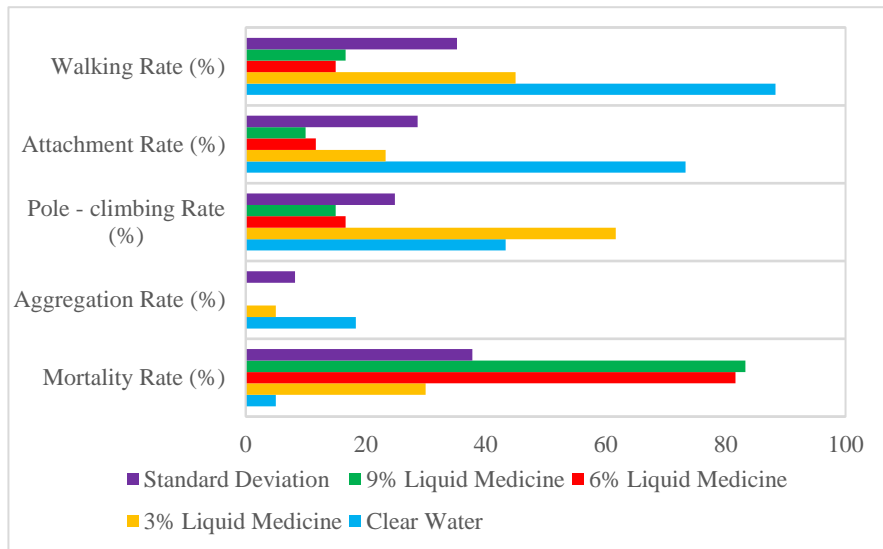


Figure 1. The toxic effect of *Ailanthus altissima* extract on red imported fire ants

As the concentration of the medicinal solution increased from 0 to 36 and then to 9 in the clear water group, the mortality rate showed a significant upward trend. However, key indicators of biological behavior, such as aggregation rate, climbing rate, attachment rate, and walking rate, all declined significantly. SPSS analysis revealed that the mortality rate in this experimental group was $F_{108.449}$, with a P value of $0.000 < 0.05$, indicating a highly significant difference between the groups with different concentrations of the medicinal solution. This result clearly demonstrates that as the concentration of the medicinal solution increases, its lethal effect on organisms and its inhibitory effect on behavior become more pronounced. In the control group, the red fire ant colony exhibited extremely active behavior, with worker ants in good condition and strong movement capabilities, performing well in all tests. However, in the experimental group, after the administration of the medicinal solution, the red fire ants' behavior became noticeably sluggish, and their activity was significantly reduced compared to the control group. This further demonstrates that the extract from the Chinese toon has a significant effect on controlling red fire ants, especially under high concentration treatment conditions, where the extract can effectively inhibit the expansion of red fire ants and may even lead to the collapse of the entire ant colony. This provides new ideas and methods for the control of red fire ants, with significant practical and application value.

3.3. The Toxic Effect of 23 Extracts of Thymol on Red Fire Ants

The trend of mortality, climbing rate, attachment rate and walking rate of red fire ants after the application of distilled water and different concentrations of extract of *Cimicifuga* was shown in Figure 2.

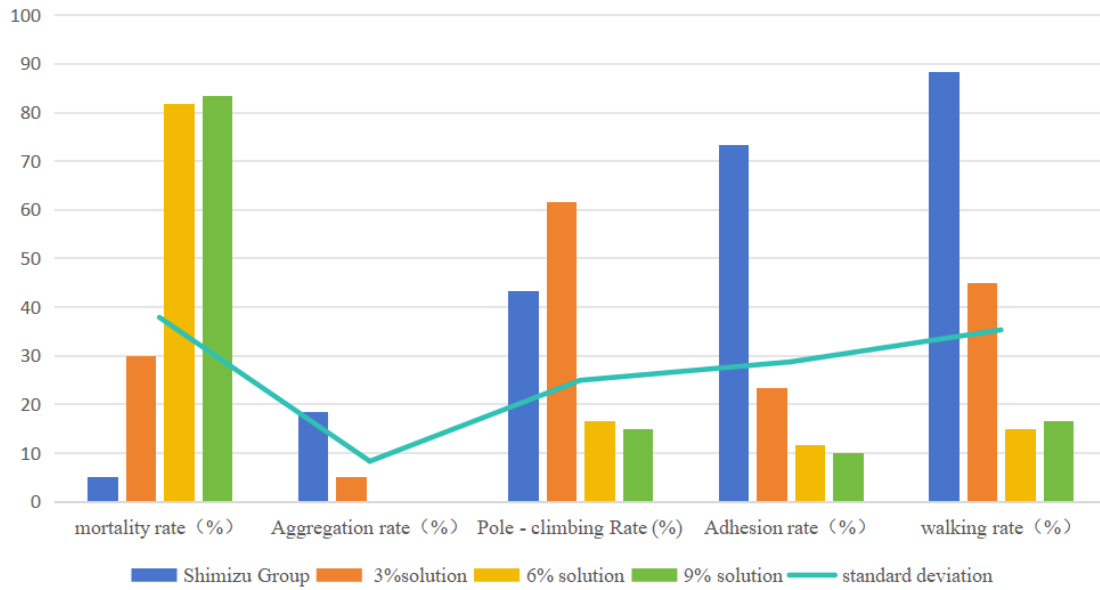


Figure 2. The toxic effect of *Satureja chinensis* extract on red imported fire ants

Comprehensive analysis of all test indicators, including mortality, aggregation rate, climbing rate, attachment rate, and walking rate, revealed a significant concentration-dependent relationship. The effects of the aggregation rate, attachment rate, and walking rate were particularly pronounced. The F value for the mortality rate in this group of experiments was 17.429, with a significance level of $0.001 < 0.05$. This indicates that different concentrations of the drug solution significantly affect mortality. It suggests that the extract from *Acorus tatarinowii* not only effectively kills red imported fire ants but also disrupts their group behavior, aggregation ability, and movement functions, including climbing, attachment, and walking, thereby impairing their survival and dispersal capabilities. This finding supports the potential use of *Acorus tatarinowii* extract as a biological control agent for red imported fire ants. For the results of the two groups at the highest concentration of the drug solution (concentration 9), see Figure 3. For the comparison of the mortality rates between the two groups at concentration 9 and the clear water control group, see Figure 4.

One-Sample Statistics				
	N	Mean	Std. Deviation	Std. Error Mean
mortalityrate	3	61.6667	49.52598	28.59384
aggregationrate	3	6.1100	10.58283	6.11000
poleclimbingrate	3	20.0000	21.27531	12.28331
attachmentrate	3	28.3333	39.19021	22.62648
walkingrate	3	35.5567	46.31434	26.73960

Figure 3. Results of single-sample T-test

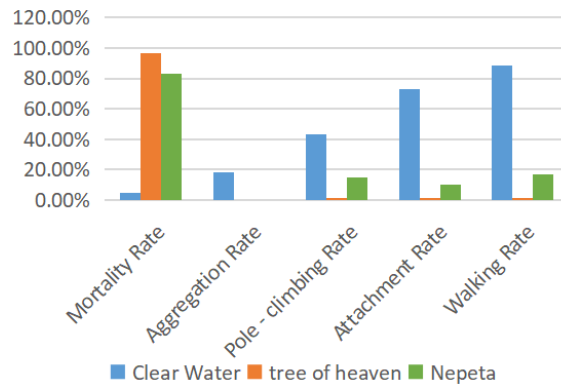


Figure 4. Comparison chart of mortality rates between two groups of 9% solution and tap water

The average mortality rate of the two experimental groups was lower than that of the control group, indicating that both plant extracts had a lethal effect on the red imported fire ants. The experimental groups also showed lower average values for aggregation rate, pole-climbing rate, attachment rate, and walking rate compared to the control group, further suggesting that the medicinal solution weakened the movement ability of the red imported fire ants to some extent. The larger standard deviation in mortality and walking rates indicates a higher dispersion of these data, suggesting more complex influencing factors. The smallest standard deviation in aggregation rate suggests relatively concentrated data, indicating simpler influencing factors. The largest standard error in mortality rate suggests poor representativeness of the sample mean, while the smallest aggregation rate suggests that the sample mean is more representative of the overall mean.

4. RESULTS AND DISCUSSION

4.1. Summary of Experimental Results

This study systematically evaluated the toxic and behavioral effects of extracts from *Toona sinensis* and *Schizonepeta tenuifolia* on red imported fire ants at various concentrations. The results showed that as the concentration increased, both extracts significantly inhibited the mortality and behavioral capabilities of the red imported fire ants, showing a clear trend of concentration dependence. Notably, the extract from *Toona sinensis* was most effective at a concentration of 9, with a mortality rate of 96.67% and almost zero aggregation, attachment, walking, and climbing rates, indicating complete loss of movement and coordination. At the same concentration, the extract from *Schizonepeta tenuifolia* had a mortality rate of 83.33%, with a significant decline in behavioral capabilities, though its overall efficacy was slightly lower than that of *Toona sinensis*. Additionally, in the medium and low concentration treatment groups, the mortality rate and behavioral changes of the red imported fire ants showed a clear stratification, confirming that these two extracts exhibit certain activity at low doses, suggesting the possibility of dose economy.

4.2. Comparison With Existing Studies

Plant extracts have been extensively studied as natural biological control methods in pest management. For instance, Li Yuling et al. (2022) noted that polyoxin and other natural products exhibit significant toxicity to red imported fire ants but are limited by high dosages and environmental instability. In contrast, the extract from *Toxicodendrum* in this study achieves near-complete lethality at a concentration of 9, outperforming most natural monomer compounds. Furthermore, Chen Jiang Lianxi et al. (2024) reported that imidacloprid, when diluted 2000 times, has a mortality rate of 825% for red imported fire ants. However, the extract from *Toxicodendrum* in this study reaches a 70% mortality rate at a concentration of 6, demonstrating competitive toxicity levels and showing potential for development as a plant-based pesticide.

4.3. Possible Mechanisms Explored

The Chinese toon contains a rich variety of volatile compounds, including quercetin and tetracyclic acids. These components have been shown to exhibit strong neurotoxic activity and feeding inhibition. They may achieve rapid poisoning and behavioral suppression by blocking the release of neurotransmitters in red fire ants or interfering with the central nervous system that regulates behavior. The extract from *Artemisia gmelinii* is rich in monoterpenes and phenolic active substances, which may primarily influence the foraging and aggregation behaviors of red fire ants through odor repulsion or mild neuroinhibition mechanisms. During experiments, it was observed that some red fire ants showed an increased rate of climbing poles in a short time under low concentrations of *Artemisia gmelinii*, possibly due to a low-dose excitation effect (hormesis), where low doses can temporarily activate the insect's motor system. This physiological basis should be further investigated in subsequent studies.

4.4. Research Value and Application Prospects

This study is the first to systematically compare the behavioral impacts of extracts from *Loropetalum chinense* and *Semenitum thunbergii* on red imported fire ants, establishing a comprehensive system for evaluating behavioral indicators. This lays the groundwork for the development of plant-based insecticides with multi-target behavioral interference capabilities. In particular, in the control of social pests like red imported fire ants, inhibiting their aggregation behavior and other group behaviors through information scent disruption is more ecologically significant than simply causing death. This approach has the potential to become a key component of new behavioral-driven ecological control strategies. Additionally, both *Loropetalum chinense* and *Semenitum thunbergii* are widely distributed native plant resources that are easily accessible and cost-effective, making them well-suited for promotion and ecological adaptation. They are especially suitable for green pest control in urban green spaces, parks, and at the boundaries of farmland.

4.5. Limitations and Future Prospects

Although this study has demonstrated the high toxicity and significant behavioral interference of the extracts from *Larix gmelinii* and *Semenitum acuminatum* against red imported fire ants under laboratory conditions, the research is still in its early stages with several areas requiring further refinement. Firstly, the active components have not been isolated, and the extract is a complex mixture, making it unclear which specific active substances are responsible for the observed effects. Future studies should focus on systematic chemical component separation and qualitative and quantitative analysis to identify the primary active substances and their content structure. Secondly, the mechanisms of action remain unclear, and there is a lack of in-depth research into the neurophysiological, biochemical metabolism, and pheromone regulation pathways of red imported fire ants, hindering a comprehensive understanding of their toxicological basis. Additionally, this study was conducted only under controlled indoor conditions and has not yet been tested in the field. Further exploration is needed to assess the stability and persistence of these extracts in natural environments to evaluate their potential for practical control. Lastly, the long-term ecological safety of the extracts on non-target organisms has not been evaluated. Future research should focus on analyzing the active components, exploring their mechanisms of action, conducting field efficacy tests, and assessing ecological safety to promote the transformation and application of *Larix gmelinii* and *Semenitum acuminatum* extracts into green, efficient, and sustainable plant-based control agents.

5. CONCLUSION

This study evaluated the toxic and behavioral interference effects of *Ailanthus altissima* (Chinese flame tree) and *Dysphania ambrosioides* (Hairy plantain) extracts on *Solenopsis invicta* (red imported

fire ant) by setting up different concentration gradients. The results showed that both plant extracts exhibit strong biological activity, with their effects significantly increasing with concentration, showing a clear concentration-dependent relationship. Specifically, the extract from *Ailanthus altissima* has a mortality rate of 96.67% at a concentration of 9, far exceeding the 83.33% achieved by *Dysphania ambrosioides*. Additionally, it shows superior performance in inhibiting the aggregation, attachment, climbing, and walking behaviors of red imported fire ants. Both extracts effectively interfere with the movement and social behavior of red imported fire ants, demonstrating significant potential as plant-based biological control agents. Compared to traditional chemical control methods, plant extracts offer advantages such as being environmentally friendly, easily accessible, and having broad-spectrum effects, making them particularly suitable for green control in ecologically sensitive areas like urban green spaces and farmland boundaries. This study not only provides new natural agent options for red imported fire ant control but also lays the experimental foundation and theoretical basis for the development of subsequent plant-based pesticides. Future research should focus on further analyzing active ingredients, exploring mechanisms of action, and conducting field studies to promote the application of these two plant extracts in the green control of red imported fire ants.

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