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Insecticidal Activity of the Leaf and Stem Water Extract of *Gelsemium elegans* against *Solenopsis invicta*

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Abstract
A comprehensive green worker ants control method that can be used to replace traditional chemical synthetic insecticides. In this study, the leaves and stems of *Gelsemium elegans* were extracted with water as the solvent, and the bioactivity of *G. elegans* against worker ants was determined by the "water tube" method. The bioassay results of insecticidal activity showed that when the time was extended to the 10th day, the mortality of worker ants treated with *G. elegans* extract reached 55.00% (1/20 leaf extract), 46.67% (1/20 stem extract) and 45.00% (1 mg/kg koumine). And the behavioral impact test results showed that the aggregation rate was reduced to 56.67% (1/100 leaf extract), 60.00% (1/100 stem extract) and 60.00% (0.5 mg/kg koumine); the climbing rate was reduced to 60.00% (1/100 leaf extract), 58.33% (1/100 stem extract) and 58.33% (0.5 mg/kg koumine). The effect on the walking ability of worker ants is obvious. The walking rate drops to 1.53 cm/s (1/100 leaf extract), 1.60 cm/s (1/100 stem extract) and 1.47 cm/s (0.5 mg/kg koumine). The leaves, stem extracts and koumine components contained in *G. elegans* can affect their behavior and show insecticidal activity. *G. elegans* might be a good natural plant resource to control red imported fire ant.

Introduction

The red imported fire ant (*Solenopsis invicta*), native from the Parana River basin of South America, is a voracious consumer of numerous other arthropod species (Nattrass et al., 2007). It also is an important agricultural and urban pest throughout most of the southeastern United States as well as in parts of several western states (Wetterer, 2011). This invasive species has overtaken and eliminated many native ant species in most areas (LeBrun et al., 2013). They first invaded mainland China in 2005 (Zhang et al., 2013) and spread widely in southern China, causing serious damage to humans, animals and the ecological environment (Kafle et al., 2013; Ascunce et al., 2011). Red imported fire ants are extremely toxic, and when the human body is smashed by red imported fire ants, it has pain like a fire, and then there will be blisters like burns. Most people only feel pain and discomfort. However, a few people are allergic to toxic proteins in venom, which can cause anaphylactic shock and risk of death.

Traditional methods for managing the red imported fire ant are used insecticides or baits, but this might lead to groundwater contamination, Non-target organism and other environmental considerations (Vogt et al., 2002). Now, with the improvement of people's quality of life, more consumers are inclined to solve pest problems safely and economically (Huang et al., 2015). Therefore, the search for safe, economical and effective prevention methods is the current priority. The use of chemical components from natural products in insect pest control is generally considered to be a safer alternative to the use of synthetic contact insecticides (Chen, 2009). Since naturally occurring chemicals have been found to have insecticidal activity, they have been widely used for pest control (Appel et al., 2004). For example, the widely used plant essential oils have proven to have significant repellent

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and toxic effects on ants，including camphor, turpentine, wintergreen, and such as d-limonene in citrus showed effective repellent or fumigation toxicity against the red imported fire ant (Tang et al., 2013).

Gelsemium, a small genus of the Loganiaceae family, includes three well-known species: *Gelsemium elegans* Benth. (*G. elegans*), native to China and Southeast Asia, *Gelsemium sempervirens* Ait. (*G. sempervirens*), and *Gelsemium rankinii* Small (*G. rankinii*), native to North America (Liu et al., 2013). They have long been used by traditional folk medicine as an effective component in relieving pain and reducing anxiety (Xu et al., 2012). Despite its toxicity, the folks use it to resist tumors according to the theory of "combat poison with poison", and there is also the effect of sedative pain, immune regulation, and rejuvenation of livestock. The primary active molecules of *G. elegans* are the alkaloids gelsemine, koumine, gelsenicine, and gelsevirine (Liu et al., 2013).

The purpose of this study was to determine the toxicity and behavioral inhibition of *G. elegans* to the red imported fire ant. In view of the extremely toxic nature of the whole plant, the leaves and stems of *G. elegans* were selected as natural sources to study the toxicity and behavioral inhibition of alkaloids on red imported fire ants. We hope to obtain a simple, economical and ecologically safe natural product to control red fire ants.

Materials and methods

Plant materials

The *G. elegans* were collected from the Insecticidal Botanical Garden at the South China Agricultural University. The length of the stem was approximately 3-12 m. Two parts of the *G. elegans* were used in the test: leaves and stem. The fresh and healthy vines were sent to the laboratory once collected. The dirt surface of the leaves was removed and the stems and leaves were separated carefully. The dried leaves and stems were gained by putting the fresh leaves and stems in a baking box at 40 °C for 5 h.

Chemicals

Koumine was purchased from Jieshikang Biotechnology Co., Ltd., Qingdao, China. HPLC-grade methanol was purchased from Yiyang Trading Co., Ltd., Guangzhou, China.

Insects

S. invicta colonies were collected from a suburb in Guangzhou. Worker ants and nest material were reared in the laboratory for bioassays in plastic containers (40 cm × 52 cm × 12 cm) coated with Teflon emulsion on the top, in which a test tube (25 mm × 200 mm) filled with water and plugged with cotton was set up in order to provide water, and the ham sausage was used as a food source. The ants were kept in a comfortable environment at 25 ± 2 °C and 70 ± 10% RH until the test was completed. The major workers applied in the experiment were 4-5 mm in length (Souto et al., 2012; Huang et al., 2016; Hu et al., 2017).

Preparation of plant extract and koumine

The dried leaves and stems were broken up with a high-speed pulverizer and then collected by filtration using a standard test sieve (mesh 60, pore diameter 0.25 mm). The powdery dried plant material was submitted the extraction of compounds with pure water at room temperature, and the aqueous solution was pumped and filtered under vacuum. After filtration, we obtained an extraction solution of 1 g leaves dissolved in 20 ml (100 ml) water, which was expressed by 1/20 (1/100). Then diluted with 1/100 of the leaf extract to get the concentration we need, such as 1/500, 1/5000. The same procedure as above was applied in order to obtain the same concentration of stem water extract. All extracts were stored at 4 °C.

We used methanol as a solvent to dissolve the koumine to obtain a standard solution, diluted with water until the methanol content is less than 0.5%. Further diluting was done to obtain 0.02, 0.05, 0.1, 0.5, 1 mg/kg of the standard aqueous solution of koumine.

Laboratory toxicity bioassays

A toxic biological test was performed according to the method described by the predecessors (Huang et al., 2015). Worker ants were placed in a 250 mL beaker [71 mm (diameter) × 97 mm] in which the vertical wall was coated with Fluon emulsion. The ants were deprived of water and food once placed in the beakers. After 6 hours of starvation, a test tube filled with extracts or standards and plugged with cotton was placed at the bottom of the beaker. All treatments were replicated thrice and each replicate included 20 worker ants. The worker ants were maintained at 25 ± 2 °C and relative humidity of 70 ± 10%. The same operation was used in the control group except that the extract or standard was changed to water and 0.5% methanol aqueous solution. The number of dead ants was recorded after 0.083, 1, 3, 5, 7 and 10 days after they were treated. The following formula was adopted to assess the mortality：

Mortality = (number of dead ants/number of total ants) × 100%

Behavior observation on aggregation ability of workers

We reduced the concentration of the treatment to test the effect of the extract on the behavior of worker ants, thus eliminated the effect of the death of the worker on the evaluation results. The treatment of the worker ants was the same as the above steps. For the behavioral impact test of worker ants, high, medium and low concentrations were selected, 1/100, 1/500, 1/5000. From these three concentrations, the range of efficacy of the extract of *G. elegans* can be roughly estimated. When more than three worker ants gathered together, and the distance between each worker ant was less than 0.5 cm, we recorded this event as aggregation. The number of ants gathered was recorded after 0.083, 1, 3, 5, 7 and 10 days after they were treated. The following formula was adopted to assess the aggregation：

Aggregation level = (number of ants gathered/number of total ants) × 100%

Behavior observation on climbing ability of workers

The concentration was similarly reduced to eliminate the effect of worker ants death on behavioral assessment, and the method of operation was the same as previously described. The worker ants were provoked by a bamboo stick (25 cm length). If the worker ants were able to climb more than 3 cm along the stick, we recorded the event as ants having climbing ability. The number of ants that could climb was recorded after 0.083, 1, 3, 5, 7 and 10 days after they were treated. The following formula was adopted to assess the climbing ability:

Climbing rate = (number of ants that can climb/number of total ants) × 100%

Behavior observation on walking ability of workers

Worker ants were treated with the same concentration and method as the above behavioral observation test, and the effects of the agents on their walking ability were observed at 0.083, 1, 3, 5, 7 and 10 days after they were treated. The worker ants were placed on the A4 cardboard marked with squares (square: 1 cm × 1 cm) and we recorded the length of the journey of the worker ants within 3 s on camera. The following formula was adopted to assess the walking speed:

Walking speed = (the length of walking distance of worker ants/three seconds)

Statistical analysis

Data were expressed as mean ± SE after statistical analysis, and data charts were generated using GraphPad Prism 8 (GraphPad Software, USA). Statistical analyses were carried out using SPSS 17.0 (SPSS Inc., Chicago). Using the Duncan-test to assess differences between the data, $p < 0.05$ was considered to be statistically significant.

Results

Insecticidal toxicity

The insecticidal toxicity test showed that the leaves and stems of *G. elegans* had effective insecticidal activity against the worker ants. In the test, it can be found that the mortality of the treated worker ants gradually increased with time (Fig 1). The mortality of worker ants in both control groups were less than 20%, which excluded the effect of worker ants physiological activity and methanol-water on the test results. For 1 d~10 d after treated, the mortality of worker ants increased from 6.67% to 55.00% (1/20 leaf extract), from 1.67% to 41.67% (1/100 leaf extract), from 0.00% to 13.33% (1/5000 leaf extract); from 1.67% to 46.67% (1/20 stem extract), from 0.00% to 30.00% (1/100 stem extract), from 0.00% to 10.00% (1/5000 stem extract); from 3.33% to 45.00% (1 mg/kg koumine), from 3.33% to 23.33% (0.1 mg/kg koumine), from 0.00% to 11.67% (0.05 mg/kg koumine). However, on day 10, red fire ants treated with 1/100 leaf extract had a mortality of 40%, which was comparable to the mortality of worker ants treated with 1/20 stem extract or 1 mg/kg koumine. Meanwhile, this result indicates that the leaf is slightly more toxic to the worker ants than the stem. On the 10th day of the experiment, there was a significant difference between the mortality of worker ants treated at different concentrations (leaf: $F=44.46$ $P<0.001$; stem: $F=28.00$ $P<0.001$; standard: $F=28.62$ $P<0.001$).

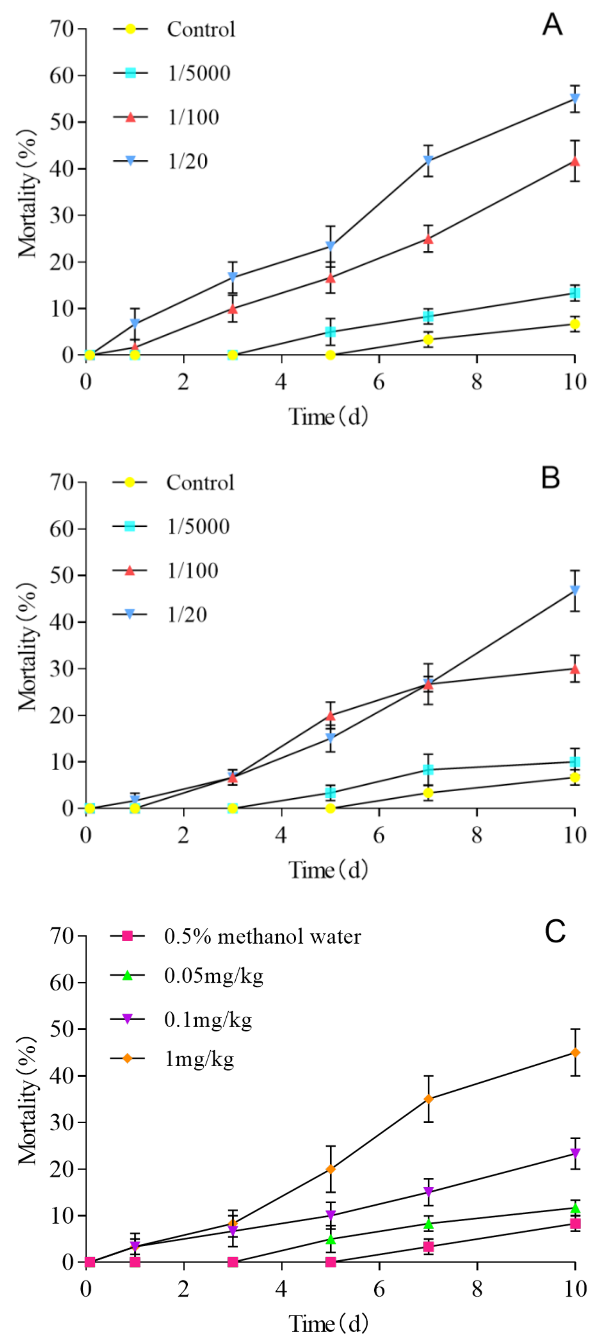

Fig 1. Mortality of worker ants after fed with leaves extract (A), stems extract (B) and koumine (C). 1/20 means the extract that 1 g of plant material dissolved in 20 ml of water, and 1/100, 1/5000 were diluted by 1/20. Each data point represents mean ± SE of three replicate beakers each containing 20 ants.

Impact on aggregating behavior

The aggregation level of worker ants all decreased over time after treatment by extracts or standard (Fig 2). For 1d~10d after treated, the aggregation level of worker ants decreased from 95.00% to 56.67% (1/100 leaf extract), from 100.00% to 66.67% (1/500 leaf extract), from 100.00% to 83.33% (1/5000 leaf extract); from 95.00% to 60.00% (1/100 stem extract), from 98.33% to 68.33% (1/500 stem extract), from 100.00% to 81.67% (1/5000 stem extract); from 95.00% to 58.33% (0.5 mg/kg koumine), from 98.33% to 75.00% (0.05 mg/kg koumine), from 100.00% to 83.33% (0.02 mg/kg koumine). From this result, it can be seen that the concentration of 1/5000 and 0.01 mg/kg has no effect on worker ants, and even 0.02 mg/kg is not effective. However, the effects of two concentrations of 1/100 and 1/500 on the aggregation behavior of worker ants were significantly higher than those in the control group. On the 10th day of the experiment, there was a significant difference between the aggregation level of worker ants treated at different concentrations (leaf: F=13.07 P=0.007; stem: F=11.73 P=0.008; standard: F=21.88 P=0.002).

Impact on climbing behavior

After treatment, when the worker ants were picked up with a long bamboo stick, the climbing ability of the worker ants was significantly reduced (Fig 3). As the treatment

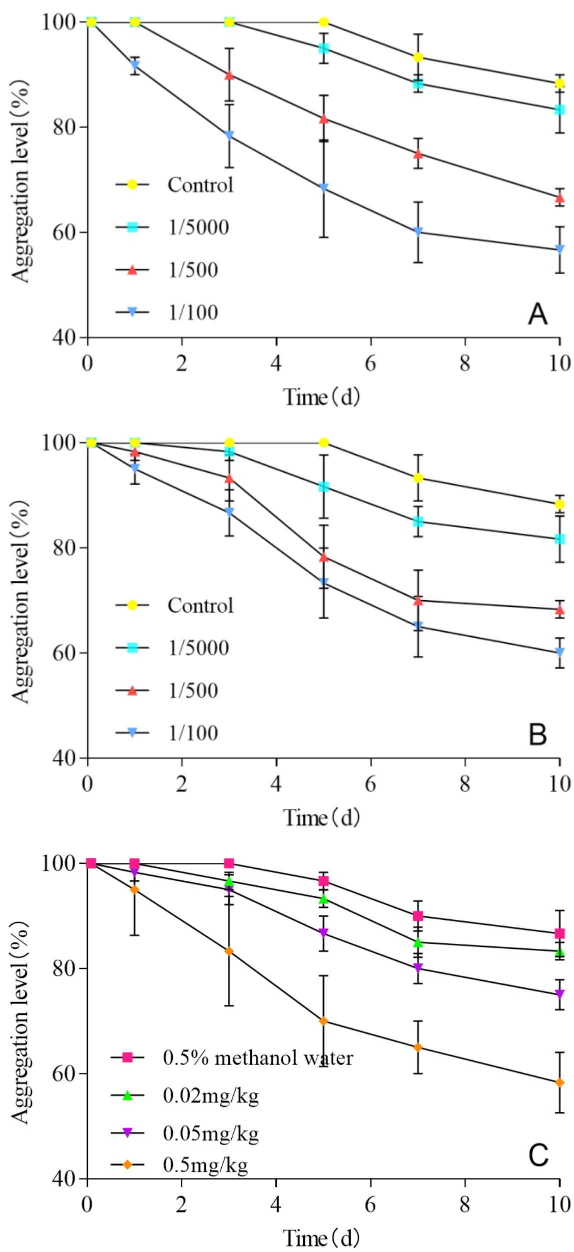

Fig 2. Effects on aggregation level of worker ants after fed with leaves extract (A), stems extract (B) and koumine (C). 1/100 means the extract that 1g of plant material dissolved in 100 ml of water, and 1/500, 1/5000 were diluted by 1/100. Each data point represents mean ± SE of three replicate beakers each containing 20 ants.

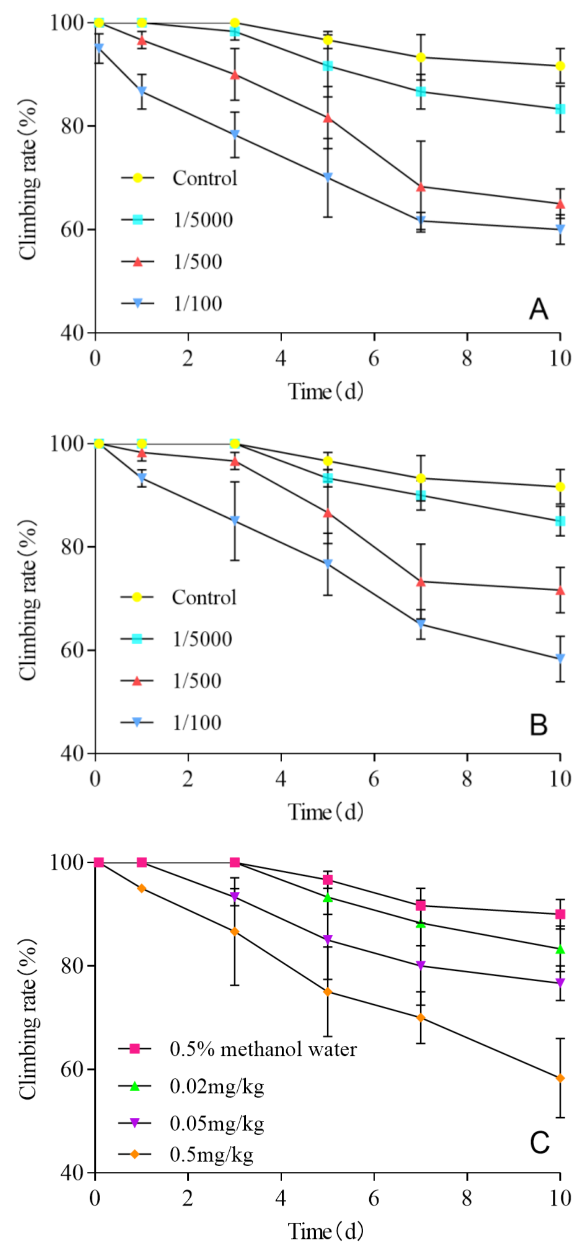

Fig 3. Effects on climbing rate of worker ants after fed with leaves extract (A), stems extract (B) and koumine (C). 1/100 means the extract that 1 g of plant material dissolved in 100 ml of water, and 1/500, 1/5000 were diluted by 1/100. Each data point represents mean ± SE of three replicate beakers each containing 20 ants.

concentration increased, the inhibitory effect on the ability of worker ants to climb was also enhanced. The climbing rate of worker ants decreased from 86.67% to 60.00% (1/100 leaf extract), from 96.67% to 65.00% (1/500 leaf extract), from 100.00% to 83.33% (1/5000 leaf extract); from 93.33% to 58.33% (1/100 stem extract), from 98.33% to 71.67% (1/500 stem extract), from 100.00% to 85.00% (1/5000 stem extract); from 95.00% to 58.33% (0.5 mg/kg koumine), from 100.00% to 76.67% (0.05 mg/kg koumine), from 100.00% to 83.33% (0.02 mg/kg koumine). On the 10^{th} day of the experiment, there was a significant difference between the climbing ability of worker ants treated at different concentrations (leaf: F=12.54 P=0.007; stem: F=11.29 P=0.009: standard: F=10.06 P=0.012).

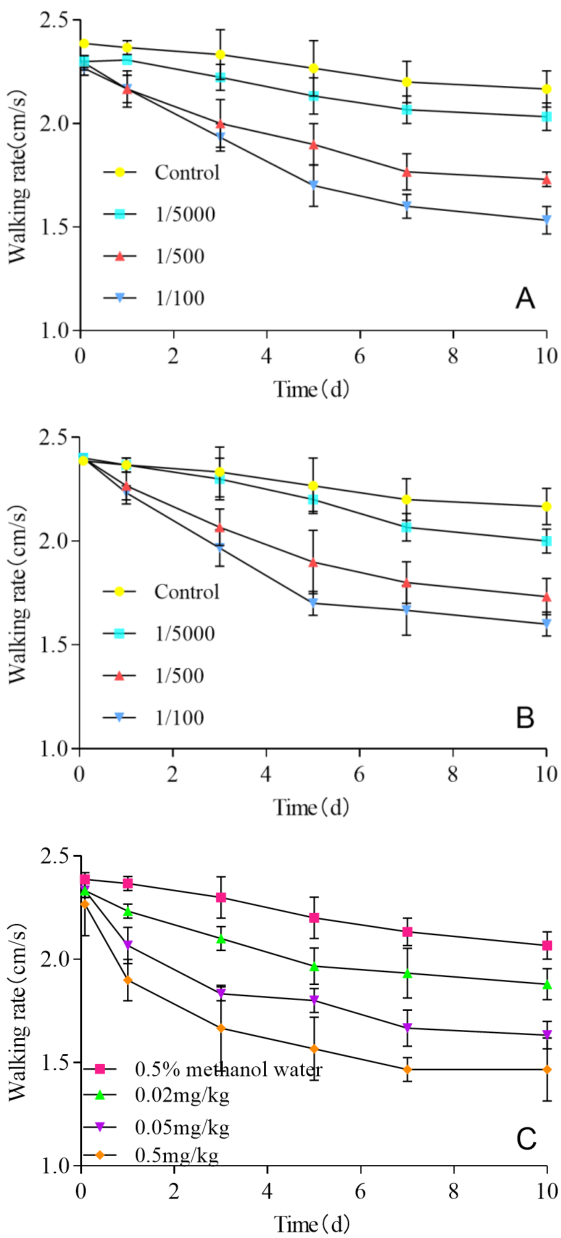

Fig 4. Effects on walking rate of worker ants after fed with leaves extract (A), stems extract (B) and koumine (C). 1/100 means the extract that 1g of plant material dissolved in 100 ml of water, and 1/500, 1/5000 were diluted by 1/100. Each data point represents mean ± SE of three replicate beakers each containing 20 ants.

Impact on walking ability

Figure 4 shows that both leaf and stem extracts have relatively high inhibitory activity on the walking of worker ants. Before the treatment, the normal red fire ant's walking speed is about 2.2~2.4 cm/s. On the 7th day of treatment, the walking ability of the worker ants tended to be stable. On day 10, the walking rate of treated worker ants decreased to 1.53 cm/s (1/100 leaf extract), 1.73 cm/s (1/500 leaf extract) and 2.03 cm/s (1/5000 leaf extraction); 1.60 cm/s (1/100 stem extract), 1.73 cm/s (1/500 stem extract) and 2.00 cm/s (1/5000 stem extract); 1.47 cm/s (0.5 mg/kg koumine), 1.63 cm/s (0.05 mg/kg koumine), 1.88 cm/s (0.02 mg/kg koumine). On the 10th day of the experiment, there was a significant difference between the walking ability of worker ants treated at different concentrations, except for standard treatment. (leaf: F=19.00 P=0.003; stem: F=8.62 P=0.017; standard: F=4.65 P=0.060).

Discussion

The results showed that both the aqueous extracts of the leaves and stems of *G. elegans* had insecticidal activity against red imported fire ants, which could reduce the aggregation level, climbing ability and walking ability of red imported fire ants. We think this may be caused by the ingredients of the koumine contained in *G.elegans*. The main component of *G. elegans* is alkaloid, and the most abundant is koumine (Xu et al., 2012). Therefore, we also studied the insecticidal activity and behavioral effects of the koumine standards on red imported fire ants, and compared the effect of the aqueous extract of plant materials, the results showed that it has a good effect. The traditional method of controlling red imported fire ants uses chemical pesticides, which will pollute the environment and endanger people's health. The use of chemicals from natural products in insect pest management is generally considered as a safer alternative to using synthetic contact insecticides (Zhang et al., 2017).

In our research team, certain components of some plants have been proved to have insecticidal activity against red imported fire ants. For example, cinnamaldehyde and eugenol contained in cinnamon have been found to have insecticidal activity against red imported fire ants (Huang et al., 2015), *Pronephrium megacuspe* (Huang et al., 2016), *Michelia alba* (Qin et al., 2018), etc. Their high concentration treatments resulted in 100% mortality of worker ants in 10 days, which is different from the lower mortality rate of our 1/20 high concentration treatment. Compared with the previous research, the method used for the insecticidal activity test this time is different. Most of the previous methods were to immerse plant insecticidal compounds in the soil where worker ants live, spray the whole body of worker ants or directly use plant residues to fumigate fire ants to test the insecticidal activity. And this time we used water as the extraction solvent, and used the "water tube" method to feed worker ants directly.

Nevertheless, we still think that using *G. elegans*, a natural plant material, to control red imported fire ants has good potential. This lower mortality rate may better control red imported fire ants. We all know that queen ant lives on food fed by worker ants. The best way to kill queen ant is through worker ants. However, red fire ants are very vigilant. When the ants die quickly, the death information will quickly spread to the entire ant nest. The *G. elegans* extract will not cause a large number of red imported fire ants to die in a short time, so it will reduce the vigilance of red imported fire ants and may kill the queen ant. In addition, the main feature of *G. elegans* extract is that it does not require any chemical solvents. It uses water as an extraction solvent and can even through boil a pot of water to extract the plant material. It is convenient and low cost because the plant can be grown all year round.

The significance of this research is to provide a new method to kill red imported fire ants. The new method is safer, more environment-friendly. The results of the insecticidal activity and behavioral effects reported in this study can provide a foundation and reference for future research on the control of red imported fire ants by *G. elegans*. The study has focused on koumine, a main insecticidal ingredient in *G. elegans*. Nonetheless, more research is needed to see if there are other chemicals in *G. elegans* that might contribute to the toxicity or inhibition of behavior on red imported fire ants.

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