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# Screening of the Acaricidal Activity of Essential Oils against *Panonychus citri* (McGregor) (Acari: Tetranychidae)

**Yijing Zhu [1], Taoqi Wu [1], Yongjian Xie [1], Yixuan Wu [1], Jianyu Deng [1], Lidong Cao [2], Zhijun Zhang [3,*] and Qiong Rao [1,*]**

[1] The Key Laboratory for Quality Improvement of Agricultural Products of Zhejiang Province, College of Advanced Agricultural Sciences, Zhejiang A & F University, Hangzhou 311300, China
[2] Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China
[3] State Key Laboratory for Managing Biotic and Chemical Threats to the Quality and Safety of Agro-Products, Institute of Plant Protection and Microbiology, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, China
* Correspondence: zhijunzhanglw@hotmail.com (Z.Z.); qiong.rao@zafu.edu.cn (Q.R.)

**Abstract:** The citrus red mite, *Panonychus citri* (McGregor), is an important pest of citrus in China, where pesticides are commonly used in citrus orchards. In order to reduce the use of chemical pesticides against *P. citri* and the development of resistance, the screening of biological control agents has attracted the attention of students. In this study, seven plant essential oils with high toxicity were selected from 40 plant essential oils by the leaf-dip bioassay, including plant essential oils of lemongrass, patchouli, juniper berry, sage, clove, frankincense, and citrus. The $LC_{50}$ after 72 h treatment were 3.198 μL/mL, 8.312 μL/mL, 3.244 μL/mL, 6.701 μL/mL, 8.350 μL/mL, 21.953 μL/mL, and 8.788 μL/mL, respectively. According to the $LT_{50}$, the essential oils' acute toxicity to *P. citri* from high to low were lemongrass, juniper berry, patchouli, citrus, sage, vetiver, and frankincense essential oils. In general, lemongrass and juniper berry essential oils have the best acaricidal effect and have high application value for the biological control of *P. citri*, which provides a basis for the development of botanical acaricides.

**Keywords:** essential oil; *Panonychus citri*; acaricidal activity; toxicity comparison; botanical acaricide



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## 1. Introduction

*Panonychus citri* (McGregor), also known as citrus red mite, is one of the most important pests in citrus orchards all over the world [1] and can harm more than one hundred different plants, including peach, pear, and prickly ash [2]. Adults and larvae of this mite could sting and suck the leaves, new shoots, and fruit skin of citrus, and in severe cases, leaves, withered shoots, and fruit would fall [3,4]. Moreover, it occurs for many generations every year, and the generations overlap, which is very serious for citrus in China [5], affecting the growth of citrus trees and the yield and quality of citrus [6]. Currently, chemical acaricides such as spirodiclofen, bifenazate, hexythiazox, pyridaben, abamectin, and fenpropathrin are mainly used to control *P. citri* [7].

The use of chemical pesticides in actual production is helpful to increase agricultural output [8]. However, people have a demand for more efficient and sustainable production of crops, thus overusing these chemicals. This situation not only caused huge economic losses, but also made the specific substances in the pesticides exert continuous selection pressure on pests, increased their adaptability and resistance [9,10], and makes the resistance traits be passed on to the next pest generations [11]. At the same time, chemical pesticides have become the main pollution sources in soil and water, destroy the ecological balance, and have high toxicity to humans and animals [12,13]. Therefore, biological control has emerged as the primary research focus in recent years.

To reduce the influence of traditional chemical pesticides, predatory natural enemies [14] and insect pathogenic microorganisms have been used for biological control of *P. citri*, such as some species of phytoseiid mites [15,16], predatory thrips [17] and *Beauveria bassiana* [18]. Besides, some natural products such as plant extracts and essential oils were also used to control pests [19]. These botanical pesticides, including veratrine [20] and matrine [21], have unique mechanisms of action, are not easy to produce drug resistance, and have the characteristics of high efficiency, low toxicity, and environmental compatibility [22]. Plant essential oil, as the secondary metabolite of plants, is a compound with special aroma, volatility, and solubility in organic solvents.

In the fields of food preservation, biomedicine, cosmetics, and agriculture, plant essential oils have been used for safe applications [23]. Essential oils are mixed with complex and unique compounds, including alkaloids, monoterpenes, phenolic acids, and aldehydes [24]. Due to different plants, different collection sites, and different extraction methods, there are great differences in the types and contents of compounds. Compared with synthetic pesticides, the cost of extracting and applying essential oils has increased. But for organic agriculture, the application of essential oils can not only solve the problem of pesticide-resistant pests but also avoid the health issues for humans and animals related to pesticide accumulation [25]. Biological pesticides synthesized from essential oils are an effective alternative in crop production and IPM [26,27].

At present, there are numerous studies and applications on the insecticidal activity of plant essential oils. When essential oils extracted from lemongrass, cinnamon, eucalyptus globulus, fennel, and pepper were mixed and compounded with soybean oil, respectively, they demonstrated high ovicidal and repellent activities against *Periplaneta americana*, with effects comparable to 10% cypermethrin [28]. Neem and tobacco extracts showed considerable mortalities of *Phenacoccus solenopsis* Tinsley and were safe for natural enemies but low as compared to the synthetic pesticides [29]. *Artemisia absinthium* essential oil caused significant acute mortalities against *Diaphorina citri*, especially carvacrol and (−)-α-bisabolol displayed synergistic effects [30]. Compound 1 is an acaricidal substance extracted from the external seed coat. Its toxicity to *P. citri* is equivalent to pyridaben, and obviously superior to omethoate, which not only has strong contact toxicity but is also quick-acting. At the same time, the compound is corrosive to *P. citri* but non-toxic to plants [31].

In this study, the acaricidal activity of 40 plant essential oils against *P. citri* was tested. The $LC_{50}$ and $LT_{50}$ of several plant essential oils against *P. citri* were further determined, and the laboratory toxicity of these plant essential oils was compared. The purpose of this study was to screen out the essential oils that had the effect of controlling *P. citri* and provide a theoretical basis for the development and application of green pesticides.

## 2. Materials and Methods

### 2.1. Testing Mites

*P. citri* was collected from Lin'an, Hangzhou, China. *P. citri* in this citrus orchard has several generations per year. The population resistance of *P. citri* was extremely high due to a large number of pesticides and frequent use. The mite was collected from the citrus orchard, which had been pesticide free for two months. The collected mites were transferred to citrus in the greenhouse to maintain the population.

### 2.2. Testing Essential Oils

Black pepper (B1), bergamot (B2), basil (B3), cinnamon (C1), clove (C2), cypress (C3), chamomile (C4), citrus (C5), eucalyptus (E1), frankincense (F1), geranium (G1), ginger (G2), grapefruit (G3), juniper berry (J1), jasmine (J2), lavender (L1), lemongrass (L2), lemon (L3), myrrh (M1), melissa (M2), niaouli (N1), neroli (N2), nutmeg (N3), origanum (O1), peppermint (P1), pine needles (P2), patchouli (P3), palmarosa (P4), ravansara (R1), rose (R2), rosemary (R3), sage (S1), sandalwood (S2), sweet fennel (S3), sweet orange (S4), tea tree (T1), thyme (T2), vanilla (V1), vetiver (V2), ylang (Y1) were purchased from ECO WILD. Tween-80 was purchased from Shanghai Macklin Biochemical Technology Co., Ltd.

### 2.3. Bioassay

The essential oils were dissolved in a 0.1% Tween-80 water solution to prepare different concentrations of the solution [32], and the solution was emulsified by ultrasonic. A leaf-dip bioassay was conducted to evaluate the effects of 40 essential oils on field-collected populations of *P. citri* [33]. The fresh, mature leaves were cut from the middle of citrus branches and made into 1 cm × 1 cm leaf discs. All leaves are washed with clean water three times and then dried to ensure that there are no arthropods on them. The leaf disc was immersed in the solution for 10 s, and allowed to naturally dry for 3 min. To prevent mites from escaping, a layer of filter paper with a diameter of 30 mm was placed at the bottom of a culture dish with a diameter of 35 mm, water was added to wet it until it was saturated but not dripping, and the leaf disc was placed on the wet filter paper with the leaf back facing up. Twenty female adult mites with similar size and high vitality on each leaf disc were picked, and the culture dish's edge was sealed with sealing film. The death of the female adult mite was recorded under the stereoscope after 6 h, 12 h, 24 h, 48 h, and 72 h. The mite's body was touched with a writing brush for judgment. If the mite foot did not move, it was deemed dead. All treatments were placed in an incubator with a temperature of 25 °C, relative humidity of 75%, and illumination of 16 h. Each treatment was repeated 3 times, and 0.1% Tween-80 aqueous solution was used as the control.

### 2.4. Data Analysis

All statistical analyses were performed using the Polo-Plus program to calculate the $LC_{50}$ and 95% confidence limits. $LT_{50}$, the toxicity regression equation, and the correlation coefficient were calculated by Excel software. GraphPad Prism 8.3.0 software was used to perform a one-way ANOVA on the group data and draw charts.

## 3. Results

### 3.1. Comparison of Lethal Toxicity of 40 Essential Oils to P. citri

The leaf-dip bioassay of *P. citri* was conducted with 1 μL/mL plant essential oil solution. The experimental results are shown in Figure 1. After 6 h and 12 h, the mortality rates of the essential oils treated with bergamot, frankincense, ginger, and myrrh differ significantly from the control ($p \leq 0.001$), showing certain acute toxicity. However, with the prolonged treatment time, the mortality rates of bergamot and frankincense oil did not differ from those of the control, and there was no significant lethal effect. After 24 h, the essential oils of citrus, ginger, myrrh, sage, and vetiver showed a certain level of toxicity, and the difference was significant ($p \leq 0.0001$) compared to the control, and the mortality rate was the highest after treatment with the myrrh essential oil. After 48 h, the mortality rate of each essential oil treatment increased significantly. After 72 h of treatment, there was a certain difference ($p \leq 0.01$) compared to 28 essential oils of the control, among which 10 essential oils, including citrus, ginger, grapefruit, juniper berry, lemon, myrrh, sage, sandalwood, sweet orange, and vetiver, had a higher mortality rate, showing an extremely significant difference ($p \leq 0.0001$) compared with the control, and the essential oils of ginger, juniper berry, and myrrh had the highest mortality rate.

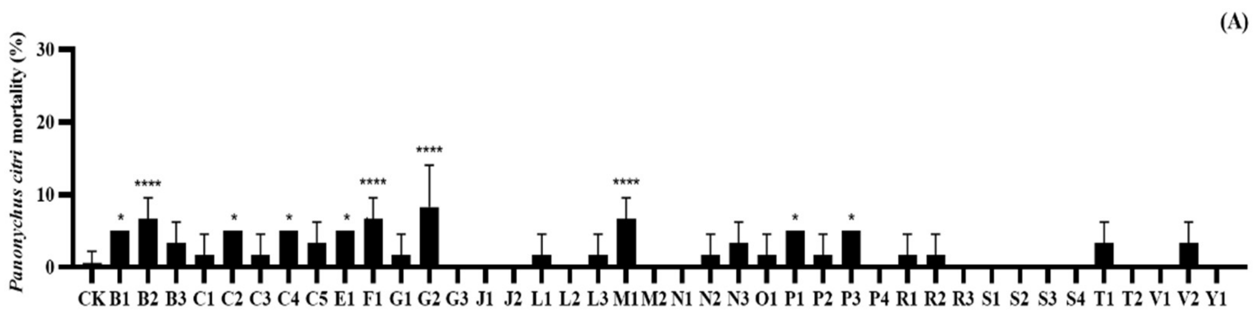


**Figure 1.** *Cont.*

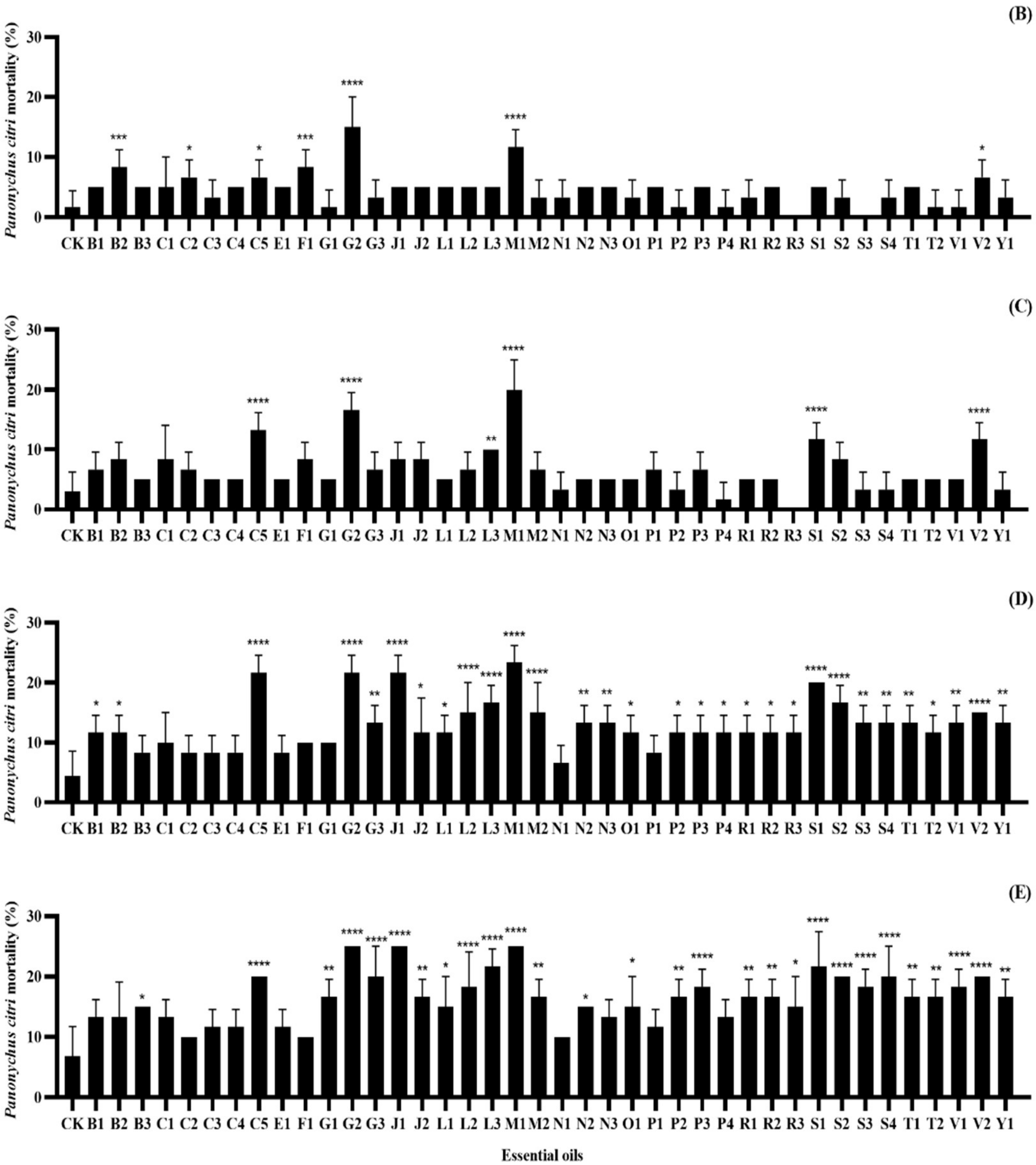


**Figure 1.** The lethal situation of 1μL/mL essential oils against *Panonychus citri* at different times. (**A**–**E**) Mortality rates of *Panonychus citri* in leaf-dip bioassay after 6 h, 12 h, 24 h, 48 h, and 72 h, respectively. The error bars denote the standard deviation. * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$.

### *3.2. Half Lethal Concentration of Seven Essential Oils to P. citri*

Through the preliminary experiment, seven plant essential oils with high toxicity to *P. citri* were further selected from 40 essential oils, including lemongrass, patchouli, juniper, sage, clove, frankincense, and citrus. The results in Table 1 show that the $LC_{50}$ of lemongrass essential oil was 4.697 μL/mL at 24 h, which was significantly lower than that of several other test essential oils and indicated a high toxicity level. After 24 h, the $LC_{50}$ of sage essential oil was the highest, at 93.384 μL/mL, and its relative toxicity was relatively poor. Considering the $LC_{50}$ values at different time points, the lower concentrations of lemongrass and juniper berry essential oils exhibited higher toxicity at 12 h, while the $LC_{50}$ values of sage and patchouli essential oils were the most affected by time, and the $LC_{50}$ values at different application time points were significantly different. The indoor toxicity of the seven essential oils to *P. citri* increased over time, and the toxicity was ranked as follows: lemongrass, juniper berry, sage, patchouli, vetiver, citrus, and frankincense at 72 h from high to low, with lemongrass and juniper berry having the best effect and the lowest $LC_{50}$.

**Table 1.** Median lethal concentration, 95% confidence limit, and toxicity regression equation of *Panonychus citri* treated with essential oils at different times.

| Essential Oils | 24 h | | 48 h | | 72 h | |
|---|---|---|---|---|---|---|
| | $LC_{50}$ 95%CL | Toxicity Regression Equation | $LC_{50}$ 95%CL | Toxicity Regression Equation | $LC_{50}$ 95%CL | Toxicity Regression Equation |
| Lemongrass | 4.697<br>3.625~7.451 | y = 1.0438ln(x) + 3.3047 | 4.157<br>3.190~6.712 | y = 0.8098ln(x) + 3.7756 | 3.198<br>2.576~4.197 | y = 0.8098ln(x) + 3.7756 |
| Patchouli | 15.591<br>10.587~27.506 | y = 0.7962ln(x) + 2.846 | 10.065<br>7.056~13.467 | y = 1.3197ln(x) + 2.3141 | 8.312<br>5.333~11.296 | y = 1.3839ln(x) + 2.5174 |
| Juniper berry | 22.484<br>8.371~1223.728 | y = 0.2607ln(x) + 4.1719 | 12.322<br>5.918~117.420 | y = 0.2883ln(x) + 4.2907 | 3.244<br>2.156~5.791 | y = 0.3765ln(x) + 4.6154 |
| Sage | 93.384<br>28.113~14526.685 | y = 0.3136ln(x) + 3.5731 | 15.753<br>10.235~35.423 | y = 0.4796ln(x) + 3.7035 | 6.701<br>4.794~10.390 | y = 0.468ln(x) +4.1938 |
| Vetiver | 49.293<br>18.928~4171.676 | y = 0.3676ln(x) + 3.5712 | 22.277<br>12.290~111.833 | y = 0.4478ln(x) + 3.6187 | 8.350<br>5.919~13.738 | y = 0.5757ln(x) + 3.8076 |
| Frankincense | 30.387<br>21.232~63.663 | y = 1.1291ln(x) + 1.2622 | 25.985<br>18.989~45.749 | y = 1.1874ln(x) + 1.2351 | 21.953<br>15.117~44.181 | y = 1.0182ln(x) + 1.9698 |
| Citrus | 62.188<br>22.156~2665.324 | y = 0.2647ln(x) + 3.536 | 19.000<br>11.419~51.234 | y = 0.3869ln(x) + 3.6866 | 8.788<br>5.245~22.605 | y = 0.4141ln(x) + 4.1372 |

### *3.3. Acute Toxicity of Seven Essential Oils to P. citri*

When treating *P. citri* with high-concentration essential oils, as shown in Table 2, the higher the concentration of the essential oil, the shorter the time to half-death ($LT_{50}$). At the concentration of 16 μL/mL, the $LT_{50}$ of the seven essential oils was all less than 50 h, with lemongrass essential oil having the lowest $LT_{50}$, and the death rate could reach half after treating 4.07 h. However, the $LT_{50}$ of frankincense oil is higher, and only after 88.96 h of treatment can the mites die by half. Other essential oils can reduce the population by half within 1–2 days. In conclusion, compared with a concentration of 1 μL/mL, most essential oils need to exert acute toxicity at a concentration of 16 μL/mL or higher. The results showed that lemongrass essential oil had the best acaricidal effect, followed by juniper berry, patchouli, citrus, sage, vetiver, and frankincense.

**Table 2.** Median lethal time of *Panonychus citri* treated with essential oils of 16 μL/mL.

| Essential Oils | $LT_{50}$ (h) |
|---|---|
| Lemongrass | 4.07 |
| Patchouli | 34.25 |
| Juniper berry | 33.00 |
| Sage | 45.70 |
| Vetiver | 49.87 |
| Frankincense | 88.96 |
| Citrus | 37.90 |

## 4. Discussion

Plant essential oil has many biological characteristics, including insecticidal, bactericidal, and herbicidal activity. *Eucalyptus saligna* leaf litter essential oil showed phytotoxicity. The effects on *Lactuca sativa* and *Amaranthus viridis* were comparable to the effects of the herbicide that was used as a positive control (Logran®) [34]. *Thymus zygis* (red thyme) essential oil possesses a broad antimicrobial spectrum and may even enhance the effect of certain antimicrobial agents [35]. The chemical components of cumin essential oil can inhibit the growth of *Panax notoginseng*-associated pathogenic fungi in vitro, and the inhibitory effect of cuminaldehyde was similar to that of hymexazol [36]. These studies verified the potential of plant essential oil as a plant insecticide, fungicide, and herbicide that is environmentally friendly and provide ideas for developing new products to control agricultural pests.

The experimental results of this study showed that essential oils of lemongrass, patchouli, juniper berry, sage, vetiver, frankincense, and citrus had great acaricidal effects on the citrus red mite, among which essential oils of lemongrass, juniper berry, patchouli, and citrus had higher acaricidal activity, and a 1.6% solution of lemongrass berry essential oil had a high mortality rate of over 50% for *P. citri* after 4 h, showing quick-acting ability.

Several studies have shown that the above essential oils also have contact-killing, fumigation, and repellent activities against other harmful insects. The lemongrass leaf extract has certain fumigation activity on *Drosophila melanogaster* and can inhibit its acetylcholinesterase and catalase activities [37]. The essential oil extracted from juniper has high larvicidal activity and repellent activity against *Aedes albopictus* [38]. Solutions with juniper essential oil concentrations of 1%, 2.5%, and 5% have significant repellent and insecticidal activity against *Rhopalosiphum padi* and *Sitobion avenae* [39]. Essential oils of vetiver, cinnamon, and lavender show high contact/fumigant toxicity against *Musca domestica* L. [40]. Ylang-ylang and frankincense essential oils showed significant insecticidal activity against *Culex quinquefasciatus* larvae and *Musca domestica* adults [41]. Five citrus essential oils have fumigant activity against the confused flour beetle *Tribolium confusum* Du Val [42]. Essential oils such as patchouli and sage have repellent activity against *Culex quinquefasciatus*, and the combination of them can effectively resist Aedes aegypti and Anopheles dirus, thus prolonging the repellent time [43]. The essential oils of patchouli, juniper, frankincense, sage, and lemongrass have repellent effects against *Lasioderma serricorne* [44]. These results are consistent with the results of this experiment, which fully prove that essential oils such as lemongrass, patchouli, juniper berry, sage, vetiver, frankincense, and citrus can be used as green pesticides to control harmful insects. These plant essential oils are a mixture of a large number of different components, mainly including linalool, limonene, patchouli alcohol, α-pinene, β-caryophyllene, α-thujone, cyclo-isolongifolene, monolinolenin, and so on [39,45–50]. Many compounds are common in several essential oils, but the content is different. They often show complex synergistic effects between components. Compared with the single application of one compound, the mixture of two or more compounds shows higher biological activity [45].

In addition, for different pests, the essential oils with insecticidal effects are different. The essential oils extracted from medicinal plants such as oregano, thyme, lavender, and mint have acaricidal activity against adults of *Tetranychus cinnabarinus* [51]. Essential oils of geranium, cedarwood, cinnamon, citronella, ginger, and lemongrass have certain acaricidal activity against adults of *T. urticae* [52]. However, in this study, the mortality of *P. citri* treated with lavender, cinnamon, mint, and geranium essential oils was not significantly different from that of the control.

In this study, the acaricidal activity and median lethal concentration of various plant essential oils against *P. citri* were determined by the leaf-dip method in the laboratory. Considering the difference of components of different essential oils in the production process, in the follow-up study, it is necessary to further analyze the main chemical components of plant essential oils that play a role in harmful mites and carry out essential oil blending and related greenhouse tests to more accurately evaluate the effects of plant essential oils on *P. citri* and citrus fruits, and thus provide a scientific basis for developing plant essential oils into green acaricides.

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