Research on the Bioactivity of Plant Essential Oils on Armyworm [Mythimna separata (Walker)] Larvae

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In order to find out the biological activity of plant essential oils on armyworm [Mythimna separata (Walker, 1865)] larvae and provide a theoretical basis for the biological control of armyworms, in this study, the antifeedant activity, repellent activity, fumigation activity, contact activity, and synergistic effect on indoxacarb of nine kinds of plant essential oils on armyworm larvae were determined. The results showed that lavender and citronella essential oils had the greatest impact on the antifeedant activity on armyworm larvae, and the antifeedant rate reached 100.00%. Meanwhile, rosemary essential oil revealed the best repellent activity on armyworm larvae with an average dwell time of 0 s at the content of 0.2%. Moreover, tea tree essential oil and lemon essential oil at the content of 2.0% had the best fumigation and contact activity against armyworm larvae, and the corrected mortality rates at 120 h were 86.67 and 66.67%, respectively. In addition, the combination of citronella essential oil and indoxacarb with the ratio of 5:1 had the best synergistic effect on armyworm larvae at 96 h, and the synergistic ratio was reached 100.00%. These findings will guide the development of new insecticides for controlling armyworm larvae.

Keywords: plant essential oil, antifeedant activity, repellent activity, fumigation activity, contact activity, synergistic effect, armyworm larvae

INTRODUCTION

The armyworm, Mythimna separata (Walker, 1865) (Lepidoptera: Noctuidae), also known as marching worms and shaving worms. It is an important migratory agricultural pest and is widely distributed worldwide (Liao et al., 2020) and hosted on grain crops, such as wheat, rice, cotton, millet, vegetables, and corn, as well as more than 104 species of plants in 16 families (Jiang et al., 2014). The armyworm has the characteristics of flocking, migratory, omnivorous, gluttonous, intermittent outbreaks, etc., has strong adaptability, prefers high-temperature and high-humidity environment, and has four to five large-scale migration processes every year. The armyworm larvae could eat up all the leaves of crops, often causing serious losses in a short period of time (Kong et al., 2019). In recent years, the insect has repeatedly appeared in high-density and concentrated damage in the northern country, posing a serious threat to food production (Duan et al., 2018). At present, the field control of armyworms is still dominated by traditional pesticides. However, the long-term application of traditional pesticides is not only destroys the ecological environment and affects the survival of natural enemies, but also significantly increases the drug resistance of armyworms to reduce the control effect (Wei et al., 2010; Dong et al., 2014; Zhao et al., 2017; Zhang et al., 2019; Liao et al., 2020).
et al., 2020). Therefore, it is of great significance to use multiple ways to coordinately regulate armyworm populations.

Plant essential oils are composed of a complex mixture of secondary metabolites, which have the advantages of being widely used, having low residues in the environment, not polluting crops, and not easy for pests to develop drug resistance. Their bioactivity research on insects has attracted increasing attention for many scholars (Sombra et al., 2020; Zhao et al., 2020; Zhang et al., 2022). The exogenous application of plant essential oils is often used for insecticidal, repellent, and antifeedant activity (Isman, 2015), for example, the repelling effect of mulberry leaf's essential oil on the red grain thief (Xu et al., 2019), the contact and repelling activity of 11 kinds of plants' essential oils on the tea orange gall mites (Li et al., 2019), the biological activity of lavender essential oil on Spodoptera litura (Zhang Z. et al., 2019), the repellent activity of lemon essential oil on the pink beetle Tribolium castaneum (Olivero-Verbel et al., 2010), and the repellent effect of citronella essential oil on the adults of Aedes aegypti (Diptera: Culicidae) (Suwansirisilp et al., 2013). In addition, some researchers have also paid attention to the research of plant essential oils on insect feeding, egg hatching, and pesticide synergy. For example, Zhang Y. et al. (2019) found that calamus essential oil had inhibitory effects on the feeding and egg hatching behavior of 2-day-old larvae of diamondback moth; Yang et al. (2021) showed that aloe vera essential oil had a good synergistic effect on the control of Spodoptera frugiperda by indoxacarb; An et al. (2012) showed that the combination of lemongrass oil and natural pyrethrins could improve its insecticidal effect; Wang et al. (2019) found that the plant's essential oil d-limonene could increase the efficacy of imidacloprid in controlling Gossypium gossypii. To sum up, plant essential oils have become a hot spot in integrated pest control, which is of great significance to the development of new pesticides for armyworm; however, only few authors have reported the relevant studies on the biological activity of armyworms (Passreiter et al., 2005; Ma et al., 2010; Cárdenas-Ortega et al., 2015; Song et al., 2020).

**MATERIALS AND METHODS**

**Test Insects**

The test armyworms were collected from wild corn fields in Guiyang City (26.387282°N, 106.625232°E), Guizhou Province, and raised to the 6th generation in the laboratory. Larvae are reared in plastic trays with 4–6 cm thick substrates and fresh and pesticide-free corn leaves, and then placed in an artificial climate box with the rearing temperature, relative humidity, and photoperiod of 24°C, 70 ± 5%, and 14L:10D, respectively. When the larvae reached 4-days-old, the well-developed armyworm larvae of basically the same size were selected for the experiments.

**Test Materials**

Basil essential oil (content ≥90%), peppermint essential oil (content ≥97%), lemon essential oil (content ≥80%), rosemary essential oil (content ≥99%), citronella essential oil (content ≥85%), frankincense essential oil (content ≥85%), eucalyptus essential oil (content ≥95%), tea tree essential oil (content ≥95%), and lavender essential oil (content ≥85%), which were proved to have good insecticidal activity (Yu et al., 2018; Sombra et al., 2020), were purchased from Guangdong Biotecotechnology Co., Ltd. (Guangdong, China). Indoxacarb EC (150 g/L) was purchased from FMC Corporation (Henan, China).

**Feeding Behavior Test on Armyworm Larvae**

Each plant's essential oil was divided into three content gradients of 2.0, 0.5, and 0.2%, respectively, with acetone according to the reported method by Sombra et al. (2020). Fresh corn leaves (5 g) with 10 μL of the test solutions of the plant's essential oils on both sides were put into a self-made, transparent, and odor-free plastic box (length 24.5 cm, width 20.5 cm, and height 6.3 cm). Acetone served as a negative control. Then, 15 4-day-old armyworm larvae, which were pre-starvation treated for 4 h, were inserted into each self-made insect box. Each treatment was repeated three times. After 24 h treatment, the antifeedant rate and mortality rate were calculated according to the calculation method of Li et al. (2014).

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\text{Antifeedant rate (\%)} = \frac{\text{Feeding quality of control group} - \text{Feeding quality of treatment group}}{\text{Feeding quality of control group}} \times 100.
\]

\[
\text{Mortality rate (\%)} = \frac{\text{Number of dead armyworm larvae}}{\text{Number of test armyworm larvae}} \times 100.
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**Behavioral Response Test on Armyworm Larvae**

The behavioral response test of 9 plant essential oils on armyworm larvae was tested according to the research method of Guarino et al. (2021). Each plant essential oil (50 μL) with the content of 2.0, 0.5, and 0.2%, respectively, was dropped in a 3 × 4 cm filter paper strip, then place the filter paper in one of the gas sources bottles, while the control gas source bottle was added with the same amount of acetone. To reduce experimental error, the filter paper was changed every 5 worms, and each treatment was repeated 30 times. The temperature of the test room was controlled at 28 ± 1°C by an air conditioner. An air pump (Aco-5505, Guangdong Haili Group Co., Ltd., all instruments are connected by rubber pipes with inner and outer diameters of 6 and 9 mm, respectively) was used to generate airflow. Then the airflow was passed through 1000 ml bottles which were filled with activated carbon to purify impurities, then passed through a 250 ml humidity bottle containing ultrapure water to increase air humidity. The airflow rate was adjusted through the glass rotor tachometer (flow rate 500 ml/min), then the airflow passed through the 250 ml gas source bottle to carry the smell and finally entered the Y-shaped pipe (stem 20 cm; arms 15 cm at 140 angles; stem internal diameter 5 cm, arms internal diameter 3.5 cm, place in an evenly lit area). After 5 min of ventilation, the armyworm larvae were placed in the middle of the main stem (3–5 cm away from the Y-pipe connection) and continued to observe for 300 s. The time when the armyworm larvae entered the experimental
group (test arm) and the control group (the armyworm larvae entered the blank arm and the stem 5 cm away from the connection) was recorded. The dwell time is calculated by the following formula.

$$\text{Average dwell time (s)} = \frac{\text{Test or control armyworm larvae active time}}{\text{Number of armyworm larvae of treatment group} + \text{Number of armyworm larvae of the control group}}.$$

**Fumigation Activity on Armyworm Larvae**

The fumigation activity of nine plant essential oils on armyworm larvae was tested according to the research method of Wu et al. (2015). Each plant essential oil (15 μL), with the contents of 2.0, 0.5, and 0.2%, respectively, was dripped in a rectangular filter paper (1.5 cm × 4 cm), then hang the filter paper vertically on the middle of a 1 L bottle which contained 30 4-day-old armyworm larvae inside. Acetone served as a negative control. Each treatment was repeated five times. After 24, 48, 72, 96, and 120 h of treatment, the corrected mortality rate is calculated using Abbott’s formula (Zhang Z. et al., 2019).

$$\text{Corrected mortality rate} (%) = \frac{\text{mortality rate of treatment group} - \text{mortality rate of the control group}}{1 - \text{mortality rate of the control group}} \times 100.$$

**Contact Activity on Armyworm Larvae**

The contact activity of nine plant essential oils on armyworm larvae was tested according to the research method of Yuan et al. (2018). Three content gradients (2.0, 0.5, and 0.2%) of each plant’s essential oil were placed on the prothorax and back of the armyworm with a micro-dropper, respectively. Then, 15 4-day-old armyworm larvae were carefully transferred to the Petri dish. Acetone served as a negative control. Three replicates were conducted for each treatment. After 24, 48, 72, and 96 h of treatment, the corrected mortality rate is determined using Abbott’s formula.

**Synergistic Ratio on Armyworm Larvae**

The synergistic effects of plant essential oils and indoxacarb on armyworm larvae were determined according to the reported method by Xiong et al. (2019). The indoxacarb EC was diluted to the content of 0.025% with the purified water and mixed with different contents of plant essential oils with the ratio of 5:1, 10:1, and 20:1, respectively. Fifteen test armyworm larvae were placed in an insect breeding box (diameter 25 cm, height 8 cm) which was lined with filter paper. Each treatment was repeated 3 times. Then, the insect breeding box was placed under the potter spray tower for quantitative spray treatment (2 ml, pressure 103.4 kPa, settling time 30 s) and placed in an industrial climate box at 25°C with the photoperiod of 14L:10D. After 24, 48, 72, and 96 h of treatment, the corrected mortality rate is determined using Abbott’s formula, and the synergistic ratios are determined by the following formula.

$$\text{Synergistic ratio} (%) = \frac{\text{corrected mortality rate of the combination of plant essential oils and indoxacarb}}{\text{corrected mortality rate of indoxacarb}} \times 100.$$
Statistical Analysis
All data in this study were analyzed using SPSS version 23.0 software (SPSS Inc., Chicago, United States). Duncan’s new complex extreme difference method was used to test the different significance of the data, $p$ values below 0.05 were considered significant differences.

RESULT
Feeding Behavior Test on Armyworm Larvae
Table 1 showed that the three content gradients (2.0, 0.5, and 0.2%) of lavender essential oil and citronella essential oil had the greatest impact on the armyworm larvae, and antifeedant rate and mortality rate are 100.00%. The antifeedant rate and mortality rate of basil essential oil, peppermint essential oil, and rosemary essential oil also reached 100% at the content levels of 2.0 and 0.5%.

Repellent Activity Test Results on Armyworm Larvae
Figure 1 showed that lavender essential oil, frankincense essential oil, and tea tree essential oil had obvious repellent effects on armyworm larvae. Among them, tea tree essential oil had an average dwell time of 0 s at the contents of 0.5 and 2.0%, meanwhile, rosemary essential oil had an average dwell time of 0 s at the content of 0.2%, indicating that tea tree essential oil and rosemary essential oil revealed the best repellent activity on armyworm larvae.

Fumigation Activity Test Results on Armyworm Larvae
It can be seen from Table 2 that the fumigation activity on armyworm larvae has gradually increased with the treatment time. Among them, tea tree essential oil with a content of 2.0% had the best fumigation activity against armyworm larvae, the
Corrected mortality rates at 48, 72, 96, and 120 h were 26.33, 48.67, 73.33, and 86.67%, respectively, followed by rosemary essential oil with the content of 2.0%, its corrected mortality rates were 26.17, 28.67, 46.33, and 64.33% at 48, 72, 96, and 120 h, respectively.

Contact Activity Test Results on Armyworm Larvae

It can be seen from Table 3, that the contact activity on armyworm larvae has gradually increased with the treatment time. Among them, basil essential oil at the content of 2.0% revealed the best contact activity on armyworm larvae with the corrected mortality rates of 40.00, 48.67, and 57.67% at 48, 72, and 96 h, respectively. Lemon essential oil with a content of 2.0% had the best contact activity (66.67%) on armyworm larvae at 120 h.

Synergistic Effect of Plant Essential Oils and Indoxacarb on Armyworm Larvae

As can be seen from Table 4, different mixture ratios of plant essential oil and indoxacarb with different synergistic effects on armyworm larvae. Among them, the combination of citronella essential oil and indoxacarb with the ratio of 5:1 had the best synergistic effect on armyworm larvae at 96 h, and the synergistic ratio was reached 100.00%, indicating that the plant essential oil could significantly improve the mortality rate of indoxacarb on armyworm larvae.

DISCUSSION

Botanical pesticides play an increasingly important role in controlling agricultural pests. The effects of essential oils on the behavior, survival, and reproduction of various pests have been extensively studied (Mossa, 2016; Pavela and Benelli, 2016; Sousa et al., 2021). At present, essential oils are considered as ideal potential green pesticides and have been well applied and recognized in forest pest control (Cetin et al., 2010). In this study, we demonstrated that nine plant essential oils, including basil essential oil, peppermint essential oil, lemon essential oil, rosemary essential oil, citronella essential oil, frankincense essential oil, eucalyptus essential oil, tea tree essential oil, and lavender essential oil, had significant biological activity against armyworm larvae. Among them, lavender essential oil and citronella essential oil had the best antifeedant effects on armyworm larvae, and the antifeedant rates in the three gradients of 2, 0.5, and 0.2% are all 100%. Zhang Y. et al. (2019) found that citronella essential oil and lemon essential oil with a concentration of 10 μL/ml had a higher antifeedant rate in a 2-day-old diamondback moth, and the antifeedant rate of calamus essential oil was 100% with the increase of concentration. Kostić et al. (2013) also found that water chestnut essential oil...
and nutmeg essential oil had strong antifeedant to gypsy moth, and when the concentration reached 0.1%, water chestnut essential oil doubled the antifeedant rate of gypsy moth larvae. Skuhrovec et al. (2020) suggested that essential oil concentrations had a significant effect on the feeding of the potato beetle *Leptinotarsa decemlineata*. Lavender and tea tree essential oils also had a significant avoidance effect on citrus psyllid adults and could inhibit the attraction of citrus leaves to psyllids (Mann et al., 2012). To sum up, it showed that the concentration of essential oil had a great influence on the feeding behavior of insects, and its antifeedant rate could increase with the increase of the concentration of essential oil within a certain concentration range. Therefore, it is necessary to add more concentration gradients of essential oils to further probe the effect of essential oil concentration on insect feeding. In addition, tea tree oil had a strong attracting effect on tobacco beetle *Lasioderma serricorne* (Fabricius) adults (Ren et al., 2022), but the attractive effect of tea tree oil on armyworm larvae in this study was extremely poor at two gradients of 2 and 0.5%.

In this study, nine plant essential oils showed significant avoidance activity against armyworm larvae. However, there were differences in the avoidance rate of armyworm larvae for each essential oil, which may be related to the volatile chemicals of plant essential oils. Maria et al. (2021) found that citronellal acid in citronella essential oil had a significant repelling effect on bed bugs, while menthone in peppermint essential oil had no apparent avoidance response to bed bugs. However, in this study, the three concentrations of peppermint essential oil produced strong avoidance activity on 4-days-old armyworm larvae, indicating that the volatile substances with repelling effect on armyworm larvae were the other main components. Kady Rocha et al. (2015) found that the main components of fennel essential oil respond differently to the behavior of *Aedes aegypti*. Taken together, it is shown that the behavioral responses of essential oils to insects are closely related to the main components of essential oils. For example, Sombra et al. (2020) believed that the behavioral changes of *Spodoptera frugiperda* caused by citronella essential oil were the effect of the main compound menthone (59.97%) and a few compounds such as geraniol (3.44%). Deletre et al. (2016) found that the main compound of citronella essential oil may react with insect olfactory receptors, leading to insect avoidance behavior. Santos et al. (2016) found that rosemary essential oil contained carvacrol (30.37%), α-himlayanene (10.38%), terpinene (7.96%), α-pinene (5.08%), and β-myrcene (3.90%), and other substances can cause muscle fatigue in caterpillars, resulting in a better insecticidal behavior.

### Table 3: The Contact Activity of Plant Essential Oils Against Armyworm Larvae.

| Plant essential oils | Content (%) | 48 h | 72 h | 96 h | 120 h |
|----------------------|-------------|------|------|------|-------|
| Basil essential oil  | 2.0         | 40.00 ± 4.04A | 48.67 ± 4.33A | 57.67 ± 9.60A | 62.00 ± 5.86AB |
| Basil essential oil  | 0.5         | 7.00 ± 0.00EFG | 16.77 ± 4.67EFG | 26.67 ± 3.76EFGH | 35.33 ± 2.33EFGH |
| Frankincense essential oil | 2.0        | 17.67 ± 4.67CDEF | 16.77 ± 4.67EFGH | 31.00 ± 5.86EFGH | 38.00 ± 5.86EFGH |
| Frankincense essential oil | 0.5         | 7.00 ± 0.00EFG | 11.00 ± 2.00HGU | 23.33 ± 2.33FEGH | 29.00 ± 2.00HGUJK |
| Eucalyptus essential oil | 2.0         | 13.00 ± 2.33G | 24.33 ± 5.93DEFG | 44.67 ± 2.33BCD | 64.67 ± 2.33A |
| Eucalyptus essential oil | 0.5         | 6.67 ± 3.76EFG | 20.00 ± 4.04EFGH | 29.00 ± 5.86EFGH | 49.00 ± 2.00BCDE |
| Citronella essential oil | 2.0         | 15.67 ± 5.93CDEF | 23.33 ± 2.33DEFG | 47.00 ± 2.00ABC | 66.67 ± 2.33A |
| Citronella essential oil | 0.5         | 6.67 ± 3.76EFG | 11.00 ± 2.33DEFG | 33.33 ± 3.76EFGH | 49.00 ± 2.00BCDE |
| Lavender essential oil | 2.0         | 6.67 ± 2.33G | 23.33 ± 2.33IJK | 13.00 ± 3.76IJ | 9.00 ± 2.00D |
| Lavender essential oil | 0.5         | 6.67 ± 3.76EFG | 13.00 ± 2.00HGU | 23.33 ± 2.33IJK | 15.67 ± 5.93CDEF |
| Citronella essential oil | 2.0         | 20.00 ± 2.00CDE | 29.00 ± 2.00CDE | 44.67 ± 2.33BCD | 55.33 ± 2.33ABC |
| Citronella essential oil | 0.5         | 26.67 ± 6.67BC | 23.33 ± 3.76CDE | 44.67 ± 2.33BCD | 55.33 ± 2.33ABC |
| Peppermint essential oil | 2.0         | 9.00 ± 2.00EFG | 17.67 ± 2.33EFGH | 29.00 ± 2.00EFGH | 33.33 ± 3.76EFGH |
| Peppermint essential oil | 0.5         | 7.00 ± 0.00EFG | 11.00 ± 2.33DEFG | 17.67 ± 3.76IJK | 22.33 ± 2.33J |
| Peppermint essential oil | 2.0         | 6.67 ± 3.76EFG | 6.67 ± 2.33IJK | 13.00 ± 3.76IJK | 22.33 ± 2.33J |
| Lemon essential oil | 2.0         | 15.67 ± 5.93CDEF | 35.67 ± 5.93BCD | 47.00 ± 0.00ABC | 66.67 ± 2.33A |
| Lemon essential oil | 0.5         | 33.33 ± 3.76AB | 46.67 ± 3.76AB | 55.33 ± 2.33ABC | 64.67 ± 2.33A |
| Lemon essential oil | 2.0         | 13.33 ± 3.76BCD | 22.33 ± 4.67DEFG | 29.00 ± 2.00EFGH | 37.67 ± 4.67DEFG |
| Rosemary essential oil | 2.0         | 20.00 ± 2.00CDE | 22.00 ± 5.86EFGH | 33.00 ± 2.00EFGH | 42.33 ± 2.33DEFG |
| Rosemary essential oil | 0.5         | 18.00 ± 5.86CDE | 24.67 ± 2.33DEFG | 40.00 ± 4.04CDE | 49.00 ± 2.00BCDE |
| Rosemary essential oil | 0.2         | 8.67 ± 3.76EFG | 22.00 ± 5.86EFGH | 33.00 ± 0.00EFGH | 44.67 ± 2.33CDEF |

*Different capital letters in the same column in the table indicate a significant difference at p < 0.05.*
in combination with pesticides (indoxacarb), and the synergistic ratio of some essential oils reaches 100%. Yu et al. (2018) also found that the synergistic ratios of basil and citronella + lemon essential oil to pesticide (butenol fipronil) were 1.3 and 1.5, respectively, which showed that essential oils had a significant synergistic effect on pesticides. Meanwhile, some studies had showed that the synergistic effect of essential oils could significantly affect the biological activity of pests (Pavela, 2014; Aungtikun et al., 2021). In addition, studies had showed that the effects of essential oils on insect bioactivity were related to insect species, treatment methods, and treatment time (Pavela et al., 2016; Giovanni et al., 2018; Lazarevi et al., 2020).

### CONCLUSION

In conclusion, 9 kinds of plant essential oils were used to explore the antifeedant activity, repellent activity, fumigation activity, contact activity, and synergistic effects on armyworm larvae. Our results showed that lavender and citronella essential oils had the greatest impact on the antifeedant activity (100.00%) on armyworm larvae at the contents of 2.0, 0.5, and 0.2%. Meanwhile, rosemary essential oil revealed the best repellent activity (average dwell time: 0 s) on armyworm larvae at the content of 0.2%. Moreover, tea tree essential oil and lemon essential oil had the best fumigation activity (86.67%) and contact activity (66.67%) against armyworm larvae at a content of 2.0%. In addition, the combination of citronella essential oil and indoxacarb with the ratio of 5:1 had the best synergistic effect (100.00%) on armyworm larvae at 96 h. Our results provide a reference for enriching the green control technology of armyworms larvae.

### DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

### AUTHOR CONTRIBUTIONS

Conceptualization: YR and TW; methodology: JZ; validation: YR, TW, and MF; formal analysis: JZ and MY; investigation: YR, TW, and MF; resources: YR; data curation: TW; writing—original draft preparation: TW; writing—review and editing: YR; visualization: YR; supervision: MY; project administration: TW; funding acquisition: YR; and insect rearing: YL, BX, WZ, LD, XZ, and JW. All authors contributed to the article and approved the submitted version.
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