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Evaluating the Repellent Efficacy of a *Vitex trifolia* L. Leaf Powder Botanical Formulation Against Oriental Fruit Flies (*Bactrocera dorsalis*)

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ABSTRACT

Fruit flies (Diptera: Tephritidae) are important horticultural crop pests that cause significant losses in both crop quality and quantity. *Vitex trifolia* L., often considered a weed, contains bioactive compounds with potential as fruit fly repellents. This research aims to evaluate the repellent activity of *V. trifolia* against oriental fruit flies (*Bactrocera dorsalis*) at various concentrations. Laboratory experiments were conducted using a completely randomized design (CRD) with four treatments (1%, 3%, 5%) and one control, each repeated five times, Testing was carried out using an olfactometer. The results showed that all tested concentrations significantly reduced *B. dorsalis* visits compared to the control (0%), with the highest repellency (65.83%) observed at the 5% concentration. A negative linear relationship was established between formulation concentration and *B. dorsalis* activity, including visits, ovipositor punctures, and larval presence. The formulation significantly decreased oviposition punctures and larval infestation, resulting in a lower percentage of infested fruits (28.57% at 5% concentration compared to 82.86% in the control). Physical and organoleptic assessments confirmed the formulation's suitability for practical application, with a moisture content of (2.66%), a light green color, and a distinctive spicy aroma. Gas Chromatography–Mass Spectrometry (GC-MS) analysis identified key bioactive compounds, including phenol, methyl β-D-glucopyranoside, vitexifolin D, and viteosin A, which are known for their repellent properties. These findings suggest that *V. trifolia* leaf powder is a promising, eco-friendly alternative for managing *B. dorsalis* infestations in horticultural crops.

1. Introduction

Bactrocera dorsalis (Diptera: Tephritidae) is a significant pest of horticultural crops that threatens global food security. Yield losses caused by fruit flies can occur in the field and at the postharvest stage (Boulahia-Kheder 2021; Cheseto *et al.* 2023). The losses incurred exceed 2 billion USD annually (Lin *et al.* 2023; Ren *et al.* 2023). In China, the loss reached 3 billion USD; in Indonesia, the loss amounted to 21.99 billion USD, with an affected area of 47,900 m^2, and 75% of the damage was to fruit crops (Zulfitriany *et al.* 2004; Vayssières *et al.* 2009; Vargas *et al.* 2015; Astriyani *et al.* 2016; Rahmawati *et al.* 2018; Sulaeha *et al.* 2020; Wang *et al.* 2021; Li *et al.* 2024).

Using synthetic pesticides for fruit fly control can damage the ecosystem by causing residues if it is not wise in its use. (Dominiak 2018; Setiawati 2018; Pei *et al.* 2023; Yan *et al.* 2024). Control efforts were also made with the attractant methyl eugenol, which only attracted male fruit flies, so female fruit flies were still attacking (Tan *et al.* 2021). The use of natural bioactive plant materials sprayed on plants is one alternative control that has the potential to be used.

Vitex trifolia L. or Legundi is a plant that belongs to the Verbenaceae Family and is categorized as a weed (Hernández *et al.* 1999; Yan *et al.* 2023; Chokpaisarn

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et al. 2024). In traditional medicine, *V. trifolia* is known for its benefits as an anti-tuberculosis, anti-cancer, and anti-fungal drug and can be used as a pesticide (Rani & Sharma 2013; Tewari *et al.* 2022; Djimabi *et al.* 2022). The *Vitex* genus contains a variety of natural bioactive compounds such as iridoids, flavonoids, diterpenoids, phytosterols, saponins, alkaloids, tannins, sterols, etc (Nyiligira *et al.* 2004; Jawalkar & Zambare 2020). Furthermore, essential oils present in all parts of the plant, especially leaves, contain sesquiterpene compounds, terpenoids, ester compounds, alkaloids (vitamin), flavonoid glycosides (attempting and 7-desmethyl artemetin), and non-flavonoid components friedelin, β-sitosterol, glucosides and hydrocarbon compounds (Asmaliyah & Wati 2010; Meng *et al.* 2023).

Studies have been conducted to prove the repellent effect of *V. trifolia* on various insects; among others, pellets of *V. trifolia* showed the ability to repel *Araecerus fasciculatus* (Coleoptera: Anthribidae) (Sjam *et al.* 2015), essential oil of *V. trifolia* was shown to have repellent *Aedes aegypti* L. (Diptera: Culicidae) (Gou *et al.* 2020; Arpiwi *et al.* 2020), *Anopheles stephensi* (Diptera: Culicidae) (Govindarajan *et al.* 2011), *Sithophilus oryzae* (Coleoptera: Curculionidae) (Jayuska *et al.* 2022).

However, little is known about the use of *V. trifolia* in powder to control fruit fly infestations. Utilizing the bioactive natural ingredients of *V. trifolia* with repellent properties in powder formulations can facilitate application and spraying on plants or fruits. Therefore, the focus of control is on developing technology to modify adult insects, preventing them from being attracted to breed on the fruit.

2. Materials and Methods

2.1. Insect Collection and Rearing

Chili fruits showing symptoms of fruit fly infestation were collected from the field. They were brought to the Natural Materials and Pesticides Laboratory, Department of Plant Pest and Disease, Faculty of Agriculture, Hasanuddin University, at a room temperature of 27.3°C and 50% R.H. The infested fruits were placed in a container containing sterile sand. Every 2–3 days, the sand was sprayed with water to keep it moist. Pupae formed in the container were filtered and transferred to a 30 × 30 × 30 cm cage containing distilled water, 10% honey, and yeast extract, which were replaced every 1–2 days to promote oviposition. Chili peppers were provided to adult flies. *B. dorsalis* from the pupae were used as test insects.

2.2. Preparation of Leaf Powder and Formulation

Young leaves of *V. trifolia* were cleaned and dried for seven days. After that, it was mashed using a blender, then filtered until a powder of uniform size was obtained and oven at a temperature of ±40°C to avoid the growth of microorganisms. Furthermore, the formulation was carried out by mixing *V. trifolia* leaf powder and carrier materials in the form of kaolin and methanol. All materials were stirred with an electric stirrer at 300 rpm for 1–2 minutes, after which evaporation was carried out for 1–2 days at room temperature and could be applied immediately, and the rest was used for storage.

2.3. Repellency Test Using an Olfactometer

The two-choice method was used to see the rejection activity of *V. trifolia* leaf powder formulations. The apparatus used was modified from Sjam *et al.* (2015) (Figure 1). The apparatus comprised a plastic tube (Ø = 7.3 cm; l = 14.5 cm) with a hole (Ø = 4.3 cm) in the center. Two plastic containers (Ø = 4.3 cm; l = 11 cm) were attached at both tube ends. For formulation testing, *V. trifolia* leaf powder was weighed according to the treatment concentrations of 1%, 3%, and 5%, mixed with 1% Teepol, stirred for ±10 seconds until homogeneous, then diluted with distilled water to the desired volume. The formulation was sprayed on the chili fruit, allowed to stand for ±1 minute, and put into the treatment plastic tube (Figure 1B), while the other plastic tube was sprayed with distilled water as a control (Figure 1C). Five pairs of six-day-old B. dorsalis were inserted into the olfactometer through the hole in the center of the plastic tube (Figure 1A). The hole was covered with a cloth. The test was repeated five times, with each replicate consisting of one olfactometer with five pairs of adults. Insects entering the plastic tube were counted, and fruit replacement was done once every 24 hours.

The activity of *B. dorsalis* adults inside the plastic tube was observed for seven days after release to calculate the repellency and visit percentages. The percentage of repellency was calculated as described by Khan (2021):

$$\text{Repellency (\%)} = [(T_a - T_b) / Ta] \times 100 \qquad (1)$$

Where:

T_a : Insect in the control
T_b : Insects in the treatment

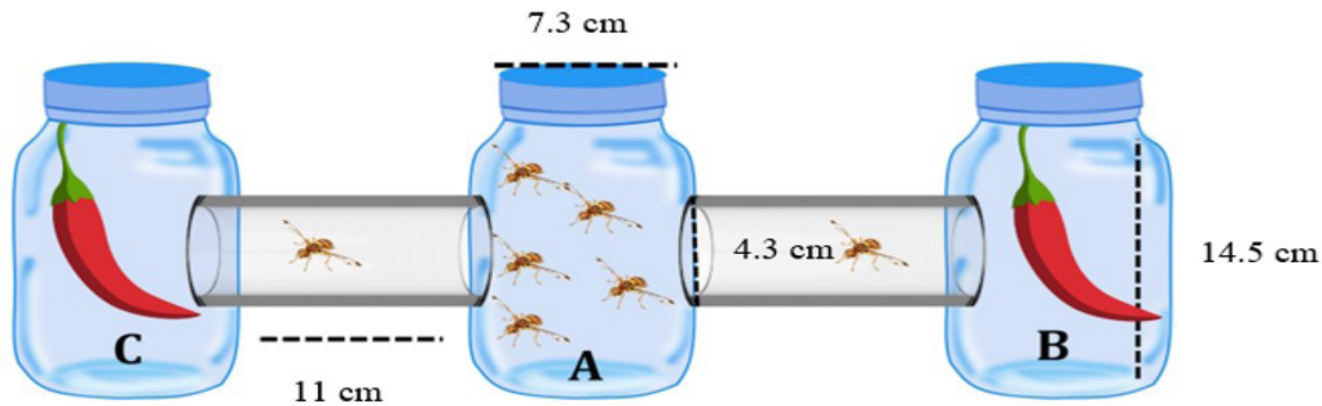

Figure 1. Olfactometer design was used for the *B. dorsalis* repellency test against leaf powder formulation

The repellent effect of the *V. trifolia* leaf powder formulation was assessed by grouping the results into the following categories, as described by Sjam *et al.* (2015):

Class 0 = Negative (-)
Class 1 = Very low (0-20%);
Class 2 = Low (20.1-40%);
Class 3 = Medium (40.1-60%);
Class 4 = High (60.1-80%);
Class 5 = Very high (80.1-100%)

The percentage of visits was calculated by Susanto *et al.* (2019) as follows:

$$\text{Visit (\%)} = [C_a / T_b] \times 100 \qquad (2)$$

Where:
C_a : Insect in the treatment
T_b : the Insects used

During the observation of repellent activity, the chili peppers were removed from the plastic tube and replaced with new ones to count the ovipositor punctures. The larval count was performed after the fruit had been incubated for seven days. Fruit infestation was then presented as a percentage. The percentage of fruit infestation was calculated as described by Wijaya *et al.* (2018):

$$\text{Fruit infestation (\%)} = [(R_a - R_b) / R_a] \times 100 \qquad (3)$$

Where:
R_a : Infested fruit
R_b : Uninfested fruit

The percentage of fruit infestation was calculated by grouping the results into the following categories, as described by Wijaya *et al.* (2018):

Light (<25%);
Medium (25 - <50%);
Heavy (50 - 75%);
Very heavy (>75%)

2.4. Characteristics of the Formulation

The characterization of *V. trifolia* leaf powder formulations can be done using organoleptic and physical tests. Organoleptic tests use human senses as the primary tool for measuring the acceptance of the formulation (Xylia *et al.* 2017). In this study, the instruments used for the organoleptic test were visual and olfactory senses. The physical test was by measuring the moisture content of the formulation (Alfiyani *et al.* 2019; Hasbullah & Mahdania 2023).

2.5. Analysis of Plant Volatile Organic Compounds

Volatile organic compounds from the methanol extract of *V. trifolia* used in the two selection tests were measured and identified using Gas Chromatography-Mass Spectrometry (GC-MS) (Gong *et al.* 2023; Gao *et al.* 2024). The analysis was conducted at the Department of Chemical Engineering, Politeknik Negeri Ujung Pandang Makassar.

2.6. Statistical Analysis

Percentage of *B. dorsalis* visits, percentage of infested fruit, and repellency effectiveness data were analyzed after transformation arcsin$\sqrt{x}$. Ovipositor punctures and larva of B. dorsalis data were analyzed after transformation $\sqrt{p+0.5}$. Data were analyzed using one-way analysis of variance (ANOVA). Tukey's test at ($P<0.05$) was applied for multiple comparisons when significant differences were found. Statistical analysis was performed using IBM

SPSS Statistics version 22.0, and graphs were created using Microsoft® Excel version 16.84.

3. Results

3.1. Fluctuation of *B. dorsalis*

The volatile compounds released by the formulation affect the visiting behavior of fruit flies and the fluctuation of *B. dorsalis* during the seven days of observation, as shown in Figure 2.

Formulation concentrations of 1%, 3%, and 5% acted as repellents against *B. dorsalis*, resulting in fewer visits compared to the 0% (control). The observed up and down pattern in the graphs is due to the experimental method, specifically the "Choice test."

3.2. Percentage of *B. dorsalis* Visits

The formulations sprayed on the test fruits exhibited a repellent effect on fruit flies at all concentrations except the control. These results are shown in Figure 3.

The percentage of visits at concentrations of 0% (control), 1%, 3%, and 5% ranged from 6.86% to 23.52%, with significant differences (P<0.05). The lowest attendance was observed at 5% concentration.

3.3. Ovipositor Puncture of *B. dorsalis*

The *V. trifolia* leaf powder formulation influenced fruit fly preferences at each concentration. It significantly affected the fruit fly puncture preferences, as shown in Figure 4.

B. dorsalis ovipositor punctures on fruits sprayed with the formulation showed a significant decrease as the treatment concentration increased. The number of punctures at the 0% treatment (control) was six, significantly different from the two punctures at the 5% concentration (P<0.05). Ovipositor punctures were found in the control compared to the treatment (P<0.05). This is because the formulation sprayed on the test fruit formed a layer on the fruit wall due to the carrier material in the form of kaolin. Kaolin mineral material has been widely used to control fruit flies, including cherry fruit flies *Rhagoletis cerasi* L., fruit flies *Drosophila suzuki*, and fruit flies *Rhagoletis pomonella* W. Kaolin is often used as a carrier material in the manufacture of fertilizers and insecticides.

3.4. Larva of *B. dorsalis*

The *V. trifolia* leaf powder formulation significantly affected the number of fruit fly larvae, as shown in Figure 5.

B. dorsalis larvae found on the fruit significantly decreased with increasing treatment concentration. The number of larvae in the 0% (control) treatment (12 individuals) was significantly different from the 3% (4 individuals), and 5% (3 individuals) concentrations (P<0.05).

3.5. Percentage of Infested Fruit

The percentage of infested fruit varied across treatments, as shown in Table 1.

The percentage of infested fruits at concentrations of 0% (control), 1%, 3%, and 5% ranged from 82.86% to 28.57%, with significant differences (P<0.05). The lowest percentage of infestation was observed at the 5% concentration. Larval damage ranged from 28.57 to 82.86%, with the highest damage observed in the control treatment (very severe category) and the lowest in the 5% concentration (moderate category) (P<0.05) (Figure 5, Table 1). The larvae's feeding activity causes fruit decay, eventually leading to rotting. Damage is categorized into external and internal. External damage includes punctures, bite marks, or wounds on the fruit surface, altering its appearance. Internal damage results from pest activity, causing physiological and biochemical changes within the fruit.

3.6. Repellency Effectiveness

The percentage of formulation repellency varied across treatments, as shown in Table 2.

The percentage repellency of the formulation at concentrations of 0% (control), 1%, 3%, and

5% ranged from 36.22 to 65.83%, with the highest repellency observed at the 5% concentration. This study indicates the potential of *V. trifolia* to be a repellent for *B. dorsalis*. The repellency of the formulation reached 36.22 to 64.83%, with the best concentration of 5% (P<0.05) (Table 2) being the highest. Insects have chemical sense organs and chemoreceptors, which are associated with the processes of taste and smell; these two processes are an important part of the insect sensory system related to various behaviors, such as feeding, mating, and feeding.

3.7. Relationship between Formulation Concentration and *B. dorsalis* Activity

The relationship between the concentration of *V. trifolia* leaf powder formulation and the activity of *B. dorsalis* is represented by a decreasing curve. The

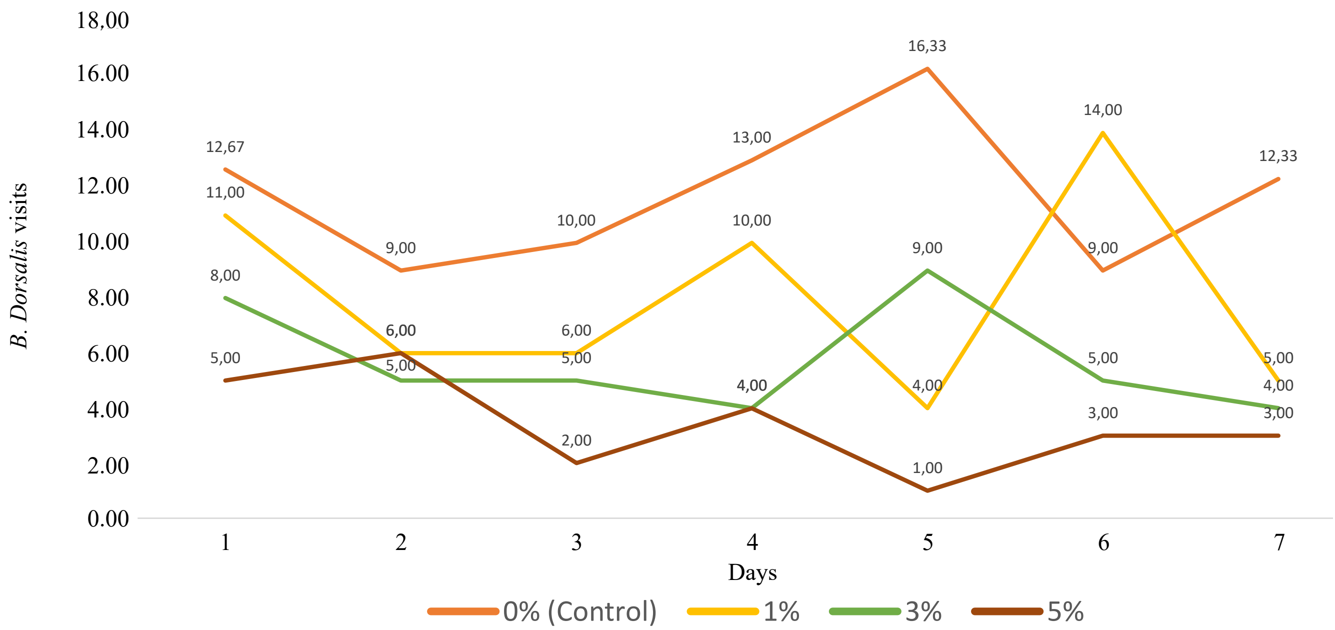

Figure 2. Fluctuation of *B. dorsalis* at various observation times

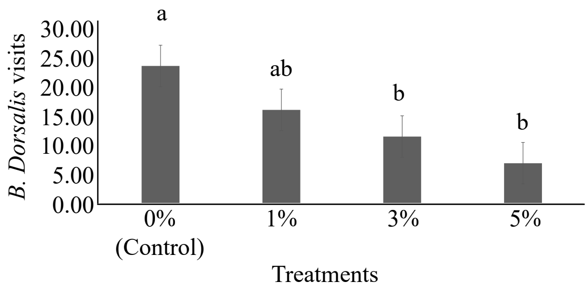

Figure 3. Visit of the percentage of *B. dorsalis*. The data were transformed by Arcsin√p before analysis. However, actual mean values are presented based on the interpretation of the transformed data. Bars with different letters are significantly different (P<0.05)

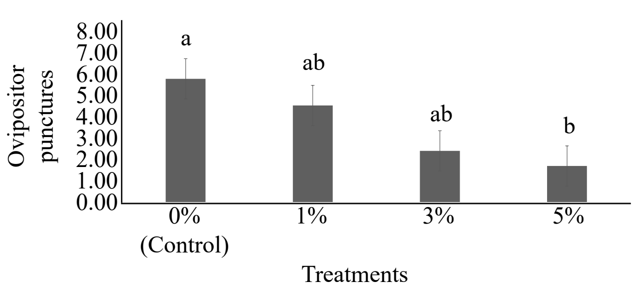

Figure 4. *B. dorsalis* ovipositor punctures. Data were √p + 0.5 transformed before analysis. However, actual mean values are presented based on the interpretation of the transformed data. Bars with different letters are significantly different (P<0.05)

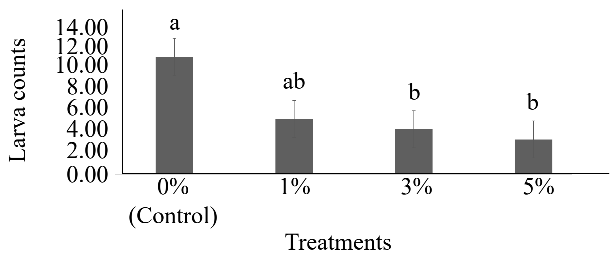

Figure 5. *B. dorsalis* larvae. Data were √p + 0.5 transformed before analysis. However, actual mean values are presented based on the interpretation of the transformed data. Bars with different letters are significantly different (P<0.05)

Table 1. Percentage of fruit damage by *B. dorsalis*

Concentration	Fruit Infestation (%) ± SD	Category
0% (Control)	82.86^{a}±24.122	Very heavy
1%	60.00^{ab}±29.378	Heavy
3%	37.14^{b}±21.379	Medium
5%	28.57^{b}±27.48	Medium

Data were Arcsin √x transformed before analysis. However, actual mean values are presented based on the interpretation of the transformed data. The different letters are significantly different (P<0.05)

Table 2. Percentage of repellency of *Vitex trifolia* L. leaf powder formulation

Concentration	Repellency (%) ± SD	Class	Category
1%	36.22 ± 13.53^{b}	3	Medium
3%	35.20 ± 13.49^{ab}	3	Medium
5%	65.83 ± 11.40^{a}	4	High

Data were Arcsin √x transformed before analysis. However, actual mean values are presented based on the interpretation of the transformed data. The different letters are significantly different ($P<0.05$)

negative linear regression equations are as follows: Y =-154.97x + 10.725 (Figure 6A), Y =-83. 414x + 5.5137 (Figure 6B), Y =-134.62x + 9.2195 (Figure 6C). These results indicate that the activity of *B. dorsalis* decreases as the concentration of *V. trifolia* increases. Based on the results of linear regression analysis (Figure 6A-C), the higher the concentration, the higher the repellency the formulation gives.

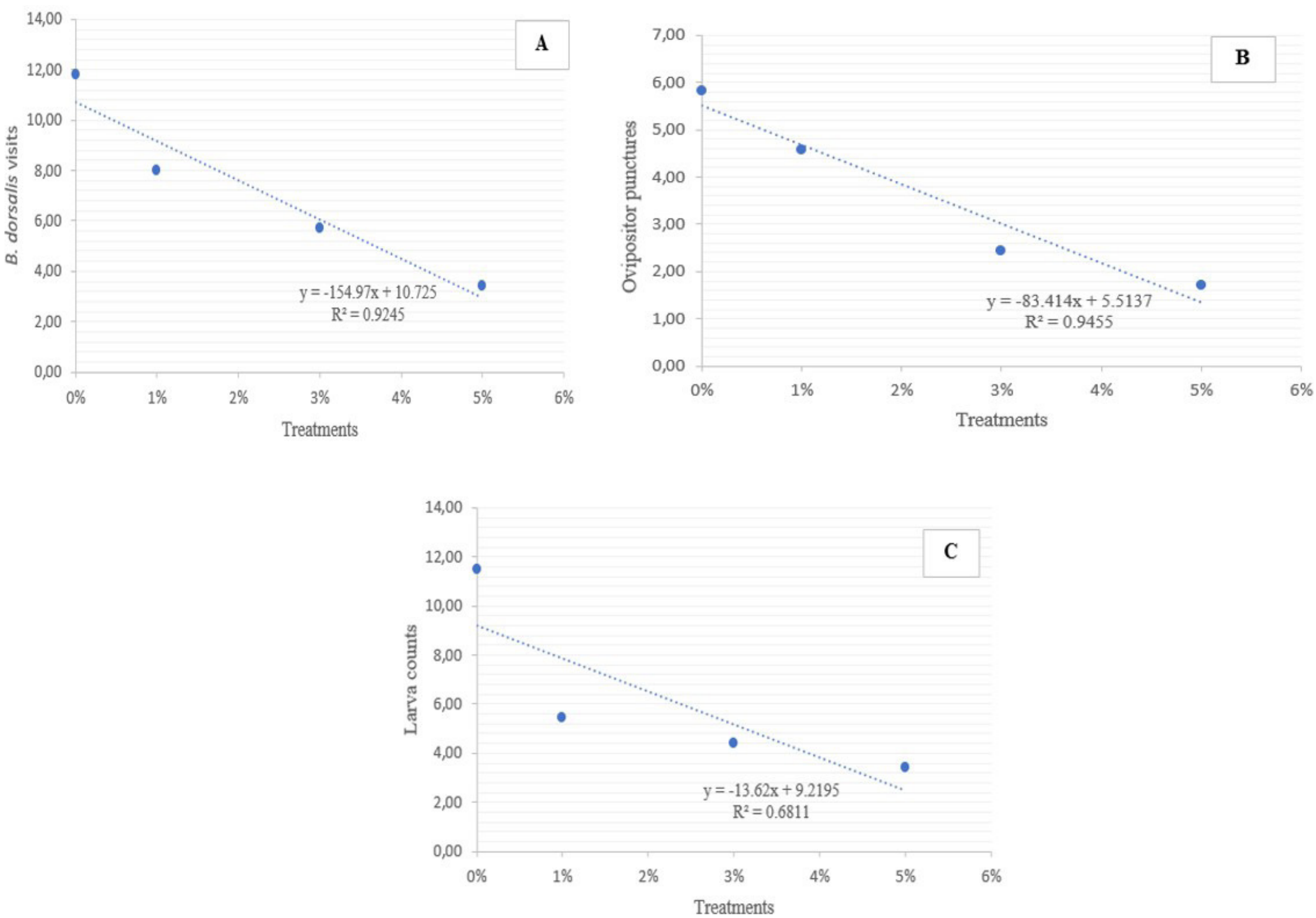

Figure 6. Relationship between the concentration of leaf powder formulation to (A) *B. dorsalis* visits, (B) ovipositor punctures, and (C) larvae counts

3.8. Characteristics of the Formulation

Physical and organoleptic testing is crucial for determining the quality of the *V. trifolia* leaf powder formulation for storage, marketing, and field application. The formulation tested had a moisture content of 2.66%, a light green color, and a distinctive spicy aroma (Table 3).

3.9. Analysis of Plant-Volatile Organic Compounds

The bioactive compound content of *V. trifolia* methanol extract was analyzed using GC–MS to identify the compounds responsible for its repellent effect on fruit flies.

4. Discussion

The analysis of the methanol extract of *V. trifolia* identified several chemical components, including phenol, β-D-glucopyrasonide, methyl (CAS), vitexfolin D, and viteosin A. These compounds belong to the classes of phenolics, flavonoids, saponins, and diterpenoids. GC–MS analysis of the *V. trifolia* methanol extract (Table 4) identified two key volatile compounds: Vitexfolin D and Viteosin A. Vitexfolin D belongs to the diterpenoid class, which, according to Luo *et al.* (2022), is reported for the first time in the Verbenaceae family. Terpenoids, the dominant compound class in the *Vitex* genus, are characteristic of this plant (Ban *et al.* 2018). Additionally, Viteosin A, classified as a flavonoid, functions as a secondary metabolite and acts as a juvenile hormone (Meng *et al.* 2023). Flavonoids interfere with fruit flies' nervous system activity through the respiratory tract, preventing egg hatching (Rina *et al.* 2019).

In conclusion, the *V. trifolia* leaf powder formulation effectively repels *B. dorsalis.* The 5% concentration yielded the best results, achieving a repellency rate of 65.83% and significantly reducing infestation levels. This eco-friendly approach could contribute to more sustainable pest management practices.

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Table 3. Physical and organoleptic test results of *V. trifolia* leaf powder formulation

Test type	Test criteria	Results
Physical	Moisture content (%)	2.66
Organoleptic	Shape	Powder
	Color	Dark green
	Scent	The characteristic scent of *V. trifolia*

Table 4. Five types of compounds with the highest percentage found in the extract of *V. trifolia*

Compound	Molecular formula	Peak	Retention time (min)	Relative (%)
Phenol	C6H6O	11	8.321	2.96
Methyl β-D-glucopyranoside	C7H14O6	33	17.910	29.85
1a,2,5,5-Tetramethyl-cis-1a,4a,5,6,7,8-hexahydro-gamma-chromene	C13H22O	34	18.563	3.12
Vitexifolin D	C19H30O4	77	30.599	1.71
Viteosin A	C22H34O5	102	38.203	8.24

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