#### RESEARCH PAPER

# Bio-attractant innovation with nano-gel technology to detect and control *Silba adipata* McAlpine on white cayenne peppers in Bali, Indonesia

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#### ABSTRACT

Silba adipata is a new pest that attacks white chili pepper in Bali, Indonesia. Most farmers control this pest with synthetic insecticides although this method poses significant risks to human health and the environment. Therefore, innovations and control technologies that are environmentally friendly and sustainable are urgently needed. Preliminary results show that *S. adipata* frequently attacks local figs (*Ficus variegata*) in Bali. Dried figs (Ficus carica) have been reported to effectively attract *S. adipata* adults. This study aims to (1) determine the phytochemical compounds in *F. variegata* fruit extracts that have the potential to act as attractants for *S. adipata* during the process of searching for and locating host plants; (2) evaluate the effectiveness and efficiency of *F. variegata* fruit extract, nano fruit extract, and nanogel fruit extract as attractants for *S. adipata* on cayenne pepper plants. The methods used to achieve these objectives were (1) LC-MS/MS analysis for phytochemical profile of *F. variegata* fruit extract includes 5-hydroxy-2-(4-hydroxyphenyl)-8,8-dimethylpirano[2,3-h]chromen-4-one, with the highest compound content (68.74%) in the fruit aged 9-16 weeks. The application of nanogel extract at a 50% concentration was highly effective and efficient as a biological attractant for *S. adipata* on white chilli plants in Bali. These findings strongly support the development of integrated pest management strategies in Indonesia.

Key words: Ficus variegata, Silba adipata, white cayenne pepper

# **INTRODUCTION**

Silba adipata McAlpine, 1956 (Diptera: Lonchaeidae) was originally found in the Mediterranean and the Middle East, where it attacked figs (*Ficus carica* L.). The pest subsequently spread to Iraq (Katsoyannos, 1983), Japan (Raz, 1998), and Turkey (Talhouk, 2003). In 2007, *S. adipata* was reported to be attacking figs in South Africa (Giliomee et al., 2007). In 2015, *S. adipata* was first reported as a pest affecting all fig plantations in Tunisia, with an attack rate of 88.17% (Abbes, 2021). By 2019, *S. adipata* was discovered in Indonesia, specifically in Banua Village, Kintamani

District, Bangli Regency, Bali Province, attacking white cayenne peppers, with an attack rate of 40.31% (Merta, 2020). Building on these findings, Yuliadhi et al. (2021) conducted further exploration of the spread of *S. adipata*, revealing that that the pest has become widespread in Bali. A key factor influencing its spread is the availability of host plant, which serve not only as a food source but also as habitats that provide shelter from natural enemies. One of the primary factors determining insect preferences for selecting host plants is the presence of sufficient nutritional content to meet their survival needs (Katayama, 2006; Supartha et al. 2023).

The process of searching for, recognizing, and accepting a host is influenced by various factors, including plant phytochemical compounds (Rosvik, 2019). Phytochemical compounds that play a significant role in the search, recognition, and suitability of host plants generally belong to the flavonoid, terpenoid, alkaloid, tannin, and steroid groups (Masriany et al., 2020). Flavonoids play multiple roles in plants, including contributing to the color and flavor of seeds, flowers, and fruits, as well as aroma (Mierziak et al., 2014). Terpenoids, on the other hand, are typically found in the leaves and fruits of higher plants (Dalimunthe

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& Rachmawan, 2017). Additionally, flavonoids also function as attractants for insects (Subramanian et al., 2007). A previous study found that attractants derived from the essential oil of nutmeg were highly effective in attracting *Bactrocera dorsalis* fruit flies (Susila et al., 2021).

Dried fig extract (*F. carica*) containing hexanol has been shown to be effective as an attractant for *S. adipata* (Abbes et al., 2021). Based on this finding, research was conducted on *S. adipata* attacks on *F. variegata*, a species commonly found in Bali. The results of the study revealed that *F. variegata* fruit was attacked by the same *S. adipata* that infests chili plants (Merta et al., 2024). Thus, it can be concluded that *S. adipata*, which attacks chilies in Bali, also utilizes *F. variegata* as an alternative host plant (Merta et al., 2024).

Research on the phytochemical content of F. variegata fruit remains limited. Therefore, phytochemical testing was conducted as a first step to identify the active compounds in F. variegata fruit with potential as attractants. While attractant compounds offer advantages, they also have limitations, particularly their volatility. These compounds are prone to rapid evaporation, making them less efficient in the field (Sulistiya, 2016). To address this issue, a nanotechnology approach can be employed to reduce the evaporation rate and enhance compound efficiency (Islamiyah & Cahyono, 2021). Nanotechnology involves engineering materials, functional structures, and devices on a nanoscale, typically between 1-100 nanometers (Duncan, 2011). Nanomaterials possess unique properties, including faster penetration and characteristics that differ significantly from their original forms.

The application of nanotechnology in attractant development has been demonstrated by Islamiyah & Cahyono (2021) on *Drosophila melanogaster* fruit flies, achieving a catch rate of 71 flies per trap over seven days. Similarly, Bhagat et al. (2013) utilized nanogel technology with the methyl eugenol pheromone compound, which proved highly effective in attracting fruit flies (*B. dorsalis*) in field settings. The use of pheromone nanogel enhances the stability of slow-release compounds, reducing the frequency of replenishment. Nuryanti et al. (2018) also demonstrated the utilization of nanoemulsion formulations of Piper retrofractum and Tagetes erecta extracts to strengthen their potential as botanical insecticides to control rice WBC.

Given these challenges and solutions, research is necessary to explore the use of nanotechnology to

improve the efficiency and effectiveness of *F. variegata* phytochemical compounds as attractants for *S. adipata* in cayenne pepper plants. The application of attractants and fig extract nanogels represents an innovative approach that could contribute significantly to the development of integrated pest management systems for these pests in the future.

# MATERIALS AND METHODS

**Research Site**. This research was conducted in several locations:

*Sampling*. Sampling of *F. variegata* fruit, used as raw material for attractants, was carried out along the Yeh Belig River in Keramas Village, Blahbatuh District, Gianyar Regency, Bali Province (8°35'50.42" S, 115°19'45.74" E). Fruits aged 9–16 weeks were selected, and 100 kg of fruit was collected using gauze bags.

**Processing**. The slicing and drying of the fruit was conducted in Munggu Village, Mengwi District, Badung Regency, Bali Province (8°36'59.37" S, 115°07'37.00" E), Indonesia. Grinding and extraction of the fruit was carried out at the Genetic Resources and Molecular Biology Laboratory at Udayana University, Indonesia.

Chemical Analysis. The LC-MS/MS assay was performed using the following equipment: The LC-System: ACQUITY UPLC®H-Class System (Waters, USA), LC Column: ACQUITY UPLC® HSS C18 (1.8  $\mu$ m 2.1 × 100 mm) (Waters, USA), and Mass Spectrometer: Xevo G2-S QTof (Waters, USA). The LC-MS/MS tests were conducted at the Forensic Laboratory of the National Police Headquarters, East Jakarta, Indonesia.

*Nanotechnology Development*. The production of nano extracts and nanogel extracts was carried out at the Research Laboratory of the Chemistry Study Program, Faculty of Mathematics and Natural Sciences (FMIPA), Udayana University.

*Field Testing*. Field tests *F. variegata* fruit extracts, nano extracts, and nanogels were conducted at a chili farm in Bonyoh Village, Kintamani District, Bangli Regency, Bali Province, Indonesia (8°17'42.14" S, 115°19'24.43" E).

**Procedure for Making Simplisia and Concentrated Extract of** *F. variegata* **Fruit.** The *F. variegata* fruit used as raw material for extracts was selected intact and fresh. The fruit was collected and thoroughly washed with running water to remove any attached dirt. Afterward, the fruit was air-dried for approximately one hour. Next, the fruit was thinly sliced using a knife to a thickness of 1 mm. The thin slices were spread on a ceramic floor covered with gauze, avoiding direct sunlight, in a room with a temperature range of 20–25 °C. Drying was carried out for 10 days until the weight became constant, producing the *simplisia*. Room temperature was measured using a Clock/Humidity HTC-1 thermometer.

The *simplisia* was then ground using a Philips 5000 Series 2L blender. The pulverisation material was sieved with a 105-mesh sieve and subsequently extracted using the maceration method (Febrina et al., 2015). Maceration process:

Prepation: Two kilograms of *F. variegata* fruit powder was placed in a 5.5 kg glass jar. A 99.5% butanol solvent was added at a ratio of 1:30 (Febrina et al., 2015).

Soaking: The mixture was soaked for four days with daily stirring for five minutes.

Filtration: The mixture was filtered every 24 hours using 110 mm filter paper to collect the filtrate. The residue from the filtration was re-macerated following the same process.

The collected filtrate was concentrated using a Buchi rotary evaporator (Swiss-made) to obtain a concentrated extract. The extract obtained from maceration was subjected to LC-MS testing, while the remainder was used to prepare nanoextract and nanogel formulations of *F. variegata* fruit extract.

LC-MS/MS Test Procedure for Concentrated Extract of *F. variegata* Fruit. Sample preparation is a critical step before injecting the sample into the chromatography system. The objective of this step is to minimize the presence of impurities that could interfere with the analysis by removing components other than the analyte. For this process, 2-5 g of F. variegata fruit extract was prepared. The preparation began with diluting the F. variegata fruit extract tenfold using methanol. Subsequently, precipitates and solid particles were separated through centrifugation. Centrifugation was performed at room temperature of 24.9 °C with a rotation speed of 14,000 rpm. For this analysis, 1 microliter of the prepared sample was taken and injected into the LC-MS/MS system. The sample analysis was carried out using the LC-MS/MS system with the following specifications: LC System: ACQUITY UPLC® H-Class System (Waters, USA), LC Column: ACQUITY UPLC® HSS C18 (1.8 µm 2.1 × 100 mm) (Waters, USA), and Mass Spectrometer: Xevo G2-S QTof (Waters, USA).

# Procedure for Making *F. variegata* Fruit Extract, Nano Extract, and Nanogel.

**Preparation of F. variegata Fruit Nano Extract.** The formulation of the *F. variegata* fruit nano extract was prepared by mixing Tween 80, VCO (Virgin Coconut Oil), PEG 400, and *F. variegata* fruit (Table 1) in a glass beaker. The mixture was stirred with a magnetic stirrer at 1000 rpm for 15 min. After stirring, distilled water was slowly added, little by little, at the same stirring speed until the total volume reached 100%.

The mixture was then subjected to ultrasonication for 2 hours without temperature treatment to prevent damage to the bioactive components of *F. variegata* fruit. The particle size of nanoemulsion was determined by analyzing the polydispersity index and droplet size distribution using a Delsa<sup>TM</sup> Nanoparticle Size

Table 1. Material composition and fruit extract formula of F. variegata

| Materials                     | Consentration formula (%) |
|-------------------------------|---------------------------|
| Fruit extract of F. variegata | 2                         |
| VCO                           | 8                         |
| Tween 80                      | 17                        |
| PEG 400                       | 9                         |
| Aquadest                      | Add 100                   |

| Table 2. Material | composition and | nanogel formula |
|-------------------|-----------------|-----------------|
|                   |                 |                 |

| Materials        | Composition formula |
|------------------|---------------------|
| Karbopol 940     | 1 g                 |
| Propylene Glikol | 5 g                 |
| TEA              | 2 g                 |
| Methylparaben    | 0,5 g               |
| Aquadest         | Add 100             |

Analyzer (Beckman Coulter, China). Measurements were performed with 4 replicates. Samples of the nanoemulsion were placed in cuvettes, and the droplet size and polydispersity index were recorded.

*Preparation of Nanogel from F. variegata Fruit Extract.* The nanogel was prepared using the casting method as follows:

Gel Base Preparation: All ingredients were weighed accordingly (Table 2). Carbophol 940 was dispersed in hot water and allowed to swell for 5 min. A mixture of Triethanolamine (TEA), propylene glycol, and methylparaben was prepared separately (mass 2). Mass 2 was added to the gel base (mass 1) and stirred until homogeneous, forming the gel base.

Addition of Nanoemulsion: The nanoemulsion of F. *variegata* fruit extract was added to the gel base at concentrations of 0%, 10%, 20%, 30%, 40%, and 50%. The mixture was stirred until homogeneous to produce the final nanogel formulations.

**Procedure for Making Plastic Traps and Installing Attractants.** Plastic traps were made using transparent plastic bottles measuring 5 cm in length and 1.4 cm in diameter. Four symmetrically placed holes, each 0.6–0.7 cm in diameter, were created around the bottle at a distance of 10 cm from the bottle cap. These holes served as entry points for *S. adipata*. A hole was also made in the bottle cap to insert a wire for hanging the trap and securing the attractant inside. A spool of cotton containing 5 g of attractant was attached to each bottle. The traps were then hung at a height of 1 m in a cayenne pepper plantation. The same procedure was followed for traps containing nano extracts and nanogel formulations of *F. variegata* fruit extract (Figure 1).

Testing Procedure for Fruit Extract Attractant,

Fruit Extract Nano, and Fruit Extract Nano Gel

in the Field. The research involved 2 factors: type of attractant and attractant concentration. Type of attractant consisted of E= F. variegata fruit extract, En= fruit nano extract, Eg=*F. variegata* fruit extract nanogel and water was used as a control. While attractant concentration consists of 0; 10%; 20%; 30%; 40%; and 50%. Each combination treatment was replicated using 18 traps, resulting in a total of 54 traps. Traps were installed 10 m apart. The experimental design followed a randomized block design (RBD) with a split-plot factorial arrangement. The experiment was repeated three times (Figure 2). Observation of the number of catches was carried out every day from the time the trap was installed until no trapped flies were found. All catches were counted both alive and dead. catch counts and records were made daily and data were analysed.

Procedure for Carrying Out F. variegata Fruit Extract, Nano Extract, and Nano Gel Extract Tests in the Field. The research consisted of two factors, namely the type of attractant and the attractant concentration. The attractant type treatment consisted of E = F. *variegata* fruit extract, En = fruit nano extract, Eg = F. variegata fruit extract nanogel and water was used as a control. While concentration consists of 0; 10%; 20%; 30%; 40%; and 50%. The combination treatment in each replication amounted to 18 traps so there was a total of 54 traps. Traps were installed with a distance between traps of 10 m. The basic design used was a randomized block design with a factorial pattern. This research was repeated three times (Figure 2). Observations of trap catches were conducted daily from the time the traps were installed until no flies were trapped. Both live and dead flies were counted. Catch data were recorded daily and analyzed. Effectiveness and efficiency were the primary variables used to assess the innovation

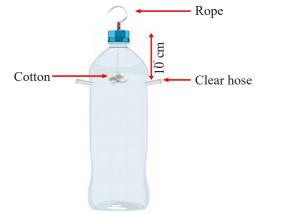


Figure 1. Plastic trap model used to trap S. adipata in the field.

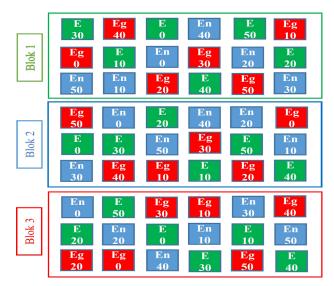


Figure 2. Layout of a field research experiment. Discription: E = F. *variegata* fruit extract, En = F. *variegata* fruit extract nanogel, Eg = F. *variegata* fruit extract nanogel.

and technology of the attractants: Effectiveness: Measured based on the number of flies caught by each type of attractant at different concentration levels. Efficiency: Measured by the duration of attractant persistence, or the length of time each attractant at different concentrations successfully caught *S. adipata* adults in the field.

#### Data Analysis.

*Phytochemical Analysis*. The data on phytochemical compounds in *F. variegata* fruit extract as an attractant were analyzed using the MassLynx v4.1 application (https://waters-masslynx-scn781.software.informer. com/4.1/). Retention times with corresponding molecular formulas were identified. The chemical name, compound class, and structural formula were determined using ChemSpider.

*Attractant Effectiveness and Efficiency*. The combined effects of attractant type and concentration (fruit extract, nano extract, and nanogel of *F. variegata* fruit extract) were were analyzed using analysis of variance (ANOVA), followed by Duncan's Multiple Range Test (DMRT) at a significance level of 5%. Data analysis was performed using IBM SPSS Statistics version 24 (SPSS 24).

*Attractant Persistence*. To persistence of each attractant was assessed by analyzing daily catch data for each concentration and attractant type. Results were presented in histogram form to visualize the duration of efficacy across treatments.

# **RESULTS AND DISCUSSION**

**Phytochemical Compound Profile of** *F. variegata* **Fruit.** To understand the content and composition of phytochemical compounds in *F. variegata* fruit, the analysis focused on fruit at different stages of development: 1–8 weeks; 9–16 weeks, and 17–22 weeks. The identification results of each fruit phenology are presented in Tables 3, 4, and 5, while LC-MS/MS chromatograms of *F. variegata* fruit extracts are shown in Figures 3, 4, and 5. The nanoparticle size of the fruit extract was found to be 604 nm.

Phytochemical Compound Profile of F. variegata Fruit Aged 1-8 Weeks. The LC-MS/MS chromatogram of F. variegata fruit extract for this age group was analyzed using the MassLynx v4.1 application (Figure 3). The analysis results provided peak retention times and corresponding molecular formulas. Using www. chemspider.com, the chemical name, compound class, and structural formula of the identified compound were determined (Table 3). At a retention time of 5.43, the compound was strongly suspected to be luteolin, with the IUPAC name 2-(3,4-dihydroxyphenyl)-5,7dihydroxychromen-4-one. Luteolin is classified as a flavonoid. This compound has also identified in plants of the genus Ficus (Sieniawska et al., 2022; Olaoluwa et al., 2022). Additionally, the LC-MS analysis of F. variegata fruit aged 1-8 weeks revealed a significant presence of the phytochemical compound 5-hydroxy2-(4-hydroxyphenyl)-8,8-dimethylpyrano[2,3-h] chromen-4-one, which constituted 48.60% of the total composition.

**Phytochemical Compound Profile of** *F. variegata* **Fruit Aged 9–16 Weeks.** Using the same method, LC-MS/ MS chromatograms were obtained for *F. variegata* fruit extract, which was analyzed using the MassLynx v4.1 application (https://waters-masslynx-scn781.software. informer.com/4.1/) (Figure 4). The analysis provided peak retention times along with their corresponding molecular formulas. The chemical name, compound class, and structural formula of the identified compound were determined using https://waters-masslynx-scn781. software.informer.com/4.1/ (Table 4).

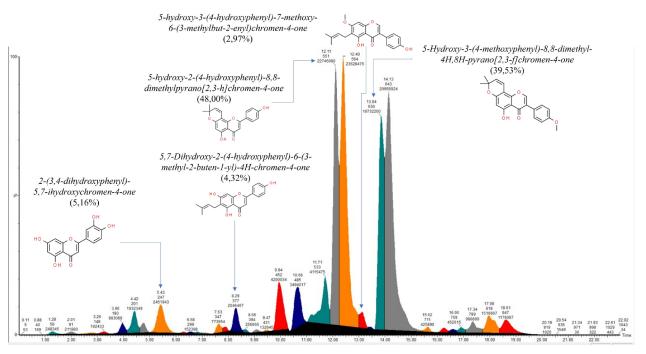


Figure 3. LC-MS/MS chromatograms of F. variegata fruit extract aged 1-8 weeks.

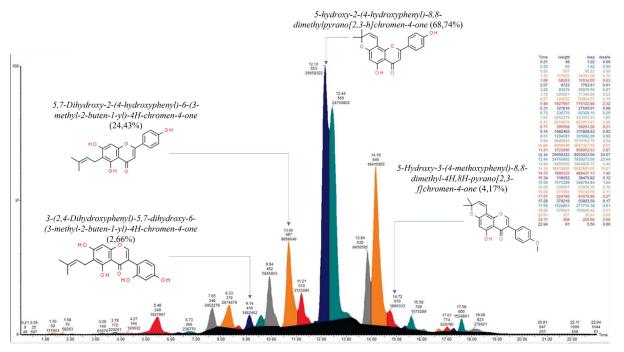


Figure 4. LC-MS/MS chromatograms of F. variegata fruit extract aged 9-16 weeks.

| Time<br>(Rt)Precursor Ions5.43287 (peak ion)8.29339 (peak ion)12.11337 (peak ion) | e Dradinat Ione     |  | Chemical Moleculer | Chemical Compound |                            |
|---|---------------------|--|--------------------|-------------------|----------------------------|
|   |                     | Chemical Name  | Formula            | Group             | Chemical Structure         |
|   | 1) 153              | Luteolin<br>IUPAC: 2-(3,4-dihydroxy-<br>phenyl)-5,7-ihydroxy-<br>chromen-4-one   | C15H1006           | Flavonoid         | HO<br>HO<br>OH<br>HO<br>OH |
|   | 1) 283, 165         | 6-Prenylapigenin<br>IUPAC: 5,7-Dihydroxy-2-<br>(4-hydroxyphenyl)-6-(3-<br>methyl-2-buten-1-yl)-4H-<br>chromen-4-one  | C20H18O5           | Flavonoid         | HO OH HO                   |
|   | () 321, 319,<br>203 | IUPAC: 5-hydroxy-2-<br>(4-hydroxyphenyl)-8,8-<br>dimethylpyrano[2,3-h]<br>chromen-4-one  | C20H16O5           | Flavonoid         | HO                         |
| 13.12 353 (peak ion)  | 1) 338, 283         | Gancaonin G<br>IUPAC: 5-hydroxy-3-<br>(4-hydroxyphenyl)-7-<br>methoxy-6-(3-methylbut-2-<br>enyl)chromen-4-one  | C21H2005           | Flavonoid         | HO                         |
| 13.84 351 (peak ion)  | () 337, 219         | <ul> <li>4'-O-Methylderrone</li> <li>IUPAC: 5-Hydroxy-</li> <li>3-(4-methoxyphenyl)-</li> <li>8,8-dimethyl-</li> <li>4H,8H-pyrano[2,3-f]</li> <li>chromen-4-one</li> </ul> | C21H18O5           | Isoflavone        |                            |

Table 3. Composition of phytochemical compounds of F. variegata fruit extract aged 1-8 weeks

Description: fruit extract analysed by LC-MS/MS

| Retention    | m/z Results (M+H)            | ts (M+H)                     |  | Chemical Molecular  | Chemical Compound           |             |
|--------------|------------------------------|------------------------------|--|---------------------|-----------------------------|-------------|
| Time<br>(Rt) | Precursor Ions               | Product Ions                 | Chemical Name  | Formula             | Group<br>Chemical Structure | Structure   |
| 9.14         | 355 (peak ion)               | 355 (peak ion) 337, 283, 165 | Luteone<br>IUPAC:<br>3-(2,4-Dihydroxyphenyl)-<br>5,7-dihydroxy-6-(3-<br>methyl-2-buten-1-yl)-4H-<br>chromen-4-one  | $C_{20}H_{18}O_{6}$ | Isoflavone                  | HO HO HO OH |
| 10.66        | 339 (peak ion)               | 283, 165                     | 6-Prenylapigenin<br>IUPAC: 5,7-Dihydroxy-2-<br>(4-hydroxyphenyl)-6-(3-<br>methyl-2-buten-1-yl)-4H-<br>chromen-4-one  | $C_{20}H_{18}O_5$   | Flavonoid                   | HO I O OH   |
| 12.09        | 337 (peak ion) 321, 319, 203 | 321, 319, 203                | IUPAC: 5-hydroxy-2-<br>(4-hydroxyphenyl)-8,8-<br>dimethylpyrano[2,3-h]<br>chromen-4-one  | $C_{20}H_{16}O_5$   | Flavonoid                   |             |
| 14.72        | 351 (peak ion)               | 337, 219                     | <ul> <li>4'-O-Methylderrone</li> <li>1UPAC: 5-Hydroxy-</li> <li>3-(4-methoxyphenyl)-</li> <li>8,8-dimethyl-</li> <li>4H,8H-pyrano[2,3-f]</li> <li>chromen-4-one</li> </ul> | $C_{21}H_{18}O_5$   | Isoflavone                  |             |

| Retention    | m/z Results (M+H) | (H+H)            |   | Chemical Molecular | Chemical Compound           |  |
|--------------|-------------------|------------------|---|--------------------|-----------------------------|--|
| Time<br>(Rt) | Precursor lons    | Product Ions     | Chemical Name   | Formula            | Group<br>Chemical Structure | Structure  |
| 10.75        | 339 (peak ion)    | 283, 165         | 6-Prenylapigenin<br>IUPAC:<br>5,7-Dihydroxy-2-(4-<br>hydroxyphenyl)-6-(3-<br>methyl-2- buten-1-yl)-4H-<br>chromen-4-one                                 | C20H18O5           | Flavonoid                   | но он он он он   |
| 10.95        | 355 (peak ion)    | 337, 283,<br>165 | Luteone<br>IUPAC: 3-(2,4-<br>Dihydroxyphenyl)-5,7-<br>dihydroxy-6-(3-methyl-2-<br>buten-1-yl)-4H-chromen-<br>4-one                                      | C20H18O6           | Isoflavone                  | HO HO HO   |
| 12.16        | 337 (peak ion)    | 321, 319,<br>203 | IUPAC: 5-hydroxy-2-(4-<br>hydroxyphenyl)-8,8-<br>dimethylpyrano[2,3- h]<br>chromen-4-one  | C20H16O5           | Flavonoid                   | He contraction of the second s |
| 13.21        | 338 (peak ion)    | 353, 283         | Gancaonin G<br>IUPAC: 5-hydroxy-3-(4-<br>hydroxyphenyl)-<br>7-methoxy-6-<br>(3-methylbut-2-enyl)<br>chromen-<br>4-one                                   | C21H20O5           | Flavonoid                   |  |
| 14.18        | 351 (peak ion)    | 337, 219         | <ul> <li>4'-O-Methylderrone</li> <li>IUPAC: 5-Hydroxy-3-(4-methoxyphenyl)-8,8-dimethyl-</li> <li>4H,8H-pyrano[2,3-f]</li> <li>chromen- 4-one</li> </ul> | C21H18O5           | Isoflavone                  |  |

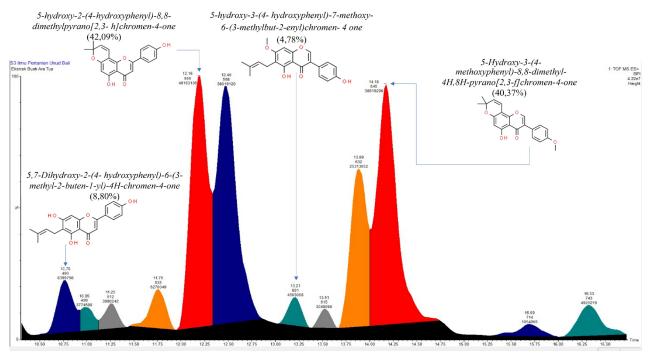


Figure 5. LC-MS/MS chromatograms of F. variegata fruit extract aged 17-22 weeks.

Based on the LC-MS test, the major phytochemical compound in *F. variegata* fruit aged 9–16 week was identified as 5-hydroxy-2-(4-hydroxyphenyl)-8,8-dimethylpyrano[2,3-h] chromen-4-one, which was present at the highest concentration, constituting 68.74% of the total composition.

**Phytochemical Compound Profile of** *F. variegata* **Fruit Aged 17–22 Weeks.** Using the same method, LC MS/MS chromatograms were obtained for *F. variegata* fruit extract, which was analyzed using the MassLynx v4.1 application (Figure 5). The analysis provided peak retention times along with their respective molecular formulas. The chemical name, compound class, and structural formula of the identified compounds were determined using www.chemspider.com.

Based on the LC-MS test, the major phytochemical compound in *F. variegata* fruit aged 17–22 weeks was identified as 5-hydroxy-2-(4-hydroxyphenyl)-8,8-dimethylpyrano[2,3-h] chromen-4-one. The concentration of this compound was 42.09%, which is lower compared to its content in fruits aged 9–16 weeks (68.74%).

The compound 5-hydroxy-2-(4-hydroxyphenyl)-8,8-dimethylpyrano[2,3-h] chromen-4-one belongs to the flavonoid group, as signifivant class of secondary metabolites in plants. Flavonoids are derivatives of 2-phenyl-benzyl- $\gamma$ -pyrone and are synthesized via the phenylpropanoid pathway.

Flavonoids perform several roles in plants,

including contributing to color, flavor in seeds, flowers, and fruits, as well as aroma (Mierziak et al., 2014). Additionally, flavonoids act as attractants for insects (Subramanian et al., 2007).

Mierziak et al. (2014) reported that flavonoids are key chemicals in regulating insect feeding and oviposition behavior. For example: 1) Compounds such as naringenin, hesperetin-7-O-rutinoside, and quercetin-3-O-rutinoside from the flavonoid group, along with other active compounds, stimulate insects oviposition, such as butterflies on young leaves of citrus plants (Ohsugi et al., 2006). Similar activity was also found in the compound luteolin 7-O-(6"-malonyl glucoside) which can stimulate oviposit of the insect *Papilio polyxenes* (Feeny et al., 1988). Meanwhile, the compound isorhamnetin glucoside has been reported to stimulate *Luehdorfia japonica* oviposition on the leaves of plants from the genus Asarum (Nishida, 1994).

Fragmentation of similar compounds carried out by Kaszynska et al. (2022) found that the precursor ion peak that appeared was (M+H) + 287 (C15H10O6) (peak ion) and the product ion was at m/z 153. The peak that appeared at m/z 153 was [(M+H)-134]+ due to the release of the C8H6O2 group. Our findings show that the compound obtained from the LC-MS/MS results of *F. variegata* fruit extract is the compound 5-hydroxy-2-(4-hydroxyphenyl)-8,8-dimethylpyrano[2,3-h] chromen-4- one from the flavonoid group. Flavonoids are one of the important secondary metabolites in plants which are derivatives of 2-phenyl-benzyl- $\gamma$ - pyrone with biosynthesis using the phenylpropanoid pathway. Flavonoids play a role in plants as forming color, flavor in seeds, flowers and fruit as well as aroma (Mierziak et al., 2014). Apart from that, flavonoids also have an odor which acts as an attractant for insects (Subramanian et al., 2007). Mierziak et al. (2014) reported that flavonoids are one of the chemicals that can regulate insect feeding and ovivosis behavior. The compounds naringenin, hesperetin-7-O-rutinoside and quercetin-3-O-rutinoside from the flavonoid group, together with other active compounds, stimulate the oviposition, such as butterflies, on young citrus leaves (Ohsugi et al., 2006); 2) Luteolin 7-O-(6"-malonyl glucoside) has been shown to stimulate oviposition by Papilio polyxenes (Feeny et al., 1988); 3) Isorhamnetin glucoside stimulates oviposition by Luehdorfia japonica on the leaves of plants from the genus Asarum (Nishida, 1994).

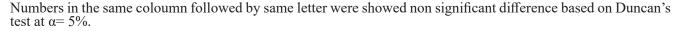
The results of this research revealed that F. variegata contains a phytochemical compound,

5-hydroxy-2-(4-hydroxyphenyl)-8,8-dimethylpyrano[2,3-h] chromen-4-one, with the highest concentration (68.74%) observed in fruits aged 9-16 weeks. The compound demonstrates strong potential as an attractant for S. *adipata*. In contrast, Abbes et al. (2021) reported that the phytochemical compound hexanol, derived from *F. carica* fruit extract, acts as an attractant for *S. adipata*. Similarly, Bhagat et al. (2013) utilized nanogel technology with the methyl eugenol pheromone compound to effectively attract fruit flies (*B. dorsalis*) in the field.

Effectiveness and Efficiency of *F. variegata* Fruit Extract, Nano extract and Nanogel extract as Attractants for *S. adipata*. The results of variance analysis showed that the type of attractant and the concentration level of the attractant compound had a significant effect on attracting *S. adipata* adults, as indicated by the trap catch data for each treatment. The results of these variables are presented in Table 6

 Table 6. The effectiveness and efficiency of traps using a combination of extract concentration levels and attractant types made from extracts, nano extracts and nanogel extracts from *F. variegata* fruit

| Treatment of      | Attractant type treatment |             |                 |
|-------------------|---------------------------|-------------|-----------------|
| concentration (%) | Extract                   | Nanoextract | Nanogel Extract |
| 0                 | 0,00 i                    | 0,00 i      | 0,00 i          |
| 10                | 4,33 h                    | 6,00 gh     | 7,00 gh         |
| 20                | 8,00 ef                   | 10,33 ef    | 13,33 de        |
| 30                | 10,67 ef                  | 14,67 d     | 20,33 c         |
| 40                | 14,00 d                   | 20,33 c     | 27,00 b         |
| 50                | 19,67 c                   | 27,00 b     | 36,00 a         |



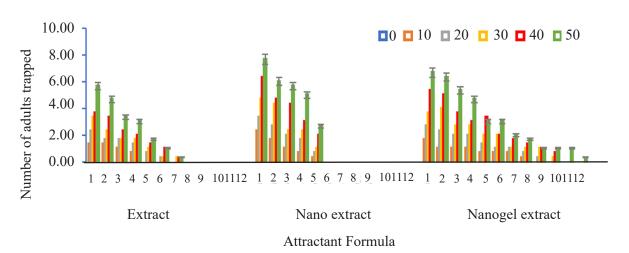


Figure 6. Effectiveness and efficiency of using traps with attractants made from extracts, nano extracts and nanogels fruit extracts of *F. variegata* on catches of adult *S. adipata* in the field.

and Figure 6. Among the three types of attractants, the nanogel extract was superior to the nano extract and *F. variegata* fruit extract at all tested concentration levels.

The most effective concentration for capturing *S*. *adipata* adults was 50%, followed by 40% and 30%. An increase in the concentration of the three attractants resulted in a corresponding increase in trap catches.

Further analysis of the combination of attractant type and concentration level revealed that the nanogel extract at a of 50% concentration was significantly more effective, capturing 36.0 adults/trap, compared to other combinations. The nanogel extract at a 40% concentration also performed well, capturing 27.00 adults/trap, which was equivalent to the nano extract at a 50% concentration.

Reducing the nanogel extract concentration to 30% resulted in a significant decrease in the number of catches to 20.33 adults/trap, a result similar to the nano extract at a 40% concentration and the fruit extract at a 50% concentration. Even at a 20% concentration, the nanogel extract caught 13.33 adults/trap, which was not significantly different from the nano extract at a 30% concentration (14.67 adults/trap). The fruit extract at a 40% concentration also yielded similar results, capturing 14.0 adults/trap (Table 6).

The lowest catches was observed with the fruit extract at a 10% concentration, capturing only 4.33 adults/trap.

# Performance and Longevity of F. variegata Nanogel

**Extract**. The nanogel extract of *F. variegata* fruit demonstrated a slow-release property, allowing the compound to vaporize gradually and last up to 12 days in the field. This slow release is comparable to the myristic acid-chitosan (MA-chitosan) nanogel of *Carum copticum* (L.) essential oil, which is effectively acted as an insecticide, lasting 21 days against *Sitophilus granaries* and up to 33 days against *Tribolium confusum*.

Similarly, methyl eugenol nanogel had a trapping duration of up to 30 days, capturing 6,636.36 fruit flies, compared to methyl eugenol without nanogel, which only caught 359 fruit flies in 7 days (Bhagat et al., 2013).

Demonstrated the effectiveness of nanotechnology when applied to nanosex pheromones for controlling the yellow rice stem borer (*Scirpophaga incertulas*). Similarly, the application of nanotechnology in attractants by Islamiyah & Cahyono (2021) was shown to be effective for *Drosophila melalogaster* fruit flies, with a catch rate of 71 flies per trap per 7 days. El-Wahab et al. (2021) also applied nanogel pheromone traps to capture *Rhynchophorus ferrugineus* beetles with highly effective results, achieving a catch rate of 4.26 individuals per day and a total catch of 55.33 individuals per trap. These results were significantly better compared to pheromones without nanotechnology, which only captured 2.69 individuals per trap per day, with a total catch of 35 individuals per trap.

Nanogel pheromones are more stable than traditional pheromones that do not utilize nanogel technology. For example, the highly volatile methyl eugenolpheromone can be protected from environmental factors such as air, water, and sunlight through the use of nanogel formulations (Ansari, 2019). This enhanced stability is crucial for improving the effectiveness of pheromone-based pest control strategies, particularly in trap-based systems, where the longevity of attractants plays a key role. In this context, Kurnianto et al. (2024) demonstrated that attractants contribute significantly to controlling coffee fruit powder pests through trap-based methods, while Kardinan & Maris (2022) highlighted their effectiveness in detecting and monitoring fruit fly pests (Bactrocera spp.). The integration of nanogel technology in pheromone formulations can further enhance the efficiency and durability of these attractants, ensuring more reliable and sustainable pest management.

#### CONCLUSION

The phytochemical profile of F. variegata fruit extract identified 5-hydroxy-2-(4-hydroxyphenyl)-8,8dimethylpirano[2,3-h] chromen-4-one as the major compound, with the highest concentration of 68.74% in fruits aged 9-16 weeks. Phytochemical compounds from F. variegata fruit extracts show significant potential as attractants for S. adipata. The application of nanogel technology with a 50% concentration of F. variegata fruit extract enhances its potential as a highly effective and efficient bio-attractant, achieving a capture rate of 36 individuals per trap using plastic bottle traps. The attractant properties of the F. variegata fruit extract nanogel remained effective for up to 12 days after installation, successfully attracting adult black flies. The innovation of using F. variegata fruit extract nanogel technology represents a novel approach for detecting and monitoring S. adipata black fly pests in white chili plants. This finding provides strong support for the advancement of integrated pest management strategies for S. adipata in chili plants in Bali and across Indonesia.

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#### **AUTHORS' CONTRIBUTIONS**

SKS and AAKD designed the research structure and methods. IMSW and DAY analyzed the data and conducted cross-references for bioactive compounds. All authors were involved in data collection and manuscript writing. SKS and IMSW proofread the manuscript and prepared the final version. All authors have read and approved the final manuscript.

# **COMPETING INTEREST**

The authors declare that there is no potential conflict of interest.

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