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Research Article

Geranium Aralia, *Polyscias guilfoylei* (W. Bull) L.H. Bailey), Leaf Extract Toxicity against Melon Fly, *Zeugodacus cucurbitae* Coquillett

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ABSTRACT

Zeugodacus cucurbitae Coquillett is one of the most detrimental pests on cucurbits. Control of this pest species often uses insecticides and *Polyscias guilfoylei* is a potential source for bio insecticides. This research aimed to study the influence of *P. guilfoylei* leaf extract application on *Z. cucurbitae* mortality and to determine the LC_{50} value. The research was conducted at Pest Invertebrate Plant Pest Science Laboratory, Department of Plant Protection, Faculty of Agriculture, Universitas Gadjah Mada. Toxicity test was done using contact, oral and residue methods by applying leaf extracts in ethanol and *n*-hexane extracts with concentrations of 0; 1.25; 2.50; 5.00; 10.00; and 20.00%. The research used a complete randomized design with six replicates. Parameters observed included the number of dead melon flies at 72 hours after treatments and LC_{50} value of each extract. Data was analyzed using Probit analysis via 1.02 version of Lenora software Polo Plus. Result showed that ethanol and *n*-hexane extracts of *P. guilfoylei* leaf using oral and residue methods affected *Z. cucurbitae* mortality. However, in contact method using ethanol and *n*-hexane extracts of *P. guilfoylei* leaf did not affect *Z. cucurbitae* mortality. LC_{50} values of ethanol extracts using oral and residual methods were 6.168% and 3.658%, respectively. LC_{50} values of *n*-hexane extracts using oral and residual method reached 5.311% and 6.607%, respectively. This research showed that ethanol and *n*-hexane extracts of *P. guilfoylei* leaf contained secondary metabolites that were toxic against melon fly *Z. cucurbitae*.

Keywords: LC_{50} value; mortality; *Polyscias guilfoylei*; *Zeugodacus cucurbitae*

INTRODUCTION

Fruit fly (Tephritidae) is one of the most detrimental pests in Indonesia and other countries for damaging high economic valued crops, such as fruits and vegetables. Damage induced by the insect, fruit fly, may result in a qualitative and quantitative financial loss (Rousse *et al.*, 2005; Copeland *et al.*, 2006; Putra & Suputa, 2013). One of the most important pest species damaging cucurbits and other vegetable crops in Hawaii and Indo-Malaya is the melon fly *Zeugodacus cucurbitae* Coquillett (Weems Jr. *et al.*, 2012). This pest mostly attacks watermelon, cucumber, melon, pumpkin and other plants (Weems, Jr. *et al.*, 2012; Mohanadas & Mukund, 2013; Mir *et al.*, 2014; Astriyani *et al.*, 2016; Agustini *et al.*, 2019; Maha *et al.*, 2019). *Z. cucurbitae* may reduce 30% up to 100% of the yields (Dhillon *et al.*, 2005). Chemically, fruit fly pest control normally rely singular use or combinations of attractants, traps, and synthetic insecticides (Sapkota *et al.*, 2010; Hasyim *et al.*, 2014, 2020; Kubar *et al.*, 2021). Continuous use of synthetic insecticides may damage yield and pollute the environment. Insecticides made from plant extracts known as botanical insecticides are now under development to solve this problem (Kardinan, 2011). Plant extracts can be used as botanical insecticides since its active ingredients are poisonous towards pests thus causing mortality (Jiang *et al.*, 2009; Duraipandiyan *et al.*, 2011; López *et al.*, 2011; Pandey *et al.*, 2014). Active compounds in plant extracts may include saponins, alkaloid, flavonoid and terpenoids. Those active compounds

could act as poisons toward plant pests (Nenaah, 2011; Silva *et al*., 2016; Attaullah *et al*., 2020; Rashwan & Hammad, 2020). A plant with potency as a botanical insecticide is geranium aralia (*Polyscias guilfoylei*). This plant is commonly used as shrubs and medicinal plants. The results of the analysis using UV-vis spectrophotometry and thin layer chromatography showed that ethanol extract of *P. guilfoylei* leaves contained organic compounds such are phenols, tannins, alkaloids, flavonoids and saponins, while *n*-hexane leaves of *P. guilfoylei* contained terpenoid, steroids, phenols, tannins, alkaloids, flavonoids and saponins (Rachmawati *et al*., 2022). Geranium aralia contained saponins that acted as molluscicides, bactericides, and fungicides (Cioffi *et al*., 2008; Sundu *et al*., 2015; Ashmawy *et al*., 2019; Anh *et al*., 2021). *P. guilfoylei* leaf extract applied to melon fly *Z. cucurbitae* may be toxic. This research aimed to learn the influence of *P. guilfoylei* leaf extract application on melon fly *B. cucurbitae* mortality and determine LC_{50}.

MATERIALS AND METHODS

Plant Origin

Polyscias guilfoylei leaf used in this research originated from Bojongmenger Village, Cijeungjing, Ciamis, West Java. The district coordinates were 7.33 SL, 108.42 EL and 124 masl. As much as 2.2 kg of leaves were collected in March 2019 and were the third to seventh leaves from the shoot tips. Collected leaves were initially identified at Plant Systematics Laboratory at the Faculty of Biology, Universitas Gadjah Mada (UGM). Leaves were dried at Phytochemical Laboratory, Department of Pharmaceutical Biology, Faculty of Pharmacy, UGM using an oven at the temperature of 50°C for three days. After dried, they were milled using a special blender for leaves to be pulverized and weighed to 350 g.

Polyscias guilfoylei Leaf Extraction

Polyscias guilfoylei leaf extraction was conducted at Phytochemical Laboratory, Department of Pharmaceutical Biology, Faculty of Pharmacy, UGM using maceration method (Harborne, 1987). *P. guilfoylei* leaf powder was diluted in two types of solvent namely, 70% ethanol and *n*-hexane. The ratio of *P guilfoylei* leaf powder to the solvent used was 1:10 (w/v). 50 g *P. guilfoylei* leaf powder was soaked with 500 ml ethanol and 300 g powder was soaked in 3 L *n*-hexane for 72 hours. Each bath was then filtrated through Buchner funnel lined with paper filter. The filtration products with ethanol solvent were then evaporated using water bath while products with *n*-hexane was evaporated in a vacuum room until thickened. As much as 16.45 g and 23.19 g of crude extracts were obtained from the ethanol solvent and *n*-hexane solvents, respectively. Each of those crude extracts was inserted to a glass bottle and kept in the refrigerator at a temperature of 10°C prior to the test. To obtain the concentration series required in this test, dilution was carried out with ethanol or *n*-hexane solvent.

Experimental Insect Rearing

Adult *Z. cucurbitae* used for experiments were reared at Pest Invertebrate Plant Pest Science Laboratory, Department of Plant Protection, Faculty of Agriculture, UGM. The initial pupae for rearing *Z. cucurbitae* were obtained from the Forecasting Center for Plant Pest Organisms (*Balai Besar Peramalan Organisme Pengganggu Tumbuhan*, BBPOPT) Jatisari, Karawang. *Z. cucurbitae* was reared on artificial diets (Chang *et al*., 2004), conforming to previously developed laboratory conforming. Insects used 7 to 10 days-old imagoes.

Toxicity Test

Toxicity test was carried out using contact, oral, and residue methods. The extracts used were ethanol and *n*-hexane extracts with concentrations of 0; 1.25; 2.50; 5.00; 10.00 and 20.00%. The experiment was set as complete randomized design and each treatment was carried out with six replicates. In contact method, 2 mL of extract concentration was sprayed using a Potter Precision Laboratory Spray Tower inside a 10 cm diameter and 5 cm high jar with a gauze was placed on top. Ten adults of *Z. cucurbitae* were placed in the jar. In the oral method, 2 mL of extracts were mixed into drinking water in each experimental jar. The mixture of extract and drinking water was left in the open air for an hour until the solvent evaporated, then inserted to 10 cm diameter and 5 cm high treatment jars with gauze placed on the lid. 10 adults of *Z. cucurbitae* aged 7 to 10 days were inserted to each jar. In residue method, 2 mL extracts were sprayed using hand sprayer to the inner wall of a 10 cm diameter and 12 cm high jar

with gauze placed on the lid. After being sprayed, the jar was left open for an hour until the solvent evaporated. Twenty individuals were introduced to the jar. Parameters observed included the number of dead insects within 72 hours after the treatment. Melon fly is considered dead when it does not respond to touches or does not move after treatment (Jiang *et al*., 2009). During the final observation, its mortality percentage was calculated.

Data Analysis

Mortality percentage was measured at 72 hours after treatment. LC_{50} value of ethanol and *n*-hexane extracts of *P. guilfoylei* leaf was calculated using Probit analysis via 1.02 version of Lenora software Polo Plus.

RESULTS AND DISCUSSION

P. guilfoylei in ethanol and *n*-hexane tested using oral and residue methods affected *Z. cucurbitae* mortality. Higher extract concentrations caused higher *Z. cucurbitae* mortality. Meanwhile in contact method, ethanol and *n*-hexane extracts did not affect *Z. cucurbitae* mortality at all (Figure 1 and 2). Toxicity test methods showed different effects on adult *Z. cucurbitae* mortality. Contact method had the lowest mortality effects on *Z. cucurbitae* imagoes compared to oral and residue methods. Residue method caused the highest mortality rate on *Z. cucurbitae* imagoes.

Extract solvents and test methods used had different effects on insect mortality. Plant extract affected insect mortality due to its metabolic compounds that act as toxic compounds towards insect. In oral and residue methods, the toxic secondary metabolic compounds were flavonoid, alkaloid, and terpenoids in *P. guilfoylei* leaf extract may induced *Z. cucurbitae* imago mortality. Secondary metabolite contained in extracts were digestive toxic that enter through consumed diets. Secondary metabolite compounds may work on digestive physiology and enzyme related to feeding inhibition (Ansante *et al*., 2017). Secondary metabolic compounds in plant can also be cytotoxic towards insect midgut epithelium. Epithelium cells in midgut are damaged thus causing mortality of that insect (Costa *et al*., 2014). In contact method, the percentage of adult *Z. cucurbitae* mortality was extremely low maybe due to insects' ability to actively ulcerate leaf extracts effects and reduce coverage on insect bodies. Volume extracts attached was only a small amount resulting in low toxicity.

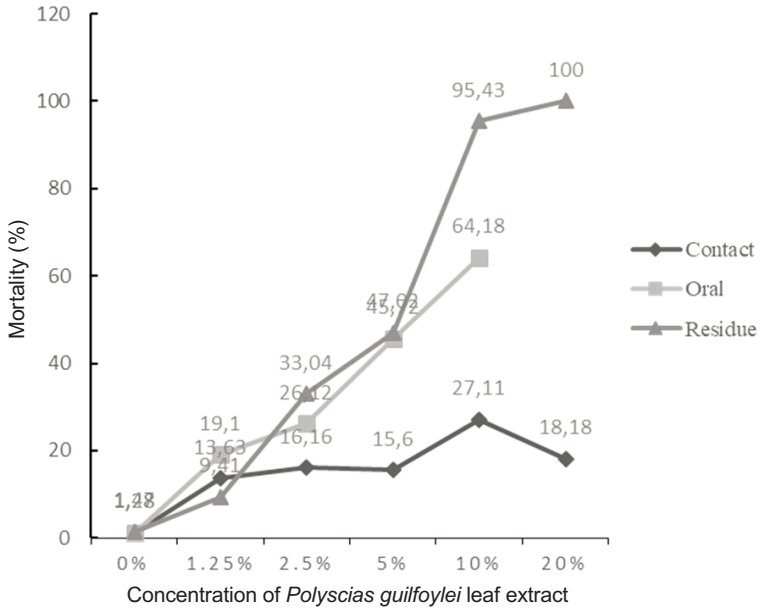

Figure 1. Melon fly *Zeugodacus cucurbitae* imago mortality after treated with ethanol extract of *Polyscias guiloylei* leaf using various testing methods
Contact method: extract was sprayed on insects using Potter Precision Laboratory Spray Tower
Oral method : extract was added to the drinking water of tested imagoes
Residue method: extract was sprayed on inner wall of the experimental jars

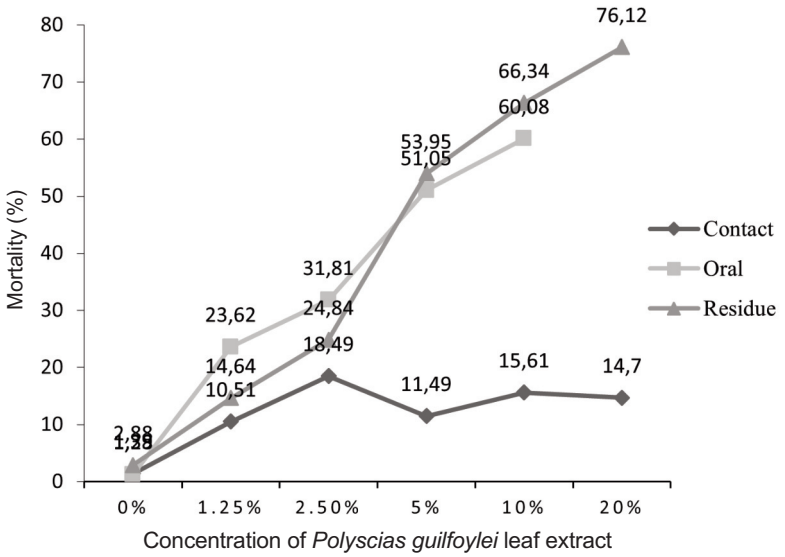

Figure 2. Melon fly *Zeugodacus cucurbitae* imago mortality after treated with n-hexane extract of *Polyscias guilfoylei* leaf using various testing methods
Contact method: extract was sprayed on insects using Potter Precision Laboratory Spray Tower
Oral method : extract was added to the drinking water of tested imagoes
Residue method: extract was sprayed on inner wall of the experimental jars

Ethanol and *n*-hexane solvents were used as control in oral and residual method toxicity tests and did not have negative effect on the mortality of *Z. cucurbitae*. This is based on results at concentration of 0%, mortality of *Z. cucurbitae* was the lowest compared to all treatments. Although at a concentration of 0% *Z. cucurbitae* mortality still occurred, it was still very low (< 3%). This indicated that ethanol and *n*-hexane solvents did not negatively affect the *Z. cucurbitae* mortality. These results were supported by preliminary research where sole application of ethanol and *n*-hexane solvents were used. The results of preliminary studies showed that the use of ethanol and *n*-hexane solvents did not negatively affect the mortality of *Z. cucurbitae* imagoes.

LC_{50} value of each extract using the same test methods were diffrerent. The LC_{50} also varied from the same extract in different test methods. LC_{50} value of ethanol extract in contact method cannot be calculated since the melon fly mortality at concentrations of 20% of ethanol extract was not different and very low. LC_{50} of ethanol extract in oral method was 6.168% (4.563–9.331), while it was 3.658% (2.085–5.372) in residue method. LC_{50} of ethanol extract in contact method cannot be calculated since the melon fly mortality at concentrations of 20% in *n*-hexane extracts was not different and very low. LC_{50} of *n*-hexane extract in oral method was 5.311% (3.877–8.152), while it reached 6.607% (5.530–7.842) in residue method (Table 1).

LC_{50} value indicate the toxicity of an extract. Higher LC_{50} value of an extract indicate lower toxicity. On the contrary, lower LC_{50} indicate higher toxicity. Extract with higher toxicity level means it is more poisonous towards insects. Toxic compounds are effective in inducing insects' mortality. It is supported by Zaka *et al.* (2019) research showing that neem essential oil and citrus extract have the lowest LC_{50} value compared to other experimental plants with 7.39 mg/L and 10.14 mg/L, respectively. This shows that the plant possessed remarkably toxic compounds that cause rapid mortality to *Tribolium confusum* (Tenebrionidae). Sayed *et al*. (2020) also stated that LC_{50} value of *Psiadia penninervia* extract influenced *Aphis craccivora* (Aphididae) mortality. *P. penninervia* extract possessed the lowest LC_{50} value compared to *Salvia officinalis, Ochradenus baccatus, Pulicaria crispa* and *Euryops arabicus* extracts. *P. penninervia* extract contained abundant amount of gallic acid that caused high *A. craccivora* mortality. Research by Hossain and Khalequzzaman (2018) showed that *Azadirachta indica*, *Persicaria hydropiper* and *Vitex negundo* extracts possessed low

Table 1. LC_{50}[a] of *Polyscias guilfoylei* leaf in ethanol and *n*-hexane solvent against melon fly *Zeugodacus cucurbitae* with various contact[b], oral[c] and residue[d] methods

Extract	Test Method	n	Slope (±SE)	LC_{50} (%) (CI 95%)
Ethanol	Contact	357	0.254 (±0.219)	Cannot be calculated
	Oral	392	1.351 (±0.211)	6.168 (4.563-9.331)
	Residue	578	3.159 (±0.289)	3.658 (2.085 – 5.372)
n-Hexane	Contact	361	0.096 (±0.232)	Cannot be calculated
	Oral	366	1.276 (±0.201)	5.311 (3.877-8.152)
	Residue	599	1.823 (±0.181)	6.607 (5.530 – 7.842)

Note:
[a] LC_{50}, concentration of extract that induces 50% experimental insects' mortality.
[b] Contact method: extract was sprayed on insects using Potter Precision Laboratory Spray Tower.
[c] Oral method: extract was added to the drinking water of tested imagoes.
[d] Residue method: extract was sprayed on innerwall of the experimental jars.
SE = Standard Error
CI = Confidence Interval

LD_{50} values indicating that the three extracts to be effective against melon fly *Z. cucurbitae* larva and pupa stadium. Toxicity test of *P. guilfoylei* leaf extracts against adult melon fly using contact method showed ineffective results. Insect mortality rates was found to be low causing difficulties to calculate LC_{50}.

CONCLUSION

Results showed ethanol and *n*-hexane extracts of *P. guilfoylei* leaves contained secondary metabolite compounds that were toxic to adult of *Z. cucurbitae*. By the residual method, the toxicity rate of ethanol extract was higher than *n*-hexane extract. However, by oral method, the toxicity rate of ethanol and *n*-hexane extracts were quite similar. The LC_{50} values of ethanol and *n*-hexane extracts tested using the residual method were 3.658% and 6.607%, respectively. While the LC_{50} values of ethanol and *n*-hexane extracts by oral method were 6.168% and 5.311%, respectively.

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