THE EFFECT OF PANGIUM SP. AND TITHONIA DIVERSIFOLIA LEAVES EXTRACT AS VEGETABLE PESTICIDES TO CROCIDOLOMIA PAVONANA (LEPIDOPTERA; PYRALIDAE) LARVA MORTALITY

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THE EFFECT OF *PANGIUM* SP. AND *TITHONIA DIVERSIFOLIA* LEAVES EXTRACT AS VEGETABLE PESTICIDES TO *CROCIDOLOMIA PAVONANA* (LEPIDOPTERA; PYRALIDAE) LARVA MORTALITY

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ABSTRACT. *Pangium* sp. and *Tithonia diversifolia* have the potential to become vegetable pesticides due to the content of secondary metabolites, such as alkaloids, tannins, flavonoids, terpenoids and saponins in their leaves, which are plant protection agents. This study aims to determine the mortality of *Crocidolomia pavonana* larvae after application of *Pangium* sp. and *Tithonia diversifolia*. *C. pavonana* is one of the main pests affecting cabbage production in North Sulawesi, Indonesia. *Pangium* sp. and *T. diversifolia* leaf extraction separately were carried out by immersion method using methanol (CH_3OH) solvent and followed by current-current distribution method. The result of crude extract was partitioned in a 95% mixture of hexane methanol (C_6H_{14} - CH_3OH). Then, the 95% methanol fraction (CH_3OH) was further partitioned with a mixture of ethyl acetate and water (EtOAc - H_2O), and the extract from the ethyl acetate fraction (EtOAc) was then used in testing as a vegetable pesticide. This study used a completely randomized design (CRD) with six treatments, namely 0% (control), 0.1%, 0.2%, 0.3%, 0.4%, 0.5% and carried out three replications, where each treatment used 10 larvae. Larval mortality observations were carried out at 24, 48, 72, 96, 120 HAA (hours after application). The results indicated that there was a mortality rate of *C. pavonana* larvae, so that the *Pangium* sp. and *T. diversifolia* have the ability to act as botanical insecticides, although *T. diversifolia* extract treatment showed a higher mortality rate, compared to *Pangium* sp. Larval mortality above 50% (LC 50) was found in P3 (0.3%) treatment after

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72 HAA, is of 53.33% (*Pangium* sp. extract) and 63.33% (*T. diversifolia* extract), successively. Then, at 120 HAA, the same larval mortality rate from both extractions in P3 treatment, increased to 76.67% using *Pangium* sp. extract, while the same mortality rate (76.67%) using *T. diversifolia* extract occurred in 96 HAA observations. ANOVA test showed significantly different results for the two extraction uses of *Pangium* sp. and *T. diversifolia*. LC 50 in *Pangium* sp. extract, at a concentration of 0.136% or 1360 ppm and in *T. diversifolia* extract of 0.1103% or 1103 ppm.

Keywords: extraction; vegetable pesticides; *Pangium* sp.; *Tithonia diversifolia*; *Crocidolomia pavonana*.

INTRODUCTION

Recently, there has been an increase in the use of chemical-based insecticides/pesticides, which are often used uncontrollably, due to the user's lack of knowledge, which results in high chemical residues in the products produced. The high frequency of pesticide use can have a negative impact on the environment and the final crop product, so that, many importing countries reject agricultural products from other countries because these products contain pesticide residues that exceed the permitted threshold. Excessive use of pesticides can also kill non-target organisms, such as when they are naturally the main plant pest enemies. Natural enemies are important for maintaining biological balance in agricultural ecosystems because they can suppress the development of pests themselves (Sembel, 2014). To overcome the problem of using chemical-based pesticides, several efforts were made, one of which was improving the quality of pesticides. One of the ways to improve the quality of pesticides is the development of vegetable pesticide formulations, namely pesticides whose basic ingredients come from plants. The chemicals contained in vegetable pesticides, especially those from plants, have bioactivity against insects, such as repellents, food inhibitors, insect growth regulators, and oviposition deterrents. Vegetable pesticides have environmentally friendly properties, which are biodegradable and safe for humans and pets. Vegetable pesticides also play a very big role in facing global problems, especially regarding the issue of agricultural commodity exports, such as limiting the maximum residue level of pesticides in agricultural export products.

Pangium edule / *Pangium* sp. is a tall tree native to the mangrove swamps of Southeast Asia (Indonesia and Papua New Guinea). It produces a large poisonous fruit (the "football fruit"), contain cyanide, which can be made edible by fermentation. On young trees the leaves have the shape of oval leaf blades. It grows in Southeast Asia, especially Indonesia and Papua New Guinea. *Pangium* sp. has a very dense plant canopy, can reach a height of 40 m and a diameter of 100 cm. The leaves have an oval shape with a width of 15 cm and a length of 20 cm, shiny dark green. This plant is known to have many benefits, both as a cooking spice, snack food, cooking oil, fish and food preservatives, medicine, fish poison, natural pesticides and woodworking (Sangi *et al.,* 2008; Ramdana and Suhartati, 2015).

Tithonia diversifolia, Family: Asteraceae, Order: Asterales, Taxon synonym: Mirasolia diversifolia Hemsl., Species: *Tithonia diversifolia;* A. Gray. *Tithonia diversifolia*, a weed plant that has many benefits, one of which is used for vegetable pesticides. This plant is very resistant to pests and diseases, and the plant is not eaten by caterpillars and insects. Initially grown in Mexico, but widely developed in tropical and sub-tropical areas, leaf-shaped crown, ribbon shape, and smooth. *Tithonia diversifolia* is a type of shrub with a height of approximately 5 m. This plant is known as a medicinal plant with erect stems that are round, woody and green. The leaves are single, 26-32 cm long, 15-25 cm wide, the tip and base of the leaves are pointed. The plant canopy is easy to prune and quick to regrow. This plant has compound flowers and is located at the end of a branch. This flower stalk is round and the petals are tubular and has fine hairs with green petals and a bright yellow crown. contains alkaloids, sesquiterpen lactones, bicyclic monoterpenes (α-pinene and β-pinene) and identified active compounds, namely flavonoids, alkaloids and tannins. The leaves of *T. diversifolia* contained the most pesticide compounds, compared to the roots and flowers (Pereira *et al.,* 1997; Moronkola *et al.,* 2007; Oyewole *et al.,* 2008; Taofik *et al.,* 2010; Odeyemi, 2014).

Crocidolomia pavonana is the main pest affecting cabbage in North Sulawesi. This pest is included in the Phylum Arthropoda, Order Lepidoptera and Family Pyralidae. The pest larvae *C. pavonana* attack *Brassicae* plants, such as cabbage (*Brassica oleracea* L., var. *capitata*), cauliflower (*B. oleracea* L., var. *botrytis*), broccoli (*B. oleracea* L., var. *itatica*) and other cabbages (*B. campestris,* var. *pekinensis*, *Brassica juncea* L., B. *juncea Coss*) and radishes (*Raphanus sativus* L.), and can live on wild mustard (*Nasturtium* sp.). The part of the cabbage plant that is attacked is the leaf part, causing the leaves to be perforated and leaving only the cabbage leaf bones (Sembel, 2014).

Grainge *et al.* (1984) reported that there were 1800 types of plants containing vegetable pesticides that could be used for pest control. Furthermore, according to Morallo-Rejesus (1986), the types of plants from the *Asteraceae*, *Fabaceae* and *Euphorbiaceae* families were reported to contain the most plant-based insecticides. As a country that has a large biodiversity, Indonesia has thousands of plants that contain botanical pesticide properties that can be used as basic materials for the manufacture of vegetable pesticides. This huge potential allows Indonesia to develop vegetable pesticides efforts, so that research activities are needed to support these efforts. This study aims to determine the mortality of *Crocidolomia pavonana* larvae after application of *Pangium* sp. and *Tithonia diversifolia* leaves extract.

MATERIALS AND METHODS

Materials

The materials used are as follows: *Pangium* sp. and *Tithonia diversifolia* leaves extract, *Crocidolomia pavonana* larvae, cultured in the laboratory to second instar. The chemicals are methanol (CH_3OH), ethyl acetate (EtOAc), hexane (C_6H_{14}), aquadest (H_2O).

Methods

Extraction

Plant extraction was carried out by immersion method using methanol (CH_3OH) solvent and followed by the counter-current distribution method. The crude extract was partitioned in a 95% mixture of hexane methanol (C_6H_{14} - CH_3OH). Then, the 95% methanol fraction (CH_3OH) was further partitioned with a mixture of ethyl acetate and water (EtOAc - H_2O) (Dadang and Prijono, 2008). The ethyl acetate (EtOAc) fraction extract obtained was then used in this test.

Propagation of Crocidolomia pavonana larvae

Host insect *C. pavonana* was collected from cabbage farms in the village of Rurukan - Tomohon, North Sulawesi - Indonesia, and was taken to the laboratory to be maintained. The eggs that appeared were then collected and placed in a petri dish covered with blotting paper. After hatching, the larvae were then transferred to a plastic box, which was also covered with blotting paper, and the larvae used for pesticide testing in this study were the second instar larvae; is inserted with cabbage, which has been dipped in the *Pangium* sp. and *T. diversifolia* leaves extract, which has been aerated for 15 min.

The study used a completely randomized design (CRD), namely six treatments: P0 (0% control), P1 (0.1%), P2 (0.2%), P3 (0.3%), P4 (0.4%), P5 (0.5%), with three replications, where each treatment used 10 larvae.

The larvae testing

Each box containing the test larvae is put with cabbage, which has been smeared separately with the extract of *Pangium* sp. and *T. diversifolia* after being aerated for 15 min. Larval mortality observations were carried out at 24, 48, 72, 96, 120 HAA (hours after application). The data obtained is then recorded. The mortality observation formula used:

$$P = a / b \times 100\%,$$

where, P: death percentage of larvae; a: the number of dead larvae; b: number of initial larvae

RESULTS AND DISCUSSION

Larval mortality above 50% from both extractions was found at 72 HAA in P3 treatment (0.3%); for *Pangium* sp. amounted to 53.33% (*Table 1*) and for *T. diversifolia* extract of 63.33% (*Table 2*). The same larval mortality rate from both extractions was equal to 76.67% for *Pangium* sp. found in treatment P3 (0.3%) at 120 HAA observations, but for *T. diversifolia* extract occurred at 96 HAA.

The feeding activity of larvae, was reported at 24 HAA for all treatments from P0 to P5, both for the use of *Pangium* sp. and of *T. diversifolia* sp. leaves without the death of the larvae.

Table 1 - Average mortality of *C. pavonana* larvae in the extraction of *Pangium* sp. leaves

HAA (hrs.)	P0 (0%)		P1 (0.1%)		P2 (0.2%)		P3 (0.3%)		P4 (0.4%)		P5 (0.5%)	
	I	%	II	%	III	%	IV	%	V	%	VI	%
24	0	0	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
48	0	0	0	0.00	1	3.33	6	20.00	7	23.33	10	33.33
72	0	0	4	13.33	5	16.67	16	53.33	17	56.67	22	73.33
96	0	0	9	30.00	12	40.00	18	60.00	20	66.67	25	83.33
120	0	0	12	40.00	17	56.67	23	76.67	24	80.00	29	96.67

Notes: HAA (hours after application); I, II, III, IV, V, VI = mortality rate P0, P1, P2, P3, P4, P5.

Table 2 - Average mortality of *C. pavonana* larvae after leaf extraction of *T. diversifolia* sp.

HAA (hrs.)	P0 (0%)		P1 (0.1%)		P2 (0.2%)		P3 (0.3%)		P4 (0.4%)		P5 (0.5%)	
	I	%	II	%	III	%	IV	%	V	%	VI	%
24	0	0	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
48	0	0	0	0.00	2	6.67	10	33.33	13	43.33	15	50.00
72	0	0	6	20.00	7	23.33	19	63.33	24	80.00	24	80.00
96	0	0	12	40.00	13	43.33	23	76.67	26	86.67	28	93.33
120	0	0	17	56.67	18	60.00	24	80.00	28	93.33	29	96.67

Notes: HAA (hours after application); I, II, III, IV, V, VI = mortality rate P0, P1, P2, P3, P4, P5.

However, on 48 HAA observations, the feeding activity decreased, and larvae mortality began in P2 treatment, but the larval mortality rate was seen to increase as the concentrations of natural pesticides increased, both for the use of *Pangium* sp. and of *T. diversifolia* sp. leaves extract. This result occurred in subsequent observations, namely in the P3 treatment, the eating activity was only found at 24 HAA, while in P4 and P5 the eating activity was not found at all observation times.

According to Salaki *et al*. (2012), the thick extract of *Pangium* sp. leaves can inhibit the feeding activity of *Plutella xylostella* larvae. *T. diversifolia* has toxic and anti-food properties (antifeedant) in insects, thus inhibiting development and breaking the insect life cycle (Ambrosio *et al.*, 2008).

Mokodompit *et al.* (2013) reported that giving *T. diversifolia* leaves extract with a concentration of 7% had an effect on the inhibition of the eating power of brown planthoppers (*Nilaparvata lugens* S.) by 88.56%.

At 24 HAA in all extraction treatments, both for the use of *Pangium* sp. and *T. diversifolia* sp. leaves extract no dead larvae were found. Dead larvae were found at 48 HAA in P2 treatment and increased at 72 HAA, 96 HAA and 120 HAA observations. The increasing of the extraction concentration, and the longer the time for larvae contamination with extraction, the higher the larval mortality rate was found. *Pangium* sp. leaves extract with a concentration of 15% given by contact, the most effective way to control stink bugs with a mortality rate of 100% and a death rate of 2.98 birds / day (Supriyadi and Setiawan, 2017), because in the leaves of *Pangium* sp. contains several chemical compounds, namely alkaloids, flavonoids, saponins, tannins, and terpenoids (Sangi *et al.*, 2008), which act as vegetable pesticides. Apart from cyanide acid, several other chemical substances are found in the fruit of *Pangium* sp. including vitamin C, iron ions, beta-carotene, hydnocarpic acid, khaulmograt acid, gloric acid, and tannins (Ramdana and Suhartati, 2015).

From the identification results using gas chromatography-mass spectroscopy (GC-MS), the active isolate hexane leaves of *Pangium* sp. contains a minimum of 11 compounds, and eight compounds were identified successfully, namely α-pinene; phytol; trimethylbenzene; nonadekena; difluoro tetradecil acetic acid; 13-hexyloxacyclotridec-10-en-2-on; 3-eicosene; diisoctyl benzene dicarboxylic acid (Mahardika *et al.*, 2014). The methanol extract of *T. diversifolia* leaves at a concentration of 1% has a digestive and contact poison effect, which is effective as a biolarvicidal against *C. bezziana* larvae, so it can cause death, decrease the weight of the pupa and block the formation of pupa and hatchability to become imago (Wardhana and Diana, 2014). Meanwhile, Pangihutan *et al.* (2016) reported that *T. diversifolia* leaf extract with a concentration of 5% that was tested on *Callosobruchus maculatus* was able to cause death up to 95% at 72 HAA, because *T. diversifolia* contains alkaloid class compounds, sesquiterpene lactones, bicyclic monoterpenes (α-pinene and β-pinene) and flavonoids that cause death in insects (Pereira *et al.,* 1997; Moronkola *et al.*, 2007; Oyewole *et al.*, 2008). Taofik *et al.* (2010) identified active compounds in the *T. diversifolia* plant, namely flavonoids, alkaloids and tannins. Odeyemi (2014) reports that the leaves of *T. diversifolia* contain the most vegetable pesticide compounds, compared to the roots and flowers, namely alkaloids, tannins, flavonoids, terpenoids and saponins. *T. diversifolia* also shows activity as antibacterial, antiprotozoa and has been tried traditionally as a natural pesticide to repel agricultural pests, grasshoppers, and ticks with quite effective results (Kuroda *et al.*, 2007; Castillo-Juárez *et al.*, 2009; Oyedokun *et al.*, 2011).

Panda and Gurdev (1995) stated that alkaloids are compounds that cause insects not to eat or are antifeedant, namely compounds that in substance do not provide resistance to eating but give insects a taste of dislike. Flavonoids function as respiratory inhibitors, in other words, these compounds can reduce the rate of chemical reactions, so that the respiration of pests is disturbed. Tannins are also able to inhibit nutrient absorption, so that it affects the ability of pests to digest food, which will eventually cause the absorption of protein in the digestive system to be disturbed (Ismarani, 2012). Due to the insecticide content in the leaf extracts of *Pangium* sp. and *T. diversifolia*, the feeding activity of the larvae is hampered, causing the larvae to die a few days afterward. The body of the dead larva changes from green to black and is slightly curved and there is no movement. To determine the effect of using *Pangium* sp. and *T. diversifolia* leaves extracts on larval mortality an ANOVA test was performed. ANOVA test shows that F-count is greater than F-table, which means that the results are significantly different in the use of the two extractions, *Pangium* sp. (*Table 3*) and *T. diversifolia* (*Table 4*). This shows that each treatment with the two extractions has a different effect on the mortality of larvae, so a further test is carried out with the LSD test (the least significant difference) (*Table 5*).

Table 3 - Analysis of variance (ANOVA) results of larvae mortality rate after application of *Pangium* sp. leaves extract

SS	Df	MS	F	*P*-value	F crit
0.66293333	5	0.132587	102.869	2E-09	3.105875
0.01546667	12	0.001289			
0.6784	17				

Table 4 - Analysis of variance (ANOVA) results of larvae mortality rate after application of *T. diversifolia* leaves extract

Source of variation	SS	Df	MS	F	*P*-value	F crit
Treatments	0.940978	5	0.188196	228.8865	1.81E-11	3.105875
Error	0.009867	12	0.000822			
Total	0.950844	17				

Lethal concentration 50 (LC 50)

Table 5 - LSD test for *Pangium* sp. and *T. diversifolia* leaves extract

Pangium sp. leaves extract			*T. diversifolia* leaves extract		
Treatments	Average	LSD Notation 0.05 (0.06386781)	Treatments	Average	LSD Notation 0.05 (0.051012)
P0 (control)	0.0	a	P0 (control)	0.0	a
P1 (0.1%)	0.166667	b	P1 (0.1%)	0.233333	b
P2 (0.2%)	0.233333	c	P2 (0.2%)	0.266667	b
P3 (0.3%)	0.42	c	P3 (0.3%)	0.506667	c
P4 (0.4%)	0.453333	d	P4 (0.4%)	0.606667	d
P5 (0.5%)	0.566667	e	P5 (0.5%)	0.64	d

LC 50 is the concentration that can cause the death of as much as 50% of the test organisms. To determine the LC 50 of the two extractions, a probit analysis is done. As shown in *Tables 6*, the probit analysis for larvae mortality analysis of *Pangium* sp. leaves extract and in *Table 7*, the probit larvae mortality analysis of *T. diversifolia* leaves extract, so that the coefficient value used for the analysis of lethal concentration 50 (LC 50) is obtained. LC 50 analysis equation for *Pangium* sp. leaves extract: y = ax + b; x = (y-b) / a; x = (5 - (- 3.0984)) / 2.5846; x = 3.13339; LC 50 = antilog (x) = 103.13339; LC 50 = antilog (x) = 1360 ppm (0.1360%).

Table 6 - Probit larva mortality analysis of *Pangium* sp. leaves extract

Concentration (%)	ppm	log (ppm)	probit	% dead	mortality	Total
0.1	1000	3.000	4.75	40.00%	12	30
0.2	2000	3.301	5.44	56.67%	17	30
0.3	3000	3.477	5.74	76.67%	23	30
0.4	4000	3.602	5.84	80.00%	24	30
0.5	5000	3.699	6.88	96.67%	29	30
	Coefficients					
Intercept	-3.0984	b				
log (ppm)	2.5846	a				

LC 50 analysis equation for *Pangium* sp. leaves extract: y = ax + b; x = (y-b) / a; x = (5 - (- 3.0984)) / 2.5846; x = 3.13339; LC 50 = antilog (x) = 103.13339; LC 50 = antilog (x) = 1360 ppm (0.1360%)

From the probit analysis obtained, that the LC 50 for *Pangium* sp. leaves extract, namely at a concentration of 1360 ppm or 0.1360%.

Table 7 - Probit larva mortality analysis of *T. diversifolia* leaves extract

Concentration (%)	ppm	log (ppm)	probit	% dead	mortality	Total
0.1	1000	3.000	5.18	56.67%	17	30
0.2	2000	3.301	5.25	60.00%	18	30
0.3	3000	3.477	5.84	80.00%	24	30
0.4	4000	3.602	6.48	93.30%	28	30
0.5	5000	3.699	6.88	96.67%	29	30
	Coefficients					
Intercept	-2.5472	b				
log (ppm)	2.4806	a				

LC 50 analysis the equation for *T. diversifolia* leaves extract: y = ax + b; x = (y-b) / a; x = (5- (2.5472)) / 2.4806; x = 3.0425; LC 50 = antilog (x) = 103.0425; LC 50 = antilog (x) = 1103 ppm (0.1103%)

LC 50 analysis the equation for *T. diversifolia* leaves extract: y = ax + b; x = (y-b) / a; x = (5- (2.5472)) / 2.4806; x = 3.0425; LC 50 = antilog (x) = 103.0425; LC 50 = antilog (x) = 1103 ppm (0.1103%).

From the probit analysis obtained, that the LC 50 for *T. diversifolia* leaves extract at a concentration of 1103 ppm or 0.1103%.

Meanwhile, lethal concentration 50 (LC 50), the concentration that can cause death as much as 50% of the test organisms, with the use of *Pangium* sp. leaves extract, namely at a concentration of 0.136% or 1360 ppm and *T. diversifolia* leaves extract of 0.1103% or 1103 ppm.

CONCLUSIONS

Pangium sp. and *T. diversifolia* leaves extract showed the ability as insecticide, but the mortality rate of *C. pavonana* larvae was higher in *T. diversifolia* leaves extract treatment, compared to *Pangium* sp. Larval mortality above 50% was found at 72 HAA treatment in P3 (0.3%) of 53.33% (*Pangium* sp. leaves extract) and 63.33% (*T. diversifolia* leaves extract), respectively. The same larval mortality rate from both extractions of 76.67% was found in P3 treatment at 120 HAA (*Pangium* sp. extract) and in P3 treatment at 96 HAA (*T. diversifolia* extract). The ANOVA test showed significantly different results in the use of the two leaves extract (*Pangium* sp. and *T. diversifolia*), namely LC 50 with *Pangium* sp. leaves extract, at a concentration of 0.136% or 1360 ppm and the extract of *T. diversifolia* leaves at 0.1103% or 1103 ppm; it means that the *T. diversifolia* leaves extract is more efficient than *Pangium* sp. leaves extract. The mortality rate of *C. pavonana* larvae was higher in using *T. diversifolia* leave extract treatment, compared to *Pangium* sp. leave extract treatment, so the use of *T. diversifolia* extract as pesticides, the concentration is lower, making it more effective as a vegetable pesticide.

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