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Bioactivity crude extracts of *Piper methysticum* Forst. F (Piperaceae) against *Plutella Xylostella* L. (Lepidoptera: Plutellidae)

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ABSTRACT

Bioactivity of n-hexane, diethyl ether, ethyl acetate, and n-butanol extract of Piper methysticum was studied to investigate antifeedant and insecticide activity, and interference of the growth of larvae Plutella xylostella L. The n-hexane, diethyl ether, ethyl acetate, and butanol extract taken from the leaves of P. methysticum were prepared and tested via the method of selected feed and non-selected feed for test on antifeedant, insecticide, and growth inhibitor of larvae P. xylostella from instar II to imago. The result seemed to reveal that the P. Methysticum leaf extract negatively affected the mortality, antifeedant, and growth and development inhibitor in larvae P. Xylostella. n-butanol extract of P. methysticum has a high mortality level 80% with LC_{50} 378.64 ppm (very toxic), and it can also lengthen the life phase of larvae up to 13.03 days, reduce the probability of formed pupa up to 13.33%, increase the probability of flaw pupa up to 63.33% and 5% for imagoes, and interfere strong antifeeding activity as much as 90.83% and weight inhibition of the larva as much as 95.23%.

Keywords: Bioactivity, *Piper methysticum*, *Plutella xylostella*, antifeedant, insecticide, growth inhibitor.

INTRODUCTION

Piper *methysticum Forst* F. or kava is categorised as a yearly plant that has been used by the peoples in Merauke, Papua Province, Indonesia for medicinal, social activity, and cultural purposes. *P. methysticum* contains chemical compound of Alkaloid (Piperidine, pipermethysticine), flavonoid, resin (kava lactone), kavain, tannin, saponin, anthraquinone, and a little volatile oil [1]. This plant could effectively function as anti-fungal, antibiotic, antiseptic, antimicrobial medication. It could also serve as narcotic and could control Altenaria solani EII, & Mart, (Pleosporaceae), Botrytis cinerea (De Bary) Whetzel, (Sclerotiniaceae), ceratocystis ulmi Buism, Sclerotinia fructicola G. Winter, (Sclerotiniaceae) [2]. Since the 20th century, kava has become popular among western people as supplementary herb which could cure anxiety and insomnia [3].

This plant extract used for insecticide has been used since 1960 until now [4]. Formerly, the mortality level of this plant was the only measurement to investigate whether the plant was useable for insecticide [5]. According to some research results, it was proven that the use of plant extract could affect the physiology of insects [6] and the behaviour of insects in term of their eating activity [7]. The secondary metabolism has nothing to do with the death of insects, but it has negative effect on biochemical function and the normal physiology of insects [8]. Secondary metabolite of the plant serves as a defence (poisonous), inhibits growth, reproduction, and other processes [9]. The insecticide coming from the plant is categorised into organic insecticide with chemical substances such as alkaloid, rotenoid, and pyrethrin which are capable of preventing or rejecting insects [10]. The family of the plants that is

considered as a potential source of organic insecticide involves Annonaceae, Asteraceae, Euphorbiaceae, Fabaceae, Meliaceae, Piperaceae, Rubiaceae, Rutaceae, Myrtaceae [2, 8, 11].

The resistance of *P. xylostella* to insecticide has occurred in 46 formula of insecticide of varied classes such as organophosphate, carbamate, pyrethroid, and organochlorine [12, 13, 14, 15] reported that *P. xylostella* was resistant to insecticide consisting of active reaction of methomyl, permethrin, methamidophos, deltramethrin and spinosad. In several research has reports on larvae *P. xylostella* feeding on cabbage leaves in Central Java and Yogyakarta, it is believed that such larvae are resistant to the insecticide containing deltramethrin [16, 17, 18]. Meanwhile, another research reported that *P. xylostella* in Pengalengan was resistant to insecticide containing fipronil, abamectin, and B. Thuringiensis [19]. In Garut, it was found that *P. xylostella* was resistant to abamectin, while it was found to be resistant to fipronil in Lembang, Pengalengan and Buleleng. Controlling pest such as *P. xylostella* mostly depends on the use of chemical insecticide. Unwise use of insecticide could cause problem in the environment, and could poison mutual insects which are beneficial for plants [20].

Uncontrolled in used of insecticide could also contribute to health problem, pollution, and ecological imbalance such as resistance and pest resurgence, and environmental damage. The damage caused by the use of synthetic insecticide triggers an attempt to find out the more effective and safer way to control pests. Therefore, attention to more envirofriendly alternative to control pests is more apparent in an attempt to reduce the frequent use of synthetic insecticide. This research was conducted to investigate the existence of potential active compound in *P.methysticum* and anfeedent, insecticide, and growth inhibitor effects on larva *P. xylostella*.

EXPERIMENTAL SECTION

Plant materials

The plant *P. methysticum* was collected from public garden of Sota village, Merauke Regency of Papua Province, Indonesia and it was then dried. The dried of plant was ground into powder until it reached 50-mesh smoothness.
Five hundred gram powder was taken from the ground samples, added with 2L methanol (1:4 v/w). Extraction was done by soaking the samples for 3 x 24 h. The extract was filtered by using filter paper which was evaporated by using rotary evaporator in the temperature of 55-60 °C with the pressure of 530-600 mmHg until it turned into thick crude extract with constant weight.

The rough extract obtained was fractionated in solvent with successive polarity level: *n*-hexane, diethyl ether, ethyl acetate, and *n*-butanol, The activity of those four fractions was then tested on larvae *P. xylostella*.

Test on insecticide activity of plant extracts

Test on insecticide activity of *P. methysticum*extract from *n*-hexane, diethyl ether, ethyl acetate and *n*-butanol to larvae *P. xylostella* collected from the field and multiplied in a laboratory was done via dipping method on the leaves. Fractionated insecticide tested was then dissolved in aquades, added with Agristick adhesive (0.5 ml/L concentration). Tween-80 was used as emulsion with 1 ml/L concentration. The positive treatment (0%) on control was done by giving aquades, added withadhesive. The negative treatment on control was done by giving aquades, added with Deltamethrin and adhesive. The introducing test result involved the tested concentration of organic insecticide from each extract. The extract concentration used in the test was 250, 500, 1000, 2000, 3000, 3500 and 4000 ppm.

The cabbage was sufficiently given as the food source for the larvae. The leaves were cut (4x4 cm), and dipped into solution for 10 seconds and dried. The cut leaves were put on petri dish with 9 cm in diameter on which thin filter paper sat. There were 10 larvae instar II put on a covered-with-filter-paper Petri dish. Each treatment was repeated six times (60 larvae *P. xylostella*/treatment).

Feeding the larvae was done for 24 h. Then. the larvae were fed without any treatment until they reached instar IV. The variables observed were those with mortality level ranging from 24-96 h after treatment, the age of larvae, the number of pupae, the flaw pupae and the number of imagoes.

To investigate the lethal concentration value (LC_{50}) of several types of solvent tested on larvae *P. xylostella*, the data on the mortality of larvae and its relation with the formula concentration of insecticide were analysed with Probit model. according to [21] via POLO PC programme [22]. The data of mortality of larvae *P. xylostella* were assessed by using the formula of Abbot [23]:

$$\mathbf{CM = \frac{\%MT - \%MC}{100 - \%MC} \times 100\ \%}$$

Where:
CM = Corrected Mortality (%)
%MT = % larval mortality in treatment;
%MC = % larval mortality in control.

The clarification of relative toxicity of chemical substance according to previous researcher [24] is presented in Table 1.

Table 1. The relationship between LC_{50} and Toxicity Classes

Toxicity rating	LC_{50} (mg/kg)
6 – Supertoxic	≤ 5
5 – Extremenly toxic	5 – 50
4 – Very Toxic	50 – 500
3 – Moderately toxic	500 – 5.000
2 – Slightly toxic	5.000 – 15.000
1– Practically nontoxic	> 15.000

Source: [24]

Evaluation of antifeedant and weight of Larvae
Antifeedant activity of crude extracts was studied using leaf disc with two methods: choice tes and non-choice test. The concentration used for testing was 250, 500, 1000, 2000, 3000, 3500, and 4000 ppm. The feed given to the treated media was placed on Petri dish covered with filter paper on which 10 larvae of each treatment were placed. It was repeated six times.

Before the leaves and larvae were placed on a Petri dish, they were scaled to figure out the fresh volume and the previous weight of the larvae. Ten pieces of leaf and 10 larvae were scaled to determine the water content. Furthermore, those samples were dried in an oven with the temperature of 100 ^{o}C for 2 days before they were scaled to figure out the dry weight. The control and experimental groups were fed for 48 hours. Then, the rest of the leaves in the experimental and control groups were scaled to obtain the amount of feed consumed.

Choice method
A Petri dish with 9 cm in diameter was covered with tissue paper on which two pieces of cabbage leaf (4x4 cm) were placed. Those two pieces of leaf came from the treated leaf and one piece of control leaf.

Non-choice method
This method was done by placing one piece of treated leaf and 1 piece of control leaf on two separated Petri dishes. Each Petri dish contained 10 larvae of *P.xylostella* instar II which just started to feed on the leaf (± 5 hours after they moulted their skin). Each treatment consisted of four-time replication. Feeding both the treated and control group was done for 24 hours until the larvae turned into imagoes.

Feeding interference (%) according to the selected *choice method* was calculated as follows:
AFI = (K-P/K) x 100%

Feeding interference with *Non-choice method* was calculated as follows:
AFI= (K-P/K+P) X 100 % [25]

Inhibition weight (%) of larvae was calculated as follows:
Iw = {(C-T) : (C-B)} x 100% [26]

AFI- Antifeeding index; K= the weight of control leaf consumed; P= the weight of treated leaf consumed; C= the weight of control larvae; T= the weight of treated larvae and; B= the previous weight of larvae.

The criterion according to Liu was applied to categorize the plants [27]:
AFI <20% - no antifeeding activity (–)
50%> AFI ≥ 20% - slightly antifeeding activity (+)
70%> AFI ≥ 50% - medium antifeeding activity (++)
AFI ≥ 70% - strong antifeeding activity (+++)

The observation involved the dry weight of remaining treated leaves and control leaves consumed by the larvae and the weight of larvae when reaching instar IV. The data of the dry weight of leaves consumed in eating interference test with selected feed method were analysed by using paired T-test with 5% significance, while the data of eating

interference test was conducted with non-selected feed method, and the weight of dried larvae was organised in completely randomised design (CRD), then analysed with variance, and followed with Interval Multiple Test by Duncan with 5% significance [28], using SAS Program [29].

RESULTS AND DISCUSSION

Mortality and Toxicity Test

The results of *P. Methysticum* extract activity test towards the mortality of larvae instar II *P. xylostella* is presented in Table 2.

Tabel 2. Percentage mortality of *P. xylostella* larvae after treatment leaf extract of *P. methyticum*

Crude ekstrak	Concentrations (ppm)	Mortality ± SD (%)			
		24 HAT[a)]	48 HAT	72 HAT	96 HAT
N-heksan	250	8.77 ± 608 a	14.29 ± 5.36 a	29.63 ± 12.83 ab	50.94 ± 6.54 b
	500	17.54 ± 10.96 a	21.43 ± 11.15 ab	37.17 ± 12.59 b	52.83 ± 11.78 b
	1000	22.81 ± 10.96 ab	32.14 ± 11.15 bc	64.82 ± 3.21 c	64.15 ± 3.27 bc
	2000	26.32 ± 13.93 ab	41.07 ± 14.17 cd	70.37 ± 3.21 c	71.70 ± 5.66 cd
	3000	38.60 ± 8.04 bc	50.00 ± 8.18de	64.82 ± 3.21 c	73.59 ± 6.54 cd
	3500	47.37 ± 10.53 cd	66.07 ± 8.18ef	75.93 ± 8.49 cd	81.13 ± 8.65 de
	4000	61.40 ± 13.25 d	75.00 ± 8.18 f	85.19 ± 3.21 d	88.68 ± 5.66 e
	Deltametrin[b)]	8.77 ± 6.08 a	18.10 ± 5.99 a	22.22 ± 9.62 a	32.08 ± 9.80 a
Etil Asetat	250	13.21 ± 8.65 a	24.53 ± 8.65 a	26.92 ± 6.66 a	3265 ± 6.12 a
	500	18.87 ± 3.27 ab	34.59 ± 7.14 ab	32.69 ± 6.62 a	42.86 ± 9.35 b
	1000	24.53 ± 6.54 ab	35.85 ± 6.54 ab	45.38 ±10.71 bc	51.02 ± 6.12 bc
	2000	33.96 ± 8.65 bc	39.62 ± 3.27 b	44.23 ± 3.33 b	59.18 ± 3.54 c
	3000	45.28 ± 3.27 cd	56.60 ± 3.27 c	55.77 ± 3.33 c	63.27 ± 6.12 d
	3500	56.60 ± 11.78 de	69.81 ± 6.54 d	73.08 ± 3.33 d	77.55 ± 3.54 e
	4000	66.04 ± 14.98 e	69.81 ± 8.65 d	80.77 ± 3.33 d	81.63 ± 0.00 e
	Deltametrin	43.40 ± 5.66 cd	43.40 ± 5.60 b	48.08 ± 5.77 bc	51.02 ± 6.12 bc
Dietil Eter	250	14.55 ± 3.15 a	24.07 ± 13.98 a	31.37 ± 14.80 ab	58.00 ± 6.00 bc
	500	20.00 ± 3.15 ab	25.93 ± 8.49 a	37.26 ± 6.79 abc	56.00 ± 3.46 b
	1000	25.45 ± 8.33 ab	29.63 ± 13.98 a	37.26 ± 12.25 abc	64.00 ± 6.00 bc
	2000	29.09 ± 5.46 ab	37.04 ± 3.21 a	47.06 ± 5.88 bc	62.00 ± 3.46 bc
	3000	34.55 ± 5.46 b	42.59 ± 8.49 a	54.90 ± 3.40 cd	68.00 ± 3.46 cd
	3500	50.60 ± 21.35 cd	61.11 ± 14.70 b	70.59 ± 15.56 de	76.00 ± 10.39 de
	4000	54.55 ± 8.33 d	72.22 ± 5.56 b	78.43 ± 3.40 e	82.00 ± 6.00 e
	Deltametrin	21.82 ± 6.30 ab	25.93 ± 3.21 a	27.45 ± 3.40 a	34.00 ± 6.00 a
N-butanol	250	39.62 ± 3.27 b	39.62 ± 3.27 b	43.14 ± 3.40 b	50.00 ± 6.93 b
	500	64.15 ± 8.65 bc	64.15 ± 8.65 c	64.71 ± 5.88 c	64.00 ± 6.00 bc
	1000	64.15 ± 31.17 bc	66.04 ± 28.30 c	70.59 ± 21.21 c	72.00 ± 19.29 cd
	2000	66.04 ± 9.80 bc	67.92 ± 6.54 c	70.59 ± 5.88 c	74.00 ± 3.46 cd
	3000	69.81 ± 8.65 c	69.81 ± 8.65 c	70.59 ± 10.19 c	74.00 ± 3.46 cd
	3500	69.81 ± 8.65 c	69.81 ± 8.65 c	72.55 ± 3.40 c	76.00 ± 6.00 cd
	4000	7736 ± 20.41 c	79.25 ± 17.29 c	78.43 ± 17.97 c	84.00 ± 15.10 d
	Deltametrin	5.66 ± 3.27 a	9.43 ± 5.66 a	11.76 ± 0.00 a	16.00 ± 6.00 a

The results are Means±SD; Means within columns foll owed by the same letters are not significantly different (p > 0.05 by DMRT) [a)]HAT = Hours After Treatment. [b)]Deltametrin = 0.6ml/100ml

The *n*-hexane, ethyl acetate, diethyl ether, and *n*-butanol extract of *P.methyticum* leaves of each concentration test with the dipping method could have significant effect on the mortality of larvae *P. xylostella* between treatments. The higher the concentration level was the higher the mortality level of the larvae would be. The mortality of larvae *P. xylostella* of 24-96 h after treatment performed the similar patterns, where the mortality chance increased time after time. The increasing mortality showed that *P. methysticum* extract by applying several kinds of solvent gradually performed identical mechanism, which could gradually kill larvae *P. xylostella*.

The mortality of four different extracts observed for 24 h ranged from 8.77-77.36%, which then increased at 48-h observation (14.29-79.25%), 72-h (26.92-85.19%), and at 96-h observation (32.65-88.68%). After 96-h treatment, *n*-hexane, ethyl acetate, diethyl ether, and *n*-butanol extract could give larvae the mortality level as much as 50.95-88.68%, 32.66-81.63%, 58-82%, and 50-85%, respectively. The mortality level of larvae tested was quite high during the beginning of the observation, and it was relatively constant in the following observation. The mortality development pattern showed that the active compound in *P. methyticum* extract performed relatively high mechanism in giving *P. xylostella* the mortality (50-88.68%).

In term of the use of the plant extract which contained bioactive compound to control pests and its effect on insects, mortality is the common and easy factor to investigate. This is known to be the characteristic of bioactive compound mechanism found in an extract. The criteria of mechanism of bioactive compound which functions as insecticide involve the following: having high toxicity level, having the ability to hamper eating activity, inhibiting the insects

to find their host plant, inhibiting spawning activities, interfering the development of eggs, interfering the development of larvae, interfering the growth of the larvae into adult phase, interfering the development of chitin, and disturbing the system of reproduction [5]

The test on toxicity was carried out to investigate the relation between concentration and mortality and to figure out the number of concentrations required to kill 50% of tested insects. The results of the toxicity test of *P. methyticum* leaf extract are presented in Table 3.

Tabel 3. Toxicity test of leaf extracts of *P. methyticum* against larvae of *P. xylostella*

Crude Ekstrak	Slope	LC_{50} (ppm)	95% confidence interval	LC_{75} (ppm)	95% confidence interval
n-hexane	1.214 ± 0.260	4.047.04	2.733.48 – 7.326.27	14.548.01	7.844.04 - 66.977.23
ethyl acetate	1.337 ± 0.364	3.020.35	1.844.66 – 4.536.17	9.653.79	5.957.43 – 36.272.71
diethyl ether	0.855 ± 0.226	5.679.28	3.216.83 – 17.679.69	34.920.85	12.893.96 – 634.664.22
n-butanol	0.809 ± 0.149	378.64	77.82 - 725.03	2.584.96	1.518.96 – 6.256.39

LC: Lethal Concentration.

The results of probit analysis revealed that the extract of *n*-butanol fraction appeared with the value of LC_{50}= 378.64 ppm or equal to 0.3%. This was lower than other fractions such as n-hexane = 4.047.04 ppm which was equal to 4%, ethyl acetate LC_{50} = 3.020.35 ppm which was equal to 3%, diethyl ether LC_{50} = 5.679.28 ppm which was equal to 5%. From the relation between LC_{50} and relative toxicity classification of chemical substance [24], it was considered that the extract of *P. methysticum* of n-butanol fraction was very toxic to larvae *P. xylostella*. While *n*-hexane, ethyl acetate, and diethyl ether fraction were moderately to slightly toxic to larvae *P.xylostella*.

When directly observed in term of eating behaviour and activity of the larvae *P. xylostella*, the larvae were different from those in control group. Those poisoned were seemingly caused by secondary metabolic compound found in the *n*-hexane, ethyl acetate, diethyl ether and n-butanol extract of *P. methysticum* leaves, which led to the disturbance of nervous system and metabolism. The alkaloid and saponin compound are known to be repellent, and they are usually used as insecticide. Weinzierl stated that one of the benefits from organic insecticide was that it could quickly stop eating activity and paralyse the insects [30], but it did not cause death in hours or days. Harborne agrees that saponin is categorised as glycoside triterpene and sterol which comprise surface active compound that can poison the insects [31]. Vincent also reports that saponin could also disturb the respiratory system of insects [32]. Robinson stated that alkaloid compound obtained from the plant extract is of saponin containing nitrogen [33]. With the existence of chemical compound in group and with nitrogen, the way the alkaloid works influences the performance of asetilkolin and the nervous system of insects [34].

Growth and Development Inhibitor Test

The results of treatment given to the concentration of *P. methysticum* extract of four different types of solvent to figure out the effect of growth and development of *P. xylostella* are presented in Table 4.

The extract of *P. methysticum* leaves significantly affected the development of larvae instar II-IV compared to control. The extract of *P. methysticum* leaves of *n*-hexane, ethyl acetate, diethyl ether, and *n*-butanol fraction would hamper the development of larvae instar II-IV as long as 1.33-2.47 days, 1.5-2.44 days, 2.06-2.67 days, and 1.90-2.32 days, respectively. The life phase of larvae would also be longer than usual such as 2.2-5.1 days, 1.37-5.93 days, 2.20-7.26 days, and 1.93-5.03 days. The results showed that the extract of *n*-hexane, ethyl acetate, diethyl ether, and n-butanol of *P. methysticum* leaves performed similar effects where they interfered the development of larvae from instar II to instar IV, and they also lengthened the life phase of the larvae up to 12.86 – 15.03 days.

The growth and the development of larvae were closely correlated with the ability of larvae to consume their food and the amount of food. The inhibited growth of larvae also interfered the development of larvae, the life cycle, and it could even cause death. Some organs attached in the body of the insects are known to be able to produce hormones which control reproduction process, skin moult, and metamorphosis. Neurosecretory cells located in the brain of insects produce one or more hormones whichhave a role in the growth and metamorphosis. Corpora allata produce a hormone called juvenile hormone (JH). This hormone could inhibit metamorphosis process in insects. Several substances, especially terpene, have similar activities to JH as metamorphosis inhibitor [35]. The extract of *P. methysticum* leaves of different types of solvent appeared to contain terpene compound (triterpenoid) and to perform the same activities as JH such as inhibiting metamorphosis so that the extract of *P. methysticum* leaves could inhibit the development period of *P. xylostella*.

Table 4. The mean age of *P. xylostella* larval stage after treatment of *P. methysticum* extract

Crude Ekstrak	Concentration (ppm)	Instar II (day)	Instar III (day)	Instar IV (day)	Age Stadia Larvae (day)
	Control	2.47 ± 0.35 a	2.43 ± 0.31 a	2.87 ± 0.35 a	7.77± 0.25a
	250	3.33 ± 0.57 bc	3.53 ± 0.26 b	3.10 ± 0.17 ab	9.97± 0.55b
	500	3.63 ± 0.24 bcd	3.60 ± 0.27 b	3.10 ± 0.20 ab	10.33± 0.57bcd
	1000	3.80 ± 0.10 bcd	3.67 ± 0.48 b	3.27 ± 0.21 ab	10.73± 0.58bcd
N-hexane	2000	3.87 ± 0.26 cd	3.77 ± 0.12 b	3.37 ± 0.21 ab	11.00± 0.40bcd
	3000	3.90 ± 0.40 cd	4.07 ± 0.16 bc	3.47 ± 0.51 ab	11.43± 0.84cd
	3500	3.93 ± 0.51 cd	4.13 ± 0.36 bc	3.80 ± 0.10 bc	11.87± 0.40de
	4000	4.13 ± 0.35 d	4.53 ± 0.36 c	4.20 ± 0.30 c	12.87± 0.65e
	Deltametrin	3.13 ± 0.40 b	2.83 ± 0.66 a	4.30 ± 0.76 c	10.27± 1.66bc
	Control	2.30 ± 0.20 a	2.43 ± 0.31 a	2.97± 0.21 a	7.70 ± 0.17 a
	250	2.83 ± 0.12 b	3.13 ± 0.06 b	3.10± 0.17 a	9.07 ± 0.15 b
	500	3.23 ± 0.23 bc	3.67 ± 0.15 c	3.10± 0.20 a	10.00 ± 0.17 c
	1000	3.47 ± 0.25 cd	3.83 ± 0.06 c	3.27± 0.21 a	10.57 ± 0.45 cd
Etil Asetat	2000	3.73 ± 0.32 de	4.03 ± 0.40 cd	3.87± 0.25 b	11.63 ± 0.49 e
	3000	3.97 ± 0.12 ef	4.30 ± 0.17 de	4.03± 0.23 b	12.30 ± 0.36 f
	3500	4.13 ± 0.21 ef	4.67 ± 0.21 ef	4.27± 0.21 bc	13.07 ± 0.21 g
	4000	4.30 ± 0.20 f	4.87 ± 0.06 f	4.47± 0.15 c	13.63 ± 0.06 g
	Deltametrin	3.20 ± 0.30 bc	3.07 ± 0.25 b	3.90± 0.30 b	10.17 ± 0.25 cd
	Control	2.47 ± 0.35 a	2.43 ± 0.31 a	2.87 ± 0.35 a	7.77± 0.25 a
	250	3.33 ± 0.57 bc	3.53 ± 0.25 b	3.10 ± 0.17 a	9.97 ± 0.55 b
	500	3.63 ± 0.23 bcd	3.60 ± 0.27 b	3.10 ± 0.20 a	10.33 ± 0.57 b
	1000	3.90 ± 0.20 bcd	3.67 ± 0.47 b	3.27 ± 0.21 ab	10.83 ± 0.51 bc
Dietil Eter	2000	4.03 ± 0.51 cd	3.90 ± 0.35 bc	3.93 ± 0.35 bc	11.87 ± 0.65 cd
	3000	4.37 ± 0.38 de	4.53 ± 0.35 cd	4.23 ± 0.31 cd	13.13 ± 0.42 de
	3500	5.03 ± 0.64 e	5.10 ± 0.46 d	3.53 ± 0.67 abc	13.67 ± 0.80 e
	4000	5.00 ± 0.44 e	5.10 ± 0.17 d	4.93 ± 0.35 d	15.03 ± 0.25 f
	Deltametrin	3.13 ± 0.40 ab	2.83 ± 0.65 a	4.30 ± 0.76 cd	10.27 ± 1.66 b
	Control	2.37 ± 0.23 a	2.77 ± 0.06 a	2.87 ± 0.35 a	8.00 ± 0.27a
	250	3.10 ± 0.40 b	3.53 ± 0.25 bc	3.30 ± 0.17 ab	9.93 ± 0.55 b
	500	3.33 ± 0.38 b	3.77 ± 0.06 cd	3.43 ± 0.42 abc	10.53 ± 0.65 bc
	1000	3.60 ± 0.36 bcd	3.80 ± 0.36 cd	3.67 ± 0.21 bcd	11.07 ± 0.42 cd
N-Butanol	2000	4.03 ± 0.51 cde	4.07 ± 0.32 de	3.67 ± 0.21 bcd	11.77 ± 0.12 d
	3000	4.13 ± 0.35 de	4.33 ± 0.15 e	4.23 ± 0.31 de	12.70 ± 0.25 e
	3500	4.23 ± 0.31 de	4.80 ± 0.27 f	4.00 ± 0.27 cd	11.07 ± 0.64 cd
	4000	4.33 ± 0.15 e	5.07 ± 0.15 f	4.77 ± 0.15 e	13.03 ± 0.31 e
	Deltametrin	3.47 ± 0.25 bc	3.30 ± 0.20 b	4.30 ± 0.76 de	14.17 ± 0.06 f

The results are Means±SD; The data is transformed to arcsin%. Within the column similar alphabets are statistically not significant (p > 0.05 by DMRT).

The percentage of pupae formed, flaw pupae, and imagoes

The results of variance analysis revealed that the application of *P. methysticum* leaf extract in all concentrations significantly influenced the percentage of pupae formed, flaw pupae and the imagoes formed (Table 5). The average percentage of pupae *P. xylostella* formed due to the application of *P. methysticum* could significantly reduce the chance of the pupae formed compared to those in control. The percentage of pupae formed in control ranged from 81.67-88.33%. The percentage of pupae formed at the highest concentration level of 4000 ppm in *n*-hexane, ethyl acetate, diethyl ether, and *n*-butanol extract was 10%, 15%, and 13.33%, respectively. The lowest percentage of pupae formed in the extract of n-hexane and n-butanol fraction was 10% and 13.33%. respectively.

The extract of *P. methysticum* leaves significantly affected the percentage of flaw pupae when compared to those in control. The number of flaw pupae in control ranged from 6-11.44% and they were significantly different from those of concentration of each extract. The percentage of flaw pupae starting from the lowest concentration (250 ppm) to the highest one (4000 ppm) in *n*-hexane, ethyl acetate, diethyl ether, and *n*-butanol extract was 30.83-61.11%, 15.81-45.08%, 18.85-66.67%, and 27.30-63.33%, respectively. The highest percentage of flaw pupae treated with extract of diethyl ether fraction of *P. methysticum* was 66.67%.

Similarly, the extract of *P. methysticum* leaves significantly affected the number of imagoes compared to those in control. In control group, the percentage of imagoes ranged from 73.33-80%, and the percentage of those treated. starting from the highest concentration (4000 ppm) to the lowest concentration (250 ppm) in *n*-hexane, ethyl acetate, diethyl ether, and *n*-butanol extract was 5-28.33%, 10-46.67%, 6.67-33.33%, and 5-25%, respectively. Meanwhile, the lowest percentage of imagoes formed treated with the extract of *n*-hexane and *n*-butanol fraction was 5%.

Table 5. Percentage of the pupae, flaw pupae and imagoes *P. xylostella* after treatment of *P. methysticum* extract

Crude Ekstrak	Concentration (ppm)	Pupae ±SD	Flaw Pupae ±SD	Imagoes±SD
	Control	88.33 ± 2.89 f	11.44 ± 6.05 a	80.00 ± 5.00 e
	250	43.33 ± 5.77 d	30.83 ± 6.29 abc	26.67 ± 7.64 c
	500	41.67 ± 10.41 d	34.44 ± 15.03 abc	28.33 ± 12.58 c
	1000	31.67 ± 2.89 cd	46.83 ± 12.22 bc	16.67 ± 2.89 bc
n-Hexane	2000	25.00 ± 5.00 bc	46.67 ± 5.77 bc	13.33 ± 2.89 b
	3000	23.33 ± 5.77 bc	47.22 ± 20.97 bc	11.67 ± 2.89 ab
	3500	16.66 ± 7.64 ab	58.89 ± 8.39 c	8.33 ± 2.89 ab
	4000	10.00 ± 5.00 a	61.11 ± 34.69 c	5.00 ± 5.00 a
	Deltametrin	60.00 ± 8.66 e	25.38 ± 4.00 ab	45.00 ± 8.66 d
	Control	81.67 ± 2.89 g	8.09 ± 3.18 a	73.33 ± 2.89 e
	250	55.00 ± 5.00 f	15.81 ± 12.30 ab	46.67 ± 10.41 d
	500	46.67 ± 7.64 ef	22.94 ± 14.22 abc	36.67 ± 12.58 cd
	1000	40.00 ± 5.00 cde	25.26 ± 3.18 abc	30.00 ± 5.00 c
Ethyl acetate	2000	33.33 ± 2.89 cd	30.16 ± 14.55 bc	23.33 ± 5.77 bc
	3000	30.00 ± 5.00 bc	44.44 ± 9.62 c	16.67 ± 7.64 ab
	3500	18.33 ± 2.89 ab	44.44 ± 19.25 c	11.67 ± 2.89 a
	4000	15.00 ± 0.00 a	45.08 ± 19.55 c	10.00 ± 5.00 a
	Deltametrin	40.00 ± 5.00 e	37.90 ± 4.77 c	25.00 ± 5.00 bc
	Control	83.33 ± 2.89 f	7.97 ± 3.30 a	75.00 ± 5.00 f
	250	35.00 ± 5.00 cd	18.65 ± 5.63 ab	33.33 ± 5.77 de
	500	36.67 ± 2.89 d	23.21 ± 9.28 ab	28.33 ± 5.77 cd
	1000	30.00 ± 5.00 cd	27.30 ± 6.76 ab	23.33 ± 2.89 cd
Diethyl ether	2000	31.67 ± 2.89 cd	26.98 ± 11.00 ab	23.33 ± 5.77 cd
	3000	26.67 ± 2.89 bc	33.33 ± 11.55 ab	16.67 ± 2.89 bc
	3500	20.00 ± 8.66 ab	50.00 ± 16.67 cb	10.00 ± 5.00 ab
	4000	15.00 ± 5.00 a	66.67 ± 28.87 c	6.67 ± 5.77 a
	Deltametrin	55.00 ± 5.00 e	21.31 ± 5.42 ab	45.00 ± 8.66 e
	Cntrol	83.33 ± 2.89 c	6.00 ± 5.89 a	78.33 ± 5.77 e
	250	35.00 ± 10.00 b	27.30 ± 6.76 abc	25.00 ± 5.00 c
	500	33.33 ± 2.89 b	40.48 ± 10.91 abc	20.00 ± 5.00 bc
	1000	20.00 ± 13.23 ab	42.22 ± 36.72 abc	10.00 ± 8.66 ab
n-Butanol	2000	21.67 ± 2.89 ab	45.00 ± 18.03 abc	10.00 ± 5.00 ab
	3000	21.67 ± 2.89 ab	52.22 ± 13.47 bc	11.67 ± 2.89 ab
	3500	20.00 ± 5.00 ab	55.00 ± 18.03 bc	10.00 ± 5.00 ab
	4000	13.33 ± 12.58 a	63.33 ± 32.15 c	5.00 ± 5.00 a
	Deltametrin	70.00 ± 5.00 a	20.78 ± 15.44 ab	48.33 ± 5.77 d

The results are Means±SD; The data is transformed to arcsin%. Within the column similar alphabets are statistically not significant (p > 0.05 by DMRT).

This condition revealed that the highest the concentration is, the lower the chance for development will be. In line with the statement of Harnoto, the small number of pupae produced was caused by the small amount of food consumed by larvae [36]. Therefore, the metamorphosis process from pre-pupa to pupa did not manage to perfectly form pupa; it could even be said the new pupa will not be formed at all. In order to prevent the toxin compound into the body of insects, the insects would reduce the amount of food consumed. so that it interfered the growth and the development of the insects.

Antifeedant Activity of Crude Extracts

The effect of *P. methyticum* extract of different types of solvent on the weight of the leaves consumed and how significant the effect was are presented in Table 6.

The test on the activity of antifeedant with the method of choice test against larvae instar II *P. xylostella* showed that at the lowest concentration (250 ppm), the number of leaves consumed by the tested larvae were 27.90-36.78 mg, and statistically, it was significantly different from those of control (50.08-56.80 mg). The concentration of *P. methyticum* extract of different types of solvent revealed that the highest the concentration of the extract was. the smallest the number of leaves was consumed. The decrease of the number of leaves consumed, starting from the lowest concentration (250 ppm) to the highest one (4000 ppm), was 1.4-18.5 times as low as those of control. Eating interference of each solvent based on the lowest concentration (250 ppm) to the highest concentration (4000 ppm) was 25.23 – 94.38%. The treatment using the method of selected extract of *P. methyticum* of *n*-hexane, ethyl acetate, and diethyl ether, and n-butanol fraction interfered eating activity as much as 88.93%, 90.83%, 93.13%, and 94.35%, respectively.

Table 6. The effect of extracts *P. methyticum* on feeding intensity (consumed leaf weight- mg) of *P. xylostella* larvae to choice test

Crude Ekstrak	Concentrate (ppm)	Consumed leaf weight (mg)[a)]		F value	AFI (%)[b)]
		Control	Treatment		
n-Hexane	250	55.70 ± 1.11 a	31.40 ± 1.08 b	22.73	40.37 a
	500	53.43 ± 2.43 a	30.23 ± 2.56 b	64.35	43.48 ab
	1000	52.53 ± 5.75 a	23.87 ± 2.23 b	10.51	54.39 b
	2000	52.57 ± 5.29 a	16.83 ± 2.61 b	9.75	73.12 c
	3000	54.27 ± 1.65 a	14.60 ± 1.30 b	89.96	81.26 cd
	3500	53.43 ± 1.44 a	10.03 ± 3.08 b	27.34	83.79 cd
	4000	52.67 ± 4.65 a	5.87 ± 1.78 b	21.34	88.93 d
Ethyl acetate	250	56.80 ± 0.10 a	27.90 ± 12.10 b	4.14	50.91 a
	500	55.53 ± 0.84 a	15.33 ± 4.28 b	13.72	72.31 b
	1000	54.27 ± 1.46 a	12.37 ± 2.01 b	24.15	77.17 bc
	2000	52.40 ± 2.01 a	8.10 ± 2.77 b	23.40	84.54 bc
	3000	46.50 ± 5.27 a	5.63 ± 1.87 b	9.99	87.50 bc
	3500	40.60 ± 7.28 a	3.90 ± 1.65 b	11.26	90.65 c
	4000	36.57 ± 5.33 a	3.37 ± 1.42 b	12.02	90.83 c
Diethyl ether	250	56.73 ± 0.17 a	35.03 ± 5.93 b	5.18	25.23 a
	500	47.08 ± 8.64 a	22.22 ± 2.65 b	10.65	43.20 b
	1000	52.08 ± 8.56 a	14.35 ± 2.38 b	9.69	72.19 c
	2000	48.43 ± 2.25 a	8.33 ± 1.85 b	31.27	82.81 d
	3000	46.15 ± 4.59 a	6.59 ± 2.14 b	14.69	84.75 d
	3500	45.12 ± 4.10 a	4.53 ± 1.12 b	19.88	89.93 de
	4000	40.35 ± 8.46 a	2.90 ± 1.46 b	10.62	93.13 e
n-Butanol	250	50.08 ± 6.67 a	36.78 ± 7.40 b	4.53	26.49 a
	500	47.90 ± 4.70 a	24.85 ± 4.25 b	18.31	48.29 b
	1000	52.08 ± 8.56 a	22.95 ± 7.13 b	15.58	56.47 bc
	2000	50.93 ± 2.93 a	17.12 ± 4.59 b	36.13	66.65 cd
	3000	51.78 ± 3.81 a	12.65 ± 4.94 b	14.32	75.61 d
	3500	51.38 ± 4.84 a	6.28 ± 1.89 b	23.49	87.87 e
	4000	50.35 ± 6.19 a	2.72 ± 0.73 b	13.88	94.38 e

The results are Means±SD;. [a)] The average of the treatment and control followed by the same letter are not significantly different at the level of the t test; [b)] Within the column similar alphabets are statistically not significant (p > 0.05 by DMRT).

Antifeedant Test of non-Choice

The treatment of *P. methyticum* extract of different types of solvent among the concentrations tested by using non-selected feed showed that it could reduce the eating activity as much as 13.07-87.69% (Table 7). The level of reduced eating activity was significantly different from that in control group. The percentage of interference in eating activity with the non-selective method for *n*-hexane, ethyl acetate, diethyl ether, and *n*-butanol extract was 86.05%, 83.60%, 87.69%, and 85.58%, respectively. The higher the concentration tested was. the stronger the effect of eating activity interference would be.

The *P. methyticum* extract of several types of solvent significantly affected the weight interference of larvae. The weight interference of *n*-hexane, ethyl acetate, diethyl ether, and *n*-butanol fraction was 94.15%, 95.23%, 93.81%, and 94.91%, respectively. The proportion of weight interference of larvae on the concentration was significantly different from that shown by the concentration below. The higher the concentration tested was, the strongest the effect of weight interference would be. It was because the amount of allelochemical compound in the concentration increased. so that the interference in eating activity was getting higher, which inhibited the larvae from gaining weight.

In general it can be concluded that the conditions with food choices or non-choices. extract *n*-hexane, ethyl acetate, ether and *n*-butanol leaves of *P. methysticum* have eaten barriers or decreased activity of eating a very strong or strong antifeeding activity (+++) fit the criteria of Liu [27]. It showed that the larvae *P.xylostella* could differentiate which part of plant was given treatment and which one was not. The active compound could work by interfering the eating activity of insects. Therefore, the larvae were not straight dead. By having low eating activity, the larvae could survive only for certain period of time.

Increasing the level of eating interference in either the selected feed method or non-selected feed method and increasing the weight interference of larvae were seemingly due to the existence of active compound from flavonoid obtained from *P. methyticum*. The compound tasted bitter and was toxic to insects [33]. Meanwhile, the flavonoid compound, according to Schoonhoven [37], alkaloid and terpenoid were potential in interfering eating activity in some insects.

Table 7. The effect of extracts *P. methyticum* on feeding intensity (consumed leaf weight- mg) and Inhibition weight of *P. xylostella* larvae to non-choice test

Crude Ekstrak	Concentrate (ppm)	Consumed leaf weight (mg)	AFI (%)	Inhibition weight (%)
n-Hexane	0	45.07 ± 8.30 f	-	-
	250	32.13 ± 0.42 e	16.76 a	24.02 a
	500	29.83 ± 5.05 de	20.71 ab	42.00 b
	1000	23.03 ± 7.56 cd	33.39 b	56.04 c
	2000	14.67 ± 6.19 bc	51.92 c	71.39 d
	3000	10.83 ± 0.99 ab	61.27 c	76.43 de
	3500	4.93 ± 1.01 a	80.32 d	84.33 ef
	4000	3.40 ± 1.21 a	86.05 d	94.15 f
Ethyl acetate	0	41.10 ± 12.32 e	-	-
	250	23.20 ± 0.61 d	28.57 a	22.65 a
	500	16.83 ± 3.38 cd	42.22 ab	55.08 b
	1000	15.50 ± 5.86 bcd	46.23 b	67.71 bc
	2000	12.27 ± 4.09 abc	54.66 bc	74.84 cd
	3000	8.97 ± 3.41 abc	64.67 cd	80.26 cd
	3500	5.70 ± 1.83 ab	75.82 de	84.81 de
	4000	3.70 ± 1.40 a	83.60 e	95.23 e
Diethyl ether	0	130.27 ± 13.99 e	-	-
	250	87.05 ± 2.42 d	19.90 a	34.36 a
	500	56.23 ± 8.80 c	39.94 b	43.65 ab
	1000	35.70 ± 6.74 b	57.17 c	58.82 bc
	2000	16.05 ± 1.24 a	78.07 d	68.38 cd
	3000	12.38 ± 3.80 a	82.75 de	76.51 cde
	3500	11.98 ± 3.66 a	83.25 de	80.35 de
	4000	8.68 ± 4.95 a	87.69 e	93.81 e
n-butanol	0	63.00 ± 1.14 g	-	-
	250	48.50 ± 3.18 f	13.07 a	25.99 a
	500	36.17 ± 3.59 e	27.17 b	43.28 b
	1000	23.80 ± 2.13 d	45.22 c	57.17 c
	2000	17.60 ± 2.95 c	56.47 d	67.28 d
	3000	11.87 ± 2.40 b	68.41 e	75.98 e
	3500	8.10 ± 3.32 ab	77.47 ef	86.14 f
	4000	5.00 ± 3.26 a	85.58 f	94.91 g

The results are Means±SD; Within the column similar alphabets are statistically not significant (p > 0.05 by DMRT).

This eating interference was due to the existence of compounds found in the extract from the food. where it worked only by shortening or stopping eating activity of the larvae. *P. methyticum* contained active compound which consisted of methysticin, dihydromethysticin, kawain, dihydrokawain, dimethoxyyangonin, and yangonin [38-39]. Bioactive compound of *P. methyticum* extract affected the response made by the senses of the body of the larvae, which detected the inhibiting substances that could stop eating activity of larvae *P. xylostella*. Bioactive compound in *P. methyticum* extract appeared to be able to distract stimulus signals that were contained in the food given. The existence of the plant given as food involved central nervous system which responded all factors that attract (attractan) and interfere/inhibit (deterrent).

CONCLUSION

The extract of *P. methysticum* leaves negatively affected the mortality, antifeedant, and growth and development interference of larvae *P. xylostella*. The extract of *P. methysticum* of *n*-butanol had higher mortality level (80%) with LC_{50} 378.64 ppm, lengthened the life phase of larvae (13.03 days), minimizing the chance of larvae formed (13.33%), increasing the number of flaw pupae (63.33%), decreasing the number of imagoes (5%), interfering strong antifeeding activity of the larvae (90.83%), and inhibit larval weight (95.23%).

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