JOINT ACTION OF THE EXTRACT MIXTURE OF *Piper aduncum* AND *Aglaia odorata* AGAINST *Plutella xylostella* (L.) (Lepidoptera: Plutellidae)

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ABSTRACT

Intensive use of synthetic insecticides has resulted in the development of resistance in *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). The development of plant-based insecticides can be an alternative strategy for pest control that is environmentally friendly and safe against natural enemies. In application, botanical insecticides can be used singly or in mixtures of extracts. Mixtures of two or more plant extracts could have additive, synergistic, or antagonistic interaction. This research sought to study the joint action of the mixtures of *Piper aduncum* and *Aglaia odorata* extracts against *P. xylostella* larvae. This research was conducted at the Insect Physiology and Toxicology Laboratory, Department of Plant Protection, Faculty of Agriculture, Bogor Agricultural University (IPB) from January to June 2023. The experiments were carried out using the leaf dipping method. Larval mortality was analyzed using the PoloPlus Ver 1.0 Program. The joint action in mixtures was determined from the combination index value at the LC_{50} and LC_{95}. The crude extract of *P. aduncum* was compatible with *A. odorata* extract at a ratio of 2:1 (w/w). Probit analysis showed that LC_{50} and LC_{95} values of the *P. aduncum* : *A. odorata* extract mixture with a concentration ratio of 2:1 (w/w) at 96 hours after treatment (HAT) were 0.06 % and 0.15 %, respectively. The hexane extract *P. aduncum* fruits (HxPa) contained 12 identified components, with notable high-abundance compounds including dillapiole at 32.3% and pentadecane at 20.3 %. In contrast, the methanol extract of *A. odorata* twigs (MtAo) was composed of 15 components, among which rocaglamide is present at 18.2%, and 2-morpholinoacetic acid at 15.2%. The components of HxPa extract that are thought to be insecticidal include dillapiole, caryophyllene, phytol, copaene, squalene, and tetracosane. Secondary metabolite compounds in MtAo extract may be associated with its content including rocaglamide, dammarane triterpene, aminopyrrolidine bis-amides, and 2-morpholinoacetic acid. A combination of HxPa and MtAo extracts has the potential to be further developed as a botanical insecticide in controlling *P. xylostella*.

Key words: botanical insecticide, dillapiole, LC_{50}, LC_{95}, mortality, rocaglamide.

INTRODUCTION

Plutella xylostella (L.) (Lepidoptera: Plutellidae) is one of the major pests in cabbage crops. *P. xylostella* originated in Europe, South Africa, or the Mediterranean region, and has now spread throughout the world (Badenes-Perez et al. 2006). *P. xylostella* larvae attack plants by feeding on leaves of cabbage crops. Larvae prefer to feed on the underside of the leaf surface, leaving the upper epidermis so that the leaf looks like a "window" (Begna and Damtew 2015). The attacks can cause severe

inhibition in crop production resulting in a high reduction in production and can cause yield losses of up to 100 % (Machekano et al. 2017; Ramzan et al. 2019; Witri and Purnomo 2021). Damage caused by larvae will reduce the quality and marketability of cabbage products.

A very common control strategy for *P. xylostella* in Indonesia is the use of synthetic insecticides (Dadang 2023). Continuous and unwise applications of synthetic insecticides cause resistance in *P. xylostella* insects. *P. xylostella* from Kupang had a high level of resistance to cypermethrin (Kotta et al. 2017). The testing of five insecticides, abamectin, chlorphenafir, emamectin benzoate, metaflumizone, and spinetoram against *P. xylostella* larvae collected from Pacet, Cipanas, and Cisarua of West Java Indonesia showed resistance to metaflumizone insecticide (Prijono et al. 2019). Therefore, to overcome the resistance problem, various efforts have been made through the search for control strategies using chemical compounds that are more environmentally friendly (Dadang 2015; Kotta et al. 2017).

The utilization of secondary plant metabolites is one alternative to manage pests that is environmentally friendly. Utilization of secondary metabolites in plant protection does not cause residues in products, persistence and the lower risk of toxic effects on nontarget organisms (Divekar et al. 2022). Some of the plant families with the most promising effects as plant-based insecticides were Meliaceae, Rutaceae, Annonaceae, Asteraceae, and Piperaceae families (Isman 2014; Nadia 2014).

Piper aduncum (Piperaceae) and *Aglaia odorata* (Meliaceae) are known as insecticides against *P. xylostella* and several other pests (Dadang 2023; Prijono 2021). *P. aduncum* was effective against *Croccidolomia pavonana* larvae (Johana et al. 2018; Lina et al. 2015), *Anticarsia gemmatalis* (Hübner) and *Spodoptera frugiperda* (Smith) (Lucena et al. 2017), *Aedes aegypti* and *Tetranychus urticae* (Durofil et al. 2021). Meanwhile, *A. odorata* was effective against *C. pavonana* larvae (Dadang et al. 2011), *Spodoptera litura* larvae (Hoesain et al. 2023), and *Earias vittella* larvae (Bharti et al. 2017). The main active component in *P. aduncum* fruit is dillapiole (Hasyim 2011). Dillapiole contains a methylenedioxyphenyl group in its structure, which is characteristic of various synergistic compounds that inhibit cytochrome P450 enzyme activity. Cytochrome P450 enzymes are responsible for metabolizing various substances, including xenobiotics, such as insecticides and plant toxins, as well as endogenous compounds (Metcalf 1967; Perry et al. 1998). These enzymes play a critical role in detoxification, growth, development, feeding, and protection against xenobiotics, contributing to pesticide resistance and tolerance to plant toxins (Feyereisen 1999; Nauen et al. 2021).

In application, botanical insecticides can be used singly or in mixtures of extracts. Mixtures of two or more plant extracts will increase the effectiveness or the spectrum of activity of botanical insecticides, overcome the limitations of raw materials, and slow down the rate of resistance (Dadang 2023). A mixture of *Tephrosia vogelii* (Fabaceae) and *P. aduncum* extracts was synergistic against the test insect *C. pavonana* in cabbage (Nailufar 2011). The mixture of *T. vogelii* and *P. aduncum* extracts in a 1:5 ratio demonstrated the highest insecticidal effectiveness, with LC_{50} and LC_{95} values of 0.014 % and 0.06 %, respectively, indicating strong synergistic activity (Lina 2014). The mixture of *P. aduncum:A. odorata* (2:1) was highly toxic against *C. pavonana* with additive mixture properties (Mahfud 2017). The synergistic nature of these compounds showed significant benefits for the future development of mixed botanical insecticides as an alternative approach to pest management. Mixtures of two or more plant-based insecticides can increase the effectiveness or improve the spectrum of activity, overcome raw material limitations and slow down the rate of resistance (Dadang 2023).This research sought to evaluate the joint action of mixtures of the hexane extract of *Piper aduncum* fruits with the methanol extract of *Aglaia odorata* twigs against *P. xylostella* larvae.

MATERIALS AND METHODS

Study site. This research was conducted at the Insect Physiology and Toxicology Laboratory, Department of Plant Protection, Faculty of Agriculture, Bogor Agricultural University (IPB). This study was conducted from January to June 2023.

Preparation of plant materials. *P. aduncum* fruits were collected from the campus area of Bogor Agricultural University (IPB), Dramaga and *A. odorata* twigs were collected from the garden area of the Agricultural Instrument Standards Agency for Spice, Medicinal and Aromatic Plants (BSIP-TROA), Bogor, Indonesia.

Preparation of plant extracts. Fresh plant materials were cut into pieces with a length of 3-5 cm and placed on a tray covered with opaque paper and allowed to air dry at a temperature of 25 ± 2 ºC without exposure to direct sunlight for 7 to 14 days and continued with oven drying at 45 ºC. The plant material was pulverized using a grinder (Retsch GmbH 5667HAAN Type SK1 Nr. 37535) to obtain a powder. Extraction of *P. aduncum* fruits and *A. odorata* twigs was carried out by maceration with periodic stirring. Each powder was soaked in organic solvent in a ratio of 1:10 (w/v). *P. aduncum* (500 g) was solvent extracted using hexane (technical grade) following the protocol of Abizar and Prijono (2010) and Hasyim (2011). *A. odorata* powder (500 g) was soaked in methanol (technical grade) Prijono et al. 2006). Soaking was done for 24 hours at room temperature (26 ± 2 ºC) and then filtered with filter paper (Whatman No. 41) using a glass funnel to get filtrate. The filtrate was evaporated using a rotary evaporator (Buchi R-100, Switzerland) at ± 40 ºC, 50 rpm, and 240 mbar pressure. The yield obtained from the fruit of *Piper aduncum* hexane extract (HxPa) and twigs of *Aglaia odorata* methanol extract (MtAo) were 5.57 % and 2.15 %, respectively based on dry weight with 3 extractions. The concentrated extract obtained was then stored in a refrigerator (± 4 ºC) until testing.

Test insects. Adult insects were obtained from the field population of cabbage plants in the IPB Experimental Farm Pasir Sarongge, Pacet District, Cianjur Regency Indonesia. *P. xylostella* rearing was conducted at the Insect Physiology and Toxicology Laboratory, Department of Plant Protection, Faculty of Agriculture, Bogor Agricultural University (IPB). Insect rearing was conducted following the procedure used by Basana and Prijono (1994). Adults of *P. xylostella* were reared in framed gauze cages (50 cm x 50 cm x 50 cm) and fed with 10 % honey solution soaked in a wad of cotton. Larvae were reared on pesticide-free broccoli leaves. Second-instar *P. xylostella* larvae of the third generation were used for bioassay due to its sensitivity that can reveal slight effects or differences in response to stimuli. Second instar larvae are generally more uniform in size and developmental stage compared to third instar larvae.

Toxicity bioassay. The botanical insecticides used in our research were three extracts: the fruit of *P. aduncum* hexane extract (HxPa), the twigs of *A. odorata* methanol extract (MtAo), and the leaves of *Dysoxylum parasiticum* methanol extract (MtDp). Among these three extracts, the two best ones, namely the hexane extract of *P. aduncum* fruit (HxPa) and the methanol extract of *A. odorata* twigs (MtAo), were selected for subsequent testing as mixed extracts. A mixture of *P. aduncum* hexane extract and the *A. odorata* methanol extract were tested at five levels with a ratio of 1:1, 1:2, 1:3, 2:1, 3:1, and control. Each extract was dissolved in methanol and Agristick 400 L was used as an emulsifier and diluted with water. Final concentration of methanol and agristick in the test mixture were 1% and 0.2%, respectively. Each treatment was replicated five times. All extract preparations were shaken with an ultrasonic shaker (NTS-1300, EYELA) to produce a homogeneous mixture. The test was conducted by leaf dipping method, adapted from IRAC method No. 18 (Abizar and Prijono 2010).

A portion of fresh broccoli leaf (4 cm x 4 cm) was dipped in the specific test extract and then air dried. Control leaves were dipped in the appropriate control solvent. Two portions of treated or control broccoli leaves were placed separately into a Petri dish (9 cm diam), and 10 *P. xylostella* larvae were placed into each Petri dish. The larvae were fed on treated leaves for 48 hours and subsequently fed on untreated leaves for an additional 24 hours (Dadang 2023). The number of dead larvae were counted at 24, 48, 72, and 96 hours after treatment (HAT).

The estimated probit regression parameters for the single extracts HxPa and MtAo against the second instar larvae of *P. xylostella* at 96 hours after treatment (HAT) using the leaf residue method, as conducted in previous research activities (Table 1), were used as a comparison.

Table 1. Toxicity of hexane extract of *P. aduncum* (HxPa) and methanol extract of *A. odorata* (MtAo) against second instar larvae of *P. xylostella* at 96 hours after treatment (HAT)

Extracts	$a^a \pm SE^c$	$b^b \pm SE^c$	$LC_{50}{}^d$ (CI[e] 95 %)(%)	$LC_{95}{}^d$ (CI[e] 95 %)(%)
HxPa	11.354 ± 0.991	5.537 ± 0.966	0.071 (0.059 - 0.079)	0.141 (0.125 - 0.177)
MtAo	4.247 ± 0.118	3.560 ± 0.400	1.627 (1.036- 2.938)	4.716 (2.719 - 61.853)

Description: a = intercept of probit regression line, b = slope of probit regression, c = standard error, d = lethal concentration (%), e = confidence interval

The concentrations of the mixtures used in each comparison differed based on the probit equations of the single extracts produced and the determined expected mortality proportions (Table 2). The expected mortality proportion of the mixture is calculated from the individual expected mortality proportions of the test insects due to HxPa treatment at concentration cPa and MtAo treatment at concentration cAo, which were calculated based on the probit regression of each single extract. The use of expected mortality helped determine the concentrations that are expected to cause 15-95 % mortality in the test insects (Dadang, 2023)

Table 2. Concentration (%) (w/v) used for toxicity testing of mixed extracts of HxPa and MtAo

Mixture ratio	Concentration of extract expected to produce mortality at various LC values				
(w/w)	LC_{15}	LC_{35}	LC_{55}	LC_{75}	LC_{95}
3:1	0.06	0.08	0.1	0.13	0.19
2:1	0.06	0.09	0.11	0.14	0.21
1:1	0.09	0.12	0.15	0.19	0.28
1:2	0.09	0.12	0.15	0.19	0.28
1:3	0.09	0.12	0.15	0.19	0.28

Quantitation of synergism, summation and antagonism. The combination index (CI) was used to evaluate possible additive, antagonistic, or synergistic interaction between the botanical insecticides in the mixture. The combination index (CI) at the LCx level was calculated by the following formula (Chou and Talalay 1984**):**

$$CI = \frac{LC_x^{a(cmp)}}{LC_x^a} + \frac{LC_x^{b(cmp)}}{LC_x^b} + \left[\frac{LC_x^{a(cmp)}}{LC_x^a} x \frac{LC_x^{b(cmp)}}{LC_x^b}\right]$$

$LCx^{a(mix)}$ and $LCx^{b(mix)}$ are LCx of extract components 1 and 2, respectively, in mixtures resulting in X mortality (e.g. 50 % and 95 %). LCx^{a} and LCx^{b} are the LCx of extract components 1 and 2, respectively, used separately.

The interaction between HxPa and MtAo extracts was determined based on the independent joint action model. This was through calculation of the combination index (CI) at both LC_{50} and LC_{95} levels. The LC value is obtained by multiplying the LCx of the mixture by the proportion of the concentration of components 1 and 2 in the mixture.

Categories of mixture interaction properties were adapted from Kosman and Cohen (1996) based on the inverse of the co-toxicity ratio value:

(1) CI < 0.5; mixture components were strongly synergistic,
(2) CI 0.5 - 0.77; mixture components were less synergistic,
(3) CI > 0.77-1.43; mixture components were additive,
(4) CI > 1.43; mixture components were antagonistic.

Chemical composition of extracts. The volatile chemical components of *P. aduncum* hexane extract was analyzed by gas chromatography mass spectrometry (GC-MS) (Agilent Technologies 7890 gas chromatograph with auto sampler and 5975 Mass Selective Detector and Chemstation Data System), under the following conditions: electron impact initiation model, electron energy 70 eV, with helium carrier gas, HP Ultra 2 column, capillary column size 30 m (length) × 0.20 mm I. D × 0.11 µm film thickness, initial column temperature of 80°C then increased at a rate of 3 ^{o}C min^{-1} until it reached 150 °C and held for 1 min, then the temperature was increased again at a rate of 20 ^{o}C min^{-1} until it reached 280 ^{o}C and held for 26 min, injection port temperature of 250 ^{o}C, ion source temperature of 230 ^{o}C, separator temperature of 280 ^{o}C, quadrupole temperature of 140 ^{o}C, constant flow column mode, column flow of 1.2 ml min^{-1}, injection volume of 5 µl, and separation ratio of 8 : 1. The GC-MS is connected to a monitor and printer that presents a chromatogram and a sequence table of pure compound types with each retention time and percentage concentration of each compound.

Meanwhile, the chemical components of *A. odorata* methanol extract was analyzed using UPLC-QToF-MS/MS (Agilent technologies, Palo Alto, CA, USA). Methanol extract (100 ppm) was prepared in dichloromethane and methanol, then injected (5 µl) into ACQUITY UPLC® H-Class System (Waters, USA) tandem Xevo G2-S QToF MS detector (Waters, USA). Samples were separated on ACQUITY BEH C18 column (1.7 µm 2.1 x 50 mm) with 0.05 % acetonitrile: 0.05 % formic acid as mobile phase, flow rate 0.2 ml/min. The UPLC-MS analysis results were processed using the Masslynx Version 4.1 application, to obtain chromatograms and mass spectra of each detected peak.

Statistical analysis. Cumulative mortality data of the test insects at 24, 48, 72, and 96 HAT were processed by probit analysis using the PoloPlus program (LeOra Software 2002-2003).

RESULTS AND DISCUSSION

Toxicity of extract mixtures. The test results of the mixture of HxPa and MtAo extract showed mortality of second instar larvae of *P. xylostella* using 2:1 (w/w) occurred at 24 HAT and continued to increase until 96 HAT. The 3:1 and 2:1 mixture ratios of HxPa and MtAo extracts at the highest concentrations of 0.19% and 0.21% caused 58% and 72% mortality, respectively at 24 HAT. The mortality increased sharply at 48 and 72 HAT. The highest concentration tested of 2:1 HxPa:MtAo extract mixture, 0.21 % (w/v), caused 100% mortality at 48 HAT, while for the 3:1 mixture, 100% larval mortality was at 72 HAT. Meanwhile, in the extract mixture of HxPa:MtAo 1:1, 1:2, and 1:3, larval mortality at the highest concentration was 78%, 86%, and 40% at 96 HAT, respectively (Fig. 1).

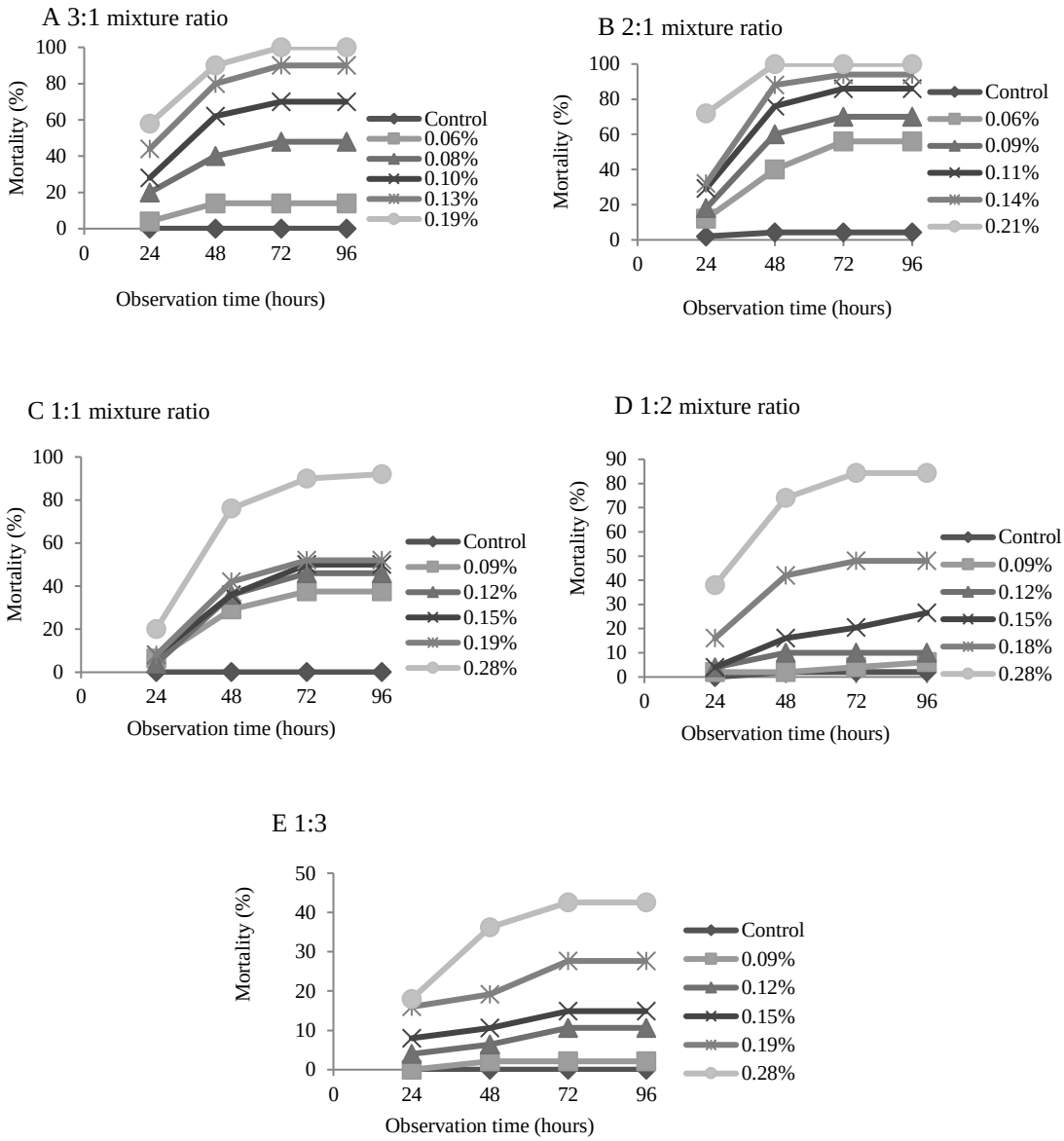

Figure 1. Cumulative mortality rate of second instar larvae of *P. xylostella* using a mixture of HxPa and MtAo at the ratio of 3:1; 2:1; 1:1; 1:2; 1:3 (w/w) at varying concentrations of extract mixtures.

Both extracts exhibited independently insecticidal properties against *P. xylostella* larvae, however HxPa extract demonstrated significantly higher activity than MtAo extract. Specifically, HxPa extract had LC_{95} 0.141 % and MtAo extract LC_{95} 4.716 % (Table 1).

Based on the results of probit analysis, the LC_{50} and LC_{95} values of extract mixtures of HxPa :MtAo 2:1 (w/w) at 48, 72 and 96 HAT were lower than other extract mixtures ratios of HxPa:MtAo, except for LC_{95} at ratio 3:1,which is lower than 2:1 ratio (Table 3).This means the ratio 2:1 was more toxic than the other mixture ratios.

This variation in toxicity between the extracts is likely due to differences in the type and concentration of active compounds present. In HxPa extract the primary insecticidal compound was dillapiole (Hasyim 2011). Dillapiole, in particular, is noted for its effective insecticidal activity against a various of pests. It acts as a neurotoxin, affecting neurotransmitter systems through mechanisms such as the inhibition of acetylcholinesterase enzymes, interference with octopamine synapses, and modulation of GABA receptors. This inhibition allows the active compound rocaglamide in the *A. odorata* extract to reach the target site and effectively cause *P. xylostella* larvae mortality. Additionally, insecticidal efficacy of dillapiole is enhanced by its synergistic interactions with other compounds. The hexane extract of *P. aduncum* fruit has high activity against *P. xylostella* larvae with LC_{50} 0.24 % and LC_{95} 0.68 % (Russianzi and Prijono 2019). A mixture of *C. soulattri* : *P. aduncum* extracts in a ratio of 1:2 at a concentration of 0.8 % caused 100 % mortality of *S. frugiperda* larvae (Widayani et al. 2023).]

Table 3. Toxicity of a mixture of *P. aduncum* fruits hexane extract (HxPa) and *A. odorata* twigs methanol extract (MtAo) against second instar *P. xylostella* larvae using leaf dipping method.

Ratio of *P. aduncum*: *A. odorata* extracts (w/w)	Observation time (HAT)[a]	a[b] ± SE[d]	b[c] ± SE[d]	LC_{50}[e] (CI[f] 95%)(%)	LC_{95}[e] (CI[f] 95%)(%)
3:1	24	2.779 ±0.525	3.418 ±0.547	0.154 (0.135-0.187)	0.466 (0.327-0.901)
	48	5.082 ±0.621	4.931 ±0.611	0.093 (0.085-0.101)	0.201(0.171-0.257)
	72	8.087 ± 0.987	7.592 ±0.887	0.086 (0.080-0.092)	0.142 (0.128-0.166)
	96	7.757 ± 0.896	0.752 ±0.714	0.084 (0.078-0.090)	0.142 (0.128-0.166)
2:1	24	2.652 ± 0.519	3.372 ± 0.566	0.164 (0.130-0.281)	0.503 (0.288-4.436)
	48	5.069 ± 0.679	4.525 ± 0.661	0.076 (0.065-0.084)	0.175(0.149-0.228)
	72	5.058 ± 0.749	4.140 ± 0.711	0.060(0.047 -0.069)	0.150 (0.127-0.200)
	96	5.058 ± 0.749	4.140 ± 0.711	0.060 (0.047-0.069)	0.150 (0.127-0.200)
1:1	24	0.147 ± 0.526	1.896 ± 0.679	0.837(0.425-33.993)	6.166 (1.400-26.90)
	48	1.471 ±0.407	2.023 ±0.495	0.187 (0.156-0.251)	1.218 (0.616-8.214)
	72	1.699 ± 0.414	2.000 ± 0.497	0.141(0.111-0.171)	0.939 (0.508-5.362)
	96	1.699 ± 0.414	2.000 ± 0.497	0.141 (0.111-0.171)	0.939 (0.508-5.362)
1:2	24	2.410 ±0.724	4.919 ± 1.070	0.324 (0.275-0.454)	0.699 (0.845-1.694)
	48	3.944 ± 0.579	5.865 ± 0.772	0.213 (0.196-0.236)	0.406(0.339-0.543)
	72	4.872 ±0.659	6.816 ± 0.867	0.193 (0.180-0.209)	0.336 (0.292-0.422)
	96	4.584 ± 0.629	6.344 ± 0.818	0.189(0.176-0.206)	0.344 (0.296-0.440)
1:3	24	0.719 ± 0.566	2.684 ± 0.757	0.540(0.357-2.084)	2.214(0.903-47.890)
	48	$1._{386}$ ± 0.508	3.226 ±0.676	0.372 (0.292-0.630)	1.203(0.687-4.514)
	72	1.597 ± 0.478	3.235 ±0.624	0.321(0.263-0.477)	1.035 (0.630-3.057)
	96	1.597 ± 0.478	3.235 ±0.624	0.321(0.263-0.477)	1.035 (0.630-3.057)

Description: a = Hours after treatment, b = Intercept of probit regression line, c = Slope of probitregression line, d = Standard error, e = Lethal concentration, f = Confidence interval.

The interaction character of mixtures of two or more different plant extracts can be synergistic, additive, or antagonistic. The extract mixtures of HxPa and MtAo at different concentration ratios demonstrated different characteristics of the combined action. The LC_{95} of the 3:1 (w/w) mixture of

HxPa and MtAo extracts showed strong synergism at 24 HAT and less synergism at 72 and 96 HAT, but at 48 HAT it showed additive character. The ratio of extract mixture at 2:1 showed strong synergistic at 24 HAT and less synergistic at 48, 72 and 96 HAT. The join action of *P. aduncum* and *A. odorata*at 1:1 was antagonistic at 24, 48, 72, and 96 HAT. Meanwhile, at the ratios of 1:2 and 1:3 showed strong synergistic character at 24 HAT and then became antagonistic as the exposure time increased (Table 4).

The mixture of HxPa : MtAo extracts at a ratio of 2:1 is the best ratio because it shows synergistic interaction characteristics and is consistent with increasing the exposure time (Table 4). A mixture of plant extracts with the right concentration ratio can be synergistic so that it can increase efficiency and effectiveness in increasing insect mortality. The mortality of the second-instar *P. xylostella* larvae was caused by the combined action of the biologically active phytochemicals present in both HxPa and MtAo.

A mixture with the appropriate ratio of two or more insecticides can increase the mortality of the test insects. Synergistic insecticides work by targeting multiple pathways, enhancing solubility and absorption, modulating distribution, and inhibiting detoxification enzymes. These mechanisms contribute collectively to the enhanced efficacy when used in combination (Ahmed 2020; Bernard 1993).

Table 4. Activity characteristics of a mixture of HxPa and MtAo extracts at different concentrations against *P. xylostella* larvae.

Ratio of *P. aduncum: A. odorata* extracts (w/w)	**Observation time (HAT)[a]**	**Combination index value**			
		LC_{50}	**Criteria**	**LC_{95}**	**Criteria**
3:1	24	0.54	LS	0.44	SS
	48	0.75	LS	0.83	AD
	72	0.88	AD	0.73	LS
	96	0.91	AD	0.77	LS
2:1	24	0.51	LS	0.43	SS
	48	0.54	LS	0.65	LS
	72	0.55	LS	0.69	LS
	96	0.58	LS	0.73	LS
1:1	24	2.05	AN	4.02	AN
	48	1.04	AD	3.47	AN
	72	1.01	AD	3.46	AN
	96	1.08	AD	3.76	AN
1:2	24	0.53	LS	0.30	SS
	48	0.82	AD	0.77	SL
	72	0.98	AD	0.81	AD
	96	2.54	AN	2.18	AN
1:3	24	0.69	LS	0.72	LS
	48	1.14	AD	1.77	AN
	72	1.33	AD	2.06	AN
	96	1.45	AN	2.30	AN

Description: a = Hours after treatment, LS = Less synergist, AD = Additive, SS = Strong synergist, AN = Antagonist

The lignan compounds contained in the HxPa extract make it synergistic when mixed with the MtAo extract. Dillapiole in *P. aduncum* fruit extract is thought to cause *P. aduncum* and *A. odorata* extract mixture to be synergistic in toxicity against *Crocidolomia odorata* (Hasyim 2011). The exposure of insects to dillapiole results in the significant changes in insect behavior due to its inhibition of acetylcholinesterase enzymes, effects on octopamine synapses, and potential modulation of GABA receptors, leading to hyperexcitability, paralysis, and altered motor functions (Fazolin et al. 2022). Dillapiole has a methylenedioxyphenyl group that can inhibit the activity of cytochrome P450 enzymes so that it can reduce the toxicity of xenobiotic compounds including insecticides (Bernard et al. 1989). This mechanism affects the phase I metabolism of xenobiotics, which is responsible for inactivating insecticides (Chellapandian et al. 2018; Fierascu 2020). This inhibition of P450 enzymes can lead to other insecticides mixed with *P. aduncum* extracts, reaching the target site. Moreover P450 enzymes play a crucial role in insecticide resistance by metabolizing and detoxifying insecticides, thereby reducing their effectiveness. These enzymes are involved in the oxidative metabolism of a wide range of xenobiotics, including insecticides. In insects, the over expression of certain P450 genes, particularly those in the CYP6 and CYP9 families, has been associated with increased resistance to various insecticides. This resistance is often due to the enhanced ability of the insects to metabolize and eliminate the insecticides, making them less toxic to the insects (Scott 2001; Zhang 2019).

Joint action of plant extract mixtures were observed in earlier studies against other insect pests. The LC_{95} level at 72 HAT, the ethyl acetate extract of *P. aduncum* (EtPa) was approximately 14.5 and 12.8 times more toxic to *C. pavonana* larvae compared to the methanol extract of *Sapindus rarak* (MeSr) and aqueous *S. rarak* (AqSr) extracts, respectively (Syahroni and Prijono 2013). When mixed in a 1:10 w/w ratio, the EtPa:MeSr extract combination was about 1.64 times more toxic to the larvae than the EtPa:AqSr extract combination at the LC_{95} level. A mixture of *P. aduncum* with *T. vogelii* and *B. javanica* works by facilitation, namely the active compound dillapiole inhibits the activity of P450 enzymes that commonly break down the toxic compounds in the insect body, so that the active compounds contained in *T. vogelii* and *B. javanica* can reach the target site (Lina 2014).

Using botanical insecticides in combinations can broaden the range of activity against target pests and potentially reduce the overall insecticide amount needed. This method is both economically and environmentally beneficial, as it lowers input costs and minimizes the environmental impact of chemical residues. Mixtures of two or more plant extracts that are synergistic mostly by inhibiting metabolic systems that will break down insecticide molecules and interfere with insecticide detoxification through their action on polysubstrate monooxygenase (PSMO) and other enzyme systems (Bernard et al. 1989). Therefore, using botanical extracts as insecticides aligns with the principles of integrated pest management (IPM) and sustainable agriculture. These natural products are often biodegradable and pose lower risks to non-target organisms, including humans, beneficial insects, and other wildlife. The significant increase in efficacy due to synergistic effects, reduction in chemical usage, and insights into resistance management are crucial findings that can influence future agricultural practices and pest control research (Dadang 2023; Fazolin et al. 2022)

Chemical components of extracts. Secondary metabolite compounds identified in the fruit HxPa extract and the twigs MtAo extract were 12 and 15 components (Tables 5 and 6). Several of these compounds were found in significant quantities. The component identified in the hexane extract of *P. aduncum* fruit was caryophyllene, pentadecane, dillapiole, copaene, phytol, trans-geranylgeraniol, tetracosane, squalene, campesterol, stigmasterol, and gamma-sitosterol. The major chemical component of the HxPa extract base on GC-MS analysis was dillapiole (32.3%) followed by pentadecane (20.3%).

Table 5. Phytochemicals identified from hexane extract of *Piper aduncum* fruits by GC-MS.

Retention time (minutes)	Compounds	Similarity (%)	Content (%)
15.009	Copaene	99	6.8
16.546	Caryophyllene	99	2.8
20.601	Pentadecane	98	20.2
26.152	Dillapiole	99	32.3
32.419	Phytol	93	4.5
32.833	trans-Geranylgeraniol	99	1.4
35.088	1-Tetracosane	99	1.0
35.750	Squalene	99	1.3
37.632	Tetracosane	98	1.0
39.439	Campesterol	99	2.5
39.894	Stigmasterol	99	2.9
40.845	gamma-Sitosterol	99	5.1

Plants in the Piperaceae family are characterized by the presence of secondary metabolite alkaloids, including piperamide compounds such as piperine, piperlongumine, dihydropiperine, dihydropiperlongumine, piperanine, and piperside. The effectiveness of *Piper* extracts as natural insecticides is associated with their piperamide (Fazolin et al. 2022). Dillapiol features a methylenedioxy-phenyl group, a characteristic structure found in various biologically active natural compounds such as lignans and other secondary metabolites. This group is also present in insecticidal alkaloids like piperine and in phenolic compounds such as safrole. The latter is used to produce the commercial synergist piperonyl butoxide. These compounds interact with cytochrome P-450 enzymes, inhibiting the activity of polysubstrate monooxygenase (PSMOs) that are responsible for metabolizing and excreting toxins in insects (Belzile et al. 2000).

Various secondary metabolites found in HxPa extracts exhibit insecticidal properties toward insects. Dillapiole was found to be the main component in HxPa extract with varying amounts ranging from 9.2% to 94.8% (Jantan et al. 1994; Santana et al. 2015; Silva et al. 2019). Rali (2007) reported that chemical analysis by GC/MS determined the volatile constituents in *P. aduncum* with the main component being dillapiole (43.3%) and other minor components such as β-caryophyllene (8.3%), piperitione (6.7%) and α-humulene (5.1%). Other compounds detected in considerable amounts were caryophyllene, copaene, 2-propenoic acid, 3-(3,4-dimethoxyphenyl)-methyl ester, phytol, campesterol, stigmasterol, and gamma-sitosterol, while other compounds were found in small amounts (<3 %). Some of these compounds showed insecticidal activities including (E)-caryophyllene and α-humulene isolated from *Commiphora leptophloeos* leaf oil significantly showed an egg-preventing effect on *Aedes aegypti* mosquitoes (Da Silva et al. 2015). Pentadecane and phytol act as natural insecticides, killing insects quickly on contact (Estrada et al. 2013). Copaene isolated from *Achillea filipendulina* is effective in controlling *Oryzaephilus surinamensis* and *Tetranychus urticae* insects (Ebadollahi et al. 2017). Squalene, contained in the *Olea europaea* (olive) plant can disrupt the integrity of cell membranes in insects, causing cellular dysfunction (Singh et al. 2012). β-sitosterol, a bioactive phytocomponent in *Thevetia neriifolia* inhibited larval growth, affecting larval and pupal development (Mishra et al. 2020).

Rocaglamide is the main component of the MtAo extract with concentration of 18.23 %. Other significant chemicals included 2-morpholinoacetic (15.19 %),1,3-bis(cyclobutylamino)-N-[(2S)-2-hydroxy-3-[6-[(4-methyl-1,3-oxazole-5yl)methoxy]-3,4-dihydro-1H-isoquinolin-2-yl]propyl]-2-azaspiro[5.7]tridecane-5-carboxamide (12.20 %,) sameridine (9.15 %), and oxo[(1-pentadecyl-4(1H)-pyridinylidene)methyl]ammonium (5.91 %) (Table 6).

Table 6. Phytochemicals identified from methanolic extract of *Aglaia odorata* twigs by LC-MS.

Retention time (minute)	Compounds	Similarity (%)	Content (%)
1.303	2-mo arpholinoaceticcid	99.94	15.2
2.223	(4R)-4-[[(5R)-5-hydroxy-5-(hydroxymethyl)-2-methoxy-3-oxocyclohexen-1-yl]amino]-5-[(2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxypentanoic acid	99.94	0.5
4.136	Antiarol	99.87	1.3
4.705	Acetamide, N-[(2R,4R,4aS,7aR)-2-[4-[(4-O-β-D-glucopyranosyl-β-D glucopyranosyl)oxy]phenyl]octahydrocyclopenta[b]pyran-4-yl]-	89.13	1.4
5.450	β-D-Glucopyranoside	95.19	1.4
7.496	Benzoic acid	99.95	1.9
8,350	Rocaglamide (1R,2R,3S,3aR,8bS)-1,8b-dihydroxy-3a-(3-hydroxy-4-methoxyphenyl)-6,8-dimethoxy-N,N-dimethyl-3-phenyl-2,3-dihydro-1H-cyclopenta[b][1]benzofuran-2-carboxamide	99.56	18.2
9.760	Nobiletin (3',4',5,6,7,8-Hexamethoxyflavone)	97.99	0.9
12.087	Phytosphingosine ((2S,3S,4R)-2-Amino-1,3,4-octadecanetriol)	100	2.3
12.945	Sameridine	97.84	9.2
13.782	Oxo[(1-pentadecyl-4(1H)-pyridinylidene)methyl]ammonium	99.90	5.9
14.506	1,3-Propanediol, 2- amino-2-(1,2,3,4-te trahydro-6-octyl-2- naphthalenyl)-	97.53	3.0
15.518	Scortechinone C	98.09	2.0
16.221	Benzenamine, N,N-didodecyl-4-[(E)-2-(4-pyridinyl)ethenyl]-	97.53	3.3
18.456	(4Z,7Z,10Z,13Z,16Z,19Z)-N-[(4E,8E,12E)-1,3-dihydroxynonadeca-4,8,12-trien-2-yl]docosa-4, 7,10,13,16,19-hexanenamide	95.21	1.9

The leaves and twigs of *A. odorata* contain the active compounds of dammarane triterpene and aminopyrrolidine bis-amides which are known to have insecticidal activity and inhibit growth as

insecticide growth regulators (IGR) against *Peridroma saucia* (H.) and *S. litura* larvae (Ishibasi et al. 1983; Janprasert et al. 1993). Organic extracts of the twigs and leaves of *Aglaia odorata* contain eight insecticidal cyclopentatetrahydrobenzofuran rocaglamide derivatives, four new cyclopentatetrahydro benzopyran aglain derivatives, as well as the known aminopyrrolidine odorine and odorinol, syringaresinol and flavonoid (Nugroho et al. 1999). The mode of action of rocaglamide compounds in insects involves direct toxicity, feeding deterrence, cellular level effects, and developmental time extension (Ebada et al. 2011). These mechanisms collectively contribute to the potent insecticidal activity of rocaglamide against various insect species. Injecting rocaglamide into the haemolymph of last instar larvae of *Spodoptera littoralis* resulted in significant toxicity, with an LC_{50} value ranging between 5.6 and 7.5 ppm (Dono et al. 2004; Ebada et al. 2011).

Phytochemical analysis on leaves of various *Aglaia* species collected in Vietnam yielded eight rocaglamide derivatives, responsible for potent insecticidal activity against *Spodoptera littoralis*, including rocaglamide A, rocaglamide I, rocaglamide W, rocaglamide AB, rocaglamide J, rocaglaol, rocaglamide S, and the novel rocaglamide AY (Ngoc et al. 2014). Rocaglamide has also been isolated from *Aglaia odorata* and *Aglaia duppereana* branches (Janpraset et al. 1993; Nugroho et al. 1997), *Aglaia elliptifolia* stems (Wu et al. 1997), and *Aglaia harmsiana* leaves (Nugroho et al. 1999). The level and effectiveness of active compounds in plants are determined by various factors, such as plant genetics and the environmental conditions experienced during their growth (Dadang 2023; Schoonhoven et al. 2005).

The main bioactive compounds in each extract play a significant role in determining the nature of their interactions, other less active compounds also contribute by modulating, stabilizing, or enhancing the overall activity through various mechanisms. This understanding highlights that botanical extracts are not simply mixtures of compounds but complex systems in which each component can play a significant role in enhancing or modulating the overall biological activity (Vaou et al. 2022).

CONCLUSION

The different ratios of extract mixtures resulted in different interactions. The 2 : 1 (w/w) mixture of HxPa and MtAo extracts showed the highest mortality against second instar larvae of *P. xylostella* than other comparisons and showed synergistic interaction. The LC_{50} and LC_{95} of the *P. aduncum*:*A. odorata* extract mixture with a concentration ratio of 2 : 1 (w/w) at 96 hours after treatment (HAT) were 0.06 % and 0.15 %, respectively. This combination has the potential to be developed further as a botanical insecticide to control *P. xylostella*.

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