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Impact of Botanical Insecticides Derived from *Pangium edule* Reinw and *annona muricata* l. Seed Extracts on the "Gay Gantung" Diamondback Moth, *Plutella xylostella* l.

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Abstract

Insecticidal properties of fractioned extracts from *Pangium edule* Reinw seed and *Annona muricata* seed against *Plutella xylostella* larvae were investigated in the laboratory. The study was initiated to investigate the possibility of using botanical pesticides to control *P.xylostella,* a serious cosmopolitan pest of crucifer plants. The study aims to determine the most effective concentration and the most active extract; to evaluate the different extract concentrations on the treated larvae; and to characterize the phytochemical contents of the most effective extracts fraction. The study was an experiment initiated by test of phytochemical screening test in order to discover the presence of secondary metabolites in the extracts. It was followed by the test of mortality of the diamondback moth larvae. Furthermore, the extracts hexane fraction and etanol fraction, were conducted with completely randomized design; The LC_{50} values were determined following probit analysis, the data were treated in the software programme IBM Statistic SPSS 20. Results showed that n-hexane fraction is the most effective againts larvae ($LC_{50\text{-}48h}$ = 12,71 mg/L) from *P.edule* seed extract, ($LC_{50\text{-}48h}$ = 50,81 mg/L) from *A.muricata* seed extract. Larva mortality was highest using 1000 ppm n-hexane fraction (96,6%) derived from *P.edule* seed extract, (93,3%) derived from *A.muricata* seed extract. The ethanol fraction tested positive for alkaloid, saponins, flavonoids, terpenoids, phenol and tannins. N-hexane fraction of *P.edule* seed extract, and *A.muricata* seed extract are an effective botanical insecticides exhibiting larvicidal and antifeedant properties against *P.xylostella* thus it can be alternative to synthethic insecticides. Results indicate that these botanical insecticides have good possibilities for control of *P.*xylostella. Further work is necessary to evaluate and characterize the active components of the extract fractions and its efficacy in the field.

Keywords: *Botanical insecticides, Pangium edule, Annona muricata, "Gay Gantung" Plutella xylostella*.

A. Introduction

Botanical insecticides represent one alternative to synthetic insecticides due to the negative effects of the latter, i.e. pest resistance, secondary pest outbreaks and effects on the environment and non-target organisms. Intensive use of synthetic insecticides to control insect pests had lead to many problems such as pest resistance and surgence, effects on non-target organisms, human exposure and environmental impacts. The negative effects have provided the impetus dor the developmentof alternatives including botancial insecticides. Botanical insecticides developed from plant extracts are less persistent in the environment and are often safer than synthetic chemicals. Botanical insecticides is an agent and a part of biological control process (Abalos, 2013; Sakul,*et al*, 2012).

Biological control is the cornerstone of any sustainable pest management strategy and an essential component of integrated pest management. Biological control is defined as the "action of parasites, predators, pathogens, chemical compounds from plant, insecticidal secondary metabolites in maintaning another organism's (the pest) density at an average lower than would occur in their absence (Charleston *et al*, 2004; Leatemia, 2003).

Integrated pest management (IPM) attempts to integrate the available pest control methods to achieve a farmer's most effective, economical and sustainable combination for a particular local situation.Emphasis is placed on biological control, host plant resistance, cultural control and other non-polluting methods. Successful IPM programs produce many benefits, including :
1) lower production costs compared with conventional pest control strategies with a high input to synthetic pesticides, 2) reduced environtment pollution, particularly improvement of soil and water quality, 3) reduced farmer and consumer risks from pesticide poisoning and related hazards, and 4) ecological sustainability by conserving natural enemy species, biodiversity and genetic diversity (Lim *et al*. 1997; Charleston *et al*, 2004).

The plant kingdom is by far the most efficient 'factory' of chemical compounds, synthesising many products that are used in the defence against herbivores. The insecticidal secondary metabolites from one plant species can be applied to other plant species to provide protection for this second plant. Extract prepared from plant (botanical pesticides) have a variety of properties including insecticidal activity, repellence to pests, antifeedant effects, insect growth regulation, toxicity to nematodes, mites and other agricultural pests, also antifungal, antiviral and antibacterial properties against pathogens (Boeke *et al.*,2001 in Charleston *et al*, 2004).
The steps involved in the development of botanical insecticides from plant extracts begin with screening of candidates for deleterious effects on insects followed by standardization of promising extracts via bioassay (Leatemia, 2003).

The "Gay Gantung" Diamondback moth, *Plutella xylostella* Linnaeus (Lepidoptera: Plutellidae), is one of the most important insect pest of Brassicaceae in the world. This species also a cosmopolitan insect pest of cruciferous plants and can be especially destructive. Adult female moths lays their eggs on the underside of leaves. The larvae hatch and feed on the parenchyma, leaving the cuticle intact, but as the plant grows the cuticle tears resulting in a characteristics holey appearance on the surface of the leaf.

There are four larval instars before pupation occurs, and at 25^{0}C the life cycle from egg to adult emergence takes approximately 24 days. The first-instar larvae mine in the spongy mesophyl tissue, whereas older larvae feed from the lower leaf surface and usually consume all tissue except the wax layer on the upper surface, thus creating the a window in the leaf (Vanlaldiki,H.*et al*., 2013; Trindade,R.C.P. *et al*.,2011; Abbasipour,H. *et al*., 2010; Charleston,D.S. *et al.,*2004).

*Plutella xylostella*is the insect pests which is cosmopolitantly distributed in. Its attacks could damage vegetables resulting in loss of quantitative and qualitative. To overcome these problems need to develop a means of pest control, which are effective but enviromental friendly. North Sulawesi has a lot of plants, which is potentially developed as a source of botanical insecticides. Pangi plant (*Pangium* edule Reinw.) is a plant species which potentially developed and effective against several types of insect pest, but testing by using crude extract can give varies results depending on the type of extract used, the test insects and environment factors. Part of body from Pangi plant as such as leaves, bark of the stem, roots, and seed have a potentially as a botanical insecticides (Sakul, *et al.,* 2012; Salaki,*et al.,2012*).

The Annonaceae (custard-apple family) is a family of almost excluscively tropical trees and shrubs. Plant parts of some species of this family have been used traditionally as insecticides. Also soursop plant (*Annona muricata* Linn.) is a potentially plant, along with their importance as sources of food materials and of popular medicaments, many members of the Annonaceae are

also valued as insecticides.For example, the powdered seeds and leaf juices of *Annona* spp are used to kill head and body mosquitoes (Trindade, *et al,* 2011; Leatemia, 2003).

Insecticidal properties of fractioned extracts from *P.edule* Reinw. seed and *A. muricata* Linn. seed against *P. xylostella* larvae were investigated in the laboratory. The study was initiated to investigate the possibility of using botanical pesticides to control *P.xylostella,* a serious cosmopolitan pest of crucifer plants. The study aims to determine the most effective concentration and the most active extract; to evaluate the different extract concentrations on the treated larvae; and to characterize the phytochemical contents of the most effective extracts fraction.

The study was an experiment initiated by test of phytochemical screening test in order to discover the presence of secondary metabolites in the extracts. It was followed by the test of mortality of the diamondback moth larvae. Toxicity of the extracts was assesed using leaf dip (leaf disc) bioassay.

B. Methodology

Insect rearingand Seedlings of cabbage plants

P.xylostella population (larval stage), was collected from cauliflower crops of Tonsealama village at the North of Tondano, Minahasa, North Sulawesi, Indonesia. For egg laying, leaves of cauliflower, *Brassica juncea* L. (Brassicaceae) were used and eggs were transfered to leaves of mentioned plant to continue their development. Insect stock was maintaned in a controlled environment at 25 ± 2^0C and 65 ± 5% relative humidity (RH). For this research, larval stage from *P.xylostella* was used the third instar larvae (age of larva is 7-12 days). Seed of cabbage plants, *B. juncea* L. (Brassicaceae) were germinated in expanded polystyrene trays containing a mixture composition consist of soils, organic fertilizer, and coconut fibre, 2 : 1 : 1) and maintained for 35 days in the greenhouse. Seedlings of cabbage plants were transplanted into polibag and planted in black plastic bags in a green house (28 ± 5^0C). To protect the plants against insect damage they were placed within a tent-loke construction made from fine netting (mesh size <1 mm). The plants were fertilised when planted, and regularly watered.

Plant material

Specimen *P.edule* Reinw. seeds and *A.muricata* L. seeds were collected from a garden Tonsealama village at the North of Tondano, Minahasa, North Sulawesi, Indonesia. The seeds were harvested and collected from trees at a height of about 3.5 m – 6.5 m, and placed in green house (28 ± 5^0C) to dry; after which they were then crushed into a crude material and stored in an airtight container until use.

Preparation of plant extract with n-hexane fraction and ethanol fraction

A crude extract of the seed of *P.edule* Reinw. and the seed of *A.muricata* L. was prepared by steeping dried seed material in n-hexane liquid and ethanol liquid. In each extraction,especially the first maceration, used 500 grams of powdered seeds*P.edule* Reinwwere extracted by maceration in 1000 ml of n-hexane at room temperature for 1 days (1 x 24 h). Also 500 grams the powdered seeds *A.muricata* L. were extracted too by maceration in 1000 ml of n-hexane at room temperature for 1 days (1 x 24 h), and after that we collected the first filtrate.

After that sediment of *P.edule* and *A.muricata* seed collected in erlenmeyer flask, this sediment were extracted again by maceration process (stage 2) in 800 ml of n-hexane at room temperature for 1 days (1 x 24 h) and after that we collected the second filtrate. The next process is collected again the sediment of *P.edule* and *A.muricata* seed in erlenmeyer flask, were extracted again by maceration process (stage 3) in 500 ml of n-hexane at room temperature for 1 days (1 x 24 h) and after that we collected the third filtrate. The filtrate of n-hexane fraction and filtrate of ethanol fraction were filtered by using the Whatmann Filter Paper and Buchener Funnel, and after that the filtrates were concentrated to dryness by a rotary evaporator under low pressured and temperature of rotary evaporator in 40^0C. Seed extract was stored in labelled bottle atrefrigerator 4^0Cuntil required for bioassay. If water remained in the concentrated crude extract, the material was store in a vacuum dessicator over silica gel.

Bioassay

A leaf dipping bioassay method (Qin *et al.*2004) can be used to assess contact toxicity. A commond assay used to assess contact as well as stomach toxicity of a compound in older larva is a lead dip bioassay. In this assay, a leaf or leaf disc is dipped in a solution of the extract being tested or dipped in the solvent alone (=control). Test insects are fed these discs and mortality recorded. Cabbage leaves were washed with distilled water and dried for about 2 hour. Four concentrations 50 ppm, 100 ppm, 500 ppm and 1000 ppm of the seed n-hexane extract and four concentrations 50 ppm, 100 ppm, 500 ppm and 1000 ppm of the seed ethanol extract, both of

specimen (*P.edule* Reinw. and *A.muricata* L.) were prepared. Cabbage leaves disks (10cm diameter) were cut with a scalpel blade from fully expanded cabbage leave grown in a green house. The disks were dipped for 30 seconds in the test solutions extract and air dried. After air-drying at the room temperature, leaves disks were then placed in a plastic cup (15-20 cm in diameter, 5-7 cm in depth). Ten third instar larvae were starved for 2 hour and then released into the plastic cup for each treatment. Both of specimen *P.edule* Reinw. and *A.muricata* L. treatments (four concentrations 50 ppm, 100 ppm, 500 ppm and 1000 ppm) were replicated three times. The cups were placed in a growth chamber at 25 ± 2^0C and 65 ± 5% relative humidity (RH). Mortalities were recorded 48 hour after treatment. Larvae were considered dead if they did not move when prodded with fine brush. Live larvae were transferred to untreated fresh cabbage leaves to continue their growth and development. The cabbage leaves were replaced with fresh ones when needed.

Statistical analysis

$LC_{50\text{-}48h}$ data values were determined following probit analysis and experimental data were subjected to one way ANOVA at 0.05 significance level using SPSS IBM-Software Ver.20. Means were then compared by Least Significance Different (LSD/BNT).

C. Result and Discussion

Results showed that larva mortality was highest using 1000 ppm n-hexane fraction (96,6%) derived from *P.edule* seed extract, (93,3%) derived from *A.muricata* seed extract.

Table 1.
The test results of mortality *P.xylostella* based on activity from *P.edule* plant seed extract fraction of n-hexane after 48 hours

Treatment	50 ppm	100 ppm	500 ppm	1000 ppm
1	3	4	9	10
2	3	5	8	10
3	2	5	8	9
Total of mortality	8	14	25	29
Average	2,667	4,667	8,333	9,667
Percentage	26,6%	46,6%	83,3%	96,6%

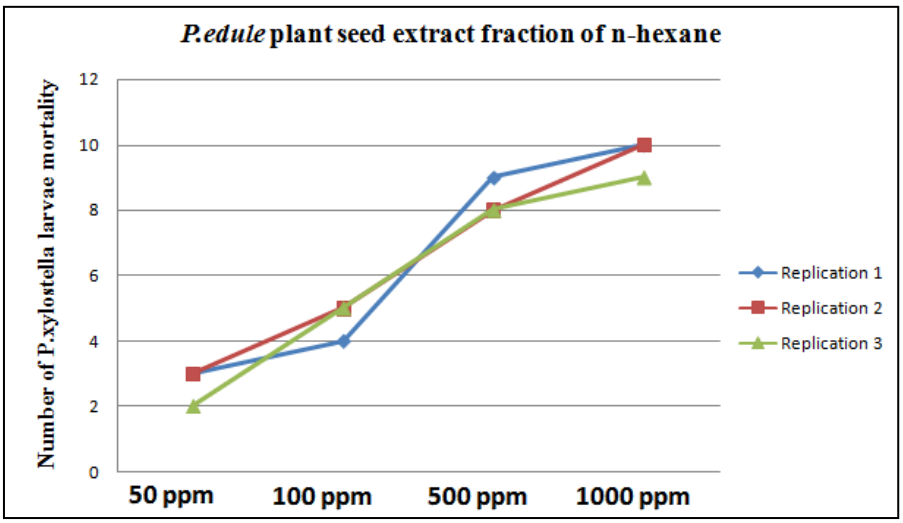

Figure.1. The test results of mortality *P.xylostella* based on activity from *P.edule* plant seed extract fraction of n-hexane after 48 hours

Table 2.
Test of Homogenity of Variances and Analysis Of Varians
From P.edule plant seed extract fraction of n-hexane after 48 hours

Test of Homogeneity of Variances

VAR00001

Levene Statistic	df1	df2	Sig.
1,055	2	9	,387

ANOVA

VAR00001

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7,200	2	3,600	,362	,706
Within Groups	89,467	9	9,941		
Total	96,667	11			

Table 3.
The test results of mortality *P.xylostella* based on activity from *A.muricata* plant seed exctract fraction of n-hexane after 48 hours

Treatment	50 ppm	100 ppm	500 ppm	1000 ppm
1	4	5	7	10
2	3	4	6	9
3	2	4	7	9
Total of mortality	9	13	20	28
Average	3,000	4,333	6,667	9,333
Percentage	30 %	43,3%	66,6%	93,3%

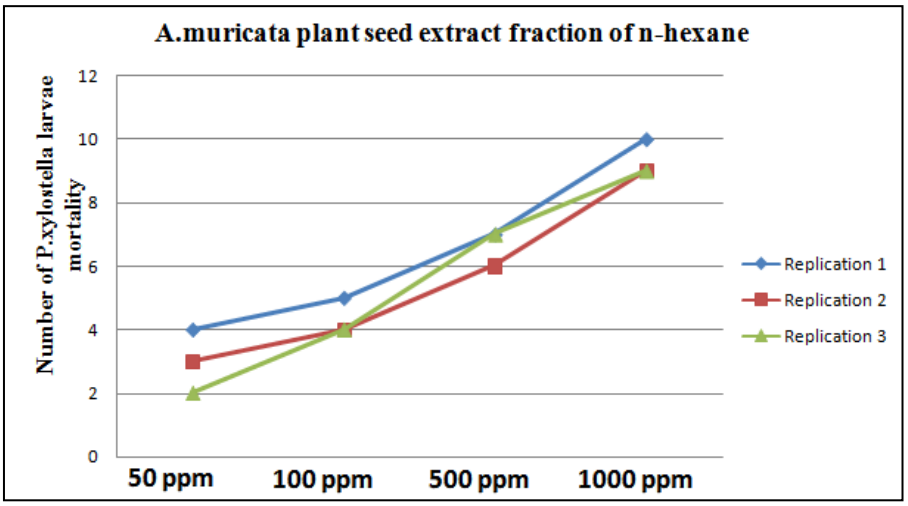

Figure.2. The test results of mortality *P.xylostella* based on activity from *A.muricata* plant seed extract fraction of n-hexane after 48 hours

Table 4.
Test of Homogenity of Variances and Analysis Of Varians
From A.muricata plant seed extract fraction of n-hexane after 48 hours

Test of Homogeneity of Variances

VAR00001

Levene Statistic	df1	df2	Sig.
,214	2	9	,811

ANOVA

VAR00001

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2,667	2	1,333	,169	,847
Within Groups	71,000	9	7,889		
Total	73,667	11			

Table 5
The test results of mortality *P.xylostella* based on activity from *P.edule* plant seed exctract fraction of ethanol

Treatment	50 ppm	100 ppm	500 ppm	1000 ppm
1	2	2	4	5
2	1	3	5	6
3	1	4	5	5
Total of mortality	4	9	14	16
Average	1,333	3,000	4,667	5,333
Percentage	13,3 %	30,0 %	46,6 %	53,3 %

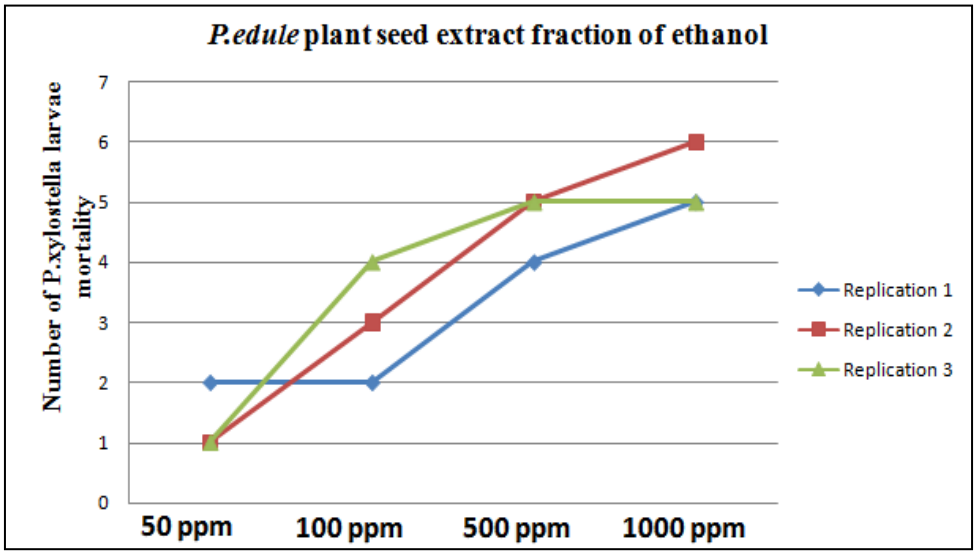

Figure.3. The test results of mortality *P.xylostella* based on activity from *P.edule*plant seed extract fraction of ethanol after 48 hours

Table 6.
Test of Homogenity of Variances and Analysis Of Varians
From *P.edule plant* seed extract fraction of n-hexane after 48 hours

Test of Homogeneity of Variances

VAR00001

Levene Statistic	df1	df2	Sig.
,394	2	9	,685

ANOVA

VAR00001

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	,667	2	,333	,093	,912
Within Groups	32,250	9	3,583		
Total	32,917	11			

Table 7.
The test results of mortality *P.xylostella* based on activity from *A.muricata* plant seed exctract fraction of etanol

Treatment	50 ppm	100 ppm	500 ppm	1000 ppm
1	1	2	3	5
2	2	2	4	4
3	2	3	4	5
Total of mortality	5	7	11	14
Average	1,667	2,333	3,667	4,667
Percentage	16,6 %	23,3%	36,6%	46,6%

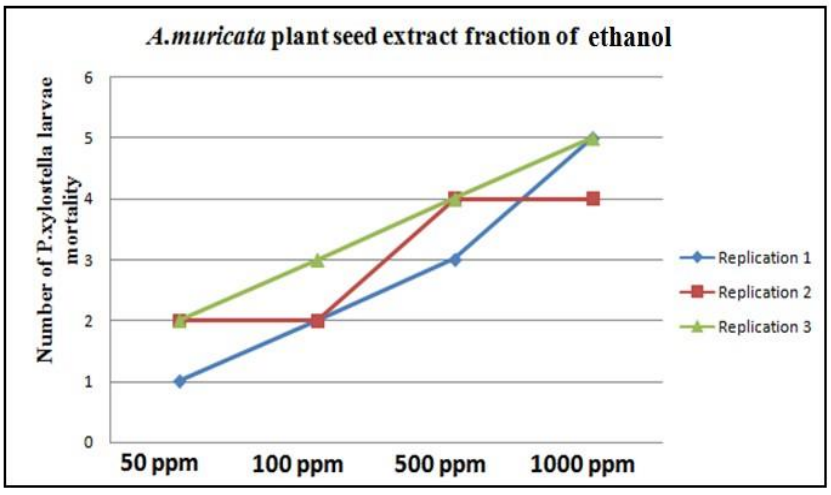

Figure.4. The test results of mortality *P.xylostella* based on activity from *A.muricata* plant seed extract fraction of ethanol after 48hours

Table 8
Test of Homogenity of Variances and Analysis Of Varians
From *P.edule plant* seed extract fraction ethanol after 48 hours

Test of Homogeneity of Variances

VAR00001

Levene Statistic	df1	df2	Sig.
,214	2	9	,811

ANOVA

VAR00001

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1,167	2	,583	,296	,751
Within Groups	17,750	9	1,972		
Total	18,917	11			

Results showed that n-hexane fraction is the most effective againts larvae ($LC_{50\text{-}48h}$ = 12,71 mg/L) from *P.edule* seed extract, ($LC_{50\text{-}48h}$ = 50,81 mg/L) from *A.muricata* seed extract. According to table 5, it mean that n-hexane fraction from both of two species *P.edule* seed extract and *A.muricata* seed extract is toxic.

Table 5. Toxicity Classification LC_{50} and Toxicity Rating (ISO,1982)

LC_{50} (mg/L)	Toxicity Rating
>10000	Non Toxic
1000 – 10000	Very low toxic
100 – 1000	Low toxic
10 – 100	Toxic
1 – 10	High Toxic
0,1 – 1	Very High Toxic
< 0,1	Extreme Toxic

Test of phytochemical screening test in order to discover the presence of secondary metabolites in the extracts. And the result is the ethanol fraction tested positive for alkaloid, saponins, flavonoids, terpenoids, phenol and tannins.

D. Conclusion

n-hexane fraction of *P.edule* seed extract, and *A.muricata* seed extract are an effective botanical insecticides exhibiting larvicidal and antifeedant properties against *P.xylostella* thus it can be alternative to against the synthethic insecticides. Results indicate that these botanical insecticides have good possibilities for control of *P.*xylostella. Further work is necessary to evaluate and characterize the active components of the extract fractions and its efficacy in the field.

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