

## **Biopesticide from Combination of Bitung (*Barringtonia Asiatica* L.Kurtz) Seed and Papaya (*Carica Papaya*) Sap**

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**Abstract :** *Crocidolomia pavonana* (Lepidoptera: Pyralidae) is one of *Brassicaceae* family plant pest such as cabbage, broccoli, cauliflower, mustard greens and turnips. Pest control using biopesticides such as *Barringtonia asiatica* L. (Kurz) has been studied. The development of biopesticides combined with papaya sap (*Carica papaya*) has never been done. The purpose of the study was to obtain a biopesticide formulation from a mixture of bitung seed (*B. asiatica*) extract and papaya (*C. papaya*) sap which can control *C. pavonana* pests on cabbage plants. This research is an experimental study using a completely randomized design. *Barringtonia asiatica* extraction using a solvent fractionation method. The bioactivity of the biopesticide formula was evaluated based on the mortality of *C. pavonana*. The compound content in biopesticides was analyzed using LCMS. The results showed that bitung seed extract could kill *C. pavonana* larvae with LC<sub>50</sub> values of 5.325 g/L. Papaya sap can increase the toxic effect of deadly bitung seed extract from *C. pavonana* larvae with LC<sub>50</sub> value of 2.390 cc/l. The LCMS analysis showed that biopesticides contained 5 compounds considered as tetraheptacontylbenzene and 4 unknown compounds. The conclusion of this study is that biopesticides from a mixture of bitung seed (*B. asiatica*) and papaya (*C. papaya*) sap were effective in controlling *C. pavonana* pest on cabbage.

**Keywords :** biopesticide, *Barringtonia asiatica*, *Carica papaya* sap, *Crocidolomia pavonana*, *Brassica oleraceae*.

### **Introduction**

Cabbage (*Brassica oleracea* var. *Capitata* L.) is one of the agricultural plants (horticulture). There are around 400 varieties of cabbage around the world in different shapes from round to cone, sizes generally ranging from ten to twenty centimeters, green, white, red, and purple leaves, and the most common are round

green. Cabbage is an economical and versatile vegetable that is easily found in supermarkets or traditional markets and provides enormous nutritional value. Because of the many benefits of cabbage plants, these plants are widely cultivated in Indonesia.

Agricultural production such as food crops, plantations and horticulture crops often decreases in terms of quality and quantity. One indicator is the presence of pest. Pest can occur starting from seed seedlings to post-harvest, or starting from the field to storage in the warehouse. The intensity of pest can vary, ranging from the intensity of minor attacks to the intensity of severe attacks. The intensity depends on several factors such as the type of pest that attacks, plants that are cultivated, the planting season and also the cultivation techniques carried out.

Efforts to develop biological control programs for tropical agricultural plant insect pests in Indonesia began to develop in the last three decades, especially after the launch of the Integrated Crop Management program in 1986. In certain regions efforts to implement pest control biodiversity has not been well implemented because of the lack of support from the local government and understanding of biological control methods that are not widely understood by the farming community. For example, control of coconut grasshopper *Sexava nubila*, in Sanger Talaud, North Sulawesi Province, which began in the early 1970s using insecticide spraying, did not produce good results.

The development of various types of pests and diseases in plants has encouraged farmers to control these pests using insecticides. Surveys conducted in North Sulawesi in 1990 showed that almost all farmers use insecticides for agricultural pest control<sup>1</sup>. The survey of vegetable farmers in Modinding in early 2007 also showed that more than 95% of farmers still use insecticides. For example, farmers in several locations in vegetable farming in North Sulawesi spray their vegetables with a mixture of several types of insecticides 3-4 times per week, even in the vegetative period, farmers spray almost every day<sup>2</sup>.

Spraying pests with high intensity causes the pests to be more resistant to these insecticides, thus requiring a higher dose of insecticide and a greater amount of treatment to kill it. As a result of excessive use of insecticides causing pollution of soil, water, air, including agricultural products. Water, food, and air contaminated with insecticide residues can cause disruption to human health. In fact, insecticide residues that enter the human body are thought to cause cancer, teratogenic or mutagenic genes.

Inappropriate use of insecticides can have a very bad impact on the environment, users and consumers. The results of several studies show that the application of synthetic insecticides has reduced natural enemy populations, especially parasitoid and predators, and insects or other useful organisms that interact with host plants, such as pollinator insects on agricultural lands. Another problem due to improper use of insecticides is that the presence of marketable agricultural commodities contains a lot of organophosphorus insecticide residues which are groups of organophosphate synthetic insecticides that are above the maximum residual limit.

Synthetic insecticides greatly help the productivity of agricultural products even though it has been realized that the negative impacts caused are not small. If synthetic insecticides are stopped drastically, it is feared that agricultural production will decline. Currently bio-insecticides are being promoted and developed that are environmentally friendly. This was done to support the implementation of Integrated Pest Management (IPM). In addition to being selective, bioinsecticides also have very short persistence levels so there is no need to worry about leaving residues in the crop.

At present there are three important natural insecticide sources that have good prospects to be developed, namely plants, soil microorganisms and marine organisms. Plant species that are known to have potential as potential bioinsecticide sources include Caricaceae, Meliaceae, Flacourtiaceae, Annonaceae, Piperaceae, Asteraceae, Zingiberaceae, Lecythidaceae and Leguminosae. One of the potential plants of the Caricaceae family is papaya (*Carica papaya* L.) and from the family Lecythidaceae is bitung (*Barringtonia asiatica* L. Kurtz). Extracts of some parts of the plant have been shown to be active as antifeedant insecticides, developmental inhibitors, and spawning inhibitors. Generally, insecticide compounds from plants are phytotoxic to agricultural crops<sup>3</sup>.

Papaya and bitung plants are currently being investigated for their insecticidal activity. Some test results show that extracts from the sap of papaya and bitung seeds can inhibit the development and growth of insects. Papaya sap has compounds of proteolytic enzymes, papain and chemopapain, glutamine

cyclotransferase, chymopapain A, B and C, peptidase A and B and lysozymes, while bitung seeds have steroid alkaloid compounds, triterpenoids, tannins, saponins, and flavonoids. In the sap of papaya, proteins and protease enzymes are defense compounds against herbivorous insects<sup>4</sup>. Flavonoids, saponins and tannins in bitung seeds are chemical compounds that act as insecticides.

Bioinsecticide testing can produce mixed results depending on the type of test material, factors in test insects and environmental factors<sup>5</sup>. The amount of active ingredients in plants can vary depending on plant genetic diversity, topographic conditions of plant origin, growth phases, and seasons when harvesting plant parts containing insecticides. How to handle these plant parts and extraction methods can influence the effectiveness of bioinsecticides obtained. Plants also have other compounds that are less active but their existence can increase the overall extract activity if together (synergy) so that insects cannot easily become resistant to plant extracts with some active ingredients because of the ability of insects to form a defense system against several different active ingredients at once smaller than a single active insecticide. In the development and use of botanical insecticides to the community more directed and recommended to a simpler way because bioinsecticides are mass-produced and have gone through laboratory processes the price becomes more expensive than synthetic insecticides. Bioinsecticides only consist of one active ingredient, this characteristic is not different from synthetic insecticides which can cause pest insects to become resistant.

## Experimental

### Standard and Chemicals

The tools used in the research are meter, name sign, handsprayer, transparent millimeter paper, calculator, camera, stationery, scales, handtractor, hoe, Rotary evaporator. The materials used in this study were bitung seeds (*B. asiatica*), papaya gum (*C. papaya*), compost fertilizer, cabbage plant seeds (Grand varieties), *C. Pavanana* larvae F. third instar, water, label name, paper.

### Sample preparation

Bitung seeds are taken from the coast of Bitung Village, South Minahasa Regency, North Sulawesi Province. Beans are peeled for the seeds. Bitung seeds are cut into pieces ( $\pm 1$  cm) and then placed on a tray covered with newsprint. Pieces of bitung seeds are left to air dry without direct sunlight. After drying, the pieces of bitung are ground using a grinder. Materials that have become powder are ready to use.

Papaya sap comes from papaya fruit which grows on the plantation in South Minahasa Regency, North Sulawesi Province. Papaya sap is taken from immature papaya. Papaya fruit is injured using a stainless steel knife, then the sap that comes out is collected using a plastic container. The papaya sap is directly used so it does not thicken.

### Extraction

1 Kg of bitung seeds powder macerated with 1 L of methanol until all components are extracted. Methanol extract was obtained, filtered and then evaporated using a rotary evaporator until a thick methanol extract was obtained. The extract was added to hexane and methanol, then the hexane fraction was taken. The hexane fraction is evaporated to obtain a hexane extract. The extract was then extracted again with ethyl acetate and aquades. Ethyl acetate fraction was taken to be evaporated to obtain ethyl acetate extract. The thick extract will be used for testing. The crude extract obtained is stored in the refrigerator at 4°C.

### Bitung seed extract solution

The bitung seed extract solution was made with concentrations of 3, 4, 5, 6 and 7 g/L. Extract of 3 g/L was made by weighing 3 g of bitung seed extract and then added 1000 mL of water. The 4 g/L extract solution was made by weighing 4 g of bitung seed extract and then adding 1000 mL of water. The 5 g/L extract solution was made by weighing 5 g of bitung seed extract and then adding 1000 mL of water. Extract of 6 g/L was made by weighing 6 mg of bitung seed extract and then added 1000 mL of water. The extract of 7 g/L was made by weighing 7 g of beetroot extract and then added 1000 mL of water. The variation in concentration is used in testing insecticide activity to get the best concentration

### ***C. pavonana* Breeding**

Breeding *C. Pavonana* was carried out at the Seed and Vegetable Garden Installation in Modinding District, North Sulawesi Province. Insect breeding follows the procedures used by Prijono and Hasan<sup>6</sup>. Imago *C. Pavonana* was kept in a plastic framed gauze cage (50 cm x 50 cm x 50 cm) and fed with a honey solution that was applied to a lump of cotton. As a place to lay eggs used cabbage leaves whose stems are inserted in a tube containing water. The group of eggs on cabbage leaves is collected every day. Towards hatching, the egg group is moved into a plastic container (28 cm x 20 cm x 5 cm) with a window screen that is lined with stencil paper, then inside is placed insecticide-free cabbage leaves as larval food. The instar III larva is used for testing. If not used for testing, larvae are further maintained in plastic containers (35 cm x 25 cm x 6 cm) with insecticide-free cabbage leaf feed. Ahead of the form, the larvae were moved into another plastic container containing sawdust as a form of medium. The pupae that are formed along with the cocoon are placed in a plastic gauze cage as above until the imago appears for further maintenance.

### **Insecticide activity against *C. Pavonana* larvae mortality**

Tests are carried out by the method of leaf dyeing. Experiments were arranged in a completely randomized design (CRD). Test insect food leaves are cut in a roundabout diameter of 5 cm. Then the leaves are dipped one by one into the suspension of the beetroot seed extract that has been made. The control leaf is dipped in aqua without extract containing solvents and emulsifiers. Excess extracts of liquid extract on the leaves are drained and the leaves are dried. This solution was tested to obtain 0-100% mortality. Each concentration and control was repeated 3 times. In each petri dish 3 larvae of *C. Pavonana* F. instar were included in 3. The larvae were treated with leaves treated and controlled for 48 hours. After that, fresh cabbage leaves were fed without treatment until the larvae were clustered. Data recorded is the number of larval mortality based on the day after treatment. Data on the percentage of larval mortality were calculated using formulas Dono and Rismanto<sup>7</sup>. Regression relationship between substance concentration and test insect mortality rate was determined by probit analysis.

### **Formulation of bitung seed and papaya sap biopesticide solution**

The mixture solution of bitung seed extract and papayasap is made from the best solution in the mortality test. The mixed solution is made with variations of papayasap. Solution I was made by adding 2 cc/l of papaya sap in a solution of beet seed extract. Solution II was made by adding 3 cc/l papaya sap in a solution of beetroot extract. Solution III was made by adding 4 cc/l of papaya gum in a solution of beet seed extract. IV solution was made by adding 5 cc/l papaya gum in a solution of beetroot extract. This mixture was made to get the best effect on the mortality of *C. pavonana*.

### **Effectivity of biopesticide from bitung seed and papaya sap formula against *C. Pavonana* larvae mortality**

Tests are carried out by the method of leaf dyeing. Experiments were arranged in a completely randomized design (CRD). Test insect food leaves are cut in a roundabout diameter of 5 cm. Then the leaves are dipped one by one into the extract suspension that has been made. Control leaves dipped in distilled water. Excess extracts of liquid extract on the leaves are drained and the leaves are dried. The best formulations of crude ethanol extract of bitung seeds and papaya sap were each tested at 5 levels of concentration which are expected to result in mortality of *C. Pavonana* larvae between 0-100%. The treatment level consists of a mixture of I-IV solutions. Each level of mixture solution and control was repeated 3 times. 30 pet larvae of *C. Pavonana* were inserted into each petri dish. Installed 3 and repeated 3 times. Larvae were fed leaves treated and controlled for 48 hours. After that, fresh cabbage leaves were fed without treatment until the larvae were clustered. Data recorded is the number of larvae mortality based on the day after treatment. Data on the percentage of larvae mortality were calculated using formulas Dono and Rismanto<sup>7</sup>.

### **Biopesticide analysis**

Compounds in a biopesticide mixture of bitung seeds (*C. pavonana*) and papaya sap (*C. papaya*) were analyzed using the LCMS method.

## Statistical analysis

All the experimental data do triplicates and the results are expressed as mean  $\pm$  SD. Analyzes were performed using SPSS software version 23.

## Result And Discussions

### Toxicity of bitung seed extract against *Crocidolomia pavonana* larvae

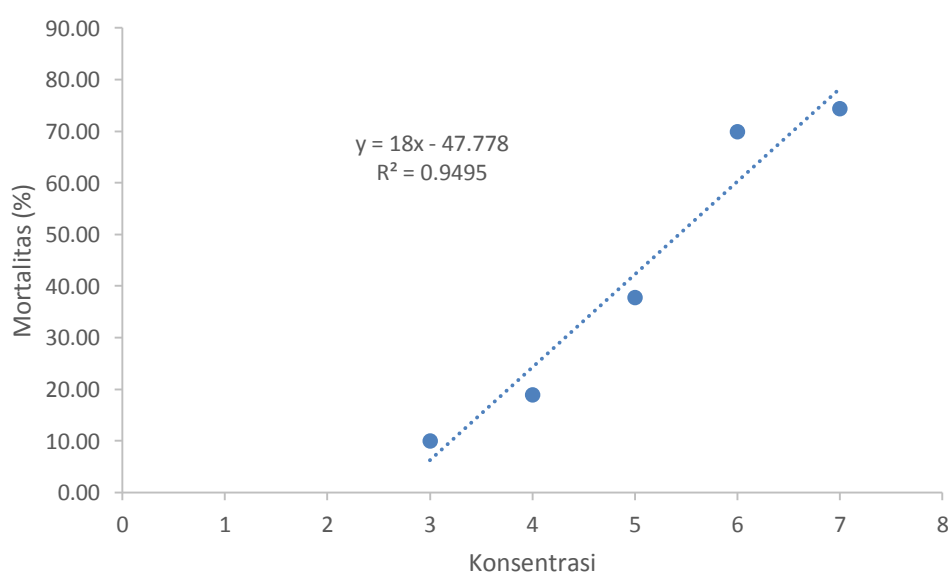
Ethyl acetate extract of bitung seeds (*Barringtonia asiatica*) showed toxic activity starting the second day after application. Bioinsecticide application of bitung seed extract by food spray method showed the highest percentage of *C. pavonana* larvae at a concentration of 7 g/L ( $74.44 \pm 6.93$ ) and the lowest percentage of *C. pavonana* at 2 g/L ( $10.00 \pm 3.33$ ). Based on the percentage of larvae mortality, the treatment of 6 g/L showed a difference of 4.44% with a treatment of 7 g/L (Table 2).

**Tabel 1. Percentage of mortality of *C. pavonana* larvae in the toxicity tes**

o	Concentration (g/L)	N	Sd	Mortality (%)
1	3	30	3,33	10,00
2	4	30	1,92	18,89
3	5	30	3,85	37,78
4	6	30	3,33	70,00
5	7	30	6,93	74,44

Based on the percentage of larvae mortality, the difference between the treatment was 6 g/L (0.006%) and 7g/L (0.007%) with the treatment of 5 g/L (0.005%), 4 g/L (0.004%) and 2 g/L (0.002%). The average mortality of larvae reached 50% when compared between the treatment of 6 g/L and 7 g/L with the treatment of 5 g/L, 4 g/L and 2 g/L. Symptoms of extract toxicity were shown by the average larvae body size smaller than the control. The abdomen is blackened, indicating damage to cells and organ tissue in the body of the larva.

The results of the probit analysis obtained the  $LC_{50}$  value of beetroot ethyl acetate extract against *C. pavonana* larvae was 5.325 mg / L.



**Figure 1. : Correlation of bitung seed extract concentration and mortality of *C. Pavonana* larvae**

Ethyl acetate extract of bitung seed has a strong toxic activity in *C. Pavonana* larvae. Insecticides are classified as having effective toxic activities if they can have a 50% mortality effect from test insects. Ethyl acetate extract of bitung seed showed the best toxicity activity in the concentration of 6 g/L and 7 g/L. The concentration of 2 g/L, 4 g/L and 5 g/L resulted in a percentage of mortality of half the treatment concentration of 6 g/L and 7 g/L. Ethyl acetate extract of bitung seeds affects the ability to eat larvae. The level of larval instar also affects the LC<sub>50</sub> extract value. The higher the instar level of the larvae of *C. pavonana*, the more vigorous survival after extract treatment.

The phytochemical groups contained in many bitung seed are reported to have toxic activity on insects<sup>8</sup>. The group of compounds that have toxic activity, which is contained in the extracts of beet seeds are alkaloids, saponins, flavonoids, tannins, volatile and benzofuran (rocaglamida)<sup>9</sup>.

The main active compounds in beet seeds are alkaloids, saponins, flavonoids, tannins, essential oils, benzofuran (rocaglamide) and some derivatives (rocaglamide) which are reported to be lethal, inhibit growth and inhibit insect feeding activities<sup>10</sup>. Saponin is a terpenoid compound which has the activity of binding to free sterols in the digestive system, so that by decreasing the number of free sterols it will affect the skin turnover process in insects<sup>11</sup>. Saponin active compounds are capable of forming foam if shaken with water and provide a bitter taste that can reduce surface tension so that it can damage the cell membrane of insects<sup>12</sup>. Tanin is one of the compounds included in the polyphenol group found in beet seeds. The mechanism of action of tannin compounds is by activating the cell lysis system because of the activation of proteolytic enzymes in the cells of the insect's body which are exposed to tannins<sup>12</sup>. Complex compounds produced from the interaction of tannins with these proteins are toxic or toxic which can play a role in inhibiting growth and reducing insect appetite through the activity of digestive enzymes. Although these compounds can inhibit the growth and eating of insects, the workings of these compounds at the cellular level of insects are not yet known.

There are several insect factors that affect the test results, namely species, age, phase of insect development, sex ratio and body size<sup>13</sup>. The difference in the amount of toxin content possessed by each type of extract is caused by different extraction methods. The crude extract still contains extract material particles both fine and coarse particles, so the chemical compounds are still bound by extract particles, while the ethyl acetate extract has gone through a process of further partitioning/separation which aims to separate the chemical compounds that are still contained in the extract rough, taking into account the polarity of the solvent to be used.

There are main active ingredients that are insecticidal. Janprasert et al<sup>14</sup> have isolated and identified the main active ingredient that is insecticidal as rocaglamide with the chemical name 1H-2,3,3a8b-tetrahydrosiklopenta [b] benzofuran, a benzofuran group. Beetroot seeds also contain rokaglamide compounds and other benzofuran compounds, namely methyl rokagloa, desmethyl rokagloa and rokagloa. Besides being insecticidal, rocaglamide also inhibits eating activities and inhibits the development of larvae. Rocaglamide has contact poison properties with LD<sub>50</sub> values of 0.32 µg/larvae and stomach poison with LD<sub>50</sub> 0.34 µg/larvae against *C.pavonana* 2 instar larvae. besides being insecticidal, rokaglamide also inhibits eating activity and inhibits the development of *C. pavonana* larvae<sup>10</sup>. The results of the study by Nugroho et al.<sup>15</sup> revealed that rokaglamide is an insecticide (LC<sub>50</sub> = 0.9%) and inhibits the development of *C. pavonana* larvae (EC<sub>50</sub> = 0.08%).

According to Proksch et al.<sup>16</sup> the intrinsic toxicity of the skirtaglamide compound was determined by the hydroxy (OH) group at R1 and R4 and the CON (CH<sub>3</sub>) 2 group on R2. Acetylation with formic acid or acetic acid at R1 and the change of group at R2 with hydrogen (H) results in a decrease in toxaglamide compound toxicity. For example, the ratio of reduction in toxicity to compounds 2 and 4 in *C. pavonana* larvae each had an LC<sub>50</sub> value of 1.5 ± 0.7% and 8.0 ± 1.4%.

The reduction in toxicity is caused by the substitution of the OH group on R1 from compound 2 with the OCOCH group 3<sup>15, 17</sup>. While the substitution of OH groups on R4 with methoxy (OCH<sub>3</sub>) can result in a skirtaglamide compound that has a marked decrease in its insecticidal properties<sup>16</sup>. In addition, changes in the heterocyclic oxygen ring (oxygen heterocycle) in the dihydrobenzofuran nucleus of the Rokaglamide compound determine its toxicity. In addition, changes in the heterocyclic oxygen ring into the ring (as in other agents or aglaforbesin) and oxepina (as in fobaglin) cause these compounds to lose their insecticidal activity<sup>16</sup>. Satasook et al.<sup>10</sup> and Nugroho et al.<sup>15</sup> reported that rocaglamide and some derivative compounds are lethal, inhibiting

growth and inhibiting insect feeding activities. From the results of the observation, it was found that there were symptoms of poisoning in the test larvae caused by bitung seed extract, ie larvae lost mobility (slow), small body size and no appetite when compared to controls and in certain parts there was a change in the larva's inner tissue. This is in accordance with what was reported by Nugroho and Proksch<sup>17</sup>, that the results of testing of bitung seed extract on *C. pavonana* can affect the physiological growth of larvae so that the larvae become sick and have little activity to eat.

The growth barriers of larvae that eat leaves using bitung seed extract may be caused by the energy allocation to detoxify toxic compounds. In addition there is an inhibition of protein synthesis due to a disruption of protein breakdown from food. In contrast to the condition of food that does not contain toxic compounds, where energy from food will be used for growth and development but with the presence of toxic compounds in food, some energy from food that should be used for growth and development is allocated for detoxifying toxic compounds<sup>18</sup>.

The presence of protein breakdown compounds can cause insects not to get enough protein from their food because food proteins are not digested so they cannot be absorbed by the body. Insect growth and development is inhibited. The occurrence of barriers to protein synthesis in the body of *C. pavonana* larvae which eat leaves treated with bitung seed extract results in the insect larvae experiencing barriers to growth and development. Syahputra<sup>19</sup> states that the influence of insecticidal compounds can result in disruption of growth and development. One of them is shown by a marked increase in mortality on the next instar which occurs before or when changing the skin.

*B. asiatica* extract has been used as bioinsectivity in *Aedes* sp. larvae<sup>20</sup>. The methanol extract of *B. asiatica* seeds is toxic to *Crociodolomia pavonana* with 0.66% LC<sub>50</sub> after 7 after treatment. Moreover, extract of *B. asiatica* also affects oviposition or behavior *C. pavonana* females lay eggs on plants<sup>21</sup>. The identification with GCMS results in three compounds of triterpenoid saponins, namely 2,4-bis- (1,1-dimethyl ethyl) -, methylcarbamate; 4-Dodecylphenol; and 2,6 bis- (1,1-dimethylethyl) -4-methyl-, methylcarbamate<sup>11</sup>. *B. asiatica* seeds contain the most active insecticidal compounds with LC<sub>50</sub> values of 290 ppm which are potential for insecticide applications. All parts of *B. asiatica* plants contain saponins which can inhibit insect feeding activities<sup>7</sup>.

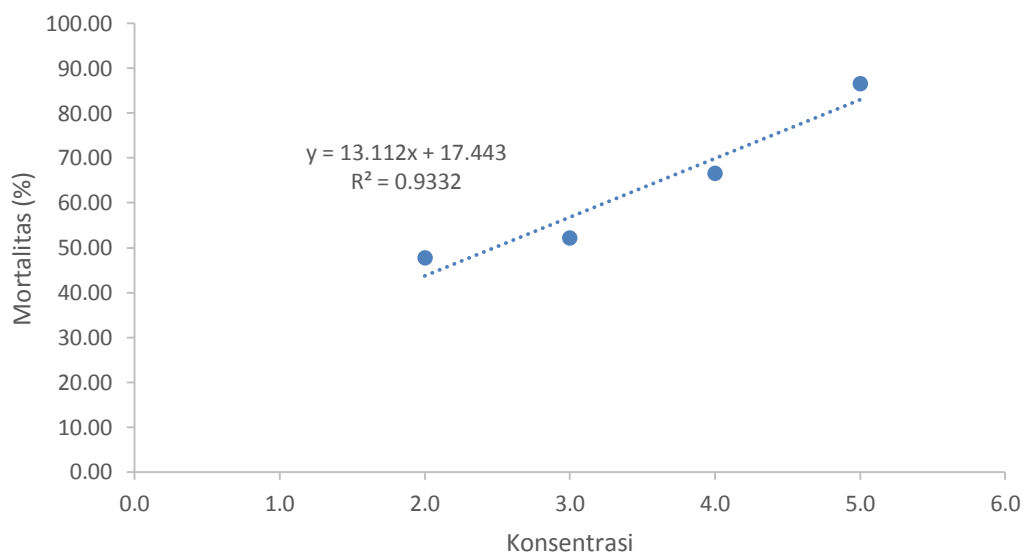
#### Toxicity of bitung (*B. asiatica*) seed extract and papaya (*C. papaya*) sap against *Crociodolomia pavonana* larvae

The LC<sub>50</sub> results obtained from the ethanol acetate seed extract of 5.325 g/L were used as a basis for combination with papaya sap. The beetroot extract is added 2-6 cc/l papaya gum. The test results with the food spray method, obtained the highest mortality of *Crociodolomia pavonana* on the bitung seed extract which added 2 cc/l papaya sap was 73.33% while the lowest mortality of *Crociodolomia pavonana* in the extract of bitung seed added by 6 cc/l papaya sap was 53.33 %. The results of this study showed the addition of papaya gum to linear bitung seed extract with the mortality of *Crociodolomia pavonana* (Table 3).

**Tabel 2. Mortality of *C. pavonana* larvae**

No	Papaya sap concentration (cc/l)	N	Sd	Mortality (%)
1	2	30	0,00	53,33
2	3	30	3,85	64,44
3	4	30	6,67	66,67
4	5	30	6,67	73,33

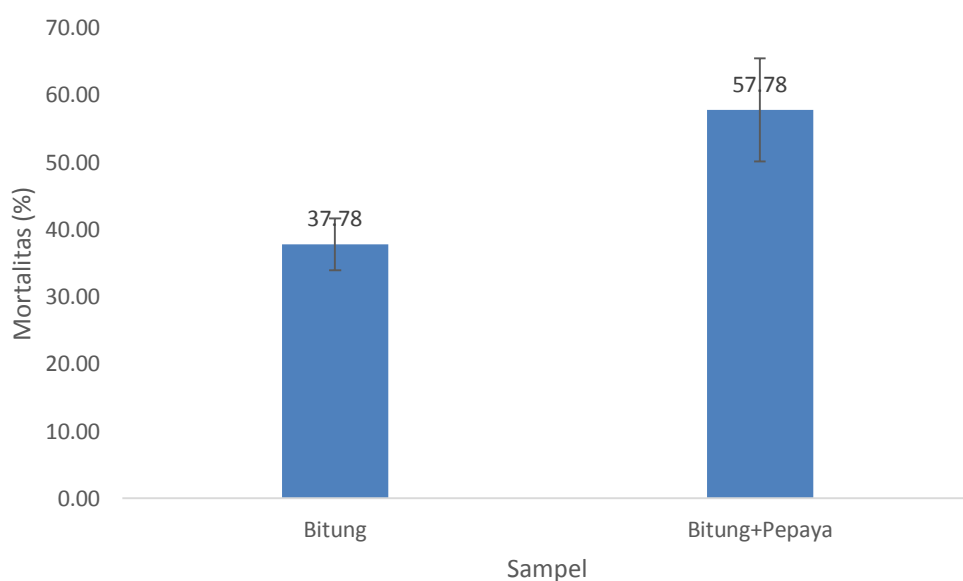
The results of the probit analysis obtained LC<sub>50</sub> values of bitung seed ethyl acetate extract which added papaya sap to *C. Pavonana* larvae was 2.39 g / L.



**Figure 2. : Correlation of concentrations of bitung seeds and papaya sap with mortality of *C. Pavonana* larvae**

The addition of papaya sap to the ethyl acetate extract increased the toxic effect on *C. pavonana* larvae. There was an increase in toxicity of bitung seed extract added with papaya sap. The addition of 2 cc/l papaya sap to the bitung seed ethyl acetate extract has increased 43.33% of the mortality of *C. pavonana* larvae compared to without the addition of papaya sap. 55% of the mortality of *C. pavonana* was compared without the addition of papaya sap. The addition of 5 cc. L papaya sap to the bitung seed ethyl acetate extract was able to increase 28.89% mortality of *C. pavonana*. The addition of 6 cc/l papaya sap to the bitung seed ethyl acetate extract can only increase 3.33% mortality of *C. pavonana* compared to without the addition of papaya sap (Fig.).

The t-test results of the treatment of ethyl acetate extract of 55,325 g/l bitung seeds + papaya sap 2,370 g/l showed a higher average mortality value of 57,78% compared to bitung seed ethyl acetate extract 5,325 g/l which was the average mortality value 37,78% (Figure 3)



**Figure 3 : Mortality test results of ethyl acetate extract of bitung seed and ethyl acetate extract of bitung seed + papaya sap**



The toxic effects of papaya sap due to the content of the compound in papaya sap are toxic to *C. pavonana*. The mechanism of action of chemical compounds in papaya sap is caused by the enzyme of papain. The papain enzyme is a proteolytic enzyme that plays a role in the breakdown of connective tissue, and has a high capacity to hydrolyze exoskeleton proteins by deciding 12 peptide bonds in the protein so that the protein will be cut off. Papain enzymes are found in all parts of papaya plants<sup>22</sup>.

The bitung seed extract and papaya sap can affect the ability of larvae to eat, the instar levels of larvae also affect the LC<sub>50</sub> value, the higher the instar level, the higher the resistance to life<sup>8</sup> this is influenced by the extract of beet seeds to cabbage larvae (*C. pavonana*). In various aspects of biology, the active components of the extract poison the insect physiology process.

### Biopesticide component

The results of analysis using LCMS mixture of bitung seed extract and papaya sap can be seen in the picture below

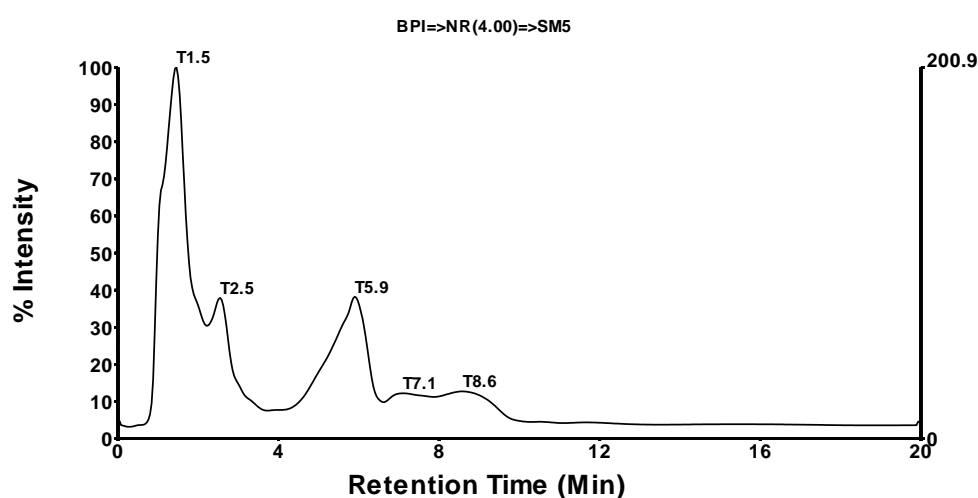


Figure 4. : LC chromatogram of biopesticide

Data analysis using LC found that the mixture of bitung extract and papaya gum contained 5 compounds which can be seen in the retention amount of 1.45; 2.54; 5.89; 7.09; and 8.59 m. The most compound quantity is shown by compounds with retention of 1.45, followed by retention of 5.89, and the lowest compound with retention of 7.09. Chemical compounds that have been separated by LC are then analyzed using MS.

Compounds with retention of 1.45 produce fractionation as can be seen in the figure below.

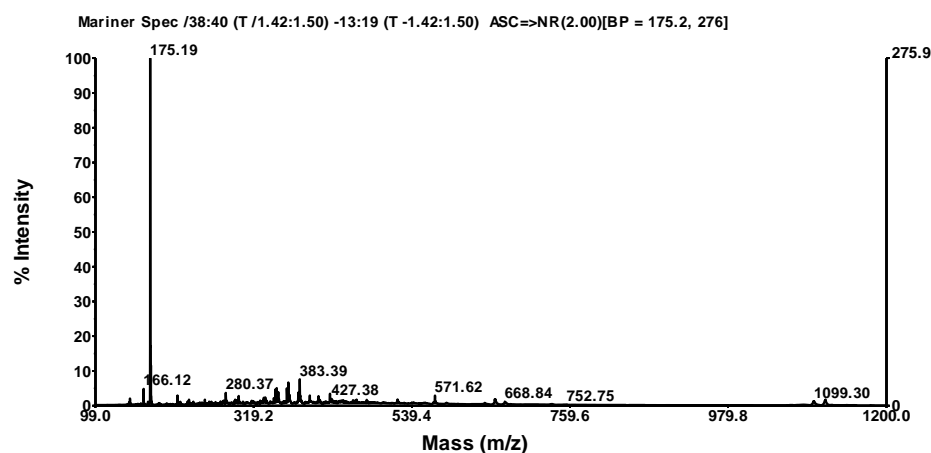


Figure5a. : Mass spectrometry analysis of compound with retention time 1,45

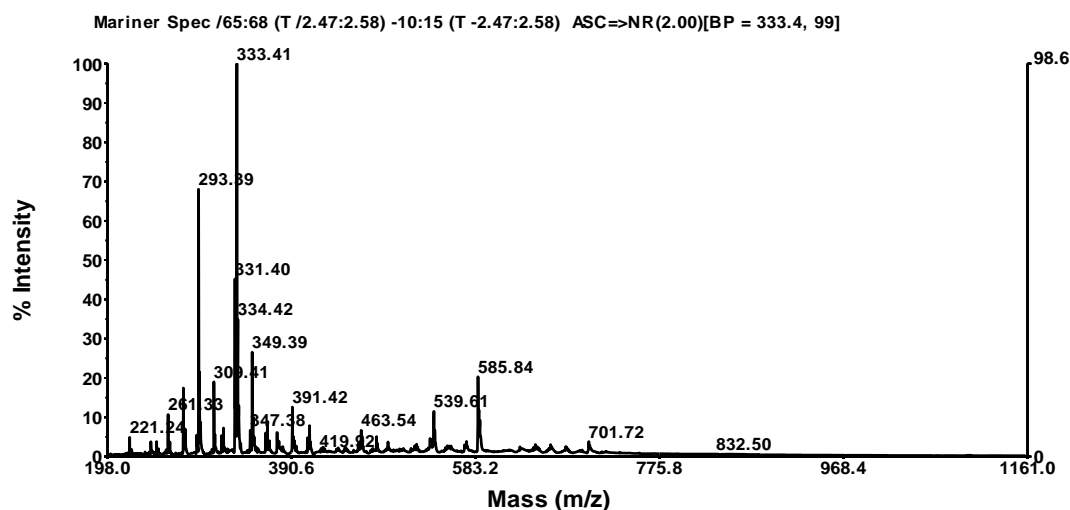


Figure5b. : Mass spectrometry analysis of compound with retention time 2,54

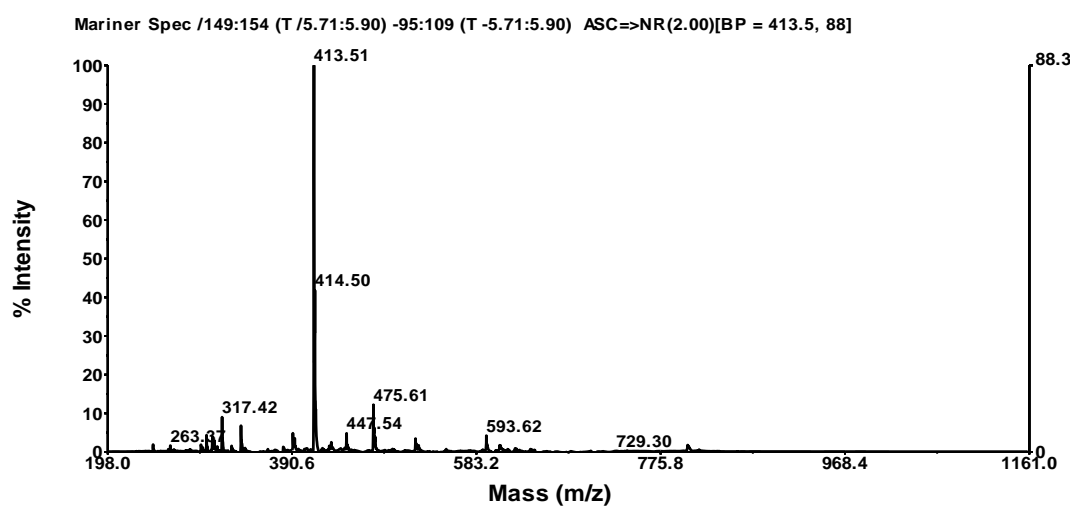


Figure5c. : Mass spectrometry analysis of compound with retention time 5,89

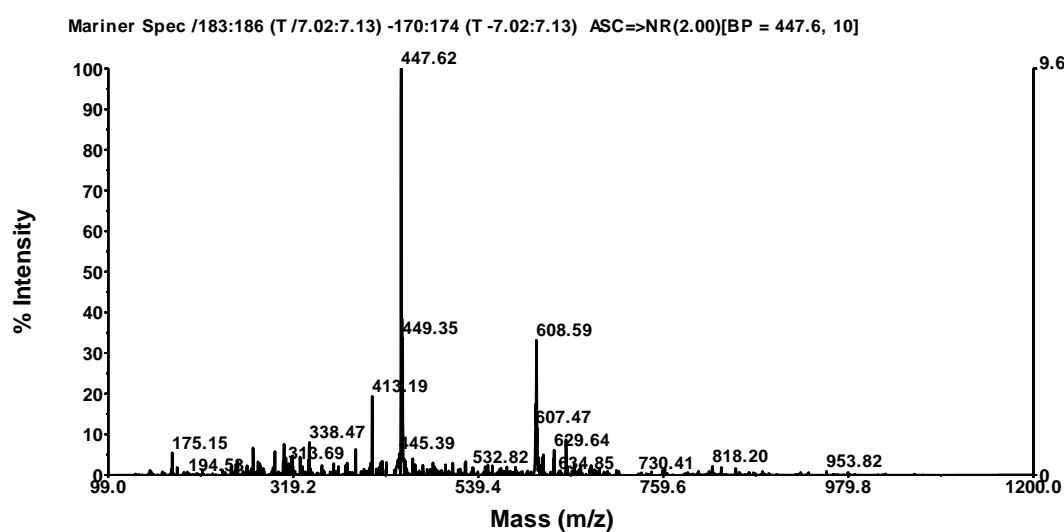
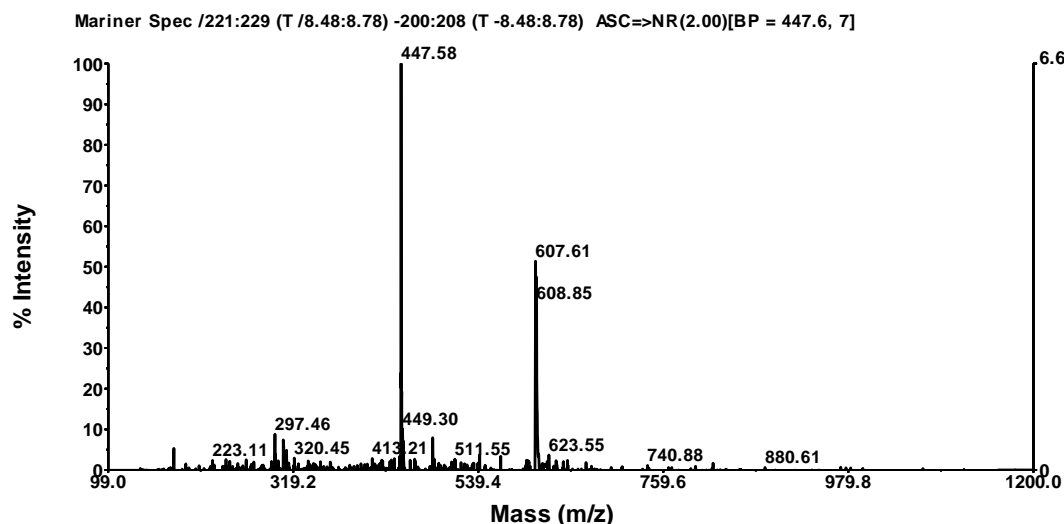


Figure5d. : Mass spectrometry analysis of compound with retention time 7,09



**Figure5e. : Mass spectrometry analysis of compound with retention time 8,59**

The above results show that compounds with retention of 1.45 have a molecular weight of 1115.20. Searching for compound-like molecular weight data with retention of 1.45 there is only 1 compound recorded in the SRR 69 NIST Chemistry WebBook. The compound is suspected to be tetraheptacontylbenzene.

Compounds with retention of 2.54 produce fractionation as can be seen in figure 5b. Compounds with retention of 5.89 produce fractionation as can be seen in figure 5c. Compounds with a retention of 7.09 produce a fractionation as can be seen in Figure 5d and a compound with retention of 8.59 produces a fractionation as can be seen in Figure 5e.

The above results indicate that the compound with retention of 1.60 has a molecular weight of 1158.18, a retention compound of 5.89 has a molecular weight of 729.30, a compound with a retention of 7.09 has a molecular weight of 1158.18 and a compound with retention of 8.59 has a molecular weight of 880.61. The search for molecular weight data similar to compounds with retention 1, 60, 5.89, 7.09 and 8.59 has not been recorded in the SRIST 69 NIST Chemistry WebBook. The compound of the compound is thought to be a new compound or the results of poor sample analysis. Poor separation causes the Mass Spectrophotometer to be insensitive to analyze.

## Conclusion

Based on the results of the study it can be concluded that the bitung seed extract can kill *C. pavonana* larvae with LC<sub>50</sub> values of 5.35 mg/L. Papaya sap can increase the toxic effect of bitung seed extract which kills *C. pavonana* larvae with a 2.5 cc LC<sub>50</sub> value. The mixture of extracts of bitung seed and papaya sap can be used as vegetable pesticides for pest control of *C. pavonana* in cabbage plants.

## Conflict of interest statement

We declare that we have no conflict of interest.

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