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Control Of The Beet Armyworm *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) Utilized Four Minahasa Plant Extracts.

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Abstract

The objective of this research was to determine the foremost effective concentration and also the most active extract of *P.edule* Reinw derived from the seed, *R.communis* derived from leaf, *Z. officinale*, and *D.elliptica* derived from the root; to evaluate the various extract concentrations on the treated larvae instar III, and to characterize the phytochemical contents of the foremost effective extracts fraction. This research using an experimental method to measure the most effective concentration and the foremost active extract. Moreover, the extracts n-hexane fraction (non-polar), and ethanol fraction (polar), were conducted with a randomized design. The study also was the check of phytochemical screening to find the presence of secondary metabolites in all extracts material. The $LC_{50\text{-}48h}$ values were determined following probit analysis to calculate the death rate of larvae instar III when treatment is given. The results showed that seed extracts of *P.edule* and root extracts of *D.elliptica* showed the very best insecticidal activity with $LC_{50\text{-}48h}$ values 12.25 mg/l and 12.30 mg/l, for twenty-four hours and forty-eight hours post-treatment, severally. The other extracts of *R.communis* with $LC_{50\text{-}48h}$ values 17.25 mg/l and *Z.officinale* $LC_{50\text{-}48h}$ values 25.50 mg/l were effectively too. The other results showed that the mortality of larvae instar III from *S.exigua* was highest utilize 1000 ppm n-hexane fraction (96.67 %) at 2 days after treatment, followed by utilizing 1000 ppm n-hexane fraction too derived from *D.elliptica* root extract (93,35%). Results conjointly showed that n-hexane fraction is that powerful against larvae of *S.exigua*, from *P.edule* n-hexane seed extract than the other potential extracts. The extracts tested positive for alkaloid, saponins, flavonoids, terpenoids, phenol, and tannins. *P.edule* seed extract and *D.elliptica* root extract was an efficient bioinsecticide exhibiting larvicidal and antifeedant properties of *S.exigua* (Hübner). Additionally work is critical to judge and characterize the active parts of the extract fractions and its effectualness within the field.

Keywords: Bioinsecticides, Ethanol fraction, n-hexane fraction, Plant extracts, Beet armyworm

INTRODUCTION

Spodoptera exigua (Hübner) (Lepidoptera: Noctuidae) common name beet armyworm is a polyphagous insect pest, which mainly attacks vegetables, flowers, and field crops. In this time, controlling of *S.exigua* is primarily dependent on chemical insecticides. As the farmers use chemical pesticides repeatedly to control the pest, the *S.exigua* has developed resistance to diverse insecticides, making its control more and more difficult (Feng,X *et al*,2012). Currently, chemical pesticides are being used to control this pest; however, they're no longer ideal because they cause environmental pollution (Huang, *et al*, 2018).

Therefore, it's far essential to use methods that will no longer pollute the environment. The important herb such as cabbages, carrots, red onion, white onion, and long green onions are horticultural plants that have a vital monetary value for farmers in North Sulawesi, Indonesia. Botanical extracts from so8me potential toxic plants can be ideal for the biocontrol of *S.exigua* (Hübner) (Lepidoptera: Noctuidae) as it is environment-friendly bioinsecticide (Sembel,D. 2010).

Botanical pesticides constitute one opportunity for artificial pesticides due to the negative outcomes of the latter i.e. pest resistance, secondary pest outbreaks, and results on the surroundings and non-target organisms. Intensive use of synthetic pesticides to control insect pests had caused many troubles such as pest resistance and resurgence, consequences on non-target organism's human exposure, and environmental impacts (Abalos *et al* 2013).

The negative results have provided the the improvement of alternatives which include botanical insecticides. Botanical pesticides evolved from plant extracts are less persistent in the surroundings and are frequently more secure than artificial chemicals. A botanical insecticide is an agent and a part of thebiological management process (Sakul *et al* 2012).

One of the most reliable alternative biological controls for management vegetable pest is the utilization of some potential local minahasa plant extract, such as *Pangium edule*, (Sakul, 2017; Manoppo, 2017), *Annona muricata* and *Derris elliptica* (Manoppo,2017; Manoppo and Sakul,2017).

There are four larval instars sometime recently pupation happens, and at 25^{0}C the ways of the life cycle from egg to individual rise takes roughly 24 days. The first-instar hatchlings mine interior the light mesophyll tissue, though more seasoned hatchlings bolster on the lower leaf floor and typically eat up all tissue the wax layer at the upper floor thus making the window inside the leaf (Vanlaldiki *et al*, 2013).

In this research, we used *S.exigua* larvae instar III (F1). Insecticidal properties of fractioned extracts from four minahasa plants using seed, leaves, and root extracts against *S.exigua* beet armyworm larvae were investigated in the biology laboratory of Manado State University.

The objective of this research was to determine the foremost effective concentration and also the most active extract of *P.edule* Reinw derived from the seed, *R.communis* derived from leaf, *Z. officinale*, and *D.elliptica* derived from the root; to evaluate the various extract concentrations on the treated larvae instar III, and to characterize the phytochemical contents of the foremost effective extracts fraction.

This research using an experimental method to measure the most effective concentration and the foremost active extract. Moreover, the extracts n-hexane fraction (non-polar), and ethanol fraction (polar), were conducted with a randomized design (Sakul,2017 ; Manoppo,2017). The study also was the check of phytochemical screening to find the presence of secondary metabolites in all extracts material. The LC50-48hr values were determined following probit analysis to calculate the death rate of larvae instar III when treatment is given.

MATERIALS AND METHODS

2.1. Insect rearing and seedlings of long green onion plants

S.exigua population (larval stage), was collected from long green onions plants of Tonsealama suburb at the North Tondano, Minahasa Region, North Sulawesi Province Indonesia, using Pheromone EXI and manual system. For egg-laying, leaves of long green onion were utilized and eggs were exchanged to clears out of the said plant to proceed with their advancement. The creepy crawly stock was kept up in a controlled environment at 25 ± 2^{0}C and 65 ± 5% relative stickiness (RH). For this inquire about, the larval arrange from *S.exigua* has utilized the third instar hatchlings (age of hatchling is 7-12 days) Filial 1.

A bunch of scallions with their roots from long green onions were sprouted in extended polystyrene plates containing a blend composition comprising of soils, natural fertilizer, and coconut fiber, (2: 1: 1) and kept up for 30 days within the nursery. After that, long green onions were transplanted into polybags and planted in black plastic bags in a greenhouse (28 ± 5^{0}C). To ensure the plants against creepy crawly harm, they were set inside a tent-loke development made from fine netting (work measure <1 mm). The plants were fertilized when planted and routinely watered.

2.2. Plant Material

The sample of *P.edule* seeds, leaf of *R.communis*, the root of *Z.officinale,* and root of *D.ellip*tica were collected from a cultivating and woodland of Tonsealama town at the North Tondano, Minahasa Region, North Sulawesi Province, Indonesia. The material derived from seeds, roots, and clears out were gathered and collected from trees at a stature of approximately 3.5 m – 6.5 m, and put within the nursery (28 ± 5^{0}C) to dry; after which they were at that point smashed into an unrefined fabric and put away in a waterproof holder until utilize.

Figure 1. Plant Material of *P.edule*, *R.communis*, *Z.officinale* and *D.elliptica* (Sakul,2017; Manoppo,2017)

2.3. Preparation of plant extract with n–hexane and ethanol fraction

An unrefined extricate of the seed of *P.edule*. leaf of *R.communis*, the root of *Z.officinale* and root of *D.elliptica* was arranged by soaking dried seed, leaf, and root material in n-hexane fluid and ethanol fluid. In each extraction, particularly the primary maceration utilized 500 grams of powdered seeds *P.edule* and leaf *R.communis* were extricated by maceration in 1000 ml of n-hexane at room temperature for 1 day (1 x 24 h). Moreover, 500 grams of the powdered root of *Z.officinale* and root of *D.elliptica* were extricated as well by maceration in 1000 ml of n-hexane at room temperature for 1 day (1 x 24 h), and after that, we collected the primary filtrate. After that silt of *P.edule* seed, leaf of *R.communis*, the root of *Z.officinale* and root of *D.elliptica* collected in an Erlenmeyer jar, these dregs was extricated once more by maceration again (arrange 2) in 800 ml of n-hexane at room temperature for 1 day (1 x 24 h) and after that, we collected the second filtrate.

The following handle is collected once more the silt of *P.edule* seed, leaf of *R.communis*, the root of *Z.officinale* and root of *D.elliptica* in an Erlenmeyer jar was extricated once more by maceration prepare (arrange 3) in 500 ml of n-hexane at room temperature for 1 day (1 x 24 h) and after that, we

collected the third filtrate. The same stage of maceration will do with the ethanol fraction. After that, the filtrate of n-hexane division and filtrate of ethanol division was sifted by utilizing the Whatman Filter Paper and Buchner Pipe (Sakul, 2017) and after that, the filtrates were concentrated to dryness by a rotational evaporator under low pressure and temperature of the revolving evaporator in 35 - 40^{0}C (48-50 rpm) (Tanor *et al*, 2014). The result of extractions derived from seed, leaf, and root was put away in a labeled bottle at fridge 4^{0}C until required for bioassay. If water remained within the concentrated rough extricate, the extracts were a store in a vacuum desiccator over silica gel.

2.4. Bioassay of Extract

A leaf plunging bioassay strategy can be utilized to evaluate contact poisonous quality. A common test utilized to survey contact as well as stomach poisonous quality of a compound in more seasoned hatchling could be a lead plunge bioassay. In this measure, a leaf or leaf plate is plunged in an arrangement of the extricate being tried or plunged within the dissolvable alone (control).

Test creepy crawlies have bolstered these circles and mortality recorded. Long Green Onion takes off were washed with refined water and dried for almost 2 hours. Four concentrations 50 ppm, 100 ppm, 500 ppm and 1000 ppm of the seed, leaf, and root utilizing n-hexane dissolvable and four concentrations 50 ppm, 100 ppm, 500 ppm and 1000 ppm of the seed, leaf, and root using ethanol solvent, both of 4 specimen plants (*P.edule* seed, leaf of *R.communis*, the root of *Z.officinale* and root of *D.elliptica*) were prepared. Long Green Onion leaves take off disks (10 cm distance across) were cut with a surgical tool edge from completely extended green onion take off developed in a nursery.

The disks were plunged for 30 seconds within the test arrangements extricate and air-dried. After air-drying at the room temperature, clears out disks were at that point set in a plastic glass (15-20 cm in distance across, 5-7 cm in profundity). Ten third instar hatchlings were starved for 2 hours and after that discharged into the plastic glass for each treatment. Both samples of extracts derived from *P.edule* seed, leaf of *R.communis*, the root of *Z.officinale*, and root of *D.elliptica* were put in four different concentrations such as 50 ppm, 100 ppm, 500 ppm, and 1000 ppm and this treatment will replicate in three times. The mugs were put in a development chamber at 25 ± 2^{0}C and 65 ± 5% relative of humidity (RH). Mortalities were recorded 48-hours after the treatment. Hatchlings were considered dead in case they did not move when nudged with a fine brush. Live hatchlings were exchanged to

untreated new long green onion takes off to proceed with their development and advancement. The long green onion clears out were supplanted with new ones when required.

2.5. Phytochemical Screening Test

Tanor *et al* (2014) argue that the chloroform division was isolated and fermented by ten drops of H_2SO_4 2M. The corrosive division, at that point, was included with Meyer and Wagner reagents. The nearness of alkaloids was apparent when the white store was made by Meyer reagent and the brown store by Wegner reagent.

The saponin test was performed by putting 1 gram of extract into a container containing 100 mL of water and was boiled for 5 minutes. At that point, it was sifted, and the filtrate was tried. Ten mL of filtrate was brooded in a closed test tube for 10 minutes. The event of saponin was identified when a steady froth/foam arrangement was made (Manoppo, 2017).

The tannin test was performed by blending 0.1 gram of extract with 2 mL of water and bubbled for 3 minutes. It was sifted, and the filtrate was included with a drop of $FeCl_3$ 1 % (b/v). The dim blue or dark color uncovered the presence of tannin (Sakul, 2017).

The triterpenoid test was performed by giving 0.1 gram of extract to 2 mL of ethanol 30%, warmed, and sifted. The filtrate was vanished and included with ether by 1:1 proportion. The ether layer was included with Lieberman Burchard reagent, three drops of acidic corrosive anhydride and one drop of concentrated H_2SO_4. The red color uncovered the nearness of the triterpenoid (Tanor *et al*, 2014).

2.6. Statistical analysis

$LC_{50\text{-}48h}$ information values were evaluated and take the decision utilizing probit analysis in SPSS and after that, the test information (data) was subjected to one way ANOVA at 0.05 significance level utilizing SPSS IBM-Software Ver.20. The data of means evaluated and take the decision again after compared by Least Significance Different (LSD).

RESULTS AND DISCUSSION

3.1. Result

All plant extracts appeared pronounced larvicidal activity and can increase the mortality of *S.exigua* larva instar III. The results showed that extracts derived from *P.edule* seed and extracts derived from of *D.elliptica* showed exhibited the highest insecticidal activity with $LC_{50\text{-}48h}$ values 12.25 mg/l and 12.30 mg/l, after 24 hours and 48-hour post-treatment, respectively (Table 1).

Table 1. Relative toxicity of *P.edule seed,* leaf of *R.communis*, the root of *Z.officinale* and root of *D.elliptica* after 24 hours and 48 hours

Treatment	LC_{50}[a] (mg/l) 24h	LC_{50}[b] (mg/l) 48h	95% Confidence Limit (mg/l)		Slope SE	Relative Toxicity
			Lower	Upper		
Pangium edule	16.25	12.25	7.95	64.7	0.667±0.164	3.64
Ricinus communis	28.78	17.25	11.25	78.89	0.767±0.161	1.43
Derris elliptica	17.59	12.30	8.68	66.5	0.652±0.180	1.83
Zingiber officinale	50.05	25.50	14.15	89.67	0.692±0.166	1.00

[a] The level concentration of extracts causing 50% death of larva *S.exigua* after 24 hours.
[b] The level concentration of extracts causing 50% death of larva *S.exigua* after 48 hours.

Meanwhile, the extracts of *R.communis* with $LC_{50\text{-}48h}$ values 17.25 mg/l and the extracts of *Z.officinale* $LC_{50\text{-}48h}$ values 25.50 mg/l were effectively and relatively potent toxicant too, and have a future possibility to use as botanical insecticides to control the beet armyworm pest. In different circumstances, all tested of extract appeared higher larvicidal action after 48 hours.

Table.2. 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

The other results conjointly appeared that n-hexane fraction is that powerful against larvae of *S.exigua*, from *P.edule* n-hexane seed extract than the other potential extracts. The mortality of larvae instar III from *S.exigua* was highest utilize 1000 ppm n-hexane fraction (96.67 %) derived from *P.edule* seed at 2 days after treatment (Figure.2) and followed by utilizing 1000 ppm n-hexane fraction too (93,35%) derived from *D.elliptica* root extract (Figure 3).

Meanwhile, the extracts utilize 1000 ppm n-hexane fraction (63,3%) derived from *R.communis* leaf (Figure.4) and 1000 ppm n-hexane fraction (53,3%) derived from *Z.officinale* root (Figure.5) were relatively potent toxicant too.

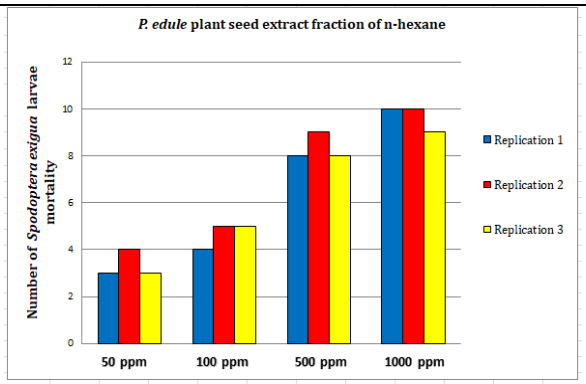

Figure.2. The test comes about of mortality S. exigua based on the action from *P.edule* seed extract utilize n-hexane fraction after 48 hours

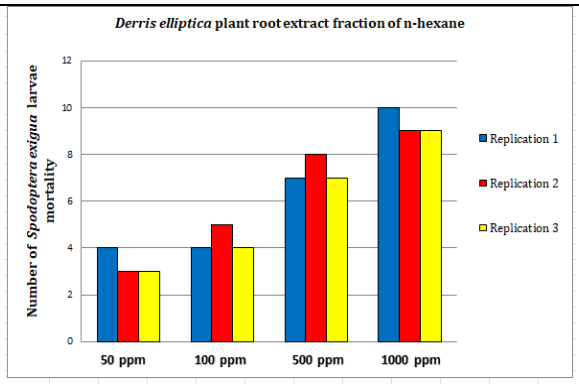

Figure.3. The test comes about of mortality *S. exigua* based on the action from *D.elliptica* root extract utilize n-hexane fraction after 48 hours

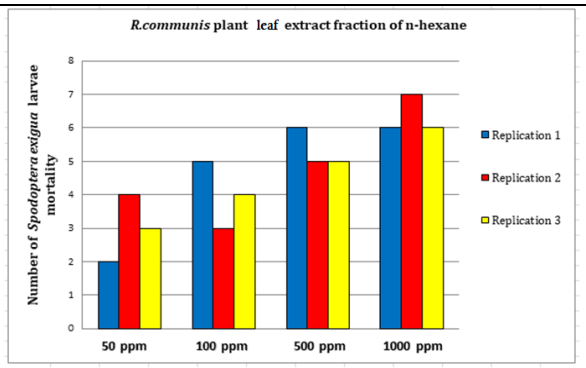

Figure.4. The test comes about of mortality S. exigua based on the action from *R.communis leaf* seed extract utilize n-hexane fraction after 48 hours

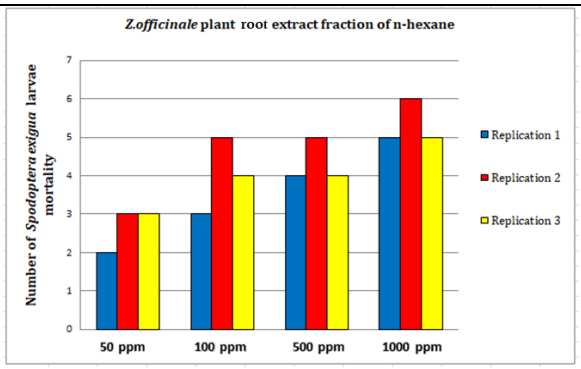

Figure.5. The test comes about of mortality S. exigua based on the action from *Z.officinale* root extract utilize n-hexane fraction after 48 hours

Table.3. The test results of phytochemical screening test

Type of Compound	Plant Seed, Leaf and Root Extract			
	Pangium edule	*Derris elliptica*	*Ricinus communis*	*Zingiber officinale*
Alkaloid Test				
- *Dragendorff Reactor*	+++	+++	++	++
- *Mayer Reactor*	+	+	-	-
- *Wegner Reactor*	+	+	-	-
Saponin Test	+++	+++	++	++
Flavonoid	++	++	++	++
Phenolic	++	++	++	++
Triterpenoid/Steroid	++	++	+++	++
Tanin	+++	++	++	++

\+ = Low Amount
++ = Adequate Amount
+++ = Large Amount

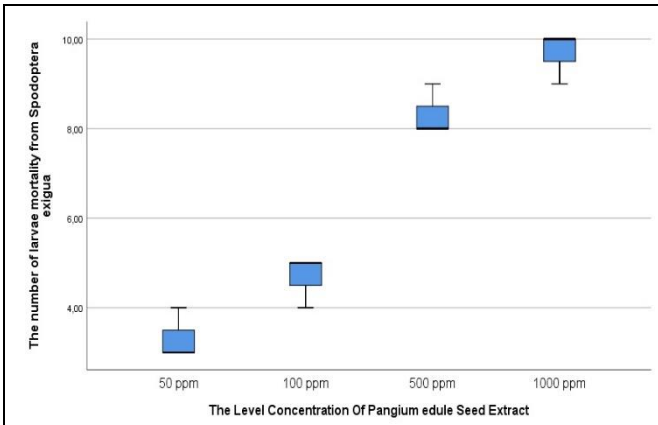

Figure.6. Box Plot Graph of Least Significance Different (α = 0.05) From *P.edule* Seed Extract

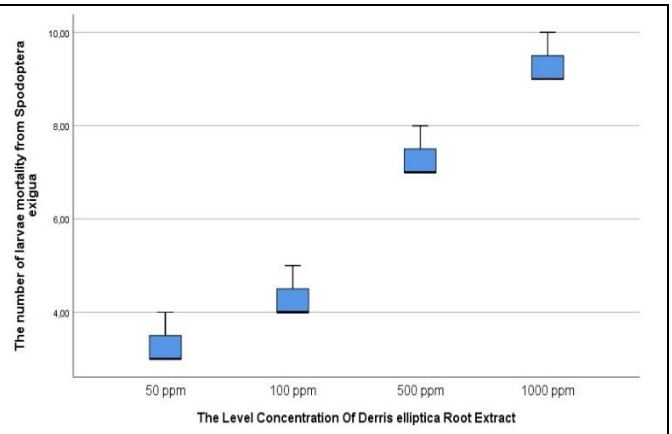

Figure.7. Box Plot Graph of Least Significance Different (α = 0.05) From *D.elliptica* Seed Extract

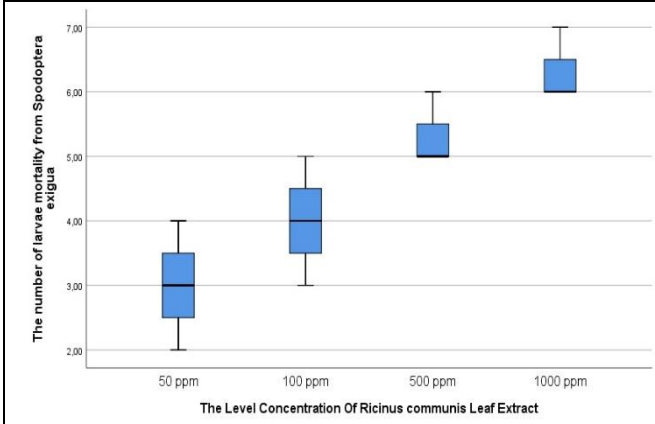

Figure.8. Box Plot Graph of Least Significance Different (α = 0.05) From *R.communis* Leaf Extract

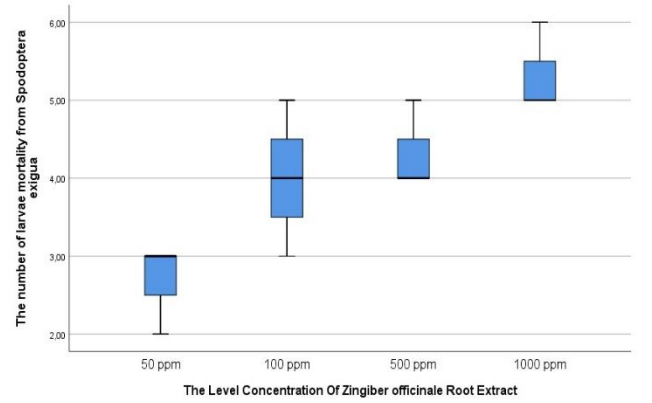

Figure.9. Box Plot Graph of Least Significance Different (α = 0.05) From *Z.officinale* Root Extract

3.2. Discussion

Beet armyworms were inexhaustible within the think about the field. The early instar stages particularly 1st and 2nd instars were found on green onion clears out while center stages instars were regularly identified interior the barrel shape clears out. The nearness of center organize or afterward arrange of beet armyworm was ordinarily related to skeletonized leaf harm (Figure 10).

Figure 10. Beet army worm on green onion leaf, a late instar III (left) (Sakul,2020.*self documentation*), and a middle instar inside the leaf (Ueno, 2015).

In this research, the process of insect nurture from *S.exigua* population (larval stage), was stockpile from welsh 'green' onion plants of Tonsealama town at the North Tondano, Minahasa Region, North Sulawesi Province Indonesia, utilize pheromone traps that contain the active element of Z-9-tetra decanol 10 µg/rubber (Feromon EXI. CV Nusagri, Bogor) (Ujiyani *et al*, 2019), listing in the Ministry of Agriculture, Republic of Indonesia and are commercialized available. Pheromones within the shape of little elastic barrels were hung on the side of green onion plants.

Figure 11. Pheromone EXI and green onion leaf in polybag

Plants commonly deliver chemical compounds to secure themselves from herbivores like creepy crawlies. There is significant proof that materials or extricates from a few plant species are valuable as botanical or natural pesticides. Botanical pesticides are possibly an elective to routine engineered pesticides since it is more often than not accepted that the common items would have lesser negative impacts on situations and human wellbeing whereas botanical pesticides are sensibly expensive and maybe viable to control target bothers (Tran *et al*, 2017.)

Therefore in this research, we found that the top peak mortality of larvae *S.exigua* reach from *P. edule* seed extract utilize 1000 ppm n-hexane fraction after 2 days treatment, with the number of death larvae is 29 from 30 larvae and it is 96,67%. After that, it followed by *D.elliptica root extract* utilize 1000 ppm n-hexane fraction, with the number of death larvae is 28 from 30 larvae and it is 93,33%. Both of two types of extracts are toxic and can increase the impermanence of larvae *S.exigua.*

Meanwhile, the other extracts derived from *R.communis* leaf utilize 1000 ppm n-hexane fraction can reach the number of death larvae is 19 from 30 larvae and it is 63,3% and the extracts derived from *Z.officinale* root utilize 1000 ppm n-hexane fraction can reach the number of death larvae is 16 from 30 larvae and it is 53,3%. We argue that both of them were relatively potent toxicants too.

In this research, we make a test to find the results of phytochemical screening test from four minahasa plants extract, we found that n-Hexane fraction contains alkaloids, tannins, triterpenoids, flavonoids, phenolic, and steroids are positive adequate amount until large amount (see.table 3). As seen on the table, the data show that alkaloid, saponin, and tannin are larger amount found in *P.edule* seed extract, and followed by *D.elliptica* root extract.

According to the other researcher, based on the test comes about of auxiliary metabolites phytochemical extricates of add up to red ginger (*Zingiber officinale* var amarum.) are alkaloids, flavonoids, triterpenoids, and phenolic. N-hexane division contains alkaloids, flavonoids, steroids, triterpenoids, and phenolic. Ethyl acetic acid derivation divisions contain alkaloids, flavonoids, triterpenoids, and phenolic. Harmfulness test appeared the mortality rate of shrimp hatchlings Artemia salina Filter utilizing Probit Examination SAS (Measurable Examination Framework) to decide the esteem of 50% Deadly Concentration (LC50), appeared that the division of n-hexane harmfulness with LC50 values of 63.8130 ppm; extricates of add up to was 71.0121 ppm and ethyl acetic acid derivation was 3821.89 ppm (Kaban *et al*, 2016).

Simultaneously, the results of research about phytochemical tests from seed tissue (endosperm) of kluwak (*P.edule*) appeared that this plant contains auxiliary metabolites alkaloids, terpenoids, saponins, flavonoid, and phenolic. n-Hexane extricates the endosperm isolated as oilseeds and analyzed utilizing Gas Chromatography-Mass Spectroscopy (GC-MS). The comes about of the investigation utilizing gas chromatography appeared 7 crests and mass spectroscopy examination uncovered several major constituents of oil compound within the frame of diethyl phthalate (2.49%), methyl palmitate (10.85%), cyclooctene pyran-1,3,5,6,7,8,9,10- hexahydro-4-isopropyl-1-phenyl (1,45%), methyl oleate (66.05%), and methyl stearate (10.85%) (Listaty *et al*,2015).

Moreover, the phytochemical examination of *P.edule* seed and *D.elliptica* root extricates, have the reason to demonstrate, there's tannin, as we know tannin have common polyphenol and carboxyl cluster with the result “browning enzymatic” that cause coloring seed to alter from white to brown. This response is catalyzed by the polifenolase enzyme. Tanin encompasses a solid characteristic particularly interaction with protein. Tanin comprises katekin, leukoantosianin, and hydroxy corrosive (galat, kafeat, and chlorogenic corrosive). Preparing phytochemical tests from n-hexane extricate and ethanol extricates of root *D.elliptica* and *P.edule* appeared positive to contain tannin, saponin, and phenolic. The

information of $LC_{50\text{-}48h}$ from n-hexane division degree with probit analysis (9,905 mg L-1) by *D.elliptica* with tall harmful category, and n-hexane division (11,574 mg L-1) by *P.edule* with the harmful category are more successful for brilliant apple snail control. The golden apple snail (*P.canaliculata*) mortality was most noteworthy utilizing 5000 ppm n-hexane division appeared 93,3% from *D.elliptica* and utilizing 5000 ppm n-hexane division appeared 63,3% from *P.edule*. In conclusion, both of extricates from *D.elliptica* roots and *P.edule* seeds has appeared strength as botanical molluscicides and it can be applied within the field (Manoppo, 2017).

The differences between treatments found in the application of *Ricinus communis* jatropha forest leaf extract with 1000 ppm n-hexane solvent with 500 ppm, showed results that were not significantly different at 0.05 alpha (α) level, while between 1000 ppm treatment with 100 and 50 ppm treatments gave different results. real.

The difference between the treatment concentration of *Zingiber officinale* red ginger root extract using 1000 ppm n-hexane solvent with 500 ppm showed no significant difference, whereas between 1000 ppm treatment with 100 and 50 ppm treatments showed significantly different results.

Treatment of Pangi seed extract (*Pangium edule*) and Tuba root (*Derris elliptica*) with 1000 ppm n-hexane solvent showed significantly different results from other extract concentration treatments, 500 ppm, 100 ppm, and 50 ppm.

Conclusion

P.edule seed extract and *D.elliptica* root extract was an efficient bioinsecticide exhibiting larvicidal and antifeedant properties of *S.exigua* (Hübner). Additionally work is critical to judge and characterize the active parts of the extract fractions and its effectualness within the field.

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