Effectiveness of n-hexane and ethanol extract of papaya (*Carica papaya* L.) leaves as shallot pest (*Spodoptera exigua* Hübner) natural insecticide

Mery Puspita Sari*, Laurentius Hartanto Nugroho, Sukirno Sukirno

Faculty of Biology, Universitas Gadjah Mada, Indonesia.
Jl. Teknika Selatan, Senolowo, Sinduadi, Mlati, Sleman City, DI-Yogyakarta 55281

**Email: merypuspita99@mail.ugm.ac.id*

Diterima 25 Juni 2022 Disetujui 28 November 2022

ABSTRACT

One of the agricultural commodities that has a high selling value and consumption level is shallot (Alium ascalonicum). However, the productivity of shallots is known to be susceptible to plant-destroying organisms, such as the shallot caterpillar (Spodoptera exigua). Currently, most shallot farmers control S. exigua using synthetic insecticides. Synthetic insecticides are poisonous and use for a long time period will cause resistance to pests and environmental pollution. One of the natural ingredients that has the potential as a botanical insecticide is papaya (Carica papaya). This study aimed to determine the effectiveness and concentration of n-hexane and ethanolic extracts of papaya leaves which are the most effective against toxicity and inhibition of the feeding power of S. exigua second instar larvae. The extract was obtained by gradual maceration of n-hexane (non polar) and ethanol (polar) solvents. Test the secondary metabolite content of papaya leaves using Thin Layer Chromatography (TLC). The results showed that the ethanol extract was the most effective extract in causing toxicity to larvae with the highest percentage of 96.67%. Meanwhile, n-hexane extract was more effective in inhibiting larval feeding than ethanol extract with the lowest feeding area of 2,27 mm. The most effective concentration of against toxicity and feeding inhibition of larvae is concentration 3%. The LC_{50} value of the ethanol extract was 0.0207% and the n-hexane extract was 0.0459%. Both extracts are known to contain compound groups, namely tannins, terpenoids, flavonoids, and alkaloids.

Keywords: shallots, Spodoptera exigua, Carica papaya*, insecticides, TLC*

INTRODUCTION

Shallots (*Allium ascalonicum* L.) is a type of horticultural vegetable that has been intensively cultivated for a long time. Shallot plantation is one of the farming businesses whose income is greater than other farming businesses, this is supported by the high price of shallots in the market. However, in recent years it is known that the productivity of shallot yields continues to decline. The reduction in the productivity of shallots is generally caused by the climate at the time of planting, including damage due to the microclimate and the attack of plant-disturbing organisms. Pests that have the potential to cause damage and decrease crop yields include the onion caterpillar (*Spodoptera exigua*). *S. exigua* is a cosmopolitan polyphagous insect pest that feeds on various types of agricultural plants (Smagghe, 2003), and develops through a metamorphosis process, and has a life cycle of 24 days through 5 instars. *S. exigua* attacks on shallots plants are characterized by visible attack symptoms on the leaves in the form of transparent white spots. Further attacks can cause the leaves to dry out and droop (DIY Agriculture Office, 2012). Yield losses by pests on shallots ranged from 20% to 100% (Udiatro et al., 2005).

To deal with pests that attack their crops, farmers generally rely on spraying synthetic pesticides. Synthetic pesticides are made of chemicals that are harmful and toxic. pesticides based on the type of target animal include insecticides, herbicides, fungicides, and so on. Synthetic pesticide residues that have entered the ecosystem can affect living organisms through the food chain where pesticides can accumulate at the highest trophic levels in the food chain (Sharma et al., 2019). Therefore, alternative solutions were developed to minimize the use of synthetic pesticides by developing and optimizing the use of botanical pesticides by utilizing plant active compounds. Papaya (*Carica papaya* L.) is one of the plants that grow in tropical areas including Indonesia. Various studies have shown that papaya leaves are believed to have high effectiveness and specific impact on nuisance organisms. The active ingredients of papaya leaves are not harmful to humans and animals, besides that the residue decomposes into non-toxic compounds so that it is safe for the environment and surrounding organisms (Hasfita et al., 2019).). These bioactive compounds can be obtained through a chemical process that is gradual extraction. Many studies of the use of this plant extract to control insects have been conducted. However, the optimation including the comparison of different solvents has not been known.

The aimed of this research was to investigate the effectiveness of n-hexane and ethanol extract of Papaya leaves to cause toxicity and antifeedant activity against second instar larvae of *S. exigua*. This study also conducted to compare the secondary metabolites profile of both extracts corresponding to their insecticide activity.

MATERIALS AND METHOD

Time and location of research

This research was conducted from September 2020 to April 2021 at the Entomology Laboratory and Biochemistry Laboratory of the Faculty Biology, Universitas Gadjah Mada.

Materials and tools

The equipments were used in this research including, erlenmeyer flask (Schoot Duran 250 ml and 500 ml), beker glass Schoot Duran 250 ml, conical flask, TLC chamber, silica gel plate GF_{254}, whatman filter paper No.1, vortex (Scilogex), micropipet (P-10, P-200, P-1000), pippet tip (10 µL, 200 µL, 1000 µL), analytical scale, Oven (Memmert). The materials were used in this research such as second instar larvae of *S. exigua*, *C. papaya* leaves, insecticide (Bongkis), n-hexane and ethanol 96%.

Methods

1. Sample Collection and Extraction of Carica papaya leaves

Leaves of *C. papaya* that has been selected were collected and rinse before dried. Dry leaves were grinded and sifted to produce leaves powder. *C. papaya* leave powder were extracted with gradual maceration technique. Two hundred gr of leaves powder were soaked in 2000 mL of n-hexane for 48 hours then filtered with Whatmann filter paper No. 1. The filtrate was evaporated to get a pure extract that would be used in the next test. residue from maceration by n-hexane solvent was reused for 96% ethanol solvent. Maceration was repeated twice for each solvent.

2. Artificial Diet Preparation for S. exigua

Artificial diet of insect was made by following Tarigan *et al.* (2020) method with some modifications. Five hundred gram of sword bean were soaked for a night then boiled. Boiled bean was drained and crushed. The dough added with 20 gr of benzoic, 2400 mL of aquades, 160 gr of yeast, 100 gr of agar, then blended until mix well. The dough was boiled and cooled till 60ºC. Twenty gr of ascorbic acid added to the dough and mixed. The dough is poured into a cup and keep it in the refrigerator.

3. Insect Rearing

Larvae of *S. exigua* were collected from shallot field fed with artificial diet until pupae stadium. After became moths, they are fed in the form of honey added to cotton. Eggs will be collected on paper. Then the eggs on the paper are taken and stored in plastic cups that have been given artificial feed. eggs that hatch and reach the second instar larval stage will be used in insecticidal activities tests.

4. Insecticidal Activities Test

The insecticidal activity test was carried out by testing the toxicity and antifeedant against the second instar larvae of *S. exigua*. Toxicity test was carried out by dripping extract on artificial feed with acetone as a negative control and insecticide as a positive control. Larval mortality was calculated after 48 hours. For the antifeedant test, the extract was dripped on shallot leaves (2 cm in diameter). The area fed by larvae was calculated after 48 hours with an image digimizer 5.3. The test was repeated 3 times for each treatment.

5. Secondary Metabolites Test

Secondary metabolites were tested by thin layer chromatography. The eluent used was n-hexane and ethyl acetate with a ratio of 6:4 for ethanol extract, and 8:2 for n-hexane extract. Two hundred and fifty mg of extract was diluted with each solvent for extraction. The extract was dripped onto the TLC plate and runned in the eluent to the upper limit line. The results were observed under UV light λ 254 nm and UV λ 365 nm. The compounds tested in this study included alkaloids, flavonoids, tannins, and terpenoids.

Data analysis

This study used an experimental design in the form of Completely Randomized (CRD) with 16 treatments, namely 7 concentration levels of two papaya leaf extracts (*C. papaya*), comparison insecticide and acetone as control. Each repeated 3 times. All the results obtained were tabulated and analyzed using SPSS version 16 software. Observation of larval mortality used Analysis of Variance (ANOVA) in order to obtain quantitative data. If there is a difference between treatments, it is continued with the Tukey-HSD test at a significance level of 5%. In testing the content of the active compound group using TLC.

RESULTS AND DISCUSSION

Based on Table 1. ethanol solvent can produce a higher yield percentage than n-hexane solvent. In the extraction process, a chemical mechanism like dissolves applies where polar compounds will dissolve in polar solvents and non-polar compounds will dissolve in non-polar solvents (Savitri et al., 2019). Therefore, it is possible that papaya leaf simplicia contains a higher polar-semi-polar active compound than nonpolar compounds so that more substances are attracted and the wet extract results obtained are also a higher when macerated using ethanol as the solvent rather than n-solvent -hexane.

Both n-hexane and ethanol extract had insecticidal activity, causing toxicity and antifeedant against second instar larvae of *S. exigua*. There was a significant difference between n-hexane and ethanol extract for causing toxicity and antifeedant against *S. exigua* larvae. However, ethanol extract exhibited higher toxicity effect and had a lower LC50 value (Table 2 and Table 3). The most effective concentration as an insecticide was at a concentration of 3% with the highest percentage of mortality in the ethanol extract of 96.67%. Nengsih and Utami (2019) stated that the higher the concentration, the higher the extract's ability as an insecticide. Mortality can be caused by the presence of toxic secondary metabolites produced by papaya leaves including alkaloids, flavonoids, tannins, and saponins (Eleazu et al., 2012). The content of these secondary metabolites is an insecticidal substance that acts as a neurotoxin (Rahman et al., 2020).

The difference in the extraction solution and the content of secondary metabolites from the extract will affect the mortality rate. This result supported by previous research. Bullangpoti et al., (2011) mentioned that the LC50 value of the ethyl acetate extract of *J. gossypifolia* leaf extract against the second instar larvae of S. exigua was 0.8644. This result was higher than the LC50 of n-hexane and ethanol extract of papaya leaves. The smaller the LC50 value of the insecticide, it means that the material is more toxic or the toxic effect is higher (Marhaen et al., 2016). It can be said that the N-hexane and ethanol extracts of papaya leaves were more toxic than the ethyl acetate extract of *J. gossypifolia* leaves.

On another hand, the use of various concentrations resulted from different mortality and antifeedant effect. The concentration treatment of the two extracts that was most effective in reducing larval feeding activity causing a decrease in the average feeding area was the highest concentration treatment of 3% with the smallest average feeding area found in the n-hexane extract treatment of 2.273 mm (Table 4). The antifeedant potential of papaya leaves supported by previous research by Rahayu et al., (2020) who conducted a papaya leaf extract antifeedant test against *S. litura*. The results showed that papaya leaf extract concentration of 50% produced an antifeedant index of 100%. The decrease in feeding area was strongly influenced by the toxic content of the extract which also affected larval mortality. Antifeedant substances can generally be obtained from the content of active compounds in plants such as alkaloids, flavonoids, terpenoids, and tannins and phenolics (Purington., 2003). In addition, Isman (2002) stated that most antifeedants have a mechanism of action by stimulating food-preventing receptors that send anti-feeding signals from the central nervous system.

Table 1. Extraction Yield of *C. papaya* leaves

Solvent	Dry weight (gram)	Extract weight (gram)	Yield percentage (%)	Colour
n-hexane	275	5,85	2,12	Brownish green
ethanol	275	6,20	2,25	Dark green

Table 2. Mortality of *S. exigua* II instar larvae

Concentration (%)	Mortality Percentage (%)	
	N-hexane	Ethanol
0,0	16,67±6,67^{a}	16,67±3,33^{a}
0,1	63.33±1,67^{b*}	71.67±1,67^{b*}
0,5	71.67±1,67^{bc*}	81.67±2,89^{bc*}
1	78.33±0^{cd*}	85,00±3,33^{bcd*}
1,5	85,00±0^{de*}	86.67±1,67^{cd*}
2	90,00±0^{de*}	93.33±0^{cd*}
2,5	90,00±1,67^{de*}	95,99±1,67^{cd*}
3	91.67±1,67^{e*}	96.67±1,67^{d*}
Insecticide	90,00±2,89^{de}	91,67±6,01^{cd}

Annotations: ^{abcde}) Number followed by the same letter within the same columns are not significantly different; *) indicates a significantly difference.

Table 3. Lethal Concentration of N-hexane and Ethanol Extract of *C. papaya*

Solvent	LC_{50}			Insecticidal activity
	Estimate	**Lower Bond**	**Upper Bond**	
N-Hexane	0,0459%	0,000066%	0,174%	Yes
Ethanol	0,0207%	0,0000009%	0,109%	Yes

Table 4. Feeding Area of Shallot Leaves

Concentration (%)	Feeding Area of Larvae (mm)	
	N-hexane	**Ethanol**
0,0	22,31±2,30^{a}	30,55±1,37^{a}
0,1	12,51±1,25^{b*}	23,69±0,72^{b*}
0,5	8,56±0,83^{bc*}	21,71±0,80^{bc*}
1	6,39±0,09^{cd*}	15,64±1,57^{cd*}
1,5	5,58±0,25^{cd*}	10,57±1,80^{de*}
2	4,40±0,49^{cd*}	8,30±1,71^{ef*}
2,5	3,82±0,76^{cd*}	7,38±1,14^{ef*}
3	2,27±0,29^{d*}	6,24±0,67^{ef*}
Insecticide	3,24±0,79^{d}	3,99±1,15^{f}

Annotations: ^{abcde}) Number followed by the same letter within the same columns are not significantly different; *) indicates a significantly difference

Based on the secondary metabolite content testing that has been carried out, it has been known that the ethanolic extract and n-hexane of papaya leaves both contain the five classes of secondary metabolites tested, namely the tannins, terpenoids, flavonoids and alkaloids. From the TLC test of the n-hexane and ethanol extracts at Figure 1, it was found that the n-hexane extract produced more stains on the alkaloid and terpenoid compounds test, so it can be assumed that the n-hexane extract had more diverse types of alkaloids and terpenoids compared to the ethanol extract. While the content of tannins, and flavonoids in the ethanol extract more stains appeared on the TLC plate so it can be assumed that the ethanol extract had more types of compounds from the tannin and flavonoid group when compared to the n-hexane extract.

In their role as insecticides, these tannins have a strong damaging effect on insect phytophagous and can reduce the efficiency of nutrient absorption, and midgut lesions in insects so that insect growth and development can be disrupted (War, 2012). Terpenoids include toxicants, antifeedants and oviposition deterrents against insects. In addition, terpenoids can also be repellant agents in insects. The effect of flavonoid content on insects is also an inhibitor of insect respiration so that it inhibits the respiratory system and causes insects to die (Wijaya, 2018). Based on the research of Simmonds & Stevenson (2001) who isolated the flavonoid content of the *Cicer arietinum* plant, the results showed that there was antifeedant activity against *S. exigua*, *S. litoris and S. frugiperda*. Alkaloids are believed to play an important role in their role as insecticides. Vien & Loc (2017) stated that papaya leaves contain alkaloid compounds with the main component called Carpain. Carpain alkaloids are toxic because they can cause nerve paralysis and heart suppression so that the larvae eventually die (Nengsih & Utami, 2019). Thus, this was consistent with the effectiveness of the two extracts in influencing mortality and feeding inhibition of the second instar larvae of *S. exigua* and the use of both types of solvents gave a significant difference to the mortality and feeding inhibition of the second instar larvae of *S. exigua*. In conclusion, higher content of secondary metabolite in ethanolic extract resulting higher toxicity and antifeedant activity.

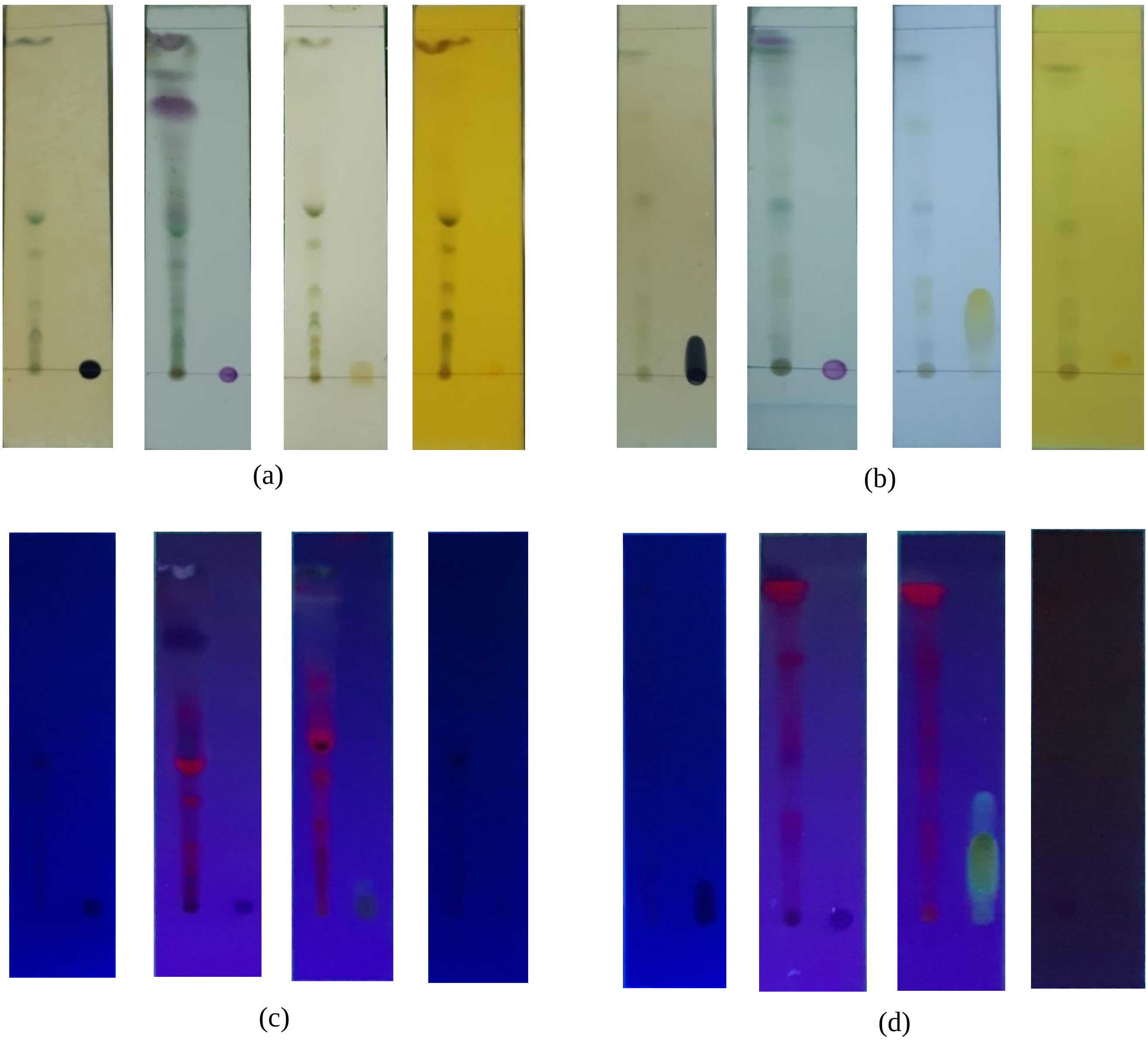

Figure 1. Secondary Metabolites Profil of *C. papaya* leaves on TLC (a) test with n-hexane extract (b) test with ethanolic extract (c) test with n-hexane extract under UV (d) test with ethanolic extract under UV (tannin test; alkaloid; terpenoid; and flavonoid test)

CONCLUSION

Based on the research that has been done, it can be concluded that n-hexane and ethanolic extract of *C. papaya* leaves had insecticidal activities both toxicity and antifeedant activity against second instar larvae of *S. exigua*. Ethanolic extract exhibited higher insecticidal activity with the value of LC50= 0,0207%. Higher activity of ethanolic extract was corresponding with higher secondary metabolites content in this extract.

ACKNOWLEDGEMENT

This research and publication was supported by the Faculty of Biology, Universitas Gadjah Mada grand under student-lecturer collaboration research grand.

REFERENCES

Bullangpoti V, Khumrungsee N, Pluempanupat W, Kainoh Y, Saguanpong U. 2011. Toxicity of Ethyl Acetate extract and Ricinine from *Jatropha gossypifolia* Senescent Leave against *Spodoptera exigua* Hubner (Lepidoptera: Noctuidae*). Journal of Pesticide Science* **36(2):** 1-4.

Dinas Pertanian DIY. 2012. Standard Operating Procedure (SOP) Bawang Merah Gunungkidul. Dinas Pertanian DIY: Yogyakarta.

Eleazu CO, Eleazu KC, Chukwuma SC. 2012. Comparative Study of The Phytochemical Composition of The Leaves of Five Nigerian Medical Plants. *Journal of Biotechnology and Pharmaceutical Research* **3(2):** 42- 46.

Hasfita F, Nasrul ZA, Lafyati. 2013. Pemanfaatan Daun Pepaya (*Carica papaya*) untuk Pembuatan Pestisida Nabati. *Jurnal Teknologi Kimia Unimal* **1(2):** 13-24.

Isman, M. 2002. Insect Antifeedant. *Pesticide Outlook*. **6:** 234-279.

Marhaen L, Aprianto SF, Hasyim A, Lukman L. 2016. Potensi Campuran *Spodoptera exigua* Nucleopolyhedrovirus (SeNPV) dengan Insektisida Botani untuk Meningkatkan Mortalitas Ulat Bawang Spodoptera exigua (Hübner) (Lepidoptera: Noctuidae) di Laboratorium. *J. Hortikultura* **26 (1):** 103-112.

Nengsih R, Utami LB. 2019. Pengendalian Ulat Grayak Bawang Merah (*S. exigua*) Menggunakan Ekstrak Metanol 70% Daun Pepaya (*Carica papaya*) dan Ekstrak Etanol 70% Umbi Gadung (Dioscorea hispida). *J. Ilmu Alam dan Tek Terapan* **1:** 12-22.

Purrington CB. 2003. Secondary Products: Antifeedant Substances in Plants. Encyclopedia of Applied Plant Sciences. 1140-1145

Rahayu SE, Leksono AS, Gama ZP, Tarno H. 2020. The Active Compounds Composition and Antifeedant Activity of Leaf Extract of Two Cultivar *Carica papaya* L. on *Spodoptera litura* F. Larvae. AIP Conference Proceedings **(1):** 2231.

Rahman A, Samharinto S, Salamiah. 2020. Mortalitas Ulat Grayak (*Spodoptera litura* F.) yang Diaplikasi dengan Berbagai Pestisida Nabati. *Proteksi Tanaman Tropika* **3(03):** 238-243.

Savitri ES, Holil K, Resmisari RS, Syarifah U, Munawaroh S. 2019. Effect of Extraction Solvent on Total Phenol, Total Flavonoid Content and Antioxidant Activities of Extract Plant *Punica granatum, Vitis vinifera* L., *Ficus carica* L. and *Olea europea*. AIP Conference Proceedings.

Sharma A, Kumar V, Shahzad B, Tanveer B, Sidhu GPS, Handa H, Kohli SK, YadaV P, Bali AS, Parihar, Dar OI, Singh K, Jastoria S, Bakshi P, Ramakrishnan M, Kumar S, R. Bhardwaj R, Ashwani, Thukral K. 2019. World Pesticide Usage and its Impacts on Ecosystem. *SN Applied Sciences* **1(11):** 1446-1485.

Simmonds MSJ, Stevenson PC. 2001. Effects of Isoflavonoids from Cicer on larvae of *Heliocoverpa armigera. J. Chem. Ecol* **27(5):** 265-277.

Smagghe G, Pineda S, Carton B, del Estal P. 2003. Toxicity and kinetics of methoxyfenozide in greenhous selected *Spodoptera exigua* (Lepidoptera: Noctuidae). *Pest Manage. Sci.* **59:** 1203–1209.

Tarigan A, Sumarmi S, Sukirno. 2020. Effectiveness of Aloe (*Aloe vera* L.) as a Protectant of *Bacillus thuringiensis* var kurstaki Againts Ultraviolet Light and Biological Control Agenst of (*Spodoptera litura* Fab.). AIP Conference Proceedings.

Udiatro BK, Setiawan W, Suryaningsih E. 2005. *Pengenalan Hama dan Penyakit pada Tanaman Bawang Merah dan Pengendaliannya.* Panduan Teknis PPT bawang merah No. 2. Balai Tanaman Sayuran: Bandung.

Vien DTH, Loc TV. 2017. Extraction and Quantification of Carpaine from *Carica* *papaya* Leaves of Vietnam. *International Journal of Environment, Agriculture and Biotechnology* (IJEAB) **2(5):** 2394-2397.

War AR, Paulraj MG, Ahmad T, Buhroo AA, Hussain B, Ignacimuthu S, Sharma HC. 2012. Mechanims of Defense againts Insect Herbivores. *Plant Signaling & Behavior* **7(10):** 1306-1320.

Wijaya IN, Wirawan IGP, Adiartayasa W. 2018. Uji Efektivitas Beberapa Konsentrasi Ekstrak Daun Kirinyuh (Chromolaena odorata L.) terhadap Perkembangan Ulat Krop Kubis (*Crocidolomia pavonana* F.). *Agrotrop* **8(1):** 11-19.