BIO Web of Conferences **117**, 01028 (2024) https://doi.org/10.1051/bioconf/202411701028
ICoLiST 2023

Histological and physiological responses of *Spodoptera litura* F. larvae after exposure to papaya leaf extract (*Carica papaya* L.)

Sofia Ery Rahayu[1*], *Amin Setyo* Leksono[2], *Zulfaidah Penata* Gama[2], and *Hagus* Tarno[3]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Malang, Malang, Indonesia
[2]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Brawijaya, Malang, Indonesia
[3]Departement of Pest and Disease, Faculty of Agriculture, Universitas Brawijaya, Malang, Indonesia

Abstract. This study aimed to analyze the histological structure of the midgut, levels of digestive enzymes, and concentrations of 20-hydroecdison in *S. litura* F. larvae after exposure to papaya leaf extract (PLE). This experimental study used *S. litura* F. instar 4th larvae exposed to 20% and 0% PLE for 24-48 hours. The larvae were dissected, and their intestines were taken to isolate intestinal protein and then to measure the levels of the protease enzyme. The larval histology was prepared by examining the larvae and taking part in the midgut of the larva measurement of 20-hydroecdison levels by isolating protein in hemolymph fluid. The results showed that the midgut epithelial cells of the control group were normal, while the larvae in the treatment group experienced disturbances, the disintegration of the peritrophic membrane, and the formation of vacuoles in the cells. The protease enzyme measurement results in the control group were 39.76 ng/ml lower than the treatment group (45.43-56.92 ng/ml). The 20-hydroekdison measurement results in the control group were 70.76 ng/ml lower than in the treatment group, which were 9060-22361.51 ng/ml. Based on the study's results, PLE can disrupt the physiology and midgut histology of *S. litura* larvae, so that it can be developed as a botanical pesticide.

1 Introduction

Currently, farmers control attacks by the armyworm S*podoptera litura* Fabricius on cultivated plants using synthetic chemical pesticides. The use of synthetic chemical pesticides by farmers often exceeds the dosage [1]. The advantage of using synthetic chemical pesticides is that they can immediately overcome pest attacks, but they have detrimental impacts on humans and the environment. These detrimental impacts can disrupt human health, cause death of non-target insects including pollinators, and create resistance in pest insect populations [2-4]. Therefore, research in the agricultural sector is developing, namely focusing on the discovery and development of alternative pesticide of natural origin that are

* Corresponding author: sofia.ery.fmipa@um.ac.id

less toxic and easily decomposed in nature. One source of pesticides is plant material. The use of plant materials as pesticides has the potential for pest attacks on plants [5,6].

Plants that can be used as sources of pesticides are very abundant. Among these plants that are easily found in the community are papaya plants. The use of papaya plants as pesticides has been carried out by several researchers. The research results showed that papaya leaf extract influenced the mortality of *Plutella xylostella* larvae [7]. Papaya leaf extract has also been studied for its effect on insect pests *Tribolium castaneum* and its results influence its growth and development [8]. This condition was also found in termite research [9]. The basis for using the papaya plant as a pesticide is its active compound content. The results of phytochemical screening show that papaya leaf extract contains various compounds including carpain alkaloids, pseudocarpain, dehydrocarpain 1 and 2 [10].

The effectiveness of papaya leaf bioinsecticides for development as bioinsecticides, apart from looking at the content and levels of active compounds, can also be supported by the response of the target animal. This response can be in the form of antifeedant activity, mortality, growth and development rate, histological disorders, and body metabolism. The entry of chemical compounds into the insect's body through its food can cause disturbances in digestive enzymes so that the process of breaking down food macromolecules into simple molecules that will be absorbed by intestinal epithelial cells is also disrupted. Bioinsecticides can interfere with the activity of digestive enzymes, as well as damage the epithelial cells of intestinal cells so that the process of food absorption is disrupted [11]. One of the digestive enzymes that is inhibited by bioinsecticides is protease.

Disruption of digestive processes and absorption results in disturbances in eating activity, behavior, growth rate, physiology, and increased mortality. Until now, no researchers have reported the use of papaya leaf extract, especially Thailand and Indonesian purple cultivars, with metabolic indicators in the form of digestive enzymes and hydroecdysone and midgut histopathology in *S. litura* Fabricius larvae. By knowing the effectiveness of papaya leaf extract (*C. papaya* L.) Thailand and Indonesian purple cultivars on *S. litura* Fabricius larvae, papaya leaves can be developed as a botanical pesticide.

2 Methods

2.1 Preparation papaya leaf extract

The leaves of the Thai and Indonesian Purple papaya cultivars were obtained from lowland areas (Nganjuk, Pare), and highland areas (Malang), East Java, Indonesia. The method for making the extract uses a maceration technique with methanol solvent. Complete procedures as presented in Rahayu et al., 2020 [10].

2.2 Testing of papaya leaf extract against intestinal protease enzymes of *S. litura* Fabricius larvae

Late 4th instar *S. litura* Fabricius larvae were starved for 4 hours then exposed to leaf feed containing 0% and 20% concentration of papaya leaf extract for 24 hours. Next, the *S. litura* Fabricius larvae were dissected, and their intestines taken. The next stage was isolation of larval intestinal proteins. The results of isolating high levels of protein after being measured using a nanodrop will be used to measure protease enzyme activity using the method in the Protease kit (G-Bioscience). The data obtained were in the form of intestinal protease enzyme levels of *S. litura* Fabricius larvae.

2.3 Testing of papaya leaf extract on the concentration of Hydroecdysone in *S. litura* Fabricius larvae

Late 4th instar *S. litura* Fabricius larvae were starved for 4 hours then exposed to leaf feed containing 0% and 20% concentration of papaya leaf extract for 24 hours. Next, the *S. litura* larvae were dissected and the hemolymph fluid was taken, then processed until dry hemolymph pellets were produced. The dry pellets were then stored in a freezer at -200C until the hydroecdysone content was measured. The method for measuring hydroecdysone levels contained in larval hemolymph follows the method in the 20 Hydroecdysone kit (Arbor Assays).

2.4 Testing of papaya leaf extract on the histopathology of the midgut of *S. litura* Fabricius larvae

Late 4th instar *S. litura* Fabricius larvae were starved for 4 hours then exposed to food leaves containing papaya leaf extract at concentrations of 0% and 20% for 48 hours. Next, the *S. litura* Fabricius larvae were dissected, and the middle intestine (midgut) was taken and fixed in 10% neutral formalin solution. The stains used are Hematoxylin and Eosin. The preparations were observed under a light microscope. Observations were made on the cells that make up the epithelium layer of the insect intestine and the peritrophic membrane.

3 Results and Discussion

Protease enzymes are very necessary for *S. litura* Fabricius larvae in the process of protein metabolism. At this stage of the research, the concentration of intestinal protease enzymes of *S. litura* Fabricius larvae was measured after the larvae were exposed to papaya leaf extract. The results of measuring the concentration of intestinal protease enzymes of *S. litura* Fabricius larvae are presented in Table 1.

Table 1. Concentration of intestinal protease enzyme of *S. litura* Fabricius larvae

Type of Extracts	Extract Concentration (%)	Protease Concentration (ng/ml)
Control	0	39,81
US	20	53,77
TS	20	53,25
UR	20	45,43
TR	20	56,92

US = Indonesian purple cultivar from middle land, TS = Thailand cultivar from middle land, UR = Indonesian purple cultivar from low land, TS = Thailand cultivar from low land, K = control, 20% = the concentration used in the study.

Based on Table 1, it shows that the concentration of protease enzymes contained in the intestines of *S. litura* Fabricius larvae in the control group was the lowest compared to *S. litura* Fabricius larvae which were given papaya leaf extract on their food leaves. The difference in the concentration of the protease enzyme in *S. litura* Fabricius larvae in the control group and the treatment group is possible due to the papain enzyme content in papaya leaf extract. Papain is a simple cysteine protease enzyme composed of 212 chains of amino acid residues with a molecular weight of 23,406 Daltons [12]. As a result of these conditions, *S. litura* larvae fed with papaya leaf extract had higher levels of intestinal protease than the control group. The higher the enzyme concentration, the higher the enzyme activity will be proportionally [13]. The function of the protease enzyme is to hydrolyze protein, so the higher the concentration of protease, the more protein will undergo hydrolysis. In addition, the higher protease production is a form of adaptation of *S. litura* larvae to protease inhibitors produced by plants. The plants themselves produce protease inhibitors which aim to inhibit herbivore attacks [14].

The growth of *S. litura* Fabricius larvae are also influenced by the hormone hydroecdysone. The hormone dissolves in the larva's hemolymph. The results of measuring the hydroecdysone concentration of *S. litura* Fabricius larvae are presented in Table 2.

Table 2. Hydroekdyson Concentration of Larva *S. litura* Fabricius

Type of Extracts	Extract Concentration (%)	Hydroecdyson Concentration (pg/ml)
Control	0	7076,33
US	20	17942,34
TS	20	9060,17
UR	20	22361,51
TR	20	12088,51

US = Indonesian purple cultivar from middle land, TS = Thailand cultivar from middle land, UR = Indonesian purple cultivar from low land, TS = Thailand cultivar from low land, K = control, 20% = the concentration used in the study.

The results of measuring the concentration of hydroecdysone in the hemolymph of *S. litura* Fabricius larvae showed that in the control group the concentration of hydroecdysone was the lowest compared to the treatment group, namely that the larvae were fed leaves given papaya leaf extract. Hydroecdysone is a stress hormone. During stressful conditions such as malnutrition, it will trigger the production and secretion of hydroecdysone 20E [15]. Based on the antifeedant test of papaya leaf extract, it shows that the Indonesian purple and Thailand cultivar papaya leaf extract has a function as an antifeedant for *S. litura* Fabricius larvae [10]. As a result, food leaves that are given papaya leaf extract tend not to be eaten by larvae, so their nutritional needs were not met. Apart from that, there is an active compound, namely phenol, in papaya leaf extract which enters the larva's body and causes oxidative stress. This is because phenolic compounds will undergo oxidation and produce ROS (reactive oxygen species) [16]. One of the responses shown by *S. litura* Fabricius larvae was increased production of hydroecdysone 20 E.

The midgut of *S. litura* Fabricius larvae are an important organ in digesting food and absorbing it. The leaves fed to *S. litura* Fabricius larvae in this study were given papaya leaf extract for 48 hours. Next, the larvae were dissected, and preparations were taken of the middle intestine to determine the tissue structure. The histology preparation of the larval midgut is presented in Fig. 1. A comparison table of the condition of the larval midgut preparations between control and treatment is presented in Table 3.

Based on the results of observations of the midgut preparations of *S. litura* Fabricius larvae from the control group, it showed that the peritrophic membrane was clearly visible limiting the lumen and epithelial cells of the middle intestine. The epithelial cells of the midgut were arranged tightly, whereas in the midgut preparations of the treated group larvae, it showed that the peritrophic membrane was disintegrating towards the intestinal lumen. The epithelial cells appear to have many vacuoles or are experiencing vacuolization and degradation so that the cells appear to move towards the intestinal lumen. The results of this study are the same as those found in the midgut conditions of *S. litura* which were exposed to *Annona squamasa* seed extract [17], where the epithelial cells were irregular, and vacuolization formed in the cell cytoplasm. The vacuolization process in the epithelial cells of the midgut of *S. litura* Fabricius larvae is possible due to the presence of papaya leaf extract which is given with the food leaves. The active compounds contained in papaya leaf extract are alkaloids in the form of carpaian, dehydrocarpain, carpain, pseudocarpain which are classified as small molecules so that these active compounds enter the midgut cells of *S. litura* Fabricius larvae through a diffusion process [18].

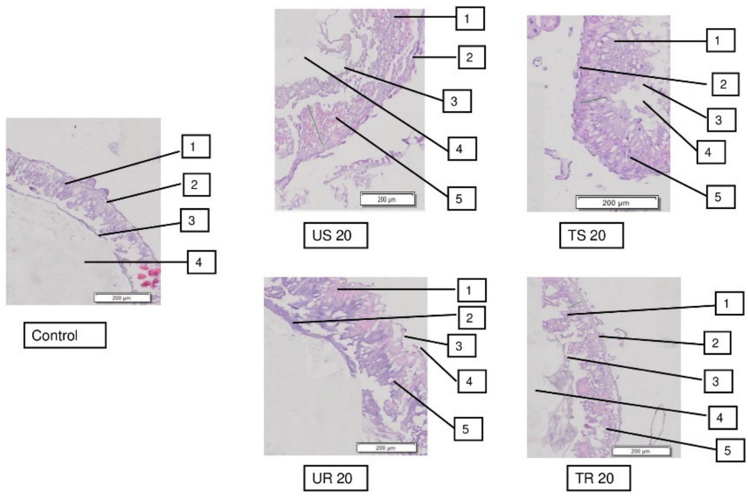

Fig. 1. Histology of the midgut of 4th instar *S. litura* Fabricius larvae at 48 hours after treatment with 0% and 20% concentration of papaya leaf extract

Image caption: 1: epithelial cells, 2: basal membrane, 3: peritrophic membrane, 4: intestinal lumen, 5: vacuole, US = Indonesian purple cultivar from middle land, TS = Thailand cultivar from middle land, UR = Indonesian purple cultivar from low land, TS = Thailand cultivar from low land, K = control, 20% = the concentration used in the study.

Table 3. Histological condition of the midgut of *S. litura* Fabricius larvae

Type of Extract	**Condition of midgut epithelial cells of *S. litura* Fabricius larvae**
Control	The epithelial cells were arranged tightly, the peritrophic membrane was clearly visible between the lumen and the epithelial cells.
US20	The cells that make up the epithelium were not arranged tightly and point towards the lumen, the peritrophic membrane leads towards the lumen, there were many vacuoles in the epithelial cells.
TS20	The cells that make up the epithelium were not tightly arranged and point towards the lumen, the peritrophic membrane leads towards the lumen, there were many vacuoles in the epithelial cells.
UR20	The cells that make up the epithelium were not arranged tightly and point towards the lumen, the peritrophic membrane leads towards the lumen, there are many vacuoles in the epithelial cells, the epithelial cells were not attached to the basement membrane.
TR20	The cells that make up the epithelium are not tightly arranged and point towards the lumen, the peritrophic membrane leads towards the lumen, there are many vacuoles in the epithelial cells.

US = Indonesian purple cultivar from middle land, TS = Thailand cultivar from middle land, UR = Indonesian purple cultivar from low land, TS = Thailand cultivar from low land, K = control, 20% = the concentration used in the study.

The flavonoid compounds contained in papaya leaf extract have very high biological activity. In the larval midgut, phenolic compounds undergo oxidation which will release reactive oxygen species (ROS) which cause oxidative stress for the larvae [16]. The presence of ROS causes the release of Ca^{2+} ions from the Endoplasmic Reticulum via the Inositol triphosphate receptor (IP3R) into the cytoplasm. The Ca^{2+} ions then enter the mitochondria through the Mitochondrial Calcium Uniporter (MCU), which causes the mitochondria to have an excess of Ca^{2+} ions. This condition causes mitochondria to not function and produces ROS. The next effect is inhibiting the proteasome and protein folding in the endoplasmic reticulum. Inhibition of the proteasome prevents the degradation of IP3R and mitochondrial MCU thereby affecting the movement of Ca^{2+} ions. This condition causes vacuolization of the endoplasmic reticulum which can ultimately cause cell death. Malfunctioning mitochondria cause the cessation of ATP synthesis. ATP is also used in ion pumps so that it ultimately influences ion transport through cell membranes and influences the ionic composition of the cytoplasm [19]. Thus, the presence of ROS in cells has an impact on the process of vacuolization and cell death.

The function of the midgut of *S. litura* larvae is very important for the life of the larvae, namely as a producer of digestive enzymes, digesting food, and absorbing nutrients. As a result of the active compounds contained in papaya leaf extract, it causes histological damage to midgut cells. This condition will disrupt the physiology of the larvae so that the growth and development of the larvae will be disrupted [20]. This situation shows that papaya leaf methanol extract can be developed into an environmentally friendly pesticide botanical product.

4 Conclusion

The results of measuring the intestinal protease enzyme concentration of *S. litura* Fabricius larvae showed that in the control group it was lower compared to the treatment group larvae which were given papaya leaf extract. The results of measuring the concentration of the 20-hydroexdison hormone in *S. litura* Fabricius larvae from the control group were lower compared to the treatment group given papaya leaf extract. The histological condition of the midgut of *S. litura* Fabricius larvae from the control group showed normal epithelial cells, whereas in the midgut of the treated group larvae the peritrophic membrane was seen to be damaged and vacuoles were formed in the epithelial cells. Papaya leaf extract can disrupt the physiology and midgut histology of *S. litura* larvae so that it can be developed as a botanical pesticide.

The authors would like to thank Universitas Negeri Malang for facilitating this research.

References

1. Ilhamiyah, Salamiah, Samharinto, H. Halim, Agroecosystem Management on Green Mustard Plants in Wetlands during Dry Season. J. Wetl. Environ. Manag. **7**, 1 (2019). http://dx.doi.org/10.20527/jwem.v7.v1.189.
2. R. Hernández-Lambraño, K. Caballero-Gallardo, J. Olivero-Verbel, Toxicity and antifeedant activity of essential oils from three aromatic plants grown in Colombia against *Euprosterna elaeasa* and *Acharia fusca* (Lepidoptera: Limacodidae). Asian Pac. J. Trop. Biomed. **4**, 9 (2014). https://doi.org/10.12980/APJTB.4.2014APJTB-2014-0178.
3. E. Kuppusamy, K.I. Dhamodharan, S. Jayakumar, Role of plants and plant based products towards the control of insect pests and vectors: A novel review. J. Coast. Life Med. **4**, 11(2016). https://doi.org/10.12980/jclm.4.2016J6-173.

4. B.S.A. Lima, F.A.D. Rocha, A. Plata-Rueda, J.C. Zanuncio, J.F.S. Cossolin, L.C. Martínez, J.E. Serrão, Abamectin induces mortality, inhibits food consumption, and causes histological changes in the midgut of the velvetbean caterpillar *Anticarsia gemmatalis* (Lepidoptera: Noctuidae). J. of Pest Science, **97** (2024). https://doi.org/10.1007/s10340-023-01642-4.
5. J.M. Adesina, K.D. Ileke, R.Yallappa, T.I. Ofuya, Insecticidal Evaluation of *Bridelia micrantha* and *Dalbergia lactea* Aqueous Extracts for The Control of *Podagrica uniforma* (Jacoby) and *Nisotra dilecta* (Jacoby) (Coleoptera: Chysomelidae) Infestation on Okra. AGRIVITA J. Agric. Sci. **38**, 3 (2016). https://doi.org/10.17503/agrivita.v38i3.845
6. A.K. Meena, R. Swaminathan, Efficacy of biopesticides against whiteflies and jasids of soybean. J. Biopestic. **11**, 1 (2018).
7. V.G. Siahaya, R.Y. Rumthe, Test of papaya leaf extract (*Carica Papaya*) against *Plutella xylostella* larvae (Lepidoptera: Plutellidae). Agrologia **3**, 2 (2018). https://doi.org/10.30598/a.v3i2.251.
8. N.Tatun, B. Vajarasathira, J. Tungjitwitayakul, S. Sakurai, Inhibitory effects of plant extracts on growth, development and α-amylase activity in the red flour beetle *Tribolium castaneum* (Coleoptera: Tenebrionidae). Eur. J. Entomol. **111**, 2 (2014). https://doi.org/10.14411/eje.2014.022.
9. A. Aihetasham, K.Z. Rasid, S.R. Hasan, I. Bodlah, Effect of *Carica papaya*, *Helianthus annus* and *Bougainvillea glabra* aqueous extracts against termite, Heterotermes indicola (Isoptera: Rhinotermitidae). Punjab Univ. J. Zool., **32**,1 (2017).
10. S.E. Rahayu, A.S. Leksono, Z.P. Gama, H. Tarno, The active compounds composition and antifeedant activity of leaf extract of two cultivar *Carica papaya* L. on *Spodoptera litura* F. Larvae. in *AIP Conference Proceedings.* Malang, Indonesia (2020) 2231 :040085. https://doi.org/10.1063/5.0002677.
11. K.R. Ranjini, J.G. Nambiar, Histopathological effects of leaf extracts of *Clerodendrum infortunatum* and *Eupatorium odoratum* on the midgut tissue of sixth instar larvae of *Orthaga exvinacea* Hampson (Lepidoptera: Pyralidae). J. Entomol. Zool. Stud. **3**, 5 (2015).
12. M. Hariono, J. Julianus, I. Djunarko, I. Hidayat, L. Adelya, F. Indayani, Z. Auw, G. Namba, P. Hariyono, The future of *Carica papaya* leaf extract as an herbal medicine product. Molecules. **26**, 22 (2021). https://doi.org/10.3390/molecules26226922.
13. H. Haryati, Y. Fujaya, E. Saade, Effect of predigested artificial diet using papain enzyme on the degree of protein hydrolysis and protease enzyme activity of mud crab (*Scylla olivacea*) larvae at zoea 2 and 3 stages. Aquac. Indones. **19**, 2 (2018). https://doi.org/10.21534/ai.v19i2.118.
14. F.A.D. Rocha, Y. B. Meriño-Cabrera, E.J.G. Pereira, J.C. Zanuncio, W. G. Campos, J.E. Serrão, and M.G.A. Oliveira, Effect of natural and artificial diets on protease activity in the midgut of *Spodoptera cosmioides* and *Spodoptera eridania* (Lepidoptera: Noctuidae) larvae. Florida Entomologist **103**, 3: 452-457 (2021). https://doi.org/10.1653/024.103.00406
15. H. Ishimoto, T. Kitamoto, Beyond molting-roles of the steroid molting hormone ecdysone in regulation of memory and sleep in adult Drosophila. Fly (Austin) **5**, 3 (2011). https://doi.org/10.4161/fly.5.3.15477
16. M. Vihakas, Flavonoids and other phenolic compounds : characterization and interactions with lepidopteran and sawfly larvae. (Doctoral Thesis). University of Turku, (2014).

17. R. Muthu, R. Visnupriya, S. Jeyarajan Nelson, D. Uma, V.P. Santana Krishnan, Insecticidal activity and changes in midgut histology of the generalist herbivore , Spodoptera litura F. (Lepidoptera : Noctuidae) in response to seed extract of Annona squamosa Linn. J. of Applied and Natural Science 15, 1 (2023). https://doi.org/10.31018/jans.v15i1.4371
18. S. Denecke, L. Swevers, V. Douris, J. Vontas, How do oral insecticidal compounds cross the insect midgut epithelium? Insect Biochem. Mol. Biol. **103,** 22-35 (2018). https://doi.org/10.1016/j.ibmb.2018.10.005.
19. A.V. Shubin, I.V. Demidyuk, A.A. Komissarov, L.M. Rafieva, S.V. Kostrov, Cytoplasmic vacuolization in cell death and survival. Oncotarget. **7,** 55863-55889 (2016). https://doi.org/10.18632/oncotarget.10150.
20. M. Mishra, K.K. Gupta, S. Kumar, Impact of the stem extract of *Thevetia neriifolia* on the feeding potential and histological architecture of the midgut epithelial tissue of early fourth instars of *Helicoverpa armigera* Hubner. International Journal of Insect Science, **7**, 53-60 (2015). https://doi:10.4137/IJIs.s29127