RESEARCH ARTICLE Indian Journal of Agricultural Research, Volume 56 Issue 6: 741-745 (December 2022)

The Bio-Insecticidal Activity of Papaya (*Carica papaya* L.) Leaves Extract against *Spodoptera litura* Fabr. (Lepidoptera: Noctuidae) Larval Growth

Sofia Ery Rahayu[1,4], Amin Setyo Leksono[2], Zulfaidah Penata Gama[2], Hagus Tarno[3]

10.18805/IJARe.AF-747

ABSTRACT

Background: Papaya leaves produce several metabolites that may be toxic to an insect pest. In East Java, it is easy to find two cultivars in the yard, plantation, and moors, such as Thailand and local papaya. However, information about their utilization as bio-pesticides remains limited. This study aimed to investigate the relationship between those papaya cultivars and the effect of their extracts on *Spodoptera litura* larvae.

Methods: The Thailand papaya cultivar (TPC) and Indonesian papaya cultivar (IPC) leaves were obtained from the medium and lowlands and were macerated with methanol until obtained a papaya leaves extract (PLE). The *S. litura* larvae on the instar phase 2 (*n= 630*) were used for treatment. The *Ricinus communis* leaves were sprayed with PLE concentrations of 0, 10, 20, 30, 40 and 50% and fed to *S. litura* larvae.

Result: Based on the secondary metabolites, the papaya cultivars form two clusters associated with its origin. The 40 and 50% concentrations of PLE significantly affect the mortality and head-capsule width of *S. litura* indicated with broken pupae and failure to transform to imago. This result signifies the potential of papaya leaves extract as bioinsecticide for *S. litura* larvae.

Key words: Bioinsecticide, Head-capsule width, Mortality, Papaya leaves, *S. litura* larvae.

INTRODUCTION

Spodoptera litura is a notorious leaf-feeding insect pest of many economically valuable crops in the Asia-Pacific region. The *S. litura* larvae attack more than 122 host plant species, including cotton, corn and soybean. The *S. litura* attack may cause leaf and pod damage and lead to a 68% reduction in soybean production (Ayudya *et al*., 2019; Motaphale *et al*., 2017). In Indonesia, *S. litura* has been distributed in 22 provinces and is reported to attack 11,163 ha/year, including soybean and mung bean plants (Melani *et al*., 2016; Indiati *et al*., 2017).

Most farmers apply chemical insecticide to control *S. litura*. However, chemical insecticide carries negative impacts, such as inducing pest resistance and population outbreak (Nandhakumar *et al*., 2020). Consequently, natural pesticides from plants can be used as an alternative pest control because they are more environmentally friendly character than those of chemical sources.

Indonesia as a tropical country has highly diverse plants used as natural pesticides. One of those plants is papaya (*Carica papaya* L.), which is massively cultivated in medium and lowlands in East Java, Indonesia. Papaya has many cultivars, such as the IPC and TPC, with the IPC having the purple petiole as its main characteristic. The previous studies demonstrated that IPC and TPC have different active compounds, including phenols, alkaloids and flavonoids that might be beneficial for inhibiting the growth and development of insects (Matsuura and Fett-Neto 2015; Aihetasham *et al*., 2017; Rahayu *et al*., 2020).

[1]Doctoral Program, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Brawijaya, Malang-65145, East Java, Indonesia.
[2]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Brawijaya, Malang-65145, East Java, Indonesia.
[3]Department Plant and Disease, Faculty of Agriculture, Universitas Brawijaya, Malang-65145, East Java, Indonesia.
[4]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Malang, Malang-65145, East Java, Indonesia.

Corresponding Author: Sofia Ery Rahayu, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Malang, Malang 65145, East Java, Indonesia.
Email: sofia.ery.fmipa@um.ac.id

How to cite this article: Rahayu, S.E., Leksono, A.S., Gama, Z.P. and Tarno, H. (2022). The Bio-Insecticidal Activity of Papaya (*Carica papaya* L.) Leaves Extract against *Spodoptera litura* Fabr. (Lepidoptera: Noctuidae) Larval Growth. Indian Journal of Agricultural Research. 56(6): 741-745. DOI: 10.18805/IJARe.AF-747.

Submitted: 20-06-2022 **Accepted:** 08-09-2022 **Online:** 20-09-2022

In addition, investigating the variety between family Caricaceae members mainly focused on its genetic features and morphology. However, the variety and relationship between the TPC and IPC based on their active compounds from different elevations have not been examined. Besides, there is lack of evidence about comparing the effectiveness of both PLE on the development and growth of *S. litura*. Therefore, this study focuses on investigating the relationship between IPC and TPC based on their active compounds, different elevations and PLE effectiveness in the development of *S. litura* larvae. Furthermore, this study aims to discover the variety and potential of that cultivar as a natural pesticide.

MATERIALS AND METHODS

Plant materials

The extract was prepared from the IPC and TPC leaves obtained from Malang and Nganjuk, East Java, Indonesia. The lowland was selected at Nganjuk at 70 m above sea level (S 07036.560′E 112001.290′), while the medium land was selected at Malang at 414 m above sea level (S 08005. 551′E 112041.175′). The leaves were washed, dried and mashed using a dry blender.

Preparation of papaya leaf extract

Papaya leaf simplicial was extracted using the maceration method with methanol solvent at a 1:10 ratio. The homogenate obtained was filtered using Whatman paper, and the residue was macerated again for 24 hours. The filtered results were mixed and concentrated by rotary evaporator. The PLE was stored at 4°C. The phytochemical screening was carried out using liquid chromatography mass spectrometry (LCMS).

Development and growth test of *S. litura* larvae

The experiment was conducted in the Department of Biology, Universitas Negeri Malang during June 2018 until June 2019. *S. litura* instar 2^{nd} larva was left starving for 4 hours and placed in the *R. communis* leaves for four days that have sprayed with 0% (control), 10%, 20%, 40% and 50% PLE of IPC and TPC. After that, the larva was given fresh *R. communis* leaves with no extract. Each extract concentration was tested into ten larvae three times, and each larva was maintained individually (*n=630 larvae*). The larvae mortality was examined from 24-96 hours post-treatment. Each of the larvae head-capsule widths was measured. The survived larvae were taken care of until they grew into a pupa. The *S. litura* pupa formed in 24 hours was weighted and observed (died or alive). The pupa was placed into a plastic glass filled with sterilized sand. They were investigated until they became imago. The hatched imago's morphology was also examined and counted.

Data analysis

The active compounds of PLE were analyzed descriptively to construct a dendrogram using Bray-Curtis analysis. The larvae head-capsule width, pupa weight and condition were analyzed using ANOVA ($p<0.05$) followed by the Duncan test if a palpable different analysis result was obtained.

RESULTS AND DISCUSSION

Dendrogram of TPC and IPC

The phytochemical screening using LCMS demonstrated that there were 62 compounds founds on TPC, while 60 were found on IPC. In comparison, two compounds were only detected on the TPC than IPC, such as p-coumaric acid and 5,7 Dimethoxycoumarin. In contrast, the IPC has four unique compounds found than TPC, including phellandrene, myrcene, linalool oxide, and caryophyllene. Similarly, a previous study reveals that the active compounds in those two cultivars differed (Rahayu *et al*., 2020). Carpaine, pseudocarpaine, dehydrocarpaine I and II, quercetin, kaempferol, luteolin-7-glucoside, isoquercitrin, gallic acid, and benzyl glucosinolate are found with a high abundance in the PLE. These similar and different compounds obtained were used to construct a dendrogram to investigate the relationship between those cultivars, as presented in Fig 1.

IPC and TPC obtained from medium areas form one cluster. The obtained similarity value from the two clusters is relatively high (95.25%). The results of Bay-Curtis analysis on the different and similar active compounds content of the PLE from IPC and TPC showed values closer to 100%, indicating their closer kinship. Further, a dendrogram constructed from the data shows that those two cultivars form

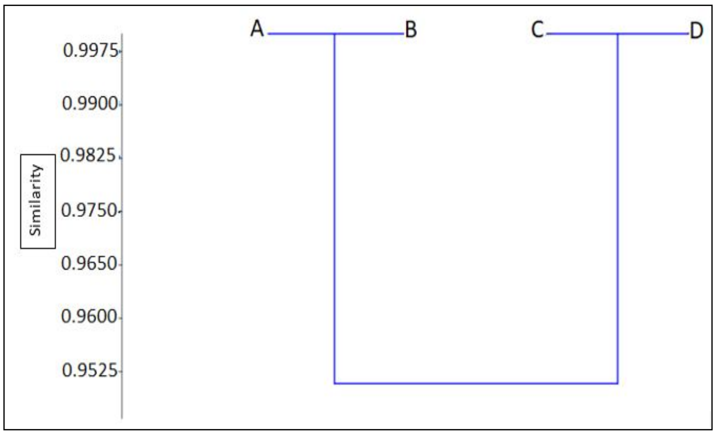

Fig 1: Dendrogram of two papaya cultivars from different height habitats based on their active compounds. A= Thailand cultivar from medium height land, B= Thailand cultivar from low land, C= Indonesia cultivar from medium land, D= Indonesia cultivar from low land.

two clusters based on its country's origin. This high similarity was observed because they are from the same species of *C. papaya*, although there were from different cultivars.

The Genus *Carica* is monotypic and only consists of one species, namely *C. papaya*, so the IPC and TPC formed one cluster in the dendrogram. Meanwhile, the morphology analysis shows that papaya has phenotypic variation in its morphological characteristics and plant stature (de Oliveira *et al*., 2010).

Larval mortality of *S. litura*

The results suggest that the treatment of PLE from IPC and TPC was not significantly different. However, the different concentrations affect the mortality of *S. litura* larvae. The PLE 40% and 50% have significantly different mortality percentages among other concentrations. More prolonged exposure to PLE results in more larvae deaths. Similarly, a higher concentration also presents a higher mortality rate of *S. litura*, as shown in Table 1.

PLE from IPC and TPC affects the mortality of *S. litura* instar 2. The highest mortality rate of *S. litura* larvae obtained in this study is below 50% (Table 1). In contrast, antifeedant test results on the *S. litura* larvae produce a high antifeedant index, 97% and 94%, respectively. Meanwhile, PLE 50% has a 100% antifeedant index (Rahayu *et al*., 2020). This result implies that PLE from both cultivars may act as an antifeedant. An ethanolic PLE suggested a high repellent activity against German cockroaches (Rahayu *et al*., 2020) or *Tribolium castaneum* (Tatun *et al*., 2014). However, the PLE effect might depend on their active compounds' abundance.

Table 1: The mortality of *S. litura* larvae after being treated by the various Papaya leaves extract concentrations.

The concentration of papaya leaves extract (%)	Mortality (%) of *S. litura* larvae after treatment	
	72 hours	96 hours
0	0.000^{a}±0.000	0.000^{a}±0.000
10	4.167^{a}±6.686	4.167^{ab}±6.686
20	3.333^{a}±4.924	6.667^{b}±6.513
30	5.000^{a}±7.977	7.500^{b}±8.660
40	16.667^{b}±12.309	22.500^{c}±13.658
50	20.833^{b}±16.214	24.167^{c}±18.809

Note: Mean followed by the same letter did not differ significantly using the Duncan test, $p<0.05$.

Development and growth of *S. litura* larvae

S. litura larvae were treated with PLE while their growth and development were observed. The growth and development indicator used was the instar larvae head-capsule width. The results showed that the larvae head-capsule width in the instar II and III treated with PLE differs from the control group (Table 2). Meanwhile, the instar IV larvae head-capsule width presents no substantial differences in all concentrations. However, in the instar V phase, the *S. litura* larvae with PLE 40% and 50% have tighter and significantly different head-capsule widths than the other concentration.

PLE of IPC and TPC contains active compounds that affect the growth and development of *S. litura* larvae. The larvae head-capsule width was used to indicate its growth and development. The larvae head-capsule width continuously grows with no expanding size in the same instar phase. Food quality substantially affects insect larval growth (Delbac *et al*., 2010), including the head-capsule width (Montezano *et al*., 2014).

The *S. litura* head-capsule width measurement indicates that the older larvae have bigger head sizes with huge cephalic and robust mandibular muscles, affecting the effectiveness of the eating strategy (Calvo and Molina 2008). PLE resulted in 2.6-2.7 mm head-capsule width on the last instar (instar 5) of *S. litura*. Similar to this result, the head-capsule width of *Spodoptera* larvae had 2.5-2.7 mm head-width (Montezano *et al*., 2014). However, the results showed that the PLE 40% and 50% generate significantly different results on the *S. litura* larvae head-capsule width. PLE produces larvae with varying head-capsule widths due to toxic active compounds, reducing the food quality for the *S. litura* larvae.

The weight of *S. litura* pupae have no difference between the control group and other groups treated with different PLE concentrations (Table 3). The average weight of the produced *S.litura* pupa was 0.30-0.34 grams. Another study also discovered similar results revealing that *Spodoptera* pupa weight is 0.3-0.4 grams (Montezano *et al*., 2014).

The average number of damaged pupae from the larvae treated with PLE 0% was substantially different from the

Table 2: The head-capsule width of *S. litura* larvae was treated by the various concentrations of Papaya leaves extract.

The concentration of papaya leaf extract (%)	Head-capsule width (mm) of S. *litura* larvae			
	2^{nd} instar	3^{rd} instar	4^{th} instar	5^{th} instar
0	0.484^{c}±0.043	0.73^{d}±0.010	1.574^{a}±0.031	2.738^{b}±0.060
10	0.472^{bc}±0.015	0.701^{c}±0.019	1.643^{a}±0.095	2.714^{b}±0.057
20	0.462^{ab}±0.014	0.699^{c}±0.027	1.631^{a}±0.790	2.692^{b}±0.087
30	0.454^{ab}±0.016	0.698^{c}±0.018	1.632^{a}±0.121	2.678^{b}±0.092
40	0.446^{a}±0.018	0.678^{b}±0.021	1.565^{a}±0.160	2.664^{a}±0.107
50	0.439^{a}±0.022	0.664^{a}±0.035	1.605^{a}±0.125	2.604^{a}±0.133

Note: Mean followed by the same letter did not differ significantly using the Duncan test, $p<0.05$.

Table 3: Weight of *S. litura* pupae after treated by various concentrations of Papaya leaves extract.

The concentration of papaya leaf extract (%)	Pupa weight (g) of *S. litura* after 24 hours pupation
0	0.3403^{a}±0.0702
10	0.3230^{a}±0.0665
20	0.3116^{a}±0.0598
30	0.3115^{a}±0.0758
40	0.3177^{a}±0.0590
50	0.3144^{a}±0.0571

Note: Mean followed by the same letter did not differ significantly using the Duncan test, p<0.05.

Table 4: The average number of pupae *S. litura* damaged and fails to hatch into an imago.

Papaya leaf extract concentration (%)	Damaged pupa average (%)	Average of pupa that failed to hatch into Imago (%)
0	0.707^{a}±0.000	0.707^{a}±0.000
10	1.481^{b}±0.249	1.439^{b}±0.537
20	1.512^{b}±0.482	1.490^{b}±0.462
30	1.424^{b}±0.392	1.518^{b}±0.350
40	1.315^{b}±0.623	1.721^{b}±0.473
50	1.210^{b}±0.469	1.337^{b}±0.481

Note: Mean followed by the same letter did not differ significantly using the Duncan test, p<0.05.

other groups treated with different PLE concentrations ($p<0.05$). Similar results have been observed in the number of pupae that failed to hatch into imago from the control group treated with PLE 0%, as shown in Table 4. Active compounds possibly caused the inability of the pupa to hatch in PLE, which affects the metabolism of *S. litura*. PLE contained flavonoids, alkaloids, phenols, saponins, and tannins (Rahayu *et al*., 2020).

PLE carries antifeedant function toward *S. litura* larvae (Rahayu *et al*. 2020), which might interrupt the physiological function or become toxic for insects, inhibiting insect growth (Kilani-Morakchi *et al*., 2021). The active compounds in the PLE might work through the food to inhibit the growth and development of *S. litura*. Flavonoids disturb the essential enzymatic reaction for insect development. Besides, a flavonoid also decreases the plants' tissue palatability toward herbivorous insects (Marques *et al*., 2016). A previous study showed that quercetin decreased the percentage of *S. litura* pupa formation (Rashwan and Hammad 2020). Meanwhile, the phenolic compound could damage the cell in its midgut. Meanwhile, tannin reduces food digestibility by binding the salivary proteins and digestive enzymes, including trypsin and chymotrypsin, to disrupt protein absorption (Rashwan and Hammad 2020).

In addition, the alkaloid compound is toxic for *S. litura*. Alkaloid has a bitter insect taste, decreasing the larvae appetite and leading to malnutrition(Matsuura and Fett-Neto 2015). Alkaloids disturbed the hormonal balance and chitin synthesis mechanism resulting in a smaller cuticle weight and an incomplete of their outer frame of the exoskeleton (Ge *et al*., 2015; Ileke and Ogungbite 2014). Alkaloids also hinder α-amylase and protease enzymes which in turn disturbing the metabolism of protein, resulting in a lack of amino acid production that is essential for the growth and development of the larvae (Zou *et al*., 2019; Sharma 2019).

The shape and size changes occur during the larvae growing process, involving the cell growth control factor and synthesis process. Ecdysteroids and insulin-like peptides (ILPs) are the growth generator in insects. Disruption of the insulin signaling causes cell size and number changes within the adult insect organs (Lin and Smagghe 2019). The observable indicators in this study are the bend and smaller wings detected in the treatment groups. Therefore, the results suggested that the PLE from the IPC and TPC might be a biopesticide toward the *S. litura* larvae by disrupting its growth and development process.

CONCLUSION

Based on its active compounds, papaya cultivars form two clusters associated with the country's origin. PLE from IPC and TPC carries the same effects on the *S. litura* larvae mortality and development. The highest *S. litura* larvae mortality rate and the smallest head capsule width was observed from the treatment using PLE 40% and 50%. Besides, PLE has no effects on the *S. litura* pupa weight but affects pupal growth and development.

Conflict of interest: None.

REFERENCES

Aihetasham, A., Rasib, K.Z., Hasan, S.R. and Bodlah, I. (2017). Effect of *Carica papaya*, *Helianthus annus* and *Bougainvillea glabraaqueous* extracts against termite, *Heterotermes indicola* (Isoptera:Rhinotermitidae). Punjab University Journal of Zoology. 32(1): 51-56.

Ayudya, D.R., Herlinda, S. and Suwandi, S. (2019). Insecticidal activity of culture filtrates from liquid medium of *Beauveria bassiana* isolates from South Sumatra (Indonesia) wetland soil against larvae of *Spodoptera litura*. Biodiversitas Journal of Biological Diversity. 20(8): 2101-2109.

Calvo, D. and Molina, J.Ma. (2008). Head capsule width and instar determination for larvae of *Streblote panda* (Lepidoptera: Lasiocampidae). Annals of the Entomological Society of America. 101(5): 881-886.

Delbac, L., Lecharpentier, P. and Thiery, D. (2010). Larval instars determination for the European Grapevine Moth (Lepidoptera: Tortricidae) based on the frequency distribution of head-capsule widths. Crop Protection. 29(6): 623-630.

Ge, Y., Liu, P., Yang, R., Zhang, L., Chen, H., Camara, I., Liu, Y. and Shi, W. (2015). Insecticidal constituents and activity of alkaloids from *Cynanchum mongolicum*. Molecules. 20(9): 17483-17492.

Ileke, K.D. and Ogungbite, O.C. (2014). Entomocidal activity of powders and extracts of four medicinal plants against *Sitophilus oryzae* (L), *Oryzaephilus mercator* (Faur) and *Ryzopertha dominica* (Fabr.). Jordan Journal of Biological Sciences. 7(1): 57-62.

Indiati, S.W., Bejo, B. and Rahayu, M. (2017). Diversity of mung bean insect pests and their natural enemies in farmers' fields in East Java, Indonesia. Biodiversitas Journal of Biological Diversity. 18(4): 1300-1307.

Kilani-Morakchi, S., Morakchi-Goudjil, H. and Sifi, K. (2021). Azadirachtin-Based Insecticide: Overview, Risk Assessments and Future Directions. Frontiers in Agronomy. 3: 676208.

Lin, X. and Smagghe, G. (2019). Roles of the insulin signaling pathway in insect development and organ growth. Peptides. 122: 169923.

Marques, T.R., Caetano, A.A., Alves, D.S., Ramos, V. de O., Simão, A.A., Carvalho, G.A. and Corrêa, A.D. (2016). *Malpighia emarginata* DC. bagasse acetone extract: Phenolic compounds and their effecton *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae). Chilean Journal of Agricultural Research. 76(1): 55-61.

Matsuura, H.N. and Fett-Neto, A.G. (2015). Plant Alkaloids: Main Features, Toxicity and Mechanisms of Action. In: Plant Toxins. [Gopalakrishnakone, P., Carlini, C.R. and Ligabue-Braun, R., eds.]. Dordrecht: Springer Netherlands. pp.1-15.

Melani, D., Himawan, T. and Afandhi, A. (2016). Bioactivity of sweet flag (*Acorus calamus* Linnaeus) essential oils against *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae). Journal of Tropical Life Science. 6(2): 86-90.

Montezano, D.G., Specht, A., Sosa-Gómez, D.R., Roque-Specht, V.F. and de Barros, N.M. (2014). Immature stages of *Spodoptera eridania* (Lepidoptera: Noctuidae): Developmental Parameters and Host Plants. Journal of Insect Science. 14(1): 1-11.

Motaphale, A.A., Bhosle, B.B. and Bawaskar, D.M. (2017). Efficacy and economics of different insecticides and bio-rational against *Spodoptera litura* (Fabr.) in Soybean. Legume Research. 41(6): 930-933.

Nandhakumar, P., Vasantha, V.S., Veilumuthu, P. and Christopher, G. (2020). 7, 8- Dihydroxyflavone, An effective natural product reduce ralstonia solanacearum populations and control tomato bacterial wilt. Indian Journal of Agricultural Research. 54(6): 731-737.

de Oliveira, E.J., Amorim, V.B.O., Matos, E.L.S., Costa, J.L., da Silva Castellen, M., Pádua, J.G. and Dantas, J.L.L. (2010). Polymorphism of microsatellite markers in papaya (*Carica papaya* L.). Plant Molecular Biology Reporter. 28(3): 519-530.

Rahayu, S.E., Leksono, A.S., Gama, Z.P. and Tarno, H. (2020). The Active Compounds Composition and Antifeedant Activity of Leaf Extract of Two Cultivar *Carica papaya* L. on *Spodoptera litura* F. larvae. In: Recent Trends in Life Sciences for Local Resource Development. Malang, Indonesia: AIP Conference Proceedings. 040085.

Rahayu, T., Darmis, A., Jannatan, R. (2020). Potency of papaya leaf (*Carica papaya* L.) as toxicant and repellent against German cockroach (*Blatella germanica*). Pakistan Journal of Biological Sciences. 23(2): 126-131.

Rashwan, R.S. and Hammad, D.M. (2020). Toxic effect of *Spirulina platensis* and *Sargassum vulgar* as natural pesticides on survival and biological characteristics of cotton leaf worm *Spodoptera littoralis*. Scientific African. 8: e00323.

Sharma, K. (2019). Purification and characterization of protease inhibitors from rice bean (*Vigna umbellata*) and their efficacy against *Spodoptera litura* (Fabricius) gut proteinases. Indian Journal of Agricultural Research. 53(5): 513-521.

Tatun, N., Vajarasathira, B., Tungjitwitayakul, J. and Sakurai, S. (2014). Inhibitory effects of plant extracts on growth, development and α-amylase activity in the red flour beetle *Tribolium castaneum* (Coleoptera: Tenebrionidae). European Journal of Entomology. 111(2): 181-188.

Zou, C., Wang, Y., Zou, H., Ding, N., Geng, N., Cao, C. and Zhang, G. (2019). Sanguinarine in *Chelidonium majus* induced antifeeding and larval lethality by suppressing food intake and digestive enzymes in *Lymantria dispar*. Pesticide Biochemistry and Physiology. 153: 9-16.