Insecticidal Activity of Crude Flower Stalk Extract of Forest Clove (*Syzygium aromaticum* L.) Against Larvae of Asian Armyworm (*Spodoptera litura* F.) (Lepidoptera: Noctuidae)

Misda Alberto Senen[1)], Johanna Audrey Leatemia[1,2*)], Betty Sahetapy[1,2)]

[1)]Study Program of Agricultural Sciences, Postgraduate Program Pattimura University. Jl. Ir. M. Putuhena, Kampus Poka, Ambon 97233
[2)]Study Program of Agrotechnology, Faculty of Agriculture, Pattimura University. Jl. Ir. M. Putuhena, Kampus Poka, Ambon 972332
*Corresponding: jaudreyleatemia@gmail.com

ABSTRACT

The intensive and prolonged use of synthetic insecticides in agriculture has resulted in multiple adverse effects, including pest resistance and resurgence, reduction of natural enemy populations, and risks to environmental and human health. Botanical insecticides offer a promising alternative for more sustainable pest control. This study evaluated the insecticidal activity of crude ethanolic extract from flower stalks of forest clove (*Syzygium aromaticum*) against the tropical armyworm, *Spodoptera litura* (Lepidoptera: Noctuidae). Laboratory bioassays were conducted to determine larval mortality and lethal concentration of 50% (LC_{50}) at 48 hours after treatment, while plastic house experiments assessed the extract's efficacy on *S. litura* infesting mustard plants. Chemical composition of the extract was analyzed using gas chromatography–mass spectrometry (GC–MS). The extract contained four major classes of secondary metabolites—terpenoids, phenolics, flavonoids, and alkaloids—with flavonoids as the dominant constituent (21.48%). Laboratory bioassays yielded an LC_{50} value of 1.5% (w/v). In plastic house trials, larval mortality reached 100% within 96 hours at 1.5% and 3% concentrations, and within 72 hours at 6%. These findings indicate that the crude extract of forest clove flower stalks possesses potent insecticidal activity and has strong potential for development as a botanical insecticide that may contribute to more environmentally compatible pest management strategies.

Keywords: *Syzygium aromaticum*; crude extract; botanical insecticide; *Spodopteralitura*; tropical armyworm

Aktivitas Insektisida Ekstrak Kasar Tangkai Bunga Cengkeh Hutan (*Syzygium aromaticum* L.) terhadap Larva Ulat Grayak (*Spodoptera litura* F.) (Lepidoptera: Noctuidae)

Penggunaan insektisida sintetis secara intensif dan berkepanjangan dalam pertanian telah menimbulkan berbagai dampak negatif, antara lain resistensi dan resurgensi hama, penurunan populasi musuh alami, serta risiko terhadap kesehatan lingkungan dan manusia. Insektisida nabati menawarkan alternatif yang menjanjikan untuk pengendalian hama yang lebih berkelanjutan. Penelitian ini mengevaluasi aktivitas insektisida ekstrak kasar etanol dari tangkai bunga cengkeh hutan (*Syzygium aromaticum*) terhadap ulat grayak (*Spodoptera litura*; Lepidoptera: Noctuidae). Uji bioasai laboratorium dilakukan untuk menentukan mortalitas larva

AGROLOGIA, Vol. 14, No. 2, pp. 168-175
e-ISSN 2580-9636
DOI: http://dx.doi.org/10.30598/ajibt.v14i2.19816

dan konsentrasi mematikan 50% (LC_{50}) pada 48 jam setelah aplikasi, sedangkan percobaan di rumah plastik digunakan untuk menilai efektivitas ekstrak terhadap *S. litura* pada tanaman sawi. Komposisi kimia ekstrak dianalisis menggunakan kromatografi gas–spektrometri massa (GC–MS). Ekstrak mengandung empat kelas utama metabolit sekunder, yaitu terpenoid, fenolik, flavonoid, dan alkaloid, dengan flavonoid sebagai senyawa dominan (21,48%). Uji bioasai di laboratorium menghasilkan nilai LC_{50} sebesar 1,5% (b/v). Dalam percobaan rumah plastik, mortalitas larva mencapai 100% dalam waktu 96 jam pada konsentrasi 1,5% dan 3%, serta dalam 72 jam pada konsentrasi 6%. Hasil ini menunjukkan bahwa ekstrak kasar tangkai bunga cengkeh hutan memiliki aktivitas insektisida yang kuat dan berpotensi besar untuk dikembangkan sebagai insektisida nabati yang lebih ramah lingkungan bagi pengelolaan hama secara berkelanjutan.

Kata kunci: *Syzygium aromaticum*; ekstrak kasar; insektisida nabati; *Spodoptera litura*; ulat grayak

INTRODUCTION

The continuous use of synthetic insecticides in agriculture has led to various negative consequences, including ecological imbalances resulting from the loss of natural enemies, the development of pest resistance and resurgence, and potential health risks to humans [1]. These concerns underscore the urgent need for safer, more environmentally friendly, and sustainable alternatives for pest control. One promising strategy is the use of botanical insecticides derived from plants that produce bioactive secondary metabolites.

Secondary metabolites such as alkaloids, flavonoids, terpenoids, saponins, and phenolics function as repellents, antifeedants, growth regulators, or toxins against insect pests [2]. Botanical insecticides are generally biodegradable, less harmful to non-target organisms, and can help reduce reliance on synthetic chemicals. Several plant families, including Asteraceae, Meliaceae, Lamiaceae, Rutaceae, and Myrtaceae, are known to produce bioactive compounds with potent insecticidal activity [3].

Within the Myrtaceae family, clove (*Syzygium aromaticum* L.) has shown considerable potential as a botanical insecticide, largely due to its high eugenol content, which may comprise up to 90% of its essential oil [4]. In addition, flavonoids and tannins in clove leaves have been associated with toxic and antifeedant effects [5]. Previous studies have reported that clove extracts caused 66.75% mortality in *Spodoptera litura* at a 10% concentration [6], over 50% mortality in *Nezara viridula* and *Callosobruchus chinensis* [7], and an LC_{50} of 0.21% against *S. litura* larvae [8].

Ecologically and historically, clove is an endemic plant of Maluku and holds high economic importance [9]. A lesser-known local variety, the forest clove (*S. aromaticum*), grows naturally in Maluku, North Maluku, and Papua. It has been cultivated in Ambon and Seram islands [10]. The flower stalks, often discarded as postharvest waste, represent a potentially valuable source of raw material for botanical insecticide development.

The Asian armyworm, *Spodoptera litura* F. (Lepidoptera: Noctuidae), is a major pest of numerous food and horticultural crops, infesting more than 40 host species, including mustard greens (*Brassica juncea* L.) [11]. Severe infestations can cause yield losses of up to 80% [12], underscoring the need for effective yet environmentally sound pest management strategies.

Therefore, the objective of this study was to evaluate the insecticidal activity of the crude ethanolic extract of forest clove flower

stalks against *S. litura* larvae and to identify its active compounds using gas chromatography–mass spectrometry (GC–MS).

MATERIALS AND METHODS

Plant extracts.

Flower stalks of forest clove (*S. aromaticum*) were collected from several trees in Hitu Village, Ambon Island. The stalks were air-dried, ground using a coffee grinder, and sieved to obtain a uniform powder. A total of 100 g of powdered material was extracted with 96% ethanol (4 × 250 mL) for four days by maceration with continuous stirring on a magnetic stirrer. The extract was vacuum filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator. The resulting crude ethanolic extract was dried and stored for subsequent bioassays and chemical analysis.

Insects

The Asian armyworm, *S. litura* F. (Lepidoptera: Noctuidae), used in this study was obtained from a laboratory culture maintained on mustard plants (*B. juncea* L.). The original colony was collected from the field in Ambon Island and maintained in the Laboratory of Plant Pest, Faculty of Agriculture, Pattimura University, under ambient room temperature conditions. The second and third generations of larvae were used for the experiments.

Leaf Spray Bioassay

This experiment was conducted to evaluate the insecticidal activity of the forest clove (*S. aromaticum*) extract against third-instar larvae of the tropical armyworm (*S. litura*). Five concentrations of aqueous emulsions of the ethanolic extract, 2.5%, 5.0%, 7.5%, 10.0%, and 12.5% (w/v), were tested. A commercial liquid dishwashing detergent (Sunlight®, Unilever Indonesia Tbk., Cikarang, West Java; containing linear alkylbenzene sulfonate) at 0.05% (w/v) was used as an emulsifier.

Fresh mustard (*B. juncea*) leaves were sprayed to runoff (approximately 15 mL per leaf) using 330 mL plastic bottle sprayers. Control leaves were sprayed with distilled water containing 0.05% (w/v) of the same detergent solution. Each treated leaf was placed individually in a 650 mL rectangular plastic cup, and the petiole was wrapped in moistened cotton to prevent desiccation.

Ten third-instar larvae were released onto each treated leaf, with three replicates (cups) per treatment. The experiment followed a Completely Randomized Design (CRD) and was maintained under ambient room temperature. Observations on larval behavioral symptoms were made at 2, 4, and 6 hours after treatment (HAT), while larval mortality was recorded at 24, 48, and 72 HAT.

Efficacy of Crude Extracts in the Plastic House

Experiments were conducted in a plastic house at the Faculty of Agriculture, Pattimura University, to evaluate the efficacy of an aqueous emulsion of the crude ethanolic flower stalk extract of forest clove (*S. aromaticum*) against larvae of the Asian armyworm (*S. litura*). Mustard (*B juncea* L. var. St) plants were grown in plastic pots (30 cm in diameter) containing a soil-to-manure mixture (2:1). Plants were watered as needed and fertilized with liquid organic fertilizer (Supermes®; PT Jenawi Suburindo Rejeki, Jakarta, Indonesia). Four-week-old plants were used for the experiment.

The aqueous emulsion of crude extract was tested at concentrations of 1.5, 3, and 6% (w/v), with Sunlight® liquid dishwashing detergent (0.05% w/v; Unilever Indonesia Tbk., Cikarang, West Java) added as an emulsifier. Water plus detergent alone served as a control, and 0.15% abamectin (Abacel® 18 EC; PT Excel Meg Indo, Jakarta, Indonesia) was used as a positive control. Treatments were arranged in a randomized block design with three blocks and three

AGROLOGIA, Vol. 14, No. 2, pp. 168-175
e-ISSN 2580-9636
DOI: http://dx.doi.org/10.30598/ajibt.v14i2.19816

plants per treatment per block. Spacing between treatments and between blocks was maintained at 50 cm and 100 cm, respectively (Figure 1).

Each plant was infested with ten third-instar *S. litura* larvae from the laboratory colony and enclosed with gauze (Figure 1). Plants were sprayed with one of the test concentrations or control solutions four hours after infestation using 330 ml plastic spray bottles until runoff (approximately 100 ml per plant). The number of surviving larvae was recorded at 1, 2, and 3 days after treatment (DAT). Differences between the initial and remaining larvae were assumed to represent larval mortality.

Figure 1. Experimental set up in the plastic house showing mustard (*B. juncea* L.) plants infested with *S. litura* larvae and covered with gauze. Treatments included the control, positive control (abamectin 0.15%), and crude ethanolic extracts of forest clove (*S. aromaticum*) flower stalks at concentrations of 1.5%, 3%, and 6% (w/v).

Phytochemical Analysis of the Extract

The crude ethanolic flower stalk extract of forest clove (*S. aromaticum*) was analyzed for its secondary metabolite composition at the Regional Health Laboratory of the Special Capital Region of Jakarta. The analysis was conducted using the gas chromatography–mass spectrometry (GC–MS) method following the laboratory's standard operating procedures.

Data Analysis

The lethal concentration causing 50% larval mortality (LC_{50}) was estimated using probit analysis [13]. Percentage mortality data were arcsine-transformed before analysis of variance (ANOVA). Differences among treatment means were determined using the Least Significant Difference (LSD) test [14]. The actual observed (untransformed) values are presented in the Results section.

RESULTS

Leaf Spray Bioassay

The effects of the forest clove flower stalk extract on *S. litura* larvae were evaluated through early symptoms and feeding behavior under controlled conditions. Within 2 hours after treatment, most larvae remained active and fed normally, though some showed reduced feeding. After 4 hours, several larvae began avoiding treated leaves. By 6 hours, larvae that continued feeding appeared weak and less responsive, while others avoided the treated area.

Larval mortality increased over time at all concentrations tested and also showed a dose-dependent increase at each exposure period, as shown in Table 1.

AGROLOGIA, Vol. 14, No. 2, pp. 168-175
e-ISSN 2580-9636
DOI: http://dx.doi.org/10.30598/ajibt.v14i2.19816

Table 1. Mortality of *S. litura* F. larvae treated with aqueous emulsion of ethanolic extract of forest clove flower stalks under laboratory conditions

Treatment	Cumulative Mortality (%) ± SE		
	24 HAT*	48 HAT	72 HAT
2,5 (% w/v)	13.33 ±0.57a**	63.33 ±0.57 a	100.00 ±0.00a
5	20.00 ±0.00ab	73.33 ±0.57 ab	100.00 ±0.00a
7,5	23.33 ±0.57 ab	83.33 ±0.57 bc	100.00 ±0.00a
10	26.67 ±0.57 bc	93.33 ±1.15 cd	100.00 ±0.00a
12.5	36.67 ±0.57 c	100.00 ±0.00 d	100.00 ±0.00a
Control	0.00	0.00	0.00

*HAT (hour After Treatment)
**** Means followed by the same letter within a column are not significantly different (p > 0.05) based on the Least Significant Difference (LSD) test.

Probit analysis of the larval mortality data in Table 1 indicated an LC_{50} of 1.5% (w/v) at 48 hours after treatment (HAT). This value was used to guide the selection of the three concentrations applied in the subsequent plastic house experiment.

Efficacy in Plastic House

Larval mortality of *S. litura* increased with both exposure time and extract concentration (Table 2), showing a clear dose–response pattern.

Table 2. Mortality of *S. litura* F. larvae treated with aqueous emulsion of ethanolic extract of forest clove flower stalks under plastic house conditions.

Treatment	Cumulative mortality (%) ± SE			
	1 DAT*	2 DAT	3 DAT	4 DAT
1,5 (%w/v)	18.89±0.78a**	52.23±0,66 a	81.11 ±0,70a	100.00±0.00a
3	23.33±0.70ab	64.67±0.52ab	93.67 ±0,78b	100.00±0.00a
6	31.11±0.78b	70.00±0.83b	100.00 ±0.00c	100.00±0.00a
0,15% abamectin	43.33±1.00c	100.00±0.00c	100.00 ±0.00c	100.00±0.00a
Control	0.00	0.00	0.00	0.00

*DAT (Day After Treatment)
** Means followed by the same letter within a column are not significantly different (p > 0.05) based on the Least Significant Difference (LSD) test.

Phytochemical Composition of the Extract

Gas chromatography–mass spectrometry (GC–MS) analysis of the crude ethanolic extract of forest clove flower stalks identified four major classes of secondary metabolites. Flavonoids were the most abundant, dominated by 4H-1-Benzopyran-4-one, 5-hydroxy-7-methoxy-2-methyl (21.48%). The total content of phenolics, alkaloids, and terpenoids was 18.06%, 13.09%, and 5.57%, respectively, with multiple compounds detected in each class (Table 3).

Table 3. Secondary Metabolite Composition of the Crude Ethanolic Extract of Forest Clove (*S. aromaticum* L.) Flower Stalks

Class	Compound	Content (%)*
Flavonoids	4H-1-Benzopyran-4-one, 5-hydroxy-7-methoxy-2-methyl-	**21.48**
Phenolics	Methyleugenol	3.14
	2',3',4' Trimethoxyacetophenone	2.80
	3-cyclohexene-1- carboxaldehyde,4-methyl-	2.39
	Ethyl 4-oxo-2-phenylpentanoate	1.99
	Hexadecanoic acid, methyl ester	1.76
	9-octadecenoic acid (Z)-,2,3- dihydroxypropyl ester	1.74
	9H-Fluoren-9-ol, 9-butyl-	1.68
	Phenol,3,5-bis(1,1-dimethulethyl)	1.42
	11-Octadecenoic acid, methyl ester	1.14
	Total Phenolics	**18.06**
Alkaloids	3-Benzylidene-2-(3-pyridyl)-1-pyrroline	11.76
	Vinbarbital	1.33
	Total Alkaloids	**13.09**
Terpenoids	Naphthalene 1,2,3,5,6,8a-hexaydro-4,7-dimethyl1-1-(1-methylethyl)-, (IS-cis)-	3.05
	Copacne	1.38
	1-cyclohexene-1-ethanol, 2,6,6 -trimethyl	1.14
	Total Terpenoids	**5.57**

*Values represent the relative abundance (%) of compounds based on GC–MS peak area.
Source: [15].

DISCUSSION

The forest clove flower stalk crude ethanolic extract consistently affected *S. litura* larvae in both laboratory and plastic-house experiments, with dose- and time-dependent mortality. In the laboratory, larvae exhibited behavioral changes, including avoidance of treated leaves and reduced activity, indicating a repellent effect likely mediated by secondary metabolites such as flavonoids [16]. Larvae that continued feeding may have tolerated or not yet been exposed to an effective dose, whereas those avoiding feeding likely detected toxic compounds through chemoreceptors [17]. Mortality increased over time, reaching 100% at higher concentrations by 48–72 hours, with physiological symptoms such as shrinkage and darkening, suggesting disruption of metabolism and tissue integrity [18].

Plastic house experiments confirmed these effects under semi-field conditions, with the highest concentration producing mortality comparable to the positive control (abamectin 0.15%), further supporting the slow-acting, dose-dependent nature of the extract.

The activity of the extract is likely due to the combined action of multiple secondary metabolites, particularly flavonoids, along with phenolics, alkaloids, and terpenoids (Table 3), rather than a single compound.

Flavonoids may act as repellents and antifeedants [19], alkaloids as neurotoxins [20], phenolics as metabolic enzyme inhibitors [21], and terpenoids as membrane or respiratory disruptors [22]. The synergistic effects of these compounds can explain both the behavioral and lethal effects observed in larvae, demonstrating the potential of this crude botanical extract as an effective pest management agent.

CONCLUSION

The crude ethanolic extract of forest clove (*S. aromaticum*) flower stalks exhibited strong insecticidal and repellent activity against *Spodoptera litura*, showing clear dose- and time-dependent effects in both laboratory and plastic house experiments. Behavioral symptoms such as reduced feeding and avoidance of treated leaves indicated repellent action, while physiological effects suggested cumulative toxicity from the extract's secondary metabolites, particularly flavonoids, along with terpenoids, phenolics, and alkaloids. Under plastic house conditions, concentrations of 3-6% (w/v) resulted in high larval mortality (≈94–100%) within 72-96 hours after treatment, comparable to the positive control. Although further evaluation on non-target organisms is needed, these findings suggest that the extract could serve as a slow-acting but effective botanical insecticide with potential application in more sustainable pest management systems.

REFERENCES

[1] S. Chowański, M. Kudlewska, P. Marciniak, and G. Rosiński, "Synthetic Insecticides – is There an Alternative". Pol. *J. Environ. Stud*. Vol. 23, no. 2 pp: 291-302, 2014.

[2] A.R. War, M.G. Paulraj, T. Ahmad, A.A. Buhroo, B. Hussain, S. Ignacimuthu, and H.C. Sharma, "Mechanisms of plant defense against insect herbivores". *Plant signaling & behavior*, vol. 7, no. 10, pp. 1306-1320, 2012.

[3] R. Pavela, and G. Benelli, "Essential oils as ecofriendly biopesticides Challenges and constraints". *Trends in plant science*, vol. *21*, *no.* 12, pp.1000-1007, 2016.

[4] A. Mu'nisa, T. Wresdiyati, N. Kusumorini, and W. Manalu, "Aktivitas antioksidan ekstrak daun cengkih". *Jurnal Veteriner,* vol. 13, no. 3, pp. 272-277, 2012.

[5] Y.P. Utami, I. Imrawati, A. Amin, and F.A. Haris, "Identifikasi Kandungan Senyawa dan Potensi Ekstrak Etanol 96% Daun Tekelan (Chromolaena odorata L.) sebagai Antioksidan Penangkal Radikal ABTS". *Journal Of Noncommunicable Diseases*, vol. 3, no. 2, pp. 72, 2023.

[6] M. Bate, "Pengaruh beberapa jenis pestisida nabati terhadap hama ulat grayak (*Spodoptera litura* F.) pada tanaman sawi (Brassica Juncea L) di lapangan". *Agrica*, vol. 12, no. 1, pp. 71-80, 2019.

[7] J.A. Leatemia, R.Y. Rumthe, dan S.H. Noya, "Pengembangan insektsida nabati berbahan baku tangkai bunga cengkih (*Syzigium Aromaticum*)". Laporan penelitian, 2017.

[8] A. Sutikno dan R. Anggriani, "Uji efektivitas konsentrasi ekstrak daun Cengkih (*Syzygium aromaticum* L.) Berpelarut organik terhadap mortalitas ulat grayak (*Spodoptera litura* F.) Hama tanaman jagung". *Jurnal Agroteknologi*, vol, 13, no. 2, pp. 61-68, 2023.

[9] A.S. Mahulette, S.Yahya, and A. Wachjar, "Physico-chemical properties of clove oil from three forest clove accession groups in Maluku". In IOP *Conference Series: Earth and Environmental Science* , vol. 418, No. 1, p. 012028, 2020

[10] A.S. Mahulette, H. Hariyadi, S. Yahya, A. Wachjar, and I. Marzuki, "Morpho-

agronomical diversity of forest clove in Moluccas, Indonesia". *Hayati Journal of Biosciences*, vol. *26, no.* 4, pp. 156-156, 2019.

[11] E. Uge, E, Yusnawan, dan Y. Baliadi, "Pengendalian ramah lingkungan hama ulat grayak (*Spodoptera litura* Fabricius) pada tanaman kedelai" *Buletin Palawija*, vol. 19, no. 1, pp. 64-80, 2021.

[12] R.D. Septian, L. Afifah, T. Surjana, N.W. Saputro, dan U. Enri, "Identifikasi dan efektivitas berbagai teknik pengendalian hama baru ulat *grayak Spodoptera frugiperda* JE smith pada tanaman jagung berbasis PHT Biointensif". *Jurnal Ilmu Pertanian Indonesia*, vol. 26, no. 4, pp. 521-529, 2021.

[13] D.J. Finney, "Probit analysis, Cambridge University Press". *Cambridge, UK*, 1-333, 1971.

[14] R.G.D. Steel, and J. H. Torrie. Principles and Procedures of Statistics: A Biometrical Approach. 2nd ed. New York: McGraw-Hill, 1980.

[15] J.A. Leatemia, J. Taribuka, dan M.A. Senen. "Proses Pembuatan Ekstrak Kasar Tangkai Bunga Cengkih Hutan (*Syzygium aromaticum* L) Sebagai Insektisida Nabati dengan metode Perendaman" Surat Pencatatan Ciptaan nomor: EC002025150411, Kementerian Hukum dan Hak Asasi Manusia Republik Indonesia, 2025.

[16] M.B. Isman, "Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world," *Annual Review of Entomology*, vol. 51, pp. 45–66, 2006.

[17] L.M. Schoonhoven, J. J. A. van Loon, and M. Dicke, *Insect–Plant Biology*, 2nd ed., Oxford: Oxford University Press, 2005.

[18] M. Dumeva, "Botanical insecticides: Mode of action and efficacy," *Journal of Plant Protection Research*, vol. 56, no. 3, pp. 215–224, 2016.

[19] J.B. Harborne, "*The Comparative Biochemistry of Flavonoids*" Academic Press, 1999

[20] S.S. Duffey, and M. J. Stout, "Antinutritive and toxic components of plant defense against insects. *Archives of Insect Biochemistry and Physiology" Published in Collaboration with the Entomological Society of America*, vol. 32, no.1, 3-37, 1996.

[21] G.A. Rosenthal and M. R. Berenbaum. "*Herbivores: Their Interactions with Secondary Plant Metabolites*". 2nd ed., Academic Press, 1992.

[22] J. Gershenzon and N. Dudareva. "The function of terpene natural products in the natural world". *Nature Chemical Biology*, vol. 3, no. 7, pp. 408–414, 2007.