

Insecticidal Activity of Selected Essential Oil Extracts Against Common Cutworm, *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae)

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Essential oils from *Alpinia pyramidata* (Blume), *Lantana camara* (Linnaeus), *Coleus amboinicus* (Loureiro) and *Curcuma longa* (Linn.) were evaluated in the laboratory for their insecticidal activities against third instar larval of common cutworm, *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae). Among the four essential oils, *Cu. longa* was the most toxic to cutworm ($LC_{50} = 5.93$ mg/mL) when applied through leaf residue film method. When applied topically, essential oil from *A. pyramidata* was the most toxic ($LD_{50} = 693.86$ μ g/g insect) which also provided the highest antifeedant activity against cutworm at 16 mg/mL acetone. Essential oil from *L. camara* ranked second in providing contact toxicity both through topical application and leaf residue film method. Essential oil from *Cu. longa* showed the highest repellency against cutworm at 16mg/mL acetone. Essential oil from *L. camara* showed remarkable insect growth regulatory activities against cutworm expressed by a high number of larval-pupal intermediates. Meanwhile, essential oil from *Cu. longa* showed high abnormalities among the pupae and adults produced. Both the latter essential oils also provided short life span of seven to eight days when applied on cutworm larvae; a normal adult lived for about nine days when provided with 10% honey solution as food. In view of their overall pesticidal properties, essential oils from *L. camara* and *Cu. longa* have potential to be exploited as botanical insecticides for cutworm management.

Key words: *Alpinia pyramidata*, botanical insecticide, *Coleus amboinicus*, *Curcuma longa*, *Lantana camara*, *Spodoptera litura*

INTRODUCTION

The common cutworm, *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) is a polyphagous insect pest that feeds on almost all kinds of green vegetation. In severe infestation, the pest can heavily damage the plant resulting to stunted growth and reduced yield (Kandagak & Khetagoudar 2013). The major line of defense employed against cutworm is the use of chemical insecticides.

However, management of the pest using synthetic chemicals has failed due to the development of insecticide resistance, pest resurgence, environmental contamination, and lethal effects on non-target organisms (Jeyasankar et al. 2014). Hence, plant products are emerging as a potential source of cutworm pest control because they are comparatively less toxic and easily biodegradable (Arivoli & Tennyson 2013).

Fan et al. (2011) investigated the topical toxicity of essential oil from black pepper, *Piper nigrum* Linnaeus

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(Piperaceae) against cutworm and found that limonene was the major compound isolated in *P. nigrum* with 35.06% of total oil followed by beta-pinene (12.95%) and linalool (9.55%). Essential oils from lagundi, *Vitex negundo* Linn. (Lamiaceae); sambong, *Blumea balsamifera* Linn. (Asteraceae); oregano, *Coleus amboinicus* Loureiro (Lamiaceae); bulak-manok, *Ageratum conyzoides* Linn. (Asteraceae); and manzanilla, *Chrysanthemum indicum* Linn. (Asteraceae) were also highly toxic against cutworm causing more than 70% mortality at 0.2 mg/mL at 24 h (Morallo-Rejesus et al. 1987). Among the 21 essential oils tested for insecticidal activity through topical application against third instar larvae of cutworm, oils from summer savory, *Satureia hortensis* Linn. (Lamiaceae); breckland thyme, *Thymus serpyllum* Linn. (Lamiaceae); and oregano, *Origanum creticum* Linn. (Lamiaceae) caused 90% larval mortality at 24 h at 100 µg per larva. The LD₅₀ value for *S. hortensis* (48.4 µg) was comparable to that of *T. vulgaris* Linn. at 46.9 µg. The monoterpene phenols thymol and carvacrol, the major constituents of the oils from *Thymus* and *Satureia* species, might be responsible for the insecticidal action (Hummelbrunner & Isman 2001).

Among the 13 plants evaluated by Javier et al. (2015) using water, vinegar and alcoholic extracts using coconut wine (lambanog), langkauas, *Alpinia pyramidata* Blume (Zingiberaceae) had the highest insecticidal activity against cabbage worm, *Crociodolomia pavonana* Zeller (Lepidoptera: Pyralidae) and *S. litura*. It also showed high insecticidal activity against flea beetles, *Phyllotreta* spp. (Coleoptera: Chrysomelidae) (Javier et al. 2015); bean beetle, *Callosobruchus chinensis* Linn. (Coleoptera: Bruchidae); and maize weevil complex, *Sitophilus* spp. (Coleoptera: Curculionidae) (Thein et al. 2013). Wine extracts of lantana, *Lantana camara* Linn.; and luyang dilaw, *Curcuma longa* Linn. were found toxic against cutworm, while water extracts of *Cu. longa* caused high mortality followed by the *Co. amboinicus* leaves. Water extracts of lantana showed antifeedant activity against cutworm. Moreover, water, coconut wine and vinegar extracts of *Cu. longa* and *Co. amboinicus* showed growth regulatory activity against cutworm (Javier et al. 2015). Essential oil from *Cu. longa* showed high topical toxicity and leaf residue film method against diamondback moth, *Plutella xylostella* Linn. (Lepidoptera: Plutellidae) at LD₅₀ and LC₅₀ values of 32.98 µg/g insect and 118.23 ppm, respectively. *Co. amboinicus* also showed high topical toxicity with LD₅₀ value of 34.71 µg/g and had remarkable insect growth regulatory activity expressed in high larval mortality and high abnormality among the pupae and adults of DBM. Meanwhile, *A. pyramidata* provided the highest antifeedant activity at 125 ppm against *P. xylostella* (Javier et al. 2016).

The rhizome of langkauas, *A. pyramidata* is used to treat diabetes, obesity and inflammatory disease (Stuartxchange

2017). Lantana, *L. camara* plant is used to treat skin diseases such as skin itches, wounds, leprosy and scabies. Plant extracts are also used to treat cancers, chicken pox, measles, asthma, ulcers, swellings, eczema, tumors, high blood pressure, bilious fevers, catarrhal infections, tetanus, rheumatism, malaria and atoxy of abdominal viscera (Ghisalberti 2007). Leaves of oregano, *Co. amboinicus* are used to treat bronchitis, asthma, chronic coughs, sores, burns and insect stings (Perry 1980). Rhizome of luyang dilaw, *Cu. longa* is used to treat different types of cancer, intestinal inflammations, ulcers and various skin diseases (Labban 2014).

This study was carried out to evaluate the insecticidal activity, antifeedant property, repellent activity and morphogenetic effects using essential oil extracts of four plants growing abundantly in the Philippines (*A. pyramidata*, *L. camara*, *Co. amboinicus* and *Cu. longa*) against third instar of cutworm. These plants were selected because of their promising insecticidal activity using crude (solvent and aqueous) extracts. Moreover, among plants extracts, essential oils are regarded as safe since they are commonly used as fragrances and as flavoring agent in food additives (Isman 2000).

MATERIALS AND METHODS

Rearing of *S. litura*

Egg masses of common cutworm were collected from the Central Experiment Station, University of the Philippines Los Baños (UPLB) either in crucifers, corn, banana, taro or legumes. Newly hatched larvae were provided with leaves of pechay, *Brassica rapa* Lour. (Brassicaceae) as food. New, fresh and clean pechay leaves were provided as necessary. Likewise, larvae were transferred into freshly cleaned and disinfected rearing trays measuring 33x25x10 cm. Fully grown larvae that were about to pupate were transferred into rearing tray with an inch layer of sterilized moist soil as pupation medium. After about three days, the pupae were collected and transferred into emergence cages measuring 43x30x30 cm to await adult emergence. The emergence cage was provided with 10% honey solution as food for emerging adults, and pechay seedlings to serve as oviposition site for the adults.

Leaves containing egg masses were collected and transferred into new and clean plastic container and were allowed to hatch. Egg masses were observed daily and hatching was recorded. F2 larvae were reared on pechay leaves and third instar larvae were used for bioassays. About 25% of the larvae were reared and allowed to pupate and emerge into adult in order to keep a continuing stock culture of the host test insect.

Preparation of Plant Samples and Essential Oil

Extraction

The plants were collected in June to September 2012. Fresh leaves of *Co. amboinicus* and citronella, *Cymbopogon nardus* Linn. (Poaceae), and rhizomes of *Cu. longa* were collected in the greenhouse of Institute of Weed Science, Entomology and Plant Pathology, College of Agriculture and Food Sciences, (IWEP, CAFS) UPLB (GPS coordinates: 14.166014, 121.239764), while leaves of *L. camara* were collected from Animal Science, UPLB near DTRI Dairy Bar (GPS coordinates: 14.156558, 121.245714). The rhizomes of *A. pyramidata* were purchased from San Agustin, Iriga City, Camarines Sur (GPS coordinates: 13.452197, 123.398899). About 5 kg of plant parts were collected per species.

Collected plants were brought to the laboratory, washed with dechlorinated water and air dried for about 10 to 20 min under room temperature of $28 \pm 1^\circ\text{C}$. The extraction procedure was conducted at the Insect Physiology Laboratory of IWEP, CAFS, UPLB. The rhizomes were chopped into small pieces, while the other plant parts were detangled and cut into smaller pieces using scissors. The fresh plant parts were homogenized in distilled water using the Nutri Tech blender-juicer set at 32000 rpm. One kg of the homogenized fresh leaves or chopped fresh rhizomes were placed in a 2-Lit round bottom flask and enough distilled water was added when needed so that it is approximately 2 cm above the level of the plant material. Essential oils were collected through steam distillation. The crude oils were placed in a vial with a pinch of anhydrous sodium sulfate to dehydrate it. The concentrated essential oil from 1 kg of fresh plant samples was measured and percent yield was determined.

Preparation of Test Solutions

Stock solutions were prepared for each crude oils using acetone as solvent. Final concentrations used in bioassay concentrations were prepared from the stock solution using acetone as diluent. The final doses and concentrations for each essential oil of the different plants against cutworm were determined from preliminary bioassays. Series of dilutions between doses and concentrations that caused 10 to 90% mortality of test insect was identified. Once the effective dose and concentration ranges are identified, six doses and concentrations were selected and tested in the first actual trial. After the first trial is completed, doses and concentrations were adjusted further in the second and third trials until the final range of doses and concentrations was determined.

Bioassay Procedures

Contact toxicity test – Topical application method. One μL of the solution of each treatment was applied equivalent to the following doses: 118, 235, 470, 941, 1882, 3764 $\mu\text{g/g}$ insect on the thoracic region of each third instar larva using

a Hamilton Repeating Dispenser plus a 10- μL microsyringe. The treatment using acetone served as a negative control, while one μL of chlorfluazuron (Atabron) at a dose of 5.88 $\mu\text{g/g}$ insect was used as the standard insecticide/ positive control for comparison. Ten larvae were used per replication and each treatment was replicated four times. In all the tests, third instar cutworm larvae with an average weight of 8.50 mg per larva were used.

Residual toxicity test - Leaf residue film method (LRFM).

Pechay leaf was cut circular using scissors measuring 8 cm in diameter and 0.50 mL of the different test solutions (2, 4, 8, 16, 32, 64 mg/mL acetone) was evenly spread on both abaxial and adaxial sides of the leaves using forceps. After air drying for about 10 min, treated leaf was placed individually in a Petri dish measuring 9 cm. Acetone and 50 μg chlorfluazuron/mL acetone (recommended rate for noctuid pest in pechay) served as the negative and positive controls, respectively. Ten larvae were used per replication and each treatment was replicated four times.

For contact and residual toxicity tests, mortality data at 24, 48 and 72 h after treatment (HAT) were subjected to probit analysis using PoloPlus© developed by LeOra Software in 2002 (Finney 1971) to determine LD_{50} and LC_{50} (dose and concentration which give 50% mortality on the test larvae).

Antifeedant and repellent activity test (No-choice test).

The pechay leaf was treated as described in the residual toxicity test. After drying, ten third instar larvae previously starved for 6 h were introduced onto the leaf disc method. Acetone and essential oil from *Cy. nardus* with concentrations same with the four essential oils (2, 4, 8 and 16 mg/mL acetone) served as the negative and positive controls, respectively (Javier et al. 2016). Crude oil from leaves of *Cy. nardus* was used as standard botanical insecticide since it has been used for over fifty years as an insect antifeedant and repellent against many insect pests. The antifeedant and repellent activity of *Cy. nardus* oil was mainly attributed to its major monoterpene constituent citronellal (Zaridah et al. 2003).

The amount of feeding in each treatment was based on percent reduction in weight of the pechay leaf consumed by the larvae after 24 h. The amount of feeding was recorded by weighing the leaf before and after the consumption. The corrected antifeedant index (Blaney et al. 1994) was calculated as follows:

$$\% \text{ Relative Feeding Index or RFI} = \frac{C-T}{C+T} \times 100$$

where C is leaf consumption in control, and T is leaf consumption in treated. The RFI measures the feeding deterrence of each test solution. The criteria used to categorize essential oils were adopted from Huang et al. (2000):

Antifeedant activity was evaluated using the following scale:

% RFI	Antifeedant activity
≥ 75	High
51 – 74	Moderate
25 – 50	Low
< 24	Very Weak

The repellent activity of extracts was evaluated by counting the number of larvae permanently moving off the treated leaf at one and six hours after exposure (Pipithsangchan & Morallo-Rejesus 2005).

Test on morphogenetic effects. The insect growth regulator (IGR) activity of the plant extracts was determined using ten third instar larvae of cutworm which were pre-weighed prior to exposure. The larvae were treated with the LD₂₅ of the crude oil extracts based on contact toxicity test using topical application at 72 HAT. Cutworm larvae were topically treated with 470 µg/g insect.

Each of the treated larvae was released inside a Petri dish containing moistened filter paper and pechay leaves as food. Each treatment was replicated four times. Pechay leaves were provided to the larvae until pupation and left over leaves and excreta were removed daily. The mortality and abnormalities were observed until adult emergence.

Statistical Analysis

For the relationship between dose and response data on mortality, antifeedant activity and repellency, Pearson's coefficients were calculated and tested using Minitab 17 (Kivuto Solutions Inc.). The response data were subjected to one-way analysis of variance (ANOVA). Significant differences between treatments were determined by Tukey's Honestly Significant Difference (HSD) for further analysis using SAS University Edition. Differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Essential Oil Extraction

Steam distillation of the *Cu. longa* produced the highest recovery of essential oil (0.426%), followed by *Co. amboinicus* (0.132%) and *A. pyramidata* (0.075%) while *L. camara* had the lowest recovery of 0.025%. Percentages were based on fresh weight basis. The amount of essential oil found in the present study is within the range obtained in most plants which is less than 2% (Koul et al. 2008).

Contact Toxicity by Topical Method

Based on LD₅₀ values, the order of decreasing toxicity of the essential oils against the cutworm larvae at 72 HAT was: *A. pyramidata* = *L. camara* = *Cu. longa* = *Co. amboinicus* (Table 1). This suggests that all the essential oils with 72 h-LD₅₀ values ranging from 694 to 1138 µg/g insect are equally toxic against the cutworm.

The activity of the different doses of the essential oils from four plant species was compared with the conventional insecticide recommended against cutworm. At 72 HAT, the highest dose (3764 µg/g) of the essential oil from *A. pyramidata* provided the highest mortality (90%) against cutworm which did not significantly differ from the mortality (97.50%) caused by chlorfluzuron at the recommended rate of 5.88 µg/g insect (Table 2).

There are very limited studies on the insecticidal activities of *A. pyramidata* in the Philippines. Recently, essential oil from *A. pyramidata* showed high topical toxicity against second instar of *P. xylostella* with an LD₅₀ of 120.08 µg/g insect (Javier et al. 2016). Essential oil from the rhizomes of *A. officinarum* Hance caused 90% knockdown in 20 min and caused 100% mortality in 24 h when sprayed against adult houseflies. Five and 10% alcoholic solutions of the oil caused 40 and 70% knockdown and 42 and 72% mortality, respectively (Abrol & Chopra 1963). Moreover, space spraying of the petroleum ether extract of the

Table 1. Lethal dose (LD₅₀) (µg/g larval body weight) of four essential oils against *Spodoptera litura* at 24, 48 and 72 h after treatment (HAT)¹.

Plant species	24 HAT	48 HAT	72 HAT	Coefficient
<i>A. pyramidata</i>	7648 (3304–63981)a	3548 (1861–15032)a	6934 (491–959)a	$y = 48.22x - 1196, R^2 = 0.8820$
<i>L. camara</i>	4079 (2341–11827)a	1874 (1248–3483)a	782 (419–1445)a	$y = 45.70x - 975, R^2 = 0.7349$
<i>Co. amboinicus</i>	6839 (3396–32084)a	2383 (1319–8637)a	1138 (612–2034)a	$y = 374.0x - 1700, R^2 = 0.2127$
<i>Cu. longa</i>	4292 (2323–5103)a	1898 (1204–3986)a	792 (534–1168)a	$y = 52.20x - 1288, R^2 = 0.8105$

¹LD₅₀ = dose which results in 50% mortality of the test larvae;

CI = class index at 95% confidence interval; HF = heterogeneity factor.

For each species, values followed by the same letter in the same column are not significantly different because of the overlapping of the confidence intervals ($P < 0.05$).

Table 2. Percent mortality of third instar larvae of *Spodoptera litura* topically applied with different doses of essential oils at 72 h after treatment¹.

Dose (µg/g)	<i>A. pyramidata</i>	<i>L. camara</i>	<i>Co. amboinicus</i>	<i>Cu. longa</i>
T1 – 118	15.00 de	15.00 cd	15.00 de	20.00 de
T2 – 235	32.50 cd	20.00 c	22.50 d	30.00 d
T3 – 470	42.50 c	55.00 b	20.00 d	32.50 d
T4 – 941	50.00 c	52.50 b	45.00 c	60.00 c
T5 – 1882	72.50 b	65.00 b	70.00 b	67.50 bc
T6 – 3764	90.00 ab	82.50 a	77.50 b	80.00 ab
T7 – Control	2.50 e	2.50 d	2.50 e	2.50 e
T8 – Chlorfluazuron (5.88)	—————→		97.50 a	←—————

¹Based on 10 larvae per replicate; each dose was replicated four times.

Means followed by the same letter(s) within the same column are not significantly different at 5% level of Tukey's HSD test.

Table 3. Lethal concentration (LC₅₀) (mg/mL acetone) of four essential oils against third instar larvae of *Spodoptera litura* at 24, 48 and 72 h after treatment (HAT)¹.

Plant species	24 HAT	48 HAT	72 HAT	Coefficient
<i>A. pyramidata</i>	129 (73.7-439)b	78.1 (50.2-185.8)b	33.3 (28.9-44.7)b	y= 2.45x + 1.02, R ² =0.9578
<i>L. camara</i>	66.0 (28.5-958)b	9.59 (4.21-19.9)a	7.12 (5.72-8.76)a	y= 2.57 + 30.9, R ² =0.6140
<i>Co. amboinicus</i>	106 (43.9-1231)b	11.4 (8.65-15.0)a	8.05 (6.41-10.0)a	y= 2.47x + 28.2, R ² =0.6394
<i>Cu. longa</i>	29.6 (18.9-59.6)a	10.9 (8.26-14.2)a	5.93 (4.81-7.24)a	y= 2.52x + 35.2, R ² =0.6507

¹LC₅₀= concentration which results in 50% mortality of the test larvae;

CI= class index at 95% confidence interval; HF = heterogeneity factor.

For each species, values followed by the same letter in the same column are not significantly different because of the overlapping of the confidence intervals (P< 0.05).

rhizomes from *A. officinarum* against houseflies at 5% in kerosene, caused 88% knockdown in 10 min and 100% mortality in 24 h (Dixit & Perti 1963). The compounds 1,8-cineole, β-bisabolene, β-caryophyllene and β-selinene were reported to be responsible for the toxicity of *A. pyramidata* against oriental fruit fly, *Bactrocera dorsalis* Hendel (Tephritidae) (Areekul et al. 1987).

Essential oils from *L. camara*, *Cu. longa* and *Co. amboinicus* provided an LD₅₀ of 85.27, 32.98 and 37.41 µg/g larval body weight respectively, on second instar of *P. xylostella* (Javier et al. 2016). Essential oil from *L. camara* was also reported to exhibit insecticidal activity against cotton stainer, housefly, corn weevil, black armyworm and lesser grain borer. It also showed LD₅₀ value of 54998 µg/g against *P. xylostella* (Morrallo-Rejesus et al. 1987).

Contact Toxicity by Leaf Residue Film Method

Based on LC₅₀ values, *Cu. longa* showed the lowest LC₅₀ value of 5.93 mg/mL acetone which did not significantly differ from *L. camara* and *Co. amboinicus* (Table 3). Essential oil from *A. pyramidata* was the least toxic among

the test plants evaluated. The LC₅₀ values of the essential oils against cutworm at 72 HAT are quite high which ranged from 5.93 to 32.29 mg/mL acetone.

The activity of the different concentrations of the essential oils from the four plants was compared with chlorfluazuron. The lowest concentration of 2 mg/mL acetone showed mortality on cutworm which did not significantly differ from the control. At 16 mg/mL acetone, all the essential oils except for *A. pyramidata* provided high contact toxicity against cutworm which does not significantly differ from chlorfluazuron at 50 µg/mL acetone (Table 4).

The present study indicates that both topical and leaf applications of *Cu. longa*, *L. camara* and *Co. amboinicus* are equally effective against cutworm. Sesquiterpene ketone *ar*-turmerone is the biologically active constituent of the *Cu. longa* rhizome based on spectroscopic analysis. Potencies varied depending on insect species and dose. *Ar*-turmerone caused 100 and 64% mortality at 1000 and 500 ppm, respectively against brown planthopper, *Nilaparvata lugens* Stal (Hemiptera: Delphacidae); and 100 and 82%

Table 4. Percent mortality of different concentrations of essential oils against third instar larvae of *Spodoptera litura* at 72 h after treatment by leaf residue film method¹.

Concentration (mg/mL acetone)	<i>A. pyramidata</i>	<i>L. camara</i>	<i>Co. amboinicus</i>	<i>Cu. longa</i>
T1 – 2	2.50 e	10.00 e	15.00 cd	17.50 e
T2 – 4	5.00 e	25.00 d	22.50 c	32.50 d
T3 – 8	15.00 de	52.50 c	40.00 b	52.50 c
T4 – 16	27.50 d	82.50 b	77.50 a	80.00 b
T5 – 32	47.50 c	92.50 ab	90.00 a	100 a
T6 – 64	75.00 b	97.50 a	92.50 a	100 a
T7 – Control	2.50 e	2.50 e	2.50 d	2.50 f
T8 - Chlorfluazuron (50 µg/mL)	90.00 a	90.00 ab	90.00 a	90.00 ab

¹Based on 10 larvae per replicate; each concentration was replicated four times.

Means followed by the same letter(s) within the same column are not significantly different at 5% level of Tukey's HSD test.

mortality at 1000 and 500 ppm, respectively against *P. xylostella*. The compound showed weak insecticidal activity against *S. litura* at 2000 ppm (Lee et al. 2001). Ar-turmerone also showed 20% larval mortality at 24 HAT against cabbage looper, *Trichoplusia ni* Hubner when applied topically at 10 µg/g insect (Tavares et al. 2013).

Bioassay of *L. camara* extracts at 40% showed 97% mortality in topical application and 100% mortality for LRFM against *S. litura* (Pratibha et al. 2011). Meanwhile, essential oils from *Co. amboinicus* showed high toxicity against larvae of *P. xylostella*, *S. exempta* Walker and *S. litura*, causing more than 90% mortality at 200 µg/g (Morrallo-Rejesus et al. 1987).

Antifeedant and Repellent Activity

An antifeedant is a behavior-modifying substance that acts directly on the chemosensilla, resulting to feeding deterrence (Isman 1994). The standard botanical insecticide, *Cy. nardus* exhibited the highest RFI against cutworm at 2 mg/mL acetone. Among the essential oils, *A. pyramidata* showed high RFI at 16 mg/mL acetone. None of the other essential oils showed potentially useful antifeedant activity (Table 5).

A. pyramidata is also an effective antifeedant against *P. xylostella* and *C. chinensis* (Dadang et al. 1998). Essential oils from *A. pyramidata* showed high RFI of 100% against second instar larvae of *P. xylostella* at 500 ppm (Javier et al. 2016).

On the other hand, repellent is a substance that causes an arthropod to make an oriented movement. Generally, insect repellents work by providing a vapor barrier deterring the arthropod from coming into contact with the surface (Brown & Herbert 1997). Among them, essential oils have been found to have repellent properties against

various insects. All concentrations of the essential oils from *Cy. nardus*, the standard botanical repellent, showed the highest repellency against cutworm larvae both at 1 and 6 h after exposure. In general, the oils from the different test plants exhibited only modest repellence, even at 16 mg/mL acetone (Table 6). Essential oils from *Cu. longa* and *A. pyramidata* showed repellent activity comparable with the standard repellent, *Cy. nardus* at 16 and 64 mg/mL acetone, respectively against cutworm.

Essential oils from *Cu. longa* and *A. pyramidata* showed 100% antifeedant activity against *P. xylostella* at 250 ppm (Javier et al. 2016). The repellent activity of *Cy. nardus* oil can be attributed to its monoterpene constituent citronellal (Zaridah et al. 2003), while turmerone and ar-turmerone are the major constituents of the essential oil of *Cu. longa* responsible for its repellent activities (Xiao et al. 2011). Mayachiew & Devahastin (2008) reported that compounds 1,8-cineole, β-bisabolene, β-caryophyllene and β-selinene, present in *A. pyramidata*, exhibit insecticidal activity against various pests.

In general, slightly higher repellency was observed at 1 h after exposure than at 6 h, suggesting that the oils are highly volatile (Javier et al. 2016). Many essential oils provided short-lasting protection that lasts for less than 2 h (Koul et al. 2008). For example, oils from *Cy. nardus*; patchouli, *Pogostemon cablin* Blanco (Lamiaceae); clove, *Syzygium aromaticum* Linn. (Myrtaceae); and prickly ash, *Zanthoxylum limonella* (Dennst.) Alston. (Rutaceae) were the most effective and provided 2 h of complete repellency against dengue mosquito, *Aedes aegypti* Linn. (Diptera: Culicidae) (Coats et al. 1991).

Pipithsangchan (1993) in a similar study reported that essential oil from sa-ra-nair, *Mentha cardiafolia* Linn.

Table 5. The relative feeding index (RFI)¹ of the third instar larvae of *Spodoptera litura* at 24 h exposure to the cabbage leaf treated with different concentrations of essential oils.

Plant Species	Concentrations (mg/ mL acetone)				Coefficient
	2	4	8	16	
<i>A. pyramidata</i>	48.13 (L) b	53.10 (M)	60.31 (M)	90.69 (H)	y= 0.833x + 57.87, R ² =0.6711
<i>L. camara</i>	52.23 (M) b	61.05 (M)	61.37 (M)	60.64 (M)	y= 0.806x + 55.62, R ² =0.7888
<i>Co. amboinicus</i>	25.65 (L) c	39.63 (L)	51.53 (M)	47.66 (L)	y= 0.440x + 37.52, R ² =0.6538
<i>Cu. longa</i>	45.33 (L) b	51.73 (M)	51.88 (M)	60.61 (M)	y= 0.8139x + 45.76, R ² =0.9860
<i>Cy. nardus</i>	89.32 (H) a	94.07 (H)	100 (H)	100 (H)	y= 0.1083x + 94.95, R ² =0.3158

¹ RFI=[(C-T)/(C+T)] x 100, where: C= leaf consumption in control; T= leaf consumption in treated. The RFI measures the feeding deterrence of each test solutions.

% RFI	Antifeedant activity
≥ 75	High (H)
51 – 74	Moderate (M)
25 – 50	Low (L)
< 24	None (N)

Based on 10 larvae per replicate; each concentration was replicated four times.

Table 6. Mean percent number of cutworm larvae repelled by different concentrations of essential oils from four plant species at one and six h after exposure.

Plant Species	Concentrations (mg/mL acetone) ¹									
	2		4		8		16		Coefficient	
	1 h	6 h	1 h	6 h	1 h	6 h	1 h	6 h	1 h	6 h
<i>A. pyramidata</i>	10.0 b	10.0 b	7.50 c	12.5 b	12.5 c	15.0 b	12.5 c	15.0 b	y=0.487x+6.44 R ² =0.9416	y=0.078x+13.36, R ² =0.2752
<i>L. camara</i>	7.50 b	12.5 b	7.50 c	15.0 b	15.0 c	15.0 b	15.0 c	15.0 b	y=0.215x+8.82 R ² =0.8215	y=0.210x+14.76, R ² =0.6747
<i>Co. amboinicus</i>	7.50 b	10.0 b	17.5 c	10.0 b	17.5 c	12.5 b	17.5 c	12.5 b	y=0.226x+13.59 R ² =0.6780	y=0.1724x+12.21, R ² =0.5279
<i>Cu. longa</i>	15.0 ab	17.5 ab	22.5 ab	15.0 b	35.0 b	20.0 b	35.0 b	20.0 b	y=0.322x+26.98 R ² =0.3731	y=0.225x+17.36, R ² =0.8477
Control	12.5 b	5.00 b	12.5 bc	5.00 b	12.5 c	5.00 b	12.5 c	5.00 b	-	-
<i>Cy. nardus</i>	27.5 a	30.0 a	27.5 a	32.5 a	50.0 a	37.5 a	50.0 a	37.5 a	y=0.317x+37.09 R ² =0.3497	y=0.196x+33.79, R ² =0.6468

¹Based on 10 larvae per replicate; each concentration was replicated four times.

Means followed by the same letter(s) within the same column are not significantly different at 5% level of Tukey's HSD test.

(Lamiaceae) exhibited higher repellent activity at 1 h after exposure than at 6 h against *P. xylostella*; however, essential oil and ethanolic extracts from kra-tue, *Zingiber serumbet* Linn. (Zingiberaceae) and wild spider flower, *Gynandropsis gynandra* Linn. (Capparaceae) exhibited higher repellency at 6 h than at 1 h.

Morphogenetic Effects

The topical application of 470 µg/g body weight of the different essential oils resulted in high cutworm larval mortality ranging from 30 to 60% and larval-pupal

intermediates of 7.5 to 12%, with the highest mortality observed on the larvae treated with essential oil from *L. camara*. From the larvae that survived, there were about 9 to 30% dead or abnormal pupae, the highest of which was observed from the larvae treated with *Cu. longa*. From the normal pupae, 20 to 57% emerged as abnormal adults, highest of which was also observed from the larvae treated with *Cu. longa* (Table 7).

Cutworm adults that emerged from normal pupae lived for 6 to 10 days in all the treatments including the control (Table 7), while the abnormal pupae did not emerge at all. Among

Table 7. Effects of essential oils on the development of cutworm at 470 µg/g.

Plant extracts (470 µg/g)	L-mortality (%)	L-P mortality (%)	Survival (%)	Abnormal Pupae (%)	Abnormal Adults (%)	Longevity (Days) ¹
<i>A. pyramidata</i>	50.00	7.50	42.50	11.76	20.00	8.75 a
<i>L. camara</i>	60.00	12.50	27.50	9.09	30.00	7.57 b
<i>Co. amboinicus</i>	30.00	10.00	60.00	16.67	35.00	9.15 a
<i>Cu. longa</i>	42.50	7.50	50.00	30.00	57.14	7.00 b
Control	7.50	0	92.50	10.00	10.00	9.24 a

¹Means followed by the same letter(s) within the same column are not significantly different at 5% level of Tukey's HSD test.

the essential oils, shortest adult lifespan was recorded on cutworm treated with *Cu. longa* which did not significantly differ from *L. camara*. Meanwhile, all the other essential oils did not significantly differ from the control.

Results showed that the essential oils exhibited insect growth regulatory activity against the third instar cutworm larvae, especially the essential oil from *L. camara* which provided high larval and larval-pupal mortality, and *Cu. longa* which provided the highest number of abnormal pupae (Figure 1) and adults. There were also pupal-adult intermediates and deformed wings from the adults that emerged from the cutworm treated with essential oils (Figure 2).

Several plants showed growth inhibitory activities against *S. litura*. The development of the different intermediates between stages such as larval-pupal and pupal-adult, and the formation



Figure 1. Normal (a) and abnormal pupae of *Spodoptera litura* that emerged from larvae treated with essential oils from *Alpinia pyramidata* (b), *Lantana camara* (c-d), *Coleus amboinicus* (e-f) and *Curcuma longa* (g).

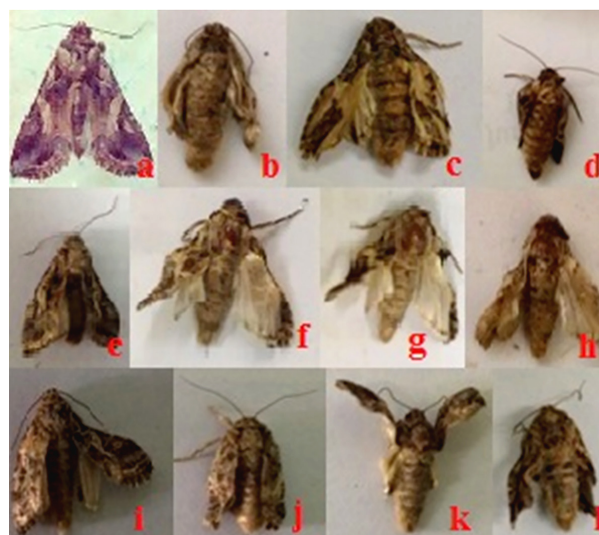


Figure 2. Normal (a) and abnormal adults that emerged from larvae treated with essential oils from *Alpinia pyramidata* (b-d), *Lantana camara* (e), *Coleus amboinicus* (f-h) and *Curcuma longa* (i-l).

of abnormal pupae and adult are also an indication of the IGR properties of the plant extracts. Such effects are also indicative of malfunctioning of endocrine system. The production of malformed larval-pupal intermediate is also observed as physiological effect of neem (Redfern et. al. 1982).

Essential oils from *L. camara* and *Cu. longa* showed IGR activities and shorter life span of the emerging adults. Botanical extracts from *L. camara* was found to be lethal to various stored grain pests and delayed their developmental stages by interfering with their apolytic and molting processes (Dwivedi & Garg 2003). Essential oil from *Cu. longa* leaves is rich in d-phellandren which acts as growth inhibitor and caused mortality against jute hairy caterpillar, *Spilosoma obliqua* Walker (Lepidoptera: Arctiidae) (Agarwal et al. 1999).

CONCLUSION

Three among the four essential oils tested were toxic against cutworms ($LC_{50} = 5.53\text{--}7.12$ mg/mL acetone) when applied through the leaf residue film method. Essential oil from *A. pyramidata* provided the highest antifeedant activity at 16 mg/mL acetone while *Cu. longa* showed some repellence at 16 mg/mL acetone. Essential oils interfered with development of cutworm larvae, especially the oil from *L. camara* that provided high larval-pupal mortality and *Cu. longa* producing the highest number of abnormal pupae and adults. Exposure of larvae to essential oils from both *L. camara* and *Cu. longa* reduced adult longevity. In view of their overall pesticidal properties, essential oils from *L. camara* and *Cu. longa* have potential to be exploited as botanical insecticides for cutworm management.

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