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Insecticidal Activity of Crude Ethanolic Extracts of Selected Philippine Plants against Diamondback Moth, *Plutella xylostella* Linnaeus

Abigaile Mia V. Javier[1]*, **Virginia R. Ocampo**[2], **Flor A. Ceballo**[2], and **Pio A. Javier**[2]

[1]Agriculture Research Section, Atomic Research Division,
Philippine Nuclear Research Institute, Department of Science and Technology,
Commonwealth Avenue, Diliman, Quezon City 1101 Philippines
[2]Institute of Weed Science, Entomology, and Plant Pathology, College of Agriculture and
Food Science, University of the Philippines Los Baños, College, Laguna 4031 Philippines

The use of plant extracts could be an alternative method to the conventional insecticides for pest control. The potential of ethanolic extracts from *Lantana camara* Linnaeus, *Coleus amboinicus* Loureiro, *Alpinia pyramidata* Blume, *Curcuma longa* Linn., and *Catharanthus roseus* Linn. as insecticide against second larval instar diamondback moth – *Plutella xylostella* Linn. – was evaluated in the laboratory through contact and residual toxicities, antifeedant activity, repellency, and growth regulator activity. Among the five plants, *L. camara* was the most toxic against *P. xylostella* through topical application (LD_{50} = 99.17 µg/g larva) and showed the highest antifeedant activity at 125 µg/mL acetone. As demonstrated using leaf residue film method, *Cu. longa* was the most toxic against *P. xylostella* (LC_{50} = 206.22 µg/mL) and also showed the highest repellency at 125 µg/mL. *Cu. longa* and *Ca. roseus* exhibited high antifeedant activity at 500 µg/mL. *L. camara* and *A. pyramidata* showed remarkable insect growth regulatory activities against *P. xylostella*. *L. camara* showed high larval and pupal mortalities, while *A. pyramidata* showed the highest number of abnormal adults produced. Among the ethanolic extracts, *L. camara* was the most promising because it consistently showed high contact toxicity plus antifeedant and remarkable insect growth regulatory activities against *P. xylostella*. Moreover, *L. camara* provided the highest ethanolic recovery (2.645%) among the test plants. In view of overall insecticidal potential of *L. camara,* can be exploited as a possible source of alternative insecticide against *P. xylostella*.

Keywords: *Alpinia pyramidata,* antifeedant, botanical insecticide, *Catharanthus roseus, Curcuma longa, Lantana camara,* repellency

INTRODUCTION

The diamondback moth (DBM), *Plutella xylostella* Linnaeus (Lepidoptera: Plutellidae), is a major pest of crucifers throughout the world. *P. xylostella* causes more than 90% crop loss in the area of outbreaks in Southeast Asia, resulting to 52% loss in marketable yield (Shelton *et al.* 2004). It is estimated that total costs associated with control of DBM is US$4 billion to US$5 billion. This estimate includes management cost due to pests, which is more of the use of insecticides, yield loss due to pest, and effect of climate. Among continents, the highest annual cost due to DBM control on cabbages based on weekly

*Corresponding author: avjavier@pnri.dost.gov.ph

application of insecticides is in Asia ($695,435,398), followed by Africa ($46,097,772) and North and Central America ($42,129,738). In the Philippines, estimated cost for DBM management on cabbages and other Brassicas based on farmer's practice is $1,440,777, while estimated cost based on weekly application of insecticide is $3,317,580 (Zalucki *et al.* 2012). The usual control method employed against *P. xylostella* includes the use of synthetic pesticides. However, the indiscriminate and improper use of chemical insecticides has led to the death of non-target organisms, environmental degradation, and emergence of insect pests that are resistant to the commonly used insecticides (Mota-Sanchez *et al.* 2002). Hence, the use of safe alternatives such as botanical products is increasing because they are effective, are often non-toxic to natural enemies, and have low environmental impact. Moreover, plant secondary chemicals can also be used as medicines, food and beverage-flavorings, fragrances, *etc.* In spite of the effectiveness and recognition of the different plant extracts that possess insecticidal properties, only few products obtained from plants are presently in use. Hence, researches on insecticidal activity of botanical extracts are needed for its development to continue to expand (Isman 2006).

It is estimated that more than 2,400 plant species from 189 plant families are rich sources of bioactive organic compounds known to have pesticidal properties (Rao *et al.* 2005, Isman 2006). A number of workers have used plant extracts against many insect pests. Crude acetone extract of *Alpinia pyramidata* Blume was found toxic (LD_{50} = 0.0854%) against *Callosobrochus chinensis* Linn. at 48 h exposure (Thein *et al.* 2013), while its essential oil was found to exhibit antifeedant activity against *P. xylostella* (Javier *et al.* 2016). *Lantana camara* Linn. is known to possess insecticidal activity especially against coleopterous stored product pests, including *Rhyzopertha dominica* Fab. and *Ca. chinensis* (Saxena *et al.* 1992). It is also effective against many mosquito species such as *Aedes aegypti* Linn., *Culex quinquefasciatus* Say, *Anopheles culicifacies* Giles, *An. fluviatilis* James, and *An. stephensi* Liston (Dua *et al.* 2003). Essential oils of *Coleus amboinicus* Loureiro and *Curcuma longa* Linn. oil extracts were reported toxic against *P. xylostella* (Javier *et al.* 2016). Morallo-Rejesus *et al.* (1990) found that *Co. amboinicus* oil extract caused 100% *Ca. chinensis* mortality and prevented adult oviposition. The ethanolic leaf extracts of *Co. amboinicus* was also found effective against third instar larvae of *An. stephensi, Cu. quinquefasciatus*, and *Ae. aegypti* (Arjunan *et al.* 2012). Ethanolic extracts from *Cu. longa* was found toxic against *Crocidolomia pavonana* Fab. when applied topically (LD_{50}= 51.00 µg/g) and through leaf residue film method (LC_{50}= 116.73 µg/mL) (Javier *et al.* 2018b). *Catharanthus roseus* Linn. extracts were reported toxic against lepidopterous pests – including *Helicoverpa armigera* Hubner (Ramya *et al.* 2008), *S. exigua* Hubn. (Feng *et al.* 2012), and *Earias vittella* Fabricius (Pavunraj *et al.* 2016).

The insecticidal activity of ethanolic extracts of five Philippine medicinal plants – including *A. pyramidata, L. camara, Co. amboinicus, Cu. longa*, and *Ca. roseus* – against *P. xylostella* are not well studied. Most of them are reported toxic against storage insect pests and mosquitoes. These plants also showed promising insecticidal activity using aqueous, alcoholic extracts using coconut wine (Javier *et al.* 2015) and essential oil extracts against *P. xylostella* (Javier *et al.* 2016), *Spodoptera litura* Fab. (Javier *et al.* 2017), and *C. pavonana* (Javier *et al.* 2018a). To evaluate the insecticidal activity of ethanolic extracts of five Philippine medicinal plants (*A. pyramidata*, *L. camara*, *Co. amboinicus*, *Cu. longa*, and *Ca. roseus*), we determined the toxicity, antifeedant property, repellent activity, and morphogenetic effects against second instar larvae of *P. xylostella*. The study was conducted at LB Uichanco Wing, Institute of Weed Science, Entomology and Plant Pathology, College of Agriculture and Food Sciences, University of the Philippines, Los Baños, (IWEP, CAFS, UPLB) College, Laguna, Philippines from Jun 2012 to Nov 2013.

MATERIALS AND METHODS

Source of Test Insect, *P. xylostella*

Larvae of *P. xylostella* were requested from BASF, Bay, Laguna, Philippines. The laboratory colony was sourced from Majayjay, Laguna and was reared in the laboratory at temperature of 26 ± 2 °C, with 60 ± 20% relative humidity, and under a photoperiod of 12L:12D – following the method of Javier *et al.* (2016). The rearing procedure was conducted at the Insect Physiology Laboratory of IWEP, CAFS.

Plant Materials Used and Extraction Method

Leaves of *L. camara*, *Co. amboinicus*, and *Cymbopogon nardus* Linn.; rhizomes of *Cu. longa*; and leaves, flowers, and stems of *Ca. roseus* were collected during Jun–Sep 2012 in areas of Bay and Los Baños, Laguna, while the rhizomes of *A. pyramidata* were from Iriga City, Camarines Sur. Plants collected were brought to the laboratory, cleaned with water, and air dried for 15 min under room temperature of 28 ± 1 ºC. The plant parts were cut into small pieces and oven-dried at 60 ºC for 48–72 h. Air-dried plant parts were ground using the Nutri Tech blender-juicer set at 32,000 rpm. The dried samples were stored in a dry place until use for extraction. Prior

to extraction, ground plant sample was placed in a clean bottle and soaked in 95% ethanol for at least 2 wk. The ethanol extract was prepared through fermentation of the ground plant sample, as shown in the flow-chart (Figure 1), using a rotary evaporator. The dried, powdered plant materials were extracted with 95% ethanol through Soxhlet extraction. The percentage recovery of the filtered ethanolic extract from 1 kg of fresh plant samples was measured and the actual percent yield was computed. The crude ethanolic extract was stored in a 50-mL centrifuge tube (Brand: Corning) and was either refrigerated before use or immediately used for bioassay.

Preparation of Test Solutions

Stock solutions and lower concentrations of each ethanolic extract were prepared using acetone as diluent. Final doses and concentrations against DBM were determined from preliminary bioassays. Series of dilutions between doses and concentrations that caused 10–90% mortality of DBM larvae was identified from the preliminary results. About three to four trials were made until the final range of doses and concentrations was determined.

Bioassay Procedures

Contact toxicity test – topical application method. Each solution of 1 µL ethanolic extract was topically applied – equivalent to doses of 16, 31, 63, 126, 251, and 502 µg/g larva – on the thoracic region of each larva using a microsyringe. Acetone and chlorfluazuron (Atabron 5 EC) at a dose of 13 µg/g larval body weight (recommended rate) served as the control and standard treatments, respectively. Ten larvae were used per replication and each ethanolic extract was replicated four times. In all the tests, second instar DBM larvae – with an average weight of 3.982 mg per larva – were used.

Residual toxicity test – leaf residue film method (LRFM). An 8 cm-diameter *pechay* leaf was cut and 0.50 mL of the different test solutions (31.25, 62.5, 125, 250, 500, and 1000 µg/mL acetone) was evenly spread on both abaxial and adaxial sides of the leaves using forceps. After air drying for about 10 min, the treated leaf was placed individually in a 9-cm Petri dish, after which ten second instar larvae were introduced. Each ethanolic extract was replicated four times. Acetone and chlorfluazuron at a concentration of 50 µg/mL acetone (recommended rate for DBM) served as control and standard treatments, respectively.

For contact and residual toxicity tests, mortality was recorded at 72 h after treatment (HAT) and probit analysis was conducted (Finney 1971) to determine the LD_{50} or LC_{50} (dose or concentration which results to 50% mortality on the test insects).

Antifeedant activity test (No-choice test). The *pechay* leaf was treated as described in the residual toxicity test. After drying, each leaf disc was placed in the Petri dish. Ten second instar DBM larvae, previously starved for 6 h, were introduced into the leaf disc. Each ethanolic extract was replicated four times. Acetone and ethanolic extract from *Cy. nardus* – with concentrations same with ethanolic extracts (125, 250, 500, and 1000 µg/mL acetone) – served as the negative and positive controls, respectively.

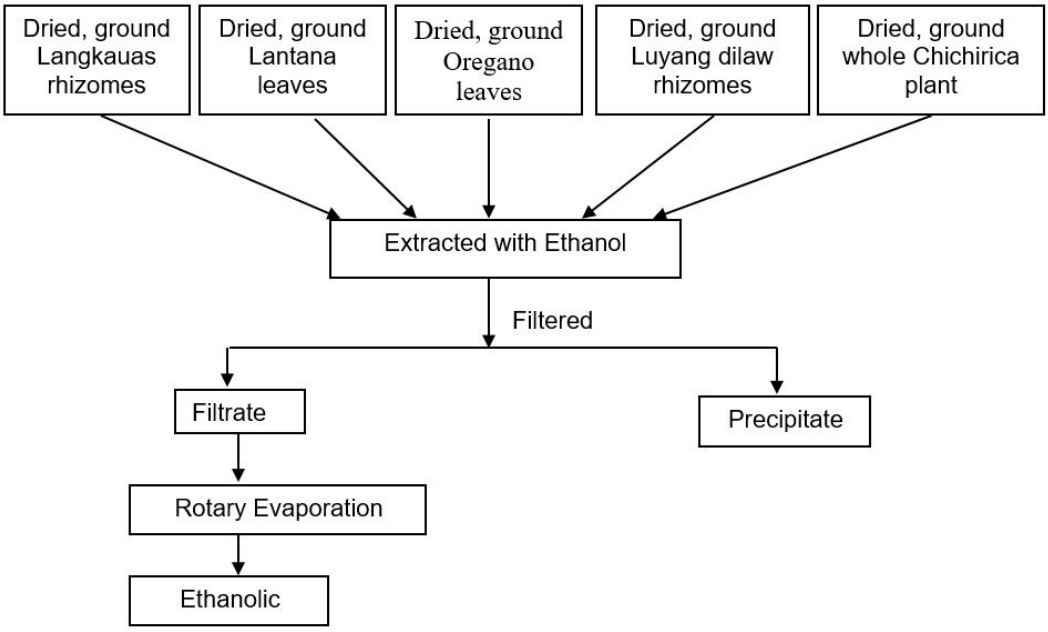

Figure 1. Schematic diagram on the extraction of ethanolic extract from 1 kg plant sample.

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 to determine antifeedant activity, as modified from Gökçe *et al.* (2009).

The corrected antifeedant index (Blaney *et al.* 1990) was calculated as follows:

$$\% \text{ Relative Feeding Index or RFI} = \frac{C - T}{C + T} \times 100 \qquad (1)$$

where C is leaf consumption in control and T is leaf consumption in treated. The RFI measures the feeding deterrence of each test solution.

Antifeedant activity was evaluated using the following scale (Huang *et al.* 2000):

% RFI	Antifeedant activity
≥75	High
51–74	Moderate
25–50	Low
<24	None

Repellency test (no-choice test). The *pechay* leaf was treated as described in the antifeedant activity test. The repellent activity of extract was evaluated by counting the number of second instar DBM larvae permanently moving off the treated leaf at 1–6 h after exposure (Pipithsangchan & Morallo-Rejesus 2005).

Test on morphogenetic effects. Ten second instar DBM larvae were treated with the LD_{25} of the ethanolic extracts based on contact toxicity test of the five extracts using topical application at 72 HAT. Larvae were topically treated with 63 µg/g body weight of the ethanolic extracts. Treated larvae were introduced inside the 9-cm Petri dish containing moistened filter paper and 8-cm *pechay* leaf as food. *Pechay* leaves were provided to the larvae until pupation, while leftover leaves and excreta were removed daily. Each ethanolic extract was replicated four times. The mortality and abnormalities were observed until adult emergence (Pipithsangchan and Morall-Rejesus 2005, Javier *et al.* 2018b).

Statistical Analysis

For the relationship between dose and response data on mortality, antifeedant activity, and repellency, Pearson's coefficients were calculated and tested. 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. Differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Ethanolic Crude Extraction

The filtered ethanolic extract from 1 kg of fresh plant samples was measured and the percent yield was computed. The fermentation of 1 kg fresh whole plant or plant part produced 0.507–2.645% ethanolic extract. The highest yield of ethanolic extract was obtained from *L. camara*, while the lowest yield was from *A. pyramidata*. Ethanolic extracts from fresh plant parts of *Ca. roseus*, *Cu. longa*, and *Co. amboincus* produced 1.991, 1.353, and 0.633% yield, respectively. The ethanolic extracts were sticky, odorless, and generally colored black (Figure 2). Tyler *et al.* (1988) reported glucosides, resin, resin combinations, hydrolysable tannins, steroids, and alkaloids inside the plant can be isolated by ethanol extraction. These might be the bioactive compounds present in the ethanolic extracts responsible for their insecticidal activity against *P. xylostella.*

Lg = *Lankauas*, *Alpinia pyramidata*
Lan = *Lantana*, *Lantana camara*
Or = Oregano, *Coleus amboinicus*
LD = *Luyang dilaw*, *Curcuma longa*
Ch = *Chichirica*, *Catharanthus roseus*.

Figure 2. Ethanolic extracts from the five plant species.

Contact Toxicity by Topical Method

Among the ethanolic extracts, *Co. amboinicus* showed the highest topical toxicity against DBM at 24 and 48 HAT. Meanwhile, *L. camara* provided the highest topical toxicity at the final hour of observation with an LD_{50} value of 99.17 µg/g larva. This is significantly similar to *Co. amboinicus* and *Cu. longa* with LD_{50} values of 117.67 and 126.03 µg/g body weight, respectively. Higher dose of the ethanolic extract from *A. pyramidata* and much higher dose from *Ca. roseus* are needed to obtain higher toxicity (Table 1).

None of the ethanolic extracts provided comparable mortality of 97.50% as chlorfluazuron at 13 µg/g larva. At 72 HAT, the highest dose (502 µg/g) of the ethanolic

Table 1. Lethal dose (LD_{50}) (µg/g larval body weight) of five ethanolic extracts against second larval instar of *Plutella xylostella* at 72 h after topical treatment[1].

Plant Species	72 HAT	Coefficient
A. pyramidata	241.21 (179.78–355.41)[b]	y = 0.1293x + 12.43, R^2 = 0.8743
L. camara	99.17 (74.48–132.29)[a]	y = 0.1333x + 27.20, R^2 = 0.7527
Co. amboinicus	117.67 (73.34–195.87)[a]	y = 0.1494x + 20.78, R^2 = 0.7741
Cu. longa	126.03 (63.20–288.87)[a]	y = 0.1325x + 22.74, R^2 = 0.6801
Ca. roseus	459.12 (218.36–4604.07)[c]	y = 0.0954x + 12.61, R^2 = 0.9358

Notes:
[1]Hypothesis of equality: Not rejected ($P > 0.05$) ($\chi^2 = 12.35$, $df = 6$, $P = 0.054$)
[1]Hypothesis of parallelism: Not rejected ($P > 0.05$) ($\chi^2 = 0.93$, $df = 3$, $P = 0.819$)
[1]LC_{50} = concentration which results in 50% mortality of the test larvae
[1]CI = class index; 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 second instar larvae of *Plutella xylostella* topically applied with different doses of ethanolic extracts at 72 h after treatment[1].

Dose (µg/g)	*A. pyramidata*	*L. camara*	*Co. amboinicus*	*Cu. longa*	*Ca. roseus*
T1 – 16	7.50[f]	12.50[de]	5.00[e]	15.00[cd]	12.50[de]
T2 – 31	7.50[f]	22.50[d]	27.50[d]	17.50[cd]	12.50[de]
T3 – 63	22.50[e]	42.50[c]	22.50[d]	20.00[c]	25.00[cd]
T4 – 126	40.00[d]	57.50[c]	60.00[c]	67.50[b]	27.50[cd]
T5 – 251	55.00[c]	77.50[b]	72.50[bc]	70.00[b]	30.00[c]
T6 – 502	70.00[b]	82.50[ab]	85.00[ab]	77.50[b]	62.50[b]
T7 – Control	2.50[f]	2.50[e]	2.50[e]	2.50[d]	2.50[e]
T8 – Chlorfluazuron (13)	97.50[a]	97.50[a]	97.50[a]	97.50[a]	97.50[a]

Notes:
[1]Based 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.

extracts from *Co. amboinicus*, *L. camara*, *Cu. longa*, *A. pyramidata*, and *Ca. roseus* provided 85, 82.50, 77.50, 70, and 62.50% mortality, respectively. The ethanolic plant extracts were less toxic than chlorfluazuron (Table 2).

In Ghana, the field application of ethanolic extract from *L. camara* in cabbage increased the yield by 25.80% by significantly reducing the number of pests such as *P. xylostella*, aphids *Brevicoryne brassicae* (Linn.), and webworm *Hellula undalis* (Fab.) (Baidoo and Adam 2012). Saponin, cardiac glycosides, and flavonoid present in ethanolic extract of leaves of *L. camara* might be responsible for its toxicity against *Aedes aegypti* Linn. and *Culex quinquefasciatus* Linn. (Kumar and Maneemegalai 2008).

Meanwhile, evaluation of the insecticidal properties of *Co. amboinicus* was focused more on its essential oil activity. Essential oil from *Co. amboinicus* provided LD_{50} values of 37.41, 782, and 304.05 µg/g body weight against second instar of *P. xylostella* larvae (Javier *et al.* 2016), third instar larvae of *S. litura* (Javier *et al.* 2017), and third instar larvae of *C. pavonana,* respectively (Javier *et al.* 2018a).

Morallo-Rejesus *et al.* (1990) confirmed that the topical application of the petroleum ether extract from *Cu. longa* provided 94% mortality of *P. xylostella* larvae. Application of essential oil from *Cu. longa* provided LD_{50} value of 32.98 µg/g against *P. xylostella* at 72 HAT (Javier *et al.* 2016).

Residual Toxicity by Leaf Residue Film Method (LFRM)

Ethanolic extracts from *Cu. longa* and *A. pyramidata* consistently showed high residual toxicity during the entire hours of observation. AT 72 HAT, *Cu. longa* and *A. pyramidata* was highly toxic with LC_{50} values of 206.22 and 209.51 µg/mL, respectively. This was followed by *A. pyramidata* and *L. camara* with LC_{50} values of 209.51 and 287.26 µg/mL, respectively. *Ca. roseus* was the least toxic among the ethanolic extracts with LC_{50} value of

408.77 µg/mL (Table 3). Different concentrations were compared with the standard insecticide, chlorfluazuron. When concentration was increased to 1000 µg/mL, all the ethanolic extracts except for *Ca. roseus* showed mortality comparable with 50 µg/mL acetone chlorfluazuron. The application of 500 µg/mL acetone *A. pyramidata* ethanolic extract showed mortality comparable with chlorfluazuron (90%) at 50 µg/mL acetone (Table 4).

Methanolic extract *ar*-turmerone from *Cu. longa* provided 82% and 100% *P. xylostella* larval mortality at 500 and 1000 ppm, respectively (Lee *et al.* 2001). Likewise, Roh (2000) found the methanol extract of *Cu. longa* rhizome to be effective against *P. xylostella* larvae. Extract from rhizomes of several species of *Curcuma* (*Cu. amada, Cu. heyneana, Cu. zedoaria*, *Cu. aromatica,* and *Cu. aeruginosa*) were effective against *P. xylostella* and *Ca. chinensis* (Linn.) (Dadang *et al.* 1998).

There are very limited reports on the insecticidal activity of the ethanolic extracts from *A. pyramidata* and *Co. amboinicus* against lepidopterous pests. Aqueous extracts from red and pink variant flowers of *A. purpurata* were toxic against fourth instar larvae of *Ae. aegypti* with LC_{50} values of 18.3 and 12.6%, respectively (Santos *et al.* 2012). In Thailand, methanolic extracts from the rhizomes of *A. galangal (=pyramidata)* exhibited remarkable toxicity against adult of *Bactrocera dorsalis* (Hendel) with LC_{50} of 5987.05 and 5652.53 ppm at 24 and 48 h, respectively (Sukhirun *et al.* 2009). Meanwhile, methanolic extracts from leaves of *Co. amboinicus* was reported to exhibit contact toxicity against *Epilachna varivestris* Mulsant at 500 ppm (Vasquez *et al.* 1999).

Antifeedant Activity

To determine the antifeedant activity of each plants against *P. xylostella,* the percent reduction in weight of the *pechay* leaf consumed by the larvae before and after 24 h was calculated. Higher RFI indicates decreased rate of feeding. The standard botanical antifeedant *Cy. nardus* exhibited high RFI of 89.17% at 125 µg/mL acetone. Among the ethanolic extracts, *L. camara* provided 100% RFI at 125 µg/mL acetone. Meanwhile, *Cu. longa* exhibited high RFI

Table 3. Lethal concentration (LC_{50}) (µg/mL acetone) of five ethanolic extracts against *Plutella xylostella* at 72 h after treatment[1].

Plant Species	72 HAT	Coefficient
A. pyramidata	209.51 (165.43–263.77)^{a}	y = 0.0807x + 22.28, R^2 = 0.7584
L. camara	287.26 (224.62–370.82)^{bc}	y= 0.0807x + 15.61, R^2 = 0.8869
Co. amboinicus	246.12 (192.47–315.60)^{ab}	y= 0.7800x + 19.84, R^2 = 0.7944
Cu. longa	206.22 (163.00–258.21)^{a}	y= 0.0828x + 22.00, R^2 = 0.7693
Ca. roseus	408.77 (260.57–772.50)^{c}	y= 0.0657x + 14.70, R^2 = 0.8503

Notes:
[1]Hypothesis of equality: Not rejected ($P > 0.05$) ($\chi^2 = 12.35$, $df = 6$, $P = 0.054$)
[1]Hypothesis of parallelism: Not rejected ($P > 0.05$) ($\chi^2 = 0.93$, $df = 3$, $P = 0.819$)
[1]LC_{50} = concentration which results in 50% mortality of the test larvae
[1]CI = class index; 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 4. Percent mortality of different concentrations of ethanolic extracts against second instar larvae of *Plutella xylostella* at 48 h after treatment by leaf residue film method[1].

Concentration (µg/mL acetone)	*A. pyramidata*	*L. camara*	*Co. amboinicus*	*Cu. longa*	*Ca. roseus*
T1 – 31.25	5.00^{c}	5.00^{d}	5.00^{d}	7.50^{e}	2.50^{d}
T2 – 62.5	15.00^{c}	15.00^{d}	15.00^{d}	15.00^{e}	12.50^{d}
T3 – 125	42.50^{b}	32.50^{c}	35.00^{c}	35.00^{d}	35.00^{c}
T4 – 250	60.00^{b}	42.50^{c}	60.00^{b}	67.50^{c}	40.00^{bc}
T5 – 500	80.00^{a}	70.00^{b}	70.00^{b}	77.50^{bc}	52.50^{b}
T6 – 1000	90.00^{a}	87.50^{a}	87.50^{a}	92.50^{a}	75.00^{a}
T7 – Control	5.00^{c}	5.00^{d}	5.00^{d}	5.00^{e}	5.00^{d}
T8 – Chlorfluazuron	90.00^{a}	90.00^{a}	90.00^{a}	90.00^{ab}	90.00^{a}
(50 µg/mL)	5.00^{c}	5.00^{d}	5.00^{d}	7.50^{e}	2.50^{d}

Notes:
[1]Based 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.

of 78.37% when the concentration was increased to 500 µg/mL acetone (Table 5).

Many researchers have reported antifeedant activity of ethanolic extracts against *P. xylostella*. The triterpenoid lantadene in *L. camara* was reported to be responsible for its antifeedant properties. Once ingested, susceptible insects cease feeding followed by starvation and finally result to death of insects (Mehta *et al.* 1995). In no-choice test, lantadene ethanolic extract at 1.6 mg/mL provided 33.1% and 62.4% antifeedant activity against the first and second instar *P. xylostella* larvae, respectively at 48 h (Dong *et al.* 2005). Meanwhile, dichloromethane extract from rhizomes of *Cu. longa* showed high antifeedant activity against second and third instar larvae of *Helicoverpa armigera* Hubner, but did not exhibit contact toxicity (Hernandez *et al.* 1992).

The results suggest that aside from toxicity, ethanolic extracts from *L. camara* and *Cu. longa* also possessed antifeedant activity against *P. xylostella* (Tables 3 and 4). *A. pyramidata* and *Co. amboinicus* exhibited more of toxicity than deterrence, while *Ca. roseus* was more deterrent than toxic. It is possible that larvae that fed on the plants treated with the *L. camara* and *Cu. longa* died through starvation. There are plant extracts that act as antifeedant which prevent feeding of pest without killing it directly, while it stays near the treated plant part and must have died through starvation. (Pavunraj *et al.* 2016, Vasquez *et al.* 1999).

Repellency

The five ethanolic extracts did not exhibit considerable repellency against *P. xylostella* larvae at 31.25 and 62.5 µg/mL. When concentration was increased to 125 µg/mL acetone, ethanolic extract from *Cu. longa* showed comparable repellency with that of *Cy. nardus* (Table 4). This suggests that when the ethanolic extracts are sprayed to crops, only about 40% of the larvae will leave the treated crops while the remaining 60% may stay and still feed on the crop. Ethanolic extracts from *L. camara, A. pyramidata,* and *Co. amboinicus* showed comparable repellency with *Cy. nardus* when concentration was at least 250 µg/mL acetone (Table 6). Repellency against larvae of *P. xylostella* was almost the same at 1 and 6 h after exposure. This suggests that ethanolic extract stays longer on the treated leaves, unlike that of volatile oils (Javier *et al.* 2016, 2017).

The crude ethanolic extracts affect an insect pest in different ways. Rajashekar *et al.* (2012) classified botanical insecticides into six groups – namely repellents, antifeedants, toxicants, protectants, chemosterilants, and growth regulators. All the crude extracts showed weak repellence. However, among the ethanolic extracts, *Cu. longa* showed consistent insecticidal activity through toxicity by LRFM and antifeedant activity, and the highest repellent activity (40%). Possible reason for the weak repellence of the other crude extracts could be the composition of the ethanolic extracts.

Terpene hydrocarbons (*e.g.*, monoterpenes and sesquiterpenes) and oxygen derivatives (*e.g.*, aldehydes and alcohols) are the main constituents possibly responsibly for the biocidal activity of *Cy. nardus*. (Doumbia *et al.* 2014). The turmerone and *ar*-turmerone are the major constituents responsible for the repellency of *Cu. longa* (Xiao *et al.* 2011). Chander *et al.* (2000) observed that turmeric extracts showed some repellency against *Tribolium castaneum* Herbst, *Oryzaephilus surinamensis* Linn., *Cryptolestes ferrugineus* Stephens,

Table 5. Percent relative feeding index (RFI)[1] of the second larval instars of *Plutella xylostella* at 24 h exposure to the cabbage leaf treated with different concentrations of ethanolic extracts.

Plant Species	Concentrations (µg/mL acetone)[2]				
	125	250	500	1000	Coefficient
A. pyramidata	15.44 (N)	48.16 (L)	44.34 (L)	70.28 (M)	y = 0.0509x + 20.68, R^2 = 0.7654
L. camara	100.00 (H)	100.00 (H)	100.00 (H)	100.00 (H)	y = 100, R^2 = #N/A
Co. amboinicus	44.75 (L)	54.33 (M)	48.41 (L)	55.89 (M)	y = 0.0088x + 46.74, R^2 = 0.4278
Cu. longa	48.36 (L)	65.33 (M)	78.37 (H)	93.30 (H)	y = 0.0470x + 49.33, R^2 = 0.9044
Ca. roseus	20.71 (N)	38.51 (L)	62.21 (M)	68.96 (M)	y = 0.0513x + 23.56, R^2 = 0.8006
Cy. nardus	89.17 (H)	96.01 (H)	100.00 (H)	100.00 (H)	y = 0.0102x + 91.50, R^2 = 0.6004

Notes:
[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 solution.

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

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

Table 6. Mean percent number of DBM larvae repelled by different concentrations of ethanolic extracts from five plant species at 1 h and 6 h after exposure.

Plant Species	Concentrations (µg/mL acetone) [1, 2]									
	125		250		500		1000		Coefficient	
	1 h	6 h	1 h	6 h	1 h	6h	1 h	6 h	1 h	6 h
A. pyramidata	20.0^{bcd}	22.5^{ab}	30.0^{ab}	30.0^{ab}	32.5^{ab}	30.0^{a}	35.0^{ab}	30.0^{ab}	y = 0.0137x + 22.94, R^2 = 0.6539	y = 0.0057x + 25.44, R^2 = 0.3507
L. camara	22.5^{bc}	27.5^{ab}	32.5^{ab}	30.0^{ab}	37.5^{ab}	37.5^{a}	35.0^{ab}	40.0^{a}	y = 0.011x + 26.74, R^2 = 0.4158	y = 0.0143x + 22.94, R^2 = 0.6539
Co. amboinicus	22.5^{bc}	17.5^{ab}	30.0^{ab}	20.0^{ab}	32.5^{ab}	25.0^{ab}	30.0^{ab}	25.0^{ab}	y = 0.0059x + 25.98, R^2 = 0.2792	y = –0.0082x + 18.04, R^2 = 0.7114
Cu. longa	40.0^{a}	30.0^{a}	37.5^{a}	32.5^{a}	40.0^{a}	40.0^{a}	37.5^{ab}	40.0^{a}	y = –0.0017x + 39.57, R^2 = 0.2174	y = 0.0113x + 30.33, R^2 = 0.7204
Ca. roseus	15.0^{cd}	10.0^{b}	15.0^{bc}	15.0^{ab}	17.5^{bc}	12.5^{b}	22.5^{bc}	20.0^{ab}	y = 0.0090x + 13.26, R^2 = 0.9797	y = 0.0086x + 9.89, R^2 = 0.7516
Control	5.00^{d}	12.5^{ab}	5.00^{c}	12.5^{b}	5.00^{c}	12.5^{b}	5.00^{c}	12.5^{b}	-	-
Cy. nardus	32.5^{ab}	30.0^{a}	42.5^{a}	32.5^{a}	47.5^{a}	35.0^{a}	47.5^{a}	35.0^{a}	y = 0.0014x + 35.98, R^2 = 0.5797	y = 0.0050x + 30.76, R^2 = 0.6648

Notes:
[1]Based on 10 larvae per replicate; each concentration was replicated four times.
[2]Means followed by the same letter(s) within the same column are not significantly different at 5% level of Tukey's HSD Test.

Sitophilus oryzae Linn., and *Corcyra cephalonica* Stainton – even after three months of aging under laboratory conditions. Lower insect counts in the most effective treatments were probably due to their repellent action. Dadang *et al.* (1998) screened several extracts from rhizomes of five *Curcuma* species against *P. xylostella* and *Ca. chinensis.*

L. camara powder at 10% w/w showed 62.5% repellency against adult *Sitophilus zeamais* Motschulsky (Ogendo *et al.* 2003). Likewise, the ethanolic extract *from L. camara* was reported to be antifeedant, repellent, and toxic against third instar larvae of the *Plecoptera reflexa* Guenee (Meshram 2000). Methanolic extracts from *A. galangal* exhibited high toxicity plus antifeedant and repellent activity against *Ca. chinensis* adults and *P. xylostella* larvae (Dadang *et al.* 1998). Ethanolic extracts from *A. pyramidata* showed 100% oviposition deterrence against *Ca. chinensis,* which was significantly higher than *Cy. nardus* and patchouli, *Pogostemon cablin* Benth. (Thein *et al.* 2013).

Insect Growth Regulatory Activity: Morphogenetic Effects

Second instar larvae of *P. xylostella* were topically applied with 63 µg/g body weight of ethanolic extracts, and mortality and abnormalities were observed until adult emergence. Larval mortality ranged 27.50– 50%, with the highest mortality observed on larvae treated with ethanolic extract from *L. camara* (Table 7). From the larvae that survived, there were about 20.69–40% dead or abnormal pupae – the highest of which was observed again from the larvae treated with ethanolic extract from *L. camara.* Percent adult abnormalities ranged 25–39.13% which was highest in *A. pyramidata* ethanolic extract. Deformed wings were observed from the adults that emerged from the larvae treated with ethanolic extracts.

Seemingly normal pupae emerged as adult and lived for 3–7 d in treated larvae and 4–5 days in the control, while abnormal pupae did not emerge at all. Among the ethanolic extracts, the shortest adult lifespan was recorded in *P. xylostella* larvae treated with *L. camara*, which did not significantly differ with that of *Co. amboinicus* and *Cu. longa* (Table 7). Abnormalities were sometimes observed but longevity was not affected. Javier *et al.* (2017) reported that ethanolic extracts showed insect growth regulatory activities expressed in high larval and pupal mortalities for *Co. amboinicus* and *Cu. longa* – as well as high number of abnormal adults for *Ca. roseus* – but did not affect the longevity of *C. pavonana*.

Among the ethanolic extracts, *L. camara* exhibited potent insect growth regulatory activity against *P. xylostella* – as shown by high larval and pupal mortalities. Exposure of larvae to ethanolic extract appeared to prevent molting of insects and majority died before pupation especially in *L. camara.* This shows physiological effects with larvae failing to initiate the larval pupal molt. Although larvae may complete their developmental stages, some may fail to pupate. Slower rate of development and failure to molt was reported in *P. xylostella* treated with neem extracts (Schmutterer 1995).

Table 7. Effects of ethanolic extracts on the development of DBM at 63 µg/g.

Plant Extracts (63 µg/g)	L-mortality (%)	Survival (%)	Abnormal Pupae	Abnormal Adults	Adult Longevity
A. pyramidata	27.50	72.50	20.69	39.13	4.62[a]
L. camara	50.00	50.00	40.00	25.00	3.86[b]
Co. amboinicus	27.50	72.50	31.03	35.00	4.00[b]
Cu. longa	30.00	70.00	28.57	25.00	4.00[b]
Ca. roseus	30.00	70.00	35.71	27.78	4.75[a]
Control	5.00	95.00	7.89	0.00	4.77[a]

Notes:
[1]Based on 10 larvae per replicate; each concentration was replicated four times.
[2]Means followed by the same letter(s) within the same column are not significantly different at 5% level of Tukey's HSD.

CONCLUSION

Among the five ethanolic extracts, *Cu. longa* was the most toxic against *P. xylostella* (LC_{50} = 203.37 µg/mL acetone) when applied through LRFM, and showed the highest repellency against DBM at 125 µg/mL acetone. *L. camara* was the most toxic against *P. xylostella* (LD_{50} = 98.78 µg/g) through topical application, and showed high antifeedant activity against the larvae at 125 µg/mL acetone. *L. camara* also showed major insect growth regulatory activity against *P. xylostella*, as expressed by high larval and pupal mortalities. Application of *Co. amboinicus* and *Cu. longa* also shortened the adult lifespan of *P. xylostella*. Highest adult abnormalities were observed in *A. pyramidata.*

L. camara is the most effective among the other ethanolic extracts against DBM because it consistently showed the highest contact toxicity and antifeedant property plus remarkable IGR activity against *P. xylostella*. It is also worthwhile to know that *L. camara* provided the highest ethanolic recovery (2.645%) among the other plants. *L. camara* is classified as invasive weed. It has extremely high seed production of 12,000 seeds from each plant per year and can survive in a wide range of climatic conditions, including drought and different soil types. Since it is widely available in the Philippines and grows throughout the year, the prospect of using this locally available plant material as a potential source of insecticide can be considered. In addition, *L. camara* is more widely used as ornamental material in the Philippines. Possible use of the plant as an insecticide will outweigh its use as an ornamental.

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