# Biological Activity of Ethanol Extracts and Essential Oils from *Curcuma longa* (Zingiberaceae), *Cymbopogon nardus* (Gramineae), and *Acorus calamus* (Acoraceae) against *Plutella xylostella* (Lepidoptera: Plutellidae)[1]

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**Abstract** The diamondback moth, *Plutella xylostella* (L.), is a major pest that has developed resistance to many groups of synthetic insecticides. Natural plant products present reliable alternatives for its management. In this study, the activities of essential oils and ethanol extracts from three medicinal plants, i.e., *Curcuma longa* L. (Zingiberaceae), *Cymbopogon nardus* (L.) Rendle (Gramineae), and *Acorus calamus* L. (Acoraceae), from Thailand against *P. xylostella* were evaluated. Four concentrations of the extracts (i.e., 0.625, 1.25, 2.5, and 5%) were tested. The essential oils and ethanol extracts of *Cu. longa*, *Cy. nardus*, and *A. calamus* exhibited different degrees of activity against *P. xylostella*. Of these, both the essential oil and ethanol extract of *A. calamus* at 5% (v/v) concentration exhibited feeding toxicity for *P. xylostella* larvae, resulting in 100% mortality 1 d after treatment, with a median lethal concentration of 0.528% for the essential oil and 1.074% for the ethanol extract. Furthermore, all concentrations of *A. calamus* ethanol extract exhibited contact toxicity to the larvae, resulting in >75% mortality 3 d after treatment. All tested extracts, except *A. calamus* ethanol extract at the 0.625% concentration, exhibited high repellent activity against *P. xylostella* larvae, while *Cu. longa* and *Cy. nardus* ethanol extracts at 5% (w/v) concentration and *A. calamus* essential oil at 2.5% (v/v) concentration deterred oviposition by *P. xylostella* adults with an effective repellency >70%. These results indicate that both essential oils and ethanol extracts of these plants have the potential to be developed as insecticides for use in integrated pest management of *P. xylostella*.

**Key Words** contact toxicity, feeding toxicity, insecticidal activities, oviposition deterrent, repellent activity

The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is an economically important pest of Brassicaceae plants worldwide. Presently, approximately US$4 billion are expended annually for the management of this insect (Sarfraz et al. 2006). Growers primarily use synthetic insecticides to protect their crops from this pest (Kibata 1996), a practice that has led to the development of resistance to many groups of chemical insecticides (Arthropod Pesticide Resistance Database 2020) and elimination of its natural enemies (Ndakidemi et al. 2016). Developing reliable alternatives to synthetic insecti-

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cides, therefore, is essential for the management of this pest (Talekar and Shelton 1993).

Botanical insecticides are traditionally a sustainable alternative for insect control. The insecticidal activities of plant compounds, such as azadirachtin (Huang et al. 2004), rotenone (Madhukar and Matsumura 1979), and nicotine (Chao and Casida 1997), have been extensively studied and are efficacious in insect pest management (Chandler et al. 2011). *Curcuma longa* L., *Cymbopogon nardus* L. Rendle, and *Acorus calamus* L. are commonly found herbs in Thailand and have activity against various insect pests. *Curcuma longa* exerts insecticidal and repellent effects on insect pests (Tavares et al. 2013). For example, it is toxic and repellent to the cutworm, *Spodoptera litura* F., and the cabbage worm, *Crocidolomia pavonana* F. (Javier et al. 2017, 2018). *Cymbopogon nardus* inhibits oviposition and has ovicidal effects on *Helicoverpa armigera* Hübner (Setiawati et al. 2011). Ilahi et al. (2019) reported that a hexane extract of *Cy. nardus* inhibited egg-laying and adult emergence in *Culex quinquefasciatus* Say at 1,000 parts per million (ppm). Extracts from rhizomes of *A. calamus* interrupted feeding and inhibited growth of cutworm larvae (Balakumbahan et al. 2010). *Acorus calamus* rhizomes extracted with methanol and hexane and applied at a concentration of 7.5% completely inhibited eclosion of *P. xylostella* eggs (Matharu and Mehta 2017).

However, the activities of such plant essential oils or ethanol extracts in controlling *P. xylostella* in Thailand need further evaluation. Our objective in the study reported herein was, thus, to determine the feeding and contact toxicities and repellent and oviposition-deterrent activities of essential oils and ethanol extracts of *Cu. longa*, *Cy. nardus*, and *A. calamus* against *P. xylostella* under laboratory conditions.

## Materials and Methods

**Rearing of *P. xylostella*.** *Plutella xylostella* larvae were collected from a field of Chinese kale, *Brassica alboglabra* L.H. Bailey (Brassicaceae), growing in Bang Bua Thong District, Nonthaburi Province, Thailand, and transported to the laboratory where they were maintained on kale at room temperature (27 $\pm$ 2°C and 10L : 14D h) at the Department of Entomology, Kasetsart University. Pupae reared from these larvae were maintained in an amber plastic cage (24 $\times$ 26 $\times$ 23 cm; width, length, height) until adult emergence. A 10% sugar solution was provided as food for the adults, and a 4-d-old Chinese kale seedling was supplied for egg-laying in each cage. Kale plants with the eggs were transferred to cylindrical glass containers (20.3 $\times$ 45.7-cm; diam, height) with fresh kale leaves for larvae emerging from the eggs as described by Auamcharoen et al. (2011). *Plutella xylostella* were reared following this procedure until the third generation when second-instar larvae were used for the bioassays.

**Preparation of plant materials and extraction.** *Cymbopogon nardus* leaves were collected from Phetchaburi Province, western Thailand, in July 2017. *Acorus calamus* rhizomes were obtained from a field in Nonthaburi Province, central Thailand, in November 2017, and *Cu. longa* rhizomes were purchased from a market in Bangkok in July 2017. All plant materials were taxonomically verified in our laboratory. Fresh rhizomes and leaves were cut into small pieces and extracted

by the water-distillation and ethanol maceration techniques for obtaining the essential oils and ethanol extracts, respectively.

In the water-distillation technique, 1 kg of fresh *Cu. longa* and *A. calamus* rhizomes and 200 g of fresh *Cy. nardus* leaves were extracted separately using a Clevenger-type apparatus. The plant samples were placed in a 5-L, round-bottom flask to which 3 L of water was then added. The plant materials were distilled for 8 h at boiling temperature to extract the essential oils as described by Sararit and Auamcharoen (2020). In the ethanol maceration technique, 1 kg of *Cu. longa* and *A. calamus* rhizomes and 300 g of *Cy. nardus* leaves were immersed separately in 2 L and 3.5 L of ethanol, respectively, at room temperature for 3 d. The ethanol solution was then filtered through a Whatman no. 1 filter paper (GE Healthcare UK Limited, Amersham Place, Little Chalfont, Buckinghamshire, U.K.) and evaporated using a rotary evaporator under reduced pressure to obtain the crude ethanol extract. All plant essential oils and ethanol extracts were stored in a refrigerator at 10–12°C until further analyses. The extract stock solutions were diluted to the different concentrations (0.625, 1.25, 2.5, and 5%) for the bioassays.

**Feeding toxicity bioassay.** Leaf discs (2-cm diam) of fresh Chinese kale leaves were placed individually in plastic cups (4 and 2.5 cm; diam × height) each containing moistened cotton and paper from straw pulp. The leaf discs were then coated with 100 µl of the ethanol extract or essential oil of *Cu. longa*, *Cy. nardus*, and *A. calamus* at the appropriate treatment concentrations. The control was treated with Tween-20® (BDH Laboratory Supplies, Poole, BH15 1TD, U.K.) (1%, v/v) in water. The solvents were allowed to evaporate at room temperature after which five, second-instar larvae were transferred to each leaf disc and the cap was placed on the plastic cup. The study was performed in a completely randomized design (CRD) with 10 replicates each of each treatment. Dead *P. xylostella* were counted 1, 2, and 3 d after initial treatment. Larvae not exhibiting any movement on light stimulation with a fine paint brush were considered dead (Silva et al. 2013).

**Contact toxicity bioassay.** One microliter of each treatment solution was applied onto the thorax of each second-instar larva using a Burkard hand microapplicator (Burkard Manufacturing Company Ltd., U.K.). The control group received 1 µl of 1% (v/v) Tween-20 in water. Five treated larvae were then placed in individual plastic cups (4 and 2.5-cm; diam × height) containing a Chinese kale leaf on a moistened cotton pad and straw paper, and the cups were covered with a cap. All treatments were arranged in a CRD with 10 replicates each. Dead *P. xylostella* larvae were recorded 1, 2, and 3 d after initial treatment.

**Repellent activity bioassay.** A Whatman No. 1 filter paper (9-cm diam) was cut in half. and one half was treated with 200 µl of the diluted extract (treatment) while the other half was treated with 200 µl of 1% (v/v) Tween-20 in water (control). Both halves were allowed to dry at room temperature and then joined using adhesive cellulose tape and placed in a plastic Petri dish (9-cm diam). Fresh Chinese kale leaf discs (2-cm diam) were placed in individual cups (2-cm diam) containing wet cotton, which were then placed on the filter paper, one cup per side, as food for *P. xylostella*. Ten, second-instar larvae were then released at the center of the filter paper disc. All treatments were arranged in a CRD with five replicates each. The number of *P. xylostella* larvae on each side of the filter paper was counted 15 and 30 min, and 1, 2, 3, 4, and 5 h after treatment. Percentage repellency (PR) was

calculated by the formula PR = 2(C−50), where C is the percentage of *P. xylostella* larvae remaining on the control side of the filter paper (Talukder and Howse 1995).

**Oviposition deterrent bioassay.** Chinese kale seeds were sown in a plastic cup (9 and 3.5-cm; diam × height) containing moistened cotton pad and tissue paper and allowed to grow for 4 d to obtain seedlings. Each cup containing the seedlings was sprayed with 500 μl of the treatment or control solutions, using a hand sprayer, and placed inside a mesh tent. Female and male adults (30 pairs) were then released inside the mesh tent and a cup of 10% sugar solution was placed at the center of the tent as the food source. All treatments were arranged in a CRD with five replicates each. *Plutella xylostella* eggs were counted after 3 d. The percentage effective repellency (ER) was calculated as % ER = (NC−NT)/NC × 100, where NC and NT indicate the number of *P. xylostella* eggs on control (NC) and treatment (NT) seedlings, respectively (Xue et al. 2001).

**Statistical analyses.** Data were analyzed by one-way analysis of variance (ANOVA) using the "agricolae" package in R (R Development Core Team 2016). Treatment means were compared using Tukey's honestly significant difference (HSD) test and contrast analysis, and differences were considered significant at $P < 0.05$. The median lethal concentration ($LC_{50}$) values were estimated by probit analysis (Finney 1971) in SPSS (Statistical Package for the Social Sciences, Version 19.0, Armonk, NY).

## Results

**Feeding toxicity bioassay.** Significant differences were observed in the mortality of *P. xylostella* larvae treated with the four concentrations of the tested plant extracts (Table 1). *Curcuma longa* essential oil and ethanol extract at concentrations of 1.25, 2.5, and 5% exhibited significantly higher toxicity than that at 0.625% concentration and that of control 3 d after treatment (essential oil: $F = 116.11$; df = 4, 45; $P < 0.001$; ethanol extract: $F = 46.144$; df = 4, 45; $P < 0.001$). The mortality levels of *P. xylostella* larvae treated with 2.5 and 5% concentrations of *C. nardus* essential oil and ethanol extract increased to 100%, 2 or 3 d after treatment. *Cymbopogon nardus* essential oil at concentrations of 2.5 and 5% presented significantly higher mortality than that at 0.625% concentration and control 3 d after treatment ($F = 53.062$; df = 4, 45; $P < 0.001$), whereas *Cy. nardus* ethanol extract at all tested concentrations exhibited significantly higher mortality than the control ($F = 269.18$; df = 4, 45; $P < 0.001$). The effects of *A. calamus* essential oil and ethanol extract increased with increasing concentration. *Plutella xylostella* larvae exhibited a mortality level of 100% after 1 d of treatment with both extracts at 5% concentration. Larval mortality in treatments with essential oil at all concentrations was significantly higher than that of the control ($F = 946.71$; df = 4, 45; $P < 0.001$), whereas mortality of larvae treated with ethanol extract at 1.25, 2.5, and 5% concentrations was significantly higher than that of larvae treated with 0.625% ethanol extract and the control ($F = 291.32$; df = 4, 45; $P < 0.001$) 3 d after treatment. The $LC_{50}$ values after 2 d of exposure to *Cu. longa* essential oil and ethanol extract were 0.53% (95% confidence interval [CI] = 0.39–0.64; Slope ± SE = 2.61 ± 0.35) and 0.62% (95% CI = 0.50–0.73; Slope ± SE = 2.94 ± 0.36), respectively, which were not statistically significant based on their overlapping 95%

**Table 1. Cumulative mean (±SE) mortality (percent) of *P. xylostella* larvae caused by essential oils and ethanol extracts of *Cu. longa*, *Cy. nardus*, and *A. calamus* on treated leaf discs.**

| Plant | Concentration (%) | Mean (±SE) Mortality (%) at d Postexposure* | | | | | |
|---|---|---|---|---|---|---|---|
| | | Essential Oil | | | Ethanol Extract | | |
| | | 1 d | 2 d | 3 d | 1 d | 2 d | 3 d |
| *Cu. longa* | 0 | 0 ± 0.0b | 2 ± 2.0c | 6 ± 3.1c | 2 ± 2.0b | 6 ± 4.3c | 18 ± 6.3c |
| | 0.625 | 24 ± 5.8b | 60 ± 7.9b | 74 ± 6.0b | 16 ± 6.5b | 54 ± 9.5b | 62 ± 8.7b |
| | 1.25 | 66 ± 7.9a | 82 ± 7.6a | 94 ± 4.3a | 20 ± 7.3b | 82 ± 9.2a | 94 ± 3.1a |
| | 2.5 | 76 ± 9.3a | 96 ± 4.0a | 98 ± 2.0a | 64 ± 8.3a | 96 ± 2.7a | 96 ± 2.7a |
| | 5 | 82 ± 5.5a | 100 ± 0.0a | 100 ± 0.0a | 68 ± 8.5a | 100 ± 0.0a | 100 ± 0.0a |
| *Cy. nardus* | 0 | 0 ± 0.0b | 2 ± 2.0c | 6 ± 3.1c | 0 ± 0.0b | 2 ± 2.0b | 6 ± 3.1b |
| | 0.625 | 6 ± 4.3b | 54 ± 13b | 70 ± 10b | 58 ± 4.7a | 86 ± 6.7a | 98 ± 2.0a |
| | 1.25 | 24 ± 6.5b | 66 ± 10.8b | 90 ± 6.1ab | 56 ± 6.5a | 92 ± 4.4a | 94 ± 4.3a |
| | 2.5 | 68 ± 8.5a | 100 ± 0.0a | 100 ± 0.0a | 64 ± 4.0a | 90 ± 3.3a | 100 ± 0.0a |
| | 5 | 64 ± 9.8a | 100 ± 0.0a | 100 ± 0.0a | 74 ± 8.5a | 100 ± 0.0a | 100 ± 0.0a |
| *A. calamus* | 0 | 0 ± 0.0c | 2 ± 2.0b | 6 ± 3.1b | 0 ± 0.0d | 2 ± 2.0c | 6 ± 3.1c |
| | 0.625 | 66 ± 7.9b | 96 ± 4.0a | 100 ± 0.0a | 36 ± 8.3c | 84 ± 5.0b | 88 ± 4.4b |
| | 1.25 | 58 ± 10.1b | 96 ± 4.0a | 100 ± 0.0a | 56 ± 7.2bc | 98 ± 2.0a | 100 ± 0.0a |
| | 2.5 | 80 ± 6.0ab | 98 ± 2.0a | 100 ± 0.0a | 68 ± 6.8b | 100 ± 0.0a | 100 ± 0.0a |
| | 5 | 100 ± 0.0a | 100 ± 0.0a | 100 ± 0.0a | 100 ± 0.0a | 100 ± 0.0a | 100 ± 0.0a |

* Treatment means followed by the same lowercase letter within a column and within a plant are not significantly different ($P > 0.05$, Tukey's HSD test, $n = 50$ larvae).

CIs. The $LC_{50}$s of *Cy. nardus* essential oil and ethanol extract were 0.462% (95% CI = 0.33–0.56; Slope ± SE = 3.25 ± 0.54) and 0.422% (95% CI = 0.01–0.89; Slope ± SE = 0.51 ± 0.19), respectively, which were not statistically significant based on their overlapping 95% CIs. The $LC_{50}$s of *A. calamus* essential oil and ethanol extract were 0.01% (95% CI = 0.00–0.12; Slope ± SE = 0.93 ± 0.46) and 0.35% (95% CI = 0.16–0.46; Slope ± SE = 3.74 ± 0.97), respectively, which were statistically significant based on their nonoverlapping 95% CIs. No significant differences, based on contrast analysis ($P > 0.05$), were observed in the cumulative mortality of *P. xylostella* larvae treated with the lowest effective concentration (1.25%) of the three tested extracts.

**Contact toxicity bioassay.** Mortality of *P. xylostella* tended to increase from 1 to 3 d after treatment (Fig. 1 A–F). The percentage mortality of *P. xylostella* treated with *C. longa* essential oil increased with increasing concentration (Fig. 1A). All tested concentrations of *Cu. longa* essential oil yielded <50% mortality 1 and 2 d after treatment. At the end of the experiment, 0.625%, 1.25%, 2.5% and 5% essential oil resulted in mean (±SE) mortality of 24.0 ± 8.8%, 54.0 ± 8.5%, 56.0 ± 10.2%, and 60.0 ± 8.9%, respectively, of which the latter three were significantly different from that of the control ($F = 6.436$; df = 4, 45; $P < 0.001$). *Plutella xylostella* larvae treated with all tested concentrations of *C. longa* ethanol extract exhibited <50% mortality 3 d after treatment (Fig. 1B). The mortality of *P. xylostella* larvae treated with *C. nardus* essential oil and ethanol extract increased slightly with increasing concentration (Fig. 1C–D). *Plutella xylostella* larvae treated with *Cy. nardus* essential oil and ethanol extract at 5% concentration exhibited mortality levels >80%, whereas those treated with the other concentrations presented <60% mortality rates 2 d after treatment. *Cymbopogon nardus* essential oil at 2.5% concentration yielded a mortality of almost 80% 3 d after treatment (Fig. 1C). Mortality of *P. xylostella* larvae treated with *A. calamus* essential oil and ethanol extract was not affected by concentration (Fig. 1E, F). Mortality in treatment with 2.5% essential oil was not significantly different from that of the control, 1 and 2 d after treatment ($P > 0.05$) (Fig. 1E). Mortality of *P. xylostella* treated with essential oil at all concentrations was <70% 3 d after treatment. On the contrary, treatment with ethanol extract at all concentrations yielded mortality levels of nearly 80 and 90%, except with the 2.5% concentration which resulted in nearly 70 and 80% mortality, 2 and 3 d after treatment, respectively (Fig. 1F). All tested concentrations of ethanol extracted yielded significantly higher *P. xylostella* mortality levels when compared with the control (Day 2: $F = 20.121$; df = 4, 45; $P < 0.001$; Day 3: $F = 37.456$; df = 4, 45; $P < 0.001$). Based on contrast analysis of cumulative percentage mortality, *Cu. longa* essential oil at 5% concentration exhibited significantly higher contact toxicity than its ethanol extract at the same concentration ($F = 5.803$; df = 1, 18; $P = 0.027$), whereas both essential oil and ethanol extract of *Cy. nardus* ($F = 0.086$; df = 1, 18; $P = 0.773$) and *A. calamus* did not result in significantly different mortality levels ($F = 3.556$; df = 1, 18; $P = 0.076$). The contact toxicity of *Cy. nardus* essential oil was significantly higher than that of *C. longa* ($F = -7.187$; df = 1, 27; $P = 0.012$), whereas that of *Cy. nardus* and *A. calamus* ethanol extracts was significantly higher than that of *Cu. longa* ($F = -40.005$; df = 1, 27; $P = 0.012$).

**Repellent activity bioassay.** The results indicated positive percentages of *P. xylostella* larval repellency with all treatments except *A. calamus* ethanol extract at

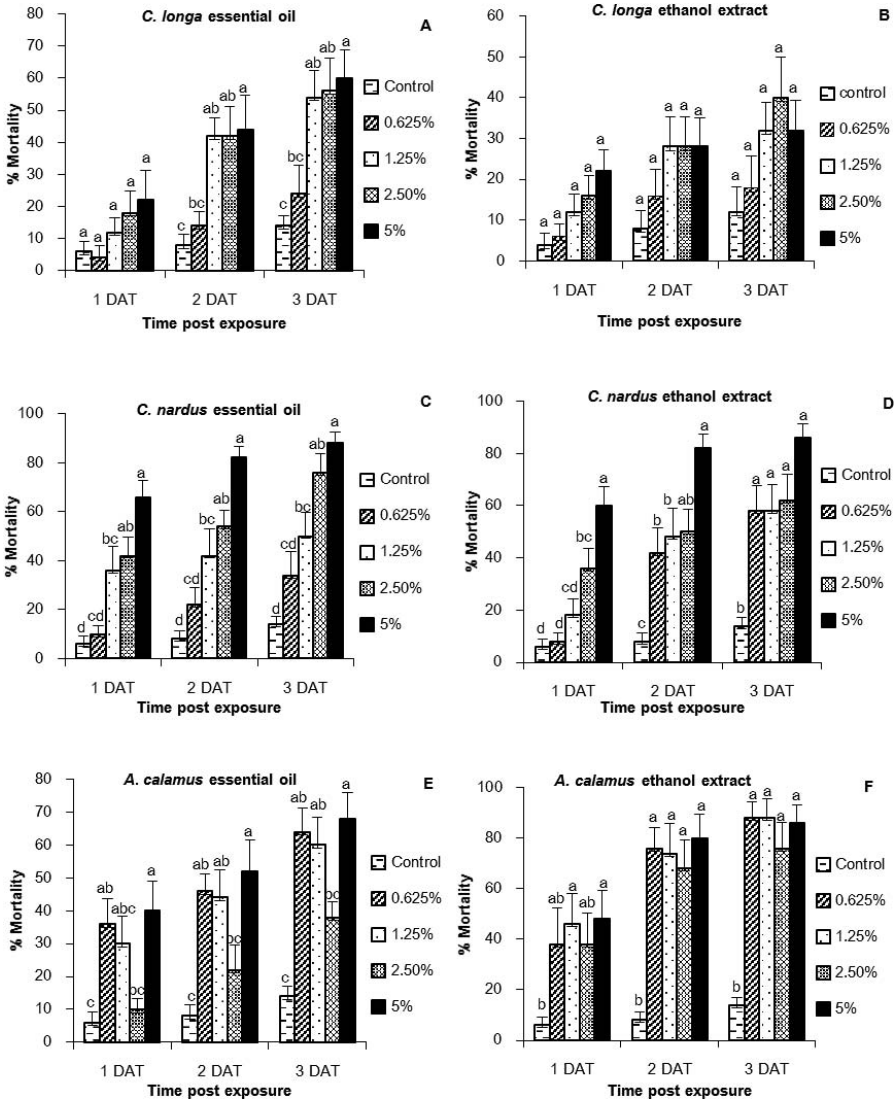


**Fig. 1. Cumulative mean (±SE) mortality (percent) of *P. xylostella* larvae treated with essential oils and ethanol extracts of *C. longa, C. nardus*, and *A. calamus.* Different letters over the bars indicate significantly different treatment means ($P < 0.05$, Tukey's HSD test, $n = 50$ larvae).**

0.625% concentration (Fig. 2A–F). The percentage repellency was variable between 15 min to 2 h after treatment, and then stabilized until 5 h after treatment. The repellency percentages did not different among the tested concentrations of most plant extracts, except *Cu. longa* essential oil (Fig. 2A) and *Cy. nardus* (Fig. 2D) and *A. calamus* ethanol extracts (Fig. 2F). The highest concentration (5%) of

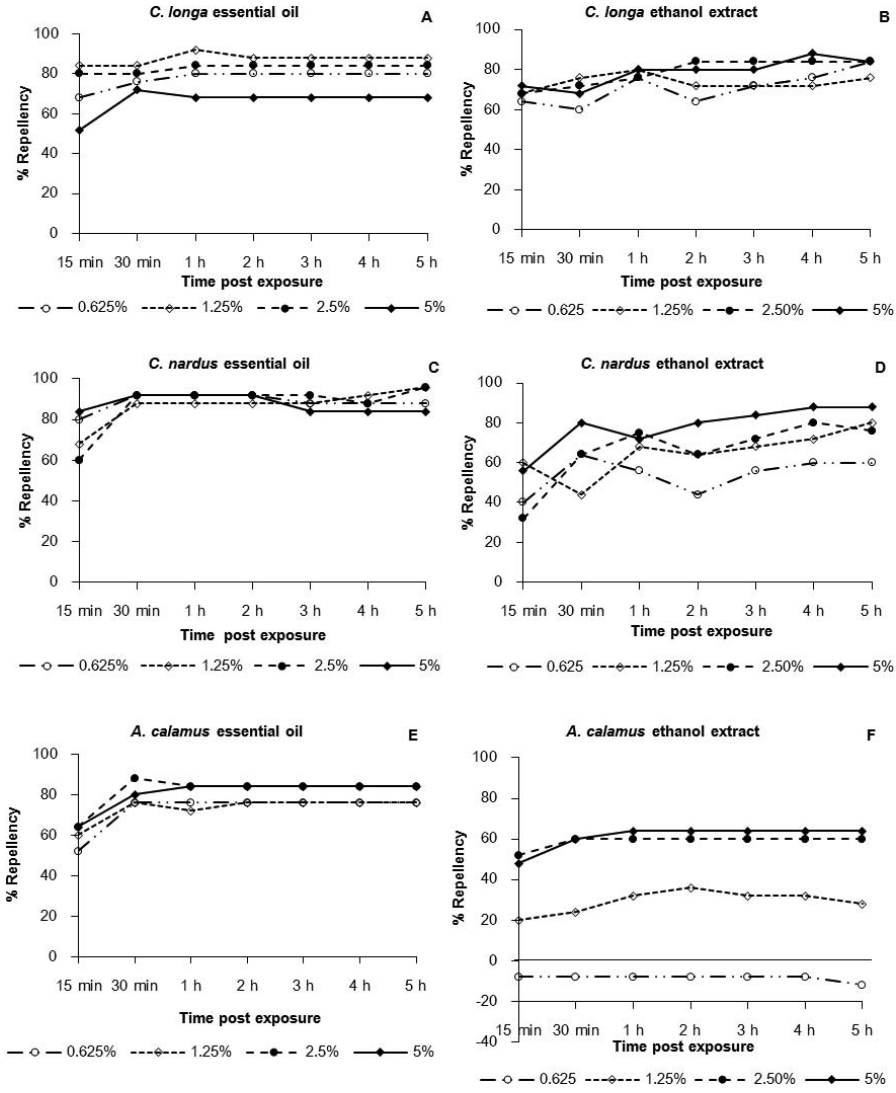


**Fig. 2. Repellency (percent) of essential oils and ethanol extracts of *Cu. longa*, *Cy. nardus*, and *A. calamus* to *P. xylostella* larvae on filter paper ($n = 50$ larvae).**

*Cu. longa* essential oil yielded a low repellency percentage (68.0% 1 h after treatment) when compared with other tested concentrations (Fig. 2A). Conversely, the lowest concentration (0.625%) of *Cy. nardus* ethanol extract resulted in a low repellency percentage (60.0% 5 h after treatment) when compared with the other concentrations 1 to 5 h after treatment (Fig. 2D). However, *A. calamus* ethanol extract at 0.625% concentration did not exhibit repellent activity (PR = −12%)

**Table 2. Mean (±SE) number of oviposited eggs of *P. xylostella* on seedlings treated with essential oils and ethanol extracts of *Cu. longa*, *Cy. nardus*, and *A. calamus*.**

| | | Essential Oil | | Ethanol Extract | |
|---|---|---|---|---|---|
| Plant | Concentration (%) | Mean (±SE) Eggs at 3 d Postexposure | Effective Repellency (%) | Mean (±SE) Eggs at 3 d Postexposure | Effective Repellency (%) |
| *Cu. longa* | 0 | 84.80 ± 26.61 | 0.00 | 84.80 ± 26.61 | 0.00 |
| | 0.625 | 106.60 ± 43.87 | −25.71 | 35.00 ± 8.98 | 58.73 |
| | 1.25 | 64.20 ± 14.38 | 24.29 | 41.80 ± 13.85 | 50.71 |
| | 2.5 | 64.80 ± 26.34 | 23.58 | 40.40 ± 11.59 | 52.36 |
| | 5 | 34.00 ± 9.70 | 59.91 | 24.20 ± 9.76 | 71.46 |
| *Cy. nardus* | 0 | 84.80 ± 26.61 | 0.00 | 84.80 ± 26.61 | 0.00 |
| | 0.625 | 58.80 ± 11.24 | 30.66 | 63.20 ± 14.13 | 25.47 |
| | 1.25 | 75.40 ± 16.58 | 11.08 | 34.00 ± 13.49 | 59.91 |
| | 2.5 | 45.00 ± 12.17 | 46.93 | 51.60 ± 25.47 | 39.15 |
| | 5 | 46.20 ± 18.04 | 45.52 | 17.80 ± 9.72 | 79.01 |
| *A. calamus* | 0 | 84.80 ± 26.61 | 0.00 | 84.80 ± 26.61 | 0.00 |
| | 0.625 | 37.20 ± 11.24 | 56.13 | 50.80 ± 16.47 | 40.09 |
| | 1.25 | 42.20 ± 12.73 | 50.24 | 46.80 ± 15.12 | 44.81 |
| | 2.5 | 24.60 ± 4.06 | 70.99 | 33.00 ± 7.27 | 61.08 |
| | 5 | 28.00 ± 12.03 | 66.98 | 27.00 ± 7.38 | 68.16 |

* Treatment means within the same column and within a plant are not differ significantly ($P > 0.05$, Tukey's HSD test, $n = 5$ seedlings).

against *P. xylostella* larvae (Fig. 2F). *Cymbopogon nardus* essential oil yielded repellency percentages ranging between 84 and 96% (Fig. 2C), followed by *C. longa* ethanol extract (76–84%) (Fig. 2B) and *A. calamus* essential oil (76–84%), 5 h after treatment (Fig. 2E).

**Oviposition deterrent bioassay.** No significant differences could be detected in the number of eggs oviposited on the control seedlings and those treated with four concentrations of the three plant essential oils and ethanol extracts ($P > 0.05$) (Table 2). Effective repellency greater than 70% was observed in the treatment with 5% *Cu. longa* (71.5%) and *Cy. nardus* (79.0%) ethanol extracts and 2.5% *A. calamus* essential oil (71.0%). Based on contrast analysis, the number of eggs on seedlings treated with the tested plant extracts at 5% concentration was not significantly different ($P > 0.05$).

## Discussion

In this study, the tested plant extracts exhibited different biological activities against *P. xylostella*, while some extracts obtained using different methods presented similar responses. Both ethanol extract and essential oil of *A. calamus*, at all tested concentrations, exhibited immediate feeding toxicity to *P. xylostella*, resulting in >80% mortality 2 d after treatment; toxicity increased with increasing concentration. Furthermore, *A. calamus* ethanol extract exhibited high contact toxicity to *P. xylostella* larvae with >75% mortality. Our results are consistent with those of Tewary et al. (2005), who reported a 30% mortality of *P. xylostella* second-instar larvae following treatment with 1% *A. calamus* essential oil residue. The high toxicity of *A. calamus* essential oil to *P. xylostella* larvae also was reported by Reddy et al. (2015) and Kumar et al. (2016).

A primary chemical compound in *A. calamus* rhizomes is benzene, 1,2,4-trimethoxy-5-(1-propenyl)-(*Z*), or β-asarone. This chemical is responsible for feeding toxicity in insects, as it destroys the intestinal wall after consumption, leading to death (Melani et al. 2016). It also exhibits contact toxicity to insects by entering the hemolymph via the cuticle and blocking acetylcholinesterase function, resulting in nervous system disorder (Oh et al. 2004).

Furthermore, *A. calamus* essential oil and ethanol extract acted as oviposition deterrents of *P. xylostella* female adults in this study. However, the repellent activity of *A. calamus* ethanol extract was low when compared to other plant extracts, resulting in <70% effective repellency at the highest concentration tested, and its activity at the lowest concentration tested did not differ from that observed with the control. Yao et al. (2008) reported that *A. calamus* ethanol extract was highly repellent, with a high contact toxicity, to *Sitophilus zeamais* Motschulsky adults.

For *C. longa*, both the essential oil and ethanol extract exhibited feeding toxicity to *P. xylostella* larvae with >80% mortality following exposure to both (1.25–5%) 2 d after treatment. Vanichpakorn et al. (2010) reported a low feeding toxicity, with <20% mortality, with 1,250 ppm *Cu. longa* acetone, 95% ethanol, ethyl acetate, and petroleum ether extracts on *P. xylostella* second-instar larvae. Both *Cu. longa* extracts in the present study yielded <60% mortality in the contact toxicity analysis. Javier et al. (2016) noted 90% mortality of *P. xylostella* larvae exposed to 251.13 μg/g of *Cu. longa* essential oil in a topical bioassay. In the present study, *Cu. longa* ethanol extract exhibited high repellent and oviposition deterrent activities to *P. xylostella* larvae and adult females, respectively. The repellency of crude extracts and essential oils of *Cu. longa* against adults of several stored-product insects has previously been evaluated (Damalas 2011). Ar-turmerone (44.4%), β-turmerone (26.5%), and α-turmerone (20.8%) have been reported in *Cu. longa* rhizomes (Ajaiyeoba et al. 2008). Of these, ar-turmerone exhibits repellent activity against *Si. zeamais*. Ten microliters of this compound mixed with 20 g of corn grain remained unaffected by pests for 45 d. Low doses of this chemical also are toxic *Si. zeamais* and *S. frugiperda* (J.E. Smith) (Tavares et al. 2013).

Our results indicated higher contact toxicity of *Cy. nardus* essential oil and ethanol extract to *P. xylostella* larvae than that of *Cu. longa*. Moreover, *Cy. nardus* extracts, particularly the essential oil, exhibited high repellent activity against *P.*

*xylostella* larvae. Kianmatee and Ranamukhaarachchi (2007), in their study on the insect pest-repellent effects of planting *Cy. nardus*, *Lycopersicon esculentum* Mill, *Capsicum frutescens* L., *Coriandrum sativum* L., *Ocimum basilicum* L., *Ocimum sanctum* L., and *Angelonia goyazensis* Benth in Chinese kale fields, reported that *Cy. nardus* was the most suited repellent for *Sp. litura* F. and *O. sanctum* was the most effective against *Phyllotreta sinuate* Stephens and *Hellula undalis* F. *Cymbopogon nardus* essential oil also has been previously recommended for repelling *Bemisia tabaci* Gennadius, *Oryzaephilus surinamensis* L., and *Si. zeamais* (Lambrano et al. 2015, Saad et al. 2017). Moreover, *Cy. nardus* essential oil at 4,000 ppm inhibited oviposition activity (53–66%) and reduced egg hatching (up to 95%) in *Helicoverpa armigera* Hübner when compared with the control (Setiawati et al. 2011). These findings are consistent with our observation that *Cy. nardus* inhibited oviposition in *P. xylostella*; however, the number of eggs laid was variable under the different concentrations tested (0.625–5%) in our study.

Our study demonstrated the activity of ethanol extracts and essential oils from *Cu. longa* rhizomes, *Cy. nardus* leaves, and *A. calamus* rhizomes against *P. xylostella*. Extracts from the same plant exhibited different levels of activity against *P. xylostella*. *Curcuma longa* essential oil and ethanol extract exhibited feeding toxicity to *P. xylostella* larvae and its ethanol extract also acted as an oviposition deterrent in female adults. *Cymbopogon nardus* essential oil and ethanol extract exhibited repellent activity and feeding toxicity to these larvae, respectively. *Acorus calamus* essential oil and ethanol extract presented feeding and contact toxicities to *P. xylostella* larvae, respectively. Thus, all tested plant materials have the potential to be developed into botanical insecticides for controlling *P. xylostella* in the field.

However, the ethanol extracts of *Cu. longa*, *Cy. nardus*, and *A. calamus* were semisolid with a dark color. Thus, application of these extracts at a high concentration may leave color residues on the leaves, which is not desirable for the producers or consumers. These may need to be applied at low concentrations. Conversely, the essential oils of *Cu. longa*, *Cy. nardus*, and *A. calamus* were light-colored liquids with a subtle fragrance. Their color is not apparent on leaves, while the odor can effectively repel insect pests. In addition, based on yield extraction, the production cost of ethanol extract is lesser than that of essential oil. These three plant species are commercially important because of their medicinal and insecticidal properties and ease of reproduction, implying that they can be field-cultivated by the producers themselves and developed into plant-derived insecticides for domestic and commercial purposes. Consequently, growers can reduce agricultural production costs by using eco-friendly botanical insecticides instead of synthetic insecticides, and may derive more benefits by cultivating high-demand industrial plants.

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