Evaluation of Insecticidal Properties of Four Essential Oils from *Illicium verum*, *Curcuma longa*, *Foeniculum vulgare*, and *Ocimum tenuiflorum* against *Spodoptera exigua*.

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Abstract. Four plant essential oils from *Illicium verum* (Star anise), *Curcuma longa* (Tumeric), *Foeniculum vulgare* (Sweet funnel), and *Ocimum tenuiflorum* (Holy basil) were tested against the 2nd instar larvae of the beet armyworm, *Spodoptera exigua* in order to evaluate their killing efficiency, and antifeedant activity by using leaf dipping bioassay. The results revealed that the essential oils from *Illicium verum* and *Curcuma longa* were the highest effective essential oils in having killing, and antifeedant property against the insect. At 24 hours, they caused 100% mortality with only 0.750% (v/v) concentration and showed the LC50 of 0.438 and 0.413%, respectively. Both essential oils at 0.500% concentration expressed the antifeedant property at 100% within 24 hours. So that, the essential oils of *Illicium verum* and *Curcuma longa* have a potential to be applied as botanical insecticide for *Spodoptera exigua* control management.

1. Background
The quantity and quality of the product are the most important output of agricultural production. The insect pest is always mentioned as the most problematic of crop production. Using the chemical pesticides has been very effective in reducing pest infection and increasing agricultural production and economic growth. However, it has an effect on the environment, reduced agricultural, negatively impacted on agricultural production, and ill-health of human (Pimentel et. al., 1992; Pimentel and Greiner, 1997). Additionally, targeted pests have developed resistance to pesticides resulting in rapid increase in pesticide use. Therefore, an increase in using pesticide for pest control has led to an increase in the poison of many species of economic pests due to the demolition of non target species and natural predators of pests and parasites (Pimentel et al., 1992) and affects the economic system. Using the synthetic insecticides causes some anxiety about their adverse effect on the environment. In the present, some farmers use the plant extracts for controlling pests. The compound of plant extracts are eco-friendly because of they are easily degraded, safe to non-target organism, and cheaper than imported insecticide (Lee, et. al., 2001).
The beet armyworm, *Spodoptera exigua*, is one of the most destructive pests of agricultural vegetables, fruits and crops because it can destroy as much as 50% of crop product (Toby, 2019). It occurs worldwide because of higher reproductive rate and migration. The larvae feed sociable by initially grinding the surface of the leaf and result to high level of economic agricultural losses. The methods of pest control are chemical, biological, and physical control. It has been heavily depending on the use synthesis pesticide. The long continued long term of problems is increasing corroboration of negative environmental, health of living things and inflexible environmental regulation of pesticides (Ozkara, et. al., 2016).

According to the following reports, herbal extracts can be used for pest control. Yinghui, et. al., 2014 showed the essential oil extracted from fructification of *Illicium verum* against the instar larvae and pupa of *Aedes albopictus* and *Culex pipens*. Jia. et. al., 2018 presented the ethanolic extracts of *Curcuma longa* root, the ar-turmerone and 8-hydroxyl-ar-turmerone of *Curcuma longa* against the 4th-instar larvae of *Culex pipens* with high efficiency after 24 h of treatment. Abbas. et. al., 2015 revealed the Arturmerone and curemimoids of *Curcuma longa* rhizome and leaf against *Aedes aegypti* and *Anopheles quadrimaculatus*. Diara, et. al., 2015 reported the essential oil of *Foeniculum vulgare* displayed strong larvicidal effect against *Aedes aegypti*. L. B. Gende, et. al., 2009 carried out the *Pimpinella anisum* and *Foeniculum vulgare* essential oils against *Paenibacillus larvae* and both oils presented great similarity in physiochemical properties values and antimicrobial activity. Sarita, et. al., 2017 demonstrated *Ocimum basilicum* leaves against *Aedes aegypti* as a suitable and eco-safe alternate to chemical insecticides as well as Zorica, et. al., 2013 showed the essential oil of sweet basil, *Ocimum basilicum* tested for their antifeedant properties against 2nd instar gypsy moth larvae, *Lymantria dispar*.

Above mentioned implies that plant extract or plant essential oils play an important role in protection methods against insect pests. In this research, the effectiveness of four essential oils, namely *Illicium verum*, *Curcuma longa*, *Foeniculum vulgare*, and *Ocimum tenuiflorum* were evaluated for their insecticidal activities against *Spodoptera exigua* by using leaf dipping bioassay.

## 2. Methods

### 2.1. Essential oils preparation

Essential oils from dried flower of star anise *Illicium verum*, fresh rhizome of tumeric, *Curcuma longa*, seed of sweet fennel, *Foeniculum vulgare*, and fresh leaves of holy basil, *Ocimum tenuiflorum* which have been previously reported to contain insecticidal properties against many insect pests were selected to test. All essential oils were purchased from Thai-China Flavours and Fragrances Industry Co. Bangkok, Thailand. Essential oils were diluted in distilled water plus tween-20 to be 0.25, 0.50, 0.75, 1.00, and 1.25% (v/v) concentrations where the control group used was 1.25 % of tween-20 plus distilled water.

### 2.2. Insect samples

The insect culture of *Spodoptera exigua* was maintained in laboratory conditions at 25°C and 12:12 light dark. The second instar larvae were used in this study.

### 2.3. Bioassay

Leaf dipping method was applied, when 3 CM diameter leaves of Chinese cabbage were dipped in 0.25, 0.50, 0.75, 1.00, and 1.25% concentrations of essential oil and also control group. After drying under shade at room temperature, five treated leaves were placed in circle box. Then, ten individuals of second instar larvae were released in each box. The mortality was observed after 24 h and Chinese cabbage leaf consumption percent was calculated over graph paper.
2.4. Data analysis
The completely randomized design (CRD) with 3 replications was applied. The data obtained were statistically analyzed by via probit analysis for LC50 values.

3. Results and discussion
The essential oils of *Illicium verum* and *Curcuma longa* treatments performed the highest killing rate against the larvae of *Spodoptera exigua* with 0.75% concentration in which caused 100% mortality at 24 hours, those showed the LC50 of 0.438 and 0.413%(v/v), respectively, whereas, the essential oils of *Foeniculum vulgare*, and *Ocimum tenuiflorum* gave the highest killing rate against the larvae of *Spodoptera exigua* with 1.25% concentration in which caused 100% mortality at 24 hours, those showed the LC50 of 0.601 and 0.646%(v/v), respectively (Table1). The essential oils from *Illicium verum* and *Curcuma longa* showed the highest antifeedant property against the larvae at the 0.50% concentration where the essential oils from *Foeniculum vulgare*, and *Ocimum tenuiflorum* at 0.75% concentration presented the highest antifeedant property against the larvae (Table2).

3.1 Table 1 The mortality of second instar larvae of *Spodeptera exigua* caused by different plant essential oils and their LC50 values.

| Essential oil          | Concentration (%), (v/v) | Average mortality percentage | LC50  |
|------------------------|---------------------------|-------------------------------|-------|
|                        | 0                         | 0.25                          | 0.50  | 0.75  | 1.00  | 1.25  |
| *Illicium verum*       | 0 ± 0.00                  | 46.66 ± 0.00                  | 66.66 ± 5.77 | 100 ± 0.00 | 100 ± 0.00 | 100 ± 0.00 | 0.438  |
| *Curcuma longa*        | 0 ± 0.00                  | 53.33 ± 0.00                  | 83.33 ± 5.77 | 100 ± 0.00 | 100 ± 0.00 | 100 ± 0.00 | 0.413  |
| *Foeniculum vulgare*   | 0 ± 0.00                  | 30.00 ± 5.77                  | 53.33 ± 5.77 | 66.66 ± 0.0 | 73.33 ± 0.0 | 100 ± 0.00 | 0.601  |
| *Ocimum tenuiflorum*   | 0 ± 0.00                  | 30.00 ± 4.71                  | 53.33 ± 5.77 | 56.66 ± 0.0 | 70.00 ± 0.0 | 100 ± 0.00 | 0.646  |

3.1 Table 2 The antifeedant activity of fresh chineese cabbage leaf after treated with different plant essential oils against *Spodoptera exigua* larvae.

| Essential oil          | Concentrations (%) | Leaf feeding area (%) |
|------------------------|--------------------|-----------------------|
|                        | 0                  | 0.25                  | 0.50  | 0.75  | 1.00  | 1.25  |
| *Illicium verum*       | 100 ± 0.00         | 8.33 ± 0.00           | 0 ± 0.00 | 0 ± 0.00 | 0 ± 0.00 | 0 ± 0.00 |
| *Curcuma longa*        | 100 ± 0.00         | 5.56 ± 0.00           | 0 ± 0.00 | 0 ± 0.00 | 0 ± 0.00 | 0 ± 0.00 |
| *Foeniculum vulgare*   | 100 ± 0.00         | 36.11 ± 0.00          | 13.89 ± 5.77 | 0 ± 0.00 | 0 ± 0.00 | 0 ± 0.00 |
| *Ocimum tenuiflorum*   | 100 ± 0.00         | 44.44 ± 4.71          | 19.44 ± 5.77 | 0 ± 0.00 | 0 ± 0.00 | 0 ± 0.00 |

Our obtained results were similar to the previous other studies, particulary their chemical compound activities such as the tumeric powder from Curcuma longa rhizome and its derivaties caused 10-20% mortality in third instar *Trichoplusia ni* at a very low dose, 10 µg/larva by contact toxicity (Wagner, et. al., 2016). A treatment of 1% (m.v-1) acetonic solution of ar-turmerone mixed in an artificial diet produced 58.3% mortality of one-day-old larvae of the fall armyworm, *Spodoptera frugiperda* after 10 days of feeding (Smith, 1797). The compound ar-turmerone caused 100 and 64% mortality of adult brown planthopper, Nilaparvata lugens (Stal., 1854). Curcuminoids of turmeric rhizome powder showed the growth inhibitory activity against the desert locust, *Schistocerca gregaria* and tumeric oil showed 10% growth inhibition and 60% nympha mortality in *Schistocerca gregaria* (Forsskal, 1775). The tumeric extract controlled the peach fruit fly and growth inhibition (Abdul, 2011). The 50 µgml-1 tumeric leaf extract showed the highest killed activity within 24 h. It may use to control instar larvae of *Aedes aegypti*. This experiment implied an eco-friendly and low-cost approach (Kalyani, et. al., 2017). The essential oil of *Illium verum* demonstrated high larvicidal activity with a LC50 < 18 mgL-1. Eucalyptol and caryophyllene compound of the *Illicium verum* extract showed the least significant
activity against mosquito larvae (Athanasios, 2012). The *Illicium verum* showed high insecticidal property against adults of *Tribolium castaneum* and *Sitophilus zeamais* by the fumigant toxicity and repellency (SH Ho., 1997). The essential oil of the *Illicium verum* was also tested against the third instar larvae of *Aedes aegypti* and presented the highest larval mortality with LC50 28.2 µg mL⁻¹ and adult mortality with LC50 10.3 µg mg female⁻¹ (Diego, 2018). The effectiveness of leaf and flower extracts of *Ocimum sanctum* against fourth instar larvae of *Aedes aegypti* and *Culex quinquefasciatus* was investigated and showed the highest larval mortality (Mohamed, 2008). This interesting result obtained when 99% mortality of *Aedes aegypti* larvae was found when it treated with 37.1 and 52.4 µL L⁻¹ of *Foeniculum vulgare* essential oils from Cape Verde and Portugal, respectively. It displayed strong larvicial effect against *Aedes aegypti* (Diara., 2015). The essential oil of *Foeniculum vulgare* seed showed larvicial activity caused 90% mortality for the fourth instars larvae of *Culex pipens* at concentration 60 mg/L after 4h (Safia, 2014). So that those two essential oils demonstrated high potential to be used as botanical insecticide. These oils contained insecticidal properties in terms of successfully killing larvae, and antifeedant effect. In the future, field study is still needed.

4. Conclusion

*Illicium verum* and *Curcuma longa* were the highest effective essential oils in having killing, and antifeedant property against the insect. At 24 hours, they caused 100% mortality with only 0.750% (v/v) concentration and showed the LC50 of 0.438 and 0.413%, respectively. Both essential oils at 0.500% concentration expressed the antifeedant property at 100% within 24 hours.

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