Cloning and Its Expression Respond to Starvation Plant Essential Oils as Growth Inhibitor against *Spodoptera Exigua*

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Abstract: One of the main problems in Chinese cabbage culture is the destruction by insect pests. Normally, the beet armyworm, Spodoptera exigua, is the major insect pest in Chinese cabbage produced in Thailand. The aim of this experiment was to study the influence of plant essential oils as growth inhibitor against the secondary stage instar larvae of the beet armyworm. The essential oils from Illicium verum, Curcuma longa, Foeniculum vulgare, and Ocimum tenuiflorum at varied concentrations; 0.00, 0.25, 0.50, 0.75, 1.00, and 1.25 % (v/v) and ten secondary stage instar larvae were used at each level of concentration in the experiment with 3 replicates. The growth stage, the development period from larva to pupa, and pupa to adult stage, and the percentage of mortality were observed. The results showed that the highest growth inhibition was at 0.75% concentration of Illicium verum and Curcuma longa. The percentage of mortality of pupa and adult stages were 100% at 0.75% concentration of *Illicium verum* and *Curcuma longa*. The development period from larvae to pupa was 5.67±5.77 days at 0.50% concentration of Illicium verum and Curcuma longa. The development period from pupa to adult was 8.67±5.77 days at 0.50% concentration of Illicium verum and Curcuma longa. The result also showed that at 0.50% concentration of Illicium verum and Curcuma longa, the development period of beet armyworm from larvae to pupa stage and from pupa to adult stage was less than the development period of the control group. As a result, the growth stage was incomplete and the survival rate was affected. Therefore, these essential oils can be developed as the effective botanical insecticides against the beet armyworm.

1 INTRODUCTION

Beet armyworms, *Spodoptera exigua*, are commonly found on several agricultural crops (Smagghe and Degheele, 1994), especially on Chinese cabbage. Controlling pests by using chemical insecticide can lead to insecticide resistance in which the pests have developed the resistance to the insecticide (Osorio et. al., 2008), resulting in an increase in the amount of chemicals used for pest control or an increase in usage of various chemicals, which will have an adverse effect on the environment and can be a cause of global warming due to higher temperature from using chemicals (Grbić et. al., 2011).

Moreover, chemical insecticide can be toxic to the environment, soil, farmers, consumers, and ecosystem because of its contamination. To lessen the impact on the environment, plant extracts and essential oils can be used as alternatives for pest management. Secondary metabolites in plant extracts have no harm to the environment, and can decompose rapidly (Croteau et. al., 20001). This study then evaluated the effectiveness of some plant essential oils as growth inhibitor against *Spodoptera exigua*.

2 MATERIALS AND METHODS

2.1 Insect and Essential Oils Preparation

In this study, beet armyworms were cultured in laboratory conditions at 25°C and 12:12 light/dark cycle. The essential oil of *Illicium verum*, *Curcuma longa*, *Foeniculum vulgare*, and *Ocimum tenuiflorum* were diluted in water plus tween-20 to obtain 0.00, 0.25, 0.50, 0.75, 1.00, and 1.25% concentrations. The essential oils used in this study were purchased from Thai-China Flavours and Fragrances Industry Co., Ltd., Bangkok, Thailand.

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2.2 Bioassay and Analysis

Brassica rapa (Chinese cabbage) leaves were cut into circles with the diameter of 3 centimeters, and were dipped into the prepared essential oils (5 Chinese cabbage leaves in circle shape for each concentration) and were put into the circle box. Ten secondary stage instar larvae were put into the circle box in which treated leaves were kept. The growth stage, the development period from larva to pupa stage, and pupa to adult stage, and the percentage of mortality at pupa and larva stage of *Spodoptera exigua* were observed.

3 RESULTS AND DISCUSSION

The result showed that the highest efficacy was at 0.75% concentration of Illicium verum and Curcuma longa essential oils and 100% inhibitory effect on the growth of the Spodoptera exigua was found at 1.25% concentration of Foeniculum vulgare, and Ocimum tenuiflorum as shown in Table 1, 2. As a result, the larvae had a less survival rate to be able to reach a pupa and adult stage, The development period from larvae to pupa stage of Illicium verum and Curcuma longa at 0.50% concentration was 5.67±5.77 days. The development period from pupa to adult stage of Illicium verum and Curcuma longa at 0.50% concentration was 8.67±5.77 days. The development period from larvae to pupa stage of Foeniculum vulgare and Ocimum tenuiflorum at 1.00% concentration was 5.67±5.77 and 6.33±5.77 days respectively. The development period from pupa to adult stage of Foeniculum vulgare and Ocimum tenuiflorum at 1.00% concentration was 9.00±0.00 and 9.33±5.77 days respectively. Therefore, the average development period from larvae to pupa stage

and from pupa to adult stage from the experiment was less than the average development period of the control treatment, which was 6.67 ± 5.77 days for larvae to pupa, and 9.67 ± 5.77 days for pupa to adult (Table 3, 4). Shorter development period resulted in incomplete growth cycle because the shorter period of molting inhibited the development of larvae into pupa and adult stage and affected the survival rate.

The result of this study was similar to the result of the previous research. For example, the turmeric oil showed repellent and growth inhibiting effect on red flour beetle. Most larva red flour beetle fail to develop into pupa stage and the delayed development affected the mortality (Jilani and Su, 1983). The Chinese star anise crude extract completely inhibited the development of housefly, Musca domestica, into pupa stage (Guntharee, 2008). Trans - anethole, the main effective chemical compound of thymol showed high toxicity to Spodoptera litura (Passreiter et. al., 2004). The essential oil of star anise lowered the growth rate of Gypsy moth (Kostić et. al., 2021). The turmeric rhizomes extract tended to have high toxicity and repellent effect against Sitophilus zeamais and Spodoptera frugiperda (Tavares et. al., 2013). Turmeric extract contained insecticidal property against dengue fever mosquito Aedes aegypti Linnaeus (Sukari et. al., 2010). Ar-turmerone, a major bioactive compound of herb, showed insecticidal activity to Nilaparvata lugens and Plutella xylostella (Lee, 2001). The turmeric extract had an effect on mortality and fecundity of Bactrocera zonata (Siddiqi et. al., 2011). (Ali et. al., 2014) reported the antifeedant, toxicant and growth regulatory effect of Curcuma longa on red flour beetle. Beside (Chowdhury et. al., 2000) presented that Curcuma longa was highly effective as growth inhibitor against Schistocerca gregaria and Dysdercus koenigii.

Table 1: The average growth inhibition of plant essential oils against Spodoptera exigua pupa.

Plant	Concentrations (%), (v/v)						
Essential	Average growth inhibition (%)						
oils	0.00	0.25	0.50	0.75	1.00	1.25	
Illicium verum	$0.00{\pm}0.00^{\text{Da}}$	50.00 ± 0.00^{Ca}	70.00 ± 0.00^{Bb}	100.00±0.00 ^{Aa}	100.00±0.00 ^{Aa}	100.00±0.00 ^{Aa}	
Curcuma longa	$0.00{\pm}0.00^{\text{Da}}$	56.67 ± 5.77^{Ca}	86.67 ± 5.77^{Ba}	100.00±0.00 ^{Aa}	100.00±0.00 ^{Aa}	100.00±0.00 ^{Aa}	
Foeniculum vulgare	$0.00{\pm}0.00^{Ea}$	33.33 ± 5.77^{Db}	60.00 ± 0.00^{Cc}	$70.00{\pm}0.00^{\rm Bb}$	76.67 ± 5.77^{Bb}	100.00±0.00 ^{Aa}	
Ocimum tenuiflorum	$0.00{\pm}0.00^{\rm Fa}$	33.33±5.77 ^{Eb}	53.33 ± 5.77^{Dd}	60.00 ± 0.00^{Cc}	$70.00{\pm}0.00^{\rm Bb}$	100.00±0.00 ^{Aa}	

Notes: Mean in a row followed by the same capital letter and means in a column followed by the same common letter are not significant different (P<0.05) according to Duncan's multiple range test.

Plant	Concentrations (%), (v/v)						
Essential	Average growth inhibition (%)						
oils	0.00	0.25	0.50	0.75	1.00	1.25	
Illicium verum	$0.00{\pm}0.00^{Da}$	$60.00{\pm}0.00^{Ca}$	$80.00{\pm}0.00^{\mathrm{Bb}}$	100.00±0.00 ^{Aa}	100.00±0.00 ^{Aa}	100.00±0.00 ^{Aa}	
Curcuma longa	$0.00{\pm}0.00^{Da}$	$60.00{\pm}0.00^{Ca}$	$90.00{\pm}0.00^{Ba}$	100.00±0.00 ^{Aa}	100.00±0.00 ^{Aa}	100.00±0.00 ^{Aa}	
Foeniculum vulgare	$0.00{\pm}0.00^{Fa}$	33.33 ± 5.77^{Eb}	60.00 ± 0.00^{Dc}	70.00 ± 0.00^{Cb}	$80.00 {\pm} 0.00^{\mathrm{Bb}}$	100.00±0.00 ^{Aa}	
Ocimum tenuiflorum	$0.00{\pm}0.00^{Fa}$	33.33±5.77 ^{Eb}	53.33±5.77 ^{Dd}	60.00 ± 0.00^{Cc}	70.00 ± 0.00^{Bc}	100.00±0.00 ^{Aa}	

Table 2: The average growth inhibition of plant essential oils against Spodoptera exigua adult.

Notes: Mean in a row followed by the same capital letter and means in a column followed by the same common letter are not significant different (P<0.05) according to Duncan's multiple range test.

Table 3: The average growth period of pupa stage of Spodoptera exigue caused by plant essential oils.

Plant	Concentrations (%), (v/v)						
essential	Average growth period (day)						
oils	0.00	0.25	0.50	0.75	1.00	1.25	
Illicium verum	$6.67{\pm}5.77^{Aa}$	6.33±5.77 ^{Aa}	5.67±5.77 ^{Bb}	$0.00{\pm}0.00^{Cb}$	$0.00{\pm}0.00^{\rm Cc}$	$0.00{\pm}0.00^{Ca}$	
Curcuma longa	$6.67{\pm}5.77^{Aa}$	6.33±5.77 ^{Aa}	5.67 ± 5.77^{Bb}	$0.00{\pm}0.00^{Cb}$	$0.00{\pm}0.00^{\rm Cc}$	$0.00{\pm}0.00^{Ca}$	
Foeniculum vulgare	$6.67{\pm}5.77^{Aa}$	$6.67{\pm}5.77^{Aa}$	6.33±5.77 ^{Aa}	6.33±5.77 ^{Aa}	5.67±5.77 ^{Bb}	$0.00{\pm}0.00^{Ca}$	
Ocimum tenuiflorum	6.67±5.77 ^{Aa}	6.67 ± 5.77^{Aa}	6.67 ± 5.77^{Aa}	6.33±5.77 ^{Aa}	6.33±5.77 ^{Aa}	$0.00{\pm}0.00^{Ca}$	

Notes: Mean in a row followed by the same capital letter and means in a column followed by the same common letter are not significant different (P<0.05) according to Duncan's multiple range test.

Table 4: The average growth period of adult stage of Spodoptera exigue caused by plant essential oils.

Plant	Concentrations (%), (v/v) Average growth period (day)					
Essential						
oils	0.00	0.25	0.50	0.75	1.00	1.25
Illicium verum	9.67±5.77 ^{Aa}	9.00±0.00 ^{Aa}	8.67±5.77 ^{Bb}	$0.00{\pm}0.00^{\rm Cb}$	$0.00{\pm}0.00^{\rm Cb}$	$0.00{\pm}0.00{}^{Ca}$
Curcuma longa	9.67±5.77 Aa	9.00±0.00 ^{Aa}	8.67±5.77 ^{Bb}	$0.00{\pm}0.00^{\mathrm{Cb}}$	$0.00{\pm}0.00^{Cb}$	$0.00{\pm}0.00{}^{Ca}$
Foeniculum vulgare	9.67±5.77 Aa	9.33±5.77 ^{Aa}	$9.33{\pm}5.77^{Aa}$	$9.33{\pm}5.77^{Aa}$	9.00±0.00 ^{Aa}	$0.00{\pm}0.00{}^{\mathrm{Ca}}$
Ocimum tenuiflorum	9.67±5.77 Aa	$9.67 \pm 5.77^{\mathrm{Aa}}$	9.33±5.77 ^{Aa}	$9.33{\pm}5.77^{Aa}$	$9.33 {\pm} 5.77^{\mathrm{Aa}}$	$0.00{\pm}0.00{}^{Ca}$

Notes: Mean in a row followed by the same capital letter and means in a column followed by the same common letter are not significant different (P<0.05) according to Duncan's multiple range test.

5 CONCLUSION

The experiment of inhibiting effect of selected plant essential oils on the growth and development of pupa and adult stages of *Spodoptera exigua* showed that the essential oils from *Illicium verum* and *Curcuma longa* had strong insecticidal activity and high potential to be developed as the botanical insecticide against beet armyworm in order to reduce the use of synthetic chemicals. However, additional study and testing should be conducted, particularly testing in the experimental plots under natural environment to confirm the effectiveness of these essential oils.

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