6. ภาคผนวก

Output จากโครงการวิจัยที่ได้รับทุนจาก สกว.

1. ผลงานตีพิมพ์ในวารสารวิชาการนานาชาติ

- Rachokarn S., N. Piyasaenghthong and **V. Bullangpoti**. 2008. Impact of botanical extracts derived from leaf extracts *Melia azedarach*L.: (Meliaceae) and *Amaranthus viridis* L. (Amaranthaceae) on population of *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) and detoxification enzyme activities. Comm. Appl. Biol. Sci, Ghent University, 73/3, 2008: 451-458.

- Phowichit S. , S. Buatippawan **and V. Bullangpoti. 2008. I**nsecticidal activity of *Jatropha ghossypifolia* L. (Euphorbiaceae) and *Cleome viscosa* L. (Capparidacae) against *Spodoptera litura* (Lepidoptera: Noctuidae). Toxicity and carboxylesterase and glutathion-S-tranferase activities studies. Comm. Appl. Biol. Sci, Ghent University, 73/3, 2008: 611-620.

- S. Siriarcharangroj, V. Chuaysuwan, C. Sudthonghong and **V. Bullangpoti.** 2008. Investigation of acute toxicity of *Jatropha gossypifolia* L. (Euphorbiaceae) and *Cleome viscosa* L. (Capparidacae) extract on guppies, *Poecilia reticulata. 2008.* Comm. Appl. Biol. Sci, Ghent University, 73/4, 2008: 871-874.

- Nutchaya Khumrungsee, **Vasakorn Bullangpoti** and Wanchai Pluempanupat. (2009) Efficiency of *Jatropha gossypifolia*(Euphorbiaceae) against *Spodoptera exigua Hubner* (Lepidoptera: Noctuidae): toxicity and its detoxification enzyme activities. KKU Sci. J.: 37 (Suppl): 50-55.

2. การนำผลงานวิจัยไปใช้ประโยชน์

- เชิงสาธารณะ

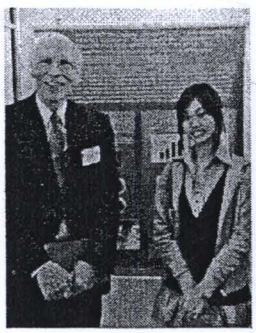
ได้นำผลงานไปเสนอในงานประชุมวิชาการระดับนานาชาติ 60th ISCP ประเทศ Belgium หัวข้อ:

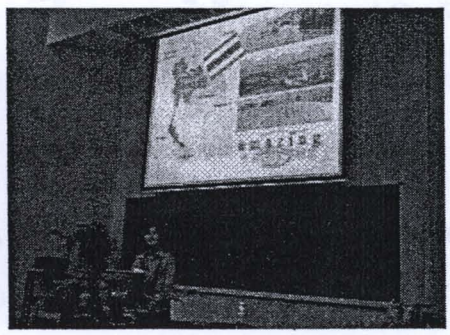
(1) Impact of botanical extracts derived from leaf extracts *Melia azedarach* L. (MELIACEAE) and *Amaranthus viridis* L.(Amaranthaceae) on population of *Spodoptera exigùa* (Hübner) (Lepidoptera: Noctuidae) and detoxification enzyme activities ในรูปแบบ Oral Presentation

(2) Investigation of acute toxicity of *Jatropha gossypifolia L. (EUPHORBIACEAE)* and *Cleome viscosa L. (CAPPARIDACAE)extract on guppies Poecilia reticulata* ในรูปแบบ Poster Presentation

(3) Effiency of *Jatropha gossypifolia L.* (Euphorbiaceae) against *Spodoptera litura (*Lepidoptera: Noctuidae*)*: Toxicity and detoxificaton enzyme activities. ในรูปแบบ Poster Presentation

(4) Effect of *Cleome viscosa, L. (*Capparidacae*)* extract on toxcity and the activity of carboxylesterase and glutathione-s-transferase in *Spodoptera litura (*Lepidoptera: Noctuidae*)*. ในรูปแบบ Poster Presentation





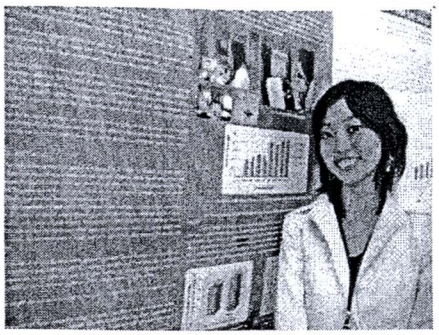
ภาพขณะนำเสนอผลงานวิจัยในงานประชุมวิชาการระดับนานาชาติ 60th ISCP ประเทศ Belgium

- ได้นำผลงานไปเสนอในงานประชุมวิชาการระดับนานาชาติ 2nd International Conference on Science and Technology for sustainable Development of the Greater Mekong Sub-region ประเทศ Vietnam

หัวข้อ:

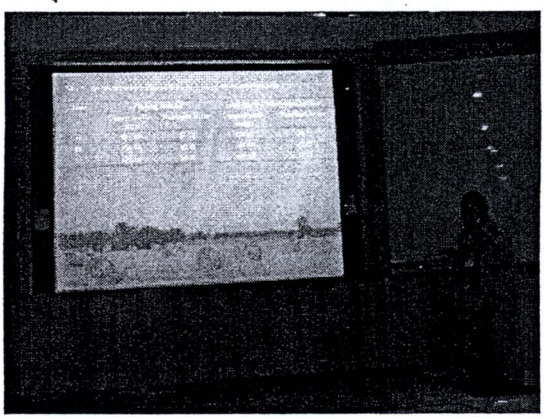
(1) Efficiency of *Jatropha gossypifolia* L. against *Spodoptera exigua* Hübner (Lepidoptera : Noctuidae) : Toxicity and detoxification enzyme activities

(2) Effects of Senescent leaf extracts from *Melia azedarach* L. (Meliaceae) on Toxicity and their detoxification activities of *Spodoptera exigua* (Hübner) (Lepidopter:Noctuidae)



ภาพขณะนำเสนอผลงานวิจัยในงานประชุมวิชาการระดับนานาชาติ2nd International Conference on Science and Technology for sustainable Development of the Greater Mekong Sub-region ประเทศ Vietnam

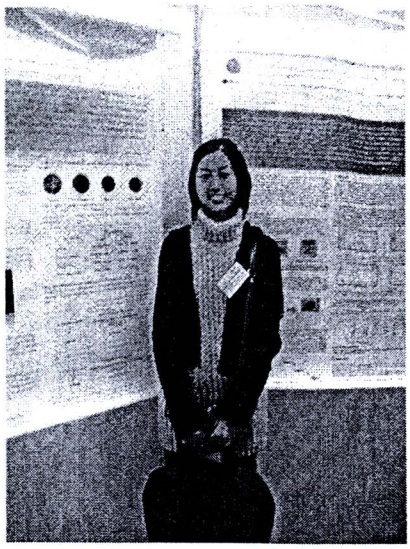
- ได้รับเชิญไปบรรยายในเรื่องสารสกัดจากพืชในประเทศไทย และบรรยายเรื่องผลการทดลองบางส่วนในโครงการ และหารือองานวิจัยในการประชุมนานาชาติในการประชุมวิชาการระดับนานาชาติ "Neem Extract and Other Botanical Insecticides: For Sustainable tool for pest control" ณ University of Tsukuba ประเทศญี่ปุ่น ในวันที่ 10-14 มีนาคม พ.ศ. 2552 โดยรับทุนจากสนับสนุนไปประชุมวิชาการครั้งนี้ จาก University of Tsukuba



ภาพขณะนำเสนอผลงานวิจัยในงานประชุมวิชาการระดับนานาชาติ Neem Extract and Other Botanical Insecticides: For Sustainable tool for pest control" ณ University of Tsukuba ประเทศญี่ปุ่น

- ได้นำผลงานไปเสนอในงานประชุมวิชาการระดับนานาชาติ 62th ISCP ประเทศ Belgium, 18May 2010

Kumrungsee1 N., **V. Bullangpoti**, W. Pluempanupat and Y. Kainoh (2010). Toxicity of *Jatropha gossypifolia L.* leaf extracts on *Spodoptera exigua (Hubner)* and *Meteorus pulchricornis*. Poster presented at 62th International Symposium on Crop Protection May 18, 2010. Belgium.



ภาพขณะนำเสนอผลงานวิจัยในงานประชุมวิชาการระดับนานาชาติ 62th ISCP ประเทศ Belgium

- ก่อเกิดการเชื่อมโยงทางวิชาการกับนักวิชาการในต่างประเทศ คือ ญี่ปุ่น อินเดีย ฝรั่งเศส เนปาล เยอรมัน อียิปต์ โดยทั้งนี้ ได้มีการนำงานวิจัยบางส่วนไปทำวิจัย ณ ประเทศญี่ปุ่น (University of Tsukuba)

(a)

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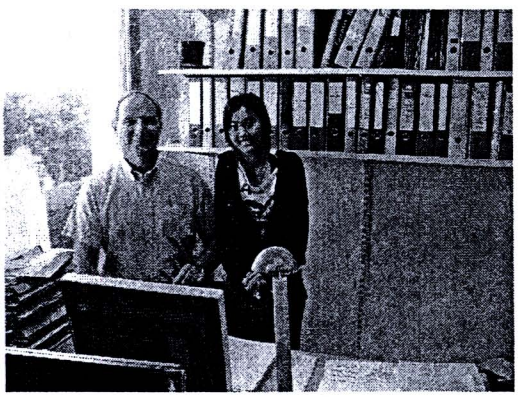

(e) (f)

ภาพการเชื่อมโยงทางวิชาการกับนักวิชาการในต่างประเทศ: (a): Egypt (b) German & India (c) Japan (US Scientist) (d) Japan & France (e-f) France

- เชิงวิชาการ (มีการพัฒนาการเรียนการสอน/สร้างนักวิจัยใหม่)

  ผลิตบัณฑิตระดับปริญาโทได้1 คน ปัญหาพิเศษนิสิตปริญญาตรี 3 คน

INSECTICIDAL ACTIVITY OF *JATROPHA GOSSYPIFOLIA* L. (EUPHORBIACEAE) AND *CLEOME VISCOSA* L. (CAPPARIDACAE) ON *SPODOPTERA LITURA* (LEPIDOPTERA: NOCTUIDAE) TOXICITY AND CARBOXYLESTERASE AND GLUTATHIONE-S-TRANSFERASE ACTIVITIES STUDIES

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SUMMARY

The naturally occurring phytocidal chemical components of some Thai plant-species are responsible for controlling and repelling insects from the host plants. The aim of this study was to evaluate the insecticidal activity of *Jatropha gossypifolia* L. leaf extracts and the senescent leaf *Cleome viscosa* L. (Capparidacae) against *S. litura* and detoxification enzyme activities. Laboratory no-choice bioassays showed treatment of second instars *Spodoptera litura* by dipping in extracts from senescent leaves of *Jatropha gossypifolia* L. at 3,000 -10,000 ppm had significant toxicity with LC_{50} of 6.56 mg/ml^{-1} (r^2 = 0.88) at 24 hours after exposure. The toxicity of *Cleome viscosa* L. extract in terms of LC50 values ca. 34 mg ml^{-1} (r^2 = 0.95) at 24 after exposure by the dipping method. Also, *S. litura* larvae surviving treatment of both extract showed a dramatic decrease in carboxylesterase and glutathione-s-transferase activities. This extract showed strong insecticidal activity and may play an alternative role as a pesticide against *Spodoptera litura*.

Key word: *Spodoptera litura*, *Jatropha gossypifolia* L., *Cleome viscose* L. , carboxylesterase, glutathione-stransferase, botanical insecticide

INTRODUCTION

The common cutworm, *Spodoptera litura* (Fabricius), is a serious pest causing enormous losses to many economically important crops such as cotton, soybean, groundnut, tobacco and vegetables (Etman and Hooper (1979); Matsuura and Naito (1997); Qin *et al.* (2000) and Qin *et al.*, (2004)). Its control has depended exclusively on application of various insecticides. As a result, many field populations of this pest have developed multiple resistances, and field control failure happens more and more frequently Armes *et al.* (1997), Kranthi *et al.* (2001), Kranthi *et al.* (2002), Murugesan and Dhingra (1995), Ranakrishnan *et al.* (1984) and Wu, *et al.* (1995). Thus, the mechanisms for multiple resistances in this pest should be declared for improvement of control effect. In Thailand, a lot of insecticidal plants have revealed good tendency for insect control namely, Capsicin from *Capsicum frutescans* L. for the control of *Sitophilus zeamais* Motschulsky (Bullangpoti *et al.*, 2002) and *Spodoptera litura* (Saisongkhroh *et al.*, 2005), Rotenone from *Annona squamosa* L. for control *Nephotettix virescens* (Srisaard *et al.*, 2005) , Pachyrhizin from *Pachyrhizus erosus* Urb) seeds for control *Aedes aegypti* (L.) (Srikhong *et al.*, 2005) and <alpha>-mangostin from *Garcinia mangostana* pericarp extract to *Nilaparvata lugens* Stal. and *Sitophilus oryzae* (Bullangpoti *et al.*, 2004, 2006 and

2007). The *Jatropha gossypifolia* L. (EUPHORBIACEAE) is Thai plant which have many chemical such as apigenin (Subramanian *et al.*, 1971); cyclogossine A (Horsten *et al.*, 1996) jatrophatrione, jatropholone (Rahman *et al.*, 1990) jatrophine, ß-sitosterol (Sengupta and Das, 1964). Moreover, this plant extract showed their pharmaceutic efficiency such as anti-alergic (Adolf *et al.*, 1984) molluscicide (Adewunmi *et al.*, 1980), repellent the oriental fruit fly (Areekul *et al.*, 1988) and insecticidal activity (Siever *et al.*, 1949)

The *Cleome viscose* L. (Capparidacae) is Thai weed which have many chemical such as stigma sterol (Gupta and Dutt, 1938), ß-sitosterol (Lin and Chen, 1975), ß-amyrin (Srivastava, 1982), benthic acid (Chauhan and Srivastava, 1979) cleomalderic acids (Jente *et al.*, 1990), cleomeolide (Burke *et al.*, 1980; Mahato *et al.*, 1979) cleomiscosin A, cleomiscosin B, cleomiscosin C (Ray *et al.*, 1980, 1985; Lee, 1984) oleic acid (Rukmini, 1978; Afaq *et al.*, 1984). The toxicity to mice showed that at dose more than 10g/kg, mouse showed no toxicity when peritoneal injection with the extract and no development effect to mice when mix 10% the extract with diet at 26 weeks

Recently, Insecticide resistance mechanism was known as it involves mainly three mechanisms, decreased penetration, enhanced detoxification, and target-site insensitivity. For detoxification enzymes always have a range of substrates, this mechanism could result in some cross-resistance among the insecticides with similar molecular structure. The main objective of this research was to develop a new botanical insecticide, the *Jatropha gossypifolia* L. extract *Cleome viscose*, L. extract for controlling S. *litura*. In addition, detoxification enzyme activities, carboxylesterase and glutathione-s-transferase (GST) were investigated using enzyme-substrate assays with a spectrophotometer.

MATERIALS AND METHODS

Rearing of insect

2nd stage of *S.litura* were received Department of Agriculture, Ministry of Agriculture and Cooperative, Thailand, S. *litura* larvae were reared in the artificial diet. Group of 10-20 neonates was placed in 5x5 cm plastic box with the mesh lid and kept in an incubator at 27°C, 70%RH with a 16h-L:8h-D photoperiod. Pupae were collected from these boxes after larvae pupated and placed into the glass jar. The paper sheets for the oviposition of adults were placed in the cage. Adults were fed with honey solution (100 gL^{-1} distilled water). Egg sheets were collected daily.

Extraction *Jatropha gossypifolia L.* and *Cleome viscose L.* method

Dried powdered senescent leaf *Jatropha gossypifolia* L. and *Cleome viscose* L. (10 kg/plant materials) were extracted using a Soxhlet extractor with 99% Ethyl alcohol as solvent. The extract was evaporated to remove solvent by the rotary evaporator (BUCHI B-850) which give the dark green sticky semi-solid crude extract resulted. Then, the extract was stored at +4°C until the preparation of the stock solution. Stock solution was prepared by weighing a certain amount of extract and diluting it in distilled water to give the various dosing concentrations as mg L^{-1}.

Bioassay of insecticide

This research using the CRD method which the 2nd stage larvae (30 individuals in each replicate) were dipped in each concentrations of crude extract from the extract which diluted with distilled water and were dipped in distilled water as control treatment for 5 second to obtain LC_{50} values. The real mortality percentages were adjusted by Abbott's formula (Matsumura, 1976)and will analyze by Probit analysis method. Insects that did not move and/or did not stay upright were inferred to be dead. Mortality was recorded for each experiment in 5 replicates at 24 and 48 hours after treatment.

Preparation of enzyme

Whole body of 2nd instars larvae were used for enzyme preparation. The whole body from five larvae was homogenized with 1000 µl homogenization buffer (0.1 M potassium phosphate buffer, pH 7.5, containing 1 mM EDTA, and 1 mM GSH). After centrifugation at 10,000*g* for 15 min, the clear supernatant was collected and used as enzyme resources for analysis of the activity of GST and carboxylesterase. All operation was carried out on ice and centrifugation at 4°C to minimize losses of enzyme activity.

Protein assay

Total protein content of the enzyme solution was determined by the Bradford method (Bradford, 1976) using bovine serum albumin as the standard.

Detoxification enzymes assay

The method reported by Oppenoorth (1979) was adopted for testing GST activity. The CDNB activity test, the reaction solution contained 20 µl of enzyme solution, 1,150 µl of 0.1 M potassium phosphate buffer pH7.5, 10 µl of 150 mM CDNB. Optical density at 340 nm was recorded at intervals of 30 s for 3 min in 25 °C using the spectrophotometer. The activity of GST was determined using the extinction coefficient of 0.000137 For CDNB. Esterase activity was determined using the method described by Han *et al.* (1998). enzyme solution (50 µl) was mixed with p-nitrophenylacetate (pNPA) (50 µl, 0.12M) and phosphate buffer (2.9 ml, 0.1M, pH7.5). Enzyme activity was measured in a Spectrophotometer (Perkin Elmer-Lamda 25) at 400 nm and 25°C using the kinetic mode for 3 min. The activity of carboxylesterase was determined using the extinction coefficient of 176.4705 For pNPA. Activity of all enzymes was analyzed by SigmaPlot software.

RESULTS AND DISCUSSION

Insecticidal activity of senescent leaf *Jatropha gossypifolia* L. and *Cleome viscose* L. extract against *S. litura*

The senescent leaf *Jatropha gossypifolia* L. extract give yield the crude extract at 22.14% w/w whereas the *Cleome viscose* L., Ethanol yielded the crude extract at 21.46% w/w. The amount of crude ethanolic extract from senescent leaf *Cleome viscose*, L. extract was greater than those produced by ethanolic extraction of nut

grass tubers (12.81% w/w) (Ruamthum, 2002) and neem seed kernels (20.12% w/w), (Visetson, 2001) but less than that from yam bean seed (34.32% w/w) (Srikong, 2005) and mangosteen pericarp extract (29.46% w/w) (Bullangpoti *et al.*, 2006, 2007). Apart from solvent type, crude extract yields can vary considerably depending on plant type, growing sources, plant part, storage conditions of the plant material, solvent, temperature and extraction method (Visetson *et al.* 2005). These parameters need to be investigated further in order to optimize main active ingredient yield.

For the toxicity result, the senescent leaf *Jatropha gossypifolia* L. extract exhibited marked insecticidal activity against cutworms following dipping method (the mortality induced by these extract was higher than 80%, with LC_{50} of 6,555.92 ppm (r^2 = 0.88) and 6,424.91 ppm (r^2 = 0.95) at 24 and 48 hours after exposure). The toxicity of common insecticides to 2nd larvae of *S. litura* was tested and the results were shown in Figure 1. The dipping of these extract induced darkening of the cutworm's color, whereas control treatment did not have such an effect (data not shown). Thus, indicating that *S. litura* mortality was highly correlated with concentration for each extract (Figure 1). In addition, a longer exposure time (48 hours) generally resulted in greater mortality than a shorter exposure time for each extract, thus indicating the importance of the period exposed to extracts to *S. litura* mortality.

Toxicity of *Cleome viscose* L. extract to *S. litura* also differed considerably among extracts' concentration as shown in Figure 2. The ethanol extract gave the highest toxicity of all extracts (LC_{50} at 24 hours after exposure = 34 mg ml^{-1} (r^2 = 0.95)). Thus, indicating that *S. litura* mortality was highly correlated with concentration for each extract (Figure 2). In addition, a longer exposure time (48 hours) generally resulted in greater mortality than a shorter exposure time for each extract, thus indicating the importance of the period exposed to extracts to *S. litura* mortality.

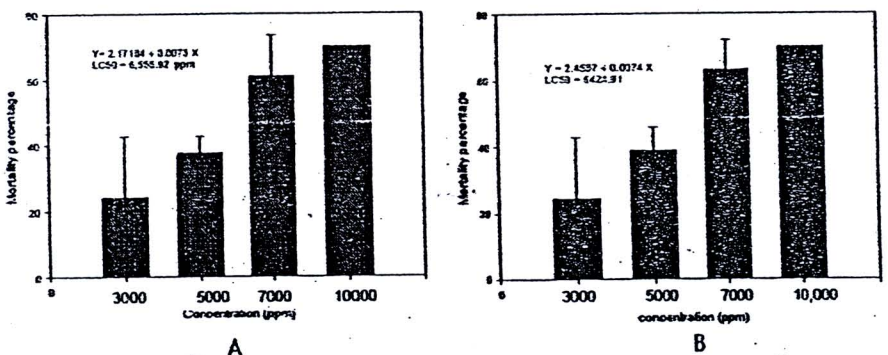
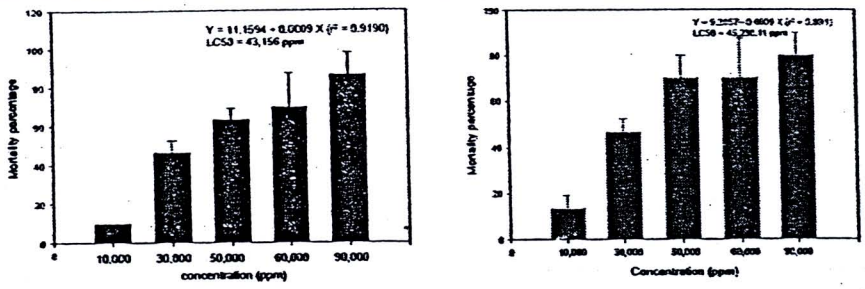


Figure 1. The toxicity of senescent leaf *Jatropha gossypifolia* L. extract against 2nd larvae of *S. litura* by dipping method after expose at 24 hours (A) and 48 hours (B)



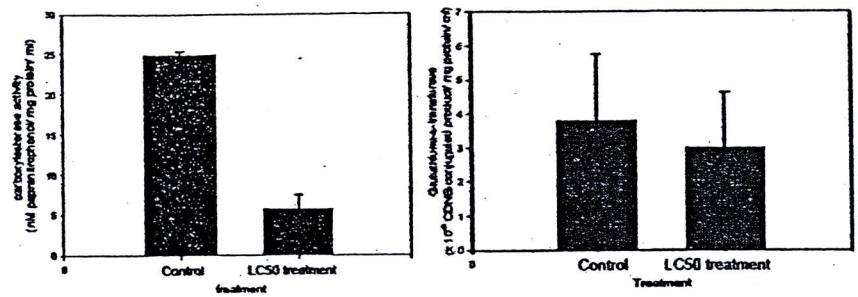
A B

Figure 2. Mortality percentage of *S. litura* after treated with dipping toxicity method with crude extract of senescent leaf *Cleome viscose* extract after 24 (A) and 48 hr. (B) under the laboratory condition

Both extract also showed a higher toxicity for *S. litura* when compared with using other botanical insecticide such as chilli extract (LC_{50} = 48.80 mg ml^{-1}) (Saisongkhroh, 2006) but less toxicity than sweet apple seed extract (LC_{50} = 16.42 mg ml^{-1}) *S. oryzae* L. using the same extract for which the LC_{50} was 52.5 mg/ml at 24 after exposure (Bullangpoti *et al.*, 2004). This difference in toxicity of the ethanol extract to same organisms may be because organisms have different detoxification mechanisms and efficiencies in avoiding or excreting xenobiotics and other toxic substances in the extract and its' relate with the evolution.

Effect of senescent leaf *Jatropha gossypifolia* L. and *Cleome viscose* L. extract against carboxylesterase and glutathione-s-transferase activities of *S. litura*

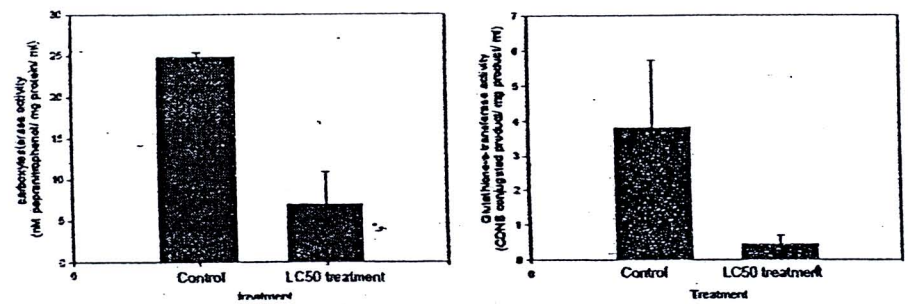
Carboxylesterase activity of *S. litura* was inhibited after treated with senescent leaf *Jatropha gossypifolia* L. extract with no significant different at 5% level using Duncan's Multiple Rang Test of protein concentration (data not shown). The correction factor when compare between control and each concentrations shows ethanolic extract can inhibit enzyme activity of 2nd instars *S. litura* larvae 4.31 fold (Figure 3). The inhibition was increased when concentration of extract increased. For GST activity of 2nd instars *S. litura larvae*, it showed efficiency of senescent leaf *Jatropha gossypifolia* L. extract same as carboxylesterase. This detoxification enzyme was inhibit after treated with senescent leaf *Jatropha gossypifolia* L. extract with no significant different at 5% level using Duncan's Multiple Rang Test of protein concentration (data not shown). The correction factor when compare between control and each concentrations shows the ethanolic extract can inhibit enzyme activity between 1.103 fold (Figure 3). The inhibition was increased when concentration of extract increased.



A B

Figure 3 (A) Carboxylesterase activity (nM paranitrophenol/mg protein/min) and **(B)** glutathione-s-transferase activity (CDNB conjugated product/mg protein/ml) of 2nd instars *S. litura* larvae after treated with LC50 concentration of senescent leaf *Jatropha gossypifolia* L. extract.

Moreover, Carboxylesterase activity of *S. litura* was inhibited after treated with senescent leaf *Cleome viscose*, L. extract with no significant different at 5% level using Duncan's Multiple Rang Test of protein concentration (data not shown). The correction factor when compare between control and each concentrations shows ethanolic extract can inhibit enzyme activity of 3rd instars *S. litura* larvae (Figure 4). The inhibition was increased when concentration of extract increased. For GST activity of 3rd instars *S. litura larvae*, it showed efficiency of senescent leaf *Cleome viscose*, L. extract same as carboxylesterase. This detoxification enzyme was inhibit after treated with senescent leaf *Cleome viscose*, L. extract with no significant different at 5% level using Duncan's Multiple Rang Test of protein concentration (data not shown). The correction factor when compare between control and each concentrations shows ethanolic extract can inhibit enzyme activity (Figure 4). The inhibition was increased when concentration of extract increased.



A B

Figure 4 (A) Carboxylesterase activity (nM paranitrophenol/mg protein/min) and **(B)** glutathione-s-transferase activity (CDNB conjugated product/mg protein/ml) of 2nd instars *S. litura* larvae after treated with LC50 concentration of senescent leaf *Cleome viscose*, L. extract.

Carboxylesterase activity seems especially important to the development and reproduction of organisms (Ezhilarasi and Subramoniam, 1984; Sayed *et al.*, 2006),

and so, the inhibition of carboxylesterase activity by the extract may have negatively affected the physiology of *S. litura*. Glutathione-s-transferase is one of the important detoxification enzymes in organisms. It can change the structural forms of xenobiotics to be more polarity via conjugation with macromolecules such as glutathione in the organism's body. Thus, this insect may use glutathione-s-transferase as one way to decrease the toxicity of compounds in the extract.

CONCLUSION

The common cutworm, *Spodoptera litura* (Fabricius), is a serious pest causing enormous losses to many economically important crops. Its control has depended exclusively on application of various insecticides. Nowadays, the naturally occurring phytocidal chemical components of some Thai plant-species are responsible for controlling and repelling insects from the host plants as shown in many research. The aim of this study was to evaluate the insecticidal activity of *Jatropha gossypifolia* L. leaf extracts and *Cleome viscose* L. against *S. litura* and detoxification enzyme activities. Laboratory bioassays showed treatment of second instars *Spodoptera litura* by dipping in extracts from senescent leaves of *Jatropha gossypifolia* L. at 3,000 -10,000 ppm had significant toxicity with LC_{50} of 6,555.92 ppm (r^2 = 0.88) and 6,424.91 ppm (r^2 = 0.95) at 24 and 48 hours after exposure. The senescent leaf *Cleome viscose* L. was investigated extract on second instars *Spodoptera litura* (Lepidoptera: Noctuidae) under the laboratory no-choice assay. The toxicity in terms of LC_{50} values ca. 34 mg ml^{-1} (r^2 = 0.95) at 24 after exposure by the dipping method. Thus, the *Jatropha grossypifolia* extract shows the higher toxicity to secondary instars larvae *S. litura* then *Cleome viscose* extract. For detoxification enzyme analysis method, *S. litura* larvae surviving treatment showed a dramatic decrease in carboxylesterase and glutathione-s-transferase activities after treated with both extracts. Results indicate that these botanical pesticides have the potential to be as the alternative control program for *Spodoptera litura*.

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IMPACT OF BOTANICAL EXTRACTS DERIVED FROM LEAF EXTRACTS *MELIA AZEDARACH* L. (MELIACEAE) AND *AMARANTHUS VIRIDIS* L. (AMARANTHACEAE) ON POPULATIONS OF *SPODOPTERA EXIGUA* (HÜBNER) (LEPIDOPTERA: NOCTUIDAE) AND DETOXIFICATION ENZYME ACTIVITIES

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SUMMARY

Spodoptera exigua (Hübner) (Lepidoptera: Noctuidae), an important insect pest of many field crops, has developed resistance to various insecticides, making its control increasingly difficult. This study explored the effects of senescent leaf *Melia azedarach* L. (Meliaceae) and *Amaranthus viridis* L. (Amaranthaeae) extract on second instar *S. exigua* larvae survival by the dipping method. We also analyzed detoxification enzyme activities of carboxylesterase and glutathione-s-transferase in *in vitro* tests with extract-treated insects. The leaf extract showed strong insecticide activity with a LC_{50} value of 9.793 mg/ml (r^2 = 0.965) and 50.5702 mg/ml (r^2 = 0.95) at 24 after exposure for *M. azedarach* L. and *A. viridis* L. extract, respectivrly but no significant increase in toxicity over time. The *M. azedarach* L. extract strongly inhibited all enzyme activities. In contrast with *A. viridis* L. extract, they inhibit only glutathione-s-transferase. This is the first report of highly effective insecticidal activity of the senescent leaf extract of *A. viridis* and *M. azedarach* L. against *S. exigua*. Both plant materials are a less expensive (0.5 $US per 1 kg leaf), suggesting this extract is a promising alternative tool for the management of this pest.

Key words: *Spodoptera exigua* (Hübner), *Melia azedarach* L., *Amaranthus viridis* L. carboxylesterase, acetylcholinesterase, glutathione-s-transferase, botanical insecticide

INTRODUCTION

The beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae), is a polyphagous pest and a tropical insect native to southeastern Asia. It is considered a major pest in many agricultural areas of the world in vegetable, field, and flower crops including corn, cotton, beet, tomato, celery, lettuce, cabbage, and alfalfa (Wang *et al.*, 2006). It was first recorded in the United States in Oregon in 1876 and has since spread throughout continental United States, Mexico, and the Caribbean (Mitchell, 1973). The beet armyworm is capable of feeding on the foliage, but most insecticide spray programs in tomato are based on insect pressure after fruit set.

Due to heavy selection pressure over the past 20 years, *S. exigua* has developed resistance to organochlorine, organophosphate, chlorinated hydrocarbons, carbamates, and pyrethroid insecticides in many countries (Moulton *et al.*, 2000). Nowadays there are several novel insecticides which show good activities against

the beet armyworm including chlorfenapyr, tebufenozide, emamectin benzoate, indoxacarb, and spinosad (Moulton *et al.*, 2000).

In Thailand, a lot of insecticidal plants have revealed good tendency for insect control namely, Capsicin from *Capsicum frutescans* L. for the control of *Sitophilus zeamais* Motschulsky (Bullangpoti *et al.*, 2002) and *Spodoptera litura* (Saisongkhroh *et al.*, 2005), Rotenone from *Annona squamosa* L. for control *Nephotettix virescens* (Srisaard *et al.*, 2005) , Pachyrhizin from *Pachyrhizus erosus* Urb) seeds for control *Aedes aegypti* (L.) (Srikhong *et al.*, 2005) and <alpha>-mangostin from *Garcinia mangostana* pericarp extract to *Nilaparvata lugens* Stal. and *Sitophilus oryzae* (Bullangpoti *et al.*, 2004, 2006 and 2007).

In this research, we are focus to develop a new insecticidal activity of leaf extract *Melia azedarach* L. (Meliaceae) and *Amaranthus viridis* L. (Amaranthaceae) on *Spodoptera exigua*. The *Melia azedarch*, also know as chinaberry or Persian lilac tree, is a deciduous tree that has long been recognized for its insecticidal properties. There such as triterpenoids, triacontane, B-sitosterol, glucose. carotenoid, meliantin, azadirachtin linoleic acid, oleic acid, myristic acid and palmitic acid. There are many research show that this plant extract have many biological activity as shown in the previous reports such as increase polymorphonucleus leucocyte and decrease lymphocyte (Benencia F. *et al.*,1992) inhibit tyrosinase(Iida K. *et al.*,1995) and aldose reductase (Shin KH *et al.*,1993) inhibit platelet activating factor(Han BH *et al.*,1994)

The second plant we analyzed their insecticidal activity on *S. exigua* is *Amaranthus viridis* L. which also know as slender amaranth, wild blite. This plant have many chemical compound such as amaranthin (Kwon *et al.*, 1997) amasterol and vitamin C. the previous report descript their biological activity as antivirus (Kwon *et al.*, 1997) inhibit tumor formation. Moreover, they have report show when inject extract which dissolve in ethanol: water (1:1) in rat, LC50 is 100 µg/ kg.

Although there are many biological activity of both extract, they are no reports describe to study their insecticidal activity on *S. exigua*. The main objective of this research would like to develop a new botanical insecticide, the *Melia azedarach* L. and *Amaranthus viridis* L. extract for controlling *S. exigua*. In addition, detoxification enzyme activities, *carboxylesterase* and glutathione-s-transferase (GST) were investigated using enzyme-substrate assays with a spectrophotometer.

MATERIALS AND METHODS

1. Rearing of insect

3rd stages of *S. exigua* were received from onion farm in Ratchaburi province, Thailand. *S. exigua* larvae were reared in the artificial diet. Group of 5-10 neonates was placed in 5 x 5 cm plastic box with the mesh lid and kept in an incubator at 27°C, 70%RH with a 16h photoperiod. Pupae were collected from these boxes after larvae pupated and placed into the glass jar. The paper sheets for the oviposition of adults were placed in the cage. Adults were fed with honey solution (100gL^{-1} distilled water). Egg sheets were collected daily.

2. Extraction method

Dried powdered senescent leaf *Melia azedarach* L. (Meliaceae) and *Amaranthus viridis* L. (Amaranthaceae) (10 kg/each) were extracted using a Soxhlet extractor with ethyl alcohol as solvent. The extract was evaporated to remove solvent by the

rotary evaporator (BUCHI B-850) which give the dark green sticky semi-solid crude extract resulted. Then, the extract was stored at +4°C until the preparation of the stock solution. Stock solution was prepared by weighing a certain amount of extract and diluting it in distilled water to give various dosing concentrations of ppm.

3. Bioassay of insecticide

In the experiments, the 2nd stage larvae (30 individuals in each replicate) were dipped in each concentrations of crude extract from the extract which diluted with distilled water and were dipped in distilled water as control treatment for 5 second to obtain LC_{50} values. The real mortality percentages were adjusted by Abbott's formula (Matsumura, 1976). Insects that did not move and/or did not stay upright were inferred to be dead. Mortality was recorded for each experiment in 5 replicates at 24 and 48 hours after treatment.

4. Preparation of enzyme

Whole body of control and treated 2nd instar larvae were used for enzyme preparation. The midgut or fat body from five larvae was homogenized with 1000 µl homogenization buffer (0.1 M potassium phosphate buffer, pH 7.5, containing 1 mM EDTA, and 1 mM GSH). After centrifugation at 10,000*g* for 15 min, the clear supernatant was collected and used as enzyme resources for analysis of the activity of GST and *carboxylesterase*. All operation was carried out on ice and centrifugation at 4 °C to minimize losses of enzyme activity.

5. Protein assay

Total protein content of the enzyme solution was determined by the Bradford method (Bradford, 1976) using bovine serum albumin as the standard.

6. Detoxification enzymes assay

The method reported by Oppenoorth (1979) was adopted for testing GST activity. The CDNB activity test, the reaction solution contained 20 µl of enzyme solution, 1,150 µl of 0.1 M potassium phosphate buffer pH 7.5, 10 µl of 150 mM CDNB. Optical density at 340 nm was recorded at intervals of 30 s for 3 min in 25°C using the spectrophotometer. The activity of GST was determined using the extinction coefficient of 0.000137 For CDNB.

Esterase activity was determined using the method described by Han *et al.* (1998). enzyme solution (50 µl) was mixed with p-nitrophenylacetate (pNPA) (50 µl, 0.12M) and phosphate buffer (2.9 ml, 0.1M, pH 7.5). Enzyme activity was measured in a Spectrophotometer (Perkin Elmer-Lamda 25) at 400 nm and 25°C using the kinetic mode for 3 min. The activity of carboxylesterase was determined using the extinction coefficient of 176.4705 For pNPA. Activity of all enzymes was analysed by SigmaPlot software.

RESULTS AND DISCUSSION

1. Insecticidal activity of leaf *Melia azedarach* L. and *Amaranthus viridis* L. extract against *S. exigua.*

The leaf *Melia azedarach* L. and *Amaranthus viridis* L. extract exhibited marked insecticidal activity against *S. exigua* following dipping method (the mortality induced by these extract was higher than 80%, with LC_{50} of 9.79 mg/ml (r^2 = 0.965) and 50.57 mg/ml (r^2 = 0.95) at 24 after exposure for *M. azedarach* L. and *A. viridis* L. extract, respectively but no significant increase in toxicity over time. The *M. azedarach* L. extract strongly inhibited all enzyme activities. The toxicity of common insecticides to 2nd larvae of *S. exigua* was tested and the results were shown in Figure 1 and 2. The dipping of these extract induced darkening of the worm's color, whereas control treatment did not have such an effect.

The results from figure 1 and 2 indicating that *S. exigua* mortality was highly correlated with concentration for each extract (Figure 1 and 2). This extract also showed a higher toxicity when compared with using other botanical insecticide such as chili extract (LC_{50} = 48.80 mg ml^{-1}) (Saisongkhroh, 2006), sweet apple seed extract (LC_{50} = 16.42 mg ml^{-1}) and mangosteen extract (LC_{50} is 5.2 for *S. oryzae* and 4.5 mg/ml for *N. lugens*) (Bullangpoti *et al.*, 2004, 2006, 2007). This difference in toxicity of the ethanol extract to same organisms may be because organisms have different detoxification mechanisms and efficiencies in avoiding or excreting xenobiotics and other toxic substances in the extract and its' relate with the evolution.

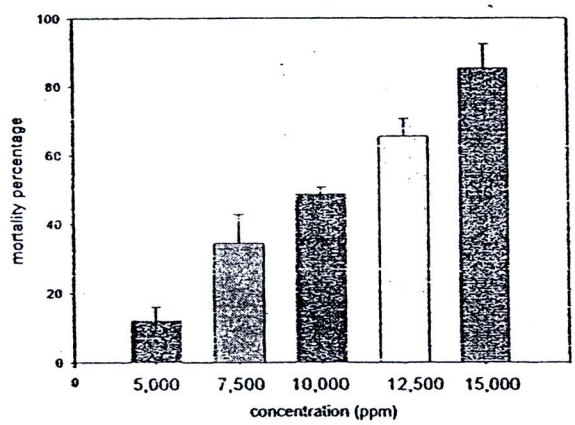

Figure 1. The toxicity of leaf *Melia azedarach* L. extract against 2nd larvae of *S. exigua* by dipping method after expose at 24 hours

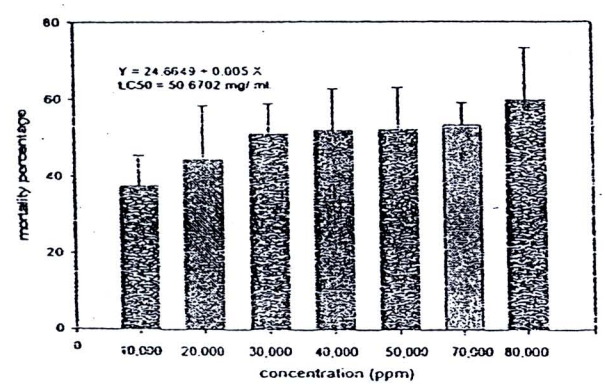

Figure 2. The toxicity of leaf *Amaranthus viridis* L. extract against 2nd larvae of *S. exigua* by dipping method after expose at 24 hours

2. Effect of leaf *Melia azedarach* L. and *Amaranthus viridis* L. extract against carboxylestrase and glutathione-s-transferase activities of *S. exigua.*

As figure 3, the carboxylesterase activity of *S. exigua* was inhibited after treated with leaf *Melia azedarach* L. extract with no significant different at 5% level using Duncan's Multiple Rang Test of protein concentration and the inhibition was increased when concentration of extract increased (data not shown); however, the larvae treated with *Amaranthus viridis* L. extract, show induce their carboxylesterase activity compare with the control. Thus, it's possible that this insect will use carboxylesterase as important detoxification enzyme to develop itself to be resistant to *Amaranthus viridis* L. extract if use this extract for long time.
For glutathione-s-transferase activity of 2nd instar *S. exigua larvae*, it showed both extract were inhibit this detoxification enzyme activity with no significant different at 5% level using Duncan's Multiple Rang Test of protein concentration (figure 4). The inhibition was increased when concentration of extract increased (data not shown).

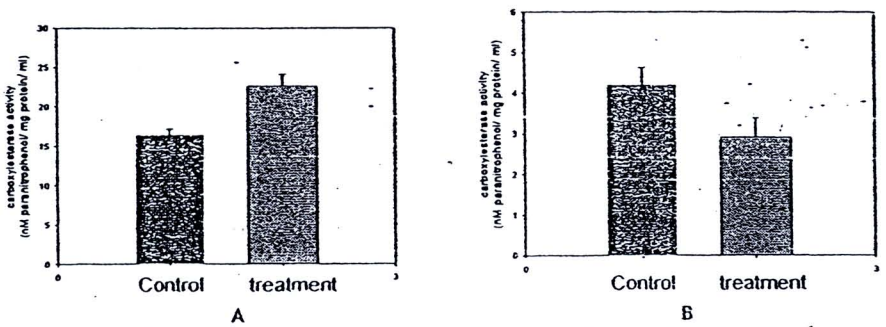

Figure 3. Carboxylesterase activity (nM paranitrophenol/mg protein/min) of 2nd instar *S. exigua* larvae after treated with LC50 concentration of (A) *Amaranthus viridis* L. and (B) *Melia azedarach* L. extract

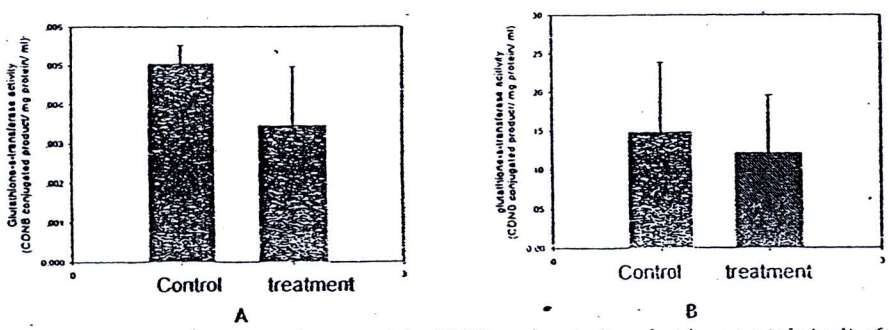

Figure 4. Glutathione-s-transferase activity (CDNB conjugated product/ mg protein/ ml) of 2nd instar *S. exigua* larvae after treated with LC50 concentration of (A) *Amaranthus viridis* L. and (B) *Melia azedarach* L. extract

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INVESTIGATION OF ACUTE TOXICITY OF *JATROPHA GOSSYPIFOLIA* L. (EUPHORBIACEAE) AND *CLEOME VISCOSA* L. (CAPPARIDACAE) EXTRACT ON GUPPIES, *POECILIA RETICULATE*

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SUMMARY

Jatropha gossypifolia L. (Etphorbiaceae) and *Cleome viscosa*, L. (Capparidacae), Thai-plant species, have phytocidal chemical components and responsible for controlling and repelling insects from the host plants. To avoid potential toxic pollutant contaminating aquatic ecosystems, this present study was investigated for acute toxicity. Guppy fish (*Poecilia reticulata*) were selected for the bioassay experiments. The experiments were repeated 5 times and the 24-h LC_{50} was determined for the guppies. The acute toxicity experiments were carried out by static method and behavioral changes in guppies were determined for each *Jatropha gossypifolia* L. and *Cleome viscosa*, L. concentration extract which extracted by Soxhlet extraction method with ethanol as solvent. Water temperature was regulated at 20 ± 1°C. Data obtained from the acute toxicity tests were evaluated using the probit analysis statistical method. The 24-h LC50 value for guppy was estimated as *ca.* 3100 ppm (r^2 = 0.95) and 5300 ppm ((r^2 = 0.96) for *Jatropha gossypifolia* L. and *Cleome viscosa*, L. extract, respectively. However, in this concentration, no mortality was observed at higher concentration for 30 second.

Key words: *Cleome viscosa*, L. (Capparidacae) extract; ; *Jatropha gossypifolia* L.; acute toxicity; Guppy; *Poecilia reticulata*; Bioassay; Behavioral effects

INTRODUCTION

Nowadays, Integrated Pest Management (IPM) is used for the control of many serious pests in Thailand. Botanical insecticides are frequently used within IPM programs in Thailand because of the great diversity of plants with pest-control properties present in the country. Numerous insecticidal plants have shown good potential for insect control, including extracts of chili (*Capsicum frutescens* L.) for the control of *Sitophilus zeamais* Motschulsky (Bullangpoti *et al.*, 2002), sweet apple (*Annona squamosa* L.) for control of *Nephotettix virescens* (Distant) (Srisaad *et al*, 2005), yam bean seed *Pachyrhizus erosua* (Urb) and Ya-Knong-Chang (*Heliotropium indicum* L.) for control of *Aedes aegypti* (Srikhong, 2005) and neem (*Azadirachta indica*) for control of *Damalinia limbata* (Habluetzel *et al.*, 2007), *Anopheles stephensi* Liston (Lucantoni *et al*, 2006) and *Spodoptera litura* Fabricius (Nathan and Kalaivani, 2006) an d mangostin for control *N. lugens* (Bullangpoti *et al.*, 2006 and 2007).

Jatropha gossypifolia L. and *Cleome viscosa* L., Thai plant species, have phytocidal chemical components and responsible for controlling and repelling many insects from the host plants (Adewunmi and Marqis, 1980; Areekul *et al.*, 1988; Siever *et*

al., 1949, Kalyanasundaram and Babu, 1982). However, both of this extract still lacks information about toxicity to aquatic organisms.
Thus, this study investigates the toxic effects of *Jatropha gossypifolia* L. and *Cleome viscosa* L. extract on guppies (*Poecilia reticulata*), the standard test species pursuant to APHA, AWWA, WEF (1998) and OECD (1993) by the determination of 24-h LC_{50} values and evaluates behavioral disorders of the fish exposed to different concentrations of the toxicant.

MATERIAL AND METHODS

About 300 adult, male guppies were obtained from a Hatchery in Bangkok, Thailand. The specimens were transported to the laboratory in appropriately aerated plastic bags. They were kept in 20 L test aquaria containing well water (pH=7.1 and dissolved OXYGEN=7.5 mg/l) and ambient water temperature (20±1°C).
The test species were selected as recommended by these standard methods (OECD, 1993; APHA, AWWA, WEF, 1998) and were put in 5 L test aquaria containing well water (pH=7.1 and dissolved OXYGEN=7.5 mg/l) and ambient water temperature (20±1 °C). The water was continuously aerated before putting in the fish to remove any residual chlorine. The aeration was stopped during dosing period. The fish were fed daily during conditioning period. Duration of the static acute bioassays was 24 h. Toxicity range finding was by pre-experiments carried out in aquaria containing 30 fish.
Different concentrations of *Jatropha gossypifolia* L. and *Cleome viscosa* L. in distilled water and control group which no treated with any extract were added to the experimental aquaria, 5L well water (pH = 7.1 and dissolved OXYGEN = 7.5 mg/l) and ambient water temperature (20±1°C). Each consisting of 30 fish, 5 replicates. The feeding was terminated 24 h and 48 h prior to the initiation of the experiment. Care was taken to keep the mortality rate under 5%. Dead fish were removed immediately and behavioral changes at each concentration were recorded.
For extraction method, dried powdered senescent leaf *Cleome viscosa*, L. (10 kg) and *Jatropha gossypifolia* L. (10 g) were extracted using a Soxhlet apparatus with Ethyl alcohol as solvent. The extract was evaporated to remove solvent by the rotary evaporator (BUCHI B-850) which give the dark green sticky semi-solid crude extract resulted of both extract. Then, both extracts were stored at +4°C until the preparation of the stock solution. Stock solution was prepared by weighing a certain amount of extract and diluting it in distilled water to give the dosing concentrations of 1000, 3000, 5000 and 7000 mg L^{-1}. LC_{50} and 95% confidence limits were calculated by a computer program (SPSS version 11.0).

RESULTS AND DISCUSSION

This research use guppy, *Poecilia reticulata* as the representative for observed the toxicity to aquatic organisms. This kind of fish is can be potential bio-indicator for urban metal pollution, especially their (1) spatial distribution over sites of all pollution region and (2) variation in metal accumulation levels deflecting the degree of pollution (Widianarko *et al.*, 2000).
The data obtained from the toxicity test of senescent leaf *Cleome viscosa*, L. and *Jatropha gossypifolia* L extract on adult male guppies (*P. reticulata*) were evaluated according to Duncan Multiple Range Test and 24-h LC_{50} value (95% confidence limits) of senescent leaf *Cleome viscosa*, L. and *Jatropha gossypifolia* L extract were 3100 mg L^{-1} and 5300 mg L^{-1}, respectively.

In the previous work, there are reported deltamethrin toxicity to *P. reticulata* as the most toxic of the pyrethroids studied (LC_{50} = 0.016 ppm), imidacloprid (over 80 ppm). (Buffin, 2003). Thus, both extract have toxicity to guppies less than the synthetic insecticides. When compare with other botanical insecticides, there are previous research showed that both *Jatropha gossypifolia* L. and *Cleome viscosa*, L extract have less toxicity to guppies such as mangostin form *Garcinia mangostana* extract (LC_{50} = 4.27 ppm) (Bullangpoti *et al.*, 2007), *Pacchyrhizus erosus* extract and seed of sweet apple extract to *Poecilia latipiana*, the LC_{50} is 0.157 ppm and 0.147 ppm, respectively.

However, in each concentration, we looking the effect of both extract when fish take it for 30 second. The result showed no mortality was observed although at higher concentration. It can be say that if the fish only pass the point contamination for short time and did not stay in that contaminated area, it will no toxicity to fish.

Thus, both *Jatropha gossypifolia* L. and *Cleome viscosa* extract seems to be friendly to fish more then other botanical insecticides. Anyway, Visetson *et al.* (2005) found that water have important role for hydrolysis of many botanical insecticides such as salinadiene from nutgrass tuber which will degradation more than 80% after go to natural environment as river at the time 12 hour. However, this result is interesting to note that only a few studies on the acute toxicity of one of the botanical pesticide, namely *Jatropha gossypifolia* L. and *Cleome viscose extract*, to fish exist in the open literature

CONCLUSION

Jatropha gossypifolia L. (Etphorbiaceae) and *Cleome viscosa*, L. (Capparidacae), Thai-plant species, have phytocidal chemical components and responsible for controlling and repelling insects from the host plants. These experiments were repeated 3 times and the 24-h LC_{50} was determined for the guppies. The acute toxicity experiments were carried out by static method and behavioral changes in guppies were determined for each *Jatropha gossypifolia* L. and *Cleome viscosa*, L. concentration extract which extracted by Soxhlet extraction method with ethanol as solvent. The 24-h LC_{50} value for guppy was estimated as *ca.* 3100 ppm ($r2$ = 0.95) and 5300 ppm (($r2$ = 0.96) for *Jatropha gossypifolia* L. and *Cleome viscosa*, L. extract, respectively. However; in this concentration, no mortality was observed at higher concentration for 30 second. This result is interesting to note that only a few studies on the acute toxicity of one of the botanical pesticide, namely *Jatropha gossypifolia* L. and *Cleome viscose extract*, to fish exist in the open literature

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ABSTRACTS

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TOXICITY OF *JATROPHA COSSYPIFOLIA* L. LEAF EXTRACTS ON *SPODOPTERA EXIGUA* (HÜBNER) AND *METEORUS PULCHRICORNIS*

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This study explored the insecticidal effects of Thai botanical, senescent leaf *Jatropha gossypifolia* extracts on second instar *Spodoptera exigua* larvae by the dipping method and topical sprayer method. The leaf crude extract was extracted using Soxhlet apparatus with ethylacetate as solvent. The leaf crude extracts showed insecticidal activity with a LC_{50} of 6182 ppm and 6182 ppm at 24 hours after treatment. In addition, this research was observed its toxicity to insect parasitoid, *Meteorus pulchricornis* by topical method. The result shows 60 percent mortality of this parasitoid species at dose up to 40,000 ppm. Thus, *Jatropha gossypifolia* leaf crude extracts can be as alternative IPM control tool for *Spodoptera exigua* which friendly to benefit insect such as *Meteorus pulchricornis*.