Note

Toxicity of ethyl acetate extract and ricinine from *Jatropha gossypifolia* senescent leaves against *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae)

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The beet armyworm (*Spodoptera exigua* (Hübner)) is one of the most important vegetable pests in Thailand. After 24 hr of both dipping and sprayer bioassay, estimation of LC₅₀ of the ethyl acetate extract of *Jatropha gossypifolia* senescent leaves demonstrated toxicity to secondary instar *S. exigua* larvae because in this stage most armyworm start to move to other plants and it is also the first susceptible stage for toxicity tests. Ricinine, the main alkaloid separated from ethyl acetate crude extract, showed toxicity on secondary instar larvae by the sprayer method with an LC₅₀ of 3,215 ppm whereas the LC₅₀ value for ethyl acetate crude extract is 8,644 ppm. Thus, the ethyl acetate crude extract of *Jatropha gossypifolia* senescent leaves may have ricinine as the active ingredient and may be used as an alternative choice for the minimal application of chemical insecticides for *Spodoptera exigua*. © Pesticide Science Society of Japan

Keywords: *Spodoptera exigua* (Hübner), Noctuidae, *Jatropha gossypifolia*, Ricinine.

Introduction

The beet armyworm (*Spodoptera exigua* Hübner) is native to southeastern Asia and is known as one of the most serious

polyphagous vegetable pests in the world. Due to heavy selection pressure over the past two decades, the pest has developed resistance to various insecticides in many countries.^{1–4)} Recently, in order to circumvent this problem, several biopesticides have been developed. Among these, several works have referred to the efficacy of semiochemicals from plant compounds.⁵⁾ Many plant extracts or allelochemicals from plant extracts are show a broad spectrum of activity against insect pests. The corresponding products have long been considered an attractive alternative to synthetic chemical pesticides for pest management because they pose little threat to the environment or to human health⁵⁾ and have low toxicity to non-target species. Hence, the use of biopesticides, specifically plant products, has gained progressively more research interest.

Several plants extracts have been evaluated for their activity against agricultural important pests for a few decades, especially in Thailand. Numerous insecticidal plants and semiochemicals from plants have been recorded as good candidates for insect control.⁶⁾ *Jatropha gossypifolia*, bellyache bush, is a tropical plant species of the Euphorbiaceae family originating from South America and used in folk medicine.⁷⁾ Various medicinal and pesticidal properties have been attributed to this species.^{8–10)} Different parts of the plant have been previously examined chemically and a review of its chemical compounds has shown that it contains mainly lignan and diterpenoid.^{11–14)}

In this paper, we present the first characterization of an alkaloid, ricinine, which we isolated from an ethyl acetate extract of senescent leaves of *J. gossypifolia*, and its toxicity effect on *S. exigua* was quantified.

Materials and Methods

1. Mass rearing of *S. exigua*

Larvae of *S. exigua* were received from the Ministry of Agriculture, Bangkok, Thailand and were reared on an artificial diet (Silkmate 2M; Nihon Nosan Kogyo Co., Ltd.) according to the modified method of Arakawa.¹⁵⁾ Groups of 5–10 neonates were placed in plastic boxes (15×21×4 cm) with a mesh lid, and reared in an environmental chamber (Sanyo) at 27°C, 70% R.H., 16L–8D photoperiod. Pupae were collected from the culture and placed in a plastic cage (15 cm high×5 cm diam.) until adult emergence. New adults were provided with 10% honey solution via a cotton pad and allowed to lay eggs on a filter-paper sheet.

2. Extraction and isolation

Dried powder of senescent leaves *Jatropha gossypifolia* (90 g) was extracted using a Soxhlet extractor with ethyl acetate as the solvent for 8 h. Extracts were concentrated into a dark-green sticky semi-solid state with a rotary evaporator (BUCHI® B-850) under reduced pressure at 60°C. The completely dried extracts (6.35 g, 7.06% wt/wt) were stored at 4°C until the preparation of a stock solution, which was prepared by weighing a specific amount of the extract and diluting it with distilled 70% ethanol

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for a series of concentrations. The ethyl acetate extract was fractionated using vacuum silica gel column chromatography (Kiesel gel 60G cat No. 7731; Merck), eluting by gradient with gradually increased polarity (5% increment) of hexane–dichloromethane, dichloromethane–ethyl acetate and ethyl acetate–ethanol, respectively. Approximately 500 ml of the eluted solution was collected from each solvent system. All fractions were checked by TLC and those with similar components were combined successively to obtain seven fractions. The toxicity of each fraction was examined using second instars larvae by the dipping method. The most active fraction was further subjected to silica gel column chromatography (Kiesel gel 60G cat No. 7734; Merck) to separate the pure compound which was used to reconfirm the toxicity by the sprayer method. The toxicity analysis method is explained in the bioassay below.

3. Structure identification

After the purification step, the structure of the isolated pure compound was identified using Nuclear Magnetic Resonance (NMR). Proton (^1H) and carbon (^{13}C) NMR spectra were recorded on a Varian Mercury-400 Plus operating at 400 (^1H) and 100 MHz (^{13}C). The chemical shifts (δ) are reported in parts per million relative to tetramethylsilane. Splitting patterns were designated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. Mass spectrometry (MS) analysis was recorded on MSD-Trap (SL).

Ricinine: ^1H NMR (400 MHz, CDCl_3) δ : 7.53 (1H, d, $J=7.6$ Hz, H6), 6.06 (1H, d, $J=7.6$ Hz, H5), 3.97 (3H, s, H9), 3.52 (3H, s, H7); ^{13}C NMR (100 MHz, CDCl_3) δ : 172.4 (C2), 161.3 (C4), 143.6 (C6), 113.7 (C3), 93.6 (C5), 88.5 (C8), 57.1 (C9), 37.5 (C7). NMR data were identical to the literature values.¹⁶⁾ APCI-MS: m/z 165 $[\text{M}+\text{H}]^+$.

4. Bioassay on *S. exigua*

The experimental study was based on a completely randomized design with five treatments of various concentrations between 500–16,000 ppm of the crude extracts and three replicates of 60 sec instars in each treatment because in this stage most armyworm start to move to other plants and it is also first susceptible stage for the toxicity test. In the dipping experiment, each 1-day-old 2nd instar was grasped with forceps and then dipped in each concentration for 5 sec. Ethanol 70% was used as a control, since each concentration was diluted with 70% ethanol. Larvae were then fed with artificial diet. In the sprayer method, 10 2nd instars were placed on filter paper in a Petri dish and sprayed with 1 ml of each concentration between 1,000–16,000 ppm. All treated larvae were transferred to a new plate and fed with artificial diet (Silkmate 2M) until 48 hr. In both experiments, insects that could not move or stay upright were considered to be dead. Mortality was recorded at 24 hr and 48 hr after treatment. Observed data were transformed by Probit analysis using the StatPlus Version 2008 Program for the analyzed LC_{50} value.

Results

Ethyl acetate extracts of *J. gossypifolia* senescent leaves exhibited

Table 1. Average ($\pm$ SD) toxicity of ethyl acetate crude extract and ricinine from *J. gossypifolia* senescent leaves on *Spodoptera exigua* 2nd instar larvae after 24 h and 48 h

Compound	Method	LC_{50} value (ppm) ^{a)}	
		24 hr	48 hr
EtOAc crude extract ^{b)}	Dipping	1,809 $\pm$ 342a	1,588 $\pm$ 295a
	Sprayer	8,644 $\pm$ 1,566a	6,027 $\pm$ 1,227b
Ricinine	Sprayer	3,215 $\pm$ 1,030a	2,087 $\pm$ 882a

^{a)} LC_{50} calculated from 60 treated larvae/replicate, 3 replicates/treatment with 70% ethanol as control for crude extract and 100% acetone for ricinine. Values with the same letter in each row were not significantly different at $P<0.05$ according to t -test. ^{b)} EtOAc=crude extract with ethyl acetate from *J. gossypifolia* senescent leaves

marked insecticidal activity against *S. exigua* larvae when the dipping method was used. The 24 hr LC_{50} and 48 hr LC_{50} of the extracts to *S. exigua* larvae showed no significant difference between these two LC_{50} values (t -value=0.8466, $df=4$, $P=0.4449$ at $P<0.05$) (Table 1). Thus, for the dipping method the exposure time may not correlate with the actual mortality although long exposure can increase mortality.

The crude extract of fraction no. 5 (40% ethyl acetate: dichloromethane–100% ethyl acetate) showed an LC_{50} value with higher toxicity against 2nd instar *S. exigua* than other fractions (Fig. 1). We then decided to analyze the chemical constituent of fraction 5 crude extract in more detail. One main alkaloid was isolated from the crude extract by column chromatography with 5% methanol in dichloromethane, and identified as ricinine using NMR and MS. The chemical structure is shown in Fig. 2. Finally, there were no significant differences between these LC_{50} values between 24 h and 48 h after *S. exigua* were exposed to ricinine (t -value=1.44, $df=4$, $P=0.7728$ at $P<0.05$) (Table 1).

Discussion

This research examined the toxicity of the crude ethyl acetate of *J. gossypifolia* and ricinine against 2nd instar *S. exigua* only. Subsequent investigation by insecticide analysis should be conducted for other larval stages; however, owing to the small size of 1st instar larvae, we were unable to evaluate moribundity to the extract in a manner identical to that used for 2nd instars. For this reason we have not presented the comparison of susceptibility of 1st instars with later instars evaluated as in the research performed by Monton *et al.*¹⁷⁾ In addition to other reasons consistent with the manufacturer's recommendation to target application toward smaller larvae, this is because most smaller larvae are always more susceptible to insecticides than later instars.¹⁷⁾ Thus, some toxicity research on *S. exigua* has started to compare insecticide toxicity with 2nd instars rather than other stages, such as Ahmed and Arif.¹⁸⁾

Toxicity levels of crude *J. gossypifolia* extract against *S. exigua*

Fig. 1. Percentage mortality ($\pm$ S.D.) of *Spodoptera exigua* after being treated with (A) fractions no.1–4 and 6–7 and (B) Fraction no. 5 of ethyl acetate *J. gossypifolia* extract by the dipping method, for which the control group was 70% ethanol.

⁽¹⁾ After monitoring the components from eluted solution using TLC, 20% dichloromethane:hexane could be separated into two fractions (I and II); similarly, 70% dichloromethane:hexane could be separated into two fractions (II and III).

Fig. 2. Structure of ricinine

appear to be higher than those obtained by Khumrunsee *et al.*¹⁹⁾ These authors used *J. gossypifolia* senescent leaf extract extracted with Soxhlet apparatus using ethanol as a solvent (LC_{50} =35,000 ppm) and varied the concentration with distilled water, and finally testing secondary *S. exigua* by the dipping method. Furthermore, the crude extract seemed to show better control efficacy than some botanical extracts by the dipping method against *S. exigua*, such as *Melia azedarach* (LC_{50} =9,793 ppm) or *Amaranthus viridis* (LC_{50} =50,702 ppm).²⁰⁾ Although all extracts listed above were collected using the same Soxhlet method as used here, Khumrunsee *et al.*¹⁹⁾ used ethanol as the extraction solvent and distilled water for dilution. Finally, the crude extract appeared less toxic than *O. canum* and *R. nasutus* methanol extract (LC_{50} =36 and 68 ppm, respectively).²¹⁾

In addition, the dipping method showed higher toxicity than the sprayer method with a significant difference (P =0.0006 and 0.0024 at P <0.05 for 24 and 48 hr after exposed) according to Duncan's New Multiple's Range Test²²⁾ (Table 1).

To compare with other insect species, our results showed that *J. gossypifolia* senescent leaf extract is a good candidate to control *S. exigua* more than *Spodoptera litura*, which demonstrated a LC_{50} of ca. 6,560 ppm.⁸⁾ Although our results were obtained using different extraction solvents than previous research, they demonstrated that ethyl acetate crude extract can control efficacy on *S. exigua* higher than the ethanol crude extract, in which the active ingredient could come from less hydrophilic compounds. The toxicity of this compound, ricinine, was investigated against *S. exigua*. The toxicity of ricinine appeared to be higher than the crude extract's efficacy, which showed an LC_{50} value of $3,215 \pm 1,030$ ppm by the sprayer method.

According to Rizwan-ul-Haq *et al.*,²³⁾ ricinine has been demonstrated to have an insecticide effect. Ricinine was also demonstrated to have high alkaloid toxicity, with the structure of a 2-pyridone ring as the tertiary base that acts almost as an insecticide. This is the first report showing that senescent leaves of *J. gossypifolia* contain ricinine; however, several studies have shown that this compound can be found in several plant species, such as *Ricinus communis*, *Piper nigrum*, *Nicotiana tabacum* or plant species belonging to the Solanaceae family. Rizwan-ul-Haq *et al.*²³⁾ studied the toxicity of ricinine against *S. exigua* and demonstrated reduction in the growth of neonate larvae up to 84.3% and an EC_{50} of 0.27 ppm. In addition, Ramos-Lopez *et al.*²⁴⁾ studied ricinine and ethyl acetate crude extract from *Ricinus communis* against *S. frugiperda* where the half-maximum larva viability concentration (LVC₅₀) was 0.38×10^3 mg ml⁻¹ for ricinine and 5.07×10^3 mg ml⁻¹ for an ethyl acetate extract of leaves, respectively. Ricinine is also toxic to various pests, e.g., *Myzus persicae* by the feeding method, in 8–24 hr²⁵⁾; however, ricinine shows less pronounced toxicity to *S. exigua* than limonoid from an acetone extract of *Citrus reticulata* seeds, which are contaminated with fungi, showing hatching inhibition with EC_{50} as 70.79 ppm and LC_{50} as 75.86 ppm.²⁶⁾ Hence, for practical application, the use of crude extract may be better than using an isolated compound because ricinine is toxic to mammals²⁷⁾ and crude extract may have other active compounds that can interact and synergize

each other to increase control effectiveness against *S. exigua*. Indeed, although secondary allelochemicals from plants are usually commercially available as single, concentrated compounds, compound mixtures have been shown to better reduce pest resistance.⁵⁾

Observations of the symptoms of dead larvae revealed various characteristics of toxicity from the ethyl acetate extract of *J. gossypifolia* senescent leaves and ricinine against *S. exigua*. Extracts did not induce a knock-down effect, as usually seen in pyrethroids; however, we observed that dead worms moved slowly and developed paralysis and enfolded bodies after treatment with all concentrations of crude extract and ricinine. This might have been due to abnormal muscular contraction, indicating a typical hyperactive neural effect of the active component(s) of the extracts. This result is the same as that reported by Ferraz *et al.*,²⁷⁾ in which a high dose of ricinine from *Ricinus communis* on mice showed effects on central nervous system, especially the cerebral cortex and hippocampus, even at low doses. This affects memory efficiency in mice. In addition, only larvae killed by ethyl acetate crude treatments showed darkened bodies, especially on the bottom, whereas control larvae were still green. Hence, the crude extract may have had some compound effects on physiological activity, such as the digestive system of *S. exigua*.

This report showed that ricinine was also included in the *J. gossypifolia* senescent leaf extract; however, this research demonstrated that ethyl acetate crude extract could control *S. exigua*, which could be used as an alternative tool for pest management as farmers produce this extract to control this pest themselves. Although this crude extract includes ricinine and the toxicity risk assessment is a concern, for practical application, using a crude extract may be better than a single chemical because compound mixtures have always reduced pest resistance better than single chemicals in previous research.

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References

- 1) G. Smagghe, T. S. Dhadialla, S. Derycke, L. Tirry and D. Degheele: *Pestic. Sci.* **54**, 27–34 (1998).
- 2) J. K. Moulton, D. A. Pepper and T. J. Dennehy: *Pest. Manag. Sci.* **56**, 842–848 (2000).
- 3) M. Takai: *Bull. Kochi Inst. Agric. Forestry Sci.* **20**, 7–10 (1988) (In Japanese).
- 4) M. Takai: *Shokubutu Boeki (Plant Protection)* **45**, 238–241 (1991) (In Japanese).
- 5) O. Koul and S. Walia: *CAB Rev. Perspect. Agric. Vet. Sci. Nutr. Nat. Res.*, **4**, 1–30 (2009).
- 6) V. Bullangpoti: *Biopestic. Int.* **5**, 75–99 (2009).
- 7) A. Catherine, C. Baraguey, A. Blon, J. Poussset and B. Bodo: *J. Nat. Prod.* **60**, 1155–1157 (1997).
- 8) S. Phowichit, S. Buatippawan and V. Bullangpoti: *Commun. Agric. Appl. Biol. Sci.* **73/3**, 611–620 (2008).
- 9) S. Siriarcharangoj, V. Chuaysuwan, C. Sudthonghong and V. Bullangpoti: *Commun. Agric. Appl. Biol. Sci.* **73/4**, 871–874 (2008).
- 10) A. Chatterjee, B. Das, N. Adityachaudhury and S. Debkirtaniya: *Indian. J. Agr. Sci.* **50**, 637–638 (1980).
- 11) B. Das and R. Das: *Phytochemistry* **40**, 931–932 (1995).
- 12) B. Das and G. Anjani: *Phytochemistry* **51**, 115–177 (1999).
- 13) J. Banerji, Das, B., Chatterjee, A. and Shoolery, J. N. (1984). Gadain, a lignan from *Jatropha gossypifolia*. *Phytochemistry* **23**, 2323–2327.
- 14) K. P. Kozhiparambil and S. Chandrasekharam: *Tetrahedron lett.* **11**, 979–980 (1979).
- 15) T. Arakawa, F. Yukuhiro and H. Noda: *Appl. Entomol. Zool.* **43**, 173–181 (2008).
- 16) C. M. Cazal, J. R. Batalhão, V. C. Domingues, O. C. Bueno, E. R. Filho, M. R. Forim, M. F. G. F. Silva, P. C. Vieira and J. B. Fernandes: *J. Chromatogr. A* **1216**, 4290–4294 (2009).
- 17) J. K. Moulton, D. A. Pepper and T. Y. Dennehy: *Pest. Manag. Sci.* **56**, 842–848 (2000).
- 18) M. Ahmed and M. I. Arif: *Crop Prot.* **29**, 1428–1433 (2010).
- 19) N. Khumrunsee, V. Bullangpoti and W. Pluempunupat: *KKU Sci. J.* **37** (Suppl.), 50–55 (2009).
- 20) S. Rachokarn, N. Piyasaengthong and V. Bullangpoti: *Commun. Agric. Appl. Biol. Sci.* **73/3**, 451–458. (2008).
- 21) C. Kamaraj, A. Rahuman and A. Bagavan: *Parasitol. Res.* **103**, 325–331 (2008).
- 22) D. B. Duncan: *Biometrics*. **13**, 164–176 (1957).
- 23) M. Rizwan-ul-Haq, B. H. Qiong, Y. H. Mei, S. L. Qing and L. Z. Wan: *J. Pest. Sci.* **82**, 1612–4758 (2009).
- 24) M. A. Ramos-López, S. Perez-G, C. Rodríguez-Hernández, P. Guevara-Fefer and M. A. Zavala Sánchez: *Afr. J. Biotechnol.* **9**, 1359–1365 (2010).
- 25) J. I. Olaifa, F. Matsumura, J. A. D. Zeveaart, C. A. Mullin and P. Charalambous: *Plant Sci.* **73**, 253–256 (1991).
- 26) N. Laohakunjit, O. Kerdchoechuen, K. Klymuk and P. Tripetch: *Agric. Sci. J. (Suppl.)*. **38**, 66–69 (2007) (in Thai with english abstract).
- 27) A. C. Ferraz, M. E. Angelucci, M. L. Da Costa, I. R. Batista, B. H. De Oliveira and C. Da Cunha: *Pharmacol. Biochem. Be.* **63**, 367–75 (1999).

1) G. Smagghe, T. S. Dhadialla, S. Derycke, L. Tirry and D.