



## Potential of *Stemona* sp. for *Plutella xylostella* control

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### ABSTRACT

The objective of this research is to assess the effect of *Stemona collinsae* extracts on reducing the major cabbage insect pest, the diamond back moth (*Plutella xylostella*). *Stemona collinsae* was collected from Phitsanulok province. The root were sequentially extracted with hexane, dichloromethane and methanol. These extracts were tested against the third instar *P. xylostella* by leaf dipping method. The highest insecticidal activity was observed from dichloromethane extract with the LC<sub>50</sub> of 0.71%. Following isolation and identification, the major active compound responsible for the insecticidal activity was suggested to be hydroxystemofoline from a molecular peak at m/z 403.2005 [M]<sup>+</sup>, calcd for C<sub>22</sub>H<sub>29</sub>O<sub>6</sub>N 403.1995 in the HR-MS spectrum. This compound can be used as a marker for standardization of stemona extracts and formulations for alternative agricultural production systems.

**Keywords:** stemonaceae, insecticidal activity, stemona alkaloids, diamondback moth, crop pest

### INTRODUCTION

Diamondback moth, *Plutella xylostella* (L.) is known as one of the most important insect pests causing severe damage to crucifers (*Brassica* spp.) worldwide especially during the rainy season in Thailand. Many synthetic insecticides were effectively used to control this pest, but intensive and excessive use of insecticides has caused development of insecticide resistance in pests and consequent pest resurgence, environmental and health hazard associated with pesticide residues. In order to reduce the pesticide uses, biopesticides have been evaluated and found effective against many pests. They are considered as safer alternative to synthetic pesticides currently in use since they have low mammal toxicity and non-persistence to the environment (Pitiyont *et al.*, 2005). Thailand, one of the tropical countries in Southeast Asia, has a wide biodiversity of tropical plants. Some botanicals showing high potential to control pests such as Sadao (*Azadiracta indica*) has been commercialized. Nevertheless, the looks for other potential plants have studied (Crouse and Sparks, 1998; Pitiyont *et al.*, 2005). Impact of botanical insecticides (Liu and Liu, 2005) and botanicals (Hermawan *et al.*, 1994; Vestrud *et al.*, 2005) on *P. xylostella* were available in the literature. *Stemona* (Stemonaceae) is the largest genus with about 32 species. It is a widely distributed perennial climbing plant in Southeast Asia, tropica Australia and North America. The roots of stemona have long been used in Chinese and Japanese traditional medicines for the treatment of respiratory diseases and as anthelmintic agents for

domestic animals. More than 80 alkaloids have been isolated from the stemona plants and their biological activities of some major compounds were described (Pilli and Ferreira, 2000). Insecticidal activity was displayed by stemofoline and its analogues, as well as by some extracts of stemona plants (Brem *et al.*, 2002). However, phytochemical investigations have been restricted to few species.

In Thailand, stemona becomes one of the popular plants used as a folk medicine and botanical pesticides. It is widely distributed across the country especially in the northeast and western part. Jiwajinda *et al.* (2001) found two stemofoline-type alkaloids (i) 16, 17-didehydro-16(E)-stemofoline and (ii) stemofoline which the compound (i) exhibited higher insecticidal and antifeedant activity against *P. xylostella* than stemofoline. The present investigation was undertaken with an objective to further study the insecticidal components from *S. collinsae* to control the major cabbage insect pest, the diamondback moth (*P. xylostella*). These bioactive compounds will be used as chemical markers for standardization of local made stemona extracts as well as developed commercial formulations so that registration of the formulation is possible for effective use by farmers in alternative agricultural production systems.

### MATERIALS AND METHODS

#### Plant material

*Stemona collinsae* was collected from Phitsanulok province, Thailand in June 2008. The plant was

taxonomically identified by the Center of Genetic Plant Diversity, Kasetsart University, Thailand.

#### Extraction, isolation and identification

*Stemona* roots were washed, dried, sliced into small pieces and ground in powder. Powder (3 kg) was sequentially extracted by maceration with hexane, dichloromethane and methanol, respectively at room temperature for 3 days per extraction. After evaporation of the solvents under reduced pressure, the crude extracts were used for the insecticidal activity to *P. xylostella* third instar larvae. Ten g portions of the crude extract which exhibited highest mortality of larvae was first fractionated on a silica gel (70-230 mesh) column chromatography and eluted with 20% step gradient of hexane in ethyl acetate and then with methanol. The most active fraction was further isolated by repeated column chromatography and purified by preparative TLC (silicagel 60 F<sub>254</sub>, Merck) using 20% ethyl acetate in methanol as the developing solution. Identification of the most active compound was carried out by spectroscopic techniques like HR-MS, IR and <sup>1</sup>NMR.

#### Insect culture and Bioassay

*Plutella xylostella* larvae were collected from fields at Bangbuathong district, Nonthaburi province and reared on chinese kale leaves at 25±2 °C, 13:11 h (L:D). Bioassay was conducted by no-choice leaf dipping method (Dadang, 1999) in a completely randomized block design. Four concentrations (0.5, 1.0, 1.5 and 2.0% w/v) of hexane, dichloromethane and methanol extracts were prepared from the respective crude extracts. Controls were acetone with 1% tween-80, the solvent used to prepare test solutions. Leaves of Chinese kale (2 cm) were dipped in different concentrations of the extracts separately for 1 min and air-dried. Thirty 3<sup>rd</sup> instar larvae of *P. xylostella* each were released in plastic cups (10 ml capacity) containing either treated or untreated (control) leaf disks as one replicate. Three replicates were used for each concentration of the extract. All bioassays were kept at 25±2 °C with a 13:11 h (L:D) photo period. Mortality was assessed after 24 h application and corrected if necessary by Abbott's formula (Abbott, 1925).

#### Statistical analyses

Data were analyzed by analysis of variance (ANOVA). Means of the treatments in each experiment were compared by DMRT at  $p < 0.05$ . LC<sub>50</sub> value was determined by probit analysis (Finney, 1971).

## RESULTS AND DISCUSSION

#### Plant characteristics

*Stemona collinsae* stems 1-2 meter, smooth; leaf heart curve, smooth slender, apex acuminate, leaf blade ovate to broadly,

**Table 1.** Efficacy of *S. collinsae* crude extracts on the percentage mortality of the 3<sup>rd</sup> instar *P. xylostella* at 24 h

Concentration (% w/v)	% mortality		
	Hexane extract	Dichloromethane extract	Methanol extract
control	0.00 ± 0.0 <sup>a</sup>	0.00 ± 0.0 <sup>a</sup>	0.00 ± 0.0 <sup>a</sup>
0.5	28.89 ± 2.1 <sup>b</sup>	43.33 ± 2.0 <sup>b</sup>	37.78 ± 1.2 <sup>b</sup>
1.0	48.89 ± 0.6 <sup>c</sup>	68.89 ± 1.2 <sup>c</sup>	66.67 ± 2.0 <sup>c</sup>
1.5	63.33 ± 2.7 <sup>d</sup>	87.78 ± 0.6 <sup>d</sup>	78.89 ± 0.6 <sup>d</sup>
2.0	70.00 ± 1.7 <sup>e</sup>	91.11 ± 0.6 <sup>e</sup>	85.56 ± 0.6 <sup>e</sup>

In a column, means ± SD followed by the same letter are not significantly different at ( $p < 0.05$ ) by DMRT

veins 9-15, crosswise throughly leaf, racemes, 1-2 flowered, flowers pass leaf bottom, pedicel round, green, smooth, 4 bracts, 4 stamens opposite, filaments with 1 ovary, anthers white-green, appendage 10-30, long - bunch, Roots 10-30 cm long, 0.6-1.2 cm diameter, light brown slender.

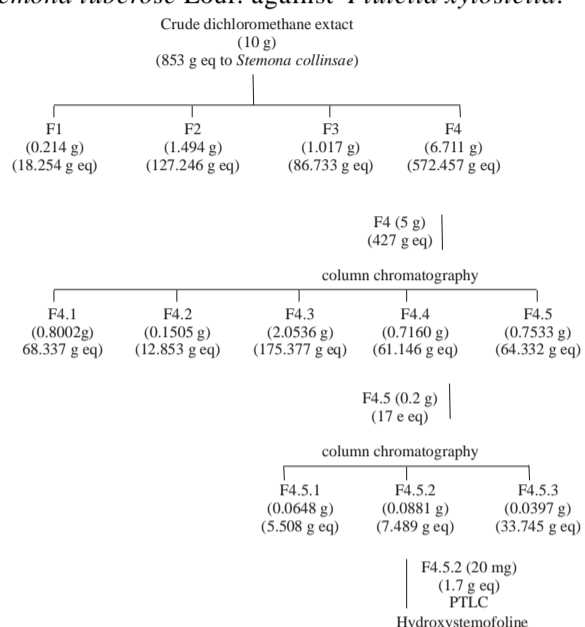
#### *Stemona collinsae* crude extracts on *Plutella xylostella*

Extraction of stemona roots (3 kg) by maceration in solvents that have different pole order from low to high yielded crude extracts as follows (w/w) : hexane, 0.35%; dichloromethane, 0.63% and methanol, 9.27%. Bioassays of the crude extracts by leaf dipping method at concentration 0.5 to 2.0% w/v revealed that there was a significant effect of concentration rate on mortality of *P. xylostella* ( $p < 0.05$ ) at 24h exposure with mortality tending to increase with increasing concentrations (Table 1). Among the crude extracts tested, the dichloromethane extract gave the highest mortality to *P. xylostella* followed by methanol and hexane extract. The extracts caused 91.11 ± 0.6%, 85.56 ± 0.6 and 70.00 ± 1.7% mortality at 2% concentration for dichloromethane, methanol and hexane, respectively (Table 1) with LC<sub>50</sub> values of 0.60, 0.71, and 1.15 % (Table 2). This result corresponds to the report of Pitiyont *et al.* (2008) and Tikum *et al.* (2008) where dichloromethane extract of the root of *Stemona burkillii* was the most effective against *Spodoptera litura* and *Spodoptera exiqua*. A similar result was reported by

**Table 2.** The median lethal concentration (LC<sub>50</sub>) of *S. collinsae* extracts on the 3<sup>rd</sup> instar *P. xylostella* larvae at 24 h

Crude extracts	LC <sub>50</sub> (% w/v)	95% Confidence Limits		Slope±S.E
		Lower	Upper	
Hexane	1.15	0.94	1.33	0.0044±0.168
Dichloromethane	0.60	0.36	0.75	0.0085±0.175
Methanol	0.71	0.45	0.88	0.0071±0.170

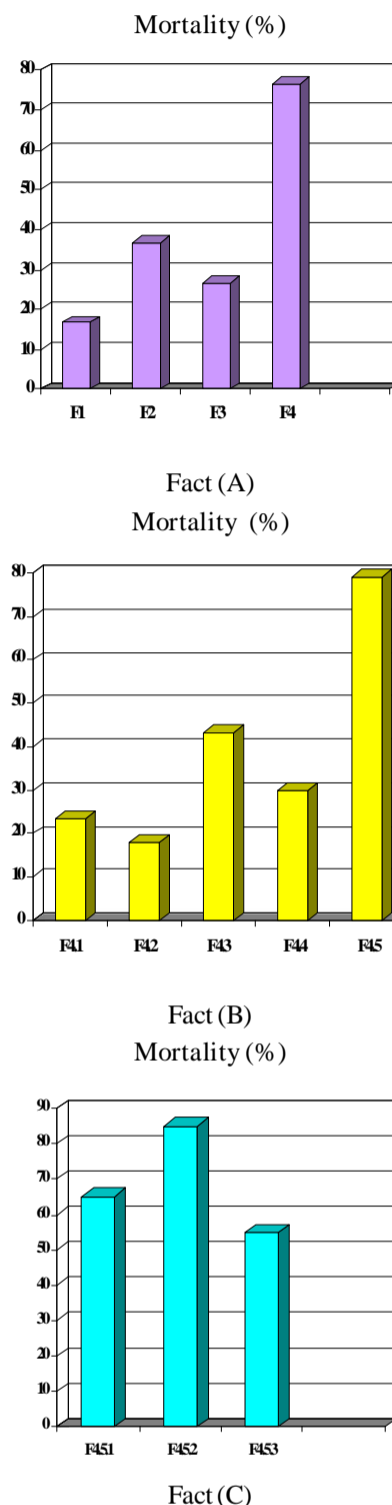
Jiyavorrnanant (2001) on the dichloromethane extract of *stemona tuberosa* Lour. against *Plutella xylostella*.



**Figure 2.** Scheme of separation of the active compound from *Stemona collinsae*

#### *Stemona collinsae* fractions on *Plutella xylostella*

Chromatographic separation of the dichloromethane extract (10 g) (853 g eq to *stemona collinsae*) on silica gel column yielded 4 fractions (F1-F4) (Figure 2). Bioassays of these fractions showed that F4 was the most toxic (76.67% mortality) at 1.5% concentration, while F1-F3 at the same concentration gave 16.67, 36.67 and 26.67% mortality, respectively. Fractionation of F4 yielded F4.1-F4.5, of which F4.5 at 1.5% concentration resulted in 78.89% larval mortality, whereas F4.1-F4.4 were comparatively less effective (Figure 3). Further separation of F4.5 by column chromatography gave 3 fractions (F4.5.1-F4.5.3). Of the three fractions isolated from F4.5, F4.5.2 showed higher mortality (85%) at 1.5% concentration, the remaining 2 fractions all showed mortality activity. This fraction was further purified by preparative thin-layer chromatography (PTLC), using 20% ethyl acetate in methanol as the developing solvent to give a purified compound ( $R_f = 0.65$ ). This compound responsible for the insecticidal activity was suggested to be hydroxystemofoline from a molecular peak at  $m/z$  403.2005  $[M]^+$ , calcd for  $C_{22}H_{29}O_6N$  403.1995 in the HR-MS spectrum. The isolated compound was possibly more toxic than two stemofoline-type alkaloids (i) 16,17-didehydro-16(E)-stemofoline and (ii) stemofoline obtained from *S. collinsae* (Jiwajinda *et al.*, 2001). These findings indicated that *Stemona collinsae* root extracts could be applicable as an alternative agent for controlling of *P. xylostella*.



**Figure 3.** Efficacy of *S. collinsae* fractions after separation by column chromatography on the percentage mortality of the 3<sup>rd</sup> instar *P. xylostella* at 24 h by F1-F4 (A), F4.1-F4.5 (B), F4.5.1-F4.5.3 (C)

In conclusion, certain concentrations of dichloromethane extract of *Stemona collinsae* roots showed pronounced insecticidal activity to the 3<sup>rd</sup> instar *Plutella xylostella* larvae. Furthermore, the study described hydroxystemofoline as a potent compound. This scientific finding supports the extensive use of this plant as a biopesticide in Thailand.

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#### REFERENCES

- Abbott, W. S. 1925. Method for computing the effectiveness of an insecticide. *Journal of Economic Entomology*, **18**: 265-267.
- Brem, B., C. Seger, T. Pacher, O. Hofer, Vajrodaya, S. and H. Greger. 2002. Feeding deterrence and contact toxicity of *stemona* alkaloids a source of potent natural insecticides. *Journal of Agricultural Food Chemistry*, **50**: 6383-6388.
- Crouse, G. D. and Sparks, T. C. 1998. Naturally derived materials as products and leads for insect control: The spinosyns. *Reviews in Toxicology*, **2**, 133-146.
- Dadang. 1999. Insect Regulatory Activity and Active Substances of Indonesian Plants Particularly to the Diamondback Moth. Ph.D. Thesis, Tokyo University of Agriculture.
- Finney, D. J. 1971. Probit analysis, 3rd ed. Cambridge University Press: London.
- Hermawan, W., Kajiyama, S., Tsukuda, R., Fujisaki, K., Kobayashi, A. and Nakasuji, F. 1994. Antifeedant and antioviposition activities of the fractions of extract from a tropical plant, *Andrographis paniculata* (Acanthaceae), against the diamondback moth, *Plutella xylostella* (Lepidoptera: Yponomeutidae). *Applied Entomology and Zoology*, **29**(4): 533-538.
- Jiwajinda, S., N. Harai, K. watanabe, V. santisopasri, N. Chuengsamarnyart, K. Koshimizu and H. Ohigashi. 2001. Occurrence of the Insecticidal 16,17-didehydro-16(E)-stemofoline in *Stemona collinsae*. *Phytochemistry*, **56**: 693-695.
- Jiyavorrnanant, T. 2001. Insecticide from *Stemona tuberosa* Lour. and *Acorus calamus* Linn. and their residues after application. M.S. Thesis, Chaing Mai University.
- Liu, T. and Liu, S. 2005. Varying effects of two lots of the botanical insecticide Neemix® 4.5 on immature survival and adult oviposition behavior of the diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae). *International Journal of Pest Management*, **51** (1): 31-35.
- Pilli, R. A. and Ferreira de Oliveira, M. C. 2000. Recent progress in the Chemistry of the *Stemona* Alkaloids. *Natural Product Report*, **17**: 117-127.
- Pitiyont, V. B. Pitiyont and Ohsawa, K. 2005. Development of Non-Tai-Yak (*Stemona* spp.) extracts for vegetable production in Thailand. *Journal of ISSAAS*, **11**(3) (suppl.): 156-160.
- Pitiyont, B., Tikum, N. and Ohsawa, K. 2008. Bioefficacy of *Stemona burkillii* extracts on *Spodoptera litura*. *Journal of ISSAAS*, **13**(3) (suppl.): 31-37.
- Tikum, N., Pitiyont, B., K. Shimomura and Ohsawa, K. 2008. Insecticidal Activity and Chemical Compositions of *Stemona burkillii* against *Spodoptera exigua* and *Spodoptera litura*. ISSAAS Congress 2008. February 23-27, 2009. Bangkok, Thailand.
- Vastrad, AS., Lingappa, S., Basavangoud, K. 2005. Ovicides for the management of insecticide resistant populations of diamondback moth, *Plutella xylostella* (Linnaeus). *Journal of Entomological Research*, **29** (1): 49-51.

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