Tropical Biomedicine 29(2): 286–296 (2012)

Larvicidal activity of a toxin from the seeds of *Jatropha curcas* Linn. against *Aedes aegypti* Linn. and *Culex quinquefasciatus* Say

Chanthakan Nuchsuk[1], Nuanchawee Wetprasit[2], Sittiruk Roytrakul[3] and Sunanta Ratanapo[1*]
[1]Department of Biochemistry, Faculty of Science, Kasetsart University, Bangkok, Thailand
[2]Department of Biotechnology, Faculty of Science, Ramkhamhaeng University, Bangkok, Thailand
[3]National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), Pathumthani, Thailand
[*]Corresponding author email: fscisnr@ku.ac.th
Received 21 January 2012; received in revised form 21 March 2012; accepted 22 April 2012

Abstract. The larvicidal effects of the crude protein extract and purified toxin, Jc-SCRIP, from the seed coat of *Jatropha curcas* Linn. against the third instar larvae of mosquitoes, *Aedes aegypti* Linn. and *Culex quinquefasciatus* Say, were investigated. This test compared the effects of the purified toxin with crude protein extracts from seed kernels of *J. curcas* and *Ricinus communis*. At various concentrations of purified toxin and crude protein extract, the larval mortality of both *Ae. aegypti* and *Cx. quinquefasciatus* were positively correlated with increased exposure time. The larvae of *Cx. quinquefasciatus* were more susceptible to the toxin and both extracts than the larvae of *Ae. aegypti*. After 24 hours of exposure, the extract showed larvicidal activity against *Ae. aegypti* and *Cx. quinquefasciatus* with LC_{50} values of 3.89 mg/ml and 0.0575 mg/ml, respectively. The toxin, Jc-SCRIP, showed larvicidal activity against *Ae. aegypti* and *Cx. quinquefasciatus* with LC_{50} values of 1.44 mg/ml and 0.0303 mg/ml, respectively. These results indicated that the crude protein extract and Jc-SCRIP were more toxic to the third instar larvae of *Cx. quinquefasciatus* than that of *Ae. aegypti*. The potent larvicidal activities of the seed coat extract and the Jc-SCRIP toxin from *J. curcas* suggest that they may be used as bioactive agents to control the mosquito population.

INTRODUCTION

Mosquitoes are well-known vectors of several endemic diseases, such as malaria, filariasis, yellow fever, dengue fever, dengue haemorrhagic fever and encephalitis. Although there are more than three thousand species of mosquitoes found throughout the world, *Aedes aegypti* and *Culex quinquefasciatus* are amongst the most important targets for population control in Thailand and other countries in the South-East Asia. *Aedes aegypti* is the primary vector of dengue. Approximately two fifths (40%) of the world's population are now at risk of contracting dengue and more than 70% of the at risk population live in the South-East Asia and Western Pacific regions (WHO & TDR, 2009). It is estimated that each year 500,000 people of the 50-100 million infected people experience severe dengue with mortality rate of about 2.5% per year (WHO, 2012). *Culex quinquefasciatus* is the vector of lymphatic filariasis, which is caused by the nematode *Wuchereria bancrofti* in tropical and sub-tropical areas. More than 1.3 billion people in 72 countries are at risk of contracting lymphatic filariasis (WHO, 2012). Approximately 65% of the at risk groups live in South-East Asia, 30% in Africa, and the remainder in other tropical countries. Over 120 million people are currently infected (WHO, 2012).

One approach to decrease the mosquito population attempts to interrupt the mosquito life cycle at the larval stage (Chowdhury *et*

al., 2008). Chemical pesticides are widely used to kill the mosquito larvae, but the residue produces environmental problem and is harmful to humans as well as animals, sometimes resulting in the widespread development of resistance to the pesticide. Plant phytochemicals, or plant extracts, have become increasingly attractive as alternative mosquito control agents because they show small or no harmful effects on non-target organisms and the environment (Chantraine *et al*., 1998; Park *et al*., 2002; Cavalcanti *et al*., 2004; Raj Mohan & Ramaswamy, 2007). However, nearly all of these phytochemicals are extracted from plants with organic solvents. Therefore, we are very interested in identifying new, effective and eco-friendly mosquito larvicide natural agents that are target specific and cheap from the aqueous extract and water-soluble bioactive proteins.

The physic nut, *Jatropha curcas* Linn, is a plant that belongs to the Euphorbiaceae family. This plant shows high agro-industrial potential in Thailand because the seed produces a non-edible oil that can be used as a biofuel. However, high numbers of seed coats are a by-product of the oil production, which become agricultural waste and seed cake for animal feed. Recently, we have purified and characterized two type 1 ribosome-inactivating proteins (type 1 RIPs) from physic nut. One of these RIPs is a toxin found in the nut kernel called curcin. Curcin has a molecular mass of 29 kDa and possesses *N*-glycosidase activity towards the 28S rRNA of rabbit reticulocytes (Lin *et al*., 2003; Kittikajhon *et al*., 2010). The other RIP is a toxin found in the nut seed coat termed Jc-SCRIP. Jc-SCRIP has a molecular mass of 38.9 kDa and possesses both *N*-glycosidase and hemagglutination activities (unpublished results). RIPs are plant toxins which function as the plant defense mechanism. They are toxic to most eukaryotes as a consequence of their *N*-glycosidase activity, which cleaves a specific adenine residue at position 4324 of eukaryotic 28S rRNA, thus inhibiting the protein synthesis (Endo & Tsurugi, 1987; Park *et al*., 2002). Some plant RIPs have been demonstrated to possess insect larvicidal activities (Zhou *et al*., 2000; Singh *et al*., 2006).

In the present study, the toxic type 1 RIP, Jc-SCRIP, was isolated from the seed coat of *J. curcas* and was evaluated for larvicidal activity against the third instar larvae of the two mosquitoes, *Ae. aegypti* and *Cx. quinquefasciatus*. The activity of Jc-SCRIP was compared to the crude proteins extracted from the seed coat and kernel of *J. curcas* and from the seed kernel of castor bean, *Ricinus communis*.

MATERIALS AND METHODS

Plant materials

Mature seeds of *J. curcas* KUBP 33 were provided by the Suwanwajokkasikit Field Crops Research Station, the Inseechandrastitya Institute for Crop Research and Development (IICRD) and Kasetsart University, Thailand. Mature seeds of *R. communis* were a gift from Assoc. Prof. Dr. Nuanchawee Wetprasit.

Preparation of crude protein extract

The mature seed coat of *J. curcas* and the seed kernels of each plant were ground and the crude protein of each sample was extracted by continuous stirring for 48 h at 4°C in a 50 mM Tris-HCl buffer (pH 7.5) containing 0.15 M NaCl (1:3 w/v). The crude protein extract was dialyzed against a 50 mM Tris-HCl buffer (pH 7.5) overnight and centrifuged at 8,000 x g for 30 min. The supernatant was stored at 4°C until use.

Purification of the seed coat toxin of *J. curcas*, Jc-SCRIP

The type 1 RIP, Jc-SCRIP, was isolated from the crude protein extract of the mature seed coat of *J. curcas*. The crude protein extract was precipitated by ammonium sulfate at 80% saturation, and the pellet was dissolved and dialyzed against a 50 mM Tris-HCl buffer (pH 7.5). The extract was applied to a DEAE Sephacel™ (GE Healthcare, Little Chalfont, UK) column (1.5 x 20 cm) that was equilibrated with the dialysis buffer. The unbound proteins were washed out with the same buffer at a flow rate of 40 ml/h. The adsorbed proteins were eluted with a step-wise application of 0-2 M NaCl in a 50 mM

Tris-HCl buffer. Fractions of 4 ml were collected, and the UV absorption was monitored at 280 nm. The unbound fractions were pooled and dialyzed against a 50 mM sodium acetate buffer (pH 5.0) before chromatography on a CM-cellulose (Sigma, St. Louis, MO) column (fast flow, 1.5 x 20 cm.) at a flow rate of 30 ml/h which was equilibrated, and the unbound proteins were washed out with the same buffer. A gradient of NaCl from 0-2 M in the same buffer was used to elute the bound fractions. Fractions containing hemagglutination and rRNA *N*-glycosidase activity were pooled, dialyzed against a 100 mM Tris-HCl buffer (pH 7.5) and stored at -20ºC.

rRNA *N*-glycosidase activity assay

The toxicity of the crude extract and the purified Jc-SCRIP toxin on eukaryote protein synthesis were determined by the modified method of Lin *et al.* (2003), employing rabbit reticulocyte lysate as the rRNA substrate. The test samples were mixed with rabbit reticulocyte lysate in 100 µl of reaction buffer (20 mM Tris-HCl buffer (pH 7.6), 50 mM KCl and 5 mM $MgCl_2$) and incubated at 37ºC for 10 min. Then, the reaction was stopped by adding 10 µl of 10% SDS. The reaction mixture was extracted with phenol/chloroform (1:1 v/v) and RNA was recovered by ethanol precipitation. The RNA pellet was dissolved and treated with 1 M aniline/0.8 M acetic acid for 10 min at 60ºC. The reaction products were separated out in a 6% polyacrylamide gel containing 7 M urea. The gel was stained with ethidium bromide and the RNA bands were visualized on a UV transilluminator.

Protein concentration

Protein concentrations were determined according to the method of Lowry *et al.* (1951), using bovine serum albumin as the standard.

Hemagglutination assay

The hemagglutinating activity of the seed coat protein against rabbit erythrocytes was determined according to Ratanapo *et al.* (1998). The hemagglutination reaction was performed in a microwell plate using a 2% (v/v) erythrocyte suspension in a 50 mM Tris-HCl buffer (pH 7.5) containing 0.15 M NaCl. The hemagglutination activity (HA) was expressed in units (U), which is the reciprocal of the highest dilution of lectin that results in complete agglutination.

Larvicidal activity assay

The toxicity of the seed coat protein on *Ae. aegypti* and *Cx. quinquefasciatus* larvae was evaluated according to Chantraine *et al.* (1998). Late third instar *Ae. aegypti* and *Cx. quinquefasciatus* larvae were procured from the Department of Medical Sciences, Ministry of Public Health. Ten healthy larvae were transferred into each plastic glass containing 10 ml of crude extract or purified protein extracts at five concentrations. Distilled water and two-fold serial dilutions of a 50 mM Tris-HCl buffer (pH 7.5) were used as controls. Food was not given to the larvae during the experiment. If the larvae could not rise to surface when probed with a needle, they were considered dead. The mortalities were recorded after 12, 24, 48, and 72 h.

Statistical analysis

Five replicates were completed for all experiments. The Abbott's formula (1925) was used to correct mortality rates: corrected mortality (%) = $[(M_{obs}–M_{control})/(100-M_{control})]x100$, where M_{obs} is mortality observed in the test and $M_{control}$ is mortality observed in the control. The lethal concentration (LC_{50}) after 24 h of exposure was calculated by probit analysis and tested using the method of Finney (1971).

RESULTS AND DISCUSSION

Jc-SCRIP is one of the toxic type 1 RIPs discovered in *J. curcas* (unpublished results). It is composed of a single chain polypeptide with a MW of 41.8 kDa as determined by SDS-PAGE. It showed hemagglutination and *N*-glycosidase activity. The *N*-glycosidase activity has been suggested to be responsible for its cytotoxicity against some human cancerous cell lines and antibacterial activity. In this study, we isolated Jc-SCRIP from the mature seed coat of *J. curcas* by ammonium sulfate precipitation, anion-

exchange on a DEAE Sephacel™ column and cation-exchange on a CM-cellulose column. The purified protein showed a 113.5-fold purification with 1.12% recovery. The purity of the protein was shown by the presence of a single band on a SDS-PAGE (data not shown). The *N*-glycosidase activity of the protein was shown by the generation of a single band of the rRNA fragment product from the 28S rRNA substrate (Fig. 1).

The toxicity of the purified Jc-SCRIP on the late third instar larvae of two mosquitoes, *Ae. aegypti* and *Cx. quinquefasciatus*, was evaluated and compared to three crude protein extracts: *J. curcas* seed coat crude protein (JSCCP), *J. curcas* seed kernel crude protein (JSKCP) and seed kernel crude protein of castor bean, *R. communis* (RSKCP). The difference in protein concentrations and exposure times required to induce the larval mortality was observed. Three crude protein extracts showed larvicidal activities against *Ae. aegypti* at a protein concentration of $\geq$ 0.375 mg/ml after 24 h of exposure (Table 1) and against *Cx. quinquefasciatus* at a protein concentration of $\geq$ 0.1875 mg/ml after 12 h of exposure (Table 2). Both larvae species remained viable in the control samples (absence of the extract or the purified protein). The Jc-SCRIP purified protein showed larvicidal activity against *Ae. aegypti* and *Cx. quinquefasciatus* at a protein concentration of $\geq$ 0.75 mg/ml after 24 h of exposure and $\geq$ 0.1875 mg/ml after 12 h of exposure, respectively. This protein induced 100% mortality in *Ae. aegypti* at a protein concentration of 3.0 mg/ml after 72 h of exposure (Table 1, Fig. 2A) and 100%

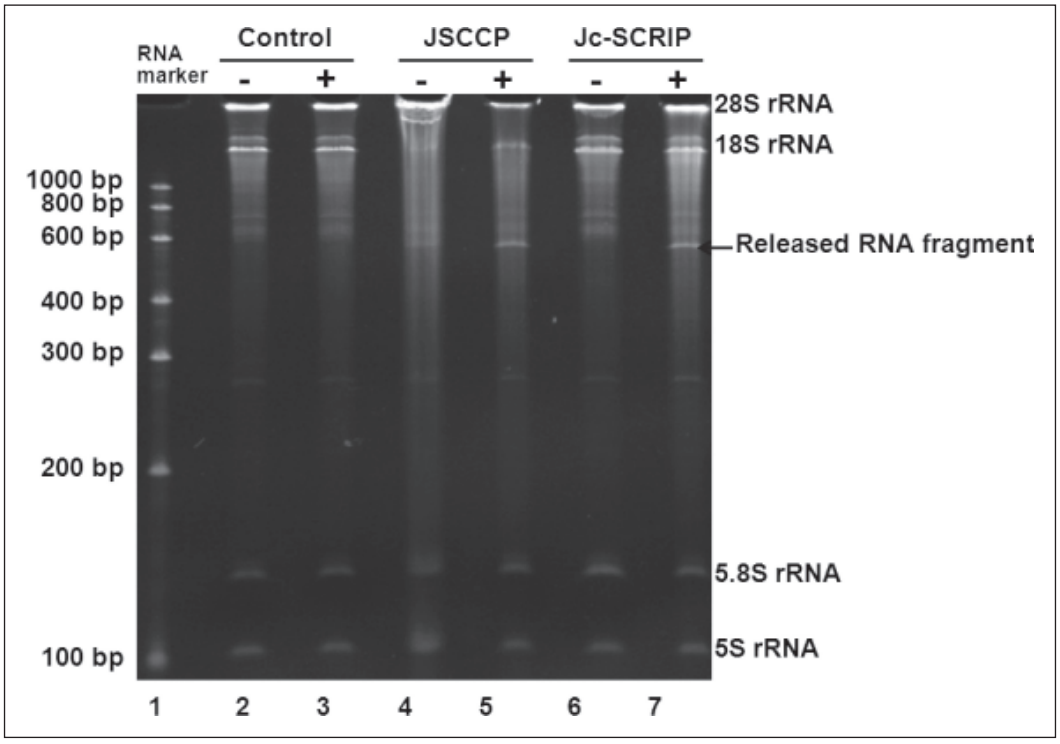

Figure 1. *N*-glycosidase activity assay. A 7.5 M urea/6% PAGE analysis of rabbit reticulocyte lysate treated with crude protein extract (JSCCP) and purified protein from the seed coat of *J. curcas* (Jc-SCRIP). '+' and '–' indicate the presence and absence of aniline treatment, respectively. Lane 1: RNA marker; Lanes 2 and 3: negative control (no protein fraction) with and without aniline treatment, respectively; Lane 4: treated with JSCCP (1 µg) without aniline treatment; Lane 5: treated with JSCCP (1 µg) with aniline treatment; Lane 6: treated with Jc-SCRIP (1 µg) without aniline treatment; Lane 7: treated with Jc-SCRIP (1 µg) with aniline treatment. The arrow indicates the released fragment after aniline treatment, which was characteristic of the *N*-glycosidase activity of plant ribosome-inactivating proteins

mortality in *Cx. quinquefasciatus* at a protein concentration of 1.5 mg/ml after 12 h of exposure (Table 2, Fig. 3A). The larvicidal activity of all crude protein extracts against *Cx. quinquefasciatus* is more robust than against *Ae. aegypti* and required lower protein concentrations and exposure times. The JSKCP and RSKCP crude protein extracts induced 100% mortality in *Cx. quinquefasciatus* at protein concentration of 1.5 mg/ml after 12 h of exposure, whereas the JSCCP crude protein extract induced 100% mortality in *Cx. quinquefasciatus* at protein concentration of 3.0 mg/ml after 12 h of exposure (Table 2, Fig. 3B-3D). However, all crude protein extracts could not induce 100% mortality in *Ae. aegypti* at any protein concentration or exposure time (Table 1, Fig. 2B-2D).

The toxicity of Jc-SCRIP and each of the crude protein extracts to both mosquito larvae was dose dependent (Fig. 2, 3). The median lethal concentration (LC_{50}) of Jc-SCRIP, JSCCP, JSKCP and RSKCP to *Ae. aegypti* larvae after 24 h of exposure was 1.44, 3.89, 2.95 and 2.24 mg protein/ml, respectively (Table 3). The LC_{50} results indicated that the larvicidal activity towards *Ae. aegypti* of the Jc-SCRIP purified protein is approximately 2.7, 2.0 and 1.5 times more toxic than JSCCP, JSKCP and RSKCP, respectively (Table 3). *Culex quinquefasciatus* larvae were more sensitive to all of the proteins tested, hence giving the lower LC_{50} values, than the *Ae. aegypti* larvae. The LC_{50} of Jc-SCRIP, JSCCP, JSKCP and RSKCP for *Cx. quinquefasciatus* larvae after 24 h of exposure were 0.0303, 0.0575, 0.0468 and 0.0389 mg protein/ml, respectively (Table 3). The LC_{50} results indicated that the larvicidal activity of the purified protein, Jc-SCRIP, against *Cx. quinquefasciatus* was approximately 1.9, 1.5 and 1.3 times more toxic than JSCCP, JSKCP and RSKCP, respectively (Table 3). The LC_{50} values indicated that Jc-SCRIP is approximately 50 times more toxic towards

Table 1. The mean % mortalities induced by plant proteins on the third instar larvae of *Aedes aegypti* at various concentrations and exposure times

Proteins	Concentration (mg/ml)	Period of Exposure (h)			
		12	24	48	72
Jc-SCRIP	0.1875	0±0.0	0±0.0	0±0.0	0±0.0
	0.375	0±0.0	0±0.0	0±0.0	0±0.0
	0.75	0±0.0	8±8.4	30±10.0	34±13.4
	1.5	0±0.0	56±8.4	70±8.9	82±8.4
	3.0	0±0.0	82±8.4	94±4.5	0±0.0
JSCCP	0.1875	0±0.0	0±0.0	0±0.0	0±0.0
	0.375	0±0.0	6±5.5	8±4.5	8±4.5
	0.75	0±0.0	12±4.5	2±8.4	2±8.4
	1.5	0±0.0	8±4.5	26±5.5	8±8.4
	3.0	0±0.0	50±7.1	64±13.4	64±13.4
JSKCP	0.1875	0±0.0	0±0.0	0±0.0	0±0.0
	0.375	0±0.0	8±4.5	18±11.0	22±13.0
	0.75	0±0.0	12±4.5	24±5.5	32±8.4
	1.5	0±0.0	24±5.5	44±8.9	54±5.5
	3.0	0±0.0	58±8.4	64±8.9	70±7.1
RSKCP	0.1875	0±0.0	0±0.0	0±0.0	0±0.0
	0.375	0±0.0	12±8.4	20±0.0	20±0.0
	0.75	0±0.0	22±8.4	36±8.4	42±8.4
	1.5	0±0.0	28±8.4	52±8.4	62±11.0
	3.0	0±0.0	66±11.4	78±13.0	86±8.9

JSCCP = *Jatropha curcas* seed coat crude protein; JSKCP = *Jatropha curcas* seed kernel crude protein; RSKCP = *Ricinus communis* seed kernel crude protein

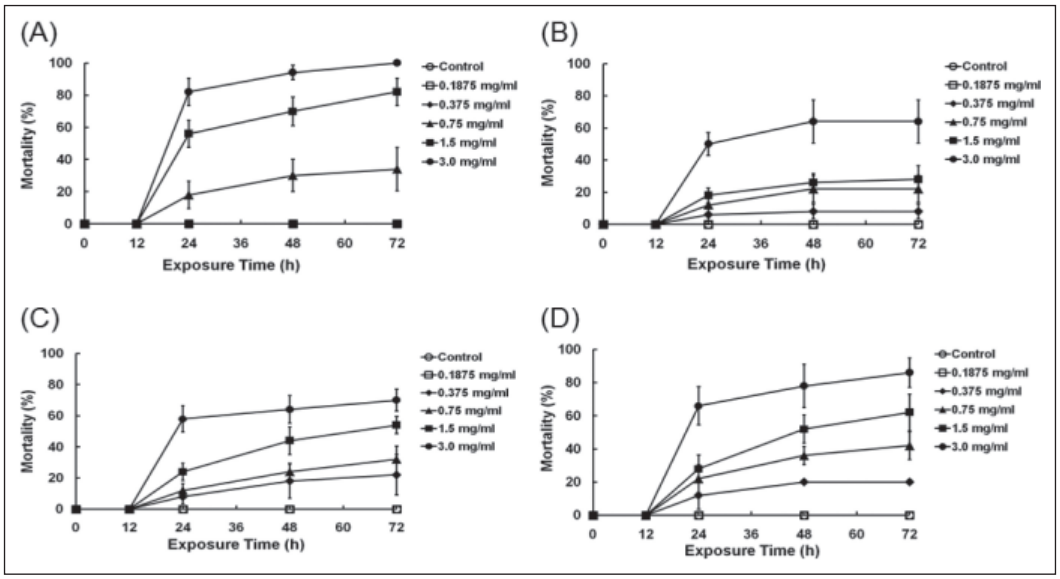

Figure 2. The larvicidal activity of the crude protein extract and purified toxin from the seed coat of *J. curcas* compared to the crude protein extract from the seed kernels of *J. curcas* and *R. communis* against the third instar larvae of *Aedes aegypti*. (A) Purified toxin from the seed coat of *J. curcas* (Jc-SCRIP). (B) *J. curcas* seed coat crude protein (JSCCP). (C) *J. curcas* seed kernel crude protein (JSKCP). (D) *R. communis* seed kernel crude protein (RSKCP)

Table 2. The mean % mortalities induced by plant proteins on the third instar larvae of *Culex quinquefascitus* at various concentrations and exposure times

Proteins	Concentration (mg/ml)	Period of Exposure (h)			
		12	24	48	72
Jc-SCRIP	0.1875	64±8.9	76±5.5	84±5.5	90±0.0
	0.375	78±8.4	86±5.5	100±0.0	100±0.0
	0.75	86±8.9	90±5.5	100±0.0	100±0.0
	1.5	100±0.0	100±0.0	100±0.0	100±0.0
	3.0	100±0.0	100±0.0	100±0.0	100±0.0
JSCCP	0.1875	42±13.0	68±11.0	72±13.0	76±13.4
	0.375	66±11.4	82±13.0	94±5.5	94±5.5
	0.75	66±11.4	86±8.9	100±0.0	100±0.0
	1.5	94±5.5	100±0.0	100±0.0	100±0.0
	3.0	100±0.0	100±0.0	100±0.0	100±0.0
JSKCP	0.1875	58±8.4	76±5.5	80±7.1	84±5.5
	0.375	72±4.5	84±5.5	88±4.5	92±4.5
	0.75	84±5.5	92±4.5	100±0.0	100±0.0
	1.5	100±0.0	100±0.0	100±0.0	100±0.0
	3.0	100±0.0	100±0.0	100±0.0	100±0.0
RSKCP	0.1875	72±13.0	80±7.1	100±0.0	100±0.0
	0.375	76±16.7	86±11.4	100±0.0	100±0.0
	0.75	88±8.4	94±5.5	100±0.0	100±0.0
	1.5	100±0.0	100±0.0	100±0.0	100±0.0
	3.0	100±0.0	100±0.0	100±0.0	100±0.0

JSCCP = *Jatropha curcas* seed coat crude protein; JSKCP = *Jatropha curcas* seed kernel crude protein; RSKCP = *Ricinus communis* seed kernel crude protein

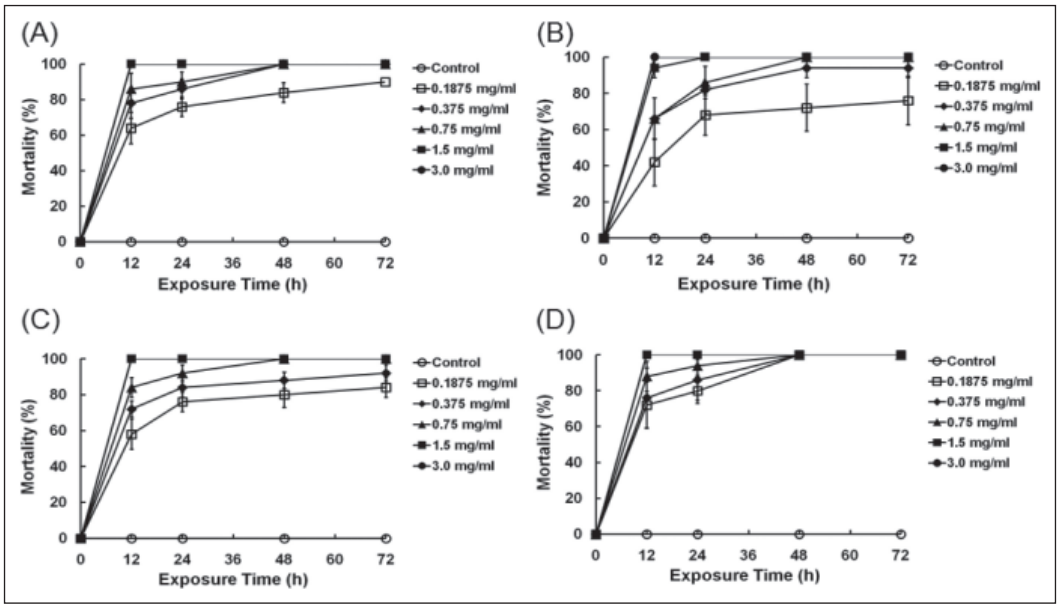

Figure 3. The larvicidal activity of the crude protein extract and purified toxin from seed coat of *J. curcas* compared to the crude protein extract from the seed kernels of *J. curcas* and *R. communis* against the third instar larvae of *Culex quinquefascitus*. (A) Purified toxin from the seed coat of *J. curcas* (Jc-SCRIP). (B) *J. curcas* seed coat crude protein (JSCCP). (C) *J. curcas* seed kernel crude protein (JSKCP). (D) *R. communis* seed kernel crude protein (RSKCP)

Table 3. The LC_{50} values of plant proteins for the third instar larvae of *Aedes aegypti* and *Culex quinquefasciatus* after 24 h of exposure

Proteins	Mosquito Species	LC_{50} (mg/ml)	Regression equations	R^2	95% confidence intervals (mg/ml)
Jc-SCRIP	*Aedes aegypti*	1.44	Y = 3.066X + 4.498	0.991	0.98 – 2.11
	Culex quinquefasciatus	0.0303	Y = 0.935X + 6.419	0.974	0.0095 – 0.097
JSCCP	*Aedes aegypti*	3.89	Y = 1.646X + 4.035	0.921	2.08 – 7.26
	Culex quinquefasciatus	0.0575	Y = 1.002X + 6.247	0.934	0.0180 – 0.184
JSKCP	*Aedes aegypti*	2.95	Y = 1.766X + 4.172	0.922	1.53 – 5.68
	Culex quinquefasciatus	0.0468	Y = 1.146X + 6.521	0.984	0.0170 – 0.129
RSKCP	*Aedes aegypti*	2.24	Y = 1.653X + 4.420	0.900	1.11 – 4.53
	Culex quinquefasciatus	0.0389	Y = 1.178X + 6.658	0.960	0.0144 – 0.100

JSCCP = *Jatropha curcas* seed coat crude protein; JSKCP = *Jatropha curcas* seed kernel crude protein; RSKCP = *Ricinus communis* seed kernel crude protein; LC_{50} = the lethal concentration at 50%; R = coefficient of regression equations

Cx. quinquefasciatus than *Ae. aegypti*. RSKCP are the most toxic towards the larvae of both mosquito species followed by JSKCP and JSCCP, respectively.

Plant RIPs have been reported to have various biological functions, including antifungal (Lam & Ng, 2001; Ng & Parkash, 2002; Parkash *et al.*, 2002), antiviral (Stirpe *et al.*, 1986; Halaweish & Zhang, 2003; Wong *et al.*, 2008) and insecticidal activities (Zhou *et al.*, 2000; Carlini & Grossi-de-Sá, 2002; Wei *et al.*, 2004; Shahidi-Noghabi *et al.*, 2008). Their toxicities may be exploited for applications in both medicine and

agriculture. To develop the use of the seed proteins from *J. curcas* in mosquito biocontrol, the type 1 RIP, Jc-SCRIP, was evaluated for its potent larvicidal activity against the third instar larvae of two mosquitoes, *Ae. aegypti* and *Cx. quinquefasciatus*, and was compared to the crude protein extracts of the plant seed coats (JSCCP) and seed kernels (JSKCP). Its larvicidal activity was also compared to the crude protein extract of another plant, *R. communis* (RSKCP), which is the source of the highly toxin protein, ricin.

The parasporal crystal of *Bacillus thuringiensis* subsp. *israelensis*, or δ-endotoxin, is one of the biopesticides used to control mosquito population (Panbangred *et al.*, 1979; Temeyer, 1984). The larvicidal activity of δ-endotoxin involves ingestion of the crystal protein (solubilized in alkaline solution) of a host to induce lysis of the midgut epithelial cells, which leads to paralysis and death of the host within a short time (Khawaled *et al.*, 1992; Cooper, 1994). The toxin is used to control highly concentrated mosquito populations of *Ae. aegypti* larvae (Liles & Dunn, 1959; Panbangred *et al.*, 1979). The toxicity of this substance is heat sensitive (Panbangred *et al.*, 1979). In addition to the bacterial toxin, a large number of plants have been examined for phytochemicals that may provide biological control of the medically important mosquitoes. However, few studies are focused on plant protein toxins. Conversely, many studies have been performed on the application of plant toxic proteins in biopesticide development (Carlini & Grossi-de-Sá, 2002; Chowdhury *et al.*, 2008; Kamaraj *et al.*, 2011). Recently, cinnamomin, a new type 2 RIP found in the seeds of the camphor tree, *Cinnamomum camphora*, has been demonstrated to have toxic effects on the larvae of the mosquito, *Culex pipines pallens* (Zhou *et al.*, 2000). The extract of the castor bean, *R. communis* has been reported to exhibit strong larvicidal effects on the diamondback moth, *Plutella xylostella*, due to the presence of the highly toxic type 2 RIP, ricin (Tounou *et al.*, 2011). Ricin belongs to the group of type 2 RIPs, which are distinguished from type 1 RIPs by the presence of the B chain (Lord *et al.*, 1994). The A chain of ricin possesses RNA *N*-glycosidase activity that results in the cleavage of a specific adenine base from ribosomal RNA, causing the inactivation of the ribosome and inhibition of protein synthesis. The lectin subunit, also known as the B chain, of ricin plays an important role of binding to the cell surface glycoconjugates of target cells and facilitates the internalization and translocation of the toxin to cytosol. The type 1 RIPs are much less cytotoxic due to the lack of the B chain. Although numerous plant RIPs and their biochemical properties were identified, the mechanism that leads to mosquito larvae toxicity is still unclear. The toxicity of those RIPs may be a result of their rRNA *N*-glycosidase activities, which inhibit protein synthesis in the larvae.

The toxicity of *J. curcas* is known to contain various toxic components, including phorbol ester, saponin, curcin, protease inhibitors and curcalonic acid (Kumar & Sharma, 2008; Acda, 2009), but no previous studies have reported the mosquito larvicidal activities of the *J. curcas* seed proteins. Many studies about the effect of methanol- and petroleum ether-extracted leaf lysates from *J. curcas* have been reported against several mosquito species, *Anopheles arabiensis*, *Anopheles stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus* (Rahuman *et al.*, 2007; Sakthivadivel & Daniel, 2008; Kovendan *et al.*, 2011; Zewdneh *et al.*, 2011). Both curcin (Lin *et al.*, 2003; Kittikajhon *et al.*, 2010) from the kernel and Jc-SCRIP (unpublished results) from the seed coat of *J. curcas* are toxic type 1 RIPs with *N*-glycosidase activity. The highly active larvicidal activity of Jc-SCRIP as well as the crude protein extracts from the seed coat (JSCCP) and seed kernel (JSKCP) of *J. curcas* may reflect the presence of the rRNA *N*-glycosidase activities. However, the exact mechanism underlying the mortality of the mosquito larvae, the ecological effects and field trials should be examined for further understanding of the use of these proteins as biopesticides for mosquito control.

To our knowledge, this is the first report describing the larvicidal activities of a type 1 RIP, Jc-SCRIP, from the seed coat of *J. curcas* compared to the crude protein extracts from the plant seed coat and seed kernel against the late third instar larvae of two medically important mosquitoes, *Ae. aegypti* and *Cx. quinquefasciatus*. In addition to being isolated from an abundant and inexpensive agricultural source, the strong larvicidal activity of JSCCP and Jc-SCRIP to the mosquito larvae of *Ae. aegypti* and *Cx. quinquefasciatus* suggests the possibility that further development of these seed proteins may provide new larvicides for use in mosquito control.

Acknowledgements. This work received financial support from the KU-Biodiesel Project of the Kasetsart University Research and Development Institute (KURDI). We are grateful to Asst. Prof. Dr. Vasakorn Bullangpoti from the Department of Zoology, Faculty of Science, Kasetsart University for her suggestion to utilize the larvicidal activity assay.

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