J. Fish. Sci. Technol. 12(1), 1-5, 2009

JOURNAL OF
FISHERIES SCIENCE
AND TECHNOLOGY

Insecticidal and Repellent Activities of Crude Saponin from the Starfish *Asterias Amurensis*

Hee Yeon Park*, Ji Young Kim, Hyun Ju Kim[1], Geon Hwi Lee[1], Jin Il Park, Chi Won Lim, Yeon Kye Kim and Ho Dong Yoon

Biotechnology Research Center, National Fisheries Research & Development Institute, Busan 619-902, Korea

[1]Laboratory of Insect Pest Management, Yeongnam Agricultural Research Institute, Milyang 629-803, Korea

Crude saponin, extracted from the starfish *Asterias amurensis*, was assessed for its capacity as a biological pesticide. As part of this analysis, its insecticidal and repellent activities, in addition to its acute and chronic toxicities were tested. In comparison with the control group, insecticidal activity of saponin against tobacco cutworm, *Spodoptera litura*, in kale, *Brassica loeracea* was low at 36.4%. Repellent activities of the extracted saponin against green peach aphid, *Myzus persicae*, and *S. litura*, on soybean leaf, *Glycine max* and kale were 65.6% and 35.0% at 1st day, and 54.5% and 30.0% at 3rd day, respectively. Acute and chronic toxicity analysis was carried out using acute immobilizatioin test and reproduction impairment test, respectively. The saponin had 48 h-EC_{50} of 65.21 μg/mL. Twenty-one day accumulative reproduction after treatment was lower in *Daphnia magna* at 7 μg/mL saponin (78 youngs), compared with the control group (129 youngs). These results indicate that the extracted saponin exhibited some toxicity and has potential as a repellent against insects.

Key words: *Asterias amurensis*, Insecticidal activity, Repellent activity, Saponin

Introduction

Organic and synthetic pesticides (organochlorine, organophosphorus, and carbamate pesticides) are increasingly being used in crop production to manage pests and improve agricultural productivity (Kianmatee and Ranamukhaarachchi, 2007). However, their persistence in the food chain is a health issue. Furthermore, pesticides overuse or misuse may induce problems in crop management such as indiscriminate destruction of non-targeted or friendly organisms, increased pest resistance to agrichemicals, and leaching of toxic residues in to the environment (Kweon et al., 1994; Jang et al., 1998). Because of these issues, the development of eco-friendly alternatives has gained increasing importance in recent years (Kwon et al., 1998; Chon et al., 2003).

It was reported that bilobilide and ginkolide A, B, and C, isolated and identified from *Gingko biloba*, have potent insecticidal activities against *Nilaparoata lugens* (Ahn et al., 1997), while essential plant oils exhibited larvicidal activities against *Culex pipiens pallens* (Kang et al., 2006). Furthermore, antifungal and insecticidal activities of ohyang (*Eugenia caryophyllata*, *Boswellia carterii*, *Agastache rugosa*, *Aristolochia contorta*, *Aquilaria agallocha*) were also reported by Chung et al. (2001).

In an effort to fine eco-friendly alternatives to synthetic pest management, marine organisms have also been the subject of numerous studies. Kim et al. (2002) looked at industrial utilization of starfish in relation to heavy metal removal, while other studies have investigated their physiological activities (Lee et al., 2002; Ross et al., 2003; Choe and Park, 2007). Furthermore, starfish powder has been shown to kill maggots in conventional toilets, and to exterminate vermins in Hokkaido, Japan, while Andersson et al. (1989) reported that starfish powder inhibits ecdysis in a fly species.

In this study, insecticidal and repellent activities of crude saponin extracted from starfish *Asterias amurensis* were examined against 3 agricultural insect pests, while toxicity of the saponin was assessed

*Corresponding author: hypark@nfrdi.go.kr

on *Daphnia magna*. Based on these analyses, the capacity of *A. amurensis* saponin as a biological pesticide was evaluated.

Materials and Methods

Extraction of *Asterias amurensis* saponin

Starfish were collected off the coast of Songjeong, Busan, and stored at -20°C. The extraction method for *A. amurensis* saponin was modified from Yasumoto et al. (1964). All solvents and reagents used for the extraction were purchased from Merck Co. Ltd. (Darmstadt, Germany). One kilogram of starfish was crushed and extracted twice with 2 L of methanol, after which the extract was concentrated (250 mL) using a rotary vacuum evaporator (EYELA, Japan). The concentration was stirred with water and benzene to remove lipid. After benzene removal, pH of the mixture was adjusted to 3 with 3 N hydrochloric acid and then neutralized using 3 N sodium hydroxide. The mixture was dialyzed through a membrane (Spectrum Laboratory Inc., USA) and then extracted with *n*-butanol. The extract was concentrated (150 mL), after which ether (50 mL) and water (300 mL) were added. After an aqueous layer was obtained, it was freeze-dried to make *A. amurensis* saponin powder (the saponin). The saponin was dissolved in water at a concentration of 5 mg of saponin/mL of water and then used in insecticidal and repellent analyses.

Insect pests

Insect pests used in the tests were green peach aphid (*Myzus persicae*), tobacco cutworm (*Spodoptera litura*), and greenhouse whitefly (*Trialeurodes vaporariorum*). All tests were performed at the laboratory of insect pest management in Yeongnam Agricultural Research Institute.

Increasing rate and developmental inhibitory analyses

After the saponin solution was sprayed on to soybean leave infested with *Myzus persicae*, their increasing rate was investigated at 1st, 3rd, 5th, and 7th days. The saponin solution was also sprayed on leaves with no insect pests, after which developmental inhibitory activity of the saponin was measured at 1st, 3rd, 5th, and 7th days.

Insecticidal activity analysis

Uniform discs, 5 cm×5 cm, were cut from leaves dipped in the saponin solution for 30 sec, ventilated with dry air and put into a petri-dish. The 3th-4th larvae were placed on leaf discs in a petri-dish and then covered. Larvae were kept at 25°C during the testing period. Counting of alive larvae were made in 1, 3, 5, and 7 days after the treatment.

Repellent activity analysis

Leaf discs were dipped in the saponin solution for 30 sec, and evaporated under a hood. Treated and untreated leaf discs were placed into a petri-dish and lined with one piece of filter paper. A petri-dish received 10 larvae and was then covered and incubated at 25°C. The number of alive larvae were counted at 1st, 3rd, and 5th days.

Saponin toxicity on *Daphnia magna*

Glass instruments were used for this analysis. *D. magna* was purchaged from Neo & Biz. Distilled water added Neo medium (Neo & Biz) was aerated to provide oxygen and adjusted to pH 8.0±0.2, after which it was used to culture the required medium. Culture medium was renewed once every two days and regularly fed *chlorella* (Neo & Biz) and YCT (Neo & Biz). Culture were maintained at 20±1°C under 16 h light : 8 h dark photoperiod. Young *D. magna*, aged less than 24 h, were separated from parent organism for all experiments.

Acute immobilizaton analysis

Acute immobilization tests were conducted using the OECD standard operating procedure (1982). Ten mL culture mediums containing various concentrations of extracted_saponin were transferred to 50 mL beakers. The tests were performed using 5 individuals in each 50 mL beaker. Immobilization was determined after 48 h. *Daphnia magna* was defined to be immobile when it was not able to swim, or if there was no observed movement of appendages or postabdomen within 15 sec after gentle stirring.

Reproduction impairment analysis

Chronic toxicity analysis was carried out using the reproduction impairment test. The concentration of extracted saponin ranged from 1/100 to 1/10 value of EC_{50}. One individual *Daphnia magna* was placed in each 50 mL beaker with the test medium. The test period lasted 21 days. Every day, the number of alive *D. magna* hatched parent organisms were recorded.

Calculation of EC_{50}

The EC_{50} (the concentration at which 50% of the examined *Daphnia magna* were immobilized) values and 95% confidence levels were determined using Microsoft Excel.

Results and Discussion

Asterias amurensis saponin extract

Extraction of *A. amurensis* saponin proceeded through several stages. The compound was freeze-dried in the last stage of extraction after the aqueous layer was obtained, ultimately yielding 0.09% of *A. amurensis* saponin powder.

The effect of extracted saponin on increase and development of insect pests

The saponin solution was sprayed on soybean leaves (*Glycine max*) infested with *Myzus persicae*, after which increasing rate of *M. persicae* was measured on the 1st, 3rd, 5th, and 7th days (Fig. 1). An untreated group was used as the control, in which increasing rates of *M. persicae* on soybean leaves were 173.4%, 46.3%, and 9.8% at 3rd, 5th, and 7th days. Increasing rates in the experimental group was low compared with the control, showing 111.3%, 4.1%, and 1.3% at 3rd, 5th, and 7th days. These data show that the extracted saponin inhibits the increase of *M. persicae* on soybean leaves.

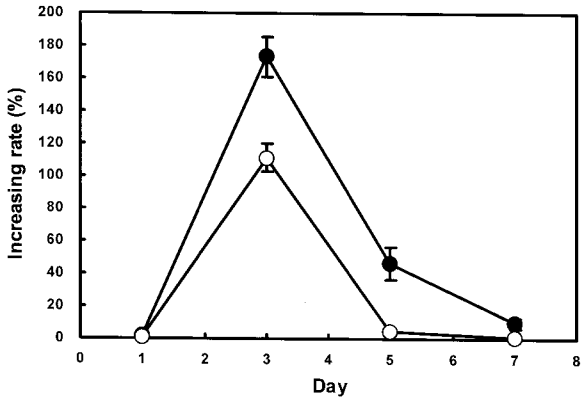

Fig. 1. Increasing rate of *Myzus persicae* on saponin sprayed soybean leaf. Values indicate means ± S.E.M. (n=5). ○, the saponin; ●, control.

To measure developmental inhibitory activity of saponin, the extracted solution was sprayed on to soybean leaves and Chinese cabbage (*Brassica campestris*) with no insect pest infections. Inhibitory activity of saponin on development of *M. persicae* on soybean leaves was highest (88.8%) on the 1st day. It decreased up to 40% after 3 days and then increased slightly. Development of *Trialeurodes vaporariorum* in chinese cabbage showed 75.0%, 48.3%, 33.1%, and 9.1% at 1st, 3rd, 5th, and 7th, respectively (Fig. 2). These results show that the extracted saponin inhibits development of insect pests, but appears to lose its effectiveness time dependently.

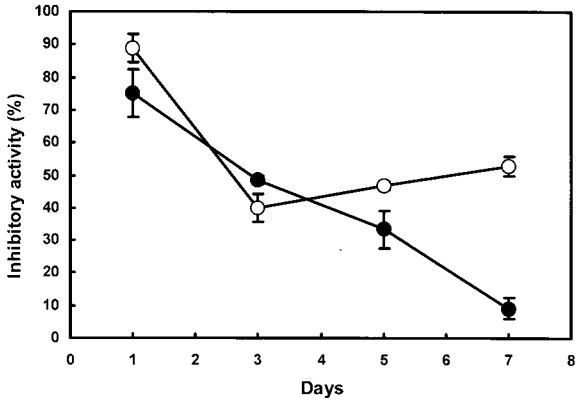

Fig. 2. Inhibitory effects of extracted saponin on *Myzus persicae* and *Trialeurodes vaporariorum* development. Values indicate means ± S.E.M. (n=5). ○, *M. persicae*; ●, *T. vaporariorum*.

Insecticidal activities of extracted saponin

Insecticidal activities of the extracted saponin against *Spodoptera litura* in kale (*Brassica oleracea*) are depicted in Fig. 3. Insecticidal activities increased after 3 days and was 36.4% at 7th days. This figure is significantly less than commercial pesticides which exhibit more than 70% activity. Kyung et al. (2002) reported that 500 ppm geranylphosphorothioate among synthesized organophophorous compounds showed 80% morality at 2rd days. Another study reported that viral insecticides formulated with *S. litura* nuclear polyhedrosis virus showed persistent 60% morality for 5 days when sprayed on surface of leaves and 12 days when sprayed on underside of leaves (Im et al., 1990). Lee et al. (2001) stated that at 5,000 ppm, insecticidal activities of ethanol extract from *Zea mays* leaves against *S. litura* were over 80%, while at 2,500 ppm, extracts from *Nelumbo nucifera* and *Z. mays* against *S. litura* were over 80%. It appears

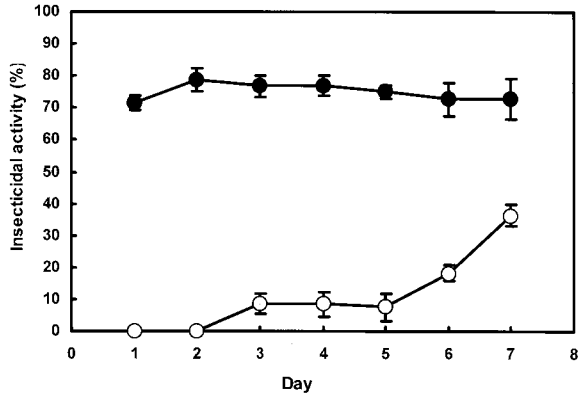

Fig. 3. Insecticidal activity of extracted saponin against *Spodoptera litura*. Values indicate means ± S.E.M. (n=5). ○, the saponin; ●, pesticide.

that insecticidal activities of biological pesticides are not significantly stronger than those of commercial pesticides. Because insecticidal activity of the extracted saponin is somewhat lower compared with those of other biological pesticides, as well as organic and synthetic pesticides, it may suggested that *Asterias amurensis* saponin is not strong enough to be used as an insecticide.

Repellent activities of the extracted saponin

Repellent activities of the saponin against *Myzus persicae* and *Spodoptera litura* in soybean leaf and kale are depicted in Fig. 4. In comparison to the control group, repellent activities of *M. persicae* and *S. litura* in soybean leaf and kale were 65.6% and 35.0%, respectively, at day 1, 54.5% and 30.0%, respectively, at day 3, and 8.7% and 30.0%, respectively, at day 5. Repellent activities of *M. persicae* decreased rapidly by the fifth day, but that of *S. litura* remained approximately at the same level during the test period. Lee et al. (2005) reported that 1/10 dilution of *Gingko biloba* extract exhibited 96.4% and 98.7% repellent activities against *M. persicae* at 1st and 2nd days, respectively. Also, Kim and Jeong (2003) reported that an essential plant oil showed strong repellent activity against *M. persicae*. Comparing our obtained results with those of other studies, the extracted saponin showed low repellent activity. However, given that the saponin concentration used in this study was low (1:200 dilution), at a stronger dilution level (1:100) it may be better utilized as a repellent.

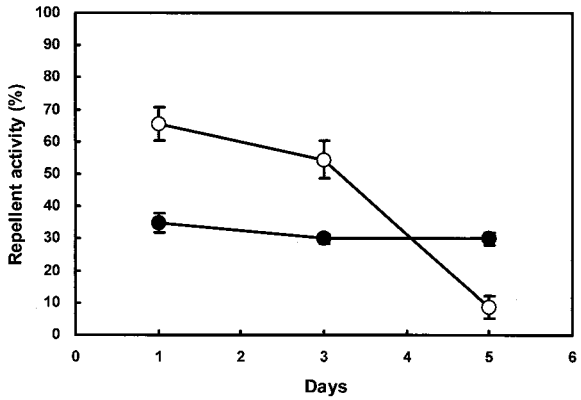

Fig. 4. Repellent activities of extracted saponin against *Myzus persicae* and *Spodoptera litura*. Values indicate means±S.E.M. (n=5). ○, *M. persicae*; ●, *S. litura*.

Toxicity of extracted saponin

Acute and chronic toxicities tests were carried out using acute immobilizatioin test and reproduction impairment test, respectively. After 48 h of exposure time, the saponin concentration from 0 to 40 μg/mL had no acute toxicity, but increased with concentration of over 40 μg/mL. The saponin resulted in 50% *Daphnia magna* immobilization at 65.21 μg/mL concentration (Fig. 5). In case of commercial pesticides, EC_{50} for benomyl, was 68.97 μg/L (Lee et al., 2007), and 0.46-0.72 μg/L and 1.50-1.56 μg/L for chlorpyrifos, and diazinon (George and Liber, 2007), respectively. According to these data, the saponin is considerably less toxic than commercial pesticides.

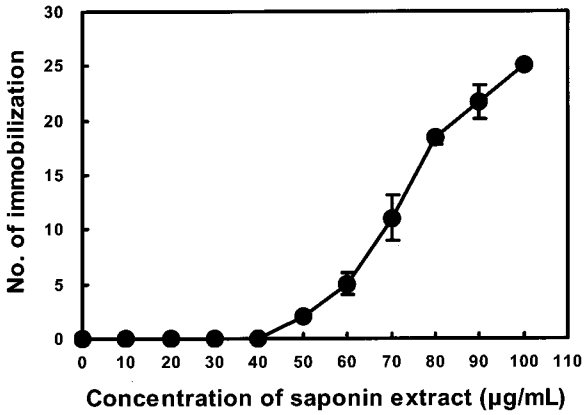

Fig. 5. Acute immobilization test of extraced saponin in *Daphnia magna*. Values indicate means ± S.E.M. (n=3).

Chronic toxicity of extracted saponin for *D. magna* was investigated (Fig. 6). The number counts of hatched *D. magna* in the experimental groups decreased with increasing saponin concentration. Reproduction of *D. magna* at 7 μg/mL was lower (78 youngs) in comparison with the control group (129

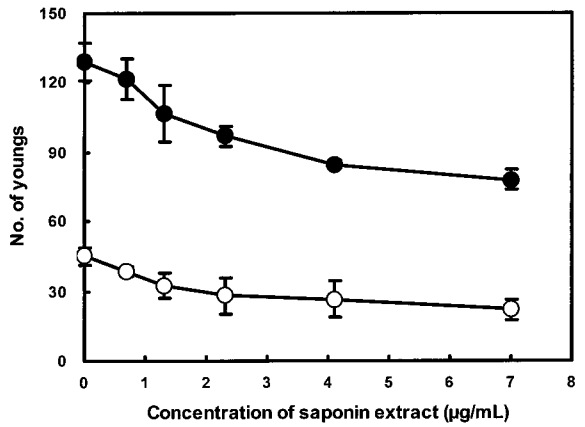

Fig. 6. Chronic reproduction impairment test of extracted saponin in *Daphnia magna* for 14 and 21 day. Values indicate means±S.E.M. (n=3). ○, 14 day; ●, 21 day.

youngs). These data indicate that saponin exhibits some toxicity and may be utilized as a repellent against insect pests.

Acknowledgments

This work was funded by a grant from the National Fisheries Research and Development Institute (RP-2008-BT-027).

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(Received 12 December 2008; Revised 25 February 2009; Accepted 17 March 2009)