

Larvicidal Activity of Constituents Identified in *Piper nigrum* L. Fruit Against the Diamondback moth, *Plutella xylostella*

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후추 열매 유래 화합물들의 배추좀나방 유충에 대한 살충 활성

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ABSTRACT: The larvicidal activities of *Piper nigrum* fruit methanol extracts and its constituents against larvae of the diamondback moth, *Plutella xylostella*, were investigated using the leaf dipping method. Administering the *P. nigrum* methanol extract resulted in 100 and 97% mortality against diamondback moth larvae at 5.0 and 2.5 mg/mL concentrations, respectively. Larvicidal activities of the *P. nigrum* fruit-derived piperidine alkaloids, piperine, and N-isobutylamide, as well as pellitorine, guineensine, pipericide, and retrofractamide A against *P. xylostella* varied according to test compound. Based on the 48 h LC₅₀ values, the most toxic compound to diamondback moth larvae was guineensine (0.013 mg/mL) followed by retrofractamide A (0.020mg/mL), pipericide (0.033mg/mL), and pellitorine (0.046 mg/mL). The LC₅₀ value of piperine was >0.5 mg/mL.

Key words: *Piper nigrum*, diamondback moth, piperine, pellitorine, guineensine, pipericide, retrofractamide A

초록: 잎침지법을 이용하여 후추 열매 메탄올 추출물 및 분리된 화합물들의 배추좀나방(*Plutellaxylostella*) 유충에 대한 살충 활성을 검정하였다. 후추 열매 메탄올 추출물은 배추좀나방 유충에 대해 5.0과 2.5 mg/mL 농도에서 각각 100%와 97%의 살충 활성을 보였다. 후추 열매에서 유래된 화합물들의 배추좀나방에 대한 살충 활성을 조사한 결과, guineensine (LC₅₀=0.013 mg/mL)의 살충 활성이 가장 높았으며, 다음으로 retrofractamide A (0.020 mg/mL), pipericide (0.033 mg/mL) 그리고 pellitorine (0.046 mg/mL) 순이었다. Piperine의 반수치사농도는 0.5 mg/mL 이상이었다.

검색어: 후추, 배추좀나방, piperine, pellitorine, guineensine, pipericide, retrofractamide A

Piper nigrum, known as black pepper, has been widely used as spices. Its taste is strongly pungent, and the odor is aromatic. Black pepper is native to India, and harvested in East and West Indies, Brazil, United States, Taiwan, Indonesia, Malay peninsula, and China. The fruit of *P. nigrum* has been used for prescribing for cholera, dyspepsia, flatulence, diarrhoea, various gastric ailments, and paralytic and arthritic disorders (Siddiquiet al., 1997). As well as used as medicine, the Piperaceae

(Pepper family) has long been used in traditional agriculture as an insect-control agent (Schultes and Raffauf, 1990). This plant species has been known to be toxic to several insect species (Freeborn and Wymore, 1929; Lathrop and Keirstead, 1946; Synerholmet al., 1945; Su, 1977; Scott and Mckibben, 1978; Miyakado and Yoshioka 1979; Miyakado et al., 1979, 1989; Park et al., 2002). The insecticidal principles of black pepper such as pipericide, dihydropipericide, guineensine, pellitorine or retrofractamide A were identified in several studies (Miyakado and Yoshioka, 1979; Miyakado et al. 1979; Park et al., 2002). This laboratory study examined the methanol extract of the fruit from *P. nigrum* for insecticidal constituents against the dia-

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mondback moth, *Plutella xylostella* larvae.

Materials and Methods

Sample preparation. The fruits (3.6 kg) of *Piper nigrum* purchased as a commercially available product were powdered with a blender, extracted twice with methanol (20 L) at room temperature for 2 d and filtered (Toyo filter paper No. 2, Toyo Roshi, Japan). The combined filtrate was concentrated in vacuo at 35°C to give a yield of 11% (based on the dry weight of the fruit). The extract (396 g) was sequentially partitioned into hexane (63.3 g), chloroform (249.5 g), ethyl acetate (19.8 g), butanol (13.8 g) and watersoluble (43.56 g) portions for subsequent bioassay with the insects used. The organic solvent portions were also concentrated to dryness by rotary evaporator at 35°C.

Chemicals and insects. In a primary screening, hexane and chloroform solvent portions showed potent insecticidal activity against diamondback moth larvae, *Plutella xylostella* (Table 1). The chloroform fraction was selected as target fraction because of its relatively higher yield than the hexane fraction. Isolation of pellitorine, guineensine, piperidine, and retrofractamide A was well described in our previous study (Park *et al.*, 2002). Piperine was purchased from Sigma (St Louis, MO, USA). *P. xylostella* larvae (3rd instar) were used in this experiment. The larvae were reared on *Brassica campestris* subsp. *napus* var. *nippooleifera* (MAKINO) seedlings. They have been maintained in the laboratory without exposure to any insecticide at 24 ±2°C, 50-60 relative humidity (RH), and a photoregime of 16 :

8 h (L : D).

Bioassay. Leaf dipping method was used to investigate the insecticidal activity. Chinese cabbage leaves were punctured with a cork borer (Ø3 cm), and test materials suspended in distilled water with Triton X-100 (Coseal Co., Seoul) added at the rate of 1 mL/L were used. Two concentrations, 5.0 and 2.5 mg/mL, for methanol extract were prepared, while the other organic fractions were tested at 2.5 mg/mL. Leaf discs were dipped in test solution for 30 sec. Controls received distilled water with Triton X-100 added at the rate of 1 mL/L. After evaporation in a draft for 2 h, twenty insects were separately placed onto the treated and the control leaf discs. Total replications were four times. Mortalities were determined 2 d after treatment. Seven to five doses (from 0.5 mg/mL to 0.007 mg/mL) of each compound were used to obtain LC₅₀ values.

Statistical analysis. The insecticidal activity was determined and transformed to arcsine values for analysis of variance (ANOVA). Treatment means were compared and separated by Scheffe's test. Means (S.E.) of untransformed data are reported. LC₅₀ values were calculated by probit analysis (SAS, 2004).

Results and Discussion

Table 1 showed the insecticidal activity of methanol extract and solvent fractions of *P. nigrum* against *P. xylostella* at 5.0 and 2.5 mg/mL concentration. Methanol extract of *P. nigrum* showed 100 and 96% insecticidal activity at 5.0 and 2.5 mg/ml, respectively. Hexane and chloroform fractions showed 100% mortality at 2.5 mg/mL. However, ethyl acetate, butanol and

Table 1. Insecticidal activity of a methanol extract and solvent fractions of *Piper nigrum* fruit against *Plutella xylostella* larvae using the leaf dipping method, at 48 h

Solvent fraction	Concentration, mg/mL	Mortality (Mean ± SE, %) ^a
MeOH	5.0	100a
MeOH	2.5	97±3.3a
Hexane	2.5	100a
Chloroform	2.5	100a
Ethyl acetate	2.5	13.3±3.3b
Butanol	2.5	7±3.3b
Water-soluble fraction	2.5	3±3.3bc
Control	-	0c

^aMeans within a column followed by the same letter are not significantly different ($P=0.05$, Scheffe test). The percentage mortality was transformed to arcsine square-root before ANOVA (SAS, 2004). Means (SE) of untransformed data are reported.

Table 2. LC₅₀ values of compounds from *Piper nigrum* fruit against *Plutella xylostella* larvae using the leaf dipping method, at 48 h.

Compound	Slope (±SE)	LC ₅₀ , mg/mL(95%CL ^a)	RT ^b	χ ²	df
Piperine	-	0.5>	-	-	-
Pellitorine	1.79±0.28	0.046 (0.033-0.061)	1.0	1.78	3
Guineensine	1.46±0.18	0.013 (0.009-0.018)	3.5	2.59	5
Pipericide	0.92±0.15	0.033 (0.019-0.053)	1.4	1.03	3
Retrofractamide A	1.45±0.19	0.020 (0.013-0.028)	2.3	0.63	5

^aConfidence limit.^bRelative Toxicity = LC₅₀ value of pellitorine/LC₅₀ value of each chemical.

water-soluble fractions exhibited 13, 7 and 3% mortality, respectively. The larvicidal activities of compounds purified from the *Piper* fruit against 3rd instar larvae of *P. xylostella* were examined by leaf dipping method (Table 2). Guineensine (LC₅₀, 0.013 mg/mL) was much more toxic than retrofractamide A, pipericide, and pellitorine. On the basis of LC₅₀ values, guineensine was about 3.5 times stronger in insecticidal activity than pellitorine. Piperine showed very low larvicidal activity against 3rd instar larvae of the diamondback moth, whereas isobutyl-containing amides pellitorine, guineensine, pipericide, and retrofractamide A produced potent larvicidal activity. These results indicated that methylenedioxyphenyl group was not essential for the toxicity against the diamondback moth larvae but isobutyl amine might play a great role in larvicidal activity. Miyakado *et al.* (1989) and Park *et al.* (2002) already reported that the isobutylamine moiety appeared to be essential for insecticidal activity against zucchini bean weevil (*Callosobruchus chinensis*) and three mosquito species such as *Culex pipiens pallens*, *Aedes aegypti* and *Aedes togoi*. Additionally, there was a difference in insecticidal activity between the isobutylamides with and without a methylenedioxyphenyl moiety. Pellitorine without methylenedioxyphenyl moiety was the least toxic followed by pipericide, retrofractamide A and guineensine with methylenedioxyphenyl moiety. Park *et al.* (2002) already reported that difference in insecticidal activity between pellitorine and other isobutylamides such as pipericide, guineensine and retrofractamide A was attributed to chemical stability. Compounds with a conjugated dienamide chromophore (-C=C-C=CONH-), such as pellitorine, are quite unstable owing to their unsaturation (Miyakado *et al.*, 1989). Our results and early study indicate that isobutylamine moiety appeared to be essential for insecticidal activity, and the methylene-

dioxyphenyl moiety improved insecticidal activity of isobutylamides by stabilizing the chemical structure. In conclusion, the *Piper* fruit-derived materials might be useful for managing *P. xylostella* larvae. Further studies on the exact mode of *Piper* derived compounds, their effect on other insect pests of the active compounds from the *Piper* fruit, formulation improving the insecticidal potency, and the safety and stability of a selected formulation for commercialization might be conducted for its practical use as a naturally occurring insect-control agent.

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