องค์ประกอบทางเคมีและฤทธิ์ฆ่าแมลงของน้ำมันหอมระเหยจากเมล็ดพริกไทยดำที่มีต่อมอดข้าวเปลือก

Chemical Composition and Insecticidal Activity of Essential Oil from *Piper nigrum* Seed against *Rhyzopertha dominica* (Coleoptera: Bostrichidae)

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บทคัดย่อ: ปัจจุบันการใช้น้ำมันหอมระเหยจากพืชเป็นทางเลือกที่ช่วยลดการใช้สารฆ่าแมลงสังเคราะห์ในการควบคุมแมลงศัตรูหลังการเก็บเกี่ยว วัตถุประสงค์ในการศึกษาครั้งนี้เพื่อจำแนกองค์ประกอบทางเคมี ตรวจสอบพิษทางสัมผัสและพิษทางการรมของน้ำมันหอมระเหยจากเมล็ดพริกไทยดำที่มีต่อมอดข้าวเปลือก *Rhyzopertha dominica* ศัตรูสำคัญของข้าวหลังการเก็บเกี่ยว ผลการศึกษาองค์ประกอบทางเคมีของน้ำมันหอมระเหยจากเมล็ดพริกไทยดำด้วยเทคนิคแก๊สโครมาโทกราฟี/แมสสเปกโทรมิเตอร์ (GC-MS) พบสารที่เป็นองค์ประกอบหลักได้แก่ β-caryophyllene (23.84%), *δ*-3-carene (20.95%), d-limonene (12.98%), β-pinene (8.15%), 1-phellandrene (6.78%) และ 3-carene (5.09%) การทดสอบพิษทางสัมผัสพบว่า น้ำมันหอมระเหยความเข้มข้น 0.63 และ 1.26 มคล./ตร.ซม. มีพิษสูงสุดทำให้มอดข้าวเปลือกตายทั้ง 100% ที่เวลา 72 ชม.หลังการทดสอบ ในขณะที่ความเข้มข้น 0.02-0.08 มคล./ตร.ซม. ไม่มีพิษต่อแมลง ส่วนการทดสอบพิษทางการรมพบว่า น้ำมันหอมระเหยความเข้มข้น 631.58 และ 842.11 มคล./ล.อากาศ มีพิษสูงสุด โดยพบการตายของมอดข้าวเปลือก 100% ที่เวลา 72 ชม.หลังการทดสอบ ส่วนความเข้มข้น 42.11 และ 84.21 มคล./ล.อากาศ ไม่มีพิษต่อมอดข้าวเปลือก สำหรับค่า LC_{50} ของพิษทางสัมผัสและพิษทางการรมที่เวลา 72 ชม.หลังการทดสอบ เท่ากับ 0.22 มคล./ตร.ซม. และ 264.16 มคล./ล.อากาศ ตามลำดับ จึงสรุปได้ว่าน้ำมันหอมระเหยจากเมล็ดพริกไทยดำมีศักยภาพในการใช้เป็นทางเลือกเพื่อควบคุมมอดข้าวเปลือก

คำสำคัญ: ฤทธิ์ฆ่าแมลง, น้ำมันหอมระเหย, พริกไทยดำ, มอดข้าวเปลือก

ABSTRACT: Currently, use of essential oils from plants has been suggested as alternative method for stored product insect pest control to reduce the use of insecticides. The objectives of the research were to identify the chemical composition of essential oil of *Piper nigrum* seed and investigate the contact and fumigant effects against adults of the lesser grain borer, *Rhyzopertha dominica*, a serious insect pest of stored rice. The major components of essential oil were β-caryophyllene (23.84%), *δ*-3-carene (20.95%), d-limonene (12.98%), β-pinene (8.15%), 1-phellandrene (6.78%) and 3-carene (5.09%). The essential oil at concentrations of 0.63 and 1.26 µL/cm^2 showed the strongest contact toxicity against *R. dominica* with mortality of 100% after exposure for 72 h. The lower concentrations (0.02-0.08 µL/cm^2) had no contact toxicity to the tested insect. For fumigant toxicity test, the essential oil at concentrations of 631.58 and 842.11 µL/L air were the most toxic with *R. dominica* mortality of 100% after exposure for 72 h while the concentrations of 42.11 and 84.21 µL/L air were ineffective. The LC_{50} values of essential oil for contact and fumigant toxicities after exposure for 72 h were 0.22 µL/cm^2 and 264.16 µL/L air, respectively. These results suggest that the essential oil of *P. nigrum* seed is a potential candidate for use as a natural insecticide for *R. dominica* control.

Keywords: insecticidal activity**,** essential oil, *Piper nigrum***,** *Rhyzopertha dominica*

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Introduction

The lesser grain borer, *Rhyzopertha dominica* (Coleoptera: Bostrichidae), is a serious post-harvest rice insect pest and broadly distributed in Thailand. Both adult and larval stages normally feed inside the rice seeds and cause great quantitative and qualitative losses (Nascimento et al., 2018). Currently, essential oils from plants constitute an alternative to conventional insecticides and are known to possess fumigant, repellent, anti-feedant, anti-ovipositant and insect growth regulatory activities against insect pests. Furthermore, they have no or little harmful effects on non-target organisms and the environment (Negahban and Moharramipour, 2007).

Piper nigrum is a well-known medicinal plant in Thailand. It possesses a wide range of biological activities including antibacterial, antifungal, antioxidative, anti-inflammatory, antitumor, antidepressant, antithyroid, antidiarrhoeal activities (Ahmad et al. 2012; Abdallah and Abdalla, 2018). Although, the bioactivity of *P. nigrum* extract against *R. dominica* and other stored product insects has been reported previously (Khani et al., 2011; Ahmad et al., 2016). However, there has been no report on the insecticidal activity of essential oil of *P. nigrum* seed against *R. dominica*. Thus, the objectives of this work were to study phytochemical analysis, the contact and fumigant toxicities of essential oil of *P. nigrum* seed against *R. dominica*.

Materials and Methods

Rearing of *R. dominica*

Adults of *R. dominica* were collected from naturally infested rice seeds from farmers' farms of Thung Song District, Nakhon Si Thammarat Province. They were reared on disinfested rice seeds variety Sang Yod in the laboratory at 26±1°C and 75% RH without exposure to insecticides. The adults were allowed for mating and oviposition for two weeks. The insect parents were separated by sieving and the rice seeds containing the eggs were kept in the same condition until adult emergence. Seven to 10-day old adults were used in all the experiments.

Extraction of Essential oil

The dried seeds of *P. nigrum* were purchased from the local market of Hat Yai District, Songkla Province. They were grounded and 500 g of powdered material were hydro-distilled in Clevenger apparatus continuously for 6 h to yield essential oil. The obtained light yellowish essential oil with 0.50% yield was stored in glass container in a refrigerator at 4°C. The constituents of essential oil were analyzed by gas chromatography-mass spectrometry (GC-MS).

Contact toxicity test

The contact toxicity of essential oil of *P. nigrum* seed against adults of *R. dominica* was conducted using an impregnated-filter paper bioassay described by Kim et al. (2003). The essential oil at 1, 2.5, 5, 10, 20, 40 and 80 µL quantities were dissolved in 1 ml of acetone. Aliquot of 1 ml of each concentration was applied to a filter paper disc of 9 cm diameter, corresponding to 0.02, 0.04, 0.08, 0.16, 0.31, 0.63 and 1.26 µL /cm^2, respectively. The treated filter paper disc was allowed to air-dry for 10 min and then placed in Petri dish. Ten unsexed adults of *R. dominica* were released on the treated filter paper disc. The control filter paper disc was treated with acetone alone. Insect mortality was recorded at 24, 48, and 72 h after treatment to determine the LC_{50} values.

Fumigant toxicity

The essential oil of *P. nigrum* seed was evaluated for fumigant toxicity against adults of *S. oryzae* according to a method described by Michelraj and Sharma (2006). A 950 ml plastic jar with screw lid was used as a fumigation chamber. A series of concentrations was prepared by diluting the amounts of 40, 50, 60, 80, 160, 320 and 400 µL in 1 ml of acetone. A filter paper disc of 5 cm diameter was treated with 0.5 ml of each concentration, corresponding to 42.11, 84.21, 168.42, 252.63, 421.05, 631.58 and 842.11 µL/ L air, respectively. After evaporating of solvent for 10 min, treated filter paper was then attached to the under surface of the lid with adhesive tape. Ten unsexed adults were transferred to a 10 ml vial and the vial was covered with fine cloth. Four vials containing the insects were placed in the fumigant chamber and considered as four replications. The lid was closed and sealed by adhesive tape to create air tight condition in the chamber. The control consisted of a similar setup but without the essential oil. Insect mortality was observed at 24, 48, and 72 h after treatment to determine the LC_{50} values.

Statistical analysis

All experiments were arranged in a completely randomized design with 4 replications. All data were analyzed with one-way ANOVA, followed by Duncan's multiple range test at $P < 0.01$. The LC_{50} values were determined by probit analysis. All values were represented as mean ± S.E.

Results

Chemical compositions of essential oil of *P. nigrum* seed

A total of 26 components were detected from essential oil of *P. nigrum* seed (data not shown). The main components were

Table 1 Main chemical compositions of essential oil of *P. nigrum* seed.

Compounds	Retention time (min)	Content (%)
3-Carene	6.76	5.09
β-Pinene	8.25	8.15
1-Phellandrene	9.34	6.78
δ-3-Carene	9.56	20.95
D-Limonene	10.35	12.98
β-Caryophyllene	29.71	23.84
β-Selinene	33.91	2.45

Table 2 Percent mortality of adults of *R. dominica* treated with essential oil of *P. nigrum* seed by contact toxicity test.

Concentration (µL /cm^2)	Mortality (mean±SE, %)[1/]		
	24 h after treatment	48 h after treatment	72 h after treatment
1.26	97.50 ± 2.50[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]
0.63	60.00 ± 7.07[b]	100.00 ± 0.00[a]	100.00 ± 0.00[a]
0.31	37.50 ± 4.79[c]	65.00 ± 2.89[b]	75.00 ± 2.89[b]
0.16	15.00 ± 6.46[d]	17.50 ± 6.29[c]	30.00 ± 4.08[c]
0.08	0.00 ± 0.00[d]	0.00 ± 0.00[d]	0.00 ± 0.00[d]
0.04	0.00 ± 0.00[d]	0.00 ± 0.00[d]	0.00 ± 0.00[d]
0.02	0.00 ± 0.00[d]	0.00 ± 0.00[d]	0.00 ± 0.00[d]
Control	0.00 ± 0.00[d]	0.00 ± 0.00[d]	0.00 ± 0.00[d]

[1/] Mortality within a column followed by the same letter are not significantly different at *P* <0.01 by DMRT.

β-caryophyllene (23.84%), δ-3-carene (20.95%), d-limonene (12.98%), β-pinene (8.15%), 1-phellandrene (6.78%), 3-carene (5.09%) and β-selinene (2.45%) (**Table 1**).

Contact toxicity

Significant differences (P < 0.01) among different concentrations of essential oil of *P. nigrum* seed were observed at 24, 48 and 72 h after treatment (**Table 2**). The mortality of *R. dominica* increased with increasing concentration and exposure time. The highest concentration (1.26 µL/cm^2) showed significantly higher toxicity against *R. dominica* than the other concentrations, with mortality of 97.50 % at 24 h. By 48 h, the concentrations of 0.63 and 1.26 µL/cm^2 achieved 100 % mortality. At 72 h, the concentrations of 0.63 and 1.26 µL/cm^2 showed the strongest contact toxicity, followed by the concentration of 0.31 µL /cm^2. The other extract had low or no contact toxicity against *R. dominica.* No mortality was observed in the control. The LC_{50} values of essential oil of *P. nigrum* seed for contact toxicity were 0.39, 0.26 and 0.22 µL/cm^2 at 24, 48 and 72 h, respectively, (**Table 4**).

Table 3 Percent mortality of adults of *R. dominica* treated with essential oil of *P. nigrum* seed by vapour toxicity test.

Concentration (µL/ L air)	Mortality (mean±SE, %)[1/]		
	24 h after treatment	48 h after treatment	72 h after treatment
842.11	77.50±4.79a	100.00±0.00a	100.00±0.00a
631.58	77.50±2.50a	95.00±2.89a	100.00±0.00a
421.05	52.50±4.79b	75.00±2.89b	80.00±5.77b
252.63	17.50±6.29c	42.50±7.50c	52.50±4.79c
168.42	2.50±2.50d	10.00±5.78d	20.00±4.08d
84.21	0.00±0.00d	0.00±0.00d	0.00±0.00e
42.11	0.00±0.00d	0.00±0.00d	0.00±0.00e
Control	0.00±0.00d	0.00±0.00d	0.00±0.00e

[1/] Mortality within a column followed by the same letter are not significantly different at *P* <0.01 by DMRT.

Fumigant toxicity

Significant differences ($P < 0.01$) among different concentrations of essential oil of *P. nigrum* seed were observed at 24, 48 and 72 h after treatment. At 24 h, the concentrations of 842.11 and 631.58 µL/L air had significantly higher fumigant toxicity against *R. dominica* than the other concentrations with mortality of 77.50%. At 48 h, the concentration of 842.11 µL/L air caused 100% mortality of *R. dominica*.

Table 4 LC_{50} values of essential oil from *P. nigrum* seed against adults of *R. dominica*

Time (h)	Contact toxicity			Fumigant toxicity		
	LC_{50} (µL/cm^2)	95% FL	χ^2(df)^b	LC_{50} (µL/ L air)	95% FL	χ^2(df)^b
24	0.39	0.26-0.67	17.22(4)	470.52	361.73-609.00	24.40(4)
48	0.26	0.20-0.33	18.29(5)	305.27	254.78-357.23	14.74(5)
72	0.22	0.17-0.28	19.34(5)	264.16	214.74-315.42	17.86(5)

FL = Feducial limit

By 72 h the strongest fumigant toxicity (100%) was obtained from the concentrations of 842.11 and 631.58 µL/L air followed by the concentrations of 421.05, 252.63 and 168.42 µL/L air, respectively. No mortality was observed in the concentration of 84.21 and 42.11 µL/L air and the control (**Table 3**). The LC_{50} values of essential oil of *P. nigrum* seed for fumigant toxicity were 470.52, 305.27 and 264.16 µL/L air at 24, 48 and 72 h, respectively, (**Table 4**).

Discussion

The use of natural products can be considered as an important alternative for the control of stored product pests. The present investigation showed that the main components found in essential oil of *P. nigrum* seed were β-caryophyllene (23.84%), δ-3-carene (20.95%), d-limonene (12.98%), β-pinene (8.15%), 1-phellandrene (6.78%), 3-carene (5.09%) and β-selinene (2.45%). These results differed from previous reports. For example, Jirovetz et al. (2002) investigated compounds in essential oils of dried fruits of *P. nigrum* from the Cameroon, and observed the presence of germacrene D (11.01%), limonene (10.26%), β-pinene (10.02%), α-phellandrene (8.35%), β-caryophyllene (7.29%), α-pinene (6.40%) and cisβ-ocimene (3.19%). The differences in essential oil compositions may be due to several factors, such as geographical location, season, environmental conditions, and the nutritional status of the plants (Ozcan and Chalchat 2006). The essential oil of *P. nigrum* seed was very effective against *R. dominica*. These findings are similar with the observation of Khani et al. (2012) who reported the 72 h LC_{50} values of essential oil of *P. nigrum* fruit in fumigant toxicity test against adults of *Sitophilus oryzae* and the third instar larvae of *Corcyra cephalonica* were 280.70 and 530.53

μL/L air. Chaubey (2017) found that essential oil of *P. nigrum* seed had strong contact toxicity against adults of *S. zeamais* with LC_{50} values of 0.208 and 0.126 μL/cm^2 at 24 and 48 h, respectively. Additionally, *P. nigrum* seed powder at concentration of 0.5% could cause 100% mortality of *R. dominica* after exposure for 14 days (Ashouri and Shayesteh, 2010).

Phytochemicals in plant have been reported to be responsible for insecticidal activity against stored product insect pests. Suthisut et al. (2011) reported contact and fumigant toxicity of β-pinene against *S. zeamais*. β-caryophyllene showed contact toxicity against both *Tribolium castaneum* and *S. oryzae* with LC_{50} values of 0.153 and 0.132 μL/cm^2 at 48 h (Chaubey, 2012). Addtionally, Liu et al. (2012) found that d-limonene had fumigant toxicity against adults of *S. zeamais* and *T. castaneum* with LC_{50} values of 33.71 mg/L and 21.24 mg/L, respectively. D-limonene also was found to possess strong contact toxicity against *T. castaneum* with LC_{50} value of 14.88 μL/cm^2 (Guo et al., 2016). Nascimento et al. (2018) reported that *R. dominica* was susceptible to limonene, α-pinene and β-pinene. Therefore, insecticidal activity of essential oil of *P. nigrum* seed may be related to these components. Moreover, Chaubey (2017) found that essential oil of *P. nigrum* seed could reduce the activity of acetylcholinesterase in *S. zeamais*. Essential oils are lipophilic in nature and can be inhaled or ingested. Thus, the rapid action against insect pests is indicative of a neurotoxic mode of action.

Conclusion

Essential oil of *P. nigrum* seed had strong contact and fumigant toxicity against adults of *R. dominica.* These findings demonstrated the potential of essential oil of *P. nigrum* seed for development into a biopesticide in the control of *R. dominica.* However, for the practical application of the essential oil, further studies on the safety to humans and on development of formulation are necessary to improve the efficacy and stability, and to reduce cost.

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