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## Potential of essential oils from *Piper nigrum* against cowpea weevil, *Callosobruchus maculatus* (Fabricius)

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Wanna, R.\*

Department of Agricultural Technology, Faculty of Technology, Maharakham University, Maha Sarakham 44150, Thailand.

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**Abstract** Essential oils from *Piper nigrum* consist of many chemical components that have properties for protecting plants from insect pests by fumigation. The potential of essential oils from dried seeds and fresh leaves of *P. nigrum* extracted by hydrodistillation was determined using Gas Chromatography-Mass Spectrometry (GC-MS). The efficacy of these essential oils against adults of *Callosobruchus maculatus* was studied using vapor-phase tests. Results indicated that major components of *P. nigrum* essential oil extracted from dried seeds included alpha-bergamotene (14.57%), caryophyllene (11.47%), beta bourbonene (8.47%), 2-nonanone (7.58%), spathulenol (6.94%) and naphthalene (5.33%). Principal compounds of *P. nigrum* essential oil extracted from fresh leaves were beta-selinene (12.26%), germacrene D (9.15%), alpha-cubebene (8.09%), 7-epi-alpha-selinene (6.70%), aromadendreneoxide-(2) (6.99%) and alpha-cadinol (5.69%). Essential oils of *P. nigrum* extracted from seeds and leaves showed strong fumigant activities at 168 h against adults of *C. maculatus*, with 100% adult mortality at 30 and 60 µL/L air, respectively. Results indicated that essential oils from seeds and leaves of *P. nigrum* showed potential for management of the *C. maculatus* population and avoided pollution risks of synthetic agricultural chemicals in stored-product insect control for protection against cowpea weevil.

**Keywords:** Insecticidal activity, *Piper nigrum*, Chemical compositions, Stored-product insect control, Cowpea weevil

### Introduction

*Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae), commonly known as the cowpea weevil is one of the most serious insect pests during seed and grain storage (Deshpande *et al.*, 2011). This species can cause damage up to 100% of legume seeds (Gbaye *et al.*, 2011) within 45 to 90 days of storage at optimum temperature of  $30 \pm 1$  °C and moisture conditions  $75 \pm 3$ % relative humidity (RH). The neonate larvae penetrate the grains causing severe damage resulting in seed or grain weight loss, poor germination ability, reduction in

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\* **Corresponding Author:** Wanna, R.; **Email:** [ruchuon.w@msu.ac.th](mailto:ruchuon.w@msu.ac.th)

nutritional value (Melo *et al.*, 2010; Oke and Akintunde, 2013), and the cowpeas become unsuitable for human or animal consumption (Elhag, 2000).

Currently, synthetic pesticide fumigants are an alternative method of increasing yields and protecting stored grain, stored food, feedstuffs, and other agricultural products from insect damage (Ghorab and Khalil, 2016). However, continuous or excessive uses of synthetic pesticides have created serious problems arising from many factors such as ozone depletion potential, environmental pollution, increasing costs of application, pesticide residue in the environment and food commodities (Ccanccapa *et al.*, 2016), insect resistance (Damalas and Eleftherohorinos, 2011) and hazardous of toxicity to non-target organisms in addition to direct toxicity to users (Isman, 2006; Ogendo *et al.*, 2008; Köhler and Triebkorn, 2013; Paoli *et al.*, 2015).

These problems have focused the need for the development of new alternatives to selective fumigant insect pest control. One approach is the use of botanical pesticides. Botanical applications are a viable alternative to synthetic chemicals, and without serious side effects as they are biodegradable and therefore do not interfere with the ecosystems (Rajendran and Sriranjini, 2008). Currently, essential oils in aromatic plants outcome from secondary metabolism are being used for insect pest management (Isman, 2006). The main compounds in these essential oils as monoterpenoids offer promising alternatives to classical fumigants (Papachristos and Stamopoulos, 2003) and impact both physiological and biological behaviors of insects such as growth rate, life span, reproduction and mortality (Pascual-Villalobos, 1996; Islam *et al.*, 2009). The toxicity of many plant essential oils and their constituents has been assessed against many of the stored-product insect pests. Studies of essential oils have stimulated the research of pest control in stored grain with very promising results. Mahmoudvand *et al.* (2011) demonstrated contact insecticidal activity of essential oils of *Lippia citrodora*, *Rosmarinus officinalis*, *Mentha piperita* and *Juniperus sabina* against *C. maculatus*, while other studies have evaluated the toxic effects of some essential oils to examine possible fumigation, contact, repellent and oviposition activities of *C. maculatus* (Wanna and Khangkhun, 2018; Wanna and Kwang-Ngoen, 2019).

*Piper nigrum* L. (black pepper) is a well-known medicinal plant in Thailand and a popular spice all over the world (Agbor *et al.*, 2006). The plant contains a variety of compounds that have medicinal, nutritive, and food industry applications. However, information on these compounds relative to their insecticidal activities is sparse. Bioactivity of *P. nigrum* extract against *Rhyzopertha dominica* and other stored-product insect pests has been previously reported (Khani *et al.*, 2011; Ahmad *et al.*, 2016). From the best of our knowledge, there are no reports of the insecticidal activity of *P. nigrum*

essential oils against *C. maculatus*. Therefore, the chemical compositions and potential of essential oils of *P. nigrum* from dried seeds and fresh leaves were investigated for their insecticidal activities against adults of *C. maculatus*.

## **Materials and methods**

### ***Insect rearing***

Cowpea weevil, *C. maculatus* (Fabricius), specimens were collected from infested mungbean seeds in grain storage at the Department of Agricultural Technology, Faculty of Technology, Mahasarakham University, Maha Sarakham Province, Thailand. Insect rearing was carried out inside a plastic bottle (diameter 15 cm, height 8 cm) under laboratory conditions ( $30\pm 5$  °C,  $70\pm 5\%$  RH and 8L: 16D photoperiods). Fifty pairs of male and female *C. maculatus* adults were reared and maintained in a plastic bottle with 1,000 g of mungbean seeds, *Vigna radiata* (L.) Wilczek. Infested seeds were removed and sterilized by freezing for 2 weeks and then left for 24 h under ambient conditions. The insects were reared and allowed to mate and oviposition under laboratory conditions. Adult *C. maculatus* were separated and females at 5 days old were used in the investigation. All experiments were performed under the same environmental conditions.

### ***Essential oil extraction***

Essential oils were extracted from dried seeds and fresh leaves of *P. nigrum* L. by the water distillation method in a modified Clevenger-type apparatus. For the water distillation process, 200 g of *P. nigrum* was weighed into the distillation flask and 600 mL of distilled water was added. The apparatus was installed using a clamp on a heating mantle for a period of 2 h. The essential oil deposited in the water was collected through an attached graduated measuring tube by opening the tap. Essential oils were removed from the remaining water after extraction by centrifuge at 8,000 rpm for 10 min and then preserved in a sealed amber glass bottle and refrigerated at 4 °C in the dark until required for use.

### ***Analysis of essential oils***

Essential oil of volatile compounds from dried seeds and fresh leaves of *P. nigrum* were analyzed with a gas chromatography-mass spectrometry (GC-MS) performed in the Clarus SQ 8 GC/MS system (PerkinElmer, MA, USA)

operating in the electron impact (EI) mode (70 eV). A Rtx-5MS capillary column (with 5% phenylmethyl polysiloxane stationary phase, 30 m x 0.25 mm, 0.25  $\mu$ m film thickness) was used. GC settings were as follows: 50  $^{\circ}$ C of initial oven temperature for 1 min, then increased to 180  $^{\circ}$ C at a rate of 10  $^{\circ}$ C/min, held for 1 min, and then raised at 3  $^{\circ}$ C/min to 240  $^{\circ}$ C for 15 min. The injector temperature was maintained at 230  $^{\circ}$ C. A sample (1  $\mu$ L, dilute to 1% with acetone) was injected with a split ratio of 1:10 with helium (flow rate of 1.0 mL/min) used as a carrier gas. Spectra were scanned from 45 to 450 m/z. Identification of essential oil compositions was applied by comparing their mass spectra with those archives in the National Institute of Standards and Technology (NIST) Mass Spectral Search Program and ChemStation Wiley Spectral Library. Essential oil compounds were assigned by comparing the substances with mass spectra more than 80% of substances with quality match. Chemical component data of essential oils from *P. nigrum* were analyzed by reading the retention time and % area.

### ***Fumigation toxicity***

Insecticidal fumigant activity of *P. nigrum* was assessed by vapor phase test. In brief, each Whatman (no.1) filter paper strip (1.5 x 5 cm) was impregnated with 100  $\mu$ L of 20, 40, 60, 80, 100 and 120  $\mu$ L/L air dilution of essential oils from dried seeds and 6, 10, 12, 16, 24 and 30  $\mu$ L/L air dilutions of essential oils from fresh leaves as previously prepared. After evaporating the solvent for 2 min, a filter paper strip was placed into a glass vials (diameter 2.5 cm x height 5 cm) hanging from the center of screw cap of a fumigation bottle (diameter 5.5 cm x height 10.5 cm) to avoid contact between insects and the filter paper strip. Ten female adults of *C. maculatus* (5-day olds) were placed inside a fumigation bottle. The cap of each fumigation bottle was tightened and maintained at  $30 \pm 5$   $^{\circ}$ C,  $70 \pm 5\%$  RH and 8L: 16D photoperiods. Control received only 100  $\mu$ L of acetone. Adult mortality was observed and recorded after 24 h to 168 h exposure. The insects were considered dead when there was no leg or antenna movement. Each treatment was repeated four times and mortality percentage was calculated using the Abbott's formula.

## **Results**

### ***Identification of compounds***

The main chemical constituents of essential oil from dried seeds of *P. nigrum* are shown in Table 1. A total number of 69 compounds was recorded.

The major 18 components were alpha-bergamotene (14.57%), followed by caryophyllene (11.47%), beta-bourbonene (8.47%), 2-nonanone (7.58%), spathulenol (6.94%), naphthalene (5.33%), bicycle [2.2.2]octane-1carboxylic acid (4.84%), beta-selinene (4.00%), 3-buten-2-one (3.43%), gamma-cadinene (3.17%), 2-undecanol (2.87%), pentadecane (2.87%), 1-tetradecyne (1.82%), alpha-selinene (1.48%), 2-propanone (1.48%), curcumene (1.42%), copaene (1.36%), and capronaldehyde (1.33%). A further 51 compounds with values of % area less than 1% were identified.

**Table 1.** Chemical compositions of essential oil from dried seeds of *Piper nigrum*

Compound	Retention time	%Area
3-Buten-2-one	1.391	3.43
Bicyclo[2.2.2]octane-1carboxylic acid	1.482	4.84
2-Propanone	1.595	1.48
Capronaldehyde	2.940	1.33
2-Nonanone	7.047	7.58
1-Tetradecyne	11.784	1.82
2-Undecanol	12.980	2.87
Copaene	14.236	1.36
Beta-bourbonene	14.379	8.47
Alpha-bergamotene	15.133	14.57
Caryophyllene	15.972	11.47
Curcumene	16.623	1.42
Naphthalene	16.770	5.33
Pentadecane	16.974	2.87
Alpha-selinene	17.188	1.48
Gamma-cadinene	17.532	3.17
Spathulenol	19.099	6.94
Beta-selinene	19.703	4.00

GC-MS analysis led to the identification and quantification of 94 compounds of essential oil from fresh leaves of *P. nigrum*. The 20 major components were beta-selinene (12.26%), germacrene D (9.15%), alpha-cubebene (8.09%), aromadendrene oxide-(2) (6.99%), 7-epi-alpha-selinene (6.70%), alpha-cadinol (5.69%), beta-bourbonene (4.75%), 2-octen-1-ol (4.44%), 7-hexadecenoic acid (4.41%), 3-hexen-1-ol (3.13%), alpha-gurjunene (2.58%), 2-propanone (2.14%), 1-cycloprop[e]azulen-7-ol (1.94%), cyclohexanol (1.76%), linalool oxide (1.72%), spathulenol (1.64%),

caryophyllene (1.28%), 1-cycloprop[e] azulene (1.21%), 9,10-dehydro-Isolongifolene (1.20%) and beta-ionone (1.10%). Further 73 compounds with values of % area less than 1% were identified (Table 2).

**Table 2.** Chemical compositions of essential oil from fresh leaves of *Piper nigrum*

Compound	Retention time	%Area
Beta-ionone	1.392	1.10
2-Propanone	1.482	2.14
3-Hexen-1-ol	2.934	3.13
2-Octen-1-ol	3.034	4.44
Linalool oxide	7.041	1.72
Alpha-cubebene	13.306	8.09
Aromadendrene oxide-(2)	14.014	6.99
Beta-bourbonene	14.381	4.75
7-Hexadecenoic acid	14.872	4.41
Alpha-gurjunene	15.128	2.58
Caryophyllene	15.345	1.28
1-Cycloprop[e]azulene	16.407	1.21
Germacrene D	16.800	9.15
Beta-selinene	17.000	12.26
Cyclohexanol	17.457	1.76
7-Epi-alpha-selinene	17.626	6.70
9,10-Dehydro-Isolongifolene	18.966	1.20
1-Cycloprop[e]azulen-7-ol	19.106	1.94
Spathulenol	20.414	1.64
Alpha-cadinol	20.519	5.69

### ***Fumigation toxicity***

Fumigant toxicity results of essential oil from dried seeds of *P. nigrum* against *C. maculatus* are presented in Table 3. Comparing of mean mortality values, when increasing the essential oil concentrations of *P. nigrum* seeds induced increasing of mortality rate of *C. maculatus* adults. Essential oil of *P. nigrum* seeds at the highest dose of 30  $\mu\text{L/L}$  air resulted in 100% adult mortality after 168 h of exposure. The highest dose of essential oil was significantly higher ( $P < 0.01$ ). Exposure with 60  $\mu\text{L/L}$  air of essential oil from fresh leaves of *P. nigrum* to adults of *C. maculatus* after 168 h of treatment

resulted in 100% mortality and caused the highest significant difference ( $P < 0.01$ ). However, there was no significant difference between 80 and 100  $\mu\text{L/L}$  air (Table 4). Two concentrations of essential oil (20 and 40  $\mu\text{L/L}$  air) were effective against *C. maculatus* at 168 h with less than 100% adult mortality. Acetone treatment also had high significant difference with more than 90% adult mortality.

**Table 3.** Adult mortality of *Callosobruchus maculatus* with fumigant toxicity treated essential oil from dried seeds of *Piper nigrum*

Conc	Mean ( $\pm$ SE) of adult mortality (%) of <i>Callosobruchus maculatus</i>						
	24 h	48 h	72 h	96 h	120 h	144 h	168 h
0	0.00 $\pm$ 0.00 <sup>b</sup>	0.00 $\pm$ 0.00 <sup>c</sup>	0.00 $\pm$ 0.00 <sup>c</sup>	0.00 $\pm$ 0.00 <sup>d</sup>	0.00 $\pm$ 0.00 <sup>d</sup>	0.00 $\pm$ 0.00 <sup>d</sup>	0.00 $\pm$ 0.00 <sup>c</sup>
6	2.50 $\pm$ 5.00 <sup>b</sup>	10.00 $\pm$ 8.16 <sup>c</sup>	20.00 $\pm$ 8.16 <sup>d</sup>	37.50 $\pm$ 9.75 <sup>c</sup>	55.00 $\pm$ 10.00 <sup>c</sup>	60.00 $\pm$ 0.00 <sup>c</sup>	80.00 $\pm$ 0.00 <sup>b</sup>
12	5.00 $\pm$ 5.77 <sup>b</sup>	22.50 $\pm$ 5.00 <sup>b</sup>	37.50 $\pm$ 9.75 <sup>c</sup>	52.50 $\pm$ 9.75 <sup>bc</sup>	57.50 $\pm$ 12.58 <sup>b</sup>	75.00 $\pm$ 10.00 <sup>b</sup>	82.50 $\pm$ 5.00 <sup>b</sup>
18	5.00 $\pm$ 10.00 <sup>b</sup>	27.50 $\pm$ 12.58 <sup>b</sup>	50.00 $\pm$ 8.16 <sup>b</sup>	60.00 $\pm$ 14.14 <sup>b</sup>	67.50 $\pm$ 5.00 <sup>b</sup>	75.00 $\pm$ 5.77 <sup>b</sup>	85.00 $\pm$ 5.77 <sup>b</sup>
24	40.00 $\pm$ 0.00 <sup>a</sup>	45.00 $\pm$ 5.77 <sup>a</sup>	57.50 $\pm$ 5.00 <sup>a</sup>	62.50 $\pm$ 9.57 <sup>ab</sup>	80.00 $\pm$ 0.00 <sup>a</sup>	82.50 $\pm$ 5.00 <sup>b</sup>	97.50 $\pm$ 5.00 <sup>a</sup>
30	40.00 $\pm$ 0.00 <sup>a</sup>	45.00 $\pm$ 5.77 <sup>a</sup>	65.00 $\pm$ 5.77 <sup>a</sup>	75.00 $\pm$ 5.77 <sup>a</sup>	90.00 $\pm$ 0.00 <sup>a</sup>	95.00 $\pm$ 5.77 <sup>a</sup>	100.00 $\pm$ 0.00 <sup>a</sup>

Conc. = concentration of *P. nigrum* ( $\mu\text{L/L}$  air)

Means within the same column followed by the same letter are not significantly different at  $p < 0.5$  by Duncan's Multiple Range Test (DMRT)

**Table 4.** Adult mortality of *Callosobruchus maculatus* with fumigant toxicity treated essential oil from fresh leaves of *Piper nigrum*

Conc.	Mean ( $\pm$ SE) of adult mortality (%) of <i>Callosobruchus maculatus</i>						
	24 h	48 h	72 h	96 h	120 h	144 h	168 h
0	0.00 $\pm$ 0.00 <sup>d</sup>	0.00 $\pm$ 0.00 <sup>c</sup>	0.00 $\pm$ 0.00 <sup>c</sup>	0.00 $\pm$ 0.00 <sup>c</sup>	0.00 $\pm$ 0.00 <sup>d</sup>	0.00 $\pm$ 0.00 <sup>c</sup>	0.00 $\pm$ 0.00 <sup>c</sup>
20	15.00 $\pm$ 5.77 <sup>bc</sup>	40.00 $\pm$ 0.00 <sup>b</sup>	50.00 $\pm$ 0.00 <sup>b</sup>	62.50 $\pm$ 9.57 <sup>ab</sup>	75.00 $\pm$ 5.77 <sup>bc</sup>	80.00 $\pm$ 0.00 <sup>b</sup>	92.50 $\pm$ 5.00 <sup>b</sup>
40	17.50 $\pm$ 5.00 <sup>c</sup>	40.00 $\pm$ 0.00 <sup>b</sup>	52.50 $\pm$ 5.00 <sup>ab</sup>	67.50 $\pm$ 5.00 <sup>b</sup>	77.50 $\pm$ 5.00 <sup>c</sup>	85.00 $\pm$ 5.77 <sup>b</sup>	97.50 $\pm$ 5.00 <sup>a</sup>
60	20.00 $\pm$ 0.00 <sup>bc</sup>	40.00 $\pm$ 0.00 <sup>b</sup>	52.50 $\pm$ 5.00 <sup>ab</sup>	72.50 $\pm$ 5.00 <sup>a</sup>	85.00 $\pm$ 5.77 <sup>abc</sup>	92.50 $\pm$ 5.00 <sup>a</sup>	100.00 $\pm$ 0.00 <sup>a</sup>
80	22.50 $\pm$ 9.57 <sup>b</sup>	45.00 $\pm$ 5.77 <sup>a</sup>	55.00 $\pm$ 5.77 <sup>ab</sup>	72.50 $\pm$ 5.00 <sup>a</sup>	82.50 $\pm$ 9.57 <sup>a</sup>	95.00 $\pm$ 5.77 <sup>a</sup>	100.00 $\pm$ 0.00 <sup>a</sup>
100	30.00 $\pm$ 0.00 <sup>a</sup>	45.00 $\pm$ 5.77 <sup>a</sup>	57.50 $\pm$ 5.00 <sup>a</sup>	75.00 $\pm$ 5.77 <sup>a</sup>	87.50 $\pm$ 5.00 <sup>ab</sup>	97.50 $\pm$ 5.00 <sup>a</sup>	100.00 $\pm$ 0.00 <sup>a</sup>

Conc. = concentration of *P. nigrum* ( $\mu\text{L/L}$  air)

Means within the same column followed by the same letter are not significantly different at  $p < 0.5$  by Duncan's Multiple Range Test (DMRT)

## Discussion

Main components in essential oil of *P. nigrum* seeds concurred with Vanichpakorn *et al.* (2019) as caryophyllene (23.84%) and beta-selinene

(2.45%). Jirovetz *et al.* (2002) investigated compounds in essential oils of dried fruits of *P. nigrum* from Cameroon and observed the presence of beta-caryophyllene (7.29%). The variation in the compositions of essential oils depends on the genetics, type and age of leaf source, environment and method of oil analysis (Lee *et al.*, 2001). Differences in essential oil compositions may be due to several factors such as geographical location, season, environmental conditions, and plant nutrition (Ozcan and Chalchat, 2006). In this study, monoterpenoids and sesquiterpenes were the main groups of constituents in *P. nigrum*. This finding was compatible with Tong and Coats (2010) who reported that the insecticidal activity of many plant essential oils might be due to monoterpenoids. Monoterpenoids were also reported as fumigants and contact toxicants on various insect pests (Rice and Coats, 1994).

Female adults of *C. maculatus* was highly sensitive to *P. nigrum* essential oils, even at low concentrations (30-60  $\mu\text{L/L}$  air) after 168 h of treatment with 100% adult mortality. Insecticidal activity varied with plant-derived materials, oil concentrations and exposure times. Here, *P. nigrum* essential oils showed greater potential as an insecticide. Several previous studies have demonstrated the distinct susceptibility of stored-product beetle species to essential oils. *Callosobruchus* species were found to be more susceptible to essential oils or their components than other insect species (Tripathi *et al.*, 2003; Lee *et al.*, 2004). Chaubey (2012) reported contact toxicity of beta-caryophyllene against both *Tribolium castaneum* and *Sitophilus oryzae*. Therefore, the insecticidal activity of essential oil from *P. nigrum* seeds may be related to these components. Moreover, Chaubey (2017) found that essential oil of *P. nigrum* seeds reduced the activity of acetylcholinesterase in *S. zeamais*.

*P. nigrum* essential oil extracted from seeds and leaves could be used as a postharvest botanical insecticide since it contains monoterpenoids and sesquiterpenes similar to conventional insecticides. *P. nigrum* essential oil was effective as an alternative control method for *C. maculatus* because it displayed insecticidal activity with 100% adult mortality after treatment for 168 h by fumigant toxicity. The results indicated that essential oils of *P. nigrum* has the potential to be a fumigated toxic agent against *C. maculatus* and reduce the environmental risks associated with the use of synthetic insecticides. However, further studies are needed to assess the safety of these essential oils before they are actually use in stored-product insect control.

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