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Chemical composition and bioactivity of essential oils from *Piper nigrum* L. and *Piper retrofractum* Vahl. against *Callosobruchus maculatus* (F.)

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Abstract

Plants containing substances with insecticidal properties can be used for pest control. Essential oils of *Piper nigrum* L. and *Piper retrofractum* Vah. from dried seeds and fresh leaves extracted by hydrodistillation was determined using Gas Chromatography-Mass Spectrometry (GC-MS). The fumigation toxicity and bioactivity bioassay were investigated using vapor-phase test. Experiment was performed under completely randomized design (CRD) with 4 replications. Adult mortality, number of eggs and adult emergence of F1 progeny of *C. maculatus* were recorded. Chemical compounds of essential oils from dried seeds and fresh leaves of *P. nigrum* and *P. retrofractum* were determined at 22-27 components (90.34-93.27%). Major compounds in dried seeds and fresh leaves of *P. nigrum* and *P. retrofractum* were alpha-bergamotene, caryophyllene, beta-selinene, germacrene D, naphthalene, undecane, 5-octadecene, cyclohexene, limonene and 2-beta-pinene. Both essential oils contained limonene, linalool, caryophyllene and naphthalene in seeds and leaves. These four components showed insecticidal properties. Essential oils at concentrations of 5 µL/L air *P. nigrum* and 10 µL/L air *P. retrofractum* extracted from dried seeds were more effective in oviposition inhibition and adult emergence of *C. maculatus* than essential oils extracted from fresh leaves. *P. nigrum* essential oil from dried seeds at a concentration of 5 µL/L air was the highest efficiency of oviposition inhibition with 55.17% and adult emergence inhibition of 71.09% of *C. maculatus*. This suggested that *P. nigrum* essential oil extracted from dried seeds could be used for protection on *C. maculatus* in stored grain.

Keywords: essential oil, *Piper* sp., oviposition, *Callosobruchus maculatus*, stored product insects.
Abbreviations: RT_retention time; Conc._concentration; LC_lethal concentration.

Introduction

Callosobruchus maculatus (Fabricius) (Coleoptera: Chrysomelidae: Bruchinae) is the most destructive stored product insect pest. This beetle can destroy up to 100% of legume seeds during storage (Gbaye et al., 2011), resulting in 2 to 5 kg of seed damage within 45 to 90 days at optimal temperature (30±1ºC) and humidity (75±3%). The neonatal larvae penetrate the grain causing serious damage, with decreased grain weight, poor germination ability and decreased nutritional value (Melo et al., 2010; Oke and Akintunde, 2013). The grain is rendered unsuitable for human or animal consumption and has poor germination capacity (Elhag, 2000). Currently, synthetic pesticides are used to protect grain, stored food, animal feed and other agricultural products from insect damage (Ghorab and Khalil, 2016). However, continuous or excessive use of synthetic pesticides causes serious problems due to many factors including the potential for ozone layer destruction, environmental pollution, increased cost of application, pesticide residues in the environment and food products (Ccanccapa et al., 2016), development of insect resistance (Damalas and Eleftherohorinos, 2011), direct toxicity hazards on users and indirect toxicity hazards on non-targets (Isman, 2006; Ogendo et al., 2008; Köhler and Triebskorn, 2013).

These problems highlight the need to develop new options as fumigants for insect control. The application of botanical pesticides is a viable alternative to synthetic chemicals with no serious side effects. These products are biodegradable and do not interfere with the ecosystem (Rajendran and Sriranjini, 2008). Currently, essential oils as secondary metabolic products in aromatic plants are used for insect pest management (Isman, 2006). In particular, monoterpenoids offer an attractive alternative to classic fumigants (Papachristos and Stamopoulos, 2003) by impacting the physiology, biology and behavior of insects such as growth rate, life cycle, reproduction and possibly death (Pascual-Villalobos, 1996; Islam et al., 2009). The chemical compounds and toxicity of essential oils from several plants have been assessed against a population of stored product insect pests.

Studies on essential oils have spurred research on pest control in stored grain with favorable results. Mahmoudvand et al. (2011) demonstrated insecticidal activity of the essential oils of *Lippia citrodora*, *Rosmarinus officinalis*, *Mentha piperita* and *Juniperus sabina* against *C. maculatus*. Previous studies also assessed the fumigant potential of *P. nigrum* essential oils from seeds and leaves against *C. maculatus*. Results showed strong fumigant activities at 168 h with 100% adult mortality at concentrations of 30 and 60 µL/L air against *C. maculatus* (Wanna, 2021). Toxic effects of some essential oils also showed fumigation, contact, repellent and oviposition activity against *C. maculatus* (Wanna and Khangkhun, 2018; Wanna and Kwang-Ngoen, 2019).

Piper is the largest genus in the Piperaceae family with approximately 1,000 species (Tebbs, 1993). Most species are distributed in the New World and the Old World, especially in Malaysia (Yuncker, 1958), with 19 species recorded in Thailand (Forest Herbarium, 2001). The genus can be easily classified based on overall morphology but species identification is difficult. The genus is characterized as monoecious or dioecious with leaves that are simple, alternate and entire. The inflorescences are spikes or catkins with dense or sparse flowers on the rachis. The flowers are unisexual or bisexual, very small, without sepals and petals and the floral bracts have different shapes. The ovary has one locule and the fruit is classified as a drupe (Suwanphakdee et al., 2006). Plants in the family Piperaceae contain many promising phytochemicals with insecticidal activity. The most well-known insecticidal compounds are derived from *Piper nigrum* L., *P. guineense* Schum and Thonn and *P. tuberculatum* Jacq. These unique *Piper* secondary plant compounds have several modes of action including synergism, contact toxicity, fumigant toxicity, oviposition inhibition, repellent and antifeedant properties (Scott et al., 2004; Khani et al., 2011; Ahmad et al., 2016; Wanna and Khangkhun, 2018; Wanna, 2021). This study identified the chemical composition and investigated the bioactivity of essential oils from dried seeds and fresh leaves of *P. nigrum* L. and *Piper retrofractum* Vahl. against *C. maculatus*.

Results

Identification of compounds

Sixty compounds were recorded in the essential oils from dried seeds and fresh leaves of *P. nigrum and P. retrofractum*. Essential oils extracted from different plant parts and species showed both similar and different chemical compositions. Twenty-six compounds (90.55%) were recorded from dried seeds of *P. nigrum* from previous report of Wanna (2021). There were 18 major compounds (88.25%), identified as alpha-bergamotene (14.68%), followed by caryophyllene (11.92%), beta-bourbonene (8.47%), 2-nonanone (7.58%), spathulenol (7.50%), 2-propanone (6.32%), naphthalene (5.73%), beta-selinene (4.94%), gamma-cadinene (3.45%), 3-buten-2-one (3.43%), 2-undecanol (2.87%), pentadecane (2.87%), 1-tetradecyne (1.82%), alpha-selinene (1.48%), curcumene (1.42%), copaene (1.36%), capronaldehyde (1.33%) and 3-hexen-1-ol (1.08%). There were 8 important compounds (3.99%) as germacrene-D (0.65%), linalool oxide (0.44%), juniper camphor (0.42%), caryophyllene oxide (0.41%), p-cymene (0.15%), linalool (0.10%), limonene (0.09%) and alpha-cubebene (0.04%). Likewise, chemical compositions in the essential oil from fresh leaves of *P. nigrum* were identified and quantified from the study results of Wanna (2021). There were 22 major compounds (90.34%) (Table 1). These were beta-selinene (12.26%), followed by 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%), alpha-gurjunene (3.81%), 3-hexen-1-ol (3.13%), caryophyllene (2.75%), 2-propanone (2.14%), naphthalene (2.12%), 1h-cycloprop[e]azulene (2.04%), cyclohexanol (1.78%), linalool oxide (1.72%), 1-naphthol (1.72%), spathulenol (1.64%), 1h-cycloprop[e]azulene (1.40%), 9,10-dehydro-isolongifolene (1.20%) and 3-buten-2-one (1.10%). There were 5 important compounds (1.31%) as beta-humulene (0.66%), followed by juniper camphor (0.38%), linalool (0.17%), limonene (0.06%) and terpineol (0.04).

Essential oil from dried seeds of *P. retrofractum* contained 22 compounds (92.78%). There were 17 major compounds (90.08%) (Table 1). These were alpha-bergamotene (15.61%), followed by naphthalene (15.40%), undecane (14.63%), 5-octadecene (10.70%), beta-farnesene (5.46%), cyclohexane (4.56%), 8-heptadecene (3.60%), 6-tetralinol (3.53%), beta-santalene (3.06%), nonadecane (3.06%), caryophyllene oxide (2.19%), copaene (2.00%), beta-bisabolene (1.55%), germacrene d (1.27%), 2-propanone (1.20%), linalool (1.18%) and pentadecane (1.08%). There were 5 important compounds (2.70%) as caryophyllene (0.98%), followed by p-cymene (0.63%), zingiberene (0.60%), limonene (0.35%) and beta-myrcene (0.14%). Essential oil from fresh leaves of *P. retrofractum* contained 22 compounds (93.27%). There were 12 major compounds (90.10%) (Table 1). These were cyclohexene (20.62%), followed by limonene (18.51%), caryophyllene (13.28%), 2-beta-pinene (10.05%), l-phellandrene (6.55%), trans-ocimene (6.26%), beta-myrcene (4.32%), benzene (3.68%), terpinene (2.29%), linalool (2.15%), caryophyllene oxide (1.24%) and beta-selinene (1.15%). There were 10 important compounds (3.17%) as beta-phellandrene (0.99%), followed by beta-elemene (0.85%), p-cymene (0.46%), camphene (0.39%), gamma-terpinene (0.32%), alpha-guriunene (0.06%), beta-farnesene (0.04%), curcumene (0.03%), naphthalene (0.02%) and linalool oxide (0.01%).

Bioactivity bioassay

Table 2 shows results of vapor phase toxicity bioassay related to the fumigant activity of essential oils from dried seeds and fresh leaves of *P. nigrum* and *P. retrofractum* against adults of *C. maculatus*. Probit analysis showed that adults of *C. maculatus* were susceptible to essential oil from dried seeds of *P. nigrum*, with highest fumigant toxicity within 72 h after treatment with LC_{50} of 20.64 µL/L air. Bioactivities of essential oils from dried seeds and fresh leaves of *P. nigrum* and *P. retrofractum* are shown in Table 3. The number of *C. maculatus* eggs on the surface of mungbean seeds significantly reduced ($p < 0.01$) at all concentrations of essential oils. Essential oil extracts from dried seeds of both *P. nigrum* and *P. retrofractum* showed high efficiency in oviposition inhibition, with adult emergence reduction significantly higher than essential oils extracted from fresh leaves, ($p < 0.01$). A concentration of 5 µL/L air from dried seeds of *P. nigrum* essential oil showed optimal oviposition and adult emergence reduction at 55.17±16.78% and 71.09±13.69, respectively,

Table 1. Chemical composition of essential oils from dried seeds and fresh leaves of *P. nigrum* and *P. retrofractum*.

No.	Compound	RT	*P. nigrum* [a]		*P. retrofractum*	
			Dried seeds	Fresh leaves	Dried seeds	Fresh leaves
1	6-tetralinol	1.389	-	-	3.53	-
2	3-buten-2-one	1.391	3.43	1.10	-	-
3	2-propanone	1.595	6.32	2.14	1.20	-
4	3-hexen-1-ol	2.934	1.08	3.13	-	-
5	capronaldehyde	2.940	1.33	-	-	-
6	2-octen-1-ol	3.034	-	4.44	-	-
7	trans-ocimene	3.990	-	-	-	6.26
8	camphene	4.220	-	-	-	0.39
9	beta-phellandrene	4.588	-	-	-	0.99
10	2-beta-pinene	4.732	-	-	-	10.05
11	terpineol	4.789	-	0.04	-	-
12	beta-myrcene	4.829	-	-	0.14	4.32
13	l-phellandrene	5.185	-	-	-	6.55
14	cyclohexene	5.362	-	-	-	20.62
15	benzene	5.551	-	-	-	3.68
16	p-cymene	5.585	0.15	-	0.63	0.46
17	limonene	5.675	0.09	0.06	0.35	18.51
18	gamma-terpinene	6.202	-	-	-	0.32
19	terpinene	6.859	-	-	-	2.29
20	linalool oxide	7.041	0.44	1.72	-	0.01
21	2-nonanone	7.047	7.58	-	-	-
22	linalool	7.064	0.10	0.17	1.18	2.15
23	1-tetradecyne	11.784	1.82	-	-	-
24	undecane	11.874	-	-	14.63	-
25	2-undecanol	12.980	2.87	-	-	-
26	alpha-cubebene	13.306	0.04	8.09	-	-
27	aromadendrene oxide-(2)	14.014	-	6.99	-	-
28	copaene	14.236	1.36	-	2.00	-
29	beta-bourbonene	14.379	8.47	4.75	-	-
30	beta-elemene	14.379	-	-	-	0.85
31	7-hexadecenoic acid	14.872	-	4.41	-	-
32	zingiberene	14.918	-	-	0.60	-
33	alpha-bergamotene	15.117	14.68	-	15.61	-
34	alpha-gurjunene	15.128	-	3.81	-	0.06
35	caryophyllene	15.221	11.92	2.75	0.98	13.28
36	beta-farnesene	15.953	-	-	5.46	0.04
37	beta-santalene	16.386	-	-	3.06	-
38	1h-cycloprop[e]azulene	16.407	-	1.40	-	-
39	5-octadecene	16.619	-	-	10.7	-
40	curcumene	16.623	1.42	-	-	0.03
41	germacrene D	16.800	0.65	9.15	1.27	-
42	naphthalene	16.840	5.73	2.12	15.4	0.02
43	pentadecane	16.974	2.87	-	1.08	-
44	beta-selinene	17.000	4.94	12.26	-	1.15
45	cyclohexane	17.184	-	-	4.56	-
46	alpha-selinene	17.188	1.48	-	-	-
47	beta-humulene	17.196	-	0.66	-	-
48	cyclohexanol	17.457	-	1.78	-	-
49	beta-bisabolene	17.527	-	-	1.55	-
50	gamma-cadinene	17.532	3.45	-	-	-
51	7-epi-alpha-selinene	17.626	-	6.70	-	-
52	9,10-dehydro-isolongifolene	18.966	-	1.20	-	-
53	spathulenol	19.099	7.50	1.64	-	-
54	1h-cycloprop[e]azulen-7-ol	19.106	-	2.04	-	-

55	1-naphthol	20.090	-	1.72	-	-
56	juniper camphor	20.108	0.42	0.38	-	-
57	alpha-cadinol	20.519	-	5.69	-	-
58	caryophyllene oxide	20.962	0.41	-	2.19	1.24
59	8-heptadecene	21.146	-	-	3.60	-
60	nonadecane	21.410	-	-	3.06	-
	Total		90.55	90.34	92.78	93.27

RT represents retention time. ^{a} Data obtained from Wanna (2021).

Table 2. Fumigant toxicity of essential oil from dried seeds and fresh leaves of *P. nigrum* and *P. retrofractum* on female adult of *C. maculatus* after 72 h exposure.

Piper sp.	Essential oil source	n	LC_{50} (µL/L air)	Regression equation	r^2
Piper nigrum	Dried seeds	240	20.64	y = 1.5999x + 16.985	0.9862
	Fresh leaves	240	22.48	y = 0.0964x + 47.833	0.9613
Piper retrofractum	Dried seeds	240	68.03	y = 0.4214x + 21.333	0.9412
	Fresh leaves	240	39.56	y = 0.1643x + 43.5	0.8891

n is the number of the tested insect population. r^2 is the coefficient of determination. LC_{50} (50% lethal concentration) is the amount of substance required to kill half of the members of a tested insect population after a specified test duration period expressed as µL/L air.

Table 3. Oviposition inhibition and adult emergence of *C. maculatus* exposed to essential oils from dried seeds and fresh leaves of *P. nigrum* and *P. retrofractum* by the vapor phase test.

Piper sp.	Essential oil source	Conc. (µL/L air)	% Oviposition inhibition	% Adult emergence
Piper nigrum	Dried seeds	5	55.17±16.78 ^{a}	71.09±13.69 ^{a}
	Fresh leaves	5	38.92±10.09 ^{b}	51.97±11.24 ^{bc}
Piper retrofractum	Dried seeds	10	39.76±9.96 ^{b}	55.04±15.06 ^{b}
	Fresh leaves	10	29.51±12.56 ^{b}	40.18±16.29 ^{c}
F-test			**	**

** represents significant difference at $p \leq 0.01$ compared with the control. Means within the same column followed by the same letter are not significantly different at $p > 0.05$ by DMRT.

while essential oils extracted from *P. nigrum* fresh leaves and *P. retrofractum* dried seeds showed similar oviposition inhibition efficiency at 38.92±10.09% and 39.76±9.96%, respectively with adult emergence 51.97±11.24% and 55.04±15.06%. Oviposition and adult emergence reduction were lower using essential oil extracted from fresh leaves of *P. retrofractum* at 29.51±12.56% and 40.18±16.29%, respectively.

Discussion

The main components from dried seeds of *P. nigrum* concurred with Jirovetz et al. (2022). They investigated compounds in essential oils of dried fruits of *P. nigrum* from Cameroon and observed the presence of beta-caryophyllene (7.29%), while Vanichpakorn et al. (2019) found beta-caryophyllene (23.84%), delta-3-carene (20.95%), d-limonene (12.98%) and beta-pinene (8.15%). By contrast, this study found low percentages of these substances as limonene (0.09%). The GC-MS results revealed the main constituents as follows: alpha-bergamotene, caryophyllene (*P. nigrum* seeds), beta-selinene, germacrene D (*P. nigrum* leaves), alpha-bergamotene, naphthalene, undecane, 5-octadecene (*P. retrofractum* seeds) and cyclohexene, limonene, caryophyllene and 2-beta-pinene (*P. retrofractum* leaves). In line with our study, Piper species have previously been reported to contain high amounts of monoterpene hydrocarbons (α-terpinene and α-terpinene), sesquiterpene hydrocarbons (β-selinene and germacrene B), oxygenated sesquiterpenes (eudesm-7(11)-en4-ol) and arylpropanoids (myristicin and elemicin) (Krinski et al., 2018). Genetics, type and age of the leaf source, environment and method of essential oil analysis may be the variables cause variations in the composition of essential oils (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).

Results indicated that essential oils from dried seeds and fresh leaves of *P. nigrum* and *P. retrofractum* slightly moderated (29-55%) oviposition inhibition of *C. maculatus*. Essential oils extracted from dried seeds of *P. nigrum* and *P. retrofractum* were more effective in oviposition inhibition and adult emergence reduction of *C. maculatus* than essential oils extracted from their fresh leaves. Efficacy of essential oil from dried seeds of *P. nigrum* with 5 µL/L gave optimal inhibition of adult emergence (71%) of *C. maculatus*. Our results concurred with Oliveira et al. (2017) who reported that essential oils of *Piper* sp. from *P. aduncum* and *P. hispidinervum* effectively reduced oviposition of *C. maculatus* at 44-66%. The essential oil of *P. aduncum* also reduced *C. maculatus* adult emergence (68%). Vanichpakorn et al. (2015) found that powdered seed from *P. nigrum* had an inhibitory effect on oviposition of *C. maculatus*, with lowest oviposition rate of 6.75 eggs/20 g seeds and highest inhibition of *C. maculatus* adult emergence of 100%, while all concentrations of *P. nigrum* seed powder did not inhibit mung bean germination and prevented damage of mung bean seeds from insect damage for up to 3 months. In this study, monoterpenoids and sesquiterpenes were the main constituents in *P. nigrum* and *P. retrofractum*. This finding was compatible with Tong and Coats (2010) who suggested that the insecticidal activity of many plants essential oils was due to monoterpenoids that were also reported as fumigants and contact toxicants on various insect pests (Rice and Coats, 1994).

Materials and Methods

Insect rearing

C. maculatus (F.) were collected from damaged mungbean seeds stored at the Department of Agricultural Technology, Faculty of Technology, Mahasarakham University, Maha Sarakham, Thailand. Insect rearing was carried out in a cylindrical plastic bottle (diameter 15 cm, height 8 cm) under laboratory conditions (30±5ºC, 70±5%RH and 8L: 16D photoperiods), following Wanna (2021). Fifty pairs of male and female adults of *C. maculatus* were raised and preserved in a plastic bottle with 1 kg of mungbean seeds (*Vigna radiata* (L.) Wilczek). Infested mungbean seeds were removed and fresh mungbean seeds were added, sterilized by freezing for 2 weeks and then left for 24 h under ambient conditions before being used for *C. maculatus* rearing. The insects were reared and allowed to mating and oviposition under laboratory conditions. Adults of *C. maculatus* were separated and newly emerged females were used in the investigation. All experiments were performed under the same environmental conditions.

Essential oil

Essential oils were extracted from dried seeds and fresh leaves of *P. nigrum* and *P. retrofractum* by the water distillation method in a modified Clevenger-type apparatus following Wanna (2021). For the water distillation process, 200 g of each *Piper* sp. were weighed into a distillation flask and added with 600 mL of distilled water. The apparatus was set up using a clamp on a heating mantle for 2 h. Essential oils deposited in the water were collected through a graduated measuring tube with the tap open, and the remaining water was removed by centrifuging at 8,000 rpm for 10 min. The essential oils were preserved in sealed amber glass bottles and kept in the refrigerator at 4ºC in the dark until required for further use.

Essential oil analysis

Chemical compounds in the essential oils from dried seeds and fresh leaves of *P. nigrum* and *P. retrofractum* were analyzed following Wanna (2021) by gas chromatography-mass spectrometry (GC-MS) performed using a Clarus SQ 8 GC/MS system (PerkinElmer, MA, USA) operating in the electron impact (EI) mode (70 eV), with a Rtx-5MS capillary column (5% phenylmethyl polysiloxane stationary phase, 30 m x 0.25 mm, 0.25 µm film thickness). GC settings were as follows: 50ºC of initial oven temperature for 1 min, then increased to 180ºC at a rate of 10ºC/min, held for 1 min, and then raised at 3ºC/min to 240 ºC for 15 min. The injector temperature was maintained at 230ºC. A sample (1 µL, diluted to 1% with acetone) was injected with a split ratio of 1:10 with helium (flow rate of 1.0 mL/min) used as the carrier gas. Spectra were scanned from 45 to 450 m/z. Essential oil compositions were identified by comparing their mass spectra with those in the archives of the National Institute of Standards and Technology (NIST) Mass Spectral Search Program and ChemStation Wiley Spectral Library. Essential oil compounds were assigned by comparing substances with mass spectra at more than 80% quality match. Chemical component data of essential oils from *P. nigrum* and *P. retrofractum* were analyzed by reading the retention time and percentage area.

Bioactivity bioassay

Essential oils from the dried seeds and fresh leaves of *P. nigrum* and *P. retrofractum* were assessed on *C. maculatus* by fumigation activity following the modified method of Wanna (2021). Fumigation toxicity of the essential oils of *P. nigrum* and *P. retrofractum* on female adults of *C. maculatus* was evaluated by the vapor phase test. Whatman (no.1) filter paper strips (1.5 cm width x 5 cm length) were impregnated with 100 µL of 6 concentrations of *P. nigrum* and *P. retrofractum* dilution of essential oils as prepared earlier. After evaporating the solvent for 2 min, the filter paper strips were placed into glass vials (2.5 cm diameter x 5 cm height) hanging from the center of screw caps of fumigation bottles (5.5 cm diameter x 10.5 cm height) to avoid contact between the insects and the filter paper strips. Ten of the newly emerged female adults of *C. maculatus* were placed inside each fumigation bottle. The bottle caps were closed tightly, with conditions maintained at 30±5°C, 70±5% relative humidity and 16L: 8D photoperiods. The control received 100 µL of acetone only. Adult mortality of *C. maculatus* was recorded after 72 h. When no leg or antennal movements were observed, the insects were dead. Oviposition inhibition of the essential oils of *P. nigrum* and *P. retrofractum* on female adults of *C. maculatus* was evaluated by the vapor phase test. Whatman (no.1) filter paper strips (1.5 cm width x 5 cm length) were impregnated with 100 µL of 5 µL/L air of *P. nigrum* and 10 µL/L air of *P. retrofractum* dilution of essential oils as prepared earlier. Forty of the newly emerged female adults of *C. maculatus* were placed inside each fumigation bottle. After 7 days, ten female adults were separated and transferred to a clean bottle with ten male adults of *C. maculatus* (5 days old) and 10 g of sterile mungbean seeds. They are allowed to breed and lay eggs for 3 days. The adults of *C. maculatus* were separated and removed from the mungbean seeds and the number of eggs was recorded. The emerging adults of F1 progeny of *C. maculatus* from the mungbean seeds were recorded. The average egg reduction and adult emergence percentage were calculated.

Data analysis

Mortality data were adjusted for control mortality according to Abbott's formula (Abbott, 1925). The mortality in the control was between 5 and 20%. The fumigation toxicity of essential oils from the dried seeds and fresh leaves of *P. nigrum* and *P. retrofractum* were assessed on *C. maculatus* was assessed for dose-mortality response using Probit analysis. Oviposition inhibition and adult emergence reduction were conducted using a completely randomized design (CRD) with four replications. Statistical analysis was performed using one-way analysis of variance (ANOVA). Data were presented as means±SE. Significant differences at p-value ≤ 0.05 among means were determined by Duncan's New Multiple Range test (DMRT).

Conclusions

Essential oils from both dried seeds and fresh leaves of *P. nigrum* and *P. retrofractum* could be used as insecticides for harvest insect pests. The chemical compounds monoterpene and sesquiterpene showed bioactivity on adults of *C. maculatus*. Essential oils from the dried seeds inhibited oviposition on *C. maculatus* adults and emergence of progeny. Results suggested high potential for use of essential oils of *P. nigrum* and *P. retrofractum* as fumigant residue agents against *C. maculatus*. However, further studies are needed to assess the safety of these essential oils before large-scale usage for insect control of stored grains.

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