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Biological activities and chemical composition of *Mentha piperita* and *Syzygium aromaticum* essential oil fractions against cowpea weevil, *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae)

Aktivitas biologis dan komposisi kimia dari fraksi minyak atsiri *Mentha piperita* dan *Syzygium aromaticum* terhadap kumbang kacang, *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae)

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ABSTRACT

Callosobruchus maculatus (Fabricius) is one of the major, common, pests of stored grains as it causes quantitative and qualitative losses in legume crops. This research sought to find the most active fraction in *Mentha piperita* and *Syzygium aromaticum* essential oils, to evaluate bioactivity of those crude essential oils and active fractions against *C. maculatus*, and to identify the compounds contained in the active fraction. The essential oils were fractionated using three solvents, namely *n*-hexane, ethyl acetate, and methanol. The *n*-hexane fraction was identified as the active fraction, causing mortality, oviposition deterrence, and ovicidal effects. In fumigation chambers, LD_{95} values of *M. piperita* (Mnf) and *S. aromaticum n*-hexane fractions (Snf) were 0.045 ml/l and 0.057 ml/l respectively. ED_{50} values for oviposition deterrence were 0.016 ml/l for Mnf and 0.022 ml/l for Snf. ED_{50} value of ovicidal effects for Mnf- and Snf-treated eggs were 0.014 ml/l for both fractions. GC-MS analysis showed 8 dominant compounds in Mnf and 5 dominant compounds in Snf. Overall it is concluded that Mnf and Snf oils have effective biological activities against stored pest *C. maculatus* and have potential to be considered as alternatives to synthetic insecticides.

Key words: ovicidal, oviposition, stored product pest, synthetic insecticide, toxicity

ABSTRAK

Callosobruchus maculatus (Fabricius) merupakan salah satu hama yang umum menyerang pada komoditas biji-bijian yang disimpan karena menyebabkan kerusakan baik secara kualitas maupun kuantitas. Tujuan penelitian ini untuk mengetahui fraksi paling aktif minyak atsiri pepermin (*Mentha piperita*) dan cengkeh (*Syzygium aromaticum*), mengevaluasi bioaktivitas dari minyak atsiri dan fraksi aktif minyak atsiri tersebut terhadap *C. maculatus*, dan mengetahui senyawa-senyawa yang terkandung di dalam fraksi aktifnya. Fraksinasi minyak atsiri pepermin dan cengkeh menggunakan tiga pelarut, yaitu *n*-hexane, *ethyl acetate*, dan methanol. Pepermin fraksi *n*-hexane merupakan fraksi aktif yang menyebabkan kematian, penghambatan aktivitas peneluran, dan berpengaruh terhadap telur yang dihasilkan (*ovicidal effect*). Nilai masing-masing LD_{95} pepermin fraksi *n*-hexane (Mnf) dan cengkeh *n*-hexane (Snf) adalah 0,045 ml/l dan 0,057 ml/l ruang fumigasi. Nilai ED Mnf untuk

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penghambatan aktivitas peneluran adalah 0,016 ml/l, sedangkan Snf adalah 0,022 ml/l. Nilai ED_{50} Mnf dan Snf yang berpengaruh terhadap telur *C. maculatus* adalah 0,014 ml/l. Hasil analisi senyawa dengan GC-MS menunjukkan delapan senyawa dominan pada Mnf dan lima senyawa dominan pada Snf. Dengan demikian, dapat disimpulkan bahwa Mnf dan Snf mepunyai aktivitas biologi yang efektif terhadap hama *C. maculatus* dan memiliki potensi untuk dipertimbangkan sebagai alternatif insektisida sintetik.

Kata kunci: hama gudang, insektisida sintetik, oviposisi, ovisidal, toksisitas

INTRODUCTION

Cowpea weevil *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae) is the common pest of stored legumes especially on mung bean and cowpea seeds because it causes quantitative and qualitative losses when, after hatching from deposited eggs, the larvae penetrate and consume the entire bean. Although infestation levels in the field are very low, insect populations grow in storage and cause 100% damage to legumes during the post-harvest period (Tembo et al. 2017). Hassan (2012) recorded 46.33 to 67.33% loss in seed weight causing lower germination rate due to infestation of cowpea weevil.

Due to the severe losses in later harvest periods, synthetic insecticides are widely used to reduce the attack of stored grain insects. The common method of controlling stored product insects is using chemical fumigants especially methyl bromide and phosphine. However, use of methyl bromide as a fumigant was restricted as it causes ozone depletion. At present, phosphine became an alternative fumigant to methyl bromide due to its high efficiency and rapid diffusion. Nevertheless, rapid development of resistance by stored-product pests is on-going and effective use of phosphine needs to be reconsidered due to this emerging problem. In this situation, natural products are considered as alternatives because of their innate biodegradability and decreased harm to non-target organisms (Prabakar & Jebanesan 2004).

The essential oils from *Syzygium aromaticum* (Family Myrtaceae) and *Mentha piperita* (Family Lamiaceae) possess many compounds with biological activity, such as repellency and developmental inhibition, resulting in their use to control stored-grain insects. Since these essential oils contain mixtures of various compounds, it will be useful to know the efficacy and toxicity of the major active components of clove and mint oils. Ramar et al. (2014) reported that clove oil showed toxic to all life stages of *Culex quinquefasciatus* Say and prominent ovicidal and oviposition response activity, and Uniyal et al. (2016) also showed the effective toxicity and oviposition deterrence of *M. piperita* to *Aedes aegypti* (Linnaeus). While other studies mentioned the effectiveness of crude essential oils in insect control, it is still need to know the efficacy of major active compounds of mint and clove fraction oils. Therefore, the objectives of this research were to find the most active fraction in clove (*S. aromaticum*) and peppermint (*M. piperita*) essential oils and to evaluate the bio-activities of crude essential oil and their active fractions against *C. maculatus*.

MATERIALS AND METHODS

Place and time

Research was carried out in the Entomology Laboratory, Southeast Asian Regional Centre for Tropical Biology (SEAMEO BIOTROP), Bogor, Indonesia, from August 2019–February 2020.

Insect rearing

Adult cowpea weevils were reared on mung bean seeds in glass jars covered with muslin cloth, then fixed with rubber bands. To have initial populations of insect adults in homogenous age, around 200 adults were introduced into the jars containing mung bean seeds for egg laying. After three days all adult insects were removed. The newly emerged weevils were used in these experiments. This rearing process was carried out until the end of experiment to use newly adult insects in tests.

Source of essential oils

Two essential oils of cloves (*S. aromaticum*) and peppermint (*M. piperita)* were obtained from the Entomology Laboratory, SEAMEO BIOTROP, Bogor, Indonesia.

Crude essential oils toxicity assay

The toxicity of peppermint and clove essential oil against *C. maculatus* was assayed by fumigation method. Doses were 0.01, 0.017, 0.025, 0.037, 0.063, 0.092 ml/l fumigation chamber for peppermint and 0.025, 0.036, 0.047, 0.06, and 0.087 ml/l fumigation chamber for clove through preliminary test. Each oil was diluted with 5 ml acetone solvent to get the solution. As much as 0.5 ml solution from each dose was applied to filter paper glued to the inside of petri dish lids. After drying for one minute, test insects were introduced to each petri dish which then was covered with its lid and sealed with plasticine. Application of acetone 0.5 ml was served as control. Mortality of test insects was observed at 72 hours after treatment. Resulting data were analyzed by probit analysis using POLO-PLUS program to determine the effect of dose.

Essential oils fractionation

The fractionation procedure was carried out according to Parwata et al. (2009); 50 ml of essential oil was put into a separating funnel, then 75 ml of *n*-hexane was added and separated it using 75 ml of water-methanol (3:2). Once two separate layers were clearly formed, the layers were collected as *n*-hexane and methanol fractions. Then the collected methanol fraction was separated again by adding 75 ml of ethyl acetate. The solution mixture was formed ethyl acetate and methanol fraction layers. The obtained three fractions were evaporated by using a rotary evaporator at 50 °C.

Fraction toxicity assay

Ethyl acetate, *n*-hexane, and methanol fractions were assayed for their toxicity by using equivalent doses. The assay and observation methods were the same as those described in the previous crude essential oil toxicity test. The following equation was used to determine the equivalent dose (Dadang 1999):

$$\text{Equivalent dose} = \frac{\text{Fraction volume (ml)}}{\text{Total volume (ml)}} \times \text{LD}_{95} \text{ crude essential oil}$$

The faction that causes the highest mortality was chosen as the active fraction. Then the advanced test was carried out to know the LD_{50} and LD_{95} value of this active fraction.

Oviposition deterrence activity

This assay was carried out with four sub-lethal doses of crude essential oil and *n*-hexane fraction. Using 100 mung bean seeds for each dose, these were treated with 0.5 ml solution. After that, beans were air-dried and put into a petri dish with one pair of *C. maculatus* adults. After 72 hrs, the eggs laid on the cowpea seeds were counted. Percentage of oviposition deterrence was calculated by using the formula of Brari & Thakur (2017).

$$\% \text{ Deterrency} = \frac{NC - NT}{NC} \times 100$$

where NC: number of eggs in control; NT: number of eggs in treatments.

Evaluation of ovicidal activity

In the ovicidal assay, twenty eggs were used for each replication. Only beans with one egg were used. Selected eggs were fumigated for 72 hours and allowed to hatch. The number of hatched eggs were recorded after 14 days of treatment. The hatching inhibition rate (% HIR) was calculated with the following formula according to Chaubey (2008).

$$\% \text{ HIR} = \frac{C_n - T_n}{C_n} \times 100$$

C_n: number of hatched eggs in untreated mung bean seeds; T_n: number of hatched egg in treated mung bean seeds.

Analysis of active fraction

The chemical composition of the active fractions of *M. piperita* and *S. aromaticum* were analyzed using gas chromatography–mass spectrometry (GC-MS) agilent technologies 7890 GC with auto sampler and 5975 MS detector and chemstation data system. The injection sample volume was 1 µl and helium was used as carrier gas with flow rate 0.6 ml/minute. Initial temperature started at 60 °C, and then it slowly raised from 2 °C/min to 150 °C in 1 minute to 20 °C/min to 210 °C for 10 minutes. The obtained chromatograms were named according to NIST17 standard database recommendations with above 90% similarity level.

Data analysis

The resulting data were analyzed with one-way ANOVA analysis and used Tukey's range test at the 5% significance level.

RESULT

Crude essential oils toxicity assay

LD_{50} and LD_{95} values of *M. piperita* oil were 0.022 ml/l and 0.063 ml/l fumigation chamber, while *S. aromaticum* oil showed 0.042 ml/l and 0.079 ml/l, respectively (Table 1). *M. piperita* oil has lower LD_{50} and LD_{95} values than *S. aromaticum* oil. *M. piperita* oil caused lowest mortality at 0.01 ml/l with 12% mortality and highest mortality 97% found at 0.063 ml/l (Figure 1). *S. aromaticum* showed lowest mortality 12% at 0.025 ml/l, while highest mortality reached to 98% at 0.087 ml/l (Figure 2).

Toxicity of essential oil fractions

In *M. piperita* oil, the *n*-hexane fraction result was 69.1% while ethyl acetate showed 31.36% and *S. aromaticum* fraction values were 64.84% for *n*-hexane and 36.28% for ethyl acetate (Table 2). For both oils, the *n*-hexane fraction was chosen as the active fraction since the mortality of *C. maculatus* was higher when exposed to this fraction than when exposed to the ethyl acetate fraction (Table 3). LD_{50} and LD_{95} values of *M. piperita n*-hexane fraction (Mnf) were 0.018 ml/l and 0.045 ml/l fumigation chamber, respectively, whereas *S. aromaticum n*-hexane fraction (Snf) showed 0.029 ml/l and 0.057 ml/l, respectively

Table 1. Lethal dose values of essential oils against *Callosobruchus maculatus*

Essential oil	Slope ± SE	LD_{50} (CI 95%) (ml/l^{air})	LD_{95} (CI 95%) (ml/l^{air})
Mentha piperita	3.45 ± 0.28	0.022 (0.020–0.024)	0.063 (0.054–0.078)
Syzygium aromaticum	6.06 ± 0.46	0.042 (0.038–0.046)	0.079 (0.067–0.100)

ml/l^{air} : ml/l fumigation chamber; CI: confdent interval; SE: standard error.

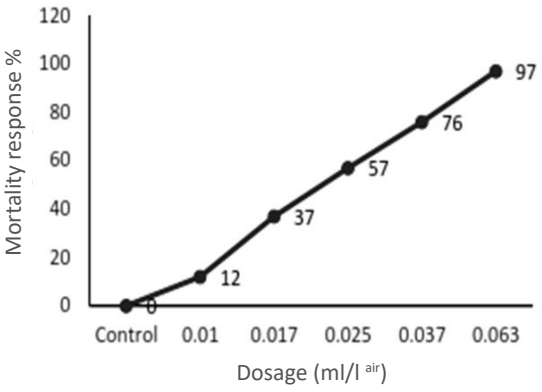

Figure 1. Mortality effect by *Mentha piperita* crude oil against *Callosobruchus maculatus* adults.

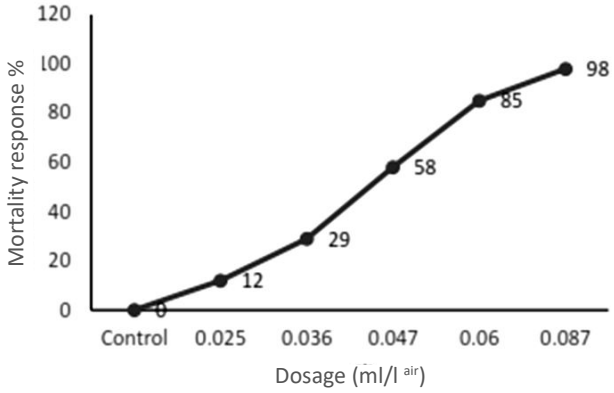

Figure 2. Mortality effect by *Syzygium aromaticum* crude oil against *Callosobruchus maculatus* adults.

Table 2. Volume of *n*-hexane, ethyl acetate and methanol fractions

Essential oil	Fraction volume		
	n-hexane (%)	Ethyl acetate (%)	Methanol (%)
Mentha piperita	69.10	31.36	0
Syzygium aromaticum	64.84	36.28	0

Table 3. Toxicity of *n*-hexane and ethyl acetate fractions against *Callosobruchus maculatus*

Essential oils	Fraction	Equivalent dose (ml/l^{air})	Mortality % ± SD
Mentha piperita	*n*-hexane	0.043	91.00 ± 0.83
	ethyl acetate	0.019	4.00 ± 1.09
Syzygium aromaticum	*n*-hexane	0.051	95.00 ± 1.41
	ethyl acetate	0.027	6.00 ± 1.09

(Table 4). Highest mortality 97% was found at 0.05 ml/l for *M.piperita n*-hexane fraction oil while *S.aromaticum n*-hexane reached to 97% mortality at 0.06 ml/l (Figure 3 and Figure 4 respectively).

Oviposition deterrence activity

For the *M. piperita* crude essential oil, the highest oviposition deterrence (71.73%) occurred with the 0.037 ml/l dose; the second highest deterrence (55.47%) occurred with the 0.025 ml/l dose. Although there is no significant difference between the two lowest doses 0.017 ml/l and 0.01 ml/l, their deterrence percentages (40.17% and 30.6%) are still significantly higher than the control. Mnf showed 64.40% deterrence at 0.028 ml/l followed by 54.37% deterrence at 0.019 ml/l, 38.87% at 0.013 ml/l and the lowest deterrence of 24.55% found at 0.007 ml/l (Table 5). ED_{50} and ED_{95} values of crude oil for oviposition deterrence were 0.02 and 0.14 ml/l, respectively, while Mnf showed 0.018 ml/l and 0.098 ml/l, respectively (Table 6).

For the *S. aromaticum* crude oil, oviposition deterrence was again dose dependent with the highest deterrence (85.73%) at the 0.06 ml/l dose followed by 0.047 ml/l and 0.036 ml/l causing 71.2% and 51.46% deterrence, respectively. The lowest deterrence (40.71%) was found at 0.025 ml/l. Snf showed the highest deterrence 83.47% at 0.04 ml/l followed by 0.03 ml/l with 64.71% while 0.023 ml/l and 0.015 ml/l showed lower deterrence of 48.88% and 30.25%, respectively (Table 5). ED_{50} and ED_{95} values of crude oil were 0.032 ml/l and 0.095 ml/l, respectively while Snf values were 0.022 ml/l and 0.067 ml/l, respectively (Table 6).

Ovicidal effect of crude essential oils and active fractions on *C. maculatus*

For the *M. piperita* crude essential oil, 0.037 ml/l showed a hatching inhibition rate (HIR) of 86.49% while the lowest HIR (17.83%) was found at 0.01 ml/l. The HIR increased with increasing dose, 0.017 ml/l caused 36.87% HIR while 0.025 ml/l showed a moderate HIR of 56.82%. Mnf oil 0.028 ml/l caused 88.17% HIR followed by 70.36% at 0.019 ml/l. Although there was no significant difference between 0.007 ml/l and 0.013 ml/l doses, their HIR were still high (34.07% and 38.35%, respectively; Table 7). ED_{50} and ED_{95} of Mnf oils were 0.014 ml/l and 0.038 ml/l, respectively while crude oil showed 0.02 ml/l and 0.052 ml/l, respectively (Table 8).

S. aromaticum crude oil demonstrated the highest HIR (98.88%) at 0.06 ml/l followed by 95% HIR at 0.047 ml/l and 72.07% HIR at 0.036

Table 4. Lethal dose values of essential oil active fractions against *Callosobruchus macultus*

Essential oil	Slope ± SE	LD_{50} (CI 95%) (ml/l^{air})	LD_{95} (CI 95%) (ml/l^{air})
Mentha piperita (*n*-hexane)	4.34 ± 0.32	0.018 (0.017–0.019)	0.045 (0.038–0.065)
Syzygium aromaticum (*n*-hexane)	5.62 ± 0.39	0.029 (0.027–0.032)	0.057 (0.050–0.072)

ml/l^{air}: ml/l fumigation chamber; CI: confdent interval; SE: standard error.

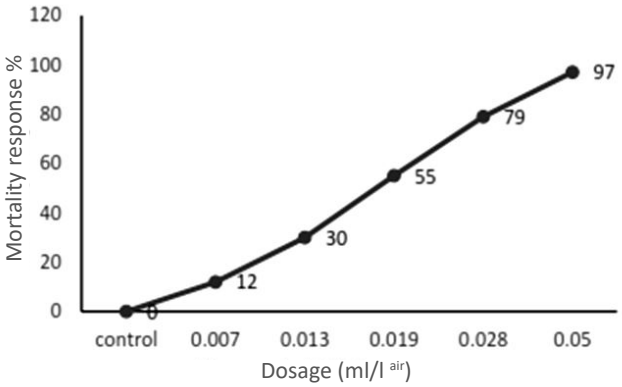

Figure 3. Mortality effect by *Mentha piperita n*-hexane fraction oil against *Callosobruchus maculatus* adults.

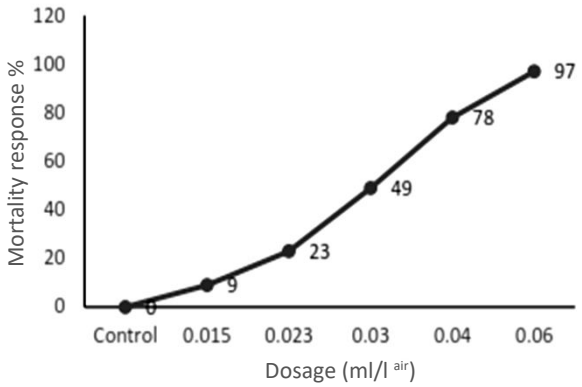

Figure 4. Mortality effect by *Syzygium aromaticum n*-hexane fraction oil against *Callosobruchus maculatus* adults.

Table 5. Oviposition deterrence effect of essential oils against *Callosobruchus maculatus*

Essential oil	Dose (ml/l^{air})	Average number of egg laid/ female	Oviposition deterrence % ± SD
Mentha piperita (crude)	0.000	57.00 ± 6.51	0.00 ± 0.00 c
	0.010	39.00 ± 2.54	30.60 ± 11.16 b
	0.017	33.40 ± 10.45	40.17 ± 22.77 b
	0.025	25.60 ± 12.23	55.47 ± 18.86 ab
	0.037	15.80 ± 1.78	71.73 ± 6.17 a
M. piperita *n*-hexane	0.000	54.80 ± 4.76	0.00 ± 0.00 d
	0.007	41.00 ± 4.35	24.55 ± 12.16 c
	0.013	33.60 ± 6.34	38.87 ± 8.32 b
	0.019	24.80 ± 2.19	54.37 ± 7.14 a
	0.028	19.40 ± 2.19	64.40 ± 4.90 a
Syzygium aromaticum (crude)	0.000	53.40 ± 5.17	0.00 ± 0.00 d
	0.025	31.60 ± 4.15	40.71 ± 6.75 c
	0.036	25.40 ± 5.54	51.46 ± 15.10 bc
	0.047	15.40 ± 6.10	71.20 ± 10.38 ab
	0.060	7.80 ± 5.76	85.73 ± 10.08 a
S. aromaticum *n*-hexane	0.000	57.40 ± 7.40	0.00 ± 0.00 d
	0.015	38.80 ± 10.44	30.25 ± 24.02 c
	0.023	28.60 ± 4.15	48.88 ± 12.97 bc
	0.030	19.80 ± 7.66	64.71 ± 15.36 ab
	0.040	9.40 ± 1.51	83.47 ± 3.02 a

Table 6. Oviposition deterrence effective dose (ED_{50} and ED_{95}) of different types of essential oils against *Callosobruchus maculatus*

Essential oils	Slope ± SE	ED_{50} (CI 95%)	ED_{95} (CI 95%) ml/l^{air}
Mentha piperita (crude oil)	1.94 ± 0.24	0.020 (0.013–0.027)	0.140 (0.060–0.380)
M. piperita (*n*-hexane)	2.24 ± 0.29	0.018 (0.016–0.020)	0.098 (0.068–0.177)
Syzygium aromatiocum (crude oil)	3.44 ± 0.36	0.032 (0.016–0.039)	0.095 (0.065–0.642)
S. aromaticum (*n*-hexane)	3.38 ± 0.32	0.022 (0.016–0.026)	0.067 (0.048–0.180)

ml/l^{air} : ml/l fumigation chamber; CI: confident Interval; SE: standard error.

ml/l. The lowest HIR (39.67%) was found at 0.025 ml/l. Snf oil reached 100% HIR at 0.04 ml/l followed by 93.62% at 0.03 ml/l, 89.04% at 0.023 ml/l. The lowest HIR (56.79%) at 0.015 ml/l is still sufficient enough to control the insect compared to control (Table 7). ED_{50} and ED_{95} values were 0.028 ml/l and 0.049 ml/l for crude oil and 0.014 and 0.029 ml/l for Snf (Table 8).

Chemical component identification active fraction oils

According to the GC-MS analysis, there were 8 dominant compounds was found in *M. piperita* *n*-hexane fraction and 5 dominant compounds in *S. aromaticum* *n*-hexane fraction (Table 9).

DISCUSSION

Crude essential oils toxicity assay

M. piperita oil has lower LD_{50} and LD_{95} values compared to *S. aromaticum* oil. It is thought to be due to the differing vapor pressures of these compounds, Baker et al. (2018) stated that *M. piperita's* vapor pressure is 0.3 mm Hg at 25 °C while *S. aromaticum's* is 0.03 mm Hg at 25 °C. Essential oils with high vapor pressures are more likely to get into the air faster than oils with a lower vapor pressure and thus more easily affect an insect's respiratory system (Hanson et al. 2016). Toxicity of essential oils is due to compounds that impair the activity of neuronal enzymes such as acetylcholinesterase (AChE) (Isman 2006). The inhibition of AChE can cause insect death

Table 7. Ovicidal rates of different type of essential oils on *Callosobruchus maculatus* eggs

Essential oil	Dose (ml/l^{air})	Number of eggs	Hatched eggs ± SD	HIR % ± SD
Mentha piperita (crude)	0.000	20	18.80 ± 1.30	0.00 ± 0.00 d
	0.010	20	15.40 ± 1.14	17.83 ± 7.30 c
	0.017	20	11.80 ± 1.64	36.87 ± 10.43 bc
	0.025	20	8.00 ± 2.00	56.82 ± 12.88 b
	0.037	20	2.60 ± 2.07	86.49 ± 10.30 a
M. piperita (*n*-hexane)	0.000	20	18.80 ± 0.83	0.00 ± 0.00 d
	0.007	20	12.40 ± 1.94	34.07 ± 9.78 c
	0.013	20	11.60 ± 1.94	38.35 ± 9.63 c
	0.019	20	5.60 ± 1.14	70.36 ± 4.86 b
	0.028	20	2.20 ± 1.92	88.17 ± 10.63 a
Syzygiumaromaticum (crude)	0.000	20	19.00 ± 1.41	0.00 ± 0.00 d
	0.025	20	11.40 ± 2.07	39.67 ± 12.16 c
	0.036	20	5.20 ± 2.38	72.07 ± 14.85 b
	0.047	20	1.00 ± 1.00	95.00 ± 5.00 a
	0.060	20	0.20 ± 0.44	98.88 ± 2.48 a
S. aromaticum (*n*-hexane)	0.000	20	18.20 ± 1.30	0.00 ± 0.00 d
	0.015	20	8.00 ± 4.06	56.79 ± 21.57 c
	0.023	20	2.00 ± 1.22	89.04 ± 6.74 b
	0.030	20	1.20 ± 1.64	93.62 ± 8.64 ab
	0.040	20	0.00 ± 0.00	100.00 ± 0.00 a

Table 8. Effective ovicidal dose (ED_{50} and ED_{95}) values of essential oils on *Callosobruchus maculatus*

Essential oils	Slope ± SE	ED_{50} (CI 95%) ml/l^{air}	ED_{95} (CI 95%) ml/l^{air}
Mentha piperita (crude)	3.92 ± 0.35	0.02 (0.015–0.025)	0.052 (0.038–0.100)
M. piperita (*n*-hexane)	3.78 ± 0.45	0.014 (0.010–0.018)	0.038 (0.026–0.160)
Syzygium aromatiocum (crude)	6.60 ± 0.70	0.028 (0.026–0.030)	0.049 (0.045–0.093)
S. aromaticum (*n*-hexane)	5.23 ± 0.70	0.014 (0.007–0.017)	0.029 (0.023–0.059)

ml/l^{air} : ml/l fumigation chamber; CI: confdent interval; SE: standard error.

Table 9. Chemical component analysis by GC-MS

Essential oil	Retention time	Chemical compound	Content percent (%)
Peppermint (*n*-hexane)	6.43	alpha-Pinene	4.47
	8.55	beta-Pinene	3.78
	11.65	Limonene	5.70
	24.59	Isomenthone	20.12
	30.19	Carane	6.17
	31.78	Dl-menthol	5.22
	34.12	Menthol	43.29
	38.54	Piperitone	1.42
Clove (*n*-hexane)	6.43	Alpha-Pinene	11.11
	32.34	Caryophyllene	17.76
	36.17	Humulene	1.94
	49.07	Caryophyllene oxide	1.13
	51.74	Eugenol	62.98

because it regulates the level of acetylcholine and terminates nerve impulses by catalyzing the hydrolysis of acetylcholine (Jankowska et al. 2018). Our observations of insects that survived the treatments indicated they were in a knockdown state and showed non-active or weak responses to touch when compared with surviving insects in the controls.

Toxicity of essential oil fractions

Fractionation of essential oils showed that both oils contain more non-polar compounds than semi-polar compounds when the *n*-hexane fraction volume is higher than ethyl acetate fraction volume. Toxicity tests also showed that mortality from *n*-hexane fractions were higher than ethyl acetate fractions. This increased effectiveness of *n*-hexane fractions seems to be due their highly volatile compounds which evaporate quickly and penetrate the insect's respiratory system during fumigation (Wiley 2014). Therefore, *n*-hexane was chosen as the active fraction after confirming its higher toxicity. LD_{50} and LD_{95} doses of the *n*-hexane fraction oil were also lower than crude essential oils on *C. maculatus*. This could be due to non-toxic chemical compounds in crude oils that dilute the effect of the toxic compounds.

Oviposition deterrence activity

Oviposition must occur before any larvae hatch and feed on the mung beans. If oviposition is prevented, it will disrupt the life cycle and population growth. Application of essential oils showed that both crude and fractionated oils can inhibit the oviposition activity of *C. maculatus* adults. The LD_{50} and LD_{95} doses are lower in *n*-hexane fractions than in the crude oils.

Singh & Pandey (2018) suggested that reductions in oviposition could be due to death of the adult females when they contact treated beans or the failure to lay eggs. Valsala & Gokuldas (2015) also mentioned that oviposition was probably regulated by the volatile compounds absorbed through the cuticle of the beans. According to Kumar et al. (2009), changes in physiology or behavior of females, following contact with essential oil compounds, may diminish their egg laying capacity. These products that cause oviposition deterrence are called behavior altering chemicals or semiochemicals and could involve ovarian changes similar to chemo-sterilizants by blocking females eggs laying (Kedia et al. 2015).

Ovicidal effect of crude essential oils and active fractions on *C. maculatus*

The present study concluded that all the tested essential oils have high egg hatching inhibition rates (HIR) although ED_{50} and ED_{95} doses of fractionated oils were lower than crude versions of both essential oil. Ovicidal effects and the occurrence of morphologically abnormal eggs may be due to toxic substances in the essential oil that enter eggs through the chorion and suppress embryonic development (Raja et al. 2001). Similar results were reported with eggs that were surface-treated with plant product essential oils allowing chemicals to penetrate the chorion (through the micropyle) leading to death (Singh & Pandey 2018). Kumar et al. (2012) also reported that ovicidal effects are due to inhibitory compounds such as phenols, flavonoids, alkaloids, tannin, saponin, glycosides, steroid, and phytosterol contained in plant essential oils. According to Singh & Pandey (2018), failure of egg hatching by *M. piperita* can occur when essential oils cause alterations in oxygen levels or surface tension within eggs.

Therefore, in general the ovicidal properties of essential oils are due to their ability to penetrate eggs and disrupt embryonic development. Ovicidal effects differ depending on the age of eggs, the ability of a toxicant reaching the target site, the length of exposure and type of essential oil, and differences in the rate of uptake or penetration of eggs.

CONCLUSION

The *n*-hexane fractions of both essential oils have insecticidal, oviposition deterrence, and hatching inhibition activities against *C. maculatus*. The *n*-hexane fraction oils are more toxic than the crude oils as both lethal and effective doses were lower than for the crude oils. There were 8 dominant compounds in Mnf oil and 5 dominant compounds in Snf oil. The *n*-hexane fractions from either source could be used as alternative insecticide to control stored pest *C. maculatus*.

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REFERENCES

Baker BP, Grant JA, Kuenen RM. 2018. Peppermint and peppermint oil profile. *Integrated Pest Management Program*. New York: Cornell Cooperative Extension. Available at: http://hdl.handle.net/1813/56135. [2 Juni 2020].

Brari J, Thakur DR. 2017. Bioefficacy of four essential oils against *Callosobruchus maculatus* (F.) (Coleoptera:Bruchidae), A seed pest of stored legumes world wide. *International Journal of Entomology Research* 2:71–75.

Chaubey MK. 2008. Fumigant toxicity of essential oils from some common spices against pulse beetle, *Callosobruchus chinensis* (Coleoptera: Bruchidae). *Journal of Oleo Science* 57:171–179. doi: https://doi.org/10.5650/jos.57.171.

Dadang. 1999. *Insect Regulatory Activity and Active Subtances of Indonesian Plants Particularly to the Diamondback Moth*. PhD Thesis. Tokyo: Tokyo University of Agriculture.

Hanson B, Bond C, Buhl K. 2016. *Pesticide Vapor Pressure Fact Sheet*; National Pesticide Information Center, Oregon State University Extension Services. Available at: http://npic.orst.edu/factsheets/vaporpressure.html. [2 Juni 2020].

Hassan NMM 2012. Determination of biochemical changes during storage of cowpea infested with the cowpea weevil (*Callosobruchus maculatus* F.) *Middle East Journal of Applied Sciences* 2: 66–70.

Isman MB. 2006. Botanical insecticides, deterrents, and repellents in modern agriculture and anincreasingly regulated world. *Annual Review of Entomology* 51:45–66. doi: https://doi.org/10.1146/annurev.ento.51.110104.151146.

Jankowska M, Rogalska J, Wuszkowska J, Stankiewicz M. 2018. Molecular targets for components of essential oils in the insect nervous system. A Review. *PMC US National Library of Medicine* 23:34. doi: https://doi.org/10.3390/molecules23010034.

Kedia A, Prakash B, Mishra PK, Singh P, Dubey NK. 2015. Botanicals as eco friendly biorational alternatives of synthetic pesticides against *Callosobruchus* spp. (Coleoptera: Bruchidae) – a review. *Journal of Food Science and Technology* 52:1239–1257. doi: https://doi.org/10.1007/s13197-013-1167-8.

Kumar A, Shukla R, Singh P, Singh AK, Dubey NK. 2009. Use of essential oil from *Mentha arvensis* L. to control storage moulds and insects in stored chickpea. *Journal of the Science of Food and Agriculture* 89:2643–2649. doi: https://doi.org/10.1002/jsfa.3768.

Kumar G, Karthik L, Rao KVB, Kirthi AV, Rahuman AA. 2012. Larvicidal, repellent and ovicidal activity of *Calotropis gigantean* against *Culex gelidus*, *Culex tritaeniorhynchus* (Diptera: Culicidae). *Journal of Agricultural Technology* 8:869–880.

Parwata IMOA, Rita WS, Yoga R. 2009. Isolasi dan uji antiradikal bebas minyak atsiri pada daun sirih (*piper betle* linn) secara spektroskopi ultra violet-tampak. *Jurnal Kimia* 3:7–13.

Raja N, Albert S, Ignacimuthu S. 2001. Effect of plant volatile oils in protecting stored cowpea *Vigna unguiculata* L. against *Callosobruchus maculatus*. *Journal of Stored Products Research* 37:127–132. doi: https://doi.org/10.1016/S0022-474X(00)00014-X.

Singh P, Pandey AK. 2018. Prospective of essential oils of the genus *Mentha* as biopesticides: A Review. *Frontiers in Plant Science* 9:1295. doi: https://doi.org/10.3389/fpls.2018.01295.

Tembo L, Pungulani L, Sohati PH, Mataa JC, Munyinda K. 2017. Resistance to *Callosobruchus maculatus* developed via gamma radiation in cowpea. *Journal of Agriculture and Crops* 3:65–71.

Valsala KK, Gokuldas MV 2015. Repellent and oviposition deterrence effects of *Clerodendrum infortunatum* on the pulse beetle *Callosobruchus chinensis* L. (Coleoptera: Bruchidae). *Journal of Entomology Studies* 3:250–253.

Wiley J. 2014. Very volatile organic compounds: An understudied class of indoor air pollutants. *Indoor Air* 26:25–38. doi: https://doi.org/10.1111/ina.12173.