THE EFFECT OF ESSENTIAL OIL MIXTURES ON MORTALITIES AND OVIPOSITION DETERRENTS OF *Crocidolomia pavonana* AND *Helopeltis antonii*

Pengaruh Campuran Minyak Atsiri terhadap Mortalitas dan Penghambatan Peneluran Crocidolomia pavonana *dan* Helopeltis antonii

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ABSTRACT

Essential oil, such as citronella, lemongrass, ageratum, and clove are known to be toxic and repel certain pests. Mixing two or more essential oils are expected increasing their insecticidal properties. The experiments aimed to examine the effect of essential oils mixtures (EOs) on mortalities and oviposition deterrents of *Crocidolomia pavonana* and *Helopeltis antonii*. The experiments were conducted at the laboratory and green house of the Indonesian Spices and Medicinal Crops Research Institute. The tested mixtures were citronella and clove (CiC); lemongrass and clove (LC); ageratum and clove (AC); individual EO; control 1 (water + emulsifier); control 2 (water) at 0.5% concentration for all treatments. The ratio used were 1:1, 1:2, 1:4, 2:1 and 4:1. Parameters observed were the mortalities of *C. pavonana* larvae and *H. antonii* nymphs; and the ovipositions deterrents of both insects. The CiC and LC mixtures gave low mortality (<50%) to *C. pavonana* larvae, while AC at ratios 1:1, 1:2,1:4 less than 20%, AC 2:1 and 4:1 >60% and the individual ageratum oil caused 77.5% mortalities. The CiC 1:1 and LC (1:1, 1:2, and 4:1) showed >40% mortalities of *H. antonii, whereas* the AC in all ratios enhanced the nymph mortalities. The number of eggs laid by *C. pavonana* at CiC and LC in all ratios were around 50-60% lower than individual EO. The mixture of AC 1:4 and 4:1 produced eggs nearly 50% lower than clove treatment. The CiC and LC at 1:1 produced the lowest numbers of egg laid by *H. antonii*, while AC 1:4 reduced 70% eggs laid compared to individual EO.

Key words: *Crocidolomia pavonana, Helopeltis antonii*, mixtures ratio

ABSTRAK

Minyak atsiri (MA) bersifat toksik dan repelen terhadap hama tertentu. Pencampuran dua atau lebih MA dapat meningkatkan daya kerja sifat insektisidanya. Percobaan bertujuan untuk mengetahui pengaruh campuran MA terhadap mortalitas dan penghambatan peneluran Crocidolomia pavonana *dan* Helopeltis antonii*. Campuran yang diuji adalah minyak serai wangi dan cengkeh (SWCK), minyak serai dapur dan cengkeh (SDCK); minyak babadotan dan cengkeh (BBCK); MA tunggal; kontrol 1 (air + emulsifier), kontrol 2 (air). Rasio campuran yang diuji adalah 1:1, 1:2, 1:4, 2:1 dan 4:1. Parameter yang diamati adalah mortalitas larva* C. pavonana *dan nimfa* H. antonii *serta penghambatan peneluran pada kedua serangga. Campuran SWCK dan SDCK memberikan mortalitas ulat* C. pavonana *<20%, tidak berbeda nyata dengan mortalitas pada perlakuan MA tunggal. Pengujian dengan campuran BBCK juga tidak menunjukkan mortalitas yang tinggi, mortalitas tertinggi diperoleh pada perlakuan minyak babadotan. Campuran SWCK 1:1 dan SDCK (1:1, 1:2, dan 4:1) memberikan mortalitas tinggi pada nimfa H. antonii dan berbeda nyata dengan hasil pengujian MA tunggal. Pengujian terhadap penghambatan peneluran* C. pavonana *menunjukkan campuran SWCK dan SDCK pada semua perbandingan menurunkan jumlah telur 50-60% dari jumlah telur pada perlakuan MA tunggal. Pada pengujian terhadap* H. antonii*, campuran SWCK dan SDCK 1:1 mengurangi jumlah telur yang dihasilkan 50% dari perlakuan MA tunggal. Campuran BBCK 1:4 menurunkan jumlah telur 70% dari jumlah telur pada perlakuan MA tunggal.*

Kata kunci: Crocidolomia pavonana*,* Helopeltis antonii*, rasio campuran*

INTRODUCTION

Crocidolomia pavonana is one of the important insect pests of Cruciferae plants. They often cause heavily damage on the plants especially in the dry season. Approximately 70% of cabbage farmers spent 25-30% of their input cost for pesticides (Dadang and Prijono 2010). On the other hand, *Helopeltis antonii* is the most important pest of cashew. The damages caused by *H. antonii* could reach 60% and higher due to plant pathogen infection following their attack. The injures as a result of *H. antonii* punctures will ease infection of *Pestalotiopsis* sp. fungi (Karmawati and Mardiningsih 2005). The unavailability of food resources during dry season will decrease and could eliminate *H. antonii* population (Siswanto *et al*. 2008).

To minimize adverse effect of synthetic insecticides, the use of botanical insecticides have been performed to control *C. pavonana* in the field. The use of *Piper retrofractum* and *Annona squamosa* (RS) extract mixtures and *Aglaia odorata* and *A*. *squamosa* (OS) extract mixtures at 0.1% were effective against *C. pavonana* larvae. Fortunately, these formulations indicated no effect on the presence of both parasitoids, *Diadegma semiclausum* and *Eriborus argentiopilosus*. The application of these formulation could reduce cabbage damage compared to deltamethrin treatment. Moreover, treatments with RS 0.1% produced the highest cabbage yield (Dadang *et al*. 2009).

The botanical pesticide to control *Helopeltis* spp. were easy to be applied by farmers and environmentally friendly (Karmawati 2010). Willis *et al*. (2013b) reported neem oil and the formulation of citronella oil + clove oil + jatropha oil significantly reduced damage intensity of *Helopeltis* sp.

Some essential oils (EOs) have been recognized as an important natural source of pesticides (Koul *et al*. 2008). EOs acted to alter insect feeding behavior, growth and development, ecdysis (molting) and behavior during mating and oviposition (Khater 2012).

EOs such as lemongrass, eucalyptus, rosemary, vetiver, clove, citronella, and thyme are known for their pest control properties (Koul *et al*. 2008). The application of citronella oil on chili pepper plants could inhibit egg laying and hatching of *Heliothis armigera* in laboratory. Futhermore, the application of citronella oil in the field reduced fruit damage of chili pepper as well as increased the yield (Setiawati *et al*. 2011). Citronella oil at 5 $ml.l^{-1}$ also was able to hamper the level of *Dasynus piperis* attack on pepper plantation in Bangka. The result of the pepper yield loss was equivalent to synthetic organophosphate insecticide (Rohimatun and Laba 2013). The other research revealed that spraying ginger plants with neem oil and citronella oil, could suppress populations of *Mimegralla coeruleifrons* 90 and 60% respectively (Balfas *et al*. 2013). The oil of citronella, lemongrass, ageratum, Java turmeric, and neem caused mortalities and oviposition deterrent to *C. pavonana* (Balfas and Mardiningsih 2016). Beside causing mortalities, EOs also can act as insect repellent such as clove extract on *Lophobaris piperis* (Wiratno 2008). In addition, eugenol in clove oil has repellent activity and fumigant action against *Tribolium castaneum* (Abo-El-Saad *et al*. 2011).

Currently, commercial plant protection products comprising proprietary mixtures of terpenes as active ingredient are available (Isman 2000). Creating the synergistic EOs combination is to reduce the dose of potentially polluting substances and the risk of resistance development (Tripathi *et al*. 2009). In addition, compared to individual usage, mixing the plant material in pest control can reduce the dependence on one plant material usage (Dadang and Prijono 2010). Moreover, a synergistic phenomenon among metabolites of EOs may result in higher biological activity, as minor constituents in low percentages may act as a synergists, enhancing the effectiveness of the major constituents through

variety of mechanisms (Barembaum 1985 *in* Khater 2012). EOs combination such as thyme, anise, and saffron have been demonstrated for its synergistic activity (Yasser 1997 *in* Tripathi *et al*. 2009).

A mixture of citronella, clove, and neem oil applied on cocoa plantations showed less yield loss caused by cocoa pod borer than individual EO (Willis *et al*. 2013a). However, there is still limited information or supporting data associated with the use of EOs as a mixture and their biological activities on arthropod insects. As a mixture, the ratio between the plant extract is very important to be examined to obtain its synergistic effect, effectiveness and efficiency in controlling pest (Dadang and Prijono 2010). In the previous study, ageratum, citronella, clove, and lemongrass indicated toxic and possed oviposition deterrent effect on *C. pavonana* (Balfas and Mardiningsih 2016). Mixing two of them in certain ratio were expected to provide higher biological action. The present study aimed to evaluate the potential of EOs mixture (ageratum, citronella, clove and lemongrass) in various ratios on the mortalities and oviposition deterrent of *C. pavonana* and *H. antonii*.

MATERIALS AND METHODS

Plant materials

The origin and distillation process of ageratum, citronella, clove, and lemongrass used in this experiment were described in Table 1. The EOs from these plants were extracted using steam distillation (Balittro 2008). The chemical contents of each EOs were analyzed with GC-MS at The Health Laboratory of Jakarta.

Essential oil mixture

Two different EOs were mixed, citronella and clove (CiC), lemongrass and clove (LC), and ageratum and clove (AC). Individual EOs (ageratum, citronella, clove, lemongrass), water + emulsifier and water were used as control. Stock solutions were prepared by mixing the two EOs at the ratio of 1:1, 1:2, 1:4, 2:1, 4:1, and single EOs. Both the mixtures or single solution were added with commercial emulsifier, wetting agent, and thickener containing alkyl glycerol ftalat 750g.l^{-1}. In every 5 ml EOs was added 400 µl alkyl glycerol ftalat (recommended dose). In all treatments, the concentration of the oil was tested at 0.5% by mixing 50 µl stock solution in 10 ml distilled water.

Preparation of targetted plants and insects

C. pavonana

Broccoli was used to feed the insects. The seeds were sown on growing media and transplanted into polybags after one month. The leaves were ready to feed insects test (*C. pavonana* larvae) at 2-3 months after transplanting. *C. pavonana* larvae were collected from broccoli plantation at Cisarua, Bogor and mass-reared in the green house of Indonesian Spice and Medicinal Research Institute (ISMCRI). The emerging adults were transferred into cages containing broccoli plants. The eggs were collected and put into small boxes. The newly emerging larvae were fed on broccoli leaves. Three-day-old larvae were used in mortality tests, while the three day-old adults were used for oviposition deterrent tests.

Table 1. Plant materials used in the study.
Tabel 1. Bahan tanaman yang digunakan dalam penelitian.

Plant material	Origin and distillation process
Clove	Leaves were obtained from Leuwiliang and distilled at The Testing Laboratory of Indonesian Spices and Medicinal Crops Research (ISMCRI), Bogor
Citronella	The leaves and stems were distilled at Manoko Research Installation, Lembang, Bandung
Lemongrass	Leaves and stems were distilled at Manoko Research Installation, Lembang, Bandung
Ageratum	Leaves, branches, and flowers were originated from Cimanggu Research Installation, Bogor and distilled at The Testing Laboratory of ISMCRI, Bogor

H. antonii

Nymph and adults of *H*. *antonii* were collected from tea plantation of PTPN VIII, Gedeh, Cianjur, West Java. They were mass-reared at the green house of ISMCRI. Cucumbers bought from Pasar Anyar market, Bogor, were washed and dried, then put in plastic container. The collected *H*. *antonii* were transferred into the container and fed on the cucumber. The cucumber were replaced with the fresh ones every three days. The three-days old nymphs were used in mortality tests, while the emerging adults were placed in one container and the three-day old ones were used in oviposition detterent tests.

The effect of EOs on mortalities of *C. pavonana* larvae and *H. antonii* nymphs

The experiments were arranged in completely randomized block designs (CRBD) with nine treatments and repeated four times. Broccoli leaves were cut 7 cm x 7 cm, then dipped in 0.5 ml solution (5 ml EOs stock solutions in 1.000 ml aquadest). The treated leaves were placed on a tray and drained up from the solution. The three-day old larvae of *C. pavonana* were placed in a small box (10 larvae/box) containing one treated leaf. The larvae mortality was observed at the first, second, and third days after treatment.

The 3/4 instar nymphs of *H. antonii* were placed in a small box with 14 cm in diameter (10 nymphs/box) and directly sprayed with the tested solution and then transferred into the container containing one cucumber and covered with cloth. The observations of nymph mortality was performed at the first, second, and third days after treatment.

The effect of EOs on oviposition deterrent

The experiments were also arranged in randomized complete block designs (RCBD) with with nine treatments and replicated four times.

C. pavonana

A total of 5-10 pairs of adult insects were put, using a plastic bootle, into a net cage containing broccoli plant to encourage the adult mating for three days. Five plants were sprayed using a small sprayer (2.3 cm in diameter and 8 cm in height) with EOs solution, then placed in the net cage contained three-days old insect. Three pairs of three-days old adult were put into the cage. The number of eggs laid by the adults was observed for eight days.

H. antonii

Newly emerged adults were paired in a container (14 cm in diameter and 13 cm in height). Cucumber was sprayed with the tested solution and dried up, then placed in a container. The three-day old adults were placed in it. The observations were done on the number of eggs laid by the adults.

Data were analyzed with analysis of variance (ANOVA) using SAS System and further analysis was completed with Duncan's multiple range test (DMRT) at 0.05 level. The effective repellency (ER) for each EOs was calculated with formula as follow (Setiawati *et al*. 2011).

$$ER\ (\%) = \frac{NC - NT}{NC} \times 100\%$$

Note/*Keterangan* :

ER = percent effective repellency/*persen effectif repellency.*

NC = numbers of egg in control (+ emulsifier)/ *jumlah telur pada kontrol (+ emulsifier).*

NT = numbers of egg in treatment/*jumlah telur dalam perawatan.*

RESULTS AND DISCUSSION

Chemical compositions of the tested essential oils

The chemical components (the content >1%) of EOs used in these studies were presented in Table 2. The ageratum oil contained 31 components, the major components were procene II (40.05%), trimethyl-8-methylene (20.23%) and demethoxyageratochromene (18.64%). The chemical composition of ageratum oil in this study was different from previous study in China. Liu and Liu (2014) reported there were 32 major com-

Table 2. Main chemical content of the essential oils used in the study.
Tabel 2. Kandungan kimia utama dari minyak atsiri yang digunakan dalam penelitian.

Essential oils	The chemical components more than 1% content
Ageratum	Trimethyl-8-methylene(20.23%), demethoxyageratochromene (18.64%). procene II (40.05%), beta selinene (1.58%), z-beta farnesene (2.3%), germacrene (1.01%), lepidozene (1.66%), alpha-farnesene (1.41%), 6-methylene-1-cyclohexenr (2.97%), p-Allylguaiacol (1.21%), n-methylaniline (1.43%)
Citronella	citronellal (44.85%), citronellol (11.92%), trans-geraniol (11.04%), tetracyclo (6.12%), isopropyl linoleate (6.12%), limonene (3.47%), 1,6-octadien-3-ol (1.03%), Isopulegol (1.12%), cyclohexene (3.47%). 2.6-octadien (2.8%), geraniol acetate (2.40%), caryophyllene (1.77%), germacrene D (1.14%), delta-cadinene (1.30%), octadecadienoic acid (2.55%), linolsaeure (2.41%), naphthalenone (1.33%), Farnesol isomer A (1.40%)
Clove	Eugenol (77.54%), beta-caryophyllene (18.38%), alpha- caryophyllen (2.18%), beta-caryophyllene epoxide (1.15%)
Lemongrass	1.6-octadiene (7.24%), citronellal (6.85%), cis-citral (20.74%). transcitral (28.58%). citronellol (5.69%), octadien-1-ol (7.91%), 5-hepten-2-one (4.03%), trimethyl-8-methylenebicyclo (2.98%), delta-cadinene 1.95%), dimethyl -2,6-octadienyl acetate (1.72%)

ponents in ageratum oil, the main components were precocene II (45.75%), precocene I (14.09%), and caryophyllene (12.13%) followed by germacrene D (4.18%) and caryophyllene oxide (4.06%).

Citronella oil consisted of 39 components, the major components were citronellal (44.85%), citronellol (11.92%), trans-geraniol (11.04%). However, Setiawati *et al*. (2011) revealed citronella oil consisted of 34 components with the major components were citronella (35.97%), nerol (17.28%), and citronellol (10.03%). As for clove oil, it contained seven components with the main components were eugenol (77.54%). It was higher than other study by Bhuiyan *et al*. (2010) which reported the eugenol content of clove oil was 74.3%. The main components of lemongrass oil were essential oil and citral content (Tajidin *et al*. 2012).

The composition of EOs varied depend on the isolation method. Distillation may influence the composition of the isolated oil, because isomerization, saponification, and other reaction might occur under distillation conditions (Tripathi *et al*. 2009). In addition, the chemical profile of plant species could vary naturally depending on geographic, genetic, climatic, and annual or seasonal factors (Koul *et al*. 2008).

The effect EOs on mortality of *C. pavonana* larvae

The results of the experiment with CiC showed that all ratios of the mixture, as well as the individual EO, generated low larva mortalities (less than 21%) on *C. pavonana* larvae at the first, second, and third days after application (Table 3).

There were no significant differences among all the EO treatments. The similar results were also shown in the experiment with LC. These suggested that combination of citronella and clove as well as lemongrass and clove oils were unable to increase the mortalities of *C. pavonana* larvae (Table 4).

However, the previous result indicated high mortalities on *C. pavonana* larvae treated with citronella and lemongrass (Balfas and Mardiningsih 2016). This may be due to the difference in the sensivity of the insect test. Furthermore, the active ingredients content in the previous study was different from this study. There was also no information on the harvesting time of the plants. Tajidin *et al*. (2012) informed that plant maturity affected essential oil and citral contents of lemongrass.

However, the highest larva mortalities was generated by ageratum, followed by the AC at ratio of 4:1 and 2:1 (Table 5). This implied ageratum oil alone was toxic to the larvae and

mixing it with clove oil had no significant effect in increasing larvae mortalities. The previous results also showed high mortality effect of ageratum oil on *Spodoptera litura* larvae and *C. pavonana* larvae at 0.5% concentration (Balfas and Willis 2009; Balfas and Mardiningsih 2016). The application of ageratum oil at 0.5% concentration on maize leaf could control 70% *Spodoptera frugiperda* (Lima *et al*. 2010). The EO of ageratum aerial parts at the flowering stage was demonstrated possessed larvicidal activity against mosquito *Aedes albopictus*. Thus, it had potential as natural larvicide for mosquito control, although still required further evaluation for its safety to human and further research to evaluate its mode of action to enhance its effectiveness against the target species (Liu and Liu 2014). Ageratum oil was also known as fumigant against insect in grain storage, *Tribolium castaneum* Herbst (Jaya *et al*. 2014).

Table 3. Mortalities of *C. pavonana* larvae at different mixture ratios of citronella and clove oil at 0.5% in water-emulsifier (v/v).

Tabel 3. Mortalitas larva C. pavonana *pada beberapa perbandingan campuran minyak serai wangi dan minyak cengkeh dalam pengemulsi 0,5%* (v/v).

Treatments (ratio)	Mortalities (%), days after treatments		
	1	2	3
Citronella : clove (1:1)	5.0 ab	10.0 ab	10.0 ab
Citronella : clove 1:2	5.0 ab	10.0 ab	12.5 ab
Citronella : clove 1:4	7.5 ab	12.5 ab	15.0 a
Citronella : clove 2:1	5.0 ab	12.5 ab	15.0 a
Citronella : clove 4:1	5.0 ab	10.0 ab	12.5 ab
Citronella	7.5 ab	10.0 ab	20.0 a
Clove	15.0 a	20.0 a	20.0 a
Control (water + emulsifier)	0.0 b	0.0 b	2.5 b
Control (water)	2.5 b	2.5 b	2.5 b
CV (%)	17.7	21.3	41.8

Note: Numbers followed by the same letters at the same column were not significantly different at DMRT 5%

Keterangan: Angka-angka yang diikuti oleh huruf yang sama pada kolom yang sama tidak berbeda nyata pada taraf DMRT 5%.

Table 4. Mortalities of *C. pavonana* larvae at different mixture ratios of lemongrass and clove oil at 0.5% in water-emulsifier (v/v).

Tabel 4. Mortalitas larva C. pavonana *pada beberapa perbandingan campuran minyak serai dapur dan minyak cengkeh dalam pengemulsi 0,5%* (v/v).

Treatments	Mortalities (%), days after treatments		
	1	2	3
Lemongrass: clove 1:1	2.5 a	2.5 a	2.5 a
Lemongrass: clove 1:2	0.0 a	0.0 a	5.0 a
Lemongrass: clove 1:4	0.0 a	2.5 a	2.5 a
Lemongrass: clove 2:1	0.0 a	0.0 a	0.0 a
Lemongrass: clove 4:1	2.5 a	5.0 a	5.0 a
Lemongrass	5.0 a	5.0 a	7.5 a
Clove	0.0 a	2.5 a	2.5 a
Control (water + emulsifier)	0.0 a	0.0 a	0.0 a
Control (water)	2.5 a	2.5 a	2.5 a
CV (%)	11.5	15.8	15.8

Note: Numbers followed by the same letters at the same column were not significantly different at DMRT 5%.

Keterangan: Angka-angka yang diikuti oleh huruf yang sama pada kolom yang sama tidak berbeda nyata pada taraf DMRT 5%.

The effect EOs on the mortality of *H. antonii* nymphs

Unlike the results test on *C. pavonana* larvae, the mixing of two EOs gave significant effect in increasing the mortalities of *H. antonii* nymphs. The CiC mixture at 1:1 gave the highest mortalities, significantly higher than citronella and clove applied individually (Table 6). The application of LC at 1:1. 1:2 and 4:1 also performed higher mortalities than the individual oil application (Table 7), The result also was similar for AC mixtures day.

Effect on oviposition deterrent of *C. pavonana*

The numbers of egg laid by *C. pavonana* adults treated with mixtures of citronella and clove oil significantly different from other treatments. Effective repellency (ER) of the mixture was 49-60%, while the ER of individual citronella and clove oil were 25.9% and 15.3% res-

Table 5. Mortalities of *C. pavonana* larvae at different mixture ratios of ageratum and clove oil at 0.5% in water-emulsifier (v/v).

Tabel 5. Mortalitas larva C. pavonana *pada beberapa perbandingan campuran minyak babadotan dan minyak cengkeh dalam pengemulsi 0,5%* (v/v).

Treatments	Mortalities (%), days after treatment		
	1	2	3
Ageratum : clove 1:1	20.0 bc	27.5 b	32.5 b
Ageratum : clove 1:2	2.5 c	2.5 c	2.5 c
Ageratum : clove 1:4	10.0 c	10.0 c	12.5 bc
Ageratum : clove 2:1	37.5 ab	57.5 a	60.0 a
Ageratum : clove 4:1	30.0 ab	62.5 a	67.5 a
Ageratum	67.5 a	72.5 a	77.5 a
Clove	10.0 c	10.0 c	12.5 c
Control (water + emulsifier)	0.0 c	0.0 c	12.5 bc
Control (water)	2.5 c	2.5	2.5 c
CV (%)	26.4	21.1	20.4

Note: Numbers followed by the same letters at the same column were not significantly different at DMRT 5%.

Keterangan: Angka-angka yang diikuti oleh huruf yang sama pada kolom yang sama tidak berbeda nyata pada taraf DMRT 5%.

Table 6. Mortalities of *H. antonii* nymphs at different mixture ratios of citronella and clove oil at 0.5% in water-emulsifier (v/v).

Tabel 6. Mortalitas nimfa H. antonii *pada beberapa perbandingan campuran minyak serai wangi dan minyak cengkeh dalam pengemulsi 0,5%* (v/v).

Teatments	Mortalities (%), days after treatment		
	1	2	3
Citronella : clove (CiC)1:1	46.0 a	58.0 a	64.0 a
Citronella : clove (CiC)1:2	4.0 bc	6.0 de	6.0 ef
Citronella : clove (CiC) 1:4	14.0 abc	20.0 ab	20.0 bcd
Citronella : clove (CiC) 2:1	16.0 abc	24.0 bc	24.0 bc
Citronella : clove (CiC) 4:1	22.0 ab	30.0 ab	30.0 b
Citronella	2.0 c	10.0 cde	12.0 cde
Clove	12.0 bc	18.0 bcd	18.0 bcde
Control (water + emulsifier)	10.0 bc	12.0 e	12.0 cde
Control (water)	0.0 c	0.0 e	0.0 def
CV (%)	29.6	22.2	19.7

Note: Numbers followed by the same letters at the same column were not significantly different at DMRT 5%.

Keterangan: Angka-angka yang diikuti oleh huruf yang sama pada kolom yang sama tidak berbeda nyata pada taraf DMRT 5%.

Table 7. Mortalities of *H. antonii* nymphs at different mixture ratios of lemongrass and clove at 0.5% in water-emulsifier (v/v).

Tabel 7. Mortalitas nimfa H. antonii *pada beberapa perbandingan campuran minyak serai dapur dan minyak cengkeh dalam pengemulsi 0,5%* (v/v).

Treatments	Mortalities (%), days after treatment		
	1	2	3
Lemongrass : clove (LC) 1:1	45.0 a	47.5 a	47.5 a
Lemongrass : clove (LC) 1:2	37.5 ab	45.0 a	47.5 a
Lemongrass : clove (LC) 1:4	20.0 bc	20.0 b	22.5 b
Lemongrass : clove (LC) 2:1	22.5 bc	32.5 ab	35.0 ab
Lemongrass : clove (LC) 4:1	50.0 a	50.0 a	50.0 a
Lemongrass	15.0 c	37.5 ab	20.0 b
Clove	32.5 ab	22.5 b	37.5 ab
Control (water + emulsifier)	0.0 d	0.0 c	2.5 c
Control (water)	0.0 d	0.0 c	0.0 c
CV (%)	26.9	27.6	25.6

Note: Numbers followed by the same letters at the same column were not significantly different at DMRT 5%.
Keterangan: Angka-angka yang diikuti oleh huruf yang sama pada kolom yang sama tidak berbeda nyata pada taraf DMRT 5%.

Table 8. Mortalities of *H. antonii* nymphs at different mixture ratios of ageratum and clove oil 0.5% in water-emulsifier (v/v).

Tabel 8. Mortalitas nimfa H. antonii *pada beberapa perbandingan campuran minyak babadotan dan minyak cengkeh dalam pengemulsi 0,5%* (v/v).

Treatments	Mortalities (%), days after treatment		
	1	2	3
Ageratum : clove (AC) 1:1	66.0 a	66.0 c	66.0 b
Ageratum : clove (AC) 1:2	38.0 bc	48.0 ab	52.0 ab
Ageratum : clove (AC) 1:4	58.0 ab	62.0 bc	66.0 a
Ageratum : clove (AC) 2:1	36.0 bc	56.0 a	56.0 b
Ageratum : clove (AC) 4:1	54.0 ab	60.0 bc	62.0 ab
Ageratum	28.0c c	36.0 bc	38.0 b
Clove	22.0 bc	36.0 bc	38.0 b
Control (water + emulsifier)	0.0 d	0.0 c	0.0 b
Control (water)	0.0 d	0.0 c	0.0 b
CV (%)	21.4	19.5	10.6

Note: Numbers followed by the same letters at the same column were not significantly different at DMRT 5%.
Keterangan: Angka-angka yang diikuti oleh huruf yang sama pada kolom yang sama tidak berbeda nyata pada taraf DMRT 5%.

pectively (Table 9). The result was similar to lemon grass and clove treatment (Table 10). All mixtures of the two EOs produced the same response in reducing numbers of egg laid by the adults. The ER of the mixture EOs were 52–56%, while ER of individual lemongrass and clove oil were 25%. The numbers of egg laid resulted from individual EO were double the mixture ones. The mixture of ageratum and clove oil at 1:4 and 4:1 resulted in the lowest the numbers of egg laid (Table 11).

Table 9. Number of *C. pavonana* egg groups laid at different mixture ratios of citronella and clove oil at 0.5% in water-emulsifier (v/v);

Tabel 9. Jumlah kelompok telur yang diletakkan C. pavonana *pada beberapa perbandingan campuran minyak serai wangi dan minyak cengkeh dalam pengemulsi 0,5%* (v/v).

Treatments	Number of eggs laid (days after treatments)				ER* (%)
	2	4	6	8	
Citronella : clove 1:1	2.25 bc	6.00 d	8.50 d	8.75 d	58.8
Citronella : clove 1:2	1.25 c	4.75 d	7.50 d	8.50 d	60.0
Citronella : clove 1:4	2.75 bc	5.25 d	8.00 d	8.75 d	58.8
Citronella : clove 2:1	2.25 bc	6.75 cd	8.75 d	10.75 d	49.4
Citronella : clove 4:1	1.50 c	4.75 d	7.75 d	9.75 d	54.1
Citronella	3.50 b	9.75 b	13.75 c	15.75 c	25.9
Clove	2.50b bc	9.25 bc	15.00 bc	18.00 bc	15.3
Control water + emulsifier)	6.50 a	14.50 a	22.00 a	23.00 a	
Control (water)	6.25 a	13.50 a	19.25 ab	21.25 ab	
CV (%)	34.15	36.16	55.41	20.88	

Note: Numbers followed by the same letters at the same column were not significantly different at DMRT 5%.

Keterangan: Angka-angka yang diikuti oleh huruf yang sama pada kolom yang sama tidak berbeda nyata pada taraf DMRT 5%.

**ER =* percent effective repellency/*persentase efektivitas repelensi.*

Table 10. Number of *C. pavonana* egg groups laid at different mixture ratios of lemongrass and clove oil at 0.5% in water-emulsifier (v/v).

Tabel 10. Jumlah kelompok telur yang diletakkan C. pavonana *pada beberapa perbandingan campuran minyak serai dapur dan minyak cengkeh dalam pengemulsi 0,5%* (v/v).

Treatments	Number of egg laid (days after treatments)				ER* (%)
	2	4	6	8	
Lemongrass : clove 1:1	2.00 cd	5.50 c	8.75 c	9.50 c	46.50
Lemongrass : clove 1:2	0.75 d	4.75 c	7.75 c	8.35 c	53.00
Lemongrass : clove 1:4	0.75 d	4.00 c	6.75 c	7.50 c	57.80
Lemongrass : clove 2:1	1.00 d	4.50 c	8.25 c	8.50 c	52.10
Lemongrass : clove 4:1	1.00 d	3.50 c	6.50 c	7.75 c	56.30
Lemongrass	3.75 bc	8.75 b	12.00 b	13.25 b	25.35
Clove	3.00 bc	8.00 b	12.50 b	13.25 b	25.35
Control (water + emulsifier)	6.75 a	12.50 a	17.25 a	19.75 a	
Control (water)	4.50 b	9.50 b	15.25 a	17.75 a	
CV (%)	47.12	20.04	17.39	17.57	

Note: Numbers followed by the same letters at the same column were not significantly different at DMRT 5%.

Keterangan: Angka-angka yang diikuti oleh huruf yang sama pada kolom yang sama tidak berbeda nyata pada taraf DMRT 5%

**ER =* percent effective repellency/*persentase efektivitas repelensi.*

Table 11. Number of *C. pavonana* egg groups laid at different mixture ratios of ageratum and clove oil at 0.5% in water-emulsifier (v/v).

Tabel 11. Jumlah kelompok telur yang diletakkan C. pavonana *pada beberapa perbandingan campuran minyak babadotan dan minyak cengkeh dalam pengemulsi 0,5%* (v/v.)

Treatments	Numbers of eggs laid (days after treatments)				ER* (%)
	2	4	6	8	
Ageratum : clove 1:1	2.50 d	6.25 b	10.75 b	13.0 bc	40.2
Ageratum : clove 1:2	5.25 bc	9.25 b	13.00 b	15.50 b	28.7
Ageratum : clove 1:4	3.50 cd	6.50 b	10.00 b	11.00 c	49.4
Ageratum : clove 2:1	4.25 cd	8.25 b	11.50 b	13.00 bc	40.2
Ageratum : clove 4:1	2.50 d	6.75 b	9.75 b	11.50 c	47.1
Ageratum	4.50 bcd	9.50 b	12.75 b	16.00 b	26.4
Clove	5.75 abc	14.75 a	19.75 a	21.75 a	10.3
Control (water)	6.75 ab	13.75 a	18.25 a	22.00 a	
Control (water+emusifier	8.00 a	14.25 a	19.25 a	24.25 a	
CV (%)	31.29	21.04	18.67	13.89	

Note: Numbers followed by the same letters at the same column were not significantly different at DMRT 5%.

Keterangan: Angka-angka yang diikuti oleh huruf yang sama pada kolom yang sama tidak berbeda nyata pada taraf DMRT 5%.

**ER =* percent effective repellency/*persentase efektivitas repelensi.*

The effect of EOs on oviposition deterrent of *H. antonii*

The mixture of citronella and clove oil 1:1 gave the highest deterrent effect on the oviposition of *H. antonii*, indicated by its lowest number of egg laid. The ER was 63.3% and much higher than other EO treatments (Table 12). The lowest number of eggs laid also was presented by lemongrass and clove 1:1 although indicated no significant differences compared to the ratio of 1:4, 2:1, and 4:1 (Table 13). All ratio of ageratum and clove mixtures were able to reduce the number of the egg laid with ERs ranged 53-76%. However, the treatments of ageratum and clove individually only produced ER 21.7% and 26.3% respectively (Table 14).

These studies revealed that mixing the EOs could increase the mortalities of *H. antonii* nymphs nearly twice than individual EO. However, the EOs mixing generated low mortalities on *C. pavonana* larvae. Cloyd *et al*. (2009) stated that the commercially plant-derived essential oil products vary in their effectiveness against certain arthropod pests. In this study, the high mortalities occurred at one day after application. There were only few additional mortalities at the second and third day after treatments. The rapid action of EOs against some pests was the result of neurotoxic mode of action by blocking neuromodulator octamine (OA) receptors (Kostyukovky *et al*. 2002 *in* Koul *et al*. 2008; Rattan 2010). The OA was a target for essential oil activity in insects due to its key roles as a neurotransmitter, neuromodulator and neurohormone in invertebrates system (Evans 1981 *in* Rattan 2010). Interrupting the OA function resulted in total disruption of nervous system in insect (Tripathi *et al*. 2009). Furthermore, this insecticidal mechanism of EO occurred on eugenol and citronella (Rattan 2010).

The mixing of EOs also reduced the number of egg laid about 50% than individual EO. Thus, the use of EO mixtures gave better control than individual EO. The EO from leaves and stem of *Piper marginatum* exhibited significant oviposition deterrent effect at 50 and 100 ppm by reducing numbers of eggs laid (<50%) by *Aedes aegypti* in glass vessels compared to control solution (Autran *et al*. 2009). Those EO mixtures are promising alternatives for pest control to mini-

Table 12. Number of *H. antonii* eggs laid at different mixture ratios of citronella and clove oil at 0.5% in water-emulsifier (v/v).

Tabel 12. Jumlah telur yang diletakkan H. antonii *pada beberapa perbandingan campuran minyak serai wangi dan minyak cengkeh dalam pengemulsi 0,5%* (v/v).

Treatments	Number of eggs laid (days after treatments)				ER* (%)
	2	4	6	Total	
Citronella : clove 1:1	7.50 c	9.50 a	7.75 c	24.75 a	63.3
Citronella : clove 1:2	15.75 bc	14.50 b	8.50 bc	41.25 b	38.9
Citronella : clove 1:4	18.25 b	16.25 bc	11.25 bc	45.75 bc	32.2
Citronella : clove 2:1	19.75 b	17.50 bc	12.25 bc	49.50 bc	26.7
Citronella : clove 4:1	15.25 bc	11.00 b	14.75 bc	41.00 b	39.3
Citronella	17.25 bc	16.50 bc	14.25 bc	48.00 bc	28,9
Clove	20.75 b	17.25 bcd	17.75 abc	53.25 bcd	21.1
Control (water + emulsifier)	24.75 ab	20.50 cd	22.25 ab	67.50 cd	
Control (water)	32.50 a	23.75 d	30.50 a	86.75 d	
CV (%)	34.15	36.16	55.41	20.28	

Note: Numbers followed by the same letters at the same column were not significantly different at DMRT 5%.

Keterangan: Angka-angka yang diikuti oleh huruf yang sama pada kolom yang sama tidak berbeda nyata pada taraf DMRT 5%.

**ER =* percent effective repellency/*persentase efektivitas repelensi.*

Table 13. Number of *H.antonii* eggs laid at different mixture ratios of lemongrass and clove oil at 0.5% in water-emulsifier (v/v).

Tabel 13. Jumlah telur yang diletakkan H. antonii *pada beberapa perbandingan campuran minyak serai dapur dan minyak cengkeh dalam pengemulsi 0,5%* (v/v).

Treatments	Numbers of eggs laid (days after treatments)				ER* (%)
	2	4	6	Total	
Lemongrass : clove 1:1	10.50 d	33.25 c	35.75 c	79.50 e	57.3
Lemongrass : clove 1:2	33.50 bc	42.00 bc	44.00 bc	122.25 cd	34.7
Lemongrass : clove 1:4	25.25 cd	31.75 c	38.50 bc	95.50 de	48.7
Lemongrass: clove 2:1	19.25 cd	35.25 bc	38.25 bc	92.75 de	50.2
Lemongrass: clove 4:1	21.50 cd	44.50 abc	46.00 abc	112.00 cde	39.9
Lemongrass	16.25 cd	44.75 abc	58.25 abc	145.75 c	21.7
Clove	34.50 bc	56.75 ab	36.25 bc	137.25 c	26.3
Control (water + emulsifier)	49.50 ab	64.75 a	46.25 ab	186.25 b	
Control (water)	61.50 a	66.50 a	57.50 a	222.00 a	
CV (%)	37.90	36.16	55.41	20.48	

Note: Numbers followed by the same letters at the same column were not significantly different at DMRT 5%.

Keterangan: Angka-angka yang diikuti oleh huruf yang sama pada kolom yang sama tidak berbeda nyata pada taraf DMRT 5%.

**ER =* percent effective repellency/*persentase efektivitas repelensi.*

Table 14. Number of *H. antonii* eggs laid at different mixture ratios of ageratum and clove oil at 0.5% in water-emulsifier (v/v)

Tabel 14. Jumlah telur yang diletakkan H. antonii *pada beberapa perbandingan campuran minyak babadotan dan minyak cengkeh dalam pengemulsi 0,5%* (v/v).

Treatments	Numbers of eggs laid (days after treatments)				ER* (%)
	2	4	6	Total	
Ageratum : clove 1:1	9.75 cd	20.00 de	18.25 bcd	48.00 d	53.5
Ageratum : clove 1:2	9.75 cd	14.50 e	14.75 cd	39.00 de	62.3
Ageratum : clove 1:4	6.50 c	9.75 e	8.75 d	25.00 e	75.8
Ageratum : clove 2:1	11.25 bcd	7.75 e	24.75 bcd	43.75 d	57.6
Ageratum : clove 4:1	5.50 cd	20.75 de	7.75 d	34.00 de	67.1
Ageratum	18.25 abc	20.75 de	32.00 abc	81.25 c	21.3
Clove	16.00 abcd	36.00 bc	27.75 abc	79.75 c	22.8
Control (water+ emulsifier)	20.25 ab	47.50 ab	35.50 ab	103.25 b	
Control (water)	24.00 a	52.25 a	43.50 a	119.75 a	
CV (%)	46.45	36.30	47.25	16.44	

Note: Numbers followed by the same letters at the same column were not significantly different at DMRT 5%.
Keterangan: Angka-angka yang diikuti oleh huruf yang sama pada kolom yang sama tidak berbeda nyata pada taraf DMRT 5%
**ER =* percent effective repellency/*persentase efektivitas repelensi.*

mize the use of synthetic insecticides. Further studies are required to evaluate their activities under field condition. Tripathi *et al*. (2009) suggested that natural ingredients must be scientifically validated for its activity, efficacy and safety before making into formulation.

CONCLUSION

Mixtures of citronella and clove oil (CiC), lemongrass and clove oil (LC) at all ratios, and ageratum and clove oil (AC) at ratios of 1:1; 1:2; and 1:4 produced <50% mortalities of *C. pavonana* larvae. However, the mixtures of AC 2:1 and 4:1 produced >50% mortalities of *C. pavonana* larvae. Moreover, the mixtures of CiC 1:1; LC 1:1, 1:2, 4:1, and AC in all ratios caused >40% mortalities of *H. antonii*. The mixtures of CiC and LC at all ratios significantly reduced around 50-60% of the number eggs laid by *C. pavonana,* while AC 1:4 and 4:1 nearly decreased 50% of the number eggs laid. Similar results were also demonstrated in the tests with *H. antonii*. The CiC and LC mixtures at the ratio of 1:1 also gave the lowest numbers of egg laid by *H. antonii*, while the mixture of AC 1:4 diminished 70% of number of eggs laid. Further studies are required to evaluate their efficacy and safety under field condition.

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