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Repellent Potency of N-Hexane Extract Leaf and Stem *Ocimum basilicum* against *Culex quinquefasciatus*

Daya Repelen Ekstrak N-Heksana Daun dan Batang Ocimum basilicum terhadap Culex quinquefasciatus

Indri Ramayanti[1,2*)], Miranti Dwi Hartanti[3], Reynaldi Aulia Rahman[4]

[1]Department of Parasitology, Faculty of Medicine, Universitas Muhammadiyah Palembang, Palembang 30116, South Sumatera, Indonesia
[2]Environmental Science Doctoral Program, Universitas Sriwijaya, Palembang 30139, South Sumatera, Indonesia
[3]Department of Pharmacology, Faculty of Medicine, Universitas Muhammadiyah Palembang, Palembang 30116, South Sumatera, Indonesia
[4]Medical Study Program, Faculty of Medicine, Universitas Muhammadiyah Palembang, Palembang 30116, South Sumatera, Indonesia
[*)]Corresponding author: indri_ramayanti@um-palembang.ac.id

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ABSTRAK

Nyamuk *Culex quinquefasciatus* merupakan vektor utama penyakit filariasis, berbagai cara yang dilakukan untuk mengontrol perkembangbiakan vektor yaitu penggunaan insektisida dari bahan alami salah satunya kemangi yang mengandung minyak atsiri dan terbukti efektif sebagai repelen terhadap nyamuk. Tujuan penelitian adalah untuk mengetahui daya repelen ekstrak n-heksana daun dan batang kemangi terhadap *Culex quinquefaciatus.* Penelitian ini merupakan penelitian eksperimental laboratorium dengan menggunakan Rancangan Acak Lengkap (RAL), dengan 3 kali replikasi pada 7 interval waktu. Konsentrasi ekstrak yaitu 15%, 25%, 35% dan kontrol negatif etanol 96%, kontrol positif repelen X yang mengandung DEET 13%. Hasil menunjukkan bahwa ekstrak n-heksana daun dan batang kemangi selama 6 jam pada semua konsentrasi mampu menolak nyamuk sampai 92.51%, analisis *one-way Anova* didapatkan nilai p= 0.000 (< 0.05) yang artinya terdapat perbedaan jumlah *C. quinquefasciatus* yang hinggap pada kain hitam pada berbagai konsentrasi ekstrak n-heksana daun dan batang kemangi, sedangkan analisis probit menunjukkan konsentrasi ekstrak yang efektif menolak hingga 90% adalah 31.52%. Ekstrak n-heksana daun dan batang kemangi efektif sebagai repelen terhadap nyamuk *C.quinquefasciatus.*

Kata kunci: filariasis, minyak atsiri, nyamuk, repelen

ABSTRACT

The *Culex quinquefasciatus* mosquito is the main vector of filariasis, various ways to control vector breeding is the use of insecticide from natural ingredients, one of which is basil containing essential oils and proven is effective as a repellent against the mosquito. The objective of this research was to determine the repellent potency of n-hexane extract of

basil leaves and stems against the *Culex quinquefasciatus*. This study was a laboratory experimental study using a completely randomized design, with 3 replications at 7 times intervals. The extract concentrations were 15%, 25%, 35 % and ethanol negative control 96%, the positive control repellent X containing 13% DEET. The results showed that the n-hexane extracts of basil leaves and stems for 6 hours at all concentrations were able to repel mosquitoes up to 92.51%, the one-way ANOVA analysis showed that the value of p= 0.000 (< 0.05), meaning that there was a difference in the number of *C. quinquefasciatus* perching on the black cloth at various concentrations of n-hexane extracts of leaves and stems of basil, while the probit analysis showed that the effective extract concentration at 90% was 31.52%. The extract of n-hexane leaves and basil stems was effective as a repellent against the *C. quinquefasciatus*.

Keywords: essential oil, filariasis, mosquitoes, repellent

INTRODUCTION

The existence of *Culex spp*. The mosquito spreads almost throughout the world, particularly those found in tropical and sub-tropical areas (Nchoutpouen et al., 2019). One of the species with the widest distribution is *Culex quinquefasciatus* (Samy et al., 2016). In Indonesia, the *Culex spp* mosquito, especially the *C. quinquefasciatus* (Say) species, is evenly distributed, especially in the areas of Sumatra, Java, Sulawesi, Kalimantan, NTT and Irian Jaya (Lee & Ryu, 2019). The *C. quinquefasciatus* mosquito is an important species and intelligent vector due to its high adaptive ability, ecological plasticity, invasive behaviour, host specificity and ability (Yee & Skiff, 2014).

C. quinquefasciatus mosquito can be prevented by using natural ingredients without having to use chemical insecticides such as repellent containing the active ingredient N, Dethyl-meta-Toluamide (DEET) (Deletre et al., 2019). Leaves, stems, seeds, and flowers from plants can be processed into mosquito repellent materials and used as botanical insecticides. (Ahmed et al., 2020; Isman, 2017) The insecticidal materials derived from plants are guaranteed safe for the environment because they decompose quickly on the ground and do not harm animals, humans or non-target insect (Amoabeng et al., 2019; Silverio et al., 2020). Plants that are potential as botanical insecticides generally have a characteristic of bitter taste because they contain alkaloids and terpenoids, a bad smell and taste a bit spicy (Campos et al., 2019; Ponsankar et al., 2016). This plant is rarely attacked by pests so it is widely used as an extract of vegetable insecticides in organic agriculture (Diop et al., 2016).

A plant considered to be a botanical insecticide is basil (*Ocimum basilicum*). It is one of the indigenous plants widely grown in Indonesia and is commonly used as a seasoning for cooking and medicine because it contains essential oils (Iskandar et al., 2020). The results of a phytochemical study on the basil have proven the existence of flavonoids, alkaloids, saponins, phenols, and essential oils containing eugenol (70.5%) as the main component (Chaudhary et al., 2020; Sankhalkar & Vernekar, 2016). The active compounds are effective as insecticides, including flavonoids functioning as respiratory toxins, saponins as stomach and contact poisons, and eugenol having a role in denaturing cytoplasmic proteins and tissue necrosis in insects (Farzaei et al., 2017; Mierziak et al., 2014) The combination of these active compounds composition hypothetically makes basil can be used as a natural alternative repellent (Dirar et al., 2019).

Based on the above introduction, the basil leaf extract in mosquito repellent preparations shows a potent activity as a vegetable insecticide. This study was aimed to find out the repellent potency of basil extract (*O. basilicum*) using an n-hexane solvent against *C. quinquefasciatus* mosquito.

MATERIALS AND METHODS

Research Design

This study was a laboratory experimental study with a Completely Randomized Design (CRD). This research was conducted at the Parasitology Laboratory of the Faculty of Medicine, Universitas Muhammadiyah Palembang from October until November 2019.

Population and Sample

The population in this study was the *C. quinquefasciatus* mosquito obtained from Lokalitbang P2B2 Entomology Laboratory Baturaja OKU, South Sumatra. The number of samples used was 375 mosquito with 3 replications at 7 times intervals. Ethical approval was obtained from the Ethical Committee of the Faculty of Medicine Muhammadiyah University Palembang.

Mosquito Rearing

C. quinquefasciatus eggs obtained from the Lokalitbang P2B2 Entomology Laboratory were hatched in a plastic tub 35x25x5 cm in size filled with aquadest three fourth of the plastic tub. The hatched larvae were reared until they become an adult mosquito. During their development, the larvae were given fed fish pellets. The rearing process was carried out in mosquito cages. After that, the female mosquito was separated from the male. The females were transferred to the test cage using an aspirator. It took 5 test cages 30 x 30 x 30 cm in size, with 25 *C. quinquefasciatus* in each cage. They were reared until they reach the age of 5-7 post-emergence days, during which time the mosquito was fed sugar solution (IAEA, 2017).

Simplicia and Extract Processing

One kg of fresh basil leaves and stems were washed and separated. Then, they were hung to dry. After the drying process, they were refined using a blender to get 500 g of simplicia powder. The extraction process was by maceration using n-hexane solvent. The simplicia powder was put into a maceration jar and two-litre of n-hexane liquid was added until the powder was immersed in the solvent. It was then stirred several times upon being left at room temperature for 24 hours. The procedure was repeated 3 times and followed with evaporation to get a thick extract. The thick extract was then diluted by adding 96% ethanol to obtain a concentration of 15%, 25% and 35%. The negative controls, which were, ethanol (96%), and n-hexane extract, were added. The positive control used was repellent X containing 13% DEET and it was added into a 25 ml spray bottle. The number of sprays was counted for each treatment.

Repellent Activity Test

The repellent activity test method was the Standard Method of Household Pesticide Efficacy Testing. The repellent test guideline was to use a black cloth the size of the test cage wall. It was attached to the surface of the wall. Into the surface of the cloth, the positive control, negative control, n-hexane was sprayed on each black cloth. Twenty-five mosquito was then put into each cage. They were observed and the number of mosquitoes that landed on the cloth for 5 minutes per hour was counted. They were counted from the first hour (immediately after application) to the sixth. The same treatment was performed using a different black cloth and different mosquito for 3 times (WHO, 2009). At the end of the test, the percentage of repulsion was assessed as the proportion of the number of a mosquito landing on the treatment cloth with the number of a mosquito landing on the control cloth. It was calculated with the following formula:

Repulsion Strength (RS) = (K-R) / K x 100%
Where:

K : Number of landing on the negative control cloth

R : The number of landing on the treated cloth

After obtaining the percentage of repellent potency at each concentration, the effective concentration was assessed to obtain a percentage of 90% repellent potency.

Data Analysis

Probit analysis was used to determine the effective concentration of 90% (EC90), i.e. the concentration of the extract that could reject the mosquito population by 90% for 6 hours of treatment with a 95% confidence level.

RESULTS

Repellent Activity

The results of statistical analysis showed the percentage repellent potency of n-hexane extracts basil (*O. basilicum*) toward the *C. quinquefasciatus* mosquito at concentrations of 15%, 25%, and 35% (Table 1), showed there was a simultaneous increase in both the mean percentage repulsion to contact with *C. quinquefasciatus* and in the concentration of n-hexane extracts at the highest concentrations of 15%, 25%, and 35%.

The number of *C. quinquefasciatus* mosquito that perched for 6 hours of treatment at a concentration of 15% was able to resist the mosquitoes that landed as much as 75%, a concentration of 25% was able to reject as much as 85.05%, and a concentration of 35% was able to reject as much as 92.51% approaching the repellent activity value of positive control.

The repulsion percentage of n-hexane extracts of basil leaves and stems with concentrations of 15%, 25%, and 30% against *C. quinquefasciatus* mosquito were then performed statistical analysis using one-way ANOVA. A one-way ANOVA test was conducted to determine the effect of n-hexane extract on the percentage of repulsion.

The results showed that there were differences in the repellent potency ($p < 0.05$) at the concentration variation (15%, 25% and 30%) at each time of the test. The difference in repulsion among the various concentrations of n-hexane extracts of leaves and basil stems was described below. The plot describes the variation in concentration with the repellent potency of n-hexane extracts at each test time period (Figure 1). It could be seen that the higher the concentration of the extract, the stronger the repulsion against *C. quinquefasciatus*.

Table 1 Repellent potency of leaves and stems extract

Repetition to-	Percentage of Repellent Potency (%)				
	15	25	35	Control (-) Etanol	Control (+) DEET 13
1	75.00	84.56	91.66	0	98.21
2	75.74	84.63	92.90	0	98.21
3	74.26	85.98	92.98	0	98.83
Mean	75.00	85.05	92.51	0	98.42

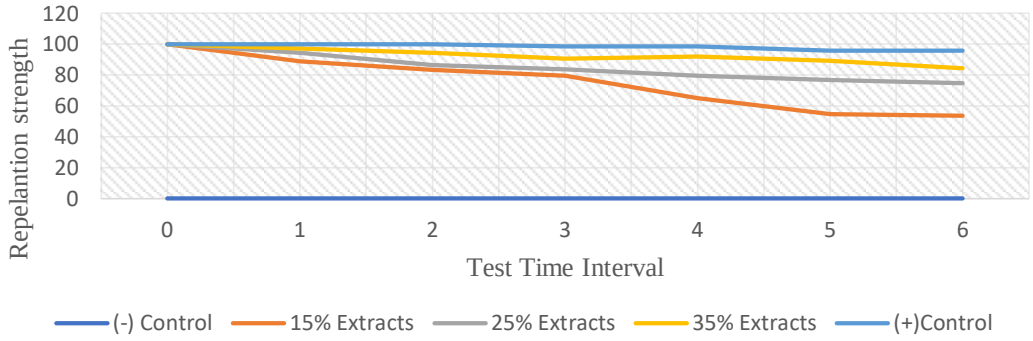

Figure 1. Concentration of N-Hexane extract from basil (*O. basilicum* Linn.) effect plot as a repellent against *C. quinquefasciatus* mosquito at seven testing time intervals

Table 2. Concentration based on probit analysis

Effectiveness (%)	Concentration (%)	Level of Significance	Interval Significance	
			Lower Limit	Upper Limit
90	31.52	95%	25.48	56.65

Effectivity Concentration

The estimation of the concentration with protective power was determined using the probit test. This study expected an Effectivity Concentration of 90% (EC_{90}). The probit analysis showed that a concentration of 31.52% within 6 hours was able to repel 90% of the tested mosquitoes (Table 2).

DISCUSSION

The number of *C. quinquefasciatus* mosquito on the black cloth as shows in Table 1. it had been known that each concentration of extract n-hexane basil leaves and stems (*O. basilicum*) has different repellent potency, while in Figure 1. how repellent potency influence *C. quinquefasciatus* mosquito, it did seem that the increase in the repellent potency of extract basil leaf and stems occurs simultaneously with how the concentration increase in the applied expositions. This trend is seen to occur continuously from the beginning to the end of the testing period (0 hours to 6 hours). This indicates that the increase in the percentage of repellent potency is directly proportional to the increase in the concentration of n-hexane extracts. The repulsion mechanism is that the higher the concentration of essential oils on the surface of the fabric that evaporates, the more active components in it which will result in the binding of the active component to the receptor (Zaniol et al., 2020). In the mosquito, their antennae and palp function as chemical sensors which are very sensitive and can easily be stimulated by chemical smells (Vinauger et al., 2018). When the resulting fragrance evaporates into the air, it will be detected by the mosquito's olfactory receptor, and it will convert it into impulses and transmit it by the sensory nerve axons to the nerve centre (brain). As a result, the mosquito will keep away from the source of smell (Deletre et al., 2016). The graph also shows that there is a difference in the repellent potency of each test time interval, in which it decreased along with the increase in the time interval of the test, and it occurred in all treatment groups. This indicates that the decrease is also influenced by the interval of observation time. The longer the time interval, the more secondary repellent metabolite compounds from the extract that evaporates which decreases the concentration of molecules in the fabric, and in turn, it causes the protection to decrease (Ntalli et al., 2019). The decrease can also be affected by the mosquito regulatory mechanism. The aroma of essential oils produced by basil extract will bind to its OBP (odorant-binding proteins) which function as carriers for odour molecules into the mosquito's olfactory receptors (Brito et al., 2016).

The odours do not always circulate in mosquito lymph vessels but can also be degraded by the enzymes known as ODEs (odour-degrading enzymes). The rate of degradation depends on the molecules that bind to the OBP. ODEs function as regulators, especially if the odour molecules that bind to OBP's are excessive (Lombardo et al., 2017). The results of statistical analysis using probit analysis at the 95% confidence level show that EC_{90} results at a concentration of 31.52% (Table 2). EC_{90} is the indication of the amount of concentration that has 90% repellent potency of the mosquito population. This study shows that the n-hexane extracts (*O. basilicum*) affected a repellent against the mosquito. The extract of n-hexane from basil (*O. basilicum*) leaves and stems contains eugenol, linalool, and geraniol

(Zahran et al., 2020). The distinctive odour from the secondary metabolites such as linalool, geraniol, and eugenol will get into the mosquito extracellularly and are then captured by chemoreceptors on the sensilla located on the mosquito antenna. These molecules will bind to OBPs (Odorant Binding Proteins), and then will be carried by OBPs through the lymph in the sensilla to ORNs (Olfactory Receptor Neurons) (Tzotzos et al., 2018).

In addition to carrying odour molecules, OBPs also function to dissolve these odour molecules and to select odour molecules to be accepted in certain ORs (olfactory receptors) (Lombardo et al., 2017). The odour will be converted into impulses transmitted by the sensory nerve axons to the nerve centre (brain) The mosquito avoid the source of the smell as the respond (Montell &Zwiebel, 2016). The odour molecule further interacts with extracellular G-protein-coupled receptors on ORs located in the dendrite of specific ORNs; where the intracellular G-protein-coupled receptors are active in turns and cause changes in the G-protein conformation (Tian et al., 2017). This causes nerve depolarization triggering the transmission of electrical impulses to the mosquito antenna lobe eliciting a rejection response or blocking the mosquito's sense of smell which ultimately acts as a barrier to the mosquito's performance to recognize its prey (Montell & Zwiebel, 2016).

Species of mosquito can affect the sensitivity in detecting odours that only low extract concentrations are effective against *C. quinquefasciatus* mosquito with an estimated EC90 at a concentration of 31.52%. The olfactory chemoreceptors of *C. quinquefasciatus* are 6,9 times more sensitive to odours than other mosquito species such as *Anopheles* and *An. albimanus* (Bohbot et al., 2011). Besides, the use of n-hexane solvent in the maceration of basil extracts also affects the bioactive compounds obtained. Several studies have shown that the use of n-hexane solvent yields more essential oils than other solvents. Studies on the effect of solvent types on the yield of citronella oil found that the best extraction technique is by using the n-hexane solvent which yields essential oil much more than using ethanol and acetone solvents (Demiray et al., 2019).

Based on the results and data analysis, it shows that the activity of the extracts waseffective as a repellent. However, the natural repellent effect against *C. quinquefasciatus* mosquito bites is lower than the synthetic one. The synthetic repellent used in this study contained 13% DEET as a positive control, with a higher repellent potency than the treatment group. Based on these observations, it is shown the effect of the extracts is lower than the synthetic repellent. The most widely used synthetic repellent contains 13% DEET. DEET is a repellent in the aromatic amide group.

The DEET compound works by blocking the receptors in the mosquito antenna, one of which is the lactic acid receptor and can interfere with the mosquito olfactory receptors that mosquito avoids the exposed surface of these compounds (Legeay et al., 2018). However, DEET is a toxin to human because its properties can be absorbed into the body through the skin. Long-term use can be toxic to humans and can cause side effects such as dystonia, seizures, slurred speech, nausea, rashes, affected motor capacity, sensory disturbances, decreased learning ability and memory impairment.

The use of a repellent containing DEET in high doses for a longer period of time can cause skin irritation, erythema, muscle cramps, and rash. Repeated use for a long time can cause systemic poisoning, which often occurs in children (Swale et al., 2014). The advantages of insecticides of essential oils derived from plants are that their spectrum of biological activity is very broad, non-toxic, systemic, compatible, easily degraded, and safer than synthetic chemicals. The insecticides made from essential oils are also safe for the environment because they are not persistent.

This is because the essential oils are easily broken down naturally that they do not last long in the water, air, soil and the body of mammals.

The essential oils are also effective against target organisms, compatible with other control methods, safe and non-toxic to non-target organisms as well as the environment and human health so that they have a huge potential to be developed as commercial plant-based insecticides (Asbahani et al., 2015).

The weakness of the insecticides containing essential oils was related to the properties of the essential oils themselves which are volatile and unstable or not resistant to sunlight. For example, cinnamaldehyde was unstable and will break down to form benzaldehyde at 60°C. However, when combined with eugenol, cinnamaldehyde was stable to a temperature of 200°C for more than 30 minutes. The effectiveness of insecticides from essential oils was generally lower, the repulsion mechanism was slower than that of synthetic chemical insecticides (Chellappandian et al., 2018). Consequently, in an insecticide formula made from active essential oils, other chemical compounds are always added to increase the stability of the active ingredient.

CONCLUSION

This study shows that the n-hexane extract from basil leaves and stems (*O. basilicum*) was effective as a *C. quinquefasciatus* mosquito repellent at a concentration of 31,52%.

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