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A.galanga (L.)-Willd Rhizome and O.sanctum L. Leave Essential Oils as Culex sp. Larvicide

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Abstract

Culex sp. can transmit diseases such as encephalitis and filariasis which is a public health problem because of elephantiasis, disability, and a neglected tropical disease. To prevent these diseases, the mosquitoes need to be eradicated and larvicides are considered to control them at their early stage. Temephos, a synthetic larvicide, could cause several disadvantage, such as potential toxicity against non-target organisms and easier-to-develop mosquito resistance. Botanical sources from Indonesian spices i.e. galangal (Alpinia galanga (L.) Willd.) and basil (Ocimum sanctum L.) are potentially used as larvicide. This study aimed to explore the larvicidal effect of their essential oils compared against temephos against Culex sp. A true experimental study was done using several concentrations and replications of these essential oils in each container, consisting of 25 third instar of Culex sp larvae for 24 hours at room temperature according to WHO procedure. The percentage of dead larvae was analyzed by ANOVA/ Kruskal Wallis. The study showed that Alpinia galanga (L.) Willd. and Ocimum sanctum L. essential oils had larvicide activity and their activity starting from 140 ppm and 400 ppm consecutively was the same as temephos 1 ppm.

Introduction

Many diseases are vector-borne diseases which are usually transmitted by bloodsucking arthropods. Mosquitoes are the best-known vectors besides other vectors. Three genera of them i.e.: Culex, Aedes, and Anopheles are the most important vector of many diseases (Osanloo *et al.*, 2019). Mosquitoes are the most dangerous animal in the world (Breedlove, 2022). *Culex sp.* besides causing itching, can transmit several fatal diseases, such as encephalitis, and also can decrease productivity by disability because of filariasis. Filariasis, especially lymphatic filariasis, is one of the important neglected tropical diseases and it becomes a problem because of the elephantiasis (WHO, 2020). Indonesia is endemic for several neglected tropical diseases including filariasis, one of the public health problems (Santoso *et al.*, 2020), and there is a target time for filariasis elimination in Indonesia (Meliyanie & Andiarsa, 2017). Elephantiasis itself could not be cured by administering medication. So, it is vital to prevent these diseases by reducing the mosquito population and this effort might support the filariasis elimination program.

Larvicide is preferred for this purpose because it reduces the population earlier by killing the larvae, and it is easier because the larvae are concentrated in breeding places and accessible before the mosquitoes become adult stadia (Osanloo *et al.*, 2019). The effectivity of temephos as a chemical/synthetic larvicide of the organophosphate group is still good, cost-effective, and recommended by WHO. However, a recent study reported detrimental

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effects on male fertility of mice in vitro which showed that temephos caused disturbance of the sperm functions by several mechanisms which significantly decreased the fertilization rate and further early embryonic development (Kim *et al.,* 2020). According to clinical studies, temephos had no significant negative effect on general human health. However, it has been proven that temephos might have mild genotoxic/teratogenic effects and could cause detrimental effects on fetal development specifically against the central nervous system to inhibit acetylcholine esterase as well as inhibition in fetal growth (Satriawan *et al.,* 2019) and has been reported the impact of exposure to pesticide, organophosphate/ carbamate, against pregnant women who worked as shallot farmers in the Middle of Java province especially at first trimester of pregnancy resulted a generation with lower IQ (Suwondo et al. 2016). Also, another study reported that there was population resistance of mosquitoes (*Aedes sp*) against temephos which reduced the impact on developmental and reproduction potential of mosquitoes (Rahim *et al.,* 2017). Resistance of mosquitoes against synthetic larvicides might be caused by several factors: it is a tendency of persistence of the synthetic larvicide for a long time in the environment causing adaptation of the mosquitoes against it. The single compound in it also causes resistance. Targeting the non-target organism in the indiscriminate use of the synthetic larvicide also becomes another risk because of the toxic residue of the synthetic larvicide (Antonio-Nkondjio *et al.,* 2018, Osanloo *et al.,* 2019). Therefore, it is wise to find another effective larvicide that is safe for non-target organisms and spared from resistant mosquitoes.

Spices such as galangal (*Alpinia galanga* (L.) Willd.) rhizome extract which flavonoid is one of the dominant compositions has been reported as a larvicide against *Aedes sp.* (Boesri *et al.,* 2015) and other spices i.e. basil (*Ocimum sp.*) leaves essential oil also has been reported about its larvicide effect which had different one according to mosquito genus and the species of Ocimum (Sneha *et al.,* 2022). These kinds of herbal are very familial especially in Indonesia because they are consumed in so many Indonesian foods. Less cloudiness usually found in essential oils applications than extracts might be another benefit. This study aimed to explore the larvicidal effect of the *Alpinia galanga* (L.) Willd rhizome and the *Ocimum sanctum* L. leaves essential oils against the third instar of *Culex sp.* larvae to be developed in further study.

METHOD

The *Culex sp.* larvae were obtained from The School of Life Science and Technology, Bandung Institute of Technology (ITB), while *Alpinia galanga* (L.) Willd. rhizome and *Ocimum sanctum* L. leaves and their essential oils were from The Taman Kebun Percobaan Manoko, Lembang, Kabupaten Bandung Barat, Indonesia, and the essential oils were made using the distillation method by the company. Temephos to be used in this experiment was Abate from BASF. Before making essential oils, these simplicia were identified by Pusat Studi Biofarmaka Tropika (*Trop BRC*), Institute for Research and Community Service, Institut Pertanian Bogor, Bogor, Indonesia. The study was done after ethical approval by The Ethical Committee of the Faculty of Medicine, Maranatha Christian University. It was a true experimental study with a completely randomized design preceded by the pre-experimental research to identify the variation of concentrations to be used. This study was done according to WHO Guidelines for Laboratory Testing of Mosquito Larvicides (WHO Communicable Disease Control, 2005). According to pre-experimental research, the range of concentrations of *Alpinia galanga* (L.) Willd rhizome essential oil (AgEO) to be used in this experiment were between 60-160 ppm: 60, 80, 100, 120, 140, and 160 ppm, while the concentrations of *Ocimum sanctum* L. leaves essential oil (OsEO) were used in concentrations 100, 200, 400, 800, and 1.600 ppm. Aquadest was used as a negative control while temephos 1 ppm was a positive control. AgEO study was done with 4 replications for 8 treatments while OsEO with 5 replications for 7 treatments. All these treatments with each replication were done in glass containers containing 100 mL treatment solutions at room temperature. Then 25 live third instar larvae of *Culex sp* were added into each container. These treatments were done for 24 hours and thereafter the percentages of dead larvae in each container were counted and analyzed using ANOVA or Kruskal Wallis according to homogeneity/ normality of the data.

RESULT AND DISCUSSION

The study of AgEO was done with 4 replications for 8 treatments: 60, 80, 100, 120, 140, and 160 ppm, with aquadest as a negative control, and temephos 1 ppm as a positive control. The result showed the percentage of dead larvae for each replication in each treatment. Because the data were not normal, it was analyzed with Kruskal Wallis and continued with Mann-Whitney.

According to probit analysis, the LC_{50} of AgEO as larvicide against *Culex sp* was 83 ppm = 0.0083% and LC_{90} was 126 ppm=0.0126%. This LC50 was less than 100 ppm. Because of this LC50, AgEO could be classified as an active larvicide against Culex sp (Gomes *et al.,* 2021, Diaz *et al.,* 2023). As shown in Table 1 below, this essential oil has a larvicidal effect starting from 60 ppm and starting from 140 ppm the effect was the same as temephos to kill the larvae. AgEO rhizome consisted of so many compounds, either major or minor, and there was synergistic interaction between them in insecticidal effect. The AgEO from Yunnan Province-China consisted of 5 major components (alpha-pinene, beta-pinene, eucalyptol, L-borneol, and alpha-terpineol) and eucalyptol and alpha-terpineol were the most active as larvicide among the major components. The major components of AgEO from Indonesia were 1,8-cineole and pinenes, while these compounds were less active than alpha-terpineol and eucalyptol. Therefore the larvicidal activity of the AgEO between these different countries might be different because of different rates of each active ingredient and the difference might depend on their geography, season, and time of harvesting (Wu *et al.,* 2014).

Another study reported that *Alpinia galanga* rhizome extract from the same country (Indonesia) showed larvicidal activity against *Aedes sp* which LC_{90} was 8.2% (Boesri *et al.,* 2015), while in this study its essential oil could kill all the *Culex sp* larvae in concentration starting from 140 ppm (=0.014%) and the LC_{90} was 0.0126%. The smaller LC_{90} of the essential oil might probably be caused by the more active compounds obtained from this preparation. The significantly less cloudy solution of the essential oil was another benefit of its application.

Essential oils are mixtures of active compounds such as alkaloids, monoterpenes, and flavonoids. Flavonoids kill the larvae by targetting the mosquito's acetylcholine esterase enzymes, while monoterpenes and alkaloids by targetting N-K-ATP-ase or K+ and Na+ channels. Using a single active compound as

TABLE 1. The Percentage of the Dead Larvae for Each Replication in Each Treatment of Various Concentrations of AgEO Compared to Aquadest (as a Negative Control) and Temephos 1 ppm (as a Positive Control)

	Percentage of The Dead Larvae from Each Treatment Group							
	I	II	III	IV	V	VI	VII	VIII
1	24	40	60	84	100	100	4	100
2	24	56	88	68	100	100	12	100
3	24	24	80	68	100	100	4	100
4	36	44	68	76	100	100	4	100
Mean ±SD	27 ± 6^{b}	41 ± 13^{b}	68 ± 12^{c}	74 ± 8^{c}	100 ± 0^{d}	100 ± 0^{d}	6 ± 4^{a}	100 ± 0^{d}

Notes: I= group treated with AgEO 60 ppm, while II, III, IV, V, VI were treated with AgEO 80. 100, 120, 140, 160 ppm consecutively. VII= group treated with aquadest only, VIII= group treated with temephos 1 ppm as a positive control. The same superscript letter means no significant difference ($p > 0,05$).

larvicide might give rise to more larvicide-resistant mosquitoes while using multiple active compounds such as compounds in essential oil that have different targets causes less resistant ones, and it might be another benefit of using essential oil as larvicide (Kim and Ahn 2017, Osanloo et al. 2019). The biodegradability of the botanical larvicide also reduces the resistance process. Essential oils are biodegradable while synthetic larvicide such as temephos tends to remain in the environment for a long time, and the larvae might adapt against this condition and then potentially become resistant to the larvicide (Osanloo *et al.,* 2019).

Larvicidal effect against *Culex sp* larvae of *Alpina galanga* rhizome dichloromethane extract continued with further bioassay-guided gradually graded fractionation of the extract also showed high effectivity and could kill 100% larvae (Abutaha & Al-Mekhlafi, 2020). So the larvicidal activity of the botanical larvicide also depends on its preparation beside its plant origin. It could be concluded that AgEO is potentially to be used as mosquito larvicide although the possibility of negative effects of this preparation should be studied further. Considering the result of our study, It is wise to do further research using concentrations between 120-140 ppm to find out the smallest concentration which still has the same effect as temephos as a standard larvicide.

Larvicidal study of OsEO was done with 5 replications for 7 treatments: 100, 200, 400, 800, and 1.600 ppm, with aquadest as negative control, and temephos 1 ppm as positive control. The result showed the percentage of dead larvae for each replication in each treatment. Because the data were not normal, it was also analyzed with Kruskal Wallis and continued with Mann-Whitney.

According to probit analysis, the LC_{90} of OsEO as *Culex sp* larvicide was 353 ppm. According to this LC, OsEO could be classified minimally as an effective larvicide. If the LC_{50} is less than 100 ppm, it could be classified as an active larvicide (Kim & Ahn, 2017). As shown in Table 2 below, this essential oil had a larvicidal effect against *Culex sp* and this effect at ≥ 400 ppm was the same as temephos larvicidal effect as a standard control which killed 100% of the larvae. This result supported the previous preliminary study which showed the potential larvicide effect of *Ocimum sanctum* leaf crude extract against *Culex sp* and *Aedes sp* larvae. Still, this last study indicated low and moderate results (Anees, 2008). Contrary to the larvicide study using ultrasound-assisted hydrodistillation essential oil of several species of Ocimum plants (*O.basilicum*, *O.canum*, *O.tenuiflorum*, *O.gratissimum*) against *Culex sp*, it was shown that all of these plants had a low LC_{50} i.e. 50-75 ppm (Sneha *et al.,* 2022). A study in India also showed that essential oil of *Ocimum sp* including *Ocimum sanctum* aerial parts as

TABLE 2. The Percentage of the Dead Larvae for Each Replication in Each Treatment of Various Concentrations of OsEO Compared to Aquadest and Temephos 1 ppm (as a Positive Control).

	Percentage of The Dead Larvae from Each Treatment Group						
	I	II	III	IV	V	VI	VII
1	50	56	100	100	100	20	100
2	76	50	100	100	100	4	100
3	56	84	100	100	100	12	100
4	56	48	100	100	100	4	100
5	40	60	80	100	100	4	100
Mean± SD	55.6±13.1[a]	59.6±14.4 [a]	96±8.9[b]	100±0 [b]	100±0 [b]	8.8±7.2[c]	100±0 [b]

Notes: I= group treated with OsEO 100 ppm, while II, III, IV, V, were treated with OsEO 200, 400, 800, and 1600 ppm consecutively. VI= group treated with aquadest only, VII= group treated with temephos 1 ppm as a positive control. The same superscript letter means no significant difference (p > 0,05).

well as their petroleum ether extracts had a lower LC_{50} against *Culex sp* which meant that they had a stronger effect as larvicide compared to our study. LC_{90} OsEO in the previous study in India was 106.66 ppm and 223.6 ppm for laboratory and field-reared *Culex sp* larvae (Rajamma *et al.,* 2011), while in our study it was 353 ppm. This might also be caused by the different geography, climate, and time to harvest (Wu *et al.,* 2014). Different extractor solutions could influence in obtaining the amount of active compounds. The major compounds that had insecticidal activity were *t*-methyl cinnamate, linalool, and estragole; *t*-methyl cinnamate was more potent than linalool while linalool was potentially better than estragole. The target of insecticidal/ larvicidal effect of these 3 compounds is the central nervous system of the insects by inhibiting acetylcholine esterase and influencing gamma-aminobutyric acid (GABA) and the result against this enzyme as well as against GABA receptor resulted in synergistic effect as an insecticide/ larvicide. Fortunately, the morphology and molecule of the GABA receptor and the molecule of the acetylcholine esterase of the insects are different against vertebrates/humans, therefore it is safe for them (Savigny *et al.,* 2021). In Brazil, OsEO could act as an insecticide against all the stadia of *Musca domestica* (Chil-Núñez *et al.,* 2020).

Because in 400 ppm the AsEO could kill 100% Culex larvae and have the same effect as temephos as a standard larvicide drug, it is also wise to consider further study using the range of concentration between 200 and 400 ppm to find out the least concentration that could still have the same larvicidal effect as temephos. Further study on whether there is any negative effect against non-target organisms also needs to be explored. All of these need to be considered before its application in the community. It showed a different LC50 than our study because of different plants origin, different preparations, different larvae stadiums, and different mosquito genera.Another spice, familiar in Indonesia, red ginger (*Zingiber Officinale* Rosc), also had been studied as a larvicide for Aedes aegyti (Boekoesoe & Ahmad, 2022). Also, other botanical sources such as Srigading (*Nyctanthes arbor*-tristis) a decorating plant easily found in Indonesia had been studied as larvicide against 4th instar Aedes aegypti (Fakhriadi *et al.,* 2023). Its leaf extract was used. Besides other factors, different active compounds obtained by different preparations i.e. juice, extract, and essential oil, could be one of the reasons for different effects. These evidences might be put in our consideration to investigate many studies ahead about natural larvicides especially using substances which are familiar to human.

CONCLUSION

AgEO and OsEO had larvicidal properties against Culex sp. and, within proper concentration, could have the same larvicidal activity as temephos, a standard synthetic larvicide, which could kill all the larvae. As we know, prevention is preferred to therapy, and the application of these essential oils might be considered to support the filariasis program to prevent the disease by killing the vector at an early stage. These essential oils need further study to determine the lowest concentration with the same effect as temephos. Although these essential oils are botanical sources, they need to be studied further also to explore any possible harm to the environment/non target organism.

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