LARVICIDAL ACTIVITIES OF *Ocimum basilicum* AND *Mentha piperita* EXTRACTS AGAINST AEDES MOSQUITO LARVAE

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ABSTRACT

Mosquito borne diseases are major health problems in tropical and subtropical countries. In addition to development of resistance in mosquito species, control measures using synthetic insecticides have led to several environmental hazards. This study investigated the larvicidal potential of *Ocimum basilicum* and *Mentha piperita,* against the Aedes mosquito larvae. Fourth instar larvae were used for larvicidal bioassay. The aqueous plant extracts of *M. piperita* and *O. basilicum* were used singly and in combination and water was used as control. The larvae were exposed to a range of concentrations of each extract under laboratory conditions and larval mortality was assessed after 24 and 48 hours exposure. After 24 hours, *M. piperita* extracts had the highest larvicidal activity (90%) at 2.0ml followed by a combination of the two extracts (76.67%). At 48 hours, *M. piperita* maintained the highest larvicidal activity. *M. piperita* and *O. basilicum* had LC_{50} of 0.01mg/ml and0.14mg/ml respectively after 48 hours of exposure. The results indicated that *M. piperita* and *O. basilicum* may play an important role in reducing the use of chemicals for mosquito control. In-depth investigations to elucidate the active ingredients of the extracts should therefore be conducted.

Keywords: Mosquitoes, Aedes mosquito, *Ocimum basilicum, Mentha piperita,* larvicidal, plant extracts.

INTRODUCTION

Mosquitoes are among the vectors responsible for the spread of some of the world's deadliest diseases. Malaria, a mosquito-borne infectious disease, is endemic in 117 countries with some 3.2 billion people living in risk areas all over the world (Hussaini et al., 2018). Mosquitoes are rural-urban insect which had been noted for their high prevalence in many developing countries (Ileke and Ogungbite, 2015). Mosquito-borne disease cause significant morbidity, mortality and economic burden to humankind (Beena, 2013). The World Health Organization has declared the mosquitoes as "public enemy number one". To prevent proliferation of mosquito borne disease and to improve the quality of environment and public health, mosquito control is essential (Anupam et al., 2012).

The major tool in mosquito control operation is the application of synthetic insecticides such as organochlorine and organophosphate compounds. But this has not been very successful due to human, technical operation, ecological and economic factors, thus there is need for alternative approaches ranging from provision of or promoting the adoption of effective and eco-friendly toxic larval control. Considering these, the application of eco-friendly alternatives such as biological control of vectors has become the central focus (Anupam et al., 2012).

Nigeria is known for high prevalence of malaria and the disease remains one of the leading causes of childhood and maternal morbidity and mortality, low productivity and reduced school attendance in Nigeria thus, there is an urgent need to develop new insecticides for controlling mosquito which are more environmentally safe, biodegradable and target-specific against mosquitoes (Sarita et al., 2014).

For centuries, medicinal plants have been used as a medicine for human diseases as they are environmentally friendly, it contains secondary metabolites and bioactive components of beneficial values. One of the best ways to control a disease is to destroy its vector's eggs or larvae as they are motionless at that stage of their life cycle. Plants and its isolated compounds are alternative agents for control of mosquitoes, because they possess bioactive chemicals, which act against limited number of species including specific target-insects and are eco-friendly (Afolabi and Aladesanmi, 2018).

Ocimum basilicum (Basil) belonging to the *Lamiaceae* family, is a perennial shrub with pleasant smell, which grows in several regions all over the world. Basil is native to tropical regions from central Africa to Southeast Asia. It is a tender plant, and is used in cuisines worldwide depending on the species and cultivar. Studies have shown many pharmacological effects in several diseases, with potent antioxidant, anti-aging, anticancer, antiviral, and antimicrobial properties (Sakr and Al-Amoudi, 2012). Basil is a member of the mint family. Sweet basil is the most common culinary basil. Physically, basils are characterized by square, branching stems, opposite leaves, brown or black seeds, and flower spikes, often referred to as the "king of the herbs (Makri and Kintzios, 2008).

Mentha piperita (Peppermint) is a hybrid mint, a cross between water mint and spear mint, indigenous to Europe and the Middle East, the plant is now widely spread and cultivated in many regions in the world. Peppermint oil extracted by steam distillation from the leaves of *Mentha piperit*a has a long tradition of medicinal use. It has high menthol content, and is often used in certain food items. It has also been reported that peppermint oil reduces colic abdominal pain and cause a major reduction in irritable bowel syndrome. The oil is also reported to contain methone and methyl esters. Methone, present in high concentration in peppermint oil is reported to act as natural pesticides (Sarita et al., 2014). It also serves as medicine due to its medicinal activities as anti-nociceptive, anti-inflammatory, antimicrobial and antioxidant properties. The presence of flavonoids such as eriocitrin, narirutin, hesperidin, luteolin-7-O-rutinoside, isorhoifolin, diosmin, rosmarinic acid exert anti-allergic effects (Barbalho et al., 2017).

M. piperita is known for having several phytochemicals, including polyphenols that are highly effective antioxidants and are less toxic than the synthetic ones. This property makes it of great interest to the Food Industry, since the phenolic compounds retard the oxidative degradation of lipids improving the quality and nutritional value of food (Barbalho et al., 2017). Several plant extracts and isolated compounds from different plant families have been evaluated for their promising larvicidal activities. About 2000 species of terrestrial plants have been reported for their insecticidal properties. Medicinal plants are more potent, eco-safe and low-cost which is easily available and have become increasingly popular as safe and biodegradable mosquito larvicides, it has less or no adverse effect to the environment (Odoh et al., 2018).

Plant based products does not have any hazardous effect on ecosystem. Recent research has proved that effectiveness of plant derived compounds, such as saponin, steroids, isoflavonoids, essential oils, alkaloids and tannins have potential mosquito larvicides. Plant secondary metabolites and their synthetic derivatives provide alternative source in the control of mosquito (Afolabi and Aladesanmi, 2018).

Synthetic organic larvicides, although efficacious to target species but can be harmful to a variety of animal life including man. There is need for larvicides of natural origin that are environmentally safe, biodegradable and target specific to combat mosquito species as vectors of some human deleterious diseases. Natural products of plants source with insecticidal properties have been used for control of a diversity of pest insect and vectors yielding different results. This study focused on the larvicidal activities of *Ocimum basilicum* and *Mentha piperita le*aves extract on the *Aedes* mosquito larvae.

MATERIALS AND METHODS

Collection of the plant materials

The fresh leaves of *O. basilicum* and *M. piperita* were collected from the environs of Ijebu Ode, Ogun State, Nigeria. It was identified and authenticated by a plant taxonomist in the Department of Botany, University of Ibadan with a voucher number UIH—22846 and UIH- 22849 for *O. basilicum* and *M. piperita* respectively.

Preparation of plant extracts

The leaves were air dried for some weeks and then crushed into coarse form using a clean mortar and pestle. Crushing of each plant was done separately. The extracts of the plant materials were obtained using the cold maceration method. Six hundred gram (600g) of each powdered plant materials (leaves)

was weighed into desiccator. Each weighed-out plant part was extracted using three litres (3L) of ethanol in tightly covered desiccator and left for 72hours at room temperature with intermittent stirring. The resultant suspensions were filtered into beakers using muslin cloth, and filtrates collected was re-filtered using Whatman No.1 filter paper into sample bottles. Each of the filtrates was concentrated in water bath at 40^0C. Final product of each plant extract was labelled and stored for further use.

Larvae culture

Stagnant water was collected from Ijagun, Ijebu Ode, Ogun State into plastic containers with net covering and kept in Biological Sciences laboratory of Tai Solarin University of Education. The eggs were left to develop into mosquito larvae which were identified and fed with fish meal. The water was changed from time to time and left until they grow into 4^{th} instar Aedes larvae stage.

Larvicidal bioassay

The larvicidal activity of the plant extract was evaluated using the procedures explained by the World Health Organization (WHO, 2005). Batches of 25 larvae were transferred to a small disposable test cups, each containing 200ml of water. The appropriate volume of dilution was added to 200ml of water in the cups to obtain the desired target dosage. Each test was conducted with three replicates with three concentrations (0.5ml 1.0ml and 2.0ml). The extracts were administered singly and in combination. Water was used as control. Mortality was recorded after 24h and again after 48hr.

Data Analysis

The larval mortality was subjected to One-way Analysis of Variance, while Duncan Multiple Range Test was used to determine the significant difference between the effects of the plant extract on the mosquito larvae at different concentrations and probit analysis was used to calculate the Lethal Concentration 50 (LC_{50}) and Lethal Concentration 90 (LC_{90}) using Statistical Package for Science Social version 22.

RESULTS

Larvicidal Activity of Extracts at 24hrs

Table 1 shows the larvicidal activity of *M. piperita* and *O. basilicum* singly and in combination 24hrs post treatment. At 2.0ml of the extracts, *M. piperita* had highest percentage mean mortality of 90.00%, followed by combination of *M. piperita* and *O. bacilicum* (76.67%). *O. basilicum* had the least larvicidal activity at different concentration of the extract (11.67%, 13.33% and 15%). There is a significant difference between the test group and the control. However, the result shows that the higher the concentration, the higher mortality rate.

Table1: Larvicidal activity of *M. piperita, O. basilicum*, and in combination against *Aedes* mosquito larvae at 24hrs

Test Groups	Concentrations		
	0.5 ML	1.0 ML	2.0 ML
M. piperita	81.67 ± 31.75^{b}	88.33 ± 12.58^{b}	90.00 ± 13.23^{b}
O. basilicum	11.67 ± 11.55^{ab}	15.00 ± 5.00^{b}	13.33 ± 2.89^{b}
M. piperita and *O. basilicum*	18.33 ± 2.89^{ab}	28.33 ± 2.89^{b}	76.67 ± 16.08^{c}
Control	20.00 ± 0.00^{a}	0.00 ± 0.00^{a}	5.00 ± 0.00^{a}

Means with same superscripts are not significantly different while those with different superscripts are significantly different ($p<0.05$).

Larvicidal Activity of Extracts at 48hrs

Table 2 shows the larvicidal activity of *M. piperita, O. basilicum* and the combination of both extracts against *Aedes* mosquitoe larvae at 48hrs post treatment. At 2.0ml of the extracts, *M. piperita* had highest percentage mean mortality of 90.00%, followed by combination of *M. piperita* and *O. basilicum* 86.67%). *O. basilicum* had the least larvicidal activity at different concentration of the

extract (26.67%, 31.67% and 20.00%). There is a significant difference between the test group and the control. The result shows that the higher the concentration, the higher the mortality rate.

Table 2: Larvicidal activity of *M. piperita, O. basilicum* and in combination against *Aedes* mosquito larvae at 48hrs

Test Groups	Concentrations		
	0.5 ML	1.0 ML	2.0 ML
M.piperita	85.00±25.98[b]	90.00±13.23[b]	90.00 ±13.23[b]
O.basilicum	26.67±2.89[a]	31.67±20.82[a]	20.00±5.00[a]
*M.piperita*and *O.basilicum*	21.67±7.64[a]	65.00±8.66[b]	86.67±12.58[c]
Control	40.00±0.00[a]	30.00±0.00[a]	20.00±0.00[a]

Means with same superscripts are not significantly different while those with different superscripts are significantly different ($p<0.05$)

LC_{50} and LC_{90} after 48 hours of Exposure

Table 3 shows the lethal concentration (50% and 90%) of *M. piperita* and *O. basilicum* singly and the combination extracts after 48 hrs exposure. The leaves extracts of the mixed plant also possess the highest larvicidal activity against *Aedes* mosquitoes with (LC_{90} 2.10mg ml^{-1}) and (LC_{50} 0.83 $mgml^{-1}$) followed by *M. piperita* with (LC_{90} 1.58$mgml^{-1}$) and (LC_{50} 0.01$mgml^{-1}$) and *O. basilicum* (LC_{90} 0.00$mgml^{-1}$) and (LC_{50} 0.14$mgml^{-1}$) against *Aedes* mosquitoes.

Table 3: LC_{50} and LC_{90} of *M. piperita* and *O. basilicum* singly and in combination after 48hrs exposure.

Test groups	$LC_{50}(mg\ ml^{-1})$	$LC_{90}(mg\ ml^{-1})$
M. piperita	0.01	1.58
O. basilicum	0.14	0.00
*M. piperita*and *O. basilicum*	0.83	2.10

DISCUSSION

The results indicated that *M. piperita* and *O. basilicum* had larvicidal activities and, therefore, may play an important role in reducing the use of chemicals for mosquito control. The leaf extract of *M. piperita* was found to be more effective when used singly compared to *O. basilicum*. More so, the combination of both extracts (*O. basilicum* and *M. piperita)* exhibited larvicidal activities against Aedes larvae.

The leaf extract of *M. piperita* was found to have more larvicidal activities on mosquito larvae when used singly and it was also observed that the toxicity of the extract increased when the larvae were exposed for a longer period. This correlates with the studies of Kumar et al., (2011), which revealed that the toxicity of *M. piperita* oil increased when the larvae were exposed to the oil for a longer duration. Also, *O. basilicum* was also found effective on larvae when used singly but not as *M. piperita*. This might be due to the chemical constituents present in it. *O. basilicum* has costituents of linalool, metylchavicol and metyl eugenol (Rajesh 2014).

In this present study, *M. piperita* was found to more effective than the leaf extract of *O. basilicum*. This supports the findings of Qureshi et al., (2017). Their studies revealed that *M. piperita* was significantly active to induce highest mortality in each larval stage of *C. quinquefastiatus* as compare to *O. basilicum.* This may be due to the phytochemicals present in *M.piperita* leaf extract. *M. piperita* possess a high concentration of menthol and menthone which are responsible for the substantial larvicidal of *Anopheles stephensis* (Barbalhoet al., 2017). *M. piperita* is characterized by its strong attractive odour and its oil has shown detrimental effect on insect especially mosquitoes and their larva (Ojewunmi et al., 2012). Ethanolic extract of *M. piperita* was significantly active to induce highest average mortality in each larval stage of *Aedes* as compared to *O. basilicum*.

Also, the combination of *O. basilicum* and *M. piperita* also showed a promising larvicidal activity on mosquitoes (*Aedes*) forming a synergy which may be due to the family the two plants belong to (Labiatae) and it might also be due to the chemical constituent present in both plants. Peppermint is mostly made up of methol and methone together with several other minor constituents including pulegone, mentofuran and limonene which makes the extract to have higher activity (Mudiganti, 2014; Loolaise et al., 2017). *O. basilicum* has a main constituent of linalool, metylchavicol and metyl eugenol (Rajesh, 2014).

M. piperita and *O. basilicum,* when mixed were found to be effective against mosquito larvae at a high concentration. This result is also in accordance with Qureshi et al., (2017). In the combination of both extract, *M. piperita* has a greater effect and high larvicidal activities over *O. basilicum*. At lower concentrations, extracts of *M. piperita* had higher lethal concentration when compared to combination of extracts of *M. piperita* and *O. basilicum.* The two plants showed promising larvicidal activities against mosquito (*Aedes*), but low larvicidal activity was observed in *O. basilicum*.

CONCLUSION

To date, many strategies have been explored to control the spread of malaria worldwide. These strategies either tend to abort the development of the *Plasmodium* parasites within the mosquito larvae or suppress the mosquito vector itself. The extracts of *O. basilicum* and *M. piperita* were shown to be quite potent as larvicidal agents against *A. aegypti* mosquito larvae, these results are very promising in creating new effective and affordable approaches to the control of *A. aegypti* mosquitoes and larvae. *M. piperita* has shown a promising larvicidal activity and the properties of a reliable natural product to combat mosquitoes. The current study provided two additional larvicidal agents of botanicals which might be used as alternative controlling agent to mosquito (Aedes) larvae.

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