Evaluation of the Insecticidal Effects of *Ocimum Sanctum* on Mosquito

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ABSTRACT

Leaves of *Ocimum sanctum* are widely grown and belong to the family Lamiaceae. Fresh *Ocimum sanctum* leaves were collected from some localities in Keffi Nasarawa State to evaluate their insecticidal properties on the larval, pupal, and adult stages of mosquito species. These stages were subjected to various concentrations of 1.0, 2.5, and 5.0g of burnt flesh, and dry leaves of *Ocimum sanctum* while Rambo (synthetic mosquito repellent) served as control at a time interval of 10 minutes. The study showed percentage mortality of 45, 72, 95, 47, 72, and 80% repellence respectively against 23, 25, 23, 23, 20, and 26 number of Anopheles mosquito on the larval stage of development. The pupal stage showed 72, 78, 80, 75, 88, and 90% against 22, 23, 25, 20, 18 and 22 respectively. The adult stage showed 40, 58, 78, 90, 95, and 95% repellence respectively against 20, 21, 22, 23, 24, and 23 numbers of mosquitoes per cage of treatment. The result suggested that a high concentration of 5.0g *O. sanctum* leaves showed greater repellent activity in all the net-containing mosquitoes but more with burnt dry leaves of *O. sanctum*. From the above, dry leaves of *O. sanctum* can be used as mosquito repellent plant-based products within a geographical populace. The study revealed that the essential oil of *O. sanctum* has an effect in all stages of the life cycle of mosquitoes revealing its potential as an effective insecticide against the parasite.

Key Words: *Ocimum sanctum, Rambo, Mosquito, Synthetic insecticide, Natural insecticide*

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INTRODUCTION

Since ancient times, mosquitoes have been known to be vectors of many disease-causing germs, and many diseases like yellow fever, zika fever, and dengue fever are transmitted to humans through them. Although chemical pesticides have been used to prevent and control the spread of these diseases, their negative impact on health and possible environmental pollution has led to the discovery of medicinal plants that have insecticidal properties [1, 2]. In the past years, it was found that thousands of plants can eliminate pests and insects as they have secondary receptors that can eliminate them. Research has proven that the essential oils extracted from the *Ocimum sanctum* have a superior ability to repel and eliminate insects [3].

Leaves of *Ocimum sanctum* also known as Krishna tulasi (Sanskri), tritiva (Malayan), tulshi (Marathi) are widely grown. They belong to the family Lamiaceae and have scented properties. The plant grows quickly and sprouts easily when grown indoors in moist soils and takes about eight weeks to grow. It does well in conditions of frost and regularly requires warm conditions to thrive. Their leaves may be green, purple, or yellow and are often called Holy Basil in **Figure 1** [4].

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Figure 1. Ocimum sanctum plant

Ocimum sanctum is an annual plant of 2 to 4 feet in height. The Genus Ocimum typically contains fragrant small herbs that have nearly 30 species which are mainly found in tropics and sub-tropics [5]. It has an erect, must-branch sub-shrub of 30-60 cm tall with simple, green leaves that are strongly scented. The two main morphotypes cultivated in India and Nepal are the ones with green leaves [6].

The study of plant materials usage from *Ocimum sanctum* against insects is necessary because of their great potential to complement the existing malaria control programs. They are used in Nigeria, India, and around the world as mosquito repellent owing to their physical features [7].

Ocimum sanctum is grown for its medical and culinary value. The herb has been known to have anti-bacterial, anti-fungal, and anti-viral properties and acts as an analgesic and rubefacient, and is highly useful in treating various types of diseases and in lowering blood glucose, especially in type 2 diabetes. The herb can also be used to treat cold, fever, dysentery, toothache, parasitic infestation, headache, and inflammation of joints and has been used post-harvest to protect crops from insect damage. It serves as a great mosquito repellent to prevent malaria and dengue fever [8].

It is a mild laxative and also, a vermicide and is used against worm infections. A tea prepared with Tulasi leaves is a common domestic remedy for milk indigestion, cough, cold, malaise, and reduced appetite. Because of its sharp pungent and hot attributes, Tulasi specifically affects the respiratory system-pranavahasrotasa by effectively liquefying the phlegm. It gives excellent results when used to treat cough because of kapha, eosinophilia, asthma, and allergic bronchitis. The juice works well to control hiccup when combined with honey. It is an effective panacea for fever especially of kapha type when given with marica fruit powder and honey. In such conditions, it effectively reduces pain and controls colds. Tulasi juice can also digest and destroy toxins [9].

The sanctum contains phytochemical compounds, the most important of which are rosmarinic acid (a phenylpropanoid) and ursolic acid (a triterpenoid). In addition, it contains volatile oils rich in β-caryophyllene and eugenol with minor terpenes like acetate methyl eugenol, β-elemene, bornyl, neral, β-pinene in addition to other substances such as apigenin, apigenin-7-O-beta- d-glucopyranoside, apigenin-7-O-beta- d-glucuronic acid, luteolin-5-O-beta-d-glucopyranoside, luteolin-7-O-beta-d-glucuronic acid 6"-methyl ester, luteolin-7-O-beta-dglucopyranoside, apigenin-7-O-beta- d -glucuronic acid 6"-methyl ester, 4-allyl-1-O-beta-dglucopyronosyl-2-ydroxybenzene and two known cerebrosides [10].

The plant contains secondary metabolites such as flavonoids, alkaloids, saponins carbohydrate glycosides steroids tannins triterpenoids, phenolic compound protein fixed oils [11].

Mosquitoes belong to the family of Nematocerid flies: the Culicidae. From various parts of the world, more than 3,500 species of mosquitoes have already been described. Some mosquitoes that bite humans routinely act as vectors for animal diseases when their habitat is disturbed, for instance, sudden deforestation [12].

Several scientists have suggested that complete mosquitoe eradication would not have serious ecological consequences but this is far from being achieved. The roles of various species of mosquito in different ecologies varies greatly and many are active agents in competing with other aquatic pests or recycling aquatic detritus. Practically, control measures focus on species of mosquito that are vectors of livestock or human disease, or that are seriously irritant pests. Some, like the genus Toxorhynchites' members, are actually beneficial predators of other mosquitoes [13].

Just like flies, mosquitoes have 4 stages in their life cycle: egg, larva, pupa, and imago or adult. The first three stages egg, larva, and pupa are largely aquatic. These stages last 5-14 days, which depend on the ambient temperature and the species, but there are exceptions that are important. In regions where some seasons are waterless or freezing, mosquitoes spend part of the year in diapauses, delay their development for months, and carry on only when there is enough warmth or water for their needs [14].

This study aims to evaluate the insecticidal effects of the fresh and dry leaves of *Ocimum sanctum* on mosquitoes which will be achieved through the following objectives:

1. To determine the insecticidal effects of both dry and fresh leaves on mosquitoes.
2. To determine the potency of the sample on mosquitoes.

MATERIALS AND METHODS

The first stage requires the sun-drying of the leaves in the laboratory. The second stage dealt with the evaluation of the fresh and dry leaves when applied to mosquitoes. And

the third stage was used to determine the efficacy of the *Ocimum sanctum* on the various stages of development of mosquitoes

Sample collection

Fresh matured leaves of *Ocimum sanctum* were collected from some environment in Keffi, Nasarawa State, Nigeria. A source of water was collected from stagnant ponds into nine (9) different containers in a net cage which was used to breed the mosquitoes for 3-5 days, allowing them to multiply in the laboratory followed by the evaluation of *Ocimum sanctum* leaves.

Sample drying

Some quantities of *Ocimum sanctum* leaves were collected and sprayed on a tray and dried in the laboratory at room temperature for two weeks.

Preparation of fresh and dry leaves

Equal grams (1.0g, 2.5g, and 5.0g) of fresh and dry leaves of *Ocimum sanctum* and synthetic repellent paper material (Rambo) were respectively measured in the laboratory using a weighing balance.

Cage experiment

The netting material used for this experiment was attached to three (3) different cages with sizes (7.5*7.5*15) and labeled as follows: A1, A2, and A3 for fresh leaves; B1, B2, and B3 for dry leaves and C1, C2 and C3 for synthetic material (Rambo). This was done for the different stages of mosquito; larva, pupa, and adult.

Mosquitoes were grown under conditions of humidity (70 ± 5%), photoperiod (12:12), and temperature (25 ± 5 °C). The female mosquitoes were fed Wistar rat blood while males were fed 10% sucrose solution.

Egg stages of mosquitoes were bred in the cages for 3 to 5 days to metamorphose into larva, pupa, and adult stages in the laboratory. The larval, pupal, and adult stages of the mosquito development were subjected to treatment with the concentration of 1.0 g, 2.5 g, and 5.0 g of fresh and dry leaves of *Ocimum sanctum* leaves by burning and synthetic repellent paper material (Rambo) was also applied by burning of the same concentration with the dry leaves of samples in a different cage labeled and effectiveness was determined.

A stopwatch was used to determine the time of application and time of mortality of the mosquitoes on treatment with the fresh, dry leaves and synthetic repellent paper (Rambo). Subsequently, evaluation of the insecticidal effects of *Ocimum sanctum* was accessed and results were noted and recorded immediately.

Both experiments were repeated to avoid bias in the evaluation of the effects observed with the application of synthetic repellent paper (Rambo).

Calculations

Abbot formula was used to determine the percentage efficacy of fresh and dry leaves of *Ocimum sanctum* as:

$$P.E = (NC - NT)/* 100\% \quad (1)$$

But the percentage repellency is calculated as:

Percentage Repellency (P.R) = C/T * 100%

Where:

C = Number of mosquito repellent
T = Total number of mosquitoes repellent per cage
NC = Number of mosquitoes repelled in control
NT = Total number of mosquitoes in the cage
P.R =Percentage Repellency

Statistical analysis

The Statistical Analysis of Variance (ANOVA) was used to calculate the significant difference between the samples on the application of *Ocimum sanctum* at ($p< 0.05$) on the different stages of development and at different concentrations.

RESULTS AND DISCUSSION

The result showed an effect of fresh *Ocimum sanctum* leaves on the larval stage of the mosquito. From the result, it could be inferred that when an increase in the weights or quantity of fresh leaves used (1.0, 2.5, and 5 grams) increased the rate of mosquito mortality were (45, 72, and 95%), respectively (P = 0.0016, F=22.493). The result of the effect of dry *Ocimum sanctum* leaves on the larval stage of mosquitoes and the mortality rates were (47, 72, and 80%), respectively (P = 0.008, F=29.9027). The result of the effect of Rambo (synthetic material) on the mosquito and the mortality rates were (47, 72, and 80%), respectively (P=0.0003, F=40.7339)

Also, the result of the effect of fresh leaves of *O. sanctum* on pupa stage of mosquito and the mortality rates were (72, 78, and 80%) respectively (P = 0.002, F = 50.16). There was a high rate of mortality (90%) that occurred when a high amount of dry leaves (5g) of O. sanctum was used on the pupal stage (P =0.000008, F =1057.6). That of Rambo at a concentration of 5g had the highest insecticidal activity on the pupa stage of mosquito (P =0.000008, F=1057).

Table 1 showed that the fresh leaves of *O. sanctum* have the strongest toxic effect at a concentration of 5g and had a mortality rate of 86% (P =0.0001, F=556.73).

Table 2 showed that 5g concentration of dry leaves of *O. sanctum* had a highly toxic effect on the adult stage of mosquito with a mortality rate of 95% (P =0.00002, F= 124.41157).

Table 3, in contrast, showed that synthetic Rambo has a more insecticidal effect on the adult stage of mosquito (P = 0.000.6, F= 124.41).

Table 1. Treatment of Adult Stage of Mosquito with Fresh Leaves of *Ocimum sanctum*

Cages	Weight (grams)	Time (Minutes)	Number	Mortality rate (%)
A1	1.0	10	11	47
A2	2.5	10	14	57
A3	5.0	10	20	86

P =0.0001, F=556.73

Table 2. Treatment of Adult Stage of Mosquito with Dry Leaves of *Ocimum sanctum*

Cages	Weight (grams)	Time (Minutes)	Number	Mortality rate (%)
BI	1.0	10	18	90
B2	2.5	10	20	95
B3	5.0	10	21	95

P =0.00002, F= 124.41157

Table 3. Treatment of Adult Stage of Mosquito with Synthetic Material (Rambo)

Cages	Weight (grams)	Time (Minutes)	Number	Mortality rate (%)
C1	1.0	10	12	63
C2	2.5	10	18	90
C3	5.0	10	20	100

P = 0.000.6, F= 124.41

Table 4 and **Figure 2** below show the mean ± standard deviation of the mortality rate values of mosquitoes on treatment with fresh and dry leaves of *Ocimum sanctum* and synthetic repellent material (Rambo).

The result showed that while dry leaves of O. sanctum have a stronger toxic effect on the larva stage of mosquito, its fresh leaves have a stronger toxic effect on the adult stage of mosquito whereas Rambo has a stronger toxic effect on the pupa stage of the mosquito.

Table 4. Result for Mortality Rate of Mosquito

Stages of Treatment	Dry Leaves of Cage A1,A2,A3	Fresh Leaves of Cages B1,B2,B3(grams)	Synthetic (Rambo) Material Cage C1,C2,C3(grams)
Larva	16.6 ±3.10	16.0± 2.33	12.0± 1.40
Pupa	18.0± 0.93	17.0 ± 1.50	19.0 ± 1.17
Adult	14.3 ± 1.72	19.6 ± 0.79	16.6± 2.21

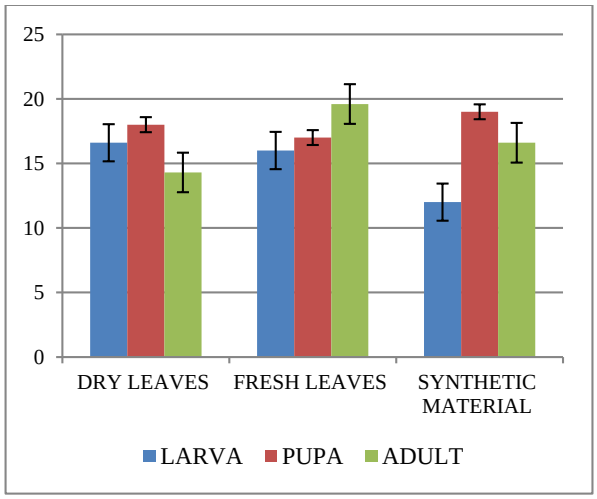

Figure 2. Mortality Rate of Mosquito

Use of plant-based repellents and increased knowledge against mosquitoes in the community suggests that local plant-derived products have some advantages over chemical insecticides or imported synthetic repellents. Synthetic chemicals like organic chlorides have been identified as ubiquitous pollutants in aquatic ecosystems and do not readily degrade in nature. Concern about the deleterious effects of synthetic insecticides and acaricides has revived scientific interest in plants as alternative insecticides for medical, protection of crop, and veterinary [15].

During the last 10 - 15 years, interest in botanical insecticides has been on the increase in search of substitutions for synthetic insecticides. The use of botanicals in insect management is not only useful for the suppression of insect population but also to maintain ecological balance. Plant-based repellents have multiple active sites in their chemical structure, which makes it difficult for development of resistance in insects and may be more economical than commercially available synthetic chemicals for use in low-income communities. Those cheap, readily available plant repellents can be promoted easily for wider community use especially in poor rural communities [16].

Medicinal plants such as *Ocimum sanctum* are the most promising source and are under extensive clinical trial for their biological activity against various stages of mosquito [17]. *Ocimum* species have been found to exhibit mosquito repellent activities against various mosquito species [18].

Malaria is a classic example of a disease that affects the productivity of individuals, families, and the world at large. It is common in the poorer and less developed countries of the world with Africa being the most affected. Other tropical areas affected include East Asia, China, and India. Thousands of synthetic insecticides have been used over the last two decades to control mosquitoes but their negative impact on health has limited their use. However,

Ocimum sanctum commonly known as holy basil has been studied for its mosquito repellent properties. Earlier studies have shown that the leaf extract of *Ocimum sanctum* protects against radiation, bone marrow, and lethality damage in mouse and has strong in vitro free radical scavenging activity [19]. More than 200 *Ocimum sanctum*-based bio-pesticides are available in the market. Ocimum products are highly photodegradable and normally degrade within a week [20].

The plant is also used for the treatment of rheumatism, high fever, epilepsy, paralysis, diarrhea, sun stoke, mental illness, gonorrhea, and influenza is also used as a spice and condiment in the southern part of Nigeria. The present work was designed to evaluate the repellent activity of the leaves extract of *Ocimum sanctum* and to explore the basis for its traditional use [21]. This was precipitated based on the fact that sweet basil has been studied for its repellent effects on *Culex pipiens* [22] which gave encouraging results.

In this study, the effect of fresh and dry leaves of *Ocimum sanctum* on evaluation has shown a drastic reduction in the number of mosquitoes at various stages upon treatment. It is observed that a 5g concentration of *Ocimum sanctum* gave a more effective treatment outcome than 1g and 2.5g on the different stages of larva, pupa, and adult mosquito. This is in agreement with Sanjaya and Safaria, [23] who decided that high extract concentrations will work faster in suppressing the activity of the nervous system of insects so that insects experience knockdown and eventually die when compared with lower concentrations.

Ocimum sanctum was studied for its physical repellent activity which showed a percentage mortality rate of 45, 72, 95, 47, 72, and 80% repellency against 22, 25, 23, 23, 20, and 26 number of mosquitoes on treatment with fresh leaves, and dry leaves were burnt around the larva stages while the synthetic material (Rambo) was used as control and it is in agreement with Singh *et al*., [24].

On pupa stage treatment there was an increasing percentage of the mortality rate of 72, 78, 80, 75, 88, and 90% repellency against 22, 23, 25, 20,18, and 22 numbers of mosquitoes per cage upon fresh and dry leaves. This is also in agreement with Singh *et al*. [24].

Adult stages of mosquitoes also showed percentage mortality of 47,58,78, 90, 95, and 95% repellency against 20, 21,22, 23, 24, 23 number of mosquitoes and synthetic repellent material (Rambo) used as control, **(Tables 1 and 2)** is in agreement with Singh *et al*. [24]. The mean and mortality rate **(Table 4)** values of mosquitoes were 16.6 ± 3.10,16.0 ± 2.33 and 12.0 ± 1.40g at larva stage on treatment with fresh and dry, and synthetic (Rambo) as control, ($p < 0.05$). At pupa, the mean and standard deviation was found to be 18.0 ± 0.93, 17.0 ± 1.50, and 19.0 ± 1.17g on treatment with fresh and dry leaves, ($p<0.05$) at different concentration. The adult stage, showed 14.3 ± 1.72, 19.6 ± 0.79 and 16.6 ± 2.21 g on treatment, ($p<0.05$).

Treatment with dry leaves clearly shows a significant difference when subjected to one-way Analysis of variance (ANOVA) when compared to the results obtained in adults, pupa, and larva stages. The concentration of 5g of dry leaves by burning shows, at ($p<0.05$) was significantly effective than fresh leaves on the stages of growth on evaluation.

A concentration of 1g and 2.5g of the sample did not show much repellent activity upon treatment with fresh, dry, and synthetic material (Rambo), but at 5g showed greater repellent activity. The high mortality rate of the leaves repellent at both high and low grams concentrations [25]. The result revealed significant ($p< 0.05$) on dry leaves application by burning and confirmed greater repellent at 5g, and not much different as of synthetic repellent paper (Rambo) on treatment. Hence, a significant difference in the three stages of mosquito is observed on evaluation on with *Ocimum sanctum* leaves both as fresh and dry by burning. This result has suggested the possibility of the leaves to give greater repellent activity on application to mosquitoes, in a similar observation with Chokechijaroenporn *et al*. [26].

The toxic effect of Ocimum is due to sanctum leaves, which are transmitted to the insect through the olfactory centers and then transmitted to the brain through the sensory axons, and because the leaves contain phytochemical compounds such as alkaloids, flavonoids, and essential oils that reach the respiratory system and affect the acetylcholine receptors in the nervous system [27].

It works by inhibiting the enzyme acetylcholinesterase, which is the most important enzyme in insects. Acetylcholine works to transmit impulses from nerve cells to muscle cells, and then Acetylcholine is converted to acetic acid and choline. And under the influence of the work of O. sanctum leaves were inhibit the acetylcholinesterase enzyme, and then the accumulation of acetylcholine occurs, and consequently, an imbalance occurs in the pulsating system of muscle cells, causing muscle spasms of the insect. In addition to the presence of flavonoids, they affect the respiratory system, affecting the breathing process, which leads to the death of the insect [28].

CONCLUSION

This present work was been designed to evaluate the mosquito repellent (insecticidal or biocidal) activity of leaves of *Ocimum sanctum* and to explore the basis for its commercial use. O. *sanctum* is classified as a biocidal plant that has effective toxicity on mosquitoes especially when used in large quantities due to the phytochemical

compounds containing it. From the study carried out, *Ocimum sanctum* is potent on different stages of mosquitoes when used in its dry form by burning.

The results show that using fresh leaves of *Ocimum sanctum* is more effective than dry leaves and synthetic material in weight against the larva stage of the Anopheles mosquito. Its effectiveness on the three stages of mosquito development: larva, pupa, and adult have shown that mosquitoes can be reduced in our environment without any fear of the photochemical effect of the insecticide used on humans. *Ocimum sanctum* has a wide range of applications. Therefore, this study has shown that although both fresh and dry leaves of *Ocimum sanctum* are effective in repelling mosquitoes, its repellent activity is more potent against mosquitoes when the dry forms are burnt but the fresh ones are more potent against the larva stages but the best insecticidal for pupa stage of mosquito is the synthetic material (Rambo).

Based on this study, the authors recommend that further research be carried out to identify the particular properties of *Ocimum sanctum* that could be harnessed to develop products that have more repellent activity and value for the Nigerian populace and Africa in general.

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