



Effect of Extracts of *Ocimum Gratissimum* L. (Scent Leaves) on Some Mosquitoe Genera in Makurdi Metropolis, Benue Nigeria

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Abstract

Ocimum gratissimum is a plant with so many uses-culinary, medicinal, pesticidal, insecticidal, aesthetic. This research was designed to find out repellence, knockdown and knock-dead activity of hot and cold aqueous and ethanol crude extracts against mosquitoes of three genera-Culex, Aedes and Anopheles sp. *Ocimum gratissimum* leaves were harvested from different locations in Makurdi. Leaves were picked, washed, and dried crispy for seven (7) days under shade. Crispy dried leaves were grinded into fine powder. The powdered was weighed and divided into four equal parts. Each part was subjected to hot and cold aqueous and ethanol extraction. Extracts were concentrated with rotatory evaporator. Concentrates were allowed to dry in the open at room temperature. 2, 3 and 5g of extracts were weighed and used to make different concentrates; each in 5ml of Absolute ethanol. These concentrates were tested against lab bred mosquitoes for repellency, knockdown and mortality, using the World Health Organization Pesticide Evaluation Scheme. The results showed complete repellence to all extract types, increased knock down with increased concentration and variable mortalities to the different extract methods. Based on these, *Ocimum gratissimum* extracts can be used locally and as an alternative to synthetic chemicals in the production biodegradable and eco-friendly mosquito repellent, and "mosquitocidal" formulations.

Keywords: *Ocimum gratissimum*; Mosquitoes; Repellant; Knockdown; Mortality

Abbreviations: CWE: Cold Water Extracts; HWE; Hot Water Extract; CEE: Cold Ethanol Extract; HEE: Hot Ethanol Extract.

Introduction

Mosquitoes are one of the most disturbing blood suckers afflicting man. Mosquitoes are very important primary hosts in the spread of malaria, yellow fever, filariasis and several arboviral infections; because females are anautogenous-requiring blood meal, before oviposition [1]. One of the important aspects of the fight against malaria and other mosquito-borne diseases is prevention of mosquito-man

contact in reducing or curbing the spread of mosquito-borne diseases [2]. Efforts have been made to prevent and or reduce mosquito contact with man. Some of these efforts include the use of untreated mosquito nets, treated mosquito nets, and treated clothing for outdoor lovers, synthetic chemical irritants (insecticides) and [3,4].

Conventionally, mosquito control relies heavily on synthetic insecticide application over decades (they have speedy act/ion and are easy to use); but, over use and injudicious application of synthetic insecticides has resulted in resistance and unwarranted toxic or lethal effects on non-target organisms, as well as environmental health problem.

Hence, there is a constant need for developing biologically active plant materials which are expected to reduce hazards to humans and other organisms by minimizing the accumulation of harmful residues in the environments insecticides. Several synthetic chemicals e.g. pyrethroids, diethyltoluamide (DEET), have been used with success in repelling mosquitoes [2]; but for their harmful effects to the environment and other important, non-target organisms, insects avoidance of treated surfaces and resistance to known and once potent insecticide/repellents; the need has arisen over the years to address the short comings of already existing vector- man control/prevention methods.

The search for potent and environmentally friendly chemical substances holds a lot of promise for the development of biologically/environmentally friendly repellents.

This study is to probe further, the claims of past laboratory reports on the insecticidal abilities of *Ocimum gratissimum*(L) extracts on mosquitoes (of a the *Aedes*, *Culex* and *Anopheles* genera). *Ocimum gratissimum* in Igbo (Eastern Nigeria) is called "nchuanwu" which means mosquito repellent, hence the need to verify the claim.

Materials and Methods

Leaf Collection

Ocimum gratissimum leaves were collected from different locations in Makurdi metropolis within the months October-November 2017. The leaves were then taken for identification at the Postgraduate Laboratory of Biological Sciences Department, of Benue State University.

Preparation of Leaf Extract

The plant material had their leaves plucked, washed in water and shade dried for 1 week [5-7]. The crispy dried leaves were then ground to fine powder, with a blender [6]. The powder was then stored in air tight containers pending the time of extraction [5,8].

Extraction

Ocimum gratissimum extraction was done according to methods of Ojo, et al. [5] Gupta, et al. [8], with modifications; Ethanol Absolute, was poured into the container, just sufficient to cover the handkerchief. The plastic was covered and left to stand for 72hours [9].

Concentration of Extracts

All the extracts- cold and hot water; cold and hot ethanol extracts where then concentrated using the Rotary

Evaporator, to recover the solvents (water and ethanol) leaving behind the various extract [8].

Larva Collection/Rearing

Larva collection and rearing was done in the months of December 2017-January 2018. With the aid of a scoop spoon, mosquito larvae of *Aedes*, *Culex* and *Anopheles* genera were carefully collected from different breeding sites, from the wild, around Makurdi. (Along UniAgric-Gbajimba Road, New Otukpo Road and behind Federal Low-cost Housing Estate, Naka road) [10].

Larva was then fed with baker's yeast, by sprinkling sparsely over the surface of the water containing larva [6]. The container of larvae was put into premade cages measuring 16x16x16 inches, with mosquito nets, to retain emerging adults [10].

After 2 days, adults begin to emerge in large numbers. Cotton balls soaked in sugar solution were attached to different points in each cage. Adults could be seen, feeding on sugar solution daubed in cotton wool Cotton balls daubed in sugar solution was changed daily to prevent microbial contamination [6,10].

Bioassay

The laboratory method used to test the efficacy of the extracts on the mosquito genera under consideration, for repellence, knockdown and knock dead rates, is the standard recommended by the World Health Organization Pesticide Evaluation Scheme [11]; as employed by Obisike, et al. [10] with modifications. Two (2g), 3g and 5g of each of the extracts were weighed into vials. The extracts were not soluble in water; so ethanol was used to dissolve the concentrations. 5mls of absolute ethanol was used to dissolve 2g, 3g and 5g of each extracts contained in each vial; for hot water, cold water, hot ethanol and cold ethanol extracts respectively. All the extracts were made ready preparatory to bioassay with an aspirator, 5 mosquitoes were collected from the holding cages. A ball of cotton was used to plug the mouth of the aspirator (to relieve the aspiration stress on the mosquitoes in the aspirator for 5 minutes). A wattmans No 1 filter paper was used as the test membrane. 2g, 3g and 5g of each extract, was rubbed onto a correspondingly labeled filter paper. The ethanol solvent allowed evaporating for 2 minutes. Standard WHOPES [11] cones were placed over the treated filter papers and taped round with masking tape to make it mosquito tight. The mosquitoes were introduced into the cones, with the aid of the aspirator. The mouth of the cone was then plugged with cotton. Mosquitoes were observed for repellence; within 3minutes of exposure to treated surface. Mosquitoes were transferred to correspondingly labeled,

transparent (disposable) cups, covered with fine net held with rubber bands. They were observed every 10 minutes for knock down, for a total period of 1hour while mortality was observed after 24hours as recommended by WHOPES [11]. The procedure was repeated for each extract concentration, for each of the 3 location in Makurdi metropolis, under consideration.

Morphological Identification

After each test, the knock-dead mosquitoes were stored in vials containing premade 70% Ethanol [12]. The labels and concentrations used during the test were maintained. Microscopic examination for the identification of the Mosquito Genera under consideration was done according to the Photographic Guide To Common mosquitoes of Florida (Cutwa and O'Meara), University of Florida, Florida Medical Entomology Laboratory, since the use of a hand lens did not

suffice.

Results

Tables 1 & 2 show the Repellence, Knockdown, and Knock-dead (Mortality) of different concentrations of Cold Water extracts (CWE) and Hot Water extract (HWE) respectively at different times and replications for locations Logo2/Kanshio, Karmen Village and UniAgric- Gbajimba Road, North Bank. There was no significant difference for repellence and knock-dead rates; except for knockdown rates that showed clear significant differences at $p = 0.008$ for CWE at the Logo/ Kanshio village.

Tables 3 & 4 show Cold Ethanol Extract (CEE) and Hot Ethanol extract (HEE) at different times and replications for Logo2/Kanshio, Karmen Village and UniAgric-Gbajimba Road, North Bank. The knockdown rate was significantly high as the concentration increases.

Concentration (g/5ml)	No of Mosquitoes	Repellence (3 mins)	Knockdown (1 hour)			Knock-dead (24hrs)
		% mean replicates	10 mins	20 mins	Total No and % at 60 mins	% mean replicate
A 2.00	5	5 (100)	1.00 ^a	1.00 ^a	1.00 ^a (6.67)	12(80.00)
A 3.00	5	5 (100)	4.00 ^b	4.00 ^b	6.00 ^b (40.00)	13(86.67)
A5.00	5	5 (100)	4.00 ^b	7.00 ^c	8.00 ^c (53.33)	14(93.33)
B 2.00	5	5 (100)	6.00 ^a	6.00 ^a	6.00 ^a (40.00)	10(66.67)
B 3.00	5	5 (100)	6.00 ^a	6.00 ^a	6.00 ^a (40.00)	10(66.67)
B 5.00	5	5 (100)	5.00 ^b	8.00 ^a	7.00 ^a (46.67)	13(86.67)
C 2.00	5	5 (100)	6.00 ^a	6.00 ^a	9.00 ^a (60.00)	15(100.00)
C 3.00	5	5 (100)	3.00 ^b	7.00 ^a	8.00 ^a (53.33)	13(86.66)
C 5.00	5	5(100)	7.00 ^a	9.00 ^a	11.00 ^b (73.33)	15(100.00)

Means with different superscript are statistically significant at $p = 0.05$.

Key: A= Logo 2 / Kanshio village, B= Karmen Village and C= UniAgric- Gbajimba Road, North Bank.

Table 1: Effects of Cold Water Extract (CWE) against Mosquitoes.

Concentration (g/5ml)	No of Mosquitoes	Repellence (3 mins)	Knockdown (1 hour)			Knock-dead (24hrs)
		% mean replicates	10 mins	20 mins	Total No and % at 60 mins	% mean replicates
A 2.00	5	5 (100)	2.00 ^a	2.00 ^a	4.00 ^b (26.67)	10(66.67)
A 3.00	5	5 (100)	5.00 ^b	5.00 ^b	5.00 ^b (33.33)	10(66.67)
A5.00	5	5 (100)	2.00 ^a	2.00 ^a	2.00 ^a (13.33)	13(86.67)
B 2.00	5	5 (100)	6.00 ^a	6.00 ^a	1.00 ^b (6.67)	10(66.67)
B 3.00	5	5 (100)	7.00 ^a	7.00 ^a	9.00 ^a (60.67)	11(73.33)
B 5.00	5	5 (100)	7.00 ^a	7.00 ^a	8.00 ^a (53.00)	13(86.67)
C 2.00	5	5 (100)	2.00 ^a	5.00 ^b	8.00 ^c (53.33)	13(86.67)
C 3.00	5	5 (100)	7.00 ^b	7.00 ^c	11.00 ^d (73.33)	13(86.67)
C 5.00	5	5 (100)	8.00 ^b	9.00 ^b	11.00 ^d (73.33)	14(93.33)

Means with different superscript are statistically significant at $p = 0.05$.

Key: A=Logo 2/Kanshio village, B=Karmen Village and C=UniAgric-Gbajimba Road, North Bank.

Table 2: Effects of Hot Water Extract (HWE) against.

Concentration (g/5ml)	No of Mosquitoes	Repellence (3 mins)	Knockdown (1 hour)			Knock-dead (24hrs)
		% mean replicates	10 mins	20 mins	Total No and % at 60 mins	% mean replicates
A 2.00	5	5 (100)	5.00 ^a	5.00 ^a	5.00 ^a (33.33)	10(66.67)
A 3.00	5	5 (100)	9.00 ^b	9.00 ^b	8.00 ^b (53.33)	12(80.00)
A5.00	5	5 (100)	10.00 ^b	10.00 ^b	11.00 ^b (73.33)	14(93.30)
B 2.00	5	5 (100)	7.00 ^a	7.00 ^a	8.00 ^a (53.33)	11(73.33)
B 3.00	5	5 (100)	6.00 ^a	8.00 ^a	9.00 ^b (60.00)	12(80.00)
B 5.00	5	5 (100)	7.00 ^a	7.00 ^a	7.00 ^a (46.66)	13(86.67)
C 2.00	5	5 (100)	5.00 ^a	7.00 ^a	9.00 ^b (60.00)	13(80.00)
C 3.00	5	5 (100)	6.00 ^a	8.00 ^b	8.00 ^b (53.33)	14(93.33)
C 5.00	5	5 (100)	5.00 ^a	7.00 ^a	9.00 ^b (60.00)	15(100.00)

Means with different superscript are statistically significant at $p=0.05$.

Key: A= Logo 2 / Kanshio village, B= Karmen Village and C= UniAgric- Gbajimba Road, North Bank.

Table 3: Effects of Cold Ethanol Extract (CEE); against Mosquitoes.

Concentration (g/5ml)	No of Mosquitoes	Repellence (3 mins)	Knockdown (1 hour)			Knock-dead (24hrs)
		% mean replicates	10 mins	20 mins	Total No and % at 60 mins	% mean replicates
A 2.00	5	5 (100)	12.00 ^a	12.00 ^a	12.00 ^a (80.00)	12(80.00)
A 3.00	5	5 (100)	7.00 ^b	8.00 ^b	11.00 ^a (73.33)	14(93.33)
A5.00	5	5 (100)	7.00 ^b	9.00 ^b	6.00 ^c (40.00)	15(100.00)
B 2.00	5	5 (100)	6.00 ^a	7.00 ^a	11.00 ^b (73.33)	12(80.00)
B 3.00	5	5 (100)	5.00 ^a	7.00 ^a	10.00 ^b (66.67)	15(100.00)
B 5.00	5	5 (100)	7.00 ^a	7.00 ^a	8.00 ^a (53.37)	15(100.00)
C 2.00	5	5 (100)	7.00 ^a	8.00 ^a	9.00 ^a (60.00)	13(86.67)
C 3.00	5	5 (100)	6.00 ^a	6.00 ^a	8.00 ^a (53.33)	13(86.67)
C 5.00	5	5 (100)	7.00 ^a	9.00 ^a	10.00 ^b (66.66)	14(93.33)

Means with different superscript are statistically significant at $p=0.05$.

Key: A= Logo 2 / Kanshio village, B= Karmen Village and C= UniAgric- Gbajimba Road, North Bank.

Table 4: Effects of Hot Ethanol Extract (HEE); against Mosquitoes.

Discussion

Malaria, dengue fever, and yellow fever, in the tropics are still serious public health concerns. These diseases are transmitted to human beings through the bite of infected female mosquitoes usually; occasionally through blood transfusion. Using repellents for prevention of infected mosquito-man contact is one of the main strategies to control or minimize incidence, morbidity and mortality of mosquito-borne diseases.

Preventing mosquito-borne diseases is important not only for people in prone areas like Nigeria but also for vulnerable visitors who tour for leisure away from their

home countries. Many mosquito formulations have been made containing essential oil of plant materials [9].

Repellence

Following the World Health Organization Pesticide Evaluation Scheme [11]; irritant or exito-repellence effect of different concentrations of hot/cold aqueous and alcohol extracts were tested against mosquitoes. At concentrations of 2, 3 and 5g/ml for all the locations(NNPC Mega station/ Kanshio, Karmen village, Behind Federal Low-cost Estate Naka road, and UniAgric-Gbajimba road, North Bank, all in Makurdi.) mosquitoes showed complete (100%) repellence to all the extract types; Cold Water Extract(CWE), Hot Water

Extract (HWE), Cold Ethanol Extracts (CEE) and Hot Ethanol Extract (HEE). This agrees with related works of Singh, et al. [13], where petroleum ether extracts of *O. sanctum*, showed repellence against mosquitoes of the *Aedes*, *Culex* and *Anopheles* genera, at all the concentrations used.

On the other hand, related studies as reported by Maia, et al. [14], showed variable repellencies of different genera and species of mosquitoes to the essential oils of different species of *Ocimum* extracts. These differences may be attributable to the proportions phytochemical constituents of the different *Ocimum* species, the concentrations of extracts used in the tastings and the nature of the research (laboratory based or field based).

Odomos (12% DEET), a conventional mosquito repellent was used as positive control to compare the test results with. Results showed complete repellence like the Odomos repellent.

Knockdown: Cold water extracts (CWE): Knockdown was also evaluated according to WHOPEs [11]. For CWE, mosquitoes from north bank showed the highest knockdown of 11(73.33%) after a 1 hour recovery period for 5g/ml concentration of CWE. Consequently, mosquitoes from Logo II/Kanshio, recorded the lowest knockdown rate 1(6.66%) at concentration of 2g/ml of CWE.

However, generally across board, knockdown trend was found to increase with increase in time and concentration with the P-values only significant for Logo II/Kanshio samples ($P < 0.05$); for other locations $P > 0.05$, hence not significant despite observable knockdown.

Hot Water Extracts (HWE)

Still following the procedure of WHOPEs, [11]. For HWE, mosquitoes from North Bank still showed the highest knockdown of 11(73.33%) after a 1 hour recovery period for 3 and 5g/ml concentration of HWE; mosquitoes from behind Federal Low-cost estate, Naka road, recorded the lowest knockdown rate 1(6.66%) at concentration of 2g/ml of HWE. The knockdown trend across board was still found to increase with increase in time and concentration; with the P-values still only significant for Logo II/Kanshio samples ($P < 0.05$; $P = 0.04$); for other locations $P > 0.05$, hence not significant despite observable knockdown.

Similarly, per time, knockdown was insignificant across the table for Karmen Village behind Federal Low-cost estate, Naka road and also not significant per concentration, down the table at 0.05 level of significance.

Cold Ethanol Extracts (CEE)

For CEE, mosquitoes from Logo 2/Kanshio, showed the highest knockdown of 11(73.33%) after a 1 hour recovery period for 5g/5ml concentration of CEE and the lowest knockdown of 5(33.33%) at 2g/ml. comparatively, for CEE, knockdown in Karmen Village and North bank had values all within the lowest and highest range above.

The difference between the mean knock down rate was not statistically significant at $P < 0.05$; $P = 0.33$. The knockdown trend across board was still found to increase with increase in time and concentration; P-values were generally not significant per time across the tables (i.e., $P > 0.05$) but for concentration down the table, P-values were generally significant down the table, for all locations.

Hot Ethanol Extracts (HEE)

The highest knockdown for HEE, was 12(80.00%) at 2g/5ml. the lowest being 6(40.00%) at 5 g/5ml. This anomaly is probably as a result of experimental error (some mosquitoes were able to temporarily recover, during the 1 hour recovery period; some were accidentally crushed or managed to escape during transfer to recovery cups after repellency testing, for the whole experiment)

Observably, HEE, has the highest set of numbers and percentages compared to the other extracts- with ranges all within 40 and 80%. P-value for knockdown was significant; $P < 0.05$; $P = 0.00$. The mean differences for knock down rates were generally not significant ($P > 0.05$) across locations, but significant for concentration down the group for Logo II/Kanshio and some significant for the other locations.

Summarily, across all locations, the number of knockdown was found to increase with increase in time and concentration. Percentage knockdown under the 60 minutes recovery period ranged from 40.00%-3.33% (CWE), 26.66%-73.33% (HWE), 33.33%-73.33% (CEE) and 40.00%-80.00% for (HEE). These agree with a related work of Obisike, et al. [10]; which stated that mosquitoes showed less than 95% knockdown to nets treated with Pyrethroids after a 60 minutes period. This is though contrary to the works of Kazembe, et al. [15], whose laboratory tests of *O. americanum* for knockdown on mosquitoes was negative; prompting further tests to see if Zimbabwean grown *Ocimum gratissimum* could knock down mosquitoes as reported in preliminary studies done in Nigeria. Ogendo, et al. [16] stated that, the actual chemical composition of *Ocimum* is a function of species, chemo type, climate, soil conditions and geographical location. This agrees with Orwa, et al. [17] who reported different Eugenol levels for different countries around the world, from extracts of *Ocimum gratissimum*. This probably explains where same plant shows negative knockdown results in some places and positive in others.

Knock-Dead (Mortality) Cold Water Extracts (CWE)

Knock-dead (mortality) was checked for after 24 hours of treatment/exposure according to WHOPEs [11] procedures. Knock-dead rates for CWE ranged from a low of 66.66%-100% for all locations. Though their p-values were statistically not significant ($P > 0.05$) for all locations; $P = 0.296$, $P = 0.512$ and $P = 0.422$ for Logo II/Kanshio and North Bank respectively mortality was recorded.

Hot Water Extracts (HWE)

Mortality for HWE, had the same least range value of 66.66% for CWE, but an upper value 93.33%, less than that of the CWE (100%) Like the mortality for CWE, the P-values for all locations were statistically insignificant as $P > 0.05$. Variance=0, so P-value couldn't be determined by the software for Logo II/Kanshio; $P = 0.58$ for Karmen Village and $P = 0.85$, North Bank respectively.

Cold Ethanol Extracts (CEE)

The range of mortality for CEE showed the highest of 100% and the lowest mortality of 66.66% across locations. It was observed that the percentage mortalities in-between the range (66.66-100%), were higher than those of CWE, and HWE. In spite of these, P-values were still statistically not significant at 0.05 level of significance.

The respective P-values are $P = 0.53$ for NNPC-Mega station; $P = 0.32$ for behin+d Federal Low-Cost, Naka road and $P = 0.31$ for North Bank respectively.

Hot Ethanol Extracts (HEE)

Knock-down rates for HEE, had the highest range of values 80.00%-100.00% across locations when compared to all the other extract types. This may be attributed to the Soxhlet extraction process with was more controlled than all other methods of extraction. HEE showed the highest knock-down rates compared to all the extract kinds for all locations. Mortality was found to increase with increasing concentration of HEE extracts.

P-values for all locations are as shown below: Logo II / Kanshio; $P = 0.07$, Karmen Village; $P = 0$, North Bank; $P = 0.85$ in general, the results discussed above agree with similar works of Obisike, et al. [11], who found knockdown to be slightly above 97% using Pyrethroids as treatment on nets for knockdown.

Generally, all the mosquitoes of the test genera showed complete repellence to the different concentrations and

extraction methods of crude extracts of *Ocimum gratissimum*, unlike some studies across Africa, using essential oil of extracts of some members of the 'mint family' which showed knockdown to specific species of mosquitoes.

Following the standard recommendations of WHOPEs [11], a good number of mosquitoes of the test genera were knocked down after a three (3) minutes exposure to extracts and sixty (60) minutes observation for recovery in holding cups. This was indicated for all the concentrations used for mosquitoes from all the locations under the research cover. The smoke toxicity test confirms the efficacy of the extracts and gives credence to the basis for folkloric use of the *Ocimum* species of plants as a mosquito repellent. All mosquitoes were knocked-dead by 30 minutes; before the recommended time of 40 minutes on exposure to the smoke from burning mosquito-cakes containing, CWE, HWE, CEE and HEE [6].

Unlike this research, that showed complete repellence of mosquitoes of the *Aedes*, *Culex* and *Anopheles* genera to all extracts of *O. gratissimum* regardless of the method of extraction, most other literatures consulted reported species-specific activity for both extracts and mosquitoes.

The mechanism of action of the formulated repellents in this study is not yet known, but other evidences suggest that repellent molecules reduce mosquito-host contacts by interacting odorant receptors, thereby affecting olfactory-driven behaviors [9].

Comparatively, repellence, knockdown and knock-dead rates seemed to be directly proportional to increasing concentration of the extracts. i.e., the effects of the extracts increased with increased concentration. Based on the total number of mosquitoes of the test genera identified by microscopy, those of the *Aedes* genera showed the most susceptibility to the extracts of *Ocimum gratissimum* with a total of 186 (34.63%); the *Anopheles* genera was least susceptible amounting to 167(31.09%). Those of the *Culex* genera stood in the middle with 184(34.26%) susceptible out of a total of 537 identified mosquitoes.

Conclusion

In conclusion, the use of repellents in this age of global travel may reduce the occurrence of mosquito-borne diseases, and impact greatly on morbidity and mortality rates. In countries like Nigeria, where conditions favor mosquito breeding for most of the year, people may use creams formulated with *O. gratissimum* alone or in combination as mosquito repellents in the place of synthetic insecticides/repellents, to increase their pleasure, comfort and stay free from mosquito-borne diseases.

For military, paramilitary and other occupationally disposed people, the use of this kind of repellents may reduce diseases and annoyance to personnel, hence impacting on productivity and output; while leaving behind no toxic after infects to humans, the environment and other non-target organisms [18].

However, removing the brown and green colors from the extracts would enhance a better cosmetic/ aesthetic appeal. Furthermore, more tests needs to be carried out to verify the range of genera/species that are susceptible to the extracts, to establish the “specificity” or “broad-spectrum” activity against mosquitoes generally.

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