Field Efficacy Evaluation of some Ethnomedicinal Plants Smoke Repellency Against *Anopheles arabiensis* and *Aedes Aaegypt*

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Research

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Abstract

Background: Plant products can either be used as an insecticide to kill larvae or adult mosquitoes or as defensive repellents against mosquito bites; depending on the type of activity they have. A present study was carried out to determine the repellence of smoke prepared from burning the plants in a traditional way in order to reduce human-mosquito.

Methods: The candidate plant species (*Azadirachta indica*, *Eucalyptus camaldulensis*, and *Ocimum forskolin*) leave was grounded and mixed together in an equal amount. The mixed plant powders were checked by the thermal expulsion and burning on the traditional stoves in the field against two important malaria vectors (Anopheles arabiensis and Aedesaegypt). A four-by-four Latin-square design was used to randomly assign treatment and control experimental huts over different nights. In the treatment and control huts, the percentage repellence of the candidate plants mixed powder smoke on mosquito was determined from mosquito catches.

Results: On direct burning of the plants' powder, the highest percentage repellency for *An. arabiensis* (94.7%, P<0.001) and *Aedesaegypt* (88.4%, P<0.001) recorded. On thermal expulsion also the highest against *An. arabiensis* (89%, P<0.001) and against *Aedesaegypt* (92%, P<0.001) were recorded. The plants' mixed powder tested by both methods of application offered significant protection (>85%) against two important mosquito species and thus has the potential to be used at least as an alternative way to control mosquito infestation in an easy manner in economic level.

Conclusion: The two methods of application may offer cost-efficient alternatives as additional household protection and as a helpful addition to bed nets, especially for the early part of the evening before bedtime.

Background

Insects are known to cause humans, animals, and plants extensive damage. The planet has well over 1 million different recognized insect species, and it is estimated that there could be as many as 10 million [1]. Mosquitoes represent the primary vector for the spread of diseases to more than 70 billion people every year around the world [2]. According to reports from the World Health Organization, malaria alone kills 30 million people per year [3]. Mosquitoes spread the arboviruses that cause yellow fever, dengue hemorrhagic fever, epidemic polyarthritis, and various forms of encephalitis [4]. Malaria kills 3 million people per year worldwide, including 1 child every 30 seconds [5]. An efficient vaccine has not been available for protection from these diseases still now, except that of yellow fever and Japanese encephalitis. Therefore, protection from mosquito bites is one of the best strategies to prevent these diseases or reduce their incidence.

DDT (dichlorodiphenyltrichloroethane) is a toxin that used widely to get rid of insects such as mosquitoes in Ethiopia. In the country, the use of DDT in malaria prevention has resulted in tremendous hope as the pesticide has been shown to be highly effective in killing the malaria vectors and interrupting their transmission due to its spatial repellence and annoying effect. In the environment however, *p,p*'-DDT is degraded into *p,p*'-DDE (1,1-Dichloro-2,2-bis(4-chlorophenyl)ethylene) and *p,p*'-DDD (1,1-Dichloro-2,2-bis(4-chlorophenyl)ethane), in which *p,p*'-DDE being more persistent than the parent compound. This persistence, caused by its high lipophilicity and low reactivity, provides the conditions necessary for bioaccumulation in organisms and biomagnification in food webs. In such a way, DDT is highly toxic to aquatic organisms, fish and amphibians, and carcinogenic and mutagenic effects in other non-target organisms [6, 7]. DDE, the main DDT metabolite, causes embryo deaths in predatory birds by thinning the eggshell [6, 8]. Lifetime DDT therapy caused a dose-related liver tumor in mice. DDT also increased lung tumor incidences in another mice study. DDE and DDD in mice are also carcinogenic [9]. According to Chen *et al*. [10], DDT and its metabolites have estrogenic activity and DDE has been shown to act as an androgen antagonist [11].

In the past few years, to overcome this problem, thousands of plants have been tested as sources of insect repellents or killers. Citronella, neem, cedar, verbenas, pennyroyal, geranium, lavender, pines, cajuput, catnip, cinnamon, rosemary, basil, thyme, allspice, garlic, and peppermint plants have been identified by means of both grassroots and scientific investigations against various hematopoietic arthropods [12, 13]. Smoke from some plants acts to repel Anopheles mosquitoes: 90.1% by *Ostostegiaintegrifolia* [14, 15], 79.8% by *Olea europaea* and 44.5% by Ocimum *suave* [16, 17]. The leaves of *Ocimumcanum* provided 63.6% protection from mosquito bites when hung fresh in the homes in Guinea Bissau, West Africa [18].

In addition to the harmful effects of synthetic products mentioned above, the malaria vectors in Ethiopia are currently developing high to moderate levels of DDT resistance and some other pyrethroids [19]. Plant-based products are believed to be safer than synthetic insecticides [20, 21]. However, plant-based products have so far received little attention in Ethiopia despite the other world. This study was carried out in this regard to evaluate the field efficacy of some ethnomedicinal plants smoke repellency against *Anopheles arabiensis* and *Aedes aegypti*by direct burning and thermal expulsion application methods in the Diguna Fango District, Wolaita, Ethiopia.

Methods

Study area

The study was conducted in small villages of Diguna Fango District located at 8.25'N and 39'01'E in Southern Region, Wolaita zone, Ethiopia. The area is located about 95 km south of the capital Addis Ababa, and 63 km from east of Wolaita Sodo town, where the Bilate River crosses the main road to Sidama, Ethiopia at an altitude of 1700 m above sea level. The Bilate River during the rainy season creates swampy and more or less permanent large pools of water. The average annual rainfall and temperature of the area are about 700 mm and 21^{0}C, respectively.

Test plants

Due to their bioactive compound content and various reports on their traditional uses as an effective vector repelling function, three candidate plant species (*Azadirachta indica*, *Eucalyptus camaldulensis*, and *Ocimum forskolin*) were selected for field efficacy studies [22–23, 16].

Preparation of plants for smoke toxicity test

The dried candidate plant species leave was collected and ground by grinder mill. Then, thoroughly mixed evenly and made available for experimentation.

Experimental houses selection

To study the repulsing effect of the smoke from three candidate plant species mixed powder on mosques, eight traditional village houses (huts) were selected from two nearby kebeles (small units of Districts); such as Fango Boloso and Fango Ofa randomly four from each of them in 20 m intervals. Four huts were used to determine the field efficacy of plants by direct burning and the other four were used to determine the efficacy of the application of plants by thermal expulsion. All experimental huts were mud-walled with grass-thatched roofs ('Sar-bet' in the Amharic language, which is the national official language of Ethiopia) in which open eaves, unscreened small holes, and doors allow ready access to mosquitoes from the shoreline of river Bilate which is extremely infested with mosquitoes and necessarily common area for their reproduction.

Repellency tests

Field evaluation of plant powder smoke repellency was performed with 3 local volunteers (only males between 15 and 25 years of age) who participated in the experimentation. On selected experimental huts, pre-weighted plants powder was put on top of a thin hot metal plate which was placed immediately above the burning charcoal in the traditional stove for thermal expulsion method and for direct smoking method; the plant powder was placed directly on the burning charcoal according to the work of Seyoum *et al*. [22, 16]. The huts were fumigated by the smoke from 7:00 p.m. to 11:00 p.m. The volunteers were sat on chairs in the treatment huts in liner by the 1-meter interval between them and collected all of the mosquitoes landing on their legs and arms for a 20 minute. Each exposure period was followed by a 5 min break before the next mosquito collection was conducted. Thus, on the first night, subjects wearing a T-shirt and shorts trousers exposed their untreated arms and legs to assess the density of biting/landing mosquitoes. The subjects (volunteers) were continuously exposed to the biting mosquito while adding 50 g of the powder directly on the traditional stove (for direct burning) and collected mosquitoes landing on exposed parts of the body using small test tubes and torchlight while being supervised by the investigators.

The same procedure was followed to check for thermal expulsion. In the case of thermal expulsion, the powder was put on top of a thin metal strip placed immediately above the lightened charcoal. The experimentation was carried out for about four hours. As a precaution, mosquitoes were immediately removed after landing, before the commencement of feeding. The hourly collections of each volunteer were counted and grouped the removed mosquitoes during the fumigation of individual huts by both methods of application based on their morphological difference for species identification. Mosquitoes have been characterized by morphological properties at the species level [24]. A four-by-four Latin-square design was used for both experiments in three treatment huts and one hut with only burning charcoal stove without any smoke treatment to serve as a control. The treatment and control huts were randomly assigned by rotation in consecutive treatment nights. The tests were carried out with an intervening period of one night to avoid the potential residual effect of the plants by periodic thermal expulsion and direct smoking. The tests were replicated for four treatment nights for both thermal expulsion and direct burning experiments.

Data analysis

The overall repellent effect of the smoke from the mixed powder of the plants was analyzed by allowing for the differences between experimental units by generalized linear modeling (ANOVA) of the relationship between mosquito collections in control (C) and in treatment huts (T). The repellence index (R) was estimated as % R = (C-T)/C × 100%, where C and T are the mean number of mosquitoes landing on the control and the treatment hunts, respectively [25]. Tukey’s test was conducted to compare responses to the smoke repellency in the landing assays by using Minitab® 18 statistical packages for Windows, version 10.

Results

The overall repulsive effect of the smoke over the four treatment nights in individual huts by two methods of application and the relative repelling effect of smoke by direct burning against two biting mosquito species (*Aedes aegypt* and *An. arabiensis*) over four treatment nights are shown in Table 1 and 2 below. The comparative repelling efficacy of smoke against two biting mosquito species (*Aedes aegypt and An. arabiensis*) over four treatment nights by direct burning is shown in Table 2. It indicated that the reduction (%) of mosquito landing within each treatment night was between 100% – 89% for *An. arabiensis* and between 100% – 75% for *Aedes aegypt*. Averagely the repellency was 94.5% and 87.5% respectively. In both species, however, the mean reductions (%) of all treatments are not significantly different from each other ($p > 0.05$).

The relative repelling efficacy of smoke against two biting mosquito species (*Aedes aegypt and An. arabiensis*) over four treatment nights by thermal expulsion is shown in Table 3 below. The result has shown that the reduction (%) of mosquito landing within each treatment night was between 97%-78% for *An. arabiensis* and between 100%-88percent for *Aedes aegypt*. Averagely the repellency was 87.5% and 94% respectively. In both species, however, the mean reduction (%) of all treatments is not significantly different from each other ($p > 0.05$).

Table 1
The overall repulsive effect of the smoke over four treatment nights in individual huts by two methods of application

Huts	Species	Total number of mosquitoes fed on volunteers over four treatment nights in individual huts				Mean ± SE
		1	2	3	4	
A_1	*An. arabiensis*	0	1	0	2	0.75 ± 0.47
	Aedes aegypt	0	0	0	0	0.00 ± 0.00
B_1	*An. arabiensis*	1	2	0	0	0.75 ± 0.47
	Aedes aegypt	0	1	3	0	1.00 ± 0.70
C_1	*An. arabiensis*	0	0	0	0	0.00 ± 0.00
	Aedes aegypt	1	0	0	0	0.25 ± 0.25
D_1	*An. arabiensis*	39	28	17	30	28.5 ± 4.51
	Aedes aegypt	16	8	12	7	10.75 ± 2.00
A_2	*An. arabiensis*	2	1	0	0	0.75 ± 0.47
	Aedes aegypt	1	0	1	0	0.50 ± 0.28
B_2	*An. Arabiensis*	0	1	3	0	1.00 ± 0.28
	Aedes aegypt	0	0	0	1	0.25 ± 0.25
C_2	*An. Arabiensis*	2	1	1	1	1.25 ± 0.25
	Aedes aegypt	0	0	0	1	0.25 ± 0.25
D_2	*An. Arabiensis*	23	31	19	36	27.25 ± 3.83
	Aedes aegypt	10	11	13	17	12.75 ± 1.54

SE: Standard error

A_1 - C_1: Treatment huts (direct burning)

A_2 - C_2: Treatment huts (thermal expulsion)

D_1: Control huts (direct burning)

D_2: Control huts (direct burning)

Table 2
The relative repelling effect of smoke by direct burning against two biting mosquito species (*Aedes aegypt* and *An. arabiensis*) over four treatment nights

Species	Reduction (%) of mosquito bites over four treatment nights (95% CI)				P-value*
	1	2	3	4	
An. arabiensis	97	89.3	100	93.3	$p > 0.05$
Aedes aegypt	93.75	87.5	75	100	$p > 0.05$
*P-Value obtained from Tukey's test at α = 0.05					

Table 3
The relative repelling efficacy of smoke against two biting mosquito species (*Aedes aegypt and An. arabiensis*) over four treatment nights by thermal expulsion

Species	Reduction (%) of mosquito bites over four treatment nights (95% CI)				P-value*
	1	2	3	4	
An. arabiensis	82.6	90.3	78.9	97.2	$p > 0.05$
Aedes aegypt	90	100	92.3	88.2	$p > 0.05$
*P-Value obtained from Tukey's test at α = 0.05					

The average number of mosquitoes collected over four nights, in both treatment as well as control huts and the mean smoke repellency index (R) of two biting insect species (*Aedes aegypt* and *An. arabiensis*) by direct burning are shown in Table 4. As shown, the mean repellency index (R) of the smoke against *An. arabiensis* was 94.73% and against *Aedes aegypt* was 88.4%. In the comparison of repellency between treatment and control groups, mosquito landing on treatment groups was reduced significantly ($p < 0.05$) and there were no significant reductions observed in control landings.

Table 4
The mean repellence index (R) of mosquitoes landing on the control and the treatment hunts by direct burning

The mosquito species	Conditions	Mean number collected	The mean repellence index (R)	P-Value*
An. arabiensis	Treatment	1.5	94.73	p 0.05
	Control	28.5		
Aedes aegypt	Treatment	1.25	88.4	p 0.05
	Control	10.75		
*P-Value obtained from Tukey’s test at α = 0.05				

The average number of mosquitoes collected over four nights, in both treatment as well as control huts and the mean smoke repellency index (R) of two biting insect species (*Aedes aegypt* and *An. arabiensis*) by thermal expulsion is shown at Table 5 below. As shown, the mean repellency index (R) of the smoke against *An. arabiensis* was 89% and against *Aedes aegypt* was 92%. In the comparison of repellency between treatment and control groups, mosquito landing on treatment groups was reduced significantly ($p < 0.05$) and there were no significant reductions observed in control landings.

Table 5
The mean repellence index (R) of mosquitoes landing on the control and the treatment huts by thermal expulsion

The mosquito species	Conditions	Mean number collected	The mean repellence index (R)	P-Value*
An. arabiensis	Treatment	3	89	p 0.05
	Control	27.25		
Aedes aegypt	Treatment	1	92	p 0.05
	Control	12.75		
*P-Value obtained from Tukey’s test at α = 0.05				

The relative efficacies of the powder smoke against two mosquito species in both direct burning and thermal expulsion methods of applications were presented above in Table 4 and 5. The provided an average repellence index (R) of both species of mosquitoes landing indirect burning methods of application were 94.73% and 88.4%, respectively, over four treatment nights. In the case of thermal explosion methods of application the mean repellency index (R) of the smoke against *An. arabiensis* was 89% and against *Aedes aegypt* was 92%. The main species presented during the study period were *Anopheles arabiensis* and *Aedes aegypt*. Of the 342 mosquito species collected in four treatment nights, *Anopheles arabiensis* constituted the bulk of the collection (70%), and *Aedes aegypt* [Rockefeller strain] comprised just 30%. The smoke from plant powder was significantly reduced the number of mosquitoes

($p < 0.05$) in both methods of application compared to the control burning charcoal alone in the traditional stove. No significant difference ($p > 0.05$) was observed in the comparison between the two methods of application (direct burning and thermal expulsion) for both mosquito species under experimentation, although, the direct burning method appeared to be a little more efficient in repelling mosquitoes (94.73–88.4% protection) than the thermal expulsion method of application (92 – 89% protection) as shown above in Tables 3 and 4.

Discussion

In Ethiopia, the use of repulsive plants to reduce human vector contact is common practice in village communities. Smoke is the most widely used means of repulsing mosquitoes. The major plants used as repellents are Ocimum species (*Ocimum forskolin, Ocimum kilimandscharicum*, and *Ocimum suave*), *Eucalyptus species*, *Eucalyptus camaldulensis*, *Lantana camara*, and *Azadirachta indica*. In this study, the repellent effect of the smoke from the leave of selected plant species (*Azadirachta indica*, *Eucalyptus camaldulensis*, and *Ocimum forskolin*) leave powder mixtures was evaluated against *Aedes aegypt* and *An. arabiensis* by direct burning and thermal expulsion methods of the application under normal field conditions. As shown in the result, the candidate test plants pose significant repellent effects both thermally expelled and directly burnt against *Aedes aegypt* and *An. arabiensis* as compared to the control of burning charcoal alone.

Plants may be alternative sources for mosquito repellent agents since they constitute a rich source of bioactive chemicals [26]. Plant products can either be used as an insecticide to kill larvae or adult mosquitoes or as defensive repellents against mosquito bites; depending on the type of activity they have [27]. Lemon eucalyptus (*Corymbia citriodora*), *Eucalyptus camaldulensis*, *Ocimum suave*, and *Ocimum basilicum* have been evaluated for their repellency by thermal expulsion of their leaves from traditional stoves against *Anopheles arabiensis* and *Anopheles phronesis* in traditional homesteads around Koka, the central part of Ethiopia [28]. In their report, *Ocimum basilicum* was found to be the most effective repellent plant against *An. arabiensis* by the direct burning application method (73.11%) followed by *Ocimum suave* (71.51%), *Corymbiacitriodora* (70.59%), and *Eucalyptus camaldulensis* (65.29%). In the thermal expulsion method, similar results were obtained as *Corymbiacitriodora* (78.69%) and *Ocimum basilicum* (78.66%) showed similar repellency effects followed by *Ocimum suave* (73.55%) and *Eucalyptus camaldulensis* (71.91%) against *An. arabiensis*. All plant species also showed over 72% repellency by the thermal expulsion application method against *An. phronesis.* The same experiment was also carried out in traditional village houses in western Kenya to evaluate the repellency of Lemon eucalyptus (*Corymbiacitriodora*) and other repellent plants (*Ocimum suave* and *O. Itilimandscharicum*) against *An. gambiae* and *An. funestus* by thermal expulsion of their leaves from traditional stoves [16]. According to their report, the highest repellency of about 49% was recorded by *Corymbiacitriodora* against *An. gambiae* and only 15% repellency against *An. funestus*.

Our results of 94.73% repellency against *An. arabiensis* and 88.4% against *Aedes aegypt*in a direct burning method, and 89% repellency against *An. arabiensis* and 92% against *Aedes aegypt* in thermal expulsion method were comparative with 78.69% repellency of *Corymbiacitriodora* against *An. arabiensis* in thermal explosion and 73.11% repellency of *O. basilicum* by direct smoking is significant and greater than those reported by Sisay *et al*. [28]. *Corymbiacitriodora's* highest repellence at 48.71% followed by an equal degree of repellence of *O. kilimandscharicum* and *O. suave* in thermal expulsions with 44.54% against *An. gambiae* and *An. funestus*is also comparative with the work of Seyom *et al*. [16]. Because under experimentation they evaluated single plant species repellence against the vector as a sole experimental source of data, however, the combined effect of different plant species repellence for the vectors understudy was evaluated in this research work.

Leaves of *Corymbiacitriodora* exhibited the highest repellency (51.3%) by direct burning, followed by leaves of *Lantana uckambensis* (33.4%) and, leaves and seeds of *Ocimum suave* 28.0%. The combination of *Ocimumkilimandscharicum* with *Lantana uckambensis* repelled 54.8% of mosquitoes by thermal expulsion in the work of Seyom *et al*. [22]. Seyoum *et al*. [23] in their article performed similar experiments in a semi-field environment in western Kenya toward *An. gambiae*. They observed the highest repellence of 74.5% and 51.3% for *Corymbiacitriodora* by thermal expulsion and direct burning, respectively. Our results by thermal expulsion and direct burning in field situations against both Anopheles and Aedes species of mosquito in the study area were found to be significant and comparable to those observed by Seyoum *et al*. [22, 23]. *Ocimumforskolei* reduced biting by over 50% against *An. arabiensis* under field conditions according to Waka *et al*. [29]. *Ocimum suave* and *Ocimumkilimandscharium* are used extensively in Tanzania and are highly effective in bioassays against a range of mosquito species as seen in Kweka *et al*. [30, 31]. In addition, there are reports of Ocimum spp. being used as mosquito repellents via burning or thermal expulsion [32].

There are well-established variations in the susceptibility of mosquito species to synthetic repellents like DEET [32]. DEET is a synthetic mosquito repellent widely used all over the world for protection against mosquito bites [33]. Because of concerns about the side effects of DEET, the U.S. Centres for Disease Control and Prevention (CDC) licensed plant-based mosquito repellents, one of which was *para*-Menthane-3,8-dio(PMD) [34]. In developed nations, PMD is successfully commercialized and is widely used [35]. Several plants and plant varieties are known to produce a range of oils and have already been shown to be effective mosquito repellents. Ongore *et al*. [36] surveyed a population in Kenya and found that 16% burned leaves of *Lantana rhodesciense* and 16% burned other waste plant materials including sisal leaves and rice husks. People burn orange peels in Sierra Leone and Ghana to flush away mosquitoes, while neem leaves (*Azadirachta indica*) and baobab tree (*Adansoni adigitata*) are burned in Ghana and Gambia [37]. The neem tree (*Azadirachta indica*) products were well-known for insect repellent and antifeedant properties long before the advent of synthetic insecticides and have already been documented using its various constituents in agriculture and other areas [38–40]. The burning of fresh and dried leaves from Lamiaceae, Poaceae, and Pinaceae around and within the home to provide protection against mosquito bites is widely used throughout rural Ethiopia [14, 15]. Some essential chemical components like eugenol, linalool, and methyl cinnamate have also been reported. These plants usually contain camphor and thymol and have mosquito repellent properties [41]. An essential oil from this plant repelled *Aedes aegypti* for about 75 min [32] and is also reported to have some insecticidal activity against a variety of insects [42]. It was also shown that *Ocimum forsltolei* reduced the indoor biting levels of *An. arabiensis* by 53% when its fresh leaves and shoots were hanging at the ends of the beds in Eritrea [29].

To our finding, no specific investigations were conducted to test the combined repelling effect of the mixed powder of the *Azadirachta indica*, *Eucalyptus camaldulensis*, and *Ocimum forskolei*to make a comparison, although both methods of application provided an important and significantly higher degree of repellence in comparison with other plant species reported (Tables 3 and 4). The higher percentage repellencies (> 85%) were generally observed in the present study might have some contributions from the burning charcoal itself used to smolder the plants. As we did in controlled huts, lightening charcoal only could have some degree of protection (approximately 20%) from mosquitoes, probably by reducing humidity near the fire [18]. Assuming the same level of repellency from charcoal alone in the present study, the actual repellencies of the test plants could be greater than 65% by both methods of applications which still are comparative with work of Seyoum *et al*. [27] (22–23, 16) and Sisay *et al*. [28]. However, charcoal, firewood or dried cow dung are usually used in traditional stoves in Ethiopia to heat the desired plant parts or incense [28], so that the additional repellents provided by them would always be advantageous.

Conclusions

The repelling effects of selected plants viz. *Azadirachta indica*, *Eucalyptus camaldulensis*, and *Ocimum forskolei* leave powder are significantly reduced two species of mosquito biting. Such plants are readily available and their application methods being simple and affordable may be useful in protecting malaria. The community-wide use of such repellent plants has the potential to complement existing control measures, such as treating mosquito nets once a month in areas where affordability of the Insecticide-treated mosquito nets (ITNs) is restricted. The two methods of application may offer cost-efficient alternatives as additional household protection and as a helpful addition to bed nets, especially for the early part of the evening before bedtime.

Declarations

Ethics approval

This study conducted in accordance with the declaration of Helsinki that provides guidance for the researcher to protect research subjects. The study was approved by the Institutional Research Review Board (IRB) of Wolaita Sodo University.

Consent to participation

Not applicable

Consent to publication

All authors agreed to the public this original research work

Data availability statement

Data sharing not applicable to this manuscript as no datasets were generated or analyzed during the current study.

Competing interests

The authors declare that they have no competing interests

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Authors' contribution statement

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Abenezer Wendimu and Wondimagegnehu Tekalign. The first draft of the manuscript was written by Abenezer Wendimu and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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