



Impact of *Ocimum basilicum* Leaf Essential Oil on The Survival and Behaviour of An Indian Strain of Dengue Vector, *Aedes aegypti* (L.)

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Abstract

Objective: *Aedes aegypti* (L.) is a well-known widely spread disease vector transmitting several diseases of concern. Current investigations were undertaken to discover the larvicidal and repellent potential of essential oil from the basil plant, *Ocimum basilicum* leaves against *Ae. aegypti* as a suitable and eco-safe alternate to chemical insecticides.

Methods: The larvicidal potential of basil oil was evaluated against *Ae. aegypti* early fourth instars using WHO protocol and LC₅₀ and LC₉₀ values were calculated. The efficacy of basil oil as mosquito repellent was assessed using the human-bait technique for 3 hours and the percent protection was calculated.

Results: Exposure of early fourth instars of *Ae. aegypti* with various concentrations of *O. basilicum* essential oil for 24 h revealed a moderate LC₅₀ and LC₉₀ value of 141.95 ppm and 445.12 ppm, respectively. The investigations also showed that oil did not have any hormono-mimetic effects as it did not result in the formation of any larval-pupal intermediates. However, the basil essential oil exhibited significant effective repellency against adult *Ae. aegypti*. Introduction of the arms of human volunteers applied with basil oil in the cages of dengue vector resulted in 100% protection till 120 min as compared to control arm which experienced 3 to 8 bites. Remarkably, the experimental arm was bitten just once in every 15 minutes in next 45 minutes after which only 2 bites were recorded until 180 min of exposure.

Conclusion: Our results recommended the efficient use of basil leaf essential oil as an efficient repellent and as a moderate larvicide agent against *Ae. aegypti*. Identification of bioactive components in the oil, their individual mode of action and synergistic effects, along with investigations on their impact on the environment and non-target organisms would help in formulating strategies for mosquito management.

Keywords

Essential oil; Basil; *Aedes*; Larvicide; Repellent; Protection

Introduction

Mosquitoes are significant disease vectors affecting human health throughout the world. A major cause of the spread of mosquito-borne diseases at such a large scale is their capacity to bite multiple individuals during a single gonotrophic cycle [1]. The dengue vector, *Aedes aegypti* is the primary vector for several tropical and subtropical diseases; yellow fever, dengue fever, dengue hemorrhagic fever, Zika fever and Chikungunya. The global estimates of World Health Organization revealed 3.9 billion people inhabiting 128 countries at the risk of dengue infection [2]. WHO reports have also indicated that at present, 47 countries are at the risk of yellow fever while 60 countries are affected by Chikungunya. A modelling study by them estimated the occurrence of 84,000–1,70,000 severe cases of yellow fever in Africa and America leading to 29, 000–60,000 deaths in 2013. Outbreaks of Zika virus disease have been recorded in Africa, the Americas, Asia and the Pacific which is on increase every year [2]. In India, dengue fever and Chikungunya are progressively becoming the imperative public health problems. The official records of the Union health ministry reported a considerable increase in the number of dengue cases in last few years. In 2015, a total of 99,913 dengue cases were reported in India, leading to 220 deaths, which respectively increased by 1.29 and 1.11-fold in 2016 [3].

The foremost approach recommended to approach to control mosquito-borne diseases primarily lies by interrupting the disease transmission cycle which can only be achieved by mosquito control. Various control measures have been devised and practiced for control of mosquitoes, such as the elimination of their breeding places; use of several biological agents - *Bacillus thuringiensis*, *B. sphaericus* var. *israelensis*, *Gambusia*, *Daphnia*, copepods, etc.; and sterile insect release method. However, the prime measures for mosquito management have always been either killing any of the life stages of mosquitoes or by repelling the adults [4]. Most commonly used method for control of mosquito vectors had at all times been the intervention through chemical insecticides-based actions, especially through indoor residual spraying (IRS) and the use of insecticide-treated bed nets (ITNs). It has resulted in the frequent and indiscriminate use of several organic insecticides such as organochlorines, carbamates, organophosphates and pyrethroids in the fields. This has been reported to cause the development of varying amount of insecticide resistance in the different life stages of mosquitoes [5-7]. In addition, many other apprehensions; such as non-degradability nature of insecticides causing destabilization of the ecosystem, their entry in the food chain, harmful effects on humans and lethality to the non-target organisms has been reported [8]. All these effects of synthetic pesticides have necessitated the need to adopt alternative innocuous and ecofriendly strategies.

The effectiveness of natural pesticides derived from the plants, particularly those which are extracted with a proper solvent and then formulated as required or essential oils distilled from the plants have been reported by many researchers as promising and advantageous alternates to synthetic insecticides against mosquitoes [9]. The botanicals are considered much safer without posing any adverse impact on humans and environment and thus, their utilization has been encouraged not only due to their eco-safety but also because of

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target specificity, degradability, higher suitability and appropriateness for rural areas [10,11].

Essential oils are fragrant oily liquids or innate volatile chemicals derived from diverse plants. On a commercial scale, essential oils are used in pharmaceuticals, as odorants in fragrances or as a flavor in many food products [8]. Essential oils have also been reported to possess antibacterial, antifungal, mosquito larvicidal, insecticidal and repellent activities [4,7,8,12-16]. Several reports are available which evidently proved the biological efficacy of several essential oils against different mosquito species at larval and adult stage [10,11,17-20].

Basil, *Ocimum basilicum* L. (Fam., Lamiaceae), vernacularly called Tulsi or Rehan is a plant native to tropical Africa, Asia and the Pacific Islands. It chemically contains a low percentage of volatile and essential oils and is used in the conventional medicine to alleviate pain, treat stress, mitigate vomiting and even as an insect repellent [21,22]. Oils from a few *Ocimum* spp. have been shown to repel insects and possess larvicidal activity against houseflies, blue bottle flies and mosquitoes. Some basil essential oils have been asserted to possess larvicidal activity against mosquito larvae [23]. The effective concentration of the basil oil to kill 90% of the larvae has been reported to range from 113-283 ppm. Basil oil has been observed to possess camphor, d-limonene, myrcene, and thymol as some of the compounds with repellent properties, while eugenol and methylchavicol present in the oil exhibit larvicidal activity.

Keeping in view the potential insect repellent potentials of *O. basilicum* essential oil against mosquitoes, the present investigations were performed to discover the probable use of the oil as a potential control agent against *Ae. aegypti*. The results of the current study may provide useful information for the development of an effective and eco-safe mosquito control agent.

Materials and Methods

Rearing of *Aedes aegypti* in the laboratory

The current investigations were performed to control dengue vector, *Ae. aegypti* maintained and reared in a laboratory at controlled conditions of $80 \pm 5\%$ RH, $28 \pm 1^\circ\text{C}$ and 14:10/Light: Dark photoperiod [24]. The adults were fed on deseeded raisins soaked in water along with which intermittent blood feed was provided to the female adults. The eggs were collected on Whatman filter paper strips lining the water-filled enamel bowl and were transferred to the enamel trays for hatching. The hatched larvae were provided a combination of live yeast and grounded dog biscuits (1:3) as food. The pupae were collected and kept in the muslin cloth cages for the emergence of adults.

Investigations on the larvicidal potential of *Ocimum basilicum* essential oil

The larvicidal potential of basil essential oil was performed on the early fourth instars of *Ae. aegypti* as per the protocol recommended by WHO with minor modifications [24]. The essential oil with 99.9% purity, extracted from the leaves of *O. basilicum*, was obtained from M/s Auroville located in Puducherry, India. The graded series of basil leaf oil was prepared with ethanol as the solvent.

For conducting the bioassay, twenty healthy and active early IV instars of *Ae. aegypti* were selected and transferred with the help of a strainer to 99 mL of distilled water taken in the plastic bowls. These were then added to a glass jar filled with 100 mL of distilled water with 1 mL of the basil oil at a particular concentration. Each

concentration had four replicates for statistical significance. Control bioassays were conducted by adding only ethanol to the water instead of the pure oil or oil-ethanol solution.

The larval mortality was recorded after 24 hours of treatment by touching the larvae gently with the help of a glass rod and observing their movement. The larvae without any indication of the movement were scored as dead. On the other hand, larvae which could move a little but did not show any kind of active wriggling or swimming movement were accounted as moribund and scored as dead.

Statistical analysis of data

The tests which had above 20% mortality in control assays and over 20% pupae formed were rejected and conducted again. The control mortality that ranged between 5-20% was corrected by Abbott's formula [25]. The data was analysed by probit regression using SPSS 19.0 Programme which transforms % mortality in probits and linearly models the normal distribution of the correlation between concentration and % mortality via a link function. The lethal concentrations (LC_{50} and LC_{90} values) with 95% confidence limit were calculated to determine the variation between the test samples. Other statistical estimates, such as standard error, regression coefficient and chi-square were also calculated.

During each larvicidal bioassay, control and experimental, the larvae were monitored carefully for behavioural modifications, if any. The observations were focused on the wriggling speed of larvae, horizontal and vertical movements, aggregation behaviour at corners and larval knockdown. The behaviour was recorded and photographed with Canon Power Shot SX50HS.

Investigations on adult repellent potential of *Ocimum basilicum* essential oil

The investigations on the repellent prospective of basil essential oil against *Ae. aegypti* were carried out using the human-bait technique. Five human volunteers (21-24 years old; 3 males and 2 females) were selected from different institutions considering their non-allergy to mosquito bites and after taking a consent letter from each of them. Laboratory reared, blood famished 3-10-days old, adult female mosquitoes were kept in separate cages ($45 \times 45 \times 40$ cm) in the groups of twenty five each.

For conducting the assays, initially, the forearm of each volunteer was meticulously cleaned, washed with fragrance-free soap, rinsed with distilled water and allowed to dry for 10 min. An area of 5×5 cm was marked by a permanent marker on both the arms of each volunteer. The marked area on one of the forearms of each volunteer was applied with 0.1 mL of *O. basilicum* essential oil. The other forearm was treated as control and applied with 0.1 mL of ethanol. The rest of each forearm area was covered leaving only the marked area open. Control and treated forearms were entered in the cage simultaneously and exposed to mosquito bites. The number of bites was calculated over 3 min, after every 15 min for continuous 3 hours from 11.30 h to 14.30 h. An attempt was considered bite only if stylets were inserted into the skin. Observations reporting no bite attempt or just landing of a mosquito on control arm were rejected and the experiment was repeated with a new set of mosquitoes to check repellence effect and ensure that biting failure was due to repellency caused by oil and not because of the mosquitoes being inclined to obtain a blood meal.

The tests were conducted thrice on different volunteers and introducing their arms in separate cages to abolish the effect of

variation in skin texture on the repellency potential of oil. The protection time was noted as the time elapsed between the application of basil oil and the time when a bite was confirmed. The percentage protection from mosquito bite was calculated as follows [4].

$$\% \text{ Protection} = \frac{C - T}{C} \times 100$$

Where, C indicates the number of bites recorded on the control arm and T denotes the number of bites scored on the treated arm.

Results

The potential of essential oil distilled from the leaves of basil plant, *O. basilicum* was investigated for larvicidal and repellency effects against dengue vector, *Ae. aegypti*. The results showed the noteworthy ability of basil essential oil as the prospective control and management agent of *Ae. aegypti* and *Aedes*-borne diseases by cidal effects or preventing its bites.

The 24 h exposure of *Ae. aegypti* early IV instars to the essential basil leaf oil resulted in LC₅₀ and LC₉₀ values of 141.95 ppm and 445.12 ppm, respectively (Table 1). The bioassay did not result in the formation of any pupa or any larval-pupal intermediate resulting in complete larval mortality. It indicates that oil did not have any hormono-mimetic effects. The control treatments also did not result in any mortality indicating the innocuous effects of solvent (Table 1).

The larvae were inspected carefully during the oil exposure period for alterations in behaviour. The observations showed that basil oil did not cause instant or rapid mortality. In fact, initial larval exposure to the essential oil did not influence the larvae, affected their movements or appearance. The first change in the larval behaviour was noticed after 20 min of treatment resulting in restlessness. The lethal effect of the basil oil was observed after approximately 30-40 min of treatment when larvae were unable to rise to the water surface, exhibited body quivers and experienced convulsions. The paralytic symptoms evidently appeared in 20% of the larvae after about two hours leading

to their mortality. Continued larval exposure to oil caused mortality in more or less 50% larvae after 8-9 h and the most of the larvae died within 14 h.

The studies performed to assess the repellency potential of basil leaf oil against adults *Ae. aegypti* revealed significantly promising and noteworthy repellency of the oil. The exposure of oil-treated arm to female *Ae. aegypti* resulted in 100% bite protection in the first 120 min as compared to the control arm applied with ethanol which experienced 3 to 8 bites and thus could not repel mosquitoes (Table 2). Further continuation of the experiment showed that the application of basil oil on the experimental arm could repel the mosquito significantly resulting in just one bite in next 45 minutes as compared to the 6 to 9 bites experienced on the control arm. However, in next 15 minutes, the repellency of oil decreased little causing 2 bites as against almost 9 bites on control arm (Table 2).

The investigations clearly revealed the 100% protection from bites of *Ae. aegypti* adults till 120 minutes which reduced gradually by 11.2% after 45 minutes. It is clear from the data that the % protection to female adults further diminished to 73% after 180 min of oil application (Table 3 and Figure 1). It is important to note that application of the *O. basilicum* oil on human skin did not induce any kind of annoyance to any volunteer not only during the experiment but also afterwards.

Discussion

The tropical and sub-tropical countries are facing a continued rise in mosquito-borne diseases each year. Though several chemical insecticides have been discovered and used for mosquito management, their sustained and regular use has caused various undesirable effects, including lethal effects on non-target beneficial organisms, adverse effects on human health, environment contamination and amplified resistance in the mosquitoes. Consequently, botanicals have become preferred and suitable mosquito control agents among researchers.

Table 1: Larvicidal effects of *Ocimum basilicum* oil (Basil oil) against early fourth instars of *Aedes aegypti*.

Concentration	LC ₅₀ (ppm)	LC ₉₀ (ppm)	χ ²	df	RC	S. E.
Lethal Concentration	141.9595	445.1186	2.738	5	2.5818	0.2460
Lower Fiducial Limit	100.823	260.6700	-	-	-	-
Upper Fiducial Limit	234.769	710.742	-	-	-	-

LC₅₀ = lethal concentration that kills 50 % of the exposed larvae, LC₉₀ = lethal concentration that kills 90 % of the exposed larvae, S.E. = Standard error, χ² = chi-square, df = degree of freedom, RC = Regression Coefficient, Test samples were transformed in to log covariant (log10), p<0.05, level of significance, Values are mean of three replicates.

Table 2: Mean number of *Aedes aegypti* bites on the arms of human volunteers after application of the ethanol (Control) or *Ocimum basilicum* essential oil (Experimental).

Time (Min.)	Mean No. of Mosquito Bites on the Control Arm	Mean No. of Mosquito Bites on the Experimental Arm
15	3.0	0.0
30	5.67	0.0
45	3.33	0.0
60	3.0	0.0
75	7.67	0.0
90	8.33	0.0
105	7.33	0.0
120	5.67	0.0
135	6.33	1.0
150	7.67	1.0
165	9.0	1.0
180	8.67	2.33

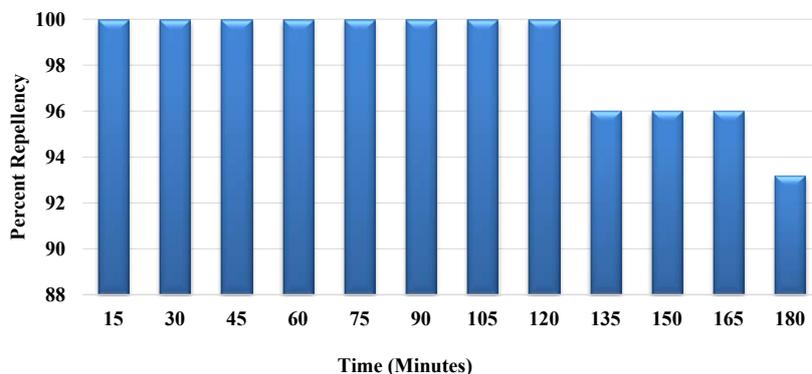


Figure 1: Decrease in percent repellency of the *Ocimum basilicum* essential oil with time against number of bites of *Aedes aegypti*.

Table 3: Percent repellency and percent protection to the *Aedes aegypti* bites after application of the *Ocimum basilicum* essential oil on the arms of human volunteers.

Time (Min.)	% Repellency after application of basil oil	% Protection after application of basil oil
15	100.0	100.0
30	100.0	100.0
45	100.0	100.0
60	100.0	100.0
75	100.0	100.0
90	100.0	100.0
105	100.0	100.0
120	100.0	100.0
135	96.0	84.2
150	96.0	86.9
165	96.0	88.8
180	93.2	73.1

Several reports exist which proved the repellent and larvicidal potency of essential oils against *Ae. aegypti* [4,8,26].

Oils from a few *Ocimum* spp. have been shown to repel insects and possess larvicidal activity against houseflies, blue bottle flies and mosquitoes [23]. However, the literature reveals that though a few reports are available on the bio-efficacy of basil leaf extracts against dengue larvae but basil essential oil has not been explored extensively against them at the larval or adult stage. Thus, present studies were conducted to evaluate the possibility of utilization of basil leaf essential oil as a larvicidal or repellent agent against *Ae. aegypti* population.

Current studies showed that 24 h exposure of *Ae. aegypti* early fourth instars to various concentrations of basil oil resulted in LC_{50} and LC_{90} value of 141.95 ppm and 445.12 ppm, respectively ($p > 0.05$). Nevertheless, a huge population of *Ae. aegypti* with massive heterogeneity, the insignificant p values and chi-square distribution obtained indicate the need for more assays with a further selection of larvae randomly and augmented replicates to ascertain the larvicidal potential of basil oil. The similar larvicidal potential of four Sudanese Accessions of *O. basilicum* essential oils was observed by Nour [27] against *Anopheles* larvae. They, however, reported varied results, the LC_{50} value ranging from 190 ppm to 300 ppm. A study conducted by Murugan [28] reported varied larvicidal activity of basil essential oil against different instars of *Ae. aegypti*. Earlier, Aidaross [29] investigated *O. basilicum* water extracts for their larvicidal activity against third instars of *Culex quinquefasciatus* and observed 50% mortality at 1000 - 10000 ppm. Another study by Anees [30] showed

that when extraction of *Ocimum sanctum* leaves with hexane, acetone, ethyl acetate, chloroform and methanol, the larvicidal activity differed against *Ae. aegypti* larvae. Likewise, Maurya [31] evaluated larvicidal effects of crude carbon tetrachloride, methanol and petroleum ether leaf extracts of *O. basilicum* against *An. stephensi* and *Cx. quinquefasciatus* and found petroleum ether extract with maximum efficacy with respective LC_{50} values of 8.29 and 87.68 ppm. Recently, Mahmoud [32] studied the effects of leaf powder and ethanolic leaf extracts of *O. asilicum* against third instars of *An. arabiensis* and reported respective LC_{50} and LC_{90} values for extract as 58mg/L and 143 mg/L; while for powder, the respective values were 9.19 g/L and 19.88 g/L.

Quite a few researchers have estimated the cidal potential of various plant essential oils against mosquito larvae. Exposure of *Ae. aegypti* early fourth instars to the *Apium graveolens* seed oil for 24 h resulted in respective LC_{50} and LC_{90} values of 16.10 and 29.08 ppm [4], the cidal effect of the oil improved by 1.2-fold with continued exposure to 48 h with a LC_{50} value of 13.22 ppm. Earlier, Kumar [8] had reported larvicidal bio-efficacy of the *Mentha piperita* (Peppermint) essential oil against dengue early fourth instars with respective LC_{50} and LC_{90} value of 111.9 ppm and 295.18 ppm when exposed to oil for 24 h. They also showed 11.8% increased the toxicity of the oil on continued larval exposure to peppermint oil for 48h. Similarly, Warikoo [15] had reported the significant efficacy of *Pinus longifolia* (Pine) essential oil against larvae of *Ae. aegypti* with an LC_{50} value of 0.330 mg/L and LC_{90} value of 1.118 mg/L. The essential oil obtained from the *Toddalia asiatica* roots and the constituents isolated from the oil have been found effective against larvae of *Ae. albopictus* [33]. Lee [34] investigated essential oils distilled from eleven medicinal plants to assess their efficacy of against *Ae. aegypti* early fourth instars and observed 100% larval mortality on exposure to 100 ppm oils. The essential oil prepared from the *Feronia limonia* leaves could cause significant larval mortality in *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* with respective LC_{50} values of 11.59, 15.03 and 22.49 ppm after 24 h [35]. Tiwary [36] investigated essential oil of *Zanthoxylum armatum* against mosquitoes and found it most effective against *Cx. quinquefasciatus* with an LC_{50} value of 49 ppm followed by decreasing efficacy against *An. stephensi* and *Ae. aegypti* with LC_{50} values ranging from 54-58 ppm.

Our studies also showed that exposure of adults *Ae. aegypti* to basil leaf essential oil could result in prospective and outstanding repellency. The application of oil on human arms provided 100% protection in the first 120 min followed by just 1 bite in the next 45

min of exposure. Earlier, Aidaross [29] reported only 58% repellent activity of *O. basilicum* essential oil against *Cx. quinquefasciatus*. Nazliniway and Masfria [37] formulated a skin lotion from different combinations of *O. basilicum* essential oil and aloe vera extract which were found to be remarkably effective. In 2015, Hassan [38] screened methanol, acetone and petroleum ether extracts of *O. basilicum* leaves for their repellency effect against *Cx. pipiens* and found petroleum ether extract to be most effective followed by acetone and methanol extracts causing 98.1%, 84.6% and 77.4% repellency, respectively at a dose of 6.7 mg/cm². Kumar [8] reported remarkable repellent properties of *M. piperita* essential oil against adults *Ae. aegypti* providing complete bite protection to human volunteers till 150 min. They recorded only 1-2 bites during the next 30 minutes, as compared to 8-9 bites scored on the control arm. Later, the authors revealed the significant repellent efficacy of the celery seed oil providing 100% protection till 165 min against adult *Ae. aegypti* [4]. The skin repellency assays performed with *Zingiber officinalis* essential oil at 1.0, 2.0, 3.0 and 4.0 mg/cm² gave 100% protection against *Cx. quinquefasciatus* up to 120 min [19]. The repellent activities of the essential oils of *Z. officinale*, *Rosmarinus officinalis* and *Cinnamomum zeylanicum* have been also reported against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* by Prajapati [39].

Amer and Mehlhorn [11] studied the repellent activity of 41 essential oils against different mosquito species. They reported the maximum protection period of 180 min and 54% repellency shown by *Glycine max* followed by 120 min protection by *Salvia sclarea* and *Mentha piperita* oils with respective 45.9 and 59.4% repellency against *Ae. aegypti*. Yang and Ma [40] investigated 5 essential oils against *Ae. albopictus* and found 99.67% repellent activity of Citronella oil with 8 h of protection period. Later, Zhu [41] reported 73% repellency of Catnip oil up to 6 hrs against *Ae. albopictus*. In 2007, Kamsuk [42] showed that essential oil of *Zanthoxylum piperitum* alone and on addition with 5% vanillin could repel *Ae. aegypti* females with respective protection times of 1.5 and 2.5 h. Tawatsin [17] studied the repellency of essential oils extracted from eighteen plant species belonging to eleven families and found them more effective against *An. dirus* and *Cx. quinquefasciatus*, providing repellency for 4.5-8 h as compared to repellency for 0.3-2.8 h against *Ae. aegypti*.

Present investigations explored the prospective role of *O. basilicum* leaf essential oil as a larvicide agent and as an effective repellent against *Ae. aegypti* and suggested the potential bio-efficacy of basil leaf oil. Nevertheless, the investigations recommend the use of basil essential oil as a more effective repellent agent than a larvicide. Use of basil essential oil in formulation of products of mosquito control could be advantageous over other control methods which can be attributed to their easy and cost-effective production, residual effectiveness under natural environment and, safety to non-targeted organisms and environment; though the efficacy may vary depending on the local weather condition, loss due to removal by abrasion and sweating, density of mosquito bites etc. Consequently, further investigations with increased replicates and indiscriminate larval selection are needed to establish its efficacy against *Ae. aegypti*. In addition, the recognition of bioactive constituents of the oil and comprehending their mode of action is critical for its use in the mosquito management program. Field trials are also recommended before the use of *O. basilicum* leaf oil against mosquitoes as an environment friendly product.

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