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Larvicidal activity of Lantana camara Leaf Extract against *Anopheles subpictus, Ades albopictus*, *Culex vishnui* (Diptera: Culicidae)

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1. INTRODUCTION:

Mosquitoes are the major public health problem throughout the world. Among the 3492 species of mosquitoes recorded worldwide, more than a hundred species are capable of transmitting various diseases in human and other vertebrates [1]. Mosquitoes transmit malaria, dengue fever, yellow fever, filariasis, Japanese encephalitis and chikungunya to humans [2]. Mosquito-borne diseases contribute significantly to disease burden, death, poverty and social debility all over the world, particularly in tropical countries. Among these diseases, malaria remains the most serious vector-borne disease affecting some 300-500 million people and 1.4 to 2.6 million deaths annually throughout the world. More than 40% of the world population lives in areas prone to malaria [3]. Dengue fever can manifest as the classic form of the diseases, which debilitates the patient for a week or more, or as the haemorrhagic form which, in many cases leads to death [4]. Chikungunya virus, a member of alpha virus genus is of considerable public health concern in Southeast Asian and African countries [5]. Mosquitoes in the larval stage are attractive target for control operation due to their low mobility in the breeding habitats and the ease to control in these habitats [6]. Measures to control the mosquito form an essential component of diseases prevention programs in developing countries. Due to increasing resistance of mosquitoes to the insecticides Lima et al., [7] has focussed interest on alternative compounds for mosquito control. Plants, being a natural source of various compounds are known to contain larvicidal agents.

2. MATERIALS AND METHODS:

2.1 selection of plant

Mature and healthy Catharanthus roseus (Figure 1) plant leaves collected from garden of Osmania University, Hyderabad, Telangana, India was taxonomical Identified and conformed at the department of botany University college of science, Osmania University Hyderabad, Telangana India. the arial parts were then washed in dechlorinated water, and dried under shade at room temperature for about 20 days.

2.2 Preparation of solvent extract of L. Camara Leaves

The dried leaves of C. roseus (500g) were powdered mechanically using a commercial electrical stainless-steel blender and extracted with 1000 ml of methanolic a Soxhlet apparatus separately until exhaustion. the extract was concentrated under reduced pressure 22-26mm hg (1mmhg=0.133kpa) at 45˚ c and the residue obtained was stored at 4˚ c.

2.3 Preparation of aqueous extract of L. Camara leaves

Around 500g air-dried C. roseus leaf powder was soaked in 2000ml of distilled water for about 24 h. the aqueous extract was then evaporated under reduced pressure in a rotary evaporator, and the residue was dissolved in a small quantity of water and subjected to freeze-drying. Freeze dried extract was collected in small glass bottles and kept at 0c for further evaluation.

2.4 phytochemical screening.

Phytochemical screening was carried out using standard procedure and the presence of several phytochemicals listed in table 1 was tested.

2.5 GC-MS Analysis

GC-MS Analysis was carried out by Department of microbiology. University college of science ,Osmania University sample ID. (mixanj .plt .ex IIX aromatics) which comprised of an auto sampler and gas chromatography interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: capillary column-624 Ms (30m x 0.32mm x 1.8m) operating in an electron mode at 70ev;helium (99.999%) was used as carrier gas at a constant flow of 1.491ml/min and injected volume of 1.0ml, injector temperature was 140c ;ion source temperature of 200c .the oven temperature was programmed from 45c mass spectrum was taken at 70eV.

2.6 FT-IR Analysis

FT-IR Analysis was carried out by central facilities research development (CFRD) Osmania University.

2.7 Selection of Mosquito species.

The mosquito species selected for the present study were An. Subpictus Ae albopictus and Cx.vishnui

An. subpictus vector of malaria and vector of some helminths [8]

Ae, albopictus vector of yellow fever, Dengue fever, Chikungunya fever

Cx.vishnui vector of JEV [9]

2.8 Mosquito Culture

Cyclic generations of the Anopheles subpictus Ades albopictus, Culex vishnui free exposure to insecticides were maintained separately in Mosquitoes (2'x2'x2') in an insectary with mean temperature of 27˚c ± 2 ˚c and a relative humidity of 70-80%the adult mosquitoes were fed on ten percent glucose solution in water. the eggs laid in ovitraps placed inside the mosquito cages were then transferred enamel larval trays, maintained in the larval raring chamber.

2.9 Larvicidal Bioassay

A total of three trails were carried out with five replicates per trail against vector mosquitoes for the following Bioassays. Toxicity assays of the crude extract were conducted separately using the fourth instar larvae of An. subpictus, Ae. albopictus and Cx. vishnui. Stock solution (1000 ppm) was prepared by dissolving 100 mg of crude extract in 1 ml acetone and volume raised to 100 ml with distilled water. From these different dilutions of (100ppm, 200 ppm, 300 ppm, 400 ppm and 500) ppm were prepared in 200 ml deionised water in 250 ml beaker and 25 fourth instar larvae were released in it and mortality as scored after 24 h. The beakers were kept in a temperature control room at 28 0 C ± 2 o C and the larvae were exposed to 200 ml water containing 0.1ml of acetone served as control. Each treatment was replicated five times [10].

2.10. Larval Susceptibility tests

The larval susceptibility tests were carried according to standard WHO procedure [11]. The extract solutions of different concentrations were prepared and larvae of An. subpictus, Ae. albopictus and Cx. vishnui, were placed in each test solution to observe the larvicidal property as per the following procedure. Groups of 25 larvae were placed in 200 ml of the extract solution. Control experiments without extract were run in parallel. The larvae in each solution were then left for 24 h and the numbers of dead larvae were counted after 24 h of exposure, and the percentage mortality was reported from the average of five replicates. Mortality was recorded when control mortality ranged from 5 – 20 per cent, it was corrected by Abbott's formula.[12].

2.11. Statistical analysis

The average larval mortality data was subjected to probit analysis for calculating LC50, LC90 and other statistics at 95% confidence limits of upper confidence limit, lower confidence limit and chi-square values were calculated using the SPSS 11.5 (Statistical Package of Social Sciences) software. Results with P< 0.05 were considered to be statistically significant [13].

Table1.Phytochemical screening of leaf extract of L. camara.

s.no	phytochemicals	Aqueous	Methanol
1	carbohydrates	++	+++
2	Tannins	-	++
3	saponins	+++	+
4	Flavonoids	++	+++
5	Alkaloids	+++	++
6	Quinones	+	+++
7	Glycosides	++	-

+++ Strongly positive ++ Positive + Trace - Not detected

Table 2. GC-MS analysis of methanol leaf extract of L. camara.

S.No	Retention Time	Compound	Peak Area (%)
1	2.624	Propanoic acid ethyl ester	1.27
2	3.457	Toluene	0.81
3	4.020	Glycolaldehyde dimer	0.03
4	5.883	Ethylbenzene	0.34
5	6.1470.15	0-xylene	0.15
6	7.7.205	Isophthalic acid	0.06
7	8.802	cyclotetrasiloxane	0.16

Table 3. Larvicidal activity of Lantana camara leaf extract against IV instar larvae of Anopheles subpictus

Extraction	Concentration (ppm)	%Mortality 24hrs	LC50 (LCL-UCL) (ppm)	LC90 (LCL-UCL) (ppm)	RA	Chi-square (Df=4)
Aqueous	Control	0.00±0.000	4.90 (51.6-54.4)	5.31 (62.1-63.9)	y=21.2+ 0.104x	27.20
	100	33.6±2.05				
	200	44.4±3.00				
	300	53.0±2.82				
	400	63.0±2.44				
	500	73.6±2.41				
Methanol	Control	0.00±0.000	5.58 (76.8-8.1)	6.18 (90.2-92.4)	y=32.0+ 0.136x	13.92
	100	49.60±2.191				
	200	59.20±3.347				
	300	78.40±4.561				
	400	91.20±3.347				
	500	100.00±0.000				

The mortality (Mean±SD) is the mean value of five replicates, control-Nil mortality; Lower confidence limit UCL; Upper confidence limit RA-regression analysis Df-degrees of freedom chi-values were significant at p<0.005 level.

Table 4. Larvicidal activity of Lantana camara leaf extract against IV instar larvae of Ae. albopictus.

Extraction	Concentration ppm	%Mortality 24hrs	LC50 (LCL-UCL) ppm	LC90 (LCL-UCL) ppm	RA	Chi-square (Df=4)
Aqueous	Control	0.00±0.000	4.05 (53.1-54.1)	5.61 (74.6-75.8)	Y=18.7+0.129x	21.06
	100	33.6±1085				
	200	44.6±2.15				
	300	53.6±1.49				
	400	75.2±1.72				
	500	82.8±0.74				
Methanol	Control	0.00±0.000	5.84 (83.1-85.8)	6.41 (88.3-90.9)	Y=43.6+0.116x	9.24
	100	60.00±4.000				
	200	67.20±3.347				
	300	84.00±4.000				
	400	89.60±3.578				
	500	100.00±0.000				

The mortality (Mean±SD) is the mean value of five replicates. control-Nil mortality, LCL; Lower confident limit UCL; Upper confident Limit, RA- regression analysis, Df-degrees of freedom, chi-square values were significant at p<0.05 level

Table 5. Larvicidal activity of Lantana camara leaf extract against IV instar larvae of Cx. Vishnui

Extraction	Concentration (ppm)	%Mortality 24hrs	LC50 (LCL-UCL) (ppm)	LC90 (LCL-UCL) (ppm)	RA	Chi-square (Df=4)
Aqueous	**Control**	0.00±0.000	4.15 (52.9-53.9)	5.63 (73.4-74.2)	Y=16.3+0.133x	26.32
	100	34.6±3.07				
	200	44.6±1.95				
	300	53.4±1.35				
	400	73.8±1.16				
	500	84.6±1.74				
Methanol	**Control**	0.00±0.000	5.47 (93.6-95.2)	6.75 (94.6-96.8)	Y=82.2+0.036x	0.88
	100	83.20±3.347				
	200	89.60±2.191				
	300	94.40±2.192				
	400	95.20±1.781				
	500	100.00±0.000				

The mortality (Mean±SD) is the mean value of five replicates. Control-Nil mortality LCL; Lower confidence limit; UCL; Upper confidence limit; RA-regression analysis Df-degrees of freedom, chi-square values ware significant at p<0.05 level

Fig 1. Lantana camara

Fig.2 GC-MS analysis of Lantana camara

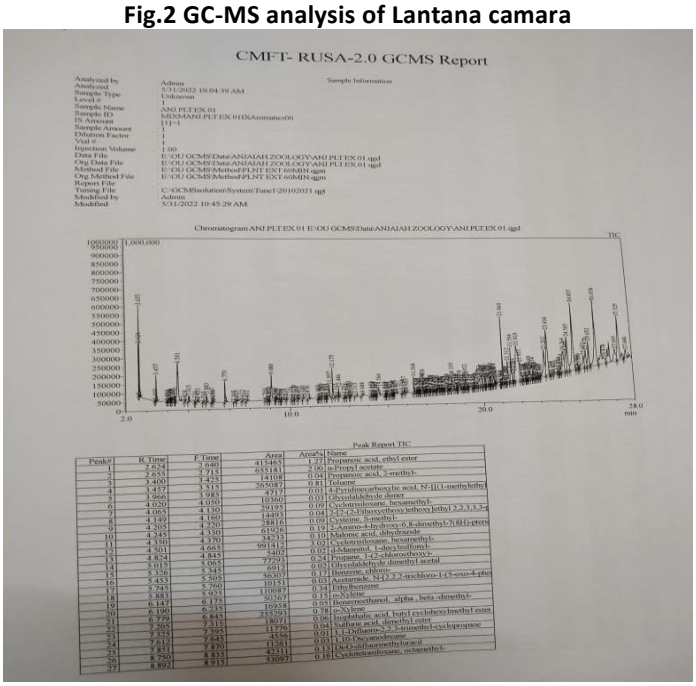


Fig-3 FT-IR analysis of Lantana camara

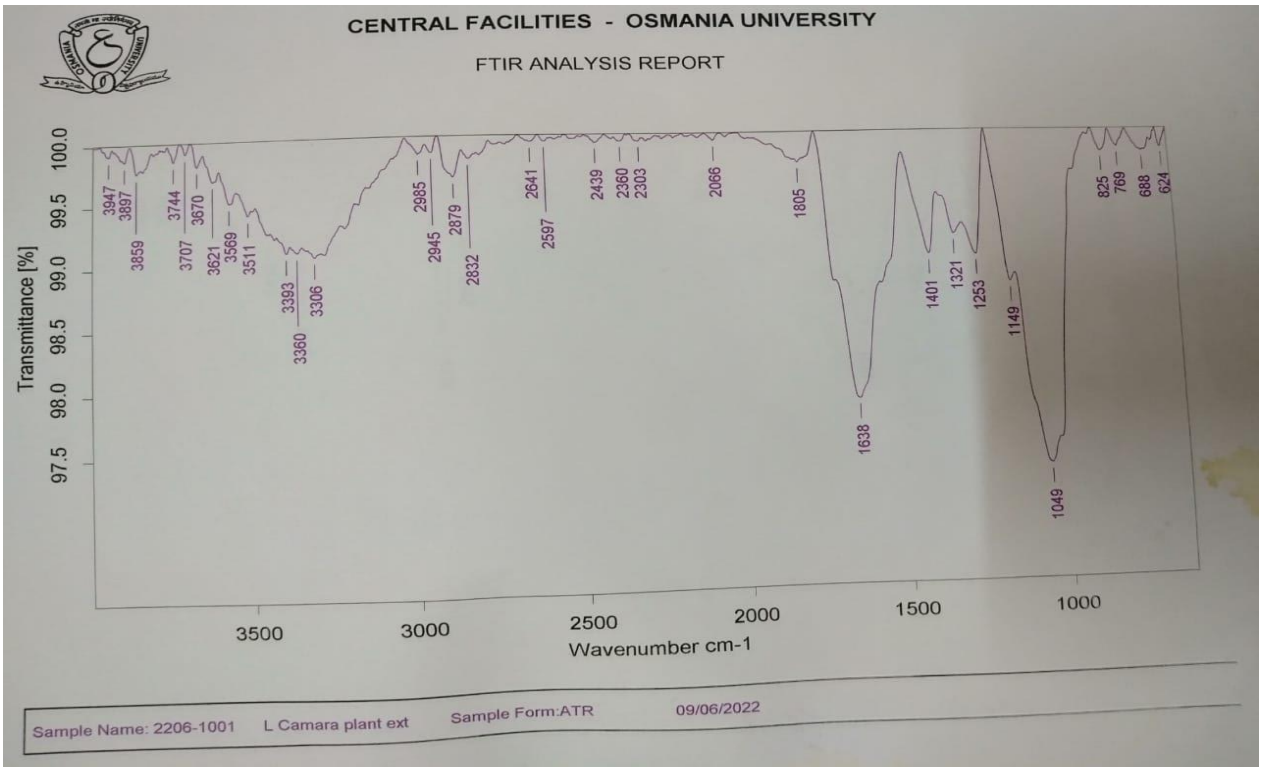


Fig.4 Larvicidal activity of plant leaf extract of L. camara against IV instar larvae of An. subpictus.

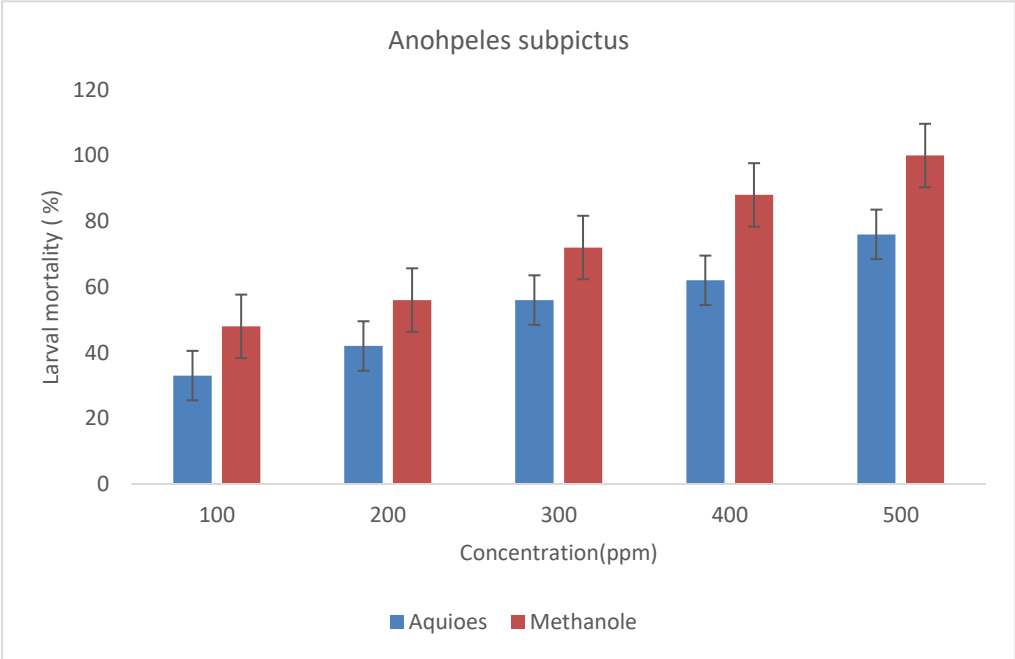


Fig.5. Larvicidal activity of plant leaf extract of Lantana camara against IV instar larvae of Ades albopictus.

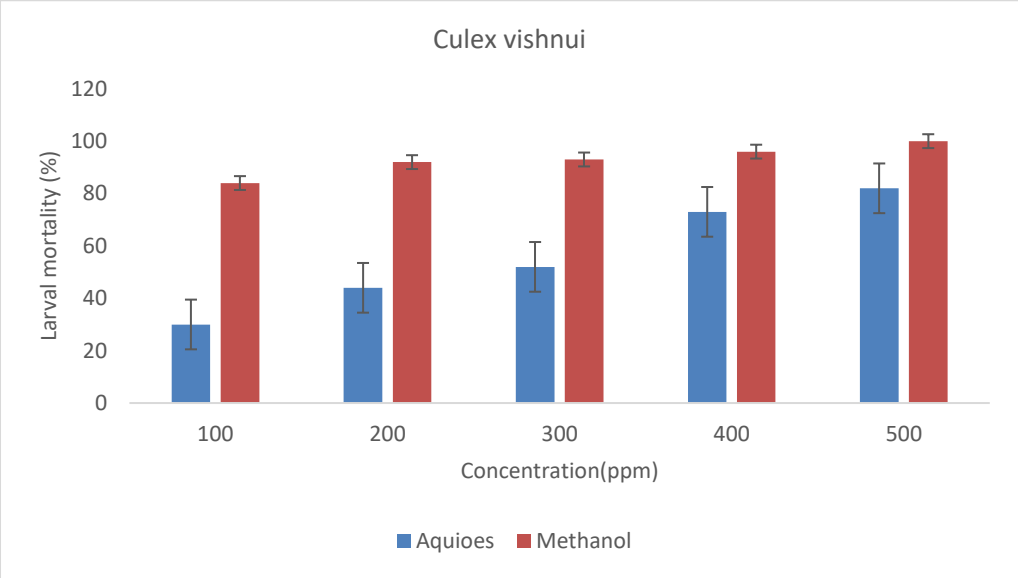
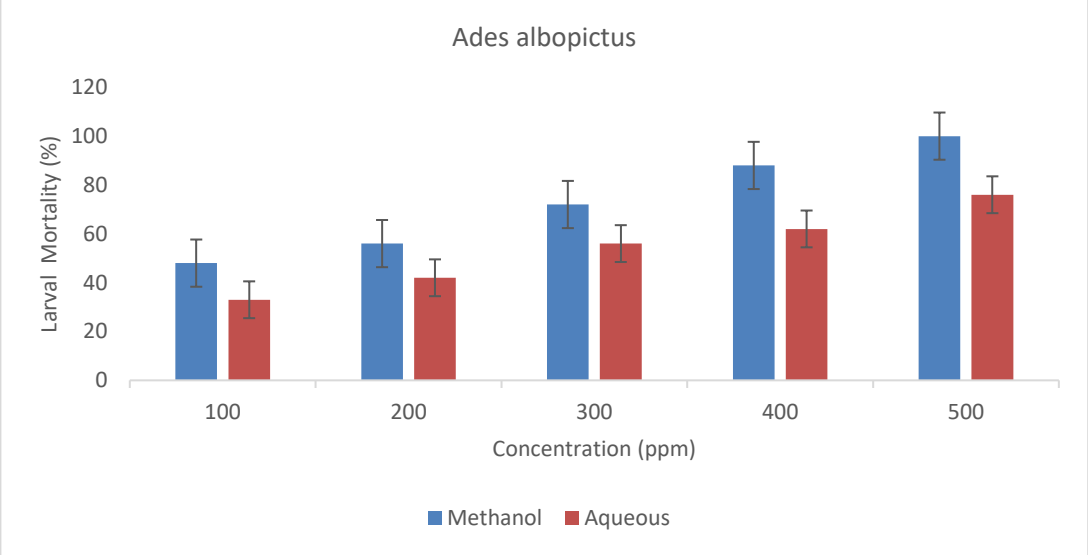


Fig 6. Larvicidal activity of plant leaf extract of L. camara against IV Instar larvae of Cx. vishnui.



3.RESULTS:

The results of phytochemical characterization of L. camara aculeata are presented in table -1 the preliminary phytochemical screening revealed the strong presence of carbohydrates, saponins, alkaloids, in Aqueous and Methanol. the other phytocompounds present were glycosides, steroids, tannins, quinones.

GC-MS Characterization of methanolic extract of L. camara aculeata are presented in Table -2 the Major compounds observed propanoic acid, toluene ethyl ester Glycoldehide dimer etc (Fig.2)

FT-IR Characterisation of methanolic extract of L. camara aculeate are presented in (Fig. 3)

Based on the probit analysis between the concentrations of plant extract against fourth instar larvae of An. subpictus, Ades albopictus, Culex visnui after 24 hrs exposure are represented in Table 3,4,5 the methanolic leaf extract was found to be more potent against an.subpictus Ades albopictus culex vishnui .with LC50 and LC90value of 5.58 ppm and 6.18 ppm and 5. 84 ppm and 5.47ppm and 6.75 when compared to Aqueous leaf extract with LC50 and LC90 value of 4.90 ppm and 5.31 and 4.05 ppm and 5.61 ppm and 4.15 ppm and 5.63 ppm against the fourth instar larvae of An.subpictus, Ae.albopictus, Cx .visnui ,other tested extracts also showed mosquito larvicidal activity at a relatively high concentration when compared to methanol plant leaf extract.

4. DISCUSSION:

The results of the present study methanolic leaf extract of L. camara shows 100% mortality at 500ppm for IV larvae of An. subpictus Ae. Albopictus Cx.vishnui. When compared to Aqueous extract of L. camara.

In this study the aqueous, methanol extracts of lantana camara leaves showed potential larvicidal activity against An. subpictus and Ae, albopictus,Cx. Vishnui. Mosquito larval control using larvicidal agents is a major component in the control of vector borne diseases. Plant as potential larvicides is considered as viable and preferred alternative in the control of the mosquito species at the community level. A large number of plant extracts have been reported to have mosquitocidal or repellent activities against mosquito vectors, but few plant products have shown practical utility for mosquito control [14]. In the present study methanolic extract of L. camara aculeata showed 100% larvicidal activity against the fourth instar larvae of Cx. quinquefasciatus when compared to Ae. aegypti and An. stephensi. Triterpenoids are generally credited with mosquito larvicidal activities [15]. Thus, high mortality rate recorded in the present study could be due to the presence of terpenoids and triterpenoids which are hydrocarbons present in the extract that inhibits the developmental stages of insects [16,17,18]. Phytochemicals derived from plant sources can act as larvicide, insect growth regulators, repellent and ovipositor attractant and have different activities which have been observed by many researches [19]. Similarly, the presence of lantadene triterpenoids and furanonaphtha quinones in Lantana sp., have been reported to have mosquito larvicidal properties [20]. DEKA [21], has reported the antifeeding and repellent effect of L. camara aculeata on a mosquito. The terpenic compounds, mainly precocenes, with their anti-juvenile hormonal activity are probably responsible for the insecticidal properties.

5. CONCLUSION:

It is evident that the plant products are emerging as a potential source of mosquito control. Crude extract or isolated bioactive compounds from the plant L. camara aculeata could be used in stagnant water bodies which are known to be the breeding grounds for the mosquitoes. The weed L. camara aculeata extracts showed promising activity in mosquito control and its commercial utilization is very much feasible.

6. ACKNOWLEDGEMENTS:

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