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Larvicidal activity of essential oils of Haridra and Tulsi against Aedes aegypti and Anopheles larvae

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ABSTRACT

An attempt is made in the present study to analyse the larvicidal activity of Haridra and Tulsi against Aedes and Anopheles larvae. Larval bioassays were carried out at different concentrations 50-500ppm. The larval mortality of fourth instar larvae of A. aegypti and Anopheles after 24h of treatment was observed separately in control, 50, 62.5, 100, 150, 250, 300 and 500 ppm concentrations. Based on the Probit analysis, Significant effect (Estimate adh_conc = 3.069, 95% C.I. [2.082, 4.057], z = 6.093, p<.05) of Haridra oil extract concentration for Aedes (adh conc) on the Probit response or mortality was observed. The median lethal Concentration, LC50= 166.353, 95% C.I. [134.745, 203.268], 90% Lethal Concentration, LC90 = 435.065, 95% C.I. [328.259, 712.462] were recorded. Statistical analysis revealed that significant effect (Estimate adt_conc = 3.369, 95% C.I. [2.229, 4.509], z = 5.791, p<.05) of Tulsi oil extract on Aedes larvae concentration (adt_conc) on the Probit response or larvae mortality (adt_mort) was observed. The median Lethal Concentration, LC50= 105.051, 95% Confidence Intervals (C.I). [81.451, 127.428], 90% Lethal Concentration, LC90 = 252.242, 95% C.I. [199.229, 376.772] of essential oil of Tulsi oil against Aedes aegypti larvae was recorded. No mortality of Aedes larvae was recorded in the control group. Larvicidal activity of both the plants against Aedes larvae at different concentration was significant. But LC50 and LC90 values of Ocimum sanctum (Tulsi) against mortality of Aedes aegypti were less as compared to Curcuma longa (Haridra). It revealed that Tulsi showed better larvicidal activity against Aedes larvae as compared to Haridra. It was also observed that LC₅₀ and LC₉₀ values of Haridra against Anopheles larvae were less than Tulsi. It revealed that Haridra showed better larvicidal activity against Anopheles larvae as compared to Tulsi.

Key words: Mosquito larvicidal activity, Haridra, Tulsi, Aedes aegypti, Anopheles.

INTRODUCTION

Mosquitoes are the most disturbing insects and are very hazardous to humanity. A single mosquito bite can cause many deadly diseases and many vector-borne diseases. Dangerous diseases like Dengue, Chikungunya,

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Malaria, Filariasis, Japanese Encephalitis, etc. spread through several mosquito species of the genera Aedes, Culex & Anopheles. Dengue is also undoubtedly one of the biggest health concerns in India.[1] NVBDCP data shows that 2017 was an even worse year: 1.88 lakh people were diagnosed with dengue and 325 were dead. NVBDCP data also reveals that over one lakh people were diagnosed and an estimated 172 people died from dengue in 2018.[2] According to the World Malaria Report 2018 of WHO, the data from 2015-2017 highlighted that no significant progress in reducing global malaria cases was made in this period. There were an estimated 219 million cases and 4, 35,000 related deaths in 2017.[3] In India, according to National Vector Borne Disease Control Programme in Uttarakhand in 2019 (prov) till November there were 10,500 cases and 8 deaths.^[4] Additionally, it has been

found that mosquitoes tend to develop resistance to many synthetic insecticides (S.R. Yankanchi et.al; 2014). [5] This study aims to provide better potency and efficacy through essential oils extracts of herbs, which are known to show improved individual as well as synergistic effects. (Sarath Mangalat et.al: 2004. Shivaii Kasthe et.al; 2015).[6] Haridra is described in Agni Purana. Haridra was used in the treatment of Prameha, Arsha, Kamala, and Vrana Ropan. In Atharvaveda, it is used for the treatment of Kushth. Various Nighantus have mentioned synonyms of Haridra like Krimighna, Jantughna, and Vishghna and effective against Krimi [insects]. [7] Curcumin (diferuloylmethane) and various volatile oils, including turmerone, atlantone and zingiberene, are Poly phenolic curcuminoids, Pharmacological actions of Haridra [Curcuma longa] are antimicrobial activity, antiviral activity, antifungal activity. Tulsi has been classified in the classical Nighantus and Samhitas of Ayurveda under different Gana or Varga (groups). Properties of Tulsi are mentioned as Vish-nashak in many Ayurvedic texts, as well as Keet-Vish Nashak properties in various formulations. The chemical composition of Tulsi [Ocimum sanctum] is a volatile oil, phenolic compounds, flavonoids Aesculectin, Aesculin, Stearic acid Aromadendrene oxide, Benzaldehyde, etc. The pharmacological actions of Tulsi [Ocimum sanctum] are antimicrobial activity, anti-inflammatory activity, and antipyretic activity. [8] By selecting these two plants due to their properties we have taken these plants for our study.

MATERIALS AND METHODS

Plants of *Haridra* and *Tulsi* were collected from Palampur, H.P, and Shirish were collected from Rishikul Haridwar. The Plants identification was done in the department of Dravyaguna, Rishikul Campus Haridwar. Essential oils of the plant materials were extracted by Clevenger Apparatus. The Clevenger apparatus was named after its inventor, Joseph Franklin Clevenger, WHO published in 1928. Parts of Clevenger Apparatus: 1. Heating mantle 2. Round bottom flask 3. Condenser 4. Clevenger part 5. Water inlet and water outlet.

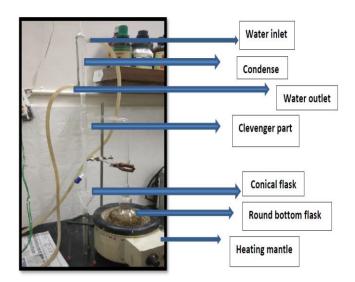


Fig. 1: Clevenger Apparatus^[9]

Samples Preparation

Rhizomes (Roots) parts of the *Haridra*, leaves of *Tulsi* and bark of Shirish were taken for the extraction of the essential oils. The plant materials were washed with distilled water to remove dust particles and shade dried. Essential oils of the plants were obtained by the hydro-distillation method. In Clevenger apparatus, the raw material of the shade dried was subjected to water distillation in a Clevenger apparatus for 7 h. The oil layer is separated from the aqueous phase using nhexane with the help of a separating funnel. The anhydrous sodium sulfate is added in hexane-oil solution to remove water content absorbed by hexane. The oil is obtained by removal of n-hexane at low temperature and the samples were kept in a refrigerator at 4ºC.

Yield of essential oils of *Haridra* (*Curcuma longa*) rhizome paste and *Tulsi* (*Ocimum sanctum*) leaves

Essential oils of *Curcuma longa* (Haldi) rhizome paste and *Ocimum sanctum* (*Tulsi*) leaves were obtained by hydro-steam distillation by Clevenger extraction methods for 4-7 hour daily for several days. A total of 250 gm of the plant materials of each plant was extracted in which 0.2gm of *Curcuma longa* and 0.1gm of *Ocimum sanctum* were obtained, which yielded 1 gm of essential oil of *Curcuma longa* and 0.5 gm of essential oil of *Ocimum sanctum* after 5 times of extraction. % Yield of essential oils of *Curcuma longa*

(Haldi) rhizome paste and *Ocimum sanctum* (*Tulsi*) leaves is given in table 1.

Table 1: Yield of essential oils of *Haridra* (*Curcuma longa*) rhizome paste and *Tulsi* (*Ocimum sanctum*) leaves.

Name of plants	Amount of plant materials extracted in per extraction (gm)*	Total amount of plant materials extracted (gm)	Total Yield of essential oils	% yield
Curcuma longa (Haldi) rhizome paste	50	250 gm	1gm	0.4
Ocimum sanctum (Tulsi) leaves	50	250 gm	0.5 gm	0.2

^{*}Plant material was extracted for 4 hour in Clevenger apparatus daily for several days.

Field collection of mosquito larvae and their rearing in laboratory

Mosquito larvae of *Aedes* and Anopheles were collected from breeding sites in different areas in Haridwar, BHEL Sector 4,5, Roadways Bus Service Station, Haridwar, Old Industrial Area of district Haridwar, Near Nigam Office, Haridwar, Village Bhogpur Tall of Chilla range, District Pauri Garhwal The larvae were brought to the laboratory and the collected mosquito larvae were identified at the Field Unit of The National Institute of Malaria Research; Haridwar. The collected mosquito larvae were brought to the laboratory and kept in the laboratory at 26±20C. The larvae were provided a mixture of dog biscuit and yeast powder in a 3:2 ratio as nutrients. The larvae were reared in the laboratory up to the 3rd and 4th instars for testing of larvicidal activity.

Bioassays test for determination of larvicidal activity

Bioassay test for determination of larvicidal activity of essential oils of the plants was performed on late 3rd and early 4th stage larvae of Anopheles and *Aedes*. Larvicidal activity of *Haridra* and *Tulsi* was performed

alone at 500 ppm, 300 ppm, 250 ppm, 150 ppm,100 ppm 62.5 ppm, and 50 ppm. Twenty mosquitoes were released into larvae. Twenty larvae of different mosquito species were placed into a 500 ml glass beaker/ bowl containing 250 ml of water. 0.1ml essential oil of the plant was dissolved in 1ml ethanol. Essential oil dissolved in ethanol was added to a 500ml glass beaker containing 250 ml at different concentrations. The mosquito larvae were exposed to different concentrations of essential oil. The test larvae were provided a mixture of dog biscuit and yeast powder in a 3:2 ratio as nutrients. Mortality of larvae was monitored within 24 hours of the exposure. All the tests were carried out in 3 replicates along with the untreated control. Data were recorded and analyzed.

OBSERVATION AND RESULTS

Larvicidal efficacy of essential oil of *Haridra* against *Aedes aegypti* larvae

Results of larvicidal activity of essential oil of *Curcuma longa* against *Aedes* larvae at different concentration is given in table-2. It was observed that 100 % mortality of *Aedes* larvae was observed at concentration of 500 ppm. Thereafter % mean mortality were 71.0 (range: 70.0-75.0), 65.0 (range: 60.0-75.0), 46.0 (40.0-50.0), 23.0 (40.0-50.0), 13.0 (10.0-15.0), 0 at concentration of 500, 300, 250, 150, 100, 62.5, 50.0 ppm respectively at 24 hour exposure of the larvae. No mortality of *Aedes* larvae were recorded in control group.

Table 2: Larvicidal activity of essential oil of *Curcuma longa* (Haldi) against *Aedes*

Concentr ation (ppm)	Replica tes	No. of larva e expos ed	Morta lity of larvae in 24 hour (Nos.)	Perce nt morta lity in 24 hr.	% correc ted mortal ity in 24 hr.	Mean % correc ted Morta lity (Rang e)*
500	R-1	20	20	100	100	100
	R-2	20	20	100	100	
	R-3	20	20	100	100	

300	R-1	20	14	70	70	71 (70-
	R-2	20	15	75	75	75)
	R-3	20	14	70	70	
250	R-1	20	12	60	60	65 (60-
	R-2	20	12	60	60	75)
	R-3	20	15	75	75	
150	R-1	20	10	50	50	46
	R-2	20	10	50	50	(40- 50)
	R-3	20	8	40	40	
100	R-1	20	5	25	50	23 (40-
	R-2	20	4	20	50	50)
	R-3	20	5	25	40	
62.5	R-1	20	2	10	10	13 (10-
	R-2	20	3	15	15	15)
	R-3	20	3	15	15	
50	R-1	20	0	0	0	0
	R-2	20	0	0	0	
	R-3	20	0	0	0	
Control	R-1	20	0	0	0	0
	R-2	20	0	0	0	
	R-3	20	0	0	0	

Larvicidal efficacy of essential oil of *Ocimum sanctum* against *Aedes aegypti* larvae:

Results of larvicidal activity of essential oil of *Ocimum* sanctum against *Aedes* larvae at different concentration is given in table-3. 100 % mortality of *Aedes* larvae was observed at concentration of 500 ppm. Thereafter % mean mortality were 95.0 (range: 90.0-100.0), 88.0 (range:85.0-90.0), 63.3 (range: 60.0-70.0), 46.0 (range:40.0-50.0) 10.0, 0 at concentration of 500, 300, 250, 150, 100, 62.5, 50.0 ppm respectively

at 24 hour exposure of the larvae. No mortality of

Aedes larvae were recorded in control group.

Table 3: Larvicidal efficacy of essential oil of *Tulsi* (*Ocimum sanctum*) against *Aedes aegypti* larvae.

(Ocimum)						
Concentr ation (ppm)	Replica tes	No. of larva e expos ed	Morta lity of larvae in 24 hour (Nos.)	Perce nt morta lity in 24 hr.	% correc ted mortal ity in 24 hr.	Mean % correc ted Morta lity (Rang e)*
500	R-1	20	20	100	100	100
	R-2	20	20	100	100	
	R-3	20	20	100	100	
300	R-1	20	19	95	95	95
	R-2	20	20	100	100	(90- 100)
	R-3	20	19	95	90	
250	R-1	20	18	90	90	88
	R-2	20	18	90	90	90)
	R-3	20	17	85	85	
150	R-1	20	14	70	70	63.3 (60- 70)
	R-2	20	12	60	60	
	R-3	20	12	60	60	
100	R-1	20	10	50	50	46
	R-2	20	10	50	50	(40- 50)
	R-3	20	08	40	40	
62.5	R-1	20	5	5	10	10
	R-2	20	5	5	10	
	R-3	20	6	6	10	
50	R-1	20	0	0	0	0
	R-2	20	0	0	0	
	R-3	20	0	0	0	
Control	R-1	20	0	0	0	0
	R-2	20	0	0	0	
	R-3	20	0	0	0	

Larvicidal efficacy of essential oil of *Haridra* against *Anopheles* larvae.

Results of larvicidal activity of essential oil of *Haridra* against *Anopheles* larvae at different concentration is given in table-4. It was observed that percent mean mortality of *Anopheles* larvae was 86.0 (range: 80.0-100.0), 81.0 (range: 70.0-90.0), 60.0 (range: 55.0-65.0), 46.0 (range: 40.0-50.0) 30.0, 0 at concentration of 500, 300, 250, 150, 100, 62.5, 50.0 ppm respectively at 24 hour exposure of the larvae. No mortality of *Aedes* larvae were recorded in control group.

Table 4: Larvicidal activity of essential oil of *Haridra* (*Curcuma longa*) against *Anopheles*.

Concentr ation (ppm)	Replica tes	No. of larva e expos ed	Morta lity of larvae in 24 hour (Nos.)	Perce nt morta lity in 24 hr.	% correc ted mortal ity in 24 hr.	Mean % correc ted Morta lity (Rang e)*
500	R-1	20	18	80	80	86
	R-2	20	18	80	80	(80- 100)
	R-3	20	20	100	100	
300	R-1	20	14	70	70	81
	R-2	20	16	85	85	(70- 90)
	R-3	20	19	95	90	
250	R-1	20	11	55	55	60
	R-2	20	12	60	60	(55- 65)
	R-3	20	13	65	65	
150	R-1	20	11	55	55	58
	R-2	20	12	60	60	(55- 60)
	R-3	20	12	60	60	
100	R-1	20	10	50	50	46
	R-2	20	10	50	50	(40- 50)
	R-3	20	8	40	40	
62.5	R-1	20	5	30	30	30
	R-2	20	5	30	30	

	R-3	20	6	30	30	
50	R-1	20	0	0	0	0
	R-2	20	0	0	0	
	R-3	20	0	0	0	
Control	R-1	20	0	0	0	0
	R-2	20	0	0	0	
	R-3	20	0	0	0	

Larvicidal efficacy of essential oil of *Tulsi* against *Anopheles* larvae

Results of Preliminary study of larvicidal efficacy of Tulsi extract oil against Anopheles larvae are shown in table-5. Results of larvicidal activity of essential oil of Tulsi against *Anopheles* larvae at different concentration is given in table-5. Mean percent mortality were 65.0 (range: 55.0-70.0), 51.0 (range: 55.0-55.0), 33.0 (range:25.0.0-40.0), 18.0 (range:15.0-20.0) 15.0 (range:10.0-15.0), 6.0 (range:5.0-10.0), 0 at concentration of 500, 300, 250, 150, 100, 62.5, 50.0 ppm respectively at 24-hour exposure of the larvae. No mortality of Anopheles larvae was recorded in control group.

Table 5: Larvicidal activity of essential oil of *Tulsi* (*Ocimum sanctum*) against *Anopheles*.

Concent ration (ppm)	Replic ates	No. of larva e expo sed	Mort ality of larva e in 24 hour (Nos.	Perce nt mort ality in 24 hr.	% corre cted mort ality in 24 hr.	Mean % corre cted Mort ality (Rang e)*
500	R-1	20	14	70	70	65 (55-
	R-2	20	11	55	55	70)
	R-3	20	14	70	70	
300	R-1	20	10	50	50	
	R-2	20	11	55	55	

	R-3	20	10	50	50	51 (50- 55)
250	R-1	20	8	40	40	33 (25-
	R-2	20	7	35	35	40)
	R-3	20	5	25	25	
150	R-1	20	4	20	20	18 (15-
	R-2	20	3	15	15	20)
	R-3	20	4	20	20	
100	R-1	20	3	15	15	15 (10- 15)
	R-2	20	2	10	10	
	R-3	20	2	10	10	
62.5	R-1	20	2	25	10	6 (5-
	R-2	20	1	30	5	10)
	R-3	20	1	25	5	
50	R-1	20	0	0	0	0
	R-2	20	0	0	0	
	R-3	20	0	0	0	
Control	R-1	20	0	0	0	0
	R-2	20	0	0	0	
	R-3	20	0	0	0	

Statistical Analysis

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The Probit model for *Haridra* oil extract on *Aedes* larvae successfully converges and finds an optimal solution after 14 iterations. The Higher the number of iterations, the higher the variability in the relationship represented by the Probit model, thus, greater attempts are required by the system to arrive at the maximum likelihood of mortality probability. Significant effect (Estimate adh_conc = 3.069, 95% C.I. [2.082, 4.057], z = 6.093, p<.05 of *Haridra* oil-extract concentration for *Aedes* (adh_conc) on the Probit

response or mortality was observed. The median lethal Concentration, LC50= 166.353, 95% C.I. [134.745, 203.268], 90% Lethal Concentration, LC90 = 435.065, 95% C.I. [328.259, 712.462] were recorded. The results are graphically depicted in Figures 2.1 and 2.2.

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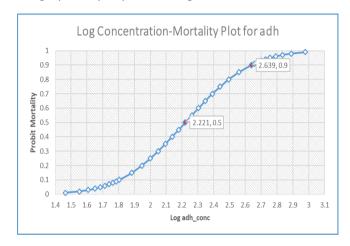


Fig. 2.1: Log Concentration-Mortality Plot of essential oil of *Haridra* against *Aedes aegypti* larvae.

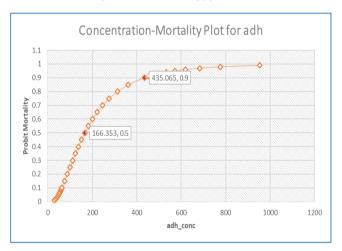


Fig. 2.2: Log Concentration-Mortality Plot of essential oil of *Curcuma longa* against *Aedes aegypti* larvae.

Statistical analysis revealed that significant effect (Estimate adt_conc = 3.369, 95% C.I. [2.229, 4.509], z = 5.791, p<.05) of Tulsi oil-extract on Aedes larvae concentration (adt_conc) on the Probit response or larvae mortality (adt_mort) was observed. The median Lethal Concentration, LC₅₀= 105.051, 95% Confidence Intervals (C.I). [81.451, 127.428], 90% Lethal Concentration, LC₉₀ = 252.242, 95% C.I. [199.229, 376.772] of essential oil of $Ocimum\ sanctum\ against\ Aedes\ aegypti\ larvae\ was\ recorded.$ The results are graphically depicted in Figures 3.1 and 3.2.

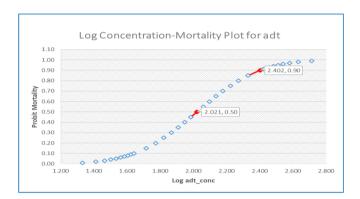


Fig. 3.1: Log Concentration-Mortality Plot of essential oil of *Ocimum sanctum* against *Aedes aegypti* larvae.

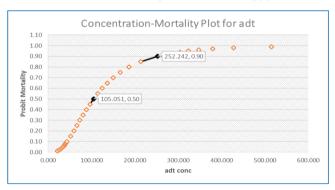


Fig. 3.2: Concentration-Mortality Plot of essential oil of *Ocimum sanctum* against *Aedes aegypti* larvae.

Essential oil of *Haridra* at different concentration shows a significant effect (Estimate anh_conc = 2.038, 95% C.I. [1.180, 2.896], z = 4.655, p < .05) against mortality of *Anopheles* Larvae on the Probit response or mortality. The median Lethal Concentration, $LC_{50} = 124.823$, 95% Confidence interval CI. [84.002, 164.336], 90% Lethal Concentration, $LC_{90} = 530.978$, 95% C.I. [349.474, 1333.827] of essential oil of *Curcuma longa* against *Anopheles* larvae was recorded. The results are graphically depicted in Figures 4.1 and 4.2.

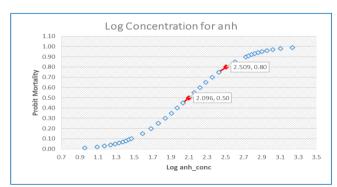


Fig. 4.1: Log Concentration-Mortality Plot of essential oil of *Curcuma longa* against *Anopheles* larvae.

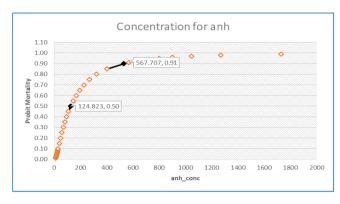


Fig. 4.2: Concentration-Mortality Plot of essential oil of *Curcuma longa* against *Anopheles* larvae.

Significant effect (Estimate ant_conc = 2.257, 95% C.I. [1.135, 3.200], z = 4.694, p<.05) of Tulsi oil-extract on Anopheles Larvae concentration (ant_conc) on the Probit response or mortality (ant mort) was observed.Median Lethal Concentration, LC_{50} = 339.419, 95% Confidence Interval (C.I.) [259.239, 533.727], 90% Lethal Concentration, LC_{90} = 1254.519, 95% C.I. [717.77, 4574.12] of essential oil of $Ocimum\ sanctum$ against Anopheles larvae were calculated. The results are graphically depicted in Figures 5.1 and 5.2.

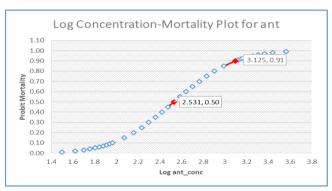


Fig. 5.1: Log Concentration-Mortality Plot of essential oil of *Ocimum sanctum* against *Anopheles* larvae.

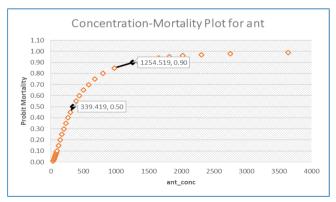
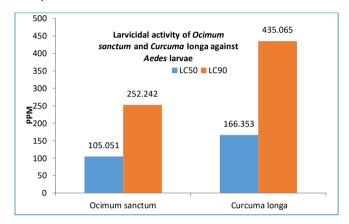


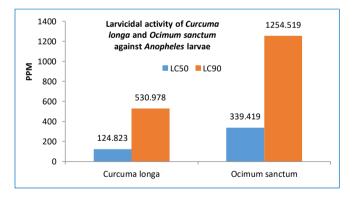
Fig. 5.2: Concentration-Mortality Plot of essential oil of *Ocimum sanctum* against *Anopheles* larvae.

DISCUSSION

Larvicidal activity of both the plants against *Aedes* larvae at different concentration was significant. But LC₅₀ and LC₉₀ values of *Ocimum sanctum (Tulsi)* against mortality of *Aedes aegypti* were less as compared to *Curcuma longa (Haridra)*. It revealed that *Tulsi* showed better larvicidal activity against *Aedes* larvae as compared to *Haridra*.



It was observed that LC₅₀ and LC₉₀ values of *Haridra* against *Anopheles* larvae were less than *Tulsi*. It revealed that *Haridra* showed better larvicidal activity against *Anopheles* larvae as compared to *Tulsi*.



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