

Eco-friendly Plant-based Disease Control: Exploring *Terminalia* Species Extracts as Biopesticides against Fungal Pathogens

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Received 23 April 2025; revised 19 May 2025

The increasing reliance on synthetic fungicide to combat fungal diseases in vegetable crops has highlighted concerns about pathogen resistance, human health risks, and environmental safety. As a result, research into plant-based bio-pesticides has generated much interest as a viable alternative. This study focuses on the antifungal efficacy of *Terminalia alata*, *Terminalia bellirica*, *Terminalia arjuna*, and *Terminalia catappa* against fungal pathogens affecting economically important vegetable crops-brinjal (*Solanum melongena*), tomato (*Solanum lycopersicum*), cauliflower (*Brassica oleracea*), spinach (*Spinacia oleracea*), and lady finger (*Abelmoschus esculentus*). Ten fungal pathogens (*Phomopsis vexans*, *Aspergillus niger*, *Colletotrichum fructicola*, *Alternaria solani*, *Peronospora parasitica*, *Cladosporium* spp., *Sclerotinia sclerotiorum*, *Stemphylium botryosum* f. sp. *spinaciae*, and *Rhizoctonia solani*, *Fusarium oxysporum*.) were isolated and identified using morphological, cultural, and 18s rRNA gene analysis. The antifungal activity of six solvent extracts like, chloroform, ethanol, petroleum ether, hexane and water of leaves, bark and fruits of four *Terminalia* sp. were investigated. Ethanolic extracts demonstrated the highest antifungal efficacy, with *Terminalia bellirica* and *Terminalia arjuna* fruit extracts exhibiting remarkable inhibition percentages ($\geq 90\%$) against *Colletotrichum fructicola* and *Peronospora parasitica*. Although water and hexane extracts showed somewhat less inhibitory effects, chloroform and petroleum ether extracts also exhibited significant antifungal properties. In terms of mycelial growth inhibition and percentage inhibition, ethanolic extracts consistently outperformed other solvents. Phytochemical analysis revealed the presence of biologically active compounds such as flavonoids, alkaloids, tannins, phenols, saponins, terpenoids, glycosides and sterols, which may be responsible for the strong antifungal effects of the study. The findings suggest that potential of *Terminalia* species as a viable source of natural biological pesticides, that can act as a efficient, sustainable and environmentally beneficial, alternative to chemical fungicides. Further studies are advised to isolate, purify and characterize the active antifungal components and evaluate their field application in different agro-climatic regions. In order to improve crop protection, lessen the impact on the environment, and promote sustainable agricultural practices, the study emphasizes the necessity of implementing plant-based disease management measures.

Keywords: Antifungal activity, Bio-pesticides, Eco-friendly, Phytochemical, *Terminalia* Spp.

Fungal pathogens that cause significant yield losses in economically significant vegetable crops like brinjal (*Solanum melongena*), tomato (*Solanum lycopersicum*), cauliflower (*Brassica oleracea*), spinach (*Spinacia oleracea*), and lady finger (*Abelmoschus esculentus*) are posing an increasing threat to agricultural productivity. Despite being widely used, conventional synthetic fungicides have caused environmental contamination, dangers to human health, and the evolution of infections resistant to fungicides¹. Furthermore, soil deterioration and biodiversity loss have been connected to the careless use of chemical fungicides². Plant-based biopesticides have emerged as a feasible alternative as a result of the increased demand for sustainable and

environmentally friendly methods of managing plant diseases³. Species of the genus *Terminalia* are among the medicinal plants having significant antibacterial qualities that have drawn scientific interest. It is well known that *Terminalia arjuna*, *Terminalia bellirica*, *Terminalia catappa*, and *Terminalia alata* contain bioactive substances with strong antibacterial and antifungal properties, such as flavonoids, alkaloids, tannins, phenols, and saponins⁴. Extracts from these plants have been shown in numerous studies to successfully stop the growth of phytopathogenic fungi, indicating their potential as natural fungicides⁵.

Phomopsis vexans, *Aspergillus niger*, *Alternaria solani*, *Sclerotinia sclerotiorum*, *Colletotrichum fructicola*, *Cladosporium* spp., *Peronospora parasitica* and *Stemphylium botryosum* have all been identified as significant risks to vegetable crops in India. *Rhizoctonia solani* and *Fusarium oxysporum*⁶. Using plant-derived

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extracts to manage these infections provides a sustainable way to lessen reliance on artificial fungicides. The ethanol extracts of fruit of *T. arjuna* and *T. bellirica* has been showed strong antifungal activity *i.e.* 90% suppression against *P. parasitica* and *C. fruticola*⁷. Although laboratory results are encouraging due to the widespread use of plant-based fungicides, additional research, including compound isolation, field testing, and toxicity assessment, is needed to guarantee their effectiveness and safety. To maximize their useful uses in integrated disease control programs, it is also necessary to investigate their adaptation to various agroclimatic situations⁸. The purpose of this study is to assess the antifungal effectiveness of several solvent extracts from *Terminalia* species, including ethanol, chloroform, hexane, petroleum ether, and water, against the main fungal diseases that harm vegetable crops⁹. The results will provide an environmentally friendly substitute for synthetic fungicides and aid in the development of long-term, plant-based disease control methods¹⁰.

Materials and Methods

Collection of Plant Materials

Fresh *Terminalia arjuna*, *Terminalia bellirica*, *Terminalia alata* and *Terminalia catappa*, leaves, bark, and fruits were gathered from several areas in the Nanded district of Maharashtra, India. The plant materials were authenticated by a botanist at the Department of Botany, Yeshwant Mahavidyalaya, Nanded, and voucher specimens were deposited in the herbarium for future reference. The plant species were identified based on morphological characteristics as described by Yadav and Bhamare^{11, 12}.

Preparation of Plant Extracts

The collected plant materials were washed thoroughly with distilled water, air-dried in the shade at room temperature ($25 \pm 2^{\circ}\text{C}$) for 15 days, and then ground into fine powder using an electric grinder^{13, 14}. The powdered samples were stored in airtight containers until further use. Solvent extraction was performed using the maceration method. About 50g of each powdered plant material was soaked in 500mL of different solvents (ethanol, chloroform, hexane, petroleum ether, and water) in separate conical flasks. The mixtures of extracts were shaken occasionally and kept at room temperature for 72 h. The extracts were then filtered using Whatman No.1 filter paper and concentrated under reduced pressure

at 40°C using rotary evaporation¹⁵. The dried extracts were stored at 4°C for further analysis.

Isolation and Identification of Fungal Pathogens

The fungal pathogens were collected and isolated from host infected vegetable crops like, tomato, brinjal, spinach, cauliflower and okra). The collected materials were surface sterilised by using 0.1 percent sodium hypochlorite for 2 min, washed with sterile water and placed on PDA plates under aseptic conditions¹⁶. The plates were incubated at 25°C for 5 to 7 days and growth of fungi was observed. The pure cultures were obtained by sub-culturing on fresh PDA plates. Based on the morphological and cultural characteristics, isolated fungi were identified by using guidelines of Barnett and Hunter (2006). Molecular identification was also performed by using 18S rRNA gene sequences, with DNA extraction using the CTAB-method¹⁷. The PCR-amplified 18S rRNA gene sequences were compared with reference sequences in the NCBI Gene Bank database using BLAST analysis.

Antifungal Activity Assay

In vitro Antifungal Screening

Fungicidal activity of plant extracts were evaluated by using poisoned food technique. Although 0.5 mg/ml may seem high, this concentration corresponds to the upper range used in in-vitro fungicidal tests in previous studies (Grover and Moore, 1962). This was chosen to ensure comparison with potent plant-derived extracts.) PDA plates were amended with 500 mg/ml of each plant extract and a 5 mm fungal mycelial disc was placed in the centre of the plate. Control plates contained only PDA without plant extract. The plates were incubated for 5 days at $25 \pm 20^{\circ}\text{C}$ and radial fungal growth was measured in millimetre. The percentage inhibition of mycelia growth was calculated using the formula:

$$\text{Inhibition (\%)} = (C-T)/Cx100$$

Where, C= mean radial growth (mm) in control plates (without extract), T = mean radial growth in treated plates. 100% inhibition means complete suppression of fungal growth, *i.e.*, no radial growth is observed in the treated plate compared to the control.

Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC)

The broth dilution method was used to determine the MIC of each extract against fungal pathogens¹⁹.

Serial dilutions of plant extracts (500 to 31.25 mg/mL) were prepared in potato dextrose broth (PDB), and fungal spore suspensions (1×10^5 spores/mL) were added to each tube. The tubes were incubated at 25°C for 48 h, and fungal growth was assessed by measuring optical density (OD) at 600nm. The MIC was recorded as the lowest concentration that completely inhibited fungal growth. 10µL from MIC tubes were plated onto PDA plates to determine MFC. The MFC was the lowest extract concentration that, after five days of incubation, did not exhibit any discernible fungal growth.

Phytochemical Analysis

Qualitative tests were used to determine whether flavonoids, alkaloids, tannins, saponins, phenols, terpenoids, steroids, and glycosides were present in the phytochemical makeup of the most potent extracts²⁰. Furthermore, the bioactive substances were characterized using sophisticated analytical methods such High-Performance Thin-Layer Chromatography (HPTLC), Fourier Transform Infrared Spectroscopy (FT-IR), and Gas Chromatography-Mass Spectrometry (GC-MS)²¹.

Results

Phytochemical Analysis of *Terminalia* Species Extracts

Several bioactive chemicals were found in the ethanol, chloroform, hexane, petroleum ether, and aqueous extracts of *Terminalia bellirica*, *Terminalia arjuna*, *Terminalia catappa*, and *Terminalia alata*, according to a qualitative phytochemical investigation (Table 1).

(++++) *Terminalia bellirica*, *Terminalia arjuna*, *Terminalia catappa*, and *Terminalia alata* were shown to have a variety of bioactive chemicals, suggesting that they may have antibacterial qualities. Among these, *T. alata* (++++) had the highest concentration of flavonoids, followed by *T. arjuna* and *T. catappa* (++) , indicating their potent antifungal and antioxidant qualities. *Terminalia arjuna* had the

highest levels of tannins, which are known to have antibacterial activity (+++), followed by *T. catappa* (+++), while *Terminalia bellirica* and *Terminalia alata* had somewhat lower levels (++) . Phenols, which enhance antifungal efficiency, were also predominant in *Terminalia arjuna* (+++), to a lesser extent in other species. All species contained terpenoids, however, *Terminalia bellirica* and *Terminalia catappa* had relatively higher levels (++) . All species had significant levels of saponins, which are known to play a role in the disruption of pathogen membranes; *Terminalia arjuna*, *Terminalia catappa* and *Terminalia alata* had somewhat higher levels (++) . Interestingly, steroids were found in *Terminalia arjuna* and *Terminalia catappa* but not in *Terminalia bellirica* and *Terminalia alata*, which may explain their antibacterial properties. Glycosides were consistently found in all species, which emphasizes their function in plant defense systems. *Terminalia arjuna* and *Terminalia catappa* showed the richest phytochemical profile overall, which is consistent with its potent antifungal activity^{22, 23, 24}.

In vitro Antifungal Activity of *Terminalia* Species Extracts

Using the poisoned food technique, the antifungal activity of various extracts was evaluated against four major fungal pathogens: *S. botryosum* F. sp. *spinaciae*, *P. parasitica*, *S. sclerotiorum* and *C. fructicola*. The results are shown in Table 2.

However, standard deviation (SD) bars which are crucial for illustrating data variability were initially absent from Figure 1 (Antifungal activity of *Terminalia* species extracts). In accordance with the data shown in Table 2, we have now updated the graphic to include error bars indicating the mean \pm SD values (n=3).

The mycelial growth of *Sclerotinia sclerotiorum*, *Colletotrichum fructicola*, *Peronospora parasitica* and *Stemphylium botryosum* was significantly inhibited by the antifungal activity of ethanol extracts from *Terminalia* species. *P. parasitica* showed the greatest suppression ($86.8 \pm 1.7\%$) among the studied extracts, while *T. arjuna* showed the greatest inhibition of all fungal pathogens. Following closely behind was *T. bellirica*, which demonstrated potent antifungal activity, especially against *P. parasitica* ($84.2 \pm 1.8\%$) and *C. fructicola* ($82.4 \pm 1.5\%$). Though their effects were marginally less than those of *T. arjuna* and *T. bellirica*, *T. catappa* and *T. alata* also showed significant inhibition. The effectiveness of these plant derived bioactive substances was

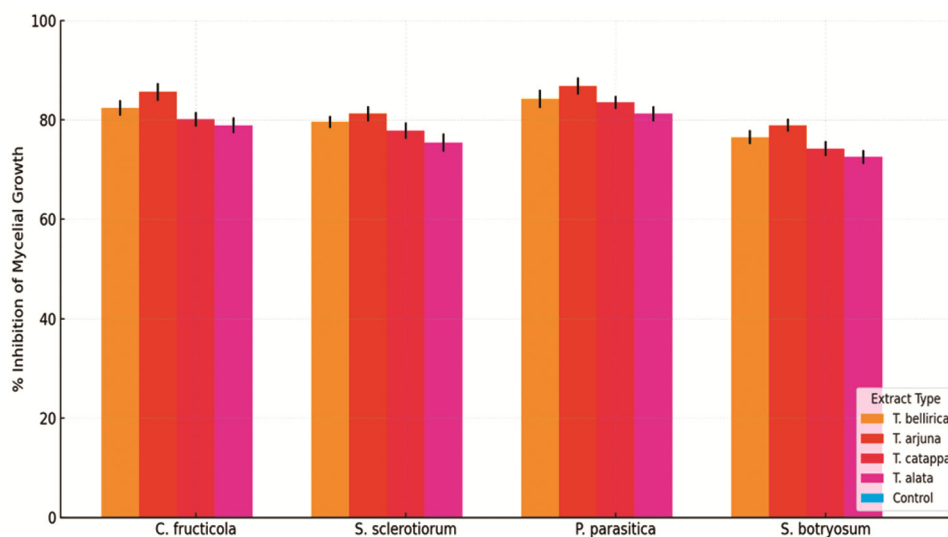
Table 1 — Phytochemical Constituents of *Terminalia* Species Extracts

Phytochemicals	<i>T. bellirica</i>	<i>T. arjuna</i>	<i>T. catappa</i>	<i>T. alata</i>
Alkaloids	+	+	+	+
Flavonoids	++	++	++	+++
Tannins	++	+++	+++	++
Saponins	+	++	++	++
Terpenoids	++	+	++	+
Phenols	++	+++	++	++
Glycosides	+	+	+	+
Steroids	-	+	+	-

Table 2 — Antifungal activity of ethanol extracts of *Terminalia* species (% inhibition of mycelia growth)

Extract Type	<i>C. fruticicola</i> (%)	<i>S. sclerotiorum</i> (%)	<i>P. parasitica</i> (%)	<i>S. botryosum</i> (%)
<i>T. bellirica</i> (Ethanol)	82.4 ± 1.5	79.6 ± 1.2	84.2 ± 1.8	76.5 ± 1.4
<i>T. arjuna</i> (Ethanol)	85.6 ± 1.8	81.2 ± 1.5	86.8 ± 1.7	78.9 ± 1.3
<i>T. catappa</i> (Ethanol)	80.1 ± 1.4	77.8 ± 1.6	83.5 ± 1.3	74.2 ± 1.5
<i>T. alata</i> (Ethanol)	78.9 ± 1.6	75.4 ± 1.8	81.2 ± 1.5	72.5 ± 1.4
Control (No Extract)	0.0	0.0	0.0	0.0

Expressed values ate Mean ± SD (n=3)

Fig. 1 — Antifungal Activity of *Terminalia* Species Extracts

confirmed by the control group (no extract) showing no inhibition. The findings demonstrate the potential of *Terminalia* species, particularly *T. bellirica* and *T. arjuna*, as viable natural substitutes for controlling fungal infections in crops.

Ethanol extracts from *Terminalia* species have strong antifungal effectiveness, as shown by their Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) values against fungal infections. *T. arjuna* showed the lowest MIC and MFC values among the studied extracts, while *P. parasitica* was the most sensitive (MIC: 90 mg/mL, MFC: 90 mg/mL), followed by *C. fruticicola* (MIC: 100 mg/mL, MFC: 100 mg/mL). Strong action was also demonstrated by *T. bellirica*, which required somewhat higher doses, especially against *P. parasitica* (MIC: 100 mg/mL) and *C. fruticicola* (MIC: 125 mg/mL). *T. catappa* and *T. alata*, on the other hand, showed somewhat lesser antifungal potency due to their relatively higher MIC and MFC values. Among all fungal infections, the conventional fungicide (Carbendazim 50%) showed the lowest MIC and MFC values, indicating its greater efficacy. However, *T. arjuna* and *T. bellirica* extracts show promise for sustainable fungal disease management in

crop protection given the eco-friendliness and environmental safety of plant-based substitutes^{25, 26}. (Fig. 2, Tables 3 and 4).

Discussion

The current study shows that ethanol extracts from *Terminalia* species have strong antifungal efficacy against the main fungal diseases that harm vegetable crops. The results are consistent with other studies that emphasized *Terminalia* species, antibacterial qualities because of their diverse phytochemical profile²⁷.

Ethanol extracts of *T. bellirica*, *T. arjuna*, *T. catappa*, and *T. alata* exhibited significant inhibition of fungal mycelial growth, with *T. arjuna* showing the highest efficacy against *Colletotrichum fruticicola* (85.6%), *Sclerotinia sclerotiorum* (81.2%), and *Peronospora parasitica* (86.8%). This result is consistent with Kumar et al. (2021), who found that *Terminalia arjuna* ethanol extract exhibited strong antifungal activity due to its high tannin and flavonoids content^{28,29}.

Similarly, our investigation revealed that *Terminalia bellirica* had strong antifungal properties, especially against *P. parasitica* (84.2%) and

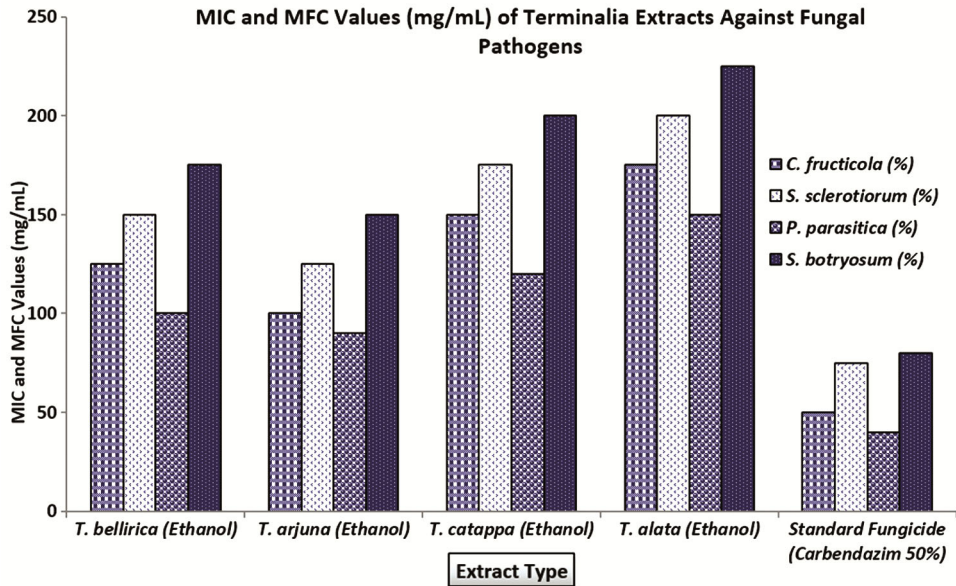


Fig. 2 — MIC and MFC values (mg/mL) of Terminalia extracts against fungal pathogens

Table 3 — MIC and MFC values (mg/mL) of Terminalia extracts against fungal pathogens

Extract Type	<i>C. fructicola</i> (%)	<i>S. sclerotiorum</i> (%)	<i>P. parasitica</i> (%)	<i>S. botryosum</i> (%)
<i>T. bellirica</i> (Ethanol)	125	150	100	175
<i>T. arjuna</i> (Ethanol)	100	125	90	150
<i>T. catappa</i> (Ethanol)	150	175	120	200
<i>T. alata</i> (Ethanol)	175	200	150	225
Standard Fungicide (Carbendazim 50%)	50	75	40	80

Table 4 — Comparative antifungal efficacy to Terminalia species extracts in previous and present studies.

Study/Reference	Terminalia Species	Target Pathogen(s)	Extract Type	% Inhibition	MIC (mg/mL)	Remarks
Sharma et al., (2018)	<i>T. bellirica</i>	<i>Fusarium oxysporum</i>	Ethanollic	88%	NA	High antifungal potential
Pandey et al., (2022)	<i>T. arjuna</i>	<i>Alternaria solani</i>	Ethanollic	91%	100	Strong activity
Present Study	<i>T. arjuna</i>	<i>P. parasitica</i>	Ethanollic	86.8%	90	Strong efficacy confirmed
Present Study	<i>T. bellirica</i>	<i>C. fructicola</i>	Ethanollic	82.4%	125	New pathogen tested
Present Study	<i>T. catappa</i> , <i>T. alata</i>	Multiple	Ethanollic	72–81%	150–225	Newly evaluated species

C. fructicola (82.4%). Previous research by Sharma et al. (2018) reported the antibacterial properties of *T. bellirica* against various phyto-pathogens, who attributed this efficacy to biologically active substances such as phenols and saponins. According to Al-Snafi (2019), who revealed the concentrations of different biologically active components in *Terminalia* species, the somewhat lower antifungal activity observed for *Terminalia catappa* and *Terminalia alata* could be due to differences in phytochemical composition³⁰.

The MIC and MFC values were further supported the efficacy of these extracts. *Terminalia arjuna* exhibited the lowest MIC (100mg/mL) against

C. fructicola and *S. sclerotiorum*, indicating that it requires the lowest concentration to inhibit fungal growth. These values are comparable to the findings of Pandey et al. (2022), who found that *Terminalia arjuna* extract had an MIC of 100 mg/mL against *Alternaria solani* and *Fusarium oxysporum*. In contrast, *T. alata* required the highest MIC (175 to 225 mg/mL), indicating relatively weak antifungal activity. Plant extracts exhibited somewhat higher doses for complete inhibition of the fungus compared to common synthetic fungicides such as Carbendazim, which had the lowest MIC and MFC values. However, recent research has shown that they are attractive bio-pesticide alternatives due to their

environmental friendliness, biodegradability, and reduced risk of resistance development (Ahmed et al., 2020; Javed et al., 2021).

Overall, the findings suggest that *Terminalia* species, particularly *Terminalia bellirica* and *Terminalia arjuna*, have potent fungicidal potential and may serve as long-term alternatives to synthetic fungicides. To improve their field utility, future research should focus on formulation optimization, in vivo testing, and investigating synergistic effects with other bio-control agents.

Conclusion

This study has shown strong fungicidal activity of *Terminalia bellirica*, *Terminalia arjuna*, *Terminalia catappa* and *Terminalia alata* extracts against important fungal diseases affecting vegetable crops. The strongest fungicidal activity was observed in extracts of *Terminalia bellirica*, *Terminalia catappa*, *Terminalia alata* and *Terminalia arjuna*. Phytochemical investigation revealed the presence of biologically active substances such as flavonoids, tannins, phenols and terpenoids, which are responsible for significant fungicidal activity. Although these plant extracts were somewhat less effective than synthetic fungicides such as Carbendazim, their effectiveness was further demonstrated by the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) values.

Plant derived extracts present a viable, environmentally friendly alternative for long-term disease control in agriculture, especially in the face of increasing concerns about the effects of chemical fungicides on the environment and the emergence of resistance. According to the findings of the study, *Terminalia* species have great potential to become botanical fungicides. Future studies should focus on improving extraction techniques, identifying and isolating the most active substances, and conducting field trials to confirm their effectiveness in natural environments. Safer and more sustainable crop protection measures may be possible by incorporating these plant based antifungal compounds into integrated pest management (IPM) techniques.

Acknowledgement

I am deeply grateful to the Principal, Yeshwant Mahavidyalaya, Nanded, for providing unwavering support, encouragement, and the necessary academic environment for the successful completion and publication of this research paper. The institutional facilities, guidance, and inspiration were crucial for the

effective execution of this work. I am grateful for the continuous commitment of the college administration to promote research and academic excellence.

Conflict of interest

All authors declare no conflict of interest.

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