**SUSTAINABLE SOLID WASTE TREATMENT AND MANAGEMENT**



# **Neem extract–blended nanocellulose derived from jackfruit peel for antibacterial packagings**

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Received: 10 January 2022 / Accepted: 18 April 2022 / Published online: 4 May 2022 © The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2022

## **Abstract**

The use of jackfruit peel as a source for natural and fully biodegradable "nanocellulose" (NC) for the production of bioplastics with *Azadirachta indica* (*A. indica*) extracts and polyethylene glycol (PEG) for the antibacterial properties is investigated. The characterization of the biocomposite using FT-IR and WXRD was reported. The physicochemical properties including thickness, moisture content, water holding capacity, swelling, porosity, and biodegradability in soil were investigated. The incorporation of *A. indica* extract revealed an increased shelf life due to the strong antibacterial activity, and these biocomposites were degraded in soil within 60 days after the end use without any harm to the environment. Jackfruit-derived nanocellulose flm blended with *A. indica* extract exhibited strong antibacterial activity against gram-positive and gram-negative food spoilage bacteria. Disc difusion assay, live/dead assay, and CFU analysis confrmed the antibacterial property of the synthesized flm. Moreover, the flms clearly prevented the bioflm formation in bacteria. Thus, the developed bioplastics can be utilized as appropriate substitutes to food packaging materials and also for biomedical applications such as wound dressings.

**Keywords** Nanocellulose · Jackfruit · *Azadirachta indica* · Antibacterial · Food packaging

Reshmy Rajasekharan, Arun Karthika Bahuleyan and Aravind Madhavan authors contributed equally.

**Highlights** • Jackfruit peel derived nanocellulose (NC) incorporated with neem extract revealed new possibilities for innovative food packagings.

• The physical and biodegradability studies showed remarkable improvements in NC biocomposites compared to pure NC.

• Neem extracts are found to be a cost-efective material for imparting antibacterial and anti-bioflm activities.

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## **Introduction**

Agricultural waste materials are of growing interest nowadays to produce value-added biocomposites for various industry applications (Bilo et al. [2018](#page-8-0); Dilamian and Noroozi [2019](#page-8-1); Reshmy et al. [2020a,](#page-8-2) [2020b,](#page-8-3) [2021a\)](#page-8-4). Among them, an interesting one is jackfruit peels. These agroresidues are composed of mainly three constituent such as cellulose, hemicellulose, and lignin (Reshmy et al. [2021b](#page-8-5)). Among them, cellulose as well as hemicellulose is carbohydrate derivatives that can be easily broken down by enzymes and/or chemical treatments followed by fermentation to produce valuable products such as bioelectricity, biofuels, biomass-derived platform chemicals, and other useful products. Recently, both the research and industrial sectors are focusing immensely on the development of bioproducts in a cheaper and easier route because of the demand for renewable resources and ecological concerns. Some cellulose derivatives are already being implemented in pharmaceuticals and scafolds, food additives, constructions, and textiles (El-Bakry et al. [2015;](#page-8-6) Arantes et al. [2020;](#page-8-7) Maraveas [2020](#page-8-8)).

Nanocellulose (NC) can be extracted from jackfruit (scientifc name: *Artocarpus heterophyllus Lam*.) agroresidue as reported by several recent studies (Dutta et al. [2011](#page-8-9); Retnowati et al. [2015](#page-8-10); Raj and Ranganathan [2018](#page-8-11); Trilokesh and Uppuluri [2019\)](#page-9-0). The peel was processed by chemical extraction routes to produce pure nanocellulose in support with steam explosion and homogenization (Reshmy et al. [2021b](#page-8-5)). NC is an extremely smart nanobiomaterial for biomedical applications due to its high porosity and suitable water retention properties, as well as its ability to mold into any form and manipulate with various bioagents. Neem extract (NM) (scientifc name: *Azadirachta indica*) are used in the present study because of their activities including antibacterial, antiviral, and antifungal properties (Sharma and Bhardwaj [2020](#page-8-12); Oyekanmi et al. [2021\)](#page-8-13). Biopolymers with several antimicrobial functionalities are signifcant in diversifying its use in various applications such as bioactive packagings, wound dressings, specialized nanopapers, and antimicrobial apparel for the biomedical industry. The use of bioagents in scientifc study is attaining traction in a variety of technical felds. However, research on the use of bio-extracts especially neem extract to impart antimicrobial properties to NC is limited. Herbal agents derived from plants are very easy to get, have low mammalian cytotoxic effects, and degrade easily in the environment, making them comparatively straightforward to exploit as environmentally acceptable alternatives to antibiotics that induce biological system and environmental adverse efects. In this context, this paper aims to report the efect of *A. indica* extract on jackfruit-derived NC along with poly ethylene glycol (PEG) as scafold for both food industry and biomedical application.

#### **Materials and methods**

The chemicals consumed in the present study were of high purity. Jackfruit peel utilized in the present work was taken from a local market at Alappuzha, Kerala, India and *A. indica* was from a nearby biodiversity spot in Kerala, India. The extraction of high-purity NC was carried out according to the previous reports using steam explosion–aided chemical treatment followed by mechanical processing (Reshmy et al. [2021b](#page-8-5)). The high-purity NC suspensions were kept at 4 °C for the production of diferent formulations. The NM extract used in the present study was prepared by soxhlet extraction of 20 g *A. indica* using 95% pure ethanol, and the two diferent compositions of this natural resin was added to impart targeted properties to NC+PEG formulations.

#### **Thin‑flm production**

Thin flms of NC, NM, and PEG systems were developed using solvent cast method. The control flm was produced by the direct evaporation of 100 mL stock NC suspension using the reported conditions. Polyethylene glycol 600 (PEG) is used in the present method as a plasticizer, and this thin flms were developed by casting the formulation including 1 g of PEG-600 dispersed in very minimum quantity of distilled water and 100 mL NC suspension. Similarly, other novel biocomposite scaffolds were developed by adding two diferent compositions such as 6% and 8% of *A. indica* extract to 100 mL of NC suspension containing 1 g PEG-600  $( NC + PEG + 6\% NM$  and  $NC + PEG + 8\% NM)$ .

#### **Characterization of thin flms**

The developed flms were characterized by sophisticated instruments, namely, Fourier transform infrared (FT-IR) and wide-angle X-ray difraction (WXRD). FT-IR (instrument: Perkin-Elmer spectrophotometer) spectra were recorded in the wave number range 400–4000 cm<sup>-1</sup> with high precision. WXRD analysis (instrument: Rigaku Minifex 600) were conducted in a step-scan mode by scanning samples under a monochromatic light with wave length 0.154 nm (scanning time of 5.0 min. and  $2\theta$  of 0–50°). The presence of characteristic peaks and functional groups was confrmed using the spectra.

#### **Physical properties of thin flms**

The physical properties such as thickness, moisture content, water holding, swelling nature, porosity, and biodegradability in soil were also investigated to study the effectiveness of new formulations in food packaging and biomedical industry. The thicknesses of the developed NC flms were

measured by digital micrometer (Model: 2K706). The thickness of flm at diferent points was measured, and the average value of about 10 measurements was reported as the thickness of the sample. Moisture content of the  $3 \times 3$  cm<sup>2</sup> thin-flm samples was analyzed using digital moisture analyzer (Model: BTS 110). The water holding capacity of the thin-film samples  $(2.5 \times 2.5 \text{ cm}^2)$  was calculated using the equation (Mathew et al. [2019](#page-8-14)):

$$
\%WHC = \frac{W2 - W1}{W2} \times 100\tag{1}
$$

 $[W_2$  wet weight of thin film,  $W_1$  initial dry weight of thin flm].

Water swelling nature of the thin flms was analyzed according to ASTM D2765-95C. The % swelling was calculated as shown in Eq. [\(2\)](#page-2-0).

$$
\%SW = \frac{W2 - W1}{W1} \times 100\tag{2}
$$

 $[W_2$  sample weight after swelling,  $W_1$  weight of the dry sample].

Porosity of the thin-flm samples was determined as per ASTM standard ASTM C20-00(2015). The porosity was determined by immersing the oven-dried sample in distilled water for 48 h and was calculated using Eq. ([3\)](#page-2-1) shown below.

$$
P = \frac{Wf - Wi}{\rho XV} \tag{3}
$$

[*Wi* initial weight of dry sample,  $W_f$  weight after immersing in distilled water, *V* volume of the flm before immersion in distilled water, *ρ* density of cellulose (standard value  $1.5 \text{ g/cm}^3$ )] (Fittipaldi et al.  $2017$ ; Sharma and Bhardwaj [2020](#page-8-12)).

Biodegradability is determined by soil burial route using ASTM D 2216. The analysis was carried out by placing the specimens  $(1 \text{ cm} \times 1 \text{ cm})$  in the soil in a pot with tiny holes and was buried approximately 10 cm in depth. Note the physical changes at regular intervals after taking out the specimens carefully and dried in oven at 65 °C for 24 h and weighed. The % weight loss was determined using Eq.  $(4)$ :

$$
\% WL = \frac{W1 - W2}{W1} \times 100\tag{4}
$$

[W<sub>1</sub> dry sample weight, W<sub>2</sub> weight of the buried sample].

#### **Colony‑forming units**

The antimicrobial activity of neem-incorporated NC composite was analyzed by CFU analysis. The assay was performed as described earlier (Reshmy et al. [2022](#page-8-16)). In short, the bacterial cultures were grown and diluted to maintain an optical density of 0.05 units. The nanocellulose composite flms (NC/NC+PEG/NC+PEG+NM[6%]/  $NC + PEG + NM[8\%]$  were positioned on culture and incubated for 24 h at 37 °C. Followed by incubation, 1 mL of culture from each group was serially diluted and plated on agar plates to count the colonies.

### **Disc difusion assay**

The antimicrobial effect of neem-incorporated NC composite was analyzed against the pathogenic *E. coli* bacteria by disc difusion method. Discs having 5-mm diameter were made from neem-incorporated NC composite and was sterilized by autoclaving. Fresh cultures of pathogenic bacteria were spread on agar plates, and the sterilized discs were positioned on them. The plates were incubated for 24 h at 37 °C. Following incubation, the zone of inhibition was imaged and measured.

#### <span id="page-2-0"></span>**Live/dead assay**

<span id="page-2-1"></span>The antimicrobial efficacy of NM incorporated NC composite were also confrmed by live-dead assay. The bacterial cells were treated with corresponding NC composites. After 24-h growth in incubator, the bacteria were stained  $(SYTO9 +$  propidium iodide  $(PI)$ ) according to manufacturer's (L7007, LIVE/DEAD kit, Thermo Fisher Scientifc, USA) instructions and the fuorescence intensity was read at 485 and 530 nm (green) and 485/630 nm (red) and green to red intensity ratio was calculated (Mah et al. [2003](#page-8-17)). Fluorescence images were captured using Nikon confocal microscope.

#### **Inhibition of bioflm formation**

Nanocellulose discs (NC/NC+PEG, NC+PEG+NM[6%], NC+PEG+NM[8%]) with diameter 50 mm was placed on the appropriate wells of 24-well plate containing 2 mL 7H9 media inoculated with 20 µL of 1% *M. smegmatis* culture  $(-1.5 \text{ OD at } 600 \text{ nm})$ . The plate was kept for incubation at 30 °C and was monitored at 4th day for bioflm formation. The images were taken at 4th day (Thibeaux et al. [2020](#page-9-1)).

# <span id="page-2-2"></span>**Result and discussions**

# **Characteristics of nanocellulose scafolds from jackfruit peel and A. indica extract**

The FT-IR spectrum of the developed NC and their composites confirmed peaks attributed to O–H and C-H stretching vibrations at an absorption region of 3406 cm<sup>-1</sup> and 2903 cm−1, respectively. The C–O–C stretching vibration due to the presence of pyranose ring was attributed to a characteristic peak at  $1056$  cm<sup>-1</sup>. The peaks correspond to -CH<sub>2</sub> wagging vibrations, C-H bending, and C–O–C asymmetric stretching of the polysaccharide moieties of the cellulose were observed at 1311 cm<sup>-1</sup>, 1370 cm<sup>-1</sup>, and 1160 cm<sup>-1</sup> respectively. The presence of  $β-(1\rightarrow 4)$ -glycosidic linkage was confirmed by the stretching peak at 898 cm<sup>-1</sup>. The WXRD spectral patterns of NC and its biocomposites showed characteristic peaks 16.4°, 22.7°, and 25.4° corresponding to the crystalline structure of the NC. The FT-IR and XRD spectra of the biocomposites are depicted in Fig. [1a](#page-3-0) and [b](#page-3-0).

The developments of novel thin flms using *A. indica* leaf extract was chosen in this study because of their prominent antibacterial and antiviral activities. Thin flms using two diferent compositions, namely, 6% and 8% of *A. indica* extract, NC and PEG were cast to produce biocomposites and investigated for their activities as a scafold for food packaging applications.

#### **Physical properties of thin flms**

Thicknesses of the thin flms were obtained in the range of 0.06 to 0.08 mm. Analysis of moisture content of the flms revealed a reduction in moisture absorption due to the incorporation of *A. indica* extract and a direct relationship with the *A. indica* extract content and moisture reduction was observed. The water holding capacity of thin flms were found to be high for pure cellulose, and the result showed a reduction in water holding and moisture absorption that could facilitate for the production of efective food packaging. A higher value of water holding capacity for pure NC thin flms reveals the hydrophilicity of NC. The strong hydrogen bonding present between the nanofbrils of NC allows water molecules to rapidly entrap well within NC framework. The mechanical properties of jackfruit peel–derived NC/PEG biocomposite were already reported by the same research group and found that the Young's modulus (2959.92 Mpa), tensile strength (88.69 MPa), and elongation at break (8.25%) were improved due to the compatibility of PEG with NC (Reshmy et al. [2021a,](#page-8-4) [b\)](#page-8-5). These improved mechanical properties along with neem extract for inducing antibacterial and anti-bioflm formation activities will make it more suitable for food packaging applications. The comparably good values of water holding, moisture content, and porosity of NC thin flms also point to the application of these NC thin flms in adsorption applications such as wound dressing, drug delivery, scafold fabrication, and facial biomasks. The above-described physical characteristics of NC biocomposites are depicted in Table [1.](#page-4-0)

The swelling in water (Fig. [2\)](#page-4-1) and the porosity (Fig. [3\)](#page-5-0) of the thin flms were also analyzed to confrm the suitability of these NC scafolds for various applications. The swelling nature of NC control flm is found to be high because of their strong hydrophilicity. The incorporation of PEG with NC results in the reduction of swelling nature due to its plasticizing nature which reduces the water uptake. In various biomedical applications, porosity is a signifcant factor for promoting cell migration and enables difusion of nutrients and oxygen within tissue. The water holding capacity and swelling are in direct relationship with porosity.

## **Biodegradability analysis**

Biodegradation of these thin flms were analyzed by soil burial method. The degradation were analyzed at regular intervals by removing the strips and dried in an oven at 65 °C for 24 h and weighed. It was seen that the degradation of pure NC flm is completed within 35 days, that of plasticized flms were happened in 45 days, and the *A. indica*



<span id="page-3-0"></span>**Fig. 1 a** FT-IR spectra of NC biocomposites. **b** XRD patterns of NC biocomposites

extract–incorporated thin flms were degraded completely only after 60 days due to the antibacterial efect of the resin. Fig. [4](#page-5-1)

# **Antibacterial action of jackfruit‑derived NC blended with A. indica extract**

Packaging of food is vital in preserving the safety and quality of foodstufs, from process and manufacture to handling and storage, until the foodstuff reaches the customers. Active food packing guarantees the quality and safeness of the food commodity through the contact between the packing flm, food items, and inner and outer environments (Realini and Marcos, 2014). The technique denotes to the integration of active chemicals or natural chemicals into packaging material. The antimicrobial active packing is a form of packing that comprises antibacterial agents that can interact with the headspace and packed food with the intention to stop

bacterial infection. The authentication of the antibacterial nature of jackfruit-derived NC blended with *A. indica* was performed by determining the growth inhibition of grampositive (*Bacillus cereus*) and gram-negative (*Escherichia. coli, Pseudomonas* sp*.*) bacteria, which are key microorganisms responsible for food spoilage in food industry (Alzohairy Mohammad [2016,](#page-8-18) Ali et al. [2021](#page-8-19)). *A. indica* extract displayed antibacterial action by in vitro and in vivo experiments due to the occurrence of several natural chemical constituents, namely, presence of bioactives like desactylimbin, triterpenes or the limonoids like desactylimpin, meliantriol, quercetin, nimbin, nimbosterol, nimbinin, and margisine. Other chemical bioactives like azadiradione, nimonol, and epoxy azadradione were also reported to have antimicrobial properties (Dai et al. [2001\)](#page-8-20). Therefore, we introduced *A. indica* to NC derived from jackfruit. The antimicrobial action of the NC composite was analyzed towards *Escherichia coli*, *Bacillus cereus*, and *Pseudomonas* sp.





<span id="page-4-1"></span>**Fig. 2** Swelling behavior of NC biocomposites in distilled water

<span id="page-4-0"></span>**Table 1** Physical characteristic of various NC biocomposites



<span id="page-5-0"></span>

Days

<span id="page-5-1"></span>

Jackfruit-derived NC with *A. indica* extract displayed high antimicrobial activity towards both gram-positive and gramnegative bacteria. A highly substantial reduction of CFU of *Escherichia coli*, *Bacillus cereus*, and *Pseudomonas* sp. (approximately 40%) after treatment with NC neem–blended composite related to the un-treated bacteria (Fig. [5a,](#page-6-0) [b](#page-6-0), [c](#page-6-0)). To reinforce our fnding, we have further demonstrated the live/dead assay of *E. coli* bacteria treated with NC blended with *A. indica* extract using SYTO9 and PI stain. The live bacterial cells were appeared green by the dye SYTO9 as this dye could attach to genomic DNA after entering through the intact cellular membrane. However, the dead bacterial cells were appeared red as the as the dye PI enter the bacterial cells via the injured cell membrane and attach to DNA after replacing SYTO9. Thus, the live/dead assay using the propidium iodide and SYTO9 demonstrated the antibacterial action of *A. indica* against *E. coli* (Fig. [6a](#page-7-0) and [b\)](#page-7-0). The disc difusion assay further demonstrated that the antimicrobial efect was dose-dependent. The diameter of the inhibition zone for the *E. coli* strain was measured to be 10 mm and 15 mm for 6% and 8% neem extract–blended NC flm discs, respectively (Figs. [6c](#page-7-0)). Thus, we proved that the synthesized nano-composite is efficient for sterile packing of food items to control bacteria triggering food contamination. A functionalized nano-crystalline cellulose modifed with aldehyde group and immobilized with nisin and lysozyme displayed antibacterial action against *Bacillus. subtilis* and *Staphylococcus aureus* was reported (Tavakolian et al. [2018\)](#page-9-2). A nanocellulose Ag-NP nanocomposite was developed and evaluated for antibacterial efect against *Staphylococcus aureus* and *E. coli* (Sarwar et al. [2018\)](#page-9-3). Panaitescu et al. [\(2018\)](#page-8-21) had constructed a PHB-based composite with zinc oxide–coated nanocellulose and nanoparticles for antibacterial action against *E. coli* and *S. aureus.* This resulted in complete inhibition of *S. aureus* growth (Panaitescu et al. [2018](#page-8-21)). Tyagi et al. [\(2019\)](#page-9-4) developed a combination of CNC and chitosan for coatings for antimicrobial and superabsorbent tissue papers. These modifcation of nanocellulose could inhibit 99% of the survival of *E. coli* (Tyagi et al. [2019\)](#page-9-4). In comparison with the above-mentioned composites of nanocellulose, our nanocellulose composite showed wide range of antibacterial action against both gram-positive and gram-negative bacteria and the antimicrobial property is plant derived instead of direct usage of a synthetic chemical compound. Thus, our NC combined with *A. indica* extract might be a good candidate to make food packing flms.

# **Jackfruit NC blended with A. indica extract showed anti‑bioflm property against Mycobacterium smegmatis**

Bioflms are highly multifaceted bacterial ecosystems created by one or more bacterial species immersed in an extracellular matrix of diferent compositions depending on the type of biomedical device or food manufacturing environment or and the colonizing species. Bacterial colonization and following bioflm formation signifcantly hamper surface activity in a plethora of situations including food packaging and medical devices (Koo et al. [2017](#page-8-22)). As a result, the growth of surfaces by bacteria has been a rigorous research aiming at fnding anti-bioflm agents for the beneft of humans. Fig. [7.](#page-7-1)

Nanocellulose derived from jackfruit blended with *A. indica* extract inhibited bioflm formation in *M. smegmatis* at the tested concentration*.* The 8% *A. indica* extract blended with NC had signifcant anti-bioflm activities compared to 6% extract; however, both extracts failed to completely inhibit *M. smegmatis* bioflm formation. However, control nanocellulose flms did not inhibit bioflm formation. Recently Hassan et al. ([2020\)](#page-8-23) have developed antibacterial nanocellulose flms by modifcation of the surface with dehydroabietic acid derivatives. Their flm reduces the number of drug-resistant *Staphylococcus aureus* and also prevented the bioflm formation in chronic wounds. Lead extracts of neem was highly efective in disrupting formation and structure of bioflms in *Pseudomonas aeruginosa* (Harjaj et al. [2013;](#page-8-24) Jack et al. [2017](#page-8-25)). Thus, neem extract–blended jackfruit NC can be a better candidate for biomedical application as wound dressing material.



<span id="page-6-0"></span>**Fig. 5** Determination of antimicrobial action of nanocellulose derived from jackfruit residue incorporated with *A. indica* extract against various pathogenic bacterial strains by colony counting method. **a**

*E. coli*, **b** *B. cereus*, and **c** *Pseudomonas* sp. # Difer signifcantly (*p* value  $\leq$  0.05) from control, NC and NC + PEG groups

<span id="page-7-0"></span>**Fig. 6** Determination of antimicrobial efficacy of nanocellulose derived from jackfruit residue incorporated with *A. indica* extract via live/dead and disc difusion methods. **a** Fluorescent images of treated bacteria. The live and dead bacteria are stained green and red, respectively. **b** Ratio of green to red fuorescence was measured in triplicates to determine the relative viability of bacteria. **c** Representative image showing the zone of inhibition produced by neem extract–incorporated nanocellulose against *E. coli*





<span id="page-7-1"></span>**Fig. 7** Nanocellulose derived from jack fruit residue incorporated with *A. indica* extract inhibits bioflm formation in M. smegmatis. M. smegmatis treated with (**a**) nanocellulose (NC), (**b**) NC with polyeth-

**Conclusion**

This is the frst report on modifcation of jackfruit peel–derived NC with the *A. indica* extract and PEG as a plasticizer for inducing antibacterial properties to pure NC. These properties showed a considerable improvement for application in food packagings by reducing the high hydrophilic tendencies of NC flms that will normally lead to spoil the preserved food commodities. All the developed biocomposites have a fully degradation ability within 60 days in soil. These flms showed remarkable antibacterial and anti-bioflm formation properties due to the incorporation of 8% *A. indica* extract compared to 6% extract. These characteristics will contribute to the design of new bioplastics for both food packagings and also for developing wound dressings biomaterial for biomedical applications.

**Acknowledgements** The authors are grateful to all researchers that contributed to the data collection required for this study. The authors Reshmy Rajasekharan and Raveendran Sindhu are thankful to the ylene glycol (PEG), (**c**) NC+PEG with 6% *A. indica* extract (NM) and (**d**) NC+PEG+NM (8%) (Reshmy et al. [2022](#page-8-16))

Department of Science and Technology (DST), Government of India, for providing the funding through Women Scientist Scheme (WOS-B), vide project grant nos: SR/WOS-B/587/2016 and SR/WOS-B/740/2016 for this research work.

**Author contribution** All authors contributed to the study conception and design. Material preparation, and writing original draft, Reshmy Rajasekharan; data collection and analysis, Arun Karthika Bahuleyan; analysis and writing original draft, Aravind Madhavan; literature survey, Eapen Philip; conceptualization, Raveendran Sindhu; revision, Parameswaran Binod; reviewing, Mukesh Kumar Awasthi; and editing, Ashok Pandey. All authors read and approved the fnal manuscript.

**Data availability** This article presents an original research work executed by the authors, so all the data presented are depending on their fndings and analysis techniques. The datasets used in this article are available from the corresponding author on reasonable request.

#### **Declarations**

**Ethics approval and consent to participate** "Not applicable", research does not report on or involve the use of any animal or human data or tissue.

**Consent for publication** Not applicable.

**Conflict of interest** The authors declare no competing interests.

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