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Research Article

Fabrication and Characterization of an Active Nanocomposite Film based on Polystyrene/Thyme/Nano ZnO for Food Packaging

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Abstract

The main objective of this research is to develop an active antifungal packaging material utilizing polystyrene as the matrix material with active agents. The uniqueness of this study lies in the use of thyme extract as a reducing agent in the green synthesis of zinc oxide nanoparticles. The nanocomposite film was fabricated by impregnating zinc oxide nanoparticles in polystyrene. The nanocomposite films were characterized using XRD, FTIR, SEM and antifungal testing. The crystallite size of the synthesized ZnO NPs was observed to be in the 20–30 nm range. The FTIR spectra revealed the presence of ZnO NPs at the peak of 1000 cm^{-1}. The morphological analysis showed the nanoparticles having a spherical shape. The results indicated that nanocomposite films exhibited excellent resistance against the fungus viz., *Pencillum sp*, *Nigrospora oryzae* and *Chaetomium oryzae*. The development of such active packaging materials with nanoparticles to preserve food grains will pave the way for a new technological path in food packaging applications.

Keywords: Nanocomposite films, Antifungal properties, Food packaging

1 Introduction

In recent years material scientists are devoting their time in developing active packing materials for enhancing the shelf life of food materials [1]. It has been reported that nanoparticles can influence the shelf life of food materials [2]. As a result, the quest for the development of novel effective antifungal agents is becoming increasingly vital [3]. Food material storage against different pathogens is becoming increasingly essential in agriculture, food technology, and biotechnology [4]. Because crop output is high during some seasons, it is critical to stockpile food grains. Mycotoxins produced by fungus have an impact on food grains such as cereals, rice, and wheat. Jute and polypropylene bags are now often used for storing food grains. Packaging is done by creating vacuum inside the bags also known as hermite packaging. Before packaging, the grains should be completely dried to avoid moisture. In such conditions, there is a requirement to prevent oxygen atmosphere and enhance carbon dioxide atmosphere within the packaging, which will limit the microorganism respiration. Polymers are low-cost materials, making polymer nanocomposites ideal for packaging applications [5].

Polymers like cellulose, Polypropylene, Polylactic acid (PLA), Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) and Poly(butylene adipate-co-terephthalate) (PBAT) are most commonly used [1], [6]–[20]. It is well known that nanoparticles exhibit excellent antibacterial and antifungal activities [1]. Because of their superior resistance to bacterial invasion, silver (AgNPs), copper (CuNPs) and titanium dioxide (TiO_2 NPs) nanoparticles have been widely employed in polymer nanocomposites [1], [2], [14], [15], [21], [22]. Zinc oxide nanoparticles exhibit a better antifungal activity when compared to their bulk counterparts. Because of their tiny size and great surface area to volume ratio, nanoparticles with a very modest loading can exhibit significant activity in nanocomposites. As a result, they are claimed to be effective fungicides in a variety of applications [23]. The United States Food and Drug Administration (FDA) has certified ZnO NPs as a safe substance since they are readily destroyed in the human body. Organic food-simulating fluids promote polymer swelling, resulting in nanoparticle migration. Cellulose based nanocomposites were fabricated by *in situ* generation in an aqueous solution having AgNPs and CuNPs. The nanocomposites exhibited the better resistance against various pathogens [1], [21]. Researchers have demonstrated the hybridization of different fillers, which can enhance the performance of nanocomposites. Poly(lactic acid) (PLA) incorporated with hybrid nanoparticles of ZnO-TiO_2 enhanced the mechanical properties and anti-bacterial performance of the nanocomposites [24]. In a similar way polypropylene incorporated with modified tamarind seed polysaccharide/AgNPs and CuNPs displayed excellent resistance against *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Bacillus licheniformis* (*B. licheniformis*)and *Staphylococcus aureus* (*S. aureus*) [14], [15]. On the other hand, ZnO NPs were added with a blend of polylactic acid/polycaprolactone. They found that the increase in filler content improved the performance of the nanocomposites which could be attributed to the better dispersion of the nanoparticles in the matrix blend [25]. In another work, the modified vegetable tanned waste leather trimming (WLT) with *in situ* generated silver based nanoparticles were synthesized in an one step thermal aided process, and the modified WLT showed outstanding antibacterial activity [26]. ZnO/Polystyrene nanocomposite thin films possessed antibacterial activity against *E. coli* and *S. aureus* bacteria [27]. Food simulants containing 4% acetic acid and hexane released ZnO nanoparticles from polypropylene food containers. Acetic acid dissolves ZnO NPs, which are then liberated as Zn^{2+} [28]. Green synthesized ZnO-NPs from diverse plant sources be more efficient against numerous fungal stains [29] and the use of plant extracts in the formulation of nanoparticles reduces the usage of hazardous chemicals used in chemical synthesis [30]. The amount of the precursor utilized during nanoparticle synthesis has a greater impact on the antifungal activity of ZnO nanoparticles. The fungal growth area is assessed to demonstrate the inhibitory impact on the fungus growth [31]. ZnO nanoparticles exhibit the action on the radial growth and dry weight of the fungus [32].

He *et al.* demonstrated two distinct antifungal effects of ZnO NPs, limiting growth via altering cellular processes and blocking the formation of fungus conidiophores and conidia [33]. Complete elimination of the antimycotic effect has been demonstrated with ZnO nanoparticles with a minimal fungicidal concentration of ZnO nanoparticles [34]. The incorporation of ZnO nanoparticles in transparent glasses with high content of ZnO (15–40 wt.%) has been developed [35]. The antifungal activity of plant extracts has been recognized for many years [30]. The optimum concentration to get better effects on ZnO nanoparticles is considered to be 5 wt.% [36].

Noorian *et al.* investigated the synergistic effects of TiO_2 NPs and *Mentha piperita* essential oil (MEO) on the performance of cassava starch films. The bionanocomposites showed good antibacterial activity against *E. coli*. The properties of the films proved that the films can be potentially used for food packaging applications [37], [38]. Similarly, Hamid *et al.* investigated the functional and antibacterial properties of potato starch films using a mixture of ZnO NPs and fennel essential oil (FEO). The results showed that as the concentration of ZnO NPs and FEO grew, so did the antibacterial activity of bionanocomposite films [39]. Researchers prepared composite films based on whey protein isolate/

pectin with betanin pigments (BP) and copper (I) oxide nanoparticles (CuO). The results revealed that the incorporation of BP and CuO nanoparticles increased the antioxidant capacity thereby enabling the films to be used for food packaging applications [40].

Naturally occurring compounds have the potential to replace synthetic fungicides. Thymol (36.81%) and ρ-cymene (30.90%) are the main components of thyme. Thymol, found in thyme essential oil has been reported to have high antifungal properties and is also deemed safe [41]. Thyme has historically been claimed to have high antifungal function owing to its high concentration of oxygenated monoterpenes (thymol) and monoterpene hydrocarbons (ρ-cymene) [42]. According to the United States Environmental Protection Agency (EPA), thymol has a low potential for toxicity and offers little danger. Thymol can be used as a pesticide, which is considered as a safe alternative to other chemical pesticides [12]. Thymol has a phenolic hydroxyl group, which aids in its antimicrobial action and also can be used as a food additive as recognized by the FDA [43]. Researchers have impregnated thymol in zein films and reported that the infusion of thymol in the films greatly improved the release performance [44]. The essential oils encapsulated nanoparticles can also enhance the food quality and safety [45].

Based on the literature survey, this research attempts to fabricate a packaging material with antifungal characteristics. Polystyrene was used as the matrix; zinc oxide nanoparticles were produced by green synthesis using thyme extract and impregnated with polystyrene to evaluate the antifungal activity. The fabricated nanocomposites were characterized by FTIR, XRD, SEM techniques. Further, the antifungal activity of the material was also evaluated.

2 Materials and Methods

The chemicals used in this study such as Zinc Chloride ($ZnCl_2$) and Sodium Hydroxide (NaOH) were procured from Ganapathy Scientific Company, Srivilliputhur. Polystyrene was supplied by Ramnath and Co. Pvt. Ltd., Madurai. Thyme solution was prepared by the procedure given below.

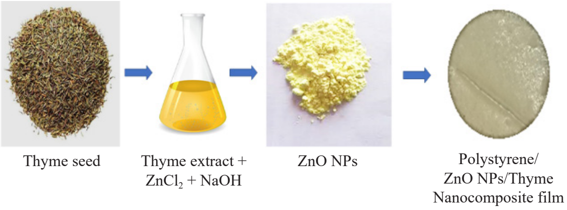

Figure 1: Synthesis and fabrication of ZnO NPs/ Polystyrene composites.

2.1 *Extraction of thyme solution*

Thyme seed was ground to form powder and 10 gm of thyme powder was mixed with 100 mL of distilled water and boiled for about 15 min. The aqueous extract was then filtered using Whatman No.1 filter paper.

2.2 *Preparation of ZnO Nanoparticles (ZnO NPs)*

0.02 M aqueous solution of $ZnCl_2$ was added to 50 mL of distilled water and was stirred vigorously at 80 °C for about 10 min. After stirring, 1.0 mL of thyme extract was added to the above solution. Then, drop by drop, 2.0 M of NaOH aqueous solution was added to the aforementioned aqueous solution, resulting in a white aqueous solution with pH 12, and the solution was agitated constantly for about 2 h. To eliminate contaminants, the precipitate was rinsed and centrifuged repeatedly with distilled water. The finished product (ZnO NPs) was obtained and dried.

2.3 *Film preparation*

Polystyrene (1 g) was added with 30 mL of toluene and was constantly stirred until the polymer was completely dissolved in the solvent. To this solution, green synthesized ZnO NPs were added at a concentration of 5 wt.% as antifungal agents. Finally, the solution is poured into a petri dish to cast the films and is allowed to dry by a slow evaporation method. The casted films were peeled off after 48 h. The process of synthesis of ZnO nanoparticles and the fabrication of nanocomposite films is presented in Figure 1.

2.4 *Characterization of the nanocomposites*

The obtained ZnO NPs were analyzed by X-ray diffraction (XRD), Scanning Electron Microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR).

2.4.1 X-ray Diffraction (XRD)

The crystalline nature of the samples was measured using an X-ray diffractometer (D8 advance ECO XRD system with SSD160 1D Detector, Bruker, USA) at a Cu-Kα radiation wavelength of 1.5406 to 1.54439 A°. The samples were scanned in 2θ range of 15–70°.

2.4.2 Fourier Transform Infrared Spectroscopy (FTIR)

The IR spectra of the samples were measured in reflection mode using a Spectrum 65, Perkin Elmer, USA, machine in the wavelength of 4000 to 500 cm^{-1}. In each case, 32 scans were taken at a resolution of 4 cm^{-1}.

2.4.3 Scanning Electron Microscopy (SEM)

The distribution and the shape of the ZnO NPs were analyzed using a Scanning Electron Microscope (EVO18, Carl Zeiss, Germany) at 20 kV.

2.4.4 Antifungal activity

Antifungal activity of ZnO NPs/Polystyrene composites was analyzed using the agar plate method against the following fungus viz., (a) *Pencillum sp*; (b) *Nigrospora oryzae* (Berk. & Br.) Petch; and (c) *Chaetomium oryzae*. All fungi were obtained from the NCIM, NCL, Pune India. Polystyrene was used as a positive control. Antifungal tests were performed with 20 µL of the extract assayed, 10 µL of a spore suspension and 70 µL of potato dextrose broth (PDB) (HiMedia, Mumbai, India). Microcultures containing 20 µL of sterile distilled water instead of a test solution were used as a negative control. All assays for antifungal activity were carried out in triplicates. The plate was incubated at 37 °C for 3 days and the zone of inhibition was measured.

3 Results and Discussions

ZnO NPs were synthesized by the reduction of Zn^{2+} using thyme extract, which contains thymol. Thymol acts as both reducing as well as the capping agent. Thymol can donate electrons to Zn^{2+}, thus forming Zn (O) nanoparticles [46].

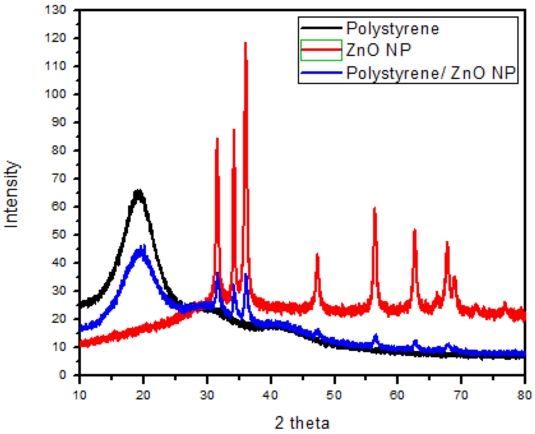

Figure 2: XRD pattern of polystyrene, ZnO NPs and polystyrene/ZnO nanocomposite.

3.1 *XRD analysis*

The X-ray diffraction patterns of polystyrene, ZnO nanoparticles and polystyrene/ZnO nanocomposite are shown in Figure 2. The intensity of both the matrix and the nanoparticles diminishes as the nanocomposite is formed. The XRD pattern of polystyrene exhibits a wide peak at $2\theta = 18.5$ suggesting the amorphous nature of the polymer, which is consistent with previous studies on polystyrene composites [47]. The XRD pattern of ZnO nanoparticles shows the reflections at 2θ values of 31.41°, 34.11°, 35.93°, 47.17°, 56.24°, 62.52° and 67.60° corresponding to the lattice planes (100), (002), (101), (102), (110), (103) and (200), respectively. This is in good agreement with the JCPDS file: 36-1451 for ZnO nanoparticles [48]. The d-spacing value increases as the composite is formed [49], and the crystallinity was found to be 40.2% with a hexagonal wurtzite structure. The crystallite size of the prepared ZnO NPs was found to be in the range of 20–30 nm, which is presented in Table 1.

Table 1: The calculated crystallite sizes and d spacing values of ZnO NPs

2θ	FWHM	Crystallite Size (nm)	d Spacing (nm)	h k l
31.41	0.433	21.2	0.284	(100)
34.11	0.312	29.5	0.263	(002)
35.93	0.425	21.8	0.25	(101)
47.17	0.468	20.59	0.192	(102)
56.24	0.476	21.03	0.163	(110)
62.52	0.520	19.85	0.148	(103)
67.60	0.468	22.73	0.138	(200)

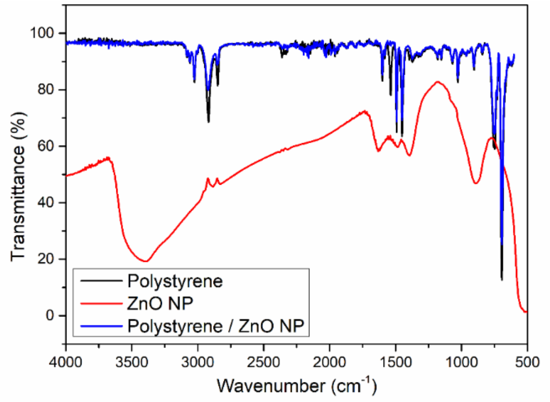

Figure 3: FTIR spectra of polystyrene, ZnO NPs and polystyrene/ZnO nanocomposite.

3.2 *FTIR analysis*

FTIR analysis was performed to identify the photochemical constituents of thyme involved in the reduction and stabilization of the nanoparticles and the FTIR spectrum is depicted in Figure 3. The various peaks and their assignments are presented in Table 2. When antimicrobials are embedded in active packaging, their mechanism of action and chemical composition become increasingly significant. Thus, bioactive substances such as essential oils can benefit customers' health. The vibrations corresponding to bioactive compounds are obtained in the FTIR spectra (Figure 3). The vibrational assignments correspond to the phenyl, methylene and methenyl groups of polystyrene film. The absorption band of ZnO NPs lies below 1000 cm^{-1} [50]. The area of the peak can be used to calculate the quantity of the compound. The peak at 1450 cm^{-1} corresponds to the benzene ring of thymol.

Table 2: Vibrational modes of polystyrene/ZnO nanocomposite

Wave Number	Vibrational Assignment
694	C=C bending
746	C-H bending
903	C-H bending
1026	S=O stretching
1450	C-H bending
1493	N-O stretching
1599	C=C stretching
2845	C-H stretching
2916	N-H stretching
3022	O-H stretching

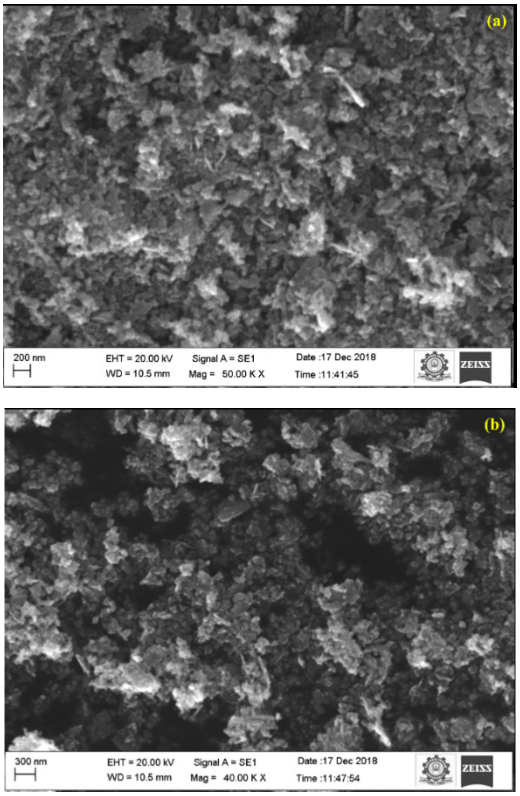

Figure 4: SEM micrographs of polystyrene/ZnO nanocomposite (a) agglomerated lumps of ZnO NPs; (b) spherical shape of ZnO NPs.

When thymol is combined with nanoparticles, the diffusion becomes extremely sluggish owing to the reason that thymol cannot permeate through the nanoparticles. Thus, zinc oxide nanoparticles act as a carrier of thymol. The chemical composition of thyme includes thymol, carvacrol, para-cymene and linalool. Thymol is found to be more effective against the fungi such as Aspergillus niger and Candida albicans [51]. Thymol is protected from various factors such as oxidation, heat, light, pH, and enzyme degradation using ZnO NPs. The strong interfacial contacts between the polymer layer and the nano reinforcement allow for the regulated release of bioactive chemicals. As a result, this packing technique prevents active chemical burst dispersion [52].

3.3 *SEM analysis*

The SEM micrographs (Figure 4) indicate that the ZnO nanoparticles are extremely tiny and heavily

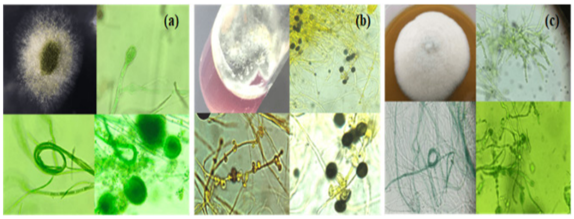

Figure 5: Different fungal strains used in the study (a) *Pencillum sp*; (b) *Nigrospora oryzae* (Berk. & Br.) Petch; and (c) *Chaetomium oryzae*.

agglomerated. The nanoparticles have a spherical form, according to the morphology. It could be ascertained from the SEM micrographs that the agglomerated lump contains multiple nanoparticle aggregates.

3.4 *Anti-fungal activity*

The prepared polystyrene/ZnO nanocomposite film was tested for antifungal potential against *Pencillium* sp, *Nigrospora oryzae* (Berk. & Br.) Petch, and *Chaetomium oryzae* [Figure 5(a)–(c)] to find the inhibitory concentration values. As a result, the quest for the development of novel effective antifungal agents is becoming increasingly vital. The antifungal studies are performed for three samples, ZnO NPs in powder form, polystyrene film without NPs and polystyrene film with NPs.

A significant antifungal activity was observed by the clear zone in the nanocomposite film. Zone of inhibition varies for different types of pathogens involved. The surface area rises as particle size decreases. As a result, nanoparticles may readily infiltrate within the bodies of the microorganisms and trigger cell death.

The ZnO NPs absorb the mycotoxins and the effect of ZnO NPs on pathogenic fungi and infestation of insects was observed [17]. Based on the previous reports, synthetic fungicides can be replaced with ZnO NPs. ZnO NPs can bind with proteins that can make adhesive interactions with cellular membranes and produce reactive oxygen species, which will cause inflammation and the destruction of cells such as mitochondria [19]. The inhibition zones indicating the antifungal activity for the different samples tested is shown in Figure 6(a)–(c) and the mechanism of antifungal activity has been depicted in Figure 7.

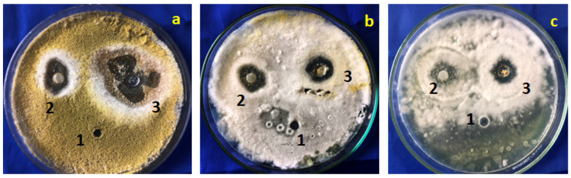

Figure 6: Antifungal activity of ZnO nanoparticle/polystyrene film against (a) *Pencillium* sp; (b) *Nigrospora oryzae* (Berk. & Br.) Petch; and (c) *Chaetomium oryzae*. Sample: 1) Control film, 2) ZnO Powder, 3) Polystyrene/ZnO nanocomposite.

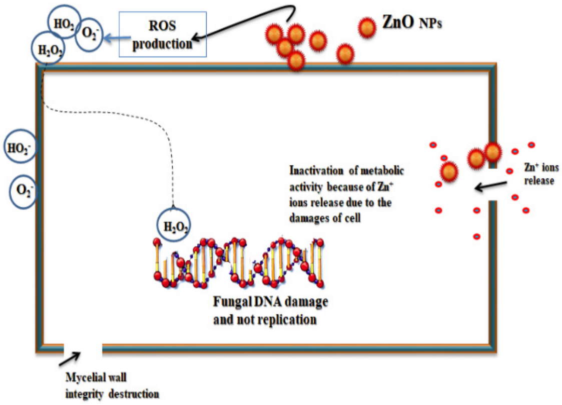

Figure 7: Mechanism of antifungal activity of ZnO NPs/polystyrene nanocomposite film.

3.5 *Mechanism of action*

The novelty of this work lies in the encapsulation of thymol in ZnO NPs, which is infused in the active packaging material. The ZnO NPs are attached to bioactive compounds of thyme by the green synthesis method. Polystyrene/ZnO/thyme nanocomposite is employed for the storage of food grains. During storage, the fungus grown on the grains degrade the polystyrene film. Now, the zinc oxide nanoparticles are released from the film in a controlled manner. Both the zinc oxide nanoparticles and the bioactive compounds of thyme participate in destroying the microorganisms [53]. Natural elements such as antioxidants and antimicrobials can be used to preserve food. However, it has been found that these materials are biodegradable in nature [54].

The direct addition of antimicrobials into the food requires huge amounts of antimicrobials but a modest amount is sufficient if they are slowly released through a packaging film. The nanoparticle loading ranges between 0.1 and 5% w/w of the packaging film. Thus, active food packaging improves the shelf life and safety of food and will be useful during food storage and transportation [55]. The nanoparticles aid in the uniform dispersion of the active ingredient within the packaging [56]. Active compounds like ZnO NPs and thymol absorb the fungus that grows in cereal grains during storage. They eliminate fungal growth and decrease lipid oxidation [56]. The mode of action of thymol is in such a way as to damage the cell membranes of microorganisms, thus it takes a short killing time [57]. The polyphenols present in thymol are responsible for their high antioxidant/antimicrobial properties [58].

4 Conclusions

ZnO NPs were green synthesized using thyme extract as a reducing agent. The ZnO NPs are impregnated with polystyrene films by a simple solution casting process. The bioactive components of thyme, which are excellent antifungal agents, are firmly linked to ZnO NPs, as evidenced by FTIR analysis. The presence of ZnO NPs inside polystyrene films is confirmed by XRD analysis. The toxicity to mycotoxins by ZnO NPs incorporated polystyrene films is observed with the agar plate method. The nanocomposite films exhibited antifungal characteristics which make them a potential candidate for the preservation of food grains in packaging applications.

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Author Contribution

T.T. and S.M.K.T.: conceptualization, methodology, data curation, writing- original draft preparation; H.N.: antifungal tests; S.M.R. and S.S.: writing- reviewing and editing.

Conflicts of Interest

The authors declare no conflict of interest.

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