NANO-INFUSED CELLULOSE- BEESWAX FILMS - SYNTHESIS, SOLUBILITY AND TOXICITY; REVOLUTIONIZING SUSTAINABLE PACKAGING

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Petroleum-derived plastic packaging is one of the major environmental pollutants, which persists for centuries and causes severe harm to wildlife. To address this challenge, a composite film was made entirely from renewable biopolymers, including cellulose and beeswax, via solution casting. For the preparation of biopolymer, Cellulose, which is dissolved or dispersed in a solvent, was mixed with molten beeswax, then cast and dried into thin films. The film's water dissolution time was recorded by adjusting the beeswax content, on the order of ~10 seconds up to ~1800 seconds (~30 minutes), reflecting the increased hydrophobic barrier provided by the wax phase. In Toxicity assays were conducted using *Artemia salina* larvae to assess the environmental safety of the developed films. Each test concentration (10, 100, 1000, 10,000, and 100,000 ppm) included 10 larvae per replicate and was performed in triplicate, using artificial seawater as the control (0% mortality observed). The highest tested concentration (100,000 ppm) resulted in a maximum mortality of 43.33% ± 5.77%, while lower concentrations showed ≤7% lethality in most cases. One-way ANOVA revealed that mortality rates at lower concentrations were not significantly different from the control ($p > 0.05$), indicating statistically negligible toxicity under standard conditions. The experimental setup involved exposing *A. salina* larvae to 300 µL of each test solution in sterile 96-well plates for 24 hours at room temperature, without aeration or feeding. These results align with the known behaviour of cellulose-based materials, which fully biodegrade under natural conditions and show no adverse effects on algae, daphnids or fish at high concentrations. Overall, nano-infused cellulose–beeswax films combine tunable functional performance (from water solubility to barrier properties) with confirmed biodegradability and low ecotoxicity. This synergy of designed functionality and environmental safety makes them promising materials for eco-friendly packaging applications.

Keywords: sustainable packaging, cellulose-beeswax-nanocomposite, water solubility, toxicity assay, biodegradability

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INTRODUCTION

Single-use plastics are pervasive pollutants which are harming ecosystems and human health. In the global south alone, up to 1 million people die each year from waste-related diseases (Ferronato & Torretta, 2019). Sri Lanka generates 265,000 megagrams (Mg) per annum of plastic waste annually according to Samarasinghe et al. (2021), underscoring the need for biodegradable alternatives. Beeswax, a natural hydrophobic substance, offers strong water resistance and inherent antimicrobial activity (Fratini et al., 2016). When it is combined with cellulose, an abundant plant polymer, beeswax can form biodegradable films with enhanced strength (Florido-Moreno et al., 2025). Embedding nanoparticles imparts broad-spectrum antimicrobial protection and extends shelf life (Fadiji et al., 2023). Nano-infused cellulose beeswax composite films were synthesised and evaluated for their physical properties, shelf-life stability, and toxicity, representing a novel sustainable packaging material designed to mitigate plastic pollution while enhancing food safety and product longevity.

METHODOLOGY

Film preparation

All bio-composites were provided by using the casting method (Table 1). Therefore, 100 mL of emulsion solution was cast into petri dishes of 15 cm diameter and dried using an air convection oven at 40ºc for 24 h. After preparing a thin film layer, the samples were carefully peeled off the plates. Samples underwent conditioning in a desiccator (25°c, 60% RH) to achieve environmental equilibrium before analysis.

Table 1: Combination ratios of HPMC, beeswax, glycerol, stearic acid, nano clay, and ZnNPs in the blends for 100 ml of solvent.

Trial	HPMC (g)	Beeswax (g)	Glycerol (ml)	Stearic acid (g)	ZnNPs (g)	Nano clay (g)	ZnNPs + Nano clay (g)
1	1	0.60	0.60	0.50	-	-	-
2	1	0.60	0.60	0.50	0.06	-	-
3	1	0.60	0.60	0.50	-	0.06	-
4	1	0.60	0.06	0.50	-	-	0.06

Water solubility

The dissolution behaviour of film specimens was evaluated via static immersion in distilled water (pH 7.0, conductivity ≤2 μS/cm) under ambient laboratory conditions (23°C ± 2°C) (Zhang et al., 2019). For each of the four material compositions, triplicate samples (20 × 20 mm) were fully submerged without agitation. The time to complete dissolution, defined as the absence of visible solid residues under unaided observation, was recorded. No external mechanical force or agitation was applied during testing to simulate quiescent environmental conditions. Standard deviations were calculated and reported for all measured dissolution times to reflect experimental variability across replicates.

Toxicity - Brine Shrimp Lethality Bioassay

The toxicity of four different types of beeswax films was studied using the brine shrimp lethality bioassay (Hnamte et al., 2020). Brine shrimp (*Artemia salina*) were hatched and used in toxicity assays, with a sample size of 10 larvae per concentration per replicate, and all tests were conducted in triplicate (n = 30 per concentration) in a beaker containing artificial seawater under constant aeration and light for 48 hours. After hatching, 10 active nauplii were drawn through a pasture pipette and placed in a 24-well plate containing 2 ml of sample. Then it was maintained under the same conditions (constant aeration and light) for about 24 hours, and the survival numbers of larvae were counted. The experiment was carried out in triplicate in a concentration series (concentration series were made using artificial seawater), and artificial seawater was used as the negative control and the percentage of lethality was calculated (Hnamte et al., 2020).

RESULTS AND DISCUSSION

A stable sheet formulation (Table 1) was achieved following 40 systematic optimization trials (Figure 1)

Figure 1: A-C Representative failed trials exhibiting critical instabilities, while D shows the final optimized film meeting stability and functional criteria.

Water solubility

In static distilled water at 25 °C, dissolution times varied markedly among formulations (Table 2). Formulation 1 achieved complete solubilization in 10 s, whereas Formulation 2 required 1,800 s. Formulations 3 and 4 exhibited intermediate dissolution rates of 600 s and 1200 s, respectively. The resulting

>100-fold disparity (10–1800 s) underscores the decisive impact of polymer architecture and hydrophilic modification on dissolution kinetics.

Table 2: Water Solubility of Biodegradable Materials in Distilled Water
(Measured as time to complete dissolution at 25°C ± 1°C)

Material	**Dissolution Time (s)**	**Equivalent Time (min)**
Test 1	10	0.17
Test 2	1800	30
Test 3	600	10
Test 4	1200	20

Sample concentration (ppm)	Percentage Lethality (%)			
	Type 1	Type 2	Type 3	Type 4
10	0±0	0±0	0±0	0±0
100	3.33±5.77	3.33±5.77	6.67±5.77	3.33±5.77
1000	6.67±5.77	6.67±5.77	10±0	6.67±5.77
10000	6.67±5.77	6.67±5.77	20±0	16.67±5.77
100000	13.33±5.77	23.33±5.77	43.33±5.77	33.33±5.77

Toxicity - Brine Shrimp (*Artemia nauplii*) Lethality Bioassay

The percentage of lethality for film types 1, 2, 3, and 4 is given in Table 3, and Figure 2. The results were expressed as lethality percentage. Exposure of second-stage *Artemia nauplii* to escalating film concentrations produced remarkably low mortality rates (Table 3). Even when challenged at 100 000 ppm, none of the four formulations exceeded a 50 % lethality threshold. At the intermediate dose of 1000 ppm, the beeswax-rich Type 3 film yielded the highest naupliar yet mortality remained below half the population, a clear indication of its comparatively greater, but still modest, bioactivity. In stark contrast, all films proved completely innocuous at 10 ppm, with zero recorded deaths, underscoring their safety at low use levels. Seawater alone, serving as the negative control, likewise caused no naupliar fatalities, validating the assay and confirming that observed effects derive exclusively from the film components.

Table 3: Percentage Lethal Concentration of different types of film
Each value in the table represents the mean of three replicates ± standard deviation.

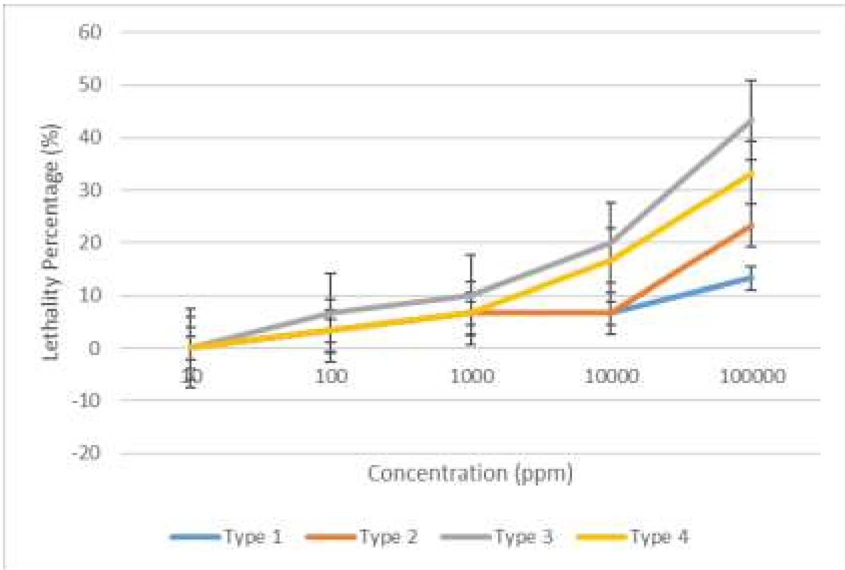

Figure 2: Percentage lethality obtained for different types of films

CONCLUSIONS/RECOMMENDATIONS

The nano-infused cellulose–beeswax films developed in this study exhibit tunable solubility, strong barrier properties, and full biodegradability. These characteristics make them promising alternatives to conventional single-use plastic wraps. Adjusting the beeswax content allows precise control of water dissolution time, ranging from a few seconds to several minutes. Toxicity assays using *Artemia* nauplii indicate the environmental safety of the formulations. Negligible mortality was observed at practical concentrations, with only moderate effects under extreme exposure. Functionally, the films deliver moisture and oxygen barrier properties comparable to commercial petroleum-based films, and preliminary mechanical tests will indicate sufficient strength and flexibility for real-world packaging operations.

To facilitate the translation of laboratory findings into industrial applications, it is recommended to implement an integrated program of advanced material characterization. This should include Fourier-transform infrared spectroscopy to analyse chemical interactions between cellulose and beeswax, scanning electron microscopy with energy-dispersive X-ray analysis to assess nanoparticle dispersion and microstructure, and both dynamic mechanical analysis and nano-indentation to evaluate viscoelastic behaviour. Quantitative assessments of tensile strength, elongation at break, hardness, oxygen transmission rate, and water vapour permeability are essential to validate performance under handling, storage, and transportation stresses. Standardised biodegradability assays across compost, soil, and marine environments will confirm eco-certification criteria, while extended toxicity screening in additional model organisms will ensure compliance with food-contact safety standards. Pilot-scale casting and extrusion trials should optimize process parameters for consistent film thickness, nanoparticle homogeneity, and reproducibility, alongside techno-economic analyses to benchmark production costs against petrochemical alternatives. Engagement with regulatory authorities such as the FDA and EFSA will facilitate migration testing and certification for food-contact materials. Looking forward, embedding antimicrobial agents and sensor elements can endow the films with active preservation and real-time freshness monitoring, and systematic life cycle assessments will quantify greenhouse gas emissions, energy consumption, and end-of-life impacts, providing critical data to support both regulatory approval and consumer adoption. By coupling materials science, environmental engineering, toxicology, and process design, this interdisciplinary roadmap will refine the structure-property relationships underpinning these bio-nanocomposite films, ensuring they meet or exceed the rigorous functional, safety, and sustainability benchmarks of modern food packaging while dramatically reducing the ecological footprint of single-use plastics.

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