Studies on biopolymer based cinnamon oil formulation to improve the shelf life of cherry tomato against *Alternaria alternata*

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Abstract. The study aimed to develop sodium alginate-based coating functionalized with cinnamon essential oils towards shelf-life extension of cherry tomatoes. Ultrasonication pretreatment and hydro distillation methods were used to extraction of essential oils from cinnamon followed by preparation of cinnamon oil nanoemulsion with tween 80 to encapsulate in sodium alginate coating. The different concentrations (2.5, 5 & 10% of cinnamon oil nanoemulsion were encapsulated in coating formulations and characterization followed by applications. The nonorange particle size (41.58 nm) with in vivo and invitro antifungal activity against *A. alternata* of nanoemulsion was confirmed. The application results showed that the encapsulation of lower concentration (2.5%) of nanoemulsion was most effective to maintain the postharvest quality of cherry tomato up to 7 days at ambient storage condition. This study concludes that biopolymer-based cinnamon oil coatings offer a promising, eco-friendly alternative to synthetic fungicides, extending the shelf life and marketability of cherry tomatoes. Future research may focus on applying this formulation to other horticultural crops and evaluating its commercial scalability and sensory impacts on produce.

1 Introduction

The postharvest losses in fruits and vegetables (30-40%) around the world are big concern due to food wastage, environment degradation and economic losses during handling, storage, and transportation [1]. Microbial degradation is the major cause of spoilage in fruits and vegetables. The fungal pathogens including *Aspergillus niger, Fusarium spp., Alternaria alternata, penicillium, Geotricum* etc, are major cause for degradation of fruits and vegetable quality during storage [2, 3]. Alternaria alternata is one of the widespread fugal pathogens, responsible for producing mycotoxin and has resulted in negative health impacts

Currently, the effects of *Alternaria alternata* are managed using synthetic fungicide, which also possess health risks [4]. However, the use of biodegradable, ecofriendly edible coating integrated with active agents including essential oil etc. is one of the major solutions to use as an alternative of the chemical fungicide to minimize microbial spoilage [5, 6].

Cherry tomatoes (*Solanum lycopersicum var. cerasiforme*) are widely consumed for their rich nutritional profile, including vitamins, antioxidants, and minerals [7]. However, their high moisture content makes them highly perishable, leading to significant postharvest losses. Several characteristics, such as a high respiration rate, relative humidity, ethylene production, temperature fluctuations, microbial deterioration, and high-water content, contribute to their short shelf life. Tomato postharvest losses are estimated to range between 20 and 65 % of total production [8] Cherry tomatoes are particularly vulnerable to postharvest fungal infections with *A. alternata.*

To manage this issue, cinnamon essential oil (CEO), known for its antifungal properties, is incorporated into a biopolymer-based coating to enhance shelf life and naturally control fungal spoilage. Cinnamon (*Cinnamomum verum*) is an aromatic spice obtained from the bark of trees belonging to the genus Cinnamomum. It is valued in the culinary, pharmaceutical, and cosmetics sectors for its antibacterial, anti- inflammatory, antioxidant, and aromatic properties [9, 10].

Cinnamon essential oil is renowned for its potent antimicrobial and antifungal activities, largely due to bioactive compounds such as cinnamaldehyde, eugenol, and linalool. These compounds have been shown to inhibit the growth of various spoilage microorganisms, including *A. alternata*.

By adding CEO in sodium alginate-based coatings can enhance their protective power, putting a barrier that shields cherry tomatoes. The nature of plant material and the extraction technique play essential roles in extraction of essential oil. Conventional methods for extracting essential oils from plant sources include

steam distillation, hydro distillation, solvent extraction, and expression. Recently, innovative techniques like ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), and supercritical fluid extraction have been developed. Among these, UAE offers advantages such as reduced time and energy consumption, increased extraction yield, and improved essential oil quality due to its cavitation, mechanical, and thermal effects. Ultrasound-assisted hydro distillation extraction (UAHDE) combines the benefits of both UAE and HD. It also results high quality oil with improved antimicrobial property, making it suitable for use in food- grade antimicrobial coatings used to prevent fungal infections. However, concerns over chemical residues, environmental pollution, and the emergence of fungicide-resistant strains have increased interest in natural alternatives. Edible coatings, which form a thin, biodegradable layer on the fruit surface, have emerged as a promising strategy for extending shelf life while maintaining quality and safety [11].

Sodium alginate, creates a semi-permeable barrier that reduces respiration rates and microbial contamination. However, sodium alginate alonehas limited antimicrobial properties, necessitating the addition of natural antimicrobial agents like essential oils. Cheng et al. [12] investigated the efficacy of chitosan and alginate-based multilayer edible coatings enriched with cinnamon essential oil microcapsules for preserving the postharvest quality of mangoes. Karthikeyan et al. [13] examined the application of 2% chitosan coatings incorporated with varying concentrations of cinnamon essential oil (0%, 0.5%, 1.0%, and 1.5%) to extend the quality and shelf life of tomatoes under ambient conditions.

This study aims to extract cinnamon essential oil (CEO) from plant bark material using a combination of ultrasonication pre-treatment and hydro-distillation and to formulate sodium alginate based edible coating incorporated with CEO. Further, to evaluate the antifungal efficacy of the formulated edible coatings against *Alternaria alternata* in cherry tomatoes through vivo experiments.

2 Materials and Methods

2.1 Materials

Fresh cinnamon bark (*Cinnamomum verum*) was procured from a local spice market in Noida, ensuring uniformity in quality, size, and maturity to minimize variability in essential oil content. Uniform size cherry tomatoes were also purchased from locat market. High-purity sodium alginate powder was obtained from Sigma Aldrich. Fungal culture, *A. alternata* was obtained from Amity Food and Agriculture Laboratory. The fungal strain was subculture and maintained on Potato Dextrose Agar (PDA) slants at 4°C until use. The experiment was performed in the Ripening and Processing laboratory of Amity University, Noida. Analytical reagent (AR) grade chemicals were used throughout the study to maintain uniformity and accuracy. Sodium alginate acted as a film-forming agent, Polysorbate 80 (Tween 80) was used as emulsifiers for the preparation of coating solution [10]. Different laboratory equipment such as Autoclave, Hot air oven, Clevenger apparatus, ultrasonication bath were utilized during experimental work.

2.2 Extraction of Cinnamon Essential Oil

2.2.1 Sample Preparation

Cinnamon bark (*Cinnamomum verum*) was first cleaned, air-dried, and ground into a coarse powder using a mechanical grinder to increase surface area for extraction. Two 100 gm samples of the ground bark were prepared. One served as the control, while the other was subjected to ultrasonic treatment. The ultrasonication process was carried out for 20 minutes at 35 °C to enhance cell wall disruption and facilitate compound release. Following sonication, the treated mixture was filtered, and the solid residue was oven-dried for 30 minutes to remove any remaining moisture. Both the control and ultrasonicated cinnamon bark powders were then stored in airtight containers and used for Hydro-distillation extraction.

2.2.2 Hydro distillation of Control Sample

For the control extraction, 100g of ground cinnamon bark was weighed and mixed with 600 ml of distilled water and assembled with a Clevenger-type hydro distillation unit. The distillation was carried out for 3 hours at 45°C, during which the essential oil vapours, carried by steam, were condensed and collected in the receiving arm of the apparatus. The essential oil, being heavier than water, accumulated at the bottom, and was carefully separated. Chen et al. [12], investigated the efficiency of ultrasound-assisted hydro distillation extraction (UAHDE) for obtaining essential oils from Cinnamomum cassia bark, comparing its performance with conventional hydro distillation (HD).

2.2.3 Ultrasonic Pretreatment and Hydro distillation

The ultrasonication of the second 100g batch was performed using an ultrasonic bath operating at a frequency of 40 kHz and a power of 150W. The ground cinnamon sample was mixed with 600 ml of distilled water in a conical flask. The flask was immersed in the ultrasonic bath and subjected to ultrasonic treatment for 30 minutes at a controlled temperature of 40°C.

Ultrasonication is a novel, green, and efficient technique that enhances the penetration of the solvent into the plant matrix by generating cavitation bubbles, thereby increasing the oil yield and preserving thermolabile bioactive compounds. After pretreatment, the

ultrasonicated mixture was immediately transferred to the hydro distillation apparatus and distilled under identical conditions as described for the control.
The essential oil was similarly collected, dried, filtered, and stored in amber vials under refrigeration for subsequent analysis and formulation. Table 1 shows Cinnamon essential oil extraction methods, including their respective sample-to-solvent ratios and referenced protocols used in the extraction process.

Table 1. Extraction of Cinnamon oil by Hydro-distillation method with and without ultrasonic pretreatment.

Extraction Techniques	Sample to solvent ration
HD(Control)	100g(sample): 600ml (Water) [12]
UPHD	100g(sample): 600ml (Water) [13]

2.3 Preparation of Cinnamon Oil Emulsion

A cinnamon oil emulsion was prepared by mixing 1 ml of cinnamon essential oil with 9 ml of distilled water containing 1.3% (w/v) Tween 80, which served as an emulsifying agent. The presence of Tween 80 facilitated the uniform dispersion of oil droplets within the aqueous phase, leading to the formation of a stable oil-in-water emulsion. The mixture was first homogenized using a magnetic stirrer at a speed of 100–200 rpm for 10 minutes to promote initial emulsification, followed by ultrasonication for 5 minutes to achieve a finer, more uniform, and stable emulsion.

2.4 Preparation of Biopolymer-Based Cinnamon Oil Formulation

2.4.1 Preparation of Sodium Alginate Solution

A 2% (w/v) stock solution of sodium alginate was prepared by gradually adding the sodium alginate powder to 100ml distilled water under continuous stirring. The solution was stirred using a magnetic stirrer until a homogenous and clear solution was obtained, ensuring complete dissolution of the biopolymer.

2.4.2 Formulation of Biopolymer Coating incorporating Cinnamon Essential Oil

A total of 20 ml of biopolymer-based film-forming formulation was prepared by mixing 17ml of sodium alginate solution, 2 ml of cinnamon essential oil and 1 ml of Tween 80. Here the concentration of the essential oil was kept at 10% (Table 2). The mixture was placed on a magnetic stirrer and stirred for several minutes until a homogeneous, white and milky emulsion was obtained, ensuring uniform dispersion of the essential oil within the matrix. Following this, the mixture was subjected to ultrasonication for 5 minutes to further enhance emulsion stability, reduce droplet size, and improve the uniformity of the film-forming solution.

Table 2. Composition of the Cinnamon oil containing biopolymer formulation.

Components	%
Aq. Alginate solution (2%)	85
Cinnamon Essential Oil	10
Tween 80	5

2.4.3 Serial Dilution of the Biopolymer based coating Formulation

The above prepared biopolymer-based coating formulation was gradually diluted with water to create a formulation with 5% (w/v) essential oil. From this, a final 2.5% (w/v) essential oil containing formulation was prepared. All three formulations with 10%, 5% and 2.5% essential oil were used for coating the cherry tomatoes. All solutions were stirred thoroughly to make sure the mixture was even and well-blended before applying to the fruits.

2.5 Characterization of CEO-based biopolymer formulation

2.5.1 Dynamic Light Scattering Analysis

The mean droplet size and Polydispersity Index (PDI) of CEO based biopolymer formulation were measured using Zetasizer Nano S90 (Malvern Instruments, Malvern, United Kingdom), operated on the principle of Dynamic light scattering (DLS), with a 90 ° scattering angle for particle size analysis. Zetasizer Nano software provided insights into the colloidal properties of nano-emulsion. 633 nm laser beam was used as light source in the instrument. A maximum of 3 mL samples is used for these measurements.

2.5.2 Stability Assay of Cinnamon essential oil Based Bioformulation

The phase stability of the CEO-based bio formulation was evaluated by vigorously shaking the mixture in a volumetric cylinder for 15 seconds, followed by allowing it to stand undisturbed for 30 minutes to observe any indication of phase separation. Solubility was assessed by mixing 1 mL of the formulation with distilled water and monitoring its miscibility. Furthermore, the thermal stability of the biofilm was tested by storing samples under two different temperature conditions ambient room temperature and elevated temperature (in an oven) for a period of 15 days, after which physical changes were observed.

2.5.3 Appearance of CEO bio-formulation

The CEO exhibits a uniform milky white appearance, indicative of fine particle dispersion throughout the medium. It has a smooth, creamy texture that is consistent and free from visible lumps or phase separation. Upon formulation, the CEO forms a fully stable, homogeneous solution, demonstrating excellent emulsification and physical stability over time. The consistency remains intact under standard storage conditions, with no signs of creaming, sedimentation, or oil separation. The visual and tactile properties suggest proper emulsifier function and optimal mixing parameters during production.

2.6 In Vitro Antifungal efficacy of Cinnamon essential oil

2.6.1 Culture Preparation

To prepare the culture medium for the growth and maintenance of *Alternaria alternata*, 12 g of Potato Dextrose Agar (PDA) powder was accurately weighed using a digital balance and dissolved in 300 ml of distilled water in a clean conical flask. From this, 25 ml of the prepared medium was carefully measured and poured into ten separate sterile conical flasks under aseptic conditions. The flasks containing the medium were then sterilized in an autoclave at 121°C for 15 minutes under a pressure of 15 psi to eliminate any microbial contaminants and maintain aseptic conditions. After sterilization, the hot medium was removed from the autoclave and allowed to cool slightly to a safe pouring temperature. Once cooled to approximately 45–50°C, the media from each flask was aseptically transferred into sterile Petri plates. To these plates, 500 µl of concentrated cinnamon essential oil emulsion (10%) and 500 µl of biopolymer-based cinnamon oil formulation (10%) were added separately, followed by gentle swirling to allow uniform mixing before the medium solidified.

2.6.2 Inoculation of Alternaria alternata

For inoculation, a distinct, morphologically intact colony of *Alternaria alternata* was carefully selected from a previously maintained pure culture on PDA slants. Using aseptic technique, a sterile inoculation loop was employed to gently extract a portion of the fungal colony. The extracted portion was then introduced into a sterilized test tube containing distilled water mixed with a small amount of Tween 80 to aid in the uniform dispersion of fungal spores. The resulting suspension was mixed gently to ensure an even distribution of spores. Subsequently, using a sterile inoculation loop, small aliquots of the prepared spore suspension were inoculated at five equidistant points on the surface of each treated and control PDA plate. This inoculation method facilitated uniform fungal growth and enabled clear observation of the inhibitory effect of the cinnamon oil formulations on the growth of *Alternaria alternata* (Fig 1).

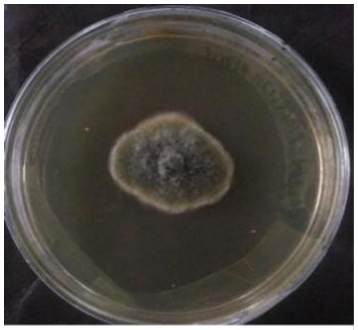


Fig. 1. *Alternaria alternata*

2.7 Application of Cinnamon oil – biopolymer-based Coating Solution

The following treatments (Table 3) were applied to cherry tomatoes to evaluate the effectiveness of biopolymer-based coatings incorporated with cinnamon essential oil (CEO). The visual of the application process on cherry tomatoes is shown in Fig. 2.

Table 3. Application of Biofilm on cherry tomatoes

Experiment	Treatment
T1R1	Biofilm with 10% CEO
T2R2	Biofilm with 10% CEO+ *A. alternata*
T3R3	Biofilm with 5% CEO+ *A. alternata*
T4R4	Biofilm with 2.5% CEO+ *A. alternata*
T5R5	Blank (Sodium Alginate)
T6R6	Blank + *A.alternata*
T7R7	Control
T8R8	Control + *A.alternata*

Fig. 2. Tomatoes after coating with the biopolymer-based cinnamon oil formulation.

3 Results and Discussions

3.1 Extraction Yield of Cinnamon Essential Oil

The extraction yield of essential oil was determined for both control and ultrasonicated samples of cinnamon bark. The oil yield was calculated based on the volume of oil obtained per 100g of dried cinnamon bark. The results demonstrated a notable difference between control and ultrasonicated hydro-distilled cinnamon oil extraction yield and time of extraction (Table 4). Ultrasonication enhanced the yield of extraction of essential oil by Clevenger apparatus at lesser time in comparison to control sample without ultrasonication. By integrating ultrasonic pretreatment into the extraction process, the method capitalizes on the physical phenomenon of acoustic cavitation, where ultrasonic waves generate microscopic bubbles in the solvent that collapse with intense energy. This cavitation effect disrupts plant cell walls, enhances the penetration of water into plant tissues, and facilitates the rapid release of intracellular essential oil components into the surrounding medium.

Table 4. Extraction yield and time of CEO by hydro-distillation method

Treatment	Sample weight	% Yield	Time
Control (Hydro distillation)	100g	1.83%	3hrs
Ultrasonic Bath + Clevenger apparatus (Hydro distillation)	100g	2.42%	2hrs30min

As a result of these enhanced mass transfer dynamics, the cinnamon bark samples pretreated with ultrasonication yielded a significantly greater volume of essential oil in a shorter time frame than those processed by conventional hydro distillation alone. Additionally, the milder temperature and reduced exposure time during UAHDE helped in preserving heat-sensitive (thermolabile) compounds such as cinnamaldehyde, eugenol, and other volatile constituents known for their potent antifungal and antioxidant activities. These preserved compounds contribute directly to the bioactivity and quality of the extracted oil.

3.2 DLS Analysis of CEO oil based bio formulation

The average diameter of Cinnamon oil based bio formulation was found to be 42.56 nm which indicates the nano-formulation of cinnamon oil in alginate biopolymer matrix. Two major peaks of particle intensity were obtained at 30.93nm and 183.3 nm. The polydispersity index (PDI) of 0.555 suggested fairly uniform particle size distribution (Table 5). Fig. 3 shows particle size distribution of CEO based bioformulation.

Table 5. Particle size analysis of CEO – based bioformulation

Z-Average (d. nm)	42.56 ± 0.5
PDI	0.555 ± 0.04
Intercept	0.659 ± 0.02

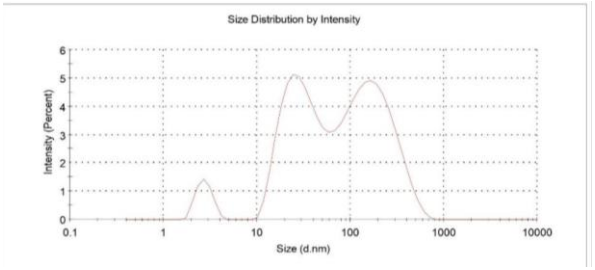
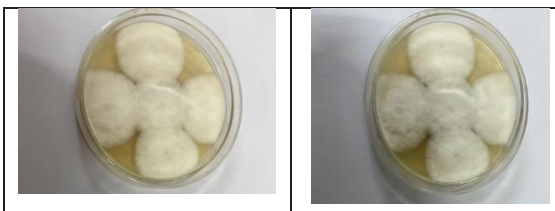


Fig. 3. Particle size distribution of CEO based bio formulation

3.3 In vitro Antifungal Properties of CEO - based Bioformulation

The fungal growth was observed in inoculated petri plates after three days of incubation. The experiment clearly showed differences in fungal growth between the treated and untreated Plates. In the control plates and blank samples which didn't contain any cinnamon oil *Alternaria alternata* grew well, forming the typical dense, spreading colonies with noticeable concentric rings. The fungal growth was consistent at all five inoculation points, confirming that the conditions supported healthy colony development in the absence of any anti-fungal agents (Fig 4). But both the plates containing cinnamon essential oil emulsion and the biopolymer-based cinnamon oil formulation completely prevented fungal growth. There were no signs of colony formation at any of the inoculation spots on these plates, and the medium remained clear throughout the incubation period.



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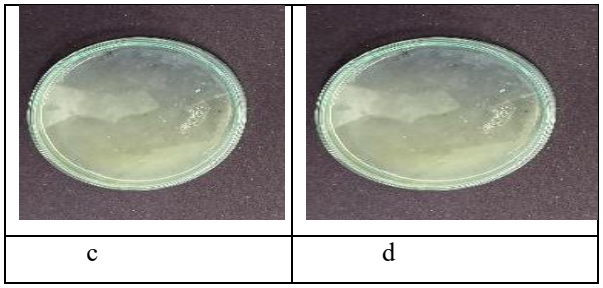

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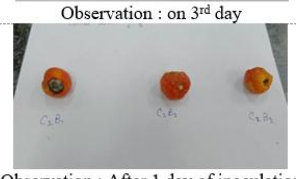
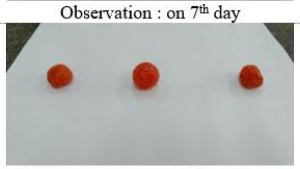
Fig. 4. Antifungal assay showing fungal growth under four treatments: (a) Control, (b)Blank (Sodium alginate), (c) CEO Emulsion at 1000 ppm, (d) CEO based bioformulation at 1000 ppm

This clear contrast highlights the strong antifungal effect of cinnamon oil and its biopolymer-based formulation against *Alternaria alternata*. The results suggest that both treatments were highly effective in completely inhibiting fungal growth under the conditions used in this study.

3.4 In vivo Antimicrobial efficacy of CEO biofilm

The antimicrobial activity of cinnamon essential oil (CEO) based biopolymer film was assessed against *Alternaria alternata* by observing fungal growth patterns on cherry tomatoes with different treatments described in Table 6. A total of eight treatments, each with two replicates, were evaluated after an incubation period at ambient conditions. Three cherry tomatoes were kept in each group. The visual observations of fungal growth, inhibition patterns, and physical changes in the biofilms were recorded meticulously up to 7 days. No fungal growth was observed throughout the incubation period for biofilm with 10% (T1), 5% (T2) even 2.5% (T3) CEO. The biopolymer films remained stable but showed noticeable shrinkage for 5% and 10% CEO containing biofilms, whereas shrinkage is moderate for 2.5% CEO containing biofilm. likely due to the high concentration of cinnamon essential oil causing film tightening. All biofilm surfaces on cherry tomatoes stayed clean and free of any fungal colony growth. Interestingly, T4 (Biofilm with 2.5% CEO + *Alternaria alternata*), i.e., treatment of cherry tomatoes with 2.5% CEO based film and inoculation with *A. Alternata* showed complete inhibition of fungal growth, no colonies appeared at any of the inoculation points. The biopolymer film remained physically stable with less shrinkage compared to the higher concentration treatments, indicating a good balance between antifungal efficacy and film integrity.
Small fungal colonies appeared on treatment with T5 (Blank Sodium Alginate Film) despite no inoculation of *A. alternata*, suggesting possible airborne contamination. The sodium alginate film showed minor shrinkage but maintained its general structure. Heavy fungal growth was observed for T7 control (no treatment), T8 control with A. Alternaria and T6 blank with A. Alternaria which was shown in Table 6.

Table 6. Effect of different treatment on cherry tomatoes after storage

Treatment	Status
Control	 Observation : on 3rd day
Control with fungus	 Observation : After 1 day of inoculation
Treatment with Biofilm with 2.5% CEO	 Observation : on 7th day
Treatment with Biofilm with 5% CEO	 Observation: on 7th day

3.5 Conclusions

The present study effectively demonstrated that ultrasound-assisted hydro distillation extraction (UAHDE) is a superior method for obtaining cinnamon essential oil (CEO) from Cinnamomum verum bark as compared to traditional hydro distillation. Moreover, UAHDE offers environmental and operational advantages, it reduces energy and water consumption, minimizes degradation of active ingredients, and shortens the overall processing time, making it a more sustainable and scalable approach for essential oil extraction in both laboratory and industrial settings. This positions UAHDE not only as an effective method for maximizing yield but also as a green technology aligned with modern trends in clean and sustainable processing. The antifungal potential of cinnamon essential oil (CEO) and its biopolymer-based film formulations exhibited good results against *Alternaria alternata*. Biofilms containing higher concentrations of CEO (10% and 5%) showed significant antifungal activity, with complete inhibition of fungal growth observed in T2R2, T3R3, and T4R4. Among all treatments, T4R4 (Biofilm with 2.5% cinnamon essential oil) emerged as the most promising. It successfully prevented fungal growth without the excessive film shrinkage observed at higher oil concentrations. This suggests that even at a lower concentration, cinnamon essential oil can provide effective antifungal protection when incorporated into a biopolymer-based film. Moreover, using 2.5% CEO makes this formulation more cost-effective and practical for larger-scale applications, reducing the overall amount of essential oil needed without compromising antifungal efficacy. This finding indicates that lower-

dose formulations like T4R4 could be an efficient, eco-friendly alternative for managing fungal contamination.

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