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PREPARATION OF BIOGENIC NANOFILM TO ENHANCE LONGEVITY OF FRUITS AND VEGETABLES

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ABSTRACT

Food produced worldwide is lost between harvest and retail. Thus, research to extend shelf-life of fruits and vegetables is essential. Our research focuses on incorporating nanotechnology to preserve fruits and vegetables, because of its remarkable properties and its various applications in the biological, medicinal, cosmetic, food, and other industries compared to physio-chemical approaches. Green synthesis of nanoparticles is preferred since it is eco friendly, economical and does not employ any harmful chemicals. Zinc oxide nanoparticles (ZnO NPs) were used because of its unique characteristics like its surface area, size, shape, low toxicity, optical properties, high binding energy and large band gap. ZnO NPs were produced using pomegranate peel aqueous extract and zinc sulphate as a precursor. The stability of ZnO NPs was validated by determining optimal density using UV-spectrophotometer. Both bacteria (*Pseudomonas aeruginosa* and *Bacillus subtilis*) and fungi (*Aspergillus sp, Colletotrichum sp, Botrytis sp* and *Diplodia sp*) were isolated from decaying fruits and vegetables. ZnO NPs was tested for its antibacterial and antifungal properties against isolated pathogens from *Dolichos lablab*, *Annona squamosa*, *Citrus sp* and *Daucus carota*. Poly vinyl alcohol ZnO NPs bio nanofilm composite was made. The fruit and vegetable samples were wrapped in bio nanofilm and tested for its preservative properties. Our bio nanofilm can be used to wrap food during transportation and long-term storage to prevent food spoilage, which may be very beneficial in the food industry as well as to agriculturalists.

Keywords: nanofilm, fruits, vegetables, ZnO nanoparticles, food preservation, shelf-life

INTRODUCTION

After harvest, horticultural produce are subjected to the active processes of senescence. The ripening process involves biochemical modifications leading to the change in its original composition, thus reducing its shelf-life and finally its market value (Ahvennainen, 1996; Boyettet *et al*., 1993; Carlin *et al*., 1990; Maria *et al*., 2016). This is the post harvest shelf life. It is determined by its overall appearance namely texture, flavor and taste. It includes a combination of sensory, biochemical, mechanical, and colorimetric measurements. The quality and shelf life of food are significantly impacted by food deterioration brought on by spoilage microorganisms during storage and distribution, and pathogen microorganisms present in food can cause a number of diseases and/or

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intoxications. The harvested fresh produce spends half of its life in transit before it reaches the market (Tingting *et al*., 2018; Abbey *et al*., 1998; Brul and Coote, 1999).

Our research focuses on incorporating nanotechnology to preserve food, because of its remarkable properties and its various applications in the biological, medicinal, cosmetic, food and other industries. In the present investigation green synthesis of nanoparticles was chosen as it has advantages over other methods (Chaudhry *et al*., 2008; Chen *et al*., 2006; Das *et al*., 2009; Sekhon and Bhupinder, 2010). Moreover, it is cheap, eco-friendly, convenient single-step method, easily scaled up for largescale synthesis and does not require high pressure, energy, temperature and toxic chemicals. It is a promising approach that allows synthesis in aqueous conditions (Wang and Xu, 2022; Shimoni, 2009; Dingman, 2008).

Zinc oxide nanoparticles (ZnO NPs) was used in the study due to its unique characteristics like its surface area, size, shape, low toxicity, optical properties, high binding energy and large band gap. ZnO NPs have several advantages like high antibacterial effectiveness at low concentrations (0.16–5.00 mmol/L), activity against a wide range of strains and are relatively cheaper (Agarwal and Happy, 2017; Bhuyan and Mishra, 2015; Pantidos, 2014). Utilizing various fruit peel extracts as reducing agents, ZnO NPs can be produced using green synthesis (Malviya *et al*., 2014). Fruit peels are a readily available, affordable, and non-toxic source of bio wastes. Hence fruit peel aqueous extracts were employed as reducing agents to produce ZnO NPs (Nuraqeelah, 2018; Sharma and Rajput, 2010). Aqueous extracts of *Punica granatum* (pomegranate) peels was used in the presence of zinc sulphate as a precursor and a source of the zinc ions. The fungi and bacteria were isolated from the fruits and vegetables, namely, *Dolichos lablab*, *Annona squamosa*, *Citrus sp* and *Daucus carota*. The antimicrobial effectiveness of ZnO NPs against isolated pathogens was investigated (Tayel and El-tras, 2011).

Nanofilms are in use as barrier materials food packaging industries to prevent spoilage by microbes and oxygen absorption (Barry-Ryan and O'Beirne, 2000; Cantwell and Suslow, 2002). These specific films are used to prevent and reduce the possibility of food drying and spoilage. These films could act as a barrier between packaged food and the external environment, reducing moisture loss and restricting the entry of oxygen [Guan, 2022; Frisvad and Samson, 2004; Hasnain and Muzamil, 2020; Huang *et al*., 2009). This type of packaging would allow for extended shelf life, keeping the food fresh and healthy for a longer period. But the underlying issue would be that plastic films coated with silicate nanoparticles pose a health risk to consumers since the composite is only formed of chemical-based elements. In light of this situation, we took the step to create "Bio nanofilms," which are biological components made of plant extract and nanoparticles (Lee *et al*., 1991). Poly vinyl alcohol (PVA) was utilized for the fabrication of ZnO NPs bio nanofilm composite from pomegranate peel aqueous extract. We chose PVA because it is an inexpensive, water-soluble synthetic polymer that is non-toxic, biocompatible, and biodegradable, and because the FDA has approved its use in food packaging (Muzafari *et al*., 2008; Nasery, 2016). The biofilm was also tested with *Solanum lycopersicum*, and *Coccinia grandis*.

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METHODOLOGY

Sample collection and extraction

Fruit peel of *Punica granatum* was obtained from local market of Chennai. It was sun dried for 48 h and ground to produce a dry powder. About 2g of it was suspended in 50ml de-ionized water and thoroughly boiled in a water bath for 30 min. The resultant solution was allowed to cool to room temperature and filtered through a filter paper. This aqueous extract was used for the biosynthesis of ZnO NPs.

Biosynthesis of ZnO NPs and verifying its stability

To carry out the biosynthesis of ZnO NPs, 15 mL of 5 M Zinc sulphate and 5 mL of aqueous extract of pomegranate were taken in a conical flask. The solution was stirred continuously in a magnetic stirrer until it turned to a milky yellow colour. The sample was stored in an airtight microfuge tube to be processed later. The ZnO NPs containing plant extract was analyzed for green synthesis under UV-spectrophotometer consecutively for 5 days.

Bacteria isolation and culture

The pure culture of *B. subtilis* and *P. aeruginosa* were prepared in nutrient broth which was used for the analysis.

Fungal isolation and culture

A total of 4 randomly selected spoilt fruits were identified for the isolation of fungi in PDA media. The plates were incubated at 28°C for 48 h. A loopful of fungal sample was inoculated into PD broth.

Antibacterial activity

The antibacterial activity of ZnO NPs was investigated using the agar well diffusion technique. Using a sterile cotton swab, the two bacterial samples, *P. aeruginosa* and *B. subtilis*, were plated on the agar surface of the two nutrient agar plates, separately. Using a sterilised gel borer, four wells were aseptically punched off on four sides of each nutrient agar plate and a fifth well was made in the centre of plate. An aliquot of 20 µl of gentamycin (1mg/ml) was pipetted into the fifth well as positive control in both plates. In both plates, ZnO NPs containing samples was pipetted into each of the four wells at concentrations of 25µl, 50µl, 75µl and100µl. In order to allow the appropriate solution to diffuse into the medium, plates were left undisturbed for 30 min and were incubated at 37°C for 24 h. Plates were observed for the formation of zones of inhibition.

Antifungal activity

ZnO NP's antifungal activity was investigated using the agar well diffusion technique. Using a sterile cotton swab, the four fungal samples from PD Broth were plated on the agar surface of the four PDA plates, separately. Using a sterilised gel borer, four wells were aseptically punched off on four sides of each PDA plate and a fifth well was made in the centre of plate. For black fungi that produce spores, Fluconazole was used as a positive control, while for white fungi that don't produce spores, Clotrimazole was used as a positive

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control. 20 µl of appropriate controls was pipetted into fifth well as positive control in appropriate plates. In all plates, ZnO NPS containing samples was pipetted into each of the four wells at concentrations of 25µl, 50µ, 75µl and 100µl. In order to allow the appropriate solution to diffuse into the medium, plates were left undisturbed for 30 min and were incubated at 28°C for 48 hours. Plates were observed for the formation of zones of inhibition.

Preparation of PVA-ZnO nanofilm composite

8 % poly vinyl alcohol (PVA) solution was prepared with deionized water by dissolving 8 g of PVA in 100 ml deionized water at 60°C in a magnetic stirrer. Then, 1:10 ratio of ZnO NPs containing plant extract was added to the PVA solution. The resultant solution was then poured into a casting plate to develop it into wrapping paper. Additional 8% PVA solution was poured onto the casting plate for future use.

Antibacterial activity of PVA-ZnO nanofilm composite

The antibacterial activity of Nano film composites was assessed using the disc diffusion method. Agar plates were divided into two equal parts and labelled with markers, with the first half bearing the label Control and second half as Treated. Using a sterile cotton swab, the two bacterial samples, *Pseudomonas aeruginosa* and *Bacillus subtilis*, were swabbed on the agar surface of the two nutrient agar plates, separately. Small pieces of nanofilm were placed on the agar surface of the two nutrient agar plates on treated part and small piece of 8% PVA film were placed on the agar surface of the nutrient agar plates on control part Plates were incubated at 37°C for 24 hours. Plates were observed for the formation of zones of inhibition.

Antifungal activity of PVA-ZnO nanofilm composite

The antifungal activity of Nano film composites was assessed using the disc diffusion method. Allfour potato dextrose agar plates were divided into two equal parts and labelled with markers, with the first half bearing the label control and second half as treated. Using a sterile cotton swab, the four fungal samples from PD Broth were swabbed on the agar surface of the four potato dextrose agar plates, separately. Small piece of nanofilm were placed on the agar surface of the four PDA plates on treated part and small piece of 8% PVA film were placed on the agar surface of the four potato dextrose agar plates on control part. Plates were incubated at 28°C for 48 hours. Plates were observed for the formation of zones of inhibition.

Evaluating Food preservation property of PVA-ZnO nanofilm composite

After successfully evaluating the antimicrobial activity of the nanofilm, it was wrapped into randomly fresh selected fruits and vegetables and also 8% PVA film was wrapped into fresh fruits and vegetables and left undisturbed until visible deterioration was evident.

RESULT & DISCUSSION

ZnO NPs synthesis

The frequency of ZnO NPs formation in pomegranate peel extract is shown in Fig 1. Using a UV spectrophotometer, the stability of the synthesis was analyzed over the course of five days. According to the concluded record of *Shamhari et al*. ZnO NPs wavelength data of

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355-380 nm, the acquired value for ZnO NPs is comparable to the wavelength range of improved UV absorption that lies within that range. Pomegranate was chosen as the sample for the antimicrobial activity process as it showed a better peak of UV absorption that fits within the range of ZnO NPs with the approximate maximum levels of stability.

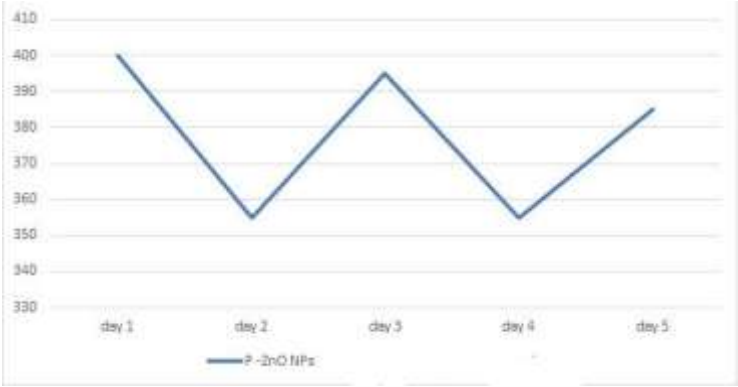

Fig1 Formation of ZnO nanoparticles

Antimicrobial activity of ZnO NPs

Both of the antibacterial and antifungal activities were performed to investigate the antimicrobial properties of ZnO NPs synthesised in pomegranate peel extract. The ZnO NPs was reported to have antibacterial and antifungal properties by *Janaki et al*. The resultant zone lengths were measured.

Antibacterial activity

The inhibition zones were formed depicting the significant level of antibacterial properties of ZnO NPs in both Gram positive (*Bacillus subtilis*) and Gram negative (*Pseudomonas aeruginosa*) bacteria (Fig 2). Both gram positive and gram negative bacteria are thought to be especially susceptible to ZnO NPs antibacterial properties (Sharma and Rajput, 2010).

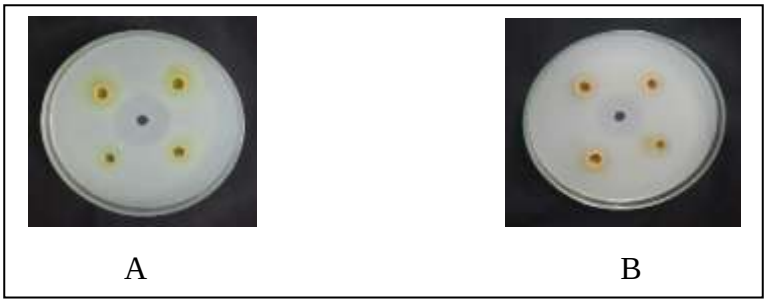

Fig 2 Zone of inhibition shown by ZnO NPs on *P. aeruginosa* (A) and *B. subtilis* (B)

Table 1 Antibacterial activity of ZnO biofilm

Treatment	Zone of inhibition				
	Control (Gentamycin) (20 µl)	ZnO biofilm (µl)			
		25	50	75	100
B. subtilis	20	6	9	10	11
P. aeruginosa	23	8	9	11	12

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Table1 depicts ZnO NPs synthesised in pomegranate peel sample showing wider inhibition zones on higher concentrations compared to lower concentrations. Bhuyan and Mishra, (2015) reported another investigation, suggesting that the concentration of NPs directly correlates with the antibacterial activity of green synthesised ZnO NPs.

Antifungal activity

The antifungal properties of ZnO NPs were evaluated with four isolated fungi (Fig 3). The resultant zone evidences the antifungal efficiency. According to Sharma et al., (2010), it is probable that the breach of the fungal cell membrane will cause a decrease in the enzymatic activity of the fungus, which will make the nanoparticles resistant to the growth.

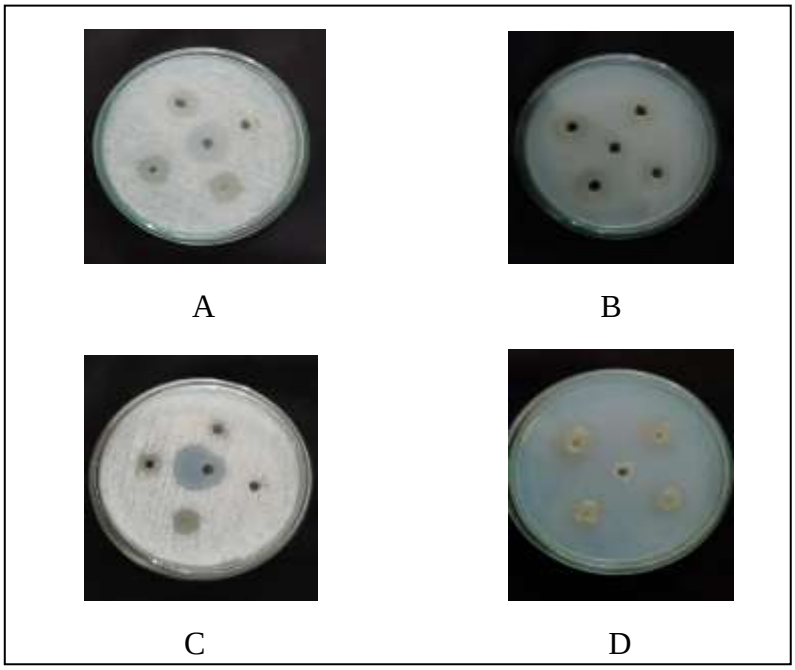

Fig 3 Zone of inhibition shown by ZnO NPs on *Aspergillus sp* (A) , *Diplodia sp* (B) *Colletotrichum sp* (C) and *Botrytis sp* (D)

Table 2 and Table 3 depicts higher the concentration higher the zone length, therefore concentration is directly proportional to zone length. The antifungal activity of ZnO NPs is concentration dependent (Sharma and Rajput, 2010).

Table 2 Antifungal activity of ZnO biofilm (Carrot and *Dolichos*)

Treatment	Zone of inhibition				
	Control (clotrimazole20 µl)	ZnO biofilm (µl)			
		25	50	75	100
Aspergillus sp	22	7	11	12	13
Colletotrichum sp	22	--	15	15	17

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Table 3 Antifungal activity of ZnO biofilm (Custard apple and Citrus)

Treatment	Zone of inhibition				
	Control (fluconazole20 µl)	ZnO biofilm (µl)			
		25	50	75	100
Diplodia sp	--	16	17	18	20
Botrytis sp	20	13	14	16	18

Preparation of bio nanofilm

Two durable sheets with medium thickness were fabricated, one of them containing pomegranate synthesised ZnO NPs expressing the anti-microbial activity (B) referred to as the treated and the other one containing only PVP (A) which is the control (Fig 4).

Fig 4 Synthesis of ZnO NPs, Control (A); treated with pomegranate (B)

Antimicrobial activity of bionano film

Antifungal activity of bionano film that will restrain the growth of food borne pathogens is shown in Fig 5.

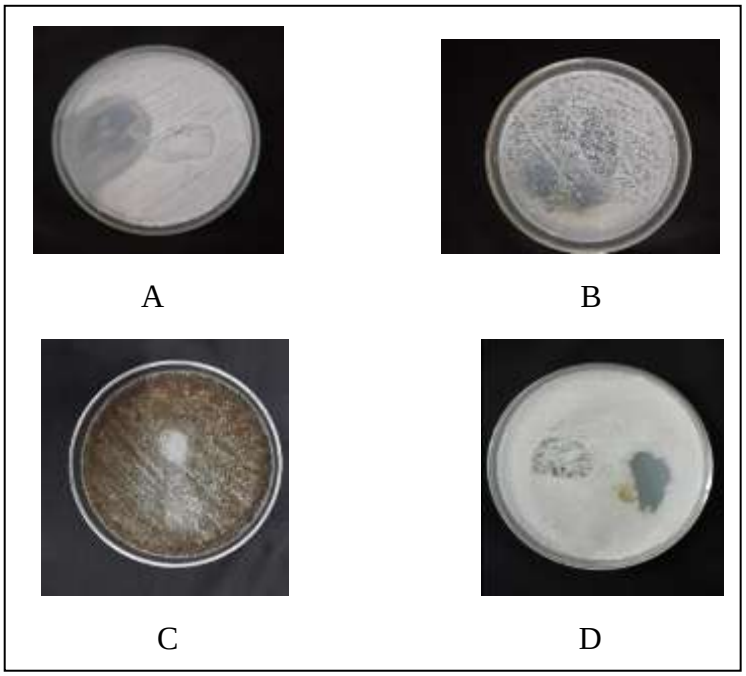

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Fig 5 Antifungal activity of ZnO NPs bio nanofilm, A-*Colletotrichum sp*; B-*Aspergillus sp*; C-*Botrytis sp*; D-*Diplodia sp*

Fig 6 Antibacterial activity of ZnO NPs bio nanofilm; A- *B. subtilis*; B- *P. aeruginosa*

Fig 6 depicts inhibition zones formed along the side of ZnO NPs incorporated bio nano film confirming the antibacterial properties. It was confirmed as the bio nano film restricts the growth of both bacteria and fungus.

Encapsulation by bio nanofilm

The unwrapped vegetable samples and the bio film with just PVA, wrapped vegetable samples were found to be decayed, when compared to bio nano film with ZnO NPs, wrapped vegetable samples remained fresh without decaying for the whole observation period (Fig 7 and Fig 8). The ZnO NPs incorporated bio nano film was a little bit yellowish when compared bio film.

Evaluating Food preservation property of PVA-ZnO nanofilm composite

The ZnO NPs incorporated bio nano film restrains the activity of bacteria and fungus that decays the food samples. The restricted activity of microbes resulted in the increase in shelf-life period of the food samples when compared to normally kept samples.

Fig 7 Effect of ZnO NPs biofilm on tomato

Fig 8 Effect of ZnO NPs biofilm on Coccinia

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CONCLUSION

This study demonstrates the eco-friendly synthesis of ZnO NPs from *Punica granatum* peel extract as well as the antibacterial efficacy of these materials against food-borne infections. Also mentioned are its applications in the production of bio nano films with features that increase shelf life. It was shown that the green ZnO NPs were fungal and bacterial resistant. Moreover, the bio nano film that was fabricated with ZnO NPs added inhibits the growth of bacteria and fungi. The created nanocomposite film could be employed as an effective packaging material to extend the shelf life of vegetables. This makes it clear that this bio nano film may also mark a breakthrough in the widespread disuse of pesticides for the preservation of fruits and vegetables. Our future research may examine the bio nano film's additional characteristics, such as water vapour permeability and tensile strength. Additionally, these possibilities for the bio-nano composite could be explored in upcoming research for the packaging of sensitive food items in the form of carry bags, zip-top bags, etc.

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