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STUDIES ON EFFECT OF NANO FILLER INCORPORATED (HYDROXYPROPYL) METHYL CELLULOSE (HPMC) POLYMER FILMS ON POST-HARVEST MANGO *(MANGIFERA INDICA* L.*)* RIPENING

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ABSTRACT

***Aim**: To study the effect of carbon-coated cobalt oxide incorporated (hydroxypropl) methyl cellulose (HPMC) nano filler films on post-harvest ripening of Mangifera indica L. **Methods:** The carbon-coated cobalt oxide nano fillers were synthesised by conventional sol-gel technique employing metal sulphate as oxidant and urea as fuel with 4.3g of yield. The synthesized nano fillers were efficiently intercalated into the particle stabilising HPMC matrix by solution casting technique and the composite obtained were characterised by UV/visible absorbance and fluorescence emission studies. **Results:** A steady increase in absorbance intensity was observed with increasing nano filler content in the films. Further, a high intense fluorescence peak at 420nm established the presence of carbon-coated cobalt oxide in the polymeric matrix. The nano filler films were found to exhibit significant effect on delaying the ripening process of Mangifera indica L thus enhancing the shelf life of the post-harvest fruit. The solution casted films with varied concentration of filler contents were then evaluated for their antimicrobial efficacy using disc diffusion technique to study their effect on food-borne diseases and poisoning. Significant antimicrobial activity was observed at 66µg of the nano filler against E.coli, B. subtilis, S. typhi and C. albicans. **Conclusion:** It is evident from the observation made in the present study that the carbon-coated cobalt oxide incorporated Hydroxy propyl methyl cellulose (HPMC) nano filler films is beneficial to the shelf life of post-harvest mangoes and significantly inhibit food borne disease causing microorganisms.*

KEY WORDS

(hydroxypropl) methyl cellulose, Mangifera indica L, antimicrobial efficacy, nano filler films, postharvest, carbon-coated cobalt oxide.

INTRODUCTION

Production of fruits and vegetables is a significant economic drive of a region. It plays vital role in food sustainability in many developing and developed countries[1]. However, post-harvest losses of fruits and vegetables are serious problem referring to quantity and quality of the produce. Some estimates suggest that about 30–40% of fruit and vegetables are lost or rejected by the consumer. In this regard, post-harvest management is recognized as one of the important areas requiring lot of attention. Post-harvest management is a set of post- production practices that includes: cleaning, washing, selection, grading, disinfection, drying, packing and storage. These eliminate undesirable elements and improve product appearance, as well as ensuring that the product complies with established quality standards for fresh and processed products. There are several post-harvest management strategies, of which chemical fumigation is widely used. Many of the post-harvest chemical fumigants currently in use, such as, ethylene dibromide (EDB), methyl bromide (MB) and ethylene oxide (ETO) are either banned or to be phased out in view of their harmful effects on human health and environment. Therefore, satisfactory long-term storage of fruits and vegetables may be at risk as traditional fumigants are phased out[2]. There is an urgent need to develop technologies to overcome post-harvest losses of fruits. One way of achieving this could be by developing feasible technology to extend the post-harvest shelf life. Similarly, there have been increasing concerns over food-borne diseases and poisoning by pathogenic microorganisms.

Mango *(Mangifera indica* L.*)*, is one of the most important and widely cultivated fruits of the tropical world. It is a seasonal fruit believed to have originated in the sub-Himalayan plains of Indian subcontinent. Botanically, this exotic fruit belongs to the family of Anacardiaceae*,* is rich source of Vitamins A, C and D. Mangoes also contains essential vitamins and dietary minerals such as vitamins A, B, B6, C, E and K and essential nutrients such as potassium, copper and 17 amino acids in good levels[3]. There are several studies reported on various methods employed to extend shelf life or reduce post-harvest losses of the mango fruit, like irradiation[4] and processing into jams[5]. However, little success has been achieved till date.

Polymers are wonderful materials attributed to their extraordinary combination of properties, low weight and ease of processing. Although these materials can be employed without any fillers, they are often loaded with inorganic fillers either to reduce cost or to improve the performance that is suitable for multifunctional applications[6].

There are different types of polymers, and among those water-soluble polymers are another important type of biodegradable polymers such as polyvinyl alcohol (PVA), polyvinyl pyrrolidone (PVP), polyethylene oxide (PEO) and (hydroxypropyl)methyl cellulose (HPMC). Now-a-days, these eco-friendly water-soluble polymers are increasingly gaining importance as they are easy to process, non-inflammable, easily available and more environmentally friendly. Polymer nanocomposites made out of such eco-friendly polymers are currently considered as the most promising material that can replace many synthetic materials currently being used. Nano particle research is currently an area of intense scientific interest due to a wide variety of potential applications. The carbon coating of cobalt nanoparticles are biologically inert and the carbon surface seems to be very effective for physical adsorption of molecules. In addition, the carbon layers isolate the particles from each other, thus avoiding the problems caused by interactions between magnetic particles, as well preventing bare metal nanoparticles from oxidation or degradation[7,8]. The present study was to investigate the effect of carbon-coated cobalt oxide incorporated (hydroxypropyl) methyl cellulose (HPMC) nano filler films on shelf life of post-harvest mangoes and food borne disease causing microorganisms.

MATERIALS AND METHODS

Collection and authentication of the fruit

The fresh Alphonso mangoes were collected directly from the horticulture farm at Yelchanahalli in the district of Mysore, Karnataka during the months of March and April. The mangoes were collected in such a way that, they retain their stalk to avoid an open space for the easy entry of microorganisms and other pollutants.

Hot water treatment

Mangoes after collection were immediately given hot water treatment at 50^0C for 5min and then mangoes were air dried to drain off any excess water.

Synthesis of carbon-coated cobalt oxide nanoparticles

The carbon-coated cobalt oxide nano fillers were synthesized according to earlier methods reported[9] with modifications by conventional sol gel technique employing metal sulphate as oxidant and urea as fuel. 0.32g of cobalt sulphate was dissolved in 100ml of distilled water to which 0.43g of urea was added, resultant solution was subjected to stirring at 40°C. Then 15ml of 0.5M ammonium hydroxide was added drop wise, resultant hydroxides were then filtered, washed with distilled water, and then dried at temperature of 60°C to obtain desired nanoparticles.

Preparation of carbon-coated cobalt oxide nanoparticles incorporated HPMC polymer films

4.75g of HPMC was weighed and transferred to 500ml beaker and 400 ml of distilled water was added. The solution was continuously stirred until HPMC dissolved and formed viscous gel. Then to the watch glass, 0.0009g of carbon-coated cobalt nanoparticles, 0.5 ml of distilled water was added to dissolve properly and transferred to 50ml of HPMC viscous gel formed earlier.

The same procedure was followed for other concentrations of carbon-coated cobalt nano particle. The film casting moulds were prepared using the hydrophobic (HPMC is hydrophilic in nature hence it does not bind to the sheet) transparent polymer sheet. Then different concentrations of nanoparticles incorporated HPMC gel is poured into the different moulds. These moulds were incubated for 2-3 days at 27±2^{0}C.

Antimicrobial activity by disc diffusion method

The antimicrobial activity of carbon-coated cobalt oxide nanoparticles incorporated HPMC polymer films against *E.coli, B. subtilis, S. typhi and C. albicans* was evaluated by disc diffusion method[10]. The nutrient agar media was prepared by dissolving 0.3% beef extract, 0.3% yeast extract, 0.5% peptone, 0.5%NaCl and 1.5%agar in 1liter of distilled water. The discs were prepared using sterile Whatmann No.1 filter paper and it was impregnated into the agar plates and the solution casted films with varied concentration of filler contents and standard antibiotic were added to the discs, a blank disc loaded without any test compound was regarded as control. For each treatment 10 replicates were maintained. The plates were incubated at 37^{0}C for 24hr and the resulting zone of inhibition was measured by comparing to the control and the standard antibiotic.

Determination of minimal inhibitory concentration (MIC)

The minimum inhibitory concentration was determined by serial dilution in the nutrient agar, with concentrations ranging from 5, 10, 20, 25, 50, 75 and 100 µg/ml. The inoculum was prepared from fresh overnight broth culture in nutrient broth. Plates were incubated for 24hr at 37^{0}C. MIC was recorded as lowest extract concentration demonstrating no visible growth in the broth[11].

Coating of nanoparticles incorporated HPMC polymer gel to unripened mangoes by dipping method

The coating of nanoparticles incorporated HPMC polymer gel to unripe mangoes was done by dipping method as described earlier[12]. The fresh mangoes collected were divided into four groups (group A-D) each containing ten mangoes. Each group of mangoes were then dipped separately in the polymer gel of concentrations 0.8mg (group A), 0.16mg (group B), 0.32mg (group C) and 0.64mg (group D) dissolved in 100 ml of HPMC. A separate 100ml HPMC gel without nanoparticles was used as control. The dipped mangoes were then analysed for any spoilage or microbial contaminants. The photographs of the dipped mangoes were recorded alongside the control and analysed for microbial contaminants. The effect of carbon-coated cobalt oxide nano fillers intercalated HPMC films on the ripening of mangoes were determined by taking 5 sets of mangoes with a control in each set uniformly.

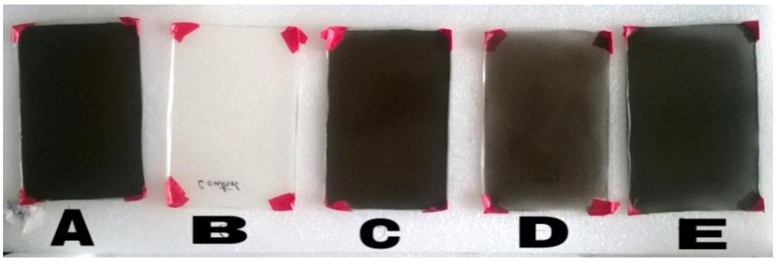
RESULTS AND DISCUSSIONS

The present study is an attempt to investigate the effect of HPMC polymer films incorporated with carbon-coated cobalt oxide nanoparticles on delayed ripening of mangoes. This would eventually result in long term storage without any preferential conditions such as low temperature and humidity. There are several studies which mention the use of HPMC film coating to increase the shelf-life of fruits including mangoes[12]. But the present study involves the use of carbon-coated cobalt oxide nanoparticles into HPMC polymer films which significantly enhance the shelf-life of mangoes by delaying the ripening process. Moreover, these carbon-coated cobalt oxide nanoparticles being inert in nature does not show any untoward activity when incorporated into HPMC polymer films.

A total 4.3g of carbon-coated cobalt oxide nanoparticles were obtained by conventional sol gel method technique using metal sulphides and urea. The sample was obtained in the form of cluster of hairs (Fig 1). Later the sample was crushed using pestle and mortar into fine powder, which was used for incorporating into HPMC polymer films. Different concentrations of nanoparticles incorporated HPMC gel was obtained as mentioned in methods (Fig 2). These polymer films were characterised by UV/visible absorbance and fluorescence emission studies (Fig 3 and Fig 4). The UV/visible absorbance studies revealed the influence of carbon-coated cobalt oxide nano fillers of the UV/visible absorbance nature of HPMC films. Furthermore, the increase in filler content increases the absorbance of HPMC films in accordance with Beer's law. The increase in nano filler content increases the number of photons trapping molecules, thereby increasing the absorbance intensity. The increase in absorbance can be attributed to the formation of cobalt oxide[13]. The highly intense fluorescence peak occurring at around 420nm establishes the presence of carbon-coated cobalt oxide in the nano polymeric matrix. Furthermore, the emission peaks are found to be blue shifted towards higher energies, while on increase in filler content owing to an increase in the defect state in the polymer matrix due to filler intercalation. The fluorescence intensity of nano composites was found to be monotonic with the filler content. The presence of fillers in heterogeneous system such as the polymer nano composites may be assessed by the electronic spectral studies. The UV/visible absorbance alongside emission are one such special technique that establishes the presence of fillers in the polymeric matrix.

Fig 1: Cluster of hairs form of the synthesized carbon-coated cobalt nanoparticles.

Fig 2: Carbon-coated cobalt nanoparticles incorporated HPMC polymer films



Different concentrations of nanoparticles incorporated HPMC polymer gel poured on the moulds. B→represents Control [polymer film without nanoparticles, D→represents 1% [0.8mg], E→represents 2% [0.16mg] and C→represents 4% [0.32mg], and A→represents 8% [0.64mg].

Fig 3: UV/visible absorbance studies of HPMC/carbon-coated cobalt oxide nanoparticles composite films.

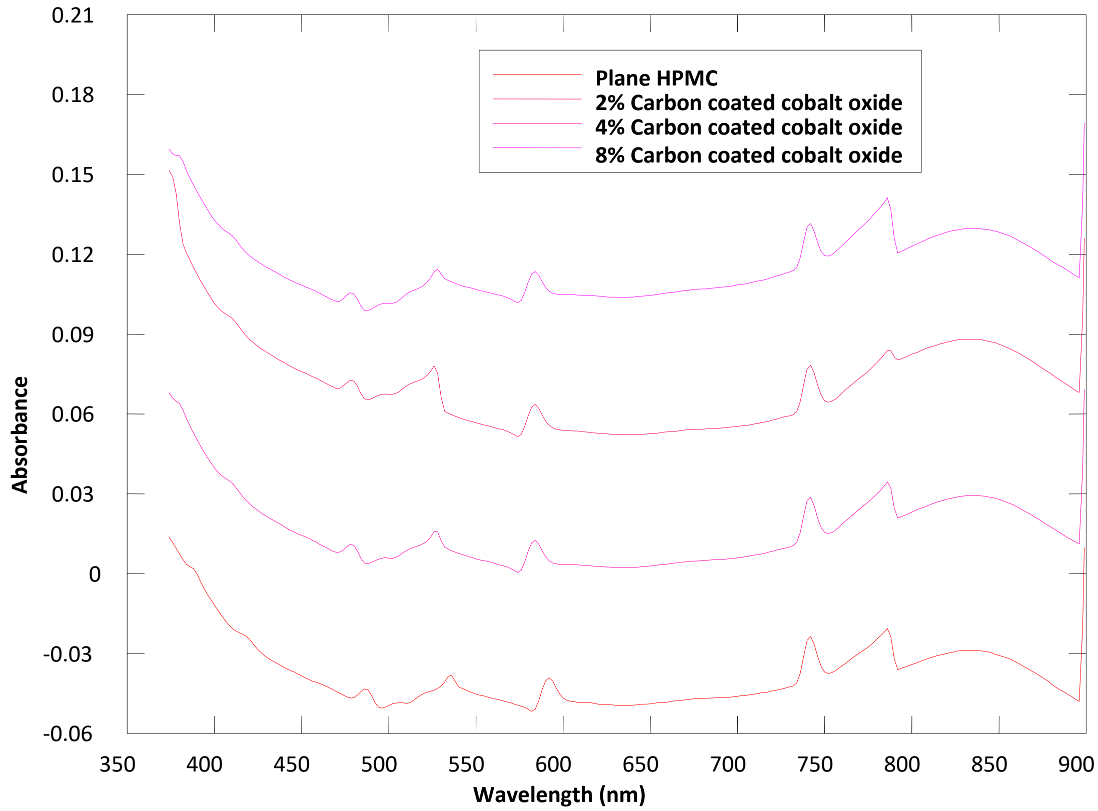


Fig 4: Fluorescence absorbance studies of HPMC/carbon-coated cobalt oxide nano composite films

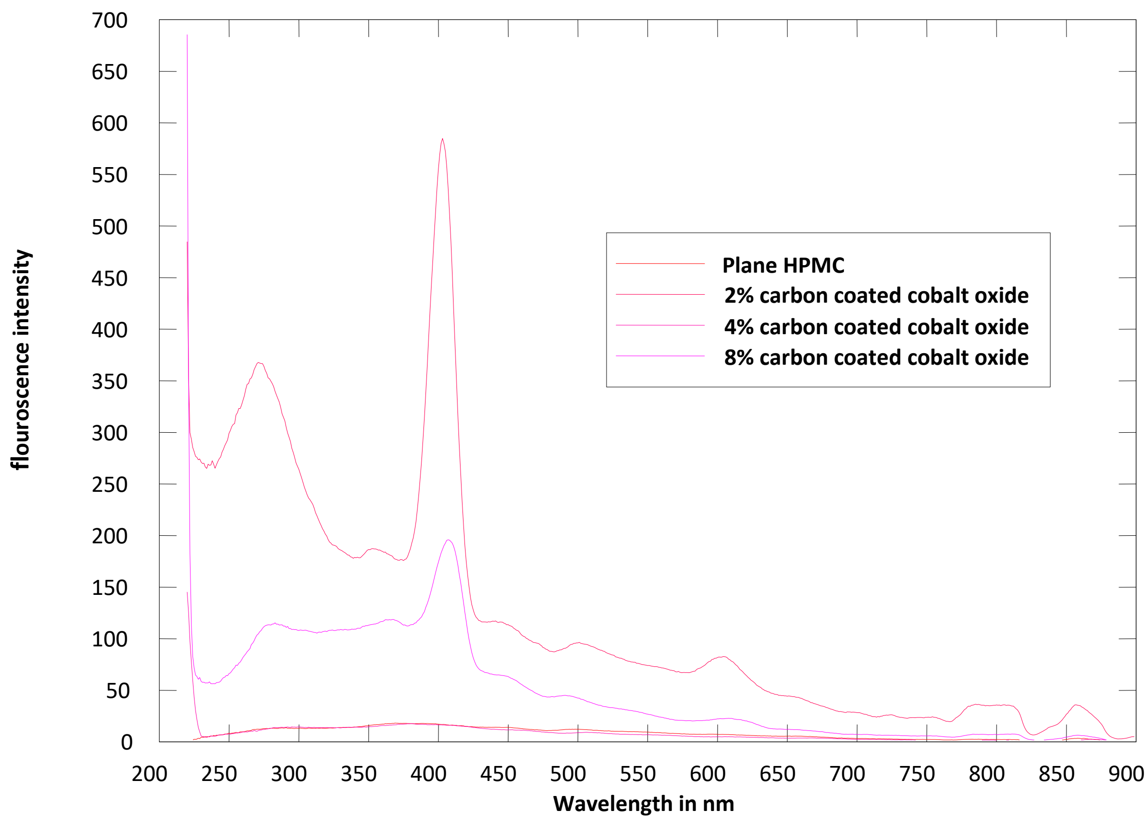


Fig 5: Image of the 5th day of mango dipped in HPMC (A→Test) and control (B) without any treatment.

The antimicrobial property of the fabricated films was evaluated by disc diffusion method. A zone of inhibition was observed around the polymer films to which the particular organism is sensitive. All the organisms were sensitive to the polymer films and hence, found to be having antimicrobial activity. The zone of inhibition was measured and tabulated (Table 1). Furthermore, the zone of inhibition increased with increasing filler content owing to an increase in filler density. The antibacterial ability is dependent on the test sample. It is believed that the metallic nano fillers react with cell walls and cytoplasmic membranes resulting in pits in the cell wall of the bacteria[14]. The MIC was found to be 11.10µg/ml, 10 µg/ml, 16.66 µg/ml, 37.50 µg/ml, and 20.30 µg/ml for *E.coli*, *B. subtilis*, *S. typhi*, *C. albicans* and *S. aureus* respectively.

Table 1: The discs were prepared using sterile Whatmann No.1 filter paper and it was impregnated into the agar plates and the solution casted films with varied concentration of filler contents and standard antibiotic were added to the discs, a blank disc loaded without any test compound was regarded as control. For each treatment 10 replicates were maintained. The plates were incubated at 37⁰C for 24hr and the resulting zone of inhibition was measured by comparing to the control and the standard antibiotic.

Concentration of carbon-coated cobalt nanoparticles incorporated HPMC polymer films in mg	Zone of inhibition in cm			
	E. coli	*B. subtilis*	*S. typhi*	*C. albicans*
0.80	0.50	1.0	0.5	0.4
0.16	0.57	1.2	0.6	0.5
0.32	0.60	1.2	0.6	0.7
0.64	0.68	1.5	0.8	0.7

The delay in ripening of mangoes was determined as mentioned in methods. Each mango which acted as test was checked daily and observation was recorded and shelf life was carefully studied with optimum incubation temperature and time. As we expected the mangoes in the control group started to spoil after the 5th day of dip coating and in contrast the mango dipped in carbon-coated cobalt oxide nanoparticles in HPMC polymer was good and could sustain for few more days (Fig 5).

CONCLUSION

The nano filler films were found to exhibit significant effect on delaying the ripening process of *Mangifera indica* L thus enhancing the shelf life of the post-harvest fruit. Significant antimicrobial activity was observed at 66µg of the nano filler against *E.coli, B. subtilis, S. typhi and C. albicans.* It is evident from the observation made in the present study that the carbon-coated cobalt oxide incorporated (hydroxypropyl) methyl cellulose (HPMC) nano filler films is beneficial to the shelf life of post-harvest mangoes and significantly inhibit food borne disease causing microorganisms.

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