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Characterization of Antifungal Edible Nano-Coating Materials Prepared by Some Waste Peel Extracts

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ABSTRACT

In developing countries, a large amount of a peel waste generated from fruits and vegetables that has led to big nutritional, economic loss and environmental problems. The present study aims to prepare edible nano-coatings, mainly chitosan nanoparticles (CsNps) developed by incorporating fruit peel extracts (pomegranate or banana) as a low-cost natural edible coating material to improve the antifungal activity and functional properties of the edible coating. The synthesized nanoparticles were characterized by Dynamic light scattering (DLS) and zeta potential (ZP), Fourier transform infrared (FTIR) spectroscopy analysis, high-resolution transmission electron microscopy (HR-TEM) and X-ray spectroscopy (XRD). Total phenolic contents (TPC) were 27.79 and 19.78 mg GAE/100g DW for pomegranate peel extract (PmPE) and banana peel extract (BaPE), respectively. While the antioxidant activity (AA) was 87.07 and 79.81% for the same extracts. The antifungal activity of CsNps coating either alone or loaded with fruit waste peel extracts (FPE) of PmPE or BaPE was evaluated against the tested phytopathogenic fungi (*Botrytis cinerea, Alternaria alternata, penicillium digitatum, Aspergillus flavus* and *Aspergillus niger*) at concentrations of 0.2, 0.4 and 0.8 mg/ml (by mycelial radial growth). The results showed that chitosan nanoparticles loaded with pomegranate peel extract (PmPE-CsNps at 0.8 mg/ml) had highly effective (100 %) which achieved completely inhibition against all tested phytopathogenic fungi. The synthesized nano-coatings were non-cytotoxic for the normal human fibroblasts (MRC-5). In conclusion, CsNps loaded with PmPE or BaPE provide an effective, low-cost, edible nanocoating with higher antifungal and antioxidant activities.

Keywords: Edible coating, Nano-Chitosan, fruit peel extract, antifungal activity, FTIR, XRD and cytotoxicity.

1. Introduction

Large quantities of wasted peel are generated from fruit and vegetable-based industries and household kitchen causing environmental serious problems because of the large number of wastes accumulated year after year. The most abundant wastes from processing of fruits and vegetables include pomace, peel, rind and seeds with a high percentage of the residues (around 40-50% of the total discards) which could be considered a good source of bioactive compounds that exhibit antioxidant and antimicrobial activities (Saini *et al*., 2019).

Pomegranate (*Punica granatum* L.) peel are one of the agricultural wastes generating 669 Kg from 1 Ton of fresh pomegranate fruit that contains 78% of peel and 22% of seeds. The pomegranate peel is rich source of various phenolic compounds that showed significant free radicle scavenging, antimicrobial and anti-atherogenic properties (Mohammed 2014; Abinaya and Harin, 2019). Banana (*Musa sapientum L*) peel comprise about 30–40% (w/w) of total weight of fresh banana that contain

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various components such as phenols, alkaloids, flavonoids and carbohydrates that are responsible for their antifungal activity (Ehiowemwenguan *et al*., 2014). Fruits and vegetables peel are novel, natural, ecofriendly and economic sources which can be used in the prevention of diseases caused by phytopathogenic fungi and they can act as a sustainable approach to improve health via food containing health-enhancing substances (Sagar *et al*., 2018).

Phytopathogenic fungi are the most common cause of plants diseases, they are widespread and very destructive to both plants and humans. During the postharvest stages, up to 25 and 50% of fruit and vegetables total production in industrialized and developing countries were lost due to fungal pathogens like *Pencillium digitatum, Altenaria alternata, Aspergillus flavus, Aspergillus niger and Botrytis cinerea* (Li Destri Nicosa *et al*., 2016).

Several methods have been used to reduce postharvest fungal spoilage, including cold storage in conventional controlled atmospheres, biological control (Wang *et al*., 2008), use of ionizing radiation (Charles *et al*., 2009), heat treatments (Zhong *et al*., 2010) and synthetic chemical fungicides. But because of their toxicity, fungi resistance and their negative impact on both the environment and human health, there is a worldwide trend to explore new alternatives. Thus, can control postharvest pathogenic diseases, giving priority to methods that reduce disease incidence and avoid negative and side effects on human health (Oliveira *et al*., 2012).

Plant extracts have proved to be good antimicrobial agents, their use for maintaining fruit quality and reducing fungal decay is often limited by application costs, reduced and inconsistent efficacy (Bautista-Banos *et al*., 2013). The use of edible films and coatings is a new environmentally-friendly alternative technology used to preserve the postharvest quality of fruits and vegetables. They provide a semi-permeable barrier to enhance food quality, safety, stability and the mechanical handling properties (Dhall, 2013). Edible films and coatings, mainly constituted by starch, cellulose derivatives, chitosan/chitin, gums, proteins (animal or vegetable) and lipids to replace commonly used waxes to extend the shelf-life of fruits, reduce moisture losses and eventually incorporate antimicrobial food additives (Aloui *et al*., 2014 and Zhang *et al*., 2016). The incorporation of these natural compounds into edible coating formulations can be an effective approach to control fruit postharvest decay by lowering the diffusion processes and maintaining high concentrations of active molecules at the fruit surface.

Chitosan is a linear polysaccharide with cationic nature and high potential to incorporate natural ingredients and approved for GRAS (General Recognition As Safe). Chitosan has some advantages, such as non-toxicity, biocompatibility, and antimicrobial properties (Keawchaoon and Yoksan, 2011). Chitosan nanoparticles (CsNps) are natural materials with excellent physicochemical properties can be prepared from chitosan through ionic gelation method which is one of the most preferred preparation methods (Oh *et al*., 2019**)**. CsNps provide the advantage of carrying natural extracts due to their higher surface-to-volume ratio. It reinforces the functionality and compatibility of the nanoparticles which shown antifungal abilities against wide varieties of microorganisms than chitosan particles (Detsi *et al*., 2020).

Few researches investigated extracts from pomegranate peel as natural inhibitors for plant pathogenic bacteria and fungi (Romeo *et al*., 2015), including *Penicillium digitatum* (Kharchoufi *et al*., 2018). Pomegranate and banana peel extracts were re-used to develop active films with high scavenging properties, antimicrobial and antimutagenic properties as well as, increasing their water vapor permeability (Kumar *et al*., 2021).

The present investigation focuses on evaluating the most effective, low-cost, antifungal, natural edible nano-coating with higher antioxidant activity to be further used in preservation of fruits and vegetables.

2. Material and Methods

2.1 Materials.

Fresh pomegranate (*Punica granatum* L*)* "Manfaloty" and banana (*Musa sapientum L*) were obtained from the Horticultural Research Institute, Agriculture Research Centre, Giza, Egypt. Low-molecular weight (LMW, MW=100-300 KDa) chitosan powder with 75–85% degree of deacetylation was purchased from Acros-organic (USA). Sodium triphosphate (STPP) and Sodium hydroxide (NaoH) were obtained from Sigma-Aldrich (USA). Acetic acid was obtained from Merck (Germany).

Normal human fibroblast cells (MRC-5) were purchased from American Type Culture Collection (ATTC).

2.2. Methods

2.2.1. Preparation of fruit peel extracts (FPE)

Pomegranate and banana fruits were freshly obtained and washed in running tap water followed by distilled water. The fruits peeled manually and the peels were cut into small pieces then dried in an air circulatory tray drier (CE3G-2, USA) at 50 °C for 24 hrs. Dried peels were powdered in a heavy-duty kitchen grinder (Moulinex grinder, 800 w, France) into a fine powder and stored in air-tight bottles. The powder (20g) was mixed with 100 ml distilled water at room temperature for 30 min with a continuous stirring. The clear extract was centrifuged at 5,000 RCF for 10 min at 5 °C using refrigerated centrifuge (3-18 Ks, sigma, Germany), then filter sterilized through a sterilized Whatman No.1 filter paper (Naveena *et al*., 2008).

2.2.2. Preparation of chitosan nanoparticles (CsNps) loaded with FPE (Fruit Peel Extracts)

Chitosan nanoparticles (CsNps) were prepared using the ionotropic gelation method of chitosan (Cs) with sodium tripolyphosphate (STPP) as a crosslinking agent. The method reported by Sreekumar *et al*., (2018) was applied with some modifications as follows: chitosan solution was prepared at concentrations of 0.2, 0.4 and 0.8 % w/v by dissolving Cs in either 1% (v/v) acetic acid solution in deionized water under magnetic stirring (800 RCF). The pH value was adjusted to pH 5.6 with NaOH (0.5 M), followed by constant stirring for 30 min. Then, 0.033 g of STPP (w/v) was added under stirring to form the nanoparticles. After 30 min of reaction, the suspension was centrifuged at 40,000 RCF and 4 °C for 1 hr and the chitosan nanoparticles (CsNps) solution was collected. For preparation of chitosan nanoparticles loaded with pomegranate peel extract (PmPE- CsNps) and chitosan-nanoparticles loaded with banana peel extract (BaPE-CsNps), 0.2 g/100 ml of PmPE and BaPE were, respectively added dropwise. The solution stirred for 30 min., centrifuged (9000 RCF) and the supernatant was taken for subsequent analysis.

2.2.3. Characterization of CsNps, PmPE-CsNps and BaPE-CsNps

The characterization procedures and experiments were performed at the Nanotechnology and Advanced Materials Central Lab. Agricultural Research center, Giza, Egypt.

2.2.3.1. Dynamic Light Scattering (DLS) analysis

The zeta potential, particle size of the synthesized nanoparticles (CsNps, PmPE-CsNps and BaPE-CsNps) were analyzed by photon correlation spectrometry and laser doppler anemometry, respectively using a Zetasizer 3000 HS (Malvern, U.K) at 25◦C in triplicate.

2.2.3.2. High-Resolution Transmission Electron Microscope (TEM)

The morphological and particles sizes of the synthesized nanoparticles of the edible coating solution were examined by using HR-TEM (Tecnai G20, FEI, Netherlands) operated at 120 kV, with maximum magnification of 600×10^3 and a resolution until 0.2 nm. A drop of an aqueous dispersion of the nonmaterial was placed on a carbon-coated copper grid and allowed to dry in air before characterization.

2.2.3.3. Fourier transforms infrared (FTIR) spectral analysis

The structural features (FTIR spectra) of the synthesized nanoparticles of the edible coating solution were performed in FTIR Spectrophotometer (Shimadzu model 8400S) in a range between 400–4000 cm^{-1} using a KBR pellet technique.

2.2.3.4. X-ray diffraction (XRD)

The crystalline structural of the synthesized nanoparticles of the edible coating solution were examined by using X-ray diffractometer (XRD, X'Pert Pro, Pan Alytical, Netherlands) equipped with Cu Kα radiation source (λ = 1.541178 Å).

2.2.4. Determination of total phenolic content (TPC) of pomegranate and banana peel extracts

Total phenolic content (TPC) was determined using Folin-Ciocalteau reagent according to the method described by Sahu and Saxena, (2013). The results were expressed as mg of gallic acid/g of extract.

2.2.5. Determination of antioxidant activity (AA) of pomegranate and banana peel extracts

The antioxidant activity of (AA) samples was determined using 2, 2'-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity according to the colorimetric method of Brand-Williams *et al*. (1995). The percentage inhibition of the DPPH radical in the samples was calculated according to the formula:

$$\text{Inhibition\%} = [(Ac\ (0) - AA\ (t)) / Ac\ (0)] \times 100 \quad (1)$$

Where: Ac (0) is the absorbance of the control at time = 0 min. AA (t) is the absorbance of the antioxidant at 1hr.

2.2.6. Antifungal activity of the edible coating solutions (mycelial radial growth)

The antifungal activity of the edible nano-coating solutions of CsNps, CsNps-PmPE and CsNps-BaPE against five phytopathogenic fungi (*B. cinerea, A. alternate, p. digitatum, A. flavus* and *A. niger*) was determined by mycelial radial growth (Tikhonov *et al*., 2006). The percentage of inhibition was calculated based on percentage inhibition of radial growth (PIRG%) as follows:

$$PIRG\% = [(R1 - R2) / R1] * 100\% \quad (2)$$

Whereby, R1 = radial growth of fungal isolate in control plate, R2 = radial growth of fungal isolate in treatment plate.

2.2.7. Assessment by light microscope

Mycelial samples, taken from the margin of 6 days old colonies grown on each coating solution amended with PDA, were observed under the light microscope (magnification 40×).

2.2.8. Cytotoxicity assay

Cytotoxicity assay of the edible nano-coating solution was evaluated against the normal human fibroblast cells (MRC-5) according to Gomha *et al*., (2015). the number of viable cells and the percentage of viability was calculated as:

$$Viability = [\ (OD_t / OD_c)] \times 100\% \quad (3)$$

Where OD_t is the mean optical density of wells treated with the tested sample and OD_c is the mean optical density of control wells.

The entire experiment was performed in triplicate and data was expressed as percentage of live cells in control and treated wells. The cytotoxic concentration of 50% induced toxicity (IC_{50}) was defined as the concentration which reduced the optical density of treated cells to 50% regarding untreated cells.

2.2.9. Statistical analysis

The statistical analysis was carried out using multi analysis of variance (ANOVA) under the significant level (0.05) using the CoStat (Ver. 6400) statistical program and data were treated as Completely Randomized Design (CRD) according to Steel *et al*., (1997). To ascertain the significant among means of different samples, LSD test was applied.

3. Results and Discussion

3.1. Determination of total phenolic content (TPC) of pomegranate and banana peel extracts

The total phenolic content of PmPE and BaPE were determined as g gallic acid/100 g dried peel and shown in Table (1). PmPE had a higher TPC (37.79 mg gallic acid equivalent (GAE)/100 mg dry weight (DW) than BaPE (27.37 mg GAE/100 g DW). These results are in agreement with the results

of Fawole *et al*., (2012) who found that the TPC of pomegranate commercially grown in South Africa was 29.5 mg GAE/100 g DW. Anal *et al*. (2014) found that TPC of banana peel extract was 35 mg GAE /g extract, respectively.

Table 1: Total phenolic content (TPC) and Antioxidant Activity (AA) of pomegranate and banana peel extract.

Fruit peel extracts	Total phenolic content (mg GAE/100 g DW)	Antioxidant activity (%)
Pomegranate peel extract (PmPE)	37.79[a]	87.07[a]
Banana peel extract (BaPE)	27.37[b]	79.81[b]

Different letters within each column indicate a significant difference.

3.2. Determination of antioxidant activity for pomegranate and banana peel extracts

The antioxidant activity (AA) of PmPE and BaPE were determined and the results are shown in Table (1). The antioxidant activity of PmPE was higher than BaPE, it recorded 87.07 and 79.81%, respectively. These were in direct proportion with the total phenolic content of the same peel extract.

Pomegranate is known to be rich in bioactive compounds such as phenolic compounds and anthocyanins, which are potent antioxidants (Pareek *et al*., 2015). These results indicated that the major component contributing to the antioxidant capacity of pomegranate is punicalagin, which is present in the peels (Morsy *et al*., 2018). These results were in compatible with those reported by Shiban *et al*., (2012) who found that the antioxidant activity as DPPH scavenging free radical activity reaction was clearly related to the total phenolics of juice powder "lyophilized" or peel powder extracts.

3.3. Characterization of CsNps and PmPE-CsNps and BaPE-CsNps

3.3.1. Dynamic Light Scattering (DSL) and zeta potential (ZP) analysis

The particle size distribution of the synthesized nano-coating (CsNps, PmPE-CsNps and BaPE-CsNps) were measured using DSL technique. The average particle size of CsNps was 20.06 nm (Figure 1 A). PmPE-CsNps and BaPE-CsNps were 37.53 and 73.78 nm, respectively (Figure 1C&E). In case of BaPE-CsNps large particles were obtained. The zeta potential measurements were 66.5, 39.0 and 29.8 mV for CsNps, PmPE-CsNps and BaPE-CsNps, respectively (Figure 1B, D&F). CsNps showed the highest (66.5 mV) ZP values, which confirms the formation of spontaneous and stable nanocomplex between chitosan and STPP. The Incorporation of PmPE into these nanoparticles achieved (39.0 mV) ZP of high degree of stability, while the incorporation of BaPE achieved (29.8 mV) ZP of moderate stability.

These results are in agreement with Shetta *et al*. (2019), who reported that ZP values > ±30 mV corresponds to the high stability where ZP of around ±20 mV indicates a moderate or short-term stability, and the ZP values around ±5 mV would result in low stability, and thereby a fast aggregation of nanoparticles.

Table 2: The PDI of the edible nano-coating solutions

Treatment	Poly dispersity index (PDI)
CsNps	0.348
PmPE-CsNps	0.387
BaPE-CsNps	0.411

CsNps, PmPE-CsNps and BaPE-CsNps showed narrow size distribution and low extent PDI recording 0.348, 0.387 and 0.411, respectively (Table 2). The lowest polydispersity index (PDI) is an indicator of the overall uniformity of nanoparticles. Higher PDI values indicate larger or aggregated particles. Lower PDIs correspond to monodispersed and small particles with no aggregation (Clayton *et al*., 2016). Loading of PmPE and BaPE increased their PDIs than the CsNps. These finding is consistent with the obtained results by Nakasato *et al*. (2017).

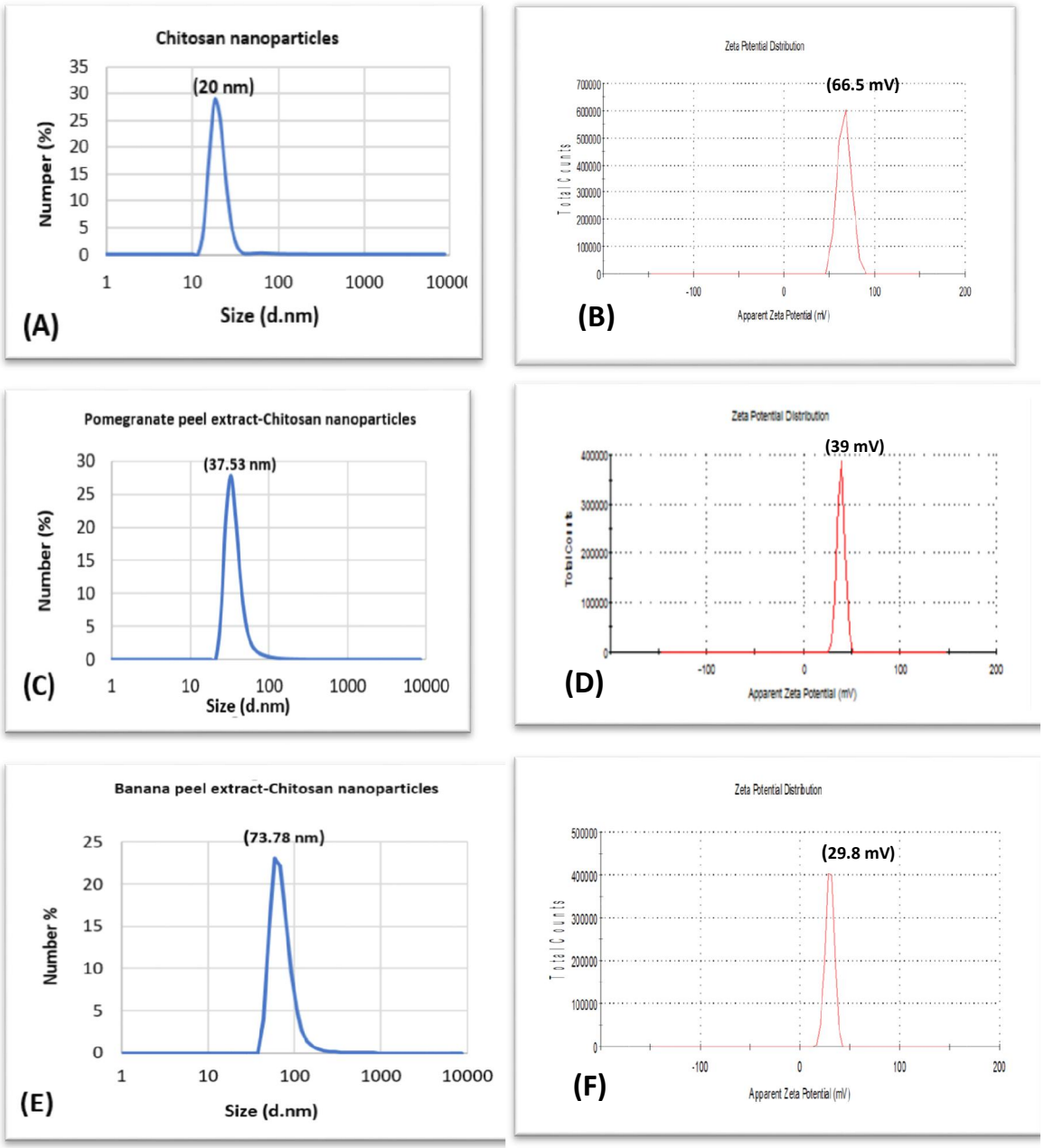

Fig. 1: Particle size distribution A), C), and E) and Zeta potential B), D), and F) of CsNps, CsNps-PmPE and BaPE- CsNps, respectively.

3.3.2. Transmission Electron Microscope (TEM)

The TEM micrograph of CsNps (Figure 2A) presents spherical shaped in range of 20-27nm. The synthesized PmPE-CsNps and BaPE-CsNps had semi spherical shape with a diameter range of 14-26 nm and 23-32 nm, respectively (Figure 2B&C). The size of the synthesized nanoparticles in TEM analysis was much smaller than the size determined by DLS analysis. The size difference between TEM and DLS analysis may be due to different principles and measuring conditions involved in these two techniques. In DLS, the hydrodynamic diameter is measured while in TEM measurement it is in dry state. This result is confirmed by Sathiyabama and Manikanadan, (2016) and Asgari-Targhi *et al*., (2018). The particle size distribution of this study meets the requirement of the nanometer scale, which may provide good interfacial interactions to strengthen the coating material (Lee *et al*., 2018).

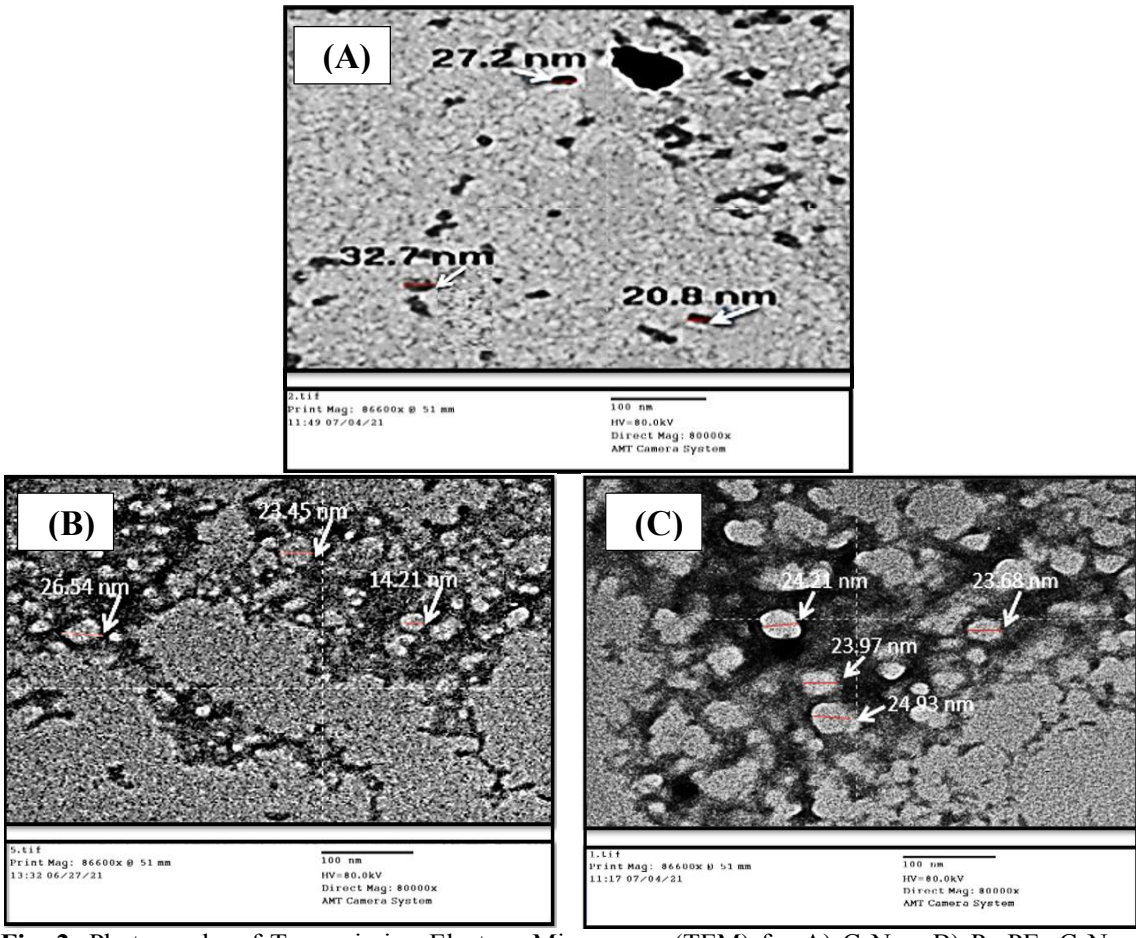

Fig. 2: Photographs of Transmission Electron Microscopy (TEM) for A) CsNps, B) PmPE- CsNps and C) BaPE-CsNps.

3.3.3. Fourier transforms infrared (FTIR) spectral analysis

Fourier transforms infrared (FTIR) analysis is used to characterize the functional groups involved in the edible nano-coating solutions (CsNps, PmPE- CsNps and BaPE-CsNps). FTIR spectra of CsNps showed a characteristic peak at 3441 cm^{-1} (for stretching vibration of OH and NH_2 due to physical interaction with STPP), 1635 cm^{-1} (for C=O bond vibrations in the amide I molecules) and at 1406 cm^{-1} (for C-C aromatic compounds) as in figure (3C). These results are reported by Soltanzadeh *et al*., (2021).

FTIR spectra of pomegranate peel and banana peel extracts are shown in Figure (3A&B). The broad peaks at 3440 and 3437cm^{-1} were ascribed to stretching vibration of N–H and O–H bands for PmPE and BaPE, respectively. They can be possibly found in tannic, ellagic and gallic acids, which had been confirmed in other studies (Ben-Ali *et al*., 2018; Licciardello *et al*., 2018 and Soltanzadeh *et al*., 2021). Small peaks obtained at 2918- 2850 cm^{-1} in PmPE and at 2923-2853 cm^{-1} in BaPE corresponds to stretching of C-H group. Gupta *et al*., (2020) revealed same peaks occur in a range of 2800 to 3000 cm^{-1}. The peaks at ranges 2071-2062, 1639-1633 and 1466-1428 cm^{-1} were Attributed to C= C, C=O (in Amide), and O-H bending, respectively.

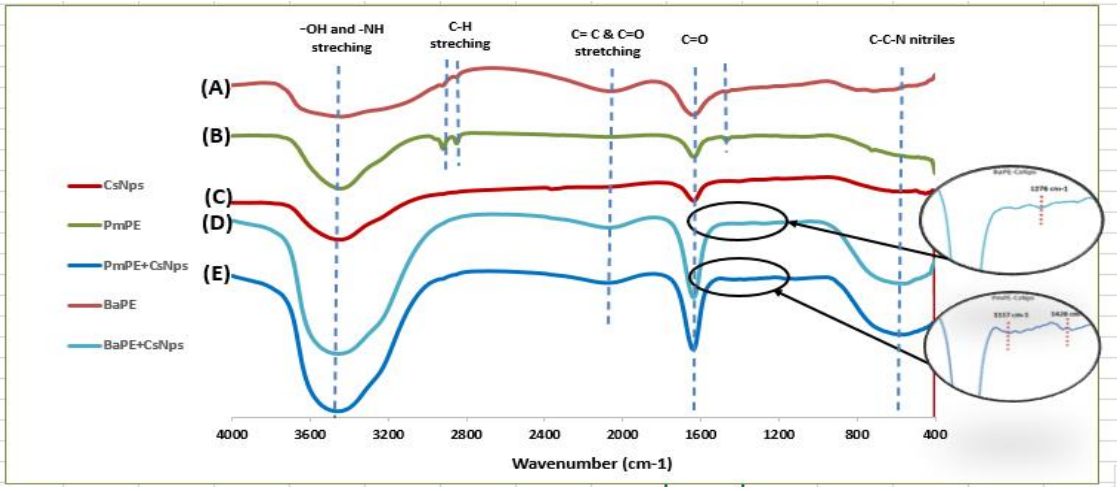

Fig. 3: FTIR spectra of (A) Banana peel extract (BaPE), (B) Pomegranate peel extract (PmPE), (C) Chitosan nanoparticles (CsNps), (D) Chitosan nanoparticles loaded with banana peel extract (BaPE-CsNps) and (E) Chitosan nanoparticles loaded with pomegranate peel extract (PmPE-CsNps).

FTIR spectra of PmPE-CsNps and BaPE-CsNps are shown in Figure (3D&E). The broad peaks that observed at 3460 cm^{-1} reveals the characteristic stretching mode of surface hydroxyl groups and adsorbed water molecules suggesting the possible interaction occurred at OH groups of polyphenols. This can be possibly found in tannic, ellagic and gallic acids, which can be formed in other studies (Ben-Ali *et al*., 2018 and Surendhiran *et al*., 2020). Furthermore, adding of these fruit peels to CsNps resulted in a significant increase in the intensity of C–H stretching bands at 3441 and 1409 cm^{-1} that have been shifted to 3460 and 1428 cm^{-1}, reflecting successful incorporation of PmPE and BaPE into CsNps. Our results were in agreement with those reported in earlier studies (Keawchaoon and Yoksan, 2011; Shetta *et al*., 2019 and Soltanzadeh *et al*., 2021).

Two new absorption peaks appeared in PmPE-CsNps at 1428.99 and 1177.55 cm^{-1} (corresponding to the presence of asymmetric bending of CH_3 and C-O stretching vibration of secondary alcohol, respectively.) A new peak appeared also, in BaPE-CsNps at 1276.64 cm^{-1} (corresponding to CH_3CO streching) confirms the presence of esters and ethers. It was observed that, the disappearance of the absorption peaks at 2361 cm^{-1} (responsible for N-H and C-O stretching) and 612.28 cm^{-1} (responsible for C-H bending). This may be due to the involvement of some functional groups through a weak electrostatic interaction or van der Waals forces (Munagapati *et al*., 2018).

3.3.4. X-ray diffraction (XRD)

X-ray diffraction was used to evaluate the crystalline structure (physical properties) of the synthesized edible nano-coating (CsNps, PmPE-CsNps and BaPE-CsNps). XRD of CsNps was characterized by a broad peak with semi-amorphous structure at range of $2\theta = 19°$ to 22° as shown in Figure (4&5A). The broadening of the peaks is due to the deformation of the crystalline regions by the increased packing of chitosan chains by ionic crosslinking. These results are confirmed by Vijayalakshmi *et al*., (2016). XRD of PmPE and BaPE (Figures 4&5B) showed an amorphous character with a flat broad peak. Diffractograms of PmPE-CsNps and BaPE-CsNps revealed two sharp diffraction peaks at 2θ=23.3°and 19.0°after the PmPE and BaPE incorporation, respectively with CsNps which indicated their crystalline structure (Figures 4&5 C). Similar results that obtained by Gupta *et al*. (2020).

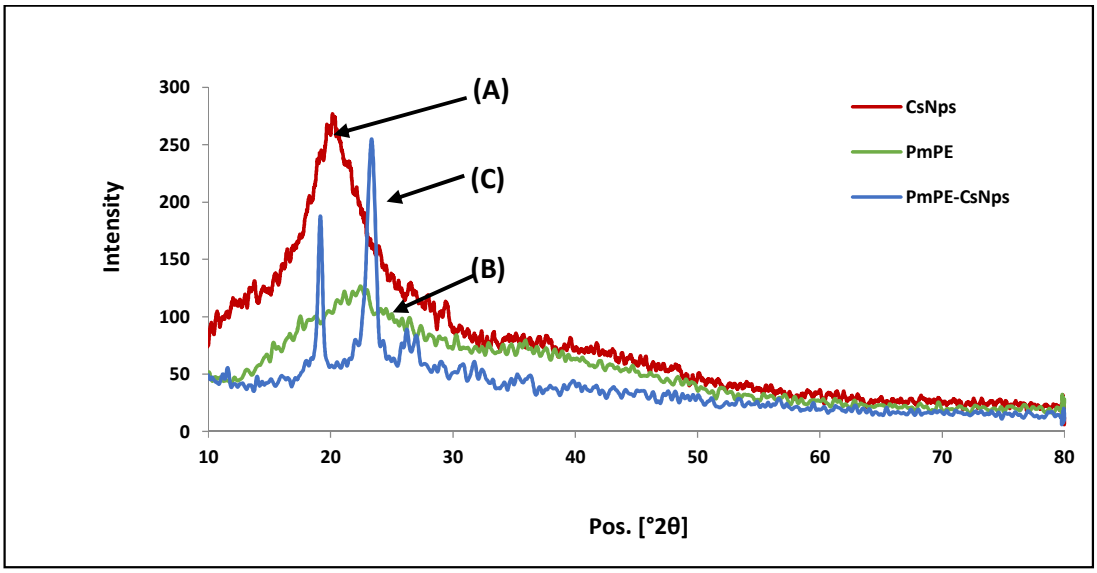

Fig. 4: X-ray diffraction profile of A) CsNps, B) PmPE and C) PmPE-CsNps.

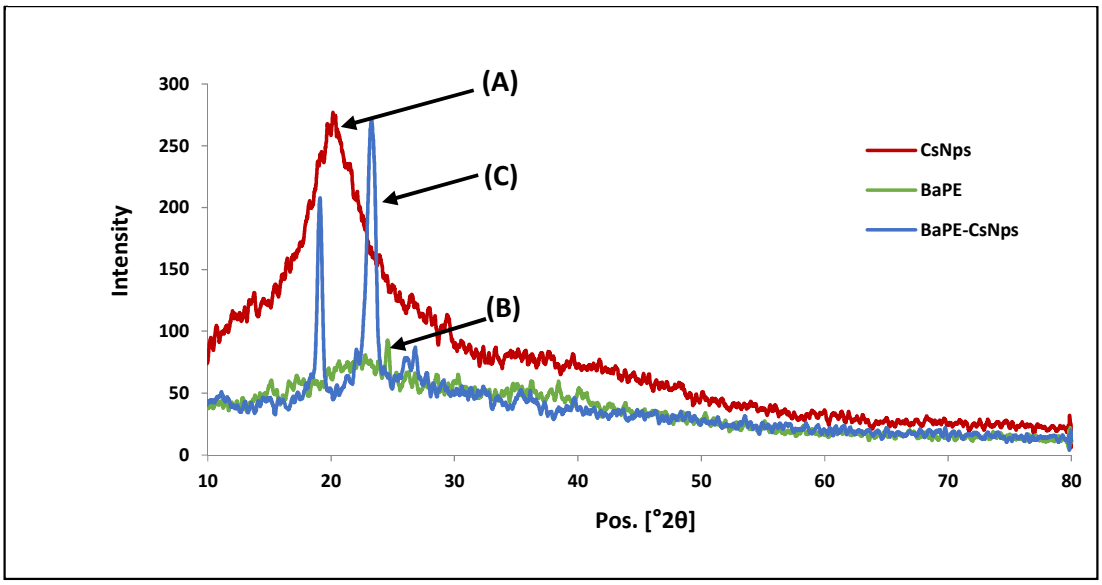

Fig. 5: X-ray diffraction profile of A) CsNps, B) BaPE and C) BaPE-CsNps

3.4. Antifungal activity of the edible coating solutions (mycelial radial growth)

The antifungal activity of different concentrations of edible nano-coating solutions (CsNps, PmPE-CsNps and BaPE-CsNps) was evaluated against some phytopathogenic fungi **(***Penicillium digitatum, Alternaria alternate, Aspergillus flavus, Aspergillus niger* and *Botrytis cinerea)* by the mycelial inhibition of radial growth method (Figure 6). The control cultures (without edible coating) of phytopathogenic fungi reached the edge of culture plates after 7-9 days of incubation.

From the results in Table (3), antifungal power of edible nano-coating solution was concentration dependent and increased by the increasing concentration of extracts. The pomegranate peel extract (PmPE-CsNps) at highest concentration (800 ppm) exhibited the strongest inhibition (100%) against all tested phytopathogenic fungi, compared with CsNps and BaPE-CsNps without any significant differences among them.

The coating solution that enriched with banana peel extract (BaPE-CsNps) was highly effective against only *A. alternate and P. digitatum,* but it was lower than PmPE-CsNps. The highest inhibition

(88.88% and 81.11%) was obtained by BaPE-CsNps against *A. alternate* at 800 and 400 ppm, respectively.

The lowest antifungal inhibition was obtained by CsNps (without peel extract) which recorded 77.77 and 74.34% against *A. alternate* at 400 and 800 ppm, respectively.

Table 3: Percentage inhibition reduction (PIRG %) on growth of the tested phytopathogenic fungi as affected by different concentrations of some edible coating treatments on PDA medium.

Phytopathogenic fungi	(PIRG%) of Edible coating solution (ppm)								
	CsNps			CsNps-PmPE			CsNps-BaPE		
	200	400	800	200	400	800	200	400	800
Alternaria alternate	72.95^{ef} ± 0.53	74.34^{e} ± 0.78	77.77^{d}± 1.88	100.0^{a} ± 0.00	100.0^{a} ± 0.00	100.0^{a} ± 0.00	74.34^{e} ± 0.78	81.11^{d} ± 0.90	88.88^{b} ± 0.91
Penicillium digitatum	64.42^{h} ± 1.74	67.02^{g} ± 1.23	71.11^{f} ± 0.00	81.11^{c} ± 0.90	88.88^{b} ± 0.91	100.0^{a} ± 0.00	71.11^{hi} ± 0.00	73.32^{e} ± 0.91	78.86^{d} ± 0.93
Aspergillus niger	00.00^{n} ± 0.00	27.75^{l} ± 0.90	36.66^{k} ± 0.90	00.00^{n} ± 0.00	66.64^{g} ± 0.90	100.0^{a} ± 0.00	00.00^{n} ± 0.00	00.00^{n} ± 0.00	00.00^{n} ± 0.00
Botrytis cinerea	00.00^{n} ± 0.00	27.75^{l} ± 0.90	36.66^{k} ± 0.90	42.22j ± 0.97	100.0^{a} ± 0.00	100.0^{a} ± 0.00	00.00^{n} ± 0.00	27.75^{l} ± 0.90	44.44^{i} ± 0.90
Aspergillus flavus	00.00^{n} ± 0.00	00.00^{n} 0.00	22.20^{m} ± 1.79	00.00^{n} ± 0.00	28.84^{l} ± 1.76	100.0^{a} ± 0.00	00.00^{n} ± 0.00	00.00^{n} ± 0.00	24.12^{m} ± 4.34

The same letters within the Table for each coating material and treatments are not significantly different at ($P<0.05$). Each value is a mean of three replicates and followed by ± standard deviation.

The antifungal activity of PmPE-CsNps may be due to the active components of the phenolic and flavonoid contents in pomegranate peel beside the antifungal activity of chitosan nanoparticles. Previous studies have shown similar effects in the domain of plant-derived compounds with antimicrobial potential, PPE has been extensively investigated for its free radical scavenging effect and strong antioxidant capacity caused by the high concentration of biologically active components, such as punicalagin, ellagic, gallic and chlorogenic acids (Elsherbiny *et al*., 2016 and Kharchoufi *et al*., 2018). Other researchers investigated that the extracts from pomegranate peel as natural inhibitors for plant pathogenic bacteria and fungi (Endo *et al*., 2010; Romeo *et al*., 2015; Elsherbiny *et al*., 2016), including *Penicillium digitatum* (Li Destri Nicosia *et al*., 2016; Kharchoufi *et al*., 2018), *Fusarium* (El-Mohamedya *et al*., 2019).

The antifungal activity of BaPE-CsNps may be due to the bioactive components as flavonoids and tannins which found in banana peel (Kapadia *et al*., 2015; Pereira and Maraschin, 2015 and Franco *et al*., 2016).

The mechanism of antifungal activity of nanocoating (CsNps, PmPE-CsNps and BaPE-CsNps) could be done by disrupting the cell membrane permeability. Furthermore, the smaller size of nanocoating material allows it to be absorbed into fungal cell thereby disrupting the membrane integrity (Yien *et al.,* 2012). This will inhibit the synthesis of mRNA and, thus, affect the production of essential proteins and enzymes (Ling *et al*., 2012).

Assessment by light microscope

The Microscopic observations of phytopathogenic fungi treated with edible nano-coating solution (CsNps, PmPE-CsNps and BaPE-CsNps) are shown in Figure (6). It was observed that all fungal cultures showed abnormal cells and cellular disorganization in presence of nano-coating solution. These changes included mycelial swelling, abnormal shapes, excessive branching and hyphal size reduction, cytoplasm aggregation, dissolution of protoplasm, large vesicles, or empty cells devoid of cytoplasm in the mycelium. Similar effects were reported by Liu *et al*., (2012) and Oliveira *et al*., (2012) for *Botrytis cinerea, Alternaria alternata* and *Penicillium expansum.*

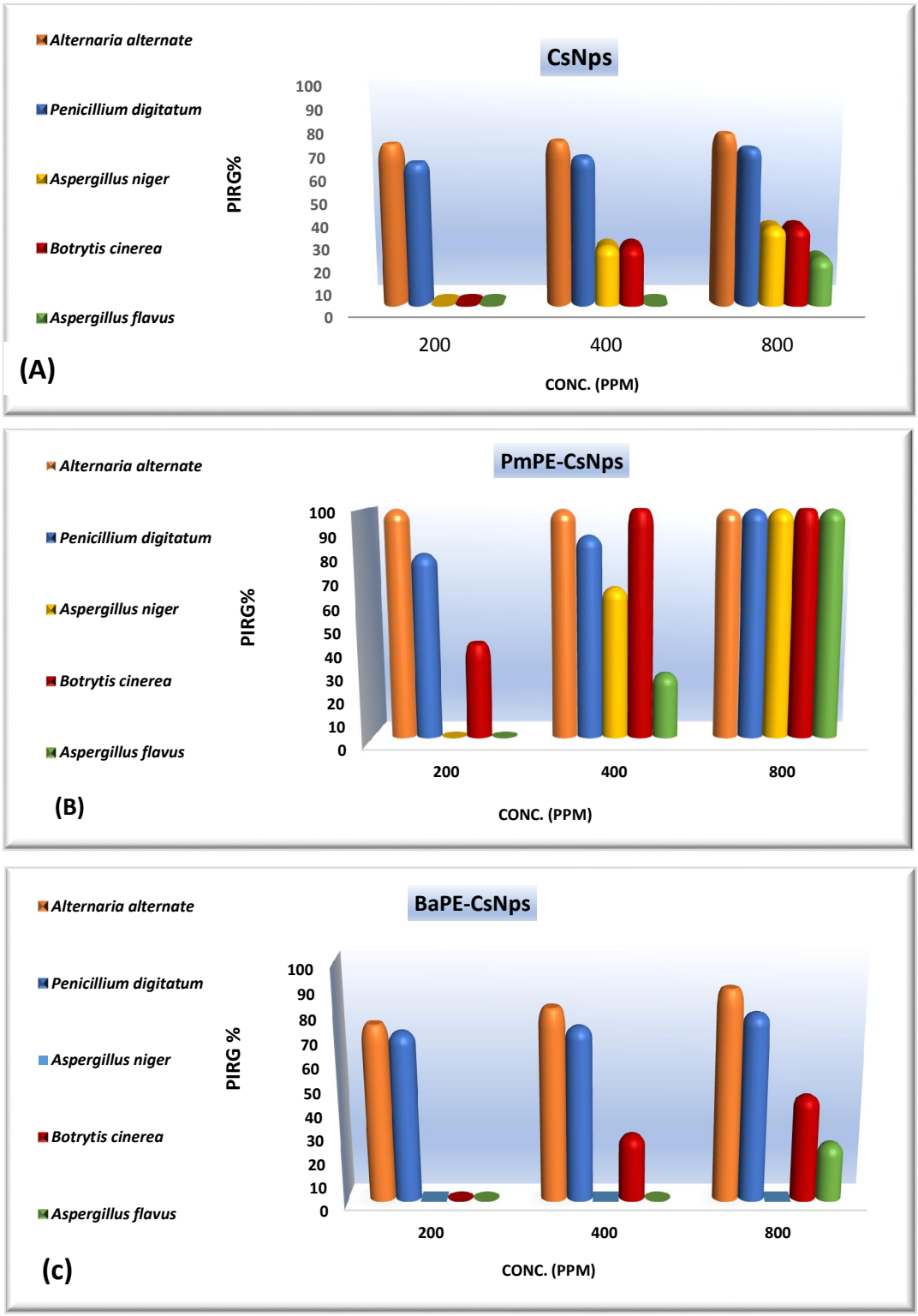

Fig. 6: Antifungal activity of edible nano-coating solution (A) CsNps, (B) PmPE-CsNps and (C) BaPE-CsNps against some phytopathogenic fungi (PIRG % means the percentage inhibition reduction).

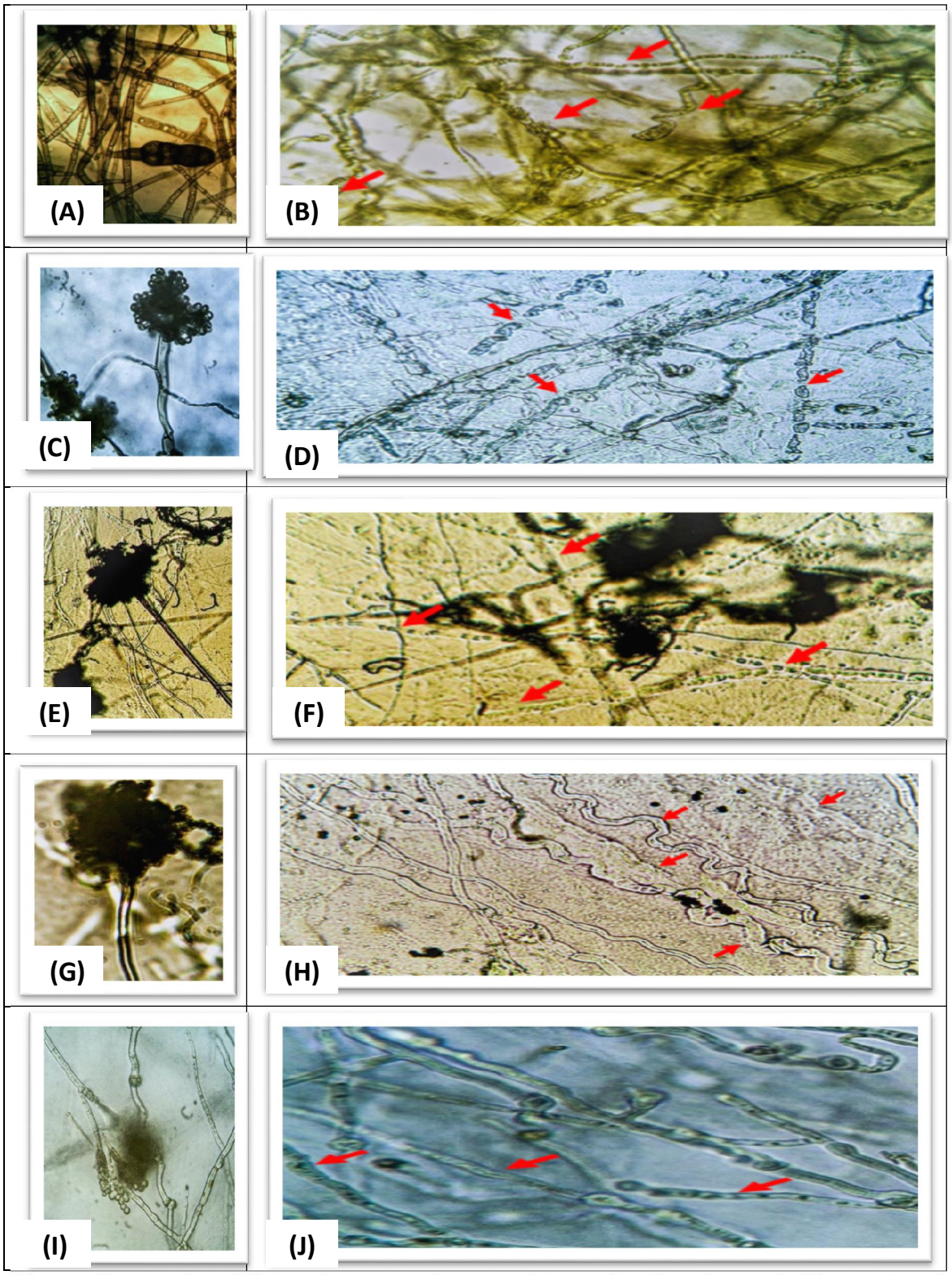

Fig. 6: Photographs of light microscopy of untreated (control) (A) and treated (B) *mycelia of Alternaria alternata,* untreated (control) (C) and treated (D) *mycelia of Botrytis cinerea*, untreated (control) (E) and treated (F) *mycelia of Aspergillus flavus* untreated (control) (G) and treated (H) *mycelia of Aspergillus niger* treated and untreated (control) (I) and treated (J) *mycelia of Penicillium digitatum* by edible nano-coating solutions.

3.5. Cytotoxicity

The cytotoxicity analysis of the edible nano-coating is essential to confirm the safety of the synthesized nanoparticles. Therefore, the cytotoxicity of the edible nano-coating solution to the normal human fibroblast cell line (MRC-5) was evaluated using MTT assay and the results are shown in Figure (7). It was observed that the IC_{50} values of CsNps, PmPE-CsNps and BaPE-CsNps were 660, 898 and 814 µg/ml, respectively. The results revealed that the synthesized nanoparticles did not render any serious impact to the MRC-5 cells and the incorporation of PmPE or BaPE into CsNps was nontoxic to cells. Previous studies reported no cytotoxicity for CsNps at a concentration less than 1 mg/ml, but this is likely due to the pH value. The present study was conducted at pH 4.8 to evaluate the acidity effect on the biocompatibility of CsNps; however, in most studies, the pH values used were 5.5–7.0. The cytotoxicity of cationic polymers depends on their surface charge. Excess positive charges on the NP surface can associate with functionally impaired cellular components (e.g., cell membranes and intracellular enzymes) (Xiao *et al*., 2017). The obtained data are in agreement with those reported by Chen *et al*., (2013) and Mina, *et al*., (2020).

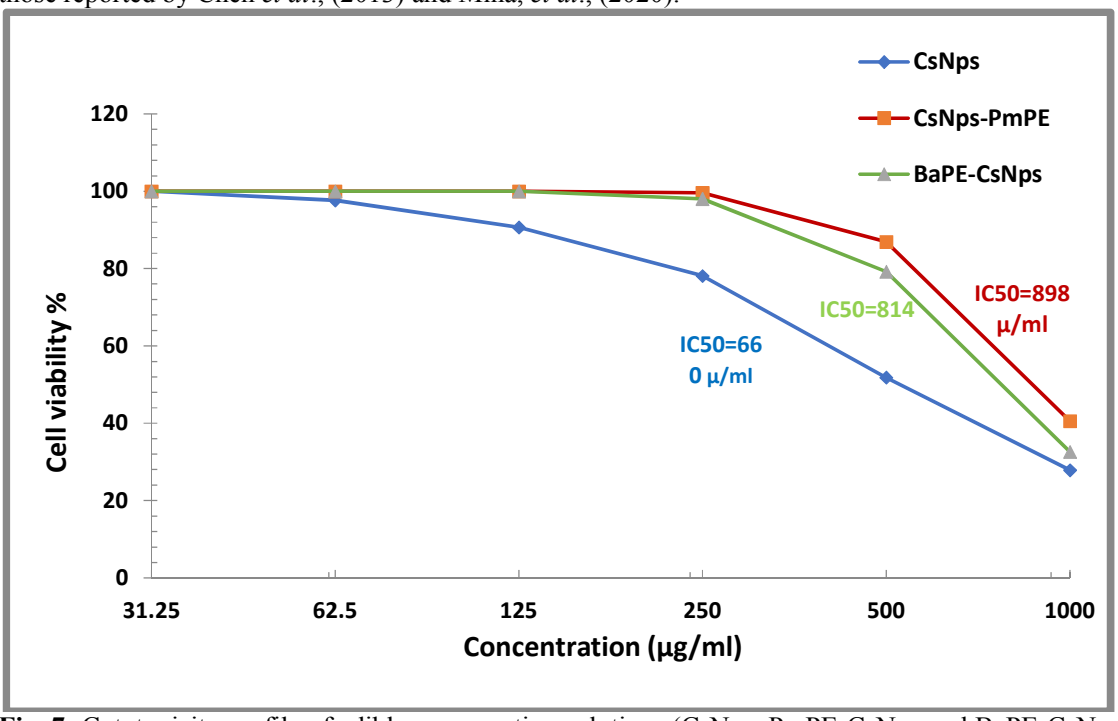

Fig. 7: Cytotoxicity profile of edible nano-coating solutions (CsNps, PmPE-CsNps and BaPE-CsNps) on cell viability of normal human fibroblast cells (MRC-5).

4. Conclusion

Fruits and vegetables processing industry produces a huge number of wastes that are rich in bioactive compounds. These bioactive compounds can be further utilized as natural antioxidant, preservative and anti-fungal agents in different industries. Fruit peel extracts (pomegranate and banana) were successfully incorporated in the edible nano-coating matrices and determined a good level of inhibition (especially PmPE-CsNps) against phytopathogenic fungi. Thus, it could control postharvest decay and manage the environmental issues related to pollution due to accumulation of peel waste. In addition to the volarization of the active components in these peel waste through nano-based coating technology. Further researches should be done to reduce the environmental pollution by extracting the bioactive compounds from fruits and vegetables waste which can further replace the synthetic antioxidant and synthetic preservatives in various industries.

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