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Fungal Growth Physicochemical Properties Inhibition by Novel Zinc Oxide/ Glutinous Tapioca Starch Composite

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ABSTRACT

Mango suffers from numerous diseases throughout its life due to fungal growth. Primarily, mango is infected with anthracnose, a disease caused by *Collectotrichum gloeosporioides*. In the present study, mango fruits were coated with zinc oxide (ZnO) and tapioca starch solution at different concentrations and stored at room temperature for a week. The growth of any black spots on the outer surface of the fruit peel was observed at two-day intervals. The mixed coating material, consisting of ZnO and tapioca starch, inhibited fungal growth. The field-emission scanning microscope (FESEM) results demonstrated that the nanoparticles were uniformly coated on the mango samples. Applying the ZnO coating also effectively maintained the quality attributes and extended the shelf life of the fruits. Consequently, the ZnO and starch coating could be an alternative safe coating technique of protecting mango fruits against anthracnose infection, hence prolonging the freshness and avoiding economic losses during transportation, marketing, and storage.

1. Introduction

Mango is among the prevalent fruits worldwide due to its attractive colour, delicious taste, excellent nutritional properties, and high international commercial value [1][2]. Nevertheless, mango fruits are decayable and mature swiftly after being harvested [3]. Moreover, producers and traders face higher losses from improper handling, packaging, storage, and poor post-harvest management [3]. Accordingly, scientists have been investigating measures to prolong the shelf life of the fruit while maintaining standard quality and flavour [3]. The fungus *Colletotrichum gloeosporides* is responsible for anthracnose, one of the significant post-harvest diseases infecting mango worldwide [4][5]. The disease is asymptomatic [4], requiring fungicide treatments either during pre- or post-harvest to reduce losses [1]. Nevertheless, the employment of fungicides

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is restricted due to health concerns. Furthermore, most mango producers in developing countries could not afford fungicides [6].

Synthetic fungicides are primarily utilized to control post-harvest diseases [7][8]. The chemicals might consist of a combination of mixtures or necessitate the sequential application of separate solutions [9]. Several fungicides have been removed from the market due to possible toxicological risks [1]. Moreover, repeated fungicide employment in packing houses has led to fungicide-resistant pathogens [1]. Recent studies have also revealed the health risks of applying synthetic fungicides to fresh fruits and vegetables pre-consumption, increasing public awareness, hence resulting in the pressure to reduce synthetic fungicides on agricultural products and the environment [1].

Nanotechnology possesses great potential in food security programs that aim to preserve agricultural and food supplies [10]. For example, an essential contribution of nanotechnology to sustainable agriculture products is nano-coating to extend the freshness of fruits and inhibit fungal attacks or growth. Consequently, food packaging with antimicrobial properties has garnered attention due to its ability to arrest or delay the microbiological decay of food products [11]. Antimicrobial substances are incorporated into packaging systems to reduce pathogen contamination risks [12][13]. Reports also documented that antimicrobial packaging improved the safety and quality of food products and enabled reduced preservatives addition [14]. Food packaging antimicrobial actions are based on agents released from the material to the surrounding space, not directly onto the food [14].

Zinc is a trace metal crucial for numerous metalloenzymes in living organisms [15] and zinc oxide (ZnO) is a multifunctional inorganic particle with antimicrobial properties. Moreover, ZnO is recognised as safe by the United States Food and Drug Administration [16] and not harmful to humans [17]. The chemical is also less toxic than silver (Ag), a substance widely employed in the food industry as a zinc supplement [15][18]. Consequently, ZnO nanoparticle applications have significantly risen in the food industry [19]. The ZnO has been incorporated into food can linings to prevent discolouration and spoilage [20]. Several studies have also proposed incorporating ZnO nanoparticles (NPs) into low-density polyethylene (LDPE), polypropylene (PP), and chitosan [20], while some reported the utilisation of chitosan films blended with different components, such as natural extracts or inorganic metal particles, as food packaging [21][22]. Furthermore, Rahman, Mujeeb, and Muraleedharan (2017) proved the efficiency of chitosan-ZnO nanocomposite films in pouches prolonging the shelf life of the raw materials assessed [23]. Nevertheless, the employment of ZnO-starch coating on fruits has yet to be reported. They had similar research but they used polyvinyl chloride films as the binder. Li *et al*. (2011) stated that the decay rate of nano-ZnO-coated PVC film apples was reduced [24].

Commonly, biopolymers are manufactured from existing polymers, including polysaccharides and proteins [25]. Among the polymers, starch, a polysaccharide, is one of the most plentiful biopolymer sources, generally found in oats (wheat, corn, and rice) and tuber plants (tapioca, potato, and sweet potato) [26]. Tapioca starch could be obtained from the cassava plant tuber (Manihot esculenta), a plant available in tropical nations [27]. Tapioca starch is amylose (19%) and amylopectin (81%), which are adequate to deliver the starch-based films developed in the present study [28]. Furthermore, starch-based films are safe, unscented, and colourless, offering potential applications in food packaging [29]. Consequently, the present study selected tapioca starch to deliver the starch-based film due to its accessibility, cost, biodegradability, and edibility [29][30]. Nevertheless, starch-based films possess several disadvantages, including restricted long-term stability and a short time frame of realistic usability brought about by water absorption.

The applications of starch films are constrained as they are brittle and profoundly and quickly dissolvable in water, attributable to the strong intermolecular relationship tendencies between starch chains due to their hygroscopic nature [29]. The water absorption property of starch influences the characteristics of the acquired films, which include poor mechanical properties regarding rigidity, Young's modulus, and prolongation at break [31]. The disadvantages could be reversed by incorporating nanosized molecules or nanofillers to frame the bio-nanocomposite films [32]. Recently, NPs have been utilized considerably in manufacturing nanocomposites. A nanocomposite is a micro-composite comprising common polymers as matrix and NPs as filler. Adding NPs could improve the qualities and functional properties, including boundary capacity, morphology, and mechanical quality of biopolymers.

The ZnO NPs produces positive effects on the mechanical and barrier properties of the nanocomposite films, which is crucial in food wrapping [33]. Nooshin et al. documented that ZnO NPs in active packaging possess the potential of inhibiting fungal growth in bread [33]. This is due to the hygroscopic effect of ZnO NPs and its positive effect on improving water vapour permeability (WVP) of the films. According to Li et al., coating fresh-cut fruits with ZnO NPs is more advantageous than typical packaging. Li *et al*. [24] also recommended ZnO coating to extend the shelf life of fruits and vegetables as it is capable of postponing aging [34]. Several coating techniques have been successfully employed on tropical fruits, such as mango and avocado. In another investigation, the natural taste of mango slices was successfully preserved with a chitosan coating. However, the colour of the chitosan-coated samples did not vary from the blank, contained less water and demonstrated a steady decay [35].

Due to the high consumption of mango in the world, this study has concerns about extending the shelf life of mango by inhibiting microbial activities. Hence, the aim of the present research is to prepare a ZnO NPs-tapioca starch solution that would inhibit fungal growth on mango samples coated with the solution, hence maintaining the quality of the fruits. The purpose of this study also was to determine the effect of physicochemical parameters (pH, Brix, and titratable acidity) on the storage for delay ripening of mango fruit.

2. Experimental Method

ZnO nanopowder <100 nm was purchased from Sigma-Aldrich Chemicals Pvt. Ltd.(USA). Tapioca starch was purchased from the market (Malaysia). The ZnO NP and tapioca starch were ball milled using planetary ball mill, model: PM 200 RESTCH. Total soluble solid of the mango samples coated with the ZnO NPs-tapioca solution was determined with a hand refractometer (Brix 0–32%, Atago, Japan). The surface morphology of mango skin has been characterized using Field Emission Scanning Electron Microscope (FESEM) (JEOL JSM-J600F).

2.1 Preparation of microorganisms

The mould was isolated from mature mango fruits and grown in dextrose agar in this study. The culture was incubated at 30°C for seven days. Subsequently, the culture stock was stored at 4°C.

2.2 Preparation of the ZnO NPs and glutinous tapioca flour

The current study employed commercially available ZnO powder under 100 nm and 99% pure. The powder was milled in a zirconia jar with zirconia balls at a ball-to-powder weight ratio of 14. The mechanical milling was conducted in a horizontal ball mill operated at 500 rpm for half an hour. Similarly, commercial glutinous tapioca flour was milled to obtain the desired nanoparticle-sized powder.

2.3 Preparation of the ZnO NPs with an edible coating solution

First, 50 ml of ZnO NPs containing 1, 1.5, 3, and 5 mg/ml were prepared. Following that, 500 ml of distilled water and starch powder solution were thoroughly mixed into the 1, 3, and 5 mg/ml ZnO NPs stocks. The mixture was then infused with edible coating (13.5 g) and constantly stirred at 100°C for two hours to induce gelatinisation [36]. In contrast, 50 ml of 1.5mg/ml ZnO NPs were only mixed with 500 ml of distilled water.

2.4 Coating and inoculating the mango samples

The mango samples utilized in the current study were washed with distilled water to remove dirt and left to dry at room temperature. Subsequently, each mango was dipped in the gelatinised ZnO NPs-tapioca solutions (1, 3, and 5 mg/ml) for five minutes. A mango was left untreated, the control. The samples were then left to dry for two hours. The previously inoculated mold was sterilized in distilled water and 10 µl was inoculated in wounds on the mango with a sterile needle. Lastly, the samples were incubated in plastic trays for seven days [36].

2.5 Disease incidence and severity

According to previous studies, anthracnose infections require observation every two days [37][38]. The current study calculated the disease incidence (%) according to Equation 1, while the severity of the infection was evaluated based on the index described in Figure 1.

$$Disease\ incidence\ (\%) = \frac{Area\ covered\ with\ black\ spots}{The\ whole\ mango\ epidermis\ area} \times 100\% \quad (1)$$

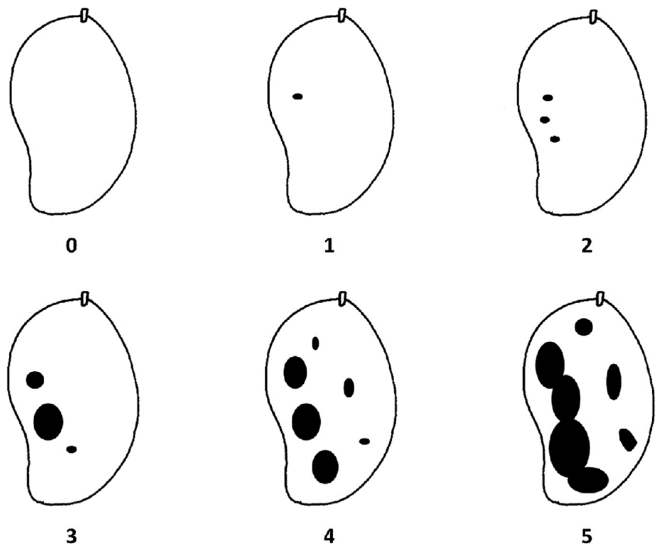


Fig. 1. The severity index of anthracnose infection on the mango samples on a scale of 0 to 5.
Note: 0 = no visible spots, 1 = one dark depressed spot 1–5 mm in diameter, 2 = two to three dark depressed spots 1–5 mm in diameter, 3 = three dark depressed spots over 5 mm in diameter, 4 = more than three dark depressed spots over 5 mm in diameter, and 5 = merged depressed spots on the fruit epidermis

2.6 Weight loss

In the present study, the weight loss of the mango samples was measured every two days. The fruits were weighed before coating with the edible ZnO NPs-tapioca solution and during storage. The weight percentage (w%) was then calculated.

2.7 Total soluble solids (TSS)

The total soluble solids (TSS) concentration of the mango samples coated with the ZnO NPs-tapioca solution was determined with a hand refractometer (Brix 0–32%, Atago, Japan) at room temperature. First, 10 g of mango flesh were homogenised in a blender and filtered. Subsequently, a drop of the sample was placed on the refractometer prism glass to obtain the direct reading. The prism was washed with distilled water and dried with tissue paper for the consequent readings. The readings were expressed in concentration per cent of TTS or ^{o}Brix.

2.8 pH

For pH determination, 10 g of mango flesh was sliced and homogenised with 100 mL distilled water in a blender before filtering the pulp. Subsequently, a pH meter was employed to determine the pH of the juice.

2.9 Titratable acidity (TA)

Titratable acidity (TA) was determined by titrating 100 mL of homogenised 10 g mango flesh with 100 mL of distilled water and 0.1 N sodium hydroxide (NaOH) solution. The solution was shaken vigorously after a few drops of phenolphthalein indicator were added. The solution was then titrated until the end-point appeared to be a permanent pink colour. The titre was recorded as the burette reading. The TA was indicated as the percentage of citric acid and calculated according to Equation 2.

$$TA\ (\%) = \frac{Acidity\ factor \times titre}{Sample} \times 100 \quad (2)$$

where the acidity factor is 0.0064.

2.10 Moisture content (MC)

In the present study, the moisture content (MC) of the samples was assessed from the change in weight of the fruits. The mangoes were oven-dried at 100℃ for 24 hours before weighing with a weight balance.

$$MC\ (\%) = \frac{Initial\ weight - Final\ weight}{Final\ weight} \times 100 \quad (3)$$

2.11 Characterization of the ZnO NPs

The ZnO NPs coating the mango samples was characterized by field-emission scanning electron microscopy (FESEM). The technique allowed morphological characterization of the mango surface. The present study performed top view and cross-section FESEM imaging at magnifications between 300–10 000×.

3. Results and Discussion

Figure 2 displays the appearance of mango skin on the (a) control, (b) coated with ZnO, and (c) coated with ZnO NPs-tapioca solution mango samples viewed through an optical microscope. The uncoated mango exhibited more black spots than the sample coated with ZnO. The diameter of the black spots observed on the ZnO-coated fruits was bigger than those on the ZnO NPs-tapioca-coated samples, 330.77 and 204.54 µm [39], respectively. The observations proved that the ZnO-glutinous tapioca starch possessed antifungal properties, which would enable in vivo antifungal actions against anthracnose pathogens.

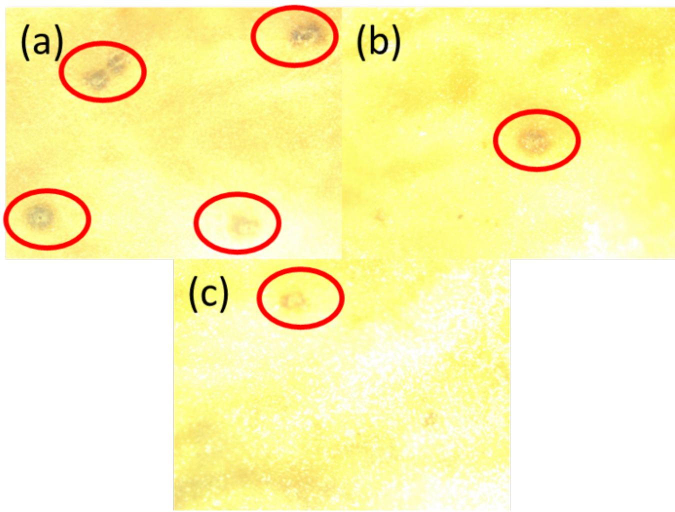
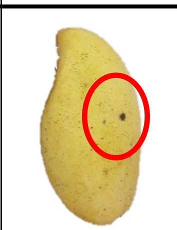
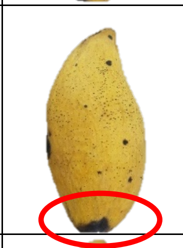
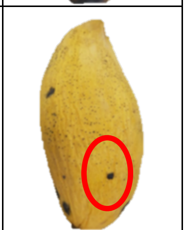


Fig. 2. The appearance of the (a) uncoated, (b) coated with ZnO, and (c) coated with the ZnO NPs-tapioca solution mango samples viewed through an optical microscope

Table 1
The initial black spots (red circles) observed on the uncoated and mangoes coated with different concentrations of ZnO and tapioca flour NPs during the 7-day storage

Sample	Condition on day				
	0	2	4	6	7
Control (uncoated)					
1 mg/ml					
3 mg/ml					
5 mg/ml					

The mango samples in the present study were visually observed for black spots. Table 1 illustrates the black spot perceived on day 7 of storage of the uncoated (control) and mango samples coated with different concentrations of ZnO-tapioca starch NPs. Only the sample coated with the 5 mg/ml ZnO- tapioca starch NPs did not rot after seven days. During the seven-day storage at room temperature, the mango samples in the current study were observed for anthracnose infection. On day 2, the control fruit exhibited disease symptoms, and its severity increased during storage. The sample coated with 1 mg/ml of ZnO and tapioca flour NPs was observed to be diseased on day 6, while the mango coated with 3 mg/ml ZnO-tapioca starch NPs exhibited infection on day 7. No black spots were discerned on the fruit coated with ZnO-tapioca starch NPs at 5 mg/ml. The disease severity percentage of the 5 mg/ml ZnO starch NPs-coated sample was also recorded lower than the uncoated mango. The observation was due to the antimicrobial agents in the ZnO NPs [39]. The results demonstrated that ZnO could retard anthracnose disease in mango fruits.

Figure 3 illustrates the weight loss percentages of the uncoated and coated mango samples during storage. The fruit coated with the 5 mg/ml ZnO-starch NPs recorded the least weight loss during the seven days. Water loss or transpiration is considered a significant factor affecting the storage life and the post-harvest quality of most fruits. In the present study, weight loss increased during storage, and a significantly ($p < 0.05$) higher weight loss percentage was documented by the 1 mg/ml ZnO-starch NPs-coated sample. The fruits coated with 5 mg/ml ZnO-tapioca starch NPs demonstrated reduced weight loss compared to those with 1 and 3 mg/ml coatings. The higher concentration of ZnO–tapioca starch NPs diminished weight loss as the tapioca starch is a polysaccharide, which works as an oxygen and moisture barrier, thus reducing water loss from the fruit [40]. Nevertheless, the tapioca starch barrier is poor compared to synthetic plastic [41], supporting the necessity of incorporating the ZnO NPs in the current study. Post-harvest water loss leads to wilting and shriveling, reducing the marketability of the products. The ZnO possesses unique traits, namely antimicrobial properties and the ability to enhance the mechanical, barrier, and thermal characteristics of the resulting film [41]. On day 7 of the storage period, the highest weight loss was recorded by the fruit coated with 1 mg/ml ZnO–tapioca starch NPs, followed by the control and 3 mg/ml ZnO–tapioca starch NPs-coated mangoes. The weight loss percentage of the 5 mg/ml ZnO-tapioca starch NPs sample was 20%.

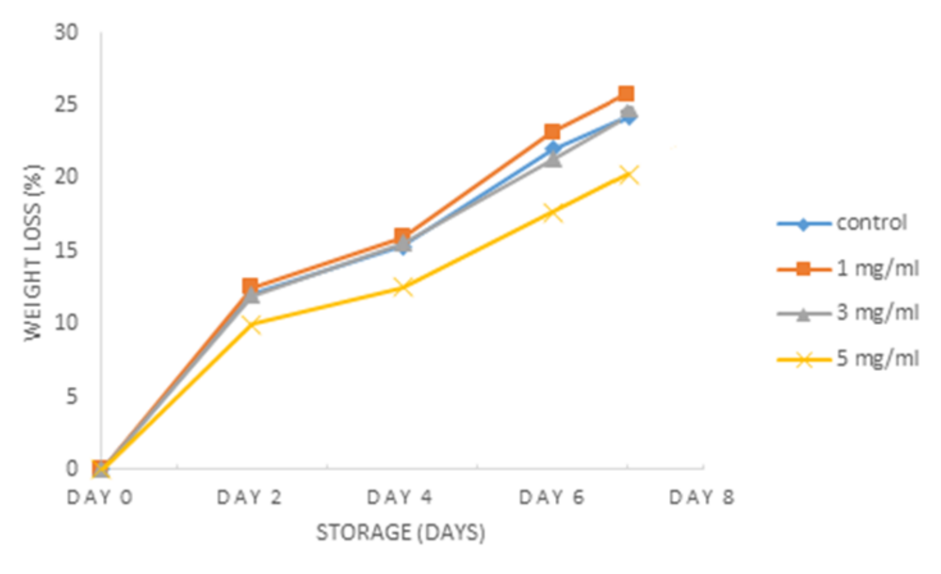


Fig. 3. The weight loss percentages of uncoated and mangoes coated at varying ZnO- tapioca flour NPs during a seven-day storage

Figure 4 displays the disease severity percentages of the mango samples during seven days of storage. The uncoated fruit and the sample coated with 5 mg/ml ZnO-tapioca flour NPs documented the highest and lowest disease severity percentages. Nevertheless, the results also demonstrated that the disease severity percentages increased to 50% on the seventh day. Nonetheless, the fruits with 1 and 3 mg/ml ZnO-tapioca flour NPs exhibited improvements on the final day of storage, at approximately 15 and 6%. Moreover, the sample with 5 mg/ml ZnO- tapioca flour NPs coat produced a superior disease severity improvement of 0.9% (see Figure 5), proving that the ZnO NPs effectively inhibited anthracnose infection on mango. Anthracnose disease in mango manifests as fungal growth visually observed as black spots on the mango surfaces [42]. The infection affects the fruit quality, damaging the income of a country [10]. Consequently, agriculturists employ fungicides to prevent anthracnose infections. Nonetheless, although fungicides could prevent diseases, the chemicals also affect the quality of the products, such as altering the skin of mangoes from green to yellow [43].

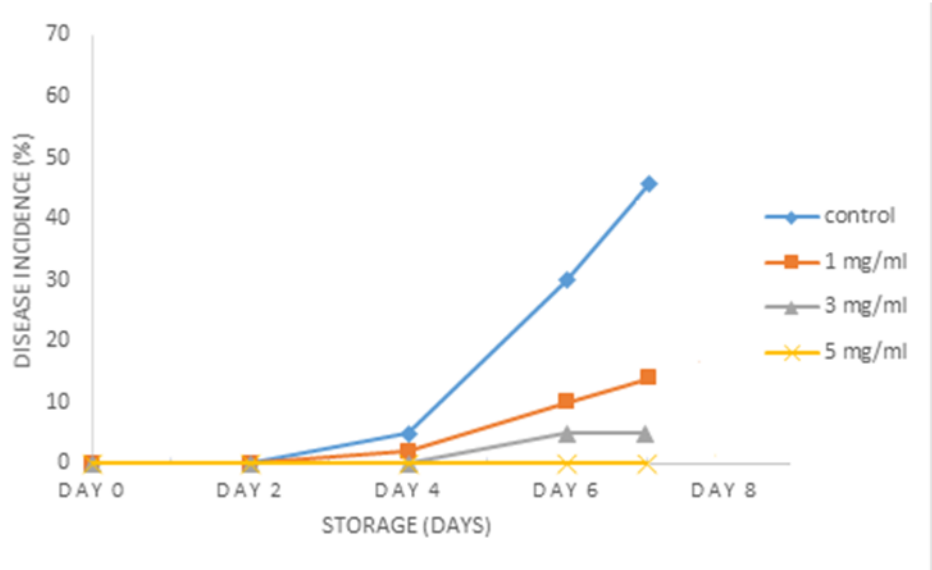


Fig. 4. The disease severity percentage of the mango fruits during seven days of storage

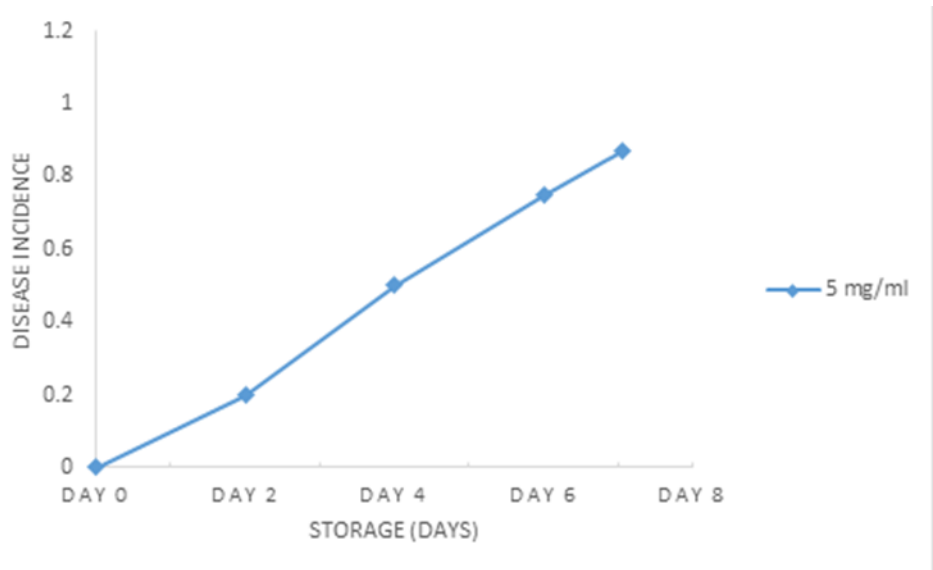


Fig. 5. The actual disease severity percentage value of the mango fruit coated with 5 mg/ml ZnO-starch flour NPs during the 7-day storage

Table 1 lists the preliminary results of the parametric measures also assessed in the current study, including MC, TA, TSS, and pH. Nonetheless, only the samples coated with <3 mg/ml of ZnO and glutinous tapioca flour were evaluated after seven days of storage. The control sample recorded a lower MC, 48%, and the highest MC, 69%, was observed in sample C (1.5 M). The TA and pH are two quality attributes of mango products. The acids primarily influence the pH of mango in the fruit. Natural acids also represent a significant taste component in mangoes. In an investigation, sugars and acids and their interactions contributed to the sweetness and sourness and the general flavour intensity of tomatoes [44]. According to Patanè, Tringali, and Sortino [45], the TA and vitamin C of tomatoes increased under water stress (50% Etc), which contrasted with the full system water treatment (100% Etc) [45]. Tomatoes irrigated with less water regulated certain metabolic activities, for example, osmotic alteration in sink organs, to build sucrose and change the rate and amount of organic acid, thus assimilating shifts to the fruit, improving solvent sugar, TSS, and TA [46]. The mechanisms observed in tomatoes are similar to mangoes, implying that low pH would lead to improved mango fruit flavour. A higher MC in sample C might be due to an increased osmotic moisture transfer from the peel to the pulp. Moreover, a barrier with superior properties from the coating material against water vapour loss enabled respiration reduction since moisture loss occurs due to changes in physiological processes during post-harvest, such as transpiration and respiration [19].

No significant difference was observed in the TSS value of the control and treated samples except for sample C. The control and treated samples recorded similar TSS data, 2.0^{o} Brix; however, sample C documented 1.8^{o} Brix (see Table 2). The low TSS concentration in sample C might be due to lower respiration levels, which might be due to the diminished hydrolysis of carbohydrates into sugar. The phenomenon was supported by a report that stated okra in nano-ZnO packaging demonstrated lower TSS compared to the control, indicating the maturity process was retarded by the edible coating that delayed respiration [19]. Sugars and organic acids are the fundamental components determining the taste of fruits. The TSS to TA proportion is the relationship between the soluble solids and titratable acidity in fruits. The measurement is one of the critical parameters used to decide the development and nature of fruits since it represents the sweetness versus the acidity of a fruit [20]. According to Lawson *et al*. [47], TSS increased while TA decreased after six days of ripening [47]. The diminishing acidity is attributable to its utilization as substrates during respiration and alteration into sugars as the ripening process progresses [20]. Nonetheless, the current study documented decreased TSS and elevated TA. The findings were due to the amount of potassium in the soil, resulting in thicker peels [48].

Table 2
The preliminary results of parametric measures, including MC, TA, TSS, and pH, of the samples coated with under 3 mg/ml ZnO and glutinous tapioca flour after seven days of storage

Sample	MC(%)	TA(%)	pH	TSS (%)
Control	48	0.448	6.524	2.0
A (0.5 M)	52	0.480	6.392	2.0
B (1.0 M)	61	0.500	6.138	2.0
C (1.5 M)	69	0.512	5.814	1.8
D (2.0 M)	55	0.506	5.746	2.0

Figure 6 displays the FESEM micrographs of the 1, 3, and 5 mg/ml ZnO NPs coatings on the mango samples observed in the present study. The 1 mg/ml coat was not uniform, while the 3 mg/ml only partially coated the fruit. The 5 mg/ml film was observed to be homogeneous. The findings demonstrated that the ZnO NPs attached to the mangoes were due to the tapioca starch, which was the binder in the present study [49]. Insufficient dipping time contributed to the non-uniformity of the coatings. The results also suggested that the ZnO NPs agglomerated, thus reducing its efficiency. Smaller and non-agglomerated ZnO particles possessed superior physical, chemical, and biological properties [50]. The films developed from starch are described as isotropic, odourless, tasteless, colourless, non-toxic, and naturally biodegradable [51]. Tapioca starch employed as the main matrix film is also relatively hydrophilic. The hydrophilic-based compound is known to upgrade the water vapour porousness of hydrocolloid films, resulting from the excellent adsorption and desorption of water atoms [52]. Simultaneously, the ZnO NPs filler upgrades the polymer hindrance properties by a convoluted pathway [52]. In future studies, the dipping period necessitates adjustments to avoid agglomeration.

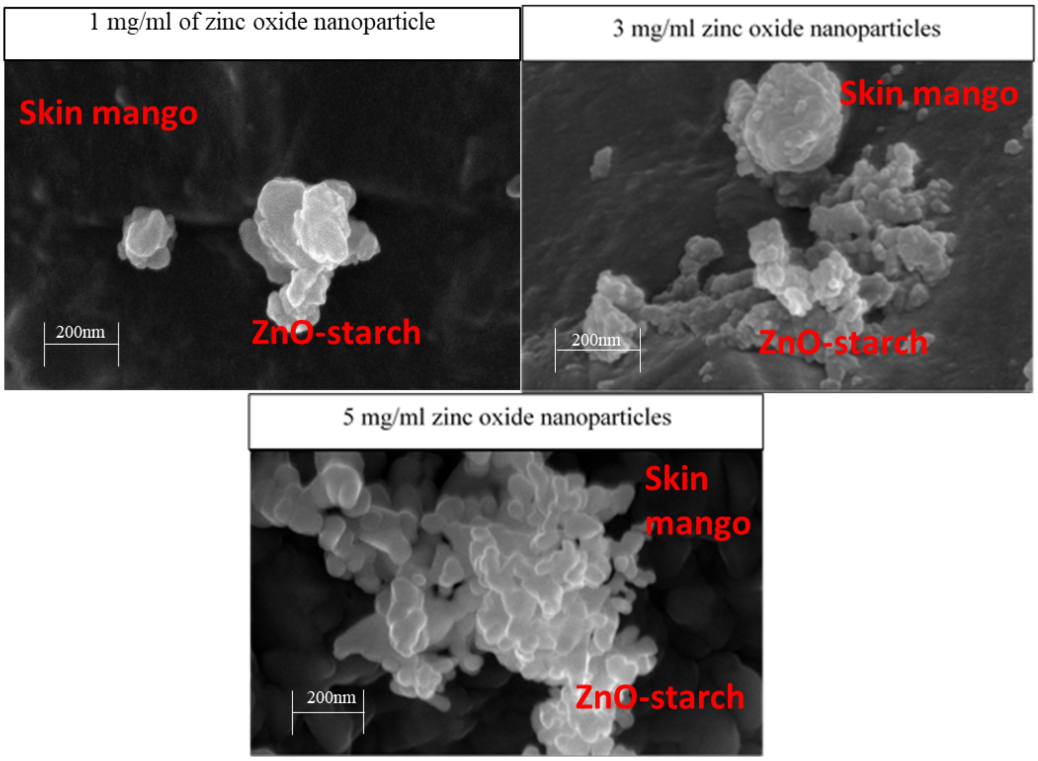


Fig. 6. The FESEM micrographs of the 1, 3, and 5 mg/ml.ZnO NPs coats on the mango samples

Based on the results, the weight loss percentage coated with 5 mg/ml ZnO-tapioca starch NPs is better than coated with 1 mg/ml and 3 mg/ml ZnO-tapioca starch NPs after 7 days. Mandal et al. reported that carboxymethyl cellulose (CMC) 1% coated mango fruits had the same weight loss percentage with control fruits [53]. Torres *et al*., [53,54] also reported that waxed tomato fruit lost less weight than control because it reduced transpiration and thus water loss. Baldwin *et al*., [2,53] discovered that mango fruit coated with carnauba wax lost less weight due to decreased moisture loss. The effect of coatings on fruit weight loss could be due to the coating producing a modified atmosphere around the fruit surface and the surrounding environment [55][56]. It can be concluded that coating with ZnO-tapioca starch NPs could delay ripening and reduce weight loss.

4. Conclusion

The present study investigated the effects of ZnO NPs and glutinous tapioca starch coating on mango fruits in preventing anthracnose disease caused by *Colletotrichum gleosporides*. The combination of 60–100 nm ZnO NPs and glutinous tapioca starch coating resulted in good physicochemical properties and inhibited the growth of microorganisms. The uncoated mango exhibited more black spots than coated with ZnO and coated with ZnO NPs-tapioca solution mango samples. Mango treated with 5 mg/ml of ZnO-tapioca starch is observed as superior to the other fruits. One of the most noticeable characteristics of physicochemical for coating ZnO NPs-tapioca samples is the appearance of black spot growth with the smallest diameter after 7 days. This study suggested that nano-ZnO-starch coated can be used for reducing postharvest deterioration, extending the shelf life and maintaining quality of mango fruit during storage.

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