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Application of ZnO-nanoparticles to manage *Rhizopus* soft rot of sweet potato and prolong shelf-life

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ABSTRACT

A reduction in crop spoilage and an increase in shelf-life is the goal of effective disease control methods. This study aimed to assess ZnO-nanoparticles (ZnO-NPs) as a safe, new protectant against *Rhizopus* soft rot of sweet potato. ZnO-NPs had a fungicidal effect against *Rhizopus stolonifer* when used at concentrations above 50 ppm. The results showed that tubers treated with ZnO-NPs exhibited fewer fungal populations (1.2 CFU per segment) than those that did not receive the treatment. Tubers infected with *Rhizopus stolonifer* and treated with ZnO-NPs showed no visible decay for up to 15 days, indicating that ZnO-NPs act as a coating layer on tuber surface. The greatest weight loss after 15 days of storage was reported in infected tubers (8.98%), followed by infected tubers treated with ZnO (6.54%) and infected tubers treated with ZnO-NPs (3.79%). The activity of cell-wall degrading enzymes, α-amylase and cellulase, were significantly increased in both infected tubers and those treated with ZnO, compared to the tubers treated with ZnO-NPs. These results confirm that coating with ZnO-NPs is an effective method of protecting sweet potato tubers from infection, maintaining their quality and increasing their shelf-life for up to 2 months in storage.

Key words: edible coating, *Rhizopus* soft rot, shelf-life, sweet potato, ZnO-nanoparticles

INTRODUCTION

A postharvest loss of fruits and vegetables refers to the proportional quality and quantity of produce lost during harvest, transportation, storage, marketing and consumption (FAO, 2011; Buzby et al., 2014). Reduction in crop losses could have a major impact on sufficiently meeting higher global food demands and food safety standards (West et al., 2014; Hertel, 2015; Reynolds et al., 2015). One of the most important factors that cause crop loss is spoilage, which is defined as any change rendering food unsuitable for human consumption. Spoilage-causing microorganisms attack fruits and vegetables after harvesting due to their nutritional richness and high moisture content. Sweet potato [*Ipomea batatas* (L. Lam)] consumption has increased due to its nutritional value, availability and health-promoting features. The most destructive postharvest disease of sweet potato is *Rhizopus* soft rot that is caused by the fungus *Rhizopus stolonifer*

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(Clark et al., 2013). Wounds on tubers are easily colonized by *Rhizopus* spores because the site of a wound releases nutrients that can be used for the growth of the pathogen (Holmes and Stange, 2002); the spores then germinate and produce white mycelia covered with black sporangiospores (Nelson, 2009). Many enzymes are produced by the pathogen, such as pectinase, amylase and cellulase, which damage tuber cell walls and enable colonization of the host (Tang et al., 2012).

The management options for postharvest control of *Rhizopus* soft rot are limited. Production of disease-resistant sweet potato cultivars is the focus of most trials. Currently, none exhibit complete resistance; however, Beauregard (the most commonly grown sweet potato cultivar) is considered the most resistant to *Rhizopus* soft rot, depending on the postharvest conditions (Clark et al., 2013). Nelson (2009) reported that there are no cultivars of sweet potato resistant to soft rot and that all cultivars can be infected in variable degrees. Another traditional method for controlling *Rhizopus* soft rot is the use of dicloran fungicides (Botran 75 W) as dips or a spray (Edmunds and Holmes, 2009). However, the use of Botran is limited, and packers usually arrange shipments without fungicide protection. At present, fungicide replacements with alternative methods are needed to control the postharvest diseases of sweet potato.

The application of nanotechnology is a promising method in future crop protection and affords a keener solution for the current problems facing the field of agriculture (Abobatta, 2018). The ability to produce different shapes and sizes of nanoparticles with antimicrobial properties is the most popular advance in nano-food science. Also, recent studies have focused on producing a safe, edible nano-coating to extend the shelf-life of fruits and vegetables. The nano-coating can be applied by spraying, dipping, brushing or panning the produce (Park et al., 2017). Such coatings can be used as antimicrobial and antioxidant layers, to prevent moisture absorption, and even as an added flavour to enhance food quality and stability (Debeaufort et al., 1998; Min and Krochta, 2005). Zinc oxide nanoparticles (ZnO-NPs) are a type of inorganic multifunctional nanoparticles that prevent microbial growth (Jin et al., 2009; Aydin and Hanley, 2010), and have strong antimicrobial activity (Jones et al., 2008). ZnO is listed by the Food and Drug Administration as a safe material, according to Regulation 21 CFR 182.899 (Xie et al., 2011). ZnO-NPs are used as food supplements or additives, as well as in packing materials and storage because very few migrate from the packaging, and the amount of soluble ionic zinc that migrates is within safety limits. Li et al. (2011) reported that a novel polyvinyl chloride film coated with ZnO nanoparticles acted as active packaging to improve the shelf-life of fresh-cut Fuji apples. ZnO nano-treatment also appears to have a positive impact on the quality of strawberry fruit during storage (Sogvar et al., 2016). Al-Naamani et al. (2018) successfully developed chitosan-ZnO-nanocomposite as a coating material, which not only kept the quality of packed okra from deteriorating but also reduced microbial growth. Therefore, the main goal of the research presented here was to assess the effects of ZnO-NPs coatings on the development of *Rhizopus* soft rot of sweet potato in order to control the disease and prolong the shelf-life of the produce during storage.

MATERIALS AND METHODS

ZnO-nanoparticles

Zinc oxide nanoparticles (ZnO-NPs) were synthesized by a wet chemical method (Lee et al., 2013). 20 mL of aqueous 1 M sodium hydroxide solution was added to 0.1 M $Zn(NO_3)_2 \cdot 6\ H_2O$ in 100 ml distilled water under flow control and slow magnetic stirring at a temperature of 50°C. The reaction mixture was maintained for 1 hour at this temperature, then allowed to settle at room temperature. The white precipitate formed was washed with distilled water to remove the ions and then centrifuged at 4000 rpm for 10 min. The resultant precipitate was dried in a hot air oven at 80°C for 6 h. The obtained white powders were analyzed by X-ray diffraction (XRD) using an X-ray diffraction meter (Shimadzu XD-3A) with Cu-Kα radiation (λ = 1.5406 Å), with 2θ ranging between 20° and 100° at a scanning rate of 0.025° per second. The morphological structures of the synthesized ZnO-NPs were visualized using a transmission electron microscope (JEOL TEM 100CXII, Tokyo, Japan) at an accelerating voltage of 200 kV.

Plant material

Two batches of sweet potato tubers [*Ipomea batatas* (L. Lam)] were collected from commercial markets in the city of Assiut, Egypt. The first batch included tubers that showed symptoms of softening and rotting and were selected for pathogen isolation. The other batch contained healthy tubers that were selected with uniformity in size, shape and colour and without any signs of damage or disease. The tubers were kept separately in sterile

polyethylene bags upon collection and immediately transferred to the laboratory.

Isolation and identification of fungal pathogen

Potato dextrose agar (PDA) was used for pathogen isolation. Rotten sweet potato tubers were washed and then cut into sections to reveal the region of rot. The tuber sections were surface sterilized by dipping completely in 70% ethanol for 1 min., 1% NaOCl solution for 3 min., 70% ethanol for 30 s. and, finally, rinsed once in sterilized distilled water (Abdel-Hafez et al., 2015). The tuber sections were placed on sterile filter paper in a laminar air-flow chamber for 10 min. to dry, and then cut into pieces (1 cm^2). The sweet potato pieces were used to inoculate PDA plates and were then incubated at 26°C until the first appearance of pathogen on sweet potato tissues. The pathogen was identified based on its morphological and microscopic features (Hernández-Lauzardo et al., 2006). The pathogenic fungal isolates (*Rhizopus* spp.) were tested for their ability to cause rot disease in healthy sweet potato tubers, as described by Anukwuorji et al. (2013).

Antifungal activity of ZnO-NPs in vitro

For the assessment of antifungal activity of ZnO-NPs, disk diffusion assays were performed using sterilized paper disks (Bauer et al., 1966). The disk diameter was about 6 mm. Six disks were placed in a 9-cm petri dish. The positions of the disks were such that the minimum centre-to-centre distance was 24 mm and the disks were no closer than 10 to 15 mm from the edge of the petri dish. Spore and sporangiospore suspensions of *Rhizopus* spp (200 µl containing approximately 10^6 spores per ml) were aseptically spread onto PDA plates. Then, disks containing freshly prepared ZnO-NPs at different concentrations (50, 100, 150, 200, 250, 300 ppm) were prepared. Fungal culture supernatants and sterile distilled water were used as controls. The plates were incubated at 26 ± 2°C for 5 days. The effects of ZnO-NPs treatments were evaluated by measuring the inhibition zones (mm) according to Andrews (2001). The assays were performed in triplicate.

Effects of ZnO-NPs against Rhizopus soft rot in postharvest management

Twenty-four healthy sweet potato tubers were surface sterilized with a sodium hypochlorite solution (5%) for 3 min., ethyl alcohol (70%) for 5 min. and, finally, washed with sterile distilled water several times. The tubers were left at room temperature to air-dry. The tubers were punctured with a sterilized cork-borer to make 5 mm diameter, 3 mm deep wounds (four wounds per tuber). The sweet potatoes were subjected to one of two treatments, which consisted of being immersed in either a freshly prepared ZnO-NPs solution (300 ppm) or bulk ZnO material (300 ppm) for 30 min. and left to air-dry in sterile conditions. The wounded tubers were then inoculated with a 50 µl suspension of *Rhizopus* spores and sporangiospores. Wounded, non-coated tubers that were not inoculated with the pathogen served as controls. The treatments were labelled as follows: T1 – healthy tubers (control), T2 – infected tubers (infected control), T3 – infected tubers treated with ZnO, and T4 – infected tubers treated with ZnO-NPs. Each treatment was performed in triplicate. All tubers were packed in sterile plastic boxes (95--98% relative humidity) and incubated at 26 ± 2°C for 15 days. The experiment was repeated twice.

Fungal growth

Samples of the control and treated sweet potato tubers (T1, T3 and T4) were tested for fungal growth. Five uniform segments from each tuber were used to inoculate PDA plates, using the surface plate method for fungal enumeration. Incubation was carried out at 26 ± 2°C for 4 days. Each treatment was performed in triplicate and the results were stated as colony forming units (CFU) per potato segment (Sogvar et al., 2016).

Pathophysiological measurements

Weight loss

The weight of sweet potato tubers in each treatment was recorded using a digital weighing balance on the day of treatment (day 0) and at each sampling time (day 5, 10 and 15). Tuber weight loss was calculated as a percentage (%) of the original tuber fresh weight using the following equations:

$$\text{Weight loss} = \text{Original weight} - \text{Present weight}$$

$$\% \text{ loss} = (\text{Weight lost} / \text{Original weight}) \times 100$$

Preparation of sweet potato extract

One gram of sweet potato tissue was ground in 10 ml of 100 mM phosphate buffer and centrifuged for 5 min. at 10,000 rpm under cooling conditions to remove plant tissue. Samples of each sweet potato were taken at the beginning of the experiment after the treatments and inoculation (day 0) and on day 5, 10 and 15 of storage. The extract was used to determine the concentration of reducing sugars, protein content and α-amylase and cellulase enzyme activities.

Assay of α-amylase activity

Amylase activity was determined as described by Kathiresan and Manivannan (2006), in a reaction mixture containing 1 ml of 100 mM phosphate buffer (pH 7), 0.5 ml of 1% starch (w/v) and 0.5 ml of crude enzyme extract. One unit of amylase activity (U) was defined as the amount of enzyme that releases one µmol of reducing sugar per min., with maltose used as a standard.

Assay of cellulase activity

Cellulase activity was determined according to Hussain et al. (2012). The assay mixture contained 1 ml of 1% carboxy methyl cellulose in phosphate buffer, pH 7, 100 mM and 1 ml of enzyme (sweet potato extract). The absorbance was measured spectrophotometrically at 540 nm against substrate-free blank. The standard curve was prepared using glucose as a reducing sugar. One cellulase unit is defined as the amount of enzyme that liberated 1 µM of reducing sugar per min. under the assay standard conditions.

Determination of protein concentration

Protein content was determined by the Coomassie brilliant blue G-250 dye binding method, as described by Bradford (1976), using bovine serum albumin (BSA) as a standard protein. The blue colour developed after 5 min. and was detected at 595 nm.

Determination of the concentration of reducing sugars

The amount of reducing sugars was determined according to the protocol used by Miller (1959), using a reagent containing dinitrosalicylic acid; glucose was used for the standard curve.

Zn analysis

Zn concentration in untreated and treated sweet potato tubers was determined in samples taken four days after the treatments. Pieces of tubers were dried at 70°C for 24 h, and then manually powdered. The tuber powder from each sample (2 g) was placed in beakers filled with 10 mL of 6 N HCl and digested on a hot plate for two hours. The digest was then cooled and filtered, and the Zn concentration (ppm) was measured by an atomic absorption spectrophotometer (AAS), Model 210 VGP Buck Scientific (Sogvar et al., 2016).

Statistical analysis

The collected data were subjected to analysis of variance (ANOVA) with SPSS (Statistical Package for the Social Sciences) software. Mean values were calculated and reported as the mean ± standard deviation (n = 3). Significance between mean values was determined by Duncan's multiple range tests.

RESULTS AND DISCUSSION

The morphological structure and the size of the synthesized ZnO-NPs were determined. The fine powder was dispersed in ethanol and examined on a carbon grid by transmission electron microscope (TEM). The TEM image revealed a uniform size and spherical shape of the ZnO-NPs (Fig. 1A). The average diameter of the homogenous ZnO nanoparticles was 18.2 ± 6.3 nm.

The XRD pattern of the ZnO nanoparticles is shown in Fig. 1B. The peaks indicated a nano-crystalline nature of ZnO and were identical to those of the hexagonal phase. The XRD chart showed the presence of peaks at angles (2θ) of around 31°, 34°, 36°, 47°, 56°, 62°, 67°, 69°, 76°, 81° and 89°, which

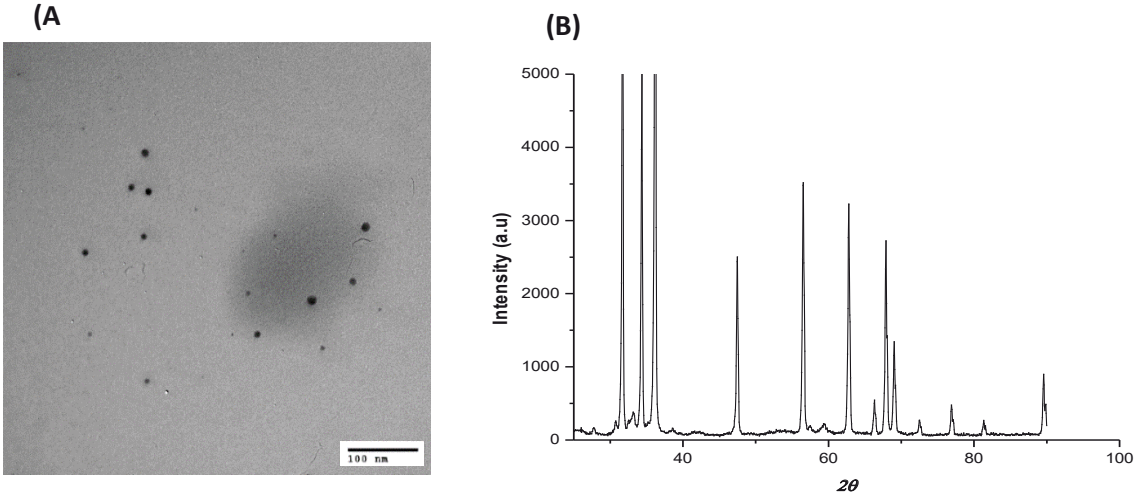

Figure 1. (A) TEM image of synthesized ZnO nanoparticles; (B) X-ray diffraction pattern of ZnO nanoparticles

correspond to reflections from 100, 002, 101, 102, 110, 103, 200, 112, 202, 104 and 203 crystal planes, respectively (Thirumavalavan et al., 2013).

Phytopathogenic fungi are usually identified on the basis of morphological characteristics. To identify fungi morphologically, the most important characteristics are those of the formation of spores and fruiting bodies (Agrios, 2001), and to a lesser extent of the mycelium; it is important to follow the keys to genera for exact identification. The *Rhizopus* sp. that was isolated from the sweet potato tubers showing the natural soft rot disease (Figs 2A and 2B) was identified as *Rhizopus stolonifer* (Ehrenb.) Vuill. based on cultural and microscopic characteristics (Figs 2C and 2D). It was characterized by a well-defined mycelia development on the growth medium (PDA) after 48 h, and by the fact that in a group of sporangiophores, the sporangia were not nodding and were almost circular in shape. *R. stolonifer* is also characterized by the formation of complex and well-developed rhizoids (Hernández-Lauzardo et al., 2006). The morphological and microscopic characterization performed in this study confirmed that *R. stolonifer* was the disease-causing pathogen.

The pathogenicity test proved that *R. stolonifer* caused the soft rot disease on the tested sweet potato tubers, colonizing the tubers after 3 days post-infection. The spores of this strain germinate, producing hyphae that penetrate the tubers. The phytopathogenic *R. stolonifer* needs wounds for infection to happen, and the wounds that occur during harvesting or packing provide the perfect conditions for infection and, consequently, development of *Rhizopus* soft rot disease (Edmunds et al., 2015). *Rhizopus* produces hydrolytic enzymes that quickly cause host discoloration and soften host tissues.

Antifungal effect of ZnO-NPs

The results showed that ZnO-NPs had a fungicidal effect against *R. stolonifer* when used at concentrations above 50 ppm. Disk diffusion testing resulted in clear zones of inhibition with diameters of 7, 8.9, 12.7, 14, and 16.8 mm for 100, 150, 200, 250 and 300 ppm ZnO-NPs, respectively (Fig. 3). By contrast, the application of ZnO concentrations did not reveal antifungal activities against *R. stolonifer*. Our results were in accordance with those of others who had demonstrated the fungicidal efficacy of ZnO-NPs against post-harvest pathogens, including *Penicillium expansum* (He et al., 2011; Yehia and Ahmed, 2013; Sardella et al., 2018) *Fusarium oxysporum* (Yehia and Ahmed, 2013) and *Botrytis cinerea* (He et al., 2011). Also, Jamdagni et al. (2018a) stated that green-synthesized ZnO-NPs had a good antifungal activity against *Alternaria alternata*, *Aspergillus niger*, *Botrytis cinerea*, *Fusarium oxysporum* and *Penicillium expansum* showing a minimum inhibitory concentration (MIC) of 64, 16, 128, 64 and 128 µg L^{-1}, respectively. Furthermore, zinc oxide nanoparticles showed strong antifungal activity against *P. expansum* when used in combination with the agricultural fungicides carbendazim and thiram (Jamdagni et al., 2018b). The antifungal effect of green-synthesized ZnO nanoparticles on the fungal pathogens *Rhizopus stolonifer* and *Aspergillus flavus* is severe due to protein leakage and leads to cell membrane damage (Gunalan et al., 2012).

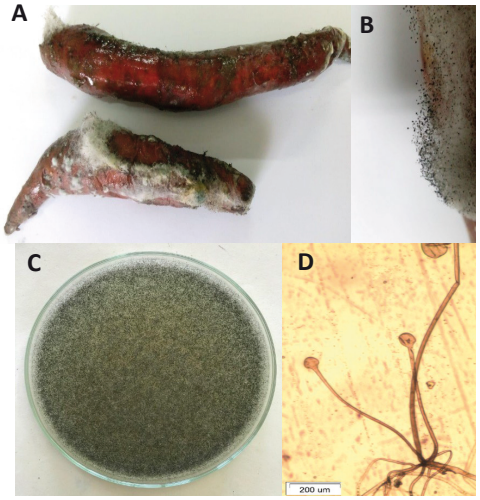

Figure 2. (A) Naturally occurring *Rhizopus* soft rot disease on sweet potato tubers; (B) *Rhizopus* sporulation – sporangia and sporangiophores are extruded like whiskers through openings and wounds on tuber surface; (C) 4-days-old culture of *Rhizopus stolonifer* at 25°C on PDA medium; (D) Microscopic features showing sporangia, sporangiophores and rhizoids

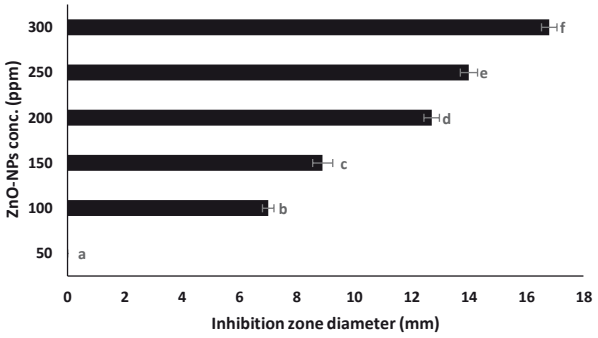

Figure 3. Antifungal activity of different concentrations of ZnO-NPs against pathogenic *Rhizopus stolonifer* as determined by the disk diffusion method

The total count of fungal populations was estimated after soaking the tubers in a solution of ZnO-NPs or bulk ZnO material (Fig. 4). The total number of fungal populations in the untreated sweet potato was 5.1 CFU per segment, which was the highest count of all the treatment groups. The lowest numbers of fungal populations were recorded in ZnO-NPs-treated tubers (1.2 CFU per segment). These results confirm that ZnO-NPs act as a capping and coating layer on tuber surfaces and exhibit antifungal properties, which is comparable with the use of metal and metal oxide nanomaterials in food systems as protective coating layers against biological deterioration (Jin et al., 2009; Emamifar et al., 2010; He and Hwang, 2016).

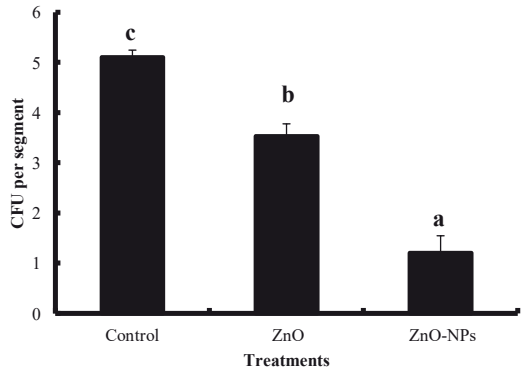

Figure 4. Total fungal counts (CFU per segment) in sweet potato tubers either untreated or treated with ZnO-nanoparticles or bulk material (ZnO)

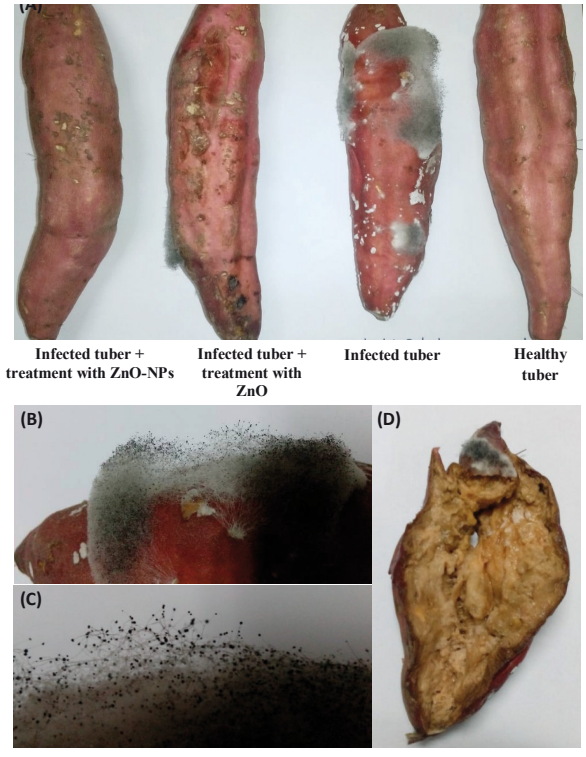

Figure 5. *Rhizopus* soft rot symptoms on sweet potato tubers. (A) Treated and untreated tubers incubated at 26 ± 2°C for 15 days; typical symptoms of *Rhizopus* soft rot disease appeared in the untreated infected tuber and the ZnO-treated infected tuber; (B-C) *Rhizopus* sporulation on the tuber surface; (D) Internal view of a tuber affected by *Rhizopus* soft rot

The effect of ZnO-NPs on *Rhizopus* soft rot of sweet potato was also evaluated. After treatment, tubers were examined for the incidence of *Rhizopus* soft rot during storage (Fig. 5). The infected sweet potato tubers were softened and watery, and all the infected tubers were completely rotted within 3 days (Fig. 5D). The colour of the infected tubers did not significantly change, while the surface ruptures were occupied with a coarse white mycelium bearing the characteristics of sporangia (Edmunds and Holmes, 2009). The spore heads were first white and later turned black, and the mycelium appeared grey; a rotting odour was also produced. The non-infected tubers were firm and exhibited no symptoms of rotting or decay. The wounded tubers inoculated with ZnO-NPs showed no development of decay or rotting. By comparison, some soft rot symptoms developed in the wounded and inoculated tubers that had been treated with ZnO as bulk material. These results verify the antifungal properties of ZnO-NPs, which could be due to the generation the reactive oxygen species (ROS) that react with cell components and cause subsequent cell death (Fu et al., 2014; Wu et al., 2014). Also, the fungal cellular function or structure can be altered due to the discharge of metal ions within the cell, outside the cell, or at the cell surface (He and Hwang, 2016).

Weight loss (%)

The percentage sweet potato weight loss increased during storage (Fig. 6). Tuber weight loss was greater in the infected tubers (not treated with ZnO-NPs) than in the treated tubers. The highest weight loss at the end of the storage period (15 days) was recorded in the infected tubers (8.98%), followed by the infected tubers treated with ZnO (6.54%). At the same time, the weight loss in the infected tubers treated with ZnO-NPs was 3.79%. Because fruits possess a short storage life, the physiological, physical, mechanical and hygienic conditions can cause significant weight loss in them between harvesting and consumption. The percentage weight loss is a vital parameter of postharvest activities. Transpiration (water loss) and respiration (carbon loss) are the main causes of weight loss (Vogler and Ernst, 1999). The results obtained showed that

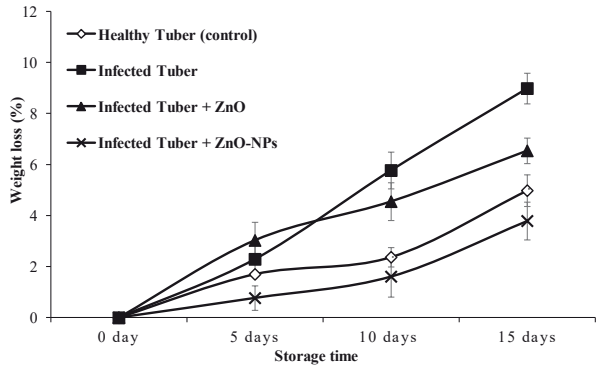

Figure 6. Weight loss (%) in sweet potato tubers, either untreated or treated with ZnO or ZnO-NPs, at different storage times. Vertical bars represent ± standard deviation (n = 3)

ZnO-NPs had a positive effect on reducing weight loss in sweet potato tubers. These findings agreed with those of other researchers, who reported that ZnO nanoparticle coating reduced weight loss in strawberries (Sogvar et al., 2016), apricot (Zhao et al., 2009), or in fresh-cut kiwifruit (Meng et al., 2014). The edible ZnO nanoparticle coating acts as a barrier to water, moisture and gas exchange, resulting in the control of weight loss. Meanwhile, ZnO-NPs could delay water transfer and reduce oxygen uptake, which in turn reduces the rate of transpiration, and the associated weight loss, from the surface of fruits (Lakshmi et al., 2018).

α-amylase and cellulase activity

The activities of α-amylase and cellulase in sweet potato tubers are shown in Fig. 7. Enzyme activity increased significantly during storage in both the infected untreated tubers and those treated with ZnO. The highest value of α-amylase activity was 41.1 U ml^{-1}, while the highest level of cellulase activity was 14.5 U ml^{-1}, both enzyme levels occurring in the infected untreated tubers. In the tubers that received treatment with ZnO-NPs after infection with the pathogen, the level of activity for both enzymes was close to the level seen in the healthy tubers. Both α-amylase and cellulase are the main plant cell wall-degrading enzymes that are excreted by the pathogen (*R. stolonifer*) so that it can use the constituents as nutrients. This process leads to spoilage of the tubers and reduces their postharvest life. The results of this study showed that the edible ZnO-NPs coating prevented fungal growth and, consequently, the activity of the enzymes in the treated tissues was very low. This finding is supported by the results reported by Li et al. (2011), who found that polyphenoloxidase and pyrogallol peroxidase activities decreased as a result of nano-packaging apple fruit. Based on these and our findings, we suggest that the use of ZnO-NPs in active packaging could be a viable alternative to the common technologies for improving the shelf-life of sweet potato tubers.

Although the action of zinc oxide on fungal growth and enzyme activities may be attributed to the effects of ZnO-NPs on chitin and glucan, as reported by Arciniegas-Grijalba et al. (2017), who recorded a noticeable thickening of the cell wall and liquefaction of the cytoplasmic contents of *Erythricium salmonicolor*. Consequently, ZnO-NPs might control or reduce the synthesis of chitin and glucan enzymes (Romero et al., 2005; Merzendorfer, 2006). These findings draw special attention to the peculiarities of the toxicity of nanoparticles and their application as fungicide (Zucolotto et al., 2013).

Protein and reducing sugar content

The initial protein content in the control tubers was 3.55 mg g^{-1} fresh weight, and there were no significant differences between the treated and untreated tubers at the beginning of the experiment (day 0; Fig. 8). Protein concentration increased during tuber storage to 5.65 mg g^{-1} FW in the infected tubers and to 4.13 mg g^{-1} FW in the infected tubers treated with ZnO, after 15 days. Meanwhile,

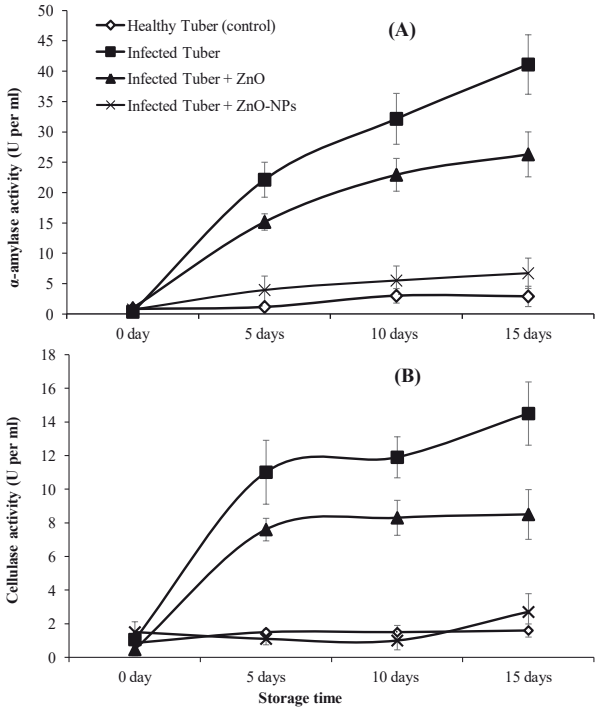

Figure 7. Alpha amylase (A) and cellulolytic (B) activities (U per ml) in sweet potato tubers, either untreated or treated with ZnO or ZnO-NPs, at different storage times. Vertical bars represent ± standard deviation (n = 3)

the effect of ZnO-NPs on tuber protein content was not significant compared with the healthy control. Similarly, the reducing sugar content of sweet potato infected by *Rhizopus* increased to 1.59 mg g^{-1} FW after 15 days of storage. The reducing sugar content of ZnO-NP-treated tubers was close to the content in the healthy controls (Fig. 9). During postharvest storage, spoilage of fruits and vegetables occurs due to many changes, such as increased rates of physiological activity, a decrease in organic acid contents and a breakdown of cell constituents due to respiration (Sharma and Singh, 2000; Ragaert et al., 2007). The edible or safety coatings during storage processes have been widely used to control postharvest diseases, maintain the quality and extend the shelf-life of fruits and vegetables (Li and Barth, 1998; Lin and Zhao, 2007).

Zinc concentrations in treated sweet potato tubers

In the current study, the sweet potato tubers treated with bulk ZnO material had the greatest Zn content at 68 ppm, while the ZnO-NP-treatment of the tubers resulted in a reduction in Zn concentration (11.59 ppm), compared to the ZnO treatment. Zn concentrations were 1.41 ppm and 0.91 ppm in the uninfected, untreated controls and the infected, untreated control, respectively. This result was in agreement with the report that ZnO nanoparticle-coated strawberries had significantly higher levels of Zn compared to untreated fruits (Sogvar et al., 2016). Zinc is an essential trace element, which is important for many enzymes in the human body, such as the DNA polymerase complex. Also, zinc has catalyzing effects on human bone formation and immune system regulation (McClung and Scrimgeour, 2005; Du et al., 2006). Since ZnO was reported to be a safe substance, it has been used as a food preservative and in food packing (Espitia et al., 2012; Sogvar et al., 2016; Galstyan et al., 2018). Interestingly, the European Commission Scientific Committee on Food (EC SCF, 2003) stated that there is no observed adverse effect level of zinc on human health of approximately 50 mg zinc per day. Also, Bonham et al. (2003) reported that 30 mg supplemental zinc exhibited no adverse effects on consumers when dietary zinc was near 10 mg. Consequently, the amount of zinc recorded in the ZnO-NP-treatment of sweet potato (11.59 ppm) meets the standard values for human consumption according to the EC SCF 2003 report. By contrast, high zinc intake may cause acute adverse effects that include nausea, vomiting, loss of appetite, abdominal cramps, diarrhea, and headaches (Solomons, 1998). Severe nausea and vomiting may be caused by the intake of 4 g of zinc gluconate (Lewis and Kokan, 1998) and chronic effects, such as low copper status, altered iron function, reduced immune function, and reduced levels of high-density lipoproteins (Hooper et al., 1980) may occur after ingesting about 150 mg zinc. The zinc concentration in the tubers treated with ZnO-NPs did not exceed the acceptable value for human consumption, making ZnO-NPs a suitable edible coating agent for prolonging the shelf-life of foods and maintaining their nutritional value. ZnO nanoparticles may be applied with washing water during storage processes of sweet potato tubers as a food surface protectant during storage.

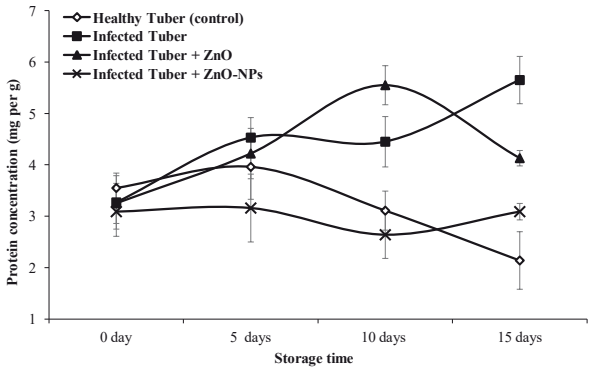

Figure 8. Protein concentration in sweet potato tubers, either untreated or treated with ZnO or ZnO-NPs, at ifferent storage times. Vertical bars represent ± standard deviation (n = 3)

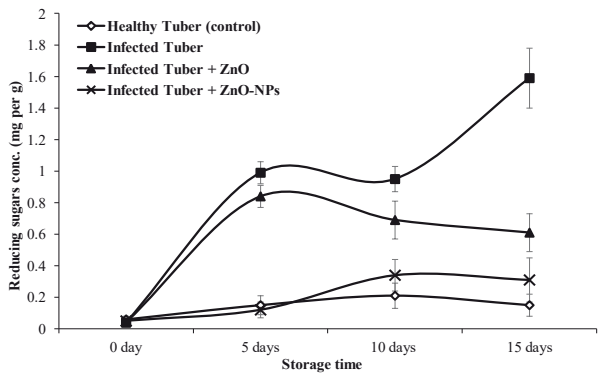

Figure 9. Reducing sugars concentration in sweet potato tubers, either untreated or treated with ZnO or ZnO-NPs, at different storage times. Vertical bars represent ± standard deviation (n = 3)

CONCLUSIONS

Our results indicate that the edible coating of ZnO-NPs has a beneficial influence against *R. stolonifer*, the causative agent of sweet potato soft rot. Treatment of sweet potato tubers with ZnO-NPs resulted in

a significant reduction in fungal populations and reduced the percentage weight loss. The infected (untreated) sweet potato tubers had the highest levels of the cell wall-degrading enzymes α-amylase and cellulase. The concentration of zinc in the tubers treated with ZnO-NPs did not exceed the allowable value. The most effective treatment for controlling *Rhizopus* soft rot of sweet potato was to use ZnO-NPs, which acted as an antifungal agent and food surface protectant during storage. ZnO-NPs are recommended for use as an edible coating of sweet potato tubers to prolong their shelf-life and maintain their nutritional value.

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AUTHOR CONTRIBUTIONS

N.N., E.H., and M.H. – suggested the idea of the work and contributed to data curation and their validation as well as writing original draft. K.E. – contributed to the formal analysis of the data. S.A. and Y.M. – contributed to the reviewing and editing the manuscript. All authors reviewed and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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