

ORIGINAL ARTICLE

Nanoactivities of natural nanomaterials rosmarinic acid, glycyrrhizic acid and glycyrrhizic acid ammonium salt against tomato phytopathogenic fungi *Alternaria alternata* and *Penicillium digitatum*

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Abstract

Black mold and green mold caused by *Alternaria alternata* and *Penicillium digitatum*, respectively, are the most important decay pathogens of tomato fruits during storage. Our research was aimed to control tomato phytopathogenic fungi *A. alternata* and *P. digitatum* *in vitro* and *in vivo* by using natural nanomaterials rosmarinic acid (RA-NPs) at concentrations of 0.3 and 0.6 mM, glycyrrhizic acid (GA-NPs) and glycyrrhizic acid ammonium salt (GAS-NPs) (0.1–0.2 mM). Characterizations of the tested nanoparticles were carried out by using dynamic light scattering which revealed that synthesized nanoparticles had particle sizes of less than 100 nm. *In vitro* studies revealed that the three tested nanoparticles reduced the growth of *A. alternata* and *P. digitatum*. Glycyrrhizic acid nanoparticles were the most effective in reducing the growth of the two tested pathogens followed by RA-NPs at 0.6 mM. Observations of *A. alternata* and *P. digitatum* by scanning electron microscopy (SEM) showed severe damage in the hyphae and deformities in the conidia due to the effect of the tested nanoparticles. *In vivo* results showed that, dipping tomato fruits as a post-harvest treatment in all of the tested nanoparticles at different concentrations, then stored at $10 \pm 1^\circ\text{C}$ and 90–95% relative humidity (RH) for 20 days greatly reduced the disease severity of infected fruits with the two tested pathogens. GA-NPs at 0.2 mM significantly reduced the development of black mold rot on tomato fruits. RA-NPs at 0.6 mM had the best effect in controlling *P. digitatum* of all naturally and artificially inoculated tomato fruits. Also, individual treatments of tomato fruits with RA-NPs, GA-NPs and GAS-NPs significantly reduced postharvest losses of fruit since they delayed decay and maintained fruit quality characteristics such as fruit firmness, titratable acidity and total soluble solids during cold storage.

Keywords: *Alternaria alternata*, natural nanoparticles, *Penicillium digitatum*, postharvest and quality, tomato fruits

Introduction

Tomatoes (*Lycopersicon esculentum* Mill.) are grown in most countries around the world. During the harvest season, tomato fruits ripen in flushes or production waves. In warm environments, fruits can be

collected once or twice a week depending on the color. In Egypt, tomatoes are available all year round, and the average yield is 30–35 ton per feddan (4,200 m²), therefore it is considered as one of the most important

vegetable crops for exportation purposes. The fruit improves the supply of vitamins and minerals in human nutrition (Anonymous 2002; Sabongari and Aliero 2004). In Egypt it is a good source of income for many farmers in both local and export trade. Tomato is susceptible to postharvest diseases caused by various pathogenic fungi. *Botrytis cinerea* (gray mold), *Rhizopus stolonifer* (soft rot), *P. digitatum* (green mold) and *A. alternata* (black mold or alternaria rot) are the most important decay pathogens of tomato causing postharvest losses at high frequency (Fatih *et al.* 2005; Abd-Allah *et al.* 2011). The diseases are favored by warm rainy weather or dew formation on the fruit surface, and its severity of infection is greater in ripe fruits than at the green stage (Pearson and Hall 1975).

Fungicides positively applied control many plant pathogenic fungi. On the other hand, fungicides pollute the environment, cause phytotoxicity to the host plant and also increase the probability of increasing fungi resistance to the fungicides. So, natural products could be applied against many fungi without harming the environment (Hahn 2014; Shoala 2018).

Rosmarinic acid (RA) is a phenolic compound and caffeic acid ester with many significant biological activities. It also has antiviral, antibacterial, anti-inflammatory and antioxidant activities (Petersen and Simmonds 2003). Rosmarinic acid is naturally produced in numerous plants of the Lamiaceae family, such as rosemary (*Rosmarinus officinalis*), common sage (*Salvia officinalis*), spanish sage (*Salvia lavandulifolia*), basil (*Ocimum tenuiflorum*), oregano (*Origanum vulgare*), marjoram (*Origanum majorana*), and lemon balm (*Melissa officinalis*) (Petersen *et al.* 2009).

Glycyrrhizic acid (GA) has been given a specified status, namely Generally Recognized as Safe (GRAS) in the USA in 1985 (Anonymous 1985). Glycyrrhizic acid is a triterpene glycoside which is naturally produced in the roots of licorice plants (*Glycyrrhiza glabra*). Glycyrrhizic acid is the most essential active constituent in the licorice root with a varied range of pharmacological and biological activities (Yong 2012).

Nanomaterials have been applied successfully against many plant pathogenic fungi. Transforming natural products to nano-size could be applied successfully to control phytopathogenic fungi without harming the environment and human beings. Natural nanoparticles could: enhance the nano-activity of natural products against phytopathogens, increase the shelf life of fruits and vegetables at room temperature, decrease the consequences and toxicity of using pesticides and reduce the negative impact of using unsafe nanomaterials (Shoala 2018).

This research was aimed to control tomato phytopathogenic fungi *A. alternata* and *P. digitatum* by using

natural nanomaterials: rosmarinic acid, glycyrrhizic acid and glycyrrhizic acid ammonium salt.

Materials and Methods

Source of the tested fungal isolates

One isolate of *A. alternata* and one isolate of *P. digitatum* were previously isolated from infected tomato fruits with black mold rot or green mold and tested for their pathogenicity at the Department of Post-Harvest Diseases, Plant Pathology Research Institute, ARC, Giza, Egypt. Purified isolates were identified on the basis of their morphological and cultural characteristics using the identification keys of Gilman (1957) and Barnett and Hunter (1972) for *A. alternata* and Pitt (2010) and NMRC (2015) for *P. digitatum*. Identification was confirmed in the Mycology Research and Disease Survey Dept., Plant Pathology Research Institute, ARC, Giza governorate, Egypt. The isolate was incubated onto potato dextrose agar (PDA) medium in Petri dishes at $25 \pm 2^\circ\text{C}$ for 7 days for further studies *in vitro* and for artificial inoculation of tomato fruits in disease control experiments.

Nano synthesis of glycyrrhizic acid

Glycyrrhizic acid was purchased from Sigma-Aldrich Company (CAS Number: 1405-86-3), and 0.2 mg of glycyrrhizic acid was dissolved in 1 ml absolute ethanol and sonicated (XUBA3Analogue Ultrasonic Bath, Grant Company) with an ultrasonic power and frequency of 50 kHz for an hour at room temperature (25°C).

Nano synthesis of glycyrrhizic acid ammonium salt

Glycyrrhizic acid ammonium salt was purchased from Sigma-Aldrich company (CAS number : 53956-04-0), and 0.1 mg of glycyrrhizic acid ammonium salt was dissolved in 1 ml absolute ethanol and sonicated (XUBA3Analogue Ultrasonic Bath, Grant Company) with an ultrasonic power and frequency of 50 kHz for an hour at room temperature (25°C).

Nano synthesis of rosmarinic acid

Rosmarinic acid was purchased from Sigma-Aldrich Company (CAS Number: 20283-92-5), and 0.1 mg of rosmarinic acid was dissolved in 1 ml absolute ethanol and sonicated (XUBA3Analogue Ultrasonic Bath, Grant Company) with an ultrasonic power and frequency of 50 kHz for an hour at room temperature (25°C).

Characterization of nanomaterials by using Dynamic light scattering (DLS)

Measurement of nano-glycyrrhizic acid nanoparticles (GA-NPs), nano-glycyrrhizic acid ammonium salt nanoparticles (GAS-NPs) and nano-rosmarinic acid nanoparticles (RA-NPs) distribution and size were performed by a dynamic light scattering method using Zetasizer Nano ZS (Malvern Instruments, UK) at room temperature. Prior to measurement, 30 μ l of the nanoparticles were diluted with 3 ml of water at 25°C. Particle size data was expressed as the mean of the Z-average of three independent batches of the nanoparticles.

Effect of some nanoparticles on growth of *Alternaria alternata* and *Penicillium digitatum* in vitro

Three nanoparticles, i.e. RA-NPs at concentrations of 0.3–0.6 mM, GA-NPs and GAS-NPs at concentrations of 0.1–0.2 mM were evaluated for their capability to suppress fungal growth of *A. alternata* and *P. digitatum* in vitro; each material was added to PDA medium. Treated or untreated medium with nanoparticles were poured into three Petri dishes per each treatment. After medium solidification, 3 mm mycelia-discs cut from the periphery of 7-day-old cultures of both pathogenic fungi were centrally placed on the surface of nanoparticle amended medium, then incubated at 24°C for 5 days. The diameter of developed colonies was measured when fungal mycelium covered one plate in the control treatment. The percentage of reduction in the colony diameter was calculated using the formula suggested by Sirirat *et al.* (2009) as follows:

$$\text{Reduction} = \frac{\Delta d_0 - \Delta d}{\Delta d} \times 100 [\%],$$

where: Δd_0 and Δd are the average diameters of the fungal colonies in the control and treatment sets, respectively.

Preparation of tissue samples for scanning electron microscopy examination

The hyphal morphological changes of *P. digitatum* and *A. alternata* by nano-rosmarinic acid at 0.6 mM, nano-glycyrrhizic acid and nano-glycyrrhizic acid ammonium salt at 0.2 mM were observed with a scanning electron microscope. The tissue samples of *P. digitatum* and *A. alternata* were prepared by cutting the agar after 4 day incubation periods, fixated by glutaraldehyde 2.5% and dehydrated by serial dilution of ethanol with agitation using an automatic tissue

processor (Leica EM TP, Leica Microsystems; Austria). Then the samples were dried using a CO₂ critical point drier (Model: Audosamdri-815, Tousimis; Rockville, Maryland, USA) and coated by gold sputter coater (SPI-Module, USA).

Microscopic examination

The coated samples were observed with a scanning electron microscopy (Model: JSM-5500 LV; JEOL LTD-Japan) by using the high vacuum mode at the Regional Center of Mycology and Biotechnology, Cairo, Egypt.

Effect of post-harvest treatments with some nanoparticles on fruit rot incidence of stored tomato fruits at 10 \pm 1°C and 90–95% RH for 20 days.

Nano-glycyrrhizic acid (GA-NPs) and nano-glycyrrhizic acid ammonium salt (GAS-NPs) at concentrations of 0.1–0.2 mM, and nano-rosmarinic acid (RA-NPs) at concentrations of 0.3–0.6 mM were tested for controlling tomato fruit rot caused by the two tested pathogens with natural and artificial infection.

Tomato (*L. esculentum* cv. Super Strain B) fruits were harvested at commercial maturity in the last week of March 2018 based on changes in skin ground color and flesh firmness from El-Qanater, a private field region in Qalubia governorate. The fruits were selected for uniform size, color and shape. The fruits were transported on the same day to the laboratory. Tomato fruits were divided into three groups; the first and second groups were washed thoroughly with tap water, sterilized in 70% ethanol for 1 min and wounded by puncturing the peel of each fruit on the equator with a template of four sterilized steel rods (2 mm deep by 0.5 mm diameter) to make a circle area with a 5 mm diameter. The third group was used without sterilization as natural infection, then left to dry under sterilized room temperature conditions.

The first and second groups were used for artificial inoculation with *A. alternata* and *P. digitatum*, respectively. The inoculum from each fungus was prepared by brushing the surface of the culture in the presence of 10 ml sterilized water per each dish and then the spore suspensions were filtered through muslin. The concentration of spore suspension was adjusted to about 4 \times 10⁵ spores \cdot ml⁻¹ using a hemocytometer. The inoculation was carried out by spraying the surface of fruits with 7-day-old cultures of fungal spore suspensions 4 \times 10⁵ spores \cdot ml⁻¹, using an atomizer. Twenty-four hours post incubation the artificially and naturally inoculated tomato fruits were dipped separately in different concentrations of all tested nanoparticle solutions for 2 min, while the control treatment was immersed in sterilized distilled water, then left to dry under sterilized room temperature conditions. Three replicates were used for each treatment.

Each replicate consisted of 15 fruits and were packed in punctured carton boxes. Naturally infected and artificially inoculated tomato fruits were stored at $10 \pm 1^\circ\text{C}$ with 90–95% RH for 20 days. Disease severity (*DS*) was calculated using the following formula according to Babalar *et al.* (2007):

$$DS = \frac{\sum(n \times v)}{4N} \times 100 [\%],$$

where: *n* – number of infected fruits in each category, *N* – total number of fruits, 4 – maximum of numerical values of symptoms categories, *v* – numerical values of symptoms category.

Fruit decay index (scores)

Decay incidence of each fruit was determined by scores. According to the amount of decay on the fruit surface, scales from 1 to 5 were given to each treatment where: 1 – normal (no decay on fruit surface), 2 – trace (up to 5% of fruit surface were decayed), 3 – slight (5–20% of fruit surface were decayed), 4 – moderate (20–50% of fruit surface were decayed), 5 – severe (>50% of fruit surface were decayed).

Effects of some nanoparticles on other tomato fruit qualities

Fruit firmness

The fruit firmness was measured on the two opposite sides of tomato fruit samples by using a hand Magness-Taylor pressure tester ($\text{lb} \cdot \text{in}^{-2}$).

Total Soluble Solids (*TSS*)

Total Soluble Solids (*TSS*) in the extracted fruit juice was measured using Digital refractometer [PR32 (0.32% Atago Paleta ATago.CO. LTD. Japan)] and the results were expressed as Brix.

Titrateable acidity (*TA*)

Five ml of extracted fruit juice was diluted to 45 ml with distilled water. Then, the extracted fruit juice was titrated with 0.1 N sodium hydroxide to a pH of 8.1. Titrateable acidity (*TA*) was determined as a percentage of citric acid by this formula:

$$TA = [(V \times N \times \text{meq})/Y] \times 100 [\%],$$

where: *V* – volume [ml] of sodium hydroxide used, *N* – sodium hydroxide normality, meq – 0.064, *Y* – volume [ml] of bulk fruit juice (Saltveit 2005).

Statistical analysis

Obtained data were statistically analyzed through Co-Stat 3.4 software as the usual technique of analysis of variance (Gomez and Gomez 1984). The mean was compared using least significant difference (*LSD*) at $p = 0.05$ as outlined by Duncan (1955).

Results and Discussion

Zeta potential analyzer for synthesized nanoparticles

The dynamic light scattering (DLS) technique was performed to understand the size distribution and the stability of prepared glycyrrhizic acid nanoparticles (GA-NPs), glycyrrhizic acid ammonium salt nanoparticles (GAS-NPs) and rosmarinic acid nanoparticles (RA-NPs) which have a size distribution range mainly within 50–65 nm, 0–20 nm and 10–30 nm, respectively, as shown in Figures 1, 2, and 3.

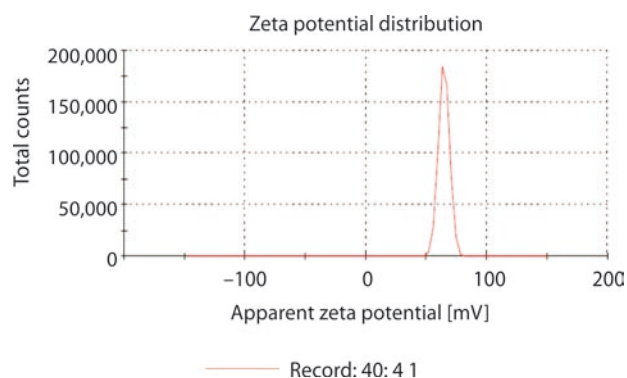


Fig. 1. Zeta potential of glycyrrhizic acid nanoparticles

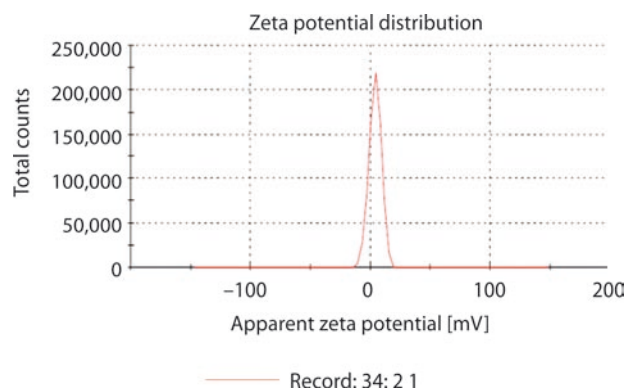


Fig. 2. Zeta potential of glycyrrhizic acid ammonium salt nanoparticles

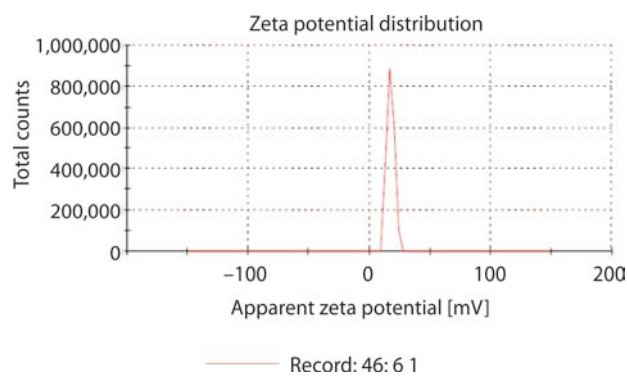


Fig. 3. Zeta potential of rosmarinic acid nanoparticles

Anti-fungal activity of selected nanoparticles against phytopathogenic fungi *Alternaria alternata* and *Penicillium digitatum*

The effects of selected nanoparticles, i.e., rosmarinic acid, glycyrrhizic acids and glycyrrhizic acid ammonium salt at different concentrations on the growth diameter of fungi are shown in Table 1. All nanoparticles at two concentrations reduced the growth of *A. alternata* and *P. digitatum* compared to the control. Data followed by the same letter are not significantly different, whereas those followed by different letters are significantly different. Glycyrrhizic acid nanoparticles were the best among all tested nanoparticles where their tested concentrations (0.1 mM and 0.2 mM) reduced the growth of *A. alternata* to 88.70 and 88.89%, respectively, and *P. digitatum* to 55.56%. It was clear from the obtained results that *A. alternata* was the most sensitive of all tested pathogens to all tested nanoparticles at various concentrations while *P. digitatum* was the least sensitive. Data also showed that, rosmarinic acid nanoparticles were less effective on the growth of *A. alternata* at 0.3 mM. Meanwhile, glycyrrhizic acid ammonium salt nanoparticles were less effective in reducing the growth of *P. digitatum* at all concentrations. Similar results were obtained by Aguilar-Mendez *et al.* (2011) who reported that silver nanoparticles significantly reduced mycelia growth in a dose-dependent manner *in vitro*. Also, Kanhed *et al.* (2014) found that copper nanoparticles showed remarkable activity against *A. alternata*. Significant inhibition in mycelia growth of *A. alternata* supplied with 15 mg · l⁻¹ silver and copper nanoparticles on PDA was reported by Ouda (2014). Silicon dioxide nanoparticle, 200 nm in size, inhibited the growth of *P. verrucosum* (Kotzybik *et al.* 2016). The growth of *P. digitatum*, *A. alternata* and *A. citri*, was significantly suppressed by silver nanoparticles in a concentration dependent way

and the maximum inhibitory effect was recorded at a concentration of 150 ppm (Abdelmalek and Salah Eldin 2016).

Scanning electron microscopy

Rosmarinic acid, glycyrrhizic acids and glycyrrhizic acid ammonium salt nanoparticles caused morphological alterations in treated mycelium of *A. alternata* and *P. digitatum* grown on PDA medium. The mycelial growth of untreated *A. alternata* and *P. digitatum* showed typical mycelial structures for both fungi (Fig. 4A and Fig. 5E, respectively). The mycelium of *A. alternata* was severely damaged in the presence of rosmarinic acid nanoparticles (Fig. 4B). But, in the presence of glycyrrhizic acid and glycyrrhizic acid ammonium salt nanoparticles severe deformities, swelling and a lot of depositions occurred on the mycelium of *A. alternata* (Fig. 4C and D).

The treatment of *P. digitatum* with glycyrrhizic acid nanoparticles caused structural changes in mycelium, cell wall deformations, intense dehydration of membranes and membrane damage (Fig. 5G). Rosmarinic acid and glycyrrhizic acid ammonium salt nanoparticles caused severe changes in mycelial structure, featured by hyphal deformation and membrane dehydration in *P. digitatum*, and significant alterations in spore form and germination (Fig. 5F and H). This is consistent with previous findings reported by Bundschuh *et al.* (2012), in this case with *Daphnia pulex*. Even low concentrations of nanoparticles (<2 mg · l⁻¹) showed significant interactions between the chitin exoskeleton of water fleas. Similar observations have been reported recently by Kotzybik *et al.* (2016) who found the effectiveness in inhibiting the growth of *P. verrucosum* with small sized nanoparticles. This strongly supports our hypothesis that the small particles penetrate into the fungal cell and disturb important cellular mechanisms.

Table 1. Effect of selected nanoparticles on the fungal growth of *Alternaria alternata* and *Penicillium digitatum*

Treatments	Concentration [mM]	Linear growth			
		<i>Alternaria alternata</i>		<i>Penicillium digitatum</i>	
		mm	Ef %	mm	Ef %
Nano-rosmarinic acid	0.3	23.67 b	73.70	53.33 b	40.74
	0.6	10.33 d	88.52	46.67 bc	48.15
Nano-glycyrrhizic acid	0.1	10.17 d	88.70	40.00 c	55.56
	0.2	10.00 d	88.89	40.00 c	55.56
Nano-glycyrrhizic acid ammonium salt	0.1	16.83 c	81.30	56.67 b	37.04
	0.2	13.50 cd	85.00	56.67 b	37.04
Control		90.00 a		90.00 a	

Fungi grown on PDA medium at 25 ± 2°C for 7 days; within each column, same letter/s indicates no significant differences among treatments ($p < 0.05$); Ef% – efficiency as percentage

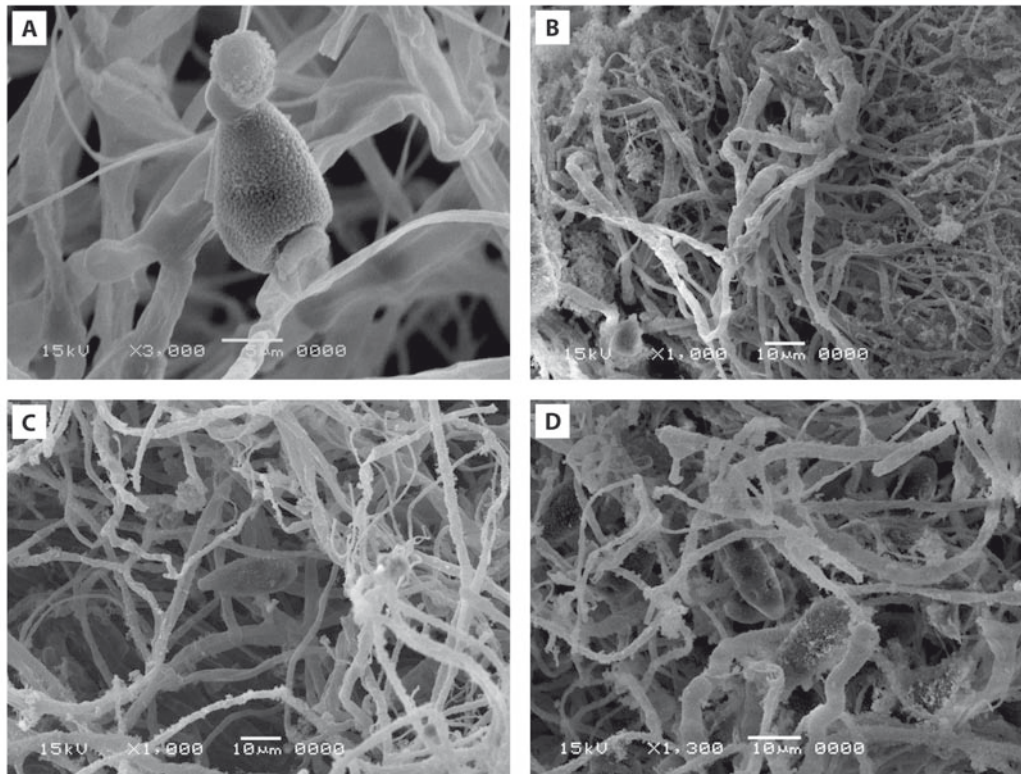


Fig. 4. Effect of some nanoparticles on the cell wall of *Alternaria alternata*. A – control, B – nano-rosmarinic acid, C – nano-glycyrrhizic acid, D – nano-glycyrrhizic acid ammonium salt

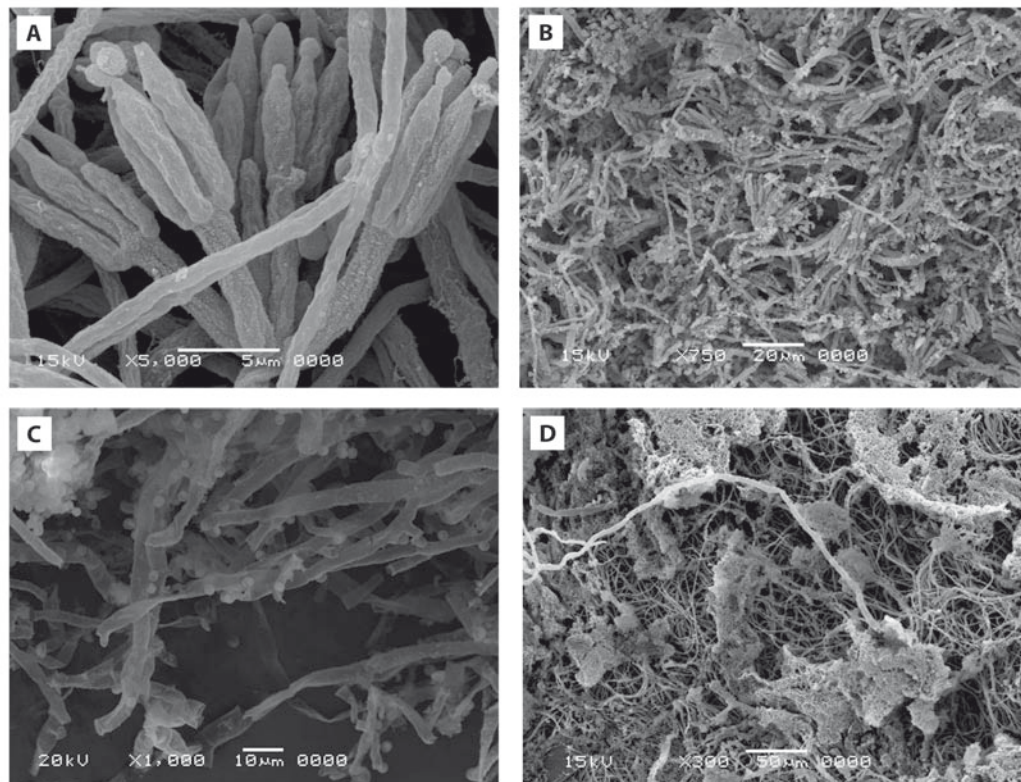


Fig. 5. Effect of some nanoparticles on the cell wall of *Penicillium digitatum*. A – control, B – nano-rosmarinic acid, C – nano-glycyrrhizic acid, D – nano-glycyrrhizic acid ammonium salt

Silver and copper nanoparticles clearly damaged the hyphae of *A. alternata* and fungal mycelia were damaged due to the effect of silver nanoparticles. Deformities in conidia were also seen (Ouda 2014).

Effects of post-harvest treatments with some nanoparticles on tomato fruit rot during storage

Treating tomato fruits with some nanoparticles (rosmarinic acid, glycyrrhizic acids and glycyrrhizic acid ammonium salt nanoparticles) as postharvest treatments reduced greatly the disease severity of fruits infected with *A. alternata* and *P. digitatum* during cold storage compared with the control (Table 2). Glycyrrhizic acid nanoparticles at 0.2 mM were the most effective of all tested treatments against *A. alternata* where it significantly reduced the development of black mold rot on tomato fruits followed by rosmarinic acid nanoparticles at 0.6 mM. Meanwhile, rosmarinic acid nanoparticles showed high efficiency in reducing the disease severity of tomato fruits naturally and artificially inoculated with *P. digitatum* compared with other treatments followed by glycyrrhizic acid ammonium salt at 0.2 mM. Glycyrrhizic acid ammonium salt nanoparticles at 0.1 mM were less effective on disease severity of *A. alternata* on tomato fruits during cold storage. Glycyrrhizic acid nanoparticles were less effective on the reduction of disease severity caused by *P. digitatum* as well as naturally infected tomato fruits. It was clear that increasing the concentration of tested nanoparticles increased gradually the effectivity of these nanoparticles in reducing the disease severity caused by *A. alternata* and *P. digitatum* on tomato fruits. This is consistent with the findings of Lamsal *et al.* (2011) who found that the efficacy of silver nanoparticles against pepper anthracnose caused by *Colletotrichum gloeosporioides* was good at 50 ppm to inhibit disease spread. Also,

Yu *et al.* (2012) found that jujube fruits coated with chitosan film with 0.04% nano-silicon dioxide showed lower red indices, decay incidence, respiration rate, and weight loss. Treating banana fruits with silver and neem-silver nanoparticles reduced the disease incidence on fruits caused by *Colletotrichum musae* (Jagana *et al.* 2017).

Effects of selected nanoparticles on the quality of postharvest tomatoes

Fruit firmness

The results showed a rapid decrease in tomato fruit firmness in the control set compared to tomato fruits treated with all tested nanoparticles, 10 days after storage. The tomato fruits treated with different nanoparticles were firmer than untreated ones.

The tomato fruits artificially inoculated with *A. alternata* recorded flesh firmness of 1.5 lb · in⁻² in the control compared to 2.40, 2.43, 2.87 lb · in⁻² in the treated fruits with nano-rosmarinic acid (0.6 mM), nano-glycyrrhizic acid (0.2 mM) and nano-glycyrrhizic acid ammonium salt (0.2 mM), respectively, 10 days after storage. Tomato fruits artificially inoculated with *P. digitatum* scored flesh firmness of 2.7 lb · in⁻² compared to 2.50, 3.40, 2.73 lb · in⁻² in the fruits treated with nano-rosmarinic acid (0.6 mM), nano-glycyrrhizic acid (0.2 mM) and nano-glycyrrhizic acid ammonium salt (0.2 mM), respectively, 10 days after storage. On the other hand tomato fruits artificially inoculated with *A. alternata* recorded flesh firmness of 3.53 lb · in⁻² in the control compared to 2.70, 2.60, 3.13 lb · in⁻² in the fruits treated with nano-rosmarinic acid (0.3 mM), nano-glycyrrhizic acid (0.1 mM) and nano-glycyrrhizic acid ammonium salt (0.1 mM), respectively, 20 days after storage. Tomato fruits artificially inoculated with *P. digitatum* scored flesh firmness of 2.87 lb · in⁻² compared to 2.23, 3.17, 3.50 lb · in⁻² in

Table 2. Effects of some nanoparticles as postharvest treatments on black mold rot and green mold development in naturally and artificially inoculated tomato fruits during storage

Treatments	Concentration [mM]	Disease severity					
		artificial infection				natural infection	
		<i>Alternaria alternata</i>		<i>Penicillium digitatum</i>		DS	Ef%
DS	Ef%	DS	Ef%				
Nano-rosmarinic acid	0.3	15.00 bc	80.00	26.67 b	65.22	15.00 b	65.38
	0.6	11.67 bc	84.44	21.67 b	71.74	11.67 b	73.08
Nano-glycyrrhizic acid	0.1	13.33 bc	82.22	30.00 b	60.87	16.67 b	61.54
	0.2	8.33 c	88.89	26.67 b	65.22	15.00 b	65.38
Nano-glycyrrhizic acid ammonium salt	0.1	20.00 b	73.33	26.67 b	65.22	15.00 b	65.38
	0.2	18.33 bc	75.56	23.33 b	69.57	13.33 b	69.23
Control		75.00 a		76.67 a		43.33 a	

Within each column, same letter/s indicates no significant differences among treatments ($p < 0.05$); Ef% – efficacy as percentage; DS – disease severity; control was infected with *Alternaria alternata* and *Penicillium digitatum* or without infection and un-treated

the fruits treated with nano-rosmarinic acid (0.6 mM), nano-glycyrrhizic acid (0.2 mM) and nano-glycyrrhizic acid ammonium salt (0.2 mM), respectively, 20 days after storage.

Naturally infected tomato fruits scored flesh firmness of 1.50 lb · in⁻², 10 days after infection compared to 4.80 lb · in⁻², and 20 days after infection. However, tomato fruits naturally infected with nanoparticles nano-rosmarinic acid (0.3 mM), nano-glycyrrhizic acid (0.2 mM) and nano-glycyrrhizic acid ammonium salt (0.2 mM) recorded fruit firmness of 2.77, 2.77 and 3.37 lb · in⁻², respectively, 10 days after storage. Tomato fruits naturally infected with nanoparticles nano-rosmarinic acid (0.6 mM), nano-glycyrrhizic acid (0.2 mM) and nano-glycyrrhizic acid ammonium salt (0.2 mM) recorded fruit firmness of 3.00, 2.70 and 2.30 lb · in⁻², respectively, 20 days after storage (Table 3).

No significant changes were observed in firmness during storage for any treatments (Table 3). The application of the Cu nanoparticles induced the production of fruits with greater firmness. Vitamin C, lycopene, and the ABTS antioxidant capacity increased compared to the control (López-Vargas *et al.* 2018). Also, Juarez-Maldonado *et al.* (2016) reported that the application of Cu NPs + chitosan increased the firmness of tomato fruits by 9%. The firmness of non-coated and coated fruits and vegetables (apples, red grapes, tomatoes and sweet peppers) was gradually decreased with increasing the storage period at cooled temperatures (Abdel Bakhy *et al.* 2018).

Total Soluble Solids (TSS)

In this study, TSS changes in tomato fruits were not significant during the storage period and no regular trend

Table 3. Changes in tomato fruit firmness (lb · in⁻²) in the tomato fruits artificially inoculated with *Alternaria alternata* and *Penicillium digitatum* fungi compared with naturally infected tomato fruits and fruits treated with selected nanoparticles

Treatments	Concentration [mM]	Artificial infection				Natural infection	
		<i>Alternaria alternata</i>		<i>Penicillium digitatum</i>		10 day storage	20 day storage
		10 day storage	20 day storage	10 day storage	20 day storage		
Nano-rosmarinic acid	0.3	2.83 ab	2.70 a	3.13 a	3.30 a	2.77 a	3.57 a
	0.6	2.40 ab	3.20 a	2.50 a	2.23 a	3.37 a	3.00 a
Nano-glycyrrhizic acid	0.1	3.05 a	2.60 a	3.37 a	3.20 a	2.80 a	3.87 a
	0.2	2.43 ab	2.93 a	3.40 a	3.17 a	2.77 a	2.70 a
Nano-glycyrrhizic acid ammonium salt	0.1	3.13 a	3.13 a	3.20 a	3.77 a	3.57 a	2.53 a
	0.2	2.87 ab	3.40 a	2.73 a	3.50 a	3.37 a	2.30 a
Control		1.50 b	3.53 a	2.17 a	2.87 a	1.30 b	4.80 a
Zero time		3.90					

Within each column, same letter/s indicates no significant differences among treatments ($p < 0.05$); control was infected with *Alternaria alternata* and *Penicillium digitatum* or without infection and un-treated

Table 4. Changes in Total Soluble Solids (TSS) percentages of tomato fruits naturally and artificially inoculated with *Alternaria alternata* and *Penicillium digitatum* and treated with some nanoparticles

Treatments	Concentration [mM]	Artificial infection				Natural infection	
		<i>Alternaria alternata</i>		<i>Penicillium digitatum</i>		10 day storage	20 day storage
		10 day storage	20 day storage	10 day storage	20 day storage		
Nano-rosmarinic acid	0.3	4.87 a	4.73 a	5.53 a	4.47 a	5.30 a	4.80 a
	0.6	4.20 a	4.23 a	4.87 a	3.87 a	4.90 ab	4.90 a
Nano-glycyrrhizic acid	0.1	4.77 a	4.80 a	5.17 a	4.50 a	5.60 a	5.10 a
	0.2	5.10 a	3.87 a	5.77 a	4.33 a	4.87 ab	3.97 a
Nano-glycyrrhizic acid ammonium salt	0.1	5.10 a	2.73 b	5.07 a	4.17 a	4.83 ab	4.83 a
	0.2	5.53 a	2.73 b	4.93 a	4.43 a	5.73 a	4.17 a
Control		4.77 a	4.40 a	4.87 a	4.23 a	4.17 b	3.77 a
Zero time		4.27					

Within each column, same letter/s indicates no significant difference among treatments ($p < 0.05$); control was infected with *Alternaria alternata* and *Penicillium digitatum* or without infection and un-treated

was observed (Table 4). But, *TSS* for all treatments decreased towards the end of storage. At harvest, the *TSS* content of tomato fruits was 4.27. At the end of storage, the highest *TSS* content in the trials was shown in the naturally infected fruits and those treated with glycyrrhizic acid nanoparticles at 0.1 mM (5.10), while the lowest *TSS* values were determined in the fruits infected by *A. alternata* and treated with glycyrrhizic acid ammonium salt nanoparticles at 0.1 mM and 0.2 mM (2.73). Also, rosmarinic acid and glycyrrhizic acid nanoparticles at low concentrations increased *TSS* content in tomato fruits naturally and artificially inoculated with *A. alternata* and *P. digitatum* compared with the control. These results showed a clear effect of the tested nanoparticles at low concentrations on decreasing fruit metabolism, leading to maintenance of respiration substrates, respiration rate and in turn to a delay of the postharvest ripening process. In this respect, glycyrrhizic acid nanoparticles at 0.1 mM were the most effective in increasing *TSS* content in tomato fruits naturally and artificially inoculated with the two tested fungi 20 days after storage compared with other treatments. Application of Cu NPs + chitosan did not generate differences in the *TSS* in tomato fruits (Juárez-Maldonado *et al.* 2016). The decrease in *TSS* can be caused by the use of simple sugars in cellular respiration (Klunklin and Savage 2017). Application of Cu NPs in chitosan-polyvinyl alcohol (Cs-PVA) hydrogels increased the soluble solid content, titratable acidity, lycopene content and total antioxidant capacity in the tomato fruits (Hernández *et al.* 2017).

Titratable acidity (TA)

The changes in the total acidity of tomato fruits were determined during the storage period. The obtained

results are recorded in Table 5. The results indicated that the total acidity gradually decreased with increasing the storage period for tomatoes. The main control of naturally infected fruits had the lower *TA* % than other control of the fruits with artificially inoculated fungi. Titratable acidity in control tomato fruits inoculated with *P. digitatum* was lower than *TA* in control tomato fruits inoculated with *A. alternata*. The decreases were significantly lower in tomato fruits treated with rosmarinic acid nanoparticles at 0.3 mM and inoculated with *A. alternata* 20 days after storage. Also, acidity losses were higher in tomato fruits naturally and artificially inoculated with *P. digitatum* 20 days after storage by the use of rosmarinic acid and glycyrrhizic acid nanoparticles at high concentrations. Glycyrrhizic acid nanoparticles at 0.1 mM achieved higher titratable acidity in tomato fruits naturally and artificially inoculated with *P. digitatum* 10 days after storage, since it maintained the *TA* content of tomato fruits at significantly higher levels. Artificial inoculation of tomato fruits with *A. alternata* caused a smaller *TA* percentage than that inoculated with *P. digitatum* with all tested treatments at low concentrations 20 days after storage. The highest *TA* content was shown in infected tomato fruits with *A. alternata* and treated with glycyrrhizic acid ammonium salt nanoparticles at 0.1 mM 10 days after storage. The results of this study are in agreement with those obtained by Konopacka and Plochanski (2004). They found that the titratable acidity of apples gradually decreased with increased storage time. On the other hand, Juárez-Maldonado *et al.* (2016) reported an increase in the *TA* in tomato fruits with the application of Cu NPs + chitosan. Also, Abdel Bakhy *et al.* (2018) reported that total acidity gradually decreased with an increased storage period for tomatoes and sweet green peppers.

Table 5. Changes in titratable acidity percentages of tomato fruits naturally and artificially inoculated with *Alternaria alternata* and *Penicillium digitatum* and treated with some nanoparticles

Treatments	Concentration [mM]	Artificial infection				Natural infection	
		<i>Alternaria alternata</i>		<i>Penicillium digitatum</i>		10 day storage	20 day storage
		10 day storage	20 day storage	10 day storage	20 day storage		
Nano-rosmarinic acid	0.3	1.17 cd	0.43 b	1.17 b	0.70 b	1.30 ab	0.80 b
	0.6	0.90 d	0.77 ab	1.27 ab	0.60 b	1.20 ab	0.63 b
Nano-glycyrrhizic acid	0.1	0.93 d	0.70 ab	1.50 a	0.77 b	1.37 a	0.77 b
	0.2	1.23 c	1.10 a	1.20 b	0.60 b	1.33 a	0.73 b
Nano-glycyrrhizic acid ammonium salt	0.1	1.87 a	0.70 ab	1.17 b	0.87 b	1.10 b	0.97 b
	0.2	1.57 b	0.70 ab	1.37 ab	1.57 a	0.87 c	1.80 a
Control		1.27 c	0.57 ab	1.37 ab	0.73 b	1.23 ab	0.43 b
Zero time		3.37					

Within each column, same letter/s indicates no significant difference among treatments ($p < 0.05$); control was infected with *Alternaria alternata* and *Penicillium digitatum* or without infection and un-treated

Conclusions

The present investigation demonstrated that all tested nanoparticles reduced the growth of *A. alternata* and *P. digitatum*. However, GA-PNs were the most effective since they significantly reduced the linear growth of *A. alternata*. Microscopic observations showed that all tested nanoparticles had detrimental effects on fungal hyphae and conidia. Also, GA-NPs showed minimum decay on tomato fruits caused by *A. alternata* at a high concentration. RA-NPs at 0.6 mM were the most effective treatment against fungal decay in response to artificial inoculation of tomato fruits with *P. digitatum* as well as naturally infected fruits. The three nanomaterials could be useful natural nanomaterials for post-harvest preservation of fruit quality.

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