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RESEARCH PAPER

Green synthesis of Copper Nanoparticles Using Rosemary Extract to Reduce Postharvest Decays Caused by *Botrytis Cinerea* in Tomato

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ABSTRACT

Gray mold disease caused by *Botrytis cinerea* is one of the serious factors that reduce storage period and marketing life of tomato. The decrease of post-harvest wastage of tomato by environmentally friendly fungicides would be of great significance to farmers and consumers. Accordingly, using of antifungal activities of various metal nanoparticles can be suitable method for the management of postharvest disease. In this study, the extract of Rosmarinus officinalis was applied to green synthesis of copper nanoparticles by fast preparation method. Characterizations of green copper nanoparticles were tested by UV–Vis absorption spectroscopy, X-ray diffraction (XRD), scanning electron microscopy (SEM) and dynamic light scattering (DLS). The size of copper nanoparticles were at about 100-300 nm. We examined the in vitro antifungal activity of green copper nanoparticles against *B.cinerea* with different concentrations and the strong inhibitory rate was observed in 15 mmol/l green copper nanoparticles at five day after inoculation. Also, the fruits quality of tomato at 10 days after artificially inoculated by *B.cinerea* and maintained at 24 °C were improved with increasing copper nanoparticles concentrations. According to our results, the green copper nanoparticles can be a good alternative to decrease the tomato fruits rots in comparison chemical fungicides.

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INTRODUCTION

Tomato (Solanum lycopersicum L.) is one of the most popular vegetable crops with a world production of more than 164 million tons per year. It is the seventh most important crop in the world after maize, rice, wheat, potatoes, soybeans and cassava. Based on FAO data in 2019, tomato production in Iran was about 5/25 million tons. Tomato is one of the very perishable fruits and it has a relatively short storage life. Depending on humidity and temperature, its quality immediately decreases and becomes unacceptable, so that post-harvest losses are on average between 24 to 40 percent in developing countries and between 2-20 percent in developed countries. Therefore, the decrease of post-harvest losses is one of the

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important programs to improve shelf life and food security in the world. Growth of fungal pathogens is the main cause of fruit rots during postharvest. Several species of fungi are responsible for this fruit rots. *Botrytis cinerea* is a fungal pathogen that leads to gray mold disease in more than 235 plant species, such as tomato, grape, strawberry, apple, and pear. It is difficult to control fungal growth of *B.cinerea* because this fungus has showed resistance to conventional fungicides. To overcome this resistance, it is important to explore novel antifungal agents, which may replace current control strategies. Many studies have shown bactericidal effects and anti-fungal activities of various metal nanoparticles, including silver, zinc, gold and copper. Among the metal nanoparticles, copper nanoparticles are one of the most extensively studied nanoparticles that can be synthesized using chemical, physical and biological approaches. Physical and chemical synthesis methods are the most common scheme for the synthesis of copper nanoparticles but these methods require toxic solvents, high-energy consumption and stabilizing agents which can cause harmful effects on health and environment. In contrast, biological synthesis methods are low cost, environmentally safe and are less toxic chemicals in the synthesis process. Microorganism cell or plant extracts are considered a reducing agent in the biological synthesis of copper nanoparticles. In plant extract, some metabolites, including terpenoids, polyphenols, sugars, alkaloids, phenolic acids, and proteins, play an important role in the bio-reduction. Many studies have shown that various plant extracts can be utilized to synthesis copper nanoparticles. In recent years, nanoparticle materials have received increasing attention due to their unique physical and chemical properties which differ significantly from their conventional counterparts [1-13].

Rosemary (Rosmarinus officinalis L.) is an evergreen shrub with fragrant needle-like leaves that are used in the medicine, cosmetic and food industries. The rosemary leaf extract is one of the major bioactive compounds which have been reported to possess many biological activities such as antibacterial, antioxidant, antifungal, etc [14].

In the present study, leaf extract of rosemary was used for the biosynthesis of copper nanoparticles and was investigated by advanced characterization techniques. Also, antifungal activity against gray mold disease was investigated by growing *Botrytis cinerea* on agar plates supplemented with different concentrations of

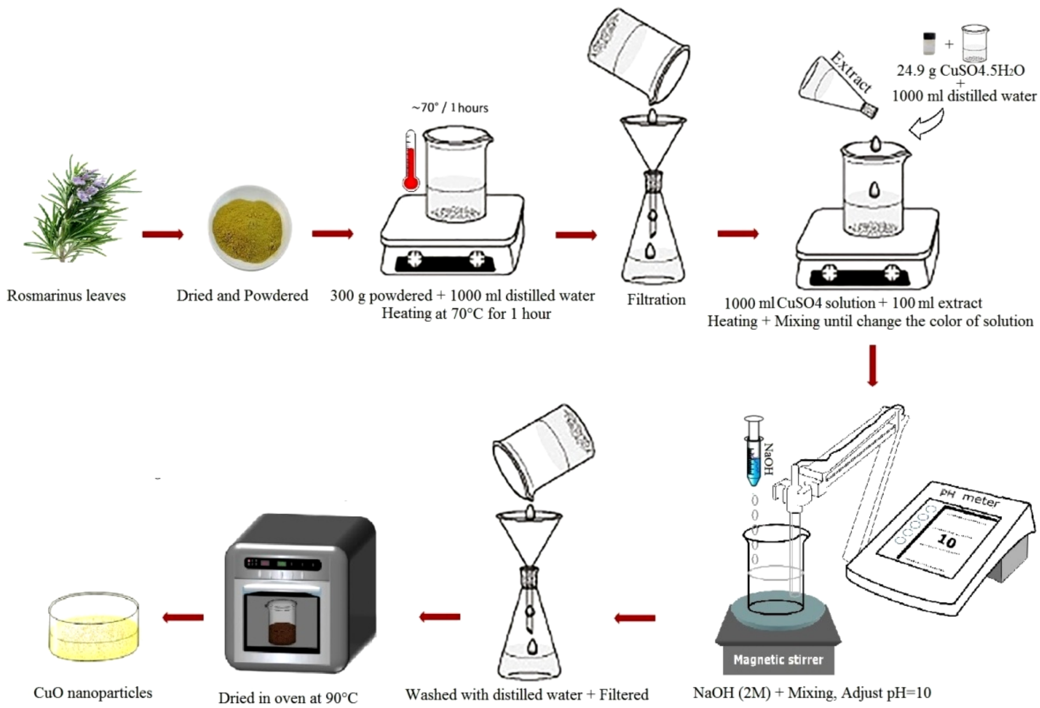

Fig. 1. Schematic presentation of copper nanoparticles synthesis using Rosemary extract

copper nanoparticles. Finally, concentrations of copper nanoparticles that can inhibit tomato fruit rots during postharvest were determined in vivo condition.

MATERIALS AND METHODS

Plant material collection and extract preparation

Rosemary (Rosmarinus officinalis L.) plant was collected from the local habitat in Markazi province, Arak, Iran. Leaves were washed with distilled water, dried at the shadow and finely powdered by the electric grinder. 300 g of powdered leaves were added to 1000 ml distilled water with the concentration of 300 g/L and boiled in at 70 °C for 1 hour. The resultant extract was filtered through sterile Whatman No. 1 filter paper and storage at 4 °C for further use (Fig. 1).

Biosynthesis of copper nanoparticles

Copper (II) sulfate ($CuSO_4$) and sodium borohydride pellets ($NaBH_4$) was purchased from Merck (Germany). To synthesis copper nanoparticles, 24.9 g of Copper (II) sulfate was dissolved in 1000 ml rosemary extract. The solution was put on the heater and heating with mixing continued until the solution color changes to bluish-green. The product was cooled at room temperature. $NaBH_4$ (2 M) was added drop-wise while stirring. A black crystalline precipitate of copper oxide was obtained, which is washed repeatedly with distilled water, filtered and dried in an oven at 90 °C to obtain the copper nanoparticles powder (Fig. 1).

Characterization of synthesized nanoparticles

The solutions were characterized by absorption spectroscopy in the UV-Vis Spectrophotometer. The morphology and size of produced nanoparticles were characterized by Scanning electron Microscopy. The formation of copper nanoparticles using X-ray diffraction (XRD) with a Ni filter and Cu Kα radiation (λ =0.15406 nm), on the PANalytical X'pert PRO powder X-Ray diffractometer. Also, dynamic light scattering (DLS) was used to determine the average size of synthesized copper nanoparticles.

In vitro antifungal assay

Pathogenic fungus of *Botrytis cinerea* was obtained from Iranian Biological Resource Center and it was cultured on PDA (Potato Dextrose Agar) at 25 ◦C in the dark. Antifungal tests were performed by the agar dilution method with some modifications [15]. The autoclaved PDA media with copper nanoparticles solution at concentrations of 0, 3, 5, 7, 11 and 15 mmol/l were poured into the Petri dishes (8 cm diameter). The fungi were inoculated after the PDA media solidified. A disc (1.5 cm) of mycelia material taken from the edge of 7-day-old fungal cultures was placed in the center of each Petri dish. The Petri dish with the inoculums was then incubated at 25 ◦C. The diameter of developed colonies was measured when fungal mycelium covered one plate in the control treatment [16]. Inhibition percentage in colony diameter was calculated using the following formula:

$$\text{Inhibition}(\%) = \frac{\Delta d_0 - \Delta d}{\Delta d_0} \times 100$$

Where: Δd_0 and Δd are the average diameters of fungal colonies in the control and treatment.

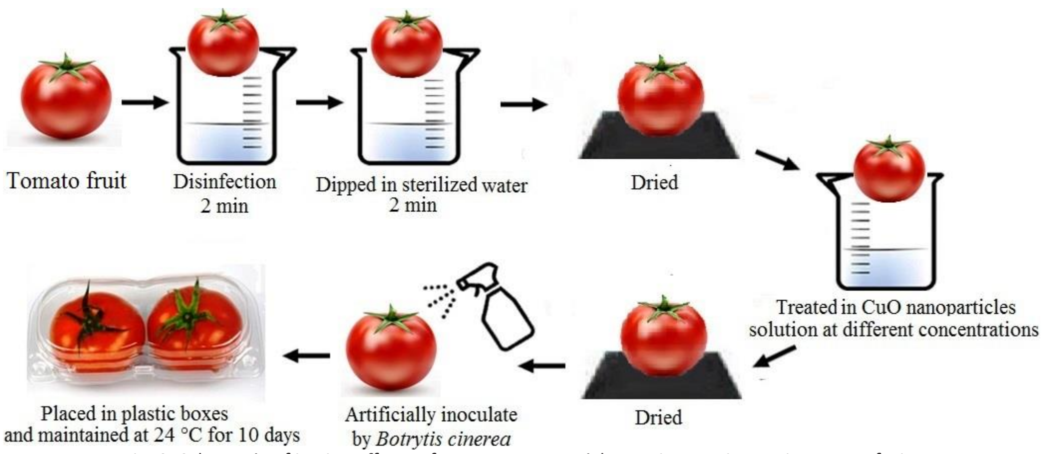

Fig. 2. Schematic of in vivo effects of copper nanoparticles against B. cinerea in tomato fruits

In vivo antifungal assay

The antifungal activity of copper nanoparticles solution was evaluated in tomato (Solanum lycopersicum "Cherry tomato") purchased in a local supermarket. Tomato fruits were properly disinfected with 0.5% (v/v) sodium hypochlorite solution for 2 min, then dipped in sterilized water for 2 min and left at room temperature until they dried completely. Subsequently, tomato fruits were treated with copper nanoparticles solution at four concentrations (0, 5, 15 and 25 mmol/l), and dried at room temperature. The tomato fruits were placed in plastic boxes to artificially inoculate by *B.cinerea* and maintained at 24 °C for 10 days. Disease incidences and symptoms of the treated tomato fruits were measured and observed 10 days after inoculation. All treatments were carried out with three replicates and three fruits for each treatment (Fig. 2). The rate of fruit rots was performed according to the method of Meena et al (2020) with slight modification [17].

Tomato fruits quality assay

The fruit firmness was measured on the two opposite sides of tomato fruit samples by using a penetrometer. Total Soluble Solids (TSS) and pH in the extracted fruit juice were measured according to the instructions mentioned in previous studies [18].

RESULTS AND DISCUSSION

UV–vis spectroscopy analysis

The UV–vis spectrum of synthesized Cu nanoparticles indicated a maximum absorbance at around 540 nm, which was specific for Cu nanoparticles (Fig. 3). Similarly, several studies have also showed the absorption spectrum between 540 and 560 nm due to the formation of Cu nanoparticles [19].

SEM images of synthesized copper nanoparticles

SEM images of copper nanoparticles synthesized with rosemary extract were showed in Fig. 4 and this image indicates that the copper nanoparticles with average diameter size about 50 nm.

XRD pattern of the synthesized copper

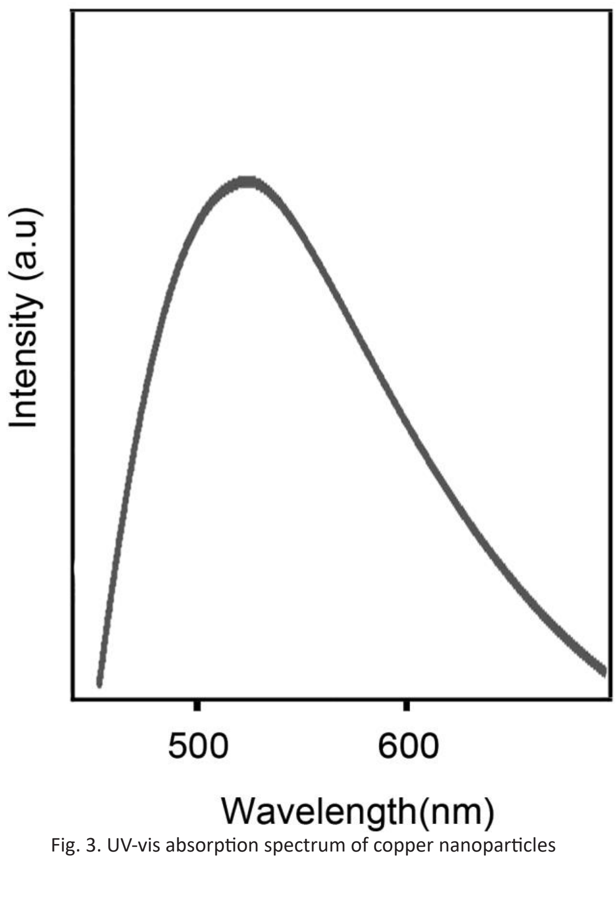

Fig. 3. UV-vis absorption spectrum of copper nanoparticles

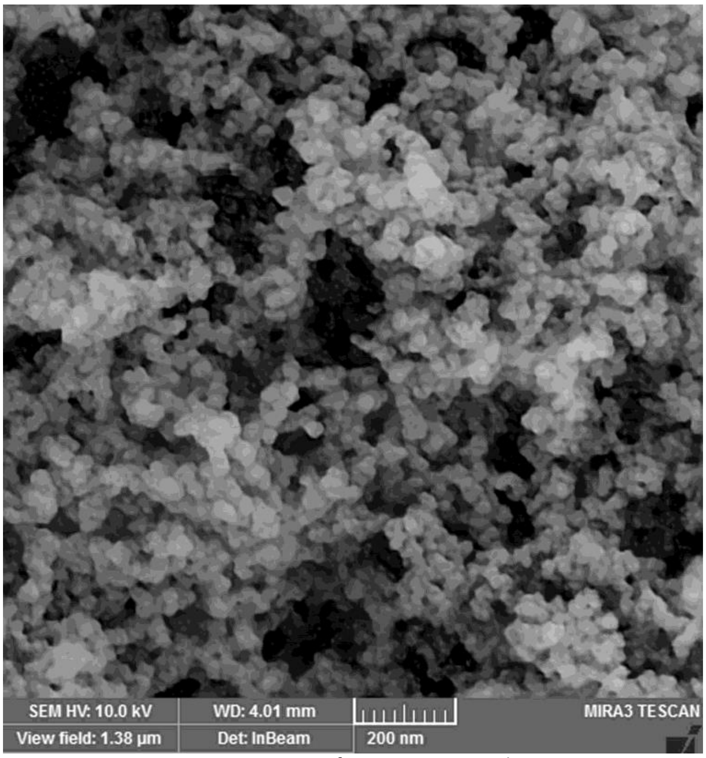

Fig. 4. SEM image of copper nanoparticles

nanoparticles

The structure and formation of copper nanoparticles from the rosemary extract were analysised by X-ray diffraction and it showed the distinctive peaks indexed to the crystal planes of face-centered cubic silver (Fig. 5). The diffraction profile had an intense peak at 2θ of 43◦, 50◦ and 4◦, corresponding to (1 1 1),(2 0 0), (2 2 0), and (3 1 1) planes respectively. Our result is in conformity with the reports of the Joint Committee on Powder (JCPDS: 03-1015).

Dynamic light scattering

Dynamic light scattering (DLS) analysis determined the average particle size distribution profile of synthesized nanoparticles and the size

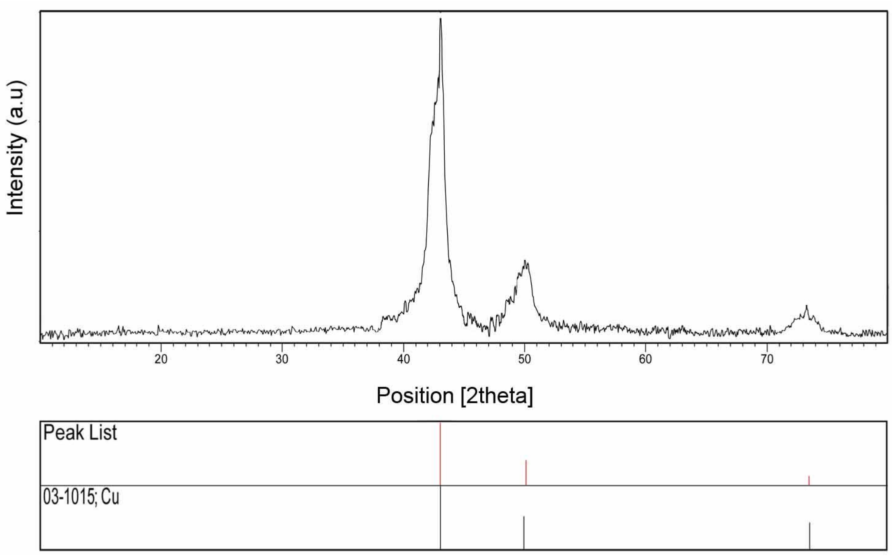

Fig. 5. XRD pattern of copper nanoparticles

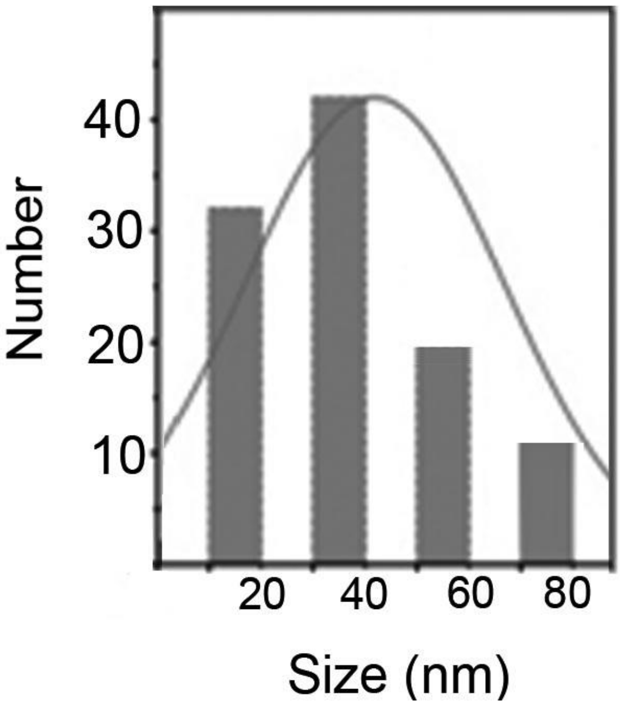

Fig. 6. Particle size distribution profile of copper nanoparticles

of copper nanoparticles were in the range 50 nm hydrodynamic diameter (Fig. 6).

In vitro inhibitory effect of copper nanoparticles against B. cinerea

Our results showed that the copper nanoparticles have significant inhibitory effect on the pathogen growth of *B. cinerea* compared to the control. In vitro culture, the inhibitory rates of 0, 3, 7, 11 and 15 mmol/l copper nanoparticles were 45.71%, 74.78%, 85.43% and 100% at five day after inoculation, respectively (Fig. 7). Various studies have been shown to copper nanoparticles have the antifungal activity against plant infecting fungi and increasing concentration of copper nanoparticles can be enhanced antifungal effectiveness [20-21]. Similar to our results, copper nanoparticles were also found to be suitable for

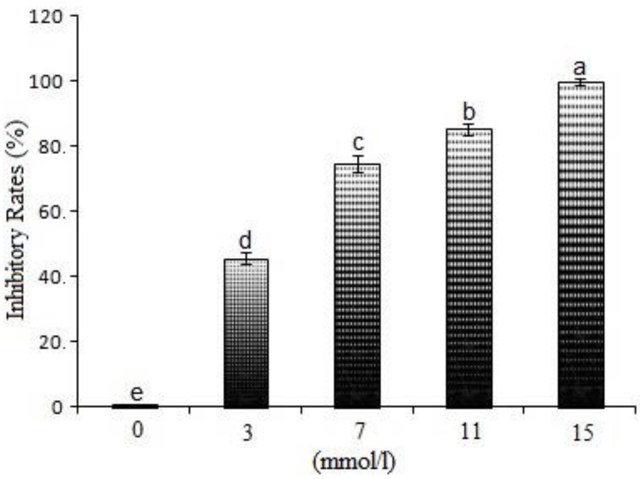

Fig. 7. Antifungal activity of copper nanoparticles concentration against B. cinerea

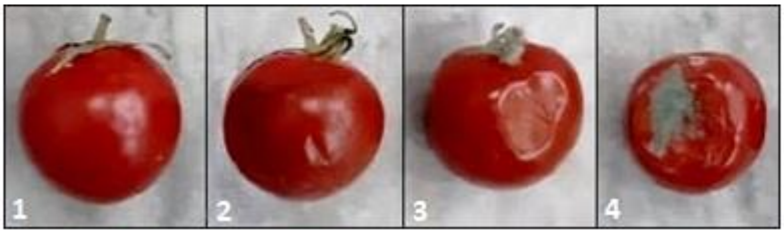

Fig. 8. Effect of copper nanoparticles on microbial decay of tomato maintained at 24 °C for 10 days. Scale ranging from 1 to 4 were given to each treatment group, where 1 = normal (no decay on fruit surface), 2 = slight (up to 5% of fruit surface decayed), 3= moderate (5–25% of fruit surface decayed) and 4 = severe (> 25% of fruit surface decayed).

Table 1. Effects of copper nanoparticles on fruit decay and quality of tomato

Treatments	Concentration (mmol/l)	DW (%)	Fruit decay	pH	TSS (%)	Fruit firmness	Vit C
Control	0	8.88a	3.1a	4.4a	3.92a	8.77b	14.17a
Copper nanoparticles	5	8.61a	2.5ab	4.1a	4.67a	8.89b	14.16a
Copper nanoparticles	15	7.65a	2.3ab	4.2a	4.83a	10.1ab	14.33a
Copper nanoparticles	25	7.27a	1.6b	4.3a	4.33a	12.4a	14.83a

Dissimilar letters in each column indicate a significant difference

the control of the fungal pathogen *Botrytis cinerea* at 15 mg/L concentration [22].

In vivo effects of copper nanoparticles on microbial decay of tomato

The microbial decay was visually inspected in stored tomatoes up to 10 days considering the extent of microbial infection on fruit surface in the scale ranging from 1 to 4 (Fig. 8). Microbial decay was increased in control tomatoes after being stored for 10 days while copper nanoparticles (0.25 %) remarkably inhibited microbial decay (Table 1). Performance of copper nanoparticles on inhibition microbial decay was not only due to the antimicrobial nature of copper but also by its effect on preventing plant cell death by enhancing plant cell immunity [17].

Effects of copper nanoparticles on fruits quality of tomato

In vivo results showed that, the fruits firmness were significantly affected by increasing copper nanoparticles concentrations, but there was no significant change in dry weight percentage, pH, total soluble solids (TSS) and vitamin C, 10 days after storage (Table 1).

Similar to our results, the application of Cu nanoparticles + chitosan increased the firmness of tomato fruits but it didn’t make differences in the total soluble solids [23]. Also, Abdel-Rahman et al (2020) reported that the tomato fruits treated with natural nanoparticles at different concentrations greatly reduced the development of black mold rot and significantly maintained fruit quality characteristics such as fruit firmness, titratable acidity and total soluble solids during cold storage [24].

CONCLUSION

In conclusion, copper nanoparticles with sizes of 70 nm were green synthesized by using the extract of Rosmarinus officinalis. Botrytis growth was completely blocked in vitro when copper nanoparticles with concentration of 15 mmol/l were added to the fungus culture medium. Also copper nanoparticles solution at 25 mmol/l were the most effective treatment against fungal decay in response to artificial inoculation of tomato fruits with *B. cinerea* and it can be useful in the control of gray mold and postharvest quality of tomato.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

REFERENCES

1. Bergougnoux V. Research review paper: The history of tomato: From domestication to biopharming. Biotechnol. Adv, 2014; 32: 170 - 189.
2. Haile A. Shelf life and quality of tomato (Lycopersicon esculentum Mill.) fruits as affected by different Packaging Materials. Afr. J. Food Sci, 2018; 12(2): 21-27.
3. Kader A.A. Increasing food availability by reducing postharvest losses of fresh produce. Int. Postharvest Symp. Acta Hort., 2005; 682: 2169–2178.
4. He L, Liu Y, Mustapha A, Lin M. Antifungal activity of zinc oxide nanoparticles against Botrytis cinerea and Penicillium expansum. Microbiol Res, 2011; 166(3):207–215.
5. Liu C, Chen L, Zhao R, Li R, Zhang Sh, Yu W, Sheng J, Shen L. Melatonin Induces Disease Resistance to Botrytis cinerea in Tomato Fruit by Activating Jasmonic Acid Signaling Pathway. J. Agric. Food Chem, 2019; 67(22): 6116-6124.
6. Stoimenov P.K, Klinger R.L, Marchin G.L, Klabunde K.J. Metal oxide nanoparticles as bactericidal agents. Langmuir, 2002; 18: 6679–6686.
7. Sivakami, M., R. Renuka, T. Thilagavathi. Green syntheses of magnetic nanoparticles via Cinnamomum verum bark extract for biological application. J. Environ. Chem, 2020; 8(5):104420.
8. Singh J, Dutta T, Kim K. H, Rawat M, Samddar P, Kumar P. Green synthesis of metals and their oxide nanoparticles: applications for environmental remediation. J Nanobiotechnology, 2018; 16(1), 1-24.
9. Ahmadian-Fard-Fini S, Salavati-Niasari M, Ghanbari D. Hydrothermal green synthesis of magnetic Fe_3O_4-carbon dots by lemon and grape fruit extracts and as a photoluminescence sensor for detecting of E. coli bacteria. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2018;203:481-93.
10. Ahmadian-Fard-Fini S, Ghanbari D, Amiri O, Salavati-Niasari M. Electro-spinning of cellulose acetate nanofibers/Fe/carbon dot as photoluminescence sensor for mercury (II) and lead (II) ions. Carbohydrate Polymers. 2020;229:115428.
11. Moradi B, Nabiyouni G, Ghanbari D. Rapid photo-degradation of toxic dye pollutants: green synthesis of mono-disperse Fe_3O_4–CeO_2 nanocomposites in the presence of lemon extract. Journal of Materials Science: Materials in Electronics. 2018; 29(13):11065-11080.
12. Etminan M, Nabiyouni G, Ghanbari D. Preparation of tin ferrite–tin oxide by hydrothermal, precipitation and auto-combustion: photo-catalyst and magnetic nanocomposites for degradation of toxic azo-dyes. Journal of Materials Science: Materials in Electronics. 2017; 29(3):1766-1776.
13. Ahmadian-Fard-Fini S, Ghanbari D, Salavati-Niasari M. Photoluminescence carbon dot as a sensor for detecting of Pseudomonas aeruginosa bacteria: Hydrothermal synthesis of magnetic hollow $NiFe_2O_4$-carbon dots nanocomposite material. Composites Part B: Engineering. 2019; 161:564-577.
14. Farshchi HK, Azizi M, Jaafari MR, Nemati SH, Fotovat A. Green synthesis of iron nanoparticles by Rosemary extract and cytotoxicity effect evaluation on cancer cell lines. Biocatal. Agric. Biotechnol, 2018; 16: 54-62.
15. Fraternale D, Giamperi L, Ricci D. Chemical composition and antifungal acivity of essential oil obtained from in virto plants of Thymus mastichina L. J. Essent. Oil Res., 2003; 15(4):278-81.
16. Sarven MS, Hao Q, Deng J, Yang F, Wang G, Xiao Y, Xiao X. Biological Control of Tomato Gray Mold Caused by Botrytis Cinerea with the Entomopathogenic Fungus Metarhizium Anisopliae. Pathogens, 2020; 9(3): 213.
17. Meena M, Pilania S, Pal A, Mandhania S, Bhushan B, Kumar S, Gohari G, Saharan V. Cu-chitosan nano-net improves keeping quality of tomato by modulating physio-biochemical responses. Scientific Reports, 2020; 10(1):1-11.
18. Salem EA, Nawito MA, Abd El-Raouf AE. Effect of silver nano-particles on gray mold of tomato fruits. J. Nanotechnol. 2019; 1(4):108-18.
19. Ramyadevi J, Jeyasubramanian K, Marikani A, Rajakumar G, Rahuman AA, Santhoshkumar T, Kirthi AV, Jayaseelan C, Marimuthu S. Copper nanoparticles synthesized by polyol process used to control hematophagous parasites. Parasitology research, 2011; 109(5):1403-15.
20. Kanhed P, Birla S, Gaikwad S, Gade A, Seabra AB, Rubilar O, Duran N, Rai M. In vitro antifungal efficacy of copper nanoparticles against selected crop pathogenic fungi. Materials Letters, 2014; 115: 13–17.
21. Giannousi K, Avramidis I, Dendrinou-Samara C. Synthesis, characterization and evaluation of copper based nanoparticles as agrochemicals against Phytophthora infestans. RSC Advances, 2013; 3(44): 21743–21752.
22. Ouda, S. M. Antifungal activity of silver and copper nanoparticles on two plant pathogens, Alternaria alternata and Botryis cinerea. Res. J. Microbiol, 2014; 9(1): 34–42.
23. Juarez-Maldonado A, Ortega-Ortíz H, Pérez-Labrada F, Cadenas-Pliego G, Benavides-Mendoza A. Cu nanoparticles absorbed on chitosan hydrogels positively alter morphological, production, and quality characteristics of tomato. J. Appl. Bot. Food Qual, 2016; 89:183–189.
24. Abdel-Rahman F. A, Rashid I.A.S.R, Shoala T.M. Nanoactivities of natural nanomaterials rosmarinic acid, glycyrrhizic acid and glycyrrhizic acid ammonium salt against tomato hytopathogenic fungi Alternaria alternata and Penicillium digitatum. J. Plant Prot. Res, 2020; 60(2):150-160.