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Effect of Chitosan nanoparticles on quality indices, metabolites, and vase life of *Rosa hybrida* cv. Black magic

Hanifeh Seyed Hajizadeh[1*], Roya Dadashzadeh[1], Sahar Azizi[1], Gholam Reza Mahdavinia[2] and Ozkan Kaya[3*]

Abstract

Background Chitosan nanoparticles (CTS-NPs) protect the active ingredients from the environment for a specific period and reduces sweating, control weight loss, delay ripening, and increase vase life. So, a factorial experiment was carried out as a randomized complete design in three replications to investigate the efficiency of CTS-NPs in quality improvement and longevity extension of cut rose flowers.

Results The 15-day maximum vase life was attained by the rose placed in a preservative solution containing 10 mg L^{-1} CTS-NPs. CTS-NPs also reduced microbial growth as compared to controls. Total phenolics, total flavonoids, and amount of anthocyanin in treated petals were dramatically increased. CTS-NPs solutions especially at 10 and 15 mg L^{-1} concentrations, markedly reduced the H_2O_2 and malondialdehyde at the end of 15th day and maintained the membrane index. The protein and carbohydrate and petals anthocyanin content and enzymatic activities such as superoxide dismutase, polyphenol oxidase, peroxidase, catalase and ascorbate peroxidase increased in cut roses placed in 10 mg L^{-1} CTS-NPs vase solution which in turn caused to increase in vase life.

Conclusion CTS-NPs especially at a level of 10 mg L^{-1} can assist plants to enhance light usage efficiency, as well as promote photosynthetic carbon fixation and the production of additional carbohydrate products for plant growth and development.

Keywords Ornamentals, Nanoparticles, Microbial proliferation, Biochemical quality, Display life

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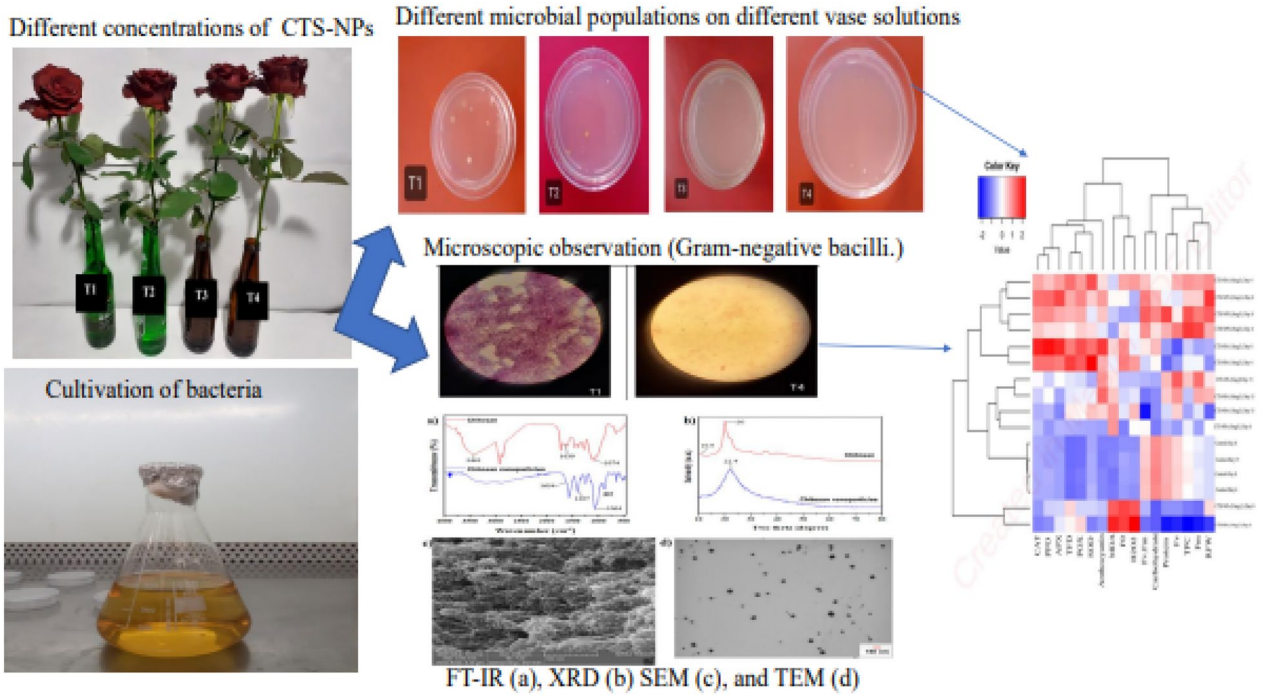
Graphical Abstract



Introduction

A rose is an ornamental shrub belongs to the Rosacea, relating to the genus *Rosa* contains more than 2000 species which most of the varieties are consumed as cut flower, indoor and outdoor plants and for food industry [1]. Roses are the most popular cut flower in floriculture sector of the world [2]. Roses are employed as decorations because of their exquisite and delightful nature, as well as their aromas. Rose plants create a stunning floral show with a variety of brilliant colors, shapes, sizes and scents. The improved longevity and quality of cut flower are preferred in the floriculture commerce industry. The main problem with cut flower is the limited postharvest life span (about 6–15 days) which is very dependent to the variety and storage conditions [3] because they are very sensitive to mechanical damage and ethylene [4] especially it is demonstrated that cut rose 'Black magic' is a short lasting cultivar [5] since the increase in expression of *RhCTR1*, through stages 2 (bud) and 8 (fully opened flower), was greater in cv. Black magic in comparison to cv. Maroussia [4]. The blocking of the vascular system owing to microbial contamination. Nonetheless, blockage, which most commonly occurs in the xylem, reduces water absorption and induces flower drooping prematurely. Apart from causing vascular constriction, bacteria create pectinases and poisonous chemicals, as well as ethylene, which accelerates senescence [6]. Stem obstruction can also be caused by extracellular polysaccharides, dead cell breakdown products and macromolecules. Additionally, microbes as well as their secretions, may result in xylem occlusions. There were some advantages in using of preservative solutions containing nanomaterials such as nanosilver had been used for some cut flowers such as freesia [7] and carnation [8]. The restriction on the application of bioactive compounds and bio-preservatives in agriculture is due to their sensitivity to oxidative stress, so, environmental stress or industrial conditions can readily degrade them. This constraint could be overcome by encapsulating bioactive compounds in polymeric casings or stores using Nano sized particles [9]. Some of the encapsulation advantages include (a) the preservation of bioactive molecules that are unstable from severe processing parameters [10], (b) increasing the foodstuff application and sensory acceptability of some plant extracts or essential oils by masking their intense bad smells [11], (c) the actual core molecule's physical qualities have changed, making it easier to handle [12], (d) creating nanoparticles with targeted distribution, (e) controlled

release, and (f) effective release to achieve long-term medicinal and functional impacts [13].

Chitosan is a cationic linear polysaccharide with higher efficiency for encapsulating natural substances. Researchers have employed chitosan to make nanoparticles in large quantities. This chemical is identified by its biocompatibility and bioactivity [14]. It also has antimicrobial characteristics, which help to prevent postharvest deterioration in horticultural crops [15]. Chitosan is also a good nanoparticle carrier because of its efficacy to overcome cellular boundaries and diffuse through epithelial cells [16]. It's also been shown to improve the formation of secondary metabolites in a variety of medicinal crops. The placement of chemical groups such as hydroxyl and amine on chitosan makes it a good substrate for complexing with other particles, resulting in more stable complexes [16]. Furthermore, functional groups are abundant, so, chitosan could be manipulated in a variety of ways to generate modified, crosslinked, carboxylated, ionic, and confined derivatives [17].

Among other techniques, the ionic gelation procedure is one of the numerous ways designed to produce chitosan nanoparticles, has gotten a lot of attention since it is a benign and controllable method. The interface between the phosphate groups (negative charge) in the sodium tripolyphosphate structure and the amino protonated groups of the chitosan is the basis for this approach [18]. Chitosan nanoparticles have the benefit of delivering natural extracts, which improve the nanoparticles' functioning and compatibility [18]. Numerous literatures had shown the effect of chitosan on the postharvest quality of flowers. It has also been applied to raise the vase life of cut flower like *Heliconia bihai* L. [19] and roses [20]. Ali et al. [21] demonstrated that applying 1-MCP or CTS-NPs after harvest is a very viable approach for preserving damask rose postharvest quality during storage.

Therefore, this research aimed to examine the efficiency of chitosan nanomaterials on the postharvest attributes and vase life of *Rosa hybrida* cv. Black magic, investigating a novel substance that could be used in cut flowers preservative solutions to enhance the longevity and quality of cut roses proceed to have a good influence on the floricultural industry globally.

Materials and methods

Preparation of Chitosan nanoparticles

Low molecular chitosan (Mw = 100 kD, DD = 85%, Purity = 97%) was purchased from Sabz Gostaresh Azin Turkan (Maragheh, Iran). Tripolyphospahte (TPP) was obtained from Merck Company (Germany). The method applied to synthesis chitosan nanoparticles was similar to Ahmadi et al. [22] report. To reach Chitosan nanoparticles, 0.1% of chitosan was synthesized by adding 1 g of chitosan powder into 1000 mL of 0.1% of acetic acid solution. After complete dissolving chitosan, 0.4 g of TPP was dissolved in 20 mL dH_2O. TPP solution was slowly added to chitosan solution while stirring (700 rpm). Afterward, according to the consumed chitosan content to prepare nanoparticles, the as-obtained nanoparticles solution was assumed as stock solution with 1000 ppm concentration.

Characterization of nanoparticles

The nanoparticles dispersion in dH_2O was transferred onto a carbon-coated copper grid and stained with 2% uranyl acetate and the TEM micrograph was taken by a transmission electron microscope (TEM, Philips CM10 operating at 60 kV). The chemical structure nanoparticles were evaluated by Fourier-transform infrared (FTIR) spectrometer (Bruker 113 V FT-IR) in KBr pellets. The crystalline structure of chitosan and its nanoparticles was studied by one-dimensional wide-angle X-ray diffraction (XRD), in which the patterns were recorded on Siemens D-500 X-ray diffractometer at a wavelength of $\lambda = 1.54$ Å (Cu-Kα), at a tube voltage of 35 kV, and a tube current of 30 mA. After coating the samples with a thin layer of gold, the nanoparticles were imaged in a scanning electron microscope/energy-dispersive.

Plant material and treatments

Rose flowers 'Black magic' was harvested from a greenhouse in Tehran, Iran at their harvestable index [23]. The flowers were re-cut to 40 cm long under distilled water and then replaced in different treatments including different concentrations of CTS-NPs (T1 = Distilled water, T2 = 5, T3 = 10 and T4 = 15 mg L^{-1}) along with 3% sucrose [7] as continuous treatment with 3 replications (2 flowers for each replication). The flowers were kept at 10 ± 2 ℃, 60% RH and 12 h light period at the intensity of 15 μM m^{-2} s^{-1} until the end of the vase life. Flowers were evaluated for their physiological and biochemical traits at sampling times of 0, 4, 8 and 15 days after harvest and at the end of experiment the microbial contamination was also measured;

Physiological and biochemical traits assay

Vase life

Vase life was measured as the days from the placement of roses within different treatments until observation symptoms such as wilting, withering, discoloration and abscission of petals and bent neck as the onset of the aging phase [24].

Microbial population

The microbial population was assayed by Plate Count Method according to the Seyed Hajizadeh et al. [25] method. In the mentioned, one ml of vase solution sample was taken from cut flowers preservative solutions. Samples were diluted up to 10^4 and were cultured on a broad spectrum nutrient agar medium at the 7th and 15th days of vase life with 3 replications. Plated samples were incubated at 37 ℃ for 48 h to allow microorganisms growth. Formed colony units after incubation were evaluated as a number of microorganisms present in preservative solutions and were recorded as colony-forming units.ml^{-1} (CFU mL^{-1}).

Relative fresh weight

The flowers relative fresh weight was assayed by a digital scale with an accuracy of 0.01 grams at the beginning of the experiment and before being placed in the solutions through the vase life duration. The relative weight of the flowers was calculated as percentage using the equation (1).

$$\mathrm{RFW} = (\mathrm{Wt}/\mathrm{Wt} = 0) \times 100 \quad (1)$$

Solution uptake

Preservative solution usage was assayed every two days after transferring from bucket to vases using the formula (2) [26].

$$Solution\ uptake\ ml.day^{(-1)}g^{(-1)}FW = \frac{(St1 - St)}{(Wt = 0)} \quad (2)$$

Chlorophyll fluorescence of rose flower leaf

The Chlorophyll parameters of *Rosa hybrida* cv. Black magic was assayed by the portable photosynthesis meter (Walz GmbH Licensing, 691090 Eichenring, Germany) during vase life. Minimal fluorescence, *F0*, was evaluated in leaves after 30 min dark incubation, and then for measuring the maximal fluorescence, *Fm*, we used the mentioned leaf samples under full light positions. Maximal variable fluorescence (*Fv*) and the photochemical performance of PSII (*Fv*/*Fm*) were then checked from the reported parameters [27].

Rose petal antioxidative enzymes

One gram of rose petals were homogenized in 5 mL of 50 mM K–phosphate buffer (pH 7.0), brought to 5 mM Na–ascorbate and 0.2 mM EDTA by the addition of concentrated stocks. The homogenate samples were centrifuged at 10,000 rpm for 15 min at 4 °C. Then the supernatant was applied for enzymes activity measurement and was carried out at 4 °C. The activity of superoxide dismutase (SOD) and catalase (CAT) was measured, as previously established by Li et al. [28]. The petals (0.5 g) were harvested and ground in liquid nitrogen and extracted with the following described method: 100 mM potassium phosphate buffer (pH 7.8) including 0.1 mM EDTA, 1% (w/v) PVP and 0.1% (v/v) Triton × 100. The extraction was centrifuged at 10,000 rpm for 15 min at 4 °C. The supernatants were collected and applied for evaluating the enzyme activities. An assay of ascorbate peroxidase was done according to the Yoshimura et al. [29] protocol. The reaction solution involved phosphate buffer (250 µL), 1 mM ascorbate (250 µL), 0.4 µM EDTA (250 µL), 190 µL ddH_2O, 10 mM trans oxide (10 µL) and 50 µL supernatant. The absorption at 290 nm for 1 min determined the enzyme activity. An extinction coefficient of 2.8 Mm^{-1} cm^{-1} for 1 min, was used to measure the enzyme activity. MacAdam et al. [30] protocol was applied to extract the peroxidase enzyme. 0.3 g of petal sample with 1500 µL buffer homogenized. The resulted solution was centrifuged for 4 min at 14,000 rpm at 4 °C. 1200 µL of buffer and 50 µL of H_2O_2 and 50 µL of guaiacol with 50 µL of supernatant were homogenized and the absorbance was recorded at 475 nm via spectrophotometer (Shimadzu, Japan). The activity of polyphenol oxidase (PPO) was measured by Nicoli et al. [31] protocol. The enzymatic solution was extracted the same as peroxidase. In this case, a reaction complex was prepared of 100 µL of enzymatic extraction, 2.5 mL of potassium phosphate buffer (pH 6.8), 200 µl of 0.02-M pyrogallol as the enzyme precursor and was recorded at 420 nm with a spectrophotometer (Shimadzu, Japan).

Hydrogen peroxide (H_2O_2)

Determination of the H_2O_2 content in rose petals was carried out following the protocol of Liu et al. [32]. So, 0.5 g samples of rose petals were ground in liquid nitrogen and a potassium phosphate buffer (pH 6.8). The extracts of the distinct samples were centrifuged at 7000 rpm for 25 min at 4 °C. A 100-µL aliquot of the supernatants was added to 1 mL of xylenol solution, mixed completely and allowed to breathe for 30 min. Then the extent of absorbance was measured by spectrophotometer (Shimadzu, Japan) at 560 nm.

Malondialdehyde (MDA)

Malondialdehyde was measured as a 2-thiobarbituric acid (TBA) reactive metabolite [33]. About 1.5 mL extraction was homogenized into 2.5 mL of 5% TBA made in 5% trichloroacetic acid. The reaction solution was warmed at 95 °C for 15 min, and then cooled

quickly. After centrifugation at 5000 rpm for 10 min, the absorbance of the supernatants was read at 532 nm.

Total phenolic compounds (TPC)

Total phenol measurement was made by Folin-Ciocalteu and gallic acid (3,4,5-trihydroxybenzoic acid) as a standard [34]. 180 µl of dH_2O was added to 5 µl of extract and then diluted in methanol and a calibration of gallic acid standard solutions of 0.0625, 0.125, 0.25, 0.5, 1, 2.5 and 5 mg/mL were prepared. After that, 1200 µl Folin-Ciocalteu (10%) was added to the solution and after 5 min, sodium carbonate (7.5%) was added. Then, samples were placed in the darkness for 30 min and the absorption was read by spectrophotometer (Shimadzu, Japan) at 760 nm. TPC were evaluated according to the gallic acid calibration (0–25 µg/mL) curve.

Total flavonoid compounds (TFC)

Total flavonoid determination was performed by the protocol using a colorimetric assay [35]. 10 µl of methanolic extract, 150 µl sodium nitrite (5%), 300 µl aluminum chloride solution and 1000 µl of acetate solution (1 M) were added and then, distilled to the amount of 5 ml. The reaction solution was kept at room temperature for 30 min. Absorbance of the reaction mixture was recorded at 380 nm. Flavonoid content was quantified using a standard quercetin curve (20–110 µg quercetin).

Protein

Protein content was done akin to the Bradford method [36] and according to the standard curves prepared with bovine serum albumin. For this purpose, Coomassie blue, in response to protein concentration, reacts with basic amino acid residues, especially arginine. The dried rose petals were added into the test tube with 2 mL of 50 mM potassium phosphate buffer at pH 7.0. Petals were centrifuged at 7000–12,000 rpm. The supernatant was recovered and centrifuged at 3000 rpm for 15 min at 4 °C. Each sample was diluted at 1:100 and recorded in triplicate in a spectrophotometer at 595 nm.

Carbohydrate

Soluble carbohydrates were extracted by anthrone reagent. In this case, 0.5 g fresh petals of roses were grounded with 5 ml ethanol. The supernatant was removed and the pellet rewashed with 5 ml ethanol 70%, and added to the last solution. The extract was centrifuged for 15 min at 4500 rpm. At the end, 3 ml anthrone reagent was added to the supernatants. Then, the amount of light absorption was recorded at a wavelength of at 625 nm [37] by a spectrophotometer (Shimadzu, Japan).

Anthocyanin

For measuring the anthocyanins, 0.1 g of fresh rose petals was grounded in 10 mL acidified methanol. The solution was centrifuged and the supernatants were kept overnight in darkness. Adsorption was read spectrophotometrically at 550 nm (Shimadzu, Japan). The concentration of anthocyanins was calculated using extinction coefficient ($\varepsilon = 33{,}000\ cm^2\ mol^{-1}$) and the following $A = \varepsilon bc$ formula [38].

Statistics

The factorial experiment was carried out based on a completely randomized design with 3 replications. Data were statistically analyzed by MSTAT-C ver 2.1 software and means were separated using the Fischer's protected least significant difference (LSD) test at $p = 0.05$.

Results

Synthesis and characterization of Chitosan nanoparticles

The structural characterization of chitosan and Chitosan nanoparticles was performed using FTIR spectra (Fig. 1a). The stretching vibration of –OH functional group is approved by appearing the band at 3441 cm^{-1}. The pyranose ring displays a peak at about 1074 cm^{-1}. The C=O stretching due to the amid I and N–H bending owing to the primary amine group show the peaks at 1639 and 1560 cm^{-1}, respectively. When the chitosan is cross-linked with TPP to attain Chitosan nanoparticles, the antisymmetric stretching of P-O-P of TPP ingredient appears at 881 cm^{-1}. In the Chitosan nanoparticles, the characteristic peaks of chitosan indicate a relative shifting to lower frequencies, showing its interaction with TPP ingredient. The crystalline structure of chitosan is confirmed by appearing the distinctive peak at $2\theta = 20°$, as shown in XRD pattern of chitosan (Fig. 1b). When chitosan is crosslinked through gelation method to obtain CTS-NPs, the crystallinity of chitosan is destroyed. Disappearing the peak at $2\theta = 20°$ confirms the presence of amorphous chitosan. A broad peak at $2\theta = 22.1$ in the XRD pattern of Chitosan nanoparticles is the common peak of polysaccharides. The surface morphology of CTS-NPs was studied by scanning electron microscopy, indicating the formation of spherical nanoparticle (Fig. 1c). The transmittance electron microscopy (TEM, Fig. 1d) confirmed the produced spherical Chitosan nanoparticles with a 190 nm-diameter size.

Vase life of cut rose 'Black magic'

Different treatments of CTS-NPs effected on the longevity of the 'Black magic' rose flower which is illustrated in Fig. 2. The highest longevity of 15 days was attained at 10 mg L^{-1} of CTS-NPs while minimum longevity of

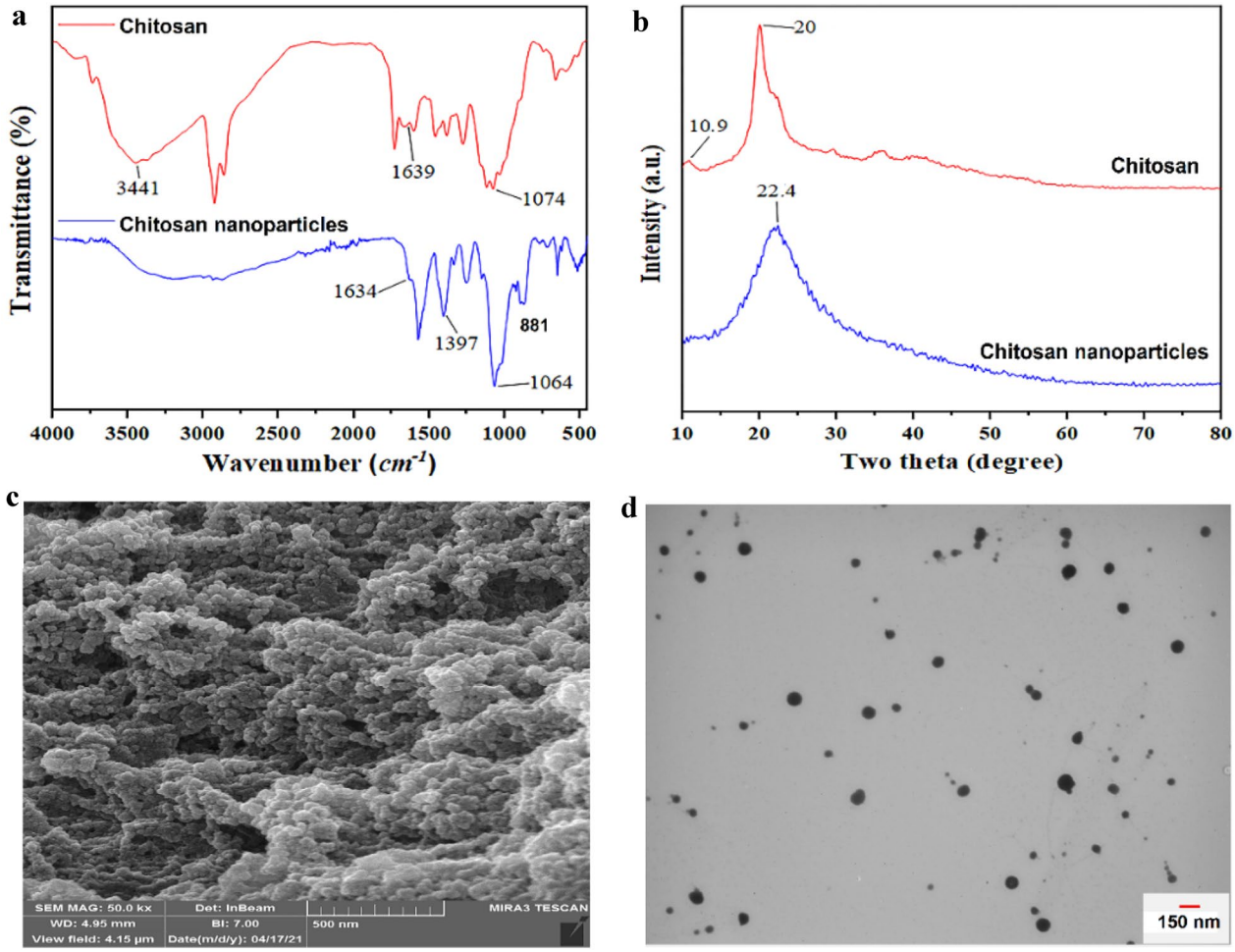


Fig. 1 FT-IR (**a**), XRD (**b**) SEM (**c**), and TEM (**d**) analysis of chitosan and chitosan nanoparticles (CTS-NPs)

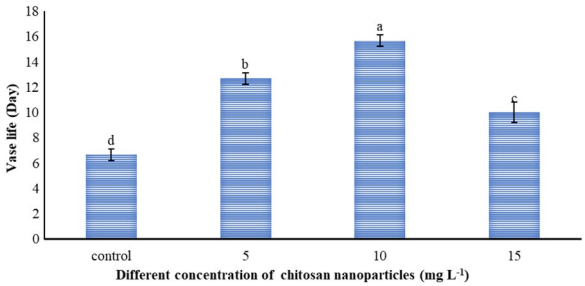


Fig. 2 Effect of chitosan nanoparticles (CTS-NPs) on cut rose 'Black magic' vase life

6 days was recorded in control as illustrated in Fig. 3. All rose flowers stems maintained in preservative solutions including different concentrations of CTS-NPs exhibit more vase life in comparison with the controls (flowers placed in distilled water).

Fig. 3 Effect of chitosan nanoparticles (CTS-NPs) on cut rose 'Black magic' on the 6th day of vase life; T1 = control (Distilled water + 3% sucrose), T2 = 5 mg. L^{-1} CTS-NPs + 3% sucrose, T3 = 10 mg. L^{-1} CTS-NPs + 3% sucrose and T4 = 15 mg. L^{-1} CTS-NPs + 3% sucrose

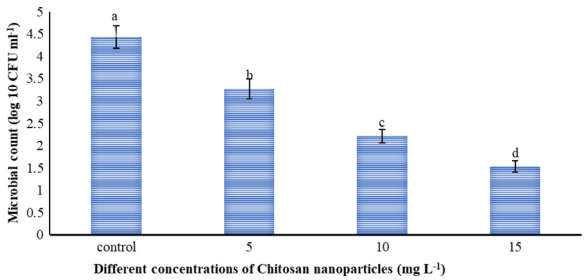


Fig. 4 Effect of chitosan nanoparticles (CTS-NPs) on cut 'Black magic' rose vase solution microbial population at day 7

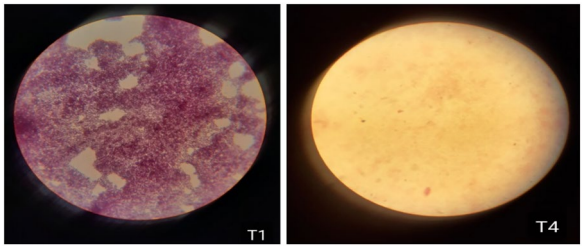


Fig. 6 Microscopic observation of T1 (distilled water + 3% sucrose) and T4 (containing 15 mg L^{-1} CTS-NPs + 3% sucrose) preservative solution

Microbial population of cut rose 'Black magic'

Results of microbial population in vase solutions are given in Figs. 4 and 5 which showed that the amount of microbial population was decreased in different CTS-NPs solutions and the mentioned inhibitory effect was in parallel with CTS-NPs concentration.

The highest mesophilic bacterial counts (4.5 Log CFU mL^{-1}) were recorded in distilled water while the lowest (1.5 Log CFU mL^{-1}) was observed in 15 mg L^{-1} CTS-NPs (T4) as shown also in microscopic picture (Fig. 6). According to the Fig. 6 contamination of the vase solutions belonged to the Gram-negative bacilli.

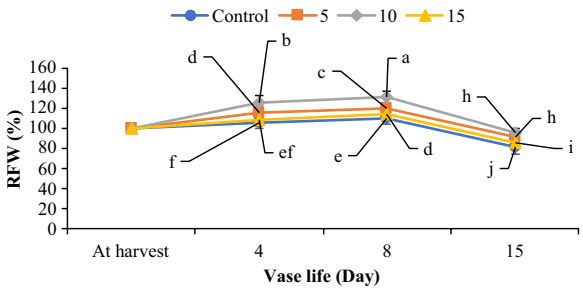


Fig. 7 Effect of chitosan nanoparticles (CTS-NPs) on relative fresh weight of cut rose 'Black magic'. Different letters indicate significant differences in each trait according to LSD test at $P < 0.05$

Relative fresh weight of cut rose 'Black magic'

Results showed that the rose 'Black magic' fresh weight increased until 8th day of vase life, then decreased on day 15 in all treatments (Figure 7). The highest (130.8%) amount of fresh weight was obtained in the concentration of 10 mg L^{-1} of CTS-NPs on the day 8, and the lowest (80.6 %) amount was observed in control flowers on the day 15. The difference between 15 mg L^{-1} CTS-NPs and control was not significant.

Solution uptake of cut rose 'Black magic'

Results showed that CTS-NPs positively effects on rose 'Black magic' quality after harvest by increasing in the rate of solution uptake which was the highest at 10 mg L^{-1} CTS-NPs. The amount of solution uptake

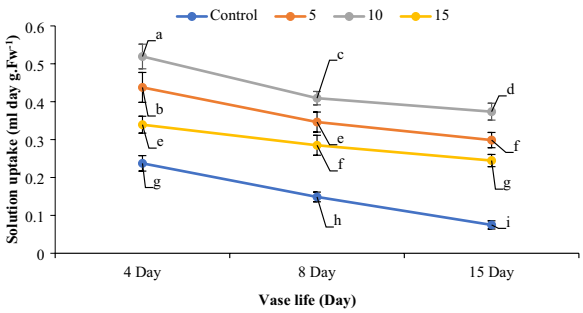


Fig. 8 Effect of chitosan nanoparticles (CTS-NPs) on vase solution uptake of cut rose 'Black magic'. Different letters indicate significant differences in each trait according to LSD test at $P < 0.05$

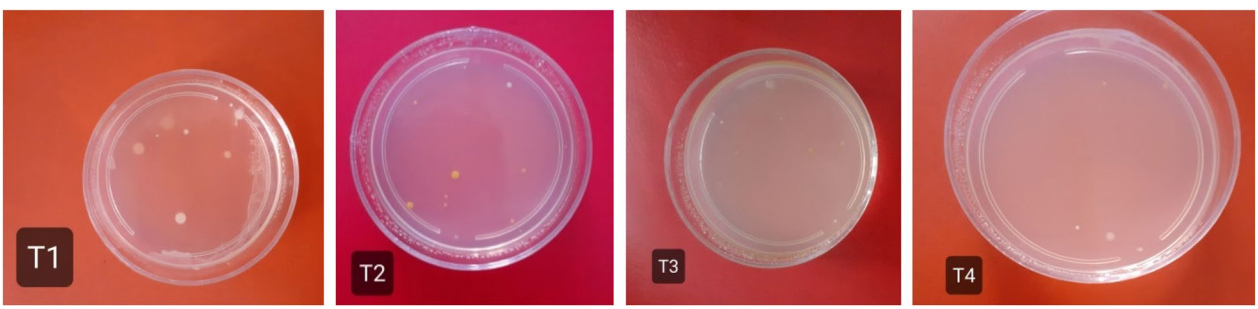


Fig. 5 Different microbial populations on different vase solutions on the 7th day

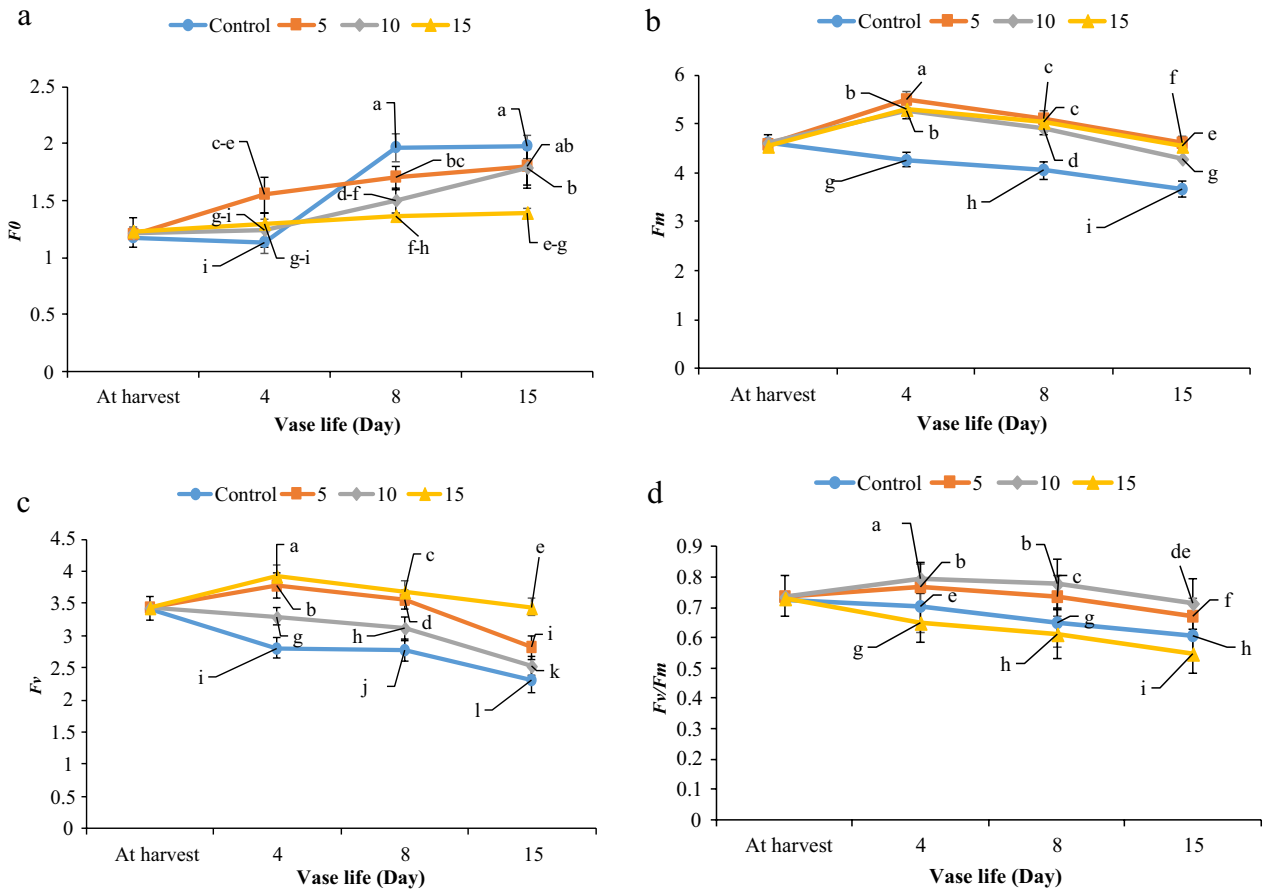


Fig. 9 Comparison between control and CTS-NPs treatments in terms of chlorophyll fluorescence *F0* (**a**), *Fm* (**b**), *Fv* (**c**), and *Fv/Fm* (**d**) of cut rose 'Black magic' during storage. Different letters indicate significant differences in each trait according to LSD test at $P < 0.05$

decreased during vase life, although all preservatives containing different concentrations of CTS-NPs had more capacity in water uptake in comparison with control flowers (Fig. 8). The highest water uptake was recorded by 10 and then 5 mg L^{-1} CTS-NPs compared to controls and flowers placed in 15 mg L^{-1} CTS-NPs preservative.

Leaf chlorophyll fluorescence of cut rose 'Black magic'

Minimal fluorescence (F0)

Data for minimal fluorescence of rose cut flowers are shown in Fig. 9a. Both vase life and CTS-NPs treatments significantly affected the rose 'Black magic' chlorophyll florescence. The highest (1.96, 1.97) minimal fluorescence was observed when CTS-NPs was applied at Control (T1) on day 8, 15 and the lowest (1.13) was calculated in control flowers on day 4.

Maximal fluorescence (Fm)

Data containing maximal fluorescence values of rose 'Black magic' is given in Fig. 9b. Among different treatments of CTS-NPs the highest (5.48) value of maximal fluorescence was in 5 mg L^{-1} Chitosan nanoparticles treatment (T2) on day 4 followed by 15 mg L^{-1} CTS-NPs (T4) (5.31) on day 4 while the lowest (3.66) in control on day 15. Maximal fluorescence of rose cut flower declined significantly during the vase life as all treatments had the highest amount of *Fm* while the lowest values was related to the controls specially at the end of vase life.

Maximal variable fluorescence (Fv)

The results showed that the highest (3.92) *Fv* value was related to a15 mg L^{-1} CTS-NPs day 4 and then decreased during postharvest life span of rose 'Black magic' as the lowest (2.31) was recorded in controls on 15th day (Fig. 9c).

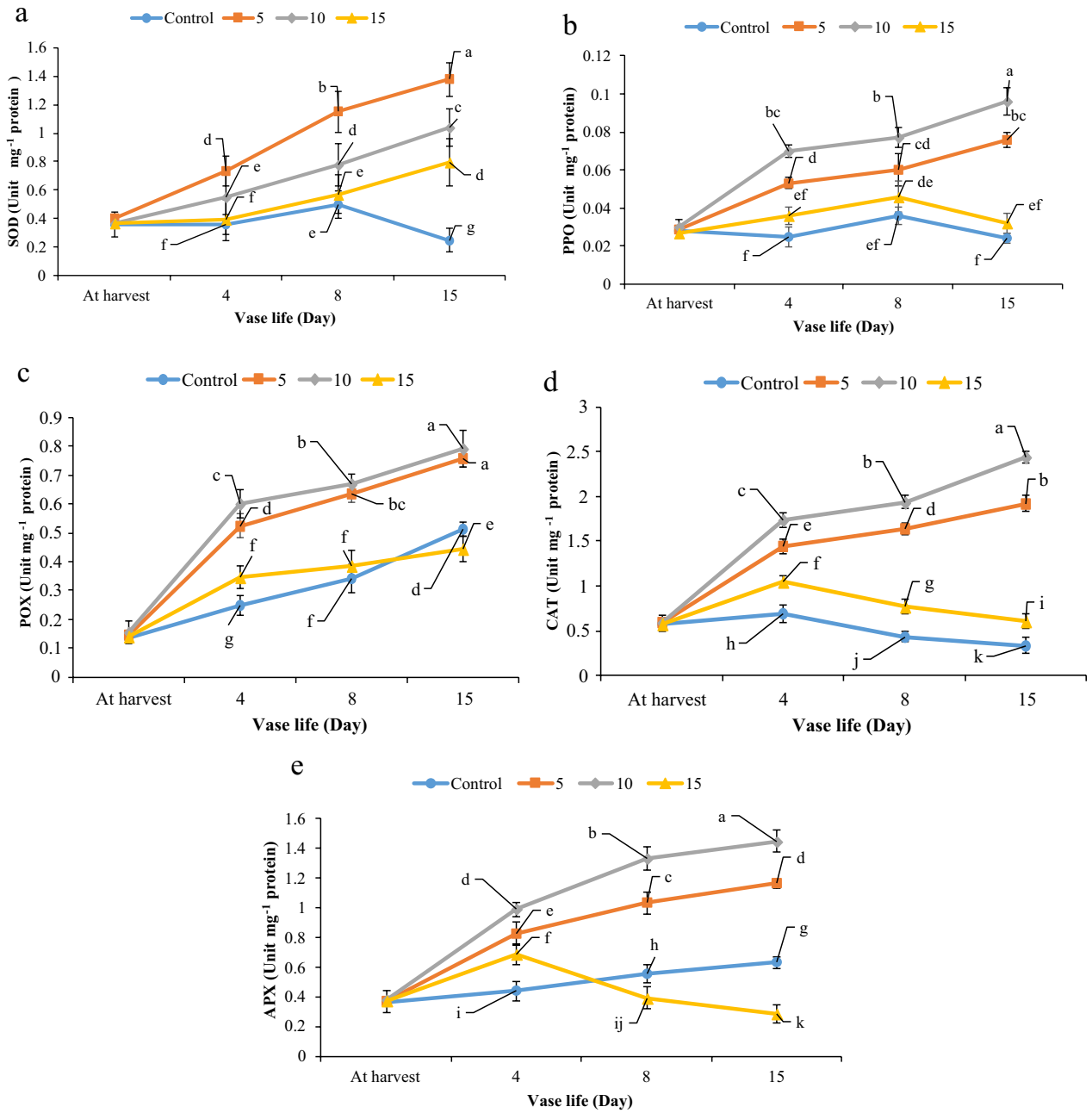


Fig. 10 Comparison between control and CTS-NPs treatments in terms of antioxidant enzymes SOD (**a**), PPO (**b**), POX (**c**). CAT (**d**) and APX (**e**) in cut rose 'Black magic' during storage. Different letters indicate significant differences in each trait according to LSD test at $P < 0.05$

Photochemical efficiency (Fv/Fm)

Data pertaining photochemical efficiency of rose during vase life is presented in Fig 9d. The effect of treatments and vase life was significantly different on photochemical efficiency of the rose. *Fv/Fm* value had a decreasing trend during vase life of rose flower in all vase solutions although flowers maintained in 10 mg L^{-1} of CTS-NPs (T3) has a higher value for *Fv/Fm* during vase life as a high (0.79) value was observed on day 4 and the lowest (0.54) value was related to the15 mg L^{-1} CTS-NPs (T4) on day 15.

Enzymatic changes of cut rose 'Black magic' petal

Superoxide dismutase (SOD)

According to the Fig. 10a, the activity of SOD had an increasing trend in all CTS-NPs treatments and decreased only in control flowers on day 15. The highest (1.37) significant value of SOD was observed in 5 mg L^{-1} of CTS-NPs treatment on day 15 followed by 10 mg L^{-1} of CTS-NPs treatment while the minimum (0.25) amount was observed in controls on day 15.

Polyphenol oxidase (PPO)

The results showed that polyphenol oxidase enzyme had an increasing trend during vase life as the highest enzyme activity was observed in a vase solution having of 10 mg L^{-1} CTS-NPs (Fig. 10b) on day 15 and the minimum enzyme activity was measured in controls and 15 mg L^{-1} CTS-NPs treatments on day 15, respectively.

Peroxidases (POX)

Data about the peroxidase activity of rose are shown in Fig. 10c. Both treatments and vase life significantly affected the POX activity of the rose. Different sampling times (At harvest, 4, 8 and 15) caused to increase in the activity of peroxidase enzyme so that, the highest (0.758, 792) increase in this enzyme was achieved by 2.7 folded compared to flowers at harvest day in flowers placed in solutions having 5 and 10 mg L^{-1} of CTS-NPs.

Catalase (CAT)

According to the Fig. 10d, the difference between flowers placed in 10 mg L^{-1} of CTS-NPs solution compared to the control and other treatments was significant, so that at this concentration, the amount of catalase had an increasing trend. Both vase life and different CTS-NPs solutions significantly affected on the longevity of rose 'Black magic'. The highest (2.43) CAT activity was observed in 10 mg L^{-1} CTS-NPs (T3) on day 15 and the minimum (0.33) amount was measured in controls on day 15. CAT enzyme also showed an increasing trend when nano chitosan application was increased and similarly it increased up to 8 days but there was a slight decline on day 15.

Ascorbate peroxidase (APX)

According to the results (Fig. 10e), the activity of ascorbate peroxidase increased during experiment except for the solution containing 15 mg L^{-1} of CTS-NPs and the maximum increase was achieved in flowers placed in solutions having 5 and 10 mg L^{-1} of CTS-NPs. CTS-NPs preservatives exhibited higher values of ascorbate peroxidase activity as compared to control. The highest (1.44) APX value was calculated in 10 mg L^{-1} CTS-NPs solution on 15th day while the lowest (0.28) APX was observed in 15 mg L^{-1} on day 15.

Hydrogen peroxide (H_2O_2) and Malondialdehyde (MDA) content of cut rose 'Black magic' petal

According to the findings the amount of MDA and H_2O_2 increased during experiment, so that the highest increase in H_2O_2 was observed in control flowers and the least increase in H_2O_2 was recorded in flowers placed in vase solutions having 10 and 15 mg L^{-1} of CTS-NPs (Fig. 11a). Different concentrations of CTS-NPs affect on H_2O_2 content significantly, as the highest (6.12) H_2O_2 was calculated in control on day 15 followed by 5 mg L^{-1} CTS-NPs treatment (5.05) on day 15 and the lowest (3.74, 3.91) was observed in solution having 10 and 15 mg L^{-1} CTS-NPs on day 4. The value of malondialdehyde in rose petals presented in Fig. 11b. Different concentrations of CTS-NPs had a significant effect on MDA values during experiment. The highest (4.32) MDA was observed in control on day 15 the same as H_2O_2, while the lowest (1.8) was calculated in 5 mg L^{-1} CTS-NPs (T2) on day 4. Although

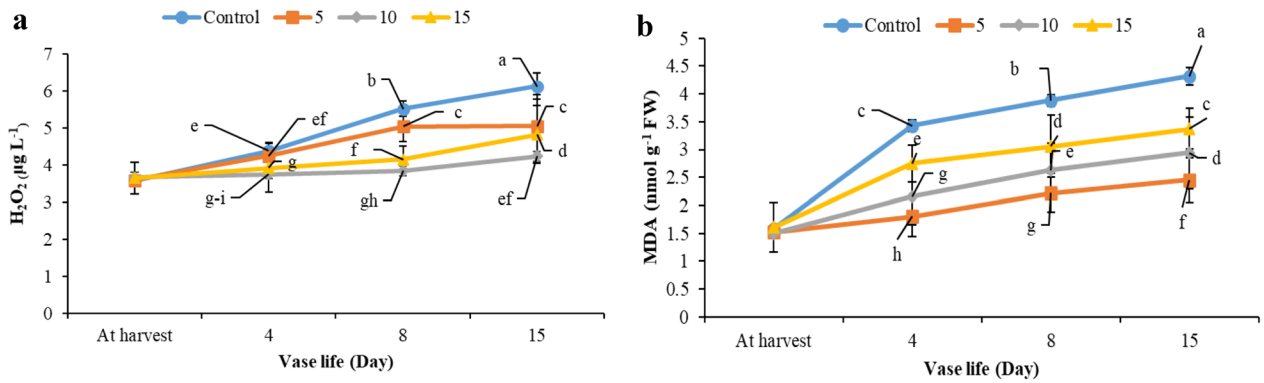


Fig. 11 Comparison between control and CTS-NPs treatments in terms of H_2O_2 (**a**) and MDA (**b**) in cut rose 'Black magic' during storage time. Different letters indicate significant differences in each trait according to LSD test at $P < 0.05$

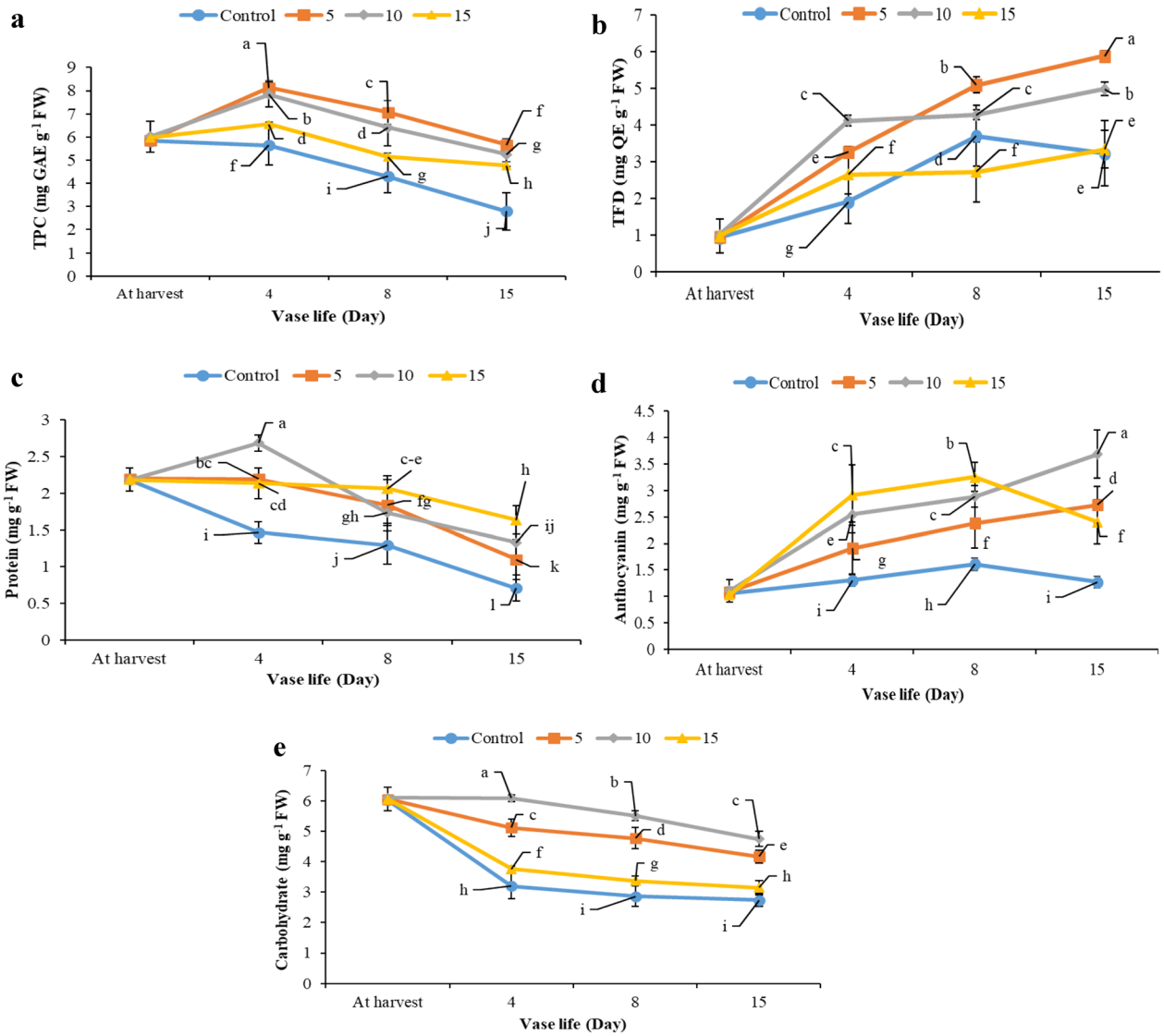


Fig. 12 Comparison between control and CTS-NPs treatments in terms of total phenol (**a**), total flavonoid (**b**), total soluble protein (**c**), carbohydrate content (**d**) and anthocyanin (**e**) in rose cut flower 'Black magic' during storage time. Different letters indicate significant differences in each trait according to LSD test at $P < 0.05$

the amount of H_2O_2 and MDA were increased during vase life, the CTS-NPs treatments markedly reduced the amount of H_2O_2 and MDA and caused to maintain the stability of membrane in comparison with the controls.

Total phenolic compounds (TPC) and total flavonoid contents (TFC) of cut rose 'Black magic' petal

It is clear that the TPC, TFC and totally the activity of antioxidants are the major post-harvest trait in 'Black magic' roses. The results of TPC and TFC of rose at different preservative solutions having CTS-NPs during vase life are presented in Figs. 12 a and b. The highest value (8.13) of TPC was evaluated in 10 mg L^{-1} of CTS-NPs solution on day 4 and the lowest (2.79) were observed in control on day 15. Total phenolic content trend increased in all treatments only until 4 days after harvest, then decreased. According to the Fig. 12b, TFC was generally increased during vase life. Flowers placed in 10 mg L^{-1} CTS-NPs had the maximum flavonoid content. The amount of flavonoids contents showed a decreasing trend in all preservative solutions during vase life. However, comparing cut roses at the end of experiment reveals that flowers maintained in solution having 10 mg L^{-1} of CTS-NPs had the maximum (5.87) TFC

compared to the flowers placed in dH_2O at the last day of experiment as they had the lowest (1.9) value of TFC.

Protein content of cut rose 'Black magic' petal

The results of CTS-NPs treatment on total soluble protein showed a decreasing trend during vase life. Flowers placed in preservative having 15 mg L^{-1} of CTS-NPs had the lowest decrease (30%) in protein content in comparison with controls at the end of 15th day while there were 83%, 99%, and 64% reduction in protein content, respectively, in solutions having dH_2O (control), 5, and 10 mg L^{-1} of CTS-NPs. The highest (2.68) protein content was calculated in 10 mg L^{-1} CTS-NPs on 4th day and the lowest (0.8) was calculated on control flowers at day 15 (Fig. 12c).

Carbohydrates content of cut rose 'Black magic' petal

Data for carbohydrates of the rose cut flower 'Black magic' are given in Fig. 12d. With the progress in flower development and senescence, the reserved carbohydrates were decreased inside the petals. The same decreasing trend is shown in the results. However, the highest value (6.09) for carbohydrates of rose was observed in CTS-NPs (10 mg L^{-1}) (T3) on day 4 and the lowest (2.87) in control flowers on day 8 which was not significantly different with 15th days of sampling time. In general, flower petals maintained in preservative having 10 mg L^{-1} CTS-NPs have the highest reserve of carbohydrate during vase life as at the end of 15th day, they have 4.74 mg g^{-1} Fw carbohydrate in petals which was higher than most of treatments at harvest day.

Anthocyanin content of cut rose 'Black magic' petal

Results indicated that the anthocyanin content was modified during longevity. Besides that, as the amounts of CTS-NPs increased, they controlled the color compounds. Data about anthocyanin of rose 'Black magic' petals on vase life are given in Fig. 12e. In this study, the amount of petal's anthocyanin increased during vase life. The highest (3.68) significant value of anthocyanin was observed in 10 mg L^{-1} (T3) on day 15 and the lowest (1.27) in controls on day 15. It was concluded that along with increase in CTS-NPs concentration in preservative solution, the overall production of carbohydrates also increased in rose petals.

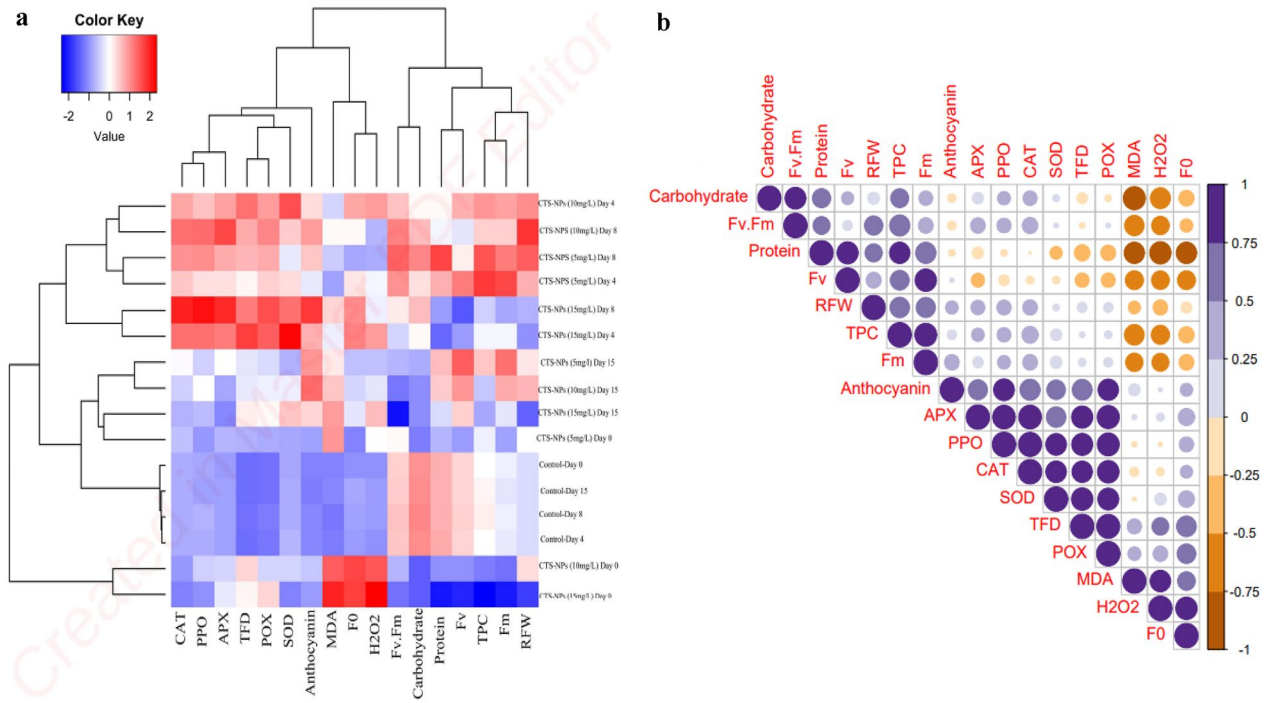


Fig. 13 The heat map (**a**) and Pearson correlation (**b**) corresponding to of cut rose 'Black magic' quality and biochemical parameters and application of postharvest treatments. Heat maps representing Relative fresh weight *RFW*, polyphenol oxidase *PPO*, superoxide dismutase *SOD*, catalase *CAT*, peroxidase *POX*, ascorbate peroxidase *APX*, total flavonoids content *TFD*, Anthocaynin, total soluble carbohydrate, total soluble protein, total phenolics content *TPC*, Hydrogen peroxide H_2O_2, Malondialdehyde *MDA* and Chlorophyll fluorescence such as *F0*, *Fm*, *Fv*, *Fv/Fm*

Heat map clustering and pearson's correlations analysis in CTS-NPs treatments on *Rosa hybrida* 'Black magic'

Due to the structured and unbiased variables concerned on this study, warmth map clustering has been used to investigate the results. Based on the warmth map clustering analysis, the results are correlated and clean discrimination into major clusters turned into determined for the different preservatives. The first cluster turned into especially composed of concentrations of chitosan nanoparticles excluding 10 and 15 mg L^{-1} CTS-NPs at harvest day, the 2d cluster consists of the control in different harvest times. Cluster analysis and dendrograms in the heat map (Fig. 13a) represented 3 major groups in the evaluated parameters of cut rose 'Black magic' placed in different chitosan nanoparticles (CTS-NPs) concentrations; Group I contained CAT, PPO, APX, TFD, POX, SOD, anthocyanin, group II contained MDA, *F0* and H_2O_2, and group III contained carbohydrate, protein, *Fv*, *Fm*, *Fv/Fm*, TPC, and RFW content (Fig. 13a).

The analysis of Pearson's correlations of some physiological and biochemical traits, of cut rose 'Black magic' are presented in Fig 13b. The findings indicated that carbohydrate, *Fv/Fm*, *Fv*, *Fm*, protein, RFW, and TPC positively correlated with each other, and also a significant positive correlated was observed among anthocyanin, APX, PPO, CAT, SOD, TFD, POX. On the other hand, MDA, H_2O_2 and *F0* negatively correlated with carbohydrate, *Fv/Fm*, *Fv*, *Fm*, protein, RFW, and TPC (Fig. 13b).

Discussion

The vase life is directly linked to the quality of flower. The finding is correlated to the study and is similar to the findings of Bañuelos-Hernández et al. [19] who investigated 20 days of vase life in *H. bihai* when chitosan was applied at a rate of 1.0% and 15 days of vase life in chitosan 1.5%, respectively. The same findings were obtained in gerbera cut flowers by Spricigo et al. [39]. One of the most important methods of nanobiotechnology is the use of nanoscience to formulate and manipulate compounds to increase quality and effect and prolong biological activity. Encapsulation of chitosan in polymer nanoparticles can improve the effectiveness of such compounds and chitosan as a biodegradable nanopolymer is highly regarded for its better encapsulation, controlled release and low toxicity. As studies have shown, nano chitosan can have a cytotoxic effect on many microorganisms [40]. Studies show that CTS-NPs can have antibacterial activity as a staphylococci agent bactericidal [41]. By entering the cell and binding to DNA, Chitosan nanoparticles inhibit RNA synthesis, resulting in the production of vital cell proteins [42] and finally extend the longevity of roses. Our results are also agree with Spricigo et al. [39] who investigated the effect of chitosan on different cut flowers and his research concluded that highest microbial growth of 4.5 Log CFU per liter was observed in control and a minimum of 2.3 Log CFU in nanochitosan (110 nm). The beneficial properties of CTS-NPs had been related to its multi-cationic nature, chain length, inhibitory synthesis of certain fungal enzymes, production of phenolic compounds and formation of structural barriers [43, 44]. There is a direct correlation between petal water content and the vase life of the cut flower. It has been stated that chitosan increases the amount of water in flowers petal and increases its lifespan [45]. The same findings were obtained by Spricigo et al. [39]. Spricigo et al. [39] who reported that the flower stems placed in nano-chitosan solution had the higher water uptake which can be because of the better absorption condition in acidified solutions and also the inhibitory effect of chitosan exerts on microbial growth and xylem obstruction. Rose stems maintained in distilled water also did not have more solution uptake compared to solutions having chitosan nanoparticles even in the initial days of vase life and their water uptake capacity loose as increasing in the longevity. Unlike the xylem sap in intact ornamentals, distilled water has no osmotically active substances [46] and the lack of ions and acids caused to decrease in water conductance and make some physiological conditions after harvest. Our findings are in line with the research work of Li et al. [47] that the light utilization efficiency of plants can be improved by chitosan and chitosan is also helpful in promoting photosynthetic carbon fixation and producing more carbohydrate products for plant growth and its developmental process. Chitosan application when applied to eggplants significantly increased its maximal fluorescence. Plants lose their chlorophyll index along with reduce in light absorbance through photo-protection mechanism during storage either by fast breakdown or slow synthesis of chlorophyll contents [48]. The maximal variable fluorescence was reported by Górnik et al. [49], who investigated that chitosan treatments on vines and other horticulture plants significantly affected their maximal variable fluorescence. Similar results were matched with the finding of Calvo-Alvarez et al. [50], who also concluded that chitosan improved maximal variable fluorescence, physiological activity and plant health in several plants. The photochemical response linked to treated roses which maintained photochemical efficiency during experiment. The present findings are in line with those of Xu and Mou [51] who stated that all treatments of chitosan significantly modified the chlorophyll index and photochemical efficiency of lettuce leaves.

The enzymes are directly linked to quality and the process of its change. Our study was similar to the research of Liu et al. Dheyab et al. [15, 16], who investigated that chitosan promoted SOD activity and reduced MDA contents of *Hydrilla verticillata* (L.f.) Royle. SOD and CAT enzymes are important during stress conditions [52] and have a key role in the normalization of the reactive oxygen species (ROS) and thus promoting the healthy growth of plants [53]. During storage, polyphenol oxidase carries the oxidation of polyphenols which causes browning and deterioration of cut flowers or vegetables [54]. Chitosan is mostly used as polysaccharide-based coatings which form a stable and rigid gel [55]. Peroxidases are the enzymes that oxidate phenols and produce compounds that are harmful to pathogens [56]. In our current study, an increasing trend was observed as the treatment and storage time proceeded, the same increasing trend was also investigated by Jail et al. [57]. These elevations in preventing cell injury of cut flowers might be because of the ability of catalase [21]. CAT enzyme by nano chitosan as compared to control treatment indicates ROS scavenging in treated flowers [58]. Our research also supported the study of Xu et al. [59] that chitosan treatments increased APX activities and prevented of the rose discoloration. The same trend was also recorded in the Damask rose cut flowers study by Ali et al. [21]. Oxidative stress impaired the functionality of the cell membrane, eventually caused to cell death [60], and it is associated with the diminished quality of crops after harvest [61]. Chitosan is a natural molecule that causes numerous biological responses in flowers [62]. It is demonstrated that reactive oxygen species has an important role in cell signal cascades, while in extreme levels have potential to damage cell organelles [63]. Application of different concentrations of CTS-NPs in flower preservative solutions caused to increase in total phenolic compounds and the antioxidative capacity shown in Fig. 13a, which led to decrease in the amount of hydrogen peroxide and also malondialdehyde in cut rose 'Black magic'. In a similar study, using of chitosan caused to reduce in lipid peroxidation and increase the stability of cell membranes in *Thymus vulgaris* [64]. The benefits of nanochitosan application were also demonstrated in decreasing hydrogen peroxide and also malondialdehyde content and prevention of oxidative stress in sweet basil during storage [65]. Numerous literatures have demonstrated the accumulation of hydrogen peroxide in plant when treated with chitosan and the accumulation of H_2O_2 is also observed in cell cultures when supplied with chitosan [66]. It is also thought that the accumulation of hydrogen peroxide can cause to the activation of antioxidative enzymes and the accumulation of compatible osmolytes such as flavonoids, phenols, tannin and phytoalexin [67]. It could be seen from the results that the use of nanochitosan can be a effective in delaying malondialdehyde production [68]. Moreover, chitosan act as a barrier to oxygen accountable for lipid peroxidation and thus maintains membrane integrity [69].

Secondary metabolites such as flavonoids, anthocyanins and carotenoids have a major effect in flower pigmentation. In this regard, the use of CTS-NPs led to increase in total phenolic compounds content of rose 'Black magic' petals compared to the controls through vase life. On the other hand, the amount of TPC and TFC showed a significant increase in flowers placed in preservative solutions having CTS-NPs. Yang et al. [70] concluded the increase in antioxidants compounds through the addition of lignin nanoparticles to films based on polyvinyl alcoholchitosan. The same findings were demonstrated by Martínez-González et al. [71] who showed the increase in TPC, TFC, and other antioxidative compounds at the last day of storage life (8th day) in comparison with coated strawberries with nanostructured chitosan compared to the untreated fruits. Total phenolics are a kind of compatible osmolytes that have a positive effect on postharvest quality factors by representing the antioxidative properties [72]. It has been demonstrated that chitosan stimulates the synthesis of TPC. Our results indicated that higher phenolic compounds were observed in control, but it is also indicated that CTS-NPs treatment preserved total phenolic over time and our study is in line with [73] that chitosan treatments strongly improved the synthesis of TPC and other antioxidants during storage. Similarly Kim et al. [74] reported that the application of chitosan to sweet basil preserved its total phenols and terpene compounds during the storage period. Our findings are related to those of Salimgandomi and Shabrangi [75] that increasing the chitosan concentration flavonoid contents of mint was also increased during storage time. The use of chitosan results in an increase in secondary metabolites particularly flavonoids and phenols which counteract the effect of free radicals and hydrogen peroxide and thus maintain the antioxidant activity [76].

According to the results from Fig. 12 decreasing trend was also observed in total protein and carbohydrates while anthocyanin amounts were increased during vase life. Any way treatments having nano chitosan preserve carbohydrates and proteins more than that in control petals. The findings are in line with the research results of [77] who observed that chitosan coatings of table grapes significantly improved the anthocyanins. The same findings were also observed by Bañuelos-Hernández et al. [19] that the application of chitosan to vase life of *H. bihai* considerably preserved the anthocyanin during storage. On the other hand, it is clear that protein has

the short half-life as it can be damaged by enzymatic cleavage. It seems that chitosan has ability to prevention of proteolysis in proteins effectively and at the same time maintain their activities [78] during longevity. Chitosans are believed to extend food shelf life by inhibition of microbial contamination and starch degradation. In this case, it is reported that chitosan with higher molecular weight is more effective in preserving food storability than chitosan with lower molecular weight [79]. So it seems that the least molecular weight of Chitosan nanoparticles will have more efficiency in preserving of cut flowers. It is demonstrated that chitosan is able to prevent of protein conformational diseases that are also related with senescence [79].

The chitosan exhibits many eliciting compounds in various plants [80]. The plant produces several defense compounds during stress conditions such as phytoalexins, pathogenesis-related proteins and anthocyanins [81], however, upon application of chitosan the production of these antioxidative compounds increases [82]. Accumulation of proteins and enzymes by chitosan has been reported in many plants like peach [83], tomato [84] and dragon fruit [85]. El-Tanahy et al. [86] proposed a study on chitosan treatments and their effect on cowpea. The results of the study were like those of ours. It was concluded that the overall production of proteins and carbohydrates increased with an increase in encapsulated-nano chitosan application. Moreover, El-Sayed et al. [76] also reported in goldenrod cut flowers that chitosan has a major role in carbohydrates modification and overall performance of cut flowers.

Conclusion

One of the most important fragrant ornamentals employed in the perfume, cosmetic, pharmaceutical industries and, especially cut flowers is *Rosa hybrida*. Yet, due to the short lasting of some cultivars such as Black magic, the postharvest quality of flower is diminished; hence, extending the cut flowers vaselife is a critical step in ensuring the crop's economic sustainability. The current study is based on the fact that application of CTS-NPs could improve the quality and increase the vaselife of flowers. The CTS-NPs was found to increased antioxidant defense mechanisms, which reduced H_2O_2 and MDA content and preserved membranes integrity especially at 10 mg L^{-1} concentrations. In rose flowers, CTS-NPs preservative solutions retained higher, total anthocyanin, total phenolic content, total flavonoids, and enzymatic activities. According to the studies, CTS-NPs has diverse activities which is related to the species and even the cultivar. The findings of this experiment mark a watershed moment in the study of CTS-NPs application in the vase solutions of ornamental plants laying the groundwork for future research into the particular mechanisms of CTS-NPs impact on cellular system.

Abbreviations

CTS-NPs	Chitosan nanoparticles
F0	Ground fluorescence in the light-adapted state
Fm	Maximum Chl fluorescence at a saturating radiation pulse in the dark-adapted state
Fv	Variable fluorescence
Fv/Fm	Maximum quantum efficiency of PSII
TPC	Total phenolic compounds
TFC	Total flavonoid compounds
SOD	Superoxide dismutase
PPO	Poly phenol oxidase
POX	Peroxidase
CAT	Catalase
APX	Ascorbate peroxidase

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Author contributions

HSH conceived and designed the experiments; RD and SA performed the experiments; SA and OK analyzed the data; GRM synthesized the chitosan nano particles; HSH and OK wrote and proof the final paper. All authors have read and agreed to the published version of the manuscript.

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Availability of data and materials

Correspondence and requests for materials should be addressed to HSH.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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