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Effect of *Colchicum Ritchii* Derived Nanoparticles on Apple Ring Rot Disease Without Changing the Taste Through the Prevented Metabolites Changes and Increased Sialic Acid Synthesis

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ABSTRACT

Objective: In this current study, we were planned to produce *Colchicum ritchii* flower extract associated calcium chloride nanoparticle (CRNPs) in a simple organic method and evaluate their apple fruit pathogenic resistance as well as their shelf-life extension properties during storage. *Methods*: This CRNPs was confirmed by analysis of various established physical and chemical methods. Moreover, the apple fruit pathogenic resistance as well as their shelf-life extension properties during storage were evaluated by standardizing methods. *Results*: Our results showed that the CRNPs were contrived and proven by physical and chemical evaluations. Moreover, the biological observation of CRNPs indicates that they have good pathogenic resistance properties against the *Botryosphaeria dothidea* without changing the taste through the prevented metabolites changes and increased sialic acid synthesis. The effective dose of pathogenic resistance in CRNPs was 40 μg/mL. The levels of defensive enzymes such as chitinase (CHI), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL), and β-1,3-glucanase (GLU) significantly increased in CRNPs treated fruits. The increasing antioxidant enzymes activities such as superoxide dismutase (SOD) and catalase (CAT) were shown in CRNPs treated apple fruit. The CRNPs-treated apple fruit was found to increase in salicylic acid (SA) and SA glucoside (SAG) levels, which might be due to increased SA synthesis. Moreover, shelf-life extension studies of fresh cut apples were conducted by immersing them in 20 and 40 μg/mL CRNPs as well as a standard solution of 2% calcium chloride for 5 min. The standard solution of calcium chloride as well the CRNPs were proved as a good preservative and they were preserved the apple fruits shelf-life up to 25 days. C*onclusion*: In this study, we have concluded that the CRNPs were produced by fusion with *Colchicum ritchii* flower extract and calcium chloride in a superlative natural method. Our results recommended that the CRNPs is acted as a best source for apple ring rot resistance without changing the taste through the prevented metabolites changes and increased sialic acid synthesis as well as the antioxidant properties. In addition, the CRNPs is superlative source for apple shelf-life extension up to 25 days without any toxicology nature.

Keywords: ***Botryosphaeria dothidea;*** **Calcium chloride;** ***Colchicum ritchii;*** **CRNPs; apple; shelf-life**

1. Introduction

Apple ring rot disease is one of the most important endemic problems in globally and its cruelly distributed on apple production region caused by *Botryosphaeria dothidea*. The characterization of this disease is shown on brown rings, concentric tan and marginally sunken lesions on the impacted fruit (Huang et al., 2021). The rate of apple ring rot incidence has been increasing marginally worldwide, and its arising biggest problem in fruit production and storage fields. In apple fruit, 50% of the infections have arisen before harvest, and another 79% have arisen on the storage stage, caused by *Botryosphaeria dothidea*. Currently, the synthetic fungicide drugs have been used popularly to control this kind of problem, even though having many health insecurities by using these fungicides because of their side effect and toxic nature as well some beneficial microbes also killed. Previous studies have demonstrated that the synthetic fungicides such as prochloraz, tebuconazole, pyrisoxazole, and fludioxonil have potential fungicidal properties; however, these fungicides need to be sprayed 10 or more times and also cause serious environmental problems and health issues (Tongtong et al., 2021; Seo et al., 2018).

Fruits and vegetables are notorious food materials, and these are unswervingly related to human health welfare. Recently, readily available fruits and vegetables have been expected and very demanded by people due to their busy schedules as well as readily available packaging with preserved nutritional benefits. Food industries are facing major problems storing fresh-cut fruits and vegetables without using toxic chemicals. Food waste is the most important problem facing people worldwide, even in Saudi Arabia (FAO, 2020). Hence, there is an urgent need for a non-toxic agent for the control of food, fruit, and vegetable waste; hence, we are concentrating on the advanced field of nanotechnology to find a new preservative agent.

Nanoparticles are the composing of numerous wide ranges of nano-sized particles that are ranging from 1 to 100 nm. These nanoparticles have various structures and shapes, including spiral, cylindrical, spherical, conical, tubular, and the like. Currently, nanoparticles are getting more attention from scientists due to their wide range of applications, including drug delivery, as well as their environmentally friendly method. In addition, these kinds of nanoparticles are considered microbial therapeutics because of their non-toxic, environment-friendly nature, improved solubility, ability to easily conjugate with hydrophilic or hydrophobic drugs, as well as their increased mutability as biological barriers. Generally, the nanoparticles are classified into several types; however, the scientists are mainly focusing on the metal and metal oxide nanoparticles, as well as the green or plant-associated metal nanoparticles. Zinc, iron aluminium, copper, silver and gold etc... are majorly using for synthesis of metal nanoparticles. The metal materials properties have been changed by the presence of oxygen called metal oxide nanoparticles which is increasing their efficiency and reactivity. Zinc oxide, iron oxide, titanium oxide, and aluminium oxide are the major metal oxide nanoparticles used by scientists for different beneficiary applications. These metal and metal oxide nanoparticles are causing different varieties of side effects on human health, particularly lung inflammation, digestive problems, and heart problems, as well as affecting the human health beneficiary microbes (Fubini et al., 2011; Soares et al., 2021). Therefore, it is essential to find an alternative method of plant associated nanoparticles to control human diseases as well as fruit and plant diseases due to their safest and environmental friendly manner.

Plant-derived nanoparticles are a very simple, costless, and friendly method that is being used to cure numerous potential therapeutic human diseases or disorders such as cardiac problems, inflammation, and cancer, as well as for antifungal, antibacterial, and antiviral effectiveness (Burketová et al., 2022; Adeyemi et al., 2022). Different varieties of medicinal plants as well as some natural sources, including fungi, algae, sea products, and bacteria, have been used to synthesize organically derived nanoparticles that have unique characterizations and medicinal applications. The different parts of plants, including the root, leaves, flowers, and seeds, play the most important role in the synthesis of nanoparticles because of their presence of primary and secondary metabolites such as flavonoids, phenolic compounds, terpinoids, steroids, vitamins, and minerals. These primary and secondary metabolites act as an oxidizing or reducing agent while synthesizing the nanoparticles. Calcium chloride associated plant nanoparticles are most importantly considerable nanoparticle for control the plant or fruit disease particularly the fruit and vegetable packaging industries because of their excellent antimicrobial nature, biocompatibility and non-toxic quality (Osuntokun 14 et al., 2018). However, the biological synthesis of calcium oxide nanoparticles from *Colchicum ritchii* flower extract and calcium chloride has not yet been inspected. 2 Hence, in our study, we planned to examine the synthesis of biological nanoparticles by using *Colchicum ritchii* flower extract and calcium chloride. This synthesized CRNPs was assessed and confirmed the nature and size by a studies of UVS, FTIR, DLS, SEM and EDx tomography.

Colchicum ritchii is a *Colchicaceae* family and it is named as a desert saffron. It is widely distributed in Jordan and Saudi Arabia. Previous studies have reported that the homoaporphine-N-oxide alkaloids and colchicine compounds are found in the Colchicum ritchii flower (Amina et al., 2021). It has been used for tradition medicines including the stomach pain, ulcer, rheumatism, arthritis, inflammation and also in abdominal colics (Amina et al., 2021). *Colchicum ritchii* is a desert saffron and it is best remedy for several illnesses such as ulcer, stomach pain, arthritis, and inflammation in traditionally, however, the scientific evidence is not yet studied and described. Hence, in this current study, we were planned to produce *Colchicum ritchii* flower extract associated calcium chloride nanoparticle in a simple organic method and confirmed this nanoparticles production by analysis of various established physical and chemical methods. Moreover, these produced nanoparticles were tested for postharvest apple fruit pathogenic resistance as well as their metabolites changes and their shelf-life extension properties during storage.

2. Materials and Methods

2.1. Chemicals, fruits and pathogen materials

All the research laboratory substances were received from Sigma Aldrich (USA), A fresh apple was purchased from a local hypermarket in Riyadh, Saudi Arabia.

2.2. Colchicum ritchii flower powder preparation

Colchicum ritchii flowers were collected from a local desert area near Riyadh, Saudi Arabia. These collected flowers were washed with running tap water followed by distilled water, which removed the dust and impurities. The washed flowers were dehydrated at room temperature for 3 days. The dried flowers were made into powder and used for further biogenic synthesis.

2.3. Colchicum ritchii derived eco-friendly calcium oxide nanoparticles production (CRNPs)

5 g of *Colchicum ritchii* flower powder was immersed in 250 mL of distilled water for 1 day, and then this extract was filtered with Whatman filter paper. This filtered extract was used for biological nanoparticle synthesis. 100 mL of *Colchicum ritchii* extract was mixed with 1 M calcium chloride (100 mL) and mixed for 20 mins with magnetic stirrer. Afterwards, sodium hydroxide (0.6 mL) was added slowly and mixed well for the next 15 min. This mixer was kept at room temperature for 1 day, then the supernatant was dispersed and washed twice with distilled water, followed by gentrification at 15, 000 rpm. Afterwards, the solution was air dried in an oven set at 37 degrees Celsius for 2 days. This CRNPs nanoparticle powder was used for all the subsequent experiments.

2.4. Nano-size confirmation and characterization studies

This synthesized CRNPs elements, morphology, and size were confirmed by UV-visible spectroscopy, dynamic light scattering (DLS) analysis, fourier transform infrared (FTIR) analysis, scanning electron microscopy (SEM), and energy dispersive X-Ray analysis (EDX) (Veeramani et al., 2020).

such as fourier transform infrared (FTIR), UV-vis spectroscopy, dynamic light scattering (DLS), scanning electron microscopy (SEM), and energy dispersive X-Ray analysis (EDX)

2.5. Botryosphaeria dothidea pathogen isolation

Botryosphaeria dothidea fungal was isolated from apple ring rot disease infected apple fruit which was found the same characteristics symptoms. The pathogen was cultured and maintained in PDA plates with potato dextrose agar, which was prepared by using 200 g potato extract, 15 g agar mixed with 1 L water, and 20 g glucose for 3 days at 4 ℃. Spore concentration was detected by hemocytometer which was adjusted with the deionized water by the concentration of 1 × 105 spores ml^{-1}. The apple fruit were purchased with the same size, freshly, same shape and without physical injuries. These apple fruits were disinfected with 2% (v/v) sodium hypochlorite and then washed with deionized water, followed by air-drying the fruits before they were used for the further experiments.

2.6. CRNPs induced resistance of apple ring rot disease against Botryosphaeria dothidea

The apple fruits were treated with CRNPs before initiation of the pathogen, and this mythology was followed by the previously described model with small modifications (Yang et al., 2017). Apple fruits were immersed for 15 mins with different concentrations of CRNPs including 10, 20, 40 and 80 μg/mL. After 15 mins the fruits were collected and air dried for 2 hr at room atmosphere. Afterwards, they were stored in plastic boxes and incubated for 48 hours at 25 ºC. The similar wounds were made by puncturing around the apple equator with a sterile borer (5 mm wide and 3 mm deep) (Zhang et al., 2016). 10 μl of *B. dothidea* was pored in each apple wound. All the treated apple fruits were kept in incubation at 25 ºC with a high range (90–95%) of humidity. The 3-, 5-, and 7-days post inoculation (dpi) disease severity and the induced resistance were calculated by the rate of incidence and lesion area. The apple ring rot incidence (infected wounds only) was calculated as in infected wound percentage and lesion diameter. The three replicates of the experiments were conducted with 10 fruits in each group.

2.7. Apple Fruit Sample Collection

The effective dose of CRNPs was carried out only for further biochemical and mechanical examinations. Fresh cut apple fruits (Cube size) were immersed for 15 mins with 40 μg/mL of CRNPs and 0.1% methanol as control fruits. After 15 mins the fruits were collected and air dried for 2 hr at room atmosphere. Afterwards, they were stored in plastic boxes and incubated for 48 hours at 25 ℃. The similar wounds were made by puncturing around the apple equator with a sterile borer (5 mm wide and 3 mm deep) (Zhang et al., 2016). 10 μl of *B. dothidea* was poured in each apple wound. All the treated apple fruits were kept in incubation at 25 ℃ with a high range (90–95%) of humidity. The fruits equator area was collected after 7 days treatment with each day intervals and cut into small species and they were stored at −86 ºC then used for further biochemical estimations. All the experiments were done three times, with five fruits in each group.

2.8. Determination of sialic acid content, Antioxidant Enzyme Activities, and Defensive Enzyme Activities

Polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL), β-1,3-glucanase (GLU), polyphenol oxidase (PPO), and β-1,3-glucanase (GLU) were measured by Zhang, Zhang et al., (2016) and Zhang et al., (2016) methods. The antioxidant assay enzymes, such as superoxide dismutase (SOD), and catalase (CAT), were assayed by assay kits. Three replicates reading were done for concordant. The SA glucoside (SAG) and sialic acid content were measured by the previously described method of Verberne et al., (2002).

2.9. Determination of total sugar, reducing sugar, sucrose, TP, TF and vitamin C

The total sugar, reducing sugar, sucrose, vitamin C, TP, and TF were estimated by Aurelie et al., (2016), Nweze et al., (2015), Folin-Ciocalteu method (Shan et al., 2005), and Ayoola et al., (2008) respectively.

2.10. Characterization and sensory analysis of apple fruit during the shelf-life extension studies

The PH, weight loss, and total soluble solid (TS) were estimated by Waghmare and Annapure, (2015) methods. Sensory analysis was assessed by Memon et al., (2015) method. A trained panel of five people was used to recognize and score the sliced apple's quality using sensory attributes.

2.11. Statistical Analysis

The mean and standard deviation (SD) were used to convey the results. ANOVA was used to analyze all of the data, followed by Duncan's multiple range tests. A P value of less than 0.05 was used to define statistical significance.

3. Results

3.1. UV-visible and FTIR analyses of CRNPs

The calcium chloride was oxidized with *Colchicum ritchii* flower extract was assayed by UV-Vis spectra and the result is shown in Fig 1. In a UV-Vis spectra peak, the absorbance was seen at 448 nm. This single absorbed peak confirmed the nanoparticles' formation.

The FTIR spectrum is one of the qualified and popularly used method for analyses the biomolecules in synthesized nanoparticles. The FTIR spectrum was done for assessing the responsible biomolecules for synthesizing calcium oxide nanoparticles from Colchicum ritchii flower extract, and the FTIR spectrum result is shown in Fig. 2. Our result showed that 3642.51, 3433.84 and 2923.94 cm^{-1} were dispensed to O–H (s) stretch. 1628.07 cm^{-1} designated to O–H stretch. 1424.36 cm^{-1} dispensed to C–H stretch. 1060.75 cm^{-1} dispensed to the C–N stretch, and 867.37, 550.75 and 455.12 cm^{-1} were assigned to the C–H stretch. These presented biomolecules were represented by the formation of Colchicum ritchii flower extract and associated nanoparticles without altering the available bioactive molecules' natural properties.

3.2. SEM and EDx as well as DLS analyses of CRNPs

SEM and EDx analyses image of CRNPs is shown in Fig 3. This result showed in crystal shape and the EDx spectrum showed the Ca and C were the majorly and others also shown such as O, Na and Sn. These available elements were evidence for the formation of *Colchicum ritchii* flower extract-associated nanoparticles without altering the available bioactive molecules' natural properties.

The CRNPs nanoparticle formation was confirmed for further analyses of DLS, as shown in Figs. 4. In figure 4, we showed the lesser zeta potential value. This lesser value has clearly indicated the nanoparticles formation in clear nano-sizes without altering available biomolecules nature.

3.3. CRNPs induced resistance of apple fruit ring rot against Botryosphaeria dothidea

The effectiveness of CRNPs on apple ring rot caused by *Botryosphaeria dothidea* and the results is shown on Fig. 5. The control fruit treated with 0.1% methanol showed the same large zones in the inoculation site of apple fruit with brown lesions. In contrast, the developed ring rot by *Botryosphaeria dothidea* significantly reduced by treatment of CRNPs. The even lower dose of CRNPs was observed better reduction properties.

3.4. Effect of CRNPs on Defensive Enzyme Activities

The defense enzyme activities such as CHI, PPO, GLU, and PAL were estimated in apple fruits with CRNPs treatment and in control apple fruits, and the results are shown in

Figs. 6. The defense enzyme activities of CHI, PPO, GLU, and PAL were significantly increased from day 1 and reached higher levels in day 7 when compared to control fruits. Hence, these results are indicating the CRNPs is having better disease resistance against the *Botryosphaeria dothidea* caused apple ring rot.

3.5. Effect of CRNPs on antioxidant markers

The antioxidant markers were estimated in control and CRNPs treated apple ring rot and the results are shown in Fig 7. The activities of SOD and CAT were significantly unchanged in control fruit; however, these enzymes' activities were significantly increased in CRNPs-treated apple fruits.

3.6. Effects of CRNPs on SA and SAG levels

The effect of CRNPs on SA and SAG levels on apple ring rot fruits were estimated, and the results are shown in Fig. 8. The SA content and the SAG level were changed moderately in control apple fruits, but the SA and the SAG level were significantly increased in CRNPs-treated apple ring-rot fruits.

3.7.Effects of CRNPs on metabolites changes

The levels of primary and secondary metabolites were assessed on normal, ring rot disease control and the CRNPs treated apple, and the results are shown in Fig. 8. The level of primary metabolites including sucrose, reducing sugar and total sugar as well as secondary metabolites including total phenolics (TP), total flavonoids (TF) and vitamin C were decreased significantly in ring rot disease control apples when compared to normal control apples and these were prohibited in CRNPs treated apples.

3.8 Shelf-life extension of CRNPs on fresh cut apples.

In this study was focused the shelf-life extension of CRNPs on sliced apple fruits during the storage and the results are showed in figures 9 and 10. The pH values of CRNPs and calcium chloride-treated sliced apple fruits were significantly increased day by day up to 25 days when compared to control. Our study has no significant weight loss was observed in CRNPs and calcium chloride treated sliced apples fruits up to 25 days. CRNPs-treated sliced apple fruits showed minimal weight loss and a larger juice drop when compared to standard calcium chloride treatment. The aroma, appearance, texture and decay were decreased significantly in 25th days of control fruits and treated with CRNPs and calcium chloride were significantly prevented to decreasing of sensory analyses.

4. Discussion

Nanotechnology is the engineering and its widely arriving in numerous sectors even in food and agricultural industries because of their exceptional usage in our daily lives. Presently, nanotechnology have been applied in food areas also with numerous applications, including acting as a pathogenic killer or inhibitor, nutrition improvement in foods and fruits, food packaging and processing, flavor inclusion in food, as well as detecting degradants, contaminants, pathogens, and allergens. Food and agricultural industries have the biggest day today because necessary services are affected by microorganisms, which damage the nature of food by decomposing. Numerous methods of nanoparticles production or synthesis have been used to kill bacteria and fungi, such as nanofibers, nanopowder, and metal and metal oxide nanoparticles. The direct use of these metal nanoparticles is toxic for humans and even causes environmental contamination. However, the natural organic nanoparticles synthesized from plant sources are not toxic, simple method and is having unique nano-size and the plentiful application in food industries. Previous studies have scrutinized the biological plant nanoparticles for their promising and potent antimicrobial and antiviral properties (Rajeshkumar et al., 2019; Fouda et al., 2022), while numerous nanoparticles have been successfully synthesized from plants and other relevant sources. Hence, our study was designed to examine the organic nanoparticles derived from plant sources and the calcium chloride. Plant is called the desert saffron and this widely used to traditional medication such as anti-inflammatory and digestive problems. In the present study, the synthesized and characterized calcium oxide nanoparticles from plants are reported from economic and ecological perspectives. It has been demonstrated that the organic synthesis of nanoparticles from the use of plant is a principal method because of their non-toxic and less economic (Fouda et al., 2022). Our results showed that the synthesis of calcium oxide nanoparticles occurred after oxidation of calcium chloride and plant extract, which has been primarily vindicated by ultraviolet–visible spectroscopy and band absorption at 448 nm. In an earlier report, it was agreed that the increasing absorption rate was associated with the increasing extract ratio, thereby confirming the systematic synthesis of nanoparticles. FTIR analysis has been done to identify the calcium ions oxidized into calcium oxide in the water extract of the plant. This analysis predicts the presence of biomolecules that are involved in the oxidation of calcium into calcium oxide in the present plant extract. In accordance with early reports, our FTIR spectrum peaks have confirmed the C = O, N–H, and OH stretching vibrations (Veeramani et al., 2020), thereby confirming the nanoparticles' stabilization because of oxidizing the calcium in the presence of plant extract. The aggregation of nanoparticles in solution were measured by DLS, and we also observed a lower zeta potential value, which thereby indicated the synthesis of nano-sized molecules. Early study has said that the nanopartilces is very sensitive to aggregation and its reported very lower zeta potential value. The majority of SEM images of nanoparticles showed their various natures, including spherical, clustered, and crystalline shapes. In our study, the crystalline structure of nanoparticles was observed in nanoscale form by SEM image. Previous studies have reported the spherical shape of calcium oxide nanoparticles by SEM analysis (Mahmoud et al., 2022). In addition, the crystalline structure of synthesized calcium oxide nanoparticles was again confirmed by EDx analysis (Khine et al., 2022). EDx peaks confirmed the major presence of Ca and C, which has been proven by the calcium oxide nanoparticle synthesis. Moreover, other elements, including O, Na, and Sn, may be due to the emission of proteins and enzymes from plant extracts, whereas the nanoparticles can be followed either by free enzymes or amino groups.

Plant or fruit disease is playing a vital issue in worldwide and currently chemical fungicidal agent or plant activator are using for control or manage these diseases especially for postharvest diseases. The synthetic chemical fungicidal can cause side effect, therefore, the natural novel promising alternative plant activator or fungicidal agent are necessary to find for preventing the postharvest diseases. Hence, we have designed to synthesize *Colchicum ritchii* flower-associated calcium oxide nanoparticles and evaluate their novel, effective, and promising alternative fungicidal agent against the apple ring rot disease. *Colchicum ritchii* is a desert saffron, and it is commonly used in traditional medicine for stomach problems and has also been scientifically proven to control acute and chronic parasitic diseases. Our study has proven that CRNPs shows the admirable potential inhibitory effect 9 of apple ring rot postharvest disease. The various three different concentrations of CRNPs were applied for control apple ring rot disease caused by *Botryosphaeria dothidea* and this effectiveness was measured by lesion diameter. All three concentrations effectively inhibited the apple ring-rod disease in a dose-dependent manner. Previous study has similar with our study that the L-arginine and melatonin are used to treat the tomato ring trot caused by *Botryosphaeria dothidea* and they have received positive inhibitory effectiveness (Dou et al., 2022). Moreover, we found that the lower two doses had no effect on the sugar and color content of apple fruits, but the third dose had a slight change in color and sugar content. We have received better inhibitory effectiveness without changing the apple fruit color and sugar content at lower doses of CRNPs; therefore, this low dose has been used for further investigation of CRNPs and prompted resistance against the *B. dothidea*-caused apple ring rot disease.

Apples is one of the nutrition rich and tasted fruits and it is widely used by peoples in worldwide. Apples are abundant in primary metabolites of carbohydrates, amino acids and proteins as well as secondary metabolites of total phenolics, flavonoids, and vitamins. It is having numerous pharmaceutical benefits including cholesterol lowered, anticancer, and antioxidant properties and these properties might be due to the presence of primary and secondary metabolites including carbohydrates, quercetin, vitamins, amino acids, catechin, chlorogenic acid and phloridzin (Huang et al., 2021). The primary metabolites of carbohydrates such as sugars and sucrose are directly associated with the fruits taste. Hence, in this study have estimated sucrose, total sugar, free sugar as well as TP, TF and the vitamin C and these levels were significantly decreased in ring rod disease. Treatment with CRNPs were significantly prevented these metabolites towards normal apples and these were supported to prevent the apple ring rod disease without changing the taste through the prevented metabolites changes.

PPO, PAL, CHI, and GLU are very important major defense enzymes, and these are used by researchers as markers to assess the induced resistance against the fungal infection. (Zhang et al., 2016; Liu et al., 2019). PAL is the most important key regulatory enzyme for the phenylpropanoid pathway that is convoluted for the biosynthesis of anthocyanin or flavonoid during fruit development (Tian et al., 2006). PAL has enhanced the phenolic compound accumulation, and these phenolic compounds are oxidized by PPO, which leads to the induction of fungal resistance. GLU and CHI are involved in the direct inhibition of fungal growth by the mechanism of induced defense chain reactions, which activate the oligosaccharide elicitors and fungal cell wall degradation (Edreva, 2005). A previous study has reported that the activities of these PPO, PAL, CHI, and GLU enzymes triggered the L-arginine-induced fungal resistance against the *B. cinerea*-caused tomato fruit (Zheng et al., 2011). In addition, the previous study also proved that these enzyme activities are upregulated by the SA-induced fungal resistance in apple fruit against the *Glomerella cingulata* infection (Zhang et al., 2016). In our study, the PPO, PAL, CHI, and GLU enzyme activities were significantly higher in patients treated with CRNPs when compared to controls. Moreover, our study has reported that CRNPs increase the SA level in apple fruit. Hence, our study has clearly proved that the CRNPs treatment induced the resistance of apple ring rot against the *B. dothidea* infection through the activation of an antifungal substance and the phenylpropanoid pathway.

SOD and CAT are playing a crucial role in controlling the reactive oxygen species or free radical generation through the antioxidant defense mechanism, which is the main mechanism for determining the resistance of apple fruit against the fungal pathogen (Mittler, 2002). Previous study reported that the plant associated nanoparticles is acted as some potent antioxidant properties which involved the inducer of resistance in apple fruit against the pathogenic microorganism (Zhao et al., 2018). In our study, the activities of SOD and CAT were significantly increased in CRNPs-treated apple fruit and maintained a higher level when compared to that of control apple fruit during storage. Our study proved that the high activities of antioxidant enzymes treated by CRNPs have played a crucial role in the scavenging of oxidative stress, which was also supported by a previous report (Tareen et al., 2012). Moreover, another study also supported the fact that the SA has inhibited the fruit ring rot disease caused by *B. dothidea* through the enhanced enzyme activities, including SOD and CAT (Zhang et al., 2016). Our study also demonstrated that the CRNPs have reduced the incidence of apple ring rot disease, which might be due to the enhanced activities of SOD and CAT.

Salicylic acid is played a crucial role for plant defense regulatory signals against the microorganism infection. Previous studies have reported that the SA has played a major role in inducing resistance against the *B. dothidea*-caused apple fruit (Zhao et al., 2020). SA and SAG content was assessed for its support of CRNPs induced resistance against *B. dothidea*-caused apple ring rot. In our study, the SA and SAG content was significantly increased in CRNPs-treated apple fruits when compared to control apple fruits. Hence, our study has proven that the CRNPs induce resistance against the apple ring rot through the increased accumulation of SA content.

Fruits and vegetables are the most important foods for humans, and they have been directly associated with numerous health benefits. It can be consumed by people in either a processed form or a fresh one. Fresh cut vegetables or fruits in a readily used and minimally processed form are most welcome by peoples due to their new life style or working style modifications. However, the food industry is facing challenging issues in maintaining the quality of the fresh-cut fruits and vegetables during storage. Hence, this study is mainly focusing on the effect of CRNPs on the shelf-life extension properties of sliced apple fruit during storage. Our results showed that the pH values of CRNPs and calcium chloride-treated sliced apple fruits were significantly higher day by day up to 25 days when compared to the control. This increased pH value of CRNPs could confirm the freshness of sliced apple fruits, which was proven by earlier studies (Heard, 2002). Weight loss measurement is a very impressive and important measurement for checking the quality of fruits and vegetables. Our study has no significant weight loss was observed in CRNPs and calcium chloride treated sliced apples fruits up to 25 days. CRNPs-treated sliced apple fruits showed minimal weight loss and a larger juice drop when compared to standard calcium chloride treatment. Hence, CRNPs is good preservative and it was synthesized in a simple lost cost method. TSS is a vital measurement to check fruit quality, and it represents the quantity of soluble solid in a fruit's liquid. TSS abnormalities can affect the fruit taste because its direct linked with the sweetness. Sensory analysis including the taste, appearance, texture, flavor and smell etc… have been using from the centuries for tolerant or refusing the food products. The aroma, appearance, texture and decay were decreased significantly in 25th days on control fruits and treated with CRNPs and calcium chloride were significantly prevented to decreasing of sensory analyses up to 25 days. Hence, our finding has proved that the CRNPs is ideal source to extend the sliced apple fruit shelf-life up to 25 days without using hazardous chemicals. Our previous study also proved that the calcium oxide nanoparticles are an ideal source for the shelf-life extension of vegetables and fruits up to 20 days (Veeramani et al., 2022).

5. Conclusion

Our study reports have concluded that the *Colchicum ritchii* is beneficial source for synthesis green calcium oxide nanoparticles. Moreover, their biological role has led to the conclusion that the CRNPs have potential antifungal properties and protect against the apple ring rot disease caused by *Botryosphaeria dothidea*. CRNPs have alleviated disease severity by increasing the activities of antioxidant enzymes and defensive enzymes including PPO, PAL, CHI, and GLU. In addition, CRNPs have increased the accumulation of SA content. Hence, our study results have concluded that the CRNPs prevent the apple ring rot disease without changing the taste through the prevented metabolites changes and increased sialic acid synthesis, increased activities of defense enzymes, and also by increasing antioxidant status. In addition, the CRNPs is superlative source for apple shelf-life extension up to 25 days without any toxicology nature.

Acknowledgements

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FIGURE 1. Spectroscopic image of CRNPs.

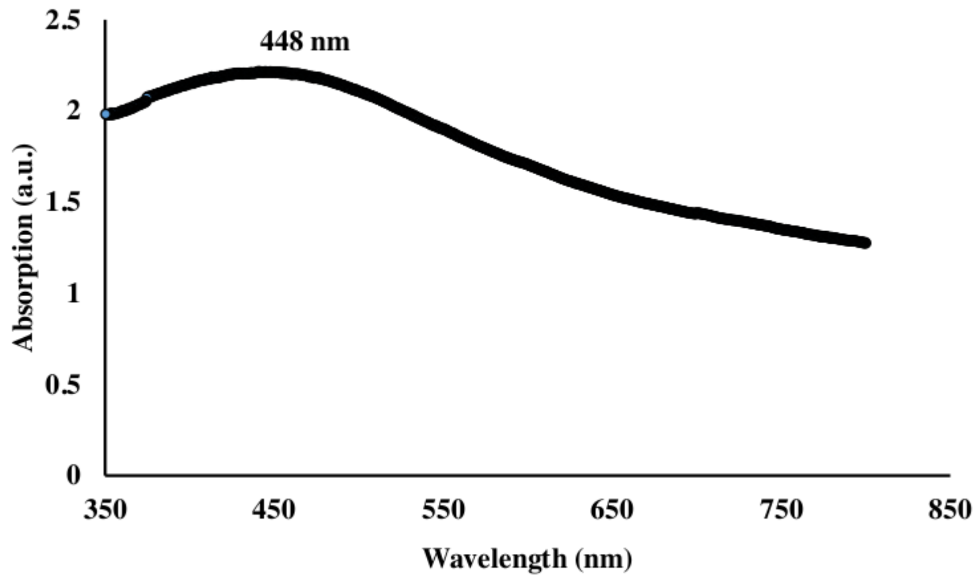


FIGURE 2. FTIR image of CRNPs.

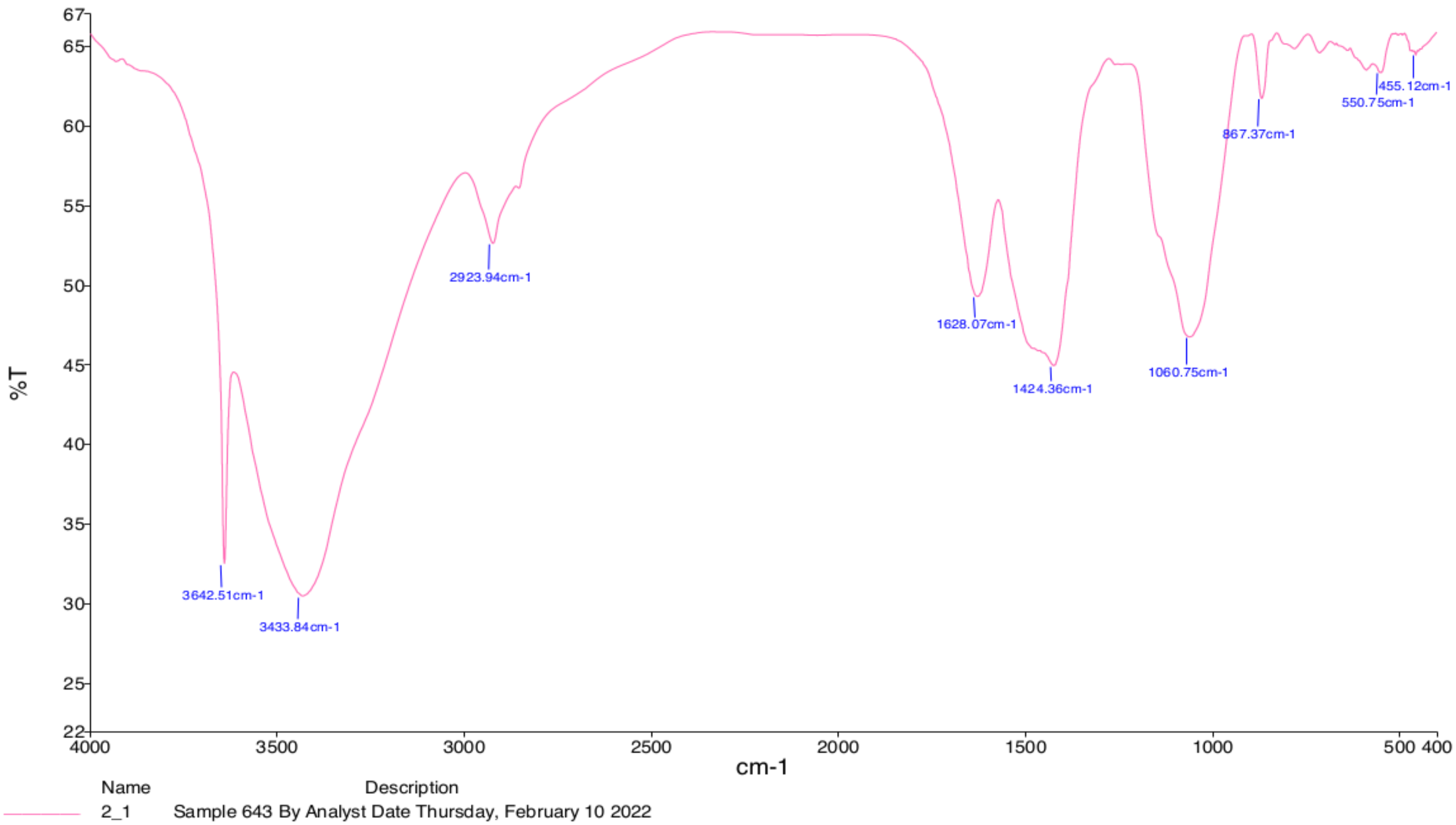


FIGURE 3. EDX image of CRNPs.

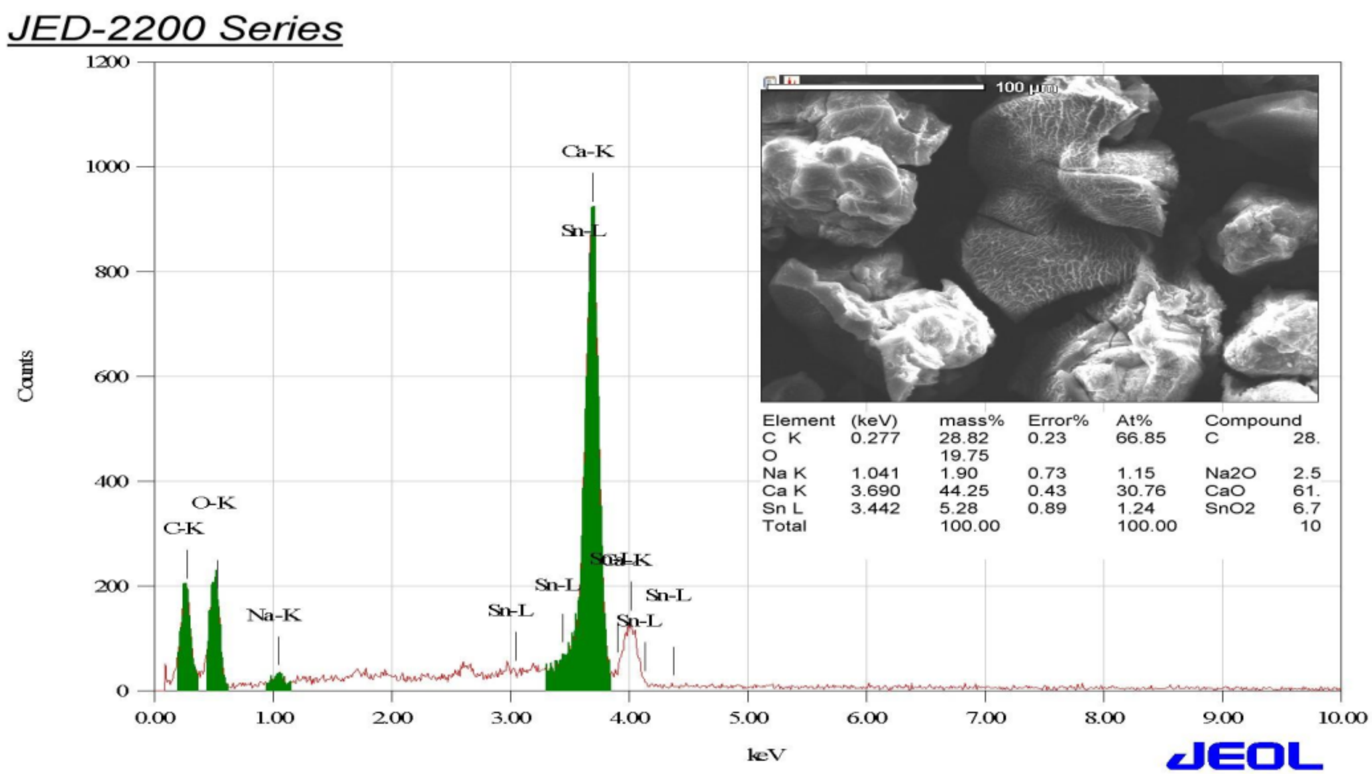


FIGURE 4. Size distribution and zeta potential images of CRNPs.

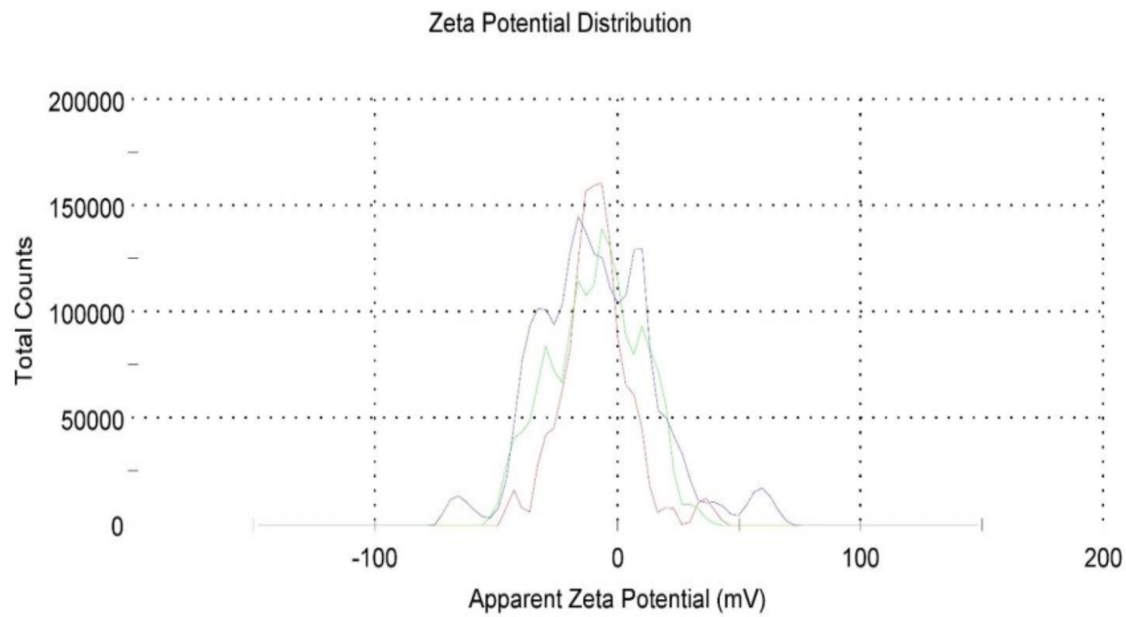
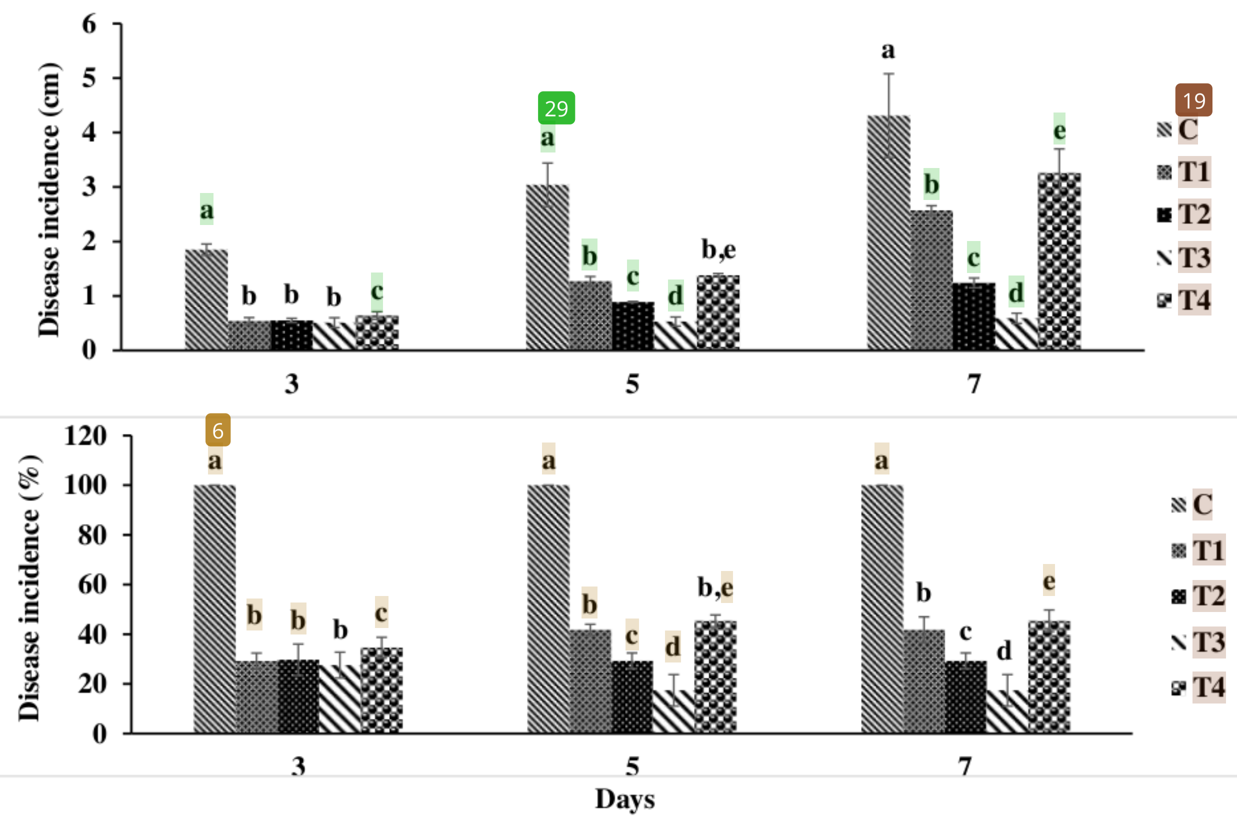
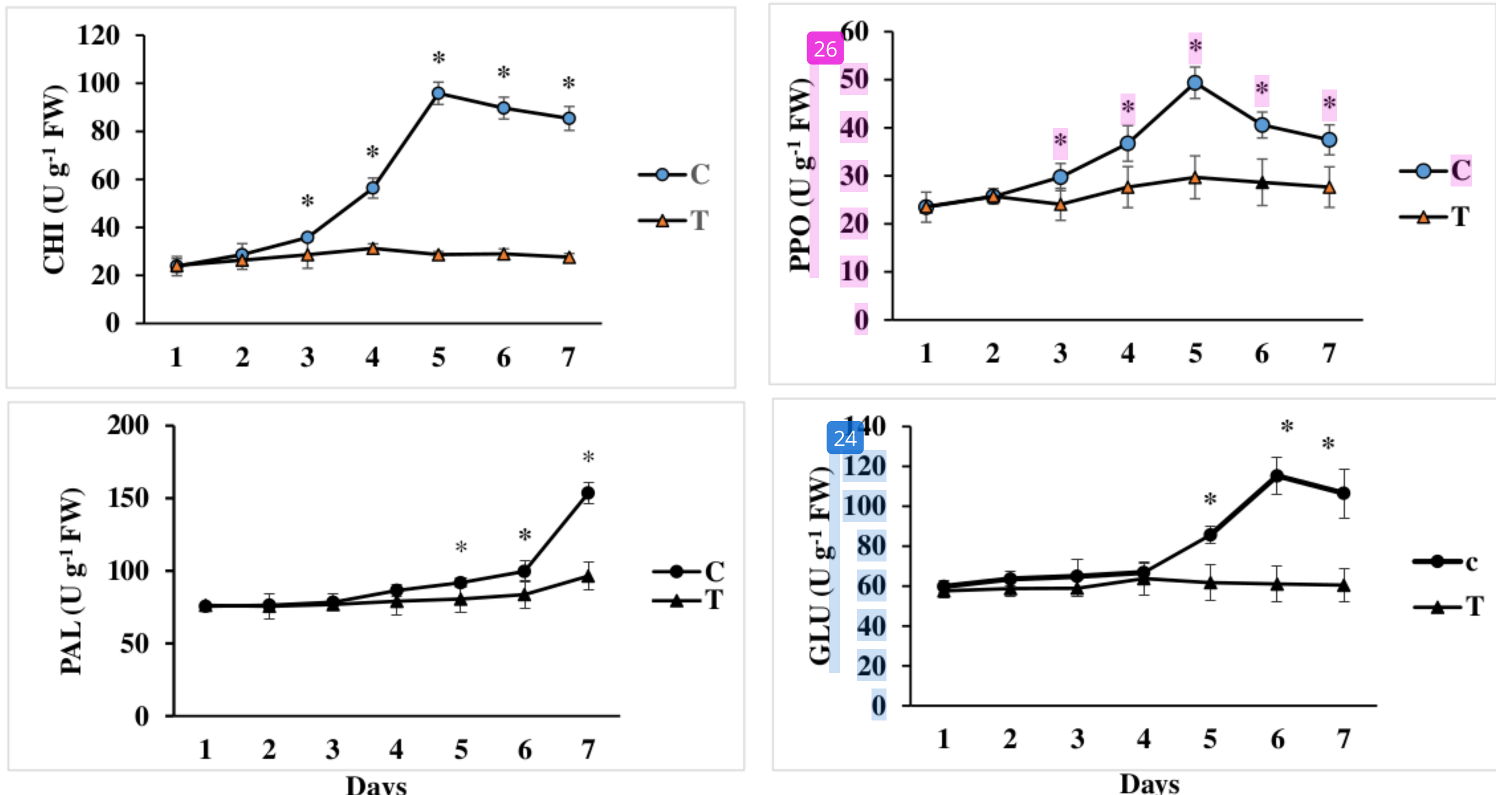


FIGURE 5. Effect of CRNPs on *Botryosphaeria dothidea* caused apple ring rod disease during after harvest storage.



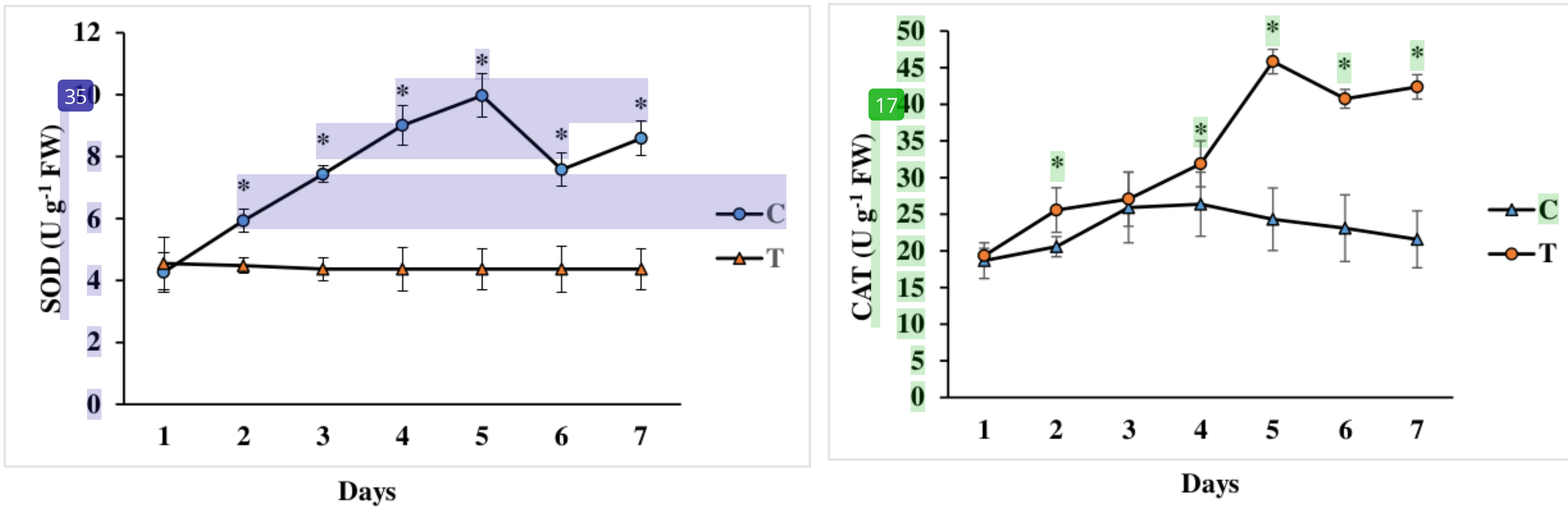
C, Control sample, T1, T2, T3 and T4 are different concentrations of CRNPs such as 10, 20, 40, and 80 $\mu g/mL$. Mean and standard deviation (SD) were used to average of three reaping values, P value of less than 0.05 was used to define statistical significance.

FIGURE 6. Effect of CRNPs on the activities of CHI, PPO, PAL, and GLU in apple fruits.



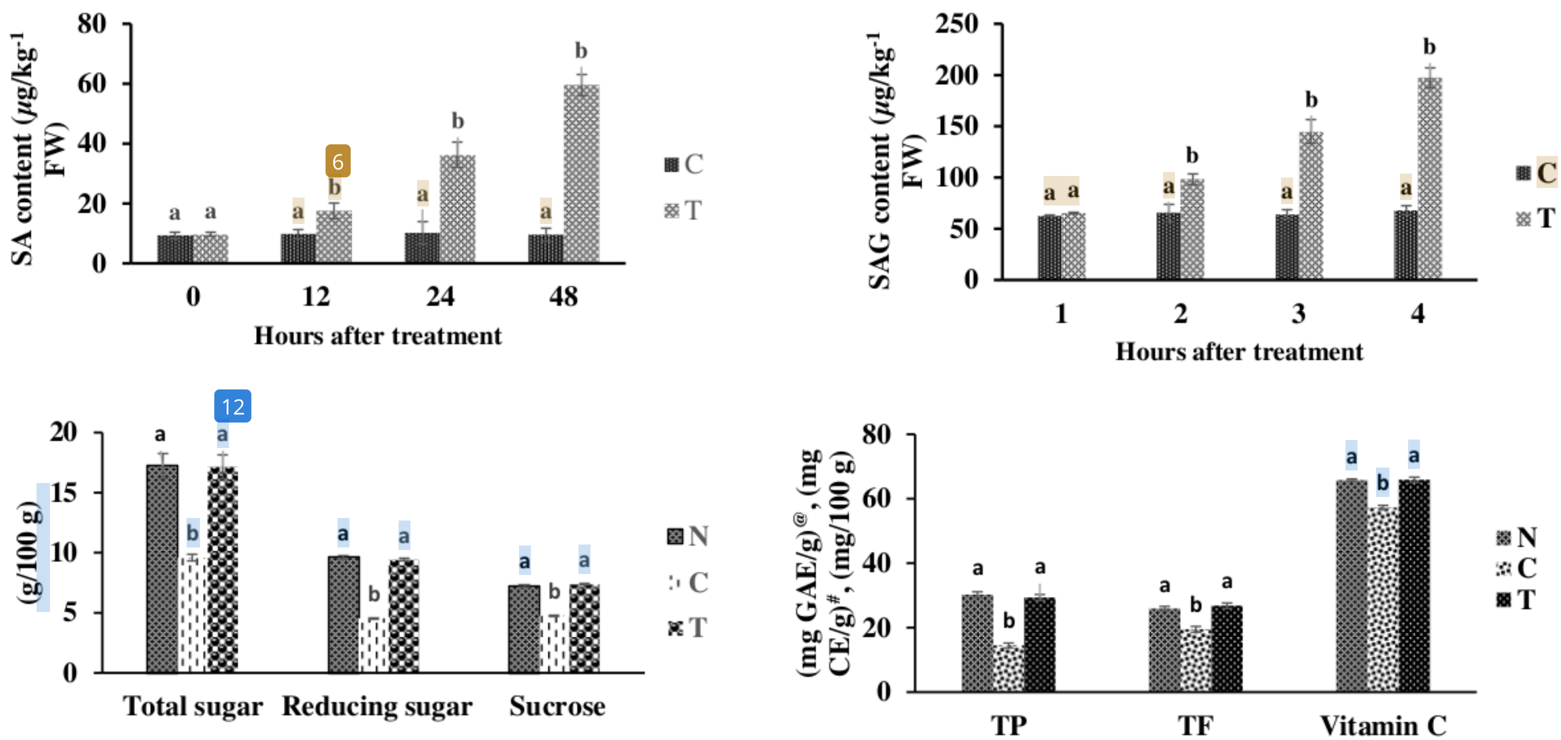
C- Control, T-40 40 µg/mL of CRNPs, Mean and standard deviation (SD) were used to average of three reaping values, P value of less than 0.05 was statistical significance from control to significant time frame.

FIGURE 7. Effect of CRNPs on the activities of SOD and CAT in apple fruits.



C- Control, T-40 40 µg/mL of CRNPs, Mean and standard deviation (SD) were used to average of three reaping values, P value of less than 0.05 was statistical significance from control to significant time frame.

FIGURE 8. Effect of CRNPs on the levels of SA and SAG content as well as primary and secondary metabolites in apple fruits.



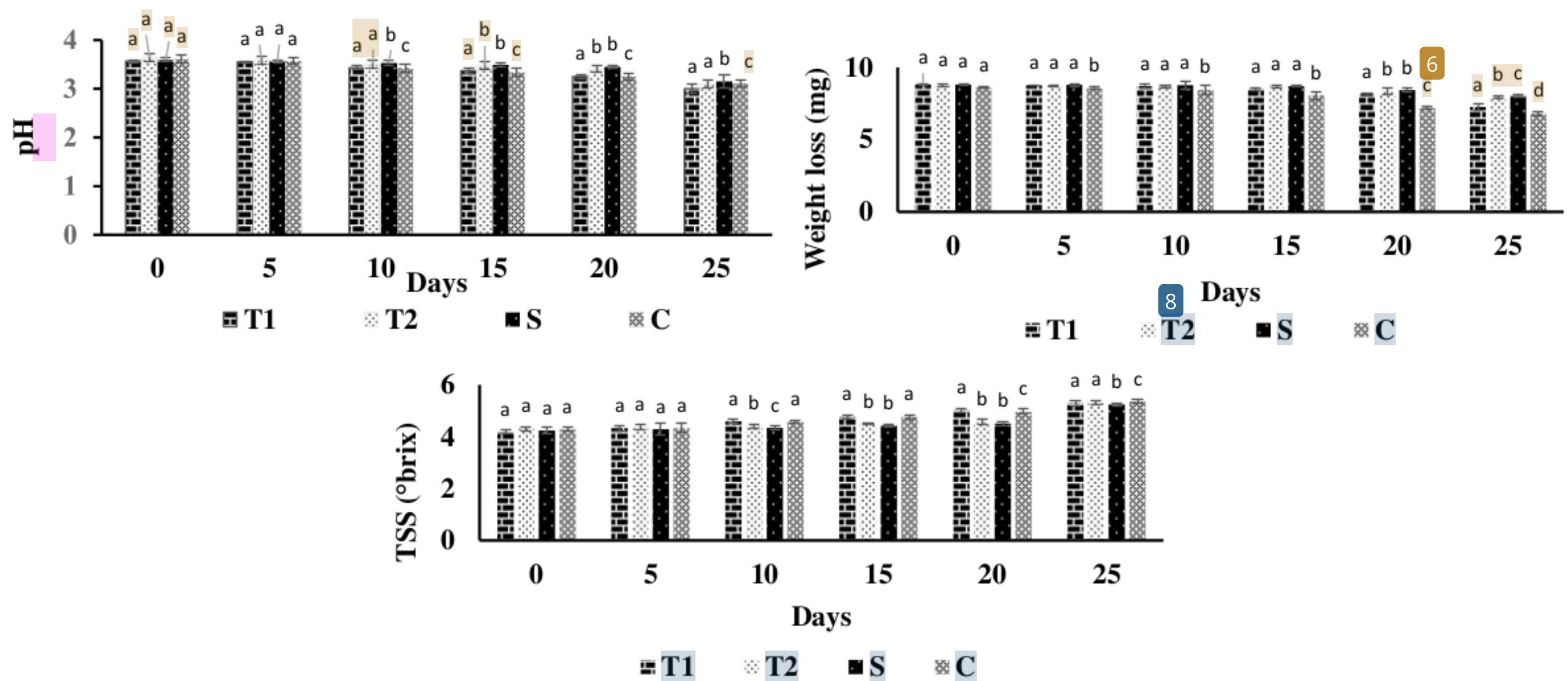
C- Control, T-40 µg/mL of CRNPs,

N-Normal Apple, C-Pathogen induced control apple; T-40 µg/mL of CRNPs

@ and # are the TP and TF expressed as gallic acid and catechin equivalents

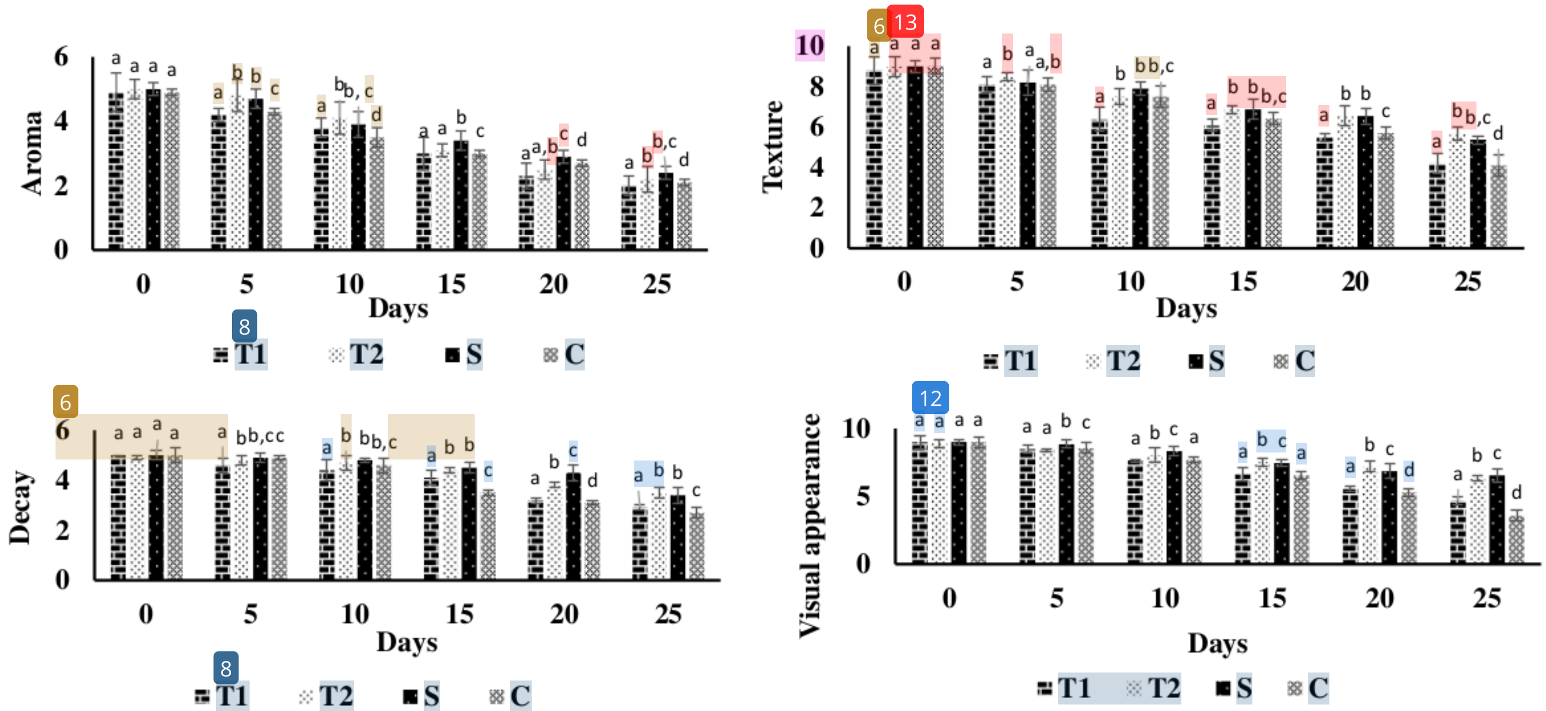
Mean and standard deviation (SD) were used to average of three reaping values, P value of less than 0.05 was statistical significance from control to significant time frame.

Figure 9. Effect of CRNPs on PH, weight loss and TS of fresh-cut apple during storage at 5 °C



T1- 20 μg/mL of CRNPs; T2 40 μg/mL of CRNPs; S-standard of 2% Calcium chloride C-Untreated control
Mean and standard deviation (SD) were used to average of three reaping values, P value of less than 0.05 was statistical significance within the same frame.

Figure 10. Effect of PH, weight loss, TSS and visual appearance of fresh-cut apple during storage at 5 °C



T1- 20 μg/mL of CRNPs; T2 40 μg/mL of CRNPs; S-Standard of 2% Calcium chloride C-Untreated control

Mean and standard deviation (SD) were used to average of three reaping values, P value of less than 0.05 was statistical significance within the same frame.

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