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Research Article

Eco-Friendly Management of Gladiolus Corm Rot and Wilt Caused by *Fusarium oxysporum* Using Green-Synthesized Silver Nanobiofungicides

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ABSTRACT

This study evaluated the potential effects of biosynthesized silver nanoparticles (BSN) produced using plant pigments from *Clitoria ternatea*, *Solanum nigrum*, *Catharanthus roseus* (CR), and *Cucumis prophetarum* at four concentrations: control, 10, 15, and 20 mg L^{-1}. Scanning electron microscopy revealed that the synthesized silver nanoparticles (AgNPs) were predominantly spherical, with an average particle size of approximately 18 nm, as confirmed by particle size distribution analysis. Fourier transform infrared spectroscopy indicated the presence of O–H (carboxylic acid) and N–O (nitro compound) functional groups, suggesting that plant biomolecules played a dual role in the reduction and stabilization of AgNPs. X-ray diffraction analysis showed four distinct diffraction peaks at 38.19° (111), 44.37° (200), 64.56° (220), and 77.47° (311), confirming the crystalline nature and high purity of the AgNPs synthesized from the plant extracts. Artificial inoculation of *Fusarium oxysporum* on gladiolus corms was conducted to assess the antifungal efficacy of BSN. Corms treated with 20 mg L^{-1} CR-based BSN exhibited dark brown lesions with approximately 90% disease suppression, reduced fungal colony size (2.36 cm and 2.14 cm), and significantly extended vase life (20 and 21 days) in the 'White Prosperity' and 'Red Advance' cultivars, respectively. BSN-coated corms stored under ambient and cold storage conditions showed minimal disease incidence, with reductions of 1.56% and 1.05% in 'White Prosperity' and 'Red Advance', respectively, at 20 mg L^{-1} CR treatment. Overall, BSN, particularly CR-based nanoparticles at 20 mg L^{-1}, proved highly effective in controlling Fusarium wilt and enhancing vase life, highlighting their potential as an eco-friendly strategy for crop protection and postharvest management in floriculture.

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Introduction

Gladiolus (*Gladiolus grandiflorus*) is a globally cultivated ornamental crop belonging to the family Iridaceae and is popularly known as the "Queen of Bulbous Flowers." It is native to the subtropical regions of South Africa (Saleem et al., 2013). The genus *Gladiolus* comprises more than 180 species and over 30,000 cultivars, of which more than 20 are commercially cultivated for cut flower production (Ranjan et al., 2010). Owing to its excellent vase life, gladiolus is widely used in bouquets, floral arrangements, borders, and bedding (Dwivedi et al., 2018). The crop is primarily grown for cut flower

production, often under protected environments such as greenhouses or polytunnels, which provide optimal conditions for flower quality and yield (Fernando et al., 2020). Gladiolus holds a significant position in the global cut flower industry. In Pakistan, the floriculture sector has substantial potential to contribute to economic growth, and farmers are increasingly shifting from traditional agronomic crops to floriculture due to its high profitability. During 2004-05, approximately 37% of farmers transitioned from conventional cropping systems to floriculture enterprises (Riaz et al., 2009).

Corm rot caused by *Fusarium oxysporum* is one of the most destructive diseases of gladiolus, leading to leaf yellowing, wilting, and dry rot at the base of the corm, ultimately reducing floral quality and market value (Khan et al., 2017). The disease is characterized by progressive discoloration of the corm, changing from light maroon to dark maroon and eventually black, followed by complete mummification. Infection in refrigerated corms can be particularly severe, often resulting in plant death and causing economic losses ranging from 60% to 100% of production (Elmer and Kamo, 2018).

Fusarium species are among the most important wilt pathogens affecting a wide range of agricultural crops (Colak and Bicici, 2013). The pathogen is both seed- and soil-borne and induces symptoms such as leaf curling, bending, arching, stunting, yellowing, and drying, accompanied by root and corm rot in both field and storage conditions. *F. oxysporum* f. sp. *gladioli* is responsible for three distinct types of corm rot in gladiolus, namely vascular corm rot, brown rot, and basal rot (Yasmin et al., 2018). Although various chemical fungicides have been employed to manage this pathogen, their indiscriminate use has resulted in environmental contamination, human health risks, and the development of fungicide-resistant pathogen strains. Furthermore, chemical residues may enter the food chain, posing serious threats to human health (Lamsal et al., 2011).

In recent years, the application of nanoparticles in agriculture has increased significantly. Due to their extremely small size, nanoparticles can easily penetrate plant cell wall pores. Silver nanoparticles (AgNPs), in particular, are capable of entering plant tissues through stomata and the bases of trichomes on leaf surfaces and can be translocated to different plant organs (Nair et al., 2010). Silver nanoparticles have been successfully evaluated in agricultural systems, where their roles in inhibiting ethylene action and delaying leaf abscission have been well documented. Furthermore, AgNPs may contribute to the regulation of plant redox status due to their electron exchange capacity with trace elements (Mukherjee and Mahapatra, 2009).

Nanoparticles can be synthesized using physical, chemical, and biological methods. Although chemical synthesis is rapid and efficient, it requires toxic reducing and capping agents for nanoparticle stabilization, leading to the generation of hazardous and non-eco-friendly byproducts. These limitations have driven increased interest in biological approaches that eliminate the need for harmful chemicals, thereby promoting the concept of "green nanotechnology." To date, several biological methods for the extracellular and intracellular synthesis of nanoparticles using microorganisms, including fungi, bacteria, and plants, have been reported (Durán et al., 2005; Huang et al., 2007). Among these, plant-mediated synthesis is considered safer, cost-effective, and environmentally friendly due to the presence of natural reducing and capping agents, while also eliminating the need for microbial isolation and maintenance (Singhal et al., 2011).

Numerous studies have demonstrated the significant antifungal, antibacterial, and antimicrobial properties of silver nanoparticles in various plant systems. However, many earlier studies relied on chemically synthesized AgNPs, raising concerns regarding environmental safety and toxicity. Given the increasing emphasis on sustainable and eco-friendly agricultural practices, there is a critical need to explore plant-based (green-synthesized) silver nanoparticles as safer alternatives. Future research should focus on optimizing plant-mediated AgNP synthesis, assessing their antimicrobial efficacy, environmental safety, and suitability for large-scale agricultural applications. Such an approach may offer a sustainable and non-toxic alternative to conventional chemical synthesis methods.

In view of the severe impact of *Fusarium* spp. on flowering plants, particularly gladiolus, the present study was designed with the objective of evaluating the efficacy of biosynthesized silver nanoparticles against *Fusarium* species causing corm rot.

Material and Methods

Pre-chilled gladiolus (*Gladiolus grandiflorus*) cultivars 'White Prosperity' and 'Advanced Red' were procured from a reliable supplier, Green Works, Lahore, Pakistan, an authorized importer of the Stoop Flower Bulb

Company, Holland. The corms were planted in sandy soil during the winter season (November) at the Lalazar Nursery Area, Gardening Wing, University of Agriculture, Faisalabad (UAF). The corms were planted at a spacing of 6 inches (plant × plant) and 18 inches (row × row).

Biosynthetic silver nanoparticles were prepared in the Nano Chemistry Laboratory, Department of Chemistry, UAF, to evaluate their effects on the vase life of gladiolus. Different concentrations of biosynthesized silver nanoparticles were used as vase solutions. Cultures of *Fusarium* spp. were obtained from the Department of Plant Pathology, UAF. The experiment was conducted at the Post-harvest and Floriculture Laboratory, Institute of Horticultural Sciences (IHS), UAF. The experimental layout followed a completely randomized design (CRD) with a factorial arrangement, comprising three replications for each treatment.

Plant material preparation

Four plant species were used for the biosynthesis of silver nanoparticles:

1. *Clitoria ternatea* (Butterfly pea)
2. *Solanum nigrum* (Blackberry nightshade)
3. *Catharanthus roseus* (Rose periwinkle)
4. *Cucumis prophetarum* (Chibber weed)

Fresh leaves were collected, thoroughly washed with distilled water, and oven-dried. After complete drying, the leaves were ground into a fine powder.

Biosynthesis of silver nanoparticles

A saturated silver nitrate ($AgNO_3$) solution was prepared by dissolving 0.31 mg of $AgNO_3$ in 3 ml of distilled water. Leaf powder (5 g) from each plant species was added to 100 ml of distilled water in a beaker and allowed to stand for 30 min. The mixture was then placed on a shaker for 2 h, followed by filtration.

The filtrate was mixed with the saturated $AgNO_3$ solution in a separate beaker and incubated at room temperature for 5 h. Subsequently, the solution was heated on a magnetic stirrer at 60°C with stirring speeds ranging from 100 to 700 rpm for 2 h. After cooling, the solution was centrifuged, and the resulting biosynthesized silver nanoparticles were collected in Petri dishes and oven-dried at 80°C for 48 h.

Stock solutions of biosynthesized silver nanoparticles were prepared by dissolving 10, 15, and 20 mg of nanoparticles in 1 L of distilled water to obtain concentrations of 10 mg L^{-1}, 15 mg L^{-1}, and 20 mg L^{-1}, respectively.

Treatments

T_0 = Control (distilled water)
T_1 = *Clitoria ternatea* (CT, 10 mg L^{-1})
T_2 = *Clitoria ternatea* (CT, 15 mg L^{-1})
T_3 = *Clitoria ternatea* (CT, 20 mg L^{-1})
T_4 = *Solanum nigrum* (SN, 10 mg L^{-1})
T_5 = *Solanum nigrum* (SN, 15 mg L^{-1})
T_6 = *Solanum nigrum* (SN, 20 mg L^{-1})
T_7 = *Catharanthus roseus* (CR, 10 mg L^{-1})
T_8 = *Catharanthus roseus* (CR, 15 mg L^{-1})
T_9 = *Catharanthus roseus* (CR, 20 mg L^{-1})
T_{10} = *Cucumis prophetarum* (CP, 10 mg L^{-1})
T_{11} = *Cucumis prophetarum* (CP, 15 mg L^{-1})
T_{12} = *Cucumis prophetarum* (CP, 20 mg L^{-1})

Characterization of biosynthesized silver nanoparticles

The biosynthesized silver nanoparticles were characterized at the Nano-Chemistry Laboratory, Department of Chemistry, University of Agriculture, Faisalabad (UAF). The nanoparticles were analyzed using scanning electron microscopy (SEM) to determine particle morphology, Fourier-transform infrared (FTIR) spectroscopy to identify functional groups involved in nanoparticle stabilization, and X-ray diffraction (XRD) to assess crystalline structure. These techniques collectively provided information on particle shape, size, surface chemistry, and crystallinity of the biosynthesized silver nanoparticles.

Application of biosynthesized silver nanoparticles as a coating on corms

Biosynthesized silver nanoparticles were applied as a coating on gladiolus corms. Artificial inoculation with *Fusarium oxysporum* f. sp. *gladioli* was performed in the fungal laboratory of the Department of Plant Pathology. The pathogen was isolated from infected host plants, purified, and maintained under low-temperature storage before use. For inoculation, the fungal culture was first grown in a growth chamber on potato dextrose agar (PDA). Healthy gladiolus corms were surface-sterilized, cut into halves, and placed in Petri dishes containing solidified agar medium. A mycelial disc of *F. oxysporum* f. sp. *gladioli* was then placed on the corm surface using the disc diffusion method. Colony characteristics of *Fusarium* spp., including colony color, size, texture, margin shape, and growth pattern, were recorded for morphological identification, recognizing that these traits may vary with species, culture medium, and environmental conditions.

Response of biosynthesized silver nanoparticle-coated corms against *Fusarium* under different storage conditions

After harvesting, gladiolus corms were dug from the field and kept at room temperature for 2-3 days. The corms were washed with distilled water and air-dried in the shade. Treatment solutions of biosynthesized silver nanoparticles were prepared using distilled water. The corms were dipped in the nanoparticle solutions for 10-15 min and then stored under two conditions: cold storage (7–12°C) and ambient/room temperature (25-30°C) for 60 days. The same treatments described in Experiment 1 were used in this experiment.

Data were recorded for morphological parameters, including corm size (cm) and corm weight (g). Biochemical analyses were conducted to determine phenolic content (mg g^{-1}), disease incidence (%), total sugars (%), reducing sugars (%), non-reducing sugars (%), and α-amylase activity (U mg^{-1} protein).

Statistical analysis

The collected data were subjected to analysis of variance (ANOVA) using Fisher's method. Treatment means were compared using the least significant difference (LSD) test at a 5% probability level (Anderson, 2001). All experiments were conducted following a completely randomized design (CRD) with a factorial arrangement and three replications per treatment.

Results

Characterization of biosynthesized silver nanoparticles

Scanning electron microscopy (SEM) was employed to evaluate the morphology and size of the biosynthesized silver nanoparticles (AgNPs) (Figure 1). The majority of the AgNPs exhibited a predominantly spherical morphology. Notably, the nanoparticles did not come into direct contact with one another, even within aggregates, suggesting effective stabilization by biomolecule-derived capping agents. Particle size distribution analysis revealed an average nanoparticle size of approximately 18 nm, as illustrated in the particle size histogram (Figure 1).

Fourier-transform infrared (FTIR) spectroscopy of the biosynthesized AgNPs revealed distinct absorption peaks at 2664.89, 2315.60, 2075.20, 1536.88, 1332.20, 1008.84, and 651.33 cm^{-1} (Figure 2). These peaks correspond to O–H stretching vibrations of carboxylic acids, O=C=O stretching of carbon dioxide, N=C=S stretching of isothiocyanates, N–O stretching of nitro compounds, O–H bending of alcohols, C–F stretching of fluoro compounds, and C–Cl stretching of halo compounds, respectively. The presence of these functional groups indicates the involvement of plant-derived biomolecules in the reduction, stabilization, and capping of AgNPs.

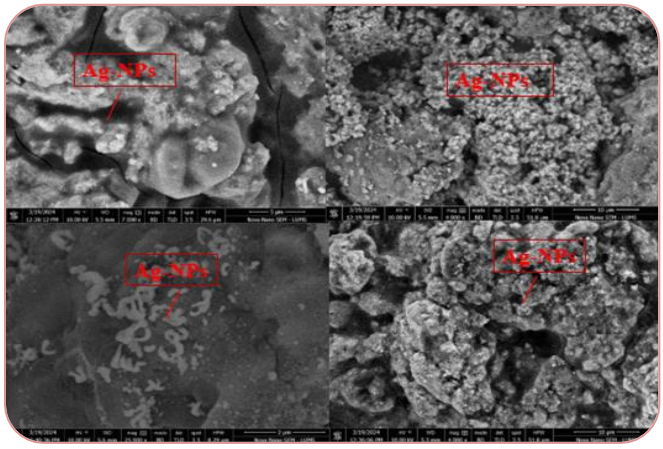

Figure 1. Electron microscopy images of biosynthesized silver nanoparticles derived from (a) *C. ternatea*, (b) *C. roseus*, (c) *C. prophetarum*, and (d) *S. nigrum*.

The crystalline nature of the AgNPs was confirmed by X-ray diffraction (XRD) analysis (Figure 3). The diffraction pattern exhibited prominent peaks at 2θ values of 38.19°, 44.37°, 64.56°, and 77.47°, corresponding to the (111), (200), (220), and (311) crystallographic planes of face-centered cubic silver. The presence of these characteristic peaks confirms the crystalline structure and high purity of the biosynthesized AgNPs.

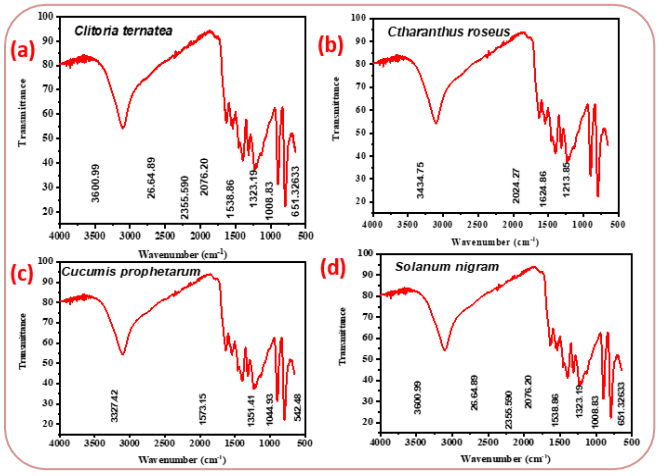

Figure 2. FTIR analysis of biosynthetic silver nanoparticles synthesized using extracts from (a) *C. ternatea*, (b) *C. roseus*, (c) *C. prophetarum*, and (d) *S. nigrum*.

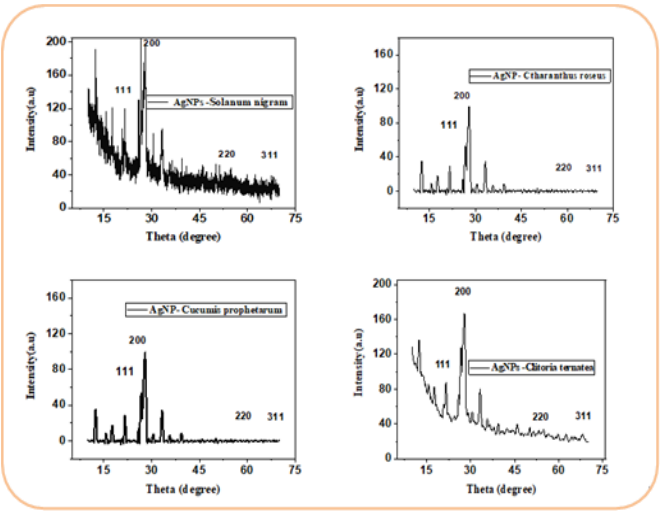

Figure 3. XRD analysis of biosynthetic silver nanoparticles synthesized using extracts from: (a) *C. prophetarum*, (b) *C. ternatea*, (c) *S. nigrum*, and (d) *C. roseus*.

Application of biosynthesized silver nanoparticles as a corm coating

Colony color and morphological characteristics

The colony color of five *Fusarium* isolates ranged from white to dirty white. Among the 31 isolates examined, pigment diffusion in the culture medium varied considerably: six isolates produced pink pigments, whereas 25 isolates exhibited dark brown pigmentation. Significant variation in colony coloration and conidial morphology among the isolates is illustrated in Figure 4.

Mycelial growth also showed pronounced variability. Some isolates exhibited dense, fluffy white mycelia, while others showed sparse white growth or a mixed pattern of white and purple mycelial development.

When *Catharanthus roseus*-mediated biosynthesized silver nanoparticles (BSN) were applied at a concentration of 20 mg L^{-1}, the corm surface initially developed a brown coloration, which later changed to light pink. In contrast, BSN synthesized from other plant extracts produced surface coloration ranging from white to pink (Figure 4).

Figure 4 further demonstrates that BSN synthesized using *C. roseus* at 20 mg L^{-1} effectively cleared *Fusarium* growth from the Petri plates, resulting in approximately 90% disease suppression in inoculated cultures. BSN derived from blackberry nightshade reduced *Fusarium* growth by approximately 60%. Similarly, BSN synthesized from butterfly pea and chibber weed achieved about 40% disease reduction each, compared with the untreated control (Figure 5).

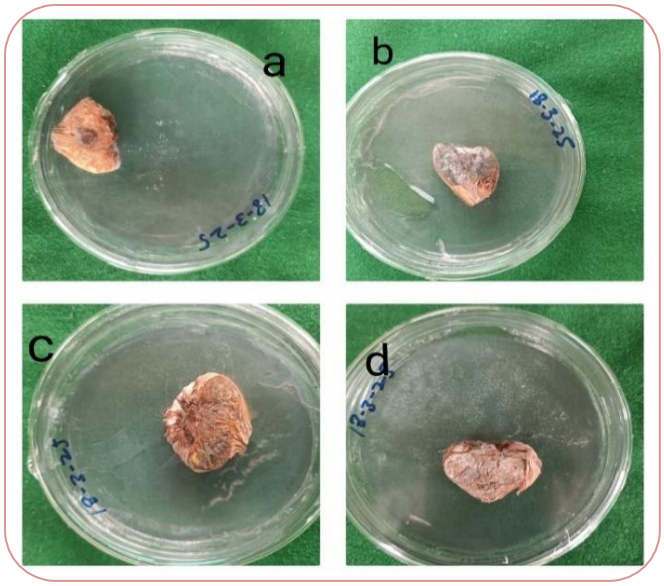

Figure 4. Effect of biosynthetic silver nanoparticles on *F. oxysporum* growth in Petri dishes over the first 7 days of observation: (a) *C. roseus*, (b) *S. nigrum*, (c) *C. ternatea*, (d) *C. prophetarum*.

Figure 5. Effect of biosynthetic silver nanoparticle treatments on *F. oxysporum* growth in inoculated petri dishes. Data for the first 20 days were compared with the untreated control.

Colony shape

All *Fusarium* isolates exhibited pronounced variation in colony morphology. Most *Fusarium* species produced distinct concentric rings upon colony maturation after 14 days of incubation. The results showed that treatment with *C. roseus*-derived biosynthesized silver nanoparticles

(BSN; 20 mg L^{-1}) coated on the corm surface markedly reduced the size of concentric rings by approximately 20% compared with the untreated control. Other biosynthesized silver nanoparticle treatments also resulted in colonies with smaller and more compact ring structures on the corm surface. Significant variation in colony shape and conidial morphology among different isolates is illustrated in Figure 6. Overall, mycelial development in *Fusarium* was highly variable, with some isolates exhibiting radial grooves, while others formed prominent concentric rings.

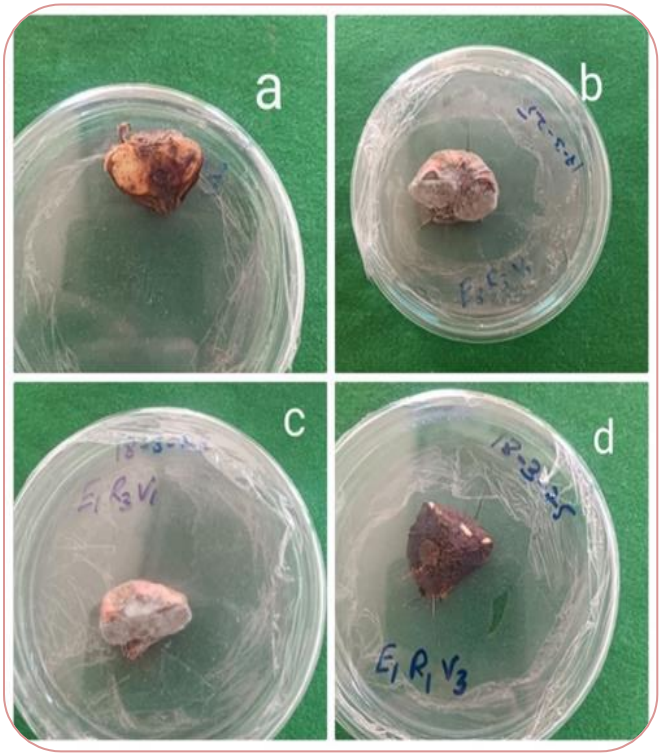

Figure 6. Effect of biosynthetic silver nanoparticle treatments on *F. oxysporum* growth on Petri dishes during the first 7 days. Treatments: (a) *C. roseus*, (b) *S. nigrum*, (c) *C. ternatea*, and (d) *C. prophetarum*.

Growth pattern

The colony growth pattern of five Fusarium isolates was fluffy. Pigment diffusion in the culture agar among the 31 isolates showed two distinct patterns: six isolates exhibited a flat type, whereas 25 showed a spreading type. Considerable variation in colony coloration and conidial morphology was observed among the isolates (Figure 7). The corms treated with a biosynthesized *C. roseus*-based silver nanoparticle (BSN) coating at 20 ml L^{-1} exhibited a clean, healthy surface free from *Fusarium* infection compared with the untreated control. In contrast, other biosynthesized silver nanoparticles showed spreading, structured colony patterns on the corm surface.

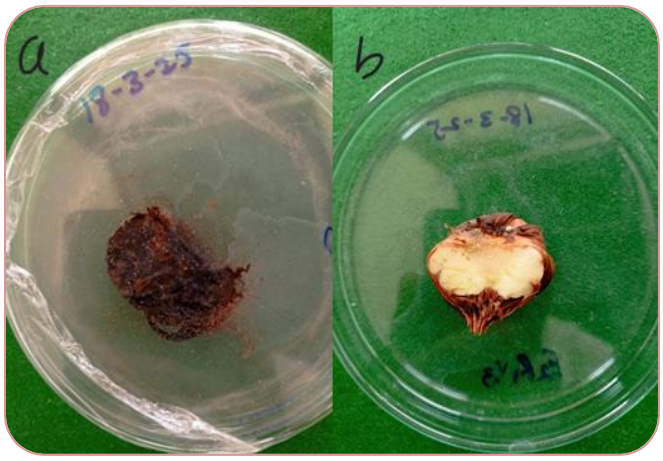

Figure 7. Comparison of the effect of biosynthetic silver nanoparticles on *F. oxysporum* growth on Petri dishes after 20 days. Treatments: (a) Control, (b) *C. roseus*.

Colony Size

The results revealed significant ($P \leq 0.05$) differences among the various treatments of biosynthesized silver nanoparticles (BSNs) and their interactions. As shown in Figure 8, BSNs synthesized using *C. roseus* at 20 mg L^{-1} and *S. nigrum* at 20 mg L^{-1} in the gladiolus cultivar 'White Prosperity' resulted in the greatest reduction in colony size compared with all other treatments. A similar reduction in colony size was also observed in the cultivar 'Red Advance'. In contrast, the highest colony size was recorded in the control treatment.

Overall, the results indicated that different concentrations of BSNs significantly reduced colony size in gladiolus flowers, whereas untreated flowers exhibited the largest colony size among all treatments. Colony size varied significantly depending on the type of plant extract used for nanoparticle synthesis and its concentration. Specifically, *C. roseus* (20 mg L^{-1}) and *S. nigrum* (20 mg L^{-1}) treatments reduced colony size in 'White Prosperity' and 'Red Advance' to 2.36 cm and 2.17 cm, and 3.15 cm and 3.23 cm, respectively.

Conversely, BSNs synthesized using *C. ternatea* at 10 mg L^{-1} showed a comparatively higher disease incidence, as indicated by larger colony sizes of 4.94 cm and 4.53 cm in the white and red cultivars, respectively. The control treatment exhibited the maximum colony size, measuring 5.11 cm in 'White Prosperity' and 5.04 cm in 'Red Advance', and was associated with the highest percentage of disease incidence.

Response of coated biosynthetic silver nanoparticles on corms under different conditions

Corm size (cm)

A significant enhancement in gladiolus corm size was observed following treatment with biosynthetic silver nanoparticles, particularly those synthesized using *C. roseus* at 20 mg L^{-1} and *S. nigrum* at 15 mg L^{-1}, compared with the untreated control. These results strongly suggest a beneficial physiological role of BSNs in corm development and storage, likely through the mitigation of structural abnormalities such as shriveling, damping-off, and mechanical damage. The superior performance of these BSN treatments, as illustrated in Figure 9, was evident in the cultivar 'White Prosperity' under both cold and room storage conditions, where corm sizes reached 6.34 cm. Similarly, *C. roseus*-derived BSNs resulted in a corm size of 5.75 cm in the cultivar 'Red Advance'. These findings highlight the critical influence of both the botanical source used for nanoparticle synthesis and the applied concentration on corm growth and quality.

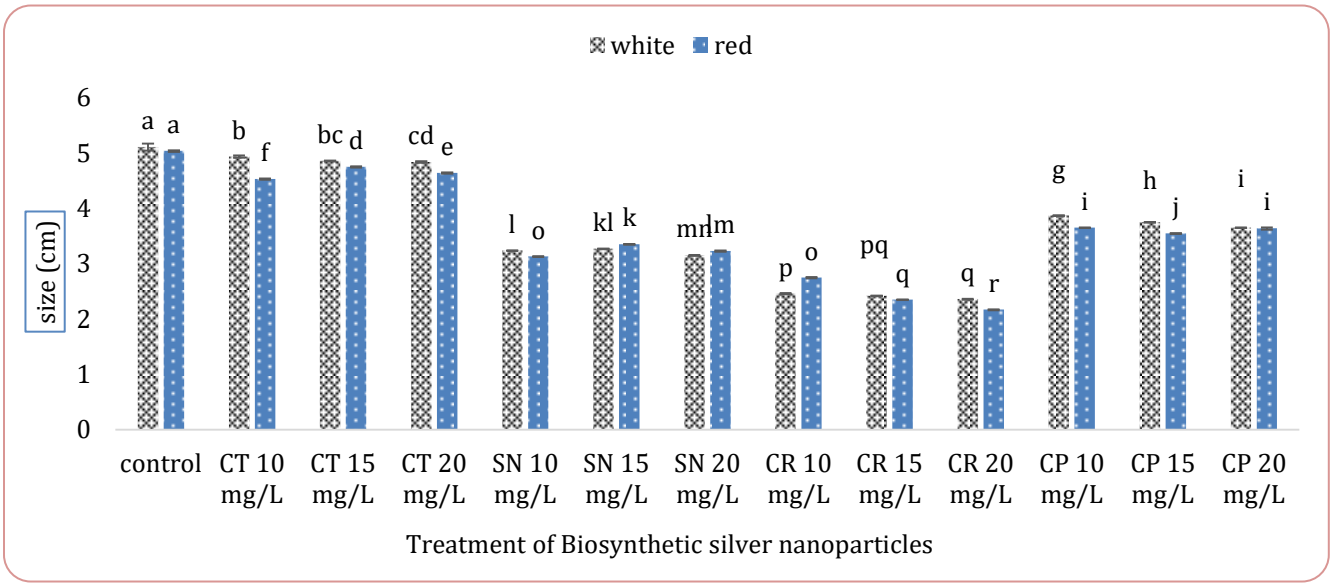

Figure 8. Effect of different concentrations of biosynthetic silver nanoparticles on *F. oxysporum* colony size (cm) in Gladiolus corms. Abbreviations: CT = *C. ternatea*, SN = *S. nigrum*, CR = *C. roseus*, CP = *C. prophetarum*.

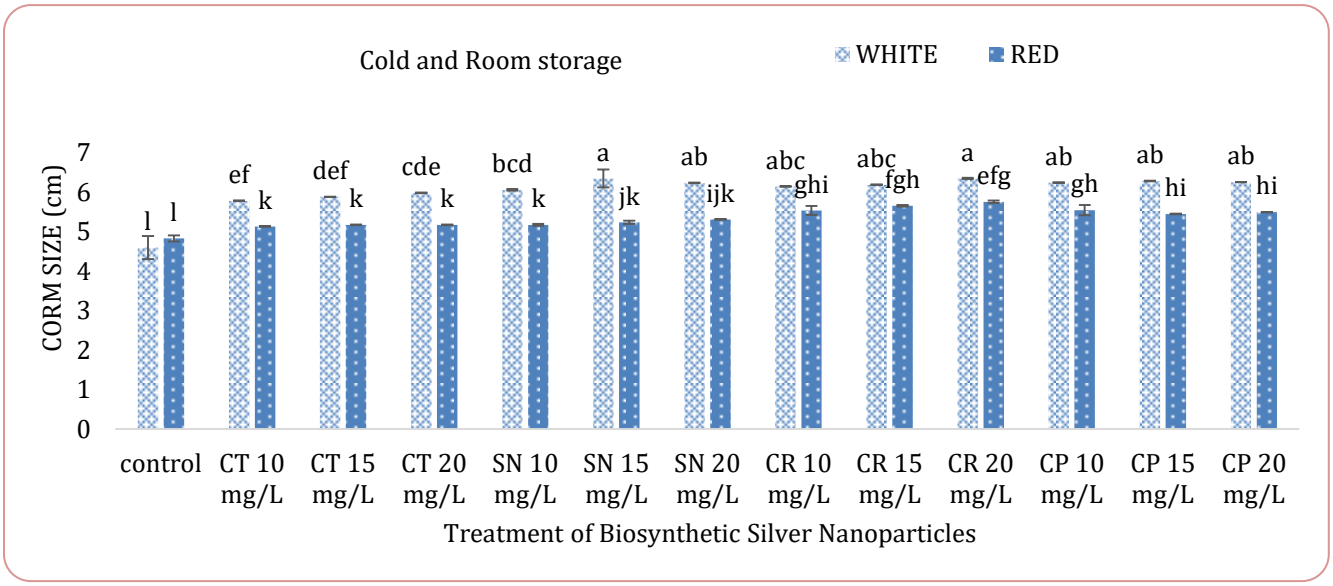

Figure 9. Effect of different concentrations of biosynthetic silver nanoparticles on corm size (cm) of Gladiolus. Treatments: CT = C. ternatea, SN = S. nigrum, CR = C. roseus, CP = *C. prophetarum*.

Weight of corms (g)

A significant enhancement in gladiolus corm weight was observed following treatment with BSN derived from *C. roseus*, particularly at a concentration of 20 mg L^{-1}. This response highlights the potential of nanoscale interventions for improving post-harvest quality and storage performance in geophytic crops. As shown in Figure 10, both White Prosperity and Red Advance cultivars produced substantially heavier corms under BSN treatments compared with the control, which consistently recorded the lowest corm weights (approximately 40.50 g and 40.33 g, respectively).

The *C. roseus*-mediated BSN at 20 mg L^{-1} resulted in the highest corm weights, reaching 69.53 g in White Prosperity and 59.90 g in Red Advance. These values were significantly higher than those of the control and all other treatment levels, including the second-best treatment (15 mg L^{-1}), which produced 68.48 g in White Prosperity.

Under room storage conditions (Figure 11), a similar trend was observed. Both cultivars treated with *C. roseus* BSN at 20 mg L^{-1} attained the maximum corm weights, measuring 62.26 g in White Prosperity and 52.43 g in Red Advance, further confirming the positive effect of this treatment on corm weight retention during storage.

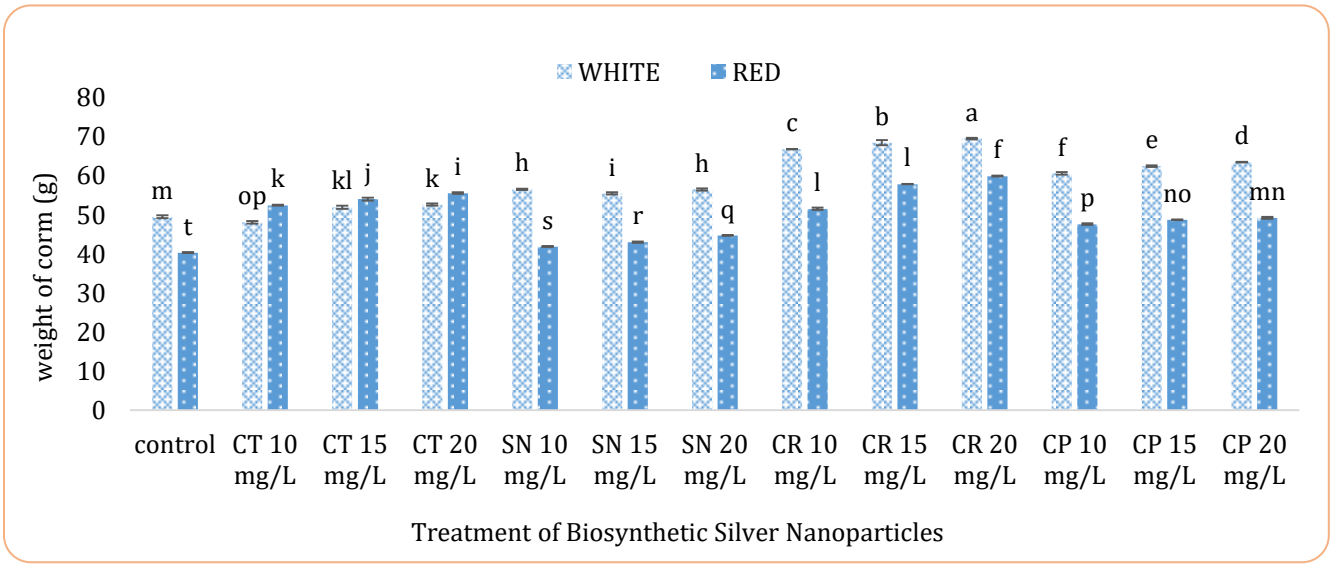

Figure 10. Effect of different concentrations of biosynthetic silver nanoparticles on the weight (g) of Gladiolus corms during cold storage.

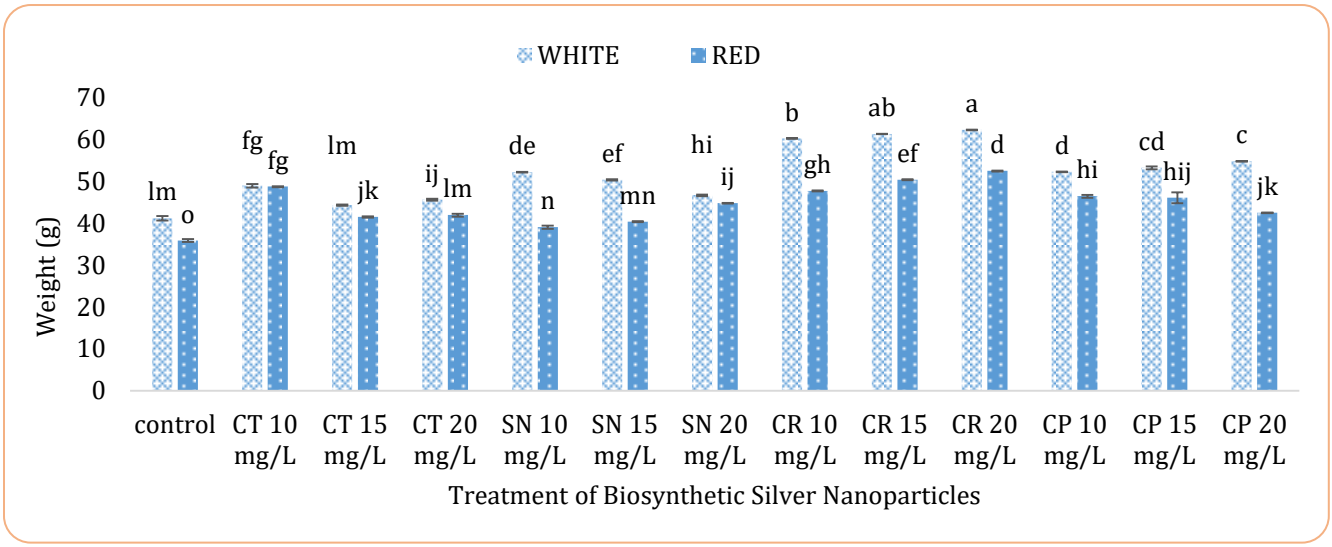

Figure 11. Effect of different concentrations of biosynthetic silver nanoparticles on gladiolus corm weight (g) during room-temperature storage.

Phenolic content (mg/g) in corms

A significant increase in phenolic content was observed in both 'White Prosperity' and 'Red Advance' gladiolus cultivars treated with BSN, particularly those synthesized from *C. roseus* (CR). This finding demonstrates the effectiveness of nanoscale elicitors in enhancing secondary metabolite production during storage. The CR-BSN treatment at 20 mg/L showed the highest phenolic contents, reaching 35.13 mg/g in White Prosperity and 34.23 mg/g in Red Advance (Figure 12), which were significantly higher ($P \leq 0.05$) than those observed at lower concentrations and in the control (T0: 11.83 mg/g and 9.4 mg/g, respectively).

Under room conditions (Figure 13), both cultivars treated with CR-BSN at 20 mg/L also achieved the highest phenolic contents, 18.56 mg/g in White Prosperity and 17.63 mg/g in Red Advance. These results align with the paradigm of nanoparticles as metabolic modulators, wherein plants adjust their secondary metabolism in response to stress.

% Disease Incidence

The results of this study demonstrate the pronounced efficacy of BSN derived from *C. roseus* in suppressing postharvest diseases in gladiolus corms during storage, outperforming both control treatments and BSN synthesized from *C. ternatea*. Specifically, BSN synthesized from *C. roseus* at 20 mg/L consistently produced the lowest disease incidence across cultivars, with minimum values of 1.57% in White Prosperity and 1.05% in Red Advance (Figure 14). Under room temperature conditions (Figure 15), the same treatment also resulted in the lowest disease incidence, recording 6.28% and 8.10% for White Prosperity and Red Advance, respectively.

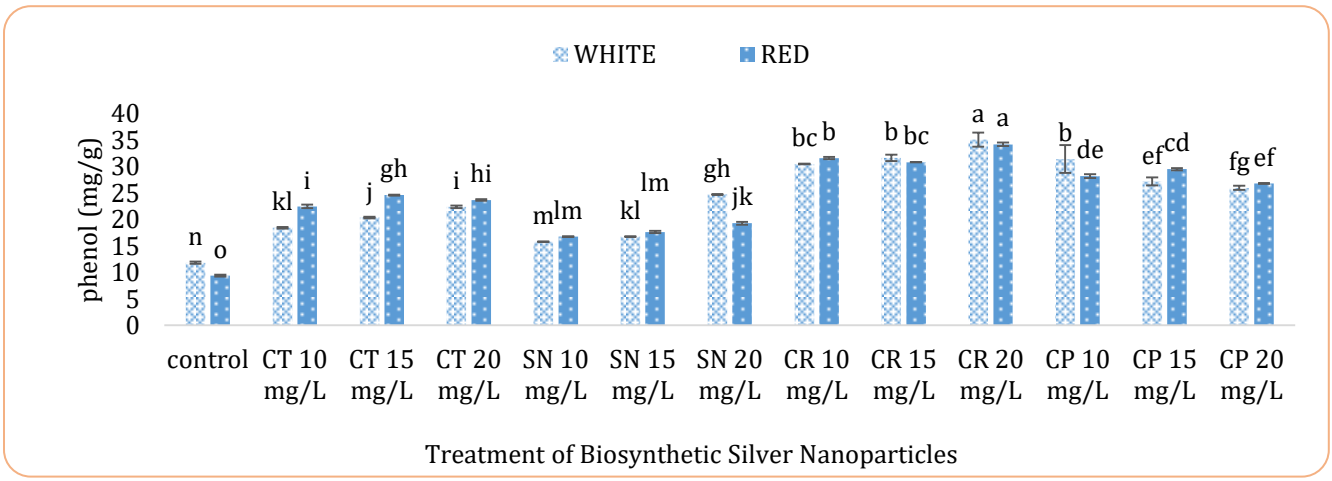

Figure 12. Effect of different concentrations of biosynthetic silver nanoparticles on phenolic content (mg/g) in gladiolus corms during cold storage.

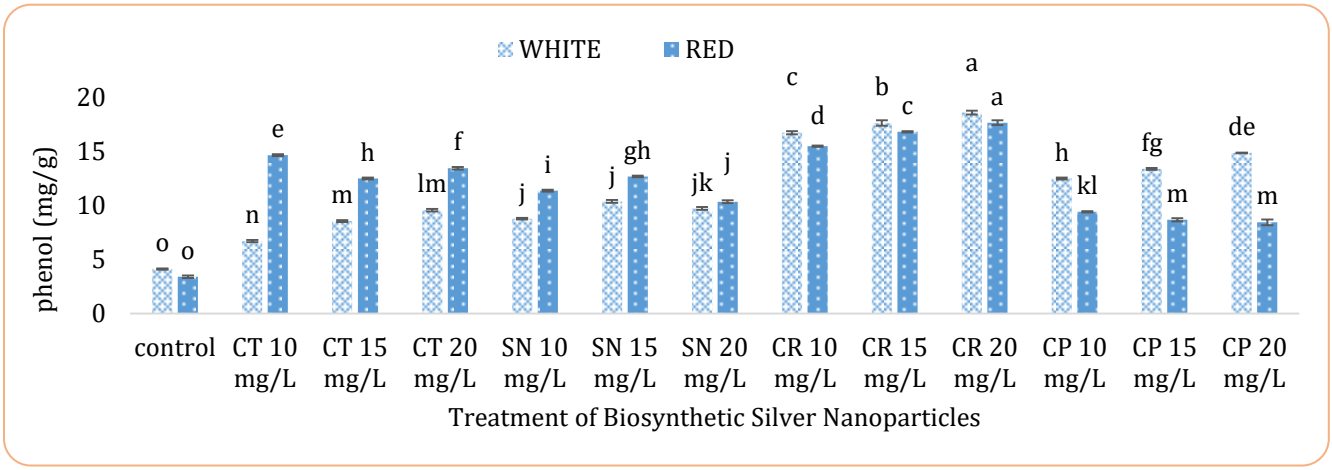

Figure 13. Effect of various concentrations of biosynthetic silver nanoparticles on phenolic content (mg/g) in gladiolus corms during cold storage.

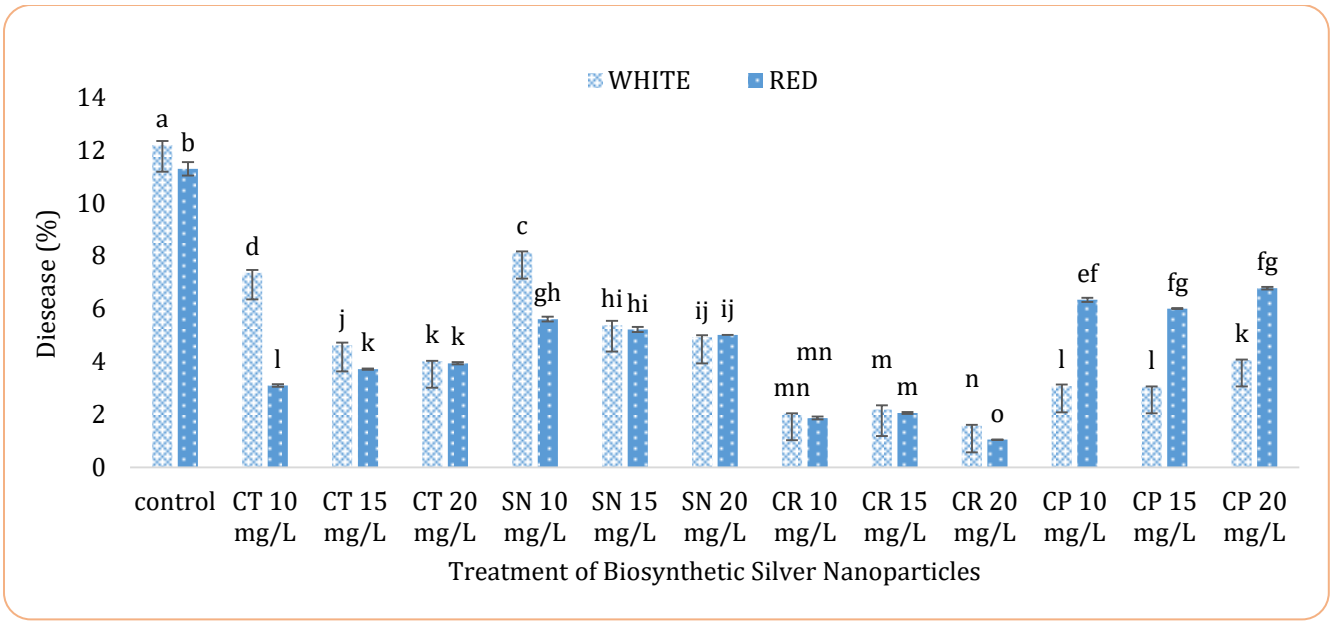

Figure 14. Effect of different concentrations of biosynthetic silver nanoparticles on disease incidence (%) in gladiolus corms during cold storage.

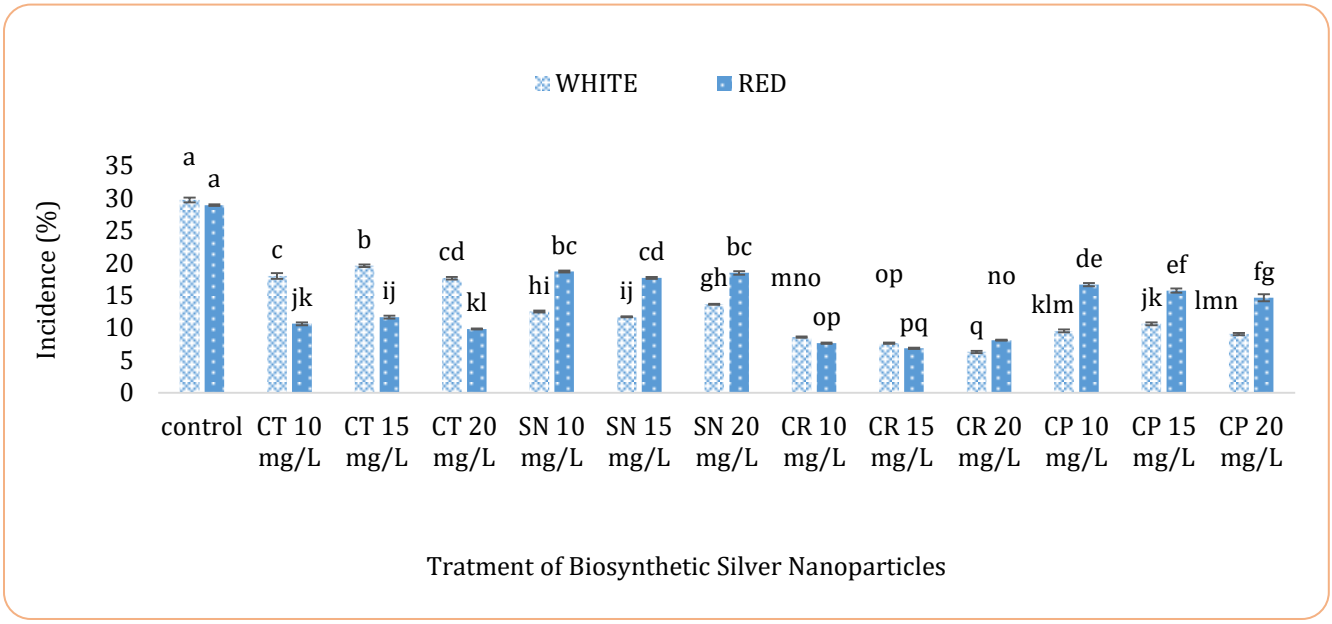

Figure 15. Effect of different concentrations of biosynthetic silver nanoparticles on disease incidence (%) in gladiolus corms during room-temperature storage.

Total sugar content (%) of corms

A significant increase in total sugar content was observed in gladiolus corms treated with BSN, particularly those synthesized using *C. roseus* extract, indicating a promising postharvest strategy to improve corm quality during storage. Among the treatments, application of *C. roseus* BSN at 20 mg/L was the most effective, resulting in the highest total sugar content in both White Prosperity (7.0%) and Red Advance (6.98%) cultivars, significantly exceeding all other treatments and the control (Figure 16). Under room temperature conditions, the same treatment (*C. roseus* BSN at 20 mg/L) also achieved the highest total sugar content in White Prosperity (5.16%) and Red Advance (5.34%) cultivars (Figure 17).

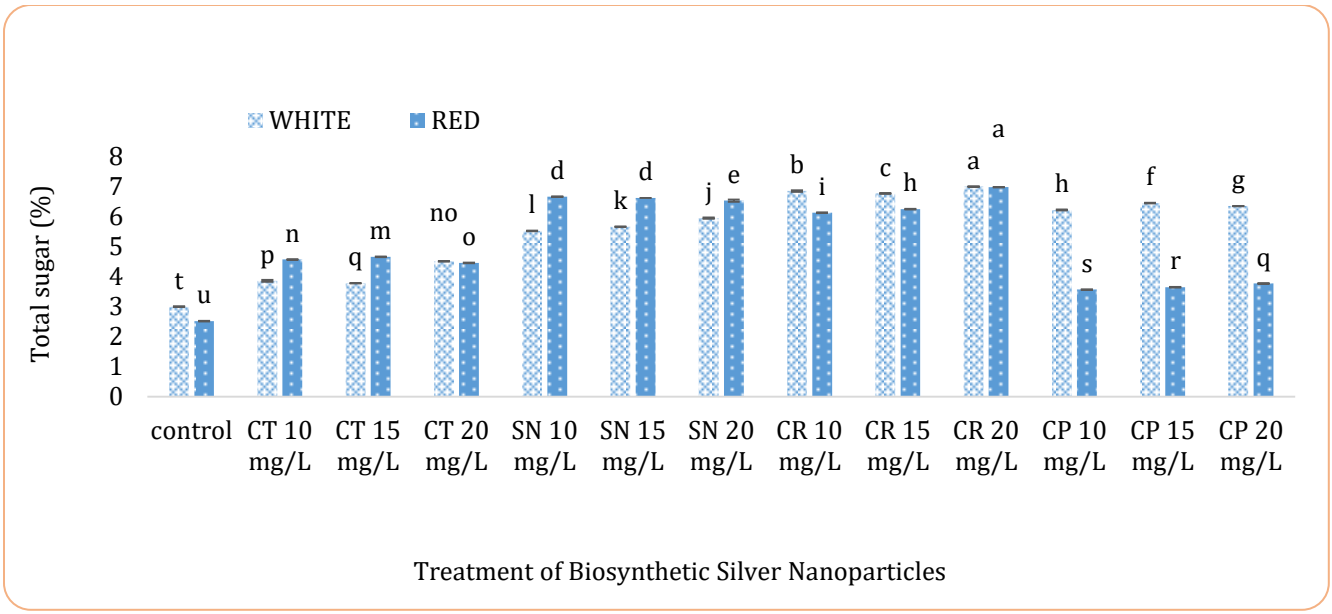

Figure 16. Effect of different concentrations of biosynthetic silver nanoparticles on total sugar content (%) in gladiolus corms during cold storage.

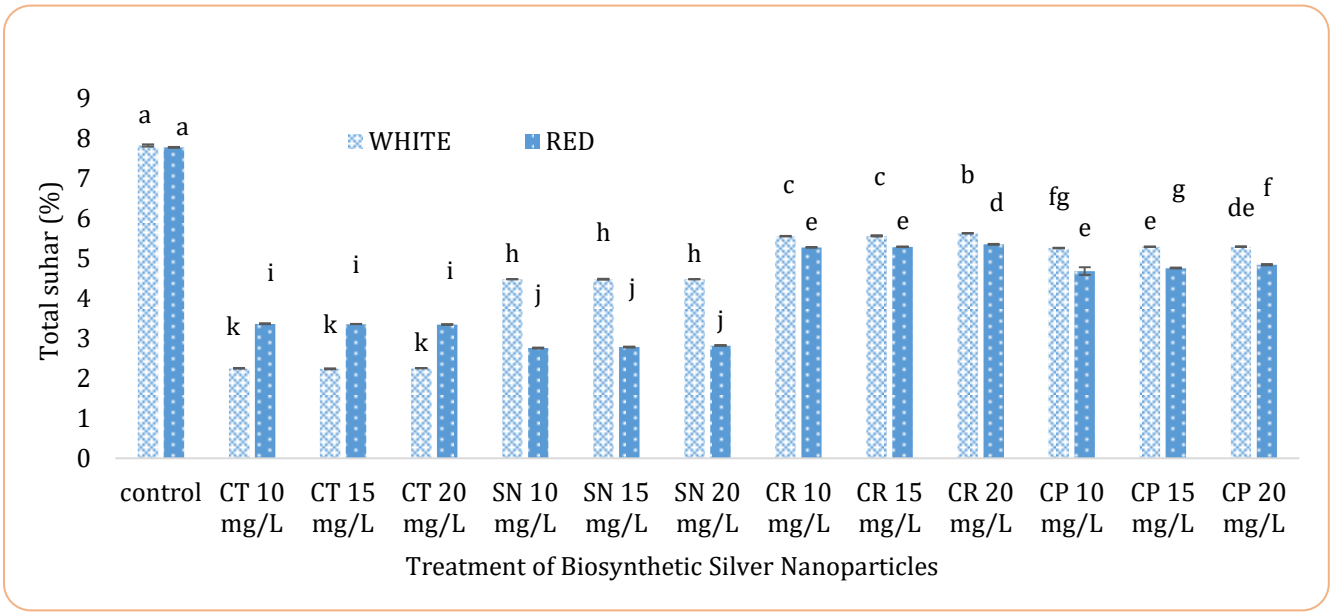

Figure 17. Effect of different concentrations of biosynthetic silver nanoparticles on total sugar content (%) in gladiolus corms during room temperature storage.

α-Amylase activity (U mg^{-1} protein)

The significant enhancement of α-amylase activity in gladiolus corms treated with BSNs synthesized from *C. roseus*, particularly in comparison to the untreated control, highlights a promising nano-priming strategy for improving post-harvest physiological processes. BSN treatments, especially *C. roseus* extracts at 15 mg/L and 20 mg/L, markedly increased α-amylase levels, reaching peaks of 7.24 U mg^{-1} and 6.92 U mg^{-1} in the White Prosperity cultivar, and 7.51 U mg^{-1} in the Red Advance cultivar (Figure 18), indicating a strong bio-stimulatory effect. Under room temperature conditions (Figure 19), both White Prosperity and Red Advance corms treated with *C. roseus* BSNs at 20 mg/L exhibited the highest α-amylase activity, measuring 12.15 U mg^{-1} and 12.03 U mg^{-1}, respectively.

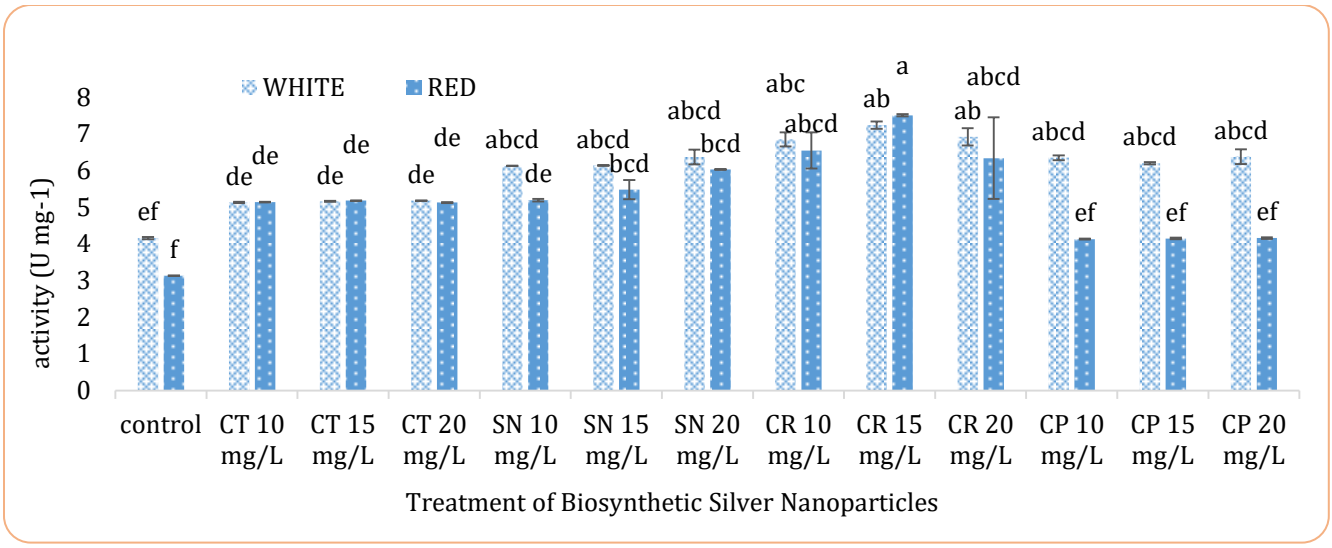

Figure 18. Effect of varying concentrations of biosynthetic silver nanoparticles on α-amylase activity (U mg^{-1} protein) in Gladiolus corms during cold storage.

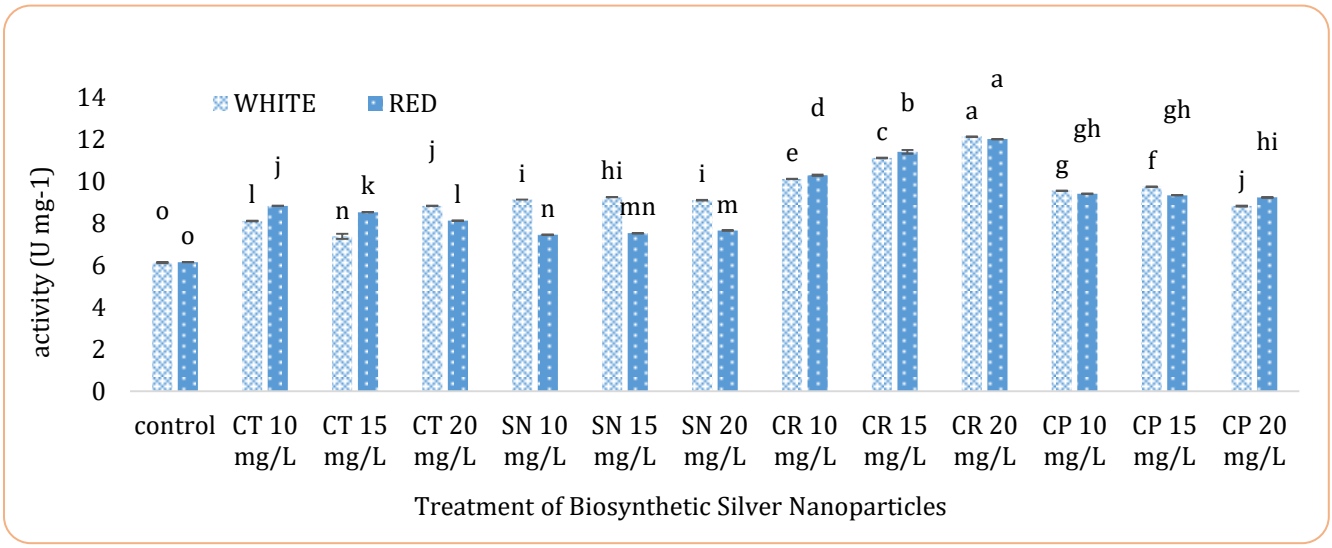

Figure 19. Effect of varying concentrations of biosynthetic silver nanoparticles on α-amylase activity (U mg^{-1} protein) in Gladiolus corms during room-temperature storage.

Non-reducing sugar content of corms

The non-reducing sugar content in gladiolus corms was significantly higher in plants treated with BSN, particularly those synthesized using *C. roseus* extract at 20 mg/L, compared to the untreated control (Figure 20). This indicates that BSN treatments effectively enhance carbohydrate storage reserves under post-harvest conditions.

Both White Prosperity and Red Advance cultivars exhibited substantial accumulation of non-reducing sugars (primarily sucrose), with White Prosperity reaching a maximum of 70.5% and Red Advance 69.3% when treated with *C. roseus* BSN at 20 mg/L (Figure 21). Under the same treatment, the corms also showed physiologically important non-reducing sugar percentages of 60.2% (White Prosperity) and 59.53% (Red Advance).

Among the BSN treatments, *C. roseus*-synthesized nanoparticles demonstrated the most pronounced effect, followed by other treatments such as . *nigrum* at 20 mg/L, which resulted in 64.33% amylase activity in White Prosperity corms. These results collectively highlight the potential of BSN, especially *C. roseus*-derived nanoparticles, in improving carbohydrate accumulation and post-harvest quality of gladiolus corms.

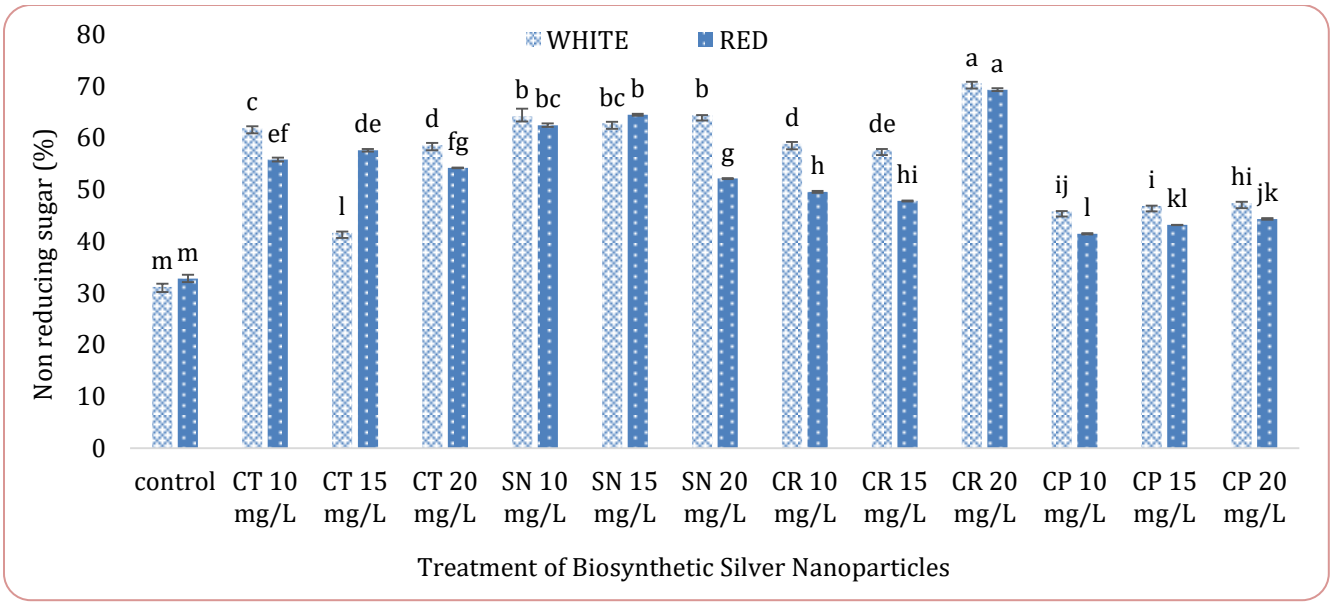

Figure 20. Effect of different concentrations of biosynthetic silver nanoparticles on non-reducing sugar (%) in Gladiolus corms during cold storage.

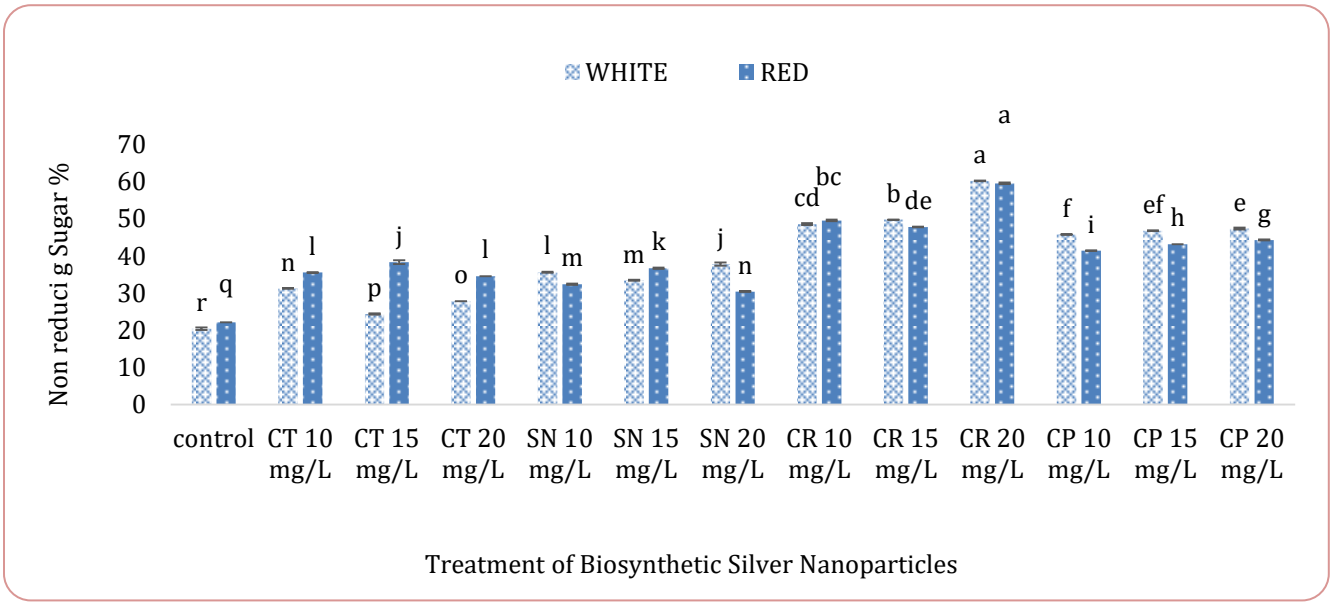

Figure 21. Effect of different concentrations of biosynthetic silver nanoparticles on non-reducing sugar (%) in Gladiolus corms during room-temperature storage.

Reducing sugar %

The reducing sugar content in gladiolus corms was significantly increased following treatment with BSNs, particularly those synthesized using *C. roseus*. This suggests a potential role of CR-BSNs in enhancing postharvest carbohydrate metabolism and improving storage quality. The CR-BSN treatment at 20 mg/L exhibited superior performance, reaching 1.76% in White Prosperity and 1.73% in Red Advance cultivars (Figure 22), significantly exceeding the control values (0.86% and 0.79%, respectively) and outperforming *C. ternatea*-BSN treatment (1.02%). Under room temperature conditions (Figure 23), both White Prosperity and Red Advance corms treated with CR-BSN at 20 mg/L achieved the highest reducing sugar content (5.21% and 4.76%, respectively), indicating a potent biological activity specific to the *C. roseus* extract or its interaction with silver nanoparticles.

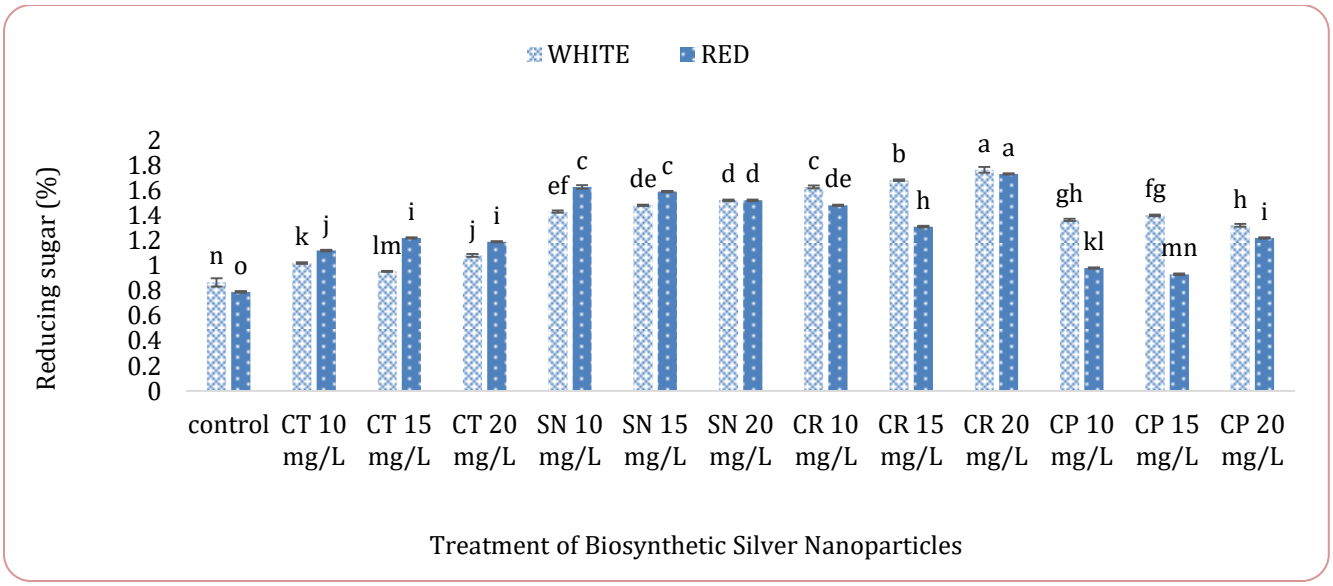

Figure 22. Effect of different concentrations of biosynthetic silver nanoparticles on reducing sugar (%) in Gladiolus corms during cold storage.

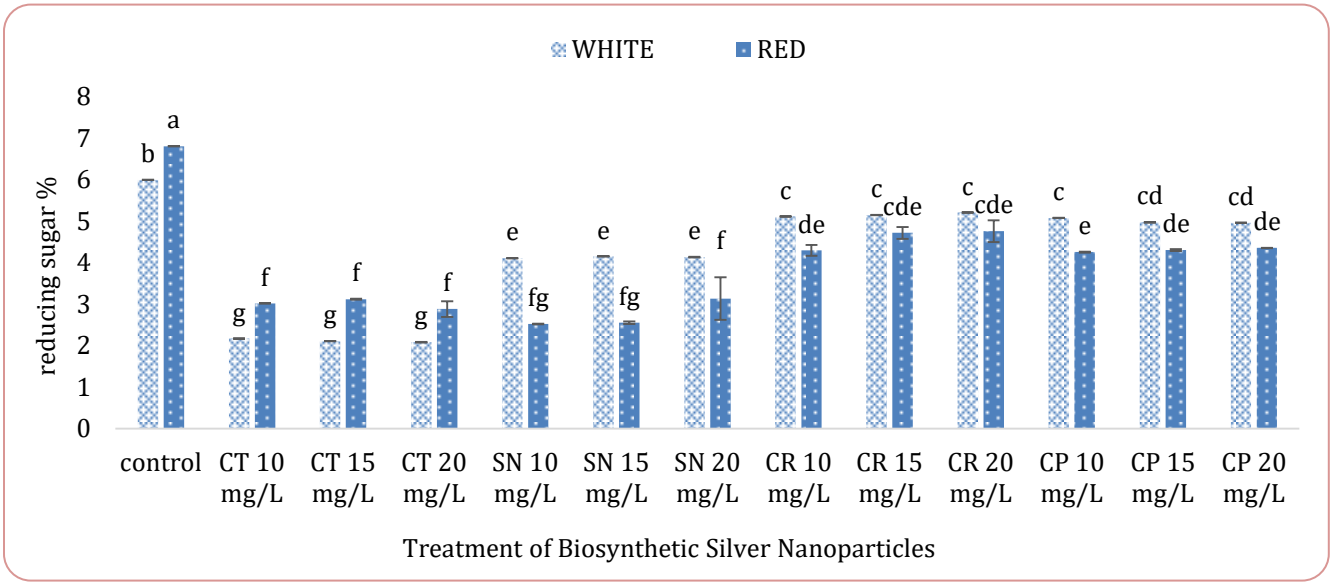

Figure 23. Effect of different concentrations of biosynthetic silver nanoparticles on reducing sugar (%) in Gladiolus corms during room-temperature storage.

Vase life (days)

The results showed significant differences ($P \leq 0.05$) among treatments with BSNPs and their interactions. As shown in Figure 24, BSNPs derived from *C. roseus* (20 mg/L) and *S. nigrum* (20 mg/L) significantly enhanced the vase life of gladiolus cultivars. Specifically, in the 'White Prosperity' cultivar, these treatments outperformed all other treatments, while in the 'Red Advance' cultivar, they also notably extended shelf life. The shortest vase life was observed in the control treatment.

Overall, the data indicate that different concentrations of BSNPs significantly increase the vase life of gladiolus flowers, with untreated flowers exhibiting the lowest vase life. Vase life varied depending on the type of plant extract and its concentration. The longest vase life was recorded for *C. roseus* at 20 mg/L, reaching 20 days in 'White Prosperity' and 21.33 days in 'Red Advance'. This was followed by *C. roseus* at 10 mg/L and 15 mg/L. *C. prophetarum* also showed a substantial increase, with a

maximum of 18 days in 'White Prosperity' and 17 days in 'Red Advance'. *S. nigrum* provided moderate improvement, extending vase life to 15.66 days in 'White Prosperity' and 12 days in 'Red Advance'. In contrast, *C. ternatea* exhibited only slight enhancement, reaching 12 days in 'White Prosperity' and 10.33 days in 'Red Advance'. The control flowers had the shortest vase life, at 9 days for both cultivars.

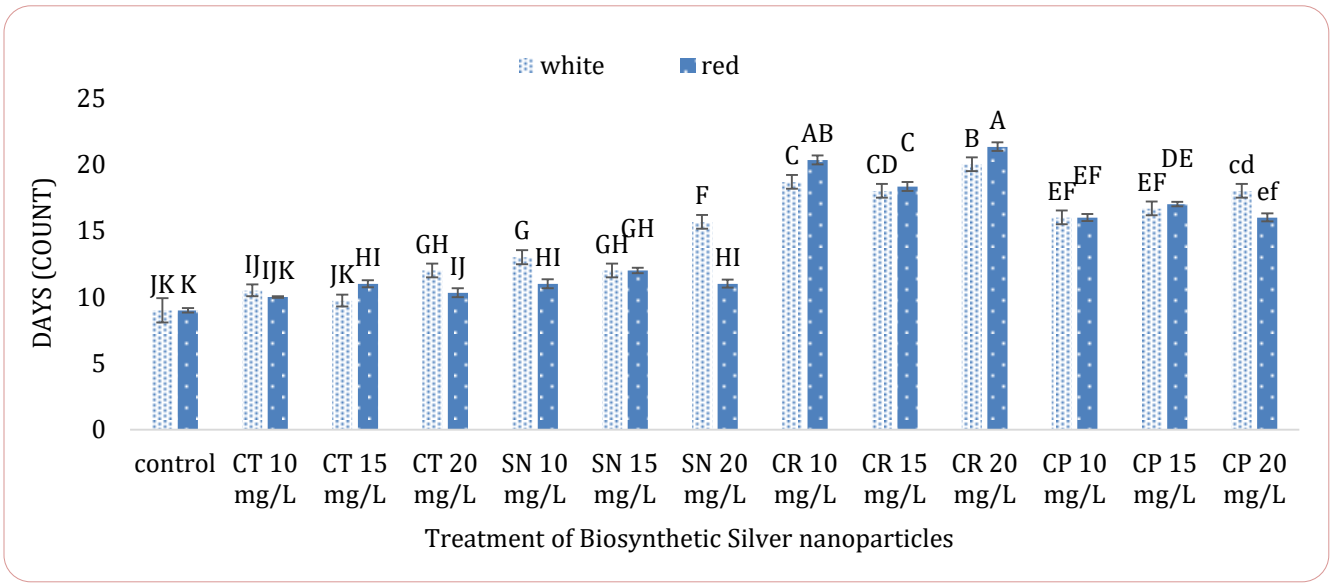

Figure 24. Effect of different concentrations of biosynthetic silver nanoparticles on the vase life (days) of Gladiolus corms.

Discussion

The cut flower industry involves the production of flowering plants and the sale of flowers harvested with stems and leaves. These cut flowers are frequently utilized for indoor decoration (Gobie, 2019). The floriculture sector is robust and expanding rapidly, with traditional markets becoming saturated while new markets emerge worldwide (Kargbo et al., 2010). Pakistan has substantial potential to earn foreign exchange with relatively low production inputs by establishing and standardizing floricultural products. Cut flower production is a key component of the floriculture industry, representing a significant share of global cultivation and ranking among the most commonly grown commercial crops (Singh et al., 2020).

In modern agriculture, nanotechnology plays a crucial role in enhancing crop productivity and stress resilience. Among various nanomaterials, biologically synthesized silver nanoparticles (AgNPs) offer innovative solutions for controlled environments and tissue culture systems, addressing common challenges such as ethylene accumulation and nutrient depletion (Mahendran et al., 2019). Silver nanoparticles, typically 10-100 nm in size, exhibit antimicrobial properties that depend on particle size, shape, and microorganism sensitivity (Lara et al., 2011). In this study, AgNPs were observed to be spherical, consistent with previous reports where plant-extract-derived nanoparticles also formed spherical structures at smaller sizes (Sati et al., 2025). Characteristic peaks in the XRD pattern confirmed the high purity and crystallinity of the synthesized AgNPs, aligning with findings from Kumar and Yadav (2009).

Gladiolus production faces significant challenges from corm rot diseases, which reduce both yield and quality. Effective management of these diseases requires integrating chemical and biological control measures for long-term efficacy (Baker et al., 2020). Treatments that can penetrate corm tissues are essential to minimize losses caused by *F. oxysporum* f. sp. *gladioli* (Chohan et al., 2011). *Fusarium* isolates display considerable phenotypic variability, including colony color, conidial size, shape, and growth patterns, ranging from fluffy, cottony, flat, spreading, to powdery morphologies (Yli-Mattila et al., 2018; Harish et al., 2023). Reducing ethylene responses in plants can help mitigate damage caused by a wide range of phytopathogens. The postharvest life of cut flowers is influenced by both biotic and abiotic factors that trigger senescence (Reid and Jiang, 2012; Mazrou et al., 2022).

The beneficial effects of BSN on gladiolus corms can be

attributed to several mechanisms. BSN may enhance nutrient uptake by altering root membrane permeability or transport processes, act as mild stressors inducing defense responses (hormesis), exhibit antimicrobial properties protecting corms from soil pathogens, or influence phytohormone activity related to sink strength and storage organ development (Mude et al., 2020; Singh et al., 2021). The concentration-dependent efficacy observed suggests an optimal threshold for physiological benefits. Increases in corm size and weight under BSN treatment likely result from these multifaceted roles. Silver nanoparticles also function as abiotic elicitors, stimulating defense pathways such as the phenylpropanoid pathway and enhancing phenolic compound synthesis (Ali et al., 2019).

The potent antifungal activity observed aligns with the broad-spectrum antimicrobial properties of AgNPs, which disrupt microbial membranes, inhibit respiration, and interfere with DNA replication (Rai et al., 2009; Ahmed et al., 2016). The enhanced performance of nanoparticles synthesized using *C. roseus* extract may be due to the extract's phytochemical composition, which could improve nanoparticle stability, bioavailability, or influence metabolic pathways favoring sugar accumulation within corm tissue (Abd-Elkareem et al., 2021). Silver nanoparticles can interact with plant biomolecules, modulating enzyme activity and gene expression related to carbohydrate metabolism. Acting as nano-elicitors, they can upregulate hydrolytic enzymes like amylase, facilitating starch breakdown into soluble sugars necessary for energy during storage (Shang et al., 2019; Das et al., 2020). Non-reducing sugars serve as stable energy reserves and osmoprotectants, crucial for corm viability, dormancy maintenance, and subsequent sprouting (Tain et al., 2018).

The superior performance of BSN synthesized using *C. roseus*, followed by . *nigrum* at 20 mg L^{-1}, suggests that the phytochemical profile of the plant extract significantly influences nanoparticle bioactivity, stability, and interactions with plant metabolic pathways (Mittal et al., 2013; Rastogi et al., 2019). The observed increase in reducing sugars may result from enhanced hydrolysis of complex carbohydrates or reduced respiratory losses during storage, contributing to improved corm vigor and sprouting potential (Ali et al., 2019; Sharma et al., 2021).

Conclusion

This study demonstrates that biosynthetic silver nanoparticles effectively prolong the vase life of gladiolus cut flowers while delaying senescence. BSN derived from *Catharanthus roseus* extract at 20 mg L^{-1} not only combats *Fusarium oxysporum* infections in corms but also improves post-harvest quality, including increased total sugar content, corm size, and weight. Future research should focus on enhancing nanoparticle stability, shelf life, and delivery methods (e.g., nano-coatings, hydroponic treatments) for field application. As a non-toxic, eco-friendly alternative, BSN offers significant promise for the floriculture industry, providing a viable replacement for synthetic pesticides. Scaling up BSN applications and evaluating long-term efficacy across diverse crops are essential for assessing cost-effectiveness and practical utility. Adoption of BSN can align floriculture practices with sustainable and environmentally responsible agricultural standards.

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Authors' Contributions

AFA and AY conceptualized and designed the study, performed the data analysis, and drafted the original manuscript. MAH and MJJ provided critical guidance, constructive feedback, and valuable suggestions to improve the content and clarity of the manuscript. All authors reviewed and approved the final version of the manuscript and agreed to be accountable for all aspects of the work, ensuring accuracy and integrity in reporting.

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Conflict of Interest

The authors declare no conflict of interest.

Sustainable Development Goals Targeted

SDG 2: Zero Hunger
SDG 12: Responsible Consumption and Production
SDG 15: Life on Land

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