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# Foliar application of chitosan improves plant biomass, physiological and biochemical attributes of rose (Gruss-an-Teplitz)

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## Abstract

Rose is an important floricultural crop that has been exploited for many uses. It is important uses in different industries include, pharmaceutical, perfumery, and food industries that manifest higher flower yield. Therefore, response of Gruss-an-Taplitz to foliar application of chitosan (Ct) solution (0, 2.5, 5, 7.5, 10 mg $L^{-1}$), was evaluated in an experimental field. Ct treatment had significant effects on studied parameters, including plant growth, pigments, enzymes, and gaseous exchange. This experiment was laid out under Randomized Complete Block Design (RCBD) using three replications per treatment. Ct (7.5 mg $L^{-1}$) significantly improved growth, in terms of higher leaf area (20.37%), plant height (20.19%), number of flowers (55.51%), flower weight (34.64%) and flower diameter (33.78%) as well as enhancing relative water contents (27.38%) with respect to control. Chlorophyll *a* (54.60%), Chlorophyll *b* (12.13%), Carotenoid (8.36%) and anthocyanins (17.09%) were also increased at 7.5 mg $L^{-1}$ Ct, which showed higher photosynthetic pigments as compared to control. Consequently, Ct (7.5 mg $L^{-1}$) treated plants showed higher enzymatic activity; CAT (9.94%), SOD (83.87%), POD (64.54%), total antioxidant (35.48%), phenolics (7.41%) and gaseous exchange; *Pn* (55.65%), *E* (31.76%), *gs* (18.38%) and *Ci* (34.17%) that improved the plant growth and productivity of Gruss-an-Taplitz. Foliar application of 7.5 mg $L^{-1}$ Ct improved biomass, water preservation, pigments, enzymatic activity, leaf gaseous exchange, and quality of Gruss-an-Taplitz plants.

**Key word:** Chitosan; enzyme activity; essential oil roses; ornamental plants; pigments

## 1. Introduction

Gruss-an-Taplitz belongs to Rosaceae family is famous among roses with high demand in the local and international markets. It is an important variety of *Rosa bourborniana* produces red color fragrant flowers, used for ornamental purposes and essential oil extraction (Sane *et al*., 2007).

Known for high demand throughout the year due to extensive use of its flowers in different occasions like marriage ceremony, death ceremony, Eid festival, valentine's day, etc. This industrially important floricultural crop is extensively used for medical, pharmaceutical and food industries. Moreover, it is commonly used as bedded or potted plant in lawns (Gulzar *et al*., 2019). Gruss-an-Taplitz could be propagated asexually using grafting, cutting and budding. Scientists are trying to enhance flowering of floricultural crops by different factors like environment, genetics, nutrition and plant growth regulators. Positive role of plant growth regulator and plant extracts for enhancing vegetative and reproductive growth in various ornamental crops have been reported by different scientists (Pervez *et al*., 2017; Hassan & Fetouh, 2019; Ahmad *et al*., 2019; Akhtar *et al*., 2021).

Ct is a polysaccharide derived from chitin, a long-chain polymer of N-acetyl-glucosamine separated from parasitic cell and the shells of marine scavengers, for example, crabs and shrimps (Badawy & Rabea, 2011). Ct is used to preserve food, medicine, limit microorganisms and enhances plant growth (Salachna & Zawadzinska, 2014). Previously, Ct has been used to increase flowering in different floricultural crops like orchid (Uthairatanakij *et al*., 2007), gladiolus (Ramos-Garcia *et al*., 2009), freesia (Salachna & Zawadzinska, 2014), sage (Vosoughi *et al*., 2018), cordyline (El-Serafy, 2020) and chrysanthemum (Elansary, 2020). Ct promotes the activities of indole acetic acid (IAA) and gibberellic acid (GA) to enhance growth in plants (Malerba & Cerana, 2018; Zhang *et al*., 2018). Similarly, Tourian *et al*. (2013) reported increased plant biomass, root length and photosynthetic pigments of plants in response to Ct application. Application of Ct also promotes the enzymatic activates that regulated different vital physiological and biochemical processes and increases flowering in plants (Hien, 2004; Hadwiger, 2013; Sharma *et al*., 2019). Likewise, Ct used in freesia to improve the growth and flowering by enhancing photosynthetic activity (Salachna & Zawadzinska, 2014). Use of Ct application is increasing in ornamental plant to extend number and period of flowering. It also extended the pot life of the flowers and number of cormlets (Ramos Garcia *et al*., 2009). Ct application in grapes have also increased anthocyanins, phenolic compounds and antioxidant potential by modulating key genes (Singh *et al*., 2019; Silva *et al*., 2020; Singh *et al*., 2020).

This study is considered to evaluate the impact of Ct as a biostimulant, on Gruss-an-Taplitz growth and flowering. We hypothesized that Ct may enhance the growth and flowering of roses. Therefore, this study was considered to investigate the effect of Ct on the vegetative and reproductive changes of Gruss-an-Taplitz plant that led to improve the production of flowers.

## 2. Materials and Methods

### 2.1 Experimental location and conditions

The study was performed in field area, Institute of Floriculture and Landscape Designing, Multan (31°30′ N, 73°10′ E, elevation 213 m) during 2019. Two-year-old Gruss-an-Teplitz plants were pruned in March 2019 to 2 feet height. Two foliar spray of different Ct levels (Control, 2.5, 5, 7.5, 10 mg $L^{-1}$) were applied to plant until runoff (20 ml/plant) by using hand sprayer after one week of pruning. For making different dilutions Ct was dissolved in 1% acetic acid solution and then diluted using distilled water. Foliar spray was applied at an interval of seven days and control plants were sprayed with distilled water at same time. The soil was loamy, having pH 8.1, organic matter 0.79%, electrical conductivity of saturated soil extract (ECe) 2.55 mS $cm^{-1}$, saturation percentage 32, total available N 0.021%, available P 6.90 mg $kg^{-1}$, and available K 230 mg $kg^{-1}$. All plants were irrigated with canal water at seven days interval through flooding and uniform cultural practices were applied. All morphological, physiological and biochemical parameters were recorded at the time of flower harvesting (30 days after Ct application). The study was arranged in RCBD and each treatment was carried out in three replications with four plants each.

2.2 Vegetative and reproductive growth analysis

Leaf area (LA) was recorded using leaf area meter (Model CT-202, CTD Inc. USA) (Carleton & Foote, 1965) and plant height (PH) by using measuring tape. Flower weight (FW) was recorded using analytical balance (G&G, JJ324BC) and flower diameter (FD) using vernier caliper (Model Number (500-196-20) Range: 0-150 mm/0-6"). The bud emergence days (BED) were counted manually.

2.3 Determination of relative water content

Turgid young leaves were used to measure leaf relative water content (RWC) using the procedure of Redondo- Gomez *et al*. (2011). Leaf fresh weight (FW) was measured immediately after harvest and turgid weight (TW) after soaking leaves in distill water for 24 hours at 4ºC. For dry weight (DW) estimation leaves were oven dried at 65°C for 72 h.

$$RWC (\%) = (FW - DW / FW) \times 100$$

2.4 Measurement of photosynthetic pigments

Leaf photosynthetic contents were determined according to Makeen *et al*. (2007). Leaf samples (0.2 g) were grinded with pistil mortar using 5 ml/L acetone (80%) solution. The mixture was placed at 4ºC overnight that further centrifuged (4℃, 9000 rpm, 15 minutes) and supernatant was used for spectrophotometer readings (663, 645 and 480 nm). Whereas, anthocyanin contents were determined by using protocol of Egbuna *et al*. (2018).

2.5 Determination of color

The leaf color *L* (brightness/lightness), *a* (redness/greenness), *b* (yellowness/blueness) was measured by using Chrome meter (CR-400 Serial Number: 2501822).

2.6 Assay of enzymatic activities

Leaf samples (1 g) were grinded and homogenized with phosphate buffer, ethylene diamine tetra acetic, $K_2HPO_4$, $KH_2PO_4$ that further centrifuged (10 min. 9000 rpm, 4ºC). The supernatant was used to assess enzymatic activities; catalase (CAT) according to the (Chance & Maehly, 1955), peroxidase (POD) according to (Zhang *et al*., 2012) and Superoxide dismutase (SOD) according to (Ekler *et al*., 1993).

2.7 Gas exchange measurement

Gas exchange attributes were measured from fully expanded leaves on a clear sunny day (12:00 to 13:00 P.M.) using a CTRAS-3 portable open-flow gas exchange system (PP Systems, Amesbury, USA, with 100 mL $min^{-1}$ mL air flow, 1200 µmol $m^{-2}$ $s^{-1}$ photon flux density, 390 ± 5 µmol $mol^{-1}$ $CO_2$ conc., and 99.9 KPa atmospheric pressure).

2.8 Statistical analysis

Data was statistically analyzed using statistix-8.1 computer software and means were compared at 5% probability level using least significant difference (LSD) test.

## 3. Results

3.1 Plant biomass

The growth attributes of Gruss-an-Taplitz were significantly ($P \leq 0.05$) improved through foliar application of Ct (Table 1). Ct at 7.5 mg $L^{-1}$ exhibited maximum LA (20.37%), PH (20.91%), FN (55.51%), FW (34.64%) and FD (33.78%) in comparison to untreated (control) plants. However, the minimum flower BED (44.68%) were observed at 7 mg $L^{-1}$ comparing control (Table 1). 10 mg $L^{-1}$ Ct increased LA (10.84%), PH (14.58%), FN (46.37%), FW (28.57%) and FD (29.60%) while decreased BED (3.23%) with respect to control.

3.2 Leaf relative water content

Different concentrations of Ct significantly ($P \leq 0.05$) enhanced RWC compared to untreated plants (control). Increased RWC was Ct dose-dependent as 7.5 mg $L^{-1}$ showed maximum RWC (27.38%) compared to control. Whereas, Ct 5 and 10 mg $L^{-1}$ improved 23.26% and 18.44% RWC compared to control respectively (Figure 1).

3.3 Pigments

Ct treated Gruss-an-taplitz plants showed a significantly ($P \leq 0.05$) higher chlorophyll contents (Figure 2 a,b,c,d).

**Table 1.** Impact of foliar application of different Ct levels (0, 2.5, 5, 7.5, 10, mg $L^{-1}$) on leaf area (LA), plant height (PH), bud emergence days (BED), flower number (FN), flower weight (FW) and flower diameter (FD) of Gruss-an-Taplitz

| Treatments (Chitosan mg $L^{-1}$) | LA ($cm^2$) | PH (cm) | BED | FN | FW (g) | FD (mm) |
|---|---|---|---|---|---|---|
| 0 | 106.19 c | 95.30 d | 6.20 a | 3.47 c | 1.00 c | 34.89 d |
| 2.5 | 118.01 b | 102.01 cd | 5.10 b | 6.27 b | 1.20 b | 46.61 c |
| 5 | 120.10 b | 104.04 c | 4.60 c | 6.87 b | 1.47 a | 48.40 bc |
| 7.5 | 133.37 a | 120.50 a | 3.43 d | 7.80 a | 1.53 a | 52.69 a |
| 10 | 119.10 b | 111.56 b | 6.00 a | 6.47 b | 1.40 a | 50.40 ab |
| *P*-value | 0.0001 | 0.0002 | 0.0000 | 0.0000 | 0.0006 | 0.0000 |
| CV | 2.64 | 3.37 | 4.40 | 7.11 | 7.05 | 3.41 |
| $LSD_{0.05}$ | 5.94 | 0.22 | 0.42 | 0.83 | 1.18 | 2.99 |

Values are mean ± SE and letters represent significant difference at $P \leq 0.05$ according to LSD test. CV, Coefficient of variation

The highest Chl *a* (54.60%), Chl *b* (12.13%), Car (8.36%) and AC (17.09%) were recorded at 7.5 mg $L^{-1}$ Ct followed by Chl *a* (21.95%), Chl *b* (8.61%), Car (4.96%) and AC (15.82 %) at 5 mg $L^{-1}$ in relation to control. Whereas, higher Ct dose (10 mg $L^{-1}$) adversely affected and reduced Chl *a* (46.43%), Chl *b* (10.07%), Car (7.17%) and AC (6.03%) compared to Ct 7.5 mg $L^{-1}$.

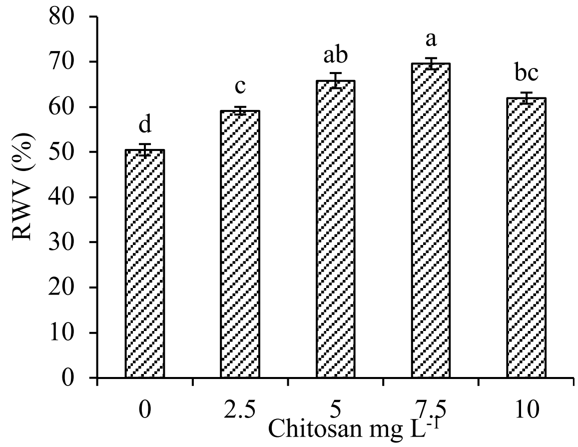


**Fig. 1.** Impact of Ct foliar application on leaf relative water contents (RWC) of Gruss an Taplitz plants. Lettering shows significance according to LSD test ($P \leq 0.05$)

3.4 Color

Plants without Ct application produced significantly light-colored leaves compared to Ct treated. Ct at 7.5 and 5 mg $L^{-1}$ produced 19.81% and 14.93% brighter leaves respectively compared to control (Figure 3 a). Green color of leaves was 93.13% and 90.91% increase in response to 7.5 and 5 mg $L^{-1}$ Ct application with respect to control (Figure 3 b). Moreover, 7.5 and 5 mg $L^{-1}$ Ct produced highest 59.91% and 59.28% yellow hue respectively in contrast to no Ct (control). (Figure 3 c).

3.5 Enzymatic activity

Enzymatic activity was significantly ($P \leq 0.05$) enhanced in Ct treated plants of Gruss-an-taplitz compared to control (Figure 4 a,b,c). Ct at 7.5 mg $L^{-1}$ increased CAT (9.94%), POD (64.54%) and SOD (83.87%) activities in relation to control. It was also noted that highest Ct dose (10 mg $L^{-1}$) significantly ($P \leq 0.05$) decreased CAT, POD and SOD to 3.66%, 30.30% and 21.43% respectively compared to 7.5 mg $L^{-1}$. Total antioxidant and phenolics were also maximum 35.48% and 7.41% respectively at 7.5 mg $L^{-1}$ Ct with respect to control. But further reduced to 25.81% (total antioxidant) and 4.81% (total phenolics) at higher Ct dose (10 mg $L^{-1}$) compared to 7.5 mg $L^{-1}$ (Figure 4 d,e).

3.6 Gaseous exchange

Gas attributes (*Pn, E, gs, Ci* and WUE) were significantly ($P \leq 0.05$) improved at all levels of Ct in comparison to control (No Ct application) (Figure 5 a,b,c). But highest *Pn* (55.65%), *E* (31.76%), and *gs* (18.38%) were recorded at Ct 7.5 mg $L^{-1}$ comparing to control. Similarly, maximum *Ci* (34.17%) and WUE (26.27%) were also noted at 7.5 mg $L^{-1}$ (Figure 5 d,e). Higher Ct dose (10 mg

L$^{-1}$) adversely affected and decreased *Pn* (28.69%), *E* (15.88%), *gs* (19.60%), *Ci* (10.06%) and WUE (12.40%) in contrast to Ct 7.5 mg L$^{-1}$.

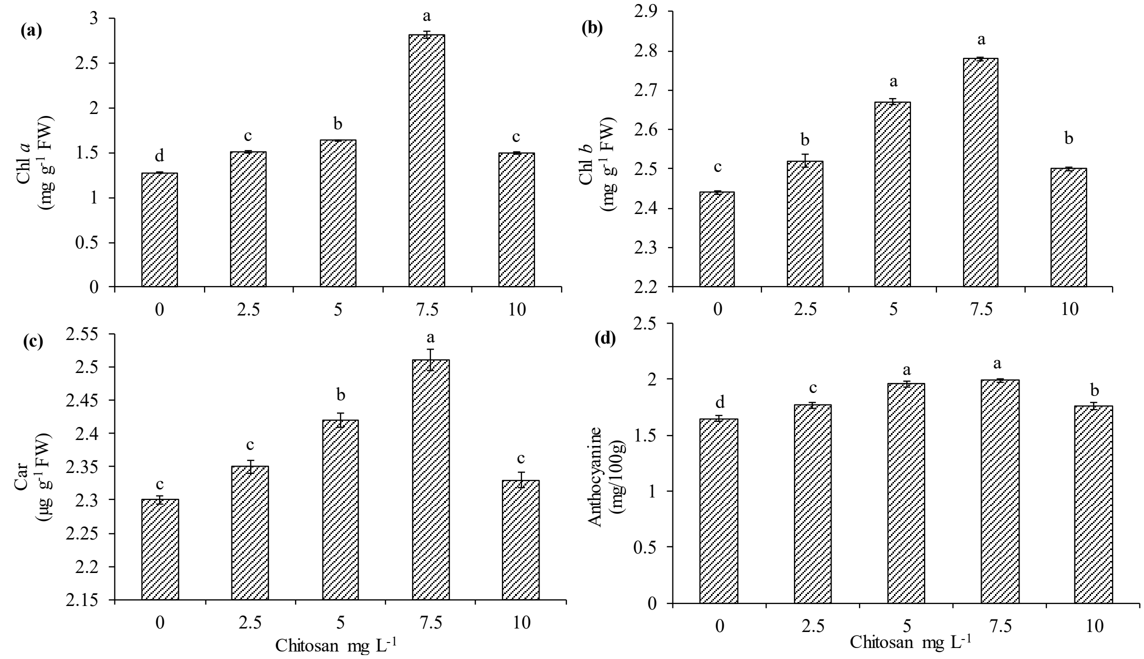


**Fig. 2.** Impact of Ct foliar application on leaf (a) chlorophyll *a* (Chl *a*), (b) chlorophyll *b* (Chl *a*), (c) carotenoids (Car) and (d) flower anthocyanins of Gruss an Taplitz plants. Lettering shows significance according to LSD test (P≤0.05)

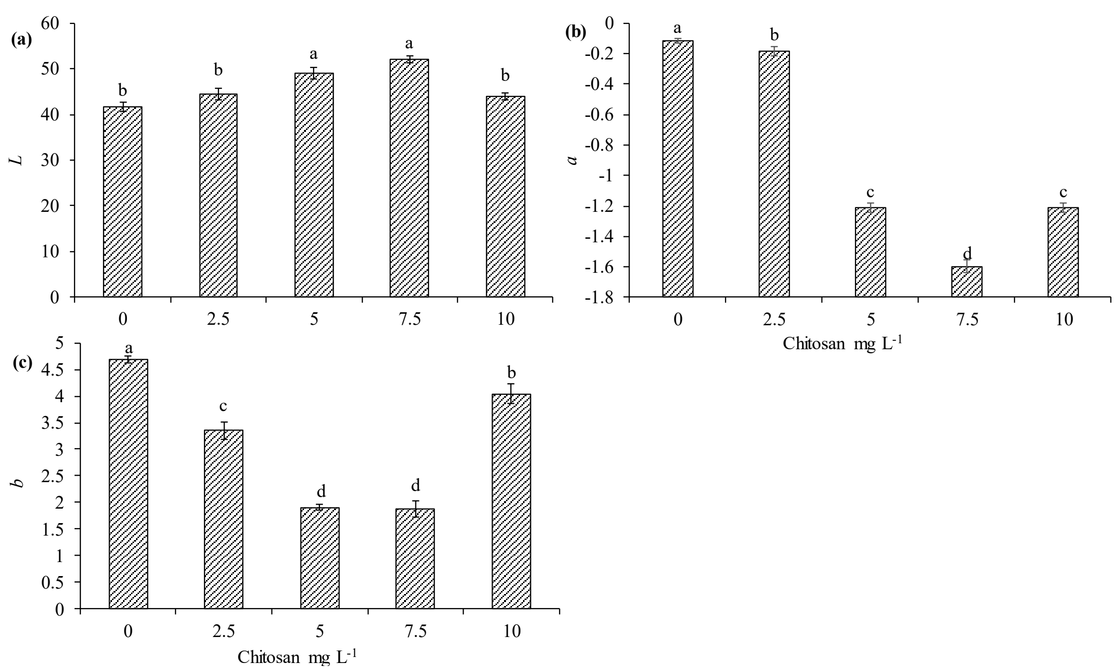


**Fig. 3.** Impact of Ct foliar application on leaf (a) brightness/lightness (*L*), (b) redness/greenness (*a*) and (c) blueness/yellowness (*b*) of Gruss an Taplitz plants. Lettering shows significance according to LSD test (P≤0.05)

## 4. Discussion

Ct improved plant growth of orchids (Nahar *et al*., 2012), freesia (Salachna & Zawadzinska, 2014), cordyline (El-Serafy, 2020) and chrysanthemum (Elansary *et al*., 2020). Present study also reported enhanced vegetative and reproductive growth of Gruss-an-Teplitz in response to foliar Ct application. Increased leaf (LA, PH) and flower (FN, FW, FD, BED) attributes showed plant response to Ct application that may be due improved nutrient availability, protein synthesis, cell growth and enzymes (Amin *et al*., 2007). Reports of Tantasawat *et al*. (2010) and Nahar *et al*. (2012) also recorded positive role of Ct on plant biomass of dendrobium and cymbidium plants respectively. Ct effectively absorbs in plant leaves, provide amino acids that enhances different metabolic processes and growth (Shibuya & Minami, 2001; Muley *et al*., 2019). Moreover, Ct facilitates biosynthesis of plant hormones (gibberellins and auxins) and provide nutrients (Nitrogen, calcium) with high stability (Uthairatanakij *et al*., 2007; Yen & Mau, 2007). Previously, El-Serafy. (2020) reported higher plant biomass due to improved nutrients uptake in cordyline after foliar Ct application. Ct treated plants produced early flowering with more flower number and flower weight, may be due to higher vegetative growth and photosynthetic activity that supports the findings of Limpanavech *et al*. (2008) in Dendrobium and Salachna & Zawadzinska. (2014) in Freezia. Similarly, Ct application produced more flowers in lisianthus (Ohta *et al*., 1999), Gerbera (Wanichpongpan *et al*., 2001), Gladiolus (Ramos-Garcia *et al*., 2009) and Freesia (Salachna & Zawadzinska, 2014).

Ct significantly increased RWC in Gruss-an-Teplitz plants is indication of higher water conservation and water use efficiency (Shehzad *et al*., 2020). Previously, El-Serafy. (2020) also observed higher RWC in leaves of cordyline in response to foliar Ct application. Ct is known to improve water uptake and water retention through maintaining membrane integrity and osmotic adjustment (Sara *et al*., 2012; Shehzad *et al*., 2020).

Foliar Ct application significantly improved leaf chlorophyll and carotenoids concentrations may be through increased enzymatic activity and gaseous exchange. Similarly, Dzung *et al*. (2011); Salachna & Zawadzinska (2014); Bistgani *et al*. (2017) and Singh *et al*. 2020 also observed improved chlorophyll in response to Ct application in coffee, freesia, thyme and grapes respectively. Higher chlorophyll pigments in the present study showed increased photosynthetic activity that significantly increased plant biomass of Gruss-an-Taplitz. Similar correlation of increased chlorophyll with improved growth in bent grass was recorded by Geng *et al*. (2020).

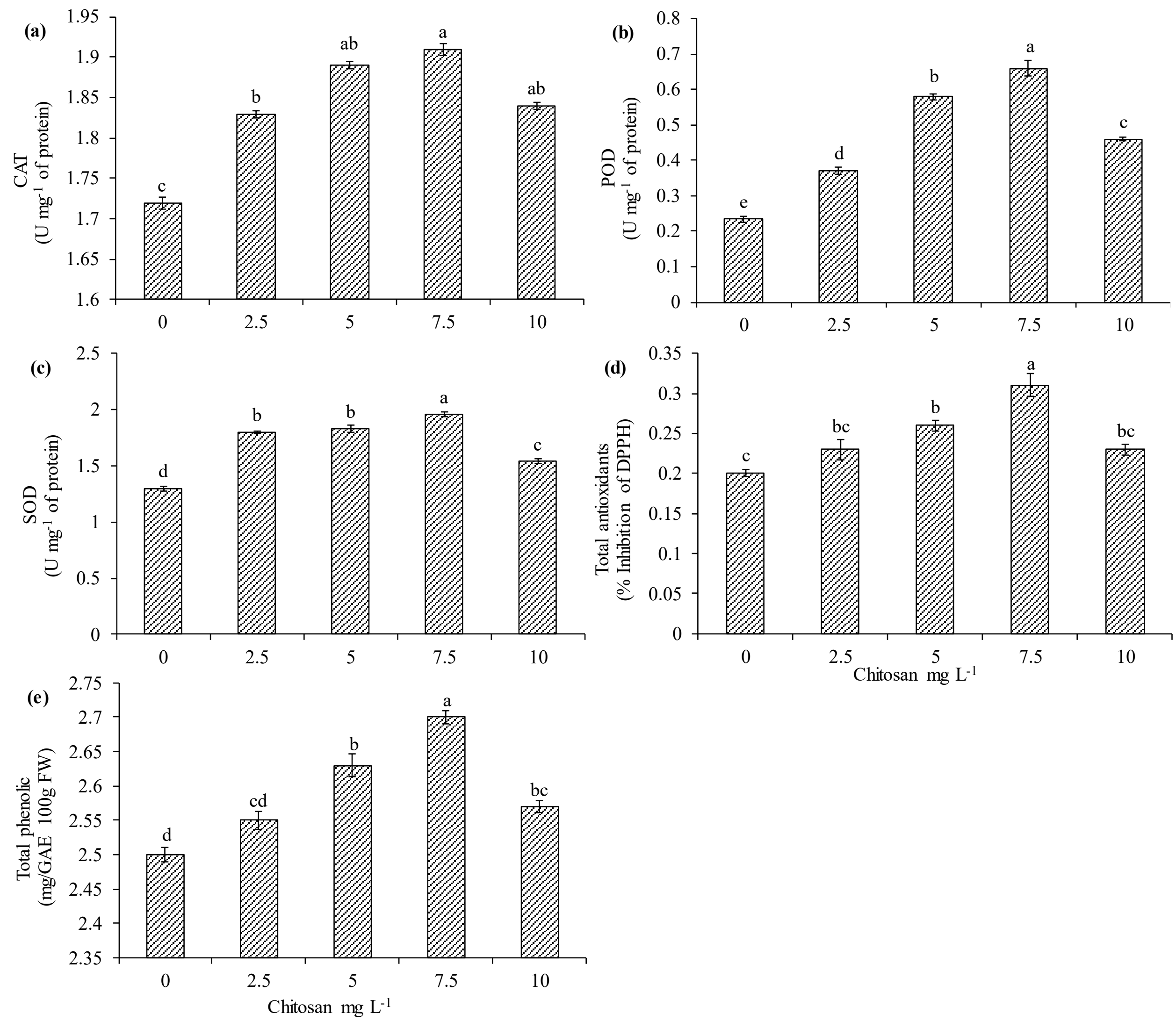


**Fig. 4.** Impact of Ct foliar application on (a) catalase (CAT), (b) peroxidase (POD), (c) superoxide dismutase (SOD), (d) total antioxidants and (e) total phenolic activity in leaves of Gruss an Taplitz plants. Lettering shows significance according to LSD test ($P \leq 0.05$)

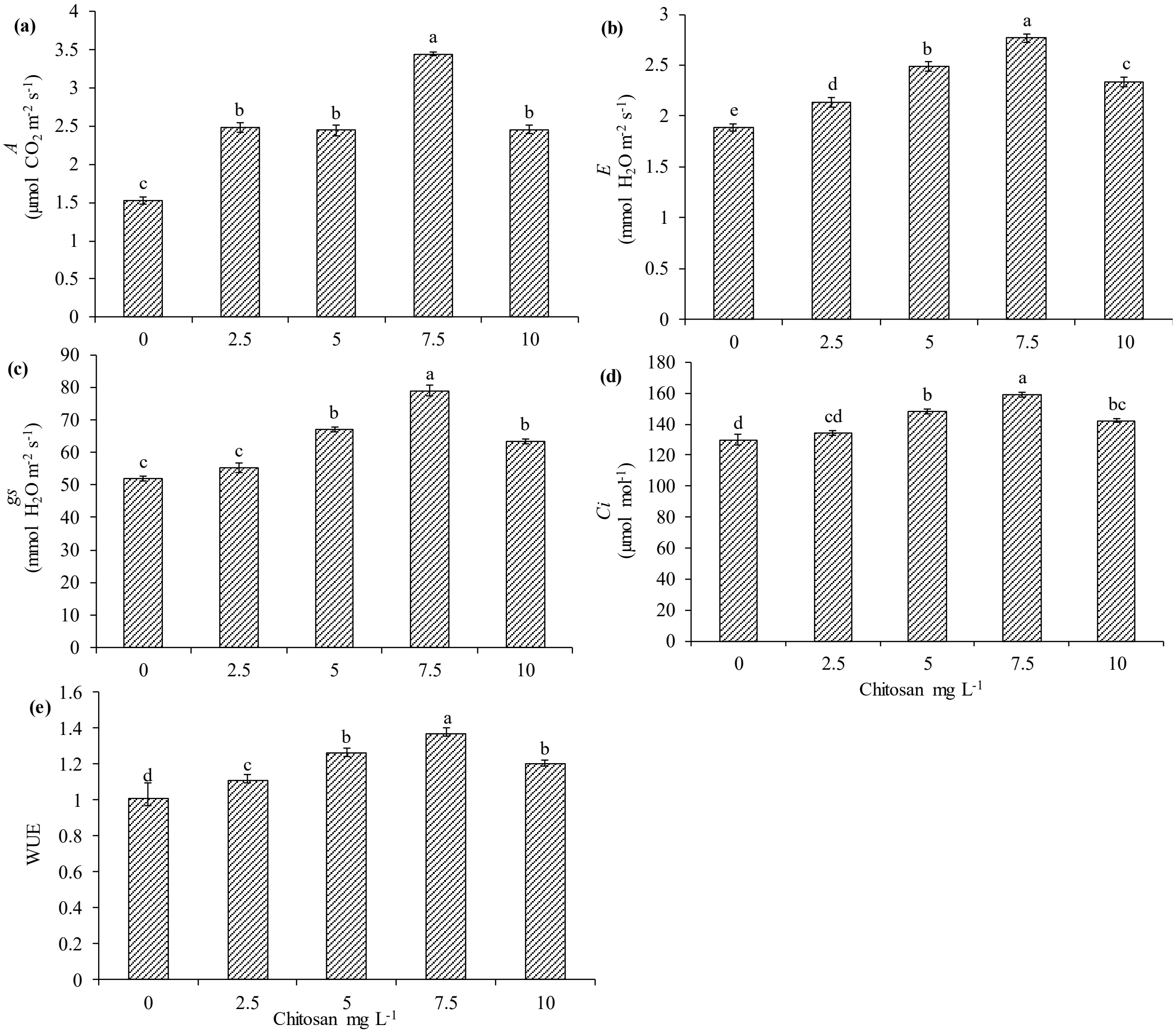


**Fig. 5.** Impact of Ct foliar application on leaf (a) photosynthetic rate (*A*), (b) transpiration rate (*E)*, (c) stomatal conductance (*g*s), (d) sub-stomatal conductance (*Ci*) and (e) water use efficiency (WUE) of Gruss an Taplitz plants. lettering shows significance according to LSD test (P≤0.05)

Ct provides extra amino groups for chlorophyll synthesis and protects the chlorophyll *a* from degradation (Wolf, 1956; Muley *et al*., 2019). Moreover, Ct increases leaf carotene contents that causes more light harvest for improved photosynthesis and ultimately higher organic matter accumulation (El-Serafy, 2020). Chlorophyll degradation is also delayed by Ct application through scavenging ROS in thylakoid membranes (Zhang *et al*., 2018).

Ct increased antioxidant activity (POD, SOD, CAT) may be due to higher photosynthetic pigments and stomatal conductance by controlling genes of nucleus and chloroplast (Choudhary *et al*., 2017; Singh *et al.,* 2019; Silva *et al.,* 2020). Previous report of Pirbalouti *et al*. (2017) also indicated higher enzymatic activity in leaves of basil species (*Ocimum ciliattum* and *O. basilicum*) after foliar application of Ct. Different Ct levels also increased biosynthesis of enzymes in leaves of cordyline (El-Serafy, 2020). Higher antioxidant activity after Ct application protects plants from lipid peroxidation and oxidative damage by detoxifying $H_2O_2$ and superoxide radicals (Shehzad *et al*., 2020).

Foliar Ct application improved gas exchange attributes ($P_N$, *E, gs, Ci,* WUE) in leaves of Gruss-an-Taplitz could explain increased RWC, photosynthesis and nutrient status of plants (Qaderi *et al*., 2019; Saifuddin *et al*., 2016). The improved stomatal conductance by Ct, increased $CO_2$ uptake and photosynthetic rate in leaves of plants may be due to higher chlorophyll concentration and enzymatic activity in photosynthetic cells (Temizel, 2015; Shehzad *et al*., 2020). Ct application produces oligomers in the cell that moves to nucleus and chloroplast for producing enzymes, enhances antioxidative and photosynthetic activities (Pichyangkura & Chadchawan, 2015). Similarly, Shehzad *et al*. (2020) recorded significantly higher gas exchange attributes in Ct treated plants of sunflower.

## 5. Conclusion

Ct foliar application on Gruss-an-Taplitz plants improved growth, photosynthesis, enzymatic activity that resulted to increase flower yield. Findings of this study showed that 7.5 mg $L^{-1}$ Ct showed maximum increase in morphological and physiological attributes that reflected increased yield of Gruss-an-Taplitz plants. Therefore, foliar spray of Ct (7.5 mg $L^{-1}$) on Gruss-an-Taplitz could be suggested commercially. But further investigation of Ct application on cut roses still needed.

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