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Article

Improvement in Physiobiochemical and Yield Characteristics of Pea Plants with Nano Silica and Melatonin under Salinity Stress Conditions

Wasimah B. Al-Shammari [1,*], Haya R. Altamimi [1] and Khaled Abdelaal [2,*]

1 Department of Biology, College of Science, University of Hail, P.O. Box 2440, Hail 55476, Saudi Arabia
2 PCRS Excellence Center, Plant Pathology and Biotechnology Lab, Department of Agricultural Botany, Faculty of Agriculture, Kafrelsheikh University, Kafrelsheikh 33516, Egypt
* Correspondence: w.alshamary@uoh.edu.sa (W.B.A.-S.); khaled.elhaies@gmail.com (K.A.)

Abstract: The effect of nano silica (50 mL L^{-1}) and melatonin (75 µM) individually or in combination in foliar applications on the morphophysiological, biochemical and yield properties of pea plants under salinity stress conditions was evaluated. Salt stress caused a remarkable decrease in the growth and yield characteristics; for example, the plant dry weight, plant height, number of flowers plant^{-1}, number of pods plant^{-1}, weight of 100 green seeds and protein concentration in the pea plants during both seasons were decreased compared with the control. Similarly, their physiobiochemical characteristics were negatively affected; chlorophyll a, chlorophyll b and the relative water content (RWC) were significantly reduced in the stressed pea plants. However, malondialdehyde (MDA), hydrogen peroxide, the electrolyte leakage (EL%), super oxide and the antioxidant components (catalase (CAT), superoxide dismutase (SOD), peroxidase (POX) and total phenolic compounds) were significantly increased when the plants were under salt stress compared with the control plants. On the other hand, the foliar application of nano silica and melatonin individually or in combination enhanced the physiobiochemical characteristics, morphological characteristics and yield of the stressed pea plants. The best treatment was the combination treatment (nano silica + melatonin), which caused significant increases in the plant dry weight, plant height, number of flowers and pods plant^{-1}, weight of 100 green seeds, protein concentration, chlorophyll concentrations and RWC in the stressed pea plants. Additionally, the combination treatment significantly decreased the EL%, MDA, $O_2\cdot-$ and H_2O_2 and adjusted the upregulation of the antioxidant enzymes, proline and total phenolic compounds in the stressed plants compared with the stressed untreated pea plants. Generally, it can be suggested that the co-application of nano silica (50 mL L^{-1}) + melatonin (75 µM) plays a positive role in alleviating the adverse impacts of salinity on pea plants by modifying the plant metabolism and regulating the antioxidant defense system as well as scavenging reactive oxygen species.

Keywords: abiotic stress; salt stress; nano silica; melatonin; chlorophyll; enzymes; reactive oxygen species

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1. Introduction

Global climate change plays a significant role in agricultural crop production. In this regard, the biotic and abiotic stress factors of climate change significantly affect the growth and yield of several crops. Biotic factors such as plant pathogens in wheat [1–3] and barley plants [4,5] and insects in vegetable crops [6,7] are very detrimental for crop production. Additionally, abiotic stress such as drought, heat and salinity negatively affect several plants. Drought and heat stress affect the growth and development of many plants such as wheat [8,9], faba bean [10], maize [11] and barley plants [12,13]. Salinity is one of the most harmful abiotic factors that affect sweet pepper [14], faba bean [15], cucumber [16] and strawberry plants [17].

All plant stages such as germination, vegetative growth and flowering are harmfully affected when plants are under salt conditions as this results in a high accumulation of solutes and oxidative stress [18,19]. Furthermore, studies on physiological factors such as the chlorophyll concentration and RWC found that they were significantly decreased in calendula under salinity stress [20]; additionally, salinity led to a reduced plant height and number of leaves [14]. Additionally, salt tress is usually associated with oxidative damage due to the gathering of ROS such as super oxide [21], which causes lipid peroxidation in numerous plants under several stress conditions [21,22]. Moreover, malondialdehyde (MDA), electrolyte leakage (EL%) and ROS are important indicators of plant health when the plant is under salinity and various stresses [23,24]. Under salinity and other stress factors, the increase in MDA and EL% was recorded due to the oxidative stress to chloroplasts, mitochondria and other organelles [14,25,26]. Additionally, the gathering of ROS is one of the most significant stress signals, especially H_2O_2 and $O_2{\cdot}-$, which increases significantly under various stresses [27–30]. This accumulation of ROS can be scavenged by the activation of antioxidant systems such as enzymatic and nonenzymatic antioxidants [30]. One of the important nonenzymatic antioxidants is proline; it plays a pivotal role as an osmoprotectant and protects the organelles against oxidative stress [31]. Additionally, the total phenolic compounds are important nonenzymatic antioxidants that play a significant role in protecting plants under stress [29]. The overproduction of enzymatic antioxidants such as CAT, POX and SOD were observed in stressed plants under various stresses to scavenge the accumulation of ROS [30,32,33].

Pisum sativum L. is an important vegetable crop grown for fresh and dry seeds; the seeds contain protein, carbohydrates, vitamins and minerals such as zinc, iron and phosphorus [34]. There are many studies that were conducted to improve the yield of peas under several stresses [35–37]. The application of nano silica and nano silver improved the growth and yield of several plants, as shown by their surface area and surface reactivity, which was greater than that of normal materials [38–40]; additionally, they had small molecular aggregates with dimensions between 1 and 100 nm. Nano silica is one of the significant nano materials in agricultural production [40], and the application of nano silica improved the quality of Larix [41]; additionally, the productivity of strawberry was increased under drought and salinity stresses [42]. Khedr et al. [43] reported that nano silica significantly improved the water relations of two wheat cultivars, such as the relative water content, during both seasons. Additionally, the highest activity of CAT and POX enzymes and the lowest values of MDA were recorded when the nano silica treatment was applied. Spraying nano silica significantly increased the No. spikes m^{-2}, 1000 grain wt, chlorophyll a and b as well as the yield of two wheat cultivars [44].

In *Duranta erecta,* applications of SiO_2NPs or AgNPs at levels of 300 mg/L led to improvements in the tolerance to salinity stress and enhanced the growth characteristics of plants [44].

Moreover, Hafez et al. [45] indicated that the application of nano materials improved the grain yield of rice plants under salinity stress. Additionally, Rashwan et al. [27] recorded the positive effect of nanoparticles on the flax yield and growth. Furthermore, the application of plant hormones is a significant method that is used to increase plant production; melatonin is one of the most important hormones and can regulate and improve plant growth [46] and yield production [47]. Additionally, the foliar application of melatonin led to an increase in plant growth and plant tolerance against stress conditions [48,49], which was associated with reactive oxygen species scavenging [48]. Many studies have stated that melatonin plays a pivotal role in scavenging reactive oxygen species, the antioxidant enzymes activity and membrane stability, and it regulates gene expression in many plants [50–52]. Under salinity conditions, a melatonin application led to increased nitrogen metabolism and improved the photosynthetic rate and mineral uptake [53]; additionally, melatonin can improve plant hormones and redox homeostasis [47,54] and consequently improve growth characteristics. The use of nano silica or melatonin to increase yield production has been studied, but few studies have been conducted on the effect of nano silica+melatonin in mitigating salt stress damages to plants. Hence, our study was carried out to assess the impact of nano silica and melatonin individually or in combination on mitigating salt stress damages in pea plants, and this was associated with improvements in the concentration of chlorophyll, RWC, regulation of the antioxidant deference system and improved seed yield characteristics.

2. Materials and Methods

2.1. Experiment Location and Treatments

Two pot experiments were conducted during the winter of 2021/2022 and 2022/2023 to study the effect of nano silica (50 mL L^{-1}) and melatonin (75 µM) on the morphophysiological and yield characteristics of pea plants (Master B cultivar) under salt conditions (50 mM and 100 mM NaCl). Saline water (50 and 100 mM NaCl) was used for irrigation 15 days after sowing, and the application of nano silica (50 mL L^{-1}) and melatonin (75 µM) were performed twice at 25 and 40 days from sowing. Morphological and physiological measurements were recorded 60 days after sowing, but the yield characteristics were recorded at 75 days. The physical and chemical characteristics of the experimental soil were analyzed as follows: N 32.1 ppm, K 291 ppm, P 10.1 ppm, pH 8.1, electrical conductivity 1.9 dS m^{-1}, soil organic matter 1.9%, silt 35.4%, sand 16.9% and clay 48.1%. The experimental design was a completely randomized design with four replicates. Pea seeds (Master B cultivar) were sown on the 2nd and 5th of November during both seasons. Each pot (40 cm diameter) contained 8 kg of soil and 5 seeds, and the seedlings were thinned to 4 seedlings 21 days after sowing. NPK fertilizers were added in two doses; the first one was added 14 days after sowing, and the second dose was added at the flowering stage. The physiological, biochemical and yield characteristics of the samples were recorded. The experiment included the following treatments:

T1: control (plants irrigated with tap water under normal conditions).
T2: plants treated with 50 mM NaCl.
T3: plants treated with 50 mM NaCl and nano silica (50 mL L^{-1}).
T4: plants treated with 50 mM NaCl and melatonin (75 µM).
T5: plants treated with 50 mM NaCl and nano silica (50 mL L^{-1}) + melatonin (75 µM).
T6: plants treated with 100 mM NaCl.
T7: plants treated with 100 mM NaCl and nano silica (50 mL L^{-1}).
T8: plants treated with 100 mM NaCl and melatonin (75 µM).
T9: plants treated with 100 mM NaCl and nano silica (50 mL L^{-1}) + melatonin (75 µM).

2.2. Morphological Parameters

The morphological parameters were determined at 60 days from sowing, and the plant height, leaves number, plant dry weight and number of flowers were recorded.

2.3. Biochemical and Physiological Parameters

2.3.1. Determination of Chlorophylls

The determination of chlorophylls was performed with pea fresh leaves by using a solution containing 95% ethanol and 80% acetone. The samples were kept in the refrigerator overnight, the reading was recorded in the extract at 663 and 645 nm and the concentration was measured [55].

2.3.2. Assay of Relative Water Content (RWC%)

The fresh leaves weight (FW) for the ten discs was recorded, and then the samples were kept in distilled water; after 1 h, the turgid weight (TW) was measured. After 24 h at 80 °C, the dry weight (DW) was recorded in the discs. The RWC% was measured as follows [56]: $RWC = (FW - DW)/(TW - DW) \times 100$.

2.3.3. Determination of Maximum Quantum Efficiency of PS II (Fv/Fm)

The Fv/Fm ratio was determined by using a portable chlorophyll fluorometer at 60 days from the sowing. The maximum efficiency of PSII was measured as the ratio of (Fv) to (Fm) [57].

2.3.4. Assay of Electrolyte Leakage (EL%)

Pea fresh leaves (20 discs) were taken to an EL assay. The EL% was determined [58] as follows: initial conductivity/final conductivity × 100.

2.3.5. Determination of Lipid Peroxidation (MDA%)

The lipid peroxidation was determined by using a spectrophotometer as follow: malondialdehyde (MDA) (nmol g^{-1} FW) = [6.45 × (A532 − A600) − (0.56 × A450)] × V −1W, where V = volume (cm^3) and W = weight (g) [59].

2.3.6. Assay of Hydrogen Peroxide (H_2O_2) and Superoxide (O_2-)

The samples of pea fresh leaves were infiltrated and incubated in the light for 140 min and 2 h, respectively. H_2O_2 and O_2- were assayed with a ChemiImager 4000 digital imaging system in nmol g^{-1} FW [60].

2.3.7. Determination of Proline Content

The proline was assayed as µg g^{-1}FW by using a spectrophotometer at 520 nm [61].

2.3.8. Determination of Total Phenolic Compounds

The total phenolic compounds in the pea leaves were determined by using the Folin–Ciocalteu reagent [62]. The extract solution (0.1 mL) containing 1000 µg of the extract was mixed with 46 mL of distilled water, and then 1 mL of the Folin–Ciocalteu reagent was added, and the samples were shaken. The mixture was allowed to react for 3 min, and then 3 mL of an aqueous solution of 2% Na_2CO_3 was added, and the incubation was performed for 2 h at room temperature. Using a spectrophotometer, the absorbance was recorded at 750 nm, and then the total phenolic compounds was recorded (µg mL^{-1} gallic acid equivalent).

2.3.9. Determination of CAT, SOD and POX Activity

In total, 500 mg of pea leaves were homogenized and centrifuged for 20 min at 4 °C (12,000× *g*), and then the supernatant was used to measure the total soluble enzyme activities [63]. The CAT activity was measured by using a spectrophotometer at 240 nm based on the consumption of H_2O_2 [64]. The SOD activity was measured at 560 nm [65]. According to Hammerschmidt et al., the peroxidase (POX) activity was measured [66].

2.4. Yield Characteristics

At harvest day (75 days from sowing), the pods number, seeds number pod^{-1}, weight of 100 green seeds and protein concentration were recorded, and the concentration of the protein was recorded according to the guidelines set by the AOAC [67].

2.5. Statistical Analysis

The results obtained during the two seasons were analyzed by using the MSTAT-C statistical software package. Data represent the mean ± SD. A Student's *t*-test was used to determine whether a significant difference ($p \leq 0.05$) existed between the mean values [68].

3. Results

3.1. Effect of Nano Silica and Melatonin on Plant Height (A), Leaves Number (B), Plant Dry Weight (g) and Flower Number Plant^{-1} (D) under Salinity Conditions

Salinity stress had negative effects on the pea plants, and the plant height, leaves number, plant dry weight and flower number were significantly decreased in the pea plants under both salinity levels (50 mM and 100 mM NaCl) during both seasons compared to the plants receiving the control treatment (Figure 1). However, a foliar application with nano silica (50 mL L^{-1}) and melatonin (75 µM) individually or in combination (nano silica + melatonin) led to a remarkable increase in the plant height, leaves number, dry weight and flower number in the stressed pea plants compared with the untreated stressed pea plants during the winter of 2021/2022 and 2022/2023. The best plant heights were recorded when the combination treatment (nano silica and melatonin) was used, followed by the melatonin treatment under salinity levels of 50 mM during the two seasons. The increase in the plant height of the stressed plants that were treated with the combination treatment was 34% and 42% during the two seasons, respectively, compared to the stressed plants that did not receive treatment. Furthermore, the high values of the plant dry weight, leaves number and flower number in the stressed pea plants (50 mM NaCl) after receiving the combination treatment were recorded, and the increase in the plant dry weight was 53% and 48% during both seasons, respectively. Additionally, the increase in the flower number during both seasons was 114% and 108%, respectively, as compared with the stressed plants that were not receiving treatments. Additionally, under the high salinity level (100 mM), the best leaves number, plant height, plant dry weight and flower number were recorded in the plants treated with the combination treatment followed by the melatonin treatment during both seasons compared to the stressed untreated plants (Figure 1).

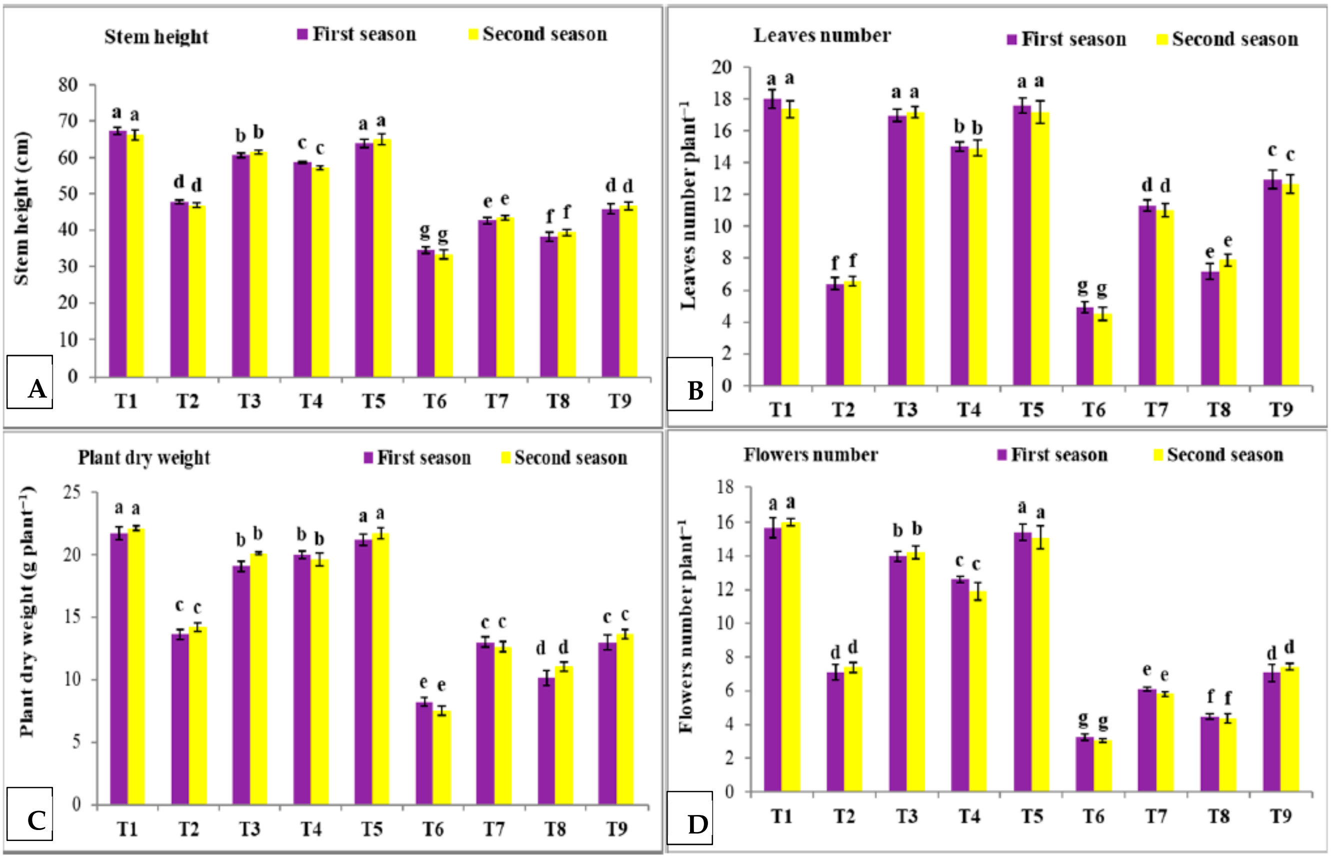

Figure 1. Effect of nano silica and melatonin on stem height (**A**), leaves number (**B**), plant dry weight (**C**) (g) and number of flowers plant^{-1} (**D**) in pea plants under salinity conditions during two seasons (2021/2022 and 2022/2023). Bars followed by different letters are significantly different according to Student's *t*-test.

T1: control (plants irrigated with tap water under normal conditions).
T2: plants irrigated with 50 mM NaCl.
T3: plants irrigated with 50 mM NaCl and treated with nano silica (50 mL L^{-1}).

T4: plants irrigated with 50 mM NaCl and treated with melatonin (75 μM).
T5: plants irrigated with 50 mM NaCl and treated with nano silica (50 mL L^{-1}) + melatonin (75 μM).
T6: plants irrigated with 100 mM NaCl.
T7: plants irrigated with 100 mM NaCl and treated with nano silica (50 mL L^{-1}).
T8: plants irrigated with 100 mM NaCl and treated with melatonin (75 μM).
T9: plants irrigated with 100 mM NaCl and treated with nano silica (50 mL L^{-1}) + melatonin (75 μM).

3.2. Effect of Nano Silica and Melatonin on Number of Pods Plant^{-1}, Number of Seeds Pod^{-1}, Weight of 100 Green Seeds and Protein Concentration in Pea Plants under Salinity Conditions

The salinity stress significantly decreased the pods number plant^{-1}, seeds number pod^{-1}, weight of 100 green seeds and protein concentration in seeds in the stressed pea plants compared with the control plants during the two seasons. Under the low salinity concentration (50 mM), the decrease in the pods number was 40% and 41% while the decrease was 53% and 54.5% under the high salinity concentration (100 mM) during both seasons, respectively, compared with the control. The number of seeds pod^{-1} was decreased significantly at the low salinity level (49.4% and 51.8%) and high salinity level (58.5% and 62.5%) during the two seasons, respectively, compared to the control plants. Additionally, the salinity level at (50 mM) significantly decreased the protein concentration (20.6% and 23.4%); however, the decrease under the high salinity level was 40.7% and 36.7% during both seasons, respectively, when compared with the control. Conversely, the application with nano silica and melatonin individually or in combination led to a significant increase in the pods number, number of seeds, weight of 100 green seeds and protein concentration in the stressed pea plants under both salinity levels. The best treatment under the two salinity levels was the combination of nano silica and melatonin, which resulted in the highest values for the pods number (53% and 40%), number of seeds (61.5% and 69%), weight of 100 green seeds (57.6% and 60.3%) and protein concentration (17% and 23%) in the stressed plants compared with the untreated plants during the two seasons, respectively, at the low salinity level (Figure 2).

T1: control (plants irrigated with tap water under normal conditions).
T2: plants irrigated with 50 mM NaCl.
T3: plants irrigated with 50 mM NaCl and treated with nano silica (50 mL L^{-1}).
T4: plants irrigated with 50 mM NaCl and treated with melatonin (75 μM).
T5: plants irrigated with 50 mM NaCl and treated with nano silica (50 mL L^{-1}) + melatonin (75 μM).
T6: plants irrigated with 100 mM NaCl.
T7: plants irrigated with 100 mM NaCl and treated with nano silica (50 mL L^{-1}).
T8: plants irrigated with 100 mM NaCl and treated with melatonin (75 μM).
T9: plants irrigated with 100 mM NaCl and treated with nano silica (50 mL L^{-1}) + melatonin (75 μM).

3.3. Effect of Nano Silica and Melatonin on Chlorophyll a and b, Maximum Quantum Efficiency of PS II and RWC in Pea Plants under Salinity Stress

Our results showed that the concentration of chlorophyll a and b, Fv/Fm and RWC significantly decreased in the stressed plants compared to the control plants during both seasons when the plants were under the two salinity levels (50 mM and 100 mM NaCl) (Figure 3). Under the low salinity level (50 mM), the decrease in chlorophyll a was 53.5% and 56.8%, chlorophyll b was 43.9% and 47.6%, Fv/Fm was 8.5% and 8.2% and RWC was 29.8% and 34.2% in the stressed pea plants compared with the control plants during both seasons, respectively. However, the decrease in these characteristics under the high salinity level (100 mM) was 67.8% and 64% in chlorophyll a, 73.2% and 71.4% in chlorophyll b, 12.2% and 11.4% in the maximum quantum efficiency of PS II (Fv/Fm) and 36.3% and 39.5 in the relative water content during the two seasons, respectively, compared with the control.

Conversely, under both salinity levels, the stressed pea plants that were treated with nano silica and melatonin individually or in combination showed a remarkable increase in chlorophyll a, b, Fv/Fm and relative water content during both seasons compared with the control. Moreover, the highest concentrations of these characteristics were recorded in the stressed pea plants that received the combination treatment (nano silica + melatonin) and were under a salinity of 50 mM. The increase in chlorophyll a was 112% and 105%, chlorophyll b was 73.9% and 79.5 and Fv/Fm was 7.8% and 10.9% while the increase in RWC was 40.7% and 49% during both seasons, respectively, compared with the stressed untreated plants.

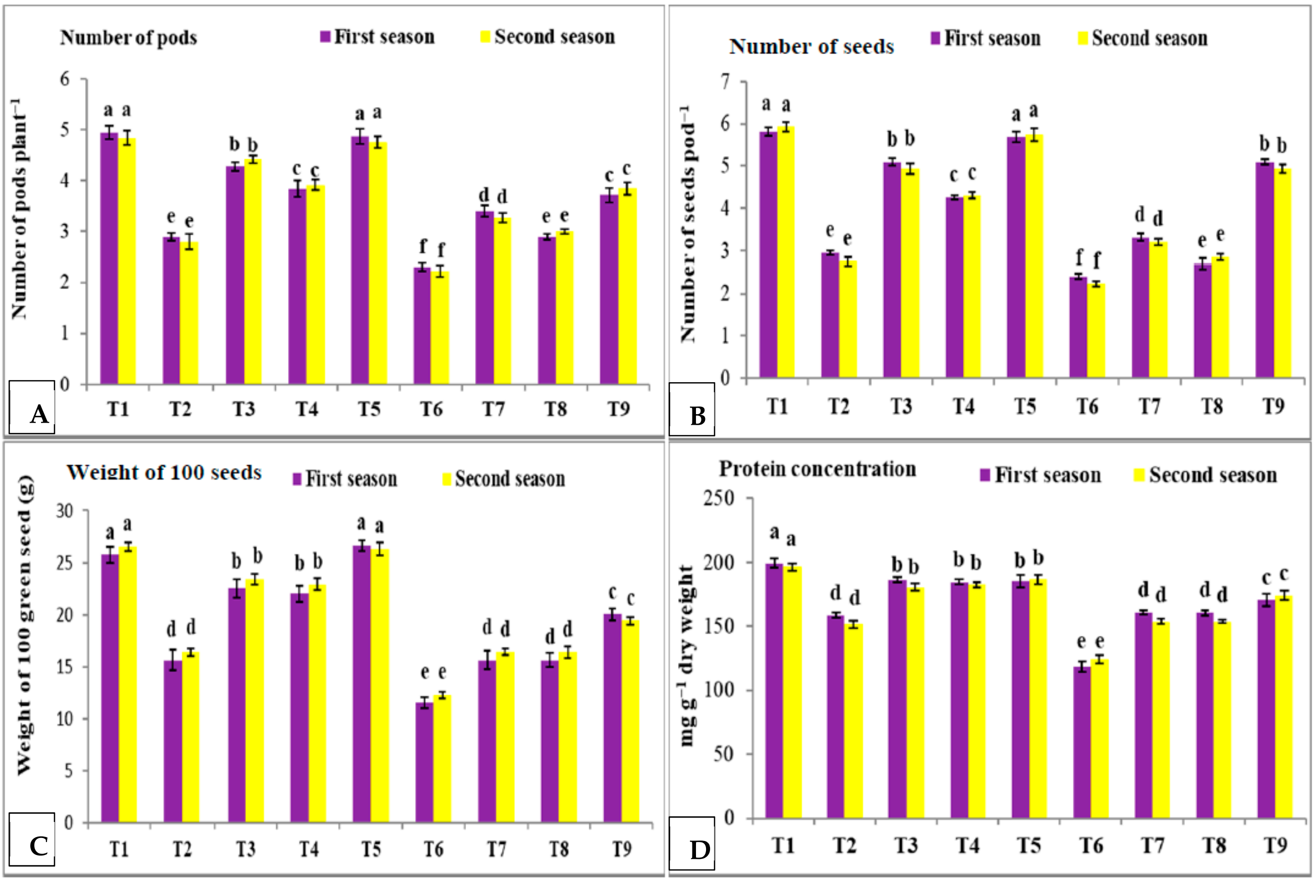

Figure 2. Effect of nano silica and melatonin on pods number plant^{-1} (**A**), seeds number pod^{-1} (**B**), weight of 100 green seeds (**C**) and protein concentration (**D**) in pea plants under salinity conditions during two seasons (2021/2022 and 2022/2023). Bars followed by different letters are significantly different according to Student's *t*-test.

T1: control (plants irrigated with tap water under normal conditions).
T2: plants irrigated with 50 mM NaCl.
T3: plants irrigated with 50 mM NaCl and treated with nano silica (50 mL L^{-1}).
T4: plants irrigated with 50 mM NaCl and treated with melatonin (75 µM).
T5: plants irrigated with 50 mM NaCl and treated with nano silica (50 mL L^{-1}) + melatonin (75 µM).
T6: plants irrigated with 100 mM NaCl.
T7: plants irrigated with 100 mM NaCl and treated with nano silica (50 mL L^{-1}).
T8: plants irrigated with 100 mM NaCl and treated with melatonin (75 µM).
T9: plants irrigated with 100 mM NaCl and treated with nano silica (50 mL L^{-1}) + melatonin (75 µM).

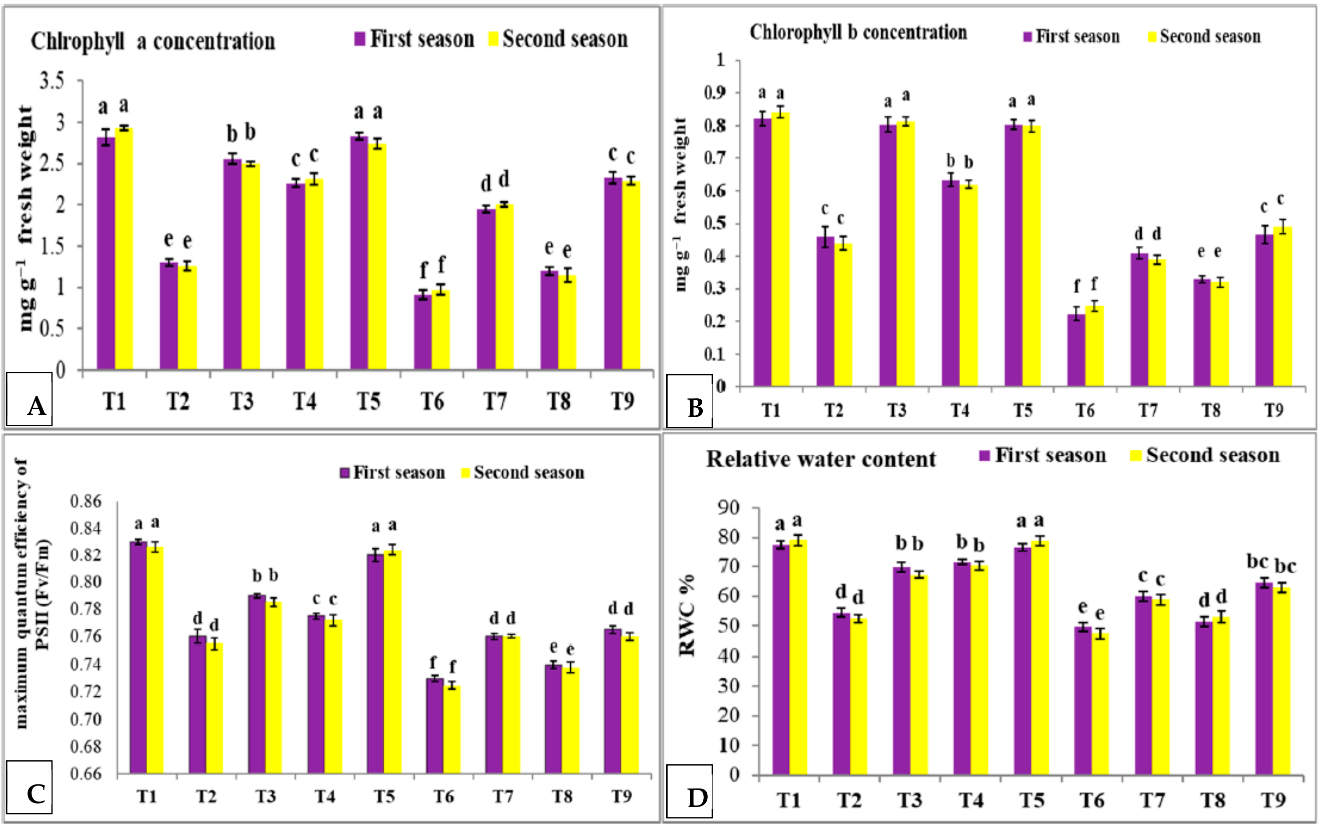

Figure 3. Effect of nano silica and melatonin on chlorophyll a (**A**), chlorophyll b (**B**), Fv/Fm (**C**) and RWC (**D**) in pea plants under salt conditions during winter of 2021/2022 and 2022/2023. Bars followed by different letters are significantly different according to Student's *t*-test.

3.4. Effect of Nano Silica and Melatonin on EL%, MDA, Superoxide Concentration and H_2O_2 Concentration in Pea Plants under Salinity Stress

The obtained results showed significant increases in the EL%, MDA, superoxide and H_2O_2 concertation as indicators of salinity stress in the stressed plants during both seasons (Figure 4). The highest levels of EL%, MDA, superoxide and H_2O_2 were noted in the stressed plants under the high salinity level (100 mM NaCl) during the two seasons. The salinity stress at 100 mM led to significant increases in the EL% (130% and 127.7%), MDA (181% and 193%), superoxide (166% and 197.2%) and H_2O_2 (184.6% and 157.1%) during both seasons, respectively, compared with the control. Regarding the effect of the foliar application with nano silica and melatonin individually or in combination, the stressed plants showed significant decreases in these characteristics under the two salinity levels during both seasons compared to the stressed untreated plants. The best treatment was the combination of nano silica + melatonin followed by melatonin and then nano silica; the decrease in the EL% was 47.1% and 42.5%, MDA was 50% and 48.5%, superoxide was 42.8% and 50.2% and H_2O_2 was 50% and 46.6% under the low salinity level during the two seasons, respectively. Furthermore, the combination treatment improved the pea status under the high salinity level and caused a significant decrease in the EL% (20% and 16.8%), MDA (26.5% and 30%), superoxide (21.5% and 23.2%) and H_2O_2 (18.5% and 21.8%) during the two seasons, respectively, compared to the stressed untreated pea.

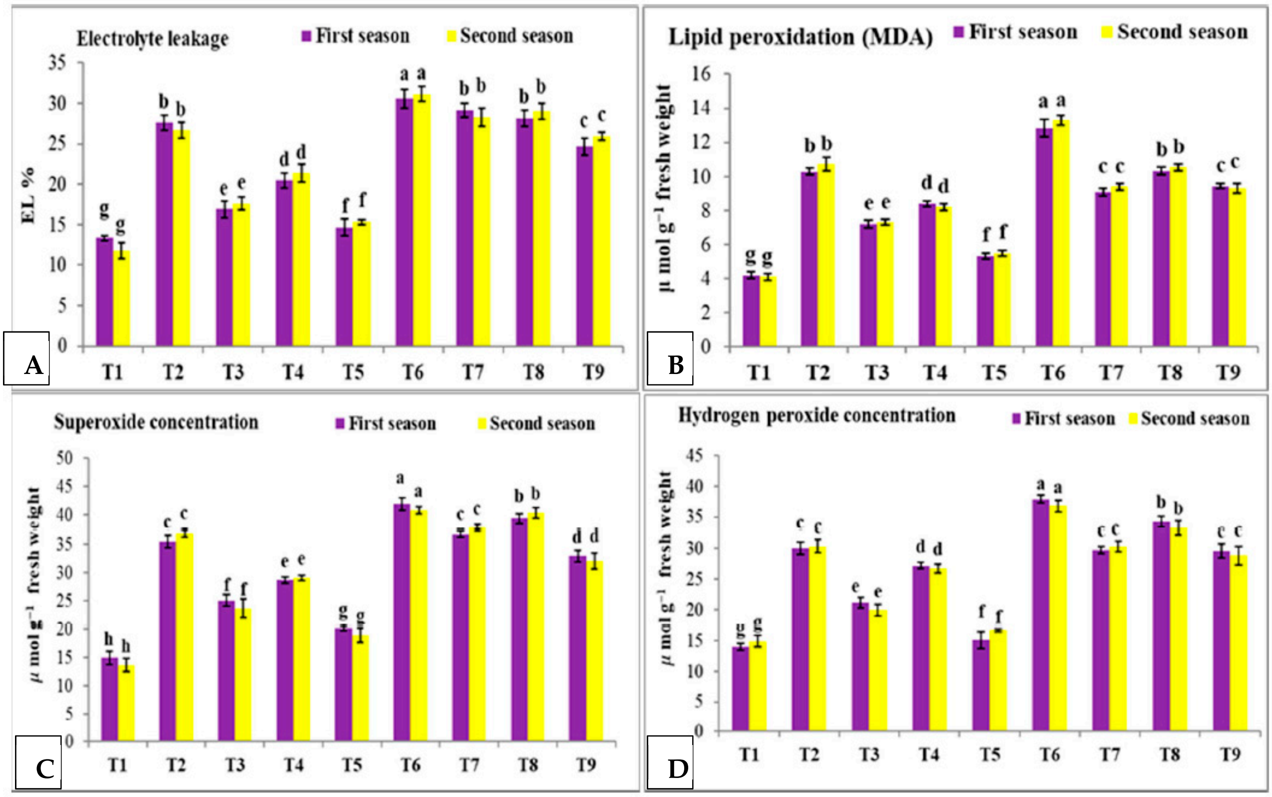

Figure 4. Effect of nano silica and melatonin on EL% (**A**), MDA (**B**), superoxide (**C**) and H_2O_2 concentration (**D**) in pea plants under salinity conditions during two seasons (2021/2022 and 2022/2023). Bars followed by different letters are significantly different according to Student's *t*-test.

T1: control (plants irrigated with tap water under normal conditions).
T2: plants irrigated with 50 mM NaCl.
T3: plants irrigated with 50 mM NaCl and treated with nano silica (50 mL L^{-1}).
T4: plants irrigated with 50 mM NaCl and treated with melatonin (75 μM).
T5: plants irrigated with 50 mM NaCl and treated with nano silica (50 mL L^{-1}) + melatonin (75 μM).
T6: plants irrigated with 100 mM NaCl.
T7: plants irrigated with 100 mM NaCl and treated with nano silica (50 mL L^{-1}).
T8: plants irrigated with 100 mM NaCl and treated with melatonin (75 μM).
T9: plants irrigated with 100 mM NaCl and treated with nano silica (50 mL L^{-1}) + melatonin (75 μM).

3.5. Effect of Nano Silica and Melatonin on CAT, SOD, POX, Total Phenolic Compounds and Proline Content in Pea Plants under Salinity Conditions

Our findings point out that the antioxidant defense system against salinity stress, which include components such as CAT, SOD, POX, total phenolic compounds and proline concentration, were significantly increased in the stressed pea plants under two salinity concentrations during both seasons (Figure 5). However, a foliar application with nano silica and melatonin individually or in combination led to the regulation of and decrease in the CAT activity in the stressed pants. The best result was recorded when the combination treatment was applied, which decreased the CAT activity to 41.2% and 36% in the stressed plants (50 mM NaCl) during both seasons, respectively; however, the decrease in CAT was 12.7% and 28.4% in the stressed plants under the high salinity level (100 mM) compared with the stressed plants that were not receiving treatments during both seasons, respectively. Additionally, the SOD activity significantly decreased according to the foliar application with various treatment, and the best treatment was the combination treatment which decreased the SOD activity to 26.14% and 32.8% during both seasons, respectively, compared with the stressed untreated pea plants under the high salinity level.

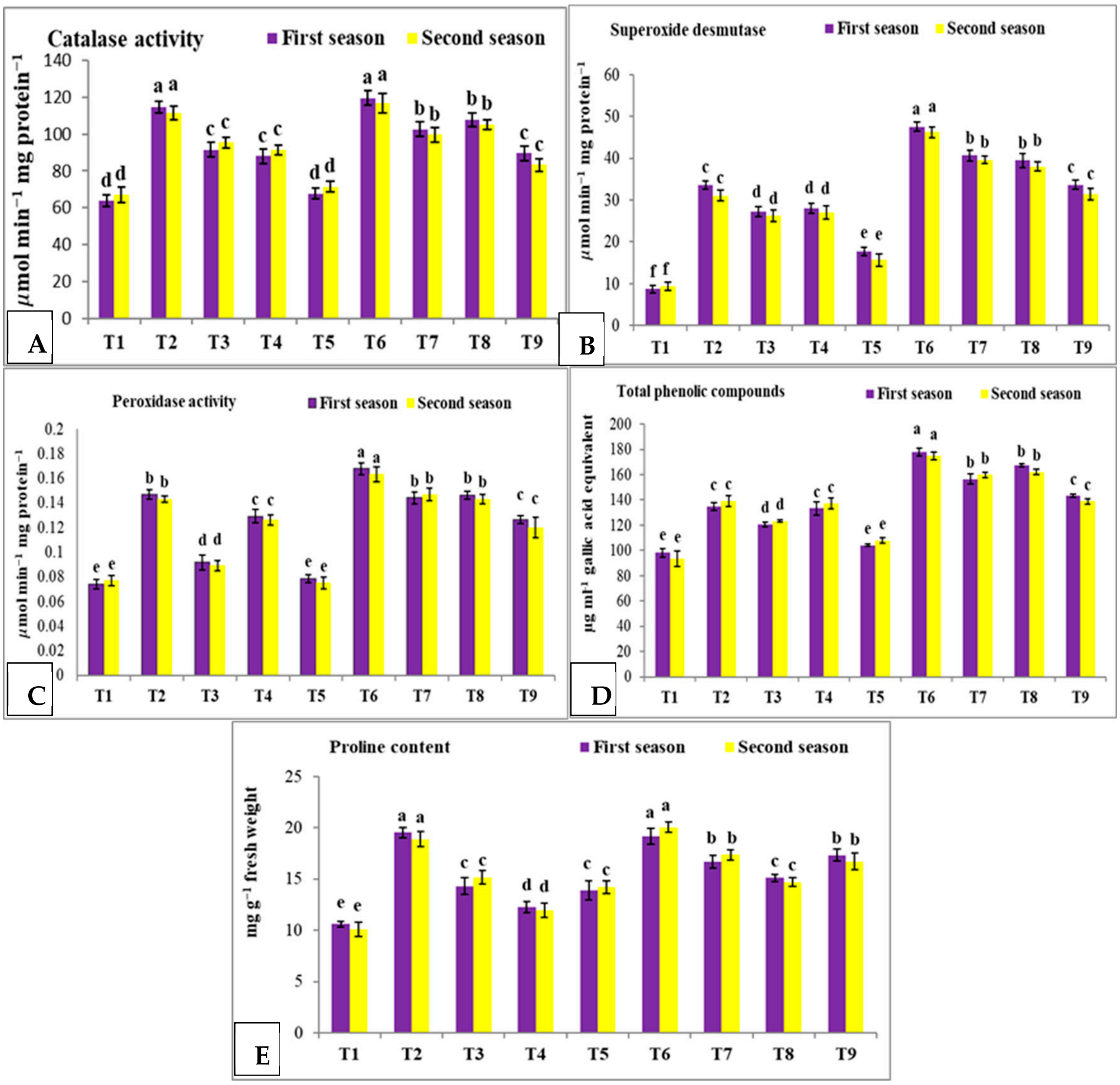

Figure 5. Effect of nano silica and melatonin on catalase (**A**), superoxide dismutase (**B**), peroxidase (**C**), total phenolic compounds (**D**) and proline (**E**) in stressed pea plants during both seasons (2021/2022 and 2022/2023). Bars followed by different letters are significantly different according to Student's *t*-test.

T1: control (plants irrigated with tap water under normal conditions).
T2: plants irrigated with 50 mM NaCl.
T3: plants irrigated with 50 mM NaCl and treated with nano silica (50 mL L^{-1}).
T4: plants irrigated with 50 mM NaCl and treated with melatonin (75 μM).
T5: plants irrigated with 50 mM NaCl and treated with nano silica (50 mL L^{-1}) + melatonin (75 μM).
T6: plants irrigated with 100 mM NaCl.
T7: plants irrigated with 100 mM NaCl and treated with nano silica (50 mL L^{-1}).
T8: plants irrigated with 100 mM NaCl and treated with melatonin (75 μM).
T9: plants irrigated with 100 mM NaCl and treated with nano silica (50 mL L^{-1}) + melatonin (75 μM).

Furthermore, the POX activity considerably decreased (24.5% and 26%) during both seasons, respectively, compared to the stressed untreated plants under the high salinity level. Our results indicated that the total phenolic compounds and proline were decreased significantly because of their foliar application with the combination of nano silica + melatonin followed by melatonin and then nano silica. The combination treatment led to a significant reduction in the total phenolic compounds (22.3% and 24.2%) and in the proline content (27.6% and 25%) under the low salinity concentration during both seasons, respectively; however, under the high salinity level, the decrease in the total phenolic compounds (19.2% and 21%) and in the proline content (10.5% and 18%) was lower compared with the stressed plants that were not receiving treatments during both seasons, respectively.

4. Discussion

Salinity is one of the most detrimental abiotic factors that harm all growth stages in many plants [14,19,45,54]. The detrimental impacts of salinity on pea plants were observed during two growing seasons, and we found that the salinity stress caused remarkable decreases in plant height, leaves number, plant dry weight and flower number plant^{-1} (Figure 1). This adverse effect of salinity may be attributed to the reduction in the water availability and toxicity of sodium chloride [69], as well as the osmotic stress induced by the influx of sodium ions, decrease in cell division and elongation, which results in a decreased number of leaves, plant height, plant dry weight and number of flowers. Similar results were recorded by some researchers when exploring several plants such as soybean [70], pepper [71], wheat [72], pea [73] and rice [74]. Conversely, the application of nano silica and melatonin individually or in combination mitigated the negative impact of salinity on the pea plant and significantly augmented the plant dry weight, leaves number and number of flowers (Figure 1). The coapplication of nano silica + melatonin gave the best results during both seasons when the plants were under a low salinity level. This helpful impact may be attributed to the effect of nano silica on the interaction between biological cells and the pathway of cellular uptake [75,76]. This result could be due to the important role of melatonin in improving the plant status during increased water availability and uptake under salinity conditions. The similar impact of melatonin was recorded in many plants [47,53].

According to the growth and yield data, such as the data on the pods number, seeds number, weight of 100 green seeds and protein concentration, it was observed that the most harmful effect was recorded when the plants were under the high salinity level (100 mM NaCl). The decrease in these characteristics may be attributed to the accumulation of ROS, which cause oxidative stress and negatively affect the metabolic activities and stability of cell membranes (Figure 2). These results are in agreement with the results obtained by Abdel-Latef and Chaoxing [77] in pepper plants and Ahmad et al. [78] in chickpea; they stated that salinity led to a significant reduction in the plant growth. This reduction in the growth may be attributed to the negative effect of salinity on water accessibility, ion uptake and ion balance in plant cells [79]. Conversely, the application of nano silica and melatonin led to a decrease in the harmful effects of salinity on pea plants and improved the pods number and weight of 100 green seeds as well as the protein content in the stressed pea plants during both seasons. The best results were documented when the plants were under the low salinity level (50 mM NaCl) and were treated with the combination of nano silica+melatonin; these results could have been due to the fact that melatonin plays an active role as a growth regulator in pea plants and increases their tolerance against salinity stress via improving mineral nutrition, water uptake and scavenging ROS. It was also reported that melatonin acts as a growth regulator in plants and plays an effective role in the biosynthesis regulation of GA4 and ABA [80,81]. Our results are in agreement with previous studies on rice plants [82,83].

The results in Figure 3 clearly showed that the chlorophyll concentration, maximum quantum efficiency of PS II and RWC were negatively affected under both salinity levels during both seasons. These results may have been due to the adverse impact of salinity on the cell cytoplasm, decrease in the enzymes activity, dehydration of the cytoplasm and chlorophyll degradation as well as the decreased cell turbidity and photosynthetic rate. Our findings are consistent with the results obtained by Damodaran et al. [84], who reported that salinity stress resulted in negative changes in membrane stability and chlorophyll concentration in mustard plants. Additionally, El-Flaah et al. [19] reported that salinity stress led to reduced chlorophyll concentrations in faba bean plants.

A significant increase in the chlorophyll concentrations, Fv/Fm and RWC was recorded in the stressed pea plants treated with nano silica and melatonin during the two seasons. The combination treatment gave the best results followed by the other treatments. The coapplication of nano silica and melatonin can decrease the detrimental effects of salt stress on pea productivity through the activation of the antioxidant defense system, regulation of auxins synthesis and stimulation of root growth. Additionally, the foliar application of SiNPs improved the water uptake and stomatal conductance, resulting in an improved photosynthetic capacity and decreased leaf abscission. Mukarram et al. [85] reported that SiNPs have a protective effect against stress conditions by improving the source–sink potential, water status, enzymatic profile and ROS production. The positive effect of melatonin was observed in cucumber plants; for example, the chlorophyll and carotenoids concentrations as well as Fv/Fm were significantly increased [86]. Additionally, in *Phaseolus vulgaris* L., melatonin led to an increased photosynthetic quantum yield (φPSII) and chlorophyll concentration [87]. The helpful impact of melatonin may be due to its protective role on the chloroplasts ultrastructure, resulting in the increase in chlorophyll concentrations under salinity conditions [88] and the increased synthesis of chlorophyll and photosystem II activity [89].

EL%, MDA, $O_2\cdot-$ and H_2O_2 concentration were significantly increased in the stressed pea plants at both salinity levels during the two seasons compared to the control (Figure 4). The increment in these characteristics as stress signals could be attributed to the damaging impact of salinity on the cell division, decrease in water availability and overproduction of hydrogen peroxide and superoxide, which negatively affected the membrane integrity and resulted in high levels of lipid peroxidation. The increase in the EL%, MDA, $O_2\cdot-$ and H_2O_2 concentration was recorded under salinity and other stress factors [21,90–94]. Our results indicated that the foliar application of nano silica and melatonin individually or in combination led to decreases in the levels of EL%, MDA, superoxide and H_2O_2 concentrations in the pea plants under both salinity levels, and the best effect was recorded with the combination treatment was applied under a low salinity level. These results might be attributed to the role that nano silica and melatonin play in protecting plants against oxidative stress by ROS scavenging because of the activation of antioxidant enzymes. In agreement with our findings, the application of melatonin decreased H_2O_2, $O_2\cdot-$, MDA and EL% in faba bean [19], *Phaseolus vulgaris* L. [87] and barley [95] under salinity conditions.

Antioxidant components such as the CAT, SOD, POX, total phenolic compounds and proline content were significantly augmented as a defense mechanism against the salinity stress compared to the control. As one of the important approaches against stress, plants accumulate antioxidant components to maintain the membrane stability and protect cells against the toxic effects of salinity stress. These components such as CAT, SOD, POX, total phenolic compounds and proline are essential in plant tolerance against various stresses [96–101]. In the same way, Abdelaal et al. [31] and Ferchichi et al. [102] stated that proline plays pivotal roles in the regulation of gene expression, redox homeostasis, cell osmotic adjustments and stabilization of proteins. The application of nano silica and melatonin individually or in combination led to an increased membrane stability, which induced the plant defenses by adjusting the enzymes activity, total phenolic compounds and proline content, which scavenged the ROS and protected the cells of the pea plants from oxidative damage under salt stress compared to the stressed pea plants that did not receive treatments. The helpful effect of SNPs on enzyme regulation was also recorded by Mukarram et al. [85], El-Shawa et al. [103] and Khedr et al. [43]. SiNPs can regulate CAT, APX and SOD activities and the AsA-GSH cycle, which improves the defense system. Generally, our results showed that the coapplication of nano silica and melatonin led to improved morphological and physiological characteristics of the stressed pea plants and increased the yield characteristics under salinity conditions.

5. Conclusions

In general, under salinity conditions, the growth and yield characteristics as well as the physiobiochemical characteristics such as the plant dry weight, number of flowers plant^{-1}, number of pods plant^{-1}, protein concentration, chlorophyll a, chlorophyll b and RWC were significantly decreased in the stressed pea plants. However, the foliar application of nano silica (50 mL L^{-1}) and melatonin (75 µM) caused a significant increase in these characteristics in the stressed pea plants during both seasons compared with the stressed untreated plants. The electrolyte leakage (EL%), malondialdehyde (MDA), hydrogen peroxide, super oxide and antioxidant components (catalase, superoxide dismutase, peroxidase and total phenolic compounds) were significantly increased under the salinity conditions compared with the control plants. On the other hand, the combination treatment caused a significant decrease in the EL%, MDA, $O_2\cdot-$ and H_2O_2 and adjusted the upregulation of the antioxidant enzymes, proline and total phenolic compounds in the stressed pea plants compared with the stressed pea plants that were not receiving treatments. Overall, it can be concluded that that the coapplication of nano silica (50 mL L^{-1}) + melatonin (75 µM) plays an effective role in decreasing the harmful impacts of salt stress on pea plants and improve the yield characteristics under salt conditions.

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