



Effects of sodium nitroprusside (SNP) application on oxidative damage in pepper (*Capsicum annuum* L.) plants under heat stress

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Isı stresi altındaki biber (*Capsicum annuum* L.) bitkilerinde sodyum nitroprussid (SNP) uygulamasının oksidatif hasar üzerine etkileri

Abstract: Heat stress is a major abiotic factor limiting plant growth and productivity. Sodium nitroprusside (SNP), a nitric oxide (NO) donor, plays an important role in regulating plant responses to abiotic stress. This study investigated the effects of SNP application on growth parameters, total chlorophyll content, and oxidative stress indicators in *Capsicum annuum* L. seedlings grown under high temperature conditions. Plants were grown at 25 °C (control) and 38 °C (heat stress) and treated with 50 and 100 µM SNP. The results showed that heat stress reduced growth parameters and total chlorophyll content, while increasing lipid peroxidation (MDA) and hydrogen peroxide (H₂O₂) accumulation. Heat stress enhanced catalase (CAT) activity. Under heat stress, 50 µM SNP alleviated oxidative damage by reducing MDA and H₂O₂ levels, whereas 100 µM SNP increased these parameters. In contrast, SNP application suppressed CAT activity in a dose-dependent manner. These findings indicate that SNP induces dose-dependent physiological and biochemical responses in *Capsicum annuum* L. seedlings under heat stress and that appropriate SNP doses may contribute to mitigating heat stress-induced damage. The results may also support the development of dose based stress management strategies for sustainable crop production under high temperature conditions.

Key words: Heat stress, *Capsicum annuum*, sodium nitroprusside (SNP), oxidative stress, growth parameters

Özet: Isı stresi, tarımsal üretimde bitki büyümesini ve verimliliğini sınırlayan önemli bir abiyotik stres faktörüdür. Nitrik oksit (NO) donörü olan sodyum nitroprussit (SNP), bitkilerde abiyotik stres yanıtlarının düzenlenmesinde rol oynayan bir bileşiktir. Bu çalışmada, yüksek sıcaklık koşullarında yetiştirilen *Capsicum annuum* L. fidelerinde SNP uygulamasının büyüme parametreleri, toplam klorofil içeriği ve oksidatif stres göstergeleri üzerindeki etkileri araştırılmıştır. Bitkiler 25 °C (kontrol) ve 38 °C (ısı stresi) koşullarında yetiştirilmiş ve 50 ile 100 µM SNP uygulanmıştır. Bulgular, ısı stresinin büyüme parametreleri ve toplam klorofil içeriğini azalttığını; lipid peroksidasyonu (MDA) ve hidrojen peroksit (H₂O₂) birikimini ise artırdığını göstermiştir. Isı stresinin katalaz (CAT) aktivitesini artırdığı belirlenmiştir. Isı stresi koşullarında 50 µM SNP uygulaması MDA ve H₂O₂ düzeylerini azaltarak oksidatif hasarı hafifletirken, 100 µM SNP bu parametreleri artırmıştır. SNP uygulamasının CAT aktivitesini dozuna bağlı olarak baskıladığı belirlenmiştir. Bu sonuçlar, SNP'nin ısı stresi altında *Capsicum annuum* L. fidelerinde doza bağlı fizyolojik ve biyokimyasal yanıtlar oluşturduğunu ve uygun dozlarda uygulandığında ısı stresinin olumsuz etkilerinin hafifletilmesine katkı sağlayabileceğini göstermektedir. Elde edilen verilerin, sıcaklık stresi koşullarında bitkisel üretime yönelik uygun doz temelli stres yönetimi yaklaşımlarının ve sürdürülebilir tarımsal stratejilerin geliştirilmesine katkı sağlayabileceği değerlendirilmektedir.

Anahtar Kelimeler: Isı stresi, *Capsicum annuum*, sodyum nitroprussid (SNP), oksidatif stres, büyüme parametreleri

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1. Introduction

Pepper (*Capsicum annuum* L.), which is widely cultivated across the globe, is a strategic species with high economic value for both fresh consumption and industrial processing (Perry et al., 2007; Kim et al., 2014; Qin et al., 2014). Ranking as the second most produced species within the Solanaceae family after the tomato, pepper holds a critical share in the global vegetable trade and food industry (Gong et al., 2022). Rich in nutritional content including vitamins C and E, carotenoids, and flavonoids, this plant also serves as a primary raw material for the pharmaceutical and cosmetic industries due to its secondary metabolites, such as capsaicin and capsanthin (Kim et al., 2014; Venier et al., 2015; Saleh et al., 2018). However, the sustainability of pepper cultivation is severely threatened by abiotic stress factors, particularly heat stress, drought, and salinity, which

are intensifying due to global climate change (Schwarz et al., 2010; Bhat et al., 2019).

Temperature increases exceeding the critical threshold of 30 °C for most crop plants trigger a series of physiological and metabolic transformations that suppress plant growth and final yield (Sun et al., 2019; Ito et al., 2020). The ideal growth range for pepper is 25–30 °C; deviations from these thermal limits significantly disrupt generative and early development stages (Venkatesh et al., 2020). Heat stress limits germination capacity, slows root shoot development, and dramatically reduces fruit set rates due to the loss of pollen viability (Saha et al., 2010; Thuy & Kenji, 2015; Rosmaina et al., 2022). Furthermore, the structural denaturation of Rubisco the key enzyme of photosynthesis at high temperatures and the inhibition of Photosystem II (PSII) activity minimize carbon fixation efficiency and

photochemical yield, leading to significant losses in plant biomass (Awasthi et al., 2014; Li et al., 2015; Zhang et al., 2020).

Environmental stress factors disrupt the delicate balance of reactive oxygen species (ROS) in plants, causing an oxidative burst. Described in the literature as a double-edged sword, ROS act as signaling molecules at low concentrations (Davletova et al., 2005); however, excessive accumulation leads to cellular membrane lipid peroxidation, protein degradation, and the destruction of vital macromolecules, thereby triggering cell death (Qin et al., 2014; Bazzaz et al., 2020; Zafar et al., 2020). To suppress this oxidative damage, plants have evolved a sophisticated antioxidant defense system consisting of enzymatic components such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and peroxidase (POD) (Shigeoka et al., 2002; Faize et al., 2011; Kotapati et al., 2017). The effectiveness of this system varies depending on the developmental stage and the heat stress severity levels (Dumanović et al., 2021; Ahmad et al., 2022).

In recent years, intensive research has been conducted on the use of endogenous gasotransmitters to strengthen the plant defense system against abiotic stresses (Yao et al., 2019). Nitric oxide (NO), a prominent molecule among these, is a key signaling transmitter that regulates all stages of the plant life cycle and modulates stress responses (El-Esawi, 2017; Kaya et al., 2019; Nabi et al., 2019; Zhou et al., 2024; Zargar et al., 2025). Sodium nitroprusside (SNP), an exogenous NO donor, easily crosses cellular membranes due to its low molecular weight and hydrophobic nature, activating defense mechanisms through two primary pathways (Kovacs et al., 2015). SNP acts directly as an antioxidant to increase ROS-scavenging capacity and indirectly as a signaling molecule that triggers defense genes (Chavoushi et al., 2019; Aras, 2021). Literature reports that SNP application prevents chlorophyll loss, stabilizes osmotic regulators like proline to maintain turgor pressure, and preserves membrane integrity, thereby alleviating the destructive effects of heat stress (Yu et al., 2013; Kharbech et al., 2020; Esim et al., 2024). Furthermore, foliar application of SNP has been shown to enhance antioxidant enzyme activities (SOD, CAT, POD) and reduce ROS accumulation, protecting cellular structures under heat stress (Kaya et al., 2019; Zhou et al., 2024; Zargar et al., 2025).

Given the projections of meteorological models indicating that extreme temperature events will continue to increase in the coming years, developing strategies to enhance heat stress tolerance in pepper production has become imperative (Fischer et al., 2021). This study investigates the effects of SNP application on the growth performance, antioxidant capacity, and yield parameters of pepper seedlings under both optimal and heat stress conditions. By evaluating the protective role of SNP in pepper plants, this research aims to fill a gap in the literature and test the hypothesis that exogenous SNP application can mitigate physiological impairments caused by heat stress and improve plant productivity.

2. Materials and Method

2.1. Plant germination and experimental design

In this study, commercially available sweet pepper (*Capsicum annuum* L.) seeds were used as plant material.

Prior to sowing, the seeds were surface-sterilized using a standard procedure to minimize microbial contamination. The sterilized seeds were sown in seedling trays filled with peat-based substrate and placed in a controlled climate cabinet. Seedlings were grown under controlled environmental conditions at 25/21 °C (day/night), 55% relative humidity, and a 16/8 h light/dark photoperiod. Illumination was provided by cabinet lamps with a light intensity of approximately 9000 lux. During the 37-day growth period, all plants were irrigated exclusively with distilled water to ensure uniform development. Fig. 1 shows images of seed sowing, seedling development at 15 and 30 days, and foliar SNP application. Following the completion of the growth period, foliar applications were initiated. Sodium nitroprusside (SNP), used as a nitric oxide donor, was applied at concentrations of 50 and 100 µM. The selected SNP concentrations were determined based on preliminary experiments in our laboratory, which indicated measurable physiological responses in sweet pepper seedlings without toxic effects, and are also supported by previous literature reporting effective use of similar concentrations under abiotic stress conditions in plants (Li et al., 2013; Iqbal et al., 2022). The solutions were freshly prepared and applied to the leaves as a foliar spray once daily for three consecutive days at 25 °C. Control plants were sprayed with distilled water only. After the third application, samples from control plants maintained at 25 °C were collected. Details of the experimental design and treatment groups are presented in Table 1. Subsequently, the remaining seedlings were subjected to heat stress by transferring the trays to a growth chamber set at 38 °C for 24 h. At the end of the heat stress treatment, plant samples were harvested for further analyses. Growth parameters, including seedling and root length, leaf and root fresh and

Table 1. Experimental design and treatment groups applied to sweet pepper (*Capsicum annuum* L.) seedlings

Group	SNP (µM)	25 °C	38 °C (24h)
C25	-	+	-
HS	-	-	+
SNP50+HS	50	-	+
SNP100+HS	100	-	+

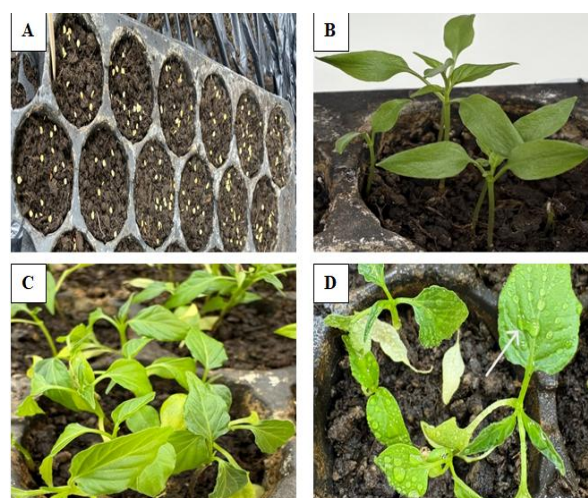


Figure 1. Experimental timeline and representative images of plant growth and treatments. (A) Seed sowing in seedling trays; (B) seedling growth at 15 days; (C) seedling growth at 30 days; (D) foliar application of SNP showing droplets on leaf surfaces

dry weights, were measured, and total chlorophyll content was determined. Additional plant tissues were immediately frozen in liquid nitrogen and stored at -80°C for the analysis of malondialdehyde (MDA), hydrogen peroxide (H_2O_2), and catalase (CAT) activity.

2.2. Assessment of plant growth traits

Seedling and root lengths were determined using randomly selected plants from each treatment group. Seedling length was measured from the soil surface to the shoot apex, while root length was measured from the soil surface to the root tip, using a ruler and recorded in centimeters (cm). The same plants were subsequently used for fresh weight determination, with leaf and root tissues weighed separately using a precision balance and expressed in grams. For dry weight analysis, the samples were oven-dried at 72°C for 48 h until a constant weight was achieved and then reweighed using a precision balance.

2.3. Determination of total chlorophyll content

Total chlorophyll content was determined according to the method described by Lichtenthaler and Wellburn (1983). Fresh leaf tissue (0.2 g) was homogenized in 15 mL of cold 100% acetone. The homogenate was centrifuged at $5000 \times g$ for 10 min, and the supernatant was collected. Absorbance values of the extracts were measured spectrophotometrically at 645, and 662 nm. Chlorophyll a (Chl a), chlorophyll b (Chl b), and total chlorophyll (Chl a + Chl b) contents were calculated using standard equations and expressed as mg g^{-1} fresh weight.

Total chlorophyll content was calculated using the following formula:

$$\text{Total chlorophyll (mg g}^{-1}\text{ FW)} = [(20.2 \times (A_{645}) + 8.02 \times (A_{662})) \times (V/1000 \times W)]$$

where A is absorbance at the specified wavelength, V is the volume of the extract (mL), and W is the fresh weight of the sample (g).

2.4. Malondialdehyde (MDA) content

Lipid peroxidation was assessed by measuring MDA levels. Approximately 0.2 g of plant tissue was homogenized in liquid nitrogen, and 2 mL of 5% trichloroacetic acid (TCA) was added to the homogenate. The mixture was centrifuged at $10,000 \times g$ for 15 min at 4°C . Then, 500 μL of the supernatant was mixed with 500 μL of 1% thiobarbituric acid (TBA) solution. The reaction mixture was incubated in a water bath at 95°C for 30 min and immediately cooled on ice to room temperature. From the cooled tubes, 200 μL of the supernatant was taken, and absorbance was measured at 532 and 600 nm using a microplate reader. MDA content was calculated using the following formula (Ananieva et al., 2004):

$$\text{MDA equivalents (nmol g}^{-1}\text{)} = [(A_{532} - A_{600}) - [(A_{440} - A_{600}) \times (0.0571)]] / 157000 \times 10^6$$

2.5. Hydrogen peroxide (H_2O_2) content

To determine H_2O_2 levels, 0.5 g of plant tissue was homogenized in 10 mL cold acetone and centrifuged at $12,000 \times g$ for 15 min. Then, 1.5 mL of the supernatant was mixed with 0.15 mL of 5% $\text{Ti}(\text{SO}_4)_2$ and 0.3 mL of 19% ammonium hydroxide (NH_4OH). After the formation of a precipitate, the mixture was centrifuged again at $10,000 \times g$ for 10 min. The resulting pellet was dissolved in 3 mL of

2 M H_2SO_4 , and the absorbance was recorded at 415 nm (Hu et al., 2005).

2.6. Catalase (CAT) activity

CAT activity was measured according to Nakano and Asada (1981) and Gong et al. (2001). Approximately 0.5 g of plant tissue was homogenized in 5 mL of cold extraction buffer containing 0.1 M KH_2PO_4 (pH 7.0), 1 mM EDTA, and 1% PVP. The homogenate was centrifuged at $15,000 \times g$ for 15 min at 4°C . The supernatant was collected, and the pellet discarded. For CAT assay, 25 μL of enzyme extract was added to a cuvette containing 1.75 mL of 103 mM KH_2PO_4 buffer and 1.5 mL of 40 mM H_2O_2 . The decomposition of H_2O_2 was monitored spectrophotometrically at 240 nm for 3 min, with absorbance readings taken every 0.5 min.

2.7. Statistical analysis

For each treatment, three independent samples (replicates) were collected, and three measurements were taken per sample, resulting in a total of nine observations per treatment. The mean values of these measurements were calculated and used for further analysis. Statistical analysis was performed using SPSS version 27 (IBM Corp., 2020). One-way analysis of variance (ANOVA) was conducted to assess differences among groups, and Duncan's multiple range test was applied to identify statistically significant differences at $p < 0.05$.

3. Results

3.1. Effects of heat stress and SNP on plant growth

The morphological appearance of representative plants at the end of the experiment illustrated the effects of heat stress and SNP treatments on plant growth (Fig. 2). Heat stress significantly impaired both seedling and root development. Compared to control conditions (25°C), exposure to heat stress (38°C) resulted in an approximately 20.8% decrease in seedling length ($p < 0.05$). Under heat stress conditions, the application of 50 μM SNP significantly alleviated this reduction, leading to an approximately 19.8% increase in seedling length compared to the heat stress control (Fig. 3). This treatment was grouped in the same statistical category (a) as the control ($p < 0.05$). In contrast, the 100 μM SNP + heat stress treatment showed no significant difference compared to the heat stress group, sharing the same statistical letter (b) ($p > 0.05$).



Figure 2. Seedling phenotypes after treatments. (A) Control at 25°C ; (B) Heat stress at 38°C ; (C) 50 μM SNP + heat stress; (D) 100 μM SNP + heat stress. The scale bar represents 5 cm.

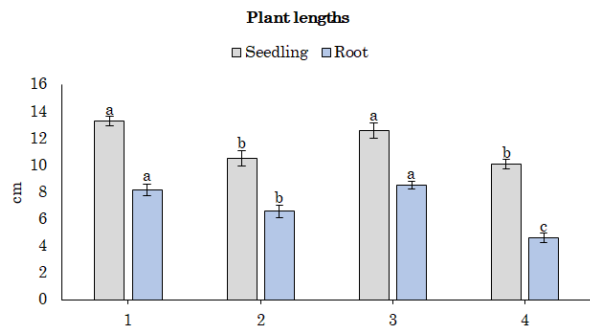


Figure 3. Seedling and root lengths. (1) 25 °C control; (2) 38 °C heat stress; (3) 50 µM SNP + heat stress; (4) 100 µM SNP + heat stress. Data are presented as mean $\pm$ SD (n=9). Different letters indicate significant differences according to Duncan's multiple range test ($p<0.05$)

Similarly, root length was negatively affected by heat stress. Compared to the 25 °C control conditions, root length decreased by approximately 19.4% under 38 °C heat stress, representing a statistically significant difference ($p<0.05$). Under heat stress conditions, the application of 50 µM SNP exerted a pronounced ameliorative effect on root development, resulting in an approximate 29.1% increase compared with the heat stress control (Fig. 3). This treatment shared the same statistical grouping (a) as the control group, indicating no significant difference between them, while it differed significantly from the heat stress group ($p<0.05$). Conversely, the 100 µM SNP + heat treatment led to a substantial reduction in root length compared with all other treatments, placing this group in a distinct statistical category (c) ($p<0.05$).

3.2. Effects of heat stress and SNP on root and leaf fresh weight

Heat stress exerted pronounced inhibitory effects on root and leaf fresh weights. Compared to control conditions (25 °C), exposure to heat stress (38 °C) resulted in an approximately 60.4% decrease in root fresh weight, representing a statistically significant difference ($p<0.05$). Under heat stress conditions, the application of 50 µM SNP significantly alleviated this reduction, leading to an approximately 123% increase in root fresh weight compared with the heat stress control (Fig. 4). This treatment shared the same statistical letter (a) as the control group, indicating no significant difference between them, while being significantly different from the heat stress group ($p<0.05$). In contrast, the 100 µM SNP + heat stress treatment showed no significant difference compared to the heat stress group, sharing the same statistical category (b) ($p>0.05$).

Similarly, leaf fresh weight was adversely affected by heat stress. Compared to control conditions (25 °C), leaf fresh weight decreased by approximately 33.3% under 38 °C heat stress, representing a statistically significant difference ($p<0.05$). Under heat stress conditions, the application of 50 µM SNP significantly alleviated this reduction, leading to an approximately 23.4% increase in leaf fresh weight compared with the heat stress control (Fig. 4). This increase was statistically significant, and the 50 µM SNP + heat stress treatment was placed in a distinct statistical group (b) compared to both the control and heat stress groups ($p<0.05$). In contrast, the 100 µM SNP + heat stress treatment exhibited a marked reduction in leaf biomass,

differing significantly from all other treatments (d) ($p<0.05$).

3.3. Effects of heat stress and SNP on root and leaf dry weight

With respect to root dry weight, exposure to 38 °C heat stress resulted in an approximately 21.3% reduction compared with the 25 °C control, representing a statistically significant difference ($p<0.05$). Under heat stress conditions, the 50 µM SNP + heat treatment led to an approximately 8.1% increase in root dry weight compared with the 38 °C control group (Fig. 5). However, this treatment shared the same statistical letter (b) as the heat stress group, indicating no significant difference ($p>0.05$). Similarly, the 100 µM SNP + heat treatment also belonged to the same statistical group (b) as the 38 °C heat stress control ($p>0.05$).

In terms of leaf dry weight, 38 °C heat stress caused a pronounced reduction of approximately 38.5% compared with the 25 °C control ($p<0.05$). Under heat stress conditions, the application of 50 µM SNP significantly increased leaf dry weight by approximately 70% relative to the 38 °C heat stress control (Fig. 5). This treatment shared the same statistical grouping letter (a) as the control group, indicating a full recovery of leaf biomass ($p<0.05$). In contrast, leaf dry weight in the 100 µM SNP + heat treatment remained at a level similar to that of the 38 °C heat stress control, sharing the same statistical category (b) ($p>0.05$).

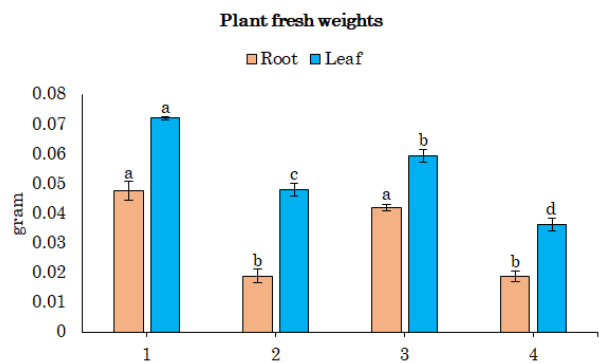


Figure 4. Fresh weights of roots and leaves. (1) 25 °C control; (2) 38 °C heat stress; (3) 50 µM SNP + heat stress; (4) 100 µM SNP + heat stress. Data are presented as mean $\pm$ SD (n=9). Different letters indicate significant differences according to Duncan's multiple range test ($p<0.05$)

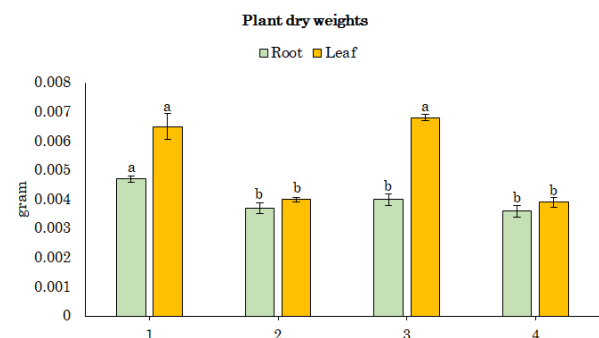


Figure 5. Dry weights of roots and leaves. (1) 25 °C control; (2) 38 °C heat stress; (3) 50 µM SNP + heat stress; (4) 100 µM SNP + heat stress. Data are presented as mean $\pm$ SD (n=9). Different letters indicate significant differences according to Duncan's multiple range test ($p<0.05$)

3.4. Effects of heat stress and SNP treatments on total chlorophyll content

In the 38 °C heat stress control, total chlorophyll content decreased by approximately 6.5% compared with the 25 °C control, representing a statistically significant difference ($p < 0.05$). Under heat stress conditions, the 50 μM SNP + heat treatment markedly increased total chlorophyll content by approximately 130% compared with the 38 °C heat stress control (Fig. 6). This value was also significantly higher than that of the 25 °C control group. This treatment differed significantly from all other treatments and was assigned to a separate statistical group (letter a) ($p < 0.05$). In contrast, the 100 μM SNP + heat treatment exhibited the lowest chlorophyll content, remaining significantly below both the 25 °C and 38 °C control groups, and forming a distinct statistical group (letter d) ($p < 0.05$).

3.5. Catalase (CAT) activity

Catalase (CAT) activity increased by approximately 43% in the 38 °C heat stress control compared with the 25 °C control, representing a statistically significant difference ($p < 0.05$). Under heat stress conditions, the 50 μM SNP + heat treatment significantly suppressed this response, leading to an approximate 87% decrease in CAT activity compared with the 38 °C heat stress control (Fig. 7). This treatment exhibited the lowest CAT activity among all treatments and was assigned to a statistically distinct group (letter d), differing significantly from all other groups ($p < 0.05$). In contrast, CAT activity in the 100 μM SNP + heat treatment was approximately 65% lower than that of

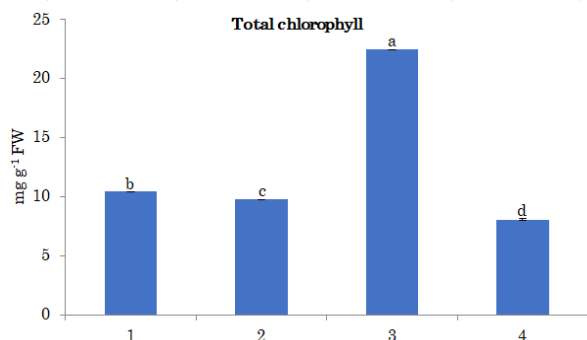


Figure 6. Total chlorophyll content. (1) 25 °C control; (2) 38 °C heat stress; (3) 50 μM SNP + heat stress; (4) 100 μM SNP + heat stress. Data are presented as mean $\pm$ SD (n=9). Different letters indicate significant differences according to Duncan's multiple range test ($p < 0.05$)

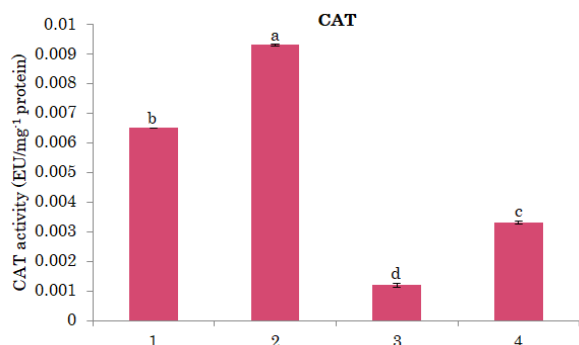


Figure 7. Catalase (CAT) activity. (1) 25 °C control; (2) 38 °C heat stress; (3) 50 μM SNP + heat stress; (4) 100 μM SNP + heat stress. Data are presented as mean $\pm$ SD (n=9). Different letters indicate significant differences according to Duncan's multiple range test ($p < 0.05$)

the 38 °C control, yet remained higher than the value observed in the 50 μM SNP + heat treatment. This treatment also formed a separate statistical group (letter c), differing significantly from both the control and the 50 μM SNP + heat groups ($p < 0.05$).

3.6. Lipid peroxidation (MDA content)

Malondialdehyde (MDA) content increased by approximately 10% in the 38 °C heat stress control compared with the 25 °C control. However, both the control and heat stress groups shared the same statistical letter (b), indicating that this numerical increase was not statistically significant ($p > 0.05$). Under heat stress conditions, the application of 50 μM SNP significantly reduced MDA content by approximately 27% compared with the 38 °C heat stress control (Fig. 8). This value was also lower than that of the 25 °C control group, and the treatment formed a statistically distinct group (letter c) ($p < 0.05$). In contrast, MDA content in the 100 μM SNP + heat treatment was approximately 59% higher than in the 38 °C control and 75% higher than in the 25 °C control. This treatment exhibited the highest MDA content among all treatments and was assigned to a separate statistical group (letter a), differing significantly from all other groups ($p < 0.05$).

3.7. Hydrogen peroxide (H₂O₂) accumulation

In the 38 °C heat stress control, H₂O₂ levels increased by approximately 54% compared with the 25 °C control, representing a statistically significant difference ($p < 0.05$). Under heat stress conditions, H₂O₂ content in the 50 μM SNP + heat treatment showed a slight numerical reduction compared with the 38 °C heat stress control (Fig. 9).



Figure 8. Malondialdehyde (MDA) content. (1) 25 °C control; (2) 38 °C heat stress; (3) 50 μM SNP + heat stress; (4) 100 μM SNP + heat stress. Data are presented as mean $\pm$ SD (n=9). Different letters indicate significant differences according to Duncan's multiple range test ($p < 0.05$)

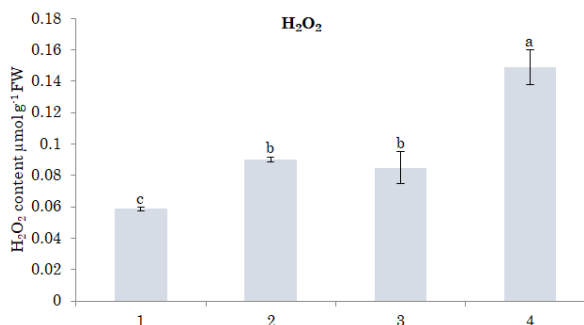


Figure 9. Hydrogen peroxide (H₂O₂) content. (1) 25 °C control; (2) 38 °C heat stress; (3) 50 μM SNP + heat stress; (4) 100 μM SNP + heat stress. Data are presented as mean $\pm$ SD (n=9). Different letters indicate significant differences according to Duncan's multiple range test ($p < 0.05$)

However, this treatment shared the same statistical letter (b) as the heat stress group, indicating that the difference was not statistically significant ($p>0.05$). In contrast, H_2O_2 content in the 100 μM SNP + heat treatment increased markedly, representing an approximate 65% increase compared with the 38 °C control and a 153% increase relative to the 25 °C control. This treatment exhibited the highest H_2O_2 content among all treatments and formed a separate statistical group (letter a) ($p<0.05$).

4. Discussions

The findings of the present study regarding the reduction in growth parameters are consistent with previous reports indicating that high temperatures restrict plant growth by suppressing cell division and elongation. In a 1-month sugarcane seedling study, Wahid (2007) applied 40°C heat stress and measured growth and metabolite changes every 12 hours, showing both immediate and delayed effects on growth and metabolites. In line with these results, Zhou et al. (2024) reported that exogenous SNP application mitigated high-temperature stress in bell pepper seedlings, maintaining normal growth by regulating amino acid metabolites and enhancing antioxidant defense, thereby preserving both root and shoot development under stress conditions. These results suggest that the impact of SNP on plant growth is concentration dependent; lower concentrations may promote growth, whereas higher concentrations may exert inhibitory effects. In agreement with this interpretation, Zhang et al. (2023) reported that SNP application significantly increased plant height in wheat under heat stress conditions, while Uchida et al. (2002) demonstrated that high SNP concentrations reduced growth rates. Since root tissues are directly exposed to environmental stress factors, root development is generally more sensitive to stress conditions than shoot growth. The results observed here are consistent with the findings reported by Iqbal et al. (2019), who demonstrated that heat stress reduced both root and shoot length in wheat. In addition, Nayanakantha et al. (2014) reported that SNP application promoted root and shoot growth, particularly enhancing root elongation; this observation is in alignment with the positive effects of low dose SNP application observed in our study. Overall, the data indicate that heat stress suppresses seedling and root development, whereas SNP applied at an appropriate dose can partially alleviate these adverse effects and support plant growth. However, higher SNP concentrations appear to exert a growth limiting effect, which is particularly pronounced in root development.

Regarding biomass production, our study indicates a strong restrictive effect of high temperature on root biomass. These results suggest that while low dose SNP application exhibits a pronounced ameliorative effect, high SNP concentrations are ineffective in promoting root development under heat stress conditions. Previous studies have reported that extreme temperature conditions lead to marked reductions in both root and shoot biomass and suppress the root to shoot ratio (Koevoets et al., 2016; Huang et al., 2012). Similarly, Zhou et al. (2024) demonstrated that SNP treatment maintained root and shoot dry weights under high temperature stress in bell pepper seedlings, highlighting the protective role of exogenous NO in mitigating heat-induced reductions in biomass. This response indicates that severe thermal stress inhibits root growth, induces unfavorable morphological changes in root

system architecture, and affects belowground tissues more severely than aboveground parts. Moreover, root tissues have been reported to exhibit greater sensitivity to heat stress than shoots, resulting in impaired root growth and functional capacity, reduced water and nutrient uptake, and ultimately decreased overall plant productivity (Huang et al., 2012).

Furthermore, the observed reduction in leaf biomass in the 100 μM SNP + heat stress treatment indicates that high SNP doses may exert inhibitory effects on leaf development. These findings are consistent with the results of Taj et al. (2024), who reported that foliar application of SNP enhanced shoot fresh weight and promoted growth in different plant species at optimal concentrations. In addition, Hameed et al. (2012) 20 hour heat stress on wheat seedlings demonstrated that heat stress significantly reduced seedling fresh weight irrespective of genotype, highlighting the importance of stress duration for physiological responses. Overall, the present study indicates that heat stress markedly reduces root and leaf biomass, whereas SNP application at an appropriate dose can partially alleviate these adverse effects and support plant growth.

The results obtained in this study also indicate that high temperature stress suppresses dry matter accumulation in root and leaf tissues. Overall, these findings demonstrate that while heat stress negatively affects dry matter accumulation, low dose SNP application (50 μM) can effectively support and maintain dry matter levels, particularly in leaf tissues. However, higher SNP doses do not appear to support this ameliorative effect. Previous studies have widely reported that high temperature stress suppresses dry matter accumulation in plants. Ashraf and Hafeez (2004) demonstrated that shoot dry weight in maize significantly decreased under high temperature conditions (38/27 °C), highlighting species-specific differences in heat stress tolerance. Similarly, Jafari and Shahsavar (2022) reported that stress conditions reduced dry weight in *Citrus aurantifolia*, while application of 25 μM SNP partially alleviated this adverse effect by preserving dry matter accumulation. In addition, Taj et al. (2024) showed that foliar application of SNP markedly stimulated root development, with significant increases in root dry weight. These findings are consistent with the present study, demonstrating that 50 μM SNP exerts a protective effect on both root and leaf dry weight under heat stress conditions and indicating that SNP applied at an appropriate dose plays an effective role in maintaining dry matter accumulation in plant tissues.

In addition to growth parameters, our findings indicate that heat stress suppresses chlorophyll accumulation; however, SNP application at an appropriate dose (50 μM) strongly compensates for chlorophyll loss by preserving and even enhancing photosynthetic pigments. Conversely, a higher SNP dose (100 μM) fails to exert such a protective effect. It is well documented that photosynthetic pigments, particularly chlorophyll a and chlorophyll b, are highly sensitive to elevated temperatures, and that heat stress accelerates pigment degradation in leaf tissues, thereby limiting photosynthetic capacity (Awasthi et al., 2014). The remarkable 130% increase in chlorophyll content observed at 50 μM SNP which the reviewer noted as a particularly interesting finding suggests that NO does not merely prevent degradation but actively stimulates chlorophyll

biosynthesis. NO has been shown to enhance the efficiency of the chlorophyll biosynthetic pathway by upregulating the expression of key enzymes such as protochlorophyllide oxidoreductase and improving the bioavailability of essential micronutrients like iron (Fe), which is a critical cofactor for chlorophyll stability (Graziano et al., 2002; Graziano & Lamattina, 2005). In this context, Li et al. (2013) reported that SNP application increased chlorophyll content in *Zingiber officinale* leaves under heat stress, prevented pigment loss, and contributed to the recovery of chlorophyll fluorescence parameters. Consistently, Zargar et al. (2025) reported that foliar application of 50 μ M SNP enhanced chlorophyll levels and photosynthetic pigments in bell pepper under heat stress, supporting improved photosynthetic efficiency. Similarly, Esringu et al. (2016) demonstrated that stress conditions significantly reduced chlorophyll a, chlorophyll b, and carotenoid contents in lettuce seedlings, whereas SNP application reversed these reductions and enhanced pigment levels. However, the contrasting sharp decline observed in the 100 μ M SNP group highlights the "hormetic" or dose dependent nature of NO. While low concentrations act as protective signals, higher concentrations can trigger nitrosative stress, leading to the overproduction of ROS that can damage thylakoid membranes and accelerate chlorophyll bleaching (Siddiqui et al., 2011). Consistent with these reports, the pronounced increase in total chlorophyll content observed with 50 μ M SNP under heat stress in the present study highlights the effective role of SNP in protecting photosynthetic pigments and maintaining the stability of the photosynthetic system under high temperature conditions.

Moving to antioxidant defense mechanisms, the results of the present study show that heat stress stimulates CAT activity, thereby activating antioxidant defense mechanisms. However, SNP application suppresses this antioxidant response in a dose dependent manner. It is well established that plants activate their antioxidant defense systems under environmental stress conditions by inducing enzymes such as CAT, SOD, APX, and GR to counterbalance the accumulation of ROS (Ahmad et al., 2016). Accordingly, Esringu et al. (2016) reported that stress conditions activated the antioxidant defense system in lettuce leaves, resulting in a significant increase in CAT activity. Similarly, Yasir et al. (2021) observed an 84% increase in CAT activity in lentil plants under stress conditions compared with the control, whereas SNP supported treatments exhibited a 29% reduction in CAT activity. Furthermore, Song et al. (2006) reported that CAT activity increased in reed calli under heat stress, with a markedly greater increase observed in heat tolerant ecotypes. Additionally, Zhou et al. (2024) demonstrated that exogenous SNP application regulated CAT and other antioxidant enzymes in bell pepper seedlings under high-temperature stress, maintaining redox balance and supporting overall stress tolerance. In the present study, CAT activity increased by 43% under 38 °C heat stress compared with the 25 °C control. Under heat stress, 50 μ M SNP treatment significantly reduced CAT activity by 87%, whereas 100 μ M SNP resulted in a 65% decrease compared with the heat stress control. This significant reduction in CAT activity following SNP application can be further explained by the direct inhibitory effect of NO on the catalase enzyme. It is well-documented that NO can reversibly inhibit CAT by binding to its iron containing heme moiety, which clarifies the observed decline in

enzymatic activity despite the overall protective role of low dose SNP (Clark et al., 2000). Corresponding H_2O_2 levels increased by approximately 54% under heat stress compared with the control. While H_2O_2 levels showed a slight numerical decrease with 50 μ M SNP, they markedly increased by 65% and 153% (relative to heat control and 25 °C control, respectively) at 100 μ M SNP. The fact that H_2O_2 levels did not decrease significantly at 50 μ M SNP despite the reduction in MDA suggests that H_2O_2 may be acting as a secondary signaling molecule. In this context, the partial persistence of H_2O_2 might work in tandem with NO to trigger downstream stress-responsive genes rather than being completely scavenged (Larkindale & Knight, 2002). Similarly, MDA content showed a numerical increase under heat stress but was significantly reduced by 27% with 50 μ M SNP and drastically increased with 100 μ M SNP. These results indicate that 50 μ M SNP partially mitigates oxidative load, whereas 100 μ M SNP may exacerbate oxidative stress, reflecting dose dependent antioxidant and prooxidant effects. Taken together with these previous studies, the reduction in CAT activity observed following SNP application in the present study is likely not indicative of suppressed antioxidant defense, but rather reflects a mitigation of stress-induced oxidative load. By reducing the overall stress severity, SNP application appears to decrease the physiological requirement for high levels of CAT mediated detoxification.

Regarding oxidative damage, our findings suggest that heat stress promotes lipid peroxidation in cellular membranes, thereby enhancing oxidative damage. Although the 38 °C heat stress resulted in a numerical increase in MDA levels compared to the 25 °C control, this difference was not statistically significant ($p > 0.05$), leading both groups to share the same statistical letter (b). These results indicate that while 50 μ M SNP application markedly reduces oxidative damage (assigned to the statistically distinct group c), a higher SNP dose (100 μ M) may exert a stimulatory effect on cellular membrane damage (assigned to group a). The findings are consistent with previous reports in the literature. Sellami and Kooli (2026) demonstrated that high temperature conditions increase oxidative damage in tomato plants, which is directly associated with elevated malondialdehyde accumulation. Similarly, Kaya et al. (2019) reported that SNP application reduced MDA content in pepper plants exposed to cadmium and lead stresses, indicating improved membrane protection. Likewise, Li et al. (2013) reported that SNP pre-treatment significantly reduced MDA content in *Zea mays* seedlings exposed to severe heat stress, indicating improved membrane protection. Yang et al. (2011) also observed that SNP application substantially decreased lipid peroxidation in chrysanthemum plants under high temperature conditions. Conversely, Wassie et al. (2019) emphasized that high temperature treatments markedly increased MDA content in heat-sensitive alfalfa cultivars, reflecting severe disruption of membrane integrity. However, the sharp contrast between the 50 μ M and 100 μ M SNP doses in our study highlights the "double-edged sword" role of NO. While low concentrations (50 μ M) act as antioxidant signals that stabilize membranes, higher concentrations (100 μ M) can transition into a prooxidant state, triggering nitrosative stress and exacerbating lipid peroxidation (Siddiqui et al., 2011). Taken together, these studies support the conclusion that low dose SNP application limits heat stress induced oxidative damage,

whereas high SNP doses may abolish this protective effect and exacerbate lipid peroxidation.

Finally, the findings demonstrate that heat stress significantly increases H_2O_2 accumulation, clearly indicating an enhanced oxidative load and ROS accumulation in plant tissues. Heat stress disrupts cellular redox homeostasis by enhancing the production of ROS, including superoxide anion radicals ($O_2^{\bullet-}$), H_2O_2 , and NO, which induce oxidative damage to cell membranes and essential macromolecules (Khan et al., 2013; Awasthi et al., 2015; Santisree et al., 2015). Numerous studies have reported that SNP application reduces H_2O_2 accumulation under heat stress. For instance, Iqbal et al. (2022) and Sehar et al. (2023) showed that SNP application suppressed H_2O_2 accumulation and contributed to the maintenance of redox homeostasis. Zargar et al. (2025) also observed that 50 μ M SNP reduced H_2O_2 content in bell pepper leaves under 40°C stress, improving oxidative stress tolerance. However, in the present study, while 50 μ M SNP application significantly reduced MDA levels, H_2O_2 accumulation was only partially limited rather than fully eliminated. This persistence suggests that H_2O_2 may function as a secondary signaling molecule rather than being merely a toxic byproduct. In this context, H_2O_2 potentially works in tandem with NO to trigger downstream stress responsive genes and enhance the plant's adaptive defense mechanisms, which explains why it was not completely scavenged (Larkindale & Knight, 2002). On the other hand, the pronounced increase in H_2O_2 content at the higher SNP dose (100 μ M) suggests that SNP may exert dose dependent antioxidant or prooxidant effects. Excessive NO release may stimulate ROS production, thereby exacerbating oxidative damage. The observed changes in CAT activity together with variations in H_2O_2 and MDA contents indicate that oxidative balance is markedly affected under heat stress. The concomitant increase in CAT activity under heat stress can be interpreted as a defense response aimed at detoxifying excessive H_2O_2 . The reduction in H_2O_2 and MDA following 50 μ M SNP application suggests a partial alleviation of oxidative load, which concurrently decreases the requirement for antioxidant defense, as reflected by lower CAT activity. In contrast, the renewed increase in H_2O_2 and MDA levels at 100 μ M SNP implies a negative impact on antioxidant balance, where CAT activity appears insufficient to counteract the elevated oxidative burden.

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Overall, these findings demonstrate that oxidative responses and antioxidant enzyme activities under heat stress are differentially regulated in a dose dependent manner by SNP application.

5. Conclusions

In this study, exposure to 38 °C caused pronounced negative effects on growth and physiological performance in *Capsicum annuum* L. seedlings. Heat stress significantly reduced seedling and root lengths as well as fresh and dry weights, decreased total chlorophyll content, and intensified oxidative stress by increasing MDA and H_2O_2 levels. The concurrent increase in CAT activity indicates that the plants activated their antioxidant defense mechanisms in response to heat-induced oxidative stress. Under these conditions, the application of 50 μ M SNP promoted seedling and root development, enhanced fresh and dry matter accumulation, and increased total chlorophyll content. Moreover, the reduction in MDA levels and the limitation of H_2O_2 accumulation demonstrate that this concentration was effective in mitigating oxidative damage. In contrast, the application of 100 μ M SNP did not result in comparable improvements; instead, it exacerbated oxidative stress indicators. Overall, these findings demonstrate that heat stress tolerance in pepper seedlings is regulated in a dose-dependent manner by SNP application. The results suggest that using SNP at an appropriate concentration can significantly contribute to maintaining plant growth and physiological balance under high temperature conditions. In this context, controlled SNP application may be considered a complementary strategy for stress management in pepper cultivation. Nevertheless, further studies across different developmental stages and growth conditions are required to fully clarify the practical applicability of this approach in broader agricultural systems.

Conflict of Interest

The authors declare that there is no conflict of interest between them.

Authors' Contributions

All author contributed equally for analyses, writing and interpretation of the article. The authors read and approved the final version of the manuscript.

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