DIPLOMA THESIS

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Mitigation of Heat Stress by Foliar Application of Chitosan on Tomato Seedlings

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1. INTRODUCTION AND OBJECTIVES

The change of climatic condition due to global warming is one of the main challenges for the horticultural sector. The growth and development of plants could be adversely affected by abiotic stresses caused by environmental factors (Mittler, 2006). Among the abiotic stresses that follow this change, heat stress is one of the critical threats. Heat stress is generally considered as increasing the temperature 10-15ºC above the optimum temperature. Constantly high temperatures exposure caused an array of morpho-anatomical, physiological, and biochemical changes in plants and may lead to a drastic reduction in economic yield (Wahid et al. 2007).

Tomato (*Solanum lycopersicum* L.) is endemic to the Andean region, South America, currently comprising Peru, Colombia, Ecuador, and Bolivia and subsequently its cultivation spread worldwide (Gerszberg et al. 2015). Today, tomato is one the most cultivated crops and its fruits are consumed worldwide with a production of more than 186 million tonnes in a cultivation area of 4.91 million hectares, becoming one of the most important vegetables in the world (FAOSTAT, 2022).

Despite tomato's ability to grow under variable climates around the world, it is a heat-sensitive crop with the optimum temperature is between 21 and 24°C (Singh et al. 2017). High temperature causes disturbances in plants, including tomato, affecting photosynthesis, respiration, water relations, membrane stability, and modulating levels of metabolites, leading to low quality and quantity of crop yield (Singh et al. 2017). At temperatures higher than 35ºC, vegetative growth, flowering, pollen viability, the number of released pollen grains, fruit development, and fruit set of tomatoes are restricted (Alsamir et al. 2021; Sato et al. 2006).

Currently, heat-tolerant varieties could be considered as one of the best solutions to deal with heat stress issues (Liaqat et al. 2019). However, thermotolerance trait acquired by plants is a cost intensive process (Wahid et al. 2007). Therefore, an inexpensive, effective, and eco-friendly material, such as chitosan, could be a potential option to cope with negative impacts of abiotic stresses (Liaqat et al. 2019).

Chitosan is polysaccharides, a derivative of chitin, obtained from crustaceans, insects, mollusks, fungus, and algae. Chitosan is harmless to plants, animals, and humans. Due to its non-toxicity, biodegradability, biocompatibility, and stimulant to cell activation and plant growth, the application of chitosan is increasing since the last decade (Elieh-Ali-Komi and Hamblin, 2016).

The main directions of using chitosan in horticultural practice are as a plant protection agent against pre-and post-harvest's diseases, enhancing biological control, support a symbiotic relation of beneficial microorganisms, and as a plant growth regulation and development (Zargar et al. 2015). Depending on its structure and concentration, plant species, and developmental stage of the plants, chitosan has been demonstrated to induce numerous biological responses in plants with positive results (Malerba and Cerana, 2016). Under drought stress, chitosan was reported to alleviate drought effects and increased the production of stress-protective metabolites (Li et al. 2017). Under salt stress, plants with chitosan treatment were found to increase the accumulation of SOD, POD, and CAT enzymes, indicating efficient detoxification of ROS, and play an important role in enhancing antioxidant enzymes (Ma et al. 2012). Under heat stress, the mechanism of chitosan is still not clearly established, however the application at different growth stages helped plants to withstand the adverse effects of the stress (Katiyar et al. 2015). Stimulating signals related to auxin and gibberellins synthesis, improving photosynthesis by increasing leaf chlorophyll content, and enhancing vegetative growth and development of plants (number of leaves, shoots length, pedicel length, plant height) might be some of its actions (Liaqat et al. 2019; Malerba and Cerana, 2016).

The objectives of this experiment were:

-To evaluate the effectiveness of chitosan application in alleviating the adverse effects of heat stress on the physiological parameters of tomato seedlings.

-To compare the effectiveness of nano-chitosan and bulk chitosan in mitigating the negative impacts of heat stress on tomato seedlings, assessing key parameters including growth, photosynthetic activity, and antioxidant defense mechanisms.

-To determine the optimal concentration of chitosan for promoting the growth and enhancing the stress tolerance of tomato seedlings under heat stress conditions.

2. LITERATURE REVIEW

2.1. Introduction of Tomato

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetables with approximately 186.1 million tonnes of fruits produced on 4.91 million ha each year. 63% of global tomato production is in Asia, while America produced 12.5%, Africa and Europe produced 12.3%, and 10.9% (FAOSTAT, 2022).

Tomato belongs to the Solanaceae family and originates from the South American Andes. In the sixteenth century, the cultivated tomato was brought to Europe by the Spanish and later introduced to southern and eastern Asia, Africa, and the Middle East (Naika et al. 2005). In the botanical classification, tomato was placed in the genus *Solanum*, along with potato. Although potato and tomato plants have similar characteristics, the flower colour (yellow for tomato and primarily white or violet for potato) and particularly the shape and opening of pollen-bearing structures that separate the two plants (Jones, 2008).

Tomato is an herbaceous perennial, but in temperate zones, it is usually grown as an annual due to frost conditions. Tomato is a day-neutral plant, flowering under conditions of either short or long days. The inflorescence is a monochasial cyme of 4 to 12 flowers. Flowers of commercial tomatoes are self-fertile, however, require physical vibration either by mechanical or insects to complete pollination (Jones, 2008).

Cultivated tomato plants are divided into indeterminate and determinate. The indeterminate type is a tall vine plant that needs to be staked, caged, or trellised. It keeps growing after flowering. The indeterminate type is for long-season production due to continuous fruit production for an extended period under favourable conditions. The determinate is a short type that does not need staking. This type stops growing after flowering. The fruits from the determinate type will last for only two or three weeks and the ripening time is faster than the indeterminate type. With the timing of fruit ripening that are almost all at once and the location of the fruits on the periphery of the plant canopy, the determinate type is suitable for mechanical harvesting (Jones, 2008).

For early varieties, the approximate time from planting to the mature stage is from 50 to 65 days, and for late varieties is around 85 to 95 days. The period from seedling to first fruit harvest will vary from 45 days to more than 100 days. In 100 to 145 days cultivar, the establishment stage (germination to initial leaves) takes place for 25 to 35 days, the vegetative stage will last for 20 to 25 days, the length of the flowering stage will be 20 to 30 days, fruit

formation duration will take 20 to 30 days, and fruit ripening will last for 15 to 20 days (Jones, 2008).

Although tomato adapts to a wide range of climatic conditions, to produce a high yield and good quality fruit, tomato requires a relatively cool and dry climate. The optimum temperature is between 21 and 24°C. If the temperature is below 10°C or above 38°C, the plant tissues will damage (Singh et al. 2017). Tomato plants require different temperatures during the growth cycle. In the stage of seed germination, the optimum temperature is around 16-29°C with the minimum is 11°C and the maximum is 34°C. The optimum temperature required for the seedling growth stage is around 21-24°C with the minimum is 18°C and the maximum is 32°C. In the fruit set stage, the optimum temperature is around 20-24°C with the minimum being 18°C and the maximum being 30°C. In the case of red colour development, tomato requires 20-24°C as the optimum temperature, 10°C as the minimum, and 30°C as the maximum temperature (Naika et al. 2005).

Tomato plants can be grown in a range of environmental settings, either in open fields or in the greenhouse. Tomato can be grown in raised garden beds with a wide range of soil mixes and is adapted to container systems in soilless media, modified soils, or hydroponics. Since tomato has a branching tap and fibrous root structure; aeration and loose soil conditions are essential for plant growth. In general, tomato grows best on fertile soils, in the medium to high range of major elements (P, K, Ca, and Mg) with a pH range of 5.5 to 6.8. Tomato is considered moderately sensitive to salinity, the maximum electrical conductivity level without a significant yield loss is around 2.5 dS/m. Tomato requires a significant quantity of water, but not in excess, since tomato roots will not function under anaerobic (water-logged) conditions (Jones, 2008).

2.2. Tomato Responses to Heat Stress

Due to climate change and global warming, heat stress become one of the most critical threats to crop production in the field. Heat stress induces metabolic disturbances that can lead to plant death. The reversibility depends on the stress level and adaptation process (Huther et al. 2013). High temperatures can cause morpho-anatomical, physiological, and biochemical changes in tomato plants, which affect plant growth and development and may lead to economic yield reduction (Singh et al. 2017). In terms of high temperature, the reproductive stage in the plant is more susceptible than the vegetative stage (Ruan et al. 2010).

Under heat stress, several traits of the plant significantly declined, such as dry mass, leaf greenness, maximum photochemical efficiency of photosystem II, photosynthetic rate, stomatal

conductance, transpiration rate, leaf chlorophyll, and carotenoid content, while the catalase and ascorbate peroxidase activities were increased (Haque et al. 2021).

The maximum photochemical efficiency of photosystem (PS) II (Fv/Fm) and photosynthesis are considered the most heat-sensitive physiological processes in plants, including tomatoes (Zhou et al. 2017). Murata et al. (2007) reported that heat stress particularly targets damage to the reaction center of PSII. The reduction of the photosynthesis rate is associated with the breakdown of chlorophyll molecules by the enzyme chlorophyllase into phytol and chlorophyllide (Mishra et al. 2017). Antioxidative enzymes, such as peroxidase (POD), superoxide dismutase (SOD), and ascorbate peroxidase (APX) increase due to heat stress, resulting in an increased level of ROS (Zhou et al. 2019).

Photosynthetic pigments, such as chlorophyll a and b, are sensitive to heat stress. Heat stress resulted in loss of plant leaf pigment and significantly damages photosynthetic activities (Awasthi et al. 2014). Zafar et al. (2017) reported that photosynthetic pigments (chlorophyll a, b, and carotenoids) decreased under heat stress in most rice cultivars.

In high moisture conditions, plants can maintain steady tissue water status regardless of temperature. However, high temperatures will harm this tendency when water is limited (Machado and Paulsen, 2001). Heat stress in tomatoes disconcerted the relations between leaf water and root hydraulic conductivity (Morales et al. 2003). During the daytime, plant transpiration induces water deficiency, causing a decrease in water potential and leading to physiological disturbance (Tsukaguchi et al. 2003).

Zhou et al. (2017) reported that tomato seedlings under heat stress (36/28°C for day/night for 4 days), showed a significant decrease in net photosynthesis rate, maximum quantum efficiency of PSII (Fv/Fm), total chlorophyll, stomatal conductance, and the length and area of stomata. Alayafi (2020) observed the effects of tomato seedlings exposure to heat stress (40 °C for 8 h) resulted in leaf curling, mild wilting; higher levels of hydrogen peroxide, lipid peroxidation, electrolyte leakage, total oxidative capacity (TOC), oxidative stress index (OSI), the expression of heat stress genes (HSP70, HSP90, HSP80) and antioxidant gene (CAT) besides the significant reduction in the photosynthetic pigments (chlorophyll-a and chlorophyll-b), anthocyanin contents, and contents of N, P, K, Na, and Mg.

Heat stress usually causes the damage of photosynthetic apparatus, decreasing transpiration due to stomatal closure and CO_2 content, inhibiting photosynthetic enzymes and ATP synthases rates, reducing leaf expansion, and accelerating senescence; consequently, plant development is hampered (Wahid, 2007; Farooq et al. 2009; Zandalinas et al. 2016). To mitigate the heat stress effect, the metabolism of carbon assimilation is altered, remobilizing plants'

starch reserve in chloroplasts by releasing energy, sugars, and derived metabolites to survive stress periods and prevent further damage (Wang et al. 2018; Raza, 2020). Photosynthesis works efficiently between temperatures of 20 and 30ºC. Above these conditions, photosynthetic rates tend to be reduced (Yamori et al. 2014).

At temperatures higher than 35ºC, Alsamir et al. (2021) and Sato et al. (2006) reported that vegetative growth of tomatoes is restricted. The increase in temperature may decrease root growth, nutrient assimilation proteins, and the concentration of nutrient uptake by roots. Under heat stress, the passive uptake and root to shoot transport of nutrients by transpiration-driven mass flow are decreased. Besides roots, shoots growth also decreased under heat stress, however, the effect is bigger in roots (Giri et al. 2017). Salah and Tardieu (1996) reported that heat stress causes a reduction in meristematic activity and in the growth of plant parts, especially leaves. Heat stress in plants arrests elongation of the cell wall and hinders cell differentiation (Potters et al. 2007). Furthermore, high temperature has been reported to affect floral abortion causing 80% flower loss in tomato plants leading to reduced fruit set (Ruan et al. 2010).

Plants that grow under extreme temperatures will gain adaptive mechanisms through osmolyte accumulation (Hare et al. 1998). Under stress, different plants may accumulate different osmolytes, such as sugars and sugar alcohols (polyols), proline, tertiary and quaternary ammonium compounds, and tertiary sulphonium compounds as a tolerance mechanism (Sairam and Tyagi, 2004). In high temperatures, any constraint in photosynthesis induces plant growth limitation (Wise et al. 2004).

To counteract the injuries of oxidative stress, plants have developed a complex defense system by producing enzymatic and non-enzymatic antioxidants. One of the non-enzymatic antioxidants is phenolic compounds which involve in stress tolerance to increase a plant's adaptability and exhibit a strong antioxidant capacity essential to scavenging (Sharma et al. 2012). The antioxidant content of tomatoes under heat stress, such as tocochromanols, increase in leaves and ascorbic acid in fruits, as a mechanism to confront the stress (Mesa et al. 2022).

In addition to physiological alterations, plants under heat stress also undergo anatomical adaptations, such as stomata numbers on the leaf surface. Generally, plants under heat stress might have higher stomatal density and smaller stomata size to improve heat resistance efficiency (Gu et al. 2016). A leaf with high density, small stomata can reduce potential conductance and increase water–use efficiency as a mechanism to cope with stress condition (Poulos et al. 2007).

2.3. Overview of Chitosan

Chitin is a structural polymer of many organisms like crustaceans, insects, molluscs, fungus, and algae (Zargar et al. 2015). Kaya et al. (2017) reported that chitin has 3 crystallographic forms, alpha (α), beta (β), and gamma (γ). The most commonly existing forms of chitin which are extracted from a large variety of organisms are the α and β. Chitin is a linear polymer consisting of 2-amino-2-deoxy-D-glucose and 2-acetamido-2-deoxy-D-glucose monomers linked through 1-4 bonds (Kaya et al, 2017).

As a chitin deacetylated form, chitosan is more functional due to its amino-based functional groups stretching along the chain (Kurita, 2006). The source and extraction method affect the molecular weight, biological and physicochemical properties, and purity of the isolated chitosan (Beaney et al. 2005). Chemically, chitosan is a linear polymer composed of D-glucosamine and N-acetyl-D-glucosamine, linked with each other through 1, 4-glycosidic bonds (Rinaudo, 2006). Chitosan exhibits three functional groups, primary hydroxyl, secondary hydroxyl, and amine groups impacted by easy chemical modification and affects the solubility and mechanical properties (Shahidi and Abuzaytoun, 2005).

Chitosan is more soluble in acidic aqueous mediums compared to chitin due to the protonation of $–NH_2$ at the C-2 position of the D-glucosamine repeat unit which induces the conversion of the polysaccharide to a polyelectrolyte in acidic media (Rinaudo, 2006). This characteristic broadens chitosan scope of applications from agriculture, medicine, process engineering, and industries (Sharif et al. 2018).

The antimicrobial activities of chitosan depend on the type of chitosan (native or modified), the degree of polymerization, the host, the chemical and/or nutrient composition of the substrates, and environmental conditions. Pentamers and heptamers (oligomeric chitosan) have been reported to exhibit better anti-fungal activity than larger units (Kulikov et al. 2006). Savard et al. (2002) reported that antimicrobial activity increased with the increase in chitosan molecular and seems to be faster on fungi and algae than on bacteria.

As an anti-virus, chitosan was shown to inhibit the systemic propagation of viruses throughout the plant and to enhance the host's hypersensitive response to infection (Pospieszny et al. 1991; Chirkov, 2002). The level of suppression of viral infections depends on the molecular weight (Kulikov et al. 2006). Chitosan also inhibits a wide range of bacteria growth (Muzzarelli et al. 1990). Quaternary ammonium salts of chitosan were shown to be effective in inhibiting the growth and development of *Escherichia coli*, especially in acidic media (Jia et al.

2001). Kim et al. (1997) reported that several derivatives chitin and chitosan were also shown to inhibit *Staphylococcus aureus*.

Rabea et al. (2005), reported that twenty-four derivatives of chitosan have a higher fungicidal activity than the native chitosan, using a radial hyphal growth bioassay of *B. cinerea* and *P. grisea*. In general, 100 µg/ml of chitosan had more fungicidal activity than chitin. Of the 46 fungi tested, chitosan inhibited 32 isolates at certain concentrations, whereas only 6 isolates were inhibited by chitin (Allan and Hadwiger, 1979). Hadrami et al. (2010) reported twenty-four chitosan derivatives were also shown to have significant insecticidal activity with the most active derivative, N-(2-chloro-6-fluorobenzyl) chitosan, causing 100% mortality of larvae. All synthesized derivatives highly inhibited larvae growth as compared to chitosan by 7% and the most active derivative was the O-(decanoyl) chitosan.

2.4. Chitosan Application in Agriculture

In agriculture practice, using chitin and chitosan have four main directions: (a) plant protection against diseases in pre-and post-harvest; (b) enhancing biological control and antagonist microorganism action; (c) support a symbiotic relation of beneficial plant microorganisms; and (d) plant growth regulation and development. Chitin and its derivatives have been employed extensively to enhance defensive mechanisms in plants (Zargar et al. 2015). Yamaguchi et al. (2000) observed N-acetyl chit oligosaccharides (oligo chitin, chitin oligosaccharides) of a specific size as potent elicitor signals in plants to protect against diseases.

In the post-harvest preservation of fruits and vegetables, the antimicrobial properties of chitosan and its film-creating aptitude grants fruits and vegetables antimicrobial protection that enhanced their shelf life (Galed et al. 2004). Chitin and chitosan in the soil increase symbiotic interactions of the plan and beneficial microorganisms, as in the case of mycorrhizas and these polysaccharides have been used to control the parasitic nematodes in soils (Zargar et al. 2015).

In plant disease control, chitosan's ability has been extensively explored. For example, Muzzarelli et al. (2001) tested the effectiveness of five chitosan derivatives to *Saprolegnia parasitica*, indicating that methyl pyrrolidinone chitosan, N-phosphonomethylchitosan, and N-carboxymethyl chitosan, as opposed to N-dicarboxymethylchitosan, did not allow the fungus to grow normally. Chitosan amendments were reported to enhance plant growth and suppress some soil-borne diseases, such as *Fusarium oxysporum* f. sp. *radicis-lycopersici* that cause root rot in tomatoes (Lafontaine and Benhamou, 1996). In terms of post-harvest diseases, using chitosan stimulates microbial degradation of pathogens (Benhamou, 2004), suggesting the alternative of pesticides on fresh produce in storage (El Ghaouth et al. 1992).

As a seed coating, Guan et al. (2009) examined chitosan's effects on prime maize seeds. It enhanced the germination index, reduced the mean germination time, and increased shoot height, root length, and shoot and root dry weights in two tested maize lines, induced a decline in malondialdehyde content, altered the relative permeability of the plasma membrane and increased the concentrations of soluble sugars and proline, and of peroxidase and catalase activities. In other studies, Reddy et al. (1999) reported that chitosan application increases wheat seed resistance to certain diseases and improves their quality and ability to germinate.

Bittelli et al. (2001) examined the potential of chitosan foliar applications on pepper transpiration as an effective anti-transpiring to preserve water resources use in agriculture. In response to chitosan treatment, the stomata of treated plants were closed, resulting in a decrease in transpiration. Bittelli et al. (2001) also reported that chitosan treatment on pepper reduced 26-43% water usage, with no change in biomass production or yield. Chitosan has also been applied as a foliar treatment to control the growth, spread, and development of many diseases involving viruses, bacteria, fungi, and pests (Rabea et al. 2003). Kowalski et al. (2006) reported that chitosan gave positive results to the yield and tuber quality of micro-propagated potatoes. Chitosan foliar spray on barley also effectively reduced the infection of powdery mildew *Blumeria graminis* f. sp. *hordei* (Faoro et al. 2008).

At an optimal concentration, chitosan application can delay disease development, reducing plant wilting (Benhamou et al. 1994). The ability of chitosan to reduce soilborne pathogens comes from the fact that it enhances plant defense responses (Bell et al. 1998).

2.5. Effect of Chitosan to Mitigate Adverse Effects of Stress

Chitin and its derivatives have evolved as natural polymers with positive responses in plants. Ali et al. (2020) reported the application of chitosan on cucumber induced growth and physiological index under heat stress. Hassnain et al. (2020) also reported that chitosan treatment alleviated drought stress and improved physiological and agronomical value of tomato.

Plants acquired their ROS scavenging mechanism through enzymatic and non-enzymatic antioxidant production (Hidangmayum et al. 2019). Higher accumulation of SOD, POD, and CAT enzymes indicates efficient detoxification of ROS. Chitosan-treated plants were found to increase the accumulation of SOD, POD, and CAT enzymes, indicating efficient detoxification of ROS, and play an important role in alleviating salt stress through enhanced antioxidant enzymes. Seeds soaked in oligochitosan at 0.0625% for 5 h, led to a significant increase in proline level (Ma et al. 2012).

The mechanism of chitosan against heat stress is not still clearly established, however, its application at different growth stages of the plants stimulated plant growth that has helped plants to withstand the adverse effects of the stress (Katiyar et al. 2015). Liaqat et al. (2019) reported that chitosan stimulated the activity of sucrose hydrolyzing enzymes in eggplant seedlings under heat stress. Chitosan application promotes auxin and gibberellins synthesis by stimulating a signal related to biosynthesis and may help plant maintain their growth and development under high-temperature stress. Under abiotic stresses, some studies reported that chitosan protect cell membranes from degradation (Liaqat et al. 2019).

Chitosan application improved photosynthesis by increasing leaf chlorophyll content that might have stabilized the cell membrane, detoxified the deleterious effects of antioxidants, and helped in cell elongation and multiplication, resulting in significant yield improvement under heat stress (Malerba and Cerana, 2016; Salachna and Zawadzińska, 2014). At vegetative stages, foliar application of chitosan enhances the plant growth and development (number of leaves, shoots length, pedicel length, plant height) which helped plants to secure fruiting and fruit yield in eggplant under heat stress (Liaqat et al. 2019).

Under heat stress conditions, proline content, glycinebetain, total soluble sugars, and total phenolics contents in leaves of eggplant genotypes decreased significantly and chitosan application chitosan improved the total phenolics level. Accumulation of total free sugar and phenolic contents serves as an adaptive mechanism in plants under heat stress and osmolytes protect the cell membrane against the adverse effects of heat stress. The application of chitosan improves the accumulation of ions such as proline that improves the membrane stability in plants. Chitosan also regulates stomatal closure, ion uptake, and transpiration, and plays a prolific role in many morphological and physiological functions of plants (Liaqat et al. 2019).

Liaqat et al. (2019) also reported that chitosan exogenous application on eggplant increased plant height, leaf chlorophyll content, and number of leaves per plant. The growth stimulatory effects of chitosan on vegetative parts of plants especially the number of leaves per plant, plant height, and leaf chlorophyll content have previously been reported in cucumber as well (Shehata et al. 2012).

2.6. Chitosan Based Nano-formulation for Abiotic Stress

Nano-based formulation is an advanced technology with a wide range of applications. In horticultural sector, nano-formulation is used as fertilizer, pesticides, and biostimulator. Nano-formulation can effectively penetrate plant surface membrane through cuticle, stomata, trichomes, hydathodes, wounds, stigma, and root junction with higher rate of mobility due to

its nano size (Hidangmayum and Dwivedi, 2022). Chitosan nanoparticles can be incorporated with other compounds as well, creating bionanoconjugation molecules containing two or more nano molecules to increase the potential biological activity of nano polymers (Mohan et al. 2023).

The application of nano-chitosan to enhance various abiotic stresses resilience in plants have been observed with positive results. Khaled et al. (2023) reported the foliar application of nano-chitosan in tomato during flowering stage under heat stress (33°C ± 2°C with 16 h of daylight and 8 h of night) improved morphological traits, such as plant height and flower numbers. Under salinity stress, Ramadan et al. (2022) observed the use of chitosan nanoparticles on *Capsicum annuum* L. significantly improved the number of leaves per plant, leaf area, and shoot fresh and dry weight per plant, chlorophyll content, and antioxidant enzymes activity. Zayed et al. (2017) reported the application of nano-chitosan on *Phaseolus vulgaris* significantly promoted seed germination, growth variables (plant height, leaf area, fresh and dry weights of the shoot and root), membrane stability index, chlorophyll a and b, catalase, proline, RWC, carotenoids, and antioxidant enzymes under salt stress.

3. MATERIAL AND METHODS

3.1. Experimental Site, Plant Material, and Management Practices

3.1.1. Time and Area of Study

The research was conducted from 9th October 2023 until 13th December 2023 at the Department of Vegetables and Mushroom Growing, Hungarian University of Agriculture and Life Sciences, Budapest.

3.1.2. Plant Material

Tomato cv. Zucchero hybrid from Blumen Company, Italy was used as the seedling material. One seed was placed per hole in seedling trays with mixture of coco peat and perlite as a substrate. Seeds were place in a phytotron chamber of 25°C temperature and 70% of air humidity. Irrigation with normal water was done. After appearing first true leaves, fertilizer with ratio 15 N: 30 P: 15 K was applied in concentration of 2 g/L. 40 seedlings were transplanted to the main pot when they reached to 2-4 true leaves stage. When seedlings have 4-5 leaves, fertilizer was converted to higher N content (14 N: 11 P: 25 K).

3.1.3. Preparation of Chitosan Solution

Chitosan with a de-acetylation degree of 85% was purchased from Sigma Chemical Company (Saint Louis, MO, USA). It was dissolved in 1.0% (w/v) acetic acid and stirred for 1 hour. The pH was subsequently adjusted to 6.0 using 2 M NaOH.

Chitosan nanoparticles in the anatase form with a purity exceeding 99% were procured from a commercial supplier (Iranian Nanomaterial Pioneers Company, Mashhad, Iran). Chitosan nanoparticles were synthesized via the ionotropic gelation method using tripolyphosphate (TPP), following the procedure outlined by Jafari et al. (2022). Initially, chitosan was dissolved as previously mentioned, and the resulting suspension was filtered. Subsequently, chitosan nanoparticles were spontaneously formed by slowly introducing 1 mL of filtered 1% TPP solution (adjusted to pH 4 using 20% acetic acid) into 10 mL of chitosan solution under continuous stirring at room temperature (25 °C) for 30 minutes. The resulting gel was then subjected to centrifugation at 8000 g for 10 minutes. After discarding the supernatant, the sediment containing nanoparticles was washed five times with double deionized water. Finally, the collected chitosan nanoparticle precipitate was dried at 60 °C. The majority of the observed nanoparticles exhibited a size smaller than 20 nm (Jafari et al., 2022).

From the stock solutions, chitosan and nano-chitosan were dissolved in 1 L of distilled water to obtain two different concentrations (100 ppm and 150 ppm).

3.1.4. Treatments

The study was conducted in a factorial experiment design, using a Completely Randomized Design (CRD) with four replications. Tomato seedlings were raised in a growth chamber (Sanyo, Japan) at temperature of 25°C ± 2°C with 13 hours of daylight and 11 hours of night and 60 ± 5% relative humidity (Figure 1).

Ten combination treatments of heat and chitosan were applied to tomato seedlings including two types of chitosan (chitosan and nano-chitosan) with 2 different concentrations of 100 ppm and 150 ppm (Table 1). Each concentration was applied manually on the tomato seedlings by foliar spray with manual pump spray after 1 month of sowing. The frequency of spray was three times per week. In the case of control seedlings, distilled water was used as a spray material.

Two different temperature treatments were applied (optimal and high) to 1 month old tomato seedlings. In optimal treatment, temperature was maintained at 25°C ± 2°C. In high temperatures, heat was applied for 4 hours per day from 10.00-11.00 at 38°C and gradually increased to 45°C for 2 hours.

Table 1: Temperature and chitosan treatments

Label	Temperature	Chitosan Form	Concentration (ppm)
OC	Optimal	Control	0
OCH100	Optimal	Chitosan	100
OCH150	Optimal	Chitosan	150
ONC100	Optimal	Nano-chitosan	100
ONC150	Optimal	Nano-chitosan	150
HC	High	Control	0
HCH100	High	Chitosan	100
HCH150	High	Chitosan	150
HNC100	High	Nano-chitosan	100
HNC150	High	Nano-chitosan	150

Figure 1: Growing tomato seedling in the growth chamber

3.2. Parameters and Instrumental Measurements

3.2.1. Shoot Length

Shoot length (cm) was measured by a ruler from the base of the plant to the tip of highest branch.

3.2.2. Relative Water Content

1 disc of fresh leaf was collected from each sample and weighted the fresh weight. The sample was promptly hydrated to full turgidity for 24 hours. After hydration, the sample was removed from water and lightly dried using tissue paper before being weighted to get turgid weight. From that point, the leaf disc was dried for 20 minutes in an oven at 70°C and then weight again to get dry weight (Sancho-Knapik et al. 2013). Relative water content (RWC) of the leaves was calculated by the following equation:

$$\text{RWC}(\%) = \frac{(\text{fresh weight-dry weight})}{(\text{turgid weight-dry weight})} \times 100$$

3.2.3. Electrolyte Leakage

3 discs of fresh leaves were collected from each sample and placed into an Erlenmeyer flask with 25 ml of distilled water and covered with aluminium foil. The initial electrical conductivity of the solution (EC_1) was determined by EC meter after 2 hours of incubation at 32°C. The final electrical conductivity (EC_2) was measured after the sample incubated in an oven at 120°C for 20 minutes and cooled down (Younis et al. 2011). The electrolyte leakage (EL) was calculated by the following formula:

$$\text{EL}(\%) = \frac{\text{EC1}}{\text{EC2}} \times 100$$

3.2.4. Chlorophyll a and Chlorophyll b

0.5 g of leaf sample was collected and extracted in 50 ml of acetone using a mortar and pastille. The mixture sample was centrifuged for 5 minutes at 5,000 rpm. The light absorbance of its supernatant was measured with a Genesys 50 UV-Vis Spectrophotometer (Thermo Fisher Scientific, USA) at the wavelengths of 644 nm, and 663 nm (Aliu et al. 2014) and calculated by the following formula:

Chlorophyll a = $(12.7*A_{663}) - (2.69*A_{644})$

Chlorophyll b = $(22.9*A_{644}) - (2.69*A_{663})$

3.2.5. SPAD

SPAD-502 chlorophyll meter (Konica Minolta, Japan) was used to measure leaf chlorophyll content in tomato seedling. The average chlorophyll result of fourth leaf was considered as the leaf SPAD value.

3.2.6. Net Photosynthesis Rate, Transpiration Rate, Water Use Efficiency

CI-340 Handheld Photosynthesis System (CID Bio-Science, USA) was used to measure net photosynthesis rate per unit leaf area of tomato seedling (Pn) and transpiration rate (E). Each treatment was measured with 3 replications. The results of both measurements were used to calculate water use efficiency (WUE) with the following formula:

$$\text{WUE} = \frac{\text{net photosynthesis rate (Pn)}}{\text{transpiration rate (E)}}$$

3.2.7. Antioxidant Capacity of FRAP

For measuring the Ferric Reducing Ability of Plasma antioxidant capacity (FRAP, μM ascorbic acid equivalent g/FW), 0.25 g of leaf sample was extracted in 2.5 ml of methanol using a mortar and pastille. The mixture was centrifuged for 2 minutes at 14,690 rpm. 50 μl supernatant sample was collected and mixed with 1.5 ml FRAP reagent (containing sodium acetate buffer 25 ml + TPTZ 2.5 ml + $FeCl_3$ 2.5 ml). The mixture was incubated for 5 minutes, and the light absorbance was measured with a Genesys 50 UV-Vis Spectrophotometer (Thermo Fisher Scientific, USA) at 593 nm.

3.2.8. Total Phenolics Content

In order to measure total phenolic content (mg gallic acid equivalent g/FW), 0.2 g of leaf sample was extracted in 2.5 ml of methanol using a mortar and pastille. The supernatant was collected and re-extracted the residue with methanol. All the collected supernatants were

evaporated in at 100ºC for 5-10 min. The evaporated residue was dissolved in a 3 ml of distilled water and centrifuged at 10,000 rpm for 5 min. 0.5 ml supernatant was collected and mixed with 2.5 ml distilled water. 0.5 ml of Folin's reagent was added and incubated for 3 min. 2 ml of 20% sodium carbonate (Na_2CO_3) solution was added to the mixture and placed for 1 minutes in boiling water. The light absorbance was measured using a Genesys 50 UV-Vis Spectrophotometer (Thermo Fisher Scientific, MA USA) at 650 nm.

3.3. Statistical Analysis

Data were analysed with Statistix 8.0 using Factorial Design analysis with 2 independent variables, 10 treatments, and 4 replications in each treatment. All-pairwise Comparisons with LSD method was used to determine the differences between all experimental groups with significance level of 95%.

4. RESULTS

4.1. Shoot Length

The shoot length (cm) of tomato seedlings was significantly affected by heat stress in comparison to control under optimal temperature (Figure 2). Foliar application of chitosan (in both concentrations and forms) under heat stress slightly improved the shoot length of tomato seedlings. The highest improvement was achieved by CH100 (19.50 cm), followed by NC100 (19.13 cm). However, the values were not significantly different than heat stress seedling without chitosan application (18.17 cm). The morphological of tomato seedlings under different temperature conditions and chitosan application can be found in Figure 3.

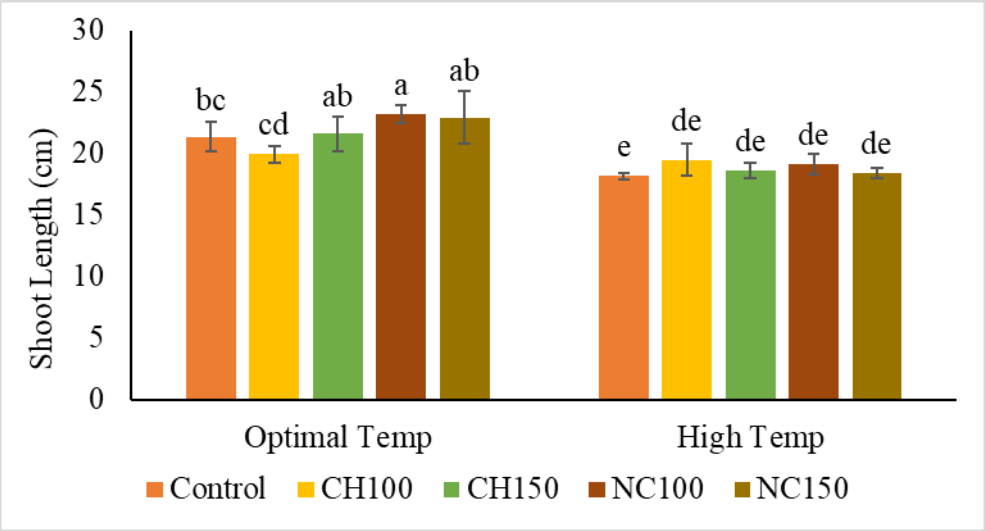

Figure 2: Effect of foliar chitosan application on shoot length of tomato seedlings under different temperature conditions, CH100 and CH150 are chitosan at 100 and 150 ppm, NC100 and NC150 are nano-chitosan at 100 and 150 ppm

Different letters indicate significant difference according to LSD test ($p < 0.05$)

OC

HC

OCH100

HCH100

OCH150

HCH150

ONC100

HNC100

ONC150 HNC150

Figure 3: Morphological effects of foliar chitosan application on tomato seedlings under different temperature conditions, OC: optimal control, OCH100: optimal chitosan 100 ppm, OCH150: optimal chitosan 150 ppm, ONC100: optimal nano-chitosan 100 ppm, ONC150: optimal nano-chitosan 150 ppm, HC: heat control, HCH100: heat chitosan 100 ppm, HCH150: heat chitosan 150 ppm, HNC100: heat nano-chitosan 100 ppm, HNC150: heat nano-chitosan 150 ppm

4.2. Relative Water Content

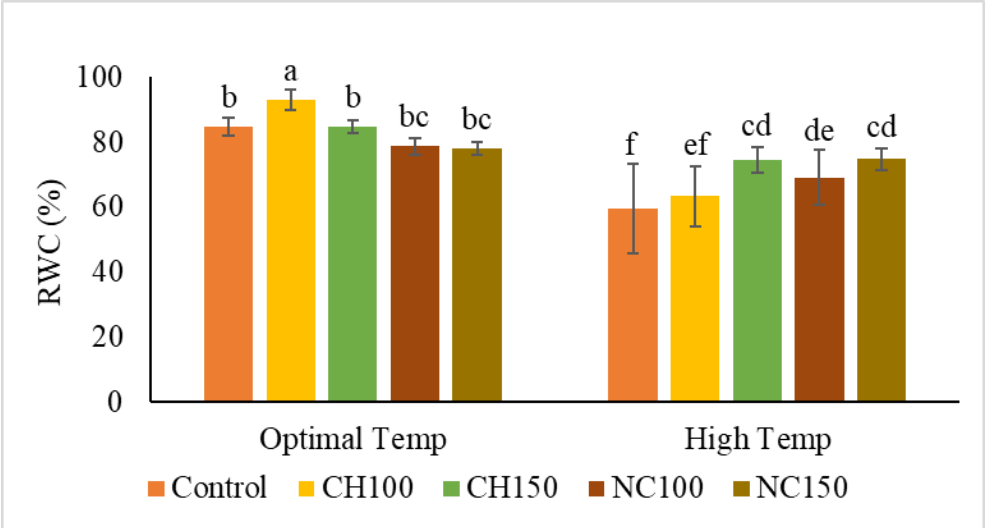

Figure 4: Effect of foliar chitosan application on relative water content (RWC) of tomato seedlings under different temperature conditions, CH100 and CH150 are chitosan at 100 and 150 ppm, NC100 and NC150 are nano-chitosan at 100 and 150 ppm

Different letters indicate significant difference according to LSD test ($p < 0.05$)

High temperature stress conditions significantly reduced RWC (-30%) in the leaves of tomato seedlings. The foliar application of chitosan under heat stress improved RWC value by 26%, 25%, and 16% in NC150, CH150, and NC100, respectively, in comparison to control (Figure 4). The highest RWC value was achieved in CH100 under optimal conditions, which was 10% higher than control.

4.3. Electrolyte Leakage

The foliar application of chitosan (in both forms and concentrations) on tomato seedlings under heat stress significantly reduced electrolyte leakage compared to control (Figure 5). The EL reductions in tomato leaves varied by 37%, 31%, 30%, and 19% in NC100, CH150, NC150, and CH100, respectively. However, under optimal temperature, chitosan application might have a negative effect in the EL as most of the treatments' value increased significantly than control, except for CH150.

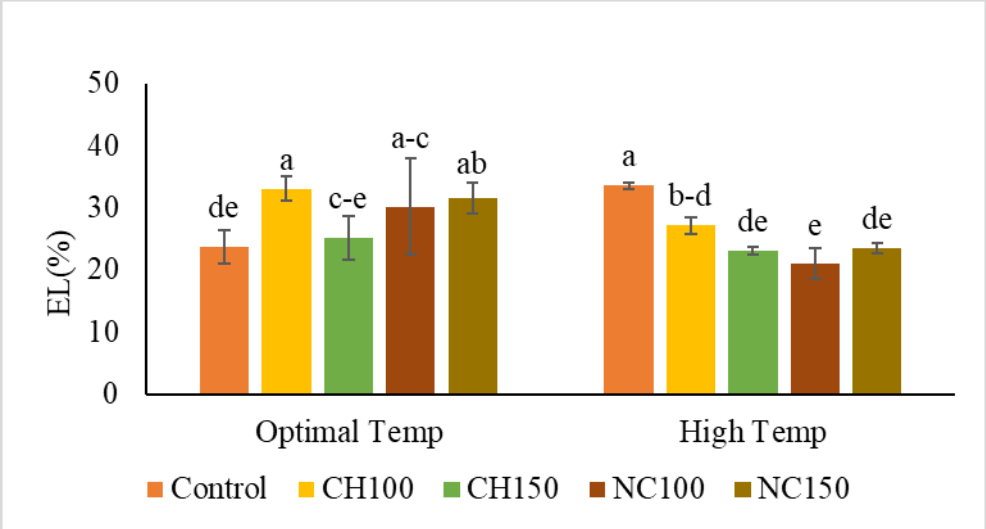

Figure 5: Effect of foliar chitosan application on electrolyte leakage (EL) of tomato seedlings under different temperature conditions, CH100 and CH150 are chitosan at 100 and 150 ppm, NC100 and NC150 are nano-chitosan at 100 and 150 ppm

Different letters indicate significant difference according to LSD test ($p < 0.05$)

4.4. Chlorophyll a and Chlorophyll b

Chlorophyll content was significantly affected by heat stress. Heat stress significantly decreased chlorophyll a content (-34%) in the tomato seedlings in comparison to optimal temperature conditions (Figure 6). Under heat stress, the foliar application of chitosan significantly improved chlorophyll a content by 45.99% and 42.19% in NC100 and CH100, respectively. Under optimal temperature, the foliar application of chitosan has no significant difference with control, except for CH100 that showed significant reduction in the result.

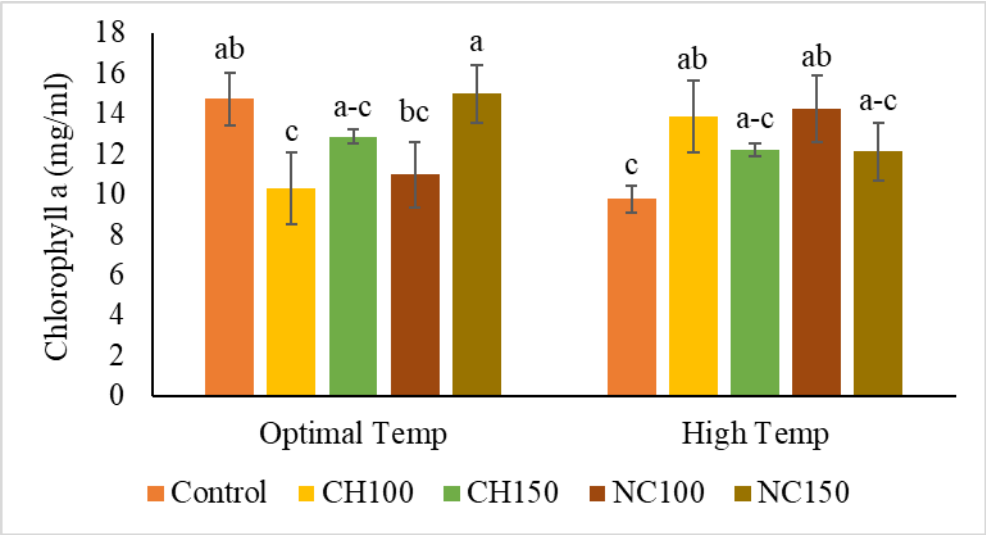

Figure 6: Effect of foliar chitosan application on chlorophyll a content of tomato seedlings under different temperature conditions, CH100 and CH150 are chitosan at 100 and 150 ppm, NC100 and NC150 are nano-chitosan at 100 and 150 ppm

Different letters indicate significant difference according to LSD test ($p< 0.05$)

The foliar application of chitosan under heat stress significantly improved chlorophyll b content in the leaf of tomato seedling in CH100 by 27.80% and NC100 by 23.57% (Figure 7). Under optimal temperature, the foliar application of chitosan showed a negative effect on chlorophyll b content, except for NC150 that slightly improved the value.

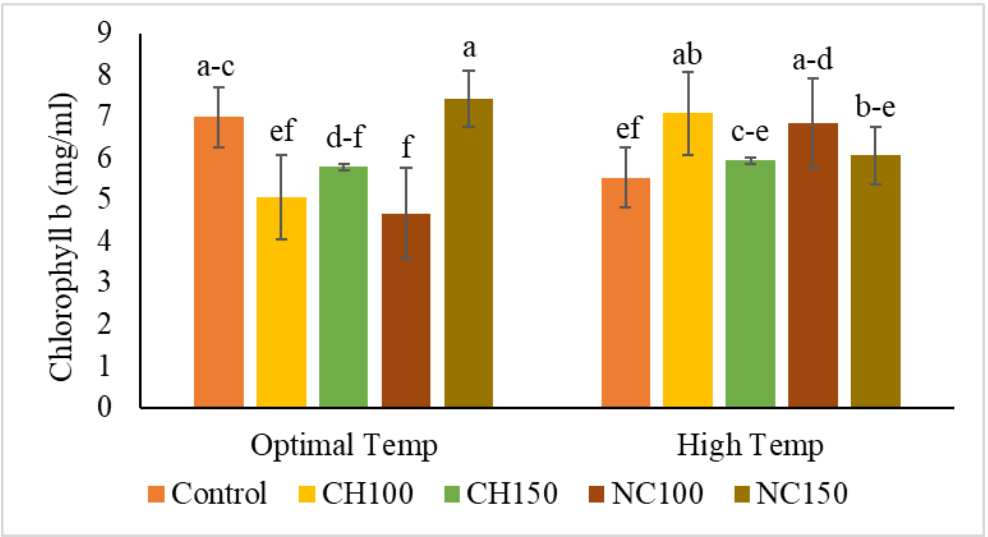

Figure 7: Effect of foliar chitosan application on chlorophyll b content of tomato seedlings under different temperature conditions, CH100 and CH150 are chitosan at 100 and 150 ppm, NC100 and NC150 are nano-chitosan at 100 and 150 ppm

Different letters indicate significant difference according to LSD test ($p< 0.05$)

4.5. SPAD

The SPAD value of control tomato seedlings were significantly reduced by heat stress in comparison to optimal temperature (Figure 8). Under heat stress, the SPAD value of control was 39.49 and with chitosan application, SPAD values were significantly improved by 6.46%, 6.18%, 5.09%, and 4.42% in NC100, CH100, NC150, and CH150, respectively. Under optimal temperature, the foliar application of chitosan in all forms was not significantly different than control.

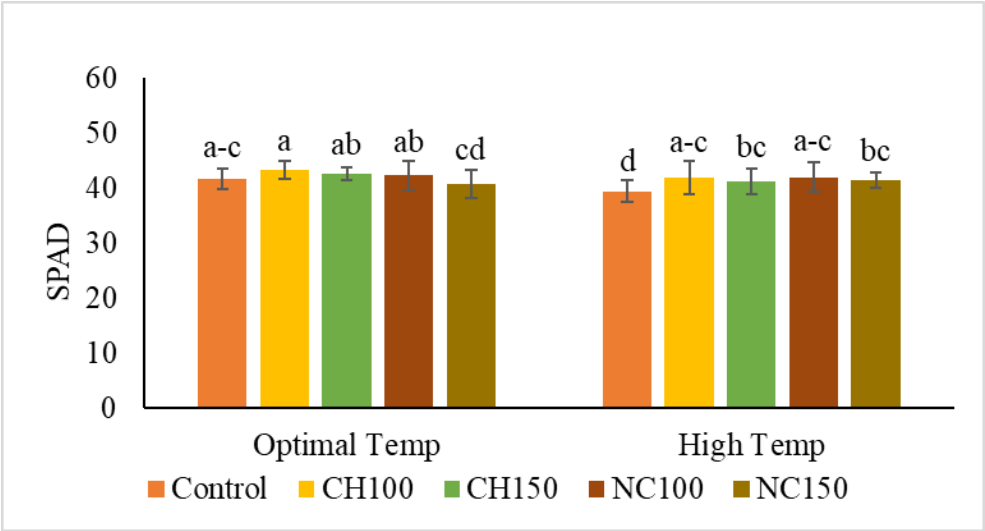

Figure 8: Effect of foliar chitosan application on SPAD of tomato seedlings under different temperature conditions, CH100 and CH150 are chitosan at 100 and 150 ppm, NC100 and NC150 are nano-chitosan at 100 and 150 ppm

Different letters indicate significant difference according to LSD test ($p < 0.05$)

4.6. Net Photosynthesis Rate, Transpiration Rate, Water Use Efficiency

The net photosynthesis rate (Pn) of tomato seedlings was negatively affected by high temperature with 60.40% reduction in comparison to control under optimal temperature (Figure 9). Under heat stress, the foliar application of chitosan 100 ppm significantly improved the Pn value by 151.30% in comparison with control. Under optimal temperature, CH150 significantly improved the net photosynthesis rate of tomato seedling by 81.59% compared to control.

The transpiration rate of control tomato seedling under heat stress was higher than optimal temperature without chitosan treatment (Figure 10). The foliar application of chitosan under heat stress significantly reduced the transpiration rate of tomato seedlings by 32.07% in NC100 and 22.60% in CH100. Under optimal temperature, the foliar applications of chitosan had no significant differences than control.

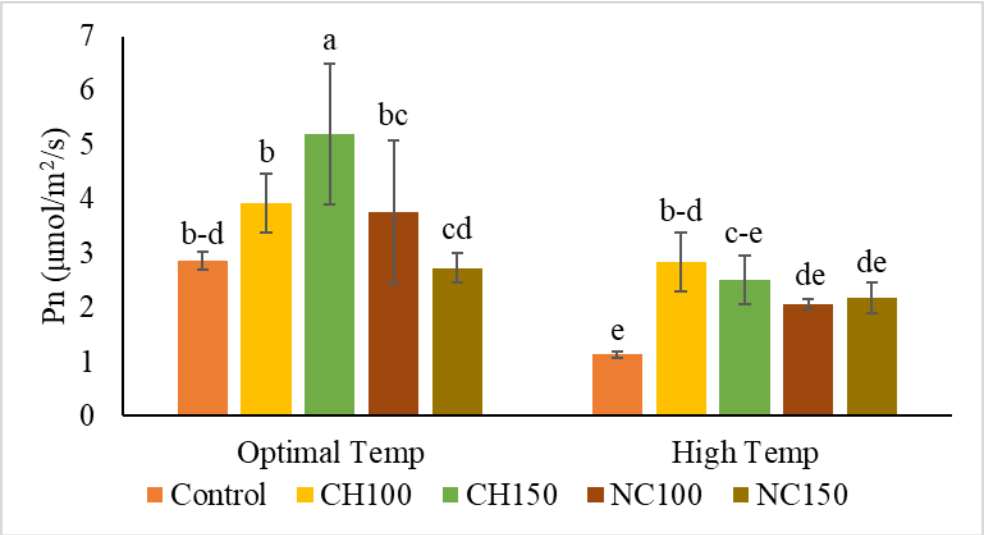

Figure 9: Effect of foliar chitosan application on net photosynthesis rate (Pn) of tomato seedlings under different temperature conditions, CH100 and CH150 are chitosan at 100 and 150 ppm, NC100 and NC150 are nano-chitosan at 100 and 150 ppm

Different letters indicate significant difference according to LSD test ($p < 0.05$)

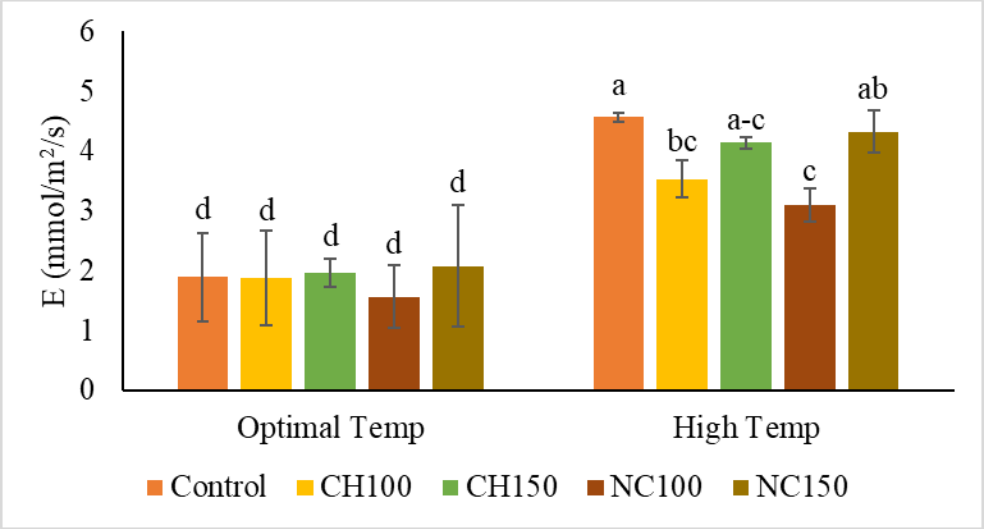

Figure 10: Effect of foliar chitosan application on transpiration rate (E) of tomato seedlings under different temperature conditions, CH100 and CH150 are chitosan at 100 and 150 ppm, NC100 and NC150 are nano-chitosan at 100 and 150 ppm

Different letters indicate significant difference according to LSD test ($p < 0.05$)

The water use efficiency (WUE) of control tomato seedling was negatively affected by high temperature conditions compared to optimal temperature (Figure 11). Under heat stress conditions, the foliar application of chitosan improved the WUE by more than twofold, however, the values were no significant difference compared to control. Under optimal

temperature, the application of CH150 and NC100 showed significant differences in WUE values by 70% and 62%, respectively, in comparison to control.

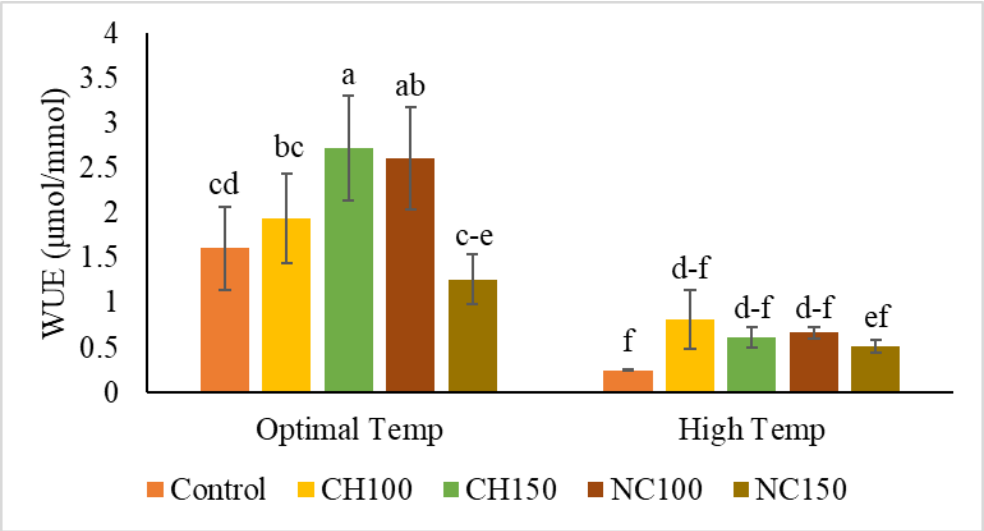

Figure 11: Effect of foliar chitosan application on water use efficiency (WUE) of tomato seedlings under different temperature conditions, CH100 and CH150 are chitosan at 100 and 150 ppm, NC100 and NC150 are nano-chitosan at 100 and 150 ppm

Different letters indicate significant difference according to LSD test ($p < 0.05$)

4.7. Antioxidant Capacity of FRAP

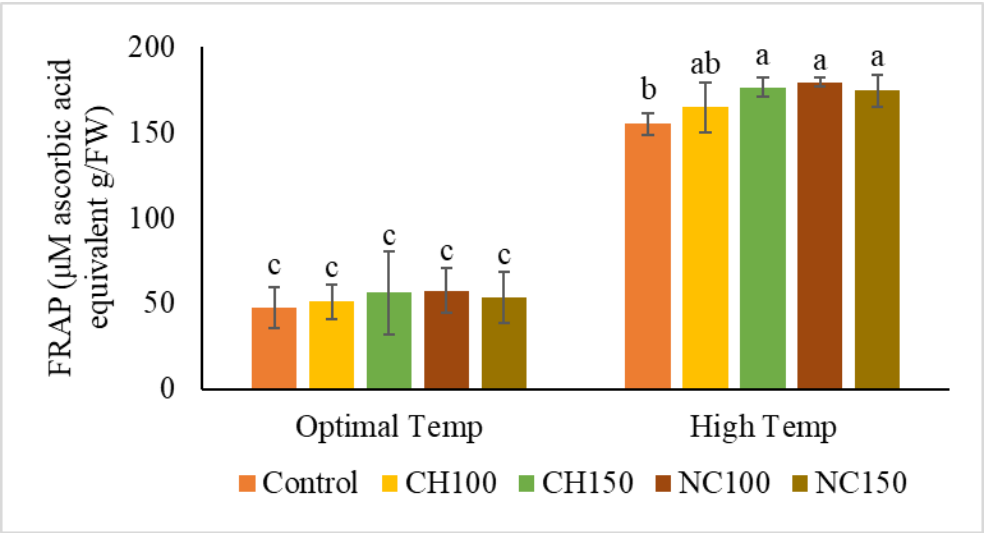

Figure 12: Effect of foliar chitosan application on antioxidant capacity (FRAP) of tomato seedlings under different temperature conditions, CH100 and CH150 are chitosan at 100 and 150 ppm, NC100 and NC150 are nano-chitosan at 100 and 150 ppm

Different letters indicate significant difference according to LSD test ($p < 0.05$)

Heat stress significantly increased the FRAP value on the leaf of tomato seedling in comparison to optimal temperature (Figure 12). Under heat stress, foliar chitosan application

significantly improved antioxidant capacity in tomato seedlings by 15.79% in NC100, 13.87% in CH150, and 12.60% in NC150. Under optimal temperature, foliar application of chitosan had no significant effect in FRAP value.

4.8. Total Phenolics Content

Heat stress increased the total phenolics content on the leaf of tomato seedlings in comparison to optimal temperature (Figure 13). Under heat stress, foliar chitosan application significantly improved the total phenolics content of tomato seedlings from 3.30 mg gallic acid equivalent g/FW in the high temperature without chitosan application to 13.01 and 10.71 mg gallic acid equivalent g/FW in CH150 and NC100, respectively. Under optimal temperature, foliar application of chitosan on tomato seedlings significantly improved the total phenolics content from 1.56 mg gallic acid equivalent g/FW in control to 7.87 and 4.81 mg gallic acid equivalent g/FW in CH150 and NC150, respectively.

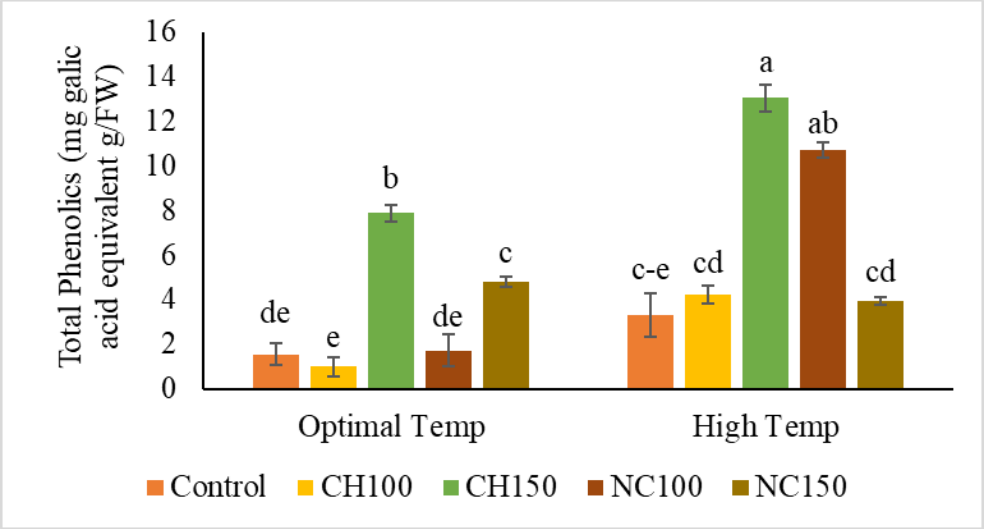

Figure 13: Effect of foliar chitosan application on total phenolics content of tomato seedlings under different temperature conditions, CH100 and CH150 are chitosan at 100 and 150 ppm, NC100 and NC150 are nano-chitosan at 100 and 150 ppm

Different letters indicate significant difference according to LSD test ($p < 0.05$)

5. DISCUSSION

Heat stress inhibit plant growth and development, causing reduction in shoot growth, root growth, plant height, and biomass (Wassie et al. 2019). In previous study, chitosan application significantly improved the shoot length of cucumber under heat stress (Ali et al. 2020). Similar result was obtained in this study, heat stress significantly reduced the shoot length and chitosan application slightly improved the shoot length of tomato seedlings. Although, there was no significant difference between chitosan application and control on the shoot length of tomato seedlings under heat stress, chitosan concentration at 100 ppm (in both forms) showed better results than at 150 ppm. These findings might be associated with the toxicity effects of chitosan at higher concentration, as Asgari-Targhi et al. (2018) reported that in *Capsicum annum,* the toxic doses of bulk (100 mgL^{-1}) or nano-chitosan (5, 10, and 20 mgL^{-1}) dramatically provoked cessation of plant growth and development.

Heat stress on plants have harmful effects on plasma membranes' function and dehydration of cytoplasm, resulting in electrolyte leakage (Hafez et al. 2020). Electrolyte leakage is an indicator to the injury occurring to plasma membrane after stress exposure. Under heat stress, proteins of the plasma membrane denature or aggregate according to the severity of stress or membrane lipids becomes hyper fluid, resulting in increased of electrolytes leakage from the membrane (Al Busaidi and Farag, 2015). The use of exogenous chitosan on tomato seedlings under heat stress conditions demonstrated a positive effect in diminishing electrolyte leakage (EL) by 19%-37%. The result is in accordance with the previous study that reported significant reductions of EL by 19%-32% with the application of chitosan on four genotypes of cucumber under heat stress (Ali et al. 2020). Under heat stress, chitosan acts as a positive regulator in osmotic adjustment by decreasing the production of lipid peroxidation, removing ROS, and improved cell membrane integrity, resulting in a decrease of electrolyte leakage (Bistgani et al. 2017).

Under heat stress, plants loss their chlorophyll as a protection mechanism to reduce light absorbance by slow synthesis or fast breakdown of chlorophyll contents (Elsheery and Cao, 2008). Chloroplasts damage might be occurred, resulting in low content of chlorophyll (Sun and Guo, 2016). In this study, chlorophyll content (SPAD) of tomato seedlings under heat stress was improved by foliar application of chitosan.

Photosynthetic pigments, such as chlorophyll a and b are sensitive to heat stress. Alayafi (2020) reported that heat treatment decreased chlorophyll a (-67%) and chlorophyll b (-77%) of tomato seedlings during 8 h/day of acute heat shock at 40°C. The reduction of photosynthetic

pigments might be attributed to chlorophyll degradation or inhibition of chlorophyll biosynthesis (Dutta et al. 2009). In this study, the foliar application of chitosan on tomato seedlings showed significant improvement in chlorophyll a and chlorophyll b content with the application of nano-chitosan 100 ppm and bulk chitosan 100 ppm, respectively. Positive effect of chitosan in chlorophyll content has previously reported in tomato under drought stress (Hassnain et al. 2020), eggplant under heat and high irradiance (Liaqat et al. 2019), and barley under drought stress (Behboudi et al. 2018) as well. Foliar application of chitosan might increase photosynthesis pigments by enhancing endogenous levels of cytokinin, which play a key role in improving chlorophyll content in leaves (Hassnain et al. 2020).

Another physiological attribute related to chlorophyll content is net photosynthesis rate (Pn). Heat stress negatively affected the net photosynthesis rate (Pn) of tomato seedlings. Similar result was observed by Camejo et al. (2005) in two tomato cultivars under heat shock treatment (45^{o}C for 2 hours). Besides the degradation of chlorophyll, Guo et al. (2022) also reported that tomato plants exposed to heat had a lower net photosynthesis rate due to the inhibition of rubisco synthesis (Calvin cycle), a vital phase in photosynthesis, at temperatures between 35 and 40°C. In other cases, closing of stomata during heat stress preventing CO_2 to enter and PSII, complex proteins that has a crucial role in the electron transport that occurs during the photochemical stage of photosynthesis, might be inhibited, causing the decrease of net photosynthesis rate under heat stress (Mathur et al. 2014).

The foliar application of chitosan on tomato seedlings under heat stress showed a positive effect in the Pn. An increase in Pn using chitosan treatment might be associated with chitosan ability to alleviate the degradation of chlorophyll, promote chlorophyll synthesis, and increase the chlorophyll content of plants under stress, as Fu et al. 2023 reported the application of chitosan on strawberry seedlings under heat stress and light stress.

In response to heat stress, plants increase their transpiration rate due to the need for cooling or the need for water conservation dictated by increasing evaporative demand (Sadok et al. 2021). The result in this study is in accordance with the previous report, as the transpiration rate of tomato seedlings under heat stress were significantly higher compared to optimal temperature. Foliar application of chitosan to reduce transpiration was observed by Bittelli et al. (2001) in pepper. Similar finding was obtained in this study by chitosan treatment, especially in NC100 (-32.07%) and CH100 (-22.60%). Nano-chitosan showed a better result than bulk chitosan due to its smaller particle size that can penetrate through plant surface membrane with higher mobility, higher surface area, resulting in increased efficiency (Hidangmayum and

Dwivedi, 2022). The reduction of transpiration might be related to stomata closure in response to chitosan treatment (Bittelli et al. 2001).

The ratio of net photosynthesis to transpiration is related to water use efficiency (WUE). An increase in WUE was reported after exogenous use of chitosan on wheat under drought stress (Farouk and EL-Merwally, 2019). Chitosan treatments in this study slightly increased WUE in tomato seedlings with no significant difference than control. This increase might be associated with chitosan ability to improve chlorophyll content and net photosynthesis rate, while reducing transpiration rate.

Heat stress is frequently associated with the reduction of water availability and high transpiration rate, affecting the reduction of relative water content (RWC). RWC reduction could be associated with the reduction growth of the roots under heat stress, limiting the supply of water and nutrient (Wassie et al. 2019). Foliar application of chitosan in different concentrations contributed to the improvement of RWC in tomato seedlings. Similar results were also observed by Hassnain et al. (2020) in tomato under water stress with chitosan treatment. Exogenous chitosan application might have the ability to expand cell layer and improved antioxidant activities to maintain RWC level under stress condition (Hassnain et al. 2020).

Plants under stress conditions will activate the defense mechanisms to scavenge reactive oxygen species (ROS) by producing enzymatic and non-enzymatic antioxidants (Sharma et al. 2012). In this study, application of chitosan on tomato seedlings significantly improved the FRAP value by 12.60%-15.79%, showing the ability of chitosan in inducing plant innate immunity by activating defense-related responses to mitigate damage caused by stress (Ji et al. 2022).

Thermal stress (35°C day/night) induces the production of phenolic compounds in tomato plants as a defense mechanism (Rivero et al. 2001). The foliar application of chitosan on tomato seedlings induced the total phenolics content. This finding is in agreement with previous study that reported an increase of the total phenolics level in eggplant as adaptive mechanism in plants under heat stress (Liaqat et al. 2019).

6. SUMMARY

Exposure of high temperature on tomato seedlings caused reductions in the shoot length, relative water content, chlorophyll a, chlorophyll b, SPAD value, net photosynthesis rate, water use efficiency and an increase of electrolyte leakage. Increasing the transpiration rate, antioxidant capacity, and total phenolics content are some of the responses of tomato seedlings to cope with heat stress conditions.

In our findings, the foliar application of chitosan has positive influences in mitigating the adverse effects of heat stress on tomato seedlings:

(1) The application of chitosan under heat stress significantly improved relative water content, chlorophyll a, chlorophyll b, SPAD value, net photosynthesis rate, antioxidant capacity, and total phenolics content, while reducing the electrolyte leakage and transpiration rate.

(2) Bulk chitosan and nano-chitosan mitigate the effects of heat stress on tomato seedlings, however nano-chitosan showed more significant results than in bulk form due to its nano size.

(3) The most effective chitosan application to significantly mitigate the adverse effects of heat stress on tomato seedlings by improving the chlorophyll a (45.99%), SPAD value (6.46%), and antioxidant capacity of FRAP (15.79%), while reducing the electrolyte leakage (-37%) and transpiration rate (-32.07%) was nano-chitosan 100 ppm (NC100). The most effective chitosan application to significantly improve chlorophyll b (27.8%) and net photosynthesis rate (151.3%) was bulk chitosan 100 ppm (CH100). The application of nano-chitosan 150 ppm (NC150) was the most effective treatment to significantly improve relative water content (26%). And to significantly improve the total phenolics content (13.01 mg gallic acid equivalent g/FW), bulk chitosan 150 ppm (CH150) was the most effective treatment.

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9. LIST OF TABLE AND FIGURES

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DECLARATION

the public access and authenticity of the thesis

Student's name:	Erika Permana Sari
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As a supervisor of Erika Permana Sari (U9GYJY), I here declare that the master's thesis has been reviewed by me, the student was informed about the requirements of literary sources management and its legal and ethical rules.

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