



A plant-based biostimulant approach to improve salt stress tolerance in tomato: The role of *Hypericum heterophyllum* Vent. extract

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Domateste tuz stresi toleransını artırmak için bitki bazlı bir biyostimülan yaklaşımı: *Hypericum heterophyllum* Vent. özütünün rolü

Abstract: Salt stress severely limits seed germination and early seedling establishment in tomato. Plant-derived extracts have recently attracted attention as eco-friendly biostimulants capable of enhancing stress tolerance. The present study investigated the effects of different concentrations of *Hypericum heterophyllum* whole-plant extract on seed germination, seedling growth, and changes in physiological and biochemical parameters in tomato under salt stress. Tomato seeds were primed with aqueous *H. heterophyllum* extract at 0.25, 0.5, 1, and 2% (w/v) and subsequently exposed to NaCl-induced (200 mM) salinity stress. Germination percentage, mean germination time, root and shoot length (at early and late seedling stages), membrane damage, total chlorophyll content, and total flavonoid and phenolic contents in tomato leaves were evaluated. Salt stress markedly reduced germination and seedling growth, whereas *H. heterophyllum* extract treatments significantly alleviated these inhibitory effects in a concentration-dependent manner. The 0.5% and 1% extract concentrations were the most effective in improving germination performance and seedling growth under salt stress. These treatments also decreased membrane damage and increased total chlorophyll content. Moreover, increasing extract concentration led to higher total phenolic and flavonoid contents under salt stress. Overall, these findings suggest that *H. heterophyllum* extract has strong potential as a natural biostimulant and could be considered a promising, sustainable strategy to enhance salt stress tolerance and improve crop establishment in agricultural production.

Key words: *Hypericum heterophyllum*, salt stress, tomato, seed priming, biostimulant

Özet: Tuz stresi, domateste tohum çimlenmesini ve erken fide oluşumunu ciddi şekilde sınırlandırmaktadır. Bitki kaynaklı özler, son zamanlarda stres toleransını artırabilen çevre dostu biyostimülanlar olarak dikkat çekmektedir. Bu çalışma, *Hypericum heterophyllum* bitkisinin tamamının farklı konsantrasyonlarının, tuz stresi koşulları altında domateste tohum çimlenmesi, fide büyümesi ve bazı fizyolojik ve biyokimyasal parametrelerdeki değişiklikler üzerindeki etkilerini araştırmıştır. Domates tohumları, %0,25, %0,5, %1 ve %2 (w/v) oranlarında sulu *H. heterophyllum* özü ile ön işleme tabi tutulmuş ve daha sonra NaCl kaynaklı (200 mM) tuz stresine maruz bırakılmıştır. Çimlenme yüzdesi, ortalama çimlenme süresi, kök ve sürgün uzunluğu (erken ve geç fide aşamalarında), membran hasarı, toplam klorofil içeriği ve domates yapraklarındaki toplam flavonoid ve fenolik içerik değerlendirilmiştir. Tuz stresi, çimlenmeyi ve fide büyümesini belirgin şekilde azaltırken, *H. heterophyllum* özü uygulamaları, bu engelleyici etkileri konsantrasyona bağlı olarak önemli ölçüde hafifletmiştir. %0,5 ve %1'lik ekstrakt konsantrasyonları, tuz stresi altında çimlenme performansını ve fide büyümesini iyileştirmede en etkili olanlardı. Bu uygulamalar ayrıca membran hasarını azalttı ve toplam klorofil içeriğini artırdı. Dahası, ekstrakt konsantrasyonunun artması, tuz stresi altında daha yüksek toplam fenolik ve flavonoid içeriğine yol açtı. Genel olarak, bu bulgular *H. heterophyllum* ekstraktının doğal bir biyostimülan olarak güçlü bir potansiyele sahip olduğunu ve tarımsal üretimde tuz stresi toleransını artırmak ve mahsul gelişimini iyileştirmek için umut vadeden, sürdürülebilir bir strateji olarak değerlendirilebileceğini göstermektedir.

Anahtar Kelimeler: *Hypericum heterophyllum*, tuz stresi, domates, tohum ön işlem, biyostimülan

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1. Introduction

Salt stress is a significant abiotic stress factor limiting agricultural yield worldwide and is becoming increasingly critical, particularly with rising soil salinity (Ondrašek et al., 2022). Salt stress suppresses early root and shoot development by reducing germination rates, hindering water uptake (osmotic effect), causing ion toxicity, and metabolic imbalances in plants (Flowers and Colmer, 2008; Munns and Tester, 2008). This leads to significant changes in plant physiological processes and ultimately reduces crop yield. Plants adapt to salt stress through mechanisms such as regulating ion homeostasis, splitting and releasing ions intracellularly, and also develop protection through the

biosynthesis of osmoregulators (Balasubramaniam et al., 2023). Intensive research is being conducted on biotechnological approaches, genetic engineering, and appropriate agricultural management strategies to mitigate the effects of salt stress. In this context, efforts are being made to minimize the negative effects of salt stress in agricultural production through multidisciplinary solutions such as the development of salt-tolerant varieties and soil-water management (Alkharabsheh et al., 2021; Zhao et al., 2021; Raza et al., 2022).

Tomatoes (*Solanum lycopersicum*) are an important crop worldwide particularly critical for yield. Moderately sensitive to salt stress, tomatoes are preferred as model

plants for studying mechanisms of salt tolerance (Guo et al., 2022). Salt stress negatively affects tomato growth and development, leading to ion toxicity, osmotic stress, and metabolic imbalances that significantly reduce yield, especially during early development (Roşca et al., 2023). Biochemical and molecular pathways, hormone applications, seed preparation techniques (priming), and genetic improvement methods play a significant role in the adaptation of tomato plants to salt stress (Berrío and Cardenal-Rubio, 2025). Tomato varieties, and selecting and improving these varieties is of strategic importance for reducing the effects of salt stress are of strategic importance in reducing the effects of salt stress in agricultural production. For these reasons, tomato is a model plant that is extensively studied in both basic research and applied agriculture due to its sensitivity to salt stress and its economic value (Bogoutdinova et al., 2024; Borromeo et al., 2023).

Natural plant extracts, when used as biostimulants, plant resistance to abiotic stresses. These extracts promote seed germination, increase stress tolerance during early developmental stages, and contain antioxidant compounds that support plant physiological processes (Shukla et al., 2019). Seaweed-based extracts, in particular, not only promote plant growth but also improve root-microbe interactions, providing a protective effect against adverse environmental conditions such as salt stress (Oosten et al., 2017). Thanks to their positive effects on plant metabolites and signalling pathways, these biostimulants regulate gene expression and strengthen stress response mechanisms. Furthermore, natural plant extracts play a significant role in sustainable agriculture as an alternative to synthetic chemicals, offering environmentally friendly solutions. Therefore, the use of biostimulants as stress-reducing agents in agricultural production is increasing and shows promise in improving yield and quality in different plant species (Ying et al., 2022; Akram et al., 2022; Asif et al., 2023; Shiade et al., 2024).

The genus *Hypericum*, particularly *Hypericum perforatum* is a plant of significant pharmaceutical and industrial importance due to its high levels of bioactive compounds (e.g., hypericin, flavonoids, and phenolic compounds). Studies on this species show an increase in the production of secondary metabolites under different stress conditions (such as drought, low temperature, and salt stress), and these compounds support the stress tolerance of plants through antioxidant and protective effects (Torun et al., 2020; Eray et al., 2020; Bruňáková et al., 2022). For example, low temperature increases hypericin biosynthesis, while drought and salt stress increase the levels of phenolic compounds and activate antioxidant defence systems (Su et al., 2021). However, the specific effects and mechanisms of *Hypericum* species against salt stress have not been sufficiently investigated in the literature, leaving a significant knowledge gap in this area (Caliskan et al., 2017). Furthermore, *H. perforatum* can enhance organogenesis and bioactive compound production through growth regulators and biotechnological applications, thereby indirectly supporting stress tolerance (Nooh et al., 2025). In conclusion, while *Hypericum* extracts have significant potential to support plant growth and stress tolerance, further research is needed, particularly on their effects under salt stress conditions.

Hypericum heterophyllum is a species endemic to Türkiye, used in traditional medicine, and contains important bioactive compounds. Analyses of its flowers have revealed high levels of phenolic compounds, particularly chlorogenic acid, and flavonoids, exhibiting strong antioxidant activity (Erenler et al., 2023). Extracts prepared in methanol and acetone have the highest phenolic and flavonoid content and have yielded effective results in various radical scavenging tests (Yaman, 2020). Furthermore, in vitro cultures of *H. heterophyllum* plants have shown high levels of phenolic acids and flavonoids, and these samples exhibit superior antioxidant capacity compared to other plant parts. Leaf and flower extracts of the species also demonstrate biological activity against pests in stored grains, with leaf extracts, in particular, possessing insecticidal potential (Yaman and Şimşek, 2022). However, cytotoxic and proliferative effects of *H. heterophyllum* have also been observed in human cells, indicating that its biological activities are complex (Öcal and Eroğlu, 2012). However, we did not find any research specifically investigating the effects of *H. heterophyllum* plant extracts on the germination of seeds from other plants. However, studies on related species, such as *H. perforatum*, show that herbal extracts can have allelopathic effects, influencing seed germination and seedling growth in various plants, sometimes inhibiting these processes, thereby revealing both physiological and biochemical impacts (Rys et al., 2022). More broadly, plant extracts from different species have been tested for their impact on seed germination, with some showing inhibitory or stimulatory effects depending on the extract and target species (Patil et al., 2018; Findura et al., 2020). The biological activity of plant extracts may vary depending on the plant species and extraction methods used. Although allelopathic interactions among plants have been widely reported in the literature, plant extracts are also known to act as biostimulants that can enhance plant growth and stress tolerance. In particular, plant-derived compounds may contribute to improved seed germination and early seedling development under adverse environmental conditions such as salinity. However, information regarding the potential effects of *H. heterophyllum* extracts under salt stress conditions remains limited. Therefore, the present study aims to evaluate the effects of *H. heterophyllum* extracts on seed germination and early seedling growth under salt stress. To address this gap, this study aimed to determine the effects of different concentrations of *H. heterophyllum* extract under salt stress on germination performance and selected physiological parameters of tomato seeds.

2. Materials and Methods

2.1. Plant material identification and collection

Hypericum heterophyllum Vent. plant material was collected from the Yaylabayır locality of Sındırgı, Balıkesir, Türkiye, at an altitude of approximately 1200 m above sea level. The plants were harvested in late June to early July 2021, during the flowering period. Voucher specimens were deposited in the KATO Herbarium of the Faculty of Forestry, Karadeniz Technical University.

2.2. Preparation of *Hypericum heterophyllum* Extract

All parts of the *H. heterophyllum* plant were thoroughly washed with distilled water and left to air dry. The plant



Figure 1. General view of the *Hypericum heterophyllum* and its natural habitat in Yaylabayır, Sındırgı-Balıkesir.

extract was prepared using a simple aqueous extraction method. For this purpose, 30 g of plant material was placed in 1.5 L of distilled water and left to extract at room temperature for 24 hours with continuous stirring. The resulting mixture was filtered through a 0.22 μ m membrane sterile extract. The filtered solution was considered the stock solution (2% w/v). This stock solution was then diluted with distilled water to prepare working concentrations of 0.25%, 0.5%, 1%, and 2% (w/v), and stored under appropriate conditions until use in experiments.

2.3. Seed material and experimental design

Tomato seeds were surface-sterilised with 1% sodium hypochlorite for 5 min, rinsed thoroughly with distilled water, and primed in the respective H. heterophyllum extract solutions (HhE) for 24 h at 25 °C in the dark. Following priming, the seeds were subjected to salt stress by applying a 200 mM NaCl solution for 24 h along with the seed-priming reagents reported in a previous study (Habibi et al., 2024), and were then incubated under controlled conditions. Seeds soaked in distilled water for the same duration as the priming treatments, without any additional application, were used as the control group. Ten primed tomato seeds from each treatment group were incubated in a petri dish under controlled conditions. Then, five germinated seeds were sown in pots containing a soil-perlite mixture, and plant growth was carried out in a growth chamber at 25 $\pm$ 2 °C with 60–70% relative humidity. After seven weeks, the plants were harvested.

2.4. Evaluation of germination

2.4.1. Germination percentage

The number of germinated seeds was recorded daily for a period of 5 days. Based on these daily observations, several germination parameters were calculated to evaluate salt tolerance. Seeds were considered germinated when the emerging radicle reached approximately 2 mm in length by the third day after sowing. In addition, seeds showing visible cotyledons by the end of the fifty days were also counted as germinated (Ranal and Santana, 2006).

2.4.2. Mean germination time

Mean Germination Time reflects the average duration required for seeds to germinate. Lower MGT values indicate a more rapid and uniform germination within the seed population (Mauromicale and Licandro, 2002).

2.5. Evaluation of seedling growth

To evaluate seedling development, ten seedlings were randomly taken from each Petri dish at the end of the

germination period. After sampling, shoot length (SL) and root length (RL) were measured and recorded in centimetres (cm). In addition, after 7 weeks (harvesting time), SL and RL were determined.

2.6. Physiological and biochemical analyses

The TBARS content of leaf tissues was determined according to the method of Heath and Packer (1968). Leaf samples (0.1 g) were homogenised in 0.1% trichloroacetic acid (TCA) and centrifuged. An aliquot of 1 mL supernatant was mixed with 0.5% thiobarbituric acid (TBA) prepared in 20% TCA, and absorbance was measured at 532 and 600 nm. Fresh leaf tissue (0.1 g) was ground in liquid nitrogen and homogenised in 80% acetone at 4 °C. The homogenate was centrifuged, and the absorbance of the supernatant was measured at 645 and 663 nm. Total chlorophyll content was calculated according to Arnon (1949). Total phenolic content was determined spectrophotometrically using the Folin–Ciocalteu method described by Singleton et al. (1999). Fresh leaf samples (0.1 g) were homogenised in 95% methanol and centrifuged. The supernatant was reacted with Folin–Ciocalteu reagent and Na₂CO₃, incubated in the dark, and the absorbance was measured at 765 nm. Total flavonoid content was determined by the colourimetric method of Kim et al. (2003). Fresh leaf samples (0.1 g) were homogenised in acidic methanol, centrifuged, and the extract was reacted sequentially with NaNO₂, AlCl₃, and NaOH. Absorbance was recorded at 510 nm, and flavonoid content was expressed as mg quercetin per 100 g fresh weight.

2.7. Statistical analysis

All experiments were performed with at least three replicates. The collected data were analysed using one-way analysis of variance (ANOVA) in SPSS software (version 23.0). Differences among treatment means were evaluated using Duncan's multiple range test.

3. Results

3.1. Effects of different HhE concentrations on germination and seedling growth under salt stress

The application of 200 mM NaCl resulted in a significant decrease in germination percentage (GP) and an increase in average germination time (MGT) compared with the control group (Table 1). GP, which was 90% in the control, decreased to 70% under salt stress, while MGT increased from 2.73 days to 4.14 days. HhE applications modulated the adverse effects of salt stress in a dose-dependent manner. Specifically, the 0.5% and 1% HhE + NaCl combinations increased GP to 80% and 85%, respectively, and reduced MGT to ~3.22 days, providing a significant

Table 1. Effects of different HhE concentrations on tomato seed germination under salt stress. Lowercase letters indicate statistically significant differences between treatment means ($p \leq 0.05$)

Applications	GP (%)	MGT (Day)
Control	90 $\pm$ 1 ^a	2.73 $\pm$ 0.1 ^e
200 mM NaCl	70 $\pm$ 3 ^c	4.14 $\pm$ 0.2 ^b
0.25% HhE+200 mM NaCl	75 $\pm$ 1 ^c	3.53 $\pm$ 0.2 ^c
0.5% HhE+200 mM NaCl	80 $\pm$ 3 ^b	3.22 $\pm$ 0.1 ^d
1% HhE+200 mM NaCl	85 $\pm$ 2 ^b	3.22 $\pm$ 0.3 ^d
2% HhE+200 mM NaCl	60 $\pm$ 3 ^d	4.19 $\pm$ 0.2 ^a

improvement compared to the NaCl group. In contrast, the 2% HhE + NaCl application reduced GP to 60% and increased MGT to 4.19 days, showing the lowest performance.

When seedling growth parameters were examined (Table 2), NaCl application reduced root length (RL) and shoot length (SL). While RL was 3.5 cm and SL was 4.0 cm in the control, RL was determined to be 2.0 cm, and SL was 3.0 cm in the NaCl group. The 0.25% HhE + NaCl treatment further suppressed growth (RL 1.8 cm; SL 2.5 cm), but the 0.5% and 1% HhE doses showed a remedial effect against salt stress. The highest RL (2.5 cm) and SL (3.2 cm) values were obtained with the 1% HhE + NaCl treatment. The 2% HhE + NaCl dose yielded the lowest values for both RL (1.5 cm) and SL (2.0 cm). These findings indicate that low and medium HhE doses support seedling development under salt stress, while high doses may have an inhibitory effect. Additionally, changes in plant shoot and root lengths are shown in Figure 2.

3.2. Effects of different HhE concentrations on seedling growth at harvest stage under salt stress

Salt stress significantly affected seedling growth parameters at the final harvest stage (Fig. 3). Exposure to 200 mM NaCl reduced both shoot length and root length compared with the control. HhE applications exhibited dose-dependent effects on seedling development under salt stress conditions. Low and moderate HhE concentrations (0.25%, 0.5%, and 1%) partially alleviated the inhibitory effects of NaCl on shoot length, with values statistically grouped and higher than the NaCl-only treatment. The most pronounced improvement was observed at 1% HhE, which resulted in the greatest shoot length among the salt-treated groups. In contrast, the 2% HhE treatment resulted in the greatest decline in shoot length. A similar trend was observed for root length. Salt stress markedly suppressed root growth, whereas 1% HhE significantly enhanced root

Table 2. Effects of different HhE concentrations on tomato seedling growth under salt stress after 5 days of germination. Lowercase letters indicate statistically significant differences between treatment means ($p \leq 0.05$)

Applications	RL(cm)	SL(cm)
Control	3.5±0.1 ^a	4±0.08 ^a
200 mM NaCl	2±0.2 ^c	3±0.1 ^c
0.25% HhE+200 mM NaCl	1.8±0.1 ^c	2.5±0.1 ^e
0.5% HhE+200 mM NaCl	2±0.1 ^c	2.8±0.07 ^d
1% HhE+200 mM NaCl	2.5±0.2 ^b	3.2±0.1 ^b
2% HhE+200 mM NaCl	1±0.1 ^d	2±0.1 ^f

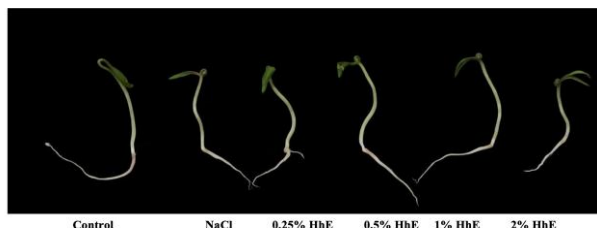


Figure 2. Representative images showing the effects of different concentrations of *H. heterophyllum* extract (HhE) on tomato seedling growth under 200 mM NaCl stress after 5 days of germination. Seeds were primed with 0, 0.25, 0.5, 1, and 2% (w/v) HhE prior to salt treatment

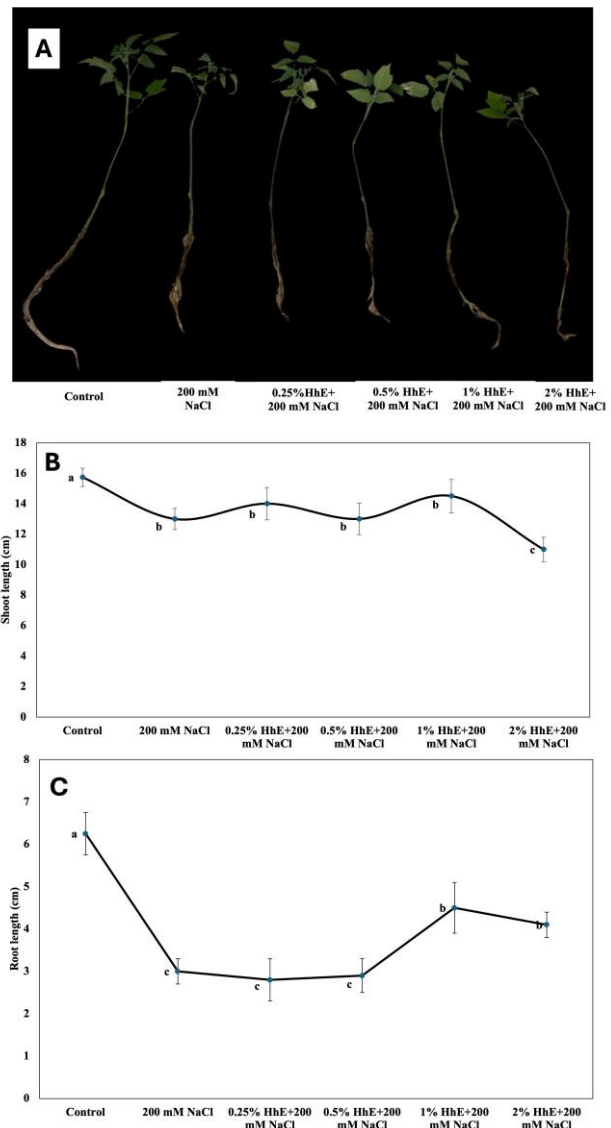


Figure 3. Effects of different concentrations of *H. heterophyllum* extract (HhE) on tomato seedling growth under salt stress (200 mM NaCl). (A) Representative images of seedlings obtained from seeds primed with 0, 0.25, 0.5, 1, and 2% (w/v) HhE. (B) Shoot length and (C) root length measurements of tomato seedlings under the same treatments. Lowercase letters indicate statistically significant differences between treatment means ($p \leq 0.05$)

length compared with the NaCl treatment. The 0.25% and 0.5% HhE concentrations did not produce a statistically significant improvement, while 2% HhE showed only a slight increase relative to NaCl. Overall, these findings indicate that HhE modulates seedling growth under salt stress in a concentration-dependent manner, with 1% HhE providing the most effective mitigation of salt-induced growth inhibition.

3.3. Physiological and biochemical responses

Salt stress and HhE treatments significantly influenced the physiological and biochemical parameters of tomato seedlings at the final harvest stage (Fig. 4). Salt stress (200 mM NaCl) markedly increased TBARS content compared with the control, indicating enhanced lipid peroxidation. HhE application modulated this response in a concentration-dependent manner. While lower concentrations (0.25% and 0.5%) slightly reduced TBARS levels relative to NaCl alone, the 1% HhE treatment

resulted in a more pronounced decrease. In contrast, the highest concentration (2% HhE) caused a substantial increase in TBARS, exceeding even the NaCl treatment (Fig. 4a). Total chlorophyll content was significantly reduced under salt stress. However, HhE treatments, particularly at 0.5% and 1%, mitigated this reduction and partially restored chlorophyll levels. The 1% HhE concentration produced the highest chlorophyll content among salt-treated plants, whereas 2% HhE led to a decline similar to or lower than the NaCl treatment (Fig. 4b). Total phenolic content increased under salt stress and was further enhanced by HhE applications. This increase was progressive with rising HhE concentrations, with the highest values observed at 2% HhE. A similar pattern was observed for total flavonoid content, which gradually and significantly increased with increasing HhE doses. The greatest flavonoid accumulation occurred at 2% HhE (Fig. 4c,d).

4. Discussions

In this study, the effects of *H. heterophyllum* extract at different concentrations under salt stress on the germination performance and physiological characteristics of tomato seeds were investigated. The results showed that extract application, especially at low and medium concentrations, could mitigate the negative effects of salt stress. Findings regarding germination percentage and germination rate revealed that 0.5% and 1% extract applications were the most effective doses. Similarly, a significant increase in root and shoot lengths was observed at these concentrations, suggesting that the extract may support early growth processes.

Salt stress causes osmotic imbalance and ion toxicity in plants, limiting germination and seedling development. The increase in germination observed in this study can be attributed to the biostimulant effects of plant extracts. Indeed, previous studies have reported that different plant extracts can have both stimulating and suppressive effects on seed germination and early development. In particular, plant extracts can alter germination and growth processes depending on concentration: low doses can stimulate

growth, while high doses can cause phytotoxicity (Patil et al., 2018; Rys et al., 2022). This is consistent with the fact that 0.5% and 1% doses yielded positive results in the present study, while higher doses showed a more limited effect.

The observed increase in root and shoot lengths may be related to components in the extract that support cellular growth. Similarly, the study by Findura et al. (2020) reported that allelopathic plant extracts can modulate germination and seedling development when used as seed-coating materials. It is also stated that some medicinal plant extracts exert regulatory effects on plant metabolism and indirectly affect growth processes.

When physiological parameters were examined, the decrease in TBARS content was particularly noteworthy in the 0.5% and 1% extract applications. The decrease in TBARS levels indicates that lipid peroxidation is reduced and, therefore, oxidative damage is limited. The increase in reactive oxygen species under salt stress can damage the membrane structure in plant cells. Therefore, the application of the extract reduces oxidative stress. These findings are consistent with those reported by Macar and Macar (2024), which showed that extracts of *Hypericum* species can reduce cellular damage under stress conditions. The observed increase in chlorophyll content is another important finding supporting the extract's protective effect. While salt stress generally reduces photosynthetic pigments, partial prevention of this loss with extract application suggests that plant metabolism is maintained in a more balanced state. However, the increase in total phenolic and flavonoid content with increasing concentration indicates that the plant's defence mechanisms are activated. Phenolic compounds and flavonoids are important metabolites that play a protective role in cells under stress conditions due to their antioxidant properties. These results are also consistent with studies reporting that plant extracts can increase secondary metabolite production. However, it should also be considered that phenolic and flavonoid compounds present in the *H. heterophyllum* extract itself may have contributed to the total amounts measured in the seedlings. Since a treatment group consisting of seedlings treated only with the extract was not included in the experimental design, it is difficult to clearly distinguish between compounds absorbed from the extract and those synthesized by the plant. Future studies including such control treatments would help to clarify this contribution. On the other hand, some studies have indicated that *Hypericum* species can exhibit allelopathic or phytotoxic effects (Alipour et al., 2013; Shurigin et al., 2018). This situation may vary depending on the extract's content and application dose. As emphasised by Rys et al. (2022), the effects of plant extracts can vary depending on species, dose, and target plant. In this context, the fact that low and medium concentrations yielded positive results in the present study, whereas higher doses did not show the same level of stimulation, supports this view. Overall, the findings show that *H. heterophyllum* extract, when applied at appropriate concentrations, can reduce the negative effects of salt stress on germination and early seedling development. When the increase in germination performance, support of root and shoot development, reduction of oxidative damage, and increase in phenolic compounds are considered together, it can be said that this extract has the potential to be used as a natural

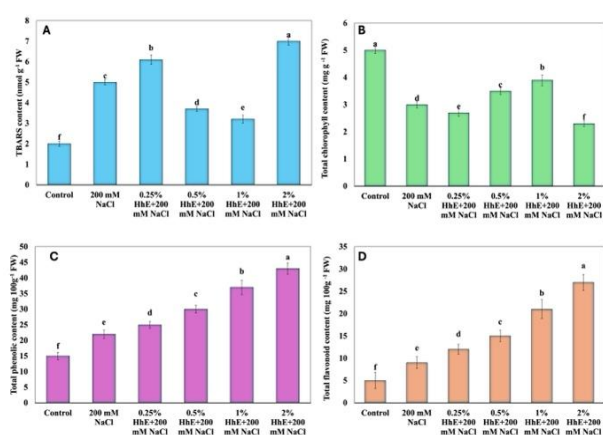


Figure 4. Effects of different concentrations of *H. heterophyllum* extract (HhE) on physiological and biochemical parameters of tomato seedlings under salt stress (200 mM NaCl). (A) TBARS content (membrane damage), (B) total chlorophyll content, (C) total phenolic content, and (D) total flavonoid content were measured in seedlings obtained from seeds primed with 0, 0.25, 0.5, 1, and 2% (w/v) HhE. Lowercase letters indicate statistically significant differences between treatment means ($p \leq 0.05$).

biostimulant. These results demonstrate that plant-derived natural products can serve as an alternative to support plant growth under stressful conditions and contribute to the literature in this field.

It has been observed that *H. heterophyllum* extract has a positive effect on the germination and growth of tomato seeds under salt stress. Extract applications at concentrations of 0.5% and 1% yielded the best results in germination percentage and root-shoot length; furthermore, TBARS content (an indicator of lipid peroxidation) decreased, and total chlorophyll content increased. Total phenol and flavonoid content increased with increasing extract concentration under salt stress, suggesting that the plant's antioxidant defence mechanisms were strengthened.

However, an interesting response was observed at the 2% extract concentration. Although this treatment showed the highest phenolic and flavonoid contents, it resulted in inhibitory effects on germination, growth, and physiological parameters. The increased TBARS levels and decreased chlorophyll content in this group indicate enhanced oxidative stress. This finding suggests that while lower concentrations of the extract may act as biostimulants, higher concentrations may exert phytotoxic or allelopathic effects. Such dose dependent responses of plant extracts have been reported in previous studies and emphasize the importance of determining optimal application concentrations. These findings are consistent with other studies supporting the protective effects of plant extracts against salt stress; for example, phytotoxic or stimulating effects of different plant extracts on seed germination and growth have been reported in the literature (Rys et al., 2022; Papoui and Koukounaras, 2025; Luong

and Luong, 2025). Furthermore, while extracts of species such as *H. perforatum* are used in weed control, it is recommended that preliminary tests be conducted to prevent adverse effects on cultivated plants (Almutairi Arabia, 2016; Rys et al., 2022). Biostimulants and microorganism inoculations have also been found effective in increasing plant resistance to salt stress; in this context, *H. heterophyllum* extract can be considered to activate biochemical defence pathways (Xu et al., 2022; Ge et al., 2023). In conclusion, *H. heterophyllum* extract can be evaluated as a potential natural agent for mitigating salt stress in tomato seeds, but optimising dosage and application method is important.

Conflict of Interest

The authors declare that there is no conflict of interest.

Authors' Contribution

ASM: Investigation, Resources, Visualization, Software, Formal Analysis, and Writing-original draft; UM: Investigation, Resources, Formal Analysis, and Validation. Authors may edit this part based on their case.

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