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Effects of Kinetin Applications on Growth and Morphological Damage in Cucumber (*Cucumis sativus* L.) Seedlings under Salt Stress Conditions

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Abstract
This study was conducted to evaluate the effects of different exogenous kinetin concentrations (10, 20, 30, 40, 50, and 60 µM) on plant growth and morphological damage in cucumber seedlings (*Cucumis sativus* L. cv. 'BT Bursalfa') under salinity stress. The experiment was carried out in a hydroponic system under controlled growth chamber conditions at Van Yuzuncu Yıl University, Faculty of Agriculture, Physiology Laboratory. Cucumber seedlings were subjected to 50 mM NaCl-induced salt stress and subsequently treated with various kinetin concentrations. Control plants were grown under optimal conditions without stress and hormone treatments. Total plant fresh weight, root length, root fresh weight, shoot fresh weight, leaf fresh weight, leaf number, plant height, shoot diameter, and internode distance were measured. Morphological damage was evaluated using a 1–5 visual scale. Data were analyzed using one-way ANOVA and Duncan's multiple range test ($p<0.05$). Results indicated that 50 mM NaCl salinity stress significantly reduced plant growth parameters and induced visual damage symptoms. Among kinetin treatments, 20 µM showed the most effective improvement in growth traits, enhancing root development and biomass accumulation under stress. Conversely, suboptimal (10 µM) and particularly supraoptimal (30–60 µM) kinetin concentrations inhibited plant growth, exacerbated morphological damage, and led to extreme phytotoxicity at 60 µM. These findings highlight the importance of optimal kinetin dosage under salinity stress conditions and demonstrate a clear biphasic (hormetic) dose-response relationship of exogenous cytokinin in cucumber seedlings.

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1. Introduction

Soil salinity stands as one of the most devastating environmental challenges restricting agricultural productivity worldwide, affecting approximately 20% of cultivated land and nearly half of all irrigated areas (Munns and Tester, 2008). The escalating salinization of agricultural lands is driven by both primary natural factors and rapid anthropogenic activities, including long-term intensive fertilizer application, improper irrigation practices, and the utilization of brackish water (Kamran et al., 2021). High salinity disrupts normal plant-water relations by inducing osmotic stress, commonly referred to as "physiological drought," which severely limits the ability of roots to extract water from the surrounding medium (Parida and Das, 2005; Kamran et al., 2021). Following this initial osmotic phase, the accumulation of toxic ions—primarily sodium (Na^+) and chloride (CI^-)—within plant tissues leads to ionic toxicity (Munns and Tester, 2008). This ionic phase results in nutrient deficiencies due to competitive uptake inhibition (such as the displacement of potassium (K^+) by the disruption of photosynthetic electron transport, and the suppression of essential metabolic pathways (Banja and Oyetunji, 2025; Kamran et al., 2021). Furthermore, salinity induces secondary oxidative stress through the excessive generation of reactive oxygen species (ROS), which triggers lipid peroxidation, membrane degradation, protein denaturation, and accelerated leaf senescence (Zhou et al., 2024). To cope with salinity-induced osmotic and ionic stress, plants have evolved complex adaptive strategies. These include the synthesis of compatible osmolytes (such as proline, soluble sugars, and proteins) for osmotic adjustment, and the upregulation of enzymatic antioxidant defense systems (including superoxide dismutase, peroxidase, and catalase) to maintain ROS homeostasis (Kamran et al., 2021). Phytohormones play a pivotal role as signaling molecules coordinating these physiological and biochemical adaptations (Hamayun et al., 2015). Cytokinins (CKs), a class of plant hormones essential for cell division, chloroplast development, nutrient mobilization, and the delay of leaf senescence, are highly sensitive to environmental stressors (Werner and Schmülling, 2009; Sharma, 2016). Under salinity stress, endogenous cytokinin biosynthesis is markedly inhibited, leading to reduced activity of the shoot apical meristem, accelerated leaf senescence, and overall stunted plant growth (Hamayun et al., 2015). Exogenous application of synthetic cytokinins, particularly kinetin (6-furfurylaminopurine), has emerged as an effective strategy to mitigate the adverse effects of salt stress in various crop species (Yasin et al., 2018). Kinetin application has been reported to restore hormonal balance, enhance chlorophyll biosynthesis, stabilize photosynthetic proteins, and stimulate the antioxidant machinery under saline conditions (Hamayun et al., 2015; Kamran et al., 2021). For instance, exogenous kinetin successfully alleviated salinity-induced growth inhibition in soybean (*Glycine max*) by modulating endogenous hormone levels (Hamayun et al., 2015), and improved growth, relative water content, and biomass production in salt-stressed black gram (*Phaseolus mungo*) (Yasin et al., 2018). However, the efficacy of exogenous kinetin is highly dependent on the concentration applied, the plant species, and the developmental stage (Sharma, 2016). While optimal concentrations of kinetin can promote cell elongation and stress tolerance, elevated or supraoptimal concentrations can exert inhibitory or even toxic effects on plant tissues (Sharma, 2016). Cucumber (*Cucumis sativus* L.) is a widely cultivated, economically vital vegetable crop that is classified as moderately sensitive to salinity (Elsheery et al., 2020). Although previous studies have investigated the general physiological responses of cucumber to salt stress (Elsheery et al., 2020; Gurmani et al., 2018), detailed investigations into the dose-dependent, biphasic (hormetic) effects of exogenous kinetin on cucumber seedlings under salinity stress remain limited. Therefore, this study was designed to evaluate the effects of a wide range of exogenous kinetin concentrations (10 to 60 µM) on the basic growth parameters and

morphological damage levels of cucumber seedlings (*Cucumis sativus* L. cv. 'BT Bursalfa') subjected to $NaCl^-$ induced salt stress. The primary objective was to identify the optimal kinetin concentration required to alleviate salinity damage Na^+ Cl^- K^+ Na^+ and to elucidate the threshold at which exogenous kinetin transitions from a protective agent to a phytotoxic inhibitor.

2. Material and Methods

2.1. Plant material and growing conditions

This research was conducted at the Physiology Laboratory of the Department of Horticulture, Faculty of Agriculture, Van Yuzuncu Yıl University. Seeds of the 'BT Bursalfa' cucumber (*Cucumis sativus* L.) cultivar were used as plant material. The experiment was conducted in a climate-controlled growth chamber using a hydroponic system (Figure 1). Seeds were germinated in plastic containers filled with a peat and pumice mixture (2:1 v/v). Nine days after germination, when cotyledon leaves had assumed horizontal orientation and the first true leaves had emerged, seedlings were transferred to modified Hoagland nutrient solution (Hoagland and Arnon, 1938). Plants were cultivated in the climate-controlled growth chamber under the following conditions:

•Temperature: 25 °C

•Photoperiod: 16 hours light / 8 hours darkness

• Light intensity: 500 µmol m^{-2} s^{-1} (daylight fluorescent lamps)

• Relative humidity: 60 %

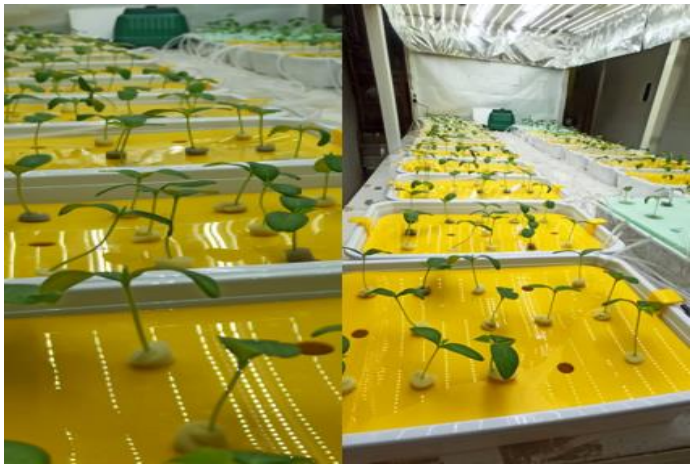

Figure 1. Laboratory view of cucumber seedlings utilized in the study

In the hydroponic system, seedlings were secured to trays placed on tables to ensure root contact with the nutrient solution. Continuous aeration of the solution was maintained throughout the study using an aquarium air pump, and nutrient solutions were replaced weekly. The composition of the nutrient solution used in the study is presented in Table 1.

Table 1. Composition of the nutrient solution (mg L^{-1}) used in the study and chemical sources

Elements	Concentration (mg L^{-1})	Macronutrient Sources	Micronutrient Sources
Nitrogen (N)	176	$Ca(NO_3)_2 \cdot 4H_2O$	$C_6H_5FeO_7 \cdot 4H_2O$
Phosphorus (P)	31	KNO_3	$MnCl_2$
Potassium (K)	146	KH_2PO_4	H_3BO_3
Magnesium (Mg)	24	$MgSO_4$	$ZnCl_2$
Calcium (Ca)	200	–	$CuCl_2 \cdot 2H_2O$
Sulfur (S)	34	–	–
Iron (Fe)	3.3	–	–
Manganese (Mn)	0.050	–	–
Boron (B)	0.457	–	–
Copper (Cu)	0.015	–	–
Zinc (Zn)	0.055	–	–

2.2. Determination of basic growth parameters

To determine the effects of treatments on cucumber plants, various morphological parameters were examined. Total plant fresh weight, root, shoot, and leaf fresh weights were measured in triplicate for each treatment group using a digital balance with 0.0001 g precision. Plant height and root length were measured using a standard ruler (cm), while shoot diameter and internode distance were measured using digital calipers (mm). Additionally, the number of leaves on each plant was recorded as a count.

2.3. Determination of salt stress damage level (1-5 Scale)

A 1-5 visual scoring scale was employed to determine the level of morphological damage in plants resulting from salt stress. This evaluation was conducted using a method developed by Uzal (2009) and modified to assess the severity of stress symptoms. Each plant was scored according to the following criteria based on observed physiological symptoms:

-Plants unaffected by salt stress, healthy (Control group).

-Local leaf yellowing and mild leaf curling.

-Pronounced leaf yellowing and necrotic spot formation at approximately 25% of leaf area.

-Necrosis affecting 50–75% of leaf surface and partial plant death.

-Severe necrosis affecting 75–100% of leaves and complete plant death.

2.4. Statistical analysis

Statistical evaluation of the obtained data was performed using one-way analysis of variance (ANOVA) to determine differences among treatments. Duncan's multiple range test was employed to group significant differences. All statistical analyses were conducted using SPSS software (Version 21.0), and the statistical significance level was set at $p<0.05$.

3. Results

The study examined the dose-dependent effects of different kinetin concentrations (10, 20, 30, 40, 50, and 60 µM) applied to cucumber seedlings under salt stress (50 mM NaCl) on plant development and stress tolerance. Experimental results were evaluated comparatively with the control group (C) and salt-treated group (S) (Figure 2, Figure 3, Figure 4).



Figure 2. General appearance of cucumber seedlings two weeks following transfer to hydroponic culture



Figure 3. Views from the stages of the application process



Figure 4. General view of plants prior to harvest (5 days post-application)

3.1. Salt stress damage level (1-5 Scale) findings

According to the 1-5 scale system employed to quantitatively evaluate salt stress-induced phytotoxicity, control group plants exhibited no stress symptoms (scale=1.0), confirming maintenance of optimal growth conditions. Plants subjected solely to 50 mM NaCl stress (T group) exhibited a scale value of 1.84, with typical salt-induced damage symptoms manifesting. Upon evaluation of the effects of exogenous kinetin applications on stress response, a positive correlation was established between increasing kinetin concentrations and damage severity. Specifically, at low kinetin concentrations (10 µM and 20 µM), scale values were measured at 1.79 and 2.16, respectively; however, when concentration was elevated to 30 µM and 40 µM levels, the damage index increased to 3.09 and 3.50, respectively. Maximum phytotoxic effects were detected at 50 µM (4.64) and 60

µM (4.82) kinetin concentrations, with the extreme toxicity induced by 60 µM kinetin concentration preventing determination of specific growth parameters (ND) (Figure 5).

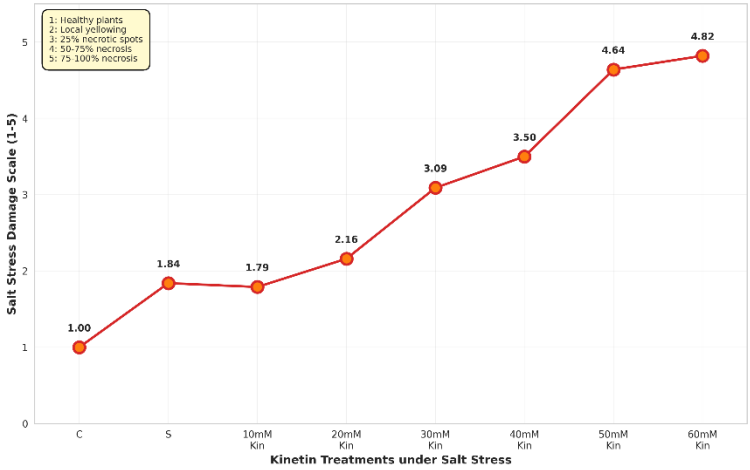

Figure 5. Effects of different kinetin concentrations on the morphological damage scale (1-5) of cucumber seedlings under 50 mM NaCl salt stress

3.2. Plant development parameters findings

Kinetin applications induced statistically significant differences ($p<0.05$) in fundamental growth parameters of cucumber seedlings (Table 2).

Table 2. Effects of kinetin treatments on plant development parameters in cucumber seedlings

Treatment	Total Plant Wt. (g)	Root Length (cm)	Root Wt. (g)	Shoot Wt. (g)	Leaf Wt. (g)	Leaf Number	Plant Height (cm)	Shoot Diameter (mm)	Internode Distance (mm)
C	15.11 a	34.83 bc	3.04 a	3.93 a	9.10 a	7.33 a	15.60 b	4.44 a	10.62 a
S	12.02 bc	34.00 c	2.57 ab	3.59 a	6.96 bc	6.00 b	18.53 a	3.89 ab	9.24 bc
10 µM Kin	7.72 d	29.67 d	1.47 d	2.54 b	4.09 e	5.67 b	12.37 d	3.31 b	10.16 ab
20 µM Kin	13.57 ab	43.17 a	2.75 a	3.91 a	7.61 b	7.67 a	16.03 b	3.62 ab	9.81 ab
30 µM Kin	11.35 c	38.33 b	2.00 bc	3.14 ab	6.05 cd	6.00 b	14.00 c	3.74 ab	5.80 d
40 µM Kin	10.61 c	33.50 c	1.71 c	2.55 b	5.45 d	5.33 b	12.67 cd	3.79 ab	8.95 bc
50 µM Kin	10.93 c	36.33 bc	1.86 c	2.95 ab	6.40 cd	5.67 b	13.90 c	3.40 b	8.48 c
60 µM Kin	ND	ND	ND	ND	ND	ND	ND	ND	ND

Means in the same column marked with different lowercase letters are significantly different ($p<0.05$, Duncan test). C: Control (no stress applied), S: 50 mM NaCl (salt stress applied), Kin: Kinetin, ND: Could not be determined due to high phytotoxicity.

Regarding total plant fresh weight, maximum biomass accumulation was recorded in the control group (15.11 g), whereas salt stress alone (S) significantly reduced this parameter to 12.02 g. Among the kinetin treatments, the 20 µM concentration (13.57 g) was the most effective in alleviating the adverse effects of salt stress, restoring the biomass to a level statistically comparable to the unstressed control (13.57 g vs. 15.11 g, $p>0.05$). Conversely, the suboptimal concentration of 10 µM resulted in a severe reduction in total plant weight (7.72 g). Supraoptimal concentrations (30–50 µM) also significantly restricted biomass accumulation, ranging from 10.61 g to 11.35 g. For root length, the highest value was recorded at 20 µM kinetin application (43.17 cm), which was statistically superior to both the control group (34.83 cm) and the salt-stressed group (34.00 cm). This represents a remarkable 27% increase in root length compared to the salt-stressed control, indicating that optimal kinetin application strongly stimulated root elongation. However, root length declined at higher concentrations, dropping to 38.33 cm at 30 µM and further down to 33.50 cm at 40 µM. A similar trend was observed for root fresh weight, where the 20 µM kinetin treatment yielded the highest weight (2.75 g) among

stressed plants, whereas the 10 µM treatment severely suppressed root biomass (1.47 g). Shoot fresh weight and leaf fresh weight both showed significant recovery under the 20 µM kinetin treatment. Shoot weight increased from 3.59 g in the salt-stressed group to 3.91 g, and leaf weight rose from 6.96 g to 7.61 g. Furthermore, the number of leaves per plant reached its maximum under the 20 µM kinetin treatment (7.67), which was significantly higher than the salt-stressed group (6.00) and even numerically exceeded the control group (7.33). Interestingly, plant height exhibited a unique pattern. The highest plant height was recorded in the salt-stressed group (18.53 cm), compared to 15.60 cm in the control and 16.03 cm in the 20 µM kinetin group. This increase under salt stress suggests a stress-induced etiolation-like elongation response. High concentrations of kinetin strongly suppressed plant height, with values declining to a range of 12.67–14.00 cm. Shoot diameter and internode distance were also significantly affected. Internode distance, which was 10.62 mm in the control, was maintained at 9.81 mm under the 20 µM kinetin treatment, but was severely compressed to 5.80 mm under the 30 µM kinetin treatment, indicating a severe dwarfing effect at higher concentrations.

4. Discussion

The results of this study clearly demonstrate that 50 mM NaCl-induced salinity stress significantly restricts the growth of cucumber seedlings, as evidenced by reductions in total plant fresh weight, root weight, and leaf number. These findings align with the established consensus that salinity stress impairs plant development through immediate osmotic effects and subsequent ionic accumulation (Parida and Das, 2005; Munns and Tester, 2008). The reduction in biomass under salt stress is primarily attributed to osmotic limitations on water uptake, which reduces cell turgor and expansion, alongside the toxic accumulation of Na^+ and Cl^- ions that impairs photosynthetic efficiency (Elsheery et al., 2020; Kamran et al., 2021). The most prominent finding of our study is the distinct biphasic (hormetic) dose-response relationship of exogenous kinetin on cucumber seedlings under salt stress. Hormesis is a biological phenomenon characterized by low-dose stimulation and high-dose inhibition (Calabrese and Baldwin, 2002). In our experiment, the 20 µM kinetin treatment acted as a highly effective biostimulant, significantly alleviating salinity-induced growth inhibition. Under 20 µM kinetin, total plant fresh weight, shoot fresh weight, leaf fresh weight, and leaf number were restored to levels close to or even exceeding those of the unstressed control. Most notably, root length was significantly enhanced (43.17 cm) compared to both the salt-stressed (34.00 cm) and control (34.83 cm) plants. This protective effect of optimal kinetin can be explained by several physiological mechanisms. Cytokinins are known to promote cell division and elongation in meristematic tissues by regulating the cell cycle (Werner and Schmülling, 2009). Under salt stress, endogenous cytokinin levels typically decline due to downregulated biosynthesis and accelerated degradation by cytokinin oxidase (Hamayun et al., 2015). This hormonal imbalance limits shoot and root growth. By exogenously supplying an optimal dose of kinetin (20 µM), we likely replenished the internal cytokinin pool, thereby maintaining cell division and expansion in both roots and shoots. Furthermore, cytokinins play an essential role in preserving chlorophyll content, stabilizing photosynthetic proteins, and delaying stress-induced leaf senescence (Sharma, 2016; Kamran et al., 2021). This is supported by the findings of Kamran et al. (2021), who reported that exogenous kinetin application under salt stress upregulated the activities of key antioxidant enzymes (SOD, POD, CAT, and APX) and increased the accumulation of compatible osmolytes (proline, soluble sugars, and proteins), thereby reducing oxidative damage and lipid peroxidation. Similarly, Yasin et al. (2018) observed that kinetin supplementation improved chlorophyll retention and relative water content under salinity. In our study, the maintenance of leaf fresh weight and leaf number under 20 µM kinetin suggests a similar preservation of photosynthetic machinery and

delay of foliar senescence. In contrast, both suboptimal (10 µM) and supraoptimal (30–60 µM) concentrations of kinetin resulted in growth inhibition and increased morphological damage. The 10 µM kinetin treatment failed to alleviate stress and actually led to the lowest total plant weight (7.72 g) and root weight (1.47 g). This indicates that an insufficient concentration of exogenous cytokinin may fail to trigger the necessary stress-adaptive pathways and could instead disrupt the delicate endogenous auxin-to-cytokinin ratio required for balanced root and shoot development (Bishopp et al., 2011). On the other hand, supraoptimal concentrations (30–60 µM) induced severe phytotoxicity, culminating in complete plant mortality at 60 µM. This high-dose inhibition is a classic characteristic of cytokinin-induced toxicity (Sharma, 2016). Cytokinins and auxins operate in a tight antagonistic and synergistic crosstalk to regulate plant development (Bishopp et al., 2011). It is well established that high concentrations of cytokinins strongly inhibit root elongation and lateral root formation by suppressing polar auxin transport and altering auxin distribution in the root apical meristem (Růžička et al., 2009; Bishopp et al., 2011). In our study, as the kinetin concentration rose from 30 to 50 µM, root length and root weight were progressively suppressed. Under salinity, where the root system is already compromised by osmotic and ionic stress, any further hormonal suppression of root development severely restricts the plant's capacity to absorb water and essential nutrients (Munns and Tester, 2008). This explains the sharp increase in the visual damage scale (reaching 4.64 at 50 µM and 4.82 at 60 µM) and the severe compression of internode distance (5.80 mm at 30 µM). Moreover, extreme concentrations of exogenous cytokinins can trigger the overproduction of stress-related hormones, particularly ethylene and abscisic acid (ABA), which promote stomatal closure, leaf abscission, and systemic necrosis (Sharma, 2016; Aktaş and Uzal, 2026). Sharma (2016) similarly documented that seed treatment with higher concentrations of kinetin exerted strong inhibitory effects on the germination and seedling growth of cucumber under normal conditions, confirming the inherent sensitivity of *Cucumis sativus* to cytokinin overload. Therefore, under salt stress, the combination of high salinity and supraoptimal kinetin concentrations creates a synergistic toxic effect, overwhelming the plant's homeostatic mechanisms and leading to severe tissue necrosis and death.

5. Conclusion

This study demonstrates that exogenous kinetin application can effectively mitigate the adverse effects of 50 mM $NaCl^-$ induced salt stress in cucumber (*Cucumis sativus* L.) seedlings, but its efficacy is strictly governed by a biphasic, dose-dependent relationship. The 20 µM kinetin concentration was identified as the optimal dosage, significantly enhancing root development, leaf retention, and overall biomass accumulation, thereby offering strong protection against salinity-induced physiological drought and chlorosis. Suboptimal (10 µM) concentrations failed to provide protective effects, while supraoptimal (30–60 µM) concentrations induced severe phytotoxicity, suppressed root growth, and led to extreme necrosis and plant mortality. These findings emphasize that precise dosage control is paramount when utilizing plant growth regulators to enhance crop stress tolerance. For practical agricultural applications under salinity, foliar application of 20 µM kinetin represents a highly promising treatment to support cucumber seedling establishment and productivity. Future research should focus on exploring the molecular crosstalk between exogenous kinetin and endogenous phytohormone pathways to further optimize application strategies in the field.

Declaration of Author Contributions

The authors declare that they have contributed equally to the article. All authors declare that they have seen/read and approved the final version of the article ready for publication.

Declaration of Conflicts of Interest

All authors declare that there is no conflict of interest related to this article.

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Ethical Committee Approval

This study did not involve human or animal subjects; therefore, ethical approval was not required.

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