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Early growth responses of selected cucurbit vegetables to salt stress

Noreen Kareem[1], Muhammad Javed[1], Madiha Rashid[1*], Zahra Noreen[1], Kakaish Raees[1], Anam Ilyas[1], Humira Ashraf[1]

[1]Department of Botany, Division of Science and Technology, University of Education, Lahore, Pakistan

Abstract
This study investigated the early growth responses of selected highly economically cucurbit vegetables under varying salt concentrations. Four potential cucurbit species (*Luffa aegyptiaca*, *Cucurbita pepo*, *Praecitrullus fistulosus*, and *Cucurbita maxima*) were grown in Petri plates in a complete randomized block design with four replications. Varying salt solutions used were: 0, 50, 100, 150 and 200 mM of NaCl. Seed germination was monitored daily. Seedlings were harvested after 10 days and their shoot and root lengths, as well as fresh and dry weights were measured. The results showed that NaCl concentrations affected all measured parameters in all four vegetables. Germination percentage and germination index decreased under high salinity levels. Overall, germination and growth of the crops were better at 50 mM NaCl than those at the other salt levels. Of the four species, *Cucurbita maxima* had the highest germination percentage and germination index at all NaCl concentrations, indicating its superior tolerance to salt stress during germination stage. *Cucurbita maxima* was also superior to all species in terms of seedling biomass produced under varying levels of NaCl. Conversely, *Cucurbita pepo* showed the lowest germination percentage and index. The study concluded that *Cucurbita maxima* was the most tolerant species to salinity stress during the early growth stages.

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Introduction

Multiple stress factors, both abiotic and biotic, frequently confront plants thriving in their natural environments (Kissoudis et al., 2014). Plants are solitary and unable to avoid a stress caused by the physical environment as well as interactions with insects and microbes like bacteria (Fadiji et al., 2023). Exogenous factors are regarded as stress in plants when they have a negative impact on production, growth or yield. Numerous fluctuations in cellular metabolism, expression of genes, crop growth rates, yields, and other factors all are consequences from a stress in plants (Zhang et al., 2020). Salt stress is also known as hyperosmotic stress because, in its initial stages the capability of roots to absorb water diminishes. This leads to increased water loss from leaves due to the accumulation of high salt concentrations in both the plant and soil (Munns, 2005). Salinity stress affects morphological, physiological and molecular functions in plants, limiting their growth and development (Soltabayeva et al., 2021). Furthermore, excessive salt content has an impact on the activity of key enzymes, stomatal conductance and photosynthetic rate (Hnilickova, et al., 2021). Moreover, salinity stress elevates the formation of reactive oxygen species (ROS), which impair

***CONTACT** Madiha Rashid, botanistpk@yahoo.com, Department of Botany, Division of Science and Technology, University of Education, Lahore, Pakistan

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several metabolic processes within the cells (Kesawat et al., 2023).

Cucurbitaceae provides a variety of potential vegetables and thus is a valuable key source of food for humans (Rolnik and Olas, 2020). Sponge gourd (*Luffa aegyptiaca*) is a creeping plant from Cucurbitaceae family and is rich in vitamins A, B_5, B_6, and C, as well as carbohydrates, manganese, potassium, copper, dietary fiber, and magnesium (https://www.healthbenefitstimes.com/luffa/). The fruit of the sponge gourd is used in cooking and for treating health disorders such as high blood sugar, constipation, and body weight. *Praecitrullus fistulosus* also called Indian squash, round melon, or apple gourd, produces fruits the size of small turnips with sunken ends (Gautam et al., 2011). In India, Pakistan and Afghanistan, *Praecitrullus fistulosus* is grown as a potential vegetable (Gautam et al., 2011). *Praecitrullus fistulosus* fruit extracts were examined for their phytochemicals, enzymatic and non-enzymatic antioxidant capacity (Bollavarapu et al., 2016).

Germination is the preliminary stage of a plant's life cycle that regulates where and when a crop can be established (Bewley and Black, 2013). Most of the metabolic processes occurring in germinating seeds differ significantly from those taking place during vegetative and adult growth stages (Hazra and Das, 2024). However, high salt content in the growth medium of seeds causes increased osmotic pressure which does not allow the transport of water into the seed, so resultantly the seed feels difficulty to germinate (Cony et al., 2006). Thus, seed germination of many crops is hampered by salinity (Khajeh-Hosseini et al., 2003; Fernández-Torquemada and Sánchez-Lizaso, 2013). Therefore, this study was conducted to determine the of impact of salinity stress on germination potential and growth of selected species of Cucurbitaceae and investigate the tolerance level of the selected cucurbit species under salinity stress.

Materials and Methods

Certified seeds of *Luffa aegyptiaca*, *Cucurbita pepo*, *Praecitrullus fistulosus* and *Cucurbita maxima* were obtained from the Ayub Agricultural Research Institute, Faisalabad. All seed samples were sterilized with 5% sodium hypochlorite solution for three minutes. Thereafter, these seeds were washed thoroughly twice with deionized water to remove the sodium hypochlorite traces, and air-dried properly.

Sowing the seeds

The sterilized seeds of each species were placed in Petri dishes on Whatman 40 filter paper wetted with distilled water and NaCl concentrations (50, 100, 150, and 200 mM). After that, the Petri dishes were placed at room temperature.
The following formula was employed to determine germination percentage.

Germination (%) = Number of seeds germinated / Total number of seeds sown × 100

Seed germination index was calculated using the below given formula:

Germination index = Count of germinated seeds at day first/number of days + count of germinated seeds at day second…………+count of germinated seed at 10th day/ Total number of days for all counts

The plants were harvested after 10 days of sowing. The roots and shoots were separated and measured. Immediately, root and shoot fresh weights were measured using an electronic balance and kept in paper bags. Subsequently, after the measurement of fresh weight, the shoot and root samples were dried in an oven at 90 °C for three days, and then their dry weights measured.

Statistical analysis

Data were analyzed by a two-way analysis of variance using the COSTAT statistical software (Cohort Software, Berkeley, California). The mean values were compared with the least significant difference test at 5% probability level following Snedecor and Cochran (1989).

Results

Impact of varying levels of salinity on seed germination and seedling growth of selected cucurbit species was evaluated in the presence of five treatments 0, 50, 100, 150, and 200 mM of NaCl.

Germination percentage

Salinity stress significantly affected seed germination across four cucurbit species, *Luffa aegyptiaca*, *Cucurbita pepo, Praecitrullus fistulosus* and *Cucurbita maxima* with variations observed depending on both the salinity level and species (**Table 1**). After two days, germination was noted in *Luffa aegyptiaca* and *Cucurbita maxima* only in 0 mM and 50 mM NaCl. After 10 days of sowing, final germination percentage was noted, all four species exhibited germination, with *Cucurbita maxima* consistently showing the highest germination rates. *Luffa aegyptiaca* reached 55%, while *Cucurbita pepo* showed 35%, *Praecitrullus fistulosus* 50%, and *Cucurbita maxima* showed 65% germination at 0 mM NaCl. By increasing salinity level, the germination percentages were found to be decreased significantly. The results indicate that increasing NaCl concentration delayed the germination process and decreased the overall germination percentage, with *Cucurbita maxima* being the most resilient species as it germinated at all salt levels (**Figure 1**) and *Cucurbita pepo* the most sensitive.

Germination index (GI)

Germination index was adversely affected by salinity stress in all four species. *Luffa aegyptiaca* showed GI as 5.27, *Cucurbita pepo* 3.92, *Praecitrullus fistulosus* 4.93, and *Cucurbita maxima* 5.94 at 0 mM NaCl. *Luffa aegyptiaca* showed GI 4.60, *Cucurbita pepo* 2.87, *Praecitrullus fistulosus* 3.92, *Cucurbita maxima* 4.93 at 50 mM salt level. *Luffa aegyptiaca* showed GI 2.39, *Cucurbita pepo* 0.957, *Praecitrullus fistulosus* 2.87, and *Cucurbita maxima* 3.35 at 100 mM NaCl. Likewise, at 150 mM NaCl, *Luffa aegyptiaca* showed GI 1.44, *Cucurbita pepo* 0.48, *Praecitrullus fistulosus* 2.39, and *Cucurbita maxima* 3.35 at 150 mM NaCl. Only *Cucurbita maxima* showed 1.44 at 200 mM NaCl. Overall, the maximum germination index was observed in *Cucurbita maxima* and the minimum germination index in *Cucurbita pepo* (**Figure 2**).

Table 1: Analysis of variance of data for seedling root length, shoot length, root fresh weight, shoot fresh weight, root dry weight and shoot dry weight of selected species of Cucurbitaceae after 10 days of growth under varying salt levels

Sources	df	RFW	SFW	RL	SL	RDW	SDW
Salt treatment	4	0.030***	0.275***	81.69***	87.01***	0.002***	0.006***
Species	3	0.003ns	0.011ns	3.97ns	1.95ns	4.08 x10^{-5}ns	5.48 x10^{-5}ns
Treatment X Species	12	0.001ns	0.008ns	2.98 ns	1.06	6.56 x10^{-5}ns	1.06 x10^{-4}ns
Residuals	60	0.001	0.016	4.42	5.53	1.52 x10^{-4}	7.12 x10^{-4}

***: level of significance at 0.001 level, ns: non-significant, df: degree of freedom, RFW: Root fresh weight, SFW: Shoot fresh weight, RL: Root length, SL: Shoot length, RDW: Root dry weight, SDW: Shoot dry weight

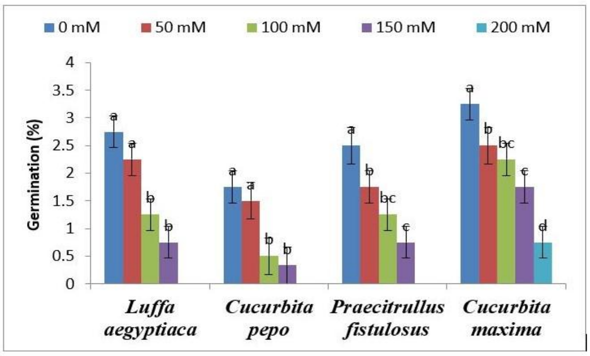

Figure 1: Influence of salinity stress on germination percentage of selected species of Cucurbitaceae after 10 days of treatments. The average values represented by the bars and labelled with different letters (a, b, c, etc), indicate statistically significant difference between each other. These differences were determined through a two-way ANOVA followed by an LSD test at 5% level.

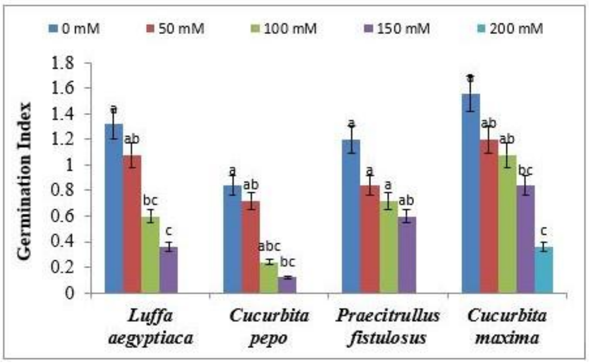

Figure 2: Influence of salinity stress on germination index of selected species of Cucurbitaceae after 10 days of treatments. The average values represented by the bars and labelled with different letters (a, b, c, etc), indicate statistically significant difference between each other. These differences were determined through a two-way ANOVA followed by an LSD test at 5% level.

Shoot length

Shoot length was negatively affected by salinity stress in all four cucurbit species. *Luffa aegyptiaca* had shoot length 5.88 cm at 0 mM NaCl and 3.33 cm at 50 mM NaCl. *Cucurbita pepo* showed shoot length 4.49 cm at 0 mM NaCl, and 2.00 cm at 50 mM NaCl. *Praecitrullus fistulosus* had shoot length 4.12 cm at 0 mM NaCl, and 3.13 cm at 50 mM NaCl (**Figure 3**). *Cucurbita maxima* displayed shoot length 6.43 cm at 0 mM NaCl, and 2.67 cm at 50 mM NaCl. Overall, *Cucurbita maxima* had the highest shoot length and *Praecitrullus fistulosus* the lowest of all species (**Figure 3**).

Root length

Salinity stress had a negative effect on the root length of all four species (**Table 1**). *Luffa aegyptiaca* showed 5.76 cm root length at 0 mM NaCl and 3.33 cm at 50 mM NaCl. The root length of *Cucurbita pepo* had been 3.125 cm at 0 mM NaCl, and 2.00 cm at 50 mM NaCl. *Praecitrullus fistulosus* had root length 3.73 cm at 0 mM NaCl, and 2.25 cm at 50 mM NaCl. *Cucurbita maxima* showed root length 7.49 cm at 0 mM NaCl and 2.63 cm at 50 mM NaCl (**Figure 4**). The results showed that *Cucurbita maxima* had the maximum root length and *Cucurbita pepo* the minimum.

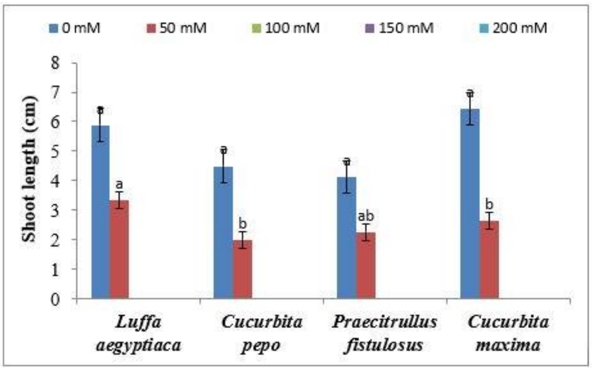

Figure 3: Influence of salinity stress on shoot length of selected species of Cucurbitaceae after 10 days of treatments. The average values represented by the bars and labelled with different letters (a, b, c, etc), indicate statistically significant difference between each other. These differences were determined through a two-way ANOVA followed by an LSD test at 5% level.

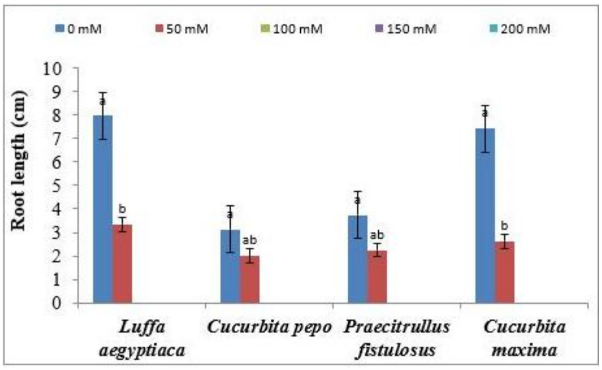

Figure 4: Influence of salinity stress on root length of selected species of Cucurbitaceae after 10 days of treatments. The average values represented by the bars and labelled with different letters (a, b, c, etc), indicate statistically significant difference between each other. These differences were determined through a two-way ANOVA followed by an LSD test at 5% level.

Shoot fresh weight

Shoot fresh weight was severely affected by salinity stress in all four Cucurbitaceae species (**Table 1**). *Luffa aegyptiaca* produced 0.34 g shoot fresh weight at 0 mM NaCl and 0.188 g at 50 mM NaCl, whereas *Cucurbita pepo* showed 0.273 g at 0 mM NaCl, and 0.125 g at 50 mM NaCl. *Praecitrullus fistulosus* showed shoot fresh weight 0.168 g at 0 mM NaCl, and 0.110 g at 50 mM NaCl, whereas that of *Cucurbita maxima* had been 0.403 g at 0 mM NaCl, and 0.130 g at 50 mM NaCl (**Figure 5**). Overall, the maximum shoot fresh weight was produced by *Luffa aegyptiaca* and the minimum by *Praecitrullus fistulosus.*

Root fresh weight

Luffa aegyptiaca had root fresh weight 0.087 g at 0 mM NaCl and 0.035 g at 50 mM NaCl, whereas *Cucurbita pepo* 0.038 g at 0 mM NaCl and 0.013 g at 50 mM NaCl. *Praecitrullus fistulosus* produced root fresh weight 0.043 g at 0 mM NaCl, and 0.007 g at 50 mM NaCl., whereas *Cucurbita maxima* 0.063 g at 0 mM NaCl and 0.024 g at 50 mM NaCl (**Figure 6**). Overall, *Cucurbita maxima* had the maximum root fresh weight and *Cucurbita pepo* the minimum.

Shoot dry weight

The shoot dry weight in *Luffa aegyptiaca* had been 0.057 g at 0 mM NaCl and 0.012 g at 50 mM NaCl, whereas *Cucurbita pepo* had 0.005 g at 0 mM NaCl, and 0.028 g at 50 mM NaCl. Likewise, the shoot dry weight of *Praecitrullus fistulosus* had shoot dry weight 0.041 g at 0 mM NaCl, and 0.018 g at 50 mM NaCl. The shoot dry weight of *Cucurbita maxima* was recorded as 0.048 g at 0 mM NaCl, and 0.008 g at 50 mM NaCl (**Figure 7**). Overall, the maximum shoot dry weight was found in *Cucurbita maxima* and the minimum in *Praecitrullus fistulosus.*

Root dry weight

Luffa aegyptiaca showed root dry weight as 0.032 g at 0 mM NaCl, and 0.012 g at 50 mM NaCl, whereas that of *Cucurbita pepo* had been 0.015 g at 0 mM NaCl and 0.010 g at 50 mM NaCl. The root dry weight of *Praecitrullus fistulosus* was 0.0175 g at 0 mM NaCl, and 0.006 g at 50 mM NaCl. *Cucurbita maxima* produced root dry weight as 0.0118 g at 0 mM NaCl, and 0.0085 g at 50 mM NaCl (**Figure 8**). The maximum root dry weight was documented in *Cucurbita maxima* and the minimum in *Cucurbita pepo.*

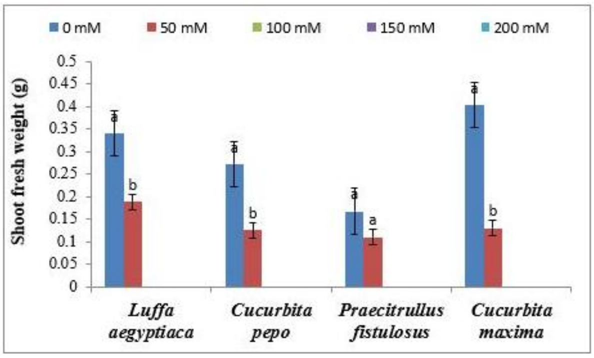

Figure 5: Influence of salinity stress on shoot fresh weight of selected species of Cucurbitaceae after 10 days of treatments. The average values represented by the bars and labelled with different letters (a, b, c, etc), indicate statistically significant difference between each other. These differences were determined through a two-way ANOVA followed by an LSD test at 5% level.

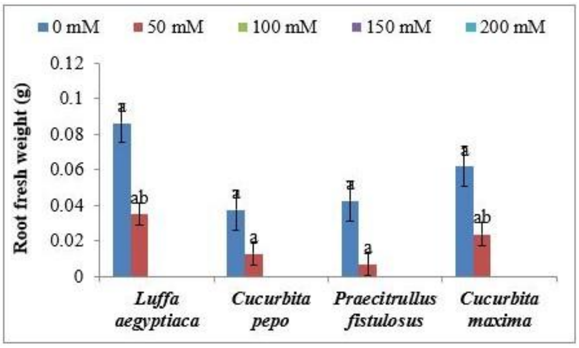

Figure 6: Influence of salinity stress on root fresh weight of selected species of Cucurbitaceae after 10 days of treatments. The average values represented by the bars and labelled with different letters (a, b, c, etc), indicate statistically significant difference between each other. These differences were determined through a two-way ANOVA followed by an LSD test at 5% level.

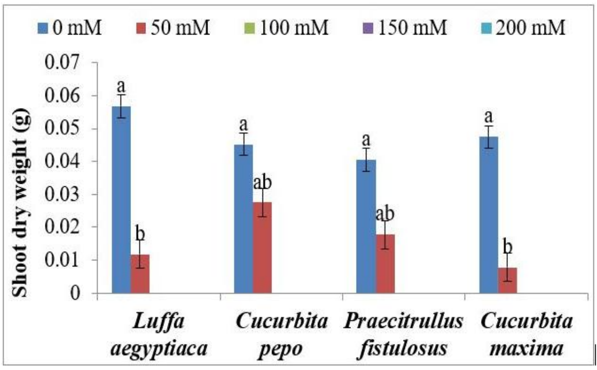

Figure 7: Influence of salinity stress on shoot dry weight of selected species of Cucurbitaceae after 10 days of treatments. The average values represented by the bars and labelled with different letters (a, b, c, etc), indicate statistically significant difference between each other. These differences were determined through a two-way ANOVA followed by an LSD test at 5% level.

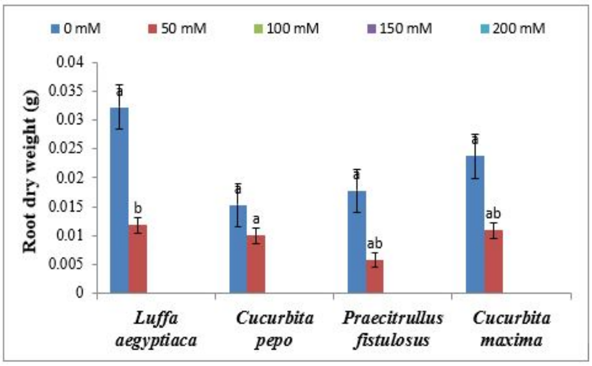

Figure 8: Influence of salinity stress on root dry weight of selected species of Cucurbitaceae after 10 days of treatments. The average values represented by the bars and labelled with different letters (a, b, c, etc), indicate statistically significant difference between each other. These differences were determined through a two-way ANOVA followed by an LSD test at 5% level.

Discussion

The germination of seeds is a crucial developmental stage for plants (Kim and Park, 2008). Utilizing the seed's stored food material is essential for seed germination. Among major environmental factors, salinity harmfully disturbs the growth and yield of plants. A significant barrier to the establishment of plants in salty environments is seed germination. Osmotic stress or particularly ion toxicity may be responsible for salt's ability to suppress seed germination (Ludwiczak et al., 2021). Salinity hinders the seedlings' ability to absorb water. This subsequently prevents the hydrolysis of seed stores, delaying and reducing seed germination (Begum et al., 2010; Haj Sghaier et al., 2022; Xiong et al., 2024). Salinity may significantly lower germination rates and percentages, which could result in uneven stand establishment and low crop yields (Liu et al., 2018). Nonetheless, when a plant exhibits strong seed germination and healthy seedling growth in salt, it can withstand the salinity indirectly significantly. Thus, these plants resultantly contribute to improved growth and productivity (Carpýcý et al., 2009).

In the current study, the results exhibited that with increase in NaCl concentration (50 mM, 100 mM, 150 mM and 200 mM), the seed germination percentage of all tested Cucurbitaceae species, *Luffa aegyptiaca*, *Cucurbita pepo, Praecitrullus fistulosus* and *Cucurbita maxima* decreased markedly. However, the maximum germination percentage was observed in *Cucurbita maxima* and the minimum in *Cucurbita pepo.* These findings can be supported by some earlier studies wherein a decline in seed germination was noted in different crops at high salt levels (Zehtab-Salmasi, 2008; Devkota and Jha, 2010). In tomato, seed germination and seedling growth were both considerably declined by the rise in the content of NaCl salt (Chakma et al., 2019). Analogously, in *Trigonella foenum-graecum* a gradual decline in seed germination percentage was observed with an elevation in salt concentrations, although there was no delay in the germination process at low concentrations of NaCl up to 40 mM, while greater concentrations of NaCl caused *Trigonella foenum-graecum* to take longer to germinate (Ratnakar and Rai, 2013). Moreover, Bina and Bostani (2017) while recording suppressed seed germination of isabgol, zucchini, and clove argued that the salt-induced decreased seed germination could be due to the reason that seeds seemingly develop an osmotically enforced dormancy.

In the current investigation, the effects of NaCl-induced osmotic potential on GP were quite noticeable. The control had the highest GP (90%) while the higher salt levels reduced GP (60%) to a considerable level. Similarly, Çamlica and Yaldiz (2017) reported that rising salinity doses led to reduce GP of sweet basil. This reduction started after 40 mM salinity level, but germination still took place at 240 mM. Likewise, Fatima et al. (2018) reported that the maximum percentage of germination in *Moringa oleifera* occurred in the control treatment, while a marked reduction in GP was observed at 10 dS m^{-1} of salt. According to Ayaz et al. (2000) various metabolic abnormalities are to blame for the decreased overall seed germination under salt stress. These findings can be supported that salinity hinders germination in two ways; first, by lowering osmotic potential, which slows or stops water uptake, and secondly, by a buildup of toxic ions that harms the growing embryo (Hassen et al., 2014).

The germination index (GI) is also an important measure of seed germination, which calculates the rate and percentage of germination (Kader, 2005). GI reflects the GP on each day along germination period. In the current study, GI was significantly affected by salinity stress in the four Cucurbitaceae species, *Luffa aegyptiaca*, *Cucurbita pepo*, *Praecitrullus fistulosus* and *Cucurbita maxima*. Overall, as the salt level increased, the GI significantly decreased in all four species. These findings can be supported by the previous findings (Kaya et al., 2008) in which NaCl caused a substantial decline in the germination index. Hassen et al. (2014) also observed a consistent decline in GI in different cultivars of pepper with increase in external salt levels.

In the current study, shoot and root lengths, and shoot and root fresh and dry weights of all four species decreased markedly with increase in external salt levels. However, different species responded differently to external saline medium. For example, of all four species, *Cucurbita maxima* had the maximum shoot length, and shoot fresh and dry weights, whereas the minimum of these attributes was observed in *Praecitrullus fistulosus.* The present findings can be supported by the findings of Naseer et al. (2022) in which all of the studied cucurbits' shoot and root lengths declined as external salt level rose, although plants with high salt levels had significantly shorter shoot and root lengths. Ahmed et al. (2019) also reported that with rising salt concentrations, *Lactuca sativa* fresh weight of both shoot and root decreased significantly.

Conclusion

It was concluded that germination percentage, germination index, shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight, and root dry weight of all four cucurbit species decreased significantly under NaCl concentrations. *Cucurbita maxima* had the maximum germination percentage, and seedling biomass, while the minimum GP was observed in *Cucurbita pepo,* and the minimum seedling biomass in *Praecitrullus fistulosus*.

Author(s), Editor(s) and Publisher's declarations

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Contribution of authors
Conceptualization: NK, MJ, MR, ZN. Conduct of experiment: NK. Data analysis: NK, ZN, AI, HA. Presentation of data: NK, ZN, KR, AI, HA. Writing—original draft preparation: NK, MJ, MR, ZN, KR. Revision of manuscript: NK, MJ, MR, ZN, HA. All authors have read and agreed to publish this article in IJAaEB.

Ethical approval
This study does not involve human/animal subjects, and thus no ethical approval is required.

Handling of bio-hazardous materials
The authors certify that all experimental materials were handled with great care during collection and experimental procedures. After completion of the study, all materials were properly discarded to minimize/eliminate any types of bio-contamination(s).

Supplementary material
No supplementary material is included with this manuscript.

Availability of primary data and materials
As per editorial policy, experimental materials, primary data, or software codes are not submitted to the publisher/Journal management. These are available with the corresponding author (s) and/or with other author(s) as declared by the corresponding author (s) of this manuscript.

Authors' consent
All authors have critically read this manuscript and agreed to publish in IJAaEB.

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