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ORIGINAL RESEARCH ARTICLE

Elevated salt stress level affected the productivity and chlorophyll content of *Centella asiatica* (L.)

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ABSTRACT

Salinity disrupts plant nutrient uptake, metabolism, and increases susceptibility to biotic stresses. This reduces nutrient use efficiency, leading to stunted growth and decreased productivity. The aim of this study is to evaluate the growth and yield performance of *Centella asiatica* L. under varying levels of salt stress. The research was conducted at the Germplasm Centre, Department of Horticulture, Patuakhali Science and Technology University. A randomized complete block design with four replications was employed, wherein five salt concentrations i.e., 0 mM, 50 mM, 100 mM, 150 mM, and 200 mM were applied in four replications on 20 pots. Data on various growth and yield parameters were taken in four installments: 21, 42, 63, and 84 days after transplanting. Results showed that the highest values for various parameters were observed in the control group (2.34 mM base value), with notable figures including number of leaves (258.5), number of runners (126.75), petiole length (9.38 cm), chlorophyll content (41.62 SPAD value), fresh weight of leaves (23.92 g), dry weight of leaves (7.97 g), fresh weight of shoot (1.84 g), and dry weight of shoot (0.61 g) at 84 DAT. Conversely, fresh weight and dry weight of roots peaked at 150 mM salt concentration (1.95 g and 0.65 g, respectively). The investigations revealed that as salinity levels increased, a gradual decline in growth parameters was observed, indicating a significant reduction in the growth and yield of *C. asiatica*. These findings highlight the sensitivity of *C. asiatica* to salt stress and underscore the importance of salinity management for optimal growth and yield.

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INTRODUCTION

Centella asiatica, commonly known as "Indian Pennywort," is a valuable medicinal herb belonging to the Apiaceae (Umbelliferae) family. Centella is a genus comprising tropical and subtropical plants with around 59 species worldwide (Torbati *et al.*, 2021). It holds significant importance in traditional medicine systems across Southeast Asian countries like India, Sri Lanka, China, Indonesia, and Malaysia, as well as in South Africa and Madagascar (Bansal *et al.*, 2024; Hossain *et al.*, 2018). *Centella asiatica* exhibits a wide range of pharmacological properties attributed to its bioactive constituents, including terpenic acids, flavonoids, and phenolic compounds (Kandasamy *et al.*, 2023; Seevaratnam *et al.*, 2012). Owing to its positive functional qualities, its use as a medicinal plant, food, and drink has enormously increased in a few years. Kaviraj (a population in the Chalna district of Bangladesh) use the entire plant to treat a variety of diseases, including dog bites, asthma, carminative, itching, leucorrhea, malaria, tumors, wounds, etc. (Muhammad & Hussain, 2010).

Salinity stands as a prominent abiotic stressor substantially impeding plant growth and yield (Gharsallah *et al.*, 2016). The persistent rise in salinity levels across cultivable areas, attributed to inadequate farming methods and climate shifts, poses dire consequences globally. Projections suggest that approximately half of the world's arable land could become unusable by the midpoint of the 21st century (Islam *et al.*, 2019). In addition, in Bangladesh, over 80% of the land is comprised of alluvial sediments from major river systems, including the Ganges and Brahmaputra, salinity poses a significant challenge to agriculture, particularly in coastal regions. Approximately 53% of coastal areas in Bangladesh are affected by salinity, leading to poor agricultural productivity and reduced cropping intensity (Haque, 2006). According to Heidari *et al.* (2008) abiotic environmental stress particularly salt has the greatest impact on medicinal plants. Despite the vital role of *C. asiatica* in traditional medicine and its potential economic value, research on its response to salinity stress remains limited.

While previous studies have investigated the effects of various environmental stressors on medicinal plants, including fertilizer, light intensity, and water stress, comprehensive research on the impact of salinity stress on *C. asiatica* is lacking. Given the increasing salinity issues in coastal regions and the potential therapeutic benefits of *C. asiatica*, there is a pressing need to explore its tolerance to salt stress. Therefore, the present study aims to evaluate the growth and yield performance of *C. asiatica* under salt stress conditions. By addressing this research gap, we aim to provide valuable insights into the adaptive mechanisms of *C. asiatica* to salinity stress, contributing to the development of strategies for its cultivation and sustainable utilization in salt-affected regions.

MATERIALS AND METHODS

Plant material and experimental design

One-and-a-half-month-old Indian pennywort (*Centella asiatica* L.) runners were used for the experiment and it was collected from Dumki Upazilla, Patuakhali-8602, Bangladesh. The experiment followed a single-factor design with five treatments: 0 mM (Control-2.34 mM base value), 50 mM, 100 mM, 150 mM, and 200 mM NaCl. The field experiment was arranged in a Randomized Complete Block Design (RCBD). Each treatment had four replications. A total of twenty pots (5 treatments × 4 replications) were used. The pots used had an inner diameter of 18 cm and an outer diameter of 20 cm, with a depth of 30 cm, including a bottom. The pots were spaced 45 cm × 45 cm apart. Harvesting and data collection were conducted in four installments: 21, 42, 63, and 84 days after transplanting.

Morphological parameters, chlorophyll content, and soil pH

After harvesting, morphological parameters, such as total number of leaves, number of runners, and petiole length were measured. The total chlorophyll content in the leaves was quantified using a BIOBASE SPAD chlorophyll meter. The fresh and dry biomass of selected cultivar was also measured. The leaf biomass, shoots biomass, and roots biomass were determined after washing with distilled water and drying them gently with tissue paper. The dry weight was determined after drying for 48 h at 80°C. Soil pH levels were determined before and after the treatment.

Statistical analysis

Data recorded on plant characteristics was subjected to statistical analysis through computer based statistical program JMP 14 (JMP®14, SAS Institute Inc., Cary, NC, 1989-2021) following the basic principles. To test the effects of treatments, data on various growth and yield parameters were subjected to analysis of variance (ANOVA) and means were compared using Tukey's honestly significant difference (HSD) post hoc test using computer based statistical program JMP 14 following the basic principles.

RESULTS AND DISCUSSION

Number of leaves and runners

The data analysis indicates significant variations in the number of leaves among the different treatments (Figure 1a). The maximum number of leaves (258.5) was observed in response to the control (2.34 mM base value) condition, followed by 50 mM (233.25) at 84 DAT, which exhibited statistically similar results. Conversely, the minimum number of leaves (43.75) was found under the highest saline condition (200 mM). These findings highlight the pronounced impact of soil salinity on leaf development in plants. Ramezanifar *et al.* (2022) reported a significant reduction in the number of leaves of spinach (*Spinacia oleracea* L.) with increasing irrigation water salinity. Similarly, significant reduction in runner count under saline soil conditions was noted compared to normal soil (Figure 1b). The highest number of runners (126.75) was observed in the control conditions (2.34 mM base value), while the lowest number of runners (21.50) was recorded in the highest saline conditions (200 mM). This substantial decrease in runner formation highlights the adverse impact of soil salinity on the vegetative propagation of plants. The observed decrease in runner formation with increasing soil salinity aligns with previous findings on the effect of salinity on plant growth Unlukara *et al.* (2008).

Length of petiole

The analysis of data reveals a significant reduction in petiole length under saline conditions (Table 1). Throughout the study period, the length of petiole was consistently highest (9.38 cm) in non-saline conditions (Control), whereas it was consistently lowest (2.71 cm) in the highest saline conditions (200 mM) at 84 DAT. This substantial decrease in petiole length under saline conditions suggests a pronounced negative impact of salt stress on plant growth and development. These findings are consistent with prior research by Wang *et al.* (2017), which also demonstrated a significant decrease in plant growth parameters under saline conditions.

Total chlorophyll content

Significant variations in total chlorophyll content, as indicated by SPAD values, were observed across different salinity treatments (Table 1). While no significant difference was detected among salt treatments at 21 DAT, notable variations were evident at 42, 63, and 84 DAT. At 84 DAT, the highest SPAD

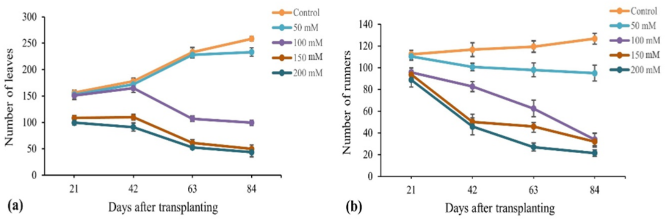

Figure 1. *Effect of salinity on number of leaves and runners of C. asiatica at different days after transplanting.*

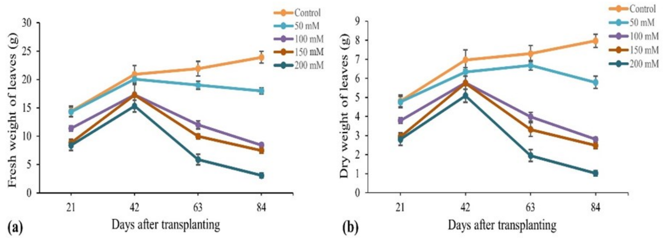

Figure 2. *Effect of salinity on fresh and dry biomass of leaves of C. asiatica at different days after transplanting.*

value (41.62) was recorded in the control conditions (2.34 mM base value), whereas the lowest value (23.39) was observed under high saline concentrations (200 mM). The observed decrease in SPAD values with increasing saline concentration aligns with the findings of Zhang *et al.* (2021).

Fresh and dry biomass of leaves per pot

Significant variations were observed in both fresh and dry weights of leaves among the treatments (Figure 2a, b). In terms of fresh weight, the control group (2.34 mM base value) displayed the highest weights, ranging from 14.42 g to 23.92 g across different time points. Following closely, the slightly saline condition (50 mM) exhibited fresh weights ranging from 14.3 g to 20.06 g at 21, 42, 63, and 84 DAT. Conversely, the lowest fresh weights were recorded in high saline concentrations (200 mM), ranging from 3.08 g to 8.41 g. The findings supported by the previous experiment of Kumar *et al.* (2021).

Fresh and dry biomass of shoot and root per plant

Salinity exerted a significant influence on both the fresh and dry weight of shoot biomass (Table 2). The highest shoot fresh weight (1.84 g) was observed in the control group (2.34 mM base value), while the lowest (1.24 g) occurred under the highest saline treatment (200 mM), indicating a 67.39% reduction compared to the control. Similarly, the highest shoot dry weight (0.61 g) was recorded in the control group, with the lowest (0.41 g) observed under the highest saline treatment, corresponding to a 67.21% reduction compared to the control. These findings align with previous studies by Al-Maskri *et al.* (2010), Ekinci *et al.* (2012), as well as investigations on sorghum and rice (Minh *et al.*, 2016).

Salinity also significantly influenced the fresh and dry weight of root biomass (Table 2). Notably, after 84 DAT, most plants succumbed to the stress conditions. Among the survivors, the highest root fresh weight (1.95 g) was observed under the highest saline treatment (150 mM), while the lowest (0.74 g) occurred in the control group. This represented a remarkable 170.20% increase compared to the control. Similarly, the highest root dry weight (0.65 g) was recorded under the highest saline treatment, with the lowest (0.24 g) observed in the c ontrol, indicating a 175% increase compared to the control. These findings suggest that certain survival mechanisms within Centella respond positively to moderate salinity levels, enhancing root biomass production. This is consistent with the findings of Hanifah & Purwestri (2021).

Soil pH and EC

There was no significant difference in the soil pH before and after salt treatment (Table 3). However, significant variations in soil EC were observed after salt treatment, while it remained non-significant before salt treatment. The lowest soil EC (0.17 dS/m) was recorded in response to the control condition (2.34 mM base value), while the highest soil EC (1.46 dS/m) was found in the highest saline treatment condition (200 mM), representing a substantial increase of 584% compared to the control. Sultana (2019) observed high concentrations of Na+, K+, and EC in salt-treated soils compared with that in the control condition.

Table 1. Effect of salinity on petiole length (cm) and total chlorophyll content (SPAD value) of *C. asiatica*.

Salinity	Petiole length at different DAT				Chlorophyll content at different DAT			
	21	42	63	84	21	42	63	84
Control	7.66 a	8.99 a	9.20 a	9.38 a	43.1	47.43 a	41.68 a	41.62 a
50 mM	7.53 a	8.86 a	8.15 ab	9.10 a	43.84	45.98 a	41.21 a	34.70 ab
100 mM	7.25 ab	8.14 ab	6.38 b	5.29 b	43.81	42.30 ab	38.73 ab	31.38 bc
150 mM	7.08 ab	7.53 b	6.16 b	5.19 b	49.66	42.76 ab	38.43 ab	29.40 bc
200 mM	5.68 b	5.42 c	3.59 c	2.71 c	48.91	38.35 b	34.14 b	23.39 c
Level of sig.	*	**	**	**	NS	*	**	**
CV (%)	14.63	7.72	8.63	5.79	11.10	11.94	5.66	7.54

In a column value having different letter (s) differ significantly, but with common letter (s) do not differ significantly at 5% and 1% levels of probability analyzed by Tukey. ** and *= Significant at 1% and 5% levels of probability, NS= Not significant, CV= Coefficient of variation.

Table 2. Effect of salinity on fresh and dry biomass of shoot & root of *C. asiatica*.

Salinity	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)
0 mM	1.84 a	0.61 a	0.74 d	0.24 d
50 mM	1.77 a	0.59 a	1.39 c	0.46 c
100 mM	1.61 b	0.53 b	1.68 b	0.56 b
150 mM	1.53 b	0.51 b	1.95 a	0.65 a
200 mM	1.24 c	0.41 c	1.26 c	0.42 c
Level of sig.	**	**	**	**
CV (%)	6.04	6.04	3.63	3.90

In a column value having different letter (s) differ significantly, but with common letter (s) do not differ significantly at 5% and 1% levels of probability analyzed by Tukey. ** = Significant at 1% level of probability, CV= Coefficient of variation.

Table 3. Effect of salinity on soil pH and EC.

Salinity	Before treatment		After treatment	
	pH	EC (dS/m)	pH	EC (dS/m)
0 mM	7.07	0.17	7.07	0.17 d
50 mM	6.97	0.19	7.04	0.93 c
100 mM	6.88	0.21	7.14	1.13 b
150 mM	6.64	0.20	7.07	1.14 b
200 mM	6.51	0.19	6.89	1.46 a
Level of sig.	NS	NS	NS	**
CV (%)	6.32	7.26	3.48	6.24

In a column value having different letter (s) differ significantly, but with common letter (s) do not differ significantly at 5% and 1% levels of probability analyzed by Tukey. ** = Significant at 1% level of probability, NS= Not significant, CV= Coefficient of variation.

Conclusion

Results of this study reveal a dose-dependent reduction in growth parameters and yield with increasing salt levels, underlining the plant's sensitivity to salinity. Notably, salt stress adversely affects leaf development, runner formation, petiole length, and chlorophyll content, hampering growth and photosynthetic capacity. While shoot biomass consistently diminishes, moderate salt levels (150 mM) surprisingly stimulate root biomass, indicating adaptive mechanisms within *C. asiatica*. Soil electrical conductivity rises with salt treatment, signifying salt accumulation, though pH remains unaffected. Notably, *C. asiatica* growth and yield performance at 50 mM salt concentrations were statistically identical to the control in most of the variables. This demonstrates that *C. asiatica* can efficiently withstand up to 50 mM salt concentrations, beyond which growth and yield sharply decline.

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DECLARATIONS

Authors contribution

Conceptualization, methodology, investigation, resources, and supervision: E.K., M.R., and L.A.L.; Formal analysis, data curation, writing—original draft, and visualization: M.M.I. and L.A.L.; Validation, writing-review and editing, resources, and software; M.M.I. and M.A.S. All authors contributed to the article and approved the submitted version

Conflicts of interest: The authors declare no conflict of interest.

Ethics approval: This study did not involve any animal or human participant and thus ethical approval was not applicable.

Consent for publication: All co-authors gave their consent to publish.

Data availability: The data that support the findings of this study are available on request from the corresponding author.

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