Innovations in Agriculture
www.innovationsagriculture.com

ISSN: 2617-1295
DOI: https://doi.org/10.25081/ia.2020.v3.2198

Research Article

Salinity induced alterations in the biochemical properties of mangrove plants

A.K. Rajalakshmi*, P. Elavazhahan, P. Manivannan

PG and Research Department of Botany, Government Arts College, C. Mutlur, Chidambaram, Tamil Nadu, India

*Corresponding Author, Email: akrajalakshmi2015@gmail. com

ARTICLE INFO

Received: August 06, 2020
Revised: October 01, 2020
Accepted: October 10, 2020
Published: October 11, 2020

Keywords:

Mangroves, salinity, saline agriculture, irrigation salinity

ABSTRACT

High concentrations of harmful ions like salt and chloride can have a direct impact on plant growth. So, the saline water irrigation in agriculture should be studied in a model system. In this study, we conducted a detailed investigation on the comparative effect of exogenous addition of different concentrations of sodium chloride on the organic components, in the seedlings of *Lumnitzera racemosa* Willd, a much commonly known mangrove species inhabiting the salt marsh ecosystem as no such detailed physiological studies have been made in this species earlier. From this study, it can be concluded that, salinity has an impact on the biochemical constituents of this important mangrove species.

INTRODUCTION

The intertidal zone along the shore is home to a collection of plants and shrubs known as mangroves [1]. Mangroves have a significant potential since they may be used to produce wood, pulp, oil, fuel, feed, and other materials [2]. In addition, they can be utilized for landscaping, dune stabilization, and land reclamation. Some of the species of halophytes and swampy plants can be found in inland marshy environments [3].

From riverine forests with salinities near or at zero parts per thousand (ppt), to fringe woods with typical salinities around 35 ppt, and even in hypersaline places where the salinity may approach 70 ppt, mangroves can be found naturally existing along a gradient of salinity [4]. Mangroves can be found growing in a variety of salinities, however some species have been found to thrive best in salinities as low as two ppt [5].

Lumnitzera racemosa Willd. (Combretaceae) as a shrub more commonly as a tree, grows luxuriantly in the salt marsh habitat of Pichavaram on the east coast of Tamil Nadu, India. Lumnitzera is an Asian genus, with species occurring from Eastern Africa to the Western Pacific [6]. This species constitutes one of the dominant mangrove flora of Pichavaram. As this species accumulate high concentrations of salts in their leaf tissues and overcome salt toxicity by developing succulence, it can be classified under succulent halophyte [7].

The concentration of the salt in the saline environment is normally measured as the chloride concentration or chlorinity and it is about 35 g/l [8]. The most important ions in salt affected soils are the sodium and chloride with concentrations of 480 and 560 mM respectively [9]. Besides Na+ and Cl- ions in the soils affected by seawater, other anions particularly, carbonates, bicarbonates and sulphates do also occur at high concentration [8,10]. The luxuriant mangrove areas need more protection and active management. The partially degraded area requires either natural regeneration or rehabilitation. The seriously degraded area need reforestation [11].

Scientists agree that mangrove areas are highly productive and comparable to good agriculture land [12,13]. During the last three decades, halophytes and the effect of salinity in general have been the subject of a number of reviews. Optimal salt concentration for growth varies with species. Different mangrove species exhibit variable growth responses to saline conditions [14]. In this present study, a detailed investigation on the comparative effect of exogenous addition of different concentrations of sodium chloride on the organic components, in the seedlings of *L. racemosa*, a much commonly known mangrove species inhabiting the salt marsh ecosystem as no such detailed physiological studies have been made in this species earlier.

MATERIALS AND METHODS

Plant collection

Seedlings of *Lumnitzera racemosa* Willd were used in the present study and collected from mangrove belt of Pichavaram, on the east coast of Tamil Nadu, India.

When the seedlings were about 10 cm, healthy plants of uniform height were screened for transplantation. The seedlings were uprooted carefully with intact root system from the mangrove belt and were washed thoroughly with fresh water. The seedlings then were planted into the individual polythene bags filled with fresh soil containing red earth, sand and farmyard manure (1:2:1).

NaCl treatment

NaCl treatment was given at various concentrations viz., 0 (control), 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 mM NaCl. Both the control and experimental plants treated with varying concentrations of NaCl were regularly irrigated with tap water. As

the objective of the present investigation was to study individually the effect of different concentrations of NaCl in *L. racemosa*. Harvesting was done on 60th, 120th and 180th day after NaCl treatment.

Biochemical analysis

Estimation of total free amino acids [15], protein [16], total sugars [17], starch [18], proline [19], glycinebetaine [20] and total phenols [21] were done by following standard methods on 60th, 120th and 180th day after NaCl treatment.

Statistical analysis

Statistical analysis was performed by following standard procedures [22].

RESULTS

Free amino acids

The results showed that the leaf had more amino acid accumulation than in the stem and root. Highest accumulation of amino acid (6.97 mg/g fr. wt.) was recorded at 800 mM NaCl on the 180th day samples and this was 49.89% over that of control plants (Table 1). The F values calculated for difference between the sampling days and between the treatments were significant at 1% level.

Protein

The highest protein content (10.98 mg/g fr. wt.) was noticed in the leaf tissue at 500 mM NaCl on the 180th day. The highest total protein content in all the three tissues (27.25 mg/g fr. wt.) was recorded at 500 mM NaCl on the 180th day samples and this was 127.13% over that of control plants (Table 2). With further increasing of NaCl salinity, the protein content was declined on all the sampling days. The F values calculated for difference between the sampling days and between the treatments were significant at 1% level.

Total sugar

The total sugar content had decreased in the leaf, stem and root with increasing NaCl salinity upto 500 mM and at higher concentrations upto 800 mM an increasing trend was noticed (Table 3). The maximum decrease recorded in the leaf, stem and root at 500 mM NaCl was 27.59%, 21.87% and 30.33% less respectively when compared to those of control plants on 180th day (Table 3). The F values calculated for difference between the sampling days and between the treatments were significant at 1% level.

Starch

The effect of sodium chloride salinity on the starch content of the leaf, stem and root was studied and the results are given in Table 4. The starch content in all the plant tissues had increased with increasing NaCl concentrations upto 500 mM. Beyond 500 mM, there was a gradual reduction in starch content on all the sampling days. The leaf had more accumulation of starch than in the stem and root in all the NaCl concentrations and all the sampling days (Table 4). The F values calculated for difference between the sampling days and between the treatments were significant at 1% level.

Proline

The leaf had always higher proline accumulation than in the stem and root. The highest proline accumulation (12.60 mg/g fr. wt.) was noticed in the leaf tissue at 800 mM NaCl on the 180th day, and this was 149% over that of control plants (Table 5). The F values calculated for difference between the sampling days and between the treatments were significant at 1% level.

Glycinebetaine

The results of glycinebetaine accumulation in the leaf, stem and root at different concentrations of NaCl are presented in Table 6. Sodium chloride salinity increased the level of glycinebetaine with increasing concentrations upto 800 mM in all the plant tissues on all the sampling days (Table 6). The increasing trend in the betaine was similar to that of proline. The leaf glycinebetaine content was always higher than that of stem and root. The maximum betaine accumulation was noticed in the leaf tissue on the 180th day samples at 800 mM NaCl and this was 154.26% higher when compared to those of control plants. The F values calculated for difference between the sampling days and between the treatments were significant at 1% level.

Table 1. Effect of NaCl on the free amino acid content of leaves, stem and root of *Lumnitzera racemosa* (mg/g fresh weight).

Concentration of NaCl (mM)	Days after NaCl treatment								
	60	120	180	60	120	180	60	120	180
	Leaves			Stem			Root		
Control	4.00	4.28	4.65	3.40	3.55	3.68	3.00	3.26	3.35
100	4.36 (+ 9.00)	4.75 (+ 10.98)	5.07 (+ 9.03)	3.56 (+ 4.70)	3.86 (+ 8.73)	4.03 (+ 9.51)	3.08 (+ 2.66)	3.38 (+ 3.68)	3.50 (+ 4.47)
200	4.77 (+ 19.25)	5.18 (+ 21.02)	5.42 (+ 16.55)	3.75 (+ 10.29)	4.10 (+ 15.49)	4.40 (+ 19.56)	3.25 (+ 8.33)	3.60 (+ 10.42)	3.84 (+ 14.62)
300	5.10 (+ 27.5)	5.60 (+ 30.84)	5.85 (+ 25.80)	4.02 (+ 18.24)	4.43 (+ 24.78)	4.65 (+ 26.35)	3.40 (+ 13.33)	3.72 (+ 14.11)	4.05 (+ 20.89)
400	5.38 (+ 34.5)	5.93 (+ 38.55)	6.20 (+ 33.33)	4.30 (+ 26.47)	4.85 (+ 36.61)	5.13 (+ 39.40)	3.63 (+ 21.00)	4.04 (+ 23.92)	4.25 (+ 26.86)
500	5.70 (+ 42.5)	6.33 (+ 47.89)	6.69 (+ 43.87)	4.74 (+ 39.41)	5.57 (+ 56.90)	5.80 (+ 57.60)	3.88 (+ 29.33)	4.36 (+ 33.74)	4.51 (+ 34.62)
600	5.86 (+ 46.5)	6.42 (+ 50.00)	6.80 (+ 46.23)	4.85 (+ 42.64)	5.68 (+ 60.00)	5.96 (+ 61.95)	3.97 (+ 32.33)	4.50 (+ 38.03)	4.63 (+ 38.20)
700	5.93 (+ 48.25)	6.58 (+ 53.73)	6.89 (+ 48.17)	4.95 (+ 45.58)	5.86 (+ 65.07)	6.10 (+ 65.76)	4.12 (+ 37.33)	4.62 (+ 41.71)	4.87 (+ 45.37)
800	6.05 (+ 51.25)	6.70 (+ 56.54)	6.97 (+ 49.89)	5.08 (+ 49.41)	5.97 (+ 67.61)	6.25 (+ 69.83)	4.25 (+ 41.66)	4.77 (+ 46.31)	5.00 (+ 49.25)
Leaves :	F1 = 314.666* F2 = 243.374*		Stem :	F1 = 65.7850* F2 = 49.0821*		Root :	F1 = 146.6018* F2 = 145.3583*		

Table 2. Effect of NaCl on the protein content of leaves, stem and root of *Lumnitzera racemosa* (mg/g fresh weight).

Concentration of NaCl (mM)	Days after NaCl treatment								
	60	120	180	60	120	180	60	120	180
	Leaves			Stem			Root		
Control	6.40	6.90	7.15	5.42	6.00	6.24	5.33	5.52	5.65
100	7.32 (+ 14.37)	7.86 (+ 13.91)	8.06 (+ 12.73)	5.79 (+ 6.82)	6.17 (+ 2.83)	6.47 (+ 3.68)	5.50 (+ 3.18)	6.10 (+ 10.50)	6.32 (+ 11.85)
200	7.96 (+ 24.37)	8.50 (+ 23.18)	8.80 (+ 23.07)	6.05 (+ 11.62)	6.52 (+ 8.66)	6.75 (+ 8.17)	5.66 (+ 6.19)	6.32 (+ 14.49)	6.68 (+ 18.23)
300	8.55 (+ 33.59)	9.08 (+ 31.59)	9.40 (+ 31.46)	6.38 (+ 17.71)	7.18 (+ 19.66)	7.40 (+ 18.58)	5.92 (+ 11.06)	6.60 (+ 19.56)	6.97 (+ 23.36)
400	9.35 (+ 46.09)	9.78 (+ 41.73)	10.05 (+ 40.55)	6.74 (+ 24.35)	7.45 (+ 24.16)	7.67 (+ 22.91)	6.25 (+ 17.26)	6.95 (+ 25.90)	7.15 (+ 26.54)
500	10.00 (+ 56.25)	10.61 (+ 53.76)	10.98 (+ 53.56)	6.96 (+ 28.41)	8.44 (+ 40.66)	8.60 (+ 37.82)	6.77 (+ 27.01)	7.40 (+ 34.05)	7.67 (+ 35.75)
600	8.42 (+ 31.56)	9.58 (+ 38.84)	9.83 (+ 37.48)	6.35 (+ 17.15)	6.80 (+ 13.66)	7.00 (+ 12.17)	5.90 (+ 10.69)	6.50 (+ 17.75)	6.88 (+ 21.76)
700	7.15 (+ 11.71)	7.63 (+ 10.57)	7.90 (+ 10.48)	5.58 (+ 2.95)	6.00 (+ 0.00)	6.32 (+ 1.28)	5.35 (+ 0.37)	6.00 (+ 8.19)	6.04 (+ 6.90)
800	6.25 (- 2.34)	6.64 (- 3.76)	6.80 (- 4.89)	5.06 (- 6.64)	5.40 (- 10.00)	6.02 (- 3.52)	4.65 (- 12.75)	4.94 (- 10.50)	5.85 (- 3.53)

Leaves : F1 = 282.8627* F2 = 87.2962* Stem : F1 = 46.5899* F2 = 48.5773* Root : F1 = 47.1857* F2 = 65.3740*

Table 3. Effect of NaCl on the total sugar content of leaves, stem and root of *Lumnitzera racemosa* (mg/g fresh weight).

Concentration of NaCl (mM)	Days after NaCl treatment								
	60	120	180	60	120	180	60	120	180
	Leaves			Stem			Root		
Control	18.50	21.00	21.67	15.60	16.88	17.60	13.40	14.90	16.25
100	17.80 (- 3.78)	20.60 (- 1.90)	21.18 (- 2.26)	14.86 (- 4.74)	16.12 (- 4.50)	17.00 (- 3.40)	12.60 (- 5.97)	14.08 (- 5.50)	15.55 (- 4.30)
200	16.30 (- 11.89)	18.38 (- 12.47)	19.25 (- 11.16)	13.75 (- 11.85)	15.35 (- 9.06)	16.60 (- 5.68)	11.20 (- 16.41)	13.10 (- 12.08)	14.31 (- 11.93)
300	15.26 (- 17.51)	17.03 (- 18.90)	18.62 (- 14.07)	12.64 (- 18.97)	14.70 (- 12.91)	15.65 (- 11.07)	10.35 (- 22.76)	12.62 (- 15.30)	13.67 (- 15.87)
400	14.05 (- 24.05)	16.25 (- 22.61)	17.33 (- 20.02)	11.50 (- 26.28)	12.60 (- 25.35)	14.33 (- 18.57)	9.06 (- 32.38)	12.00 (- 19.46)	13.11 (- 19.32)
500	12.25 (- 33.78)	14.37 (- 31.57)	15.69 (- 27.59)	09.44 (- 39.48)	10.98 (- 34.95)	13.75 (- 21.87)	8.00 (- 40.29)	10.65 (- 28.52)	11.32 (- 30.33)
600	13.30 (- 28.10)	15.68 (- 25.33)	17.60 (- 18.78)	10.67 (- 31.60)	12.90 (- 23.57)	15.61 (- 11.30)	9.67 (- 27.83)	11.95 (- 19.80)	13.68 (- 15.81)
700	14.70 (- 20.54)	16.95 (- 19.28)	18.76 (- 13.42)	12.30 (- 21.15)	14.26 (- 15.52)	16.78 (- 4.65)	11.00 (- 17.91)	13.64 (- 8.46)	14.92 (- 8.18)
800	15.85 (- 14.32)	18.66 (- 11.14)	19.35 (- 10.70)	13.80 (- 11.53)	15.94 (- 5.56)	17.12 (- 2.72)	12.06 (- 10.00)	14.01 (- 5.97)	15.66 (- 3.63)

Leaves : F1 = 131.6602* F2 = 305.2864* Stem : F1 = 27.0623* F2 = 76.5837* Root : F1 = 71.6434* F2 = 285.5531*

Total phenol

The results on the effect of sodium chloride on the total phenol content of the leaf, stem and root are given in Table 7. The phenol content was increased with increasing NaCl salinity upto 500 mM in all the plant tissues. The stem had more phenol content than the leaf and root. The highest phenol content (14.50 mg/g fr. wt.) was recorded in the stem of *L. racemosa* at 500 mM NaCl on the 180th day. The increase was 61.11% over to that of control plants. The F values calculated for difference between the sampling days and between the treatments were significant at 1% level.

DISCUSSION

Free amino acids

A gradual increase in free amino acid content was noticed with increasing concentrations of NaCl salinity upto 800mM. Free amino acids have been reported to accumulate in halophytes subjected to saline stress [23]. Accumulation of free amino acids occurred in many plants in response to changing osmotic potentials in their external environment by osmotic adjustment of their cellular contents [24].

Reports showed some similarity as in certain other halophytes such as *Atriplex satidea* [25] and Avicennia officinalis [26]. The increased level of free amino acid pool during salt treatment in different plant species contradict with decreased amino acid pool by salinity in *Aegiceras corniculatum* [27].

An increase in the free amino acids at higher salinity levels may be due to degradation of protein [28]. In the present study, the gradual increase in the free amino acid content under salt treatments can also be correlated to the decrease in protein content at higher salinity concentrations. This may be attributed to increased degradation of protein at high salinity levels [29].

Table 4. Effect of NaCl on the starch content of leaves, stem and root of Lumnitzera racemosa (mg/g fresh weight)

Concentration of NaCl (mM)	Days after NaCl treatment								
	60	120	180	60	120	180	60	120	180
	Leaves			Stem			Root		
Control	20.60	22.35	24.00	18.28	20.00	21.06	15.80	18.32	19.86
100	21.20 (+ 2.91)	23.94 (+ 7.11)	25.67 (+ 6.95)	18.96 (+ 3.71)	21.17 (+ 5.85)	22.00 (+ 4.46)	16.60 (+ 5.06)	19.83 (+ 8.24)	21.08 (+ 6.14)
200	22.35 (+ 8.49)	25.36 (+ 13.46)	26.38 (+ 9.91)	19.95 (+ 9.13)	22.95 (+ 14.75)	24.03 (+ 14.10)	17.90 (+ 13.29)	21.00 (+ 14.62)	22.62 (+ 13.89)
300	24.90 (+ 20.87)	27.00 (+ 20.80)	28.06 (+ 16.91)	21.80 (+ 19.25)	24.08 (+ 20.40)	25.93 (+ 23.12)	19.27 (+ 21.96)	22.05 (+ 20.36)	24.34 (+ 22.55)
400	27.50 (+ 33.49)	29.35 (+ 31.31)	32.66 (+ 36.08)	23.25 (+ 27.18)	26.33 (+ 31.65)	28.08 (+ 33.33)	20.86 (+ 32.02)	24.08 (+ 31.44)	25.86 (+ 30.21)
500	30.80 (+ 49.51)	33.60 (+ 50.33)	34.42 (+ 43.41)	26.70 (+ 46.06)	29.06 (+ 45.30)	30.94 (+ 46.91)	22.35 (+ 41.45)	26.65 (+ 45.46)	28.00 (+ 40.98)
600	25.65 (+ 24.51)	26.34 (+ 17.85)	30.27 (+ 26.12)	22.30 (+ 21.99)	23.17 (+ 15.85)	25.00 (+ 18.70)	18.00 (+ 13.92)	22.06 (+ 20.41)	23.02 (+ 15.91)
700	20.04 (- 2.71)	22.05 (- 1.34)	23.00 (- 4.16)	17.35 (- 5.08)	19.66 (- 1.70)	20.17 (- 4.22)	15.05 (- 4.74)	18.06 (- 1.41)	19.67 (- 0.95)
800	18.00 (- 12.62)	20.87 (- 6.62)	22.16 (- 7.66)	15.50 (- 15.20)	17.00 (- 15.00)	18.05 (- 14.29)	13.80 (- 12.65)	16.84 (- 8.07)	18.00 (- 9.36)

Leaves : F1 = 130.4144* F2 = 91.2448* Stem: F1 = 166.7564* F2 = 111.615* Root : F1 = 234.6732* F2 = 454.156*

Table 5. Effect of NaCl on the proline content of leaves, stem and root of *Lumnitzera racemosa* (mg/g fresh weight).

Concentration of NaCl (mM)	Days after NaCl treatment								
	60	120	180	60	120	180	60	120	180
	Leaves			Stem			Root		
Control	4.05	4.80	5.06	3.35	4.00	4.36	2.82	3.67	3.85
100	4.34 (+ 7.16)	5.10 (+ 6.25)	5.66 (+ 11.85)	3.62 (+ 8.05)	4.54 (+ 13.5)	5.00 (+ 14.67)	3.13 (+ 10.99)	4.06 (+ 10.62)	4.38 (+ 13.76)
200	4.78 (+ 18.02)	5.64 (+ 17.50)	5.93 (+ 17.19)	3.98 (+ 18.80)	4.89 (+ 22.25)	5.41 (+ 24.08)	3.45 (+ 22.34)	4.50 (+ 22.61)	4.96 (+ 28.83)
300	5.36 (+ 32.34)	6.47 (+ 34.79)	6.90 (+ 36.36)	4.55 (+ 35.82)	5.60 (+ 40.00)	5.97 (+ 36.92)	3.92 (+ 39.007)	4.83 (+ 31.60)	5.36 (+ 39.22)
400	6.00 (+ 48.14)	7.05 (+ 46.87)	7.56 (+ 49.40)	4.96 (+ 48.05)	5.88 (+ 47.00)	6.35 (+ 45.64)	4.28 (+ 51.77)	5.06 (+ 37.87)	5.85 (+ 51.94)
500	6.65 (+ 64.19)	8.00 (+ 66.66)	8.63 (+ 70.55)	5.64 (+ 68.35)	6.43 (+ 60.75)	6.85 (+ 57.11)	4.66 (+ 65.24)	5.56 (+ 51.49)	6.04 (+ 56.88)
600	7.48 (+ 84.69)	9.30 (+ 93.75)	10.00 (+ 97.62)	5.87 (+ 75.22)	6.75 (+ 68.75)	7.13 (+ 63.53)	4.96 (+ 75.88)	5.84 (+ 59.12)	6.35 (+ 64.93)
700	8.07 (+ 99.25)	10.55 (+ 119.79)	11.80 (+ 133.20)	6.44 (+ 92.23)	7.08 (+ 77.00)	7.49 (+ 71.78)	5.29 (+ 87.58)	5.99 (+ 63.21)	6.60 (+ 71.42)
800	8.85 (+ 118.51)	11.70 (+ 143.75)	12.60 (+ 149.01)	6.90 (+ 105.97)	7.50 (+ 87.50)	7.86 (+ 80.27)	5.45 (+ 93.26)	6.15 (+ 67.57)	6.91 (+ 79.48)

Leaves : F1 = 52.4356* F2 = 33.653* Stem : F1 = 423.2271* F2 = 341.0243* Root : F1 = 214.638* F2 = 340.1484*

Protein

The sodium chloride salinity upto the optimum level increased the protein content in *L. racemosa* and decreasing trend was noticed with increasing salinity. Similar observations were noticed under seawater and various salinity concentrations in certain halophytes such as *Ceriops roxburghiana* [30] and in *Rhizophora apiculata* [31].

Carbohydrates

The total sugar content decreased with increasing sodium chloride salinity, and the starch content of the various plant tissues increased in *L. racemosa*. The reverse situation was noticed at concentrations beyond the optimum level. The role of salt up to a specific level on the stomatal opening has been implicated in the decrease in sugar and rise in starch content [32]. When the high molecular weight carbohydrates are released, they are transformed into soluble forms, such as sucrose, glucose, and fructose, which are easily transported to locations where they are needed for growth [33].

Many physiological and biochemical processes in plants are impacted by salinity stress, which changes some metabolic pathways. The buildup of sugars and several other organic solutes is one of the main impacts of those affecting carbohydrate metabolism [34].

Studies on seagrass carbohydrates have revealed that soluble sugar concentration decreases as salinity rises. Carbohydrates are probably transformed into other organic components during salt stress, which would help these plants adjust to their osmotic environment. The observed decrease in sucrose-P synthase activity in seagrass subjected to increased salinities provides more support for this [35].

Table 6. Effect of NaCl on the glycinebetaine content of leaves, stem and root of *Lumnitzera racemosa* (mg/g fresh weight).

Concentration of NaCl (mM)	Days after NaCl treatment								
	60	120	180	60	120	180	60	120	180
	Leaves			Stem			Root		
Control	2.50	2.80	2.93	2.06	2.45	2.66	1.80	2.00	2.15
100	2.84 (+ 13.60)	3.55 (+ 26.78)	4.01 (+ 36.86)	2.25 (+ 9.22)	2.77 (+ 13.06)	3.00 (+ 12.78)	2.05 (+ 13.88)	2.65 (+ 32.50)	2.90 (+ 34.88)
200	3.17 (+ 26.80)	4.00 (+ 42.85)	4.47 (+ 52.55)	2.60 (+ 26.21)	3.30 (+ 34.69)	3.70 (+ 39.09)	2.18 (+ 21.11)	2.80 (+ 40.00)	3.10 (+ 44.18)
300	3.58 (+ 43.20)	4.61 (+ 64.64)	5.02 (+ 71.33)	3.07 (+ 49.02)	3.80 (+ 55.10)	4.15 (+ 56.01)	2.64 (+ 46.66)	3.33 (+ 66.50)	3.56 (+ 65.58)
400	4.50 (+ 80.00)	5.45 (+ 94.64)	5.94 (+ 102.73)	3.65 (+ 77.18)	4.42 (+ 80.40)	4.78 (+ 79.69)	2.90 (+ 61.11)	3.64 (+ 82.00)	3.95 (+ 83.72)
500	5.60 (+ 124.00)	6.47 (+ 131.07)	6.85 (+ 133.78)	4.24 (+ 105.82)	4.88 (+ 99.18)	5.15 (+ 93.60)	3.32 (+ 84.44)	4.00 (+ 100.00)	4.34 (+ 101.86)
600	5.88 (+ 135.20)	6.72 (+ 140.00)	7.04 (+ 140.27)	4.40 (+ 113.92)	5.14 (+ 109.79)	5.41 (+ 103.38)	3.46 (+ 92.22)	4.19 (+ 109.50)	4.58 (+ 113.02)
700	6.16 (+ 146.40)	7.00 (+ 150.00)	7.25 (+ 147.44)	4.68 (+ 127.18)	5.26 (+ 114.69)	5.58 (+ 109.77)	3.64 (+ 102.22)	4.35 (+ 117.50)	4.66 (+ 116.74)
800	6.30 (+ 152.00)	7.22 (+ 157.85)	7.45 (+ 154.26)	4.90 (+ 137.86)	5.37 (+ 119.18)	5.69 (+ 113.90)	3.80 (+ 111.11)	4.52 (+ 126.00)	4.78 (+ 122.32)

Leaves : F1 = 302.6655* Stem : F1 = 407.905* Root : F1 = 149.116*
F2 = 126.9569* F2 = 218.6504* F2 = 139.8846*

Table 7. Effect of NaCl on the total phenol content of the leaves, stem and root of *Lumnitzera racemosa* (mg/g fresh weight).

Concentration of NaCl (mM)	Days after NaCl treatment								
	60	120	180	60	120	180	60	120	180
	Leaves			Stem			Root		
Control	6.00	6.54	7.06	8.05	8.66	9.00	5.40	5.86	6.90
100	6.35 (+ 5.83)	7.00 (+ 7.03)	9.10 (+ 28.89)	8.62 (+ 7.08)	9.18 (+ 6.00)	12.10 (+ 34.44)	5.80 (+ 7.40)	6.45 (+ 10.06)	8.44 (+ 22.31)
200	7.05 (+ 17.5)	7.65 (+ 16.97)	10.40 (+ 47.30)	9.25 (+ 14.90)	10.00 (+ 15.47)	12.86 (+ 42.88)	6.30 (+ 16.66)	6.97 (+ 18.94)	8.95 (+ 29.71)
300	7.66 (+ 27.66)	8.50 (+ 29.96)	10.96 (+ 55.24)	9.68 (+ 20.24)	10.86 (+ 25.40)	13.27 (+ 47.44)	6.68 (+ 23.70)	7.69 (+ 31.22)	9.58 (+ 38.84)
400	8.30 (+ 38.33)	9.75 (+ 49.08)	12.05 (+ 70.67)	10.65 (+ 32.29)	11.30 (+ 30.48)	13.94 (+ 54.88)	7.26 (+ 34.44)	8.33 (+ 42.15)	10.16 (+ 47.24)
500	9.00 (+ 50.00)	10.46 (+ 59.93)	13.60 (+ 92.63)	11.28 (+ 40.12)	11.75 (+ 35.68)	14.50 (+ 61.11)	7.75 (+ 43.51)	8.70 (+ 48.46)	10.87 (+ 57.53)
600	8.06 (+ 34.33)	9.20 (+ 40.67)	11.34 (+ 60.62)	9.77 (+ 21.36)	10.07 (+ 16.28)	12.60 (+ 40.00)	7.00 (+ 29.62)	8.00 (+ 36.51)	9.80 (+ 42.02)
700	7.04 (+ 17.33)	8.78 (+ 34.25)	10.30 (+ 45.89)	9.00 (+ 11.80)	9.80 (+ 13.16)	12.07 (+ 34.11)	6.25 (+ 15.74)	7.52 (+ 28.32)	9.03 (+ 30.86)
800	6.55 (+ 9.16)	7.67 (+ 17.27)	9.25 (+ 31.01)	8.64 (+ 7.32)	9.08 (+ 4.84)	9.03 (+ 0.33)	6.00 (+ 11.11)	7.23 (+ 23.37)	8.40 (+ 21.73)

Leaves : F1 = 19.9958* Stem : F1 = 11.0480* Root : F1 = 34.5412*
F2 = 80.2436* F2 = 39.7060* F2 = 215.873*

Proline

A substantial increase in the proline content of the plant tissues of *L. racemosa* was noticed with increasing sodium chloride salinity upto 800 mM. The leaf tissue had more proline content than stem and root tissues. Increasing proline levels in the leaves of *L. racemosa* are in consistence with other reports in different species [31] and *Sesuvium portulacastrum* [36]. This is persumably achieved by inducing proline biosynthetic enzymes and representing synthesis of proline catabolising enzymes [37].

Glycinebetaine

With increasing NaCl concentrations up to 800 mM, *L. racemosa* accumulated the glycinebetaine content. In *Halopyrum mucronatum* species of Chenopodiaceae [38], stress induced glycinebetaine is adaptive, since it may function as a non-toxic cytoplasmic osmoticum or an osmoprotectant and may act as a compatible solute. The present results indicate that a passive relationship between salt concentration and accumulation of betaine and is also reported for plants such as *Atriplex barchyana* [39] and in sugar beet [40].

Total phenol

The leaf, stem and root issues of *L. racemosa* were used for the estimation of total phenol. The results of the present investigation clearly showed that the phenol content was high in the stem tissue than in leaf and root tissues, at the optimum salt concentration and low phenol was recorded at very high salinity concentrations. The study on the nature of distribution of phenols in different seasons in majority of mangrove species, the total phenol was high in the monsoon season [41]. The higher amount of phenols in the monsoon season in most members of mangrove species studied, could be correlated with the low temperature and high rainfall prevailing in this season because of the lowest soil salinity due to heavy discharge of freshwater in the estuary.

The mangrove species which possess higher amount of phenols are actually growing in saline marshy habitat. In this habitat, there

is a rich microbial and fungal flora which may cause pathogenesis in plants. The literature survey on the role of the phenols shows that they are protective agents based on their chemical nature and play a significant role directly or indirectly in physiological process such as growth and development, besides acting as auto-toxic and alleopathic agents [42]. The present study revealed that the high phenol content seen at low salinity level was evident to prevent the phenolic attack by microbes and fungi in *L. racemosa* seedlings.

CONCLUSION

From this study it can be concluded that the biochemical constitution of *L. recemosa* seedlings are affected due to the exogenous application of salinity. This should be taken care while planning future breeding practices, and also saline water irrigation practices in crops.

REFERENCES

1. Khairnar SO, Solanki BV, Junwei L. Mangrove ecosystem-its threats and conservation. Aquafind, College of Fisheries. Ocean University of China. 2019;
2. Bandaranayake W. Traditional and medicinal uses of mangroves. Mangroves and salt marshes. 1998;2:133–48.
3. Abd El-Gawad AM, El-Amier YA. Anatomical features of three perennial swampy plants of Poaceae, grown on the water stream banks in Nile Delta, Egypt. J Med Bot. 2018;1:58. http://dx.doi.org/10.25081/jmb.2017.v1.863
4. Odum WE, Mcivor CC, Smith TJ. The ecology of the mangroves of south Florida: a community profile. The Service. 1982.
5. Barik J, Mukhopadhyay A, Ghosh T, Mukhopadhyay SK, Chowdhury SM, Hazra S. Mangrove species distribution and water salinity: an indicator species approach to Sundarban. J Coast Conserv. 2018;22(2):361–8. http://dx.doi.org/10.1007/s11852-017-0584-7
6. Soumya K, Srivani A, Mohan GK. Insights into phyto pharmaceutical studies of the under-utilized mangrove species of *Lumnitzera racemosa* wild: A review. Int J Herb Med. 2022;10(6):25–31. http://dx.doi.org/10.22271/flora.2022.v10.i6a.839
7. Abobatta WF. Plant responses and tolerance to extreme salinity: Learning from halophyte tolerance to extreme salinity. Salt and Drought Stress Tolerance in Plants: Signaling Networks and Adaptive Mechanisms. 2020:177–210.
8. Bottomley DJ, Conrad Gregoire D, Raven KG. Saline ground waters and brines in the Canadian Shield: Geochemical and isotopic evidence for a residual evaporite brine component. Geochim Cosmochim Acta. 1994;58(5):1483–98. http://dx.doi.org/10.1016/0016-7037(94)90551-7
9. Kawtar B. Trifolium isthmocarpum Brot, a salt-tolerant wild leguminous forage crop in salt-affected soils. J Stress Physiol Biochem. 2013;9(3):299–317.
10. Ravikumar P, Somashekar RK. A geochemical assessment of coastal groundwater quality in the Varahi river basin, Udupi District, Karnataka State, India. Arab J Geosci. 2013;6(6):1855–70. http://dx.doi.org/10.1007/s12517-011-0470-9
11. Hardwick K, Healey JR, Elliott S, Blakesley D. Research needs for restoring seasonal tropical forests in Thailand: accelerated natural regeneration. New Forests. 2004;27(3):285–302. http://dx.doi.org/10.1023/b:nefo.0000022228.08887.d2
12. Alongi DM. Carbon sequestration in mangrove forests. Carbon Manag. 2012;3(3):313–22. http://dx.doi.org/10.4155/cmt.12.20
13. Shrestha S, Miranda I, Kumar A, Pardo MLE, Dahal S, Rashid T, et al. Identifying and forecasting potential biophysical risk areas within a tropical mangrove ecosystem using multi-sensor data. Int J Appl Earth Obs Geoinf. 2019;74:281–94. http://dx.doi.org/10.1016/j.jag.2018.09.017
14. Krauss KW, Lovelock CE, McKee KL, López-Hoffman L, Ewe SML, Sousa WP. Environmental drivers in mangrove establishment and early development: A review. Aquat Bot. 2008;89(2):105–27. http://dx.doi.org/10.1016/j.aquabot.2007.12.014
15. Moore S, Stein WH. Photometric nin-hydrin method for use in the chromatography of amino acids. J Biol Chem. 1948;176:367–88.
16. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193(1):265–75. http://dx.doi.org/10.1016/s0021-9258(19)52451-6
17. Nelson N. A photometric adaptation of the Somogyis method for the determination of reducing sugar. Anal Chem. 1944;31:426–8.
18. Summer JB, Somers CF. Laboratory experiment in biological chemistry. New York: Academic Press; 1949.
19. Bates LS, Waldren RP, Teare ID. Rapid determination of the free proline in water stress studies. Plant Soil. 1973;38:205–8.
20. Grieve CM, Grattan SR. Rapid assay for determination of water soluble quaternary ammonium compounds. Plant Soil. 1983;70(2):303–7. http://dx.doi.org/10.1007/bf02374789
21. Bray HG, Thorpe WV. Analysis of phenolic compounds of interest in metabolism. Methods Biochem Anal. 1954;1:27–52. http://dx.doi.org/10.1002/9780470110171.ch2
22. Cox DF, Gomez KA, Gomez AA. Statistical procedures for agricultural research. J Am Stat Assoc. 1985;80(390):486. http://dx.doi.org/10.2307/2287932
23. Bar-Nun N, Poljakoff-Mayber A. Salinity stress and the control of proline in roots of Pisum sativum and *Tamaria tetragyna*. Ann Bot. 1977;41:173–9.
24. Greenway H, Munns R. Mechanism of salt tolerance in non-halophytes. Annu Rev Plant Physiol. 1980;31:149–90.
25. Flowers TJ, Dalmond D. Protein synthesis in halophytes: The influence of potassium, sodium and magnesium in vitro. Plant Soil. 1992;146(1–2):153–61. http://dx.doi.org/10.1007/bf00012008
26. Ranganathan R, Sheela R, Venkatesan A, Ravindran KC. Seawater induced changes in organic constituents of *Avicennia officinalis* L. J Ind Bot Soc. 2001;80:285–7.
27. Parida AK, Das AB, Sanada Y, Mohanty P. Effects of salinity on biochemical components of the mangrove, *Aegiceras corniculatum*. Aquat Bot. 2004;80(2):77–87. http://dx.doi.org/10.1016/j.aquabot.2004.07.005
28. Shamsutdinow Z, Sh NA, Myasoedov LC, Kalinkina OK, Baburina TG, Naumova V. Effect of soil salinity on contents and sets of amino acids in halophytes. Probl Desert Develop. 1995;3:66–7.
29. Zahra S, Amin B, Ali VM, Ali Y, Mehdi Y. The salicylic acid effect on the tomato (*Lycopersicum esculentum* Mill.) sugar, protein and proline contents under salinity stress (NaCl). J Biophysics Struct Biol. 2010;2:35–41.
30. Rajesh A, Arumugam R, Venkatesalu V. Responses of *Ceriops Roxburghiana* to NaCl Stress. Biol Plant. 1999;42(1):143–8. http://dx.doi.org/10.1023/a:1002189425061
31. Das, Parida, Basak, Das. Studies on pigments, proteins and photosynthetic rates in some mangroves and mangrove associates from Bhitarkanika, Orissa. Mar Biol. 2002;141(3):415–22. http://dx.doi.org/10.1007/s00227-002-0847-0
32. Parida AK, Veerabathini SK, Kumari A, Agarwal PK. Physiological, anatomical and metabolic implications of salt tolerance in the halophyte *Salvadora persica* under hydroponic culture condition. Front Plant Sci. 2016;7:351. http://dx.doi.org/10.3389/fpls.2016.00351
33. Hartmann H, Trumbore S. Understanding the roles of nonstructural carbohydrates in forest trees-from what we can measure to what we want to know. New Phytol. 2016;211(2):386–403.
34. Kameli A, Lösel DM. Growth and sugar accumulation in durum wheat plants under water stress. New Phytol. 1996;132(1):57–62. http://dx.doi.org/10.1111/j.1469-8137.1996.tb04508.x
35. Touchette BW. Seagrass-salinity interactions: Physiological mechanisms used by submersed marine angiosperms for a life at sea. J Exp Mar Bio Ecol. 2007;350(1–2):194–215. http://dx.doi.org/10.1016/j.jembe.2007.05.037

36. Slama I, Ghnaya T, Messedi D, Hessini K, Labidi N, Savoure A, et al. Effect of sodium chloride on the response of the halophyte species *Sesuvium portulacastrum* grown in mannital-induced water stress. J Plant Res. 2007;120(2):291–9.
37. Yoshiba Y, Kiyosue T, Nakashima K, Yamaguchi-Shinozaki K, Shinozaki K. Regulation of levels of proline as an osmolyte in plants under water stress. Plant Cell Physiol. 1997;38(10):1095–102. http://dx.doi.org/10.1093/oxfordjournals.pcp.a029093
38. Marcum KB, Murdoch CL. Salt tolerance of the coastal salt marsh grass, *Sporobolus virginicus* (L.) kunth. New Phytol. 1992;120(2):281–8. http://dx.doi.org/10.1111/j.1469-8137.1992.tb05665.x
39. Nerd A, Pasternak D. Growth, ion accumulation and nitrogen fractioning in *Atriplex barchayana* grown at various salinities. J Range Manage. 1992;45:164–6.
40. Chołuj D, Karwowska R, Ciszewska A, Jasińska M. Influence of long-term drought stress on osmolyte accumulation in sugar beet (*Beta vulgaris* L.) plants. Acta Physiol Plant. 2008;30(5):679–87. Available from: http://dx.doi.org/10.1007/s11738-008-0166-2
41. Marchand C, Disnar JR, Lallier-Vergès E, Lottier N. Early diagenesis of carbohydrates and lignin in mangrove sediments subject to variable redox conditions (French Guiana). *Geochim Cosmochim* Acta. 2005;69(1):131–42. http://dx.doi.org/10.1016/j.gca.2004.06.016
42. Cheynier V, Comte G, Davies KM, Lattanzio V, Martens S. Plant phenolics: recent advances on their biosynthesis, genetics, and ecophysiology. Plant Physiol Biochem. 2013;72:1–20. http://dx.doi.org/10.1016/j.plaphy.2013.05.009