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Salinity stress effects on the growth, morphological, physiological, and biochemical properties of Melia (*Melia dubia* Cav.) plant

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Abstract: Salinity stress severely affects the growth, physiological and developmental processes in plant species. *Melia dubia* is an ecologically and economically important tree species of the Indian subcontinent. However, systematic information with respect to the species salt tolerance potential is completely lacking. Under salt stress conditions, determining suitable soil EC range is required for the better survival, growth and productivity of the tree species. In present study, we investigated the effects of different soil salinity (EC 4, 8, and 12) levels on the ion homeostasis, physio-biochemistry, morphology, and growth of *M. dubia* plant. Results revealed that increase in soil salinity causes higher Na^+ content and Na^+/K^+ ratio, while lower K^+ content, in the leaf tissues of *M. dubia*. The physiological processes such as the photosynthetic rate, stomatal conductance, internal CO_2 concentration, and transpiration rate were adversely affected with the increased salt stress levels. Morphological parameters, such as internodal length, petiole length, leaf length, and leaf width also decreased ($P<0.05$) under saline stress conditions. Results further indicated that salinity levels significantly ($P<0.05$) affected the *M. dubia* growth, and the growth rate was found optimum upto 8 EC, thereafter it slightly decreased with the increased salt stress to 12 EC. Our findings showed that increased salinity stress causes significant changes in the physiological, morphological, and growth pattern of *M. dubia*. Therefore, based on present experiment, we found *M. dubia* suitable for the salt affected soils of EC 8 with optimum growth rate and at EC 12 with the moderate (20–25%) growth reduction.

Keywords: *Melia*, salt stress, growth, physiology, phenology, biochemical

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Introduction

Plants growing under field conditions suffer from variety of stresses that severely affects their growth and productivity (Yuan et al., 2019). Among the environmental stressors, soil salts is one of the most detrimental abiotic stresses in plants (Flowers, 2004; Munns & Tester, 2008). More importantly, area under salt-affected soils is expanding and spreading not only in India but also in the different regions of the world (Zhang, 2014; Bhardwaj et al., 2019). Even though limited land resource, available land is affected by the soil salts that consequently affects the biological processes and economic yield of plants. Moreover, due to shrinking agriculture land, rising tree products demand, decreasing crop productivity

and increasing salinization globally, strong need are thereby felt to bring these lands under commercial tree plantations (Kumar et al., 2020).

Salt stress imposes more severe damage to plants as result of combination of ion toxicity, osmotic stress, nutrient deficiency, water imbalance, and unfavorable soil structure (Zhao, 2016; Sjoman et al., 2021). Saline soils mostly contain Na^+, K^+, Ca^{2+} and Mg^{2+} cations, and Cl^- and SO_4^{2-} anions, and the increased anions concentration leads to the high soil EC in the saline conditions (Zhang et al., 2006). High salt stress induced by non-essential toxic element in glycophytes modifies the various physio-biochemical and morphological traits, and reduces the growth of the exposed plants (Parida et al., 2019). Once tree species are exposed to high salt stress, their growth and productivity are severely affected, however, different soil EC levels can have contrasting effect on the plant physiological and growth processes (Banyal et al., 2017). Based on salt tolerance, tree species can be categorized as sensitive, moderately tolerant, highly tolerant and extremely high tolerant (Tomar et al., 2003; Dagar, 2014). Therefore, determining optimum range of salt tolerance in commercially important tree species is urgently required for obtaining the greater ecological and economic benefits, and for enhancing the overall productivity of salt affected soils.

Melia dubia Cav. is grown extensively in the tropical and subtropical regions of India, South East Asia, China, and Australia (Kumar et al., 2017). Species can be grown in all soil types of tropics and subtropics regions, receiving more than 500 mm annual rainfall, although species survival has also been reported in the drier areas receiving a low annual rainfall (200 mm) (Pradeep, 2015). It is a fast growing, multipurpose, industrially important, and has high ecological and economic value, which is extensively cultivated in different agroforestry systems of the several countries (Kumar et al., 2021a). In India, this species is expanding rapidly due to greater demand and acceptability among the farmers and industries. Because, Melia is used mostly in pulpwood, plywood, light timber, and medicinal industry, species is expected to rapidly occupy large areas of fertile and marginal salt affected lands (Parthiban et al., 2009). Therefore, *M. dubia* is considered as an outstanding tree for the plantation and agroforestry, as well as a good model plant among tree species for understanding the salt tolerance.

Understanding the salt tolerance potential of *Melia* is required to improve the land productivity, which is an important challenge for the researchers to cope with the issue of salt stress induced land degradation. The demand for the tree based products is increasing exponentially, and with the limited land availability need is thereby felt to bring unproductive salt affected soils under commercial tree plantations. Still, industries and stakeholders have not adopted Melia in salt affected soils; hence, strong opportunity exits to explore species potential in the such soils. Previous studies on the effect of salinity stress on Melia growth have been reported (Kumar et al., 2121b); however, no systematic information about the biochemical, physiological, morphological, phenological and growth aspect related to the salinity has been provided. A combination of these approaches may provide better insight in the salinity tolerance threshold of the species. The question about the range of salinity stress tolerance of *Melia* for achieving the best ecological and economic benefits is still left to be resolved. Furthermore, it would be interesting to determine the change in the biochemical, physiological and phenological at different EC levels. Assessment of growth performance under different salinity stress levels needs to be quantified to assess species suitability in salt affected soils to plan the suitable afforestation measures. The accomplishment of information about these issues will provide idea regarding the salt tolerance potential of Melia. Based on above studies, we hypothesized that soil salt causes accumulation of toxic ions that may results in a decline in the physiological process, alteration in phenology and thereby reduced plant growth of *M. dubia*. Therefore, the objectives of study were to observe the effect of salinity (i) on change in the ion homeostasis, physiological and biochemical parameters, and (ii) on the morphological modification, phenological altercation and growth dynamics of *M. dubia*. In the current investigation, we have first time reported an integrated study on change in biochemical, physiological, morphological, and growth pattern of *M. dubia* under long-term exposure to salinity stress to obtain an insight into the salt tolerance and physio-biochemical processes in species with a future aim to develop salt tolerant germplasm.

Materials and Methods

Plant material

Seeds of *M. dubia* were collected from US Nagar, Uttarakhand (29°03'N; 79°27'E at 298 m asl) during February 2018. Seeds were sown in germination beds (soil and sand) and watered daily till the completion of germination. After 10 days, the seedlings were transplanted in polybags containing sand, soil and FYM (1:1:1) and watered every day.

Experimental site

The experiment was conducted during 2018–2019 & 2019–2020 at ICAR-CSSRI, Karnal, India

(29°84'30"N, 76°85'80"E) situated at 245 m above mean sea level. The climate of area is semi-arid characterized by winters from November to March and summers from April to October. A mean monthly maximum temperature of 38.6 °C during May, and the minimum of 5.8 °C were recorded during the January. The mean annual precipitation was 1388 mm, with 80% receives during the rainy season (July–September). Experiment was started during August–September (rainy season) which continued during October–November (autumn), December–January (winter), and February–March (spring), and terminated during April (summer) of the following year.

Seedling transplanting

For salinity treatments, the soil brought from outside was sieved, made homogenous and 10 kg soils filled in the each pots. In Aug 2018 and 2019, three-month-old uniformly grown saplings of *M. dubia* were transplanted in plastic pots. Fifteen seedlings (replication) were planted in each treatment, and a total 60 seedlings were planted in the three EC (4, 8, and 12) levels and in control. Seedlings were uniformly watered (every alternate day in summer and after four days in winter) and weeding operation was carried out every week.

Imposition of salt stress

We maintained three levels of salinity irrigation water stresses i.e. 4, 8, 12 EC dS/m and a control. We brought saline water of 16 EC dS/m from outside and stored in a plastic container for watering the seedlings. The required quantity of good quality water (2 EC) mixed with the saline water (16EC) for making the 4, 8, and 12 EC salinity levels. We alternatively applied saline and good quality water to distribute salt uniformly and to prevent excess accumulation of salts than the required in the treatments. Ionic composition of saline water used is presented in Table 1. We regularly monitored ECe of the soils for maintaining the above salinity levels. Beside EC, a control (EC=2.2; pH 7.7) was also maintained which was irrigated with the good quality water.

Measurement of soil EC

The soil ECe (4, 8, and 12) of all the levels and control were measured every month. For that, 20 g of the dried, grinded, and sieved soil mixed with the 40 mL distilled water to make suspension. Electrical conductance of soil saturation paste extract was measured using EC meter (Eutech Instruments, CON 700) in accordance with USSL (1954).

Table 1. Ionic composition of saline water used in the study

EC dSm^{-1}	Ions (meq/L)		
	Na^+	K^+	$Ca^{2+}+Mg^{2+}$
Control	3.64±0.54	0.18±0.01	6.52±1.57
4	26.84±4.51	0.21±0.02	13.10±3.54
8	58.64±8.41	0.35±0.02	30.14±6.87
12	110.54±14.50	0.49±0.04	50.47±12.40

Ionic analysis

Fresh leaf samples were brought to laboratory, cleaned with distilled water, air dried and stored in paper envelopes. These leaf samples were oven dried at 60 °C to a constant weight. The 0.1 g dried samples were grinded and homogenized to fine power using the pestle and mortar and placed in a 25 mL volumetric flask. In each flask, 10 mL di-acidic mixture (HNO_3:$HClO_4$, 3:1, volume ratio) added and kept overnight, and next day sample were digested at 420 °C. The cooled samples were filtered through Whatman paper no. 42, and final volume made to 50 ml by adding the deionized water. Aliquots of this solution were used for the determination of ions viz., Na^+, K^+ by flame photometer (Systronics, 128) and Ca^{2+}, Mg^{2+}, Zn^{2+}, Mn^{2+}, Fe^{2+} and Cu^{2+} by atomic absorption spectrometry (ZEEnit 700 P, analytikjena, Germany).

Physiological measurement

Plant physiological parameters, such as intracellular CO_2 concentration, photosynthetic rate, stomatal conductance, and transpiration rate were measured as the indicators of abiotic stress in plants. The observation was conducted on the fully developed leaves grown at different salinity levels. The measurements were carried out using LI-6800 Portable Photosynthesis System at the tri-monthly interval (Oct, Jan, April). For chlorophyll analysis, 200 mg fresh leaves samples were taken and mixed with 3 ml cooled acetone (80%) in test tubes and centrifuged at 10000 rpm for 10 minutes. The centrifuges supernatant was extracted and stored in the test tube and kept overnight after adding 10 ml acetone. The absorbance of the samples was recorded at 645 and 663 nm in spectrophotometer. The chlorophyll concentration was calculated using the procedure given by Arnon's (1949). For determination of relative water content, top visible leaves were removed in morning and stored in ice humidified polythene bags. Fresh sample weight was recorded after removing all the dust particle, and sample were then soaked in distilled water for four hours. After this, leaves were dried using bloating paper and turgid weight was recorded. These leaves were further dried in oven at 60 °C for four hours to record the dry weight. The relative water content was measured using the procedure described by Barrs and Weatherly (1962). For analyzing the membrane injury, 10 ml distilled water were added in ice-humidified samples for three hours and then initial electric conductivity of the solution were measured. Further, samples were kept in the water bath for 50 minutes to achieve the total killing

tissue of tissues. The sample was cooled, and the final electric conductivity was recorded. The membrane stability was measured adopting the methodology described by Dionisio-Sene and Tobita (1962).

Protein starch and sugar analysis

The total soluble sugar was estimated as per the methodology described by Yemm and willis (1954). For this, 200 mg leaf sample was grinded in 2.5 ml ethanol (80%) and 100 µl extract was dissolved in 5 ml anthrone reagent (0.4%). The mixture was heated in the water bath at 60 °C for 10 minutes. After cooling, the absorbance was recorded at 620 nm against anthrone reagent (blank). The pellets of same extract were used for estimation of starch as per procedure described by Hassid and Neufeld (1964). The pellets were hydrolyzed with 2 ml perchloric acid (0.2 N), and allowed to stand for 24 hours at 4 °C. After centrifugation at 5000 rpm for 15 minutes, 50 µl of the supernatant was dissolved in 5 ml anthrone reagent (0.4%). The mixture was then heated at 60 °C for 10 minutes in the water bath and after cooling, absorbance was recorded at 620 nm against anthrone reagent (blank). For protein content estimation, 3 ml Bradford was added to 100 µl aliquot from the protein extract prepared in 1 N NaOH, and absorbance was recorded at 595 nm after 2 minutes and before 1 hour against the Bradford reagent (blank) as per the procedure described by Bradford (1976).

Morphology and growth measurement

The growth and morphological traits such as seedling height, collar diameter, number of leaves and branches, leaf length, leaf width, internodal length, and petiole length of each *M. dubia* seedlings were examined monthly during August to April, each during 2018–2019 and 2019–2020. Plant height was measured from base to apical shoot tips using a measurement scale. Collar diameter was measured at the base using a digital vernier caliper. The longest leaf at the first node below the apical meristem of shoots was used for the measurement of maximum leaf length (mm), maximum leaf width (mm) and petiole length (mm) using the measurement scale. Additionally, three random seedlings were measured for internode length (mm) between the third and fourth fully extended nodes from the apical meristem. Number of branches per plant, number of leaves per branch and bud formation was determined quantitatively. Apical shoot initiation measured when minimum of 0.5 cm shoot emerged after one week of first shoot appearance. All the parameters were recorded for each EC level and in control.

Experimental design and data analysis

All results were analyzed for three salinity levels and in control treatments. The data were subjected to one-way analysis of variance using SPSS 17.0 software (SPSS, Chicago, IL, USA). A p value smaller than 0.05 considered being statistically significant. Principal component analysis was performed for the thirty-two variable to determine factors contributing for the maximum variance using the component matrix value (MV).

Results

Salinity stress affect cations accumulation

We estimated the cations concentrations in leaves, stem, and root under varying salinity levels. Our results indicated that with the increased salinity stress, the Na^+ concentration of leaves, stem and root increased, while opposite trend was observed for the K^+ concentration ($P<0.05$; Table 2). Likewise, with the increasing soil salinity, the Ca^{2+} concentration decreased, while variable trends for Mg^{2+} content was observed in all the tissues. Among the

Table 2. Effects of salinity on mineral ion contents of leaf, stem, and root of *M. dubia* seedlings. (mean ± SD, n=15)

Plant tissue	Salinity level (dS/m)	Na^+ (mg g^{-1})	K^+ (mg g^{-1})	Ca^{2+} (mg g^{-1})	Mg^{2+} (mg g^{-1})	Zn^{2+} (µg g^{-1})	Cu^{2+} (µg g^{-1})	Fe^{2+} (µg g^{-1})	Mn^{2+} (µg g^{-1})
Leaf	Control	$2.68^{c}\pm0.32$	**$20.27^{a}\pm3.54$**	**$7.17^{a}\pm1.54$**	$1.61^{b}\pm0.25$	**$12.78^{a}\pm4.15$**	$0.92^{b}\pm0.24$	**$52.86^{a}\pm6.38$**	**$2.96^{a}\pm0.54$**
	EC 4	$5.29^{b}\pm0.42$	$9.16^{b}\pm1.58$	$5.01^{b}\pm0.78$	$1.79^{b}\pm0.39$	$10.07^{b}\pm3.24$	$1.02^{b}\pm0.34$	$39.11^{b}\pm8.54$	$2.34^{b}\pm0.44$
	EC 8	$6.89^{ab}\pm1.10$	$3.06^{c}\pm0.54$	$3.03^{c}\pm0.54$	$1.97^{a}\pm0.41$	$6.97^{c}\pm2.45$	$1.22^{a}\pm0.29$	$33.88^{bc}\pm6.85$	$2.20^{b}\pm0.34$
	EC 12	**$8.40^{a}\pm0.56$**	$2.80^{c}\pm0.41$	$2.95^{c}\pm0.47$	**$2.02^{a}\pm0.56$**	$5.45^{c}\pm1.54$	**$1.26^{a}\pm0.39$**	$26.20^{c}\pm4.56$	$1.76^{c}\pm0.41$
Stem	Control	$2.26^{c}\pm0.24$	**$13.99^{a}\pm2.58$**	$1.61^{b}\pm0.23$	$0.59^{c}\pm0.18$	$7.85^{a}\pm2.65$	$1.05^{b}\pm0.25$	$38.41^{ab}\pm4.12$	$2.42^{a}\pm0.36$
	EC 4	$3.19^{c}\pm0.34$	$4.44^{b}\pm0.58$	$1.78^{b}\pm0.33$	$0.79^{c}\pm0.26$	**$8.17^{a}\pm3.24$**	$0.93^{b}\pm0.36$	$12.75^{c}\pm3.01$	**$2.47^{a}\pm0.34$**
	EC 8	$5.01^{b}\pm0.85$	$3.44^{b}\pm0.56$	$1.75^{b}\pm0.28$	$1.04^{b}\pm0.24$	$5.03^{b}\pm1.74$	$0.79^{c}\pm0.24$	$32.25^{b}\pm4.68$	$1.81^{b}\pm0.26$
	EC 12	**$8.10^{a}\pm0.66$**	$3.03^{b}\pm0.45$	**$3.50^{a}\pm0.76$**	**$2.29^{a}\pm0.57$**	$3.15^{c}\pm0.57$	**$1.64^{a}\pm0.29$**	**$45.03^{a}\pm5.98$**	$1.06^{c}\pm0.21$
Root	Control	$1.37^{d}\pm0.22$	**$6.76^{a}\pm1.25$**	**$3.78^{a}\pm0.67$**	$1.12^{b}\pm0.24$	**$9.28^{a}\pm2.65$**	$1.59^{b}\pm0.38$	$34.76^{b}\pm3.65$	**$3.37^{a}\pm0.84$**
	EC 4	$4.86^{c}\pm0.51$	$6.15^{a}\pm1.12$	$2.85^{b}\pm0.57$	$1.29^{b}\pm0.33$	$8.39^{a}\pm1.65$	$1.28^{c}\pm0.41$	$35.58^{b}\pm4.85$	$2.54^{b}\pm0.64$
	EC 8	$6.89^{b}\pm0.39$	$3.74^{b}\pm0.54$	$2.88^{b}\pm0.52$	**$1.88^{a}\pm0.42$**	$6.26^{b}\pm2.01$	$1.67^{b}\pm0.33$	$12.48^{c}\pm2.65$	$1.33^{c}\pm0.54$
	EC 12	**$7.33^{a}\pm1.25$**	$3.92^{b}\pm0.48$	$1.58^{c}\pm0.28$	$1.81^{a}\pm0.52$	$5.61^{b}\pm1.78$	**$2.38^{a}\pm0.68$**	**$36.17^{a}\pm6.40$**	$1.39^{c}\pm0.32$

Means with similar latter are non-significantly different at $P<0.05$).

micronutrients, increase in salinity causes decrease in Zn^{2+} and Cu^{2+}, while variable trends for Fe^{2+} and Mn^{2+} concentration were observed in the three plant parts. The findings showed that the salt stress affected the cations accumulation in *M. dubia* seedlings.

Soil salinity induces change in physiological processes

We observed that all the physiological processes significantly declined along with the increased salt stress in plants. With increasing salinity levels from control to EC12, we observed a decrease ($P<0.05$; Table 3) in the photosynthetic rate, stomatal conductance, internal CO_2 concentration, and transpiration rate. Likewise, increase in salinity stress from EC 4 to 12 dS/m causes consistently decline in the chlorophyll content, relative water content, and increase in membrane injury of leaves ($P<0.05$; Fig. 5), compared to control. These results indicated that salt treatment induced changes in physiological processes in the *M. dubia* seedlings.

Salinity stress altered the plant biochemical

We analyzed changes in biochemical parameters of *M. dubia* leaves under the salt stress by growing seedlings at different levels of salinity (EC). The results revealed that the non-significant ($P<0.05$; Fig. 5) change in total starch was observed with the increasing salinity stress. Likewise, value of total sugar declined ($P<0.05$; Fig. 2) with increased EC from 4 to 12 dS/m, compared to control (EC 2 dS/m). Likewise, protein content decreased to the level of 2.5% (4 dS/m), 3.5% (8 dS/m), and 20% (12 dS/m) with the increasing salt stress, compared to control. Therefore, salt treatments altered the biochemical characteristics in the *M. dubia*.

Salinity stress modifies the plant morphology

We observed the effect of salt stress on morphological and phenological parameters of *Melia* under varying level of salinity stress. These findings explained that salinity levels of EC 4, 8, and 12 dS/m reduced the internodal length (6–52%), petiole length (2–39%), leaf length (7–23%) and width (3–22%) in *Melia*, compared to control ($P<0.05$; Fig. 4). Likewise, during spring season, the bud formation and apical shoot initiation was observed earlier at control (pH 7.7; EC 2 dS/m), compared to rest of the salt stress levels. Therefore, our investigation demonstrated that the morphological parameters of *M. dubia* are modified under the salt stress conditions.

Growth and Biomass of Melia decreases under salinity stress

To investigate whether salt stress could influence seedling growth, we detected changes in diameter and height growth increment of seedlings at different levels of salt stress (EC) for the seven months. Our results observed that the reduction ($P<0.05$; Fig. 1 & 2) in height and diameter growth was minimum at EC 4 and maximum at EC12 dS/m, compared to control (EC 2). In quantitative terms, height and diameter growth at EC 4, 8, and 12 decreased by 4%, 10%, 25%, and 5%, 9%, 23%, respectively, compared to control (EC 2 dS/m). The values of the number of branches per plant and leaves per branch under salinity stress showed a decreasing trend initially and increasing trend later, which was related to the season of a month, except for increasing trend from September to October. We found that the branches (no.) and leaves (no.) significantly ($P<0.05$; Fig. 1) reduced with increased salinity level from control to EC 12. Present outcome also explained that leaves, stem and biomass of seedlings were adversely affected at different salinity levels (EC), compared to control, and the decrease in ($P<0.05$; Fig. 3) these

Table 4. Proportion of total variability among different variable as explained by the principal components analysis

Component	Initial Eigenvalues		
	Total	% of Variance	Cumulative %
1	19.927	62.271	62.271
2	2.924	9.139	71.409
3	2.552	7.974	79.383
4	1.948	6.086	85.469
5	1.366	4.269	89.738
6	1.226	3.832	93.570
7	0.907	2.834	96.403
8	0.518	1.617	98.021
9	0.272	0.849	98.870
10	0.226	0.706	99.575
11	0.136	0.425	100.000

Table 3. Effect of salinity on photosynthetic rate, stomatal conductance, internal CO_2 concentration and transpiration rate in *M. dubia* (mean± SD, n=15).

	Photosynthetic rate (μ mol m^{-2} s^{-1})	Stomatal conductance (mol m^{-2} s^{-1})	Transpiration rate (m mol m^{-2} s^{-1})	Internal CO_2 concentration (ppm)
	Salinity levels			
Control	35.5^{a}±3.5	0.51^{a}±0.4	8.1^{a}±1.2	135.9^{a}±3.4
EC 4	34.9^{a}±4.2	0.48^{a}±0.3	6.0^{bc}±2.5	124.0^{b}±5.2
EC 8	29.1^{b}±4.9	0.33^{b}±0.3	6.1^{bc}±1.8	119.5^{b}±4.8
EC 12	24.7^{bc}±3.2	0.29^{b}±0.3	4.8^{c}±2.2	115.8^{c}±3.5

Means with similar latter are non-significantly different at $P<0.05$).

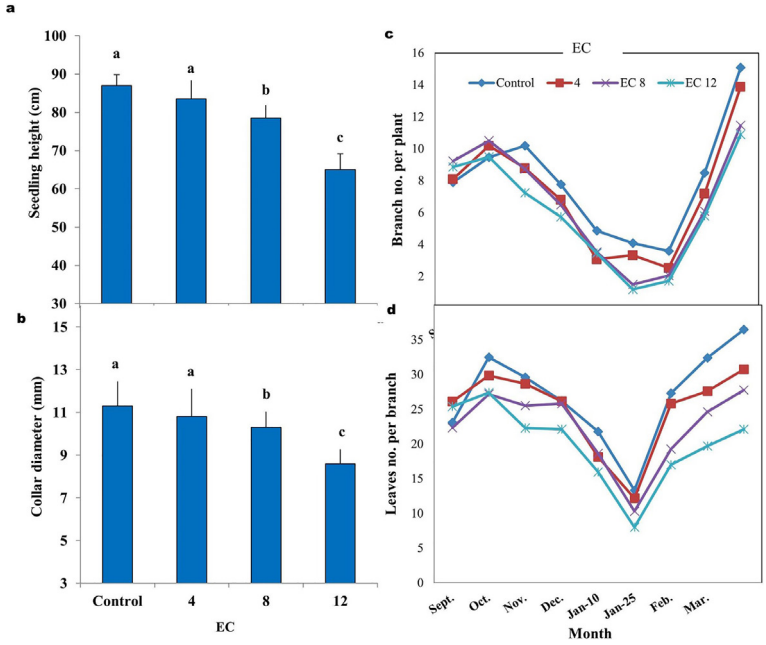

Fig 1. Effect of salinity (EC) on plant height (a), collar diameter growth (b), number of branches (c) and leaves (d) of *M. dubia* during different season (mean, n=15). Bar with similar latter are non-significantly different at P<0.05)

parameters were highest (16–32%) at EC 12, moderate (15–25%) at EC 8 and low (5–10%) at EC 4. These findings showed that the salinity stress adversely affected the growth and biomass of *M. dubia*.

Principal component analysis

Principal Components Analysis (PCA) is used to determine the most important factor (variable) contributing the variance and to approximate the relationships among a set of variables. The first eleven PCs together explained 84.02% of the total variation for different factors. The first principal component explained 62% of the entire variation, while first six PCs explained 93.5% of the total variation (Table 4). The variable , such as K^+, apical shoot initiation, Fe^{2+}, Zn^{2+}, Mn^{2+}, internodal length, petiole length, diameter, Ca^{2+}, Na^+, transpiration rate, total biomass, leaf biomass, bud initiation and height were the major contributor for the total variance in PCA1 with the matrix value >0.9 (Table 5). In second PCA, variable such as membrane injury, total starch, relative water content and chlorophyll content were the major

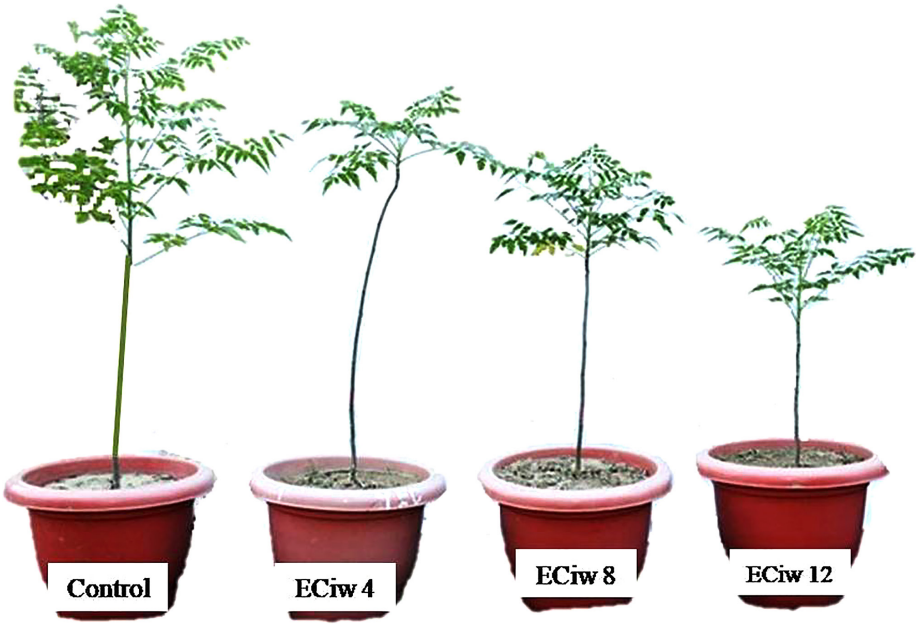

Fig. 2. Effect of salinity stress on performance of *M. dubia*

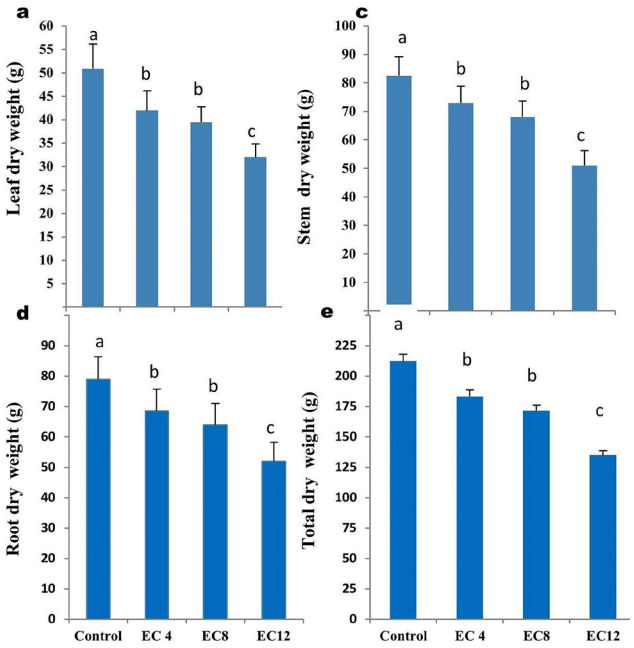

Fig 3. Salinity response of *M. dubia* for leaf biomass (a), root biomass (b), stem biomass (c) and total biomass (d) (mean, n=15). Bar with similar latter are non-significantly different at P<0.05)

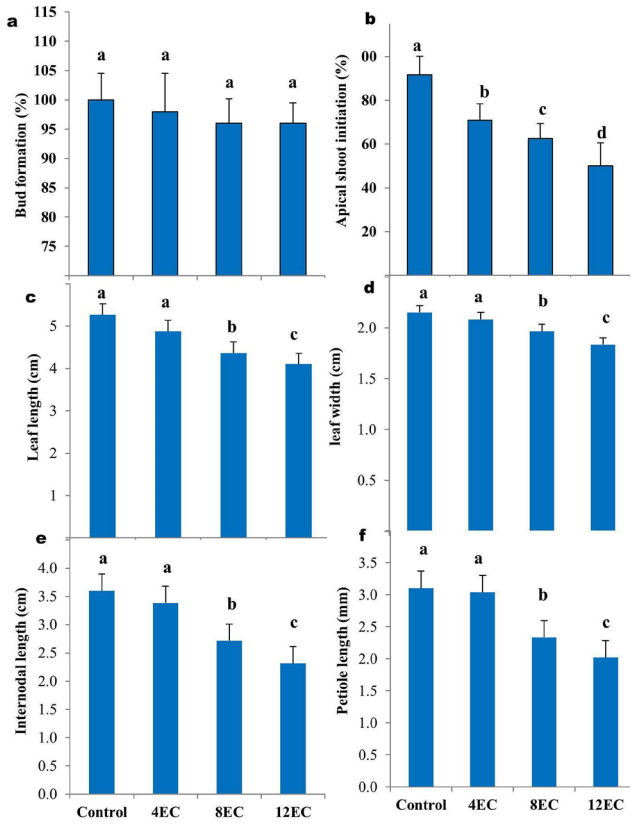

Fig 4. Effect of salinity on bud formation (a), apical shoot initiation (b), leaf length (a), leaf width (d), internodal length (e), and petiole length (f) (mean, n=15). Bar with similar latter are non-significantly different at P<0.05)

Table 5. Component matrix value of major 6 principal components for different factors

Factor	Component					
	1	2	3	4	5	6
K^+	.981	.108	−.018	.093	.008	.036
Apical shoot initiation	**.977**	.043	.102	.056	−.060	−.095
Fe^{2+}	**.976**	.036	.131	.022	.092	−.013
Zn^{2+}	**.973**	−.056	.050	.165	.103	.056
Mn^{2+}	**.963**	.072	.001	.027	−.059	−.194
Internodal length	**.961**	.014	−.066	.161	.068	.169
Petiole length	**.944**	−.195	−.120	.178	.069	.093
Diameter	**.933**	−.098	.014	.087	.162	−.153
Ca^{2+}	**.928**	−.116	.210	.098	.172	.003
Na^+	**−.927**	.012	.168	−.165	−.023	−.215
Transpiration rate	**.927**	−.033	.060	−.088	−.223	−.188
Total biomass	**.909**	−.136	−.162	.079	−.173	−.123
Leaf biomass	**.904**	.317	.002	−.078	−.112	.007
Bud initiation	**.901**	−.124	.075	.176	−.170	.057
Height	**.900**	.006	.157	−.020	.106	−.180
Root biomass	.899	.171	−.092	.144	.044	−.154
Shoot biomass	.892	.037	−.163	.095	−.288	−.074
Photosynthesis	.890	.060	−.281	.019	−.148	.249
Total sugar	.888	.007	.169	−.167	−.027	**−.373**
Stomatal conductance	.870	.069	−.005	.138	.275	**.351**
Cu^{2+}	−.809	.351	−.112	.229	−.251	.066
Mg^{2+}	−.753	−.365	.080	.241	.299	**−.341**
Leaf length	.633	.371	.378	−.171	**.401**	.026
Internal CO_2 conc.	−.581	.363	−.238	**.546**	−.142	.180
Membrane injury	.296	**.796**	.254	−.149	−.101	−.090
Total starch	−.031	**.670**	**.534**	.297	−.218	.279
Relative water content	−.118	**−.602**	**.558**	.201	.065	**.453**
Leaf width	.490	−.499	−.375	.321	−.253	.013
Protein	.088	−.160	**.864**	−.300	−.149	.111
No. of Branches	.257	.178	−.436	−.749	−.228	.218
No. of leaves	.561	−.281	−.292	**−.565**	.200	.351
Chlorophyll content	−.144	**.556**	−.433	.117	**.621**	.028

contributor (matrix value >0.5) for the variance. The variance for PCA3 (MV>0.5), PCA4 (MV>0.5), PCA5 (MV>0.4) and PCA6 (MV>0.3) were contributed mainly by three (total starch, relative water content and protein), two (Internal CO_2 conc. and no. of leaves), two (leaf length and chlorophyll content), and four variable (Total sugar, stomatal conductance, Mg^{2+}, and relative water content), respectively.

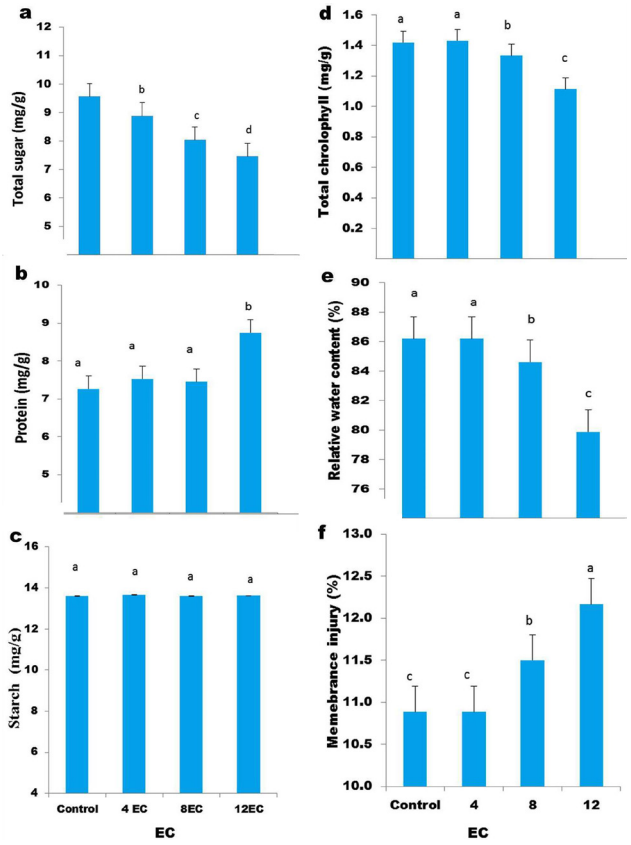

Fig 5. Salinity induced changes in total sugar (a), protein (b), starch (c), chlorophyll (d), relative water content (e), and membrane injury (f) (mean, n=15). Bar with similar latter are non-significantly different at P<0.05)

Discussion

Salinity induced salt stress affects the survival, growth, and developmental processes of plant species (Banyal et al., 2017). Proper functioning of all the structural attributes and physiological processes of plants is required to produce the essential energy for their proper growth and development. However, once plants are exposed to salt stress, accumulation of toxic ions causes the physiological disruption, biochemical alteration, and morphological modification that affect the overall growth and development of plants (Fig. 6). Therefore, assessing species salt tolerance and determining suitable EC range for tree optimum growth is essentially required for planning afforestation programmes in the salt affected soils.

When plants are subjected to salt stress, ion homeostasis and nutrient imbalance are the primary physio-biochemical changes occur in plants. Present analysis revealed that with the increase in soil salinity from control to 12 EC, the Na^+, Cu^{2+}, and Mg^{2+} concentration increased, while a decrease in K^+, Ca^{2+}, Zn^{2+}, Fe^{2+} and Mn^{2+} concentration was observed. It is well established that high concentrations of Na^+ can inhibit K^+ and Ca^{2+} uptake, indicating existence of antagonism between these monovalent ions (Lemos et al., 2008). Our results are similar to the existing reports regarding the reduced uptake of K^+ and Ca^{2+} at moderate and high salt stress conditions; indicating the competition between the ions for the similar binding site at the exchange complex. Present findings showed decrease in K^+ content with the increasing Na^+ content; suggesting the competition between the both ions and replacement of K^+ by Na^+ at the reaction center. The physio-chemical similarity between both ions promotes ionic competition for binding sites on the membrane transporters (Zhu, 2003). Results further showed that per cent increase in Na^+ ions was greater at high salinity level (EC 12), compared to rest of the EC levels and control. The high salt stress in plant growth medium reduces K^+ content, and enhance Na^+ uptake and accumulation, causing the efflux and leakage of K^+ ions from the plant cells (Azooz et al., 2015). Under such conditions, Na^+ content rises above that of K^+ which causes the poor nutrient uptake and lack of Na^+/K^+ homeostasis (Latef et al., 2016). A slightly higher EC than the ideal level of ions toxicity adversely affects the physio-biochemical processes, including mineral uptake, photosynthesis, membrane integrity and plants growth (Chen et al., 2012 and Hazman et al., 2016). A critical limit of Na^+ in leaf tissue need to be fixed, above which it has toxic effect on the plant growth and development. The Na^+ ions damage various plant systems that absorb and utilize Ca^{2+} and K^+ ions in plant tissues, thereby adversely affects the plant growth and development, as both ions are essential for the various physio-biochemical processes (Hilge, 2012; Demidchik & Maathuis, 2007). Results further showed that with increased salinity stress, the Ca^{2+} content decreased in leaf tissues, while opposite trend was noticed for Mg^{2+} concentration. As both ions showed competitiveness to each other at tissue and cell level, the increase in Mg^{2+} concentration causes Ca^{2+} deficiency that could adversely affect the plant physiological processes. The decrease in Ca^{2+} with increased salt stress alters the cell wall rigidity and plasma membrane integrity (Maathuis, 2009). During this period, Ca^{2+} transporters play an important role in regulating cellular Ca^{2+} levels to cope with salinity stresses. Moreover, the increased Mg^{2+} content in salt stressed plants benefits the chlorophyll synthesis, enzyme activation, and the stabilization of nucleotides and nucleic acids to cope the salt stresses (Maathuis, 2009). Hence, increased accumulation of toxic ions and nutrient imbalance may greatly affect the physiological processes in *Melia*.

Maintaining structural integrity and orderliness of the chloroplast is necessary for the proper functioning of plants physiological processes. It is observed that many stressors induces structural changes in photosynthetic apparatus, that reduces the photochemical efficiency and electron transport activity of

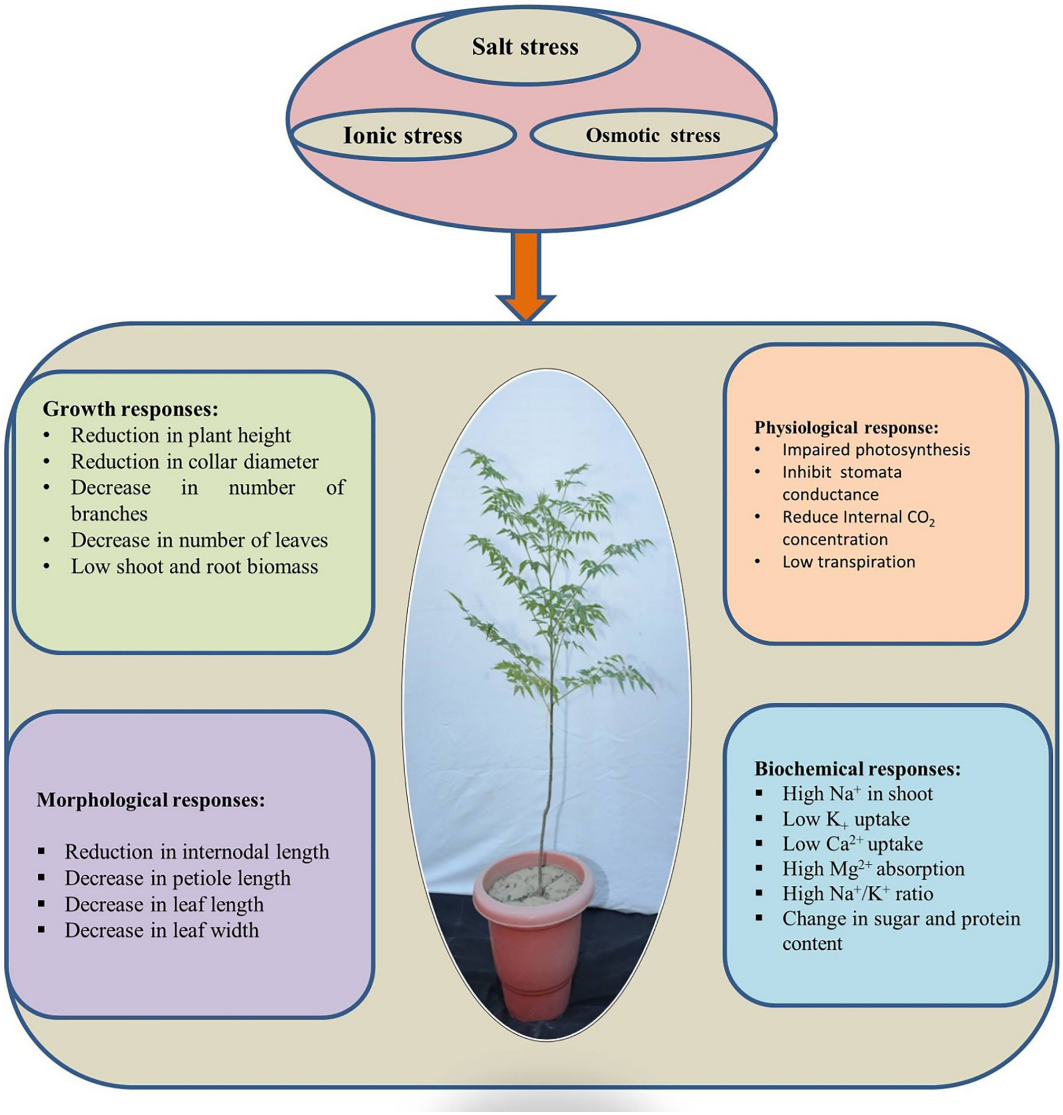

Fig. 6. Effect of salinity stress on growth, physiological, morphological and biochemical properties of *M. dubia*

chlorpophyll (Mittal et al., 2012). Our findings indicated that increase in salinity stress adversely affected the chlorophyll content, leading to decline in the photosynthetic rate, stomatal conductance, internal CO_2 concentration, and transpiration rate, and the extent of the reduction was dependent on the salt strength. Zollinger (2007) also reported that salt-water irrigation adversely affects the photosynthetic rate and stomatal conductance in *Echinacea purpurea*. The alteration in physiological processes under salt stress can be attributed to the both stomatal and non-stomatal limitations (Thiem, 2020), as plants tend to close their stomata in response to the specific-ion accumulations. Results further explained that salt stress reduces the relative water content and enhances the stress injury that subsequently leads to decrease in the rate of photosynthesis, internal CO_2 concentration and transpiration. Several previous studies have also highlighted the degradation of plants physiological processes with the increased accumulation of toxic ions under salt stress (Arias-Moreno et al., 2017; Gao et al., 2015; Shu et al., 2013; Mitsuya et al., 2000 & Yamane et al., 2004). The adverse effect on physiological processes with increased salt stress leads to the reduction in total protein and sugar in the plant tissues.

It was noticed that salt stress modified the morphological parameters of *M. dubia,* and the increase in salinity stress caused reduction in the leaf length, leaf width, petiole length, and internodal length. The ionic imbalance and lesser water availability with increasing salinity levels adversely affected these parameters, which ultimately produces the alteration in physio-biochemical processes and morphological parameters of Melia. Similarly, Kotagiri and Kolluru (2017) reported that salt stress decreases the plant leaf area to make osmotic adjustment by carbohydrates accumulation in the tissues. As leaves are directly involved in regulating all the plant physiological processes, therefore,

determining critical salinity tolerance level is crucial, and beyond which salinity stress severely affects these processes, leading to the adverse effect on the plant growth and development. In present study, upto EC 8 dS/m, all the morphological parameters were non-significantly affected, above this, salinity had the significant negative effect on the various plant processes. Similarly, Yu et al. (2015) and Mzabri et al. (2017) also reported the adverse effect of salt stress on the plant morphological parameters. Results further explained that both the phenological parameters i.e. number of leaves and branches of *Melia* declined during each month with the increasing levels of salinity. The decrease in number of leaves and branches at EC 12 was more pronounced than the rest of EC levels and control. The greater uniformity in the saplings during initial months was responsible for depicting overlapping trends between the treatments for both the parameters. However, after November, as a consequence of salt stress, difference subsequently become more and more clear till the termination of experiment in April. The toxic effect of variable Na^+ ions concentration at each salinity level causes difference in the leaf and branch senescence (Munns & James, 2003). The change in regional climate viz. summer, autumn, winter, and spring were responsible for monthly variation in the phenological parameters. Senescence (leaves and branches) was initiated during October and both was minimum during January (peak winter), afterwards their re-emergence initiated during mid-Febuary (spring season). Such change in phenological behavior under salt stress has been previously described in various crop plants (Kim et al., 2007; Leila et al., 2016). Moreover, in addition to climatic parameters, results explained that salt stress also played important role in regulating the plant phenology. The phenological changes under salt stress could potentially affect the plants reproductive biology and development processes (Ryu et al., 2014). Another interesting phenomenon observed was the post winter apical shoot and bud initiation, which might also serve as a good indicator of salt tolerance in the species. Delayed apical shoots initiation with increased salt stress might lead to substantial reduction in the plant growth during the growing season. Thus, timing of shoot initiation can indirectly provide idea about the range of salt tolerance and subsequent seasonal growth pattern in tree species. Moreover, understanding the relationship between environmental stress and plant phenology is vital for future genetic manipulation to increase plant productivity under the changing climate (Kazan & Lyons, 2016). Therefore, alteration in the seedlings morphology and phenology under salt stress could affect the overall development processes in *Melia*.

We further observed the effect of salinity stress on growth and biomass of *M. dubia* to assess the species salt tolerance potential. Results showed that height and diameter growth were almost consistently declined in response to salt stress treatments. Under saline conditions, decrease in growth was non-significant up to EC 8 dS/m, while significant at EC 12 dS/m, indicating that *M. dubia* could successfully grow under moderate saline stress conditions. However, decrease in growth at EC 12 dS/m showed non-suitability of species at high salt stress (>12 ds/m) conditions. Results further showed that upto EC 8 dS/m, this species can be grown successfully without any substantial reduction in growth and hence; upto these levels best benefits can be obtained from the species. It can be grown at EC 12 dS/m with 20–30% reduction in the growth and biomass. Similar findings were reported by Banyal et al. (2018) who reported that growth increment in *Melia* decreases with the increased salinity stress. Although reduction in the trees growth with increased salt stress is well proven by the previous researchers in the region (Minhas et al., 1997; Tomar et al., 1998; Tomar et al., 2003; Singh et al., 2011). *M. dubia*, being industrially important species owing to its fast growth, greater ecological and economic benefits under pure plantation or agroforestry can be obtained within 7–8 years in such soils, except at the high salt stress. Specifically, at high salt stress, plantation restoration benefits will not be achieved in reasonable time period and best to the stakeholders' expectation in the absence of reclamation measures (Kumar et al., 2021a). Moreover, *M. dubia* has showed good growth potential in fertile, marginal and degraded soils conditions. Developing Melia based agroforestry practises/systems in salt affected soils could be helpful in enhancing the productivity, sustainability and climate resilience of agroecosystems (Newete et al., 2020). Standardization of plantation technology, cultural practises, water and nutrient management could further increase the species growth and productivity under salt stress. However, present findings needs to be confirmed and validated from the long-term field results for obtaining more precise information on the productivity and restoration potential of *Melia* in salt affected soils. Further, understanding physiological mechanism and undertaking genetic improvement work for enhancing salt tolerance might play important role in species success on the salt affected soils. Therefore, the salt tolerance of *M. dubia* showed strong potential in restoration and sustainable utilization of the salt affected soils.

Conclusion

The present study demonstrated an overview of the salinity stress effect on the growth and physio-biochemical responses of *M. dubia*. Based on the findings,

we recommend *M. dubia* for salt affected soils of EC 8 ds/m without any substantial effect on species growth rate and of EC 12 ds/m with 20–25% reduction in the growth rate. Therefore, the present outcome provided an insight into the salinity tolerance and change in physiological processes in the species which could be useful for devising the tree improvement strategies. Elucidating physiological mechanism, assessing biochemical changes, and identifying plant metabolites will provide ideas about species salt tolerance and futuristic strategies for developing salt tolerant crop through breeding and improvement approaches.

Declarations

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Conflict of interest

Author (s) doesn't have conflict of interest.

Availability of data and material

Raw data is available with ICAR-CSSRI, Karnal, India.

Code availability

N.A.

Authors' contributions

Raj Kumar: Conceptualization, Investigation, Data collection, Original draft preparation, Methodology. Rakesh Banyal: Investigation and data analysis. Awtar Singh: Investigation and editing. R K Yadav: Supervision and Editing. P C Sharma: Conceptualization and methodology.

Compliance with ethical standards

Consent to participate and publication

All authors given their consent for participation and publication.

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