EFFECT OF SALINITY ON GROWTH, PHYSIOLOGICAL AND BIOCHEMICAL PROCESSES OF COCONUT SEEDLINGS (*Cocos nucifera* L).

BY

ARYA SANTHOSH

(2014- 20-120)

ACADEMY OF CLIMATE CHANGE EDUCATION AND RESEARCH

VELLANIKKARA THRISSUR – 680656

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THESIS

Submitted in partial fulfillment of the requirement

for the degree of

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Faculty of Agriculture

Kerala Agricultural University, Thrissur

ACADEMY OF CLIMATE CHANGE EDUCATION AND RESEARCH

VELLANIKKARA THRISSUR – 680656

KERALA INDIA

2020

DECLARATION

I hereby declare that the thesis entitled **Effect of salinity on growth, physiological and biochemical processes coconut seedlings (*Cocos nucifera* L.)** is a bonafide record of research work done by me during the course of research and the thesis has not been previously formed the basis for the award to me any degree, diploma, fellowship or other similar title, of any other University or Society.

Arya Santhosh
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Date:

CERTIFICATE

Certified that this thesis entitled **Effect of salinity on growth, physiological and biochemical processes of coconut seedlings (*Cocos nucifera L*)** is a record of research work done independently by Miss Arya Santhosh. under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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Dedicated to my parents

and

brother

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ABBREVIATIONS

ICAR	Indian Council of Agricultural Research
CPCRI	Central Plantation Crop Research Institute
ACCER	Academy of Climate Change Education and Research
IPCC	Inter governmental Panel on Climate Change
MGD	Malayan Green Dwarf
Rs	Stomatal resistance
CI	Chlorophyll Index
OP	Osmotic potential
ROS	Reactive Oxygen Species
SOD	Superoxide dismutase
POD	Peroxidase
PPO	Polyphenol oxidase
RWP	Relative Water Potential

PVPP	Poly Vinyl Polypyrrolidone
NBT	Nitro Blue Tetrazolium
OD	Optical Density
FCR	Folin Ciocalteu Reagent
P_N	Photosynthesis
Tr	Transpiration
Gs	Stomatal conductance
NS	Non Significant
EDTA	Ethylenediamine tetraacetic acid
s.act	Specific activity
CD	Critical Difference
ppm	Parts per million

CHAPTER 1

INTRODUCTION

The coconut (*Cocos nucifera)* had served the humanity for more than three millennia. The coconut palm is widely distributed along the tropical countries .It has been grown around in 94 countries. India occupies the second position in the world with an annual production of 15 billion coconuts. India with 15.6 % of coconut cultivable land contributes to 24.1% of coconut production at global level. The coconut is mainly classified into two categories, tall and dwarf. The coconut is mainly found near in costal climates where it is known to adapt and tolerate to salt sprays and brackish soils

Climate change is considered as an important environmental concern of this century. In recent decades, climate change and climate-change induced variability are subjects of worldwide discussion and Climate related disasters such as droughts, floods, ice storms, storm surges, dust storms, hail storm, landslides, heat and cold waves and thunder clouds are not uncommon over one or another region of the world.

Sea level rise has been a major problem caused by climate change and global warming. As per the process-based model projections, for the period 2081 to 2100, with reference to 1986-2005, global mean sea level rise is likely to be 0.45 to 0.82 m for representative concentration pathway (RCP) 8.5. According to the report of IPCC (2013), a little rise of 0.66 m sea level can potentially deluge the low lying wetlands, erode the coastal shores, greatly augment the salinity stress, along with an increase in coastal salt water tables, and aggravate the coastal flooding all leading to the salt water intrusion into estuaries and aquifer. The majority of the vulnerable coastal regions are within 1 m elevation of sea level. In India analysis of tide gauge data divulge that an increase of 1.92

mm/year of sea level in the main coconut growing regions of east coast in Bay of Bengal. In the west coast (Arabian Sea), the increase of 1.72 mm/year is observed which are in accordance with the global estimate of 1-2 mm/year.

Sea water has a salinity of about 3.5% (35g/l, 599 mM) predominantly comprising dissolved salts of Cl^-, Na^+, SO_4^- etc. with a conductivity of about 53 dS m^{-1}. Coconut is considered as a moderately salt tolerant. Nevertheless, little is known about the sea water concentration coconut seedlings could tolerate. Few studies to understand the impact of salinity in coconut are either on soil with fixed salinity levels or with the application of common salt. Undoubtedly, salinity stress greatly modulates the photosynthetic efficiency of the plants since it directly affects the frequency of stomatal closure/opening in the photochemical and biochemical processes. Furthermore, accumulation of Na^+ ions in plant tissues severely impedes the growth and physiology of the plants leading to impaired morpho- physiological functions. The various adaptive mechanisms of plants towards salinity stress including osmotic adjustment-effectively accumulating compatible low molecular weight solutes to reduce the osmotic potential, enzymatic and non-enzymatic anti-oxidants scavenging reactive oxygen species (ROS), maintenance of cell turgor etc are poorly understood in coconut. Therefore, the response of dwarf coconut seedlings to varying concentration of sea water substitution in a hydroponic system and its influence on morpho-physiological and biochemical processes were investigated.

Objectives

1. To study the effect of salinity on growth, physiological and biochemical processes.

2. To decipher the mechanisms of salinity tolerance at physiological and biochemical level

CHAPTER 2

REVIEW OF LITERATURE

2.1 CLIMATE CHANGE

"Climate change is a change of climate which is attributed directly or indirectly to human activity that alters the composition of the global atmosphere and which is in addition to natural climate variability observed over comparable time periods (UNFCC)". Climate change becomes a global problem with unique characteristic and involves complex interactions between climatic, environmental, economic political and technological processes. Climate change besides being characterized with an increase in average temperatures are also associated with other untoward events such as a shift in the composition of wild life population, changes in the habitats, increase in sea levels, and host of other unwanted impacts. These extraordinary set of changes are occurring owing to greater accumulation of greenhouse gases in the atmosphere causing the phenomenon of global warming.

2.2 CLIMATE CHANGE AND SEA LEVEL RISE

Sea level rise has been a major problem caused by climate change and global warming. Eventhough rise in sea level is considered a major challenge arising due to global warming phenomenon; it continues to be one of the least investigated areas in the field. Sea level rise could be attributed to two major reasons (i) greater degree of shrinkage in ice of mountain glaciers, polar ice sheets which discharges large quantum of water to the oceans (ii) as the greater increase in the ocean temperatures causes the warmer water to expand. The continents are trapping the rising sea water within the basin causing a major rise in sea water levels. Also there are instances of low lying areas in the coastal belts, delta regions sinking due to rise in sea water level (called subsidence).

Trapped within a basin bounded by the continents, the water has nowhere to go but up. In some parts of the world, especially low-lying river deltas, local land is sinking (known as subsidence)—making sea levels that much higher.

Climate change has a significant effect on islands and their populations, resulting in the destruction of their livelihoods and genetic variability of the living organisms. The instability in the climatic conditions causes changes in the sea levels, thereby extremely affecting the low lying coastal regions and the island ecosystems. The rise in sea level is an important outcome of climate change and is currently the world's most important problem. In comparison to global emissions, the emission of greenhouse gases from small islands is marginal, however the threat to these small island nations is very high. Moreover an increase of 30 cm in the sea level will amount to substantial increase in flood intensity (of 36-58 percent) along the coastal regions (IPCC, 2018). The majority of the coastal regions that are most vulnerable to sea level rise are within 1 m of sea level elevation (Lazrus, 2012).

The islands like Maldives, Marshall Islands, Federated States of Micronesia, Kiribati, Tuvalu and Arctic islands such as Shishmaref and small islands in Nunavut are becoming derelict owing to the rise in sea levels (Marino *et al*. 2009). Also, some of the coastal regions of Bangladesh are being severely afflicted due to sea level rise. IPCCs projections have foreseen scenarios such as an increment of 0.3-1.1 m in sea levels by the year 2100 (a best estimate of 0.66 m rise). It will result in flooding of low-lying wetlands, erosion of sea shores, enhanced soil salinity, rise in the water tables of the coastal regions, and intensify the storm damage. Nicholls and Leatherman (1996) have analyzed the sea level rise and concluded that there was an increase of 0.1 and 0.2 m in sea level during the 20th century. However analysis based on the tide gauge data revealed that the rate of mean sea level rise was in the range of 1.0-2.0 mm/year compared to an average rate of about 0.1-0.2 mm/year during the last 3,000 years. The intrusion of salt water into estuaries and aquifers is a significant consequence of sea level rise. FAO studies point out that of the total world land 6% is affected by salinity

and thus salinity becomes a major limiting factor to crop growth and productivity. Salinity is one of the main abiotic stressor that affects the crop production. Almost 7% of total land area, and one fifth of the cultivated land and one half of the irrigated land area are afflicted by the salinity stress (Kibria *et al.*, 2017). Thus on a global scale the total area of soils afflicted by salt is 830 million hectare (Munns & Tester., 2008)".

2.3 SALINITY

Salinity is defined the quantum of the dissolved salts in the sea water which is presented as S%. It is defined as the approximation of weight (g), in vacuo, of the solids in 1 Kg of sea water. Solids are required to be dried to a constant weight at a high temperature of 480°C, and the organic matter fully decomposed or oxidized, the ions of bromide and iodide are represented as chloride and carbonates are converted in to the oxides.

Salinity is generally obtained from the unit-less conductivity measurements and it is also called as Practical salinity (PS). Thus, the conductance of the sample is compared in relation with the standards such as sea water. These derivations compare the specific conductance of the sample to salinity standards such as sea water. The dissolved salts generally contribute to the salinity of sea water. Hence the sea water with a designated practical salinity of 35 predominantly comprises ions such as chloride, sodium, magnesium, sulfate, calcium, potassium, bicarbonate etc.

Table 2.1.Composition of sea water (Hem, 1985)

Constituent	Concentration (mg/liter)
Cl	19500
Na	10500
So_4	2700
Mg	1350
Ca	410
HCO_3	145
K	390
B	4.5
Zn	0.01
Fe	0.003
Mn	0.002
Cu	0.003

2.4 CHLORINITY

The halide ions of the sea water (except fluoride) are calculated by argentimetry. Hence, the chloride ion concentration or percentage of sea water (in vacuo weighing) is mentioned as 0.3285 times the quantum of silver precipitated as silver halides from the defined quantum of sea water (usually 1 Kg). By measuring salinity and chlorinity in sea water collected from different parts of the world Knudsen formulated an empirical relation between salinity and chlorinity in 1889 (Lyman. and Fleming, 1940) .The modified form of Knudsen' formula :

$$\mathbf{S\ \% = 1.80655\ Cl}$$

2.5 CHLOROSITY

The chlorosity value (Cl/liter) is defined as the quantum of chloride ions determined by following the volumetric methods and thus it represents the chloride ions per unit volume of seq water (1000 mL) at a defined and all the weighing performed in vacuo.

2.6 SALINITY AND COCONUT

Around one fifth of the world's biodiversity is found in island ecosystem (in 180,000 islands) (Kier et al. 2009) which also accounts for little over 50% of coconut diversity (Bellard *et al*., 2012). Major portion of the coconut plantations are in the coastal zones and many of the coconut growing regions are islands. Incidentally among the ten insular biodiversity hotspots (Bellard *et al*., 2014), eight belongs to the 3927 islands, including major coconut growing regions. The number of coconut islands with submergence risk varies from 231 to 700 depending the level of sea rise. Sea water incursion has not only contributes to the high salinisation of soils and fresh water bodies but also affects the coconut production scenario in in Polynesian islands of the Tuamotu Archipelago (Prades and Ollivier, 2013)".

Coconut palms are often found along tropical, sandy shorelines and tolerate salt spray and brackish soil. It is a major crop species that known to be having the potential for growing in the areas which are affected by salts. This is also true in the case of high saline areas such as in coastal lines. The moderate salt tolerance of coconut (Ferreira neto *et al*., 2007; Marinho *et al*., 2006) gives this crop the potential to be used in revegetation programs of salt-affected areas. "Some studies also point out the adaptation of coconut to irrigation with saline water. (Marinho *et al*.,2006)". The coconut palms are found near coastal and sandy beaches. Coconut palms can grow in saline conditions by developing a series of adaptive mechanisms. But it is little known about the adaptive mechanisms.

2.7 EFFECT OF SALINITY ON PLANTS

The prevalence of excessive amounts of salts is responsible for the water salinity. "The major effects of salt stress are decreasing of the water potential and induces ion imbalance / disturbances in ion homeostasis and toxicity.(Parida and das 2005)". This altered water status contributes to initial growth reduction and decrease in plant productivity. The retarded growth of plants could be attributed to the osmotic potential of soil water (Flowers *et al*.,1977;Greenway and

Munns.,1980*)*. Salinity stress modulates all the major plant developmental pathways such as overall growth, photosynthesis, metabolic pathways such as protein synthesis, lipid metabolism and energy pathways. Studies on the effect of salinity on coconut are very much limited. So this study is very much relevant under changing climate scenario.

2.8 EFFECT OF SALINTY ON GROWTH

Plants are highly susceptible to saline stress in their early vegetative growth phase. The decline of plant growth due to the salinity varies among the speices of same crop and even among the varieties of the same species (Bolarian *et al*.,1991 ;Ghoulam *et al*., 2002)". Salinity stress causes a clear stunting of plant growth (Hernandez *et al*. 1995). The reduction of plant growth leading to cessation is the immediate response of salt stress. Salinity also leads to significant reduction in the fresh and dry weights of all major plant parts. (Hernandez *et al*,.1993). The optimum growth of plants was observed when the sea water concentration is at 50%s and growth of *Rhizophoro mucronata* severely declines if salinity increases(Aziz and Khan 2001). Eventhough fresh and dry weights of *Salicornia rubra* increases with salinity optimal growth was observed when salinity levels are at 200 Mm NaCl and with the further increase in salt concentration the growth declines. As reported by Mohammad *et al* (1998), in radish total plant dry weight decreases at high salinity which could be due to the greater reduction of leaf area and reduction in stomatal conductance. The small leaf area at high salinity could be attributed to the reduced specific leaf area and increased tuber formation starting at a smaller plant size at high salinity and increasing salinity will results in the sizable reductions in shoot weight, plant height of leaves in tomato.

Interestingly the toxicity effect of the accumulated ions is not the due to its effect on the plant growth rather they influence the turgor, activities of specific enzymes and the process of photosynthesis (Munns ,1992). Hence, the initial deceleration in growth of plants is due to the greater decrease in water potential which ultimately leads to specific effects such salt injury and death of cells due to accumulation of toxic ions in the cell wall or cytoplasm. A major physical response of the plants to salinity stress is substantial reduction in the total leaf area and thus reduced leaf growth (Munns and Termaat 1986). Thus, this phenomenon of reduction in the total leaf area is a basic avoidance mechanism utilized by the plants to minimize the water loss due to transpiration due to closed stomata, However, it causes the retention of ions in the roots to the level of toxic concentration leading to deficiency of theses ions on the aerial plant parts (Munns and Tester 2008). Effect of salinity stress is manifested as reduced stem growth, decreased leaf and stem culminating in the reduced aerial plant parts.

Munns et al., 2000 demonstrated that shoot and root growth are permanently affected in the salt sensitive plants within few hours of salt stress treatment. Interestingly, this effect on the plant growth is independent of the Na+ concentration but depends on the osmolarity of the external solution (Munns *et al.*, 2000). Moreover, accumulation of Na^+ in the leaf tissues causes necrosis of older tissues starting from the tips and margins of the leaf surface and working back in to the leaf. Munns (2002), have shown that the growth and reduction of yield of plants could be attributed to the reduced leaf time of the individual leaves, thereby severely affecting the the net productivity and overall yield of the crop. Also, the rate of accumulation of Na^+ in the leaves and the efficiency of its compartmentalization determine the duration of the effect of Na^+ specific damage. The Na+ specific damage generally gets superimposed on the osmotic effects of the NaCl and it varies with the species.

2.9 EFFECT OF SALINITY ON IONIC LEVELS AND NUTRITIONAL BALANCE

"Production of crops will be severely affected by nutritional disorders caused by salinity. The interactions between the salinity and mineral nutrition of the crops are very complex (Grattan and Grieve 1999). The nutritional disorders observed in the plants could be due to the effect of salinity on the availability of nutrients, competitions in the nutrient uptake their transport and further distribution within the plant systems (Rogers *et al.*2003). Furthermore, the high uptake of NaCl leads to the competition in the uptake of other nutrients such as K^+ , causing the K^+ deficiency. Expectedly, increased treatment of NaCl causes an increase in Na^+ and Cl^- ions whereas it is accompanied with a huge decrease in Ca^{2+}, K^+ and Mg^+ concentrations (Khan *et al.*, 1999)".

In glycophytes, Na^+ and Cl^- accumulation is correlated with the sensitivity to salt stress. "In a study conducted by Lima *et al.* (2016) in dwarf coconut seedlings of cv.ano verde, there was a greater accumulation of Na^+ ions in the roots and in shoots the contents remained low and also there is an ionic competition between Na^+ and K^+ after 120 days of treatment with brackish water with linear response of increasing contents in the roots". "In a study conducted by Remison *et al*(1988), the P content of the coconut seedlings was decreased with salinity". Shukla and Mukhi. (1979) have reasoned that the antagonistic relationships between the cations could be due to the physiological processes that occur during the various processes such as nutrient absorption, thier translocation from the root to shoot. This relationship between the various plant nutrients ensures that the plants could withstand very high concentrations of nutrients in the root zone. The retention of Na^+ ions may be a mechanism of tolerance to salinity and one of the effects of high soil Na^+ is the deficiency of the other nutrients (Aquino *et al.* 2007). Other nutrient deficiencies can also occur owing to the elevated [Na^+] which interferes with the transporter proteins in the plasma membrane of roots thereby obstructing the uptake of nutrients. For instance, K^+ selective ion channels inhibiting the root growth, osmotic effects of Na^+ and the

detrimental effects of Na^+ on soil structure are observed." According to Taiz and zeiger .(2013) and Hebbar *et al*(2000), even though Na^+ ions can substitute for K^+ ions the maintenance of lower Na^+/K^+ ratio in the leaf tissues reduces the harmful effects of saline stress on the production and metabolism of photoassimilates (Aquino *et al*.,2007)". "In a study conducted by Lima et al. (2016) in dwarf coconut seedlings of cv.ano verde , there was a greater accumulation of Na^+ ions in the roots and in shoots the contents remained low and also there is an ionic competition between Na^+ and K^+". Plants have to balance their nutrient uptake for maintaining normal metabolism hence they are involved in the inhibition of nutrient uptake and consequent translocation of toxic elements such as Na+ however, accelerated uptake of metabolically important nutrients (K^+ by K^+ and Na^+) are also and are transported through H^+ pumps.

K^+ ion is an indispensable component of the cells in order to maintain the nccessary osmotic balance, stomatal closure and it acts as co-enzymes of many essential enzymes (Yeo, 1998). Also it was observed that high concentration of stomatal K^+ is mandatory for the proper functioning of the photosynthesis (Chow *et al*). Thus the deficiency of these vital macro nutrients will seriously affect the plant metabolism. Similar observations has been recorded in the studies conducted by Lima *et al*.(2017) and Ferreira Neto *et al*.(2007), suggesting the ionic competition between potassium and sodium in the process of absorption by the roots and or in the differences in the retention rate during the transport to the shoots and leaves". In guava salinity stress causes an increase in levels of Na^+ and Cl^- in guava and the highest accumulation of these ion was in the leaves followed by the roots The concentration of Ca^{2+} ions are found to be stable in the roots but decreases to great extent in the stems and leaves. Also, the K^+ concentration decreases with an increased salinity stress ,particularly in leaves whereas , Mg^{2+} concentrations are not affected by salinity in the stems and roots but decrease in the leaves of guava .Decreases of Ca^{2+} and Mg^{2+} content of leaves have also been noticed as a result of salt accumulation in some species suggesting decreased chlorophyll content (Parida *et al*.,2004)". "In a

study conducted by Ferriaria *et al.*(2014) , it was found that the N, K and Ca contents in the plant decreased with the increasing electrical conductivity and increase in the levels of Cl and Na. Antagonistic relations can be observed between Cl and N, Na and K and Na and Ca" . Moreover the availability of phosphorous becomes greatly reduced in the saline soils owing to many factors such as (a) the effect of ionic strength that reduces the activity of PO43−, (b) the process of sorption which controls the phosphate concentrations in soil solution and (c) the decreased solubility of Ca-P minerals. With an increase in salinity stress, the P concentration of the seedlings decreased to great extent with a concomitant increase in P of the root of the seedlings (Remison *et al.*,1988). However, it must be acknowledged that the interactions between P and salinity stress is very complex process and mechanisms that explain the underlying changes of P uptake due to salinity stress is virtually non-existent for various species of plants(Grattan and Grieve, 1992)". " It is known that P concentration is related to the photosynthesis rate (Overlach *et al.*, 1993) and; therefore, reduced the plant growth can be very well correlated to the decreasing contents of P in the plants". The phosphate content of many cultivated crops decreases with an increase in salinity stress *(*Qadir and Schubert 2002)". "Calcium is important during salt stress in many aspects of plant growth and metabolism including metabolic functions such as maintaining the membrane integrity (Rengel 1992), cellular signaling and osmoregulation processes (Mansfield *et al.*, 1990) and influencing K/Na selectivity (Cramer *et al.*, 1987)". Owing to various factors such as ionic interactions, precipitation process and increased ionic strength the processs of uptake of Ca^{2+} from the soil solution is severely impeded and resulting in reduced activity of Ca^{2+} (Janzen & Chang 1987, Garg & Gupta 1997). Here an antagonistic relationship exists between sodium and calcium. As a result, Na induced Ca deficiency in plants occurs. "Uptake of Ca^{2+} from the soil solution may decrease because of ion interactions, precipitation and increases in ionic strength that reduce the activity of Ca^{2+} (Janzen & Chang 1987, Garg & Gupta 1997)". Similar observations has been recorded in the studies conducted by Lima *et al.*(2017) and Ferreira Neto *et al.*(2007). Hirpara *et al.*,(2005) also

recorded a decrease in the uptake of calcium when the seedlings of Butea monosperma (Fabaceae) are subjected to salt stress. Similar results has been has been recorded by Remision *et al*(1998). Talei *et al*(2012) in the study conducted in the medicinal plant “Andrographis paniculata Nees” , recorded similar observations of declining uptake of magnesium with the increasing salinity. Mg^{2+} has an important role in chlorophyll structure and as an enzyme co-factor. “Another importance of Mg2+ in plants is in the export of photosynthetic pigments which leads to chlorophyll reductiona and reduction in photosynthetic rate in Mg^{2+} deficient source leaves (Marschner and Cakmak, 1989)”.

N accumulation in plants is reduced by salinity owing to reduced shoot nitrate accumulation arising from the enhanced accumulation of chloride ions (Feigin 1985: Garg *et al.* 1993, Torres & Bingham 1973; Garg & Gupta 1997). Uptake of boron increases with salinity stress and soluble Boron is toxic in the intracellular concentration (Loomis & Durst., 1992). Wimmer *et al.*(2002) reasoned that the enhanced supply of B could results in an increased influx of boric acid into the cell, leading to high concentrations of borate due to high pH which forms complexed with many intracellular ligands such as pyridine nucleotide coenzymes (NAD^+), ATP, RNA and several sugars (Ralston & Hunt., 2000). The relatively simpler structural variations observed upon binding of boron to these molecules will eventually make then lose their functional viability or alter the activities of enzymes leading to greater disruption of metabolic functions.

2.10 IONIC TOXICITY

The proper ion ratios are the important physiological parameter for the plant to maintain its normal growth and development (Wang *et al*., 2003). Enhanced salt uptake leads to conditions of specific ion toxicities such as toxic levels of Na^+, Cl^- or sulphate (SO_4^{2-}) which ultimately arrest the uptake of essential elements such as phosphorus (P), potassium (K^+), nitrogen (N), and calcium (

Ca^{2+}) (Zhu , 2001).The deleterious effects of Na^+ ions with respect to the maintenance of structural and functional integrity are known (Kurth., et al; 1986). Salinity stress causes increase in the concentration of Na^+ and Cl^- ions in the roots, stems and leaves of *Atriplex griffithii* suggesting a positive relationship between Na^+ and Cl^- concentration. Furthermore the metabolic toxicity of Na^+ could be attributed to its efficiency in competing with the cellular K^+ for the binding sites indispensable for various cellular functions (Tester and davenport, 2002). According to Bhandal and Malik (1998), more than 50 enzymes are activated by K^+ and Na^+ cannot substitute the role and s a result of high Na^+: K^+ ratios can disrupt various enzymes processes in the cytoplasm. Since most of the plants accumulate both the ions (Na^+ and Cl^-) in toxic levels in their shoot tissues Cl^- toxicity is an important cause of growth reduction. According to Tavakkoli *et al.* (2011), the high content of Na^+ severely reduced the concentrations of K^+ and Ca^{2+} uptake and thereby afflicting the photosynthetic process owing to reduced stomatal conductance. On the other hand, high high Cl^- concentrations generally impair the photosynthetic capacity due to non-stomatal effects and chlorophyll degradation.

The concentrations of Na^+ and Cl^- ions are high in saline soils however the role of Cl^- ions in reducing the growth parameters is comprehended well compared to that of Na^+ in many crops. However, salt tolerance research in cereals have been concentrated mostly on the effect of Na^+ with little regard to Cl^- toxicity (Teakle and Tyerman, 2010). However the concentrations of both the ions [Na^+ and Cl^-] are important since their toxic level accumulation severely impairs the metabolism (Xu *et al.*, 2000; White and Broadley, 2001; Tavakkoli *et al.*, 2010a, b; Teakle and Tyerman, 2010)". In some plants the concentrations of Na^+, rather than Cl^- are negatively associated with salt tolerance (Kingsbury and Epstein, 1986; Kinraide, 1999; Lin and Kao, 2001), whereas in other species the control of Cl^- transport and Cl^- exclusion from the shoot tissues are correlated with salinity tolerance (Martin and Koebner, 1995; Luo *et al.*, 2005; Islam *et al.*, 2007; Teakle *et al.*, 2007; Aydi et al., 2008; Tavakkoli *et al.*, 2010a, b).

"Cramer et al. (1985) reported that a primary response of root cells to salt stress involves competitive displacement by sodium of essential calcium ions from attachment sites at the outer plasma membrane surface". This would then lead to reductions in membrane integrity and function e.g. loss of sodium exclusion capacity, increased leakage of essential solutes, turgor losses and consequent growth inhibition. However subsequent reports indicated that salinity induced calcium release from internal membranes of root cells rather than plasma membrane surfaces (Lynch *et al*., 1987; Lynch and Lauchli, 1988). Moreover even high sodium concentrations did not appear to compete effectively with calcium ions in initially saturating plasma membrane binding sites in maize roots (Zidan *et al*., 1991). Additional evidence against the hypothesis that salinity necessarily leads to loss of plasma membrane integrity and function came from a study of the effects of salinity on the capacity of root epidermal cells to acidify their surrounding cell walls (Zidan *et aI*., 1990). Wall acidification (e.g. via plasma membrane proton pumping ATPases) is, according to the acid growth hypothesis (Rayle and Cleland, 1992) one of the essential prerequisites for cell expansion growth. Disruption of transmembrane proton transport and wall acidification, as a result of membrane disruption by salinity, would therefore lead automatically to a restriction of expansion growth. However, capacity for progressive wall acidification by growing epidermal cells in maize root tips was not inhibited by growth in NaCI solutions with or without extra calcium. Similar results were obtained when root tip tissues were assayed for capacity to change the colour of bromocresol purple pH indicator in agar gels. Presumably therefore, this membrane function was relatively unaffected by levels of salinity which clearly inhibited growth. Thus membrane disruption as a result of competitive displacement by sodium of calcium bound to the plasma membrane surface, did not appear to be a primary response of growing maize root cells to salinity(Zidan *et aI*., 1991)..

2.11 EFFECT OF SALINITY ON WATER REALTIONS AND GAS EXCHANGE PARAMETERS

Water potential becomes a major physiological parameter for determining the water content of the plants. "According to studies Romcao – Aranda *et al* .(2014) the increase in the salt content in the root zone will decrease in the water potential of leaves ". Many studies reveals that osmotic potential and water potential became more negative by increasing salt concentration and the turgor pressure increases (Ghoulam *et al*., 2002; Gulzar *et al*., 2003; Romero-Aranda *et al*., 2001). In a study conducted by Khan *et al*(2001) in *Cucumis sativa* , it is found out that the water potential will decreases linearly as the salinity level increases. In sugar beet varieties , the relative water content decreased because of the application of salt (Ghoulam *et al*; 2002).

Salinity causes a reduction in the stomatal conductance and evaporation. This results in the entering of some toxic ions into the root zone and to the plants. "Decreases in stomatal conductance due to salinity have been found in several different plant species (Gómez *et al*., 2013)". Studies proved that stomatal closure at the beginning of salinity exposure can contribute to a decline in the flow of toxic ions within the transpiration stream (Kerstiens *et al*.,2002 , Veselov *et al*., 2008 , Vysotskaya *et a l*., 2010). "According to Koyoro(2006) reductions in stomatal conductance represent an adaptive mechanism for coping with excessive salt, reducing the salt load of leaves and helping increase longevity by maintaining salts at sub toxic levels for longer than would occur if transpiration rates were not diminished". "Decreases in stomatal conductance are one of the causes of photosynthesis (PN) decline, although photo inhibition or increases in mesophyll resistance may also play an important role when stress is more severe or prolonged (Flexas, *et al*.,2004)".

2.12 SALINTY AND OXIDATIVE STRESS

"In the normal conditions, the plants are capable of withstanding the deleterious effects of ROS by the synthesis of antioxidants .If the antioxidants produced is not sufficient for the neutralization of the ROS , it will accumulate and result in an oxidative stress .This may occurs when the plants are grown under abiotic stresses such as salinity (Scandalios ,2002)". Salinity leads to the accumulation of ROS (Reactive oxygen species) ; which ultimately results in the inactivation of enzymes , lipid peroxidation , damage of DNA etc. "Salinity stress can results in the stomatal closure , which leads to the reduced carbon dioxide availability and inhibits the carbon fixation, exposing the chloroplasts to excessive excitation energy , finally leads to the increased production of ROS such as hydrogen peroxide (H_2O_2 , superoxide (O_2^-),), hydroxyl radical (OH^-)and singlet oxygen(Parida and Das 2005; Ahmad and Sharma 2008)".

In a study conducted in wheat seedlings , salinity treatments of E_c .5.4 and 10.6 ds m^{-1} , for 60 days causes increase in lipid peroxidation (Sairam *et al*.,2002). When the plants are subjected to environmental stresses such as salinity, the production of ROS is the major reason for the decrease in crop productivity ((Halliwell and Gutteridge 1989 ; Asarda 1994).

2.13 EFFECT OF SALINITY ON PHYSIOLOGY OF PLANTS

2.13.1 Effect of salinity on photosynthesis

"Plant growth and thus biomass production can be very well correlated to net photosynthetic rate. The effect of salt stress may be either long term or short term. After the exposure to saline stress, the short term effect occurs within few hours or in one to two days . After several days, the long term effect occurs. The salt accumulation in the younger leaves results in the decline in carbon assimilation (Munns and Termatt ,1986)". Salinity cause a sudden decrease in the stomatal aperature of the plants. Stomatal responses of the plants are caused by the osmotic effect of the salt present in the root zone (Munns and Tester

2005). As a result of the decrease in the stomatal conductance, there will be a reduction of CO_2 assimilation and the respiration rate(Marier and Zozor 1996 ; Romero – Aranda 2001). Under the salt stress, the rate of reduction in the photosynthesis will depend on salt concentration and species (Parida *et al*., 2004). In a study conducted in two cultivars of wheat grown under saline conditions, expressed two stages of photosynthetic reduction . In the first stage, photosynthetic reduction was slower and during the second phase , the reduction was rapid , followed by a significant decline of energy conversion efficiency of photosystem II (Murnaka *et al*, 2002). According to Iyengar and Reddy (1996) , under the saline conditions , the decrease in photosynthetic rate is a result of various factors such as

1. Cell membrane dehydration and reduction in the permeability of CO_2 because of the reduced water availability and higher osmotic potential and as a result of photosynthetic electron transport declines .

2. Reduction of CO_2 supply due to the stomatal closure and results in the reduced availability of CO_2 for the carboxylation reactions.

3. Enzyme activities and cytoplasmic structure will change as a result of saline stress.

2.13.2 Effect of salinity on photosynthetic pigments

Chlorophyll is one of the most important plant pigments, supporting photosynthetic ability. Chlorophyll content can vary due to salinity level, eventually affecting plant growth and development (Kibira etal.,2017).According to Wang and Nil .(2000) , the chlorophyll content of the leaves of Amaranthus grown under the saline treatment does not show an variation. But generally the content of chlorophyll of leaves will show a decrease in the saline conditions . There is a decrease in the chl - *a* , chl - *b* content of the leaves of B . parviflora under long term saline conditions. The decrease in chlorophyll content is an indicative response across different plants subjected to salinity stress (Roy and

Basu., 2008). Parida and Das (2005) suggested that the decrease in chlorophyll content in response to salt stress is a widespread phenomenon. Chen and Yu (2007) also observed a significant decrease in chlorophyll content at high NaCl level

2.13.3 Effect of salinity on chlorophyll fluorescence

Analyzing chlorophyll fluorescence is a very useful technique in plant physiological studies which can provide useful information about the state of PSII (Maxwell and Johnson 2000). According to Nobel (1999) , of the total solar radiation absorbed only less than one percent is utilized for the photosynthesis and the rest of the absorbed radiation will be discharged . The fluorescence is the most effective processes in PS II (Torabi 2014). Chlorophyll fluorescence can be used as an indicator for detecting stress (Pospil and Dau 2000) .F_v is the difference between the maximum fluorescence signal (F_m) and the background level signal (F_0) and the ratio denotes the maximum quantum yield of PS II and it is highly related to the quantum yield of net photosynthesis (Maxwell and Johnson 2006). During salinity stress a decrease in the photochemical quenching parameters such as F_v / F_m and in the electron transport rate has been observed under various studies (Acosta et al.,2015). The response to saline stress is correlated with decreases in PSII efficiency and increases in non–photochemical parameters as a mechanism to safely dissipate excess energy. Salt stress was found to reduce ETR in a salt-sensitive rice cultivar, whereas only a slight reduction in ETR occurred in a salt-tolerant cultivar..According to Morali and ismail(2007) the effect of NaCl stress on non-photochemical quenching parameters was somewhat different, since qN increased more substantially in the salt-tolerant cultivar than in the salt-sensitive cultivar. In salt-sensitive plants, salt stress, besides reducing photochemical-quenching parameters and ETR, also reduces non-photochemical quenching parameters (Shu *et al*.,2013). In some of

the plants, when subjected to a salinity of 200 mM or more there is a sharp reduction of F_v / F_m ratio (Zhuo *et al*., 2007).

2.13.4 Effect of salinity on stomatal conductance of plants

"There is are strong evidence that stomatal regulation of vapour loss is extremely sensitive to short-term salt stress (Cochard et al.,2002 , Meinzer 2002 , Bunce,2006)". Some studies based on water relations in some barley cultivars with different drought resistance levels showed that lower stomatal conductance and transpiration at the beginning of salt exposure contributed to higher salt tolerance in terms of improved extension growth and less accumulation of toxic ions (Vysotskaya *et al*.,2010). "According to Koyoro (2006) reduction in stomatal conductance represents an adaptive mechanism for coping with excessive salt, reducing the salt load of leaves and helping increase longevity by maintaining salts at subtoxic levels for longer than would occur if transpiration rates were not diminished". "Decreases in stomatal conductance are one of the causes of photosynthesis (PN) decline, although photoinhibition or increases in mesophyll resistance may also play an important role when stress is more severe or prolonged (Flexas ,*et al* 2006)". "A steep decline in the stomatal conductance and decrease in the rate of transpiration are probably the first line of defense of the plants in response to increased salinity, alone or associated with other stress factors (Suarez 2011)". Similar observations has been recorded in the studies conducted by Medeiros et al(2018).

2.14 EFFECT OF SALINITY ON BIOCHEMSIRY OF PLANTS

2.14.1 Effect of salinity on carbohydrates

"During the saline stress, an increase in soluble sugars is reported in glycophytes and this contributes upto 50% increase in osmotic potential (Parveiz and satyawati 2008)". "According to parida *et al*(2002) , the carbohydrates including disaccharide , monosaccharides and polysaccharides accumulate and has a vital role in carbon storage, osmoprotection, osmotic adjustment and free

radical scavenging". "Accumulation of soluble carbohydrates as response to salinity and drought has been reported in various studies despite a significant decrease in net CO2 assimilation rate (Murakeozy et al., 2003)". "Researchers found that under saline conditions there are differences between cultivars and accession in terms of the amount of sugar .(Ashraf and Tufail ., 1995)". "In a study conducted in sunflower, Ashraf and Tufail (1995) determined the total soluble sugar content in five accessions differing in salinity tolerance. The results indicate that although the total soluble sugar content increased significantly in all five lines with increasing salt in the growth medium, the salt tolerant lines had generally greater soluble sugars than the salt sensitive ones". "During the imposition of saline stress to tomato , there is an increase in the content of soluble sugars and total saccharides (Khavari-Nejad and Mostofi 1998)". "Under the saline stress, in the leaves of Bruguiera pariflora , there is an increase in the content of reducing and non reducing sugars (Parida *et al*., 2002)".

" As a result of various abiotic stresses, 'trehalose', a disaccharide accumulates in many organisms and it is known to be an osmolyte and an osmoprotectant (Ashraf 1994 ; Crowe et al.,1984)". It protects membranes and proteins in cells exposed to stresses that cause water deficit (Hounsa *et al*., 1998 ; Garcia *et al*.,1997). "According to Yamada et al(2003) trehalose has a suppressive effect on apoptotic cell death".

2.14.2 Effect of salinity on amino acids

When the plants are exposed to salt stress , amino acids including arginine , alanine, leucine and proline etc accumulates. "The major amino acid that accumulates in the plants as result of salt stress is proline (Torabi *et al*., 2010)". In the cytosol the proline accumulates and accomplishes the role of osmotic adjustment. "The accumulation affects the maintenance and also alleviate the effects of salt accumulation on the cell membrane interruption (Mamsour 1998)". "Proline synthesis is a non-specific response to low growth medium water potential (Ashraf 1994) and it also regulates the accumulation of useable N,

osmotically very active and also contributes to membrane stability (Whn 1981; Ashraf 1994; Lone *et al*., 1987; Hanson *et al*., 1994)". "Even at its supra optimal level it does not suppress the enzyme activity (Dubey 1997) and can also act a regulatory molecule able to activate multiple responses that are the components of the adaptation process". "Researches also reported that the accumulation of proline in some plant is a consequence of salt injury and not an indicator for the salt tolerance (De – Lacerda *et al*., 2003)".

2.14.3 Effect of salinity on phenolic compounds

"Phenolic acids are secondary metabolites extensively spread throughout the plant kingdom(Tomas-Barberan *et al*.,2001)". Secondary metabolites especially phenolic compounds play an indispensable role as antioxidants quenching reactive oxygen species and supporting the plants to deal with the high temperature, salinity or other induced oxidative stress (Gould *et al*.,2002). Moreover phenolic compounds are vital for the optimal plant growth and reproduction, and are produced in high concentrations when plants are subjected to unfavorable environmental factors (light, chilling, salinity etc and to defend injured plants(Kefeli *et al*.,2003). High accumulation of phenolic compounds in plant plays an imperitative role physiological role in overcoming salinity – induced oxidative stress (Minh etal .,2016). Accumulation of phenolic compounds in artichoke leaf subjected to moderate salinity stress has been reported by Hanen *et al*. (2008). Also increase in phenolic compounds response to salinity stress has also been documented from the extracts of various plant tissues (Agastian *et al*., 2000; Muthukumarasamy *et al*., 2000). In contrast, the total phenolic contents of 5- and 7-day-old radish sprouts were profoundly declined when subjected to moderate salinity (Yuan *et al*., 2010).

2.14.3 Effect of salinity on anti-oxidant enzymes

"The activity of antioxidant enzymes was reported to increase under salinity in wheat shoot (Meneguzzo et al,1999; Sai ram and Srivastava ,2002)". "At higher NaCl concentration(100 mM) it seems that such resistance to oxidate stress may be overcome leading to growth reductions (Agarwal and Joshi ,2002; Pandey and Agarwal 2002)". "Increase in peroxidase(POX) activity is supposed to overcome to damage to the tissue metabolism by reducing toxic levels of H_2O_2 produced during cell metabolism and protection against oxidative stress (Rasheed and Mukerji ,1991;Scalet *et al*., 1995; Gosset *et al*., 1996 ; Dionisio – sese and Tobita 1998, sudhakar *et al*., 2001)". According to Agarwal and Pandey (2002), the activity of peroxidase enzyme has been enhanced owing to salt stress imply it has effective ROS (H_2O_2)scavenging mechanism so as to impart tolerance against NaCl oxidative stress. The POX and PPO are the two major enzymatic antioxidants that are involved in the oxidation of phenolic compounds hence may play an important role as defense against salt stress (Sheen and calvert.,1969). Furthermore the enhanced enzymatic activities of SOD,POX and PPO during salt (NaCl) stress indicate the stronger inter-relationship between the phenomenon of salt tolerance and enzymatic antioxidant defense systems.

CHAPTER 3

MATERIALS AND METHODS

A Study was conducted to determine the effect of salinity on growth parameters, physiological and morphological processes of coconut seedlings and to decipher the mechanisms of tolerance at physiological and biochemical level.

3.1. LOCATION AND GEOGRAPHY

The experiment was conducted at the net house of ICAR- Central Plantation Crop Research Institute (ICAR-CPCRI), Kasaragod, Kerala, India. CPCRI is located at 12° 18' N latitude and 75° E latitude at an altitude of 10.7m above mean sea level.

3.2 .EXPERIMENTAL SETUP

The study was conducted in a controlled hydroponic system that eliminates the confounding effects of drought and limited nutrients. For the present study a dwarf coconut variety MGD (Malayan green dwarf) was selected. It is one of the popular dwarf varieties cultivated in India as well as in the world.

The experiment was conducted in large drums of approximate 60 liters capacity. The outer surface of the drums was painted black in order to prevent the entry of sunlight that results in the growth of algae in the medium. Five month old uniform MGD seedlings were selected for the experiment after the initial screening processes. The pots were filled with 40 liter water enriched with 1 strength Hoagland solution. The selected seedlings were transplanted to the pot on 5 July 2018. The water in the pots were adequately aerated by the air pumps connected to a 15 HP suction pump which ensured continuous aeration from 8.30 am morning to 5pm evening. These plants were grown in a net house, top covered with polythene sheets to protect from the rain. The plants were allowed to establish in hydroponics till the imposition of treatment.

In order to study the effect of sea water on coconut seedlings the following treatment combinations were selected.

Table 3.1 Treatments involving different volume of sea water substitution

Treatments	Amount of irrigation water added (liter)	Amount of Sea water(liter)
T1 (control)	40	0
T2 (10% sea water) ,10 %S	36	4
T3 (25% sea water) ,25 %S	30	10
T4 (50% sea water) , 50% S	20	20
T5 (75% sea water) , 75 % S	10	30
T6 (100%sea water) ,100 %S	0	40

Replications: Each treatment was replicated thrice

Each pot with the above treatments was supplemented with 1 strength Hoagland solution with the following composition. All together there were 18 pots. Every 15 day the solution in the pot is replaced with fresh solution after a thorough washing**.**

3.3 CHEMICAL REQUIRMENTS

Hoagland solution

Each time @ 40 L/pot, 720 l of fresh Hoagland solution was prepared to be filled in 18 pots. The following are the different chemicals stock and volume required for the preparation of 720 liters

Chemicals required

Macronutrients

Potassium nitrate (KNO_3) - 5 ml /liter , for 18 plants 5 x 720 = 3.6 liters

Calcium nitrate ($CaNO_3$) - 5 ml/liter , for 20 plants 5 x 720 = 3.6 liters

Magnesium sulphate ($MgSO_4$) - 2 ml/liter , for 20 plants 2 x 720 = 1.4 litres

Potassium di-hydrogen phosphate($KHPO_4$) -1 ml/liter , for 20 plants 1 x 720= 720 mL

KNO_3

1 M in 1liter = 101.10 g in 1000 mL

1 M in 4liter = 101.10 x 3.6 =363.96g

$CaNO_3$

1M in 1000 ml = 236.16

1 M in 3.6 liter = 236 .16 x 3.6 = 850 .17 g

$MgSO_4$

1 M in 1 liter = 246.48 g in 1000 mL

1 M in 1.44 liter= 354.93 g

$KHPO_4$

1 M in 1 liter= 136.09 g in 1000 mL

1 M in 720 ml= 97.98 g

Micronutrients

Micronutrients required to prepare a stock of 2 liter

Potassium Chloride (KCl) - 3.728 g

Boric acid (H_3BO_3) -1.546 g

Manganese Sulphate ($MnSO_4 .2H_2O$)-0.238 g

Zinc Sulphate Heptahydrate ($ZnSO_4$ .7H2O)-0.576 g

Copper Sulphate ($CuSO_4$ .5H20)-0.124 g

Molybdic acid (H_2MoO_4)- 0.080 g

80 ml of micro nutrients is applied to each plant.

EDTA Ferric mono sodium salt-19.71 g/liter

20 ml Fe- EDTA is applied to each plant.

Table 3.2 Composition of Hoagland solution

Chemical	Nutrient required for 720 liter(g)	Stock applied for 40 liter(mL)
	Macro nutrients	
KNO_3	363.96	200
$CaNO_3$	850 .17	200
$MgSO_4$	354.93	80
$KHPO_4$	97.98	40
	Micro nutrients	
KCl	3.728	80
H_3BO_3	1.546	
$MnSO_4.2H_2O$	0.238	
$ZnSO_4$ .7H2O	0.576	
$CuSO_4$ .5H20	0.124	
H_2MoO_4	0.080	
EDTA	19.71g/liter	20

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3.4 TREATMENT IMPOSITION

The sea water treatment imposition was started on 5 November 2018 after the initial establishment of seedlings in hydroponics for a period of four months. Sea water brought from Arabian sea was added to each treatment as specified above (Table 2) to get the set concentrations of 0%, 10% , 25%, 50% and 100% sea water substitution. In all there were 18 seedlings with 6 treatments and 3 replications .The plants were arranged randomly in 3 rows as shown in the Fig. 1 below. Whenever the nutrient solution of pots was replaced by fresh one, from each bucket 45 ml sample was taken for water analysis and the same treatment samples is mixed and analyzed as a single sample.

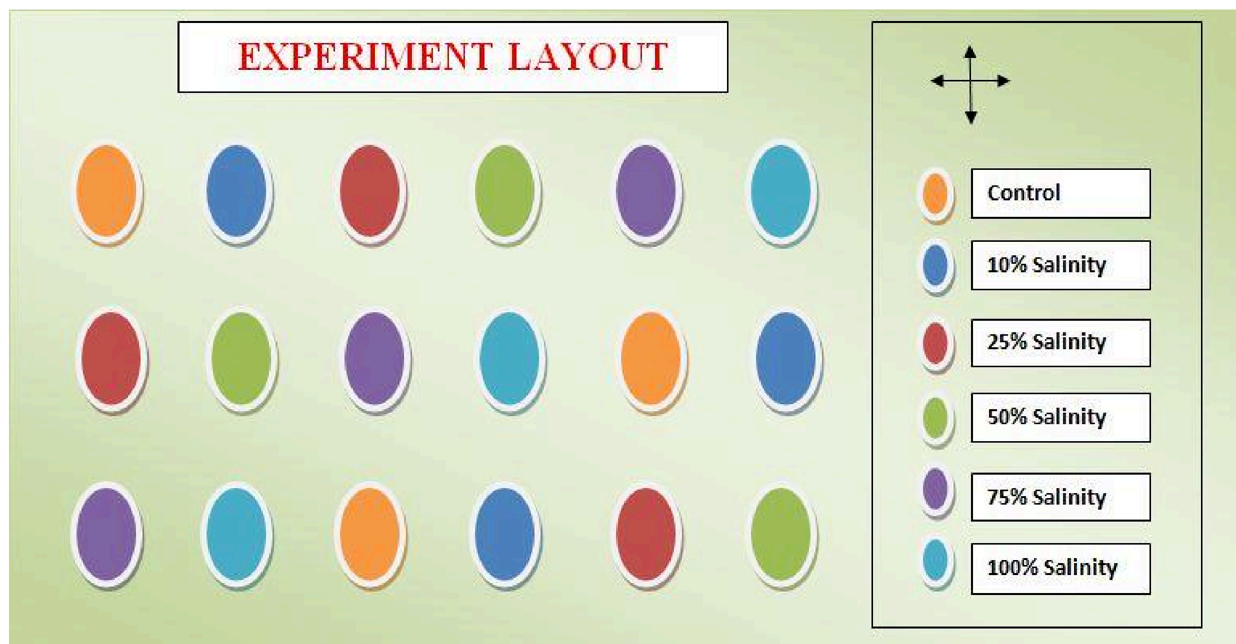

Fig.3.1. Experimental set up

Observations

Just before the imposition of treatments the following initial observations were made.

Nutrient content of water was measured.

Initial leaf sample was collected for the nutrient analysis.

Morphological observations of plant: Plant height, collar girth, number of leaves, fresh weight of plant, leaf area, root number and root length

Time course observations during treatment period

Pot water: At two to three week interval the volume of unutilized water in the pot was measured and its nutrient content was analyzed to calculate the rate of uptake of water and nutrients.

Plant growth: Plant height, collar girth, number of leaves, fresh weight of plant, leaf area, root number and root length

Physiological observations: Photosynthesis, stomatal conductance, transpiration, stomatal resistance, chlorophyll index, chlorophyll fluorescence, leaf water potential.

Biochemical observations: Total sugar, reducing sugar, free amino acids, antioxidants, total phenols, Super oxide dismutase (SOD), peroxidase and polyphenol oxidase, membrane stability index, chlorophyll content.

At the time of termination of experiment the following observations were recorded

Fresh weight and dry weight of plant parts *viz*. leaf lamina, midrib

shoot, root, Nutrient content of plant parts *viz*. root, shoot, leaf lamina, midrib

Fig 3.2 Collecting sea water from CPCRI beach

Fig3.3.Filling pipe water and adding nutrients

Fig 3.4 Morphological measurements

3.5 MORPHOLOGICAL OBSERVATIONS

3.5.1 Plant height

Height of the plant is taken from the base (just above the nut) of the seedling to the tip of the fully expanded leaf and expressed in centimeters.

3.5.2 Number of leaves and Total Leaf Area

Total number of leaves and the whole leaf area of each the seedlings were recorded. For estimating leaf area of an individual leaf, the length and width of each fresh leaf was taken. Length of the leaf was measured from the point of origin of leaflets in the leaf to the tip of petiole of that leaf and the width was measured by stretching the leaf lets on the either side of the petiole to maximum. Leaf area was estimated by using the linear regression equations of Mathes et al, (1989), $y = 5.9647 + 0.6314\,x$,$y = 3.9325 + 0.7044\,x$ and $y = 8.4507 + 0.6798\,x$; where x is the product of length and breadth at the broadest position of the leaflet and y is the area of the leaflet.

3.5.3 Plant weight

The total fresh weight of plants including the nut is measured using a weighing balance and it is expressed in kilograms. At the termination of the experiment, on 10.05.2019 plant parts were separated as root, shoot, leaf lamina and midrib and their fresh weight was recorded. Parts were initially solar dried for two weeks and one day before taking dry weight it was oven dried at $60^{o}C$ for 8 hours.

3.5.4 Number of roots and maximum root length

Total numbers of roots of the seedlings were recorded and maximum root length was measured in centimeters and documented.

3.5.5 Collar Girth

The circumference of shoot at a marked fixed point just above the nut was measured using a measuring tape and recorded as collar girth. It was expressed in centimeters.

3.6. PHYSIOLOGICAL PARAMETERS

3.6.1 Photosynthesis, Transpiration

Photosynthetic rate, stomatal conductance and transpiration rate of fully opened top most leaf was measured using portable Infra –Red Gas Analyzer (IRGA, LICOR -6400XT, USA) which has leaf chamber. The observations were made between 9.30 AM to 11 AM on clear sunny day and leaf gas exchange parameters were estimated at a fixed light intensity of PAR 1000 µmol $m^{-2}s^{-1}$. The IRGA was calibrated for zero CO2. The sample IRGA and the reference were matched for CO2 and water concentrations at regular intervals by using the instrument at match mode. The leaflet was clipped into the leaf chamber and the observations were recorded after the internal CO2 concentration became stable . All observations were downloaded to the PC using the software 'L16400TERM ' and files were opened in MS –EXCEL for the analysis.

3.5.2 Stomatal Resistance

The stomatal resistance of the top most fully opened leaf was estimated using the porometer (Porometer AP4, USA) which measures the stomatal resistance , light intensity and leaf temperature. The instrument was calibrated before taking the observations using the calibration plate and silica gel. The equipment was taken to the field on a clear sunny day between 9.30 am and 11 am and the sensor clip was clipped to the middle of the leaflet of the topmost fully opened leaf. The measurement was taken until we get consecutive same reading for the leaf. The readings were saved and later downloaded for data analysis.

3.5.3 Chlorophyll Fluorescence

Chlorophyll fluorescence was recorded using chlorophyll fluorometer (Opti-sciences -30p, USA). The leaflets were adapted to dark before taking the observations. The dark adaptation clips with a 4 mm aperture size having a sliding shutter (which prevents the entry of light) was used for the dark adaptation process. Clips was plugged on to the surface of the middle leaflet of fully opened first leaf and the leaflets were adapted to dark for half an hour. After that the sample probe was inserted into the clip . Then the shutter was opened and the measurement was recorded. Minimal fluorescence(F_0), Maximum fluorescence (F□) and Fv/F□ (ratio of variable fluorescence and maximal fluorescence) were recorded in a file and the data was retrieved using hyper – terminal software. Measurements were taken from at least 3 plants.

3.5.6 Leaf water potential

The leaf water potential in coconut seedlings was measured on the middle leaflets of the top most fully opened leaf using LWP meter (Skye SKPM 1400, UK). The measurements were taken between 9.30 and 11.30 am. The instrument was taken to the field and it was connected to 2 L nitrogen cylinder through connecting hose. Instrument was switched on and 0 to 20 bar pressure mode was selected since it gives 0.01 bar resolution. Display was set to zero by turning the adjusting knob. Leaflet from top leaf was cut and a small portion of leaf lamina was removed from leaflet base to help insert midrib into the hole of chamber lid. The lid with leaflet was placed in leaf chamber and was made air tight. Pressure inside the chamber was increased using the nitrogen gas. The pressure was recorded when the water oozed out from the tip of midrib and expressed in bars. A magnifying lens was used to visualize water oozing. The chamber pressure was then released by exhausting the gas using a knob

3.7 BIOCHEMICAL PARAMETERS

Leaflets from fully opened leaf of the seedlings were used for biochemical analysis. The samples were collected from the field in ice bags before 10 AM in the morning.

3.7.1 Ethanol (80%) extraction of leaf tissue

0.5 g of leaf tissue was ground in 10 ml of 80% ethanol. Then the extract was subjected to rotation for 30 minutes in rotospin. After that the extract was allowed for sonication for 30 minutes. Then it was centrifuged at 6000 rpm for 10 -15 minutes. After the centrifugation the supernatant was collected. To the pellet 5 ml 80% alcohol was added and the extraction procedure was repeated. After that the supernatant was pooled and evaporated at 80 °C - 90°**C** in the water bath until a drop was left. Finally the extract was dissolved at 80 °C and made up with 10 ml of distilled water. This leaf tissue extract was used for the estimation of biochemical parameters such as total sugars, reducing sugars, total phenol, free amino acids and antioxidant potential etc.

3.7.2 Estimation of total sugars

Estimation of total sugar was performed following the phenol sulphuric acid protocol suggested by Dubois *et al.*, 1956. Briefly, concentrated sulphuric acid used breaks down any polysaccharides, oligosaccharides, and disaccharides of the samples to monosaccharides. Then, Pentoses (5-carbon compounds) are dehydrated to furfural, and hexoses (6-carbon compounds) to hydroxymethyl furfural. These compounds then react with phenol to produce a yellow-gold color which was measured spectrophotometrically at 490 nm. The quantity of the sugars was estimated using glucose as standard.

3.7.3 Estimation of reducing sugar

Quantitative estimation of reducing sugars was done following the method suggested by Nelson and Somogyi (Somogyi ,1952). Briefly, reducing sugars in the samples when heated with alkaline copper tartrate solution reduce the copper from the cupric to cuprous state and thus cuprous oxide is formed. When cuprous oxide is treated with arsenomolybdate the reduction of molybdic acid to molybdenum blue takes place. The blue color developed is compared with a set of standards (glucose) in a spectrophotometer (Shimadzu UV 160A) at 620nm.

3.7.4 Estimation of free amino acids

Total free amino acids were estimated following the ninhydrin method developed by Moore and stein, 1954. Ninhydrin reacts with all those substances containing primary amino groups, such as amino acids, amino sugars, aliphatic amines. The total free amino acids in the samples were estimated by spectrophotometric measurement at 570 nm and using the standard leucine (1mg/1ml).

3.7.5 Estimation of total phenols

Total phenols was estimated by using Folins ciocalteus method (Bray and Thorpe,1954). The Folin–Ciocalteu reaction is an antioxidant assay based on electron transfer that measures the reductive capacity of an antioxidant. In this method, the polyphenolic fraction in the plant extracts reacts with a specific redox reagent (Folin-Ciocalteu reagent) to form a blue complex that was quantified by visible-light spectrophotometry (Shimadzu UV 160A). The polyphenols content of the samples were expressed as catechol equivalent /g F.W.

3.7.6 Determination of Antioxidant activity

Cupric ion reducing antioxidant capacity (CUPRAC) Cupric ion reducing capacity was assay was done by the method described by Apak *et al*(2004). This assay is based on the reduction reaction of Cu (II) to Cu(I) by the combined

action of all the anti-oxidants present in the aliquot of sample extract. It involves mixing the sample extracts with solutions of $CuCl_2$, neocuproine, and ammonium acetate at pH 7, and measuring the absorbance at 450 nm after 30 min. The anti-oxidant potential of the plant extracts was estimated as Trolox equivalent.

Ferric reducing anti oxidant power (FRAP)

In the ferric reducing antioxidant power (FRAP) assay antioxidant components of the plant extracts act as reductants in a redox-linked colorimetric reaction, wherein Fe^{3+} (Ferric ion) is reduced to $Fe2^{+}$ (Ferrous ion). Ferric (Fe3+) to ferrous (Fe2+) ion reduction at low pH causes formation of a colored ferrous-probe complex from a colorless ferric-probe complex. FRAP values were obtained by comparing the absorbance change at 593 nm in the sample reaction mixtures with those containing ferrous ions in known concentration (Benzie and Strain 1996).

3.7.7 Enzyme extraction from leaf tissue

Leaf tissue enzyme extract was prepared in sodium phosphate buffer (0.1M, pH 7.6) by following the method of Chempakam et al. (1993) which was standardized for coconut leaf tissue. Around 0.5 g of leaf tissue was ground in 15 ml sodium phosphate buffer, along with PVPP and sand. The extract was centrifuged at 4ºC for 20 min and the supernatant obtained was pooled for the various enzyme analyses.

3.7.8 Superoxide dismutase assay (Beauchamp & Fridovich, 1971)

The crude enzyme extracts of the plant parts are used in SOD assay suggested by Beauchamp & Fridovich, 1971. The principle of this assay is spectrophotometric measurement of the reduction of Nitro blue tetrazolium by O_2^- which is inhibited in the presence of super oxide dismutase enzyme from the plant extract.

The following reagents were added: potassium phosphate buffer (0.1 M pH 7.8)-1.6 ml; nitro blue tetrazolium (NBT) solution (conc)-0.3 ml; methionine(0.13 M) – 0.3 ml; EDTA (0.0127)0.3 ml; enzyme extract 0.1 ml; sodium carbonate

(1.5 M) 0.1 ml and the reaction was started by adding riboflavin (0.00129M). After adding the riboflavin, the test tubes were incubated in florescent light for 30 minutes. Another set of test tubes were incubated at dark condition for 30 minutes After 30 minutes the optical density at 560 nm was measured using visible spectrophotometer against reagent blank and the specific activity was measured.

3.7.9 Polyphenol oxide Assay

Five milliliters of the assay mixture for the polyphenols oxidase activity comprised: 125 µmoles of phosphate buffer, pH 6.8, 50 µ moles of pyrogallol, and 0.1 ml of the diluted enzyme extract. This was incubated for 5 min at 25º C after which the reaction was stopped by adding 0.5 ml of 5% (v/v) H_2SO_4. For control reactions the same procedure was followed however enzyme extract was added after the termination of reaction with sulphuric acid (Kar and Mishra 1976).

3.7.10 Peroxidase assay

Three milliliters of the assay mixture for the peroxidase activity comprised ; Sodium phosphate buffer (0.1 M , pH 7.6) - 2.7 ml; 0.2 % dianizidin - 0.1 ml; 0.5 % hydrogen peroxide - 0.1 ml; and 0.1 ml of enzyme extract. The reagents and the enzyme extract were added one after another. The change in optical density was measured continuosly for 3 minutes. The enzyme activity was calculated as follows:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{420}/20\text{ sec Test Sample} - \Delta A_{420}/20\text{ sec Blank})(3)(df)}{(12)\ (0.1)}$$

3.7.11 Estimation of chlorophyll

Around 0.5 g of finely cut sample of leaf was weighed into a clean motor and pestle. The tissue was ground to a fine pulp with the addition of 20 ml of 80% acetone. The extract was centrifuged at 5000 rpm for 5 minutes and the supernatant was transferred into a 100 ml volumetric flask. The residue was ground with 20 ml of 80% acetone, centrifuged and the supernatant was taken in the volumetric flask. The volume was made upto to 100 ml with 80% acetone. The absorbance of the solution at 645 and 663 nm was taken against the solvent (80 % acetone) blank.

3.7.12. Estimation of membrane stability index

Coconut leaf membrane stability was determined following the method of Jamil et al. 2012. Conductivity measured (µS cm-1) were recorded with a conductivity meter. Eight leaflet segments of 3 cm^2 area each were immersed overnight at 25°C in 25ml of distilled water in a beaker and the initial conductivity was measured (C1). Then the leaf with water is placed in boiling water bath for 30 minutes, and cooled to room temperature. Conductivity was again measured (C2). Conductivity of double distilled water is also measured and was considered as blank (C0). The membrane stability index was calculated using the following formula.

Membrane stability index (MSI) =1 –[$(C1-C0)$ $(C2-C0)$] 100

3.8 NUTRIENT ANALYSIS

3.8.1 Water analysis

The initial and final water samples were analyzed for estimating the nutrient uptake by the plants. In the initial sampling of water, the samples from each 3 replications is mixed well and analyzed as a representative sample. For the final sampling, the 100 ml of water remained in each drum is collected and analyzed.

3.8.11Determination of P_H and E_c

The pH and E_c of the water sample is determined by using a pH meter. pH meter is first calibrated using the buffer solutions of known pH .

3.8.1.2 Determination of bicarbonates

10 ml of water sample was pipetted out into in a clean conical flask and it was diluted by adding about 25 ml of distilled water. 2-3 drops of phenolphthalein (0.25%) was added . Titrate it against standard sulphuric acid solution (0.02 N)till the solution becomes colourless. As there is no appearance of pink colour after adding the phenolphthalein, 2-3 drops of methyl orange (0.5%) was added in this colourless solution and it was titrated against standard sulphuric acid, till the yellow colour changes into rosy red . The volume of sulphuric acid consumed was noted each time.

Amount of bicarbonate = ((0.00122*CON :H_2SO_4)/0.1*TV/ VOL: ALQ *10^^{6}

3.8.1.3 Determination of chloride

2 ml of the water sample was pipette out into a clean 250 ml conical flask and it was diluted by adding about 25 ml distilled water. After adding 2-3 drops of potassium chromate indicator (5%) appearance of dark yellow color), it was

titrated against the standard silver nitrate solution(0.05N) with continuous stirring till the first brick red tinge appears

Amount of chloride (PPM) =((0.00355*CON:$AGNO_3$)/0.1)*(TV/VOL OF ALQ)*10^6

3.8.1.4 Determination of sulphate

5 ml of the water sample was pipette out into a 25 ml volumetric flask. 1 ml of gum acacia and 1 g barium chloride crystals were added and the contents were shaked well. The volume was made upto 25 ml by adding distilled water. Flask was inverted several times and turbidity was measured with a spectrophotometer at 490 nm .

3.8.1.5. Determination of calcium and magnesium

Determination calcium and magnesium in water sample is by complexometric titration using ethylene diamine tetra acetic acid (EDTA)(Schwarzenbach et al.,1946).

Estimation of total amount of calcium and magnesium

1 ml of water sample was pipetted out into a clean porcelain dish and the sample was diluted by adding about 25 ml of distilled water .1 ml of ammonium chloride - ammonium hydroxide buffer and 3- 4 drops of EBT indicator were added . It will give wine red color. The titration was carried out against EDTA till color changes to blue or bluish green. The volume (ml) of EDTA used was noted as A .

Procedure for the estimation of calcium alone

1 ml of another water sample was pipette out into another porcelain dish and it was by adding 25 ml distilled water . 5 ml of NaOH and a pinch of ammonium perpurate powder were added . Here the original colour was orange red. Then it was titrated against EDTA till the colour changes to purple. The volume of EDTA used was noted as B.

3.8.1.6 Determination of potassium and sodium

Potassium

Procedure

The amount of potassium present in the water sample was determined by using flame photometry

Instrumentation

A fine aerosol is formed and the atoms get excited by taking energy from flame created by mixture of liquid petroleum gas mixed with air. The isolation of radiation of desired wavelength .The isolation of radiation can also be achieved by a prism or monochromater. Radiation is measured either by photocell or photomultiplier tube .The concentration of K is measured by comparing the radiation emitted by a known standard with that of the sample.

Sodium

The procedure for Na is similar to that of potassium.

3.8.1.7 Determination of phosphorus

10 ml of water samples and the standard P solutions was pipette out into 50 mL conical flasks. 10 ml of Reagent B was added. The volume was made upto to 50 ml with distilled water and the samples and standards were incubated for 30 minutes for the development of blue color. The absorbance of colour developed at 660 nm wavelength was measured using a spectrophotometer.

Reagent A was prepared by dissolving 12g ammonium paramolybdate in 250 mL of distilled water and 0.2908 g of potassium antimony tartarate in 100 mL of distilled water. These dissolved reagents are added to 1 litre of 5 N sulphuric acid. The reagent was mix thoroughly and diluted to 2 litre with distilled water. Reagent B was freshly prepared as required by dissolving 0.523 g of ascorbic acid in 200 mL of reagent A .

3.7.1.8 Determination of ammonical nitrogen and nitrate nitrogen

Nitrate nitrogen and ammonical nitrogen in water samples is determined titrimetrically after distilling NH_3 in boric acid. This method has the advantage of permitting the estimation of both ammonical and nitrate nitrogen in the same aliquot. There is no hindrance due to coloured samples if distilled and NH_3 determined titrimetrically.

10 ml of water sample was pipetted out into the distillation flask. 20 ml of boric acid solution with mixed indicator was added into 250 ml conical flask and put beneath the condenser .The flask was stoppered and the ammonia was steam distilled into the boric acid solution. Distillated it according the pre-setted program. This distillate was titrated with 0.02 N sulphuric acid till the pinkish colour appears. Blank was also carried out simultaneously.

Nitrate Nitrogen

Same reagents used for ammonium determination was used. In addition, Devarda's alloy (50 Cu:5Al :5Zn) which is finely ground to pass 0.15 mm sieve was used.

A fresh water sample (10ml) was added into the distillation flask and reagents were added as given under ammoniacal determination along with 0.2 g of Devarda's alloy. The ammonia was distilled into fresh boric acid (with mixed indicator) and the titration was done as described earlier.

3.8.1.10 Determination of Iron, manganese, zinc and copper

The micronutrients such as iron, manganese, zinc and copper can be determined with the help of Atomic spectrophotometer(ASS).

3.8.1.11 Estimation of boron

Procedure

1 ml of water sample and standards into were pipetted out into 25 ml volumetric flasks. To that 2 ml of buffer(buffer solution was prepared by dissolving 62.5 g of ammonium acetate and 3.75 g of EDTA disodium salt in 100 ml of distilled water. To that 31.5 mLof glacial acetic acid was added slowly and mix thoroughly.) and 2 ml azomethine - H reagent was added .The samples and standards were incubated at room temperature for 30 minutes for the colour development .After the incubation, absorbance at 420 nm was taken on a spectrophotometer.

3.8.2 Plant sample analysis

3.8.2.1 Digestion of the sample

For the analysis of nutrients other than N, the plant material was digested in a diacid mixture.

Diacid digestion: The diacid digestion was carried out using a 9:4 mixture of nitric acid : perchloric acid . 0.5 g of ground plant material was placed in 100 ml volumetric flask. To this, 10 ml of acid mixture was added and the contents of the flask were mixed by swirling . The flask was placed on a low temperature until the production of red NO_3 fumes ceases . The contents were further evaporated until the volume is reduced to 3 to 5 ml. The completion of digestion was confirmed when the liquid becomes colourless.

After the flask is cooled, 20 ml of distilled water was added. The volume was made up to 100 ml with distilled water and the solution was filtered through whatman No.1 filter paper. Estimation of potassium, sodium, calcium, magnesium, boron, sulphur, iron, zinc ,manganese and copper was done according to same procedure as followed for the plant water samples .

3.8.2.2 Estimation of phosphorus

Procedure

5 ml of aliquots were pipette out into 50 ml standard flasks .10 ml vandate reagent molybdate reagent was added and the volume was made up to 50 ml with distilled water and the contents were mixed well by inverted shaking. The samples and standards were incubated for 30 minutes for the development of yellow colour. After the incubation absorbance at 420 nm was taken on a spectrophotometer.

3.8.2.3 Total nitrogen in plant samples

Apparatus

Block -digestor, Kjeldahl distillation unit, conical flask, pipette, measuring cylinder etc

1. Sulphuric acid – salicylic acid

1 g of salicylic acid was dissolved in 30 ml of concentrated sulphuric acid

2. Digestion mixture

Mix 25 g of K_2SO_4 with 5 g of $CuSO_45H_2O$ and 0.5 g of metallic selenium powder by grinding in a mortar.

3. Sodium Hydroxide solution (NaOH) ,40%

4. Mixed indicator

0.14 g of bromocresol green + 0.098 of methyl red in 140 ml of 95% ethanol

4. Boric acid solution

Dissolve 40 g of boric acid in distilled water and dilute the contents to 900 ml . Add 20 ml bromocresol green + methyl red mixed indicator solution. Then add 0.1 N NaOH drop wise till solution becomes reddish purple. Make up the volume to one liter.

Procedure

Digestion

1.0.5 g of dry plant material was weighed and transfered quantitatively into a 100 ml digestion tube .

2. 3 g catalyst mixture was added.

3.10 ml concentrated sulphuric acid containing salicylic acid was added using a dispenser and the contents were mixed well.

4. The tubes were placed in a block digestor that was set at 100 ^{o}C for 20 minutes and digested at low flame and the tubes were removed to wash down any material adhering to the neck of the tube with the same concentrated sulphuric acid. The tube contents were agitated and the tubes were placed back to the block digestor set at 380 ^{o}C for 2 hours.

5. After the digestion is completed, the tubes were removed and allowed to cool

Distillation

1.10 digests were taken and transferred to vacuum jacket of micro – kjeldaghl distillation apparatus. 10 ml of 4 % boric acid solution is taken containing bromocresol green and methyl red indicator , to which the condenser outlet of the flask was dipped .After adding the aliquot , the funnel of the apparatus was washed with 2 -3 of deionized water and 10 ml of 40 % solution was added.

2. ml of aliquot was distilled into the flask containing 10 ml boric acid. After the distillation was completed, the boric acid was titrated 0.02 N sulphuric acid . The blank was also runned simultaneously.

CHAPTER 4

RESULTS

An investigation entitled “Effect of salinity on growth, physiological and biochemical processes of coconut (*Cocos nucifera*) seedlings was conducted at ICAR–CPCRI, Kasaragod to decipher the biochemical and physiological basis of salinity tolerance. For this present research, 18 uniform seedlings of Malayan green dwarf (MGD) variety were selected and salinity stress was imposed. After subjecting the seedlings to salinity stress morphological, physiological, biochemical parameters were measured. Water and plant nutrient analysis were also conducted as part of the study. The results obtained from the study are described below.

4.1. MORPHOLOGICAL RESPONSES

Morphological measurements such as plant height, number of green leaves, leaf area and collar girth are important indicators of the effect of salt stress on plant growth. Significant differences in the morphological features of the seedlings were documented when the coconut seedlings were subjected to salinity treatment. Seedlings grown in nutrient solutions substituted with sea water at the levels of 10% to 100% showed significant differences in the change in plant height compared to the control seedlings where no substitution of sea water was made in nutrient growth solution. Level of significance is indicated by p vale and the treatment means were compared by CD values

Table 4.1Morphological parameters of the MGD seedlings before starting salinity treatment

SI NO	Total fresh weight(kg)	Total height(cm)	No of leaves	Colar diameter	No of main roots	root length(cm)	Leaf area(Cm²)
T1R1	3.714	157.5	7	27	10	28 5	22251.58
T1R2	4.162	187	7	34	24	40	28659
T1R3	3.874	190	6	31.5	11	10 5	28152.82
T2R1	4 154	171	8	30	23	27 5	26208 67
T2R2	2.432	179.5	5	25.2	9	18	27651.98
T2R3	3.974	201	6	32	17	17 5	34796.07
T3R1	3 758	157 5	6	34	13	22 5	22759 56
T3R2	3.626	150	5	20	4	8 5	40796.39
T3R3	2.706	211	5	27.5	3	5	39772.61
T4R1	3 028	164	7	30	22	14	21301 31
T4R2	4.608	165.5	9	34.2	14	25 5	39277.18
T4R3	2.44	195	5	22	2	7 5	27214.29
T5R1	4.4	152.5	8	30	18	22 5	40387.34
T5R2	4.716	202	6	33	11	10	29860.28
T5R3	3.936	207.5	7	35	7	6 2	39735.12
T6R1	3.658	184.5	7	30	19	15	46842.76
T6R2	3 158	177	5	28	16	15 5	34685 11
T6R3	3.764	207.5	5	33	17	16	34858.24

Table 4.2 Morphological parameters of the MGD seedlings subjected to salinity treatments **(**10% to 100% S refers to the various salinity stress treatments of 10% to 100% substitution of sea water, respectively in the nutrient solution of the seedlings**).**

Treatments	**Change in Plant height (cm)**	**No. of fresh leaves**	**Leaf area (cm^2)**	**Max. root length (cm)**	**No. of roots**	**Collar girth (cm)**
Control	43.33^{A}	9^{A}	55166^{AB}	46.66^{A}	44.00^{A}	44.16^{A}
10 % S	30.50^{AB}	8^{A}	51078^{AB}	50.00^{A}	38.33^{A}	44.33^{A}
25% S	5.50^{CB}	5^{B}	34431^{CB}	44.66^{A}	16.00^{B}	37.73^{AB}
50 %S	-2.03^{CB}	3^{C}	20381^{CD}	12.33^{B}	6.00^{BC}	31.06^{BC}
75%S	-8.83^{C}	2^{C}	3717^{ED}	7.500^{B}	6.00^{BC}	24.00^{C}
100%S	-24.70^{C}	2^{C}	66^{E}	8.16^{B}	1.00^{C}	29.33^{BC}
CD at 5 %	33.35	1.28	16466.28	17.68	11.83	7.55
P value	0.0095	<.0001	<.0001	0.0005	<.0001	0.0016

Table 4.3 Total fresh weight (kg) and dry weight (kg) of coconut seedlings under various salinity treatments

Treatments	**Total fresh weight(Kg)**	**Total dry weight(Kg)**	**Root dry weight(Kg)**	**Shoot dry weight(Kg)**	**Leaf dry Weight(Kg)**
Control	5.71^{A}	1.22^{A}	0.09^{A}	0.56^{A}	0.56^{A}
10 % S	5.56^{A}	1.12^{A}	0.08^{A}	0.50^{A}	0.53^{A}
25% S	3.01^{B}	0.65^{B}	0.07^{A}	0.29^{B}	0.28^{B}
50 %S	1.48^{BC}	0.34^{BC}	0.01^{B}	0.16^{BC}	0.16^{B}
75%S	0.76^{C}	0.24^{C}	0.01^{B}	0.07^{C}	0.16^{B}
100%S	0.92^{C}	0.25^{C}	0.007^{B}	0.07^{C}	0.17^{B}
CD at 5 %	1.67	0.04	0.051	0.012	0.03
P value	<.0001	<.0001	0.0069	0.0003	<.0001

4.1.1 Plant height

The results have shown that salinity treatment significantly affected the height of the seedlings. The change in plant height (Final height – initial height) between the control and 10% of sea water substitution in the nutrient solution did not have any significant difference (table 4.1 and Fig4.1). However, at 25% sea water treatment and beyond at high salinity stress (50-100% salt water substitutions) the height of the plants were significantly reduced. Fig. 4.1 and 4.2 clearly depicted that at 10% S the increase in plant height was on par with control but the increase was significantly low as the days progressed at 25% and beyond. The total height increase of the control seedlings was 43.3 cm. At 10% and 25% sea water exposures, the height increased by 30.5 cm and 5.5 cm respectively. At 25 % of the sea water substitution a sharp reduction in the total height was observed. It indicated that virtually there was no growth throughout the experiment. Seedlings subjected to 50 % of the sea water substitution showed 2.03 cm reduction indicating that the leaves were completely dried due to the salinity effects and the

total height was reduced. At the stress levels of 75 % and 100% sea water substitutions, the mean reduction in total height was at 8.83 and 24.7 cm respectively.

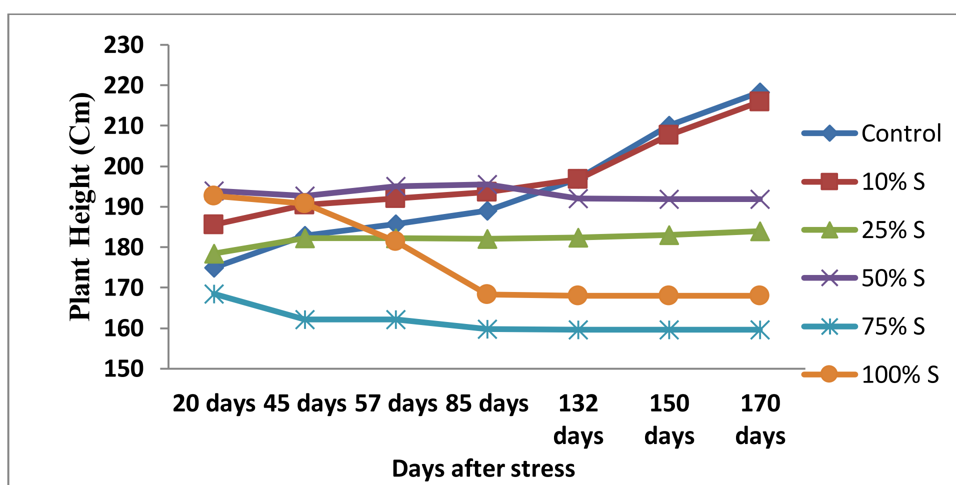

Fig.4.1. Time course measurement of total height of the coconut seedlings under various salinity treatments

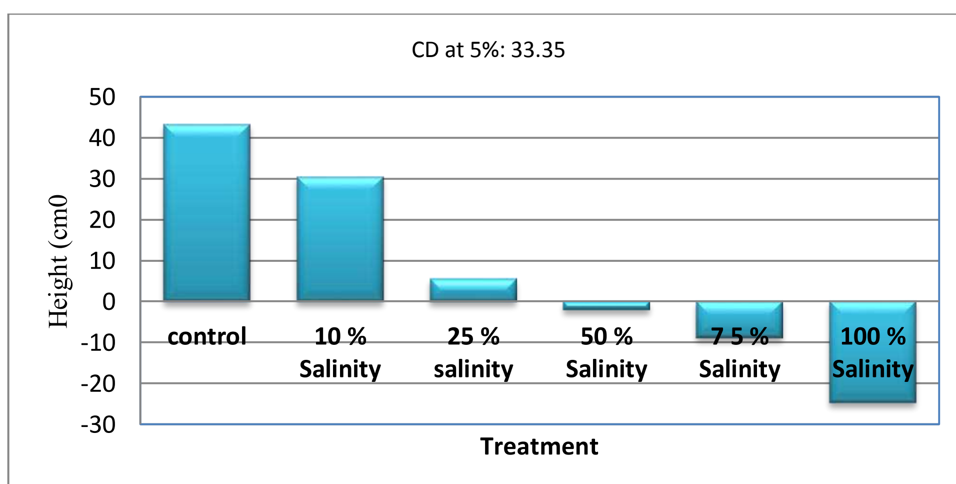

Fig.4.2 Change (final height –initial height) in plant height of the coconut seedlings with various salinity treatments.

4.1.2 Number of fresh leaves

Expectedly, number of green leaves was more in the seedlings grown as control compared to the salinity treatments. At the end of the experiment there were nearly 8 leaves in these treatments. It was only 5 leaves at 25% S and sharply declined at higher salinity levels (Table 4.1 and Fig 4.3)

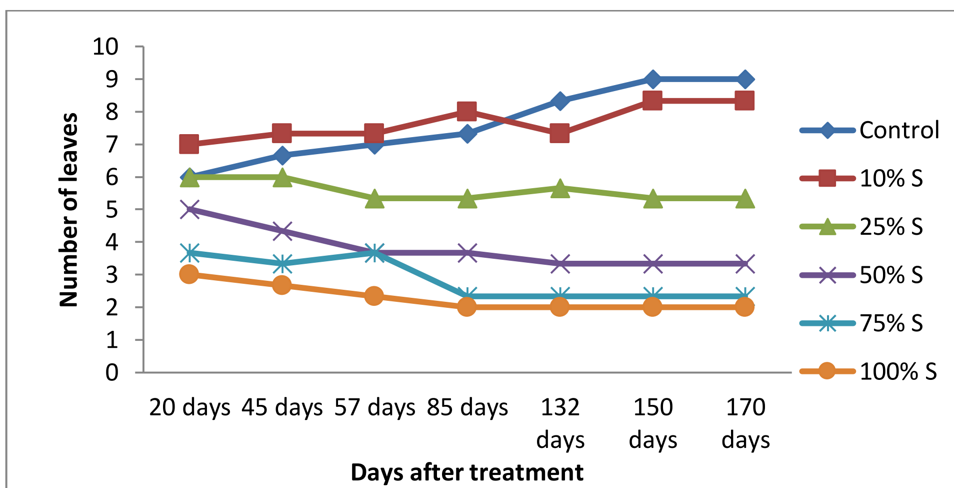

Fig.4.3 Time course measurement of number of fresh leaves of coconut seedlings under various salinity treatments.

4.1.3 Leaf area

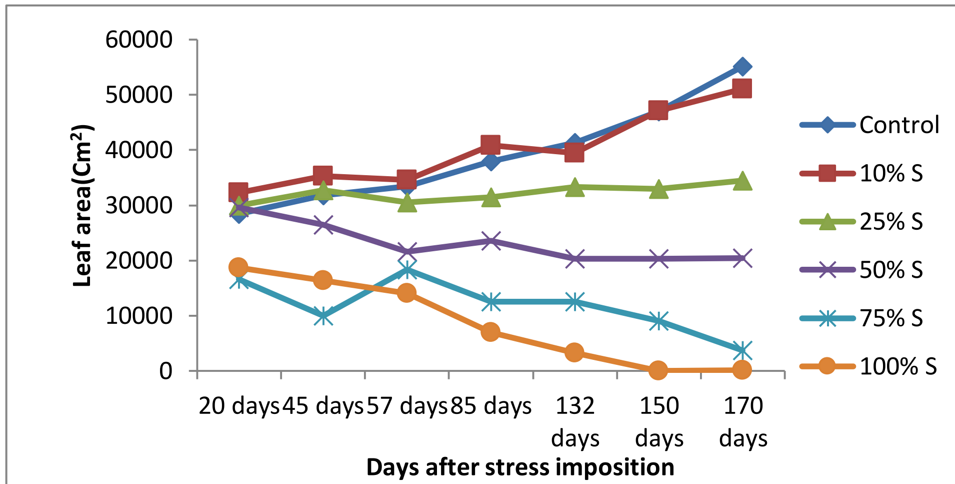

Fig 4.4 Time course measurement of mean leaf area of coconut seedlings grown under various salinity treatments

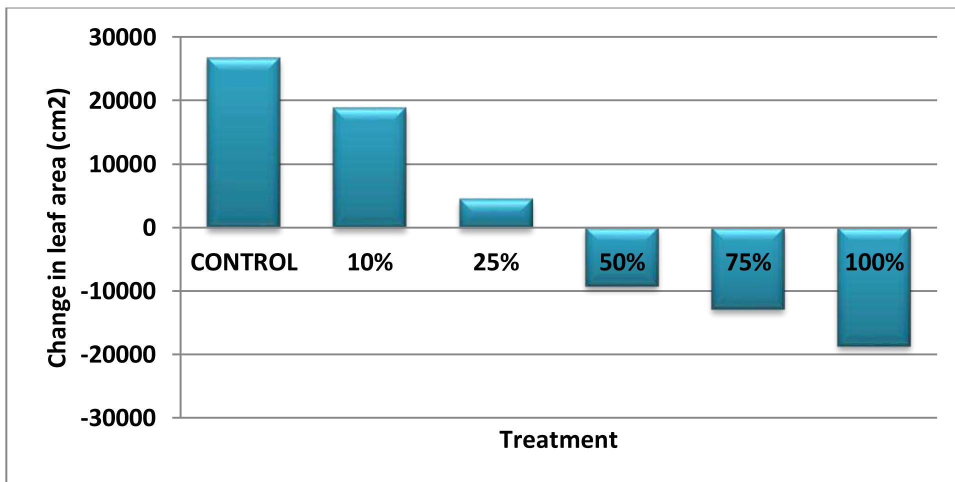

Fig.4.5 Change in leaf area of coconut seedlings from the period of starting of the experiment till the termination of experiment

Leaf area across various treatments showed a significant variation (Fig.4.4 & 4.5). In control seedlings and seedlings at 10% S at the end of the experiment it was on par and had risen to around 53000 cm2 (Fig. 4.4). The rate of increase was almost on par in both these treatments. The control plants showed the highest increase in leaf area during the period of experimentation (26738.55 cm^2) . During the same time at 10 % S there was a mean increase of 18839.5 cm^2 in leaf area (Fig 4.5). At 25 % salinity level there was an increase of 4150.6cm^2 thus seedlings at 25 % salinity treatment showed a significant reduction in the leaf area. At 50 % salinity level, there was a mean reduction of 9235.42 cm^2 in the leaf area. Similarly, seedlings at 75 % and 100% salinity treatments showed that their leaf areas were reduced by 12863.9 cm^2 and 18668.2 cm^2, respectively. Results indicate that leaf area is an important and sensitive growth parameter that is severely affected due to increase in salinity in the root zone of the coconut seedlings.

4.1.4 Total fresh weight

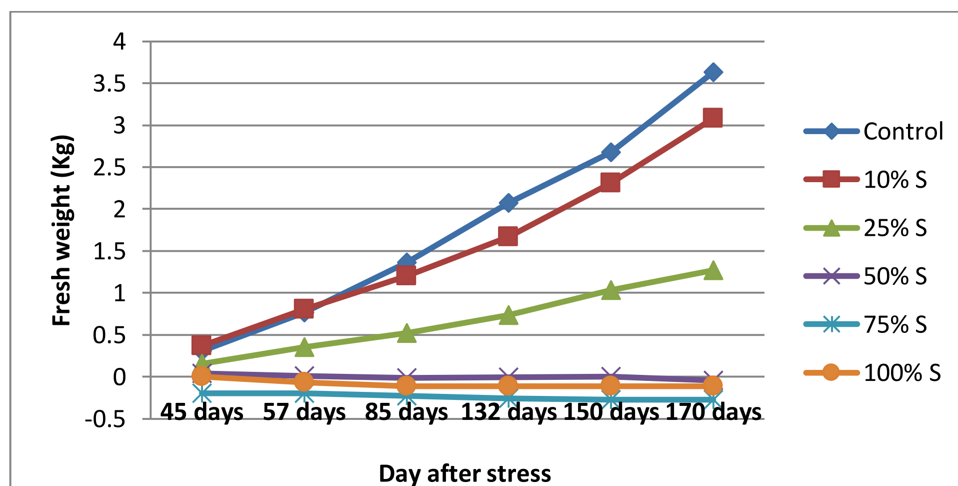

Fig.4.6. Time course increment in the total fresh weight of the coconut seedlings with salinity stress

The total fresh weight of the coconut seedlings across various treatments showed a significant variation. The control seedlings without any interference from the salt have a mean increase of 3.63 Kg in total fresh weight. Seedlings at 10 % saline water substitution had an increase of 3.07 Kg whereas, seedlings subjected to 25 % of the salinity treatment showed an increase of only 1.26 Kg from its initial stage. Beyond the salt stress of 25% salt water or sea water substitution, seedlings showed a greater reduction in total weight. When seedlings were subjected to 50 % of salinity stress, there is a reduction of 0.04 Kg in the fresh weight whereas seedlings grown at 75 % and 100% of sea water substitution in the nutrient solution exhibited a reduction of 3.29 Kg and 3.13 Kg of fresh weights, respectively.

4.1.5 Maximum root length

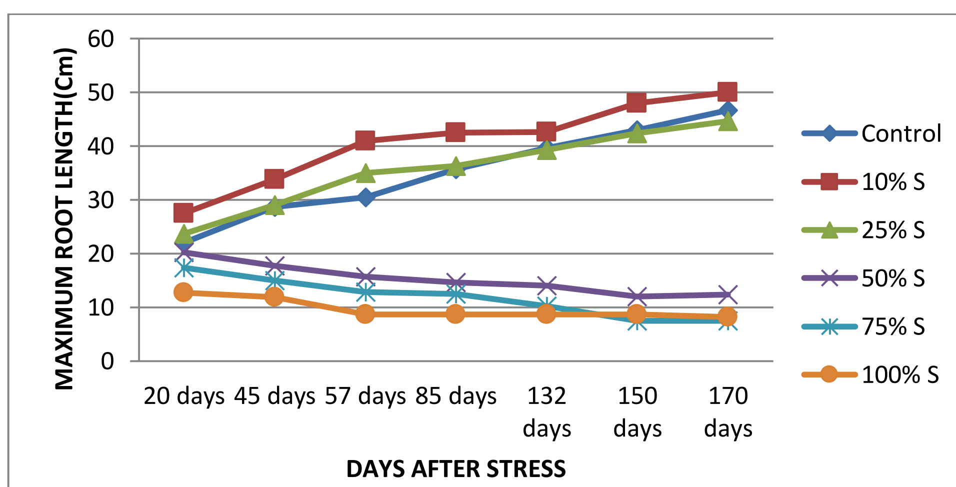

Fig.4.7 Time course measurement of maximum root length of the coconut seedlings under various salinity treatments

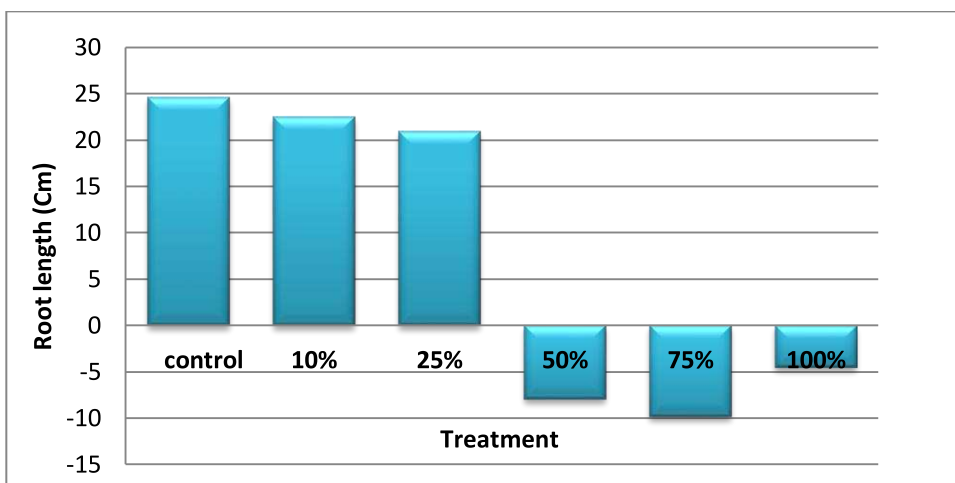

Fig 4.8 Change in the maximum root length of the coconut seedlings under various salinity treatments

The observation of the maximum root length of the seedlings revealed that the control seedlings had a mean increase of 24.6 cm during the period of experimentation (Fig 4.9). For the seedlings grown at 10 % of salinity stress, the increase in root length was reduced to 22.5 cm.

4.1.6 Root number

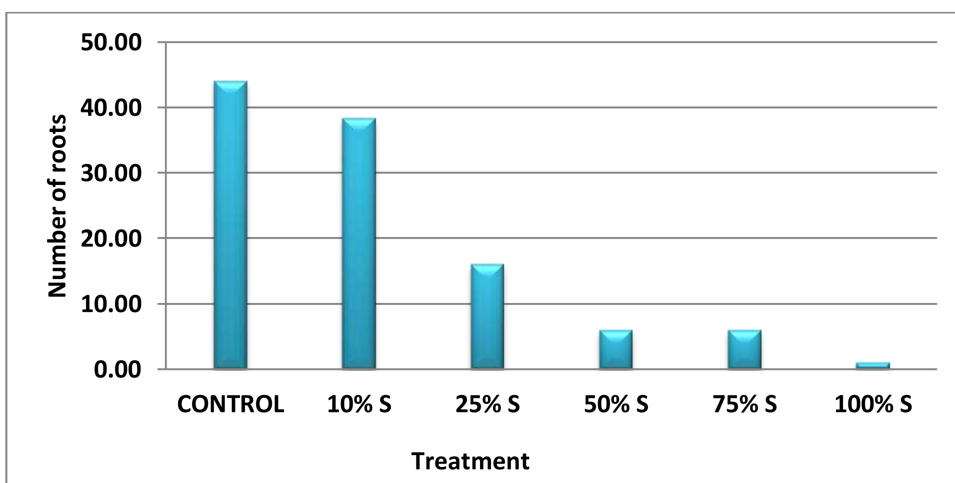

Fig 4.9 Number of roots of coconut seedlings under various treatments of salinity

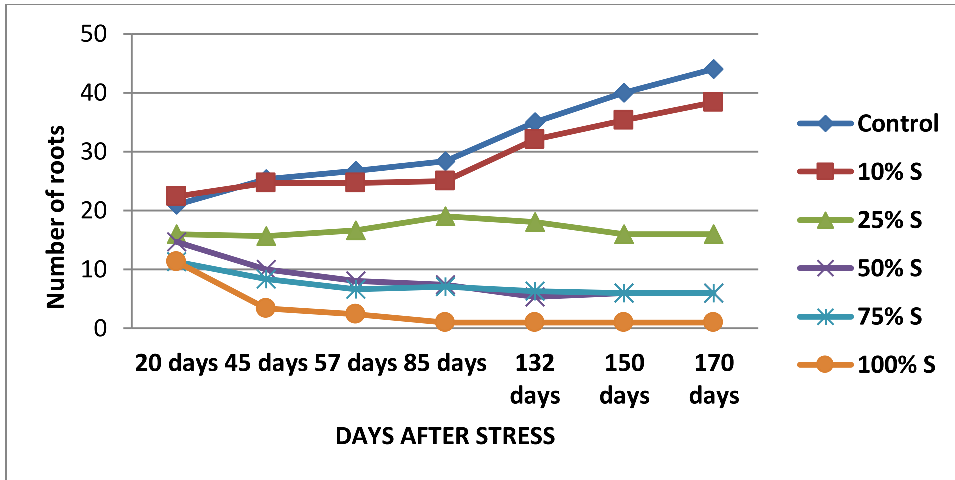

Fig 4.10 Time course measurement of number of roots of coconut seedlings under various salinity treatments

The observations regarding the root growth parameters across various treatments were recorded. The control coconut seedlings had 44 roots during the treatment period. Seedlings at 10% and 25% of the salinity treatment, had 33 and 16 root respectively. Seedlings 25 % salinity showed no signs of root development as roots have started rotting and not able to withstand salinity stress.

4.1.7 Collar Girth

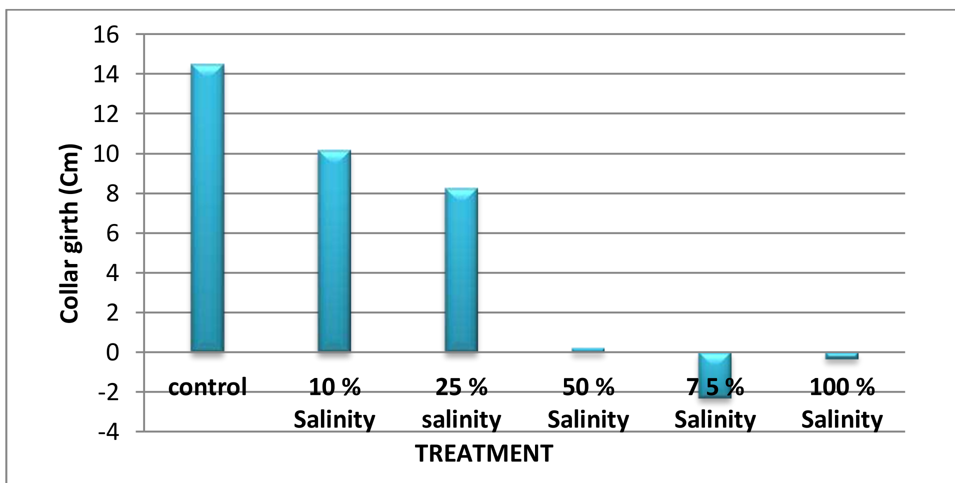

Fig 4.11 Mean change in the collar girth of coconut seedlings under various salinity treatments

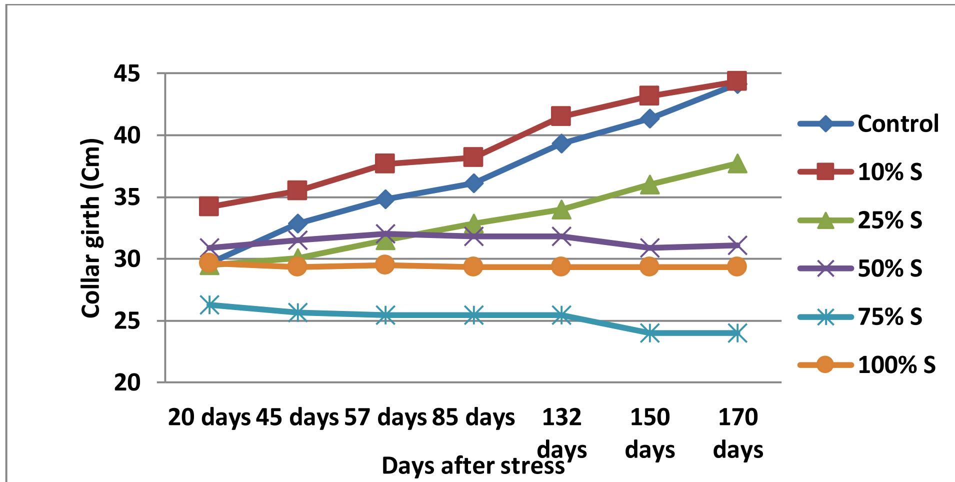

Fig. 4.12 Time course measurement of collar girth of coconut seedlings under various salinity treatments

The collar girth of the coconut seedlings across various treatments showed a significant variation (Fig 4.12 and 4.13). Like the other morphological parameters, control seedlings showed a maximum increase in collar girth (14.5 cm). At 10 % salinity treatment there is a mean increase of 10.13 cm. Nonetheless, seedlings grown at 25 % salinity stress showed only an increase of 8.23 cm and the increase in collar girth of seedlings grown under 50 % salinity treatment stress was a meager 0.2 cm.

4.1.8 Total dry weight

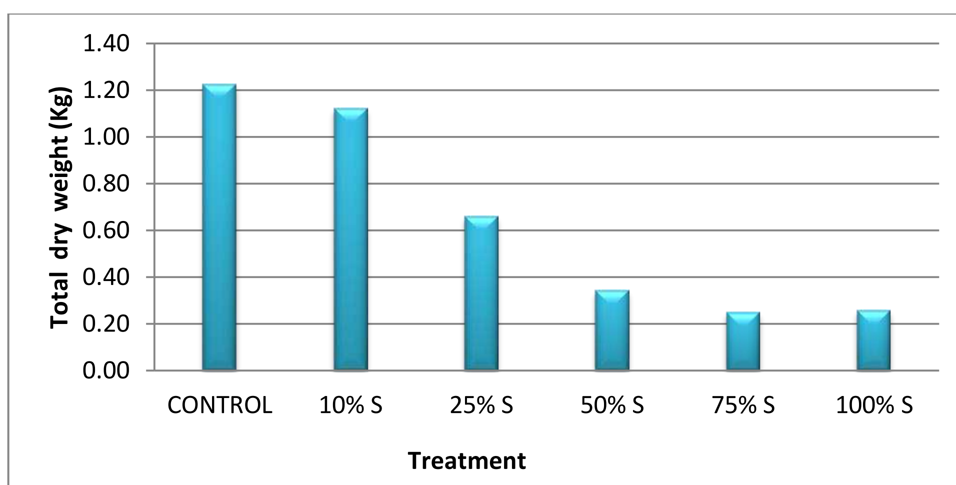

Fig 4.13 Total dry weight of seedlings subjected to various salinity stresses after the end of the experiments.

Total dry biomass of coconut seedlings at control and 10% S treatment were on par and it was 1.22 and 1.12 kg respectively (Fig 14). However at 25% S it was reduced by 47% and beyond that at higher salinity biomass accumulation was negligible. This is quite evident from the Figs. 4.15 and 4.16 plants grown with sea water substitution for 3 months and 6 months respectively.

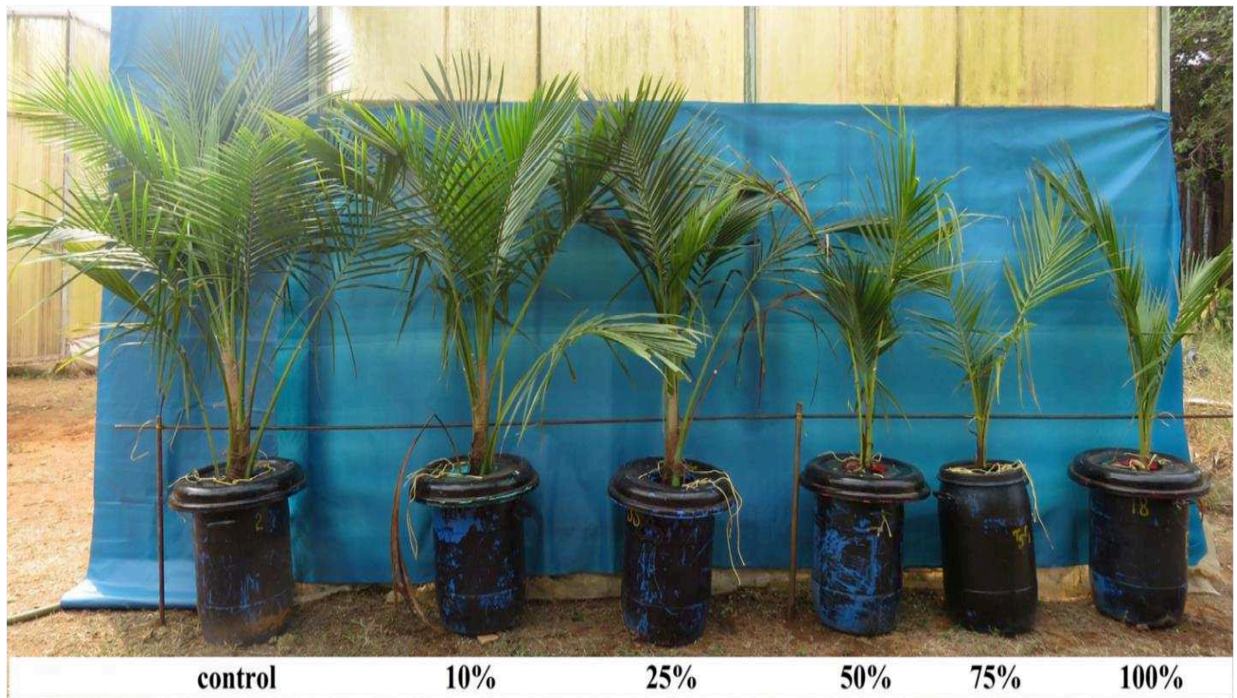

Fig 4.14 Growth of seedlings after 3 months of salinity treatment (percentages refer to the quantum of sea water substitution to the nutrient solution)

Fig 4.15 Growth of seedlings after 6 months of salinity treatment (percentages refer to the quantum of sea water substitution to the nutrient solution)

4.2 PYSIOLOGICAL RESPONES

Significant decline in photosynthesis (p<.0001), stomatal conductance (p<.0001), transpiration (p<.0001), stomatal resistance (p<.0001), water potential (p=.009), chlorophyll fluorescence (p=.001) and chlorophyll index (p<.0001) were recorded (Table 4.3). Excluding the physiological parameters such as (photosynthetic rate, stomatal conductance and transpiration rate) all other parameters were on par at 10% S with control plants.

Table 4.4 Physiological responses of the coconut seedlings subjected to various salinity treatments

Treatment	**Stomatal resistance (cm^{-1})**	**Chlorophyll fluorescence**	**Chlorophyll Index (CI)**	**Photosynthesis (µmole m^{-2} s^{-1})**	**Stomatal conductance (mole m^{-2}s^{-1})**	**Transpiration (m mole m^{-2}s^{-1})**	**Water potential (bars)**
Control	1 88^{C}	0.788^{A}	57 90^{A}	6.29^{A}	0.091^{A}	2.75^{A}	9.93^{B}
10% S	5 38^{C}	0.787^{A}	58 23^{A}	4.12^{B}	0.053^{B}	1.70^{B}	10 06^{B}
25% S	21 42^{C}	0.774^{AB}	57 43^{A}	2.89^{C}	0.041^{B}	1.13^{C}	10 93^{B}
50%S	94 00^{B}	0.733^{AB}	49 37^{B}	0.54^{D}	0.013^{C}	0.44^{D}	13 95^{AB}
75%S	121 00^{AB}	0.686^{B}	42 23^{B}	-1.41^{E}	0.015^{C}	0.49^{D}	19 01^{A}
100%S	134.33^{A}	0.573^{C}	36 10^{D}	-1.54^{E}	0.009^{C}	0.28^{D}	18 18^{A}
CD at 5 %	67 43	0.35	4.63	0.68	0.02	0.45	5.40
P value	< 0001	0 0015	< 0001	< 0001	< 0001	< 0001	0 0098

4.2.1 Stomatal resistance

The control seedlings had a mean stomatal resistance of 1.78 cm^{-1}. Seedlings at 10 % salinity treatment showed a slight but non-significant increase in stomatal resistance to 3.23 s cm^{-1}. Seedlings at 25 % of salinity treatment showed increased stomatal resistance of 14.23 s cm^{-1}. Seedlings beyond 25% of salinity stress showed a sharp increase in stomatal resistance. Seedlings at 50 %, 75% and 100% salinity stresses, the stomatal resistance was 40.11 s cm^{-1} 138.83s cm^{-1} and 242.22 s cm^{-1} respectively (Fig.4.17)

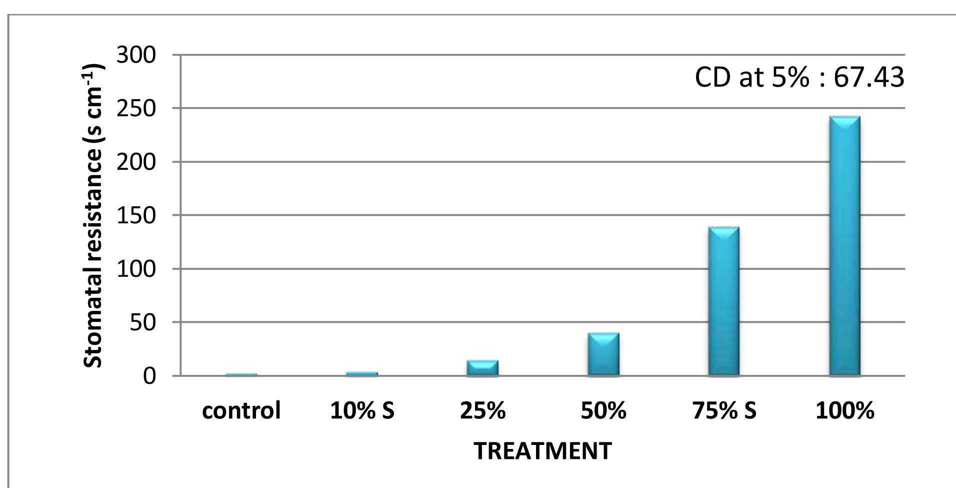

Fig 4.16 Stomatal resistance of coconut seedlings under various treatments

4.2.2 Chlorophyll fluorescence (Fv/Fm ratio)

Chlorophyll fluorescence was up to 50% S was on par with control plants (Table 4.18). The control seedlings showed a highest chlorophyll fluorescence (0.802) followed by 10% salinity (0.801). At 25 % treatment, it was reduced to 0.770 and at 50 % and 75 % salinity treatments chlorophyll fluorescence was further reduced to 0.735 and 0.635, respectively. Expectedly, seedlings at 100 % salinity stress showed the least chlorophyll fluorescence value of 0.597.

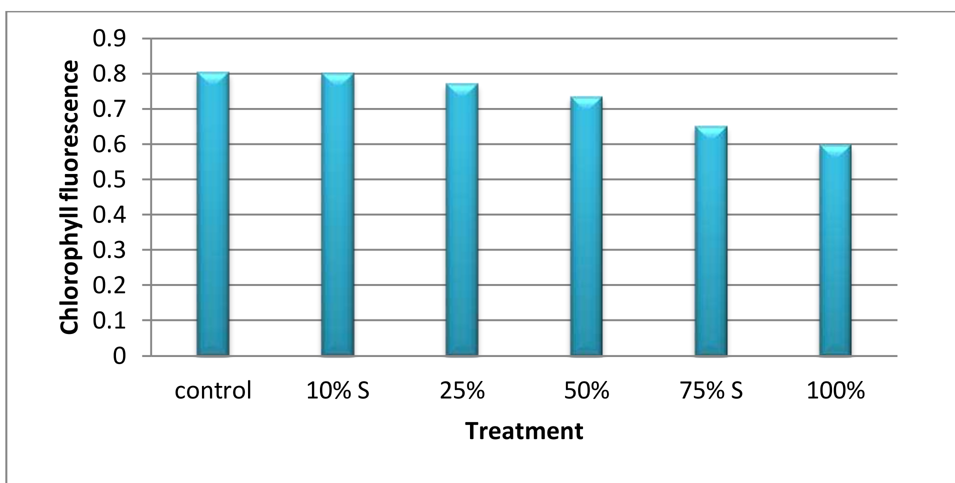

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Fig 4.17 Chlorophyll fluorescence of coconut seedlings under various treatments

4.2.3 Chlorophyll Index (CI)

Similar to chlorophyll fluorescence, the chlorophyll index up to 25%S was on par with control plants (Table 4.19). The control seedlings showed the highest value of chlorophyll index (58.66) followed by seedlings that underwent 10% salinity treatment (57.76). Chlorophyll index was further reduced to 57 and 49.46, respectively in the seedlings subjected to 25 % and 50 % of salinity stresses. Seedlings at 75 % and 100% of the salinity treatments showed the lowest CI values of 41.36 and 36.13 respectively.

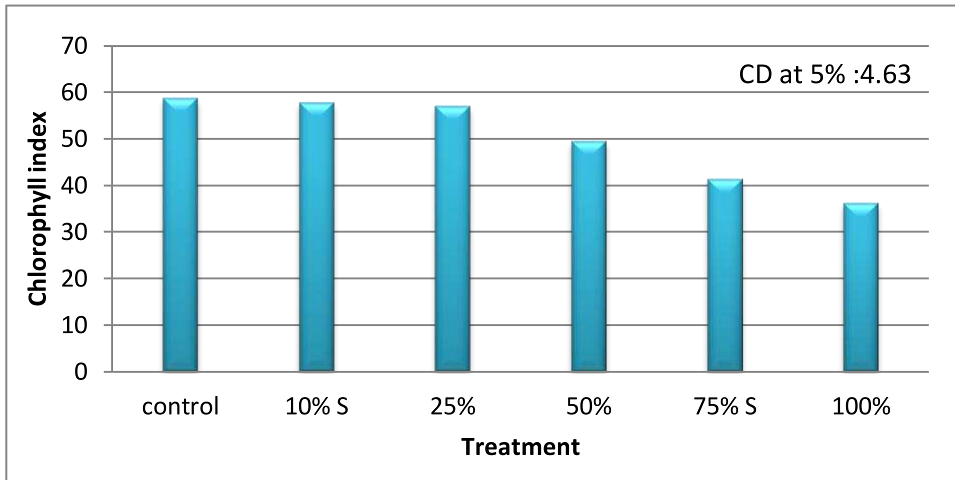

Fig 4.18 Chlorophyll index of the coconut seedlings under various salinity stresses

4.2.4 Net photosynthesis (Pn)

The control seedlings showed a high photosynthetic rate of 6.83 µmole m^{-2} s^{-1}. Seedlings at 10 % salinity stress exhibited a photosynthetic rate of 4.5 µmole m^{-2} s^{-1}. Photosynthetic rates of the seedlings were further reduced to 3.41 in seedlings at 25 % of salinity treatment. At 50 % salinity the photosynthetic rate was 2.19 µmole m^{-2} s^{-1} whereas seedlings of 75 % and 100 % salinity stresses showed the lowest photosynthetic rate of 0.21 and -1.54 µmole m^{-2} s^{-1}, respectively (Fig 4.20).

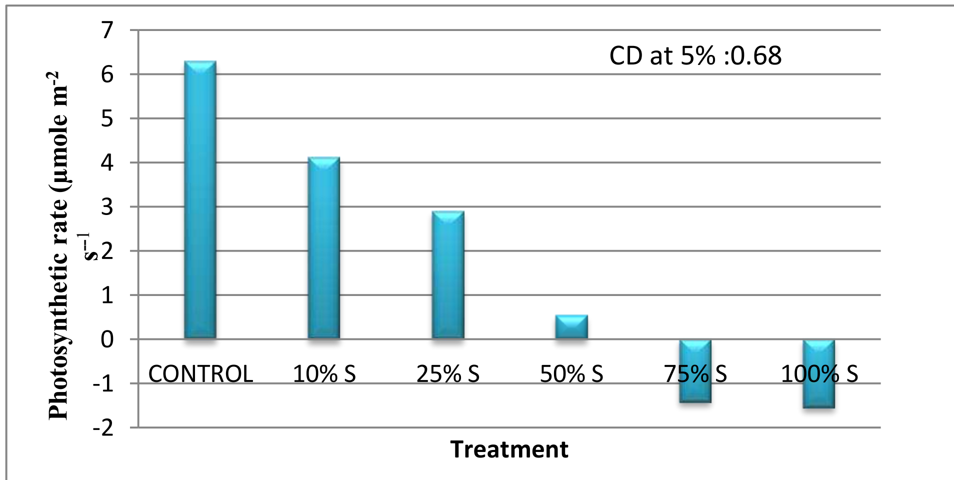

Fig 4.19 Net photosynthetic rate of coconut seedlings under various treatments

4.2.5 Stomatal conductance (gs)

The control coconut seedlings showed a maximum stomatal conductance of (0.091 mole $m^{-2}s^{-1}$) during the treatment period. Seedlings at 10 % of salinity stress, showed that the stomatal conductance was reduced to 0.053 mole $m^{-2}s^{-1}$ and the conductance was further reduced with the seedlings (0.041 mole $m^{-2}s^{-1}$) that were subjected to 25 % salinity. Seedlings at salinity stress beyond 25 % of sea water substitutions, a sharp reduction in stomatal conductance was observed. Seedlings of 50 % salinity treatment, showed a stomatal conductance of 0.015 mole $m^{-2}s^{-1}$. The lowest values of stomatal conductance were exhibited by the seedlings that underwent 75% and 100 % salinity treatments (0.013 and 0.009 mole $m^{-2}s^{-1}$).

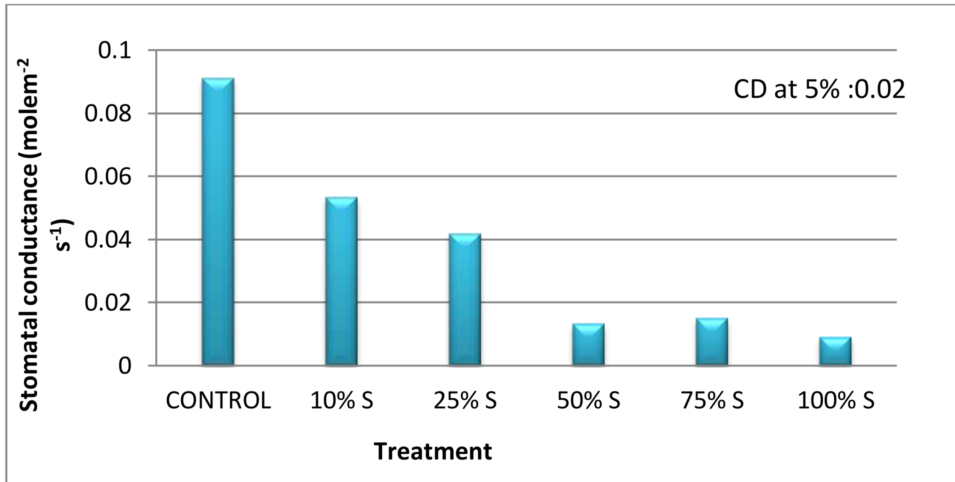

Fig 4.20 Stomatal conductance of coconut seedlings under various treatments

4.2.6 Transpiration (Tr)

The mean transpiration rate of the control seedlings was found to be 1.265 m mole m $^{-2}s^{-1}$. The decrease in the transpiration rate of the seedlings with the increase in the salinity stress of the seedlings was very significant. Seedlings at 10 % salinity treatment showed a reduction of the mean transpiration rate to 0.967 m mole m $^{-2}s^{-1}$. Seedlings that underwent 25 % salinity treatment showed further reduction of transpiration rate to 0.839 m mole m $^{-2}s^{-1}$. Beyond 25 % of the salinity stress a sudden decrease in the transpiration rate was observed. Seedlings at 50 % salinity stress showed the transpiration rate of 0.228 m mole m $^{-2}s^{-1}$ whereas the lowest values of transpiration rate were observed in the seedlings subjected to 75 % (0.077 m mole m $^{-2}s^{-1}$) and 100 % (-1.543 m mole m $^{-2}s^{-1}$) salinity treatments, respectively.

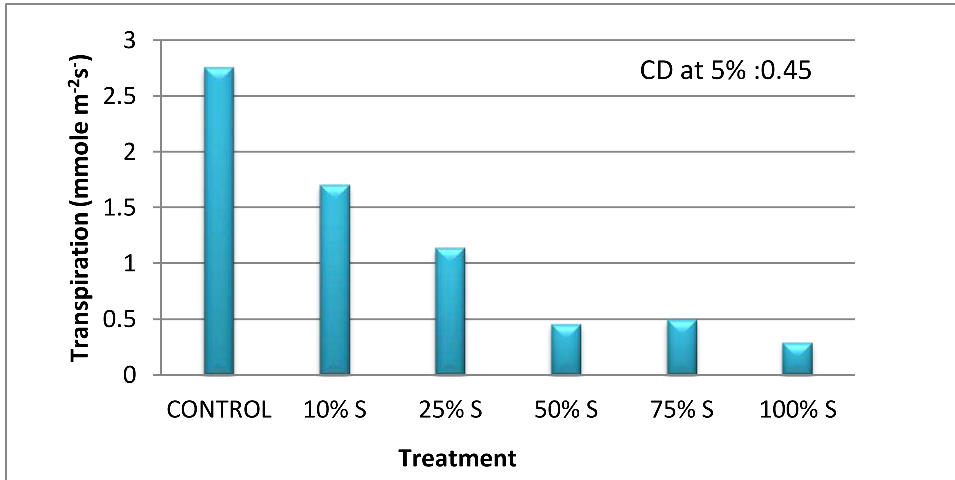

Fig 4.21 Transpiration rate of coconut seedlings under various treatments

4.2.7 Leaf water potential (LWP)

The leaf water potential of the control coconut seedlings was high (-9.93 bars). At 10% S and 25% S it was on par with control plants. Seedlings at 10% of salinity stress showed a leaf water potential of -10.06 bars whereas seedlings subjected to 25 % salinity stress the leaf water potential was -10.93 bars and for the seedlings that at 50 % salinity treatment it has become -13.95 bars. The highest values of leaf water potential were recorded in the seedlings that were subjected to 75 % and 100 % treatments (-19.91 and -18.18 bars respectively).

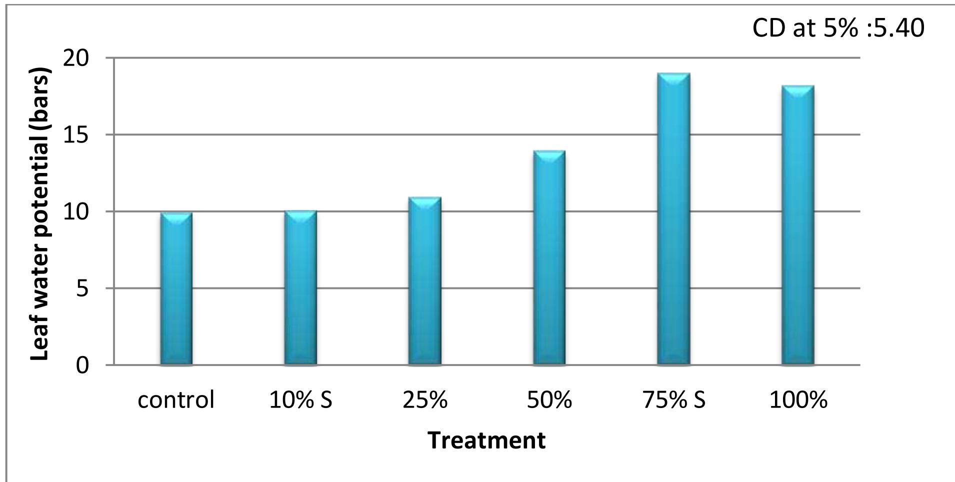

Fig 4.22 Leaf water potential (-bars) of coconut seedlings under various treatments

4.3 BIOCHEMICAL RESPONSE

Biochemical measurements like chlorophyll content, total sugar, reducing sugar, soluble protein, and total phenol content were measured in coconut seedlings that were subjected to salinity stresses. The resultant values are presented below.

Table 4.5 Biochemical responses of coconut seedlings under coconut seedlings

Treatment	Total sugar (g 100 g^{-1} fresh leaf tissue)	Reducing sugar (g 100 g^{-1} fresh leaf tissue)	Free amino acids (mg100 g^{-1} fresh leaf tissue	Total poly phenols (mg GAE 100g^{-1} fresh leaf tissue)	Anti oxidant capacity (mg trolox eq.)	
					CUPRAC	FRAP
Control	0.866	0 204	0 281 ^{C}	5.573 ^{C}	22.01 ^{D}	5.83
10% S	0.883	0 289	0 254 ^{C}	4.4821 ^{C}	25.75 ^{CD}	6.12
25% S	0 888	0 400	0 533 ^{B}	6 1482 ^{CB}	2642 ^{CD}	8 02
50%S	0.961	0 446	0 633 ^{AB}	6.5286 ^{CB}	29.61 ^{CB}	8.04
75%S	1.203	0 673	0 600 ^{AB}	8.4714 ^{AB}	33.05 ^{AB}	8.91
100%S	1.202	0 588	0 773 ^{A}	9.3696 ^{A}	36.580 ^{A}	10.17
CD at 5 %	NS	NS	0 15	1 63	4 15	NS
P value	0.27	0 22	0 0005	0.014	0 007	0.067

4.3.1 Total soluble sugar

The control seedlings showed the least value of soluble sugar value (0.865 g 100 g^{-1} fresh leaf tissue and the amount of soluble sugar increased as the salinity stress increased. However there was no significant effect of salinity on sugar content The highest accumulation of total soluble sugar was recorded in 75 % and 100 % salinity treatment respectively.

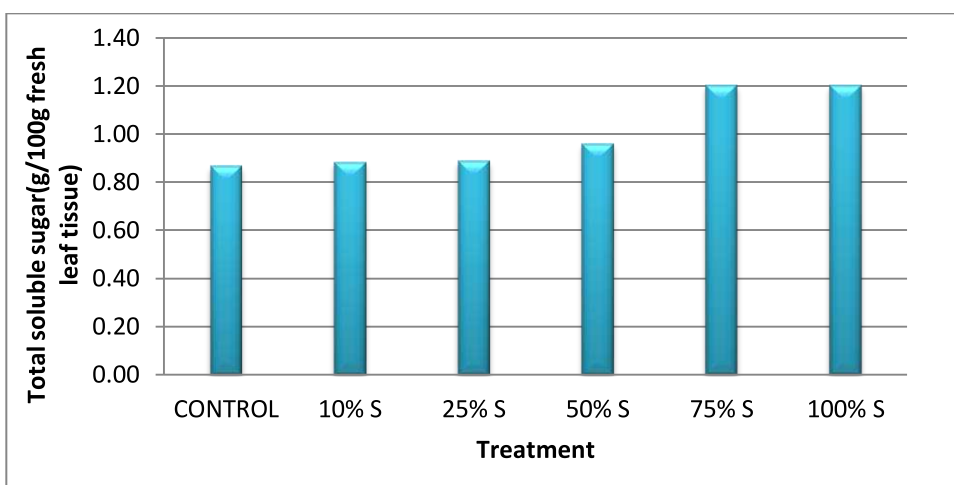

Fig 4.23 Total sugar content of coconut seedlings under various treatments

4.3.2 Reducing sugar

Similar to the total soluble sugar, the reducing sugar content of leaves also increased with an increase in salinity stress(Fig 4.25) but it was not significant (table 4.4). The maximum reducing sugar content was observed in the seedlings that underwent 75 % of salinity stress (0.673g100 g^{-1} fresh leaf tissue). As the salinity stress level decreased, the reducing sugar content also decreased.

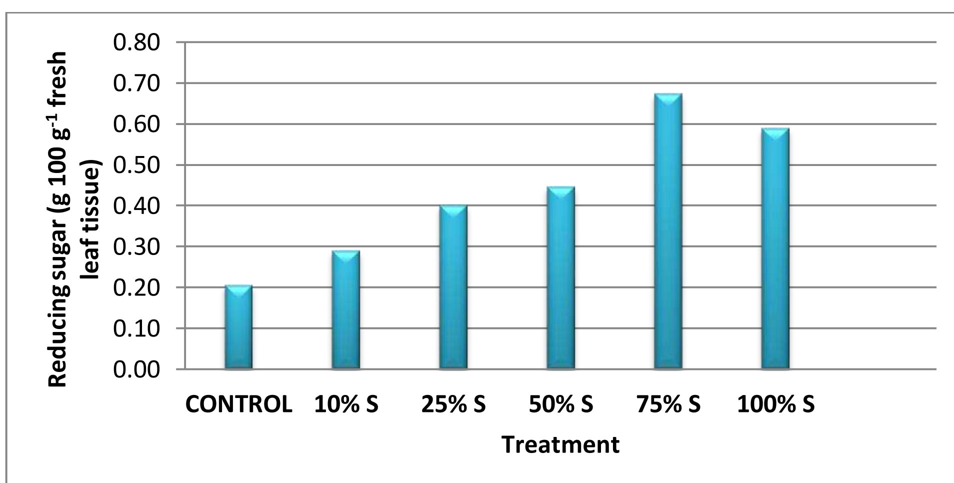

Fig 4.24 Reducing sugar content of coconut seedlings under various treatments

4.3.3 Free amino acids

A gradual build up of free amino acids was documented in the leaves of coconut seedlings subjected to salinity stress. At 10% S it was on par with control and at 25% significantly increased. The maximum accumulation of free amino acids was detected in the seedlings that have at 100 % salinity treatment (0.774 mg100 g^{-1} fresh leaf tissue).

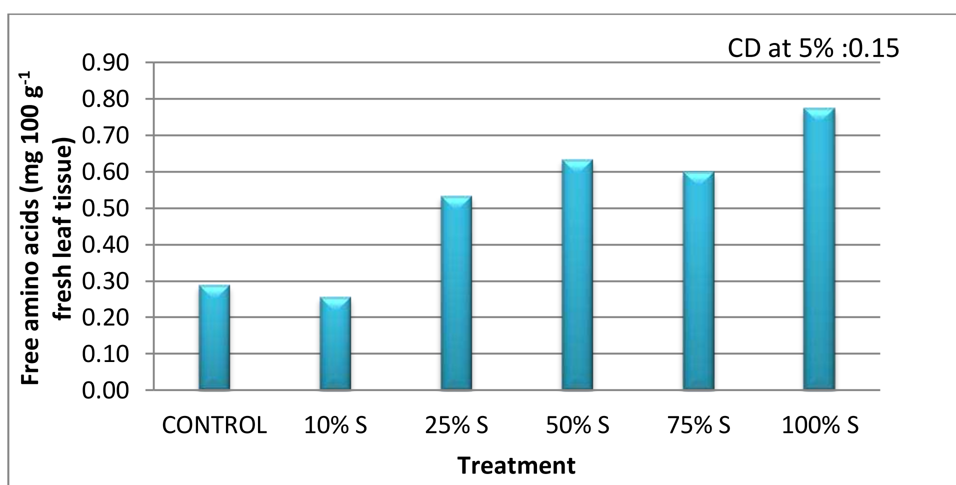

Fig. 4.25 Free amino acid content of coconut seedlings under various salinity treatments

4.3.4 Total phenol

The total phenol content increased significantly as the stress increased. However, up to 50% salinity level the phenol content was on par with control plants. The maximum phenol content was in the 100 % salinity treatment.

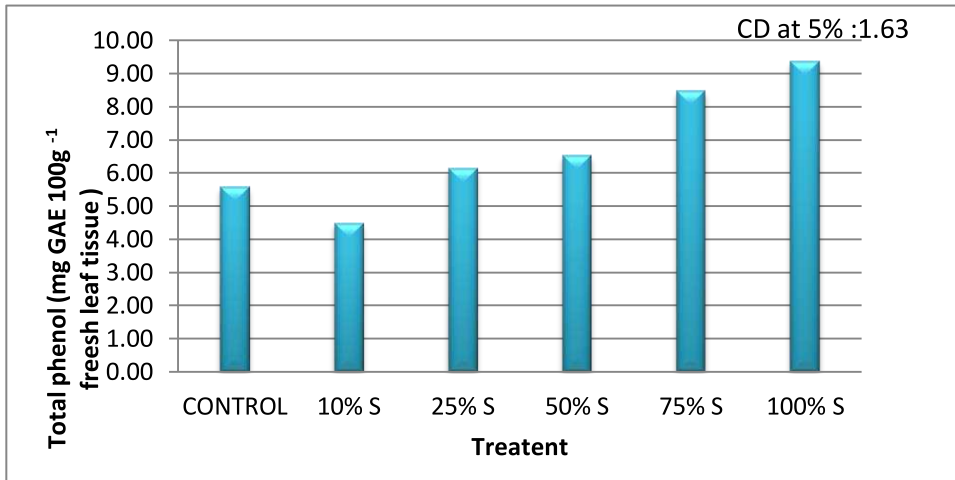

Fig 4.26 Total phenol content of coconut seedlings under various salinity treatments

4.3.5 Antioxidant activity

The antioxidant activity in the leaves was measured in terms of reducing power using the methods of FRAP and CUPRAC. In CUPRAC, a significant increase in the antioxidant activity was observed with the increase in stress, but it was not significant up to 50% salinity treatment. The maximum value was recorded in the 100 % salinity. In the FRAP method there was no significant variation among various treatments.

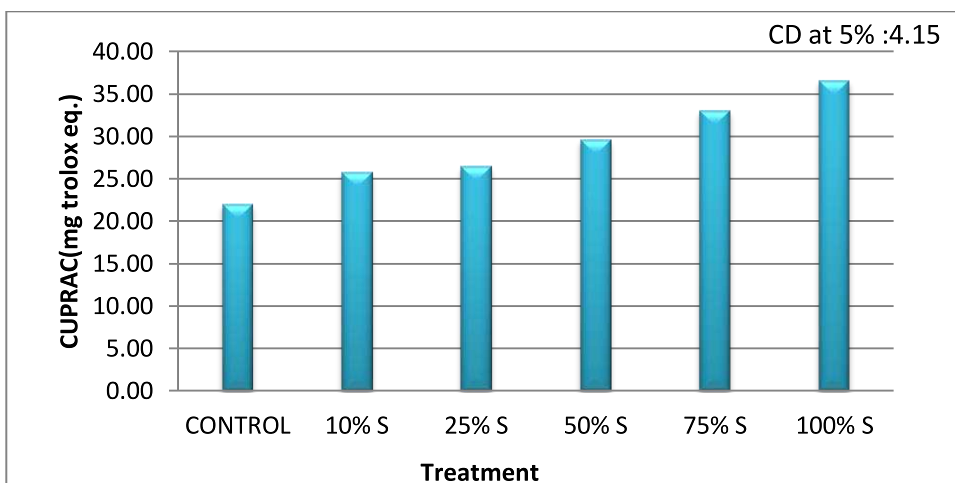

Fig 4.27.Antioxidant capacity (CUPRAC) of various salinity treatments

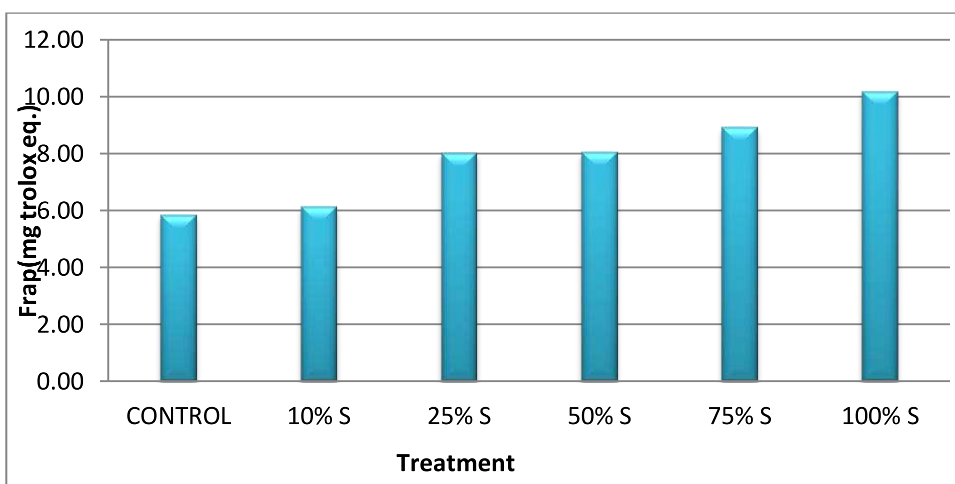

Fig 4.28 .Antioxidant capacity (FRAP) of various salinity treatments.

Table 4.6 SOD, POD, PPO, MSI, chlorophyll content and RWC of coconut seedlings under various salinity treatments

Treatment	SOD (μg g^{-1} protein)	POD (μg g^{-1} protein)	PPO (μg g^{-1} protein)	MSI (%)	Chlorophyll content (Mg/g of fresh leaf tissue)	RWC (%)
Control	111.59 ^{C}	0.84	0.495^{B}	80.57	1.47	69.49
10 % S	118.70 ^{C}	1.27	0.81 ^{B}	78.97	1.46	67.66
25% S	162.37 ^{B}	1.83	1.5750 ^{A}	74.67	1.52	60.34
50 %S	174.01^{AB}	1.39	1.6200 ^{A}	67.35	1.00	59.11
75%S	175.63 ^{AB}	1.65	1.7550 ^{A}	66.02	0.60	56.08
100%S	188.57 ^{A}	1.98	2.1600 ^{A}	62.99	0.54	55.16
CD at 5 %	15.85	NS	0.40	3.86	0.28	0.86
P value	0.0006	0.240	0.0026	<.0001	<.0001	0.0148

4.3.6 Super oxide dismutase

Super-oxide dismutase (SOD) enzyme is a major anti-oxidant enzyme that plays greater role in scavenging reactive oxygen species (ROS) generated during stress. The super oxide dismutase activity increased as the stress increased. The maximum SOD activity was recorded in 100 % seedlings (188μg g^{-1} protein). And the mean activity for the control seedlings was only 111 μg g^{-1} protein which was on par with the activity of seedlings at 10%S.

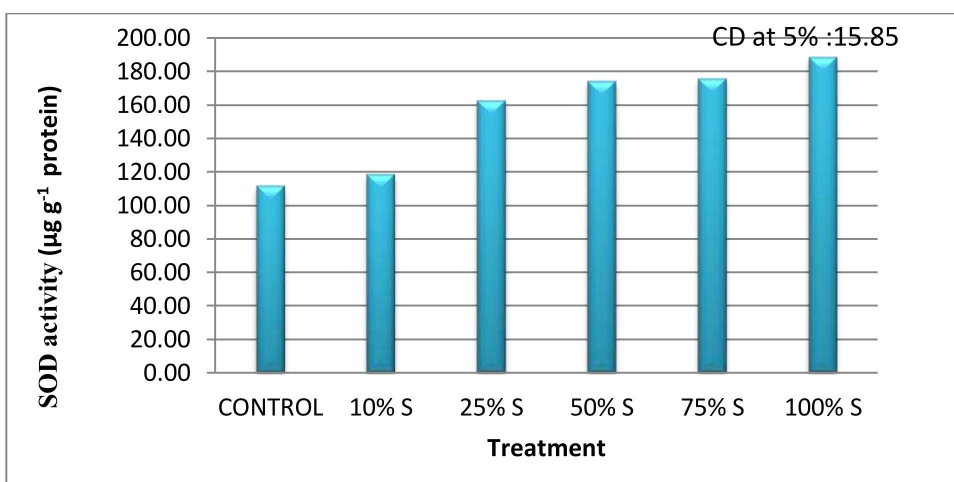

Fig 4.29 SOD activity of coconut seedlings under various salinity treatments

4.3.7 Peroxidase

The peroxidase activity did not show any significant variation across various treatments. The minimum activity of POD was recorded in control seedlings. Then as the stress increases, there was an increase in the activity of POD (Fig 4.31).

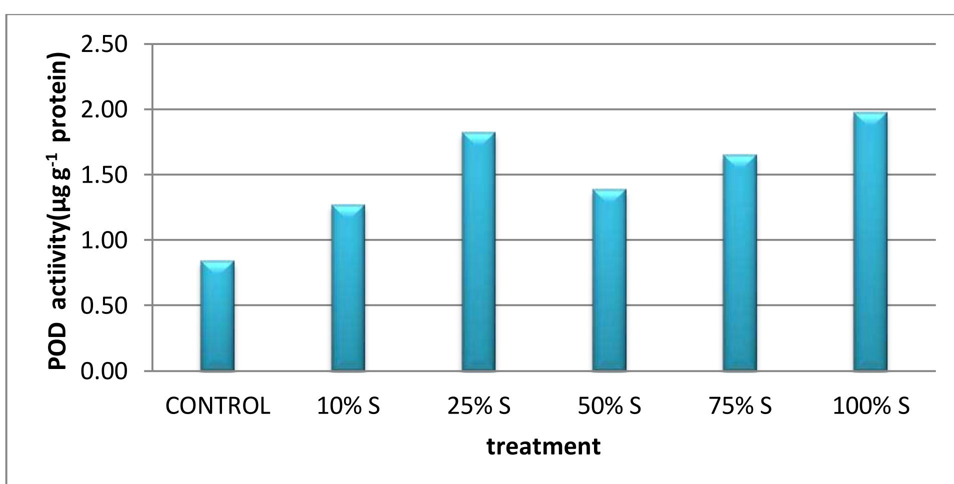

Fig 4.30 POD activity of coconut seedlings under various salinity treatments

4.3.8 Poly phenol oxidase

In plants, polyphenol oxidase (PPO) is involved in the oxidation of monophenols and/or odiphenols to o-quinones with the concomitant reduction of oxygen to water causing protein complexing and brown melanin pigments. The highest activity of poly phenol oxidase was observed in 100 % salinity treatment. The activity of the enzyme was significantly reduced as the stress reduced.

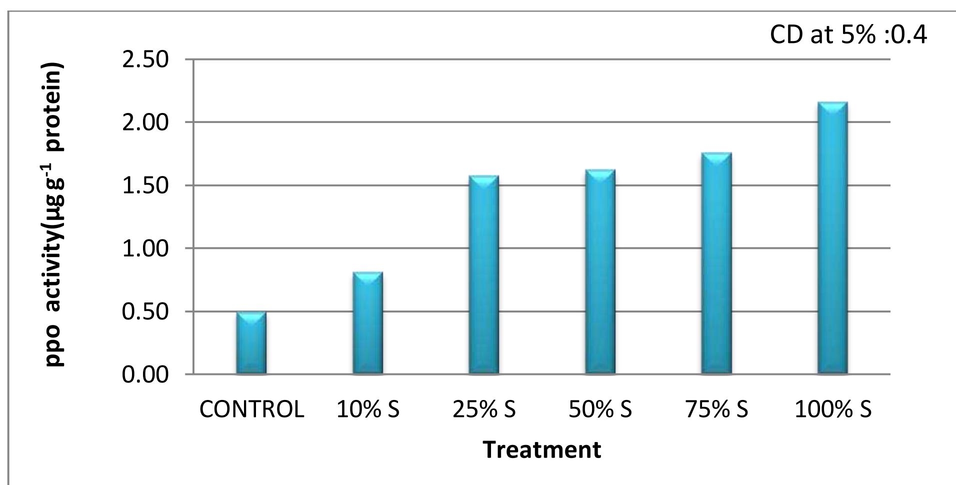

Fig 4.31 PPO activity of coconut seedlings under various salinity treatments

4.3.9 Membrane stability index

MSI was 80% in control plants and it decreased marginally at 10 and 25% S but was not significant. At 50% S it was reduced to 63% and was significant (Fig 4.32).

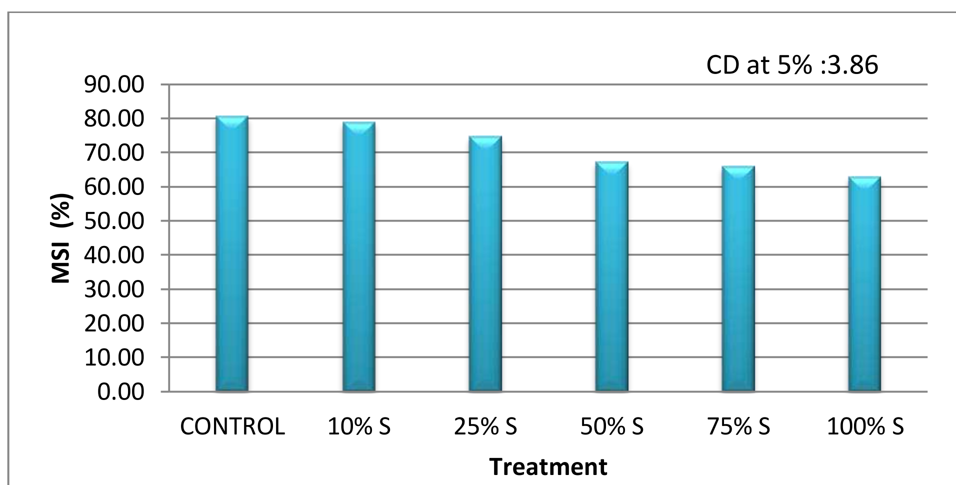

Fig 4.32 Membrane stability index of coconut seedlings under various salinity treatments

4.3.10 Relative water content

The control seedlings has the highest relative water content of (69.49 %). Then as the stress increased the relative water content reduced significantly. At 10% S the decrease was not significant. At 100 % salinity, the relative water content was only 55 .15.

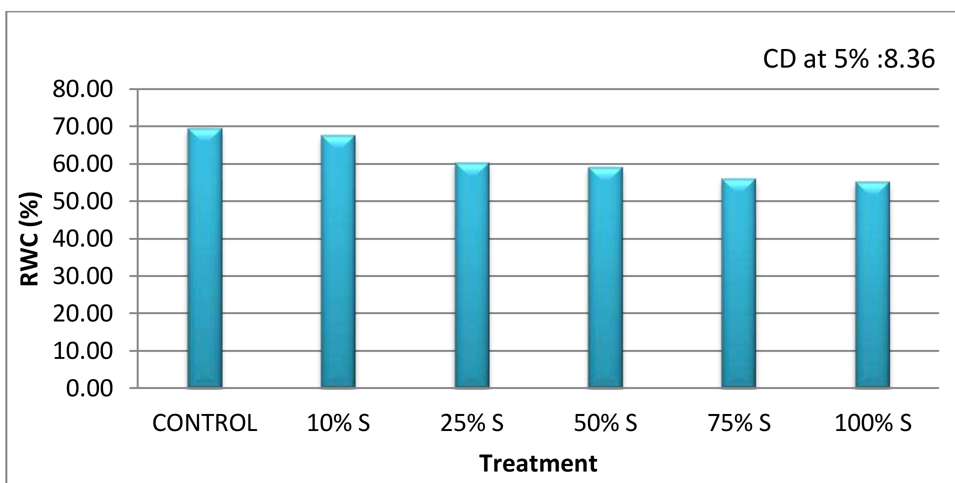

Fig 4.33Relative water content of coconut seedlings under various salinity treatments

4.3.11 Chlorophyll content

Chlorophyll content was 1.4 mg/g leaf tissue in control plants. It was on par at 10 and 25%S salinity treatments. But at 50% and beyond it significantly declined (Fig 4.34).

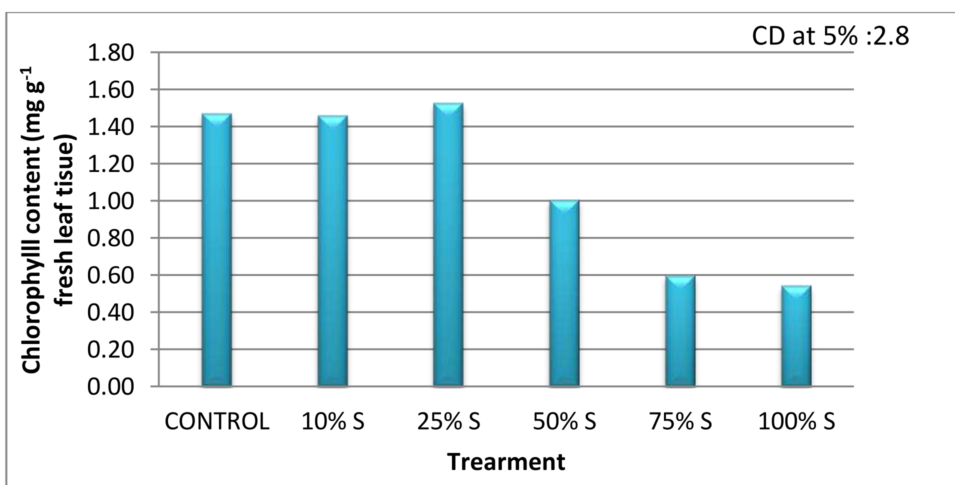

Fig 4.34 chlorophyll content of coconut seedlings under various salinity treatments

4.4 NUTRIENT ANAYSIS

The nutrient content of sea water, nutrient solution in the pots (initial and at the time of replacement), and the plant parts at the termination of experiment were quantified in order to study the nutrient uptake pattern in different treatments. Plant parts such as root , shoot , leaf and mid rib were analyzed .The results are presented below.

Table 4.7 Sea water analysis

Constituent	Composition of standard sea water (PPM) - Reference	Sample result (PPM)
Cl	19500	19258
Na	10500	10625
SO_4	2700	2799
Mg	1350	1344
Ca	410	400
K	390	395
HCO_3	142	146
B	4.35	4.1
P	0.09	0.08
Zn	0.01	0.009
Fe	0.003	0.002
Mn	0.002	0.002
Cu	0.003	0.004

The Arabian Sea water used in the experiment is rich in Cl, followed by Na and SO4 which is almost similar to the composition of standard sea water.

Nutrient analysis of plant parts

Nutrient content of root (Table 4.7), shoot (Table 4.8), leaf (Table 4.9), mid rib(4.10) and total plant uptake are presented below. Most of the major nutrients in roots were significantly declined with increasing salinity except S. Na content at 10%S was on par with control but at higher concentration it further increased (Table). Almost similar trend was seen in shoot but the decline in K was less and there was a significant increase in Na even at 10% S. Leaf K though significantly declined with 10%S but value was less and similarly the Na accumulation was relatively low compared to other plant parts. Majority of leaf macro and micro nutrients were more stable up to 10 %S beyond that they were significantly low. As a consequence of better compartmentalization of nutrients the total plant uptake of K and Na did not differ significantly up to 10%S beyond that it declined significantly.

Table .4.8 Root analysis

Treatment	K(%)	Na(%)	P(%)	S(%)	Ca(%)	Mg(%)	B(PPM)	Fe(PPM)	Mn(PPM)	Zn(PPM)	Cu(PPM)	N(PPM)
Control	1.88 ^{A}	1.07 ^{D}	0.81 ^{A}	0.21^{D}	0.64^{A}	0.384 ^{A}	0.002 ^{C}	449.4	75.58	80.20	29.30	4.61^{A}
10 % S	0.80 ^{B}	1.84 ^{D}	0.54 ^{B}	0.28^{DC}	0.53 ^{B}	0.320^{B}	0.002^{C}	524.26	70.56	108.77	17.14	3.64A^{B}
25% S	0.60 ^{C}	2.64 ^{B}	0.53^{B}	0.36^{DC}	0.45 ^{B}	0.256^{C}	0.004^{BC}	473.46	129.48	100.3	13.86	3.34^{BC}
50 %S	0.36^{D}	3.14 ^{B}	0.48 ^{B}	0.50 ^{B}	0.34 ^{C}	0.208^{C}	0.005^{B}	857.93	130.63	67.37	44.33	3.17^{BC}
75%S	0.23^{D}	4.15 ^{A}	0.44 ^{B}	0.86 ^{A}	0.18 ^{D}	0.128 ^{D}	0.005^{B}	905.06	60.6	72.47	40.26	2.98^{C}
100%S	0.10^{E}	4.02 ^{A}	0.49 ^{B}	0.44^{BC}	0.32 ^{C}	0.072 ^{D}	0.008 ^{A}	21.08	132.89	49.03	15.4	3.36^{BC}
CD at 5 %	0.15	0.62	0.22	0.15	0.086	0.054	22.43	NS	NS	NS	NS	
P value	<.0001	<.0001	0.0484	<.0001	<.0001	<.0001	0.0011	0.0121		0.4916	0.5223	0.0002

Table.4.9 Shoot analysis

Treatment	K(%)	Na(%)	P(%)	S(%)	Ca(%)	Mg(%)	B(PPM)	Fe(PPM)	Mn(PPM)	Zn(PPM)	Cu(PPM)	N(%)
Control	1.97^{A}	1.34^{D}	0.22^{A}	0.03^{C}	0.45^{A}	0.40^{A}	25.06^{C}	385.11	30.24^{CB}	36.67	4.74	3.73
10 % S	1.64^{B}	2.59^{C}	0.16^{B}	0.06^{C}	0.37^{B}	0.35^{B}	26.63^{C}	232.15	41.81^{AB}	30.18	6.71	3.58
25% S	1.22^{C}	3.29^{B}	0.12^{C}	0.10^{B}	0.29^{C}	0.20^{C}	30.53^{C}	328.34	44.07^{A}	36.69	4.48	4.01
50 %S	0.95^{D}	3.68^{AB}	0.121^{C}	0.13^{B}	0.24^{CD}	0.16^{D}	45.26^{B}	419.24	26.93^{C}	41.64	6.02	3.49
75%S	0.69^{E}	4.00^{A}	0.11^{C}	0.25^{A}	0.21^{ED}	0.14^{D}	61.00^{B}	249.68	23.88^{C}	26.51	7.74	3.34
100%S	0.54^{E}	2.01^{C}	0.04^{D}	0.13^{B}	0.16^{E}	0.09^{E}	31.93^{C}	263.68	25.74^{C}	19.21	2.94	3.41
CD at 5 %	0.23	0.61	0.034	0.038	0.064	0.038	7.17	NS	11.62	NS	NS	NS
P value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.29	0.01	0.41	0.42	0.35

Table 4.10 Leaf analysis

Treatment	K(%)	Na(%)	P(%)	S(%)	Ca(%)	Mg(%)	B(PPM)	Mn(PPM)	Zn(PPM)	Cu(PPM)	N
Control	1.74^{A}	0.86^{D}	0.23^{A}	0.03^{C}	0.42^{A}	0.33^{A}	15.80^{C}	248.97	22.32	6.92	2.74
10 % S	1.55^{B}	1.00^{C}	0.18^{B}	0.04^{C}	0.42^{A}	0.25^{B}	25.06^{BC}	127.63	17.39	6.62	2.48
25% S	1.42^{B}	1.10^{BC}	0.15^{BC}	0.10^{B}	0.32^{B}	0.22^{B}	30.36^{B}	154.22	23.05	8.40	2.93
50 %S	1.17^{C}	1.21^{B}	0.14^{C}	0.12^{B}	0.21^{C}	0.20^{B}	43.06^{A}	140.24	19.57	6.36	2.48
75%S	0.75^{D}	1.34^{A}	0.10^{D}	0.15^{A}	0.21^{C}	0.12^{C}	46.70^{A}	231.67	30.50	7.54	2.22
100%S	0.53^{E}	1.07^{C}	0.06^{E}	0.12^{B}	0.16^{C}	0.09^{C}	33.13^{B}	192.32	24.63	6.63	2.16
CD at 5 %	0.15	0.12	0.029	0.024	0.086	0.062	9.15	NS	NS	NS	NS
P value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0001	0.51	0.22	0.14	0.2022

Table 4.11 Mid rib analysis

Treatment	K(%)	Na(%)	P(%)	S(%)	Ca(%)	Mg(%)	B(PPM)	Fe(PPM)	Mn(PPM)	Zn(PPM)	Cu(PPM)
Control	1.98^{A}	1.48^{C}	0.15^{A}	0.03^{E}	0.15^{A}	0.22^{A}	17.26^{E}	343.62	24.12	18.62	5.23
10 % S	1.57^{B}	1.86^{BC}	0.13^{B}	0.05^{D}	0.13^{B}	0.16^{B}	24.03^{D}	243.21	34.19	29.58	6.06
25% S	1.24^{C}	2.08^{B}	0.12^{B}	0.07^{C}	0.12^{B}	0.14^{B}	29.76^{C}	328.34	39.98	30.78	5.54
50 %S	1.03^{CD}	2.79^{A}	0.10^{C}	0.11^{B}	0.10^{C}	0.09^{C}	34.26^{C}	419.24	25.72	17.80	4.96
75%S	0.86^{ED}	3.27^{A}	0.09^{C}	0.14^{A}	0.09^{C}	0.09^{C}	40.50^{B}	249.68	31.77	21.23	5.91
100%S	0.73^{E}	2.15^{B}	0.05^{D}	0.09^{B}	0.05^{D}	0.06^{C}	45.76^{A}	258.30	36.62	17.08	8.40
CD at 5 %	0.21	0.49	0.017	0.017	0.057	0.034	4.40	NS	NS	NS	NS
P value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.23	0.1374	0.5057	0.50

Table 4.12 Total nutrient uptake

Treatment	K(g)	Na(g)	P(g)	S(g)	Ca(%)	Mg(g)	B(g)	Fe(g)	Mn(g)	Zn(g)	Cu(PPM)	N(g)
Control	20.49^{A}	12.42^{AB}	3.08^{A}	0.54^{AB}	5.19^{A}	4.22^{A}	0.024^{A}	0.35^{A}	0.145	0.040^{A}	0.008^{A}	36.34^{A}
10 % S	14.85^{A}	18.54^{A}	1.99^{B}	0.74^{A}	4.08^{A}	3.10^{A}	0.025^{A}	0.25^{B}	0.081	0.032^{AB}	0.007^{AB}	30.81^{A}
25% S	7.240^{B}	13.94^{A}	1.12^{BC}	0.82^{A}	1.84^{B}	1.35^{B}	0.018^{AB}	0.19^{B}	0.053	0.0214^{CB}	0.004^{CB}	20.28^{B}
50 %S	2.711^{BC}	7.47^{CB}	0.40^{C}	0.40^{CB}	0.62^{B}	0.47^{B}	0.012^{CB}	0.10^{C}	0.018	0.009^{C}	0.0023^{C}	8.30^{C}
75%S	1.093^{C}	4.38^{C}	0.21^{C}	0.41^{CB}	0.32^{B}	0.21^{B}	0.008^{CB}	0.04^{C}	0.019	0.005^{C}	0.001^{C}	4.49^{C}
100%S	0.65^{C}	2.28^{C}	0.09^{C}	0.19^{C}	0.22^{B}	0.12^{B}	0.004^{C}	0.03^{C}	0.011	0.003^{C}	0.0006^{C}	3.94^{C}
CD at 5 %	5.64	5.83	1.03	0.30	1.61	1.39	0.00	0.07	NS	0.01	0.00	10
P value	<.0001	0.0006	0.0003	0.0083	<.0001	0.0001	0.0065	<.0001	0.068	0.0028	0.026	<.0001

Table 4.13 Water analysis (Initial nutrient content –final nutrient content)

Treatment	K(PPM)	Na(PPM)	S(PPM)	P(PPM)	B(PPM)	Fe(PPM)	Cu(PPM)	Ca(PPM)	Mg(PPM)	N(PPM)	Zn(PPM)	Mn	Cl(PPM)	Bicarbonate
Control	2694.66 ^{A}	1354.75 ^{B}	7864.69	239.96 ^{A}	2.95 ^{C}	17.74 ^{C}	0.06 ^{B}	2219.66 ^{A}	720.40 ^{A}	1627.54	10.44	6.61 ^{AB}	51090.41	333.67
10 % S	2447.33 ^{AB}	2945.75 ^{B}	18754.06	132.14 ^{B}	0.24 ^{C}	24.33 ^{B}	0.31 ^{B}	3199.00 ^{A}	704.80 ^{A}	2207.56	14.17	6.68 ^{A}	135565.625	302.56
25% S	1070.50 ^{CB}	1974.25 ^{B}	27568.13	81.15 ^{C}	0.88 ^{C}	15.21 ^{C}	0.17 ^{B}	753.66 ^{B}	548.60 ^{AB}	950.27	15.66	5.52 ^{CB}	724658.542	244
50 %S	295.91 ^{C}	3704.16 ^{B}	67442.34	34.65 ^{CD}	1.80 ^{C}	18.97 ^{C}	0.03 ^{B}	743.00 ^{B}	509.80 ^{AB}	464.89	12.19	5.20 ^{C}	154114.375	337.33
75%S	144.00 ^{C}	3602.08 ^{B}	105931.88	15.19 ^{D}	17.14 ^{B}	24.42 ^{B}	0.13 ^{B}	387.66 ^{B}	428.00 ^{B}	408.47	11.30	4.98 ^{C}	172219.375	176.9
100%S	57.16 ^{C}	6025.00 ^{A}	116550.00	4.43 ^{D}	65.55 ^{A}	39.66 ^{A}	0.64 ^{A}	450.66 ^{B}	342.00 ^{B}	465.22	8.829	3.35 ^{D}	181981.875	99.43
CD at 5 %	1457.0	2229.5	9721.7	46.0	13.4	4.1	0.3	13.4	228.3	1425.7	9.6	1.1	703266.9	426.5
P value	0.0057	0.0123	0.3420	<.0001	<.0001	<.0001	0.0086	0.0008	0.0261	0.0964	0.7024	0.0003	0.4244	0.1818

Plant Uptake of nutrients

4.4.1 Potassium

Fig.4.35 shows the potassium uptake by the plant across various treatments with the increase in salinity. K was around 20 g in control plants which was reduced to 14.55 g at 10% S and 7.8 g at 25%S. At 50% S and beyond K uptake was markedly low.

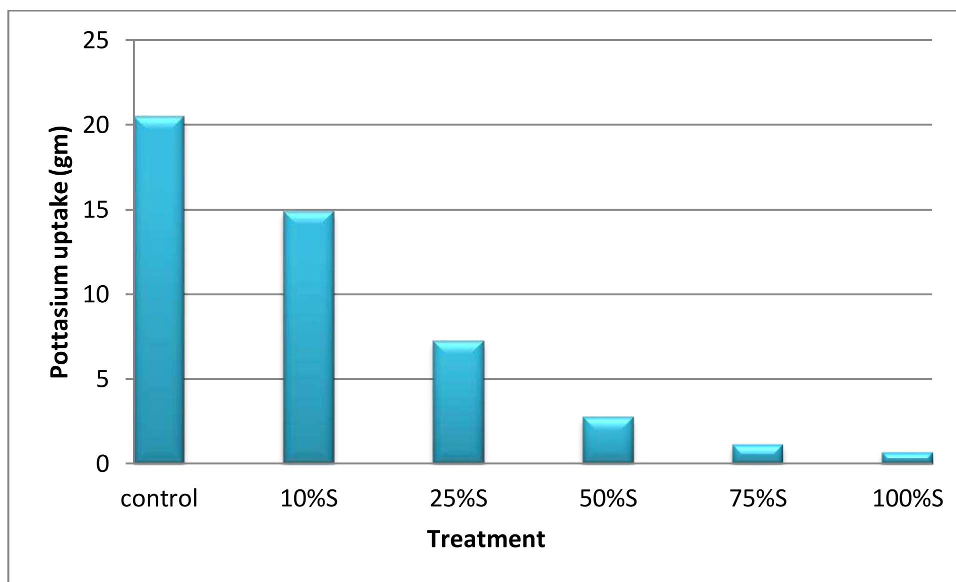

Fig 4.35 Potassium uptake of coconut seedlings under various salinity treatment

4.4.2 Sodium

Fig 4.36 shows the uptake of sodium across different salinity treatments. Uptake was high at 10% S (18.54 g) followed by 25%S (13.94 g) and control (12.42 g). At 50% and beyond it was markedly low.

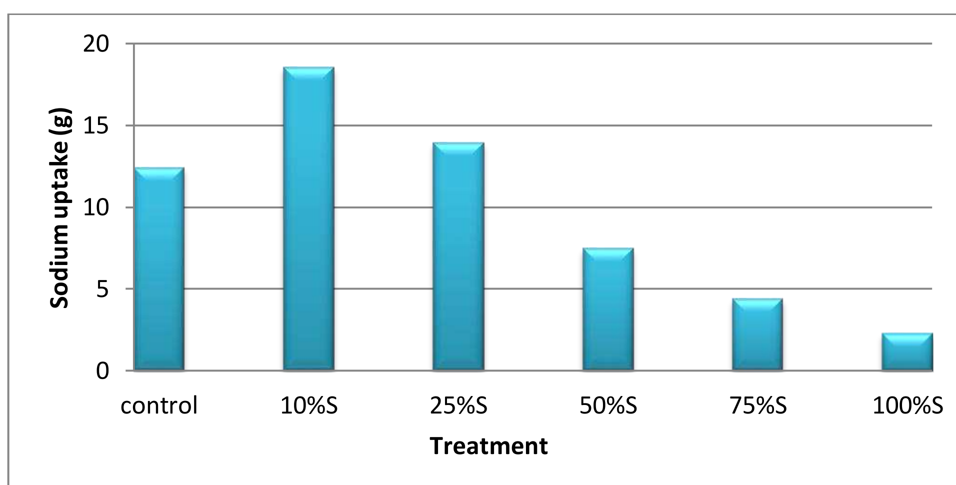

Fig 4.35 Sodium uptake of coconut seedlings under various salinity treatments

4.4.3 Phosphorus

The content of phosphorus, one of the major macro nutrient in the plant samples decreases significantly with the increasing salinity. Fig shows the P uptake of plants at different salinity levels. In control plants it was 3,0 g followed by 2.0 g at 10%S and 1.1 g at 25%S.

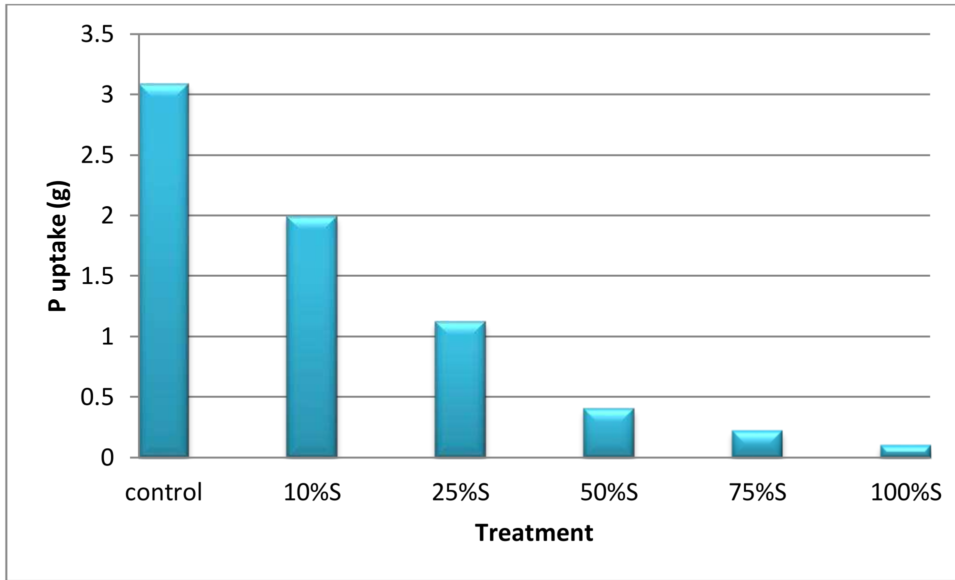

Fig.4.36 Phosphorus uptake of coconut seedlings under various salinity treatments

4.4.4 Sulphur

The content of sulphur is significantly increased with the salinity. The highest accumulation was found in 25 % salinity treatment (0.82 g) followed by 10 %. The content of sulphur was low in 100 % salinity treatment(0.22 g) , when compared to 75 % salinity treatment(0.32), similar in the case of sodium . This may be because of the seedlings wilted before the termination of treatment.

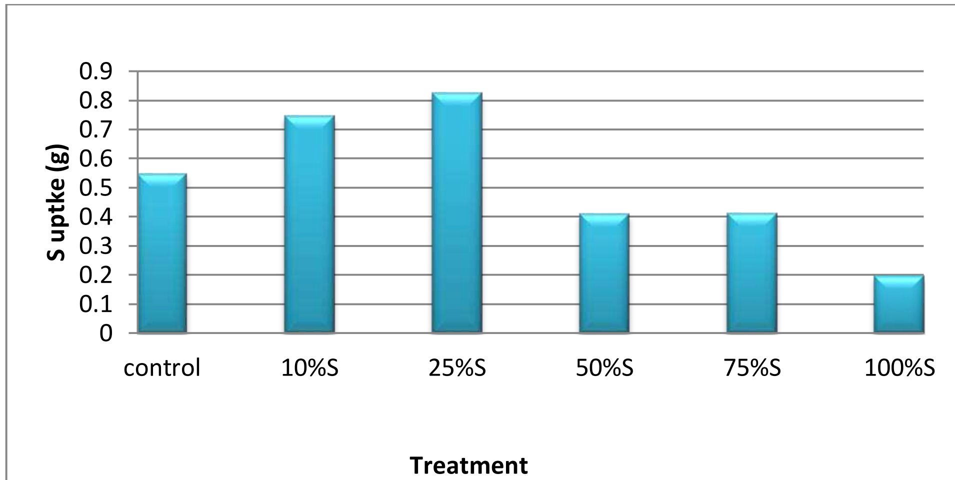

Fig 4.37 Sulphur uptake coconut seedlings under various salinity treatments

4.4.5 Calcium

The uptake of calcium was significantly reduced as the salinity increased. The highest content of this macro nutrient was recorded in the control seedlings (5.19 g) followed by 10% salinity treated seedlings(4.08 g) and in 25% salinity treated seedlings it was (1.84 g).

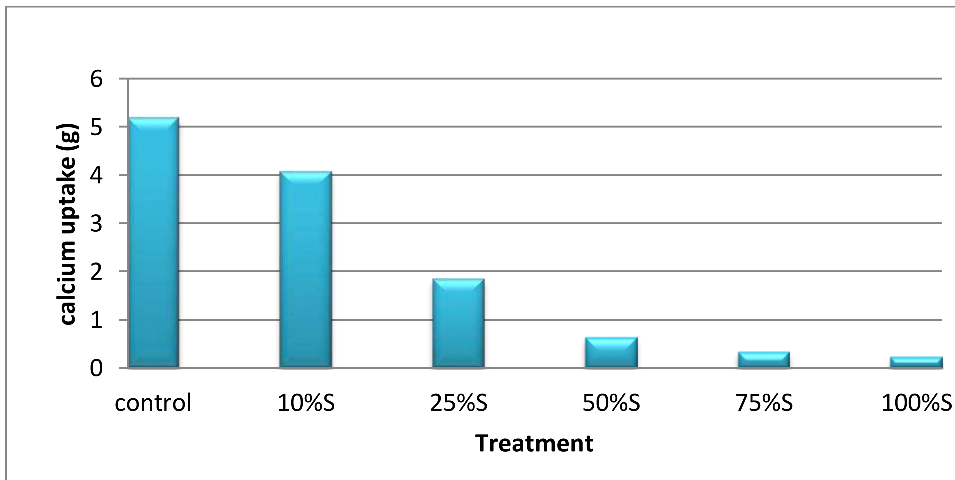

Fig 4.38 Calcium uptake of coconut seedlings under various salinity treatments

4.4.6 Magnesium

The uptake of magnesium was also significantly reduced with the increasing salinity. The maximum uptake was recorded in control seedlings (4.2gm) followed by 10 % salinity treated seedlings (3.10 g) and 25% salinity treated seedlings (1.35gm).

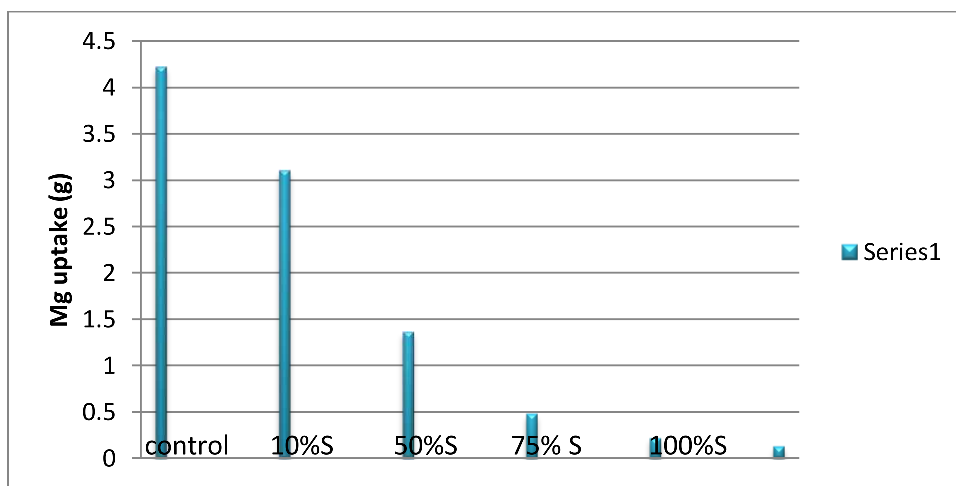

Fig 4.40 Magnesium uptake of various plant parts such as root, shoot, mid rib of coconut seedlings under various salinity treatment

4.4.7 Nitrogen

The nitrate uptake was also decreased with the increased salinity. The uptake was maximum in control seedlings (36.34gm) and 10 % salinity treatment (30.81gm) and the uptake was significantly reduced at 25% salinity treatment (20.28gm) and beyond.

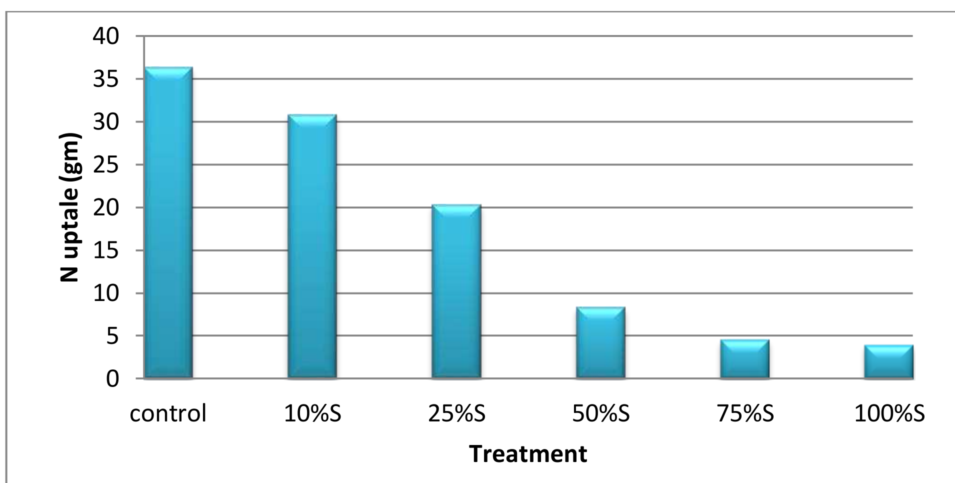

Fig 4.41 Nitrogen uptake of various plant parts such as root, shoot, mid rib of coconut seedlings under various salinity treatments

4.4.8 Boron

The boron uptake was also decreasing with the increased salinity. The maximum uptake was recorded in control seedlings (0.024 g) followed by 10% salinity treated seedlings(

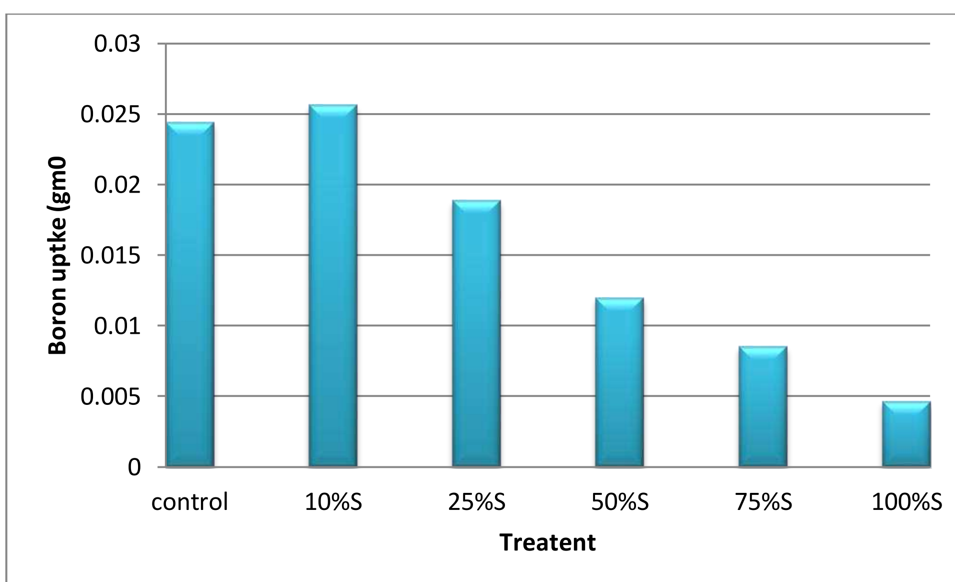

Fig 4.42 Boron uptake of coconut seedlings under various salinity treatments

4.4.9 K/Na Ratio

The K^+ content in root, shoot and leaf was 1.88, 1.97 and 1.74%, respectively. Sea water treatment significantly declined the K^+ content of root ($p<.0001$), shoot ($p<.0001$) and leaf ($p<.0001$). In comparison with the leaf of the control seedlings, leaves subjected to 10% S, K^+ content was reduced by 57% in root, 17% in shoot and only 11% in leaf. On the other hand there was significant increase in Na^+ accumulation, 72% in root ($p<.0001$), 93% in shoot ($p<.0001$) and only 16% in leaf ($p<.0001$) at 10% S. With the increasing sea water substitution there was significant and steep decline of K^+ content in root and shoot while the decrease was marginal in leaf and it was vice versa for Na^+ content. Consequently, the K^+/Na^+ ratio was high in leaf in all the sea water treatments followed by shoot and was the least in roots (Fig.4.43).

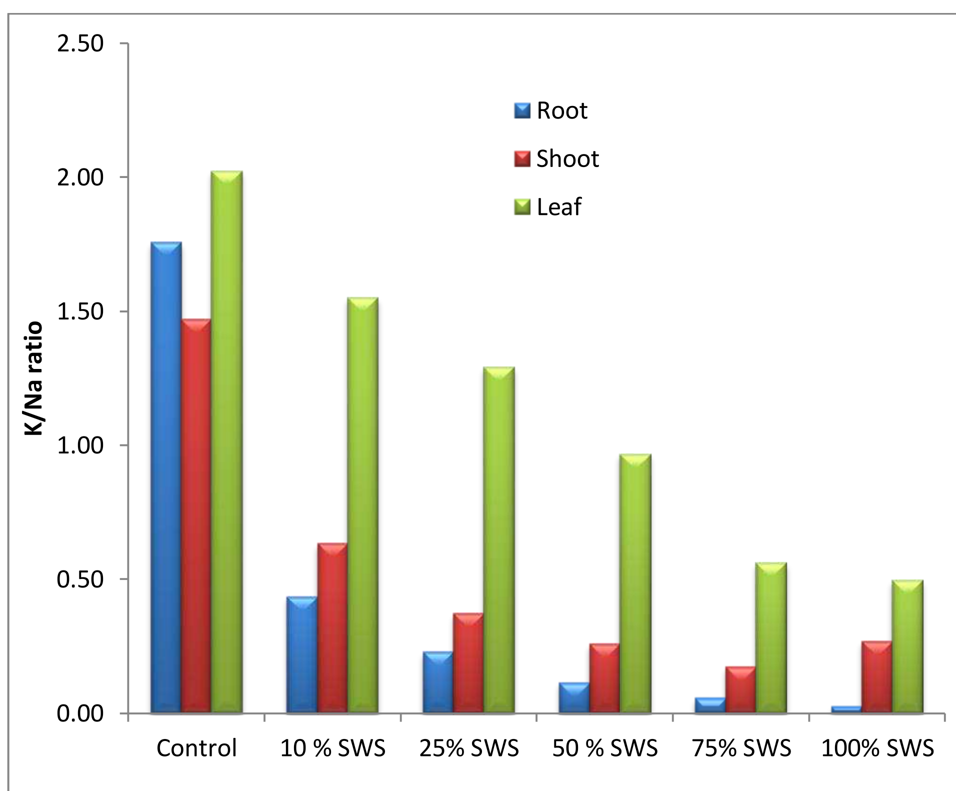

Fig.4.43 K/Na ratio of root, shoot and leaf of coconut seedlings with increasing level of sea water substitution

CHAPTER 5

DISCUSSION

Coconut is largely grown in coastal belts and islands (Cogent 2017) led to believe that it is salt tolerant and hence expected that the damage by either sea water inundation or contamination of subsurface/underground water due to rising sea level under climate change would be minimal. This is mainly because there are no systematic studies delineating the response of coconut to either inundation or contamination by the sea water. In this study we made an attempt to assess the response of coconut seedlings to sea water in a hydroponic system. The water collected from Arabian sea which was used in the experiment comprised mainly of Cl^-, Na^+, SO_4^{2-}, Mg^{2+} etc. and their concentration was well within the range of the composition of the reference sea water (Millero et al. 2008). The pH of the sea water was slightly alkaline (7.63) and EC was 53.78 d Sm^{-1}, the value is in conformity with earlier reports.

To investigate the response of coconut to sea water, we chose treatments of a wider range involving control (100% Hoagland solution), followed by 10%, 25%, 50%, 75% and 100% substitution of Hoagland solution by sea water which was equivalent to 2.17, 8.32, 16.32, 30.03, 42.14 and 53.69 dSm^{-1} EC respectively. One strength Hoagland's nutrient solution has EC of is 2.0 dSm^{-1} (Jong and Lersel 2002), but in our control pots it was 2.17, due to the possible contribution of ions from the irrigation water used for the preparation of Hoagland's nutrient solution. Further, this is the first report of salt tolerance of coconut seedlings that was conducted in a controlled hydroponic system that eliminates the confounding effects of drought and limited nutrients. Coconut responded very well to hydroponic system and the growth was comparable to the growth of seedlings of similar age in potted plants raised in soil (Hebbar *et al*2013).

Exposing coconut seedlings to 100%, 75% and 50% sea water severely injured the root and above ground parts. At the earliest observation made on 24

DAT, some of the morphological parameters like root length, leaf area, collar girth and fresh weight of whole plant started showing severe decline due to faster senescence of existing and slow emergence of fresh roots and leaves. Seedlings could survive 50% S without any additional increase in accumulation of fresh weight. On the other hand at 10% S which corresponds to EC of 8.32 dSm^{-1} all the growth parameters and biomass accumulation was similar to the control seedlings. New leaf emergence, leaf area expansion and growth were normal. In earlier studies conducted either in salt affected fields or potted plants and through application of saline water it was observed that full establishment of dwarf coconut seedlings happened at 6.5 d S m-1 (Silva *et al*2017) and 5.2 dSm-1 (Lima et al. 2017), beyond that growth is suppressed. Nevertheless, in this study we demonstrated that there was no significant effect on growth and biomass accumulation of coconut seedlings when sea water substitution raised the EC upto 8.32 dSm^{-1}. However, seedlings grown at 25% sea water substitution (EC 16.32 dSm^{-1}) had 47% decline in biomass, which is in agreement with earlier findings (Lima et al. 2017). In these plants root elongation rate and also root weight was on par with control or 10% S, but shoot growth increased at a decreasing rate which resulted in 47% less dry mass as that of control. It is also evident from biomass fraction in different parts, at 25% S it was 0.11:0.45:0.43 and at control or 10% S it was 0.07:0.45:0.47 in roots: shoot: leaf respectively, suggesting greater allocation of biomass to roots under salinity could be an adaptive mechanism in coconut. Thus, the results further corroborate the plasticity in the root architecture of coconut seedlings in countering the severe abiotic stresses(Silva et al. 2017). An important morphological response of seedlings to the salinity stress is its greater decrease in the total leaf area and during initial stages of stress a substantial decrease in the leaf growth was also observed (Munns and Termaat 1986) By reducing the leaf area, seedlings or plants effectively utilize a drought avoidance mechanism of reducing the water loss due to transpiration. As a result of this mechanism, greater accumulation of toxic ions in the roots of plants under stress was observed thereby reducing the accumulation of these toxic ions in the aerial plant parts.

Leaf water potential did not differ significantly up to 25% S from control. However there was a significant decline in stomatal conductance at 10% S, while the stomatal resistance increased to 5.4 s cm^{-1} as against 1.88 in control, though the difference was non-significant, implying the induction of stomatal closure in coconut without a decline in leaf water potential, supporting the alternately held view that stress induced signals generated in roots (could be either chemicals like ABA or electrical potential) might have regulated stomatal movement in coconut with salinity (Zhang and Davies 1991; Hebbar *et al*1994). From the data it is clear that the stomatal closure prevented the transport of water vapor and CO_2, and thus photosynthesis and transpiration decreased significantly. [P_N] decreased by 19 and 43% at 10% S and 25% S, respectively. However, at 10% S [P_N] did not significantly influence the biomass production as the reduction was not significant. Chlorophyll index (CI) a measure of chlorophyll content and chlorophyll fluorescence did not vary significantly up to 25% S suggesting the maximum quantum yield of PSII (Fv/Fm) was not significantly affected. It was deduced that photochemical and biochemical processes of photosynthesis, leaf area reduction, stomatal closure and loss of chlorophyll are attributed to the decreased biomass or carbon assimilation capacity of the plants (Taiz et al.2015, Medeiros et al. 2018). Another important adaptive mechanism of salt tolerance is substantial reduction of stomatal conductance so as to maintain salt concentration of leaves at a sub-toxic level for relatively long duration than would occur in the absence of reduction of transpiration rates (Koyoro 2006).

Leaf total sugar and reducing sugar as osmotic solutes did not increase significantly. Total phenols, antioxidants and free radical scavenging enzymes like SOD and PPO increased significantly with sea water treatment but their protective role was minimal beyond 25% S. The uptake of K by plants was severely hindered in the presence of sea water, and hence its content declined significantly in different plant parts ($p<.001$). On the other hand Na^+ content increased significantly ($p<.0001$). Though earlier workers reported positive response of coconut to small quantity of NaCl application (Manciot *et al*1979),

but in general Na^+ could substitute K^+ only to a certain extent because at high Na concentrations cause deleterious effect and it cannot perform the role of K^+ (Hebbar et al. 2000). Excess accumulation of intracellular Na^+ has detrimental effects *in vivo*such as stimulation of cytosolic K^+ efflux causing severe imbalances in the cellular homeostasis, oxidative stress responsiveness, hindrances in the intracellular functions of Ca^{2+} and K^+ ions, leading to deficiency in the nutrient contents, and impaired growth and death of plant cells (Tester and Davenport 2003, Munns and Tester 2008, Craig Plett and Møller 2010, Cabot et al. 2014). A key salt tolerance trait is relatively high cytosolic K^+/Na^+ ratio hence plants regulate the Na^+ uptake and transport during salinity stress with an ultimate objective of maintaining high tissue K^+/Na^+ ratios (Shabala and Pottosin, 2014). In coconut most of the Na taken up by the plant in sea water treatment is accumulated in root and shoot and very little is transported to leaves while maximum K was transported to leaves resulted in maintenance of balanced K/Na ratio in leaves which may be a salinity tolerance mechanism in coconut **.**

CHAPTER 6

SUMMARY

An experiment was conducted at CPCRI Kasaragod to study the response of coconut variety Malayan Green dwarf (MGD) to salinity. The observations on morphological, physiological and biochemical parameters were recorded for seven months. In this study we imposed the sea water treatments in hydroponically grown coconut seedlings of variety MGD (Malayan green dwarf) and monitored the morphology and physiological parameters.

The salient findings are summarized as follows:

Stomatal parameters were more sensitive to salinity in coconut. This was reflected in significant reduction in photosynthesis even at 10%sea water . Photosynthesis (Pn) was significantly reduced to 34%, 54% and 91% at sea water substitution of 10%, 25% and 50% respectively. Leaf water potential was not affected up to 25%. The biomass accumulation of control and 10% sea water substitution were not significantly different. But at 25% sea water treatment, biomass accumulation reduced to 46% and beyond 50% sea water substitution it shows more than 70% reduction in biomass. Root number, collar girth, leaf number and leaf area were decreased with increasing sea water substitution level. The number of roots was more sensitive to the salinity than the maximum root length. The results indicate that at 25 % sea water substitution and beyond morphological, physiological and biochemical parameters were significantly affected. From the present study it is clear that coconut seedlings could moderately tolerate the substitution of sea water up to 10% and at 25% salinity there was 47% decrease in biomass. Hence, in future experiments there is a need to study the response at narrow ranges of sea water substitution. Further experiments are also need to be conducted to study the response of tall varieties which are known to have better adaptability to abiotic stresses.

As one third of coconut is grown in coastal areas mainly in Islands any increase in sea water levels owing to climate change and its consequences like inundation or contamination of underground water with salinity will affect coconut growth and productivity. In some of the west coast of India coconut farmers are already facing the ground water contamination with saline water. Therefore identification of salt tolerant phenotypic traits and its utilization either to breed adaptive genotypes or evolve suitable agronomic management practices so as to minimize the losses under salinity is vital.

REFERENCES

Acosta-Motos, J.R., Díaz-Vivancos, P., Álvarez, S., Fernández-García, N., Sánchez-Blanco, M.J.,and Hernández, J.A.2015.NaCl-induced physiological and biochemical adaptative mechanism in the ornamental Myrtus cummunis L. plants. *J. Plant Physiol.*183: 41–51.

Acosta-Motos, J.R., Díaz-Vivancos, P., Álvarez, S., Fernández-García, N., Sánchez-Blanco, M.J., and Hernández, J.A.2015.Physiological and biochemical mechanisms of the ornamental Eugeniamyrtifolia L. plants for coping with NaCl stress and recovery.. *Planta*.242: 829–846.

Adams, P., Thomas, J., Vernon, D., Bohnert, H., and Jensen, R.1992. Distinct cellular and organismic responses to salt stress. *Plant.Cell Physiol.*33:1215-1223.

Agarwal, S. and Joshi, G., 2002. Effect of salinity stress on the growth and nitrate reductase activity of Lens culinaris seedlings. *Ann. agr. Res*, *2*..322-326.

Agastian, P., Kingsley, S.J. and Vivekanandan, M., 2000. Effect of salinity on photosynthesis and biochemical characteristics in mulberry genotypes. *Photosynthetica*.*38*(2):287-290.

Ahmad, P., Sarwat, M. and Sharma, S., 2008. Reactive oxygen species, antioxidants and signaling in plants. *J.Plant Biol*, *51*(3):167-173.

Apak, R., Güçlü, K., Özyürek, M. and Karademir, S.E. 2004. Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. *J.agri. food .chem.52*(26):7970-7981.

Aquino., Alexandre, J., and Silva., de .2007. Growth, dry matter partition and Na^+, K^+ and Cl^- retention in two sorghum genotypes irrigated with saline water. *Rev. Bras. Ciênc. Solo* .31:(5)961-971

Asada, K. 1994. Production and action of active oxygen species in photosynthetic tissues. *Causes of photooxidative stress and amelioration of defense systems in plants*, pp.77-104.

Ashra, M.2001. Relationships between growth and gas exchange characteristics in some salttolerant amphidiploid Brassica species in relation to their diploid parents. *Environ. Exp. Bot.* 45(2):155-163.

Ashraf, M, and Khanum, A.1997.Relationship between ion accumulation and growth in two spring wheat lines differing in salt tolerance at different growth stages. *J. Agron and Crop Sci.* 178:39-52

Ashraf, M. and O'leary, J.W., 1996. Responses of some newly developed salt- tolerant genotypes of spring wheat to salt stress: 1. Yield components and ion distribution. *J.Agron.Crop Sci*, 176(2): 91-101.

Ashraf, M. and Tufail, M. 1995. Variation in salinity tolerance in sunflower (Helianthus annum L.). *J . Agron.Crop Sci*, *174*(5).351-362.

Ashraf, M. 1994.Organic substances responsible for salt tolerance in Eruca sativa. *Biologia Plantarum*.36(2):255-259.

Ashraf, M.Y. and Wu, L.1994. Breeding for salinity tolerance in plants. *Critical Reviews in Plant Sci* .13(1).17-42.

Ashraf,M. 1994. Organic substances responsible for salt tolerance in Eruca sativa. *Biol. Plant*. 36 (2) 255–259.

Ashraf., M. and Harris.P.G.C. 2004. Biochemical indicators of salinity tolerance in plant. *Plant Sci.* 166:3-16

Aydi, S., Sassi, S. and Abdelly, C. 2008. Growth, nitrogen fixation and ion distribution in Medicago truncatula subjected to salt stress. *Plant and Soil*.312(1-2):.59.

Aziz, I. and Khan, M.A. 2001. Experimental assessment of salinity tolerance of Ceriops tagal seedlings and saplings from the Indus delta, Pakistan. *Aquatic Botany*, *70*(3), pp.259-268.

Basu, S., Sarkar, S.N., SenGupta, D.N. and Roy-Choudhury, A. 2008. Comparative physiological and molecular responses of a common aromatic indica rice cultivar to high salinity with non-aromatic indica rice cultivars.

Beauchamp, C. and Fridovich, I.. 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Anal. biochem. 44*(1).276-287.

Bellard, C., Bertelsmeier, C., Leadley P., Thuiller, W, Courchamp F .2012. Impacts of climate change on the future of biodiversity. *Ecol Lett* 15:365–377

Bellard, C., Leclerc, C., Leroy, B., Bakkenes, M., Veloz, S., Thuiller, W. and Courchamp, F., 2014. Vulnerability of biodiversity hotspots to global change. *Global Ecol and Biogeography*, *23*(12), pp.1376-1386.

Benzie, I.F. and Strain, J.J., 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal.biochemi. 239*(1).70-76.

Bhandal, I.S. and Malik, C.P., 1988. Potassium estimation, uptake, and its role in the physiology and metabolism of flowering plants. In *International review of cytology* (Vol. 110, pp. 205-254). Academic Press.

Bohnert, H., Nelson, D., and Jensen, R.1995).Adaptations to environmental stresses.*Plant Cell* 7:1099-1111.

Bolarian, M., Fernandez, F., Cruz,V.,and Cuartero,J. 1991. Salinity tolerance in four wild tomato species using vegetative yield-salinity response curves. *J. A. Soc. Hortic.Sci*. 116: 286–290.

Bray,H.G. and Thorpe, W.V. 1954. Analysis of phenolic compounds of interest in metabolism.*Methods in Biochem Anal*.27-52.

Bunce, J.A. How do leaf hydraulics limit stomatal conductance at high water vapour pressure deficits?. 2006. *Plant Cell Environ*. 29: 1644–1650.

Cabot, C., Sibole, J.V., Barceló, J. and Poschenrieder, C., 2014. Lessons from crop plants struggling with salinity. *Plant Sci*, *226*, pp.2-13.

Carden D.E., Walker D.J., Flowers T.J. & Miller A.J.2003. Single- cell measurements of the contributions of cytosolic Na+ and K+ to salt tolerance. *Plant Physiol*. 131: 676–683.

Chempakam, B., Kastui Bai, K.V. and Rajagopal, V. 1993. Lipid peroxidation in relation to drought tolerance in coconut (*Cocos nucifera* L.). *J. Plant Physiol and Biochem.* 20(1).5-10.

Chen, Y.F., Ai, C.X., Lin, Q.W., Xu, H. and Shen, Y.H., 2007. Effect of salinity stress on the activities of phenoloxidase and superoxide dismutase of the serum tissue and organ of mud crab, Scylla serrata. *J. Ocean. Taiwan Strait*, *26*(4) .567.

Cochard, H., Coll, L., Roux, X.L., and Ameglio, T. Unraveling the effects of plant hydraulics on stomatal closure during water stress in walnut. 2002.*Plant Phys*. 128: 282–290.

COGENT. 2017. A Global Strategy for the Conservation and Use of Coconut Genetic Resources, 2018-2028. (R. Bourdeix and A. Prades, compilers). Bioversity International, Montpellier, France.

CRAIG PLETT, D.A.R.R.E.N. and Møller, I.S., 2010. Na+ transport in glycophytic plants: what we know and would like to know. *Plant. Cell. Environ*, *33*(4), pp.612-626.

Craigpellet, D.A.R.R.E.N. and Møller, I.S., 2010. Na+ transport in glycophytic plants: what we know and would like to know. *Plant, cell . environ*, *33*(4), pp.612-626.

Cram, W.J., 1976. Negative feedback regulation of transport in cells. The maintenance of turgor, volume and nutrient supply. In *Transport in plants II* (pp. 284-316). Springer, Berlin, Heidelberg.

Cramer, G.R. 2002.Sodium–calcium interactions under salinity stress. In Salinity: Environment–Plants–Molecules; Läuchli, A., Lüttge, U., Eds.; Kluwer: Dordrecht, The Netherlands, pp. 205–227. 74.

Cramer, G.R., Läuchli, A. and Polito, V.S., 1985. Displacement of Ca2+ by Na+ from the plasmalemma of root cells: A primary response to salt stress?. *Plant physiol. 79*(1):.207-211.

Crowe, J.H., Crowe, L.M. and Chapman, D., 1984. Preservation of membranes in anhydrobiotic organisms: the role of trehalose. *Sci*, *223*. pp.701-703.

Cushman, J.C., Meyer, G., Michalowski, C.B., Schmitt, J.M. and Bohnert, H.J., 1989. Salt stress leads to differential expression of two isogenes of phosphoenolpyruvate carboxylase during Crassulacean acid metabolism induction in the common ice plant. *Plant Cell*, *1*(7), pp.715-725.

Dajic ,Z.2006.Salt Stress//Physiology and Molecular Biology of Stress Tolerance in Plants/ Rao,M. K.V., Raghavendra ,A.S., Reddy.,J. K., Dordrech.t(Eds): Springer-Verlag. pp: 41-99

de Lacerda, C.F., Cambraia, J., Oliva, M.A., Ruiz, H.A. and Prisco, J.T., 2003. Solute accumulation and distribution during shoot and leaf development in two sorghum genotypes under salt stress. *Environ.Exp Botony*, *49*(2), pp.107-120.

de Medeiros, W.J.F., de Oliveira, F.Í.F., de Lacerda, C.F., de Sousa, C.H.C., Cavalcante, L.F., da Silva, A.R.A. and da Silva Ferreira, J.F., 2018. Isolated and combined effects of soil salinity and waterlogging in seedlings of 'Green Dwarf'coconut. *Semina:s*, *Agric.sci39*(4), pp.1459-1468.

Dietz, K., Tavakoli, N., Kluge, C., Mimura ,T., Sharma ,S., Harris ,G., Chardonnens ,A., and Golldack, D. 2001. Significance of the V-type ATPase for the adaptation to stressful growth conditions and its regulation on the molecular and biochemical level. *J.Exp. Bot.*52:1969-1980

Dietz, K.J., Tavakoli, N., Kluge, C., Mimura, T., Sharma, S.S., Harris, G.C., Chardonnens, A.N. and Golldack, D., 2001. Significance of the V- type ATPase for the adaptation to stressful growth conditions and its regulation on the molecular and biochemical level. *J.Exp.Bot*, *52*(363), pp.1969-1980.

Dionisio-Sese, M.L. and Tobita, S., 1998. Antioxidant responses of rice seedlings to salinity stress. *Plant science*, *135*(1):1-9.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P. A. and Smith, F. 1956. Colorimetric method for determination of sugars and related substances. *J. Analy Chem*. 28:350-356.

Feigin, A., 1985. Fertilization management of crops irrigated with saline water. In *Biosalinity in Action: Bioproduction with Saline Water* (pp. 285-299). Springer, Dordrecht.

Ferreira Neto, M., Gheyi, H. R.,Fernandes, P. D.,Holanda., J. S .,and Blanco, F. F. Leaf emission ,ionic relations and production of coconut palms irrigated with saline water. *Rural sci* 37 6 PP 1675 -1681

Flexas, J., Bota, J., Galmes, J., Medrano, H. and Ribas- Carbó, M., 2006. Keeping a positive carbon balance under adverse conditions: responses of photosynthesis and respiration to water stress. *Physiologia Plantarum*, *127*(3), pp.343-352.

Flexas, J.,Bota, J., Loreto, F., Cornic, G., and Sharkey, T.D. 2004. Diffusive and metabolic limitations to photosynthesis under drought and salinity in C3 *plants. Plant Biol*., 6: 269–279.

Flowers, T., Troke, P., and Yeo., A.1977.The mechanism of salt tolerance in halophytes. *Annual Rev of Plant Physiol* 8:89-121.

Garcia, A.B., Engler, J.D.A., Iyer, S., Gerats, T., Van Montagu, M. and Caplan, A.B., 1997. Effects of osmoprotectants upon NaCl stress in rice. *Plant physiol*, *115*(1).159-169.

Garg, B.K. and Gupta, I.C., 1997. *Saline wastelands environment and plant growth*. Scientific Publishers.

Garg, B.K., Vyas, S.P., Kathju, S., Lahiri, A.N., Mali, P.C. and Sharma, P.C., 1993. Salinity- fertility interaction on growth, mineral composition and nitrogen metabolism of Indian mustard. *J. Plant Nutrition*, *16*(9), pp.1637-1650.

Ghoulam, C., Foursy ,A., and Fares, K.2002. Effects of salt stress on growth, inorganic ions and proline accumulation in relation to osmotic adjustment in five sugar beet cultivars. *Environ.Exp. Bot*. 47:39-50

Gómez-Bellot, M.J., Álvarez, S., Castillo, M., Bañón, S., Ortuño, M.F., Sánchez-Blanco, M.J. Water relations, nutrient content and developmental responses of Euonymus plants irrigated with water of different degrees of salinity and quality. 2013. *J. Plant Res*. 126: 567–576.

Gosset D.R., Banks S.W., Millholon E.P., Lucas C. 1996. Antioxidant response to NaCl stress in control and an NaCl-tolerant cotton line grown in the presence of paraquat, buthionine sulfoximine and exogenous glutathione. *Plan Physiol*. 112: 803–809.

Gould, K.S., McKelvie, J. and Markham, K.R., 2002. Do anthocyanins function as antioxidants in leaves? Imaging of H2O2 in red and green leaves after mechanical injury. *Plant, Cell.Environ. 25*(10):1261-1269.

Grattan , S.R. and Grieve ,C.M.1999. Salinity-mineral nutrient relations in horticultural crops. *Sci Horti* .78:127–135.

Greenway, H and Munns, R.1980.Mechanisms of salt tolerance in nonhalophytes. *Annual Rev of Plant Physiol* 31:149-190

Gulzar, S., Khan, M.A. and Ungar, I.A., 2003. Salt tolerance of a coastal salt marsh grass. *Commun. Soil Sci. Plant Anal*, *34*(17-18), pp.2595-2605.

Halliwell, B and Gutteridge, J.M., 1989. 1 Iron toxicity and oxygen radicals. *Bailliere's clinic. haematol*, *2*(2), pp.195-256.

Hanen, F., Ksouri, R., Megdiche, W., Trabelsi, N., Boulaaba, M. and Abdelly, C., 2008. Effect of salinity on growth, leaf-phenolic content and antioxidant scavenging activity in Cynara cardunculus L. In *Biosaline agriculture and high salinity tolerance* (pp. 335-343). Birkhäuser Basel.

Hanson, A.D. and Burnet, M., 1994. Evolution and metabolic engineering of osmoprotectant accumulation in higher plants. In *Biochemical and cellular mechanisms of stress tolerance in plants* (pp. 291-302). Springer, Berlin, Heidelberg.

Hebbar, K.B., Balasimha, D. and Thomas, G.V., 2013. Plantation crops response to climate change: coconut perspective. In *Climate-Resilient Horticulture: Adaptation and Mitigation Strategies* (pp. 177-187). Springer, India.

Hebbar, K.B., Sashidhar, V.R., Udayakumar, M., Devendra, R. and Rao, R.N., 1994. A comparative assessment of water use efficiency in groundnut (Arachis hypogaea) grown in containers and in the field under water-limited conditions. *The Journal of Agricultural Science*, *122*(3), pp.429-434.

Hebbar, K.B., Venugopalan, M.V. and Rao, M.R.K., 2000. Effect of salinity on cotton growth and development: sodium cannot substitute for potassium in cotton. *J. Plant Biol*, *27*(3), pp.271-276.

Hem, J.D., 1985. *Study and interpretation of the chemical characteristics of natural water* (Vol. 2254). Department of the Interior, US Geological Survey.

Hernandez, J., Olmos, E., Corpas, F., Sevilla, F.,and Del Rio L.1995. Salt-induced oxidative stress in chloroplasts of pea plants. *Plant Sci*. 105:151-67.

Hirpara, K.D., Ramoliya, P.J., Patel, A.D. and Pandey, A.N., 2005. Effect of salinisation of soil on growth and macro-and micro-nutrient accumulation in seedlings of Butea monosperma (Fabaceae). In *Anales de biología* . 27, pp. 3-14.

Hounsa, C.G., Brandt, E.V., Thevelein, J., Hohmann, S. and Prior, B.A., 1998. Role of trehalose in survival of Saccharomyces cerevisiae under osmotic stress. *Microbiol*, *144*(3).671-680.

IPCC (2018). Summary for Policymakers. In IPCC, M. Allen, M. Babiker, Y. Chen, H. Coninck, S. Connors, et al. (Eds.), *Global Warming of 1.5°C: An IPCC Special Report on the impacts of global warming of 1.5°C above pre- industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty* (pp. 1– 32). Geneva:World Meteorological Organization.

IPCC, 2013: Climate Change 2013: The Physical Sciences Basis, Contribution of Working Group 1to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change (Stoker,,T.,D.Q in , G. K. Plauner, M. Tignor, S. K. Allen, J. Boschung, A. Nauels. Y. Xia, V. Bex and P. M. Midgley (eds). Cambridge University Press, Cambridge, United Kingdom and New York. NY. USA. 1535 pp.

Islam S, Malik AI, Islam AKMR, Colmer TD. 2007. Salt tolerance in a Hordeum marinum–Triticum aestivum amphiploid, and its parents. *J. Exp.Bot* .58:1219–1229.

Iyengar, E.R.R. and Reddy, M.P., 1996. Photosynthesis in highly salt tolerant plants. *Handbook of photosynthesis. Marshal Dekar, Baten Rose, USA*, *909.book of photosynth*. Marcel Dekker, New York, pp 897–909 JacobyRP,MillarAH,TaylorNL(2010)

Jamil, M., Ashraf, M., Rehman, S., Ahmad, M. and Rha, E.S., 2012. Salinity induced changes in cell membrane stability, protein and RNA contents. *African. J. Biotech*, *11*(24), pp.6476-6483.

Janzen, H.H. and Chang, C., 1987. Cation nutrition of barley as influenced by soil solution composition in a saline soil. *Canadian J. Soil Sci*, *67*(3), pp.619-629.

Jong-Goo K and van Lersel M W 2002 *J. Plant Nutrition* 25.387-403

Kar, M. and Mishra, D., 1976. Catalase, peroxidase, and polyphenoloxidase activities during rice leaf senescence. *Plant physiol*, *57*(2), pp.315-319.

Kefeli, V.I., Kalevitch, M.V. and Borsari, B., 2003. Phenolic cycle in plants and environment. *J. Cell Mol. Biol*, *2*(1), pp.13-18.

Kerstiens, G., Tych, W., Robinson, M.F., and Mansfield, T.A. 2002 Sodium-related partial stomatal closure and salt tolerance of Aster tripolium. *New Phytol*. 153: 509–515.

Khan ,M.A., Ungar, I.A., and Showalter, A.2000.Effects of sodium chloride treatments on growth and ion accumulation of the halophyte Haloxylon recurvum. Commun.Soil Sci.*Plant Anal.*31:2763-2774

Khan, M.A., Ungar, I.A. and Showalter, A.M., 1999. Effects of salinity on growth, ion content, and osmotic relations in Halopyrum mucronatum (L.) Stapf. *J. Plant Nutrition*, *22*(1), pp.191-204.

Khavari-Nejad R. and Mostofi ,Y.1998. Effects of NaCl on photosynthetic pigments saccharides, and chloroplast ultra structure in leaves of tomato cultivars.*Photosynthetica* .35:151-154

Khavari-Nejad R. and Chaparzadeh, N.1998.The effects of NaCl and CaCl2 on photosynthesis and growth of alfalfa plants. *Photosynthetica*. 35:461-466.

Kibria, M.G., Hossain, M., Murata, Y. and Hoque, M.A., 2017. Antioxidant defense mechanisms of salinity tolerance in rice genotypes. *Rice* Sci.24(3), pp.155-162.

Kier, G., Kreft, H., Lee, T.M., Jetz, W., Ibisch, P.L., Nowicki, C., Mutke, J. and Barthlott, W., 2009. A global assessment of endemism and species richness across island and mainland regions. *Proceedings of the National Academy of Sciences*, *106*(23), pp.9322-9327.

Kingsbury, R.W. and Epstein, E., 1986. Salt sensitivity in wheat: a case for specific ion toxicity. *Plant Physiol. 80*(3):pp.651-654.

Kinraide, T.B., 1999. Interactions among Ca2+, Na+ and K+ in salinity toxicity: quantitative resolution of multiple toxic and ameliorative effects. *J. Exp. Bot.*, *50*(338):1495-1505.

Koyro, H.W. 2006. Effect of salinity of growth, photosynthesis, water relations and solute composition of the potential cash crop halophyte Plantago coronopus (L.). *Environ. Exp. Bot*. 56: 136–146.

Kurth, E., Cramer, G.R., Läuchli, A. and Epstein, E., 1986. Effects of NaCl and CaCl2 on cell enlargement and cell production in cotton roots. *Plant Physiol*, *82*(4), pp.1102-1106.

Lacerda, C. F., Cambraia, J., Oliva, M. A., Ruiz, H. A., and Prisco, J. T. 2003.Solute accumulation and distribution during shoot and leaf development in two sorghum genotypes under salt stress. *Environ . Exp. Bot*. 49107-120,

Lazrus, H., 2012. Sea change: island communities and climate change. *Annual Review of Anthropology*, *41*, pp.285-301.

Lee, M.H.,Cho, E.J., Wi, S.G., Bae, H., Kim, J.E., Cho, J.Y., Lee, S.,Kim, J.H., Chung, B.Y. Divergences in morphological changes and antioxidant responses in salt-

tolerant and salt-sensitive rice seedlings after salt stress.2013. *Plant Physiol. Biochem*.70:325–335.

Levitt ,J. 1980. Responses of plants to environmental stresses, Volume 1, chilling freezing, and high temperature stresses. Academic Press: New York.

Lin, C.C. and Kao, C.H., 2001. Relative importance of Na+, Cl–, and abscisic acid in NaCl induced inhibition of root growth of rice seedlings. *Plant and Soil*. *237*(1):165-171.

Lone, M.I., Kueh, J.S.H., Wyn Jones, R.G. and Bright, S.W.J., 1987. Influence of proline and glycinebetaine on salt tolerance of cultured barley embryos. *J of Experimental Botany*.*38*(3).479-490.

Loomis, W.D. and Durst, R.W., 1992. Chemistry and biology of boron. *BioFactors (Oxford, England)*, *3*(4), pp.229-239.

Luo Q, Yu B, Liu Y. Differential sensitivity to chloride and sodium ions in seedlings of Glycine max and G. soja under NaCl stress. 2005 *J.plant.phyisol*. 162(9):1003-12.

Lyman, J. and Fleming, R.H., 1940. Composition of sea water. *J Mar Res*, *3*(2), pp.134-146.

Lynch, J. and Läuchli, A., 1988. Salinity affects intracellular calcium in corn root protoplasts. *Plant Physiol. 87*(2):351-356.

Lynch, J., Cramer, G.R. and Läuchli, A., 1987. Salinity reduces membrane-associated calcium in corn root protoplasts. *Plant Physiol. 83*(2).390-394.

Mairnho, Francisco JL; GHEYI, Hans R. and FERNANDES, Pedro D .. Germination and formation of coconut seedlings irrigated with saline waters. *Rev. bras. eng. agricultural. ambien*

Manciot, R., Ollagnier, M. and Ochs, R., 1979. Mineral nutrition and fertilizion of coconut around the world. *Oleagineux*, *34*(11), pp.499-515.

Mansfield, T.A., Hetherington, A.M. and Atkinson, C.J., 1990. Some current aspects of stomatal physiology. *Annual rev. plant biolo 41*(1), pp.55-75.

Mansour, M.M.F., 1998. Protection of plasma membrane of onion epidermal cells by glycinebetaine and proline against NaCl stress. *Plant Physiol .Biochem*, *36*(10), pp.767-772.

Marcum ,K.and Pessarakli ,M.2006.Salinity tolerance and salt gland excretion efficiency of Bermuda grass turf cultivars. *Crop Sci*. 46:2571-2575.

Maricle, B., Lee ,R., Hellquist, C., Kiirats ,O., and Edwards ,G.2007.Effects of salinity on chlorophyll fluorescence and CO2 fixation in C4 estuarine grasses. *Photosynthetica*.45:433-440.

Marinho, FJL, Gheyi, HR, Fernandes, PD, Holanda, JSD and Ferreira Neto, M., 2006. Cultivation of coconut 'Anão Verde' irrigated with saline waters. *Brazilian.Agric.Resareach*, *41* (8), pp.1277-1284.

Marler, T.and Zozor ,Y.1996. Salinity influences photosynthetic characteristics, water relations, and foliar mineral composition of Annona squamosa L. American Society for Horticultural Science (USA).

Marschner, H. and Cakmak, I., 1989. High light intensity enhances chlorosis and necrosis in leaves of zinc, potassium, and magnesium deficient bean (Phaseolus vulgaris) plants. *J. Plant physiol*, *134*(3), pp.308-315.

Martin, P.K. and Koebner, R.M.D., 1995. Sodium and chloride ions contribute synergistically to salt toxicity in wheat. *Biologia Plantarum*, *37*(2):.265-271

Mathes, D.T., Liyanage, L.V.K. and Randeni, G., 1989. A method for determining leaf area of one, two and three year old coconut seedlings (Var. CRIC 60). In *Cocos* (Vol. 7, pp. 21-25).

Maxwell ,K. and Johnson ,G.2000.Chlorophyll fluorescence--a practical guide. *J.Exp.Bot*. 51:659-664

Meinzer, F.C. Co-ordination of vapour and liquid phase water transport properties in plants. 2002. *Plant Cell Environ* . 25: 265–274.

Meneguzzo, S., Navam-Izzo, F. and Izzo, R., 1999. Antioxidative responses of shoots and roots of wheat to increasing NaCI concentrations. *Journal of plant physiol.155*(2).274-280.

Millero, F.J., Feistel, R., Wright, D.G. and McDougall, T.J., 2008. The composition of Standard Seawater and the definition of the Reference-Composition Salinity Scale. *Deep Sea Research Part I: Oceanographic Research Papers*, *55*(1), pp.50-72.

Minh, L.T., Khang, D.T., Ha, P.T.T., Tuyen, P.T., Minh, T.N., Quan, N.V. and Xuan, T.D., 2016. Effects of salinity stress on growth and phenolics of rice (Oryza sativa L.). *Int.Lett.Nat Sci. 57.*

Mohammad, M., Shibli, R., Ajlouni, M. and Nimri, L., 1998. Tomato root and shoot responses to salt stress under different levels of phosphorus nutrition. *Journal of plant nutrition*, *21*(8), pp.1667-1680.

Moore, S. and Stein, W.H., 1954. A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. *J.Biol Chem.211*..907-913.

Moradi, F. and Ismail, A.M., 2007. Responses of photosynthesis, chlorophyll fluorescence and ROS-scavenging systems to salt stress during seedling and reproductive stages in rice. *Annals of botany*, *99*(6), pp.1161-1173..

Munns, R. and Termaat, A., 1986. Whole-plant responses to salinity. *Functional Plant Biol*, *13*(1), pp.143-160.

Munns, R. and Tester, M., 2008. Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.*, *59*, pp.651-681.

Munns, R., 2002. Salinity, growth and phytohormones. In *Salinity: environment-plants-molecules* (pp. 271-290). Springer, Dordrecht.

Munns, R., Passioura, J.B., Guo, J., Chazen, O. and Cramer, G.R., 2000. Water relations and leaf expansion: importance of time scale. *J.Exp. Botany*, *51*(350), pp.1495-1504.

Munns,R.,and Tester,M .2008 .Mechanisms of salinity of salinity tolerance.*Annu.Rev.Plant Biol*.59:51-681

Munns, R., 1992. A leaf elongation assay detects an unknown growth inhibitor in xylem sap from wheat and barley. *Functional Plant Biology*, *19*(2), pp.127-135.

Murakeözy, É.P., Nagy, Z., Duhazé, C., Bouchereau, A. and Tuba, Z., 2003. Seasonal changes in the levels of compatible osmolytes in three halophytic species of inland saline vegetation in Hungary. *J . plant physiol.*, *160*(4), pp.395-401.

Muranaka, S., Shimizu, K. and Kato, M., 2002. A salt-tolerant cultivar of wheat maintains photosynthetic activity by suppressing sodium uptake. *Photosynthetica*, *40*(4), pp.505-515..

Muthukumarasamy, M., Gupta, S.D. and Panneerselvam, R., 2000. Enhancement of peroxidase, polyphenol oxidase and superoxide dismutase activities by triadimefon in NaCl stressed Raphanus sativus L. *Biologia Plantarum*, *43*(2), pp.317-320.

Nicholls, R.J. and Leatherman, S.P., 1996. Adapting to sea- level rise: Relative sea- level trends to 2100 for the United States. *Coastal Management*, *24*(4), pp.301-324.

Nobel, P.1999.Physicochemical & environmental plant physiology Academic Press New York

Noble, C.L. and Rogers, M.E., 1992. Arguments for the use of physiological criteria for improving the salt tolerance in crops. *Plant and soil*, *146*(1-2), pp.99-107.

Omami ,N.E. 2005.Response of amaranth to salinity stress.235p, Thesis (Ph. D.). University of Pretoria etd. Pretoria. South Africa.

Overlach, S., Diekmann, W. and Raschke, K., 1993. Phosphate translocator of isolated guard-cell chloroplasts from Pisum sativum L. transports glucose-6-phosphate. *Plant physiol*, *101*(4), pp.1201-1207.

Pandey, V. and Agarwal, S., 2002. Amino transferases and nitrate reductase activity in Cassia angustifolia seedlings as affected by salinity stress. *Indian. Plant Physiol.*

Parida ,A.K., Das ,A.B.,and Mittra., B .2004. Effects of salt on growth, ion accumulation photosynthesis and leaf anatomy of the mangrove, Bruguiera parviflora. *Trees-Struct Funct* 18:167–174

Parida, A., Das, A, and Das, P.2002. NaCl stress causes changes in photosynthetic pigments, proteins, and other metabolic components in the leaves of a true mangrove, Bruguiera parviflora, in hydroponic cultures. *J. Plant Biol.* 45:28-3

Parida,A.K and Das, A.B .2005.Salt tolerance and salinity effect on plants: a review. *Ecotoxicol Environ Saf.* 60:324–349

Parvaiz, A Satyawati .,S. 2008. Salt stress and phyto-biochemical responses of plants-a review. *Plant Soil Environ* .54:89-96.

Pospil, P.,and Dau ,H.2000. Chlorophyll fluorescence transients of Photosystem II membrane particles as a tool for studying photosynthetic oxygen evolution. Photosynthesis Research 65:41-52

Prades, A and Ollivier, J., 2013. Expertise in the coconut palm sector in French Polynesia..

Qadir, M.and Schubert, S.2002. Degradation processes and nutrient constraints in sodic soils. *Land Degrad Dev* 13:275–294

R.G. Wyn Jones, J. Gorham, E. McDonnell, Organic and inorganic solute contents as selection criteria for salt tolerance in the Triticeae, in: R. Staples, G.H. Toennissen (Eds.), Salinity Tolerance in Plants: Strategies for Crop Improvement, Wiley, New York, 1984, pp. 189–203.

R.S. Dubey, Photosynthesis in plants under stressful conditions, in: M. Pessarakli (Ed.), Handbook of Photosynthesis, Marcel Dekker, New York, 1997. 859–875.

Ralston, N. and Hunt, C., 2000. Boron incorporation by RAW 264.7 macrophages as indicated by differences in intracellular and extracellular boron concentrations. *Journal of Federation of American Societies for Exp. Biol*, *14*, p.A539.

Rasheed, P., Mukerji, S.1991.Changes in catalase and ascorbic acid oxidase activities in response to lead nitrate treatments in mung bean.*Indian J. Plant Physio*l. 34.143-146,.

Rayle, D.L. and Cleland, R.E., 1992. The Acid Growth Theory of auxin-induced cell elongation is alive and well. *Plant physiol*.*99*(4):1271-1274.

Reddy, M. P. ,Sanish, S., and Iyengar. E. R. R.1992.Photosynthetic studies and compartmentation of ions in different tissues of Salicornia brachiata Roxb. under saline conditions. *Photosynthetica*.26:173–179.

Remision, S. U. and Iremiren, O. G. 1990. Effect of salinity on the performance of coconut seedlings in two contrasting soils. *cocos*.8: 33-3.

Remision, S. U.1988. Effect of salinity on nutrient content of the leaves of the coconut seedlings. *Plant and Soil* .109:135- 138.

Rengasamy,P. and Olsson, K.A.Irrigation and sodicity J. *Soil Res*.1993. 31: 821–837.

Rengel, Z., 1992. The role of calcium in salt toxicity. *Plant, Cell & Environ 15*(6), pp.625-632.

Rogers ,M ., Noble, C.,Halloran, G.,and Nicolas, M. 1997. Selecting for salt tolerance in white clover (Trifolium repens): chloride ion exclusion and its heritability. *New Phytologist* 135:645654.

Rogers, M.E., Grieve ,C.M., and Shannon, M.C.2003. Plant growth and ion relations in lucerne(MedicagosativaL.)in response of the combined effects of NaCl and P. *Plant Soil.* 253:187–194 7 y Tolerance in Rice Genotype

Romero-Aranda ,R., Soria,T., and Cuartero ,J. 2001. Tomato plant-water uptake and plant-water relationships under saline growth conditions. *Plant Sci.* 160:265-272

Romero-Aranda, M.R., Jurado, O. and Cuartero, J., 2006. Silicon alleviates the deleterious salt effect on tomato plant growth by improving plant water status. *J. plant physiol 163*(8), pp.847-855.

Romero-Aranda, R., Soria, T. and Cuartero, J., 2001. Tomato plant-water uptake and plant-water relationships under saline growth conditions. *Plant sci*, *160*(2), pp.265-272.

Roy, C.A. and Basu, S., 2008. Over expression of an abiotic stress responsive plant protein in bacteria E. coli. *African J. Plant Biotech*, *7*, pp.3231-3234.

Sacher ,R.,and Staples ,R.1985. Inositol and sugars in adaptation of tomato to salt. *Plant Physiol*. 77:206-210.

Sairam, R. K., Rao, K. V., & Srivastava, G. C. 2002). Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. *Plant sci. 163*(5). 1037-1046.

Sairam, R.K. and Srivastava, G.C., 2002. Changes in antioxidant activity in sub-cellular fractions of tolerant and susceptible wheat genotypes in response to long term salt stress. *Plant Sci*, *162*(6), pp.897-904.

Scalet, M., Federico, R., Guido, M.C. and Manes, F., 1995. Peroxidase activity and polyamine changes in response to ozone and simulated acid rain in Aleppo pine needles. *Environ.Exp.Bot.35*(3):417-425.

Scandalios, J.G., 2002. The rise of ROS. *Trends in biochem.sci*, *27*(9), pp.483-486.

Schubert, S. and Läuchli, A., 1990. Sodium exclusion mechanisms at the root surface of two maize cultivars. In *Genetic Aspects of Plant Mineral Nutrition* (pp. 183-187). Springer, Dordrecht.

Schwarzenbach, G., Biedermann, W. and Bangerter, F., 1946. New, Simple Titration Methods for Determining the Hardness of Water. *Helv. chim. acta*, *29*, p.811.

Shabala, S. and Pottosin, I., 2014. Regulation of potassium transport in plants under hostile conditions: implications for abiotic and biotic stress tolerance. *Physiologia plantarum*, *151*(3), pp.257-279.

Shannon ,M.C., Grieve, C.M., and Francois., L.E. 1994. Whole-plant response to salinity. Plant environmental interactions. New York, Marcel-Decker: 199-244.

Shannon,M .and Grieve., C. 1998. Tolerance of vegetable crops to salinity. *Sci Horti*. 78:5-38.

Sheen, S.J. and Calvert, J., 1969. Studies on polyphenol content, activities and isozymes of polyphenol oxidase and peroxidase during air-curing in three tobacco types. *Plant physiol.44*(2):199-204.

Shu, S., Yuan, L.Y., Guo, S.R., Sun, J., and Yuan, Y.H. Effects of exogenous spermine on chlorophyll fluorescence, antioxidant system and ultrastructure of chloroplasts in Cucumissativus L. under salt stress. *Plant.Physiol. Biochem*. 2013, 63, 209–216. [CrossRef] [PubMed]

Shukla, U.C. and Mukhi, A.K., 1979. Sodium, Potassium, and Zinc Relationship in Corn 1. *Agron. J.71*(2),..235-237.

Silva, A. R. A., Bezara, L. M. F., Lacerda. DF.C., Sousa, D.C.H.C., Bezar, A. M. 2017. Physiological Responses of Dwarf Coconut Plants Under Water Deficit in Salt-Affected Soils. *Revista. Caatinga.* 30 (2): 447-457.

Somogyi, M. 1952. Estimation of sugars by colorimetric method. *J. Biol Chem*. 200:245.

Suárez, N., 2011. Effects of short-and long-term salinity on leaf water relations, gas exchange, and growth in Ipomoea pes-caprae. *Flora-Morpho. Distribution,Function.Ecol.Plants*, *206*(3), pp.267-275.

Sudhakar, C., Lakshmi, A. and Giridarakumar, S., 2001. Changes in the antioxidant enzyme efficacy in two high yielding genotypes of mulberry (Morus alba L.) under NaCl salinity. *Plant Sci*, *161*(3), pp.613-619.

Taiz, L., Zeiger, E. and Maffei, M., 2013. *Fisiologia vegetale*. Piccin.

Taiz, L., Zeiger, E., Møller, I.M. and Murphy, A., 2015. Plant physiology and development.

Talei, D., Kadir, M.A., Yusop, M.K., Valdiani, A. and Abdullah, M.P., 2012. Salinity effects on macro and micronutrients uptake in medicinal plant King of Bitters (Andrographis paniculata Nees.). *Plant Omics J*, *5*(3).271-278.

Tattini,M., Gucci, R., Coradeschi,M.A.,Ponzio,C.,and Everard, J.D.1995.Growth,gas exchange and ion content in Oleaeuropaea plants during salinity stress and subsequent relief.*Plant. Physiol*. 95:203–210.

Tavakkoli, E., Rengasamy, P. and McDonald, G.K., 2010. High concentrations of Na+ and Cl–ions in soil solution have simultaneous detrimental effects on growth of faba bean under salinity stress. *J.Exp.Bot.61*(15)..4449-4459.

Tavakkoli, E., Rengasamy, P. and McDonald, G.K., 2010. The response of barley to salinity stress differs between hydroponic and soil systems. *Funct.Plant Biol. 37*(7):621-633.

Teakle, N.L. and Tyerman, S.D., 2010. Mechanisms of Cl- transport contributing to salt tolerance. *Plant cell.Environ.33*(4):566-589.

Teakle, N.L., Flowers, T.J., Real, D. and Colmer, T.D., 2007. Lotus tenuis tolerates the interactive effects of salinity and waterlogging by 'excluding'Na+ and Cl− from the xylem. *J. Exp. Bot*, *58*(8), pp.2169-2180.

Tester, M. and Davenport, R., 2003. Na+ tolerance and Na+ transport in higher plants. *Annals.Bot*, *91*(5):503-527

Tomás- Barberán, F.A. and Espín, J.C., 2001. Phenolic compounds and related enzymes as determinants of quality in fruits and vegetables. *J.Sci. Food . Agri. 81*(9):853-876.

Torabi, M., Halim ,R. A., Sinniah ,U. R.,and Choukan R.2010.Influence of salinity on the germination of Iranian alfalfa ecotypes. *African. J.of Agri.Research* 6(19): 4624-4630.

Torres, C. and Bingham, F.T., 1973. Salt tolerance of Mexican wheat I. effect of NO_3 and NaClon mineral nutrition, growth, and grain production of four wheats. *Soil. Sci. Soc. Am. Proc. 37,711-715.*

Veselov, D.S., Sharipova, G.V., Veselov, S.U., and Kudoyarova, G.R. The Effects of NaCl Treatment on Water Relations, Growth, and ABA Content in Barley Cultivars Differing in Drought Tolerance. .2008. *J. Plant Growth Regul*. 27: 380.

Vysotskaya, L., Hedley, P.E., Sharipova, G., Veselov, D., Kudoyarova, G., Morris, J., and Jones, H.G. Effect of salinity on water relations of wild barley plants differing in salt tolerance. 2010. AoB Plants [CrossRef

Wang ,W., Vinocur., B., and Altman., A. 2003. Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. *Planta* .218:1–14

Wang ,Y.,and Nil ,N.2000. Changes in chlorophyll, ribulosebiphosphate carboxylase oxygenase, glycine betaine content, photosynthesis and transpiration in Amaranthus tricolor leaves during salt stress *J. Hortic. Sci. Biotechnol* .75:623-627.

White, P.J. and Broadley, M.R., 2001. Chloride in soils and its uptake and movement within the plant: a review. *Annals. Bot. 88*(6):.967-988.

Wimmer, M.A., Mühling, K.H., Läuchli, A., Brown, P.H. and Goldbach, H.E., 2003. The interaction between salinity and boron toxicity affects the subcellular distribution of ions and proteins in wheat leaves. *Plant, Cell & Environment*, *26*(8), pp.1267-1274.

Wyn Jones, R.G., 1978. Salt stress and comparative physiology in the Gramineae. IV. Comparison of salt stress in Spartina x towsendii and three barley cultivars. *J. Plant Physiol.*5.839-850.

Wyn Jones, R.G., 1981. Salt tolerance. *Physiol Processes Limiting Plant Productivity*.

Xu, G., Magen, H., Tarchitzky, J. and Kafkafi, U., 1999. Advances in chloride nutrition of plants. In *Advances in Agronomy* (Vol. 68, pp. 97-150). Academic Press.

Yeo, A., 1998. Molecular biology of salt tolerance in the context of whole-plant physiology. *J. Exp. Botany*, *49*(323), pp.915-929.

Yuan, G., Wang, X., Guo, R. and Wang, Q., 2010. Effect of salt stress on phenolic compounds, glucosinolates, myrosinase and antioxidant activity in radish sprouts. *Food Chem.121*(4):1014-1019.

Zhanj, J. and Davies, W.J., 1991. Antitranspirant activity in xylem sap of maize plants. *Journal of Experimental Botany*, *42*(3), pp.317-321.

Zhao, G., Ma, B., and Ren., C.2007. Growth, gas exchange, chlorophyll fluorescence, and ion content of naked oat in response to salinity. *Crop Sci*. 47:123-131

Zhu, B., Su, J., Chang, M., Verma, D.P.S., Fan, Y.L. and Wu, R., 1998. Overexpression of a Δ1-pyrroline-5-carboxylate synthetase gene and analysis of tolerance to water-and salt-stress in transgenic rice. *Plant Sci*, *139*(1), pp.41-48.

Zhu, J. 2001. Plant salt tolerance. *Trends in plant sci*.6:66-71.

Zhu, J.K., 2001. Plant salt tolerance. *Trends in plant sci 6*(2), pp.66-71.

Zidan ,I., Jacoby .,B, Ravina, I, Neumann, P.M.1991.Sodium does not compete with calcium in saturating plasma membrane sites regulating 22Na influx in salinized maize roots. *Plant Physiol*.96(1):331-4.

Zidan, I., Azaizeh, H. and Neumann, P.M., 1990. Does salinity reduce growth in maize root epidermal cells by inhibiting their capacity for cell wall acidification?. *Plant Physiol. 93*(1):7-11.

EFFECT OF SALINITY ON GROWTH, PHYSIOLOGICAL AND BIOCHEMICAL PROCESSES OF COCONUT SEEDLINGS (*Cocos nucifera* L).

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ABSTRACT

Under the climate change the envisaged sea level rise would inundate large area of coconut plantation and affect the growth and production. In this study an attempt is made to study the response of coconut seedlings to different levels of sea water substitution. Hydroponically grown coconut seedlings of variety MGD (Malayan green dwarf) were exposed to 0, 10, 25, 50, 75 and 100% substitution of sea water. Growth and most of the physiological and biochemical parameters were almost on par up to 10% substation of sea water from control. At 25% and beyond the above parameters were significantly reduced. However photosynthesis was more sensitive and declined even at 10%. Leaf water potential was not affected up to 25% suggesting that the decline in photosynthesis was due to the effect of specific ions and not due to water deficit effect. In fact there was significant accumulation of sodium, boron, chlorine and sulphur and a concomitant decline in some of the essential elements like potassium, nitrogen, phosphorus , calcium ,magnesium and micro nutrients such as iron, copper, manganese and zinc. At 25% sea water treatment, biomass accumulation reduced to 46% and beyond 50% sea water substitution it shows more than 70% reduction in biomass thus suggesting dwarf variety like MGD could tolerate 10% substitution of sea water.