# STUDIES ON EFFECT OF SALT STRESS ON SOME MEDICINAL PLANTS

**S.P Kiran kumari [1], V. Sridevi* [2], M.V.V. Chandana Lakshmi [3]**

[1,2,3] Centre for Biotechnology, Department of Chemical Engineering, A.U College Of Engineering(A) Andhra University, Visakhapatnam 530003, Andhra Pradesh, India

**Abstract:**
Climatic change has become increasingly recognized as one of the greatest challenges to humankind and all other life on earth. The productivity of plants is greatly affected by various environmental stresses. Plant stress is a condition where excessive salts in soil solution cause inhibition of plant growth or plant death. Salinity stress negatively impacts agricultural yield throughout the world affecting production whether it is for subsistence or economic gain. The medicinal properties of plant species have made an outstanding contribution in the origin and evolution of many traditional therapies. In India, many government and non-government organizations have had focused attention on improving the medicinal plants sector. So the study is related to effect of salt stress on our selected medicinal plants which may help upto some extent for their cultivation. *Azadirachta indica* , *Cassia fistula*, *Catharanthus roseus*, *Aloe barbadensis*, and *Ocimum sanctum* were selected for our study and there biochemical parameters like total chlorophyll content and total carbohydrate content were estimated to know the salt tolerance among those plants. Through our field experiment *Azadirachta indica* showed the highest tolerance towards salinity both by morphological parameters and by biochemical parameters and remaining all four get wilted and the quality reduced gradually.

**Keywords:** Biochemical parameters , Chlorophyll content ,Medicinal plants, Salt stress.

## 1 Introduction

India is well known for the usage of medicinal plants in curing various diseases from ancient times .It has been estimated that in developed countries such as united states , plant drugs constitute as much as 25% of the total drugs, while in fast developing countries such as china and India it is as much as 80%. As medicinal plants play a vital role in Indian medicine so there is a need to know about the factors effecting their growth for the large scale cultivation. Plant growth and productivity are greatly affected by environmental stresses such as dehydration, high salinity, low temperature and biotic pathogen infection[1]. Salinity stress is one among the various environmental stresses .Salinity stress negatively impacts agricultural yield throughout the world affecting production whether it is for subsistence or economic gain. Salt stress negatively presents an increase threat to plant agriculture. This impact has been studied upon some selected medicinal plants to know the serious impact of salt stress among those plants which has a lot of significance in the Indian system of medicine[2-3].

### Medicinal plants

FIG:1 *Azadirachta indica*

***Azadirachta indica*** (**Neem**) is a tree in the mahogany family Meliaceae. It is one of two species in the genus ***Azadirachta***, and is native to India growing in tropical and semi-tropical regions. Its fruits and seeds are the source of neem oil. In East Africa it is also known as *Muarubaini* (Swahili), which means *the tree of the 40*, as it is said to treat 40 different diseases, and in Somalia it is known as "Geed Hindi" which means "the Indian tree".Neem is a fast-growing tree that can reach a height of 15–20 metres (49–66 ft), rarely to 35–40 metres (115–130 ft). It is evergreen, but in severe drought it may shed most or nearly all of its leaves. The branches are wide spread. The fairly dense crown is roundish or oval and may reach the diameter of 15–20 metres (49–66 ft) in old, free-standing specimens[5]. All parts of the tree are said to have medicinal properties (seeds, leaves, flowers and bark) and are used for preparing many different medical preparations. The chemical constituents nimbidin and nimbin have some spermicidal activity . Neem oil is used for preparing cosmetics (soap, neem shampoo, balms and creams such as Margo soap) and many oral health products. Besides its

use in traditional Indian medicine, the neem tree is of great importance for its anti-desertification properties and possibly as a good carbon dioxide sink[7-8].

FIG:2 *Cassia fistula*

*Cassia* ***fistula***, known as the **golden shower tree** and other names, is a flowering plant in the family Fabaceae, native to southern Asia, from southern Pakistan east through India to Myanmar and south to Sri Lanka. It is associated with the Mullai region of Sangam landscape. It is the national tree of Thailand, and its flower is Thailand's national flower. It is also state flower of Kerala in India and of immense importance amongst Malayali population. It is a popular ornamental plant and is an herbal medicine. In Ayurvedic medicine, golden shower tree is known as *aragvadha*, meaning "disease killer". The root is considered a very strong purgative, and self-medication or any use without medical supervision is strongly advised against in Ayurvedic texts[9-10].

FIG:3 *Aloe barbadensis*

***Aloe barbadensis*** **,**common name ***Aloe vera*** , pronounced, also known as the **true aloe** or **medicinal aloe**, is a species of succulent plant in the genus *Aloe* that is believed to have originated in the Sudan. *Aloe vera* grows in arid climates and is widely distributed in Africa, India, Nepal and other arid areas. The species is frequently cited as being used in herbal medicine. Many scientific studies on the use of extracts of *Aloe vera* have been undertaken, some of them conflicting. There is some preliminary evidence that

*Aloe vera* extracts may be useful in the treatment of wound and burn healing, minor skin infections, sebaceous cysts, diabetes, and elevated blood lipids in humans[11-15]. These positive effects are thought to be due to the presence of compounds such as polysaccharides, mannans, anthraquinones, and lectins.

FIG:4 *Catharanthus roseus*

***Catharanthus roseus*** (**Madagascar Periwinkle**) is a species of *Catharanthus* native and endemic to Madagascar. Synonyms include *Vinca rosea* (the basionym), *Ammocallis rosea*, and *Lochnera rosea*; other English names occasionally used include Cape Periwinkle, Rose Periwinkle, Rosy Periwinkle, and "Old-maid". In the wild, it is an endangered plant; the main cause of decline is habitat destruction by slash and burn agriculture. It is also however widely cultivated and is naturalised in subtropical and tropical areas of the world. The species has long been cultivated for herbal medicine and as an ornamental plant. In traditional Chinese medicine, extracts from it have been used to treat numerous diseases, including diabetes, malaria, and Hodgkin's disease. The substances vinblastine and vincristine extracted from the plant are used in the treatment of leukemia[16].

These are the plants which are subjected to salt stress to know the salinity withstanding capability and also verifying the biochemical changes in these plants due to the presence of excess salt in the soil.

## 2 Materials and methods

The selected medicinal plants were daily subjected to 1000mg of Nacl salt for 20 days after sown. Their respective control plants without the subjection to salt stress were sown in separate place so that it will be helpful for the comparision of salt stress on these subjected plants.

### 2.1 Soil chemical analysis

**2.1.1 Determination of soil pH:** Significance of pH lies in its influence on availability of soil nutrients, solubility of toxic nutrients elements in the soil , physical breakdown of root cells, cation exchange capacity in soils whose colloids(clay/humus) are pH-dependent, and on biological activity. At high pH values, availability of phosphorus(p) and most micronutrients, except boron(B) and molybdenum (MO),tends to decrease. Thus , soil pH is one of the most common measurements in soil laboratories[17]. It reflects whether a soil is acid , neutral, basic or alkaline. Procedure for determining soil pH in a 1:1 (soil: water) suspension is McKeague, 1978 and McLean,1982 methodolgy.

**2.1.2 Determination of soil Electrical conductivity:** The methodology of EC measurement followed by Richards and et al., 1954 was used by us.

**2.2 Plant Morphological parameters:**

**2.2.1 Determination of plant height:** Plant height was measured from the cotyledonary node to the growing tip and expressed in centimeters.

**2.2.2 Determination of leaf area :** The leaves from five randomly selected branches were used for the estimation of leaf area. Leaf area was computed by using disc method and expressed as $cm^2 plant^{-1}$.

**2.3 Plant biochemical parameters:**

**2.3.1 Estimation of total carbohydrates content in plant leaves:**

Plant sample preparation:

i) Plant leaves should be collected. Oven dried at 70 °C for 72 hrs.
ii) Grinded to fine powder.
iii) 5gms of plant powder was weighed and crushed in 25ml of sterile water.
iv) Boiled at 50-60 °C for 30 minutes on water bath.
v) It was used filtered through Whattman No.1 filter paper.
vi) The filterate was centrifuged at 2500 rpm for 15 minutes and filterate was stored in sterile bottles at 5°C for further use.

Reagent preparation: 0.2 gms of anthrone was dissolved in 100ml of concentrated Sulphuric acid. Fresh solution was prepared just before the use.

Procedure:

i) 1ml of the aliquot was taken in a test tube.
ii) The volume was made upto 2.5 ml with distilled water.
iii) All the test tubes were kept in the ice bath and to which 5ml of anthrone reagent was added slowly.
iv) Contents were stirred gently with a glass rod and heated on boiling water bath exactly for 7.5 mins and cooled immediately on ice bath.
v) After cooling, the absorbance of the solution were measured at 630nm against the blank in a spectrophotometer (Elico SL-177) and the sugar content was calculated through the standard curve.

Standard curve:

i) 100 mg of glucose was dissolved in little quality of water and make up to 100 ml to get a stock solution.
ii) From this, different concentration were made from 10-100mg/ml by diluting and used for standard curve.

**2.3.2 Estimation of total chlorophyll content in plant leaves:**

To the estimate the amount of chlorophyll in plant leaves Arnon methodology (1949) was used[18].

i) 0.25 gms of fresh leaves was weighed and homogenized with pure 80% acetone.
ii) Extract was filtered using whatman No.1filter paper.
iii) Wash 2-3 times using 80% acetone.
iv) Finally made the volume to 25 ml
v) Read the absorbance at 645 and 663 nm

Calculate the total chlorophyll content by using the Formulae:

Total chlorophyll content present in A/g tissue = $20.2(A_{645}) + 8.02(A_{663})$ *V/1000*W

A= absorbance at specific wavelength

V= final volume of chlorophyll extract in 80% acetone

W= Fresh weight of tissue extracted

## 3. Results and Discussion:

### 3.1 Soil chemical analysis:

| Soil sampl | pH | E.C |
|---|---|---|
| Before salt stress | 6 | 1.23dsm$^{-1}$ |
| After salt stress | 7.9 | 0.93dsm$^{-1}$ |

**Table 1. Compares the pH and electrical conductivity of the soil before and the after the experimental work**

### 3.2 Plant Morphological parameters:

#### 3.2.1 Determination of plant height:

| PLANTS | 5$^{th}$ day | 10$^{th}$ day | 15$^{th}$ day | 20$^{th}$ day |
|---|---|---|---|---|
| *Azadirachta indica* | C-46<br>S -47 | C-46<br>S -48 | C-47<br>S -49 | C-48<br>S -**51** |
| *Cassia fistula* | C-45<br>S -45 | C-46<br>S -45 | C-47<br>S -45.5 | C-48<br>S -46 |
| *Aloe barbadensis* | C-20<br>S -20 | C-21<br>S -20 | C-22.5<br>S -21 | C-24<br>S -21 |
| *Catharanthus roseus* | C-13<br>S -12 | C-14<br>S -13 | C-14<br>S -13 | C-16<br>S -14 |

Plant height was estimated for 21 days and the height in centimeters was estimated with a gap period of 5 days(Table.2)

**Table 2.The observed plant height for 20 days in an interval of 5 days where C- indicates control and S- indicates salt stress on their respective plants.**

Table 2, shows the parameter in terms of growth where we can see clearly the inhibitory effect of salt in all plants , but astonishingly *Azadirachta indica* shows the growth effect when compared with another plants while all other plants height were less when compared to their respective control plants.

**3.2.2 Determination of leaf area:**

Leaf area was determined in as $cm^2plant^{-1}$[19]**.** Here the control plants $20^{th}$ day area is tabulated so that direct identification can be done , at the same time the $5^{th}$ -$15^{th}$ day leaves area is also tabulated so that the increase rate of area can be easily noticed(Table.3).

| PLANTS | Control value on $20^{th}$ day | $5^{th}$ day | $10^{th}$ day | $15^{th}$ day | $20^{th}$ day |
|---|---|---|---|---|---|
| *Azadirachta indica* | **7** | 7 | 7.85 | 7.99 | **8.5** |
| *Cassia fistula* | 4.5 | 4.5 | 4.22 | 4.55 | 4.44 |
| *Aloe barbadensis* | 15 | 15 | 14 | 13 | 14 |
| *Catharanthus roseus* | 12 | 12 | 13 | 13 | 14 |

**Table 3.It shows the leaf area of the plant leaves which is a morphological parameter which helps to indicate the plant survival.**

**3.3 Plant biochemical parameters:**

**3.3.1 Total carbohydrates content in plant leaves:**

| Plants | Control on $20^{th}$ day | $5^{th}$ day | $10^{th}$ day | $15^{th}$ day | $20^{th}$ day |
|---|---|---|---|---|---|
| *Azadirachta indica* | **1.98** | 1.888 | 1.87 | 1.47 | **1.57** |
| *Cassia fistula* | 1.45 | 1.23 | 1.22 | 1.20 | 0.878 |
| *Aloe barbadensis* | 1.23 | 1.01 | 0.67 | 0.64 | 0.56 |
| *Catharanthus roseus* | 1.23 | 1.21 | 1.023 | 0.98 | 0.77 |

**Table 4 .Total carbohydrate content of the selected medicinal plants were tabulated with respect to five days of interval**

Total carbohydrates content was estimated by Anthrone method, and the total carbohydrate content of all the plants with respect to their days of interval is tabulated here(Table 4).We found that more amount of carbohydrate was found in *Azadirachta indica* control plant and also the salt stress effected *Azadirachta indica* has nearby amount of carbohydrates in it [20,21].It resembles that *Azadirachta indica* has some internal tolerance towards salt stress.

**3.2.2 Total chlorophyll content in plant leaves:**

| Plants | Control on 20th day | 5th day | 10th day | 15th day | 20th day |
|---|---|---|---|---|---|
| *Azadirachta indica* | **1.98** | 1.78 | 1.88 | 1.91 | **2.012** |
| *Cassia fistula* | 1.33 | 1.23 | 1.12 | 1.01 | 0.98 |
| *Aloe barbadensis* | 1.23 | 0.98 | 0.96 | 0.88 | 0.77 |
| *Catharanthus roseus* | 1.42 | 1.09 | 1.02 | 0.98 | 0.92 |

**Table 5.Chlorophyll content of the selected medicinal plants in an interval of five days are tabulated here.**

In the chlorophyll content estimation of the selected plants for 20 days, in the interval of five days , we found high amount of chlorophyll in *Azadirachta indica*, which is amazing phenomenon when compared to other plants. Whereas total chlorophyll content is an indicative of photosynthetic and metabolic activity and it was found high in *Azadirachta indica* ,and the other plants like *Cassia fistula ,Aloe barbadensis, Catharanthus roseus* lack the ability to withstand the salinity condition.

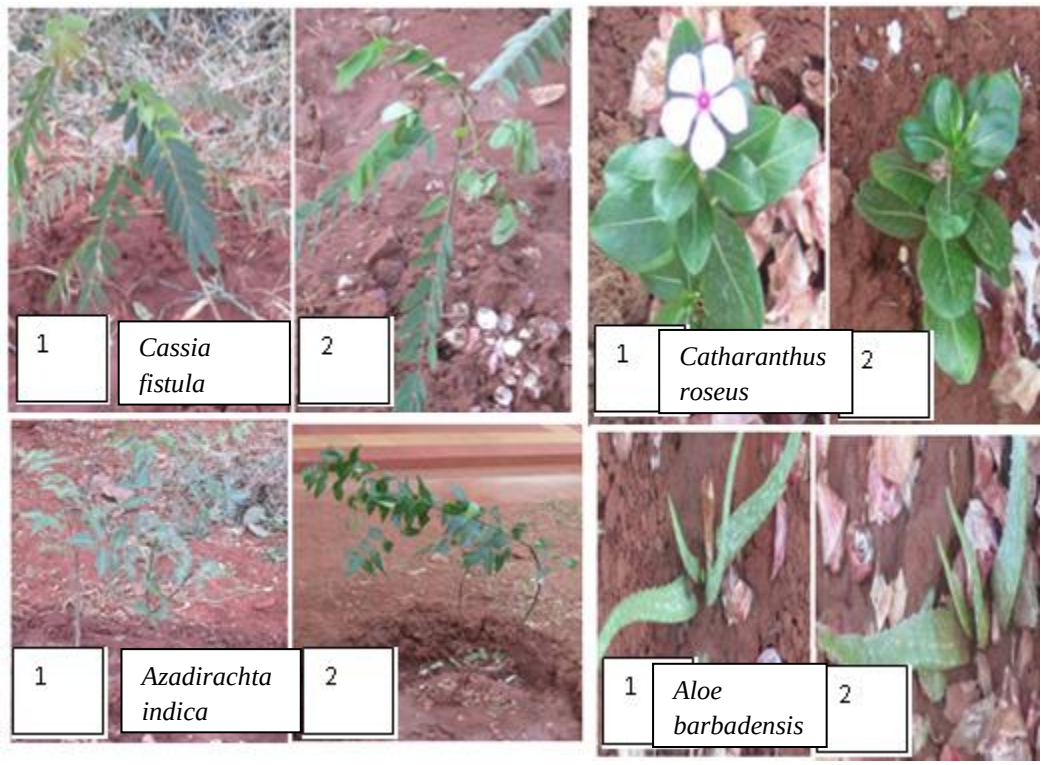


Figure 5. 1- indicates the control plant,2- indicates salt stress effected plant. The figure consist of *Cassia fistula and Catharanthus roseus in the first row, and Azadirachta indica and ,Aloe barbadensis* in the second row

From the above experimental work it was determined that *Azadirachta indica* which a most commonly used plant in medicinal works has the capability to survive in salinity conditions too. Its large scale cultivation is also easy in saline conditions as it has saline conditions withstanding property, while other plants have showed the suppressed growth conditions.

## 4.CONCLUSION:

*Azadirachta indica* has an ability to withstand salinity, was determined by some morphological and biochemical parameters. Whereas the salts stress varies from one plant to another which can be clearly observed from the experimental work which has been done. Good agricultural practice is specific for each country because of some differences in geoclimate, length of vegetative, time, precipitation, temperature and quality of soil[23-25]. Therefore there is a necessity for verification and adaptation of each plant in natural conditions of each region. By having advance research on these plants by gathering the genetic work and molecular work on these plants in addition to the morphological and biochemical parameters , new genetically manipulated traits can generated. This approach is promising, considering the novel approaches in combining genetic, physiological , biochemical and molecular techniques which results a excellent future of plants. One day soon, crops will be altered to survive and produce maximum yield grown under minimal conditions. The problem of salt stress will be alleviated and farmers will be satisfied[26].

## 5.REFERENCES

[1] J.P. Thornber, R.S. Alberte, F.A. Hunter, J.A. Shiozawa and K.S. Kan. *Brookhaven Symp. Biol.*, 1977, pp132–148.
[2] J. Argyroudi-Akoyunoglou and H. Thomou. *FEBS Lett.*, 1981, Vol 135, pp177–181.
[3] F.A. Wollman and P. Bennoun. *Biochim. Biophys. Acta*, 1982, Vol 680 , pp352–360.
[4] D. Ish-Shalom and I. Ohad. *Biochim. Biophys. Acta*,1983,Vol 722 , pp 498–507.
[5] J.M. Anderson, J.S. Brown, E. Lam and R. Malkin. *Photochem. Photobiol*, 1983,Vol 38 , pp205–210.
[6] P. Haworth, J.L. Watson and C.J. Arntzen. *Biochim. Biophys. Acta.*, 1983,Vol 724 , pp 151–158.
[7] N.H. Chua. *Methods Enzymol*,1980,Vol 69 , pp434–446
[8] D.I. Arnon. *Plant Physiol.*,1949, Vol 24 , pp1–15.
[9] E. Lam, W. Ortiz, S. Mayfield and R. Malkin, *Plant Physiol*,1984.
[10] J.E. Mullet, J.J. Burke and C.J. Arntzen. *Plant Physiol.*, 1980,Vol 65, pp823–827.
[11] G.W. Schmidt, S.G. Bartlett, A.R. Grossman, A.R. Cashmore and N.H. Chua. *J. Cell Biol*, 1981, Vol 91 , pp. 468–478.
[12] Ahuja AK: In *Himalayan Medicinal Plants: Potential and Prospects*. Edited by Samant SS, Dhar U, Palni LMS. Nainital: *Gyanodaya Prakashan*, 2001,pp1-21.
[13] Anonymous: *National Health Policy.* New Delhi: Ministry of Health and Family Welfare, Government of India; 1983.
[14] Kala CP: *International Journal of Sustainable Development and World Ecology* 2004, **11**(2)**:**205-210.
[15] Tolia RS.,*Patwari, Gharat and Chai*. Dehradun: Bishen Singh Mahendra Pal Singh; 2004
[16] Rawat RBS, Uniyal RC: Status of medicinal and aromatic plants sector in Uttaranchal: initiatives taken by the Government of India. *Financing Agriculture* 2004, Vol 36, pp7-13.
[17] Jain AP, Kumar H in: R and D funding in Himalayan region in India: A comparison. *Hima-Paryavaran* 1994, Vol 6, pp10-11.
[18] Kumar R., Medicinal, aromatic and herbal crops. *Financing Agriculture* 2004,Vol 36, pp3-5.
[19] Prahalathan S , Export potential of Indian medicinal plants and products. *FinancingAgriculture* 2004, Vol 36, pp33-36.
[20] Kaushik P, Dhiman AK in: *Medicinal Plants and Raw Drugs of India.* Dehradun: Bishen Singh Mahendra Pal Singh, 1999.
[21] Olsen CS, Larsen HO in: Alpine medicinal plant trade and Himalayan mountain livelihood strategies. *The Geographical Journal* 2003, pp169-243
[22] Joshi P, Dhawan V., in : *Swertia chirayita* – an overview, *Current Science* 2005, Vol 89, pp 635-640.
[23] Kumar S, *The Economic Plants of North East India.* Jodhpur: Scientific Publisher, 2000.
[24] Anonymous: *Herbals in India: Opportunities, Challenges and Initiatives by NABARD.* National Bank for Agriculture and Rural Development, India, 2004.
[25] Jain SK., Dictionary of Indian Folk Medicine and Ethnobotnay. New Delhi: Deep Publications, 1991.
[26] R. K. Sairam, and Aruna Tyagi, *Curent Science*, 2004,Vol 86, No. 3.