PHYSIOLOGICAL STUDIES IN SAFFLOWER (*CARTHAMUS TINCTORIUS* L.) Cv. BHIMA UNDER SALINE CONDITIONS

A THESIS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY IN BOTANY

SUBMITTED BY

NEETA N. BANKAR

UNDER THE GUIDANCE OF

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(M.Sc., Ph.D.)

DEPARTMENT OF BOTANY
UNIVERSITY OF PUNE
PUNE - 411 007

★ [JULY 1999] ★

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29/7/99

Dr. M. G. Shitole
Guide

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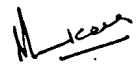

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INTRODUCTION

CHAPTER II

SAFFLOWER AT A GLANCE AND SCOPE OF PRESENT INVESTIGATION

1) INTRODUCTION

In a world where the population is increasing at an alarming rate, the pressure on agriculture to produce large amounts of products is unrelenting. There is also an increasing concern that the means employed in the recent past to achieve high yield of crops are becoming unsustainable because of their disastrous long term ecological consequences (Boulter,1995).

Today, man has to face many complex problems as demand for food has increased in world, because of rise in population. Therefore, it has become necessary to increase crop production substantially throughout the world which is possible by agriculture. The agricultural research programs are playing an important role in production of new and better varieties and strains of crop plants. Thus, it is necessary for plant physiologists to undertake research programs to increase crop yield.

Humans are directly or indirectly dependent upon plants for food and food is directly or indirectly produced by photosynthetic activity. About 70% of earth is covered by oceans and 30 % is covered by land. One third of the land area is semi arid or arid and half of this area has saline soil.

There are many reasons for soil salinization. One of the reasons is increasing use of poor quality water, continuous addition of waste salts to our environment and increasing contamination of underground water sources (Somers, 1979). Another reason is excessive presence of sodium salts like chlorides, sulphates, carbonates and magnesium.

High concentrations of salts result in water deficit in apoplasts and injures subcellular organelles disrupting functions like photosynthesis, respiration and affect synthesis of nucleic acids, proteins and hormones which results in poor germination, poor crop growth and yield. As salinity decreases, permeability of roots to water absorption is increased.

However, there are certain salt tolerant crops eg- barley, sugarbeet, cotton; paddy and wheat are medium tolerant and beans, celery, pea are salt sensitive crops. Salt tolerant varieties possess the ability to reduce intracellular accumulation of sodium and chloride ions. In halophytes, however, intracellular salt concentration is reduced because of secretion of salt through salt glands through the shoots or by developing succulence. It has been suggested that ability of the plant to tolerate salinity may enhance agricultural productivity.

Salinity problems occur in both arid and irrigated areas of the world. Irrigated area in 103 countries totalled 203 million hectares and if 25 % of the land is saline (Thorne and Peterson, 1955) it works out to be 50 million hectares (Carter, 1975). About 7 million hectares of productive land is saline (Champagnol, 1979). Saline, alkali and saline alkali soils are found in all the states of India. In Maharashtra, salinity is caused due to tidal action of sea water in coastal regions and due to improper irrigation practices in the plains (Bowa, 1981). Due to these reasons, lands were damaged due to development of salinity in soil because of which land has been rendered uncultivable (Kakade, 1968). Soil in the districts of Ahmednagar, Pune, Satara, Sangli and Sholapur is saline. As more importance is given to increasing irrigation facilities, more area becomes saline which requires immediate attention (Wadkar, 1976).

Saline soil is predominant in sodium and chloride ions which contributes to the salinity of the soil. Salt stress results in poor growth and productivity, if soil contains excess (about 0.1 %) of soluble salts. Soil salinity also affects plant morphology, anatomy and physiology. However, reclamation measures were suggested by Puri (1934) and Basu (1950). NaCl has been demonstrated to be damaging to germination and plant development (Bliss et al. 1986; Jeschke and Wolf, 1988) and toxic effects asssociated with excess salinity are described (Greenway and Munns, 1980).

To overcome the problems of salinity, use of suitable agronomic practices, selection, breeding of well adapted crops or varieties can be done. Conventional plant breeding methods have been successful in increasing salinity tolerance of some crop plants (Shannon, 1984). In addition, plant tissue culture and genetic engineering offer alternative strategies to improving the salinity tolerance of a given crop plant (Stavarek and Rains, 1984; Hanson, 1984). Commercial crop yield for establishing salt tolerance is one of the remedies according to Mass and Hoffman (1977). It is very important to investigate the physiological basis of salt tolerance, in order to study the aspect of salt tolerance which can help to solve salinity problem. One of the useful strategies to combat soil salinity is to select salt tolerant crop. The variability in salt tolerance among varieties of crops offers excellent ground for growers to grow salt tolerant cultures to increase agricultural productivity under unfavourable environment like salinity.

According to Wadleigh and Gauch (1944), Magistad (1945), Brown and Hayward (1956) and Bernstein (1961), accumulation of soluble salts in soil increases osmotic pressure of soil solution reducing uptake of water and nutrients by plants. On the other hand , plants absorb constituents of saline solutions at different degrees which brings a

toxic or nutritional effect on the plants which is known as 'specific ion effect' (Eaton, 1942, Uvhits,1946). This view was supported by (Berg, 1950, Russel, 1950, Bernstein and Ayers; 1953).

Excess accumulation of salts under saline conditions disturbs dynamics of normal plant life due to effect of 'salt injury'. However, Russel (1950) and Kakade (1968) observed reduction in growth and yield in salt tolerant plants without any salt injury. Besides , the plants differ in their sensitiveness towards a particular saline condition within the genera, species and varieties (Bhardwaj, 1958, Torres and Bingham, 1973). Maas and Nieman (1978) and Lauchli and Epstein (1984) stated that such studies will help in the selection and breeding of crops for salt tolerance, for example, rice, sorghum, barley, wheat, corn, cotton, tomato and mung bean. Safflower and bajra are being tested. Such work is going on in Australia, Egypt, Israel, Italy, Korea, Kuwait, Pakistan, Philipines, Rumania, Russia, Spain, Taiwan, Tunisia and USA (Somers, 1979).

In India, about 7.5 million hectares of land has become salty due to faulty water management and excessive use of fertilizers (Abroal, 1984). Keeping this view in mind, it was invisaged to study salt tolerance of safflower Cv. Bhima. This cultivar is recent promising and is recommended for growing in the state of Maharashtra.

A) SAFFLOWER

Safflower is known by different names that is kusumbha in sanskrit, kusum or kusumphuli in Bengali, kardai or Kurdi in Marathi, kusumbo in Gujarati, Sendurakam in Tamil, kushumba in Telgu, kusumbe or kusum in Kannada and kasumba in Punjabi. It is also known as false saffron, bastard saffron, thistle saffron, dyers saffron, kszhirak. Ghurtum and suff.

a) Geographic origin

Carthamus tinctorius L., safflower belongs to family Asteraceae. There are approximately 25 valid species in the genus and they are distributed across Spain, North Africa, West Asia and India. Many species are indigenous to the Mediterranean region. The probable origin of cultivated safflower is a region bounded by eastern Mediterranean and the Persian Gulf (Knowles, 1969). Cultivated safflower is supposed to have originated either from saffron thistle or from wild safflower in the mountainous regions of Abyssinia and Afganisthan (Chavan, 1960). According to Vaviloe, the three centres of origin of safflower are India, Afganisthan and Ethiopia. Decandale was of the opinion that Arabia was the most probable centre of origin (ICAR, 1980).

Fragments of safflower plants and safflower seeds have been found in some of the ancient Egyptian tombs. Safflower has been domesticated for the orange dye which can be obtained from its florets and the clothes of mummies found in Egyptian tombs have been dyed saffron using this dye. Safflower was probably introduced to Egypt from the Euphrates. A bundle of single safflower flowers was found with the Eighteenth Dynasty mummy of Amenophis I (BC 1600) and was so well preserved that it could be accurately identified (Schweinfurth, 1887). An accurately dated revenue papyrus of

Ptolemy II (BC 259 - BC 258) states that the king had a complete monopoly on the production and marketing of certain vegetable oils including safflower (Keimer, 1924). Hasselquist (1762) while on a visit to Egypt, noted that the dye was exported to Italy, France and England where it was used for colouring and in the preparation of cheese.

Safflower is the latinized version of the Arab word quartum or qurtum which alludes to the colour of the dye obtained from flower. 'Usfar' is probably the origin of the English name 'Safflower via various written forms of usfar, affore, asfiore, saffiore. Safflower is mentioned as a medicinal plant in De Materia Medica written by a Greek physician, Pedanius (60 AD).

Safflower was introduced to England via Egypt (The Botanical Register) and was used as a food colouring and dye. Safflower has been used as a source of a dye from ancient times by carpet weavers of Iran and Afganisthan and was probably introduced into southern Russian regions from here. In ancient Indian Sanskrit, safflower has been described as kusumbha, from which the word 'kusum' is derived. Safflower oil was regarded as a purgative and it was used in a manner similar to castor oil. Florets of safflower are added to rice, bread and pickles to give them an attractive orange colour. The tubular florets are commonly used as an adulterant for true saffron or as a less costly substitute.

Safflower is considered to have been introduced to China around 200 - 300 AD as a dye. From China it was introduced to Japan. An early reference to safflower in USA is a research report of the University of California early this century (Weiss, 1983).

b) Distribution, Area and Production

Around 50 % of the world's production of safflower is in India. An area of about 0.9 million hectare annually produces a safflower crop fluctuating between 0.35 to 0.50 million tonnes (Virender Malik, 1995). Besides India, USA, Mexico, Ethiopia, Russia and Australia also cultivate safflower.

In India the states of Maharashtra, Karnataka and Andhra Pradesh are the dominant safflower producers (74 % of the area and 69 % of the production). In Maharashtra, safflower is cultivated mainly in the districts of Ahmednagar, Aurangabad, Beed, Latur, Osmanabad, Parbhani, Pune and Solapur (Vaidya et al., 1978).

In India when seed oil is the object, yields are about 90 - 130 kg florets / hectare and 440 to 660 kg of seed / hectare (C.S.I.R., 1948-1976). In Maharashtra state 310,000 hectares of land is under safflower cultivation from which 47,000 tonnes of seeds are harvested.

c) Climate, soils, season and rotations

In Maharashtra, Gujarat and southern India safflower is cultivated as a rain fed crop, but in other parts of the country, it is generally cultivated as an irrigated crop. It's rainfall requirement is between 62.5 to 100 cm.

Safflower is considered to be drought resistant as it is capable of tapping subsoil moisture or seepage not normally available to the majority of other crops. This may be because of its deep and efficient rooting system. Fully grown plants are extremely wind resistant and even after the seeds are mature, there is little loss from lodging and

shattering. It is a day neutral plant. However, varieties may show adaptation to specific photo periods and short photo period can prolong the rosette stage. From an assessment of available data, growing plants are damaged by temperature approaching freezing and even when there is little visual effect the yield can be drastically reduced by a fall in temperature. Frost on maturing crops affects yield and oil content of seeds. Thus, growth period from stem elongation to maturity should be frost free (Weiss, 1983). There could be an interaction between high temperature and soil moisture and the shortage of the moisture exacerbating effects of high temperature. There could be an interaction between high temperature and high humidity resulting in reduction of seed yield (Zimmermann, 1978).

Safflower can be grown in soils with pH values between 5 to 8. For commercial production safflower highest yields can be obtained on fairly deep, well drained and somewhat sandy loams of neutral reaction. Irrespective of their fertility shallow soils seldom produce high yields and this is invariably due to insufficient moisture. Dense soils retards soil growth. Acid soil can increase possibility of attack by Fusarium root rot. The major production, in India, is on typical black cotton soils with the rest of the production being grown on loams and light alluvial soils under dry and irrigated conditions. The crop shows a good response to nitrogenous fertilizers. Excessive rainfall or humidity increases possibility from fungal diseases and water logging due to poor drainage, even for short periods of time, drastically reduces seed yield.

Safflower, mixed with another crop, is grown as a single rabi crop of the year, following the kharif cotton crop taken during the previous year and followed by the kharif jowar during the next year. On heavy soils it is taken after an early kharif crop such as green

gram, black gram, coriander or early groundnut. On lighter soils safflower is usually rotated with jowar, wheat, bajra or even rice.

d) Cultivation of safflower

Being an oil seed crop, it is grown with rabi crop like jowar, wheat, barley or gram. Because of its spiny nature it is also grown as a border crop to protect the main crops against cattle trespass. Three rows of safflower are planted after nine to twelve or more rows of main crop. For the production of dye it is cultivated as an entire crop.

i) Pre cultivation :-

It is sown in October to November and harvested in March to April. Late varieties are harvested by the end of April. The land is ploughed once or twice after rains and the clods are crushed. Manure is applied as per the requirement of main crop.

ii) Sowing :-

Healthy, plump and improved seeds are selected. It is sown in moist soil at a depth of 3-5 cm in one to three rows 45 - 55 cm apart alternately with 6 to 12 or more rows of the main crop. Planking is necessary after sowing to ensure proper germination. The seed rate varies from 5 - 12 kg / hectare depending on soil fertility and the nature of the crop. Plants growing very close to each other have a tendency to develop thinner stem or a superficial root system and less number of flowers.

iii) After care :-

Interculturing is done two to three times at fortnightly intervals when the plant is 7.5 cm high. Pure crop is given one or two weeding on 20^{th} and 45^{th} day after sowing. An application of 20 to 45 kg of nitrogen per hectare results in a substantial increase in the yield. Mixed crop shares the preparatory tillage, manuring and cultivation given to the main crop. The young crop is nipped at the top after two months which encourages

branching which is meant for inducing more flowering which results in greater production of seed.

iv) Harvesting :-

The safflower crop ripens in 110 to 120 days after sowing. The crop is harvested after the main crop by pulling out the plant when there is dew on the drop.

v) Yield :-

Average yield of seed for entire crop is 200 - 300 kg per acre while that of the mixed crop is 50 kg per acre. An irrigated crop to which 5000 kg of manure is added yields 750 - 1000 kg of seed per acre. The oil content of seed varies from 20 - 30 %. 41 kg of seed yields about 8 kg of oil, 14 kg oil cake and 19 kg husk.

e) Morphology :-

Safflower is highly branched, herbaceous, annual, varying in height from 30 - 60 cm in dwarf types and 90 - 150 cm in tall types. It has fleshy trap roots producing thin horizontal laterals. The stem is stiff, cylindrical, smooth, glabrous, grey, green to white in colour. The stem has fine longitudinal grooves and becomes brittle when mature. The leaves are alternate, large, lanceolate, deeply serrated on lower stem. The lower leaves are spineless while upper leaves have spiny tips. The leaf size and shape varies between varieties from 2.5 - 5.0 cm broad and 10 - 15 cm long.

The inflorescence consists of numerous florets collected together on flattened receptacle which is covered by several layers of involucral bracts, outer ring being heavily spined. It protects the developing inflorescence. Individual florets have bracts in form of hairs. Flowers are yellow to orange red. Each capitulum contains 100 florets, outer sterile ray florets and inner fertile bisexual disc florets. Pollination is carried out by

honey bees at the time of flowering. The fruit is achene. The fruits are smooth, angular, glabrous, obovoid truncate at the top with four bosses. The number of seeds per capitulum varies from 18 - 23.

f) Varieties under cultivation:-

Safflower plants are grouped into two broad varieties - those having spiny leaves and those with spineless or sparingly spiny leaves. The spiny varieties are oil yielding forms having yellow flowers while the spineless varieties are the dye yielding types having orange flowers or yellow flowers tinged with scarlet.

Various varieties recommended for different states of India are as follows (Rajan, 1974):-

State	Improved variety
Andhra Pradesh	'7-13-3'
Gujarat	'N.62-3'
Haryana	'A 300', 'N. 62-8'
Karnataka	'17-3-3'
Madhya Pradesh	N. 62-8'
Maharashtra	'N. 62-8', '116-42'
Rajasthan	'A. 300', 'N. 62-8'
Tamil Nadu	'K 1', '6503'
Uttar Pradesh (Eastern)	'S 2-27', 'N. 62-8', '6503'
West Bengal	'N. 62-8'

Some promising varieties of safflower along with days for maturity, yield and oil content are given in the table below :-

State	Variety	Days to maturity	Yield (kg per hectare)	Oil content
Andhra Pradesh	'Manjra' (C 438)	110	1200	32.0 %
Karnataka	'A - 1'	125	800 - 850	30.8 %
	'A - 300'	125	750 - 800	31.9 %
Madhya Pradesh	'N0, 7'	140	700 - 850	30.3 %
Maharashtra	'Tara'	120 - 125	1200 - 1400	32.5 %
	'N. 62-8'	130 - 135	1400 - 1600	30.0 %
	'Bhima'	110-120	1400 - 1500	30.0 %
	'Nag-7'	135 - 140	1000 - 1250	30.0 %
Tamil Nadu	'K- 1'	120	600 - 800	30.5 %

'Bhima' is the second highest yielding variety and is recommended for cultivation in Ahmednagar, Pune, Sangli, Satara, Solapur districts and Marathwada regions.

g) Economic importance

Safflower crop is an economically important crop since many centuries. It yields the following commercial products such as safflower oil, safflower dye, oil cake, green vegetable, fodder, medicinal uses and other uses.

i) Safflower oil :-

Safflower oil is an important industrial product. It is cultivated for edible oil obtained from its seed. It contains higher percentage of essential unsaturated fatty acids and a lower percentage of saturated fatty acids than other vegetable seed oils. The oil is light coloured and easily clarified. Safflower oil lowers blood cholesterol levels and is used to treat heart diseases. The highest yielding variety is Tara and Bhima yields 30 %. The oil content of the seed depends on the nature of the soil and climate. The component fatty acids of oil are myristic acid (with lauric and lower acids) 1.5%, palmitic 3.0%, stearic 1.0%, arachidic 0.5%, oleic 33.0% and linoleic acid 61.8%.

The safflower oil is mainly used for culinary purpose, burning of lamps and manufacture of soap. It is used as an adulterant of sesame oil and ghee. It is an ingredient of macassr hair oil. The 'sweet oil' of Mumbai is obtained by crushing groundnut, sesame and safflower seeds. Under certain conditions the oil develops an unpleasant flavour which is corrected by using extract of turmeric , clove, nutmeg fruit , pepper, red chillies, cinnamon leaves, betel leaves and dry ginger. It is used in paints, varnishes and linoleum. A gelatinous roghan is prepared by heating the safflower oil to high temperature of 150 °C for two hours and then pouring into cold water. This product is used as glass cement and is a substitute for plaster of Paris. The safflower oil is also used for making waterproof cloth.

ii) Safflower dye :-

The florets contain two colouring materials, carthamidin, cartharmin and iso-carthmin, of which carthamidin is yellow and water soluble while cartharmin is orange-red dye, insoluble in water, but soluble in alkaline solution. Cartharmin is found in florets and

ranges from 0.3 - 0.6 % and imparts bright red colour to cotton and silk fabrics. The yellow colouring matter is present in the range of 26 - 36 % in the florets. Safflower dye is used in India to dye clothing used for ceremonial purposes, toys, cosmetics, artificial decorations, food and confectionery, etc. Along with starch and talc it is used for preparing rouge.

iii) Safflower cake :-

The oil cake obtained from decorticated seeds is used as cattle feed while the cake obtained form un-decorticated seeds is used as manure. The oil cake contains fats, proteins, carbohydrates, fibres, ash, nitrogen, potash and phosphoric acid. If used as an organic fertilizer, it improves the physical condition of heavy soils. The cake from decorticated seeds is fed to lambs and poultry.

iv) Green vegetable :-

The tender shoots of the plant are eaten as pot herb and salad. Young leaves contain iron and carotene.

v) Fodder :-

The green safflower yields a fodder of 20 tonnes per acre,which is relished by cattle. Fresh safflower hay, cut before flowering, is relished by sheep. Safflower is also made into silage and fed to hogs in foreign countries.

vi) Medicinal uses :-

Safflower is used as a folk medicine for treating various diseases especially inflammatory tumours of the liver (Hartwell, 1967-1971). Flowers are diaphoretic, emmenagogue, laxative, sedative, stimulant, and in large doses laxative; used as a substitute for adulterant for saffron,in treating measles, scarlatina and other exanthematous diseases. Charred safflower oil is used for rheumatism and sores; seeds, diuretic and tonic (C.S.I.R. 1948-1976). In China, it is prescribed as uterine

astringent in dysmenorrhea (Keys, 1976). In Iran, the oil is used as asalve for sprains and rheumatism. (James A. Duke 1984, Handbook of Energy crops unpublished updated on 9.8.97)

The dye obtained from the flower is also used as cathartic, antioxidant, anti-inflammatory, analgesic, anticonvulsant, antithrombosis and diuretic (Verma et al., 1997).

vii) Other uses :-

It is suggested that hull may be used in the manufacture of cellulose, insulation, abrasives, etc. The seeds are eaten by several wild birds, fowls, parrots, pheasants and turkeys. Seeds, both edible and nutritious, are eaten roasted or fried and used in chutney in India. A brew made from foliage is said to prevent abortion. Powder of dried safflower leaves is used for curdling milk (Weiss, 1983).

B) SCOPE OF THE PRESENT INVESTIGATION AND WORK DONE ON SAFFLOWER UNDER SALINE CONDITIONS.

According to Ghorashy et al. 1972, in three varieties 'Ute', Iranian local 3151 and Iranian local 2811 of safflower, germination reduced with increasing salinity from 0 to 1 % NaCl. However, Iranian local 3151 showed least reduction in percent germination as compared with the other two of NaCl concentrations greater than 1 %. Francois and Bernstein (1964), observed that yield decline of safflower was 10 % at 7 mmhos per cm and 20 - 25 % at 11 mmhos per cm, thus, categorising safflower under tolerant group. Salt tolerance during germination was half that of later stages of growth. According to Yermanos et al. (1964), soil salinity depressed oil content, seed weight, protein content in seed which contributed to total yield of the tertiary heads. It increased percent hull

content of the seed but did not affect the fatty acid composition of the oil. These results indicated varietal difference in salt tolerance capacity of safflower. Iyengar et al. (1977) observed that, in safflower, plumule growth was more affected than radicle, when subjected to various dilutions of sea water during germination stage.

Darra et al. (1978) reported that safflower, when treated with NaCl, Na_2SO_4 and $CaCl_2$ 2:1:1 ratio gave 100% germination upto 12.0 mmhos/cm and at 16.0 mmhos/cm germination, percentage was reduced by 10% and at 25.0 mmhos/cm germination percentage was reduced by 90.0%. These observations are similar to those given by Francois and Bernstein (1964). Similar results were given by Janardhan et al. (1986), where the reduction in yield in safflower ranged from 0.2 to 31.4 % at 8 mmhos/cm and 9.1 to 68.1% at 12 mmhos/cm. Also, they reported that the varieties (A-300, 7-13-3, US-10) of safflower which exhibited less reduction in yield under saline conditions accumulated relatively lower amounts of Na and maintained higher K and higher K:Na ratios in leaves. The yield reduction of safflower under salinity is caused by a decrease in number of heads and yield of seed per head, the latter being affected by seed weight and not by seed number (Francois and Bernstein, 1964, Yermonas et al., 1964). Based on the observations of Mass and Hoffmann (1977) and Bresler et al. (1982) , safflower was categorised under moderately salt tolerant plant with salinity threshold at ECe of 6.5 mmhos/cm and 50% reduction at ECe 12.0 $mScm^{-1}$.

According to Nieman et al. (1988), when Carthamus tinctorius L. Cv. Gila was subjected to salt stress (51.0 mol m^{-3} NaCl plus 25.5 mol m^{-3} $CaCl_2$) and analysed for nucleotide by HPLC, it was found that safflower had switched to flower bud formation and salt stress reduced fresh shoot yield by half. Salt also reduced ATP pool and ATP/ADP ratios in source leaves. It had little or no effect on ATP or other nucleotide

pools in safflower buds. The UDPG pool was not affected in source leaves of safflower buds. Salt stress had little or no effect on UDPG, hexose or ester phosphate in source leaves or buds. Stress did not affect assimilation of photosynthate in more tolerant safflower. Irving et al. (1988), reported that increasing salinity in irrigation water decreased seed yield, plant height, oil content and increased growth rate in salt stressed plants. Fatty acid composition of high linoleate safflower oil was not altered with increasing salinity. Fatty acid composition was altered in the high oleate cultivar, resulting in depressed oleic acid content in the oil. Cl, Ca and Na increased while P and Mg decreased in leaf tissues with increasing salinity levels.

According to Goswami et al. (1978) variety Nagpur-7 proved to be highly salt tolerant followed by 319-12 at higher levels of salt content. The germination percentage of Nagpur-7 was 53.3% at (20 mmhos/cm) while N-62-8 and safflower 6503 were found to be least salt tolerant and were of 23.3% and 25% respectively at salinity level of 20 mmhos/cm. Results of Kole and Gupta (1982) revealed that high concentration of NaCl (50 and 200 mM) inhibited germination percent, shoot / root length, whereas , low concentrations (5 mM) promoted same in safflower Cv. N-140. Soluble carbohydrates decreased at 200 mM and increased at 5 mM in embryo axis and cotyledons and amino acids increased significantly. Nakhlawy and Fawal (1989) indicated that growth of safflower seedlings was affected severely by NaCl than Na_2SO_4.

Application of saline water of electrical conductivity 4, 8, 12, 16 and 24 ds/m reduced seed yield by 9, 25.4, 34.6 and 45.0 % in vertic ustochrept soil and by 2.8, 6.2, 11.8, 23.7 and 37.8 % in Fluventic Eutrochrept soil with significant yield reduction with 8 ds/m in 'Bhima' Cv. of safflower (Carthamus tinctorius L.) (Singh et al., 1995).

From all these above mentioned references, it is clear that work done on safflower under saline conditions is scanty or meagre. This fact prompted us to select work on physiological studies in safflower Cv. Bhima under saline conditions. Cultivar Bhima is a recent promising cultivar. This cultivar is recommended for the state of Maharashtra. More than 7.5 million hectares of land is saline in India. In the state of Maharashtra, about 60,000 hectares of land has gone out of cultivation due to salinity (Abroal,1984). Major saline lands of Maharashtra have become salty either due to NaCl or Na_2SO_4 or both. Therefore, it was proposed to study effect of chloride and sulphate salinizations on growth and yield of safflower Cv.Bhima. In order to explain adaptations to salinity, several physiological processes such as mineral nutrition, organic metabolism, photosynthesis, photosynthetic and oxidative enzymes and protein profile were proposed to investigate under saline and control conditions with the hope that such studies will be useful in choosing cultivars by farmers to grow under saline conditions as well as it will be useful in understanding adaptive mechanisms in Cv. Bhima of safflower in response to NaCl and Na_2SO_4 salinizations.

CHAPTER III

GROWTH AND PRODUCTIVITY UNDER SALINE CONDITIONS

1) INTRODUCTION

Growth of agricultural crops is studied under natural conditions since long time. Many factors are responsible for alterations in the growth of the plant which may be beneficial or may be hazardous to the plant ,for instance,light, temperature, water, chemicals ,etc. For centuries man has known that the deleterious effects of heat waves, droughts, mineral deficiency, disease, epidemics, climatic conditions and salinity in soil on crop plants and the development of environmental stress in nature is a natural event (Poljakoff- Mayber, 1982).

The physical and chemical nature of soil affects the plant growth. Various minerals and inorganic elements are essential for normal growth of the plant. Most of the soils of Indo - Gangetic belt are alkaline, while those of the other region are saline. (Yadav, 1980). Saline soils affect the absorption of minerals leading to abnormalities in plant metabolism which affects growth and yield. According to (Abroal et al.,1984) in India, about 7.5 million hectare of productive land has become salty due to heavy use of chemicals, fertilizers, over irrigation and poor drainage. In Maharashtra, about 3.4 lakh hectare of land is saline. The distribution and causes for the development of saline and alkaline soils in Maharashtra has been investigated by several investigators.

The ability of the plant to tolerate high concentrations of salt is salt tolerance. It is expressed with reference to salinity level which causes decline in the yield. Crop plants show greater spectrum of salt tolerance from very sensitive to fairly tolerant. Thus, salt tolerance varies from species to species and within different varieties of same species (Bernstien and Ayers, 1953; Pearson et al., 1966; Torres and Bingham, 1973; Shannon, 1978 and Rathert, 1982 (abc)).Therefore, in the present investigation

attempts have been made to study the effects of sodium chloride and sodium sulphate salts on growth and productivity of safflower Cv. Bhima.

2) MATERIALS AND METHODS

The plants of Carthamus tinctorius L. Var. Bhima were grown in cement pots in the research laboratory, Botany Department, University of Pune. An artificially salinized pot culture method was followed for salt stress studies experiment. (USDA handbook No. 60, 1954). The pure certified seeds of Var. Bhima were obtained from the College of Agriculture, Pune-7.

A. Experiment A:- Preparation and maintenance of salinity in pot culture.

a] Preparation of pots : - Forty two square cement pots of 25cm x 30cm were selected and filled with 5 Kg of homogeneously mixed air dried soil and well rotted compost in 3 : 1 proportion . The pots did not have any drainage hole. Three sets were prepared . Each set had fourteen pots.

b] Sowing of seeds:- In each pot, thirty seeds were sown at equal distance and depth. The pots were watered every day on the basis of water holding capacity of soil. After seven days of germination, uniform eight seedlings were maintained by removing others.

c] Salt treatment:- After fifteen days of germination , plants were treated with sodium chloride and sodium sulphate, except for the controls. At the same time, the control pots were irrigated with equal amount of tap water. The treatments were given to the plants according to the description given in the USDA Handbook No. 60 (1954).

i) Sodium chloride treatment :- Three sets of pots were treated with sodium chloride and calcium chloride salt solution. The Electrical conductivity of soil in pot was raised from ECe 5.0, 7.5, 10.0, 12.5, 15.0 and 17.5 $mScm^{-1}$(USDA Hand book No. 60) by adding required amount of salts.

ii) Sodium sulphate treatment :- Three sets of pots were treated with sodium sulphate and calcium chloride salt solution. The Electrical conductivity of soil in pot was raised from ECe 5.0, 7.5, 10.0, 12.5, 15.0 and 17.5 $mScm^{-1}$(USDA Hand book No. 60) by adding required amount of salts.

d] Salinity status :- The electrical conductivity (of soil saturation extract) level was maintained constant by adjusting salt solutions in the soil from time to time. For this, soil samples from 20 cm depth of the pots were collected after seven days and air dried. Their soil saturation extracts were subjected to ECe tests expressed in terms of $mScm^{-1}$ at 25 °C (Wadleigh and Gauch, 1944).

All treatments were set in three multiples . 6 X 3 sets for NaCl treatment and 6 X 3 sets for Na_2SO_4 treatments were set with 1 X 3 sets of control pots. The concentrations of salts were calculated according to the water holding capacity of the soil. The Calcium Chloride salt was added to each pot during each treatment for the maintenance of constant pH (USDA Hand book No. 60 ,1954). Each pot was treated with salt solution while control was irrigated with equal amounts of tap water. On the basis of water holding capacity of the soil, each pot was irrigated. Required ECe was maintained throughout the experiment by analysing ECe of each pot per week and by adding required amounts of respective salts. Eight plants were grown in each pot.

Growth in terms of height was studied at 30, 60 and 90 days of germination. Productivity per plant was studied at flowering stage as well as at post harvest.

B. Experiment B :- Growth Observations.

Following growth observations were recorded after 30, 60 and 90 days of germination.

a)Height:-

Height of the main shoot was measured from the soil surface to the base of the uppermost leaf or to the base of capitulum after 30, 60 and 90 days of germination.

b) Total leaf area :-

The total mean leaf area per plant for each salinity treatment and control was calculated after 30 and 90 days of germination by using graph papers (Sestak et al., 1971).

c) Dry matter at flowering :-

The productivity per plant was studied at flowering by following Sestak et al. (1971) method. Plants were uprooted , cleaned with tap water and then with distilled water. With the help of blotting paper, plants were made dry. Each plant organ was separated, its fresh weight was taken and kept for drying in Oven at 60° C till constant weight (Sestak et al., 1971).

d) Yield and Post harvest studies :-

At maturity watering to the crop was stopped and post harvest studies were done when plants were completely dried in the pots. The post harvest studies were done according to Sestak et al. (1971) method.

3) RESULTS

a) Height

The effect of increasing levels of NaCl and Na_2SO_4 on height, are recorded in the Tables 1,2 and Figs 1a,1b, 2a,2b , after 30, 60 and 90 days of germination. The results indicated that low concentrations (ECe 5.0 to 10.0 $mScm^{-1}$) of NaCl stimulate, while high concentrations (ECe 15.0 to 17.5 $mScm^{-1}$) of it inhibit growth in height at 30 days of germination. However, after 60 days of growth, height was decreased linearly with increasing levels of NaCl ,thereby ,indicating that all the levels of salinity inhibit growth in height of safflower. At 90 days of growth, height was less than the control at all levels of chloride salinization. Plants growing at ECe 12.5 to 17.5 $mScm^{-1}$ died during 60 to 90 days of growth. Thus, at 90 days of growth plants could survive upto ECe 10.0 $mScm^{-1}$ of NaCl while at all higher levels plants died indicating its more susceptibility to NaCl during flowering phase of the life cycle. At ECe 10.0 $mScm^{-1}$ plants died during 90 to 115 days of growth.

With increasing levels of sulphate salt, height increased linearly upto ECe 10.0 $mScm^{-1}$ of Na_2SO_4 at 30 days of germination. At ECe 10.0 $mScm^{-1}$, it was more than the control but was less than that of ECe 7.5 $mScm^{-1}$. At higher (ECe 12.5 to 17.5 $mScm^{-1}$) levels height was linearly decreased with increasing levels of sulphate salinizations.

At 60 days of growth, height was more than the control upto ECe 10.0 $mScm^{-1}$ while it was less than the control in plants growing at ECe 12.5 to 17.5 $mScm^{-1}$. At 90 days of growth, height was more than the control upto ECe 10.0 $mScm^{-1}$ and was less than the control at ECe 12.5 $mScm^{-1}$. During 60 to 90 days of growth, plants growing at ECe 15.0 to 17.5 $mScm^{-1}$ could not survive, while plants growing at ECe 12.5 $mScm^{-1}$ died

Table. 1.

Effect of increasing concentration of NaCl salinity on height of Carthamus tinctorius L. Var. Bhima

Treatment ECe $mScm^{-1}$	Height after 30 days of germination in cm	Height after 60 days of germination in cm	Height after 90 days of germination in cm
Control 0.44	11.480	23.373	54.197
NaCl 5.00	11.507	22.800	52.377
NaCl 7.50	13.063	21.463	50.513
NaCl 10.00	12.017	20.367	49.510
NaCl 12.50	11.471	19.910	23.41
NaCl 15.00	11.267	17.833	-
NaCl 17.50	11.117	17.717	-
SEM	7.47	13.31	36.54
LSD	23.02	41.02	112.61
SD±	± 0.639	± 2.019	± 1.929

LSD at 5% level of significance

- Plants died during 60 to 90 days of growth

Table. 2.

Effect of increasing concentration of Na_2SO_4 salinity on height of Carthamus tinctorius L. Var. Bhima

Treatment ECe $mScm^{-1}$	Height after 30 days of germination in cm	Height after 60 days of germination in cm	Height after 90 days of germination in cm
Control 0.44	11.480	23.373	54.197
Na_2SO_4 5.00	14.503	25.290	56.163
Na_2SO_4 7.50	16.727	24.833	55.150
Na_2SO_4 10.00	14.283	23.733	54.420
Na_2SO_4 12.50	11.470	19.967	31.810
Na_2SO_4 15.00	10.260	14.940	18.31
Na_2SO_4 17.50	9.837	12.517	-
SEM	8.42	14.00	35.51
LSD	25.97	43.16	109.45
SD±	± 2.40	± 3.68	± 9.422

LSD at 5% level of significance

- Plants died during 60 to 90 days of growth

Fig. 1a.

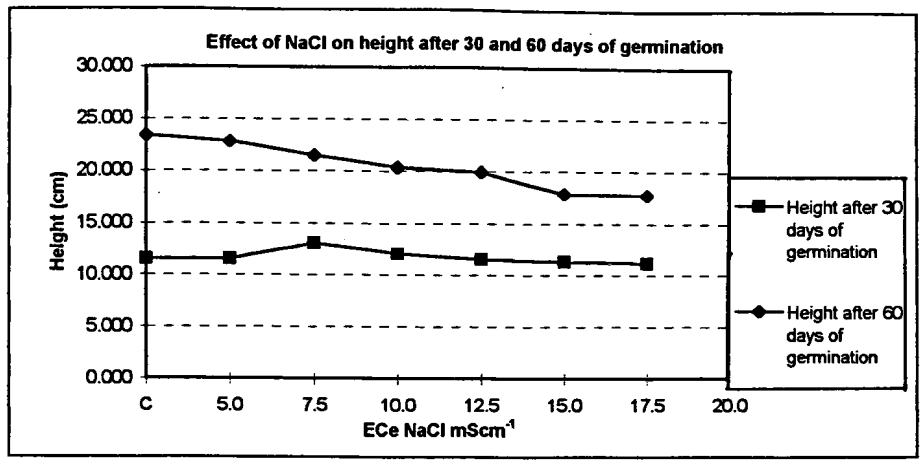

Effect of NaCl on height after 30 and 60 days of germination
30.000
25.000
20.000
15.000
10.000
5.000
0.000
Height (cm)
C
5.0
7.5
10.0
12.5
15.0
17.5
20.0
ECe NaCl mScm-1
Height after 30 days of germination
Height after 60 days of germination

Fig. 1b.

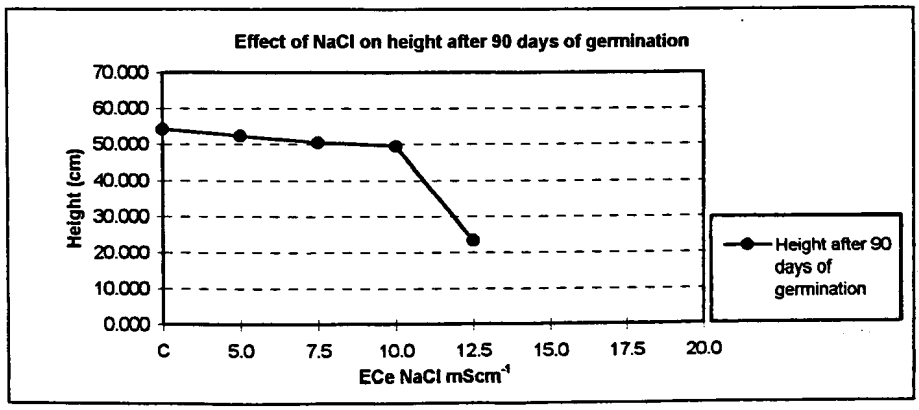

Effect of NaCl on height after 90 days of germination
70.000
60.000
50.000
40.000
30.000
20.000
10.000
0.000
Height (cm)
C
5.0
7.5
10.0
12.5
15.0
17.5
20.0
ECe NaCl mScm-1
Height after 90 days of germination

Fig. 2a.

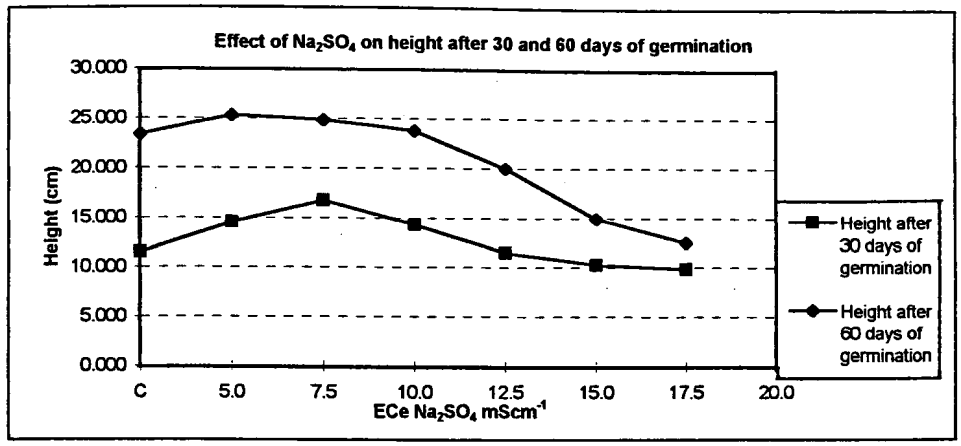
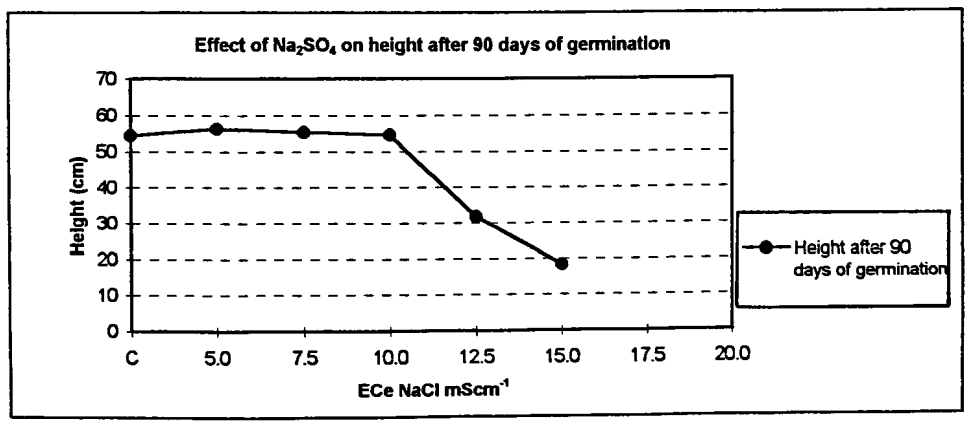

Effect of Na_2SO_4 on height after 30 and 60 days of germination
30.000
25.000
20.000
15.000
10.000
5.000
0.000
Height (cm)
C
5.0
7.5
10.0
12.5
15.0
17.5
20.0
ECe Na_2SO_4 $mScm^{-1}$
Height after 30 days of germination
Height after 60 days of germination

Fig. 2b.


Effect of Na_2SO_4 on height after 90 days of germination
70
60
50
40
30
20
10
0
Height (cm)
C
5.0
7.5
10.0
12.5
15.0
17.5
20.0
ECe NaCl $mScm^{-1}$
Height after 90 days of germination

after 90 days of growth. Thus, low levels of sulphate stimulate while high levels of it inhibit growth in height in Carthamus tinctorius Cv. Bhima.

b) Leaf area

Effect of increasing concentrations of NaCl and Na_2SO_4 salinity's on leaf area per plant are depicted in Tables 3,4 and Fig. 3,4.

The leaf area of Carthamus tinctorius Cv. Bhima was calculated after 30 and 90 days of growth. From the results, it is clear that at 30 days of growth the total leaf area per plant was increased upto ECe 12.5 $mScm^{-1}$ of NaCl over the control. However, at high (ECe 15.0 and 17.5 $mScm^{-1}$) levels of NaCl, it was less than the control. Thus, it was observed that low concentrations of NaCl stimulate formation of leaf area while high concentrations of it inhibit the same in safflower Cv. Bhima. At 90 days of growth leaf area was more than the control upto ECe 7.5 $mScm^{-1}$ of NaCl, while it was less than the control at ECe 10 $mScm^{-1}$.

Under sodium sulphate salinity, at 30 days of growth, leaf area was less than the control which reflects that all levels of Na_2SO_4 inhibit formation of leaf area in safflower Cv. Bhima. At 90 days of growth, leaf area was also less than the control at all levels of Na_2SO_4 salinity. Thus, all levels of Na_2SO_4 inhibit leaf area growth.

c) Productivity at flowering (dry matter)

Effect of increasing levels of NaCl and Na_2SO_4 salinity's on root, stem and leaves per plant and average total (R+S+L) dry weight per plant is recorded in Tables 5,6. Fig. 5,6.

Table. 3.

Effect of increasing concentrations of NaCl on leaf area of Carthamus tinctorius L. Cv. Bhima

Treatment ECe $mScm^{-1}$	Leaf area cm^2 / plant			
	After 30 days of germination	% of control	After 90 days of germination	% of control
Control 0.44	273.3	100.00	245.2	100.00
NaCl 5.0	360.2	131.80	302.2	123.25
NaCl 7.5	325.8	119.21	269.8	110.03
NaCl 10.0	368.2	134.72	169.3	69.00
NaCl 12.5	294.6	107.79	-	-
NaCl 15.0	265.8	97.26	-	-
NaCl 17.5	120.5	44.09	-	-
SEM	200.59		111.61	
LSD	618.15		343.94	
SD±	± 41.08		± 24.75	

LSD at 5% level of significance

- Plants died during 60 to 90 days of growth

Table. 4.

Effect of increasing concentrations of Na_2SO_4 on leaf area of Carthamus tinctorius Cv. Bhima

Treatment ECemScm^{-1}	Leaf area cm^2 / plant			
	After 30 days of germination	% of control	After 90 days of germination	% of control
Control 0.44	273.3	100.0	245.2	100.00
Na_2SO_4 5.0	225.0	82.3	244.8	99.83
Na_2SO_4 7.5	242.2	88.6	235.1	95.88
Na_2SO_4 10.0	228.1	83.5	220.8	89.72
Na_2SO_4 12.5	234.2	85.7	180.8	73.73
Na_2SO_4 15.0	187.0	68.4	-	-
Na_2SO_4 17.5	124.8	45.7	-	-
SEM	147.38		114.80	
LSD	454.16		353.78	
SD±	± 26.24		± 10.35	

LSD at 5% level of significance

- Plants died during 60 to 90 days of growth

Fig. 3.

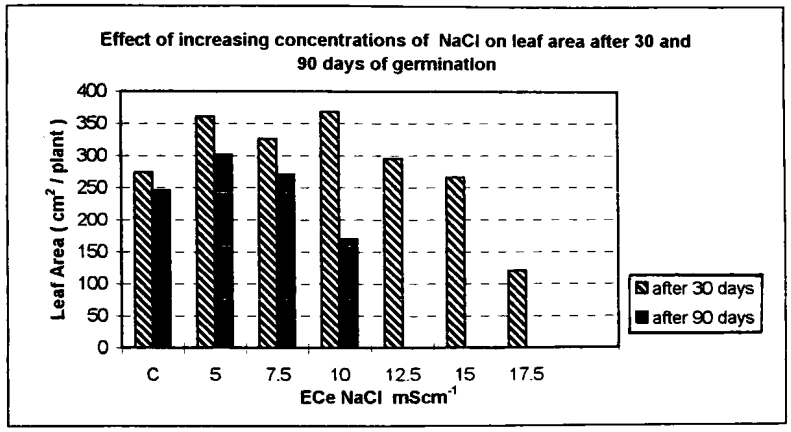

Effect of increasing concentrations of NaCl on leaf area after 30 and 90 days of germination
Leaf Area (cm² / plant)
400
350
300
250
200
150
100
50
0
C
5
7.5
10
12.5
15
17.5
ECe NaCl mScm⁻¹
after 30 days
after 90 days

Fig. 4.

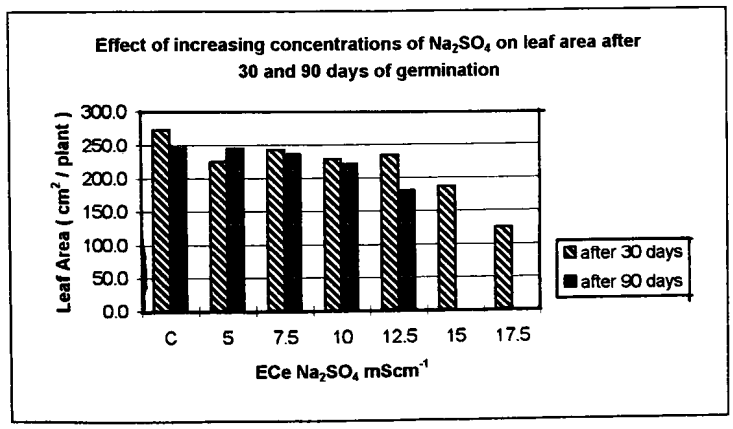

Effect of increasing concentrations of Na₂SO₄ on leaf area after 30 and 90 days of germination
Leaf Area (cm² / plant)
300.0
250.0
200.0
150.0
100.0
50.0
0.0
C
5
7.5
10
12.5
15
17.5
ECe Na₂SO₄ mScm⁻¹
after 30 days
after 90 days

Table.5.

Effect of NaCl salinity on productivity of root, stem, leaf , total plant, days of flowering and maturity in Carthamus tinctorius L. Var. Bhima at flowering

Treatment ECe $mScm^{-1}$		Total root (R) weight/ plant (g)	Total stem (S) weight/ plant (g)	Total leaf (L) weight/ plant (g)	Total plant (g) (R + S + L)	Total as % of the Control	Days for flowering	Days for maturity
Control	0.44	0.20	0.52	0.58	1.30	100.00	88	111
NaCl	5.0	0.16	0.48	0.61	1.25	96.15	81	109
NaCl	7.5	0.14	0.42	0.50	1.06	81.53	85	110
NaCl	10.0	0.13	0.29	0.24	0.66	50.76	83	107
NaCl	12.5	0.04	0.12	0.10	0.26	20.00	**77	-
NaCl	15.0 *	0.01	0.08	0.07	0.16	12.30	-	-
SEM		0.009	0.008	0.006				
LSD		0.029	0.027	0.020				
SD±		±0.06	± 0.17	±0.22				

LSD at 5% level of significance

*Plants died during 60 to 90 days of growth.

**Plants died within 15 days after flowering.

Table.6.

Effect of Na_2SO_4 salinity on productivity of root, stem, leaf , total plant, days of flowering and maturity in Carthamus tinctorius L. Var. Bhima at flowering

Treatment ECe $mScm^{-1}$		Total root (R) weight/ plant (g)	Total stem (S) weight/ plant (g)	Total leaf (L) weight/ plant (g)	Total plant mean in (g) (R + S + L)	Total as % of the Control	Days for flowering	Days for maturity
Control	0.44	0.20	0.52	0.58	1.30	100.00	88	111
Na_2SO_4	5.0	0.26	0.72	0.71	1.69	130.00	92	114
Na_2SO_4	7.5	0.32	0.75	0.80	1.87	143.84	95	117
Na_2SO_4	10.0	0.16	0.53	0.47	1.16	89.23	91	112
Na_2SO_4	12.5	0.10	0.22	0.17	0.49	37.69	83	106
Na_2SO_4	15.0 *	0.01	0.10	0.08	0.19	14.61	**75	-
SEM		0.008	0.006	0.008				
LSD		0.025	0.021	0.025				
SD±		±0.10	± 0.24	±0.27				

LSD at 5% level of significance

*Plants died during 60 to 90 days of growth.

**Plants died within 15 days after flowering.

Fig. 5.

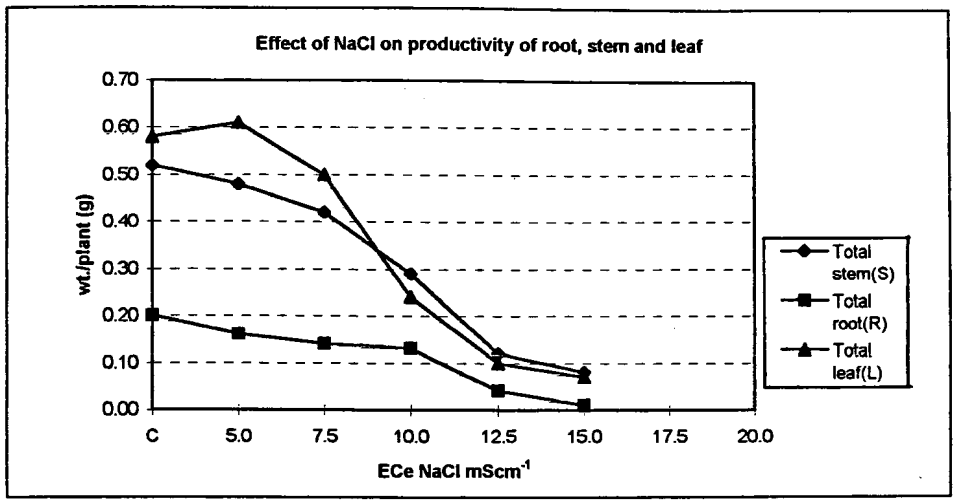
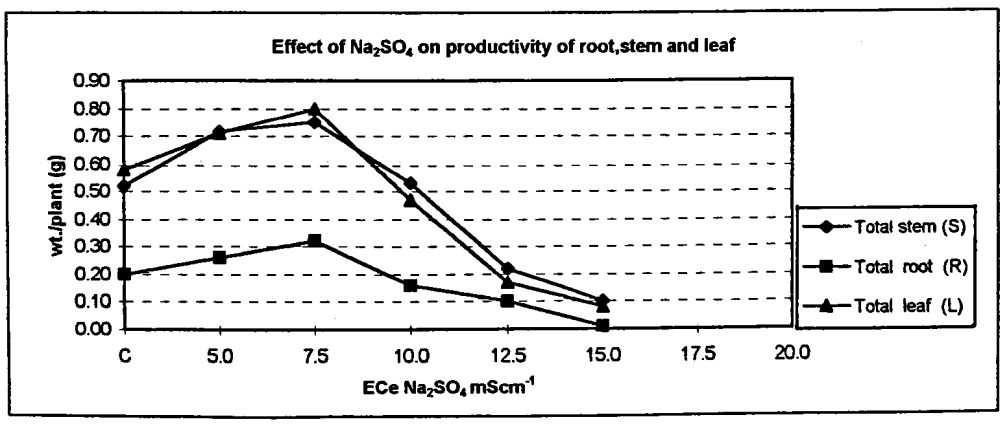

Effect of NaCl on productivity of root, stem and leaf
wt./plant (g)
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
C
5.0
7.5
10.0
12.5
15.0
17.5
20.0
ECe NaCl mScm-1
Total stem(S)
Total root(R)
Total leaf(L)

Fig. 6.


Effect of Na2SO4 on productivity of root,stem and leaf
wt./plant (g)
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
C
5.0
7.5
10.0
12.5
15.0
17.5
20.0
ECe Na2SO4 mScm-1
Total stem (S)
Total root (R)
Total leaf (L)

i) Root

From the observations (Tables. 5,6) , it is clear that there is decrease in root weight with increasing sodium chloride salinity. However, under sulphate salinity, root weight increased at ECe 5.0 $mScm^{-1}$and ECe 7.5 $mScm^{-1}$ and decreased at all higher levels. Thus, all levels of chloride inhibit, while low levels of sulphate stimulate and high levels of it inhibit root growth indicating different effect of sulphate and chloride salinity on root growth.

ii) Stem

Reduction in stem weight per plant (Tables.5,6) was observed with increasing concentrations of sodium chloride. On the contrary, under sulphate salinity, the stem weight increased in plants grown upto ECe 10.0 $mScm^{-1}$ and reduced at all higher concentrations of sulphate salinity which reflects that low levels of sulphate stimulate and high levels of it inhibit stem growth.

iii) Leaves

Average leaf weight per plant (Tables.5,6) increased over the control in plants growing at low (ECe 5.0 $mScm^{-1}$) NaCl salinity. However, at high (ECe 7.5 to 15.0 $mScm^{-1}$) levels leaf weight decreased with increasing salinity levels. Under sulphate salinization, leaf weight increased at low (ECe 5.0 and 7.5 $mScm^{-1}$) but decreased at all higher levels. Thus, low levels of both the salts stimulated, while high concentrations of them inhibited growth of leaves in Carthamus tinctorius Cv. Bhima.

iv) Total (R+S+L) DM per plant at flowering

It was less than the control at all levels of chloride (Tables. 5,6) , whereas, it was increased in plants growing upto ECe 7.5 $mSCm^{-1}$ of sulphate and was less than the control at all higher levels. Thus, all high levels of chloride were detrimental to safflower

while low levels of sulphate stimulate growth and high levels of it inhibit growth in safflower Cv. Bhima. The dry matter per plant was very less at ECe 10.0 to 15.0 $mScm^{-1}$ of NaCl and at ECe 12.5 and 15.0 $mScm^{-1}$ of Na_2SO_4 because plants growing at ECe 12.5 to 17.5 $mScm^{-1}$ of NaCl died during 60 to 90 days of growth and plants growing at ECe 12.5 to 17.5 $mScm^{-1}$ of sulphate also died during 60 to 90 days of growth.

v) Moisture percentage at flowering

The results of moisture percentage at flowering stage are indicated in Tables 7, 8. Under NaCl salinity in roots, the moisture percentage decreased with increasing salinity. Under sulphate salinity, the moisture percentage increased upto ECe 7.5 $mScm^{-1}$ and decreased further with increasing salinity. In stem, it decreased with increasing NaCl and Na_2SO_4 salinity. In leaf, the moisture percentage decreased under NaCl salinity but under Na_2SO_4 salinity moisture percentage increased upto ECe 10.0 $mScm^{-1}$ and decreased further at high salinity levels. However,moisture content of total plant decreased under both the salinity's.

d) Post harvest studies

Effect of increasing concentrations of NaCl and Na_2SO_4 on root, stem, leaf, husk and grain weight per plant are given in Tables. 9,10. .

The root and stem weight linearly decreased with increasing concentrations of NaCl upto ECe 10.0 $mScm^{-1}$. The leaf and grain weight was slightly more than the control at ECe 5.0 $mScm^{-1}$ of NaCl. The husk weight was less than the control at all levels of NaCl salinity. The average total of plant weight increased at ECe 5.0 $mScm^{-1}$ and decreased linearly with increase in chloride salinizations at higher levels.

Table. 7.

Effect of increasing concentrations of NaCl on moisture percentage in root, stem, leaf and total plant in Carthamus tinctorius L. Cv. Bhima

	% moisture			
Treatment ECe $mScm^{-1}$	Root	Stem	leaf	Total Plant (R + S + L)
Control 0.44	90.47	88.69	94.82	91.32
NaCl 5.0	88.00	88.70	94.10	90.26
NaCl 7.5	87.70	85.50	93.10	88.76
NaCl 10.0	83.69	84.26	90.20	86.03
NaCl 12.5	78.00	81.30	88.10	82.46
NaCl 15.0	-	-	-	-

- Plants died during 60 - 90 days of growth

R - root S - stem L - leaf

Table. 8.

Effect of increasing concentrations of Na_2SO_4 on moisture percentage in root, stem, leaf and total plant in Carthamus tinctorius L. Cv. Bhima

	% moisture			
Treatment ECe $mScm^{-1}$	Root	Stem	leaf	Total Plant (R + S + L)
Control 0.44	90.47	88.69	94.82	91.32
Na_2SO_4 5.0	91.20	87.00	94.90	91.03
Na_2SO_4 7.5	91.40	85.60	94.86	90.62
Na_2SO_4 10.0	90.10	85.20	94.83	90.03
Na_2SO_4 12.5	88.00	84.19	91.71	87.63
Na_2SO_4 15.0	85.00	82.69	89.26	84.61

R - root S - stem L - leaf

PLATE 1 Photograph showing effect of increasing concentrations of NaCl salinity in Carthamus tinctorius L. Cv. Bhima, on growth.

PLATE 2 Photograph showing effect of increasing concentrations of Na_2SO_4 salinity in Carthamus tinctorius L. Cv. Bhima, on growth.

PLATE-1

PLATE-2

Table. 9.

Effect of increasing concentrations of NaCl on root, stem, leaf, husk and grain weight at maturity in Carthamus tinctorius L. Cv. Bhima

Treatment ECe $mScm^{-1}$	Root	Stem	Leaf	Husk	Grain wt.	% of control	Total plant (R+S+L+H+G)	% of control
Control 0.44	0.25	0.58	0.60	0.7	1.1	100.0	0.646	100.0
NaCl 5.0	0.20	0.52	0.63	0.6	1.4	127.3	0.670	103.7
NaCl 7.5	0.16	0.51	0.52	0.6	0.7	63.6	0.498	77.1
NaCl 10.0 *	0.14	0.38	0.26	0.6	0.5	45.5	0.376	58.2
NaCl 12.5	-	-	-	-	-	-	-	-
NaCl 15.0	-	-	-	-	-	-	-	-
SEM	0.135	0.355	0.37	0.44		0.700		
LSD	0.418	1.09	1.14	1.36		2.15		
SD±	±0.04	± 0.07	±0.152	±0.06		± 0.36		

LSD at 5% level of significance

*Plants died after 90 days of growth and seeds were not viable.

-Values are given in g / plant (dry wt.)

Table. 10.

Effect of increasing concentrations of Na_2SO_4 on root, stem, leaf, husk and grain weight at maturity in Carthamus tinctorius Cv. Bhima

Treatment ECe $mScm^{-1}$	Root	Stem	Leaf	Husk	Grain wt.	% of control	Total plant (R+S+L+H+G)	% of control
Control	0.25	0.58	0.60	0.7	1.10	100.0	0.646	100.0
Na_2SO_4 5.0	0.28	0.75	0.73	5.2	1.35	122.7	1.662	257.3
Na_2SO_4 7.5	0.36	0.78	0.82	5.2	1.56	141.8	1.744	270.0
Na_2SO_4 10.0	0.30	0.76	0.61	3.6	1.01	91.8	1.256	194.4
Na_2SO_4 12.5*	0.13	0.28	0.19	0.6	0.40	36.4	0.320	49.5
N a_2SO_4 15.0	-	-	-	-	-	-	-	-
SEM	0.200	0.46	0.44	2.60		0.815		
LSD	0.617	1.43	1.36	8.03		2.510.		
SD±	±0.07	± 0.19	± 0.22	±2.12		±0.4		

LSD at 5% level of significance

*Plants died after 90 days of growth and seeds were not viable.

-Values are given in g / plant (dry wt.)

Under sulphate salinity, the root, stem, leaf and husk weight increased upto ECe 10.0 $mScm^{-1}$ and decreased at ECe 12.5 $mScm^{-1}$. The grain weight was more than the control upto ECe 7.5 $mScm^{-1}$ and decreased at ECe 10.0 and 12.5 $mScm^{-1}$. The average total of the plant was increased upto ECe 10.0 $mScm^{-1}$ and decreased at ECe 12.5 $mScm^{-1}$ of Na_2SO_4. This fact suggested that plants growing upto ECe 5.0 $mScm^{-1}$ of NaCl and upto ECe 10.0 $mScm^{-1}$ of Na_2SO_4 become more resistant to both salts after flowering.

4) DISCUSSION

a) Height

Salt stress may reduce plant growth by causing water deficits, ion toxicity, ion imbalance or a combination of any of these adverse factors when plants are exposed to salinity. In glykic plants, reduction in growth occurred due to salinity (Hayward , 1955; Bernstein and Hayward, 1958 and Eaton et al., 1971). The vegetative growth is reduced, with the increase in osmotic potential of the substrate which is proportional to the amount of salt present in the substrate (Bernstein, 1964). The soil containing 0.1 % of NaCl showed 50% reduction in growth of tomatoes (Strogonov, 1964). Decrease in height with increasing salinity has been reported in many other crops and plants by several workers like Kaddah et al. (1973) and El-Shouny (1976) in rice; Longnecker (1973); Abdel-Wahab and Al-Juboory (1975) and Malofeev et al.(1979) in cotton; Bhardwaj (1960); Joshi (1976) and Soliman et al. (1978) in wheat and barley; Ansari (1972) in Brassica and Joolka and Singh (1979) in citrus.

Salim and Pitman (1983) reported reduction in growth in Vigna radiata which was due to high K, Na, and Cl contents in shoots. Gomes et al. (1983) reported delay in seedling

growth in Vigna unguiculata. Decrease in growth with increasing levels of salinity upto 350 $mmol^{-1}$ was noted in Spinacia oleraceae by Kaiser et al. (1983). Kawasaki and Moritsugu (1983) had reported inhibitory effect of high concentrations of NaCl and PEG on growth of Phaseolus vulgaris, Zea mays and Sorghum vulgare. In Pisum sativum, shoot growth was inhibited at 192 mM NaCl (Hasson and Mayber, 1983).

Growth was reduced by 22% under salt stress in Trifolium alexandrinum (Winter, 1984). Abdel-Rahman and Abdel-Hadi (1984) reported consistent reduction in growth in cowpea under saline conditions. Bhatti et al. (1983) investigated that upto 150 mM NaCl and KCl in soil had little effect on growth, however, $CaCl_2$ depressed growth strongly in kaller grass. In Avicennia marina , decline in growth was due to high concentrations of Na and / or Cl but in Rhizophora stylosa growth was poor at high salinity due to water stress (Clough 1984). Sharma et al. (1984) reported growth inhibition by NaCl in wheat seedlings, whereas, Poly Ethylene Glycol (PEG) was detrimental to resistant Var. (Karchia - 65). Kent and Lauchli (1985) reported reduction in root growth in 7 - 9 days old seedlings of Gossypium hirsutum. Termatt et al. (1985) tested the hypothesis of reduced rates by ~20 % in roots of Triticum aestivum and Hordeum vulgare at 100 mM NaCl, which was due to inadequate turgor in expanding cell of leaves. Robinson et al. (1985) reported growth reduction in Suaeda australis seedlings at high salinities at 50 to 150 mM NaCl. Decrease in growth in Cajanus indicus and Sesamum indicum was reported under NaCl salinity by Rao (1985). Sharma and Garg (1985) observed reduction in growth in terms of parameters such as fresh and dry weight, plant height, leaf number and leaf area in wheat under saline conditions. Ashraf and Bradshaw (1986) observed reduction and variation in capacity of salt tolerance in seedlings of Agrostis stolonifera L., A Capillaris L, Holeum lanatus, Lolium perenne L, Dactylis glomerata L, Festura rubra L and puccicnellia distans L.

According to Cramer et al. (1986), root growth of seedlings of Gossypium hirsutum was inhibited at high NaCl concentrations when supplemented with Ca.

Reduction in height was reported by Azhas and McNielly (1987) in Sorghum bicolor; Larher et al. (1987) in Vigna unguiculata; Abdel - Rahman (1987) in red radish; Yassen et al. (1987) in barley and Jeschke and Wolf (1988) in Ricinus communis. Gomes and Sadek (1988) observed inhibition in growth of seedlings in Vigna unguiculata L. warp which was due to osmosis. Kayani and Rehman (1988) found significant reduction in shoot growth with increasing salinity in Zea mays L. Rapid decrease in growth rate in wheat and Lophopyrum elongatum was observed under saline conditions (Schachtman et al., 1989).

Blits and Gallagher (1990) reported that when Kosteletzkya virginia (L) Prest. was grown in nutrient solutions of 0, 85, 170 and 255 mol m^{-3} NaCl, growth was reduced at 170 and 255 mol m^{-3} NaCl progressively. Decrease in relative growth rates was observed at and above 300 mol m^{-3} NaCl that is when plants were subjected to 0-420 $molm^{-3}$ NaCl. However, salinity range 0-200 $molm^{-3}$ NaCl did not affect growth rate in Diplachne fusca L. P.Beav.ex Roemer and Schultes (Myers et al., 1990).

Hatzmann (1991) found suppression in growth by salt treatment at different (50 and 100 mM)concentrations in Opuntia ficus indica (L) Miller. Brugnoli and Lauteri (1991) reported that plant growth was strongly reduced by salinity in cotton and bean plants at different concentrations from 10 day and until mature reproductive structures were formed. Reduction in relative growth rate with increasing salinity levels in growth medium was observed in Phaseolus vulgaris (Abbas et al., 1991). Haddad and Coudret (1991) observed that growth was strongly affected by increased (upto 150 mM) NaCl

concentrations after 21 days of growth in culture medium which was due to water deficit linked to a drop in water(Ψ) and osmotic (Π) potentials in Triticum cultivars Clercal and Beagle. Growth was however increased by more than 30 % when medium was supplied with KCl or $CaCl_2$ which suggests that KCl or $CaCl_2$ decrease toxicity of NaCl. Kalaji and Nalborczyk (1991) reported decrease in growth rate in 8 barley cultivars under salinity stress and on the basis of their tolerance they have classified cultivars into 3 groups that is tolerant (Aksad 60, Rihane, Furat 1); medium (Ars, sth,215, cerice) and sensitive (Arabian black, Beecher). Shitole and Shinde (1991) observed decrease in height with increasing levels of chloride and sulphate salinity in Carica papaya Cv. Ranchi.

Grattan and Grieve (1992) found reduction in growth under saline conditions due to low nutrient ion activities and extreme ratios of Na/Ca, Na/K, Ca/Mg and Cl/NO_3, resulting in nutritional disorders. Considerable decrease in growth of the seedlings of Brassica juncea at varying concentrations of NaCl (0, 100, 150 and 200 mM) took place (Mohanty and Saradhi, 1992). Ashraf and Naqvi (1992) reported that low amounts of Ca cause growth inhibition in salt affected soils in four Brassica species thereby indicating need of sufficient amount of Ca for salt tolerance in Brassica.

Blits and Gallagher (1993) reported that growth in cell cultures of Kosteletzkya viriginia L. was inhibited at high 225 $molm^{-3}$ due to increase in Na content. Tipirdamaz and Karakullukcu (1993) observed that 150 mM salt concentration along with 10 mM proline or glycine betaine decreased growth in invitro cultured tomato embryo which indicated that proline or glycine betaine when provided externally could not help in increasing salt tolerance. Growth was inhibited in broad bean and Triticum vulgaris plants by NaCl salinity (El-Samad and Abd, 1993). He and Cramer (1993) reported that relative growth

rate in Brassica napus and B. carinata was reduced due to salinization. Marcar (1993) observed reduction in seedling growth when Eucalyptus species were treated with 150 or 100 mol m^{-3} NaCl with or with out water logging. At 100 NaCl mol m^{-3} pretreatment with water logging improved growth. E. camaldulensis showed least growth reduction with addition of S, W and S X W treatment. Storey et al. (1993) studied in laboratory and reported that, root growth of Melanthera biflora (Asteraceae) was inhibited by salt above 50 mol m^{-3} but survived upto 400 mol m^{-3} salinity level.

Zakharin (1994) demonstrated that plant growth could be delayed or stopped by high (100 to 200 mM) NaCl concentrations as well as low (10 to 20 mM) NaCl concentrations resulting in substantial organ shortening. Elimination of salt stress led to elongation and original growth due to osmotic pressure. Ortiz (1994) reported retardation of growth and then restored growth to reach steady state growth rate in Phaseolus vulgaris plants under saline conditions. Also leaf growth was interrupted due to water storage in roots. Meinzer et al. (1994) observed decline in shoot growth rate as concentration of NaCl (. EC 2.0, 4.0, 8.0, 12.0 dSm^{-1}) of irrigation solution increased above 2 dSm^{-1} and growth rate was high in sugarcane resistant cultivar (H69-8235) at all salinity levels. Hamada and Enany (1994) reported that increasing salinity upto 80 mM Na showed reduction in growth in broad bean leaves.

El-Samad and Abd (1995) reported that salinity affected growth in Cucumis sativus, however Sodium pyruvate ameliorated adverse effects of NaCl. Reinhardt and Rose (1995) observed that salinity stress delayed primary root growth and reduced peak elongation rates, without changing general primary root growth. Lateral root growth of Gossypium hirsutum L. Cv. Acala SJ-2 was inhibited by salinity than primary root growth. Elongation of lateral roots was more inhibited by salinity than their initiation and

emergence. Huang and Redmann (1995) investigated the role of Ca under saline conditions in Hordeum vulgare and reported that increased Ca in growth medium increased the salt tolerance in barley. Muthuchelian et al. (1996) showed significant reduction in growth rate in Erythrina variegata Linn seedlings when grown under low (100 mM NaCl) and high (250 mM NaCl) salinity for 10 days. Lin and kao (1996) reported inhibition of root growth in rice seedlings under NaCl stress.

Morabito et al. (1996) reported that in Eucalyptus microtheca clone 42, growth was delayed while in clone 43 shoot length growth was completely inhibited during salt stress. Zhang et al. (1996) showed that germination percentage was reduced and growth was inhibited under NaCl stress in Eleucine coracana seedlings. Guerrier (1996) found that after six days of salinization relative growth rates of both, Lycopersicon pimpinellifolium and L. esculentum, decreased significantly in whole plants. Hamada (1996) reported that growth was retarded with rise in NaCl/ drought or NaCl and drought in wheat plants. Misra et al. (1996) found that higher salinity level (200 mM NaCl) reduced seed germination and seeding growth due to reduction in root or shoot elongation and dry mass accumulation. However, 20 mM NaCl increased seedling length and biomass accumulation over the control in 2 Brassica juncea L. cultivars Kranti and T-59. Okuba and Utsunomiya (1996) observed suppression in growth when plotted Ficus carica L. Cv. Masui Dauphine cuttings were subjected to 0-50 mM NaCl solution for four weeks.

Botella et al. (1997) reported that salinity significantly decreased shoot growth when Zea mays L. plants were grown in ½ Hoagland nutrient solution which was 0.1 m mol/L which was probably due to salinity induced K deficiency. Lechno and E-Telor (1997) reported reduced growth in cucumber seedlings grown in hydroponic solution for 1 to 2

weeks to a final concentration of 100 mmol/L for 4 days. According to Gucci et al. (1997) growth parameters were completely inhibited at 300 mM external NaCl for 64 days in Phillyrea species.

Misra et al. (1997) reported that leaf growth of resistant Oryza sativa Cv. Damodar was less affected than susceptible Cv. Jaya under NaCl stress. Al-Yemeny and Basahy (1997) found marked growth reduction at 16 and 20 mmhos/cm upto 40% in root, 73% in shoot and 54% in stem and 58% in root dry weight in Cyamopsis tetragonoloba (L) Taubert. Garg et al. (1997) observed that increasing NaCl concentration (0, 50, 100 and 150 mM) progressively decreased growth of Cyamopsis tetragonoloba (L)Taubert. However, supplemental Ca (2.5 and 5.0mM) which ameliorated adverse effect of NaCl. Muthukumaraswamy and Pannerselvam (1997) found marked reduction in growth over the control in root, stem and leaf of green gram at 100 and 150 mM NaCl treatment. According to Wang and Zhao Ke-Fu (1997) root growth decreases in corn due to NaCl stress. Converso et al. (1997) observed inhibition of growth in stems of wheat plants under salt stress. Sharma (1997) observed that plant growth declined with increasing levels of salinity in CSG 8890 and CSG 8927 genotypes of chickpea.

There are few reports of increase in height under saline conditions in glycophytes. Nieman (1962) suggested stimulation of growth in garden beet and spinach by 0.2 % NaCl. Das and Mehrotra (1971) reported that salinity accelerated growth in barley, oat, rice, maize, Sorghum and cotton. However, 0.6% salinity proved to be critical salinity level for barley, oat, maize and Sorghum and 0.8% for rice and cotton. Growth stimulation by low concentration of NaCl (0.01 and 0.05 M) and inhibition by high concentrations was reported in sugarcane Var. Co 740 by Nimbalkar and Joshi (1975). Similar results were reported in cowpea plants by Imamil Huq and Larher (1984).

Jeschke et al. (1983) found an increase in growth at 10 and 50 mM Na salts due to efficient K uptake under NaCl and Na_2SO_4 salinity's in Atriplex hortensis. Clough (1984) observed stimulation in growth in seedlings of Avicennia marina and Rhizophora stylosa in 25% of sea water. According to Huq et al. (1985) at low salinities (10 mM) relative growth rate of Vigna sinensis was greater than Phaseolus aureus and at 100 mM relative growth rate was same for both species indicating that both the plants were more resistant at younger stage. Eshel (1985) observed 90% acceleration in growth in presence of Na, tested at all concentrations of NaCl and Na_2SO_4 at 125 eq m^{-3} indicating more resistance to NaCl at younger stage.

Waisel (1985) reported stimulation of growth at low levels of salinity's in halophytic rhodes grass (Chloris gayana Kunth Cv.). Harvey (1985) observed considerable growth in Zea mays under 100 mol m^{-3} NaCl. According to Cramer et al. (1986) root growth of Gossypium hirsutum was stimulated by low NaCl (25 mM) concentration.

Clipson (1987) reported that growth rates increased in presence of salt in Suaeda maritima L. Dunn seedlings in 0 - 200 mol m^{-3} salinity. Abdel Rahman (1987) observed acceleration of main stem growth in dwarf bean varieties in Var. Balady (upto 20 m eq) while in variety black valentine the concentration of NaCl was higher (60 m eq). Abdel-Rahman (1987) found that salinity promoted growth in cowpea while in calabrese, effect was promotive or depressive depending on the concentration of NaCl. Results of Larher et al. (1987) revealed that 10 mM NaCl promoted growth in Vigna unguiculata, Vigna faba and Trigonella foenum graceum. Results of Blits and Gallagher (1990) suggested that when Kosteletzkya virginia L. Prest were grown in nutrient solutions of 0, 85, 170, 255 mol m^{-3} NaCl then the growth was stimulated by 85 mol m^{-3} NaCl.

Reinhardt and Rose (1995) reported daily primary root elongation gradually increased to maximum then declined close to zero in dark grown seedlings in Gossypium hirsutum L. Cv. Acala SJ-2. Guerrier (1996) observed that short term salt exposure (4-6 days) of salinization enhanced relative growth rates of Lycopersicon pimpinellifolium and not in L. esculentum. Kohl (1997) reported growth enhancement by NaCl in inland population upto 200 mM NaCl in Armeria maritima (Mill). Venkatesan et al. (1997) reported that growth was promoted by exogenous addition of NaCl upto 200 mM in Ipomoea pes-capre sweet plants. Khan et al. (1997) reported that plant height, shoot and root growth was increased with increasing salinity (0, 50, 100, 150 and 200 mM NaCl concentration in 3 cultivars of rice. Saha and Gupta (1997) found that 5 mhos/cm NaCl promoted growth in sunflower seedlings. From all these results it is clear that many glykic plants need Na and Cl in small amounts for their growth which is clear from the observations that low concentrations of NaCl stimulate growth in many glykic plants.

Tyerman et al. (1984) reported that leaf growth was unaffected by 13 % to 15 % of salinity in Posidonia australia. Results of Yeo and Flowers (1985) suggested no effect on growth at a range of Na / Ca (5.25), but reported marginal increase in NaCl entry to shoot in Oryza sativa. Aslam et al., (1986) observed that growth of Atriplex amnicola at 400 mol m^{-3} NaCl was not limited by availability of photosynthate in the plant as a whole. However, there is no growth limitation due to inadequate organic solutes for osmotic regulations. Ohta et al. (1987) observed that Na is required for normal growth of Amaranthus tricolor L. seedlings. Flowers et al. (1990) reported that in Oryza members salt concentrations upto 20 % did not affect growth adversely.

Marcar (1993) reported that salt and water logging alone did not affect seedling growth of Eucalyptus species. Sadek (1993) observed that growth was not affected by salinity in Atriplex halimus var. Schweinfurthii. Fernandez et al. (1996) reported that plant growth was unaffected upto NaCl 150 mol m^{-3} in Lupinus albus L. studied over six day period. Thus in many plants salinity does not affect growth.

Kaavari - Nejad and Najafi (1990) reported that in Helianthus annus Cv. Record growth was adversely affected by NaCl salinity at 25 and 50 mM. Salt solutions with or without $CaCl_2$ at 17 h light period 100 wm^{-2} PAR day / night and temperature of 24 / 18 ^{0}C. Zallag et al. (1990) suggested that shoot mean relative growth rates were similar for both control and plants growing at 300 mol m^{-3} NaCl. Matoh and Murata (1990) found the beneficial effect of sodium on growth of Panicum coloratum walt Cv. Kabulabula,. Zhao and Munns (1992) provided evidence that plant growth of barley and salt brush is controlled by a message given from roots under increasing NaCl concentration. Amzallag and Lerner (1994) observed that 150 and 300mM NaCl inhibit growth in Sorghum bicolor L. Moench genotypes.

Thus among glykic plants the effect of salt is not uniform. In some plants all the concentrations of NaCl are toxic while in some plants low concentrations of NaCl stimulate while high concentrations of it , inhibit growth.

Results of the present investigation (Table.1 ; Fig.1a,b) revealed that growth in height was stimulated upto ECe 10 $mScm^{-1}$ of NaCl at 30 days of germination. However, it was less than the control at 60 and 90 days of germination which clearly indicated that all levels of NaCl salt were toxic for growth in height in this plant. Further plants could

survive and complete life cycle upto ECe 7.5 $mScm^{-1}$ of NaCl. Plants growing at ECe 12.5 to 17.5 $mScm^{-1}$ of NaCl died during 60 to 90 days of growth.

Under sulphate salinization (Table. 2 ;Fig. 2a,b), height was more than the control upto ECe 10.0 $mScm^{-1}$ at 30 days of growth and upto ECe 7.5 $mScm^{-1}$ at 60 and upto Ece 10.0 $mScm^{-1}$ at 90 days of growth which clearly indicated that low concentrations of Na_2SO_4 are required for growth and development of this crop. However, high concentrations of it are detrimental. Plants could survive and complete life cycle upto ECe 10.0 $mScm^{-1}$ of Na_2SO_4. Plants growing at ECe 15.0 and 17.5 $mScm^{-1}$ of Na_2SO_4 died during 60 to 90 days of growth. Thus survival range is wider under sulphate salt rather than chloride salt in Carthamus tinctorius Var. Bhima.

Reduction in height at all levels of chloride and at all higher levels of sulphate must be due to combination of osmotic and specific ion effect because moisture content (Tables. 7, 8) was less than the control and Na and Cl content (Tables. 11 - 16) was more than the control. At low concentrations of sulphate increase in height must be due to stimulatory effect of Na and SO_4 on growth because content of both the minerals was more than the control and moisture content of leaf was also more than the control (Table. 8).

b) Leaf Area

It has been observed that efficiency of energy conversion is less in young leaves as they have less leaf area to carry out high rates of photosynthesis and other leaves which are senescent. The rates of important physiological processes such as photosynthesis, transpiration and final yield of the plant depends on area of green leaves exposed to radiant energy (Date et al., 1980) .

With an increase in salinity, leaf area and number decreases (Kale ,1962; Ahmed ,1965; Sharma et al., 1977 and St. Omer and William.,1980). Reduction in growth due to reduced leaf area by 20 to 40 % in bean plants under saline conditions was observed by Meiri and Poljakoff-Mayber (1970). Productivity in corn, bean and sunflower is affected as a result of decreased leaf area due to salinity (Ch'Yung and Lapina 1974), Decreased leaf area , leaf number and fresh and dry weight in kidney bean (Heikal, 1976); in rice (El-keredy and El-Showry, 1976); in citrus (Joolka and Singh, 1979) in cotton (Malofeev et al., 1979), under saline conditions have been reported. Chavan (1980) observed considerable decrease in leaf area in ragi at 100 mM NaCl. Robinson et al. (1983) subjected Spinacea oleraceae to 25 mM to 200mM NaCl salinity and observed decrease in leaf area .

Longstreth et al. (1984) showed that when alligator weed, Alternanthera philoxeroides was grown at different concentrations of NaCl, then plants produced less leaf area / unit dry weight with increase in salinity (to conserve water). Yasseen et al. (1987) observed decrease in leaf area by salinity in two barley cultivars (Black local and Arivat) by reducing number and volume of cells. Schubert and Lauchli (1986) in wheat and Blits and Gallagher (1989) in a dicot halophyte made similar observations as Chavan (1980). Blits and Gallagher (1990) observed decrease in leaf production and leaf area in Kosteletzkya virginia (L) Prest, a dicot halophyte subjected to nutrient of 0, 85, 170, 255 mol m^{-3} NaCl.Myers et al. (1990) reported decrease in leaf area in Diplachne fusca L. (beetle grass) when subjected to NaCl (0-420 mol m^{-3}). Brugnoli and Lauteri (1991) reported reduction in leaf area development in Gossypium hirsutum L. and Phaseolus vulgaris L. at different NaCl concentrations from 10 day old seedling till mature reproductive structure formation. Kalagi and Nalborczyk (1991) observed reduction in

leaf area under salinity stress which classified 8 barley cultivars into 3 groups. Tolerant (Aksad 60, Rihane, Furat 1), medium (Ars, sth, 215 cerice) and sensitive (Arabian lack, Beecher). Zhao and Munns (1992) found decrease in leaf area and relative leaf expansion rate in barley and salt brush under increasing NaCl saline conditions where this rate is suggested to be controlled by factor originating from roots associated with concentrations of water status of roots. Alarcon et al. (1993) reported that salinity of 0, 40, 70 and 210 mM NaCl reduced leaf area in Lycopersicon esculentum. Sharma (1996) observed reduced leaf area and number of litters in Triticum durum L. Cv. HD 4502 under steady state NaCl salinities (1.6, 12.0, and 16.0 dsm^{-1}) for 8 weeks. Salinity reduced leaf area by 35-75 per cent in different sugarcane varieties (Sharma, 1997).

Nunes et al. (1984) demonstrated that low Na concentration in root medium of intact and decapitate young sugar beet (Beta vulgaris) cultivar Kaueply grown under controlled conditions modified leaf water relations and increased leaf area. Jeschke et al. (1984) observed that leaf size was stimulated at 10 mM and 50 mm of (NaCl, Na_2SO_4) salts in Atriplex hortensis . Kayani and Mujeeb-ur-Rehman (1988) reported in crease in leaf area with increasing salinity in Zea mays L. Cv. Sunahry. Hwang and Chen (1995) studied histology of leaves and roots of Kandelia candel seedlings and reported greater leaf area and thickness at 50 and 100 mM NaCl. According to Venkatesan et al (1997) leaf area increases in plants (Ipomea pes- caprae sweet) grown upto 20 mM of NaCl.

Our results of present investigations indicate that (Table. 3 ; Fig.3) at 30 days of growth total leaf area per plant was increased upto ECe 12.5 $mScm^{-1}$ of NaCl, however, it was less than control at higher salinity level (ECe 15.0 and 17.5 $mScm^{-1}$). At 90 days, leaf area was more than the control upto ECe 7.5 $mScm^{-1}$. Under sulphate salinity (Table. 4;

Fig. 4) , at 30 days of growth, leaf area was less than the control and at 90 days of growth, leaf area was more than the control at ECe 5 $mScm^{-1}$ and less than the control at all higher levels of Na_2SO_4 salinity. Thus, all concentrations of NaCl inhibit while low concentrations of Na_2SO_4 stimulate and high concentrations of it inhibit leaf development in safflower Cv.Bhima.

c) Productivity (Dry matter)

The total area of photosynthetic organs and their efficiency to receive solar energy gave an idea about total dry matter production (biomass) which gives the idea about carbon budget and productivity of the plant (Sestak et al., 1971).

To study the effect of salinity on plants, it is necessary to consider parameters such as total dry matter production and yieldper plant.

Results of several workers (Ayers et al., 1943; Gauch and Wadleigh, 1944, 1945; Bernstein and Hayward, 1958; Lunt et al., 1961; Lunin et al., 1961; Greenway et al., 1966; Nieman and Poulsen, 1967; Meire and Poljakoff-Mayber, 1969; Lahaye and Epstein, 1971; Meire et al., 1971; Neiman and Poulsen, 1971; Ayoub, 1974; Heikel, 1976; Lessani and Marschner, 1978; El-Kurouri, 1979; Sameni et al., 1980) suggested that salinity reduces growth and dry matter production per plant in many crop plants.

A decrease in dry matter production due to increased salinity was reported by Kale (1962) in rice; Heikal (1975, 1976) in safflower, sunflower and Kidney bean; (Paliwal et al., 1976) in barley and Dahiya and Singh (1976) in Pea. Mahajan and Sonar (1980) observed decrease in dry matter with increasing salinity where the effect was more hazardous under chloride compared to sulphate salinity. Wagnet et al. (1980) reported

similar results. With increase in NaCl from 0 to 18 mM, a decrease in shoot dry weight took place in barley (Dale et al., 1980).

Robinson et al. (1983) observed that in Spinacia oleraceae there was decrease in fresh and dry weight (more than 50%) under salt (0 to 200 mM) stress. Mehta and Bharti (1983) reported that chloride salinity reduced fresh and dry weight of cotyledons whereas sulphate salinity had opposite effect. Karadge and Chavan (1983) found 40% reduction in dry matter in Sesbania aculeata. Winter (1984) observed 70% reduction in dry matter production in Trifolium alexandrinum at 50 mM NaCl stress.

Bhatti et al. (1983) reported decrease in dry weight of Diplachne fusca (Kaller grass) at 150 mM NaCl/ KCl. Noble et al. (1984) observed decrease in stem biomass in seedlings of cactus (Cereus validus). Haworth, when treated with NaCl 400 mM. Clough (1984) found fall in dry matter production between 25 and 50% seawater in Rhizophora stylosa. Great reduction in fresh weight was observed at 200 $molm^{-3}$ NaCl in Gossypium hirsutum (Kent and Lauchli, 1985); in tomato (Raymond, 1985); in tobacco (Flower et al.,1986); in grapes (Arbabzadeh and Dutt,1987); in cowpea Kannan and Ramani, (1988) and in Pistachio cultivars (sepaskhan and Maftown, 1988) under saline conditions.

Seemann and Critchley (1985) showed that dry and fresh weight decreased with increasing external NaCl concentration ranging from 0 to150 mM in Phaseolus vulgaris. Robinson et al. (1985) reported decrease in growth on basis of fresh/ dry weight in halophyte Suaeda australis at 50-150 mM NaCl. Jeschke et al. (1986) observed linear decrease in dry matter of shoots and root with increasing (1, 5, 10, 25 and 40 mol m^{-3}) external NaCl in nodulated Lupinus albus. According to Naidoo (1986) decrease in root

mass at low external osmotic potential in Rhizophora macronata occurred. Safaraliev et al. (1987) found significant decrease in dry matter at 0.6% salinity in maize seedlings. Younis et al. (1987) subjected seeds of flax, cotton and castor beans to 0.5 and 1 % NaCl for 12 days and observed continued decrease in dry matter.

Pessarakli et al. (1989) reported that increasing pressure of culture solution decreased total dry matter production when grown under control and NaCl salinized (3, 6 and 9 bars osmotic pressures) in Zea mays . Cv." Florida stay sweet". According to Khan and Ashraf (1988) depression in dry matter yield in 4 varieties of sorghum took place when subjected to tolerant, moderately tolerant and sensitive NaCl salinity. In Cicer arietinum, NaCl significantly decreased shoot and root dry weight; total nodule number per plant, nodule weight and average nodule weight (El-Sheikh and Wood, 1990). Myers et al. (1990) reported decrease in plant dry weight at and above 300 $molm^{-3}$ NaCl when plants were subjected to 0-420 $molm^{-3}$ NaCl in Diplachne fusca L.P.Beave-ex Roemer and Schultes. Kalaji and Nalborczyk (1991) observed decrease in dry mass under salinity stress which classified 8 barley cultivars into 3 groups; Tolerant (Aksad 60, Rihane, Furat 1); medium (Ars, sth, 215 Cerice) and sensitive (Arabian Black, Beecher). Hatzmann (1991) added 50 and 100mM NaCl to nutrient solutions and reported suppression in dry matter in Opuntia ficus indica. Shitole and Shinde (1991) reported decrease in dry matter production with increasing levels of NaCl and Na_2SO_4 salinities in Carica papaya cv. Ranchi. Zhao and Munns (1992) found decrease in dry weight in barley and saltbrush with increasing NaCl concentrations while Ashraf and Naqvi (1992) reported lowest plant dry biomass in 4 species of Brassica napus at varying Na/Ca ratios of NaCl (15, 30, 60, 120 and 150 mM) concentrations. Wilson et al. (1992) reported low overall fresh weight in 25 days old plants of Phaseolus vulgaris Cv. Stringless green pod with full salt treatment for 7 days by 33% compared to control.

Shoot fresh weight decreased by 40% compared with 22% for roots thus increasing root:shoot ratio from 0.7 to 0.9. Banuls and Millo (1992) reported decrease in dry matter in Citrus sinensis Osbeck Cv. Hamlin due to accumulation of Cl ions under salinity stress. Mirza and Tariq (1992) found decrease in dry weight of shoots and roots of Sesbania sesban with increasing salinity levels (0 to 2 %) of NaCl in sandy clay loam soil (ECe 1 dSm^{-1}). A decrease in fresh and dry weight of root and coleoptile with increasing levels of NaCl was reported by Chippa et al. (1992) in Pearl millet. In Apium graveolus, total shoot weight and root growth was severly reduced at all salt concentrations (Everard et al., 1992).

Storey et al. (1993) reported decrease in fresh weight / dry weight ratio in Melanthera biflora (Asteraceae) with increasing salt stress (50 mol m^{-3}) to (400 mol m^{-3}) due to increase in onium compound (quaternary ammonium and /or tertiary sulphonium). Cachorro et al. (1993) observed decrease in plant weight at 25 mM NaCl concentration in Phaseolus vulgaris .

According to Alarcon et al. (1994), reduction in leaf and shoot dry weight in Lycopersicon esculentum at 0, 70, 140 and 210 mM NaCl occured. Mansour (1994) reported more pronounced decrease in fresh mass of sensitive cultivar of 10-d-old wheat seedlings in presence of 100 mM NaCl for 7days. Gouia et al. (1994), observed reduction in dry mass after 20 days at 50 and 100 mM NaCl by 48% and 55% in bean and 6% and 14% in cotton in culture solution. According to Sharma (1995) biomass declined with increasing salinity in salt sensitivity variety of wheat. Zhao et al. (1995) reported inhibition of dry weight production by 100-500 mmol/L KCl in Suaeda salsa, Atriplex centralasiatica and Limonium bicolor. Cayuela et al. (1996) reported low reduction in shoot and root dry weights in primed seed of tomato with 6M NaCl at

different harvests (30, 45, and 60 days after sowing). Lopez and Satti (1996) observed reduction in root volume and fresh weight in 5 cultivars of tomato at 150 $molm^{-3}$ NaCl. Misra et al. (1996) reported reduced fresh mass with increasing NaCl salinity (0.0, 0.5, 1.0, 2.0, 3.0 percent m/v) in 2 cultivars, Sujata and Cv.K851 of Vigna radiata. Muthuchelian et al. (1996) showed reduction in biomass production in Erythrina variegata seedlings grown under low (100 mM NaCl) and high (250 mM NaCl) salinity for 10 days. Al-Yemeny and Basahy (1997) found reduction by 58% in dry weight in Cyamopsis tetragonoloba Taubert at 16 and 20 mmhos/cm salinity levels. Converso et al. (1997) observed decrease in fresh weight of stems and leaves under salt stress in wheat plants .According to Maliwal (1997), dry mass decreased with increasing salt concentrations in 5 varieties of wheat. Saha and Gupta (1997) reported decline in dry matter production with increasing NaCl salinity in sunflower seedlings.

On the other hand, increased dry matter production at low and moderate salinity concentrations was reported by Nimbalkar and Joshi (1975) in sugarcane; St. Omer et al. (1980) in Jaumea carnosa; Nukaya (1983) in musk melon; Matoh et al. (1986) in Atriplex gmelini; Mahmood and Malik (1987) in Atriplex rhagodioides; Weimberg and Shannon (1988) in tall wheat grass and Alka et al. (1982) in barley.

Jeschke and Stelter (1983) observed substantial stimulation in dry matter production at 10 mM and 50 mM Na salts under mild conditions of NaCl and Na_2SO_4 in Atriplex hortensis. Nunes et al. (1984) demonstrated increased dry weight at low Na concentrations in young Beta vulgaris Cultivar Kauepdy. Seemann and Critchley (1985) found that at 0 - 150 mM NaCl concentrations, root / shoot ratio increased in Phaseolus vulgaris Cv. Hawkesbury Wonder. Eshel (1985) suggested that fresh weight of Suaeda monoica and Suaeda aegypica was raised by NaCl, 5 to 10 times respectively. In these

plants,NaCl stimulated growth and plants stored more amount of water. In seedlings of coastal halophyte Atriplex gmelini, maximum dry weight yield was obtained at both low and high NaCl concentrations. Matoh et al., (1986) and Kunth et al. (1986) observed increase in both length and weight of primary root at 25 -100 mM NaCl in presence of 10 mM Ca in Gossypium hirsutum L.(Cv Acala SI - 2) grown hydroponically.

NG and BH (1987) reported increase in dry weight of nodules, shoots and roots and nitrogen content of shoots at 50-100 mM NaCl in Casuarina equisitifolia. Stiborova et al. (1987) observed increase in root/shoot ratio at 0-100 mM NaCl concentration in Hordeum vulgare and Zea mays, indicating that shoot growth was more affected than root growth. Tirmizi et al. (1991) found that increasing NaCl treatments increased dry matter production in Hippophae rhamnoides L.

Ashraf and Naqvi (1991) observed that in Leptochloa fusca L. Kunth highest fresh and dry biomass production at varying Na/Ca ratio under different salt treatment of 24, 49, 99 and 199 at constant concentrations of 200 $molm^{-3}$ NaCl. Further, Ashraf and Naqvi (1992) reported that in Brassica carinata plant dry biomass was greater than other species at varying Na/Ca ratios in 150 NaCl solutions of 15, 30, 60 and 120. Khan et.al. (1992) observed that Sorghum Var. IS-1347 yielded more biomass than IS-4807 when grown in series of NaCl concentration in nutrient culture solution. Reddy et al (1992) reported increase in biomass production, % moisture and succulence in different tissues of Salicornia branchiata, Roxb. under saline conditions (seawater irrigation).

Tipirdamaz and Karakullukcu (1993) observed increase in stem weight at 150 mM NaCl concentration along with application of 10 mM proline/ Glycinebetaine in invitro cultured tomato embryo. Everard et al. (1994) reported increase in shoot fresh weight at low NaCl concentration of root zone salinity (25mM NaCl) compared to control and growth

continued at further higher salinity's in celery Apium graveolus. Ashraf (1994) reported increase in dry matter production in oil seed salt tolerant (Eruca sativa) at 0, 100, 200 or 300 mol m^{-3} NaCl. Ashraf and Fatima (1995) observed that two tolerant accessions (260622 and 305167) of safflower (Carthamus tinctorius L.) when grown in soil salinized with 0, 70, 140 and 210 mol m^{-3} NaCl produced greater fresh and dry biomass. Reiman and Breckle (1995) reported increase in dry weight by 50 $mmol^{-1}$ NaCl in ssp tragus (L.) and not in ssp ruthenica of Salsola Kali L. when subjected to 200 $mmol^{-1}$ NaCl.

Studies of Cayuela et al. (1996) indicated positive effect of seed priming with 6M NaCl on shoot and dry weight at different harvests (30, 45 and 60 days) after sowing at 140mM NaCl in Lycopersicon esculentum Mill. Cv. Pera. According to Muthuchelian et al. (1996) increase in biomass was partially ameliorated by triacontanol (1 mg/ kg) in Erythrina variegata Linn. seedlings grown under low (100 mM NaCl) and high (250 mM NaCl) salinity for 10 days. Kohl (1997) reported increase in shoot root dry weight in Armeria maritima (Mill) upto 200 mM NaCl in Inland populations, which grew better upto 40 mM NaCl compared to control. Venkatesan et al (1997) reported increased fresh weight upto 200 mM NaCl in Ipomoea pes caprae sweet.Mohanty and Saradhi (1992) reported no significant change in dry weight at varying concentrations of NaCl (0, 100, 150 and 200 mM) in Brassica juncea Cv. DIRA 367.

Thus in several glykic plants,salinity inhibits growth. However, there are many glykic plants in which dry matter production per plant increases by low levels of salts which is clear from the results of several workers cited above.This fact indicated that, for many glykic plants, Na and Cl are essential for growth but their high concentrations are inhibitory.

Results of the present investigation (Tables. 9,10) revealed that all concentrations of NaCl inhibit total dry matter production per plant. However, total dry matter production per plant increased in plants grown upto ECe 7.5 $mScm^{-1}$ of Na_2SO_4 and at higher (ECe 10.0 to 15.0 $mScm^{-1}$) levels of sulphate, dry matter per plant was less than the control. Further, this cultivar Carthamus could survive and complete its life cycle upto ECe 7.5 $mScm^{-1}$ of chloride and upto ECe 10.0 $mScm^{-1}$ of sulphate. Thus, higher levels of NaCl are more toxic than higher levels of sulphate.

d) Post harvest

Salinity affects plant growth (Epstein, 1962; Greenway, 1973 and Rai, 1977) and induces changes in anatomy and morphology of stems and leaves (Poljakoff- Mayber et al., 1975; Strogonov, 1962; Waisel, 1972 and Hayward and Long, 1941). Bhardwaj (1960) observed that yield was reduced from 7.2 to 2.1 g per plant in wheat and gram under saline conditions. Asana and Kale (1965) reported that grain yield /1000 grain weight were reduced but grain number was not affected. Jadhav (1969) mentioned that yield of jowar was reduced at ECe 2.6 to ECe 4.4 levels but the grain number was stable. Shalhevet et al. (1969) observed 50 % reduction in the yield of Arachis hypogea, under artificially salinized conditions (4.7 ECe). Venkateswarlu et al. (1972) stated that at ECe 4.5 $mScm^{-1}$ the yield was reduced by 25 to 30 % in rice. Longneckar (1973) noted reduction in total number of fruiting forms, total number of matured balls, weight of both, seed and lint per ball and fiber length in cotton due to salt stress. Ch'Yung and Lapina (1974) have traced the effect of salinization in corn, sunflower and bean and recorded reduced productivity. Starck et al. (1975) and Udovenko (1973, 1975 and 1977) suggested that reduction in economic yield is related to greater reduction of translocation of photosynthate in bean plants due to NaCl treatment.

Paliwal et al. (1976) reported reduction in dry weight of tops and grain yield in barley due to increased salt concentration of irrigation water. Seed yield per plant and 1000 grain weight of barley were decreased when grown with sea water (Iyengar et al., 1977). Ayoub (1977) found 50 % reduction in the seed yield at soil salinity of about 3.9 mmhos / cm in lentil. A decrease in seed yield and seed weight and soybean due to salt stress was evident in the experiments of Sepaskhan (1977). Sourour et al. (1975) reported that the negative effect of salinity resulted in reduction of average number of tillers and spikes per plant, total yield (grain + straw) and also grain size of cotton. Dhir et al. (1977) and Ansari et al. (1977) reported decrease in grain yield due to salinity. Murthy et al. (1979) reported that Na of 23.49 meq 100^{-1} g in leaf reduced the grain yield by 61 % of the control while 50 % decrement in grain yield was associated with 19.2 meq 100^{-1} g of Na and K/Na ratio 5 in the leaves of wheat. They attributed the reduction in the yield at high concentration of Na in wheat leaves induced by salinity. The grain yield was more seriously affected than other morphological characteristics in barley when grown under salinization (Kumar et al., 1981). Hassan (1981) observed that spraying of rice plant with NaCl solution during flowering reduced flower growth, pod and seed growth. Cerda et al. (1982) and Sharma et al. (1997) registered reduction in seed yield due to salt stress in pea and chick pea respectively. Nukaya et al. (1982) reported reduction in pod and seed yield of soybean. West and Francios (1982) and Wagnet et al. (1983) reported marked reduction in pod and seed yield in pea and Phaseolus sp. under saline conditions. Ioneva and Spiridonov (1985) found change in total length of main root and lateral roots (wheat and corn) in 14 days exposed young plants of wheat Var. Bezostaya 1, Corn hybrid KVS-701 at 25, 50, 100 and 200 mmol NaCl in Hoagland's mixture. Jeschke et al. (1986) observed that at 1, 5, 10, 25 and 40 mol m^{-3} NaCl the relative growth rate of roots was affected more than in shoots of nodulated Lupines albus Cv. Ultra. Tirmizi et al. (1991) reported reduction in root

length, increased root to shoot ratio relative to control in Hippophae rhamnoides L. under NaCl stress. Khan et al. (1995) observed decrease in biological yield at high levels of NaCl salt (0-100 $mmol^{-1}$) and was more suppressive than Na_2SO_4 (0 -50 $mmol^{-1}$). Khan et.al. (1995) reported reduction in biological yield after 4 weeks of growth at high level of NaCl in Sorghum bicolor L. Moench Cv. IS-4807.

According to Siddiqui and Krishnamoorthy (1995), salinity inhibited flowering and yield of cowpea and gram. However, when salinity exceeded 15 meq salts/l , plants either did not survive or failed to flower or yield. Application of B9 alleviated deleterious effects of salinity. Misra et al (1996) found that due to reduced root and shoot elongation, growth and dry mass accumulation was reduced at high salinity level (200 mM NaCl) in Brassica juncea L. cultivars Kranti and T-59. However, at low salinity level (20 mM NaCl), reverse effect was observed. Savvas and Lenz (1996) studied effects of 20, 40 and 60 mM NaCl on eggplants and reported reduction in yield when grown in closed sand culture system for 6 months. Misra et al. (1996) reported reduced shoot and root length at 0, 0.5, 1.0, 2.0, 3.0 % w/v NaCl salinity in cultivars, Sujata and Cv. K-851 of Vigna radiata L. seedlings.

According to Patil et al. (1996) irrigating the maize crop with saline water significantly reduced grain yield at high (4 ds/m) levels of salinity. Okubo and Utsunomya (1996) reported that stem elongation was suppressed by increasing concentration from 0-50 mM NaCl in Ficus carica L. Cv. Masui Dauphine cuttings. According to Gucci et al. (1997), increasing salinity levels inhibited shoot elongation by 50% at 123 and 135 mM external NaCl after 31 and 123 days of salt treatment in Phillyrea species. Garg et al (1997) reported that increasing NaCl concentration (0, 50, 100 and 150 mM) decreased seed yield in Cyamopsis tetragonoloba Taub. Gadallah and Ramadan (1997) observed

that shoot and root length decreased in salt stress plants compared to unstressed plants in Carthamus tinctorius L. Maliwal (1997) reported low reduction in grain yield in Karchia-65 and highest reduction in grain yield in J-405. Var. of wheat and observed that chloride salinity reduced yield more than sulphate salinity. Salinity decreased yield of sugarcane crops (Sharma, 1997).Reeve et al. (1948) found that yield of wheat was increased from 0.7 to 42.6 bushels per acre as ECe was reduced from 40 mmhos / cm to 4 mmhos/cm. Hamid and Talibudeen (1976) noted that Na salt in the soil benefits the yield in barley and sugar beet. Singh and Chandra (1980) observed that hybrids of Pennisetum typhoides survived and yielded better at ECe 20.0 than ECe 10.0 and 15.0 and grain yield increased over the control at ECe 5.0. Hussain (1981) suggested that ECe 4.0 mmhos / cm may be utilized without excessive loss of yield for barley crop. Ashraf and Fatima (1995) reported greater seed yield in two tolerant accessions of safflower at salinity levels of 0, 70, 140 and 210 mol m^{-3} NaCl.

Results of the present investigation (Tables. 5, 6) indicate that safflower plants flower earlier (77 to 88 days) under NaCl salinity than sulphate salinity (88 to 75 days). However, the plants mature within 95 to 115 days under both the salinity's. At maturity, under both salinizations, the content of root, stem and leaf weight increased slightly than at flowering. The husk weight was less than the control under chloride and sulphate salinizations. Under NaCl salinity the grain weight increased at low (ECe 5.0 $mScm^{-1}$ (Table. 9) and decreased at high (upto 10.0 $mScm^{-1}$) whereas,under Na_2SO_4 salinity the grain weight was increased upto ECe 7.5 $mScm^{-1}$ (Table 10) and decreased at high (ECe 10.0 and 12.5 $mScm^{-1}$) salinity. Under NaCl salinity the total DM per plant (Root + Stem + Leaf + Husk + Grain) (Table. 9) was increased at ECe 5.0 $mScm^{-1}$ and decreased at high (upto ECe 10.0 $mScm^{-1}$) levels. The plants died after 90 days of growth and their seeds were not viable (Table. 9) at ECe 10.0 $mScm^{-1}$. Under Na_2SO_4

salinity, the total DM per plant (Root + Stem + Leaf + Husk + Grain) was increased upto ECe 10.0 $mScm^{-1}$ and decreased at ECe 12.5 $mScm^{-1}$ (Table. 10). The plants died after 90 days of growth and their seeds were not viable at ECe 12.5 $mScm^{-1}$ (Table.10). Thus, this plant can be grown upto ECe 7.5 $mScm^{-1}$ of NaCl with 77% overall productivity and 63.6% of grain productivity. While under sulphate salinity, this plant can be grown profitably upto ECe 10.0 $mScm^{-1}$ of Na_2SO_4, where overall productivity was 194.4% of the control and the grain productivity was 91.8% of the control. This plant can grown upto ECe 10.0 $mScm^{1}$, where its grain productivity was 91.8% of the control. Highest (141.8% of the control) grain productivity was at ECe 7.5$mScm^{-1}$ of Na_2SO_4.

Thus, low levels of sulphate are essential for growth of this plant and this plant Carthamus tinctorius L. Cv.Bhima is moderately salt tolerant.

CHAPTER IV

MINERAL METABOLISM UNDER SALINE CONDITIONS

1) INTRODUCTION

Today greater attention is given to problem on salt affected soils. According to Ayadi and Hamza (1984), presence of NaCl in a medium modifies nutritional requirements of plants. The response of plant in relation to mineral status varies not only from one crop to the other but also changes with varieties of the same crop. The regulation of the transport and distribution of the ions in the various organs of the plants and within the cells is an essential component of the mechanism of salt tolerance. Various aspects of salt tolerance remain uncertain because of the lack of information on the concentration of salt ions in the various compartments (Osmond et al., 1980). However, energy crisis has become the catalyst for recent interests in the genetic improvement of salt tolerance and in the uptake and utilization of essential nutrients. Common interest in both has resulted because nutrient imbalance is a possible effect of high salt concentration (Epstien , 1972 ; Nieman and Shannon; 1976). Cell membranes are likely target for the toxic effects of NaCl (Bliss et al., 1986), since proton fluxes are a response to salt stress (Reinhold et al., 1984). Wiedders (1992) suggested that saline and osmotic solutions were eliciting a biochemical or physical signal in the root which resulted in an increase in plasma membrane permeability and thus an extracellular ion accumulation within leaf mesophyll tissue of Pisum sativum. Observations made by Joshi et. al. (1987) indicated that specific ion effect in proline content was high under NaCl salinity and low under Na_2SO_4. With new dimensions to research technology coming into being , it is but natural that 'Stress Physiology' studies also incorporate the same but at the same time give priority to fundamental and applied aspects. In order to envision the problem at the whole plant level, it is necessary to determine salt tolerance limits and study the interrelations of ion status with other plant metabolism. With this

view in mind, macro and micro nutrients were analysed in Carthamus tinctorius Var. Bhima. under saline conditions.

2) MATERIALS AND METHODS

Plants which were grown in pots under chloride and sulphate salinizations served as source for mineral studies. At the time of flowering these plants were harvested, separated into individual plant parts, sun dried and finally oven dried to constant weight (Sestak et al., 1971). The dried plant parts were subjected to tri acid digestion as per the method given by Chapman and Pratt (1961). Sodium and Potassium were estimated Spectrophotometrically (Model CL:22A, ELICO). Phosphorus was determined by Vanado - molybdate method of Sekine et al. (1972). Chloride was estimated by the method of Volhard (1960), while sulphate by the method of Chapman and Pratt (1961) and elements like Ca, Mg, Mn, Fe, Ni, Zn by using Atomic absorption spectro-photometer, Model - 300, (USA). All the elements were estimated twice from the vegetative plant parts like root, stem and fully expanded third leaves. From each sample all the elements were determined thrice. Thus, the mean value recorded is the mean of three determinations.

3) RESULTS

Effect of increasing levels of NaCl and Na_2SO_4 salinity's on mineral nutrition of Carthamus tinctorius Var. Bhima from the results are recorded in (Tables. 11-16 ; Fig. 7-12).

A) SODIUM

Results of sodium content under saline conditions in Carthamus tinctorius Var. Bhima. are given in (Tables.11-16 ; Fig. 7-12).

Table. 11.

Effect of increasing concentrations of NaCl on minerals in root in Carthamus tinctorius L. Cv. Bhima

Minerals (mg /100 g) ECe $mScm^{-1}$	Na	K	K/Na	Cl	SO_4	Ca	PO_4	Zn	Fe	Mn
Control 0.44	1260	82.0	0.0651	316.00	340.00	3334	440	289	1.73	2.0
NaCl 5.0	1570	97.0	0.0618	1046.0	345.10	3916	450	210	1.96	3.2
NaCl 7.5	1920	100	0.0521	2646.0	356.00	4153	456	188	2.48	3.4
NaCl 10.0	2010	170	0.0846	3890.0	360.20	4316	396	170	2.76	4.2
NaCl 12.5*	2510	275	0.1096	3990.0	365.30	4450	321	150	1.90	5.4
NaCl 15.0*	2972	390	0.1312	4500.0	372.80	2610	200	128	1.83	7.1
SEM	0.07	1.53	0.06	104.36	252.28	27.24	2.74	1.38	1.47	0.03
LSD	0.23	4.73	0.19	321.55	777.43	83.96	8.45	4.27	4.54	0.09
SD±	±5.84	±1.15	± 0.02	±26.73	±11.76	± 6.73	± 0.94	± 0.53	±0.36	±0.01

* Analysis was done from dead plant material

Table. 12.

Effect of increasing concentrations of Na_2SO_4 on minerals in root in Carthamus tinctorius L. Cv. Bhima

Minerals (mg / 100g) ECe $mScm^{-1}$	Na	K	K/Na	Cl	SO_4	Ca	PO_4	Zn	Fe	Mn
Control 0.44	1260	82.0	0.065	316.00	340.00	3334	440	289	1.73	2.0
Na_2SO_4 5.0	1320	76.0	0.057	947.00	363.20	4321	457	300	2.50	6.1
Na_2SO_4 7.5	1540	65.0	0.042	1567.0	370.60	4560	478	311	3.66	8.7
Na_2SO_4 10.0	1780	56.0	0.031	2823.0	376.70	4719	490	295	3.89	3.6
Na_2SO_4 12.5	2090	32.0	0.015	3600.0	380.20	4516	556	215	2.75	2.5
Na_2SO_4 15.0*	2660	18.0	0.006	3950.0	382.80	4419	620	122	2.08	2.1
SEM	0.30	0.42	0.02	94.83	260.9	30.65	3.61	1.86	2.03	0.03
LSD	0.94	1.29	0.09	292.23	804.1	94.45	11.3	5.75	6.27	0.10
SD±	±5.00	± 0.23	± 0.02	±14.73	±15.05	± 4.78	± 0.6	± 0.69	±0.80	±0.02

Analysis was done from dead plant material

Table. 13.

Effect of increasing concentrations of NaCl salinity on mineral content of stem (upper, middle and lower parts) in Carthamus tinctorius L. Cv. Bhima

Mineral s (mg / 100g) ECe $mScm^{-1}$	Na	TS Na	K	TS K	K/Na	TS K/Na	Cl	TS Cl	SO_4	TS SO_4	Ca	TS Ca	PO_4	TS PO_4	Zn	TS Zn	Fe	TS Fe	Mn	TS Mn
Control (stem U.P.)	420		260		0.619		250		120.60		3700		460		153		6.00		5.6	
Control (stem M.P.)	520		15.0		0.029		243		126.00		3966		320		136		4.02		12.2	
Control (stem L.P.)	400	447	15.0	97.0	0.038	0.228	241	244.66	112.00	119.53	6820	4829	720	500	130	140	4.01	4.68	37.4	0.184
NaCl 5.0 U.P.	440		190		0.432		851		121.50		2919		460		190		7.08		7.9	
NaCl 5.0 M.P.	1260		30.0		0.024		846		143.00		4140		1460		270		8.73		19.4	
NaCl 5.0 L.P.	680	793	30.0	83.0	0.044	0.167	844	847.00	115.00	126.50	7817	4959	730	883	288	249	9.80	8.54	15.7	14.3
NaCl 7.5 U.P.	560		130		0.232		967		115.80		2719		490		211		5.16		6.6	
NaCl 7.5 M.P.	1400		50.0		0.036		952		128.00		4960		1500		290		8.87		19.8	
NaCl 7.5 L.P.	1470	1143	52.0	77.0	0.035	0.101	951	956.66	119.00	120.93	8670	5450	735	908	388	296	9.90	7.98	15.9	14.1
NaCl 10.0 U.P.	902		1.00		0.111		1074		110.60		2616		500		225		4.70		6.1	
NaCl 10.0 M.P.	1720		60.0		0.035		1063		115.00		5215		1556		295		8.91		20.1	
NaCl 10.0 L.P.	2090	1571	60.0	73.0	0.029	0.058	1060	1065.66	115.00	113.53	9610	5814	740	932	396	305	9.90	7.84	16.0	14.1
NaCl 12.5 U.P.	960		70.0		0.073		1192		107.00		2501		530		250		4.57		3.2	
NaCl 12.5 M.P.	1890		60.0		0.032		1173		110.00		6015		1571		310		8.96		21.0	
NaCl 12.5 L.P.	2740	1863	82.0	71.0	0.030	0.045	1169	1178.00	109.00	108.67	10117	6211	760	954	399	320	9.96	7.83	16.8	13.7
NaCl 15.0 U.P.	1240		40.0		0.032		1210		90.00		2420		660		303		4.32		2.9	
NaCl 15.0 M.P.	2620		63.0		0.024		1206		101.00		6217		1660		360		9.10		21.6	
NaCl 15.0 L.P.	3600	2487	100	68.0	0.028	0.028	1202	1206.00	80.00	90.33	10294	6310	920	1080	363	342	9.34	7.59	17.5	14.0
	LSD	SEM	LSD	SEM	LSD	SEM	LSD	SEM	LSD	SEM	LSD	SEM	LSD	SEM	LSD	SEM	LSD	SEM	LSD	SEM
U.P.	0.33	0.10	0.28	0.09	0.71	0.23	167.80	54.45	242.80	78.79	61.99	20.11	11.35	3.68	4.94	1.60	11.75	3.81	0.12	0.04
M.P.	36.99	12.00	0.25	0.08	0.06	0.02	146.52	47.54	264.24	85.75	112.36	36.46	30.90	10.05	6.21	2.01	18.08	5.87	0.41	0.13
L.P.	46.75	15.52	0.13	0.04	0.07	0.02	140.64	45.64	237.70	77.16	195.59	63.47	16.80	5.45	7.43	2.41	19.5	6.33	0.46	0.15

U.P. - Upper Part M.P.- Middle Part L.P. - Lower Part TS -Total stem

Table. 14.

ffect of increasing concentrations of Na_2SO_4 salinity on mineral content of stem (upper, middle and lower parts) in Carthamus tinctorius L. Cv. Bhima

Minerals (mg / 100g) ECe mScm^{-1}	Na	TS Na	K	TS K	K/Na	TS K/Na	Cl	TS Cl	SO_4	TS SO_4	Ca	TS Ca	PO_4	TS PO_4	Zn	TS Zn	Fe	TS Fe	Mn	TS Mn
Control (stem U.P.)	420		260		0.619		250		120.60		3700		460		153		6.00		5.6	
Control (stem M.P.)	520		15.0		0.029		243		126.00		3966		320		136		4.02		12.2	
Control (stem L.P.)	400	447	15.0	97.0	0.038	0.228	241	244.66	112.00	119.53	6820	4829	720	500	130	140	4.01	4.68	37.4	18.4
Na_2SO_4 5.0 U.P.	450		270		0.600		752		155.00		8500		510		166		7.76		11.6	
Na_2SO_4 5.0 M.P.	570		25.0		0.044		748		140.00		9010		1218		267		5.90		15.6	
Na_2SO_4 5.0 L.P.	410	477	30.0	108	0.073	0.239	746	748.66	145.00	146.67	9620	9043	710	813	288	240	6.20	6.62	31.6	19.6
Na_2SO_4 7.5 U.P.	560		290		0.518		853		163.00		8500		520		188		9.50		11.4	
Na_2SO_4 7.5 M.P.	860		30.0		0.035		856		153.00		9270		1920		280		6.08		12.2	
Na_2SO_4 7.5 L.P.	1320	913	390	237	0.295	0.283	848	852.33	160.00	158.67	9860	9210	720	1053	320	263	6.99	7.52	20.4	14.7
Na_2SO_4 10.0 U.P.	780		301		0.386		969		171.00		8777		569		210		6.66		10.0	
Na_2SO_4 10.0 M.P.	1013		35.0		0.035		960		160.00		9310		2121		296		7.15		13.0	
Na_2SO_4 10.0 L.P.	1590	1128	390	242	0.245	0.222	955	960.66	170.00	167.00	9720	9269	790	1160	410	305	7.50	7.10	20.4	14.5
Na_2SO_4 12.5 U.P.	1023		340		0.332		1074		180.00		8819		591		216		6.05		8.0	
Na_2SO_4 12.5 M.P.	1320		50.0		0.038		1064		170.00		9420		2125		310		7.50		13.5	
Na_2SO_4 12.5 L.P.	1960	1434	396	262	0.202	0.191	1057	1065.00	185.00	178.33	9860	9366	802	1173	410	312	7.70	7.08	20.2	13.9
Na_2SO_4 15.0 U.P.	1360		360		0.265		1181		188.20		5470		600		119		5.97		8.4	
Na_2SO_4 15.0 M.P.	1640		80.0		0.049		1169		193.00		6093		42.80		186		7.88		15.6	
Na_2SO_4 15.0 L.P.	3640	2213	580	340	0.159	0.158	1163	1171.00	195.00	192.07	8890	6818	2053	2311	517	274	9.46	7.77	20.0	14.7
	LSD	SEM	LSD	SEM	LSD	SEM	LSD	SEM	LSD	SEM	LSD	SEM	LSD	SEM	LSD	SEM	LSD	SEM	LSD	SEM
U.P.	18.24	5.92	0.29	0.09	3.23	1.04	142.14	46.12	358.31	116.2	164.7	53.46	11.85	3.84	3.9	1.26	15.46	5.02	0.20	0.06
M.P.	23.18	7.52	0.09	0.03	0.08	0.02	125.19	40.62	345.26	112.0	176.9	57.43	50.79	16.48	5.54	1.79	14.39	4.67	0.29	0.09
L.P.	41.34	13.41	0.07	0.07	0.41	0.13	113.7	36.89	356.2	115.5	203.9	66.43	23.55	7.64	8.14	2.64	15.49	5.02	0.55	0.18

U.P - Upper Part M.P - Middle Part L.P - Lower Part TS- Total stem

Table ,15.

Effect of increasing NaCl salinity on mineral content of leaves and crown leaves in Carthamus tinctorius L. Cv. Bhima

Minerals (mg / 100g) ECe mScm^{-1}	Na	LCL Na	K	LCL K	K/Na	LCL K/Na	Cl	LCL Cl	SO_4	LCL SO_4	Ca	LCL Ca	PO_4	LCL PO_4	Zn	LCL Zn	Fe	LCL Fe	Mn	LCL Mn
Control 0.44 Lvs.	1100		380		0.345		143.60		120.80		3308		400		105		1.26		7.6	
Control 0.44 Cr.lvs.	940	1020	410	395	0.436	0.391	142.10	142.85	125.90	123.35	3413	3361	280	340	157	131	0.76	1.01	2.1	4.9
NaCl 6.0 Lvs.	1260		320		0.254		445.20		125.30		3910		316		256		2.10		6.1	
NaCl 6.0 Cr.lvs.	1020	1140	389	355	0.381	0.318	443.50	444.35	130.20	127.75	3626	3768	316	316	140	198	1.12	1.61	4.9	5.5
NaCl 7.5 Lvs.	1380		260		0.188		550.80		122.60		4120		250		311		3.10		5.2	
NaCl 7.5 Cr.lvs.	1160	1270	290	275	0.250	0.219	548.21	549.51	133.70	128.15	3920	4020	350	300	102	207	1.50	2.30	6.6	5.9
NaCl 10.0 Lvs.	1490		160		0.107		753.20		120.20		4320		220		275		3.50		4.0	
NaCl 10.0 Cr.lvs.	1220	1355	160	160	0.131	0.119	752.00	752.60	130.20	125.20	4010	4165	250	235	100	188	1.50	2.50	4.7	4.4
NaCl 12.5 Lvs.	1570		120		0.076		861.30		117.20		4110		180		246		3.79		3.1	
NaCl 12.5 Cr.lvs.	1210	1390	75.0	98.0	0.062	0.069	858.20	859.75	128.20	122.70	3890	4000	200	190	90.0	168	1.20	2.50	3.5	3.3
NaCl 15.0 Lvs.	1620		90.0		0.056		1073.80		115.80		3180		108		222		4.17		2.7	
NaCl 15.0 Cr.lvs.	1200	1410	48.0	69.0	0.040	0.048	1076.20	1075.00	110.10	112.95	3320	3250	120	114	70.0	146	1.02	2.60	3.0	2.9
LSD Lvs.	30.52		0.41		0.003		0.24		0.34		0.20		0.32		0.02		0.19		0.29	
SD± Lvs.	±1.30		±1.25		±0.10		±10.62		±3.26		±4.4		±0.97		±0.66		±1.04		±0.17	
SEM Lvs.	0.42		0.13		0.001		0.07		0.11		0.06		0.10		0.008		0.06		0.09	
LSD Cr. lvs.	0.177		0.16		0.028		0.38		0.26		0.39		0.41		0.12		0.26		0.007	
SD± Cr. lvs.	±1.10		±1.47		±0.15		±11.88		±7.87		±2.65		±0.80		±0.31		±0.29		±0.01	
SEM Cr. lvs.	0.05		0.05		0.009		0.12		0.08		0.12		0.13		0.04		0.08		0.002	

Lvs. - Leaves Cr. lvs. - Crown leaves LCL - Leaves + Crown leaves

Table . 16.

Effect of increasing Na_2SO_4 salinity on mineral content of leaves and crown leaves in Carthamus tinctorius L. Cv. Bhima

Minerals (mg / 100 g) ECe mScm^{-1}	Na	LCL Na	K	LCL K	K/Na	LCL K/Na	Cl	LCL Cl	SO_4	LCL SO_4	Ca	LCL Ca	PO_4	LCL PO_4	Zn	LCL Zn	Fe	LCL Fe	Mn	LC L Mn
Control 0.44 Lvs.	1100		380		0.345		143.60		120.8		3308		400		105		1.26		7.6	
Control 0.44 Cr.lvs.	940	1020	410	395	0.436	0.391	142.10	142.85	125.9	123.3	3413	3361	280	340	157	131	0.76	1.01	2.1	4.9
Na_2SO_4 5.0 Lvs.	1160		350		0.302		330.70		129.0		4120		355		270		2.19		7.1	
Na_2SO_4 5.0 Cr.lvs.	916	1038	256	303	0.279	0.291	328.20	329.45	135.0	132.0	4810	4465	395	375	166	218	1.69	1.94	2.9	5.0
Na_2SO_4 7.5 Lvs.	1380		250		0.181		443.60		131.0		4480		376		308		3.03		6.7	
Na_2SO_4 7.5 Cr.lvs.	1010	1195	160	205	0.158	0.170	440.10	441.85	141.0	136.0	5020	4750	410	393	170	239	1.59	2.31	3.0	4.9
Na_2SO_4 10.0 Lvs.	1470		210		0.143		548.30		141.0		4120		295		296		3.26		5.6	
Na_2SO_4 10.0 Cr.lvs.	1090	1280	140	175	0.128	0.136	544.20	546.25	155.0	148.0	4610	4365	310	303	189	243	1.51	2.39	2.6	4.1
Na_2SO_4 12.5 Lvs.	1560		190		0.122		769.90		130.0		3960		250		210		2.16		4.1	
Na_2SO_4 12.5 Cr.lvs.	1120	1340	120	155	0.107	0.114	766.30	768.10	161.0	145.5	4010	3985	210	230	196	203	1.21	1.69	1.7	2.9
Na_2SO_4 15.0 Lvs.	1610		121		0.075		872.10		126.0		3564		160		085		1.75		3.2	
Na_2SO_4 15.0 Cr.lvs.	1130	1370	110	116	0.097	0.086	868.60	870.35	149.0	137.5	2308	2936	136	148	229	157	0.66	1.21	1.1	2.2
LSD Lvs.	0.35		0.25		0.003		0.34		1.78		0.20		0.11		0.17		0.03		0.29	
SD±Lvs.	±0.86		±0.93		±0.09		±15.31		±6.32		±3.97		±0.85		±0.91		±0.71		±0.17	
SEM Lvs.	0.11		0.08		0.001		0.11		0.58		0.06		0.03		0.05		0.01		0.09	
LSD Cr. lvs.	0.17		0.21		0.003		0.54		2.58		0.24		0.20		0.07		0.03		0.10	
SD± Cr. lvs.	±1.10		±1.07		±0.12		±14.88		±12.33		±5.5		±0.99		±0.24		±0.41		±0.08	
SEM Cr. lvs.	0.05		0.07		0.001		0.17		0.83		0.07		0.06		0.025		0.01		0.03	

Lvs. - Leaves Cr. lvs. - Crown leaves LCL - Lvs. + Cr. lvs.

Table. 17.

A comparative study of the effect of NaCl salinity on Na, K, K/Na, Cl and SO_4 content in Carthamus tinctorius L. Cv. Bhima

Minerals (mg / 100 g)	Na				K				K/Na				Cl				SO_4			
ECe $mScm^{-1}$	Root	Stem	Leaves	(R+S+L)	Root	Stem	Leaves	(R+S+L)	Root	Stem	Leaves	(R+S+L)	Root	Stem	Leaves	(R+S+L)	Root	Stem	Leaves	(R+S+L)
Control 0.44	1260	447	1020	909	82.0	97.0	395	191	0.0651	0.228	0.391	0.2280	316	244.66	142.86	234.50	340.0	119.53	123.35	194.29
NaCl 5.0	1570	793	1140	1168	97.0	83.0	355	178	0.0618	0.167	0.318	0.1822	1046	847.00	144.36	779.12	345.1	126.50	127.75	199.78
NaCl 7.5	1920	1143	1270	1444	100	77.0	275	151	0.0521	0.101	0.219	0.1240	2646	956.66	749.51	1450.72	356.0	120.93	128.15	201.69
NaCl 10.0	2010	1571	1355	1645	170	73.0	160	134	0.0846	0.058	0.119	0.0872	3890	1015.66	752.60	1886.08	360.2	113.53	125.20	199.64
NaCl 12.5	2510	1863	1390	1921	275	71.0	98.0	148	0.1096	0.045	0.069	0.0745	3990	1178.00	859.75	2009.25	365.3	108.67	122.70	198.69
NaCl 15.0	2972	2487	1410	2290	390	68.0	69.0	176	0.1312	0.028	0.048	0.0690	4500	1206.00	1075.0	2260.33	372.8	90.33	112.95	192.03

R- Root S- Stem L- Leaves

Table. 18.

A comparative study of the effect of NaCl salinity on Ca, PO_4, Zn, Fe and Mn content in Carthamus tinctorius L.Cv. Bhima

Mineral s (mg / 100 g)	Ca				PO_4				Zn				Fe				Mn			
ECe $mScm^{-1}$	Root	Stem	Leaves	(R+S+L)	Root	Stem	Leaves	(R+S+L)	Root	Stem	Leaves	(R+S+L)	Root	Stem	Leaves	(R+S+L)	Root	Stem	Leaves	(R+S+L)
Control 0.44	3334	4829	3361	3841	440	500	340	427	289	140	131	187	1.73	4.68	1.01	2.47	2.0	18.4	4.9	8.43
NaCl 5.0	3916	4959	3768	4214	450	883	316	550	210	250	198	219	1.96	8.64	1.61	4.04	3.2	14.3	5.5	7.67
NaCl 7.5	4153	5450	4020	4541	456	908	300	555	188	300	270	251	2.48	7.98	2.30	4.25	3.4	14.1	5.9	7.80
NaCl 10.0	4316	5814	4165	4765	396	932	235	521	170	310	188	221	2.76	7.84	2.50	4.37	4.2	14.1	4.4	7.57
NaCl 12.5	4450	6211	4000	4887	321	954	190	488	150	320	168	213	1.90	7.83	2.50	4.08	5.4	13.7	3.3	7.47
NaCl 15.0	2610	6310	3250	4057	200	1080	114	465	128	340	146	205	1.83	7.59	2.60	3.91	7.1	14.0	2.9	8.00

R- Root S- Stem L- Leaves

Table. 19.

A comparative study of the effect of Na_2SO_4 salinity on Na, K, K/Na, Cl and SO_4 content in Carthamus tinctorius L. Cv. Bhima

Minerals (mg / 100 g)	Na				K				K/Na				Cl				SO_4			
ECe $mScm^{-1}$	Root	Stem	Leaves	(R+S+L)	Root	Stem	Leaves	(R+S+L)	Root	Stem	Leaves	(R+S+L)	Root	Stem	Leaves	(R+S+L)	Root	Stem	Leaves	(R+S+L)
Control 0.44	1260	4.47	1020	909	82.0	97.0	395	191	0.0651	0.228	0.391	0.2280	316	244.66	142.86	234.50	340.00	119.53	123.35	194.29
Na_2SO_4 5.0	1320	477	1038	945	76.0	108	303	162	0.0576	0.239	0.291	0.1958	947	748.66	329.45	675.36	363.20	146.67	132.00	213.96
Na_2SO_4 7.5	1540	913	1195	1216	65.0	237	205	169	0.0422	0.283	0.170	0.1650	1567	852.33	441.85	953.72	370.60	158.67	136.00	221.76
Na_2SO_4 10.0	1780	1128	1280	1396	56.0	242	175	158	0.0315	0.222	0.136	0.1298	2823	960.66	546.25	1443.30	376.70	167.00	148.00	230.57
Na_2SO_4 12.5	2090	1434	1340	1621	32.0	262	155	150	0.0153	0.191	0.114	0.14166	3600	1065.0	768.10	1811.03	380.20	178.33	145.50	234.68
Na_2SO_4 15.0	2660	2213	1370	2081	18.0	340	116	158	0.0068	0.158	0.080	0.0817	3950	1071.0	870.35	1963.78	382.80	192.07	137.50	237.46

R- Root S- Stem L- Leaves

Table. 20.

A comparative study of the effect of Na_2SO_4 salinity on Ca, PO_4, Zn, Fe and Mn content in Carthamus tinctorius L. Cv. Bhima

Minerals (mg / 100 g)	Ca				PO_4				Zn				Fe				Mn			
ECe $mScm^{-1}$	Root	Stem	Leaves	(R+S+L)	Root	Stem	Leaves	(R+S+L)	Root	Stem	Leaves	(R+S+L)	Root	Stem	Leaves	(R+S+L)	Root	Stem	Leaves	(R+S+L)
Control 0.44	3334	4829	3361	3841	440	500	340	427	289	140	131	187	1.73	4.68	1.01	2.47	2.0	18.0	4.9	8.30
Na_2SO_4 5.0	4321	9043	4465	5943	457	813	375	548	300	240	218	253	2.50	6.62	1.94	3.69	6.1	20.0	5.0	0.37
Na_2SO_4 7.5	4560	9210	4750	6173	478	1053	393	641	311	263	239	271	3.66	7.52	2.31	4.50	8.7	15.0	4.9	9.53
Na_2SO_4 10.0	4719	9269	4365	6118	490	1160	303	651	295	305	243	281	3.89	7.10	2.39	4.46	3.6	14.0	4.1	7.23
Na_2SO_4 12.5	4516	9366	3985	5956	556	1173	230	653	215	312	203	243	2.75	7.08	1.69	3.84	2.5	14.0	2.9	6.47
Na_2SO_4 15.0	4419	6818	2936	4724	620	2311	148	1026	122	274	157	184	2.08	7.77	1.21	3.68	2.1	15.0	2.2	6.43

R- Root S- Stem L- Leaves

Fig. 7a.

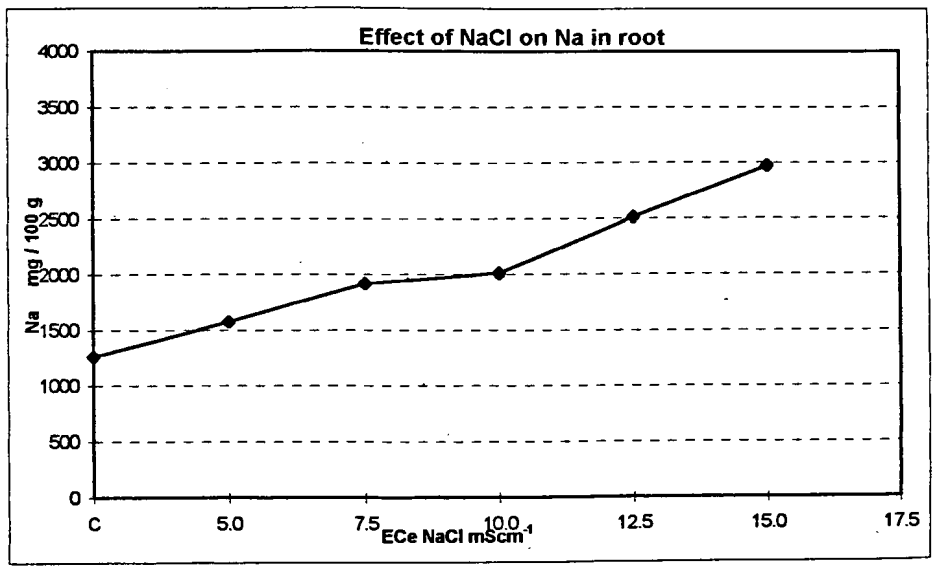

Effect of NaCl on Na in root
Na mg / 100 g
4000
3500
3000
2500
2000
1500
1000
500
0
C
5.0
7.5
10.0
12.5
15.0
17.5
ECe NaCl mScm-1

Fig. 7b.

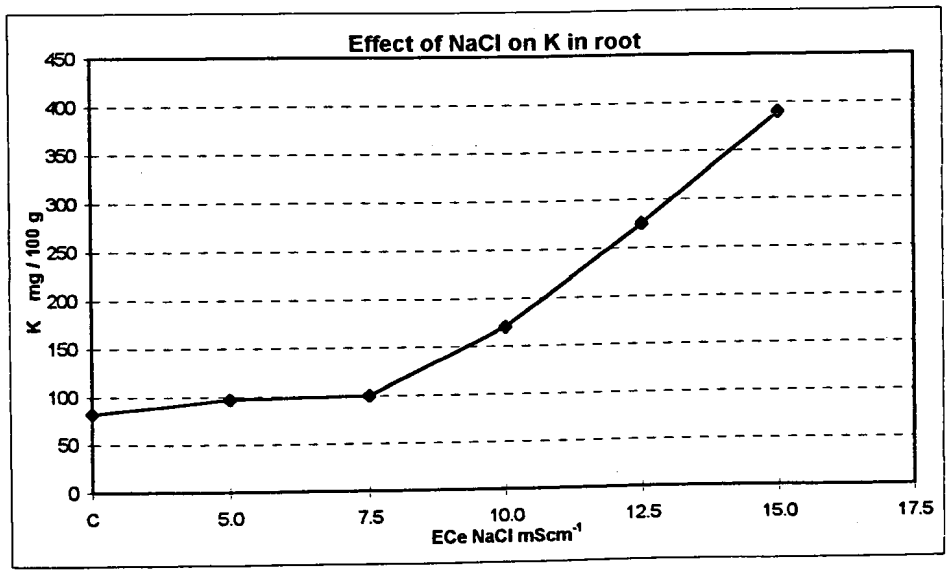

Effect of NaCl on K in root
K mg / 100 g
450
400
350
300
250
200
150
100
50
0
C
5.0
7.5
10.0
12.5
15.0
17.5
ECe NaCl mScm-1

Fig. 7c.

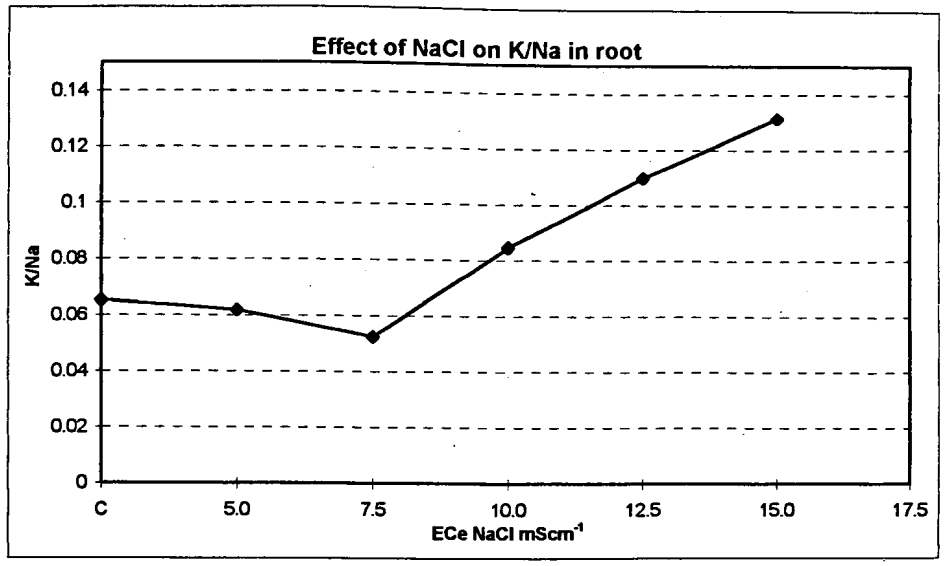

Effect of NaCl on K/Na in root
K/Na
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
C
5.0
7.5
10.0
12.5
15.0
17.5
ECe NaCl mScm-1

Fig. 7d.

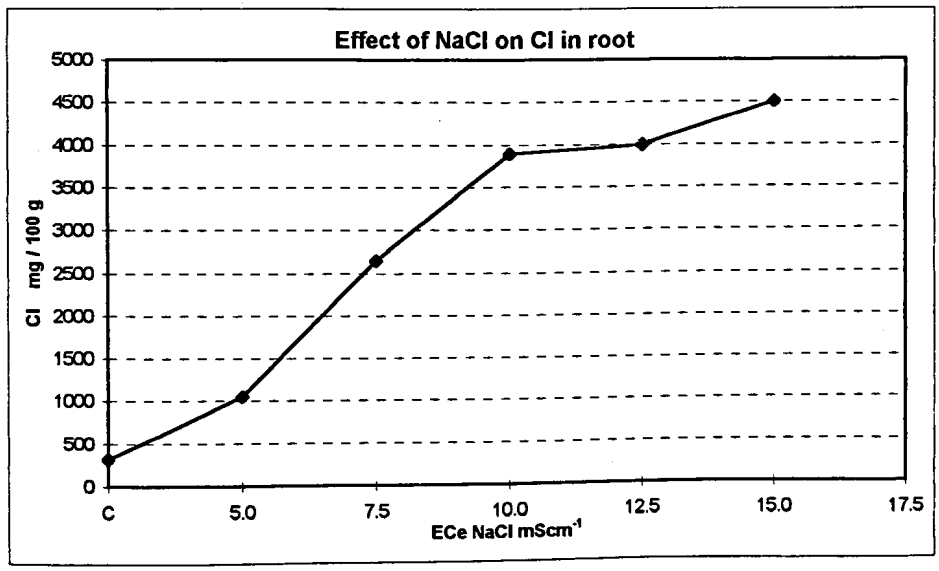

Effect of NaCl on Cl in root
Cl mg / 100 g
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
C
5.0
7.5
10.0
12.5
15.0
17.5
ECe NaCl mScm-1

Fig. 7e.

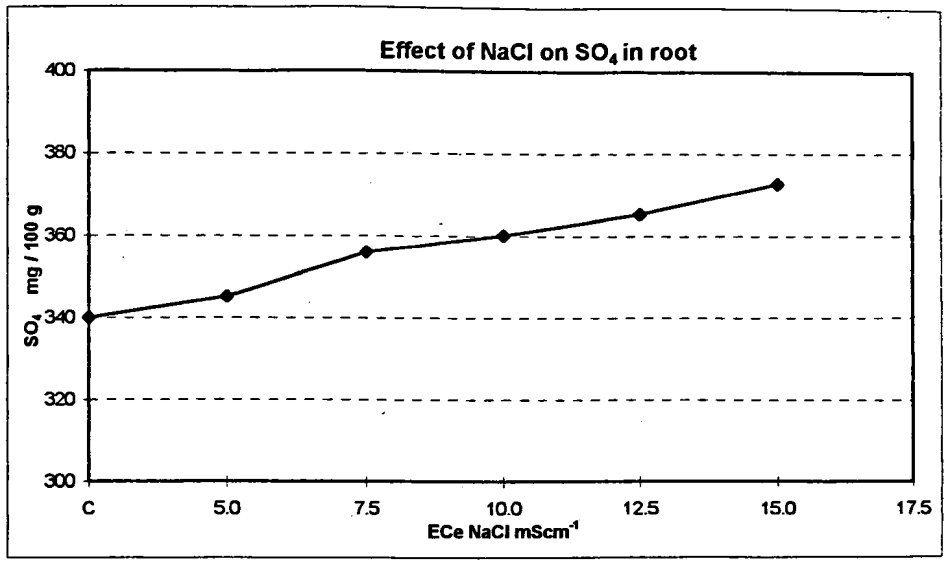

Effect of NaCl on SO_4 in root
400
380
360
340
320
300
SO_4 mg / 100 g
C
5.0
7.5
10.0
12.5
15.0
17.5
ECe NaCl $mScm^{-1}$

Fig. 7f.

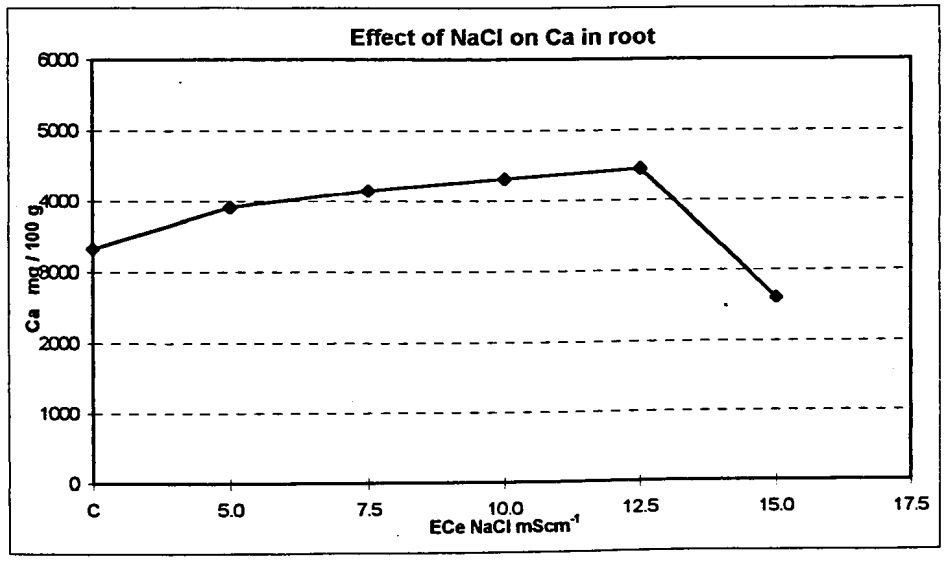

Effect of NaCl on Ca in root
6000
5000
4000
3000
2000
1000
0
Ca mg / 100 g
C
5.0
7.5
10.0
12.5
15.0
17.5
ECe NaCl $mScm^{-1}$

Fig. 7g.

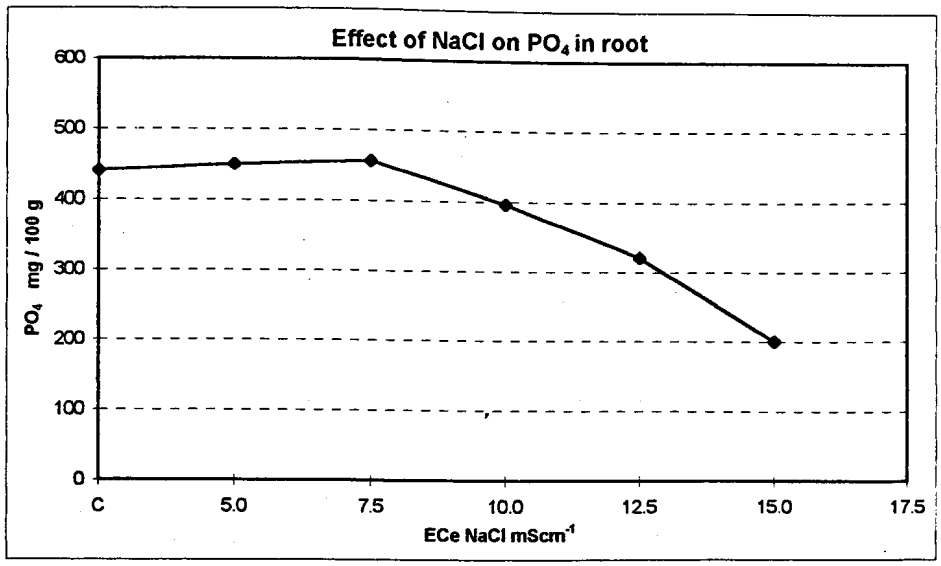

Effect of NaCl on PO_4 in root
600
500
400
300
200
100
0
PO_4 mg / 100 g
C
5.0
7.5
10.0
12.5
15.0
17.5
ECe NaCl mScm^{-1}

Fig. 7h.

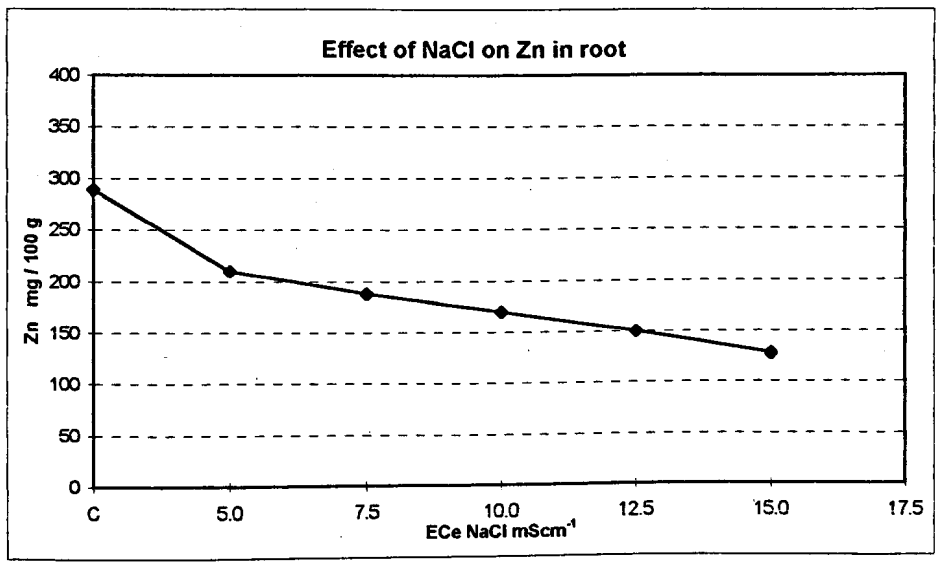

Effect of NaCl on Zn in root
400
350
300
250
200
150
100
50
0
Zn mg / 100 g
C
5.0
7.5
10.0
12.5
15.0
17.5
ECe NaCl mScm^{-1}

Fig. 7i.

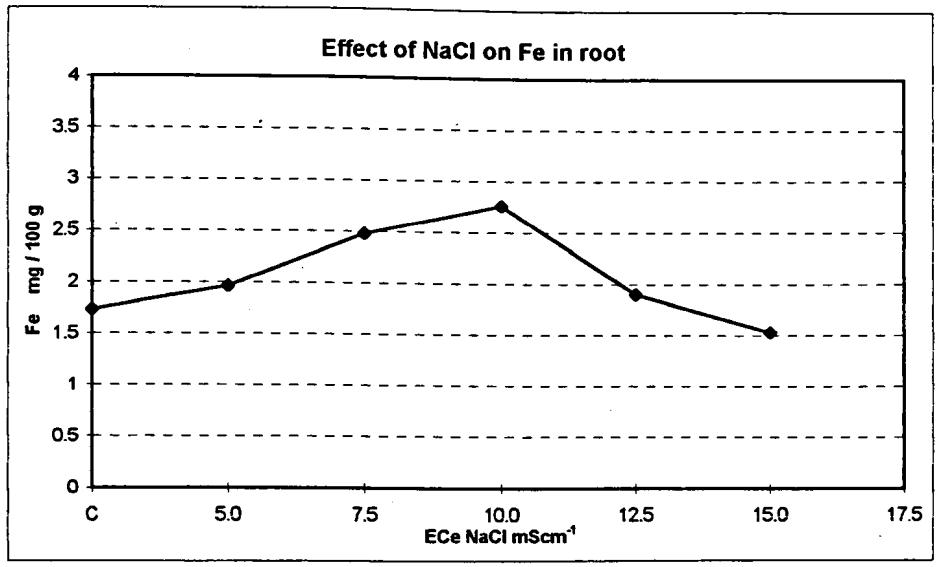

Effect of NaCl on Fe in root
Fe mg / 100 g
4
3.5
3
2.5
2
1.5
1
0.5
0
C
5.0
7.5
10.0
12.5
15.0
17.5
ECe NaCl mScm-1

Fig. 7i.

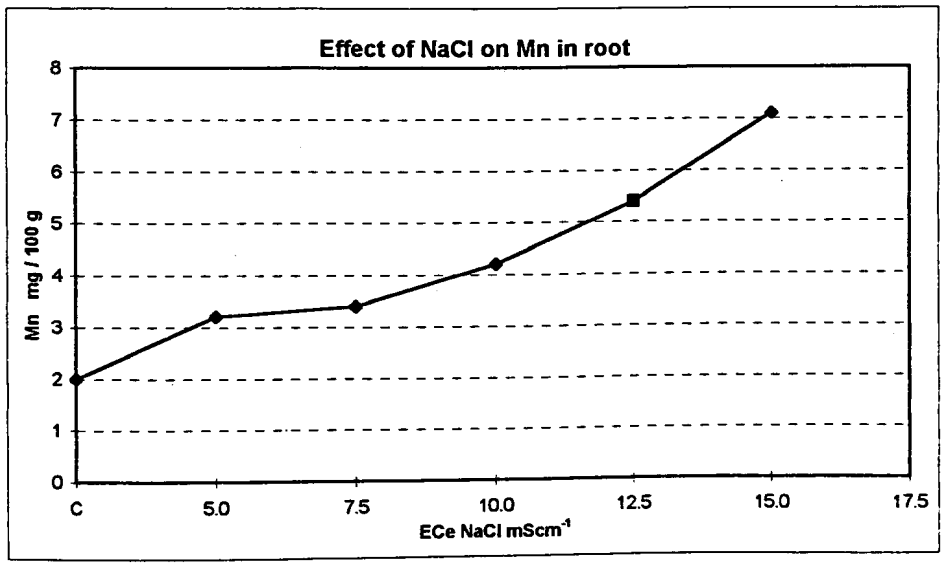

Effect of NaCl on Mn in root
Mn mg / 100 g
8
7
6
5
4
3
2
1
0
C
5.0
7.5
10.0
12.5
15.0
17.5
ECe NaCl mScm-1

Fig. 8a.

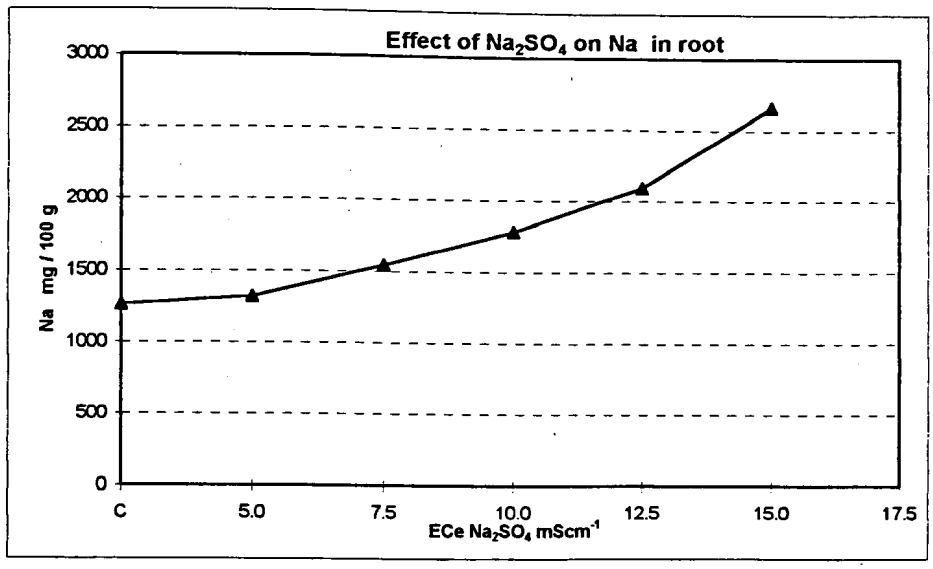

Effect of Na_2SO_4 on Na in root
Na mg / 100 g
3000
2500
2000
1500
1000
500
0
C
5.0
7.5
10.0
12.5
15.0
17.5
ECe Na_2SO_4 $mScm^{-1}$

Fig. 8b.

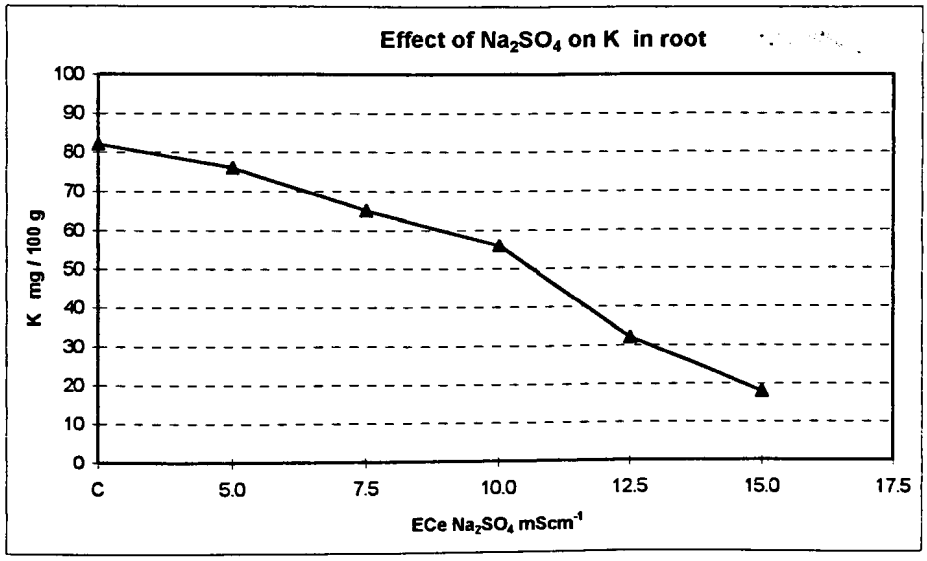

Effect of Na_2SO_4 on K in root
K mg / 100 g
100
90
80
70
60
50
40
30
20
10
0
C
5.0
7.5
10.0
12.5
15.0
17.5
ECe Na_2SO_4 $mScm^{-1}$

Fig. 8c.

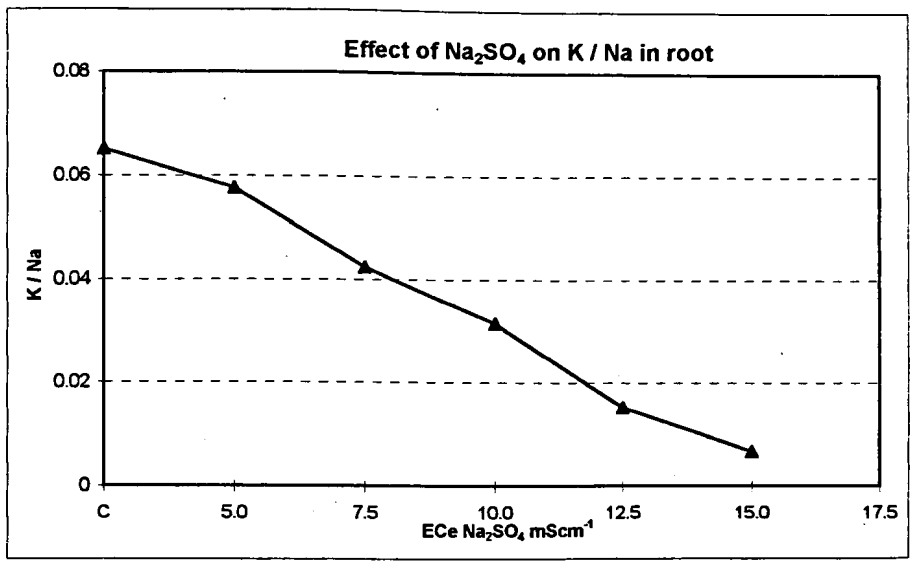

Effect of Na_2SO_4 on K / Na in root
0.08
0.06
0.04
0.02
0
K / Na
C
5.0
7.5
10.0
12.5
15.0
17.5
ECe Na_2SO_4 mScm^{-1}

Fig. 8d.

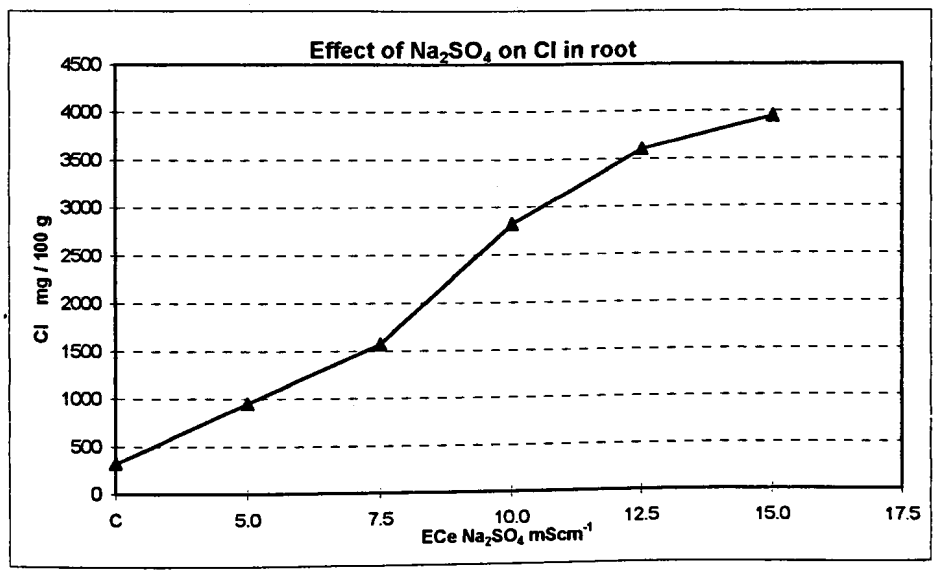

Effect of Na_2SO_4 on Cl in root
4500
4000
3500
3000
2500
2000
1500
1000
500
0
Cl mg / 100 g
C
5.0
7.5
10.0
12.5
15.0
17.5
ECe Na_2SO_4 mScm^{-1}

Fig. 8e.

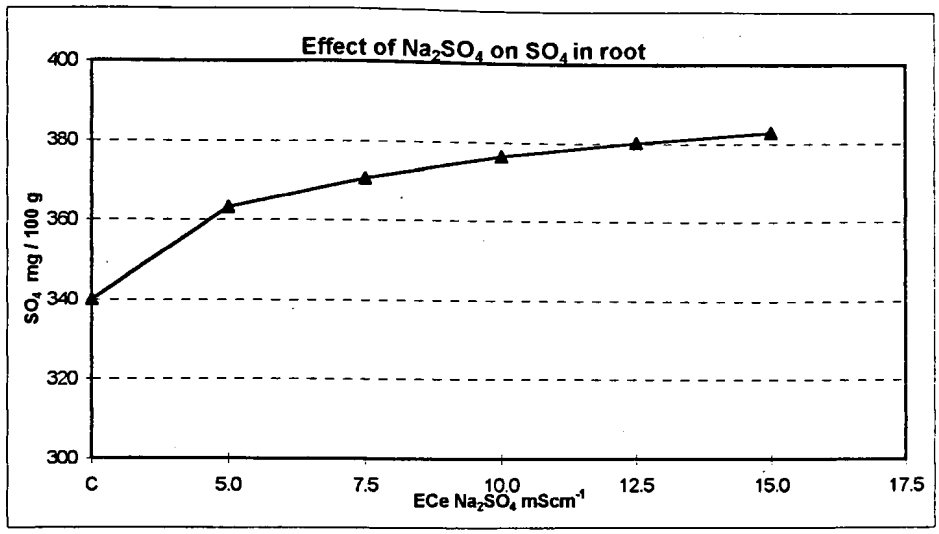

Effect of Na_2SO_4 on SO_4 in root
400
380
360
340
320
300
SO_4 mg / 100 g
C
5.0
7.5
10.0
12.5
15.0
17.5
ECe Na_2SO_4 mScm^{-1}

Fig. 8f.

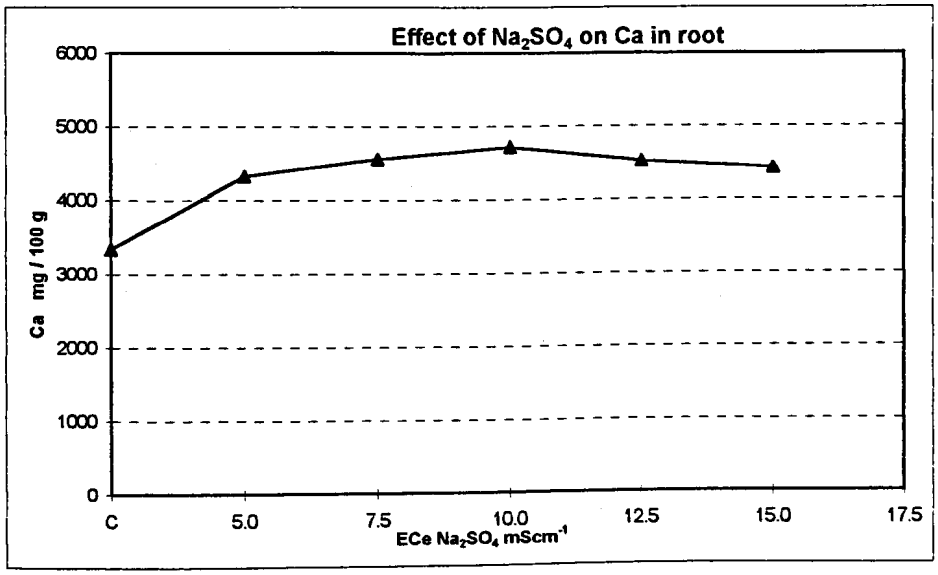

Effect of Na_2SO_4 on Ca in root
6000
5000
4000
3000
2000
1000
0
Ca mg / 100 g
C
5.0
7.5
10.0
12.5
15.0
17.5
ECe Na_2SO_4 mScm^{-1}

Fig. 8g.

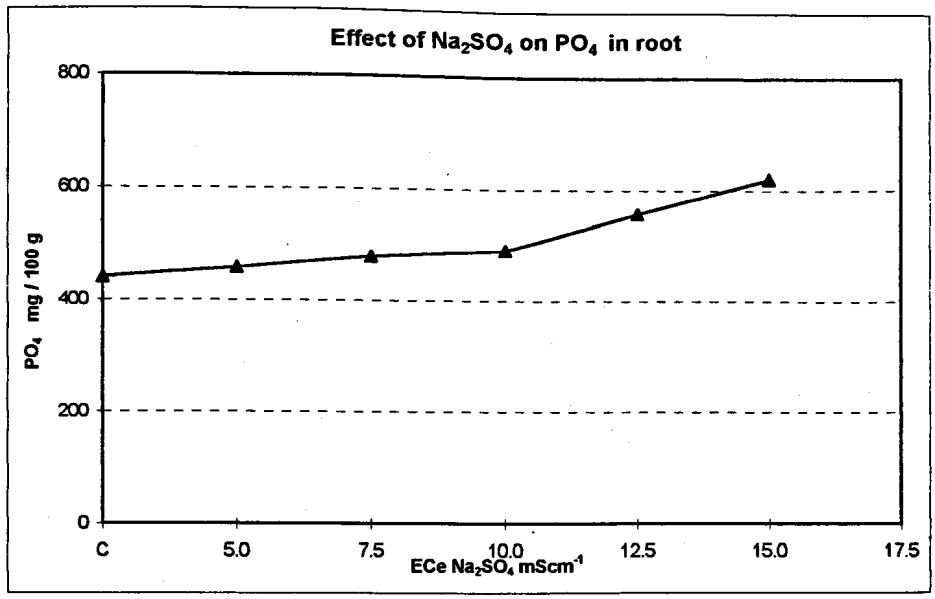

Effect of Na_2SO_4 on PO_4 in root
800
600
400
200
0
PO_4 mg / 100 g
C
5.0
7.5
10.0
12.5
15.0
17.5
ECe Na_2SO_4 mScm^{-1}

Fig. 8h.

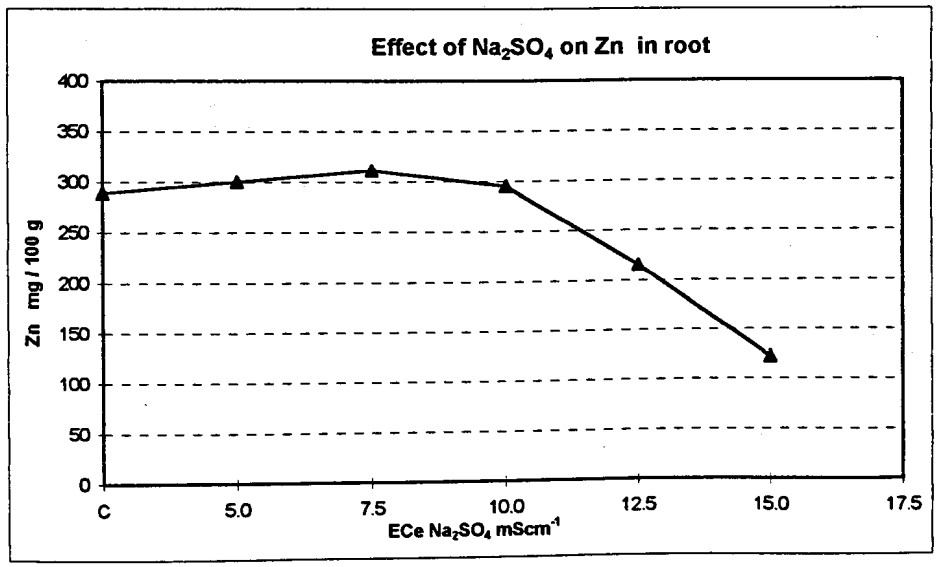

Effect of Na_2SO_4 on Zn in root
400
350
300
250
200
150
100
50
0
Zn mg / 100 g
C
5.0
7.5
10.0
12.5
15.0
17.5
ECe Na_2SO_4 mScm^{-1}

Fig. 8i.

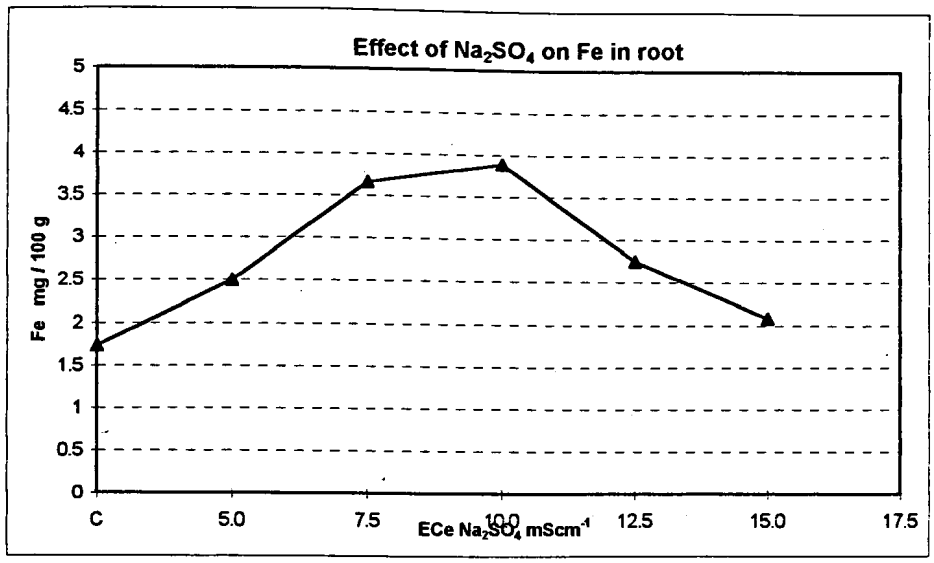

Effect of Na_2SO_4 on Fe in root
Fe mg / 100 g
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
C
5.0
7.5
10.0
12.5
15.0
17.5
ECe Na_2SO_4 mScm^{-1}

Fig. 8j.

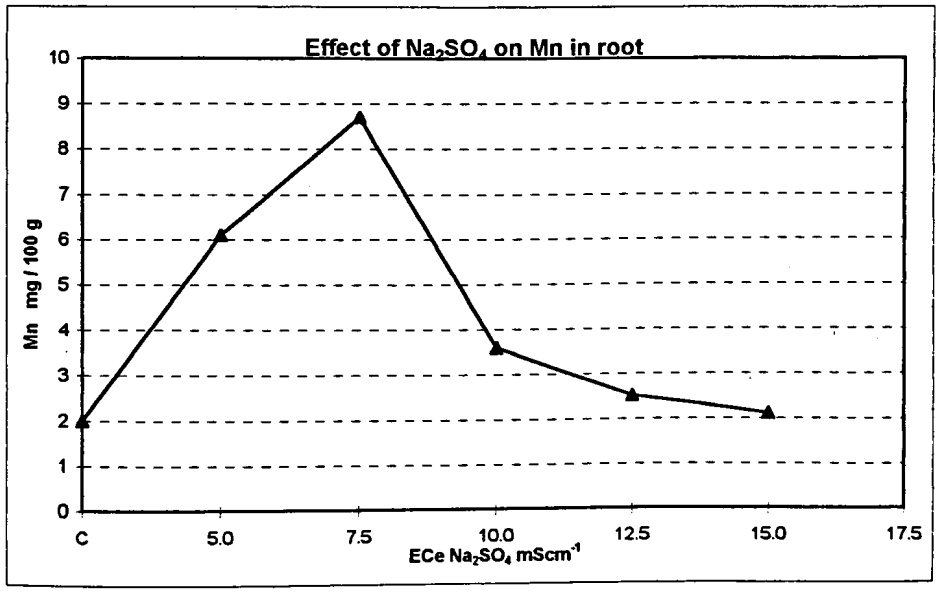
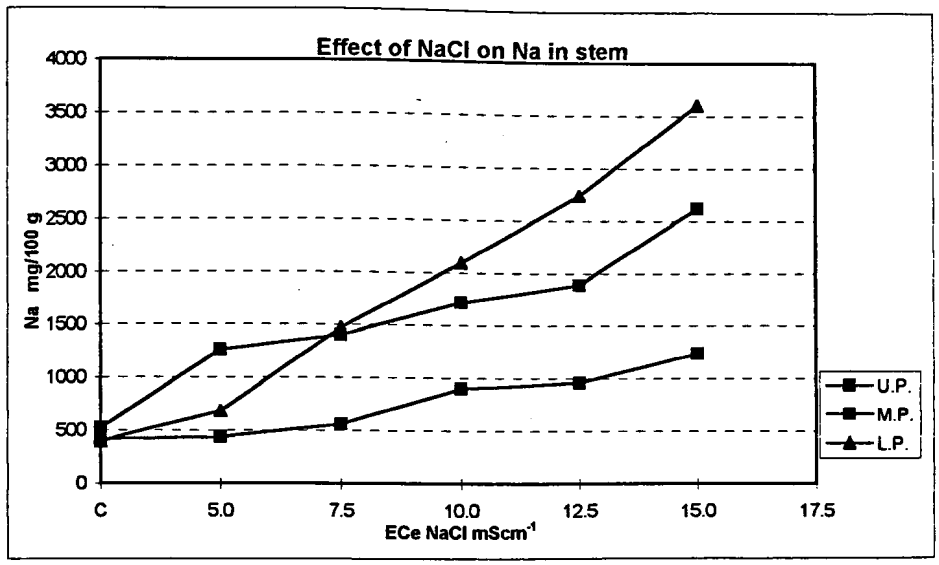
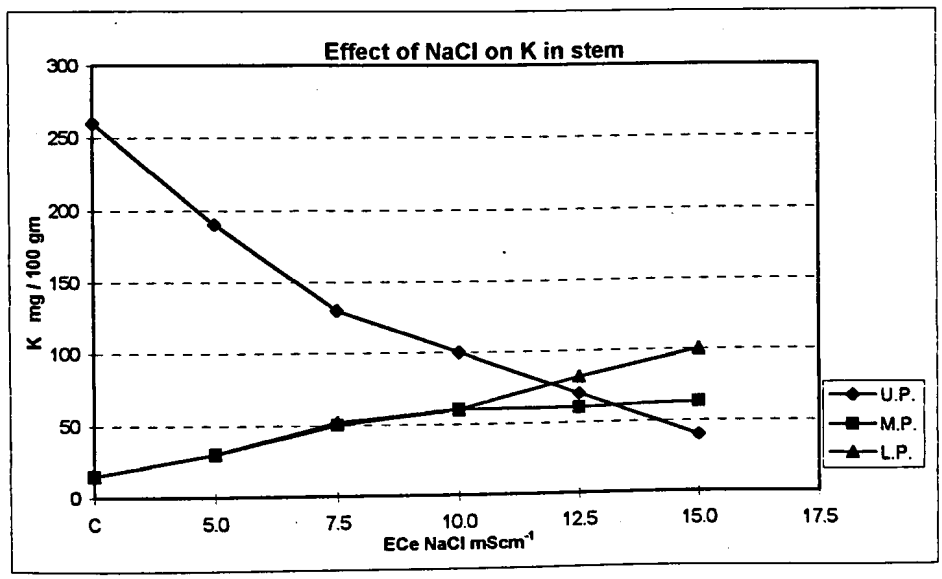

Effect of Na_2SO_4 on Mn in root
Mn mg / 100 g
10
9
8
7
6
5
4
3
2
1
0
C
5.0
7.5
10.0
12.5
15.0
17.5
ECe Na_2SO_4 mScm^{-1}

Fig. 9a.



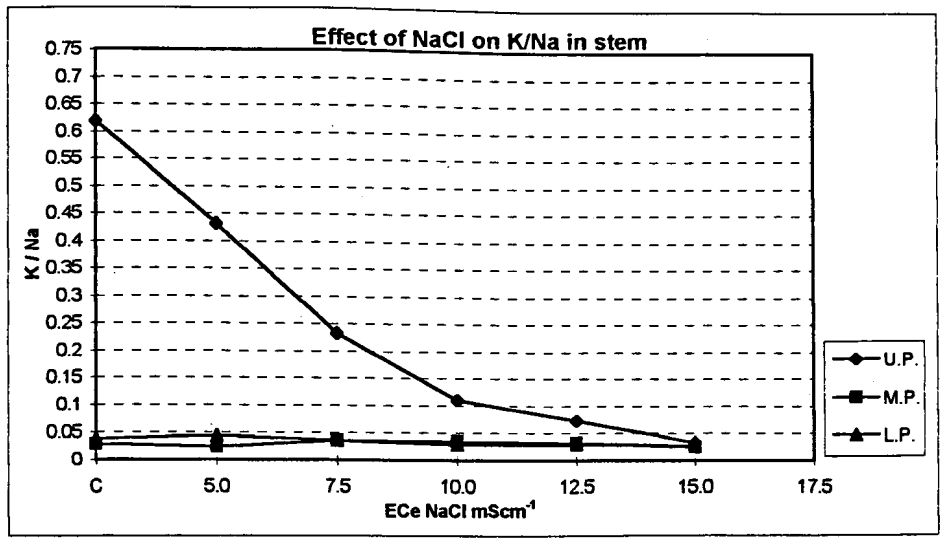
U.P. - Upper part M.P. - Middle part L.P. - Lower part

Fig. 9b.



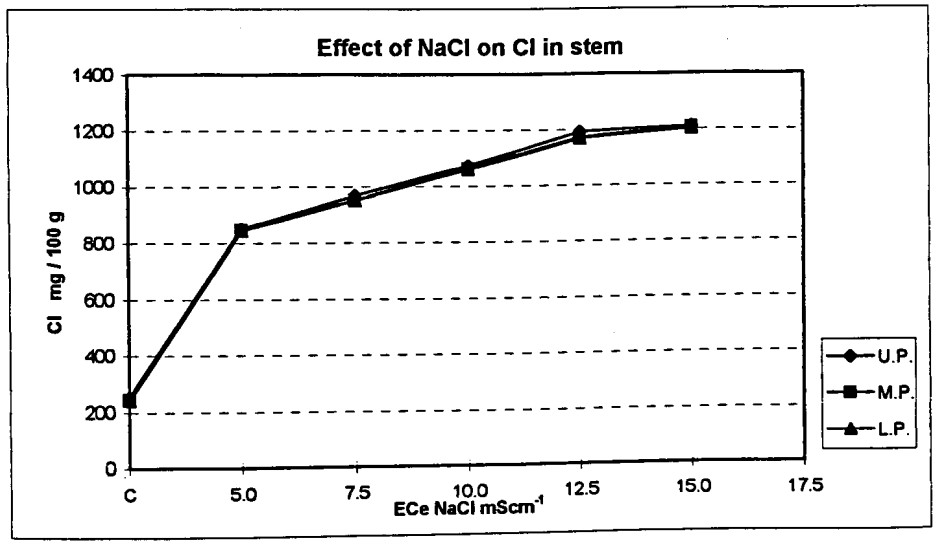
U.P. - Upper part M.P. - Middle part L.P. - Lower part

Fig. 9c.



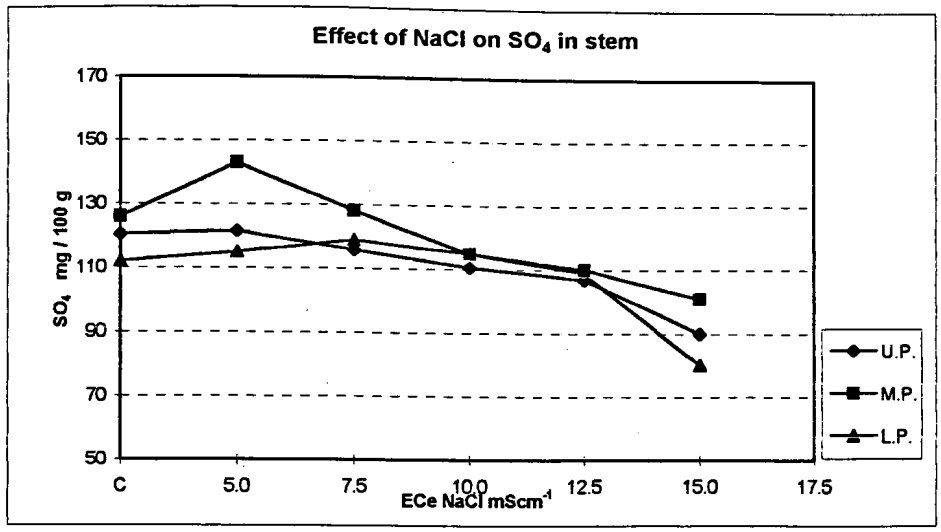
U.P. - Upper part M.P. - Middle part L.P. - Lower part

Fig. 9d.



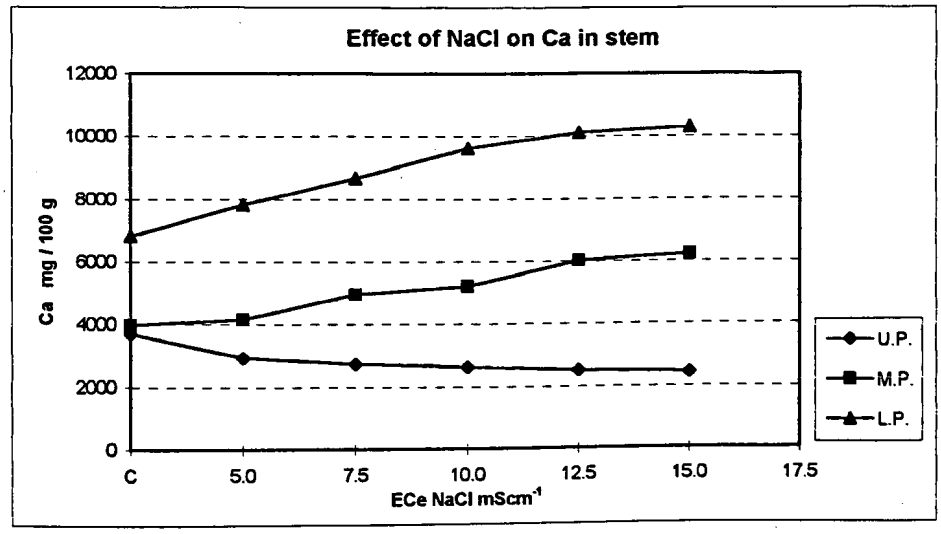
U.P. - Upper part M.P. - Middle part L.P. - Lower part

Fig . 9e.



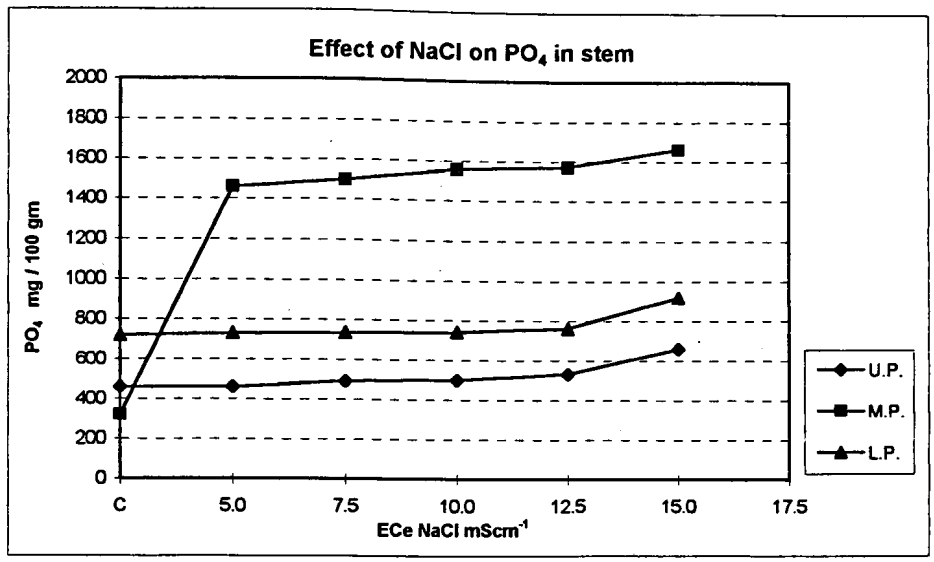
U.P. - Upper part M.P. - Middle part L.P. - Lower part

Fig. 9f.



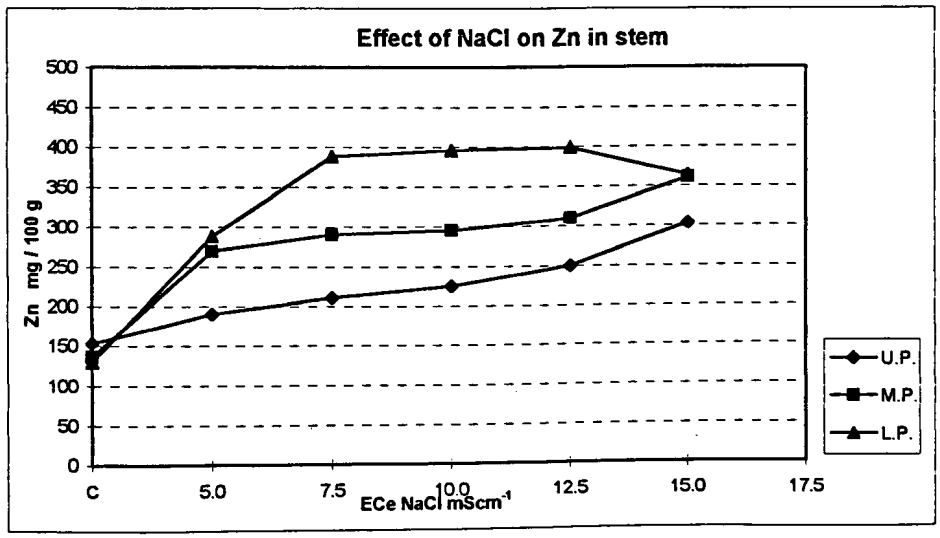
U.P. - Upper part M.P. - Middle part L.P. - Lower part

Fig. 9g.



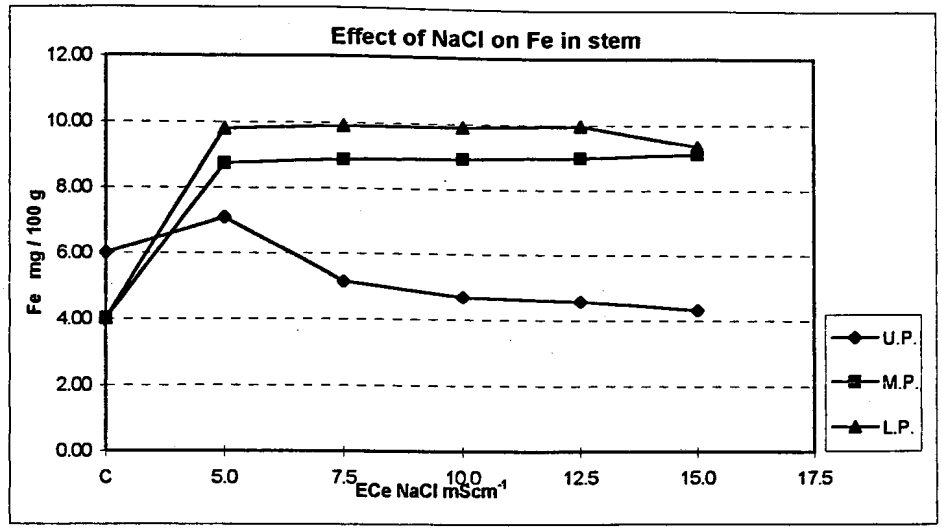
U.P. - Upper part M.P. - Middle part L.P. - Lower part

Fig. 9h.



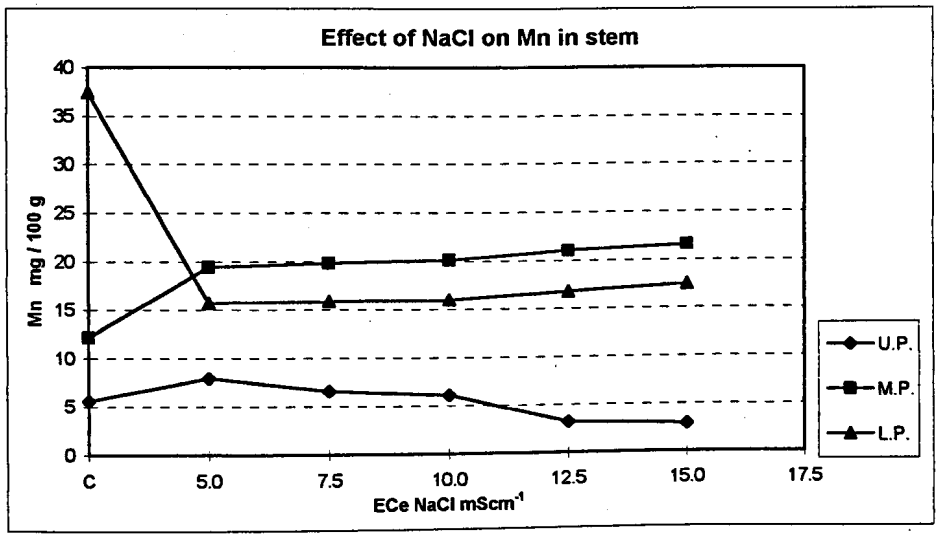
U.P. - Upper part M.P. - Middle part L.P. - Lower part

Fig. 9i.



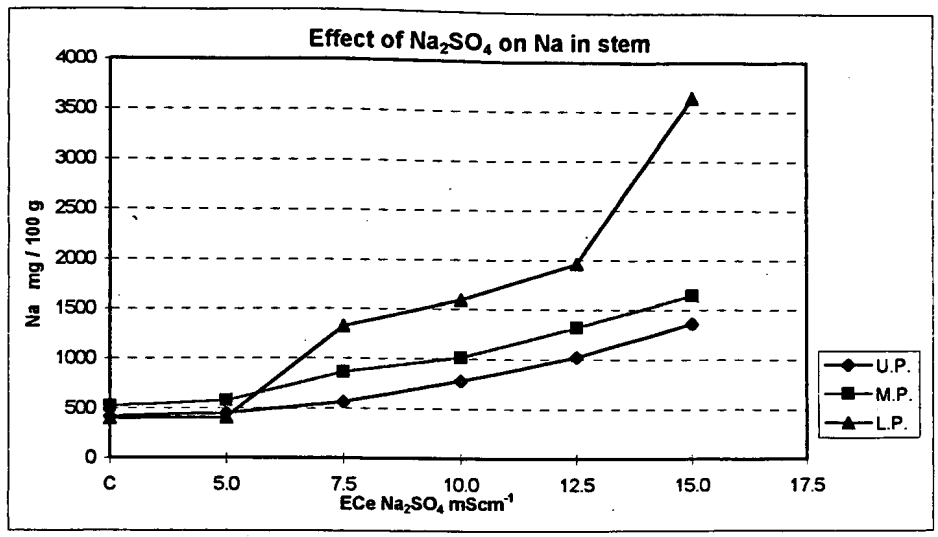
U.P. - Upper part M.P. - Middle part L.P. - Lower part

Fig. 9j.



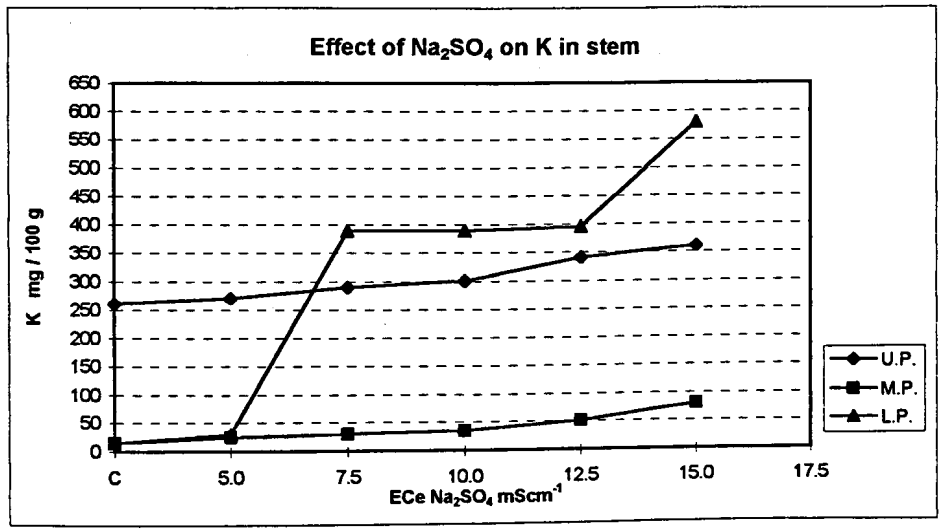
U.P. - Upper part M.P. - Middle part L.P. - Lower part

Fig.10a.



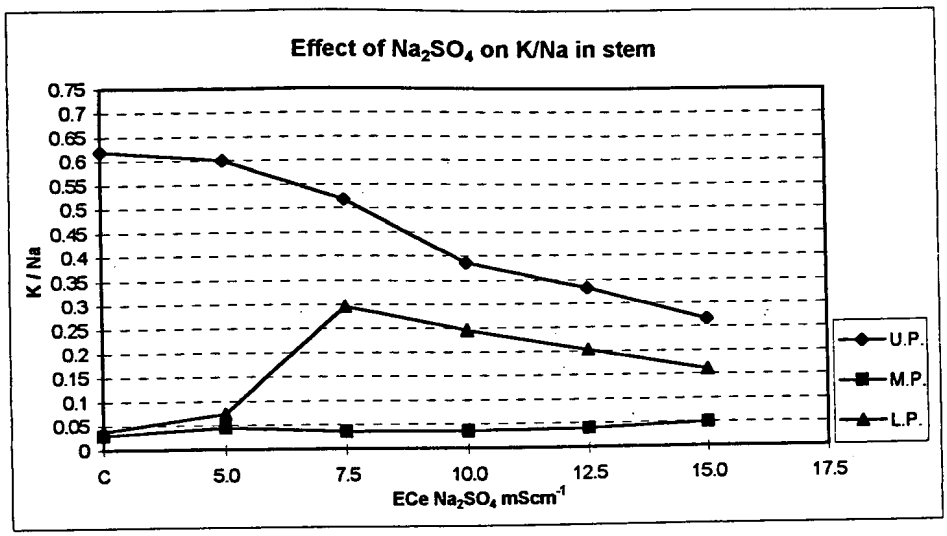
U.P. - Upper part M.P. - Middle part L.P. - Lower part

Fig.10b.



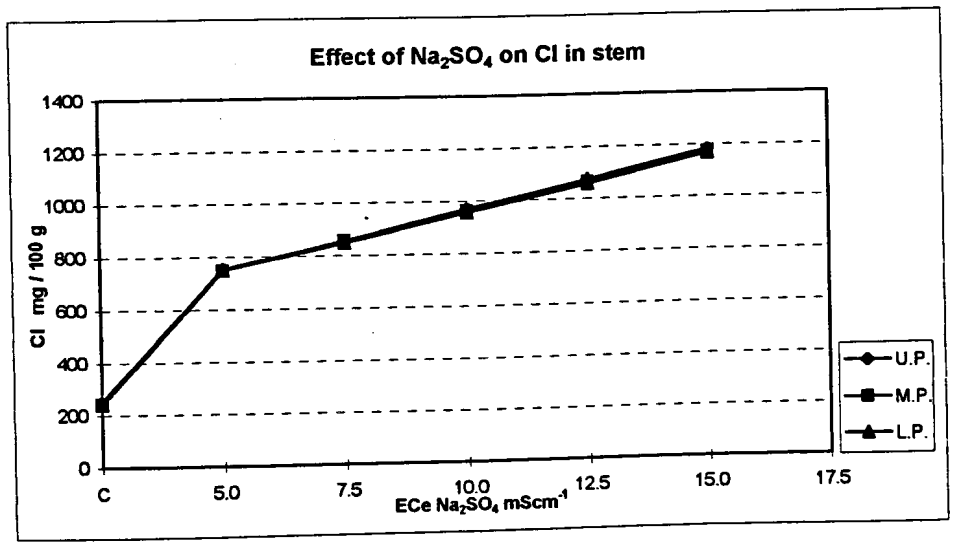
U.P. - Upper part M.P. - Middle part L.P. - Lower part

Fig.10c.



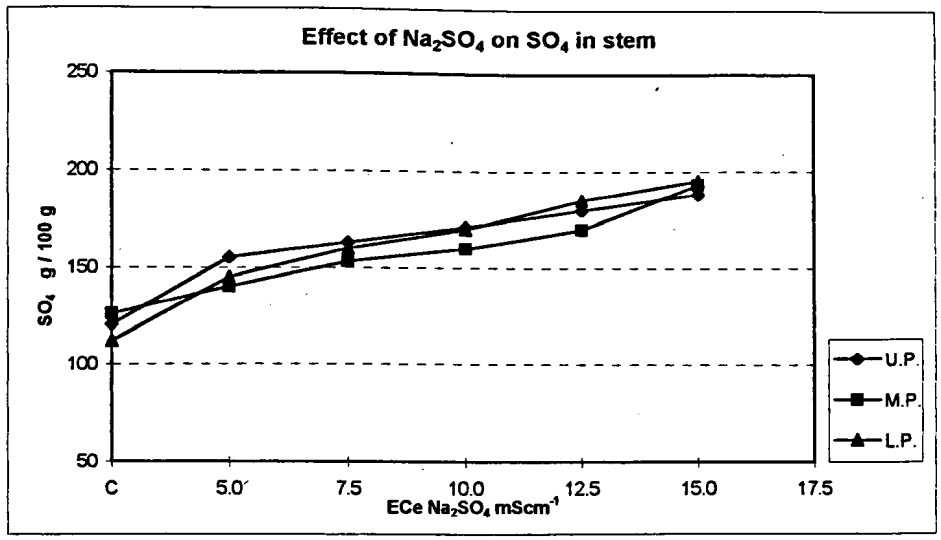
U.P. - Upper part M.P. - Middle part L.P. - Lower part

Fig.10d.



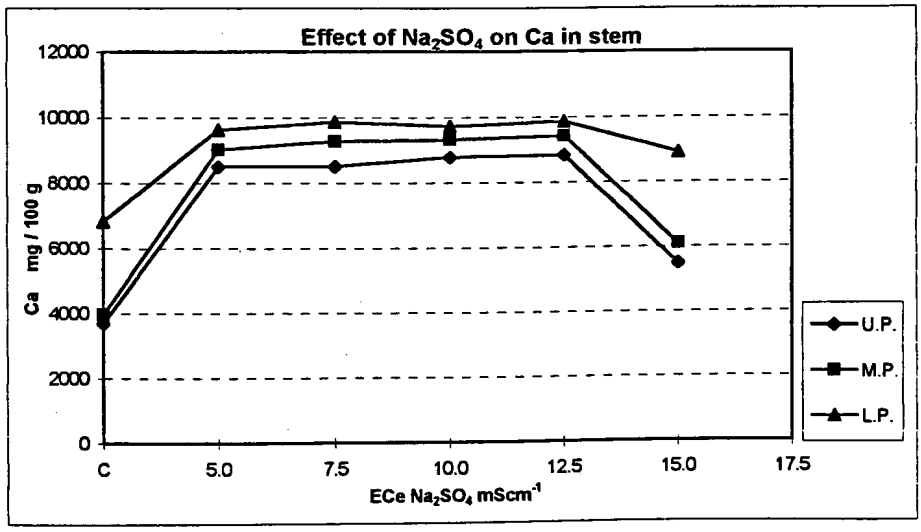
U.P. - Upper part M.P. - Middle part L.P. - Lower part

Fig.10e.



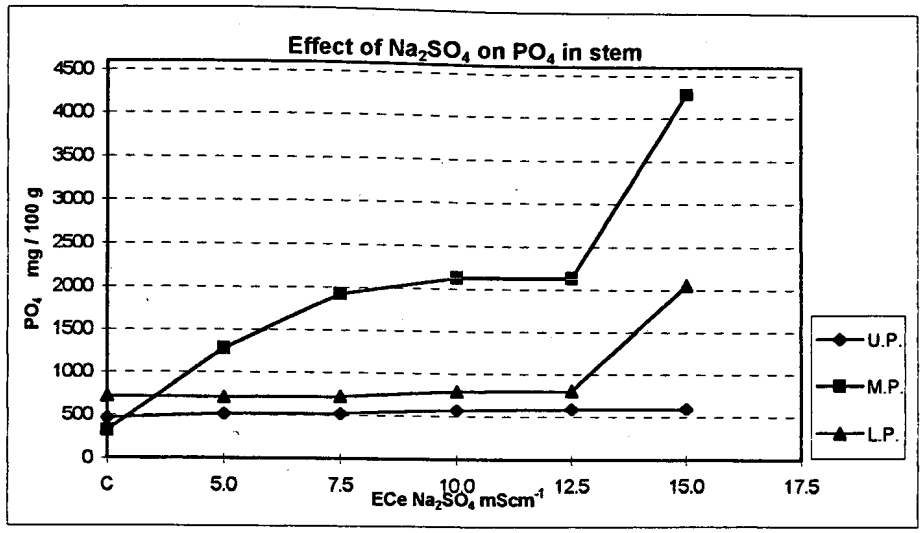
U.P. - Upper part M.P. - Middle part L.P. - Lower part

Fig.10f.



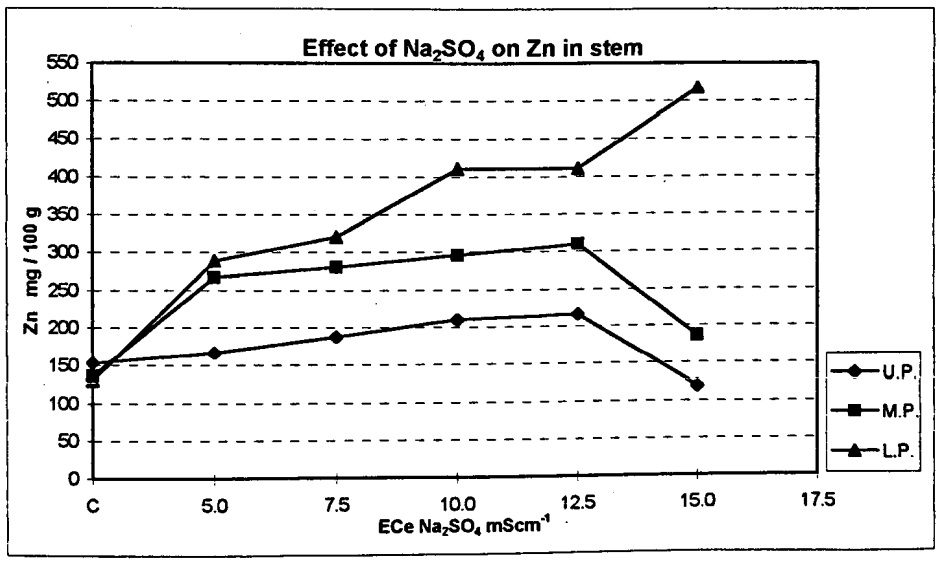
U.P. - Upper part M.P. - Middle part L.P. - Lower part

Fig.10g.



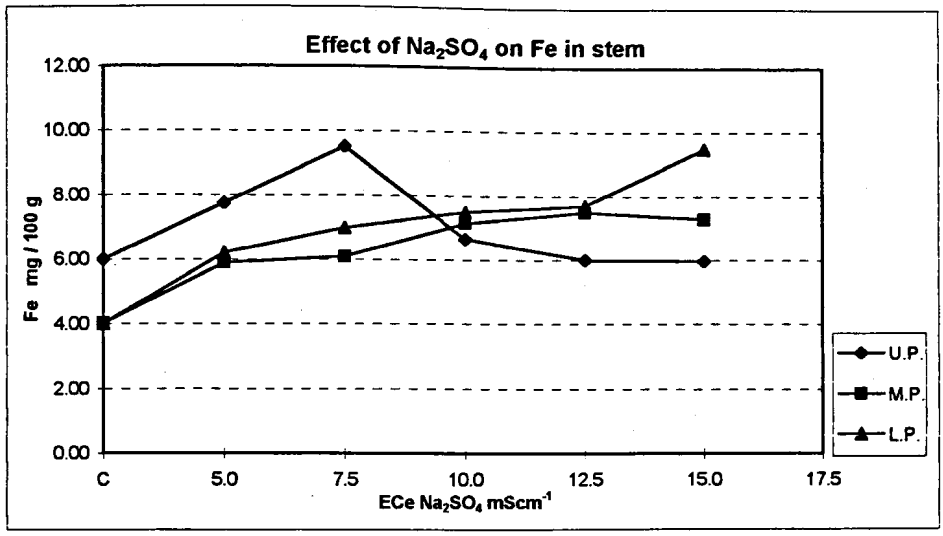
U.P. - Upper part M.P. - Middle part L.P. - Lower part

Fig.10h.



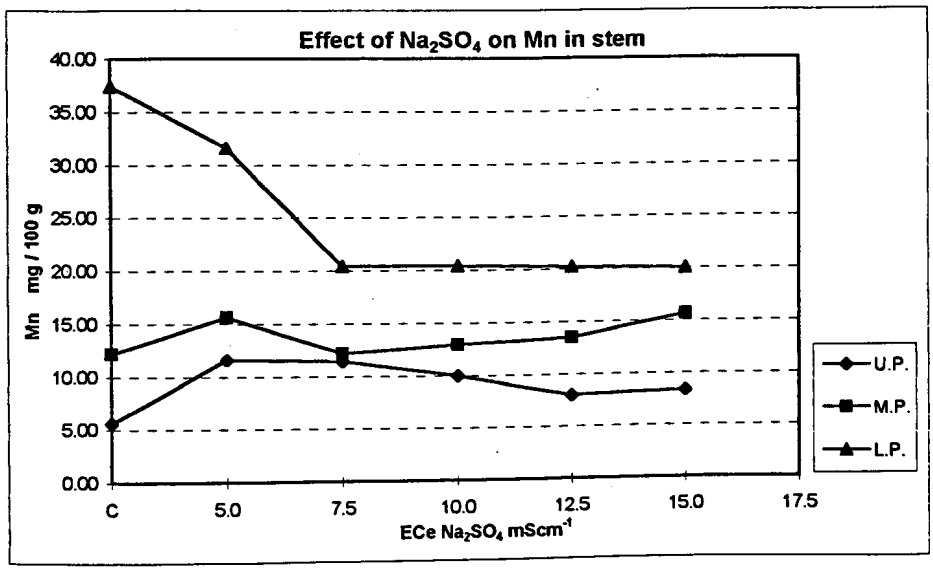
U.P. - Upper part M.P. - Middle part L.P. - Lower part

Fig.10i.



U.P. - Upper part M.P. - Middle part L.P. - Lower part

Fig.10j.



U.P. - Upper part M.P. - Middle part L.P. - Lower part

Fig. 11a.

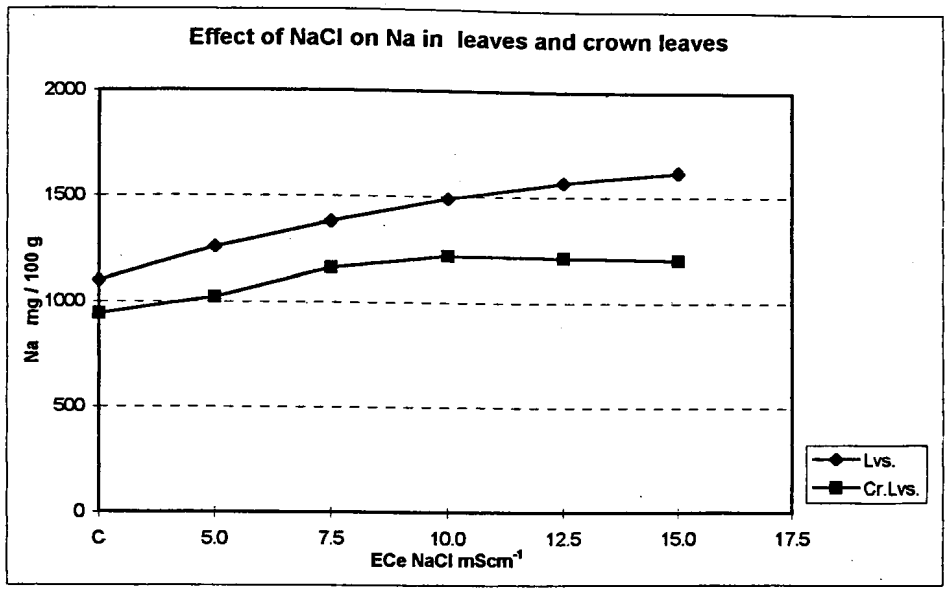


Fig. 11b.

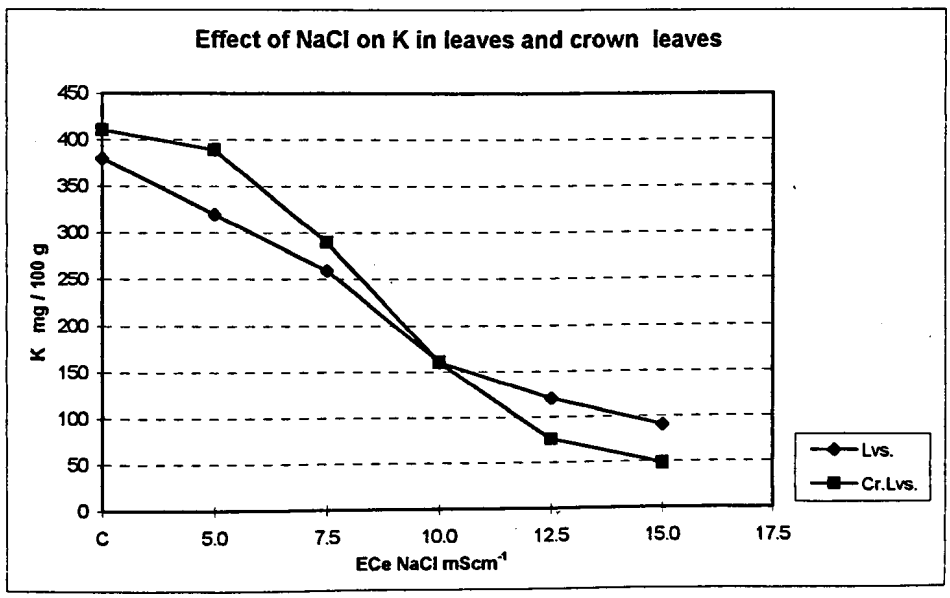


Fig.11c.

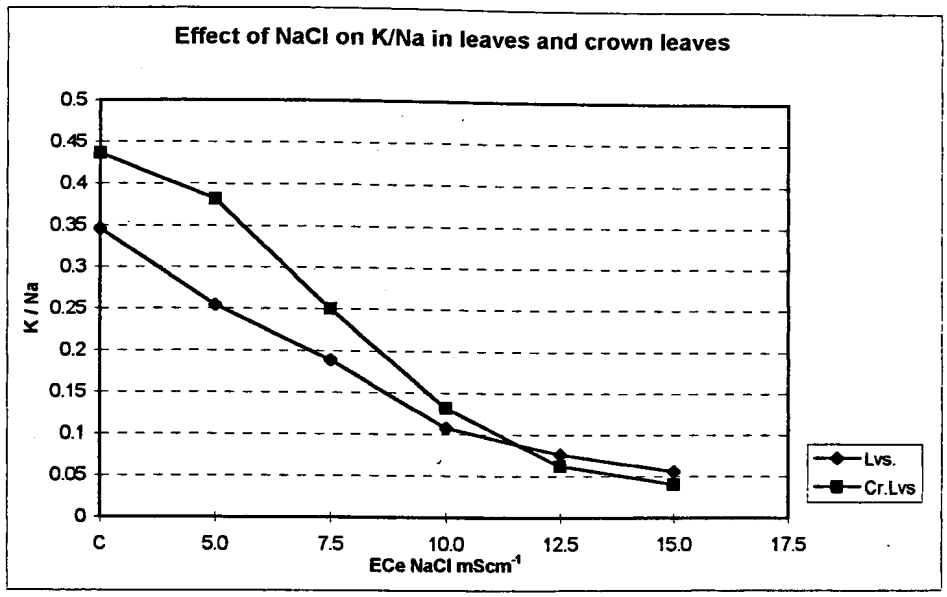


Fig. 11d.

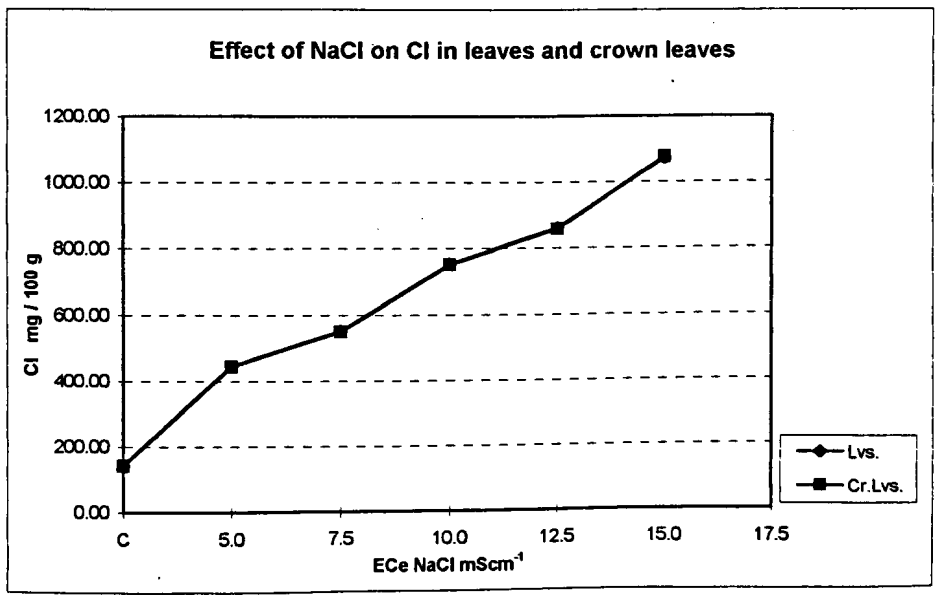


Fig. 11e.

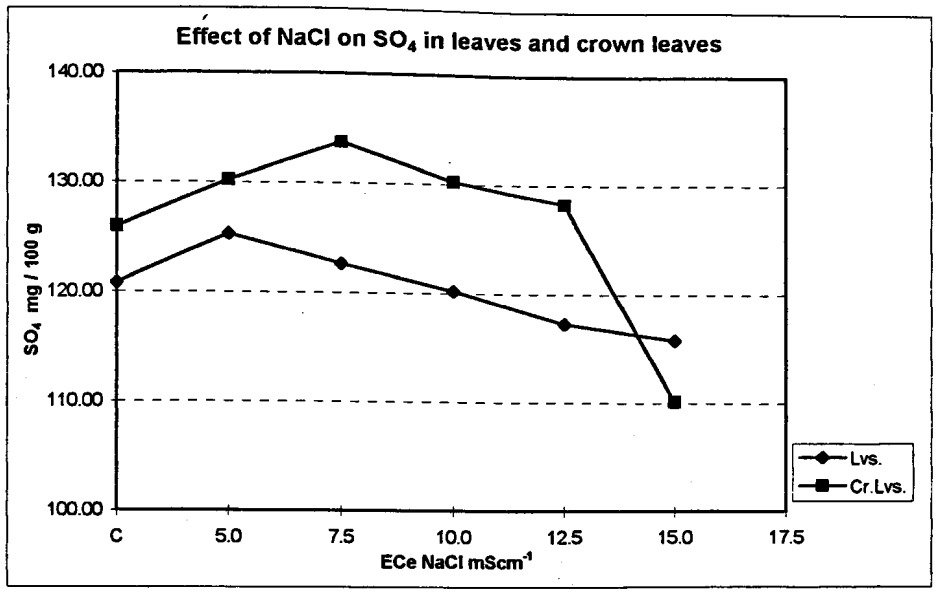


Fig. 11f.

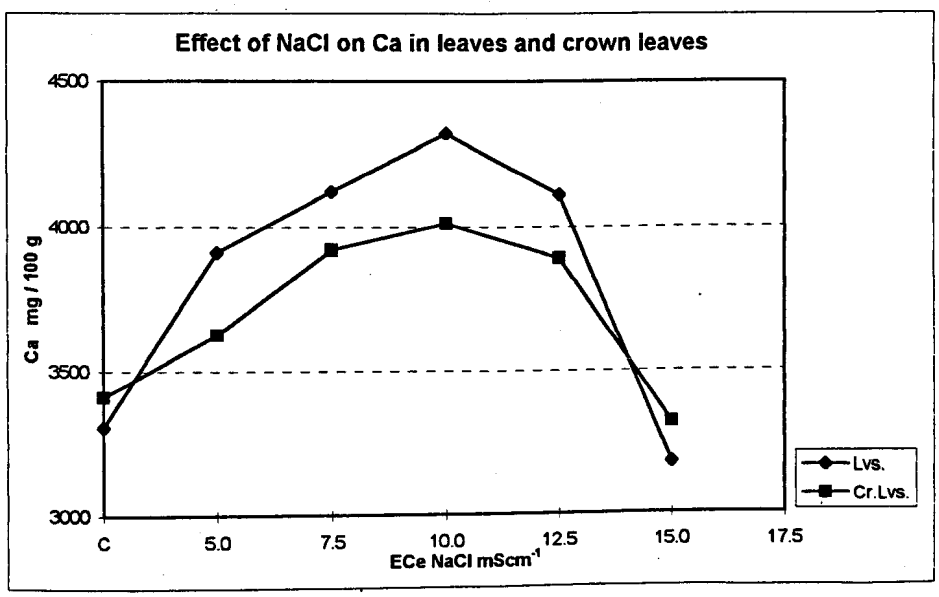


Fig. 11g.

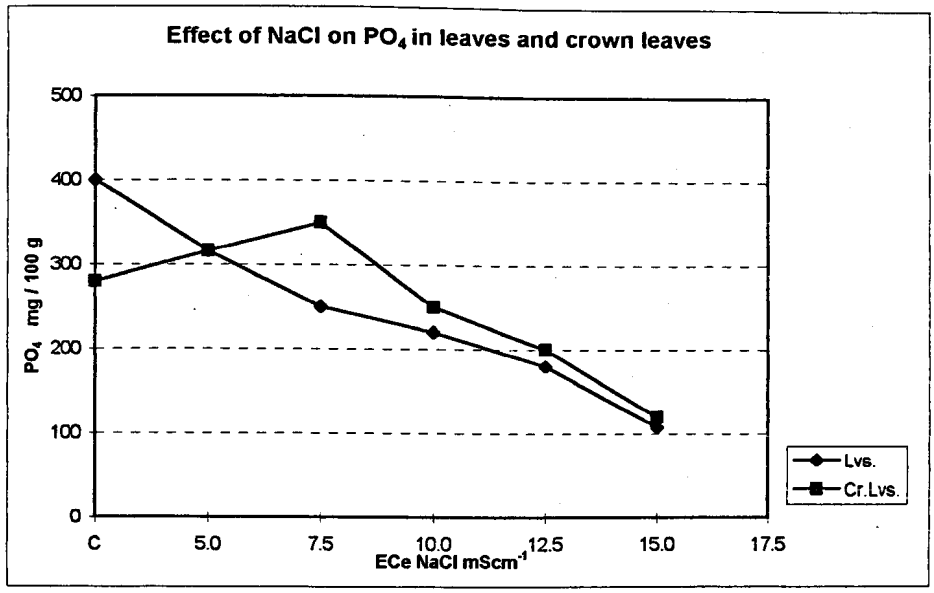


Fig. 11h.

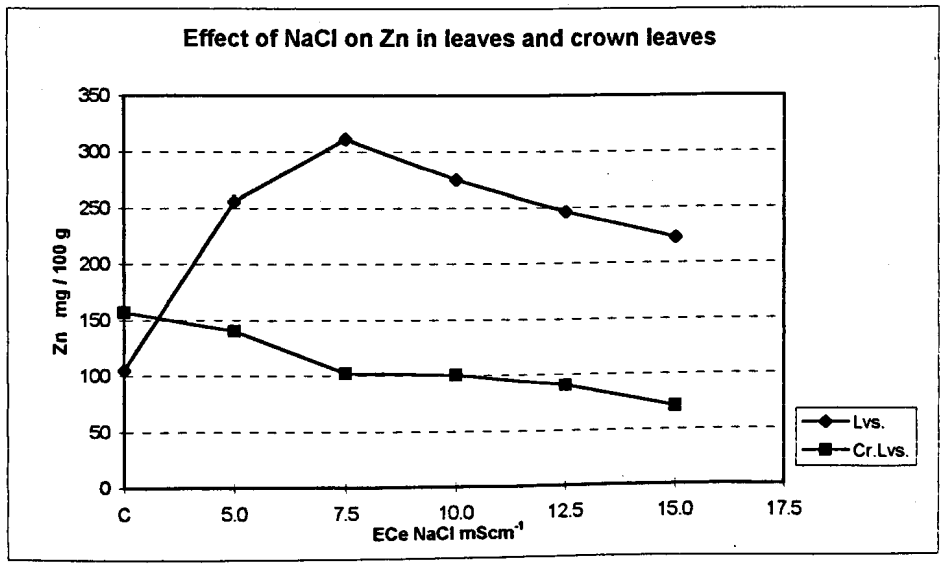


Fig. 11i.

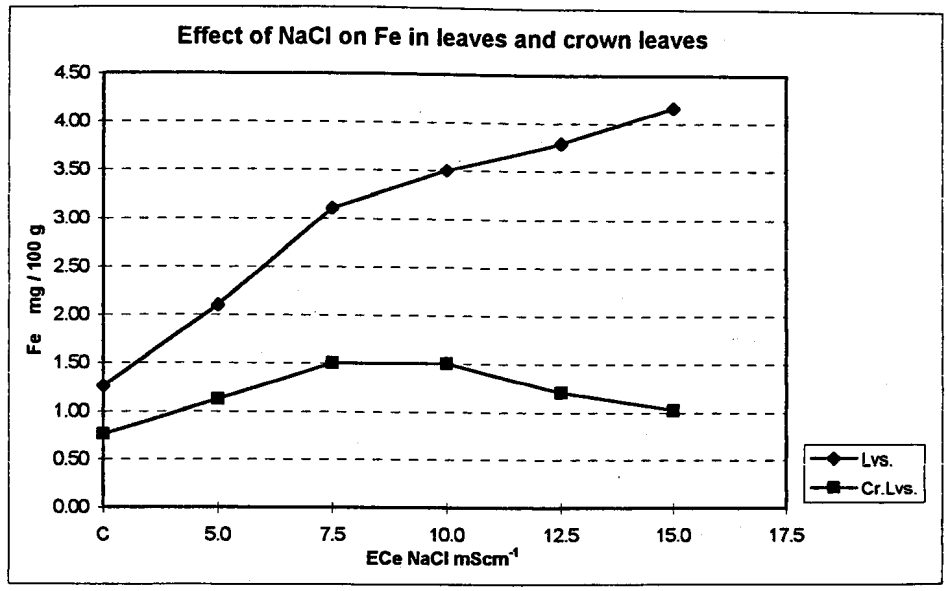


Fig.11j.

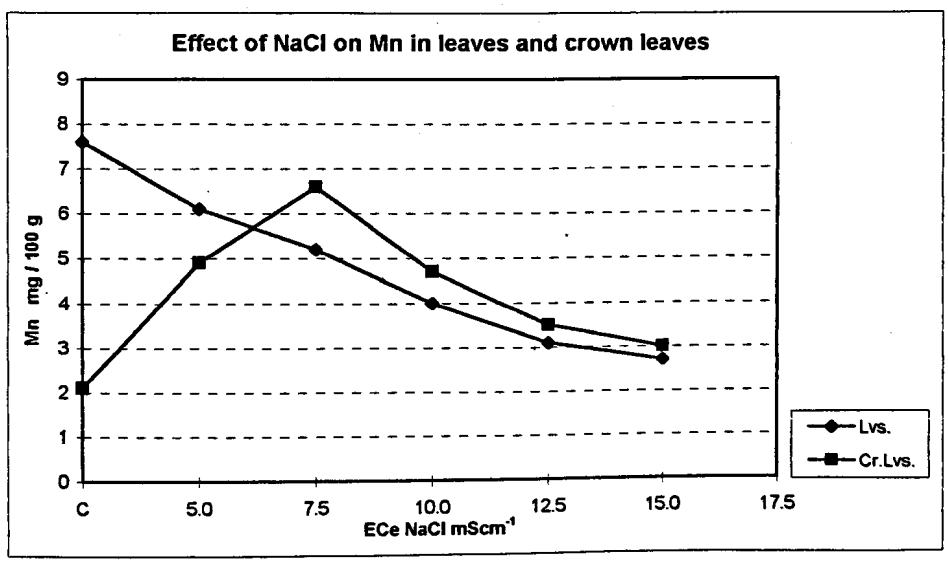


Fig. 12a.

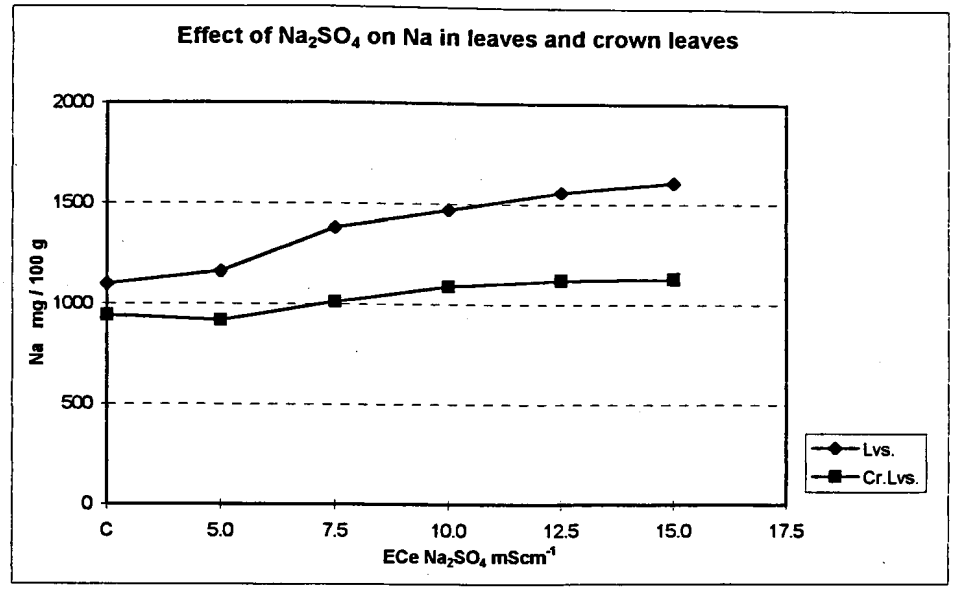


Fig. 12b.

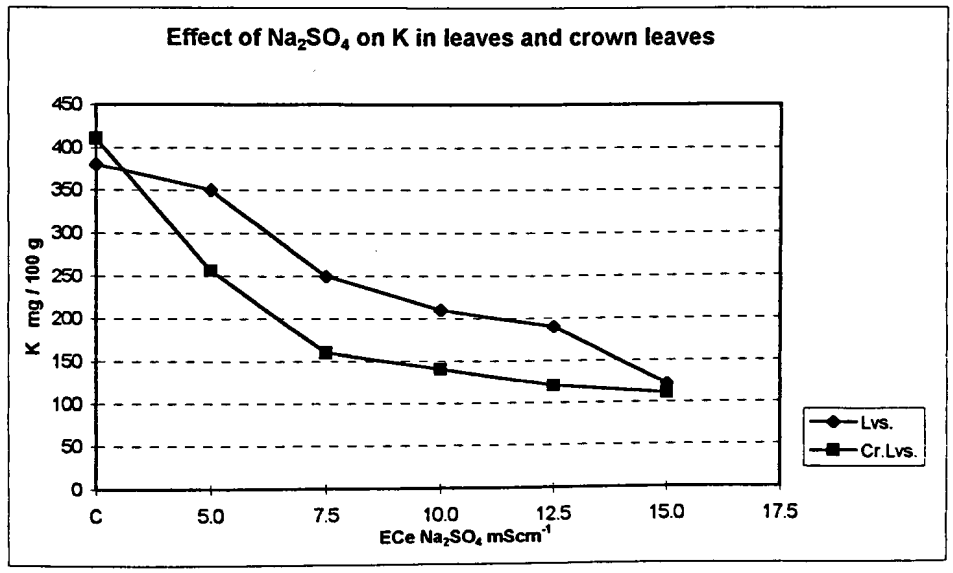


Fig. 12c.

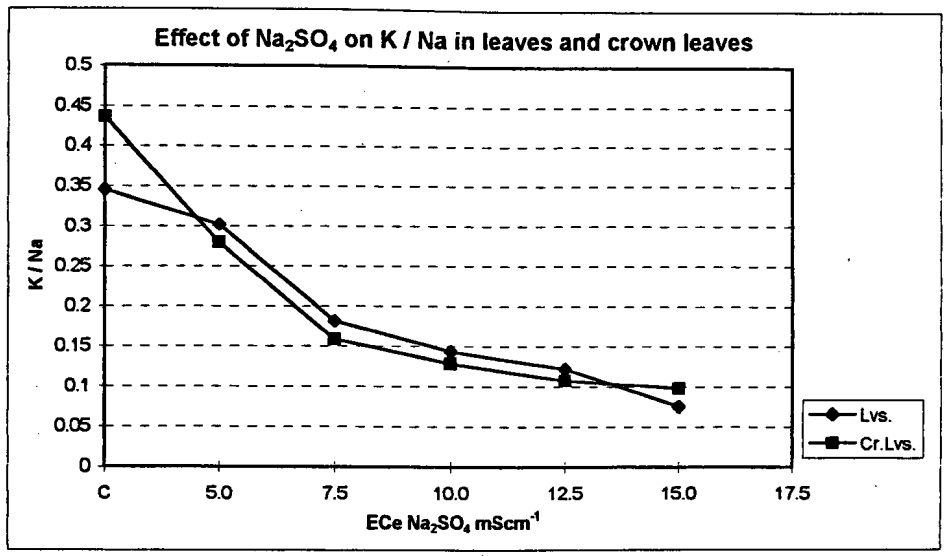


Fig. 12d.

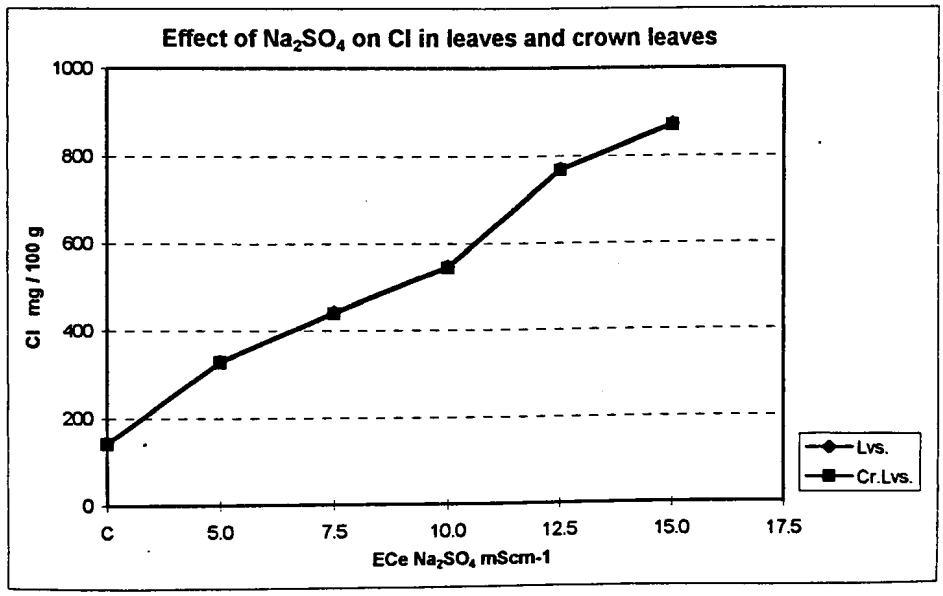


Fig. 12e.

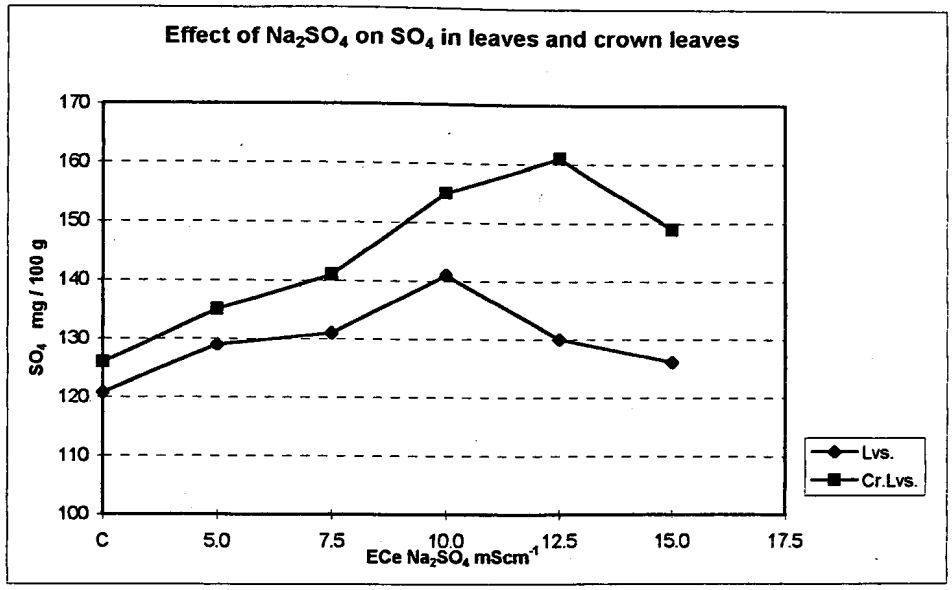


Fig. 12f.

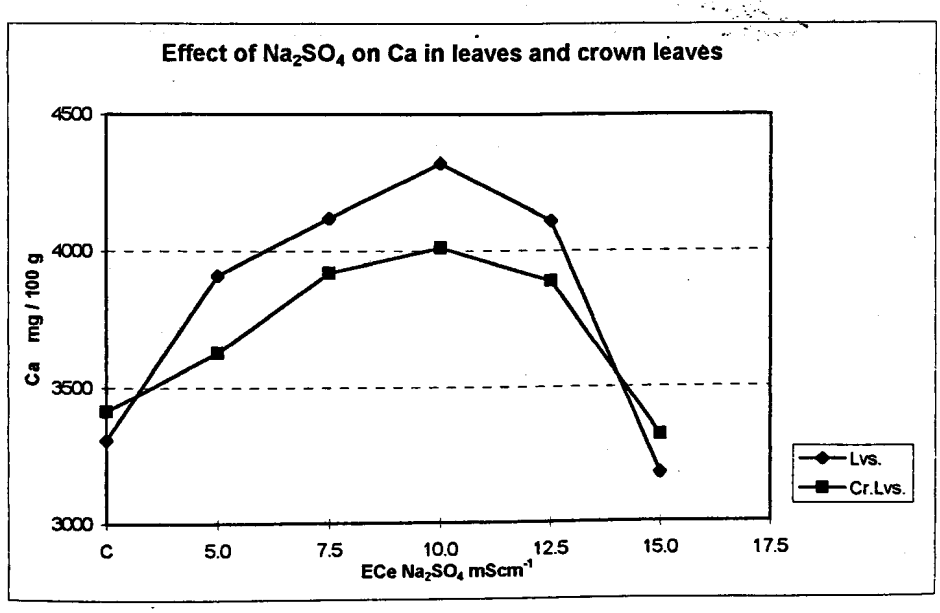


Fig. 12g.

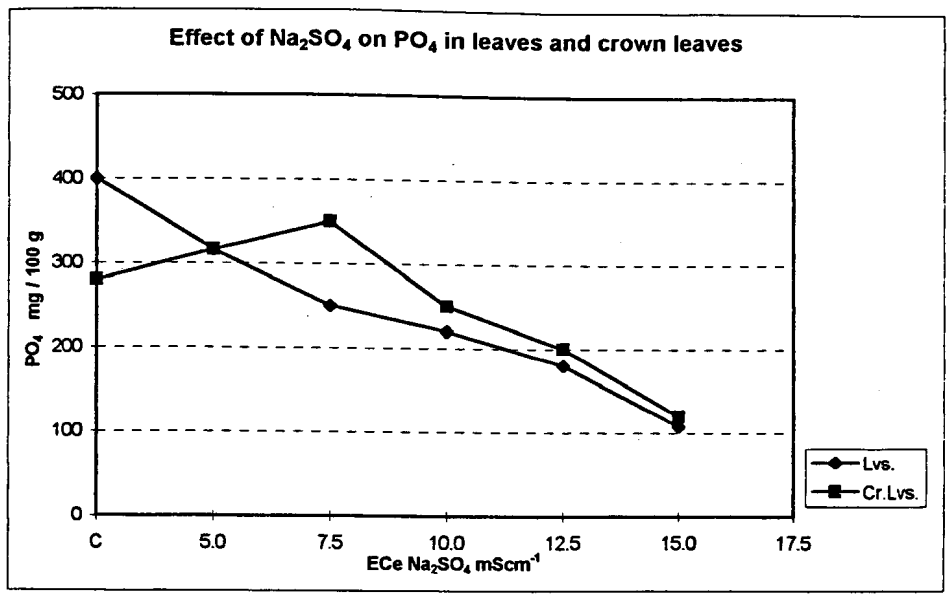


Fig. 12h.

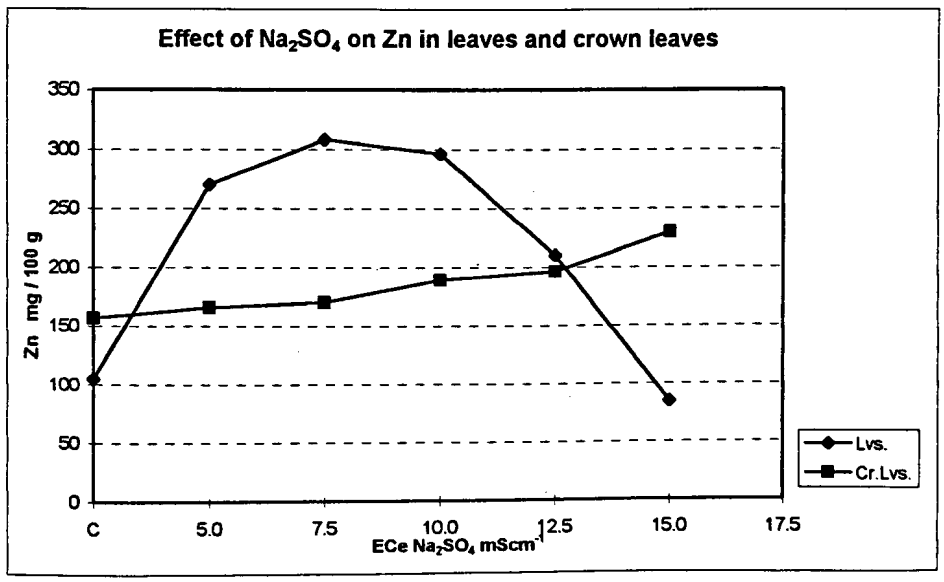


Fig. 12i.

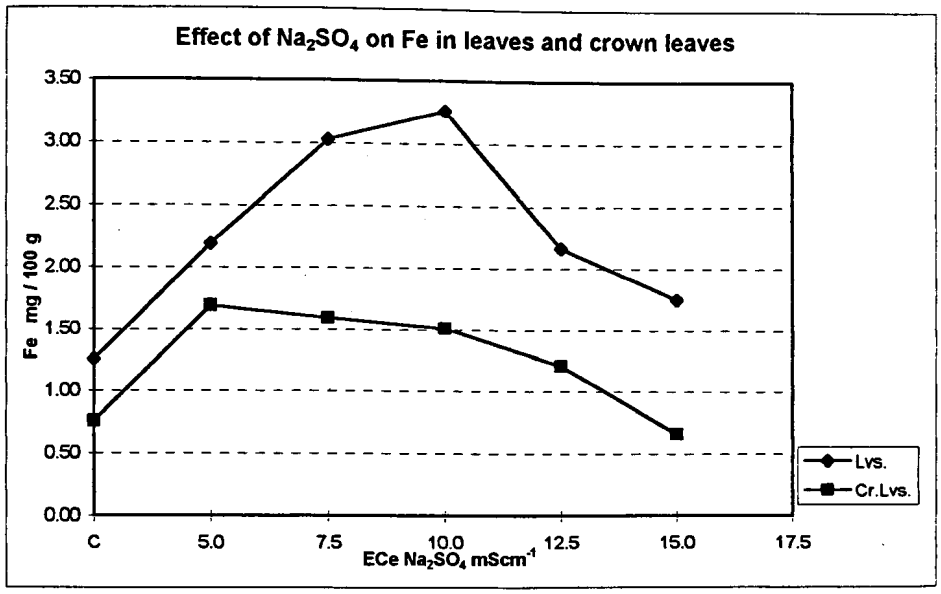
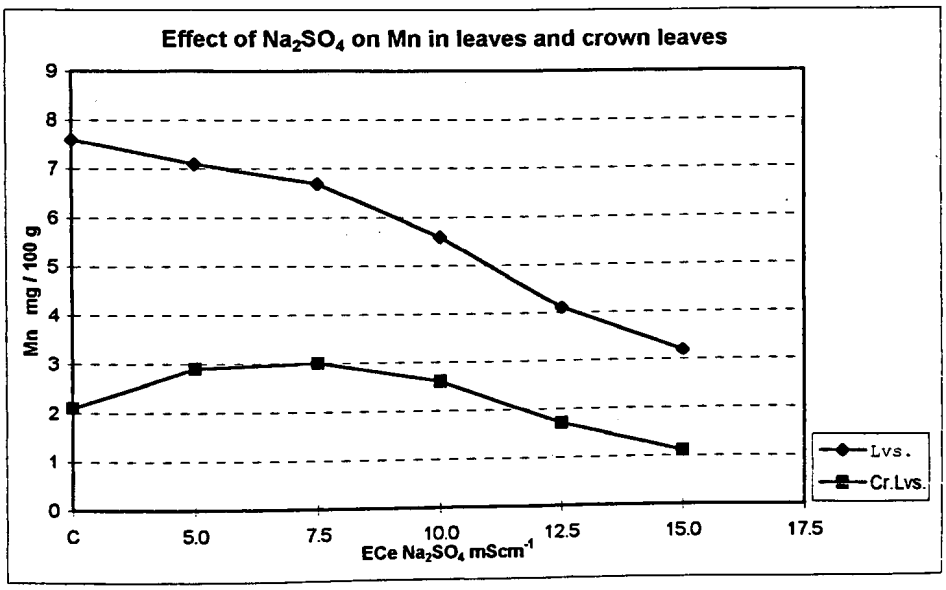


Fig. 12j.



I) ROOTS

Under NaCl and Na_2SO_4 treatment (Tables 7,8; Fig.7a -12a) sodium content increased with increasing saline concentration , which clearly indicated that Na uptake was stimulated by all levels of both the salts.

II) STEM

It is clear from Tables. 13,14; Fig.9a,10a that, in upper, middle and lower part of stem, Na was found to be linearly increasing under NaCl and Na_2SO_4 treatments which revealed that all levels of both the salts stimulate Na uptake.

Na was stored more in the middle part of the stem at lower levels of both the salinity's while it was more in the lower portion of the stem at higher levels of both the salinity's thereby,indicating that middle part of the stem acts as a storehouse for Na at lower levels of both the salinity's while lower portion of the stem acts as a storage organ for Na at higher levels of both the salts. Thus, mechanism of storing Na in stem is same under both the salinity's in Carthamus tinctorius Var. Bhima.

The average total Na of the stem increased linearly with increasing NaCl and Na_2SO_4 salinity's, suggesting stimulating effect of all levels of both salinizations on the uptake.

III) LEAVES AND CROWN LEAVES

Under NaCl and Na_2SO_4 salinity's, (Tables 15-16 ;Fig.11a,12a), it was observed that Na content increased with increasing salinity in leaves. In crown leaves, however, Na content was less compared to lower leaves under NaCl salinity but Na content accumulation was more than the control at all levels of NaCl. Under Na_2SO_4 salinity, Na

content was less than the control at low (ECe 5.0 $mScm^{-1}$) and then increased with increasing levels of salinity.

The average Na of total plant (Tables.17-19) increased with increasing levels of chloride and sulphate salinity. Na content was highest in roots at all levels of NaCl and Na_2SO_4 salinity's indicating that maximum Na is stored in roots of Carthamus tinctorius L. Cv. Bhima at all levels of both the salinity's.

B) POTASSIUM

Results of potassium content in various parts of the root, stem, leaves and crown leaves are depicted in Tables.11-16 ; Fig.7b -12b.

I) ROOTS

In roots (Table.11,12 ; Fig. 7b,8b), under sodium chloride treatment, accumulation of K content was found to be increasing with increasing NaCl treatment but under sodium sulphate salinity, K content linearly decreased with increasing salinity.

II) STEM

Under sodium chloride treatment (Table.13 ; Fig. 9b), K content was decreased with increase of salinity in upper part of stem. In the middle and lower part of stem, K content increased with increasing levels of chloride salinity. Under sodium sulphate salinity, (Table.14 ; Fig. 10b) in upper part of stem, K content increased with increasing salinity. In middle and lower part of stem, also, accumulation of K was more at all concentrations of salinity's.

In control, K was more in upper part of stem than middle and lower part of stem. Similarly, K was more in upper part of stems in plants treated upto ECe 10.0 $mScm^{-1}$ of NaCl. However, interestingly, it was more in lower part of stem in plants treated at ECe 12.5 and 15.0 $mScm^{-1}$ of NaCl. Thus, storage of K in stems is different at low and high levels of NaCl salinity.

Under sulphate salinity also, at lower concentrations (ECe 5.0 $mScm^{-1}$), K was more in the upper part of stem while it was more in the lower part of stem at all higher concentrations of Na_2SO_4 thereby indicating that K storage in stem is similar under chloride and sulphate salinizations.

III) LEAVES AND CROWN LEAVES

In the control, K content of crown leaves was more than that of the other leaves.Under both salinity's, K content (Tables.15,16; Fig.11b,12b) decreased with increasing salinity in leaves and crown leaves.

In the control, K content (Tables.17,19) value was highest in leaf than root and average stem values indicating that K is maintained more in leaves than in stems and roots in Carthamus tinctorius Var. Bhima, under saline conditions.

Under chloride salinization, K was more in leaves at low (upto ECe 7.5 $mScm^{-1}$) concentrations while it was more in roots at high (ECe 10.0 to 15.0 $mScm^{-1}$) concentrations indicating that at low salinity's, K is stored in leaves while at all high salinity's K is stored in roots. Under sulphate salinization, at low concentrations (ECe 5.0 $mScm^{-1}$), K was high in leaves while at high (ECe 7.5 to 15.0 $mScm^{-1}$) concentrations it was more in stems reflecting that K is stored more in leaves at low

sulphate salinity levels while it was stored in stem at high concentrations. Thus, K metabolism is different under chloride and sulphate salinizations in Carthamus tinctorius Var. Bhima.

Average K content of total (Root + Stem + Leaf) plant was less than the control at all levels of NaCl and Na_2SO_4 reflecting that K uptake fails in Carthamus tinctorius Var. Bhima at all levels of salinity.

C) K/Na RATIO

Results of K/Na ratio under saline conditions in Carthamus tinctorius Var. Bhima. are given in Tables. 11-16; Fig. 7c-12c.

I) ROOTS

In case of roots (Table. 11 and Fig. 7c), K/Na ratio was decreased at low (ECe 5.0 and 7.5 $mScm^{-1}$) and increased over the control at high (ECe 10.0 to 15.0 $mScm^{-1}$) concentrations of NaCl. However, under Na_2SO_4 salinity (Table.12 ; Fig. 8c), K/Na ratio decreased over the control with increasing salinity, indicating that low levels of chloride and all levels of Na_2SO_4 favour more accumulation of Na than K.

II) STEM

In upper part of stem (Tables.13,14 ; Fig. 9c,10c), K/Na ratio linearly decreased with increasing levels of NaCl and Na_2SO_4. In middle part ,it was less than that of the control at low (ECe 5.0 $mScm^{-1}$) levels while it was more than the control at high (ECe 7.5 to 12.0 $mScm^{-1}$) levels of NaCl. In middle part, the content was more than the control at all Na_2SO_4 concentrations. Thus, K/Na ratio was different under chloride and sulphate

salinizations. In lower part of the stem, the K/Na ratio was slightly more at low (ECe 5.0 $mScm^{-1}$) level of NaCl, but was less than the control at all higher levels of NaCl salinity. However, under sulphate treatment the K/Na ratio was more than the control at all levels of Na_2SO_4 salinity.

The average K/Na of total stem, under chloride treatment, decreased with increasing salinity. However, under sulphate treatment, K/Na ratio was more than control at low (ECe 5.0 and 7.5 $mScm^{-1}$) and less than the control at high salinity levels.

III) LEAVES AND CROWN LEAVES

In leaves and crown leaves, K/Na ratio (Tables.15,16 ; Fig. 11c,12c) decreased under both salinity's thereby indicating inhibition of K uptake.

The K/Na ratio of average total plant (Tables. 17,19) decreased with increase in salinity of NaCl and Na_2SO_4. Thus, Na uptake was increased over K uptake in Carthamus tinctorius Var. Bhima under both the salinizations.

D) CHLORIDE

Results of chloride content in various parts of the root, stem, leaves and crown leaves are depicted in Tables. 11 -16 ; Fig. 7d-12d.

I) ROOTS

In roots (Tables.11,12 ; Fig. 7d,8d), Cl content was linearly increased with increase in levels of both the salts. It is observed that Cl concentration was higher in roots than in stem and leaves.

II) STEM

Observations (Tables.13,14; Fig. 9d,10d) indicated that in all parts of stem Cl content linearly increased under chloride and sulphate salinizations. Its content was more under chloride salinization than sulphate which is obvious. Chloride content was highest in upper part of the stem under control as well as under all levels of both the salinizations.

III) LEAVES AND CROWN LEAVES

Under NaCl treatment (Table.15 ; Fig.11d), chloride content linearly increased with increasing levels of NaCl salt. Under Na_2SO_4 treatment (Table.16 ; Fig.12d), Cl content slightly decreased upto ECe 5.0 $mScm^{-1}$ in leaves and upto ECe 7.5 $mScm^{-1}$ in crown leaves in Carthamus tinctorius L .Var. Bhima.

Chloride content of total plant (Tables. 17,19) was increased with increase in levels of both the salts except at ECe 5.0$mScm^{-1}$ of Na_2SO_4 where it was slightly less than control indicating that all levels of both the salts, stimulate chloride uptake in Carthamus tinctorius Var. Bhima.

E) SULPHATE

Results of sulphate content in various parts of safflower under chloride and sulphate salinity's are recorded in Tables.11-16 ; Fig.7e-12e.

I) ROOTS

Under NaCl and Na_2SO_4 treatment (Tables.11,12 ; Fig. 7e,8e), content of sulphate was three times more in roots than stem and leaves. Sulphate content was linearly

increased with increasing concentrations of both the salts thereby indicating that all concentrations of both the salts stimulate sulphate accumulation in roots of safflower Cv. Bhima. Its content was more in roots than in leaves and stem under control conditions. Same pattern of sulphate accumulation was observed at all levels of both the salts which reflects that the pattern of sulphate accumulation is same in safflower Cv. Bhima irrespective of growth conditions.

II) STEM

In upper part of stem (Tables.13,14 ; Fig.9e,10e), at ECe 5.0 $mScm^{-1}$ of NaCl, SO_4 content was slightly more than the control while at all other concentrations it was gradually decreased with increase in NaCl concentrations. Under sulphate salinity, its content gradually increased with increasing concentrations of Na_2SO_4 which indicated that all concentrations of both the salts stimulate sulphate accumulation in upper part of the stem. Its content was always more under sulphate salinization than chloride which is obvious.

In middle part of the stem, its content was more than the control at low (ECe 5.0 and 7.5 $mScm^{-1}$) concentrations while at high (ECe 10.0 to 15.0 $mScm^{-1}$) it was decreased linearly with increase in levels of chloride salinization. Thus low concentrations of NaCl stimulate accumulation of sulphate while high concentrations of the same inhibit accumulation of sulphate in middle part of stem. At all levels of sulphate salinization, its content was linearly increased with increase in concentrations of sulphate in the soil which reflects that all levels of sulphate salinization in the soil stimulate sulphate accumulation in the middle part of the stem.

In lower part of the stem, sulphate content was more than the control in plants grown upto ECe 10.0 $mScm^{-1}$ of chloride while it was less than the control at very high (ECe 12.5 to 15.0 $mScm^{-1}$) of chloride indicating that wider range of chloride salinization enhance accumulation of sulphate in lower part of stem. Under sulphate salinization, its content was increased gradually with increase in levels of sulphate salinization which is obvious.

Average sulphate content of total stem was more than the control in stem of plants grown upto ECe 7.5 $mScm^{-1}$ of chloride while it was less than the control at all higher concentrations of NaCl thereby indicating that low concentration of NaCl stimulates while high concentration of NaCl inhibits accumulation of sulphate in the stem of safflower Cv. Bhima.

Under sulphate salinization its content was increased linearly with increase in concentrations of sulphate in the growth medium indicating that all levels of sulphate favours accumulation of sulphate in stem of safflower Cv. Bhima.Sulphate content was more in middle part of stem under control conditions. Similar was the case under chloride salinization whereas it was more in upper part of stem under low concentrations of sulphate salinization thereby indicating different pattern of sulphate accumulation is stem under chloride and sulphate salinizations.

III) LEAVES AND CROWN LEAVES

The sulphate content was more than the control in leaves upto ECe 7.5 $mScm^{-1}$ while its accumulation decreased at higher salinity levels of NaCl (Tables.15-16;Fig. 11e,12e). However, it was observed that sulphate accumulation was stimulated at all

levels of sulphate salinization. Highest sulphate content was recorded at ECe 10.0 $mScm^{-1}$ of sulphate which reflects that all levels of sulphate salinization stimulate uptake of sulphate.

In crown leaves, sulphate content was high than leaves under NaCl and Na_2SO_4 treatment. Sulphate content was more than the control upto ECe 12.5 $mScm^{-1}$ of NaCl and decreased at ECe 15.0 $mScm^{-1}$ of NaCl. Under sulphate treatment however, content of sulphate was higher as compared to NaCl treatment which is obvious. Sulphate content was more than the control at all levels of sulphate salinization. Highest content was recorded at ECe 12.5 $mScm^{-1}$ of sulphate.

Average total sulphate content of total plant (Root + Stem + Leaves) (Table. 17,19) was more than in plants grown upto ECe 12.5 $mScm^{-1}$ of chloride while it was less than control in plants grown at ECe 15.0 $mScm^{-1}$ suggesting that low concentrations of NaCl stimulate sulphate uptake in Carthamus tinctorius Cv. Bhima. Its content was more than the control at all levels of sulphate salinization indicating that all concentrations of sulphate salt stimulate sulphate uptake in safflower Cv. Bhima.

F) CALCIUM

Results on effect of chloride and sulphate salinity's on Ca content are recorded in Tables. 11-16 ; Fig. 7f-12f.

I) ROOTS

Under NaCl treatment (Tables. 11,12 ; Fig. 7f,8f) , Ca content increased linearly in plants grown upto ECe 12.5 $mScm^{-1}$ and it was less than the control at ECe 15.0

$mScm^{-1}$. Thus, low concentrations of NaCl stimulate while high concentrations of it inhibit Ca uptake in Carthamus tinctorius Var. Bhima. Ca content was more than the control at all levels of Na_2SO_4. In Carthamus tinctorius, Ca content was more under sulphate salinity than under chloride salinity.

II) STEM

Under control conditions (Tables. 13,14 ; Fig. 9f,10f) , Ca was highest in the lower part of the stem. Similar was the condition under chloride and sulphate salinization indicating that distribution of Ca in stem is same under non-saline and saline conditions in Carthamus tinctorius Var. Bhima.The content of Ca was found to be decreasing with increasing concentrations in upper part of stem under NaCl salinity. However, it was linearly increasing in middle and lower part of stem under chloride salinity. Under sulphate salinization, Ca content was more than the control in all parts of the stem.

The average content of Ca in the total stem increased with increasing levels of both the salinity's. Average Ca content in the stem was more under sulphate salinization than under chloride salinization reflecting that more Ca is stored in stem under sulphate salinization.

III) LEAVES AND CROWN LEAVES

Under control conditions, Ca was more in crown leaves than normal leaves indicating its role in grain feeding (Tables. 15,16 ;Fig. 11f,12f).

In leaves and crown leaves, the content of Ca was more than the control upto ECe 12.5 $mScm^{-1}$ and was less than the control at a high (ECe 15.0 $mScm^{-1}$) levels of NaCl and

Na_2SO_4. However, Ca content in the leaves was more than the control at all concentrations of both the salts except at ECe 15.0 $mScm^{-1}$ of NaCl and ECe 15.0 $mScm^{-1}$ of sulphate in crown leaves where it was less than the control. Highest Ca content was recorded at ECe 10.0 $mScm^{-1}$ of NaCl and at ECe 7.5 $mScm^{-1}$ of Na_2SO_4 in both the leaves. Thus, the trend of Ca storage in both the leaves was similar under both salinity's.

The average Ca content of leaves and crown leaves was more than the control under both salinizations except at ECe 15.0 $mScm^{-1}$ of NaCl and Na_2SO_4 where it was slightly less than the control. Under control conditions, Ca content was more in stems than in leaves and roots under both the salinity's.

The average Ca in the total plant (Tables.18-20), was more than the control at all levels of chloride and sulphate salinity indicating that Ca uptake is stimulated under both the salinity's in Carthamus tinctorius Cv. Bhima.

G) PHOSPHORUS

Results of P content in various parts of root, stem, leaves and crown leaves are given in Tables. 11,16; Fig.9g,12g.

I) ROOTS

Phosphorus content (Tables. 11,12; Fig.7g,8g) was enhanced in plants grown upto ECe 7.5 $mScm^{-1}$ whereas it decreased at higher salinity's (ECe 10.0 to 15.0 $mScm^{-1}$) of NaCl treatment. Under sulphate treatment, accumulation increased with increasing salinity. Thus, at low levels of chloride and at all levels of sulphate salinization P

accumulation was stimulated in roots. Its content was more in roots under sulphate salinization than under chloride salinization which indicated that sulphate salinity favours more accumulation of P in roots than chloride salinity.

II) STEM

Phosphate content (Tables. 13,14 ; Fig. 9g,10g) in upper, middle and lower part of the stem linearly increased with increasing levels of NaCl and Na_2SO_4 salinity. In middle part of the stem, phosphate accumulation was approximately four times more under NaCl treatment when compared to the control. Under control conditions, its content was highest in lower part of the stem while its content was lowest in middle part of the stem. Under all levels of chloride and sulphate salinity's, P accumulation was highest in middle part and lowest in upper part of stem thereby suggesting that phosphorus accumulation is different under control and saline conditions in safflower Cv. Bhima.

The average P content of stem increased with increasing levels of chloride and sulphate salinization indicating that all levels of both the salts favour phosphate accumulation in Carthamus tinctorius Var. Bhima. Its content was more under sulphate salinity than under chloride salinity suggesting that sulphate salinity is more favourable than chloride salinity for accumulation of P in the stems of safflower Cv. Bhima.

III) LEAVES AND CROWN LEAVES

In leaves, phosphorus content (Tables. 15,16 ; Fig. 11g,12g), linearly decreased with increasing levels of chloride and sulphate salinizations.

In crown leaves, however, phosphorus accumulation was stimulated at low (ECe 5.0 and 7.5 $mScm^{-1}$) and decreased at all higher levels of NaCl salinity. Under sulphate salinity , P accumulation was stimulated upto ECe 10.0 $mScm^{-1}$ and decreased at all higher levels of Na_2SO_4.

Average P content of the total plant (Tables. 18,20) was more than control at all levels of chloride and sulphate salinizations indicating that uptake of P is stimulated in safflower Cv. Bhima.

H) ZINC

Results of Zinc content under saline conditions in Carthamus tinctorius Var. Bhima. are given in Tables. 11-16 ; Fig. 7h-12h.

I) ROOTS

Under NaCl treatment Zn content (Tables. 11,12 ; Fig. 7h,8h), decreased linearly with increasing salinity levels. However, under sulphate treatment, Zn content was more than the control in plants grown upto ECe 10.0 $mScm^{-1}$ and was less than the control at ECe 12.5 to 15.0 $mScm^{-1}$.

II) STEM

Results of Zn content (Tables.13,14 and Fig. 9h,10h) indicated that under control conditions, Zn is more in the upper part of the stem while its content was least in the lower part of the stem. However, under both the saline conditions it was more in the lower part of the stem which indicated that Zn translocation within the stem is inhibited or excess Zn is stored more in the lower part of the stem. Zinc content was increased

with increasing salinity in upper, middle and lower part of stem over the control under both the salinizations. This indicated that all levels of both the salinizations enhanced Zn accumulation in stem of Carthamus tinctorius Var. Bhima.

III) LEAVES AND CROWN LEAVES

In leaves, under both the salinizations (Tables. 15,16 ; Fig. 11h, 12h), Zn content was more than the control at all concentrations. Highest Zn content was recorded at ECe 7.5 $mScm^{-1}$ of both the salts. Thus, all concentrations of chloride and sulphate salinizations stimulate uptake of Zn.

In crown leaves, under NaCl salinization, Zn content decreased while under sulphate salinizations, its content increased with increase in level of salt. Thus, Zn metabolism in leaves and crown leaves is different under chloride and sulphate salinizations in Carthamus tinctorius Var. Bhima.

The average (Tables.18,20) Zn content of the total plant increased under both chloride and sulphate salinizations,indicating that all levels of both the salts stimulate Zn uptake in safflower Cv.Bhima.

I) IRON

Results of Iron content in root, stem and leaves under various levels of both the salinizations are presented in Tables.11-16 ; Fig. 7i-12i.

I) ROOTS

Fe accumulation (Tables. 11,12 ;Figs. 7i, 8i) was more in roots than the control at all levels of both the salts.

II) STEM

In upper part of stem (Tables. 13,14, Fig.9i,10i) , the content of Fe was more than the control at ECe 5.0 $mScm^{-1}$ of NaCl and it was less than the control at high chloride salinity levels while its content was more than the control at all levels of sulphate salinity's. However, in middle and lower part of stem the content of Fe was approximately twice as compared to control and it linearly increased with increasing salinity levels under chloride and sulphate salinizations. Thus, high levels of both the salinity's favour Fe accumulation in middle and lower part of the stem. Average Fe content in the stem was more than the control under both the salinizations.

III) LEAVES AND CROWN LEAVES

In leaves and crown leaves (Tables.15,16 ; Fig.11i,12i), Fe accumulation increased with increasing chloride salinity. Average Fe content of total plant (Tables.18,20) was more than the control in plants grown at all levels of both the salts. The highest Fe content was observed at ECe 10.0 $mScm^{-1}$ of NaCl and ECe 7.5 $mScm^{-1}$ of Na_2SO_4. This fact suggests that all levels of both the salts stimulate uptake of iron in Carthamus tinctorius Var. Bhima.

J) MANGANESE

Results of Mn content in root, stem, leaves and crown leaves are given in Tables.15,16; Fig.11j,12j.

I) ROOTS

Under NaCl and Na_2SO_4 treatments, Mn content was more than the control at all salinity levels reflecting that all levels of both the salts favour accumulation of Mn in roots.

II) STEM

Under control conditions, Mn content was more in lower part of the stem than middle and upper part. However, its content was more in the middle part of the stem than lower and upper part under chloride salinization which clearly indicates that maximum Mn is stored in the middle part of the stem under chloride salinization.

Mn content is more than the control upto ECe 10.0 $mScm^{-1}$ and its content decreased further with increasing levels of salinity in upper part of stem under NaCl treatment. Mn of middle part of the stem linearly increased with increasing concentration of NaCl salinity. In lower part, the content was less than the control at all concentrations of NaCl salinity. Under sulphate treatment, in upper part of stem Mn content was more than the control at all levels of salinity. Highest Mn content was recorded at ECe 5.0 $mScm^{-1}$ in the middle part of stem. In lower part of stem Mn content was less compared to control.

Average total content of total stem decreased with increasing salinity under all levels of NaCl salinity whereas under sulphate salinity, it was more at low (ECe 5.0 $mScm^{-1}$) and was less than the control at all higher levels.

III) LEAVES AND CROWN LEAVES

In leaves, Mn content decreased with increasing chloride and sulphate salinity's.

In crown leaves, the content increases at all salinity levels under chloride treatment. Under sulphate treatment, however, its content was more than the control and was less at higher salinity levels (ECe 12.5 and ECe 15.0 $mScm^{-1}$).

Average Mn content of total plant was less than the control under NaCl salinity. However, it was more at low (ECe 5.0 and 7.5 $mScm^{-1}$) levels of Na_2SO_4 and was less at higher levels. This fact suggested that chloride salinity inhibits Fe uptake while low levels of sulphate stimulate and high levels of it inhibit the same.

4) DISCUSSION

A) SODIUM

Sodium is a microelement which is essential in certain species and it is essential in Atriplex vesicaria, Halogeton glomeratus. Sodium can partially replace potassium in many reactions known to require potassium . Sodium is apparently necessary for C_4 carbon fixation in certain plant species. Aeluropus litoralis (C_4), a halophyte, is found to fix carbon through C_3 pathway when depleted of sodium (Shomer-illan and Waisel;1973,1976). If C_3 plant is grown in presence of sodium, the photosynthetic pathway is shifted to C_4 mode (Shitole and Joshi;1984). It is demonstrated that sodium influences the balance between PEP-Case and RUBISCO in maize. Certain CAM plants show requirement for sodium for the expression of crassulacean acid metabolism pathway when plants are treated with NaCl, CO_2 uptake is increased in the

dark with increase in malate content in the leaves. Sodium plays a role in maintaining a favourable water balance in plants (Willert et al., 1976).

There are several reports where sodium content has been reduced in many plants under saline conditions. Karadge and Chavan (1983) found less Na uptake than Cl uptake in Sesbania aculeata at ECe 10 and 15 $mScm^{-1}$ of NaCl. Robinson and Downtown (1984) reported less amount of Na (40 mM NaCl) in Spinacia oleraceae, Beta vulgaris, Pisum sativum under saline conditions. Eshel (1985) observed that Suaeda monoica and S. aegyptica exhibited Na deficiency and had low selectivity of K Vs Na than Salsola Kali. Hajibagheri et al. (1987) reported decrease in Na concentration in vacuoles inwards from epidermal cells in salt treated (200 mol m^{-3} NaCl) roots of salt sensitive Var. (LG_H). Solomon et al. (1987) observed depression in Na accumulation in roots of Pisum sativum (v. Alaska) under NaCl stress. Abdel Rahman (1987) reported decrease in Na content in calabrese and red radish leaves under saline conditions. Jeschke and Wolf (1988) observed that when Ricinus communis L.was grown at 160 mol m^{-3} NaCl, Na concentration in phloem sap (collected from young petioles) was 2.5 times lower than older petioles or from stem base. Hajibagheri et al. (1989) reported that resistance to salinity was developed by low Na fluxes under NaCl treatment. Matoh and Murata (1990) noted that deficiency of Na affects rate of photosynthesis in Panicum coloratum walt. Cv. Kabulabula. According to Wolf et al. (1986,1990) Na concentration in xylem decreased from base of leaf to the tip and also decreased with leaf age .In phloem sap, Na decreased from 21 $molm^{-3}$ at the tip to 7.5 $molm^{-3}$ NaCl at base in Hordeum vulgare Cv. California Mariout. Gorham et al. (1990) reported low Na in Aegilops squarrosa in presence of salt. Ashraf and Naqvi (1991) observed decrease in shoot Na in Cenchrus pennisetiformis at highest Na/Ca ratio. when plants were subjected to different Na/Ca ratio of 24, 49, 99,

199 at constant concentration of 200 mol m^{-3} concluding plant to be intermediate tolerant to low Ca concentration of saline growth medium. Glenn et al. (1992) observed low Na levels in leaf and stem tissues in 3 sub species (Canascens, macropoda, linearis) when grown in 22, 180, 540 and 720 mol m^{-3} NaCl at harvest concluding the high tolerance of species was not necessarily dependent on high levels of Na accumulation. Khan et al. (1992) reported that in Sorghum Var IS-1347 Na uptake in shoot was restricted and reverse was observed in IS-4807 and IS-1347 had high osmolalities concurrent with high relative water content El-Samad and ABD (1993) observed decrease in Na content in Triticum vulgaris L. plants under NaCl salinity. Marcar (1993) reported reduction in leaf Na content under 100 mol m^{-3} NaCl and pretreated waterlogging conditions in Eucalyptus globulus. Alarcon et al. (1993) revealed that at low salinity level, osmotic adjustment was based on exclusion of Na with marked energy savings in Lycopersicon pennelli Cv. PE-47. At high salinity, species accommodate stress through higher adjustment. Lycopersicon esculentum carried out osmotic adjustment based on important contribution of organic solutes. Yin et al. (1993) reported that in Anerolepidium chinensis Kitag at salt content of 0.088 % to 1.63 %, Na content decreased in plant tissue, with increase in organic solute at Na concentration > 80 u mol g in soil. Lu and Wang (1993) reported that Na concentration of Triticum aestivum roots cultured in Hoagland solution containing NaCl with $CaCl_2$ supplement was low and the relative permeability of Plasma membrane and efflux of Na were reduced.

Ortiz et al (1994) noted decrease in Na content with increased K and Ca content in expanding leaves of Phaseolus vulgaris L. Cv. Contender under NaCl shock. El-Samad and Abd (1995) reported that salinity affects sodium content in Cucumis sativus. Colmer et al. (1995) observed that Na level was low in younger ones in genotype of

wheat grown for 18 days in saline (1.25mM and salinized 200 mM NaCl) medium indicating that low Na levels contributed to NaCl tolerance. Huang and Redmann (1995) reported that transport of Na from roots to shoots was low in wild barley than in Harrington under salt stress. Zhao et al. (1995) reported that in halophyte Suaeda salsa, Atriplex centralasiatica and Suaeda bicolor treated with different concentrations of KCl and isosmotic NaCl then at 100-400 mmol / L KCl, Na decreased proving that growth inhibition was due to Na deficiency. Fernandez (1996) observed that Na ions were largely excluded from infected zone of nodules of Lupinus albus L. under high salinity levels. Shukla and Singh (1996) reported marked reduction in sodium with increase in salinity and sodicity levels in Aegle marmelos Correa.//

Increase in sodium content in different parts has been reported by Strogonov (1964), Meiri et al. (1971); Ackerson and Younger (1975); Lessani and Marschner (1978); Clemes et al. (1983) and Gorham et al. (1985). Robinson et al. (1983) reported increase in chloroplast Na at 0 to 200 mM NaCl stress with increments of 25 mM in Spinacea oleraceae. Pakroo and Kashirad (1981) observed increase in Na content at 0 to 1.5 ppm NaCl in sunflower. Salim and Pitman (1983) observed increase in Na content with increasing levels of salinity in Vigna radiata. Buhl et al. (1983) reported increase in Na content when cut ends of of Hordium vulgare cultivar Larker were placed in NaCl solution. Kaiser et al. (1983) grew Spinacea oleraceae hydroponically and reported that at 350 $mmol^{-1}$ NaCl concentration, Na increased 60 fold which is pre-requisite for high photosynthetic capacity under salt stress. Merrier (1984) found that low NaCl concentrations around root medium accumulate greatest quantities of Na in tomato. Joshi (1984) observed more Na under Na_2SO_4 salinity in pigeon pea. Robinson and Downtown. (1984) reported high levels of Na in leaves of Spinach at 200 mM NaCl. According to Abdel- Rehman and Abdel- Haji (1984) increase in Na contents in leaves

of Vigna sinensis Cv. Balady occurs under saline conditions, when compared to control. When NaCl was increased upto 600 mM in daily increments to 50 mM in Cereus validus, Na concentration in roots increased from 7 to 100 mm and concentration of Na in shoots increased from 8 to 17 mm (Nobel et al., 1984). Gorham et al. (1985) observed greater uptake of Na under salt stress in Elymus dahurius. Sharma et al. (1984) reported that excessive accumulation of Na ions resulted in poor performance in salt sensitive var (HD-4502) of wheat seedlings. Downtown and Millhouse (1985) found accumulation of Na in barley leaves and exudation from leaves in vitis or bean. Huq et al. (1985) have reported that Na absorption was at maximum during first 15-20 days of culture in Vigna sinensis and Phaseolus aureus at two levels of salinities for 35 days. High accumulation of Na was avoided in organs with less vacuolated tissues, roots, hypocotyls and lower part of stem stored large quantity of Na. In Vigna sinensis, Na accumulated was evenly distributed among various organs while in Phaseolus aureus it was concentrated in roots. Seemann and Critchley (1985) observed that increase in Na content in Phaseolus vulgaris at 0-150 mM NaCl concentration. Eshel (1985) reported that Na at macronutrient level has effect on physiological process in Suaeda aegyptica under saline conditions. Harvey (1985) observed that in Zea mays when grown at </= 100 mol m^{-3} NaCl increase in Na in leaves reduced growth and its concentration increased in cytoplasm and cell walls. Robinson et al. (1985) found that when seedlings of halophyte, Suada australis were grown hydroponically in nutrient solution of 0 - 600 mM NaCl then Na increased reaching 800 mM. Leaf Na was low (< 10mM) which accumulated in chloroplasts containing 70 - 140 mM Na despite increase in salinity by 70 fold in the ion in leaf indicating that concentration of Na is regulated in chloroplast and accumulated at low levels in chloroplast and exuded when Na is high. Eddin et al. (1986) reported that Anthrocnemum fruticosum, a halophyte, under NaCl stress becomes more secculent with Na accumulation and Na was found to be more in

shoots than Cl. Harvey and Thorpe (1986) studied ion distribution in wheat leaf mesophyll cells at 100 mol m^{-3} NaCl and reported high concentration of Na in cytoplasm. Increase in Na concentration in Salicornia brachiata at 20 to 50 $mScm^{-1}$ was reported by Joshi (1986). Jeschke et al. (1986) reported that concentration of Na in root bleeding (xylem) sap and shoot tissues increased proportional to applied NaCl indicating limited entry of Na into root and xylem stream. Demming and Winter (1986) observed increase in Na in total leaf sap of Mesembryanthemum crystallinum grown at 400 mM NaCl. Jeschke et al. (1986) reported that in slightly vascuolated root tips of Atriplex amnicola , Na was only 40mol m^{-3} NaCl and in highly vascuolated root tissues, Na was higher at 200 and 400 $molm^{-3}$ NaCl than at 25 $molm^{-3}$ NaCl. Nigwekar and Chavan (1987) in Dolicos biflorous found higher accumulation of sodium in roots and stems. Clipson and Flowers (1987) reported that mean Na concentration in xylem was maximum at 56 mol m^{-3} Na with external salinity of 200 mol m^{-3}. Na concentration in xylem was greater in dark than light at all external salinities in Suaeda maritima seedlings. Na was excluded from transpiration stream as salinity increased . Hajibagheri et al. (1987) observed that salinity induced greater increase (about 1.7 times) in cytoplasmic Na concentration in salt sensitive variety (LG_{11}) than resistant variety (Across 8024 and Prolador) in root cells of maize at 200 mol m^{-3} NaCl. Ohta et al. (1987) reported increase in Na concentration from 0.4 mM to 2-3 mM within 24 h in leaves of 30 day old seedlings of Amaranthus tricolor L. when grown under saline conditions. Weimberg (1987) observed that Na was 10 times higher in Triticum turgidum than T. aestivum at low levels of salinity stress. Joshi and Iyenger (1987) reported massive accumulation of Na in Suaeda nudiflora at sea water salinity stress (10-40 $mScm^{-1}$) causing variations in Ca, Mg and SO_4. Bowman (1988) reported increase in leaf Na concentration only in inland population of Andropogon glomeratus, but were higher at all salinities in marsh population which contrasts with the studies of

salt tolerance in other non- halophytic grasses. Jeschke and Wolf (1988), observed that when Ricinus communis was grown at 160 $molm^{-3}$ NaCl , internal concentration of Na increased and Na concentration in stem and petiole was higher than leaf blades. Na concentration increased in xylem and phloem saps with external NaCl. Thus, at high external salinity, non uniform distribution of Na within shoot and leaf was maintained. Joneva (1988) reported competitive relations between K and Na in roots at all NaCl concentrations in wheat at 0.1 - 100 mM NaCl concentration and 6h exposure. In tomato roots, competitiveness was manifested at low NaCl. In maize under low NaCl concentration, competitiveness was compared which is a important factor for plant resistance to salinity. Munns et al. (1988) observed high Na concentration in expanding tissues (150 $molm^{-3}$) and low Na concentration in dividing tissues indicating that growth of shoot is not controlled by local concentration of Na and or Cl in Lupinus albus.

Khan and Ashraf (1988) reported retention of Na in roots of tolerant sorghum Var and was translocated in limited amounts to shoots and reverse was observed in sensitive var. Schroeppel- Meier et al. (1988) exposed Spinacea oleraceae Var Yates to NaCl or $NaNO_3$ upto final concentration of 300 mM/ Litre at constant Ca concentration and reported that plants took up larger amounts of Na (upto 400 mM/ L). Schachtman et al. (1989) observed that under saline conditions, transport rates from roots to shoots of Na reached higher levels in wheat cultivar. Huang and Steveninck (1989) reported greater accumulation of Na in mesophyll of the sheath than in blade of Hordeum vulgare seedlings (Cv. California Maiout and Clipper) with 100 mM NaCl for 1 day or 50 mM for 4 days. Plaut et al. (1991) reported that in mesophyll cells of leaves of cowpea (Vigna unguiculata L. Wasp) under 173 moles / cubic meter of NaCl, high accumulation of Na inhibited photosynthesis. Blits and Gallaghar (1990) found increased Na content in shoots of Kosteletzkya virginica with increasing salinity. Torres et al. (1989) observed

that when Lycopersicon esculentum was grown at 100 mM NaCl, Na content increased in roots, hypocotyl and cotyledon. Zoldos et al. (1990) observed increase in Na concentration in rice seedlings with increasing salinity. Flowers et al. (1991) reported accumulation of sodium ions in leaves of rice under saline conditions. Cramer et al. (1991) found increase in Na over time in Hordeum vulgare L (M72) grown for 25 days under NaCl or KCl (125 mM) concentration. According to Jeschke and Pate (1991), when Ricinus communis L. plants were exposed to mean salinity stress of 128 molm^{-3} NaCl, Na was retained in the root and lateral uptake from xylem by hypocotyl, stem internodes and petioles led to low intake by young leaf laminae and substantial cycling from older leaves back to the root, at late vegetative growth. Tirmizi et al. (1991) reported that increasing NaCl concentration enhanced Na uptake in leaves of Hippophae rhamnoides L. Shitole and Shinde (1991) observed increase in Na inroots under chloride salinity and Na in petiole under sulphate salinity in Carica papaya Cv. Ranchi. Hartzmann et al. (1991) reported accumulation of Na in roots at NaCl 50 and 100 mM concentration in Opuntia ficus indica (L) Miller. Warwick and Halloran (1991) found that Na concentration were higher in leaf sheath than in leaf blade of Diplachne fusca L. indicating that leaves have capacity to sequester high levels of Na in sheath and blade as well as maintaining high selectivity for K over Na when plants were subjected to salinity upto 400 mol m^{-3} NaCl. Results of the present investigation (Table.10 ;Fig.8a) also suggested that Na content was more in roots than stem and leaves under both the salinities indicating that roots have capacity to sequester high levels of Na in roots of Carthamus tinctorius Var. Bhima.

Plaut and Federman (1991) have reported accumulation of Na in salinity acclimated plants. Asharf and Naqvi (1991) have observed increase in shoot Na concentration in Leptochloa fusca (L) Kunth at different Na/Ca ratio of 24, 49, 99, 199 at constant

concentration of 200 mol m^{-3} concluding that the plant was tolerant to low Ca concentration of saline growth medium. According to Abbas et al. (1991) there was continuos increase in Na concentration in all organs of Phaseolus vulgaris with increasing salinity levels in growth medium with more profound increase in roots than in leaves. Reverse was observed when medium was desalinized. Haddad and Coudret (1991) reported accumulation of Na in aboveground parts of 2 triticale cultivars Clercal and Beagel after 21 days of growth in NaCl containing nutrient medium. Dutt et al. (1991) reported accumulation of high amounts of Na with increasing salinity levels from 4, 8, 12, 16, 20 dS /m in Casuarina equisetifolia Forst. Zsoldos et al. (1992) reported marked increase in internal Na concentration of root in 6 days old wheat (Triticum aestivum L.Cv. Gkszeq) concluding that salt tolerance of wheat is related to restricting transport of Na to shoot at low and moderate level where it is highly toxic. El-sayed and Kirkwood (1992) reported accumulation of Na in Glycine max L. cells adapted to NaCl salinity up to 680 mM. However, ions did not contribute to osmotic adjustment during culture growth cycle. Sharma and Kumar (1992) reported retention of Na concentration by roots on a dry mass basis in one month old plants of two chickpea (Cicer arietinum) cultivars under 4 and 8 dS/m and concluded that expression of ion concentration on tissue water basis appears to be more useful than dry matter basis. Cachorro et al. (1993) found increase in Na concentration in leaves of Phaseolus vulgaris with increase in NaCl concentration in nutrient solution. Blits and Gallagher (1993) observed increase in Na content under extreme salinity reducing growth in cell culture of Kosteletzkya virginia L. Malvacea. Tipirdamaz and Karakullukcu (1993)observed increase in Na concentration with increase in NaCl concentration when tomato embryos were cultured in vitro with 150 mM NaCl along with 10 mM proline / glycinebetain. Marcar (1993) reported accumulation of leaf and stem Na concentration at 150 mol m^{-3} and pretreated waterlogging condition in Eucalyptus species. Yin et al.

(1993) observed that Na was rapidly absorbed from soil in roots and older leaves with exclusion of K. When Na concentration in soil was < 20 u mol/g for osmotic adjustment in Aneuro lepidium Chinensis Kilag under saline treatment of 0.088 % to 1.63% . Tillering buds also showed accumulation of Na. Fedina et al. (1993) reported that in 10 day old Pisum L. Cv. Ran 1 plants pretreated with proline (10^{-6} M or 10^{-5} M) for 24 hours before salinization with 50 mM NaCl for two days resulting in increase in Na content in root indicating that proline alleviated inhibitory effect of NaCl. Storey et al. (1993) reported accumulation of Na upto 400 mol m^{-3} salinity resulting in increase in external osmotic pressure in leaf of Melanthera biflora. Ramanjulu et al. (1993) found increase in Na content in Mulberry Var. Mysore local tissue under NaCl treatment.

Zhong and Lauchli (1994) reported that deposition rate of Na was increased greatly by 150 mM NaCl supplemented with $CaCl_2$ and reduced by 10 mM Ca in cotton Gossypium hirsutum L.Cv. Acala SJ-2. Hamada and Enany (1994) have reported increase in Na in roots and shoots of broad bean and pea plants with increasing salinity. Gouia et al. (1994) reported accumulation of 10% Na in shoots and 60% in roots of bean at 50 mM NaCl. Nabil and Coudret (1995) observed that accumulation of Na ions was useful for osmotic adjustment in Acacia nilotica subspecies cupressiformis and tomentosa when subjected to 0, 75, 100, 200 mM stress. Ayala and O' Leary (1995) have reported increased sodium concentration in shoots and roots when Salicornia bigelovii Torr. plants were grown in 5, 200,600 mol m^{-3} NaCl. Ashraf and Fatima (1995) reported that salt tolerant (260622 and 305167) accessions of Carthamus tinctorius L. accumulated significantly greater Na in leaves compared to salt - sensitive (199952 and 170274) accessions. Therefore, salt tolerance of safflower is associated with inclusion of Na in leaves. Khan et al. (1995) observed that Na concentration were 29-36 times higher in leaves , stem and root of Sorghum bicolor L. Moench Cv. IS- 4807 raised at NaCl (100

mmol L^{-1}). At Na_2SO_4 stress (50 mmol L^{-1}) leaf, Na was 21-23 times than the control. Na uptake was less in Na_2SO_4 than in NaCl stressed plants. Reimann and Breckle (1995) found high accumulation of sodium in Salsola kali L. in ssp. tragus than in ssp. ruthenia. Sharma (1995) observed increase in Na in all parts of wheat especially in roots and stem under salinity stress. Colmer et al. (1995) reported that Na level was highest in oldest leaf blade. Na declined sap (sap osmotic potential) in wheat genotype for 18 days in non saline (1.25 mM Na and salinized 200 mM NaCl) conditions. Aldesuquy (1995) reported induced accumulation of Na at various stages of flag leaf development of Triticum aestivum L. at 33 or 66 mM NaCl treatment . Huang and Redmann (1995) observed that salt stressed barley roots accumulated more Na than in shoots . Tattini et al. (1995) found that Na concentration in leaves of Olea europaea L. Cv. Frantoio plants treated with 200 mM NaCl reached maximum levels of 211 and 388 mM at end of first salinity and relief period respectively indicating that inhibitory effects of salinization can be reversed, when salinity is relieved despite accumulation of toxic ions (Na , Cl) in leaf. Sehmer et al. (1995) studied effect of NaCl salinity on young pedunculate oak trees and reported increase in Na content especially in first flush leaves. According to Patil et al. (1996), leaf sodium content increased markedly in maize leaves at higher salinity levels. Morabito et al. (1996) reported that sodium uptake in roots was 2.5 times higher in more tolerant clone 42 than in clone 43 in Eucalyptus microthera at 200 mM NaCl. Erdie et al. (1996) reported higher level of Na accumulation in maize roots than in Sorghum. Cayuela et al.(1996) reported higher accumulation of Na at 70 and 140mM Nacl from prunned seedlings with 6M Nacl in roots of Lycopersicon esculentum Mill. Cv. Pera. Zhang et al. (1996) found increase in Na content in roots of Eleusine Coracana seedlings grown in Hoagland solution containing NaCl, when compared to control and efflux % of Na increased. Guerrier (1996) reported increased accumulation of Na uptake rates under short -term salt

exposure to Lycopersicon pimpinellifolium and L. esculentum. Hamada (1996) reported that Na content in the shoots and roots of wheat plants were increased with increasing salinity. Na accumulated preferentially in old rather than in young leaf blades in salt stressed barley plants. Savvas and Lenz (1996) observed increase in Na and Cl concentration in roots and older leaves of egg plant under 20,40,60 mM NaCl salinity and in fruits and young leaves it was low. Sharma (1996) reported increased concentration of Na in flag (Triticum durum L. Cv. HD4508) leaves exposed to 1.6, 12 and 16 dSm^{-1} salinity levels. However, the oldest leaf contained 6.8 times more Na than young leaf. Okuba and Utsunomiya (1996) studied that Na ions were accumulated in leaves and stems rather than in roots with increasing 0-50 mM NaCl concentration in Ficus carica L. cuttings for weeks. However, Na content was less in latex than in leaves/stems, suggesting high capacity for exclusion of Na by membranes of laticiferous cells. Garg et al. (1997) have reported that Na concentration increased in shoots in cluster bean in presence of the Ca 2.5 and 5.0mM with increasing NaCl (0, 50,100 and 150 mM) concentrations. Venkatesan et al. (1997) observed increase in Na content upto 600 mM NaCl in Ipomoea caprae sweet tissue. Khan et al. (1997) reported increase of Na in 3 cultivars of rice under saline conditions. Maliwal (1997) found that absorption of Na increased with increasing chloride salt concentration than sulphate salinity (0.78 to 15.40 ds/m) in five wheat varieties. Rodriguez et al. (1997) reported that, in apical 0 to 3 mm zone, Na concentration was 160 to 180 $\mu mol\ mL^{-1}$, and in 3 to 10 mm zone, Na concentration increased over the control by about 26 $\mu mol\ mL^{-1}$ at 12h whereas in >10 mm zone, Na concentration increased by 70 $\mu mol\ mL^{-1}$ that is 4.5 fold increase, when maize seedlings were treated with 100 mM NaCl. Saha and Gupta (1997) observed increase in Na concentration with increasing NaCl salinity in sunflower seedlings. Sharma (1995) found increased Na concentration in 2 genotypes of

chickpea with increasing levels of salinity. According to Yuncai and Schmidhalter (1998), Na concentration ($mmol.kg^{-1}$fresh.weight.h^{-1}) was high at 120 mM NaCl than at 0 mM NaCl along leaf axis from leaf base of wheat and local net deposition rates of Na ($mmol.kg^{-1}$fresh.weight.h^{-1}) in actively elongating zone were enhanced by 120 mM NaCl. However, higher Na tissue concentration did not result in ion toxicity in growing leaves but could cause ion imbalance.

Abdel- Rehman (1987) reported no effect of salinity on sodium content in cowpea. Banuls and Millo (1992) observed that leaf Na concentration of 478 mM did not affect dry matter, defoliation, photosynthesis and stomatal conductance in Citrus sinensis [L] Osbeck Cv. Hamlin. Yamanouchi et al. (1989) reported that at 40 mM NaCl , Na ion was absorbed with Cl which occurs due to difference in salinity tolerance which is due to power of their water absorption at higher osmotic medium. Bernstein et al. (1995) concluded that Na is not the cause of inhibition of growth in salt affected tissue of Sorghum bicolor [L.] Moench. Cv. 'Nk 265' leaves at 1 or patter 100 mol m^{-3} NaCl salinity.

Results of the present investigation (Tables. 17-20) indicated that maximum Na is stored in roots at all levels of NaCl and Na_2SO_4 salinity's. In stem and leaves also Na increased with increasing levels of both the salts because, under saline stress proton gradients may supply the driving force for the transport of Na from the cytoplasm to the vacuole or extemal medium (Bruggemann and Janiesch, 1989; Matoh et al.,1989). Gorham and coworkers (1990) suggested that the better growth of Hordeum vulgare and of Triticum durum leaves with similar salt concentrations may be a result of better compartmentation of Na, Cl and K between different tissues or between different compartments within cells. This is apparently achieved by mechanism that regulate

K/Na selectivity and Cl uptake across the plasma membrane and compartmentalize Na and Cl in the vacuole (Flowers et al., 1977; Greenway and Munns, 1980; Jeschke, 1984 and Binzel et al., 1988). It is found that safflower plants survive upto ECe 10.0 $mScm^{-1}$ of NaCl and Na_2SO_4 by storing more Na in roots (Tables. 17,19). At all levels of Na_2SO_4, Na content in leaves (Tables. 17,19) increased, however, productivity was more than control upto ECe 7.5 $mScm^{-1}$ of Na_2SO_4 and upto ECe 7.5 $mScm^{-1}$ of NaCl (Tables. 5,6) which indicated that safflower Cv. Bhima maintains more productivity by storing Na in compartments of cells.

B) POTASSIUM

Potassium is known to have multiple functions. Evans and Sorger (1966), indicated that probably it provides the necessary ionic environment for preserving the proper three-dimensional structure of enzymes for optimal activity. Besides, K plays an important role in physiological and biochemical functions. Potassium is linked to carbohydrate metabolism and it is essential for translocation of sugar. Spanner (1958) proposed a mechanism of K circulation around the sieve plate for increasing translocation of sugar in sieve plate which actually favors sugar translocation. Thus any factor that increases potassium transport could alter the electro-osmotic potential between sieve tubes and influence sugar translocation. K is essential for respiratory metabolism that is, CO_2 uptake(Walker and Ward, 1974); CO_2 liberation (Jackson and Volk,1968; Barenkiewics,1978) as well as activities of TCA cycle and oxidation phosphorylation enzymes (Okamoto,1969).

Potassium serves as a counter ion to hydrogen in the ion transport across the thylakoid membrane in photosynthetic processes. This enables efficient energy transfer between

the photosystems, affects chlorophyll biosynthesis and chloroplast ultrastructure. K by its role in ATPase activity may be involved in ion transport across biological membranes. The rate of N turnover and protein synthesis in intact plants depends on K content (Koch and Peoples,1979). The role of K in growth and metabolism makes it difficult to trace a specific and casual relationship between K nutrition and the response mechanism. The effect of K deficiency on photosynthesis is not so expressive as that of N or P (Natr,1972.1975).

Stomatal opening in higher plants requires potassium. It has been estimated by X-ray electron probe microanalyser, that guard cells of opened stomata contains relatively high concentrations of K as compared to closed stomata (Humble and Raschke ,1971). There is an influx of potassium ion into the guard cells at the expense of ATP, in vacuoles which result in osmotic swelling of guard cell, due to which opening of stomata takes place. Potassium also plays role in regulation of water in plant cells. Under water stress, absorption of K selectively prevents water loss (Kremer et.al.,1972).

Role of K in synthesis and maximal activity of many enzymes has been implicated (Catsky et al.,1987). K plays role as cellular cation . High concentration of K are required for active conformation of many enzymes participating in intermediatory metabolism and biosynthesis. The reactions involved in the phosphorylation of carboxyl groups and interconversions of enol-keto intermediates are activated by potassium. Potassium is required by the enzyme acetic thiokinase from spinach leaves for maximal activity (Hiatt and Evans, 1960). Potassium might act as a regulator of the enzyme pyruvate kinase through repression of synthesis of the enzyme (Sugiyama et al., 1968). Folic acid metabolism and γ-glutamylcysteine synthesis requires potassium. K is

required by enzyme succinyl-CoA synthetase isolated from tobacco for maximal activity (Bush,1969). Nitrate reductase formation in rice seedlings requires K (Oji and Izawa, 1969). There is absolute requirement for potassium by starch synthetase isolated from sweet corn (Nitsos and Evans,1969).

Robinson et al. (1983) reported decrease in leaf K at NaCl (0 to 200 mM) stress with increments of 25 mM and K concentration in chloroplast decreased by 50 % in Spinacea oleraceae. Tsenov et al. (1983) reported decrease in K content in Pea shoot apices under 0.4 and 0.8 % NaCl salinization. Kawasaki and Moritsugu (1983) observed that depressive effect of K content in Phaseolus vulgaris, Zea mays and Sorghum vulgare was more severe with NaCl than PEG. Robinson and Downtown (1984) reported decrease in K at 200 mM NaCl concentration in Spinacea oleraceae. Abdel-Rahman, and Abdel-Hadi (1984) reported decrease in K contents in leaves of Vigna sinensis Cv. Balady under saline conditions. Gorham et al. (1984) observed greater loss of K from shoots of Elymus dahurius under salt stress. Abdel - Rahman and Hadi (1984) reported decrease in K content in Vigna sinensis Cv. Balady compared to control. Eshel (1985) reported that K was lowered due to Na presence in Sauaeda aegyptica under saline conditions of KCl and NaCl. Eshel (1985) showed that Salsola monaica and Salsola aegyptiea revealed low selectivity of K compared to Na when treated with half strength Hoagland's nutrient solution (control) and control +150 mol m^{-3} KCl or NaCl. Robinson et al. (1985) observed that when seedlings of halophyte Suaeda australis were grown at 0 - 600 mM NaCl, leaf K concentration decreased with increasing salinity. Clipson and Flowers (1987) reported that mean K concentration in xylem declined as external NaCl concentration increased though selectivity of K increased at higher salinities. Solomon et al. (1987) observed depression in K accumulation in roots of Pisum sativum Cv. Alaska under NaCl stress. Abdel-Rehman

(1987) reported decrease in K content in calabrease and red radish leaves under saline conditions. Weimberg (1987) found that in Tritium turgidum ,K concentration decreased with increasing Na concentration but their sum remained constant at all salinity levels but in T. aestivum , K decreased more rapidly than Na. Clipson (1987) reported decline in K content in seedlings of Suaeda maritima L. Dum at 0 - 200 molm^{-3} salinity for 11 d period. Hansen and Munns (1988) found that NaCl salinity strongly depressed K uptake in leaves in Leucaena leucocephala. Jeschke and Wolf (1988) observed that when Ricinus communis L. was grown at 160 mol m^{-3} NaCl , K concentration in petiole or stem decreased. Schachtman et al. (1989) observed less accumulation of K in shoots of Triticum aestivum (hexaploid) Cvs Chinesespring and in wild salt tolerant Lophopyrum elongatum when grown under saline and non-saline conditions. Torres et al. (1989) observed that when Lycospersicon esculentum were grown at 100 mM NaCl, then K content decreased in roots, hypocotyl and cotyledon. Shaddad (1990) reported that K content lowered significantly with increasing NaCl salinization in Raphanus sativus plants, which was counteracted by proline spraying (200 g m^{-3}). Zoldos et al. (1992) reported that K uptake of root in absence of Ca declined significantly with increasing salinity in 6d old wheat (Triticum aestivum L. Cv. GK szeged) seedlings. Wolf et al. (1986, 1990) observed that K concentration decreased from base to the leaf tip in xylem and also decreased with leaf age. In phloem sap K declined from tip to the base of leaves in control and salt treated Hordeum vulgare Cv. California Mariout. Ashraf and Naqvi (1991) reported decrease in root K , concentration at 3 higher Ca/Na ratios of 24, 49, 99 and 199 in Panicum turgidum. Abbas et al. (1991) reported decrease in K concentration with increasing salinity levels in growth medium except in 2nd and 3rd trifoliate leaves in Phaseolus vulgaris This effect was reversed when medium was desalinized. Haddad and Coudret (1991) reported reduction in K in above ground parts of 2 triticale cultivars Clercal and Beagel after 21 days of growth at 150

mM NaCl in nutrient medium. However, uptake was improved more in Clercal than Beagel with addition of KCl to NaCl containing medium. Sharma and Kumar (1992) observed retention of K concentration in roots. Leaves and nodules received K preferentially on a dry mass basis in one month old plants of 2 chick pea (Cicer arietinum L.) cultivars under 4 and 8 dSm^{-1} and concluded that expression of ion concentration on a tissue water basis is more useful than dry matter basis. Tipirdamaz and Karakullukcu (1993) reported decrease in K ion content when invitro cultured tomato embryo were treated with 150 mM salt concentration along with 10 mM proline /glycinebetaine. Ramanjulu et al. (1993) reported decrease in K content under varied concentration of NaCl in mulberry Var Mysore local. El-Samad and Abd (1993) reported decrease in K ion content in Triticum vulgaris L. plants under NaCl salinity. Yin et al. (1993) reported that in Aneurolepidium chinensis ,K was excluded with Na accumulation when Na concentration in soil was < 20 u mol / g accompanied by increase in organic solutes at Na concentration > 80 u mol / g. K distributed in young leaves was metabolically active and tillering buds absorb and accumulated K ion. Taleisnik and Grunberg (1994) found lower K uptake rate in Ace and Edlkarin cultivars of Lycospersiion esculentum especially in Cv. Ace. at 25 or 100 mM NaCl when compared to control. Ortiz (1994) reported increase in K concentration with decreased Na concentration in expanding leaves of Phaseolus vulgaris L. Cv. Contender under NaCl shock. Zhong and Lauchli (1994) reported reduction in deposition rate of K in Gossypium hirsutum L. Cv. Acala SJ-2. At 150 mM NaCl selectivity of K Vs Na of root enhanced in apical 2 mm region by 10 mM Ca which declined with distance from root tip concluding that supplemented Ca alleviated inhibitory effects of NaCl by maintaining plasma- membrane selectivity of K over Na. Reimann and Breckle (1995) reported decrease in potassium content in Salsola kali L. at 200 mol m^{-3} NaCl. Aldesuquy (1995) reported decreased K content at various stages

of flag leaf development of Tritium aestivum L. at 33 or 66 mM NaCl treatement. Bernstein et al. (1995) investigated that K concentration and deposition rate was decreased due to salinity in elongation zone in Sorghum bicolor (L.) Moench. Cv. NK 265 leaves at 1 or 100 mol m^{-3} NaCl. El-Samad and Abd (1995) reported that salinity affected K content in Cucumis sativus. Spraying the shoot system with sodium pyruvate ameliorated adverse effects of NaCl salinity. Khan et al. (1995) found loss of K in leaves, stem and roots of Sorghum bicolor L. Moench. Cv.IS-4807, which was accompanied by accumulation of Na. Loss of K was less in Na_2SO_4 (0-50 mmol L^{-1}) than in NaCl (0-100 mmol L^{-1}) stressed plants. According to Patil et al. (1996) leaf K content decreased in maize leaves at higher salinity levels. Guerrier (1996) reported drastic reduction in K uptake rates during long term salt exposure in Lycopersicon pimpinellifolium and L. esculentum in all parts. Savvas and Lenz (1996) observed that NaCl salinity (20,40 and 60 mM) reduced K in roots and older leaves in eggplants grown in closed sand culture system for 6 months. Shukla and Singh (1996) found marked reduction in K content with increase in salinity and sodicity levels in Aegle marmelos. Correa. Lopez and Satti (1996) observed reduction in potassium concentration when 5 tomato cultivars were treated with 50 mM NaCl. Botella et al. (1997) reported that NaCl reduced K net uptake rates and translocation from root to shoot resulting in lower K shoot content and high K root content when 1/2 Hoagland nutrient solution was supplemented with low (0.1 mmol / L) and high NaCl salinity (100 mmol / L) in Zea mays L. plants. The inhibitory effect of salinity on K translocation was strong with low K concentration in the nutrient solution . Net uptake of K was dependent on K concentration in root medium and on K status of root. Abd-El Samad and Shaddad (1997) reported that sensitivity of soybean cultivars Cv. Kent was due to decreased K content under NaCl stress. Khan et al. (1997) observed that K

accumulation decreased in three cultivars of rice subjected to 0 to 200 mM NaCl concentrations.

Several references (Strogonov, 1964; Mieri et al., 1971; Tal, 1971; Lashon and Atansiu., 1972; Siegel et al., 1980; Brun, 1987) indicated that K uptake increases under saline conditions in some plants. Guerrier (1983) reported high rate of entry of K at any given NaCl concentration in excised roots of Raphanus sativus Cv Rond 2 gros bout blanc. Jeschke et al. (1983) observed remarkable preference for K in roots and hypocotyl of Atriplex hortensis under mild conditions of NaCl and Na_2SO_4. Kaiser et al. (1983) grew Spinacea oleraceae hydroponically at 350 $mmol^{-1}$ and reported that in controlled plants K was equally distributed between chloroplasts and extra chloplastic space. Kawasaki and Moritsugu (1983) reported more absorption of K under NaCl than in PEG in Phaseolus vulgaris, Zea mays and Sorghum vulgare. They reported that high NaCl concentration inhibited absorption and translocation of K in absence of Ca in excised roots of Hordeum vulgare. Bhatti et al. (1983) observed that Diplacne fusca (kaller grass) under saline conditions shows strong selectivity for K over Na in top portions and does not affect plants response to Na and also did not interact with Na . Harivandi et al. (1983) reported no marked difference in accumulation of K and pattern of accumulation was not uniform in roots, shoots and entire plant but as salt solution concentration increased, ion accumulation also increased in Puccinia distans L. Parl and P. lemmonii (Vasey) at 42 meq/l of sulphate salt K_2SO_4 and 56 meq/l or less of chloride salt (KCl). According to Robinson and Downtown (1984), K was the predominant monovalent cation at 160-200 mM in Spinacea oleraceae, Beta vulgaris and Pisum sativum. The rate of K absorption was at maximum during the first fifteen to twenty days of culture of Vigna sinensis and Phaseolus aureus under saline conditions. (Huq et al.; 1985). Moreover K accumulation was evenly distributed among various

organs in Vigna sinensis while in Phaseolus aureus it was concentrated more in the root. Seemann and Critchley (1985) have observed that K concentration increased to lesser extent in Phaseolus vulgaris at 0-150 mM NaCl concentration and increase in K balanced leaf Cl. Increase in K concentration in Salicornia brachiata at 20-50 mM NaCl was reported by (Joshi, 1986). Harvey and Thorpe (1986) studied ion distribution in wheat leaf mesophyll cells at 100 mol m^{-3} NaCl and reported high concentration of K in cytoplasm. Jeschke et al. (1986) reported that in slightly vacuolated root tips, K concentration was not affected between 25 and 400 mol m^{-3} NaCl but in highly vacuolated root tissues, it was higher at 200 and 400 mol m^{-3} NaCl than the plants grown at 25 mol m^{-3} NaCl. Increase in K content of the leaves was reported by Pezeskhi et al. (1988) in Taxodium under saline conditions. Thus, non uniform distribution of K within shoot and leaf was maintained when exposed to high external salinity. Jeschke and Wolf (1988) observed that when Ricinus communis L. was grown at 160 mol m^{-3} NaCl , K concentration increased in leaf blades. K concentration in both long distant transport fluids were maintained at high levels. Khan and Ashraf (1988) reported increase in K with decrease in NaCl in four varieties of Sorghum. Hajibagheri et al. (1989) observed that resistance to salt in Zea mays appears to be due to high K fluxes and cytoplasmic concentration. Gorham et al. (1990) observed high leaf K concentration in Aegilops squarrosa under salt stress. According to Cramer et al. (1991) increased K over time in Hordeum vulgare L. (M72) for 29 days under NaCl or KCl (125 mM) treatment. Increasing NaCl concentration enhanced K uptake in leaves of Hippophae rhamnoides L (Tirmizi et al., 1991). Jeschke and Pate (1991) revealed that K was prominently deposited in leaves with high mobility of K in phloem and high rates of cycling through leaves and downward translocation of K providing root with excess of K at late vegetative growth of Ricinus communis plants exposed to 128 mol m^{-3} NaCl. Shitole and Shinde (1991) observed that Cv. Ranchi of papaya has efficient

K uptake mechanism under saline conditions. Glenn et al. (1992) reported high K levels and low Na: K ratios in leaf and stem tissues in Canascens, macropoda, linearis subsp of Atriplex canascens when grown in 22, 180, 150 and 720 $molm^{-3}$ NaCl at harvest. Similarly Khan et al. (1992) reported that Sorghum Var.IS-1347 maintained high K content under saline conditions. Cachorro et al. (1993) observed significant increase in K concentration with increase in salinity in Phaseolus vulgaris L. Blits and Gallagher (1993) reported high K uptake in salt treated cell cultures of Kosteletzkya virginia L. at high salinity levels of 225 $molm^{-3}$ NaCl. Storey et al. (1993) reported that shoot K concentration was maintained over a range of salinities upto 400 $molm^{-3}$ followed by increase in external osmotic pressure in Melanthera biflora Asteraceae. Lu and Wang (1993) showed that K content of roots cultured in Hoagland solution containing NaCl with $CaCl_2$ supplement was high and the efflux % of K was reduced in Triticum aestivum. Hamada and Enany (1994) reported an increase in K content with increasing salinization in broad bean. Gouia et al. (1994) observed decrease in K concentration in shoot of bean plants at 150 mM NaCl. Zhao et al. (1995) observed that in Suaeda salsa, Atriplex centralasiatica and Suaeda bicolor when treated with different concentrations of KCl and isosmotic NaCl, content of K increased. Nabil and Coudret (1995) attributed a role for osmotic adjustment for K in Acacia nilotica subspecies cupressiformis and tomentosa when subjected to 0, 75, 100, 200 mM NaCl stress and at 100 mM NaCl cupressiformis showed higher absorption of K than in tomentosa. Ayala and O' Leary (1995) reported that K content was highly concentrated in shoots and roots at 5 $molm^{-3}$ Morabito et al. (1996) reported that in clone 42, K content was higher than clone 43 of Eucalyptus microtheca at 200 mM NaCl. Erdei et al. (1996) reported that under NaCl and PEG - 6000 treatments, internal K concentration was higher in Sorghum roots compared to maize. Zhang et al. (1996) observed that efflux of K increases in Eleusine coracana L. under NaCl stress. Guerrier

(1996) reported increased K uptake rates under short term salt exposure in all parts of Lycopersicon pimpinellifolium. and L. esculentum , indicating their adaptive responses during short term salt exposure.

Abdel El-Samad and Shaddad (1997) reported accumulation of K in soybean cultivars, Clark and Forest . Venkatesan et al. (1997) found increase in K content in tissues of Ipomoea pes- caprae sweet plants grown upto 200 mM NaCl. Rodriquez et al. (1997) observed that K uptake increases in maize seedlings under saline conditions. According to Yuncai and Schmidhalter (1998), K concentration (mmol. Kg^{-1} fresh weight.) was high at 120 mM NaCl than at 0 mM NaCl along axis leaf base of wheat.

Adverse effects of salinity on K content have been reported by Gauch and Wadleigh (1944) in bean, Sarin (1961 (a,b)) in Cicer arietinum, Imamul Huq and Larher (1983) in Phaseolus aureus and Mishra and Shitole (1986) in Oat. Nobel et al. (1984) reported that when NaCl was increased upto 600 mM in daily increments to 50 mM in Cereus validus then K concentration remained near 26 mM in cell sap. Harvey (1985) observed that K level was maintained at 79 $molm^{-3}$ which was compatible with biochemical functions in Zea mays grown at salinity <= 100 $molm^{-3}$ NaCl. Abdel-Rahman (1987) reported no effect of salinity on potassium content in cowpea leaves. Ashraf and Naqvi (1991) reported that root and shoot K remained unchanged at varying Na/Ca ratios in Panicum turgidum under constant salt treatment of 200 $molm^{-3}$. Yin et al. (1993) reported that K remained constant when Na concentration in soil was 20-80 umol/g in Aneurolepidium chinensis Kilag under 0.088 % to 1.63 % concentration of salt. Hamada and Enany (1994) reported that K content remained unaffected by salinity. Ashraf and Fatima (1995) observed no difference in K concentration in salt tolerant and salt sensitive safflower under 0, 70, 120 and 140 mol m^{-3} NaCl salinity's. According to

Sharma (1995), K concentration of plant parts of wheat under salt stress was not much affected.

Our results (Tables. 17,19) indicated that in general the K content in safflower Cv. Bhima was less than the control at all levels of NaCl and Na_2SO_4 reflecting that K uptake fails under saline conditions in Carthamus tinctorius L. Cv. Bhima.

C) K/Na RATIO

Taleisnik-Gertel et al. (1983) showed that ion balance (K:Na) in detained leaves and apices of Lycopersicon esculentum, L peruvianum (L) Mill and Solanum pennelli was different from attached parts which may be due to severance of excised parts from major sites controlling balance of ions in whole plants. However, according to Huq et al. (1985) at no stage endogenous K:Na ratio falls below 1.0 in Vigna sinensis and Phaseolus aureus at 2 levels of salinity's for 35 days. Wethered and Jennings (1985) reported that K:Na ratio was around unity irrespective of external salinity in Traustochytrium aureum and T. roseum. Joly Robert (1989) reported Na:K ratio to be 34 in soybean root system after 6 weeks of growth in 25% artificial sea water. Ratio was maintained due to secretion of ions from leaves which is important in salt-tolerance of leaves. Blits and Gallagher (1990) observed that Kosteletzkya virginia (L) Prest in nutrient solutions of 0, 85, 170, 255 $molm^{-3}$ NaCl showed affinity for K with foliar K levels compared to control after initial decrease suggesting selective uptake and transport foliar compartmentation of Na and K in opposite directions along shoot axis and regulation of leaf salt loads over time prevent build up toxic concentration which is favourable to establish favorable K and Na relations under saline conditions. Demming and Winter (1986) reported that Na /K ratio of chloroplast to be 1 at 20 mM NaCl and

Na /Cl ratio to be 5 at 400 mM NaCl in root medium of Mesembryanthemum crystallinum.

A decrease in K:Na ratio was observed by Jeschke et al. (1983) in Atriplex hortensis in shoot with increasing leaf age. Karadge and Chavan (1983) reported that K:Na ratio was maintained at fairly constant levels in leaflet but was reduced in leaf rachis, stem and roots in Sesbania aculeata at ECe 10 $mScm^{-1}$ and 15 $mScm^{-1}$ salinity levels. Jeschke et al. (1986) investigated that when Atriplex amnicola was grown at 25, 200 or 400 $molm^{-3}$ NaCl treatment, it was found that in highly vacuolated root tissues K:Na ratio decreased with age.

Flowers et al. (1990) observed high Na:K ratio, i.e. 34, after 6 weeks of growth of Oryza in 25% artificial sea water. This ratio was maintained in leaves due to secretion of ions from leaves. Baset and Arju (1990) have reported low K:Na ratio in mid portion of 3rd youngest leaf in two rice varieties grown in nutrient solution without and with 50 mM NaCl. Roy et al. (1993) observed that 30 mM L-proline with 100 mM NaCl salinity reduced K:Na ratio indicating that 30 mM L-proline alleviates salinity stress in rice seedlings of salt sensitive cultivars of Oryza sativa. According to Aldesuquey (1995), K:Na ratio decreases at various stages of flag leaf development of Triticum aestivum L. at 33 or 66 mM NaCl treatment. Zhang (1996) observed enhanced Na:K ratio in Eleucine coracana L. under NaCl stress.

According to Jeschke et al. (1986) K:Na ratio was higher in leaflets than in adjoining petiole and stem segments and in younger than in older parts of the shoot suggesting capacity of Na retention in stem and selectivity in K mobilization to young tissues with increasing salinity (NaCl 1,5,10,25,40 $molm^{-3}$) in Lupinus albus cultivar Ultra.

Hajibagheri et al. (1987) observed that mean K:Na ratio in cytoplasm of cortical cells in salt -resistant variety grown for 15 days at 100 $molm^{-3}$ NaCl was twice compared to salt - sensitive variety (LG_{11}) which clearly indicated that salt resistant variety has selective Na absorption mechanism. According to Blits and Gallagher (1993) Na/K ratio was low in salt treated cultures of Kosteletzkya virginia L. Malvaceae at high external salinities at 225 mol m^{-3} NaCl. Haddad and Coudret (1991) reported higher K:Na ratio with addition of KCl or $CaCl_2$ in NaCl containing nutrient medium in 2 Tritical cultivars Clercal and Beagle indicating that K/Na selectivity ratio is critical for salinity tolerance. Taleisnik and Grunberg (1994) reported that K/Na selectivity ratios were higher in Edkui than in Ace cultivar of Lycopersicon esculentum at 25 or 100 mM NaCl. Contrary to this, Huang and Redmann (1995) found high K:Na ratio in control wild barley plants than Harrington. Sharma (1995) reported increase in K/Na ratio in all parts of plant with highest concentration in root and lowest concentration in leaves of wheat under salinity stress. Erdei et al. (1996) reported increase in internal K concentration resulting in two fold higher K:Na ratio in sorghum roots compared to maize under saline conditions. Khan et al. (1995) reported higher K/Na in tolerant cultivar of rice under NaCl treatment of 0 to 200 mM , indicating efficient uptake of K under saline conditions.

Recently, Ashraf and Fatima (1995) found no difference in K:Na ratio at 0, 70, 140 and 120 $molm^{-3}$ NaCl salinities in salt tolerant and salt sensitive varieties of safflower plants. Reimann and Breckle (1995) reported correlation between salt tolerance and K:Na ratio in leaves of Salsola Kali L. at 200 mmol L^{-1} NaCl.

From all these references, it is clear that in many plants K:Na ratio increases under saline conditions. Generally, these plants are salt susceptible and lack selective

absorption mechanisms. However, Kharchia variety of rice maintains good growth with very high K:Na ratio which is due to its genetic potential (Joshi et.al., 1973).

There are several plants in which K:Na ratio decreases under saline conditions indicating that such plants absorb more K and less Na under saline conditions i.e. they possess selective absorption mechanism. These plants are salt resistant. Another group of salt resistant plants maintain more or less constant K:Na ratio by absorbing minerals selectively or by excreting Na. Results of the present investigation on Carthamus tinctorius Var. Bhima (Tables. 17-20) revealed that average K:Na ratio of total plant decreased with increase in concentrations of both the salts which indicated that there is no selective absorption mechanism in Carthamus tinctorius Cv. Bhima. However, the plant has the ability to maintain more growth with less K:Na ratio which is the genetic potential of the plant.

D) CHLORIDE

Though chloride is not a very essential micronutrient in higher plants, it is known to perform some important functions. Chloride ion, is involved in primary process of oxygen evolution. PSII contains one or more proteins containing manganese called manganese protein which is involved directly in the first step of H_2O_2 oxidation. It is thought that a chloride ion bridges two Mn together. During stomatal opening in light there is an influx of both potassium and chloride ions into the guard cells from subsidiary cells, giving osmotic potentiality to the guard cells. However, exact role of Cl ion in stomatal opening is not clear.

Sharma and Kumar (1992) reported retention of Cl concentration by roots, keeping leaves free of ion accumulation on a dry matter basis and not on tissue water basis in

one month old plants of 2 chickpea (Cicer arietinum L) cultivar under 4 and 8 dSm^{-1} NaCl and concluded that expression of ion concentration on a tissue water basis is more useful than on dry matter basis. Khan et al. (1992) reported that in Sorghum Var. IS-1347. uptake of Cl was restricted in root and reverse was observed in Var.IS-4807 and IS-1347. He Tie and Cramer (1993) observed decrease in Cl in two brassica species during first 5 days of sea water salinization. According to Marcar (1993) Cl in leaves of Eucalyptus globulus reduces under 100 $molm^{-3}$ NaCl. Lu and Wang (1993) reported that Cl concentration of Triticum aestivum roots cultured in Hoagland solution containing NaCl and supplemented with $CaCl_2$ was low and relative permeability to plasma membrane and efflux of Cl were reduced.

Gauch and Wadleigh (1943, 1945) observed high chloride accumulation in the leaves than in stem and roots of salinized bean plants. Lauchli and Wanke (1979) in salt sensitive soybean variety 'Jackson' have found accumulation of chloride in all shoot parts particularly in leaves. While the moderately tolerant variety 'Lee' accumulated in roots only. Increased chloride content was reported by Imamul Huq and Larher (1984) in non-nodulated Phaseolus aureus, Nigvekar and Chavan (1987) in Dolichos biflorus; Hansen and Munns (1988), in Leucaena leucocephala and Matoh et al. (1988), in Phragmites communis under saline conditions. Black (1956) reported that high concentration of Cl in leaves of halophytes might be due to the passive flow of Cl. Greenway (1965) has recognized two types of Cl uptake mechanisms. One is, Active absorption affected by temperature and the other is Passive occurring along with transpiration rate. According to Meiri and Poljakoff-Meyber (1969) accumulation of Cl in leaves is rate of salinization. They observed that rapid rather than slow salinization of the medium enhanced the Cl accumulation in bean leaves. Iyengar (1978a,b) reported that accumulation of ^{32}Cl was independent of excessive Cl in the medium. Clough

(1984) reported increase in Cl concentration in Avicennia marina with increasing salinity. Munns et al.(1988) observed high Cl concentration in expanding tissues (150 $molm^{-3}$) and low in dividing tissues suggesting that growth of shoot is not controlled by chloride concentration in Lupinus albus. Zoldos et al. (1990) found increase in Cl with increasing salinity in rice seedlings.

Plaut and Federman (1991) reported accumulation of chloride in salinity acclimated plants decreasing leaf osmotic potential in cotton leaves. Ashraf and Naqvi (1991) observed increase in shoot chloride concentration in Cenchrus pennisetiformis Hochst and Steud at highest external Na/Ca ratio when plant was subjected to different Na/Ca ratios of 24, 49, 99 and 199. Warwick and Halloran (1991) found that Cl concentration were higher in sheath than in leaf blade in Diplachnae fusca L. indicating that leaves have capacity to sequester high levels of chloride in sheath and blade, when plants were subjected to salinity upto 400 $molm^{-3}$ NaCl. Hatzmann et al. (1991) reported accumulation of large amounts of Cl in Opuntia cladodes under two different concentrations of NaCl (50 and 100 mM). According to Shitole and Shinde (1991) papaya Cv. Ranchi stored more Cl in petioles under NaCl and Na_2SO_4 salinity. Abbas et al. (1991) reported continuos increase in Cl concentration in all organs of Phaseolus vulgaris with increasing salinity levels in growth medium. Results of Jeschke and Pate (1991) revealed that Cl was deposited more or less uniformly in stem, petiolated leaf lamina tissues rather than the roots at late vegetative growth of Ricinus communis plants exposed to 128 $molm^{-3}$ NaCl. El-Sayed and Kirkwood (1992) observed accumulation of Cl in Glycine max L. cells adapted to NaCl salinity upto 680 mM, however iron accumulation did not contribute to osmotic adjustment during culture growth cycle. Marcar (1993) found accumulation of leaf and stem chloride concentration at 150 NaCl $molm^{-3}$ in Eucalyptus species. Tipirdamaz and Karakullukcu

(1993) observed increase in Cl concentration when tomato embryos were cultured in vitro with 150 mM NaCl along with 10 mM proline or glycinebetaine. He Tie and Cramer (1993) reported increase in Cl content in shoots of two brassica species during first five days of sea water salinization. Storey et al. (1993) observed that accumulation of shoot Cl was followed by increase in external osmotic pressure in Melanthera biflora (Asteraceae) upto salinity of 400 $molm^{-3}$. Gouia et al. (1994) found accumulation of Cl in all parts of bean plants at 50 mM NaCl. Nabil and Coudret (1995) reported that accumulation of Cl ions was useful in osmotic adjustment in Acacia nilotica sub-species Cupressiformis and Tomentosa when subjected to 0, 75, 100, 200 mM NaCl stress. Khan et al. (1995) reported that chloride concentration were 29 to 36 times higher in leaves, stems and roots of Sorghum bicolor L. Moench Cv. IS-4807 raised at 100 $mmolL^{-1}$ NaCl. Cayuela et al.(1996) reported high chloride accumulation in roots of plant Lycopersicon esculentum Mill. Cv. Pera seedlings at 70 and 140 mM NaCl. Nakamura et al. (1996) reported that Cl accumulation preferentially in old leaves rather than young leaf blades in salt stressed barley plants. Savvas and Lenz (1996) observed accumulation of Cl in photosynthetically active leaves under 20, 40, 60 mM NaCl salinity in eggplants grown in closed sand culture system for 6 months. According to Sharma (1996) Cl concentration in flag (Triticum durum L.Cv.HD 4508) leaves increased when exposed to 1.6, 12.0 and 16.0 dSm^{-1} salinity levels, however, oldest leaf contained 6-8 times more Cl than young leaf. Okuba and Utsunomiya (1996) reported that Cl ions were accumulated in leaves and stems than in roots with increasing 0-50 mM NaCl concentration in Ficus carica L. cuttings, however, Cl content in latex was equal to that of leaves /stems. Venkatesan et al. (1997) reported increased Cl content upto 600 mM NaCl in Ipomoea pes- caprae sweet plants. Maliwal (1997) reported that chloride absorption increased with increasing salt concentrations (0.78 to 15.4 ds/m) in 5 wheat varieties. Rodriquez et al. (1997) found that concentration of Cl

increased rapidly in response to salt shock (NaCl 100 mM) in 0 to 3 mm in root of maize seedlings. On the other hand, Saha and Gupta (1997) reported increase in chloride concentration with increasing NaCl salinity's in sunflower seedlings. Sharma (1997) observed increase in Cl concentration with increasing levels of salinity in 2 genotypes of chickpea. According to Yuncai and Schmidhalter (1998) Cl concentration (mmol. Kg^{-1} fresh weight.h^{-1}) was high at 120 mM than at 0 mM NaCl along leaf axis from leaf base of wheat and local net deposition rates of Cl (mmol. Kg^{-1} fresh weight.h^{-1}) in actively elongating zone were enhanced by 120 mM NaCl. Higher Cl tissue concentration did not result in ion toxicity in growing leaves but could have caused ion imbalance.

Alarcon et al. (1993) observed that, at low salinity, Lycopersicon pennelli Cv. PE-47 carried out osmotic adjustment, based on exclusion of Cl with marked energy savings. Under highest salinity ,species accommodate stress through a higher adjustment and in L. esculentum osmotic adjustment was based on important contribution of organic solutes. Fernandez et al. (1996) found that chloride ions penetrated into nodules of Lupinus albus L. under saline conditions.

Kumar et al. (1994) reported no change in chloride concentration within 3 hours of desalinization in sugarcane leaves under saline conditions. Ashraf and Fatima (1995) found that salt tolerant (260622 and 305167) and salt sensitive (199952 and 170274) accessions of Carthamus tinctorius L. did not differ in Cl concentrations,under saline conditions.

For the first time Terry (1976, 1977) observed that Cl is essential for growth. He has observed that when plants are grown in chloride free medium there is 40% reduction in growth. However, till today nobody has given any major role for chloride in plants,

except for photosynthesis. It is present in minute quantities in water evolving complex of photosynthetic electron transport chains.

Results of present investigation (Tables.17,19) show that chloride uptake was stimulated at all levels of chloride and sulphate salinizations in Carthamus tinctorius Cv. Bhima, which indicated that in C. tinctorius Cv. Bhima, there is no selective absorption mechanism.

E) SULPHATE

Sulphur is a constituent of amino acids like cysteine, cystine and methionine. Thus, it participates in protein structure. The absorbed SO_4 is converted to activated form, which is then reduced and incorporated in above amino acids, finally forming protein structure, which is stabilized because sulphur forms cross-links in protein molecule. Sulphur is a constituent of biotin , thiamine, coenzyme A and lipoic acid which are involved in cellular metabolism. Sulphur along with iron forms Fe-S centers of many enzymes, which are important in photosynthesis, respiration, N_2 metabolism,etc. Sulphur is a component of S-adenosyl-methionine, which is involved in lignin and sterol biosynthesis. It is a constituent of volatile oils which give characteristic pungent odours to cruciferous plants like onion, garlic, etc.

Sulphur is considered to be the fourth major nutrient of the plant (Nyborg and Bently, 1971). Different aspects of sulphur effects have been studied and reviewed by workers like Coleman (1966), Kanwar and Randhawa (1967), Muth and Oldfield (1970), Vimel (1972), Tisdale (1977) and Singh (1984) who reported that when plant is supplied with additional sulphur at an early stage then it produces more dry matter.

Gauch and Wadleigh (1944) reported that sulphate is absorbed in much smaller quantities than chloride but produces equivalent effects on growth of bean plants. In 1945, they observed exponential increase in sulphate content in leaves and roots with increasing concentration of sulphate in solution whereas the stem showed linear increase. According to Hayward and Wadleigh (1949) sulphate ion restricts the absorption of Ca while it promotes uptake of Na. At the same time Richards (1954) reported limited uptake of Ca under sulphate salinity which is responsible for the sensitivity of the plants because stimulated uptake of Na disturbs cationic balance in salinized plant tissues.

Sulphate content in bean (Meiri et al., 1971); in sugarcane leaves (Syed and Swaify, 1973; Naik 1980); in leaves of pigeonpea (Deshpande, 1981); in soybean (Nukaya et al., 1982); in roots of bean plants (Hajrasuliha ,1980); in Cucumis melo (Nukaya et al., 1984); and in leaves of Puncia granatum (Doring and Ludders,1987) was found increased with increasing sulphate salinization. Salinity alters permeability of the membranes (Mengel and Kirkby, 1979), which may be responsible for more mineral ion uptake.

Khan et al. (1995) reported that SO_4 content was 21-23 times higher than control plants, under Na_2SO_4 stress (50 mmol L^{-1}) in Sorghum bicolor Moench Cv. IS-4807. Acording to Datta et.al.(1995), sulphate salinity was more detrimental than chloride in barley cultivars.

Our results (Tables.17,19) suggested that low concentrations of NaCl stimulate sulphate uptake in safflower Cv. Bhima, whereas all concentrations of Na_2SO_4 stimulated sulphate uptake.

F) CALCIUM

Ca functions both as structural component and as a cofactor for certain enzymes. Ca is involved in the metabolism or formation of the nucleus and mitochondria (Bidwell, 1979). Protein phosphorylation is an important mechanism by which Ca and Calmodulin regulate biochemical events inside the cell (Cohen, 1982). Protein phosphorylation in higher plants has been reported for nuclear protein (Murray et al., 1978; Trewat, 1979; Lin et al., 1980), chloroplast proteins (Bennet, 1977), ribosomal proteins (Keates and Trewavas, 1974; Gowda and Pillay, 1980) and for cytokinin binding protein (Polya and Davies, 1983).

The role of Ca and Calmodulin is known in regulating protein phosphorylation in plants (Hetherington and Trewavas, 1982; Poyla and Davies, 1983; Ranjeva et al., 1983; Salimath and Marme, 1983; Veluthambi and Povaiah, 1984a). According to Veluthambi and Povaiah (1984b) Ca and Calmodulin regulates phosphorylation of membrane and soluble proteins in corn coleoptiles. Ca stimulates several protein synthesizing enzymes (Ranjeva et al., 1983).

A considerable part of Ca is associated with the structure and is required to bind pectate polysaccharides to form a new middle lamella in the cell plate arising between daughter cells. Several plants have crystalline calcium in the form of salts for eg. calcium oxalate, carbonates, phosphates or sulphates, silicate, tartarate and malate.

However, little is known about the crystals and their role. Cell wall extension induced by CO_2 and low pH is reduced when Ca is added in experimental solution (Evans et al., 1971) , supporting the hypothesis that calcium in cell influences cell wall rigidity through its effect on ionic bridging between cellular molecules.

The primary function of Ca in plants is in membrane stabilization (Christiansen and Foy, 1979). How Ca stabilizes membranes is not known but (Garrad and Humphrey, 1967) suggested a theory that Ca binds ionic groups of the membranes to form structural bridges between structural components thereby maintaining a selective permeability by pore radius and surface charge reactions; and also thereby maintaining structural membrane integrity. Ca is involved in 3-D arrangement of membranes. Calcium salt of lecithin, a lipid compound is involved in the formation or organization of cell membranes (Hewitt,1963).

Few reports indicated that Ca plays a role of activator in certain enzymes like amylase (Chrispeels and Verner, 1967), ATPase (Dodds and Ellis, 1966) and phospholipase (Nelson et al., 1977). The involvement of Ca in membrane form and function has the potential of affecting large number of membrane bound enzymes and metabolic reactions. Thus, Ca becomes important to plants growing under saline environment (Rains, 1972). External application of Ca protects the plants from damage caused to plants exposed to saline conditions (Ayoub, 1974; Kawasaki and Moritsugu, 1978a). Addition of Ca reduces adverse effects of salinity on plants (Lattaye and Epstein, 1969; Cramer et al .,1990). Exogenous Ca reduces the perception of stress by the cytoplasm. It has been suggested that Ca displaces Na from the plasmalemma of salt stressed root cell, thus decreasing the influx of ions into the cytoplasm (Cramer et al.,1985; Lynch et al.,1987). Extra Ca added to the medium possibly has some role in maintaining

membrane integrity, which contributes to the ability of different plants to resist salt stress. The presence of extra Ca in the solution lowers response to osmotic stress.

Kawasaki and Moritsugu (1983) observed depressive effect of Ca content in Phaseolus vulgaris, Zea mays and Sorghum vulgare was more severe with NaCl than PEG. Torres et al. (1989) observed that when Lycopersicon esculentum were grown at 100 mM NaCl, Ca content decreased in hypocotyl and roots. Shaddad (1990) reported lowering of Ca element in Raphanus sativus plants with increase in NaCl salinity which was counteracted by proline spraying at low and moderate salinity. Cramer et al. (1991) reported reduced Ca uptake in Hordeum vulgare L.(M72) when treated with NaCl or KCl (125 mM) with or without supplemented with Ca and suggested that supplemental Ca increased Ca concentration which was positively correlated with growth and total sum of cations concentration was negatively correlated with growth. Ashraf and Naqvi (1991) observed decrease in Ca concentration under saline medium in 3 grass sp. Cenchrus pennisetiformis Hochst and steud, Leptochloa fusca L. Kunth and Panicum turgidum Forssk., which was assessed after 7 weeks of growth in sand culture. Abbas et al. (1991) reported decrease in Ca concentration with increasing salinity levels in Phaseolus vulgaris leaves. This effect was reversed when medium was desalinized. El-Samad and Abd (1993) reported decrease in Ca content in Triticum vulgaris L. under NaCl salinity. This effect of NaCl was ameliorated by irrigating the soil with $CaCl_2$ or KCl resulting in increase in Ca content. Gouia et al. (1994) observed decrease in Ca cations in bean plants at 50 mM NaCl. He Tie et al. (1994) have reported that deposition rates of Ca was reduced throughout growth zone at 150 mM NaCl supplemented with $CaCl_2$ in Gossypium hirsutum L.Cv. Acala SJ-2.Huang and Redmann (1995) observed that cultivated barley had lower Ca content than wild barley under saline conditions. Lopez and satti (1996) found reduction in calcium concentration when 5 cultivars of tomato

were subjected to 50 mM NaCl. Khan et al. (1997) reported decrease in calcium concentration under NaCl stress (0-200 mM) in rice cultivars.

Khan and Ashraf (1988) reported increase in Ca with decrease in NaCl in all 4 varieties of Sorghum. Lynch et al. (1987) observed elevation in cytoplasmic Ca activity at high concentration of NaCl in Zea mays L. Cv. Pioneer 3377. Shitole and Shinde (1991) found that salt stress caused accumulation of calcium in all parts of papaya plant.Tirmizi et al. (1991) reported enhanced Ca uptake with increasing NaCl concentration in leaves of Hippophae rhamnoides L. Lu and Wang (1993) observed high Ca content in roots cultured in Hoagland solution containing NaCl supplemented with $CaCl_2$ and relative efflux of Ca increased slightly. They concluded that Ca may regulate absorption of inorganic ions, a possible mechanism by which Ca enhanced resistance of plants under salt stress. Hamada and Enany (1994) found increased Ca concentration in shoot and root with increasing salinization in broad bean. Ayala and O'leary (1995) showed that calcium concentrations were high in shoots and roots at NaCl 5.0 mol m^{-3} when Salicornia bigelovii Torr. plants were grown at 5.0, 200.0 or 600.0 mol m^{-3}. However, 600 $molm^{-3}$ NaCl caused calcium deficiencies in plants. Morabito et al. (1996) reported increase in Ca content in Clone 42 than in Clone 43 of Eucalyptus microtheca at 200 mM NaCl. Abd-El-Samad and Shaddad (1997) have observed tolerance of soyabean cultivars Cv. Clark to osmotic potential in soil (-1800 kPa) and Cv. Forest to osmotic potential in soil (-1500 kPa) was due to accumulation of Ca content in plants.. According to Venkatesan et al. (1997) Ca content in tissues of Ipomoea pes-Caprae sweet increased upto 200 mM NaCl. Garg et al. (1997) reported that supplemental Ca (2.5 mM and 5.0 mM) ameliorated adverse effects of NaCl by increasing calcium uptake in Cluster bean (Cyamopsis tetragonoloba Taub) which indicated that Ca antagonized toxic effects of NaCl. According to Yuncai and

Schmidhalter (1998) Ca concentration (mmol.kg^{-1}.fresh weight.h^{-1}) was high at 120 mM than at 0 mM NaCl along leaf axis from leaf base of wheat and local net deposition rates of Cl (mmol. Kg^{-1} fresh weight.h^{-1}) in actively elongating zone were enhanced by 120 mM NaCl. However, high concentration of Cl in tissue cause ion imbalance but did not result in ion toxicity in growing leaves.

Banuls and Millo (1992) reported that calcium (as calcium acetate) increased growth, stomatal conductance and photosynthesis under salt stress in Citrus sinensis [L] Osbeck Cv. Hamlin. Hamada and Enany (1994) observed that calcium content remained unaffected by salinity in pea plants. Lin and Kao (1995) found that high levels of salinity (50mM NaCl) inhibited growth of rice roots. Root growth was improved by addition of $CaCl_2$. The inhibition was counteracted by induction of NaCl via decrease in root Na levels. Ashraf and Fatima (1995) reported no difference in calcium concentration in salt tolerance and salt-sensitive safflower at 0, 70, 140 and 120 molm^{-3} NaCl. Bernstein et al. (1995) investigated that Ca caused leaf growth inhibition in Sorghum bicolor [L] Moench. Cv. 'NK 265' leaves at 1 or patter 100 mol m^{-3} NaCl salinity. However, in variety Bhima of safflower (Tables. 18,20) calcium uptake was stimulated under chloride and sulphate salinizations, which indicates that this plant has an ability to uptake more Ca under saline conditions.

G) PHOSPHORUS

Reduction in P content under sulphate salinization was reported by Strogonov (1964), Taha et al (1972), Heikal et al (1980), Dravid and Goswami (1987) in chickpea, Treeby et al (1988) in lupine roots and Dravid and Goswami (1988) in rice. Abdel-Rahman (1987), observed decrease in leaf P content in cowpea calabrease and red radish. Shitole and Shinde (1991) observed reduction in P uptake under chloride and sulphate

salinity's . El-Samad and Abd (1993) reported decrease in P content in Triticum vulgaris L. under NaCl salinity. This effect of NaCl was ameliorated by irrigating the soil with $CaCl_2$ or KCl resulting in increase in P content which might explain its role in osmotic adjustment. El-Samad and Abd (1995) found that salinity affected P content in Cucumis sativus plants. Spraying the shoot system with sodium pyruvate ameliorated adverse effects of NaCl salinity. Savvas and Lenz (1996) observed that NaCl (20, 40, 60 mM NaCl) reduced P in roots and older petioles of egg plants grown in closed sand culture system for six months. Shukla and Singh (1996) reported marked reduction in P with increase in salinity and sodicity levels in Aegle marmelos correa.

Ferguson and Hedlin (1963) have studied the influence of NaCl and Na_2SO_4 salts on P absorbtion in barley and found that P absorbtion was favoured at lower concentration (upto 6 mm hos cm) of the salts while it was adversely affected at high concentrations. As compared to sulphate salinity, chloride salinity was much effective in retaining the entry of ^{32}P into plant parts (Matukhin and Zhuskovskaya, 1961). Rate of entry of this element into organs of barley and tomato depends on type of salinity in soil. Asana and Kale (1965) have found an increase in P content in wheat while Gates et al. (1966b) have reported 100 % increase in roots of Glycine javanica under saline conditions. Chavan and Karadge (1980) have reported increased P uptake. Bernstein et al. (1974) have stated that increasing levels of P aggravate salt injury in corn and decrease salt tolerance but they could not establish a definite relationship between P content and salt tolerance in corn. A higher increase in P content (100%) was responsible for toxicity and death of Glycine javanica under saline conditions (Gates et al., 1966b). Wilson et al. (1970) has suggested that higher P content was closely related to salt tolerance of Glycine falcata.

Shimose (1972) observed no significant effect of Na_2SO_4 treatment on P contents of barley , wheat and asparagus. Similar observations were made by Matar et al. (1975). Abdel-Rahman (1987), found no effect on P content in leaves of cowpea under NaCl salinity.

Results of the present investigation reveal that P content (Tables. 18,20) was more than the control at all levels of NaCl and Na_2SO_4 indicating that P uptake is stimulated in safflower Cv. Bhima. This fact reflects that this cultivar of safflower has efficient phosphorus uptake mechanism under saline conditions.

H) ZINC

Zinc is found to play role in IAA synthesis from its precursor tryptophan. (Skoog,1940) suggested that zinc prevented oxidation of auxin, so through tryptophan synthesis, zinc affects auxin levels. Zinc is essential for the activity of many enzymes like pyridine, nuleotide dehydrogenases, alcohol dehydrogenase, glucose-6-phosphate and trios phosphate dehydogenases. Zn is involved in binding NAD to enzyme protein. It is required by the enzyme phosphodiesterase. The enzyme carbonic anhydrase requires Zn for maximal activity. Zinc acts as a metal activator of this enzyme. Zinc induces de novo synthesis of cytochrome. It participates in chlorophyll formation and prevents chlorophyll destruction.

Early reports indicated both positive and adverse effects on zinc uptake in plants under saline conditions. A positive correlation between soil salinity and zinc was reported by Hassan et al. (1970) in the leaves and stems of corn and barley. An increase in Zn

content under salt stress was reported by Wallace et al. (1980) in bush bean plant; D'Arrigo et al. (1983) in bean; El-Sherbieny et al. (1986), in medium salinity levels in shoot and spike 4 of wheat cultivars. Low zinc content was evident in the experiments of Bhatti and Sarwar (1977); Sonoda and Hara (1981), in bean; Pakroo and Kashirad (1981) in sunflower under chloride-salinity; Patil and Patil (1983), in Jamun; Murumkar and Chavan (1986) and Dravid and Goswami (1987), in chickpea; Dravid and Goswami (1988), in rice reported decrease in zinc uptake under saline conditions.

Cramer et al. (1991) observed increase in Zn over time in Hordeum vulgare L. (M72) when treated for 25 days with NaCl or KCl (125 mM). Tirmizi et al. (1991) reported enhanced Zn uptake with increasing concentration of NaCl in leaves of Hippophae rhamnoides L. According to Venkatesan et al. (1997) Zinc content increases in plants grown upto optimum salt level in Ipomoea pes-caprae sweet plants. Gadallah and Ramadan (1997) reported that high concentration of Zinc in Carthamus tinctorius L. improved growth of roots and enhanced xylem formation in NaCl stressed Carthamus tinctorius plants.

Results of the present investigation indicated that under NaCl treatment in roots (Table.11), Zn is decreased whereas in stem (Table.13) and in leaves (Table.15), Zn content increases with increasing salinity. In crown leaves (Table.15), its content decreased. Under sulphate salinity, however, in roots, low levels stimulated while high levels (Table.12) decreased Zn content. In stem and leaves (Tables.14,16) Zn content increased with increasing levels of salinity in Carthamus tinctorius Cv. Bhima. Thus,all levels of both salinity's stimulate Zn uptake in this cultivar of safflower.

I) IRON

Iron is a micro nutrient or element essential in enzyme systems where it performs haem or haemin functions as prosthetic groups. It is involved in photo oxidation reduction reactions via ferrodoxin and in chlorophyll synthesis (Spiller and Terry, 1980). Ferrodoxin contains 10 atoms of Iron per molecule of which 8 are probably attached to sulpha hydryl groups in the ferrous form. There are evidences for the involvement of ferrous ion in the condensation of succinic acid and glycine to form 8-amino-laevulinic acid (ALA) which then condenses to form pyrrole groups. Ferrodoxin is also involved in the reduction of nitrite to ammonia (Losada et al., 1963) and in nitrogen fixation by bacteria. An important enzyme system in nitrogen fixation, denitrogenase contains a co-factor composed of molybdenum, iron and sulphur in 1:8:6 proportion (Shah and Brill, 1977). Another component of nitrogenase, di-nitrogenase reductase contains four iron atoms and four acid labile sulphur atoms per molecule (Emerich and Evans, 1980). There are reports of both iron chlorosis as well as iron toxicity in crop plants raised under different field conditions (Tanaka and Yoshida, 1970; Kanwar and Randhawa, 1967; Mehrotra et al.,1971). Agarwala et al. (1965) determined the threshold values of iron deficiency in culture solutions varying from 0.6 to 1.12 ppm, while for plants the range is between 35 to 150 ppm. For different crops the optimum requirement of Fe varied from 52 to 300 ppm.

Fe is found to be associated with porphyrins in the form of cytochromes, which are necessary for the electron transport system in mitochondria as well as chloroplasts. Fe is a component of ferrodoxin which is required for light reactions of photosynthesis. Fe

is a constituent of the enzymes catalase and peroxidase which involve molecular oxygen directly in oxidation reduction reactions. Fe is essential for chlorophyll synthesis but there the effect is uncertain. Ferrous form of iron is required for the aconitase reaction in TCA cycle. Ferric form of iron is required for amylase synthesis in GA treated barley aleuron layer. Iron is identified as a component of various flavoproteins active in biological oxidations. Fe is a constituent of leghaemoglobin which is found in roots.

Several scientists (Sarin, 1961a,b; Strogonov, 1964; Rehman et al., 1972; Shimose, 1972; Deshpande, 1981) reported decrease in Fe content under saline conditions. Medium levels of salinity resulted in highest content of Fe while higher levels of salinity, decreased Fe content of shoots of Sankha 8, Sonalika, Sakha 3 and Soltane cultivars of wheat (El-Sherbiery et al., 1986).

On the contrary, increased iron content have been observed by Mass et al. (1972) in the roots and tops of tomato and soybean; Dahiya and Singh (1976) in pea; Pandey and Khanna (1979) in beans; Karadge and Chavan (1980) in peanut; Nigwekar and Chavan (1987) in Dolichos biflorus and El-Sherbieny et al. (1986) in wheat cultivars under saline conditions. Karadge and Chavan (1983) reported accumulation of Fe in salt stessed roots of Sesbania aculeata at 10 $mScm^{-1}$ and 15 $mScm^{-1}$ discussing salt tolerance capacity of plant. Cramer et al. (1991) observed increase in Fe in Hordeum vulgare L. (M72) grown for 29 days and treated with NaCl or KCl (125mM). Tirmizi et al. (1991) have found enhanced Fe uptake with increasing concentration in leaves of Hippophae rhamnoides L. According to Venkatesan et al. (!997) increase in Fe content was reported upto optimum salt level in Ipomoea pes-caprae sweet plants.

Pitman (1972) has speculated that iron uptake and transport is mediated through two active stages in the shoot of barley seedlings. Increase in Fe content under saline conditions may be due to abrupt changes in membrane permeability. Niazi and Ahmed (1984) reported that uptake of Fe ions in leaves remained unaffected in tomato plants.

Results of the present investigation (Tables.11-16 ; Fig.7i-12i) revealed that Fe uptake is stimulated at all levels of both the salts in Carthamus tinctorius L. Var. Bhima. This stimulation of Fe uptake may be due to abrupt changes in membrane permeability under saline conditions as suggested by Pitman (1972). Results also indicated that Fe is not much stored in roots but it is translocated to stem. Within the stem Fe is more retained in lower and middle part under both the salinizations indicating the presence of some regulatory mechanism within the stem.

J) MANGANESE

Manganese is involved in oxidation reduction, decarboxylation and hydrolysis as a cofactor. It is associated with photosynthesis, respiration, oxidation of carbohydrates, IAA and activation of enzymes of nitrogen metabolism.

Inhibition of Mn uptake under saline conditions was reported by Shimose (1973) in barley; Dahiya and Singh (1976) in pea; and Deshpande (1981) in pigeonpea. El-Sherbieny et al. (1986) have observed that medium concentration of NaCl stimulated Mn uptake while high concentration inhibited the same. Also no characteristic relationship could be observed between salt tolerance capacity of wheat cultivars and Mn content under saline conditions. According to Mass et al. (1972) the change in Mn uptake can be explained by some abrupt change in membrane permeability permitting

an increased diffusive influx and sequestering of metal ions by the roots due to salt treatment. Decreased Mn uptake at all levels of chloride and sulphate salinization must have imbalanced oxidation, reduction, decarboxylation and hydrolysis, photosynthesis, respiration, oxidation of carbohydrates and IAA and activities of enzymes of nitrogen metabolism (Bidwell, 1979). Cramer et al. (1991) grew Hordeum vulgare L. (M72) for 25 days and treated with NaCl or KCl (125 mM) and reported decline in Mn concentration over time in shoot below 50 mmol/g fresh weight.

Stimulation of Mn uptake under saline conditions was reported by Mass et al. (1972) in tomato and soybean; Pandey and Khanna (1979) in bean; Chavan and Karadge (1980), in peanut; D'Arrigo et al. (1983) in Phaseolus vulgaris; Martinez et al. (1987), in cucumber and Bhandari (1988) in chilli. Venkatesan et al. (1997) found increase in Mn content upto optimum salt level in Ipomoea pes-caprae sweet plants.Naizi and Ahmed (1984) observed that Mn content of stem, leaves and fruits of tomato under NaCl salinity treatments remained unaffected.

Results of present investigation (Tables.11-16 ; Fig. 7j -12j) indicated that Mn content was enhanced in roots whereas, in stem and leaves, it was decreased under chloride salinization. However, under sulphate salinization in roots and stem low concentrations enhanced while high concentrations reduced Mn content. In leaves and crown leaves, Mn content of total plant decreased with increasing levels of chloride salinity, while its content was increased at low levels of sulphate and decreased at high levels of sulphate and at all levels of chloride. This fact suggests that all levels of chloride and high levels of sulphate inhibit Mn uptake while low levels of sulphate stimulate Mn uptake.

CHAPTER V

ORGANIC METABOLISM UNDER SALINE CONDITIONS

1) INTRODUCTION

Growth parameters are phenotypical indicators of salt tolerance. It can be stated that salt induced changes in the growth pattern which are manifestations of the metabolic disorders caused due to salinity stress bring about quantitative and qualitative changes in organic constituents of the plants.

Salt rich environments affect organic constituents quantitatively and qualitatively. However, quantitative changes induced by salinity have been proved to be protective in function like proline accumulation under adverse conditions of salt stress (Stogonov, 1964). Also, accumulation of toxic intermediates of metabolism in salt stressed plants can cause growth inhibition. Synthesis of organic molecules like proline, sugars and glycine betaine are leading osmoregulators. The accumulation of putative compatible solutes may be due to metabolic perturbations or slower growth resulting from osmotic stress, rather than being an adaptation to it (Jones et al., 1980). Hence, the designation of compounds as compatible solutes is often speculative (Greenway and Munns, 1980). Growth potential of any plant in the prevalent environment is a sum total of the physiological mechanism, taking place in sequence, that is assimilation, translocation and utilisation. It has been hypothesised by Nieman and Mass (1978) that the production and use of energy by salt affected plants is closely correlated with salt tolerance. Earlier literature indicated that very little is known about the mechanism of salt tolerance in safflower. Hence, in the present investigation , an attempt to study productivity,chlorophyll content, carbohydrate content, organic acids (Titratable Acid Number), proline and protein content , under control and saline conditions in safflower has been made.

2) MATERIALS AND METHODS

Plants were grown in pots and treated with NaCl and Na_2SO_4 salts (as given in Chapter II). Quantitative estimation of organic constituents like chlorophylls was done by Arnon's (1949) method; total organic acids (TAN) by Thomas and Beevers (1949); proline by Bates et al. (1973); carbohydrates (reducing, non-reducing sugars, starch) by Nelson's (1944) method and proteins by modified Lowry's method given by Miller (1959).

For chlorophylls and TAN, fifth fully expanded leaves were selected at the time of flowering. Proline was estimated from all the organs by using fresh material at the time of flowering and values were converted on dry mass basis. Carbohydrate and protein were estimated from fifth fully expanded leaf, root and stem parts, which were dried at 80° C in oven till constant weight (Sestak, 1971).The experiments were run in triplicate.

3) RESULTS

A) CHLOROPHYLLS

Results of chlorophyll content are given in Tables.21,22;Fig.13,14. From the observations, it is clear that chlorophyll a content increased at all levels of NaCl and Na_2SO_4. Its content was maximum at ECe 7.5 $mScm^{-1}$ of NaCl and of Na_2SO_4. Similarly chlorophyll b content was more than that the control at all levels of both the salts. It was maximum at ECe 5.0 $mScm^{-1}$ of NaCl and of Na_2SO_4. Thus, all levels of both the salts stimulate biosynthesis of chlorophylls a and b in Carthamus tinctorius L. Cv. Bhima.

The ratio of chlorophyll a to chlorophyll b was more than the control at all levels of chloride and sulphate salinity's except at ECe 5.0 $mScm^{-1}$ of Na_2SO_4 where it was less than the control. The ratio was highest at ECe 7.5 $mScm^{-1}$ of chloride and at ECe 10.0

Table. 21.

Effect of increasing concentrations of NaCl on total chlorophyll content in leaves of

Carthamus tinctorius L. Var. Bhima

Treatment ECe $mScm^{-1}$	Chlorophyll a	Chlorophyll b	Chlorophyll a / b	% of Control a / b	Chlorophyll (a + b)	% of Control (a + b)
Control 0.44	48.75	50.42	0.96	100.00	99.17	100.0
NaCl 5.0	111.73	86.67	1.28	133.30	198.40	200.0
NaCl 7.5	141.62	77.67	1.82	188.50	219.29	221.1
NaCl 10.0*	82.42	73.27	1.12	116.30	155.69	156.9
NaCl 12.5**	-	-	-	-	-	-
NaCl 15.0	-	-	-	-	-	-
SEM	0.341	14.19				
LSD	1.053	43.73				
SD±	±35.97	±25.84				

Results are expressed in g/100g fresh weight

* Plants died after 90 days

** Plants died before flowering

Table. 22.

Effect of increasing concentrations of Na_2SO_4 on total chlorophyll content in leaves of

Carthamus tinctorius L. Var. Bhima

Treatment ECe $mScm^{-1}$	Chlorophyll a	Chlorophyll b	Chlorophyll a / b	% of Control a / b	Chlorophyll (a + b)	% of Control (a + b)
Control 0.44	48.75	50.42	0.96	100.00	99.17	100.00
Na_2SO_4 5.0	64.61	117.02	0.55	57.09	181.63	183.15
Na_2SO_4 7.5	121.19	97.04	1.24	129.13	218.23	220.05
Na_2SO_4 10.0	87.54	68.62	1.27	131.91	156.16	157.46
Na_2SO_4 12.5*	75.44	63.77	1.18	122.32	139.21	140.37
Na_2SO_4 15.0 **	-	-	-	-	-	-
SEM	2.95	0.17				
LSD	9.09	0.54				
SD±	±25.35	±25.03				

Results are expressed in g/100g fresh weight

* Plants died after 90 days

** Plants died before flowering

Fig. 13.

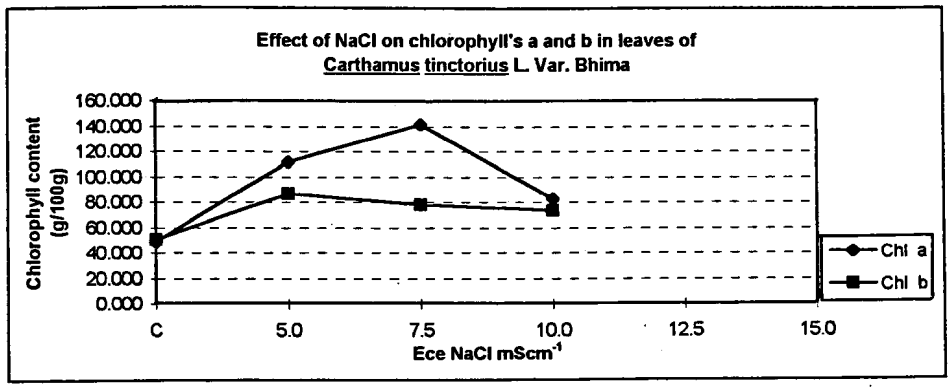
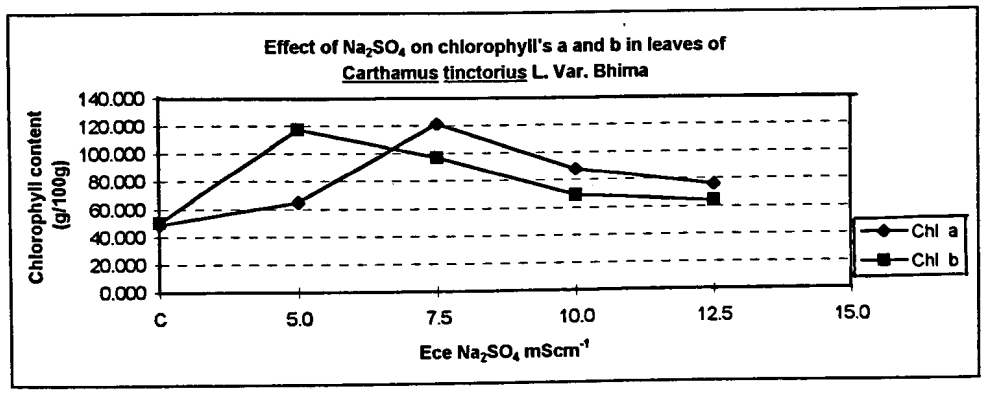

Effect of NaCl on chlorophyll's a and b in leaves of
Carthamus tinctorius L. Var. Bhima
Chlorophyll content (g/100g)
160.000
140.000
120.000
100.000
80.000
60.000
40.000
20.000
0.000
C
5.0
7.5
10.0
12.5
15.0
Ece NaCl mScm-1
Chl a
Chl b

Fig.14.


Effect of Na2SO4 on chlorophyll's a and b in leaves of
Carthamus tinctorius L. Var. Bhima
Chlorophyll content (g/100g)
140.000
120.000
100.000
80.000
60.000
40.000
20.000
0.000
C
5.0
7.5
10.0
12.5
15.0
Ece Na2SO4 mScm-1
Chl a
Chl b

$mScm^{-1}$ of sulphate. This indicates that all levels of chloride and sulphate stimulate chlorophyll a biosynthesis than chlorophyll b biosynthesis except at ECe 5.0 $mScm^{-1}$ of Na_2SO_4 where biosynthesis of chlorophyll b was more stimulated than biosynthesis of chlorophyll a. Thus, effect of low and high concentrations of Na_2SO_4 are different on chlorophyll a and chlorophyll b biosynthesis. The total chlorophylls were also observed more than the control at all levels of NaCl and Na_2SO_4 salinity's and maximum total chlorophyll's were recorded at ECe 7.5 $mScm^{-1}$ of NaCl and Na_2SO_4. This indicates that all the levels of chloride and sulphate salinity's stimulate chlorophyll synthesis in Carthamus tinctorius L. Var. Bhima.

B) TAN

Results of total organic acids (TAN) are depicted in Tables. 23,24; Fig.15,16.It is evident from the results presented in the Table. 23 ; Fig. 15, that in the morning at 0600 hours, in the afternoon at 1200 hours and in the evening at 1800 hours, TAN values decreased with increasing levels of NaCl. However, under sulphate salinizations, TAN values increased at low levels and decreased at higher levels.

TAN values under control, as well as under both salinizations, were minimum in the morning at 0600 hours and maximum in the afternoon at 1200 hours and intermediate in the evening at 1800 hours which indicated that there is no crassulacean acid metabolism (CAM) under control as well as saline conditions in safflower Cv. Bhima.

C) CARBOHYDRATES

Results of carbohydrate contents in root, stem and leaves of Carthamus tinctorius Cv. Bhima are given in Tables. 25-30 ; Fig. 17-22.

Table. 23.

Effect of increasing concentrations of NaCl on TAN content in leaves of Carthamus tinctorius L. Var. Bhima

	Titratable Acid Number (TAN)		
Treatment ECe $mScm^{-1}$	At 0600 hours	At 1200 hours	At 1800 hours
Control 0.44	69.02	141.60	73.75
NaCl 5.0	61.86	75.50	60.16
NaCl 7.5	60.00	73.29	63.65
NaCl 10.0	57.70	71.00	60.00
NaCl 12.5	-	-	-
NaCl 15.0	-	-	-
SEM	0.7	5.00	0.017
LSD	2.17	15.43	0.055
SD±	± 4.54	± 33.74	±5.84

Table. 24.

Effect of increasing concentrations of Na_2SO_4 on TAN content in leaves of Carthamus tinctorius L. Var. Bhima

	Titratable Acid number (TAN)		
Treatment Ece $mScm^{-1}$	At 0600 hours	At 1200 hours	At 1800 hours
Control 0.44	69.02	141.60	73.75
Na_2SO_4 5.0	77.08	165.26	82.65
Na_2SO_4 7.5	75.29	96.70	75.57
Na_2SO_4 10.0	70.48	90.20	74.87
Na_2SO_4 12.5	65.00	70.36	67.53
Na_2SO_4 15.0	-	-	-
SEM	0.0	0.26	0.09
LSD	0.21	0.8	0.29
SD±	± 4.50	± 36.31	±5.05

Fig. 15.

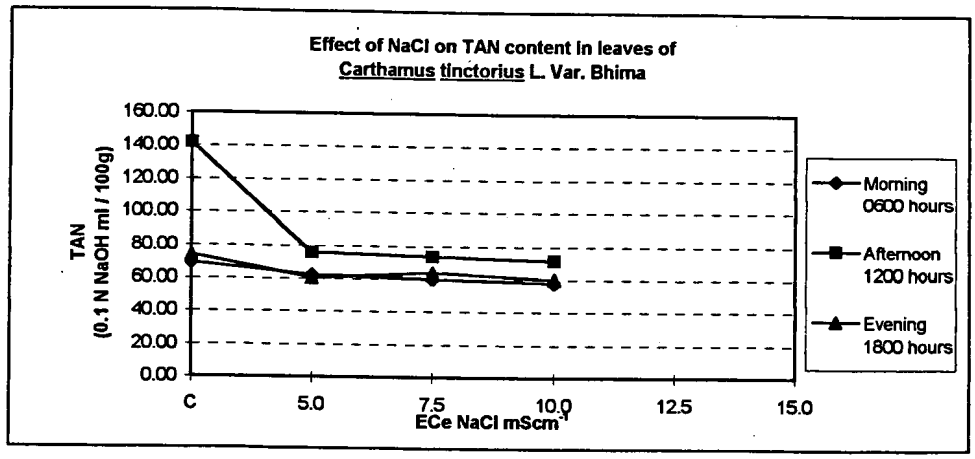

Effect of NaCl on TAN content in leaves of
Carthamus tinctorius L. Var. Bhima
TAN
(0.1 N NaOH ml / 100g)
160.00
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
C
5.0
7.5
10.0
12.5
15.0
ECe NaCl mScm-1
Morning
0600 hours
Afternoon
1200 hours
Evening
1800 hours

Fig. 16.

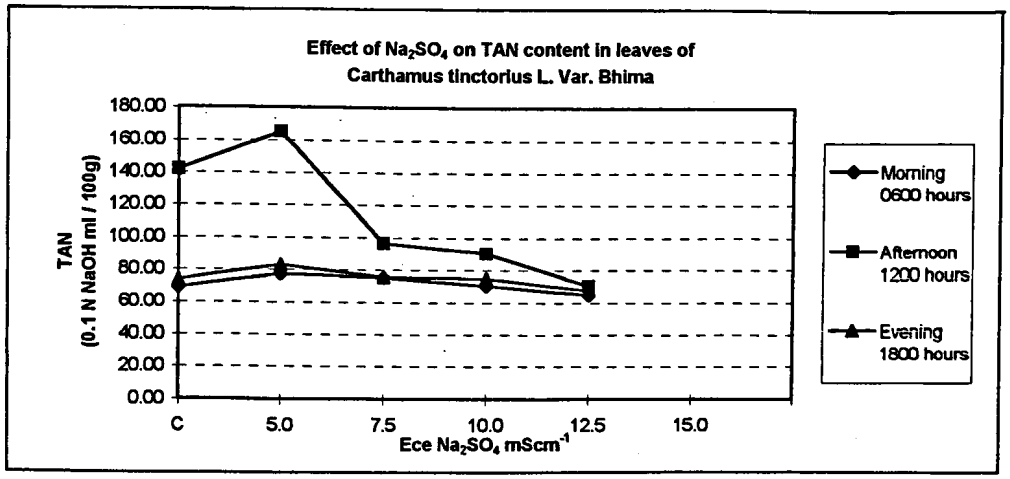

Effect of Na2SO4 on TAN content in leaves of
Carthamus tinctorius L. Var. Bhima
TAN
(0.1 N NaOH ml / 100g)
180.00
160.00
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
C
5.0
7.5
10.0
12.5
15.0
Ece Na2SO4 mScm-1
Morning
0600 hours
Afternoon
1200 hours
Evening
1800 hours

Table. 25.

Effect of increasing concentrations of NaCl on reducing, non-reducing sugars and starch content in roots of Carthamus tinctorius L.Var. Bhima

Treatment Ece $mScm^{-1}$	Reducing sugars	Non-reducing sugars	Starch	Total carbohydrates
Control 0.44	105.00	71.50	626.00	802.50
NaCl 5.0	223.00	113.80	531.00	867.80
NaCl 7.5	95.70	105.00	426.80	627.50
NaCl 10.0	71.40	103.00	328.00	502.40
NaCl 12.5*	24.00	10.00	301.20	335.20
NaCl 15.0*	12.30	9.20	200.20	221.70
SEM	1.06	0.97	10.03	
LSD	3.29	3.00	30.92	
SD±	± 71.13	±45.10	± 146.7	

Results are expressed in mg /100 g

*Estimated from dead plant parts

Table. 26.

Effect of increasing concentrations of Na_2SO_4 on reducing, non-reducing sugars and starch content in roots of Carthamus tinctorius L. Var. Bhima

Treatment Ece $mScm^{-1}$	Reducing sugars	Non-reducing sugars	Starch	Total carbohydrates
Control 0.44	105.00	71.50	626.00	802.50
Na_2SO_4 5.0	109.60	168.00	688.00	965.60
Na_2SO_4 7.5	203.30	158.20	699.00	1060.50
Na_2SO_4 10.0	142.90	138.20	728.00	1009.10
Na_2SO_4 12.5	134.60	155.50	752.00	1042.10
Na_2SO_4 15.0*	134.00	152.80	786.00	1072.80
SEM	0.64	1.03	1.23	
LSD	1.98	3.17	3.79	
SD±	±33.17	±46.27	± 52.18	

Results are expressed in mg / 100 g

*Estimated from dead plant parts

Fig. 17.

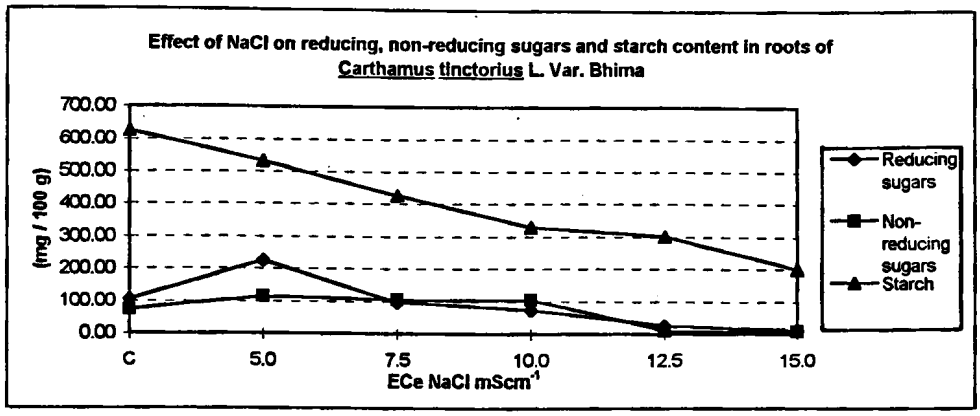

Effect of NaCl on reducing, non-reducing sugars and starch content in roots of Carthamus tinctorius L. Var. Bhima
700.00
600.00
500.00
400.00
300.00
200.00
100.00
0.00
(mg / 100 g)
C
5.0
7.5
10.0
12.5
15.0
ECe NaCl mScm-1
Reducing sugars
Non-reducing sugars
Starch

Fig. 18.

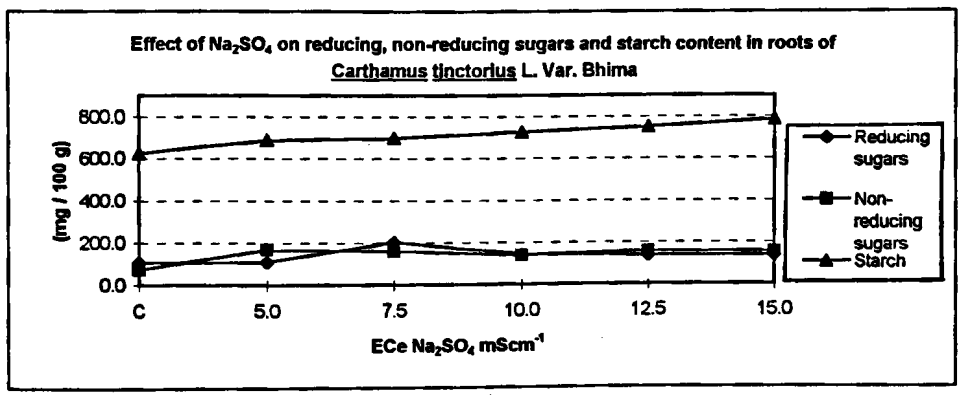

Effect of Na2SO4 on reducing, non-reducing sugars and starch content in roots of Carthamus tinctorius L. Var. Bhima
800.0
600.0
400.0
200.0
0.0
(mg / 100 g)
C
5.0
7.5
10.0
12.5
15.0
ECe Na2SO4 mScm-1
Reducing sugars
Non-reducing sugars
Starch

Table. 27.

Effect of increasing concentrations of NaCl salinity on Reducing sugars, Non-Reducing sugars and Starch in stem (upper, middle and lower part) in Carthamus tinctorius L. Cv. Bhima

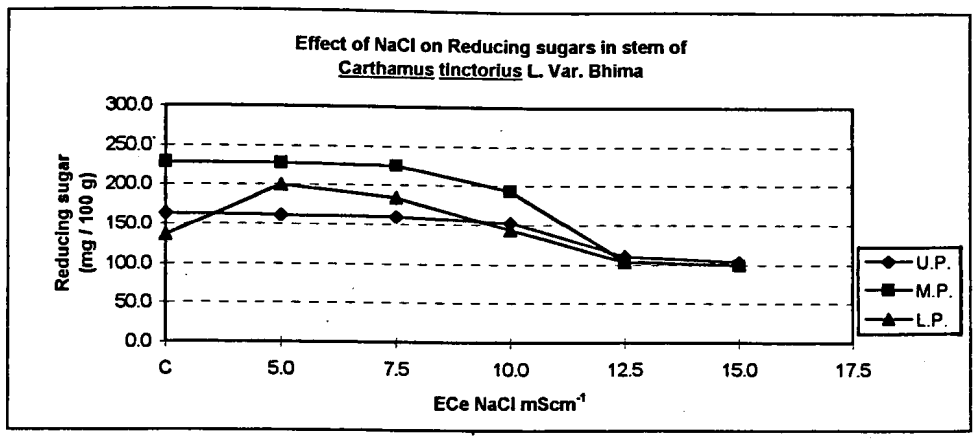
Carbohydrates (mg/100g) Treatment ECe $mScm^{-1}$	RS	RS (TS)	NRS	NRS(TS)	S	S (TS)	TC (RS+NRS+S)	TC(TS)
Control 0.44 (stem U.P.)	163.1		24.6		148.0		335.7	
Control 0.44 (stem M.P.)	228.5		46.2		557.0		831.7	
Control 0.44 (stem L.P.)	136.1	175.9	24.3	31.7	38.4	247.8	406.4	524.6
NaCl 5.0 U.P.	161.5		24.6		398.0		584.1	
NaCl 5.0 M.P.	228.0		54.6		679.0		961.6	
NaCl 5.0 L.P.	199.4	196.3	26.0	35.1	29.6	368.9	486.4	677.4
NaCl 7.5 U.P.	160.0		43.2		436.0		639.2	
NaCl 7.5 M.P.	225.0		74.6		698.0		997.6	
NaCl 7.5 L.P.	184.3	189.8	28.6	48.8	25.9	386.6	477.4	704.7
NaCl 10.0 U.P.	152.5		25.0		476.0		653.5	
NaCl 10.0 M.P.	193.0		96.5		594.0		883.5	
NaCl 10.0 L.P.	143.6	163.0	14.3	45.3	24.2	364.7	390.4	642.5
NaCl 12.5 U.P.*	110.7		22.5		567.0		700.2	
NaCl 12.5 M.P.*	104.0		22.5		555.0		681.5	
NaCl 12.5 L.P.*	103.2	106.0	19.0	21.3	21.8	381.3	271.3	551.0
NaCl 15.0 U.P.*	103.0		19.9		542.0		664.9	
NaCl 15.0 M.P.*	99.0		19.2		538.0		656.2	
NaCl 15.0 L.P.*	100.0	100.7	9.0	16.0	18.3	366.1	244.0	521.7
	SEM	LSD	SEM	LSD	SEM	LSD		
U.P.**	0.74	2.30	0.09	0.29	1.76	5.43		
M.P.**	1.49	4.59	0.10	0.33	0.88	2.71		
L.P.**	0.13	0.40	0.11	0.34	0.08	0.26		

RS - Reducing Sugars NRS - Non reducing Sugars S -Starch TC-Total Carbohydrates TS- Total stem

U.P. - Upper part M.P. - Middle part L.P - Lower part

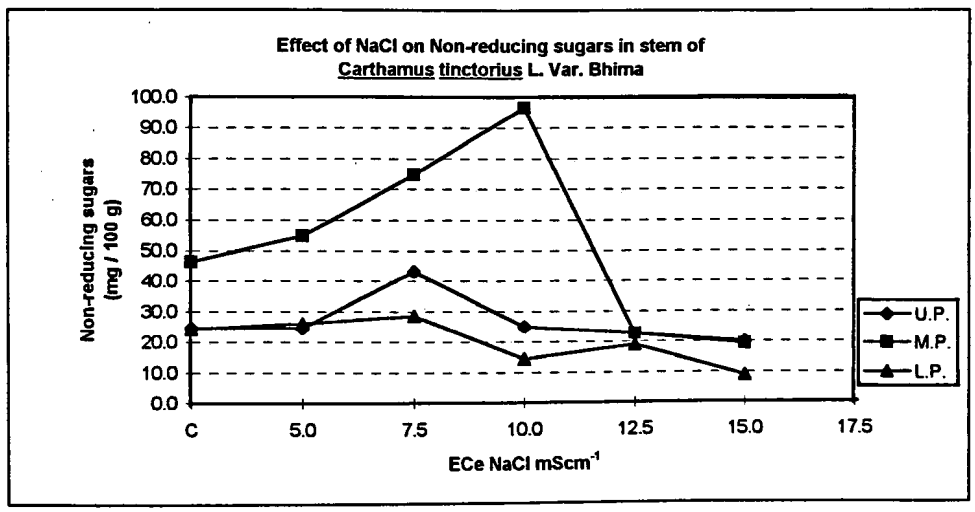
* Dried plant material ** Plants died within three days

Fig. 19a.



U.P. - Upper Part M.P. - Middle Part L.P. - Lower Part

Fig. 19b.



U.P. - Upper Part M.P. - Middle Part L.P. - Lower Part

Table. 28.

Effect of increasing concentrations of Na_2SO_4 salinity on Reducing sugars, Non-Reducing sugars and Starch in stem (upper, middle and lower parts) in Carthamus tinctorius L. Cv. Bhima

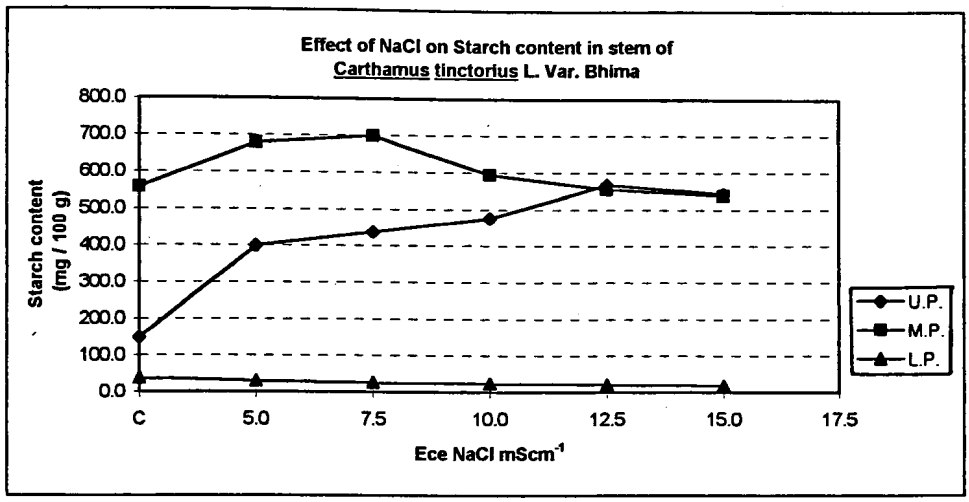
Carbohydrates (mg/100g) Treatment ECe $mScm^{-1}$	RS	RS(TS)	NRS	NRS(TS)	S	S(TS)	TC (RS+NRS+S)	TC(TS)
Control 0.44(stem U.P.)	163.1		24.6		148.0		335.7	
Control 0.44(stem M.P.)	228.5		46.2		557.0		831.7	
Control 0.44(stem L.P.)	136.1	175.9	24.3	31.7	38.4	247.8	198.8	455.4
Na_2SO_4 5.0 U.P.	330.0		560.0		228.0		111.8	
Na_2SO_4 5.0 M.P.	300.0		462.0		216.4		978.4	
Na_2SO_4 5.0 L.P.	340.0	323.3	462.0	494.7	271.2	238.5	1073.2	721.1
Na_2SO_4 7.5 U.P.	400.0		580.0		345.0		1325.0	
Na_2SO_4 7.5 M.P.	570.0		625.0		260.2		1455.2	
Na_2SO_4 7.5 L.P.	440.0	470.0	497.0	567.3	291.1	298.8	1228.1	1336.1
Na_2SO_4 10.0 U.P.	660.0		540.0		270.7		1470,7	
Na_2SO_4 10.0 M.P.	460.0		462.0		217.1		1139.1	
Na_2SO_4 10.0 L.P.	400.0	506.7	460.0	487.3	209.2	232.3	1069.2	1226.3
Na_2SO_4 12.5 U.P.	384.0		540.0		256.3		1180.3	
Na_2SO_4 12.5 M.P.	425.0		440.0		220.3		1085.3	
Na_2SO_4 12.5 L.P.	386.0	398.3	430.0	470.0	193.2	223.3	1009.2	1091.6
Na_2SO_4 15.0 U.P.*	350.0		508.0		242.8		1100.8	
Na_2SO_4 15.0 M.P.*	400.0		410.0		145.0		955.0	
Na_2SO_4 15.0 L.P.*	360.0	370.0	412.0	443.3	164.8	184.2	936.8	997.53
	SEM	LSD	SEM	LSD	SEM	LSD		
U.P.**	0.78	2.24	0.76	2.34	1.09	3.38		
M.P.**	0.59	1.84	1.35	4.16	0.31	0.96		
L.P.**	0.70	2.15	1.05	3.25	0.11	0.34		

RS - Reducing Sugars NRS - Non reducing Sugars S -Starch TC-Total Carbohydrates TS- Total stem

U.P. - Upper part M.P. - Middle part L.P - Lower part

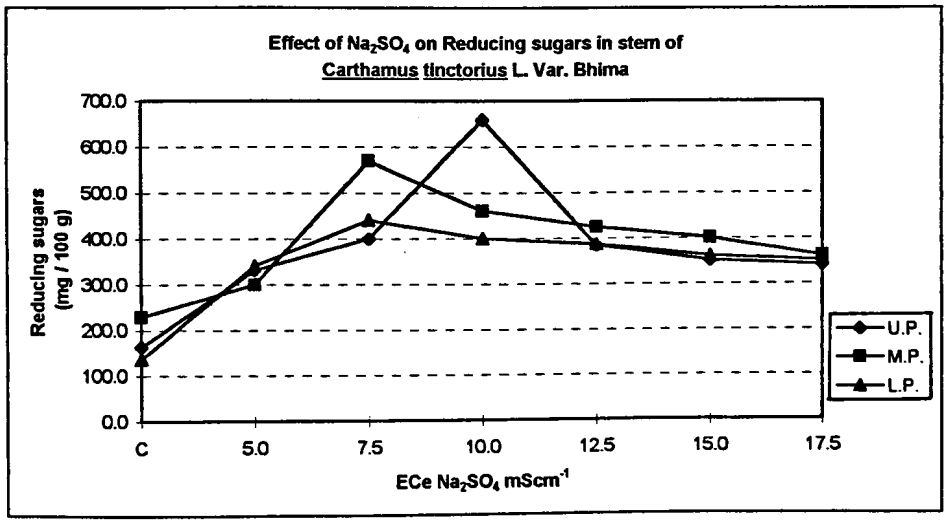
* Dried plant material ** Plants died within three days

Fig. 19c.



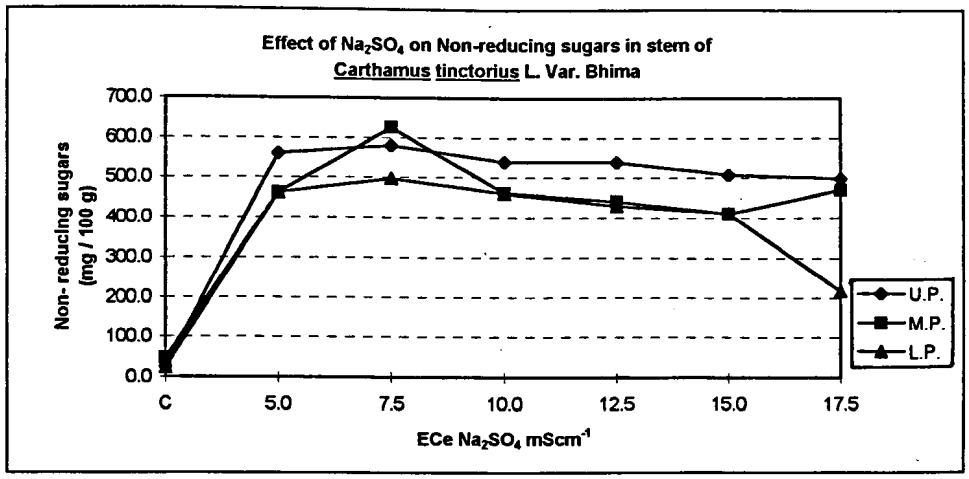
U.P. - Upper Part M.P. - Middle Part L.P. - Lower Part

Fig. 20a.



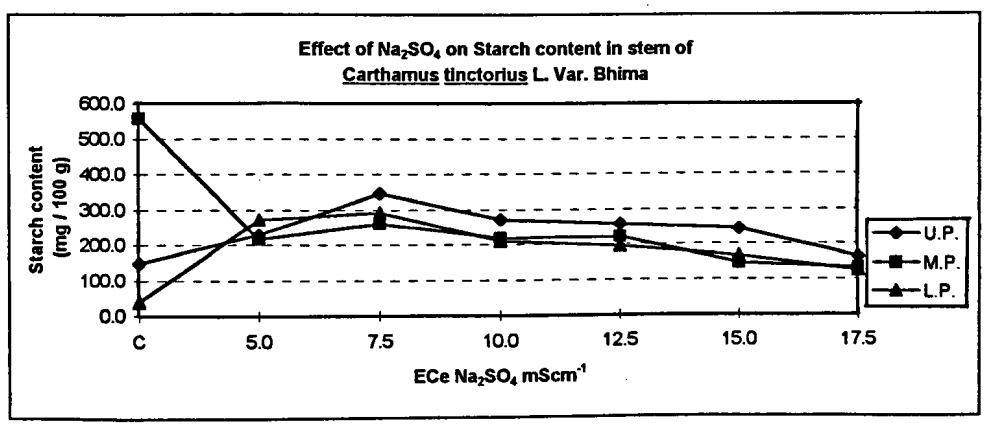
U.P. - Upper Part M.P. - Middle Part L.P. - Lower Part

Fig. 20b.



U.P. - Upper Part M.P. - Middle Part L.P. - Lower Part

Fig. 20c.



U.P. - Upper Part M.P. - Middle Part L.P. - Lower Part

Table. 29.

Effect of increasing concentrations of NaCl on reducing, non-reducing sugars and starch content in leaves of Carthamus tinctorius L. Var. Bhima

Treatment Ece $mScm^{-1}$	Reducing sugars	Non-reducing sugars	Starch	Total carbohydrates
Control 0.44	4.62	27.00	276.80	308.42
NaCl 5.0	5.92	50.00	261.00	316.92
NaCl 7.5	6.58	90.20	230.30	327.08
NaCl 10.0	7.68	131.00	217.10	355.82
NaCl 12.5*	7.58	52.80	100.00	160.38
NaCl 15.0*	7.70	43.80	94.40	145.90
SEM	0.02	0.67	0.07	
LSD	0.08	2.08	0.23	
SD±	±1.16	± 35.8	± 75.05	

Results are expressed in mg / 100 g

* Estimated from dried plant material

Table. 30.

Effect of increasing concentration of Na_2SO_4 on reducing, non-reducing sugars and starch content in leaves of Carthamus tinctorius L. Var. Bhima

Treatment Ece $mScm^{-1}$	Reducing sugars	Non-reducing sugars	Starch	Total carbohydrates
Control 0.44	4.62	27.00	276.80	308.42
Na_2SO_4 5.0	14.3	19.04	525.40	558.74
Na_2SO_4 7.5	14.6	14.07	484.20	512.87
Na_2SO_4 10.0	13.3	14.00	405.80	433.10
Na_2SO_4 12.5	12.1	8.90	304.40	325.40
Na_2SO_4 15.0*	11.1	0.93	275.60	287.63
SEM	0.08	0.33	0.08	
LSD	0.25	1.03	0.25	
SD±	±3.48	± 8.32	± 102.78	

Results are expressed in mg / 100 g

* Estimated from dried plant material

Fig. 21.

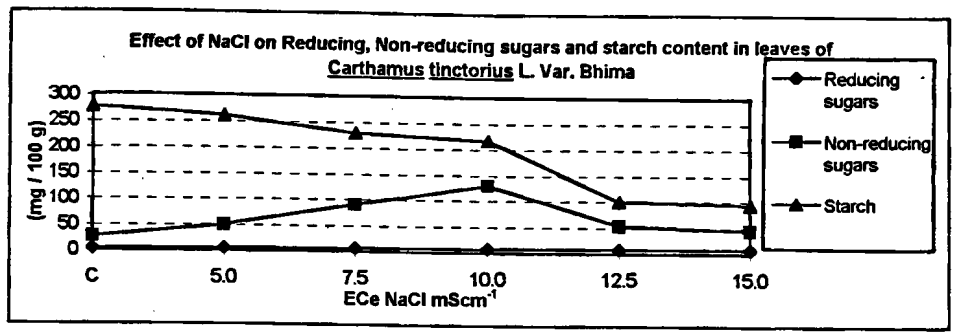
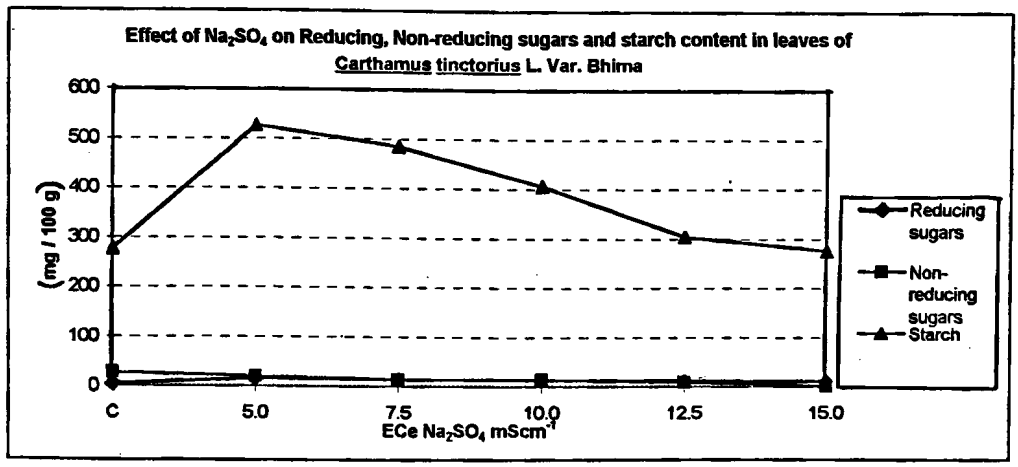

Effect of NaCl on Reducing, Non-reducing sugars and starch content in leaves of
Carthamus tinctorius L. Var. Bhima
(mg / 100 g)
300
250
200
150
100
50
0
C
5.0
7.5
10.0
12.5
15.0
ECe NaCl mScm-1
Reducing sugars
Non-reducing sugars
Starch

Fig. 22.


Effect of Na2SO4 on Reducing, Non-reducing sugars and starch content in leaves of
Carthamus tinctorius L. Var. Bhima
(mg / 100 g)
600
500
400
300
200
100
0
C
5.0
7.5
10.0
12.5
15.0
ECe Na2SO4 mScm-1
Reducing sugars
Non-reducing sugars
Starch

I) ROOTS

The reducing sugar content increased only at ECe 5.0 $mScm^{-1}$ of NaCl and decreased at all higher levels (Tables. 25,26 ; Fig. 17,18).Under sulphate treatment,reducing sugar increased over the control at all levels.

The non reducing sugars, increased upto ECe 10.0 $mScm^{-1}$ of NaCl and decreased at all higher levels. However, under Na_2SO_4 treatment, content was more than the control at all levels of salt. Maximum content was recorded at ECe 5.0 $mScm^{-1}$.

The starch content decreased with increasing NaCl treatments and increased with increasing Na_2SO_4 treatment. The total carbohydrate content increased upto ECe 5.0 $mScm^{-1}$ of NaCl and decreased at higher levels of NaCl, while under Na_2SO_4 treatment total carbohydrate content was more than the control at all levels.

II) STEM

The results on stem (upper, middle, lower part) and carbohydrate content are given in Tables. 27,28 ; Fig.19 abc , 20 abc.

In upper part of stem, reducing sugar decreased with increasing NaCl concentrations, whereas, under Na_2SO_4 concentrations, reducing sugar was more than the control at all levels and maximum content was present at ECe 10.0 $mScm^{-1}$ of Na_2SO_4. The non reducing sugars, under NaCl were more than the control upto ECe 10.0 $mScm^{-1}$ and decreased at high (ECe12.5 to 15.0 $mScm^{-1}$) levels. Under sulphate treatment, non reducing content was more than the control at all levels and the maximum content was observed at ECe 7.5 $mScm^{-1}$ of Na_2SO_4.

The starch content was more than the control at all levels of NaCl and Na_2SO_4 treatments. Maximum starch content was present at ECe 12.5 $mScm^{-1}$ NaCl and ECe 7.5 $mScm^{-1}$ Na_2SO_4.

The total carbohydrate content in upper part of stem was more than the control at all levels. Of NaCl and Na_2SO_4 Maximum content was at ECe 10.0 $mScm^{-1}$ of Na_2SO_4.

In middle part of stem, reducing sugar content decreased with increasing NaCl salinity. However, under Na_2SO_4 salinization, content was more than the control at all levels and maximum reducing sugar was present at ECe 7.5 $mScm^{-1}$ of Na_2SO_4

The non reducing sugars increased upto ECe 10.0 $mScm^{-1}$ of NaCl and decreased at all higher levels. However, under Na_2SO_4 treatment, content was more than the control at all levels and maximum content was found at ECe 7.5 $mScm^{-1}$ of Na_2SO_4 indicating that ECe 7.5 $mScm^{-1}$ is optimum for synthesis of non-reducing sugars.

The starch content was increased upto ECe 10.0 $mScm^{-1}$ and decreased with increasing NaCl concentrations and the content was found to be less than the control at all levels of sulphate treatments.

The total carbohydrate content, in middle part of stem, was more than the control upto ECe 10.0 $mScm^{-1}$ and decreased at higher NaCl concentrations. However, under Na_2SO_4 treatments, total carbohydrates were more than the control at all levels and maximum content was found at ECe 7.5 $mScm^{-1}$ which clearly indicates that this salinity level is most optimum for the synthesis of carbohydrates.

In lower part of stem, reducing sugar increased upto ECe 10.0 $mScm^{-1}$ of NaCl and reduced with increasing chloride treatment whereas, the content was more than the control at all levels of sulphate salinizations, with maximum content at ECe 7.5 $mScm^{-1}$ of Na_2SO_4.

The non reducing sugar, increased upto ECe 7.5 $mScm^{-1}$ of NaCl and decreased at higher salinity levels (ECe 10.0 to 15.0 $mScm^{-1}$). Under Na_2SO_4 treatments, content was more than the control at all levels and maximum content was found at ECe 7.5 $mScm^{-1}$ of Na_2SO_4.

The starch content decreased with increasing NaCl concentrations and under Na_2SO_4 its content was more than the control at all the levels and maximum content was found at ECe 7.5 $mScm^{-1}$.

The total carbohydrate content in lower part of stem was more than the control at low (ECe 5.0 and 7.5 $mScm^{-1}$) under NaCl treatment. However, the total carbohydrate content was more than the control at all levels of Na_2SO_4 and the maximum content was at ECe 7.5 $mScm^{-1}$ of Na_2SO_4.

III) LEAVES

The results of reducing sugars, non reducing sugars and starch are depicted in Tables. 29,30 and Fig. 21,22, which indicated that reducing sugar content was more than the control at all levels of NaCl and Na_2SO_4 treatments. Maximum content was at ECe 10.0 $mScm^{-1}$ of NaCl and at 7.5 $mScm^{-1}$ of Na_2SO_4 treatments.

The non reducing sugar was more than the control at all levels of NaCl and maximum content was recorded at ECe 10.0 $mScm^{-1}$. However, under Na_2SO_4 treatment, content decreased with increasing salinity.

The starch content decreased with increasing NaCl concentrations. However, starch content was more than control at all levels of sulphate salinization with maximum content at ECe 5.0 $mScm^{-1}$ which reflects that ECe 5.0 $mScm^{-1}$ of sulphate is optimum level for synthesis of starch.

The average total carbohydrate content of roots, stem and leaves (Tables. 29,30) increased at low (ECe 5.0 to 10.0 $mScm^{-1}$) and decreased at high (ECe 12.5 to 15.0 $mScm^{-1}$) levels of NaCl. Under Na_2SO_4 treatment, the average total carbohydrate content of the total plant was more than the control upto ECe 12.5 $mScm^{-1}$ and less at higher salinity levels. Thus, low levels stimulate while higher levels of both the salts inhibit carbohydrate biosynthesis in Carthamus tinctorius Cv. Bhima.

D) PROLINE

Results of effect of increasing concentrations of sodium chloride and sodium sulphate on proline content in roots, stem and leaves are presented in Tables. 31,32 ; Fig. 23ab,24ab.

I) ROOTS

From the results presented in Tables. 31,32 ; Fig.23a,24a, it is clear that proline content in roots increased linearly with increasing con`centrations of both the salts suggesting

Table. 31.

Effect of increasing concentrations of NaCl on proline content in roots, stem and leaves of Carthamus tinctorius L. Var. Bhima

	Roots	Stem				Leaves	(R+S+L)
Treatment ECe $mScm^{-1}$		U.P.	M.P.	L.P.	Average Stem		
Control 0.44	3.58	2.39	2.61	1.80	2.26	3.17	3.01
NaCl 5.0	4.38	3.01	4.98	1.90	3.29	5.20	4.29
NaCl 7.5	4.50	0.91	4.96	5.03	3.63	6.24	4.79
NaCl 10.0	4.98	0.91	4.27	5.19	3.45	7.66	5.36
NaCl 12.5	5.13	1.43	5.88	5.96	4.42	9.96	6.50
NaCl 15.0	5.72	1.77	6.19	7.50	5.15	11.81	7.56
SEM	0.21	0.009	0.005	0.05	0.01		
LSD	0.65	0.02	0.01	0.16	0.03		
SD±	±0.83	±0.78	± 1.2	±2.13	±0.93		

Results are expressed in mg / g

U.P. - Upper Part M.P. - Middle Part L.P. -Lower Part

Table. 32.

Effect of increasing concentrations of Na_2SO_4 on proline content in roots, stem and leaves of Carthamus tinctorius L.Var. Bhima

	Roots	Stem				Leaves	(R+S+L)
Treatment ECe $mScm^{-1}$		U.P.	M.P.	L.P.	Average Stem		
Control 0.44	3.58	2.39	2.61	1.80	2.26	3.17	3.01
Na_2SO_4 5.0	5.10	3.17	4.66	1.97	3.26	8.30	5.55
Na_2SO_4 7.5	5.40	4.10	3.97	5.50	4.52	8.90	6.27
Na_2SO_4 10.0	6.16	5.24	4.96	5.64	5.28	9.34	6.92
Na_2SO_4 12.5	7.16	3.52	5.16	6.19	4.95	9.48	7.20
Na_2SO_4 15.0	7.34	3.20	6.59	7.81	5.86	9.60	7.60
SEM	0.04	0.08	0.007	0.034	0.08		
LSD	0.14	0.26	0.022	0.10	0.25		
SD±	±1.32	±0.92	±1.24	±2.26	±2.67		

Results are expressed in mg / g

U.P. - Upper Part M.P. - Middle Part L.P. -Lower Part

Fig. 23a.

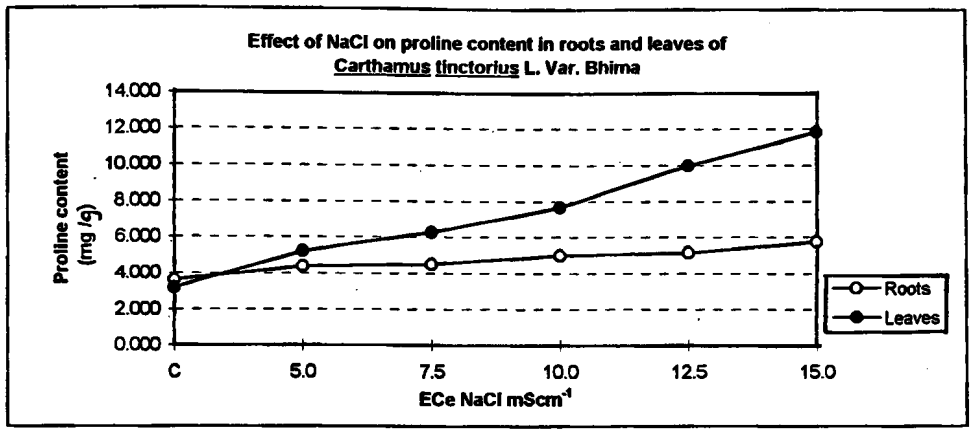
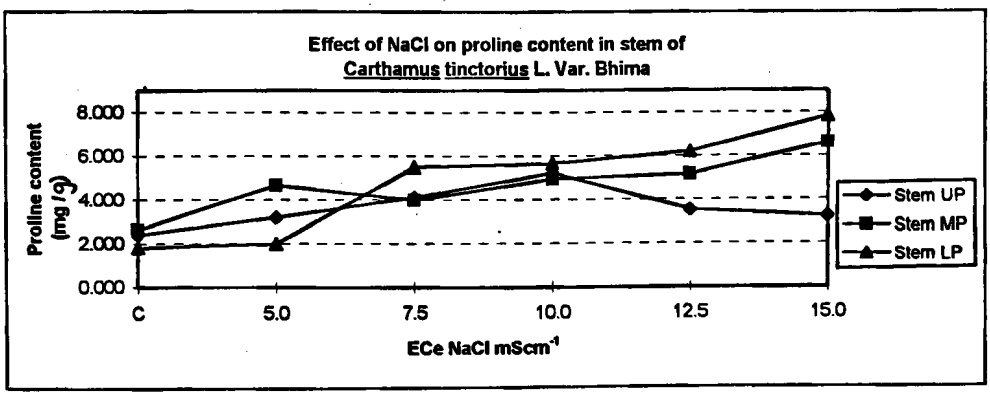


Fig. 23b.



UP -Upper part MP - Middle part LP - Lower part

Fig. 24a.

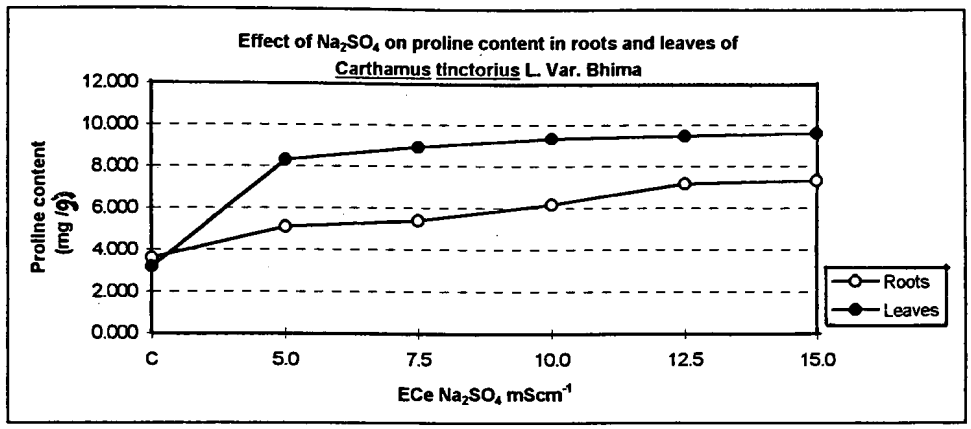
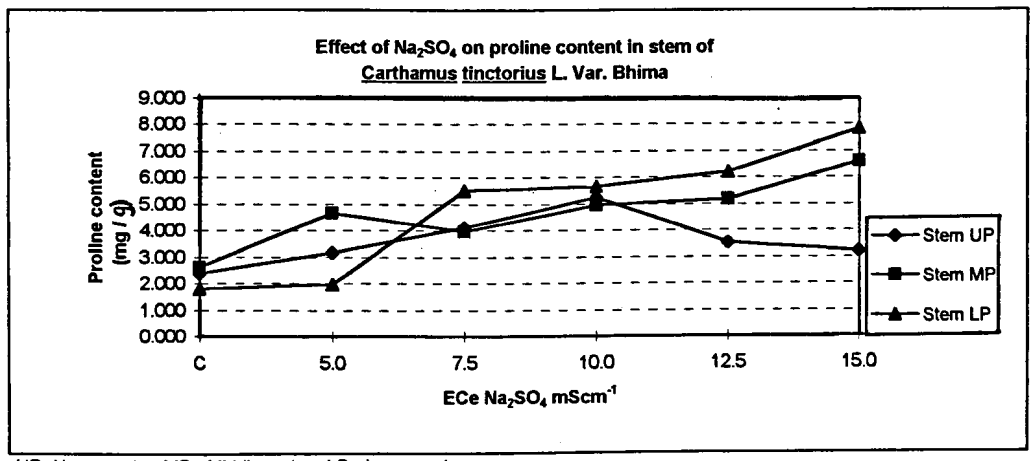


Fig. 24b.



UP -Upper part MP - Middle part LP - Lower part

that proline plays an important role in salt tolerance under both the salts in Carthamus tinctorius Cv. Bhima.

II) STEM

In upper part of stem, accumulation of proline was increased only upto ECe 5 $mScm^{-1}$ of NaCl and at all higher levels content of proline was less than the control(Table.31 ; Fig.23b). Under Na_2SO_4 treatment(Table.32 ;Fig.24b) its content was more than the control in plants grown at all levels of salts. Highest content of proline was recorded at ECe 10.0 $mScm^{-1}$ of Na_2SO_4.

In middle part of stem, proline was found to be accumulated with increasing salinity under NaCl and Na_2SO_4 treatments. In lower part of stem, its content was linearly increased with increase in concentrations of both the salts. As a result, average total proline content of stem was linearly increased with increase in concentrations of both the salts thereby indicating that proline plays significant role in salt tolerance in safflower Cv. Bhima. In control , proline was highest in middle part followed by upper part and it was consistent at ECe 5 $mScm^{-1}$ of both the salts. However, at high ECe 7.5 to 15 $mScm^{-1}$ of both the salts, proline content was highest in lower part, medium in middle part and lowest in the upper part which clearly suggested that pattern of proline accumulation in stem changes under saline conditions.

III) LEAVES

In leaves(Tables.31,32;Figs.23a,24a), proline content increased linearly with increasing concentrations of both the salts thereby indicating that all levels of both the salts stimulate proline biosynthesis in Carthamus tinctorius Cv. Bhima. Average proline content of total plant increased with increasing concentrations of NaCl and Na_2SO_4 ,

which indicated that proline plays significant role for salt tolerance under both the salinizations.

E) PROTEINS

Results of effect of increasing concentrations of NaCl and Na_2SO_4 salinizations on protein content in roots, stem and leaves is depicted in Tables. 33,34 ; Fig. 25ab,26ab.

I) ROOTS

In roots (Tables. 33,34;Fig.25a,26a), protein content decreased with increasing NaCl concentrations while its content increased at low levels (ECe 5.0 and 7.5 $mScm^{-1}$) of Na_2SO_4 and decreased at all higher (ECe 10.0 to 15.0 $mScm^{-1}$) levels of Na_2SO_4. These results suggest that all concentrations of NaCl inhibit protein biosynthesis in roots. Low concentrations of Na_2SO_4 stimulate while high concentrations of it inhibit protein biosynthesis in roots of safflower Cv. Bhima.

II) STEM

In upper, middle and lower parts of stem (Tables. 33,34;Fig.25b,26b), protein content decreased under NaCl salinization, while protein content enhanced at low (5.0 and 7.5 $mScm^{-1}$) levels of Na_2SO_4 and was inhibited at high (10.0 to 15.0 $mScm^{-1}$) concentrations of Na_2SO_4. Thus, it can be concluded that NaCl reduced protein content in all parts of the stem while low concentration of Na_2SO_4 enhanced and high concentrations of it decreased protein content in all parts of the stem. Average total protein content in stem decreased linearly with increase in NaCl concentrations whereas, its content increased at low levels (ECe 5.0 and 7.5 $mScm^{-1}$) of Na_2SO_4 and decreased at all higher concentrations, thereby indicating that all concentrations of

Table. 33.

Effect of increasing concentrations of NaCl on protein content in roots, stem and leaves of Carthamus tinctorius L.Var. Bhima

	Roots	Stem				Leaves	(R+S+L)
Treatment ECe $mScm^{-1}$		U.P.	M.P.	L.P.	Average Stem		
Control 0.44	10.30	21.84	19.00	20.10	20.31	11.48	14.03
NaCl 5.0	8.74	20.70	17.28	19.60	19.19	9.84	12.59
NaCl 7.5	8.04	19.30	16.90	18.20	18.13	7.81	11.33
NaCl 10.0	7.79	17.80	15.90	18.20	17.30	6.92	10.67
NaCl 12.5*	7.69	16.03	15.70	17.96	16.56	6.00	10.08
NaCl 15.0*	7.32	16.02	15.85	15.90	15.92	6.00	9.75
SEM	0.12	0.09	0.001	0.07		0.07	
LSD	0.37	0.29	0.004	0.24		0.21	
SD±	±1.03	±2.26	±1.18	±1.38		±1.56	

Results are expressed in mg / g fresh weight

Estimated from dried plant material

U.P. - Upper Part M.P. - Middle Part L.P. - Lower Part

Table. 34.

Effect of increasing concentrations of Na_2SO_4 on protein content in roots, stem and leaves of Carthamus tinctorius L. Var. Bhima

	Roots	Stem				Leaves	(R+S+L)
Treatment ECe $mScm^{-1}$		U.P.	M.P.	L.P.	Average Stem		
Control 0.44	10.30	21.84	19.00	20.10	20.31	11.48	14.03
Na_2SO_4 5.0	10.98	23.57	20.27	20.96	21.60	10.16	14.25
Na_2SO_4 7.5	11.92	24.2	21.24	20.9	22.11	10.10	14.71
Na_2SO_4 10.0	9.61	21.1	18.2	19.1	19.46	9.86	12.98
Na_2SO_4 12.5	9.25	17.02	15.6	18	16.87	8.72	11.61
Na_2SO_4 15.0*	8.27	16.16	15.13	16.12	15.80	8.00	10.69
SEM	0.12	0.07	0.06	0.06		0.05	
LSD	0.37	0.24	0.19	0.19		0.17	
SD±	±1.23	±3.14	±2.31	±1.76		±2.4	

Results are expressed in mg / g fresh weight

* Estimated from dried plant material

U.P. - Upper Part M.P. - Middle Part L.P. - Lower Part

Fig. 25a.

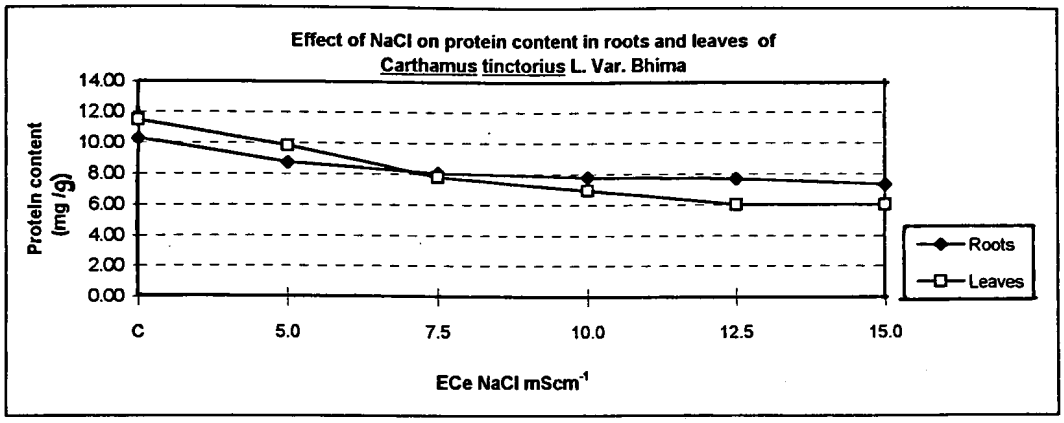
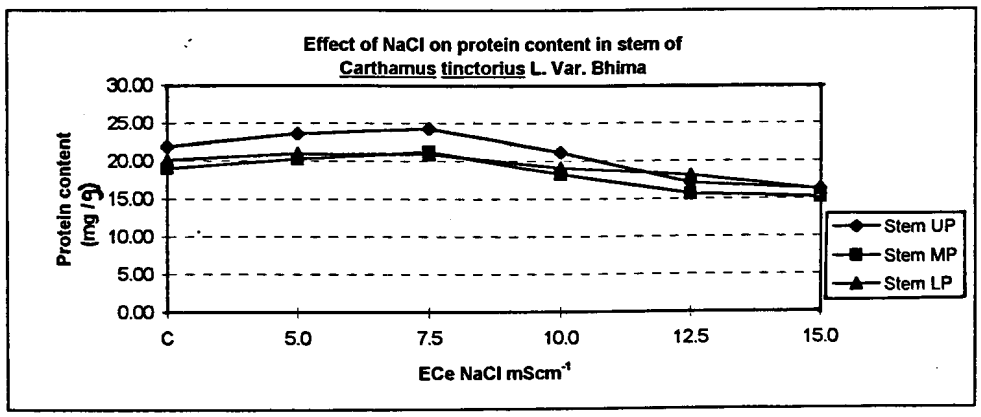


Fig. 25b.



UP -Upper part MP - Middle part LP - Lower part

Fig. 26a.

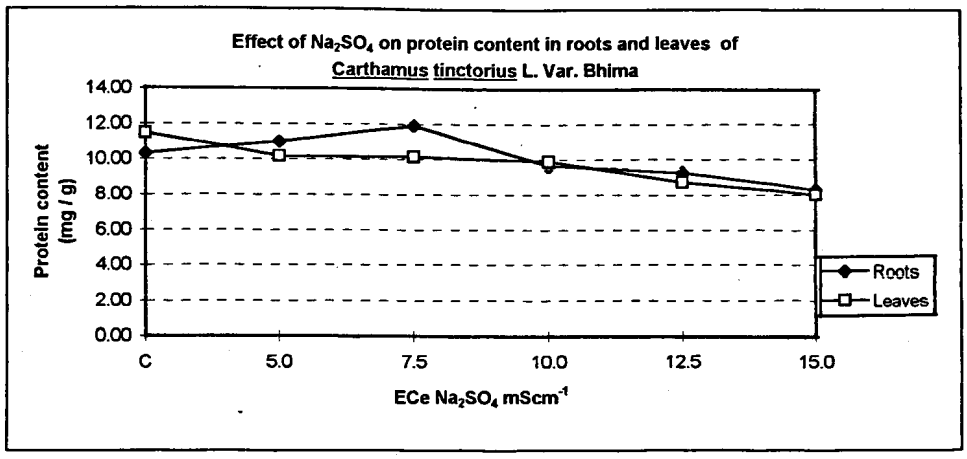
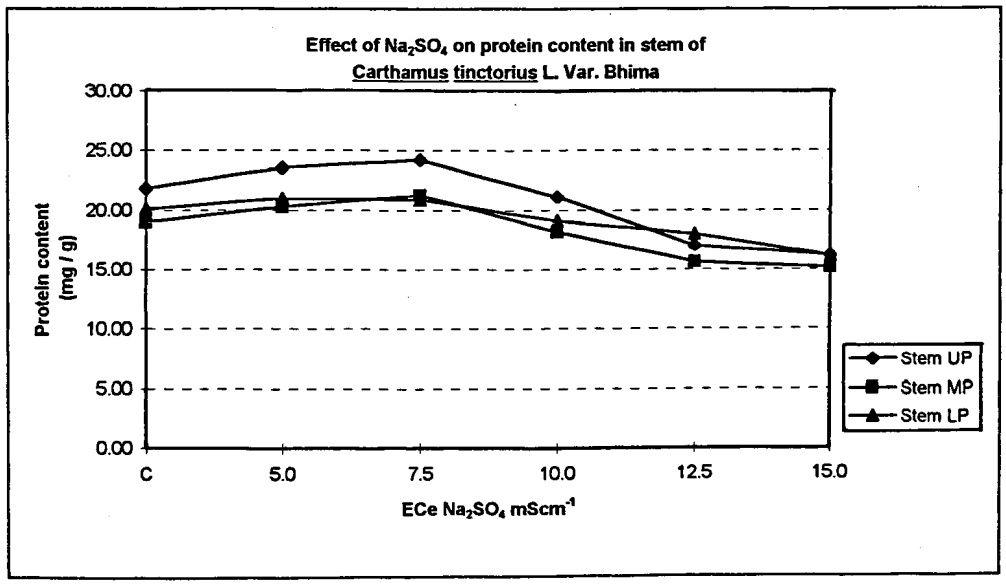


Fig. 26b.



UP -Upper part MP - Middle part LP - Lower part

NaCl inhibit while low concentrations of Na_2SO_4 stimulate and high concentrations of Na_2SO_4 inhibit protein biosynthesis in stem of safflower Cv. Bhima.

III) LEAVES

It is observed that protein content in leaves (Tables. 33,34;Fig.25a,26a) decreased at all levels of both the salts thereby indicating that all the concentrations of both the salts inhibit protein synthesis in leaves of Carthamus tinctorius Cv. Bhima.

Average protein content total plant (R+S+L) (Tables.33,34) was less than the control at all levels of NaCl while its content was more than that of the control in plants grown upto ECe 7.5 $mScm^{-1}$ of Na_2SO_4 and was less than the control at all high levels of Na_2SO_4 thereby indicating that all concentrations of NaCl inhibit while low concentrations of Na_2SO_4 stimulate and high concentrations of Na_2SO_4 inhibit protein biosynthesis of total plant.

4) DISCUSSION

A) CHLOROPHYLLS :

Interference with the photosynthetic machinery with increasing chloride ions resulting in reduced chlorophyll's has been reported by Baslavaskya (1936). Similar results were given by Ibragimov and Azimov (1975) in cotton; Divate and Pandey (1981) in grapes; Stiborova et al. (1987) in barley and maize; Reddy and Vora (1986) in bajra and Ashraf (1989) in cultivars of black gram. Tiku (1976) reported increased chlorophyll content in Salicornia rubra and a decrease in Distichillis stricta, with increase in salinity. This indicated that among halophtes also salinity effect is different on chlorophyll biosynthesis.

Alina et al. (1984) examined various chloroplast fractions from pea seedlings and reported suppression of chlorophyll by NaCl at high light intensity, the effect being greater in membrane fractions than in whole organelles. Stiborova et al. (1987) reported decrease in chlorophyll content in Hordeum vulgare L. and Zea mays at NaCl concentrations of 0-100 mM. Ashraf and Naqvi (1992) observed decrease in chlorophyll b and total chlorophyll in Brassica compestris and B. napus at highest Na/Ca ratio.

Saha and Gupta (1993) reported that salinity induced decrease in chlorophyll in sensitive variety of mung bean. Peiris and Ranasinghe (1993) found low chlorophyll a/b ratio in Nona bokra variety of rice under saline (EC 5.2 ds/m) than non-saline (control) conditions. Ramanjulu et al. (1993) observed decrease in total chlorophylls(chlorophyll a and chlorophyll b) content under varied concentrations of NaCl in mulberry Var. Mysore local. El-Samad and Abd (1993) observed decrease in chlorophyll content and carotenoids due to NaCl salinity in broad bean plants. The same effect was observed in Triticum vulgaris L. plants.

Results of El-Samad and Abd (1995) revealed decrease in chlorophyll content and carotenoids under salinity in Cucumis sativus and spraying of sodium pyruvate ameliorated adverse effects of NaCl. Singh and Dubey (1995) reported decrease in chlorophyll a and chlorophyll b contents in salt sensitive cultivar Ratna and Jaya than in tolerant cultivars CSR-1 and CSR-3 of rice at moderate salinity (7 ds/m). Reddy and Vora (1986) reported that saline soil (0.2 and 0.4 % NaCl) reduced chlorophyll a, chlorophyll b and chlorophylls (a+b). According to Popova et al. (1995) chlorophyll content decreases in Hordeum vulgare L. when NaCl stress was imposed through the root medium for a period of 8 days. Lutts and Bouharmont (1996) reported that NaCl hastened naturally occurring senescence of Oryza sativa L. leaves which normally

appear during leaf ontogeny and decreased chlorophyll concentration. Misra et al. (1997) observed decrease in chlorophyll and carotenoid content in Oryza sativa L. Cv. Jaya in 15 day old seedlings under NaCl stress. Muthukumaraswamy and Panneerselvam (1997) reported marked reduction in chlorophyll at 100 and 150 mM NaCl over the control in stem and leaf of green gram. Thus, in general chlorophyll content decreased in several plants under saline conditions.

Enhancement of chlorophyll synthesis by NaCl salinity in rice (Pearson and Bernstein, 1959); barley (Greenway, 1962); and wheat (Passera and Albuzio, 1978) has been reported. Bhosale (1974) observed stimulation of chlorophyll synthesis by Na_2SO_4 treatment in a mangrove, Aegiceras majus. According to Udovenko et al. (1971) salinization does not decrease the chlorophyll content but weakens the stability of their binding to the proteins in the chloro-protein complex. They further stated that salinity brought about the migration of chloroplasts into the lower part of the cells of palisade and spongy parenchyma, that considerably inhibited the photochemical activity of chlorophyll under salinity. Bhivare et al. (1988) reported decreased chlorophyll content under chloride and increased chlorophyll content under sulphate treatment which reflects that sulphate and chloride salinity act differently in some plants.

Baset and Arju (1990) reported high chlorophyll concentration in tolerant variety of Oryza sativa in mid portion of third youngest leaf, after 3 days. when treated with 50 mM NaCl. Mohanty and Saradhi (1992) observed high chlorophyll a content in cotyledonary leaves of seedlings raised at varying concentrations of NaCl (0, 100, 150 and 200 mM) while chlorophyll b showed no significant variation till the eleventh day of growth. The high chlorophyll a /chlorophyll b ratio reflected changes in photosynthetic antennae size. According to Reddy et al. (1992) in sea water treated Salicornia

branchiata Roxbirgi plants, increase in chlorophyll (a + b) upto concentrations of 1547.71 $MeQm^{-3}$) in whole plant and in separated Pallisade and mesophyll, chlorophylls increased which also indicated that NaCl stimulates chlorophyll biosynthesis in mangroves.

Peiris and Ranasinghe (1993) reported high amount of total chlorophylls and chlorophyll a in varieties of rice, Nona bokra (salt tolerant) and IR 5931-110-1 (salt sensitive) under saline (EC 5.2 ds/m) than under non saline (control) conditions. Chlorophyll b was high in Nona bokra and Chlorophyll a/b ratio was high in IR 5931-110-1 compared to control. Hamada and Enany (1994) observed increase in chlorophylls and carotenoids in broad bean leaves under saline conditions. Hamada (1996) found increase in chlorophlls in wheat plants subjected to salinity or drought or both. Misra et al. (!997) reported that NaCl increased chlorophyll and carotenoids content of 25 day old seedlings of both Oryza sativa L. Cv. Damodar and Jaya.

Asraf and Naqvi (1992) found no change in chlorophyll b and total chlorophyll at highest Na/Ca ratio in Brassica carinata and B. napus. Hamada and Enany (1994) reported that chlorophylls and carotenoids content remained more or less unchanged in pea plants, with rise in salinization upto 80 mM NaCl. Results of the present investigation on Carthamus tinctorius Cv. Bhima (Tables. 21,22; Figs.13,14) revealed that all concentrations of both the salts stimulate chlorophyll in Carthamus tinctorius Cv. Bhima, however, productivity at flowering (Table.6) was more than the control upto ECe 7.5 $mScm^{-1}$ of Na_2SO_4 and decreased at higher salinity. Under NaCl treatment, productivity at flowering (Table. 5) decreased with increasing salt concentrations, thereby indicating that increased chlorophylls at all levels of NaCl could not help for maintaining productivity. However, one of the reasons for increased productivity at low

levels of Na_2SO_4 must be the increased chlorophyll content. At higher levels of Na_2SO_4, increased chlorophyll content could not help for increasing productivity at flowering. At maturity, increased chlorophylls definitely increased productivity at ECe 5.0 $mScm^{-1}$ of NaCl and upto ECe 10.0 $mScm^{-1}$ of Na_2SO_4 (Tables. 9,10). Thus, there is a correlation between chlorophyll content and productivity at maturity at low levels of both the salts but increased chlorophylls at higher levels of both the salts could not help in maintaining growth. //

B) TAN (Titratable Acid Number)

TAN represents organic acids in the cell. One of the salt tolerance mechanism is to neutralise the salts by synthesizing more amounts of organic acids. By accumulating more organic acids, plants neutralize toxic effects of Na by binding Na with organic acids. Hence, its toxicity decreases. Thus, by synthesizing more organic acids plants tolerate salty environment (Strogonov, 1964).

Strogonov et al. (1970) postulated that organic acids play a protective role in plants under saline conditions. Accoding to Cram (1976) when salinity consists predominantly of monovalent cations and divalent anions for example, Na_2SO_4 then cation uptake rate exceeds those of anions and ionic balance is achieved by the synthesis and accumulation of organic acids. Ackerson and Youngner (1975) have suggested that salinity tolerance of bermuda grass might be facilitated by redistribution of increased concentrations of organic acids in the cell sap. One of the numerous adaptive responses of plants to salinity is the binding of the mineral elements to organic acids in plant cells (Strogonov, 1964; 1974).

Decrease in TAN values under saline conditions have been observed by Azizbekova and Rasulova (1972) in cotton; Flowers and Hall (1978) in Suaeda maritima and Abdel Rasoul et al. (1980) in Portulaca. Increase in TAN values by Wallace et al. (1982) in Atriplex polycarpa and Krishnamurthy et al. (1987) in salt tolerant Au I, Co 43, CSC 1 varieties of rice were recorded. Srogonov (1964, 1974) have observed increase in organic acid due to NaCl and Na_2SO_4 salinity's in maize. Karmarkar (1965) in Bryophyllum pinnatum noted increase in TAN at 6.00 and 7.00 PM due to NaCl and Na_2SO_4 salinization in sand culture experiments. Shetty (1971) recorded increase in TAN of leaves of Acrosticum at 20 mM NaCl treatment. Nimbalkar (1973) reported considerable increase in TAN value at 150 mM NaCl treated sugarcane leaves. According to Downtown and Loveys (1978,1981), increase in titratable acidity in grapeberry occurs due to salinity. Thus, increase in organic acids may play important role in ionic balance of plants under saline conditions where Na uptake increases. Chavan (1980) observed an initial increase of TAN at lower (20mM) concentrations of NaCl and Na_2SO_4 salts and an inhibition at higher concentration of the salts in ragi.

Results of Bhandari (1988) in Capsicum Cv. NP 46A and Cv. Pant C1; and Shukla (1995) in Salvadora persica indicated that in these plants there is inhibition of organic acid biosynthesis under saline conditions.

Results of the present investigation (Tables. 23, 24 ; Fig.15,16) revealed that TAN decreases at all levels of NaCl which reflects that mechanism of neutralisation of Na with organic acids is not present in Carthamus tinctorius Cv. Bhima. However, this mechanism of salt tolerance is present at lower levels of sulphate salinizations and is absent at higher levels of the sulphate salinizations.

C) CARBOHYDRATES

Decreased total sugar and starch content was reported by El-Saidi and Hawash (1971) in Hibiscus subdariffa; Nanawati and Maliwal (1973) in tomato; Matar et al. (1975) in spinach and lettuce. Deshpande and Nimbalkar (1982) in pigeon pea observed decrease in total sugars and starch content and increase in reducing sugar under saline conditions. Decrease in starch content occurs in salt stressed leaves of Sesbania aculeata at 10 $mScm^{-1}$ and 15 $mScm^{-1}$ (Karadge and Chavan, 1983). Rathert (1984) observed decrease in leaf sucrose and starch level in soybean and cotton under saline stress. Shaddad (1990) reported decrease in saccharide content in Raphanus sativus plants with rise in salinization by NaCl. Singh et al. (1990) found decrease in total, reducing and non reducing sugars with more harmful effect on reducing saccharides with increasing salt concentration (NaCl, $CaCl_2$, Na_2SO_4) and PEG 6000 in Pisum sativum L. during early seedling growth. El-Samad and ABD (1993) reported decrease in saccharide contents in broad bean plants under NaCl salinity and effect of NaCl was ameliorated by foliar application of NaH_2PO_4 and $NaNO_3$ resulting in increase in saccharides. Similar effect of NaCl was found to be ameliorated by $CaCl_2$ or KCl in Triticum vulgaris L. which resulted in increase in saccharides. Results of Saha and Gupta (1993) revealed that salinity decreases soluble carbohydrate level which was partially negated by pre-treatment of retardant in sensitive variety of mung bean.

Everard et al. (1994) observed decrease in sucrose and starch in leaf tissues of celery Apium graveolus L. at intermediate NaCl level (100 mM NaCl) of root zone salinity due to shifts in photosynthetic carbon partition. El-Samad and Abd (1995) also observed decrease in sacharides in Cucumis plants and spraying the shoot system with sodium pyruvate ameliorated effects of NaCl salinity. Chandrashekar and Sandhyarani (1995)

found decrease in sugar and starch content under 0, 0.2, 0.4, 0.6 and 0.8 % NaCl salinity in Crotolaria striata Dc. Tattini et al. (1996) observed decrease in starch content with increase in salinity (NaCl 0-200 mM) for 28-32 days followed by 28 to 30 days of relief from salinity over two growing seasons in Olea europaea. Abd-El Samad and Shaddad (1997) reported that sensitivity of soybean cultivar was due to decreased saccharide content under NaCl salinity. Muthukumaraswamy and Paneerselvam (1997) found that NaCl salinity decreased accumulation of starch, sugar content and activity of α-amylase in radish seedlings. Salinity reduced sucrose content in sugarcane (Sharma, 1997).

Hayward and Long (1941); Gauch and Eaton (1942);Bernstein and Hayward (1958); Strogonov(1964) reported increase in glucose and fructose in maize sap due to salinity. Increase in reducing sugar under NaCl salinity is clearly indicated by the work of Shetty (1971); Meiri et al. (1971) in bean; Nimbalkar and Joshi (1975) in sugarcane; El-Shourbagy and Kishk (1975) and Petolino and Leone (1980). Low salt concentrations resulting in increase in reducing sugars is one of the causative factors of stimulated growth. El-Sharkawi and Salama (1977) have suggested that in Linum increased synthesis of soluble sugars and probably nitrogen metabolites are means of adjustment to salt stress. Albert and Falter (1979) have reported an increase in the soluble carbohydrate content in the salt affected leaves. According to them carbohydrates play an important role in the osmotic adjustment under salt stress. Aleschin et al. (1984) have considered carbohydrates as criteria for salt tolerance in rice cultivars. In more salt resistant tomatoes under maximum salinizations (0.5 % of Cl), starch content increased compared to control (Kabuzenko and Ponomarova, 1980). In roots of Hedysarum coronarium, the amount of sucrose increased with increase in NaCl concentration (Soltani et al., 1981). Munns et al. (1982) reported increase in

soluble carbohydrate with increased NaCl in elongating leaf tissue of barley while starch did no change. Rathert (1983) observed increase in sugar and starch in leaves of cotton. According to Genkel and Bakanova (1974) indicated that low concentrations of NaCl stimulate sugar content while KCl and Na_2SO_4 did not have the same effect. Appreciable increase in reducing sugars is expected in salinity experiments for halogen induced increased respiration and degradation of carbohydrates of higher order.

Soluble sugar content increased in roots and leaves due to salt stress in salt stressed leaves of Sesbania aculeata at 10.0 $mScm^{-1}$ and 15.0 $mScm^{-1}$ (Karadge and Chavan, 1983). Imamul and Larher (1984) found accumulation of soluble sugars at 150 mM NaCl concentration indicating its role as principle organic osmotica. Rathert (1984) observed increase in leaf sucrose and starch levels in bushbean and rice leaves and root under saline stress. McNulty (1985) reported small increase in sugar (+3 mM) in halophyte Salicornia europaea L. Spp. Rubra, at 100 mM NaCl exposure. Krishnamurthy et al. (1986) observed increase in soluble sugars and total carbohydrates in rice varieties.

According to Corchete and Guerra (1986) sucrose accumulates in embryonic axes of Leus culinaris cultivar Castellana at different concentrations of NaCl. Ashraf and Naqvi (1992) observed that total shoot sugars increased at highest Na/Ca ratio whereas root sugars increased with increasing external Na/Ca ratio in three Brassica species except in B. compestris. Cachorro et al. (1993) reported that in Phaseolus vulgaris at 50 mM NaCl concentration, total sugars did not differ much from control plants, however, at 100 mM NaCl concentrations, significant increase in total sugars was observed.

Kumar et al. (1994) subjected plants of sugarcane Cv.Co.1148 to salinization with 200 meq L^{-1} of Cl type salts mixture (having Na, Ca and Mg as 3:1:1 and Cl and SO_4 as 4:1 on m eq. Bases) for two weeks or more and reported increase in soluble sugars concentration by 55% in recently matured blades (soluble tissues) and 22.5 % in elongated sheath bases (sink tissues). The sugar content dropped in elongating sheath by 43 % after desalinisation. Ashraf (1994) reported greater amounts of soluble sugars in leaves of Erica sativa in salt tolerant populations at salinities 0,100,200,300 $molm^{-3}$ NaCl compared to non-tolerant populations indicating that these compounds are important components of salt tolerance. Ashraf and Fatima (1995) observed greater accumulation of soluble carbohydrates in leaves of salt tolerant accession 260622 under 70, 140 and 210 $molm^{-3}$ NaCl salinity compared to other salt sensitive accessions. Chandrashekar and Sandhyarani (1995) found increase in starch content with increasing salinity levels (0, 0.2, 0.4, 0.6 and 0.8 % NaCl) in Crotolaria striata DC. Khan et al. (1995) reported that sugars were enhanced under NaCl (0-100 mmol L^{-1}) and Na_2SO_4 (0-50 mmol L^{-1}) salinity in Sorghum bicolor (L) Moench. Cv. IS-4807. According to Muthuchelian et al. (1996) salt stress (100 mM to 250 mM) increased starch and saccharide contents in Erythrina variegata seedlings. Cayuela (1996) reported significant increase in sugars in seeds primed with 6M NaCl in salt treated leaves (70 and 140 mM NaCl) after 30 days of sowing which increased with longer treatment time in Lycopersicon esculentum Mill.Cv. Pera. Tattini et al. (1996) found increase in soluble carbohydrates under NaCl (0-200 mM) grown plants for 28-32 days followed by 28-30 days of relief from salinity over 2 growing seasons in Olea europea L. Misra et al. (1996) observed increase in total soluble saccharides in root and shoot in dark in NaCl stressed (0, 0.5, 1, 2, 3 % w/v) seedlings of two cultivars Cv. Sujata and Cv. K 851 of Vigna radiata L. seedlings. Fernandez et al. (1996) reported increase in sucrose and decrease in nodular starch and 77 % decrease in sucrose synthetase

activity over a period of 6 days in Lupinus albus L.at 150 mol m^{-3} NaCl concluding that though the supply of carbohydrates from source tissues is reduced the sink make use of sugars under saline conditions.

Petolino and Leone (1980) and Staples and Sacher (1981) have reported that starch content did not change significantly in Phaseolus and total sugar content remained unchanged in Lycopersicon esculentum by salt treatments. Results of Zidan and Elewa (1995) revealed that soluble carbohydrate remained unchanged under low and moderate levels of NaCl in four plant species of Umbelliferae.

From the above references it is clear that in some plants soluble sugar increases while in others , it decreases and in some other plants its content remains unaffected. In general, plants use soluble sugars as osmoticum under saline conditions. Hence, the plants which can tolerate low or medium levels of salt stress synthesise more soluble sugars and tolerate salt stress. The plants which fail to increase soluble sugars biosynthesis could not tolerate salts. Results of the present investigations (Table 20 to 25 and Figure 20 to 25), revealed that in roots carbohydrate content decreased with increasing NaCl salinity. However, under sulphate salinity, carbohydrate synthesis was stimulated at ECe 5.0 $mScm^{-1}$ and at all higher salinity levels, content was less than the control. In stem, carbohydrate content was more than the control at all levels of NaCl salinization and maximum content was recorded at ECe 7.5 $mScm^{-1}$. Under Na_2SO_4 salinization, content was less than the control at all levels of Na_2SO_4. In leaves, carbohydrate content, increased upto ECe 10.0 $mScm^{-1}$ and decreased further under NaCl salinity. Under Na_2SO_4 treatment, carbohydrate content increased upto ECe 12.5 $mScm^{-1}$ and decreased at higher salinity levels.

D) PROLINE

Plants accumulate a number of osmoprotective substances in response to NaCl stress, one of them being proline. Plant cells adjust to the imbalance in water relations through osmotic adjustments utilising both organic solutes and ions (Rhodes, 1987; Mathius et al, 1992). Salinity induced reduction in growth is consequences of several physiological responses including modification of ion balance, water status, mineral nutrition, stomatal behaviour and photosynthetic efficiency (Flowers et al., 1977; Munns and Termaat, 1986). Proline accumulation is known to have a variety of roles under salt stress. Palfi et al. (1974) reported that proline increases the amount of bound water in the leaves. A correlation between proline content and salt tolerance has been reported by Goas (1968). Stewart and Lee (1974) feel that proline may be functioning as a source of solute for intra cellular osmotic adjustment while Schobert and Tschesch (1979) and Pollard and Wyn Jones (1979) reported that it acts as a protective agent of enzymes and cellular structures. It's high concentration in the cells (upto 0.5 M) could not inhibit the activity of enzymes, therefore, it acts as a good osmoregulator (Greenway and Munns, 1980). According to Aspinall and Paleg, 1981; Chandler and Thorpe, 1986; proline protects the plant tissues against stress by acting as an N-storage compound, osmosolute and hydrophobic protectant for enzymes and cellular structure. Moftah and Michel (1987) reported that proline accumulation was not a sensitive indicator of salt stress in soybean cultivars.

Decrease in proline content was reported by Shevyakova and Komizerko (1969) in cabbage leaf callus; Totawat and Saxena (1971) in Phaseolus aureus; Stewart and Lee (1974) in Plantago; Joshi and Naik (1980) in sugarcane; Reddy and Vora (1983) in Pennisetum typhoides and Devitt et al. (1987) in wheat grain. Jeschke et al. (1986)

reported that proline was minor constituent of xylem and phloem at highest salinities in nodulated Lupinus albus cultivar Ultra under 1, 5, 10, 25, and 40 molm^{-3} NaCl. Kishore et al. (1987) observed decrease in proline content in leaves of Perlette grapevines subjected to salt stress of 0.15 % chloride, 0.23 % sulphates and 0.30 % carbonates but later accumulated in all vines which survived the stress applied. Fedina et al. (1993) observed that in 10 days old Pisum sativum L. Cv. Ran 1 when pretreated for 24 hours with proline (10^{-6} M or 10^{-5} M) before salinization and with 50 mM NaCl for 2 days resulted in decreased Na accumulation in shoot. Dutt et al. (1991) observed decrease in proline accumulation and water status at increasing salinity levels (4, 8, 12, 16,20 dS/m) in Casuarina equisetifolia Forst. Banuls and Millo (1992) found decrease in proline accumulation in leaves of Citrus sinensis [L] Osbeck Cv. Hamlin under saline stress; due to addition of Ca (as calcium acetate). Belkhodja and Saadi (1993) reported that in stem and root of intermediate growth lines of Vicia faba L. salinity ($NaCl+CaCl_2$) 50 and 200 meqL^{-1} decreases proline accumulation. This comparison is very important in stems than in roots without salt. Manetas et al. (1986) reported that proline did not protect PEP Carboxylase against NaCl that is it was inhibitory in Cynodon dactylon and Sporobolus pungens (Poaceae). Colmer et al. (1995) reported lower proline levels in younger ones which contributed to NaCl tolerance in amphiploid genotype of wheat when grown for 18 days in non saline (1.25 mM Na) and salinized (200 mM NaCl) soils.

Accumulation of proline under salt stress was reported by Strogonov (1964); Palfi and Juhasz (1970) and Chu et al. (1976). Bar-Nun and Poljakoff-Mayber (1977) observed that proline effectively counter balanced the inhibitory effect of NaCl on pea seed germination and root growth. In glycophytes, increased proline under saline conditions is evident from the reports of Downton and Loveys (1981); Dix and Pearce (1981);

Larher et al. (1992); Roeb et al. (1982), Murray and Ayers (1986) and Misra and Shitole (1986). Similar observations were made by Anthony and Anthony (1978) on marshy halophytes; Rao et al. (1981) in Cajanus cajan; Huq and Larher (1985) in Phaseolus aureus and Vigna sinensis; Gupta et al. (1987) in Pearl millet and Ashraf (1989) in Vigna mungo.

Buhl et al. (1983) observed that when cut ends of barley (Hordeum vulgare cultivar Larker) leaves, were placed in NaCl solution then proline accumulates over 12 hours at average rate of 0.6 μ mol/h/g fresh weight and when 14C proline was added in separate experiments ,salt treatments increased proline synthesis indicating that proline accumulated under different stresses. Considerable increase in proline in salt stressed leaves of Sesbania aculeata plants took place at 10 $mScm^{-1}$ and 15 $mScm^{-1}$ salinity levels (Karadge and Chavan, 1983). Hasson and Mayber (1983) observed accumulation of proline in Pisum sativum plants grown at 192 mM NaCl.

Gorham et al. (1984) reported high concentrations of proline present in different tissues of in salt stressed plants Triticum species. Imamul and Larher (1984) reported that free proline behaved as a stress indicator in NaCl 150 mM treated Vigna sinensis. Huq et al. (1985) observed accumulation of proline more in younger parts, in Phaseolus aureus than in Vigna sinensis which depends on time and which directly correlated to water deficit and K content but non to on Na content, when these plants were acclimatised at two levels of salinity's for 35 days. McNulty (1985) reported small increase in proline content at 5 mM and at 100 mM NaCl at 72 hours in Salicornia europaea L. Supp. Rubra. By 24 hours Proline increased to 208 mM. Wethered and Jennings (1985) observed increase in Proline with increasing salinity in Thraustochytrium aureum and T. roseum.

Manetas et al. (1986) reported high levels of proline in Cynodan dactylon and Sporobolus pungens (Poaeae) under saline conditions, when compared to Chenopodiaceae free proline in non stressed plants. Increased concentration of proline was reported at salinities 20 to 50 $mScm^{-1}$ in Salicornia brachiata (Joshi, 1986). Gorham et al. (1986) observed inverse relationship between proline and glycinebetaine contents of different tissues with proline levels higher in older leaves in Thinopyrum scirpeum, concentration of both compounds was high in salt stressed plants. Joshi and Iyengar (1987) reported increase in Proline at higher salinity in secculent leaves of Suaeda nudiflora Moq. when subjected to 10-40 $mScm^{-1}$ sea water salinity stress. Moftah and Michel (1987) reported that leaf proline in Soybean cultivar. Ranson was 0.4 micro mole/g fresh weight at or below 20 mM NaCl ad did not exceed 0.5 micro mole/g. fresh weight at 100 mM NaCl and in Bragg cultivar leaf proline was near 1.2 and 1.9 micro mole/g. fresh weight when stress caused injury indication that accumulation of proline is not a sensitive indicator of salt stress soybean plant.

Bowman (1988) reported increase in proline concentration at high salinity level and did not differ significantly between population in 2 naturally occurring populations of 4 non halophyte Andropogon glomeratus. Tipirdamaz and Cakirlar (1990) reported increase in Proline content of leaf, stem and root in Gerek 79 and Bezostaya 1 varieties of wheat plant subjected to NaCl salinity. Shaddad (1990) has reported lowering of P content in Raphanus sativus plants with increase in salinization levels using NaCl which was counteracted by spraying proline (200 gm^{-3}).

Singh et al. (1990) reported increase in free proline content with increasing salt concentration (NaCl, $CaCl_2$, Na_2SO_4) and PEG-6000 in Pisum sativum L. during early

seedling growth. Abbas et al. (1991) found increase in proline content in all organs of Phaseolus vulgaris with increasing salinity levels in growth medium. Maslenkova et al. (1992) observed 8 fold increase in proline content in salt treated barley (Hordeum vulgare L.) seedlings while studying soluble and thylakoid membrane proteins. Lu and Ying Yi (1992) observed increase in free proline content with decreasing osmotic potential of external solution or prolonged culture line in leaves of wheat seedlings. This accumulation may be related to alterations of ultrastructure of mitochondria and low proline oxidase activity. Ashraf and Naqvi (1992) reported increase in proline content at highest Na/Ca ratio in Brassica species. According to Thomas et al. (1992) salt stress induces accumulation of proline prior to switch from C_3 to CAM in Mesembryanthemum Crystallinum. and exogenous applied abscisic acid was a poor substitute for NaCl in inducing proline . Roy et al. (1993) observed that 30 mM L-proline alleviates salinity stress of 100 mM NaCl of salt sensitive cultivar of Oryza sativa.

Sadek (1993) reported accumulation of proline in Atriplex halimus Var. Schweinfurttii under NaCl salinity. Yin et al. (1993) observed accumulation of proline for osmotic adjustment when Na concentration increased. According to them at Na concentration 20-80 μmol / g proline content increased in primary leaves, mature leaves and stems of Anerolepidium chinensis at 0.088 % -1.63 % salt concentration. Fedina et al. (1993) found that in 10 days old Pisum sativum L. Cv. Ran 1 plants when treated for 24 hours with proline (10^{-6} M or 10^{-5} M) before salinization with 50 mM NaCl for 2 days resulted in increase in endogenous free proline content. Cachorro et al. (1993) reported that at 50 mM NaCl concentration plants did not differ from control plants in proline concentration but in 100 mM NaCl concentration total sugars and proline increased significantly. Blits and Gallagher (1993) reported increase in free proline in salt stressed cell cultures of Kosteletzkya virginia L. Malvaceae at high 225 mol m^{-3} NaCl. Tipirdamaz and

Karakullukcu (1993) observed increase in endogenous proline and glycinebetaine content in invitro cultured tomato embryo exposed to saline conditions. Belkhodja and Saadi (1993) found that local variety of Vicia faba L. accumulated proline in roots with NaCl+$CaCl_2$ 50 and 100 meq of salt L^{-1} v/v and determinate growth lines accumulates proline in stem with increasing salt concentration. Ramanjulu et al. (1993) reported increase in proline content in mulberry Var. Mysore tissues under NaCl treatment. According to Zidan and Elewa (1995), proline content increased progressively in four plant species of Umbelliferae, when treated with NaCl.

Ashraf (1994) reported greater amounts of proline in leaves of salt tolerant population at salinities 0, 200, 300 $molm^{-3}$ NaCl in oil seed crop Erica sativa, when compared to non tolerant population indicating that these compounds are important components of salt tolerance. Yurkevich and Potopals' Kii (1994) found that proline concentration increased both in shoots and roots in all variants of rye plants under sea water salts of varying concentrations. Tetraploid rye shoots accumulated more proline than roots concluding that proline accumulation rate in shoots and roots of rye with different paddy level correlated with peculiarities of definite rye varieties and forms. Venkatasalu et al. (1994) reported accumulation of proline and glycinebetaine contents with increasing NaCl salinity in Sesuvium portulacastrum L. a salt marsh halophyte. Durgaprasad et al. (1996) observed that NaCl salinity increased proline accumulation in two cultivars of Glycine max L.Merrill (Co-1) and ADT-1, which may serve as osmoticum to protect cell organelles and enzymes Co-1 was slightly salinity tolerant than ADT-1. According to Chandrashekar and Sandhyarani (1996) higher proline and low epicuticular wax (ECW) accumulation in young leaves compared to mature leaves of Crotolaria striata DC. Saline habitat appears to help the plants in tolerating the stress by acting as compatible solute and ECW content on surface helps to conserve water. Misra et al. (1996)

reported accumulation of proline in root and shoot of two cultivars of Vigna radiata L. Cv. Sujata and K-851 in dark under 0, 0.5, 1, 2 , 3 % w/v NaCl stress and proline accumulation in root was 4-5 times higher than shoot. Chandrashekar and Sandhyarani (1996) found increase in proline content with increasing salinity levels (0, 0.2, 0.4, 0.6, 0.8 % in Crotolaria striata DC seeds. Lin and Kao (1996) observed accumulation of proline in roots of rice seedlings. Abd-El samad and Shaddad (1997) reported that due to accumulation of proline Soybean Cv. Clark tolerated NaCl salinity upto osmotic potential in soil -1800 kpa and Cv. Forest to -1500 kPa. Muthukumaraswamy and Paneerselvam (1997) observed increase in proline and glycinebetaine content at 100 and 150 mM NaCl over control in green gram. Muthukumaraswamy et al. (1997) reported increased proline in all parts of chickpea seedlings under saline condition compared to control. Garcia et al. (1997) reported accumulation of proline in response to NaCl stress in Oryza sativa L.

Lin and Kao (1995) reported that application of L-proline and D-asparagine reduced shoot growth under NaCl stress. L-proline, glycine betaine, D and L-asparagine enhanced NaCl inhibition of root growth in rice seedlings. Ashraf and Fatima (1995) observed no difference in leaf proline in salt tolerant accession and other accessions in safflower. Garcia et al. (1997) observed that proline either has no effect or in some cases promotes effect of NaCl on growth, inhibition, chlorophyll loss and induction of highly sensitive marker for plant stress, the osmotically regulated Sa/T gene. However, high concentration (10mM) of proline prevents NaCl induced chlorophyll loss in blades, preserves its integrity and enhances growth. Proline does not play an important role in salt tolerance in rice.

Increased proline content in all organs of safflower Cv. Bhima (Tables. 20-25; Fig. 23 (a,b), 24(a,b)) suggested that proline plays an important role for salt tolerance in Carthamus tinctorius Var. Bhima. From the results of present investigation, it is clear that increased proline content under NaCl saline conditions did not help for maintaining growth because productivity (DM/ plant) (Tables. 5,6 ;Figs. 5,6) at flowering was less than the control at all levels of NaCl salinity. Thus, it appears that increased proline content in safflower Cv. Bhima under NaCl salinity helps for survival and not for maintaining growth. However, increased proline at ECe 5.0 mScm-1 of NaCl helps for increasing productivity (Table.9) at maturity of this variety. Under sulphate salinizations, proline content (Table.25 ;Fig.24ab) as well as productivity that is dry matter per plant (Tables. 5, 6 ; Fig.5,6) was more than the control at flowering which indicated that proline definitely helps for maintaining growth under low levels of sulphate salinizations in Bhima cultivar of safflower.

However, at higher levels of sulphate proline (Table.25 ; Fig.24ab) content was much more than the control but productivity (Tables. 5,6 ; Fig. 5,6) at flowering was less than the control indicating that proline helps for survival. Increased proline helps for increasing productivity (Table.10) at maturity in plants grown upto ECe 10.0 $mScm^{-1}$ of Na_2SO_4. Thus, safflower Cv. Bhima adapts to low levels of both the salts by increase in proline. Increased levels of proline at higher concentrations of both the salts could not help for maintaining growth but must be helping for survival as suggested by Greenway and Munns (1980).

E) PROTEINS

Protein synthesis and turnover in growing plants is a basic component of metabolic regulation which provides a way for varying the enzymatic complement during response

to environmental conditions (Huffakar and Peterson, 1974). Miniberg and Le-Zu (1974) observed that moderate salinity increases protein content in bean leaves. Decreased protein content under salt stress has been reported and attributed to reduced synthesis and accelerated proteolysis (Levitt, 1972; Prisco and O' Leary, 1970); Bernstein, 1975, Sheoran, 1975). Rakova et al. (1969) observed that in pea roots sodium salts inhibited synthesis as well as hydrolysis of basic proteins. The decrease in protein synthesis may be due to decreased availability of amino acids and denaturation of amino acids and protein synthesis under saline conditions (Levitt, 1972). Alina et al. (1984) examined various chloroplast fractions from pea seedlings and reported suppression of protein content by NaCl at high light intensity, the effect being greater in membrane fractions than in whole organelles. Decrease in protein content under saline conditions was reported by Lal and Bhardwaj (1987) in Pea; Jena and Rao (1988) in rice, Cerda and Martinez (1988) in tomato and cucumber. Remison et al. (1988) in coconut seedlings and Hafeez et al.(1988) in Vigna radiata reported decreased protein content, nitrogen reductase activity and nitrogen fixation. Shaddad (1990) reported lowering of protein contents in Raphanus sativus plants with rise in NaCl salinizaiton. Miteva (1992) observed decrease in level of total soluble proteins on a fresh weight basis by 64% than that of control in 7 day old leaves of Hordeum vulgare L. Var. Alfa at 100 mM NaCl. Saha and Gupta (1993) reported salinity induced decrease in protein in sensitive variety of mung bean. El-Samad and Abd (1993) observed decrease in protein content with increase in NaCl content in broad bean plants which was ameliorated with foliar application of NaH_2PO_4 and $NaNO_3$ causing increase in protein content. The same effect was observed in Triticum vulgaris L. plants but here $CaCl_2$ or KCl ameliorated adverse effects of NaCl salinity resulting in increase in protein content. Ramanjulu et al. (1994) reported decrease in total protein content of NaCl stressed Morus alba with increase in amino acids and ammonia.

Popova et al. (1995) observed decrease in leaf protein content when NaCl stress was imposed on root medium for a period of 8 days in Hordeum vulgare L. plants. Lutts and Buharmont (1996) reported that NaCl hastened naturally occurring senescence of Oryza sativa L. leaves (which normally appear during leaf ontogeny),which decreased protein content. Abd El-Samad and Shaddad (1997) observed that sensitivity of soybean Cv. Kent was associated with decrease in protein content under NaCl salinity. Muthukumaraswamy et al. (1997) observed that protein content decreased in root, shoot and leaf in chick pea seedlings under NaCl salinity.

Strogonov (1964) reported higher protein content in maize under sulphate salinity. According to him in general, salt tolerant plant maintain protein synthesis under saline conditions while salt susceptible plants could not maintain protein synthesis under saline conditions. Shevyakova and Leonovo (1975) have noticed that NaCl and Na_2SO_4 at concentrations of 1 to 3.25 % Na per unit dry weight stimulated protein synthesis. Ashour et al. (1977) and Siegel et al. (1980) have reported the same under chloride salinity in wheat and corn respectively. Stiborova et al. (1987) reported increase in protein content in roots of Hordeum vulgare and Zea mays L. at NaCl concentration of 0-100 mM. Ashraf (1989) reported increase in leaf protein content in Cv's Candhari Mash and Mash 654 of Vigna mungo. Plaut and Federman (1991) concluded that cotton leaves acclimated to salinity of -0.3 to -0.6 mega pascal by synthesizing more protein content per unit leaf area. Ashraf and Naqvi (1992) reported increase in soluble proteins in Brassica napus at highest Na/Ca ratio whereas osmotica remained unchanged. According to Ventakesalu et al. (1994) , increase in protein content upto 600 mM NaCl in Sesuvium portulacastrum L. a salt marsh halophyte.

Chandrashekhar and Sandhyarani (1995) reported increase in protein content with increasing NaCl salinity (0, 0.2, 0.4, 0.6 and 0.8 %) concentration in Crotolaria striata DC. Kord and Khalil (1995) observed increase in protein when pea seeds were soaked for 5 days in 10-2 M NaCl which arrested germination and water uptake. According to Muthuchelian et al. (1996) salt stress (100 mM to 250 mM NaCl) increased protein content in Erythrina variegata seedlings. Abd El-Samad and Shaddad (1997) reported that due to accumulation of soluble proteins, soybean Cv. Clark tolerated NaCl salinity upto osmotic potential in soil -1800 kPa and Cv. Forest to -1500 kPa. Thus, in many plants protein biosynthesis is stimulated under saline conditions.

Binett and Boucaud (1982) reported that salinity did not induce detectable changes in protein content of Phaseolus vulgaris. Stiborova et al. (1987) observed that salinity had little effect on protein content in shoots of Hordeum vulgare L. and Zea mays L. at NaCl concentrations of 0-100 mM. Ashraf and Fatima (1995) found no difference in leaf soluble proteins in salt tolerant accession and other accessions of safflower. Zidan and Elewa (1995) revealed that soluble protein in anise and coriander remained unchanged under low and moderate levels of NaCl.

Results of the present investigation (Tables.24,25 ; Fig.25ab) in safflower Cv. Bhima revealed that , protein content of total plant decreased at all levels of NaCl salinity. These results are on similar lines of the results of Shaddad (1990); Miteva (1992); and El-Samad and Abd (1993); Ramanjulu et al. (1994); Popova et al. (1995); Lutts and Buharmont (1996); Saha and Gupta (1997) and Muthukumaraswamy et al.,(1997). However, low levels of sulphate (upto ECe 7.5 $mScm^{-1}$) increased protein content in root and stem and high levels of it decreased protein content. In leaves, all levels of

Na_2SO_4 inhibited protein accumulation. Bidwell (1979) suggests that for efficient action of protein synthesising enzymes, K is required which regulated protein conformation causing exposure of active stress. The failure of uptake of K, under saline conditions, might be one of the causative factors for decreased protein synthesis under saline conditions in this cultivar of safflower.

CHAPTER VI

STOMATAL STUDIES UNDER SALINE CONDITIONS

1) INTRODUCTION

Stomata are known to play a pivotal role in productivity of plants. Thus, it is necessary to study stomatal characteristics. The plants require special adaptive mechanisms to help the plant survive under saline stress. Leaves are the major sites of transpiration and photosynthesis in higher plants. In relation to salinity induced water stress, one might expect the principal structural and metabolic modifications in leaves to be associated with a tendency to minimize transpiration rate and the occurrence of photosynthetic pathways with high water use efficiency.

Stomata are widespread on all aerial parts of the plant. Stomata being an indispensible organ of the plant, their role also being unique, it is but natural that their performance under natural and treated conditions be studied. Hence, detailed study of stomatal rhythms, stomatal frequency and stomatal aperture enabled us to study their behaviour under salt influence.

2) MATERIALS AND METHODS

At the time of flowering stomatal studies that is, stomatal index, stomatal number and stomatal frequency were studied by using fifth fully expanded leaf of control and treated plants. The stomatal studies were done by following the method of Stoddard (1965). The index and frequency were determined by using method of Trease and Evans (1972). The width of the stomatal aperture was measured every hour using ocular meter which was pre-calibrated using a stage micrometer. The peelings were observed under the microscope using thin films obtained from nail-polish (Sampson, 1961). In this method , nail polish was applied to the middle portion of the leaf. After

drying the nail polish , films were removed. Every time two impressions were taken and on each impression five different unit areas were marked out and the number of stomata were counted and studied. The stomata were counted from three leaves and the stomatal frequency was studied. Stomatal rhythms and the width of aperture for 18 hour cycle were also studied by taking epidermal leaf peelings of both surfaces and observing them every hour under the microscope. The values are average of three measurements taken from three different leaves.

3) RESULTS

A. Stomatal Index and Stomatal number

Results of effect of increasing concentrations of chloride and sulphate salinity's on stomatal Index and Stomatal number are presented in Tables. 35, 36.

The stomatal Index of upper surface and lower surface decreased gradually with increase in levels of NaCl salt. However, under the influence of Na_2SO_4 salt, stomatal index of upper surface and lower surface increased upto ECe 7.5 $mScm^{-1}$ and decreased further at all higher salinity levels.

The stomatal number of upper and lower surface decreased with increasing levels of both the salts in leaves of Carthamus tinctorius L.Cv. Bhima. These results clearly indicated that all levels of both the salts inhibit development of stomata.

B. Stomatal Rhythms

From the results recorded in Tables. 37, 38, it is clear that under control conditions, stomata of lower surface open during 9 am to 11 a.m. and during 4 to 6 p.m., while on

Table. 35.

Effect of increasing concentrations of NaCl on stomatal index and stomatal number in Carthamus tinctorius L.Cv. Bhima

Treatment	Stomatal index		Stomatal number/ mm^2	
ECe $mScm^{-1}$	U.S	L.S	U.S	L.S
Control 0.44	44.70	47.56	224.00	212.00
NaCl 5.0	44.50	47.37	175.00	208.00
NaCl 7.5	37.90	46.52	172.00	205.00
NaCl 10.0	36.17	41.41	164.60	180.00
NaCl 12.5	-	-	-	-
SEM	1.96	1.39	34.8	31.18
LSD	6.05	4.30	107.3	96.10
SD±	± 4.07	± 3.28	56.97	48.32

U.S - Upper surface L.S - Lower surface
-Plants survived upto 60 to 90 days of growth

Table. 36.

Effect of increasing concentrations of Na_2SO_4 on stomatal index and stomatal number in Carthamus tinctorius L.Cv. Bhima

Treatment	Stomatal index		Stomatal number/ mm^2	
ECe mScm-1	U.S	L.S	U.S	L.S
Control	44.70	47.56	224.00	212.00
Na2SO4 5.0	44.81	48.36	210.30	178.00
Na2SO4 7.5	48.82	48.52	193.30	175.00
Na2SO4 10.0	44.44	39.41	183.00	172.33
Na2SO4 12.5	40.25	39.58	172.00	164.00
Na2SO4 15.0	-	-	-	-
SEM	2.43	3.01	31.18	
LSD	7.49	9.29	96.10	
SD±	± 4.54	± 6.20	48.32	

U.S - Upper surface L.S - Lower surface
-Plants survived upto 60 to 90 days of growth

Table. 37.

Effect of increasing concentrations of NaCl on Stomatal aperture in Carthamus tinctorius L. Cv. Bhima

Time (hr)	0600		0700		0800		0900		1000		1100		1200	
Treatment ECe $mScm^{-1}$	US	LS	US	LS	US	LS	US	LS	US	LS	US	LS	US	LS
Control 0.44	-	-	-	-	9.36	-	9.36	9.24	9.24	9.32	9.36	9.36	-	-
NaCl 5.0	-	-	-	-	-	-	6.24	6.24	6.42	6.12	-	-	-	-
NaCl 7.5	-	-	-	-	-	-	6.24	6.24	6.52	6.24	-	-	-	-
NaCl 10.0	-	-	-	-	-	-	6.24	6.24	6.61	6.12	-	-	-	-
NaCl 12.5														

Time (hr)	1300		1400		1500		1600		1700		1800		1900		2000	
Treatment ECe $mScm^{-1}$	US	LS	US	LS	US	LS	US	LS	US	LS	US	LS	US	LS	US	LS
Control	-	-	-	-	6.24	-	6.24	6.24	9.36	9.36	6.24	6.24	-	-	-	-
NaCl 5.0	-	-	-	-	-		6.24	6.24	9.36	9.36	6.24	6.24	6.24	6.24	-	-
NaCl 7.5	-	-	-	-	-	-	6.24	6.24	9.36	9.36	6.24	6.24	6.24	6.24	-	-
NaCl 10.0	-	-	-	-	-	-	6.24	6.24	9.36	9.36	6.24	6.24	6.24	6.24	-	-
NaCl 12.5																

-Stomata closed.
US - Upper Surface LS - Lower Surface
Width of stomatal aperture expressed in U.

Table. 38.

Effect of increasing concentrations of Na_2SO_4 on stomatal aperture in Carthamus tinctorius L. Cv. Bhima

Time (hr)	600		700		800		900		1000		1100		1200	
Treatment ECe $mScm^{-1}$	US	LS	US	LS	US	LS	US	LS	US	LS	US	LS	US	LS
Control	-	-	-	-	9.36	-	9.36	9.24	9.24	9.32	9.36	9.36	-	-
Na_2SO_4 5.0	-	-	-	-	9.36	-	6.24	9.36	6.24	6.24	-	-	-	-
Na_2SO_4 7.5	-	-	-	-	6.24	-	6.24	6.24	6.24	6.24	-	-	-	-
Na_2SO_4 10.0	-	-	-	-	3.12	-	6.24	6.24	6.24	6.24	-	-	-	-
Na_2SO_4 12.5	-	-	-	-	3.12	-	6.24	6.24	6.24	6.24	-	-	-	-
Na_2SO_4 15.0														

Time (hr)	1300		1400		1500		1600		1700		1800		1900		2000	
Treatment ECe $mScm^{-1}$	US	LS	US	LS	US	LS	US	LS	US	LS	US	LS	US	LS	US	LS
Control	-	-	-	-	6.24	-	6.24	6.24	9.36	9.36	6.24	6.24	-	-	-	-
Na_2SO_4 5.0	-	-	-	-	-	6.24	6.24	6.24	9.36	9.36	6.24	6.24	6.24	6.24	-	-
Na_2SO_4 7.5	-	-	-	-	-	6.24	6.24	6.24	9.36	9.36	6.24	6.24	6.24	6.24	-	-
Na_2SO_4 10.0	-	-	-	-	-	-	6.24	6.24	9.36	9.36	6.24	6.24	6.24	6.24	-	-
Na_2SO_4 12.5	-	-	-	-	-	-	6.24	6.24	9.36	9.36	6.24	6.24	6.24	6.24	-	-
Na_2SO_4 15.0																

-Stomata closed.
US - Upper Surface LS - Lower Surface
Width of stomatal aperture expressed in U.

upper surface stomata was open during 8 to 11 a.m. and 4 to 6 p.m. This showed two peaks of opening like a typical C_3 plant. Under both the salinity's stomata remained open during 9 to 10 a.m. and 4 to 7 p.m. Thus, stomatal rhythm was altered under both the salinity's.

4) DISCUSSION

There are few reports on effect of salinity on stomatal frequency. Sharada Devi and Rao (1978,1980) studied the effect of salinity on stomatal frequency and characteristics of stomata in groundnut and reported that both frequency, index and rhythm was altered by salinity. It is well known that stress induces shift in rhythm in stomatal movement (Huber and Sankhla, 1972; Joshi et al., 1976; Fischer et al.,1978). But correlation of tissue K with the frequency of stomata due to salinity is not much known.

Gill and Dutta (1982) in barley and Patil et al. (1983) in sorghum hybrid C5H5 have reported decrease in stomatal number under saline conditions which helps for reducing transpiration under saline conditions. Strogonov (1964) and Waisel (1972), while indexing the common effects of salinity referred to the reduced number of stomata per unit area in the context of diminishing size of the organ. According to Strogonov (1974), cells failed to divide under stress conditions rather than elongate and this leads to reduced frequency of stomata per unit area. Robinson et al. (1983) noted that, when Spinacea oleraceae plants were subjected to final concentration of 200 mM, stomatal conductance was reduced by 70 % which decreased actual photosynthesis. Longstreth et al. (1984) observed decline in stomatal conductance in parallel with net rate of CO_2 uptake (P_a) which did not result in intercellular CO_2 concentration in Alternanthera philoxeroides, an alligator weed, at 0-400 NaCl range. Rao (1985) reported decrease in

stomatal opening in Cajanus indicus spreng var. LRG-30 and Sesamum indicum L. var. TMV 1 under NaCl salinity. The stomata were wide open during the early hours of the day. Guy and Reid (1986) observed that depressed intercellular CO_2 concentration which decreased stomatal conductance coupled with small effects of intrinsic photosynthetic capacity in Puccinella muttalliana (Schutes) Hitch.

Pezeshki et al.(1988) flooded Taxodium distichum L. with tap water (control) and salinity of 50 $molm^{-3}$ and observed a decline in stomatal conductance. Flanagan and Jafferies (1989) grew Plantago maritima at low and high salinity levels and observed that, 50 $molm^{-3}$ to 300 $molm^{-3}$ salinity, resulted in reduction of stomatal conductance. Shaddad (1990) reported lowering in stomatal frequency in Raphanus sativus plants with increasing NaCl salinity which was counteracted by addition of proline solution (200 g m^{-3}) at low and moderate salinity levels. Brugnoli and Lauteri (1991) observed a reduction in stomatal conductance in Gossypium hirsutum L. and Phaseolus vulgaris at different NaCl concentrations from 10 day old seedlings till mature reproductive structure formation. Banuls and Millo (1992) reported decrease in stomatal conductance due to accumulation of chloride ions in Citrus sinensis L. osbeck Cv. Hamlim under salt stress but was increased due to Ca (as calcium acetate). Marcar (1993) found reduction in stomatal conductance when eucalyptus plants were treated with 150 or 100 $molm^{-3}$ NaCl with water logging for 4 weeks. Everard (1994) observed a decrease in stomatal conductance at intermidiate (100 m M NaCl) in root zone salinity in celery (Apium graveolus L.)compared to control. Meinzer et al. (1994) reported decline in stomatal conductance (gs) at EC 2, 4, 8, 12 dsm^{-1} of NaCl of irrigation solution increased above 2 ds/m and gs was high in sugarcane resistant cultivar (H69-8335) at all levels of salinity but declined less sharply with increasing salinity.

According to Perera et al. (1994) stomatal opening was suppressed by increasing NaCl concentration in Aster tripolium which has no glands or cannot excrete salt explaining that salt accumulation in cell vacuoles increase Na ions in apoplast around guard cells causing partial closure reducing transpiration and increasing water use efficiency, reducing flow of salt to leaves and not affecting new photosynthates synthesis and growth. Ayala and O'Leary (1995) observed a decreased stomatal conductance with increasing salinity (5, 200 or 600 $molm^{-3}$ NaCl) which increased transpiration rate at low salinity level in Salicornia bigelovii Torr. plants. Lakshmi et al. (1996) reported decrease in stomatal conductance (gs) in Morus alba L. cultivars (S-30 and K-2) under saline conditions.

Rao and Gururaja (1985) found that Cajanus cajan leaves had widest stomatal opening during early hours of the day. Kayani and Rehman (1988) observed that stomatal number, stomatal size and stomatal index increased with increased salinity in Zea mays L. Cv. sunahry. Ziska et al. (1990) reported that during fruit ripening and expansion period increase in stomatal conductance resulted in leaf water loss at field salinity conditions. Sharma (1996) reported that stomatal conductance (gs) was highest in Triticum durum L. Cv. HD 4502 leaves , which declined in the middle and fully expanded leaves and was minimum in the oldest leaves under steady state salinity's (1.6, 12.0 and 16.0 dsm^{-1}) for eight weeks.

Results of present investigation (Tables.37,38) in Carthamus tinctorius L.Cv. Bhima revealed that pattern of stomatal opening is similar to that of C_3 plants and it showed two peaks of opening under control and saline conditions. Under control conditions, stomata opened at 9 a.m. and closed at 12 noon in the morning and in the evening

opened at 3 p.m. and closed at 6 p.m. Under both the salinity's, stomata of upper and lower surfaces opened at 9 a.m. and closed at 11 a.m. and in the evening opened at 3 p.m. and closed at 7 p.m. Thus, during morning session stomata closed one hour earlier, while in evening session they closed one hour late. Thus, total opening time was similar, however, stomata were closed one hour earlier that is at 11 a.m., when there was more heat. The stomata remained closed when the heat was maximum. Thus, plants adapt to saline conditions by conserving water. Plants also adapt to saline conditions by decreasing number of stomata which leads to conservation of water.

CHAPTER VII

PHOTOSYNTHETIC ENZYMES UNDER SALINE CONDITIONS

1) INTRODUCTION

Plants regulate various aspects of their growth in a synchronized form with a high degree of organisation involving co-ordination of many components. Regulation of various metabolic processes have direct control with respect to regulation of catalysis, action and transport and to understand the metabolic activities, it becomes necessary to study multi-enzyme systems because metabolic processes in plant system occur due to specific enzyme activity.

In living cells, the intense chemical activity occurs due to activity of numerous specific enzymes which leads to consideration of interdependence of physiological processes (Street and Cockburn, 1972). Under saline conditions, growth is not only related to osmotic and nutritional effects but also to the disturbances in their normal physiological and metabolic processes (Strogonov and Henckel, 1961; Gauch and Eaton,1942). Also under salt stress, it is indicated that salt induces decrease or increase in enzyme activity (Hasson-Porath and Poljakoff-Mayber, 1968), which in turn reflects several metabolic processes.

The biochemical processes inside the leaf cells generally regulate the growth and development of plants. But toxicity influences early metabolic changes, such as synthesis of enzyme, to a greater extent (Malik and Sahukat, 1986). The adaptation of glycophytes to saline soil in adverse conditions is possible mainly because of changeability of their metabolism and chemical properties of the protoplasm (Strogonov and Heinke, 1961). Oxidative, photosynthetic and photorespiratory enzymes are given importance because of their various interrelationships in the process of growth and

development. Physiologists and biochemists have tried to correlate the possible role of these enzymes to relative metabolic processes of the plants under saline conditions.

RuBP-case is the main enzyme of CO_2 fixation in the C_3 plants while PEP-Case and RuBP-Case are important enzymes of the C_4 plants. C_3 pathway of photosynthesis is always associated with photorespiration. Therefore, photorespiratory enzymes like Phosphoglycolate phosphatase which are responsible for conversion of phosphoglycolate into glycolate. Glycolate oxidase is a key photorespiratory enzyme and converts glycolate into glyoxylate.

Peroxidase is an oxidative enzyme and it is also essential for the conversion of H_2O_2 into H_2O and (O) in photorespiration. According to Strogonov (1964) peroxidase plays an important role in adaptation of plants to saline conditions by regulating toxic accumulation of H_2O_2. Hence it was proposed to study the activity of peroxidase in Carthamus tinctorius Cv. Bhima to understand the mechanism of salt tolerance. Pyruvate Pi dikinase, an important enzyme of C_4 pathway was also studied.

We have tried to correlate the role of enzymes with various processes like photosynthesis and photorespiration in Carthamus tinctorius Cv. Bhima.

2) MATERIALS AND METHODS

The activities of the photosynthetic and photorespiratory enzymes, that is RuBP-Case (Rubisco), PEP-Case, phosphoglycolate phosphatase, glycolate oxidase (glycolate oxygen reductase) and peroxidase were estimated at the time of flowering. The fifth (fully expanded leaf) was used for enzyme studies of the control and treated plants. The plants that were grown in pots (as described in Chapter-II) was the source material

for enzyme studies. Enzyme extraction and assay was done by using standard methods.

A. PHOTOSYNTHETIC ENZYMES

a) RuBP-Carboxylase (E.C. 4.1.1.39) and b) PEP-Carboxylase (E.C. 4.1.1.31)

i) Enzyme extraction

The isolation of RuBP-Carboxylase (Rubisco) and PEP-Carboxylase was done by the method of Kluge and Osmond (1972).

The extraction and assay was performed at 0-4^{o} C. The material was homogenised in ice cold buffer (pH 8.2) containing 200 mM Bicine, 170 mM 2- ME, 10mM $MgCl_2$ and 16 g / L PVP (Molecular. Weight. 10,000). To this 50 mM $NaHCO_3$ was added (Tabita et al., 1976). The homogenate was filtered through 4 layers of muslin cloth and centrifuged at 30,000 X g for 20 min. The supernatant was used for RuBP-Case and PEP-Case enzyme activity.

Protein estimation was done by Kluge and Osmond (1972) method. An aliquot of an enzyme extract was mixed with three volumes of 10% TCA to precipitate proteins together with PVP, which was re-dissolved by adding 70% ethanol and stirring till the volume was doubled. This was kept overnight to precipitate. After centrifugation, the protein was washed thrice with water and estimated by Lowry et al. (1951) method.

Specific activities of enzymes were expressed in units per mg protein per minute (Recker, 1957). All the experiments were run in triplicate.

ii) Assay of RuBP-Case

RuBP-Carboxylase enzyme was assayed by following the method of Kluge and Osmond (1972). Enzyme activity was measured by activating enzyme by pre incubation with 20 μ moles $MgCl_2$ at pH 8.2 and by exposing to light (Lorimer et al., 1977). The final volume of assay medium was made to 3 ml which contained 50 μ moles Bicine, 50 μ moles MES, 30 μ moles $MgCl_2$, 10 μ moles 2 ME, 6 μ moles ATP, 50 μ moles $NaHCO_3$, 0.2 μ moles RuBP , 1 unit PG Kinase, 4.5 units GAP Dehydrogenase and 0.25 μ moles NADH at pH 8.2. The reaction was initiated by addition of substrate (RuBP). The change in Optical Density per min. was measured on Shimadzu UV-210, a double beam spectro photo meter at 340 nm.

iii) Assay of PEP-Case

PEP-Carboxylase was carboxylated resulting into reduction of oxalo acetate by Kluge and Osmond (1972) method. The final volume of assay medium was made to 3 ml which contained 50 μ moles Bicine, 50 μ moles MES, 10 μ moles $MgCl_2$, 10 μ moles 2 ME, 2 μ moles $NaHCO_3$, 1 μ moles PEP units and 0.083 μ moles NADH at pH 8.2. External MDH was not added to the reaction mixture (Codd and Steward, 1973). The change in optical density per min was measured on Shimadzu UV-210, a double beam spectro photo meter at 340 nm.

B. PHOTORESPIRATORY ENZYMES

c) Phospo glycolate phosphatase (E.C.3.1.3.18)

i) Enzyme extraction

This enzyme was extracted as follows. 0.1 g of material was cut into pieces and crushed in chilled buffer 0.01 M Tris-HCl pH 6.9. The extract was centrifuged at 8000 X g for 10 min at 4°C. The volume of the clear supernatant was noted and used as a source of enzymes.

ii) Assay of Phospo glycolate phosphatase

The total 12 ml of assay mixture contained 0.01 M Tris HCl buffer, 100 μ M phospho glycolate 100 mM $MgCl_2$, 10% TCA, ANSA Reagent. After incubation at 37°C for 30 min the reaction was terminated with 1 ml of 10% TCA. The assay mixture was centrifuged to remove the precipitate. The released phosphorus was measured. The optical density was measured at 625 nm.

Proteins were estimated by following Kluge and Osmond (1972) method.

d) Gycolate-oxidase (E.C. 1.1.3.1)

i) Enzyme extraction

This enzyme extraction was done by the method of Hess and Tolbert (1967). 1 g of leaf material was homogenised in 10 ml of 0.1 M cold. phosphate buffer (pH 8.3) containing 0.25 mM EDTA and 0.1 M cystein at 4°C. The homogenate was filtered through four layers of muslin cloth. From the filtrate an aliquot was saved for protein estimation which was done according to Lowry et al. (1951) method. The remaining

filtrate was centrifuged at 2-4°C at 15,000 x g for 15 minutes. The clear supernatant was used as the enzyme source.

ii) Assay of Gycolate-oxidase

The assay was carried out by the method of Patil and Hegde (1981). The total 3 ml assay mixture contained 2.4 ml of 0.1 M phosphate buffer (pH 8.3), 0.1 ml of 0.1 M phenyl hydrazine hydrochloride (pH 6.8) , 0.1 ml of 0.1 M cysteine hydrochloride, 0.3 ml of 0.1 M sodium glycolate and 0.1 ml of enzyme extract. The reaction was initiated by adding the substrate (Glycolate). The activity of this enzyme was measured spectro photometrically at 324 nm. The change in Optical density. per min due to formation of glyoxylate phenyl hydrazone was recorded at 0 minute and after 10 minute on a Shimadzu UV-210, a double beam spectrophotometer.

e) Peroxidase (E.C.1.11.1.7)

i) Enzyme extraction

Activity of peroxidase was studied at the time of flowering in Carthamus tinctorius Cv. Bhima under control and saline conditions. This enzyme was extracted by the Vidyasekharan and Durairaj (1973) method. The fifth leaves from the top were selected, washed, blotted dry and weighed leaves were crushed in chilled mortar in 0.1 M phosphate buffer (pH 7.0). The extract was centrifuged in cold at 16000 X g for 10 min and clear supernatant was used as the enzyme source.

ii) Assay of Peroxidase

This enzyme was assayed by the Vidyasekharan and Durairaj (1973) method. The total assay mixture of 4 ml contained 2 ml of phosphate buffer (pH 7.0), 1 ml of freshly

prepared 10 mM Guaicol, 0.01 ml of 20 mM H_2O_2, 1 ml of enzyme extract. Optical density was measured at 470 nm at 0 min and after 1 min intervals upto 5 min.

f) Pyruvate Pi orthophosphate dikinase (Pyruvate Pi dikinase, PPDK) (E.C. 2.7.91)

i) Enzyme extraction

The enzyme Pyruvate pidikinase was extracted and assayed by Sugiyama et al.(1979) method. Five leaves from top of shoot were selected, washed and blotted dry. 0.1 g of material was weighed. The leaves were crushed in cold Tris-HCl buffer 100 mM (pH 8.0, 5 ml), 10 mM $MgCl_2$, 1mM EDTA, 0.5% sodium ascorbate, 2%(W/V) polyvinyl pyrrolidine and 10mM 2-Mercaptoethanol. The crude extract was passed through 80 µm net and centrifuged at 10,000 x g for 10 min at 22°C. The homogenate was centrifuged in cold centrifuge at 4°C for 10 min at 8000 g. The supernatant was separated and the volume of extract was noted.

ii) Assay of Pyruvate Pi dikinase

The total assay mixture of 1.5 ml contained Tris buffer 100 mM pH 8.0, 10 mM $MgCl_2$, 0.1 mM EDTA, 1.25 mM sodium pyruvate, 5mM DTT, 0.2 mM NADH, 2.5 mM K_2HPO_4, 50 mM $NaHCO_3$, 1.25 mM ATP, 2 units PEP-Carboxylase enzyme and 2 units malate Dehydrogenase Protein concentration was determined according to the method of Lowry et al. (1951). The specific activity of enzyme was determined as change in u moles/ mg protein/ min.

3) RESULTS

The results of enzymes in vivo studies under NaCl and Na_2SO_4 salinity's are given in Tables.39,40 ;Fig.27abc,28abc and in vitro studies under both the salinity's are given in Tables.41,42.

a) RuBP-Carboxylase and b) PEP-Carboxylase

i) In vivo effect of NaCl and Na_2SO_4 on activities of RuBP- Case and PEP- Case in safflower Cv. Bhima.

From the results presented in Tables. 39, 40; Fig. 27ab,28ab , it is clear that activity of RuBP-Case in plants grown at all levels of NaCl, decreased linearly with increasing salinity levels. Under sulphate treatment, however, the activity increases upto ECe 7.5 $mScm^{-1}$ but decreased at higher salinity levels which reflects that all levels of NaCl inhibit while low levels of Na_2SO_4 stimulate and high levels of it inhibit activity of Rubisco. Thus ,effect of NaCl and Na_2SO_4 is different.

The activity of PEP-Case was observed to be more than the control at all levels of both the salinity's. Highest activity of PEP-Case was found at ECe 15.0 $mScm^{-1}$. These results indicate that all levels of both the salts stimulate activity of PEP carboxylase in this plant. The ratio of RuBP-Case and PEP-Case decreased with increasing NaCl and Na_2SO_4 salinity's. Thus, the results indicated that saline conditions alter the ratio of RuBP-Case to PEP-Case, which reflects that effect of salt is different on RuBP-Case and PEP -carboxylase .Under control conditions , ratio of RuBP-Case to PEP-Case was 6.44 indicating its C_3 nature while under both the saline conditions, its ratio altered. This suggested that effect of both the salts is different on activities of RuBP-Case and PEP-case.

Table. 39.

A comparativestudy of RuBP-Case, PEP-Case, RuBP-Case/PEP-Case, PGP, Glycolate Oxidase, Peroxidase and Pyruvate Pi dikinase enzyme activities in vivo under NaCl salinity

Enzyme	RuBP-Carboxylase		PEP-Carboxylase		RuBP-Carboxylase / PEP-Carboxylase Ratio		Phosphoglcolate Phosphatase		Glycolate Oxidase		Peroxidase		Pyruvate Pi dikinase	
In vivo treatment $mScm^{-1}$	Activity	% of control	Activity	% of control	Activity	% of control	Activity	% of control	Activity	% of control	Activity	% of control	Activity	% of control
Control 0.44	270.73	100.00	42.03	100.00	6.44	100.00	74.35	100.00	12.50	100.00	408.00	100.00	0.102	100.00
NaCl 5.0	246.39	91.00	48.54	115.48	5.07	78.72	78.45	105.51	13.70	105.51	448.00	109.80	0.169	165.68
NaCl 7.5	217.54	80.34	53.62	127.57	2.66	41.30	11.64	15.66	18.40	15.66	230.00	56.37	0.056	54.90
NaCl 10.0	155.09	57.28	68.93	164.00	2.24	34.78	126.82	170.57	19.10	170.57	176.00	43.13	0.041	40.19
NaCl 12.5	71.78	26.51	71.78	170.78	1.00	15.52	139.00	186.95	20.70	186.95	182.00	44.60	0.028	27.45
NaCl 15.0	70.38	25.99	75.56	179.77	0.93	14.44	154.70	208.07	30.10	208.07	125.00	30.63	0.010	9.80
SD±	± 81.90		±12.98		±2.10		± 49.87		31.90 ±		±125.5		± 0.05	

specific activity is expressed as μ mole / mg protein / min.

Table. 40.

A comparative study of RuBP-Case, PEP-Case, RuBP-Case/PEP-Case, PGP, Glycolate Oxidase, Peroxidase and Pyruvate Pi dikinase enzyme activities in vivo under Na_2SO_4 salinity

Enzyme	RuBP-Carboxylase		PEP-Carboxylase		RuBP-Carboxylase / PEP-Carboxylase Ratio		Phosphoglycolate Phosphotase		Glycolate Oxidase		Peroxidase		Pyruvate Pi dikinase	
In vivo treatment $mScm^{-1}$	Activity	% of control	Activity	% of control	Activity	% of control	Activity	% of control	Activity	% of control	Activity	% of control	Activity	% of control
Control 0.44	270.73	100.00	42.03	100.00	6.44	100.00	74.35	100.00	12.50	100.00	408.00	100.00	0.102	100.00
Na_2SO_4 5.0*	287.24	106.09	51.26	121.96	2.35	19.40	82.50	110.96	13.40	107.20	440.00	107.84	0.199	195.09
Na_2SO_4 7.5	321.54	118.76	69.21	164.66	1.95	30.27	88.99	119.69	13.60	108.80	477.00	116.91	0.185	181.37
Na_2SO_4 10.0	262.88	97.100	74.02	176.11	3.55	55.12	91.59	123.19	14.10	112.80	545.00	133.58	0.123	120.58
Na_2SO_4 12.5	187.31	69.18	75.56	179.77	2.47	38.35	93.60	125.89	15.00	120.00	683.00	167.40	0.108	105.88
Na_2SO_4 15.0	98.50	36.38	78.33	186.36	1.25	19.40	112.49	151.30	16.90	135.20	588.00	144.11	0.091	88.23
SD±	± 76.45		±13.83		±1.68		± 32.46		±32.74		±101.46		± 0.05	

specific activity is expressed as μ mole / mg protein / min.

Table. 41.

A comparative study of RuBP-Case, PEP-Case, RuBP-Case/PEP-Case, PGP, Glycolate Oxidase, Peroxidase and Pyruvate Pi dikinase enzyme activities in vitro under NaCl salinity

In vitro Treatment	Enzyme Activity						
Concentration (mM)	RuBP-Carboxylase	PEP-Carboxylase	RuBP-Case / PEP-Case	Phosphoglyco Phosphatase	Glycolate Oxidase	Peroxidase	Pyruvate Pi dikinase
Control	270.73	42.03	6.44	74.34	12.50	408.00	0.102
NaCl 1.0	255.64	44.00	5.81	89.25	14.20	430.10	0.109
NaCl 5.0	300.73	54.00	5.56	101.10	16.40	445.00	0.115
NaCl 7.5	306.68	64.00	4.79	136.47	25.20	460.10	0.156
NaCl 10.0	340.65	76.21	4.46	148.68	30.40	480.12	0.111
NaCl 15.0	369.81	81.25	4.55	186.31	36.20	510.10	0.101
NaCl 20.0	275.32	78.32	3.51	192.00	39.10	570.12	0.097
NaCl 25.0	240.11	75.52	3.17	201.10	31.03	600.20	0.081
NaCl 30.0	223.20	66.56	3.35	208.00	29.20	611.12	0.076
NaCl 50.0	210.12	44.52	4.71	200.11	24.30	725.20	0.067
NaCl 60.0	198.35	34.12	5.81	170.15	16.40	670.10	0.054
NaCl 90.0	183.20	29.58	6.19	139.00	15.20	650.12	0.049
NaCl 100.0	175.00	23.00	7.60	101.00	10.20	600.10	0.036
NaCl 120.0	170.40	14.42	11.81	90.12	8.90	570.12	0.021
NaCl 150.0	161.60	11.00	14.69	72.34	8.90	451.20	0.016
NaCl 200.0	-	-	-	61.00	8.90	415.11	0.005
NaCl 300.0	-	-	-	54.12	7.40	402.10	0.002
NaCl 400.0	-	-	-	51.15	6.20	312.15	0.001
NaCl 600.0	-	-	-	39.76	6.10	300.00	0.001
NaCl 700.0	-	-		30.12	5.70	280.11	0.001
NaCl 800.0	-	-	-	25.11	5.40	250.19	-
NaCl 1000.0	-	-	-	-	4.40	200.00	-

specific activity expressed as μ mole / mg protein / min.

- NaCl concentration not used.

Table. 42.

A comparative study of RuBP-Case, PEP-Case, RuBP-Case/PEP-Case, PGP, Glycolate Oxidase Peroxidase and Pyruvate Pi dikinase enzyme activities in vitro under Na_2SO_4 salinity

In vitro Treatment	Enzyme Activity						
Concentration (mM)	RuBP-Carboxylase	PEP-Carboxylase	RuBP-Case/ PEP-Case	Phosphoglyco Phosphatase	Glycolate Oxidase	Peroxidase	Pyruvate Pidikinase
Control 0.44	270.73	42.03	6.44	74.34	12.50	408.00	0.102
Na_2SO_4 1.0	281.12	43.12	6.53	75.95	13.60	435.00	0.119
Na_2SO_4 5.0	285.10	45.12	6.31	76.13	15.40	445.50	0.120
Na_2SO_4 7.5	278.17	50.10	5.55	79.20	16.20	450.10	0.131
Na_2SO_4 10.0	277.12	61.12	4.53	81.12	17.40	463.00	0.134
Na_2SO_4 15.0	269.00	70.10	3.83	89.20	20.50	471.10	0.135
Na_2SO_4 20.0	260.00	81.60	3.18	73.12	21.12	475.12	0.135
Na_2SO_4 25.0	254.00	70.10	3.62	71.10	22.13	460.11	0.140
Na_2SO_4 30.0	251.00	65.90	3.80	69.20	23.20	490.92	0.143
Na_2SO_4 50.0	240.00	55.10	4.35	69.20	25.40	510.11	0.162
Na_2SO_4 60.0	230.12	46.11	4.99	66.10	21.10	560.15	0.150
Na_2SO_4 90.0	210.60	43.12	4.88	63.86	19.12	570.81	0.139
Na_2SO_4 100.0	205.90	41.20	4.99	61.12	16.40	590.11	0.120
Na_2SO_4 120.0	201.00	36.00	5.58	60.12	15.50	581.12	0.110
Na_2SO_4 150.0	159.24	35.10	4.53	50.70	14.30	563.17	0.101
Na_2SO_4 200.0	-	-	-	49.10	13.20	540.11	0.096
Na_2SO_4 300.0	-	-	-	48.00	12.40	490.10	0.082
Na_2SO_4 400.0	-	-	-	46.00	11.20	470.11	0.072
Na_2SO_4 500.0	-	-	-	45.10	10.10	430.12	0.061
Na_2SO_4 700.0	-	-	-	41.00	9.20	410.00	0.042
Na_2SO_4 800.0	-	-	-	39.00	7.30	405.00	0.031
Na_2SO_4 1000.0	-	-	-	36.00	7.30	300.11	0.023

specific activity expressed as μ mole / mg protein / min.

- Na_2SO_4 concentration not used.

Fig. 27a.

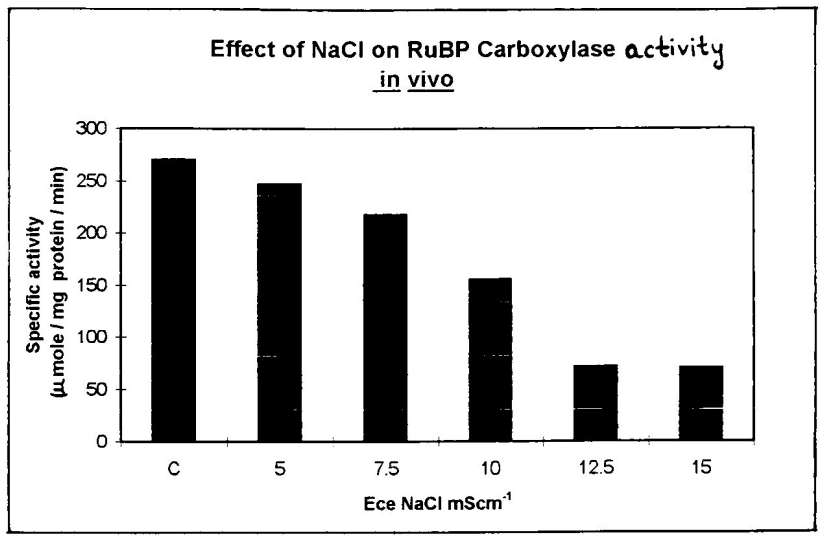


Fig. 27b.

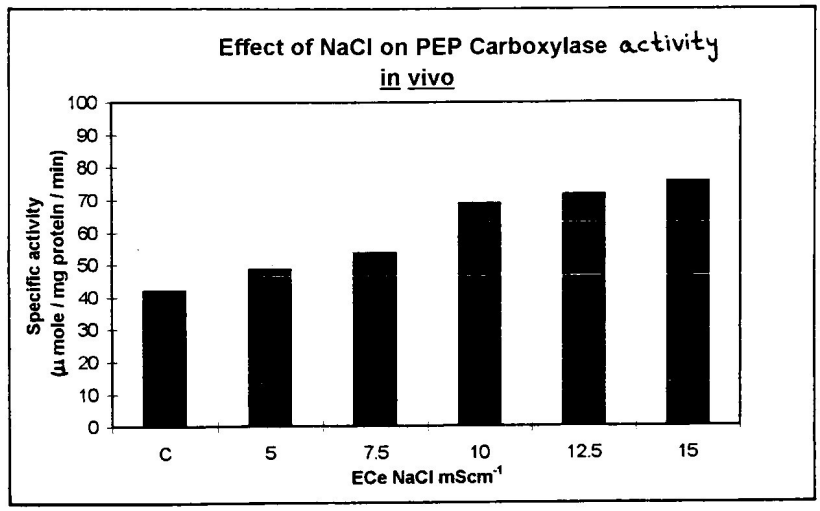


Fig. 27c.

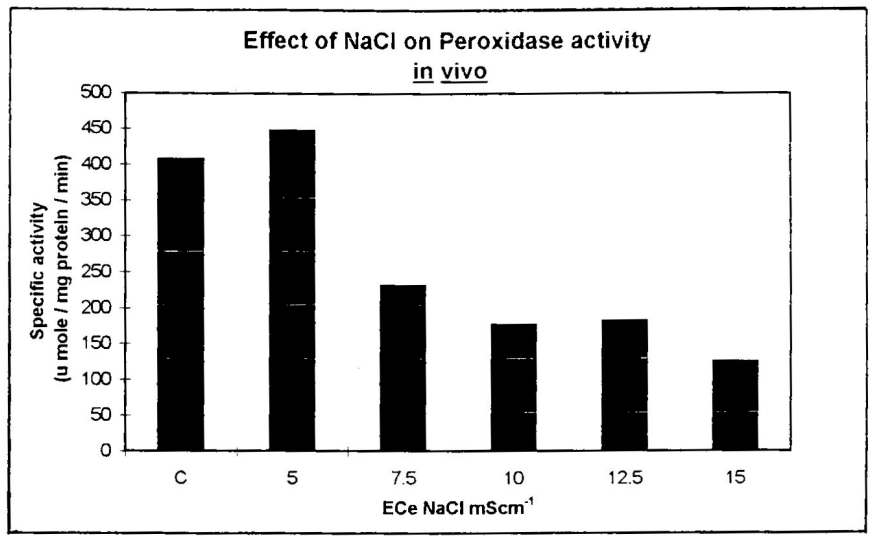


Fig. 28a.

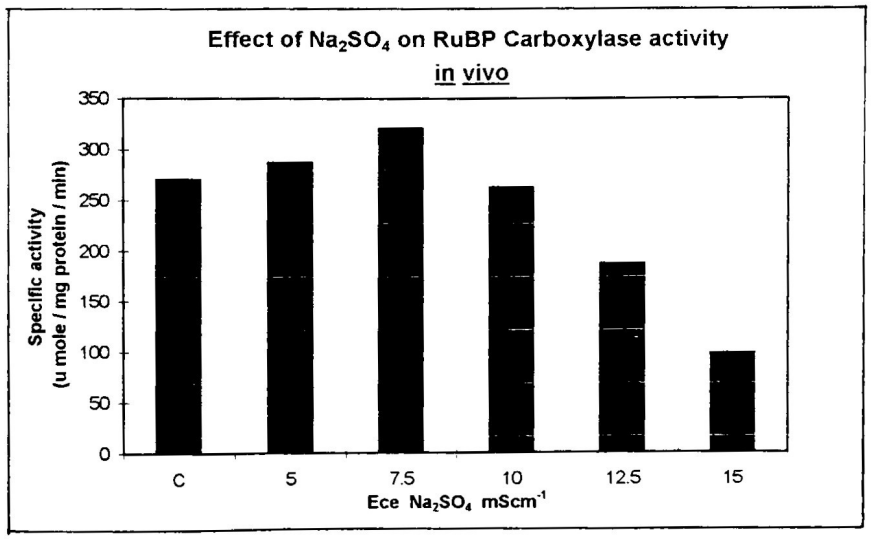


Fig. 28b.

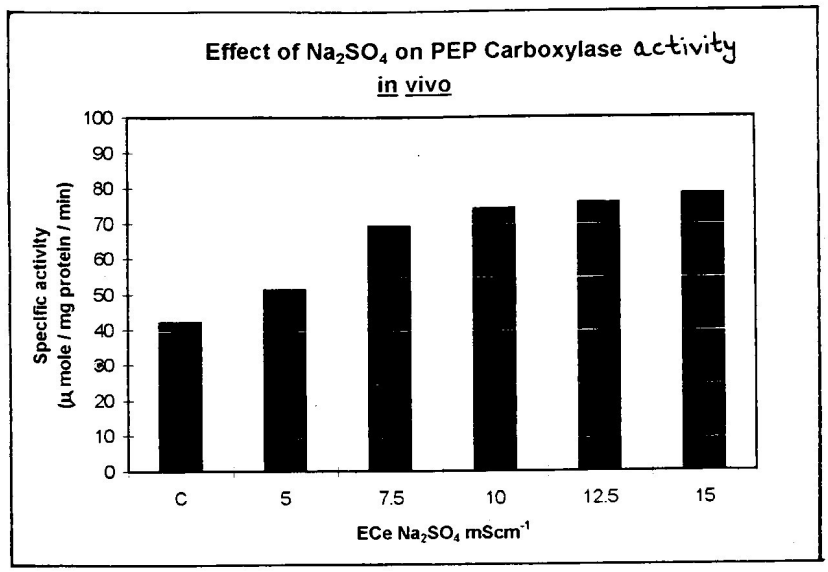
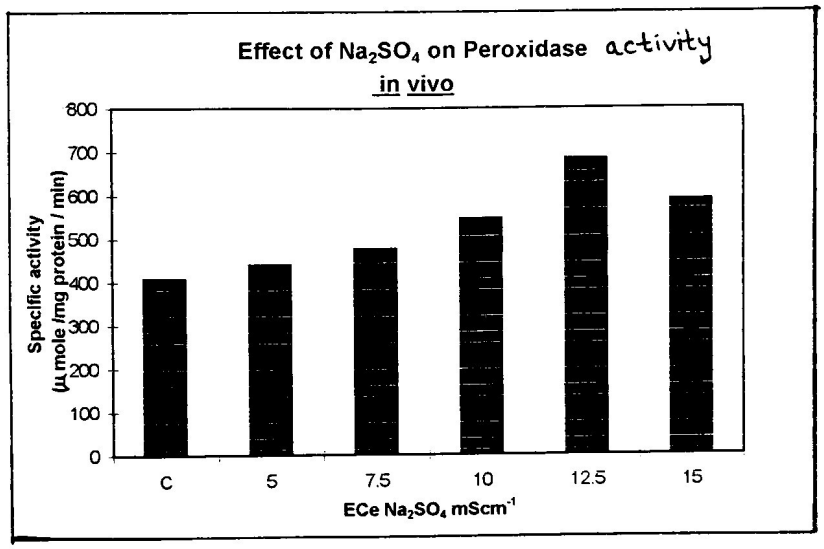


Fig. 28c.



ii) In vitro effect of NaCl and Na_2SO_4 on activities of RuBP- Case and PEP- Case in safflower Cv. Bhima.

The results depicted in Tables. 41, 42, suggest that addition of NaCl upto 20 mM, stimulated activity of RuBP-Case and addition of 25 to 150 mM of NaCl inhibited the same. Results also revealed that addition of Na_2SO_4 upto 10 mM stimulates while higher concentrations of it inhibit the activity of RuBP-Case.

The activity of PEP-Case was more than the control upto 50 mM of NaCl and upto 90 mM Na_2SO_4 salinity levels and its activity decreased at all higher levels of both the salts. Thus the results indicated that low concentrations of both the salts stimulate activities of RuBP-Case and PEP-Case while high concentrations of them inhibit activity of both the enzymes.

The ratio of RuBP-Case to PEP-Case is observed to be less than that in the control upto 90 mM NaCl and upto 150 mM Na_2SO_4 except at 1.0 mM, suggesting that PEP-Case is more tolerant to sulphate and chloride treatment than RuBP-Case.

c) Phospho glycolate phosphatase (PGP)

i) In vivo effect of NaCl and Na_2SO_4 on activity of phosphoglycolate phosphatase in safflower Cv. Bhima.

Under NaCl and Na_2SO_4 treatment (Tables. 39,40), PGP activity was more than the control in plants grown at all levels of both the salts which indicated that all levels of both the salts stimulated activity of PGP.

ii) In vitro effect of NaCl and Na_2SO_4 on activity of phosphoglycolate phosphotase in safflower Cv. Bhima.

In vitro conditions (Tables. 41,42), indicated that under NaCl conditions activity of PGP was more than the control upto 120 mM of NaCl, but decreases further with increasing NaCl levels. Its highest activity was observed at 30 mM of NaCl. Addition of Na_2SO_4 upto 15.0 mM stimulates its activity while at all higher (20-1000 mM) levels activity was inhibited.

d) Glycolate-oxidase

i) In vivo effect of NaCl and Na_2SO_4 on activity of glycolate oxidase in safflower Cv. Bhima.

In vivo effect of NaCl and Na_2SO_4, depicted in Tables. 39,40 , indicated that activity of glycolate oxidase increased linearly with increase in concentration of NaCl and Na_2SO_4. Thus, all levels of salt stimulate the activity of glycolate oxidase in Carthamus tinctorius Var. Bhima.

ii) In vitro effect of NaCl and Na_2SO_4 on activity of glycolate oxidase in safflower Cv. Bhima.

Results given in Tables. 41,42, suggest that highest activity of glycolate oxidase was recorded at 20 mM NaCl and decreased at all higher levels (upto 1000 mM). Addition of Na_2SO_4 upto 200 mM, to the reaction mixture resulted in increase in activity of glycolate oxidase. while addition of higher (300 to 1000 mM) concentrations of it inhibited the same which reflects that low concentrations of Na_2SO_4 stimulate activity of glycolate oxidase. Thus, in vivo results of glycolate oxidase were confirmed by in vitro studies.

e) Peroxidase

i) In vivo effect of NaCl and Na_2SO_4 on activity of peroxidase in safflower Cv. Bhima.

From the observations given in Table.39; Fig. 27c , it is clear that activity of peroxidase increases in plants grown upto ECe 5.0 $mScm^{-1}$ NaCl followed by sudden decrease at higher salinity levels. Under Na_2SO_4 treatment (Tables.40 ; Fig.28c) , however, enzyme activity increased linearly with increasing salinity levels indicating that low levels of NaCl and all levels of sulphate stimulate peroxidase activity whereas high levels of NaCl inhibited peroxidase activity. Thus, peroxidase plays an important role for salt tolerance under both the salinizations.

ii) In vitro effect of NaCl and Na_2SO_4 on activity of peroxidase in safflower Cv. Bhima.

From the results (Tables. 41,42), it is clear that activity of enzyme is stimulated upto 400mM NaCl and highest activity was recorded at 200 mM NaCl and it was decreased at all high concentrations (upto 1000 mM) of NaCl. Under sulphate treatment, its activity increased upto 700mM of Na_2SO_4 and decreased at all higher (upto 1000 mM) concentrations of it .

f) Pyruvate Pi orthophosphate dikinase (Pyruvate Pi dikinase ,PPDK)

i) In vivo effect of NaCl and Na_2SO_4 on activity of PPDK in safflower Cv. Bhima.

According to the results presented in Tables. 39,40, the activity of enzyme was stimulated at low (ECe 5.0 $mScm^{-1}$) NaCl concentrations, while it decreased at all higher salinity levels. Under Na_2SO_4 treatment, the activity increased with increasing

concentrations of salt upto ECe 12.5 $mScm^{-1}$ and decreased at very high (ECe 15.0 $mScm^{-1}$) levels of Na_2SO_4. Highest activity was recorded at ECe 5.0 $mScm^{-1}$ indicating that it is the best concentration of both the salts for activity of this enzyme.

ii) In vitro effects of NaCl and Na_2SO_4 on activity of PPDK in safflower Cv. Bhima.

Effect of in vitro NaCl (Tables. 41,42), indicated that activity of PPDK increased upto 10mM of NaCl. Addition of higher concentration of NaCl decreased activity of the same. Highest activity of PPDK was recorded at 10mM of NaCl. Addition of Na_2SO_4 stimulate the activity of PPDK upto 120 mM of Na_2SO_4. Highest activity was recorded at 50 mM of Na_2SO_4 . At all higher concnetrations its activity was less than the control.

4) DISCUSSION

a) RuBP-Carboxylase and b) PEP-Carboxylase

i) In vivo effect of NaCl and Na_2SO_4 on activities of RuBP-Case and PEP-Case:-

From the results (Tables. 39,40 ;Fig. 27ab,28ab) , it is clear that the ratio of RuBP-Case to PEP-Case is 6.44 under the control conditions indicating that it is a C_3 plant. All plants contain RuBP-Case (Rubisco) and PEP-Case (Melzer and O'leary, 1987) but their proportions differ with different photosynthetic pathways. They also reported that, in the C_4 plants, the ratio of these two enzymes is almost 1.0 while the C_3 plants have excess (15:1) of Rubisco over PEP-Case (Melzer and O'leary, 1987). PEP-Carboxylase enzyme is responsible for CO_2 fixation into oxaloacetate and plays multitude of metabolic roles in plants (Latzke and Kelly, 1983); it is of pivotal importance in C_4 photosynthesis , Crassulacean acid metabolism (Kluge,1983) and guard cell function (Willmer, 1983). It has been shown that light and darkness affect PEP-Case in a way commensurate with metabolic requirements (Manetas, 1982; Winter, 1982;

Karabourniotis et al. 1983, 1985; Huber and Sugiyama, 1986), but the biochemical mechanism remains largely unknown.

According to Poljakoff and Mayber (1982), all the enzymes do not behave identically under saline conditions and the enzymes located in certain places or on certain membranes in the cell may be salt tolerant while others may be salt sensitive. Monovalent ions frequently result in shifting the pH optimum of an enzymatic reaction (Dixon and Webb, 1955). Enzymes from the salt tolerant species of higher plants do not differ from those of salt sensitive species (Treichel et al., 1974; Greenway and Osmond, 1972; Flowers, 1972). Sankla and Huber (1974) studied the effect of NaCl ($1.7 \times 10^{-3} \times 1.7 \times 10^{-2}$ M) on the activities of photosynthetic enzymes in wheat, Lemna minor and Pennisetum typhoides and reported that salt tolerance of RuBP-Case and PEP- Case varies with species.

Weimberg (1975) have observed that the activities of RuBP-Case decreased when Pisum sativum was grown at 100 mM NaCl. According to Passera and Albuzio (1978) when Triticum durum and Triticum aestivum were subjected to 25 mM and 50mM NaCl concentrations, RuBP-Case and PEP-Case activities were affected differently by salt concentrations increased. RuBP-Case activity reached to maximum at 25 mM NaCl whereas PEP-Case increased gradually with increasing salt concentrations.The loss of RuBP-Case at higher concentrations of NaCl may be due to increased photorespiration and stimulated activity of RuBP-oxygenase under salt stress. It was observed by Seeman and Critchley (1985) that even through chlorophyll contents increased, photosynthetic CO_2 assimilation was reduced under salt stress. It was a consequence in the apparent efficiency of RuBP-Case rather than a reduction in the leaf content of the enzyme. This was consistent of increased chloride levels in the leaf chloroplast or

cytosol in the salt stressed Phaselous vulgaris. Rao (1985) reported decrease in RuBP-Case activity under salt stress in Cajanus indicus Sprang. Var. LRG-30 and Sesbania indicum L. Var. TMU-1.

It was reported by Seeman and Sharkey (1986) that salinization lowered RuBP pool size in Phaseolus vulgaris. The biochemical basis for this reduction under salt stress is unknown. One of the reasons may be due to inhibition of ATP synthesis under saline conditions. In addition, the rate of photosynthesis at any given pool size was lower for leaflets from the salinized plants than the control leaves. Thus, it can be concluded that salinity reduces the photosynthetic capacity of leaves by reducing pool of RuBP as an effect on the RuBP regeneration capacity and secondly, by reducing the activity of RuBP-Case by an unknown mechanism when RuBP is in limited supply. Salinity also has other effects on RuBP-Case which cannot be assessed by in vitro techniques (Jefferey et al., 1986).

Guy and Reid (1986) observed decrease in RuBP-Case activity with increase in salinity, though level was not typical of C_3 plant in Puccinella nuttalliana (Shultes) Hitch. Stiborova et al. (1987) grew Hordeum vulgare and Zea mays in NaCl concentrations of 0-100 mM and observed decrease, by salinity stress (upto 70% of the control). Taleisnik (1987) reported decrease in RuBP Case activity in tomato under in vivo saline conditions. Miteva (1992) observed decrease in Rubisco level by 20% than that of control plants in seven day old leaves of Hordeum vulgare L. Var. Alpfa under 100 mM NaCl. Solomon et al. (1994) found that NaCl inhibited Rubisco activity which was ameliorated by N-Methyl L-Proline fully at 200 mM NaCl. Popova et al. (1995) reported that NaCl stress imposed through root medium for eight days, decreased the activity of RuBP-Case in Hordeum vulgare L. Sudhakar et al.(1997) studied response of few

Calvin cycle enzymes to salinity shock in vitro and observed decline in RuBP Carboxylase activity in 10 day old seedlings of horsegram subjected to NaCl or Na_2SO_4 treatment in vitro indicating that RuBP- Case was more sensitive to salt shock than other enzymes and NaCl was more toxic compared to Na_2SO_4.

Shomer-illan et al. (1979) reported rise in the yield of Chloris gayana under saline conditions due to increased activity of key photosynthetic enzymes, RuBP-Case suggesting that the enzymatic systems were not damaged under high salt treatments and the potential photosynthetic capacity remained practically unaffected. Joshi et al. (1980) have reported increased RuBP Case activity under salt stress in sugarbeet and attributed to its salt tolerant nature. Plaut and Federman (1991) concluded that cotton leaves acclimated to salinity of -0.3 and -0.6 mega pascal was interpreted in light of enhanced RuBP-Case activity. Thus, in some plants lower salt concentrations increase activity of Rubisco.

According to Greenway and Osmond (1972) Na_2SO_4 salinity causes inhibition of PEP-Case in Atriplex sporgiosa and Phaseolus vulgaris. Further, they reported that Na_2SO_4 salt inhibits PEP-Case activity more than KCl and NaCl in these plants and concluded that RuBP-Case was less sensitive to salt than PEP-Case. A coastal C_3 halophyte when grown at 100 M NaCl in the growth medium yielded 30 % higher rate of PEP Case activity than did salt depleted plants (Beer et al.,1975) They further reported that the enzyme activity was stimulated with NaCl addition to the reaction mixture in concentrations upto 200 mM. Shomer-illan et al. (1975) have reported that Suaeda monoica grown at 100 mM NaCl concentration showed PEP Case activity of 0.4 μ moles CO_2 / mg protein / min while salt depleted plants showed an activity of 0.34 μ

moles CO_2 / mg protein / min which is about 15 % less. Weimberg (1975) has observed that the activities of PEP Case decrease (that is inhibited than the control) when Pisum sativum was grown at 100 mM NaCl. Naik (1980) observed more inhibition of PEP-Case activity under SO_4 salinity than Cl salinization in sugarcane. Stiborova et al. (1987) grew Hordeum vulgare and Zea mays in NaCl concentration of 0-100 mM and observed that PEP-Case from Z. mays was inhibited to 32% of the control by NaCl.

In vivo studies of Willert (1975) and Willert et al. (1976) on Mesembryanthemum crystallinum have revealed that PEP-Case activity increases under NaCl treatment suggesting that NaCl is necessary for the synthesis of PEP Case and in its presence PEP Case is induced in the plants. Shomer -Ilan et al. (1979) reported rise in the yield of Chloris gayana under saline conditions due to increased activity of key photosynthetic enzymes, PEP-Case suggesting that the enzymatic systems were not damaged under high salt treatments and the potential photosynthetic capacity remained practically unaffected.

Guy and Reid (1986) observed increase in PEP-Case activity with salinity, though the level was not typical of C_3 plant in Puccinella nuttalliana (Shultes Hitch). Hoefner et al. (1987) reported approximately 40 fold increase in PEP-Case activity in NaCl (500 mM) treated Mesembryanthemum crystallinum L. plants. De novo synthesis of PEP-Case protein was shown by immuno-precipitation by newly synthesised, radioactively labelled protein in leaf discs from salt treated plants. Non treated plants showed low levels of enzymes and low rates of PEP-Case synthesis. Saitou et al. (1991) observed increase in PEP-Case activity with increase in malate content in leaves of Mesembryanthemum crystallinum L. at the end of dark period and increase was accompanied by De novo synthesis of protein. Thomas et al. (1992) reported accumulation of specific isoform of

enzyme PEP-Case prior to the switch from C_3 to CAM under salt stress and exogenous application of abscisic acid was found to be a poor substitute for NaCl in inducing CAM specific PEP-Case accumulation. According to Li and Chollet (1994), when Mesembryanthemum crystallinum was irrigated with 0.5 M NaCl then PEP-Case protein level and PEP-Case-K activity increased after 2 days treatment and continued to rise for 8 days attaining 14 and 8 fold increase in leaves harvested at night only. Popova et al. (1995) reported that activity of PEP-Case was two fold over the control under NaCl stress imposed through root medium for a period of eight days in Hordeum vulgare L.

Manetas et al. (1986) reported that under saline conditions PEP-Case activity was physiologically affected by betaine which was compatible with PEP-Case extracted from Salsola kali and proline which did not protect enzyme against NaCl in Cynodon dactylon and Sporobolus pungeus Poaceae indicating that osmoregulators could by compatible with cytoplasmic enzymes but also promote or inhibit enzyme activity.

Results of the present investigation on Carthamus tinctorius Cv. Bhima indicated that when plants are grown at ECe 5.0 to 15 $mScm^{-1}$ of NaCl, activity of RuBP-Case decreased with increase in concentrations of NaCl in the growth medium. However, plants could maintain more productivity per plant (Table. 9) at maturity with less RuBP-Case activity (Table. 39) .Its activity was more than the control at low concentrations (ECe 5.0 to 7.5 $mScm^{-1}$)of Na_2SO_4 while at high (ECe 10 to 15 $mScm^{-1}$) concentrations it was inhibited. Thus, all concentrations of NaCl inhibit while low concentrations of Na_2SO_4 stimulate and high concentrations of it inhibit RuBP-Case in Carthamus tinctorius Cv. Bhima. Increased activity of RuBP-Case at ECe 5 to 7.5 $mScm^{-1}$ positively correlates with increased productivity in plants grown upto ECe 7.5 $mScm^{-1}$ of Na_2SO_4 (Table. 6) at flowering and its increased activity also correlates well with increased grain

yield (Table. 10) and total productivity per plant. At ECe 10.0 $mScm^{-1}$ of Na_2SO_4, total dry matter per plant was also more (194.4 % of the control) than the control (Table.10) at maturity. However, activity of RuBP-case was slightly less than the control (Table.40). Decreased activity of RuBP-Case at this level of Na_2SO_4, decreased grain yield per plant.

Activity of PEP-Case was more than the control in plants grown at all concentrations of NaCl and Na_2SO_4 (Tables.39,40) indicating that all concentrations of both the salts stimulated activity of PEP-Case which is similar to the results of several scientists (Beer et al.,1975; Shomer- Ilan et al., 1975; Willert (1975) and Willert et al., 1976; Shomer - Ilan et al., 1979; Guy and Reid 1986; Hoefner et al., 1987; Saitou et al., 1991; Popova et al. 1995).

ii) In vitro effect of NaCl and Na_2SO_4 on activities of RuBP-Case and PEP-Case:-

Holm Hansen et al. (1958) and Karmarkar and Joshi (1968) reported stimulation in carboxylation by addition of salt in growth medium. Increase in NaCl salt concentrations in the reaction medium inhibits activities of various enzymes (Ting and Osmond, 1973 and Flowers et al., 1977).

Johnson et al. (1968) reported in vitro inhibition of RuBP-Case by 3.75M NaCl. However, results obtained by (Shitole and Joshi, 1984) indicated that high concentrations of NaCl in assay medium inhibited the activity of RuBP-Case enzyme in fresh water algae Pithophora oedogonia and Oedogonium abbreviatum. Taleisnik (1987) reported decrease in RuBP-Case activity in tomato under in vitro conditions. Sudhakar et al. (1997) found that in ten days old seedlings of horsegram, addition of NaCl and Na_2SO_4 in the reaction medium caused decline in activities of RuBP-Case,

R-5-P kinase and R-5-P isomerase. Low concentration of Na_2SO_4 did not alter activities of any of the above enzymes. Further, NaCl was more toxic than Na_2SO_4 to the enzyme.

In Enteromorpha tubulosa, addition of low concentrations (upto 2 mM) of NaCl to reaction mixture stimulated RuBP-Case (Karekar, 1974). Triechel (1974,1975) has worked out effects of NaCl on in vitro activity of PEP- Case and RuBP-Case isolated from various halophytes and reported that the salt tolerance of both the enzymes varies with the species. According to Shitole and Joshi (1984), low concentration of NaCl (1 to 3 mM) in assay medium stimulated activity of RuBP- Case in fresh water algae Pithophora oedogonia and Oedogonium abbreviatum.

According to the relevant literature (O'Leary, 1982; Andreo et al. 1987), PEP-Case is a homo-tetramer with molecular weight around 400,000 and has unclear allosteric properties. O'Leary (1982) extracted PEP-Case from a C_3 halophyte Cakile maritima and showed that it is a salt tolerant as well as salt requiring enzyme. PEP-Case reveals its allosteric nature under specific conditions only (O'Leary, 1982). The number of PEP molecules bound to the enzyme, their concentration and the organisation of PEP around it may be the main factors in determining the allosteric behaviour of PEP-Case (Coombs et al., 1973). Following pre-treatment with PEP, the enzyme may become loaded with it and at this stage the allosteric nature of the enzyme becomes unclear. When NaCl is added to the assay medium, the properties of the enzymes change, NaCl modifies V_{max}, K and pH indicating that NaCl affects the binding of PEP to the enzyme protein and thus acts as an allosteric effector (Shomer-Ilan et al., 1985). Addition of moderate concentrations of NaCl change the tertiary and quaternary structure of the protein to give an active form which implies that binding of PEP to the

enzyme is affected by NaCl and co-operativity increases. Supra optimal concentrations of NaCl may cause hysteresis and consequently inactivation of the enzyme and decrease in its activity (Mevarech and Neumann, 1977; Shomer - Ilan et.al., 1985).

In vitro studies of Willert (1975) and Willert et al. (1976) on Mesembryanthemum crystallinum have reported that PEP-Case activity increases under NaCl treatment suggesting that NaCl is neccesary for the synthesis of PEP- Case and in its presence PEP- Case is induced in the plants. In Enteromorpha tubulosa, addition of NaCl (upto 20 mM) to reaction mixture stimulated PEP- Case (Karekar, 1974). According to Shitole and Joshi (1984) PEP- Case activity increased with increase in NaCl concentration in reaction mixture from 1 to 25 mM in fresh water algae Pithophora oedogonia and Oedogonium abbreviatum. Results of the present investigation (Table. 41,42) in Carthamus tinctorius Cv. Bhima revealed that low concentrations (upto 20 mM) of NaCl and Na_2SO_4 (upto 10 mM) stimulate activity of RuBP-Case while high concentrations of both the salts inhibit activity of the same. Addition of NaCl upto 90 mM stimulate activity of PEP-Case whereas,at all higher concentrations of both the salts inhibit the same.

c) Phospho Glycolate Phosphatase

It is a photorespiratory enzyme . There are few reports given by Austenfeld (1976) and Waghmode and Joshi (1979) indicating that salinity inhibits photorespiration which is activity of glycolate oxidase in many mangroves. Results of the present investigation (Tables. 39,40) revealed that activity of this enzyme increased in plants grown at all levels of NaCl and Na_2SO_4. This effect is confirmed by in vitro studies (Tables. 41,42). There are no references on the effect of salinity on activity of phosphoglycolate phosphatase. Results from our laboratory (Shukla, 1995) indicated that activity of this

enzyme increases under saline conditions. Thus, increased activity of this enzyme must have increased photorespiration in safflower Cv. Bhima. , under saline conditions.

d) Glycolate-Oxidase

In vivo and in vitro effect of NaCl and Na_2SO_4 on activities of glycolate oxidase:-

The path of glycolate synthesis by RuBP oxygenase is now well established (Ogren, 1975; Lorimer et al., 1977). It is accepted that glycolate is a substrate for photorespiration. Glycolate is oxidised to glyoxylate in presence of glycolate oxidase. By measuring the activity of RuBP oxygenase and glycolate oxidase, one can determine the rate of photorespiration. Also, increase in photorespiration can be accounted for increased activity of glycolate oxidase provided there is no multiple enzyme for the oxidation of glycolate (Havir, 1993).

Austenfeld (1976) reported greater reduction (34%) in glycolate oxidase in Pisum sativum at 1 M NaCl concentration than in Salicornia species (10%), while Na_2SO_4 salinity caused remarkable enhancement of it in both the plants. Downton (1977) and Walker et al. (1981) have reported an enhancement of photorespiration in grapevine due to salt stress. Passera and Albuzio (1978) observed stimulation of RuBP-Case oxygenase due to salt stress in wheat species which was associated with higher light to dark CO_2 evolution (L/D ratio). They concluded that there is a strong correlation between RuBP oxygenase activity and light / dark evolution of CO_2 in wheat plants under saline conditions. There was no detection of correlation between glycolate oxydase activity and light / dark CO_2 evolution.

In halophytic plants like Ceriops, Lumnitzera and Aeluropus, (Waghmode and Joshi; 1979) observed inhibition of glycolate oxydase by 47.6 %, 8.33 % and 47.36 %

respectively at higher salinity level of NaCl. Patil (1980) has observed an adverse effect of NaCl salinity on the photorespiratory ratio and glycolate oxidase activity in Parthenium hysterophorus. Murumkar et al. (1985) reported that activity of glycolate oxydase was slightly stimulated by salt stress in Cicer and highly in Arachis. Salt stress increases oxygenase activity of RuBP carboxylase in Andropogon glomeratus (Bowman, 1987). Thus effect of salt on glycolate oxydase activity differs from plant to plant. Fedina et al. (1993) reported that in 10 days old Pisum sativum L. Cv. Ran 1 plants treated for 24 hours with proline (10^{-6} M or 10^{-3} M) before salinization with 50 mM NaCl for 2 days resulted in an increase of glycolate oxidase activity which indicates that salinity inhibits glycolate oxidase and proline protects this enzyme under saline conditions.

The results of the present investigation (Tables. 41,42) in Carthamus tinctorius L. Cv. Bhima , indicated that activity of glycolate oxidase increases at all levels of NaCl and Na_2SO_4 salinity's. However, dry matter per plant (Table. 6,10) was more than the control in plants grown at ECe 5.0 and 7.5 $mScm^{-1}$ of sulphate which cannot be correlated. It may be due to more activity of RuBP carbooxylase at these salinity levels (Table. 40). At higher concentrations of sulphate increase in activity is correlated with decrease in productivity. This increased activity of glycolate oxidase is correlated with decreased productivity at all levels of NaCl (Tables. 5, 6 ; Fig. 5,6). Stimulatory effect of NaCl and Na_2SO_4 on activities of glycolate oxidase is confirmed by in vitro studies where activity of glycolate oxidase was more than the control upto 10 mM of NaCl and 5mM of Na_2SO_4 . At higher levels of both the salts activity was inhibited. This must be due to direct contact of enzyme with the salts and high concentrations of the salts.

e) Peroxidase

In vivo and in vitro effect of NaCl and Na_2SO_4 on activity of peroxidase:-

Inhibition of peroxidase activity was found by Vasile (1963) at high concentrations of NaCl and Na_2SO_4 in pea, corn and wheat. Flowers (1972) observed the same result in pea with 0.34 M NaCl. Malik and Sahaukat (1986) found decrease in peroxidase activity at -2 and -4 bar NaCl in wheat. El-Fouly and Jung (1970) reported that peroxidase activity was not affected by lower concentrations of NaCl, but was decreased at higher concentrations. Chavan (1980) observed stimulation in peroxidase activity at lower NaCl and Na_2SO_4 salinity but decrease at higher concentrations of both salts in ragi.

Kalir and Poljakoff-Mayber (1983) reported that peroxidase was not affected under saline conditions. Siegal et al. (1982) remarked that magnitude of peroxidase response was not related to the degree of fresh weight inhibition under saline media in Cucumis and Brassica. Guerrier (1987) found no relation between enzyme activity of peroxidase and catalase and protein levels in young plants but a good correlation was observed between enzymes and Na content of seedlings.

Increased peroxidase activity has been observed in plants due to NaCl and Na_2SO_4 salinity's by a number of workers (Strogonov, 1964; Weimberg, 1970; Kleinkopf and Wallace, 1974; Tesu et al., 1979 and Chavan, 1980). Peroxidase is an indicator of respiration rate (Paul and Mukherji, 1972; Tregubenko et al., 1974) and increase in peroxidase activity can be taken as a reliable indicator of leaf senescence (Perish, 1968). According to Horovitz et al. (1968) deficiencies of nitrogen, phosphorous and potassium activates peroxidase activity, thus stimulating respiration. Baba et al. (1964), remarked that increase in respiration rate due to peroxidase was 'wasteful'.Thus, Kleinkopf and Wallace (1974) asserted that growth depression brought about by

salinity in Tamarix ramosissima was mainly due to energy losses through increased respiration. However, Strogonov (1964) and Aleshin et al. (1971) believed that increasing peroxidase activity in cells plays an adaptive role in plants under salt stress conditions, who further explained that toxic H_2O_2 gets accumulated in cells under NaCl stress and to neutralise the toxic effect, peroxidase and catalase activities increase in salt tolerant plants. Gettys et al. (1980) studied salt tolerance of Spartina alterniflora and in vitro effect of NaCl on activity of peroxidase and malate dehydrogenase in the halophytes Spartina alterniflora and Spartina patens and noted that peroxidase was much more tolerant to salt than malate dehydrogenase, which must be due to peroxidase being in saltier cellular compartments than in MDH. Recently, Garcia and Ochoa (1991) investigated effect of salinity on peroxidase activity in leaves of Prosopis articulata plants growing in conductivity range of 1000-18000 μ Mhos. They observed poor correlation between peroxidase activity and electrical conductivities supporting plant growth. This led them to suggest that mechanism of salt tolerance varies from plant to plant. Gossett and Marney, (1994) observed significant increase in peroxidase activity in vitro at 150 mM compared to 0 mM NaCl in callus of salt tolerant cotton cultivar.

Suncho (1996) reported increase in peroxidase activity as well as increase of neutral and basic peroxidase isoenzymes which reflected the changed mechanical property of cell wall which in turn could be related to salt adaptation process in medium sized tomato cells adapted to NaCl in culture media, compared to control. Saha and Gupta (1997) reported increase in peroxidase activity with increasing NaCl salinity in sunflower seedlings. Results of the present investigation revealed that under in vivo conditions, (Tables. 41,42) activity of peroxidase increases in plants grown at ECe 5 $mScm^{-1}$ of NaCl and at all levels of Na_2SO_4 indicating that low concentrations of NaCl and wider

range of Na_2SO_4 stimulate activity of peroxidase in Carthamus tinctorius Var. Bhima. These results are further confirmed by in vitro studies (Tables. 41,42). This fact suggests that Cv. Bhima of safflower adapts to low levels of NaCl and at wider range of Na_2SO_4 by increasing activity of peroxidase.

f) Pyruvate pi dikinase (PPDK)

In vivo and in vitro effect of NaCl and Na_2SO_4 on activity of pyruvate pi dikinase:-

PPDK is an essential enzyme in the pathway of photosynthetic CO_2 assimilation in the C_4 plants. The distinctive feature of C_4 plants such as maize, sugarcane, Atriplex semibauata and Amaranthus palmeri is the presence in their leaves of high activities of pyruvate pidikinase (Hatch and Slack, 1967), an enzyme capable of forming PEP from pyruvate according to the following equation.

$$ATP + Pi + Pyruvate \xrightarrow{Mg^{2+}} AMP + Ppi + PEP.$$

Hatch and Slack (1968) first identified enzyme activity during investigations on photosynthesis by leaves of tropical grasses and has been since found in other C_4 plants (Johnson and Hatch, 1968) with CAM (Kluge and Osmond, 1972; Sugiyama and Hatch,1981). In contrast, no appreciable activity has been detected in the leaves of C_3 plants such as wheat and oat (Hatch and Slack, 1967; Johnson and Hatch, 1968). It was generally accepted that C_3 plants lacked the enzyme until Duffus and Rosie (1973) presented evidence for its occurance in the green pericarp of barley; in immature wheat grains (Meyer et al., 1982) and in young leaves of tobacco (Kisaki et al.,1973); epidermal strips of Commelina benghalensis (Das and Raghavendra, 1977) and in Vicia faba L. in both mesophyll and guard cell protoplasts (Schnabl., 1981). Kazuko and Bassham (!983,1984) found that amount of enzyme / mg of soluble protein in C_3 plants (wheat, pea, green bean, plum and castor bean) and in some C_3 leaves (tobacco, spinach, sunflower and wheat) was less than that of C_4 leaves maize kernels

and suggested that PPDK may be involved in mechanisms of amino acid interconversion during seed development. It has been identified in the green grains of eight cereal grasses, most of which are classified as C_3 plants.

The role of PPDK in metabolism of C_3 leaves is not well estabilished. It has been proposed that there may be involvement of PPDK in control of stomatal opening through a participation in exchange in ion transport in to guard cells (Burnell, 1986; Ricardo and Rees, 1970). PPDK may be involved in control of guard cell shrinking and swelling via malate transport in wheat plants (Schnabl, 1981). In seeds, PPDK may supply PEP for photosynthetic and dark CO_2 fixation. PPDK enzyme plays role in metabolism of cereal grains of C_3 grasses. The most obvious possibility is that like C_4 plants, it generates CO_2 the acceptor (PEP) for PEP-Carboxylase which is active in this tissue. These two enzymes (PPDK and PEP-Case) together with ample transaminase activity reported in pericarps, permit the net synthesis of aspartate from CO_2 respired by the developing seed and alanine supplied by the xylem sap.

According to Aoyagi and Bassham (1983), PPDK amount in wheat tissues is approximately $1/70^{th}$ than found in maize leaf. The investigations made by Meyer et al. (1978) confirms that immature wheat grains contain a pyruvate pidikinase similar to that present in the leaves of C_4 plants and raised the possibility that the enzyme is widely distributed in the grains of C_3 grasses. PPDK appears to be widely distributed among C_3 plants. The negative results from the past efforts to detect enzyme activity may be due to a variety of causes including variability in amount due to age of leaves and other physiological conditions, inactivation during extraction and lack of purification.

Hermaan (1984) measured activity of PPDK in leaf extracts in Flaveria ceronquistic (C_3); F. pubescens; F. anomala (C_3-C_4); F. brownii; F. palmori; F. trinervia and F. autralasica (C_4) and suggested that PPDK is light activated reaching maximum activity at noon in F. brownii and in contrast, very early after beginning of light phase in F. pubescence. Bauve (1984), reported that activity of PPDK in C_3 species Moricandia moricandioides, M. fortida, C_3-C_4 intermediates, M. sinacia, M. arvensis and M. spinosa fall in ranges typical for C_3 photosynthetic plants. PPDK activity in Panicum miliodes was similar to Moricandia moricandioides, C_3-C_4 intermediate species. However, it was much lower than previously reported by other laboratories. Saitou et al (1991) reported incease in PPDK with increase in malate content in leaves at the end of dark period in Mesembryanthemum crystallinum L. leaves under NaCl treatment and this increase was accompanied by De novo synthesis of proteins. Results of the present investigations (Tables. 39-42 ;Fig. 27abc ;28abc), revealed that this enzyme is present in this plant. Its activity is low in this variety Bhima which is similar to the results given by Aoyagi and Bassham (1983) in wheat. Activity of PPDK was increased at low levels (ECe 5.0 $mScm^{-1}$) of NaCl and upto ECe 12.5 $mScm^{-1}$ of Na_2SO_4 which suggested that both the salts stimulate activity of PPDK. Results are further confirmed by in vitro studies with NaCl and Na_2SO_4. In safflower, PPDK may be involved in mechanism of amino acid interconversions and in guard cell functions as suggested by Kazuko and Bassham (1984) and Schnabl (1981).

CHAPTER VIII

PROTEIN PROFILE UNDER SALINE CONDITIONS

1) INTRODUCTION

Analysis of proteins in their nature form is carried out in polyacrylamide buffer gel. The classical disc electrophoresis using cylindrical gels has been described by Davis in 1964. Different stress factors could cause different response by same protein polypeptide. The recent studies suggest that the changes in protein polypeptide content is related to stress tolerance or resistance metabolism. There are certain stress proteins (Hurkmann and Tanaka, 1987) which help for salt tolerance. Therefore, it was proposed to study protein profile during germination in Carthamus tinctorius L. Cv. Bhima under saline conditions.

2) MATERIALS AND METHODS

The seeds of Carthamus tinctorius L. Cv. Bhima were collected from Agriculture college, Pune-411007. First seeds were washed with tap water and then with 0.1% $HgCl_2$ and again washed with distilled water. The seeds were germinated in control (water), 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 % of NaCl and Na_2SO_4. The first emerging leaves were selected for analysis of proteins. The protein profile was studied by following method of Hurkmann and Tanaka (1987).

One gram of material was homogenised in chilled mortar and pestle in ice cold buffer containing 50mM Tris-HCl buffer , pH 8.0 containing 10 mM EDTA and 0.5 M NaCl. The extract was centrifuged at 13000 rpm for 30 min at 4^0 C. The supernatent was the source of protein. Protein content from the samples was estimated by Lowry's method. Equal quantity of sample and buffer was loaded in the well of 12.5 percent SDS gel.

PLATE 3 Photograph showing effect of increasing concentrations of NaCl on Relative mobility in Carthamus tinctorius L. Cv. Bhima, on growth.

PLATE 4 Photograph showing effect of increasing concentrations of Na_2SO_4 on Relative mobility in Carthamus tinctorius L. Cv. Bhima, on growth.

PLATE-3

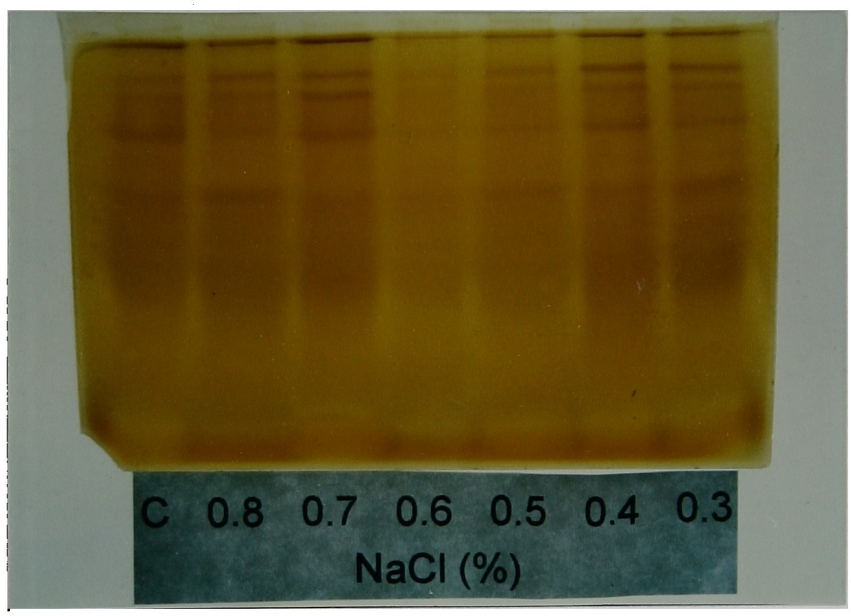


PLATE-4

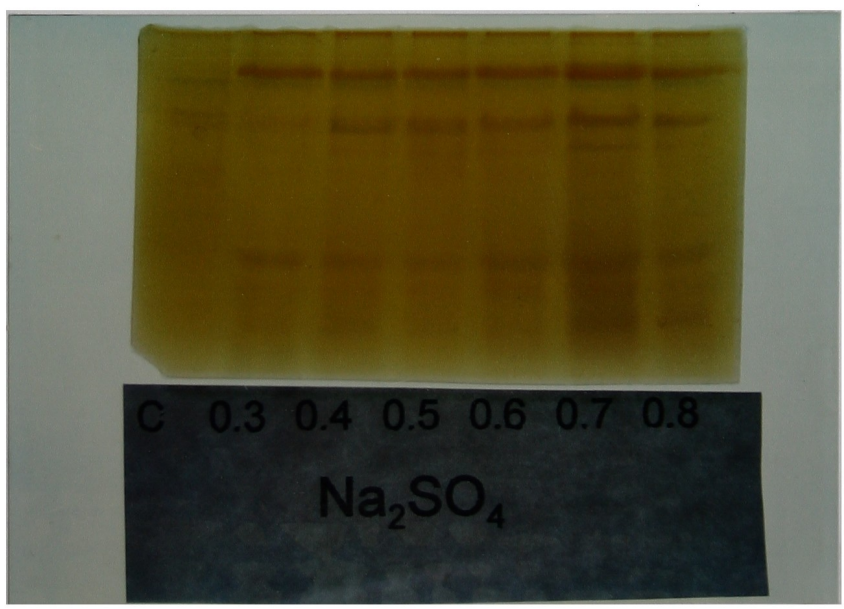


Table. 43.

Effect of increasing concentrations of NaCl on the Rm of total soluble proteins in Carthamus tinctorius L. Cv. Bhima.

BandNo. (cm)	Control	0.30%	0.40%	0.50%	0.60%	0.70%	0.80%
Band 1	0.091	0.091	0.091	0.091	0.091	0.091	0.091
Band 2	0.145	0.145	0.145	0.145	0.145	0.145	0.145
Band 3	0.218	0.218	0.218	0.218	0.218	0.218	0.218
Band 4	0.382	0.382	0.382	0.382	0.382	0.382	0.382

Total gel run 5.5 cm

Table. 44.

Effect of increasing concentrations of Na_2SO_4 on the Rm of total soluble proteins in Carthamus tinctorius L. Cv. Bhima.

Band No. (cm)	Control	0.30%	0.40%	0.50%	0.60%	0.70%	0.80%
Band 1	-	-	-	-	0.196	0.196	-
Band 2		0.980	0.980	0.980	0.980	0.980	0.980
Band 3	0.137	0.137	0.137	0.137	0.137	0.137	0.137
Band 4	0.255	0.255	0.255	0.255	0.255	0.255	0.255
Band 5	0.353	0.353	0.353	0.353	0.353	0.353	0.353
Band 6	-	0.667	0.667	0.667	0.667	0.667	0.667

Total gel run 5.1 cm
-Absence of bands

Fig. 29.
Effect of increasing concentrations of NaCl on protein profile in Carthamus tinctorius L. Cv. Bhima.

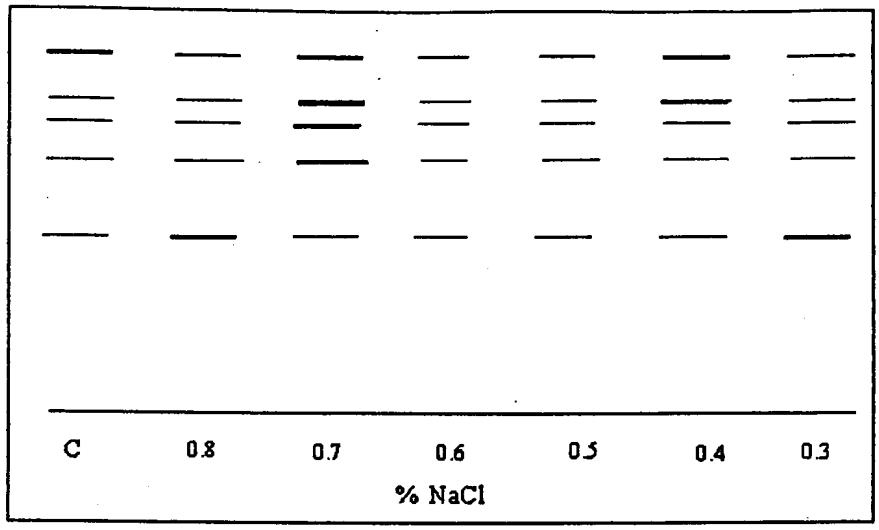
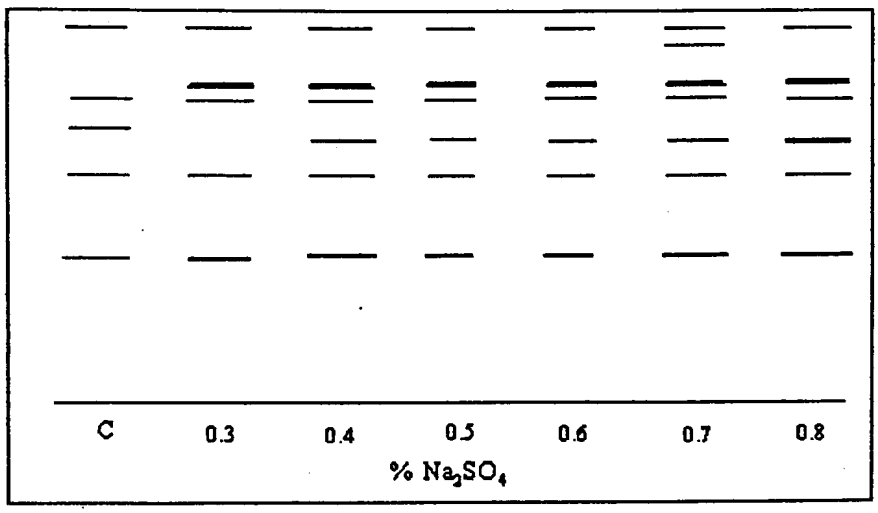


Fig. 30.
Effect of increasing concentrations of Na_2SO_4 on protein profile in Carthamus tinctorius L. Cv. Bhima.



The gel was allowed to run and after complete running, gel was separated from the glass plates carefully and further was stained using silver stain to visualise fractionated polypeptides.

3) RESULTS

Results of protein profile under increasing concentrations of NaCl and Na_2SO_4 are represented in Tables. 43, 44 ; Fig. 29,30.

From the results presented in Fig. 43, it is clear that the number of polypeptides present in control at all levels of NaCl are similar. Under saline conditions ,synthesis of these polypeptides is imbalanced or impaired that is, some are more synthesized than the control while some are less synthesized. Thus there is no quanlitative change under sodium chloride salinization in Carthamus tinctorius L. Cv. Bhima. Contrary to this, under sulphate salinizations two new polypeptides are present in all treated seedlings. These two polypeptides should be stress proteins. In addition to this at 0.6% and 0.7% of Na_2SO_4, one or more extra polypeptide is also present.Thus safflower Cv. Bhima tolerates sulphate salinizations by synthesizing stress proteins. However, stress proteins do not play a role for salt tolerance under chloride salinizations.

4) DISCUSSION

Several stress proteins synthesized in response to stress have been reported (Amer and Reinhold, 1973; Cooper and TDH HO,1983; Singh et al., 1983 and Hasegawa et al., 1984). Stress alters protein synthesis pattern in plants (Cooper and TDH. HO, 1983). Salt stress has been also suggested to change to cope with stress (Singh et al., 1984). It is not known whether the specific changes in particular proteins are responsible to

particular plant responses. The control mechanism and the sequence in which stress imposes its regulation remains obscure. Stress induced proteins in response to stress has been worked out by Ericson and Alfinito (1984) and Singh et al., (1985) in tobacco cell culture. According to them several proteins are found to be more abundant in salt adapted cells and another protein of 26 kd is unique in salt adapted cells. When the salt adapted cells are transferred to the low salt concentration medium, the levels of 20 and 32 kd proteins return to those in non adapted cells. However, the 26 kd protein is present for at least two passages. The 20 kd protein has 4% hydroxy proline and 11.3% proline, which is unusually high, yet less than the hydroxy proline rich glycoproteins in cell walls. Bressan's group (unpublished, cf. Sachs and TDH HO, 1986) has purified the 26 kd protein and found that this protein is also induced by ABA which accumulates in the salt adapted cells. The physiological role of these proteins in yet not known.

King et al. (1986) while working with NaCl induced protein in suspension cultures of Nicotiana tobacum reported that accumulation of 26 kd protein is dependent upon the growth phase of cultures and also osmotic stress. Lowering in the water potential of the medium resulted in an increase of 26,000 D protein. Their findings supported the theory that the accumulation of protein is determined by the osmotic strength of the medium. They also suggested that 26,000 D protein is involved in the response to salt in the whole plant of Nicotiana tobaccum. Hurkmann and Tanaka (1987) have observed formation of stress proteins. According to them such proteins could be the result of lowered water potential, Na ion toxicity or the reflection of general stress response. Furthermore, the alteration in the pattern of net protein synthesis caused by NaCl may be due to changes in the efficiency of mRNA translocation or regulation of mRNA

transcription or because of altered rates of protein degradation which could not be confirmed.

The physiological significance of such changes explains their role in adaptation process by regulating protein synthesis. Plants have been shown to exhibit metabolic changes along with the morphological ones in response to their changed surroundings. Such changes are believed to be adaptive which make the organism to survive and grow in the presence of NaCl salt, is largely as a result of altered gene expression. (Singh et al., 1985). These expressional changes seem to help in correlating the level of different gene products, that is , protein and or mRNA and the degree of adaptations to NaCl.

Alina et al. (1985) reported alterations in protein composition of pea chloroplasts ribosomes of 10 day old plants in 2-D polyacrylamide gel electrophoresis. Alkaline and acidic proteins of pea chloroplasts ribosomes on Cl differ from those of control ribosomes in electrophoretic migration and quality. The alterations in the protein composition of ribosomes show the modifications of ribosome proteins in pea chloroplasts under Cl salinity. Ball and Anderson (1986) observed that NaCl induced depletion of 23 and 17 kDa proteins from PSII enriched membrane sheets of thylakoids isolated in salt tolerant mangroove Avicennia marina and salt sensitive pea Pisum sativum. Hurkmann et al. (1989) compared the effect of salt (200 mM) NaCl for 6 days on polycrylamide and transtable mRNA in roots of a salt tolerant and salt sensitive cultivar of barley Hordeum vulgare L. tolerant Cv.CM.72 and sensitive Cv. Prato using 2-D polyacrylamide gel electrophoresis. The results indicated that the patterns of invivo labelled polypeptides and invitro products of CM72 and Prato were quantitatively same. Salt did not induce the synthesis of unique polypepetide or transferable mRNAs and also did not cause any to disappear. This could not identify salt tolerance in barley.

Hurkmann et al. (1991) isolated and identified germin like (GS1 and GS2) polypeptides, the 26 kilodalton isoelectric point 6.3 and 6.5 and observed increase of germin like polypeptide in mature barley roots during salt stress. Maslenkovo et al. (1992) reported that in Hordeum vulgare seeedlings , NaCl stress induced marked quantitative and qualitative changes in polypeptide profiles. Concerning mainly the proteins with equal mobility. The thylakoid polypeptide band intensity was at 55.57 kD. The relative share of some polypeptides with apparent molecular masses above 66 kDa and of polypeptide with low molecular mass in region of 20.5 and 15.0 kD was enhanced. One new band at 31.0 and 31.5 kD was well expressed at 25 and 250 micromolar Jasmonic acid concentration and became discernible in 10 mM NaCl treated plants. Enhanced levels of 60, 47, 34, and 30 kD polypeptide and reduced levels of 55 and 15 kD polypeptide was present in NaCl treated plants. One new polypeptide of 25-1 kD was observed in NaCl treated plants.

Miteva et al.(1992) observed that in Hordeum vulgare L. Var Alfa, salinity (100 mM NaCl) induced marked quantitative and qualitative changes in polypeptide profiles of soluble leaf proteins. Enhanced levels of 76, 60, 47, 43 and 30 kD polypeptides and reduced levels of 55 and 15 kD polypeptides were observed in NaCl treated plants. A new polypeptide of 26-25 kD was found in NaCl treated samples concluding that salinity inhibits synthesis of Rubisco. Lopez et al. (1994) isolated salt responsive proteins by comparative 2D-PAGE analysis in Raphanus sativus. They isolated three polypepetides of 22, 28 and 28.5 kDa and subjected to microsequencing and studied homology further. Uma et al. (1995) reported synthesis of stress induced proteins in two contrasting genotypes (Eleucine coracana Gaetm) at 200 mm NaCl. Proteins with apparent molecular weight of 70-72, 52, 37, 34 and 23 kDa were synthesised in highly

responsive genotype (GE 415) and poorly responsive (VL481) genotype. However, GE-415 synthesized a 54 kDa protein that was not observed in VL-481. Synthesis of stress protein is correlated with observed variation in acquired tolerance of two genotypes.

Maslenkova et al. (1995) observed protein patterns of thylakoid membranes in barley seedlings on SDS-PAGE stained with comassie blue which revealed that the relative amount of a number of polypeptides were altered under NaCl solutions (20-100 mM). Popova et al. (1995) reported that ABA and NaCl induced marked quantitative and qualitative changes in the polypeptide profiles concerning mainly the proteins with equal mobility in Hordeum vulgare L. Morabito et al.(1996) observed that salinity had significant effects on content of one predominant polypeptide of molecular weight 18 kDa induced by salt stress in roots of clone 43 of Eucalyptus microtheca. In clone 42, polypeptide was present in low amounts in control and salt treatment increased its synthesis. Wang and Ming (1996) analysed protein polypeptide contents in Lathyrus sylvestris L. under salt stress, water stress and temperature 40^{0} C by 2D-electrophoresis and reported (1) identical changes in some protein polypeptide content. (2) similar effects on some proteins by two different factors were found. Munoz et al. (1997) observed three predominant polypeptides with apparent molecular weights of 50, 34 and 25 kDa in Prosopis alba, P. fruticosa and P. chilensis respectively in hypocotyls and roots by SDS-PAGE analysis in NaCl solution and sea water treatments. However, 60 kDa polypeptide disappeared as a response to salinity. A 37 kDa phospho protein and HAL-1, a salt stress responsive protein were detected in roots of P. fruticosa under saline conditions.

Results of present investigation (Table.43;Fig.29), revealed that under NaCl salinity, there was no qualitative variation in protein profile. These results are similar to those given by Anantani and Vaidya (1982) where they did not find much variation in number of bands in Phormidium except for appearance and disappearance of bands and shift in Rm values. Under sulphate salinity, changes do occur with respect to number of bands compared to control. The appearance and disappearance of bands were observed under sulphate salinity only(Table.44;Fig.30). This indicates that stress alters gene expression. The number of bands as well as the Rm values differed. Differences in Rm values could be due to shift in mobolities of few of them which were shown by their disappearance in some cases. The changed protein profile signifies response to salt by an adaptation process as suggested by Singh et al. (1987). Their role in adptation can be claimed based on its increased growth at lower concentrations and survival at higher concentrations.

Kursanov (1967) and Hurkmann and Tanaka (1987) have expressed the possibility of Na ion toxicity. According to Kursanov (1967), in a given fraction accumulation of protein can be attributed to a depression of some genes by excess ions in the cells. The change in the protein profile in safflower (Fig. 30) can be attributed to increased Na, as the plant accumulates Na with increasing concentrations of Na_2SO_4. The appearance and disappearance of bands under Na_2SO_4 stress is for adaptation under saline conditions. However, under NaCl stress, (Fig. 29) there was not much significant change in band pattern. The preliminary work on qualitative changes at protein level suggests that gene expression alters under Na_2SO_4 saline conditions . The alteration in the protein profile under sulphate conditions suggests its contribution in the salt tolerance mechanism of safflower Cv. Bhima.

CHAPTER IX

SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

India is one of the major countries producing safflower on large scale. Safflower 'Carthamus tinctorius L.' is an economically important oil yielding crop. In India, about 7.5 million hectare of productive land has become salty due to heavy use of chemicals, fertilizers, over irrigation and poor drainage, out of which, in Maharashtra, about 3.4 lakh hectare of land is saline. One of the useful strategies to combat soil salinity is to select salt tolerant crop.

As the work done on Carthamus tinctorius L. under saline conditions is scanty and there is no work on Carthamus tinctorius L. Cv. Bhima under saline conditions, therefore, it was proposed to study the physiological basis of safflower under saline conditions.Variety Bhima is a recommended variety in Maharashtra for cultivation which is a high oil yielding crop.

To study the salinity effects, 15 day old seedlings were treated with with salt treatments Chapter III, where seedlings were treated with increasing (5.0, 7.5, 10.0, 12.5, 15.0 and 17.5 $mScm^{-1}$) ECe levels of NaCl and Na_2SO_4 separately because major salinity in the state of Maharashtra is either due to chloride or sulphate salt. All the parameters were studied in triplicates by using standard analytical methods. The results obtained are summarised in brief as follows:

I) GROWTH AND PRODUCTIVITY UNDER SALINE CONDITIONS.

1) Height was measured after 30, 60 and 90 days of germination (Table.1; Fig.1a,b). Results indicated that low concentrations (ECe 5.0 to 10.0 $mScm^{-1}$) of NaCl stimulate while high concentrations (ECe 15.0 to 17.5 $mScm^{-1}$) of it inhibited growth in height at

30 days of germination. However, after 60 days of growth, height was decreased linearly with increasing levels of NaCl thereby indicating that all the levels of salinity inhibit growth in height of safflower. At 90 days of growth, height was less than the control at all levels of chloride salinization, thereby indicating that all levels of NaCl inhibit growth in height . Plants growing at ECe 15.0 to 17.5 $mScm^{-1}$ died during 60 to 90 days of growth. Thus, at 90 days of growth plants could survive upto ECe 12.0 $mScm^{-1}$ of NaCl while at all higher levels plants died indicating its more susceptibility to NaCl during flowering phase of the life cycle. At ECe 10.0 to 12.5 $mScm^{-1}$ plants died during 90 to 115 days of growth. Thus plants could survive upto Ece 7.5 $mScm^{-1}$ of NaCl.

With increasing levels of sulphate salt (Table. 2; Fig.2a,b), height increased linearly upto ECe 10.0 $mScm^{-1}$ of Na_2SO_4 at 30 days of germination. At ECe 10.0 $mScm^{-1}$ it was more than the control but was less than that of ECe 7.5 $mScm^{-1}$. At higher (ECe 12.5 to 17.5 $mScm^{-1}$) levels height was linearly decreased with increasing levels of sulphate salinizations.

At 60 days of growth, height was more than the control upto ECe 10.0 $mScm^{-1}$ while it was less than the control in plants growing at ECe 12.5 to 17.5 $mScm^{-1}$. At 90 days of growth height was more than the control upto ECe 10.0 $mScm^{-1}$ and was less than the control at ECe 12.5 $mScm^{-1}$. During 60 to 90 days of growth, plants growing at ECe 15.0 to 17.5 $mScm^{-1}$ could not survive, while plants growing at ECe 12.5 $mScm^{-1}$ died after 90 days of growth. Thus, low levels of sulphate stimulated while high levels of it inhibited growth in height in Carthamus tinctorius Cv. Bhima and this plant could survive upto Ece 10.0 $mScm^{-1}$ of Na_2SO_4.

2) LEAF AREA

The leaf area was studied after 30 and 90 days of germination (Table.3 ;Fig.3). Results suggested that at 30 days of germination, the total leaf area per plant was increased upto ECe 12.5 $mScm^{-1}$ of NaCl over the control. However, at high (ECe 15.0 and 17.5 $mScm^{-1}$) levels of NaCl, it was less than the control. Thus, low concentrations of NaCl stimulate formation of leaf area while high concentrations of it inhibit the same in safflower Cv. Bhima. At 90 days of growth leaf area was more than the control upto ECe 7.5 $mScm^{-1}$ of NaCl. Under sodium sulphate salinity, at 30 days of growth (Table. 4 ;Fig. 4), leaf area was less than the control which reflects that all levels of Na_2SO_4 inhibit formation of leaf area in safflower Cv. Bhima. At 90 days of growth, leaf area was less than the control at all levels of Na_2SO_4 salinity. Thus, all levels of Na_2SO_4 inhibited leaf area growth.

3) PRODUCTIVITY AT FLOWERING (DRY MATTER)

Results of productivity (DM) shown in Table. 5 ; Fig.5, suggested that all levels of NaCl salinity decreased DM of root and stem. However, leaf weight per plant increased over the control in plants growing at low (ECe 5.0 $mScm^{-1}$) NaCl salinity and at high (ECe 7.5 to 15.0 $mScm^{-1}$) levels, it decreased with increasing salinity levels. Thus,low levels of NaCl stimulated leaf growth while high levels of it inhibited the same.

Under sulphate salinity (Table. 6; Fig.6), root and leaf weight increased at ECe 5.0 and 7.5 $mScm^{-1}$ and decreased at all higher levels. The stem weight increased in plants grown upto ECe 10.0 $mScm^{-1}$ and reduced at all higher concentrations of sulphate

salinity which reflects that low levels of sulphate stimulate and high levels of it inhibit stem, leaf and root growth.

The average total DM per plant (Tables. 5,6 ;Fig.5,6) was less than the control at all levels of chloride whereas it was increased in plants growing upto ECe 7.5 $mSCm^{-1}$ of sulphate and was less than the control at all higher levels. Thus, at flowering all levels of chloride were detrimental to safflower while low levels of sulphate stimulate growth and high levels of it, inhibit growth in safflower Cv. Bhima. The dry matter per plant was very less at ECe 12.5 to 15.0 $mScm^{1}$ of NaCl and at ECe 15.0 $mScm^{-1}$ of Na_2SO_4 because plants growing at ECe 15.0 $mScm^{-1}$ of NaCl died during 60 to 90 days of growth and plants growing at ECe 15.0 to 17.5 $mScm^{-1}$ of sulphate also died during 60 to 90 days of growth.

4) POST HARVEST

The root and stem weight linearly decreased with increasing concentrations of NaCl upto ECe 10.0 $mScm^{-1}$. The leaf and grain weight was slightly more than the control at ECe 5.0 $mScm^{-1}$ of NaCl. The husk weight was less than the control at all levels of NaCl salinity.The average DM per plant was increased at ECe 5.0 $mScm^{-1}$ and decreased linearly with increase in chloride salinizations (Table.9). Under sulphate salinity, the root, stem, leaf and husk weight (Table.10) increased upto ECe 10.0 $mScm^{-1}$ and decreased at ECe 12.5 $mScm^{-1}$. However, the grain weight was more than the control upto ECe 7.5 $mScm^{-1}$ and decreased at ECe 10.0 and 12.5 $mScm^{-1}$. The average total DM per plant was increased upto ECe 10.0 $mScm^{-1}$ and decreased at ECe 12.5 $mScm^{-1}$ of Na_2SO_4. This variety of safflower (Table. 9) can be cultivated with more grain (127.5% of control) yield at Ece 5.0 $mScm^{-1}$ of NaCl and crop can be grown

upto Ece 10.0 $mScm^{-1}$ of NaCl with 45.5 % of the control productivity. Under sulphate salinity also, this variety showed more grain yield per plant at Ece 5 $mScm^{-1}$ (122% of the control) and at Ece 7.5 $mScm^{-1}$ (141.5% of the control), at low levels of sulphate. It also produced considerable grain yield at Ece 10.0 $mScm^{-1}$(98.8% of the control) and at Ece 12.5 $mScm^{-1}$ (36.4% of the control). Thus, this crop can be profitably cultivated at very low (upto ECe 5.0 $mScm^{-1}$) levels of NaCl and upto moderate (upto ECe 7.5 $mScm^{-1}$) levels of sulphate. Results also revealed that plants could survive and complete their life cycle upto ECe 7.5 $mScm^{-1}$ of chloride and upto ECe 10.0 $mScm^{-1}$ of sulphate. Higher levels of chloride are more detrimental than higher levels of sulphate to Carthamus tinctorius L. Cv. Bhima. Further, it was observed that low levels of sulphate are essential for growth and development of this crop as the grain yield per plant (Tables. 9,10) was cosiderably higher in plants grown upto ECe 7.5 $mScm^{-1}$ of sulphate.

II) MINERAL METABOLISM UNDER SALINE CONDITIONS

1) SODIUM

Na content of root (Tables.11,12 ; Fig.7a,8a),increased under both the salinity's. It's content was more than the stem and leaves which indicated that roots act as storage organs for Na in safflower under both the salinity's.

In stem (in upper, middle and lower part of stem), Na was linearly increased under NaCl and Na_2SO_4 treatments(Table.13). Na was stored more in the middle part of the stem at lower levels of both the salinity's while it was more in the lower portion of the stem at higher levels of both the salinity's thereby indicating that middle part of the stem acts as a storehouse for Na at lower levels of both the salinity's while lower

portion of the stem acts as a storage organ for Na at higher levels of both the salts. Thus mechanism of storing Na in stem is same under both the salinity's in Carthamus tinctorius Var. Bhima. The average total of the stem Na increased linearly with increasing NaCl and Na_2SO_4 salinity's.

In leaves (Tables.15,16:Figs.11a,12a), under NaCl and Na_2SO_4 salinity's, it was observed that sodium content increased with increasing salinity. In crown leaves, however Na content was less compared to lower leaves under NaCl salinity but Na content accumulation was more than control at all levels of NaCl.

The average Na content (Tables.17,19) of the total plant increased with increasing levels of chloride and sulphate salinity thereby indicating safflower Cv. Bhima does not have selective absorbtion mechanisms under saline conditions. However, it adapts to saline conditions by storing more Na in roots and stem under both the salinizations.

2) POTASSIUM

In roots (Table.11;Fig.11b), under sodium chloride treatment, accumulation of K content was found to be increasing with increasing NaCl treatment but under sodium sulphate salinity, K content linearly decreased with increasing salinity. In stem (Table. 13), under sodium chloride treatment, in upper part, its content was less than the control while its content was more than the control in the middle and lower part of stem. However, K content of total stem was less than the control at all levels of NaCl. Under sulphate salinization (Table.14), it was more than the control at all at levels of Na_2SO_4 in upper, middle and lower part of stem. As a result, K content of the total stem increased at all levels of sulphate salinization.

In leaves and crown leaves (Tables.15,16 ; Fig. 11b,12b), under both salinity's, K content decreased with increasing salinity. In the control and at levels of both the salts, the K content value was highest in leaf than root and average stem values indicating that K is more in leaves than stems and roots in Carthamus tinctorius Var. Bhima. Results clearly indicated that under chloride salinization K was more in leaves at low (upto ECe 7.5 $mScm^{-1}$) concentrations while it was more in roots at high (ECe 10.0 to 15.0 $mScm^{-1}$) concentrations indicating at low salinity's more K is translocated to leaves while at all high salinities K is retained in roots. Under sulphate salinization, at low concentrations (ECe 5.0 $mScm^{-1}$), K was high in leaves while at high (ECe 7.5 to 15.0 $mScm^{-1}$) concentrations it was more in stems reflecting that more K is translocated to leaves at low levels of Na_2SO_4,while reverse is the case at higher levels. Thus, K translocation from root to leaf is affected under chloride and sulphate salinizations in Carthamus tinctorius Var. Bhima.

Average K content of total plant (Root + Stem +Leaf) , (Tables.17,19) was less than the control at all levels of NaCl and Na_2SO_4 reflecting that K uptake fails in Carthamus tinctorius Var. Bhima at all levels of salinity.

3) CHLORIDE

Results of chloride content (Tables.11-16) in various parts of the root, stem, leaves and crown leaves suggested that in roots Cl content was linearly increased with increase in levels of both the salts. It is observed that Cl content was higher in roots than in stem and leaves thereby indicating that root acts as storage organ for chloride under both salinizations.

In all parts of stem, Cl content linearly increased under chloride and sulphate salinizations. Its content was more under chloride salinization than sulphate which is obvious. Chloride content was highest in upper part of the stem under control as well as under all levels of both the salinizations which reflect that after roots upper part of stem acts as storage organ for chloride.

In leaves and crown leaves (Tables.15,16;Fig.11d, 12d) under NaCl and Na_2SO_4 treatment, chloride content linearly increased with increasing levels of both the salts.

Chloride content of total plant (Tables. 17,19) was increased with increase in levels of both the salts indicating that all levels of both the salts, stimulate chloride uptake in Carthamus tinctorius Var. Bhima and this plant does not have selective absorption mechanism. However, it adapts to saline conditions by storing more chloride in the roots.

4) SULPHATE

Results of sulphate content (Tables.11 -16) in various parts of safflower under chloride and sulphate salinity suggested that, under NaCl and Na_2SO_4 treatment content of sulphate was three times more in roots than stem and leaves which reflects that roots act as storage organ for sulphate under both the salinizations. Sulphate content was linearly increased with increasing concentrations of both the salts thereby indicating that all levels of sulphate and chloride stimulate sulphate uptake in safflower Cv. Bhima.

5) CALCIUM

Results obtained in Table.11 indicated that in roots under NaCl treatment, Ca content increased linearly in plants grown upto ECe 12.5 $mScm^{-1}$ and it was less than the control at ECe 15.0 $mScm^{-1}$. Ca content was more than the control at all levels of Na_2SO_4.

The Ca content of of total stem increased with increasing levels of both the salinity's. Average Ca content of total stem was more under sulphate salinization than under chloride salinization reflecting that more Ca is stored in stem under sulphate salinization than chloride.

In leaves and crown leaves (Tables.15,16 ;Fig.15f,16f) the content of Ca was more than the control upto ECe 12.5 $mScm^{-1}$ and was less than the control at high (ECe 15.0 $mScm^{-1}$) levels of NaCl and Na_2SO_4.

The average Ca content of the total plant (Tables. 18,20) was more than the control at all levels of chloride and sulphate salinity indicating that plants adapt to saline conditions by maintaining efficient Ca uptake.

6) PHOSPHORUS

Average P content (Tables.18,20) the plant was more than control at all levels of chloride and sulphate salinizations indicating that safflower Cv. Bhima has efficient phosphate uptake mechanism under both the salinity's.

In leaves (Table.15,16), the average P content was linearly decreased with increasing levels of chloride and sulphate salinizations. In crown leaves, however, P accumulation was stimulated at low (ECe 5.0 and 7.5 $mScm^{-1}$) level and decreased at all higher levels of NaCl salinity. Under sulphate salinity, P accumulation was stimulated upto ECe 10.0 $mScm^{-1}$ and decreased at all higher levels of Na_2SO_4. Thus, translocation of phosphorus from roots and stem to leaves reduced under both the salinizations which must be one of the reasons for decreased productivity at all higher levels of both the salts.

7) ZINC

In leaves (Tables.15,16), under both the salinizations, Zn content was more than the control at all concentrations. In crown leaves, under NaCl salinization, Zn content decreases while under sulphate salinizations, Zn content increases with increase in level of salt. Thus, Zn metabolism in leaves and crown leaves is different under chloride and sulphate salinizations in Carthamus tinctorius Var. Bhima.

The average (Tables.18,20) zinc content of the total plant increased under both chloride and sulphate salinizations which indicated that zinc uptake stimulates under both the salinizations.

8) IRON

Fe accumulation was more in roots than the control at all levels of both the salts (Tables.11,12 ; Fig. 7i, 8i) thereby indicating that roots act as storage organ for Fe.

Average Fe content in the stem was more than the control under both the salinizations. In leaves and crown leaves under chloride and sulphate salinity (Tables.15,16 ; Fig. 11i, 12i), Fe accumulation increased with increasing salinity. Average Fe content of total plant (Tables.18,20) was more than the control in plants grown at all levels of both the salts.This fact suggests that all levels of both the salts stimulate uptake of iron in Carthamus tinctorius Var. Bhima.

9) MANGANESE

In leaves (Tables.15,16 ; Fig.11j,12j),Mn content decreased with increasing chloride and sulphate salinity's. In crown leaves, under chloride treatment the content increased at all salinity levels in comparision to control. Under sulphate treatment, however, content was less at higher salinity levels (ECe 12.5 and ECe 15.0 $mScm^{-1}$).

Average Mn content (Tables. 18,20) of total plant was less than the control under NaCl salinity. However, the average total Mn content of total plant was more at low (ECe 5.0 and 7.5 $mScm^{-1}$) of Na_2SO_4 and was less at higher levels. Thus, Mn uptake was different under chloride and sulphate salinizations.

III) ORGANIC METABOLISM UNDER SALINE CONDITIONS

1) CHLOROPHYLLS

From the observations (Tables.21,22 ; Fig.13,14), it is clear that chlorophyll a content increased at all levels of NaCl and Na_2SO_4. Its content was maximum at ECe 7.5 $mScm^{-1}$ of NaCl and of Na_2SO_4. Similarly, chlorophyll b content was more than that the control at all levels of both the salts. It was maximum at ECe 5.0 $mScm^{-1}$ of NaCl and of

Na_2SO_4. Thus, all levels of both the salts stimulate biosynthesis of chlorophyll's a and b in Carthamus tinctorius L. Cv. Bhima.

The ratio of chlorophyll a to chlorophyll b was more than the control at all levels of chloride and sulphate salinity's except at ECe 5.0 $mScm^{-1}$ of Na_2SO_4 where it was less than the control. The ratio was highest at ECe 7.5 $mScm^{-1}$ of chloride and at ECe 10.0 $mScm^{-1}$ of sulphate. This indicates that all levels of chloride and sulphate stimulate chlorophyll a biosynthesis than chlorophyll b biosynthesis except at ECe 5.0 $mScm^{-1}$ of Na_2SO_4 where biosynthesis of chlorophyll b was more stimulated than biosynthesis of chlorophyll a. Thus, effect of low and high concentrations of Na_2SO_4 are different on chlorophyll a and chlorophyll b biosynthesis. The total chlorophylls were also found to be more than the control at all levels of NaCl and Na_2SO_4 salinity's and maximum total chlorophylls were recorded at ECe 7.5 $mScm^{-1}$ of NaCl and Na_2SO_4. This indicates that all the levels of chloride and sulphate salinity's stimulate chlorophyll synthesis in Carthamus tinctorius L. Var. Bhima.

However, productivity (Tables.5,6 ; Fig.5,6) at flowering was less than control at all levels of chloride, which reflects that eventhough chlorophylls play major role in the process of photosynthesis there is no direct correlation between total chlorophylls and productivity (rate of photosynthesis) in safflower under chloride salinity. Increased chlorophylls at low levels of Na_2SO_4 defintely increase productivity (Table.6 ; Fig.6) at flowering which indicated that there is correlation between photosynthesis and total chlorophylls. At higher levels of sulphate increased chlorophylls could not increase productivity in safflower Cv. Bhima. However, increased total chlorophylls increased productivity per plant at maturity at low levels (ECe 5.0 $mScm^{-1}$) of NaCl and upto ECe 10.0 $mScm^{-1}$ of Na_2SO_4 (Tables. 9,10). This indicated that increased chlorophylls at low levels of chloride and at moderate levels of sulphate increase productivity at maturity.

Thus, this plant adapts to saline conditions by synthesizing more chlorophylls at all levels of both the salts.

The moisture percentage (Tables.7,8) under NaCl treatment decreased in root and leaf but increased at low (ECe 5.0 $mScm^{-1}$) of NaCl. The total plant moisture content decreased with increasing salinity. Under sulphate salinity, moisture percentage decreased in root and stem, however, in leaf it increased at low (ECe 5.0 $mScm^{-1}$) of Na_2SO_4.

2) TAN

Results of total organic acids (TAN) (Tables.23,24) revealed that in the morning at 0600 hours, afternoon at 1200 hours and evening at 1800 hours, TAN values decreased with increasing levels of NaCl. However, under sulphate salinizations, TAN values increased at low levels and decreased at higher levels thereby indicating that plants tolerated low levels of sulphate by increasing organic acids.

TAN values under control, as well as under both salinizations, were minimum in the morning at 0600 hours and maximum in the afternoon at 1200 hours and intermediate in the evening at 1800 hours which indicated that there is no Crassulacean acid metabolism (CAM) under control as well as under saline conditions in safflower Cv. Bhima.

3) CARBOHYDRATES

Results of carbohydrate (Tables.25-30 ; Fig.17 -22) content in root of Carthamus tinctorius Cv. Bhima. indicated that the reducing sugars content increased only at ECe

5.0 $mScm^{-1}$ of NaCl and decreased at all higher levels. Under sulphate treatment, reducing sugar increased over the control at all levels. The non reducing sugars increased upto ECe 10.0 $mScm^{-1}$ of NaCl and decreased further at higher levels. However, under Na_2SO_4 treatment, its content was more than the control at all levels of salt. Maximum content was recorded at ECe 5.0 $mScm^{-1}$. This fact suggested that safflower Cv. Bhima adapts to all levels of sulphate salinizations and low levels of chloride salinity by increasing non-reducing sugars which are safe osmo-regulators. The starch content decreased with increasing NaCl and Na_2SO_4 treatments. The total carbohydrate content increased upto ECe 5.0 $mScm^{-1}$ of NaCl and decreased further at higher levels of NaCl, while under Na_2SO_4 treatment total carbohydrate content was more than the control at all levels. This fact revealed that this plant has an ability to maintain carbohydrate metabolism at all levels of sulphate salinizations.

The results of stem (upper, middle, lower part) indicated (Tables.27,28) that in upper part of stem, reducing sugar decreased with increasing NaCl concentrations, whereas, under Na_2SO_4 concentrations, reducing sugar was more than the control at all levels and maximum content was present at ECe 10.0 $mScm^{-1}$ of Na_2SO_4. The non reducing sugars, under NaCl were more than the control upto ECe 10.0 $mScm^{-1}$ and decreased at high (ECe12.5 to 15.0 $mScm^{-1}$) levels. Under sulphate treatment, non reducing sugar content was more than the control at all levels and the maximum content was observed at ECe 7.5 $mScm^{-1}$ of Na_2SO_4. The starch content was more than the control at all levels of NaCl and Na_2SO_4 treatments. Maximum starch content was present at ECe 12.5 $mScm^{-1}$ NaCl and ECe 7.5 $mScm^{-1}$ Na_2SO_4.

The total carbohydrate content in upper part of stem (Table.27) was more than the control at all levels of NaCl and Na_2SO_4 . Maximum content was at ECe 10.0 $mScm^{-1}$ of

Na_2SO_4. In middle part of stem (Table.27), reducing sugar content decreased with increasing NaCl salinity. However, under Na_2SO_4 salinization, content was more than the control at all levels and maximum reducing sugar was present at ECe 7.5 $mScm^{-1}$ of Na_2SO_4 .The non reducing sugars increased upto ECe 10.0 $mScm^{-1}$ of NaCl and decreased at all higher levels. However, under Na_2SO_4 treatment, content was more than the control at all levels and maximum content was found at ECe 7.5 $mScm^{-1}$ of Na_2SO_4 indicating that ECe 7.5 $mScm^{-1}$ is optimum. The starch content was increased upto ECe 10.0 $mScm^{-1}$ and decreased further with increasing NaCl concentrations and the content was found to be less than the control at all levels of sulphate treatments. Thus, sugar content was different in different parts of stem.

The total carbohydrate content in middle part of stem, was more than the control upto ECe 10.0 $mScm^{-1}$ and decreased at higher NaCl concentrations. However, under Na_2SO_4 treatment, total carbohydrates were more than the control at all levels and maximum content was found at ECe 7.5 $mScm^{-1}$ which clearly indicated that these salinity levels are most optimum for the synthesis of carbohydrates.

In lower part of stem, reducing sugar increased upto ECe 10.0 $mScm^{-1}$ of NaCl and then further decreased with increasing chloride treatment whereas, the content was more than the control at all levels of sulphate salinizations, with maximum content at ECe 7.5 $mScm^{-1}$ Na_2SO_4 .The non reducing sugar increased upto ECe 7.5 $mScm^{-1}$ of NaCl and decreased at higher salinity levels (ECe 7.5 to 10.0 $mScm^{-1}$). Under Na_2SO_4 treatments, content was more than the control at all levels and maximum content was found at ECe 7.5 $mScm^{-1}$ of Na_2SO_4. The starch content (Tables.25-30) decreased with increasing NaCl.and Na_2SO_4 concentrations.

The total carbohydrate content in lower part of stem was more than the control at low ECe 5.0 and 7.5 $mScm^{-1}$ levels of NaCl. However, the total carbohydrate content was more than the control at all levels of Na_2SO_4 and the maximum content was at ECe 7.5 $mScm^{-1}$ of Na_2SO_4.

In leaves and crown leaves (Tables. 29, 30), reducing sugar content was more than the control at all levels of NaCl and Na_2SO_4 treatments. Maximum content was at ECe 10.0 $mScm^{-1}$ of NaCl and Na_2SO_4 treatments.The non reducing sugar were more than control at all levels of NaCl and maximum content was recorded at ECe 10.0 $mScm^{-1}$. However, under Na_2SO_4 treatment, its content decreased with increasing salinity.The starch content decreased with increasing NaCl concentrations. However, starch content was more than control at all levels of sulphate salinization with maximum content at ECe 5.0 $mScm^{-1}$ which reflects that ECe 5.0 $mScm^{-1}$ of sulphate is optimum level for synthesis of starch.

The average total carbohydrate (Tables.25-30 ;Fig.17-22) content of total plant increased at low (ECe 5.0 to 10.0 $mScm^{-1)}$) and decreased at high (ECe 12.5 to 15.0 $mScm^{-1}$) levels of NaCl. Under Na_2SO_4 treatment, the average total carbohydrate content was more than the control upto ECe 12.5 $mScm^{-1}$ and was less at higher salinity levels. Increased carbohydrate content of total plant upto ECe 10.0 $mScm^{-1}$ of chloride could not help in maintaining productivity (Tables.5,6) at flowering because productivity under NaCl salinity was less than the control. However, increased carbohydrate content definitely helped for maintaining more productivity at maturity at Ece 5.0 $mScm^{-1}$ of chloride (Table. 9). Increased carbohydrate content upto ECe 12.5 $mScm^{-1}$ of sulphate helped for increasing productivity at flowering in plants grown upto

ECe 7.5 $mScm^{-1}$ of sulphate and at maturity (Table. 10) in plants grown upto ECe 10.0 $mScm^{-1}$ of Na_2SO_4.

4) PROLINE

From the results(Tables.31,32 ; Fig. 23ab,24ab), it is clear that proline content in roots increased linearly with increasing concentrations of both the salts suggesting that proline plays an important role in salt tolerance under both the salts in Carthamus tinctorius Cv. Bhima.

In upper part of stem, accumulation of proline was increased only upto ECe 5 $mScm^{-1}$ of NaCl and decreased at all higher levels of chloride. Under Na_2SO_4 treatment, its content was more than the control in plants grown at all levels of salts. Highest content of proline was recorded at ECe 10.0 $mScm^{-1}$ of Na_2SO_4.

In middle part of stem, proline was more than the control at all levels of NaCl and Na_2SO_4 treatments. In lower part of stem, its content was linearly increased with increase in concentrations of both the salts. As a result, average total proline content of total stem was linearly increased with increase in concentrations of both the salts,thereby, indicating that proline plays significant role in salt tolerance in safflower Cv. Bhima. In control, proline was highest in middle part followed by upper part while this trend was consistent at ECe 5.0 $mScm^{-1}$ of both the salts. However, at high ECe 7.5 to 15.0 $mScm^{-1}$ of both the salts, proline content was highest in the lower part, medium in the middle part and lowest in the upper part which clearly suggested that pattern of proline accumulation in stem changes under saline conditions.

In leaves, proline content increased linearly with increasing concentrations of both the salts ,thereby, indicating that all levels of both the salts stimulate proline biosynthesis in Carthamus tinctorius Cv. Bhima.

Proline content of total plant increased with increasing concentrations of NaCl and Na_2SO_4 which clearly suggested that the plant adapted to both the salinizations by synthesisizing more proline. Increased proline content helps in maintaining more productivity upto ECe 10.0 $mScm^{-1}$ under sulphate salinizations and at ECe 5.0 $mScm^{-1}$ of NaCl at maturity because productivity (Tables. 9,10) was more than the control. However, increased proline at higher levels of chloride and sulphate helps for survival rather than maintaining growth.

5) PROTEINS

The results in Tables. 33,34 indicated that in roots, protein content decreased with increasing NaCl concentrations while its content increased at low levels (ECe 5.0 and 7.5 $mScm^{-1}$) of Na_2SO_4 and decreased at all higher (ECe 10.0 to 15.0 $mScm^{-1}$) Na_2SO_4 levels. These results suggest that all concentrations of NaCl inhibit protein biosynthesis in roots. Low concentrations of Na_2SO_4 stimulate while high concentrations of it, inhibit protein biosynthesis in roots of safflower Cv. Bhima.

In upper, middle and lower parts of stem, protein content decreased under NaCl salinization, while protein content increased at a low (Ece 5.0 and 7.5 $mScm^{-1}$) levels of Na_2SO_4 and was inhibited at high (Ece 10.0 to 15.0 $mScm^{-1}$) concentrations of Na_2SO_4. Thus, it can be concluded that NaCl reduced protein content in all parts of the stem while low concentration of Na_2SO_4 enhanced the content and high concentrations of it

decreased protein content in all parts of the stem. Average total protein content in total stem decreased linearly with increase in NaCl concentrations whereas its content increased at low ECe 5.0 and 7.5 $mScm^{-1}$ levels of Na_2SO_4 and decreased at all higher concentrations, thereby, indicating that all concentrations of NaCl reduce while low concentrations of Na_2SO_4 enhance the total protein content in stem. High concentrations of Na_2SO_4 reduced protein content in stem of safflower Cv. Bhima. It was observed that protein content (Tables.33,34 ; Fig. 25,26) decreased at all levels of both the salts.

Protein content of average total plant (R+S+L) (Tables. 33, 34), was less than the control at all levels of NaCl while its content was more that the control in plants grown upto ECe 7.5 $mScm^{-1}$ of Na_2SO_4 and was less than the control at all high levels of Na_2SO_4 ,thereby, indicating that all concentrations of NaCl inhibit while low concentrations of Na_2SO_4 stimulate and high concentrations of Na_2SO_4 inhibit protein biosynthesis of safflower Cv. Bhima..

IV) PHOTOSYNTHETIC AND OXIDATIVE ENZYMES UNDER SALINE CONDITIONS.

1) RuBP-Carboxylase and 2) PEP-Carboxylase

a) In vivo effect of NaCl and Na_2SO_4 on activity of RuBP-Case (Table.39 ; Fig.27a, 28(a)) indicated that activity of RuBP-Case decreased linearly with increasing NaCl salinity. Under sulphate treatment however, the activity increased upto ECe 7.5 $mScm^{-1}$ but decreased at higher salinity levels which reflects that all levels of NaCl inhibit while low levels of Na_2SO_4 stimulate and high levels of it, inhibit activity of Rubisco. Thus, effect of NaCl and Na_2SO_4 is different. Increased activity of RuBP-Case correlates with increased productivity at low (upto ECe 10.0 $mScm^{-1}$) levels of Na_2SO_4 (Table. 10).

Slight decrease in activity of RuBP-Case did not affect productivity at ECe 5.0 $mScm^{-1}$ of NaCl (Table. 9). One of the reasons for decreased productivity at higher levels of both the salts is decreased activity of RuBP-Case.

The activity of PEP-Case (Tables.39, 40 ; Fig. 27b,28b) was more than the control at all levels of both the salinity's. Highest activity of PEP-Case was at ECe 15.0 $mScm^{-1}$ of both the salts. These results indicated that all levels of both the salts stimulate activity of PEP carboxylase in this plant. The ratio of RuBP-Case and PEP-Case decreased with increasing levels of both the salts. Thus, the results indicated that saline conditions alter the ratio of RuBP-Case to PEP-Case and activity of PEP-Case increases under saline conditions. However, its importance at this stage is unclear because safflower is a C_3 plant. The ratio of RubP-Case to PEP-Case was 6.24 under control conditions which indicated that safflower Cv. Bhima is a C_3 plant.

b) Addition of NaCl, upto 20 mM (Table.41) in the reaction mixture, stimulates activity of RuBP-Case and addition of 25 to 150 mM of NaCl inhibits the same. Results (Table. 42) also revealed that addition of Na_2SO_4 upto 10.0 mM stimulates while higher concentrations of it inhibit the activity of RuBP-Case.

The activity of PEP-Case (Tables. 41,42) was more than the control upto 50 mM of NaCl and upto 90 mM Na_2SO_4 salinity levels and further activity of enzymes decreased at higher levels of both the salts. Thus, the results indicated that low concentrations of both the salts stimulated activities of RuBP-Case and PEP-Case while high concentrations of them inhibit activity of both the enzymes and PEP-Case is more salt tolerant than RuBP-Case. These results confirmed in vivo effect of both the salts on activities of both the enzymes.

3) Phospho glycolate phosphatase (PGP)

a) In vivo effect of NaCl (Table.39) and Na_2SO_4 (Table.40) on activity of phosphoglycolate phosphotase in safflower Cv. Bhima indicated that under NaCl and Na_2SO_4 treatment, PGP activity was more than the control in plants grown at all levels of both the salts which indicated that all levels of both the salts stimulate activity of PGP.

b) In vitro effect of NaCl and Na_2SO_4 (Tables.41,42) on activity of phosphoglycolate phosphotase indicated that under NaCl conditions activity of PGP was more than the control upto 120 mM of NaCl, but decreased further with increasing NaCl levels. Addition of Na_2SO_4 upto 15 mM stimulated its activity while at all higher (20-1000 mM) levels it inhibited its activity.

4) Glycolate-oxidase

a) In vivo effect of NaCl and Na_2SO_4 (Tables.39,40) on activity of glycolate oxidase suggested that the activity was linearly increased with increase in concentration of NaCl and Na_2SO_4 treatments. Thus, all levels of salt stimulate the activity of glycolate oxidase in Carthamus tinctorius Var. Bhima.

b) In vitro effect of NaCl and Na_2SO_4 (Tables.41,42) on activity of glycolate oxidase revealed that activity of glycolate oxidase was more than the control upto 90 mM NaCl and decreased at all higher concentrations. Addition of Na_2SO_4, upto 200 mM to the reaction mixture resulted in increase in activity of glycolate oxidase while addition of higher (200 mM to 1000 mM) concentrations of it inhibited the same, which reflects that

low concentrations of NaCl and Na_2SO_4 stimulate activity of glycolate oxidase. Thus, in vivo results of glycolate oxidase were confirmed by in vitro studies. It is clear from these results that activities of all photorespiratory enzymes increases at all levels of both the salts. However, productivity (Tables. 9, 10) at maturity per plant was more than the control upto ECe 5.0 $mScm^{-1}$ of NaCl and upto ECe 7.5 $mScm^{-1}$ of Na_2SO_4 (grain weight per plant) and upto ECe 10.0 $mScm^{-1}$ of Na_2SO_4 (DM per plant) which reflects that increased photorespiration could not decrease productivity at low levels of both the salts. This increase in photorespiration must be accompanied with much more increase in photosynthesis at these levels. At higher levels of both the salts increase in activities of these enzymes correlates well with decrease in productivity. (Tables.9,10) at maturity.

5) Peroxidase

a) In vivo effect of NaCl and Na_2SO_4 (Tables.39,40;Fig. 27c,28c) on activity of peroxidase in safflower Cv. Bhima indicated that activity of peroxidase increases in plants grown upto ECe 5.0 $mScm^{-1}$ of NaCl followed by sudden decrease at higher salinity levels. Under Na_2SO_4 treatment, however, enzyme activity increased linearly with increasing salinity levels indicating that low levels of NaCl and all levels of sulphate stimulate peroxidase activity whereas high levels of NaCl inhibited peroxidase activity. Thus ,peroxidase plays an important role for salt tolerance at low levels of NaCl and at all levels of sulphate. This enzyme must be destroying accumulation of toxic H_2O_2 produced during increased photorespiration under saline conditions.

b) In vitro effect of NaCl and Na_2SO_4 on activity of peroxidase indicated (Tables. 41,42) that activity of enzyme was stimulated upto 200mM NaCl and further it was decreased at all high concentrations (upto 1000 mM) of NaCl. Under sulphate treatment, its activity

increased upto 700 mM of Na_2SO_4 and decreased at all higher (upto 1000 mM) concentrations of it.

In vivo effect of NaCl on activity of peroxidase (Table. 39) indicated that its activity increases in plants grown upto ECe 5.0 $mScm^{-1}$ of NaCl and at higher levels, it decreases. However, in vitro effect of NaCl indicated that a wider range of NaCl concentration (upto 200 mM) stimulates activity of peroxidase. The reason for this at present is obscure.

6) Pyruvate Pi dikinase (orthophosphate dikinase) (PPDK)

a) In vivo effect of NaCl and Na_2SO_4 (Tables. 39, 40) on activity of PPDK revealed that the activity of enzyme is stimulated at low (ECe 5.0 $mScm^{-1}$) levels of NaCl concentrations, while it decreased at all higher salinity levels. Under Na_2SO_4 treatment, the activity of enzyme increased with increasing concentrations of salt upto ECe 12.5 $mScm^{-1}$. Thus, a wider range of Na_2SO_4 concentration favors activity of PPDK.

b) In vitro effect of NaCl and Na_2SO_4 (Tables. 41,42) on activity of PPDK reflects that its activity was increased upto 10 mM of NaCl and upto 120 mM of Na_2SO_4 ,which confirms in vivo effect of both the salts on activity of PPDK.

V) STOMATAL STUDIES UNDER SALINE CONDITIONS.

A. Stomatal Index and stomatal number

The Stomatal Index (Tables. 35, 36). of upper surface decreased gradually with increase in levels of NaCl salt. However, under Na_2SO_4 salt, stomatal index of upper surface and lower surface increased upto ECe 7.5 $mScm^{-1}$ and decreased at all higher salinity levels.

The stomatal number of upper and lower surface decreased with increasing levels of both the salts. These results clearly indicated that all levels of both the salts inhibit development of stomata. Thus, by decrease in stomatal number plants adapt to saline conditions i.e. by reducing transpiration.

B. Stomatal Rhythms

Under control conditions (Tables. 37, 38) stomata of lower surface opened during 9 to 11 a.m. and during 4 to 6 p.m., while on upper surface stomata was open during 8 to 11 a.m. and 3 to 6 p.m. This showed two peaks of opening like a typical C_3 plant. Under both the salinity's of upper and lower surfaces stomata remained open during 9 to 10 a.m. and 4 to 7 p.m. Thus, stomatal rhythm was altered under both the salinity's and major adapation was to close stomata at 11 am i.e. one hour earlier than the control. This indicates that when there was more heat (between 10 to 11 am) plants closed stomata and conserved water. From all these results, it is clear that safflower Cv. Bhima adapts to wider range of sulphate salinity and can be grown profitably upto ECe 7.5 $mScm^{-1}$ of Na_2SO_4 and it can survive and complete a life cycle upto ECe 10.5 $mScm^{-1}$. It also adapts to low levels of NaCl. It can be grown profitably upto ECe 5.0 $mScm^{-1}$ of NaCl and can survive and complete a life cycle upto ECe 7.5 $mScm^{-1}$. The mechanism of salt tolerance is differentiated under chloride and sulphate salinity.

I) PROTEIN PROFILE AND ISOENZYME PATTERN UNDER SALINE CONDITIONS

Results of protein profile (Tables.43,44 ; Fig.29,30) under increasing concentrations of NaCl and Na_2SO_4 indicated that the number of polypeptides present in control and at all levels of NaCl are similar. Under saline conditions synthesis of these polypeptides is

imbalanced or impaired ,i.e. some are more synthesized than the control while some are less synthesized. Thus, there is no qualitative change under sodium chloride salinization in Carthamus tinctorius L. Cv. Bhima. Contrary to this, under sulphate salinizations two new polypeptides are present in all treated seedlings. These two polypeptides should be stress proteins. In addition to this at 0.6 % and 0.7% of Na_2SO_4, one (Rm=0.196) polypeptide is also present. Thus, safflower Cv. Bhima tolerates sulphate salinizations by synthesising stress proteins. From all these results, it is clear that safflower Cv. Bhima can be cultivated on sulphate saline lands with more productivity per plant (Table. 10), ie. Upto ECe 7.5 $mScm^{-1}$ of Na_2SO_4 (grain weight per plant =141.8% of the control , DM per plant =270% of the control) and with 91.8% grain yield per plant upto ECe 10.0 $mScm^{-1}$ of Na_2SO_4. This plant adapts to sulphate salinizations by several mechanisms mentioned above. This cultivar could be grown with more productivity (Table. 9) at maturity upto ECe 5.0 $mScm^{-1}$ of NaCl (grain weight per plant = 127% of the control, DM per plant = 103.7% of the control). It can be cultivated upto ECe 7.5 $mScm^{-1}$ of NaCl with grain weight = 63.6% of the control per plant.

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