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RESEARCH ARTICLE

EFFECT OF Na_2SO_4 SALINITY ON GROWTH, CHLOROPHYLL CONTENT, POLYPHENOLS AND PROLINE CONTENTS OF *TRIANTHEMA PORTULACASTRUM* L.

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Abstract

The effect of Na_2SO_4 salinity (sand culture) on root length, shoot length, total length (Height of Plant), root / shoot ratio, biomass (fresh and dry wt.) and chlorophylls, polyphenols and proline contents of a sodium loving plant Trianthema portulacastrum L. has been investigated. The plants were raised from seeds in acid free silica sand culture with Hoagland nutrient medium and treated with 0.0 (control), 100, 200 and 300mM Na_2SO_4 for two months. The plants exhibited better performance in growth and development under low salinity (100mM Na_2SO_4) level, 300mM salt concentration was slightly harmful. Amount of total chlorophylls, chl. at and chl. b, was significantly reduced under increasing salinity levels. Polyphenol level was increased with increasing salt level in the medium. Proline content was also significantly increased under increasing Na_2SO_4 salinity level. Trianthema portulacastrum L. appears to be moderately tolerant to Na_2SO_4 salinit.

Introduction:-

Soil salinity problem has been widely discussed all over the world. Salinity is generally defined as the presence of the excessive amount of soluble salt that affects the normal functions in plant growth and development. Soil salinity affects large areas of the cultivated land in more than 100 countries (Rengasamy 2006).Area of saline soil is increasing day by day.Some of the productive soils are converted into the saline soils due to over irrigation,improper water management, poor drainage, excess use of fertilizers. The ions responsible for salination are Na^+, K^+, $Ca^{2,}$ Mg^{2+}, and Cl^-

Depending upon the nature of salt the salinity is chloride salinity, sulphate salinity, and carbonate salinity. Salt stress has negative effects on plant growth and development, such as seed germination and seedling growth, reduction in water potential Nitrogen metabolism and photosynthetic enzymes are affected, stimulates respiratory enzymes. To face the problem of salinity it is necessary to understood, the effect of salinity on plant different adaptations developed by plants growing under salt stress conditions and mechanism of salt tolerance in them.

The present work was conducted in order to study the effect of Na_2SO_4 sand culture on growth and development, chlorophyll content polyphenol content and proline accumulation in *Trianthema portulacastrum* L.

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Material and methods :-

Plantlets of *Trianthema portulacastrum* L were raised from the seeds in Hoagland solution in acid free silica sand. The plants were stabilized for 20 days and then treated with 0.00 (Control), 100, 200, 300 mM Na_2SO_4 for two months. The treatmentwere given twice a week, alternating with water – to check the loss of water from pots and to avoid excess accumulation saltin the pots. Drainage of the waterand salt solution was well maintained. Two months after the growth in saline media the plants were analyzed for different parameters.

Growth analysis:-

For growth analysis 5 plants from every treatment pot were carefully uprooted and washed with water . Water drops on the plant surface were removed using blotting paper. Each plant was then analysed for root length, shoot length and total length of plants.

The fresh weight of each plant from each treatment was also measured. After recording fresh weight the respective plants were kept in oven separately at 60^0 C till they gave constant dry weights. Which were recorded.

With the help of dry weight and fresh weight, moisture percentage was calculated by using formula-

$$\text{Moisture percentage} = \frac{freshwt.-drywt}{freshwt} \times 100$$

All the results were statistically analysed.

Photosynthetic pigments:-

Chlorophylls:-

Chlorophylls were estimated from fresh leaves following the method by Arnon (1949). The plant material was homogenized in 80% acetone at 0-4^0C in dark with addition of a pinch of magnesium carbonate, to protect and stabilize the chlorophylls. The extract was filtered through Whatman No. 1 filter paper under suction. The residue was washed 3-4 times with 80% acetone. The filtrate and washings were collected together and final volume was made to 100ml with 80% acetone. The absorbance was read at 663 and 645nm respectively for chlorophyll a and b on UV-VIS spectrophotometer (Shimadzu – UV 190) using 80% acetone as blank. The chlorophyll a, chlorophyll b, and total chlorophylls were calculated by the following formula –

Chlorophyll a = (12.7 x $^A663$) - (2.69 x $^A645$) = ……. X

Chlorophyll b = (22.9 x $^A645$ - (4.68 x $^A663$) = ….. Y

Total chlorophylls = (8.02 x $^A663$) + (20.2 x $^A645$) = …..Z

$$\text{Chlorophyll a / chlorophyll b / Total chlorophylls} = \frac{X\ /\ Y\ /Z\ x\ volume\ of\ extract\ \ x\ 100}{1000\ x\ weight\ of\ plant\ mater\ in\ (g)}$$

(mg 100^{-1}g fresh tissue)

Polyphenols:-

Polyphenols were estimated according to Folin and Denis (1915) from fresh leaves. The plant material was homogenized in mortar with pestle in 30ml 80% acetone. The extract was filter through Whatman no.1 filter paper under suction. The residue was washed 3-4 times with 80% acetone. The filtrate and washings were collected together and final volume was made to 50ml with 80% acetone. 2ml of this extract was mixed with 10 ml 20% Na_2CO_3 solution volume was made to 35ml with distilled water and 2ml Folin Denis reagent (100g sodium tungstate and 20g phosphomolybdic acid dissolved in 800 ml distilled water to which 50ml 85% phosphoric acid were mixed and mixture was refluxed for 24 hours) was added to it. Final volume was made to 50 ml with distilled water. Absorbance was read at 660nm on UV Spectrophotometer (Shimadzu). A blank was prepared without polyphenols.Standard curve of polyphenols (tannic acid, 10mg / 100ml) was obtained and polyphenol content were calculated, and the values are expressed as mg 100^{-1} fresh tissue.

Proline:-

Free proline content of leaves was estimated following the method by Bates et al. (1973) 0.5g of plant material washed dried and cut into small pieces. The material was homogenized in 10ml of 3% sulphosalicylic acid and then filtered through Whatman no.1 filter paper.

4ml of the filtrate was mixed with 2ml acid ninhydrin reagent. (Mixture of 2.5g Ninhydrin, 60ml Glycial acetic acid and 40ml, 6M Orthophosphoric acid heated for 5-10 min) and 2ml acetic acid. The contents were boiled on water bath for 1hours and cooled rapidly in ice bath. 4ml Tolune was then added and absorbance of Tolune chromophore at room temperature was recorded at 520nm against tolune blank. Standard curve of proline (0.1mg/ml) was prepared taking different concentrations as 0.2, 0.4, 0.6, 0.8ml of L-proline.

Results and Discussion:-

Growth analysis:-

Analysis of growth is the fundamental key for characterization of a plants response to an environmental stress. The effect of Na2SO4 salinity on growth and development of *Trianthema portulacastrum* L. plants under saline condition is given in Table No.1. It is evident that Na_2SO_4 salinity has caused an increase in root length, shoot length, biomass production and moisture content of plants under lower concentration of Na_2SO_4(100mM) and decreased under the higher concentrations (200 and 300mM). The root to shoot ratio has clearly indicated that salinity mainly affects the root growth. From the present results it was observed that the growth of *Trianthema portulacastrum* L was stimulated at lower levels of Na_2SO_4salinity only. It is interesting to note that even though there was decrease in the growth of the plants (under higher level of salinity i.e.200 and 300mM), these were not only surviving but also growing well except that there was stunted growth. According to Hameda et al.(2013) salinity treatments (NaCl and Na_2SO_4) reduced plant growth of *Vicia faba* L.

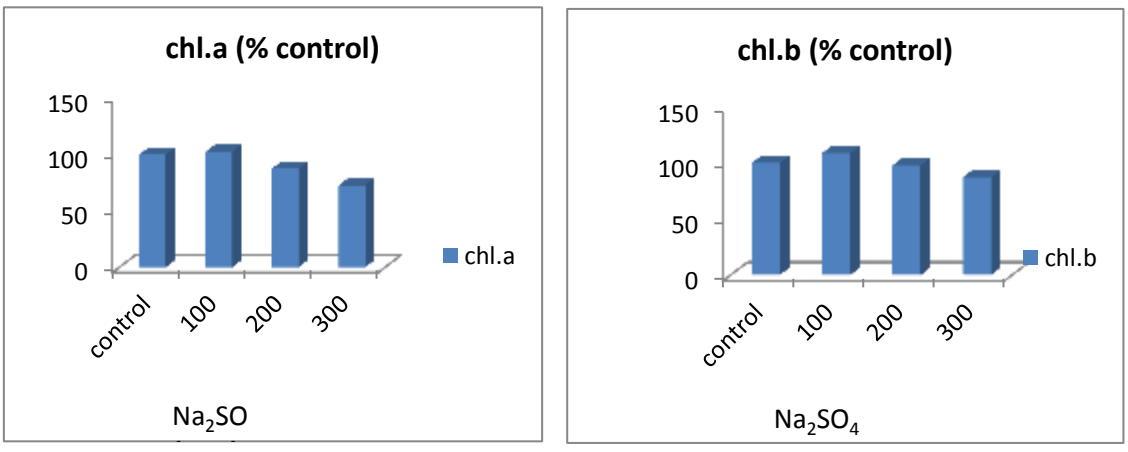
Table No. 1:- Influence of Na_2SO_4 Salinity on growth and Development of *T. portulacastrum*. grown in sand culture.

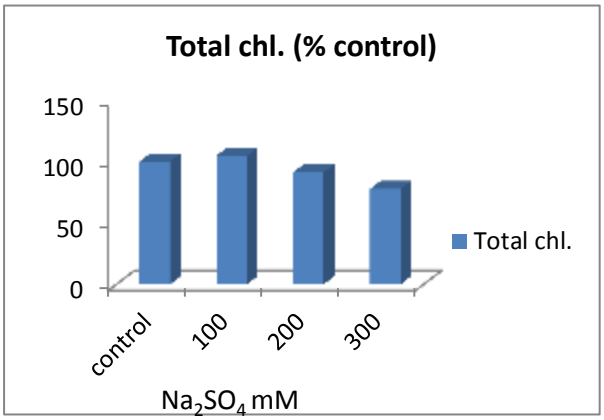
Treatment Na_2SO_4 mM	Root Length (cm.)	Shoot Length (cm.)	Total Length (cm.)	Root / Shoot Ratio	Fresh wt. (g/plant)	Dry wt. (g/plant)	Moisture %
00	8.12 ± 1.67	39.4 ± 8.20	47.52 ±9.65	0.21 ±0.02	16.14 ±9.51	1.84 ±1.07	88.59 ±0.53
100	**8.20 ± 1.64**	**43.60 ± 8.29**	**51.80 ±7.98**	**0.18 ±0.05**	**20.04 ±6.66**	**1.92 ±0.59**	**90.41 ±0.69**
200	6.80 ± 1.20	28.80 ±2.46	35.60 ±3.47	0.24 ±.02	9.11 ±2.30	1.27 ±0.26	86.05 ±1.14
300	6.50 ± 1.51	20.8o ±5.22	27.30 ±6.10	0.32 ±0.07	4.52 ±1.7	0.71 ±0.22	84.29 ±2.37

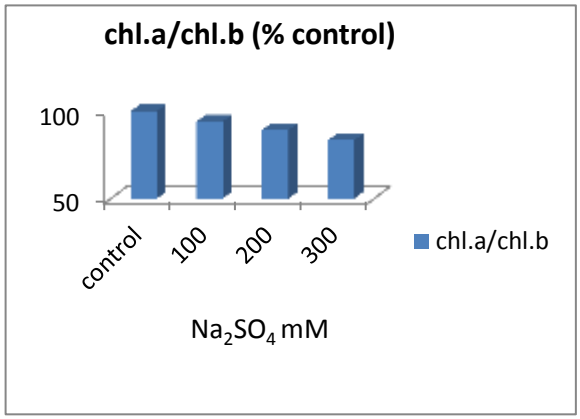
Chlorophyll Content :-

The chlorophyll a, chlorophyll b, and total chlorophyll contents of leaves were increased under low level of salinity and that decreased under higher level of salinity. Chlorophyll a / b ratio was decreased with increasing level of salinity. This reduction in chlorophyll content might have affected the production of dry matter in the leaves. Appearance of the plants grown at higher levels of stress clearly indicated the adverse effect of salinity on the photosynthetic pigments in the foliage. According to Khan et al. (2013), total leaf chlorophyll content was significantly decreased with increasing NaCl levels. The decrease in chlorophyll content of plants affected by salt may be due to inhibition of chlorophyll synthesis, together with the activation of its degradation by the enzyme chlorophyllase (Santos, 2004).

Fig No.1:-Influence of Na_2SO_4 Salinity on Chlorophyll content of the leaves of *T. portulacastrum L.* grown in sand culture.







Total Chl. content of leaves under control=119.17mg/100g fresh tissue

The Chl. a content of leaves under control=77.75mg/100g fresh tissue

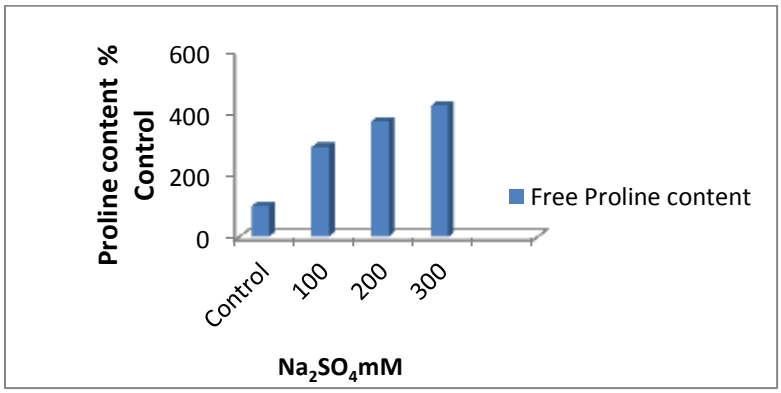
The Chl. a/b ratio in the leaves under control= 1.87

The Chl. b content of leaves under control=41.45mg/100g fresh tissue

Free Proline Content:-

Proline accumulation was increased due to salt treatment. Proline is an important secondary amino acid. Generally it is accumulated in the tissue of plant growing under salt stress. The accumulated proline helps in osmotic adjustments of such plants. It acts as an enzyme protector. Gaikwad (2005) has made similar type of observation in *Catharanthus*. An increase in proline content of *Trianthema portulacastrum* L. might be for osmotic adjustment under salinity stress. Alavi and Ranjbar (2012) also observed increase in proline content due to salinity in Rape seed (*Brassica napus* L). Proline is generally considered as a good indicator of environmental stress (Clausen, 2005).

Fig. No. 2:- Influence of Na_2SO_4 Salinity on Proline content of the leaves of *T. portulacastrum* L. grown in sand culture.



The Proline content of leaves of *Trianthema portulacastrum* under control =5.4m g/100g fresh tissue

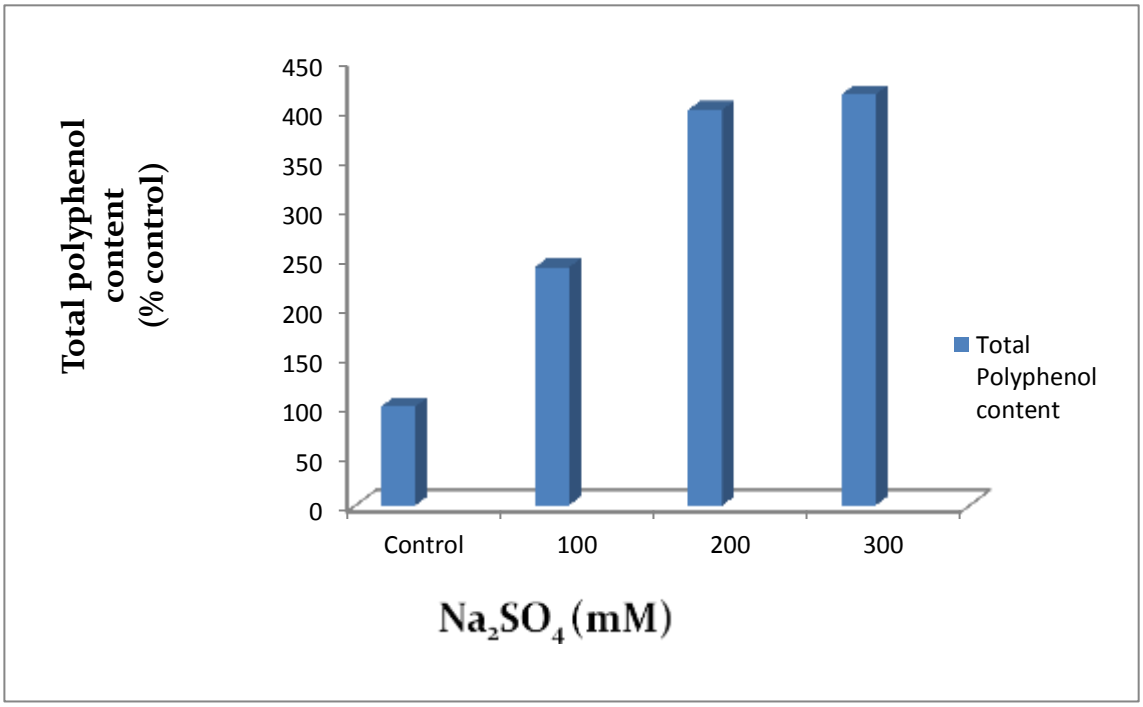
Polyphenols:-

Influence of Na_2SO_4 salinity on polyphenol contents of the leaves is shown in fig (3)

In the present study the polyphenol content was increased with increasing the level of salinity. It indicates that there is an induction of secondary metabolism by salinity which might have helped the plant to tolerate such on adverse condition. Accumulation of polyphenols due to salt stress was noticed by many workers. Parida et al. (2002)

observed an accumulation of polyphenols in *Brughira parviflora* with the increasing levels of salinity. According to them such accumulation of polyphenols played akey role in the plants towards the stress tolerance. Different plant processes such as photosynthesis, chlorophyll synthesis, water relation, protein synthesis, respiration, membrane permeability are sometimes affected by these phenolic compounds.

Fig. No. 3:-Influence of Na_2SO_4 Salinity on Polyphenol content of the leaves of *T. portulacastrum L.*



The Polyphenol content of leaves of *Trianthema portulacastrum* under control =0.32 g/100g fresh tissue.

Conclusion:-

Trianthema portulacastrum L. appears to be moderately tolerant to salinity because various growth parameters of this plant are affected under high salinity levels.

Increase in polyphenol content and proline accumulation probably help the plant to tolerate such an adverse condition.

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