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STUDIES OF PHYSICAL AND METABOLIC RESPONSES TO SALINITY STRESS IN FENUGREEK

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ABSTRACT:

The herbage vegetables like *Trigonella foenum-graecum* Linn. (Vern. Methi, Eng: Fenugreek), is green vegetable whose leaves are packed with essential nutrients. The ability of plants to tolerate and thrive in saline soils of semi arid region like Patan district is of great importance in agriculture, since it indicates that the affected plants have genetic potential of salt tolerance.

The soil characteristic was evaluated as it influences the plant growth and development. These included Physical parameters like Soil Texture, Soil Temperature and Soil Density. The Chemical parameter tested were Soil Salinity, Water Holding Capacity, Soil pH and Moisture Content. Total Dissolved Salt (TDS) of the irrigated water was also measured.

The uniform seeds of three varieties of this plant were sown in polyethylene bags. Sodium chloride (NaCl) was added to the soil and salinity was maintained at 0.35(control), 2.78, 5.52, 8.58, 11.15 and 13.8 dS/m. The plants were cultivated using normal agronomic practice. The Physiological and Biochemical Analysis of plant including germination studies were carried out under the above salinity stress. The percentage germination decreased with the increase in salinity. The Physiological parameters included Root length, Shoot length, Leaf number per plant, fresh weight and dry weight of stem, root and leaf. The difference in fresh and dry weight gave biomass. All the above parameter decreased with the increase in salinity. The Biochemical parameters included estimation of Total Carbohydrate, Total Protein, Total free Amino

Acids, Proline, Chlorophyll a, Chlorophyll b, and Total Chlorophyll. All the above Biochemical decreased with the increase in salinity except Proline which increased along with the increase in salinity. The variety of the plant which performed better on all the above parameter was ascertained for further recommendation for cultivation.

KEY WORDS: *Salinity, Fenugreek, Physiological parameter, Biochemical parameter, Patan.*

INTRODUCTION:

Soil is a very specific component of the biosphere because it is not only a geochemical sink for contaminants, but also acts as a natural buffer controlling the transport of chemical elements and substances to the atmosphere, hydrosphere, and biota. However, the most important role of soil is its productivity, which is basic for the survival of humans. Plants depend on it for their nutrients, water supply and anchorage.

The Salinity of the soil relates to accumulation of salts[1]. Salinity is known to induce stress in plants; hence the ability of plants to tolerate and thrive in saline soils is of great importance in agriculture, since it indicates that the affected plants have genetic potential of salt tolerance, which is highly desirable trait. (Francois and Mass, 1994[2], Mahmood *et al*., 2000[3]). Sruvastanv & Jana, (1984)[4] and Shannon (1984)[5] attribute the lack of salinity cultivars to inadequate means of detecting and measuring plant response to salinity and ineffective selection methods. Bernstein and Hawward[6] and Strongnov[7] have attributed the deleterious effect of salinity as it increases the osmotic pressure of rooting medium and consequently decreases the availability of soil water to plants. The salt-induced water deficit is one of the major constraints for plant growth in saline soil. Root zone salinity can rapidly inhibit root growth and in turn their capacity to uptake water and essential mineral nutrients from the soil. (Neumann, 1995)[8]. Richard H.Niemen (1962)[9] studied effect of sodium chloride on growth, photosynthesis and respiration and found growth decreased with increasing salt concentration. Salt induced oxidative stress is one of the most important factors that affect plants. Proline plays a major role in the anti-oxidative stress as a hydroxyl radical scavenger (Smirnoff & Cumbes 1989[10], Matysik *et al*., 2002[11]). Hydroxyl radical (OH°) are produced as a result of oxidative stress are harmful and can rapidly react with all types of bio-molecules, such as DNA, proteins and lipids leading to radical chain processes, cross linking, peroxidation, membrane leakage, production of toxic compound and finally cell death. (Hallewelland Gutteridge 1989[12], Davies 1995[13]). Proline protects membranes and protein against the effect of high concentration of inorganic ions (Gibson *et al* 1984[14]). It has been widely noted

that proline accumulation can be used as a salt stress parameter (Ramanjuja & Sudhakar 2001[15], Madan *et al* 1995[16]). The response of plants to excess NaCl is complex and involves changes in their morphology, physiology and metabolism (Hilal *et al.* 1998)[17]

STUDY AREA:

Patan district is located in northern part of the Gujarat state. It is located 23^0 41' to 23^0 55' North Latitude and 71^0 31' to 72^0 20' East Longitude. As per data available with office of the District Agriculture Officer- Patan, the area is covered by deposits comprises of alternated bands of sand and clay. The alluvium mainly consists of sand, gravel, clay, pebbles, *kankars*, etc. The soil of the district consists of Black soil, medium black soil, Loam soil, sandy soil and saline soil. The quality of ground-water deteriorates progressively from east to west with an increase in chloride and Total Dissolved Salts (TDS). The Kutch and North Gujarat region are ecoclimatically classified as arid and semi-arid region respectively. Holland and Cristle[19], Eriksson[20], concluded that one of the important factors responsible for further spread of salinity in non-saline region is the wind borne salt particles from adjoining sea shores or salty deserts. The conclusion support increasing salinity in North Gujarat due to its proximity with the desert of Kuchchh.

The climate is characterized by a hot summer and general dryness in the major part of the year. The year may be divided into four seasons. The cold season is from December to February. The hot season from March to the middle of June followed by the south-west monsoon season which continues up to about the end of the September, about 96 percent of the rainfall is received during this season. October and November constitute the post-monsoon or transition period. Average rainfall for last ten years is 641.8 mm.

General Vegetation in North Gujarat has been classified in 'India desert' by Clark (1898).[21]Main crop of Patan district are pearl millet, castor, pulses, cotton, sorghum, and fennel in *kharif* season while in Rabi season mustard, cumin, gram, carrot, potato, leafy vegetable and wheat are the major crop.

MATERIAL AND METHODS:

Soil and Water Analysis

It is important to evaluate the characteristic of the soil which provide anchorage and nutrition to the plant, thereby influencing the plant growth, and development. The top 15 cm surface soil was collected from an agricultural field and various soil parameters[22, 23, 24] which can be classified into Physical and Chemical Parameters were determined as follow. Physical Parameter included Soil

Texture, Soil Temperature and Soil Density while Chemical Parameter tested were Soil Salinity, Water Holding Capacity, Soil pH and Moisture Content

Physical Parameters

(a) Soil Texture

Soil texture was determined by the use of sieves of different number of meshes. This is a standard method. 100 gm soil is passed successively through a series of sieves with different size (diameter) of their meshes (pores), as given in table 1 below, beginning with maximum mesh size. Thus different fraction (different-sized particles), from the sample is separated. Texture is determined according to their proportion as given in table 2.

As shown in Table 1, the Sand is 44% while Clay and Silt is 44.8%. Therefore it can be inferred from Table 2, that the soil is Loam Soil.

(b) Soil Temperature

Soil thermometer is inserted into the soil and reading is recorded after 15 minutes. The temperature of the soil was 26°C.

(c) Soil Density

Definitive volume of soil is weighted in gram and density is derived in gm/cm^3. A box 58.5 (3 x 3 x 6.5) cubic cm was filled with soil. The weight of the soil was measured after taking out from the box. The weight was 55 gm. The Soil Density was calculated by dividing the weigh of the soil (55 gm) by Volume of the soil (58.5 cm^3) which came out to be 0.940 gm/ cm^3.

Chemical Parameters

(a) Soil Salinity

This characteristic of soils relates to their salt content. These salts usually involve sodium chloride, but other salts also occur. Soil salinity can be measured by determining the electrical conductivity of a solution using Electrical Conductivity Meter. When a voltage is applied across a substance, an electric current will only flow if the substance conducts electricity. When salts dissolve in water, ions are formed and the solution (the electrolyte) will conduct electricity. As a general rule, the higher the concentration of ions in solution (i.e, the higher the salt concentration) the better the solution conducts electricity; in other words, its electrical conductivity increases. Electrical conductivity is often expressed in units such as deciSiemens per metre (dS/m) or millisiemens per cm (mS/cm). Surface soil was collected, air dried and passed through a 2 mm sieve. Soil suspension was prepared in distilled water at 1:2 soil: water ratio. The suspension was

shaken and allowed to stand overnight. Thereafter, electric conductivity of the supernatant solution was determined with the conductivity meter. (Ramoliya et al. 2004).[25]

(b) Water Holding Capacity

Small holes are picked in the base of the tin box. Air dried and sieved soil is filled in the box. Water is added so that the soil gets saturated. The tin is kept in slanting position and hanged to a stand with the help of a string. Extra water comes out of the perforations at the base. It is allowed to stand for a while. When water drops stop coming out, the soil is removed and weighed immediately. This weight was 15.4 gm. Afterward the soil is kept in a hot air oven and dried at 105°C for 48 hrs. The sample is cooled in a desiccators and weight is recorded which was 10 gm. The difference in the weight in soil sample before and after heating gives the amount of water in the soil or water holding capacity of the soil which came out to be 5.4 gm. Water holding capacity per gram is calculated as 0.54. (5.4/10). Also the percentage of water retained by the soil is calculated as 54 %. (0.54 x 100). This is generally referred to as the field capacity of the soil.[23]

(c) Soil pH

The soil pH is found using pH meter. The soil solution is prepared after saturating the soil with water. The extract is used for pH measurement. The electrode of the pH meter is dipped in the solution and reading which is displayed in the meter indicates pH of the soil. The pH of the soil was 8.

(d) Moisture Content

It is generally estimated as percentage moisture content on oven dry weight basis. The soil sample of known weight (10 gm) is kept in hot air oven at a temperature of 105-110°C for 24 hours. The sample is reweighed (9.53 gm) and the loss in weight is found as 0.47 gm (10-9.53). The moisture content of the soil (dry weight basis) is calculated in percentage as 4.93% (0.47/9.53 x 100). [23]

(e) Total dissolved solids (TDS) of irrigation water

The dissolved mineral in irrigated water contributes to the overall salinity of the soil. They can be measured in terms of Total dissolved solids. (TDS). Total dissolved solids denote mainly the various kinds of minerals present in the water sample. They do not contain any gaseous or colloidal fraction. TDS was measured using Microprocessor Based Conductivity/TDS Meter. The electric conductivity of the irrigation water used was 1.5 dS/m which is equal to 960 ppm of dissolved salt. (APHA 1998).[26]

(f) Salinization of Soil

Surface soil was collected air dried and passed through a 2 mm sieve. Six lots of soil, 9 kg each, were separately spread, on polyethylene sheets. Sodium chloride (NaCl) amounting to 18, 36, 54, 72, and 90 gm was then thoroughly mixed with soil of five lots, respectively to give electrical conductivities of 2.78, 5.52, 8.58, 11.15 and 13.8 dS/m. There was no addition of NaCl to sixth lot of soil that served as a control. The electrical conductivity of control soil was 0.35 dS/m and this value is equal to 3.83 mM salinity. The method for determining electrical conductivity is described before.

Physiological and Biochemical Analysis of Plant including germination studies

Eighteen polyethylene bags were taken and small holes at regular interval were made for aeration and drainage of excessive water. Three kg of soil each with 0.35, 2.78, 5.52, 8.58, 11.15 and 13.8 dS/m soil salinity were filled in each polyethylene bag. Irrigation water was added to each bag to bring the soils to field capacity and soils were allowed to dry for seven days. Soils were racked using fingers and seeds of three varieties of *Trigonella foenum-graecum* Linn. viz, Desi, Javra, and Kitchen Garden were sown in bag containing soil of different salinity as mentioned above. Fifty seeds were sown in each bag and percentage germination was recorded after 24, 48, 72, 96 and 120 hrs (ISTA-International Seed Testing Association, 1985)[27].

Irrigation of soil with required amount of water was taken as a measure to control the level of soil salinity. For each salinity level, about 50 gm surfaces soil was carefully taken out from each bag at the interval of one month during the course of experiment and salinity was measured. Salinity of salinized soil became insignificantly lower (0.2 to 0.4 dS/m) until the experiment was terminated. The plants were cultivated using normal agronomic practice.

Methods of growth analysis of Gregory (1921[28], 1926[29]) and Hunt (1978)[30] were used for growth study. **Physiological** and **Biochemical** parameters were ascertained. Physiological parameters were Root length (cm), Shoot length (cm), Leaf number per plant, fresh weight (g/plant) and Dry weigh (g/plant) of stem, root and leaf. The difference in fresh and dry weight would give biomass. The dry weight was obtained after keeping the respective plant material in hot air oven at 80°C for 48 hrs. These parameters for different Salinity level were determined once plant attains maturity which was approximately 3 to 4 month.

Following Biochemical parameters were ascertained by the method given below after the plant attains maturity as mentioned above.

Estimation of Total Carbohydrates by DNS method (Miller, G.L, 1972)[31]

Standard curve of glucose solution

100 mg of glucose was dissolved in 100 ml of distilled water. DNS reagent is prepared by dissolving 1 gm of 3, 5- dinitrosalisalic acid, 30 gm of sodium potassium tartrate and 1.6 gm of sodium hydroxide in water to make the volume up to 100 ml. Different aliquots of glucose solution was taken and distilled water was added to make final volume 1 ml as shown in the observation table. 1 ml DNS reagent was added in all the test tube. The tubes were kept in water bath for 10 min and O.D. was taken at 540 nm. A proper blank without glucose is used. Under alkaline condition the sugar reacts with DNS to produce brown colour complex. Intensity of colour is measured at 540 nm and is directly proportional to its concentration. Absorbance was plotted against glucose solution. (Annexure 1)

Extraction and Estimation of Total Carbohydrate from Plant material

One gm plant material was crushed in hot 80% ethanol with mortar and pestle. It was then centrifuged and supernatant was collected. Ten ml 80% hot ethanol was added to the residue and again centrifuged. Supernatant was collected. Both the above supernatants were evaporated by keeping them on water bath at 80°C. 10 ml distilled water was added to dissolve the sugar. One ml of aliquots was taken in a test tube and One ml of distilled water was added. One ml of DNS reagent was added and the test tubes were kept for 10 minute in boiling water bath. Total volume was made 10 ml. with distilled water and O.D. was measured at 540 nm as shown in the observation table. Total Carbohydrate was estimated using Standard Curve by plotting the value of O.D. with corresponding concentration of sugar (µg/ml) as shown in observation table and finally calculated in mg for 10 ml extracted sample of carbohydrate which correspond to one gm plant material (mg/gm).

Calculation

Absorbance corresponds to 1 ml of test = A µg/ml

10 ml contains = B µg (A x 10)

Now µg is converted into mg by dividing it by 1000 as 1mg = 1000 µg and the result so obtained is show in the following observation table.

Estimation of Total protein by method of Lowry et al. (1951)[32]

Standard Curve of Protein

Preparation of Reagent

1. Reagent A: 2% Alkaline Na_2CO_3

0.1 N NaOH is prepared by dissolving 400 mg NaOH in 100 ml distilled water and 2 gm Na_2CO_3 is dissolved in 100 ml of 0.1 N NaOH.

2. Reagent B: 0.5% $CuSO_4$ solution in 1% Sodium Potassium tartrate (Na-K)

500 mg of $CuSO_4$ is dissolved in 100 ml of distilled Water and 1 gm Na-K is added. The solution is prepared fresh.

3. Reagent C:

100 ml Reagent A is Mixed with 2 ml Reagent B, just prior to use.

4. Reagent D: Folin-Ciocalteau reagent.

5 ml Folin reagent is diluted by adding equal amount of distilled water (5 ml.)

5. Preparation of Standard Protein Solution:

Stock solution of Bovine Serum albumin (BSA) is prepared by dissolving 200 mg (BSA) in 100 ml of distilled water. Working solution is prepared by taking 10 ml stock solution and mixing it in 90 ml distilled water.

Procedure:

Different aliquots of standard solution ranging from 0.1 ml to 1 ml are taken and distilled water is added in each test tube containing the above solution to make up the volume to 1 ml. Final volume 4 ml is made by adding distilled water. 5.5 ml alkaline mix (Reagent C) is added in each test tube, mixed well and allowed to stand at room temperature for 15 minute. 0.5 ml diluted folin reagent (Reagent D) is added to each tube, mixing rapidly after each addition. The tubes are left as such for 30 minutes at room temperature and the blue colour formed is measured at 650 nm. A proper blank without the protein is used. The standard graph is prepared by plotting the value of O.D with corresponding concentration of protein as shown in the following observation table. (Annexure 2)

Extraction and Estimation of Protein from Plant material

One gm plant material is crushed in 10 ml 80% alcohol and boiled at 100°C for 2-3 minute. It was kept overnight in fridge. It was centrifuged for 15 minute and supernatant was separated. 10 ml 5% perchloric acid is added to the residue and centrifuged. The residue was dissolved in 5 ml 1 N NaOH by shaking it for 30 minute and centrifuged. The supernatant collected (10 ml) was used as protein sample. One ml protein sample was taken and final volume was made 4 ml with distilled water. 5.5 ml alkaline mix (Reagent C) is added in each test tube, mixed well and allowed to stand at room temperature for 15 minute. 0.5 ml diluted folin reagent (Reagent D) is added to each tube, mixing rapidly after each addition. The tubes are left as such for 30 minutes at room temperature

and the blue O.D was taken at 650 nm. Total Protein was estimated using Standard Curve by plotting the value of O.D. with corresponding concentration of Protein in µg/ml as shown in observation table and finally calculated in mg for 10 ml protein sample obtained which corresponds to one gm plant material (mg/gm).

Calculation

Absorbance corresponds to 1 ml of test = A µg/ml

10 ml contains = B µg (A x 10)

Now µg is converted into mg by dividing it by 1000 as 1mg = 1000 µg and the result so obtained is show in the following observation table.

Estimation of Total free Amino Acids by Ninhydrin method (Moore S. and Stein W.H.-1948)[33]

Standard Curve of Glutamic acid

20 mg of Glutamic acid is dissolved in 50 ml distilled water to give 400 µg/ml concentration. Ninhydrin solution is prepared by dissolving 0.3 g ninhydrin in 100 ml n-butanol and 3 ml glacial acetic acid. Glutamic acid solution of different concentration is prepared by diluting the stock solution (0.5 to 3.0 ml). Final volume 4 ml is made with distilled water in all the test tube. 1 ml ninhydrin solution is added in each test tube. Reagent blank is prepared by mixing 1 ml ninhydrin solution in 4 ml distilled water. All the test tube were heated on a boiling water bath for 20 minute. After cooling absorbance was read at 540 nm using reagent blank as reference. Standard curve was prepared by plotting the absorbances against the amount of Glutiamic acid. (Annexure 3)

Extraction and Estimation of Total free amino acid

One gm plant material is crushed in 10 ml 80% ethanol in a mortar with a spatula of sand. The homogenate is centrifuged. Supernatant is collected and the residue is homogenized 2 or 3 more time with few ml ethanol. The homogentate is again centrifuged and the supernatant is collected. The whole process is again repeated and all the supernatant is pooled together. Excess of ethanol is evaporated on a water bath to make one ml solution. One ml plant extract is taken and distilled water is added to make up final volume to 4 ml. One ml ninhydrin solution is added to each test tube and all the test tubes were heated on a boiling water bath for 20 minute. After cooling absorbance was read at 540 nm. Total free amino acids (equivalent to glutamic acid) was estimated using Standard Curve by plotting the value of O.D. with corresponding concentration of glutamic acid in µg/ml which was converted into mg/gm plant material as one ml extraction was

obtained from one gm plant material and µg is converted into mg by dividing by 1000 as shown in observation table.

Estimation of Proline (Bates et al - 1973)[34]

Standard curve of Proline

20 mg of is Proline dissolved in 50 ml distilled water to give 400 µg/ml concentration. Ninhydrin solution is prepared by dissolving 0.3 g ninhydrin in 100 ml n-butanol and 3 ml glacial acetic acid. Proline solution of different concentration is prepared by diluting the stock solution (0.5 to 3.0 ml). Final volume 4 ml is made with distilled water in all the test tube. 1 ml ninhydrin solution is added in each test tube. Reagent blank is prepared by mixing 1 ml ninhydrin solution in 4 ml distilled water. All the test tube were heated on a boiling water bath for 20 minute. After cooling absorbance was read at 420 nm using reagent blank as reference. Standard curve was prepared by plotting the absorbances against the amount of Proline. (Annexure 4)

Extraction and Estimation of Proline

One gm plant material is crushed in 10 ml 80% ethanol in a mortar with a spatula of sand. The homogenate is centrifuged. Supernatant is collected and the residue is homogenized 2 or 3 more time with few ml ethanol. The homogentate is again centrifuged and the supernatant is collected. The whole process is again repeated and all the supernatant is pooled together. Excess of ethanol is evaporated on a water bath to make one ml solution. One ml plant extract is taken and distilled water is added to make up final volume to 4 ml. One ml ninhydrin solution is added to each test tube and all the test tubes were heated on a boiling water bath for 20 minute. After cooling absorbance was read at 420 nm. Proline was estimated using Standard Curve by plotting the value of O.D. with corresponding concentration of Proline in µg/ml which was converted into mg/gm plant material as one ml extraction was obtained from one gm plant material and µg is converted into mg by dividing by 1000 as shown in observation table.

Estimation of Chlorophyll a, Chlorophyll b and Total Chlorophyll by method given by Arnon, D.I., (1949)[35]

One gm leaf was cut into small pieces and grinded with a pinch of acid washed sand and pinch of $CaCO_3$. 5-10 ml of 80% acetone was added and made a paste. The paste in 10 ml of 80% acetone was centrifuged. The whole content (Total volume) was made to 25 ml with supernatant. The optical density was taken with the spectrophotometer at the wavelength A_{645} and A_{663} and using the following formula calculation was done.

Chlorophyll a = 12.7 (A_{663}) – 2.69 (A_{645}) x V /1000 x 1/W

Chlorophyll b = 22.9 (A_{645}) – 4.68 (A_{663}) x V/1000 x 1/W

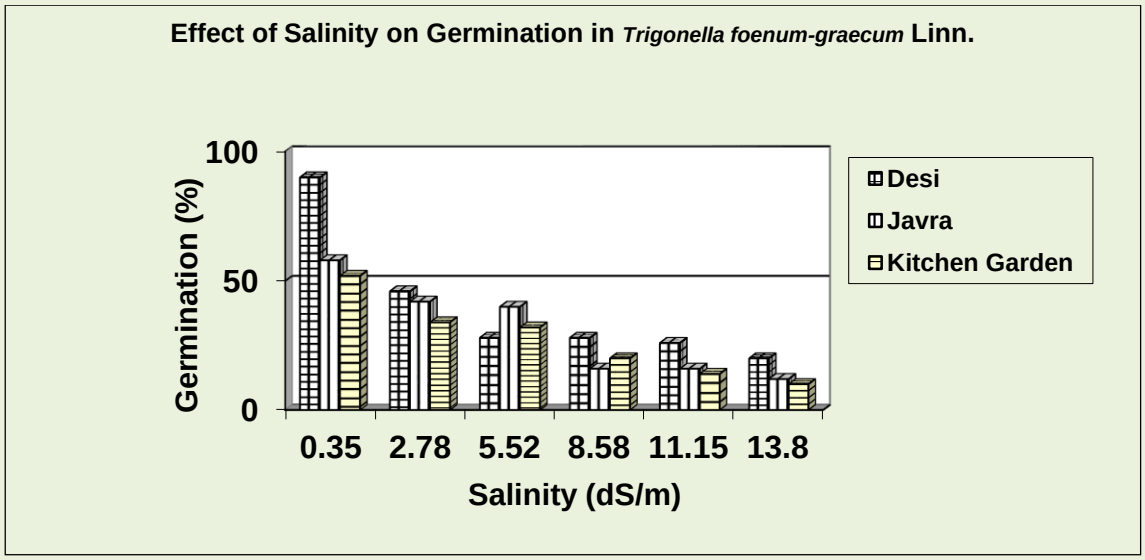
Total Chlorophyll = 20.2 (A_{645}) + 8.02 (A_{663}) x V/1000 x 1/W

Where V = Total Volume (25 ml) W = Weight of Plant Sample i.e. leaf (1 gm)

RESULTS AND DISCUSSION:

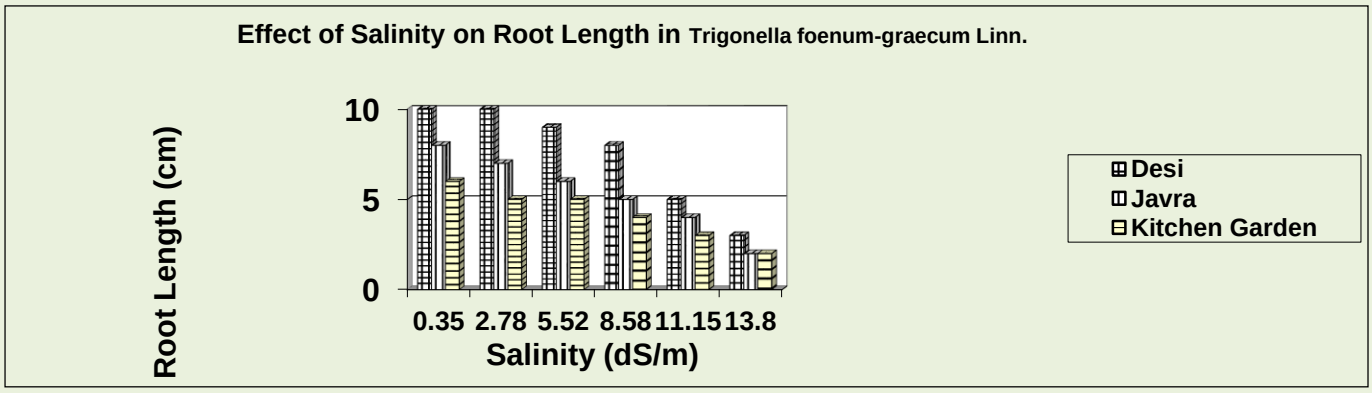
Effect of salinity of germination

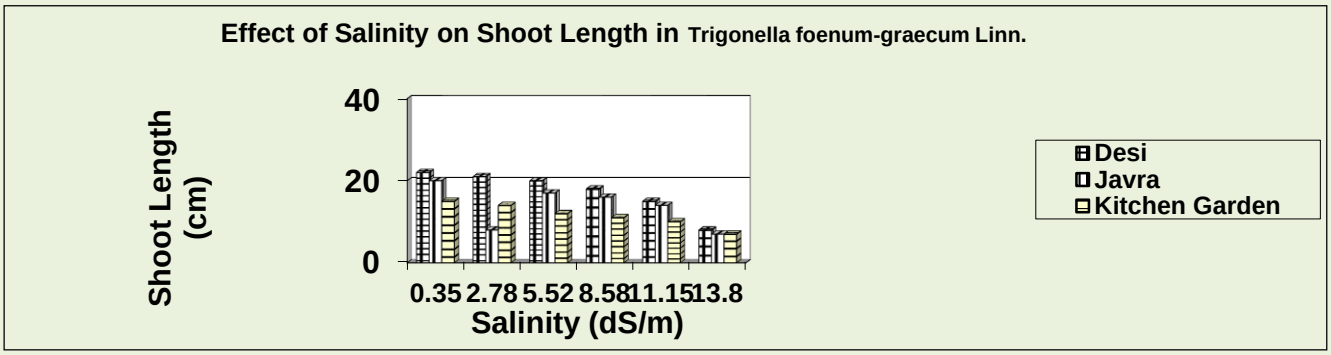
There was a significant reduction in seed germination with increasing salt salinity in all variety, however comparatively; the ability to withstand salinity was more in Desi, followed by Javra and lastly Kitchen Garden as shown in observation table and graph.

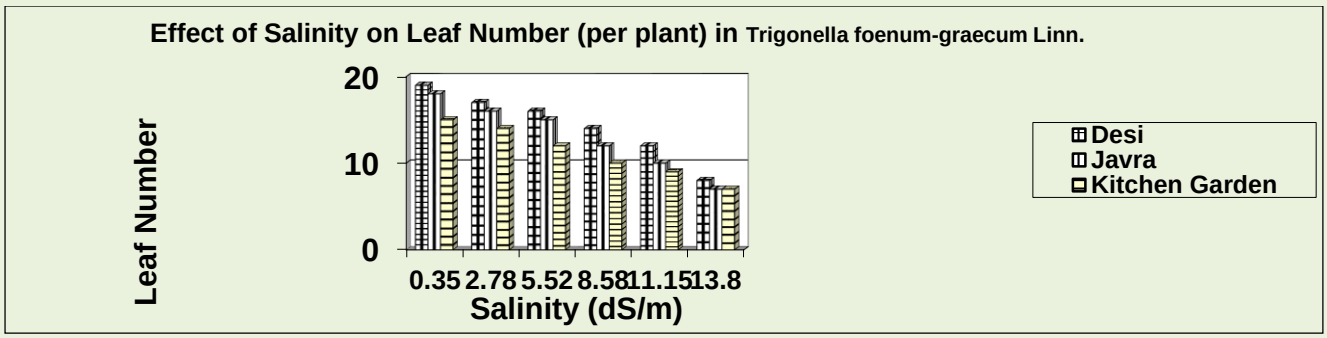


Effect of salinity on Physiological Parameter such as Root length, Shoot length and Leaf number per plant

Increasing soil salinity significantly retarded elongation of stem and root. The number of leaf per plant also decreased with increase in salinity. Comparatively; the ability to withstand salinity for all the three above mentioned physiological parameter was more in Desi, followed by Javra and lastly Kitchen Garden as shown in observation table and graphs.

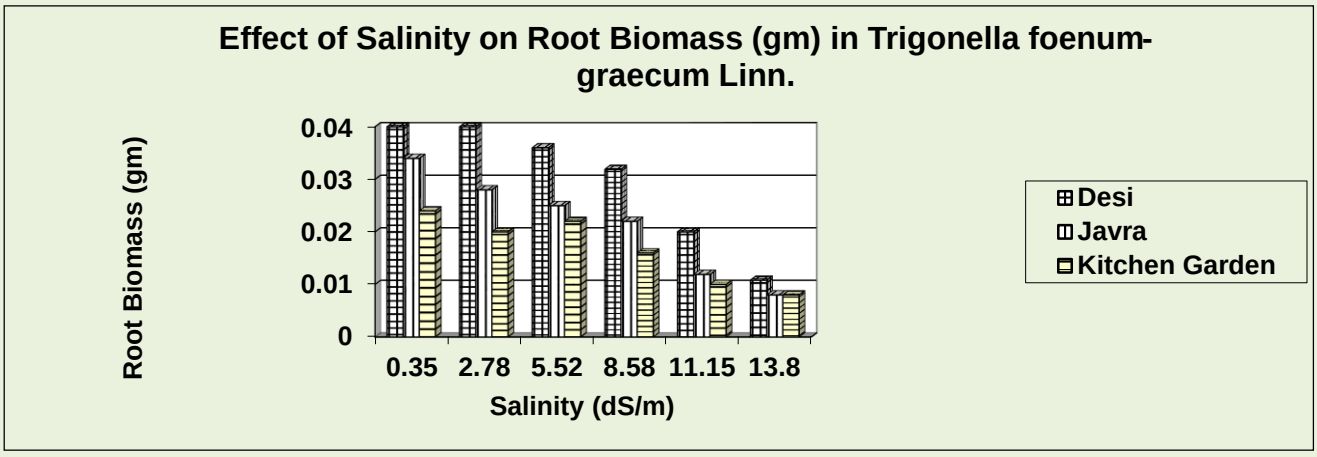


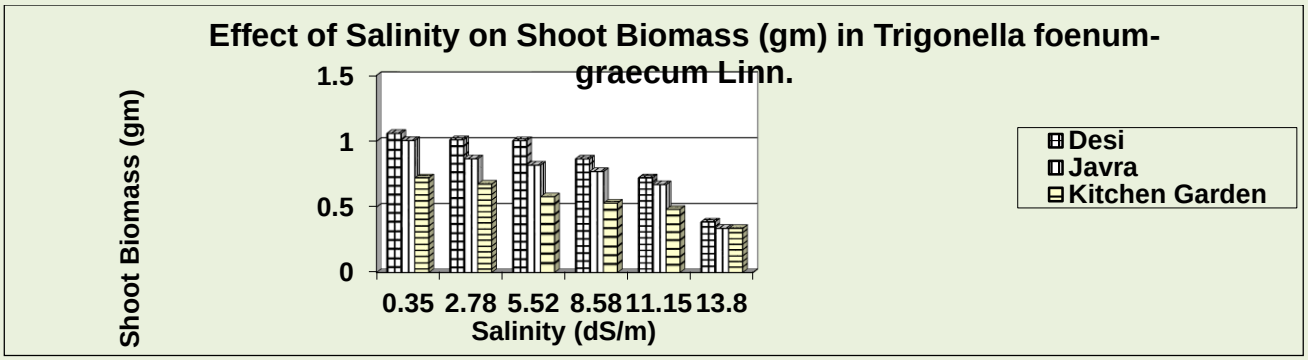


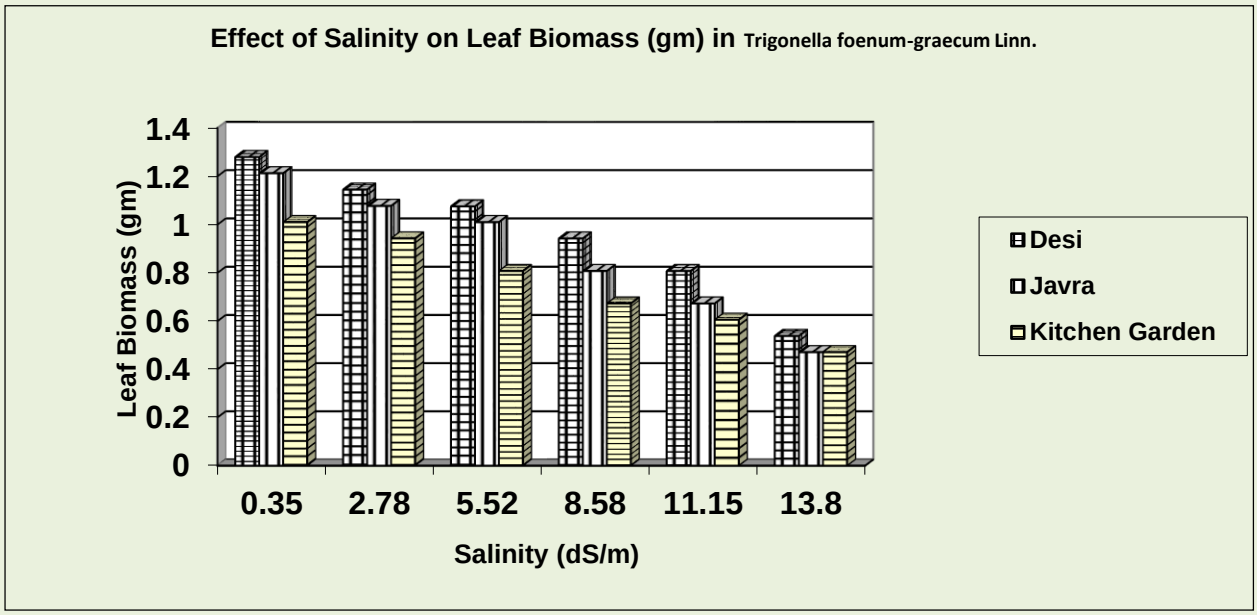


Effect of salinity on Physiological Parameter i.e. Biomass of root, shoot and leaf

Biomass of root, shoot and leaf decreased significantly in response to increasing concentration of salt. Comparatively; the ability to withstand salinity for all the three above mentioned physiological parameter was more in Desi, followed by Javra and lastly Kitchen Garden as shown in observation table and graphs.

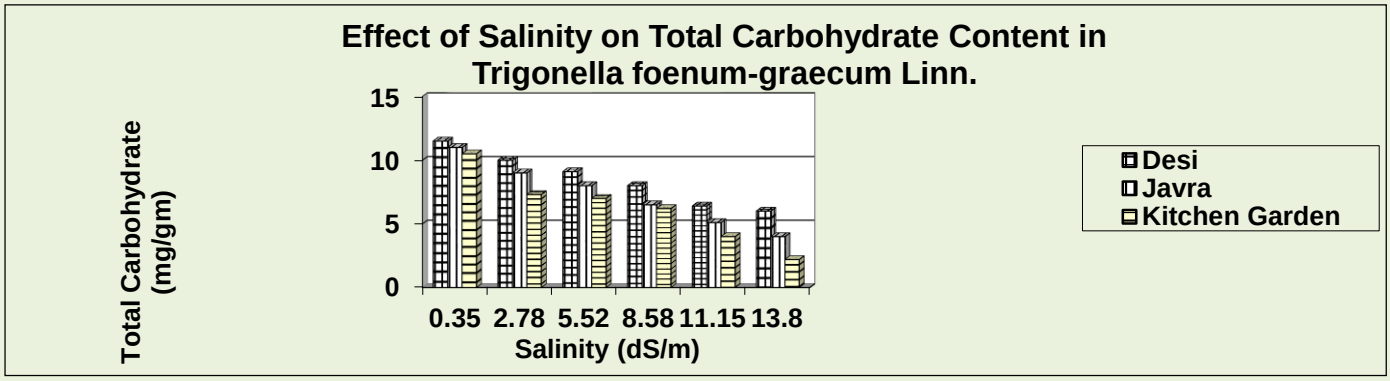






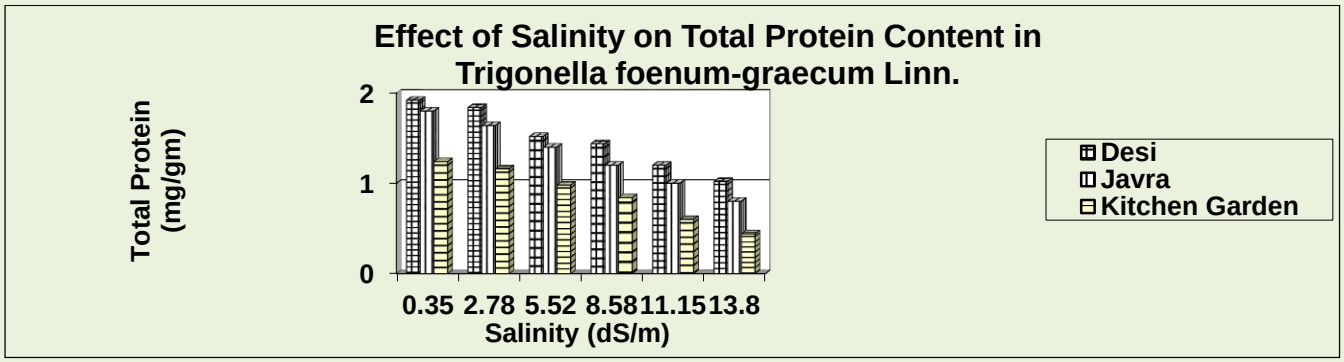
Effect of salinity on Total Carbohydrate Content

Total Carbohydrate content decreased with the increase in salinity. Comparatively; the ability to assimilate total carbohydrate was more in Desi, followed by Javra and lastly Kitchen Garden as shown in observation table no. 5 and following graph.



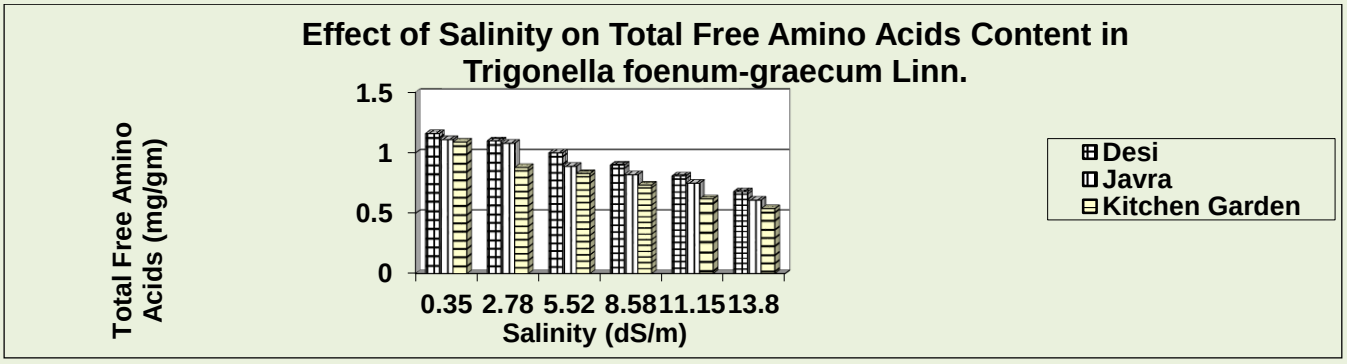
Effect of salinity on Total Protein Content

Total Protein content decreased with the increase in salinity. Comparatively; the ability to assimilate total protein was more in Desi, followed by Javra and lastly Kitchen Garden as shown in observation table no. 8 and following graph.



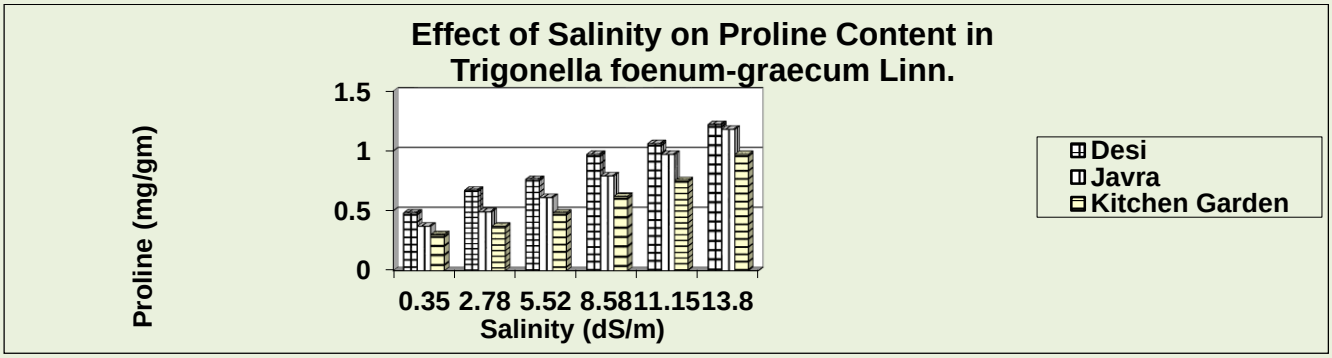
Effect of salinity on Total free Amino Acids Content

Total free amino acids content decreased with the increase in salinity. Comparatively; the ability to assimilate total free amino acids was more in Desi, followed by Javra and lastly Kitchen Garden as shown in observation table no. 10 and following graph.



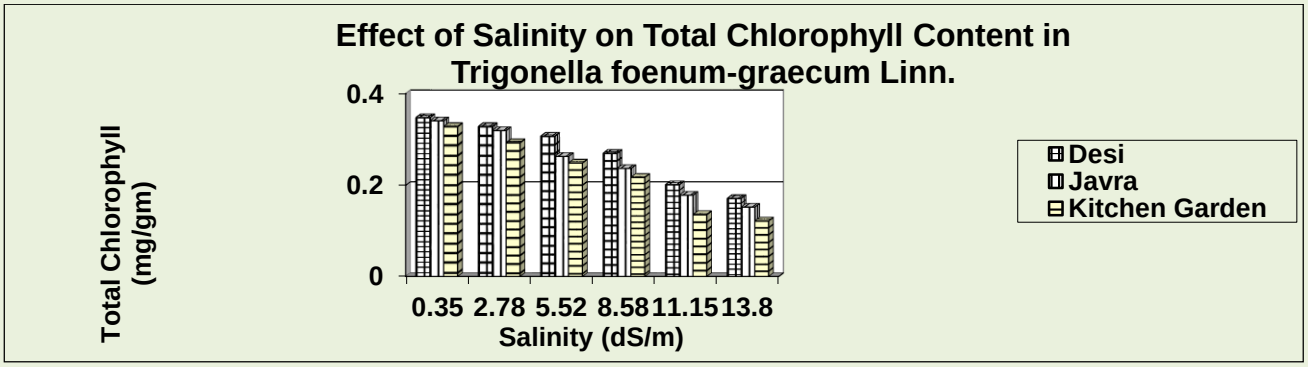
Effect of salinity on Proline Content

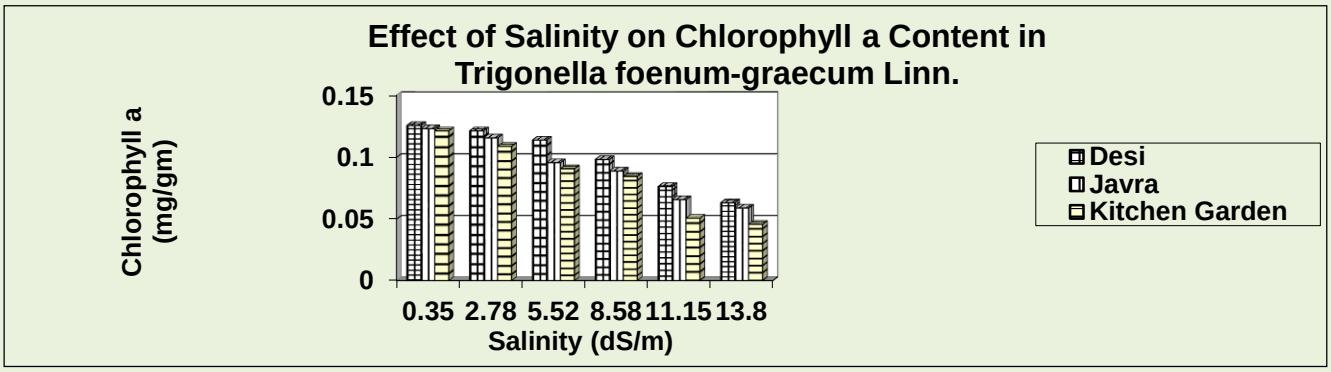
Proline content increased with the increase in salinity. Comparatively; the ability to assimilate proline was more in Desi, followed by Javra and lastly Kitchen Garden as shown in observation table no. 12 and following graph.

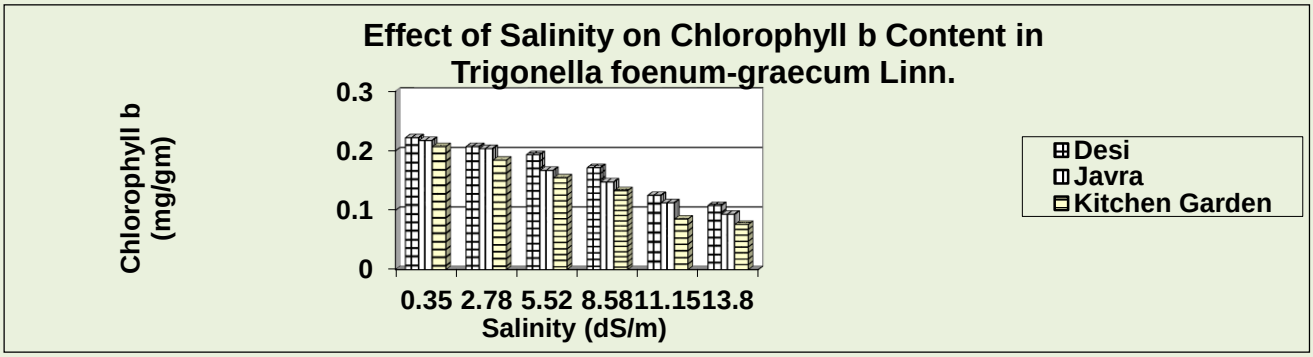


Effect of salinity on Chlorophyll Content

Effect of salinity on assimilation of Total Chlorophyll, Chlorophyll a, and Chlorophyll b was ascertained. They decreased with the increase in salinity. Comparatively; the ability to assimilate these chlorophylls was more in Desi, followed by Javra and lastly Kitchen Garden as shown in observation table no. 13 and following graphs.







DISCUSSION:

Salt stress can damage plants in various ways. High salt concentrations and high sodium concentrations in particular, will alter the structure of soils. Because porosity of the soil is decreased, both aeration and hydraulic conductance of soils can be adversely affected. High salt concentrations are also inextricably linked with water stress. High salt concentrations generate low soil water potentials, a form of physiological draught, that make it increasingly difficult for the plant to acquire both water and nutrients. Because they share the common element of osmotic stress, drought and high salt evoke similar responses as well. The solutes contributing to osmotic adjustment include proline, betaine and sorbitol that accumulate in response to water stress. Another form of injury involves toxicity effects of specific ions, especially Na^+ and Cl^-. Although the precise mechanism for injury is not yet understood, excess Na^+ might cause problems with membranes, enzyme inhibition, or general metabolic dysfunction. High salt suppresses growth and reduces carbon assimilation. (William G. Hopkins and Norman P.A.. Hunner, 2004).[36] Reductions in carbon assimilation is due to reduced photosynthesis as chlorophyll content decreases with increase in salinity. Salt stress evokes changes in the pattern of protein synthesis, suggesting that new genes may be transcribed or, at least, the products of some genes are increase and other decreased. Total Protein and Free Amino acid decreased while Proline increased with increase in salinity. The increase of proline content with increasing Na^+ concentration indicates that higher proline accumulation alleviates NaCl stress.

CONCLUSION:

Results of the present investigation, on the effect of salinity on three variety of *Trigonella foenum-graecum* Linn. viz, Desi, Javra and Kitchen Garden, as can be ascertained from germination studies, as well as physiological and chemical parameter such as nutrient accumulation like carbohydrate, protein etc, shows Desi, performed better on all the parameters followed by Javra and lastly Kitchen Garden. So for the Agriculture purpose Desi should be given first preference followed by Javra and lastly Kitchen Garden as per the order of salt tolerance as mentioned above.

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Table 1: Different Soil Particle Size.

Diameter of the particle in mm	Classification of Particle (weight of particle separated)
Less than 0.002	Clay (33.5 gm)
0.002-0.02	Silt (11.3 gm)
0.02-0.20	Fine sand (35.7 gm)
0.20-2.00	Coarse sand (8.3 gm)
2.0-5.0	Fine gravel (4.2 gm)
More than 5.0	Coarse gravel and stone (6.8gm)

Table 2: Different Soil Textural Group.

Relative Proportion of different-sized mineral particles	Textural group
85% Sand + 15% Clay or Silt or both	Sandy Soil
70% Sand + 30% Clay or Silt or both	Loamy Sand
50% Sand + 50% Clay or Silt or both	Loam Soil
90% Silt + 10% Sand	Silt

Table 3: Observation Table for Standard curve of glucose solution

Sr.No.	Vol. of Sugar Solution (ml)	Sugar Conc. (µg/ml)	Vol. of D.W. to make up to 1 ml.	DNS reagent (ml.)	Duration in boiling water bath (min.)	Total vol. 10 ml made with D.W.	O.D. at 540 nm.
1	Blank	- -	1.0	1	10	8	--
2	0.1	100	0.9	1	10	8	0.050
3	0.2	200	0.8	1	10	8	0.125
4	0.3	300	0.7	1	10	8	0.150
5	0.4	400	0.6	1	10	8	0.200
6	0.5	500	0.5	1	10	8	0.265
7	0.6	600	0.4	1	10	8	0.300
8	0.7	700	0.3	1	10	8	0.350
9	0.8	800	0.2	1	10	8	0.420
10	0.9	900	0.1	1	10	8	0.465
11	1.0	1000	0.0	1	10	8	0.525

Table 4: Observation Table for estimation of Total Carbohydrate per ml of test as derived from standard graph

Sr. No	Salinity (dS/m)	Variety	Vol. of Sugar sample (ml.)	Vol. of D.W. (ml.)	DNS reagent (ml.)	Duration in boiling water bath (min.)	Total vol. 10 ml made with D.W.	O.D at 540 nm	Total Carboh--ydrate (µg/ml)
1	0.35 (Control)	Desi	1	1	1	10	7	0.575	1150
		Javra	1	1	1	10	7	0.550	1100
		Kitchen Garden	1	1	1	10	7	0.525	1050
2	2.78 (2g/kg NaCl)	Desi	1	1	1	10	7	0.500	1000
		Javra	1	1	1	10	7	0.450	900
		Kitchen Garden	1	1	1	10	7	0.365	730
3	5.52 (4g/kg NaCl)	Desi	1	1	1	10	7	0.455	910
		Javra	1	1	1	10	7	0.400	800
		Kitchen Garden	1	1	1	10	7	0.350	700
4	8.58 (6g/kg NaCl)	Desi	1	1	1	10	7	0.400	800
		Javra	1	1	1	10	7	0.325	650
		Kitchen Garden	1	1	1	10	7	0.310	620
5	11.15 (8g/kg NaCl)	Desi	1	1	1	10	7	0.320	640
		Javra	1	1	1	10	7	0.255	510
		Kitchen Garden	1	1	1	10	7	0.200	400
6	13.8 (10g/kg NaCl)	Desi	1	1	1	10	7	0.300	600
		Javra	1	1	1	10	7	0.200	400
		Kitchen Garden	1	1	1	10	7	0.110	220

Table 5: Observation Table for estimation of Total Carbohydrate per gm plant material

Sr. No	Salinity (dS/m)	Variety	Total Carbohydrate (mg/gm)
1	0.35 (Control)	Desi	11.50
		Javra	11
		Kitchen Garden	10.50
2	2.78 (2g/kg NaCl)	Desi	10
		Javra	9
		Kitchen Garden	7.30
3	5.52 (4g/kg NaCl)	Desi	9.10
		Javra	8
		Kitchen Garden	7
4	8.58 (6g/kg NaCl)	Desi	8
		Javra	6.50
		Kitchen Garden	6.20
5	11.15 (8g/kg NaCl)	Desi	6.40
		Javra	5.10
		Kitchen Garden	4
6	13.8 (10g/kg NaCl)	Desi	6
		Javra	4
		Kitchen Garden	2.20

Table 6: Observation Table for Standard curve of Protein

Sr.No.	Working solution (ml)	Conc. of protein (µg/ml)	Vol.(ml) of D.W to make up to 4 ml.	Reagent C (ml)	Incubation	Reagent D (ml)	Time allowed for reaction	O.D. at 650 nm
B	Blank	- -	4.0	5.5 ml added to each test tube.	Incubation for 15 minutes at room temperature.	0.5 ml added to each test tube	Allowed to react for 30 min at room temp.	- -
1	0.1	20	3.9					0.050
2	0.2	40	3.8					0.100
3	0.3	60	3.7					0.145
4	0.4	80	3.6					0.200
5	0.5	100	3.5					0.255
6	0.6	120	3.4					0.290
7	0.7	140	3.3					0.350
8	0.8	160	3.2					0.410
9	0.9	180	3.1					0.450
10	1.0	200	3.0					0.485

Table 7: Observation Table for estimation of Total Protein per ml of test as derived from standard graph

Sr. No	Salinity (dS/m)	Variety	Protein sample (ml)	D.W (ml) to make 4 ml.	Reagent C (ml)	Incubation (min.)	Reagent D (ml)	Incubation (min.)	O.D at 650 nm.	Total Protein (µg/ml)
1	0.35 (Control)	Desi	1	3	5.5	15	0.5	30	0.480	192
		Javra	1	3	5.5	15	0.5	30	0.450	180
		Kitchen Garden	1	3	5.5	15	0.5	30	0.310	124
2	2.78 (2g/kg NaCl)	Desi	1	3	5.5	15	0.5	30	0.460	184
		Javra	1	3	5.5	15	0.5	30	0.410	164
		Kitchen Garden	1	3	5.5	15	0.5	30	0.290	116
3	5.52 (4g/kg NaCl)	Desi	1	3	5.5	15	0.5	30	0.380	152
		Javra	1	3	5.5	15	0.5	30	0.350	140
		Kitchen Garden	1	3	5.5	15	0.5	30	0.245	98
4	8.58 (6g/kg NaCl)	Desi	1	3	5.5	15	0.5	30	0.360	144
		Javra	1	3	5.5	15	0.5	30	0.300	120
		Kitchen Garden	1	3	5.5	15	0.5	30	0.210	84
5	11.15 (8g/kg NaCl)	Desi	1	3	5.5	15	0.5	30	0.300	120
		Javra	1	3	5.5	15	0.5	30	0.250	100
		Kitchen Garden	1	3	5.5	15	0.5	30	0.150	60
6	13.8 (10g/kg NaCl)	Desi	1	3	5.5	15	0.5	30	0.255	102
		Javra	1	3	5.5	15	0.5	30	0.200	80
		Kitchen Garden	1	3	5.5	15	0.5	30	0.110	44

Table 8: Observation Table for estimation of Total Protein per gm plant material

Sr. No	Salinity (dS/m)	Variety	Total Protein (mg/gm)
1	0.35 (Control)	Desi	1.92
		Javra	1.80
		Kitchen Garden	1.24
2	2.78 (2g/kg NaCl)	Desi	1.84
		Javra	1.64
		Kitchen Garden	1.16
3	5.52 (4g/kg NaCl)	Desi	1.52
		Javra	1.40
		Kitchen Garden	0.98
4	8.58 (6g/kg NaCl)	Desi	1.44
		Javra	1.20
		Kitchen Garden	0.84
5	11.15 (8g/kg NaCl)	Desi	1.20
		Javra	1
		Kitchen Garden	0.60
6	13.8 (10g/kg NaCl)	Desi	1.02
		Javra	0.80
		Kitchen Garden	0.44

Table 9: Observation Table for Standard curve of Total free amino acids

Sr.No.	Vol. of amino acid (ml)	Conc. of amino acids (µg/ml)	Vol.(ml) of D.W to make up to 4 ml.	Ninhydrin solution (ml)	Duration in boiling water bath (min.)	O.D at 540 nm
B	Blank	--	4.0	1	20	--
1	0.5	200	3.5	1	20	0.150
2	1.0	400	3.0	1	20	0.300
3	1.5	600	2.5	1	20	0.425
4	2.0	800	2.0	1	20	0.585
5	2.5	1000	1.5	1	20	0.710
6	3.0	1200	1.0	1	20	0.875

Table 10: Observation Table for estimation of Total Free Amino Acid per gm plant material

Sr. No	Salinity (dS/m)	Variety	Vol. of amino acid extract (ml)	Vol.(ml) of D.W to make up to 4 ml.	Ninhydrin solution (ml)	Duration in boiling water bath (min.)	O.D at 540 nm.	Total free amino acid (µg/ml)	Total free amino acid (mg/gm)
1	0.35 (Control)	Desi	1	3	1	20	0.850	1160	1.160
		Javra	1	3	1	20	0.815	1110	1.110
		Kitchen Garden	1	3	1	20	0.800	1090	1.090
2	2.78 (2g/kg NaCl)	Desi	1	3	1	20	0.810	1100	1.100
		Javra	1	3	1	20	0.790	1080	1.080
		Kitchen Garden	1	3	1	20	0.645	880	0.880
		Desi	1	3	1	20	0.730	1000	1

3	5.52 (4g/kg NaCl)	Javra	1	3	1	20	0.650	890	0.890
		Kitchen Garden	1	3	1	20	0.610	830	0.830
4	8.58 (6g/kg NaCl)	Desi	1	3	1	20	0.655	900	0.900
		Javra	1	3	1	20	0.605	820	0.820
		Kitchen Garden	1	3	1	20	0.540	735	0.735
5	11.15 (8g/kg NaCl)	Desi	1	3	1	20	0.600	810	0.810
		Javra	1	3	1	20	0.550	750	0.750
		Kitchen Garden	1	3	1	20	0.455	620	0.620
6	13.8 (10g/kg NaCl)	Desi	1	3	1	20	0.500	680	0.680
		Javra	1	3	1	20	0.450	610	0.610
		Kitchen Garden	1	3	1	20	0.400	540	0.540

Table 11: Observation Table for Standard curve of Proline

Sr.No.	Vol. of amino acid (ml)	Conc. of amino acids (µg/ml)	Vol.(ml) of D.W to make up to 4 ml.	Ninhydrin solution (ml)	Duration in boiling water bath (min.)	**O.D at 420 nm**
B	Blank	--	4.0	1	20	--
1	0.5	200	3.5	1	20	0.130
2	1.0	400	3.0	1	20	0.255
3	1.5	600	2.5	1	20	0.40
4	2.0	800	2.0	1	20	0.540
5	2.5	1000	1.5	1	20	0.665
6	3.0	1200	1.0	1	20	0.80

Table 12: Observation Table for estimation of Proline per gm plant material

Sr. No	Salinity (dS/m)	Variety	Vol. of amino acid extract (ml)	Vol.(ml) of D.W to make up to 4 ml.	Ninhydrin solution (ml)	Duration in boiling water bath (min.)	O.D at 420 nm.	Proline (µg/ml)	**Proline (mg/gm)**
1	0.35 (Control)	Desi	1	3	1	20	0.320	480	0.480
		Javra	1	3	1	20	0.250	370	0.370
		Kitchen Garden	1	3	1	20	0.205	300	0.300
2	2.78 (2g/kg NaCl)	Desi	1	3	1	20	0.450	670	0.670
		Javra	1	3	1	20	0.330	490	0.490
		Kitchen Garden	1	3	1	20	0.250	370	0.370
3	5.52 (4g/kg NaCl)	Desi	1	3	1	20	0.505	760	0.760
		Javra	1	3	1	20	0.410	610	0.610
		Kitchen Garden	1	3	1	20	0.320	480	0.480
4	8.58 (6g/kg NaCl)	Desi	1	3	1	20	0.650	970	0.970
		Javra	1	3	1	20	0.535	790	0.790
		Kitchen Garden	1	3	1	20	0.415	620	0.620
5	11.15 (8g/kg NaCl)	Desi	1	3	1	20	0.705	1060	1.060
		Javra	1	3	1	20	0.650	970	0.970
		Kitchen Garden	1	3	1	20	0.500	750	0.750

6	13.8 (10g/kg NaCl)	Desi	1	3	1	20	0.815	1220	1.220
		Javra	1	3	1	20	0.790	1180	1.180
		Kitchen Garden	1	3	1	20	0.650	970	0.970

Table 13: Observation Table for Estimation of Chlorophyll a, Chlorophyll b and Total Chlorophyll

Sr. No	Salinity (dS/m)	Variety	O.D 645	O.D 663	Chlorophyll a (mg/gm)	Chlorophyll b (mg/gm)	**Total Chlorophyll (mg/gm)**
1	0.35 (Control)	Desi	0.49	0.50	0.1257	0.2220	0.3477
		Javra	0.48	0.49	0.1232	0.2174	0.3406
		Kitchen Garden	0.46	0.48	0.1214	0.2071	0.3285
2	2.78 (2g/kg NaCl)	Desi	0.46	0.48	0.1214	0.2071	0.3285
		Javra	0.45	0.46	0.1157	0.2038	0.3194
		Kitchen Garden	0.41	0.43	0.1089	0.1844	0.2932
3	5.52 (4g/kg NaCl)	Desi	0.43	0.45	0.1139	0.1935	0.3073
		Javra	0.37	0.38	0.0957	0.1673	0.2630
		Kitchen Garden	0.35	0.36	0.09076	0.1547	0.2489
4	8.58 (6g/kg NaCl)	Desi	0.38	0.39	0.09827	0.1719	0.2700
		Javra	0.33	0.35	0.08893	0.1479	0.2368
		Kitchen Garden	0.30	0.33	0.0846	0.1331	0.2176
5	11.15 (8g/kg NaCl)	Desi	0.28	0.30	0.07642	0.1252	0.2015
		Javra	0.25	0.26	0.06573	0.1127	0.1783
		Kitchen Garden	0.19	0.20	0.05072	0.0853	0.1360
6	13.8 (10g/kg NaCl)	Desi	0.24	0.25	0.06323	0.1081	0.1713
		Javra	0.21	0.23	0.05890	0.0933	0.1521
		Kitchen Garden	0.17	0.18	0.04571	0.0762	0.1219

Table 14: Observation Table for Percentage Germination (%). Total 50 Seed were taken.

Sr. No.	Salinity (dS/m)	Variety	24 hrs		48 hrs.		72 hrs.		96 hrs		**120 hrs.**	
			No. of Seed germ.	Germination (%)	No. of Seed germ.	Germination (%)	No. of Seed germ.	Germination (%)	No. of Seed germ.	Germination (%)	No. of Seed germ.	Germination (%)
1	0.35 (Control)	Desi	0	0	40	80	43	86	45	90	45	90
		Javra	0	0	25	50	26	52	27	54	29	58
		Kitchen Garden	0	0	21	42	22	44	25	50	26	52
2	2.78 (2g/kg NaCl)	Desi	0	0	17	34	18	36	20	40	23	46
		Javra	0	0	20	40	20	40	21	42	21	42
		Kitchen Garden	0	0	14	28	15	30	16	32	17	34
3	5.52 (4g/kg NaCl)	Desi	0	0	13	26	14	28	14	28	14	28
		Javra	0	0	17	34	17	34	18	36	20	40
		Kitchen Garden	0	0	14	28	14	28	15	30	16	32

4	8.58 (6g/kg NaCl)	Desi	0	0	11	22	13	26	14	28	14	28
		Javra	0	0	4	8	5	10	6	12	8	16
		Kitchen Garden	0	0	7	14	8	16	9	18	10	20
5	11.15 (8g/kg NaCl)	Desi	0	0	12	24	12	24	12	24	13	26
		Javra	0	0	6	12	7	14	8	16	8	16
		Kitchen Garden	0	0	4	8	6	12	6	12	7	14
6	13.8 (10g/kg NaCl)	Desi	0	0	7	14	8	16	10	20	10	20
		Javra	0	0	2	4	4	8	5	10	6	12
		Kitchen Garden	0	0	3	6	4	8	4	8	5	10

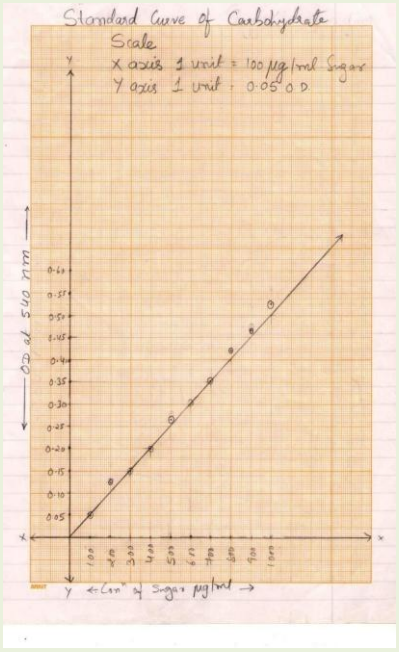
Table 15: Observation Table for Physiological Parameter such as Root length, Shoot length and Leaf number per plant

Sr.No	Salinity (dS/m)	Variety	Root Length (cm)	Shoot Length (cm)	**Leaf No. (per plant)**
1	0.35 (Control)	Desi	10	22	19
		Javra	8	20	18
		Kitchen Garden	6	15	15
2	2.78 (2g/kg NaCl)	Desi	10	21	17
		Javra	7	8	16
		Kitchen Garden	5	14	14
3	5.52 (4g/kg NaCl)	Desi	9	20	16
		Javra	6	17	15
		Kitchen Garden	5	12	12
4	8.58 (6g/kg NaCl)	Desi	8	18	14
		Javra	5	16	12
		Kitchen Garden	4	11	10
5	11.15 (8g/kg NaCl)	Desi	5	15	12
		Javra	4	14	10
		Kitchen Garden	3	10	9
6	13.8 (10g/kg NaCl)	Desi	3	8	8
		Javra	2	7	7
		Kitchen Garden	2	7	7

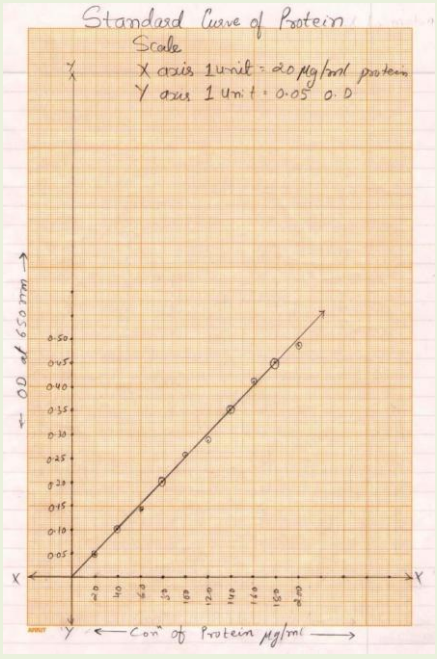
Table 16: Observation Table for Biomass of stem, root and leaf

Sr. No	Salinity (dS/m)	Variety	Fresh weight (gm)			Dry weight (gm)			**Biomass (Difference) (gm)**		
			Root	Shoot	Leaf	Root	Shoot	Leaf	Root	Shoot	Leaf
1	0.35 (Control)	Desi	0.060	1.17	1.57	0.020	0.11	0.290	0.04	1.06	1.28
		Javra	0.050	1.1063	1.488	0.016	0.100	0.275	0.034	1.0063	1.213
		Kitchen Garden	0.036	0.7977	1.2394	0.012	0.075	0.2289	0.024	0.7227	1.0105
2	2.78 (2g/kg NaCl)	Desi	0.061	1.1168	1.4047	0.021	0.105	0.2594	0.04	1.0118	1.1453
		Javra	0.042	0.9572	1.322	0.014	0.090	0.2442	0.028	0.8672	1.0778
		Kitchen Garden	0.030	0.7445	1.1568	0.010	0.070	0.2136	0.02	0.6745	0.9432
3	5.52 (4g/kg NaCl)	Desi	0.054	1.1060	1.320	0.018	0.101	0.2441	0.036	1.005	1.0759
		Javra	0.036	0.9040	1.2392	0.011	0.085	0.2286	0.025	0.819	1.0106
		Kitchen Garden	0.032	0.6381	0.9915	0.010	0.06	0.1831	0.022	0.5781	0.8084
4	8.58 (6g/kg NaCl)	Desi	0.048	0.9510	1.1566	0.0161	0.091	0.2135	0.0319	0.866	0.9431
		Javra	0.033	0.8509	0.9914	0.011	0.08	0.1830	0.022	0.7709	0.8084
		Kitchen Garden	0.024	0.5849	0.8263	0.008	0.055	0.1526	0.016	0.5299	0.6737
5	11.15 (8g/kg	Desi	0.030	0.7975	0.9915	0.010	0.0752	0.1834	0.02	0.7223	0.8081
		Javra	0.020	0.7444	0.8261	0.0081	0.073	0.1525	0.0119	0.6714	0.6736
		Kitchen	0.016	0.5318	0.7436	0.006	0.050	0.1373	0.010	0.4818	0.6063

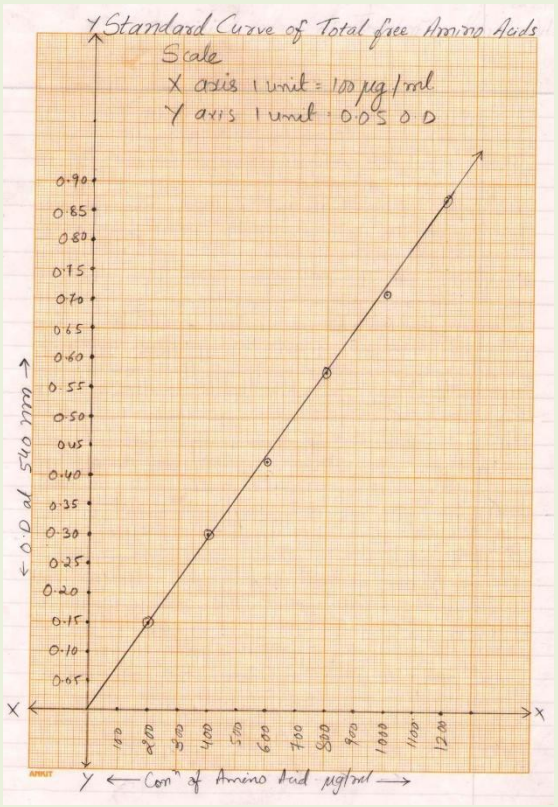
	NaCl)	Garden									
6	13.8 (10g/kg NaCl)	Desi	0.017	0.4254	0.6610	0.0062	0.04	0.1221	0.0108	0.3854	0.5389
		Javra	0.012	0.3722	0.5784	0.0040	0.035	0.1068	0.008	0.3372	0.4716
		Kitchen Garden	0.013	0.3720	0.5782	0.005	0.0353	0.1066	0.008	0.3367	0.4716



Annexure 1



Annexure 2



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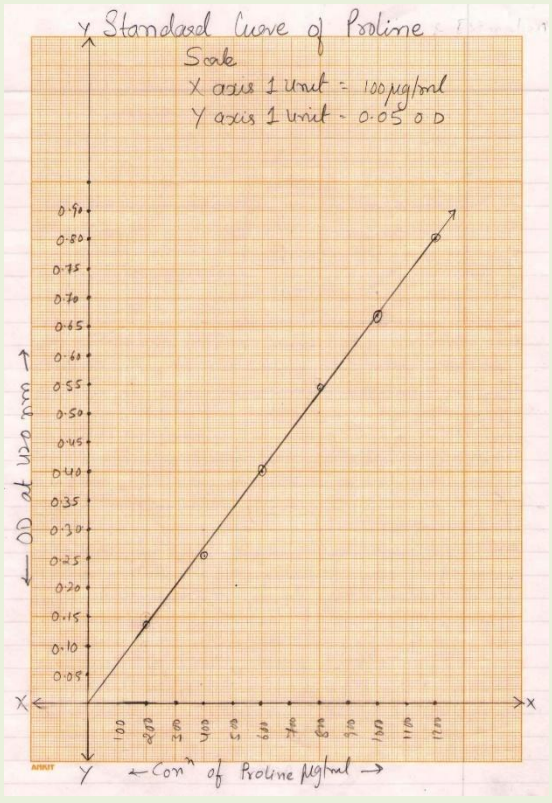

Annexure 4