MATERIAL AND METHODS

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A) Material

The germinated mother rhizome of 2 varieties of Curcuma longa - Sugamdhum and Erode were sown in soil culture in earthenware pots. The plants were stabilized for 3 months in well fertilized soil and under well irrigation. These plants were then grown in increasing saline media in different pots. The plants were treated with NaCl so that the level of salinity in the pots was at 0, 0.05, 0.1, 0.15, and 0.2 molar. To maintain the salinity level, to avoid accumulation of excess salts and to check the loss of water due to evaporation in the medium the plants were watered regularly alternating with salt treatment plants were treated twice a week with equal amount of salt solutions. After two months of growth under saline conditions the plant were used for the experimental analysis. The control plants were grown in the soil without any addition of salt solution.

For the analytical purposes plant parts such as leaves and rhizomes and roots were separated, thoroughly washed with tap water and then with distilled water to remove the dust if any and blotted to dry. Plants parts were then subjected to oven at $80^{o}C$ for about a week till a constant weight of the dried material was obtained.

B) Method :

For the estimation of inorganic constituents an acid digest from the oven dried plant material was used. The material was digest

ed following the method of sekin et al., (1965).

Na^+ and K^+ were estimated flame photometrically following the procedure standardized in our laboratory Cl^- were estimated following the method by Immamul Huq and Larher (1983) with a slight modifications and estimated according to the method of Chapman and Pratt (1961).

P^{5+} was estimated following the method by Sekin et al. (1965) spectrophotometrically.

Fe^{3+} was estimated following the method by Durie et al (1965) spectrophotometrically.

Ca^{2+}, Mg^{2+}, Mn^{2+}, Cu^{2+} and Zn^{2+} were estimated following the standard procedure on Atomic Absorption spectrophotometer. (Elmer, Perkin, Model).

PHOTOSYNTHETIC PIGMENTS :

The Chlorophylls from the leaves were estimated following the methods of Arnon (1949). For this young and mature leaves were washed first with tap water and then with distilled water, blotted to dry and cut into small pieces. This material was homogenised in 80% acetone and filtered through whatman No. 1 filter paper under suction. The residue was washed throughly 2-3 times with 80% acetone, collecting all the washings in the same containes. Final volume was made to 100 ml with 80% acetone. Absorbance was read at 663 and 645 for Chlorophyll and Chlorophyll 'b' respectively on spectrophotometer. The chlorophyll 'a'

Chlorophyll 'b' and total chlorophylls were calculated by the formula suggested by Arnon (1949).

Chlorophyll "a" $12.7 \times A\ 663 - 2.69 \times A\ 645 = x$

Chlorophyll "b" $22.9 \times A\ 645 - 4.68 \times A\ 663 = Y$

Total Chlorophyll $8.02 \times A\ 663 + 20.2 \times A\ 645 = Z$

TITRATABLE ACID NUMBER :

The method described by Thomas and Beevers (1949) was followed to determine TAN. Fresh leaf tissue was washed andrinsed with distilled water and blotted to dryness. It was accurately weighed (0.5 g) and boiled for half an hour in 100 ml distilled water. After cooling it was filtered through cheese cloth and volume was made to 50 ml with distilled water. The filterate was then titrated against standardized NaoH (N/40) using phenolpthalein as an indicator. TAN represents the number of ml of deci normal NaOH required to neutralize the acid present in 100 g fresh tissue.

TAN value was calculated using the following formula :

$$\frac{\text{Vol. of oxalic acid taken of titration} \times \text{Total vol. of Extract ml} \times \text{Extract tit.n reading ml} \times 100}{\text{Titration reading ml} \times \text{Weight of Plant material in g} \times \text{Vol. of Ext. taken for tit.n} \times 4}$$

CARBOHYDRATES :

SOLUBLE SUGARS :

The sugars were estimated following the method of Nelson (1944). The soluble carbohydrates were extracted from 0.5 g oven dried

plant material with alcohol. The extract was filtered through Buchners funnel using Whatman no 1 filter paper. The filterate thus obtained was condensed on water bath to about 5 ml. To this 2.3 lead acetate and potassium oxalate (1:1) were added and 50 ml of distilled water was added and aliquot was filtered. The volume of filterate was measured and it served as on extract for determination of reducing sugar. A known volume of this extract (20ml) was hydrolysed with 2 ml conc HCl by autoclaving at 15 lbs atm pressure for half an hour. The content was cooled neutralised with anhydrous sodium carbonate and filtered. The volume of the filterate was measured and this filterate was used for the estimation of total (reducing + non reducing) sugar.

STARCH :

For estimation of starch, the insoluble residue obtained at the beginning after filtering the alcoholic extract was transferred to a 100 ml capacity conical flask with 50 ml distilled water and 5 ml conc. HCl. The same was hydrolysed at 15 lbs atmosphere pressure for half and hour and cooled to room temperature neutralised by addition of anhydrous Na_2CO_3 and filtered. The volume of the filterate was measured at this contains reducing sugars (glucose) formed as a result of hydrolysis of starch. These sugars represent the starch content in this residue.

The sugars from all these filterates were estimated by determining the reducing power by employing arsenomolybdate reagent introduced by Nelson (1944).

For the Calorimetric determination of the cupros oxide formed in the oxidation of sugars by alkaline copper tartarate reagent. For this 0.5 ml aliquots (Rs and Ts) and 0.1 ml aliquot for starch were taken in test tubes along with different concentration of standard glucose soln (0.1 mg/ml) in other test tubes. To the requisite amount of distilled H_2O was added to make final volume 10 ml. In case of blank instead of filterate or standard glucose distilled water was added to begin with the reaction. One ml of somogys alkaline copper tartarate reagent (4g $CuSO_4$ $5H_2O$, 24 g anhydrous Na_2SO_4 dissolved in 1 litre distilled water) was added in each test tube and all these reaction mixtures were transferred to boiling water bath for 10 min. After cooling to room temperature 1 ml Arsenomolybdate reagent (125 g NH_4Mo_4 in 450 ml water to which 21 ml conc. H_2SO_4 were added followed by 3 g Na_2HSO_4, $7H_2O$ dissolved in 25 ml water. These ingredients were mixed well and digested for 48 hrs at 37°C in incubator before use) was added to each reaction mixture which were further diluted to 10 ml with distilled water. After 10 min. absorbance was read at 560 nm on spectrophotometer.

Using calibration curve of standard glucose the sugar percentage in the above three fractions were calculated values of soluble sugars are expressed as g $100g^{-1}$ dry tissue.

TOTAL POLYPHENOLS :

Total Polyphenols were determined according to the method of Follin and Dennis (1915 . Dry plant material (0.5 gm) was homogenised in 30 ml of 80% acetone and filtered through Buchners

funnel. The residue was washed several times with 80% acetone and the final volume was made 100 ml with 80% acetone. Two ml extract along with a series of standard (std. tannic acid, 0.1 mg per ml) were taken in separate. Nesseler's tubes and to each tube 10 ml of 20%. Na_2CO_3 and 2 ml Follin Dennis reagent (100 g Na tungstate mixed with 20 g Phosphomolybdic acid in about 800 ml distilled water to this 200ml 25% phosphoric acid was added and the mixture was refluxed for 2.1/2 hrs cooled to room temperature and volume made to 100 ml with distilled water) was added. Final volume was made 50 ml with distilled water. After 20 min. absorbance was read at 660 nm using reagent blank. Total Polyphenols were calculated with the help of standard curve of Tannic acid and expressed as g 100 g^{-1} dry tissue.

PROLINE :

Proline content from the leaves, rhizome and roots of turmeric determined according to the method of Bates et al. (1973). For this 0.5 g oven dried Plant material was homogenised in 10 ml Sulphosalicylic acid (3%) throughly and then filterate was reacted with 2 ml glacial acetic acid and 2 ml acid ninhydrin reagent prepared by warming 1.2 g ninhydrin in 30 ml glacial acetic acid and 20 ml 6M Phosphoric acid with agitation, cooled and stored at 4°C in a test tube for 1 hour at 100°C in boiling water bath similar procedure was followed for another set of test tubes containing various concentrations of standard proline solution (0.1 mg proline/ml). After boiling the reaction was terminated by transferring the test tube immediately to ice bath. To this 4

ml of Tolune was added and mixed vigourously for 15-20 sec. Reaction mixtures werre then brought to room temperature and absorbance of toluene chromophore was measured at 520 nm using toluene, blank. Proline concentration was calculated from calibration and final values were expressed as g 100 g^{-1} dry tissue.

TOTAL NITROGEN :

Plant grown under different salt treatment were analysed for nitrogen following the method of Hawk et al., (1946) Ovendried powdered plant material (0.1 g) was taken in Kjeldalhs flask with a pinch of micro salt (200 g K_2SO_4 (1:1) was added. Few glass beads were added to avoid bumping and the material was digested on low flame. After complete digestion a faint yellow solution was obtained which was cooled to room temperature and transferred to Volumetric flask, diluted to 100 ml with distilled water.

In Nesseler's tubes 1 ml of plant extract and different conc of standard ammonium sulphate soln (0.2369 of Ammonium sulphate dissolved in distilled water and few drops of H_2SO_4 were added. The volume was made 1000 ml with distilled water. This solution contains 0.05 mg of nitrogen per ml) are taken. In control tube 1 ml distilled water was taken. To this 1 drop of 8% $KHSO_4$ was added and volume was made 35 ml with distilled water. To this 15 ml Nesselers reagent was added. (Reagent A: 7 g KI + 10 g HgI_2 in 40 ml d/w) (Reagent B: 10 g NaOH in 50 ml water. A abd B are to be mixed in proportion of 4:5 at the time of estimation. The reation between the sample and the reagent gives the product

NH_4HgI_3 which has Orange brown colour. This colour was measured after 15 min at 520 nm on spectrophotometer.

MINERAL NUTRITION:

Procedure for Acid digestion method:

Take 0.5 gm Ovendried plant material i.e. leaves, Roots and rhizome. Then it was transferred to 100 ml capacity beakers. 20 ml of Conc HNO_3 were the poured into the beaker. Then allow it to initiate for 30 min. The heat the beakers gradually to dissolve each and every particle of material. Cool to room temperature. Then add 10 ml 72% Perchloric acid.

Again heat gradually to a colourless solution condense the volume upto 1 to 2 ml cool it. Then add distilled waterr and adjust the volume to 100 ml. Keep this extract overnight on next day filter the extract through Whatman filter paper No. 1 and use as source of plant extract for Iron and Phosphorus.

Calculation : 1 ml std. 'Fe' solution = 0.1 mg Fe.

1 ml std. 'P' solution = 0.025 mg P.

SODIUM, POTASSIUM :

These cations were estimated according to standard flame photometric process employing Toshniwala Flame photometer for standarization of various concentrations of Na (10 ppm), K (20ppm) and Ca (200 ppm) from NaCl and KCl and $CaCl_2$ respectively were prepared using these standard solutions, standard curves for these elements were prepared using flame photometer with specific filters. The plant extract was subjected to same procedure. Calcium, Magnesium, Copper, Zinc and Mangenese are estimated by

Atomic Absorption Spectrophotometer.

PHOSPHORUS :

For estimation of Phosphorus method of Sekine et al., (1965) was followed. Here Phosphorus reacts with "Molybdate Vanadate Reagent" to give yellow colour complex. By estimating colorometrically the intensity of the colour developed and by comparing it with the colour intensity to known standards, Phosphorus content was estimated.

Take 2 ml of acid digest in a test tube, 2 ml of 2N HNO_3 were added followed by 1 ml of Molybdate Vanadate reagent (A-25 g Ammonium molybdate in 500 ml of distalled water B-1.25 g Ammonium Vanadate in 500 ml in HNO_3 A and B are mixed) and adjust the volume 10 ml. with d/w. The ingredients were mixed well and allowed to react for 20 minutes. After 20 minute colour intensity was measured at 420 nm using a reaction blank containing no phosphorus. Calibration Curve of standard Phosphorus was prepared from standard Phosphorus solution (0.110g KH_2PO_4 per litre = 0.025 mg P^{5+} ml^{-1}) taking different concentrations 0.025, 0.05, 0.1, 0.2, and 0.4 mg of Phosphorus. Other steps being essentially similar to the one described above with the help of standard curve, amount of Phosphorus in the Plant material was calculated.

IRON :

Fe^{3+} was determined by the method described by Durie et al. (1965). In this method ferric ions are reduced to ferrous ions. The colour developed between the latter and O-phenonthroline is read at 520 nm.

Five ml. of acid digest and 5 ml of standard iron solution (3 µg $Fe_2 O_3$ ml^{-1}) were taken separately into 50 ml marked test tubes. One more 50 ml marked test tubes was reserved for reagent blank containing no iron solution. To this 10 ml of hydroxylamine hydrochloride (10 percent W/V) were added followed by small piece of congo red paper. It was mixed well. The acetate buffer solution (140 g sodium acetate in distilled water + 60 ml acetic acid, diluted to 1 litre with distilled water) was added dropwise until the indicator paper changed just from blue to red. Eight ml of O-phenonthroline (0.25 percent W/V) were then added, volume was adjusted to 50 ml with distilled water and optical density was read at 520 nm against reagent blank.

By comparing the optical density of the test solution with that of standard $Fe_2 O_3$ solutions, total amount of Fe in the plant material was calculated.

CHLORIDES :

Chlorides were extracted according to the method described by Imamul Huq and Larher (1963) with slight modifications and estimated according to the method of Chapman and Pratl (1961). The chlorides were extracted to distilled water at 45°C for 1 hour and add hot distilled water to prevent drying. After cooling the extract was filtered through a layer of muslin cloth. The filterate was collected in 50 ml. volumetric flask and volume was made with distilled water. For this 10 ml extract was taken for titration against standardized $AgNO_3$. Few drops of acetic acid

(20%) soln (dilute 200 ml conc. acetic acid with 800 ml of distilled water) were added to the filterate until the pH of the solution was 6 to 7. Then five drops of Potassium chromate soln (1%) was added and titrated with standardized 0.05 N silver nitrate. (Dissolve 8.5 g AR grade $AgNO_3$ in distilled water. Transfer to 1 litre volumetric flask and make up to volume with distilled water until the first permenent reddish brown colour appears).

Standardization : 10 ml of 0.1N Sodium Chloride standard was taken into erlemeyer flask and add 50 ml of distilled water. This standard was then titrated with the Prepared Silver Nitrate Solution.

1 ml 0.05N $AgNO_3$ = 1.77 mg of Chlorides.

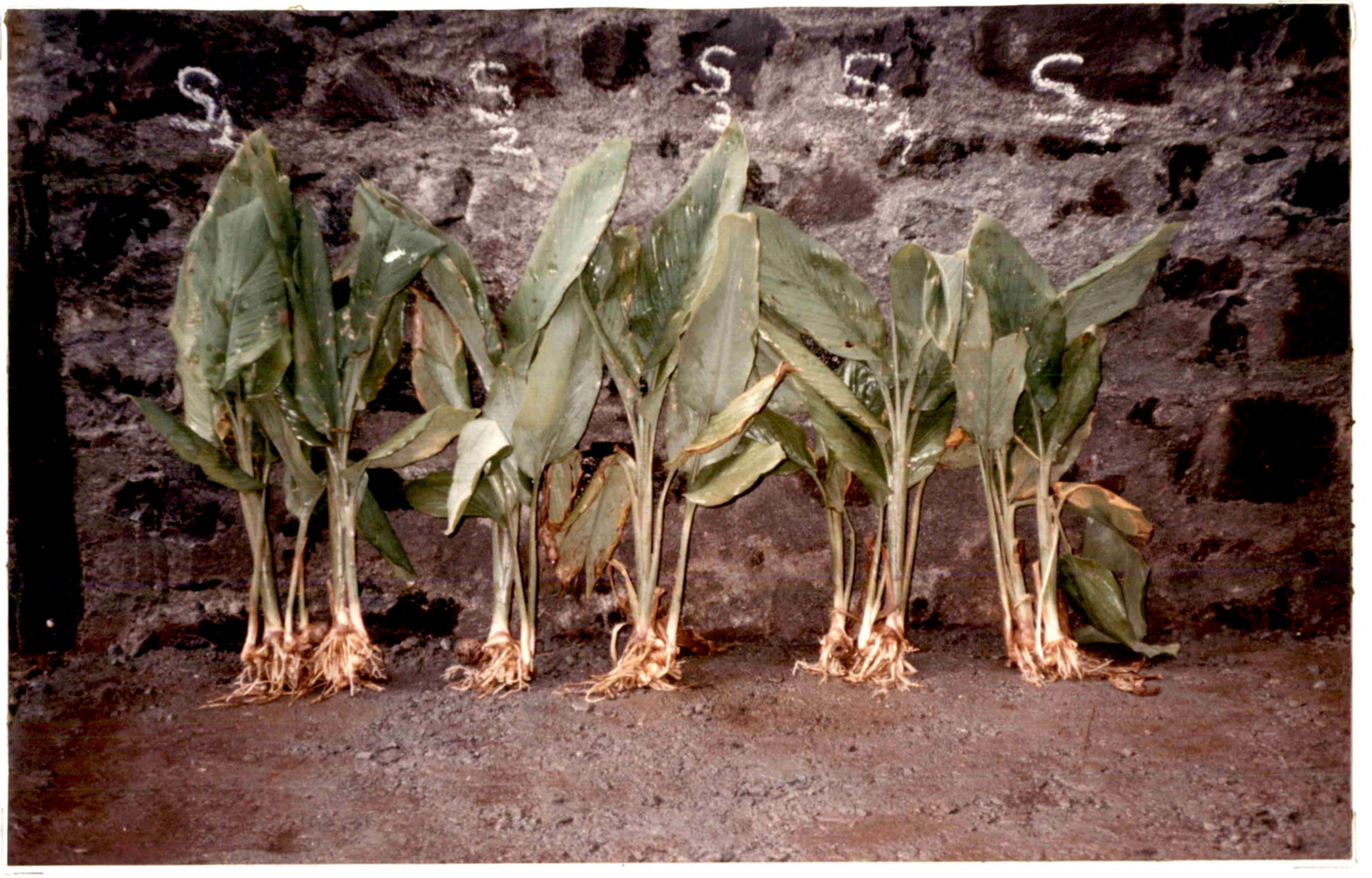
CURCUMIN :

Estimation of curcumin from plant sample is determined by "ASTA official analytical methods of the American Spice Trade Association".

0.1 gm of turmeric powder was extracted in 30 ml of alcohol and refluxed for 2 1/2 hours. Cool the extract and filter quantitatively into a 100 ml. volumetric flask. Then the extract was filered and washed thoroughly and diluted to the mark with alcohol. 20 ml. of filtered extract was pipetted out into a 250 ml. volumetric flask and diluted to volume with alcohol. The the absorbance of the extract and Std. solution was measured at 425 nm in against an alcohol as a blank.

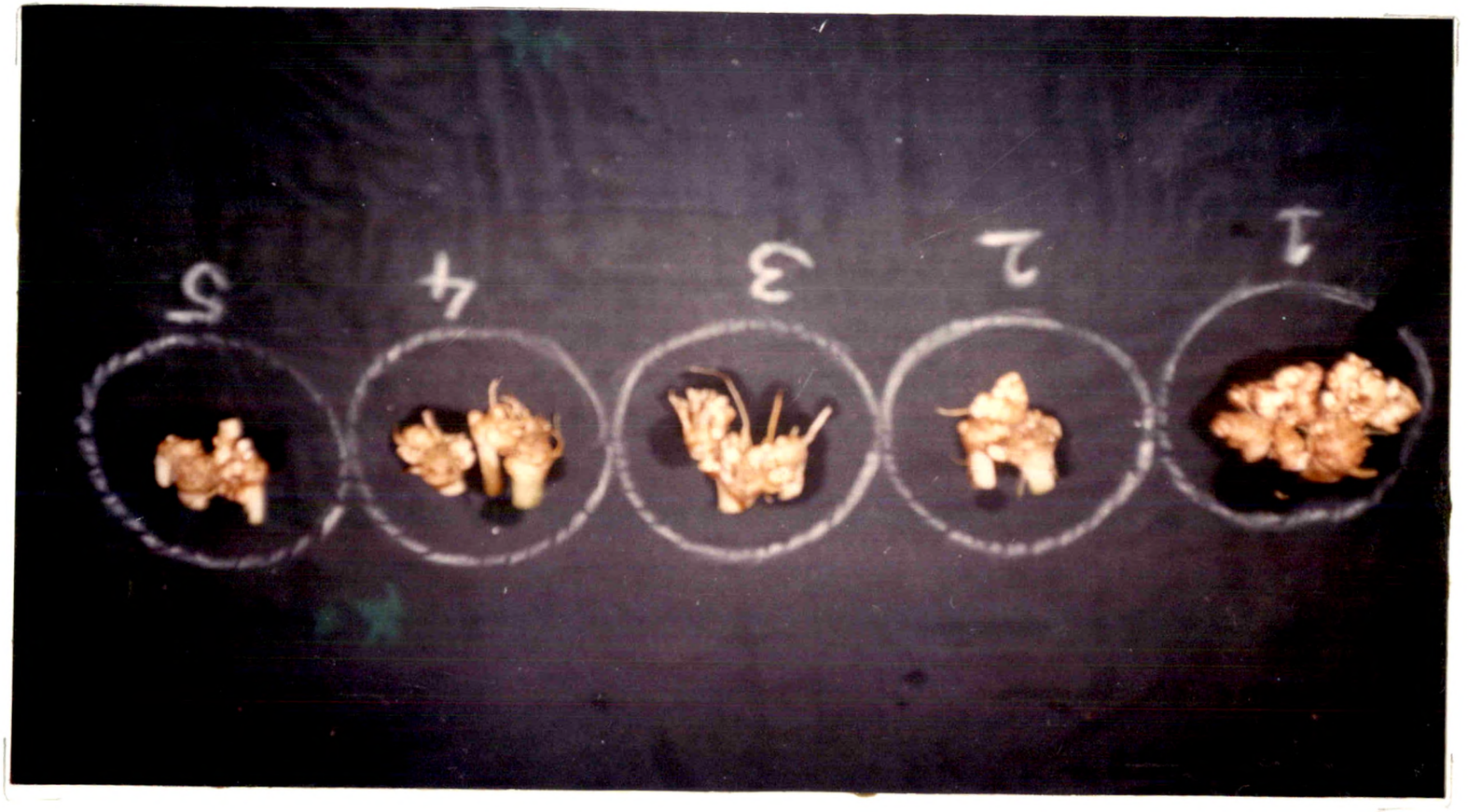
Formula :

$$\text{Curcumin \%} = \frac{\text{Absorbance of extract at 425 nm}}{\text{Sample Weight}} \times 100$$

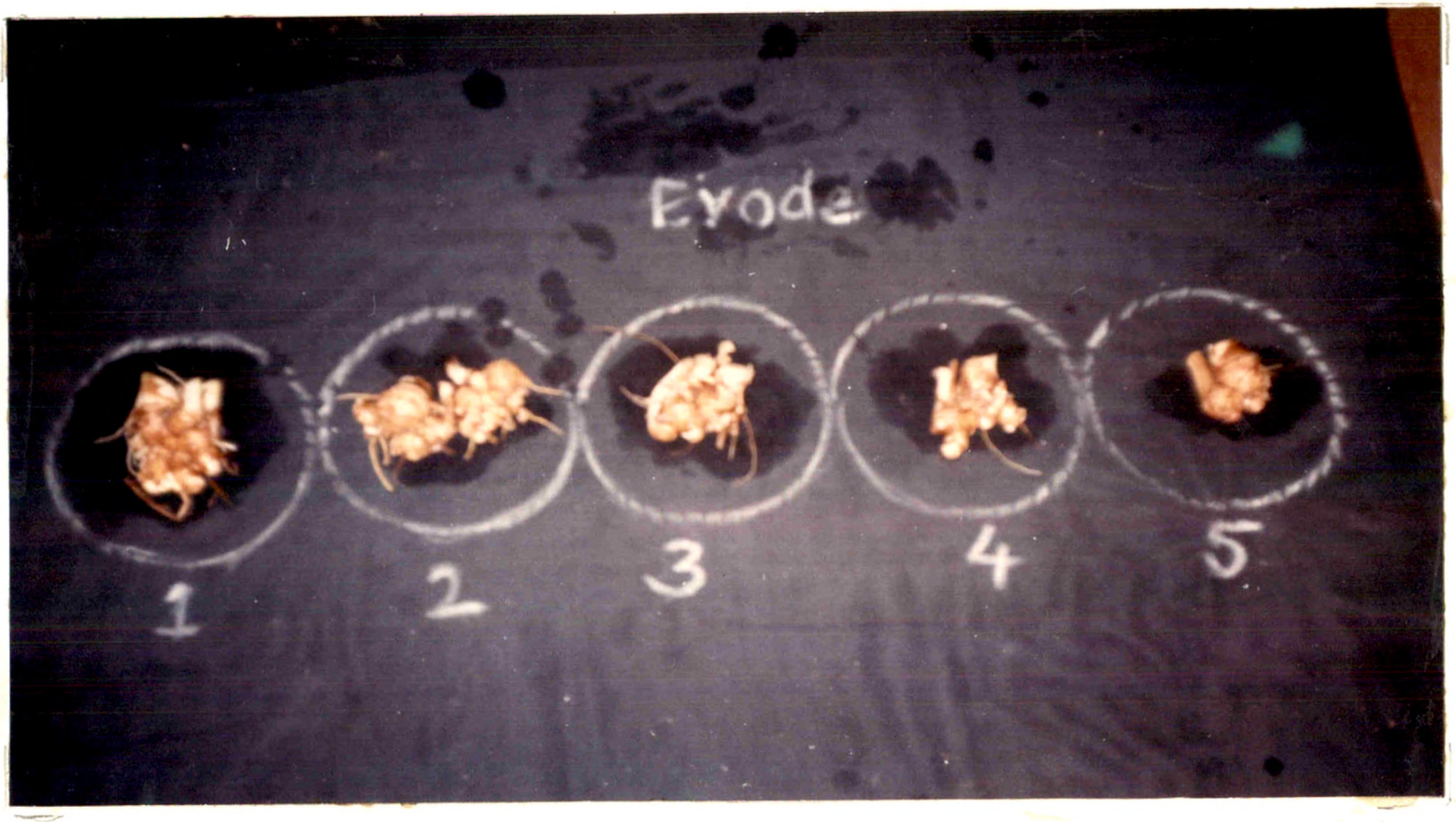


EFFECT OF NaCl SALINITY ON Curcuma longa - SUGAMDHUM VARIETY.

EFFECT OF NaCl SALINITY ON Curcuma longa -ERODE VARIETY.



EFFECT OF NaCl SALINITY ON Curcuma longa -SUGAMDHUM RHIZOME.



EFFECT OF NaCl SALINITY ON Curcuma longa -ERODE RHIZOME.