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ORIGINAL ARTICLE

Modulation of Plant growth parameters by salinity stress, exogenous supply of Ascorbic acid and various water stress by phytorid waste water treatment plant in *Cardamine hirsuta*L.

Mona Kejariwal[1] Harshada Gowda[2] and Dale Rodriguez[3]
1: Assistant Professor, Department of Botany, R.D. & S. H. National College, Bandra (w), Mumbai-50
2 & 3: M.sc Biotechnology Student, Department of Biotechnology, R.D. & S. H. National College, Bandra (w), Mumbai-50

ABSTRACT
Cardamine hirsutais one of the most recently explored plant model system as a substitute for its own comrade Arabidopsis thaliana. They both belong to Brassicaceae family. To establish as plant model, a plant should have a petite life cycle, considering the importance of completing it for experimental life span. Arabidopsis is not easily available in India while Cardamineis easily available as a weed in various part of India including Mumbai, Maharashtra. The study focuses on the stress physiology experiments done on Cardaminehirsuta, like salt stress, water from a Phytoridwater recycling plant and exogenous supply of ascorbic acid. Various growth parameters and phytochemical assays were evaluated to check the effect on plant'sgrowth.The plant showed responses within three-four days once it attains 5th leaf stage after 30-40days of germination at various salt, water stress and ascorbic acid concentrations. The plant can withstand upto 120 µm of salt concentration more than 25 days. Plant showed remarkable growth with alleviated protein levels and phytochemical contents due to exogenous supply of ascorbic acid. In untreated water from Phytorid, plant showed proper growth with higher production of protein and other phytochemicals. The study revealed that Cardaminehirsutacan be show significant variation with various stress treatments.
Keywords*: Cardamine hirsuta, waste water treatment*

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INTRODUCTION

Weed, which shows vigorous growth, tends to overgrow or choke out more desirable plants, is a plant that is not considered where it is growing [1]. They have a controversial nature i.e. despite the negative impacts of weeds, some plants usually thought of as weeds may actually provide some benefits [2].One of such weeds is *Cardaminehirsuta* L., commonly called hairy bittercress or popping cress for its explosive seed dispersal, which is a very close relative of one of the most explored plant model system *Arabidopsis thaliana*.

Cardaminehirsuta shares many of the desirable traits found in the model species *A. thaliana*: it is a diploid and self-compatible annual plant with an abundant seed set, an 8-week seed-to-seed generation time and a small rosette growth habit that is amenable to large-scale cultivation. The C. hirsuta genome is estimated to be 1.5 times that of A. thaliana and, importantly, C. hirsuta can be efficiently transformed by floral dip using Agrobacterium tumefaciens (e.g. transformation efficiency: A. thaliana Col-0, 0.3%; C. hirsuta Ox, 0.1%) [3].

Cardaminehirsuta L. is one of the beneficial weed plant.It is erect, slender, small, annual, herb, branching from the base and usually hairless except at the base. Up to 300 mm tall. The leaves have 1-7 leaflets. The flowers are complete, bisexual i.e., with functional male (androecium) and female (gynoecium), white in colour including stamens, carpels and ovary. Flowers are sessile or almost sessile.Fruities of this plant are

erect pods[4].The seed are borne in siliquae which, often burst explosively when touched (explosive dehiscence), sending the seeds flying far from the parent plant. This plant grows best in cool, damp places and in recently disturbed soil [5].

Cardaminehirsuta and *Arabidopsis thaliana* showed their lineage long back around 13 million years ago (Mya), when *A. thaliana* and *Arabidopsis lyrata* diverged, and 34–43 Mya when the split between *Arabidopsis* and the *Brassica* complex has been estimated [6-8]. Earlier studies focused on the genetic causes on leaf shape development in *C. hirsute and A. thaliana* , which has turned up as a very important genetic experimental tool in developmental pathways of leaf pattern and shape evolution studies [6-9]. *Cardaminehirsuta* exhibited many of its traits like *A. thaliana*: it is a diploid and self-compatible annual plant with an abundant seed set, an 8-week seed-to-seed generation time and a small rosette growth habit that is amenable to large-scale cultivation [3]. The *C. hirsuta* genome is estimated to be 1.5 times that of *A. thaliana* [10] and, importantly, *C. hirsuta* can be efficiently transformed by floral dip using *Agrobacterium tumefaciens* (e.g. transformation efficiency: *A. thaliana* Col-0, 0.3%; *C. hirsuta* Ox, 0.1%). This experimental tractability allows large-scale genetic screens and cross-species tests of gene function to be performed, in addition to sophisticated molecular genetic approaches that use the multitude of transgenic tools available in *A. thaliana* to manipulate and visualise gene expression and generate mosaics of gene function [3].

Figure-1. Fully grown Cardamine hirsuta plant

The chemical consituents of Cardamine include alkaloids, flavonoids, phenolic acids, terpenes, steroids, fatty acids, fatty acid methyl esters, triglycerides, amino acids, some other metabolites, and elements. The main constituent of importance is glucosinolates which belongs to the alkaloids[11].

Plant stress measurement is the quantification of environmental effects on plant health. Plant stress measurement usually focuses on taking measurements from living plants. It can involve visual assessments of plant vitality, however, more recently the focus has moved to the use of instruments and protocols that reveal the response of particular processes within the plant. In light of this, our research aims to study the modulation of plant growth parameters by salinity stress, exogenous supply of ascorbic acid and the effects of various types water stress on *Cardamine hirsuta* L. Here, we describe *C. hirsuta* as an experimental system with the functional tools to ask questions about the overall impact on various vital parameters like protein, ascorbic acid and various phytochemicals. Here we have used waste water from Phytorid plant contains many waste from biology laboratory, salt stressand exogenous supply of ascorbic acid to modulate growth parameter in *Cardaminehirsuta*. Where the scientific community is depicting this plant as very good genetic model, the purpose of the study is to step further and establish this plant as a plant stress physiology model due to its small life span and easy availability as a common weed. Here we add a note on Phytorid, which is a subsurface mixed flow constructed wetland system (SSFCW) developed and internationally patented by National Environmental Engineering Research Institute (NEERI) Nagpur with successful demonstration in the field for more than 8 years of continuous operation as a stand-alone sewage treatment system. 'Phytorid Technology' can treat the wastewaters by naturally without the addition of chemicals. It has been accomplished with the use of aquatic or semi aquatic plants along with their associated biota.The plant is successfully installed in R.D. & S.H. National College and S.W.A. Science College as a waste water treatment plant for biology and biotechnology labs. It is working successfully in the college and treated water is used to water garden plants. As an academician we want to establish a model plant system to have a check on the maintenance of this Phytorid plant

within six months and the study suggested that *Cardaminehirsuta* is best suited for untreated water stress tolerance experiments.

MATERIALS AND METHODS

Plant Material Collection

Seeds of *Cardaminehirsuta* L. which were laid out at HBSCE campus, TIFR, Mankhurd were allowed to germinate and grown for next 30 – 40 days till the 5th leaf was seen. These fresh, young saplings were collected directly from the campus with the help of shovel without damaging their roots and individual saplings with roots in the soil were placed in a large tray having dimensions of length=37cm; breadth=25cm and height=5cm. Around 180 – 200 ppsaplings of *Cardaminehirsuta* L. were collected in 3 trays and were carried to the Research Lab of R. D. National College, Bandra.

Preparation and Planting of Saplings

Small transparent plastic cups of dimensions length = 8cm; width = 6cm and volume of cup = 48ml were perforated manually using thick hot pointers for extra water to drain off. Moist red soil was taken from the campus of R. D. National College, Bandra and was filled upto 4 cm in these cups as the root system of *Cardaminehirsuta* L. is tap root system is not deeper but is upto 2 – 3 cms. The saplings brought from HBSCE campus were sorted and the good whole plant of 5 leaf stage was planted one in each cup. 9 trays containing 15 cups each were prepared and labelled as follows:

- Tray 1 – 60 mm NaCl
- Tray 2 – 120 mm NaCl
- Tray 3 – 240 mm NaCl
- Tray 4 – 0.1 mm Ascorbic acid
- Tray 5 – 0.5 mm Ascorbic acid
- Tray 6 – 1 mm Ascorbic acid
- Tray 7 – Untreated water
- Tray 8 – Treated water
- Tray 9 – Control

3.3 Treatments

The saplings were divided in three parts, each part was subjected to different type of the treatmemt. The saplings were irrigated everyday with 5 ml of the given below solutions at the same time of the day for a duration of 25 days. The volume of the solution was decided on the retention capacity of the soil and the plant. Specimens were collected on every 5th day from the start of treatment. Thus, in 25 days 5 such set of specimen for each part was obtained.

- Part I : Salinity Stress

Plants were divided into three groups each group comprising one of the concentrations of sodium chloride. The concentration was as such 60µM, 120µMand 240µMfor each group which consisted 15 plants in each tray . The control plants only received the Municipal water.

- Part II : Exogenous supply of Ascorbic Acid(A.A.)

Plants were divided into three groups each group comprising one of the concentrations of Ascorbic acid. The concentration was as such 0.1 µM, 0.5 µM and 1 µMfor each group which consisted 15 plants in each tray. The control plants only received the Municipal water.

- Part III : Various water stress by Phytorid waste water treatment plant

Plants were divided into three groups each group comprising different type of water. The different type of water was as such Untreated Water (U.W.), Treated Water (T.W.) and Municipal water (M.W.) [12] for each group which consisted 15 plants in each tray.The Untreated Water and Treated Water was taken from the upper and the lower tank of the Phytorid waste water treatment plant respectively. The control plants only received the Municipal water which was obtained from the tank. Multiple parameters such as Total Hardness, Calcium Hardness, Alkalinity, Chloride, Sulphite, Residual Free Chlorine, Fluoride, Iron and Nitrite for all the three types of water was tested using the Water Testing Kits of HiMedia Laboratories

Chemical Content

Photosynthetic pigments

Extraction and estimation of chlorophyll

Chlorophyll extraction from fresh leaf material was carried out with 80% acetone (buffered to pH 7.8 with phosphate buffer). The chlorophyll-a, chlorophyll-band total chlorophyll measurements were done by using a spectrophotometer zeroed at 663 nm and 645 nm using 80 percent acetone as blank.

Chlorophyll contents were calculated according to Arnon's method [13] and chlorophyll *a/b* ratios were determined.

Estimation of carotenoids

Total carotenoids were measured according to Jenson [14]. Plant material was crushed in mortar and pestle with 80 per cent acetone. The homogenate was centrifuged and supernatant thus collected was used for measuring the absorbance at 450 nm, The carotenoids present in the extract were calculated using the following formula:

$$C = \frac{D \times V \times F \times 10}{2500}$$

Where C: Total amount of carotenoid in mg; D: Absorbance at 450 in a cuvette of 1 cm length path; V: Volume of original extract in ml; 2500: average extinction coefficient of the pigment and F: Path length.

3.4.2. Protein

- Extraction of protein

0.02grams of fresh tissue from each group, taken from the third and fourth leaf,
was extracted by grinding in a mortar using 2ml of Phosphate Buffer Solution(PBS). The extract was filtered using a glass funnel with Whatman filter No. 1 and collected in a test tube. The samples of all three types were collected and in all 9 samples were obtained.

Estimation of soluble proteins

Bradford method (1976)[15] was used for the estimation of soluble proteins. 0.1 ml of aliquot was mixed with 5.0ml of coomassie brilliant blue reagent and the absorbance was recorded within 2-60 min at 595 nm against blank in which 100 mM/l phosphate buffer, pH 7.0 was added instead of aliquot. Reference cure was prepared by using defatted bovine serum albumin (0.02-0.2 mg/0.1ml) as standard protein. Coomassie brilliant blue reagent was prepared by dissolving 100 mg of coomassie brilliant blue G-200 (sigma) in 50 ml of 95 percent ethanol. 100ml of 85 percent phosphoric acid was added to this and the resulting solution was diluted to a final volume of 1.0 liter with distilled water.

Ascorbic Acid

The ascorbic acid (AA) concentration was measured by using the 2,6-dichlorophenol-indophenol (DCPIP) photometric method of Guri (1983)[16]. Fresh leaves (0.02gms) were quickly detached and homogenized in 1 mL of ice-cold 0.0005 mol/L EDTA solution containing 3 % trichloro-acetic acid (TCA) for 1–2 min. The homogenate was quickly filtered through Whatman No.1 filter paper and brought up to 2 ml with EDTA-TCA (Ethylenediaminetetraacetic acid- Trichloroacetic acid) extracting solution. The reaction mixture contained 3mL of DCPIP reagent and 2mL of filtered leaf extract. The absorbance was measured quickly at 512 nm spectrophotometrically. The concentration of ascorbic acid was determined from a standard curve that was prepared previously using various known concentrations of ascorbic acid i.e.0.01, 0.05, 0.075 and 0.1. DCPIP reagent was prepared by dissolving13 mg of DCPIP and 3 g of reagent grade anhydrous sodium acetate in 1L of distilled water.

RESULTS

A total of three replicates were chosen for each morphological observations (at an average of three plants per replica) of each group. In the morphological observations the plant was visually observed for the live, wilted and dead plants. The morphological observations was carried out on every 5 days interval up till 5 sets of observations were obtained. The images of the morphological observations were taken.

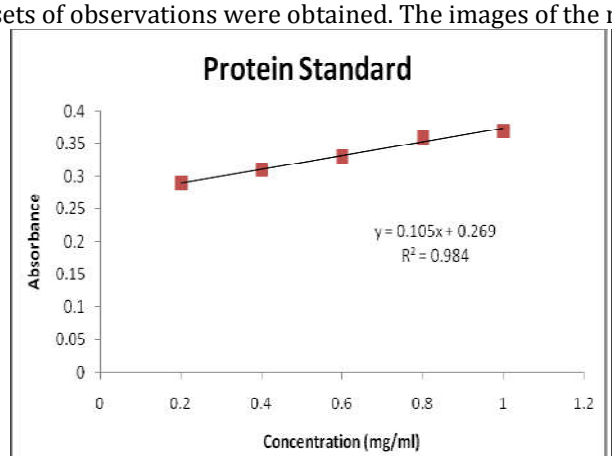

Figure 2. Standard Protein graph (595nm)

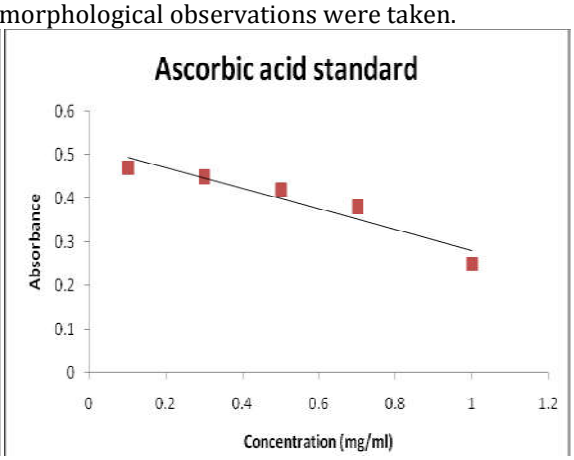

Figure 3. Standard Ascorbic Acid graph (512nm)

The standard graphs of the protein content and ascorbic acid content was obtained for the control samples(fig.2 & 3)respectively.

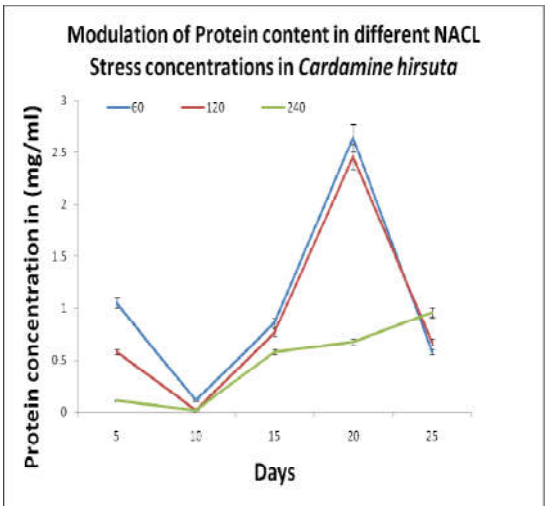

Figure 4. Day wise protein content in salt stressed Cardaminehirsuta plants. *Blue=60mM NaCl, Red=120mM NaCl& Green=240mM NaCl & Green=240mM NaCl.

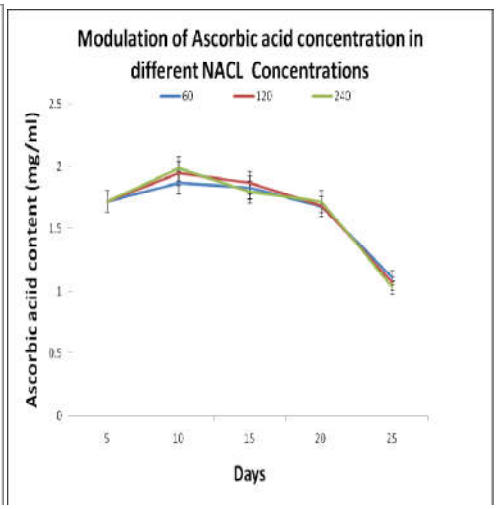

Figure 5. Day wise Ascorbic Acid content in on salt stressed Cardaminehirsuta plants. *Blue=60mM NaCl, Red=120mM NaCl

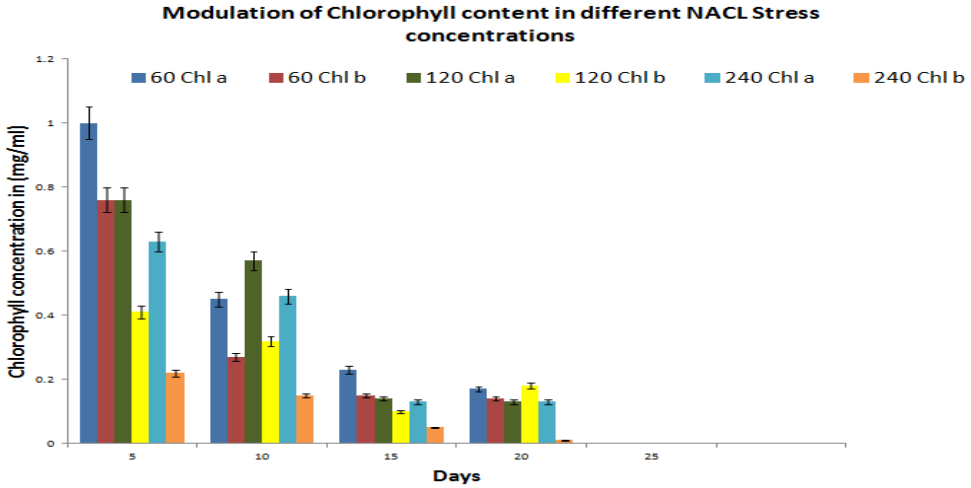

Figure 6. Day wise chlorophyll content a & b in salt stressed Cardaminehirsuta plants. *Purple=60mM NaClChl a, Red=60mM NaClChl b, Green=120mM NaClChl a, Yellow=120mM NaClChlb,Blue= 240mM NaClChl a & Orange=240mM NaClChl b.

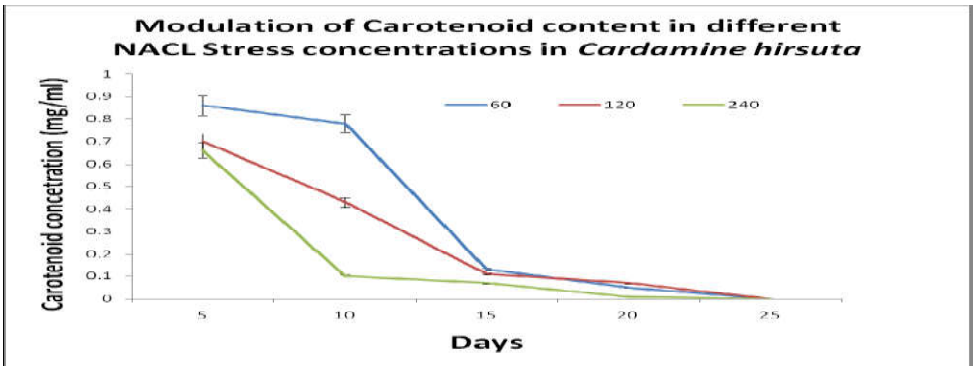

Figure 7 . Day wise carotenoid content in salt stressed Cardaminehirsuta plants. *Blue=60mM NaCl, Red=120mM NaCl& Green=240mM NaCl.

When the *Cardaminehirsuta* plants were checked for protein content, they showed a decrease in the initial treatment where as it was obsereved an increase and gradual decrease at the end of the treatment(Fig.4). The ascorbic content was stable and a decrease by the end of the treatment(Fig.5). The photosynthetic content of chlorohpylla & b and carotenoids showed a decrease at the end of the treatment (Fig.6 & 7).

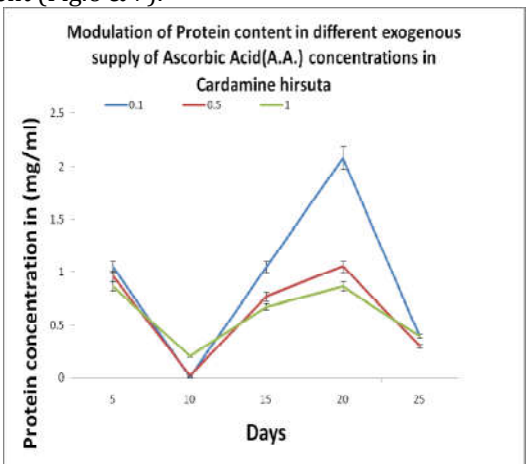

Figure 8 . Day wise protein content in A. Cardaminehirsuta plants.in A. *Blue=0.1mM A.A, Red=0.5mM A.A & Green=1mM A.A.

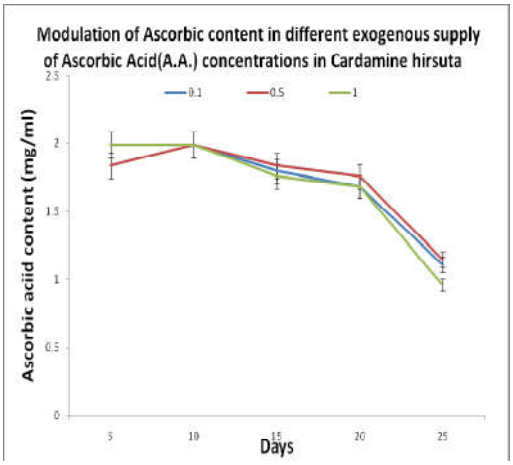

Figure 9. Day wise Ascorbic acid contentstressed A stressed Cardaminehirsuta plants. *Blue=0.1mM A.A, Red=0.5mM A.A & Green=1mM A.A

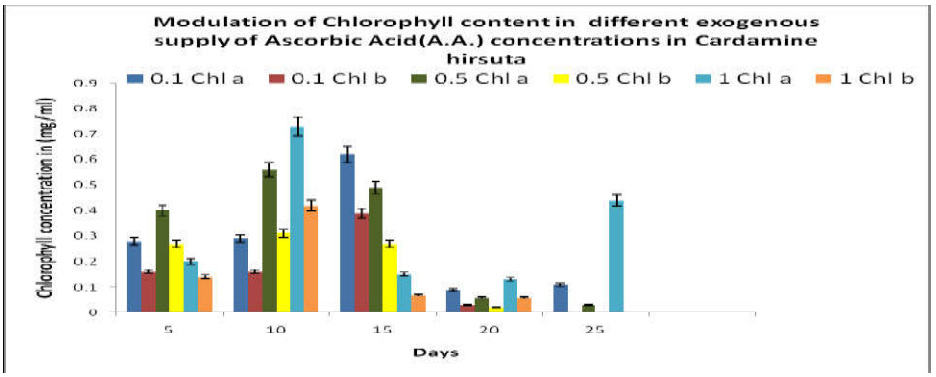

Figure 10. Day wise chlorophyll content a & b in A.A stressed Cardaminehirsuta plants. *Purple=0.1mM Chla A.A, Red=0.1mM A.A Chl b, Green=0.5mM A.A Chl a, Yellow=0.5mM Chl a, Blue=1mM A.A Chl a & Orange=1mM Chl b.

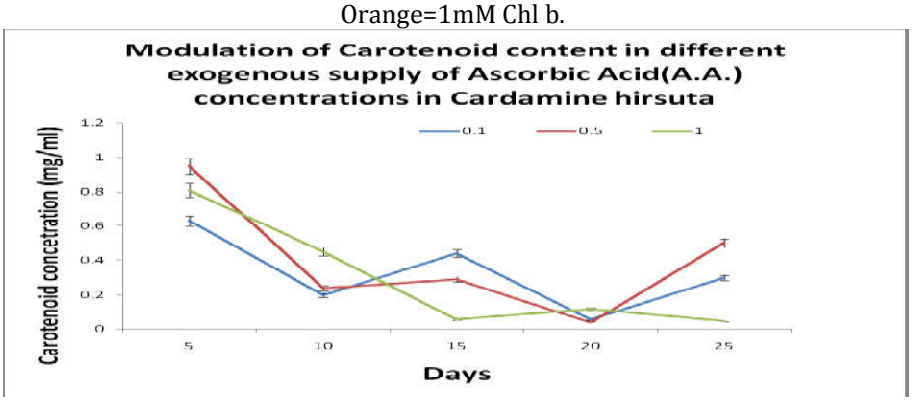

Figure 11. Day wise carotenoid content in A.A stressed Cardaminehirsuta plants. *Blue=0.1mM A.A, Red=0.5mM A.A & Green=1mM A.A.

The protein content of these plants showed initial decrease and gradual increase and again decrease by the end of the treatment (Fig,8), while the A.A content of these plants showed alleviated concentration for a long time and decrease by the end of the treatment (Fig,9). The photosynthetic pigments chlorophyll a &

b at the mid of the treatment showed increase whereas the carotenoid content had a fluctuating increase and decrease content in them. Thus the photosynthetic content was not stable(fig,810&11).

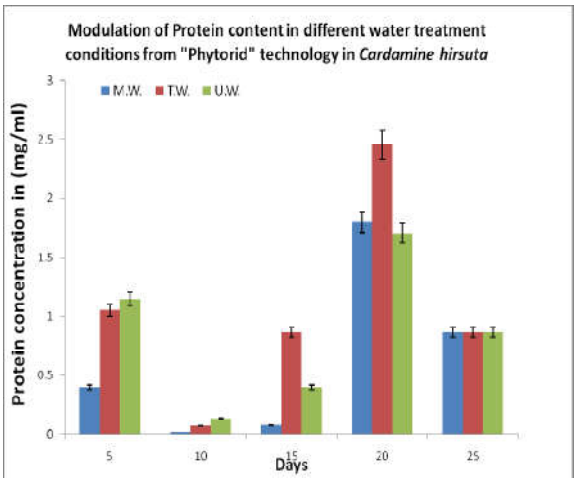

Figure 12. Day wise protein content in Phytorid water stressed Cardaminehirsuta plants. *Blue=Muncipal water, Red=Treated water& Green=Untreated water.

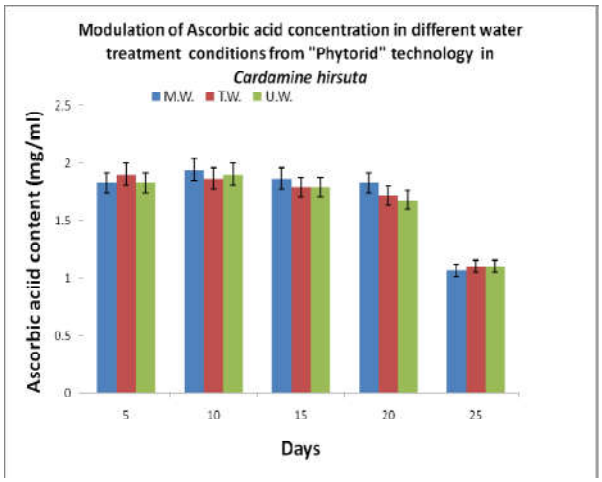

Figure 13. Day wise Ascorbic acid content in Phytorid water stressed Cardamine hirsuta plants. . *Blue=Muncipal water, Red=Treated water & Green=Untreated water.

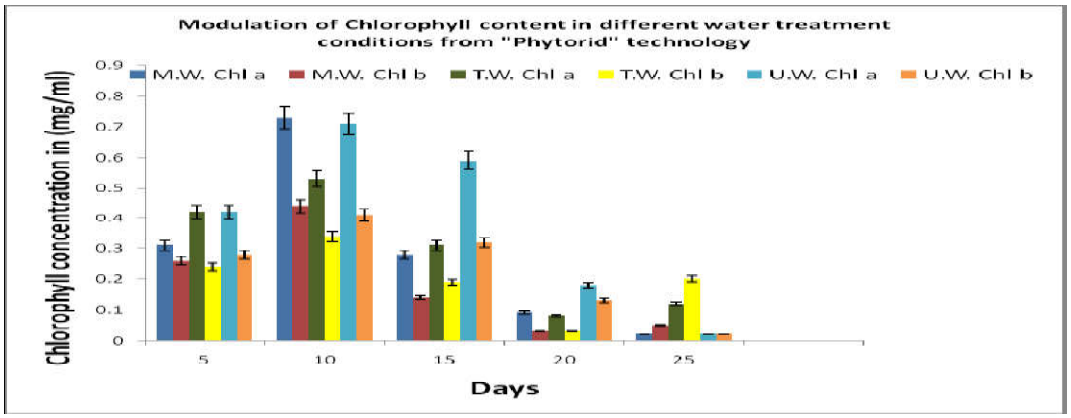

Figure 14. Day wise Chlorophyll a & b content in Phytorid water stressed Cardaminehirsuta plants. . *Purple=Muncipal water Chl a, Red=Muncipal water Chlb,Green=Tap water Chl a, Yellow= Treated water Chl b, Blue= Untreated water Chl a & Orange= Untreated water Chl b.

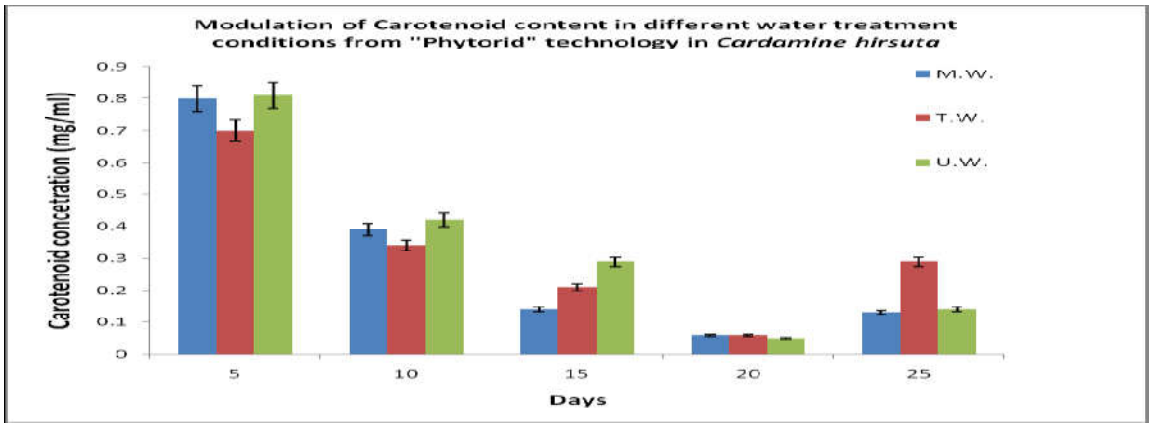

Figure 15. Day wise carotenoid content in Phytorid water stressed Cardaminehirsuta plants. *Blue= Control muncipal water, Red=Treated Phytorid water & Green=Untreated water.

The protein content showed a steap decrease and an increase at the end of the treatment(fig.12). Where as the ascorbic acid was almost constant throughout the treatment(fig.13). The photosynthetic pigmemts carotenoids decrease stEEply whereas chlorophyll a & b increased and decreased at the end of the treatment(fig.14 &.15).

DISCUSSION AND CONCLUSION

CardamineHirsuta was subjected to different concentration of NaCl, shoowd significant variation with different concentration of salinity stress. The plant which were subjected to 60Mm of NaCl stress showed the survival rate of 50% in the 25 days treatment where as those subjected to 120Mm and 240Mm of NaCl stress showed the 100% mortality within 5-10 days of treatment respectively. The plant (*Cardaminehirsuta L*) growth depends on the lower concentrations of NaCl and higher concentration blocks the photosynthetic process which effects the metabolism and further development of the plants. The threshold concentration for*Cardaminehirsuta* plants under salinity stress is about 60 µM, and concertation above 120 µM improves fatal for the plant. The plant respond well with different salinity concertation which gives us an indication that it can be established as a model plant for the salinity stress molecular mechanisms.

The plants that were subjected to different concentration of ascorbic acid exogenously. The plants were subjected to different concentration of A.A 0.1µM, 0.5µM& 1µM respectively. The growth was best observed in 0.1µM while 0.5µM& 1µM moderate growth of the plant was observed. When *Cardaminehirsuta*plant was treated with A.A it showed a positive result which report that A.A acts as a growth factor for the plant the most effective concentration was 0.1mM, however higher concentration of A.A is 0.5mM,1.0mM showed a moderate growth development while 0.1mM showed a high growth rate and higher metabolism of plants hence lower concentration of exogenous supply of A.A was found to be effective. For further scope A.A can be supplemented in still more less concentration and growth rate can be observed.

*Cardaminehirsuta*was also treated with different types of Phytorid recycling waste water. When the plants was subjected to different types of water i.eMuncipal, Treated Phytorid water & Untreated water, the best growth of the plant was observed in unfiltered water. The untreated water which contain waste from the laboratory can accelerate the growth of due to extra supply of some important nutrients and other factors. Since *Cardaminehirsuta* is weed and it grows profusely if proper environmental conditions and nutirients are suppled therefore it showed high affinity towards untreated water. Further research is needed to ear mark the exact reasons and factors for its increased groth. Since chemicals and altered ph of the untreated water can cause stress in the plant, the protein and phytochemical content of the plants becomes high with such treatment. In tap water and filtered water of the Phytorid recycling waste water, *Cardamine* showed moderate growth with altered phytochemical content and proein content which further support our theory for induced stress though unknown factors in untreated water. More different parameters of stress can applied to the experimental plant and futher research can be carried out.

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