



Chromium-induced growth inhibition and alteration of biochemical parameters in *Ocimum basilicum* L.

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ABSTRACT

The hydroponically grown plants of *Ocimum basilicum* L. were exposed to varying levels of $K_2Cr_2O_7$ (0, 5, 10, 25 μ M). The plants were tested for various morphological and biochemical parameters on 3rd and 5th day after treatment. Chromium (Cr) resulted in reduction of plant length and biomass. The deleterious effects of the hexavalent chromium on *O. basilicum* were further confirmed by the reductions in chlorophyll a and b contents, soluble protein and while as the free amino acid and proline contents were increased.

The study concludes that chromium causes stress in the *Ocimum basilicum* plants and thus alters various morphological and biochemical parameters.

Introduction

Ocimum basilicum is used as antispasmodic, aromatic, carminative and digestive. It has been used to treat fever, abdominal cramps, nausea, migraine, insomnia, depression, dysentery and diarrhea (Marwat et al., 2011). Due to rapid industrial growth, plants are under the stress because of many abiotic stresses. Heavy metal pollution is one of the most vital stresses causing low productivity in plants (Jabeen et al., 2009). Various lethal heavy metals such as Cd, Cr, Ni, Hg etc enter the biosphere from different sources, the most common of which are the metal smelters, emissions from industries as well as indiscriminate use of fertilizers and pesticides in agriculture (Hu et al., 2013). Heavy metals alter the metabolism in plants. At greater concentrations, these are very toxic and hinder the normal growth of plants. Heavy metals

damage the molecular structure of plant cells either directly or by generation of ROS (Diwan et al., 2008), including the free radicals and highly reactive non-radical molecules like H_2O_2 and singlet oxygen (1O_2). Chromium (Cr) can prove to be a harmful heavy metal and thus its consequence on plants has been well researched by agricultural scientists. The various other sources of Chromium in the soil are leather tanning industry, textile industry, besides the carpet and electroplating industries (Cao et al., 2017). There is an estimation of about 3550 metric tons anthropogenic release of Cr in the world fresh water bodies. The major two stable chemical forms of chromium in the environment are Cr (III) and Cr (VI) amongst which the most toxic is Cr (VI) because of its carcinogenic properties (Wang et al., 2012).

Cr phytotoxicity causes a decrease in the seed germination, reduced root growth, induces chlorosis in leaves and reduces biomass (Vajpayee et al., 2001). It can degrade the photosynthetic pigments, cause nutrient balance, enhance the antioxidant enzymes, and induce the oxidative stress in plants in addition to causing a change in the ultrastructure of membrane and chloroplast (Ashfaq et al., 2017).

However, plants are naturally blessed with a defense system which comprises of enzymatic and non-enzymatic antioxidants which defends the different important physiological processes from damage by the ROS released in the plants under heavy metal stresses (Rai et al., 2004). The various enzymatic antioxidants are superoxide dismutase, catalase and glutathione transferase, various enzymes of

Ascorbate-Glutathione cycle like ascorbate peroxidase, glutathione reductase. Ascorbate and glutathione, the non-enzymatic components of the antioxidative defense system have been known to increase under metal stress (Diwan et al., 2008)

This study was aimed at finding the effect of chromium on the growth and biochemical parameters of the plant. Further studies on the antioxidant defense system of the plants under various chromium treatments and the concentration of chromium in various plant parts of the treated plants need to be done to ensure whether the plant defense system can render the plant resistance against heavy metal stress. The screening of the plant for chromium effect on the secondary metabolite of this medicinal plant will ensure the possibility of using this plant for chromium phytoremediation in affected soils.

Materials and Methods:

Plant description:

Ocimum basilicum L. is an annual herbaceous plant from the Lamiaceae family. It is used as a spice, drug, and tea etc. It has many phytochemicals and has been used against digestive ailments, rheumatoid arthritis, depression, paralysis, cough and many other problems.

Plant Culture:

Seeds of *Ocimum basilicum* L were collected from the lawns of College of Science and Arts, Addarb, Jazan University, Saudi Arabia. They were grown on petridishes using filter paper and seedlings of almost same size were transferred to Hoagland's nutrient

solution which contained 3 mM KNO₃, 1 mM NH₄H₂PO₄, 50 μM KCl, 2 mM Ca (NO₃)₂, 25 μM H₃BO₃, 2 μM ZnCl₂, 2 μM MnCl₂, 0.5 μM (NH₄)₆MO₇O₂₄, 0.5 μM CuCl₂, 1 mM MgSO₄ and 20 μM Na₂ FeEDTA, and pH of this nutrient medium was maintained at 6.5 ±0.1 by NaOH. 10-day old seedlings were treated with 5, 10 and 25 μM K₂Cr₂O₇ while as the plants without any chromium concentration served as a control. Seedlings were analyzed for various parameters on 3rd and 5th days after treatment.

A. Growth Parameters:

The 10-day old seedlings of *Ocimum basilicum* L. were treated with various doses of chromium and the plants were harvested three and five days after treatment. The whole plant length was measured in 'cm' while as the plant biomass was measured in 'g' for control and treated plants.

B. Biochemical parameters:

1. Pigment concentrations

Hiscox and Israelstam's (1979) method was used to estimate the pigment concentration in the samples.

The method involves the estimation of plant pigments without maceration. Leaves were washed with distilled water (DDW) and chopped. 100 mg of chopped leaf material was taken in vials in triplicates and 10 ml of Dimethyl sulphoxide was added to each vial which was heated in oven at 65° C. After 30 min, the vials were taken out and the OD of the solution was measured at 663 and 645 nm.

$$\text{Chlorophyll } a \text{ (mg g}^{-1} \text{ fw)} = \frac{12.3 (A_{663}) - 0.86 (A_{645})}{d \times 1000 \times W} \times V$$

$$\text{Chlorophyll } b \text{ (mg g}^{-1} \text{ fw)} = \frac{19.3 (A_{645}) - 3.60 (A_{663})}{d \times 1000 \times W} \times V$$

Where d= distance traveled by the light path, W = weight of the leaf material taken, V = volume of the extract and A = Absorbance

2. **Soluble protein content:** Bradford (1976) protocol was used for protein estimation.

Reagent preparation:

(a) Extraction buffer (0.1-M/ pH 7.2 Phosphate buffer)

The solution of 0.1 M monobasic potassium phosphate (KH_2PO_4) was made by mixing 1.36 g of KH_2PO_4 in DDW and volume was completed to 100 ml. 0.1 M dibasic potassium phosphate (K_2HPO_4) solution was made by dissolution of 1.74 g of K_2HPO_4 in DDW and volume was made to 100 ml. Two components of the buffer were mixed in an appropriate amount to maintain the pH of buffer 7.2 that was monitored with the pH meter.

(b) 10% Trichloroacetic acid (TCA)

10 g of TCA was mixed in DDW to a total volume of 100 ml.

(c) 0.1N- Sodium hydroxide (NaOH)

0.4 g of NaOH was mixed in DDW to a total volume of 100 ml.

(d) Bradford's reagent

100 mg of Coomassie brilliant blue G-250 (CBBG-250) was mixed in 50 ml of 90 % ethanol. Add 100 ml of 85 % O-phosphoric acid to this solution and stir well on a magnetic stirrer. This was diluted to a final volume of 1000 ml with DDW and again stirred well. This solution was filtered carefully in dark by using Whatmann filter Paper No. 42 to remove the undissolved particles of dye. The final concentration of ingredients was 0.01 % CBBG-250, 4.75 % ethanol and 8.5 % O-phosphoric acid.

Procedure:

0.2 g of fresh and clean and chopped leaf material was homogenized in 2 ml 0.1 M/pH 7.2 phosphate buffer with the help of a pre-cooled mortar and pestle. Homogenate was transferred to the pre-cooled centrifuge tube and the centrifugation was done at 5000 rpm for 10 min. 1.0 ml of supernatant was added to equal amount of chilled 10 % Trichloroacetic acid (TCA) in a microfuge. It was centrifuged for 10 min at 3300 rpm and the supernatant obtained was thrown while as the remaining pellet was washed with acetone. 1 ml of 0.1 N NaOH was used to dissolve the pellet. 0.5 ml Bradford's reagent was added to 0.1 ml aliquot and was subjected to vortex. The tubes were

left to let the color develop for 10 min. OD was taken at 595 nm on spectrophotometer.

BSA was used as the standard to calculate protein concentrations and the protein content was expressed as mg g^{-1} FW.

3. Free Amino Acids

The estimation of free amino acids was done by the method of Lee and Takahashi's method (1966).

Reagent preparation:

(a) Citrate buffer (0.5 M/pH 5.5)

0.5 M citric acid solution was prepared by dissolving 52.339 g citric acid in DDW and volume was made to 500 ml. 0.5 M sodium citrate solution was prepared by dissolving 73.53 g of sodium citrate in DDW and volume was made to 500 ml. Two components of the buffer were mixed in an appropriate amount to maintain the pH of buffer 5.5 and was monitored with the pH meter.

(b) 55 % Glycerol

It was made by dissolving 55 ml of glycerol to 45 ml of DDW.

(c) 1.0 % Ninhydrin solution

Ninhydrin solution was prepared by mixing 1 g ninhydrin in 0.5 M citrate buffer to a final volume of 100 ml.

Procedure:

Fresh chopped leaves of weight 0.5 g were homogenated in 5 ml absolute ethanol and were poured in to the centrifuge tubes. Centrifugation was done for 10 min at 5000 rpm at temperature of 4° C. The supernatant was heated for 1 hr at 80° C so that the alcohol gets evaporated. 10 ml 0.5 M/5.5 pH citrate buffer was used to dissolve the pellet. 0.5 ml aliquot from this was added to 0.5 ml Ninhydrin and 1.5 ml 55 % Glycerol. The vials were heated at 100° C for 20 minutes and a purple colour was noticed. The final volume was made 6 ml with the addition of citrate buffer. The blue color appeared remain stable for 1 hr. Optical density was measured at 570 nm on spectrophotometer.

The standard curve was made by using Glycine solution as the standard and the results were expressed as mg g^{-1} FW.

4. Proline Content

Proline content estimation was done by the protocol of Bates et al. (1973).

Reagent preparation:

(a) 3 % (w / v) Sulphosalicylic acid

3 g of Sulphosalicylic acid was mixed in DDW to make a volume of 100 ml.

(b) 6 N Orthophosphoric acid

It was prepared by mixing 38.10 ml of Orthophosphoric acid in 61.90 ml of DDW.

(c) Ninhydrin solution

1.25 g ninhydrin was added to 30 ml GAA in addition to 20 ml 6 N orthophosphoric acid and agitated continuously till dissolved. The total volume was made 100 ml by adding distilled water.

Procedure:

Fresh leaves of weight 0.5 g were ground by mortar and pestle. Centrifugation of the mixture was done for 10 min duration at 5000 rpm. The 0.2 ml supernatant was added to 2 ml ninhydrin and 2 ml GAA. The solution was boiled at 100 ° C and later transferred to ice bath to stop the reaction. Each test tube was added with 4 ml of toluene and vortexed for 10-15 sec in order to facilitate quick diffusion / movement of chromophores from the aqueous phase to non-aqueous phase. The upper layer of toluene was transferred to a cuvette and OD was taken at 520 nm on spectrophotometer using toluene as blank. The standard curve was made by using L-proline as a standard and the results were recorded by the unit mg g⁻¹ FW.

Results and discussion

Growth Parameters

Growth parameters of *Ocimum basilicum* were studied by the parameter of whole plant length and the plant dry weight. The results of these parameters are shown in figure 1 and 2. Chromium treatments resulted in decrease of whole plant at both the stages of sampling. The highest decrease was found at Cr-25 which was 29.29% and 65.76% on 3 and 5 days of treatment respectively. Plant biomass was not affected by Cr-5 treatment but the increase in treatment dose reduced the plant biomass by

maximum 54.13% at Cr-25 on 3rd day after treatment. At 5th day after treatment, a gradual decrease was noticed in the plant biomass with the maximum (78%) recorded at Cr-25 treatment. Our results were in conformity with the results of Vajpayee et al. (2001) who noticed a similar Cr-inhibited growth in *Vallesneria spirralis* L. plants. The inhibited growth due to chromium treatments may be because of inhibited cell division (Diwan et al., 2008).

Pigment Concentrations

The chlorophyll a and b contents were measured and recorded in figures 3 and 4. Three days after treatment, the chlorophyll a content was increased by Cr-10 treatment and decreased with other treatments. The maximum 21% decrease was found with Cr-25 treatment. Five days after treatment, there was a decrease irrespective of the chromium treatments, the maximum decrease was recorded at Cr-10(46%). Chlorophyll b showed a decrease with all Cr treatments except at Cr-10 concentration where it showed an increase of 18% at 3DAT while as on 5DAT, it showed a gradual decrease except at Cr-5 while it showed a slight (3%) increase. This falls in line with the study of Appenroth et al. (2003) in the plant *Spirodela polyrhiza*. The decrease in pigment concentration may be due to the degradation of amino-levulinic acid dehydratase, which in turn causes a reduction in prothobilinogen necessary for chl biosynthesis, thereby affecting the amino levulinic acid (ALA) utilization.

Soluble Protein Content:

Chromium treatments caused a decrease in the soluble protein content (Fig 5). The maximum percentage decrease was found with Cr-25 on both the days of sampling, 3DAT and 5DAT. The respective percentage decrease was 56% and 57% for 3DAT and 5DAT. Similar decrease in soluble protein content was witnessed by Vajpayee et al. (2001) in *Vallesneria spirralis*. The decrease in the soluble protein content may be because of increased proteolysis under stress.

Free Amino Acid:

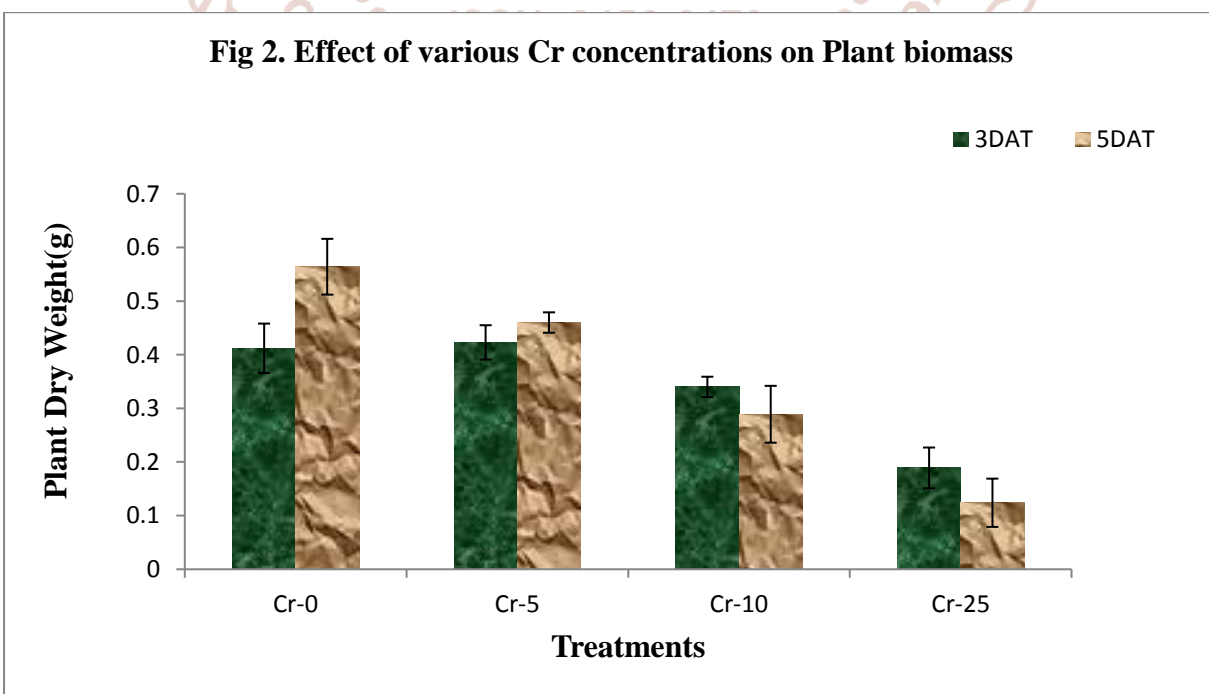
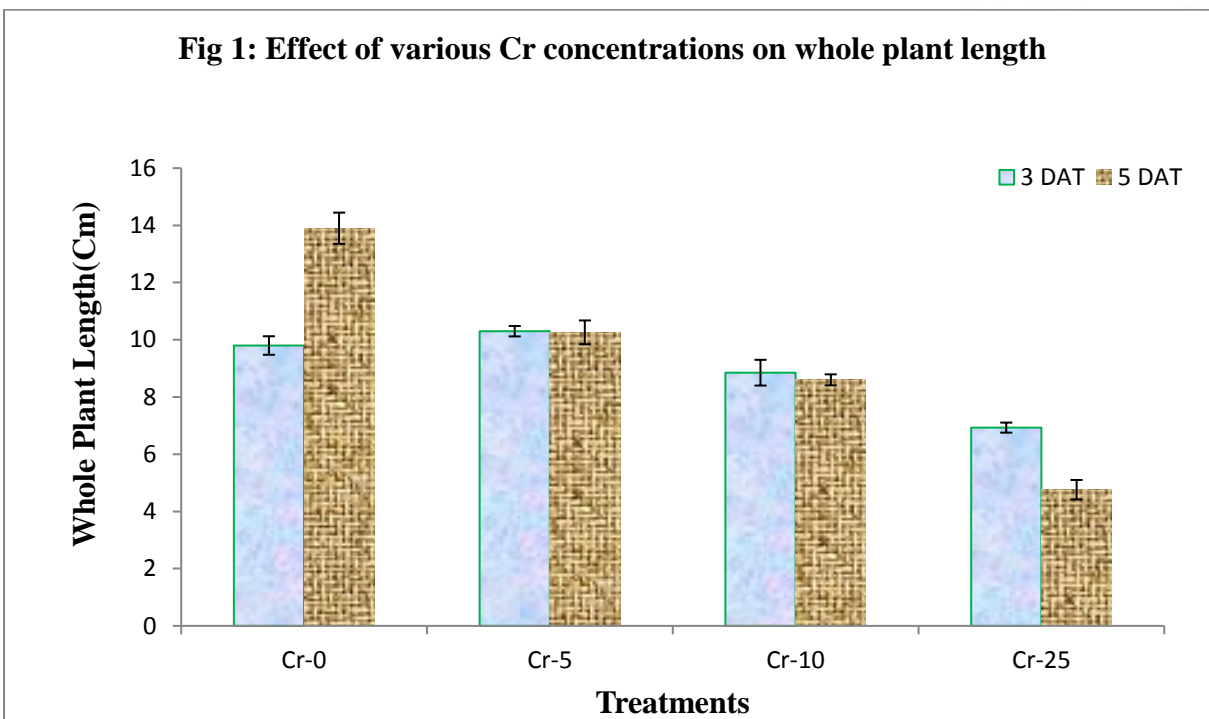
Free amino acid content saw a gradual increase on 3DAT where it ranged from 0.4-6% from Cr5-Cr25 (Fig 6). On fifth day of sampling, maximum increase was found with Cr-10 (35%). Diwan et al. (2012) also witnessed an increase in free amino acid content in *Brassica juncea* plants when subjected to Cr stress.

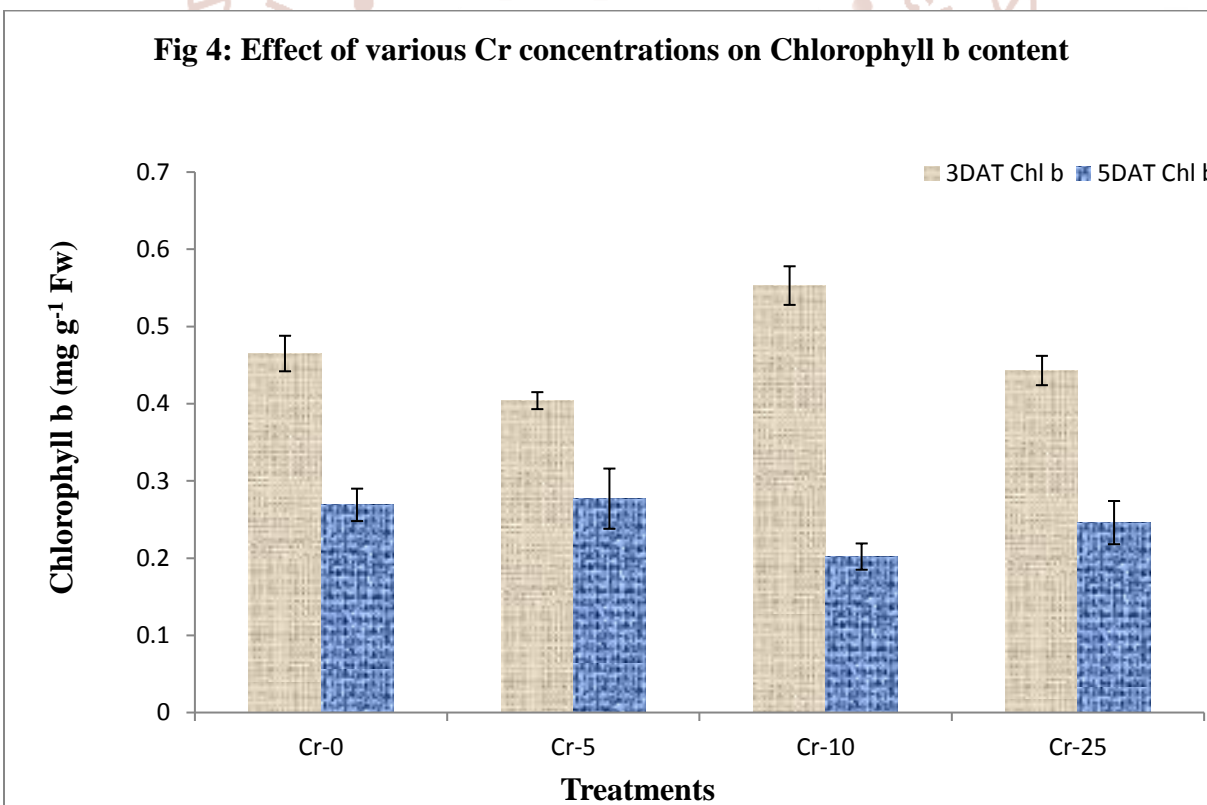
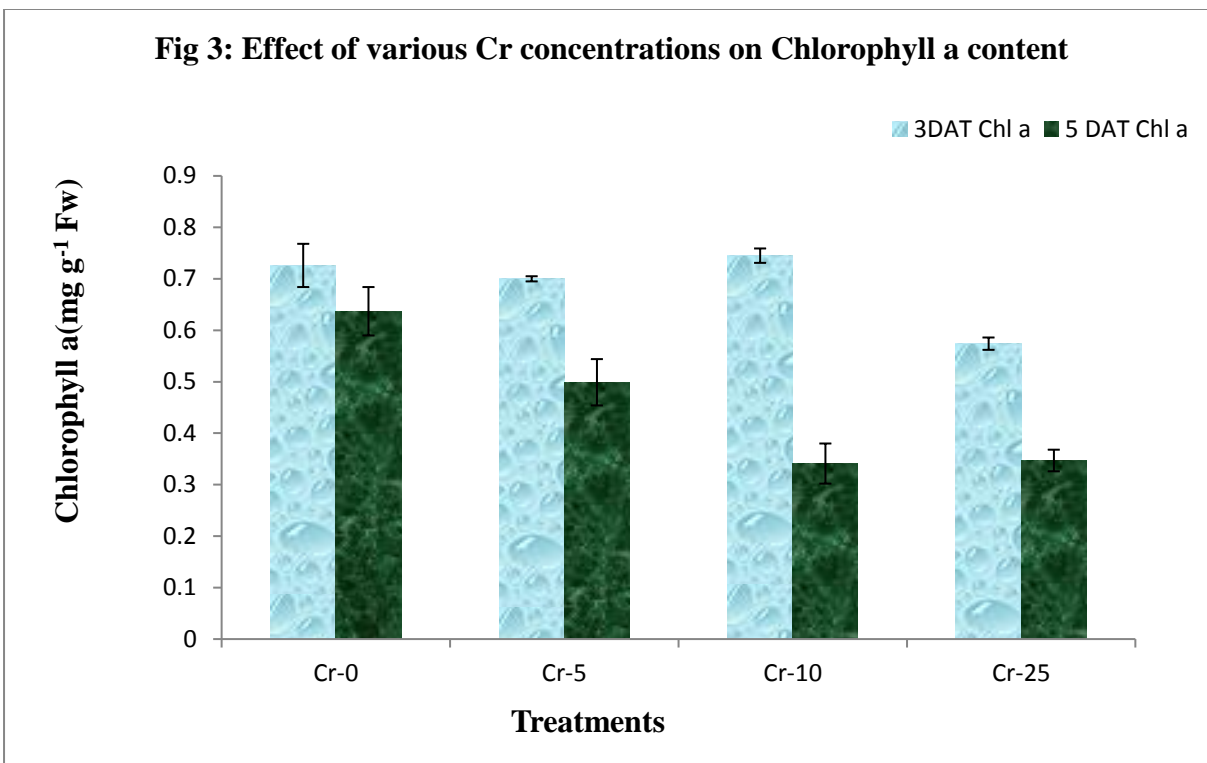
The reason for increased free amino acid content may be either due to decreased protein synthesis or increased proteolysis.

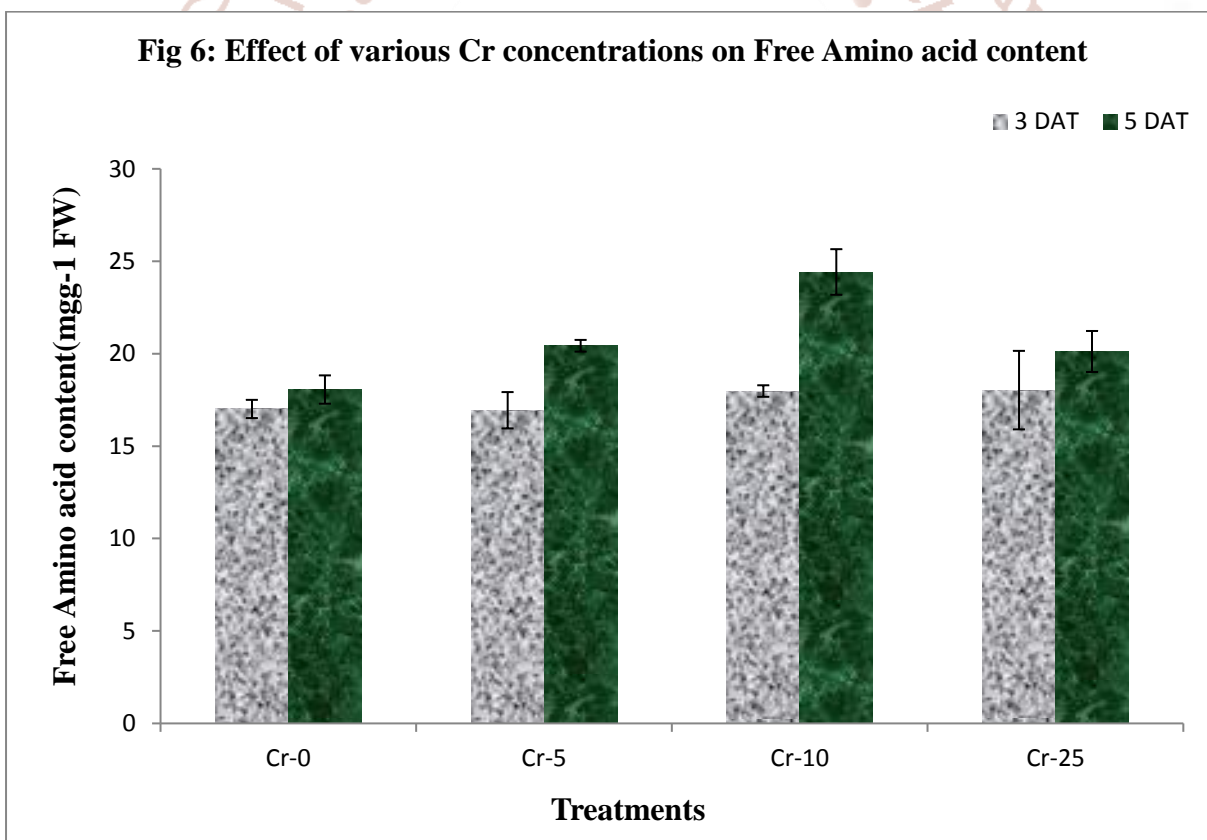
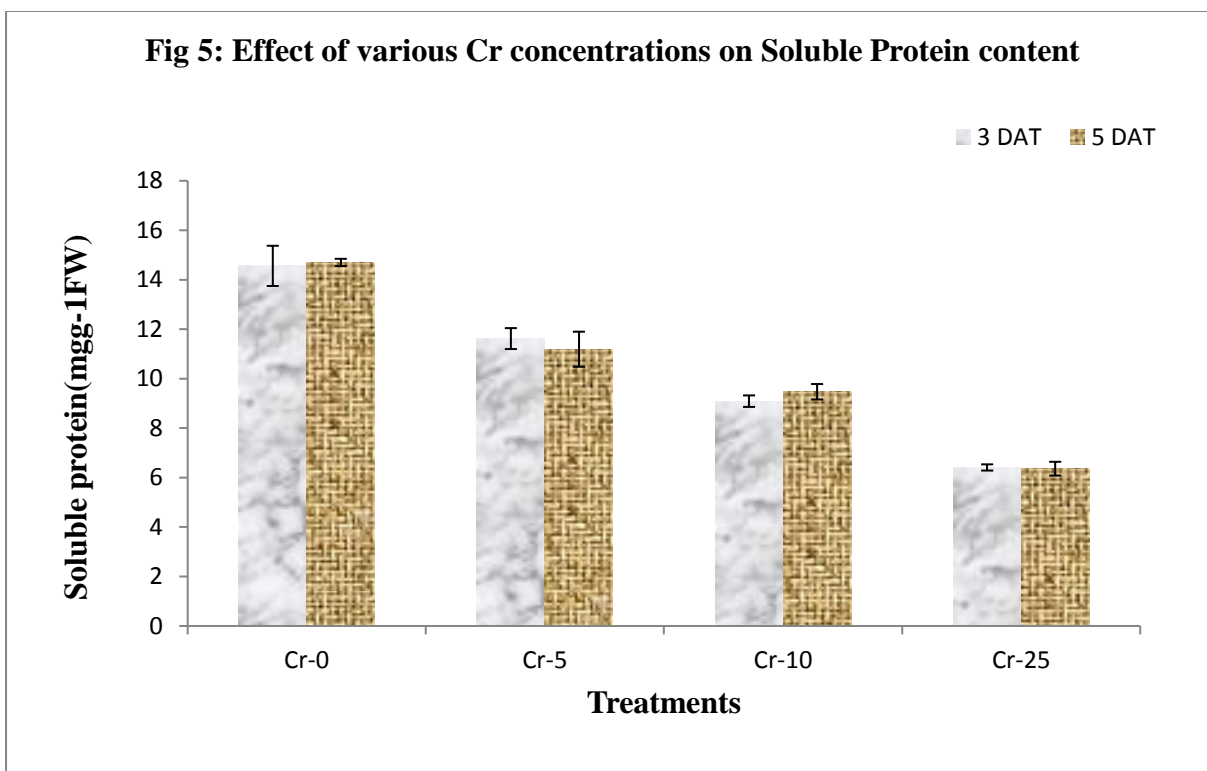
Proline Content:

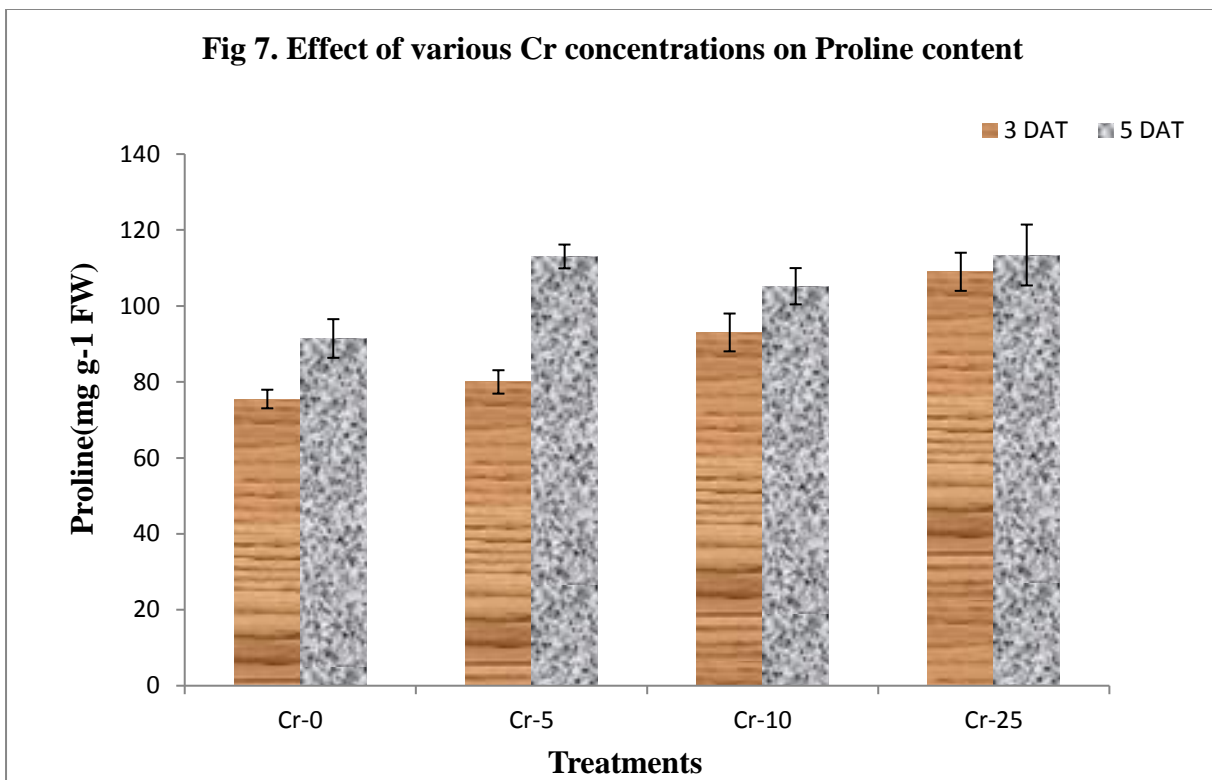
Proline content in response to various Cr stresses showed a gradual increase on 3DAT (6-44%) while as

on 5DAT, the content was maximum (24%) at Cr-25 concentration (Fig 7). Alia and Saradhi (1991) have also acquired similar increase in proline accumulation in response to heavy metal stress. Plants respond various stresses by inducing accumulation of some metabolites, the most obvious are generally amino acid and proline in particular (Aleksza et al., 2017)









Conclusion:

From this study, we conclude that Chromium affects the growth and biochemical parameters in *Ocimum basilicum*. The magnitude of the effect is directly proportional to the amount of stress imposed at different growth stages of the plants. Further studies on the antioxidant defense system of the plants under various chromium treatments and the concentration of chromium in various plant parts of the treated plants need to be done to ensure whether the plant defense system can render the plant resistance against heavy metal stress. The screening of the plant for chromium effect on the secondary metabolite of this medicinal plant will ensure the possibility of using this plant for chromium phytoremediation in affected soils.

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