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Study of Biochemistry of *Cicer arietinum* Crop Irrigated with Industrial waste water of Kota, Rajasthan

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ABSTRACT

The present study was conducted to determine the effect of industrial effluents on biochemical parameters of *Cicer arietinum* crop. Water samples were collected from a common outlet of different industries of Kota. The biochemical parameters studied were phenol, protein, starch, total soluble sugar, pigments (chlorophyll a, chlorophyll b, carotenoid, and total chlorophyll) and antioxidative enzymes like catalase and peroxidase. These parameters were compared between plants grown in industrial waste water and controls. Results showed that industrial waste water irrigated crops significantly affect some biochemical contents. Result showed that total chlorophyll, protein and catalase content is decreased in plants irrigated with waste water in comparison to that with control water crop, Whereas starch , total soluble sugar, carotenoid, phenol and peroxidase contents were increased in plants irrigated with waste water in comparison to that with control water crop. Significant changes are seen in total carbohydrate content of industrial waste water irrigated crop as compared to control. Study showed that industrial waste water irrigation stress induces the changes in biochemical parameters and antioxidative enzymes content in *Cicer* crop.

Introduction

Industrial effluents are constantly adding up toxic substances into the ground water reservoir at a very high rate, especially in industrial zones. Since most of the wastewater is being discharged into the surrounding water bodies, which disturbs the ecological balance and deteriorates the water quality. **[1]**

Effluents from industries which are normally considered as the main industrial pollutants containing organic and inorganic compounds are discharged into the nearby water bodies. It makes the water bodies toxic as various industries discharge the suspended solids, toxic chemicals, oils, greases, dyes, radioactive wastes and thermal pollutants. As a result the high level of pollutants mainly organic matter in river water causes an increase in BOD, COD, TDS TSS etc. It makes the water unsuitable for drinking, irrigation or for other uses. It has been found that the growth, yield and soil health get reduced when the farmers use the effluents for irrigation of the cultivated land (Nandy and Kaul.1994). **[2]**

The use of such industrial waste water in irrigation system definitely provide some nutrients to enhance the fertility of soil but also deposit toxicants that change soil properties in the long run. This necessitates a detailed scientific study before any specific waste can be used for irrigation for a particular crop and environmental conditions. Heavy metal pollution is a serious threat to the environment due to the fact that they cannot be degraded, rather they persist and are accumulated, hence pose severe affects on all life forms. They can cause adverse toxic effects on the plants growing in the affected area leading to a decrease in agricultural productivity. **[3]**

Much of biochemistry deals with the structures, functions and interactions of biological macromolecules, such as proteins, phenols, carbohydrates and lipids, which provide the structure of cells and perform many of the functions associated with life. So, it was proposed to compare the data on biochemical aspects in the plants grown under control and industrial waste waters.

To investigate the effect of effluents present in the industrial waste water, the present study was proposed. The very common crops *Cicer arietinum* grown in the Kota region was selected to study biochemical characteristics of the plants irrigated with control water and industrial waste water.

Study Area

The district Kota lies between 24°25’ and 25°51’ North latitudes and 75°31’ and 77°26’ East longitudes with total area of 5767.97 Sq Km. “Kota City” is located at extreme South of it at 25°11’ North latitude and 75°51’ East longitude occupying total area of 238.59 Sq Km with average height 253.30 meters from sea level. . Kota is a prime industrial town of Rajasthan with historical importance of its own. In last decade, Kota city has emerged as “educational city” of India mainly because of its excellence in coaching for entrance examination.**[4]**

Few large scale industries including DCM Shriram Consolidated Limited (DSCL), Multimetals Limited, Samtel Glass Limited, Chambal Fertilizers and Chemicals Limited (CFCL), Shriram Fertilizers and Metal India, Shriram Rayons and a number of Kota stone cutting polishing units further enhance the heavy metal burden in the Atmosphere. Many large and small scale industries are present due to availability of river water and power.**[5]**

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Fig 1. Industrial waste water outlet site

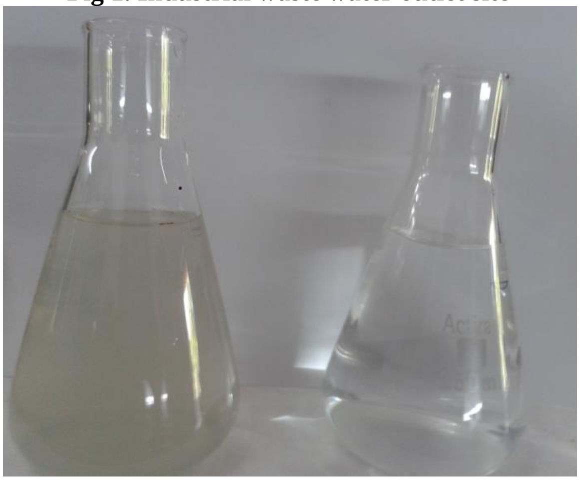

Fig 2. Industrial waste water control water

Material and Methods

Experimental plant: Cicer arietinum

Fig 3. *Cicer* waste water crop and *Cicer* control water crop

Collection of plants and water samples

The study was conducted with waste water released from industries at Kota, Rajasthan. Waste water samples were collected from common outlet point in Kansua nalla of combined effluents from industries and water sample of control water was collected from tap water. Effluent samples were collected in plastic container of 5-liter capacity. The seeds of *Cicer arietinum* (Gram) are purchased from registered seed center.

Two plots of 6.5 × 4.5 m^2 size were prepared. Seeds of cicer were sown in each plot. One plot was irrigated with control water and other with industrial waste water. Uniform irrigation schedule was followed at both plots to maintain similar moisture condition throughout the growth of plants. Names of the two plots were given as *Cicer* control and *Cicer* waste water plot. Plant samples collected were washed with distilled water to remove dust particles. Fresh leaf material was used for estimation of different biochemical contents.

Experiments

Fresh leaves were used for estimation of chlorophyll, protein, phenol, carbohydrate, starch and the two antioxidative enzymes viz., peroxidase and catalase.

Chlorophyll a, chlorophyll b, carotenoid and total chlorophyll was estimated by Arnon's method (1949). **[6]** Protein content in leaves was measured by method described by Lowry *et al* (1951). **[7]** Phenol content in leaf was measured by method described by Bray and Thorpe (1954) . **[8]** Carbohydrate was estimated in leaf by phenol- sulphuric acid method (Dubois *et al)* 1951.**[9] [10]** Peroxidase and catalase in leaf was estimated by methods used by Putter and Aebi. [11][12]

Observation Table

Table 1. Showing comparison of biochemical characteristics of *Cicer arietinum* crop irrigated with control water and industrial waste water

S No.	Parameters	Cicer (Control)	Cicer (Waste water)
1	Chlorophyll a(mg/gm)	4.88±0.15	4.94±0.14
2	Chlorophyll b(mg/gm)	4.85±0.20	4.61±0.33
3	Total Chlorophyll (mg/gm)	9.73±0.06	9.55±0.19
4	Carotenoid (mg/gm)	3.44±0.18	4.00±0.11
5	Protein (mg/gm)	2.88±0.19	1.96±0.09
6	Phenol (mg/gm)	0.11±0.43	0.15±0.01
7	Total Soluble Sugar (mg/gm)	1.93±0.23	13.33±1.45
8	Starch (mg/gm)	0.95±0.17	2.03±0.28
9	Catalase µMolar/L/gm	0.53±0.14	0.45±0.17
10	Peroxidase µMolar/L/gm	1.90±0.12	2.75±0.27

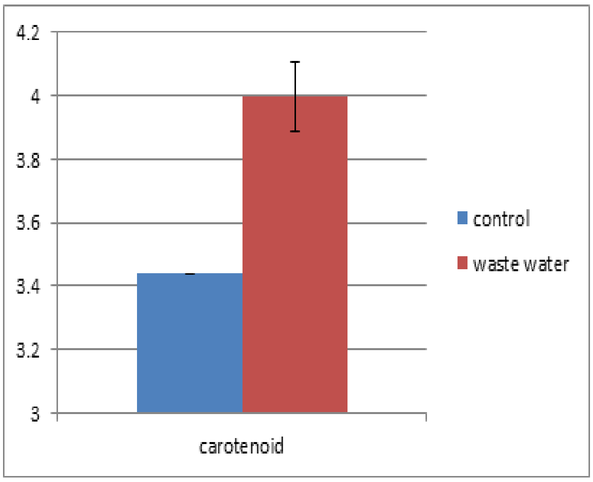

Fig 3

In *Cicer* crop the carotenoid, phenol and starch amount in industrial waste water irrigated crop was 4.00±0.11, 0.15±0.01 and 2.03±0.28mg/gm respectively, whereas control water irrigated crop have values as 3.44±0.18, 0.11±0.43 and 0.95±0.17mg/gm respectively. This shows that increased amount of carotenoid, phenol and starch is found in crop irrigated with industrial waste water. Carotenoid is photosynthetic pigment, also functions as non-enzymatic antioxidant protecting plants from oxidative stress by changing the physical properties of photosynthetic membranes with involvement of xanthophyll cycle. **[13]**An increase in carotenoid content was suggested to be a defense strategy of the plants to combat metal stress. [14]

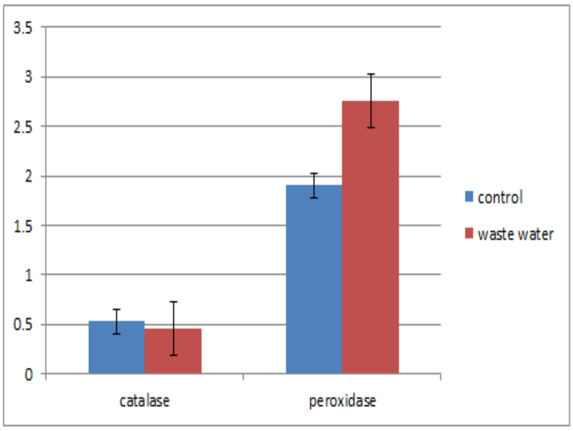

Fig 4

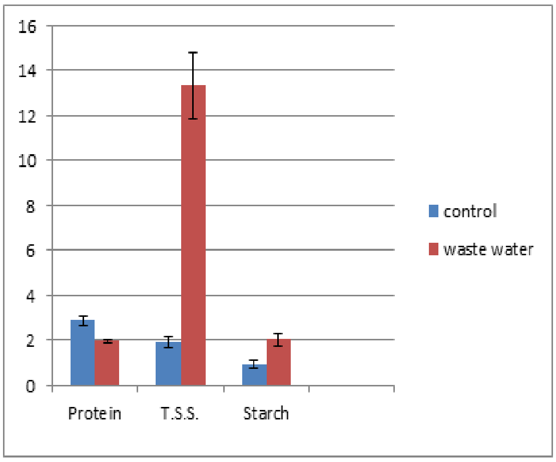

Fig 5

Drastic increase is seen in total soluble sugar value. Total soluble sugar value is 1.93±0.23mg/gm in control crop and 13.33±1.45mg/gm in industrial waste water irrigated crop. Significant increase in total soluble sugar content is seen in industrial waste water irrigated crop. Earlier studies have shown that the enrichment of soluble sugars, in addition to its osmotic index, may protect the membranes from drying out. It has been shown that certain sugars such as trehalose, by binding to membrane lipids, could stabilize the membrane structure. [15]

Our results are consistent with those of Bekhouche [16] which show that irrigation with wastewater resulted in different wheat varieties, a high activity peroxydatique resulting in increased levels of soluble sugars. These results appear to be consistent with many previous studies where the accumulation of osmolytes (sugars and proline) turns out to be a reliable indicator of stress tolerance in general. Among the possible explanations, waste water is loaded with organic matter and decaying detritus are the major source of nitrogen molecules composed of amino acids (proline), proteins or even chlorophylls. Mineralization of nitrogenous substances from wastewater is a participation in the size of general explanation of the mechanism of tolerance. Studies showed that stress induces the decline in protein contents in plants but increase in soluble sugar content. [17][18][19]

The amount of chlorophyll and protein are 9.73±0.06 and 2.88±0.19mg/gm in control crop, whereas 9.55±0.19 and 1.96±0.09mg/gm in industrial waste water irrigated crop. This shows decrease in chlorophyll content in industrial water treated crop as compared to control. Changes in total chlorophyll concentration indicate that the chlorophyll synthesizing capacity of the crop has diminished affecting the overall photosynthetic process. [20]

The inhibition of chlorophyll synthesis probably results from the Cu-induced inhibition of ALA-dehydrates reported by Scarponi and Perucci (1984). Izawa (1997) suggested that the inhibition of chlorophyll may be due to the induced inhibition of electron transport system in PS - II. [21][22]

The lower amount of protein in crop irrigated with industrial waste water indicate breakdown of soluble protein, increased activity of protease or other catabolic enzymes (Mishra et al., 2009). [23]

Reduced biomolecules (protein and total chlorophyll) in plants treated with industrial effluent could be due to the diversion of metabolites to other synthesis processes, failure of antioxidative systems and produced stresses against heavy metals, and generation of reactive oxygen species (Pandey and Pathak, 2006). [24]

The catalase and peroxidase value in control water crop was 0.53±0.14 and 1.90±0.12 µMolar/L/gm, whereas in industrial waste water irrigated crop it is 0.45±0.17 and 2.75±0.27 µMolar/L/gm respectively. In *Cicer* crop increased peroxidase enzyme activity and decreased catalase enzyme activity is seen. Balasimha (1982) reported that peroxidase plays a vital role in IAA and chlorophyll degradation. Thus observed increase in peroxidase activity can be correlated with the observed reduction in chlorophyll content, fresh weight and biomass. [25]

Peroxidases play a significant role in defense against oxidative stress and are suggested to be indicators of metal toxicity. [26]

Antioxidant systems enzyme's, are activated and produced against heavy metal stress, damage of plasma membrane and generation of ROS, H2O2. Several cellular functions have also been described for plant catalase. It neutralizes H2O2 Which is produced during photorespiration and acyl CoA oxidation of fatty acids (Yamaguchi and Aso, 1977).[27][28]

According to Panda and Patra (2000) chromium ions increased the catalase activity in younger leaves while the activity decreased in older ones. [29]

Result

Studies have been shown that stress induces the decline in protein contents in Cicer crop but increase in soluble sugar content (Rong Guo *et al.*, 2007) **[30].** Significant change in carbohydrate content is seen in crop. Protein, chlorophyll and catalase value decreases in *Cicer* crop irrigated with industrial waste water whereas carotenoid, phenol, carbohydrate and peroxidase quantity increases in industrial waste water treated crop.

The study concluded that, common effluent of various nature of industries (from Kota district of Rajasthan), was contaminated with heavy metals. It posed toxic effects on biochemical responses of *Cicer arietinum* crop grown in Kota region. So industrial waste water should not be used directly for irrigation purpose. It may have some beneficiary effects on crop, but can also adversely affect the crop enzymes and nutrients. It is suggested that waste water have to be diluted before it is used for irrigation. After dilution, the effluent characteristics will become within the prescribed limits and pollution load of the effluent decreases.

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