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PHOTOCATALYTIC DEGRADATION OF INDIGO CARMINE DYE SOLUTION AND ITS EFFECT ON GROWTH AND BIOCHEMICAL PARAMETERS OF *ALLIUM CEPA* (ONION)

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ABSTRACT

Titanium dioxide is known as an effective semiconductor for photodegradation of dyes in aqueous solution using visible light. Natural sunlight was used to photodegrade Indigo carmine dye solution. This solution was used for growth of onion bulbs. Comparisons between untreated and treated dye solutions were made on the basis of certain growth and biochemical parameters in *Allium cepa* (onion). An overall increase in growth and biochemical parameters was observed in onion bulbs grown in TiO_2 treated dye solution. It has been established that irrigation of vegetables with untreated industrial waste water causes accumulation of heavy metals in plants, which are toxic and cause cessation of crop growth, affects seed germination, lowers crop yield and results in various human health problems demanding that either this practice must be dejected or must be followed with some suitable management. The crops should be irrigated with waste water only after proper treatment. Thus, photoassisted degradation of the wastewaters containing dyes using low cost semiconductor material is a promising technique to treat waste water.

Key words: Indigo carmine, Photocatalysis, Biochemical parameters, *Allium cepa*.

INTRODUCTION

Wastewaters from various industries, factories, laboratories, etc. are serious problems to the environment. The discharged wastes containing dyes are toxic to microorganisms, aquatic life and human beings[1,2].

Most textile dyes are photocatalytically stable and refractory towards chemical oxidation, and these characteristics render them resistant towards decolourization by conventional biochemical and physico-chemical methods. Gupta and Nathawat[3] studied the effect of textile effluent on germination and seedling growth of peas (*Pisum sativum*). Khan and Jain[4] observed effects of textile industry waste on growth and some biological parameters of wheat (*Triticum aestivum* var Raj. 3077). Nirmalarani and Janardhanan[5] studied the effect of South India viscose factory effluents on seed germination, seedling growth and chloroplast pigment content in five varieties of maize (*Zea mays*). Yousaf et al.[6] conducted an experiment with 5 varieties of *Glycine max* (soyabean) and reported that seedling lengths were longer with application of paper and board industry effluent as compared to the textile industry effluent application.

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Many researchers have established that effluent waters containing dyes at lower concentration have promoting effect on plant growth. However, as the amount of effluents was increased it had a negative impact on plant growth. At low concentration seed germination is not affected but has inhibitory effect at higher concentrations. Kaushik et al.[7] found growth promoting effect of treated textile waste, when applied at low concentration. Ameta et al.[8] studied that there was a prominent growth, increased sugar, protein percentage and chlorophyll contents in onion grown with photocatalytically treated effluent.

Degradation of dyes in industrial wastewaters has therefore received increasing attention and some methods of remediation have been preferred. It has been noted that traditional wastewater treatment technologies have proven to be markedly ineffective for handling wastewater of synthetic textile dyes because of the chemical stability of these pollutants.

Systematic investigations on photbleaching of dyes have also been carried out. Rao et al.[9] carried out photobleaching of crystal violet whereas Lakshmi et al.[10] reported titanium oxide mediated photocatalytic degradation of methylene blue. Photocatalytic oxidation of methylene blue on thin film TiO_2 was observed by Matthews[11] while Artemev et al.[12] carried out photocatalytic decomposition of methylene blue in ozonized aqueous TiO_2 and Nb_2O_5 suspensions.

Recent studies have been devoted to the use of photocatalysis in the removal of dyes from wastewaters, particularly, because of the ability of this method to completely mineralize the target pollutants. It is possible to combine photocatalysis with conventional biological treatment for the remedy of waste water containing generally non-biodegradable dyes. The treated water can be used for plant growth thus making it possible to reuse the water.

EXPERIMENTAL

Material and methods

Indigo carmine dye was procured from HiMedia. Semiconductor TiO_2 used was procured from Merck.

General procedure

Dye solutions were prepared in distilled water so that final concentration of the dye solution was 1.0×10^{-5} M. This solution was further divided into two parts. Half of the solution was kept as such and covered with black paper or plastic. This solution was used as untreated dye solution. The other half of the solution was treated photocatalytically in the presence of semiconductor TiO_2. 0.5 g of semiconductor was added and the beaker was placed in sunlight. It was stirred at regular intervals as TiO_2 particles get settled at the bottom of the container in some time. Complete disappearance of colour indicates photobleaching. Any loss in the volume of solution is made up using distilled water. It was then filtered and used as treated dye solution. Control experiments were carried out using distilled water as medium.

Onion bulbs of roughly same size and weight were used for experiments. Root and shoot growth of *Allium cepa* bulbs grown in treated and untreated solutions. Growth of root and shoot was measured per day in centimeters. Number of root or shoot per onion bulb was reported as root and shoot density for each onion bulb.

Equal amount of treated and untreated solutions were filled in separate cylindrical jars. Onion bulbs of same size and mass were used. Each bulb was cleaned and any dried roots from the bulb before were neatly cut with a blade. Bulb was placed in the cylindrical jar such that the root bearing tip of bulb was just immersed in the solution and upper part was exposed to the air. The level of the solution was marked on the

cylinder at start of the experiment. The set up was exposed to normal light and temperature. The root and shoot lengths were measured daily in centimeters. Also the number of roots and number of leaves emerged per onion bulb were also recorded daily and reported.

Detection method

Protein content was measured using Kjeldahl method for determination of protein as per Indian Standard Institute.

Sugar content was measured using Anthrone reagent. Extraction of Sugar: 0.5 g of dried and ground tissue was extracted with with 20 mL of 80% ethanol and after boiling for two minutes the supernatant was collected into a beaker. This was repeated 4 times and supernatant collected into same beaker. The volume was made up to 100 mL with distilled water. Determination of total sugar: 1 mL of sugar extract from above was added to 4 mL of 0.2% anthrone reagent (placed in ice cold water). Anthrone reagent was prepared by dissolving 200 mg anthrone in 100 mL conc. H_2SO_4. Absorbance was measured at 620 nm.

Chlorophyll content was measured using Arnon method. 1 g of finely cut tissue was ground in a clean mortar and pestle with 20% acetone. This was centrifuge at 5000 rpm for 5 mins. Grinding and extraction was repeated till a clear residue is obtained. The mortar and pestle were washed thoroughly with 80% acetone and washings collected in 100 mL volumetric flask. The volume was made 80% acetone. Absorbance was read at 645 nm and 663 nm against solvent blank (80% acetone). Amount of chlorophyll is calculated as follows:

$$\text{mg chl a/ gm tissue} = [(12.7)\,(A_{663}) - 2.69\,(A_{645})] * [V/\,(1000*W)]$$

$$\text{mg chl b/ gm tissue} = [(22.9)\,(A_{645}) - 4.68\,(A_{663})] * [V/\,(1000*W)]$$

$$\text{Total chl/ gm tissue} = [(20.2)\,(A_{645}) + 8.02\,(A_{663})] * [V/\,(1000*W)]$$

A = Absorbance at specific wavelength

V = Final vol. of chl. extract in 80% acetone

W = Fresh wt of tissue

RESULTS AND DISCUSSION

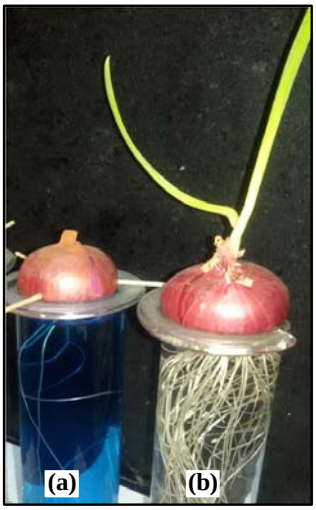

Fig. 1: Growth of onion bulb in (a) indigo carmine and (b) photocatalytically treated solution

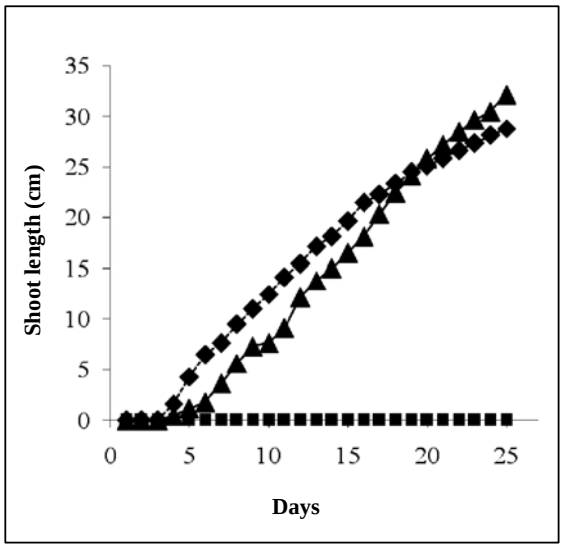

Fig. 2: Shoot growth of onion bulb

Table 1: Shoot growth of onion bulb

Days	Shoot length (cm)					
	Control		Untreated		Treated	
	Mean	SD	Mean	SD	Mean	SD
1	0.00	0.00	0.00	0.00	0.00	0.00
2	0.00	0.00	0.00	0.00	0.00	0.00
3	0.00	0.00	0.00	0.00	0.00	0.00
4	1.67	0.24	0.00	0.00	0.50	0.71
5	4.33	0.62	0.00	0.00	1.17	1.03
6	6.50	0.82	0.00	0.00	1.83	1.43
7	7.67	0.85	0.00	0.00	3.67	1.55
8	9.50	0.82	0.00	0.00	5.67	1.18
9	11.00	0.71	0.00	0.00	7.33	2.09
10	12.50	0.82	0.00	0.00	7.67	1.18
11	14.17	0.47	0.00	0.00	9.17	1.43
12	15.50	0.82	0.00	0.00	12.17	2.49
13	17.17	0.47	0.00	0.00	13.83	2.72
14	18.17	0.62	0.00	0.00	15.00	2.27
15	19.67	1.03	0.00	0.00	16.50	2.27
16	21.50	0.82	0.00	0.00	18.17	2.25
17	22.33	0.62	0.00	0.00	20.33	2.09
18	23.33	0.62	0.00	0.00	22.50	2.16
19	24.50	0.82	0.00	0.00	24.17	1.55
20	25.17	1.25	0.00	0.00	25.83	1.70

Cont…

Days	Shoot length (cm)					
	Control		Untreated		Treated	
	Mean	SD	Mean	SD	Mean	SD
21	25.83	1.31	0.00	0.00	27.17	1.25
22	26.67	1.55	0.00	0.00	28.50	0.41
23	27.33	1.65	0.00	0.00	29.67	0.47
24	28.17	1.55	0.00	0.00	30.50	0.41
25	28.83	1.03	0.00	0.00	32.17	0.85

Table 2: Shoot density of onion bulb

Days	No. of leaves per Onion bulb					
	Control		Untreated		Treated	
	Mean	SD	Mean	SD	Mean	SD
1	0.00	0.00	0.00	0.00	0.00	0.00
2	0.00	0.00	0.00	0.00	0.00	0.00
3	0.00	0.00	0.00	0.00	0.00	0.00
4	2.33	0.47	0.00	0.00	0.33	0.47
5	3.00	0.00	0.00	0.00	0.67	0.47
6	3.33	0.47	0.00	0.00	0.67	0.47
7	4.33	0.47	0.00	0.00	2.00	1.41
8	4.33	0.47	0.00	0.00	7.33	1.25
9	4.67	0.47	0.00	0.00	8.00	1.63
10	5.00	0.82	0.00	0.00	9.00	0.82
11	5.00	0.82	0.00	0.00	9.33	0.94
12	5.33	0.47	0.00	0.00	10.33	0.47
13	5.67	0.47	0.00	0.00	11.00	0.82
14	5.67	0.47	0.00	0.00	12.00	0.82
15	6.33	0.47	0.00	0.00	13.67	0.47
16	7.33	0.47	0.00	0.00	13.67	0.47
17	7.67	0.47	0.00	0.00	14.67	0.47
18	8.33	0.94	0.00	0.00	15.67	0.94
19	9.33	0.94	0.00	0.00	16.00	0.82
20	10.33	0.94	0.00	0.00	17.67	1.25
21	10.33	0.94	0.00	0.00	18.33	0.47
22	10.33	0.94	0.00	0.00	18.33	0.47
23	10.67	0.47	0.00	0.00	18.33	0.47
24	12.00	0.82	0.00	0.00	19.00	0.82
25	12.00	0.82	0.00	0.00	19.33	0.94

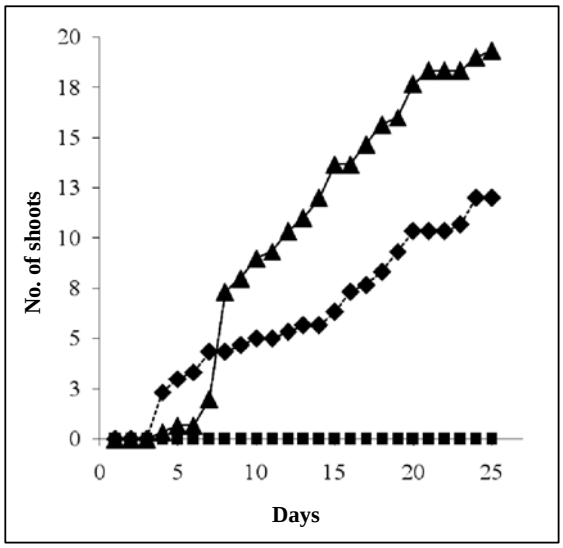

Fig. 3: Shoot density of onion bulb

Table 3: Root growth of onion bulb

Days	Root length (cm)					
	Control		Untreated		Treated	
	Mean	SD	Mean	SD	Mean	SD
1	0.00	0.00	0.00	0.00	0.00	0.00
2	0.67	0.24	0.00	0.00	0.00	0.00
3	1.50	0.00	0.00	0.00	0.00	0.00
4	2.50	0.71	0.00	0.00	0.00	0.00
5	4.00	0.71	0.00	0.00	0.67	0.62
6	4.83	0.85	0.00	0.00	1.83	0.24
7	5.50	0.82	0.00	0.00	3.17	0.24
8	6.00	0.71	0.00	0.00	4.00	0.41
9	6.50	0.71	0.00	0.00	4.17	1.25
10	7.17	0.62	0.00	0.00	4.83	1.03
11	7.83	0.85	0.00	0.00	5.50	0.82
12	8.50	0.71	0.00	0.00	6.33	1.31
13	9.50	0.71	0.00	0.00	7.17	1.25
14	9.83	0.94	0.00	0.00	7.17	1.25
15	10.50	1.08	0.00	0.00	8.33	0.85
16	10.83	0.94	0.00	0.00	9.17	0.62
17	11.50	1.08	0.00	0.00	9.83	0.85
18	12.17	1.18	0.00	0.00	11.00	0.71
19	12.50	1.08	0.00	0.00	12.17	0.62

Cont…

Days	Root length (cm)					
	Control		Untreated		Treated	
	Mean	SD	Mean	SD	Mean	SD
20	13.17	1.18	0.00	0.00	13.67	0.62
21	13.50	1.08	0.00	0.00	14.83	0.62
22	14.00	1.08	0.00	0.00	15.83	0.47
23	14.33	1.03	0.00	0.00	17.50	0.41
24	14.67	1.25	0.00	0.00	18.67	0.24
25	15.00	1.22	0.00	0.00	19.00	0.41

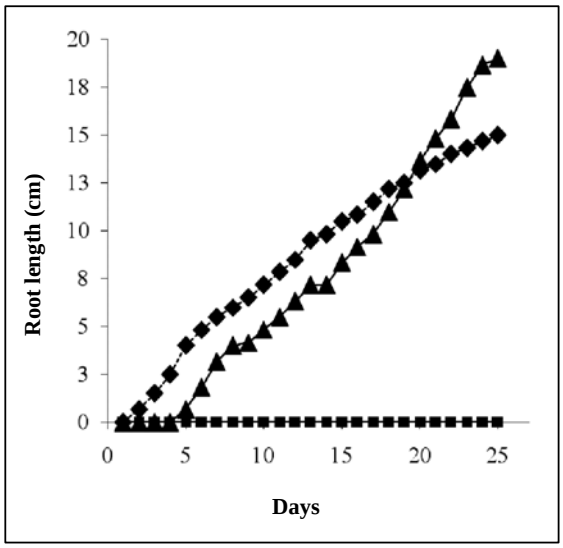

Fig. 4: Root growth of onion bulb

Table 4: Root density of onion bulb

Days	No. of Roots per Onion bulb					
	Control		Untreated		Treated	
	Mean	SD	Mean	SD	Mean	SD
1	0.00	0.00	0.00	0.00	0.00	0.00
2	1.33	0.47	0.00	0.00	0.00	0.00
3	2.50	0.41	0.00	0.00	0.00	0.00
4	3.33	0.47	0.00	0.00	0.00	0.00
5	3.67	0.47	0.00	0.00	2.00	1.63
6	3.67	0.47	0.00	0.00	2.67	0.24
7	4.67	0.47	0.00	0.00	3.00	1.41
8	5.00	0.00	0.00	0.00	4.00	0.82

Cont…

Days	No. of Roots per Onion bulb					
	Control		Untreated		Treated	
	Mean	SD	Mean	SD	Mean	SD
9	5.00	0.00	0.00	0.00	4.00	0.82
10	5.33	0.47	0.00	0.00	4.33	0.94
11	6.67	0.47	0.00	0.00	7.00	0.82
12	7.67	0.47	0.00	0.00	9.00	0.82
13	8.00	0.00	0.00	0.00	11.33	0.47
14	9.00	0.00	0.00	0.00	13.67	0.47
15	9.00	0.82	0.00	0.00	17.00	0.82
16	10.00	0.00	0.00	0.00	19.00	0.82
17	11.33	0.47	0.00	0.00	19.00	0.82
18	11.67	0.47	0.00	0.00	19.67	1.25
19	13.00	0.82	0.00	0.00	20.00	1.41
20	13.67	0.47	0.00	0.00	20.33	0.62
21	14.67	0.47	0.00	0.00	21.00	1.63
22	15.00	0.82	0.00	0.00	21.00	1.63
23	15.00	0.82	0.00	0.00	21.67	0.94
24	15.00	0.82	0.00	0.00	22.00	0.82
25	15.00	0.82	0.00	0.00	22.33	1.25

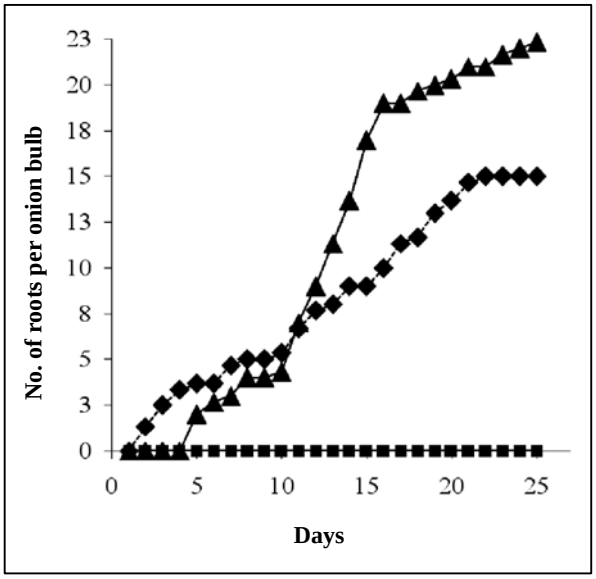

Fig. 5: Root density of onion bulb

No growth was there in the root and shoot of onion bulb placed in the untreated dye solution. However, root and shoot growth was observed in onion bulbs placed in treated dye solution.

Table 5: Chlorophyll contents of onion shoots

Sample	Chlorophyll content (mg/g)		
	Chl a	Chl b	Total Chl
Control	0.18	0.17	0.36
Treated	0.14	0.08	0.22

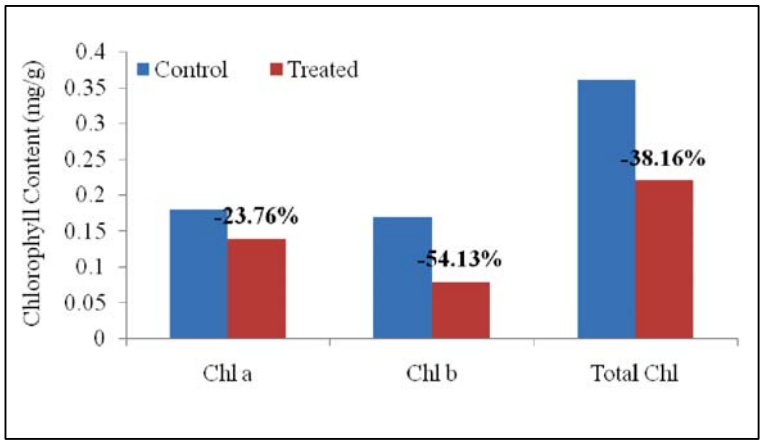

Fig. 6: Chlorophyll contents of onion shoot

It can be seen from Fig 6 and Table 5, that there is a decrease in the Chl a, Chl b and total chlorophyll content of the onion bulbs grown in treated indigo carmine solution.

Table 6: Sugar and protein contents of shoot

Sample	% Sugar content	% Protein content
Control	9.35	2.83
Treated	17.75	2.00

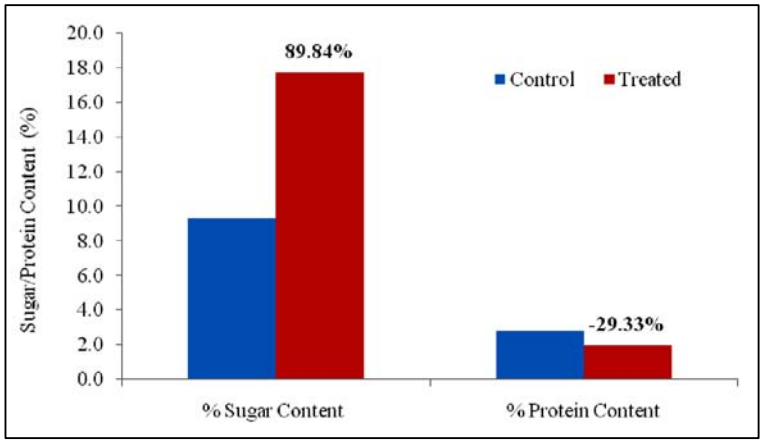

Fig. 7: Sugar and protein contents of onion shoot

There was a great increase in the sugar content of shoot of the onion bulb grown in treated indigo carmine solution. However, the same was not observed for protein concentration where a decrease was seen (Fig. 7 and Table 6)

Table 7: Sugar and protein contents of root

Sample	% Sugar content	% Protein content
Control	1.09	1.20
Treated	6.30	1.90

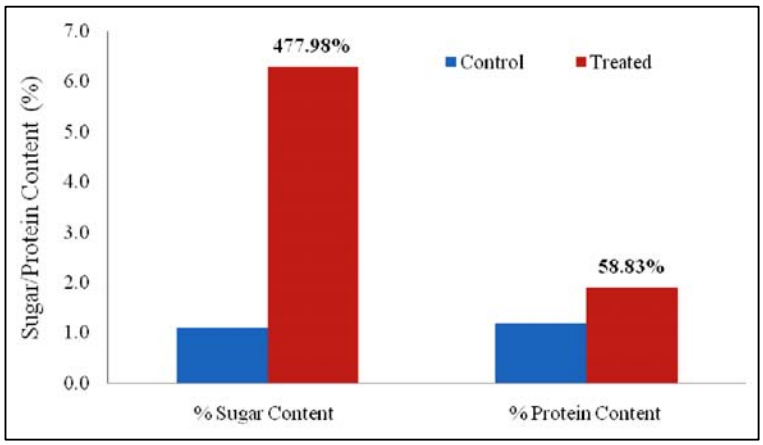

Fig. 8: Sugar and protein contents of onion shoot

An increase in the sugar as well as protein content of root of the onion bulb grown in treated indigo carmine solution was observed (Fig. 8 and Table 7)

CONCLUSION

Shoot and root growth and density

When onion bulbs were grown in untreated and photocatalytically treated indigo carmine solution, it was observed that there was no root and shoot growth in onion bulb placed in untreated dye solution. But good growth was observed in onion bulb grown in treated indigo carmine solution. This confirms the fact that the presence of dissolved dyes in solution is detrimental for plant growth and development. However, when onion bulbs were placed in treated indigo carmine solution good growth of root and shoot was observed. Profuse rooting was observed. There was 11.59% increase in shoot length and 61.08% increase in shoot density of the onion bulbs grown in treated indigo carmine solution as compared to that of onion bulbs grown in control.

Similarly, there was 26.67% increase in root length and 48.89% increase in root density of the onion bulbs grown in treated indigo carmine solution, respectively as compared to control.

The presence of dye molecules in the solution is detrimental for onion bulb growth, but in photocatalytically treated solution, the toxic dye molecules are converted in simpler inorganic counterparts, which enhance root and shoot growth. Not only there is an increase in length but also an increase in number of roots and shoots in onion bulbs grown in photocatalytically treated indigo carmine solution as compared to the onion bulbs grown in control solutions.

Chlorophyll contents of shoot

The chlorophyll content of the shoot of onion bulbs grown in treated was compared with that of control group. It was observed that there was decrease in chlorophyll contents of shoots of onion bulb grown

in treated dye solution. The decrease was by 23.76% in Chl a, 54.13% in Chl b and 38.56% in total chlorophyll contents. This suggests the presence of chemical entities, which may be harmful for chlorophyll synthesis.

Sugar and protein contents of shoot

89.84% Increase in sugar content and 29.33% decrease in protein content were observed in the shoot of onion bulbs grown in treated indigo carmine solution.

Sugar and protein contents of root

A very high 477.98% increase in sugar content and 58.33% increase in protein content were observed in the root of onion bulbs grown in treated indigo carmine solution.

Thus, effluent containing dye water from dyeing industries should be photocatalytically treated using low cost semiconductor material and then reused for irrigation purposes. This can also result in the increase of yield of crop plants. This method can aid in use of large amount of water, which is major cause of pollution and is wasted otherwise.

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