Acta Chimica & Pharmaceutica Indica

Acta Chim. Pharm. Indica: 6(3), 2016, 92-103

ISSN 2277-288X

A STUDY ON THE EFFECT OF UNTREATED AND TiO_2 TREATED ORANGE G SOLUTION ON THE GROWTH AND BIOCHEMICAL PARAMETERS OF *ALLIUM CEPA* (ONION)

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(*Received : 24.06.2016; Accepted : 04.07.2016*)

ABSTRACT

The present study aims at using dye industry effluents for crop irrigation following treatment by low cost semiconductor material. Orange G dye solution was used and treated with TiO_2 under sunlight. The resulting photobleached solution was used for growth of analysis studies in *Allium cepa*. Growth parameters for onion bulb like shoot growth, shoot density, root growth and root density were reported. The effect of this photocatalytic treatment was observed in terms of certain biochemical parameters like chlorophyll, sugar and protein content. The findings of the investigation reinforce the fact that effluents from dyeing industries can be reused effectively.

Key words: Photocatalysis, Biochemical parameters, Orange G, *Allium cepa*.

INTRODUCTION

Effluents from various industries, factories, laboratories, etc. pose a serious threat to the environment. The discharged wastes containing dyes are toxic to microorganisms, aquatic life and human beings[1]. Degradation of dyes in industrial wastewaters has therefore received increasing attention and some methods of remediation have been preferred. Traditional physical techniques like adsorption on activated carbon, ultra filtration, reverse osmosis, coagulation by chemical agents, ion exchange on synthetic adsorbent resins, etc. are a few methods which only succeed in transferring these organic compounds from water to another phase, this creating secondary pollution. This requires a further treatment of solid-wastes and regeneration of the adsorbent, which adds more cost to the process. Microbiological or enzymatic decomposition, biodegradation, ozonation and advanced oxidation processes reactions have also been used for removal of dyes[2, 3]. 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. 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. 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[4].

The science of photocatalytic treatment of waste water is still in its primary stages. Research in the field has shown promising applications and this tool can be used in handling problems worldwide related to

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environmental pollution, waste water treatment, energy crisis etc. Photocatalysis may be termed as a photo induced reaction, which is accelerated by the presence of a catalyst.

Photo induced electron transfer reactions have attracted the attention of photo-chemists all over the world, because these reactions are capable of converting toxic compounds into non-toxic or less toxic materials. The photocatalytic bleaching of dye using low cost semiconducting materials (such as TiO_2, ZnO, Fe_2O_3, CdS, and ZnS) has opened new directions for the treatment of wastewater from dyeing, printing and textile industries. The treated water which is otherwise unfit for human use may be made available for different purposes like cleaning, waste land irrigation, etc., which is not possible with coloured water.

Earlier studies have shown that 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. Exhaustive amount of work has been done on photocatalytic degradation of various dyes.

Ameta et al.[5] used zinc oxide particulate system as a photocatalyst for photobleaching of basic blue 24 dye, whereas Ozkan et al.[6] used TiO_2 particles for degradation of azo dye. Orange II was degraded by TiO_2-mediated photocatalytic degradation in the presence of Mn^{2+} in solution by Mu et al.[7] The effect of photocatalytic degradation of azo dye acid red 14 in water on ZnO as an alternative catalyst to TiO_2 was studied by Daneshwar et al.[8] Ameta et al.[9] studied the use of semiconducting iron(III) oxide in photocatalytic bleaching of some dyes.

Despite all these the possibility of making use of treated waste water needs to be explored in detail. Few earlier studies have indicated that photocatalytically treated waste water can enhance plant growth and seed germination as compared to untreated water. This may be due to the fact that nitrogen content of the water is increased, which supports plant growth. Effluents from the dyeing and textile industry should be treated before disposal so that quality of water may be restored required for normal growth of the plants.[10-12]

In the present study, water containing Orange G dye was treated in the presence of sunlight and TiO_2 as semiconductor. The effect of treated and untreated water was observed on root and shoot growth in *Allium cepa* bulbs (onion). The effects were tested in terms of certain biochemical parameters like carbohydrate, protein and chlorophyll content of the plant shoot. Growth parameters for onion root and shoot were observed. Control experiments were carried out using water medium for preparing the solution.

EXPERIMENTAL

Material and methods

Orange G dye was procured from Hi Media. 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) orange G and (b) photocatalytically treated solution

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	1.83	0.85
5	4.33	0.62	0.00	0.00	3.67	1.43
6	6.50	0.82	0.00	0.00	4.83	2.05
7	7.67	0.85	0.00	0.00	6.67	2.09
8	9.50	0.82	0.00	0.00	8.83	2.05
9	11.00	0.71	0.00	0.00	10.33	2.49
10	12.50	0.82	0.00	0.00	12.00	2.55
11	14.17	0.47	0.00	0.00	14.00	2.48
12	15.50	0.82	0.00	0.00	15.67	3.01
13	17.17	0.47	0.00	0.00	16.83	3.09
14	18.17	0.62	0.00	0.00	17.83	3.06
15	19.67	1.03	0.00	0.00	19.67	3.70
16	21.50	0.82	0.00	0.00	21.33	3.47

Cont…

Days	Shoot length (cm)					
	Control		Untreated		Treated	
	Mean	SD	Mean	SD	Mean	SD
17	22.33	0.62	0.00	0.00	22.50	3.94
18	23.33	0.62	0.00	0.00	24.17	3.70
19	24.50	0.82	0.00	0.00	24.67	3.40
20	25.17	1.25	0.00	0.00	25.83	3.47
21	25.83	1.31	0.00	0.00	27.67	3.30
22	26.67	1.55	0.00	0.00	28.33	3.42
23	27.33	1.65	0.00	0.00	29.67	3.70
24	28.17	1.55	0.00	0.00	30.50	3.24
25	28.83	1.03	0.00	0.00	31.00	3.24

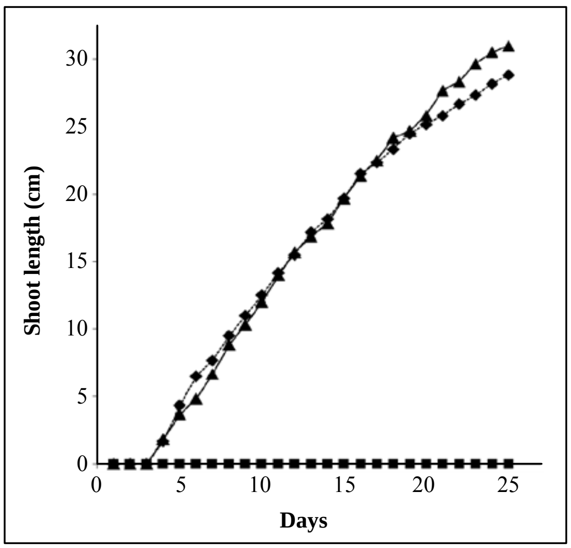

Fig. 2: Shoot growth of onion bulbs

Table 2: Shoot density of onion bulbs

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	1.00	0.00
5	3.00	0.00	0.00	0.00	1.00	0.00

Cont…

Days	No. of leaves per Onion bulb					
	Control		Untreated		Treated	
	Mean	SD	Mean	SD	Mean	SD
6	3.33	0.47	0.00	0.00	1.00	0.00
7	4.33	0.47	0.00	0.00	1.67	0.47
8	4.33	0.47	0.00	0.00	2.00	0.00
9	4.67	0.47	0.00	0.00	3.00	0.82
10	5.00	0.82	0.00	0.00	3.00	0.82
11	5.00	0.82	0.00	0.00	3.67	0.47
12	5.33	0.47	0.00	0.00	5.33	0.47
13	5.67	0.47	0.00	0.00	6.67	0.47
14	5.67	0.47	0.00	0.00	8.67	0.47
15	6.33	0.47	0.00	0.00	9.67	1.25
16	7.33	0.47	0.00	0.00	11.67	1.25
17	7.67	0.47	0.00	0.00	13.67	1.25
18	8.33	0.94	0.00	0.00	15.67	1.25
19	9.33	0.94	0.00	0.00	17.33	1.70
20	10.33	0.94	0.00	0.00	20.00	0.82
21	10.33	0.94	0.00	0.00	20.67	1.25
22	10.33	0.94	0.00	0.00	21.00	1.63
23	10.67	0.47	0.00	0.00	22.00	1.63
24	12.00	0.82	0.00	0.00	22.00	1.63
25	12.00	0.82	0.00	0.00	22.00	1.63

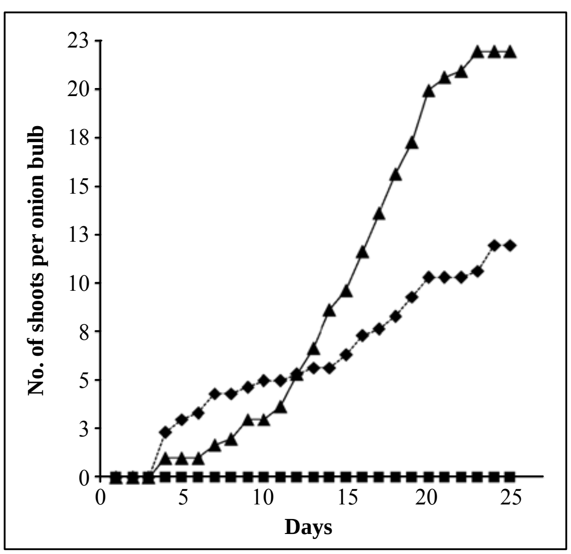

Fig. 3: Shoot density of onion bulbs

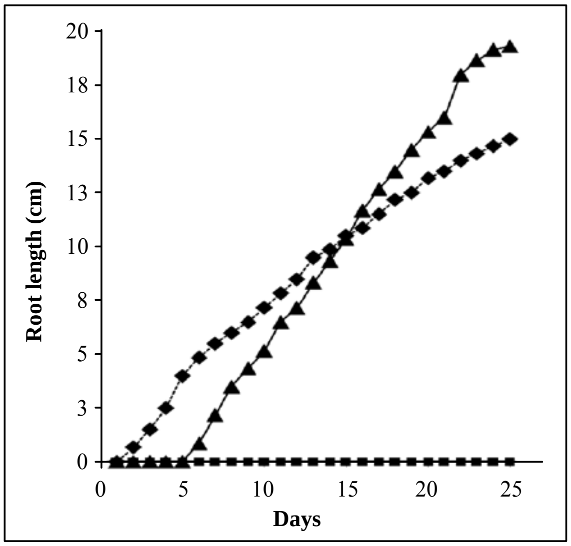

Fig. 4: Root growth of onion bulb

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	0.00	0.00
6	4.83	0.85	0.00	0.00	0.83	0.62
7	5.50	0.82	0.00	0.00	2.17	0.24
8	6.00	0.71	0.00	0.00	3.50	0.71
9	6.50	0.71	0.00	0.00	4.33	1.18
10	7.17	0.62	0.00	0.00	5.17	0.94
11	7.83	0.85	0.00	0.00	6.50	1.22
12	8.50	0.71	0.00	0.00	7.17	1.03
13	9.50	0.71	0.00	0.00	8.33	0.62
14	9.83	0.94	0.00	0.00	9.33	1.03
15	10.50	1.08	0.00	0.00	10.33	1.03
16	10.83	0.94	0.00	0.00	11.67	0.62
17	11.50	1.08	0.00	0.00	12.67	0.62
18	12.17	1.18	0.00	0.00	13.50	0.41
19	12.50	1.08	0.00	0.00	14.50	0.41

Cont…

Days	Root length (cm)					
	Control		Untreated		Treated	
	Mean	SD	Mean	SD	Mean	SD
20	13.17	1.18	0.00	0.00	15.33	0.24
21	13.50	1.08	0.00	0.00	16.00	0.41
22	14.00	1.08	0.00	0.00	18.00	0.41
23	14.33	1.03	0.00	0.00	18.67	0.62
24	14.67	1.25	0.00	0.00	19.17	0.62
25	15.00	1.22	0.00	0.00	19.33	0.85

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	0.00	0.00
6	3.67	0.47	0.00	0.00	0.67	0.62
7	4.67	0.47	0.00	0.00	1.33	0.47
8	5.00	0.00	0.00	0.00	2.67	0.47
9	5.00	0.00	0.00	0.00	4.33	0.47
10	5.33	0.47	0.00	0.00	6.00	0.82
11	6.67	0.47	0.00	0.00	7.00	0.82
12	7.67	0.47	0.00	0.00	8.00	0.82
13	8.00	0.00	0.00	0.00	8.67	0.47
14	9.00	0.00	0.00	0.00	9.33	0.47
15	9.00	0.82	0.00	0.00	10.00	0.82
16	10.00	0.00	0.00	0.00	11.00	0.82
17	11.33	0.47	0.00	0.00	11.67	1.25
18	11.67	0.47	0.00	0.00	12.67	1.25
19	13.00	0.82	0.00	0.00	14.00	1.41
20	13.67	0.47	0.00	0.00	15.00	0.24
21	14.67	0.47	0.00	0.00	16.00	1.41
22	15.00	0.82	0.00	0.00	16.33	1.70
23	15.00	0.82	0.00	0.00	16.67	1.89
24	15.00	0.82	0.00	0.00	17.00	1.41
25	15.00	0.82	0.00	0.00	17.33	1.70

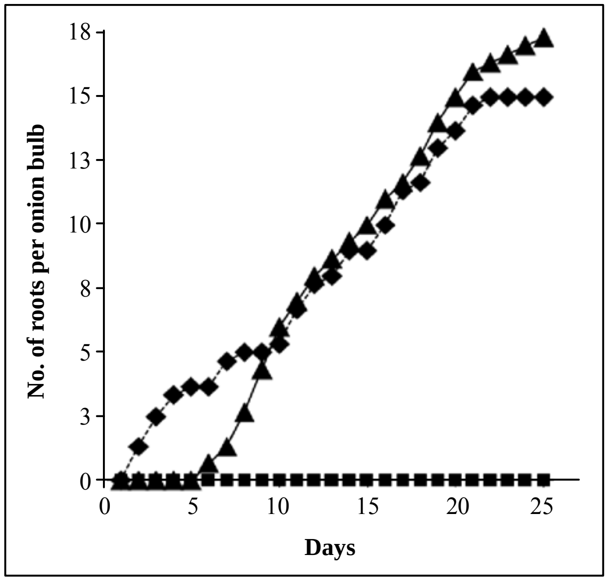

Fig. 5: Root density of onion bulb

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

Table 5: Chlorophyll contents of onion shoot

Sample	Chlorophyll content (mg/g)		
	Chl a	Chl b	Total Chl
Control	0.18	0.17	0.36
Treated	0.26	0.15	0.41

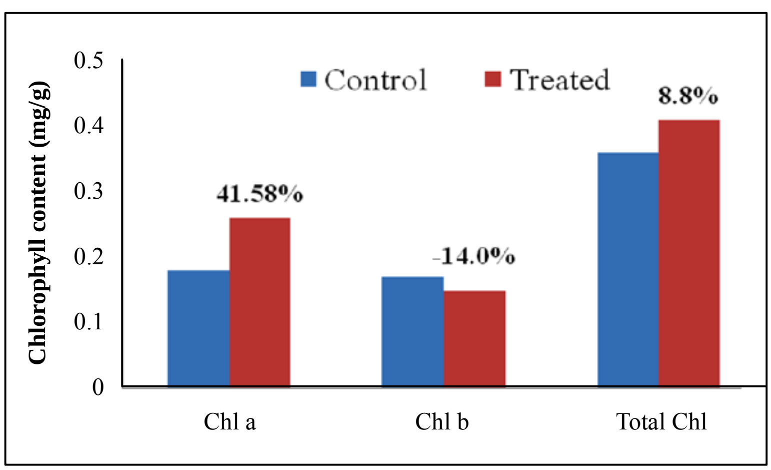

Fig. 6: Chlorophyll contents of onion shoot

There is an increase in Chl a and total chlorophyll content of shoot of onion bulbs grown in treated dye solution, but a decrease in Chl b was observed.

Table 6: Sugar and protein contents of onion shoot

Sample	% Sugar content	% Protein content
Control	9.35	2.83
Treated	18.30	2.10

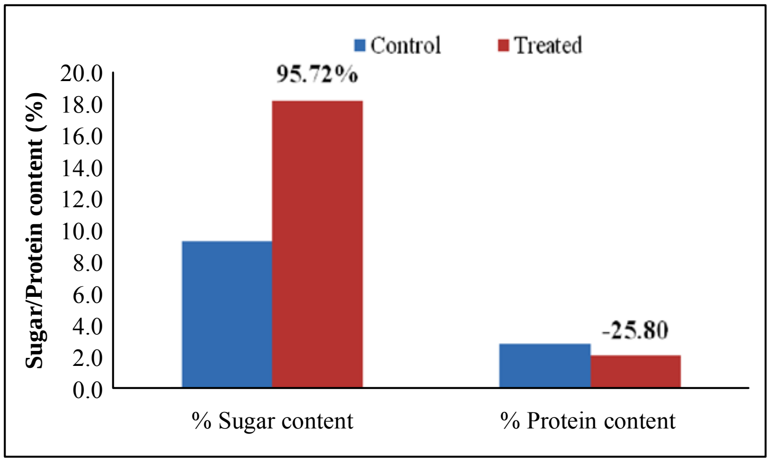

Fig. 7: Sugar and protein contents of onion shoot

There is an increase in the sugar content of shoot of the onion bulb grown in treated dye solution. However, a slight decrease in the protein concentration was observed.

Table 7: Sugar and protein contents of onion root

Sample	% Sugar content	% Protein content
Control	1.09	1.20
Treated	2.45	2.30

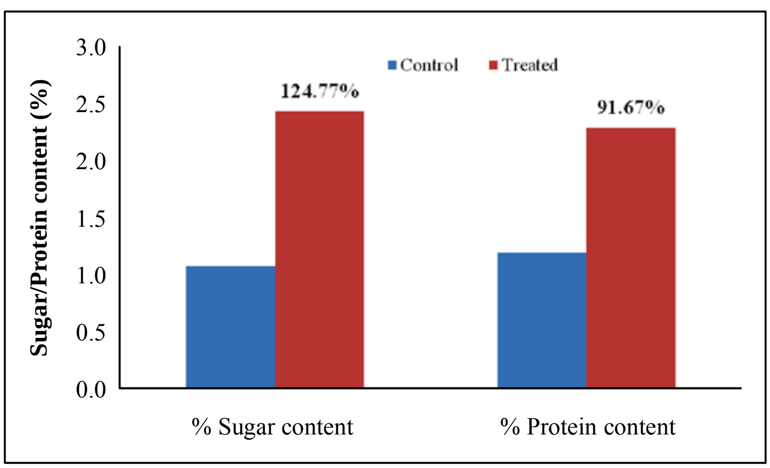

Fig. 8: Sugar and protein contents of onion shoot

An increase in the sugar and protein content of root of the onion bulb grown in treated dye solution was seen.

CONCLUSION

Shoot and root growth and density

When onion bulbs were grown in untreated and photocatalytically treated orange G solutions, no root and shoot growth was observed in onion bulbs placed in untreated dye solution. But good growth was observed in onion bulb grown in treated dye solution. This repeats the findings of earlier experiments that, the presence of dissolved dyes in solution is detrimental for plant growth and development. However, when onion bulbs were placed in TiO_2 treated Orange G solution good growth of root and shoot was observed. Profuse rooting and shoot growth was observed. There was 7.53% increase in shoot length and 44.42% increase in shoot density of the onion bulbs grown in treated Orange G solution as compared to that of onion bulbs grown in control.

Similarly, there was 28.89% increase in root length and 15.56% increase in root density of the onion bulbs grown in treated Orange G solution respectively, as compared to control.

The presence of dye molecules in the solution is harmful for onion bulb growth, but in photocatalytically treated solution, the same molecules are converted in simpler inorganic counterparts which promote root and shoot growth. Not only there is increase in length but also increase in number of roots and shoots in onion bulbs grown in photocatalytically treated Orange G as compared to the onion bulbs grown in control solutions.

Chlorophyll contents of shoot

The chlorophyll contents of the shoot of onion bulbs grown in treated was compared with that of control group. It was observed that there was an increase in individual Chl a, Chl b and total chlorophyll contents of shoots of onion bulb grown in treated orange G dye solution. The increase was by 41.58% in Chl a and by 8.8% in total chlorophyll contents. However, there was a decrease in Chl b by 14.0%.

Sugar and protein contents of shoot

95.72% increase in sugar content and 25.8% decrease in protein content were observed in the shoot of onion bulbs grown in treated orange G solution.

Sugar and protein contents of root

124.77% increase in sugar content and 91.67% increase in protein content were observed in the root of onion bulbs grown in treated orange G solution.

Thus, effluent containing dye water can be reused for irrigation purposes after suitable treatment. In monsoon dependent countries like India, reuse of waste water for irrigation of various crops is very effective method for fulfilling our water and food demands. From the results of current investigations, it can be concluded that waste water from dyeing industries can be successfully used, but only after proper photocatalytic treatment.

REFERENCES

1. Ratna and B. S. Padhi, Int. J. Environ. Sci., **3(3)**, 941-955 (2012).
2. Y. Slokar, L. Majcen and A. Marechal, Dyes Pigm., **37(4)**, 335-356 (1998).
3. M. N. V. R. Kumar, T. R. Sridhar, K. D. Bhavani and P. K. Dutta, Colorage, **40**, 25-34 (1998).

4. R. W. Matthews, Water Res., **20(5)**, 569-578 (1986).
5. R. Ameta, C. Kumari, C. V. Bhatt and S. C. Ameta, Ind. Quim., **33**, 36-39 (1998).
6. A. Ozkan, M. H. Ozkan, R. Gurkan, M. Akcay and M. Sokmen, J. Photochem. Photobiol. A: Chem., **163(1-2)**, 29-35 (2004).
7. Y. Mu, H. Q. Yu, J. C. Zheng and S. J. Zhang, J. Photochem. Photobiol. A: Chem., **163(3)**, 311-316 (2004).
8. N. Daneshwar, D. Salari and A. R. Khataee, J. Photochem. Photobiol. A: Chem, **162(2)**, 317-322 (2004).
9. R. Ameta, J. Vardia, P. B. Punjabi and S. C. Ameta, Indian J. Chem. Tech., **13**, 114-118 (2006).
10. S. C. Ameta, P. B. Punjabi, S. Kothari and A. Sancheti, Polln. Res., **22(3)**, 389-392 (2003).
11. N. Puvaneswari, J. Muthukrishnan and P. Gunasekaran, Indian J. Exper. Biol., **(44)**, 618-626 (2006).
12. M. R. Mehta, N. Goyal, K. N. Salvi and V. P. Prajapati, Acta. Chim. Pharm. Indica, **2(2)**, 95-100 (2012).