

Impact of Textile Dyeing Effluents on Germination and Seedlings of Country Beans (*Lablab niger* var. *typicus*)

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Abstract

An experiment has been completed on the impact of dying effluents on germination and seedlings of country beans (*Lablab niger* var. *typicus* Medikus, Fabaceae). Seven types of dyeing effluents have been taken as treatment variables, and their effects on the germination of country beans at the early growth stage compared. The treatment of neutralized effluent water (D₅) enhanced the germination percentage remarkably (100%) as well as statistically identical with the result of underground water (D₁) whereas the treatments of effluent from 2nd wash after Bath (D₄) and mixed effluent from ETP showed poor germination (73.33%). Second wash after scouring and bleaching treatment (D₂) showed a good result. All results have been considered on the basis of germination energy, relative germination rate, effluents injury rate and germination velocity. Neutralized effluent water (D₅) performed better at the seedling growth period of country bean plants. But D₄ treatment performed very poorly among all parameters. So, it can be suggested that, neutralized effluent water should be applied as irrigation water for the purpose of crop production as well as country bean cultivation and a sustainable environment.

Key words: Country bean/ Effluents/ Germination/ Seedling growth/ Textile

1. Introduction

Country bean (*Lablab niger* var. *typicus*) is one of the most ancient types of bean among cultivated plants and is presently grown throughout the tropical regions of Asia, Africa and America. It is

cultivated either, exclusively or mixed with other crops, such as finger millet, groundnut, castor, corn or sorghum. It is also grown in home garden areas. It is a multipurpose crop grown for pulse, vegetable and forage. The crop is mainly grown for its green pods, while the dry

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seeds are used in various vegetable preparations. It is one of the major sources of protein in the diet of South India. *Leguminous crops* like country beans play a vital role to meet the protein requirement. Beans contain 20-30% protein on a dry weight basis which is nearly three times than that of most other cereals. In Bangladesh, the total land area under bean cultivation is 15385 hectares and the production was 83,000 metric tons during 2006-2007 (BBS, 2008). It also contains appreciable amounts of thiamin, riboflavin, niacin, vitamin C, and iron 0.1, 0.06, 0.7, 9.0, and 41.7 mg/100 gm, respectively (Rehana, 2006). Periphery and urban areas are used by farmers for cultivation of country bean vegetables in Bangladesh.

Textile dying effluents are discharged in agriculture crop areas without treatment. Treating waste effluents is a significant factor for the cultivation of crops and environment. Moreover, the economy of Bangladesh is predominantly based on agriculture but, in the race towards industrialization, industry is expanding at an ever increasing pace. The major industries are; textiles, leather tanning, fertilizer production, sugar refining, chemical, pharmaceutical, and oil refining. Among

these industries, textile factories are rapidly expanding day by day. There are 1821 small and large knit dyeing industries in Bangladesh (BKMEA, 2012). These industries are a major source of effluent due to the nature of their operations which requires high volumes of water that eventually results in high wastewater generation. The most common textile wet processing setup consists of; desizing, scouring, bleaching, mercerizing and dyeing, as well as the finishing process. Dyeing is the process of adding color to the fibers, which normally requires a large volume of water. It has been estimated that for dyeing, 1 Kg of cotton with reactive dyes requires an average of 70-150 L water (Chakraborty *et al.*, 2003). The scouring effluents are strongly alkaline. Dyes are carbon based organic compounds while pigments are normally inorganic compounds often involving heavy toxic metals (i.e. chromium, copper zinc, lead or nickel). Most of the dyeing factories discharge their effluent into the environment either, after partial treatment or no treatment at all. The utility of municipal and industrial waste water for irrigation to crops is well documented (Singh *et al.*, 2006; Nath *et al.*, 2007). The use of such wastewater in irrigation systems definitely provide some

nutrients to enhance the fertility of soil but it also deposits toxicants that change soil properties in the long run. Furthermore, it also causes phyto-toxicity resulting from the intoxication of living tissues by substances accumulated from the growth medium (Chang *et al.*, 1992). However, the adverse effect of textile effluents on plants depends on the type of species, and the types and concentrations of toxic materials in the effluent.

Mehta and Bhardwaj (2012) described the effect of industrial effluents on seed germination and seedling growth of *Vigna radiata* and *Cicer arietinum*. Germination percentage and seedling growth of both plants showed considerable reduction in the case of untreated effluents. Root and shoot length of *Vigna* seedling were reduced by 58.66% to 69.06% respectively, while in *Cicer* the reduction was between 53.62% and 67.91% in untreated effluent. Minimum reduction in root and shoot length was observed in treated effluent in both *Vigna* and *Cicer*. Maximum phytotoxicity was observed in untreated effluent of *Vigna* and *Cicer*. Treated effluent showed minimum phytotoxicity. Seed germination and plant growth bioassays are the most common techniques used to evaluate phytotoxicity

(Kapanen and Itäavaara, 2001). Such types of work have been performed in a scattered way in many countries of the world rather than those in our country, for instance, industrial effluent reduced the germination percentage of kidney beans (*Phaseolus aureus* Rexburg, Fabaceae) and ladies' fingers (*Abelmoschus esculentus* L. Moench, Malvaceae) (Mohammad and Khan, 1985). While *Cicer arietinum* L. (Fabaceae), even highly diluted industrial effluent (5% of industrial effluent) adversely reduced the seed germination (Dayama, 1987). In contrast, 50% diluted textile effluent increased the seed germination, total sugars, starch, reducing sugars and chlorophyll of groundnut seedlings (Swaminathan and Vaidheeswaram, 1991). Similarly, the effect of dye factory effluent was studied with respect to germination and growth of Bengal gram *C. arietinum*. In lower concentrations, the germination percentage and growth are relatively higher than the control, but a gradual decrease of germination and seedling growth occurs with an increase in effluent concentration. The best germination, seedling growth, number of root nodules, yield and biochemical attributes was observed in 20% concentration with growth promoting

effects and significantly better than the control sample. Beyond 20% effluent, root and shoot length decreased. Thus the dye factory effluent that should be safely used for irrigation purposes with proper treatment and dilution is at 20% (Kathirvel, 2012). However, with the advent of modern wastewater treatment processes in the early 20th century, industrialized countries began to establish regulatory frameworks for treatment of wastewater for the purpose of irrigation & the environment. These frameworks continued to evolve over time, but still rely heavily on capital-intensive wastewater treatment as the principal intervention for protecting public health and the environment. Developing countries like Bangladesh lack the financial and institutional capacity to build and operate sophisticated wastewater treatment facilities; indeed, universal wastewater treatment has still not been achieved in many industrialized countries due to financial constraints. So, alternative complementary solutions are needed to maintain the sustainability of agriculture through reutilizing discharged wastewater from industries after taking into consideration the benefit to the environment, crops, and public health as well. Moreover, in many regions, as

freshwater sources become scarcer, wastewater use has become an attractive option for conserving and expanding available water supplies. In principle, wastewater might be used for all purposes after being given appropriate treatment. Because Country bean (*Lablab niger* var. *typicus* Medikus, Fabaceae) is one of the most popular legume vegetables grown in Bangladesh, it is very often cultivated in effluent discharge areas. The neutralized effluent water produced better germination and seedling growth of country bean. It had no impact on the sustainable environment. The present study suggested that neutralization stages of effluent water should be applied for irrigation purposes for crop production as well as country bean cultivation and a sustainable environment.

2. Materials and Methods

2.1 Collection of Textile Effluents

Different stages of textile dying effluents were collected in pre-cleaned plastic bottles. The effluents were stored at 4 °C to avoid changes of their physiochemical properties. Various physio-chemical characteristics were analyzed at the laboratory of Dhaka University of Engineering Technology

(DUET), Gazipur, Bangladesh. pH and temperature of wastewater were determined by portable HACH pH meter following the procedure of Quirk & Schofield (1955). Biochemical oxygen demand (BOD) was measured by dilution method (APHA, 1998). Dissolved oxygen (DO), Chemical oxygen demand (COD) and chloride were measured by chemical, dichromate digestion and Mohr's method, respectively. Total Suspended solid (TSS) was measured gravimetrically while suspended solids (SS) was obtained by subtracting the TDS from TS. Other parameters such as color, nitrate (NO_3^-), sulphate (SO_4^{2-}), phosphate (PO_4^{3-}) were detected by Spectrophotometer. Electrical conductivity was also determined by conductivity meter (EC150, HACH). Heavy metals of Cu, Co, Cr, Ni, Cd, Pb and Zn were detected by Atomic absorption spectrophotometer (SPECTRA A.A-55B, VARIAN, Australia) as per standard methods.

2.2 Germination Experiments

The germination test was conducted following the prescribed method in ISTA rules (Anonymous, 1999) in the Department of Horticulture, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh. All seeds

were surface-sterilized with 1% sodium hypochlorite (NaOCl) for one minute to prevent any fungal contamination, thereafter the seeds were washed with distilled water. Germination was recorded daily at a fixed hourly rate and the emergence of radical was taken as a criterion of germination. Germination percentage, germination energy, germination index, relative germination rate and relative effluent-injury rate were recorded (Table 3).

2.3 Pot Experiment

Four hundred viable seeds of country bean were randomly selected from the stock. Twenty seeds were sown in each of four plastic pots (10 cm diameter and 10 cm height) and filled with required ordinary garden soil and washed well by tap water and then pour distilled water as to flush through all the salts that were present in the soil. The pots were irrigated with underground water as the control treatment and seven different stages of dying effluent. 250 ml. of each stage of effluent were applied. This experiment was prolonged up to the standard age of transplantation (third leaf stage). For observation of growth, seedlings were picked from each of the poly bags and then; length of the root; shoot, leaf length, leaf width, as well as

dry fresh weight were recorded at the termination of the experiment. Then individual plants were picked up and kept at 70 °C in oven for 3 days. Then their dry weights were recorded. This process was replicated three times and the experiment was laid out following the randomized complete block design (RCBD). The values were subjected to one-way analysis of variance (ANOVA) and DMRT for comparison of means to determine statistical significance. Statistical analysis was performed using MSTAT-C program.

3. Results

3.1 Physio-chemical parameters of textile dying effluents

Physio-chemical parameters of textile dying effluents have been detected at the laboratory. The pH range of effluent samples were within permissible limits of 6.8 to 8.8, except second wash after scouring & bleaching and mixed effluent from ETP. Chemical oxygen demand (COD) of underground water was below that of the standard limit but others were above. A similar trend was also found for BOD results except, tube well water (underground water), second wash water after scouring & bleaching and second wash after soaping. Electrical

conductivity exceeded standard limits as the treatment of second wash after BD and mixed effluent from ETP. A similar result was found with the chloride content. Total Dissolved Solid (TDS) was found to be within irrigation standard except, 2nd wash after BD and mixed effluent samples. Total Suspended Solid (TSS) was lower than the maximum samples than irrigation standard. Moreover, other element contents such as sulphate, nitrate, phosphate, lead, copper, nickel, cobalt, chromium and zinc were within the standard limits for irrigation purpose (Table 1).

3.2 Germination percentage at DAS

Germination% of seeds was recorded at specific times of the day. During this time, seeds were considered germinated when their radical length was more than 3 mm. Counting continued to count more germinated seeds and the results of different days of observation were converted into percentage. The highest percentage of germination was observed in D₂ treatment at three days after sowing and the lowest was in D₄ treatment which was identical with D₈ treatment for the same date of observation and the figures were 33.33 and 13.33% accordingly. On the other hand, after four

days of sowing, the highest result was found from D₂ treatment which was similar with D₆ (66.67%). Treatment D₄ showed the lowest result (26.67%). Moreover, after five days of sowing, the

highest (D₅) and lowest (D₄) germination percentage was found (93.33%) and 53.33%, successively. A similar trend was also observed after six days of observation (Figure 1).

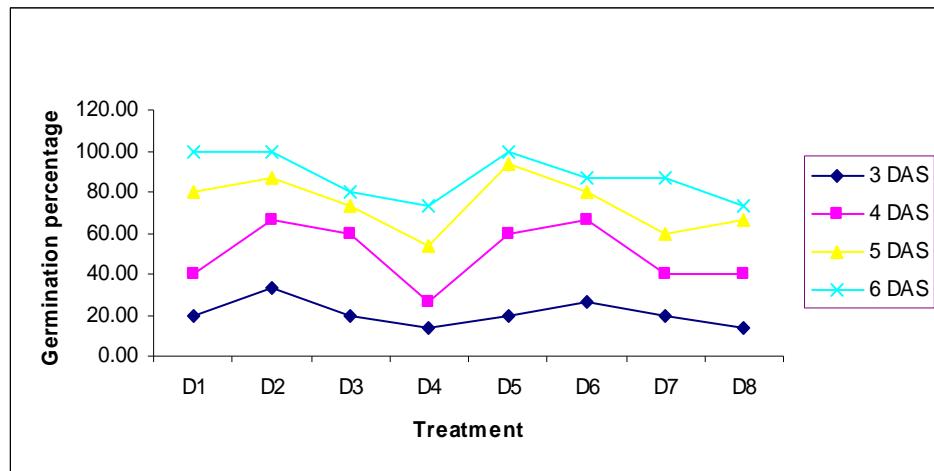
Table 1: Physio-chemical parameters of dying effluents

Parameters	Irrigation std. (NEQS)	Treatments								Mixed effluent from ETP
		D ₁	D ₂	D ₃	D ₄	D ₅	D ₆	D ₇	D ₈	
pH	6-9	6.8	9.4	6.3	8.8	7.1	7.4	7.3	9.5	
DO (mg/L)	4.5-8	4.1	5.85	6.12	4.58	5.0	5.80	4.77	0.19	
Color (pt.co-unit)	7	16	97	67	477	367	171	348	1038	
TDS (mg/L)	2100	300	910	650	2170	1840	470	1290	3320	
TSS (mg/L)	200	30	40	50	60	60	40	40	310	
E. conductivity	1200	350	850	900	1350	550	480	700	4200	
BOD (mg/L)	250	57	35	Nil	411	Nil	214	350	900	
COD (mg/L)	400	310	454	1374	450	525	630	199	478	
Nitrate (NO₃) (mg/L)	10	ND	0.8	0	0.6	0	0.3	0.8	0.8	
Phosphate (PO₄³⁻)(mg/L)	15	ND	0.52	0.81	0.23	0.27	0.19	1.06	0.40	
Sulphate (SO₄²⁻) (mg/L)	1000	ND	9.0	1.0	44	0	1.0	1.0	80	
Chloride (Cl) (mg/L)	1000	31	08	05	2500	58	64	42	2700	
Copper (Cu) (ppm)	1.0	ND	ND	ND	0.299	0.681	0.005	0.006	0.111	
Cobalt (Co) ppm	NGVS	ND	ND	ND	0.369	0.138	ND	ND	0.255	
Zinc (Zn) ppm	5	ND	ND	ND	0.914	0.192	0.316	0.384	0.354	
Lead (Pb) ppm	0.5	ND	ND	ND	0.0236	0.008	0.0232	0.0192	0.0263	
Ni ppm	1.0	ND	ND	ND	0.0045	0.0027	0.0229	0.0262	0.0237	
Cr ppm	1.0	ND	ND	ND	0.0168	0.0039	0.0326	0.0406	0.0306	
Cd ppm	0.1	ND	ND	ND	0.0046	0.0008	0.0022	0.0026	0.0078	

Note: ND = Not determined

NEQS = (National Environment Quality Standards)

NGVS = No guideline value set

**Figure 1:** Germination percentage at DAS

3.3 Germination attributes of seed

Germination attributes of country bean were affected by textile dyeing effluents. The maximum seed germination has been recorded in the treatment of D₅ and D₂ that were statistically identical with the control treatment. The minimum was at D₄ and D₈ as compared to others and the values were 100% and 73.33%, respectively. D₂ considered as an efficient treatment as

compared to others whereas estimated to 0.62, 1.00, 1.00 and 61.78 of germination race, germination energy, relative germination rate and germination index, accordingly. Eventually, statistically more or less similar results were found within the treatment of D₅ and D₄. Significantly lower values for all parameters of seed quality were observed (Table 2).

Table 2: Effect of dyeing effluents on seed quality of country bean

Treatment	Germination %	r	GE	RGR	GI
D ₁	100.00a	0.49abc	1.00a	1.00a	49.33abc
D ₂	100.00 a	0.62a	1.00a	1.00a	61.78a
D ₃	80.00b	0.50abc	0.80b	0.80b	49.67abc
D ₄	73.33 b	0.34d	0.73b	0.73b	34.00d
D ₅	100.00 a	0.57ab	1.00a	1.00a	57.00ab
D ₆	86.67 ab	0.56ab	0.87ab	0.87ab	56.00ab
D ₇	86.67 ab	0.43bcd	0.87ab	0.87ab	43.11bcd
D ₈	73.33 b	0.38cd	0.73b	0.73b	40.11cd
CV (%)	11.70	16.33	11.70	11.70	15.43
SE \pm	5.91	0.05	0.06	0.06	4.35

Note * same letter present within the column shows non significant at 5% level.

r = coefficient velocity of germination, GE = Germination Energy, RGR = Relative Germination Rate, GI = Germination Index.

3.4 Relative effluent injury rate

The germination process of country bean was greatly affected by different effluent treatments compared with the control treatment of ground water. The germination of treated seeds with D₃, D₄, D₆, D₇ and D₈ treatment were highly affected

whereas zero injuries were found in the treated seeds of D₂ and D₅ (Figure 2). Consequently, the germination percentage of D₂ and D₅ treated seeds were the highest. The treatment of D₄ & D₈ effluent stage possessed the lowest germination ranking followed by the others.

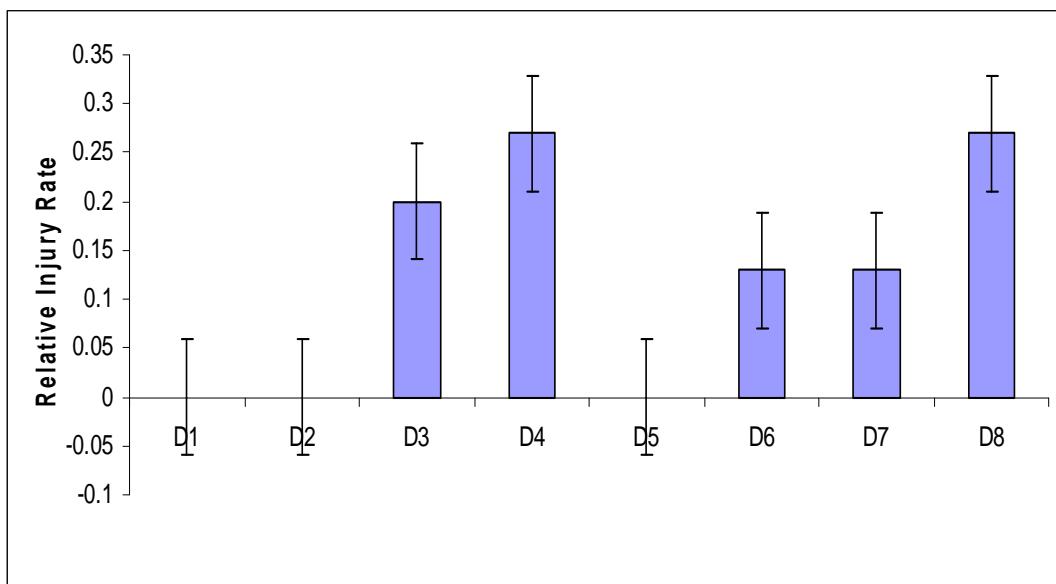


Figure 2: Effect of dying effluents on relative injury rate.

3.5 Seedling growth parameters

A significant difference in shoot length, fresh weight and dry weight of individual seedlings were observed, whereas the rest of the characteristics showed statistically insignificant results. The highest value of shoot length (23.47 cm), root length (12.96 cm), seedling length (35.48 cm), fresh weight per plant (0.46 g), dry weight per plant (0.15 g), water percentage (78.31%) and dry matter percentage (37.56%) were recorded for

the treatments of D₅, D₆, D₅, D₄, D₈, D₇ and D₈, successively. On the contrary, the lowest results were produced by D₇, D₁, D₇, D₂, D₅, D₈ and D₄ accordingly and these constituted as 17.26 cm, 10.18 cm, 28.04 cm, 0.23 g, 0.07 g, 62.44% and 22.13%, respectively for the same attributes. However, in general among the treatments D₅ performed better than that of other treatments (Table 3).

Table 3: Effect of dyeing effluents on seedling growth

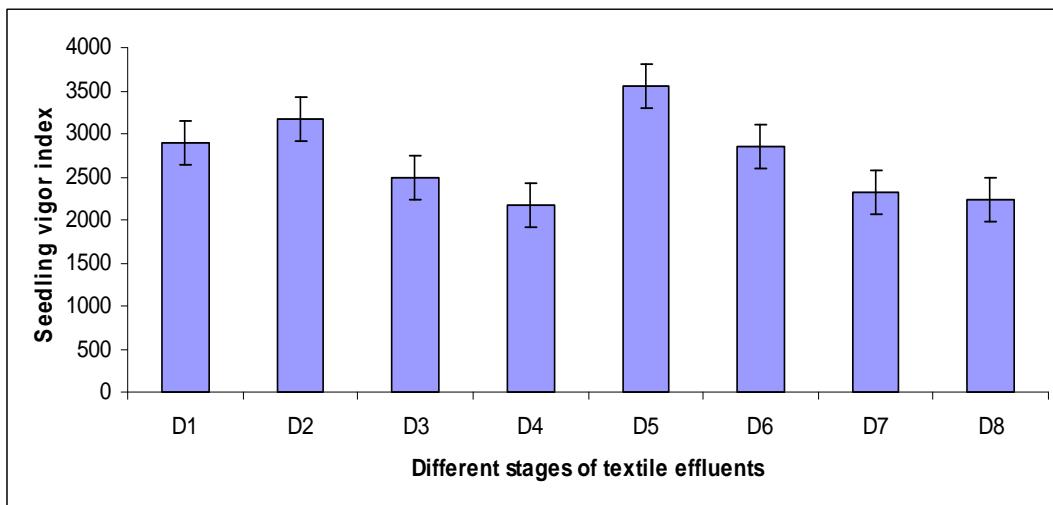
Treatment	SL (cm)	RL (cm)	Seedling length (cm)	FW (g)	DW (g)	W (%)	DM (%)
D ₁	18.81ab	10.18ns	28.99	0.28ab	0.09ab	66.33	33.67
D ₂	20.48ab	11.19 ns	31.67	0.23b	0.08b	64.06	35.94
D ₃	19.85ab	11.38 ns	31.22	0.42ab	0.12ab	71.06	28.94
D ₄	17.26ab	12.11 ns	29.90	0.46a	0.09ab	77.87	22.13
D ₅	23.47a	12.01 ns	35.48	0.30ab	0.07b	75.06	24.94
D ₆	19.69ab	12.96 ns	32.65	0.43a	0.12ab	70.47	29.53
D ₇	17.79ab	10.78 ns	28.04	0.40ab	0.08b	78.31	21.69
D ₈	18.7ab	11.31 ns	30.05	0.39ab	0.15a	62.44	37.56
CV (%)	15.36	16.87	13.57	26.83	35.13	12.89	31.10
SE±	1.73	1.12	2.43	0.06	0.02	5.26	5.26

Note *same letter present within the column shows non significant at 5% level.
 SL= Shoot length, RL= Root length, FW= Fresh weight, DW= Dry weight, W%= Water percentage, DM % = Dry matter percentage

3.6 Seedling vigor index

Seed vigor index has been observed where a significant difference exists among the different stages of textile waste water (Figure 3). The highest seed vigor index was

observed in D₅ treatment (3548) whereas the lowest was in D₄ treatment (2175). However, in the neutralizing stage of textile dyeing (D₅) effluent showed a 22.38% higher seed vigor than that of the control treatment (2899).

**Figure 3:** Effect of dyeing effluents on seed vigor of country bean.

3.7 Leaf length and width

Although the leaf length and width did not vary significantly with different wastewater, the highest leaf length and width of country beans were found in D₃ treated

plants followed by others (Figure 4). Looking at the other samples, it is clear that, D₄ treated seed produced the smallest leaf length and width.

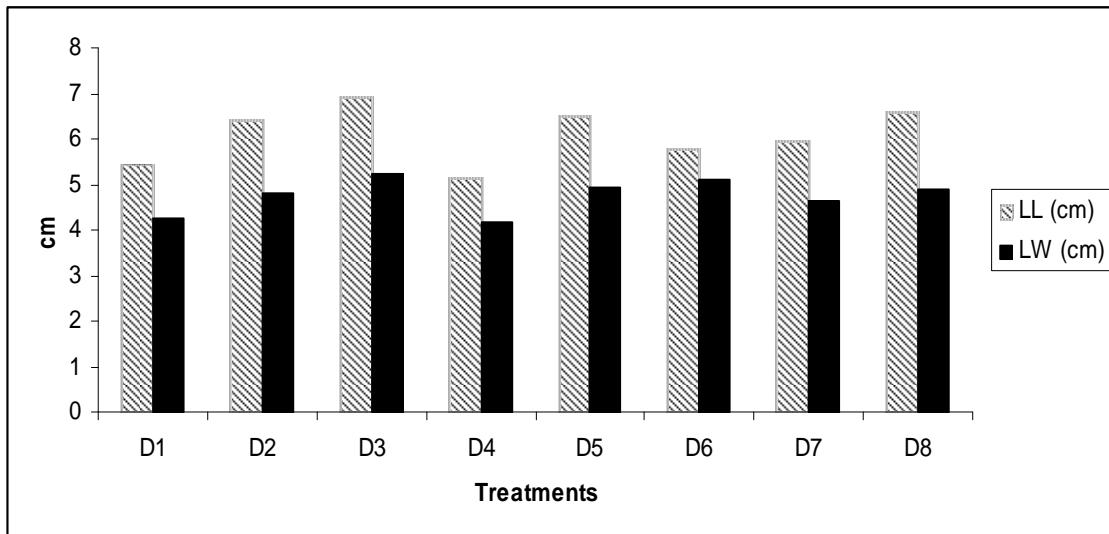


Figure 4: Leaf length and width of effluent affected country bean

4. Discussion

Dying effluents are one of the major threats for crop production. These effluents contain trace metals, high value of BOD, TDS, TSS, COD & DO that threaten germination and seedling growth of country bean vegetables. Dying effluent was collected at different stages. Neutralization stages of effluent did not constrain germination and seedling growth of country beans. Most of the stages of dying effluent were a threat to country bean production (Table 1 & Figure 1, 2 3). In this context, shoots and seedlings vigor were affected. Some researchers found that germination is directly affected by the presence of higher salt/metal concentrations (Baruah and Das, 1997), while others described the

inhibitory (Crowe *et al.*, 2002) as well as stimulatory (Yousaf *et al.*, 2010) effects of various effluents on the germination of a number of plant species. Considering the fact, that higher concentration of effluent decreases enzymatic activities are considered as one of the causes of biochemical changes which might disrupt germination and seedling growth (Murkumar and Chavan, 1987). The application of textile dyeing wastewater into soil for irrigation purposes raises the soil pH, EC and SAR values which reduce nutrient uptake by vegetative growth of plants. Consequently, it reduces the growth rate and results in smaller leaves, shorter height, and sometimes fewer leaves. On the contrary, physio-chemical properties such as Cu, Zn, Fe, Mn, Cd and Pb were present in varying concentrations in the

dying effluents. These metals restricted plant physiological and growth processes. Unsatisfactory growth appeared due to irrigation water with metal contaminants in sunflower plants (*Helianthus annuus* L., Asteraceae) (Andaleeb *et al.*, 2008).

However, sulphate, phosphate and nitrate contents salts were present in dying effluents. But the range was below that of the standard limit in these samples (Table 1). These type of salts are constraints for seed germination and growth. Similarly, inhibition of seed germination in untreated effluents due to greater amount of dissolved solids that increases the salinity and conductivity of the absorbed solute by seed before germination (Gautam *et al.* 1992; Singh *et al.* 2006). Higher salt content of untreated effluent also changes the osmotic potential outside the seed thereby reducing the amount of water absorbed by the seed which results in retardation of seed germination (Adriano *et al.*, 1973). Increase of germination percentage in treated effluent due to lower salt concentration that has created favorable environmental conditions for germination and utilization of nutrients present in the effluent (Kannan and Oblisamy, 1992; Indra and Ravi, 2009).

The major adverse and toxic effects on the growth performance and yield of certain vegetables i.e. spinach, lettuce, carrot, radish and sugar beet, due to presence of higher concentration of pH, SAR value and heavy metals in municipal wastewater (Tamoutsidis *et al.*, 2002). Ahmed *et al.*, (2011) also reported that higher concentrations of heavy metals in wastewater are potent in retarding plant growth and development and adversely affect the yield. Disturbance of nutrient uptake and metabolism due to increased metal content in the growth environment, caused reduction in overall growth of many plants has been observed (Panda and Chowdhury, 2005). Similarly, reduction in seedling (root and shoot) lengths with elevated amounts of total dissolved solids at higher concentrations. This could also be related to the fact that some of the nutrients present in the effluents are essentials but at above the levels of a particular concentration, they become hazardous. Tannery effluents caused a reduction in germination, growth of sunflower parameters along with other parameters like chlorophyll content, protein, carbohydrate content, etc. (Hussain *et al.*, 2010).

Besides, shoot and root weight of *Amaranthus gangeticus* L. (Amaranthaceae) decreased significantly when Pb was applied above 40 and 60 mg.kg⁻¹, respectively, from that of the control (Kibria et al., 2009). The decreased shoot and root biomass of *Spinacia oleracea* L. (Amaranthaceae) might be due to interference of Pb with physiological processes of the plant. Lead phytotoxicity involves inhibition of enzyme activities, disturbed mineral nutrition, water imbalance, and change in hormonal status and alteration in membrane permeability (Sharma and Dubey, 2005). These disorders upset normal physiological activities of the plant resulting in low productivity. The reduction of biomass by Pb toxicity could be direct consequences of the inhibition of chlorophyll synthesis and photosynthesis (Chatterjee et al., 2004). Textile industries are major sources of these effluents due to the nature of their operations which requires high volumes of water that eventually result in high waste water generation (Ghoreishi and Haghishi, 2003). Industrial effluents possess various organic and inorganic chemical compounds. The presence of these chemicals will cause detrimental effects on the development of plants, germination process and growth of seedlings.

Therefore, neutralization of effluent water should be considered for crop production, reuse and sustainable livelihoods. Therefore, it is necessary to study the impact of effluents on crop systems before being recommended for irrigation (Thamizhiniyan et al., 2009). In this respect, continuous research on the hazards of effluent will play a significant role for sustainable environments.

5. Conclusions

It can be concluded that physico-chemical characteristics of the effluents that exceeded the standard limit have a negative impact on germination and seedling growth. But neutralized effluent water does not have a negative impact on the germination percentage, germination energy, relative germination rate and relative effluent injury rate of country bean seeds, or its seedling growth. Thereby, disposal of these effluents through proper treatment might have a positive result from the view of producing a sustainable environment. Consequently, farmers who are adjacent to textile industry areas will benefit by using treated effluent for production of crops that will minimize the use of fresh

underground water for dying and other purposes.

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