Impact of Dye Effluent on Seed Germination, Seedling Growth and Chlorophyll Content of Soybean (*Glycine max* L.)

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Abstract: ***Textile dyeing industry is one of the major water consuming and high polluting industries in India. Untreated industrial effluent discharged into ecosystem pose a serious problem to the aqua living organisms, plants and human being. Effluent in higher concentration affects the soil and causes heavy damage to crop growth. In the present study effects of dyeing effluent on seed germination and seedling growth of soybean (Glycine max L.) has been carried out at different concentration (20, 40, 60, 80, 100%) of the effluent collected from dyeing industries at Kaithun region, Kota and control was maintained. Seed germination percentage of Glycine max was not significantly affected at 20% treatment level where as higher concentration of dyeing effluent negatively affects the germination percentage of seeds. Seeding growth (Shoot and Root lengths) and Chlorophyll content (Chlorophyll a and b) have found to increase at 20% and 40% of treatment level. The root length was found to increase 4.4% and 2.2% and shoot length was found to increase 1.5% and no change at treatment level 20% and 40% respectively than control. Due to increase in concentration of effluent at 60% treatment level and above a gradual decline of seedling growth was observed. At 100% treatment level root length and shoot length were decrease 57.7% and 58.7% respectively in comparison to control. Chlorophyll content was also adversely affected due to higher concentration of dyeing effluent. The present study revealed that Glycine max L. (soybean) is susceptible to dyeing industrial effluent.***

Keywords: Dye Industrial Effluent, Physico-chemical analysis, Soybean seeds, Germination percentage, Seedling growth, Chlorophyll a and b

1. Introduction

The unplanned rapid industrial growth is the main cause for the environmental pollution. Each industry is associated with an emission of many pollutants. Textile industries are large industrial consumers of water as well as producers of waste water with the increased demand for textile products. The most potential and hazardous source of water and soil pollution are industrial effluents. One major group of contaminants in effluent is dyes from dying and printing industries which make it aesthetically unacceptable and toxic depending on the chemicals. The waste water from textile dying industries is a complex mixture of many polluting substances ranging from organo-chloride based waste to heavy metals associated with dye and dying process (Correia et al. 1994). These contain heavy metals poisonous compounds and nutrients which affect plant and soil in number of ways. These toxic chemicals presents in effluent caused reduction in cell activities, retardation of growth, various deficiencies and diseases when accumulated in cells of living being (Patel et al. 2008 and Naik et al. 2009). Industrial effluents are constantly adding up toxic substances into the ground water reservoir at a very high rate especially in industrial zones (Babiker et al. 2004). The industrial pollutants caused the alteration in Physico-chemical and biological properties of the environment. Dye waste water has also been found toxic to several crop plants (Parameswari M. 2014). The presence of heavy metals and toxic chemicals will show detrimental effects on the development of plants, germination process and growth of seedlings (Singh et al. 2004 and Nath et al. 2007). Effluent in higher concentration affect the soil and causes heavy damage to the crop growth conditions. The use of such waste water in irrigation system definitely provides some nutrients to enhance the fertility of soil but it also deposits toxicants that change soil properties in the long run. The low amount of O_2 in dissolved form due to the presence of high concentration of solid in the effluent reduces the energy supply through anaerobic respiration resulting in restriction of growth of seedlings (Sazena et al. 1986). The improper and indiscriminate disposal of textile effluents in natural waters and land in posing serious problems (Kaushik et al. 2004). The exposure of lower concentration of effluent to the seedling shows growth promotion, over all development of the seedling and chlorophyll content. Reduction in seed germination percentage at higher concentration of effluent may be due to the higher amounts of solids presents in the effluent, which causes changes in osmotic relationship of the seed and water (Prabhakar et al. 2006). In the present study an attempt was made to analyze the impact of dying effluent, collected from dying and printing industries at Kaithoon region, Kota, on some growth parameters of soybean (*Glycine max* L.)

2. Material and Methods

Experimental Plant- Soybean (*Glycine max* L.)

Soybean is a leguminous vegetable of the pea family that grows in tropical, subtropical, and temperate climates. It consists of more than 36% protein, 30% carbohydrates, and excellent amounts of dietary fiber, vitamins, and minerals. It also consists of 20% oil, which makes it the most important crop for producing edible oil. Soybean produces significantly more protein in per acre than most other uses of land. Soy varies in growth and habit. The height of the plant varies from less than 0.2 to 2.0 m (0.66 to 6.56 ft). Soybean contains symbiotic bacteria called *Rhizobia* within nodules of their root system. These bacteria have the special ability of fixing nitrogen from atmosphere.

1) Collection of Effluent sample-

The effluent samples were collected from small dying and printing units from kaithoon region, Kota, Rajasthan. Samples were collected in sterilized wide mouth plastic bottles and were stored at 4^0 C temperature to avoid any changes on its characteristics. To evaluate the effects of dyeing effluent on *Glycine max* L. , solution of different concentrations of effluent were prepared.

Treatment Level

C- Control (100% Distilled water)
T_1- Effluent: Distilled water (20% + 80%)
T_2- Effluent: Distilled water (40% + 60%)
T_3- Effluent: Distilled water (60% + 40%)
T_4- Effluent: Distilled water (80% + 20%)
T_5- Effluent (100%)

For germination tests, seeds were sterilized with 0.1% w/v mercuric chloride ($Hgcl_2$) solution for 5 minutes to remove microbes and then washed three times with sterile distilled water. 20 seeds of *Glycine max* L. were placed in sterilized glass Petri dishes of uniform size lined with filter paper discs. These filter discs were then moistened with 5 ml of distilled water for control and with the same quantity of various concentrations of the textile effluent (20%, 40%, 60%, 80%, 100%). The Petri dishes were incubated at room temperature. 5 ml of respective dilutions were sprayed for consecutive 6 days. Germination was recorded daily. All the levels were carried out in triplicate. On the 7^{th} day, germination percentage was calculated and various growth parameters and chlorophyll content were evaluated on 14^{th} day of the experiment.

- **Germination percentage-** The formula given by Rehman et al.(1998) was used to estimate germination percentage.

$$\text{Germination percentage} = \frac{\text{No. of seeds germinated}}{\text{Total no. of seeds}} \times 100$$

- **Root and Shoot length-** Length of root and shoot of seedlings were calculated by using the standard centimeter scale.
- **Vigour index-** The formula suggested by Abdul-Baki and Anderson (1973) was used to calculate vigour index.
  Vigour index = germination percentage X (root length* + shoot length*)
  (* indicate that length of root and shoot in cm.)
- **Fresh and Dry Weight of seeds –** Ten seeds of each treatment were weighted in order to determine the fresh weight and then dried in oven at 80^0 C for 24 hrs. to obtain dry weight. Fresh weight and Dry weight were recorded in mg.
- **Chlorophyll estimation-** The estimation of chlorophyll content was done according to Arnon's method (1949).

$$\text{Chlorophyll a (mg/g)} = \frac{12.7\, A_{663} - 2.69\, A_{645}}{A \times 1000 \times W} \times V$$

$$\text{Chlorophyll B (mg/g)} = \frac{22.9\, A_{645} - 4.68\, A_{663}}{A \times 1000 \times W} \times V$$

Where
A_{663}= Absorbance at wavelength 663 nm
A_{645}= Absorbance at wavelength 645 nm
V= Volume of the extract in ml
A = Length of light path in the cuvette (1cm)
W= Fresh weight of the samples in gm.

3. Results and Discussion

Table 1 is showing effect of different dilution percentage of dying and printing effluent on germination percentage, vigour index, root length, shoot length, fresh and dry weight, chlorophyll a and b content in *Glycine max* L.

Germination percentage in untreated seedling (control) of *Glycine max* was 93% while the germination percentage of seedlings treated with 20% dilution was 96% in comparison to control. At high concentration of treatment levels 40%, 60%, 80% and 100% the germination percentage were decreased 70%, 56%, 40% and 33% respectively. The maximum decrease was found at 100% (33%) effluent concentration.

Vigour index in untreated seedling (control) of *Glycine max* L. was 1590.3 while the vigour index of seedlings treated with 20% dilution was increased 1680 in comparison to control. At high concentration of treatment levels 40%, 60%, 80% and 100% the vigour index were decreased 1204, 856.8, 436 and 234.3 respectively. The maximum reduction was found 85.26% at 100% effluent concentration.

Root length in untreated seedling (control) of *Glycine max* L. was 4.5cm while the root length of seedlings treated with 20% and 40% dilution were increased 4.7 and 4.6 cm in comparison to control. At high concentration of treatment levels 60%, 80% and 100% the root length were decreased 3.4, 2.1 and 1.9 cm respectively. The maximum reduction was found 57.7% at 100% effluent concentration.

Shoot length in untreated seedling (control) of *Glycine max* L.was 12.6 cm while the root length of seedlings treated with 20% and 40% dilution were increased 12.8 cm and no change at 40% in comparison to control. At high concentration of treatment levels 60%, 80% and 100% the soot length were decreased 3.4, 2.1 and 1.9 cm respectively. The maximum reduction was found 57.7% at 100% effluent concentration.

Fresh weight in untreated seedling (control) of *Glycine max* was 3.10 gm while the fresh weight of seedlings treated with 20% and 40% dilution were increased 3.26 and 3.15 gm in comparison to control. At high concentration of treatment levels 60%, 80% and 100% the fresh weight were decreased 2.98, 2.42 and 2.10 gm respectively. The maximum reduction was found 32.25% at 100% effluent concentration.

Dry weight in untreated seedling (control) of *Glycine max* L. was 1.90 gm while the dry weight of seedlings treated with 20% and 40% dilution were increased 1.98 and 1.93 gm in comparison to control. At high concentration of treatment levels 60%, 80% and 100% the dry weight were decreased 1.51, 1.26 and 1.09 gm respectively. The maximum reduction was found 42.63% at 100% effluent concentration.

Chlorophyll a content in untreated seedling (control) of *Glycine max* L. was 5.06 mg/gm while the Chlorophyll a of seedlings treated with 20% and 40% dilution were increased 5.11 and 5.08 mg/gm in comparison to control. At high concentration of treatment levels 60%, 80% and 100% the Chlorophyll a were decreased 4.86, 3.87 and 2.42 mg/gm respectively. The maximum reduction was found 52.1% at 100% effluent concentration.

Chlorophyll b content in untreated seedling (control) of *Glycine max* L. was 5.02 mg/gm while the Chlorophyll b of seedlings treated with 20% and 40% dilution were increased 5.07 and 5.05 mg/gm in comparison to control. At high concentration of treatment levels 60%, 80% and 100% the Chlorophyll b were decreased 3.71, 2.69 and 1.80 mg/gm respectively. The maximum reduction was found 64.1% at 100% effluent concentration.

Present study result revealed that effluent of dying industries inhibit seed germination and seedling growth. It may be due to the presence of toxic elements and metal ions in the corresponding industrial effluents (Rohit et al. 2013). The higher p^H, BOD, COD and other higher organic loads that cause adverse effect on germination (Panday et al. 2004 and Saddaqat et al. 2006. This could be related to the fact that some of the nutrients present in the effluent are essential but in higher concentration they become hazardous and toxic to the soybean plant (Ravi et al. 2014). The exposure of lower concentration of effluent to the seedling shows growth promotion, over all development of the seedling and chlorophyll content. Reduction in seed germination percentage at higher concentration of effluent may be due to the higher amount of solids presents in the effluent, which causes in the osmotic relationship of the seed and water.

Present study supported with the views of Sundaramoorthy et al. (2000) who investigated that the percentage of seed germination and seedling growth was maximum at 10% diluted effluent than the control while undiluted effluent showed inhibitory effects . Hussain et al. (2013) also reported that diluted effluent (25%) increase the growth parameters and pigments in the Maize seedlings. Kathirvel (2012) also supported present study who investigated that at 20% concentration of effluent, the plant showed maximum seed germination, root and shoot length than the control and the maximum chlorophyll content were also reported at 20% effluent concentration which could be due to the best growth of seedling at this concentration. Present study is also supported by Mayuri et al. (2015) who reported that the best germination and seedling growth was observed in 25% concentration with growth promoting effect and significantly better than control. Beyond 25% effluent germination percentage and seedling growth decreased gradually. Divyaprivas et al. (2014) also concluded that germination percentage and other biochemical contents were high at 30% effluent dilution in comparable with control but beyond 30% dilution all parameters were decreased and at 100% effluent treatment the seed germination was completely inhibited in *Cicer arientum.*

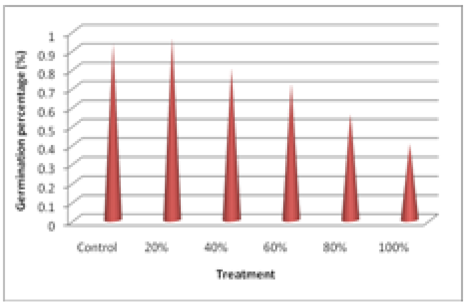
4. Conclusion

The present study concluded that the dying effluent waste significantly influence growth parameters of soybean (*Glycine max* L.). The collected effluent sample contains anion which can be beneficial for plant growth but its excessive level could be toxic, retard the growth of the plants. Reduction in seed germination percentage at higher concentration of effluent may be due to the higher amount of solids present in the effluent, which causes changes in the osmotic relationship of the seed and water. The results suggested that dye industrial effluent (20%) could be used for irrigation of soybean crops.

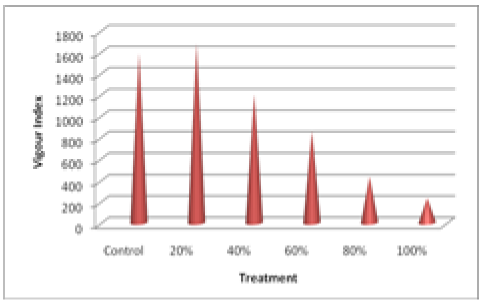
Table 1: Effect of dye effluent on some growth parameters of *Glycine max* L

S. No.	Treatment	Seed germination Percentage (7th days)	Vigour index	Seedling growth (14th days)		Weight		Chlorophyll Content (mg/gm)	
				Shoot length (cm.) Avg.	Root length (cm) Avg.	Fresh weight (gm)	Dry weight (gm)	Chlorophyll a (mg/gm)	Chlorophyll b (mg/gm)
1.	Control	93	1590.3	12.6	4.5	3.10	1.90	5.06	5.02
2.	20%	96	1680(5.4%)*	12.8 (1.5%)**	4.7(4.4%)**	3.26(5.1%)**	1.98(9.2%)**	5.11(0.98%)**	5.07(0.99%)**
3.	40%	70	1204(24.2%)*	12.6	4.6(2.2%)**	3.15(1.5%)**	1.93(1.5%)**	5.08(0.39%)**	5.05(0.59%)**
4.	60%	56	856.8(46.1%)*	11.9 (5.5%)*	3.4(24.4%)*	2.98(3.8%)*	1.51(20.5%)*	4.86(3.95%)*	3.71(26.09%)*
5.	80%	40	436(72.5%)*	8.8 (30.1%)*	2.1(53.3%)*	2.42(21.9%)*	1.26(33.6%)*	3.87(23.5%)*	2.69(46.4%)*
6.	100%	33	234.3(85.2%)*	5.2 (58.7%)*	1.9(57.7%)*	2.10(32.2%)*	1.09(42.6%)*	2.42(52.1%)*	1.80(64.1%)*

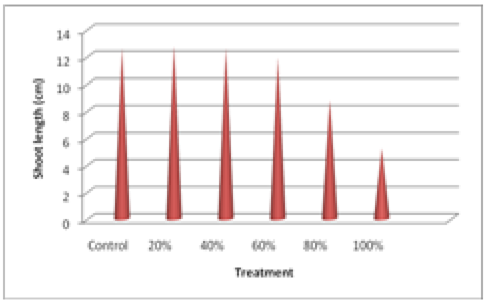
* Figures in parenthesis showed decrease over control.
** Figures in parenthesis showed increase over control



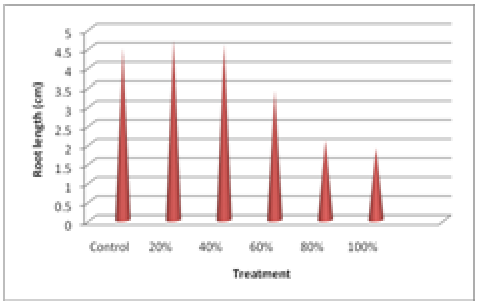
Germination Percentage (%)



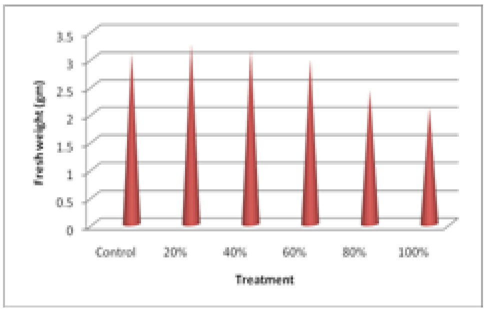
Vigour Index



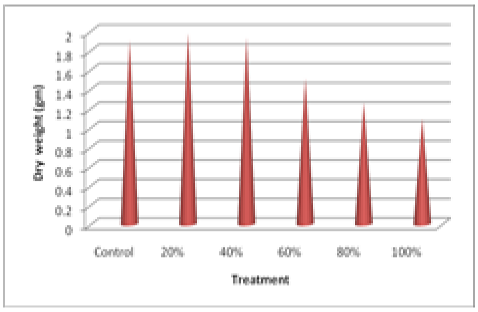
Shoot length (cm)



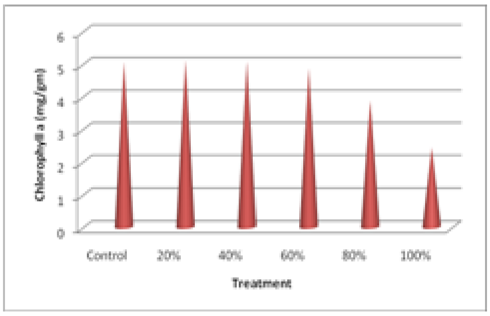
Root length (cm)



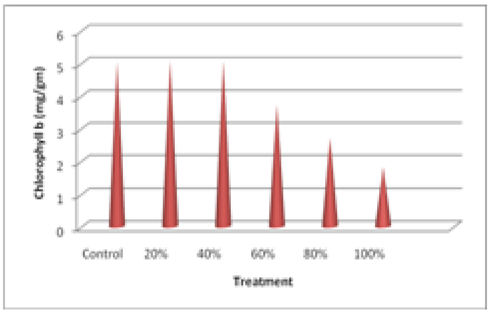
Fresh weight (gm)



Dry weight (gm)



Chlorophyll a (mg/gm)



Chlorophyll b (mg/gm)

Graph showing impact of dying and printing effluent on seed germination,seedling growth and chlorophyll content of Soybean (*Glycine max* L.).

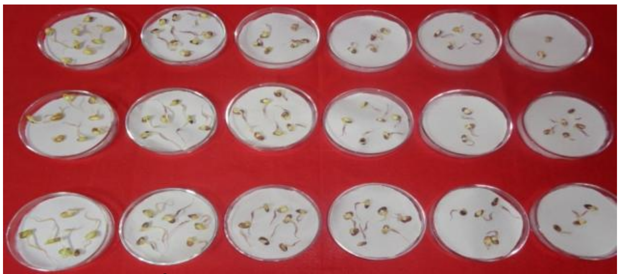


Figure: 3rd day germination state in *Glycine max*

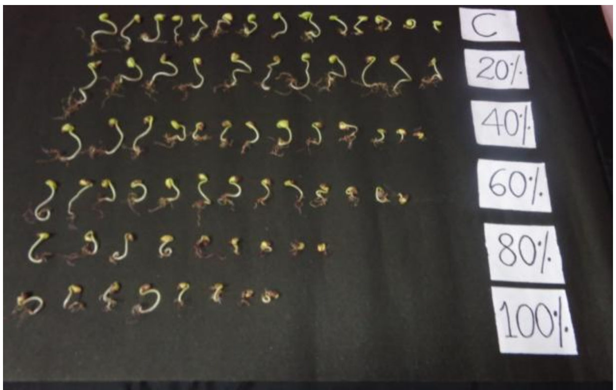


Figure 7: 14th day root length of *Glycine max*

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