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Soil Irrigation Effect of Dyeing and Printing Effluent on Changes of Growth and Biochemical Contents of *Medicago sativa* L.

Pratibha Mahawar[1], Azra Akhtar[2]
[1]*Research Scholar,* [2]*Associate Professor, Department of Botany, Government College, Kota, Rajasthan, India*

Abstract: ***The effect of dyeing and printing effluent on plant growth and biochemical constituents of Medicago sativa L. var. T9 was studied in a pot culture experiment. In the pot culture experiment, alfalfa plants were grown up to 60 days, in the soil irrigated with different concentrations of dyeing and printing effluent (viz. 20%, 40%, 60%, 80% & 100%v/v). Each pot containing sterilized soil. 20 Seeds were sown in each pot. All pots were irrigated (200ml) with respective concentration of test solutions daily. Plants were thinned to a maximum of three per pots, after a week of germination. The higher dyeing and printing effluent concentrations (above 40%) were found to affect plant growth and decreased chlorophyll-a, chlorophyll-b and total chlorophyll, caroteinoids, free sugar, amino acids and protein contents, but diluted effluent (up to 40%) favored the plant growth and biochemical contents.***

Keywords: dyeing and printing industrial effluents, growth, biochemical etc.

I. INTRODUCTION

The rapid industrialization has led to the enormous amount of discharge of wastes from industries. Textile dyeing and printing industries require large amount of water and consequently generate an equally large amount of waste water carrying high concentrations of the dyes resulting in the pollution of surface waters and also ground water upon percolation. Besides that, they also cause major loss to crops, vegetables and stored organic materials. Recently, the use of dye factory effluent for growing crops is considered as one of the viable alternative for its disposal (Chidambaram pillai and Shunmugasundaram (2008) but also had adverse effect on plant growth and tissue metabolism. The waste water treatment system in Indian industries is recommended to be essentially installed to meet the waste water discharge norms, but presently only 10% of the waste water generated is treated and the rest of untreated water is discharged into nearby water bodies (Mehta *et. al*., 2012). Such as use of dye stuffs in textile paper, paint and printing industries and improper disposal of these stuffs into the water sources cause serious problem of pollution and health hazards due to presence of heavy metals above permissible limits (Khan *et. al*., 2001). In the present investigation, an attempt has been made to study the effects of dyeing and printing effluent on the growth, chlorophyll, carotenoid, free sugar, amino acids and proteins contents of Alfalfa plants.

II. MATERIALS AND METHODS

A. Seed Materials

Working sample certified seeds were collected from seed certification and testing laboratory, Bajrang nagar, Kota, Rajasthan. Seeds with uniform size, color and weight were chosen for the experimental purpose.

B. Collection Of Effluent From The Dyeing And Printing Industrial Effluent

The effluent samples were collected in a pre-cleaned, plastic container from the point of disposal from dyeing and printing small industries located at Kaithun, Kota, India. The collected effluent was stored at 5°C to maintain its original characteristics (APHA 1998).

C. Experimental Soil

The soil used in the experiment was collected in polythene covers near by the dyeing and printing small industries and soil sterilization was done before experimental set up.

D. Treatment Level

1) C- Control (100% Tap water)
2) T_1- Effluent: Tap water (20% + 80%)
3) T_2- Effluent: Tap water (40% + 60%)
4) T_3- Effluent: Tap water (60% + 40%)
5) T_4- Effluent: Tap water (80% + 20%)
6) T_5- Effluent (100%)

E. Experimental Design

The various treatment levels (20%, 40%, 60%, 80% and 100%) of textile dyeing and printing effluent solution were prepared and used for Pot culture studies. Twenty seeds were sown in each of triplets in control and various treatment levels. The pots were irrigated with equal volumes of various treatment levels of dyeing and printing effluent. Control set was irrigated with equal volume of tap water.

F. Growth Analysis

The plant samples were collected on 30^{th} and 60^{th} days after sowing. Germinated plants were collected from the pot to analyze for the various growth parameters such as Root length (cm) and Shoot length (cm), Fresh weight (g/plant), Dry weight (g) and Vigour index.

G. Biochemical Estimations

Leaves of control and treated plants were used for the estimation of Chlorophyll-a, Chlorophyll-b, total Chlorophyll and Carotenoid content was measured according to Arnon 1949 and total Protein content in seeds was estimated by Lowry *et al*., 1951. Free amino acid content was estimated by Moore and Stein method (1944). Free sugar was estimated by Nelson (1944).

III. RESULTS AND DISCUSSION

Effect on Growth, Pigment and Biochemical contents of *Medicago sativa* L. at 30 day of sowing and after completion of life cycle:-

Table 1 and 2 are showing effect of different dilution percentage of dyeing and printing effluent on vigour index, root length, shoot length, fresh and dry weight, chlorophyll a and b, total chlorophyll, carotenoid, amino acid, protein and free sugar content in *Medicago sativa* L. at 30 day of sowing and after completion of life cycle:-

A gradual decline in shoot length of *Medicago sativa* L. was observed with increasing concentration of dyeing and printing effluent (Table 1). At 30 DAS, the average highest shoot length was recorded in T_1 (20%) treatment level (24.53cm), followed by T_2 (40%) treatment level (20.76cm) and control (19.56cm). At T_1 and T_2 treatment level shoot length was increased 20.40% and 6.13% respectively as compare to control.

Higher concentration of effluent had maximum inhibitory effect i.e. T_5 (100%) treatment level, where shoot length was 15.06cm which is 23.00% reduced in comparison to control. Similarly, At 60 DAS the average highest shoot length was recorded in T_1 (20%) treatment level (30.91 cm), followed by T_2 (40%) treatment level (27.3 cm) and control (25.76 cm). At T_1 and T_2 treatment level shoot length was increased 19.99% and 5.98% respectively as compare to control. Higher concentration of effluent had maximum inhibitory effect i.e. at T_5 (100%) treatment level, where shoot length was 20.14cm which is 21.82% reduced in comparison to control.

The effect of highly diluted effluent on root length in *Medicago sativa* L. shows stimulatory effect rather than inhibitory effect. At T_1 (20%) treatment level root length was 18.6cm followed by control 15.73cm, Percentage enhancement was found 18.24% in treatment level T_1 (20%) over control.

While highly reducing value of root length was found in T_5 (100%) treatment level (9.83cm) followed by gradual decreasing values in increasing concentration of effluent at treatment level T_2 (40%), T_3 (60%) and T_4 (80%) i.e. 15.43cm, 15.2cm and 12.73cm respectively.

Similarly, At 60 DAS root length was relatively enhanced by T_1 (20%) that showed maximum value was obtained 21.42cm followed by T_2 (40%) treatment level 18.92cm while slight reduction in value of root length was obtained in control (16.96cm). Inhibition of root length were obtained from treatment level T_3 (60%), T_4 (80%) and T_5 (100%) i.e. 13.81cm, 12.94cm and 10.91cm respectively.

In case of fresh weight of shoots in *Medicago sativa* L., minimum value was observed on 30 days at treatment level T_5 (100% raw effluent) i.e. 9.02 gm/plant which was 31.87% decreased in comparison to control 13.24gm/plant while maximum value of fresh weight of shoot was observed at T_1 (20%) and T_2 (40%) treatment level i.e. 16.2 gm/plant and 15.22 gm/plant which was 22.35% and 14.95% respectively increased when compared with control (13.24 gm/plant). In case of fresh weight of shoots, maximum reductive value was observed at 60 days after sowing in treatment level T_5 (100%) raw effluent 8.54 g/plant (33.54%) while maximum value of fresh weight of shoots was found in T_1 (20%) treatment level 14.52 g/plant (13%) followed by control 12.85g/plant.

Whereas gradual decline occurred in fresh weight values in treatment level T_2 (40%), T_3 (60%) and T_4 treatment level (80%) i.e. 12.21g/plant (4.98%), 11.04g (14.09%) and 9.80g (23.74%) respectively in comparison to control

Maximum fresh weight of plant roots treated with dyeing and printing effluent was found in treatment level T_1 (20%) and T_2 (40%) (9.43 gm/plant and 8.08 gm/plant respectively) which were 30.06% and 11.44% increased as compared with control (7.25 gm/plant).

The fresh weight of root was decreased with the increasing concentration of effluent where fresh weight of root was 6.13 gm/plant, 4.81 gm/plant and 4.09 gm/plant at T_3, T_4 and T_5 treatment level respectively.

Minimum value of fresh weight of roots in *Medicago sativa* L., was observed at treatment level T_5 (100% raw effluent) i.e. 5.02 gm/plant which was 45.13% decreased in comparison to control while maximum value of fresh weight of root was observed at T_1 (20%) and T_2 (40%) treatment level i.e. 11.85 gm/plant and 10.18 gm/plant which was 29.51% and 11.26% respectively increased when compared with control (9.15 gm/plant).

Observations showed maximum values of dry weight of shoot at 20% (T_1) effluent concentration i.e. 3.83 gm/plant which are 26.40% increased as compared with control 3.03 gm/plant. The dry weight of shoot inhibit when plants treated with higher concentration of effluent.

Reduction in percentage increased from T_3 to T_5 i.e. 4.62%, 14.19% and 30.36% respectively. Similarly, At 60 DAS dry weight of shoot showed maximum values at 20% (T_1) effluent concentration 3.26 gm/plant (11.56%) followed by T_2 (40%) effluent concentration 2.92 gm/plant and control 2.76 gm/plant.

The values of dry weight of shoot decreased with the increasing treatment level. The maximum percentage reduction 41.32% (1.18 gm/plant) was observed at 100% treatment level (T_5 raw effluent).

Dry weight of roots was relatively enhanced by 20% (T_1) that showed maximum value of dry weight of root i.e. 2.19 gm/plant followed by T_2 treatment level i.e. 2.09 gm/plant.

At T_1 treatment level dry weight of root was increased 19.67% over control. Maximum reduction in dry weight of root was recorded at T_5 treatment level (1.14 gm/plant) which is 37.70% reduced when compared with control. Similarly, At 60 DAS maximum value of dry weight of plant roots treated by dyeing and printing effluent was found in treatment level T_1 (20%) i.e. 1.62g per plant followed by T_2 (40%) treatment level 1.34g per plant and control 1.19 gm/plant. Percentage enhancement was found 32.33% in T_1 and 20.30% in T_2 over control.

The vigour index was decrease with the increasing concentration of effluent. The minimum value of vigour index was found at T_5 (100%) treatment level i.e. 1869.75 which was 44.36% decreased as compared with control 3360.90. At T_1 (20%) and T_2 (40%) treatment level vigour index value of *Medicago sativa* L. plant was higher than control. The percentage increase was 18.60% and 2.80% over control at T_1 and T_2 treatment level respectively. Similarly, At 60 DAS the minimum value of vigour index was found at T_5 (100%) treatment level i.e. 2974.90 which was 23.91% decreased as compared with control 3909.51. At T_1 (20%) and T_2 (40%) treatment level vigour index value of *Medicago sativa* L. plant was higher than control. The percentage increase was 15.46% and 7.78% over control at T_1 and T_2 treatment level respectively.

The present investigation might be related to reduction in seedling (root and shoot) lengths with the 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 level of a particular concentration, that was become hazardous.

The presence of optimum level of nutrients in the lower concentration of dye effluent might have increased the Fresh Weight and Dry Weight of crop plant.

The reduction in dry weight of plant material may be due to the poor growth under effluent irrigation (Balashouri and Prameela Devi., 1994). The decreased in shoot length, root length, fresh weight and dry weight were recorded. It may be due to the presence of toxic pollutants in the effluent. The same result affects the respiration of the root (Singh et. al., 1985).

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Table 1: Effect of dyeing and printing effluent on seedling growth of *Medicago sativa* L. after 30 day of sowing
(Values are mean ± Standard Deviation of 3 replicates)

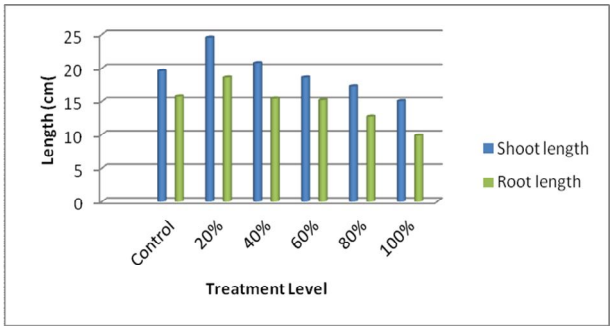
S. No.	Treatment Level	Shoot Length (cm)	Root Length (cm)	Shoot Fresh Weight (gm/plant)	Root Fresh Weight (gm/plant)	Shoot Dry Weight (gm/plant)	Root Dry Weight (gm/plant)	Vigour Index
1.	Control	19.56±0.0599	15.73±0.0331	13.24±0.01010	7.25±0.0210	3.03±0.0580	1.83±0.0501	3360.90±4.4219
2.	T_1	24.53±0.0360 (+20.40%)**	18.6±0.0274 (+18.24%)**	16.2±0.0351 (+22.35%)**	9.43±0.01515 (+30.06%)**	3.83±0.0195 (+26.40%)**	2.19±0.0200 (+19.67%)**	3986.13±3.5412 (+18.60%)**
3.	T_2	20.76±0.3512 (+6.13%)**	15.43±0.0311 (-1.90%)*	15.22±0.0099 (+14.95%)**	8.08±0.0251 (+11.44%)**	3.01±0.0511 (-0.66%)*	2.09±0.0119 (+14.20%)**	3455.24±3.8911 (+2.80%)**
4.	T_3	18.6±0.2517 (-4.90%)*	15.2±0.0405 (-3.36%)*	12.73±0.0115 (-3.85%)*	6.13±0.0704 (-15.44%)*	2.89±0.0325 (-4.62%)*	1.51±0.0119 (-17.48%)*	2707.24±3.9608 (-19.44%)*
5.	T_4	17.33±0.4583 (-11.40%)*	12.73±0.01155 (-19.07%)*	10.26±0.0154 (-22.50%)*	4.81±0.0501 (-33.65%)*	2.60±0.0115 (-14.19%)*	1.38±0.0577 (-24.59%)*	2119.23±1.4811 (-36.94%)*
6.	T_5	15.06±0.3 (-23.00%)*	9.83±0.0200 (-37.50%)*	9.02±0.0191 (-31.87%)*	4.09±0.0112 (-43.58%)*	2.11±0.0415 (-30.36%)*	1.14±0.0173 (-37.70%)*	1869.75±2.9373 (-44.36%)*

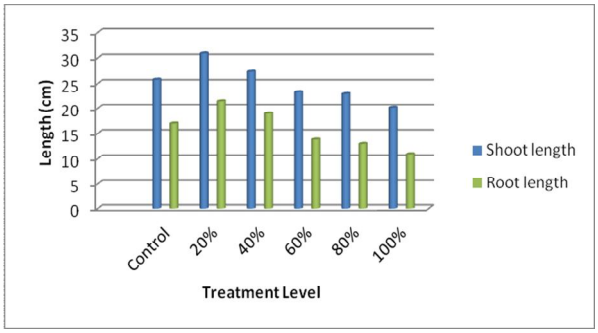
*Figures in parentheses represent % decrease over control.
**Figures in parentheses represent % increase over control.

Table 2: Effect of dyeing and printing effluent on seedling growth of *Medicago sativa* L. after completion of life cycle (60 DAS)
(Values are mean ± Standard Deviation of 3 replicates)

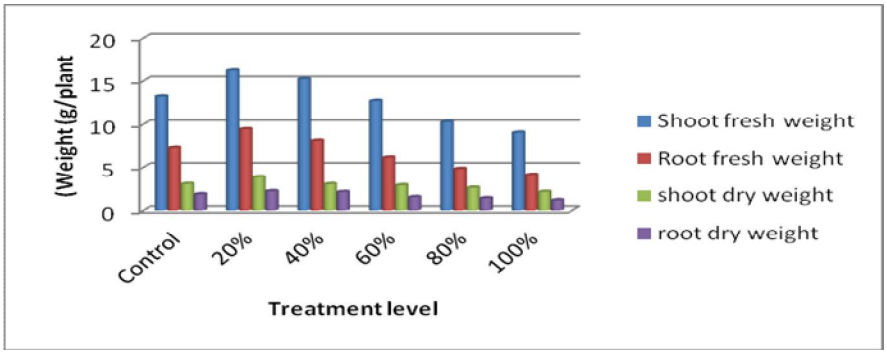
S. No.	Treatment Level	Shoot Length (cm)	Root Length (cm)	Shoot Fresh Weight (gm/plant)	Root Fresh Weight (gm/plant)	Shoot Dry Weight (gm/plant)	Root Dry Weight (gm/plant)	Vigour Index
1.	Control	25.76±0.0471	16.96±0.0377	12.85±0.1608	9.15±0.0410	2.76±0.0577	1.19±0.2081	3909.51±6.5079
2.	T_1	30.91±0.1885 (19.99%)**	21.42±0.0169 (10.36%)**	14.52±0.0188 (12.99%)**	11.85±0.0216 (29.50%)**	3.26±0.2516 (18.11%)**	1.62±0.1154 (36.13%)**	4513.90±3.7913 (15.45%)**
3.	T_2	27.3±0.0816 (5.97)**	18.92±0.0286 (5.22%)**	12.21±0.0294 (4.98%)*	10.18±0.0205 (11.25%)**	2.92±0.2 (5.79%)**	1.34±0.1732 (12.60%)**	4213.84±5.1705 (7.78%)**
4.	T_3	23.32±0.0124 (9.47)*	13.81±0.0124 (9.35%)**	11.04±0.1219 (14.08%)*	8.68±0.0339 (5.13%)*	2.18±0.0999 (21.01%)*	1.05±0.0264 (11.76%)*	3521.94±5.5423 (9.91%)*
5.	T_4	22.94±0.0094 (10.94%)*	12.94±0.0081 (20.29%)*	9.80±0.0047 (23.73%)*	7.22±0.0249 (21.09%)*	1.76±0.0305 (36.23%)*	0.81±0.0321 (31.93%)*	3119.56±4.5439 (20.20%)*
6.	T_5	20.14±0.0449 (21.81%)*	10.91±0.1515 (26.53%)*	8.54±0.0496 (33.54%)*	5.02±0.0725 (45.13%)*	1.18±0.2309 (57.24%)*	0.74±0.0351 (37.81%)*	2974.90±4.2129 (23.90%)*

*Figures in parentheses represent % decrease over control.
**Figures in parentheses represent % increase over control.

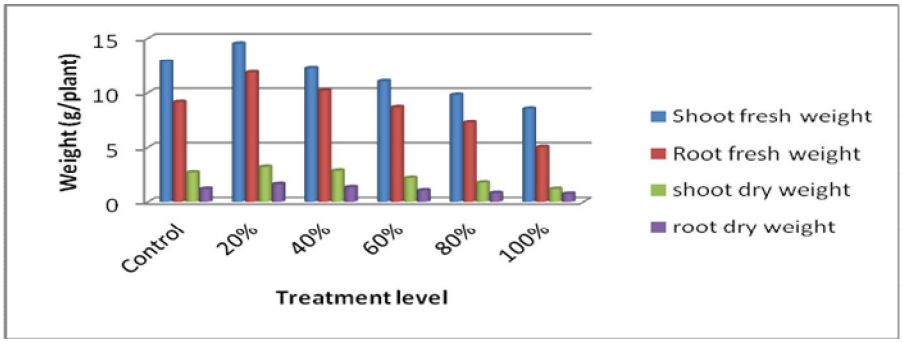




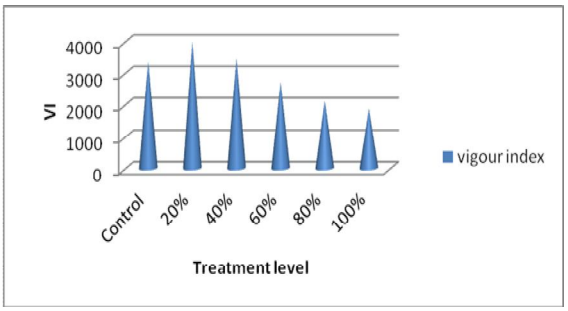
(A) Shoot and Root length (30 DAS) (B) Shoot and Root length (60 DAS)



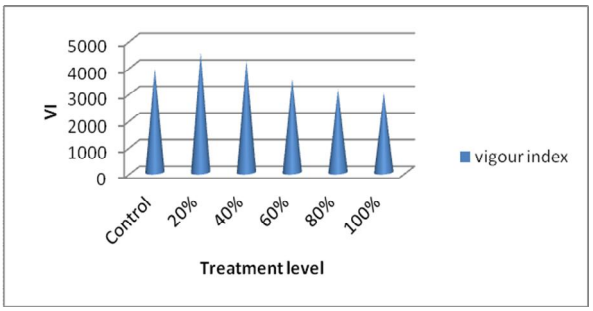
(C) Fresh and Dry Weight of Shoot and Root (30 DAS)



(D) Fresh and Dry Weight of Shoot and Root (60 DAS)



(E)Vigour Index (30 DAS)



(F) Vigour Index (60 DAS)

Increase level of pigment content in *Medicago sativa* L. was recorded when effluent was diluted 80% with the normal water i.e. T_1 treatment level. The chlorophyll 'a' was found maximum at T_1 treatment level i.e. 4.36 mg/gm fresh weight which was 4.05% higher than control 4.19 mg/gm fresh weight. A gradual decline was observed with the higher concentration of effluent from T_3 to T_5 treatment level. The maximum reduction in chlorophyll 'a' was 47.97% (2.18 mg/gm fresh weight) at T_5 treatment level when compared with control. Similarly, at 60 DAS, The chlorophyll 'a' was found maximum at T_1 treatment level i.e. 6.08 mg/gm fresh weight which was 12.59% higher than control 5.4 mg/gm fresh weight. A gradual decline was observed with the higher concentration of effluent from T_3 to T_5 treatment level. The maximum reduction in chlorophyll 'a' was 4.44% (17.77 mg/gm fresh weight) at T_5 treatment level when compared with control.

Chlorophyll b content in untreated seedling (control) of *Medicago sativa* L. was 3.21 mg/gm fresh weight while the Chlorophyll b of seedlings treated with 20% and 40% dilution were increased 4.26 and 3.65 mg/gm fresh weight in comparison to control. At high concentration of treatment levels 60%, 80% and 100% the Chlorophyll b were decreased 3.18, 2.71 and 2.07 mg/gm fresh weight respectively. The maximum reduction was found 35.51% at 100% effluent concentration. Similarly, at 60 DAS Chlorophyll b content in untreated seedling (control) of *Medicago sativa* L. was 9.2 mg/gm while the Chlorophyll b of seedlings treated with 20% and 40% dilution were increased 11.20 and 9.41 mg/gm in comparison to control. At high concentration of treatment levels 60%, 80% and 100% the Chlorophyll b were decreased 9.04, 8.68 and 7.88 mg/gm respectively. The maximum reduction was found 14.34% at 100% effluent concentration (Table 3).

Total Chlorophyll content in untreated seedling (control) of *Medicago sativa* L. was 8.37 mg/gm while the Total Chlorophyll of seedlings treated with 20% and 40% dilution were increased 9.40 and 8.71 mg/gm in comparison to control. At high concentration of treatment levels 60%, 80% and 100% the Total Chlorophyll was decreased 7.02, 6.46 and 5.85 mg/gm respectively. The maximum reduction was found 30.10% at 100% effluent concentration. Similarly, at 60 DAS, Total Chlorophyll content in untreated seedling (control) of *Medicago sativa* L. was 14.61 mg/gm while the Total Chlorophyll of seedlings treated with 20% and 40% dilution were increased 17.27 and 14.81 mg/gm in comparison to control. At high concentration of treatment levels 60%, 80% and 100% the Total Chlorophyll was decreased 14.49, 14.26 and 12.45 mg/gm respectively. The maximum reduction was found 14.78% at 100% effluent concentration (Table 3).

Maximum carotenoid content was recorded at T_1 treatment level (3.19 mg/gm fresh weight) that was 8.13% enhance over control (2.95 mg/gm fresh weight). The minimum value of carotenoid content was at T_5 treatment level (2.33 mg/gm fresh weight) which was 21.01% decreased as compared with control. Maximum carotenoid content was recorded at T_1 treatment level (3.63 mg/gm fresh weight) that was 19.01% enhance over control (3.05 mg/gm fresh weight). The minimum value of carotenoid content was at T_5 treatment level (1.6 mg/gm fresh weight) which was 47.54% decreased as compared with control (Table 3).

Mishra and Bera (1995) have also reported that leaf chlorophyll and leaf area were reduced in higher concentrations of effluent, while these parameters were enhanced at lower concentrations. Chlorophyll content indicates the rate of photosynthetic activity of the plant, which in turn reflects upon the nutrient intake of the plant. Chlorophyll a, b and total chlorophyll contents of plants increased based upon the concentrations of effluent. Reduction in chlorophyll content induced by effluent may be associated with the mineral ions (Gadallah, 1999). This might be due to enhancing influence of increased nitrogen on carotenoid synthesis (Cottenie, 1973).

Table 3: Effect of dyeing and printing effluent on pigment content (mg g–1 fresh weight) of *Medicago sativa* L. after 30 day of sowing and after completion of life cycle (60 DAS)
(Values are mean ± Standard Deviation of 3 replicates)

S. No	Treatment Level	Chlorophyll a (mg/gm f.wt.)		Chlorophyll b (mg/gm f.wt.)		Total Chlorophyll (mg/gm f.wt.)		Carotenoid (mg/gm f.wt.)	
		30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS
1.	Control	4.19±0.019	5.4±0.099	3.21±0.015	9.2±0.057	8.37±0.032	14.61±0.011	2.95±0.015	3.05±0.029
2.	T_1	4.36±0.251 (+4.05%)**	6.08±0.015 (+12.59%)**	4.26±0.026 (+32.71%)**	11.20±0.010 (+21.73%)**	9.40±0.036 (+12.30%)**	17.27±0.043 (+18.20%)**	3.19±0.017 (+8.13%)**	3.63±0.009 (+19.01%)**
3.	T_2	4.27±0.020 (+1.90%)**	5.52±0.030 (+2.22%)**	3.65±0.030 (+13.70%)**	9.41±0.020 (+2.28%)**	8.71±0.045 (+4.06%)**	14.81±0.023 (+1.36%)**	3.08±0.029 (+4.40%)**	3.13±0.015 (+2.62%)**
4.	T_3	3.64±0.037 (-13.12%)*	5.35±0.019 (-0.92%)*	3.18±0.045 (-0.93%)*	9.04±1.192 (-1.73%)*	7.02±0.009 (-16.12%)*	14.49±0.017 (-0.82%)*	2.90±0.005 (-1.69%)*	2.59±0.055 (-15.08%)*
5.	T_4	3.01±0.011 (-28.16%)*	4.68±0.025 (-13.33%)*	2.71±0.035 (-15.57%)*	8.68±0.026 (-5.65%)*	6.46±0.040 (-22.81%)*	14.26±0.009 (-2.39%)*	2.84±0.041 (-3.72%)*	1.92±0.010 (-37.04%)*
6.	T_5	2.18±0.058 (-47.97%)*	4.44±0.028 (-17.77%)*	2.07±0.020 (-35.51%)*	7.88±0.015 (-14.34%)*	5.85±0.028 (-30.10%)*	12.45±0.005 (-14.78%)*	2.33±0.020 (-21.01%)*	1.6±0.020 (-47.54%)*

*Figures in parentheses represent % decrease over control.
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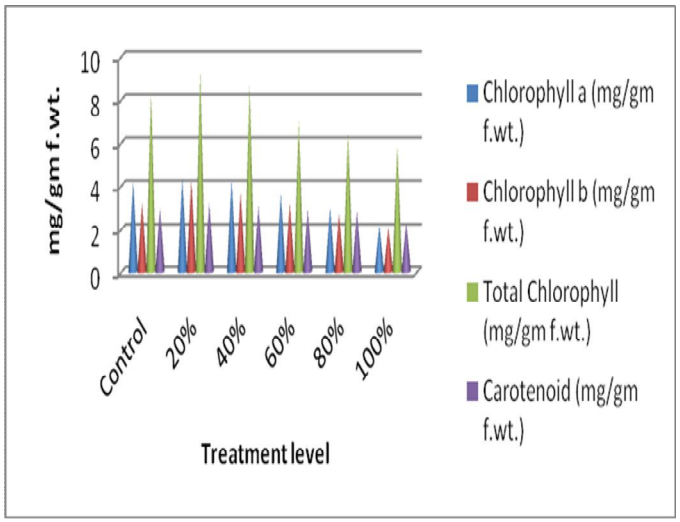
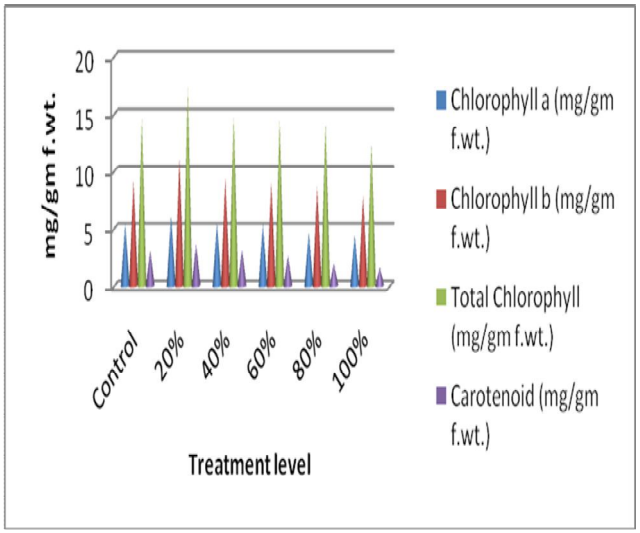


(A) Pigment Content (30 DAS)



(B) Pigment Content (60 DAS)

A. Free Amino acid

Medicago sativa L. plants when treated with various concentration of dyeing and printing effluent the free amino acid ranged between 7.15 mg/gm fresh weight to 5.91 mg/gm fresh weight. Highest amount (7.15 mg/gm fresh weight) of free amino acid was observed at T_1 treatment level which was 7.51% increased over control. A gradual decline in free amino acid content was observed with the increasing concentration of effluent. At T_3, T_4 and T_5 the free amino acid was 6.44 mg/gm fresh weight, 6.18 mg/gm fresh weight, 5.91 mg/gm fresh weight respectively which were 3.15%, 7.06% and 11.12% reduced when compared with control (6.65 mg/gm fresh weight). The difference in the value of free amino acid at T_2 (6.69 mg/gm fresh weight) and control is negligible (Table 4).

B. Protein

The protein content in *Medicago sativa* L. was negatively affected when treated with higher concentration of dying and printing effluent (Table 4). As effluent concentration increased from T_3 (60%) to T_5 (100%) the protein content was adversely affected. The maximum reduction was found at T_5 (100% effluent) treatment level i.e. 12.23 mg/gm fresh weight which decreased 6.42% as compared with control. Increased protein content was recorded at T_1 treatment level i.e. 13.21 mg/gm fresh weight which enhance 1.07% over control (13.07 mg/gm fresh weight). The value of protein was approximately similar in plants treated with 40% effluent (13.09 mg/gm fresh weight) and control.

C. Free Sugar

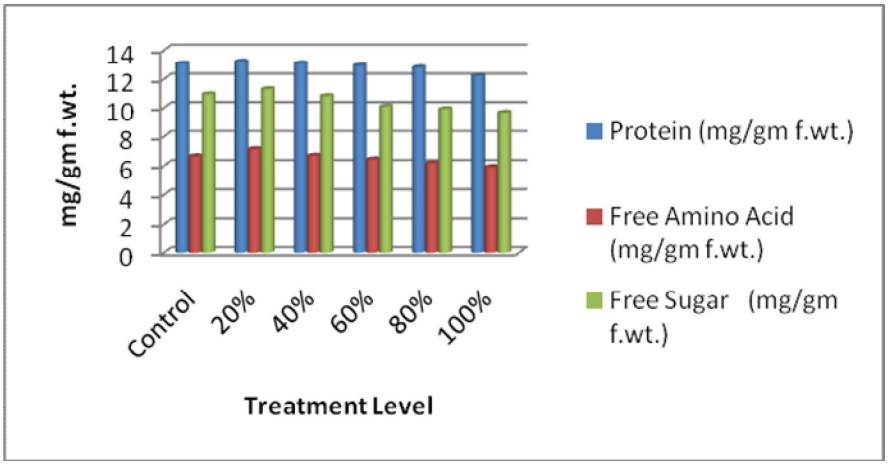
Free sugar in treated plant was also adversely affected with the increasing concentration of effluent. The maximum (11.30 mg/gm fresh weight) free sugar content was recorded at T_1 treatment level which was 3.29% higher when compared with control 10.94 mg/gm fresh weight. At T_2 treatment level also free sugar content was 1.18% increased over the control. The minimum (9.66 mg/gm fresh weight) free sugar content was recorded at T_5 treatment level which was 11.70% decreased when compared with control (Table 4).

Similarly, the protein content was decreased in all concentration of dye effluent compared to control, followed by treated effluent. The amount of carbohydrate, protein and total free amino acids were comparable with control, their amounts were increased in the 30% effluent treated seeds (Divyapriya et. al., 2014). Swaminathan and Vaidheeswarn (1991) analyzed biochemical changes in peanut crops, and observed that 50% diluted textile effluent increased the seed germination, total sugars, amino acids, phenol and proline starch, protein and chlorophyll than control (distilled water) of peanut seedlings. These studies showed that effects of an industrial effluent vary from crop to crop. So it is essential to study the effect of industrial effluents on individual crops before their disposal in agricultural fields. Extensive efforts have been made by workers to find out proper dilution for different industrial effluent, which can be used for irrigational purpose (Sisodia and Bedi, 1985; Srivastava and Sahai, 1987).

Table 4: Effect of dyeing and printing effluent on Biochemical content (mg g–1 fresh weight) of *Medicago sativa* L. after completion of life cycle (60 DAS)
(Values are mean ± Standard Deviation of 3 replicates)

S. No.	Treatment Level	Protein (mg/gm f.wt.)	Free Amino Acid (mg/gm f.wt.)	Free Sugar (mg/gm f.wt.)
1	Control	13.07±0.023	6.65±0.030	10.94±0.015
2	T_1	13.21±0.005 (+1.07%)**	7.15±0.017 (+7.51%)**	11.30±0.100 (+3.29%)**
3	T_2	13.09±0.015 (+0.15%)**	6.69±0.035 (+0.60%)**	10.81±0.005 (-1.18%)*
4	T_3	12.94±0.036 (-0.99%)*	6.44±0.051 (-3.15%)*	10.08±0.020 (-7.86%)*
5	T_4	12.81±0.032 (-1.98%)*	6.18±0.029 (-7.06%)*	9.91±0.015 (-9.41%)*
6	T_5	12.23±0.009 (-6.42%)*	5.91±0.005 (-11.12%)*	9.66±0.017 (-11.70%)*

*Figures in parentheses represent % decrease over control.
**Figures in parentheses represent % increase over



Biochemical content

(A) (B)

Medicago sativa L. Showing Growth in Pot

IV. CONCLUSION

From the results it can be concluded that seedling growth and biochemical parameters affected when treated with dyeing and printing effluent. The effect of dyeing effluent on seedling growth and biochemical parameters of *Medicago sativa* L. was analyzed in the present investigation. Seedling growth in the legume plant was enhanced at T_1 (20%) treatment level increasingly as compared to the control. With the increasing concentration of effluent seedling growth, pigment content, free sugar, protein and amino acid content were reduced or negatively affected. It can be concluded that dyeing effluent as such inhibit the growth whereas with the dilution it promotes the growth parameters of *Medicago sativa* L. Concentrated dyeing effluent was heavily loaded with pollutants which negatively affect plant growth and biochemical contents by interfering with nutrient uptake and physiological process. However, on dilution toxic effects of the effluent were reduced and its effects on growth, physiological and biochemical parameters could be stimulatory rather inhibitory. The untreated dyeing industry effluent could possibly lead to soil deterioration and low productivity. In conclusion, dyeing industry effluent at various concentrations influences seedling growth of *Medicago sativa* L. However the effects vary from crop to crop because each plant species has its own tolerance of the different effluent concentrations.

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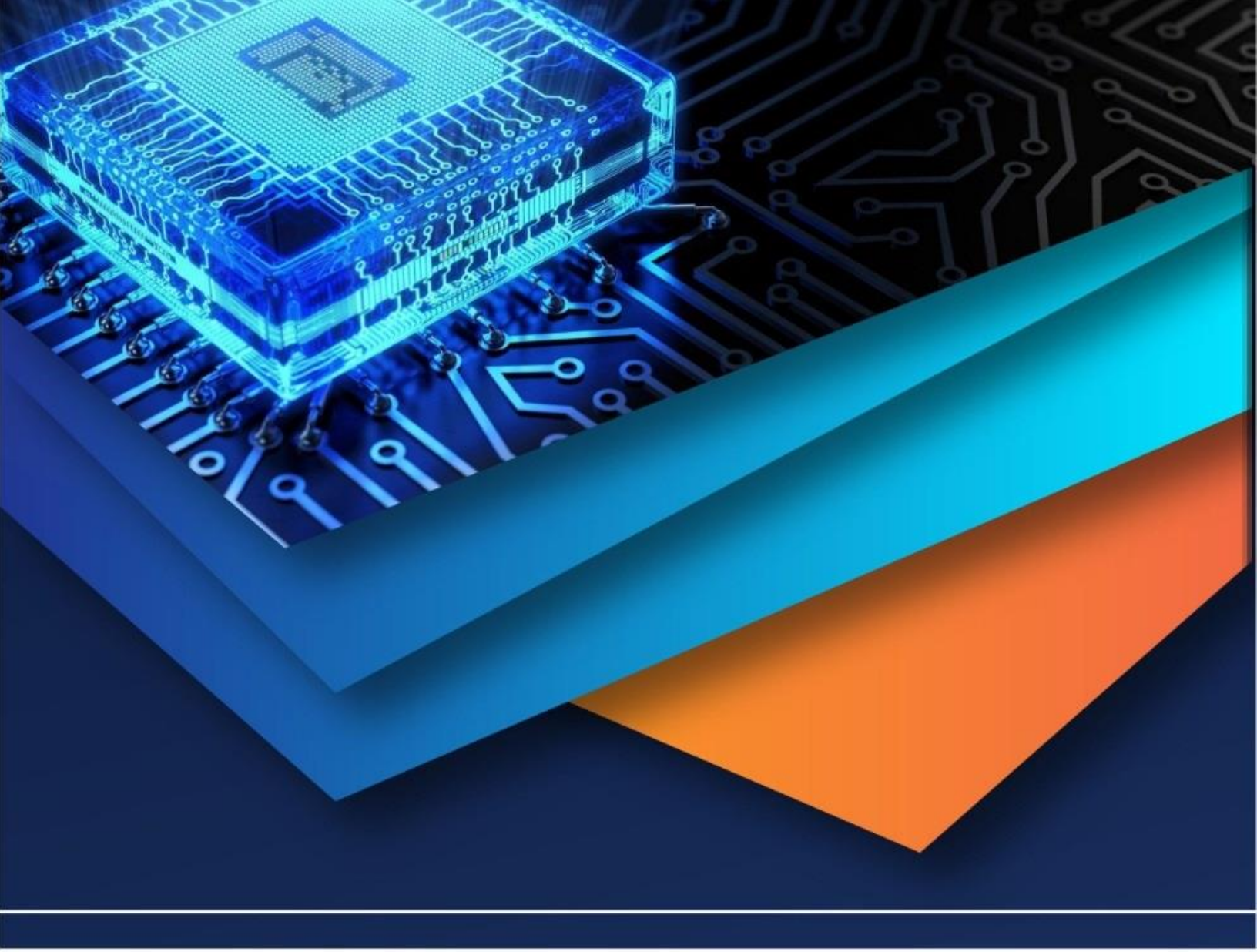

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