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Impact of tannery effluent on growth and biochemical constituents of tomato (*Solanum lycopersicum* L.)

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Abstract

The objective of this piece of work was to assess phytotoxicity and effects on biochemical contents of *Solanum lycopersicum* seedlings cultivated under different concentration levels of tannery effluent (0, 10, 25, 50, 75 and 100 percent) and chromium (2.5, 5, 10, 25 and 50 mg/l). Chromium is among the most hazardous heavy metals found in tannery wastewater. As a result, the various chromium concentrations adopted in the current investigation were analyzed. When compared to control and other effluent treatments, the lower concentration 10 percent of tannery effluent improves tomato growth, photosynthetic pigments, and biochemical alterations such as amino acid, protein, sugar, and proline content. The noxious effects of chromium reduced the growth and other aspects gradually reduced in comparison to control.

Keywords: tannery effluent, phytotoxicity, chlorophyll, proline, chromium, photosynthetic pigments

Introduction

The discharge of various industrial effluents, containing substances ranges from simple nutrients to highly toxic substances, is currently causing an acute pollution problem in our environment. Pollution causes widespread denaturation of the environment, endangering the existence of all organisms. Tannery industry is recognized worldwide as a major industry that is poses most detrimental effects to the environment. They discharge large volumes of effluents that are far from acceptable into waterways (UNIDO, 2005) [14] containing heavy metals such as chromium. Organic compounds found in tannery effluents include protein, fatty acids, tannins, sulphates, hydrocarbons, phenol, detergent, oil, and grease. Tannery industries discharge effluents directly onto agricultural land or the surface of bodies of water, which eventually leach into groundwater, contaminating it with toxic mineral components and causing a slew of well-documented problems in living beings. Chromium exists in many oxidation states of which only Cr(VI) and Cr(III) ions are the most stable under environmental circumstances, chromium (III) is required in the human body, but in very small amounts and relatively immobile, slightly acidic to alkaline and chemically more stable than Cr(VI) and less bioavailable, due to its negligibility and permeable nature to biomembrane. As compared to Cr(III), Cr(VI) is more mobile and water soluble, whereas chromium is also used as a pigment. Chrome yellow, made of lead chromate, was once widely used as a pigment, but its use has declined significantly due to environmental concerns because it contains lead, a toxic material. Cr(III) is primarily required for lipid and sugar metabolism. Hexavalent chromium, on the other hand, is toxic, mutagenic, and carcinogenic (Alpha, 1998). Chromate salts have also been linked to allergic reactions in some people. Because of these dreadful environmental concerns, many countries have placed restrictions on the use of certain chromium compounds.

Materials and Methods

The current study was carried out to arbitrate the effect of different concentrations of tannery effluent (TE) and chromium on tomato seed germination, seedling growth, and biochemical changes in (*Solanum lycopersicum* L.). They were grown the laboratory for one week at temperatures ranging from 30-34^{0}C.

Effluent samples and chromium preparation

The tannery effluent collected from industry located at Vellore, Tamil Nadu, India, was transferred to the Ecology Laboratory, Department of Botany, Annamalai University and stored in a refrigerator at 0-5 degrees Celsius for analysis. The physico-chemical properties of an effluent sample were determined using APHA standard methods.

For chromium treatment, the analytical reagent potassium dichromate ($K_2Cr_2O_7$) was used. In 1000 ml of distilled water, a known weight (5.912 g) of potassium dichromate salt was dissolved. Following concentrations (2.5, 5, 10, 25, 50 mg/L) of were prepared from this stock solution and used in the experiments.

Seed materials

Experimental seeds of *Solanum lycopersicum* were collected from Aduthurai Seed Research Centre, Aduthurai, Tanjore district, Tamil Nadu, India.

Experimental Setup

Germination test accomplished out on tomato seeds that had been exposed to various concentrations of effluent and chromium solutions. Surface sterilized seeds were evenly distributed in petriplates stuffed with filter paper and moistened with tannery effluent concentrations of 10, 25, 50, 75, and 100%. In the case of chromium treatment, the surface sterilized seeds were treated with chromium at various concentrations (2.5, 5, 10, 25, 50 ml/l). Only tap water was administered to control plants. For each treatment, five replicates were kept.

Estimation of photosynthetic pigment and biochemicals

Fresh leaf material from both effluents and chromium-treated seedlings was extracted using 80 percent acetone, and the absorbance was measured in a UV Spectrophotometer at 645, 663, and 480 nm. The pigments chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids were calculated using the methods described by Arnon (1949) [2] and Kirk and Allen (1965) [4]. The protein content was determined using the Lowry *et al*. (1951) method [5]. Moore and Stein (1948) [6] determined the amino acid content. Nelson's (1944) standard method was used to estimate sugars [11].

Statistical analysis

The experiments were carried out using a Completely Randomized Design (CRD). Each treatment was carried out in triplicate, and the Standard Deviation (SD) was computed. The values presented were the mean and ± (SD).

Results and Discussion

Physico-chemical properties of effluent

Table 1 shows the results of the physicochemical characterization of the tannery effluent. The effluent was grey in colour, acidic (pH 4.1-5.7), and contained a higher amount of total dissolved solids (10520 mg/l). The effluent had an electrical conductivity of 15480 M mhos/cm, with a higher biological oxygen demand (940 mg/l) and chemical oxygen demand (4500 mg/l). In addition, tannery effluent contained significant amounts of calcium (640 mg/l), magnesium (367 mg/l), sodium (1900 mg/l), sulphate (2850 mg/l), and chromium (14.34 mg/l). Because tanneries use sodium chloride as a raw material, untreated industrial effluent producing nitrogenous material may also contribute significantly to an increase in nitrate (Mohamed *et al*., 2004) [8]. The tannery effluent's pH acidity, excessive hardness, total suspended solids, BOD, COD, and high salt content indicate that the effluent is highly polluted. Our findings were in consistent with previous findings (Augusthy and Annsherin, 2001).

Table 1: Physico-chemical characteristics of raw tannery effluent and tannery effluent treated experimental soil

S. No.	Physico-chemical parameters	Raw effluent	BIS limits IS 2490-2009	Tannery effluent treated soil
1	Colour	Brown	-	-
2	Odour	Offensive	-	-
3	Turbidity	Turbid	-	-
4	pH	11.4	5.5-9.0	9.3
5	Electrical conductivity (dSm^{-1})	8.99	-	7.45
6	Total hardness (mg/l)	761.00	100	-
7	Total dissolved solids (mg/l)	4133.00	2100	-
8	Total suspended solids (mg/l)	1884.00	100	-
9	Alkalinity	1618.00	NM	-
10	Biological oxygen demand	739.00	30	-
11	Nitrogen (meq/l)	35.88	NM	29.64 (meq/l)
12	Phosphorus (meq/l)	17.85	NM	16.88 (meq/l)
13	Potassium (meq/l)	25.72	NM	19.12 (meq/l)
14	Sodium (meq/l)	149.87	NM	135.10 (meq/l)
15	Chloride (meq/l)	123.63	NM	110.00 (meq/l)
16	Calcium (meq/l)	9.90	NM	8.15 (meq/l)
17	Magnesium (meq/l)	21.84	NM	18.85 (meq/l)
18	Chromium (mg/l)	122.40	2.0	104.00 (mg/kg dr. wt.)
19	Cadmium (mg/l)	78.20	2.0	69.85 (mg/kg dr. wt.)
20	Copper (mg/l)	134.91	NM	114.90 (mg/kg dr. wt.)
21	Lead (mg/l)	155.88	NM	118.60 (mg/kg dr. wt.)
22	Zinc (mg/l)	167.50	1.0	145.12 (mg/kg dr. wt.)

NM – Not mentioned

Germination and seedling growth

Table 2 displays the germination percentage and germination properties. Table 2 shows the plumule and radical length of tomato seeds treated with various concentrations of tannery effluent. The highest seed germination percentage, plumule length, and radical lengths were observed in 10% tannery effluent concentration, followed by control. In a 10% concentration of tannery effluent treatment, the percentages of phytotoxicity values were negative. Simultaneously, as effluent concentrations increased, the germination percentage, plumule length, and radical length decreased gradually.

Table 3 shows the effect of different chromium concentrations on the germination percentage, plumule length, and radical length of tomato seedlings at the same time. There is a solid decrease in germination percentage, plumule length, radical length, fresh weight in chromium treatment and percentage of phytotoxicity of tomato seedling were increased gradually as compared to control. In the present investigation there was no germination recorded beyond the 50 mg/l of chromium concentrations. The increase in germination percentage with respect to control at lower concentrations indicates that the effluent treatment stimulated the dormant seeds seeds in the lot. In lower concentrations, nutrients such as nitrogen, phosphorus, and others present in the diluted effluent may have played a role in promoting plant growth. Similarly, higher concentrations of tannery effluent inhibited tomato germination. It might be due to the effect of higher levels of total solids and heavy metals on seed germination during effluent treatment. The salt content outside the seed is known to act as a liming factor, causing less water absorption by osmosis and inhibiting seed germination (Liu *et al*., 1993).

Germination percentage of chromium treated tomato seeds significantly reduced with the increasing concentration of chromium. The reduction in seed germination caused by chromium stress could be due to chromium's depressive effect on amylase activity and the subsequent transport of sugars to embryo axes. On the other hand, Cr treatment caused increase in protease activity. It also helped to reduce the germination of Cr-treated seeds. It has been analyzed that chromium treatment causes a decrease in both protease and amylase, which is one of the important factors for germination inhibition (Nath *et al.*, 2005). The growth of seedlings was significantly reduced as tannery effluent concentrations increased (Table 4). It could be due to the presence of an excess of trace elements in the effluent. Under higher concentrations of effluent treatment, seed germination would receive a low amount of oxygen, which could have limited energy supply and slowed seedling growth and development. Plant growth inhibition may be caused by chromosomal aberrations that inhibit cell division. Poor seedling growth in chromium treatment may be due to insufficient starch breakdown by amylase activity.

Table 2: Effect of various concentration of tannery effluent in germination behaviour for tomato seedlings

Chromium concentration (mg/l)	Germination percentage	Vigour index	Tolerance index	Percentage phytotoxicity
Control	90.0±4.50	1467.0±73.55	–	–
10	95.0±4.85	1832.0±89.62	1.289±0.061	-9.87±0.448
25	85.0±4.01	1329.0±56.80	0.928±0.052	7.69±0.394
50	75.0±4.35	1104.0±46.20	0.822±0.041	16.86±0.922
75	60.0±3.50	907.0±37.07	0.716±0.037	29.48±1.153
100	55.0±2.75	644.0±27.80	0.642±0.032	41.00±1.667

± Standard deviation

Table 3: Effect of various concentration of chromium behaviour of tomato seedlings

Chromium concentration (mg/l)	Germination percentage	Vigour index	Tolerance index	Percentage phytotoxicity
Control	90.0±4.50	1020±54.0	–	–
10	85±4.25	0970±45.6	0.841±0.040	4.0±0.200
25	75±3.80	920±40.2	0.797±0.031	15.0±0.685
50	60±3.00	860±36.90	0.680±0.027	27.0±1.345
75	45±2.20	770±33.50	0.520±0.024	34.0±1.770
100	20±1.90	560±26.97	0.445±0.0180	49.3±2.460

± Standard deviation

Fresh and dry weight

The fresh and dry weight of tomato seedlings grown in various concentrations of effluent are presented in Table 5. Upto 10%, fresh and dry weights were increased and significantly decreased on increasing concentrations. The fresh weight and dry weight of the plant were also found to decrease as the chromium concentrations increased, as shown in Table 4. The presence of optimal nutrient levels in the 10% tannery effluent concentration effluent may have increased the fresh and dry weight of crop plants. The decrease in plant dry weight might be attributed to poor growth under effluent exposure. Heavy-metal stress, particularly the presence of Cr, has a negative effect on plant dry mass in a fundamentally indirect manner.

Table 4: Plumule of radicals growth of *Solanum lycopersicum* under different concentration of tannery and chromium treatment

Effluent concentration (%)	Tannery effluent		Chromium concentrations (mg/l)	Chromium	
	Plumule	Radical		Plumule	Radical
Control	7.9±0.39	6.45±0.32	Control	6.95±0.35	6.10±0.30
10	9.2±0.04	7.10±0.35	2.5	6.10±0.30	5.65±0.28
25	8.4±0.04	6.80±0.34	5	5.36±0.26	5.10±0.25
50	7.6±0.38	6.20±0.31	10	4.97±0.24	4.40±0.22
75	6.4±0.32	5.25±0.26	25	3.28±0.16	3.10±0.15
100	5.8±0.29	4.22±0.21	50	1.26±0.06	1.15±0.05

± Standard deviation

Table 5: Effect of tannery effluent treatment of fresh and dry weight of *Solanum lycopersicum*

Effluent Concentrations (%)	Tannery effluent		Chromium concentrations (mg/l)	Chromium	
	Fresh weight (mg g^{-1})	Dry weight (mg g^{-1})		Fresh weight (mg g^{-1})	Dry weight (mg g^{-1})
Control	0.478±0.023	0.174±0.008	Control	0.478±0.023	0.174±0.008
10	0.410±0.025	0.132±0.006	2.5	0.433±0.021	0.144±0.007
25	0.382±0.019	0.110±0.005	5	0.400±0.02	0.121±0.006
50	0.344±0.019	0.944±0.004	10	0.386±0.019	0.092±0.004
75	0.299±0.014	0.766±0.003	25	0.340±0.017	0.066±0.003
100	0.267±0.0133	0.527±0.002	50	0.296±0.014	0.044±0.002

± Standard deviation

Photosynthetic pigments

When seedlings were grown in a 10% concentration of tannery effluent, the pigment content such as chlorophyll and carotenoids increased when compared to the control (Table 6). It could be because of the beneficial effect of elements present in effluent on the pigment system. Increasing concentrations of effluent might be destabilizing chloroplast and protein which might deplete the pigment content. The chlorophyll molecules may be converted into phaeophytin, at low pH, magnesium is replaced by hydrogen ions. Magnesium is a component of the chlorophyll molecule and is also necessary for the structural integrity of the chloroplast. Magnesium deficiency and the resulting chlorosis reduced the photosynthetic rate, lowering the carbohydrate and starch content of the crop plant (Vajpayee *et al*., 2000) [15]. In the current study, chlorophyll 'b' and total chlorophyll contents were measured (Table 7). This could be accredited to the toxicity of chromium to the test plant's chlorophyll biosynthesis. In chromium-treated *Nymphaea alba*, -amino laevulinic acid dehydratase activity was reduced, resulting in lower levels of photosynthetic pigments (Singh and Sinha, 2005). Furthermore, lipid peroxidation resulted in the degradation of photosynthetic pigments (Shankar *et al*., 2005). The decrease in photosynthetic pigments could also be attributed to the disruption of chloroplast phosphorylation observed in several plants (Halliwell, 1987) [7]. The decrease in chlorophyll content may be due to distortion of chloroplast structure. Table 7 represents the comparative studies of total chlorophyll and carotenoid contents of tomato grown under different concentrations of chromium treatment. Carotenoid, a nonenzymatic antioxidant, is a part of photosynthetic pigment. It is essential for the protection of chlorophyll pigment under stress conditions by reducing photodynamic reactions that replace peroxidation (Vajpayee *et al*., 2001) [16].

An increase in carotenoid content is thought to be a plant defence mechanism against metal stress. According to our findings, the carotenoid content of *Vallisnaria spiralis* exposed to treatment increases (Magre and Khillare, 2016) [18].

Table 6: Effect tannery effluent on chlorophyll, carotenoid, protein amino acid and sugar content of *Solanum lycopersicum*

Concentrations	**Chlorophyll (mg g^{-1} fr. wt.)**			**Carotenoid (mg g^{-1} fr. wt.)**	**Protein (mg g^{-1} fr. wt.)**		**Amino acid (mg g^{-1} fr. wt.)**		**Sugar (mg g^{-1} fr. wt.)**	
	Chlorophyll ‘a’	**Chlorophyll ‘b’**	**Total chlorophyll**		**Shoot**	**Root**	**Shoot**	**Root**	**Shoot**	**Root**
Control	0.198±0.009	0.110±0.005	0.308±0.015	0.170±0.095	1.916±0.095	1.204±0.060	0.978±0.048	0.710±0.035	1.520±0.076	0.418±0.020
2.5	0.236±0.011	0.149±0.007	0.385±0.019	0.192±0.123	2.468±0.123	1.649±0.082	1.164±0.058	0.833±0.041	2.010±0.100	0.976±0.048
5	0.212±0.010	0.116±0.005	0.328±0.016	0.146±0.107	2.140±0.107	1.344±0.067	0.927±0.046	0.550±0.027	1.898±0.090	0.714±0.035
10	0.193±0.009	0.098±0.004	0.291±0.145	0.120±0.091	1.822±0.091	1.040±0.052	0.768±0.038	0.410±0.020	1.710±0.085	0.628±0.031
25	0.130±0.006	0.073±0.003	0.203±0.010	0.101±0.079	1.598±0.079	0.076±0.003	0.667±0.033	0.320±0.016	1.440±0.072	0.440±0.022
50	0.087±0.004	0.044±0.002	0.131±0.004	0.086±0.050	1.010±0.050	0.034±0.001	0.410±0.020	0.225±0.011	1.200±0.060	0.320±0.016

± Standard deviation

Table 7: Effect chromium treatment on chlorophyll, carotenoid, protein, amino acid and sugar content of *Solanum lycopersicum*

Concentrations	**Chlorophyll (mg g^{-1} fr. wt.)**			**Carotenoid (mg g^{-1} fr. wt.)**	**Protein (mg g^{-1} fr. wt.)**		**Amino acid (mg g^{-1} fr. wt.)**		**Sugar (mg g^{-1} fr. wt.)**	
	Chlorophyll ‘a’	**Chlorophyll ‘b’**	**Total chlorophyll**		**Shoot**	**Root**	**Shoot**	**Root**	**Shoot**	**Root**
Control	0.138±0.006	0.097±0.004	0.235±0.011	0.127±0.006	0.073±0.003	0.043±0.002	0.120±0.006	0.091±0.004	1.102±0.055	0.760±0.038
2.5	0.104±0.005	0.073±0.003	0.177±0.008	0.112±0.005	0.062±0.003	0.039±0.001	0.104±0.005	0.080±0.004	0.920±0.046	0.690±0.034
5	0.094±0.004	0.051±0.002	0.145±0.007	0.092±0.004	0.059±0.002	0.032±0.001	0.090±0.004	0.070±0.003	0.876±0.043	0.626±0.031
10	0.080±0.004	0.044±0.002	0.125±0.006	0.074±0.003	0.044±0.002	0.029±0.001	0.081±0.004	0.066±0.003	0.721±0.036	0.520±0.026
25	0.067±0.003	0.037±0.002	0.104±0.005	0.066±0.003	0.037±0.001	0.024±0.001	0.077±0.003	0.054±0.002	0.533±0.026	0.344±0.017
50	0.044±0.002	0.027±0.001	0.071±0.003	0.043±0.002	0.030±0.001	0.019±0.001	0.066±0.003	0.049±0.002	0.501±0.025	0.289±0.014

± Standard deviation

Estimation of protein and amino acid

Table 6 represents the amount protein and amino acid of tomato under tannery effluent stress. The protein content of seedlings increased at lower tannery effluent concentrations. The highest protein content was found in tannery effluent at 10% concentration. It could be because the plant absorbs the majority of the necessary elements. At the same time, as the concentration of tannery effluent increased, so did the protein content. It has been proclaimed that high concentrations of various cations and anions in effluent caused a decrease in protein content (Vajpayee *et al*., 2000) [15]. A sufficient amount of magnesium is also required to maintain the structure of ribosomes and ribonucleic protein bodies, which are required for protein synthesis. There was a gradual decline in protein and increase in amino acid contents of tomato when exposed to different concentration of chromium (Table 6). Under chromium stress, protein content may degrade due to increased activity of protease or other catabolic enzymes (Magrae and Khillare 2016) [18]. The sulphydryl group of protein may be reduced during heavy metal transport in plants, causing a negative effect in the normal protein form.

Estimation of sugar

The highest sugar content of the seedling was observed at 10% tannery effluent concentration, and above that, the sugar content decreased as tannery effluent concentration increased (Table 5). The trend could be attributed to the lower starch content in higher concentration effluent treatment, implying starch metabolism and poor sugar translocation to the growing part. The excess nitrogen, sodium, and chloride in the effluent inhibit the plant's uptake of other elements such as magnesium, potassium, and phosphorus (Sangeetha and Saravanan, 2018). Magnesium deficiency and the resulting chlorosis reduced photosynthetic rate, lowering crop plant carbohydrate and starch content.

With increasing chromium concentrations, the sugar content decreased (Table 6). The decrease in sugar formation could be linked to lower rates of photochemical activity and chlorophyll synthesis. Sugar formation loss could also be ascribed to the metabolism of sugar for energy requirement when the plants were stressed. It could also be due to an imbalance, which could eventually lead to carbohydrate depletion (Balusamy *et al*., 2019) [19].

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